“Design, Synthesis and Biological Evaluation of Novel and Selective DPP-IV Inhibitors for the Treatment of Type-2 Diabetes’’

A Thesis Submitted to
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For the Degree of

Doctor of Philosophy

in

Chemistry

BY
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CERTIFICATE

This is to certify that the thesis entitled **"Design, Synthesis and Biological Evaluation of Novel and Selective DPP-IV Inhibitors for the Treatment of Type-2 Diabetes''** which is being submitted to The Maharaja Sayajirao University of Baroda, Vadodara for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the original research work conducted by **Mr. Pradipsinh A. Jadav** under our supervision and guidance at Zydus Research Centre, Ahmedabad and the work embodied in this thesis has not formed earlier the basis for the award of any degree or similar title of this or any other university or examining body.

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DECLARATION

I hereby declare that the topic entitled **"Design, Synthesis and Biological Evaluation of Novel and Selective DPP-IV Inhibitors for the Treatment of Type-2 Diabetes''** submitted herewith to The Maharaja Sayajirao University of Baroda, Vadodara for the fulfillment for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the work carried out by me at Chemistry Department, Zydus research Centre, Ahmedabad.

The result of this work has not been previously submitted for any degree/fellowship to any university or institution.

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Place:

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PREFACE

This thesis is the outcome of my Ph.D. work carried out at Zydus Research Centre and the Department of Chemistry, The Maharaja Sayajirao University of Baroda, Vadodara, India.

The thesis consists of four major sections, Introduction, Designing, Results & discussion, experimental and overall summary part which cover various aspects of metabolic diseases and development of DPP-IV inhibitors for the treatment of the Type 2 diabetes. Three papers have been published in international journals.

The '**Introduction**" section deals with the general information about metabolic diseases, wherein detailed pathophysiology and the current therapeutic treatment options are discussed with its limitation, followed by an introduction to DPP-IV inhibitors as a target for the treatment of diabetes.

The "**Designing of DPP-IV inhibitors**" section deals with the strategies and rationale for designing novel and sub-type selective DPP-IV inhibitors.

The "**Results & Discussion**" section describes the synthesis, biological activities and molecular modeling studies of the novel compounds.

In the "**Experimental**" section, detailed procedures for the synthesis of the compounds as well as the characterization data are presented. The details of various biological experiments are also described in this section.

The copy of spectra (ESI-MS, HPLC, ^{1}H-NMR & ^{13}C-NMR) of most of the compounds from each series (intermediates and final compounds) are included at the end of the thesis, followed by copies of our publications.

Working for this thesis has been a great learning experience for me. Understanding the physiological pathway involved in metabolic syndrome and the biological roles of DPP-IV in this complex disease was very interesting and stimulative. Molecular modeling experiments provided good learning and were instrumental in understanding the ligand receptor interaction and structural requirements of the compounds to be synthesized. Presenting the work in the form of publications was equally a good learning experience.

Since this work has been carried out at Zydus Research Centre which is an industrial R&D centre as a medicinal chemist, it gives me a feeling of satisfaction that my drug design strategies and the studies described in this thesis may form the basis for the development of novel DPP-IV inhibitors. The feeling of satisfaction is not only for the scientific outcome of the research work but also as it contributes for the social cause, since the ultimate need for the treatment of metabolic disorders such as diabetes. My philosophy is aline with the mission statement of my organization which says that,

"*ZRC aims to be the most admired pharmaceutical research centre for innovation in life science dedicated to alleviating human suffering*"

Human suffering is increasing day by day owing to various life threatening diseases and due to absence of treatment or resistance to treatment. Current understanding of metabolic diseases and treatment options are good but not adequate enough. Hence every endeavor in the direction of developing novel therapies in this area would be a significant contribution towards alleviating human suffering.

Pradipsinh A. Jadav

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*Finally, no words can express the feeling towards my spouse-****Sandhya****, daughters-****Shruja*** *&* ***Netra****, parents-****Arjunsinh*** *&* ***Manharba****, parents in law-* ***Harisinhji Solanki*** *&* ***Padmaba*** *and other family members, who have contributed and sacrified a lot to reach me at this stage and will always remain a sole source of inspiration in my life.*

Pradipsinh A. Jadav

Dedicating to my lovely;

Wife: Sandhya

Daughters: Shruja & Netra

Abbreviations used

MS	Metabolic syndrome
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
CAD	coronary artery disease
GIT	Gastrointestinal tract
T1DM	Type I diabetes mellitus
IDDM	Insulin-dependent diabetes mellitus
T2DM	Type II diabetes mellitus
GDM	Gestational diabetes mellitus
GLP-1	Glucagon Like Peptide-1
GIP	Gastric inhibitory polypeptide
DPP-IV	Dipeptidyl peptidase type IV
PC2	prohormone convertase-2
NEP	Neutral endopeptidases
GI	Gastrointestinal
PPARγ	Peroxisome proliferators-activated receptor gamma
11β-HSD-1	11β-hydroxysteroid dehydrogenase type-1
FBPase	Fructose 1 6-bisphosphatase
GSK-3	Glycogen synthase kinase-3
SGLT2	Sodium-dependent glucose cotransporters 2
PTP-1B	Protein tyrosine phosphatases 1B
USFDA	United States Food and Drug Administration
CD26	Cluster of differentiation 26
FAP	Fibroblast activation protein
POP	Prolyl oligopeptidase
QPP	Quiescent cell prolin dipeptidase
NPY	Neuropeptide Y
I-TAC	Interferon-inducible T-cell *α* chemoattractant
GHRH	Growth-hormone-releasing hormone
IL-1	Interleukin 1
CYP	Cytochrome P450
BMS	Bristol-Myers Squibb
HbA1c	Hemoglobin A1c
DDI	Drug-drug interactions

IDT	Idiosyncratic drug toxicity
IR	Insulin receptor
IRTK	Insulin receptor tyrosine kinase
IRS	Insulin receptor substrate
GLUT-4	Glucose transporter-4
MOA	Mechanism of action
β-PPA	β-phenyl phenyl alanine
GABA	γ-Amino butanoic acid
PABA	*Para*-Amino benzoic acid
PMB	*Para*-Methoxy benzyl
Aib	Amino isobutyric acid
Phg	Phenyl glycine
Chg	Cyclohexyl glycine
Phe	Phenyl alanine
His	Histidine
Ala	Alanine
Gly	Glycine
Arg	Arginine
Ile	Isolucine
Leu	Leucine
Val	Valine
Ser	Serine
Asp	Aspartic acid
Lys	Lysine
Asn	Aspargine
Tyr	Tyrosine
Trp	Tryptophan
IZD	Isothiazolidinone
TZD	Thiadiazolidinone
PK	Pharmacokinetic
PD	Pharmacodynamic
SAR	Structure activity relationship
DAST	Diethylaminosulfur trifluoride
HOBt	1-Hydroxy benzotriazole
Na_2CO_3	Sodium carbonate
NaHMDS	Sodium hexamethyldisilazane

NHS	N-Hydroxy succinimide
Cbz-Cl	Carboobenzoxy Chloride/ Benzyl Chloroformate
Fmoc-OSu	N-(9-Fluorenylmethoxycarbonyloxy) succinimide
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
$Pd(OH)_2$	Palladium hydroxide
BF_3-Et_2O	Boron trifluoride diethyl etherate
$LiAlH_4$	Lithium aluminiumhydride
$SOCl_2$	Thionyl chloride
THF	Tetrahydrofuran
NaH	Sodium hydride
CBr_4	Carbon tetrabromide
$NaBH_4$	Sodium borohydride
PPh_3	Triphenyl phosphine
DMF	*N*,*N'*-dimethyl formamide
DCM	Dichloromethane
H_2	Hydrogen
HI	Hydroiodic acid
OGTT	Oral glucose tolerance test
IPGTT	Intraperitoneal glucose tolerance test
IC_{50}	half maximal inhibitory concentration
i.v.	Intravenous
i.p.	Intraperitoneal
p.o.	Oral
%F	Oral bioavailability
AUC	Area under curve
IFD	induced-fit docking
ACN	Acetonitrile
D. M.	Demineralized /Deionized
DIC	1,3-diisopropylcarbodiimide
SPPS	Solid Phase Peptide Synthesis
EDC	Ethylene dichloride
HPLC	High-performance liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry

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Chapter I:

Introduction

"Imagination is more important than knowledge."-Einstein

1. Introduction

1.1. Metabolic syndrome

The term “Metabolic syndrome” (MS) refers to a cluster of medical disorders such as, hyperinsulinemia, hyperglycemia, dyslipidemia, high blood pressure, insulin resistance and obesity. Metabolic syndrome increases the risk of developing cardiovascular diseases and diabetes. The incidences of metabolic syndrome have reached global epidemic proportions **[1-2]**. Metabolic syndrome is also known as metabolic syndrome X, cardiometabolic syndrome, syndrome X, insulin resistance syndrome, Reaven’s syndrome (named after Gerald Reaven) and CHAOS (in Australia).

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health and is a major risk factor for developing the other metabolic diseases. Obesity as a metabolic disorder was reported in 1947 **[3]** and subsequently described as a syndrome, which comprised of hypertension, hyperglycemia **[4]**. After a gap of four decades, in 1988 a cluster of risk factors for diabetes and cardiovascular diseases were defined and was named as Syndrome X **[5]**. Consequently, patients with metabolic syndrome are at increased risk of micro- and macro-vascular complications (e.g. coronary artery disease (CAD), stroke, renal failure, blindness and lower extremity amputation) as diabetes progress **[6-7]**.

1.2. Diabetes

Diabetes mellitus is a group of metabolic diseases in which hyperglycemia arises as a result of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both **[8]**. Diabetes is an ailment in which the body does not produce or properly use insulin. Insulin is a regulatory hormone required for energy management. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 220 million people in 2010 and it is expected to increase to 366 million people by 2030 **[9]**. Many people also have other abnormalities of glucose metabolism (sometimes called “prediabetes”) manifest either as impaired fasting glucose (IFG) levels or as impaired glucose tolerance

(IGT). The criteria for diagnosis of diabetes and prediabetes conditions are summarized in **Table 1**.

Table 1. Diagnostic Criteria for Diabetes Mellitus and Prediabetes conditions

Types of Diabetes	Pre prandial fasting plasma glucose mg/dL (mmol/L)	Post-prendial plasma glucose mg/dL (mmol/L)
Normal	**< 110 (< 6.1)**	**< 140 (< 7.8)**
Impaired fasting glucose (IFG)	**≥ 100 (≥ 6.1) &< 120 (< 7.0)**	**< 140 (< 7.8)**
Impaired glucose tolerance (IGT)	**< 126 (< 7.0)**	**≥ 140 (≥ 7.8)**
Diabetes mellitus	**≥ 126 (≥ 7.0)**	**≥ 200 (≥ 11.1)**

Majority of diabetic patients can be treated with the agents that reduce hepatic glucose production (glucagon antagonist), reduce glucose absorption from gastrointestinal track (GIT), stimulate β-cell function (insulin secretagogues) or with the agents that enhance the tissue sensitivity of the patients towards insulin (insulin sensitizers). The drugs presently used to treat diabetes include α-glucosidase inhibitors, insulin sensitizers, insulin secretagogues and K_{ATP} channel blockers **[10]**. However, almost one-half of diabetic subjects lose their response to these agents, over a period of time and thereby to insulin therapy. Insulin treatment has several drawbacks, it is injectable, causes hypoglycemia and weight gain **[11]**.

1.2.1. Types of diabetes

Although several pathogenic processes are involved in the development of diabetes, the vast majority of cases fall into two main categories: Type 1 diabetes

and Type 2 diabetes. Gestational diabetes, yet another type of diabetes diagnosed in pregnant women.

1.2.1.1. Type 1 diabetes mellitus (T1DM)

Type 1 diabetes occurs usually due to an immune-mediated destruction of pancreatic islet β-cells with consequent insulin deficiency. Although usually having an abrupt clinical onset, the disease process unfolds slowly, with progressive loss of β-cells. In T1DM, >90% β-cells are destroyed by autoimmune-mediated islet cell destruction, and hence T1DM patient rely on insulin injections for survival. T1DM is usually diagnosed in children and young adults, it is also called as juvenile diabetes or insulin-dependent diabetes mellitus (IDDM). Conditions associated with T1DM include hyperglycemia and ketoacidosis (**Figure 1**). T1DM increases risk for many serious complications. Some complications of T1DM includes: heart disease (cardiovascular disease), blindness (retinopathy), nerve damage (neuropathy), kidney damage (nephropathy), foot and skin complications and depression.

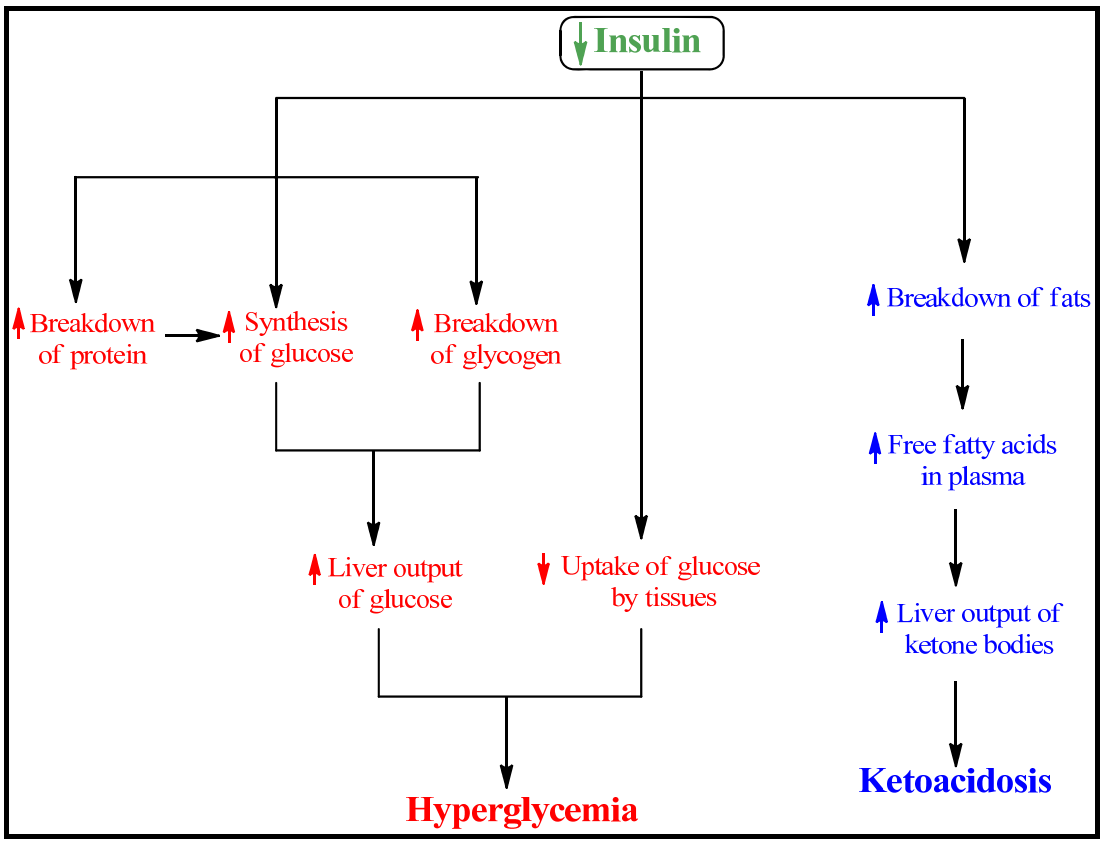


Figure 1. Metabolic Changes in T1DM

1.2.1.2. Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM), the most common type of diabetes usually occurs due to insulin resistance, defect in the insulin production or increase in the hepatic glucose production and is usually associated with dyslipidemia, hypertension and obesity **[12]**.

In T2DM, >50% of β-cells are already lost at the time of diagnosis continue to decline throughout the course of T2DM, mainly due to apoptosis **[13]**. As depicted in **Figure 2**, insulin resistance arises as a consequence of multiple factors such as sedentary lifestyle, aging and obesity which results in hyperglycemia, blood pressure elevation, and dyslipidemia. The important contributing factors for T2DM include resistance to insulin, increased hepatic glucose production, decreased insulin-mediated glucose transport into adipose tissues and impaired β-cell function leading to loss of early phase of insulin release.

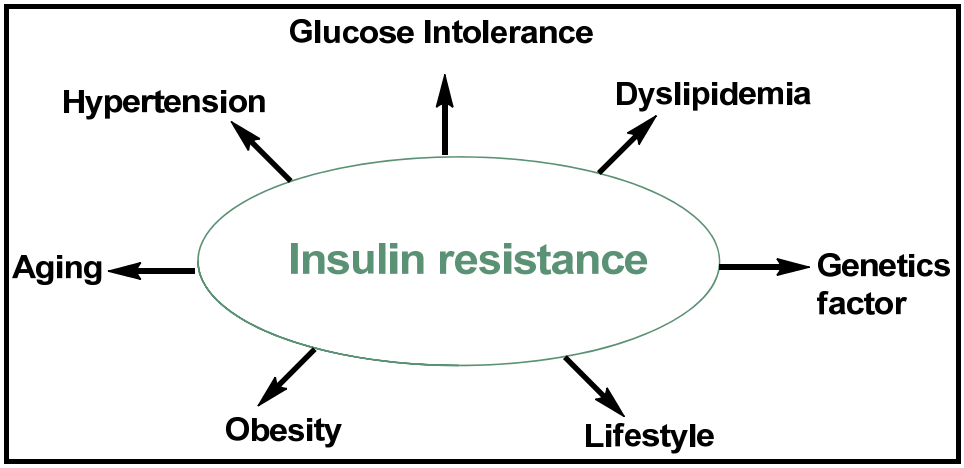


Figure 2. Causes and consequences of Insulin resistance

In T2DM, patients begin with insulin resistance and often treated with various oral antihyperglycemic agents; however, over a period of time, almost one-half of T2DM subjects lose their response to these agents and thereby require insulin therapy **[14]**. The decline in β-cells in T2DM drives the progressive deterioration in glycemic control and develops secondary complications.

1.2.1.3. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy (especially during third trimester of pregnancy). Gestational diabetes occurs when

the body of a pregnant woman does not secrete excess insulin required during pregnancy leading to increased blood sugar levels.

Gestational diabetes generally has few symptoms and it is most commonly diagnosed by screening during pregnancy. Diagnostic tests detect inappropriately high levels of glucose in blood samples. Gestational diabetes affects 3-10% of pregnancies, depending on the population studied **[15]**. In general, babies born to mothers with gestational diabetes are typically at increased risk of problems such as being large for gestational age (which may lead to delivery complications), low blood sugar, and jaundice. Gestational diabetes is a treatable condition and women who have adequate control of glucose levels can effectively decrease these risks **[16]**.

1.3. Pathogenesis of T2DM

The pathological sequence of T2DM is complex and involves many different elements that act together and make T2DM condition more complex (**Figure 3**). As described earlier, T2DM is characterized by varying degree of insulin resistance and insulin deficiency. It isthought that the earliest defect in the pathogenesis of T2DM is impaired insulin action or insulin resistance.

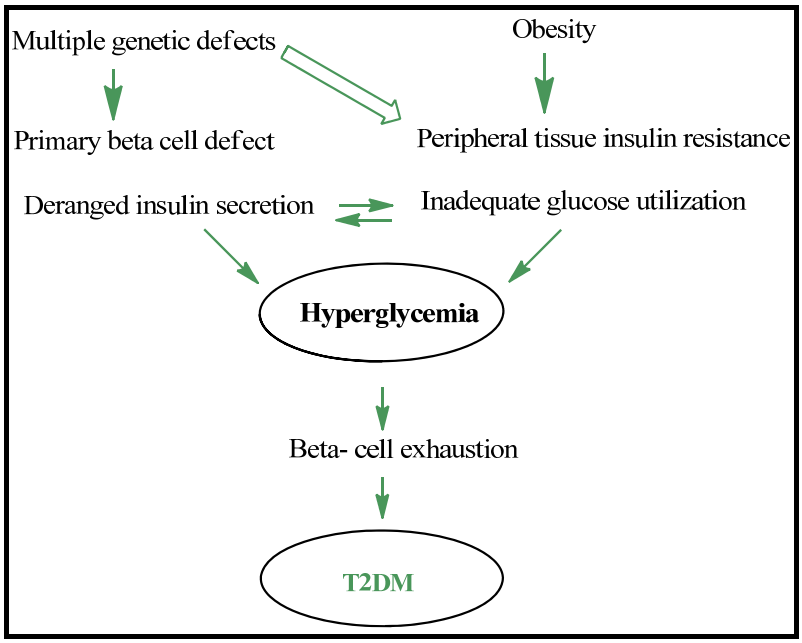


Figure 3. Proposed Pathogenesis of Type 2 Diabetes

Resistance to the action of insulin results in impaired insulin mediated glucose uptake by muscles, incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance, beta islet cells will increase the amount of insulin secreted.

Along with different factors, both endogenous hormone Glucagon like peptide-1 (GLP-1) and glucagon play an important role in pathogenesis of T2DM **[17]**. GLP-1 (7-36) amide is a product of the preproglucagon gene, which is secreted from intestinal L-cells, in response to the ingestion of food. Endogenous GLP-1 binds to a membrane GLP-1 receptor. As a consequence of this, insulin release from the pancreatic β-cells is increased **[17]**. The major problem of GLP-1 is its shorter half life. Glucagon (29 amino acid peptide) hormone is produced from proglucagon in pancreatic α-cell by prohormone convertase-2(PC2)**[17]**. The main physiological role of glucagon is to stimulate hepatic glucose output, thereby leading to hyperglycemia. Therefore two defects, insulin resistance and insulin deficiency are responsible factors for the development of T2DM.

1.4. Current & Newer therapies for the treatment of T2DM

1.4.1. Current therapies for the treatment of T2DM

The cornerstone of treatment and prevention of T2DM is lifestyle modification through increased physical activity and attention to food intake, particularly among the subjects, where in weight loss is the principal goal. When lifestyle modifications do not result in normalization or near normalization of metabolic abnormalities, pharmacological therapy is required.Based on route of administration, current therapies are divided in two groups, (1) Injectable and (2) Oral therapies.

1.4.1.1. Injectable therapies for the treatment of T2DM

Insulin facilitates glucose entry into adipose tissues, muscles, and liver by stimulating several enzymatic reactions that start at the insulin receptors. The stimulation of an intrinsic tyrosine kinase of the insulin receptor results in an increase in membrane phosphorylation that consequently increases the membrane permeability to glucose through a complicated cascade of intracellular events. Currently available injectable analogues are divided into two groups, (1) insulin analogues and (2) incretin mimetics as shown in **Table 2**. Further, insulin analogues are sub-divided into three groups depending upon their duration of action.

As described earlier, in T1DM >90% β-cells are destroyed and hence T1DM patients rely on insulin injection for survival. While T2DM begins with insulin resistance and over a period of time almost one-half of T2DM patients lose their response to antihyperglycemic agents and thereby require insulin therapy **[13]**.

Table 2. Insulin analogues and incretin mimetics.

Drugs	Source	Trade names
Insulin product **Fast acting:** **Regular**	Recombinant DNA Pork	Humulin R, Novolin R Iletin II
Lispro	Recombinant DNA	Humalog, Humalog, Lispro-PFC
Aspart	Recombinant DNA	Novolog, Flexpen
Glulisine	Recombinant DNA	Apidra
Intermediate acting: **Isophane Insulin**	Pork	Iletin II NPH Purified Pork
Isophane Insulin	Recombinant DNA	Humulin N, Novolin N
Insulin zinc	Pork	Iletin II Lente
Insulin humane zinc	Recombinant DNA	Humulin L, Novolin Ge Lente
Long acting: **Extended insulin human zinc suspension**	Recombinant DNA	Humulin U, Novolin ge Ultralente
Insulin glargine	Recombinant DNA	Lantus
Incretin mimetics **Exenatide**	Saliva of the Gila monster	Byetta
Liraglutide	----	Victoza

The effect of insulin on glucose uptake and metabolism is shown in **Figure 4**. Secreted insulin binds to its receptor, which in turn starts many protein activation cascades. These cascades include translocation of Glut-4 transporter to the plasma

membrane and influx of glucose, glycogen synthesis, glycolysis and fatty acid synthesis. The main action of insulin on cells includes increased glycogen synthesis, increased fatty acid synthesis, increased esterification of fatty acid, decreased proteolysis, decreased lipolysis and decreased gluconeogenesis**[18]**.

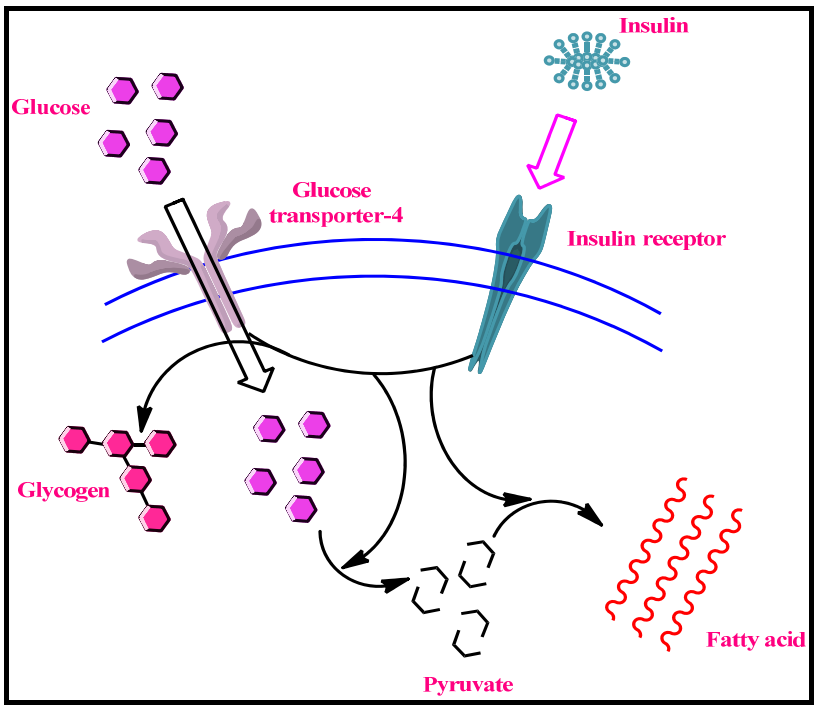


Figure 4. Mode of action of insulin on glucose uptake

Exenatide and liraglutide belong to group of incretin mimetics. Exenatide is a 39-amino-acid peptide, an insulin secretagogues, with glucoregulatory effects. It bears a 50% amino acid homology to GLP-1 and it has a longer half-life. Liraglutide is an acylated human GLP-1 receptor agonist, with a 97% amino acid sequence identity to endogenous human GLP-1(7-37).

Liraglutide is stable against metabolic degradation by both peptidases,dipeptidyl peptidase IV (DPP-IV) and neutral endopeptidases (NEP). Exenatide augments pancreas response **[19]** (i.e. increases insulin secretion) in response to eating meals; the result is the release of a higher, more appropriate amount of insulin that helps lowering the rise in blood sugar. It also suppresses pancreatic release of glucagon in response to eating. Exenatide helps to slow down gastric emptying and reduces liver fat content. While liraglutide acts in a glucose-dependent manner, meaning it will stimulate insulin secretion only when blood glucose levels are higher than normal. It has the potential for inhibiting apoptosis and stimulating regeneration of beta cells. It

decreases appetite and maintains body weight and lowers blood triglyceride levels**[20]**.

These injectable therapies (Insulin &incretin mimetics) have several drawbacks, it is injectable, produces hypoglycemia and causes weight gain, which is believed to be a potential cause for the development of diabetes complications **[21]**. Thus, there is an urgent need to develop some oral antihyperglycemic agents that can complement with the existing injectable therapies.

1.4.1.2. Oral antidiabetic agents for the treatment of T2DM

Before 1995, sulfonylureas were the only oral antidiabetic agents available for the treatment of T2DM. Since 1995, there has been an explosion of introduction of new classes of pharmacologic agents. Currently available oral antidiabetic therapies includes agents which cause insulin production (sulfonylureas, secretagogues); agents which decrease hepatic glucose production (biguanides); agents which act as insulin sensitizers (glitazones) and R-glucosidase inhibitors, which are listed in **Table 3**.

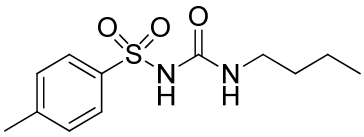
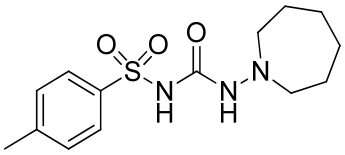
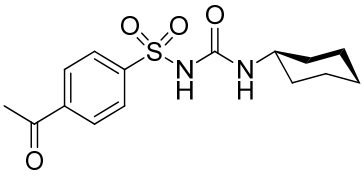
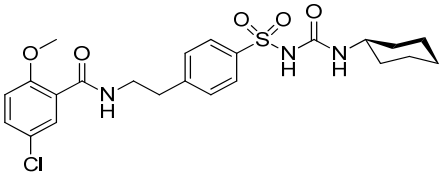
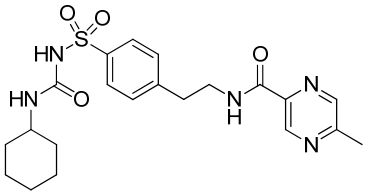
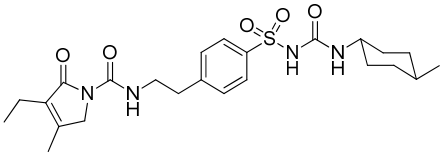
The usual treatment strategy in T2DM is to start with either metformin **[24]** or a secretagogue **[23]**. If adequate control is still not achieved, the second step is to add a complementary drug, i.e. one working by a different pathway. The most common such combination is metformin plus a secretagogue. If adequate glycemic control is still not attained, the choice is to add a third class of oral drugs (e.g. glitazone or glucosidase inhibitors).

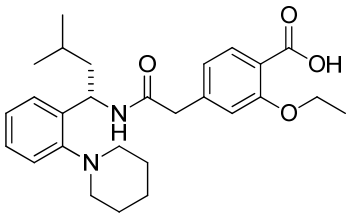
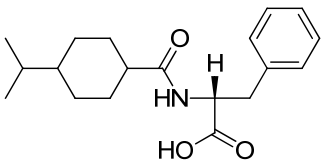
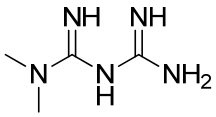
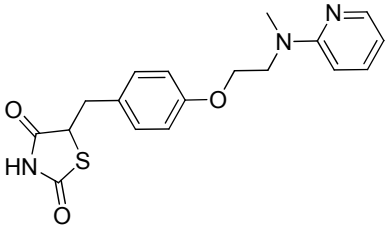
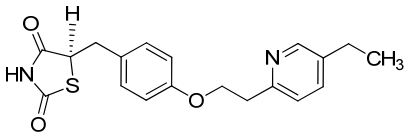
However, most of the oral antihyperglycemic agents are also associated with side effects and adverse events. In case of sulfonylureas, they work by stimulating endogenous release of insulin and exhibit hypoglycemia as the major side effects. The other adverse effect consists of digestive manifestation (nausea, epigastic pain, liver pain) and of hematological manifestations (pancytopenia, autoimmune hemolytic anemia, thrombocytopenia) **[22]**.

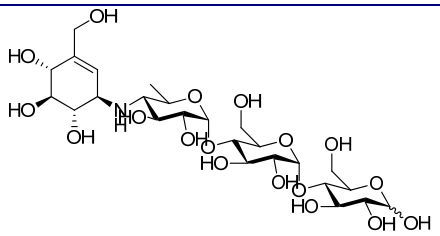
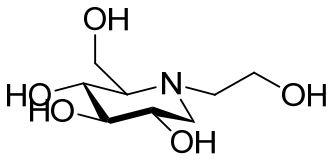
Biguanides reduce hepatic glucose output and increase uptake of glucose by periphery. They are associated with side effects such as digestive manifestations especially epigastric pain and diarrhea. Lactic acidosis is another adverse effect associated with biguanides. The major side effects associated with glitazones are mild edema of the lower limbs, through the loss of elimination of salt and water. The other adverse effect is decrease in hemoglobin, with the appearance of anemia.

Glitazones can also cause hypercholesterolemia and triglycerides disorders. Alpha glucosidase inhibitors exhibit side effects such as weight gain, abdominal bloating, flatulence, abdominal discomfort and diarrhea.

Table 3. Orally administered antidiabetic agents for T2DM treatment

Drug class	Agent (Brand name)	Structure
Sulfonylureas[22]		
First generation	Tolbutamide (Oramide, Orinase)	
	Tolazamide (Tolamide, Tolinase)	
Second generation	Acetohexamide (Demylor)	
	Glyburide (Micronase, Diabeta)	
	Glipizide (Glucotrol)	
Third generation	Glimepride (Amaryl)	
M.O.A- Stimulating insulin production by inhibiting the K_{ATP} channel in pancreatic β- cells		

Non-Sulfonylurea[23] **Secretagogues**	Repaglinide (Prandin)	
	Nateglinide (Starlix)	
M.O.A-enhance insulin secretion in pancreatic β- cells		
Biguanides[24]	Metformin (Glucophage)	
M.O.A- Decreases insulin resistance in liver		
Insulin receptors sensitizers[25]	Rosiglitazone (Avandia)	
	Pioglitazone (Actors)	
M.O.A- stimulates PPAR-γ and PPAR-α, reduces insulin resistance in the liver and peripheral tissues		

Alpha-Glucosidase Inhibitors[26]	Acarbose (Prelose)	
	Miglitol (Glyset)	
M.O.A- Reduces intestinal glucose absorption in G.I tract		

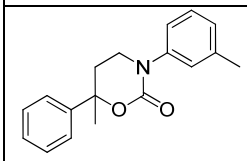
1.4.2. Newer therapies for the treatment of T2DM

As described earlier, most of the injectable and oral therapies exhibit serious side effects and adverse events such as hypoglycemia, GI side effects, lactate production, fluid retention, hepatotoxicity, allergic reaction and cardiovascular effects. Also long term use exhibits side effects such as body weight gain and progressive loss of β-cell function. To overcome the side effects and safety concern of these current drugs, several newer pharmacologic approaches have been developed for the safe and effective treatment of T2DM. Currently several new therapies are in various stages of clinical development for the treatment of T2DM as shown in **Table 4**.

Table 4. New therapies under clinical development for the treatment of T2DM

Target	Structure/ Name	Company	Clinical status	Ref.
GLP-1 agonist*	ZYOG1	Zydus Cadila	Phase I	**[27]**
	PB-1023	PhaseBio	Phase II	**[28]**
	NNC-0113-0987	Novo Nordisk	Phase I	**[29]**
	NOX-G15	Noxxon	Preclinical	**[30]**

Target	Structure/ Name	Company	Clinical status	Ref.
DPP-IV Inhibitors *	Sitagliptin(MK-0431)	Merck	Launched	**[31]**
	Vidagliptin (LAF-237)	Novartis	Launched	**[32-35]**
	Saxagliptin(BMS-477118)	Astra-zeneca/BMS	Launched	**[36-38]**
	Linagliptin(BI-1356)	Boehringer Ingelheim/Eli Lilly	Launched	**[39-40]**
	Alogliptin(SYR-322)	Takeda	Launched	**[41]**
	Anagliptin(SK-0403)	Kowa JW Pharmaceutical	Launched	**[42-43]**
	Gemigliptin(LC15-0444)	LG Life Sciences	Launched	**[44-45]**
	Teneligliptin(MP-513)	Mitsubishi Tanabe Pharma	Launched	**[46-48]**
	Melogliptin(GRC-8200)	Glenmark	Phase II	**[49]**
	Gosogliptin(PF-734200)	SatRx	Phase II	**[50-52]**
	Trelagliptin(SYR-472)	Takeda/Furiex	Phase III	**[53]**
	ARI-2243	Arisaph Pharmaceutical	Phase I	**[54-55]**
	Omarigliptin(MK-3102)	Merck & Co	Phase III	**[56]**
	Evogliptin(DA-1229)	Dong-A	Phase II	**[57-58]**

Target	Structure/Name	Company	Clinical status	Ref.
FBPaseinhibitors**	CS-917	Metabasis Therapeutics	Discontinued(Phase II)	**[59]**
GSK-3 inhibitors**	DM-199	DiaMedica	Preclinical	**[60]**
	DM-204	DiaMedica	Preclinical	**[61]**
11β-HSD-1 inhibitors**	BMS-770767	Bristol-Mayers Squibb	Phase II	**[62]**
	RG-4929	Roche	Phase II	**[63]**
	RG-7234	Roche	Phase I	**[63]**
	BVT-3498	Biovitrum	Discontinued Phase II	**[63]**
	PF-915275	Pfizer	Discontinued Phase I	**[64]**
		Vitae Pharmaceutical	Preclinical	**[65]**
	INCB-13739	Incyte Corporation	Phase IIb	**[66]**
	INCB-20817	Incyte Corporation	Discontinued Phase I	**[67]**
	AZD4017	AstraZeneca	Phase I	**[68]**
	AMG-221	Biovitrum	Phase I	**[69]**

Target	Structure/Name	Company	Clinical status	Ref.
SGLT2 Inhibitors*	Canagliflozin(TA-7284)	Mitsubishi Tanabe Pharma	Under development	**[70]**
	Dapagliflozin(TA-7284)	Bristol-Myers Squibb	Pre-registration	**[71]**
	Empagliflozin(BI-10773)	Boehringer Ingelheim	Phase III	**[72-73]**
	Tofogliflozin(RG-7201)	Roche	Phase III	**[74]**
	Remogliflozin etabonate(KGT-1681)	Kissei	Phase II	**[75-78]**
	Ipragliflozin(ASP-1941)	Kotobuki Pharmaceutical	Phase III	**[79]**
	Luseogliflozin(TS-071)	Taisho	Phase III	**[80-81]**
	SBM-TFC-039	Sirona Biochem	Preclinical	**[82]**
	THR-1474	Theracos	Phase II	**[83]**
	Ertugliflozin(PF-04971729)	Pfizer	Phase II	**[84-86]**
	EGT-0001442	Theracos	Phase II	**[87]**

Target	Structure/ Name	Company	Clinical status	Ref.
PPARγ dual agonist**	Efatutazone(CS-7017)	Daiichi Sanky	Phase II	**[88]**
	Chiglitzar(CS-0038)	Chipscreen Biosciences	Phase III	**[89]**
	Libeglitazone(CKD-501)	Chong Kun Dang	Phase III	**[90-91]**
	PPARγ agonist	Takeda	Preclinical	**[92-93]**
PTP-1B Inhibitors*	Ertiprotafib	Wyeth Pharmaceutical	Discontinued	**[94]**
	TTP-814	Trans Tech Pharma	Phase II	**[95]**
	ISIS-PTP1BRx	Isis Pharmaceutical	Phase I	**[96]**
	PTP1B Inhibitors	Advinus	Preclinical	**[97]**

GLP-1: Glucagon-like peptide 1; **DPP-IV**: Dipeptidyl peptidase IV; **PPARγ:** Peroxisome proliferators-activated receptor gamma; **11β-HSD-1**:11β-hydroxysteroid dehydrogenase type-1; **FBPase**: Fructose 1 6-bisphosphatase ; **GSK-3**: Glycogen synthase kinase-3; **SGLT2**: Sodium-dependent glucose cotransporters; **PTP-1B**: Protein tyrosine phosphatases 1B.* Injectable; ** Oral

As described in **Table 4**, currently several new therapies are in various stages of clinical development for the treatment of T2DM. Among these new therapies, DPP-IV inhibitors and PTP-1B inhibitors are most promising. Endogenous dipeptidyl peptidase type IV (DPP-IV) enzyme has been shown to be a key physiological regulator of incretin activity. DPP-IV is a serine protease and *in vivo*, it inactivates both the incretin hormones GLP-1 & Gastric inhibitory peptide (GIP) **[98-100]**, which in-turn stimulates glucose dependent insulin secretion. Thus, inhibition of DPP-IV activity results in increased level of intact bioactive GIP and GLP-1 peptides, which cause an increase in the amount of post prandial insulin release from β-cell of the islets, thereby, it acts as an antidiabetic agent.

Protein tyrosine phosphatase-1B (PTP-1B) enzyme leads to dephosphorylation of insulin receptor and acts as a negative regulator in insulin signaling pathway **[101-102]**. The two different reports suggest that PTP-1B knock-out mice showed improved insulin sensitivity **[103-104]**. Thus, inhibition of PTP-1B could be the most effective and safe target for the treatment of T2DM.

Among the newer therapies, GLP-1 agonists have not been very successful due to little risk of hypoglycemia **[105]**, gastrointestinal side effects **[106]** and their injectable route of administration. Main side effect of PPARγ dual agonist is water retention, leading to edema, an increased risk of coronary heart disease **[107-108]**. Compounds of FBPase inhibitors show hypoglycemia as a major side effect **[73]**. Inhibitors of GSK-3 are associated with side effects such as neuronal disorders **[109]**, while inhibitors SGLT2 and 11β-HSD-1 have low potential for hypoglycemia.

As discuss earlier, inhibitors of DPP-IV and PTP-1B are the most upcoming therapies for T2DM treatment. Many PTP-1B inhibitors are being manufactured and studied. However, the drawbacks of PTP1B inhibitors include their low affinity, selectivity and membrane permeability**[110-111]**.While, treatment of T2DM with DPP-IV inhibition is clinically proven therapy and several DPP-IV inhibitors are in market **[112]**.Long-term inhibition of DPP-IV improves glucose tolerance and preservesislet function in mice**[113]**..Apart from T2DM, DPP-IV inhibitors are also believed to be useful for several other related disease conditions such as diabetic dyslipidemia, conditions of impaired glucose tolerance (IGT), conditions of impaired fasting plasma glucose (IFG), metabolic acidosis and ketosis, appetite regulation and obesity**[114-118]**.

Hence, DPP-IV inhibitors are considered as one of the best validated biological target for T2DM.

Overall among different therapies such as agents which cause insulin secretion, agents which decrease hepatic glucose production and agents which decrease insulin resistance, it can be concluded that the insulin resistance is the most dominant cause for T2DM, hence increasing insulin sensitivity can form a promising therapy for the treatment of T2DM. Various drugs are available that directly or indirectly increase insulin sensitivity but their high cost, selectivity and route of administration are limiting factors. This invites research to develop small molecule based DPP-IV inhibitors which could be safe and cost effective. There are currently eight DPP-IV inhibitors approved worldwide with several more on the way.

Though efficacious and safer treatment are available in the market under DPP-IV tag, considering the seriousness of the growing prevalence of diabetes worldwide, particular research efforts being made from both the academia and the pharmaceutical industry to develop long acting DPP-IV inhibitors. Thereby to meet the regulatory compliances of USFDA for the drug evaluated with regards to their cardiovascular safety as well as to additional effects that DPP-IV inhibition may exert other than antidiabetic effect.

The research presented in this thesis focus on the synthesis, biological evaluation of potent, selective and orally bioavailable DPP-IV inhibitors. In the next section, an overview on DPP-IV inhibitorsare presented.

1.5.Introduction to Dipeptidyl Peptidase IV (DPP-IV) inhibitors

1.5.1.DPP-IV and their importance

The incretin mimeticsglucagon-like peptide 1 (GLP-1) and glucose-dependent gastric inhibitory polypeptide (GIP) are released from the L-cell of intestineupon injestion of food **[119-122]**. Thesehormones regulate insulin secretion in a glucose-dependent manner. GLP-1 has many roles in the human body; it stimulates insulin biosynthesis, inhibits glucagon secretion, slows gastric emptying, reduces appetite and stimulates regeneration of islet β-cells. GIP and GLP-1 have extremely short plasma half-lives, get inactivated by DPP-IV enzyme**[123-124]**.

Dipeptidyl peptidase-IV (DPP-IV), also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26) (Enzyme Commission no.: E.C. 3.4.14.5). Itis a serine protease enzyme,which cleaves the N-terminal dipeptide (X-Ala or X-Pro), from target polypeptides, such as chemokines and peptide hormones**[125]**. DPP-IV a multifunctional type II cell surface glycoprotein, is widely expressed in a variety of cell types, particularly on differential epithelial cells of the intestine, liver, prostate tissue, corpus luteum, and kidney proximal tubules**[126-127]** as well as leukocyte subsets**[128]**, such as T-helper lympho- cytes, and subsets of macrophages **[129]**, and a soluble form is reported to be present in plasma and urine **[130]**.Endogenous dipeptidyl peptidase IV (DPP-IV) enzyme has been shown to be a key physiological regulator of incretin activity. In vivo, DPP-IV enzyme inactivates both the incretin hormones (GLP-1 & GIP), which in-turn stimulates glucose dependent insulin secretion. DPP-IV enzyme selectively cleaves first two amino acids (His-Ala) of 29 amino acid GLP-1 peptide and thereby makes it inactive which are eliminated via kidney (**Figure 5**)**[131]**. Potential inhibition of DPP-IV

enzyme may prolong half life of these incretins, thereby helps in glucose homeostasis.

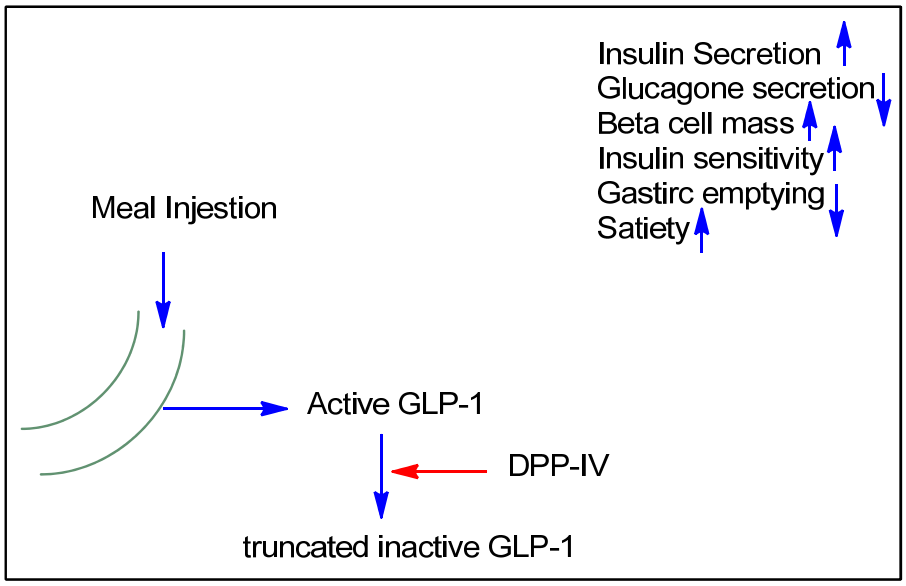


Figure 5. Secretion of GLP-1 after meal ingestion and metabolism by DPP-IV enzyme

The DPP-IV enzyme is a transmembrane glycoprotein, consist of 766 amino acids. It has two subunits and exhibit 85% homology, with its isoforms. The DPP-IV enzyme is consists of three parts; a cytoplasmic tail, a transmembrane region and an extracellular part. The extracellular part is divided into a catalytic domain and an eight-bladed β-propeller domain. The latter contributes to the inhibitor binding site.The extracellular domain of DPP-IV enzyme contains 22 hydrophobic residues of N-terminus which helps to anchor with the cell membrane. The catalytic site of DPP-IV enzyme consists of GWSYG pentapeptide sequence and a catalytic triade S630, Asp708 and His740 forms a binding domain (**Figure 6**).

Structure of DPP-IV enzyme resembles with several isoforms of other protease enzymes, such as DPP-6/8/9, QPP, FAP (fibroblast activation protein)and POP. Potential functions of the various members of the DPP gene family and their relevance with DPP-IV enzyme have been described in the following section.

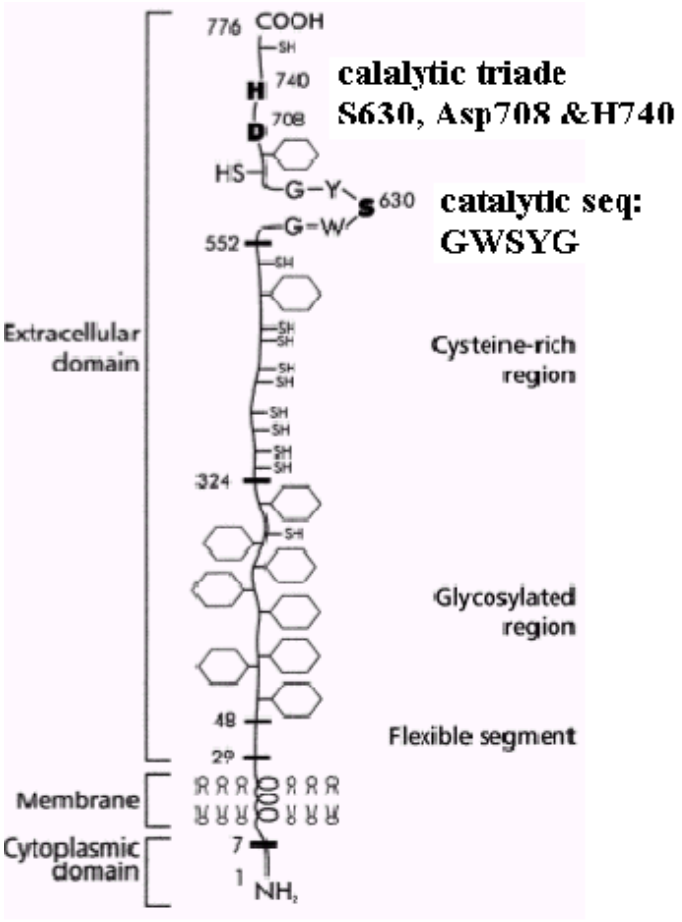


Figure 6: Structure of DPP-IV enzyme

1.5.2.Dipeptidyl peptidase family

The DPP family (family S9), a subfamily of the prolyl oligopeptidase superfamily, includes four enzymes, DPP-IV, FAP, DPP-8 and DPP-9, and two non-enzymes, DPP-IV-like protein-6 (DPP-6, DPL-1 or DPP-X) and DPP-10 (DPL-2)(**Figure 7**)**[132-133]**.

Members of the DPP-IV family preferentially cleave Xaa-Pro- and Xaa- Ala-dipeptides (where Xaa is any amino acid except proline) from theN-terminus of proteins **[134]**. The DPP-IV family differentiates itself from the prolyl oligopeptidase superfamily by the presence of two glutamate residueslocated within the catalytic pocket, which are essential for enzymatic activity **[135]**.

The enzyme FAP, also known as seprase, is the most similar family member to DPP-IV, as it shares a 52% amino acid identity (human enzymes) and similar substrate specificity. Despite these similarities, DPP-IV and FAP differ markedly in their expression patterns. FAP expression is confined predominantly to activated

fibroblasts in diseased tissue, such as fibrotic and epithelial tumours, and invasive cancers, and may be important in wound healing **[136]**.

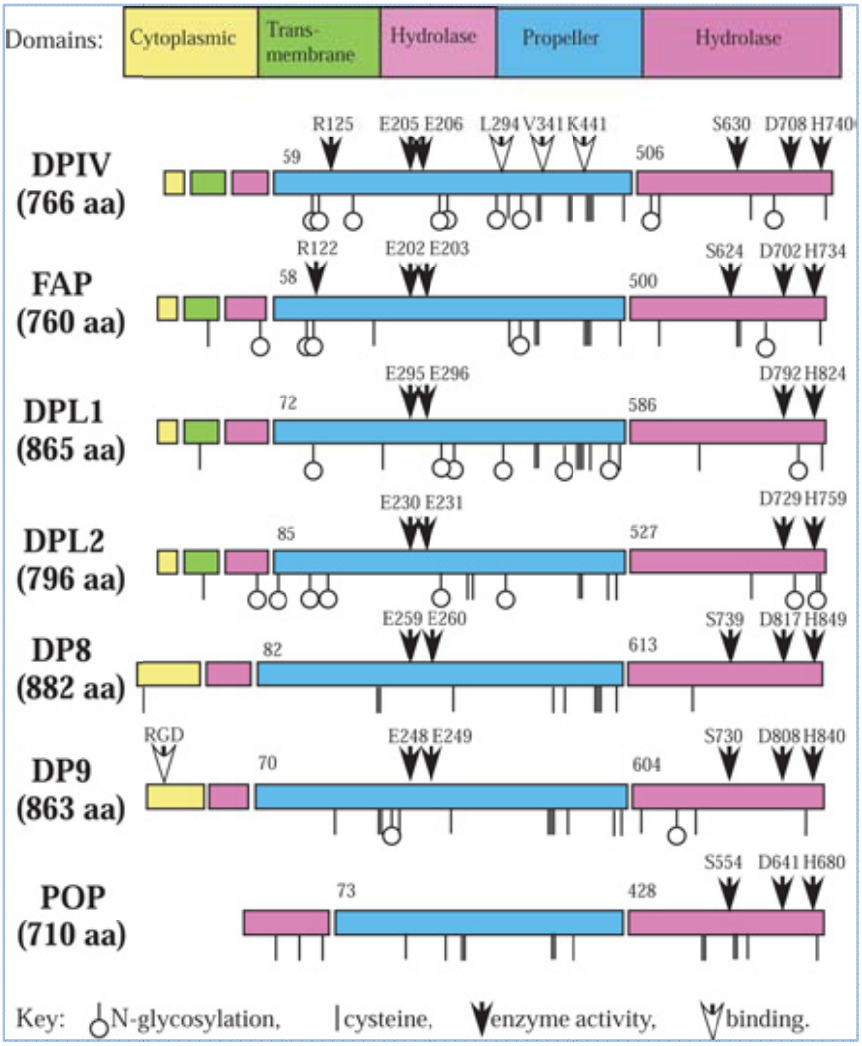


Figure 7: Schematic presentation of the proteins of the DPP family

The other two catalytically active DPP-IV family members, DPP-8 and DPP-9, share 61% amino acid identity with each other, and a 26 and 21% amino acid identity with the protein sequences of DPP-IVand FAP respectively (human enzymes) **[137]**. In contrast with DPP-IVand FAP, which have an extracellular catalytic domain, bothDPP-8 and DPP-9 proteins are localized to the cytoplasm **[137]**. DPP-8 expression is upregulated in activated T-cells. DPP-8 and DPP-9 enzyme activity has been detected in human blood lymphocytes and monocytes **[138]**, High levels ofDPP-9 are found in cancer cells, normal skeletal muscle, the heart and liver. DPP-8 and DPP-9 hydrolyse *H*-Ala-Pro- and *H*-Gly-Pro-derived substrates, although with less efficiency than DPP-IV. *In vitro* peptide substrates of DPP-8 or DPP-9 identified to date include GLP-1, GLP-2, NPY (neuropeptide Y), PYY (peptide YY), SDF-1, IP-10 (interferon-*γ* - induced protein-10) and I-TAC (interferon-inducible T-cell *α*

chemoattractant) **[139-140]**. However, a physiological substrate or role for the DPP activity of either DPP-8 or DPP-9 remains to be demonstrated *in vivo*. However, as the *β*-propeller domain of DPP-IV, DPP-8 and DPP-9 is not as conserved as the *α*/*β*-hydrolase domain and the active sites of DPP-IV, DPP-8 and DPP-9 differ **[141-143]**, hence selective inhibition of DPP-IV may beachieved.

Prolyl oligopeptidase (POP),also known as Prolyl endopeptidases (PEP/PREP)is a group of aminopeptidases and endopeptidases able to hydrolyse the postproline bond, belongs to the DPP gene family. POP bears significant structural homology with the α/β hydrolase fold of DPP-IV**[144]**.POP are involved in the maturation and degradation of peptide hormones and neuropeptides**[145]**. Because of its action on neuropeptides, POP is considered to be involved in processes such as learning, memory, and depression.

Two enzymatically inactive proteins (i.e. DPL1/DPP-6 & DPL2/ DPP-10) closely related to DPP-IV lack catalytic activity due to mutations of thecatalytic serine residue and its neighbouring tryptophan residue, giving a surrounding sequence of Gly-Lys-Asp-Tyr-Gly-Gly instead of the motif Gly-Trp-Ser-Tyr-Gly- Gly.The absence of catalytic activity in DPL1 and DPL2 is also attributed to a number of amino acid substitutions in the catalytic pocket **[146]**.
DPP-II (also known as quiescent cell proline dipeptidase QPP/ DPP-7) shows DPP-IV like peptidase activity although it belongs to another family (family S28) **[147].**

1.5.3.Role of DPP-IV in metabolic diseases

As discussed earlier DPP-IV enzyme rapidly degrades bioactiveincretin hormones GLP-1, and GIP to their inactive metabolites. Both these incretins are important regulator of glucose metabolism. Competitive inhibition of DPP-IV increases the half-life and bioavailability of active incretin hormones, enhancing theirbiological effect (**Figure 8)[148-149]**.

Also, DPP-IV enzyme cleaves many other substrates, such as NPY, GHRH, IL-1 and IL-2, Bradykinin, endomorphin-1 and substance-P (**Table 5**)**[150-151]**. Inhibition of QPP led to reticulocytopenia, while DPP-8/9 inhibition results into thrombocytopenia**[152]**. Thus other than regulating the levels of endogenous incretin hormones such as GLP-1 and GIP, DPP-IV enzyme play crucial role in controlling the lymphocyte and cell growth, T-cell activation, metastasis, inflammation and immune function of body.

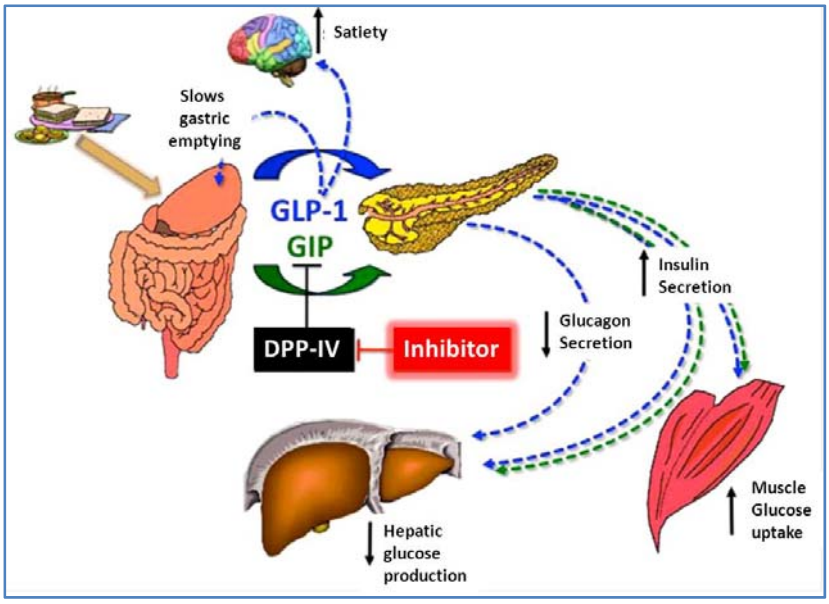


Figure 8: Effect of DPP-IV and its inhibition on physiology of incretin system.

Table 5. Somenatural substrates of DPP-IV

Substrate	N-terminus	Reference
GLP-1	His-Ala-Glu-	**[153]**
GLP-2	His-Ala-Asp-	**[154]**
GIP	Tyr-Ala-Asp-	**[153]**
GRP	Val-Pro-Leu-	**[155-156]**
Substance P	Arg-Pro-Lys-	**[155]**
NPY	Tyr-Pro-Ser-	**[157]**
PACAP38	His-Ser-Asp-	**[156,158]**
IGF-1	Gly-Pro-Glu-	**[159]**
Prolactin	Thr-Pro-Val-	**[155]**
hCG*α*	Ala-Pro-Asp-	**[155]**
GHRF	Tyr-Ala-Glu-	**[153,158]**
LH*α*	Phe-Pro-Asn-	**[159]**
Thyrotropin *α*	Phe-Pro-Asp-	**[159]**
Peptide histidine methionine	His-Ala-Asp-	**[153,158]**
Enkephalins	Tyr-Pro-Val-	**[160]**
Vasostatin-1	Leu-Pro-Val-	**[161]**

Hence, while developing new class of DPP IV inhibitors for the treatment of diabetes, it is essential to consider selectivity of DPP IV inhibitor over other serine protease enzymes and also substrate specificity is crucial, so as to develop safe and effective DPP IV inhibitor based antidiabetic agents.

1.6.Crystal structure of DPP-IV

The seven DPP-IV crystal structures reported till date reflect tremendous global interest in the pharmaceuticaldesign of DPP-IV inhibitors **[162-168]**.Human DPP-IV has a short cytoplasmic tail of 6 amino acids, a 22-amino acid hydrophobic transmembrane region, and a 738-amino acid extracellular domain with ten potential glycosylation sites **[169]**.

The DPP-IV glycoprotein is a homodimer (**Figure 9**). Each monomer subunit consists of two domains, an *α*/*β*-hydrolase domain and a *β*-propeller domain, that enclose a large cavity of approx. 30–45A°in diameter.Dimerization is also observed in solution under various conditions, soluble DPP-IV forms a symmetric assembly as a dimer of dimers, which is required for activity (**Figure 10**). The main DPP-IV structural features includes (A) catalytic or*α*/*β*-hydrolase domain (B) *β*-propeller domain (C) active site and (D) substrate binding site.

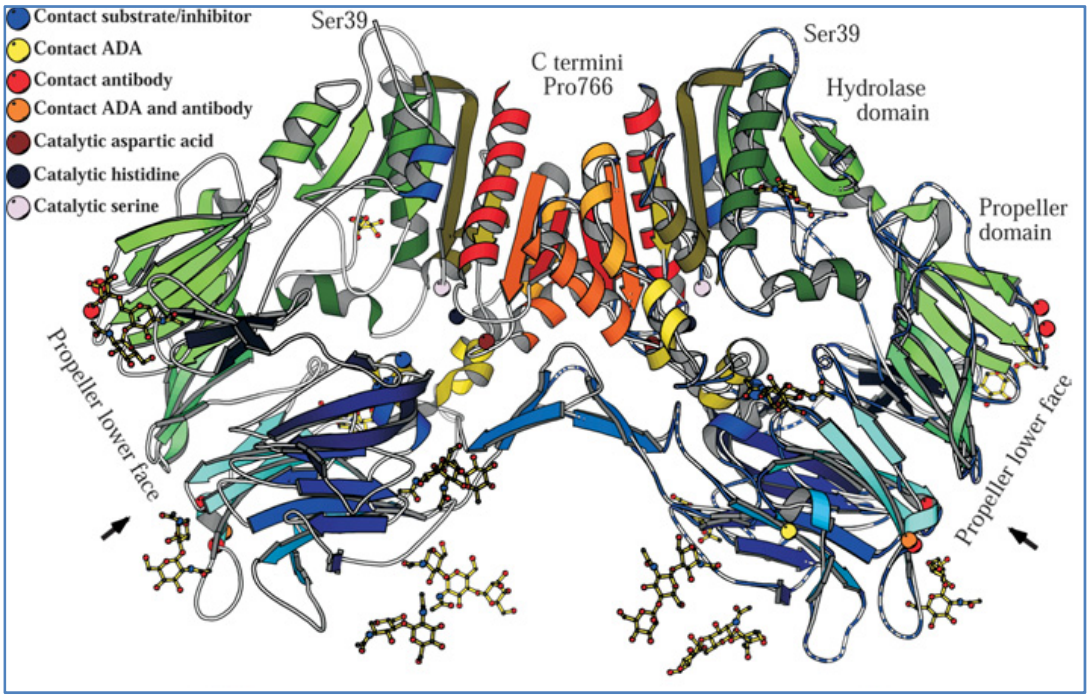


Figure 9:crystal structure of DPP-IV homodimer

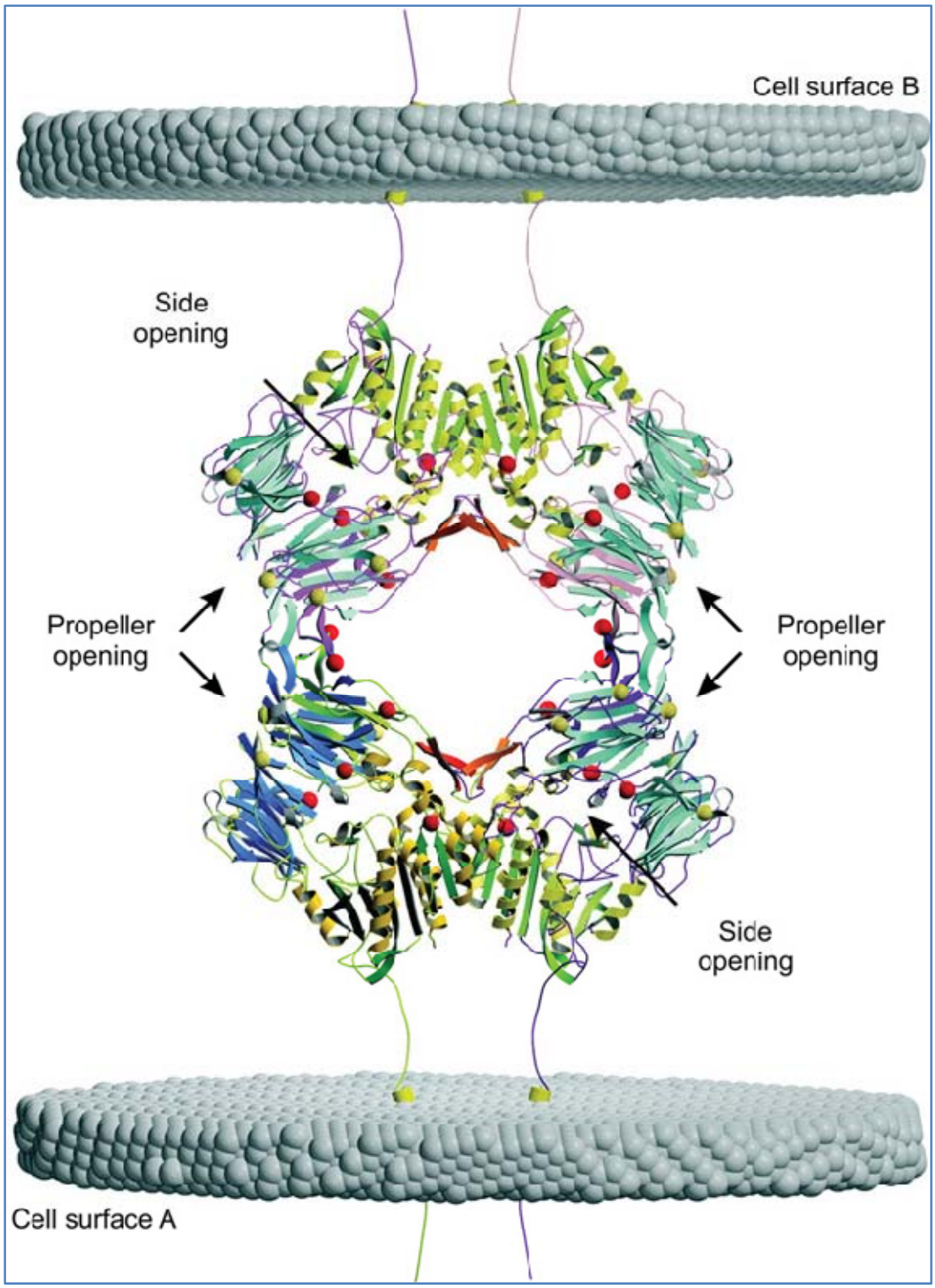


Figure 10:DPP-IV dimer of dimers

(A) **Catalytic domain**: catalytic or*α*/*β*-hydrolase domain is built up of residues Gln508- Pro766and contains a central eight-stranded *β*sheet that is flanked by 12 helices known as the *α*/*β*-hydrolase fold. Superposition of the central *α*helix,

carriesthe catalytic Ser630.The catalytic domain is connected to the βpropeller by an N-terminal 15-residue linker.

(B)***β*-propeller domain**:The βpropeller domain is formed by residues Lys56–Asn497. The preceding N-terminal residues Ser39- Leu55 form a loop structure with a small αhelix at the surface and in close proximity to the first residues of the catalytic domain. The βpropeller domain consists of an 8-fold repeat of a four-stranded antiparallel βsheet motif (**Figure 11**).The βpropeller domainform a significant part of the substrate binding site and are mainly responsible for the substrate specificity.

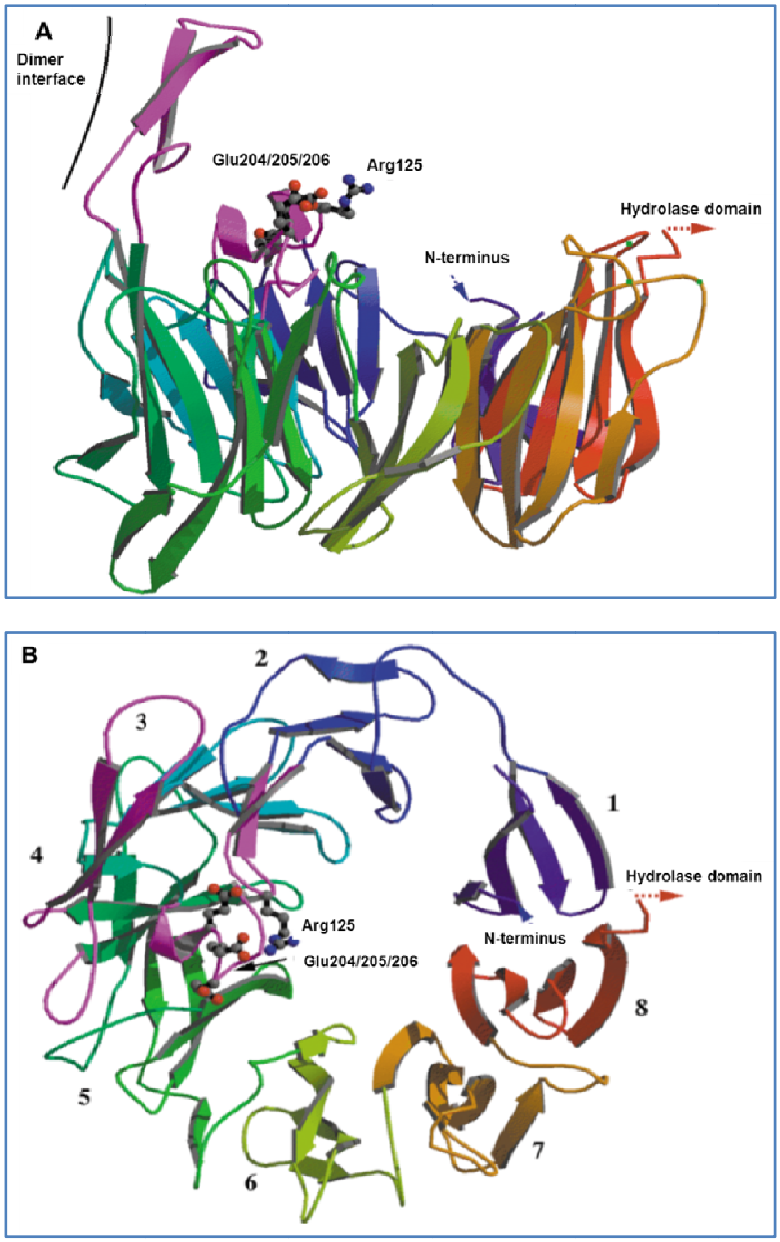


Figure 11:(A) β propeller domain of DPP-IV (B) β propeller domain of DPP-IV rotated 90°

(C)**Active site**:Active site bears the catalytic triad (Ser630, Asp708, and His740), which is located in a large cavity at the interface of the two domains. Ser630 is found at the tip of a very sharp turn between *β*strand 5 and helix C, called the nucleophile elbow, which is a characteristic of hydrolases of the *α*/*β* type **[170]**. The serine hydroxy group is well exposed to solvent and hydrogen bonded to the catalytic imidazole group of His740 on one side and accessible to the substrate on the other side. His740 is found in the middle of a loop between *β*strand 8 and helix F.One of the oxygen atoms of Asp708 is hydrogen bonded to His740 and completes the catalytic triad. The other oxygen atom of the carboxylate group of Asp708 is coordinated by two main chain NH groups of Val711 and Asn710. Thus, the location and geometry of the triad are very similar to that of the classical serine peptidases **[170]**.

Furthermore, the structure shows that Gly628 and Gly632 are important for the formation of the sharp turn to bring the catalytic residue Ser630 in the correct position. This is in accordance with mutagenesis studies on rat DPP-IV showing that the sequence Gly628-X-Ser630-Tyr631-Gly632 is essential for DPP-IV activity**[171]**.

(D) **Substrate binding site**: Combination of the selected residues of the above three structural motif of DPP-IV forms the substrate binding site. The substrate binding site of DPP-IV is indicated by the bound diprotin A (Ile-Pro-Ile), which is a substrate leading to an apparent competitive inhibition (**Figure 12**)**[172]**.

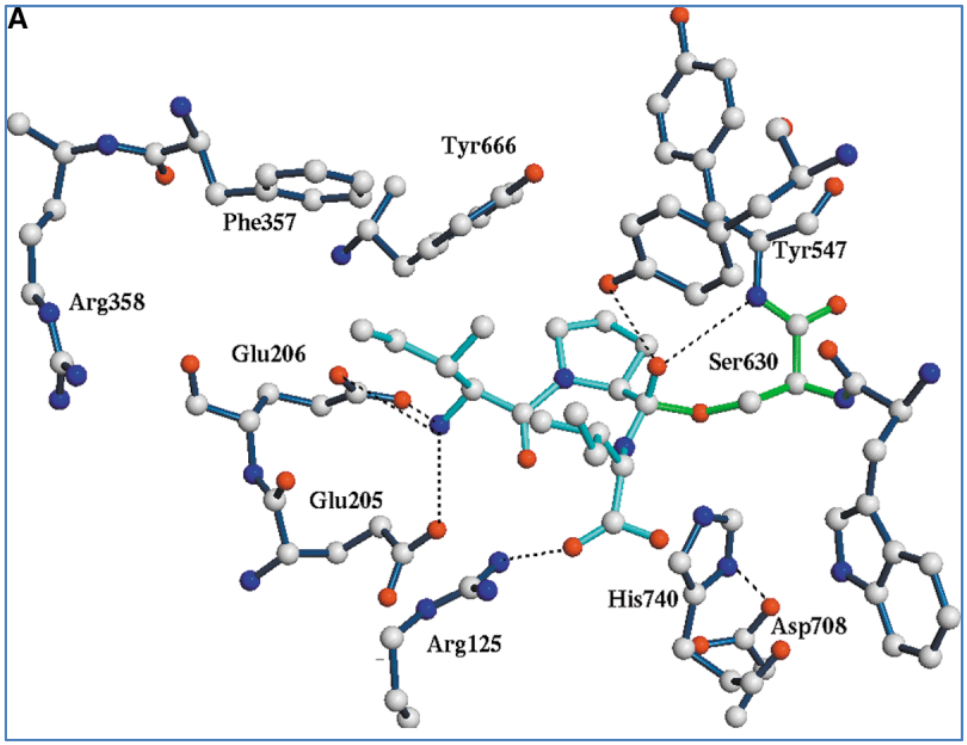


Figure 12: Active site of DPP-IV with substrate Diprotin A.

The ligand is covalently bound to the active site Ser630 of the enzyme in both subunits. The N-terminal Ile (P2) and Pro residues (P1) of ligand are well defined and enable interaction with the substrate binding site **[173]**. The side chain Nof the catalytic His740 is in hydrogen bonding distance to the NH group of P1 and to the oxygenof the Ser630 side chain. The S1 pocket is formed by Val711, Val656, Tyr662, Tyr666, Trp659, and Tyr631, which shape a well-defined hydrophobic pocket that would be filled by proline much better than by alanine. A major contribution to binding to the pyrrolidine ring of Pro is achieved by ring stacking to Tyr662. Essential for substrate binding and catalysis is the N terminus of the substrates, which has to be unprotected and protonated **[174-176]**.

The diprotin A complex shows that the terminal $-NH_3^+$ group is held very precisely in position by strong interactions with the carboxylates of the double Glu motif, Glu205 and Glu206, as well as the OH of Tyr662. A third glutamate, Glu204 stabilizes this substrate recognition site by a hydrogen bonding network with the backbone NH of Arg125, His126, and Ser127 as well as the hydroxy group of Ser127. This additional structural element in the exopeptidase is very important for substrate selectivity. The importance of the glutamate residues is confirmed by single point mutations that abolish DPP- IV activity **[177]**. Thus, the double Glu motif is a recognition site for the N terminus of substrates and restricts the cleavage to dipeptides, and the S1 pocket provides an optimal binding to proline and alanine residues, leading to a highly specific peptidase.

Hence, detailed structural characteristics of the DPP-IV binding site to identify the molecular interaction that are most important for tight enzyme-inhibitor binding, which indeed leads to selectivity as well as subnanomolar activity is described in the following section.

1.7.Molecular recognition of ligands in DPP-IV

Concomitant with a large variety of published small molecule DPP-IV inhibitors almost one hundred and five co-crystalstructures have been released to the public till April 2014. **[178]**The structural characteristics of the DPP-IV binding site is discussed based upon the available X-ray information (bioinformatics) and pharmacokinetic data together with structure-activity relationship data of the published DPP-IV ligands/inhibitors. This section is divided into several subparagraphs that separately discuss the different interaction motifs used byDPP-IV ligands.

Catalytic Ser630 and Oxyanion Hole: The catalytic machinery of DPP-IV involves a serinenucleophile within the catalytic triad Ser-Asp-His, whosesequential order,

however, is inverse to that found inclassical serine proteases (His-Asp-Ser) **[179]**. Several earlyinhibitors have been developed that use an electrophilicgroup, mainly a nitrile, to interact covalently with Ser630**[180-181]**. The concept of covalent binding to nitriles is wellknown from cysteine protease inhibitors **[182]**. X-ray studiesconfirmed that the nitrile carbon atom changes its hybridizationstate and is in covalent bond distance from the oxygen atom of the Ser630 side chain **[183]**. Theincrease in binding affinity with the additional -CN group issubstantial, leading to an up to 1000-fold tighter binding to the enzyme **[117,184]**. Enzymatic and biophysical studies revealed that the covalent interaction is reversible and that the activity of the enzyme is regenerated upon release of the inhibitor **[184]**. Based upon this phenomenon nitrile group containg inhibitors are classified as reversible inhibitors.

Apart from the transition state mimetics that covalently bind to Ser630, few inhibitors use the oxyanion hole, which is composed of the backbone NH of Tyr631 and the side chain OH of Tyr547, for binding. The only ligands for which this interaction is confirmed by crystal structures are the xanthines and the related pyrimidine-2,4-dione**[185]**, in which a carbonyl group accepts a hydrogen bond from the amide NH of Tyr631. As hydrogen bonding is very sensitive to a correct geometry few chemotypes are apparently able to interact with the hydrogen bond donor arrangement of the oxyanion hole.

S1 Pocket: The specificity pocket S1 is composed of the side chains of Tyr631, Val656, Trp659, Tyr662, Tyr666, and Val711 and it is highly hydrophobic. Overlays of the existing X-ray structures reveal very littlechanges in size and shape of the pocket demonstrating its high specificity for proline residues. The rigidity of this pocket was probed by several groups through modification of the ring size of P1 fragments. The close-up view of the S1 reveals a small hydrophobic niche in the back and suggests to introduce some asymmetry into the P1 fragment to mimic the shape of this pocket. This higher asymmetry can be achieved by introducing a sulfur atom into a 5-membered ring, as illustrated by the thiazolidine, which is approximately 2-fold more active than the corresponding pyrrolidine **[186]**. Hulin *et al*. performed a fluorine scan around the pyrrolidine ring in the cyclohexylglycine amide series and found that the activity highly depends on the position and stereo-configuration of the fluorine substitution **[187]**. A maximal gain in Ki of ~4-fold compared to the unsubstituted pyrrolidine could be achieved. As fluorine occupies little more space than hydrogen this high sensitivity underlines the stringent shape constraints of the S1 pocket.A considerably larger gain in binding affinity compared to the pyrrolidines could be achieved by small substituents on aromatic rings in the S1 pocket **[188-190]**. Slightly bigger substituents than fluorine, such as chlorine or

methyl, are best - in the optimal *para* position - and improve the IC50 compared to the unsubstituted phenyl by a factor 30-40. Substitutions in the *meta* position lead to repulsive interactions with the enzyme and are less favorable. It is noteworthy that also some polar groups such as pyridine and lactam **are** tolerated in the S1 pocket leading to compounds with an overall more balanced polarity pattern.

P2 Amide Recognition: Arg125, Asn710: The carbonyl of the amide bond connecting the N-terminus with the P1 residue in DPP-IV substrates is located in a polar, "electrophilic" environment consisting of the side chains of Arg125 and Asn710. Conversion of the amide to a thioamide leads to a reduction of affinity and replacing the amide by a methylene unit makes the molecule inactive, confirming the favorable electrostatic interaction of the carbonyl dipole with the protein environment **[186]**. Attempts in the cyanopyrrolidine series to mimic the geometry and dipole effect of an amide linker by a *trans*fluoroolefin lead to a reduction of potency **[117]** Merckreported a 3-4 fold tighter binding to DPP-IV substitutingthe phenyl in various β-phenethylamine series with a fluorineat the *ortho*-position **[189, 191]**. While the terminal amidegroup of Asn710 is slightly rotated and not involved in ahydrogen bond with the ligand, a favorable electrostatic interaction between the positively charged Arg125 and the$C^{\delta+}$-$F^{\delta-}$dipole moment remains. Using another *ortho*substituentwith high dipole moment, Takeda reported afavorable interaction of their 2-cyano group with Arg125 inthe pyrimidine-2,4-dione series**[185].** For the aminopyrimidinessmall *ortho*-substitutions (-Me, -Cl,-OMe) on the 6-phenyl moiety lead to 17-28 fold lower IC_{50}values compared to the unsubstituted ring. The consistentgain in affinity with these three substituents indicates thatthe change of torsional angle between the pyrimidine and 6-phenyl rings due to *ortho*-substitution might be acontributing factor for better protein-ligand fit in this series**[188]**.

Overall placing hydrophobic and/or electronegative ligand atoms at a very precise location in the vicinity of Arg125 and Asn710 is rewarded with substantial affinity gains.

N-Terminal Recognition: Glu205, Glu206, Tyr662: Hydrogen bonding interactions with the side chains ofthe two glutamate residues 205 and 206 are, besides the filling of the S1 pocket, the second most important anchor point for inhibitor binding. Secondary and primary amines are recognized by DPP-IV in this region, and for the latter one a third hydrogen bond is formed, typically to Tyr662. This interaction substitutes the binding of the N-terminus of peptide substrates. The positions of the basic nitrogen atoms in the different crystal structures overlap within a sphere of only 1.2 Å, making this a very tight pharmacophore constraint. Destruction of the hydrogen bond network through alkylation of the amino group abolishes activity

[192]. Furthermore, variation of the basicity of the amine in a Roche cyanopyrrolidine series revealed a sharp drop in binding affinity by 2 orders of magnitude when the pKa was lowered from 7.3 to 6.0 **[193]**. An interesting class ofcompounds without basic nitrogen are the carbamoyltriazoles from Eisai**[194]**. Hence presence of primary or secondary amine group in the ligand provides substantial enhancement of the inhibition, However amide linkage or substituted amine group containg substrates can also accommodate this region.

Additional Interactions: Phe 357, Tyr 547 and Arg 358: High nanomolar affinity can be achieved by interactionswith the S1 pocket, the Glu dyad, and the P2 amiderecognition site. However, furtheraffinity gains require either covalent binding to the Ser630residue or the exploitation of additional protein-ligandinteractions. Prime candidates which are used in almost alllow nanomolar DPP-IV inhibitors are the phenyl rings of thetwo residues Phe357 and Tyr547. These are 6-10 Å awayfrom S1 pocket and Glu dyad and exposed to the ligandbinding site. π-πstackinginteractions between each of the two residues with differentaromatic ligand fragments improves the binding affinity of the ligand. Alternative to aromatic-aromatic interactions, hydrophobic contacts between Phe357, Tyr547andlarge aliphatic groups, such as adamantyl can be used to achieve low nanomolar IC_{50} values.

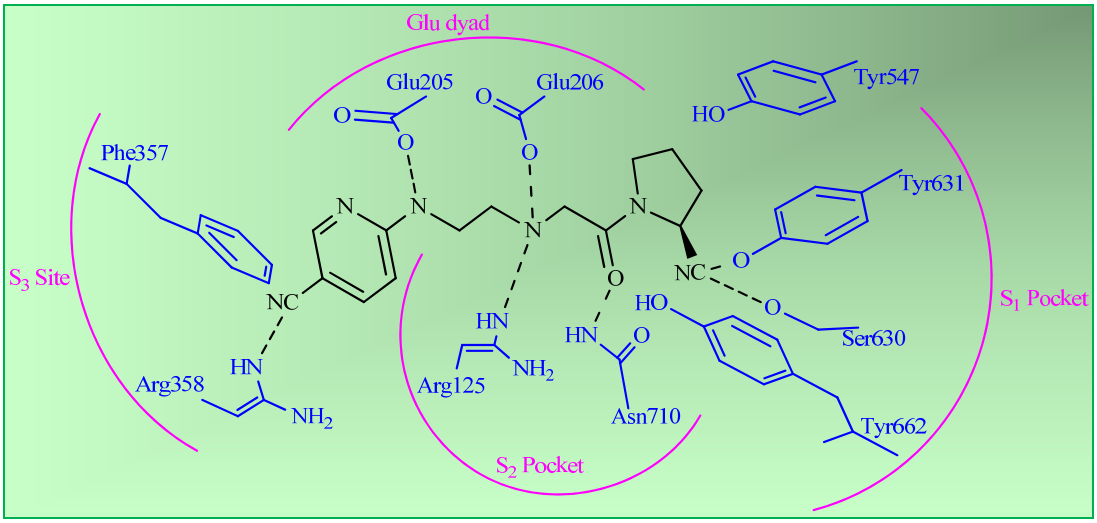


Figure-13: Structural features required for selective DPP-IV inhibitor

Lastly, the presence of Arg358 in close proximity to Phe357 makes the positively charged side chain an additional interaction partner for substituents on ligand aromatic rings. the observed SAR for several series interacting with Phe357 indicates that additional binding free energy can be gained by optimizing the electrostatics in this region. For example, placing electronegative groups such as trifluoromethyl orfluorine next to the positive charge of Arg358 led to a 4-fold increase

in binding in sitagliptin and in thecyanopyrrolidines each,as well as in a potential back up molecule to sitagliptin **[31,183,195]**.

Hence, to achieve subnanomolar potency and selectivity over other serine proteases substrate should have all favourable interaction with above discussed residues of DPP-IV enzyme (**Figure-13**).

1.8.Mechanism of action of DPP-IV inhibitors

As described earlier DPP-IV enzyme selectively cleaves the N-terminal dipeptide from the penultimate position of GLP-1 and GIP thus makes them inactive**[196]**.Competitive inhibiton of the DPP-IV enzyme blocks the degradation of these incretin hormones and extend the duration of action of endogenous GLP-1, thereby stimulating insulin secretion, inhibiting glucagon release and slowing gastric emptying.**[197-198]**.

Inhibition of DPP-IV enzyme activity, using suitable DPP-IV enzyme inhibitor likely to increase the levels (prolong half-life) of endogenous intact and bioactive GIP and GLP-1 peptides which in-turn increase the insulin secretion and decrease the blood glucose, thereby, it acts as antidiabetic agents (**Figure 14**).

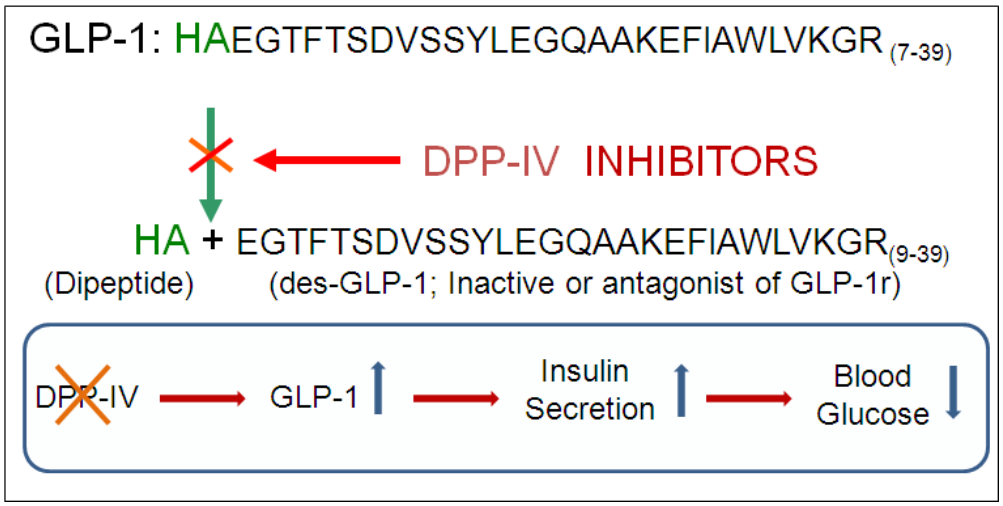


Figure 14. Effect of DPP-IV inhibition

1.9.Challenges in developing potent and selective DPP-IV inhibitors

As discussed earlier in section **1.5.2.** DPP-IV enzyme resembles with several other closely related serine proteases so development of small molecules as selective inhibitors of DPP-IVbecome a major challenge.Although the in vivo function of other members of DPP family, that is, DPP-2, DPP-8, DPP-9 etc. are largely unknown, the physiological effects of their inhibition has been documented in the literature**[199]**.For example, inhibition of DPP-2 has been shown to result in the

apoptosis of quiescent T cells. Selective inhibition of DPP-8/DPP-9 in animals resulted in severe toxic reactions, including alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies and increased mortality**[200]**.Notably, it has been shown very recently that inhibition of DPP-8 and DPP-9 did not lead to organ toxicities and mortality in rodents and thus, a mechanism other than DPP-8/DPP-9 inhibition has been suggested to be responsible for the observed toxicities associated with the inhibitors of DPP-8/DPP-9**[201]**.Nevertheless, in view of likely toxic side effects associated with the inhibition of other members of DPP family it has become necessary to design selective inhibitors targeting DPP-IV.

Experimental observation indicated that the S2 pocket of DPP-IV might be similar to that of DPP-8 and consequently inhibitors designed for DPP-IV might show an inhibitory effect on DPP-8 as well. However, extensive SAR work has proved that desired selectivity for DPP-IV inhibition can be achieved via introducing appropriate substituents or groupsthat can attribute all favourable interactions as discussed in the above section **1.7**. Nevertheless, once synthesized, it has therefore become mandatory to determine the DPP-IV selectivity of an inhibitor over closely related other DPPs.

Furthermore, astudy has demonstrated that high levels of GLP-1 should be maintained for 24 h for optimal glycemic control**[202]**.Thus, in addition to focusing on potency and selectivity, development of longacting inhibitors is also desirable that could potentially provide maximal efficacy, particularly in patients suffering from severe diabetes (e.g., HbA1c >9%).

1.10.Overview on DPP-IV inhibitors under recent development

The clinical success of the gliptins following the initial approval of Sitagliptin has stimulated the field todevelop and evaluate additional DPP IV inhibitors in order to obtain drugs with improved properties. Several excellent reviews have been published covering the development of potential new therapeutic DPP IV inhibitors**[203-206]**.

Though BMS discovered Saxagliptin, their continued interest in DPP-IV inhibition as a therapy for type 2 diabetes prompted discovery of the azolopyrimidine based **compound A [207]** and**compound B [208]** as a potent and selective DPP-IV inhibitors(**Figure 15**).

After the development of first FDA approved DPP-IV inhibitor Sitagliptin to treat T2DM, Merck endeavour for batter medication through DPP-IV inhibition led the development of **compound C[209]**which showed good potency (IC_{50}=6nM)and

selectivity but the shortfall with the compound was poor calcium channel and Cyp2D6 selectivity. In continued efforts they develop **compound D [210]**with good pharmacokinetic and pharmacodynamic profile having >1000 fold selectivity for L-type calcium channel and Cyp2D6 with respect to its intrinsic DPP-IV potency.Unfortunately**compound D** showed poor bioavailability in higher animal model (i.e. monkey F%=11%) so it was unable to transform to the clinic for medication and dropped in phase-II.HoweverMerck's indefinite interest in research led the discovery of Omarigliptin **[56]**having all favourable attributes for good medicationand currently it is in phase III clinical trials.

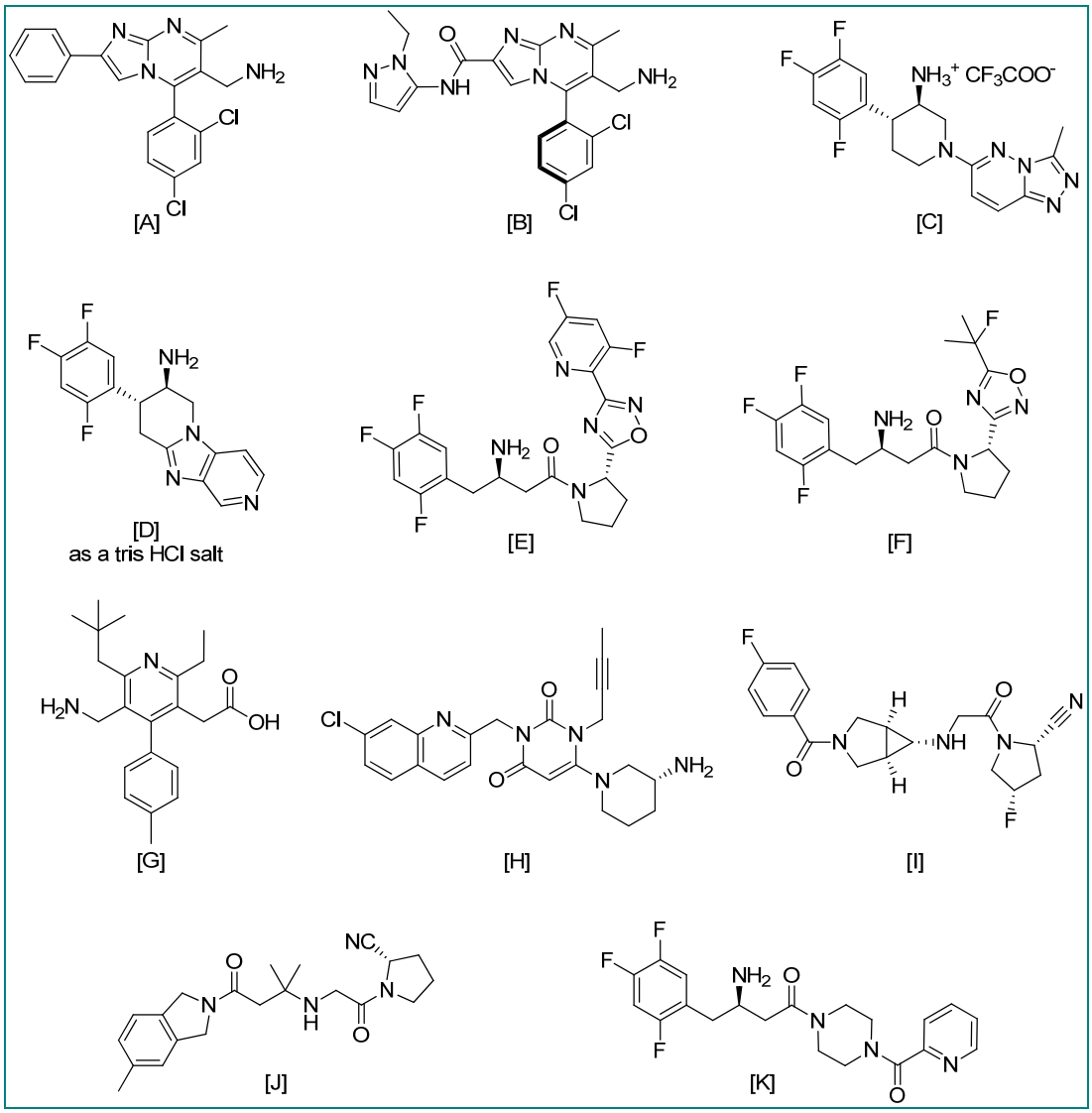


Figure 15. Potent DPP-IV inhibitors under recent development

Santhera Pharmaceuticals from Switzerland reported **compound E** and **compound F**by structural modification in a series of β-homophenylalanine based

DPP-IV inhibitors with good pk profile and efficacy**[211]**. However further evaluation focused on preclinical safety and more comprehensive efficacy profiling is in progress.

Takeda Pharmaceutical from Japan is still in search of even batter treatment for the T2DM though received FDA approval for the drug namely Alogliptin and in phase III clinical trial entity Trelagliptin, they reported **compound G**viz TAK-100 as a potent and selective DPP-IV inhibitor currently in phase I **[212]**.

Recently a group of researchers from Jiangsu Chia-Tai Tianqing Pharmaceutical Co. Ltd. China reported **compound H** derived from Alogliptin through pharmacophore hybridization with low nanomolar potency(IC_{50}=0.4nM)**[213]**.

A group of scientist from Ranbaxy Research Laboratories, India reported **compound I**as a potent, selective and slow binding inhibitor of DPP-IV**[214]**.

Sanwa Kagaku Kenkyusho company from Japan reported isoindiline based **compound J** as a highly potent DPP-IV inhibitor, however due to its high clearance rate further evaluation in this series has been abandoned**[215]**.

A research group from Seoul National University-Korea reported **compound K** as a potent DPP-IV inhibitor showing longer duration of action and no CYP inhibition up to 50μM **[216]**.

Although the bibliography on DPP-IV inhibitors is rich, active research continues on this subject. Currently 35 compounds are in preclinical development having IC_{50}≤6nM. Major players include AstraZeneca Plc., Boehringer Ingelheim GmbH, Bristol-Myers Squibb, Eli Lilly and Company, Merck & Co Inc., Mitsubishi Tanabe Pharma Corp., Novartis AG, Takeda Pharmaceutical Company Limited, Cadila Healthcare Ltd., Phenomix, Lupin limited, L G Life Sciences etc, and have filed no. of patent applictions like WO2006009886, WO2006127530, WO2006039325, WO2006098342, WO2006127287, WO2007053819, US20070082932, WO2007015767, WO2007099385, WO2008119005, WO2008087560, WO2008077597, WO2008130151, WO2008017670, WO2009111239, WO2009082134, WO2009093269, WO2009003681, WO2009045476, WO2009068531, WO2010146597, WO2010029422, WO2011103256, WO2011146358, WO2012118945, WO2013122920, WO2014061031.

Although eight gliptins have reached the market, they have done so recently, and so the long-term adverse effects of these drugs are still unknown. Until now, secondary effectshave been attributed to the off-target repercussion of themolecules. Hence, research has been focused on the discovery of potent, selective and long acting DPP-IV inhibitors with minimal off target activity.

In this regard, knowing the potential of DPP-IV inhibitor target, we also attempted to design novel series of DPP-IV inhibitor and these design strategy is described in next section.

1.11.Introduction to Cytochrome P450 (CYPs) and its importance

The cytochrome P450 system is a group of enzymes, found mainly in the liver and gut mucosa, that plays a crucial role in controlling the concentrations of many endogenous substances and drugs.There are 18 CYP families bearing 43 subfamilies**[217]**. CYPs enzymes are mainly essential for the detoxification and the metabolism of drugs. CYP subfamilies involved in drug metabolism includes CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. However CYP2D6 and CYP3A4 are the major drug-metabolizing enzymes in humans. Diabetic patients are treated with a number of otherdrugs in addition to antidiabetic drugs, including anti-hypertensive and lipid-lowering agents. Notably, more than 50% of these drugs are metabolized by CYP3A4 or CYP2D6 enzymes. Drugs can inhibit (decrease), induce (increase) CYP metabolism or may act as a substratefor CYP enzymes. Inhibition of CYP metabolism will likely increase the affected drug's systemic concentrations, whereas induction of metabolism often reduces systemic concentrations**[218]**.

CYP3A4 or CYP2D6 inhibition and induction is clinically relevant to diabetic patients, especially whentreated with antidiabetic agents such as Sulfonylureas, Metformin and Meglitanides. For example, Sulfonylureas are known substrates of CYP. Thus inducers and inhibitors of CYP can affect the metabolism of Sulfonylureas. Similarly, Repaglinide is metabolized by the CYP3A4 and a serious drug-drug interactions (DDI) may occur when it is coadministered with CYP inhibitor, such as Gemfibrozil (triglyceride lowering agent), as it increases eightfold exposure of Repaglinide. Thus, CYP inhibition/ induction can havesignificant consequences on other antidiabetic drugs that are metabolized by these enzymes, which may result in DDI and idiosyncratic drug toxicity (IDT)**[219-220]**.

Hence, knowing the clinical importance of the CYP enzyme, for particular drug like new chemical entity it is essential to examine its effect on CYP enzyme inhibition.

1.12.Conclusion

Diabetes mellitus is the most prevalent and serious metabolic disorder. Among the T1DM & T2DM, T2DM is one of the major public health challenges of 21st century. Currently available therapies have several drawbackstherefore various new therapies are being developed, among which DPP-IV inhibitors are the most promising approach for the safe and effective treatment of Type 2 diabetes. However achieving selectivity and oral bioavailibity with longer duration of action are major challenges with the design & development of DPP-IV inhibitors. To address these concern, in the next section, we have designed novel series of DPP-IV inhibitors to develop second generation therapies for the treatment of T2DM.

Chapter II:

Designing Strategy Of DPP-IV inhibitors

"An investment in knowledge pays the best interest."

-Benjamin Franklin

2. Design strategy of DPP-IV inhibitors

2.1. Orally active, potent and selective DPP-IV inhibitors

As mention earlier, DPP IV enzyme selectively cleaves the N-terminal dipeptides (X-Ala or X-Pro) from target polypeptides, such as GLP-1 and GIP **[125,134]**. Also, structure of DPP-IV enzyme resembles with several other protease enzymes and it exhibit broad substrate specificity. Thus, in order to develop selective DPP-IV inhibitor, we decided to design dipeptide based DPP-IV inhibitors, based upon the sequence homology of first two amino acids of GLP-1 peptide (His-Ala) and SAR study of DPP-IV inhibitors, which are in clinic or in clinical development **[221-224]**.

Most of the DPP-IV inhibitors, which are in clinical development were designed based upon the SAR study of dipeptide substrate recognized by DPP-IV enzyme (**Figure 16**). A key feature in most of the DPP-IV inhibitors, which are under development include incorporation of cyanopyrrolidine ring system, attached to sterically hindered group such as adamantyl, with -CH_2- spacer/linker **[32,36,42,46,49,50]**.

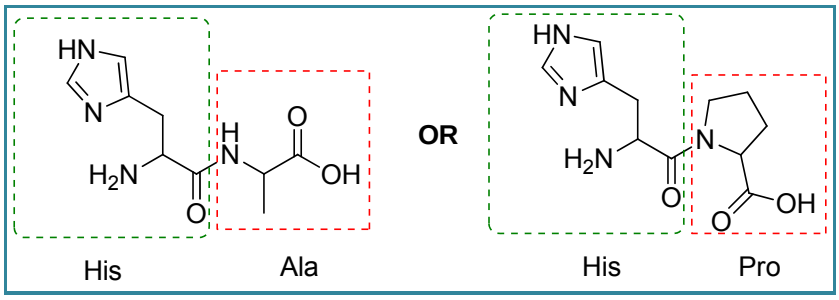


Figure 16: Structure of dipeptide substrate recognized by DPP IV enzyme

Earlier several attempts have been made to develop dipeptide based DPP-IV inhibitors **[203,225-227]**. In general, pyrrolidine or thiazolidine based DPP-IV inhibitors were found to be very potent and selective but under in vivo condition, they were found to be metabolically unstable, mainly due to cyclisation (**Figure 17**). To overcome ring cyclisation problem under basic condition, sterically hindered bulky substitution were introduced in new class of DPP-IV inhibitors.

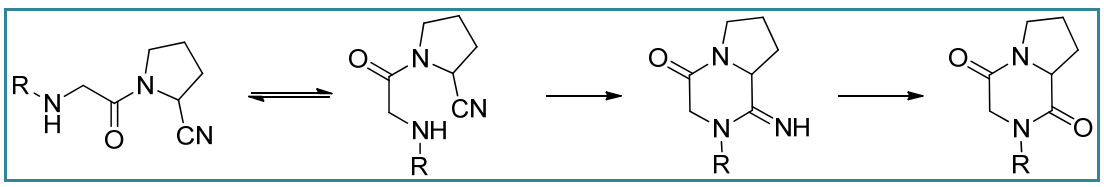


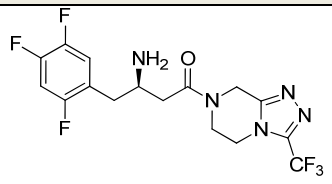
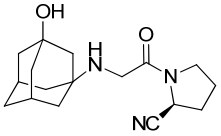
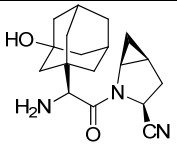
Figure 17: Cyclic amide or diketopiperazine formation

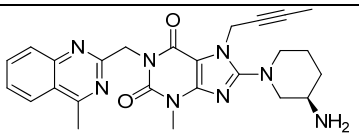
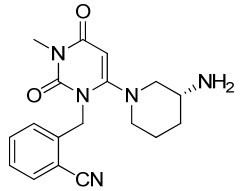
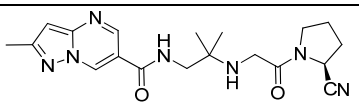
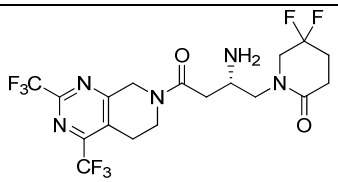
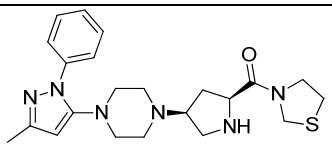
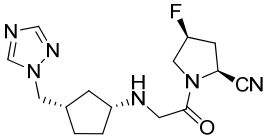
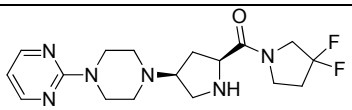
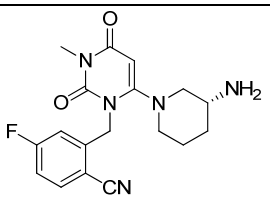
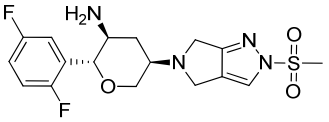
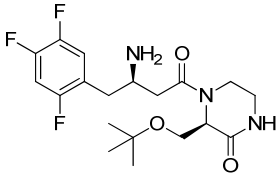
Among the DPP-IV inhibitors launched or in active development (**Table 6**), vildagliptin, saxagliptin, anagliptin, teneligliptin, melogliptin and gosogliptin are peptide mimetic compounds, which have been discovered by replacing segments of peptide-based substrates **[228]**. All these compounds contains pyrrolidine ring system bearing either –CN and/or –F substituent at specific positions on the basis of SAR **[229-230]**. As nitrile and fluoro groups are important for high potency; analogs with similar structures, but without a nitrile/fluoro in this position are 2-3 orders of magnitudes less active. Since pyrrolidine best fits in S1 pocket and nitrile group covalently interact with the catalytically active serine hydroxyl (Ser630), imparts high potency.

However, sitagliptin, alogliptin and linagliptin are non-peptide mimetic compounds, which have been discovered by optimization of the initial lead compounds identified by random screening **[228]**. Therefore, their chemical structures are diverse, suggesting that each of their binding modes in DPP-IV would be unique **[231]**. Fluoro or nitrile substituted phenyl ring in sitagliptin, alogliptin, trelagliptin, omarigliptin and evogliptin occupies the S1 pocket, where it provide tight binding by additional interaction with Tyr666.

Binding of DPP-IV inhibitors to S3 site play important role in increasing the selectivity of the inhibitor over other related enzymes. Compounds which are recently launched and in active development, contains various hetero aromatic substituents, which fit in to S3 site and give binding interaction with Phe357 and Arg358 **[232]**.

Table 6. DPP-IV inhibitors in clinic and in active development.

Sr. No.	Name	Structure	Company	Clinical status	Ref.
1	Sitagliptin (MK-0431)		Merck	Launched	**[31]**
2	Vidagliptin (LAF-237)		Novartis	Launched	**[32]**
3	Saxagliptin (BMS-477118)		Astra-zeneca / BMS	Launched	**[36]**

4	Linagliptin (BI-1356)		Boehringer Ingelheim / Eli Lilly	Launched	**[39]**
5	Alogliptin (SYR-322)		Takeda	Launched	**[41]**
6	Anagliptin (SK-0403)		Kowa JW Pharma	Launched	**[42]**
7	Gemigliptin (LC15-0444)		LG Life Sciences	Launched	**[44]**
8	Teneligliptin (MP-513)		Mitsubishi Tanabe	Launched	**[46]**
9	Melogliptin (GRC-8200)		Glenmark	Phase II	**[49]**
10	Gosogliptin (PF-734200)		SatRx	Phase II	**[50]**
11	Trelagliptin (SYR-472)		Takeda / Furiex	Phase III	**[53]**
12	Omarigliptin (MK-3102)		Merck	Phase III	**[56]**
13	Evogliptin (DA-1229)		Dong-A Pharma	Phase II	**[57]**

Hence, keeping in mind the SAR study of DPP-IV inhibitors developed and in active development, novel DPP-IV inhibitors are designed as illustrated in sections **2.1.1. -2.1.3.**

2.1.1. Rational for designing cyanopyrrolidine containing peptidomimetic based DPP- IV inhibitors. (First series)

As discussed above to develop dipeptide based DPP-IV inhibitors 2-cyano-pyrrolidine based DPP-IV inhibitors have been studied most extensively because cyanopyrrolidine ring system not only mimic the proline ring system, but also the presence of nitrile on the five membered ring provides nanomolar inhibition of DPP-IV and the metabolic stability favors oral administration of cyanopyrrolidine based DPP-IV inhibitors. Hence to overcome ring cyclisation problem under basic condition and chemical instability, sterically hindered bulky substitution and pyrrolidine ring with various substituents were introduced into the new class of dipeptide based DPP-IV inhibitors **[204]**.

Among various DPP-IV inhibitors reported in the literature, NVP-DPP728, closely resembles with dipeptide substrate and therefore it was found to be very potent and selective (reported EC_{50} value 7 nM and > 15000 fold selective) **[233]**. Furthermore, crystal structure of DPP-IV enzyme interaction with derivative of NVP-DPP728 at catalytic site, is reported in the literature indicated that that there are two hydrophobic binding pockets located at catalytic site and electrophilic groups are essential for hydrogen binding **[234]**.

Thus based upon SAR study of NVP-DPP728 and past developmental scenario, we have designed new series of dipeptide based DPP-IV inhibitors, which mainly consist of five member proline ring system, attached to sterically hindered aromatic acid, with suitable linker. These Novel dipeptide based DPP-IV inhibitors were prepared, either by varying the length of linker with carbon chain or aromatic ring as a spacer and electrophilic functionality on proline ring (amide/nitrile) or electron withdrawing / donating groups on sterically hindered aromatic ring system (**Figure 18**).

Compounds represented by general structure **I** and **II** were designed in close analogy to NVP-DPP728 for the reason that it attenuate all the favorable enzyme interactions for binding and activation to achieve nanomolar potency. Pyrrolidinecarboxamides **I** and **II** were specifically designed to minimize the intra-molecular cyclisation (i.e. cyclic amide or diketopiperazine formation). However it is known that phenomenon is more prone in dipeptidic smaller compounds so knowing the fact that the presence of nitrile on the five membered proline ring provides

nanomolar inhibition of DPP-IV we designed pyrrolidinecarbonitriles **III** and **IV** tripeptide based peptidomimetics.

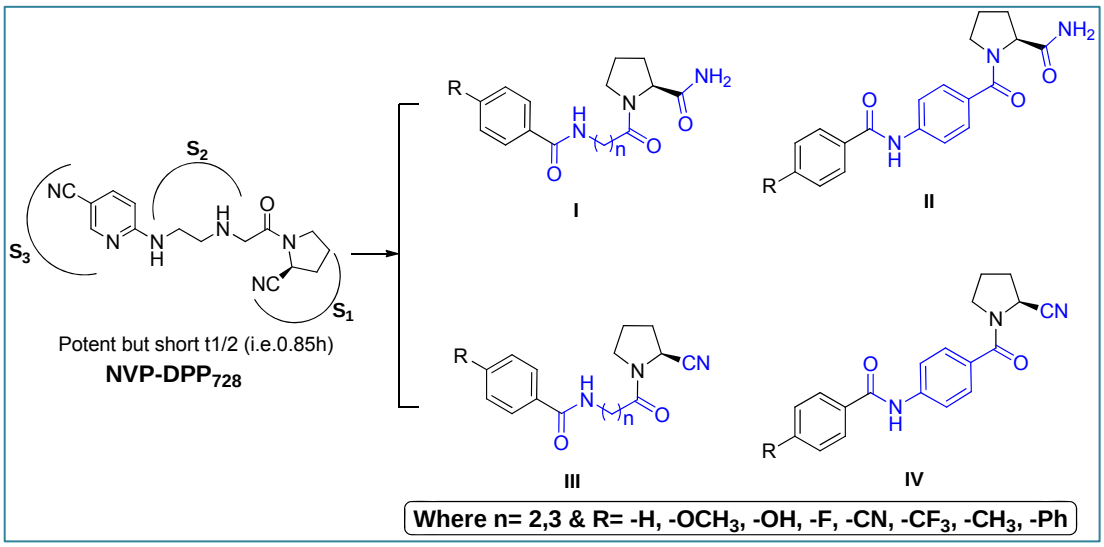


Figure 18: Design strategy of cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors.

Thus, in this series, all favorable structure components of compound NVP-DPP728 essential for the key interaction with DPP-IV were retained, except suitable changes were made to improve potency, selectivity and pharmacokinetic profile. In this, series, we planned to prepare total thirty compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b**, their synthetic schemes are explained in **Chemistry section 3.1.1**.

2.1.2. Rational for designing peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities. (Second series)

However, upon secondary profiling of lead compound of the first series **17c**, CYP3A4 and CYP2D6 inhibitions (IC_{50}: 1.1 and 1.9 mM respectively) were observed, which halted its further preclinical development because CYP inhibition/ induction can have significant consequences on antidiabetic drugs that are metabolized by these enzymes, which may result in drug-drug interaction (DDI) and idiosyncratic drug toxicity (IDT). Hence new series have been designed and synthesized based upon the following rationale.

Affinity of a molecule for CYP can be attenuated by increasing / decreasing the carbon chain length **[235]**. So to overcome CYP liabilities, amino-alkyl spacer (-$(CH_2)_3$-; 3C) of compound **17c** was specifically reduced from 3C to 2C (-$(CH_2)_2$-) (i.e. **17a**) and 1C (-CH_2-) (i.e. **27a**) and the resulting molecules were examined for CYP

inhibitions. Here the chain length has been reduced for the reason that all DPP-IV inhibitors reported in literature are very small molecules in bulk with shorter length to preserve the potency such as Vildagliptin, Saxagliptine, Alogliptin etc. The reduction of amino-alkyl spacer attenuates CYP inhibitions but led to a significant drop in DPP-IV inhibitory activity.

Further to improve DPP-IV inhibitory activity of **27a**, two series (**27b-j** and **34a-m**) of structurally constrained cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors were designed (**Figure 19**). In the first series suitable modifications were carried out on 1C amino-alkyl spacer of **27a** and altogether nine compounds (**27b-j**) were prepared by linking ring A with ring B, using various α-substituted amino acids as spacers. In the second series, thirteen compounds (**34a-m**) were prepared by modifying the best compound obtained from first series (i.e. **27j**), specifically by carrying out suitable changes over ring -A and –B, taking in to consideration the effect of substituents extensively being used in the DPP-IV drug discovery research **[229-230]**.

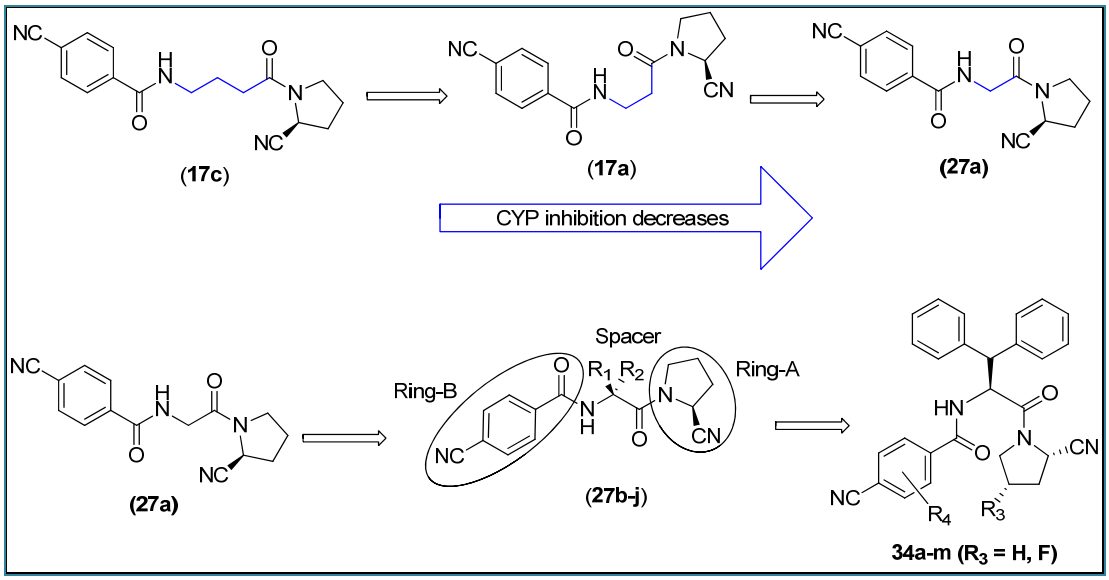


Figure 19: Design strategy of peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities.

R_1 and R_2 together represent substituted α-amino acids with absolute (S) stereo configuration (**Figure 20**). Substituent R_4 in ring-B together represent substituted 4-Cyano benzoic acids selected from the group given in **Figure 21**.

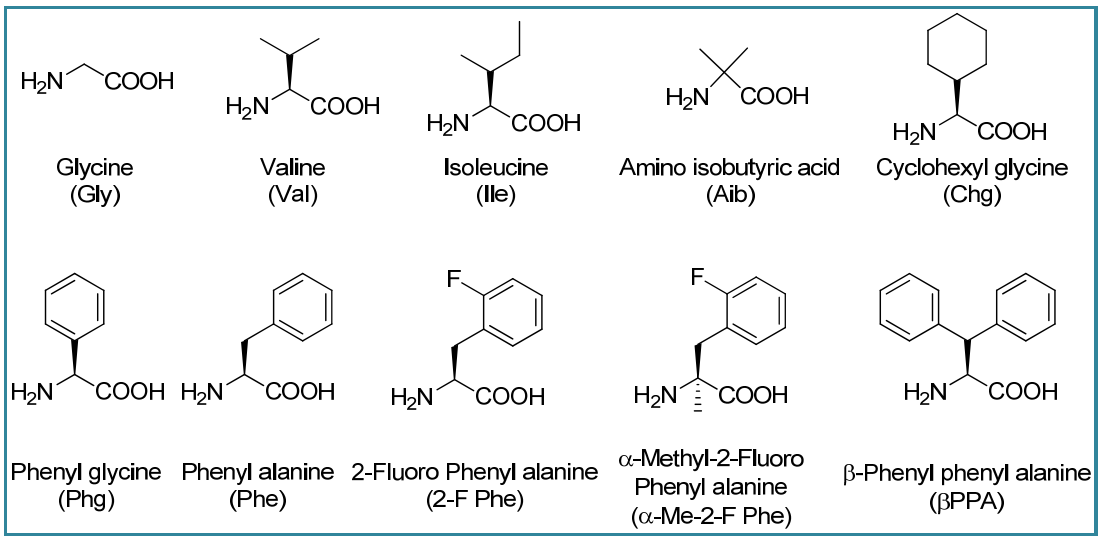


Figure 20: Structures of α-amino acids used as a linker.

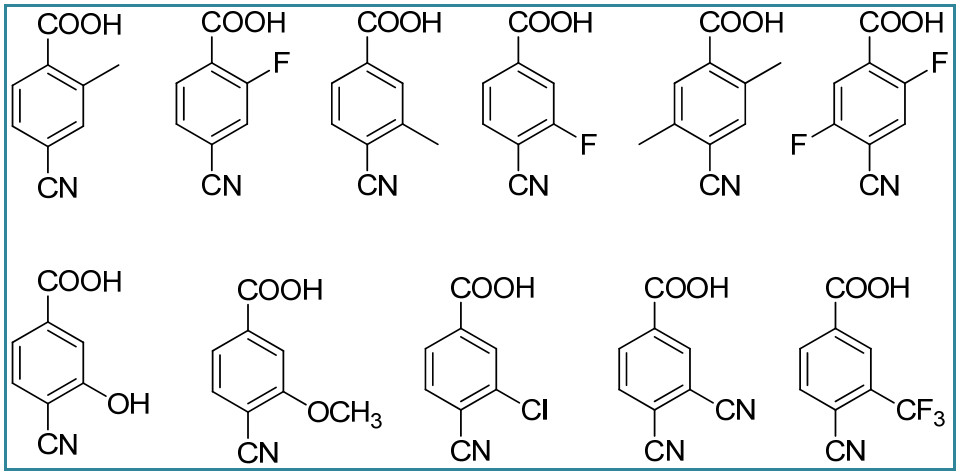


Figure 21: Structures of substituted 4-Cyano benzoic acids

In this series, we planned total twenty-three compounds as two different series **27a-j** and **34a-m**, their synthetic methodology and chemical characterization are explained in details in **Chemistry section 3.2.1**.

2.1.3. Rational for designing of aminomethylpiperidone based DPP-IV inhibitors. (Third series)

Taking into consideration journey of Merck Sharp & Dohme Corp. to develop potent and selective DPP-IV inhibitors with improved pharmacokinetic profile, we have designed aminomethylpiperidone based DPP-IV inhibitors (**Figure 22**).

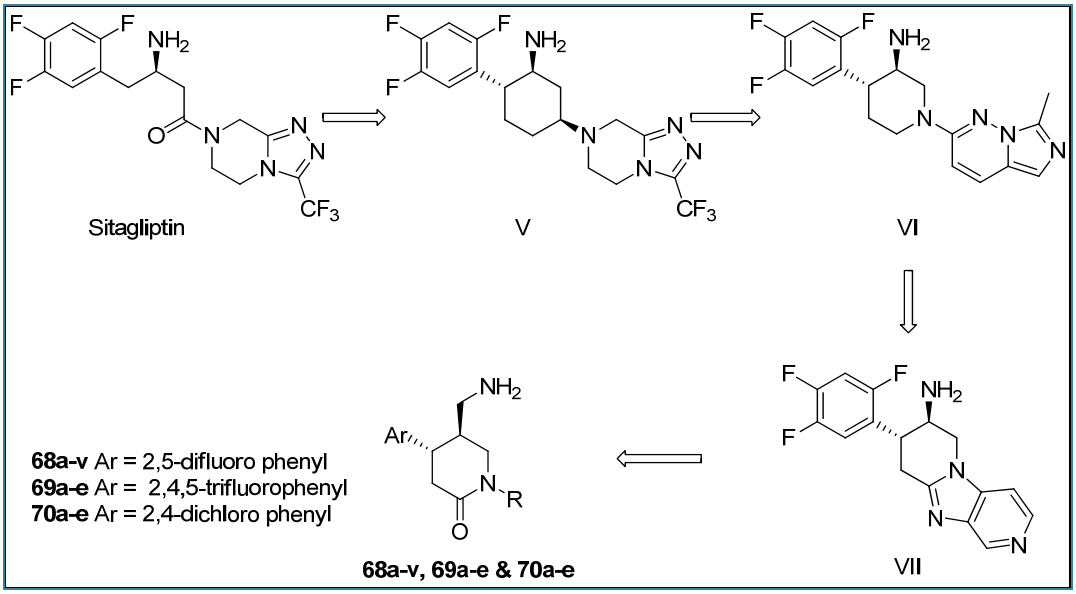


Figure 22: Design strategy of aminomethylpiperidone based DPP-IV inhibitors.

During their journey to improve PK profile Merck has come up with compound **VII** having extended $t_{1/2}$ and almost double bioavailability as compared to Sitagliptin **[195,209-210,236]**. So considering all structural modification done by Merck and extending the scope of novelty with rationale, compounds (**68a-v**, **69a-e** and **70a-e)** were designed based on the piperidone skeleton and anticipated that the aminomethyl and the amide groups of the piperidone ring may contribute improved pharmacokinetic and pharmacodynamic effects, along with the potent and selective DPP-IV inhibitory activity.

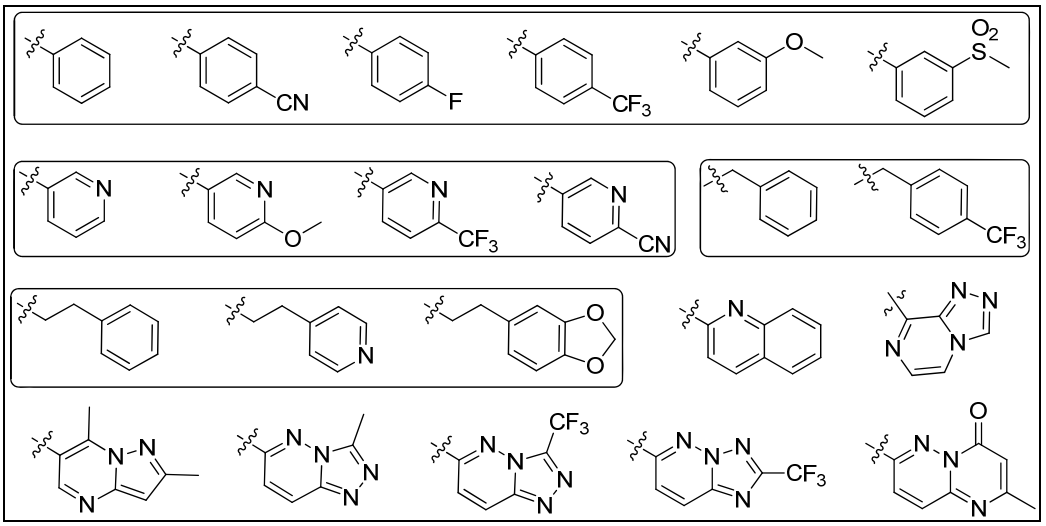


Figure 23: Substituents used for synthesis of aminomethylpiperidone based DPP-IV inhibitors.

Substituent R in novel aminomethylpiperidones **68a-v**, **69a-e** and **70a-e** was selected from the given group (**Figure 23**).

In this series we planned to prepare total thirty two compounds as three different series viz **68a-v**, **69a-e** and **70a-e**, their synthetic methodology and chemical characterization are explained in details in **Chemistry section 3.3.1**.

2.1.4. Conclusion

In the present investigation, total three series (First series: cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors, second series: peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities and Third series: aminomethylpiperidone based DPP-IV inhibitors) were designed as potent and selective DPP-IV inhibitors for the safe and effective treatment of metabolic diseases such as T2DM. Altogether eighty five compounds were planned, mainly as DPP-IV inhibitors. All the test compounds were synthesized and well characterized, subjected for *in vitro*, *in vivo* and pharmacokinetic (PK) studies and results are summarized in the results and discussion section.

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Chapter III:

Results & Discussion

"Seven Deadly Sins: Wealth without work, Pleasure without conscience, Science without humanity, Knowledge without character, Politics without principle, Commerce without morality, Worship without sacrifice." — Mahatma Gandhi

3. Results and discussion

As described in the previous chapter, we designed and synthesized three novel series of DPP-IV inhibitors, the first series was cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors, second series was peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities and third series was aminomethylpiperidone based DPP-IV inhibitors. All synthesized compounds were purified, characterized and subjected for *in-vitro* DPP-IV inhibition study to establish Structure Activity Relationship (SAR) of individual series. Selected short listed most potent compounds from each series were subjected for *in vitro* selectivity over related serine proteases. The most potent and selective compounds were further subjected for *in vivo* antidiabetic activity. Selected short listed compounds (most potent compounds : both *in vitro* & *in vivo*) were also subjected for PK studies.

In this section, we summarized results and discussion of :

a) Cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors (First series)

b) Peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities (Second series)

c) Aminomethylpiperidone based DPP-IV inhibitors (Third series), in following sections:

- Synthesis of three different series (Chemistry)
- *In vitro* DPP-IV inhibitory activity, selectivity and SAR
- *In vitro* CYP inhibition study
- *In vivo* (antidiabetic activity) evaluation of DPP-IV inhibitors
- PK studies of selected compounds
- Docking studies

3.1. Cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors (First series)

3.1.1. Chemistry

In the previous section rational for designing cyanopyrrolidine containing peptidomimetic based potent DPP-IV inhibitors has been described, wherein we intended to synthesize the compounds represented by general structures **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** (**Figure 24**). Synthetic methodology was designed based on the retrosynthetic analysis and the schemes are described below. Synthetic method reported in literature were adapted for the synthesis of title compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** respectively. All compounds were synthesized following the procedure reported earlier in literature by choosing the appropriate starting materials and optimizing reaction conditions.

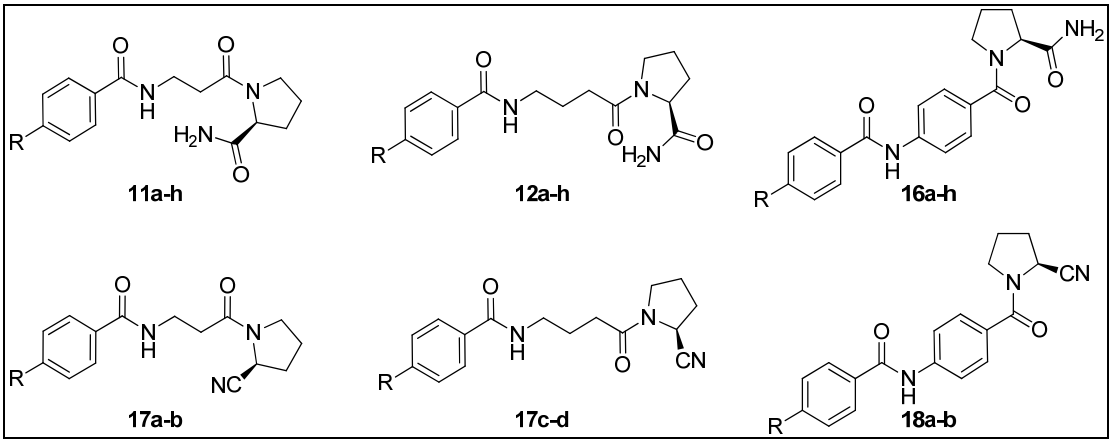
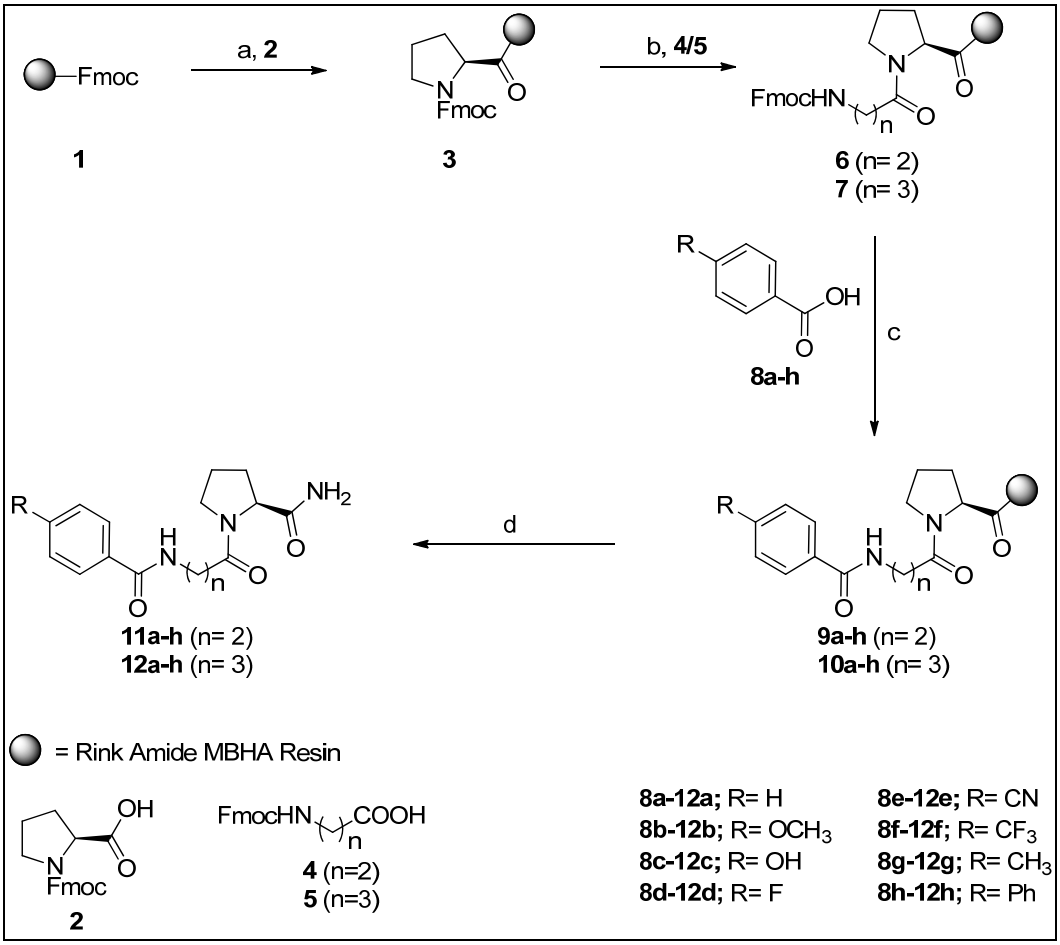


Figure 24. cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors

Synthesis of designed compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** is illustrated in **Schemes 1-3.** Novel peptidomimetics were synthesized using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach **[237]**, starting from commercially available Fmoc Rink Amide MBHA resin **1**. Deprotection of **1** with 20% piperidine in DMF and 1,3-diisopropylcarbodiimide (DIC) coupling with Fmoc-protected natural amino acid Proline **2** gives the resin-bouned Fmoc-protected amino acid **3**. Deprotection of **3** with 20% piperidine in DMF and DIC coupling with Fmoc protected unnatural aminoacids β-alanine (Fmoc-NH-$(CH_2)_2$-COOH) **4** , γ-amino butanoic acid (Fmoc-NH-$(CH_2)_3$-COOH) **5** or *p*-amino benzoic acid (PABA) **13** gives Fmoc-protected resin bound dipeptide **6**, **7** or **14** respectively. Deprotection of **6**, **7** and **14** with 20% piperidine in DMF and DIC

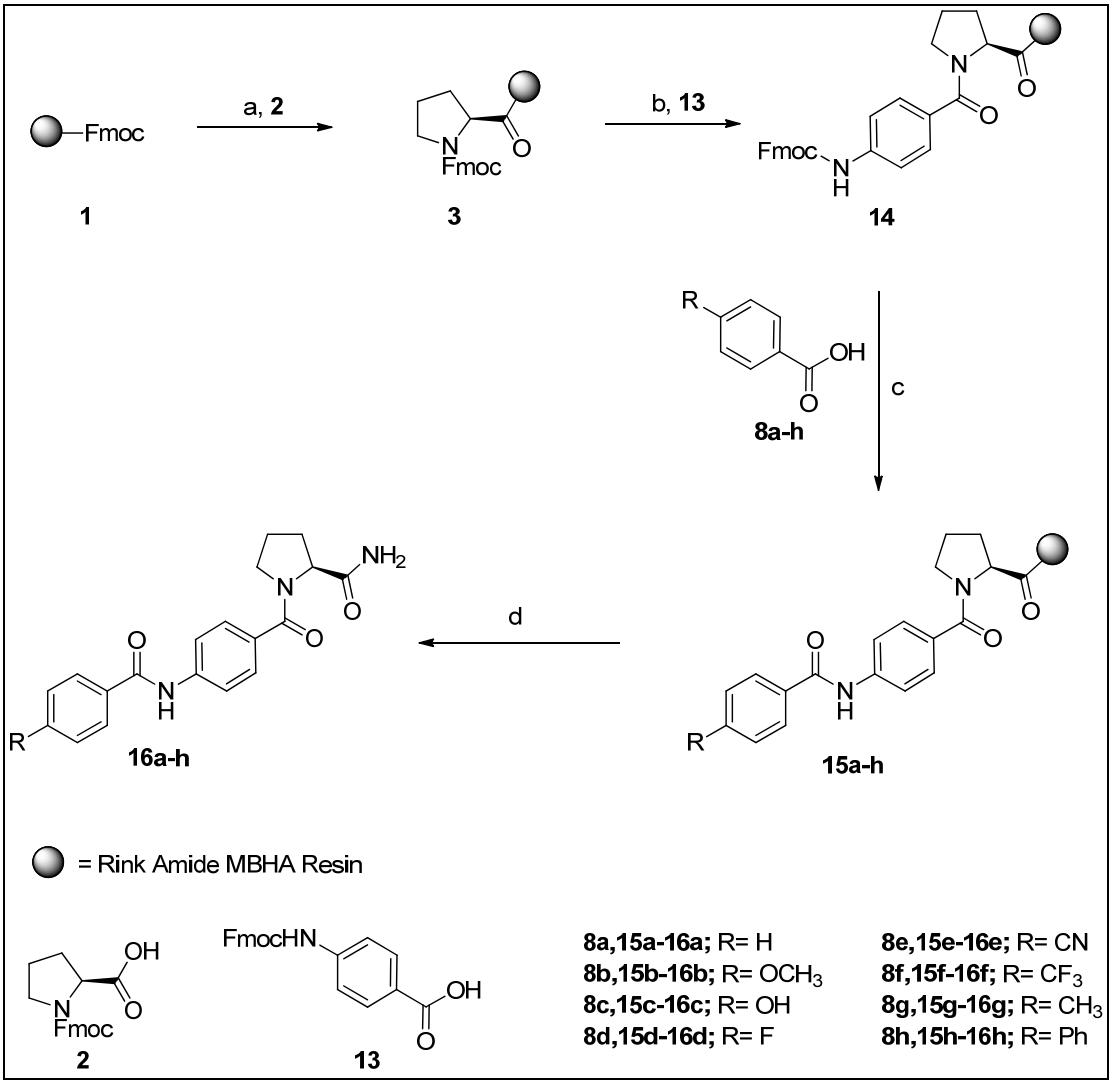
coupling with substituted benzoic acids **8a-h** gives fully-protected resin bounded peptoides **9a-h**, **10a-h** and **15a-h**. Final cleavage from resin was achieved by treatment with cleavage mixture of TFA, H_2O and Triisopropylsilane to give pyrrolidinecarboxamide based peptidomimetics **11a-h** ,**12a-h** and **16a-h**. Dehydration of pyrrolidinecarboxamide based peptidomimetics **11e-f**, **12e-f** and **16e-f** using trifluoroacetic anhydride at room temperature provided cyanopyrrolidin based peptidomimetics **17a-d** and **18a-b**.

Crude peptidomimetics thus obtained were purified using semi-preparative HPLC on a Shimadzu model LC-8A liquid chromatography. Desired fractions were pooled together, frozen and lyophilized to give title compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b**.



Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-NH-$(CH_2)_n$-COOH (**4**/**5**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. Substituted benzoic acids (**8a-h**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 25°C, 3h.

Scheme 1. Synthetic methods for the preparation of title compounds **11a-h** and **12a-h**

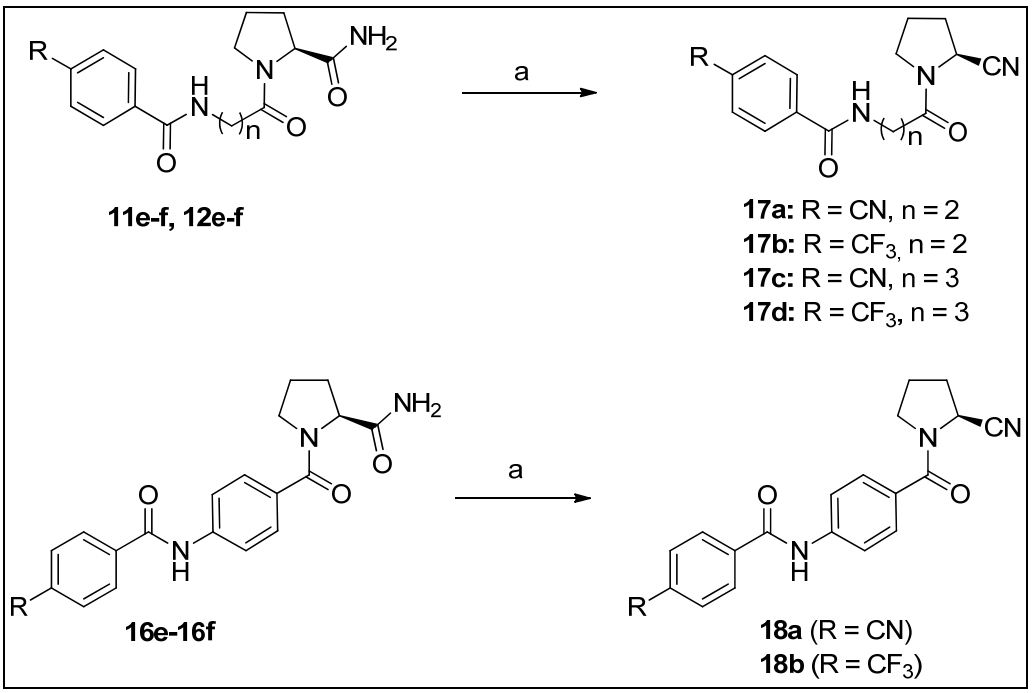


Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-PABA-COOH (**13**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. Substituted benzoic acids (**8a-h**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 25°C, 3h.

Scheme 2. Synthetic methods for the preparation of title compounds **16a-h**.

For the preparation of title compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** (**Scheme 1**-**3**), we need to first synthesize Fmoc derivative of the commercially available unnatural amino acids proline **19**, β-alanine **20**, γ-amino butanoic acid **21** and *p*-amino benzoic acid **22**, synthesis of which is outline in **scheme 4**.

Substituted benzoic acids **8a-h** used for the synthesis of peptidomimetics **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** were procured from the commercial bulk supplier and used as such without doing any modification or purification.

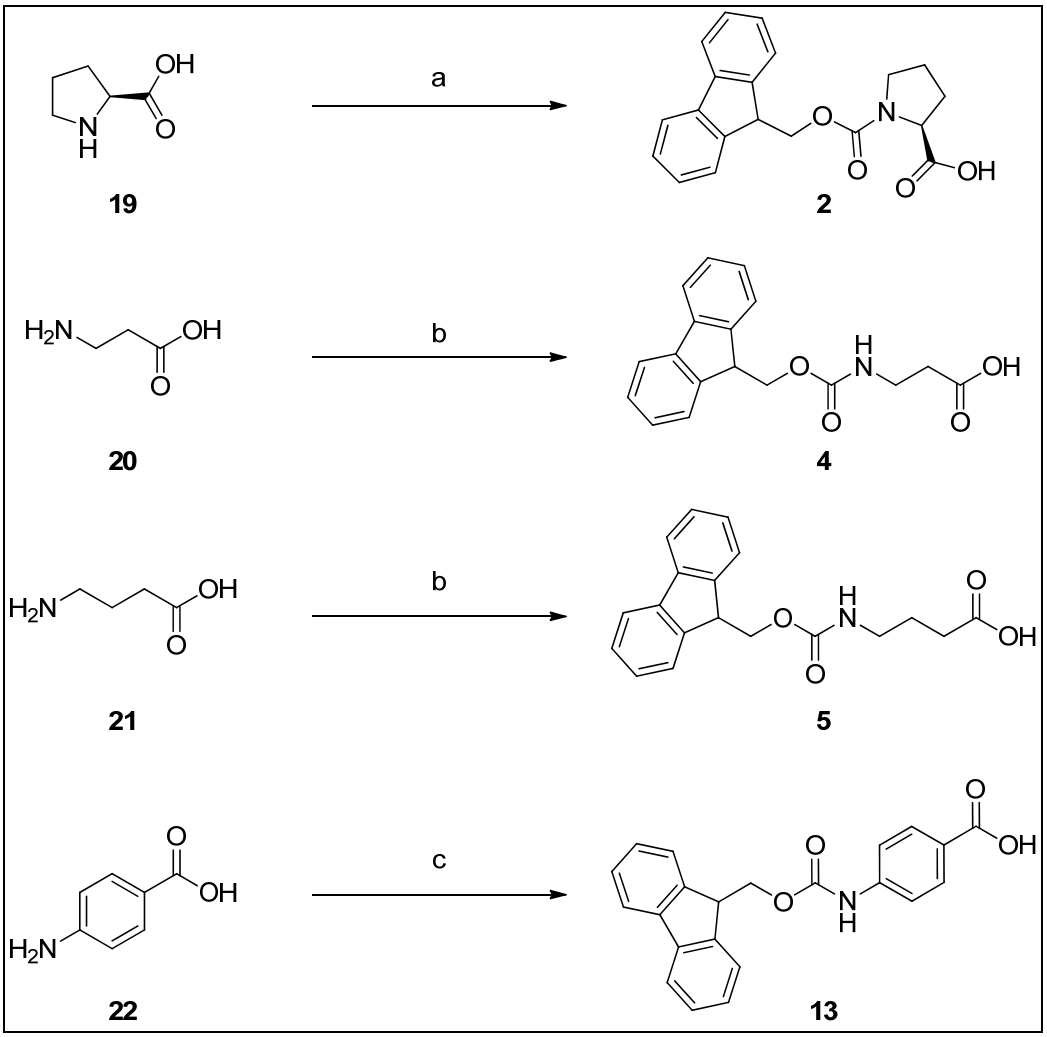


Reagents and conditions: (a) TFAA, CH_2Cl_2, 25°C, 6h.

Scheme 3. Synthetic methods for the preparation of title compounds **17a-d** and **18a-b**.

Synthesis of Fmoc deravitives of unnatural amino acids (2,4,5 and13)

In general synthesis of N-Fmoc protected amino acid is a major challenge due to racimization possibility as well as due to their zwitter ionic nature isolation problem arise. Several methods are reported in the literature for the Fmoc protection of amino acids, among which few most efficient high yielding methods are mention here. Douglass Taber et al reported convenient synthetic route to an enantiomerically pure Fmoc α-amino acid **[238]**. Manoj Gawande et al reported Fmoc protection without using any base (to avoid any racemization) at higher temperature to give desired compounds with high yield 80-95% and enantiomeric purity **[239]**. Carpino et al Fmoc-O-Su and Na_2CO_3 in a 1:1 H_2O:dioxane mixture where as more efficient method then this used by Jeffrey M. Dener utilised Fmoc-O-Su and $KHCO_3$ in a 1:1 H_2O:acetonitrile mixture **[240-241]**. Here we used different literature methods depending upon the nature of amino acids as outlined in **scheme 4** by optimizing reaction condition.



Reagents and conditions: (a) N-hydroxy succinamide, Fmoc-Cl, Na_2CO_3, Water:Acetone, 0°C-25°C, 15h (b) Fmoc-Cl, Na_2CO_3, Water:1,4 Dioxane, 25°C, 15h. (c) Fmoc-OSu, NEt_3, Water:Acetonitrile, 25°C, 3h.

Scheme 4. Different conditions for the synthesis of Fmoc protected amino acids.

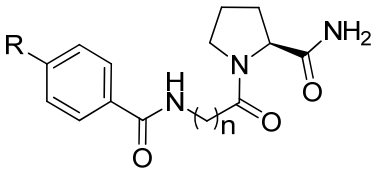
However, method developed by Carpino et al is used extensively as a generlised method for the synthesis of Fmoc protected aminoacids **[240]**.

3.1.2. *In vitro* DPP-IV inhibitory activity, selectivity and structure activity relationship (SAR)

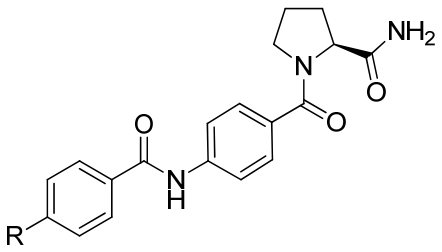
All the novel peptidomimetics compounds prepared above as two different series, pyrrolidincarboxamide containing (**11a-h**, **12a-h** and **16a-h)**, and cyanopyrrolidine containg (**17a-d** and **18a-b**) peptidomimetics were subjected for the *in vitro* DPP-IV

inhibitory activity in order to establish the structure–activity relationship (SAR) using fluorescence-based assay (details experimental protocol is given in **experimental section 5.2.1**) **[242]**. As depicted in **Table 7-8**, depending upon the nature of substitution, all the compounds showed different degree of DPP-IV inhibition (IC_{50}).

Table 7: *In vitro* DPP-IV inhibitory activity of peptidomimetics **11a-h**, **12a-h** and **16a-h**∗



11a-h (n= 2) ; 12a-h (n= 3)



16a-h

S. No	R	DPP-IV inhibition**	S. No	R	DPP-IV inhibition**
11a	*-H*	320 ± 29	**12e**	*-CN*	26 ± 3.1
11b	*-OCH_3*	890 ± 21	**12f**	*-CF_3*	19 ± 2.3
11c	*-OH*	863 ± 18	**12g**	*-CH_3*	694 ± 14
11d	*-F*	93 ± 9.3	**12h**	*-Ph*	104 ± 16
11e	*-CN*	31 ± 2.5	**16a**	*-H*	311 ± 24
11f	*-CF_3*	28 ± 1.7	**16b**	*-OCH_3*	879 ± 22
11g	*-CH_3*	715 ± 27	**16c**	*-OH*	863 ± 13
11h	*-Ph*	107 ± 19	**16d**	*-F*	100 ± 8.5
12a	*-H*	298 ± 19	**16e**	*-CN*	34 ± 7.6
12b	*-OCH_3*	869 ± 43	**16f**	*-CF_3*	31 ± 8.3
12c	*-OH*	843 ± 26	**16g**	*-CH_3*	723 ± 21
12d	*-F*	74 ± 11	**16h**	*-Ph*	116 ± 13

∗DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software

∗∗ DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3)

As described earlier in designing strategy section of DPP-IV inhibitors, novel peptidomimetics of the first series were prepared by linking basic pharmacophore, a pyrrrolidine ring system (proline) with substituted benzoic acids, using suitable spacers (i.e. set-1: β-Ala (**11a-h**); set-2: GABA (**12a-h**) and set-3: PABA (**16a-h**)). In the second series (pyrrolidinecarbonitriles), six compounds (**17a-d** and **18a,b**) were prepared by replacing pyrrolidinecarboxamides with pyrrolidinecarbonitriles.

Within the first series (**11a-h**, **12a**-**h**, **16a**-**h**), the set-1 (**11a-h**) containing *β*-alanine spacer attached to *para*-substituted benzamides, showed diverse DPP-IV inhibitory activity depending on the nature of substituents at the *para*-position. Compounds with electron donating groups (**11b**: -OMe and **11c**: -OH) showed weak inhibitory activity relative to that of un-substituted (**11a**: -H), whereas compounds with electron withdrawing groups (**11d**; **11e** and **11f**) showed good DPP-IV inhibitory activity. Among the **11d**, **11e** and **11f** tested, **11e** and **11f** showed improved DPP-IV inhibitory activity, which could be due to increase in the electronegativity at *para*-position of benzamide. Aliphatic substitution at *para* position (**11g**; -CH_3) showed weak inhibitory activity, while aromatic substitution at *para* position (**11h**; -Ph) showed moderate DPP-IV inhibitory activity. The second and third set of compounds (**12a**-**h**, **16a**-**h**) comprising GABA and PABA spacers attached to *para*-substituted benzamides, showed similar trend in DPP-IV inhibitory activity as observed with first set of compounds, with respect to nature of *para*-substituents.

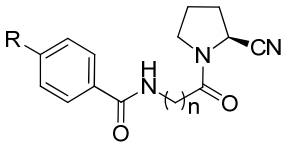
The first series was specifically designed to understand the role of spacer and effect of *para*-substituents on benzamide. The SAR study of first series reveals that the DPP-IV inhibitory activity of test compounds drastically varies with *para*-substituents, whereas alteration in spacers (aliphatic with two/ three carbon chain-length versus aromatic) do not exhibit significant change. In general, neutral effect of spacers on inhibitory activity might be due to the flexibility in S2 pocket and stapled orientation of Glu-dyad. Substituents on *para*-position of benzamide altered inhibitory activity to greater extent because in S3 pocket, para-substituents play crucial role for its interaction with Ser209, Arg358 and Phe357.

From first series, altogether in three different sets, **11e**, **11f**, **12e**, **12f**, **16e** and **16f** (*para*-nitrile/ trifluoromethyl benzamide) were identified as primary lead compounds. Further to study effect of nitrile group on pyrrolidine ring system, a second series (**17a**-**d** and **18a**-**b**) was prepared by replacing pyrrolidinecarboxamides of first series lead compounds with pyrrolidinecarbonitriles.

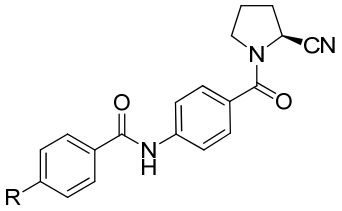
As depicted in **Table 8**, all the six compounds (**17a**-**d** and **18a**-**b**) from second series showed potent inhibitory activity and was found to be comparable with standard compounds (NVP-DPP728 and Vildagliptin) **[32, 233]**. Compared to first series (pyrrolidinecarboxamides), significant improvement in the inhibitory activity was observed with second series (pyrrolidinecarbonitriles) of compounds (**17a**-**d** and **18a**-**b**), which could be due to the favorable interactions of pyrrolidinecarbonitriles with the key

residues of S1 pocket. Among six compounds tested (second series), **17c** and **17d** were found to be equipotent as Vildagliptin.

Table 8: *In vitro* DPP-IV inhibitory activity and selectivity of peptidomimetics **17a-d** & **18a-b**∗



17a-b (n= 2); 17c-d (n= 3)



18a-b

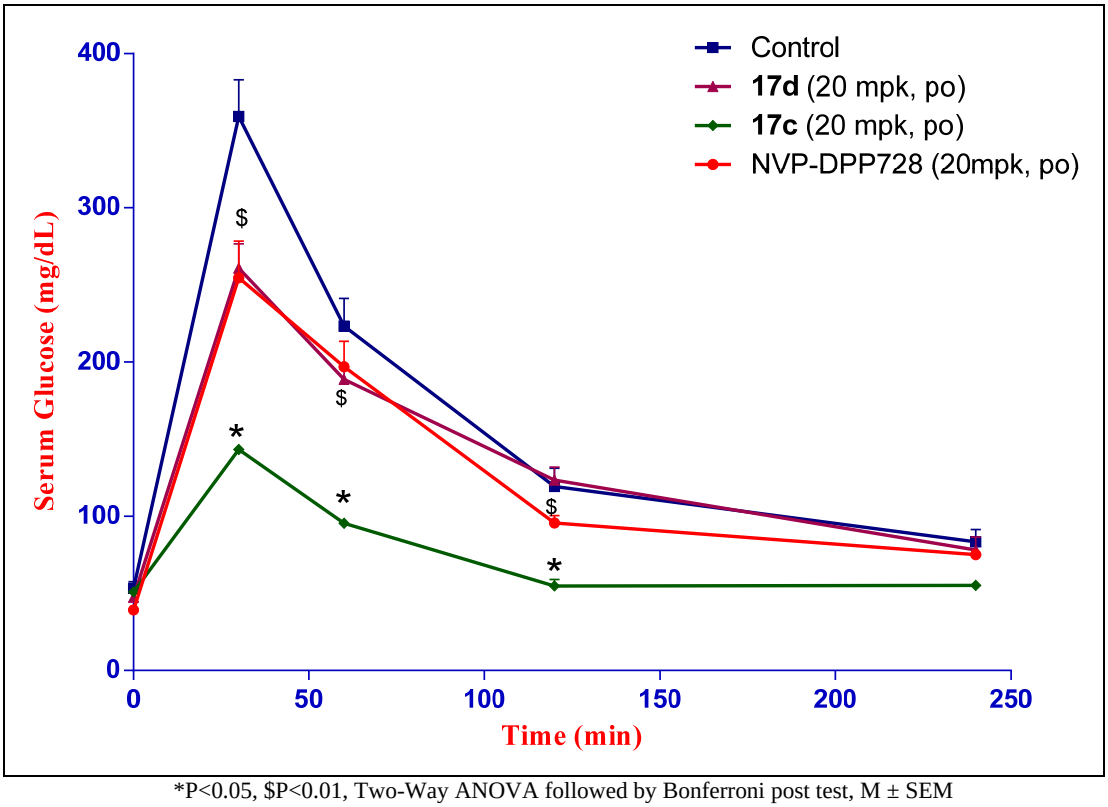
S. No	R	DPP-IV**	DPP2$	DPP8$	DPP9$
17a	-CN	10.3 ± 1.9	---	---	---
17b	$-CF_3$	13.2 ± 2.3	---	---	---
17c	-CN	2.3 ± 0.9	>25,000	>15,000	>15,000
17d	$-CF_3$	3.8 ± 0.5	>25,000	>15,000	>15,000
18a	-CN	11.6 ± 1.6	---	---	---
18b	$-CF_3$	14.3 ± 2.5	---	---	---
NVP-DPP728#		7.2 ± 1.3	>25,000	>15,000	>15,000
Vildagliptin#		3.2 ± 0.5	---	---	---

*DPP-IV inhibitory activity determined by fluorescence-based assay.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3).
$ DPP2, DPP8 and DPP9 inhibitory activity represented as fold-selectivity wrt DPP-IV inhibitory activity.
# Reported literature values for NVP-DPP728 and Vildagliptin are 7±1.7 and 2.7±0.1 respectively.

The *in vitro* selectivity over serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for most potent compounds (**17c** and **17d**) and the fold-selectivity values are listed in **Table 8** (details experimental protocol is given in **experimental section 5.2.1**). Compounds **17c** and **17d** showed >25000-fold selectivity over DPP-2 and >15000-fold selectivity over DPP-8 and DPP-9, which was found to be comparable with reference standard compounds (NVP-DDP728). Among all the compounds tested, **17c** and **17d** were found most potent and selective, hence subjected for pharmacodynamic (PD) as well as pharmacokinetic (PK) profiling studies in animal models.

3.1.3. *In vivo* antidiabetic activity of selected compounds (17c and 17d)

The *in vivo* antidiabetic activity of **17c**, **17d** and NVP-DPP728 (@ 20 mg/kg, p.o.) was evaluated in male C57BL/6J mice, using IPGTT (intraperitoneal glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose up to 240 min; mg/dL) are shown in **Figure 25** (details experimental protocol is given in **experimental section 5.2.3**) **[243-244]**. Compound **17c** showed good oral antidiabetic activity (% Decrease in AUC glucose 54.9 ± 3.86), whereas **17d** and NVP-DPP728 (positive control) showed moderate activity upon oral administration (% Decrease in AUC glucose 17.4 ± 5.35 and 21.5 ± 6.1 respectively). In C57 mice (IPGTT protocol), it was interesting to observe that **17c** showed suppression in the blood glucose at all the time points (30, 60, 120 and 240 min) compared to vehicle control, while **17d** and NVP-DPP728 showed reduction in blood glucose at 30 and 60 minutes.

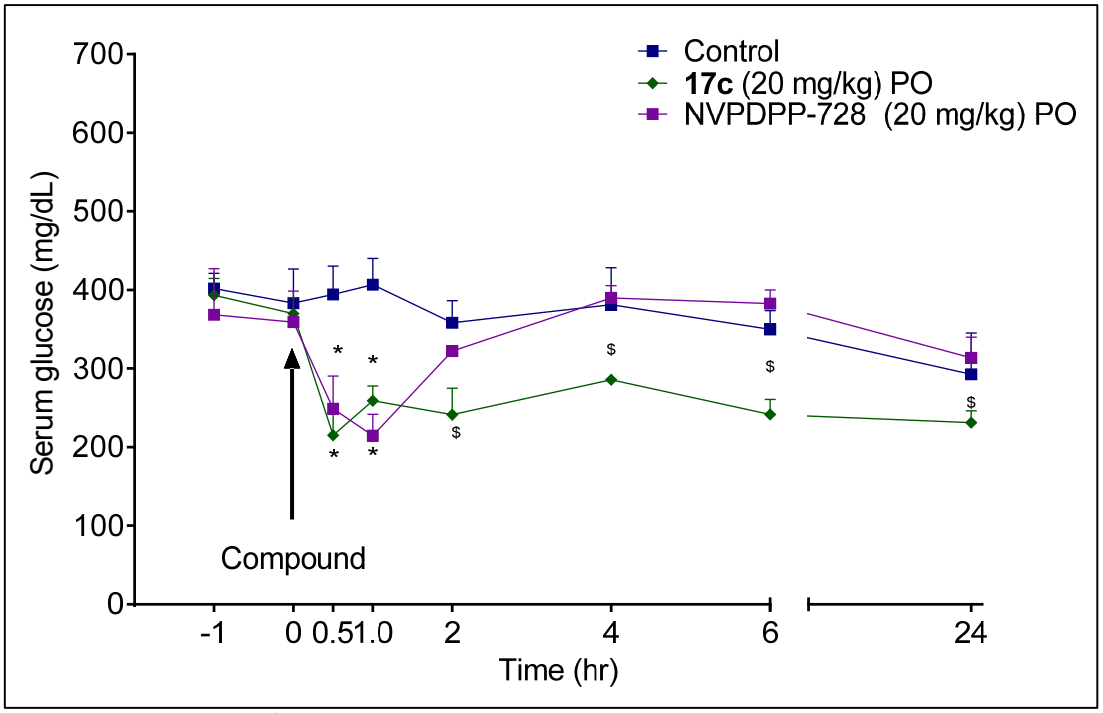


*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 25. *In vivo* antidiabetic activity of **17c**, **17d** and NVP-DPP728 in C57 mice (OGTT)

Further to understand the duration of action and effect of test compounds on post-prandial glucose excursion, single dose (@ 20 mg/kg, p.o.) antidiabetic activity of

17c and NVP-DPP728 was evaluated in fed-db/db mice (hyperglycemic animals) for 24h (**Figure 26**). Under fed condition, compared to vehicle control, NVP-DPP728 and **17c** showed good antidiabetic activity (% Decrease in AUC glucose 31.4±8.7 and 33.5 ± 7.4, respectively) up to 2h. However, **17c** showed sustained suppression in serum glucose levels for > 8h (% Decrease in AUC glucose, 14.9 ± 6.3 for NVP-DPP728 and 30.8±6.2 for **17c**, after 8h).



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 26. *In vivo* antidiabetic activity of **17c** and NVP-DPP728 in db/db mice

3.1.4. Pharmacokinetic (PK) studies of selected compounds (**17c** and **17d**)

A comparative single dose (20 mg/kg i.v. or p.o.) pharmacokinetic (PK) profile of **17c, 17d** and NVP-DPP728 was evaluated in male C57BL/6J mice (n=6) and the various PK parameters such as Tmax, $t_{1/2}$, Cmax, AUC and %F were recorded as shown in **Table 9** (details experimental protocol is given in **experimental Section 5.3**). In PK study, all the test compounds showed rapid t_{max}, good C_{max} and oral bioavailability (%F ~ 63 to 72 %). Compound **17c** showed higher area under the curve (AUC: > 2-fold compared to **17d** and NVP-DPP728) and extended half-life ($t_{1/2}$: >7h compared to **17d** and NVP-DPP728). Thus improved pharmacokinetic profile of compound **17c** justifies its potent and extended pharmacodynamic effects (antidiabetic activity) in C57 and db/db mice, when administered orally.

Table 9: Pharmacokinetic study parameters[a] of **17c, 17d** and **NVP-DPP728**

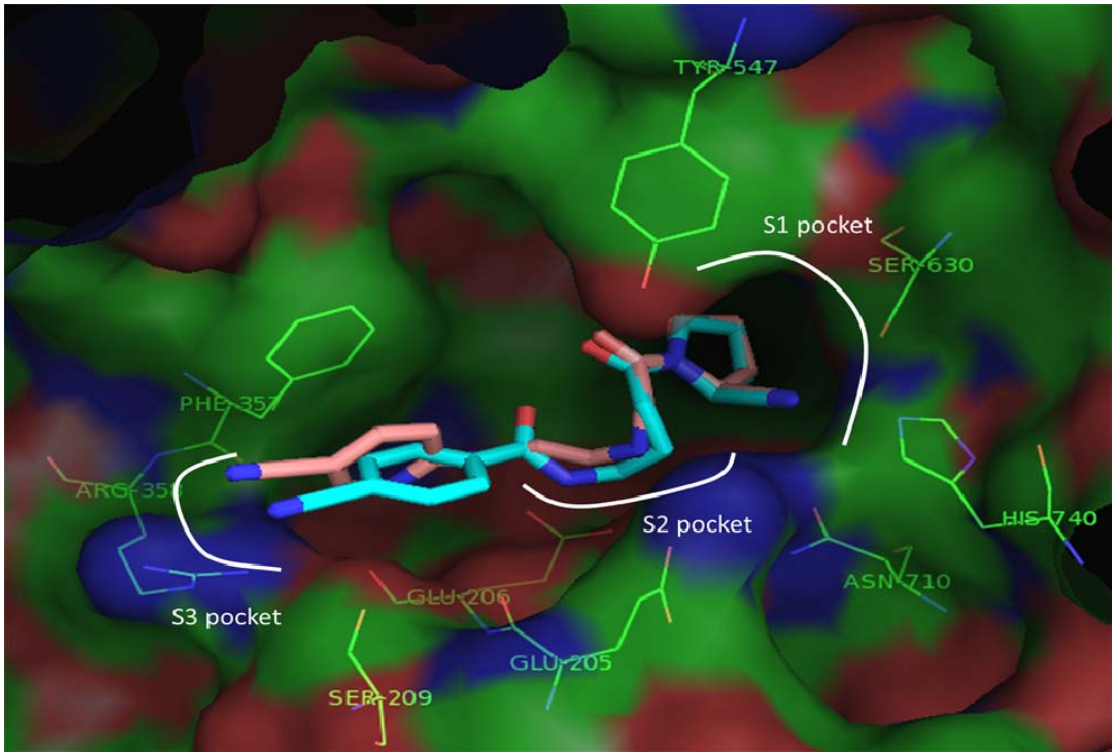
Compd	Tmax (h)	Cmax (mg/ml)	$T_{1/2}$ (h)	AUC (0-α) h mg/ml	%F*
17c	0.29 ± 0.11	7.1 ± 0.83	7.99 ± 0.33	14.3 ± 1.13	72.5%
17d	0.28 ± 0.10	5.9 ± 0.88	0.99 ± 0.14	6.89 ± 1.21	63.1%
NVP-DPP728	0.32 ± 0.08	6.2 ± 0.91	0.88 ± 0.11	6.49 ± 1.11	65%

[a]In male C57BL/6J mice (n=6), compounds were administered orally (p.o) at 20 mg/kg dose and plasma concentration was analyzed by LC-MS, values indicate Mean ± SD.
* Oral bioavailability (%F) was calculated wrt to iv AUC (**17c**: 11.02 ± 0.11; **17d**: 10.92 ± 0.12 & NVP-DPP728: 9.98 ± 0.09 h µg/ml) administered at 20 mg/kg dose, iv.

3.1.5. Molecular docking study of peptidomimetic 17c

The molecular docking analysis of **17c** and NVP-DPP728 was carried out using extra precision (XP) Glide docking method, to understand its critical interactions with all the three binding sites (S1, S2 and S3) of DPP-IV enzyme (**Figure 27**) **[245-246]**. The crystal structure of the DPP-IV enzyme (PDB ID: 2I03) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box. For docking study, the ligands were minimized by applying an OPLS-AA forcefield, using ligprep module of Schrödinger (details experimental protocol is given in **experimental section 5.4**).

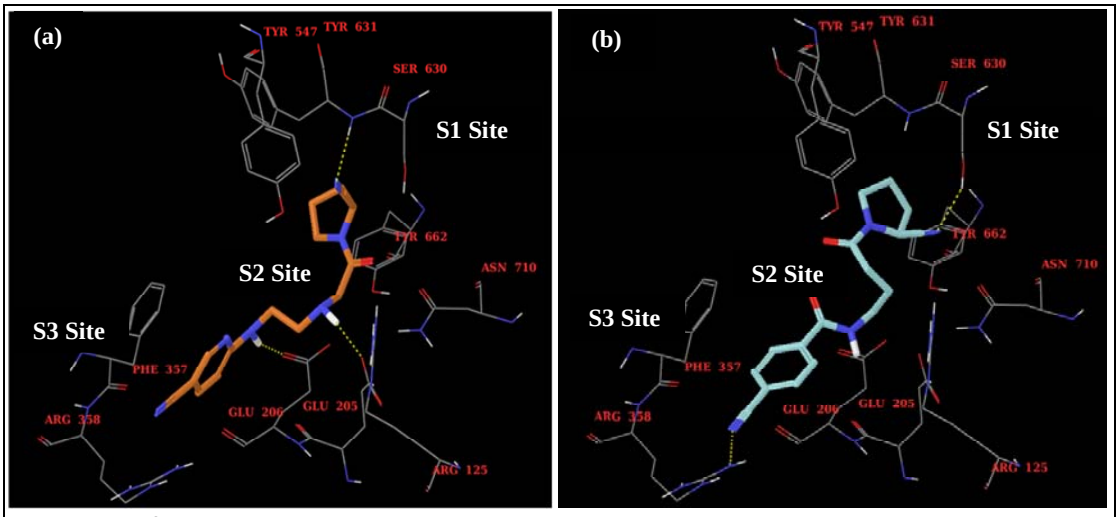
The overlay of binding poses of **17c** (Turquoise) and NVP-DPP728 (Rose) in the DPP-IV active site is shown in **Figure 27** (the molecular surface is shown in Green, the inhibitor in stick representation). The docking study results illustrate that both the compounds interact closely with key residues of S1 (cyanopyrrolidine-CN form covalent bond with OH-group of side-chain of Ser630); S2 (benzamide-NH form H-bonding with C=O groups of side-chains of Glu205 and Glu206 dyad) and S3 (aromatic–CN forms H-bonding with the NH of guanidine side-chain of Arg358) pockets (**Figure 28**).



Binding pose of compound **17c** (Turquoise) and **NVP-DPP728** (Rose) in the DPP-IV active site is indicated (Surface view: Green),
wherein both compounds interact closely with key residues of site S1, S2 and S3.

Figure 27: Key interactions of compound **17c** and NVP-DPP728 (Overlay pose) with active sites of DPP-IV enzyme

Incorporation of GABA linkage (spacer) in **17c** allows it to adopt new confirmation, which favors covalent interaction of cyanopyrrolidine ring with Ser630 (S1 pocket), strong H-bonding of back-bone amide with Glu dyad (S2 pocket) and *para*-nitrile benzamide with Arg358, including aromatic π- π stacking with Phe357 in S3 pocket. As observed with NVP-DPP728, **17c** docks very well into all the three sites (S1, S2 and S3) of DPP-IV crystal structure and these favorable interactions of **17c** with all the three sites of DPP-IV enzyme support its potent *in vitro* DPP-IV inhibitory activity and excellent selectivity over other protease.



Binding pose of **NVP-DPP728** (orange) and **17c** (Turquoise) in the DPP-IV active site is indicated, wherein both compounds interact closely with key residues of site S1, S2 and S3. In site S1, cyanopyrrolidine-CN form covalent bond with OH of side chain of Ser630; S2 site, benzamide-NH…C=O groups of side chains of Glu205 & Glu206 dyad and in S3 site, Aromatic –CN…NH of guanidine side chain of Arg358.

Figure 28: Key interactions of compound **17c** and NVP-DPP728 with active sites of DPP-IV enzyme

3.1.6. Conclusion

In summary, we report here SPPS approach to discover cyanopyrrolidine containing peptidomimetic based potent DPP-IV inhibitors. Total thirty peptidomimetics have been synthesized. The peptidomimetics consisting of *para*-nitrile/-trifluoromethyl benzamide attached to cyanopyrrolidine ring with GABA spacer showed excellent *in vitro* potency and selectivity over other serine protease, due to its favorable orientation across all the three binding sites. The lead compound **17c** showed sustained suppression of pre- and post-prandial blood glucose levels (*in vivo*), which correlates with its extended half-life.

3.2. Peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities (Second series)

3.2.1. Chemistry

As discussed earlier in designing section **2.1.2.** this series was designed specially to overcome CYP activity, as because of it further development of the lead compound **17c** of the first series has been halted. Based upon the designing herein we intended to synthesize the compounds represented by general structures **27a-j** and **34a-**

m (**Figure 24**). Synthetic methodology was designed based on the retrosynthetic analysis and the schemes are described below. Synthetic method reported in literature were adapted for the synthesis of title compounds **27a-j** and **34a-m**. All compounds were synthesized following the procedure reported earlier in literature by choosing the appropriate starting materials and optimizing reaction conditions.

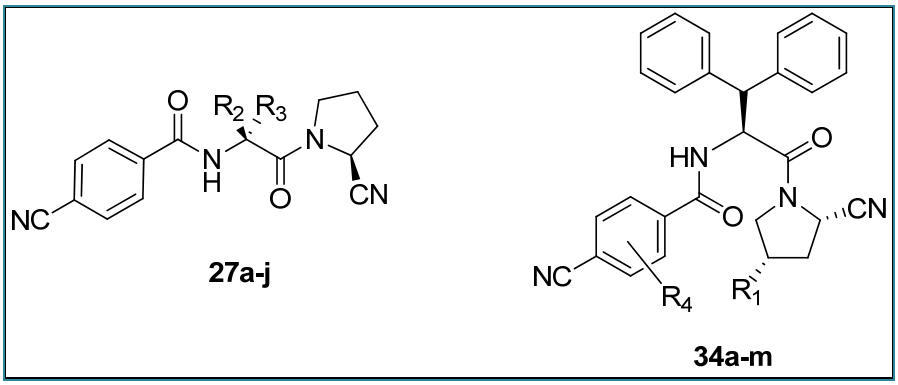
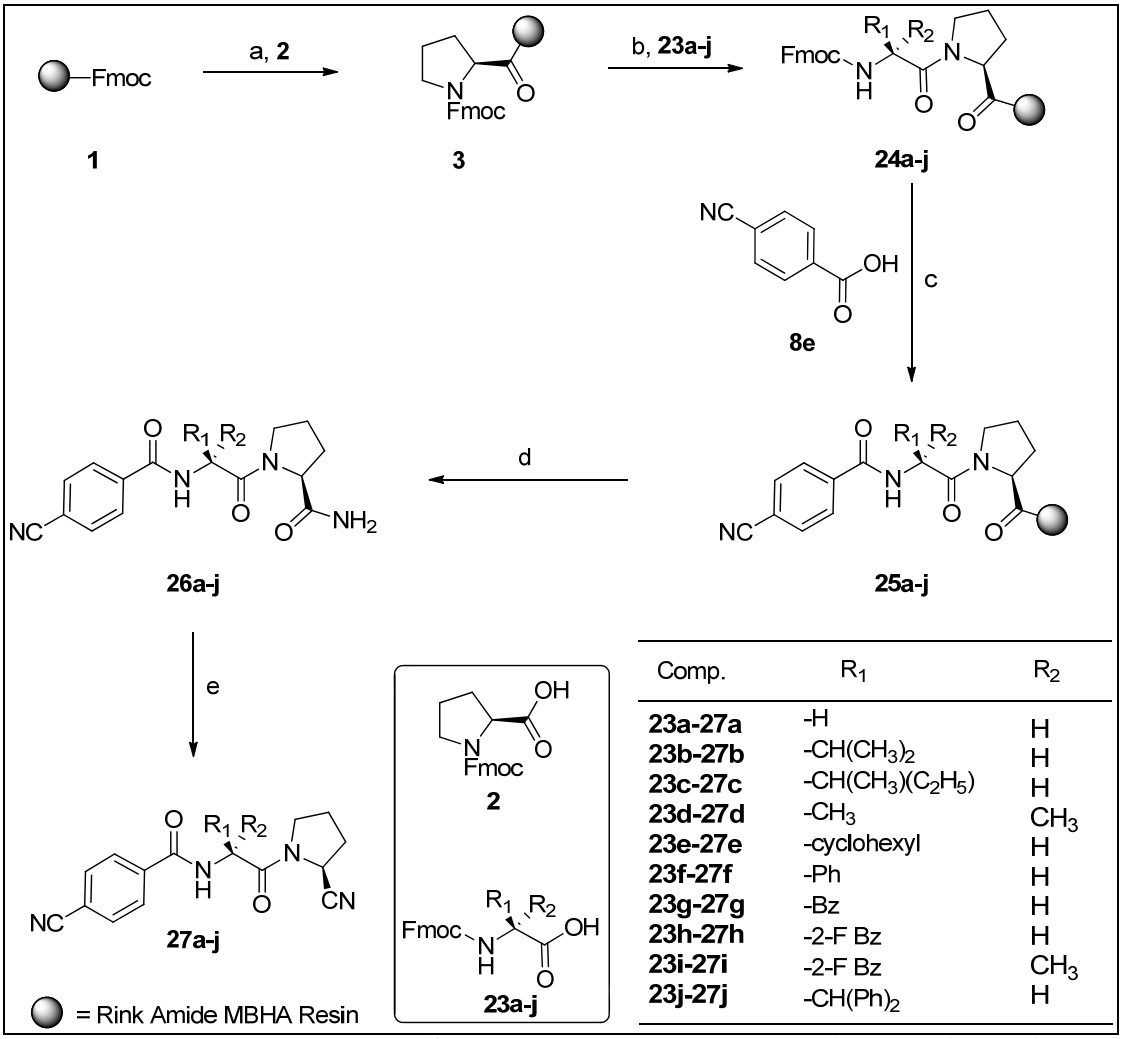


Figure 29. Peptidomimetics based DPP-IV inhibitors.

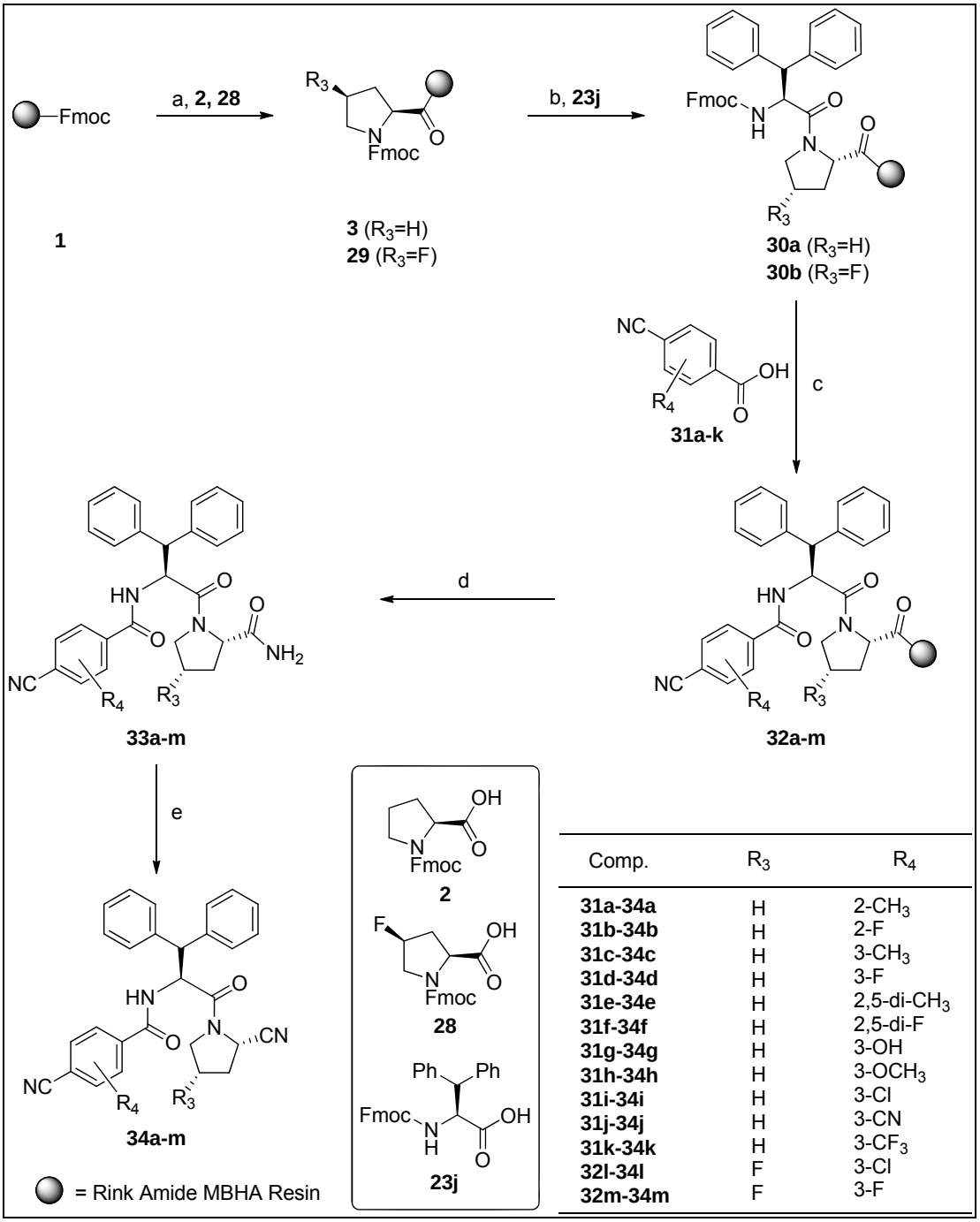
Synthesis of designed peptidomimetics **27a-j** and **34a-m** was carried out using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach illustrated in **Schemes 5-6 [237]**, starting from commercially available Rink-amide MBHA resin **1**, Deprotection of **1** with 20% piperidine in DMF and 1,3-diisopropylcarbodiimide (DIC) coupling with Fmoc-protected amino acid Proline **2** or 4-Fluoro proline **28** gave the resin-bouned Fmoc-protected amino acid **3** and **29**. Which upon deprotection with piperidine (20% DMF) and 1,3-diisopropycarbodiimide (DIC) coupling with Fmoc-protected amino acids **23a-j** provided the resin-bound Fmoc-protected dipeptides **24a-j** and **30a-b**. Deprotection of **24a-j** and **30a-b** with piperidine (20% DMF) and DIC coupling with substituted benzoic acids **8e** or **31a-k** gave resin-bound tripeptides **25a-j** and **32a-m**, which upon Trifluroacetic acid (TFA) mediated cleavage gives pyrrolidinecarboxamides (**26a-j** and **33a-m**). Trifluroacetic anhydride (TFAA) mediated dehydration of pyrrolidinecarboxamides **(26a-j** and **33a-m**) afforded title compounds as pyrrolidinecarbonitriles (**27a-j** and **34a-m**). All the test compounds obtained were purified by preparative HPLC (yield 70-85%; HPLC purity >97%) and characterized by various spectroscopic techniques.



Comp.	R_1	R_2
23a-27a	-H	H
23b-27b	$-CH(CH_3)_2$	H
23c-27c	$-CH(CH_3)(C_2H_5)$	H
23d-27d	$-CH_3$	CH_3
23e-27e	-cyclohexyl	H
23f-27f	-Ph	H
23g-27g	-Bz	H
23h-27h	-2-F Bz	H
23i-27i	-2-F Bz	CH_3
23j-27j	$-CH(Ph)_2$	H

Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-NH-(CHR_1R_2)-COOH (**23a-j**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. *p*-cyano benzoic acid (**8e**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 3h. (e) TFAA, CH_2Cl_2, 25°C, 6h.

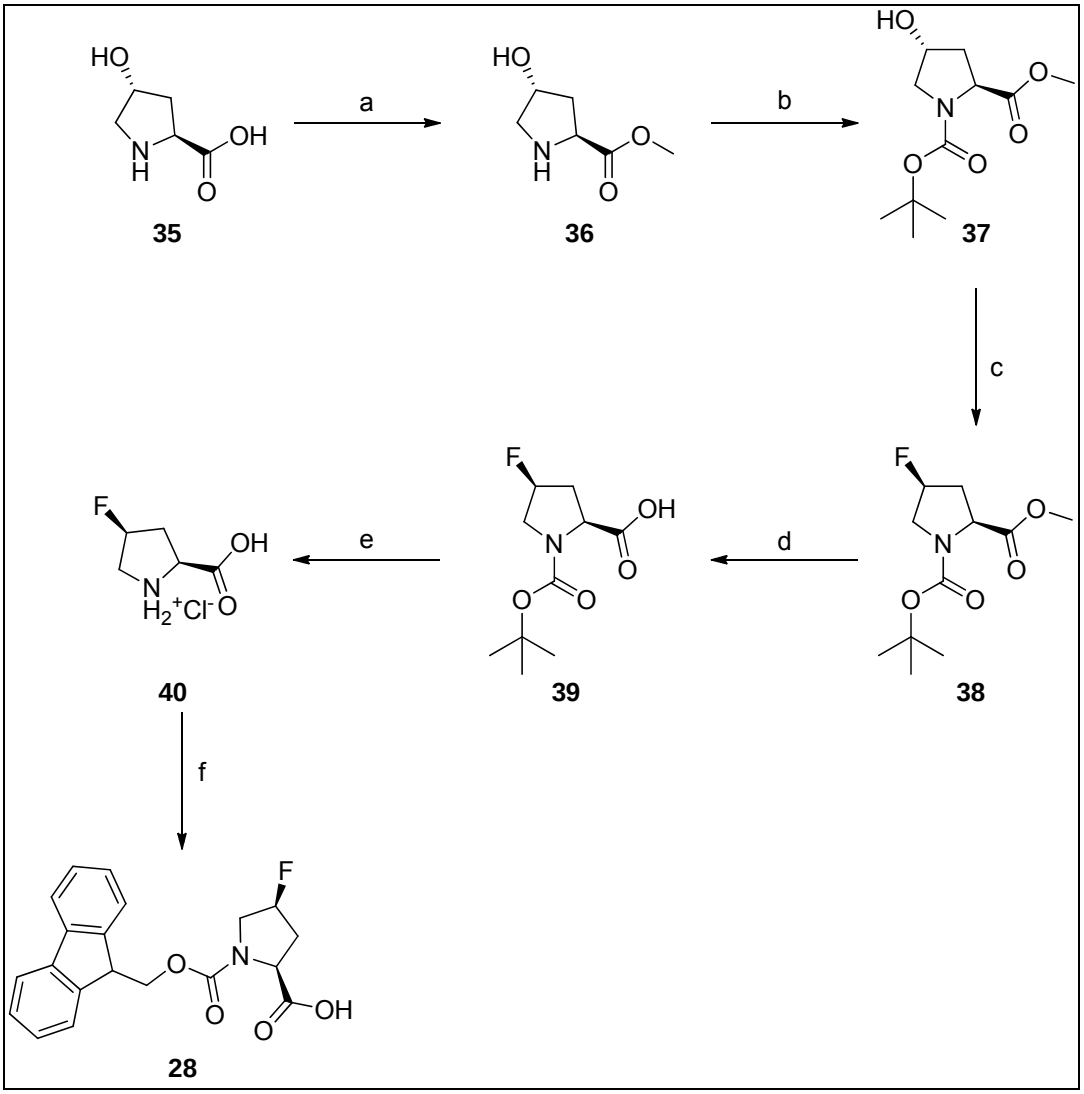
Scheme 5. Synthetic methods for the preparation of peptidomimetics **27a-j**



Comp.	R_3	R_4
31a-34a	H	2-CH_3
31b-34b	H	2-F
31c-34c	H	3-CH_3
31d-34d	H	3-F
31e-34e	H	2,5-di-CH_3
31f-34f	H	2,5-di-F
31g-34g	H	3-OH
31h-34h	H	3-OCH_3
31i-34i	H	3-Cl
31j-34j	H	3-CN
31k-34k	H	3-CF_3
32l-34l	F	3-Cl
32m-34m	F	3-F

Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**)/Fmoc-4-F-Pro-OH (**28**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-NH-($CHPh_2$)-COOH (**23j**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. Substituted benzoic acids (**30a-k**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 3h. (e) TFAA, CH_2Cl_2, 25°C, 6h.

Scheme 6. Synthetic methods for the preparation of peptidomimetics **34a-m**

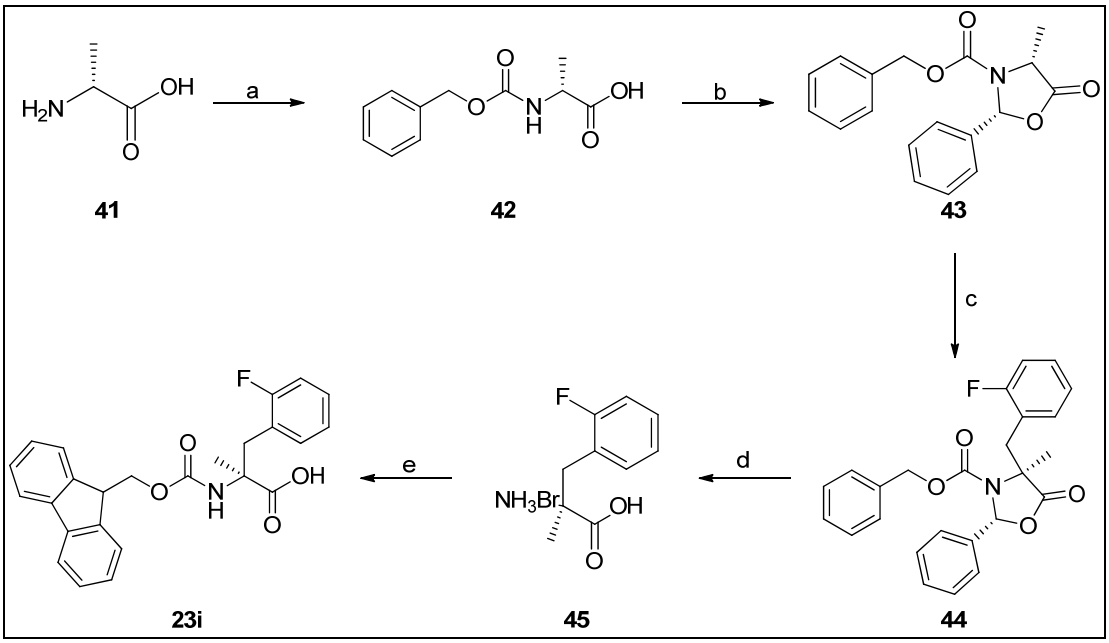


Reagents and conditions: (a) $SOCl_2$, MeOH, Reflux, 15h (b) BOC-anhydride, NEt_3, Water:Acetonitile, 0°C-25°C. 15h (c) DAST, dry DCM, -78°C-25°C, 5h (d) $NaOH_{(aq)}$, THF: MeOH (e) 4M HCl in 1,4-Dioxane, DCM, 25°C (f) N-hydroxy succinamide, Fmoc-Cl, Na_2CO_3, Water:Acetone, 0°C-25°C, 15h

Scheme 7. Synthetic methods for the preparation of unnatural amino acid **28**

However amino acids **23a-h** are commercially available so procured from commercial source and used as such. Synthesis of the unnatural aminoacids **23i-j** used to synthesize novel peptidomimetics **27a-j** and **34a-m** is illustrated in **schemes 7-9**. Christophe Dugave et al reported synthesis of 4-Fluoro prolines from commercially available trans-4-hydroxy proline methyl ester with optimum yield **[247]**. Mukund Chorghade et al reported synthesis of fluoro prolines from trans-4-hydroxy proline using tetrabutyl ammonium fluoride as a fluorine source but the yields were low **[248]**. Weiping

Zhuang et al synthesized fluoro prolines with good yields as well as chiral purity using perfluoro-1-butanesulfonyl fluoride and tetrabutylammonium triphenyldifluorosilicate (PBSF-TBAT) as a fluoride source **[249]**. Here we used method of Christophe Dugave et al for the synthesis of chiral pure Fmoc protected (2S, 4S) 4-hydroxy proline **28** by optimizing the reaction conditions (**Scheme-7**).



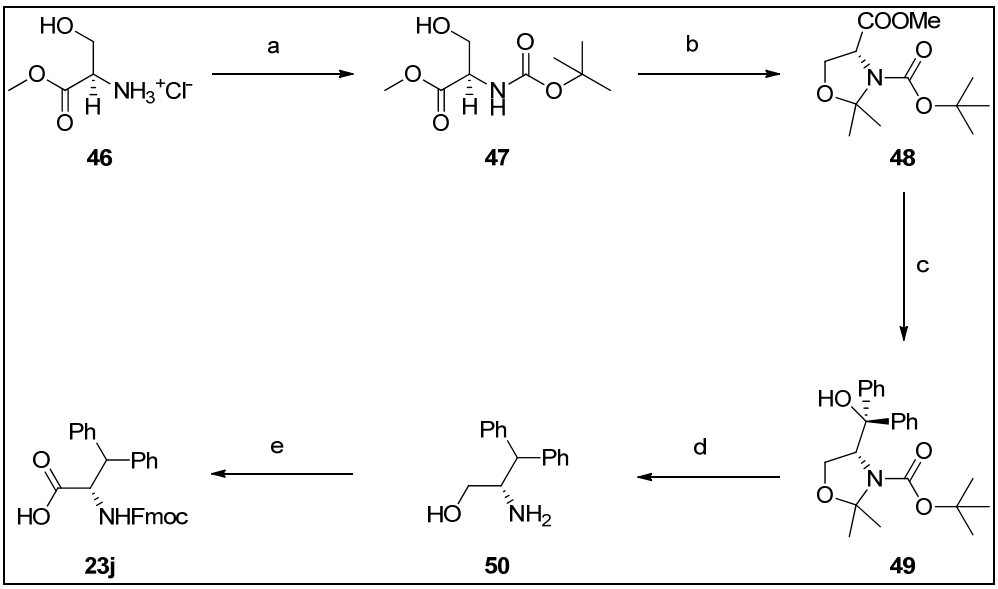
Reagents and conditions: (a) $NaOH_{(aq)}$, Cbz-Cl, Acetonitrile, 0°C-25°C, 15h (b) Benzaldehyde dimethyl acetal, $SOCl_2$, $ZnCl_2$, THF, 0°C 4h (c) 2-F Benzyl bromide, KHMDS, THF, -27°C (d) 33%HBr in CH_3COOH, CH_3COOH, 25°C, 20h (e) Fmoc-Cl, Na_2CO_3, Water:1,4-Dioxane, 0°C-25°C, 15h.

Scheme 8. Synthetic methods for the preparation of unnatural amino acid **23i**

For the synthesis of α-methyl amino acids various methods are reported in the literature **[250]**. Roy Storcken et al reported a chemoenzymatic approach to the synthesis of functionalized α-methyl α-substituted amino acids which involves amidase-mediated enzymatic resolution and cross-metathesis **[251]**. Ta-Jung Lu et al reported asymmetric synthesis of α-methyl-α-amino acids via diastereoselective alkylation of (1*S*)-(+)-3-Carene derived tricyclic iminolactone with high enantiopurity **[252]**. Martin O'Donnell et al reported enantioselective synthesis of α-methyl amino acid via phase-transfer catalysis **[253]**. Franklin Davis et al reported sulfinimine-mediated asymmetric strecker synthesis of the synthesis of α-alkyl α-amino acids **[254]**. Peng-Fei Xu et al. reported synthesis of α,α-disubstituted α-amino acids by diastereoselective alkylation of camphor-based tricyclic iminolactone **[255]**.

Here we used enetioselective and high yielding method of Suresh Kapadia et al for the synthesis of amino acid **23i** as shown in **scheme-8** by modifying the reaction conditions **[256]**.

Soledad Royo et al reported high yielding racemic synthesis and a very efficient resolution procedure for synthesis of enantiomerically pure unnatural amino acid β-phenyl phenyl alanine (β-PPA) **23j [257]**. Mukund Sibl et al have reported convenient synthesis of antipode of **23j** using chiral auxiliary starting from L-serine methyl ester hydrochloride in good yield and chiral purity **[258]**. Ari Koskinen et al reported very good scalable method for the preparation of enantiomer of **23j [259]**, Here we adopted this method of Ari Koskinen et al for the synthesis of **23j** by replacing the starting material with its antipode and optimizing the reaction conditions to obtaine **23j** with desire absolute (S) configuration (**scheme-9**) **[259]**.



Reagents and conditions: (a) BOC-anhydride, NEt_3, Water:Acetonitrile, 0°C-25°C. 15h (b) 2,2-Dimethoxypropane, BF_3-Et_2O, Acetone, 25°C, 3h (c) PhMgBr, THF, 0°C, 5h (d) i. H_2, $Pd(OH)_2$/C, HCOOH, 60°C, 5h, ii. NaOH, MeOH:Water, reflux, 15h (e) i. Fmoc-Cl, Na_2CO_3, Water:1,4-Dioxane, 0°C-25°C, 15h, ii. CrO_3, H_2SO_4, Acetone, 0°C, 3h.

Scheme 9. Synthetic methods for the preparation of unnatural amino acid **23j**

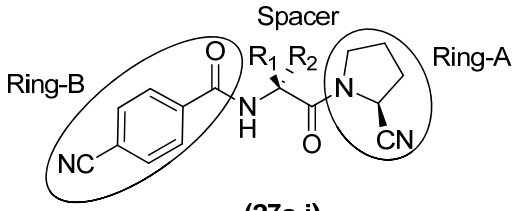
Synthesis of **23j** was accomplished with commercially available D-Serine methyl ester hydrochloride **46**, making Garner's aldehyde type chiral auxiliary derivative **48** by adopting method reported by Andrew Campbell et al and performing necessary optimization in reaction conditions **[260]**.

However various substituted 4-cyano benzoic acids **31a-k** used for the synthesis of peptidimimetics **27a-j** and **34a-m** have been procured from the commercial suppliers and no attempt have been made for their synthesis.

3.2.2. *In vitro* DPP-IV inhibitory activity, selectivity and structure activity relationship (SAR)

The *in vitro* DPP-IV inhibitory activity was determined in order to establish the structure–activity relationship (SAR) using fluorescence-based assay (details experimental protocol is given in **experimental Section 5.2.1.**) **[242]**. As shown in **Table 10-11**, two series of peptidomimetics (**27a-j** and **34a-m**) were prepared and depending on the nature of substitutions, different degree of DPP-IV inhibitory activity was observed.

Table 10: *In vitro* DPP-IV inhibitory activity of peptidomimetics **27a-j**∗



(27a-j)

S. No	R_1	R_2	Amino acids[$]	DPP-IV inhibition[**]
27a	H	-H	Gly	722 ± 3.4
27b	$-CH(CH_3)_2$	-H	Val	74 ± 2.4
27c	$-CH(CH_3)(C_2H_5)$	-H	Ile	39 ± 1.2
27d	$-CH_3$	$-CH_3$	Aib	157 ± 3.3
27e	cyclohexyl	-H	Chg	97 ± 2.7
27f	-Ph	-H	Phg	463 ± 3.8
27g	-Bz	-H	Phe	239 ± 1.9
27h	2-F Bz	-H	2-F Phe	197 ± 3.6
27i	2-F Bz	$-CH_3$	α-Me-2-F Phe	137 ± 4.9
27j	$-CH(Ph)_2$	-H	βPPA	**27 ± 1.6**
Vildagliptin	--	--	--	3.2 ± 0.5
17c	--	--	--	2.3 ± 0.9

∗DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software

∗∗ DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3)

[$] **$R_1$, R_2** together represents amino acids with absolute (S) stereo configuration

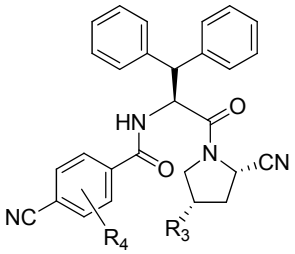
In the first series, upon linking cyanopyrrolidine (ring A) with *para*-cyanobenzoic acid (ring B), using α-substituted amino acid spacers (Val; **27b**, Ile; **27c** or cyclohexyl glycine (Chg); **27e**), compounds **27b**, **27c** and **27e** showed moderate DPP-IV inhibitory activities. When amino-isobutyric acid (Aib); **27d** or α-methyl-2-fluoro phenyl alanine (α-Me-2-F Phe); **27i**, were introduced as spacer, the resulting compounds however showed weak *in vitro* activities. The compounds **27f**, **27g** and **27h** containing phenyl glycine (Phg), phenyl alanine (Phe) and 2-fluoro phenyl alanine (2-F-Phe) respectively as spacers were also found to be the least potent. However compound **27j** with β-phenyl phenyl alanine (β-PPA) showed the highest DPP-IV inhibitory activity (IC_{50}: 27 nM) within the series.

The first series was specifically designed as analogs of **27a**, to understand the role of α-substituents on 1C amino-alkyl spacer so as to get the low nM DPP-IV inhibitory activity. The SAR study of first series reveals that the DPP-IV inhibitory activity of test compounds drastically varies with the nature of α-substituents and among various substituents screened, β-PPA was found to be favorable. It appears that the DPP-IV enzyme accepts changes in limited steric bulk at S2 binding pocket, which might be due to the stapled orientation of Glu-dyad in S2 pocket.

Compound **27j** was identified as primary hit from the first series. Further to improve DPP-IV inhibitory activity of **27j**, second series (**34a-m**) was designed, specifically by carrying out suitable changes over ring-A and -B of **27j** and in second series, five sets of compounds were prepared (**Table 11**). Substitutions were carried out in set-1 (**34a** and **34b**) on 2^{nd} position, in set-2 (**34c** and **34d**) on 3^{rd} position and in set-3 (**34e** and **34f**) on 2^{nd} and 5^{th} positions of cyano-benzamide (ring-A) , either with electron withdrawing (EW) or electron donating (ED) groups. In set-4 (**34g**-**34k**), substitutions were carried out specifically on 3^{rd} position of cyano-benzamide (ring-A). Finally, based upon the literature presidencies (favorable substitution of 4F- pyrrolidine in Denagliptin), set-5 (**34l** and **34m**) was prepared by substituting 4^{th} position of cyano-pyrrolidine (ring-B) with fluoro group, to improve the DPP-IV inhibitory activity.

All the test compounds from the second series showed significant DPP-IV inhibitory activities. Set-1 and 2 showed improved but similar DPP-IV inhibitory activities, irrespective of electron withdrawing (EW) or electron donating (ED) nature of the substituents. Compare to Set-1 and 3, Set-2 showed very good DPP-IV inhibitory activities.

Table 11: *In vitro* DPP-IV inhibitory activity of peptidomimetics **34a-m**∗



34a-m

S. No	R_3	R_4	DPP-IV inhibition**	DPP2$	DPP8$	DPP9$
34a	-H	2-CH_3	34 ± 2.9	---	---	---
34b	-H	2-F	22 ± 1.7	---	---	---
34c	-H	3-CH_3	18 ± 1.3	---	---	---
34d	-H	3-F	9.6 ± 0.6	>25,000	>15,000	>15,000
34e	-H	2,5-di-CH_3	31 ± 2.4	---	---	---
34f	-H	2,5-di-F	19 ± 0.7	---	---	---
34g	-H	3-OH	28 ± 2.7	---	---	---
34h	-H	3-OCH_3	23 ± 1.9	---	---	---
34i	-H	3-Cl	11 ± 0.8	>25,000	>15,000	>15,000
34j	-H	3-CN	17 ± 1.3	---	---	---
34k	-H	3-CF_3	14 ± 2.1	---	---	---
34l	-F	3-Cl	**4.2 ± 0.7**	>25,000	>15,000	>15,000
34m	-F	3-F	**2.7 ± 0.3**	>25,000	>15,000	>15,000
Denagliptin* **		--	19 ± 3.2	---	---	---
17c	--	--	2.3 ± 0.9	>25,000	>15,000	>15,000

∗DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.

** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3).

$ DPP2, DPP8 and DPP9 inhibitory activity represented as fold-selectivity wrt DPP-IV inhibitory activity.

*** Reported literature value for Denagliptin 22 nM (Ref: **[261]**)

Based on these results, further changes were made only at 3rd position of cyano-benzamide, as set-4 (**34g**-**34k**). In set-4, compounds **34j and 34k** with EW groups at *meta* position of cyano-benzamide showed higher DPP-IV inhibitory activities than compounds **34g and 34h**, with ED groups. Among all the compounds tested from second series, halo substituted compounds (**34d** and **34i**) showed excellent DPP-IV inhibitory activities (IC_{50}: 9.6 and 11 nM respectively). The 4-fluoropyrrolidine-carbonitrile derivatives (**34l** and **34m**, set-5) of **34d** and **34i** showed further improvement in DPP-IV inhibitory activities (IC_{50}: 4.2 and 2.7 nM respectively, similar to the best lead compound **17c** of our first designed series-1 discussed in section **3.1**), which could be due to the

favorable interactions of 4-fluoro pyrrolidine-carbonitrile with the key residues of S1 pocket.

The *in vitro* selectivity over serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for most potent compounds **34d**, **34i**, **34l** and **34m** (fold-selectivity listed in **Table 11**) **[242]**. All the test compounds showed >25000-fold selectivity over DPP-2 and >15000-fold selectivity over DPP-8 and DPP-9, which was found to be comparable with reference standard compound **17c**. Among all the compounds tested, **34l** and **34m** were found to be most potent and selective.

3.2.3. *In vitro* CYP inhibition study of selected peptidomimetics.

To assess the CYP liabilities of these peptidomimetics, **34l** and **34m** were subjected for CYP3A4 and CYP2D6 inhibition studies (details experimental protocol is given in **experimental Section 5.2.2.**). Both the test compounds were found to be devoid of CYP3A4 and CYP2D6 inhibition up to 100 mM concentrations **[262]**.

3.2.4. Molecular docking study of lead peptidomimetic 34m

The molecular docking analysis of **27a**, **34m** and **Denagliptin** was carried out using extra precision (XP) Glide docking method (**Figure 30**), to understand their critical interactions with all the three binding sites (S1, S2 and S3) of DPP-IV enzyme (**Figure 31**; binding poses overlay of **27a** (Turquoise), **34m** (Brown) and **Denagliptin** (Rose)). The crystal structure of the DPP-IV enzyme (PDB ID: 2I03) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger (details experimental protocol is given in **experimental section 5.4**) **[245-246]**.

The results of docking studies illustrate that all the three compounds interact closely with the key residues of S1 pocket (as per literature presidencies, cyanopyrrolidine-CN may form covalent bond with OH-group of side-chain of Ser_{630}). In S_2 pocket, benzamide-NH of **34m** and α-amino group of Denagliptin forms H-bonding with C=O groups of side-chains of Glu205 and Glu206 dyad, while benzamide-NH of **27a** flip away from the Glu dyad. Compound **34m** interact closely in S3 pocket (aromatic–CN forms H-bonding with the NH of guanidine side-chain of Arg_{358}), while **27a** interact

weakly with the key residues of S2 and S3 pockets, which may justify its weak *in vitro* DPP-IV inhibitory activity.

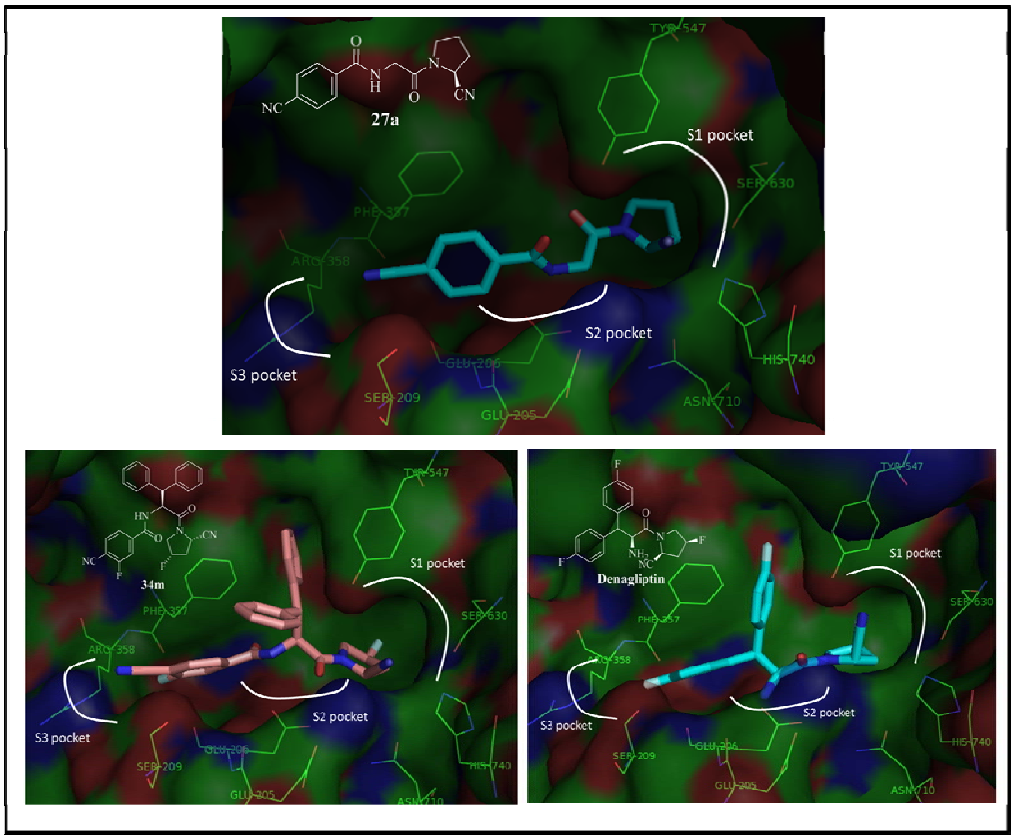
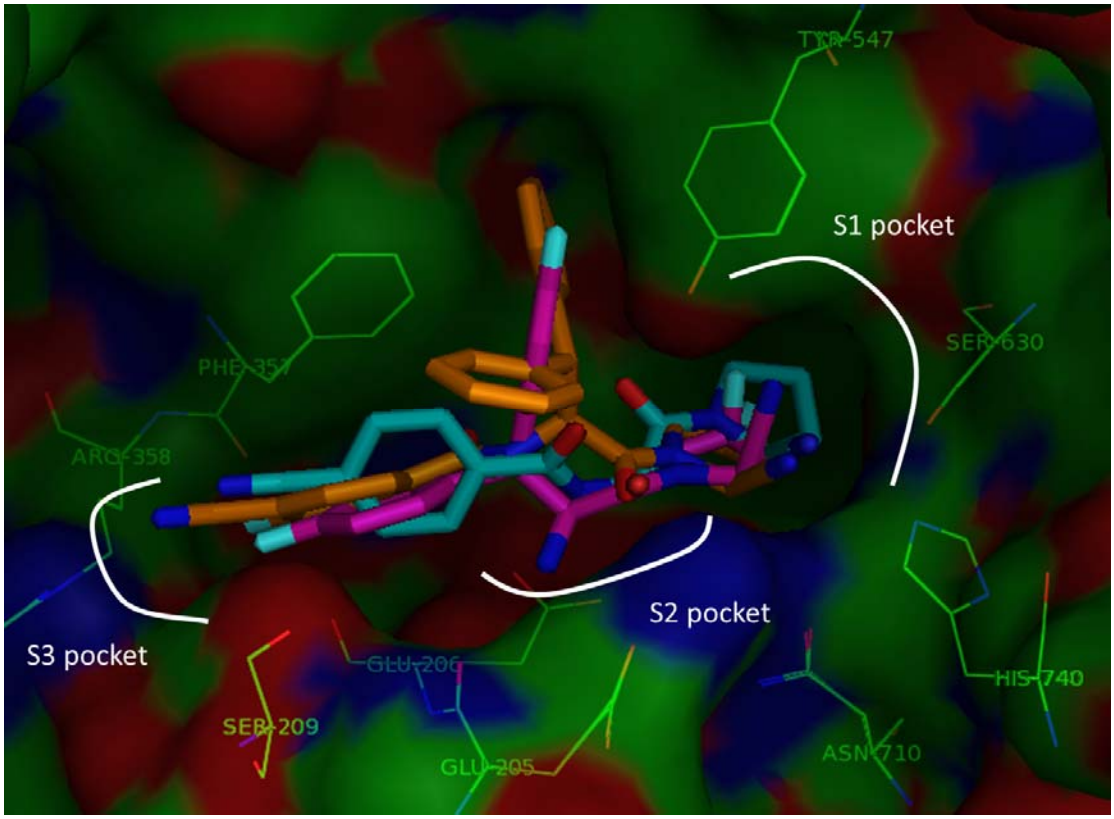


Figure 30: Key interactions of compounds **27a**, **34m** and **Denagliptin** with active sites of DPP-IV enzyme

Incorporation of β-PPA linkage (spacer) in **34m** allows it to adopt new confirmation, which may favors covalent interaction of cyanopyrrolidine ring with Ser630 (S1 pocket, covalent interaction of cyanopyrrolidine ring, as reported for cyanopyrrolidine derivatives), strong H-bonding of back-bone benzamide-NH with Glu dyad (S2 pocket) and *para*-nitrile benzamide with Arg358, including aromatic π- π stacking of benzamide with Phe357 in S3 pocket. As observed with Denagliptin, **34m** docks very well into all the three sites (S1, S2 and S3) of DPP-IV crystal structure and these favorable interactions of **34m** across all the three sites of DPP-IV enzyme support its potent *in vitro* DPP-IV inhibitory activity and excellent selectivity over other protease.



Binding pose of compound **27a** (Turquoise), **34m** (Brown) and **Denagliptin** (Rose) in the DPP-IV active site is indicated (Surface view: Green),
wherein compounds **34m** and **Denagliptin** interacts closely with key residues of site S1, S2 and S3.

Figure 31: Overlay binding pose of compounds **27a**, **34m** and **Denagliptin** with active sites of DPP-IV enzyme

3.2.5. Conclusion

In summary, we have reported discovery of peptidomimetic based cyanopyrrolidines derivatives as potent and selective inhibitors of DPP-IV and devoid of CYP liabilities. Novel peptidomimetics **34l** and **34m** showed excellent *in vitro* potency and selectivity over other serine proteases, due to their favorable orientations across all the three binding sites. Thus we successfully overcome the CYP inhibition problem arise with the lead compound **17c** of the first series by modifying it to novel peptidomimetic **34m** with no CYP inhibition up to 100 mM concentrations.

3.3. Aminomethylpiperidone based DPP-IV inhibitors (Third series)

3.3.1. Chemistry

As discussed in designing section **2.1.3** this series was specifically designed to develop potent and selective DPP-IV inhibitors with improved pharmacokinetic profile. wherein we intended to synthesize the compounds represented by general structures **68a-v**, **69a-e** and **70a-e** (**Figure 32**). Synthetic methodology was designed based on the retrosynthetic analysis and the schemes are described below. Synthetic method reported in literature were adapted for the synthesis of title compounds **68a-v**, **69a-e** and **70a-e**. All compounds were synthesized following the procedure reported earlier in literature by choosing the appropriate starting materials and optimizing reaction conditions.

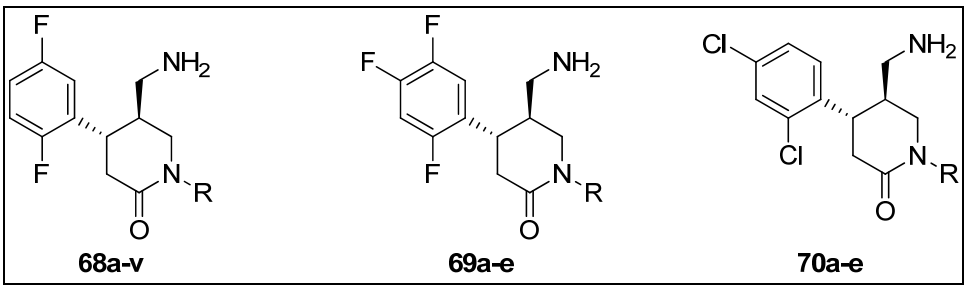
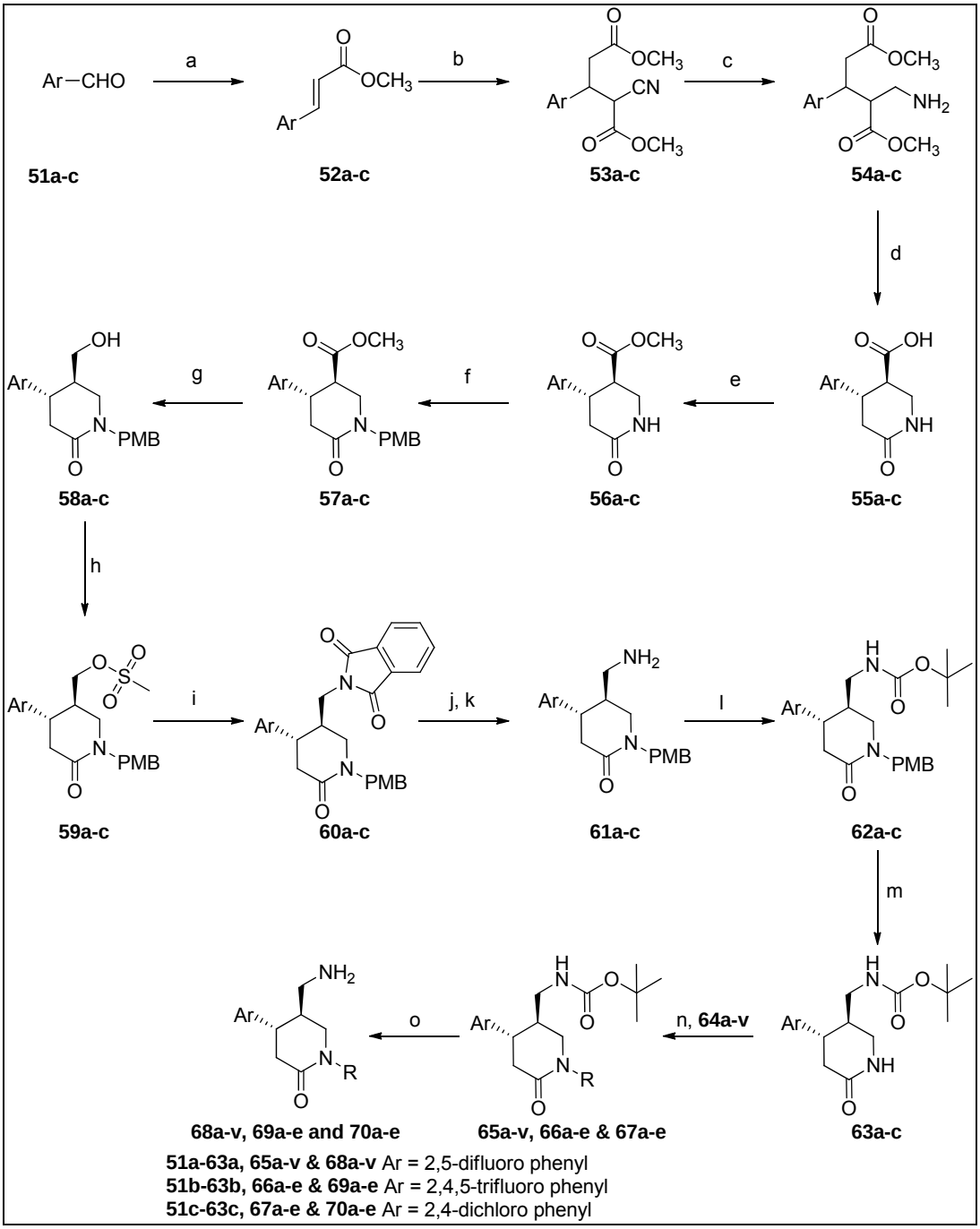


Figure 32. Aminomethyl-piperidone based DPP-IV inhibitors

As depicted in **Scheme-10**, synthesis of the aminomethyl-piperidones based DPP-IV inhibitors (**68a-v**, **69a-e** and **70a-e**) commenced with a Horner-Wadsworth-Emmons reaction of aldehydes **51a-c**, followed by Michael addition, to get diester (**53a-c**). Reduction of nitrile group of **53a-c** by hydrogenation, using Adam's catalyst, followed by cyclization and ester regeneration by trimethylsilyldiazomethane yielded piperidone-carboxylate (**56a-c**), with >85% *trans* selectivity **[263]**. *Trans* racemic mixture [(3R, 4S) and (3S, 4R)] of (**56a-c**) were isolated in pure form by removing corresponding *cis* racemic mixture [(3R, 4R) and (3S, 4S)], by column chromatography (mobile phase: 0-3% methanol in DCM, using 100-200 mesh silica gel). Amide *–NH* protection of *trans* racemic **56a-c** with *para*-methoxy benzyl (PMB) group and reduction of ester with lithium aluminium hydride ($LiAlH_4$) provided *trans* racemic alcohol (**58a-c**). Subsequently, **58a-c** were converted to a good leaving group (methanesulfonate derivatives **59a-c**), which upon treatment with potassium phthalimide via Gabriel synthesis type reaction lead to the formation of *trans* racemic phthalimido-piperidones (**60a-c**).



Reagents and conditions: (a) $(Et_2O)_2POCH_2COOMe$, Na_2CO_3, EtOH (b) $NCCH_2COOMe$, NaOMe, MeOH (c) H_2, PtO_2, HCl, MeOH (d) K_2CO_3, Toluene/MeOH (e) Me_3SiCHN_2, Et_2O/MeOH (f) PMB-Br, NaHMDS, THF/DMF(4:1), -78°C (g) $LiAlH_4$, THF, 0°C (h) CH_3SO_2Cl, NEt_3, DCM, 0°C (i) Pottassium phthalimide, DMF, 90°C (j) NH_2-NH_2, EtOH, 25°C (k) Chiral resolution: D-tartaric acid, MeOH. (l) Boc_2O, NEt_3, THF/H_2O(3:2), 25°C (m) CAN, CH_3CN/H_2O(3:1), 25°C (n) R-X (**64a-v**), CuI, K_2CO_3/K_3PO_4, N,N'-dimethylethylenediamine, Toluene, Reflux *or* R-X (**64a-v**), NaH, DMF, 0°C-25°C (o) Conc. HCl/EtOAc(1:3), -50°C, 2h, 0°C, 1h.

Scheme 10. Synthetic methods for the preparation of aminomethylpiperidones **68a-v**, **69a-e** and **70a-e**

Hydrazinolysis of phthalimido group of **60a-c** provided *trans* racemic aminopiperidones (**61a-c**). *Trans* racemic **61a-c** was subjected for chiral resolution using D-tartaric acid, to get enantiomerically pure (4S, 5S) desired piperidones (**61a-c)** as a tartrate salt with >97% ee. Protection of primary amine with Boc-group and subsequent oxidative removal of PMB group gave Boc-aminopiperidones (**63a-c**). Various haloheterocycles/ halo-aromatics of the interest **64a-v** (**Figure 33**) were coupled with Boc-aminopiperidones (**63a-c**) by Goldberg reaction **[264]** or by nucleophilic substitution, followed by Boc-deprotection lead to the formation of chiral pure (4S, 5S) aminomethyl-piperidones (**68a-v**, **69a-e** and **70a-e**) **[265-266]**. All the test compounds obtained were purified by preparative HPLC (yield 70–85%; HPLC purity >97% and chiral purity >97%ee) and characterized by various spectroscopic techniques (^{13}C NMR, ^{1}H NMR and ESI MS).

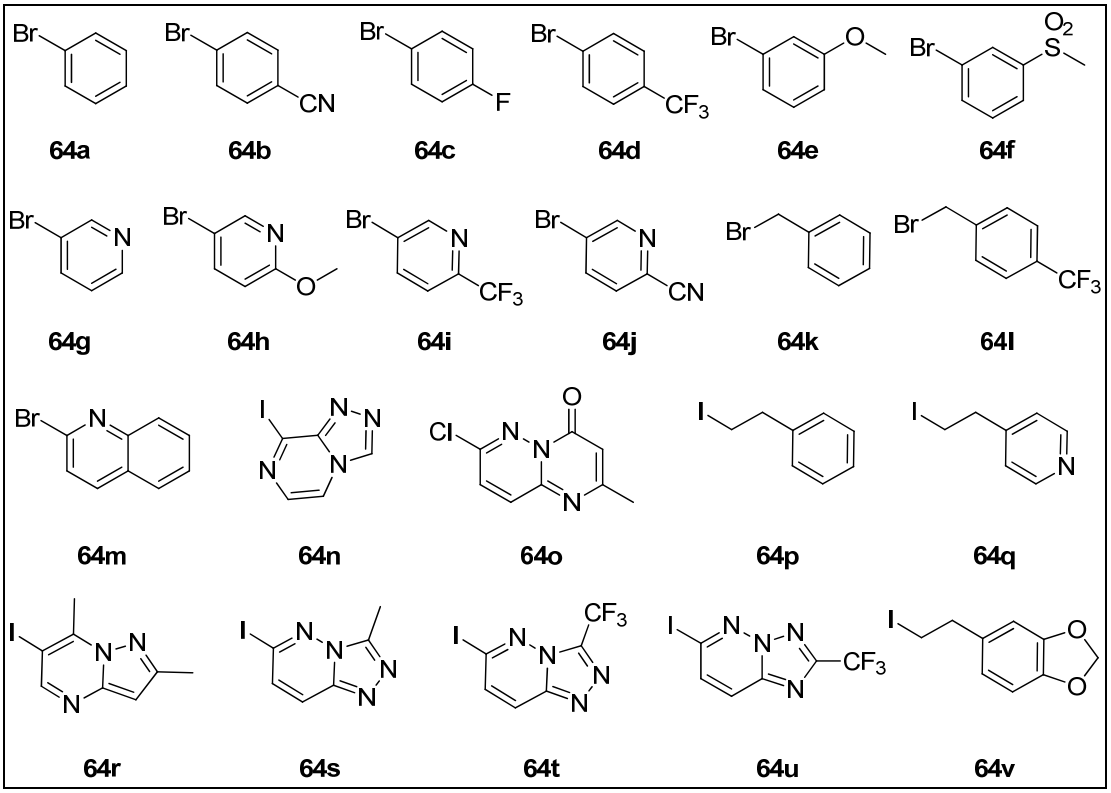
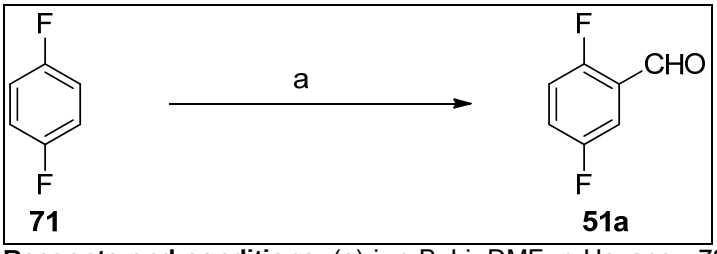


Figure 33. Structures of halo-heterocycles and halo-aromatics **64a-v**

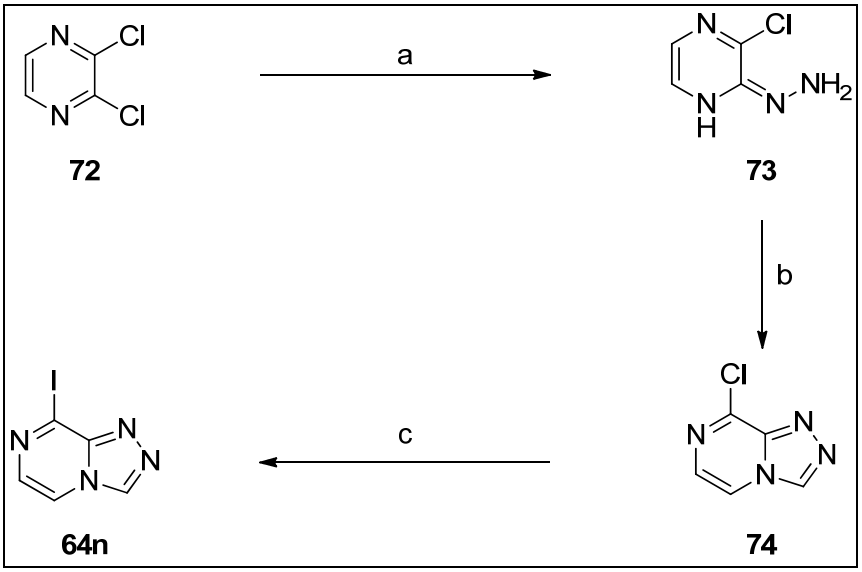
As shown in **shceme-11** substituted bezaldehyde **51a** was synthesized from its difluoro benzene precursor **71** by formylation via Vilsmeier-Haack type reaction, using the method reported by Anthony David et al in good yield **[267]**. Whereas substituted

bezaldehydes **51b-c** used as starting material for the synthesis of aminomethyl-piperidones **69a-e** and **70a-e** were procured from the commercial bulk suppliers. Various halo-heterocycles or halo-aromatics **64a-v** required for the synthesis of aminomethyl-piperidones **68a-v**, **69a-e** and **70a-e** have been either synthesized by reported literature procedures by choosing the appropriate starting materials and optimizing reaction conditions or procured from the commercial suppliers **[268]**. However halo-aromatics and halo-heterocycle **64a-m** and **64p-q** were procured from the commercial bulk suppliers. Whereas halo-heterocycles **64n-o** and **64r-v** were synthesized by different synthetic methodologies reported in the literature (**Schemes 12-17**).



Reagents and conditions: (a) i. n-BuLi, DMF, n-Hexane, -78°C, 1h, < -65°C 1h. ii. CH_3COOH, Water, -65°C to 5°C .

Scheme 11. Synthetic method for the preparation of benzaldehyde **51a**

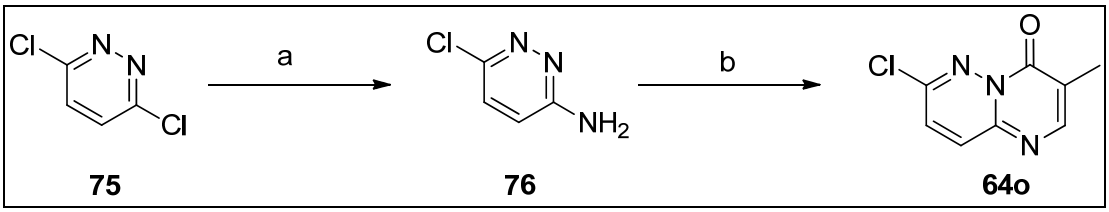


Reagents and conditions: (a) Hydrazin-hydrate, EtOH, Reflux, 2h (b) $CH(OEt)_3$, Xylene, Reflux, 4h. (c) HI, Reflux, 3days.

Scheme 12. Synthetic method for the preparation of halo-heterocycle **64n**

Heterocycle **64n** has been synthesized by various methods in the literature, here we adopted the best optimized reaction condition of each step reported in the literature for the sythesis of **64n** as outlined in **scheme 12 [31,269-271]**.

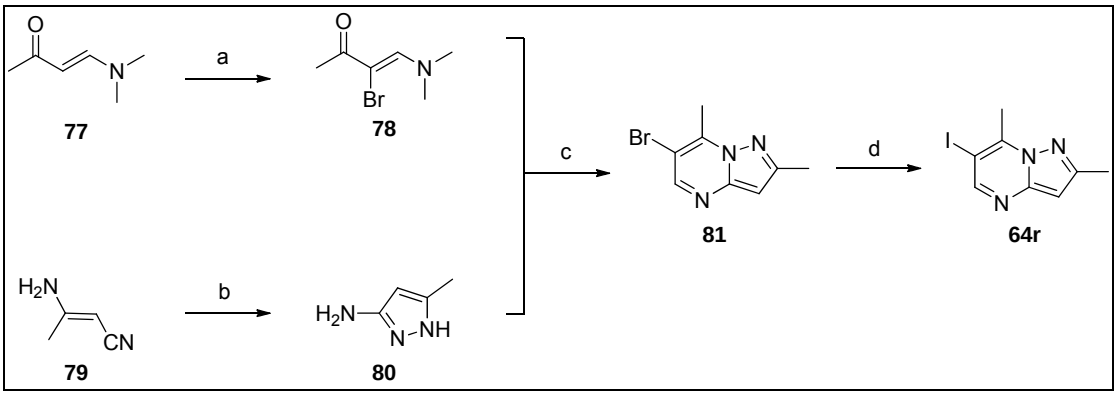
Synthesis of **64o** was carried out by making variation of a rout described by Avellana et al as illustrated in **scheme 13 [272]**.



Reagents and conditions: (a) NH_4OH, 130°C, 10bar pressure, 48h (b) $CH_3COCH_2COOC_2H_5$, benzyl alcohol, Reflux, 24h.

Scheme 13. Synthetic method for the preparation of halo-heterocycle **64o**

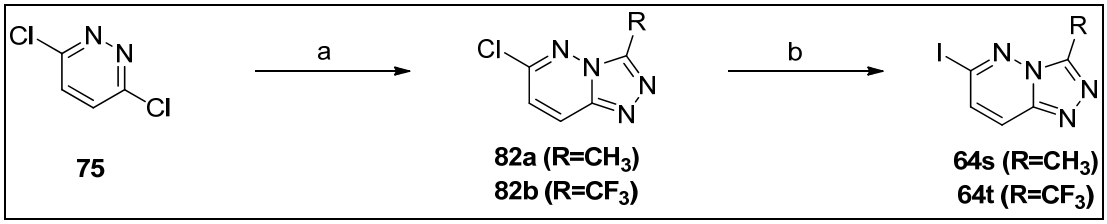
Synthesis of **64r** (**scheme 14**) was accomplised with commercially available starting materials **77** (Which can also be prepared by method of Keisuke Suzuki et al **[273]**) and 3-Aminocrotononitrile **79** by modifying the literature procedure reported by Gerald Shipps et al and Nam et al **[274-275]**.



Reagents and conditions: (a) i. Br_2, DCM, 0°C, 1h ii. NEt_3, Et_2O, 0°C, 1h (b) Hydrazine hydrate, Water, Reflux, 8h (c) 33% HBr in CH_3COOH, EtOH, CH_3COOH, Reflux, 3h. (d) HI, Reflux, 3days.

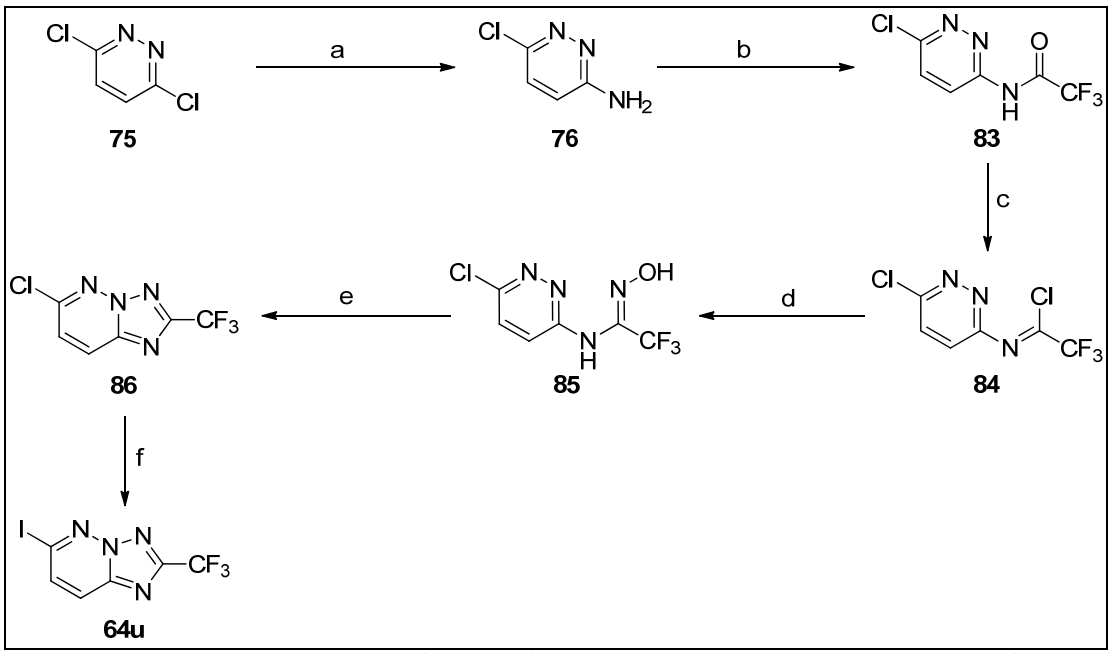
Scheme 14. Synthetic method for the preparation of halo-heterocycle **64r**

Synthesis of **64s-t** was accomplished with 3,6-Dichloropyridazine **75** according to the method reported by Jason Cox et al without making any modification of reaction procedure (**scheme 15**)**[265, 271]**.



Reagents and conditions: (a) $CH_3CONHNH_2/CF_3CONHNH_2$, Butanol, Reflux, 24h. (b) HI, Reflux, 3days.

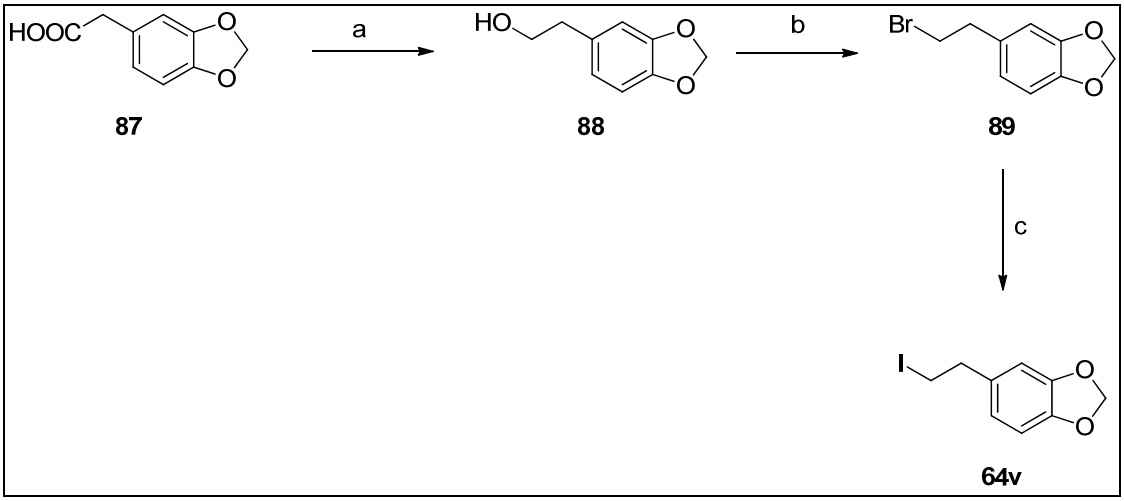
Scheme 15. Synthetic method for the preparation of halo-heterocycle **64s-t**



Reagents and conditions: (a) NH_4OH, 130°C, 10bar pressure, 48h (b) TFAA, NEt_3, DCM, 25°C, 24h (c) PCl_5, EDC, Reflux, 6h (d) $NH_2OH{\cdot}HCl$, 25°C, 3h (e) H_3PO_4, 150°C, 4h. (f) HI, Reflux, 3days.

Scheme 16. Synthetic method for the preparation of halo-heterocycle **64u**

For the synthesis of halo-heterocycle **64u** (**Scheme 16**), commercially available 3,6-Dichloropyridazine **75** was converted to 3-Amino-6-chloropyridazine **76** by heating with liq. ammonia under high pressure, which upon treatment with trifluoroacetic anhydride provided acyl derivative **83**. Compound **83** upon treatment with PCl_5 gave imidoylchloride **84**, which was converted to hydroxyme **85** and subsequent treatment with polyphosphoric acid provided halo-heterocycle **86**, Heterocycle **86** upon treatment with hydro iodic acid and basic workup gave desired compound **64u [265, 271]**.



Reagents and conditions: (a) $LiAlH_4$, THF, 0°C, 1h, 25°C, 5h (b) CBr_4, PPh_3, ACN, 25°C , 15h (c) NaI, Acetone, Reflux, 24h.

Scheme 17. Synthetic method for the preparation of halo-heterocycle **64v**

Various routs are reported in the literature for the synthesis of benzo-dioxole derivative **64v [276-278]**. Here we synthesized **64v** by method as illustrated in **scheme-17** starting from commercially available benzo-dioxole acetic acid **87** (procured from Taizhou Bolon Pharmachem CO. LTD. Chaina.). Compound **87** was reduced to alcohol derivative **88** by using the method reported by Patrick Bailey et al by optimizing the reaction condition **[277]**. Further compound **88** was converted to benzo-dioxole derivative **64v** through its corresponding halo derivative **89** using the method reported by Saurabh Shahane et al by modifying the reaction conditions **[278]**.

3.3.2. *In vitro* DPP-IV inhibitory activity, selectivity and structure activity relationship (SAR)

The *in vitro* DPP-IV inhibitory activity was determined in order to establish the structure-activity relationship (SAR) using fluorescence-based assay (details experimental protocol is given in **experimental Section 5.2.1.**) **[242]**. Three sets of the aminomethyl-piperidones (**68a-v**, **69a-e** and **70a-e**) were prepared (**Table 12**). In the first set (Ar = 2,5-difluoro phenyl), 22 compounds (**68a-v**) were prepared by coupling 2,5-difluoro phenyl-aminopiperidone (**63a**) with various halo-heterocycles/halo-aromatics. In the second set (Ar = 2,4,5-trifluoro phenyl), 5 compounds (**69a-e**) were prepared by replacing 2,5-difluoro phenyl with 2,4,5-trifluoro phenyl, while in third set (Ar = 2,4-

dichloro phenyl), 5 compounds (**70a-e**) were prepared by replacing 2,5-difluoro phenyl with 2,4-dichloro phenyl. All the test compounds showed varying degrees of DPP-IV inhibitory activity (IC_{50}), depending on the nature of the substituents.

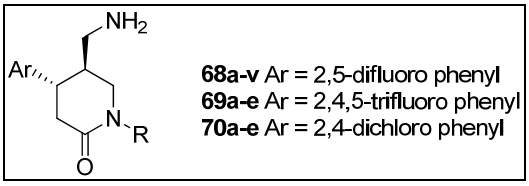
Within the first set (**68a-v**), test compounds showed diverse DPP-IV inhibitory activity depending on the nature of substituents on piperidone ring system. Compounds with electron withdrawing groups (**68b**: -CN, **68c**: -F and **68d**: -CF_3) at *para*-position of phenyl ring system showed improved DPP-IV inhibitory activity, compared to unsubstituted derivative (R=-Ph; **68a**). Compounds with electron donating groups (**68e**: -OMe and **68f**: -SO_2-Me) at *para*-position of phenyl ring showed further improvement in *in vitro* DPP-IV inhibitory activity.

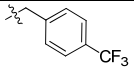
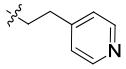
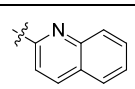
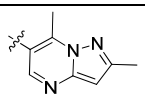
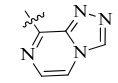
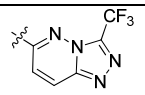
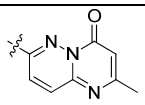
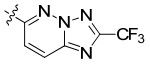
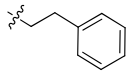
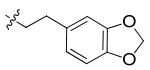
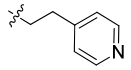
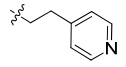
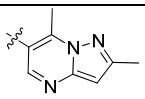
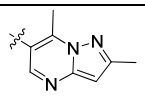
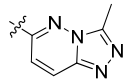
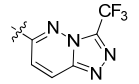
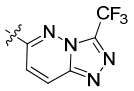
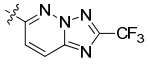
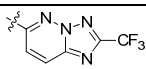
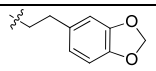
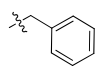
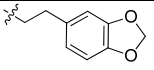
Replacement of phenyl ring system with 3-pyridyl (**68g**) and further substitutions with electron donating (**68h**) and withdrawing (**68i** and **68j**) groups at para-position showed moderate DPP-IV inhibitory activity. Replacement of phenyl ring system with quinoline (**68m**), triazolo[4,3-a]pyrazine (**68n**), 2-methyl-pyrimido[1,2-b]pyridazinone (**68o**), benzyl (**68k**) and further substitutions with electron withdrawing (**68l**) groups at *para*-position showed moderate DPP-IV inhibitory activity. Substitutions with ethylbenzene (**68p**), ethylpyridine (**68q**), dimethylpyrazolo[1,5-a]-pyrimidine (**68r**), 3-methyl-triazolo[4,3-b]pyridazine (**68s**), 3-trifluoromethyl-triazolo[4,3-b]pyridazine (**68t**) and 2-trifluoromethyl-triazolo[1,5-b]pyridazine (**66u**) showed good DPP-IV inhibitory activity, while **68v** (methylenedioxy phenethyl) showed superior DPP-IV inhibitory activity (IC_{50}: 8.5±0.4 nM), compared to Sitagliptin (IC_{50}: 18±2.4 nM).

In the second set (**69a-e**, Ar = 2,4,5-trifluoro phenyl), all the five compounds showed good activity, but compared to 2,5-difluoro phenyl series (Set-1 analogs, **68q**, **68r**, **68t**, **68u** and **68v**), *in vitro* DPP-IV inhibition were found to be bit weaker, while in set three (**70a**-**e**, Ar = 2,4-dichloro phenyl), *in vitro* DPP-IV inhibition were found to be slight weaker than set-1 and set-2 corresponding analogs. Thus the nature and position of halogen atom on aromatic ring system contributed significantly towards *in vitro* DPP-IV inhibition.

Further the activity difference of the corresponding analogues in all three series compounds could be due to difference in the binding inter action of the aromatic rings in S1 pocket of the DPP-IV enzyme. 2,5-difluoro phenyl might be best fitting in the S1 pocket compared to the 2,4,5-trifluoro phenyl , which could be further binding batter than 2,4-dichloro phenyl ring.

Table-12: *In-vitro* DPP-IV inhibitory activity of aminomethyl-piperidones (**68a-v**, **69a-e** & **70a-e**).



Comp.	R	IC_{50} (nM)*	Comp.	R	IC_{50} (nM)*	Comp.	R	IC_{50} (nM)*
68a		1436±12	**68l**		910±3.1	**69a**		157±4.1
68b		378±1.4	**68m**		1034±21	**69b**		119±1.0
68c		382±4.5	**68n**		1023±3.1	**69c**		125±2.7
68d		342±3.3	**68o**		997±13.5	**69d**		111±2.1
68e		217±8.6	**68p**		119±4.2	**69e**		19±5.1
68f		193±8.4	**68q**		**84±2.6**	**70a**		197±4.2
68g		1388±5.9	**68r**		**77.6±1.2**	**70b**		148±3.7
68h		452±3.7	**68s**		122±3.2	**70c**		134±7.3
68i		443±5.3	**68t**		**79±0.2**	**70d**		137±9.6
68j		404±7.7	**68u**		**74±0.9**	**70e**		43±3.2
68k		885±11.2	**68v**		**8.5±0.4**	**Sitagliptin**	--	18±2.4

∗DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software

∗∗ DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3)

Overall 32 compounds screened for *in vitro* DPP-IV inhibition and here we identified compound **68v** as the most potent molecule among all the three series. Further, *in vitro* selectivity over other related serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for **68v** and it showed >5000-fold selectivity over DPP-2 and >10,000-fold selectivity over DPP-8 and DPP-9 **[242]**.

3.3.3. *In vitro* CYP inhibition study of aminomethyl-pipyridone 68v.

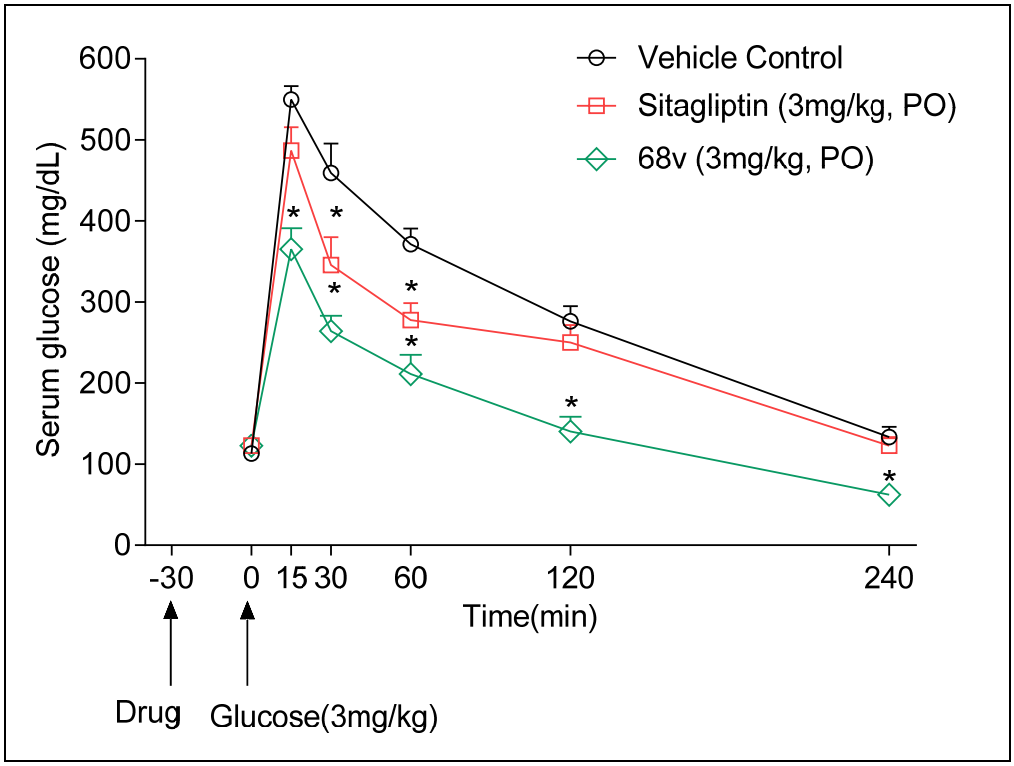
To assess the CYP liabilities, **68v** was subjected for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition studies (@1, 10 and 100 μM concentrations) and the test compound **68v** was found to be devoid of CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition up to 100 μM concentrations (details experimental protocol is given in **experimental Section 5.2.2.**).

3.3.4. *In vivo* antidiabetic activity of aminomethyl-pipyridone 68v.

Detailed pharmacodynamic (PD) profiling of **68v** was carried out. The *in vivo* antidiabetic activity of **68v** and Sitagliptin (@ 3 mg/kg, po) was evaluated in male C57BL/6J mice, using OGTT (oral glucose tolerance test) protocol (details experimental protocol is given in **experimental Section 5.2.3.**) and changes in serum glucose levels (AUC glucose up to 240 min; mg/dL) was estimated (**Figure 34**) **[243-244]**.

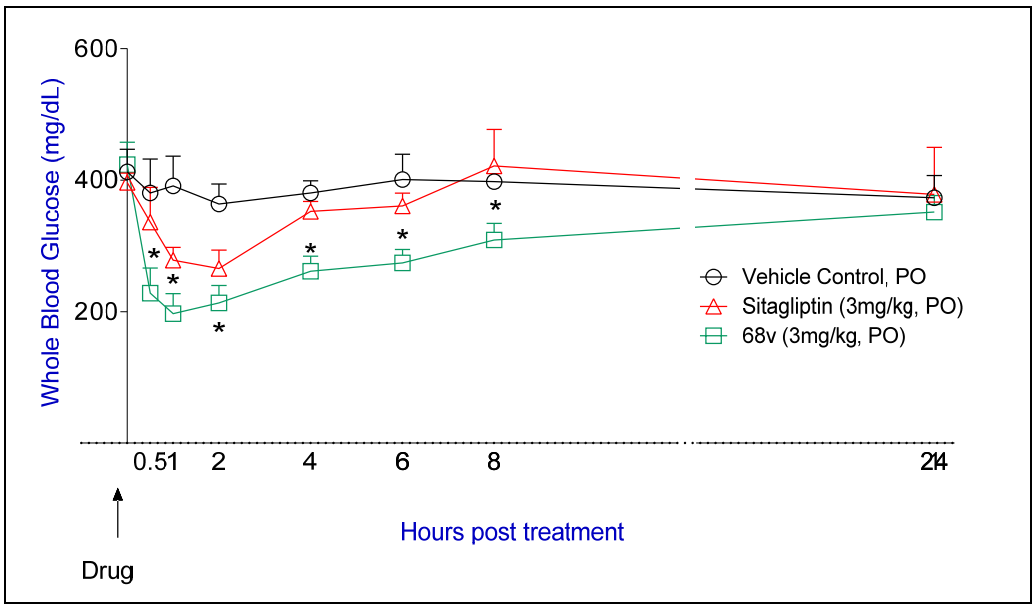
Compound **68v** showed good oral antidiabetic activity (% decrease in AUC glucose 38.9 ± 5.20), which was found to be better than Sitagliptin (% decrease in AUC glucose 17.9 ± 4.58). In C57 mice, it was interesting to observe that **68v** showed suppression in the blood glucose at all the time points (15, 30, 60, 120 and 240 min) compared to vehicle control, while sitagliptin showed blood glucose reduction only at 30 and 60 min.

Here this study reviles that compound **68v** might have a tendency of sustained release. Hence to understand its duration of action a separate study has been conducted in fed db/db mice for antidiabetic activity followed by pharmacokinetic (PK) study.



*P<0.05, Two-Way ANOVA followed by Bonferroni posttest, Mean ± SEM

Figure 34: *In vivo* antidiabetic activity of **68v** and Sitagliptin in C57 mice (OGTT)



*P<0.05, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 35: In vivo antidiabetic activity of **68v** and Sitagliptin in db/db mice

Further to understand the duration of action and effect of **68v** on post-prandial glucose excursion, single dose (@ 3 mg/kg, po) antidiabetic activity of **68v** and sitagliptin was evaluated in fed-db/db mice (hyperglycemic animals) for 24 h (**Figure 35**). Under fed condition, compared to vehicle control, **68v** and sitagliptin showed good antidiabetic activity (% decrease in AUC glucose 38.29 ± 12.13 and 20.80 ± 11.06, respectively) up to 2 h. However, **68v** showed prolonged suppression of serum glucose levels (% decrease in AUC glucose 20.62 ± 7.05 for **68v** and 1.48 ± 11.84 for sitagliptin, up to 24 h).

3.3.5. Pharmacokinetic (PK) studies of aminomethyl-pipyridone 68v.

A comparative single dose (3 mg/kg iv or po) PK profile of **68v** and Sitagliptin was evaluated in male C57BL/6J mice (n = 6) and the various PK parameters (T_{max}, $T_{1/2}$, C_{max}, AUC and %F) were recorded (**Table 13**) (details experimental protocol is given in **experimental Section 5.3**).

In PK study, **68v** showed rapid Tmax, higher AUC (~twofold compared to sitagliptin), extended half-life ($T_{1/2}$: >8 h) compared to sitagliptin and good oral bioavailability (%F:79.5%). Compound **68v** showed extended half-life and higher AUC, which could be due to its low clearance compared to sitagliptin (elimination rate constant (kel; h^{-1}), 0.12 ± 0.02 for **68v** and 0.84 ± 0.14 for sitagliptin). Thus improved pharmacokinetic profile of compound **68v** justifies its potent and prolonged antidiabetic activity in C57 and db/db mice.

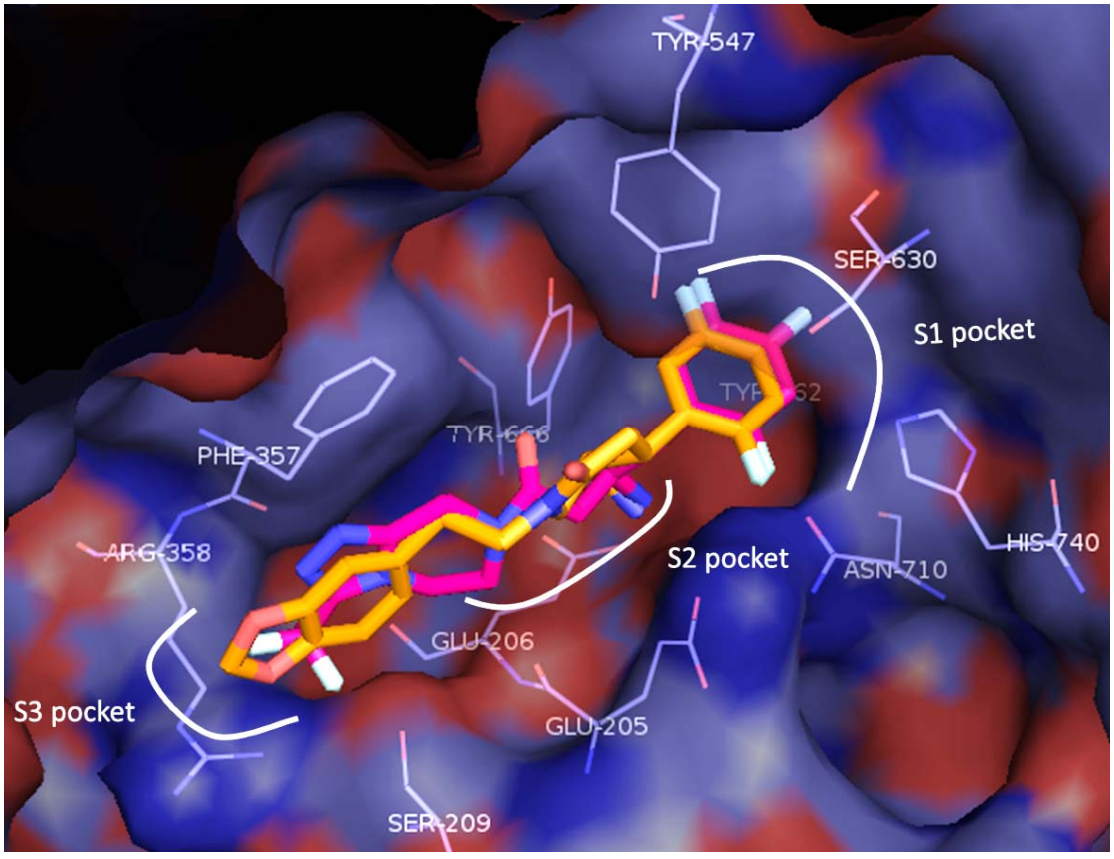
Table 13: Pharmacokinetic study parameters[a] of **68v** and **Sitagliptin**

Compd	Tmax (h)	Cmax (mg/ml)	$T_{1/2}$ (h)	AUC (0-α) h mg/ml	%F*
68v	0.28 ± 0.12	0.42 ± 0.03	8.99 ± 0.31	1.07 ± 0.09	79.5%
Sitagliptin	0.31 ± 0.10	0.31 ± 0.01	1.56 ± 0.11	0.56 ± 0.02	75.9%

[a]In male C57BL/6J mice (n=6), compounds were administered orally (p.o) at 2 mg/kg dose and plasma concentration was analyzed by LC-MS, values indicate Mean ± SD.
* Oral bioavailability (%F) was calculated wrt to iv AUC (**68v**: 1.19 ± 0.08 & sitagliptin: 0.66 ± 0.09 h µg/ml) administered at 2 mg/kg dose, iv.

3.3.6. Molecular docking study of aminomethyl-pipyridone 68v

The molecular docking analysis of **68v** and sitagliptin, in the binding pocket of DPP-IV was carried out using extra precision (XP) Glide docking method (**Figure 36**) (details experimental protocol is given in **experimental section 5.4.**) **[245-246]**. The X-ray structure of the DPP-IV enzyme (PDB ID: 2OQI) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. For docking study, the ligands were geometrically optimized and prepared by using ligprep module of Schrödinger.



Binding pose of compound **68v** (Orange) and **Sitagliptin** (Maroon) in the DPP-IV active site is indicated (Surface view: Blue), wherein both compounds interact closely with key residues of site S_1, S_2 and S_3.

Figure 36: Key interactions of compound **68v** and Sitagliptin with active sites of DPP-IV enzyme

The overlay of binding poses of **68v** (Orange) and Sitagliptin (Maroon) in the DPP-IV active site is shown in **Figure 36**. As observed with sitagliptin, **68v** docks very well into all the three sites (G-scores -11.81 (9/9) and -10.99 (9/9) for **68v** and sitagliptin respectively). Although, G-score of **68v** and sitagliptin are comparable, however, *in vitro*, DPP-IV IC_{50} of **68v** is half of that of sitagliptin, which could be due to favorable interactions of **68v**, in all the three binding pockets. Di-fluoro-phenyl ring of **68v** occupies S1 pocket. In S2 pocket, aminomethyl groups of piperidone ring forms H-bonding with the side-chains of Glu205 and Glu206 dyad, while methylenedioxy phenyl ring of **68v** accommodates very well in S3 pocket, which together supports excellent *in vitro* DPP-IV activity and selectivity of **68v** over other protease.

3.3.7. Conclusion

Various gliptins, currently used in the clinic (Sitagliptin, Vildagliptin, Saxagliptin, Alogliptin and Linagliptin), exhibit short half-life thereby requires once or twice daily drug administration **[279-280]**. Further to regulate the pre- and post-prandial blood glucose and thereby to control HbA1c, several long-acting DPP-IV inhibitors (Omarigliptin and Trelagliptin) are under developments, as once-weekly drugs **[205]**. Their clinical efficacy and side effects profile appear to be comparable with other gliptins in the class, however, their infrequent dosing creates a niche and promotes patients compliance. In this context, overall pre-clinical profile of **68v** demonstrated added advantages over currently practiced gliptins and appears to serve as long-acting DPP-IV inhibitors.

Here we report discovery of novel aminomethyl-piperidone derivatives as potent, selective and long acting DPP-IV inhibitors for the treatment of T2DM. The lead compound **68v** ((4S,5S)-5-(aminomethyl)-1-(2-(benzo[d] [1,3]dioxol-5-yl)ethyl)-4-(2,5-difluorophenyl)-piperidin-2-one) showed prolonged suppression of pre-and post-prandial blood glucose levels (*in vivo*), which correlates with its extended PK profile.

Chapter IV:

Overall summary

and

Future prospects

"The scientist is not a person who gives the right answers, he's one who asks the right questions." — Claude Lévi-Strauss

4. Overall Summary and Future Plan

4.1 Overall summary of current investigation

T2DM is a complex multifactorial disease affecting the length and quality of life of diabetic patients. T2DM is characterized by hyperglycemia, insulin resistance and/or impaired insulin secretion. T2DM remains a leading cause of cardiovascular disorders, blindness, end-stage renal failure, amputations and hospitalizations. The economic burden for healthcare systems is rising sharply, owing to the costs associated with the treatment of diabetes and its complications. Treatment of patients with T2DM strives for normalization of hyperglycemia by a combination of medical nutrition therapy, physical activity and pharmacological agents. Patient education and empowerment are essential components of therapy **[281]**. Most patients require continuous treatment in order to maintain normal or near-normal glycemia. T2DM is a progressive disorder accompanied by deterioration in β cell function and insulin resistance. Despite this fact, there is now clear evidence that tight control of blood glucose significantly reduces the risk of complications of diabetes.

The DPP-IV inhibitors are well known oral hypoglycemic drugs which have been in clinical use for the past 8 years. The DPP-IV inhibitors are safe, weight neutral and widely prescribed. There are currently eight gliptins registered worldwide with several more in advanced stages of development. However no gliptin is there in market for long term treatment of T2DM. Further, In vivo evidence in experimental animal models and in initial clinical trials has demonstrated that DPP IV inhibitors have therapeutic potential in the long term chronic treatment of T2DM, delaying disease progression and decreasing the circulating levels of glycated hemoglobin (HbA1c), There are two gliptins in advanced stages of clinical development which can be registered for once-weekly dosing regimen.

In the present investigation altogether three series of DPP-IV inhibitors were designed. In the first series, cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors, total thirty compounds were prepared. In the second series, peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities, total twenty three compounds were prepared. In the third series, aminomethylpiperidone based DPP-IV inhibitors, total thirty two compounds were prepared. Altogether eighty five compounds were synthesized, purified, characterized and subjected for *in vitro* DPP-IV inhibitory activity. The most potent selected DPP-IV inhibitors from each series were further

subjected for the *in vitro* selectivity over other serine proteases (especially over DPP-2, DPP-8 and DPP-9). From each series, the most potent and selective compounds were subjected for the *in vivo* antidiabetic activity followed by PK studies. Compounds of all the three series were found to be potent and selective DPP-IV inhibitors.

In the first series, pyrrolidine carboxamide compounds **11e**, **11f**, **12e**, **12f**, **16e** and **16f** (*para*-nitrile/ trifluoromethyl benzamide) were identified as primary lead compounds. These lead compounds were transformed to their respective nitrile derivatives to give potent DPP-IV inhibitors **17a**-**d** and **18a**-**b**. Among these cyanopyrrolidene based peptidomimetics, compounds **17c** and **17d** showed excellent DPP-IV inhibition (*in vitro*) along with selectivity over other related serine proteases. therefore **17c** and **17d** was considered as optimize lead in this series.

Results of *in vitro* DPP-IV inhibitory activity, *in vivo* pharmacodynamic study and molecular docking studies of **17c** and **17d** clearly demonstrated that the potency of cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors can be modulated by introducing nitrile group (-CN) at the C2 carbon of the pyrrolidine ring, which binds to the S1 pocket of the DPP-IV enzyme. Further selectivity can be modulated by introducing -CN or $-CF_3$ group at para position of the benzamide ring, which binds to S3 site of the DPP-IV enzyme. It was observed that introduction of suitable spacer (i.e. GABA: γ-amino butyric acid), which links the S1 and S3 pocket binding component of the ligand, contributed significantly towards improvement in the *in vivo* DPP-IV inhibitory activity, which could be correlated with its improved oral bioavailability. The pharmacodynamic study of **17c** demonstrated excellent *in vitro* DPP-IV inhibitory activity and >15,000 fold selectivity against related enzymes with sustained suppression of pre- and post-prandial blood glucose levels (*in vivo*). In PK studies, compound **17c** showed higher oral bioavailability with extended $T_{1/2}$, indicating that compound **17c** can be considered as the promising candidate for effective treatment of T2DM and need to subject for further pre-clinical evaluation.

As discussed earlier in designing section, second series was planned to overcome CYP activity associated with the lead compound **17c** of the first series. In this regard initial attempts were made to reduce CYP activity, by introducing suitable spacers (substituted α-amino acid) of reduced chain length to link cyanopyrrolidine (ring A) with *para*-cyanobenzoic acid (ring B) of the lead molecule (**17c** of the first series) **[235]**. Compound **27j** was identified as primary hit from this series. Further to improve DPP-IV potency of **27j**, changes were done in *para*-cyanobenzamide ring, which lead to potent

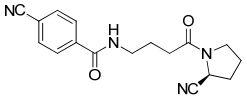
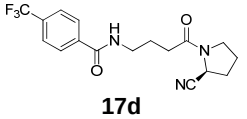
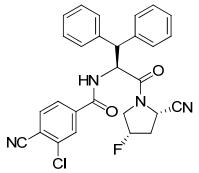
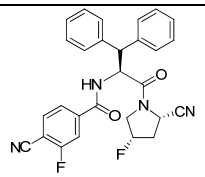
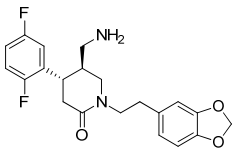
compounds **34d** and **34i**. The high potency of compounds **34d** and **34i** could be because, introduction of sterically less bulky halo atoms (i.e. –F/-Cl) specifically at meta position of the benzamide ring (might be best fit in S3 site and imparts tight binding of the molecule with DPP-IV enzyme in all the three binding sites). Again based upon the literature presidencies **[229-230]**, we made change in pyrrolidine ring system by introducing *cis*-4 fluoro substituent and thereby we identified compounds **34l** and **34m** as the most potent compounds from this series. Lead compound **34m** showed in vitro DPP-IV inhibition equivalent to lead compound **17c** of the first series with >15,000 fold selectivity over other related serine protease (DPP2, DPP8 and DPP9) and showed no CYP inhibition up to 100μM concentration.

In the third series, modification of the lead compound **VII** developed by Merck Sharp & Dohme Corp. was carried out by enhancing the spatial position of $–NH_2$ group by introducing methylene group (i.e. amino methyl group) and rupturing tricyclic ring from the 5-membered imidazole ring to get flexible structure keeping other component intact. We anticipated that the presence and the position of the primary amine might be critical for the inhibitory activity. By making this suitable scaffold changes and incorporating widely used halo-aromatics and halo-heterocycle, we identified progressive lead compound **68v** in this series.

Compound **68v** showed *in vitro* DPP-IV inhibitory activity 2-fold more than Sitagliptin and >5000 fold selectivity over DPP2, DPP8 and DPP9 enzyme. Further PD study of compound **68v** revels prolonged suppression of pre-and post-prandial blood glucose levels (*in vivo*), which correlates with its extended PK profile.

Shortlisted lead compounds from all the three series are listed in **Table 14**. Among all the series, compound **68v** from the third series turn out as the best compound in terms of preclinical profiling. Compound **68v** showed extended $T_{1/2}$ of ~9 h and bioavailability of ~80% with prolong suppression of serum glucose levels ~20% up to 24h, which reports discovery of compound **68v**, a novel aminomethyl-piperidone derivative as potent, selective and long acting DPP-IV inhibitor for the treatment of T2DM.

Table. 14 Short listed lead compounds from all the three series.

Series	Lead molecule	*In vitro* DPP-IV (IC_{50} nM)	*In vitro* fold selectivity			*In vivo* IPGTT (OGTT) %Glucose reduction	PK $T_{1/2}$ (h) (%F)
			DPP2	DPP8	DPP9		
1	 **17c**	2.3±0.9	>25,000	>15,000	>15,000	54.9±3.86 (33.5±7.4) at 20mpk	7.99±0.3 (72.5%)
	 **17d**	3.8±0.5	>25,000	>15,000	>15,000	17.4±5.35 at 20 mpk	0.99±0.1 (63.1%)
2	 **34l**	4.2±0.7	>25,000	>15,000	>15,000	NA	NA
	 **34m**	2.7±0.3	>25,000	>15,000	>15,000	NA	NA
3	 **68v**	8.5±0.4	>5000	>10,000	>10,000	(38.9±5.2) at 3 mpk	8.99±0.3 (79.5%)

4.2 Future Plan

From the third series, compound **68v** showed excellent DPP-IV inhibitory activity (*in vitro*) & antidiabetic activity (*in vivo*). The PK profile of **68v** was found to be satisfactory to represent it as a promising long acting DPP-IV inhibitor to work on. Future work includes some additional safety study and pre-clinical studies before it has been subjected for clinical development. Compound **68v** should be subjected for chronic efficacy studies and for long term toxicological evaluation, along with its PK profile in higher animals such as dog or monkey.

Furthermore, during the development of compound **68v**, it has been observed that compounds of this series accommodate more flexibility with the halo aromatics which

binds in a far region of DPP-IV enzyme S3 site. Further modification can be accomplished by incorporating alicyclic ring systems that can mimic the size of the acyclic part in halo-aromatic of **68v**. This new structural aspect may lead to change in molecular conformation analogues to **68v** but with more effective binding interaction with the enzyme. So by incorporating heterocycles which have been used widely for the development of DPP-IV inhibitors, in the form fused with alicyclic ring systems expands further scope for new series development. Main focus of developing new series will be towards improving the PK profile to get batter DPP-IV inhibitor that can turn as a once a week regimen (**Figure 37**).

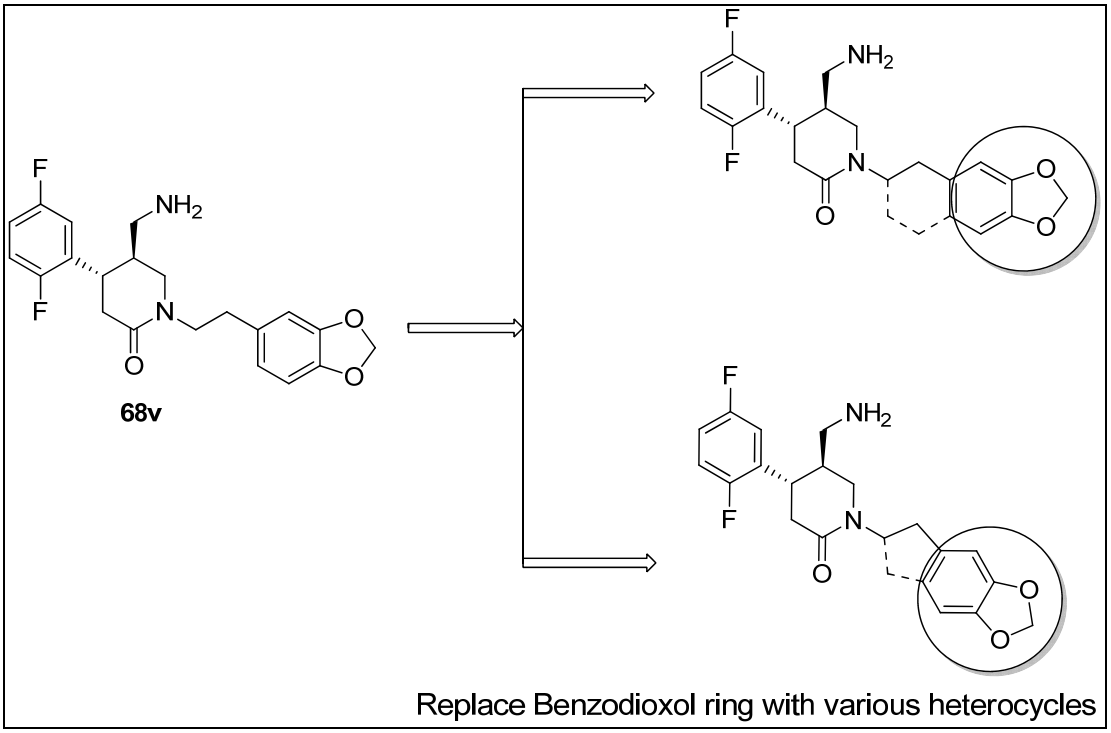


Figure 37: New series development based on lead molecule **68v**

Chapter V:

Experimental

"Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning." — Albert Einstein,

5. Experimental

5.1 Chemistry

5.1.1. Materials and Methods

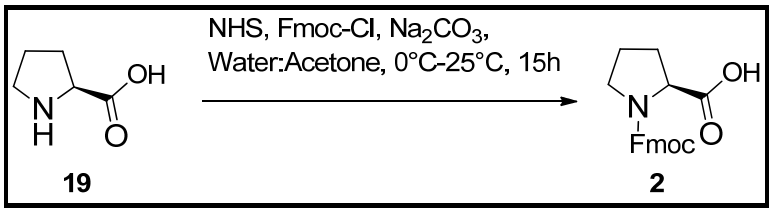
All the reagents used for the synthesis were purchased from Sigma Aldrich Company Limited, Dorset and were used without further purification. Solvents were procured from commercial source and used after distilling or drying according to the known methods. All the air and/or moisture sensitive reactions were carried out in dry solvents under nitrogen atmosphere. Melting points were recorded in open glass capillaries, using scientific melting point apparatus and are uncorrected.

The ^{1}H NMR spectra were recorded on a Bruker Avance-400 (400 MHz) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS either in CD_3OD, DMSO-d_6 or $CDCl_3$. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). ^{13}C NMR spectra were recorded on Bruker Avance-400 at 100 MHz either in $CDCl_3$ or Acetone-d_6. Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer. Elemental analyses were carried out using a Perkin-Elmer 2400 CHN analyzer.

HPLC analyses were carried out at λ_{max} 220 nm using column. Progress of the reactions was monitored by TLC using precoated TLC plates (E. Merck Kieselgel 60 F254) and the spots were visualized in UV and/or in iodine vapours. The chromatographic purification was performed on silica gel (200-400 mesh). Few compounds were directly used for next step without purification and analysis. Detailed synthetic procedures and characterization data of all the final compounds and intermediates are described in this chapter.

5.1.2. Experimental Details : Cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors (First series)

5.1.2.1. (S)-1-(((9H-Fluoren-9-yl)methoxy)carbonyl)pyrrolidine-2-carboxylic acid (Fmoc-Pro-OH) (2)



N-Hydroxy succinamide (5.5 g) was charged in a 1L R B flask, to it was added Na_2CO_3 (3.7 g) dissolved in D.M. water (45 ml) and stirred for 15 minutes. Cool it to 0 ^oC to 5 ^oC using ice-salt bath and was added Fmoc-chloride (11.3 g) dissolved in acetone (45 ml) to the reaction mixture drop wise within 20 to 30 minutes maintaining temperature 0^oC-5^oC. The reaction mixture was stirred at 0 ^oC-5 ^oC for 30 minutes. Free amino acid L-Proline **19** (5 g) was dissolved in Na_2CO_3 (10.18 g in 45 ml D. M. water) solution followed by addition of acetone (45 ml). This solution was added to the reaction mixture using addition funnel within 5 to 10 minutes at temperature 0 ^oC-5 ^oC. The reaction mixture was stirred at this temperature for 30 minutes, after which the temperature was increased gradually to 25 ^oC and was stirred for 15hours.

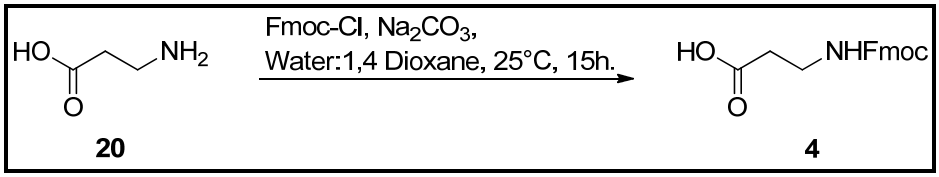
After completion of the reaction (TLC), it was poured into D.M.water (1L) and was basified with 1M Na_2CO_3 till pH increased to10. The resulting mixture was extracted with diisopropyl ether (2 X 150 ml) to remove the non-polar impurities. The aqueous layer was acidified with 1M HCl solution tol pH ~2 and was extracted with ethyl acetate (2 X 150 ml). The combined organic layers were washed with D.M.water (1 X 150 ml) and saturated NaCl solution (1 X 150 ml) and filtered through Hyflo supercel using filter flask and Buchner funnel) and dried over anhydrous Na_2SO_4 (~10 g) , and evaporated to dryness. Crude residue thus obtained was purified by column chromatography using 100-200 mesh size silica gel as a stationary phase and 0-40% Ethyl acetate in Dichloromethane as an eluting system to give 12.01 g (82% yield) of the title product **2** as a white amorphous solid. mp: 116-117 ^oC; Purity by HPLC: 96%.

ESI/MS (m/z) : 338.4 $(M+H)^+$. **Mol. Wt.** = 337.3 g

^{1}HNMR (400 MHz, DMSO-d_6) : δ 1.81-1.86 (m, 2H), 1.94-1.96 (m, 1H), 2.14-2.29 (m, 1H), 3.33-3.45 (m, 2H), 4.18-4.27 (m, 2H), 4.33 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.8Hz), 7.31-

7.35 (m, 2H), 7.36-7.43 (m. 2H), 7.63-7.67 (m, 2H), 7.89 (t, 2H, J = 6.4Hz), 12.69 (bs, 1H, -COO**H**)

5.1.2.2.1. 3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (Fmoc-βAla-OH) (4)



20 **4**

4.0 g (45 mmol) of β-Alanine was suspended in 1,4-dioxane (40 ml) in a R B Flask, and 11.9 g (112 mmol) of Na_2CO_3 dissolved in D. M. water (80 ml) was added in a single portion to give clear solution. To this mixture was added 12.2 g (47 mmol) of Fmoc-Cl dissolved in 1,4-dioxane (20 ml) dropwise over a period of 30min.(During addition solid precipitated in reaction mixture). The mixture was stirred at room temperature for 15h.

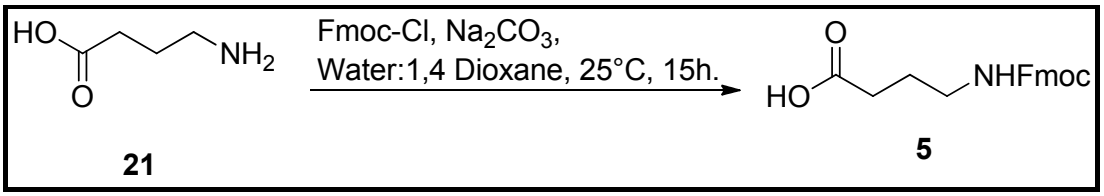
After completion (TLC), the reaction mixture was poured in water (200 ml), and was extracted with ether (3X100 ml) to remove unwanted impurities. Aqueous layer was then acidified with sat. citric acid to pH 3 and extracted with ethyl acetate (3X150 ml), combined organic layers were washed sequentially with water (1X150 ml) and brine (1x150 ml) solution. Organic layer was then dried over anhy. Na_2SO_4, filtered and evaporated to dryness to give thick gummy residue.

The residue thus obtained was purified by column chromatography using 100-200 mesh silica as stationary phase and 0-0.5 % MeOH in $CHCl_3$ as eluting system to give 8.85 g (63% yield) of desired product **4** as a white solid. Mp: 144-145 °C; Purity by HPLC: 97.4% AUC.

ESI/MS (m/z) : 312.4 $(M+H)^+$. **Mol. Wt.** = 311.3 g

^{1}HNMR (400 MHz, DMSO-d_6) : δ 2.37 (t, 2H, J = 6.9 Hz), 3.15-3.21 (m, 2H), 4.16-4.21 (t, 1H, J = 6.6 Hz), 4.28 (d, 2H, J = 6.6 Hz), 7.28-7.35 (m, 3H), 7.40 (t, 2H, J = 6.9 Hz), 7.68 (d, 2H, J = 7.3 Hz), 7.88 (d, 2H, J = 7.3 Hz), 12.21 (bs, 1H, -COO**H**).

5.1.2.2.2. 4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)butanoic acid (Fmoc-GABA-OH) (5)



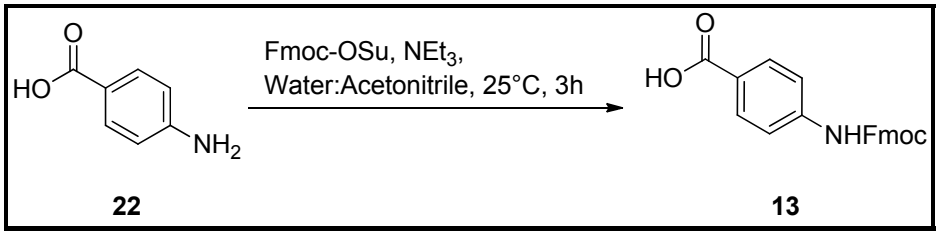
21 **5**

5 (1.2 g) was prepared by means of the general procedure described in **5.1.2.2.1.** with 78% yield as a white solid. 168-170 °C; Purity by HPLC: 98.3% AUC.

ESI/MS (m/z) : 326.4 $(M+H)^+$. **Mol. Wt.** = 325.4 g

^{1}HNMR (400 MHz, $CDCl_3$) : δ 1.98 (t, 2H, J=6.5 Hz), 2.71 (t, 2H, J=6.5 Hz), 3.32 (d, 2H, J=6.5 Hz), 4.21 (t, 2H, J=6.3 Hz), 4.45 (d, 2H, J=6.3 Hz), 4.85 (bs, 1H, -N**H**), 7.31 (t, 2H, J=7.5 Hz), 7.40 (t, 2H, J=7.5 Hz), 7.59 (d, 2H, J=7.5 Hz), 7.77 (d, 2H, J=7.5 Hz), 11.98 (bs, 1H, -COO**H**).

5.1.2.3. 4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)benzoic acid (Fmoc-PABA-OH) (13)



22 **13**

5.0 g (36.5 mmol) of *p*-amino benzoic acid **22** was suspended in a 250 ml R.B. Flask containing 25 ml of D. M. water. To this suspension added triethylamine (5.07 ml, 36.5 mmol) in a single portion and stirred at room temperature for 30min. To this content added acetonitrile (25ml) followed by solid *N*-(9-Fluorenylmethoxycarbonyloxy) succinimide (Fmoc-Osu, 10.24 g, 30.4 mmol). Most of the solid dissolved within 30 min., reaction mixture became stirrable thick gel. Reaction mixture was stirred for 3h at room temperature to completion (TLC).

Acetonitrile of the reaction mixture was removed under reduced pressure and was extracted with ether to remove impurities if any. Aqueous layers were then acidified with 6N HCl to pH 2 and extracted with ethyl acetate (3X100 ml), combined organic layer was washed with water (1X100 ml) and brine (1X100 ml), dried over anhy. Na_2SO_4, filtered and solvent was removed under reduced pressure to give 11.66 g (89% yield) of

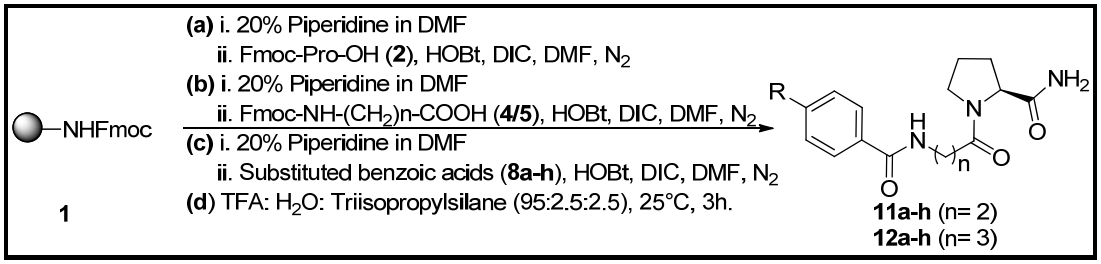
the desired compound **13** as an off white solid. 268 °C dec.; Purity by HPLC: 96.8% AUC.

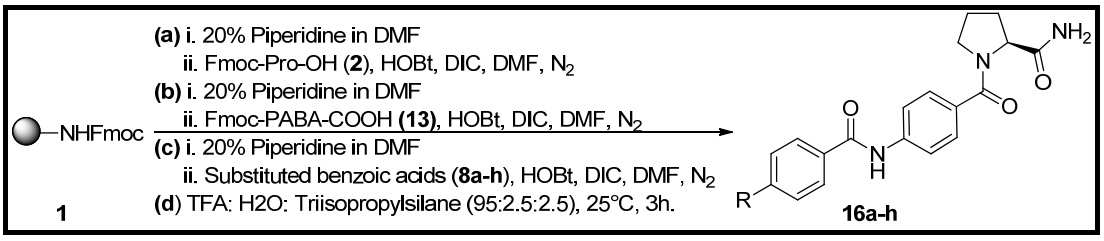
ESI/MS (m/z) : 360.3 $(M+H)^+$. **Mol. Wt.** = 359.2 g

^{1}HNMR (400 MHz, DMSO-d_6) : δ 4.29 (t, 1H, J = 6.5 Hz), 4.50 (d, 2H, J = 6.5 Hz), 7.36 (m, 4H), 7.42-7.51 (m, 3H), 7.72 (d, 2H, J = 7.2 Hz), 7.81 (d, 2H, J = 8.6 Hz), 7.88 (d, 2H, J = 7.4 Hz), 12.64 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 47.0, 66.2, 117.8, 120.6, 124.8, 125.5, 127.6, 128.2, 130.9, 142.3, 144.1, 153.7, 167.4.

5.1.3. General procedure for the synthesis of compounds (11a-h, 12a-h and 16a-h)





All the above compounds were synthesized using Fmoc based solid-phase peptide synthesis protocol (SPPS). Fmoc protected Rink amide MBHA resin **1** (750 mg, 0.58 mmol/g) was swollen in DMF for 20 min and washed with DMF (3 X 25 ml). Fmoc group of the resin was removed by agitating peptidyl resin in 20% piperidine solution (25 ml, 1 X 5 min and 1 X 30 min) for the next coupling reaction. Fmoc-Pro-OH **2** was coupled to the deprotected resin by agitating its pre-activated solution under N_2 atmosphere. [i.e. Fmoc-Pro-OH (4 eq), HOBt (4 eq), and 1,3-diisopropyl carbidiimide (DIC) (4 eq) in DMF (5ml) for 30 min)]. After completion of the coupling reaction confirmed by Kaiser ninhydrin and TNBS tests, peptidyl resin was washed with DMF, DCM and ether (3 X 25 ml each). However if coupling found incomplete by Kaiser ninhydrin test then coupling reaction was repeated by performing one more coupling cycle. Fmoc group of Fmoc-Pro-OH **2** coupled resin was then deprotected with 20% piperidine solution (25 ml, 1 X 5 min and 1 X 30 min). Fmoc-β-Ala-OH **4**/ Fmoc-GABA-

OH **5**/ Fmoc-PABA-OH **13** were coupled to the peptidyl resin by agitating their respective preactivated solutions under N_2 atmosphere. (i.e. Fmoc-XX-OH (4 equiv), HOBt (4 equiv), and DIC (4 equiv) in DMF (5 ml) for 30 min.). After completion of the coupling reaction confirmed by Kaiser ninhydrin and TNBS tests, peptidyl resin was washed with DMF, DCM and ether (3 X 25 ml each) and treated with 20% piperidine solution (25 ml, 1 X 5 min and 1 X 30 min) to remove Fmoc group. Peptidyl resin was then washed with DMF, DCM and ether (3 X 25 ml each) and swollen in DMF for 30 min for the coupling of substituted benzoic acids **8a-h**. Substituted benzoic acids **8a-h** were incorporated to the respective peptidyl resin by agitating peptidyl rasine in the preactivated coupling solution of respective benzoic acid under N_2 atm. over a period of 2-4hrs. Completion of coupling was confirmed by Kaiser ninhydrin and TNBS tests, whenever coupling was found incomplete one more coupling cycle was performed. Then, the resin was washed with DMF, DCM and ether (5 X 25ml each) and dried under vacuum for the global cleavage to get the desired peptidomimetics.

Cleavage;

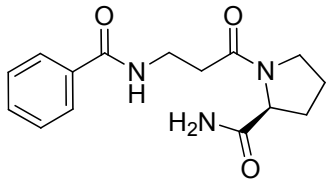
The desired peptidomimetics were cleaved and deprotected from their respective peptidyl-resins by treatment with TFA cleavage mixture as follows. A solution of TFA / Water / Triisopropylsilane (95: 2.5: 2.5) (10 ml / 100 mg of peptidyl-resin) was added to peptidyl-resins and the mixture was kept at room temperature with occasional stirring. The resin was filtered, washed with a cleavage mixture and the combined filtrate was evaporated to dryness. Residue obtained was titrated with ether (20 ml each) to yield crude compounds, typically in >100% yield (Ca 200-230 mg). Crude compounds thus obtained were purified by preparative HPLC as follows:

Purification;

Preparative HPLC was carried out on a Shimadzu LC-8A liquid chromatograph. A solution of crude compounds dissolved in water: acetonotile (ACN) (1:1, 5ml) or Methanol (5ml) was injected into a semi-prep column (Luna 10 μ; C_{18}; 210-220 nm), dimension 250 X 21.20 mm and eluted with a linear gradient of ACN in water, both buffered with 0.1 % TFA, using a flow rate of 15 ml / min, with effluent monitoring by PDA detector at 220 nm. A typical gradient of 20 % to 70 % of water-ACN mixture, buffered with 0.1 % TFA was used, over a period of 100 minutes, with 1% gradient change per minute. The desired product eluted were collected in a single 50-80 ml fraction and pure peptidomimetics **11a-h, 12a-h** and **16a-h** were obtained as white solids

either by lyophilisation of respective HPLC fractions or by normal work up procedure after evaporation of ACN from the fractions and extraction with DCM.

5.1.3.1. (S)-1-(3-Benzamidopropanoyl)pyrrolidine-2-carboxamide (11a)



11a (200 mg, 82%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 153-155 °C; Purity by HPLC: 99.05% AUC.

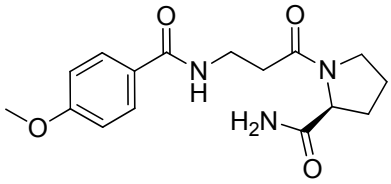
ESI/MS (m/z) : 290.1 $(M+H)^+$. **Mol. Wt.** = 289.3 g

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.96-2.13 (m, 3H), 2.19- 2.24 (m, 1H), 2.63-2.71 (m, 2H), 3.32-3.67(m, 4H), 4.38-4.41 (dd, 1H, J_1 = 3.6Hz, J_2 = 8.2Hz), 7.56-8.05 (m, 5H);

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.8, 23.8, 33.4, 38.7, 42.6, 61.5, 126.7, 128.6, 131.6, 138.4, 167.8, 176.4, 178.1.

Analysis : Mol. Formula: $C_{15}H_{19}N_3O_3$:
Calcd.: C 62.27, H 6.62, N 14.52.
Found: C 62.25, H 6.59, N 14.48.

5.1.3.2. (S)-1-(3-(4-Methoxybenzamido)propanoyl)pyrrolidine-2-carboxamide (11b)



11b (190 mg, 79%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 156-158 °C; Purity by HPLC: 99.21% AUC.

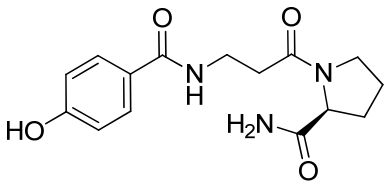
ESI/MS (m/z) : 320.3 $(M+H)^+$. **Mol. Wt.** = 319.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.84-2.03 (m, 3H), 2.17- 2.21 (m, 1H), 2.68-2.72 (m, 2H), 3.31-3.68 (m, 4H), 3.67 (s, 3H), 4.39-4.42 (dd, 1H, J_1 = 3.6Hz, J_2 = 8.4Hz), 6.82 (d, 2H, J = 8.8Hz), 7.68 (d, 2H, J = 8.8Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 18.7, 23.4, 35.2, 38.7, 43.2, 55.8, 61.2, 115.4, 125.2, 127.6, 163.8, 167.3, 176.4, 178.3.

Analysis : Mol. Formula: $C_{16}H_{21}N_3O_4$
Calcd.: C 60.17, H 6.63, N 13.16.
Found: C 60.16, H 6.64, N 13.14.

5.1.3.3. (S)-1-(3-(4-Hydroxybenzamido)propanoyl)pyrrolidine-2-carboxamide (11c)



11c (188 mg, 78%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 143-145 °C; Purity by HPLC: 99.46% AUC.

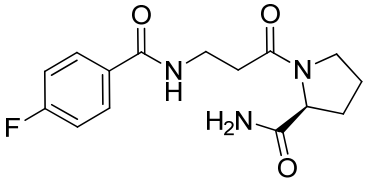
ESI/MS (m/z) : 306.5 $(M+H)^+$. **Mol. Wt.** = 305.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.87-2.08 (m, 3H), 2.19- 2.23 (m, 1H), 2.65-2.71 (m, 2H), 3.31-3.65(m, 4H), 4.38-4.41 (dd, 1H, J_1 = 3.8Hz, J_2 = 8.2Hz), 6.93 (d, 2H, J = 9.2Hz), 7.75 (d, 2H, J = 9.2Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.4, 23.6, 34.8, 39.4, 42.8, 61.3, 116.3, 126.5, 129.2, 161.4, 167.7, 176.3, 177.9.

Analysis : Mol. Formula: $C_{15}H_{19}N_3O_4$
Calcd.: C 59.01, H 6.27, N 13.76.
Found: C 58.98, H 6.24, N 13.73.

5.1.3.4. (S)-1-(3-(4-Fluorobenzamido)propanoyl)pyrrolidine-2-carboxamide (11d)



11d (184 mg, 75%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 165-167 °C; Purity by HPLC: 99.07% AUC.

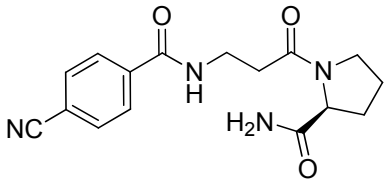
ESI/MS (m/z) : 308.2 $(M+H)^+$. **Mol. Wt.** = 307.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.93-2.15 (m, 3H), 2.19- 2.26 (m, 1H), 2.67-2.71 (m, 2H), 3.32-3.64(m, 4H), 4.39-4.42 (m, 1H), 7.21 (m, 2H), 7.89 (m, 2H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.7, 23.4, 33.9, 39.2, 42.3, 61.5, 115.4 (d, J = 19.2Hz), 128.7 (d, J = 4.2Hz), 129.3, 166.2 (d, J = 246Hz), 167.8, 176.4, 178.1.

Analysis : Mol. Formula: $C_{15}H_{18}FN_3O_3$
Calcd.: C 58.62, H 5.90, N 13.67.
Found: C 58.64, H 5.87, N 13.66.

5.1.3.5. (S)-1-(3-(4-Cyanobenzamido)propanoyl)pyrrolidine-2-carboxamide (11e)



11e (192 mg, 83%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 147-149 °C; Purity by HPLC: 99.23% AUC.

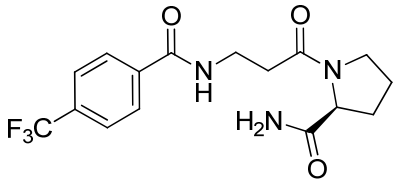
ESI/MS (m/z) : 315.5 $(M+H)^+$. **Mol. Wt.** = 314.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.89-2.10 (m, 3H), 2.17- 2.23 (m, 1H), 2.64-2.70 (m, 2H), 3.31-3.65(m, 4H), 4.39-4.41 (m, 1H), 7.53 (d, 2H, J = 8.4Hz), 8.34 (d, 2H, J = 8.4Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.5, 23.3, 33.7, 38.8, 42.4, 61.4, 115.8, 116.9, 128.4, 131.8, 137.6, 167.6, 176.4, 177.9.

Analysis : Mol. Formula: $C_{16}H_{18}N_4O_3$
Calcd.: C 61.13, H 5.77, N 17.82.
Found: C 61.16, H 5.79, N 17.85.

5.1.3.6. (S)-1-(3-(4-(Trifluoromethyl)benzamido)propanoyl)pyrrolidine-2-carboxamide (11f)



11f (195 mg, 80%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 169-171 °C; Purity by HPLC: 99.04% AUC.

ESI/MS (m/z) : 358.4 $(M+H)^+$. **Mol. Wt.** = 357.2 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.95-2.10 (m, 3H), 2.18- 2.23 (m, 1H), 2.64-2.71 (m, 2H), 3.31-3.65(m, 4H), 4.38-4.42 (dd, 1H, J_1 = 3.4Hz, J_2 = 8.0Hz), 7.74 (d, 2H, J = 8.8Hz), 7.89 (d, 2H, J = 8.8Hz).

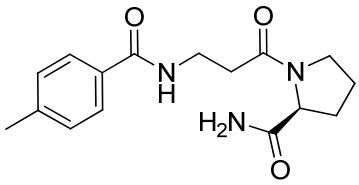
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.6, 23.3, 33.6, 39.4, 42.8, 61.3, 120.2, 125.7 (q, J = 271.3Hz), 128.5, 135.4 (q, J = 30.4Hz), 137.3, 167.2, 176.2, 178.3.

Analysis : Mol. Formula: $C_{16}H_{18}F_3N_3O_3$

Calcd.: C 53.78, H 5.08, N 11.76.

Found: C 53.74, H 5.06, N 11.73.

5.1.3.7. (S)-1-(3-(4-Methylbenzamido)propanoyl)pyrrolidine-2-carboxamide (11g)



11g (186 mg, 78%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 138-140 °C; Purity by HPLC: 99.13% AUC.

ESI/MS (m/z) : 304.5 $(M+H)^+$. **Mol. Wt.** = 303.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.96-2.11 (m, 3H), 2.17- 2.21 (m, 1H), 2.39 (s, 3H), 2.65-2.72 (m, 2H), 3.31-3.67(m, 4H), 4.39-4.43 (dd, 1H, J_1 = 3.6Hz, J_2 = 8.2Hz), 7.31 (d, 2H, J = 8.6Hz), 7.84 (d, 2H, J = 8.6Hz).

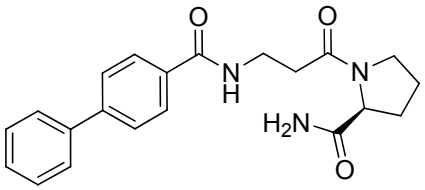
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.9, 21.3, 23.5, 33.4, 39.3, 42.5, 61.5, 128.3, 129.6, 131.2, 140.1, 167.6, 176.5, 177.8.

Analysis : Mol. Formula: $C_{16}H_{21}N_3O_3$

Calcd.: C 63.35, H 6.98, N 13.85.

Found: C 63.37, H 6.95, N 13.83.

5.1.3.8. (S)-1-(3-([1,1'-Biphenyl]-4-ylcarboxamido)propanoyl)pyrrolidine-2-carboxamide (11h)



11h (206 mg, 84%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 179-181 °C; Purity by HPLC: 99.17% AUC.

ESI/MS (m/z) : 366.2 $(M+H)^+$. **Mol. Wt.** = 365.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.97-2.12 (m, 3H), 2.19- 2.23 (m, 1H), 2.63-2.71 (m, 2H), 3.32-3.67(m, 4H), 4.38-4.41 (dd, 1H, J_1 = 3.6Hz, J_2 = 8.4Hz), 7.23-7.51 (m, 5H), 7.63 (d, 2H, J = 9.0Hz), 7.89 (d, 2H, J = 9.0Hz).

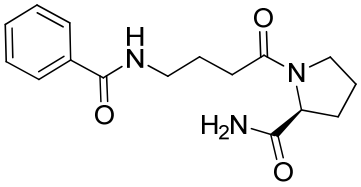
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.6, 23.3, 33.2, 39.7, 42.3, 61.3, 127.3, 127.5, 127.6, 127.9, 128.1, 128.6, 129.3, 132.7, 137.2, 139.8, 167.8, 176.3, 178.2.

Analysis : Mol. Formula: $C_{21}H_{23}N_3O_3$

Calcd.: C 69.02, H 6.34, N 11.50.

Found: C 68.99, H 6.37, N 11.47.

5.1.3.9. (S)-1-(4-Benzamidobutanoyl)pyrrolidine-2-carboxamide (12a)



12a (178 mg, 73%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 172-175 °C; Purity by HPLC: 99.47% AUC.

ESI/MS (m/z) : 304.2 $(M+H)^+$. **Mol. Wt.** = 303.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.83-1.87 (m, 3H), 1.93- 2.03 (m, 1H), 2.15-2.21 (m, 2H), 2.32-2.44 (m, 2H), 3.21-3.25 (m, 2H), 3.36-3.41 (m, 1H), 3.51-3.55 (m, 1H), 4.42-4.47 (dd, 1H, J_1 = 3.8Hz, J_2 = 8.2Hz), 7.67-8.04 (m, 5H).

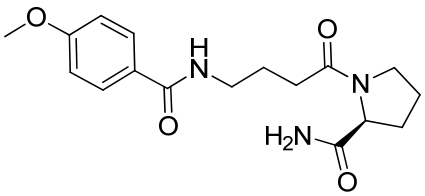
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.9, 23.3, 28.2, 29.4, 42.2, 42.9, 61.3, 126.5, 128.9, 131.9, 137.7, 167.9, 176.5, 176.7.

Analysis : Mol. Formula: $C_{16}H_{21}N_3O_3$

Calcd.: C 63.35, H 6.98, N 13.85.

Found: C 63.33, H 7.02, N 13.82.

5.1.3.10. (S)-1-(4-(4-Methoxybenzamido)butanoyl)pyrrolidine-2-carboxamide (12b)



12b (158 mg, 69%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 123-125 °C; Purity by HPLC: 99.64% AUC.

ESI/MS (m/z) : 334.2 $(M+H)^+$. **Mol. Wt.** = 333.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.82-1.89 (m, 3H), 1.92- 2.04 (m, 1H), 2.13-2.24 (m, 2H), 2.32-2.41 (m, 2H), 3.21-3.26 (m, 2H), 3.38-3.43 (m, 1H), 3.53-3.59 (m, 1H), 3.69 (s, 3H), 4.41-4.49 (dd, 1H, J_1 = 4.0Hz, J_2 = 7.8Hz), 6.93 (d, 2H, J = 8.4Hz), 7.65 (d, 2H, J = 8.4Hz).

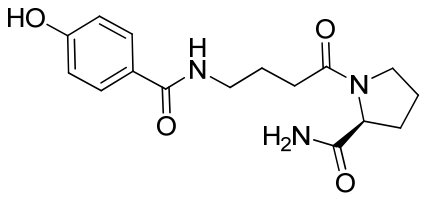
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.5, 23.3, 28.1, 29.4, 42.1, 43.9, 55.3, 61.3, 115.1, 125.6, 128.2, 164.8, 167.6, 176.4, 177.1.

Analysis : Mol. Formula: $C_{17}H_{23}N_3O_4$

Calcd.: C 61.25, H 6.95, N 12.60.

Found: C 61.28, H 6.98, N 12.61.

5.1.3.11. (S)-1-(4-(4-Hydroxybenzamido)butanoyl)pyrrolidine-2-carboxamide (12c)



12c (196 mg, 81%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 116-118 °C; Purity by HPLC: 99.28% AUC.

ESI/MS (m/z) : 320.2 $(M+H)^+$. **Mol. Wt.** = 319.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.84-1.89 (m, 3H), 1.91- 2.05 (m, 1H), 2.15-2.24 (m, 2H), 2.31-2.42 (m, 2H), 3.21-3.28 (m, 2H), 3.39-3.43 (m, 1H), 3.53-3.57 (m, 1H), 4.41-4.47 (dd, 1H, J_1 = 3.8Hz, J_2 = 7.6Hz), 6.98 (d, 2H, J = 8.6Hz), 7.71 (d, 2H, J = 8.6Hz).

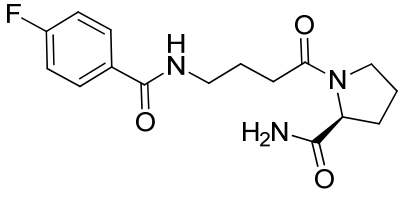
^{13}C NMR (100 MHz, Methanol-d_6): δ 19.6, 23.5, 27.9, 29.2, 42.3, 43.6, 61.4, 115.9, 125.8, 128.9, 161.3, 167.8, 176.2, 177.0.

Analysis : Mol. Formula: $C_{16}H_{21}N_3O_4$

Calcd.: C 60.17, H 6.63, N 13.16.

Found: C 60.19, H 6.66, N 13.14.

5.1.3.12. (S)-1-(4-(4-Fluorobenzamido)butanoyl)pyrrolidine-2-carboxamide (12d)



12d (196 mg, 85%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 132-134 °C; Purity by HPLC: 99.79% AUC.

ESI/MS (m/z) : 322.1 $(M+H)^+$. **Mol. Wt.** = 321.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.86-1.89 (m, 3H), 1.93- 2.04 (m, 1H), 2.14-2.24 (m, 2H), 2.29-2.41 (m, 2H), 3.22-3.29 (m, 2H), 3.37-3.41 (m, 1H), 3.52-3.57 (m, 1H), 4.41-4.46 (dd, 1H, J_1 = 3.6Hz, J_2 = 7.8Hz), 6.98 (m, 2H), 7.71 (m, 2H).

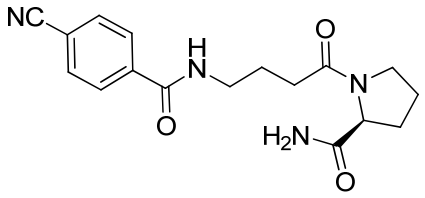
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.5, 22.8, 27.6, 28.9, 42.3, 43.1, 61.2, 115.8 (d, J = 21Hz), 129.2 (d, J = 3.8Hz), 129.5, 164.5 (d, J = 249Hz), 167.4, 176.2, 177.2.

Analysis : Mol. Formula: $C_{16}H_{20}FN_3O_3$

Calcd.: C 59.80, H 6.27, N 13.08.

Found: C 59.81, H 6.29, N 13.05.

5.1.3.13. (S)-1-(4-(4-Cyanobenzamido)butanoyl)pyrrolidine-2-carboxamide (12e)



12e (189 mg, 83%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 149-151 °C; Purity by HPLC: 99.04% AUC.

ESI/MS (m/z) : 329.6 $(M+H)^+$. **Mol. Wt.** = 328.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.83-1.88 (m, 3H), 1.91- 2.04 (m, 1H), 2.13-2.23 (m, 2H), 2.32-2.45 (m, 2H), 3.20-3.27 (m, 2H), 3.38-3.42 (m, 1H), 3.52-3.56 (m, 1H), 4.42-4.48 (dd, 1H, J_1 = 4.0Hz, J_2 = 8.2Hz), 7.72 (d, 2H, J = 8.4Hz), 8.21 (d, 2H, J = 8.4Hz).

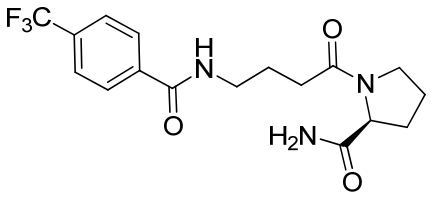
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.7, 23.4, 27.8, 29.1, 42.1, 42.9, 61.4, 115.8, 117.3, 128.1, 132.3, 136.8, 167.8, 176.3, 176.8.

Analysis : Mol. Formula: $C_{17}H_{20}N_4O_3$

Calcd.: C 62.18, H 6.14, N 17.06.

Found: C 62.14, H 6.12, N 17.04.

5.1.3.14. (S)-1-(4-(4-(Trifluoromethyl)benzamido)butanoyl)pyrrolidine-2-carboxamide (12f)



12f (183 mg, 78%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 193-195 °C; Purity by HPLC: 99.19% AUC.

ESI/MS (m/z) : 372.2 $(M+H)^+$. **Mol. Wt.** = 371.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.82-1.87 (m, 3H), 1.91- 2.02 (m, 1H), 2.17-2.21 (m, 2H), 2.32-2.43 (m, 2H), 3.20-3.23 (m, 2H), 3.34-3.41 (m, 1H), 3.49-3.53 (m, 1H), 4.40-4.46 (dd, 1H, J_1 = 4.0Hz, J_2 = 7.8Hz), 7.69 (d, 2H, J = 8.6Hz), 7.93 (d, 2H, J = 8.6Hz).

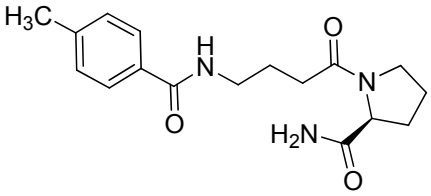
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.6, 22.9, 27.9, 29.2, 42.1, 42.3, 61.5, 119.7, 125.8 (q, J = 271.4Hz), 128.1, 133.8 (q, J = 29.8Hz), 137.2, 167.7, 176.4, 177.3.

Analysis : Mol. Formula: $C_{17}H_{20}F_3N_3O_3$

Calcd.: C 54.98, H 5.43, N 11.32.

Found: C 55.01, H 5.44, N 11.30.

5.1.3.15. (S)-1-(4-(4-Methylbenzamido)butanoyl)pyrrolidine-2-carboxamide (12g)



12g (181 mg, 72%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 145-147 °C; Purity by HPLC: 99.48% AUC.

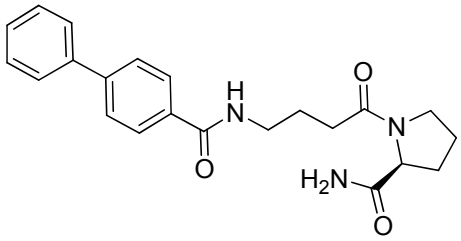
ESI/MS (m/z) : 318.6 $(M+H)^+$. **Mol. Wt.** = 317.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.81-1.86 (m, 3H), 1.92- 2.03 (m, 1H), 2.16-2.23 (m, 2H), 2.31-2.44 (m, 3H), 3.21-3.26 (m, 2H), 3.36-3.42 (m, 1H), 3.51-3.54 (m, 1H), 4.41-4.45 (dd, 1H, J_1 = 3.6Hz, J_2 = 7.6Hz), 7.33 (d, 2H, J = 8.4Hz), 7.86 (d, 2H, J = 8.4Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.7, 21.3, 23.2, 28.4, 29.1, 42.2, 42.5, 61.5, 128.1, 130.2, 130.9, 141.7, 167.6, 176.3, 177.4.

Analysis : Mol. Formula: $C_{17}H_{23}N_3O_3$
Calcd.: C 64.33, H 7.30, N 13.24.
Found: C 64.29, H 7.32, N 13.20.

5.1.3.16. (S)-1-(4-([1,1'-Biphenyl]-4-ylcarboxamido)butanoyl)pyrrolidine-2-carboxamide (12h)



12h (206 mg, 84%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 204-206 °C; Purity by HPLC: 99.23% AUC.

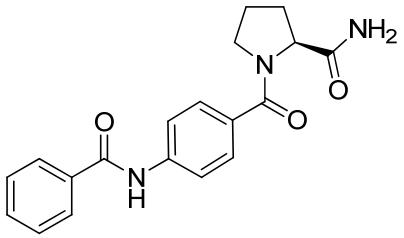
ESI/MS (m/z) : 380.3 $(M+H)^+$. **Mol. Wt.** = 379.5 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.79-1.84 (m, 3H), 1.90- 2.02 (m, 1H), 2.17-2.23 (m, 2H), 2.31-2.45 (m, 2H), 3.23-3.27 (m, 2H), 3.34-3.41 (m, 1H), 3.51-3.56 (m, 1H), 4.42-4.47 (dd, 1H, J_1 = 3.8Hz, J_2 = 7.8Hz), 7.24-7.49 (m, 5H), 7.65 (d, 2H, J = 8.7Hz), 7.98 (d, 2H, J = 8.7Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.7, 23.3, 27.9, 28.7, 42.1, 42.8, 61.3, 127.5, 127.7, 127.8, 128.0, 128.1, 128.7, 129.2, 132.6, 137.4, 140.3, 167.7, 176.4, 177.1.

Analysis : Mol. Formula: $C_{22}H_{25}N_3O_3$
Calcd.: C 69.64, H 6.64, N 11.07.
Found: C 69.61, H 6.63, N 11.05.

5.1.3.17. (S)-1-(4-Benzamidobenzoyl)pyrrolidine-2-carboxamide (16a)



16a (184 mg, 83%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 184-186 °C; Purity by HPLC: 99.07% AUC.

ESI/MS (m/z) : 338.5 $(M+H)^+$. **Mol. Wt.** = 337.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.63-1.85 (m, 3H), 1.87- 1.98 (m, 1H), 3.31-3.42 (m, 1H), 3.45-3.51 (m, 1H), 4.64-4.70 (dd, 1H, J = 4.8Hz, J = 7.6Hz), 7.62-7.94 (m, 7H), 8.07 (d, 2H, J = 7.8Hz).

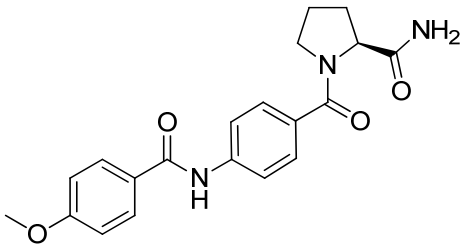
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.9, 23.3, 42.9, 61.8, 121.3, 124.1, 127.3, 128.4, 128.7, 129.5, 130.5, 131.6, 137.8, 140.3, 165.4, 169.9, 176.4.

Analysis : Mol. Formula: $C_{19}H_{19}N_3O_3$

Calcd.: C 67.64, H 5.68, N 12.46.

Found: C 67.61, H 5.65, N 12.42.

5.1.3.18. (S)-1-(4-(4-Methoxybenzamido)benzoyl)pyrrolidine-2-carboxamide (16b)



16b (173 mg, 79%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 158-160 °C; Purity by HPLC: 99.49% AUC.

ESI/MS (m/z) : 368.2 $(M+H)^+$. **Mol. Wt.** = 367.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.63-1.85 (m, 3H), 1.87- 1.98 (m, 1H), 3.34-3.43 (m, 1H), 3.47-3.52 (m, 1H), 3.68 (s, 3H), 4.63-4.72 (dd, 1H, J_1 = 5.2Hz, J_2 = 7.6Hz), 7.12 (d, 2H, J = 8.4Hz), 7.61 (d, 2H, J = 8.8Hz), 7.74 (d, 2H, J = 8.4Hz), 7.79 (d, 2H, J = 8.8Hz).

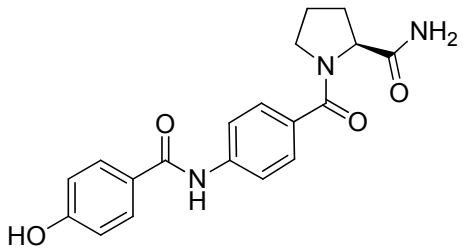
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.8, 23.4, 42.8, 54.8, 61.7, 114.7, 119.8, 126.3, 127.4, 128.4, 130.4, 140.1, 164.8, 165.3, 170.9, 176.6.

Analysis : Mol. Formula: $C_{20}H_{21}N_3O_4$

Calcd.: C 65.38, H 5.76, N 11.44.

Found: C 65.40, H 5.77, N 11.42.

5.1.3.19. (S)-1-(4-(4-Hydroxybenzamido)benzoyl)pyrrolidine-2-carboxamide (16c)



16c (176 mg, 77%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 146-148 °C; Purity by HPLC: 99.76% AUC.

ESI/MS (m/z) : 354.6 $(M+H)^+$. **Mol. Wt.** = 353.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.65-1.87 (m, 3H), 1.89- 1.97 (m, 1H), 3.31-3.42 (m, 1H), 3.45-3.51 (m, 1H), 4.64-4.71 (dd, 1H, J_1 = 5.0Hz, J_2 = 7.8Hz), 7.08 (d, 2H, J = 8.7Hz), 7.63 (d, 2H, J = 8.6Hz), 7.76 (d, 2H, J = 8.7Hz), 7.81 (d, 2H, J = 8.6Hz).

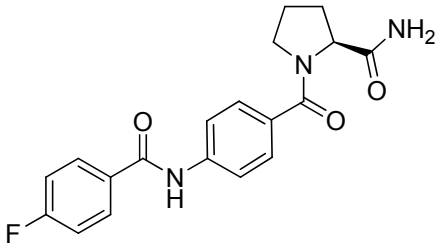
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.9, 23.3, 42.9, 61.9, 116.3, 120.3, 126.5, 127.6, 128.9, 130.7, 139.8, 161.2, 165.3, 170.4, 176.3.

Analysis : Mol. Formula: $C_{19}H_{19}N_3O_4$

Calcd.: C 64.58, H 5.42, N 11.89.

Found: C 64.54, H 5.40, N 11.86.

5.1.3.20. (S)-1-(4-(4-Fluorobenzamido)benzoyl)pyrrolidine-2-carboxamide (16d)



16d (211 mg, 85%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 168-170 °C; Purity by HPLC: 99.17% AUC.

ESI/MS (m/z) : 355.3 $(M+H)^+$. **Mol. Wt.** = 355.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.66-1.89 (m, 3H), 1.90- 1.97 (m, 1H), 3.32-3.41 (m, 1H), 3.46-3.51 (m, 1H), 4.63-4.70 (dd, 1H, J_1 = 5.2Hz, J_2 = 7.4Hz), 7.19 (d, 2H, J = 8.4Hz), 7.68 (d, 2H, J = 8.6Hz), 7.73 (d, 2H, J = 8.4Hz), 7.81 (d, 2H, J = 8.6Hz).

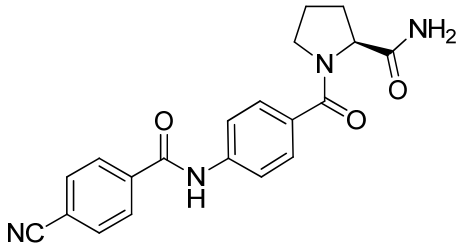
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.7, 23.1, 42.8, 61.5, 116.1(d, J = 21Hz), 120.4, 127.4, 129.2 (d, J = 4.2Hz), 129.9, 130.5, 139.6, 165.3, 165.8 (d, J = 249Hz), 169.9, 176.2.

Analysis : Mol. Formula: $C_{19}H_{18}FN_3O_3$

Calcd.: C 64.22, H 5.11, N 11.82.

Found: C 64.19, H 5.07, N 11.83.

5.1.3.21. (S)-1-(4-(4-Cyanobenzamido)benzoyl)pyrrolidine-2-carboxamide (16e)



16e (203 mg, 79%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 137-139 °C; Purity by HPLC: 99.06% AUC.

ESI/MS (m/z) : 363.3 $(M+H)^+$. **Mol. Wt.** = 362.4 g.

^{1}H NMR (400 MHz, Methanol-d$_4$): δ = 1.64-1.86 (m, 3H), 1.89- 1.98 (m, 1H), 3.32-3.42 (m, 1H), 3.43-3.49 (m, 1H), 4.64-4.70 (m, 1H), 7.68 (d, 2H, J = 8.4Hz), 7.71 (d, 2H, J = 8.6Hz), 7.82 (d, 2H, J = 8.4Hz), 7.99 (d, 2H, J = 8.6Hz).

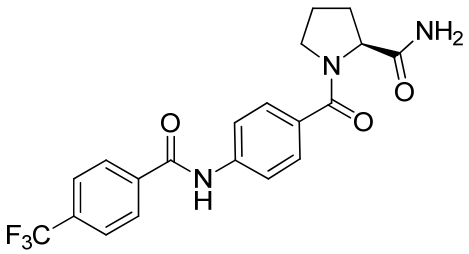
^{13}C NMR (100 MHz, Methanol-d$_4$): δ 19.8, 23.4, 42.7, 61.6, 116.2, 116.7, 120.4, 127.5, 128.2, 130.7, 133.4, 138.4, 139.5, 165.1, 169.8, 176.1.

Analysis : Mol. Formula: $C_{20}H_{18}N_3O_4$

Calcd.: C 66.29, H 5.01, N 15.46.

Found: C 66.31, H 5.05, N 15.42.

5.1.3.22. (S)-1-(4-(4-(Trifluoromethyl)benzamido)benzoyl)pyrrolidine-2-carboxamide (16f)



16f (209 mg, 80%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 169-171 °C; Purity by HPLC: 99.18% AUC.

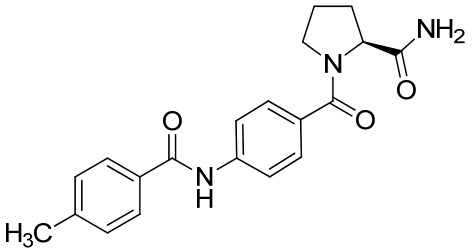
ESI/MS (m/z) : 406.2 $(M+H)^+$. **Mol. Wt.** = 405.4 g.

^{1}H NMR (400 MHz, Methanol-d$_4$): δ = 1.65-1.86 (m, 3H), 1.88- 1.97 (m, 1H), 3.31-3.43 (m, 1H), 3.45-3.50 (m, 1H), 4.63-4.72 (dd, 1H, J = 5.0Hz, J = 7.4Hz), 7.62 (d, 2H, J = 8.6Hz), 7.68 (d, 2H, J = 8.9Hz), 7.83 (d, 2H, J = 8.6Hz), 7.87 (d, 2H, J = 8.9Hz).

^{13}C NMR (100 MHz, Methanol-d$_4$): δ 19.9, 23.4, 43.1, 61.9, 119.7, 120.4, 125.7(q, J = 271.6Hz), 127.9, 128.0, 130.3, 135.0 (q, J= 28.6Hz), 136.6, 139.4, 165.2, 169.7, 177.1.

Analysis : Mol. Formula: $C_{20}H_{18}F_3N_3O_3$
Calcd.: C 59.26, H 4.48, N 10.37.
Found: 59.23, H 4.44, N 10.34.

5.1.3.23. (S)-1-(4-(4-Methylbenzamido)benzoyl)pyrrolidine-2-carboxamide (16g)



16g (201 mg, 78%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 192-194 °C; Purity by HPLC: 99.26% AUC.

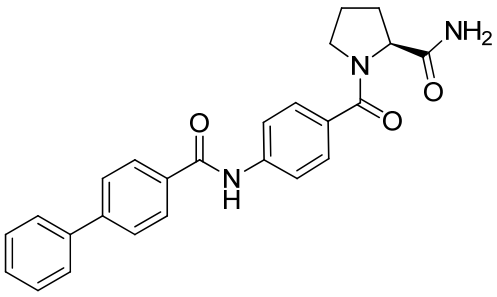
ESI/MS (m/z) : 352.3 $(M+H)^+$. **Mol. Wt.** = 351.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.63-1.86 (m, 3H), 1.87- 1.96 (m, 1H), 2.37 (s, 3H), 3.32-3.43 (m, 1H), 3.43-3.52 (m, 1H), 4.64-4.72 (dd, 1H, J = 5.1Hz, J = 7.6Hz), 7.31 (d, 2H, J = 8.8Hz), 7.63 (d, 2H, J = 8.6Hz), 7.69 (d, 2H, J = 8.8Hz), 7.89 (d, 2H, J = 8.6Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.8, 21.2, 23.2, 43.3, 61.7, 120.2, 127.7, 127.9, 129.0, 130.2, 130.6, 139.0, 141.3, 165.4, 170.0, 176.9.

Analysis : Mol. Formula: $C_{20}H_{21}N_3O_3$
Calcd.: C 68.36, H 6.02, N 11.96.
Found: C 68.38, H 6.05, N 11.93.

5.1.3.24. (S)-1-(4-([1,1'-Biphenyl]-4-ylcarboxamido)benzoyl)pyrrolidine-2-carboxamide (16h)



16h (223 mg, 83%) was prepared by means of the general procedure described in **5.1.3.** as a white solid. 223-225 °C; Purity by HPLC: 99.73% AUC.

ESI/MS (m/z) : 414.7 $(M+H)^+$. **Mol. Wt.** = 413.5 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.64-1.84 (m, 3H), 1.86- 1.95 (m, 1H), 3.30-3.42 (m, 1H), 3.45-3.53 (m, 1H), 4.63-4.70 (dd, 1H, J = 4.8Hz, J = 7.8Hz), 7.23-7.49 (m, 5H), 7.63 (d, 2H, J = 8.6Hz), 7.65 (d, 2H, J = 8.7Hz), 7.91 (d, 2H, J = 8.6Hz), 7.98 (d, 2H, J = 8.7Hz).

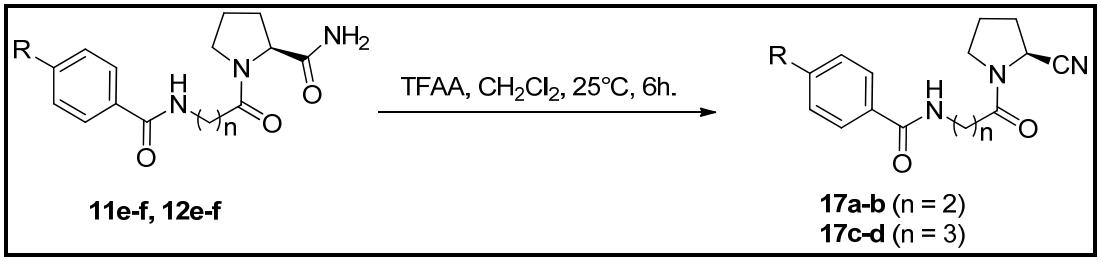
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.9, 23.3, 42.7, 61.9, 120.4, 127.3, 127.5, 127.7, 127.8, 127.9, 128.0, 129.2, 130.4, 133.1, 137.0, 139.1, 140.6, 165.4, 169.9, 176.7.

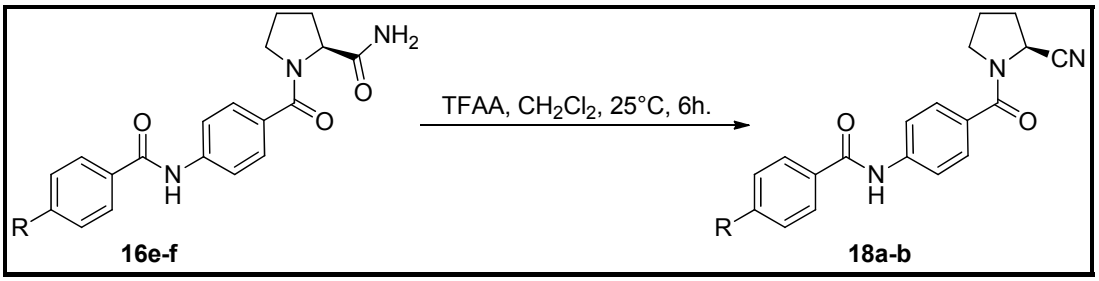
Analysis : Mol. Formula: $C_{25}H_{23}N_3O_3$

Calcd.: C 72.62, H 5.61, N 10.16.

Found: C 72.59, H 5.58, N 10.13.

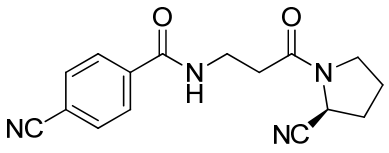
5.1.4. General procedure for the synthesis of compounds (17a-d and 18a-b)





To a solution of respective amide compounds **11e-f**, **12e-f** and **16e-f** (0.25 mmol) in dry CH_2Cl_2 (2.5 ml) at 0 °C was added dropwise trifluoroacetic anhydride (0.5 mmol) and the mixture was gradually warm to room temperature and stirred for 6hrs. After completion of reaction solvent was evaporated under reduced pressure and the residue thus obtained was subjected for purification by preparative HPLC method as described earlier in section **5.1.3.-purification**.

5.1.4.1. (S)-4-Cyano-N-(3-(2-cyanopyrrolidin-1-yl)-3-oxopropyl)benzamide (17a)



17a (260 mg, 85%) was prepared by means of the general procedure described in **5.1.4.** as a white solid. 84-86 °C; Purity by HPLC: 99.83% AUC.

ESI/MS (m/z) : 297.1 $(M+H)^+$. **Mol. Wt.** = 296.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.17-2.19 (m, 2H), 2.24- 2.28 (m, 2H), 2.73-2.77 (m, 2H), 3.55-3.62 (m, 1H), 3.63-3.68 (m, 2H), 3.71-3.76 (m, 1H), 4.74-4.87 (dd, 1H, J_1 = 5.0Hz, J_2 = 7.6Hz), 7.71 (d, 2H, J = 8.4Hz), 8.02 (d, 2H, J = 8.4Hz).

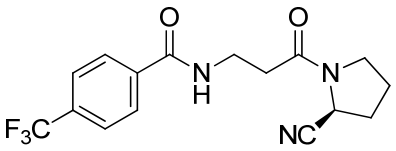
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.8, 23.1, 34.3, 39.2, 42.2, 43.6, 116.1, 116.4, 117.9, 127.9, 133.2, 137.7, 167.6, 177.4.

Analysis : Mol. Formula: $C_{16}H_{16}N_4O_2$

Calcd.: C 64.85, H 5.44, N 18.91.

Found: C 64.87, H 5.43, N 18.93.

5.1.4.2. (S)-N-(3-(2-Cyanopyrrolidin-1-yl)-3-oxopropyl)-4-(trifluoromethyl) benzamide (17b)



17b (280 mg, 81%) was prepared by means of the general procedure described in **5.1.4.** as a white solid. 77-79 °C; Purity by HPLC: 99.74% AUC.

ESI/MS (m/z) : 340.2 $(M+H)^+$. **Mol. Wt.** = 339.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.14-2.16 (m, 2H), 2.23- 2.26 (m, 2H), 2.71-2.76 (m, 2H), 3.54-3.59 (m, 1H), 3.64-3.67 (m, 2H), 3.69-3.73 (m, 1H), 4.74-4.87 (dd, 1H, J_1 = 5.2Hz, J_2 = 7.8Hz), 7.68 (d, 2H, J = 8.9Hz), 7.89 (d, 2H, J = 8.9Hz).

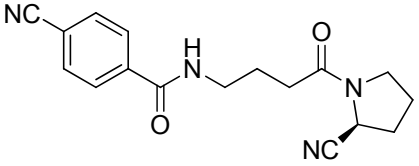
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.5, 23.2, 34.8, 38.9, 42.4, 43.7, 118.1, 119.4, 125.6 (q, J = 268.4Hz), 127.3, 134.5 (q, J = 26.7Hz), 137.0, 167.3, 177.8.

Analysis : Mol. Formula: $C_{16}H_{16}F_3N_3O_2$

Calcd.: C 56.64, H 4.75, N 12.38.

Found: C 56.60, H 4.73, N 12.35.

5.1.4.3. (S)-4-Cyano-N-(4-(2-cyanopyrrolidin-1-yl)-4-oxobutyl)benzamide (17c)



17c (284 mg, 81%) was prepared by means of the general procedure described in **5.1.4.** as a white solid. 97-99 °C; Purity by HPLC: 99.56% AUC.

ESI/MS (m/z) : 311.3 $(M+H)^+$. **Mol. Wt.** = 310.4 g.

^{1}H NMR (400 MHz, Methanol-d$_4$): δ = 1.71-1.76 (m, 2H), 1.88- 2.01 (m, 2H), 2.11-2.15 (m, 2H), 2.35-2.39 (m, 2H), 3.23-3.31 (m, 2H), 3.42-3.45 (m, 1H), 3.57-3.64 (m, 1H), 4.71-4.73 (dd, 1H, J_1 = 4.2Hz, J_2 = 7.6Hz), 7.70 (d, 2H, J = 8.4Hz), 7.99 (d, 2H, J = 8.4Hz).

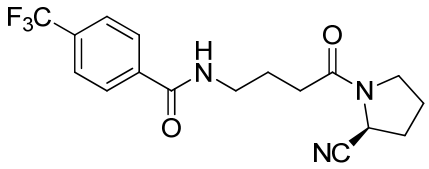
^{13}C NMR (100 MHz, Methanol-d$_4$): δ 19.9, 23.1, 27.8, 29.2, 42.2, 42.7, 43.2, 116.2, 116.7, 117.8, 127.9, 133.3, 137.6, 167.9, 176.6.

Analysis : Mol. Formula: $C_{17}H_{18}N_4O_2$

Calcd.: C 65.79, H 5.85, N 18.05.

Found: C 65.76, H 5.85, N 18.04.

5.1.4.4. (S)-N-(4-(2-Cyanopyrrolidin-1-yl)-4-oxobutyl)-4-(trifluoromethyl)benzamide (17d)



17d (278 mg, 79%) was prepared by means of the general procedure described in **5.1.4.** as a white solid. 82-84 °C; Purity by HPLC: 99.27% AUC.

ESI/MS (m/z) : 354.2 $(M+H)^+$. **Mol. Wt.** = 353.3 g.

^{1}H NMR (400 MHz, Methanol-d$_4$): δ = 1.73-1.77 (m, 2H), 1.89- 2.03 (m, 2H), 2.10-2.13 (m, 2H), 2.34-2.39 (m, 2H), 3.23-3.30 (m, 2H), 3.41-3.45 (m, 1H), 3.56-3.63 (m, 1H), 4.70-4.73 (dd, 1H, J_1 = 4.0Hz, J_2 = 7.6Hz), 7.67 (d, 2H, J = 8.6Hz), 7.96 (d, 2H, J = 8.6Hz).

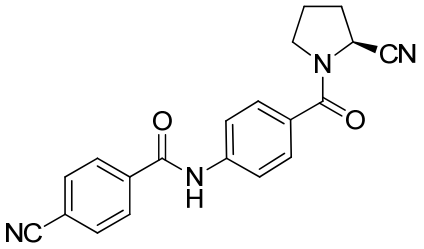
^{13}C NMR (100 MHz, Methanol-d$_4$): δ 19.8, 22.9, 27.9, 29.0, 42.2, 42.9, 43.4, 118.0, 119.5, 125.8 (q, J = 269.2Hz), 127.8, 133.8 (q, J = 28.4Hz), 137.0, 167.8, 176.4.

Analysis : Mol. Formula: $C_{17}H_{18}F_3N_3O_2$

Calcd.: C 57.79, H 5.13, N 11.89.

Found: C 57.77, H 5.10, N 11.85.

5.1.4.5. (S)-4-Cyano-N-(4-(2-cyanopyrrolidine-1-carbonyl)phenyl)benzamide (18a)



18a (211 mg, 85%) was prepared by means of the general procedure described in **5.1.4.** as a white solid. 109-111 °C; Purity by HPLC: 99.68% AUC.

ESI/MS (m/z) : 345.6 $(M+H)^+$. **Mol. Wt.** = 344.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ 1.96-2.03 (m, 2H), 2.14-2.23 (m, 1H), 2.27-2.35 (m, 1H), 3.50-3.55 (m, 1H), 3.62-3.68 (m, 1H), 4.84-4.89 (dd, 1H, J = 5.0Hz, J = 7.8Hz), 7.69 (d, 2H, J = 8.4Hz), 7.73 (d, 2H, J = 8.6Hz), 7.82 (d, 2H, J = 8.4Hz), 7.98 (d, 2H, J = 8.6Hz).

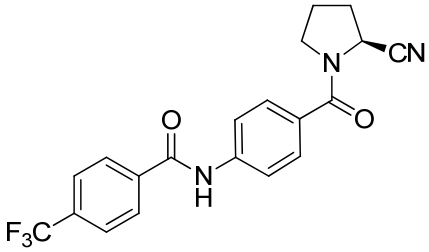
^{13}C NMR (100 MHz, Methanol-d_4): δ 19.8, 22.9, 42.7, 43.9, 116.0, 116.4, 117.8, 120.3, 128.1, 128.3, 130.1, 131.9, 136.8, 139.3, 165.2, 169.6.

Analysis : Mol. Formula: $C_{20}H_{16}N_4O_2$

Calcd.: C 69.76, H 4.68, N 16.27.

Found: C 69.73, H 4.65, N 16.29.

5.1.4.6. (S)-N-(4-(2-Cyanopyrrolidine-1-carbonyl)phenyl)-4-(trifluoromethyl) benzamide (18b)



18a (224 mg, 83%) was prepared by means of the general procedure described in **5.1.4.** as a white solid. 132-134 °C; Purity by HPLC: 99.36% AUC.

ESI/MS (m/z) : 388.2 $(M+H)^+$. **Mol. Wt.** = 387.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ 1.95-2.01 (m, 2H), 2.13-2.21 (m, 1H), 2.27-2.36 (m, 1H), 3.49-3.55 (m, 1H), 3.61-3.67 (m, 1H), 4.85-4.89 (dd, 1H, J = 5.2Hz, J = 7.8Hz), 7.65 (d, 2H, J = 8.4Hz), 7.69 (d, 2H, J = 8.8Hz), 7.82 (d, 2H, J = 8.4Hz), 7.89 (d, 2H, J = 8.8Hz).

^{13}C NMR (100 MHz, Methanol-d_4): δ 19.9, 23.1, 42.8, 43.9, 117.9, 119.5, 120.3, 125.5 (q, J = 270.8Hz), 127.9, 128.0, 130.2, 134.8 (q, J = 29.3Hz), 136.7, 139.4, 165.4, 169.8.

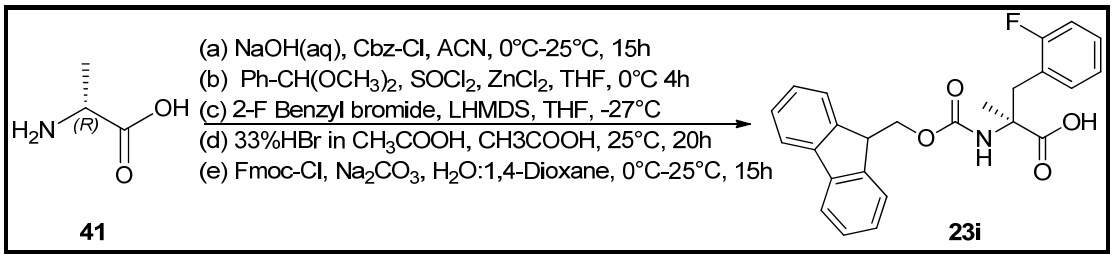
Analysis : Mol. Formula: $C_{20}H_{16}F_3N_3O_2$

Calcd.: C 62.01, H 4.16, N 10.85.

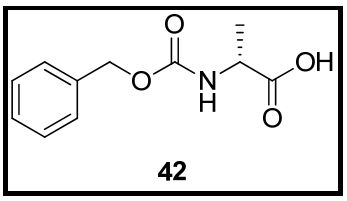
Found: C 61.97, H 4.15, N 10.83.

5.1.5. Experimental Details : Peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities (Second series)

5.1.5.1. (S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-fluorophenyl)-2-methyl propanoic acid (Fmoc-αMe-2F-Phe-OH)(23i)



Step a: (R)-2-(((Benzyloxy)carbonyl)amino)propanoic acid (42)



In a 500ml R.B. Flask was placed D-alanine **41** (10 g, 112 mmol), to it added 112.5 ml 2N NaOH (8.98 g, 224 mmol) solution at 0°C-5°C, (clear solution obtained.) followed by acetonitrile (100 ml) and stirred at 0°C-5°C for 10 min. Charged to this content 57.5 ml of 50% benzylchloroformate solution in toluene (28.7 g, 168 mmol) maintaining temperature 0°C-5°C over a period 30min. Reaction mixture was then slowly brought to room temperature and stirred for 15h.

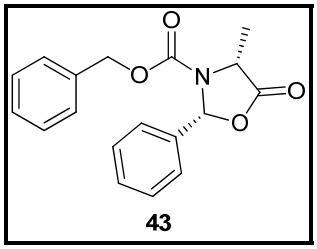
After completion of reaction (TLC), solvent of the reaction mixture was evaporated under reduced pressure and diluted with D. M. water (150 ml). This aqueous layer was extracted with ether (2X100 ml) to remove impurities. Aqueous layer was then acidified to pH 2 with 2N HCl and extracted with ethyl acetate (3X100 ml). Combined organic layers were washed with water and brine solution, dried over anhy. Na_2SO_4, filtered and evaporated to dryness. Crude residue thus obtain was purified by column chromatography using 100-200 mesh silica as a stationary phase and 0-30% ethyl

acetate in n-Hexane as mobile phase. Pure fractions were collected, combined and evaporated to dryness to give 18 g (72% yield) of the desired product Cbz-D-alanine **42** as a white powder. Mp: 83-84 ^{o}C, Purity by HPLC: 97.6% AUC.

ESI/MS (m/z) : 3 $(M+H)^{+}$. **Mol. Wt.** = 223.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (d, 3H, J = 7.35Hz), 4.45 (m, 1H), 5.12 (s, 2H), 5.32 (d, 1H, J = 7.26Hz, -N**H**), 7.32-7.35 (m, 5H), 7.98 (bs, 1H, -COO**H**).

Step b: (2R,4R)-Benzyl 4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (43)



43

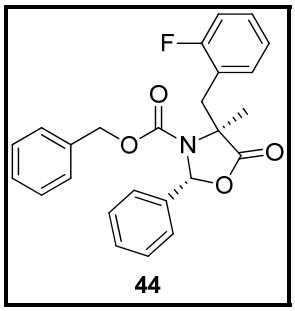
In a 250 ml 3-neck R. B. Flask was placed Cbz-D-Ala-OH **42** (18 g, 80.7 mmol) prepared in **step a**, and was dissolved in dry THF (135 ml) under nitrogen atmosphere. To the clear solution obtained was added 12.1 ml benzaldehyde dimethylacetal (12.26 g, 80.7 mmol) and cooled to 0^{o}C-5^{o}C using ice-bath. To this reaction mixture was charged dropwise $SOCl_2$ (5.85 ml, 80.7 mmol) maintaining temperature 0^{o}C-5^{o}C. After 5 min. charged anhy. $ZnCl_2$ (11.0 g, 80.7 mmol) portionwise.(Observation: colourless clear reaction mixture turned light yellow clear solution) Reaction mixture was stirred at this temperature for 3h then again charged sequentially $SOCl_2$ (1.2 ml, 16.2 mmol) and $ZnCl_2$ (2.2 g, 16.2 mmol) and stirred for additional 1h at 0^{o}C-5^{o}C.

After completion of reaction, reaction mixture was quenched with D. M. water (100 ml) in such a way that temperature should not rise above 10^{o}C. Compound was extracted with ether (3X100 ml), washed with water (100 ml) till became neutral to pH, washed with sat. $NaHCO_3$ (1X100 ml) and brine (1X100 ml). Organic layer was dried over anhy. Na_2SO_4, and evaporated to dryness, residue obtained was titurated with n-hexane (2X50 ml), solid precipitated, solvent was decanted and dried the solid under reduced pressure to give 17.07 g (68% yield) of (2R,4R)-benzyl 4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate **43** as a white solid. The product was used as such in the next reaction step without any further purification. Purity by HPLC: 89.4% AUC.

ESI/MS (m/z) : 312.2 $(M+H)^{+}$. **Mol. Wt.** = 311.3 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58 (d, 3H, J = 6.9Hz), 4.49 (q, 1H, J = 6.9Hz), 5.15 (s, 2H), 6.64 (s, 1H), 7.25-7.40 (m, 10H).

Step c: (2R,4S)-Benzyl 4-(2-fluorobenzyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (44)



44

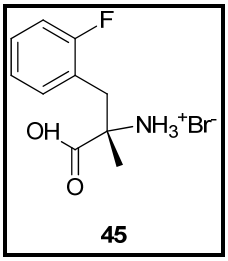
In a 250 ml R. B. Flask equipped with nitrogen inlet charged dry THF (170 ml) through septum and was cooled to -27 ^{o}C. To this added 115 ml solution of 0.5 M KHMDS in toluene (11.45 g, 57.4 mmol) at -27 ^{o}C. To this reaction mixture was charged dropwise premixed solution of oxazolidinone **43** (17 g, 54.6 mmol) and 2-fluoro benzyl bromide (6.6 ml, 54.6 mmol) dissolved in 35 ml of dry THF over a period of 30 min. maintaining temperature -30 ^{o}C to -28 ^{o}C. Stirred the reaction mixture at this temperature for 1h and then at room temperature for 1h. Reaction was monitored by TLC.

After completion of the reaction, the reaction mixture was poured in to an ice cold sat. $NaHCO_3$ solution (250 ml), extracted with ether (3X150 ml), washed the organic layer with water (1X150 ml) and brine (1X150 ml). Solvent was evaporated to dryness after drying over anhy. Na_2SO_4 to give 21.3 g (92% yield) of (2R,4S)-benzyl 4-(2-fluorobenzyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate **44** as a light yellow coloured thick oil. Purity by HPLC: 83.2% AUC.

ESI/MS (m/z) : 420.1 $(M+H)^+$, 443.8 $(M+Na)^+$ **Mol. Wt.** = 419.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 (s, 1.5H), 2.02 (s, 1.5H), 2.98 (d, 0.5H, J = 13.95Hz), 3.09 (d, 0.5H, J = 13.86Hz), 3.66 (d, 0.5H, J = 13.98Hz), 3.91 (d, 0.5H, J = 13.89Hz), 5.15 (s, 1H), 5.26 (s, 1H), 5.53 (s, 0.5H), 5.60 (s, 0.5H), 6.80 (d, 1H, J = 7.02Hz), 7.09-7.78 (m, 13H).

Step d: (S)-2-Amino-3-(2-fluorophenyl)-2-methylpropanoic acid hydrobromide (45)



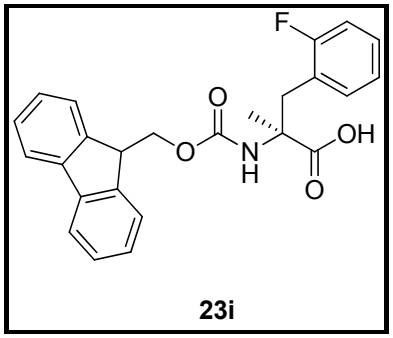
45

21 g, (50.1 mmol) of (2R,4S)-benzyl 4-(2-fluorobenzyl)-4-methyl-5-oxo-2-phenyl oxazolidine-3-carboxylate **44** prepared in above step was placed in single neck 100 ml R. B. Flask, to it added glacial acetic acid (42 ml) and stirred at room temperature for 15 min. To this reaction mixture was added 26 ml 33% HBr in acetic acid (9.22 g, 115.2 mmol) and stirred at room temperature for 20h. Acetic acid from the reaction mixture was distilled off and to the residue left was added D. M. water (168 ml) followed by addition of 2M $HBr_{(aq)}$ (32 ml, 62.6 mmol). This reaction content was extracted with ether (3X100 ml) to remove impurities. Aqueous layer was then evaporated to dryness and residue obtained was triturated with dry ether (3X100 ml) to give 10 g (72% yield) of (S)-2-amino-3-(2-fluorophenyl)-2-methylpropanoic acid hydrobromide **45** as a cream coloured crystalline solid. Mp: 248 ^{o}C dec., Purity by HPLC: 96.2% AUC.

ESI/MS (m/z) : 198.3 $(M+H)^+$. **Mol. Wt. =** 197.2 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 1.44 (s, 3H), 3.15 (m, 2H), 7.04-728 (m, 4H), 8.33 (bs, 3H, -N**H**$_3^+$), 13.62 (bs, 1H, -COO**H**).

Step d: (S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-fluorophenyl)-2-methyl propanoic acid (23i)



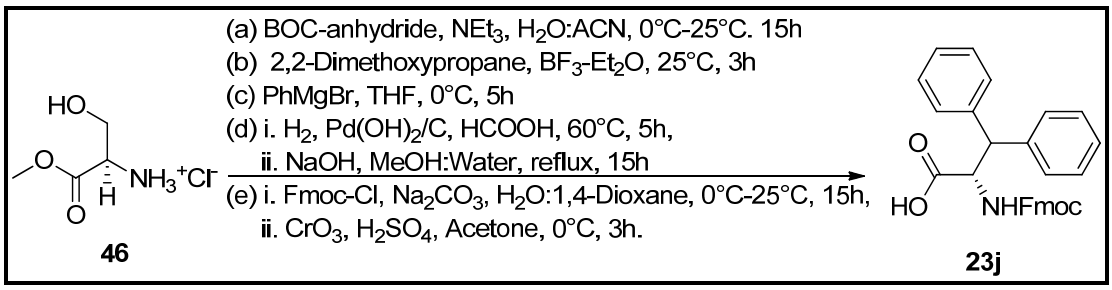
23i

(S)-2-Amino-3-(2-fluorophenyl)-2-methylpropanoic acid hydrobromide **45** was converted to its Fmoc derivative **23i** with 87% yield by using method reported for the synthesis of compound **4** as described in **section 5.1.2.2.1.** as a white fluffy solid. Mp: 187-189 ^{o}C, Purity by HPLC: 99.3% AUC, 98.45% ee., Specific optical rotation (SOR): -14.48^{o} (C=1% in $CHCl_3$ at 25 ^{o}C)

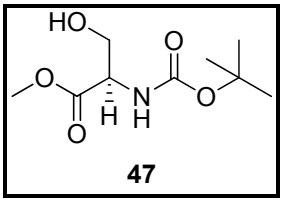
ESI/MS (m/z) : 420.3 $(M+H)^+$. **Mol. Wt. =** 419.4 g.

^{1}H NMR (400 MHz, CDCl$_3$): δ 1.60 (bs, 3H), 3.35-3.43 (m, 2H), 4.24 (t, 1H, J = 4.95Hz), 4.42 (bs, 2H), 5.38 (s, 1H, -N**H**), 6.97-7.00 (m, 3H), 7.05-7.18 (m, 1H), 7.19-7.31 (m, 2H), 7.39 (t, 2H, J = 5.4Hz), 7.57 (t, 2H, J = 6.3Hz), 7.76 (d, 2H, J = 5.4Hz), 8.70 (bs, 1H, -COO**H**).

5.1.6. (S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3,3-diphenylpropanoic acid (Fmoc-βPPA-OH)(23j)



Step a: (R)-Methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate (47)



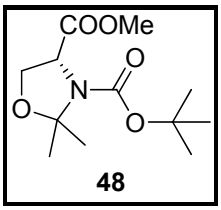
In a single neck R. B. Flash was placed 10 g (64.3 mmol) of D-serine methyl ester hydrochloride **46**, acetonitrile (100 ml) and D. M. water (100 ml). To the suspension formed was added triethylamine (22.4 ml, 160.75 mmol) at room temperature. The reaction mixture was stirred for 30 min. and then cool to 0°C-5°C and charged to it BOC-anhydride (17.8 ml, 77.4 mmol) dropwise over a period of 45 min. Reaction mixture was then brought to room temperature and stirred for 15h.

After completion of reaction (TLC), solvent was removed under reduced pressure, followed by addition of ethyl acetate (150 ml), D. M. Water (150 ml), layers were separated and organic layer was washed with water (1X100 ml) and brine (1X100 ml). Organic layer was dried over anhy. Na_2SO_4 and evaporated to dryness. Crude residue thus obtained was purified by column chromatography using 100-200 mesh silica as stationary phase and 0-20% ethyl acetate in n-hexane as an eluting system. Pure fractions were collected and evaporated to dryness to give 11.86 g (84% yield) of (R)-methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate **47** as a colourless viscous liquid.

ESI/MS (m/z) : 220.3 $(M+H)^+$. **Mol. Wt.** = 219.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (s, 9H), 3.63 (s, 3H), 3.84 (dd, 1H, J1 = 3.2Hz, J2 = 11.2Hz), 4.05 (m, 1H), 4.32-4.39 (m, 1H), 5.91 (bs, 1H, -N**H**), 6.65 (bs, 1H, -O**H**).

Step b: (R)-3-tert-Butyl 4-methyl-2,2-dimethyloxazolidine-3,4-dicarboxylate (48)



48

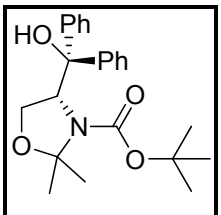
11 g (50.2 mmol) of product prepared in above step (R)-methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate **47** was placed in a two neck R. B. Flask and was dissolved in acetone (160 ml 2,2-dimethoxy propane (50 ml) and catalytic amount of $BF_3{\cdot}Et_2O$ (0.4 ml) were added at room temperature. Reaction mixture changed from colourless to dark yellow clear solution. Reaction was stirred at room temperature for 3h. Reaction was monitored by TLC spotting.

After completion of reaction (TLC), triethylamine (1.0 ml) was added to quench the reaction, solvent was then evaporated to dryness. Crude residue thus obtained was purified by column chromatography (100-200 mesh silica gel, 0-30% Ethyl acetate in n-Hexane) to give 10.2 g (78% yield) of the title compound **48** as a colourless thick oil.

ESI/MS (m/z) : 260.5 $(M+H)^+$. **Mol. Wt. =** 259.3 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.35 (s, 9H), 1.51 (s, 6H), 3.67 (s, 3H), 4.03-4.07 (m, 2H), 4.35-4.39 (m, 1H).

Step c: (R)-tert-Butyl 4-(hydroxydiphenylmethyl)-2,2-dimethyloxazolidine-3-carboxylate (49)



49

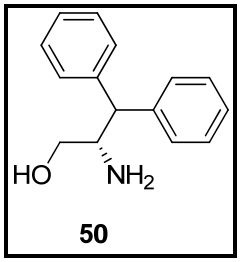
In a 500 ml 3-neck R. B. Flask were placed Magnesium metal (5.09 g, 212.3 mmol), dry THF (250 ml) and a small crystal of iodine. To this content bromobenzene (33.3 g, 193 mmol) was added dropwise with gentle heating of the R. B. flask. The reaction got initiated, remaining amount of bromobenzene was added cautiously so as to control the rate. After the Mg metal gets consumed, reaction content was stirred for additional 1h at room temperature. To the freshly prepared Grignard reagent was added 10 g (38.61 mmol) of the (R)-3-tert-butyl-4-methyl-2,2-dimethyloxazolidine-3,4-dicarboxylate **48**

dissolved in dry THF (50 ml) dropwise through pressure equalizing dropping funnel in an inert atmosphere at room temperature over a period of 30min. After completion of the addition, reaction mixture was stirred at room temperature for further 5h.

After completion of reaction, reaction mixture was quenched with sat. NH_4Cl solution (300 ml) and extracted with ethyl acetate (3X250 ml). Combined organic extracts were washed with sat. $Na_2S_2O_3$ solution (1X250 ml) and brine (1X250 ml). Organic layer was dried over anhy. Na_2SO_4 and evaporated to dryness to give 11.98 g (81% yield) of (R)-tert-butyl-4-(hydroxydiphenylmethyl)-2,2-dimethyloxazolidine-3-carboxylate **49** as a light yellow thick oil. Crude product thus obtained was used as such for the next reaction step. Purity by HPLC: 78.6% AUC.

ESI/MS (m/z) : 384.4 $(M+H)^+$. **Mol. Wt. =** 383.5 g.

Step d: (S)-2-amino-3,3-diphenylpropan-1-ol (50)



50

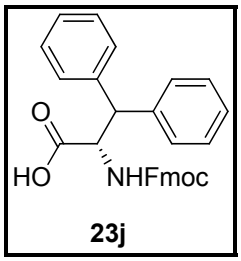
Deoxygenation of the tertiary hydroxyl group in the compound **49** was accomplished as described here. 11 g (28.7 mmol) of (R)-tert-butyl-4-(hydroxydiphenylmethyl)-2,2-dimethyloxazolidine-3-carboxylate **49** was placed in a Parr hydrogenation apparatus (preferably Autoclave) dissolved in formic acid (55 ml), to it added 20% $Pd(OH)_2$ on carbon (2.2 g, 20% by wt. of starting material) and hydrogenated at 60 ^oC under 10 bar pressure for 5h. Reaction mixture was then filtered through Hyflo supercel and evaporated to dryness. To the residue thus obtained was added NaOH (11.5 g, 287.2 mmol) dissolved in D. M. Water (125 ml), followed by addition of Methanol (125 ml), and then refluxed for 15h.

After completion of the reaction, solvent was evaporated to dryness under reduced pressure and residue was partition between ethyl acetate (200 ml) and sat. brine solution (100 ml). Layers were separated and aqueous layer was extracted repeatedly with ethyl acetate (3X100 ml). Combined organic layers were dried over anhy. Na_2SO_4 and evaporated to dryness to give 5.3 g (81% yield) of (S)-2-amino-

3,3-diphenylpropan-1-ol **50** as an off white solid. Product thus obtained was used as such for the next reaction step. Mp: 198-202 °C, Purity by HPLC: 83.6% AUC.

ESI/MS (m/z) : 228.4 $(M+H)^+$. **Mol. Wt.** = 227.3 g.

Step e: (S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3,3-diphenylpropanoic acid (23j)



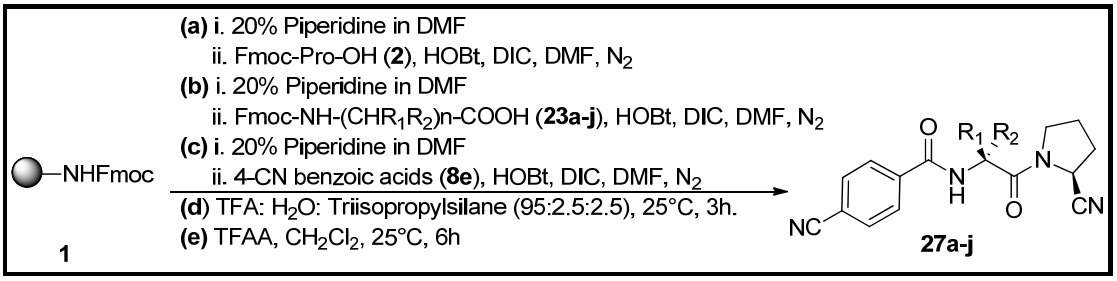
23j

23j (7.26 g) was prepared by using the method described for the synthesis of compound **4** as illustrated in **section 5.1.2.2.1** with 81% yield as a white solid. Mp: 127-128 °C, Purity by HPLC: 99.07% AUC, Specific optical rotation SOR $[\alpha]_D$= -12° (C=1% in Chloroform).

ESI/MS (m/z) : 464.3 $(M+H)^+$. **Mol. Wt.** = 463.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.24 (t, 1H, J = 6.6 Hz), 4.38 (d, 2H, J = 6.6 Hz), 4.44 (d, 1H, J = 7.3 Hz), 4.76 (d, 1H, J = 8.8 Hz), 6.93 (d, 1H, J = 7.3 Hz, -N**H**), 7.14-7.29 (m, 12H), 7.41 (t, 2H, J = 6.8 Hz), 7.64 (d, 2H, J = 7.3 Hz), 7.82 (d, 2H, J = 7.2 Hz), 12.43 (bs, 1H, -COO**H**).

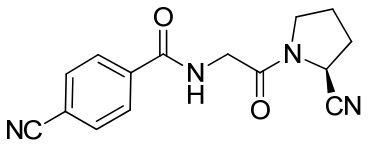
5.1.7. General procedure for the synthesis of compounds (27a-j)



The compounds **27a-j** were prepared using Fmoc based Solid Phase Peptide Synthesis (SPPS) approach using the practical methodology described for the synthesis of compounds **11a-h**, **12a-h** and **16a-h** as illustrated in experimental **section 5.1.3.** The crude carboxamides were obtained after global cleavage from the peptidyl resin were then transformed to respective nitrile derivative by dehydration using the method

describe for the synthesis of compounds **17a-d** and **18a-b** as illustrated in experimental **section 5.1.4.** and purified by the method described in **section 5.1.3.-Purification** to give desired compounds **27a-j**.

5.1.7.1 (S)-4-Cyano-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)benzamide (27a)



27a (230 mg, 81%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 159-161 °C; Purity by HPLC: 97.65% AUC.

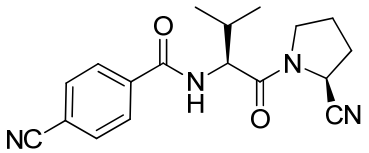
ESI/MS (m/z) : 283.1 $(M+H)^+$. **Mol. Wt.** = 282.3 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.76-1.94 (m, 3H), 2.09- 2.14 (m, 1H), 3.28-3.39 (m, 1H), 3.41-3.53 (m, 1H), 4.42 (d, 2H, J = 8.8Hz), 4.86 (dd, 1H, J_1 = 4.2Hz, J_2 = 7.6Hz), 8.12 (d, 2H, J = 8.4Hz), 8.28 (d, 2H, J = 8.4Hz), 8.72 (t, 1H, J = 8.8Hz, -NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ 22.8, 30.2, 41.6, 48.2, 50.1, 116.9, 117.2, 119.3, 126.7, 128.6, 138.4, 166.8, 167.2.

Analysis : Mol. Formula: $C_{15}H_{14}N_4O_2$
Calcd.: C 63.82, H 5.00, N 19.85.
Found: C 63.85, H 5.01, N 19.81.

5.1.7.2 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (27b)



27b (223 mg, 79%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 146-148 °C; Purity by HPLC: 98.21% AUC.

ESI/MS (m/z) : 325.3 $(M+H)^+$. **Mol. Wt.** = 324.2 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.943 (d, 3H, J = 6.4Hz), 0.991 (d, 3H, J = 6.4Hz), 1.77-1.85 (m, 2H), 1.92-2.03 (m, 2H), 2.12-2.18 (m, 1H), 3.62-3.66 (m, 1H), 3.84-3.89

(m, 1H), 4.43 (t, 1H, J = 8.6Hz), 4.89 (dd, 1H, J_1 = 4.4Hz, J_2 = 8.4Hz), 7.98 (d, 2H, J = 8.6Hz), 8.18 (d, 2H, J = 8.6Hz), 8.53 (d, 1H, J = 8.4Hz, -NH).

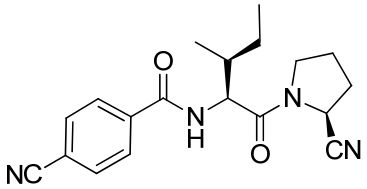
^{13}C NMR (100 MHz, DMSO-d_6): δ 18.5, 18.7, 22.4, 30.4, 32.1, 48.1, 50.3, 60.8, 116.7, 116.9, 117.8, 127.2, 127.9, 137.8, 167.3, 173.1.

Analysis : Mol. Formula: $C_{18}H_{20}N_4O_2$

Calcd.: C 66.65, H 6.21, N 17.27.

Found: C 66.62, H 6.19, N 17.23.

5.1.7.3. 4-Cyano-N-((2S,3S)-1-((S)-2-cyanopyrrolidin-1-yl)-3-methyl-1-oxopentan-2-yl) benzamide (27c)



27c (231 mg, 84%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 183-185 °C; Purity by HPLC: 99.41% AUC.

ESI/MS (m/z) : 339.1 $(M+H)^+$. **Mol. Wt.** = 338.2 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.825 (t, 3H, J = 7.4Hz), 0.955 (d, 3H, J = 6.8Hz), 1.09-1.21 (m, 1H), 1.52-1.63 (m, 1H), 1.77-1.85 (m, 2H), 1.93-2.05 (m, 3H), 3.61-3.65 (m, 1H), 3.87-3.93 (m, 1H), 4.49 (dd, 1H, J_1 = 8.0Hz, J_2 = 9.6Hz), 4.91 (dd, 1H, J_1 = 4.4Hz, J_2 = 8.0Hz), 8.02 (d, 2H, J = 8.4Hz), 8.17 (d, 2H, J = 8.4Hz), 8.58 (d, 1H, J = 8.0Hz, -NH).

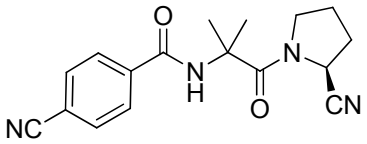
^{13}C NMR (100 MHz, DMSO-d_6): δ 10.7, 15.4, 22.6, 24.5, 31.1, 38.2, 48.3, 50.7, 58.3, 116.9, 117.2, 117.8, 127.2, 131.9, 138.3, 167.4, 172.9.

Analysis : Mol. Formula: $C_{19}H_{22}N_4O_2$

Calcd.: C 67.44, H 6.55, N 16.56.

Found: C 67.45, H 6.52, N 16.54.

5.1.7.4. (S)-4-Cyano-N-(1-(2-cyanopyrrolidin-1-yl)-2-methyl-1-oxopropan-2-yl) benzamide (27d)



27d (198 mg, 78%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 165-167 °C; Purity by HPLC: 97.87% AUC.

ESI/MS (m/z) : 311.2 $(M+H)^+$. **Mol. Wt.** = 310.1 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.41 (s, 3H), 1.45 (s, 3H), 1.67-1.91 (m, 3H), 2.01-2.09 (m, 1H), 3.49-3.53 (m, 1H), 3.55-3.72 (m, 1H), 4.86 (dd, 1H, J_1 = 5.6Hz, J_2 = 8.4Hz), 8.07 (d, 2H, J = 8.6Hz), 8.19 (d, 2H, J = 8.6Hz), 9.21 (s, 1H, -NH).

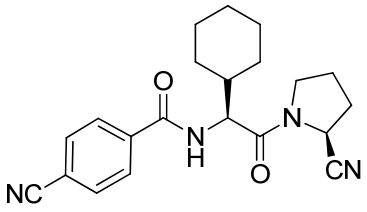
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.6, 26.7, 30.9, 48.5, 50.6, 67.3, 116.8, 117.1, 117.6, 127.6, 132.1, 138.7, 167.2, 172.9.

Analysis : Mol. Formula: $C_{17}H_{18}N_4O_2$

Calcd.: C 65.79, H 5.85, N 18.05.

Found: C 65.76, H 5.87, N 18.04.

5.1.7.5. 4-Cyano-N-((S)-2-((S)-2-cyanopyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl) benzamide (27e)



27e (174 mg, 83%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 143-145 °C; Purity by HPLC: 97.25% AUC.

ESI/MS (m/z) : 365.1 $(M+H)^+$. **Mol. Wt.** = 364.4 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.17-1.24 (m, 10H), 1.53-1.65 (m, 1H), 1.74-1.91 (m, 2H), 2.28-2.42 (m, 2H), 3.43-3.55 (m, 1H), 3.62-3.67 (m, 1H), 4.46 (dd, 1H, J_1 = 8.2Hz, J_2 = 9.2Hz), 4.92 (dd, 1H, J_1 = 4.8Hz, J_2 = 8.2Hz), 7.97 (d, 2H, J = 8.4Hz), 8.13 (d, 2H, J = 8.4Hz), 9.18 (d, 1H, J = 8.2Hz, -NH).

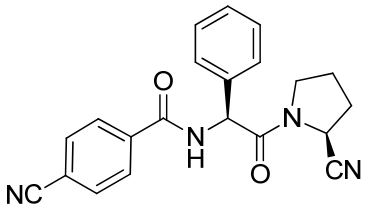
^{13}C NMR (100 MHz, DMSO-d_6): δ 21.8, 25.3, 25.4, 26.7, 27.9, 30.7, 32.8, 48.3, 50.7, 56.4, 116.9, 117.7, 118.3, 127.4, 132.3, 138.5, 167.4, 172.5.

Analysis : Mol. Formula: $C_{21}H_{24}N_4O_2$

Calcd.: C 69.21, H 6.64, N 15.24.

Found: C 69.23, H 6.60, N 15.21.

5.1.7.6. 4-Cyano-N-((S)-2-((S)-2-cyanopyrrolidin-1-yl)-2-oxo-1-phenylethyl) benzamide (27f)



27f (178 mg, 80%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 182-184 °C; Purity by HPLC: 99.08% AUC.

ESI/MS (m/z) : 359.2 $(M+H)^+$. **Mol. Wt.** = 358.4 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.68-1.93 (m, 3H), 2.01-2.11 (m, 1H), 3.41-3.53 (m, 1H), 3.62-3.65 (m, 1H), 4.92 (dd, 1H, J_1 = 5.2Hz, J_2 = 8.0Hz), 5.21 (d, 1H, J = 8.2Hz), 7.21-7.30 (m, 5H), 8.04 (d, 2H, J = 8.4Hz), 8.19 (d, 2H, J = 8.4Hz), 8.96 (d, 1H, J = 8.2Hz, -NH).

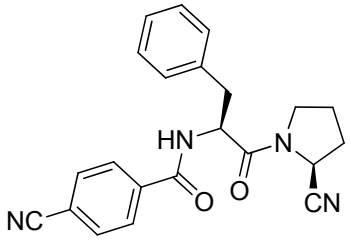
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.5, 31.2, 48.7, 50.4, 59,2, 116.8, 117.4, 118.6, 126.2, 127.3, 127.4, 128.8, 129.3, 132.3, 136.3, 138.5, 167.4, 172.7.

Analysis : Mol. Formula: $C_{21}H_{18}N_4O_2$

Calcd.: C 70.38, H 5.06, N 15.63.

Found: C 70.35, H 5.04, N 15.59.

5.1.7.7. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl) benzamide (27g)



27g (164 mg, 77%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 184-186 °C; Purity by HPLC: 98.53% AUC.

ESI/MS (m/z) : 373.5 $(M+H)^+$. **Mol. Wt.** = 372.4 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.67-1.93 (m, 3H), 2.01-2.08 (m, 1H), 2.98 (dd, 1H, J_1 = 8.0Hz, J_2 = 13.2Hz), 3.06 (dd, 1H, J_1 = 7.2Hz, J_2 = 13.2Hz), 3.37-3.46 (m, 1H), 3.58-3.63 (m, 1H), 4.74-4.78 (m, 1H), 4.87 (dd, 1H, J_1 = 4.8Hz, J_2 = 8.0Hz), 7.23-7.34 (m, 5H), 8.02 (d, 2H, J = 8.6Hz), 8.17 (d, 2H, J = 8.6Hz), 8.96 (d, 1H, J = 8.2Hz, -NH).

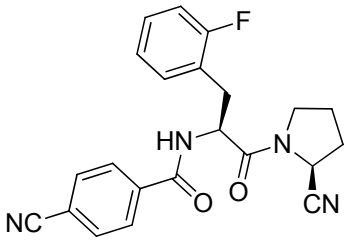
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.3, 31.4, 37.9, 48.7, 50.4, 56,2, 117.0, 117.6, 118.5, 126.2, 127.4, 127.6, 128.8, 129.1, 132.1, 136.3, 138.6, 167.4, 173.9.

Analysis : Mol. Formula: $C_{22}H_{20}N_4O_2$

Calcd.: C 70.95, H 5.41, N 15.04.

Found: C 70.92, H 5.39, N 15.01.

5.1.7.8. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-3-(2-fluorophenyl)-1-oxopropan-2-yl)benzamide (27h)



27h (221 mg, 85%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 208-209 °C; Purity by HPLC: 99.27% AUC.

ESI/MS (m/z) : 391.2 $(M+H)^+$. **Mol. Wt.** = 390.4 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.78-1.81 (m, 1H), 1.83-1.97 (m, 1H), 2.02-2.15 (m, 2H), 2.96 (dd, 1H, J_1 = 7.8Hz, J_2 = 13.2Hz), 3.08 (dd, 1H, J_1 = 7.2Hz, J_2 = 13.2Hz), 3.39-3.46 (m, 1H), 3.53-3.64 (m, 1H), 4.75-4.78 (m, 1H), 4.92 (dd, 1H, J_1 = 5.2Hz, J_2 = 8.2Hz), 6.98-7.13 (m, 3H), 7.19-7.26 (m, 1H), 7.92 (d, 2H, J = 8.2Hz), 8.11 (d, 2H, J = 8.2Hz), 9.01 (d, 1H, J = 8.0Hz, -NH).

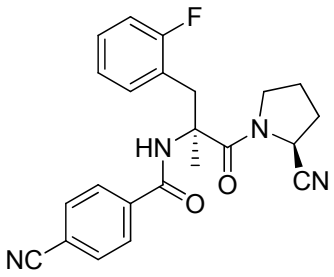
^{13}C NMR (100 MHz, DMSO-d_6): δ 21.9, 30.6, 31.2, 48.6, 50.3, 56,2, 115.4 (d, J = 31Hz), 117.0, 117.6, 118.5, 125.3, 126.1, 127.7(d, J = 14Hz), 128.2, 128.7, 129.1, 132.1, 132.7, 138.7, 162.4 (d, J= 243Hz), 167.3, 173.2.

Analysis : Mol. Formula: $C_{22}H_{19}FN_4O_2$

Calcd.: C 67.68, H 4.91, N 14.35.

Found: C 67.63, H 4.93, N 14.32.

5.1.7.9. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-3-(2-fluorophenyl)-2-methyl-1-oxopropan-2-yl)benzamide (27i)



27i (179 mg, 77%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 173-175 °C; Purity by HPLC: 98.42% AUC.

ESI/MS (m/z) : 405.2 $(M+H)^+$. **Mol. Wt.** = 404.4 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.27 (s, 3H), 1.69-1.78 (m, 1H), 1.81-1.95 (m, 1H), 2.01-2.15 (m, 2H), 3.02 (d, 1H, J = 13.6Hz), 3.13 (d, 1H, J = 13.6Hz), 3.41-3.48 (m, 1H), 3.52-3.63 (m, 1H), 4.91 (dd, 1H, J_1 = 4.8Hz, J_2 = 8.0Hz), 6.96-7.10 (m, 3H), 7.22-7.27 (m, 1H), 7.98 (d, 2H, J = 8.4Hz), 8.19 (d, 2H, J = 8.4Hz), 8.76 (bs, 1H, -NH).

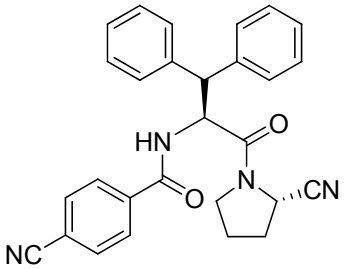
^{13}C NMR (100 MHz, DMSO-d_6): δ 21.9, 23.7, 30.4, 34.2, 48.6, 50.5, 69,2, 115.6 (d, J = 30Hz), 117.2, 117.7, 118.5, 125.3, 126.2, 127.5(d, J = 14.2Hz), 128.3, 128.7, 129.3, 132.4, 132.7, 138.7, 162.8 (d, J = 247Hz), 167.8, 172.9.

Analysis : Mol. Formula: $C_{23}H_{21}FN_4O_2$

Calcd.: C 68.30, H 5.23, N 13.85.

Found: C 68.33, H 5.19, N 13.82.

5.1.7.10. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)benzamide (27j)



27j (233 mg, 84%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 129-131 °C; Purity by HPLC: 98.65% AUC.

ESI/MS (m/z) : 449.3 $(M+H)^+$. **Mol. Wt.** = 448.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.68-1.83 (m, 3H), 1.85-1.91 (m, 1H), 3.61-3.65 (m, 1H), 3.98-4.01 (m, 1H), 4.63 (d, 1H, J = 11.6Hz), 4.87-4.92 (m, 1H), 5.74 (dd, 1H, J_1 = 8.8Hz, J_2 = 11.6Hz), 7.02-7.28 (m, 6H), 7.37-7.41 (m, 4H), 7.76 (d, 2H, J = 7.6Hz), 7.84 (d, 2H, J = 7.6Hz), 9.14 (d, 1H, J = 8.8Hz, -NH).

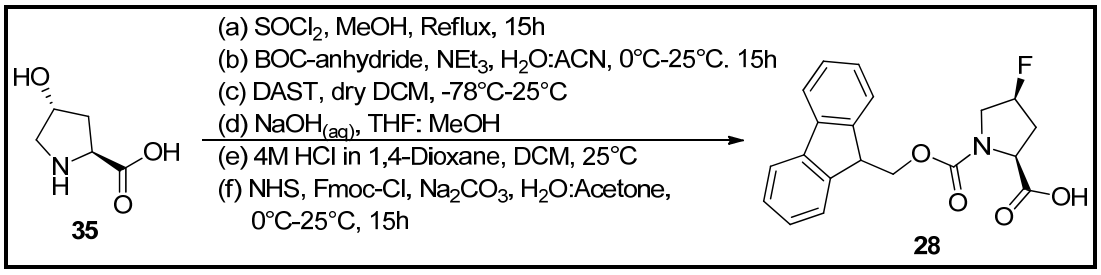
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 30.4, 42.4, 48.9, 51.3, 61.8, 116.4, 117.1, 117.6, 126.4, 126.6, 127.7, 127.8, 128.2, 128.7, 129.2, 129.3, 132.3, 137.9, 139.2, 167.6, 173.4.

Analysis : Mol. Formula: $C_{28}H_{24}N_4O_2$

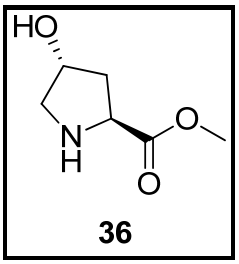
Calcd.: C 74.98, H 5.39, N 12.49.

Found: C 75.01, H 5.41, N 12.47.

5.1.8. (2S,4S)-1-(((9H-Fluoren-9-yl)methoxy)carbonyl)-4-fluoropyrrolidine-2-carboxylic acid (Fmoc-*Cis* 4F-Pro-OH) (28)



Step a: (2S,4R)-methyl 4-hydroxypyrrolidine-2-carboxylate (36)



10 g (76.33 mmol) of commercially available *trans*-4-Hydroxy proline **35** was suspended in 250 ml R. B. Flask containing dry Methanol (150 ml) and cooled to 0°C-5°C using ice-bath. $SOCl_2$ (16.6 ml, 229 mmol) was added dropwise over a period of 30min. Reaction

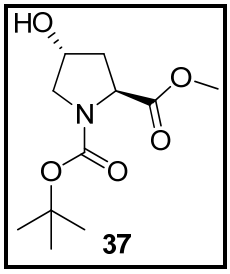
mixture was then brought to room temperature gradually and refluxed for 15h. Reaction was monitored by TLC.

After completion of the reaction, solvent was evaporated to dryness and residue obtained was triturated with n-hexane (3X150 ml) and di-isopropyl ether (3X150 ml) respectively. Crystalline solid obtained after decanting the solvent used for titration was dried well under reduced pressure using high vacuum pump to provide 9.85 g (89% Yield) hydrochloride salt of (2S,4R)-methyl 4-hydroxypyrrolidine-2-carboxylate **36** as a free flowing shiny crystalline solid. Mp: 268 °C dec.

ESI/MS (m/z) : 146.3 $(M+H)^+$. **Mol. Wt. =** 145.2 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 1.84 (bs, 1H, -O**H**), 1.98-2.19 (m, 2H), 2.73-2.78 (m, 1H), 3.02-3.23 (m, 2H), 3.47-3.51 (m, 1H), 3.66 (s, 3H), 6.93 (bs, 2H, >N**H$_2^+$**).

Step b: (2S,4R)-1-tert-Butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate (37)



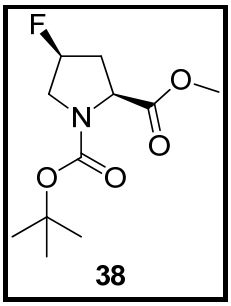
37

Titled compound **37** (11.06 g) was prepared by using the method describe for the synthesis of compound **47** as illustrated in experimental section **5.1.6. step b.** with 83% yield as a white solid. Mp: 96-97 °C, SOR $[\alpha]_D$= -65° (C=1% in Chloroform).

ESI/MS (m/z) : 246.1 $(M+H)^+$. **Mol. Wt.** = 245.3 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 1.49 (s, 9H), 1.94-2.15 (m, 2H), 2.34 (bs, 1H, -O**H**), 2.68-2.72 (m, 1H), 3.03-3.21 (m, 2H), 3.34-3.39 (m, 1H), 3.67 (s, 3H).

Step c: (2S,4S)-1-tert-Butyl 2-methyl 4-fluoropyrrolidine-1,2-dicarboxylate (38)



38

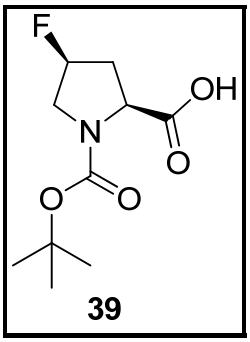
In a 2-neck R. B. Flask equipped with nitrogen inlet was charged 11 g (44.89 mmol) of product prepared in above step (2S,4R)-1-tert-butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate **37**, dissolved in dry DCM (165 ml) and cooled to -78 ^{o}C using dry ice-acetone bath. To this reaction mixture was added diethylaminosulfur trifluoride (DAST) (14.8 ml, 112.24 mmol) dropwise over a period of 45 min. Reaction mixture was then stirred at same temperature for 1h. Reaction mixture was then brought gradually to room temperature and stirred for 5h.

After completion of reaction, the mixture was poured in ice cold water (150 ml), followed by DCM (100 ml) and stirred, layers were separated and organic layer was washed with D. M. water (2X200 ml) and brine (1X100 ml). Organic layer was dried over anhy. Na_2SO_4 and evaporated to dryness. Thr residue thus obtained was purified by column chromatography to give 7.5 g (68% Yield) of (2S,4S)-1-tert-butyl 2-methyl 4-fluoropyrrolidine-1,2-dicarboxylate **38** as a light yellow oil.

ESI/MS (m/z) : 248.4 $(M+H)^+$. **Mol. Wt. =** 247.3 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.42 (s, 9H), 1.98-2.15 (m, 2H), 3.24 (m, 1H), 3.42-3.59 (m, 2H), 3.71 (s, 3H), 4.68-4.73 (m, 1H).

Step d: (2S,4S)-1-(tert-Butoxycarbonyl)-4-fluoropyrrolidine-2-carboxylic acid (39)



39

7.0 g (28.34 mmol) of (2S,4S)-1-tert-butyl 2-methyl-4-fluoropyrrolidine-1,2-dicarboxylate **38** was placed in a 150 ml 2-neck R. B. Flask, to it added methanol (35 ml), THF (35 ml) and stirred at room temperature for 5min. To the clear solution obtained was charged NaOH (1.7 g, 42.51 mmol) dissolved in D. M. Water (35 ml) at room temperature and stirred the reaction mixture for 3h.

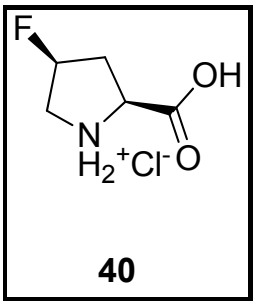
After completion of the reaction (TLC), solvent was evaporated under reduced pressure and residue thus obtained was diluted with D. M. water (125 ml). This aqueous content was extracted with ether (3X100 ml) to remove impurities if any. Aqueous layer

was then acidified to pH 4 with citric acid and extracted with ethyl acetate (3X100 ml). Combined extracts were washed successively with water (2X100 ml) and brine (1X100 ml), dried over anhy. Na_2SO_4 and concentrated under reduced pressure to give 6.47 g (98% Yield) of (2S,4S)-1-(tert-butoxycarbonyl)-4-fluoropyrrolidine-2-carboxylic acid **39** as a white wax.

ESI/MS (m/z) : 234.3 $(M+H)^+$. **Mol. Wt. =** 233.2 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 1.44 (s, 9H), 1.89-2.12 (m, 2H), 3.31-3.38 (m, 1H), 3.43-3.57 (m, 2H), 4.79-4.82 (m, 1H), 12.31 (bs, 1H, -COO**H**).

Step d: (2S,4S)-2-Carboxy-4-fluoropyrrolidin-1-ium chloride (40)



40

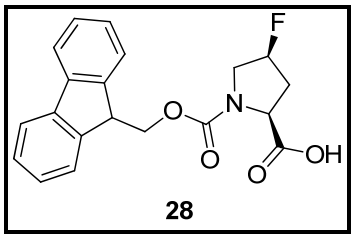
6.0 g (25.75 mmol) of (2S,4S)-1-(tert-butoxycarbonyl)-4-fluoropyrrolidine-2-carboxylic acid **39** was dissolved in DCM (72 ml) and cooled to 10 ^oC. To this reaction mixture charged in-house prepared solution of 3.2M HCl in 1,4-dioxane (ml, mmol) dropwise within 15min. Reaction mixture was then brought to room temperature and stirred for 3h.

After completion of the reaction, solvent was evaporated to dryness under reduced pressure. Residue obtained was triturated with dry ether (3X75 ml), solvent was decanted and the solid precipitated was dried well under high vacuum to give 4.3 g (99% Yield) hydrochloride salt of (2S,4S)-4-fluoropyrrolidine-2-carboxylic acid **40** as a white solid.

ESI/MS (m/z) : 134.1 $(M+H)^+$. **Mol. Wt. =** 133.2 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 2.18 (m, 1H), 2.21-2.27 (m, 1H), 3.32-3.37 (m, 1H), 3.48-3.57 (m, 2H), 4.49-4.58 (m, 1H), 7.21 (bs, 2H, -N**H$_2^+$**), 12.07 (bs, 1H, -COO**H**).

Step e: (2S,4S)-1-(((9H-Fluoren-9-yl)methoxy)carbonyl)-4-fluoropyrrolidine-2-carboxylic acid (28)



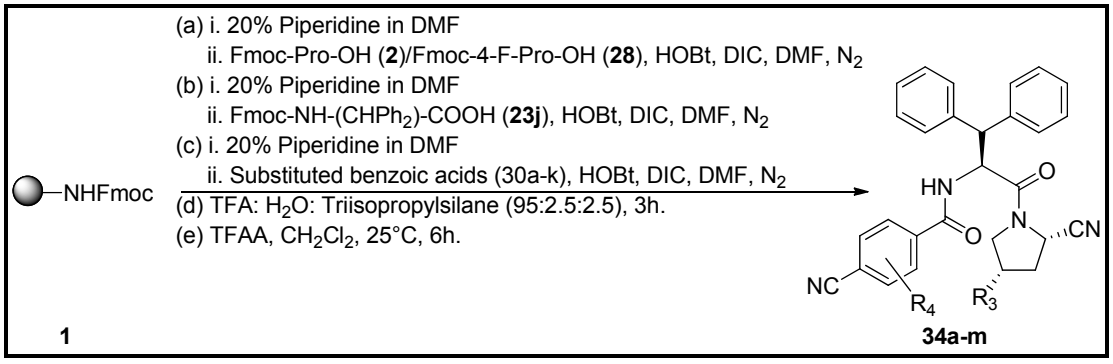
28

Compound **28** (7.22 g) was prepared according to the procedure described for the synthesis of compound **2** as illustrated in experimental **section 5.1.2.1.** with 85% yield as a white solid. Purity by HPLC: 98.37% AUC.

ESI/MS (m/z) : 356.2 $(M+H)^+$. **Mol. Wt. =** 355.4 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.89-1.94 (m, 1H), 2.21-2.26 (m, 1H), 3.31-3.36 (m, 1H), 3.48-3.57 (m, 2H), 4.43-4.56 (m, 2H), 4.92-5.01 (m, 2H), 7.34 (t, 2H, J=7.8 Hz), 7.42 (t, 2H, J=7.6 Hz), 7.59 (d, 2H, J=7.5 Hz), 7.72 (d, 2H, J=7.5 Hz), 11.94 (bs, 1H, -COO**H**).

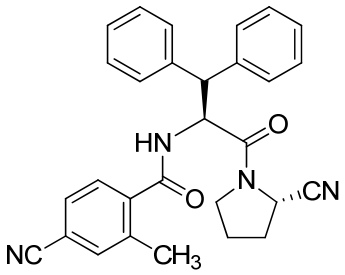
5.1.9. General procedure for the synthesis of compounds (34a-m)



1 **34a-m**

Compounds **34a-m** were prepared by Fmoc based Solid Phase Peptide Synthesis (SPPS) approach using the practical methodology described for the synthesis of compounds **11a-h**, **12a-h** and **16a-h** as illustrated in experimental **section 5.1.3.** The crude carboxamides **33a-m** obtained after global cleavage from the peptidyl resin were then transformed to respective nitrile derivative by dehydration using the method describe for the synthesis of compounds **17a-d** and **18a-b** as illustrated in experimental **section 5.1.4.** and purified by the method described in **section 5.1.3.-Purification** to give desired compounds **34a-m**.

5.1.9.1. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-2-methylbenzamide (34a)



34a (202 mg, 81%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 126-128 °C; Purity by HPLC: 97.88% AUC.

ESI/MS (m/z) : 462.7 $(M+H)^+$. **Mol. Wt.** = 462.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.78-1.82 (m, 3H), 1.85-1.93 (m, 1H), 2.46 (s, 3H), 3.61-3.65 (m, 1H), 3.98-4.01 (m, 1H), 4.51 (d, 1H, J = 11.6Hz), 4.84 (dd, 1H, J_1 = 2.8Hz, J_2 = 8.0Hz), 5.72 (dd, 1H, J_1 = 9.2Hz, J_2 = 11.6Hz), 7.01-7.19 (m, 7H), 7.22-7.39 (m, 5H), 7.56 (s, 1H), 9.01 (d, 1H, J = 8.8Hz, -NH).

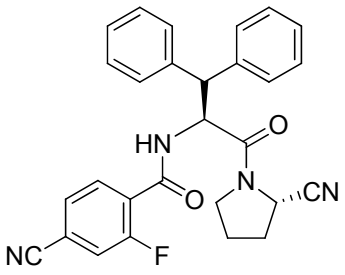
^{13}C NMR (100 MHz, DMSO-d_6): δ 18.2, 22.3, 30.2, 42.1, 48.6, 51.0, 60.4, 116.1, 116.9, 117.8, 126.2, 126.5, 127.8, 128.1, 128.2, 128.7, 129.2, 129.3, 133.3, 137.9, 140.2, 143.3, 167.4, 173.3.

Analysis : Mol. Formula: $C_{29}H_{26}N_4O_2$

Calcd.: C 75.30, H 5.67, N 12.11.

Found: C 75.28, H 5.64, N 12.10.

5.1.9.2. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-2-fluorobenzamide (34b)



34b (202 mg, 85%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 142-144 °C; Purity by HPLC: 98.79% AUC.

ESI/MS (m/z) : 467.3 $(M+H)^+$. **Mol. Wt.** = 466.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.81-1.92 (m, 3H), 1.93-1.98 (m, 1H), 3.62-3.65 (m, 1H), 4.00-4.04 (m, 1H), 4.53 (d, 1H, J = 11.6Hz), 4.87 (dd, 1H, J_1 = 2.8Hz, J_2 = 8.0Hz), 5.69 (dd, 1H, J_1 = 9.0Hz, J_2 = 11.6Hz), 7.07-7.18 (m, 3H), 7.22-7.31 (m, 5H), 7.39-7.75 (m, 4H), 7.92-7.96 (m, 1H), 9.01 (d, 1H, J = 8.8Hz, -NH).

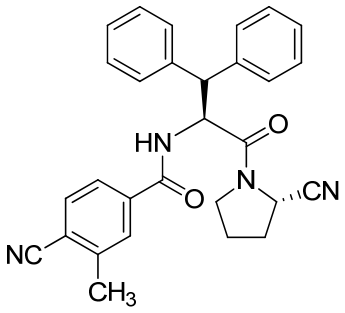
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.4, 30.4, 42.2, 48.7, 50.8, 60.1, 116.1(d, J = 28Hz), 116.9, 117.8, 119,5, 126.2, 126.5, 127.6, 128.2, 128.4, 128.7, 129.2, 129.3, 129.8, 140.3, 158.7(d, J = 248Hz), 167.4, 173.3.

Analysis : Mol. Formula: $C_{28}H_{23}FN_4O_2$

Calcd.: C 72.09, H 4.97, N 12.01.

Found: C 72.05, H 4.95, N 11.98.

5.1.9.3. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-3-methylbenzamide (34c)



34c (211 mg, 83%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 169-171 °C; Purity by HPLC: 97.14% AUC.

ESI/MS (m/z) : 463.6 $(M+H)^+$. **Mol. Wt.** = 462.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.76-1.81 (m, 3H), 1.84-1.92 (m, 1H), 2.49 (s, 3H), 3.62-3.65 (m, 1H), 3.89-3.95 (m, 1H), 4.54 (d, 1H, J = 11.6Hz), 4.84-4.93 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.0Hz), 5.72 (dd, 1H, J_1 = 9.2Hz, J_2 = 11.6Hz), 7.03-7.22 (m, 7H), 7.25-7.39 (m, 5H), 7.96 (s, 1H), 9.03 (d, 1H, J = 8.4Hz, -NH).

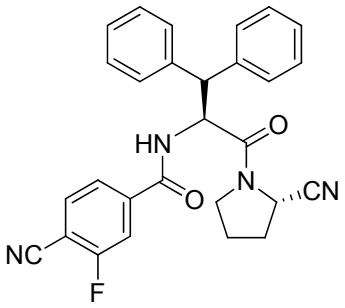
^{13}C NMR (100 MHz, DMSO-d_6): δ 18.1, 22.4, 30.5, 42.1, 48.5, 51.1, 59.7, 116.2, 116.7, 117.8, 126.1, 126.4, 127.7, 128.1, 128.2, 128.6, 129.1, 129.3, 133.2, 137.8, 140.2, 143.2, 167.4, 172.9.

Analysis : Mol. Formula: $C_{29}H_{26}N_4O_2$

Calcd.: C 75.30, H 5.67, N 12.11.

Found: C 75.27, H 5.66, N 12.14.

5.1.9.4. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-3-fluorobenzamide (34d)



34d (184 mg, 79%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 197-198 °C; Purity by HPLC: 98.39% AUC.

ESI/MS (m/z) : 466.2 $(M+H)^+$. **Mol. Wt.** = 466.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.82-1.93 (m, 3H), 1.95-2.04 (m, 1H), 3.59-3.63 (m, 1H), 3.97-4.02 (m, 1H), 4.53 (d, 1H, J = 11.6Hz), 4.87-4.91 (dd, 1H, J_1 = 3.8Hz, J_2 = 8.0Hz), 5.64 (dd, 1H, J_1 = 8.8Hz, J_2 = 11.6Hz), 7.09-7.18 (m, 3H), 7.21-7.33 (m, 4H), 7.38-7.76 (m, 4H), 7.82-7.91 (m, 2H), 7.93-7.96 (m, 1H), 9.04 (d, 1H, J = 8.6Hz, -NH).

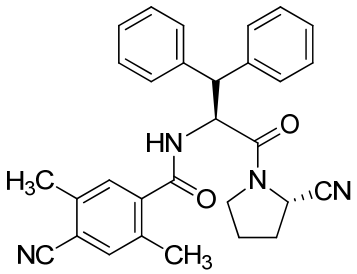
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.4, 30.4, 42.2, 48.6, 50.5, 59.7, 113.8 (d, J = 30Hz), 116.9, 117.8, 121,5, 126.2, 126.5, 127.8, 128.1, 128.2, 128.4, 128.7, 129.2, 129.3, 129.8, 140.1, 140.3, 161.2 (d, J = 253Hz), 167.5, 173.1.

Analysis : Mol. Formula: $C_{28}H_{23}FN_4O_2$

Calcd.: C 72.09, H 4.97, N 12.01.

Found: C 72.07, H 4.98, N 12.03.

5.1.9.5. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-2,5-dimethylbenzamide (34e)



34e (172 mg, 75%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 144-147 °C; Purity by HPLC: 98.48% AUC.

ESI/MS (m/z) : 477.8 $(M+H)^+$. **Mol. Wt.** = 476.6 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.78-1.82 (m, 3H), 1.84-1.92 (m, 1H), 2.44 (s, 3H), 2.49 (s, 3H), 3.61-3.65 (m, 1H), 3.97-4.01 (m, 1H), 4.52 (d, 1H, J = 11.4Hz), 4.85 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.0Hz), 5.71 (dd, 1H, J_1 = 9.0Hz, J_2 = 11.4Hz), 7.09-7.24 (m, 6H), 7.29-7.42 (m, 4H), 7.76 (s, 1H), 7.98 (s, 1H), 9.03 (d, 1H, J = 8.2Hz, -NH).

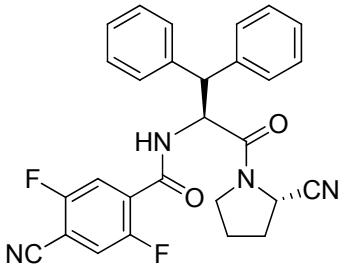
^{13}C NMR (100 MHz, DMSO-d_6): δ 17.6, 19.1, 22.3, 30.3, 42.1, 48.6, 50.3, 60.1, 115.9, 116.9, 117.8, 126.2, 126.5, 128.2, 128.3, 128.5, 128.7, 129.2, 129.3, 133.3, 139.3, 139.7, 140.2, 140.3, 167.4, 173.3.

Analysis : Mol. Formula: $C_{30}H_{28}N_4O_2$

Calcd.: C 75.61, H 5.92, N 11.76.

Found: C 75.58, H 5.89, N 11.73.

5.1.9.6. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-2,5-difluorobenzamide (34f)



34e (237 mg, 85%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 214-216 °C; Purity by HPLC: 98.73% AUC.

ESI/MS (m/z) : 485.4 $(M+H)^+$. **Mol. Wt.** = 484.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.79-1.89 (m, 3H), 1.92-2.01 (m, 1H), 3.60-3.63 (m, 1H), 3.98-4.03 (m, 1H), 4.53 (d, 1H, J = 11.6Hz), 4.87 (dd, 1H, J_1 = 2.8Hz, J_2 = 8.0Hz), 5.64 (dd, 1H, J_1 = 8.4Hz, J_2 = 11.6Hz), 7.07-7.16 (m, 3H), 7.21-7.33 (m, 5H), 7.38-7.76 (m, 3H), 7.81-7.89 (m, 1H), 9.03 (d, 1H, J = 8.2Hz, -NH).

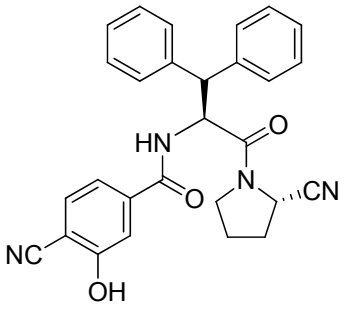
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 30.4, 42.1, 48.7, 50.7, 59.9, 114.8 (d, J = 30Hz), 116.3, 117.6, 120.6 (d, J = 23Hz), 121,5, 126.2, 126.5, 128.2, 128.4, 128.6, 129.2, 129.3, 131.3, 140.2, 155.3 (d, J = 248Hz), 156.9 (d, J = 253Hz), 167.5, 173.1.

Analysis : Mol. Formula: $C_{28}H_{22}F_2N_4O_2$

Calcd.: C 69.41, H 4.58, N 11.56.

Found: C 69.43, H 4.57, N 11.53.

5.1.9.7. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-3-hydroxybenzamide (34g)



34g (184 mg, 81%) was prepared by using general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 234-236 °C; Purity by HPLC: 99.17% AUC.

ESI/MS (m/z) : 465.6 $(M+H)^+$. **Mol. Wt.** = 464.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.79-1.84 (m, 3H), 1.88-1.94 (m, 1H), 3.60-3.65 (m, 1H), 3.97-4.02 (m, 1H), 4.53 (d, 1H, J = 11.6Hz), 4.86 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.0Hz), 5.71 (dd, 1H, J_1 = 8.8Hz, J_2 = 11.6Hz), 7.01-7.15 (m, 4H), 7.18-7.23 (m, 2H), 7.27-7.39 (m, 4H), 7.64 (d, 1H, J = 7.8Hz), 7.74-7.79 (m, 2H), 9.01 (d, 1H, J = 8.6Hz, -NH), 9.51 (s, 1H, -OH).

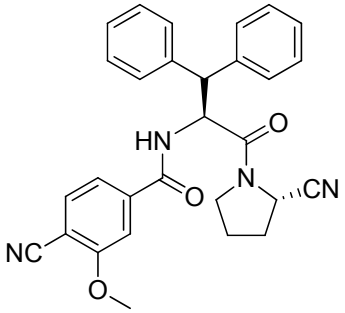
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 30.4, 42.3, 48.3, 51.1, 59.8, 106.5, 113.4, 115.9, 116.5, 120.7, 126.2, 126.5, 127.8, 128.1, 128.3, 128.7, 129.2, 129.3, 133.8, 140.2, 141.3, 159.4, 167.5, 173.2.

Analysis : Mol. Formula: $C_{28}H_{24}N_4O_3$

Calcd.: C 72.40, H 5.21, N 12.06.

Found: C 72.38, H 5.23, N 12.03.

5.1.9.8. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-3-methoxybenzamide (34h)



34h (178 mg, 79%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 158-160 °C; Purity by HPLC: 97.74% AUC.

ESI/MS (m/z) : 479.6 $(M+H)^+$. **Mol. Wt.** = 478.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.81-1.86 (m, 3H), 1.89-1.94 (m, 1H), 3.61-3.66 (m, 1H), 3.84 (s, 3H), 3.99-4.03 (m, 1H), 4.56 (d, 1H, J = 11.6Hz), 4.89 (dd, 1H, J_1 = 3.4Hz, J_2 = 8.2Hz), 5.73 (dd, 1H, J_1 = 8.6Hz, J_2 = 11.6Hz), 7.04-7.25 (m, 6H), 7.27-7.38 (m, 4H), 7.73 (d, 1H, J = 8.4Hz), 7.76-7.79 (m, 2H), 9.03 (d, 1H, J = 8.0Hz, -NH).

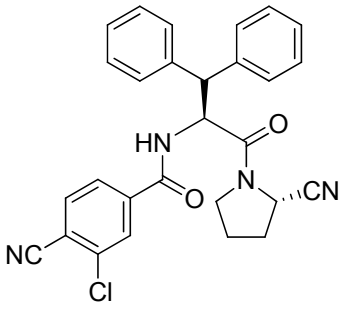
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.3, 30.3, 42.5, 48.2, 51.0, 55.7, 59.9, 104.8, 112.5, 115.7, 116.3, 120.6, 126.2, 126.5, 127.8, 128.1, 128.3, 128.7, 129.2, 129.3, 133.3, 140.2, 141.3, 163.4, 167.3, 173.5.

Analysis : Mol. Formula: $C_{29}H_{26}N_4O_3$

Calcd.: C 72.79, H 5.48, N 11.71.

Found: C 72.76, H 5.46, N 11.68.

5.1.9.9. 3-Chloro-4-cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)benzamide (34i)



34i (154 mg, 85%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 166-168 °C; Purity by HPLC: 99.06% AUC.

ESI/MS (m/z) : 483.6 $(M+H)^+$. **Mol. Wt.** = 483.0 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.83-1.87 (m, 3H), 1.89-1.95 (m, 1H), 3.60-3.64 (m, 1H), 4.01-4.03 (m, 1H), 4.56 (d, 1H, J = 11.4Hz), 4.92 (dd, 1H, J_1 = 3.4Hz, J_2 = 8.2Hz), 5.72 (dd, 1H, J_1 = 8.8Hz, J_2 = 11.4Hz), 7.07-7.25 (m, 6H), 7.28-7.36 (m, 4H), 7.76 (d, 1H, J = 7.8Hz), 8.09-8.12 (m, 2H), 8.98 (d, 1H, J = 8.2Hz, -NH).

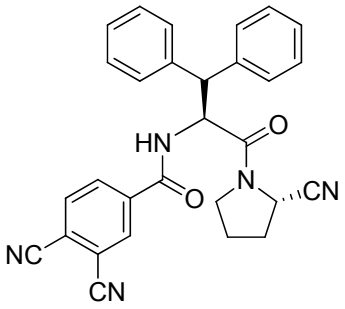
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 30.2, 42.1, 48.2, 50.4, 59.7, 115.7, 116.3, 116.8, 126.3, 126.4, 127.9, 128.2, 128.3, 128.5, 128.6, 129.2, 129.4, 133.8, 134.5, 139.8, 141.3, 167.4, 173.2.

Analysis : Mol. Formula: $C_{28}H_{23}ClN_4O_2$

Calcd.: C 69.63, H 4.80, N 11.60.

Found: C 69.59, H 4.78, N 11.61.

5.1.9.10. 3,4-Dicyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenyl propan-2-yl)benzamide (34j)



34j (169 mg, 82%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 198-201 °C; Purity by HPLC: 98.37% AUC.

ESI/MS (m/z) : 474.7 $(M+H)^+$. **Mol. Wt.** = 473.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.78-1.84 (m, 3H), 1.87-1.95 (m, 1H), 3.58-3.63 (m, 1H), 3.98-4.03 (m, 1H), 4.56 (d, 1H, J = 11.6Hz), 4.89 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.4Hz), 5.75 (dd, 1H, J_1 = 8.6Hz, J_2 = 11.6Hz), 7.09-7.25 (m, 6H), 7.27-7.36 (m, 4H), 7.96 (d, 1H, J = 7.4Hz), 8.24 (d, 1H, J = 7.4Hz), 8.63 (s, 1H), 9.03 (d, 1H, J = 8.0Hz, -NH).

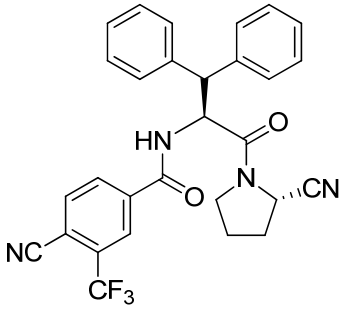
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 30.3, 42.4, 48.3, 50.1, 60.1, 115.7, 115.8, 116.4, 119.8, 126.2, 126.3, 127.9, 128.0, 128.3, 128.4, 128.6, 129.2, 129.4, 131.3, 131.8, 132.5, 139.3, 141.3, 167.5, 173.3.

Analysis : Mol. Formula: $C_{29}H_{23}N_5O_2$

Calcd.: C 73.56, H 4.90, N 14.79.

Found: C 73.53, H 4.87, N 14.82.

5.1.9.11. 4-Cyano-N-((S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)-3-(trifluoromethyl)benzamide (34k)



34k (178 mg, 79%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 137-139 °C; Purity by HPLC: 97.46% AUC.

ESI/MS (m/z) : 517.4 $(M+H)^+$. **Mol. Wt.** = 516.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.81-1.86 (m, 3H), 1.87-1.94 (m, 1H), 3.60-3.63 (m, 1H), 3.99-4.03 (m, 1H), 4.58 (d, 1H, J = 11.6Hz), 4.89 (dd, 1H, J_1 = 2.8Hz, J_2 = 8.8Hz), 5.73 (dd, 1H, J_1 = 8.4Hz, J_2 = 11.6Hz), 7.09-7.18 (m, 4H), 7.21-7.28 (m, 2H), 7.29-7.35 (m, 4H), 7.76 (d, 1H, J = 7.8Hz), 8.24-8.31 (m, 2H), 9.07 (d, 1H, J = 8.4Hz, -NH).

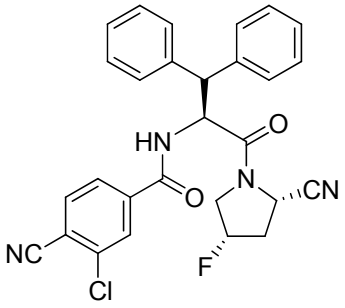
^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 30.1, 42.5, 48.5, 50.2, 60.0, 112.7, 115.6, 116.4, 119.4 (d, J = 28.4Hz), 124.3, 126.2, 126.3, 127.9, 128.0, 128.3, 128.4, 129.0, 129.2, 129.4, 131.3, 132.5, 134.5 (d, J = 248Hz), 138.9, 140.7, 167.3, 173.2.

Analysis : Mol. Formula: $C_{29}H_{23}F_3N_4O_2$

Calcd.: C 67.43, H 4.49, N 10.85.

Found: C 67.39, H 4.51, N 10.82.

5.1.9.12. 3-Chloro-4-cyano-N-((S)-1-((2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl)-1-oxo-3,3-diphenylpropan-2-yl)benzamide (34l)



34l (198 mg, 72%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 129-131 °C; Purity by HPLC: 99.38% AUC.

ESI/MS (m/z) : 501.7 $(M+H)^+$. **Mol. Wt.** = 501.0 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.29-2.41 (m, 2H), 3.64-3.68 (m, 1H), 4.01-4.04 (m, 1H), 4.57 (d, 1H, J = 11.6Hz), 4.91 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.8Hz), 5.21 (m, 1H), 5.74 (dd, 1H, J_1 = 8.2Hz, J_2 = 11.6Hz), 7.13-7.22 (m, 3H), 7.25-7.31 (m, 3H), 7.34-7.41 (m, 4H), 7.74 (d, 1H, J = 8.4Hz), 8.11-8.24 (m, 2H), 9.04 (d, 1H, J = 8.2Hz, -NH).

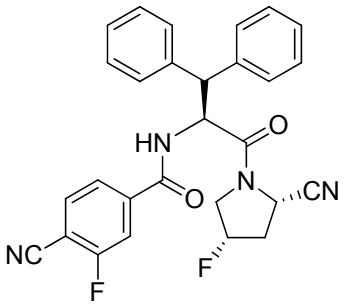
^{13}C NMR (100 MHz, DMSO-d_6): δ 28.1 (d, J = 23Hz), 42.6, 43.2, 49.8, 60.1, 91.2 (d, J = 177Hz), 115.7, 116.3, 116.8, 126.2, 126.3, 126.7, 127.9, 128.1, 128.3, 128.4, 129.0, 129.2, 129.4, 133.5, 134.7, 139.8, 140.5, 140.6, 167.4, 173.3.

Analysis : Mol. Formula: $C_{28}H_{22}ClFN_4O_2$

Calcd.: C 67.13, H 4.43, N 11.18.

Found: 67.14, H 4.44, N 11.15.

5.1.9.13. 4-Cyano-N-((S)-1-((2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl)-1-oxo-3,3-diphenyl propan-2-yl)-3-fluorobenzamide (34m)



34m (186 mg, 78%) was prepared by means of the general procedure described in section **5.1.3.** and section **5.1.4.** as a white solid. 179-182 °C; Purity by HPLC: 98.76% AUC.

ESI/MS (m/z) : 485.4 $(M+H)^+$. **Mol. Wt.** = 484.5 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.27-2.43 (m, 2H), 3.62-3.67 (m, 1H), 3.99-4.02 (m, 1H), 4.56 (d, 1H, J = 11.6Hz), 4.97 (dd, 1H, J_1 = 3.2Hz, J_2 = 8.8Hz), 5.21 (m, 1H), 5.73 (dd, 1H, J_1= 8.4Hz, J_2 = 11.6Hz), 7.10-7.28 (m, 4H), 7.31-7.36 (m, 2H), 7.37-7.41 (m, 4H), 7.76-7.79 (m, 2H), 7.87 (d, 1H, J = 8.6Hz), 9.01 (d, 1H, J = 8.4Hz, -NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ 28.2 (d, J = 20Hz), 42.4, 43.6, 49.9, 59.8, 91.6 (d, J = 178Hz), 113.7 (d, J = 31Hz), 115.6 (d, J = 3.8Hz), 116.4, 121.4, 123.9, 126.3, 126.7, 127.9, 128.1, 128.3, 128.4, 129.2, 129.4, 133.7, 139.9, 140.5, 140.7, 161.3 (d, J = 252Hz), 167.4, 173.3.

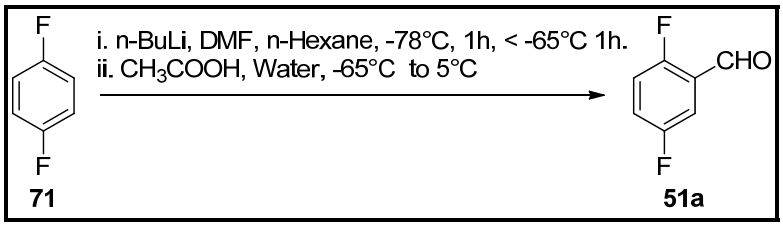
Analysis : Mol. Formula: $C_{28}H_{22}F_2N_4O_2$

Calcd.: C 69.41, H 4.58, N 11.56.

Found: 69.39, H 4.56, N 11.52.

5.1.10. Experimental Details : Aminomethylpiperidone based DPP-IV inhibitors (Third series)

5.1.10.1. 2,5-Difluorobenzaldehyde (51a)

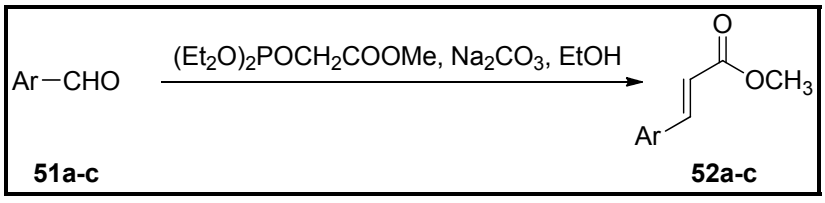


A solution of 1,4-difluorobenzene **71** (10 g, 87.6 mmol) in THF (175 ml, 17.5 vol) was stirred under nitrogen and cooled to -78°C. n-Butyllithium (82 ml of 1.6 M solution in hexanes, 131.4 mmol, 1.5 eq) was added to the reaction mixture over a period of 30 minutes maintaining the temperature below -65°C. After an hour DMF (10.16 ml, 131.4 mmol, 1.5 eq) was added within 10 min. maintaining the temperature below -65° C. The reaction mixture was quenched after 10 minutes by the addition of acetic acid (17.5 ml, 1.75 eq) directly followed by D. M. water (440 ml, 36.4 vol). This caused the reaction to exotherm to 5°C. The cold bath was then removed, *tert*-Butyl methyl ether (TBME) (220 ml, 22 vol) added and the reaction was stirred for 5 minutes. The layers were separated and the aqueous layer was extracted with TBME (2×220 ml). Combined organic layers were washed with 0.2 M HCl (1X220 ml), sat. Na_2CO_3 (1X220 ml) and brine (1X220 ml, 22 vol), dried over Na_2SO_4 and concentrated on a rotary evaporator to afford the crude product (11.8 g). Column chromatographic purification gave 7.8 g (62% Yield) of 2,5-difluorobenzaldehyde **51a** as a pale yellow oil. Purity by HPLC: 98.3% AUC.

ESI/MS (m/z) : 143.2 $(M+H)^+$. **Mol. Wt. =** 142.1 g

^{1}HNMR (400 MHz, DMSO-d_6) : δ 7.15-7.20 (m, 1H), 7.26-7.33 (m, 1H), 7.51-7.55 (m, 1H), 10.32 (s, 1H, -C**H**O)

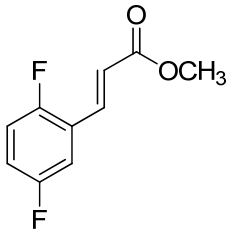
5.1.11. General procedure for the synthesis of compounds (52a-c)



Synthesis of **52a-c** was commenced with Horner-Wadsworth-Emmons reaction of aldehydes **51a-c** by using protocol reported by J. Villieras et.al. **[282]**. 50.0 mmol of

aldehyde **51a-c** was placed in a 50 ml single neck R. B. Flask, to it was added rectified spirit (1 vol by weight of starting material) and stirred at room temperature. To this clear solution was charged triethyl phosphonoacetate (1.05 eq) in a single portion followed by addition of 3M K_2CO_3 solution (2.0 eq). Reaction mixture was stirred at room temperature for 2h. After completion of reaction(TLC), reaction mixture was diluted with D. M. water (140 ml) and extracted with ethyl acetate (3X100 ml). Combined organic extracts were washed with brine (1X100 ml), dried over anhy. Na_2SO_4 and evaporated to dryness under reduced pressure. Crude residue thus obtained was purified by column chromatography (100-200 mesh silica gel, 0-20% ethyl acetate in n-hexane) to give desired (E)-methyl acrylates **52a-c**.

5.1.11.1. (E)-Methyl 3-(2,5-difluorophenyl)acrylate (52a)

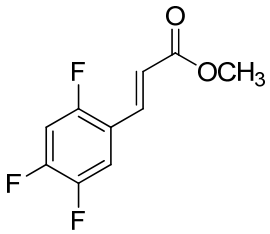


52a (7 g, 81%) was prepared from **51a** by means of the general procedure described in **5.1.11.** as a colourless oil; Purity by HPLC: 99.55% AUC.

ESI/MS (m/z) : 198.7 $(M+H)^+$. **Mol. Wt. =** 198.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.72 (s, 3H), 6.26 (d, 1H, J =13.6Hz), 7.05-7.11 (m, 2H), 7.26-7.39 (m, 1H), 7.93 (d, 1H, J = 13.6Hz).

5.1.11.2. (E)-Methyl 3-(2,4,5-trifluorophenyl)acrylate (52b)

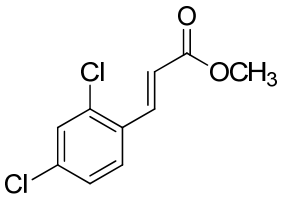


52b (8.3 g, 86%) was prepared from **51b** by means of the general procedure described in **5.1.11.** as a colourless oil; Purity by HPLC: 96.71% AUC.

ESI/MS (m/z) : 217.3 $(M+H)^+$. **Mol. Wt. =** 216.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.71 (s, 3H), 6.27 (d, 1H, J =14.0Hz), 7.26-7.30 (m, 1H), 7.43-7.50 (m, 1H), 7.89 (d, 1H, J = 14.0Hz).

5.1.11.3. (E)-Methyl 3-(2,4-dichlorophenyl)acrylate (52c)

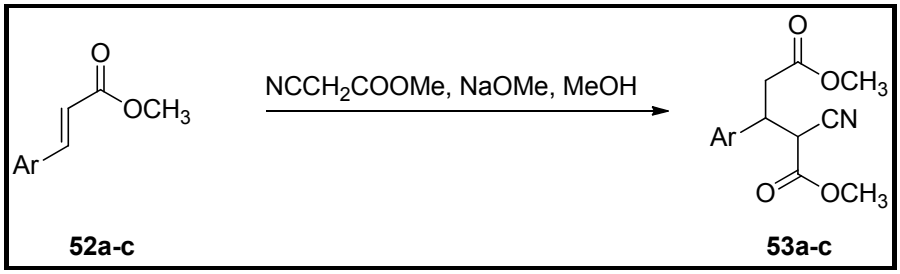


52c (8.3 g, 86%) was prepared from **51c** by means of the general procedure described in **5.1.11.** as a colourless oil; Purity by HPLC: 97.43% AUC.

ESI/MS (m/z) : 231.7 $(M+H)^+$. **Mol. Wt.** = 231.1 g.

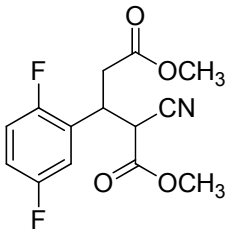
^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.77 (s, 3H), 6.24 (d, 1H, J =13.6Hz), 7.4 (dd, 1H, J_1 = 8.4Hz, J_2 = 2.0Hz), 7.46 (d, 1H, J = 8.4Hz), 7.53 (d, 1H, J = 1.6Hz), 7.97 (d, 1H, J = 13.6Hz).

5.1.12. General procedure for the synthesis of compounds (53a-c)



To a solution of sodium methoxide (1.03 eq by wt. of starting material) in 200 ml of methanol was added (1.0 eq) of methyl cyanoacetate and the mixture was stirred at ambient temperature for 30 min. To this solution was added (E)-methyl acrylates **52a-c** (62 mmol) 50 ml of methanol and the resulting yellow mixture was heated to reflux for 6 h. The mixture was then quenched at ambient temperature with 1N $HCl_{(aq)}$ (100 ml) and concentrated to remove methanol. The resulting mixture was extracted with ethyl acetate (3X300 ml) portions, and the organic phases combined and washed sequentially with 1N HCl, saturated aqueous $NaHCO_3$ solution, and brine (1X100 ml each), dried over magnesium sulfate, filtered, and evaporated in vacuo to yield a viscous oil. The crude material was purified by flash chromatography (230-400 silica gel, 0 to 25% ethyl acetate/hexanes gradient) to give the title compounds 3-aryl-2-cyanopentane diesters **53a-c** as a mixture of stereoisomers.

5.1.12.1. Dimethyl 2-cyano-3-(2,5-difluorophenyl)pentanedioate (53a)



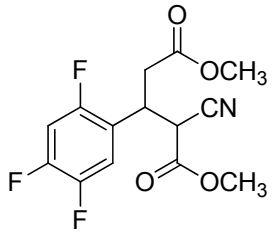
53a (14.9 g, 83%) was prepared from **52a** by means of the general procedure described in **5.1.12.** as light yellow coloured oil; Purity by HPLC: 99.22% AUC.

ESI/MS (m/z) : 298.2 $(M+H)^+$. **Mol. Wt.** = 297.3 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.48-2.52 (m, 2H), 3.63 (s, 3H), 3.72 (s, 3H), 3.94 (d, 1H, J = 8.6 Hz), 4.35-4.41 (m, 1H), 6.92-6.98 (m, 2H), 7.08-7.14 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 29.4, 37.6, 40.2, 51.3, 52.7, 108.9 (d, J = 178Hz), 113.7 (d, J = 31Hz), 115.6 (d, J = 3.8Hz), 116.4, 136.7, 158.2 (d, J = 246Hz), 161.3 (d, J = 252Hz), 167.4, 170.3.

5.1.12.2. Dimethyl 2-cyano-3-(2,4,5-trifluorophenyl)pentanedioate (53b)

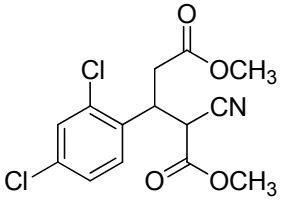


53b (13.6 g, 73%) was prepared from **52b** by means of the general procedure described in **5.1.12.** as light yellow coloured oil; Purity by HPLC: 97.34% AUC.

ESI/MS (m/z) : 316.1 $(M+H)^+$. **Mol. Wt.** = 315.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.82-3.05 (m, 2H), 3.64 (s, 3H), 3.69 (s, 3H), 3.91 (d, 1H, J = 9.2 Hz), 4.33-4.40 (m, 1H), 7.26-7.31 (m, 1H), 7.43-7.52 (m, 1H).

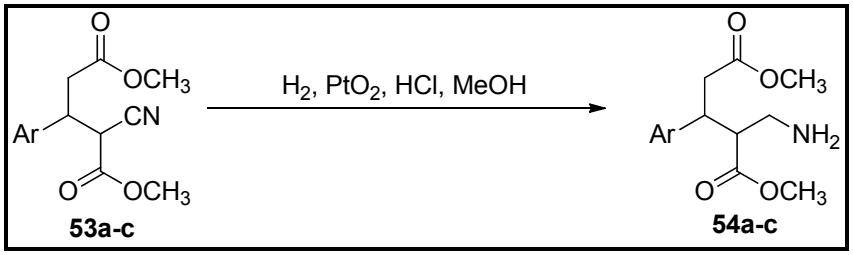
5.1.12.3. Dimethyl 2-cyano-3-(2,4-dichlorophenyl)pentanedioate (53c)



53c (14.2 g, 78%) was prepared from **52c** by means of the general procedure described in **5.1.12.** as light yellow coloured oil; Purity by HPLC: 96.84% AUC.
ESI/MS (m/z) : 331.1 $(M+H)^+$. **Mol. Wt.** = 330.2 g.

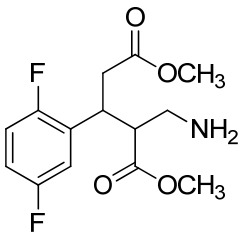
^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.68-2.91 (m, 2H), 3.62 (s, 3H), 3.71 (s, 3H), 3.87 (d, 1H, J = 9.4 Hz), 4.23-4.37 (m, 1H), 7.21 (d, 1H, J = 8.2 Hz), 7.34 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.2 Hz), 7.65 (d, 1H, J = 2.2 Hz).

5.1.13. General procedure for the synthesis of compounds (54a-c)



To 450 ml of methanol at 0 ^{0}C was carefully added acetyl chloride (30 ml) and the resulting solution was allowed to stir at ambient temperature for 30 min. The resulting solution was added to 3-aryl-2-cyanopentane diesters **53a-c** (43 mmol) and the reaction mixture was then shaken with 5.0 g of platinum oxide under 50 psi of hydrogen for 20 h. The mixture was filtered through a pad of Celite and the filter cake washed with methanol and dichloromethane. The combined filtrate and washings were concentrated under reduced pressure. Residue obtained was triturated with ether (3X100 ml), solid obtained was dried well to give the compounds **54a-c** as a hydrochloride salt, which were neutralized with sat. $NaHCO_3$ solution and extracted with DCM to give free amines **54a-c**.

5.1.13.1. Dimethyl 2-(aminomethyl)-3-(2,5-difluorophenyl)pentanedioate (54a)



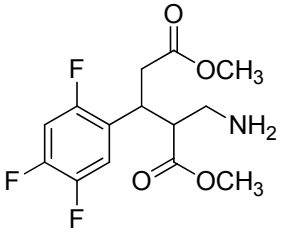
54a (11.4 g, 69%) was prepared from **53a** by means of the general procedure described in **5.1.13.** as a white solid; mp: 139-141^{0}C Purity by HPLC: 98.97% AUC.

ESI/MS (m/z) : 302.1 $(M+H)^+$. **Mol. Wt.** = 301.3 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.45-2.49 (m, 2H), 2.79 (d, 1H, J = 7.6 Hz), 2.97-3.04 (m, 1H), 3.14 (d, 1H, J = 7.8 Hz), 3.63 (s, 3H), 3.78 (s, 3H), 3.87 (d, 1H, J = 9.4 Hz), 4.35-4.41 (m, 1H), 6.92-6.98 (m, 2H), 7.08-7.14 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 38.7, 40.3, 51.3, 52.3, 53.1, 114.2 (d, J = 29Hz), 116.6 (d, J = 3.8Hz), 117.4(d, J = 178Hz), 154.2 (d, J = 242Hz), 157.3 (d, J = 247Hz), 166.8, 167.3.

5.1.13.2. Dimethyl 2-(aminomethyl)-3-(2,4,5-trifluorophenyl)pentanedioate (54b)

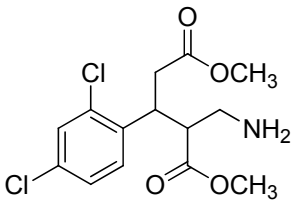


54b (12.4 g, 72%) was prepared from **53b** by means of the general procedure described in **5.1.13.** as a white solid; mp: 157-159^{0}C Purity by HPLC: 95.29% AUC.

ESI/MS (m/z) : 320.1 $(M+H)^+$. **Mol. Wt.** = 319.3 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.45-2.47 (m, 2H), 2.77 (d, 1H, J = 7.8 Hz), 2.97-3.02 (m, 1H), 3.14 (d, 1H, J = 7.8 Hz), 3.64 (s, 3H), 3.77 (s, 3H), 3.86 (d, 1H, J = 8.8 Hz), 4.35-4.41 (m, 1H), 6.92-7.01 (m, 1H), 7.11-7.24 (m, 1H).

5.1.13.3. Dimethyl 2-(aminomethyl)-3-(2,4-dichlorophenyl)pentanedioate (54c)

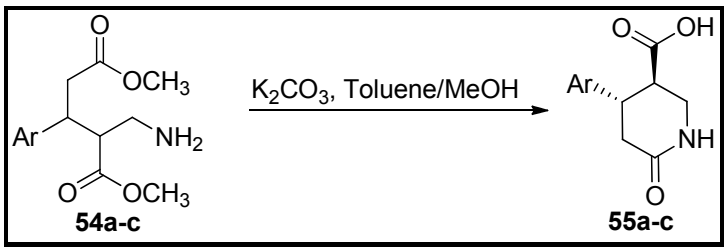


54c (11.9 g, 64%) was prepared from **53c** by means of the general procedure described in **5.1.13.** as a thick wax; Purity by HPLC: 95.29% AUC.

ESI/MS (m/z) : 320.1 $(M+H)^+$. **Mol. Wt.** = 319.3 g.

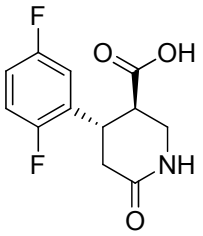
^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43-2.45 (m, 2H), 2.67-2.72 (m, 1H), 2.96-3.02 (m, 1H), 3.15 (d, 1H, J = 7.6 Hz), 3.65 (s, 3H), 3.74 (s, 3H), 3.92 (d, 1H, J = 8.9 Hz), 4.36-4.42 (m, 1H), 7.23 (d, 1H, J = 8.4 Hz), 7.37 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.2 Hz), 7.63 (d, 1H, J = 2.0 Hz).

5.1.14. General procedure for the synthesis of compounds (55a-c)



2-Aminomethyl-3-aryl pentane diesters **54a-c** (50 mmol) prepared in **section 5.1.13.** were taken up in 400 ml of 1:1 methanol/toluene with K_2CO_3 (28 g, 200 mmol). The resulting mixture was heated to reflux for 4 h, then cooled to 0 ^{0}C and quenched with 1N $HCl_{(aq)}$ until the solution was acidic to pH paper. The resulting mixture was then extracted with mixture of 3: 1 chloroform/isopropyl alcohol (5X300 ml) and the organic phases combined and washed with brine (1X300 ml), dried over anhy. Na_2SO_4, filtered, and evaporated in vacuo to yield the compounds **55a-c**. Compounds prepared were used as such without any purification in the next reaction step.

5.1.14.1. *trans*-4-(2,5-Difluorophenyl)-6-oxopiperidine-3-carboxylic acid (55a)



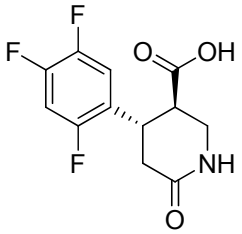
55a (9.3 g, 61%) was prepared from **54a** by means of the general procedure described in **5.1.14.** as a white solid; Mp: 206-208^{0}C Purity by HPLC: 95.98% AUC.

ESI/MS (m/z) : 256.1 $(M+H)^+$. **Mol. Wt.** = 255.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.59 (dd, 1H, J_1 = 10.8Hz, J_2 = 18.0Hz), 2.75 (dd, 1H, J_1 = 9.6Hz, J_2 = 18.2Hz), 3.21 (dd, 1H, J_1 = 10.8Hz, J_2 = 9.6Hz), 3.37-3.49 (m, 2H), 3.59-3.62 (m, 1H), 6.08 (bs, 1H), 6.92-6.99 (m, 2H), 7.09-7.11 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 36.5, 38.4, 46.3, 48.9, 105.4 (d, J = 23Hz), 116.4 (d, J = 3.8Hz), 117.2 (d, J = 178Hz), 127.3 (d, J = 21Hz), 148.2 (d, J = 246Hz), 154.3 (d, J = 253Hz), 170.8, 178.6.

5.1.14.2. *trans*-6-Oxo-4-(2,4,5-trifluorophenyl)piperidine-3-carboxylic acid (55b)

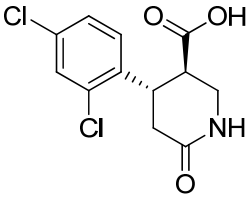


55b (10.1 g, 64%) was prepared from **54b** by means of the general procedure described in **5.1.14.** as a white solid; Mp: 197-199^{0}C Purity by HPLC: 97.31% AUC.

ESI/MS (m/z) : 273.9 $(M+H)^+$. **Mol. Wt.** = 273.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.54 (dd, 1H, J_1 = 10.6Hz, J_2 = 16.4Hz), 2.73 (dd, 1H, J_1 = 9.8Hz, J_2 = 16.2Hz), 3.18 (dd, 1H, J_1 = 10.8Hz, J_2 = 9.6Hz), 3.35-3.46 (m, 2H), 3.56-3.59 (m, 1H), 6.02 (bs, 1H), 6.92-7.03 (m, 1H), 7.09-7.21 (m, 1H).

5.1.14.3. *trans*-6-Oxo-4-(2,4-dichlorophenyl)piperidine-3-carboxylic acid (55c)

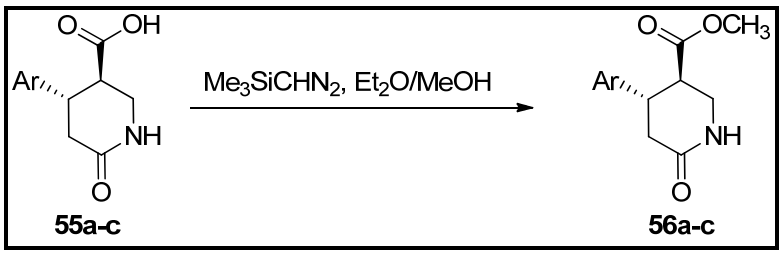


55c (8.3 g, 54%) was prepared from **54c** by means of the general procedure described in **5.1.14.** as an off white solid; Mp: 171-174^{0}C Purity by HPLC: 95.46% AUC.

ESI/MS (m/z) : 288.6 $(M+H)^+$. **Mol. Wt.** = 288.1 g.

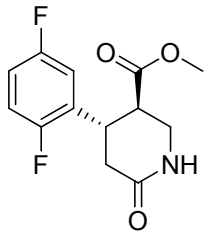
^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.56 (dd, 1H, J_1 = 9.8Hz, J_2 = 15.2Hz), 2.76 (dd, 1H, J_1 = 10.2Hz, J_2 = 15.2Hz), 3.19 (dd, 1H, J_1 = 10.4Hz, J_2 = 9.6Hz), 3.37-3.48 (m, 2H), 3.56-3.61 (m, 1H), 6.08 (bs, 1H), 7.25 (d, 1H, J = 8.4 Hz), 7.37 (m, 1H), 7.67 (d, 1H, J = 2.0 Hz).

5.1.15. General procedure for the synthesis of compounds (56a-c)



Compounds **56a-c** were prepared by using literature reported method **[283]**. The Compounds **55a-c** (75 mmol) prepared in section **5.1.14.** were dissolved in a mixture of 1 : 1 diethyl ether/methanol (500 ml) and cooled to 0 ^{0}C. To this solution was added 75 ml solution of 2M trimethylsilyldiazomethane in hexane (150 mmol) dropwise until a yellow colour persisted. After warming to room temperature, the solution was stirred for an additional 2 h, and then concentrated in vacuo. The compounds **56a-c** were collected as a colorless crystalline solid (obtained as *cis*: *trans* mixture having >85% *trans* stereo configuration) which were purified by column chromatography to remove the *cis* isomer.

5.1.15.1. Methyl-*trans*-4-(2,5-difluorophenyl)-6-oxopiperidine-3-carboxylate (56a)



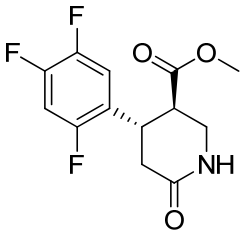
56a (9.8 g, 89%) was prepared from **55a** by means of the general procedure described in **5.1.15.** as a white solid; Mp: 174-177^{0}C Purity by HPLC: 98.40% AUC.

ESI/MS (m/z) : 270.3 $(M+H)^+$. **Mol. Wt.** = 269.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42 (dd, 1H, J_1 = 9.6Hz, J_2 = 14.8Hz), 2.58 (dd, 1H, J_1 = 9.2Hz, J_2 = 14.8Hz), 3.28 (dd, 1H, J_1 = 9.2Hz, J_2 = 9.6Hz), 3.36-3.49 (m, 2H), 3.59-3.63 (m, 1H), 3.77 (s, 3H), 6.90-6.99 (m, 3H), 7.07-7.12 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 37.5, 40.6, 47.5, 49.2, 52.2, 106.7 (d, J = 28Hz), 116.9 (d, J = 3.8Hz), 117.2 (d, J = 38Hz), 121.3 (d, J = 21Hz), 148.2 (d, J = 249Hz), 157.3 (d, J = 250Hz), 166.6, 170.6.

5.1.15.2. Methyl-*trans*-6-oxo-4-(2,4,5-trifluorophenyl)piperidine-3-carboxylate (56b)

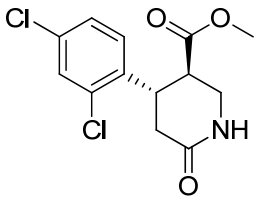


56a (9.3 g, 87%) was prepared from **55b** by means of the general procedure described in **5.1.15.** as a white solid; Mp: 162-164^{0}C Purity by HPLC: 97.87% AUC.

ESI/MS (m/z) : 288.3 $(M+H)^+$. **Mol. Wt.** = 287.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.44 (m, 1H), 2.54 (dd, 1H, J_1 = 9.6Hz, J_2 = 15.2Hz), 3.27 (dd, 1H, J_1 = 9.4Hz, J_2 = 9.6Hz), 3.36-3.47 (m, 2H), 3.56-3.62 (m, 1H), 3.78 (s, 3H), 6.37 (bs, 1H), 6.96-7.13 (m, 2H).

5.1.15.c. Methyl-*trans*-4-(2,4-dichlorophenyl)-6-oxopiperidine-3-carboxylate (56c)

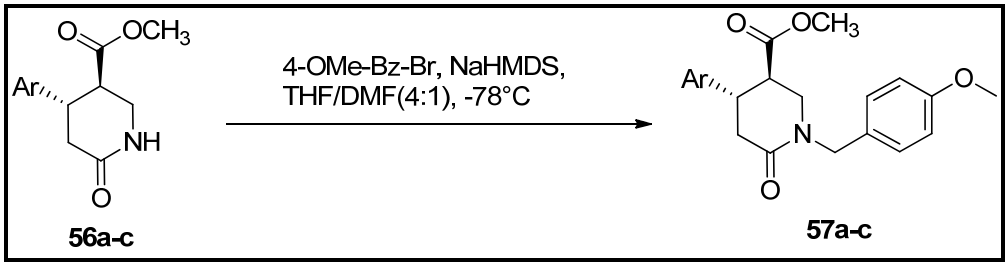


56c (10.6 g, 93%) was prepared from **55c** by means of the general procedure described in **5.1.15.** as a white solid; Mp: 137-139^{0}C Purity by HPLC: 96.82% AUC.

ESI/MS (m/z) : 303.3 $(M+H)^+$. **Mol. Wt.** = 302.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.47 (dd, 1H, J_1 = 9.4Hz, J_2 = 13.8Hz), 2.54 (dd, 1H, J_1 = 10.4Hz, J_2 = 13.8Hz), 3.25 (dd, 1H, J_1 = 9.4Hz, J_2 = 10.6Hz), 3.34-3.51 (m, 2H), 3.56-3.61 (m, 1H), 3.77 (s, 3H), 6.83 (bs, 1H), 7.45 (d, 1H, J = 8.4Hz), 7.51 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.6Hz), 7.92 (d, 1H, J = 1.6Hz).

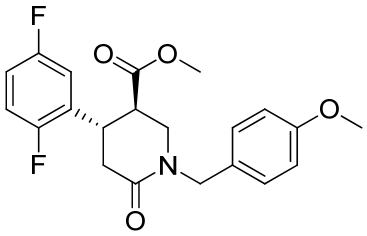
5.1.16. General procedure for the synthesis of compounds (57a-c)



To a stirred solution of compounds **56a-c** (28 mmol) in a mixture of 4:1 THF/DMF (150 ml) at -78 ^{0}C was added 32 ml solution of 1M sodium bis(trimethylsilyl)amide in THF (32 mmol) and the resulting solution was stirred at -78 ^{0}C for 30 min. *p*-methoxy benzyl bromide (35 mmol) was then added and the resulting solution was stirred at 0^{0}C for 60 min, then allowed to warm to ambient temperature over 12 h. The mixture was quenched with 1N $HCl_{(aq)}$ (100 ml) and concentrated to remove the THF. The resulting mixture was extracted with ethyl acetate (3X300 ml), and the organic phases combined and washed sequentially with 1N HCl, saturated $NaHCO_3$ solution, and brine (1X100 ml each). The organic phase was dried over anhy. Na_2SO_4 and evaporated in vacuo to yield

viscous oil. The crude material was purified by flash chromatography (230-400 mesh silica gel, 0 to 50% ethyl acetate/hexanes gradient) to give the compounds **57a-c**.

5.1.16.1. Methyl-*trans*-4-(2,5-difluorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (57a)



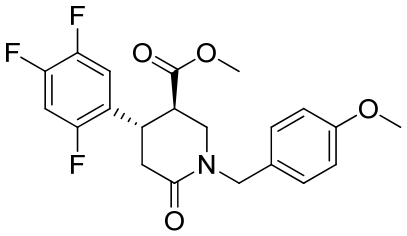
57a (8.3 g, 73%) was prepared from **56a** by means of the general procedure described in **5.1.16.** as a colourless thick oil; Purity by HPLC: 99.60% AUC.

ESI/MS (m/z) : 390.1 $(M+H)^+$. **Mol. Wt.** = 389.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.56 (dd, 1H, J_1 = 9.8Hz, J_2 = 14.2Hz), 2.69 (dd, 1H, J_1 = 8.1Hz, J_2 = 14.2Hz), 3.21 (dd, 1H, J_1 = 8.4Hz, J_2 = 9.8Hz), 3.40-3.47 (m, 1H), 3.57-3.59 (m, 2H), 3.77 (s, 3H), 3.79 (s, 3H), 4.36 (d, 1H, J = 9.6Hz), 4.44 (d, 1H, J = 9.6Hz), 6.90-7.01 (m, 4H), 7.11-7.15 (m, 1H), 7.23 (d, 2H, J = 7.4Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ 37.9, 40.3, 47.5, 49.2, 49.8, 52.2, 56.7, 106.7 (d, J = 28Hz), 114.3, 116.4, 116.9 (d, J = 3.2Hz), 117.2 (d, J = 39Hz), 126.3 (d, J = 23Hz), 128.3, 131.3, 148.2 (d, J = 247Hz), 157.5 (d, J = 252Hz), 166.6, 170.6.

5.1.16.2. Methyl-*trans*-1-(4-methoxybenzyl)-6-oxo-4-(2,4,5-trifluorophenyl) piperidine-3-carboxylate (57b)



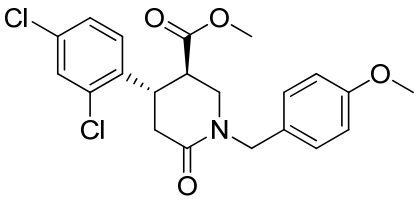
57b (8.9 g, 81%) was prepared from **56b** by means of the general procedure described in **5.1.16.** as a colourless thick oil; Purity by HPLC: 97.21% AUC.

ESI/MS (m/z) : 408.1 $(M+H)^+$. **Mol. Wt.** = 407.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.58 (dd, 1H, J_1 = 9.4Hz, J_2 = 14.2Hz), 2.67 (dd, 1H, J_1 = 8.4Hz, J_2 = 14.2Hz), 3.22 (dd, 1H, J_1 = 8.4Hz, J_2 = 9.4Hz), 3.42-3.49 (m, 1H), 3.57-3.61

(m, 2H), 3.78 (s, 3H), 3.79 (s, 3H), 4.37 (d, 1H, J = 9.4Hz), 4.43 (d, 1H, J = 9.4Hz), 6.92-7.03 (m, 3H), 7.09-7.10 (m, 1H), 7.11-7.15 (m, 1H), 7.23 (d, 2H, J = 7.8Hz).

5.1.16.3. Methyl-*trans*-4-(2,4-dichlorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (57c)

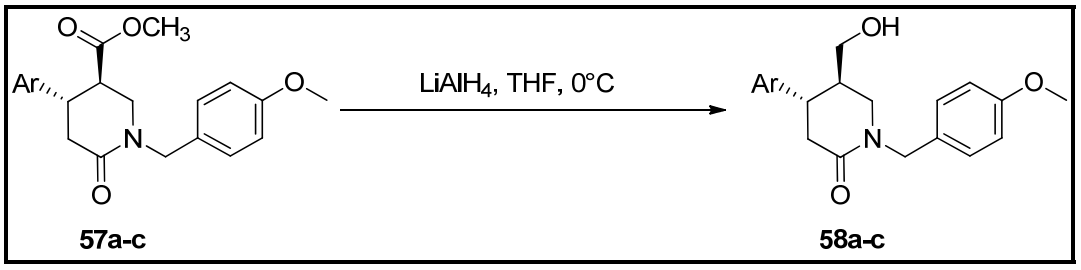


57c (7.3 g, 68%) was prepared from **56c** by means of the general procedure described in **5.1.16.** as a colourless thick oil; Purity by HPLC: 98.24% AUC.

ESI/MS (m/z) : 423.4 $(M+H)^+$. **Mol. Wt.** = 422.3 g.

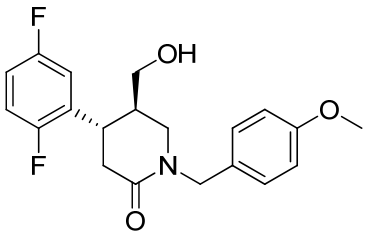
^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.56 (m, 1H), 2.63 (dd, 1H, J_1 = 8.6Hz, J_2 = 14.2Hz), 3.19 (dd, 1H, J_1 = 8.6Hz, J_2 = 9.6Hz), 3.42-3.49 (m, 1H), 3.56-3.63 (m, 2H), 3.76 (s, 3H), 3.78 (s, 3H), 4.36 (d, 1H, J = 9.4Hz), 4.43 (d, 1H, J = 9.4Hz), 6.92-7.03 (m, 3H), 7.18 (d, 2H, J = 7.8Hz), 7.23 (d, 2H, J = 7.8Hz) 7.45 (d, 1H, J = 8.4Hz), 7.52 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.6Hz), 7.92 (d, 1H, J = 1.6Hz).

5.1.17. General procedure for the synthesis of compounds (58a-c)



A solution of compounds **57a-c** (20 mmol) in dry THF (105 ml) was cooled to 0 ^{0}C. To the clear solution obtained was charged $LiAlH_4$ (24 mmol) in portions. Each portion was added after hydrogen gas evolution ceased. Reaction mixture was stirred at 0 ^{0}C for 2h. After completion of reaction, reaction mixture was quenched with sat. Na_2SO_4 solution (5 ml). Stirred the content at room temperature for 30 min., diluted it with DCM (100 ml). Reaction content was then filtered through celite and filtrate was dried over anhy. Na_2SO_4 and evaporated to dryness under reduced pressure to give title compounds **58a-c**.

5.1.17.1. *trans*-4-(2,5-Difluorophenyl)-5-(hydroxymethyl)-1-(4-methoxybenzyl) piperidin-2-one (58a)



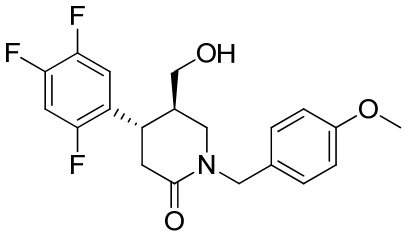
58a (6.9 g, 98%) was prepared by means of the general procedure described in **5.1.17.** as a colourless thick oil; Purity by HPLC: 98.37% AUC.

ESI/MS (m/z) : 362.2 $(M+H)^+$. **Mol. Wt.** = 361.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36-2.40 (m, 2H), 2.50-2.59 (m, 2H), 2.94-2.95 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.32 (m,2H), 3.51-3.59 (m, 2H), 3.80 (s, 3H), 4.29 (d, 1H, J = 9.6Hz), 4.32 (d, 1H, J = 9.6Hz), 7.21-7.25 (m, 4H), 7.39-7.44 (m, 1H), 7.91 (d, 2H, J = 7.2Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ 37.7, 40.3, 47.5, 49.2, 49.8, 56.7, 62.3, 104.7 (d, J = 28Hz), 114.3, 116.4, 117.9 (d, J = 3.2Hz), 118.2 (d, J = 38Hz), 126.5 (d, J = 27Hz), 128.3, 131.3, 148.2 (d, J = 252Hz), 155.5 (d, J = 258Hz), 158.4, 166.6.

5.1.17.2. *trans*-5-(Hydroxymethyl)-1-(4-methoxybenzyl)-4-(2,4,5-trifluorophenyl) piperidin-2-one (58b)

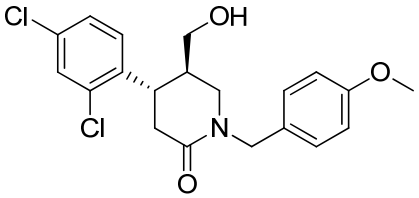


58b (7.1 g, 96%) was prepared by means of the general procedure described in **5.1.17.** as a colourless thick oil; Purity by HPLC: 97.45% AUC.

ESI/MS (m/z) : 380.2 $(M+H)^+$. **Mol. Wt.** = 379.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.35-2.40 (m, 2H), 2.51-2.59 (m, 2H), 2.91-2.96 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.32 (m,2H), 3.51-3.57 (m, 2H), 3.79 (s, 3H), 4.28 (d, 1H, J = 9.6Hz), 4.32 (d, 1H, J = 9.6Hz), 7.26-7.33 (m, 3H), 7.43-7.52 (m, 1H), 7.91 (d, 2H, J = 7.8Hz).

5.1.17.2. *trans*-4-(2,4-Dichlorophenyl)-5-(hydroxymethyl)-1-(4-methoxybenzyl) piperidin-2-one (58c)

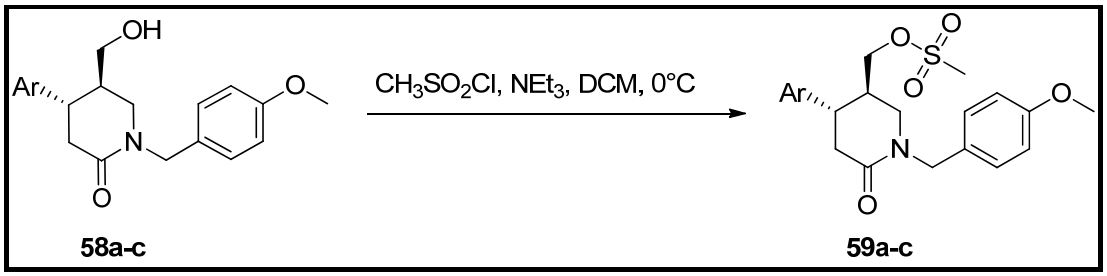


58c (7.3 g, 97%) was prepared by means of the general procedure described in **5.1.17.** as a colourless thick oil; Purity by HPLC: 97.67% AUC.

ESI/MS (m/z) : 394.7 $(M+H)^+$. **Mol. Wt.** = 394.3 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.37-2.42 (m, 2H), 2.51-2.58 (m, 2H), 2.92-2.96 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.34 (m,2H), 3.51-3.57 (m, 2H), 3.79 (s, 3H), 4.29 (d, 1H, J = 9.6Hz), 4.31 (d, 1H, J = 9.6Hz), 7.21 (d, 1H, J = 8.2 Hz), 7.26 (d, 2H, J = 7.8Hz), 7.34 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.2 Hz), 7.65 (d, 1H, J = 2.2 Hz), 7.91 (d, 2H, J = 7.8Hz).

5.1.18. General procedure for the synthesis of compounds (59a-c)

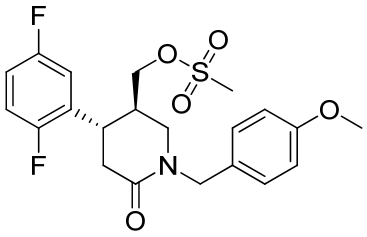


58a-c **59a-c**

To a solution of compounds **58a-c** (18 mmol) in dry DCM (85 ml) was added triethyl amine (27 mmol) in a single portion. Reaction mixture was cooled to 0 ^{0}C and charged to it methane sulfonyl chloride (21.6 mmol) dropwise over a period of 15 min. After completion of addition, reaction mixture was stirred at the same temperature for 2h.

After completion of reaction, reaction mixture was poured in to D. M. water (100 ml), layers were separated and aqueous layer was extracted with DCM (1X100 ml). Combined organic extracts were washed with D. M. water (3X150 ml), sat. $NaHCO_3$ solution (1X150 ml) and brine (1X100 ml). Organic layer was dried over anhy. Na_2SO_4 and solvent was removed under reduced pressure to give mesylate compounds **59a-c** as colourless oil.

5.1.18.1. *trans*-4-(2,5-Difluorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl)methyl methanesulfonate (59a)



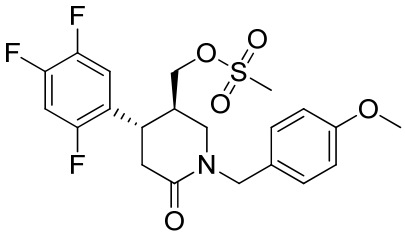
59a (8.7 g, 94%) was prepared from **58a** by means of the general procedure described in **5.1.18.** as a colourless thick oil; Purity by HPLC: 97.04% AUC.

ESI/MS (m/z) : 440.3 $(M+H)^+$. **Mol. Wt.** = 439.5 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36-2.40 (m, 2H), 2.52 (t, 1H, J = 16.3Hz), 2.55-2.58 (m, 4H), 2.94-2.95 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.32 (m,2H), 3.51-3.59 (m, 2H), 3.81 (s, 3H), 4.29 (d, 1H, J = 9.6Hz), 4.31 (d, 1H, J = 9.6Hz), 7.21-7.25 (m, 4H), 7.40-7.44 (m, 1H), 7.93 (d, 2H, J = 7.4Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ 37.7, 40.3, 41.5, 43.2, 49.8, 56.7, 67.3, 106.6 (d, J = 23Hz), 114.4, 116.7, 117.7 (d, J = 2.8Hz), 118.2 (d, J = 34Hz), 127.1 (d, J = 28Hz), 128.5, 131.3, 148.7 (d, J = 248Hz), 157.0 (d, J = 258Hz), 158.4, 166.7.

5.1.18.2. *trans*-1-(4-Methoxybenzyl)-6-oxo-4-(2,4,5-trifluorophenyl)piperidin-3-yl)methyl methanesulfonate (59b)

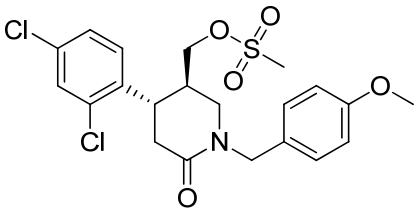


59b (7.6 g, 96%) was prepared from **58b** by means of the general procedure described in **5.1.18.** as a colourless thick oil; Purity by HPLC: 98.21% AUC.

ESI/MS (m/z) : 458.6 $(M+H)^+$. **Mol. Wt.** = 457.5 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.35-2.40 (m, 2H), 2.52-2.54 (m, 1H), 2.55-2.57 (m, 1H), 2.59 (s, 3H), 2.94-2.97 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.32 (m,2H), 3.51-3.59 (m, 2H), 3.81 (s, 3H), 4.28 (d, 1H, J = 9.4Hz), 4.30 (d, 1H, J = 9.4Hz), 6.92-7.03 (m, 1H), 7.09-7.21 (m, 1H),7.22 (d, 2H, J = 7.8Hz), 7.93 (d, 2H, J = 7.8Hz).

5.1.18.3. *trans*-4-(2,4-dichlorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl)methyl methanesulfonate (59c)

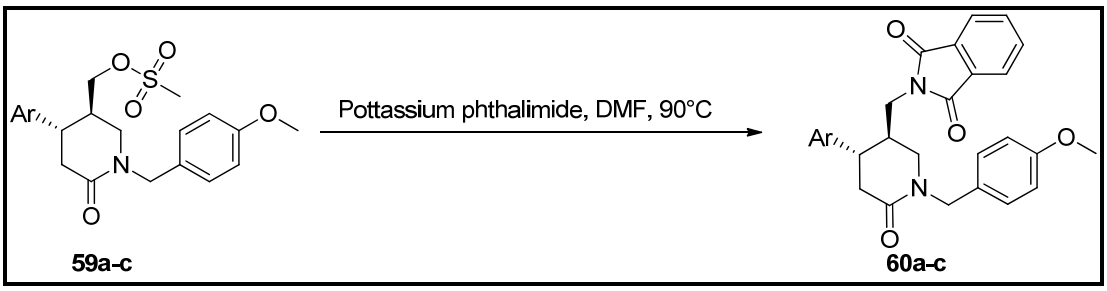


59c (8.1 g, 96%) was prepared from **58c** by means of the general procedure described in **5.1.18.** as a colourless thick oil; Purity by HPLC: 97.84% AUC.

ESI/MS (m/z) : 473.1 $(M+H)^+$. **Mol. Wt.** = 472.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33-2.40 (m, 2H), 2.48-2.52 (m, 1H), 2.55-2.57 (m, 1H), 2.61 (s, 3H), 2.94-2.97 (m, 1H), 3.09-3.12 (m, 1H), 3.29-3.32 (m,2H), 3.51-3.59 (m, 2H), 3.80 (s, 3H), 4.29 (d, 1H, J = 9.4Hz), 4.30 (d, 1H, J = 9.4Hz), 7.22 (d, 2H, J = 7.8Hz), 7.45 (d, 1H, J = 8.4Hz), 7.51 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.6Hz), 7.92 (d, 1H, J = 1.6Hz), 7.93 (d, 2H, J = 7.8Hz).

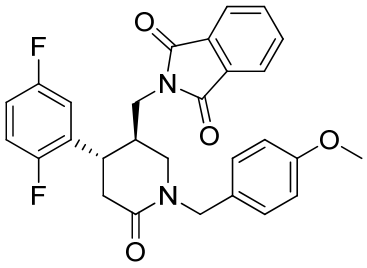
5.1.19. General procedure for the synthesis of compounds (60a-c)



To a solution of the compounds **59a-c** (16 mmol) in dry DMF (85 ml) was charged pottassium phthalimide (20.8 mmol) in a single portion under nitrogen atmosphere at room temperature. Reaction mixture was then heated to 90 °C for 8h.

After completion of reaction (TLC), reaction mixture was cool to room temperature and poured in ice cold D. M. water (425 ml). solid precipitates was filtered through Wattman filter paper and washed with plenty of D. M. water. Solid was then dried under suction to give desired title compounds **60a-c [284]**.

5.1.19.1. *trans*-2-((4-(2,5-Difluorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl)methyl) isoindoline-1,3-dione (60a)



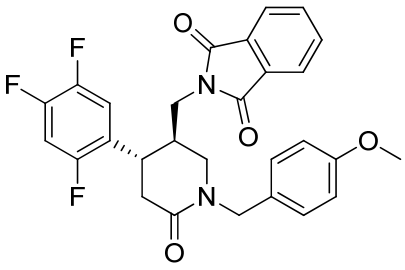
60a (5.3 g, 68%) was prepared from **59a** by means of the general procedure described in **5.1.19.** as a White solid; Mp: 214-217^{0}C, Purity by HPLC: 98.57% AUC.

ESI/MS (m/z) : 490.6 $(M+H)^+$. **Mol. Wt.** = 490.5 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36-2.39 (m, 1H), 2.41-2.56 (m, 1H), 2.89-2.92 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.32 (m,2H), 3.53-3.59 (m, 2H), 3.79 (s, 3H), 4.36 (d, 1H, J = 9.6Hz), 4.44 (d, 1H, J = 9.6Hz), 6.90-7.01 (m, 4H), 7.09-7.12 (m, 1H), 7. 23 (d, 2H, J = 7.4Hz), 7.81-7.87 (m, 4H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 38.1, 41.3, 42.2, 42.5, 49.8, 51.7, 56.7, 109.6 (d, J = 23Hz), 114.4, 116.7, 117.7 (d, J = 2.8Hz), 118.2 (d, J = 34Hz), 122.8, 127.1 (d, J = 29Hz), 128.5, 131.3, 131.7, 132.1, 148.3 (d, J = 248Hz), 157.3 (d, J = 258Hz), 158.9, 166.7, 168.2.

5.1.19.2. *trans*-2-((1-(4-Methoxybenzyl)-6-oxo-4-(2,4,5-trifluorophenyl)piperidin-3-yl) methyl)isoindoline-1,3-dione (60b)

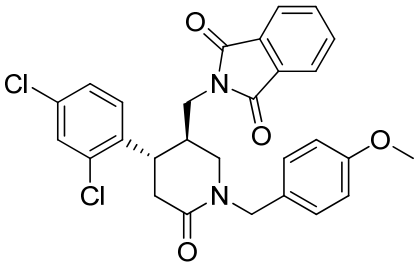


60b (6.0 g, 75%) was prepared from **59b** by means of the general procedure described in **5.1.19.** as a White solid; Mp: 221-224^{0}C, Purity by HPLC: 96.52% AUC.

ESI/MS (m/z) : 509.3 $(M+H)^+$. **Mol. Wt.** = 508.5 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36-2.39 (m, 1H), 2.41-2.56 (m, 1H), 2.89-2.92 (m, 1H), 3.09-3.12 (m, 1H), 3.30-3.32 (m,2H), 3.53-3.59 (m, 2H), 3.79 (s, 3H), 4.34 (d, 1H, J = 9.4Hz), 4.43 (d, 1H, J = 9.4Hz), 7.23 (d, 2H, J = 7.4Hz), 7.26-7.33 (m, 3H), 7.43-7.52 (m, 1H), 7.83-7.89 (m, 4H).

5.1.19.3. *trans*-2-((4-(2,4-Dichlorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl)methyl) isoindoline-1,3-dione (60c)

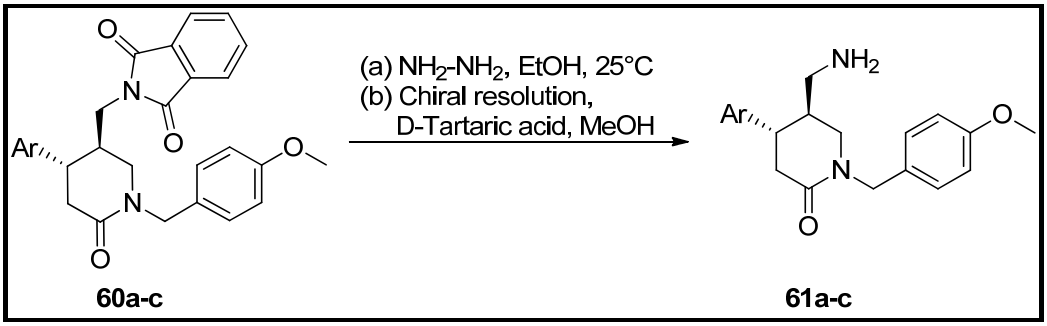


60c (6.2 g, 72%) was prepared from **59c** by means of the general procedure described in **5.1.19.** as a White solid; Mp: 178-186^{0}C (No clear melting point), Purity by HPLC: 97.21% AUC.

ESI/MS (m/z) : 523.9 $(M+H)^+$. **Mol. Wt.** = 523.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.32-2.37 (m, 1H), 2.39-2.52 (m, 1H), 2.87-2.91 (m, 1H), 3.07-3.10 (m, 1H), 3.28-3.31 (m,2H), 3.49-3.52 (m, 2H), 3.77 (s, 3H), 4.31 (d, 1H, J = 9.6Hz), 4.39 (d, 1H, J = 9.6Hz), 7.21 (d, 1H, J = 8.2 Hz), 7.23 (d, 2H, J = 7.4Hz), 7.29-7.37 (m, 3H), 7.65 (d, 1H, J = 2.2 Hz), 7.83-7.91 (m, 4H).

5.1.20. General procedure for the synthesis of compounds (61a-c)



Deprotection of phthalimido group was accomplished by treatment with hydrazine-hydrate **[285]**. Phthalimido compounds **60a-c** (15 mmol) prepared above were dissolved in ethanol (75 ml). To the clear solution obtained was added hydrazine-hydrate (75 mmol) and reaction mixture was stirred at room temperature for 5h.

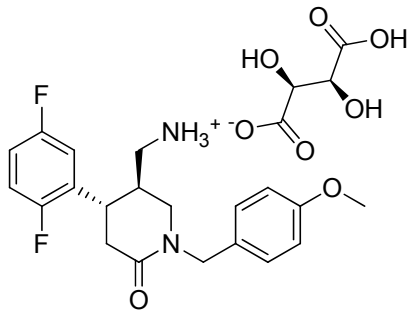
After completion of reaction (TLC), D. M. water (150 ml) was added to the reaction mixture and ethanol was removed under reduced pressure. Aqueous layer was then extracted with ethyl acetate (4X100 ml). Combined organic layers were washed with brine (1X100 ml). Organic layer was then dried over anhy. Na_2SO_4 and concentrated under reduced pressure to give fairly pure (>95% purity) *trans*- racemic

compounds **61a-c**. However this deprotection of phthlimide group to get primary amine can also be accomplish by the mild condition reported by John Osby et al **[286]**.

Chiral resolution:

Amino-methyl pipyridones **61a-c** prepared above were dissolved in methanol (100 ml)- and D-Tartaric acid (16.5 mmol) was added at room temperature and the reaction mixture was stirred for 15h at the same temperature. White solid precipitated in the reaction mixture was filtered through hot filter paper and washed it with methanol (2X100 ml) and dried under suction. Solid was then transferred in drying tray and dried in an air drier at 50^0C till constant weight obtained (approximately 5h). Thus tartrate salt of compounds **61a-c** was obtained as a white free flowing powder with absolute stereo configuration (4S, 5S).

5.1.20.1. ((3S,4S)-4-(2,5-Difluorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl) methanaminium (2S,3S)-3-carboxy-2,3-dihydroxypropanoate (61a)



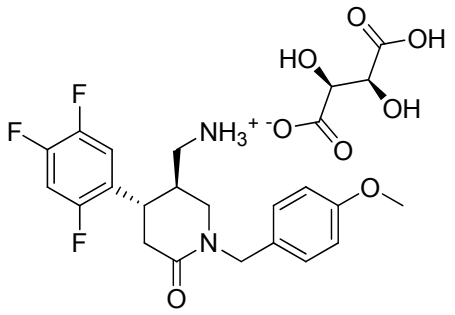
61a (2.48 g, 41%) was prepared from **60a** by means of the general procedure described in **5.1.20.** as a white solid; Mp: 198-201^{0}C, Purity by HPLC: 98.94% AUC, Chiral purity:98.18%ee AUC.

ESI/MS (m/z) : 361.4 $(M+H)^+$. **Mol. Wt.** = 360.4 g.

^{1}H NMR (400 MHz, D_2O): δ = 2.34-2.39 (m, 1H), 2.41-2.53 (m, 1H), 2.89-2.93 (m, 1H), 3.09-3.13 (m, 1H), 3.31-3.34 (m,2H), 3.51-3.57 (m, 2H), 3.79 (s, 3H), 4.36 (d, 1H, J = 9.6Hz), 4.44 (d, 1H, J = 9.6Hz), 4.49 (s, 2H), 6.93-7.04 (m, 4H), 7.09-7.12 (m, 1H), 7. 23 (d, 2H, J = 7.4Hz).

^{13}C NMR (100 MHz, D_2O): δ 37.2, 40.3, 40.9, 45.3, 49.4, 49.9, 55.7, 78.3, 109.6 (d, J = 27Hz), 114.4, 116.7, 117.7 (d, J = 2.8Hz), 118.2 (d, J = 32Hz), 127.1 (d, J = 26Hz), 128.3, 131.4, 148.6 (d, J = 248Hz), 156.3 (d, J = 252Hz), 158.9, 166.7, 176.3.2.

5.1.20.2. ((3S,4S)-1-(4-Methoxybenzyl)-6-oxo-4-(2,4,5-trifluorophenyl)piperidin-3-yl)methanaminium (2S,3S)-3-carboxy-2,3-dihydroxypropanoate (61b)

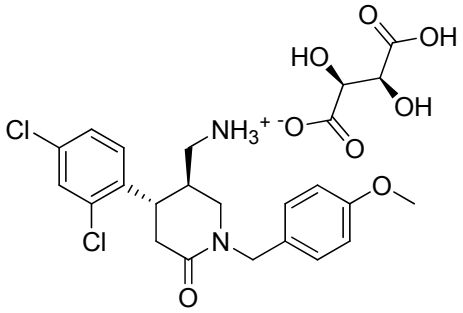


61b (2.17 g, 38%) was prepared from **60b** by means of the general procedure described in **5.1.20.** as a white solid; Mp: 217-219^{0}C, Purity by HPLC: 99.04% AUC, Chiral purity:98.47%ee AUC.

ESI/MS (m/z) : 379.2 $(M+H)^+$. **Mol. Wt.** = 378.4 g.

^{1}H NMR (400 MHz, D_2O): δ = 2.32-2.37 (m, 1H), 2.41-2.53 (m, 1H), 2.92-2.97 (m, 1H), 3.09-3.15 (m, 1H), 3.31-3.34 (m,2H), 3.49-3.56 (m, 2H), 3.77 (s, 3H), 4.33 (d, 1H, J = 9.6Hz), 4.41 (d, 1H, J = 9.6Hz), 4.49 (s, 2H), 6.96-7.07 (m, 1H), 7.09-7.19 (m, 3H), 7. 26 (d, 2H, J = 7.4Hz).

5.1.20.3. ((3S,4S)-4-(2,4-Dichlorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl)methanaminium (2S,3S)-3-carboxy-2,3-dihydroxypropanoate (61c)

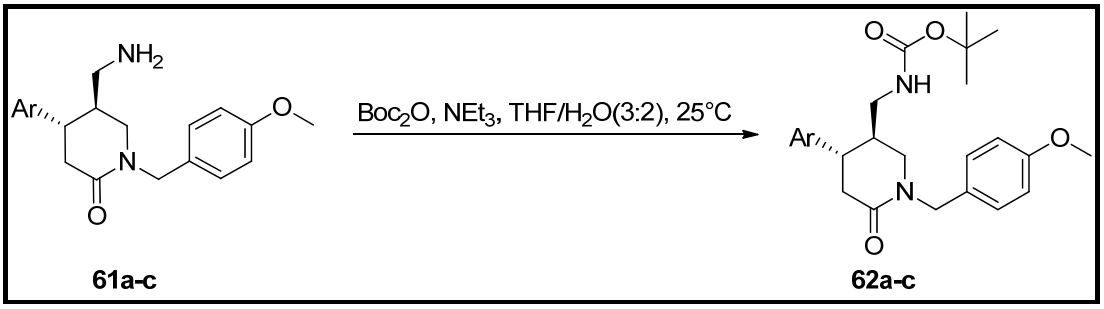


61c (3.2 g, 40%) was prepared **60c** by means of the general procedure described in **5.1.20.** as a white solid; Mp: 168-171^{0}C, Purity by HPLC: 98.24% AUC, Chiral purity:99.07%ee AUC.

ESI/MS (m/z) : 394.0 $(M+H)^+$. **Mol. Wt.** = 393.3 g.

^{1}H NMR (400 MHz, D_2O): δ = 2.33-2.37 (m, 1H), 2.41-2.53 (m, 1H), 2.92-2.96 (m, 1H), 3.09-3.15 (m, 1H), 3.31-3.34 (m,2H), 3.49-3.56 (m, 2H), 3.78 (s, 3H), 4.34 (d, 1H, J = 9.6Hz), 4.44 (d, 1H, J = 9.6Hz), 4.48 (s, 2H), 7.16-7.21 (m, 3H), 7. 26 (d, 2H, J = 7.4Hz), 7.34 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.2 Hz), 7.65 (d, 1H, J = 2.2 Hz).

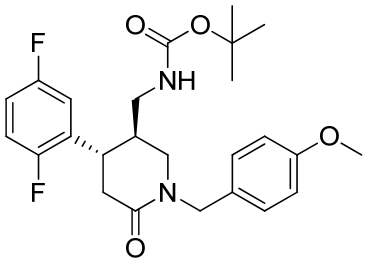
5.1.21. General procedure for the synthesis of compounds (62a-c)



Chiral pure compounds **61a-c** (10 mmol) were dissolved in a mixture of 3:2 THF/D. M. water (30 ml) and triethyl amine (30 mmol) was added in a single portion. Reaction mixture was stirred for 15 min. at room temperature then cool to 0 $^{\circ}$C and charged to it Boc-anhydride (12 mmol) dropwise over a period of 15min. Reaction mixture was then gradually brought to room temperature and stirred at the same temperature for 15h.

After completion of reaction, solvent of the reaction mixture was evaporated under reduced pressure and diluted with D. M. water (100 ml). Aqueous layer was then extracted with ethyl acetate (3X75 ml). Combined organic layers were washed with water (2X75 ml), 1N Hall (1X75 ml), water (1X75 ml) and brine (1X75 ml). Organic layer was dried over any. Na_2SO_4 and solvent was removed under reduced pressure to give the compounds **62a-c**, which were used for next reaction step without any purification.

5.1.21.1. tert-Butyl (((3S, 4S)-4-(2, 5-difluorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl) methyl) carbamate (62a)



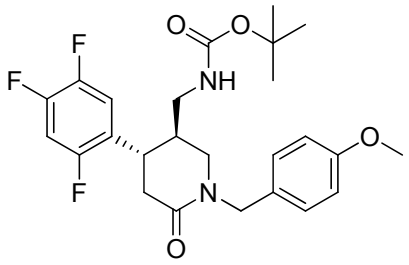
62a (3.2 g, 98%) was prepared from **61a** by means of the general procedure described in **5.1.21.** as a White solid; MP: 187-189^{0}C, Purity by HPLC: 97.31% AUC.

ESI/MS (m/z) : 461.5 $(M+H)^+$. **Mol. Wt.** = 460.5 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.39 (s, 9H), 2.34-2.49 (m, 2H), 2.89-2.93 (m, 1H), 3.09-3.13 (m, 1H), 3.31-3.34 (m,2H), 3.51-3.57 (m, 2H), 3.80 (s, 3H), 4.34 (d, 1H, J = 9.6Hz), 4.42 (d, 1H, J = 9.6Hz), 4.48 (bs, 1H), 6.85-6.94 (m, 4H), 7.09-7.12 (m, 1H), 7. 24 (d, 2H, J = 7.8Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2, 37.5, 40.6, 40.8, 42.3, 42.8, 49.9, 56.7, 80.3, 110.2 (d, J = 24Hz), 114.3, 116.5, 117.3 (d, J = 2.4Hz), 118.6 (d, J = 38Hz), 127.3 (d, J = 23Hz), 128.3, 132.0, 148.3 (d, J = 253Hz), 156.3 (d, J = 250Hz), 158.9, 166.7.

5.1.21.2. tert-Butyl (((3S,4S)-1-(4-methoxybenzyl)-6-oxo-4-(2,4,5-trifluorophenyl) piperidin-3-yl)methyl)carbamate (62b)

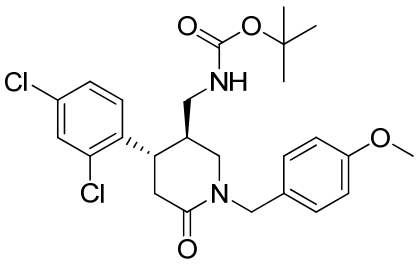


62b (2.9 g, 97%) was prepared from **61b** by means of the general procedure described in **5.1.21.** as a White solid; Mp: 199-203^{0}C, Purity by HPLC: 98.06% AUC.

ESI/MS (m/z) : 479.4 $(M+H)^+$. **Mol. Wt.** = 478.5 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (s, 9H), 2.33-2.49 (m, 2H), 2.89-2.94 (m, 1H), 3.10-3.15 (m, 1H), 3.31-3.38 (m,2H), 3.49-3.57 (m, 2H), 3.80 (s, 3H), 4.32 (d, 1H, J = 9.4Hz), 4.42 (d, 1H, J = 9.4Hz), 4.48 (bs, 1H), 6.92-7.03 (m, 3H), 7.09-7.21 (m, 1H), 7. 24 (d, 2H, J = 7.8Hz).

5.1.21.3. tert-Butyl (((3S,4S)-4-(2,4-dichlorophenyl)-1-(4-methoxybenzyl)-6-oxopiperidin-3-yl)methyl)carbamate (62c)



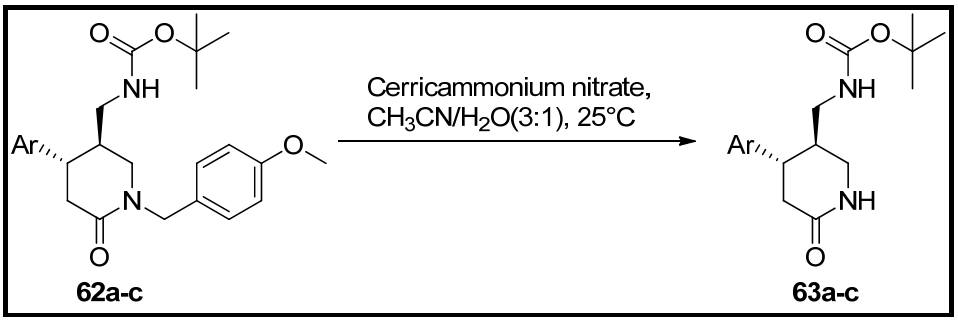
62c (3.1 g, 98%) was prepared from **61c** by means of the general procedure described in **5.1.21.** as a White solid; Mp: 211-213^{0}C, Purity by HPLC: 97.66% AUC.

ESI/MS (m/z) : 493.9 $(M+H)^+$. **Mol. Wt.** = 493.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (s, 9H), 2.31-2.49 (m, 2H), 2.89-2.94 (m, 1H), 3.09-3.15 (m, 1H), 3.31-3.38 (m,2H), 3.49-3.56 (m, 2H), 3.79 (s, 3H), 4.32 (d, 1H, J = 9.4Hz),

4.42 (d, 1H, J = 9.4Hz), 4.46 (bs, 1H), 6.93 (d, 2H, J = 7.8 Hz), 7.23 (d, 1H, J = 8.4 Hz), 7. 24 (d, 2H, J = 7.8Hz), 7.37 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.2 Hz), 7.63 (d, 1H, J = 2.0 Hz).

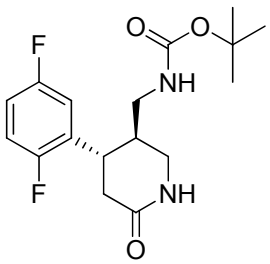
5.1.22. General procedure for the synthesis of compounds (63a-c)



Oxidative removal of *p*-methoxy benzyl group of compounds **62a-c** prepared in experimental section **5.1.21.** was accomplished using the method reported by Masanori Yamaura et al **[287]**. Compounds **62a-c** (8 mmol) were dissolved in a mixture of acetonitrile (100 ml) and D. M. water (33 ml) and aqueous 0.25M ceric ammonium nitrate solution (32 mmol) was added in a single portion and stirred at room temperature for 2h.

After completion of reaction (TLC), the reaction mixture was diluted with D. M. water (100 ml) and extracted with ethyl acetate (6X100 ml). Combined organic layers were washed with sat. $NaHCO_3$ solution (2X100 ml) and brine (1X100 ml). Organic layer was dried over anhy. Na_2SO_4 and solvent was removed under reduced pressure. Crude product thus obtained was purified by column chromatography (stationary phase: 100-200 mesh silica, Mobile phase: 0-3% Methanol in DCM gradient) to give title compounds **63a-c** as a white solid.

5.1.22.1. tert-Butyl (((3R,4S)-4-(2,5-difluorophenyl)-6-oxopiperidin-3-yl)methyl) carbamate (63a)



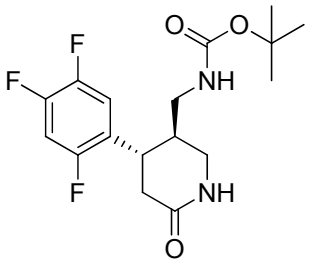
63a (1.98 g, 68%) was prepared from **62a** by means of the general procedure described in **5.1.22.** as a White solid; Mp: 243-246^{0}C, Purity by HPLC: 98.27% AUC.

ESI/MS (m/z) : 341.2 $(M+H)^+$. **Mol. Wt.** = 340.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.39 (s, 9H), 2.34-2.49 (m, 2H), 2.89-2.93 (m, 1H), 3.09-3.13 (m, 1H), 3.31-3.34 (m,2H), 3.51-3.57 (m, 2H), 4.48 (bs, 1H), 6.08 (bs, 1H), 6.92-6.99 (m, 2H), 7.09-7.12 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2, 36.4, 38.5, 41.3, 41.8, 46.1, 80.3, 109.8 (d, J = 24Hz), 117.3 (d, J = 2.4Hz), 118.6 (d, J = 39Hz), 127.3 (d, J = 23Hz), 149.3 (d, J = 253Hz), 156.3 (d, J = 250Hz), 159.3, 170.6.

5.1.22.2. tert-Butyl (((3R,4S)-6-oxo-4-(2,4,5-trifluorophenyl)piperidin-3-yl)methyl) carbamate (63b)

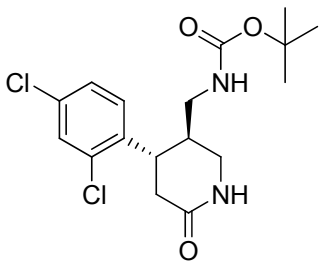


63b (2.1 g, 72%) was prepared from **62b** by means of the general procedure described in **5.1.22.** as a White solid; Mp: 278-281^{0}C, Purity by HPLC: 97.14% AUC.

ESI/MS (m/z) : 359.3 $(M+H)^+$. **Mol. Wt.** = 358.4 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43 (s, 9H), 2.34-2.49 (m, 2H), 2.89-2.93 (m, 1H), 3.10-3.16 (m, 1H), 3.31-3.34 (m,2H), 3.51-3.57 (m, 2H), 4.52 (bs, 1H), 6.09 (bs, 1H), 6.96-7.04 (m, 1H), 7.09-7.11 (m, 1H).

5.1.22.3. tert-Butyl (((3R,4S)-4-(2,4-dichlorophenyl)-6-oxopiperidin-3-yl)methyl) carbamate (63c)

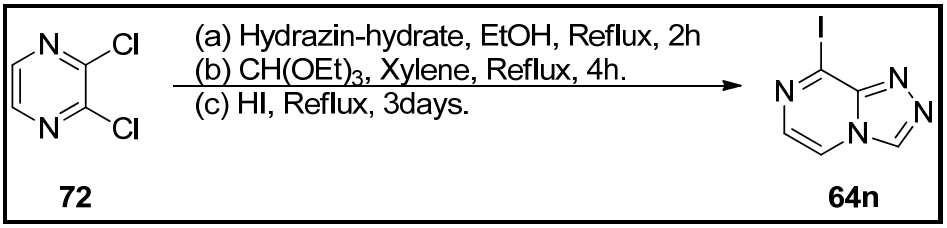


63c (1.8 g, 62%) was prepared from **62c** by means of the general procedure described in **5.1.22.** as a White solid; Mp: 185-189^{0}C, Purity by HPLC: 96.76% AUC.

ESI/MS (m/z) : 373.9 $(M+H)^+$. **Mol. Wt.** = 373.3 g.

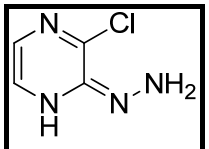
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.41 (s, 9H), 2.34-2.49 (m, 2H), 2.89-2.93 (m, 1H), 3.09-3.15 (m, 1H), 3.31-3.34 (m,2H), 3.51-3.57 (m, 2H), 4.49 (bs, 1H), 6.08 (bs, 1H), 7.21 (d, 1H, J = 8.4 Hz), 7.34 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.4 Hz), 7.63 (d, 1H, J = 2.0 Hz).

5.1.23. Procedure for the synthesis of 8-Iodo-[1,2,4]triazolo[4,3-a]pyrazine (64n)



72 **64n**

Step a: (E)-3-Chloro-2-hydrazono-1,2-dihydropyrazine (73)



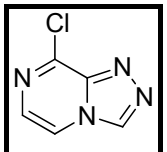
73

2,3-Dichloropyrazine **72** (4 ml, 26 mmol) was dissolved in 95% ethanol (8 ml) and to this was added, dropwise and with stirring, hydrazine anhydrous (4 ml, 134 mmol). During the addition of the Hydrazine the solution became warm and yellowish. Reaction mixture was refluxed for 2h. After completion and cooling of this mixture in an ice bath, the resulting solid material was isolated by filtration, washed with cold aqueous 95% ethanol and dried well under suction to give 3.02 g (73% yield) of (E)-3-chloro-2-hydrazono-1,2-dihydropyrazine **73** as white crystals. Purity by HPLC: 97.67% AUC.

ESI/MS (m/z) : 145.3 $(M+H)^+$. **Mol. Wt.** = 144.6 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.94 (d, 1H, J = 7.8 Hz), 7.21 (bs, 2H, -NH-N**H_2**), 8.12 (d, 1H, J = 7.8 Hz), 12.89 (s, 1H, -N**H**-NH_2)

Step b: 8-Chloro-[1,2,4]triazolo[4,3-a]pyrazine (74)



74

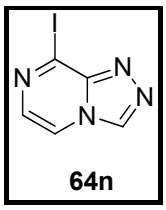
To (E)-3-chloro-2-hydrazono-1,2-dihydropyrazine **73** (2.0 g, 13.84 mmol), prepared in **step a** was added trimethyl orthoformate (30 ml). After refluxing for 10 h, the reaction

was cooled to room temperature and the precipitated product was filtered. Washed the product with ether and dried well under suction to give 1.27 g (62% yield) of the title compound **74** as a white solid. Purity by HPLC: 95.27% AUC.

ESI/MS (m/z) : 155.0 $(M+H)^+$. **Mol. Wt.** = 154.6 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, 2H, J = 5.2 Hz), 8.50 (d, 2H, J = 5.2 Hz). 9.40 (s, 1H).

Step c: 8-Iodo-[1,2,4]triazolo[4,3-a]pyrazine (64n)



64n

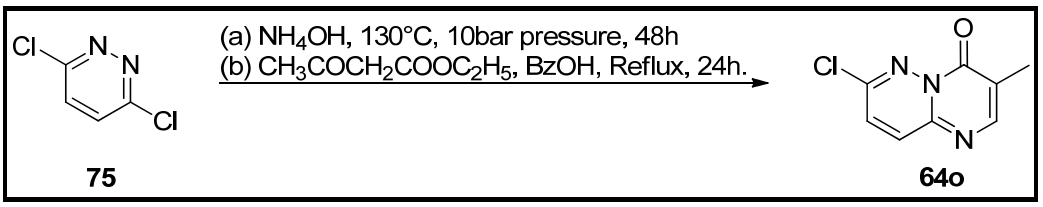
In a 25 ml 2-neck R. B. Flask placed 8-chloro-[1,2,4]triazolo[4,3-a]pyrazine **74** (7.75 mmol), to it charged sodium iodide (11.64 mmol) and Hydroiodic acid (57wt% in water, stabilized with <1.5%hypophosphorous acid) (77.5 mmol). Reaction mixture was heated to reflux and stirred at reflux temperature for 3 days.

After completion of reaction, reaction mixture was cooled to room temperature and basified with 25% aq. NaOH. Aqueous layer was then extracted with DCM (4X 50 ml). dried over anhy. Na_2SO_4 and concentrated under reduced pressure. Crude product thus obtained was crystallized from IPA to give 1.44 g (76% yield) of the title compound **64n** as a light yellow solid. Purity by HPLC: 97.43% AUC.

ESI/MS (m/z) : 247.1 $(M+H)^+$. **Mol. Wt.** = 246.0 g.

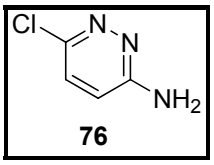
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, 2H, J = 5.0 Hz), 8.63 (d, 2H, J = 5.0 Hz). 9.41 (s, 1H).

5.1.24. Procedure for the synthesis of 7-Chloro-3-methyl-4H-pyrimido[1,2-b]pyridazin-4-one (64o)



75 **64o**

Step a: 6-Chloropyridazin-3-amine (76)



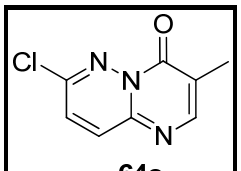
76

3,6-Dichloropyridazine **75** (33.5 mmol) was placed in an autoclave of 300 ml capacity. To it charged ethanol (50 ml) and liquor NH_3 (100 ml, 20 vol. by wt. of starting material) and the clear solution formed was heated to 130 $^{\circ}$C. Reaction mixture was stirred at same temperature for 48h under 10bar pressure. After completion, Crystals precipitated in the reaction mixture were filtered and washed with cold ethanol (2X20 ml). Filtrate was concentrated to a volume of 40 ml under reduced pressure, solid precipitated from filtrate was filtered and washed with ethanol (2X10 ml). Combined solid was dried well under reduced pressure till constant weight to give 3.37 g (78% Yield) of 6-chloropyridazin-3-amine **76** as an off white crystalline solid. Mp: 226-228^{0}C, Purity by HPLC: 96.43% AUC.

ESI/MS (m/z) : 130.4 $(M+H)^+$. **Mol. Wt. =** 129.5 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 6.61 (bs, 2H), 6.83 (d, 1H, J = 9.2 Hz), 7.36 (d, 1H, J = 9.2 Hz). 7.81 (bs, 2H).

Step b: 7-Chloro-3-methyl-4H-pyrimido[1,2-b]pyridazin-4-one (64o)



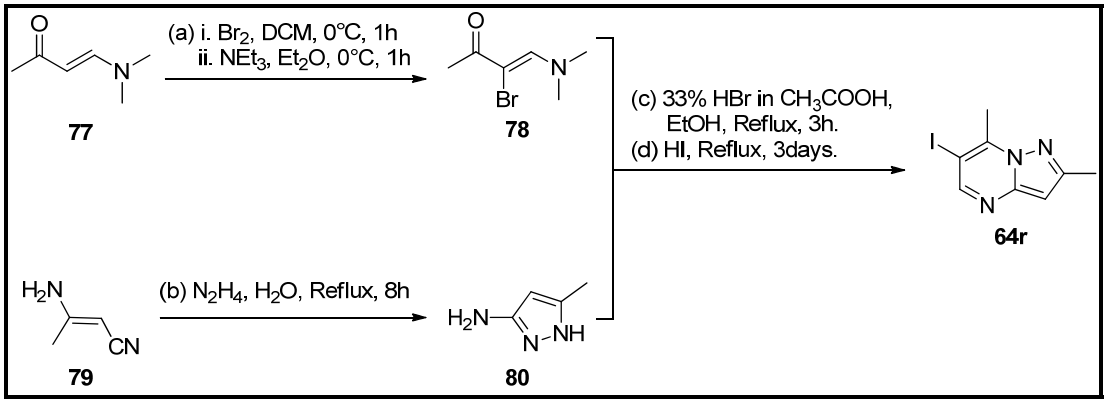
64o

7-Chloro-3-methyl-4H-pyrimido[1,2-b]pyridazin-4-one **64o** was prepared by optimizing a route reported by Avellana et al **[272]**. To a stirred solution of 6-chloropyridazin-3-amine **76** (3.0 g, 23.1 mmol) in benzyl alcohol (15 ml) was added ethyl acetoacetate (4.41 ml, 34.65 mmol). The resulting solution was refluxed for 24 h then evaporated in vacuo to yield a crude solid which was purified by flash chromatography (230-400 mesh silica gel, 0 to 80% ethyl acetate/hexanes gradient) to give 7-chloro-3-methyl-4H-pyrimido[1,2-b]pyridazin-4-one **64o** as a light yellow crystalline solid. Mp: 193-196^{0}C dec., Purity by HPLC: 98.27% AUC.

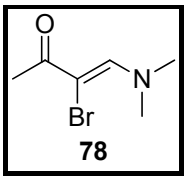
ESI/MS (m/z) : 196.3 $(M+H)^+$. **Mol. Wt. =** 195.6 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H), 6.84 (d, 1H, J = 8.2 Hz), 7.06 (d, 1H, J = 8.2 Hz). 7.68 (s, 1H).

5.1.25. procedure for the synthesis of 6-Iodo-2,7-dimethylpyrazolo[1,5-a]pyrimidine (64r)



Step a: 3-Bromo-4-(dimethylamino)but-3-en-2-one (78)

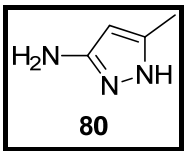


To an ice cold solution of (E)-4-(dimethylamino)but-3-en-2-one (5.0 g, 44.25 mmol) in DCM (50 ml) was added dropwise Bromine (2.71 ml, 48.67 mmol) via an addition funnel. The reaction mixture was stirred at 0 °C for 0.5 h and then Triethylamine (7.4 ml, 48.67 mmol) in 10 ml of ether was added dropwise. The mixture was stirred at 0 °C for 1 h and allowed to warm up to room temperature. A light yellow solid precipitated from the solution, and it was filtered. The filtrate was then concentrated to give 7.05 g (82% Yield) of 3-Bromo-4-(dimethylamino)but-3-en-2-one **78** as a yellow solid. The product was used in the next reaction without any further purification. Mp: 89-93^{0}C, Purity by HPLC: 93.47% AUC.

ESI/MS (m/z) : 193.0 $(M+H)^+$. **Mol. Wt.** = 192.1 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.31 (s, 3H), 2.93 (s, 6H), 7.32 (s, 1H).

Step b: 5-Methyl-1H-pyrazol-3-amine (80)



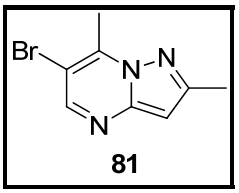
80

3-Aminocrotonnitrile **79** (5.0 g, 61 mmol) was dissolved in ethanol (25 ml) at room temperature and 85% hydrazine-hydrate (80 ml) was added there to, followed by stirring the mixture at room temperature. The mixture was heated to an internal temperature of 65 °C and stirring was conducted for 12 hours. After cooling the mixture to room temperature, the solvent was distilled off under reduced pressure to obtain 4.55 g (77% Yield) of 5-methyl-1H-pyrazol-3-amine **80** as a brown oily compound. The product was used in the next reaction without further purification. Purity by HPLC: 95.28% AUC.

ESI/MS (m/z) : 98.3 $(M+H)^+$. **Mol. Wt. =** 97.1 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (3H, s), 5.42 (1H, s).

Step c: 6-Bromo-2,7-dimethylpyrazolo[1,5-a]pyrimidine (81)



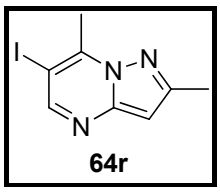
81

To a solution of 3-bromo-4-(dimethylamino)but-3-en-2-one **78** (4.0 g, 20.8 mmol) and 5-methyl-1H-pyrazol-3-amine **80** (2.02 g, 20.8 mmol) in ethanol (50 ml) was added 33% HBr in acetic acid solution (2.6 ml, mmol) and the resulting mixture was heated at reflux for 3 h. The reaction mixture was cooled to room temperature and concentrated to give brown residue which was titurated with 30% ethyl acetate in hexane (100 ml) to give a precipitates which were filtered, and then washed with ethyl acetate (2X50 ml). The combined organic extracts were evaporated to give orange solid which was chromatographed (100-200 mesh silica gel, 0% to 10% ethyl acetate in DCM gradient) to give 2.64 g (57% Yield) of 6-bromo-2,7-dimethylpyrazolo[1,5-a]pyrimidine **81** as a yellow solid. Mp: 232-234^{0}C, Purity by HPLC: 97.14% AUC.

ESI/MS (m/z) : 227.0 $(M+H)^+$. **Mol. Wt. =** 226.1 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (3H, s), 2.67 (s, 3H), 5.69 (1H, s), 8.52 (s, 1H).

Step d: 6-Iodo-2,7-dimethylpyrazolo[1,5-a]pyrimidine (64r)



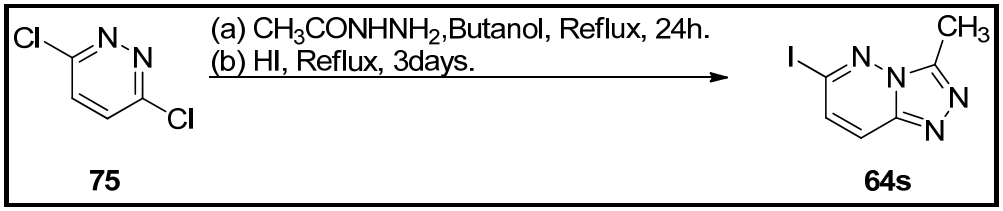
64r

64r (1.6 g, 68%) was prepared by means of the general procedure described in experimental **section 5.1.23. step-c** as a yellow solid; Mp: 187-189^{0}C, Purity by HPLC: 97.36% AUC.

ESI/MS (m/z) : 274.2 $(M+H)^+$. **Mol. Wt. =** 273.1 g.

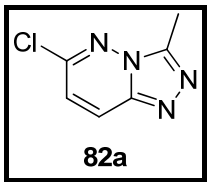
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (3H, s), 2.68 (s, 3H), 5.69 (1H, s), 8.63 (s, 1H).

5.1.26. Procedure for the synthesis of 6-Iodo-3-methyl-[1,2,4]triazolo[4,3-b]pyridazine (64s)



75 **64s**

Step a: 6-Chloro-3-methyl-[1,2,4]triazolo[4,3-b]pyridazine (82a)



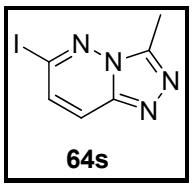
82a

To a stirred solution of 3,6-dichloropyridazine **75** (2.0 g, 13.4 mmol) in butanol (10 ml) was added 500 mg (6.7 mmol) of acetic hydrazide (1.0 g, 13.4 mmol) and the resulting solution was stirred under nitrogen at refluxed for 24 h. The reaction mixture was then cooled to ambient temperature, filtered, and the resulting precipitates were washed with ethyl acetate and methanol. The combined filtrate and washings were concentrated and dissolved in mixture of 10:1 chloroform/methanol (250 ml) then washed with brine solution (2X100 ml). The organic phase was then dried over anhy. Na_2SO_4, filtered, and evaporated in vacuo to yield a yellow solid. The crude material was purified by flash chromatography (230-400 mesh silica gel, 0 to 80% ethyl acetate/hexanes gradient) to give 1.56 g (69% Yield) of the title compound **82a** as light yellow crystalline solid.. Mp: 163-165^{0}C, Purity by HPLC: 98.79% AUC.

ESI/MS (m/z) : 168.9 $(M+H)^+$. **Mol. Wt.** = 168.6 g.

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.67 (3H, s), 7.24 (d, 1H, J = 9.6 Hz), 8.43 (d, 1H, J = 9.6 Hz).

Step b: 6-Iodo-3-methyl-[1,2,4]triazolo[4,3-b]pyridazine (64s)



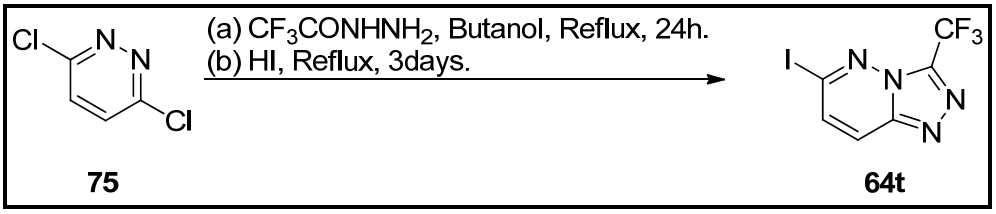
64s

64s (1.2 g, 64%) was prepared by means of the general procedure described in experimental **section 5.1.23. step-c** as a light yellow solid; Mp: 194-197^{0}C, Purity by HPLC: 96.62% AUC.

ESI/MS (m/z) : 260.8 $(M+H)^+$. **Mol. Wt.** = 260.0 g.

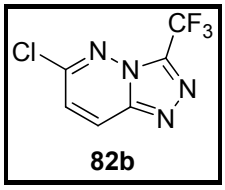
^{1}H NMR (400 MHz, DMSO-d_6): δ 2.67 (3H, s), 7.26 (d, 1H, J = 9.8 Hz), 8.44 (d, 1H, J = 9.8 Hz).

5.1.27. Procedure for the synthesis of 6-Iodo-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazine (64t)



75 **64t**

Step a: 6-Chloro-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazine (82b)



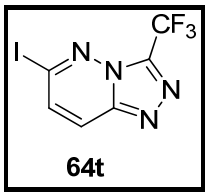
82b

82b (2.3 g, 64%) was prepared using 2,2,2-trifluoro acetic hydrazide by means of the general procedure described in experimental **section 5.1.23. step-c** as a white solid; Mp: 159-161^{0}C, Purity by HPLC: 95.78% AUC.

ESI/MS (m/z) : 223.2 $(M+H)^+$. **Mol. Wt.** = 222.6 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, 1H, J = 9.8 Hz), 8.46 (d, 1H, J = 9.8 Hz).

Step b: 6-Iodo-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazine (64t)



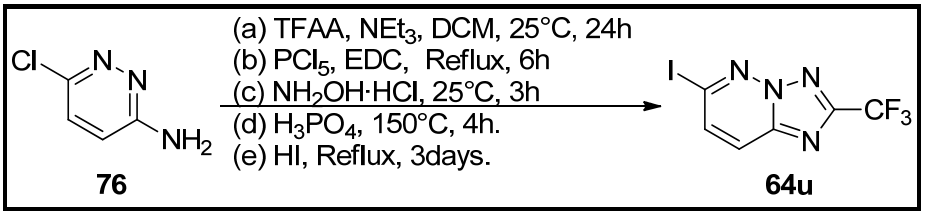
64t

64t (1.03 g, 67%) was prepared by means of the general procedure described in experimental **section 5.1.23. step-c** as a light yellow solid; Mp: 208-210^{0}C, Purity by HPLC: 95.32% AUC.

ESI/MS (m/z) : 314.9 $(M+H)^+$. **Mol. Wt.** = 314.0 g.

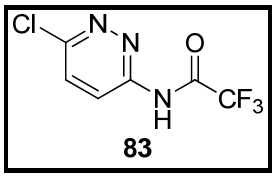
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (d, 1H, J = 9.8 Hz), 8.46 (d, 1H, J = 9.8 Hz).

5.1.28. Procedure for the synthesis of 6-Iodo-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-b]pyridazine (64u)



76 **64u**

Step a: N-(6-Chloropyridazin-3-yl)-2,2,2-trifluoroacetamide (83)



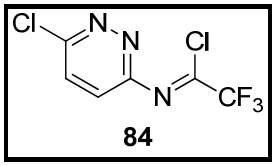
83

To a solution of 6-chloropyridazin-3-amine **76** (4 g, 30 mmol) and triethylamine (4.8 ml, 34 mmol) in DCM (150 ml) at 0 ^{0}C was carefully added trifluoroacetic acid anhydride (4.2 ml, 30 mmol). The resulting solution was allowed to warm to ambient temperature then concentrated in vacuo and dissolved in a mixture of 3: 1 chloroform/IPA (200 ml). The resulting solution was washed sequentially with saturated $NaHCO_3$ solution and brine solution (200 ml each). The organic phase was then dried over anhy. Na_2SO_4 and evaporated in vacuo to yield a crude oil which was purified by column chromatography to give 6.2 g (89% yield) of N-(6-chloropyridazin-3-yl)-2,2,2-trifluoroacetamide **83** as a colourless oil; Purity by HPLC: 99.03% AUC.

ESI/MS (m/z) : 242.9 $(M+NH_4)^+$. **Mol. Wt.** = 225.6 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, 1H, J = 9.6 Hz), 8.46 (d, 1H, J = 9.6 Hz), 9.16 (bs, 1H).

Step b: (Z)-N-(6-Chloropyridazin-3-yl)-2,2,2-trifluoroacetimidoyl chloride (84)

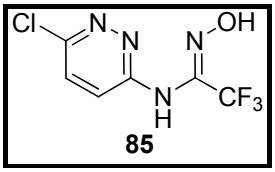


84

A solution of N-(6-chloropyridazin-3-yl)-2,2,2-trifluoroacetamide **83** (3.8 g, 17 mmol) and phosphorous pentachloride (4.2 g, 22 mmol) in dichloroethane (200 ml) was heated at reflux temperature under nitrogen. After 6 h the reaction was cooled to ambient temperature then concentrated in vacuo to give 3.87 g (95% yield) of (Z)-N-(6-chloropyridazin-3-yl)-2,2,2-trifluoroacetimidoyl chloride **84** as a yellow oil. The product was used in the next reaction without any further purification.

ESI/MS (m/z) : 244.7 $(M+H)^+$. **Mol. Wt.** = 244.0 g.

Step c: (E)-N-(6-Chloropyridazin-3-yl)-2,2,2-trifluoro-N'-hydroxyacetimidamide (85)



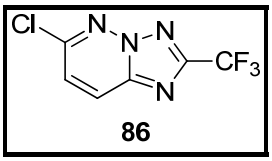
85

To a solution of the crude product (Z)-N-(6-chloropyridazin-3-yl)-2,2,2-trifluoroacetimidoyl chloride **84** (3.0 g, 12.3 mmol), in dry THF (150 ml) was carefully added hydroxylamine-hydrochloride (1.03 g, 14.7 mmol). The resulting solution was stirred under nitrogen at room temperature. After 1 h the solution was concentrated in vacuo then dissolved in 100 ml ethyl acetate and washed sequentially with saturated $NaHCO_3$ solution and brine (100 ml each). The organic phase was then dried over anhy. Na_2SO_4 and evaporated in vacuo to give 2.63 g (89% Yield) of (E)-N-(6-chloropyridazin-3-yl)-2,2,2-trifluoro-N'-hydroxyacetimidamide **85** as a white solid; Mp: 259-261^{0}C, Purity by HPLC: 97.83% AUC.

ESI/MS (m/z) : 241.0 $(M+H)^+$. **Mol. Wt.** = 240.6 g.

^{1}H NMR (400 MHz, DMSO-*d_6*): δ 7.11 (d, 1H, J = 9.2 Hz), 7.69 (d, 1H, J = 9.2 Hz), 9.78 (bs, 1H), 12.32 (bs, 1H).

Step d: 6-Chloro-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-b]pyridazine (86)



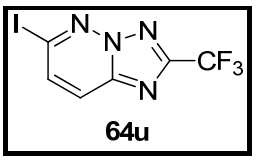
86

To (E)-N-(6-Chloropyridazin-3-yl)-2,2,2-trifluoro-N'-hydroxyacetimidamide **85** (2.2 g, 9.0 mmol) prepared in **Step C** was added concentrated polyphosphoric acid (2.0 ml) and the resulting mixture was stirred under nitrogen at 150 °C for 4 h. The reaction mixture was then cooled to 0 °C and quenched carefully with concentrated ammonium hydroxide solution until the solution was basic by pH paper. The resulting solution was then extracted with ethyl acetate (3X100 ml), and the organic phases combined and washed with brine solution (1X250 ml). The organic phase was then dried over anhy. Na_2SO_4, filtered, and evaporated in vacuum to yield a crude oil. The crude material was purified by flash chromatography (100-200 mesh silica gel, 0 to 80% ethyl acetate/Hexanes gradient) to give 1.59 g (78% Yield) of 6-chloro-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-b]pyridazine **86** as a yellow crystalline solid. Mp: 274-276^{0}C, Purity by HPLC: 99.15% AUC.

ESI/MS (m/z) : 247.0 $(M+Na)^+$. **Mol. Wt.** = 222.6 g.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.06 (d, 1H, J = 9.2 Hz), 8.70 (d, 1H, J = 9.2 Hz).

Step e: 6-Iodo-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-b]pyridazine (64u)



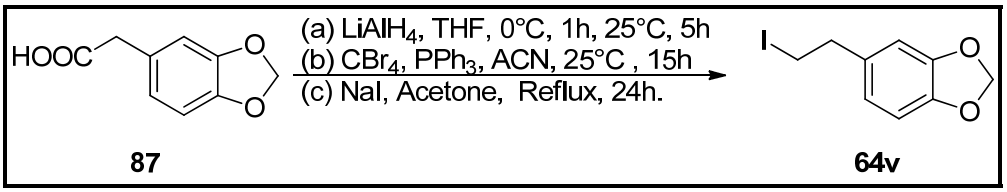
64u

64u (1.5 g, 67%) was prepared by means of the general procedure described in experimental **section 5.1.23. step-c** as a light yellow solid; Mp: 287-293^{0}C dec., Purity by HPLC: 97.63% AUC.

ESI/MS (m/z) : 314.5 $(M+H)^+$. **Mol. Wt.** = 314.0 g.

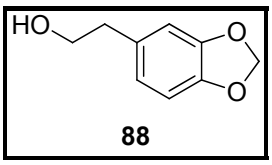
^{1}H NMR (400 MHz, CDCl$_3$): δ 8.07 (d, 1H, J = 9.2 Hz), 8.73 (d, 1H, J = 9.6 Hz).

5.1.29. Procedure for the synthesis of 5-(2-Iodoethyl)benzo[d][1,3]dioxole (64v)



87 **64v**

Step a: 2-(Benzo[d][1,3]dioxol-5-yl)ethanol (88)



88

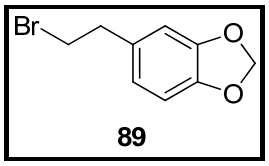
2-(Benzo[d][1,3]dioxol-5-yl)acetic acid **87** (5.0 g, 27.8 mmol) was dissolved in dry THF (60 ml), cooled to 0^0C and $LiAlH_4$ (2.3 g, 62.2 mmol) was added in portions under nitrogen atmosphere. Reaction mixture was stirred at same temperature for 1h then brought to room temperature and stirred for 4h.

After completion of reaction, reaction mixture was quenched with sat. Na_2SO_4 solution (10 ml), diluted with DCM and was filtered through celite. Filtrate was evaporated to dryness to give 3.69 g (80% Yield) of 2-(benzo[d][1,3]dioxol-5-yl)ethanol **88** as a colourless oil. Purity by HPLC; 94.89% AUC.

ESI/MS (m/z) : 167.1 $(M+H)^+$. **Mol. Wt.** = 166.2 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.85 (t, 2H, J = 7.4Hz), 3.46 (t, 2H, J = 7.4Hz), 5.91 (s, 2H), 6.33 (dd, 1H, J_1 = 2.4Hz, J_2 = 8.4Hz), 6.55 (d, 1H, J= 2.4Hz), 6.70 (d, 1H, J= 8.4Hz).

Step b: 5-(2-Bromoethyl)benzo[d][1,3]dioxole (89)



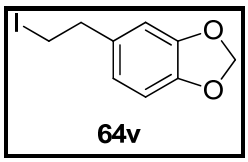
89

To a solution of 2-(benzo[d][1,3]dioxol-5-yl)ethanol **88** (3.5 g, 21.08 mmol) and Triphenylphosphene (4.97 g, 18.97 mmol) in acetonitrile (42 ml) was added carbon tetrabromide (7.13 g, 21.5 mmol) in portions. Resulting solution was stirred at room temperature for 15h. After completion of the reaction, solvent of the reaction mixture was evaporated under reduced pressure and the residue obtained was purified by column chromatography (100-200 mesh silica gel, 0 to 30% ethyl acetate/hexanes gradient) to yield 3.96 g (82% Yield) of 5-(2-bromoethyl)benzo[d][1,3]dioxole **89** as a colourless oil. Purity by HPLC: 96.31% AUC.

ESI/MS (m/z) : 228.4 $(M+H)^+$. **Mol. Wt.** = 229.1 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.05 (t, 2H, J = 7.4Hz), 3.51 (t, 2H, J = 7.4Hz), 5.93 (s, 2H), 6.32 (dd, 1H, J_1 = 2.4Hz, J_2 = 8.4Hz), 6.57 (d, 1H, J= 2.4Hz), 6.71 (d, 1H, J= 8.4Hz).

Step c: 5-(2-Iodoethyl)benzo[d][1,3]dioxole (64v)



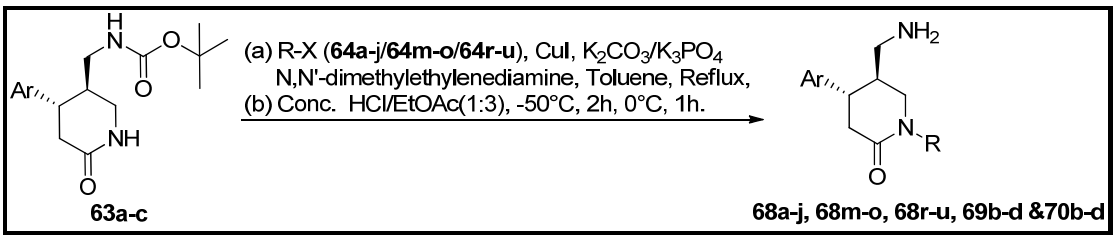
64v

To a solution of 5-(2-bromoethyl)benzo[d][1,3]dioxole **89** (3.5 g, 15.3 mmol) in acetone (52.5 ml) was added sodium iodide (11.5 g, 76.5 mmol). Reaction content was refluxed for 12h when additional sodium iodide (5.575 g, 38.25 mmol) was added and refluxed for 24h. After completion of reaction, solvent of the reaction mixture was evaporated under reduced pressure and the residue obtained was purified by column chromatography (100-200 mesh silica gel, 0 to 30% ethyl acetate/hexanes gradient) to give 3.92 g (92% Yield) of 5-(2-iodoethyl)benzo[d][1,3]dioxole **64v** as a colourless oil. Purity by HPLC: 97.86% AUC.

ESI/MS (m/z) : 277.3 $(M+H)^+$. **Mol. Wt.** = 276.1 g.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.07 (t, 2H, J = 7.6Hz), 3.69 (t, 2H, J = 7.6Hz), 5.98 (s, 2H), 6.32 (dd, 1H, J_1 = 1.8Hz, J_2 = 8.4Hz), 6.57 (d, 1H, J= 1.8Hz), 6.72 (d, 1H, J= 8.4Hz).

5.1.30. General procedure for the synthesis of compounds (68a-j, 68m-o, 68r-u, 69b-d & 70b-d)



63a-c **68a-j, 68m-o, 68r-u, 69b-d &70b-d**

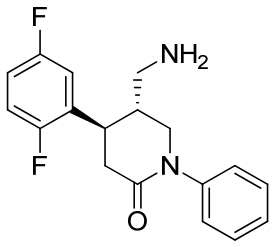
Synthesis of amino-methyl pipyridones **68a-j, 68m-o, 68r-u, 69b-d & 70b-d** was accomplished with Goldberg reaction reported by Klapars et al **[264]**. In a 50 ml 2-neck R. B. Flask placed compound **63a-c** (5 mmol), respective halo-aromatics **64a-j**, **64m-o** or **64r-u** (7.5 mmol), dry K_2CO_3 (10.5 mmol) and CuI (0.5 mmol) followed by addition of dry toluene (20 ml). Reaction mixture was purged with nitrogen and stirred for 30 min. at room temperature then N,N'-dimethyl ethylenediamine (1.0 mmol) was added and reaction mixture was purged again with nitrogen. Reaction mixture was then refluxed for 15h.

After completion of the reaction mixture was diluted with D. M. water (50 ml) and extracted with ethyl acetate (3X50 ml). Combined organic extracts were washed with 1N HCl (1X50 ml), sat. $NaHCO_3$ solution (1X50 ml) and brine (1X50 ml). Organic layer was dried over anhy. Na_2SO_4 and solvent was removed under reduced pressure to give amide -NH alkylated product with 65-80% yield.

Crude product thus obtained was dissolved in ethyl acetate (45 ml) and concentrated HCl (15 ml) was added at -50^{0}C and the reaction mixture was stirred at same temperature for 2h. Temperature of the reaction mixture was then gradually increased to 0 $^\circ$C and stirred for additional 1h at this temperature. After completion of reaction, reaction mixture was basified with sat. $NaHCO_3$ solution till pH 9 and extracted with ethyl acetate (3X50 ml). Combined organic extracts were washed with water (1X100 ml) and brine (1X100 ml). Organic layer was then dried over anhy. Na_2SO_4 and evaporated to dryness.

Crude residue thus obtain was purified by preparative HPLC method using the procedure as described in experimental **section 5.1.3.-Purification** to give desired amino-methyl pipyridones **68a-j, 68m-o, 68r-u, 69b-d & 70b-d**.

5.1.30.1. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-phenylpiperidin-2-one (68a)



68a (510 mg, 72%) was prepared by means of the general procedure as in section **5.1.30.** as a white solid. 179-182 °C; Purity by HPLC: 98.76% AUC, Chiral purity: 97.2%ee AUC.

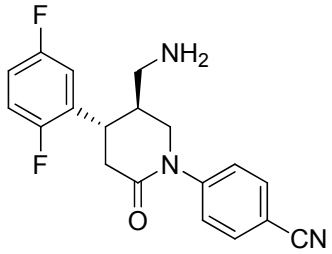
ESI/MS (m/z) : 485.4 $(M+H)^+$. **Mol. Wt.** = 484.5 g.

^{1}H NMR (400 MHz, Methanol-d$_4$): δ 2.33-2.38 (m, 1H), 2.41-2.49 (m, 3H), 2.73-2.78 (m, 1H), 3.25 (dd, J_1 = 10Hz, J_2 = 12Hz, 1H), 3.45-3.52 (m, 2H), 7.01-7.15 (m, 4H), 7.20 (dd, J_1 = 3.6Hz, J_2 = 8.2Hz, 1H), 7.22-7.25 (m, 2H), 7.28-7.31 (m, 1H).

^{13}C NMR (100 MHz, Methanol-d$_4$): δ 36.5, 40.3, 41.2, 47.3, 52.1, 115.4 (d, J = 25.1Hz), 117.7 (dd, J_1 = 24.9Hz, J_2 = 8.8Hz), 118.5 (dd, J_1 = 24.1Hz, J_2 = 9.0Hz), 119.3, 123.7, 127.6, 128.2, 155.2 (d, J = 241.3Hz), 157.6 (d, J = 240.1Hz), 168.7.

Analysis : Mol. Formula: $C_{18}H_{18}F_2N_2O$
Calcd.: C 68.34, H 5.74, N 8.86.
Found: C 68.30, H 5.77, N 8.83.

5.1.30.2. 4-((4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-2-oxopiperidin-1-yl) benzonitrile (68b)



68b (524 mg, 78%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 166-168 °C; Purity by HPLC: 99.1% AUC, Chiral purity: 97.7%ee AUC.

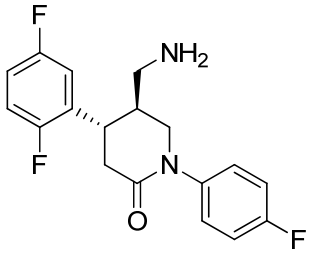
ESI-MS: $[M+H]^+$ = 342.1 *m/z*. **Mol.Wt** = 341.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.37 (m, 1H), 2.41-2.48 (m, 3H), 2.74-2.79 (m, 1H), 3.25-3.30 (m, 1H), 3.47-3.54 (m, 2H), 7.22-7.27 (m, 2H), 7.29-7.33 (m, 1H), 7.53 (d, J = 8.1Hz, 2H), 7.81 (d, J = 8.1Hz, 2H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 36.9, 40.4, 41.7, 47.9, 53.2, 111.3, 115.3 (d, J = 24.9Hz), 117.9 (dd, J_1 = 24.9Hz, J_2 = 8.8Hz), 118.4 (dd, J_1 = 24.3Hz, J_2 = 8.8Hz), 118.7, 122.7, 127.6, 128.2, 132.4, 155.6 (d, J = 240.8Hz), 157.9 (d, J = 240.3Hz), 169.8.

Analysis : Mol. Formula: $C_{19}H_{17}F_2N_3O$
Calcd.: C 66.85, H 5.02, N 12.31.
Found: C 66.86, H 5.07, N 12.34.

5.1.30.3. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(4-fluorophenyl) piperidin-2-one (68c)



68c (563 mg, 87%) was prepared using general procedure described in section **5.1.30.** as a white solid. 201-203 °C; Purity by HPLC: 98.6% AUC, Chiral purity: 98.7%ee AUC.

ESI-MS: $[M+H]^+$ = 335.5 *m/z*. **Mol.Wt** = 334.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.29-2.35 (m, 1H), 2.43-2.45 (m, 2H), 2.51-2.57 (m, 1H), 2.75-2.79 (m, 1H), 3.27 (dd, J_1 = 9.8Hz, J_2 = 12.1Hz, 1H), 3.46-3.52 (m, 2H), 7.22-7.25 (m, 2H), 7.28-7.31 (m, 3H), 7.39 (d, J = 7.8Hz, 2H).

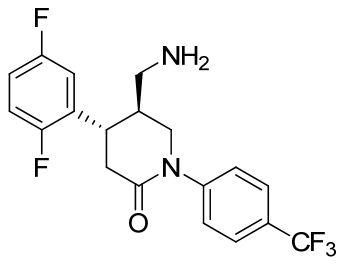
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.6, 40.1, 41.2, 47.5, 53.4, 115.4 (d, J = 25.2Hz), 115.7, 117.6 (dd, J_1 = 24.9Hz, J_2 = 9.0Hz), 118.6 (dd, J_1 = 24.4Hz, J_2 = 9.0Hz), 119.3, 123.7, 135.4, 155.2 (d, J = 241.3Hz), 157.6 (d, J = 240.1Hz), 161.2 (d, 237.2Hz), 170.4.

Analysis : Mol. Formula: $C_{18}H_{17}F_3N_2O$

Calcd.: C 64.66, H 5.13, N 8.38.

Found: C 64.61, H 5.17, N 8.34.

5.1.30.4. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(4-(trifluoromethyl)-phenyl) piperidin-2-one (68d)



68d (570 mg, 85%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 193-195 °C; Purity by HPLC: 97.7% AUC, Chiral purity: 97.9%ee AUC.

ESI-MS: $[M+H]^+$ = 385.2 *m/z*. **Mol.Wt** = 384.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.34-2.39 (m, 1H), 2.41-2.47 (m, 3H), 2.72-2.78 (m, 1H), 3.27 (dd, J_1 = 10Hz, J_2 = 11.8Hz, 1H), 3.45-3.52 (m, 2H), 7.13 (d, J = 7.6Hz, 2H), 7.22-7.25 (m, 2H), 7.28-7.31 (m, 1H), 7.53 (2H, J = 7.6Hz, 2H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 36.9, 40.7, 41.3, 47.3, 53.2, 115.5 (d, J = 25.1Hz), 117.7 (dd, J_1 = 25.0Hz, J_2 = 8.8Hz), 118.5 (dd, J_1 = 24.3Hz, J_2 = 9.0Hz), 123.8, 124.5, 125.4, 127.6, 128.2, 132.6, 143.2, 155.2 (d, J = 241.3Hz), 157.6 (d, J = 240.1Hz), 170.7.

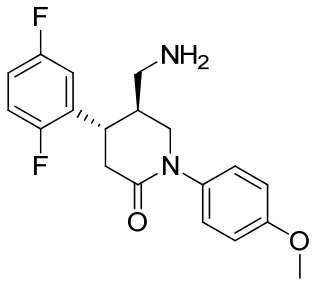
Anal. Calcd. for $C_{19}H_{17}F_5N_2O$: C 59.37, H 4.46, N 7.29; Found: C 59.33, H 4.42, N 7.26.

Analysis : Mol. Formula: $C_{19}H_{17}F_5N_2O$

Calcd.: C 59.37, H 4.46, N 7.29.

Found: C 59.33, H 4.42, N 7.26.

5.1.30.5. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(4-methoxyphenyl) piperidin-2-one (68e)



68e (538 mg, 83%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 175-178 °C; Purity by HPLC: 98.3% AUC, Chiral purity: 97.1%ee AUC.

ESI-MS: $[M+H]^+$ = 347.5 *m/z*. **Mol.Wt** = 346.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.37 (m, 1H), 2.43-2.48 (m, 3H), 2.72-2.79 (m, 1H), 3.25-3.29 (m, 1H), 3.47-3.54 (m, 2H), 3.81 (s, 3H), 7.11 (d, J = 8.2 Hz, 2H), 7.22-7.27 (m, 2H), 7.29-7.39 (m, 3H).

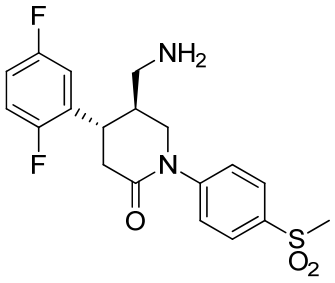
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.7, 40.2, 41.6, 47.9, 53.3, 56.4, 114.8, 115.5 (d, J = 25.1Hz), 117.9 (dd, J_1 = 25.0Hz, J_2 = 9.0Hz), 118.7 (dd, J_1 = 24.3Hz, J_2 = 8.8Hz), 122.7, 123.4, 128.2, 132.1, 155.6 (d, J = 239.8Hz), 157.4 (d, J = 240.1Hz), 159.2, 168.5.

Analysis : Mol. Formula: $C_{19}H_{20}F_2N_2O_2$

Calcd.: C 65.88, H 5.82, N 8.09.

Found: C 65.85, H 5.79, N 8.07.

5.1.30.6. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(4-(methylsulfonyl) phenyl) piperidin-2-one (68f)



68f (567 mg, 80%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 213-214 °C; Purity by HPLC: 99.2% AUC, Chiral purity: 97.6%ee AUC.

ESI-MS: $[M+H]^+$ = 395.3 *m/z*. **Mol.Wt** = 394.5 g,

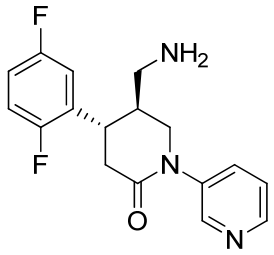
^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.37 (m, 1H), 2.43-2.48 (m, 3H), 2.72-2.79 (m, 1H), 3.25-3.29 (m, 1H), 3.32 (s, 3H), 3.47-3.54 (m, 2H), 7.09 (d, J = 7.8 Hz, 2H), 7.22-7.27 (m, 2H), 7.29-7.39 (m, 1H), 7.47 (d, J = 7.8 Hz, 2H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 36.8, 40.1, 41.6, 47.3, 47.9, 53.3, 115.5 (d, J = 25.3Hz), 117.9 (dd, J_1 = 25.2Hz, J_2 = 9.0Hz), 118.6 (dd, J_1 = 24.7Hz, J_2 = 8.8Hz), 122.5, 123.5, 128.4, 136.7, 146.3, 155.6 (d, J = 239.8Hz), 157.4 (d, J = 240.1Hz), 168.2.

Anal. Calcd. for $C_{19}H_{20}F_2N_2O_3S$: C 57.86, H 5.11, N 7.10, S 8.13; Found: C 57.85, H 5.09, N 7.14, S 8.12.

Analysis : Mol. Formula: $C_{19}H_{20}F_2N_2O_3S$
Calcd.: C 57.86, H 5.11, N 7.10, S 8.13.
Found: C 57.85, H 5.09, N 7.14, S 8.12.

5.1.30.7. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(pyridin-3-yl)piperidin-2-one (68g)



68g (610 mg, 73%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 168-170 °C; Purity by HPLC: 98.5% AUC, Chiral purity: 98.4%ee AUC.

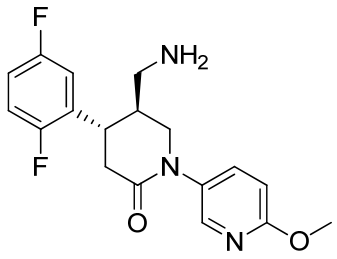
ESI-MS: $[M+H]^+$ = 318.5 *m/z*. **Mol.Wt** = 317.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.34-2.38 (m, 1H), 2.42-2.49 (m, 3H), 2.73-2.78 (m, 1H), 3.27 (dd, J_1 = 10Hz, J_2 = 12Hz, 1H), 3.47-3.52 (m, 2H), 7.22-7.25 (m, 2H), 7.28-7.31 (m, 1H), 7.42 (d, J = 5.2Hz, 1H), 8.27-8.34 (m, 2H), 8.89 (s, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 36.8, 40.2, 41.2, 47.3, 52.1, 115.4 (d, J = 25.1Hz), 117.7 (dd, J_1 = 24.9Hz, J_2 = 8.8Hz), 118.5 (dd, J_1 = 24.1Hz, J_2 = 9.0Hz), 123.4, 123.7, 125.6, 141.6, 143.2, 150.8, 155.2 (d, J = 237.9Hz), 157.6 (d, J = 240.3Hz), 169.5.

Analysis : Mol. Formula: $C_{17}H_{17}F_2N_3O$
Calcd.: C 64.34, H 5.40, N 13.24.
Found: C 64.38, H 5.42, N 13.21.

5.1.30.8. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(6-methoxypyridin-3-yl) piperidin-2-one (68h)



68h (515 mg, 73%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 174-176 °C; Purity by HPLC: 99.7% AUC, Chiral purity: 98.6%ee AUC.

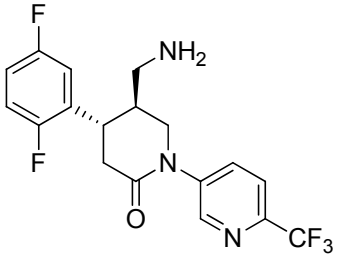
ESI-MS: $[M+H]^+$ = 348.5 *m/z*. **Mol.Wt** = 347.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.42-2.49 (m, 3H), 2.73-2.78 (m, 1H), 3.25-3.29 (m, 1H), 3.47-3.52 (m, 2H), 3.81 (s, 3H), 7.11 (d, J = 6.4 Hz, 1H), 7.22-7.25 (m, 3H), 7.28-7.31 (m, 1H), 8.15 (d, J = 6.4Hz, 1H);

^{13}C NMR (100 MHz, Methanol-d_4): δ 36.8, 40.2, 41.2, 47.3, 52.1, 54.8, 11.6, 115.4, 115.5, 115.9, 117.6 (dd, J_1 = 23.9Hz, J_2 = 8.8Hz), 118.5 (dd, J_1 = 24.4Hz, J_2 = 8.8Hz), 123.4, 128.3, 139.6, 143.2, 150.8, 155.2 (d, J = 239.7Hz), 157.6 (d, J = 241.0Hz), 159.6, 169.5;

Analysis : Mol. Formula: $C_{18}H_{19}F_2N_3O_2$
Calcd.: C 62.24, H 5.51, N 12.10.
Found: C 62.28, H 5.54, N 12.14.

5.1.30.9. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(6-(trifluoromethyl) pyridin-3-yl)piperidin-2-one (68i)



68i (640 mg, 79%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 231-233 °C; Purity by HPLC: 99.4% AUC, Chiral purity: 99.2%ee AUC.

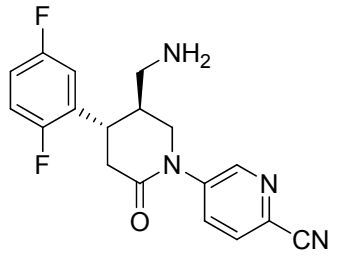
ESI-MS: $[M+H]^+$ = 386.2 *m/z*. **Mol.Wt** = 385.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.39 (m, 1H), 2.42-2.49 (m, 3H), 2.73-2.78 (m, 1H), 3.24-3.29 (m, 1H), 3.49-3.55 (m, 2H), 7.22-7.25 (m, 2H), 7.28-7.38 (m, 2H), 8.01 (d, J = 5.6 Hz, 1H), 8.12 (s, 1H);

^{13}C NMR (100 MHz, Methanol-d_4): δ 36.4, 40.3, 41.2, 47.4, 51.9, 115.4 (d, J = 25.0Hz), 117.7 (dd, J_1 = 25.1Hz, J_2 = 8.9Hz), 118.5 (dd, J_1 = 24.8Hz, J_2 = 9.0Hz), 119.8, 123.4, 124.7, 125.6, 126.9, 137.8, 139.4, 155.2 (d, J = 241.5Hz), 157.6 (d, J = 240.8Hz), 170.2;

Analysis : Mol. Formula: $C_{18}H_{16}F_5N_3O$
Calcd.: C 56.11, H 4.19, N 10.90.
Found: C 56.08, H 4.17, N 10.93.

5.1.30.10. 5-((4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-2-oxopiperidin-1-yl) picolinonitrile (68j)



68j (610 mg, 81%) was prepared by means of the general procedure described in section **5.1.30.** as a white solid. 123-125 °C; Purity by HPLC: 97.8% AUC, Chiral purity: 98.7%ee AUC.

ESI-MS: $[M+H]^+$ = 343.5 *m/z*. **Mol.Wt** = 342.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.34-2.39 (m, 1H), 2.41-2.49 (m, 3H), 2.73-2.78 (m, 1H), 3.27 (dd, J_1 = 9.8Hz, J_2 = 12Hz, 1H), 3.47-3.53 (m, 2H), 7.23-7.25 (m, 2H), 7.27-7.38 (m, 2H), 7.74 (d, J = 6.4Hz, 1H), 8.41 (d, J =6.4 Hz, 1H), 8.47 (s, 1H).

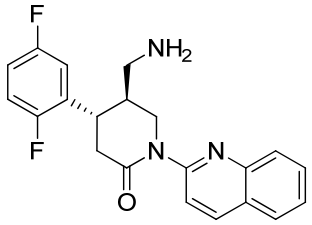
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.9, 40.4, 41.6, 47.3, 51.7, 115.6 (d, J = 24.8Hz), 117.4, 117.8 (dd, J_1 = 25.3Hz, J_2 = 9.0Hz), 118.5 (dd, J_1 = 24.8Hz, J_2 = 9.0Hz), 123.4, 127.9, 128.4, 129.4, 133.8, 141.5, 156.1 (d, J = 240.5Hz), 158.2 (d, J = 241.3Hz), 168.6.

Analysis : Mol. Formula: $C_{18}H_{16}F_2N_4O$

Calcd.: C 63.15, H 4.71, N 16.37.

Found: C 63.18, H 4.74, N 16.39.

5.1.30.11. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(quinolin-2-yl) piperidin-2-one (68m)



68m (570 mg, 81%) was prepared by means of the general procedure described in section **5.1.30.** as a thick oil. Purity by HPLC: 97.2% AUC, Chiral purity: 97.0%ee AUC.

ESI-MS: $[M+H]^+$ = 368.3 *m/z*. **Mol.Wt** = 367.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.34-2.39 (m, 1H), 2.43-2.47 (m, 3H), 2.71-2.76 (m, 1H), 3.25-3.29 (m, 1H), 3.47-3.53 (m, 2H), 7.23-7.27 (m, 2H), 7.29-7.34 (m, 1H), 7.43-7.79 (m, 4H), 7.98 (d, J = 6.8Hz, 1H), 8.09 (d, J= 7.6Hz, 1H).

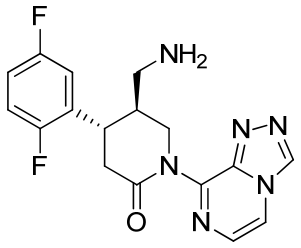
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.7, 40.1, 41.9, 47.2, 51.7, 112.1, 115.4 (d, J = 25.1Hz), 117.6 (dd, J_1 = 24.8Hz, J_2 = 8.8Hz), 118.7 (dd, J_1 = 25.2Hz, J_2 = 9.0Hz), 121.4, 122.3, 123.4, 125.8, 126.6, 129.6, 137.5, 146.8, 156.3 (d, J = 237.5Hz), 157.6 (d, J = 239.6Hz), 165.3, 169.4.

Analysis : Mol. Formula: $C_{21}H_{19}F_2N_3O$

Calcd.: C 68.65, H 5.21, N 11.44.

Found: C 68.61, H 5.23, N 11.40.

5.1.30.12. (4S,5S)-1-([1,2,4]Triazolo[4,3-a]pyrazin-8-yl)-5-(aminomethyl)-4-(2,5-difluorophenyl)piperidin-2-one (68n)



68n (680 mg, 94%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 196-197 °C. Purity by HPLC: 99.1% AUC, Chiral purity: 98.4%ee AUC.

ESI-MS: $[M+H]^+$ = 359.4 *m/z*. **Mol.Wt** = 358.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.39 (m, 1H), 2.43-2.48 (m, 3H), 2.73-2.79 (m, 1H), 3.24-3.29 (m, 1H), 3.47-3.53 (m, 2H), 7.23-7.27 (m, 2H), 7.29-7.34 (m, 1H), 7.89 (d, J = 7.6Hz, 1H), 8.07 (d, J = 7.6Hz, 1H), 9.01 (s, 1H).

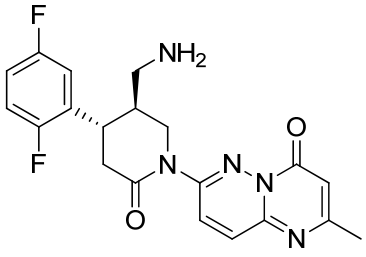
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.8, 40.6, 41.9, 47.4, 52.3, 114.8, 115.6 (d, J = 25.0Hz), 117.6 (dd, J_1 = 24.9Hz, J_2 = 9.0Hz), 118.7 (dd, J_1 = 25.0Hz, J_2 = 8.6Hz), 123.4, 126.6, 137.2, 146.9, 147.3, 156.5 (d, J = 241.5Hz), 157.6 (d, J = 242.1Hz), 169.7.

Analysis : Mol. Formula: $C_{17}H_{16}F_2N_6O$

Calcd.: C 56.98, H 4.50, N 23.45.

Found: C 57.01, H 4.53, N 23.42.

5.1.30.13. 7-((4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-2-oxopiperidin-1-yl)-2-methyl-4H-pyrimido[1,2-b]pyridazin-4-one (68o)



68o (520 mg, 75%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 165-167 °C. Purity by HPLC: 97.0% AUC, Chiral purity: 97.6%ee AUC.

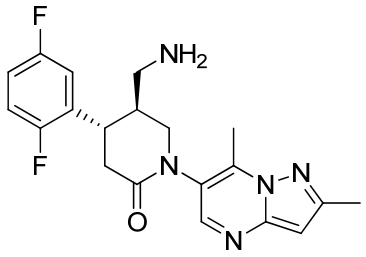
ESI-MS: $[M+H]^+$ = 400.2 *m/z*. **Mol.Wt** = 399.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.29 (s, 3H), 2.34-2.39 (m, 1H), 2.45-2.52 (m, 3H), 2.71-2.77 (m, 1H), 3.25-3.29 (m, 1H), 3.47-3.53 (m, 2H), 6.19 (s, 1H), 6.32 (s, 2H), 7.23-7.28 (m, 2H), 7.29-7.34 (m, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 28.3, 36.6, 40.3, 41.7, 46.9, 52.3, 110.8, 115.6 (d, J = 25.2Hz), 117.6 (dd, J_1 = 24.8Hz, J_2 = 9.1Hz), 118.6 (dd, J_1 = 24.9Hz, J_2 = 9.0Hz), 123.4, 130.2, 135.8, 148.9, 153.8, 156.5 (d, J = 241.5Hz), 158.2, 157.6 (d, J = 242.1Hz), 164.6, 168.8.

Analysis : Mol. Formula: $C_{20}H_{19}F_2N_5O_2$
Calcd.: C 60.14, H 4.79, N 17.53.
Found: C 60.10, H 4.82, N 17.50.

5.1.30.14. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(2,7-dimethylpyrazolo [1,5-a]pyrimidin-6-yl)piperidin-2-one (68r)



68r (575 mg, 73%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 187-188 °C. Purity by HPLC: 99.7% AUC, Chiral purity: 98.8%ee AUC.

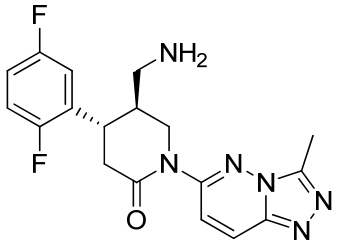
ESI-MS: $[M+H]^+$ = 386.5 *m/z*. **Mol.Wt** = 385.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.27 (s, 3H), 2.32 (s, 3H), 2.34-2.39 (m, 1H), 2.43-2.48 (m, 3H), 2.71-2.79 (m, 1H), 3.24-3.29 (m, 1H), 3.47-3.52 (m, 2H), 6.71 (s, 1H), 7.23-7.27 (m, 2H), 7.29-7.32 (m, 1H), 9.13 (s, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 16.7, 19.2, 37.9, 41.4, 41.7, 47.4, 52.3, 98.8, 115.4 (d, J = 24.3Hz), 117.6 (dd, J_1 = 25.3Hz, J_2 = 8.8Hz), 118.6 (dd, J_1 = 24.3Hz, J_2 = 8.8Hz), 123.4, 125.9, 148.9, 150.6, 155.3, 156.7 (d, J = 242.4Hz), 157.2, 157.8 (d, J = 242.7Hz), 169.9.

Analysis : Mol. Formula: for $C_{20}H_{21}F_2N_5O$
Calcd.: C 62.33, H 5.49, N 18.17.
Found: C 62.33, H 5.49, N 18.17.

5.1.30.15. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(3-methyl-[1,2,4] triazolo[4,3-b]pyridazin-6-yl)piperidin-2-one (68s)



68s (612 mg, 79%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 148-151 °C. Purity by HPLC: 98.9% AUC, Chiral purity: 97.6%ee AUC.

ESI-MS: $[M+H]^+$ = 373.5 *m/z*. **Mol.Wt** = 372.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.39 (m, 1H), 2.41 (s, 3H), 2.43-2.47 (m, 3H), 2.73-2.79 (m, 1H), 3.24-3.27 (m, 1H), 3.46-3.52 (m, 2H), 7.23-7.27 (m, 2H), 7.29-7.32 (m, 1H), 8.71 (s, 1H), 9.01 (s, 1H).

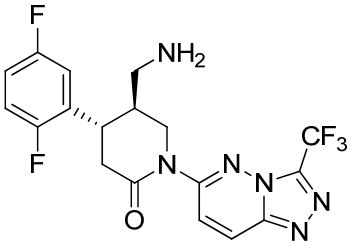
^{13}C NMR (100 MHz, Methanol-d_4): δ 14.3, 37.9, 41.2, 41.9, 47.4, 52.3, 115.6 (d, J = 25.2Hz), 117.6 (dd, J_1 = 25.3Hz, J_2 = 8.9Hz), 118.6 (dd, J_1 = 24.6Hz, J_2 = 8.8Hz), 123.4, 124.0, 124.6, 137.1, 143.9, 154.7, 156.7 (d, J = 241.0Hz), 157.6 (d, J = 241.3Hz), 169.5.

Analysis : Mol. Formula: for $C_{18}H_{18}F_2N_6O$

Calcd.: C 58.06, H 4.87, N 22.57.

Found: C 58.02, H 4.85, N 22.59.

5.1.30.16. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-2-one (68t)



68t (540 mg, 87%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 126-128 °C. Purity by HPLC: 97.6% AUC, Chiral purity: 97.5%ee AUC.

ESI-MS: $[M+H]^+$ = 427.4 *m/z*. **Mol.Wt** = 426.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.38 (m, 1H), 2.43-2.48 (m, 3H), 2.74-2.81 (m, 1H), 3.25-3.28 (m, 1H), 3.47-3.54 (m, 2H), 7.23-7.27 (m, 2H), 7.29-7.32 (m, 1H), 8.64 (d, J = 6.4Hz, 1H), 9.01 (d, J = 6.4Hz, 1H).

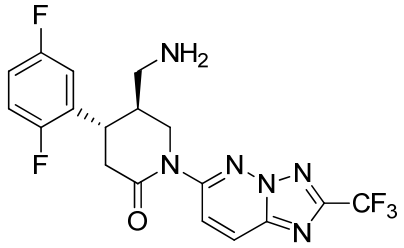
^{13}C NMR (100 MHz, Methanol-d_4): δ 37.8, 41.2, 41.6, 47.3, 52.7, 114.7, 115.6 (d, J = 25.8Hz), 117.6 (dd, J_1 = 24.6Hz, J_2 = 9.0Hz), 118.6 (dd, J_1 = 25.6Hz, J_2 = 8.7Hz), 123.4, 123.9, 124.7, 143.6, 154.6, 156.4 (d, J = 240.8Hz), 157.7 (d, J = 241.2Hz), 159.6, 169.8.

Analysis : Mol. Formula: for $C_{18}H_{15}F_5N_6O$

Calcd.: C 50.71, H 3.55, N 19.71.

Found: C 50.73, H 3.57, N 19.69.

5.1.30.17. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(2-(trifluoromethyl)-[1,2,4] triazolo[1,5-b]pyridazin-6-yl)piperidin-2-one (68u)



68u (630 mg, 95%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 186-187 °C. Purity by HPLC: 99.1% AUC, Chiral purity: 98.9%ee AUC.

ESI-MS: $[M+H]^+$ = 427.5 *m/z*. **Mol.Wt** = 426.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.39 (m, 1H), 2.41-2.47 (m, 3H), 2.72-2.78 (m, 1H), 3.24-3.27 (m, 1H), 3.45-3.50 (m, 2H), 7.22-7.27 (m, 2H), 7.29-7.31 (m, 1H), 8.57 (d, J = 6.8Hz, 1H), 8.78 (d, J = 6.8Hz, 1H).

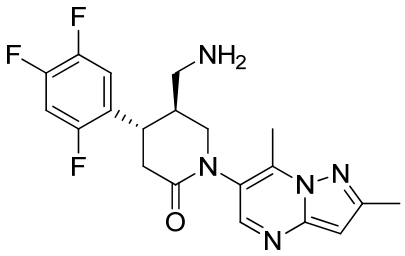
^{13}C NMR (100 MHz, Methanol-d_4): δ 37.9, 41.2, 41.7, 47.2, 52.7, 115.6 (d, J = 25.8Hz), 117.6 (dd, J_1 = 24.6Hz, J_2 = 9.0Hz), 118.6 (dd, J_1 = 25.6Hz, J_2 = 8.7Hz), 123.4, 123.7, 126.4, 144.6, 147.6, 154.3, 156.5 (d, J = 240.2Hz), 157.7 (d, J = 241.0Hz), 169.7.

Analysis : Mol. Formula: for $C_{18}H_{15}F_5N_6O$

Calcd.: C 50.71, H 3.55, N 19.71.

Found: C 50.74, H 3.56, N 19.67.

5.1.30.18. (4S,5S)-5-(Aminomethyl)-1-(2,7-dimethylpyrazolo[1,5-a]pyrimidin-6-yl)-4-(2,4,5-trifluorophenyl)piperidin-2-one (69b)



69b (560 mg, 76%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 197-199 °C. Purity by HPLC: 99.6% AUC, Chiral purity: 97.6%ee AUC.

ESI-MS: $[M+H]^+$ = 404.5 *m/z*. **Mol.Wt** = 403.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.28 (s, 3H), 2.31 (s, 3H), 2.34-2.39 (m, 1H), 2.43-2.48 (m, 3H), 2.71-2.79 (m, 1H), 3.24-3.29 (m, 1H), 3.47-3.52 (m, 2H), 6.74 (s, 1H), 7.34-7.40 (m, 1H), 7.49-7.54 (m, 1H), 9.14 (s, 1H).

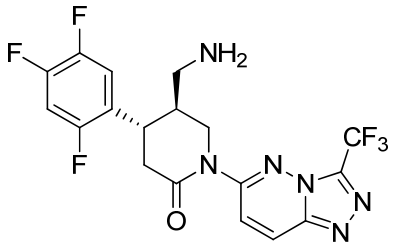
^{13}C NMR (100 MHz, Methanol-d_4): δ 16.8, 19.2, 37.8, 41.2, 41.7, 47.4, 52.3, 101.2, 105.6 (dd, J_1 = 21.3Hz, J_2 = 27.6Hz), 116.4 (dd, J_1 = 19.9Hz, J_2 = 5.8Hz), 123.2 (dt, J_1= 15.4Hz, J_2 = 5.0Hz), 125.6, 145.9 (ddd, J_1 = 246.4Hz, J_2 = 12.5Hz, J_3 = 3.8Hz), 149.6, 150.2 (dt, J_1 = 251.6Hz, J_2 = 13.4Hz), 150.8, 154.4, 156.3 (ddd, J_1 = 246.3Hz, J_2 = 10.3Hz, J_3 = 3.0Hz), 157.3, 167.6.

Analysis : Mol. Formula: for $C_{20}H_{20}F_3N_5O$

Calcd.: C 59.55, H 5.00, N 17.36.

Found: C 59.57, H 5.03, N 17.33.

5.1.30.19. (4S,5S)-5-(Aminomethyl)-1-(3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-4-(2,4,5-trifluorophenyl)piperidin-2-one (69c)



69c (586 mg, 87%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 212-215 °C. Purity by HPLC: 97.7% AUC, Chiral purity: 98.1%ee AUC.

ESI-MS: $[M+H]^+$ = 445.4 *m/z*. **Mol.Wt** = 444.3 g.

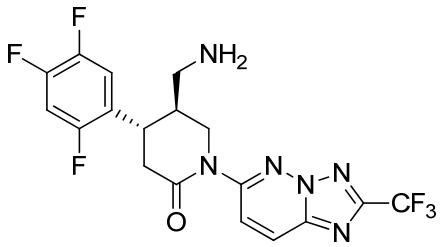
^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.43-2.47 (m, 3H), 2.73-2.79 (m, 1H), 3.25-3.28 (m, 1H), 3.47-3.54 (m, 2H), 7.32-7.40 (m, 1H), 7.46-7.54 (m, 1H), 8.62 (d, J = 6.2Hz, 1H), 8.95 (d, J = 6.2Hz, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 37.8, 41.2, 41.7, 47.4, 52.3, 105.6 (dd, J_1 = 21.3Hz, J_2 = 28.2Hz), 114.5, 116.4 (dd, J_1 = 19.8Hz, J_2 = 5.4Hz), 123.3 (dt, J_1= 15.4Hz, J_2 = 5.0Hz), 123.8, 124.6, 143.5, 146.2 (ddd, J_1 = 246.4Hz, J_2 = 12.5Hz, J_3 = 3.8Hz), 150.4 (dt, J_1 = 251.6Hz, J_2 = 13.4Hz), 154.3, 156.5 (ddd, J_1 = 246.3Hz, J_2 = 10.3Hz, J_3 = 3.0Hz), 159.8, 166.8.

Anal. Calcd. for $C_{18}H_{14}F_6N_6O$: C 48.66, H 3.18, N 18.91; Found: C 48.63, H 3.15, N 18.88.

Analysis : Mol. Formula: for $C_{18}H_{14}F_6N_6O$
Calcd.: C 48.66, H 3.18, N 18.91.
Found: C 48.63, H 3.15, N 18.88.

5.1.30.20. (4S,5S)-5-(Aminomethyl)-1-(2-(trifluoromethyl)-[1,2,4]triazolo[1,5-b]pyridazin-6-yl)-4-(2,4,5-trifluorophenyl)piperidin-2-one (69d)



69d (670 mg, 93%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 137-139 °C. Purity by HPLC: 98.1% AUC, Chiral purity: 97.2%ee AUC.

ESI-MS: $[M+H]^+$ = 445.5 *m/z*. **Mol.Wt** = 444.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.39 (m, 1H), 2.41-2.47 (m, 3H), 2.72-2.78 (m, 1H), 3.24-3.27 (m, 1H), 3.45-3.50 (m, 2H), 7.34-7.40 (m, 1H), 7.49-7.54 (m, 1H), 8.57 (d, J = 6.4Hz, 1H), 8.77 (d, J = 6.4Hz, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 37.9, 41.2, 41.8, 47.4, 52.7, 105.6 (dd, J_1 = 20.8Hz, J_2 = 28.6Hz), 116.5 (dd, J_1 = 20.4Hz, J_2 = 5.4Hz), 119.7, 123.3 (dt, J_1= 15.4Hz, J_2 = 5.2Hz), 123.6, 126.4, 144.6, 146.3 (ddd, J_1 = 246.4Hz, J_2 = 12.7Hz, J_3 = 3.8Hz),

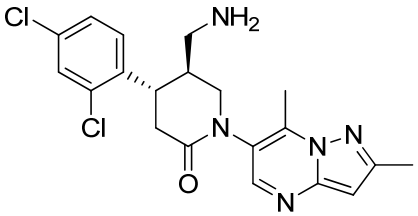
147.7, 150.6 (dt, J_1 = 251.4Hz, J_2 = 13.4Hz), 154.3, 156.4 (ddd, J_1 = 246.2Hz, J_2 = 10.3Hz, J_3 = 2.8Hz), 167.3.

Analysis : Mol. Formula: for $C_{18}H_{14}F_6N_6O$

Calcd.: C 48.66, H 3.18, N 18.91.

Found: C 48.67, H 3.16, N 18.89.

5.1.30.21. (4S,5S)-5-(Aminomethyl)-4-(2,4-dichlorophenyl)-1-(2,7-dimethylpyrazolo [1,5-a]pyrimidin-6-yl)piperidin-2-one (70b)



70b (550 mg, 86%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 132-134 °C. Purity by HPLC: 98.2% AUC, Chiral purity: 97.8%ee AUC.

ESI-MS: $[M+H]^+$ = 418.9 *m/z*. **Mol.Wt** = 418.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.26 (s, 3H), 2.31 (s, 3H), 2.34-2.37 (m, 1H), 2.43-2.48 (m, 3H), 2.71-2.79 (m, 1H), 3.25 (dd, J_1 = 10Hz, J_2 = 11.8Hz, 1H), 3.47-3.52 (m, 2H), 6.74 (s, 1H), 7.38 (dd, J_1 = 8.2Hz, J_2 = 2.2Hz, 1H), 7.44 (d, J = 8.2Hz, 1H), 7.53 (d, J = 2.2Hz, 1H), 9.14 (s, 1H).

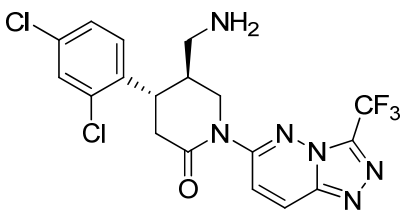
^{13}C NMR (100 MHz, Methanol-d_4): δ 16.8, 19.2, 37.8, 41.2, 41.7, 47.4, 52.3, 98.7, 125.6, 126.4, 127.4, 130.1, 132.2, 132.7, 133.8, 149.6, 150.8, 154.4, 157.3, 167.6.

Analysis : Mol. Formula: for $C_{20}H_{21}Cl_2N_5O$

Calcd.: C 57.42, H 5.06, N 16.74.

Found: C 57.39, H 5.02, N 16.72.

5.1.30.22. (4S,5S)-5-(Aminomethyl)-4-(2,4-dichlorophenyl)-1-(3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-2-one (70c)



70c (620 mg, 75%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 109-111 °C. Purity by HPLC: 99.1% AUC, Chiral purity: 99.3%ee AUC.

ESI-MS: $[M+H]^+$ = 459.9 *m/z*. **Mol.Wt** = 459.2 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.33-2.37 (m, 1H), 2.43-2.47 (m, 3H), 2.73-2.78 (m, 1H), 3.25-3.28 (m, 1H), 3.47-3.54 (m, 2H), 7.36 (dd, J_1 = 8.4Hz, J_2 = 1.8Hz, 1H), 7.43 (d, J = 8.4Hz, 1H), 7.53 (d, J = 2.0Hz, 1H), 8.58 (d, J = 5.8Hz, 1H), 8.89 (d, J = 5.8Hz, 1H).

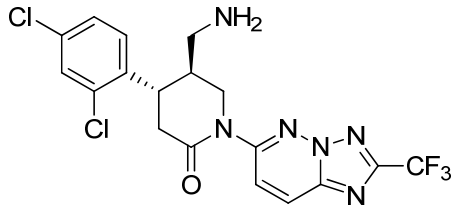
^{13}C NMR (100 MHz, Methanol-d_4): δ 37.9, 41.3, 41.7, 47.4, 52.3, 114.6, 123.8, 124.6, 126.4, 127.2, 130.3, 132.3, 132.7, 133.6, 143.5, 154.4, 159.8, 167.5.

Analysis : Mol. Formula: for $C_{18}H_{15}Cl_2F_3N_6O$

Calcd.: C 47.07, H 3.29, N 18.30.

Found: C 47.04, H 3.26, N 18.28.

5.1.30.23. (4S,5S)-5-(Aminomethyl)-4-(2,4-dichlorophenyl)-1-(2-(trifluoromethyl)-[1,2,4]triazolo[1,5-b]pyridazin-6-yl)piperidin-2-one (70d)



70d (530 mg, 89%) was prepared by means of the general procedure described in section **5.1.30.** as a White solid; mp: 164-166 °C. Purity by HPLC: 98.7% AUC, Chiral purity: 98.9%ee AUC.

ESI-MS: $[M+H]^+$ = 460.1 *m/z*. **Mol.Wt** = 459.2 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.31-2.36 (m, 1H), 2.41-2.46 (m, 3H), 2.72-2.78 (m, 1H), 3.27 (dd, J_1 = 10.4Hz, J_2 = 12Hz, 1H), 3.45-3.50 (m, 2H), 7.34 (dd, J_1 = 8.6Hz, J_2 = 2.0Hz, 1H), 7.43 (d, J = 8.6Hz, 1H), 7.53 (d, J = 2.0Hz, 1H), 8.49 (d, J = 6.2Hz, 1H), 8.72 (d, J = 6.2Hz, 1H).

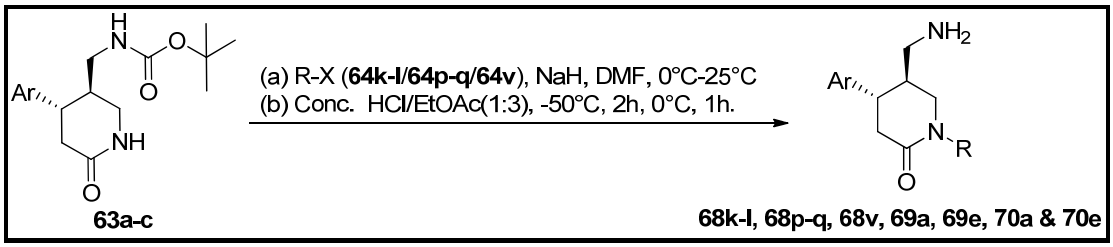
^{13}C NMR (100 MHz, Methanol-d_4): δ 37.9, 41.2, 41.8, 47.4, 52.7, 119.7, 123.6, 126.2, 126.4, 127.7, 130.6, 132.3, 132.8, 133.8, 144.6, 147.6, 154.3, 167.3.

Analysis : Mol. Formula: for $C_{18}H_{15}Cl_2F_3N_6O$

Calcd.: C 47.07, H 3.29, N 18.30.

Found: C 47.09, H 3.32, N 18.27.

5.1.31. General procedure for the synthesis of compounds (68k-l, 68p-q, 68v, 69a, 69e, 70a & 70e)



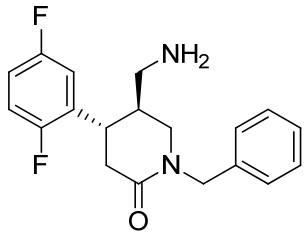
To a solution of compound **63a-c** (5 mmol) in dry DMF (25 ml) was added sodium hydride (6 mmol) in portions at 0^0C. After all the sodium hydride was added to the reaction mixture, the reaction was brought to room temperature gradually and stirred for 2h at same temperature. Reaction was cooled again to 0 °C and a solution of halo-aromatics of the interest **64k-l**, **64p-q** or **64v** dissolved in DMF (5 ml) was added dropwise over a period of 15min. Reaction was stirred at same temperature for 1h and then at room temperature for 2h.

After completion of the reaction, reaction mixture was poured in cold D. M. water (100 ml) and extracted with ethyl acetate (3X100 ml). Combined organic extracts were washed with water (2X100 ml) and brine (1X100 ml). Organic layer was then dried over anhy. Na_2SO_4 and evaporated under reduced pressure to give amide -NH alkylated product with 65-80% yield.

Crude product thus obtain was dissolved in ethyl acetate (45 ml), and concentrated HCl (15 ml) was added at -50^0C and the reaction mixture was stirred at the same temperature for 2h. Temperature of the reaction mixture was then gradually increased to 0^0C and stirred for additional 1h at this temperature. After completion of the reaction, reaction mixture was basified with sat. $NaHCO_3$ solution till pH 9 and extracted with Ethyl acetate (3X50 ml). Combined organic extracts were washed with water (1X100 ml) and brine (1X100 ml). Organic layer was then dried over anhy. Na_2SO_4 and evaporated to dryness.

Crude residue thus obtained was purified by preparative HPLC method using the procedure as illustrated in experimental **section 5.1.3.-Purification** to give desired amino-methyl pipyridones **68k-l, 68p-q, 68v, 69a, 69e, 70a & 70e**.

5.1.31.1. (4S,5S)-5-(Aminomethyl)-1-benzyl-4-(2,5-difluorophenyl)piperidin-2-one (68k)



68k (640 mg, 93%) was prepared by means of the general procedure described in section **5.1.31.** as a white solid. 119-121 °C; Purity by HPLC: 98.2% AUC, Chiral purity: 98.3%ee AUC.

ESI/MS (m/z) : 331.2 $(M+H)^+$. **Mol. Wt.** = 330.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.34-2.39 (m, 1H), 2.43-2.47 (m, 3H), 2.71-2.76 (m, 1H), 3.23 (dd, J_1 = 10.2Hz, J_2 = 11.8Hz, 1H), 3.47-3.53 (m, 2H), 4.82 (s, 2H), 7.21-7.27 (m, 4H), 7.29-7.41 (m, 4H).

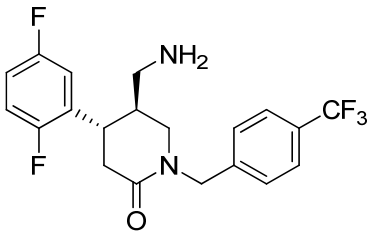
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.9, 40.2, 41.6, 47.2, 51.7, 52.6, 115.6 (d, J = 24.9Hz), 117.8 (dd, J_1 = 25.3Hz, J_2 = 8.8Hz), 118.7 (dd, J_1 = 24.9Hz, J_2 = 9.0Hz), 123.4, 127.2, 127.9, 128.8, 136.5, 156.3 (d, J = 237.5Hz), 157.6 (d, J = 240.1Hz), 169.2.

Analysis : Mol. Formula: $C_{19}H_{20}F_2N_2O$

Calcd.: C 69.07, H 6.10, N 8.48.

Found: C 69.09, H 6.13, N 8.45.

5.1.31.2. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(4-(trifluoromethyl) benzyl) piperidin-2-one (68l)



68l (580 mg, 85%) was prepared by means of the general procedure described in section **5.1.31.** as a white solid. 147-149 °C; Purity by HPLC: 97.7% AUC, Chiral purity: 97.5%ee AUC.

ESI/MS (m/z) : 399.2 $(M+H)^+$. **Mol. Wt.** = 398.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.42-2.47 (m, 3H), 2.73-2.78 (m, 1H), 3.23 (dd, J_1 = 10Hz, J_2 = 11.8Hz, 1H), 3.47-3.53 (m, 2H), 4.83 (s, 2H), 7.09 (d, J = 8.2Hz, 2H), 7.21-7.27 (m, 2H), 7.29-7.34 (m, 1H),7.47 (d, J = 8.2Hz, 2H).

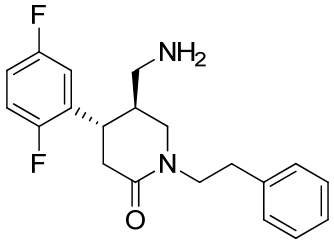
^{13}C NMR (100 MHz, Methanol-d_4): δ 36.9, 40.2, 41.6, 47.2, 51.7, 52.7, 115.6 (d, J = 24.9Hz), 117.8 (dd, J_1 = 25.3Hz, J_2 = 8.8Hz), 118.7 (dd, J_1 = 24.9Hz, J_2 = 9.0Hz), 123.4, 124.3, 124.8, 129.3, 129.8, 139.5, 156.3 (d, J = 237.5Hz), 157.6 (d, J = 240.1Hz), 169.2.

Analysis : Mol. Formula: $C_{20}H_{19}F_5N_2O$

Calcd.: C 60.30, H 4.81, N 5.02.

Found: C 60.33, H 4.85, N 5.06.

5.1.31.3. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-phenethylpiperidin-2-one (68p)



68p (648 mg, 93%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 197-198 °C. Purity by HPLC: 99.5% AUC, Chiral purity: 98.7%ee AUC.

ESI-MS: $[M+H]^+$ = 345.3 *m/z*. **Mol.Wt** = 344.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.43-2.48 (m, 3H), 2.72-2.85 (m, 3H), 3.24-3.27 (m, 1H), 3.47-3.53 (m, 2H), 3.63 (t, J = 9.6Hz, 2H), 7.23-7.27 (m, 3H), 7.29-7.32 (m, 4H), 7.43 (m, 1H).

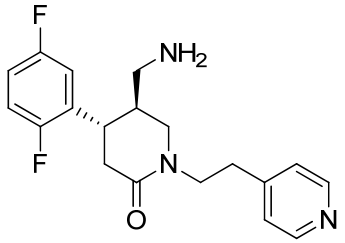
^{13}C NMR (100 MHz, Methanol-d_4): δ 34.3, 37.3, 41.2, 41.9, 46.9, 49.8, 52.3, 115.6 (d, J = 25.2Hz), 117.6 (dd, J_1 = 23.8Hz, J_2 = 7.8Hz), 118.6 (dd, J_1 = 24.3Hz, J_2 = 8.4Hz), 123.4, 125.6, 127.9, 128.4, 138.9, 156.5 (d, J = 240.3Hz), 157.6 (d, J = 240.1Hz), 170.1.

Analysis : Mol. Formula: $C_{20}H_{22}F_2N_2O$

Calcd.: C 69.75, H 6.44, N 8.13.

Found: C 69.77, H 6.47, N 8.10.

5.1.31.4. (4S,5S)-5-(Aminomethyl)-4-(2,5-difluorophenyl)-1-(2-(pyridin-4-yl)ethyl) piperidin-2-one (68q)



68q (544 mg, 88%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 167-169 °C. Purity by HPLC: 97.3% AUC, Chiral purity: 97.2%ee AUC.

ESI-MS: $[M+H]^+$ = 346.5 *m/z*. **Mol.Wt** = 345.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.29-2.35 (m, 1H), 2.41-2.46 (m, 3H), 2.71-2.79 (m, 3H), 3.25-3.29 (m, 1H), 3.47-3.52 (m, 2H), 3.59 (t, J = 8.8Hz, 2H), 7.18 (d, J = 5.6Hz, 2H), 7.23-7.27 (m, 2H), 7.29-7.32 (m, 1H), 8.49 (d, J = 5.6Hz, 2H).

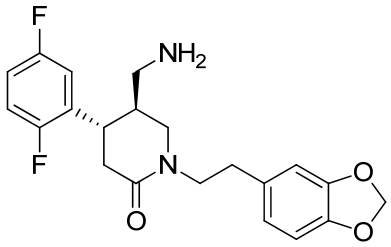
^{13}C NMR (100 MHz, Methanol-d_4): δ 34.2, 38.3, 41.2, 41.9, 47.4, 49.4, 52.3, 115.4 (d, J = 24.8Hz), 117.7 (dd, J_1 = 25.3Hz, J_2 = 8.8Hz), 118.6 (dd, J_1 = 24.3Hz, J_2 = 8.4Hz), 123.4, 124.1, 148.3, 149.6, 156.7 (d, J = 242.4Hz), 157.8 (d, J = 242.7Hz), 169.7.

Analysis : Mol. Formula: $C_{19}H_{21}F_2N_3O$

Calcd.: C 66.07, H 6.13, N 12.17.

Found: C 66.09, H 6.10, N 12.14.

5.1.31.5. (4S,5S)-5-(Aminomethyl)-1-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-4-(2,5-difluorophenyl)piperidin-2-one (68v)



68v (524 mg, 85%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 177-179 °C. Purity by HPLC: 99.6% AUC, Chiral purity: 99.1%ee AUC.

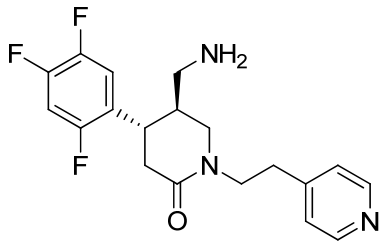
ESI-MS: $[M+H]^+$ = 389.5 *m/z*. **Mol.Wt** = 388.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.43-2.47 (m, 3H), 2.72-2.87 (m, 3H), 3.25-3.29 (m, 1H), 3.47-3.53 (m, 2H), 3.64 (t, J = 9.6Hz, 2H), 5.97 (s, 2H), 6.63-6.72 (m, 3H), 7.23-7.27 (m, 2H), 7.29-7.32 (m, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 34.5, 37.9, 41.3, 41.7, 46.9, 49.7, 52.2, 101.4, 107.6, 109.4, 115.6 (d, J = 25.2Hz), 117.6 (dd, J_1 = 23.8Hz, J_2 = 7.8Hz), 118.6 (dd, J_1 = 24.3Hz, J_2 = 8.4Hz), 122.8, 123.4, 130.9, 146.4, 148.8, 156.5 (d, J = 240.3Hz), 157.6 (d, J = 240.1Hz), 170.2.

Analysis : Mol. Formula: $C_{21}H_{22}F_2N_2O_3$
Calcd.: C 64.94, H 5.71, N 7.21.
Found: C 64.98, H 5.74, N 7.18.

5.1.31.6. (4S,5S)-5-(Aminomethyl)-1-(2-(pyridin-4-yl)ethyl)-4-(2,4,5-trifluorophenyl) piperidin-2-one (69a)



69a (510 mg, 80%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 169-171 °C. Purity by HPLC: 98.8% AUC, Chiral purity: 98.3%ee AUC.

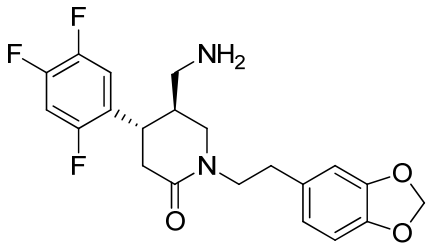
ESI-MS: $[M+H]^+$ = 364.5 *m/z*. **Mol.Wt** = 363.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.41-2.46 (m, 3H), 2.71-2.83 (m, 3H), 3.25-3.29 (m, 1H), 3.47-3.52 (m, 2H), 3.59 (t, J = 8.8Hz, 2H), 7.21 (d, J = 6.8Hz, 2H), 7.34-7.40 (m, 1H), 7.49-7.54 (m, 1H), 8.47 (d, J = 6.8Hz, 2H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 34.2, 39.3, 42.2, 43.5, 47.4, 49.4, 52.7, 105.6 (dd, J_1 = 21.1Hz, J_2 = 28.3Hz), 116.4 (dd, J_1 = 19.9Hz, J_2 = 5.8Hz), 123.2 (dt, J_1= 15.6Hz, J_2 = 5.2Hz), 123.8, 145.9 (ddd, J_1 = 246.2Hz, J_2 = 12.5Hz, J_3 = 3.8Hz), 148.4, 149.5, 150.2 (dt, J_1 = 251.3Hz, J_2 = 13.6Hz), 156.3 (ddd, J_1 = 245.1Hz, J_2 = 10.3Hz, J_3 = 2.7Hz), 167.8.

Analysis : Mol. Formula: $C_{19}H_{20}F_3N_3O$
Calcd.: C 62.80, H 5.55, N 11.56.
Found: C 62.83, H 5.57, N 11.53.

5.1.31.7. (4S,5S)-5-(Aminomethyl)-1-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-4-(2,4,5-trifluorophenyl)piperidin-2-one (69e)



69e (524 mg, 74%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 121-123 °C. Purity by HPLC: 97.2% AUC, Chiral purity: 98.5%ee AUC.

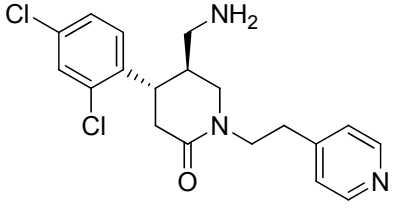
ESI-MS: $[M+H]^+$ = 407.5 *m/z*. **Mol.Wt** = 406.4 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.38 (m, 1H), 2.43-2.47 (m, 3H), 2.72-2.87 (m, 3H), 3.25-3.29 (m, 1H), 3.47-3.53 (m, 2H), 3.62 (t, J = 10.2Hz, 2H), 5.96 (s, 2H), 6.63-6.72 (m, 3H), 7.37-7.52 (m, 2H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 34.4, 37.9, 41.3, 41.7, 46.9, 49.7, 52.2, 101.4, 107.6, 109.4, 105.4 (dd, J_1 = 21.2Hz, J_2 = 28.2Hz), 116.4 (dd, J_1 = 19.9Hz, J_2 = 5.8Hz), 122.6, 123.2 (dt, J_1= 18.6Hz, J_2 = 5.2Hz), 130.4, 145.9 (ddd, J_1 = 246.2Hz, J_2 = 12.5Hz, J_3 = 3.8Hz), 146.6, 148.3, 150.2 (dt, J_1 = 251.3Hz, J_2 = 13.6Hz), 156.4 (ddd, J_1 = 245.4Hz, J_2 = 10.4Hz, J_3 = 2.7Hz), 168.3.

Analysis : Mol. Formula: $C_{21}H_{21}F_3N_2O_3$
Calcd.: C 62.06, H 5.21, N 6.89.
Found: 62.02, H 5.18, N 6.92.

5.1.31.8. (4S,5S)-5-(Aminomethyl)-4-(2,4-dichlorophenyl)-1-(2-(pyridin-4-yl)ethyl) piperidin-2-one (70a)



70a (530mg, 79%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 94-96 °C. Purity by HPLC: 98.6% AUC, Chiral purity: 98.3%ee AUC.

ESI-MS: $[M+H]^+$ = 378.5 *m/z*. **Mol.Wt** = 378.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.32-2.37 (m, 1H), 2.41-2.46 (m, 3H), 2.71-2.83 (m, 3H), 3.25-3.29 (m, 1H), 3.47-3.52 (m, 2H), 3.59 (t, J = 8.8Hz, 2H), 7.24 (d, J = 6.8Hz, 2H), 7.37 (dd, J_1 = 8.2Hz, J_2 = 2.0Hz, 1H), 7.44 (d, J = 8.2Hz, 1H), 7.53 (d, J = 2.0Hz, 1H), 8.44 (d, J = 6.8Hz, 2H);

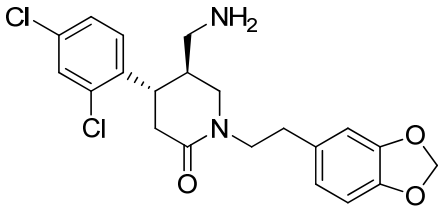
^{13}C NMR (100 MHz, Methanol-d_4): δ 34.2, 38.4, 41.6, 42.3, 46.9, 49.8, 52.6, 123.4, 126.3, 127.8, 130.4, 132.2, 132.6, 133.9, 148.3, 149.2, 166.7;

Analysis : Mol. Formula: $C_{19}H_{21}Cl_2N_3O_2$

Calcd.: C 60.32, H 5.60, N 11.11.

Found: C 60.36, H 5.63, N 11.10.

5.1.31.9. (4S,5S)-5-(Aminomethyl)-1-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-4-(2,4-dichlorophenyl)piperidin-2-one (70e)



70e (610mg, 83%) was prepared by means of the general procedure described in section **5.1.31.** as a White solid; mp: 132-134 °C. Purity by HPLC: 98.5% AUC, Chiral purity: 97.1%ee AUC.

ESI-MS: $[M+H]^+$ = 421.4 *m/z*. **Mol.Wt** = 421.3 g.

^{1}H NMR (400 MHz, Methanol-d_4): δ = 2.29-2.35 (m, 1H), 2.41-2.45 (m, 3H), 2.72-2.84 (m, 3H), 3.25-3.29 (m, 1H), 3.47-3.53 (m, 2H), 3.62 (t, J = 9.8Hz, 2H), 5.98 (s, 2H), 6.64-6.72 (m, 3H), 7.38 (dd, J_1 = 8.4Hz, J_2 = 2.0Hz, 1H), 7.44 (d, J = 8.4Hz, 1H), 7.53 (d, J = 2.0Hz, 1H).

^{13}C NMR (100 MHz, Methanol-d_4): δ 34.4, 38.4, 41.6, 42.3, 46.9, 49.8, 52.6, 101.3, 107.6, 109.4, 122.6, 126.3, 127.8, 130.4, 132.2, 132.6, 133.9, 146.6, 148.3, 168.9.

Analysis : Mol. Formula: $C_{21}H_{22}Cl_2N_2O_3$

Calcd.: C 59.87, H 5.26, N 6.65.

Found: C 59.89, H 5.24, N 6.68.

5.2. Biology

5.2.1. DPP-IV inhibitory activity and selectivity over other serine protease (*in vitro*)

Enzyme activity was determined by a fluorescence-based assay, adapted from the work of Blackmon et al **[242]**. H-Glycine-Proline-7-amino-4-methyl coumarin (Gly-Pro-AMC, 200 µM) (Bachem, PA) was used as substrate (which is cleaved by the enzyme to release the fluorescent aminomethylcoumarin (AMC)), and soluble human protein (DPP-IV enzyme) produced in a baculovirus expression system (Bac-To-Bac; Life Technologies), was used as the enzyme source. H-Gly-Pro-AMC (200 µM) was incubated with DPP-IV enzyme in the presence of various concentrations of test compounds. Reaction was carried out at pH 7.8 (HEPES buffer 25 mM containing 1.0% BSA, 140 mM NaCl, 16 mM $MgCl_2$, 2.8% DMSO) in a total volume of 100 µl at 25°C for 30 min., in the dark. Reaction was terminated with acetic acid (25 µl of 25% solution). Activity (fluorescence) was measured (expressed as fluorescent units (FU)) in a Spectra Max fluorometer (Molecular Devices, Sunnyvale CA) by exciting at 380 nm, and measuring emission at 460 nm. IC_{50} values were determined for test compounds using Graph Pad prism software.

DPP8.

Compounds were tested against human DPP8 (baculovirus) in a continuous fluorescent assay in 50 mmol/l sodium phosphate buffer, pH 8.0, and 0.1 mg/ml BSA, using Ala-Pro-7-amino-4-trifluormethylcoumarin as substrate at 100 µmol/l at 37°C for 15 min (excitation/emission: 400/505 nm).

DPP9.

Compounds were tested against human DPP9 (baculovirus) in a continuous fluorescent assay in 100 mmol/l Tris/HCl buffer, pH 7.4, and 0.1 mg/ml BSA, using Gly-Pro-AMC as substrate at 100 µmol/l at 37°C for 30 min (excitation/emission: 360/460 nm).

QPP/DPP2.

Compounds were tested against human QPP (baculovirus) in a continuous fluorescent assay in 100 mmol/l cacodylate buffer, pH 5.5, and 0.1 mg/ml BSA, using Nle-Pro-AMC as substrate at 5 µmol/l at 37°C for 15 min (excitation/emission: 360/460 nm).

Data analysis:

To measure the inhibition constants, serial dilutions of inhibitor were added to reactions containing enzyme and substrate. IC_{50} values were determined by a fit of the reaction rates to a three-parameter Hill equation by nonlinear regression. The data are reported

as percentage inhibition calculated as follows: %Inhibition = 100 (1 – (V_t/V_c)), where V_t is the rate of reaction of treated sample and V_c is the rate of reaction of control sample.

5.2.2. CYP inhibition study (*in vitro*)

For CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition studies, Human liver microsomes (0.2 mg/ml), Testosterone (50 µM) / Dextromethorphan (5 µM) respectively, as probe substrates, potassium phosphate buffer (0.1 M; pH 7.4) and NADPH (1mM) were incubated with different concentrations of test compounds (@1, 10 and 100 µM concentrations) at 37^{o}C for 10 min., enzyme activity (% of control) was determined and IC_{50} values were calculated **[262]**.

5.2.3. Pharmacodinemic study (Antidiabetic activity) (*in vivo*)

The *in- vivo* glucose lowering properties of some of the test compounds and standards were evaluated in db/db animal models as described below. Study was conducted in male C57BL/6J (using IPGTT protocol) or without glucose load, in db/db mice (age 8-12weeks). All animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'. Two days prior to the study, the animals were randomized and divided into 2 groups (n = 6), based upon their fed glucose levels. Animals were left for 2 days under acclimatization and maintained on a standard diet. On the day of experiment, food was withdrawn from all the cages, water was given *ad-libitum* and were kept for overnight fasting. Briefly, in IPGTT protocol (C57 mice) overnight fasted mice were dosed orally (p.o.) with the test compounds (x mg/kg), 0.5 h prior to the intraperitoneal (i.p.) glucose load (1.5 g/kg), while in db/db mice, fed mice were dosed orally (p.o.) with the test compounds (x mg/kg) and the blood samples were collected at various time points. Blood samples were centrifuged and the separated serum was immediately subjected for the glucose estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, California). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (Ver 4.0) was used to plot an area under the curve (0-240 min AUC). The AUC obtained from graphs were analyzed for two-way ANOVA, followed by Bonferroni post test, using Graph Pad prism software **[243-244]**.

5.3. Pharmacokinetic study (PK study)

Briefly, for single dose PK study, test compounds were administered orally / iv on a body weight basis (x mg/kg) to overnight fasted male C57BL/6J mice. Serial blood samples were collected in micro-centrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 36 and 48h post-dose after compounds administration. Approximately 0.2 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at -70 °C and the concentrations of compounds in plasma were determined by the LC-MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0 x 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters, such as Tmax, $t_{1/2}$, Cmax, AUC and %F were calculated using a non-compartmental model of WinNonlin software version 5.2.1.

5.4. Docking study

The molecular docking analysis of potent compounds from all the three series and standard compounds was carried out using extra precision (XP) Glide docking method, to understand its critical interactions with all the three binding sites (S1, S2 and S3) of DPP-IV enzyme. The crystal structure of the DPP-IV enzyme (PDB ID: 2I03/ 2AJL/2OQI) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box.

For docking experiments all the compounds were geometry optimized using the Optimized Potentials for Liquid Simulations-all atom (OPLS-AA) force field **[288]** with the steepest descent followed by a truncated Newton conjugate gradient protocol as implemented in Macromodel. DPP-IV was optimized for docking using the protein preparation wizard provided by Schrodinger LLC **[246]**. Partial atomic charges for compounds as well as protein were assigned according to the OPLS-AA force field. The extra precision (XP) Glide docking method was then used to dock all compounds into the catalytic site of DPP-IV **[245]**. Grids for Glide docking were calculated using the bound inhibitor as the reference of catalytic site in the DPP-IV. Upon completion of each docking calculation, 50 poses per ligand were allowed to generate. The top-scored pose was chosen using a Glidescore (Gscore) function **[289]**. The docking method was further validated by docking NVP-DPP728 and the binding mode was found similar as reported earlier.

Spectral data

"To kill an error is as good a service as, and sometimes even better than, the establishing of a new truth or fact."

- Charles Darwin

6. Spectral Data

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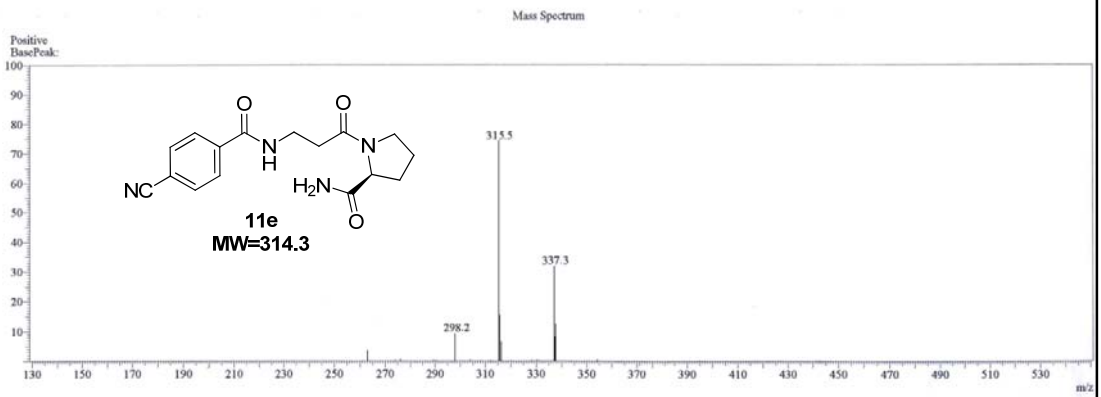
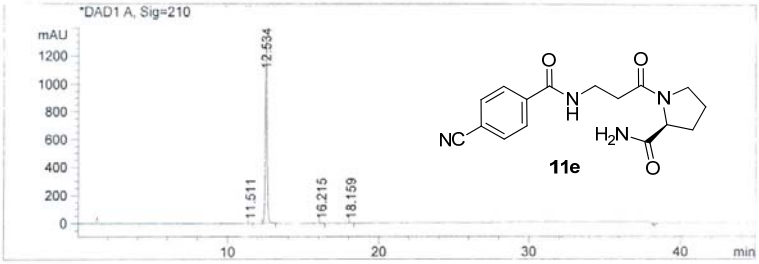
Mass Spectrum



11e
MW=314.3

ANALYSED BY : CHAUHAN JIGNESH

Method Info : Column : YMC TriartC18 (150*4.6mm), 5µ
Mobile Phase : 10mmK2HPO4 (pH:7.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



11e

Area Percent Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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Signal has been modified after loading from rawdata file!

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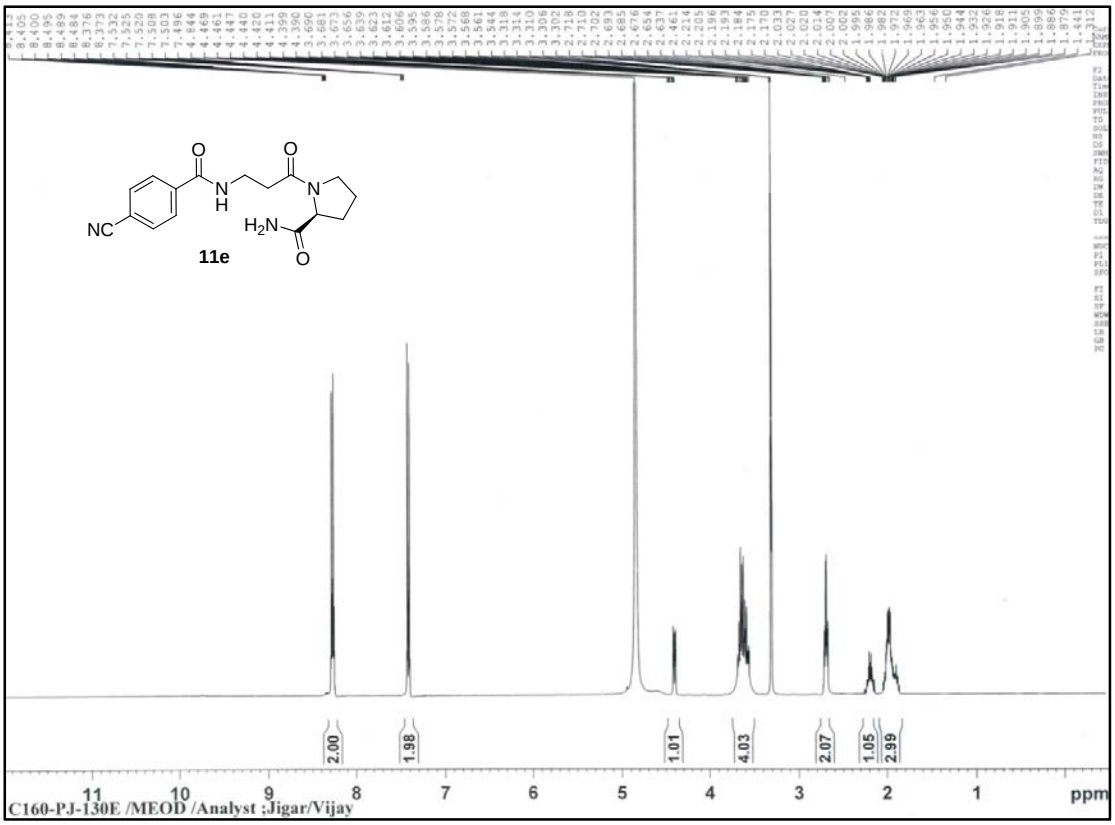
2	12.534	BBA	0.1218	1.07533e4	1337.84204	99.2339
3	16.215	BBA	0.1358	9.65967	1.04549	0.5896
4	18.159	BBA	0.1177	9.37443	1.24685	0.0870
Totals :				1.07766e4	1340.64338	

*** End of Report ***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N
H
O
N
H_2N
O
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1.98
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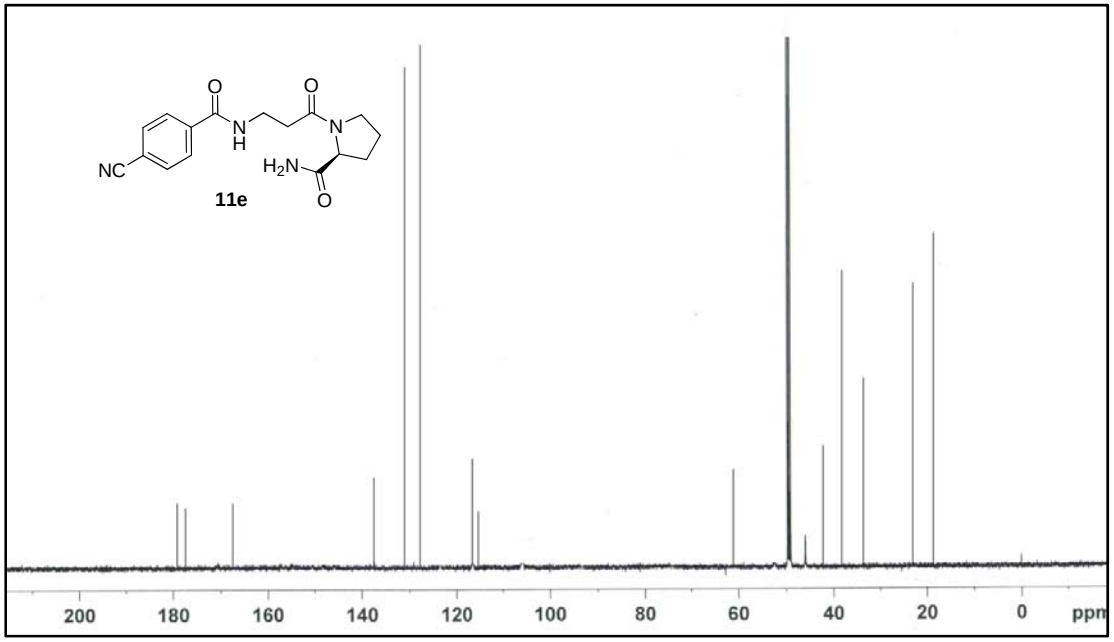
1.05
2.99
11 10 9 8 7 6 5 4 3 2 1 ppm
C160-PJ-130E /MEOD /Analyst :Jigar/Vijay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O
N
H
O
N
H_2N
O
11e
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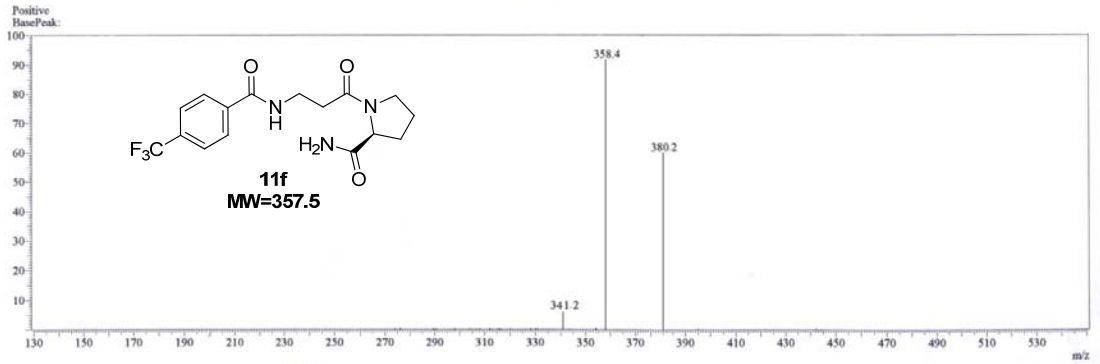
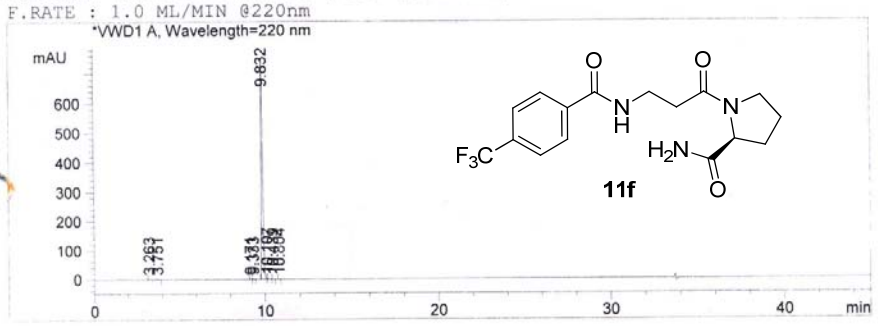
Mass Spectrum



11f
MW=357.5

ANALYSED BY : CHAUHAN JIGNESH

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



11f

Area Percent Report

Sorted By : Signal
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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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Signal has been modified after loading from rawdata file!

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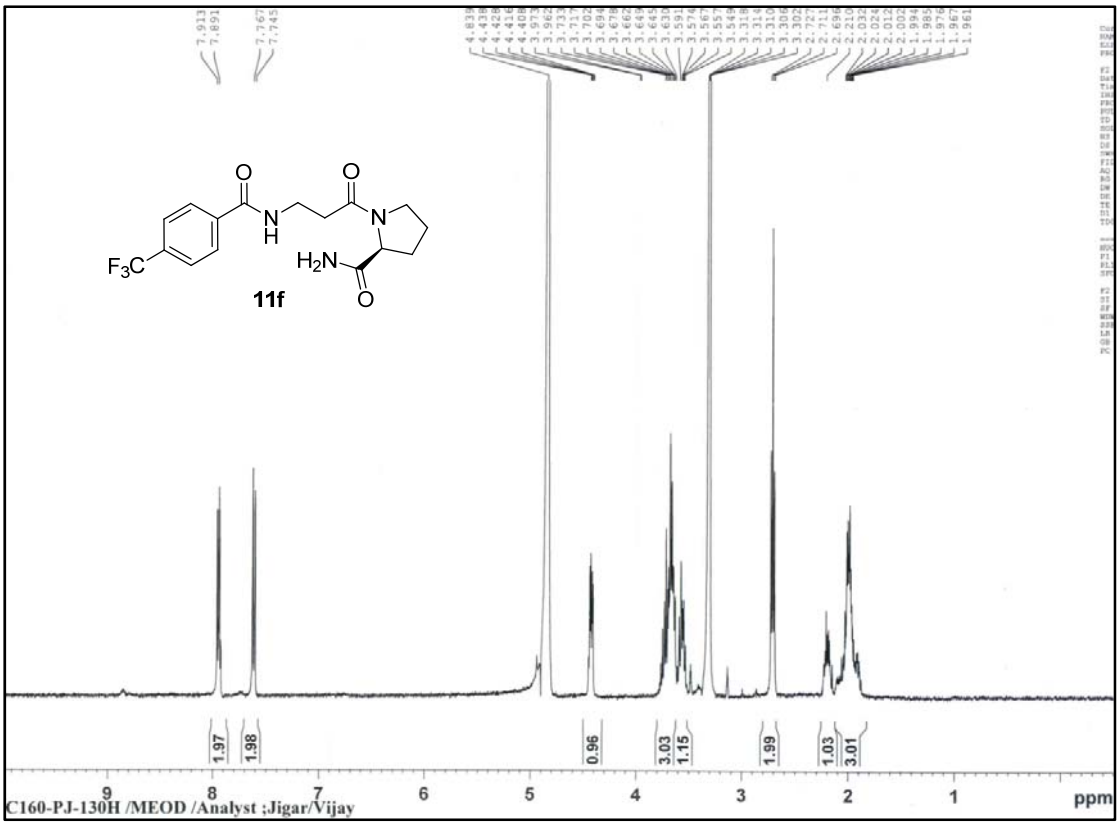
3	9.171	PV	0.0821	2.58589	4.77705e-1	0.0573
4	9.333	VBA	0.0800	2.01834	3.85258e-1	0.0447
5	9.832	MF R	0.0992	4463.96680	749.99207	99.0423
6	10.107	FM R	0.0905	13.13297	2.41764	0.2912
7	10.439	BB	0.0875	1.70522	2.84125e-1	0.0378
8	10.804	BBA	0.0931	1.07646	1.91847e-1	0.0239
Totals :				4510.32066	757.92334	

Results obtained with enhanced integrator!

*** End of Report ***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1.15
1.99
1.03
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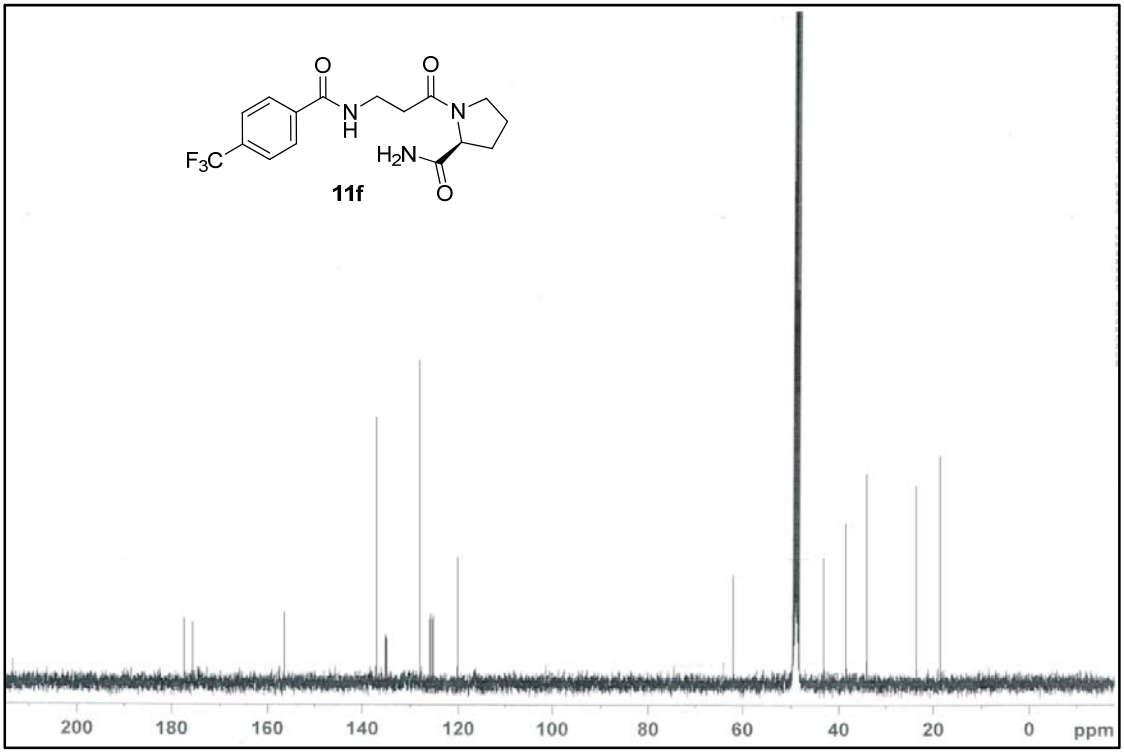
C160-PJ-130H /MEOD /Analyst ;Jigar/Vijay
ppm


11f
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40
20
0
ppm

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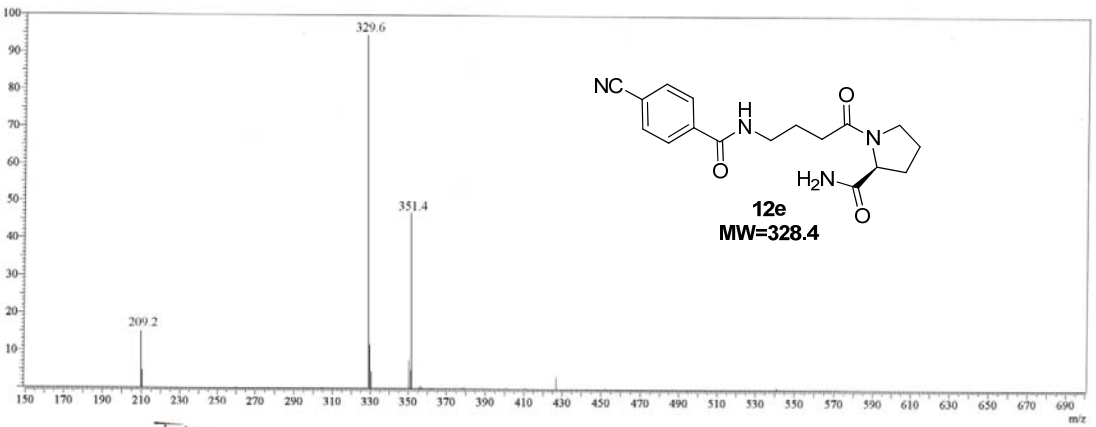
Mass Spectrum

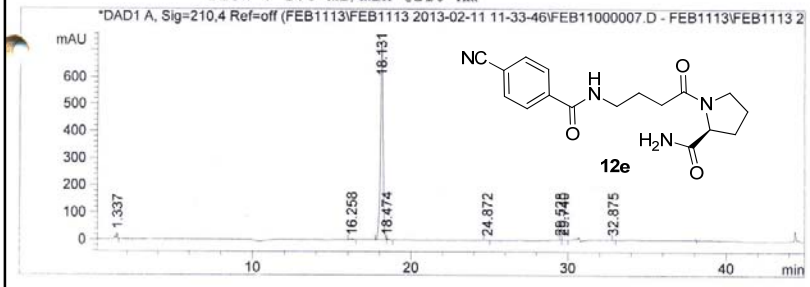
Positive
BasePeak:



12e
MW=328.4

ANALYSED BY : JIGNESH CHAUHAN

Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



12e

Area Percent Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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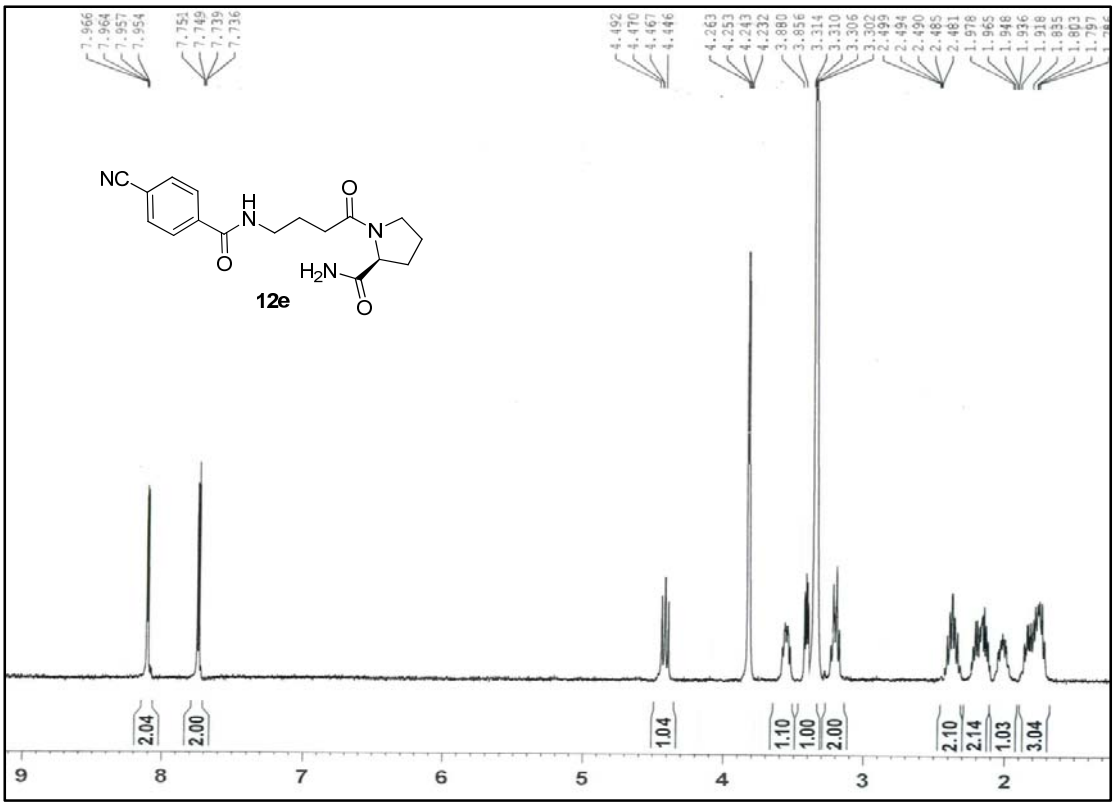
1	1.337	BBA	0.0718	81.75259	17.72912	0.9780
2	16.258	BBA	0.1923	25.93850	2.15735	0.3103
3	18.131	MF R	0.1880	8223.08203	729.08527	99.0402
4	18.474	FM R	0.1136	16.33422	2.39602	0.7954
5	24.872	MM R	0.1859	4.63921	4.15861e-1	0.0555
6	29.528	BV	0.0865	1.98986	3.38825e-1	0.0238
7	29.740	VB R	0.1483	4.24995	4.18112e-1	0.0508
8	32.875	BB	0.0862	1.33533	2.28394e-1	0.0860

Totals : 8359.32169 752.76895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3.04
9
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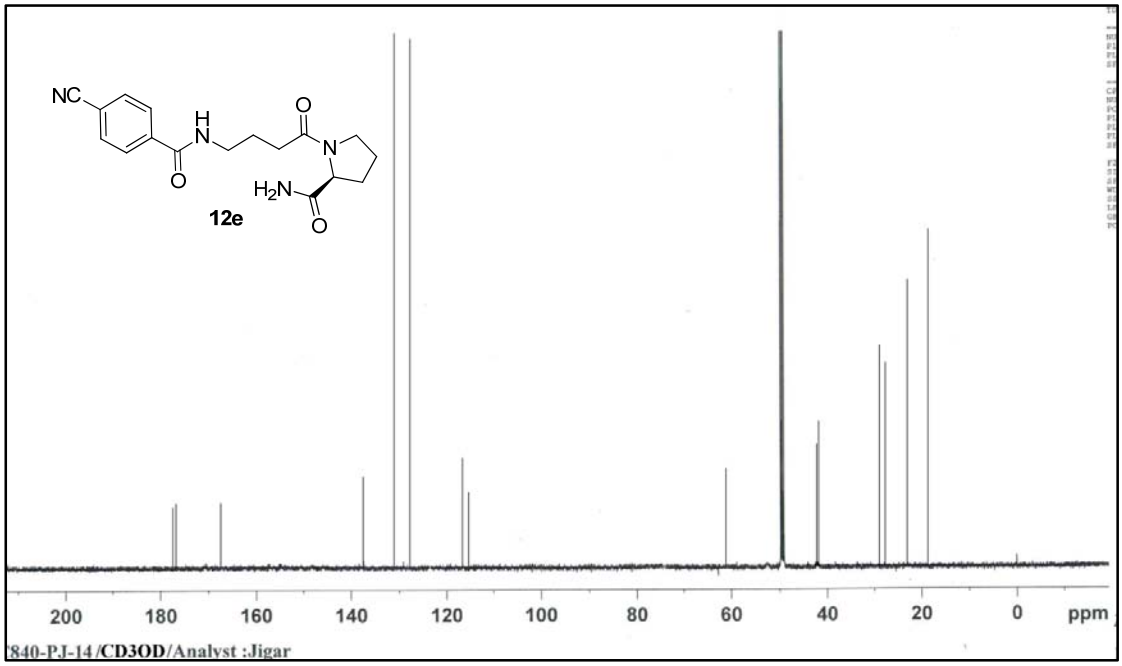
6
5
4
3
2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120
100
80
60
40
20
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ppm
840-PJ-14/CD3OD/Analyst :Jigar

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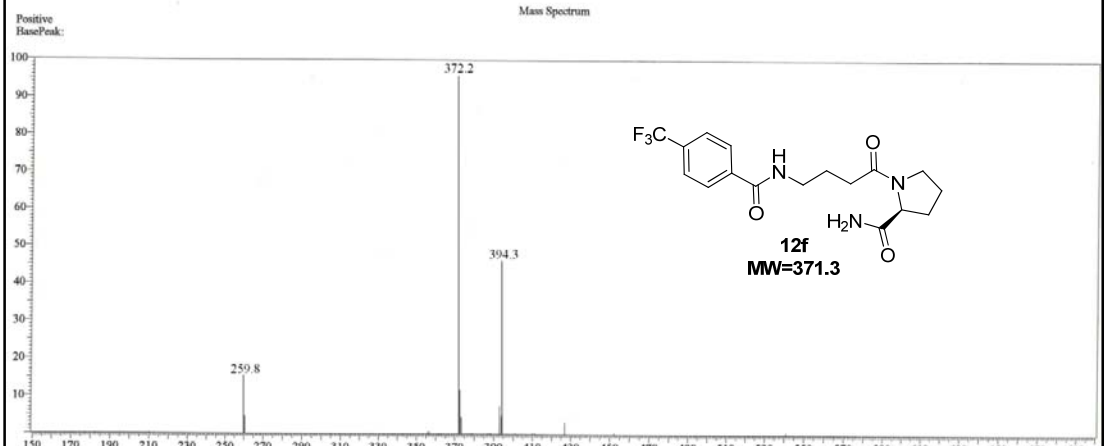
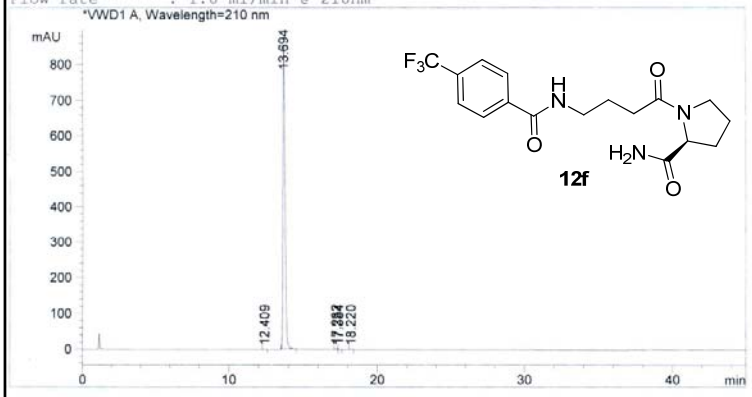
Mass Spectrum

Positive
BasePeak:



ANALYSED BY : JIGNESH CHAUHAN

Column : YMC Triart C18(150*4.6mm) 5.0 µ
Mobile phase : 10mM K2HPO4 (pH-7.0) : ACN (Gradient)
Flow rate : 1.0 ml/min @ 210nm



Area Percent Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=210 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
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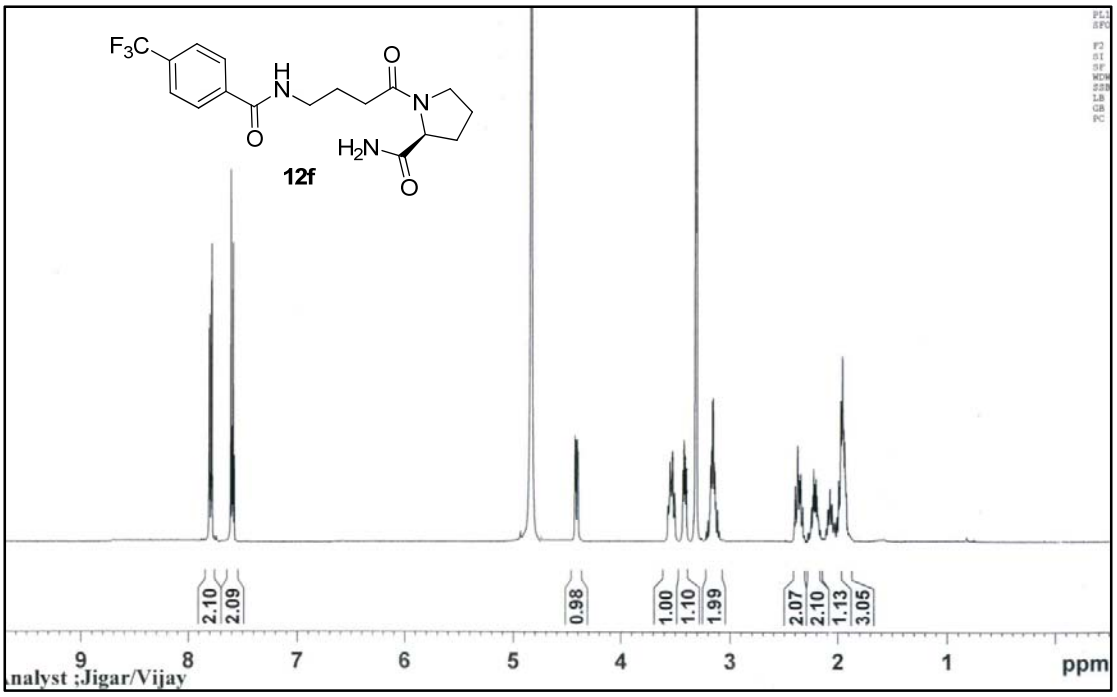
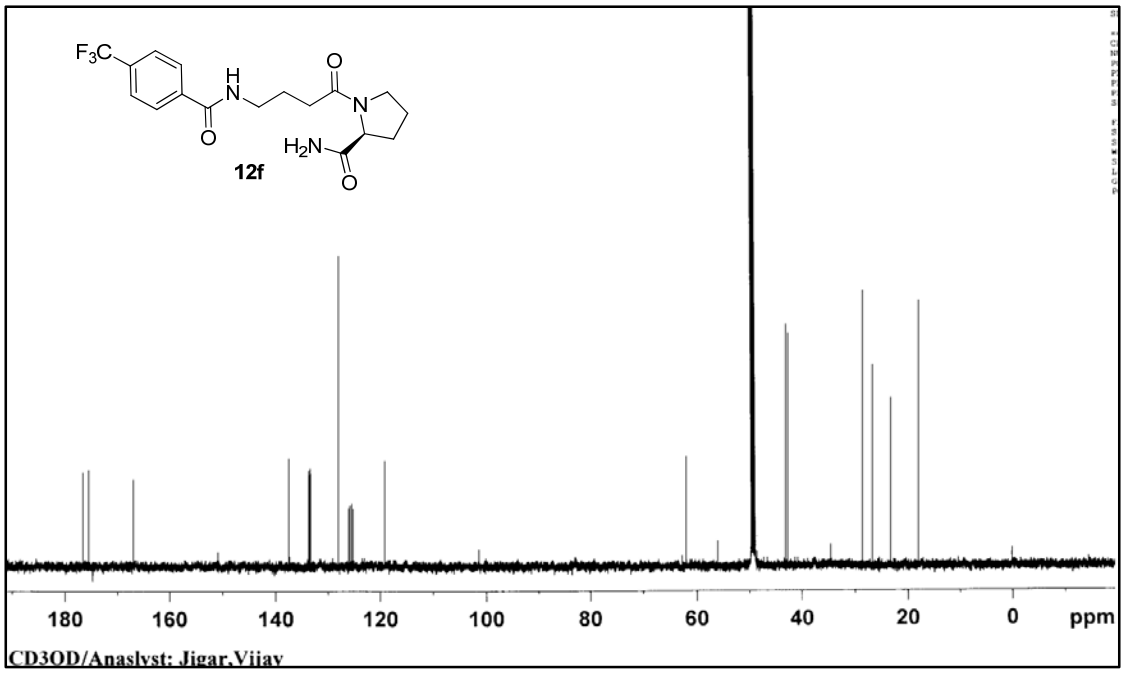
2	13.694	BBA	0.1526	8526.40625	856.35291	99.1949
3	17.232	MF R	0.1430	16.55033	1.00836	0.2833
4	17.384	FM R	0.1333	8.03497	1.92463	0.3938
5	18.220	BBA	0.1227	5.39725	6.94865e-1	0.0630
Totals :				8561.94334	860.64165	

Results obtained with enhanced integrator!

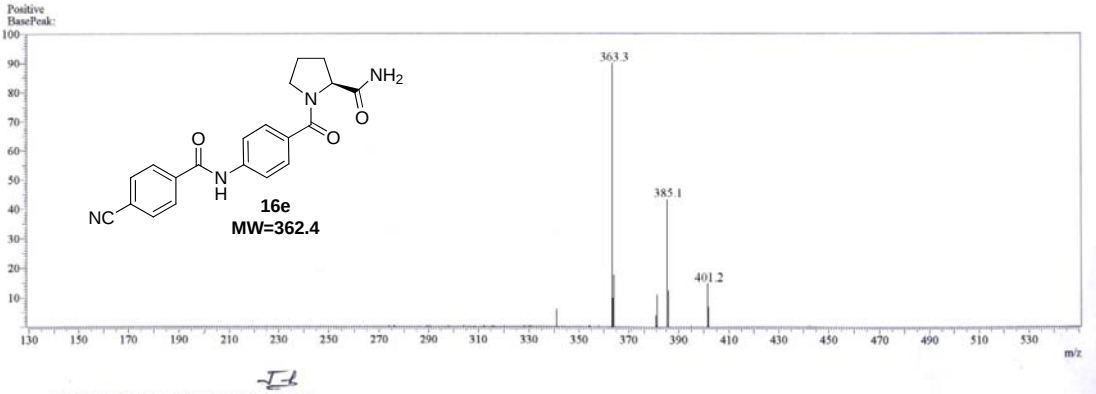
*** End of Report ***


F3C
H
N
O
N
H2N
O
O
12f
2.10
2.09
0.98
1.00
1.10
1.99
2.07
2.10
1.13
3.05
9
8
7
6
5
4
3
2
1
ppm
nalyst ;Jigar/Vijay


F3C
H
N
O
O
N
H2N
O
12f
180
160
140
120
100
80
60
40
20
0
ppm
CD3OD/Anaslyst: Jigar,Vijay

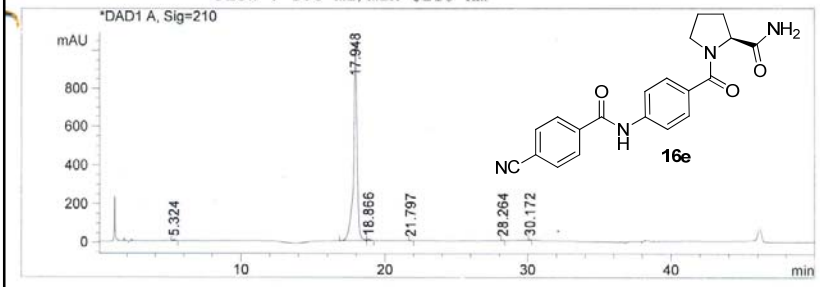
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum



ANALYSED BY : CHAUHAN JIGNESH

Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



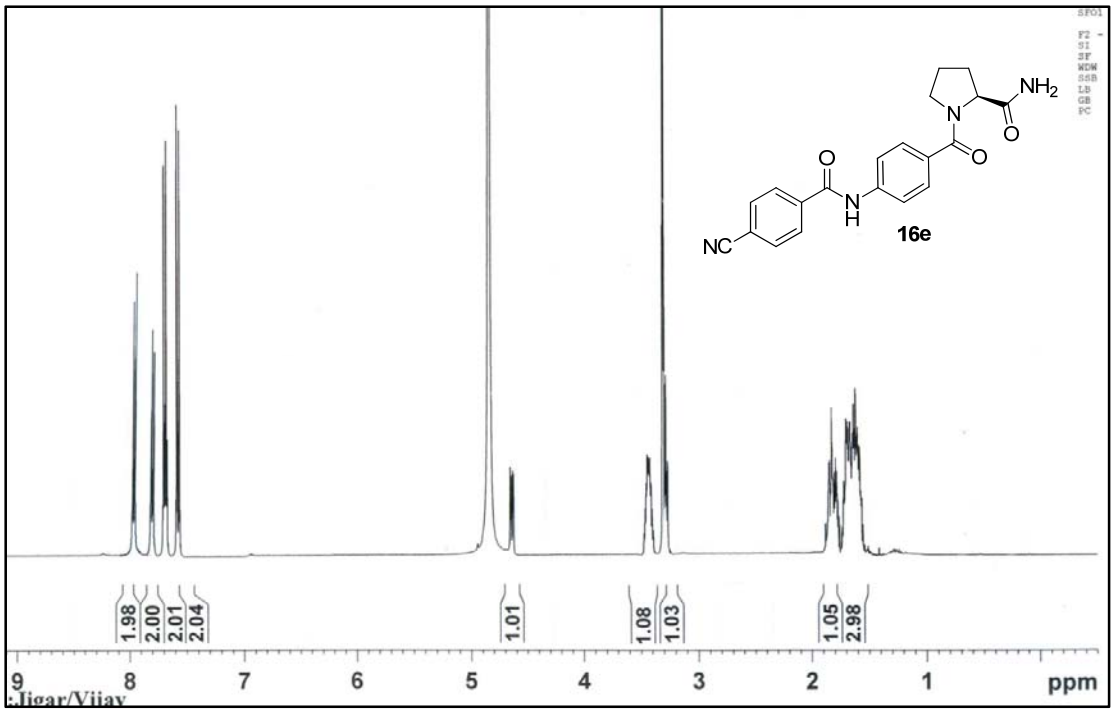
Area Percent Report

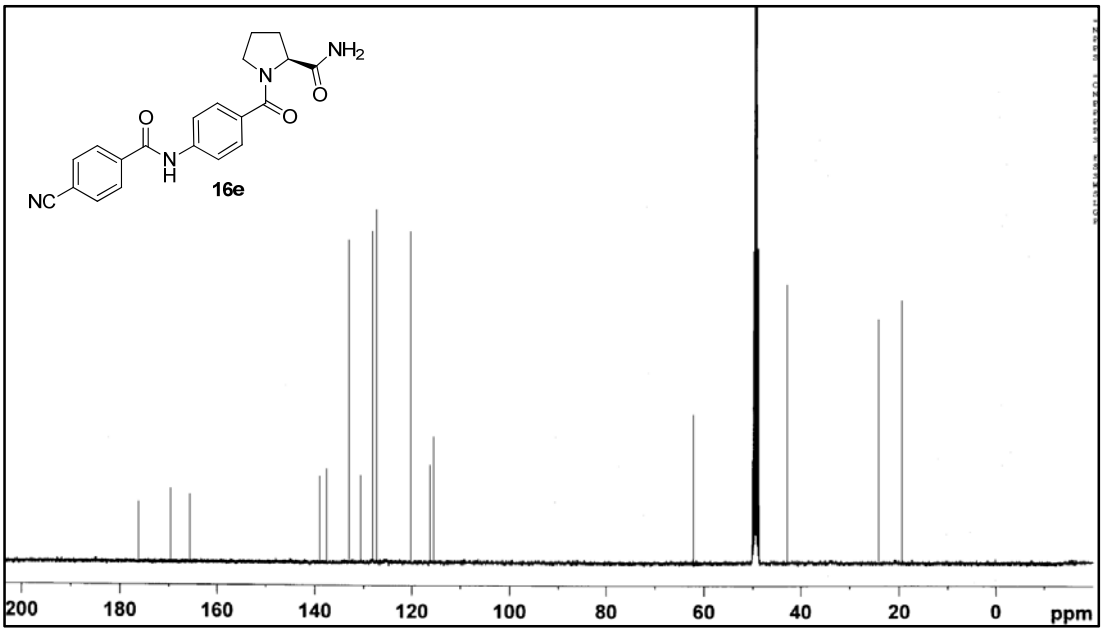
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.324	BBA	0.1852	46.67568	4.02887	0.2037
2	17.948	BV	0.2416	1.75228e4	1034.49072	99.0617
3	18.866	VBA	0.1956	93.73508	6.85016	0.5296
4	21.797	BBA	0.1375	11.17355	1.10956	0.0631
5	28.264	BBA	0.1141	15.17448	1.96532	0.0857
6	30.172	BBA	0.0788	9.93271	1.97134	0.0561
Totals :				1.76994e4	1050.41598	

*** End of Report ***


NH2
O
N
O
O
N
H
NC
16e
1.98
2.00
2.01
2.04
1.01
1.08
1.03
1.05
2.98
9
8
7
6
5
4
3
2
1
ppm
Jigar/Vijay


NH2
N
O
O
O
N
H
NC
16e
200
180
160
140
120
100
80
60
40
20
0
ppm

ZYDUS RESEARCH CENTRE
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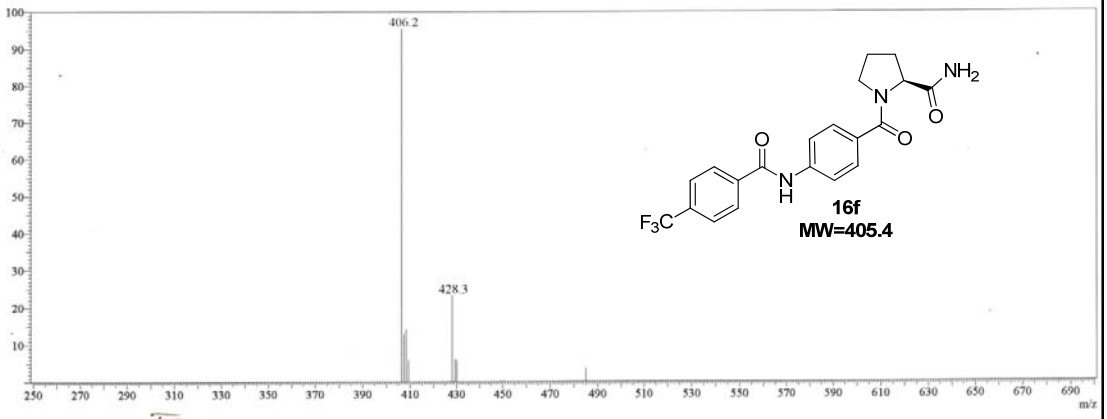
Mass Spectrum

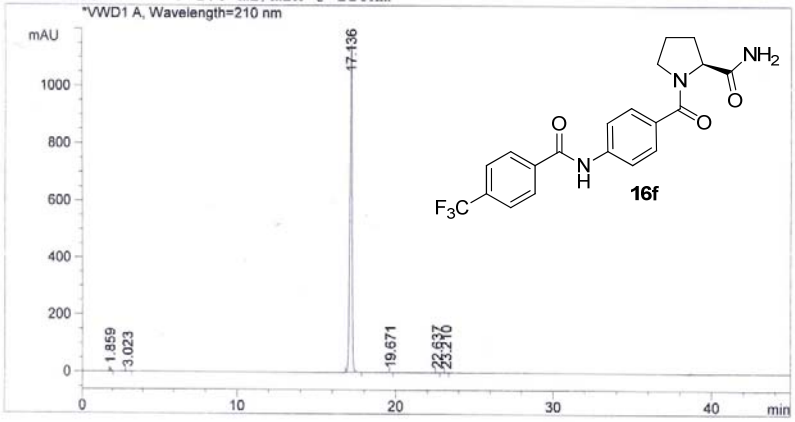
Positive
BasePeak:



16f
MW=405.4

ANALYSED BY : JIGNESH CHAUHAN

Column : YMC Triart C18(150*4.6mm) 5.0 µ
Mobile phase : 10mM K2HPO4 (pH-7.0) : ACN (Gradient)
Flow rate : 1.0 ml/min @ 210nm



16f

Area Percent Report

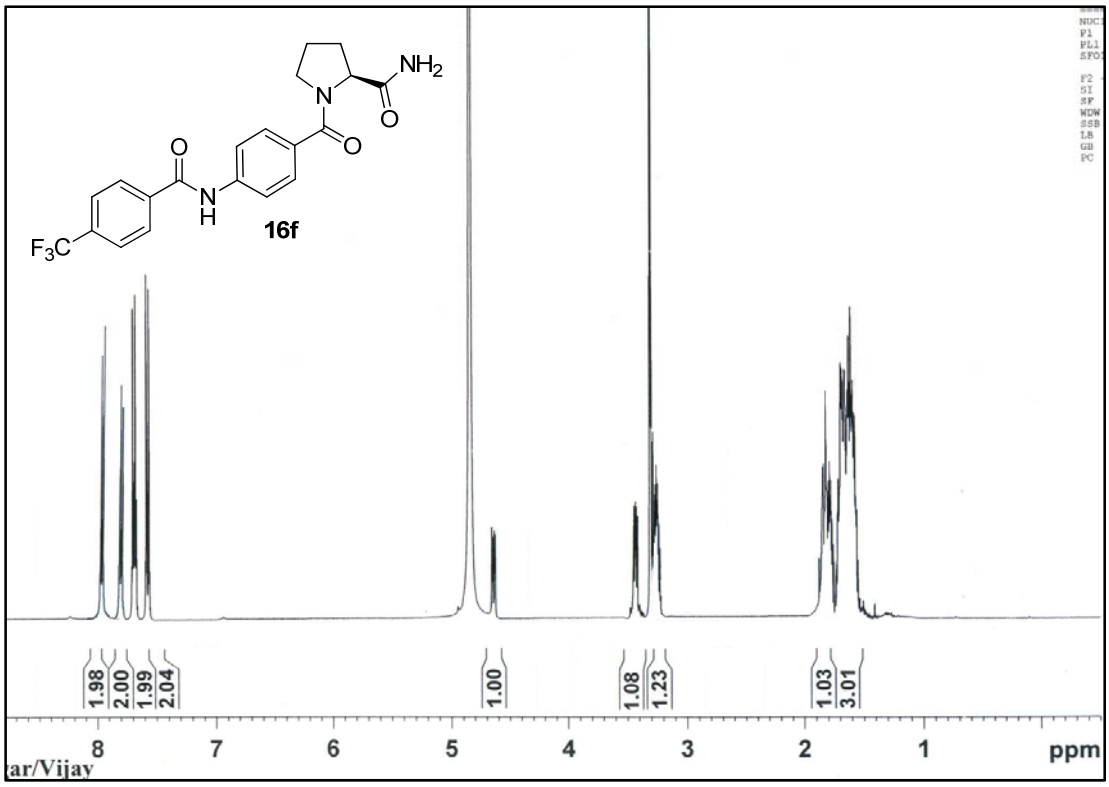
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

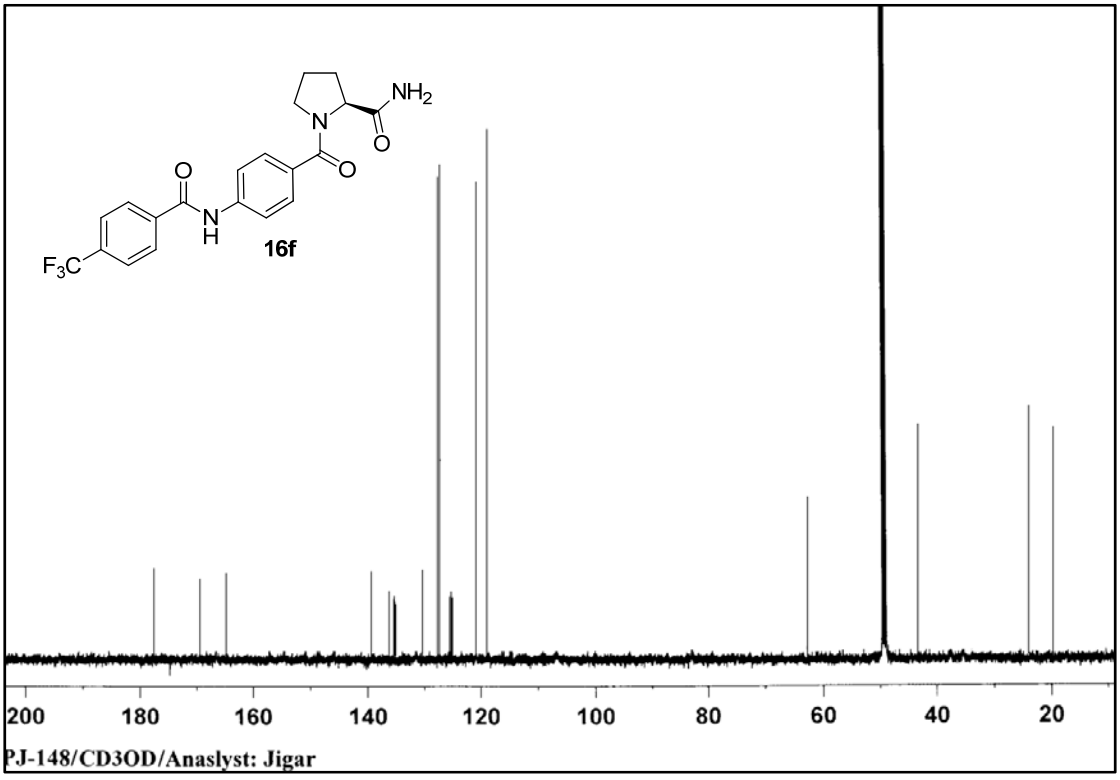
Signal 1: VWD1 A, Wavelength=210 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.859	BBA	0.0786	61.02678	11.92249	0.5585
2	3.023	BBA	0.1752	7.44498	6.39295e-1	0.0681
3	17.136	BBA	0.1454	1.08374e4	1144.73767	99.1830
4	19.671	BP	0.0987	1.33308	1.94963e-1	0.0877
5	22.637	BBA	0.1146	6.08490	8.50215e-1	0.0557
6	23.210	BBA	0.0982	1.30493	1.75799e-1	0.0228
Totals :				1.09266e4	1160.23052	

Results obtained with enhanced integrator!

*** End of Report ***


NH2
O
N
O
O
N
H
F3C
16f
1.98
2.00
1.99
2.04
1.00
1.08
1.23
1.03
3.01
8
7
6
5
4
3
2
1
ppm
ar/Vijay


NH2
O
N
O
O
N
H
F3C
16f
200
180
160
140
120
100
80
60
40
20
PJ-148/CD3OD/Anaslyst: Jigar

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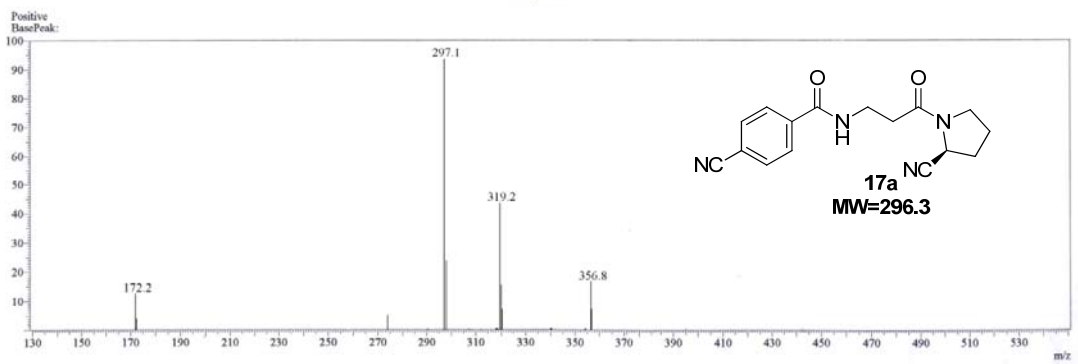
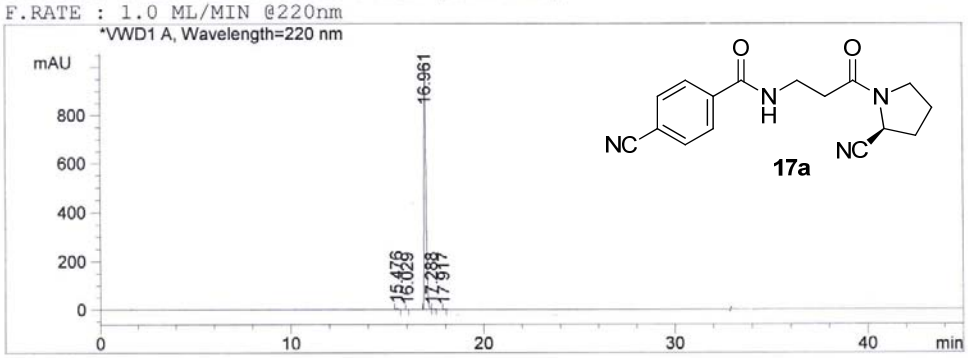
Mass Spectrum



17a
MW=296.3

ANALYSED BY : CHAUHAN JIGNESH

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



17a

Area Percent Report

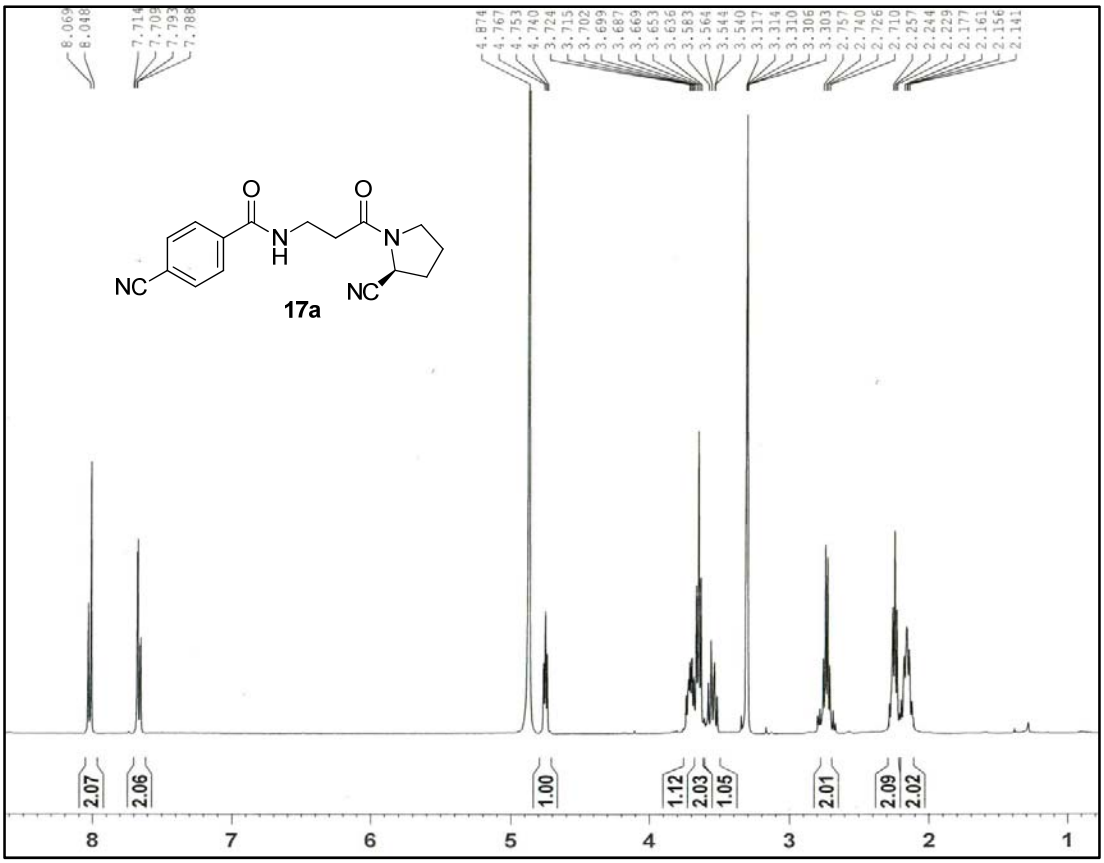
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

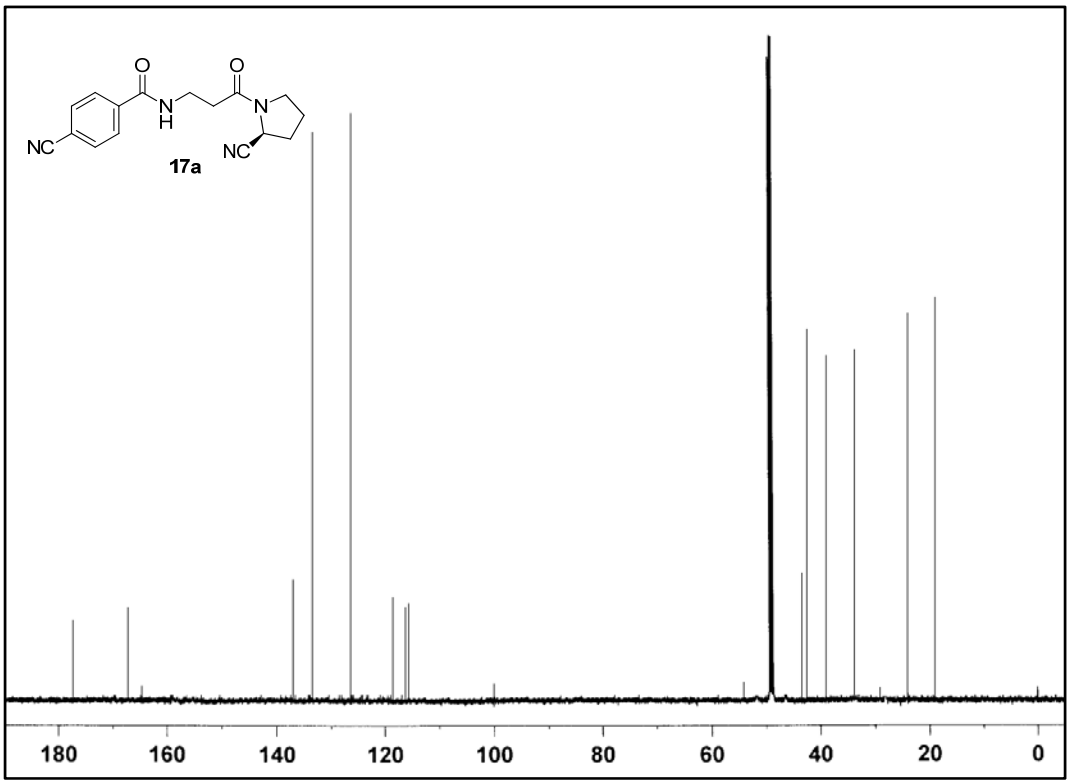
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	15.476	BBA	0.1001	39.52889	6.00477	0.0618
2	16.029	BBA	0.0742	1.08086	1.96956e-1	0.0156
3	16.961	MF R	0.1129	6838.54736	1009.79938	99.8344
4	17.288	FM R	0.0795	6.02183	1.26204	0.0871
5	17.917	MM R	0.0990	1.15465	1.94389e-1	0.0167
Totals :				6911.57454	1021.28646	

Results obtained with enhanced integrator!

*** End of Report ***


17a
2.07
2.06
1.00
1.12
2.03
1.05
2.01
2.09
2.02


17a

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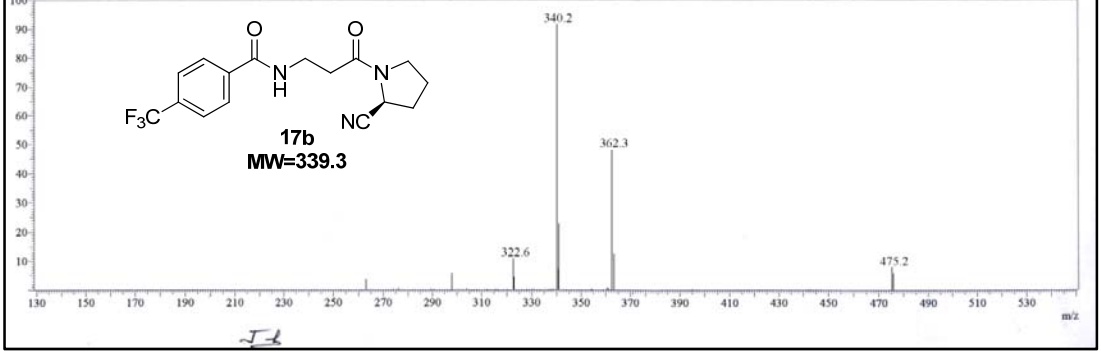
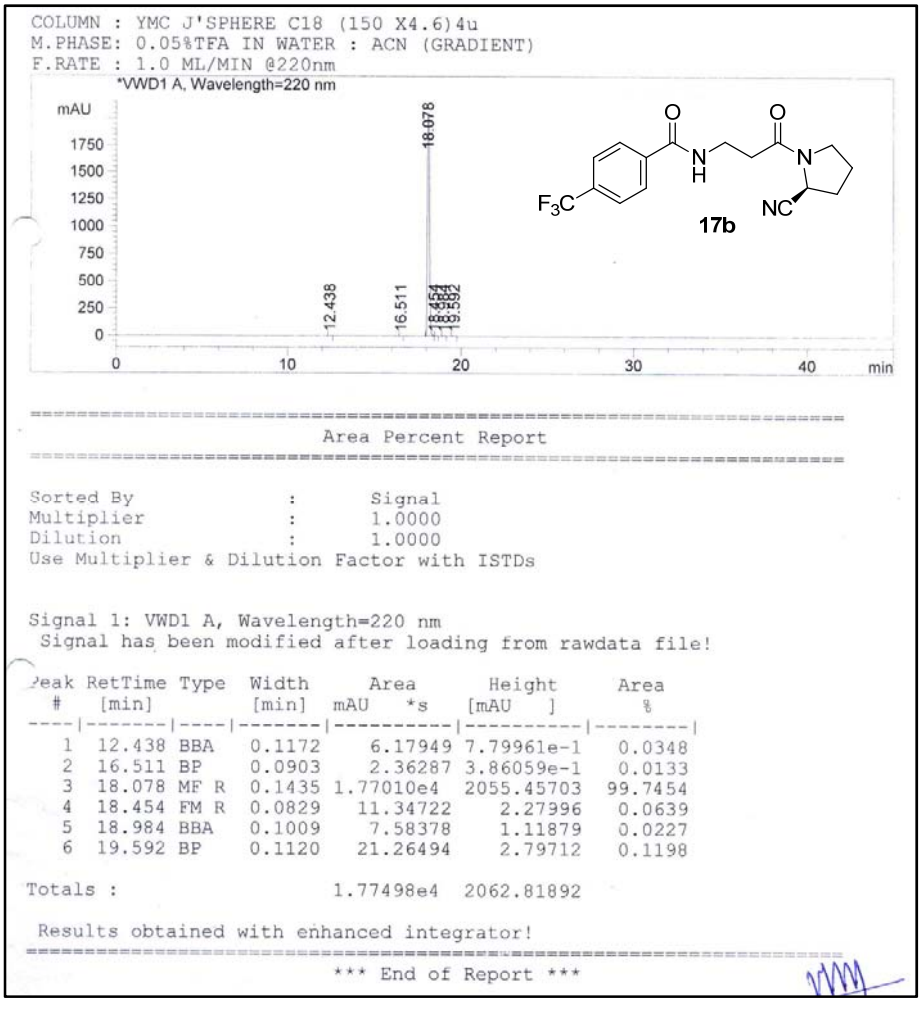
Mass Spectrum





Area Percent Report

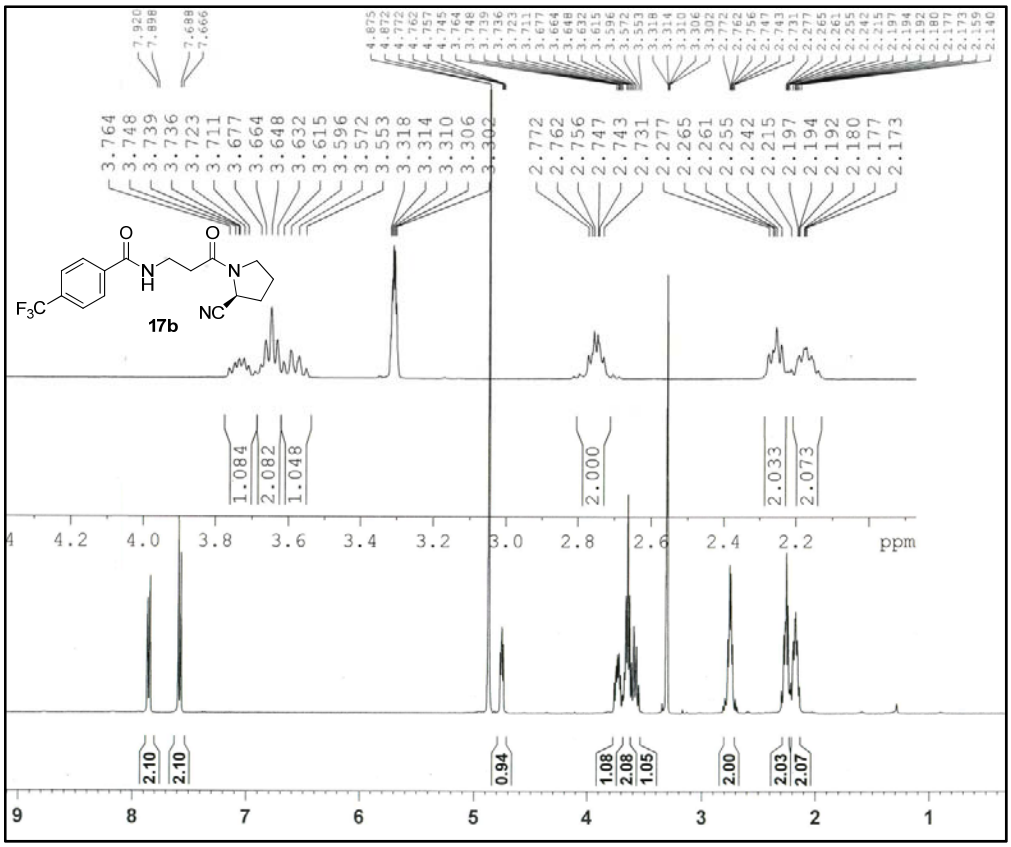
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

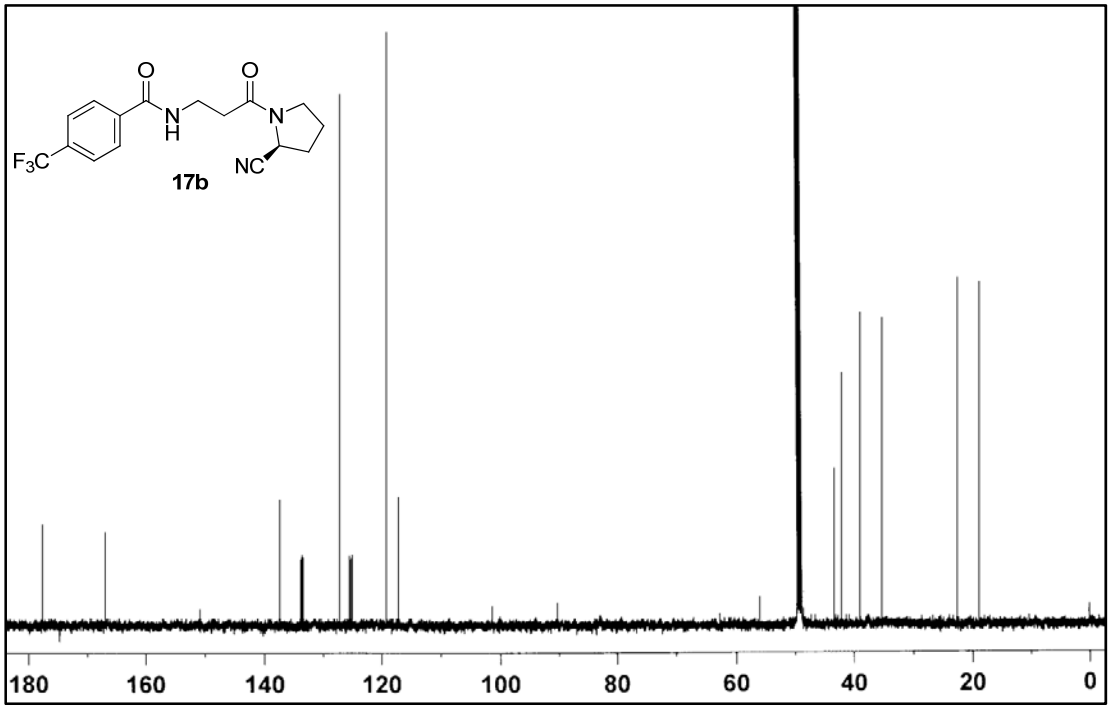
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	12.438	BBA	0.1172	6.17949	7.79961e-1	0.0348
2	16.511	BP	0.0903	2.36287	3.86059e-1	0.0133
3	18.078	MF R	0.1435	1.77010e4	2055.45703	99.7454
4	18.454	FM R	0.0829	11.34722	2.27996	0.0639
5	18.984	BBA	0.1009	7.58378	1.11879	0.0227
6	19.592	BP	0.1120	21.26494	2.79712	0.1198
Totals :				1.77498e4	2062.81892	

Results obtained with enhanced integrator!

*** End of Report ***


17b
ppm


17b

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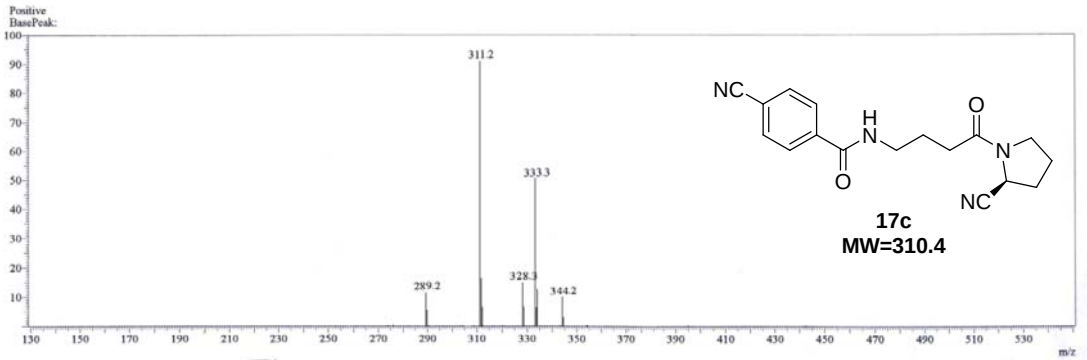
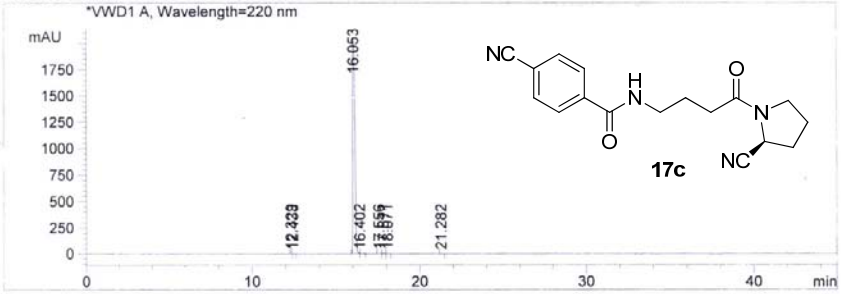
Mass Spectrum



17c
MW=310.4

ANALYSED BY : CHAUHAN JIGNESH

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



17c

Area Percent Report

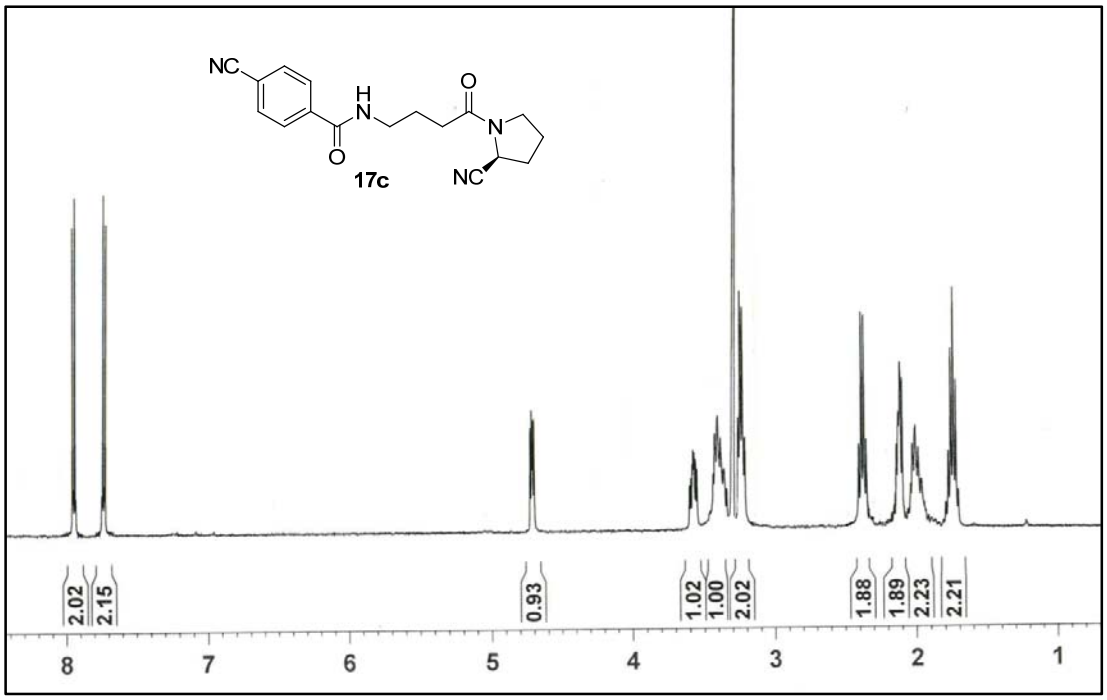
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	12.329	PV	0.0521	2.29090	6.60629e-1	0.0122
2	12.438	VBA	0.1078	3.20680	4.20807e-1	0.0171
3	16.053	MF R	0.1523	1.86815e4	2044.28528	99.5618
4	16.402	FM R	0.1087	26.34491	4.03911	0.1404
5	17.556	VP	0.1084	3.91575	5.36818e-1	0.0209
6	17.811	VV	0.0998	29.81113	4.46001	0.1589
7	18.071	VBA	0.1022	10.29938	1.49576	0.0549
8	21.282	BBA	0.1159	6.34651	8.39083e-1	0.0338
Totals :				1.87637e4	2056.73750	

Results obtained with enhanced integrator!

*** End of Report ***


NC
H
N
O
O
N
NC
17c
2.02
2.15
0.93
1.02
1.00
2.02
1.88
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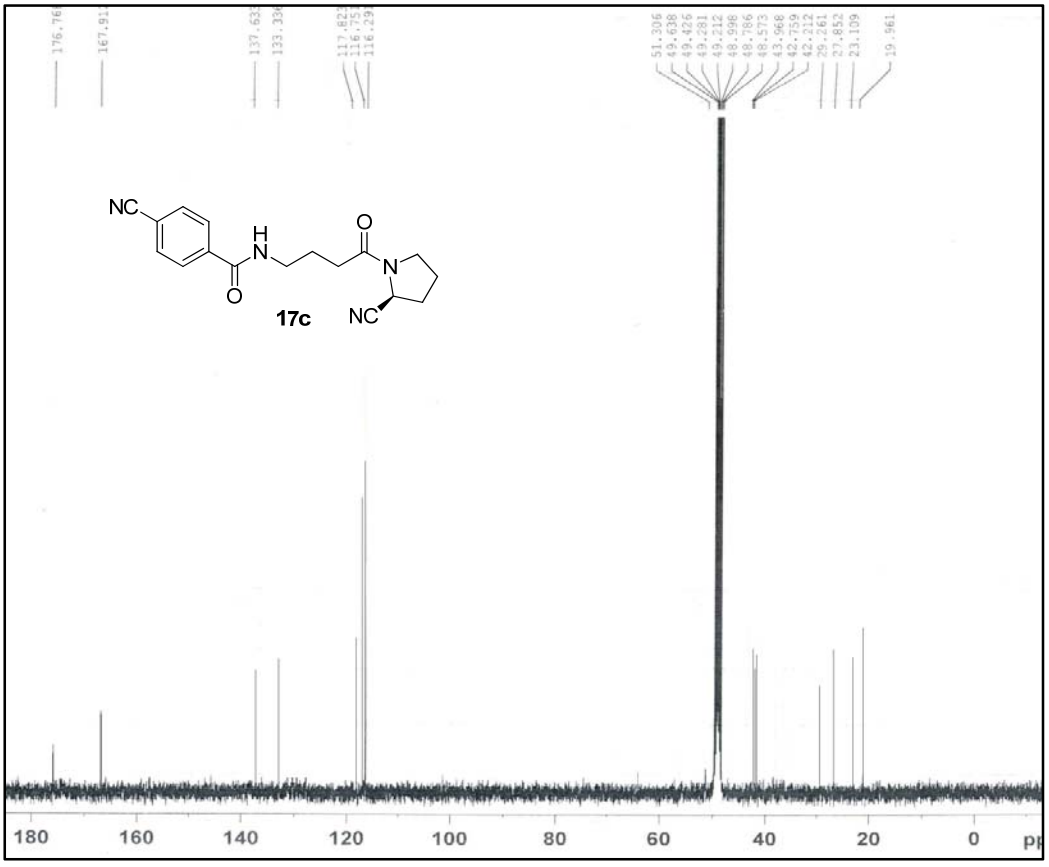
2.21
8
7
6
5
4
3
2
1


NC
H
N
O
O
N
NC
17c
180
160
140
120
100
80
60
40
20
0

ZYDUS RESEARCH CENTRE
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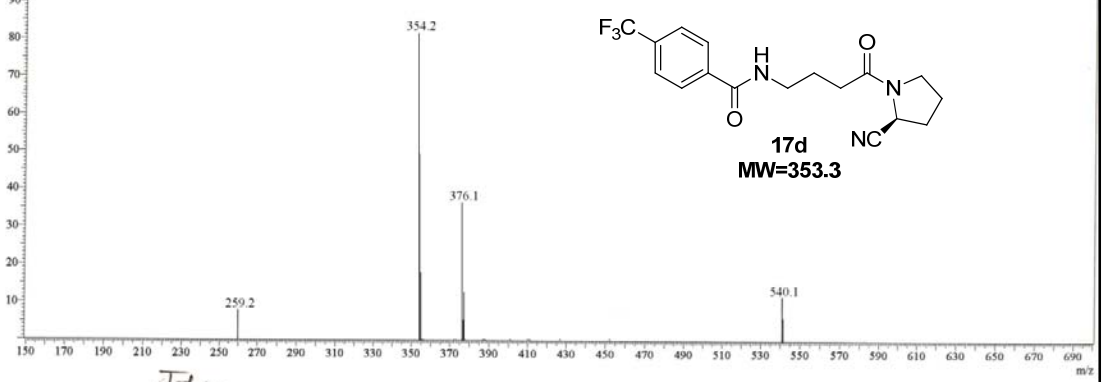
Mass Spectrum

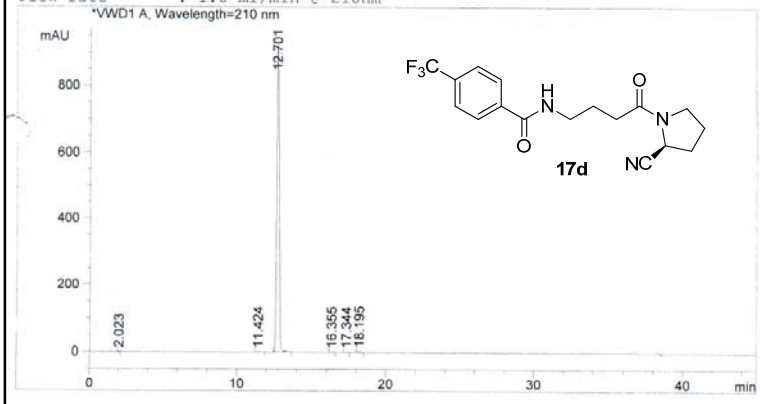
Positive
BasePeak:



17d
MW=353.3

ANALYSED BY : JIGNESH CHAUHAN

Column : YMC Triart C18(150*4.6mm) 5.0 µ
Mobile phase : 10mM K2HPO4 (pH-7.0) : ACN (Gradient)
Flow rate : 1.0 ml/min @ 210nm



17d

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

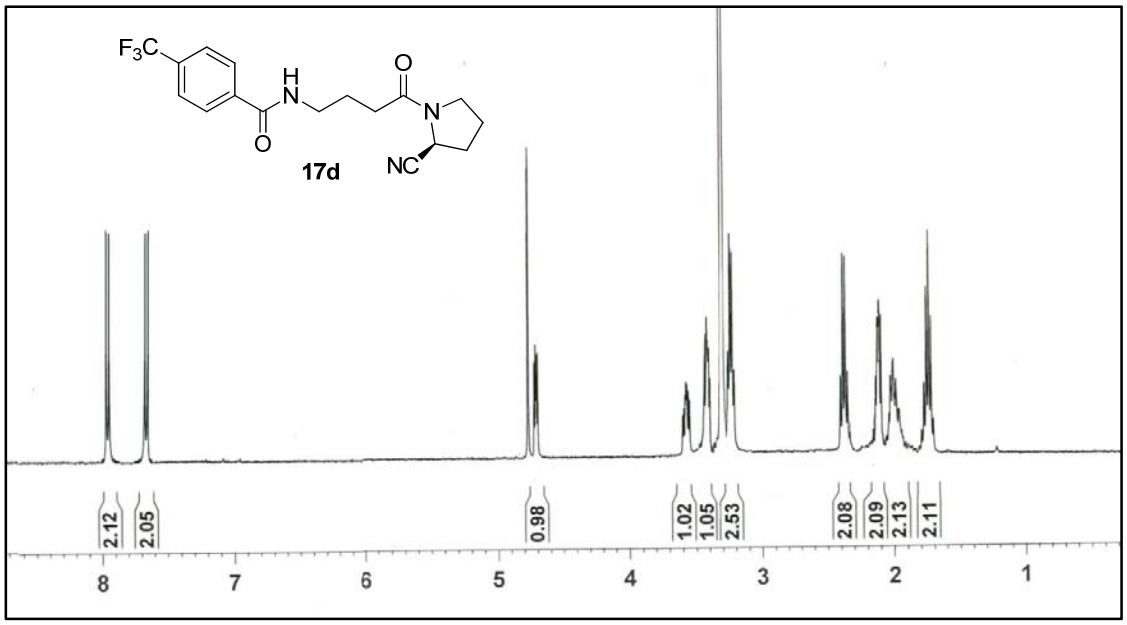
Signal 1: VWD1 A, Wavelength=210 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	2.023	BBA	0.0690	1.33871	3.02824e-1	0.0123
2	11.424	BP	0.2344	19.89976	1.13113	0.1824
3	12.701	BB	0.1922	1.08307e4	923.38245	99.2799
4	16.355	BBA	0.1758	11.65487	9.85785e-1	0.1068
5	17.344	BBA	0.1484	8.70161	8.30637e-1	0.0798
6	18.195	BBA	0.1836	36.95856	3.28803	0.3388
Totals :				1.09093e4	929.92086	

Results obtained with enhanced integrator!

*** End of Report ***

2010/11/


F3C
H
N
O
O
N
NC
17d
2.12
2.05
0.98
1.02
1.05
2.53
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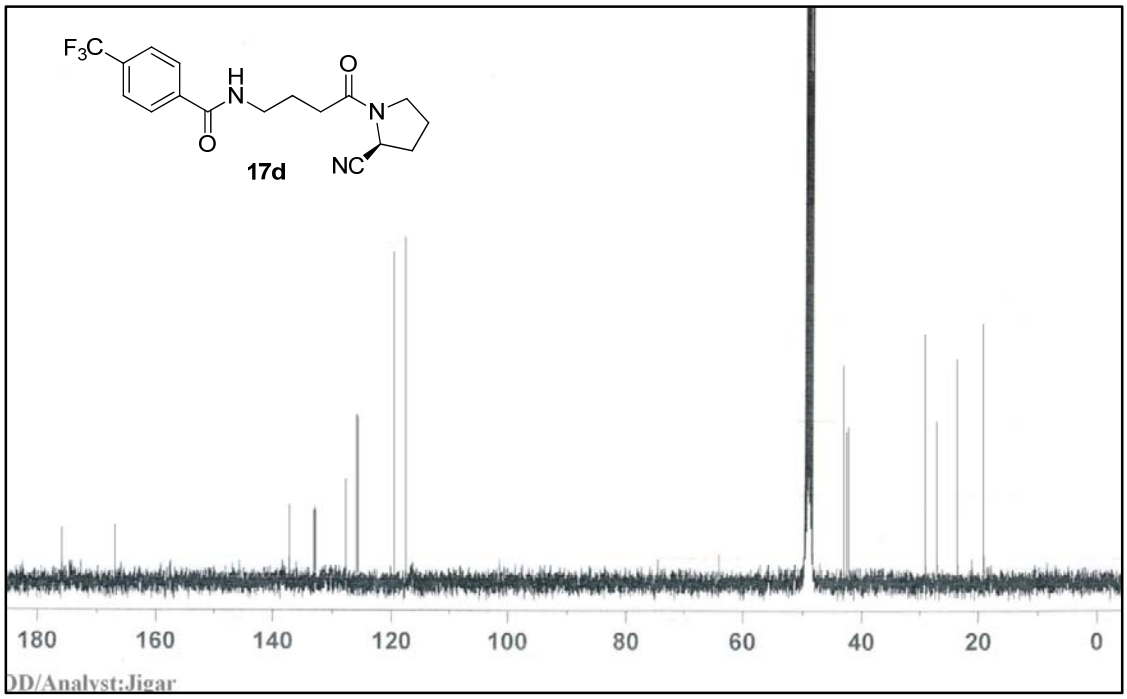
2.11
8
7
6
5
4
3
2
1


F3C
H
N
O
O
N
NC
17d
180
160
140
120
100
80
60
40
20
0
OD/Analyst:Jigar

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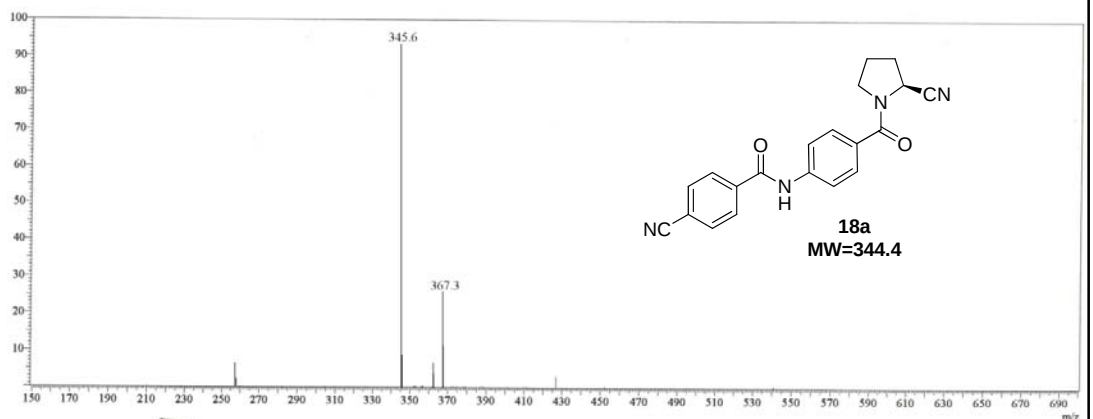
Mass Spectrum

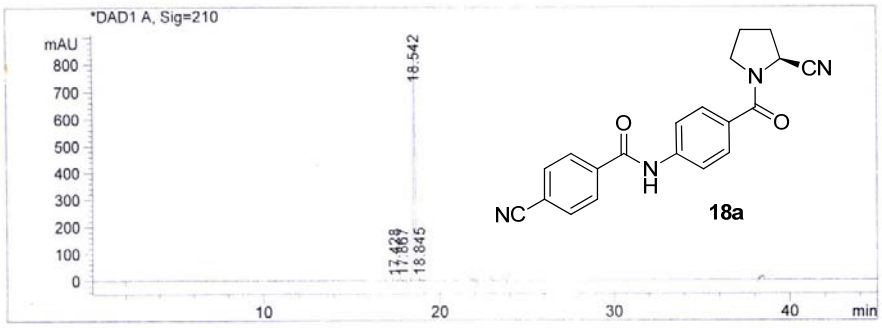
Positive
BasePeak:



18a
MW=344.4

ANALYSED BY : JIGNESH CHAUHAN

Method Info : Column : YMC Triart C18 (150*4.6mm), 5µ
Mobile Phase : 10mm K2HPO4 (pH:7.0) : ACN (Gradient)
Flow : 1.0 ml/min @ 210 nm



18a

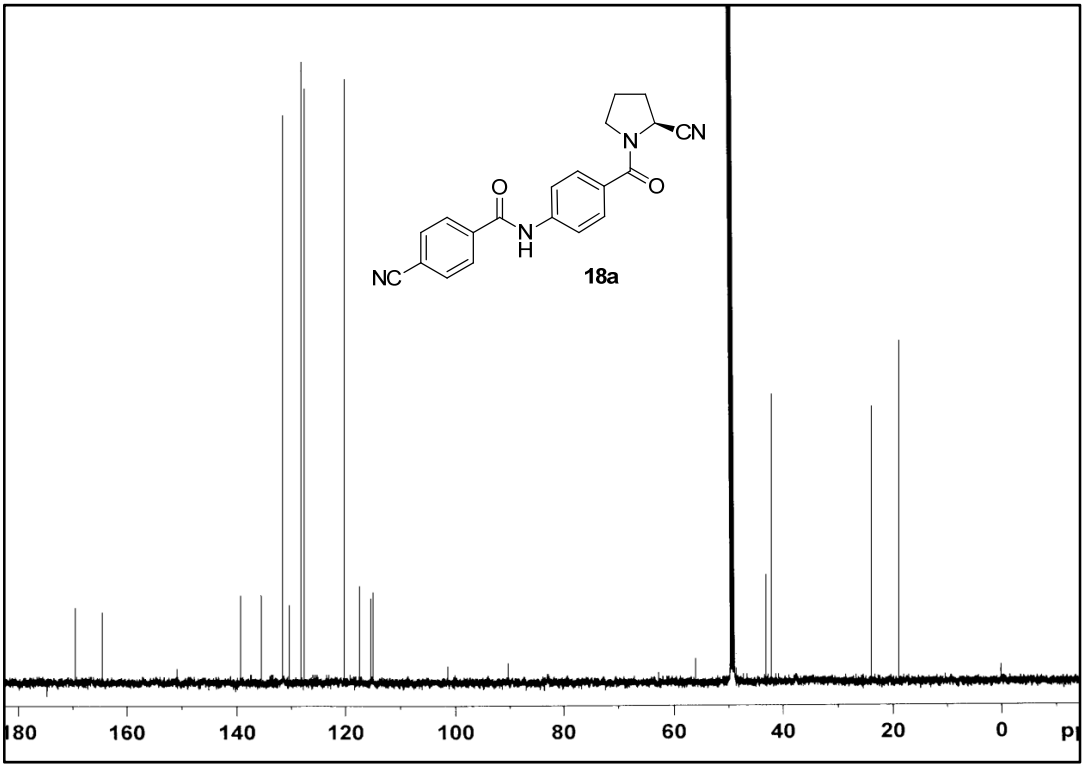
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1						
2	17.428	BBA	0.1129	2.62836	3.52878e-1	0.0376
3	17.867	BBA	0.1169	13.19701	1.77217	0.0606
4	18.542	MF R	0.1314	6867.09814	871.20312	99.6836
5	18.845	FM R	0.1077	22.26688	3.44451	0.2182
Totals :				998.41 95	893.53858	

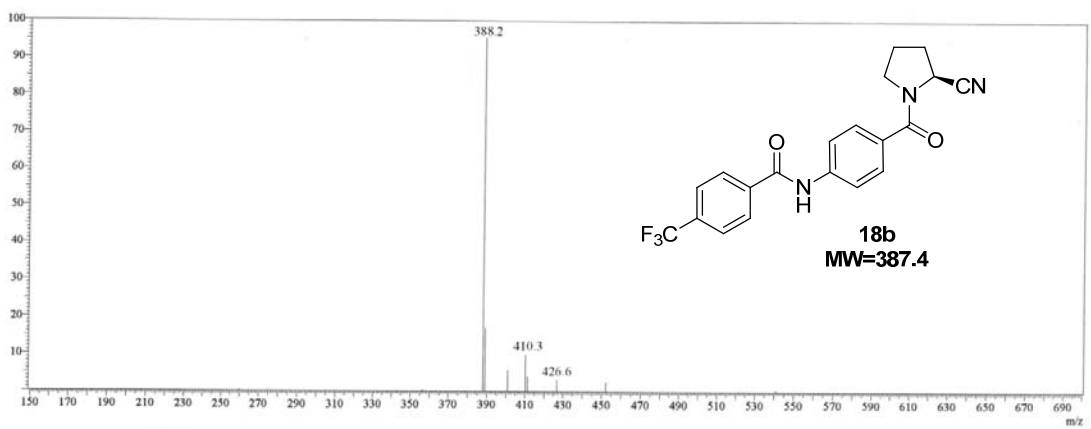

8.013
7.885
7.882
7.880
7.874
7.867
7.846
7.663
4.884
4.871
4.865
4.852
3.677
3.661
3.653
3.637
3.620
3.557
3.540
3.532
3.523
3.515
3.498
3.317
3.314
3.310
3.306
3.303
2.343
2.326
2.312
2.294
2.207
2.191
2.178
2.162
2.146
2.130
1.992
1.976
1.959
1.942
1.925
CN
N
O
O
N
H
NC
18a
2.03
2.02
2.08
2.09
0.95
1.04
1.05
1.02
1.01
1.98
8
7
6
5
4
3
2
1
ppm


CN
N
O
O
N
H
NC
18a
180
160
140
120
100
80
60
40
20
0

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum

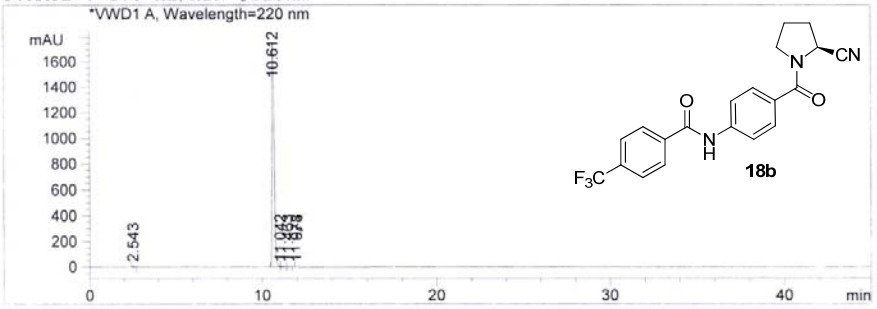
Positive
BasePeak:



18b
MW=387.4

ANALYSED BY : JIGNESH CHAUHAN

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



18b

Area Percent Report

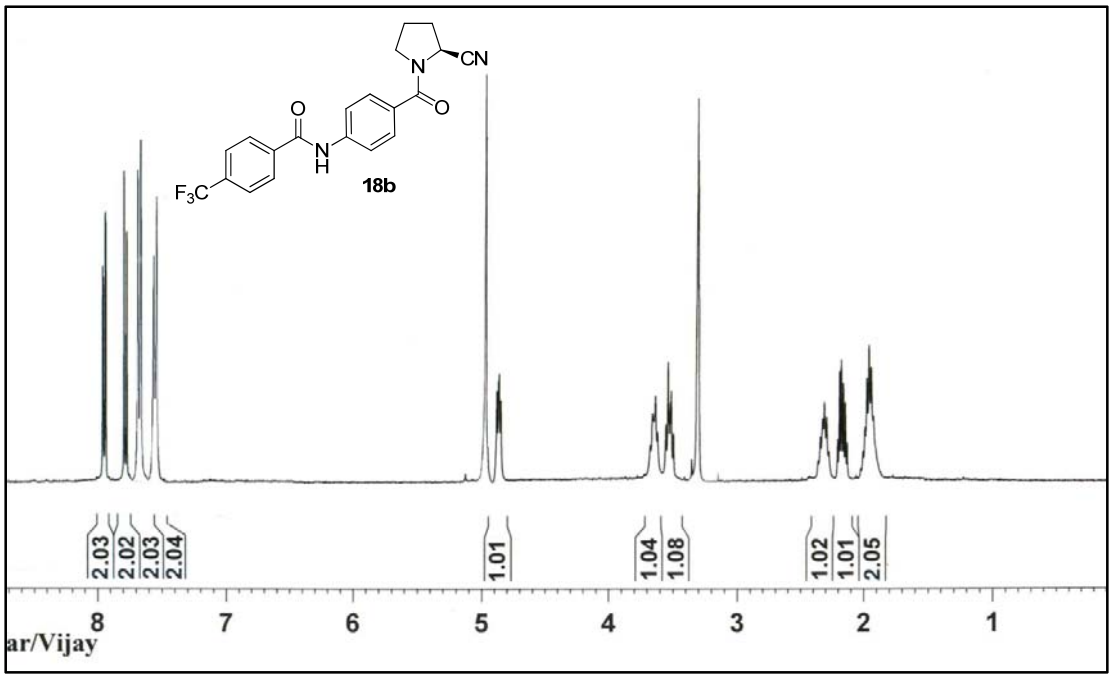
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

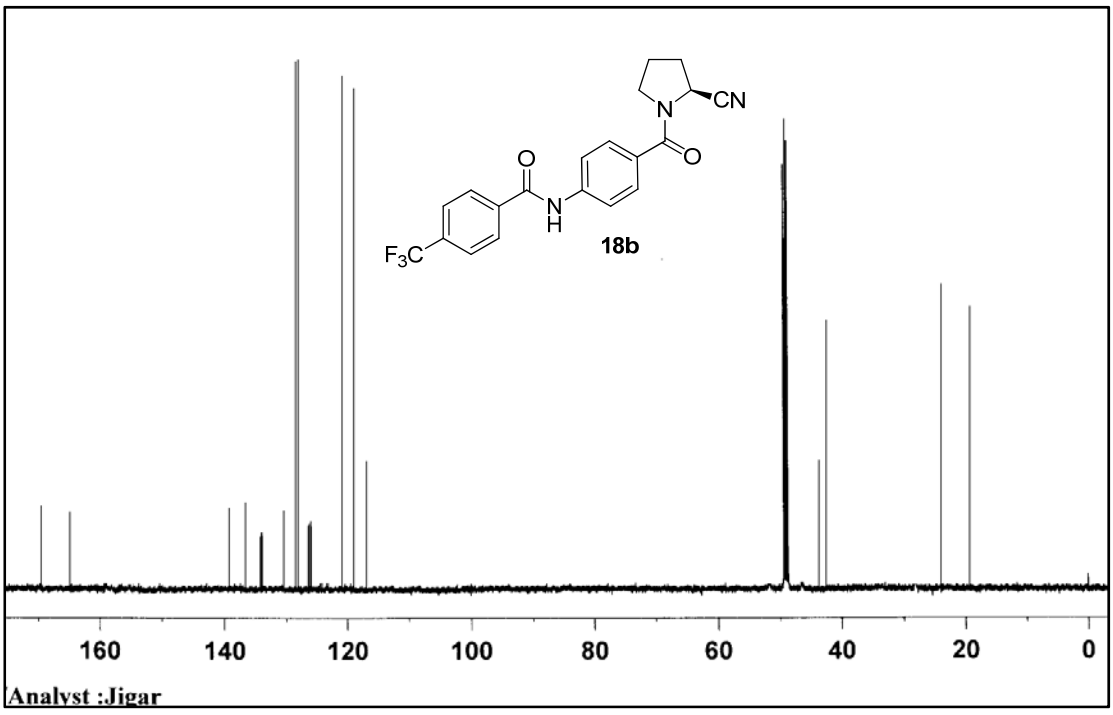
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	2.543	PBA	0.0955	34.75888	5.40170	0.2253
2	10.612	MF R	0.1450	1.53229e4	1761.73853	99.3601
3	11.042	FM R	0.1593	35.82122	3.74806	0.2922
4	11.463	BBA	0.1399	8.47246	8.27341e-1	0.1224
Totals :				1.54247e4	1775.40741	

Results obtained with enhanced integrator!

*** End of Report ***


CN
N
O
O
N
H
F3C
18b
2.03
2.02
2.03
2.04
1.01
1.04
1.08
1.02
1.01
2.05
8
7
6
5
4
3
2
1
ar/Vijay


CN
N
O
O
N
H
F3C
18b
160
140
120
100
80
60
40
20
0
Analyst :Jigar

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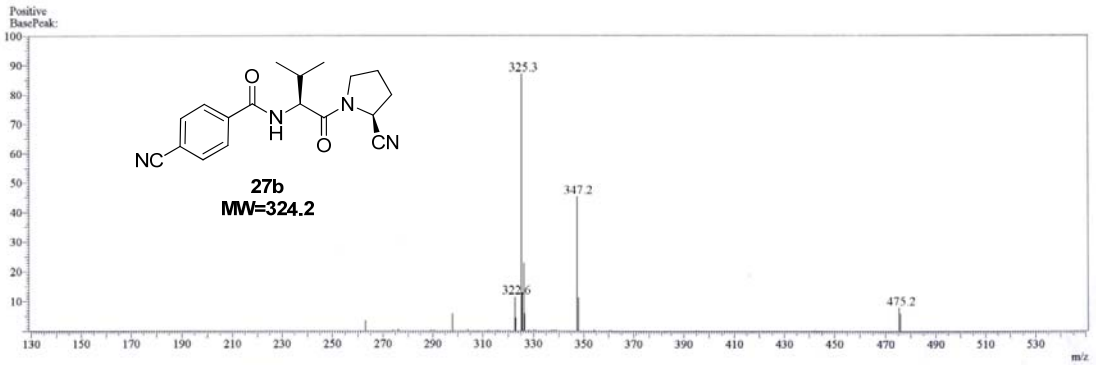
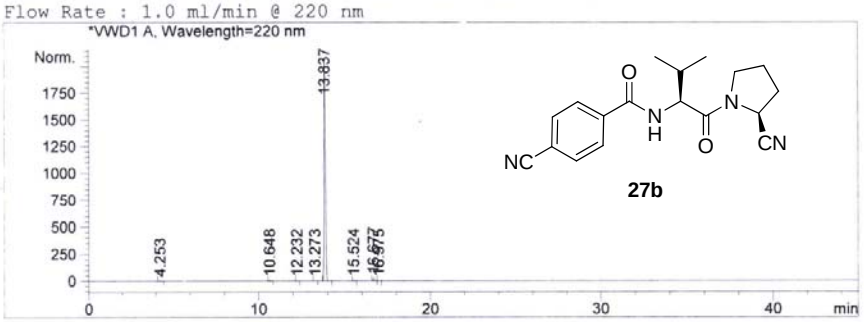
Mass Spectrum



27b
MW=324.2

ANALYSED BY : CHAUHAN JIGNESH

Column : YMC J'sphere C18-(150 X4.6mm)4.0 µ
Mobile Phase : 0.05 % TFA in Water : ACN (Gradient)
Flow Rate : 1.0 ml/min @ 220 nm



27b

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

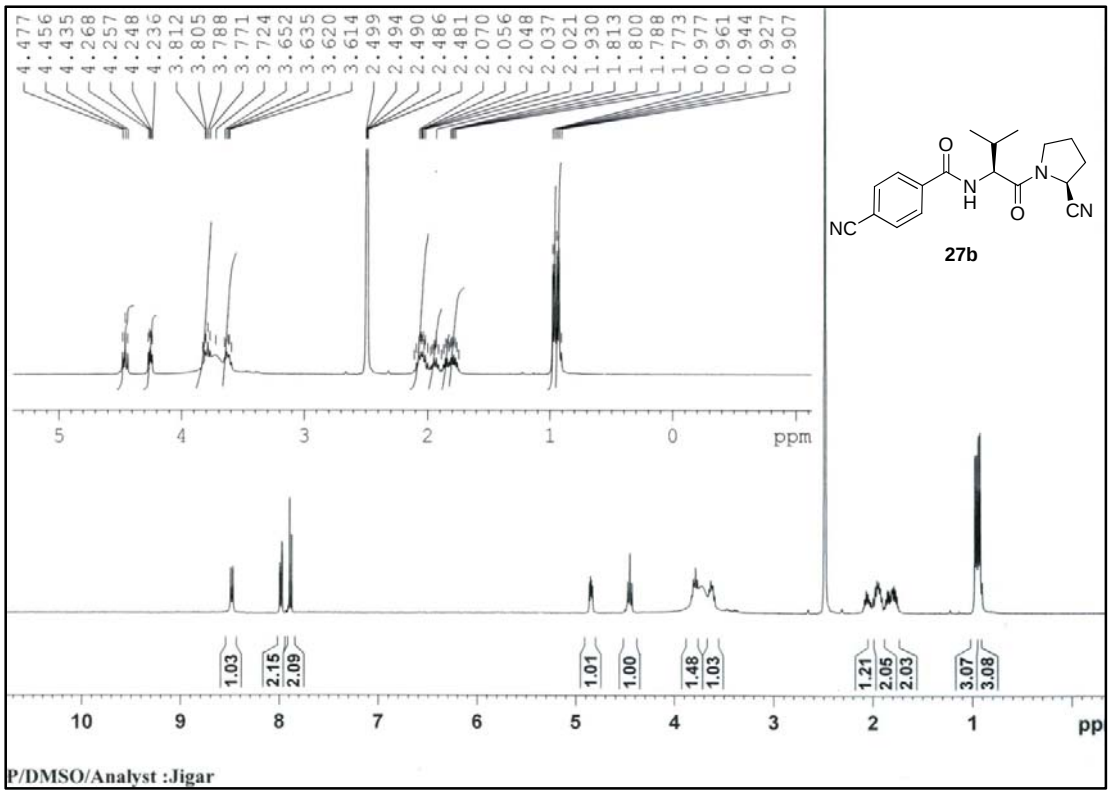
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

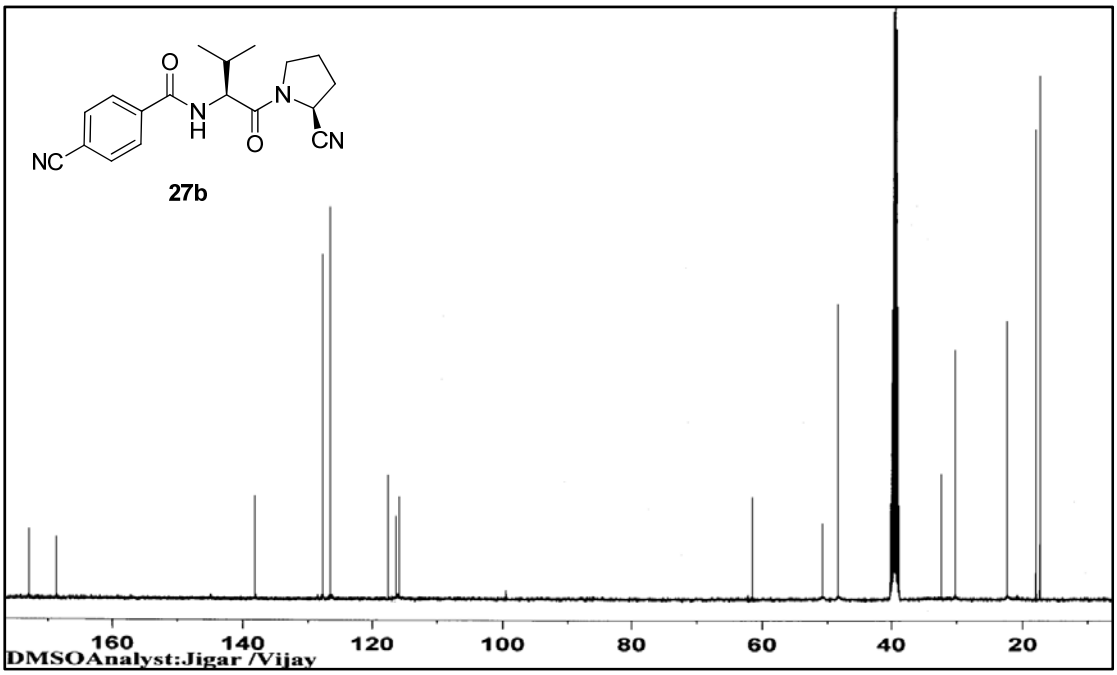
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	4.253	BB	0.1217	18.47021	2.36540	0.1518
2	10.648	BB	0.1061	27.33073	3.99180	0.2246
3	12.232	PP	0.0853	8.62247	1.58564	0.0709
4	13.273	BB	0.0924	4.76349	8.03809e-1	0.0392
5	13.837	PB	0.0906	1.19515e4	2070.37305	98.2152
6	15.524	BP	0.0877	34.87373	6.18126	0.2866
7	16.677	BB	0.0886	115.75317	20.21747	0.9514
8	16.975	BBA	0.0917	4.89842	8.18157e-1	0.0403
Totals :				1.21662e4	2106.33659	

Results obtained with enhanced integrator!

==========

*** End of Report ***


4.477
4.456
4.435
4.268
4.257
4.248
4.236
3.812
3.805
3.788
3.771
3.724
3.652
3.635
3.620
3.614
2.499
2.494
2.490
2.486
2.481
2.070
2.056
2.048
2.037
2.021
1.930
1.813
1.800
1.788
1.773
0.977
0.961
0.944
0.927
0.907
ppm
1.03
2.15
2.09
1.01
1.00
1.48
1.03
1.21
2.05
2.03
3.07
3.08
P/DMSO/Analyst :Jigar


DMSOAnalyst:Jigar /Vijay

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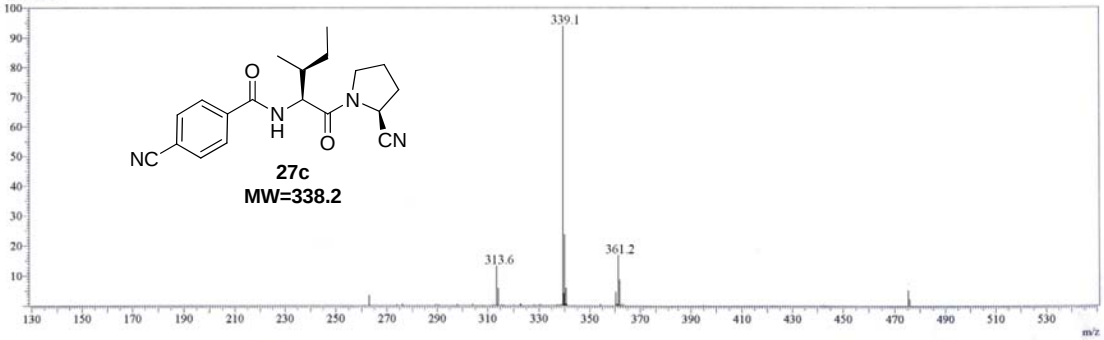
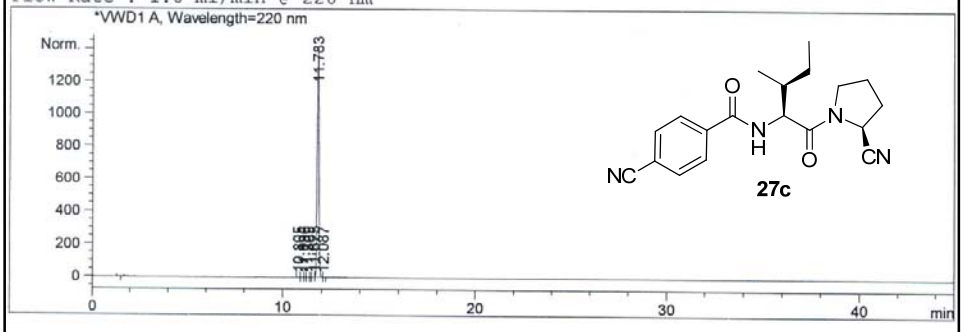
Mass Spectrum



27c
MW=338.2

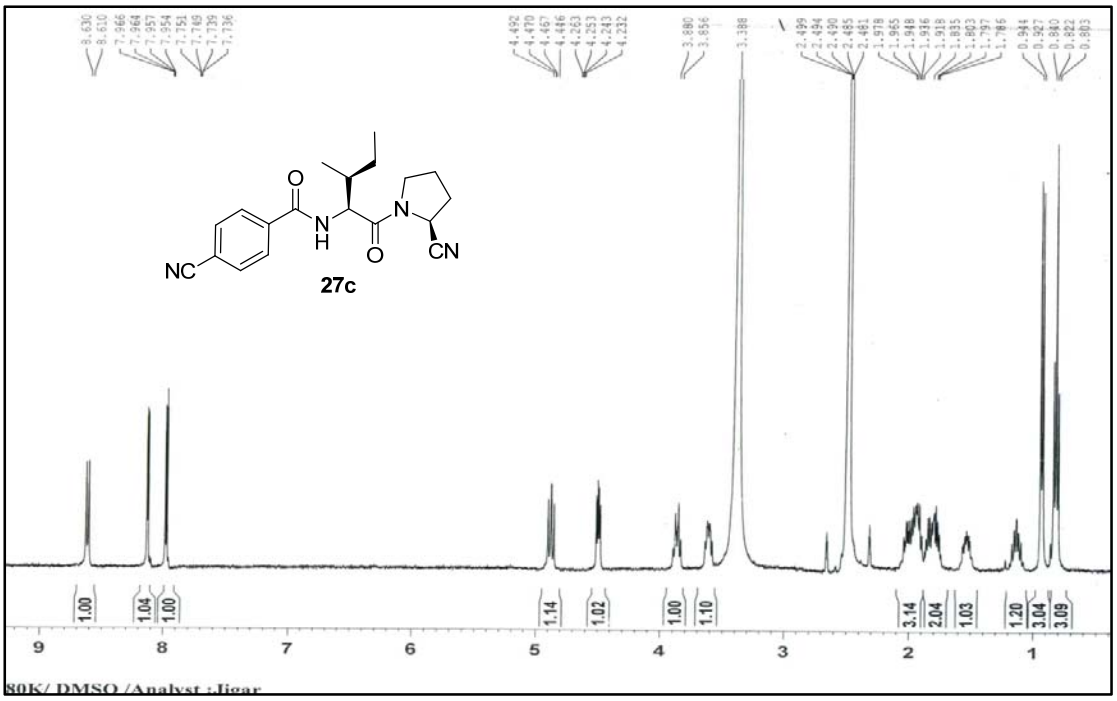
ANALYSED BY : CHAUHAN JIGNESH

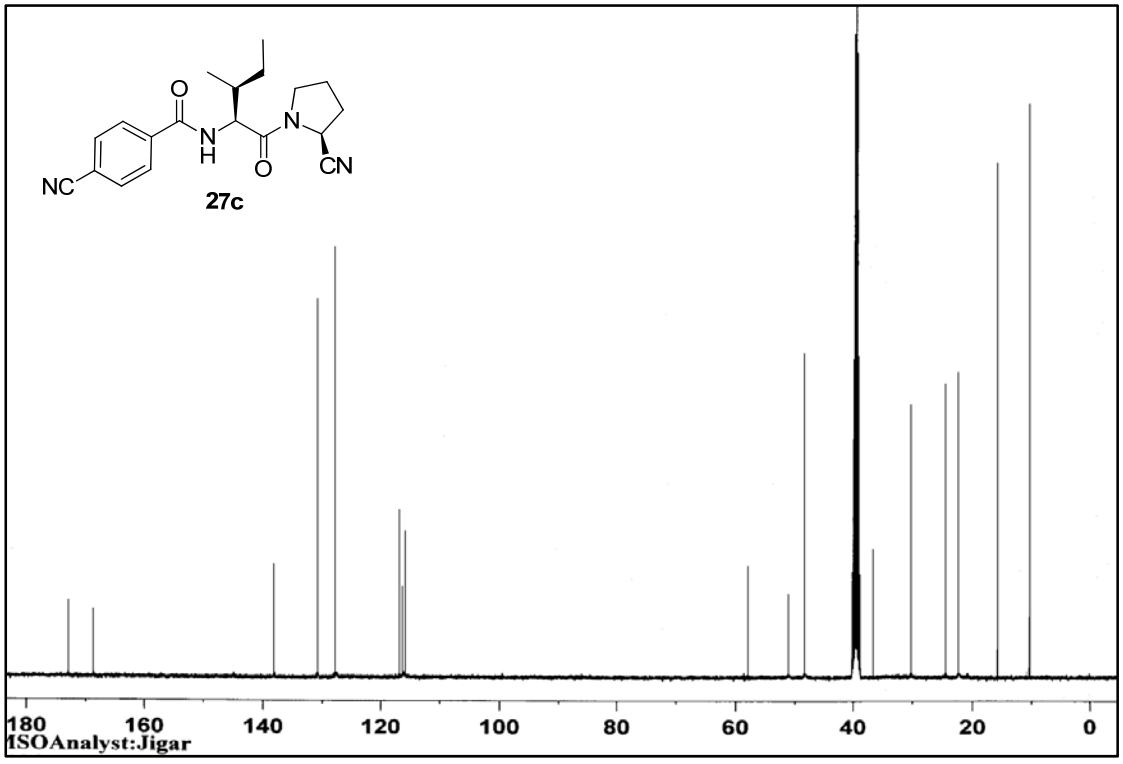
```
Column : YMC J'sphere C18-(150 X4.6mm)4.0 µ
Mobile Phase : 0.05 % TFA in Water : ACN (Gradient)
Flow Rate : 1.0 ml/min @ 220 nm
```



27c

```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=220 nm
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]  mAU   *s    [mAU   ]     %
----|-------|----|-------|----------|----------|--------|
   1  10.805 BV    0.0846    1.29288    1.01697   0.0802
   2  11.405 FM R  0.0603    1.70330 4.70730e-1   0.0184
   3  11.623 VV    0.0922   17.92708    3.10287   0.1932
   4  11.783 MF R  0.1091 9231.68555 1410.32227  99.4134
   5  12.087 FM R  0.0519    1.40855 4.52009e-1   0.0752

Totals :                  9280.55322 1418.92352

 Results obtained with enhanced integrator!
==========================================================================
                        *** End of Report ***
```


27c
80K/ DMSO /Analyst :Jigar


27c
1SOAnalyst:Jigar

ZYDUS RESEARCH CENTRE
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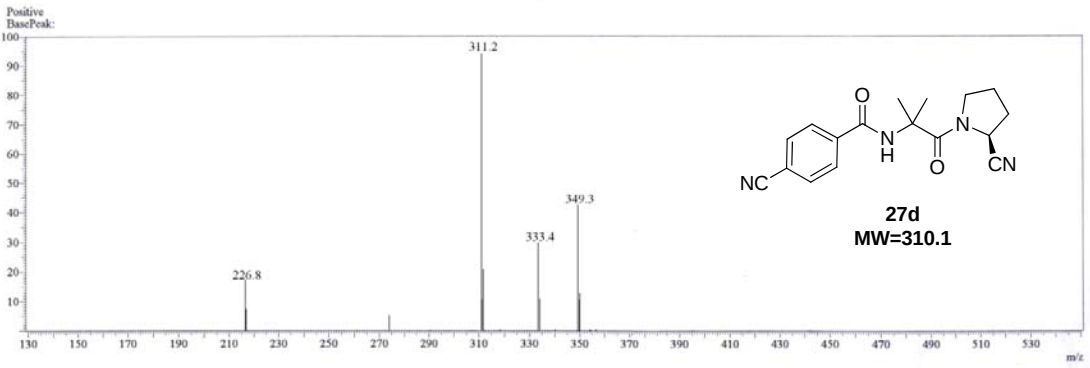
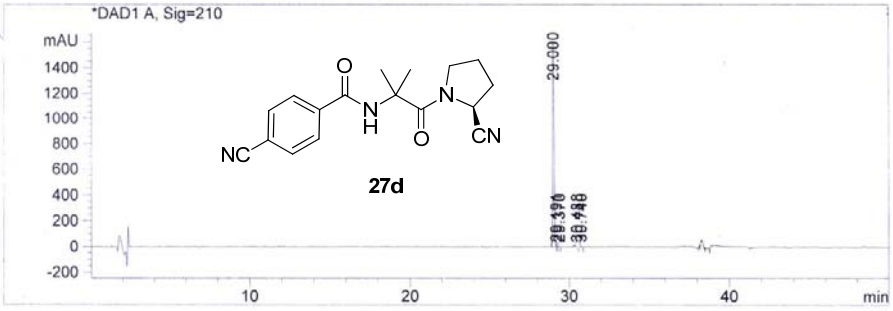
Mass Spectrum



27d
MW=310.1

Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



27d

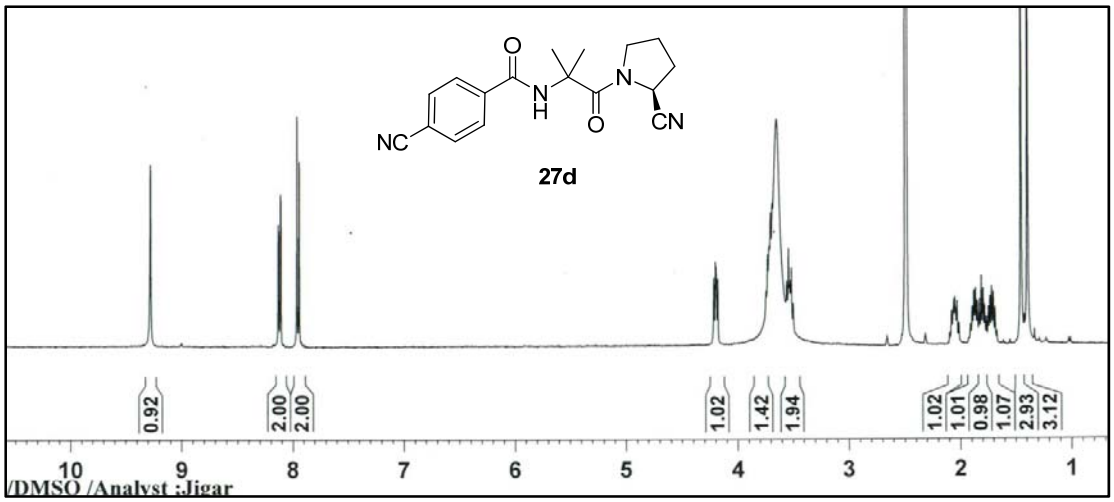
Area Percent Report

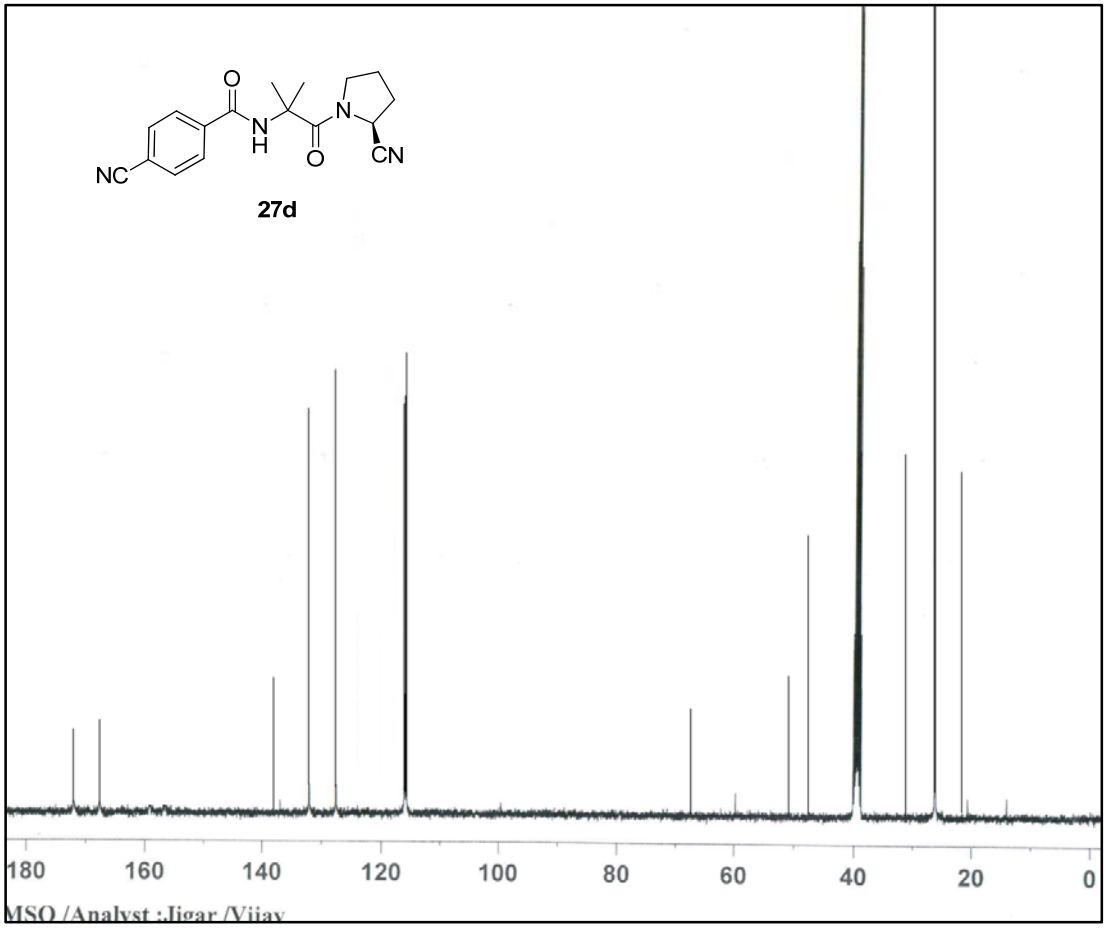
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	29.000	MF R	0.0966	9255.99316	1597.73279	97.8713
2	29.191	FM R	0.0301	3.37071	1.86419	1.0363
3	29.370	VBA	0.0720	7.92173	1.77629	0.0553
4	30.488	BV	0.0772	7.02734	1.43407	1.0057
5	30.740	BBA	0.0802	12.20884	2.36601	0.0315
Totals :				9286.52179	1605.17335	

*** End of Report ***


O
N
H
O
N
CN
NC
27d
0.92
2.00
2.00
1.02
1.42
1.94
1.02
1.01
0.98
1.07
2.93
3.12
10
9
8
7
6
5
4
3
2
1
/DMSO /Analyst :Jigar


O
N
H
O
N
CN
NC
27d
180
160
140
120
100
80
60
40
20
0
MSO /Analyst :Jigar /Vijay

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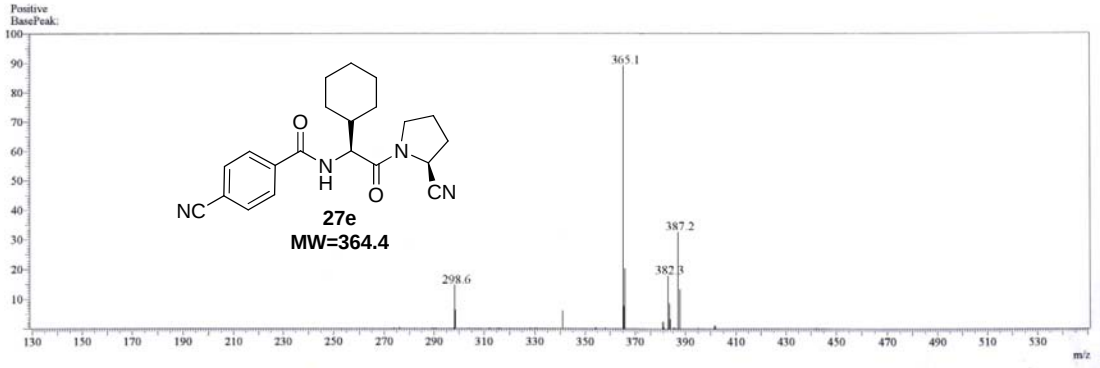
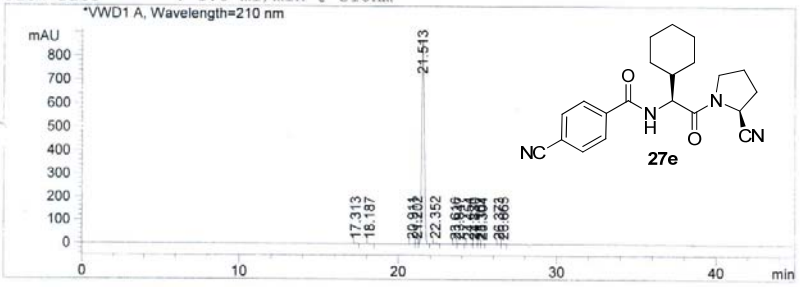
Mass Spectrum



27e
MW=364.4

ANALYSED BY : CHAUHAN JIGNESH

Column : YMC Triart C18(150*4.6mm) 5.0 µ
Mobile phase : 10mM K2HPO4 (pH-7.0) : ACN (Gradient)
Flow rate : 1.0 ml/min @ 210nm



27e

Area Percent Report

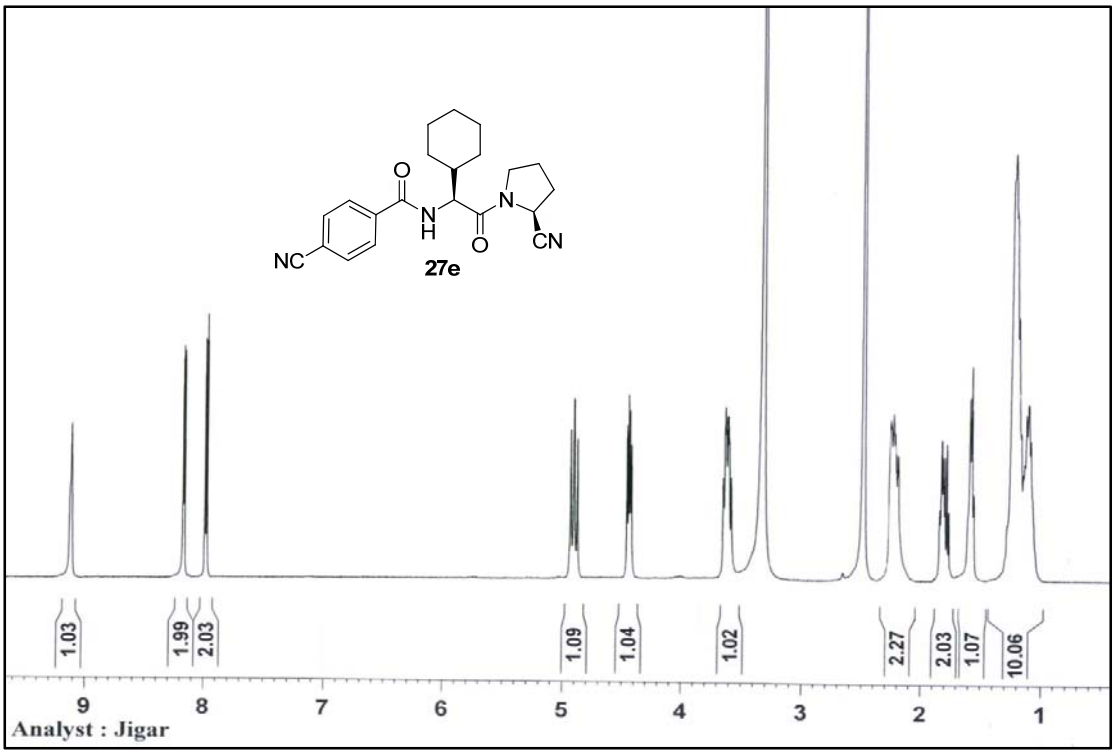
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

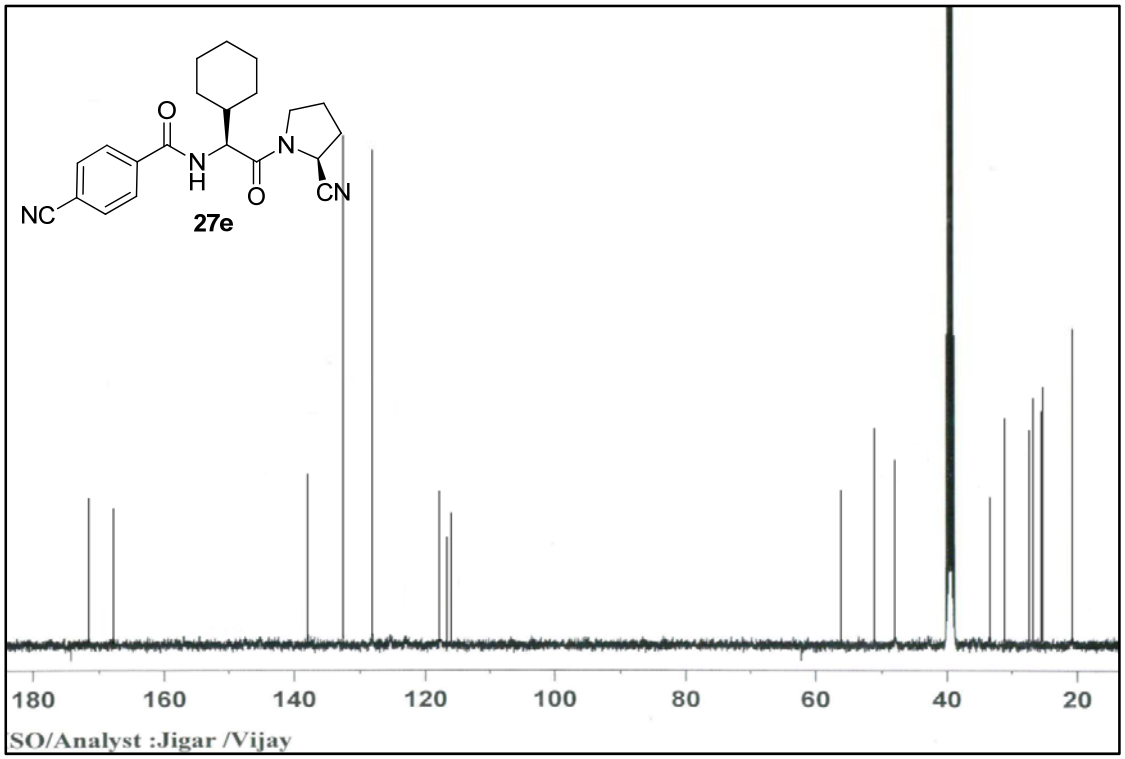
Signal 1: VWD1 A, Wavelength=210 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	17.313	BBA	0.1554	3.43358	3.20880e-1	0.0338
2	18.187	BBA	0.1835	7.74479	6.20335e-1	0.0762
3	20.911	BV	0.1762	16.02354	1.15641	0.1577
4	21.202	VV	0.1346	37.50934	4.20641	0.3691
5	21.513	VB	0.1933	9971.72949	870.54291	97.2589
6	22.352	BBA	0.1630	47.89588	4.62628	0.4713
7	23.616	BV	0.1425	5.64655	6.04333e-1	0.0556
8	23.847	VBA	0.1710	33.31367	2.68511	0.3278
9	24.454	BP	0.1893	7.86530	5.78148e-1	0.0774
10	24.830	VBA	0.1265	4.57712	5.74416e-1	0.0450
11	25.162	MF R	0.1136	1.76670	2.59098e-1	0.8774
12	25.304	FM R	0.1417	3.36392	3.95720e-1	0.0331
13	26.373	PV	0.1088	6.95019	9.65018e-1	0.0684
14	26.665	VBA	0.1300	15.08382	1.71949	0.1484
Totals :				1.01629e4	889.25455	

Results obtained with enhanced integrator!

*** End of Report ***


NC
O
N
H
O
N
CN
27e
1.03
1.99
2.03
1.09
1.04
1.02
2.27
2.03
1.07
10.06
9
8
7
6
5
4
3
2
1
Analyst : Jigar


NC
O
N
H
O
N
CN
27e
180
160
140
120
100
80
60
40
20
SO/Analyst :Jigar /Vijay

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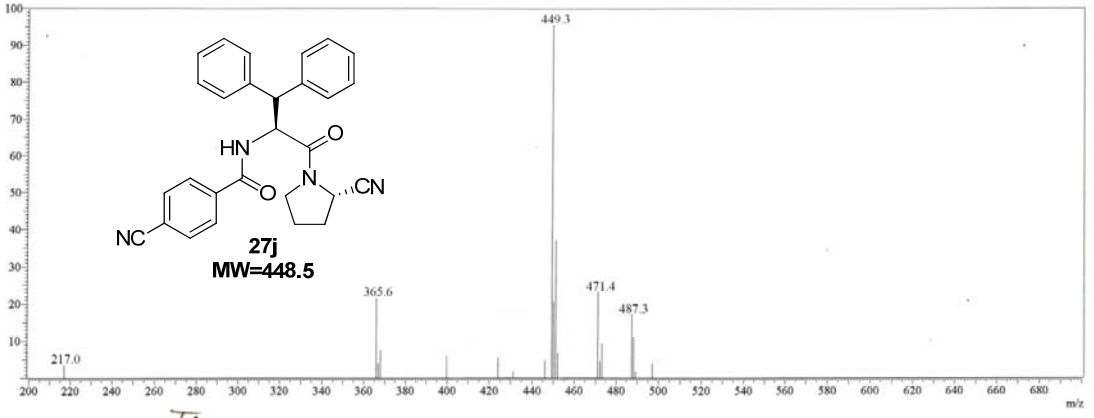
Mass Spectrum

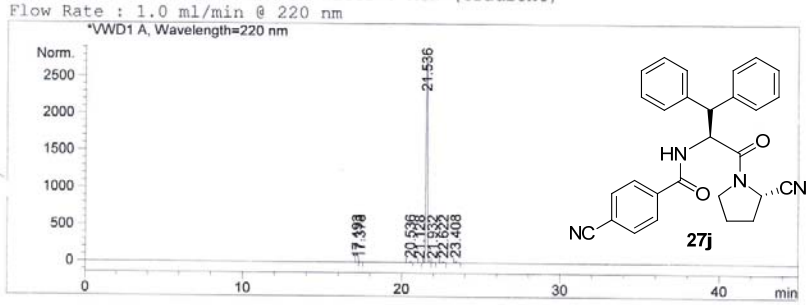
Positive
BasePeak:



27j
MW=448.5

ANALYSED BY : JIGNESH CHAUHAN

Column : YMC J'sphere C18-(150 X4.6mm)4.0 µ
Mobile Phase : 0.05 % TFA in Water : ACN (Gradient)
Flow Rate : 1.0 ml/min @ 220 nm



27j

Area Percent Report

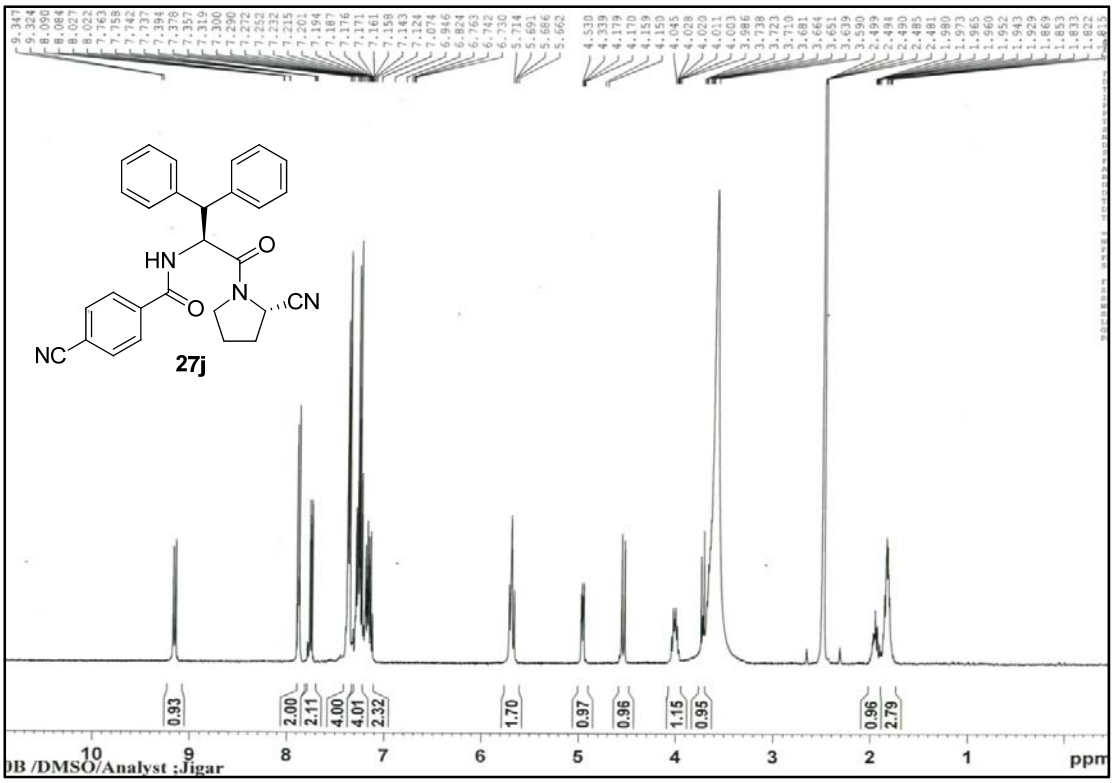
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

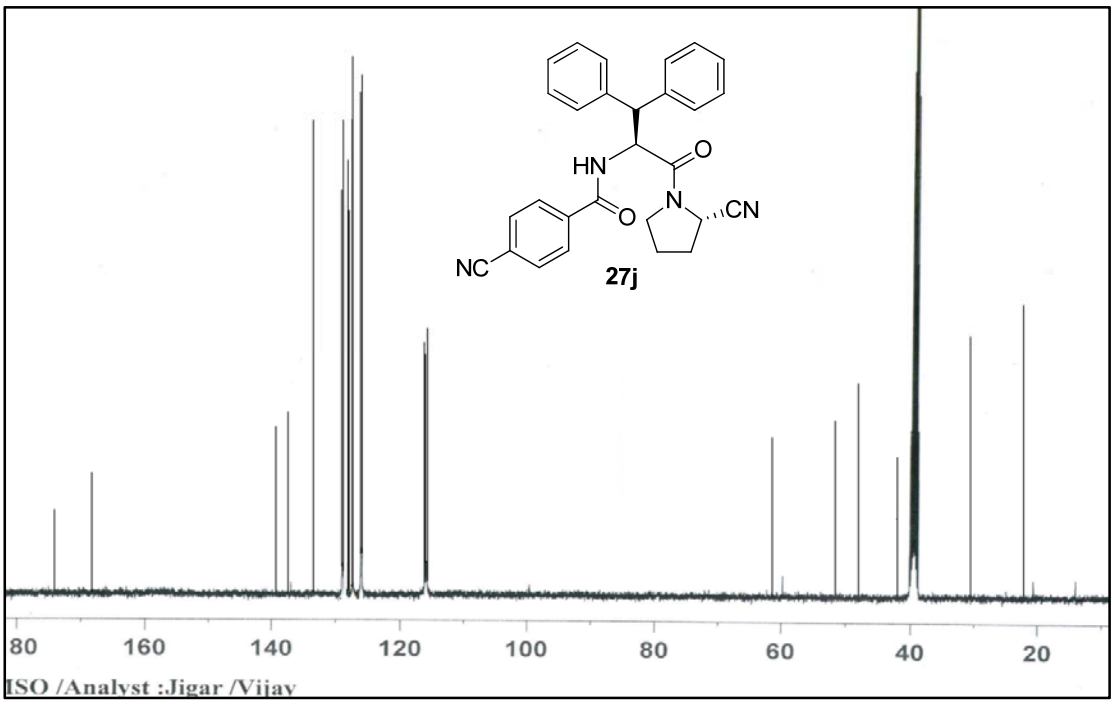
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	17.193	BV	0.0939	3.33542	5.62532e-1	0.0165
2	17.378	VP	0.0955	16.48300	2.71936	0.0814
3	20.536	BP R	0.0955	61.51388	10.02730	0.3039
4	21.128	BBA	0.0834	2.67624	4.95730e-1	0.0132
5	21.536	BV	0.1176	1.99946e4	2773.76318	98.6585
6	21.932	VBA	0.1196	17.50287	2.15367	0.0878
7	22.622	BP R	0.0967	56.86963	9.14135	0.2810
8	23.408	BB	0.0925	86.81881	14.34307	0.4290
Totals :				2.02398e4	2813.20621	

Results obtained with enhanced integrator!

*** End of Report ***

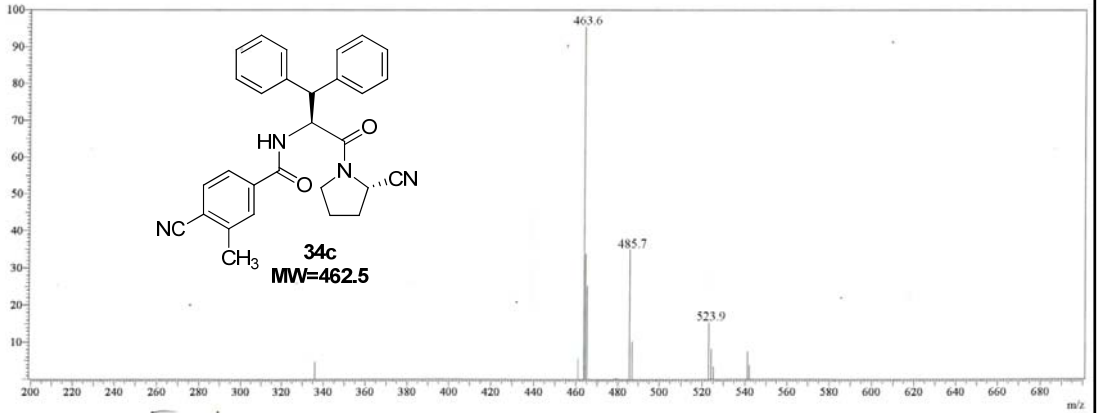

HN
O
N
CN
O
NC
27j
0.93
2.00
2.11
4.00
4.01
2.32
1.70
0.97
0.96
1.15
0.95
0.96
2.79
10
9
8
7
6
5
4
3
2
1
ppm
DB /DMSO/Analyst ;Jigar


HN
O
N
CN
O
NC
27j
80
160
140
120
100
80
60
40
20
ISO /Analyst :Jigar /Vijay

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum

Positive
BasePeak:



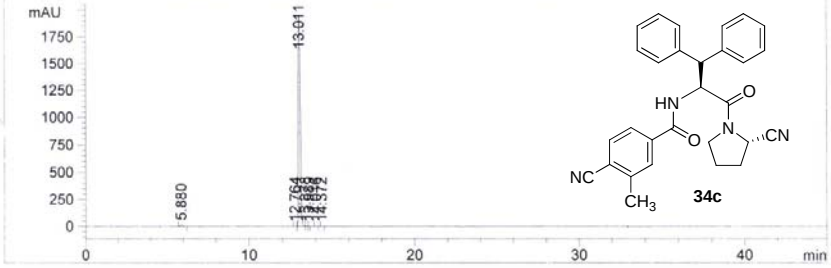
34c
MW=462.5

03/07/10

ANALYSED BY : JIGNESH CHAUHAN

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm

*VWD1 A, Wavelength=220 nm (MAR1513\MAR15011.D - MAR1513\MAR15010.D)



34c

Area Percent Report

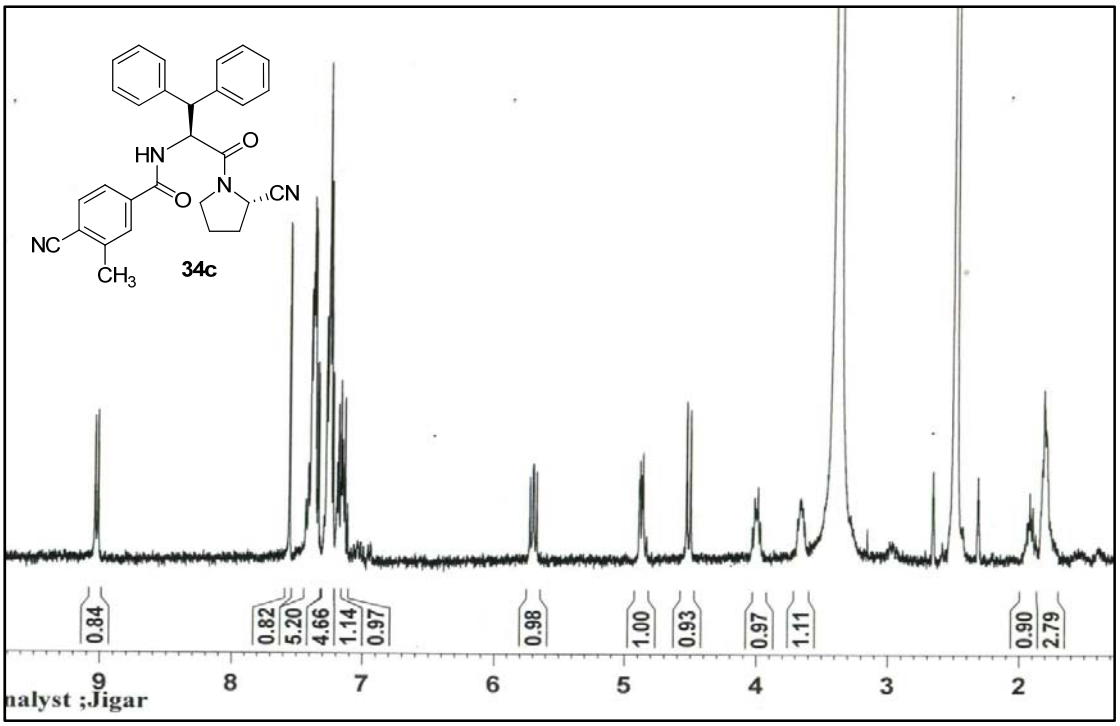
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

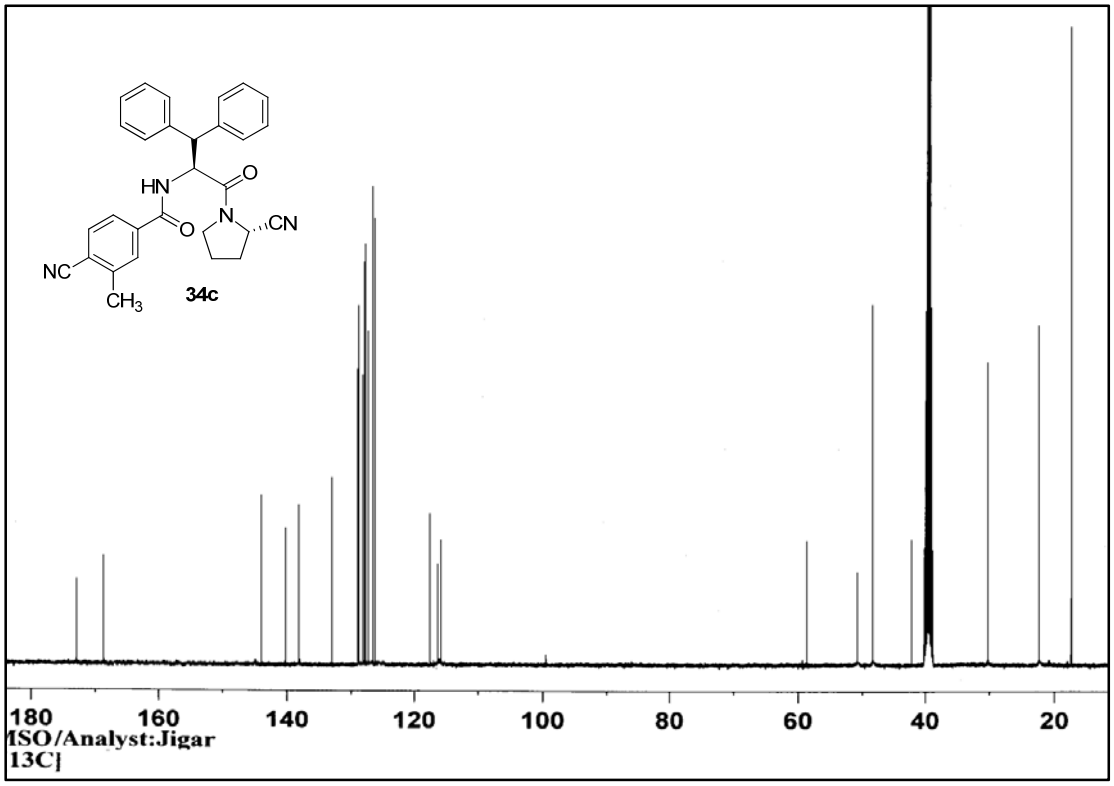
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	5.880	BBA	0.1602	177.15422	16.50138	1.0995
2	12.764	BV	0.1020	6.57375	9.74003e-1	0.0408
3	13.011	MF R	0.1331	1.56359e4	1957.34802	97.1423
4	13.378	FM R	0.0901	16.81635	3.11017	0.1044
5	13.589	VBA	0.0570	4.11689	1.09436	0.0256
6	14.016	PBA	0.0900	7.52201	1.31656	0.0467
7	14.372	PBA	0.0999	4.58343	7.25743e-1	0.0284
Totals :				1.61125e4	2018.33694	

Results obtained with enhanced integrator!

*** End of Report ***


HN
O
N
CN
O
NC
CH3
34c
0.84
0.82
5.20
4.66
1.14
0.97
0.98
1.00
0.93
0.97
1.11
0.90
2.79
9
8
7
6
5
4
3
2
nalyst ;Jigar


HN
O
N
CN
O
NC
CH3
34c
180
160
140
120
100
80
60
40
20
ISO/Analyst:Jigar
13C]

ZYDUS RESEARCH CENTRE
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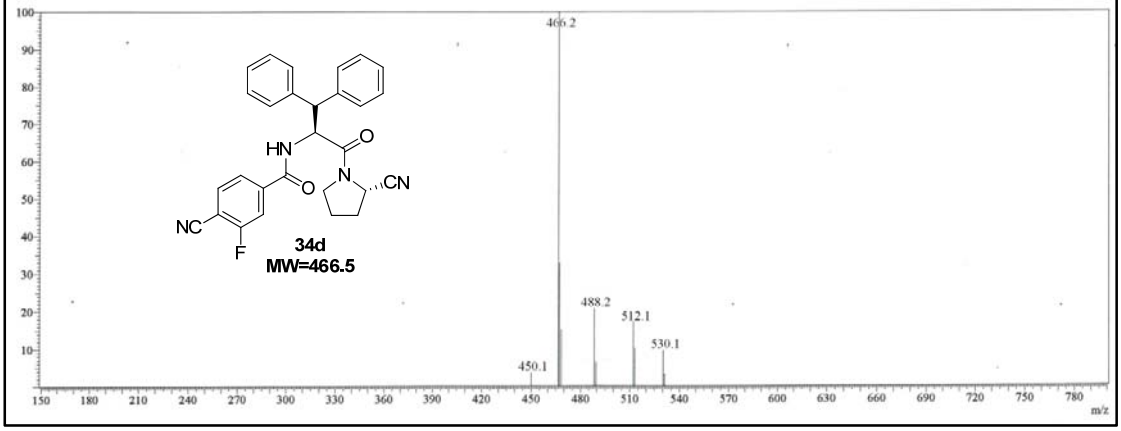
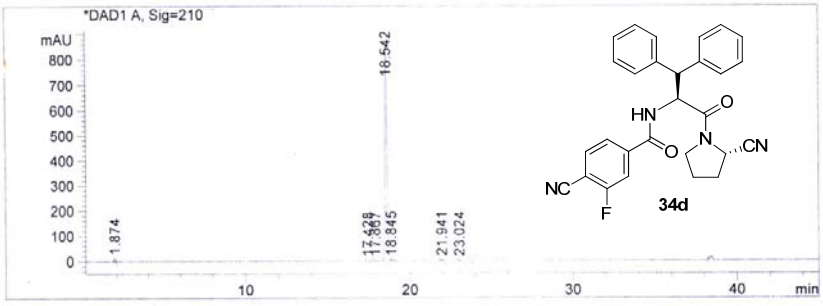
Mass Spectrum

Positive
BasePeak:



Method Info : Column : YMC Triart C18 (150*4.6mm), 5µ
Mobile Phase : 10mm K2HPO4 (pH:7.0) : ACN (Gradient)
Flow : 1.0 ml/min @ 210 nm

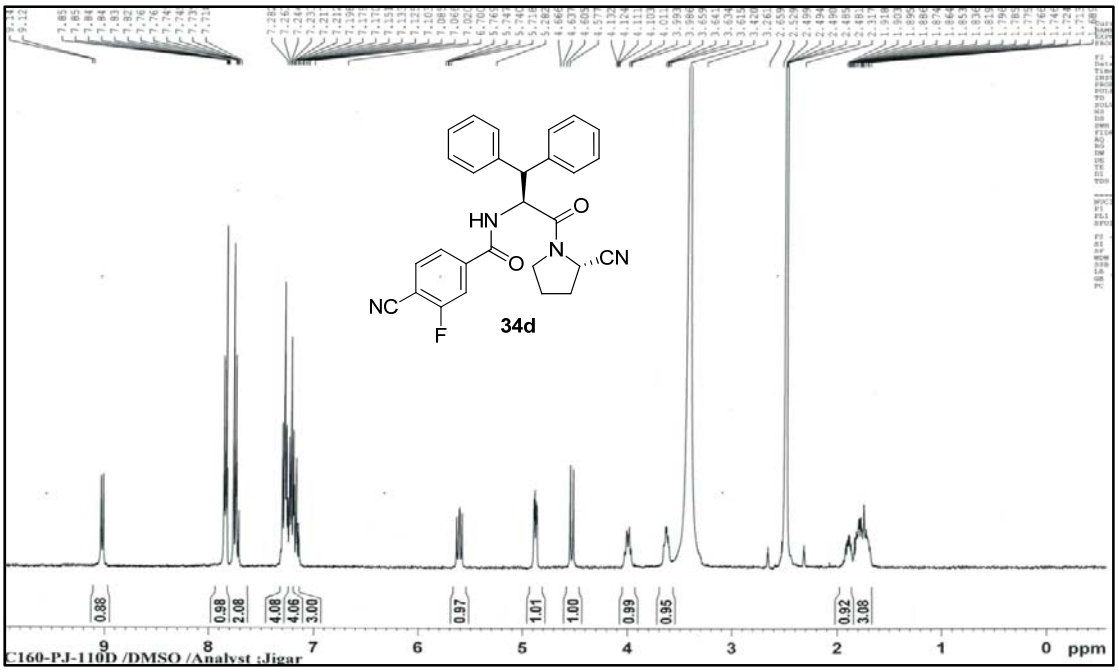


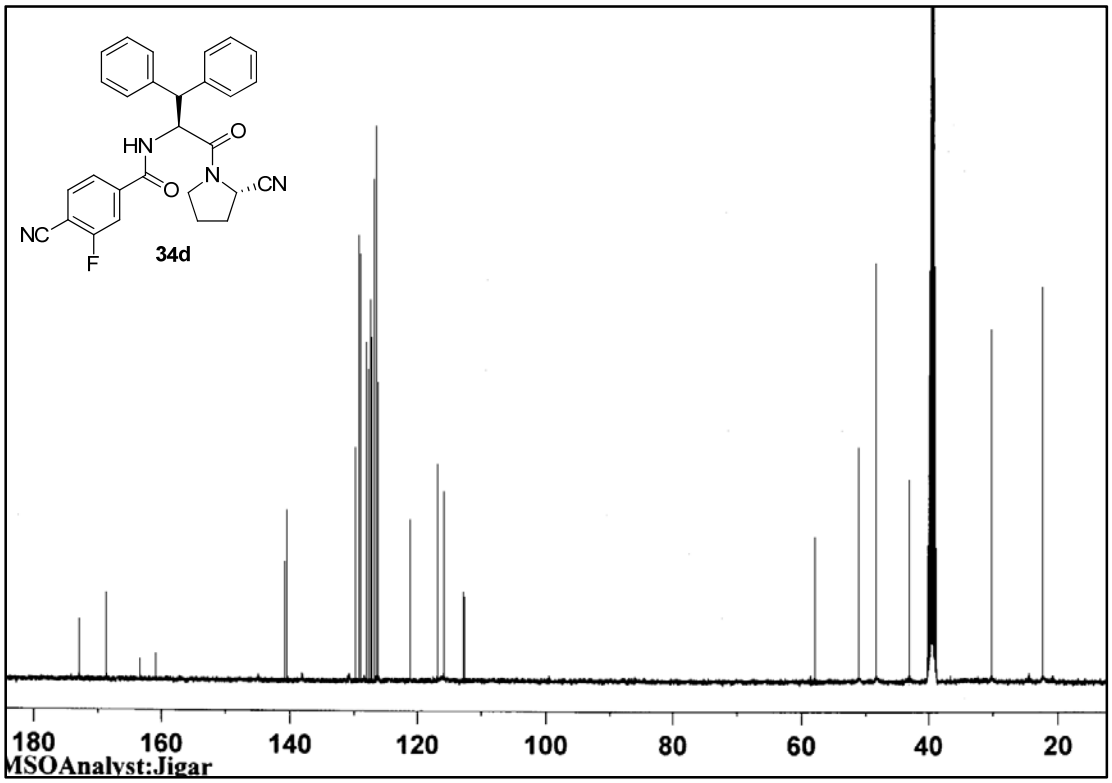
Area Percent Report

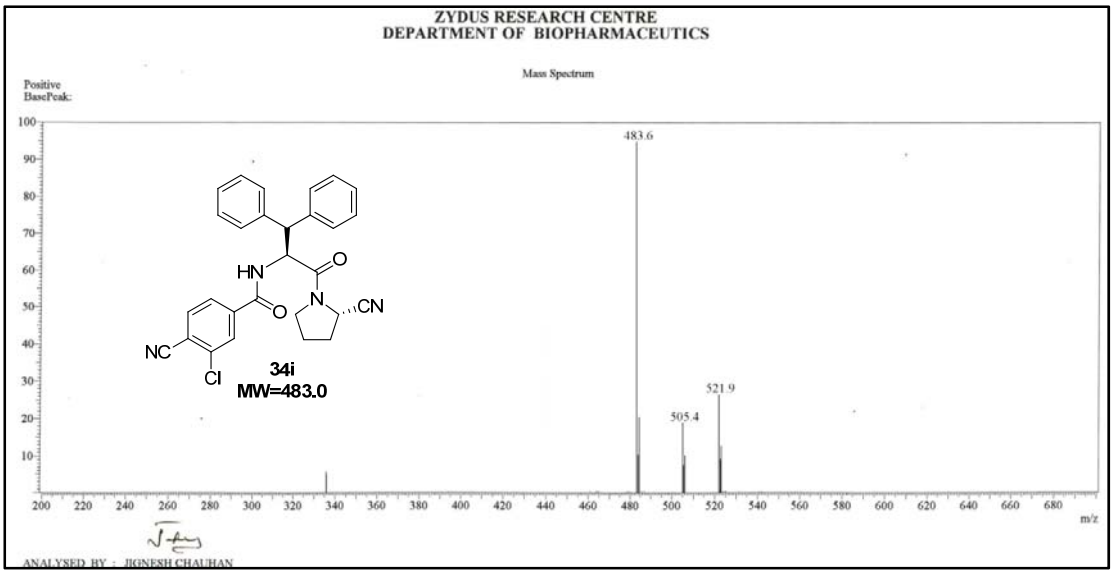
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

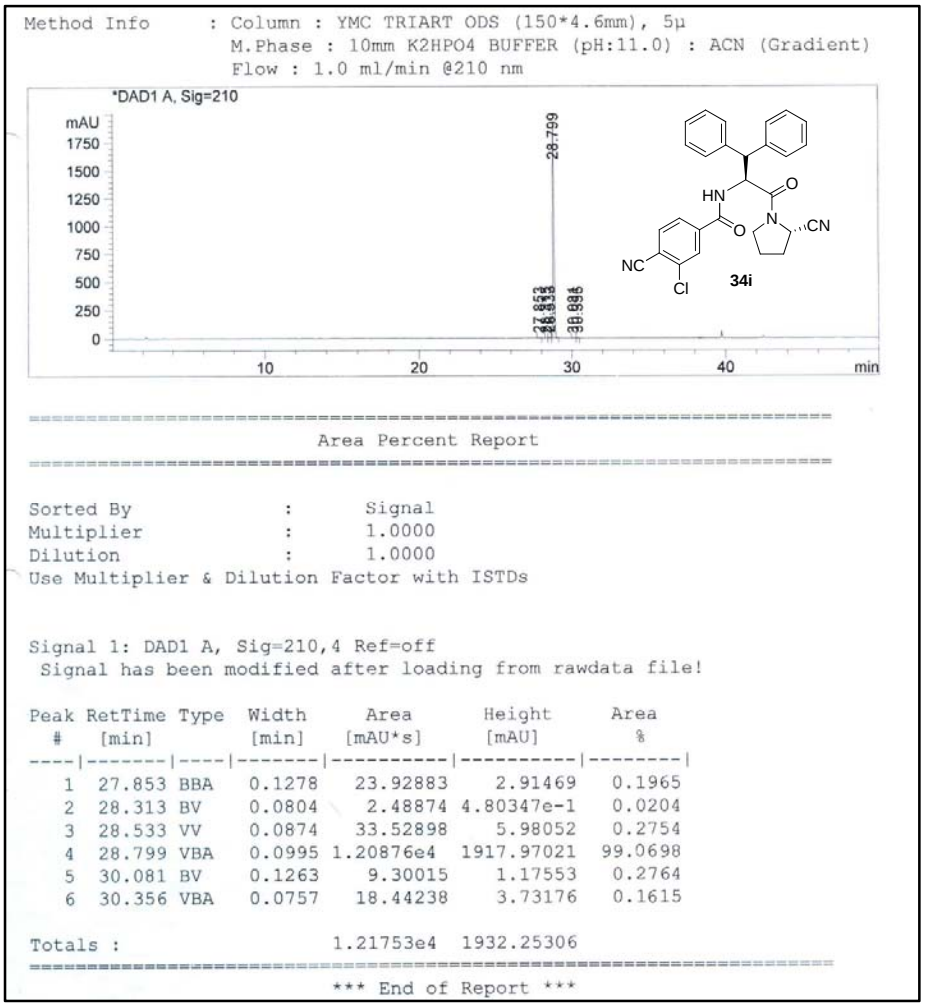
Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.874	BBA	0.0802	53.90536	10.80079	0.7703
2	17.428	BBA	0.1129	2.62836	3.52878e-1	0.0376
3	17.867	BBA	0.1169	13.19701	1.77217	0.1886
4	18.542	MF R	0.1314	6867.09814	871.20312	98.3936
5	18.845	FM R	0.1077	22.26688	3.44451	0.3182
6	21.941	BBA	0.1095	27.00157	3.77369	0.3858
7	23.024	BBA	0.0811	8.68366	1.55532	0.1241
Totals :				6998.41295	893.53858	


HN
O
O
N
CN
NC
F
34d
0.88
0.98
2.08
4.08
4.06
3.00
0.97
1.01
1.00
0.99
0.95
0.92
3.08
9
8
7
6
5
4
3
2
1
0
ppm
C160-PJ-110D /DMSO /Analyst :Jigar


HN
O
O
N
CN
NC
F
34d
180
160
140
120
100
80
60
40
20
MSOAnalyst:Jigar





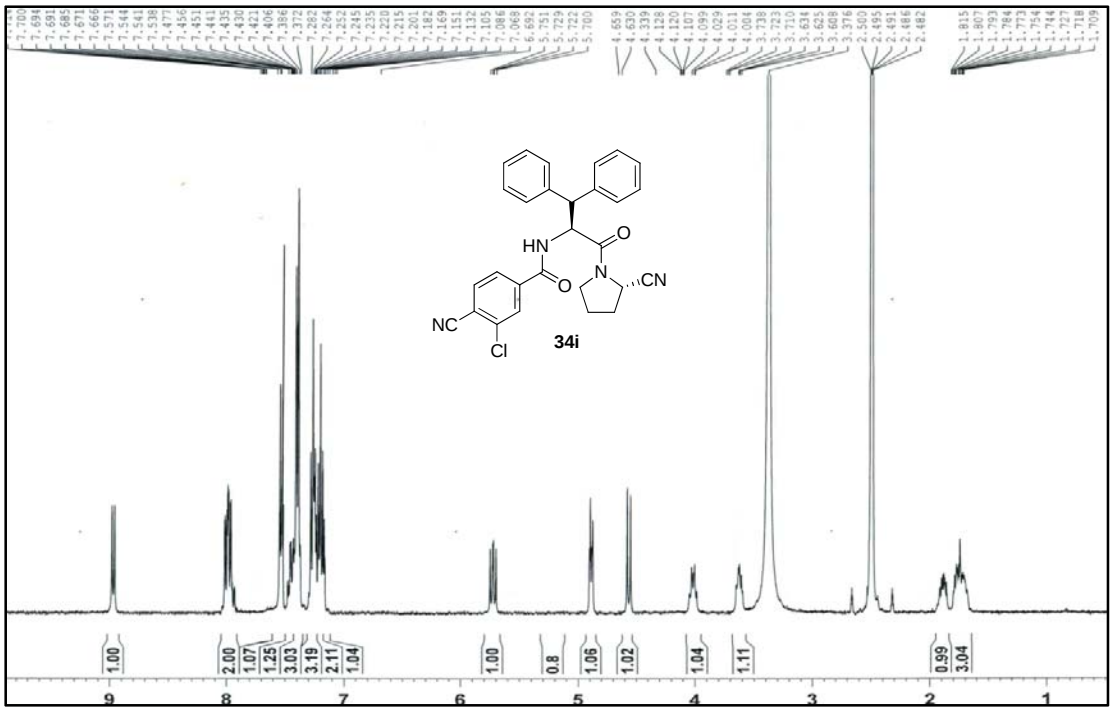
Area Percent Report

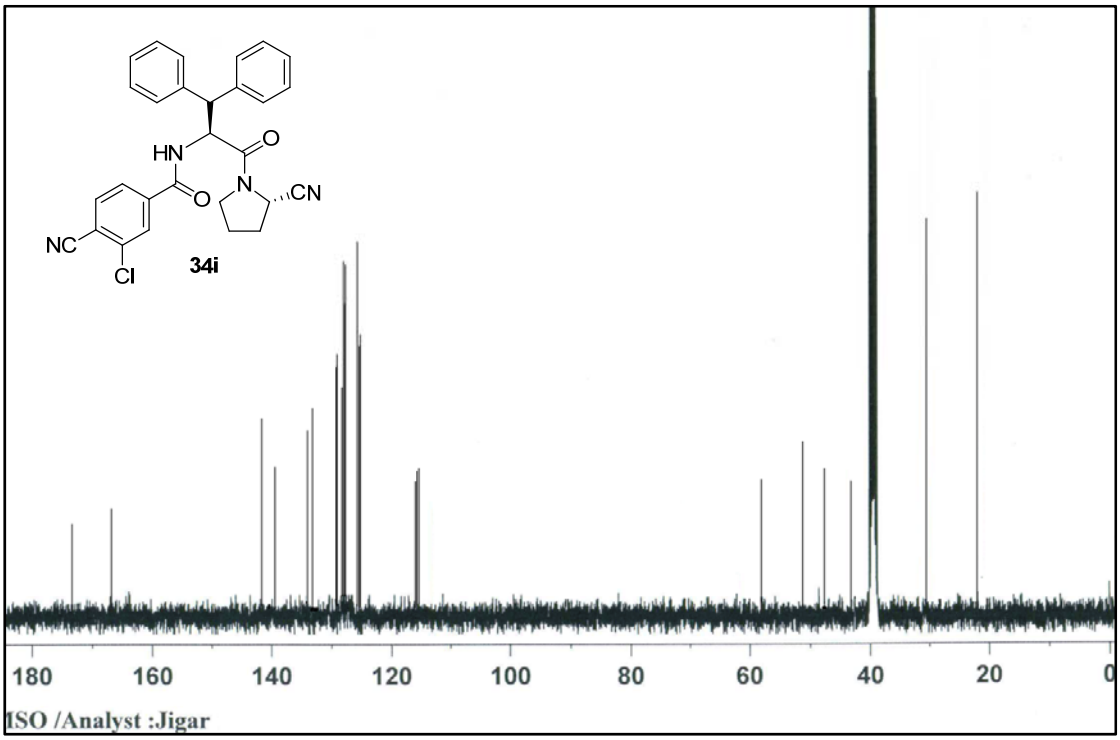
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	27.853	BBA	0.1278	23.92883	2.91469	0.1965
2	28.313	BV	0.0804	2.48874	4.80347e-1	0.0204
3	28.533	VV	0.0874	33.52898	5.98052	0.2754
4	28.799	VBA	0.0995	1.20876e4	1917.97021	99.0698
5	30.081	BV	0.1263	9.30015	1.17553	0.2764
6	30.356	VBA	0.0757	18.44238	3.73176	0.1615
Totals :				1.21753e4	1932.25306	

*** End of Report ***


HN
O
O
N
CN
NC
Cl
34i
1.00
2.00
1.07
1.25
3.03
3.19
2.11
1.04
1.00
0.8
1.06
1.02
1.04
1.11
0.99
3.04
9
8
7
6
5
4
3
2
1


HN
O
O
N
CN
NC
Cl
34i
180
160
140
120
100
80
60
40
20
0
ISO /Analyst :Jigar

ZYDUS RESEARCH CENTRE
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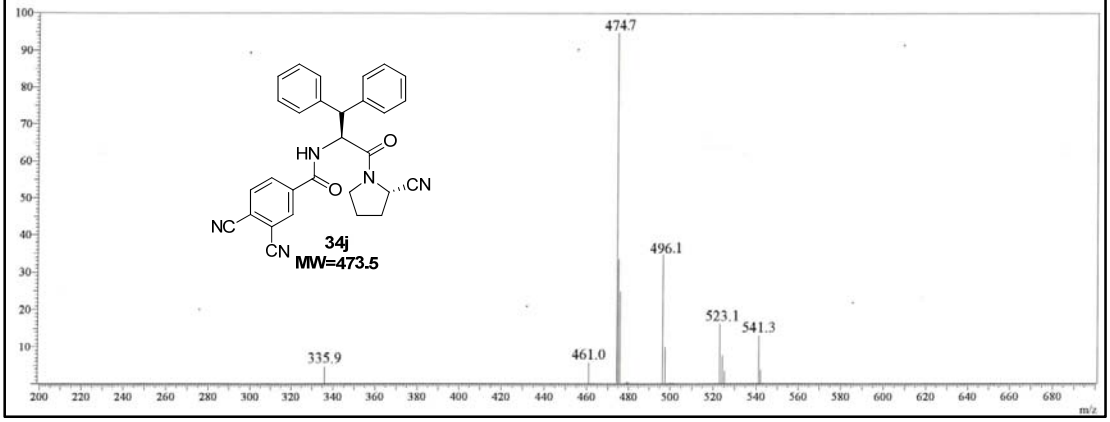
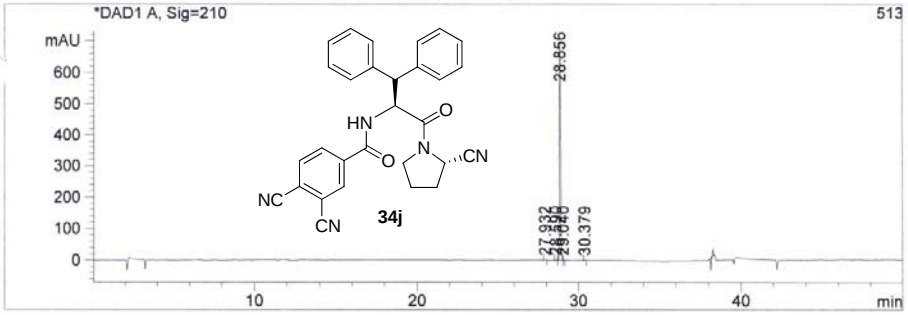
Mass Spectrum

Positive
BasePeak:



Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



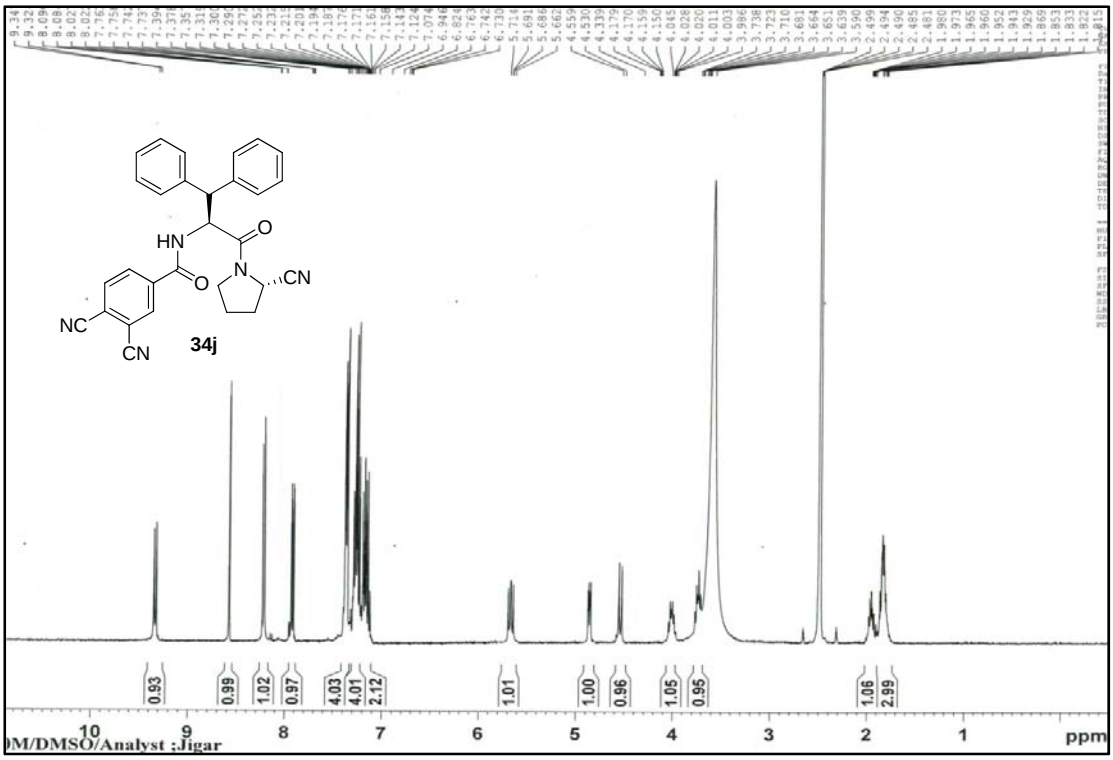
Area Percent Report

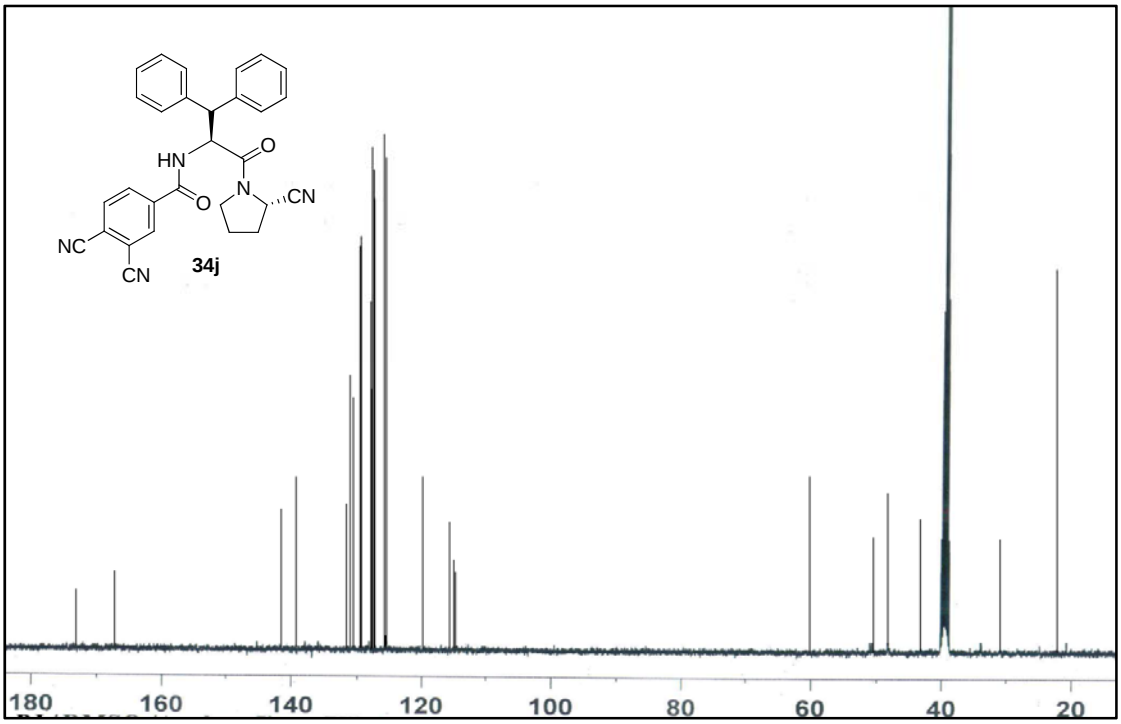
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	27.932	BBA	0.0826	2.55833	4.22955e-1	0.0134
2	28.590	BV	0.0826	5.50684	1.05991	0.8381
3	28.856	MF R	0.0833	3471.77319	694.54993	98.3739
4	29.040	FM R	0.0385	1.65588	7.16403e-1	0.5475
5	30.379	BBA	0.0629	4.43594	1.10452	0.2273
Totals :				3485.93018	697.85372	

*** End of Report ***


HN
O
O
N
CN
NC
CN
34j
0.93
0.99
1.02
0.97
4.03
4.01
2.12
1.01
1.00
0.96
1.05
0.95
1.06
2.99
10
9
8
7
6
5
4
3
2
1
ppm


HN
O
O
N
CN
NC
CN
34j
180
160
140
120
100
80
60
40
20

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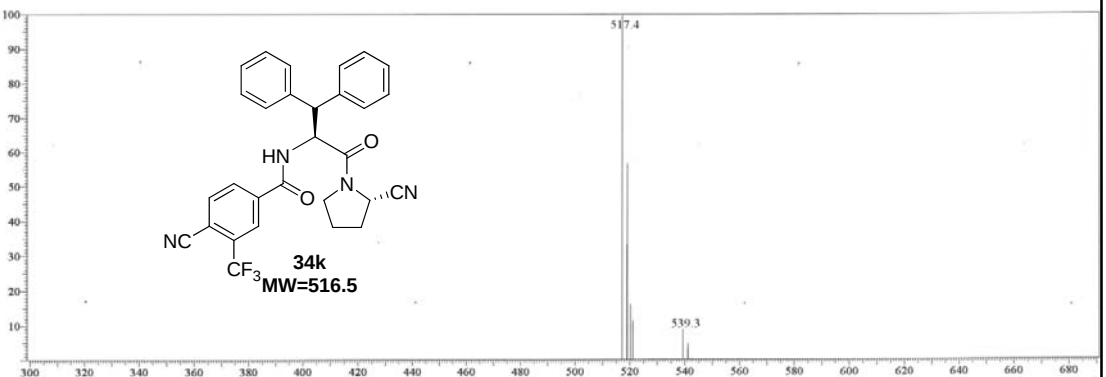
Mass Spectrum

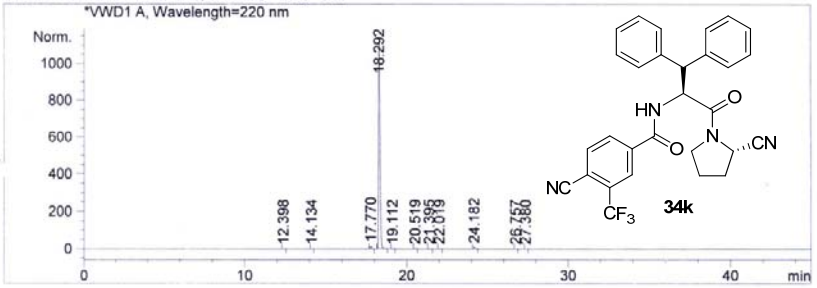
Positive
BasePeak:



34k
MW=516.5

ANALYSED BY : JIGNESH CHAUHAN

Column : YMC J'sphere C18-(150 X4.6mm)4.0 µ
Mobile Phase : 0.05 % TFA in Water : ACN (Gradient)
Flow Rate : 1.0 ml/min @ 220 nm



34k

Area Percent Report

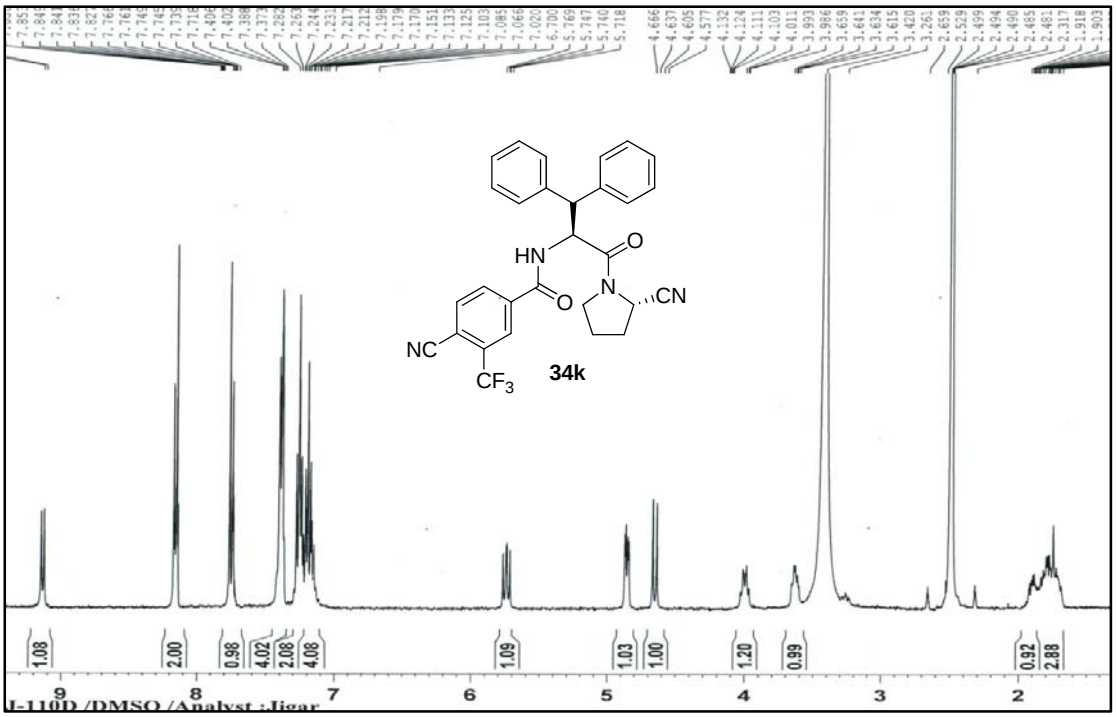
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

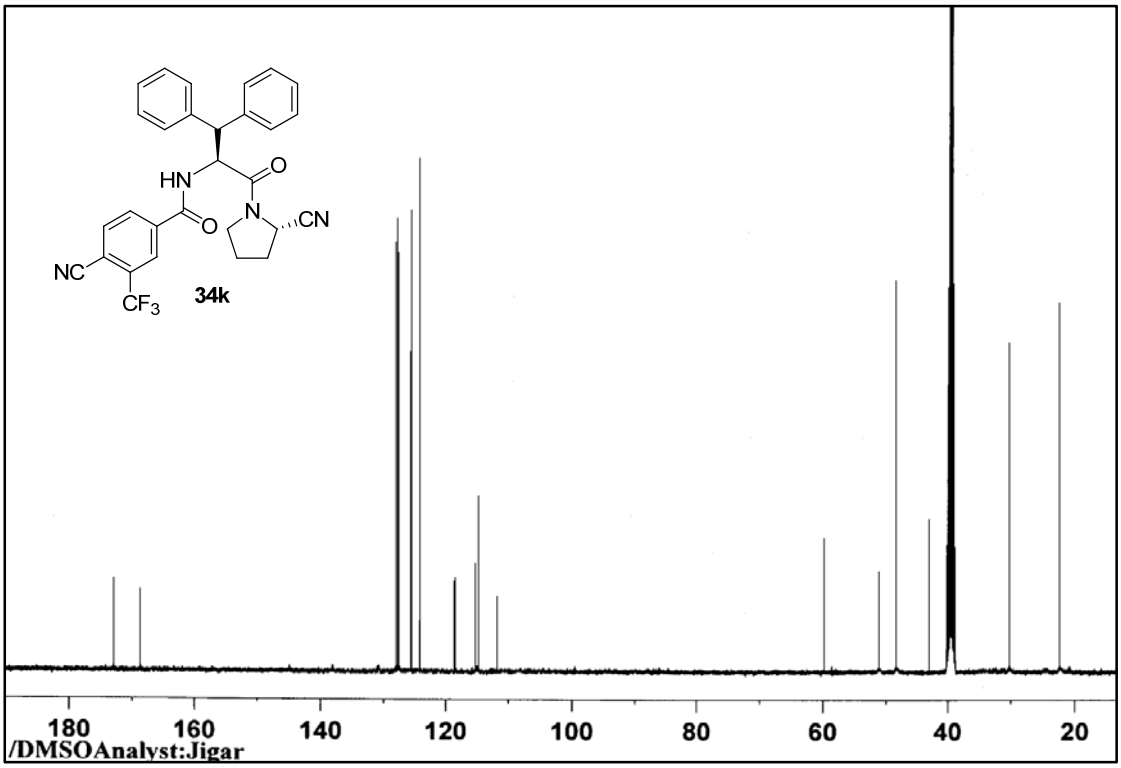
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	12.398	BBA	0.1009	1.84486	2.77456e-1	0.0257
2	14.134	BBA	0.0791	9.21952e-1	1.74605e-1	0.0129
3	17.770	BB	0.0930	83.15585	13.63450	1.1601
4	18.292	PB	0.0925	6989.45459	1131.47021	97.4614
5	19.112	BB	0.0887	2.08129	3.47968e-1	0.0290
6	20.519	PBA	0.0913	11.96158	2.05086	0.1669
7	21.395	BBA	0.1002	11.86269	1.79846	0.1655
8	22.019	BB	0.0959	9.22294	1.45314	0.1287
9	24.182	PB	0.0929	52.63739	8.63725	0.7344
10	26.757	BP	0.0786	2.97472	5.81801e-1	0.0415
11	27.380	BP	0.0842	1.71694	3.06885e-1	0.0240
Totals :				7167.83479	1160.73314	

Results obtained with enhanced integrator!

*** End of Report ***


HN
O
O
N
CN
NC
CF3
34k
1.08
2.00
0.98
4.02
2.08
4.08
1.09
1.03
1.00
1.20
0.99
0.92
2.88
9
8
7
6
5
4
3
2


HN
O
O
N
CN
NC
CF3
34k
180
160
140
120
100
80
60
40
20
/DMSOAnalyst:Jigar

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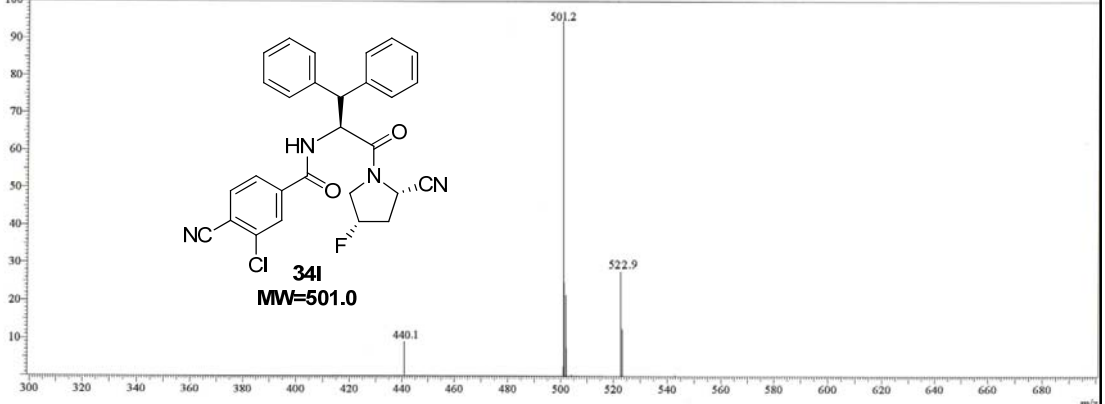
Mass Spectrum

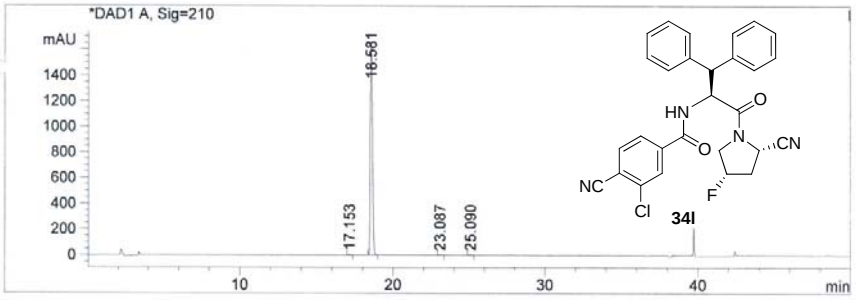
Positive
BasePeak:501.25



34l
MW=501.0

ANALYSED BY : JIGNESH CHAUHAN

Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



Area Percent Report

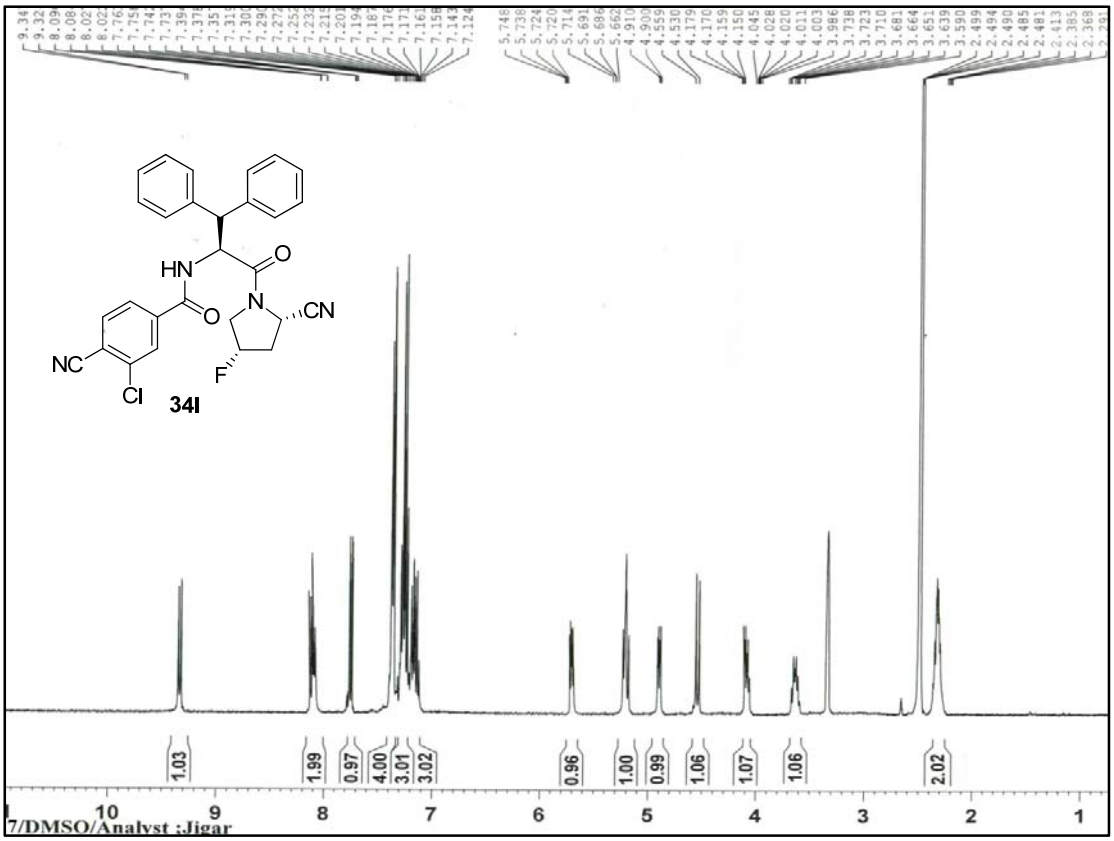
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

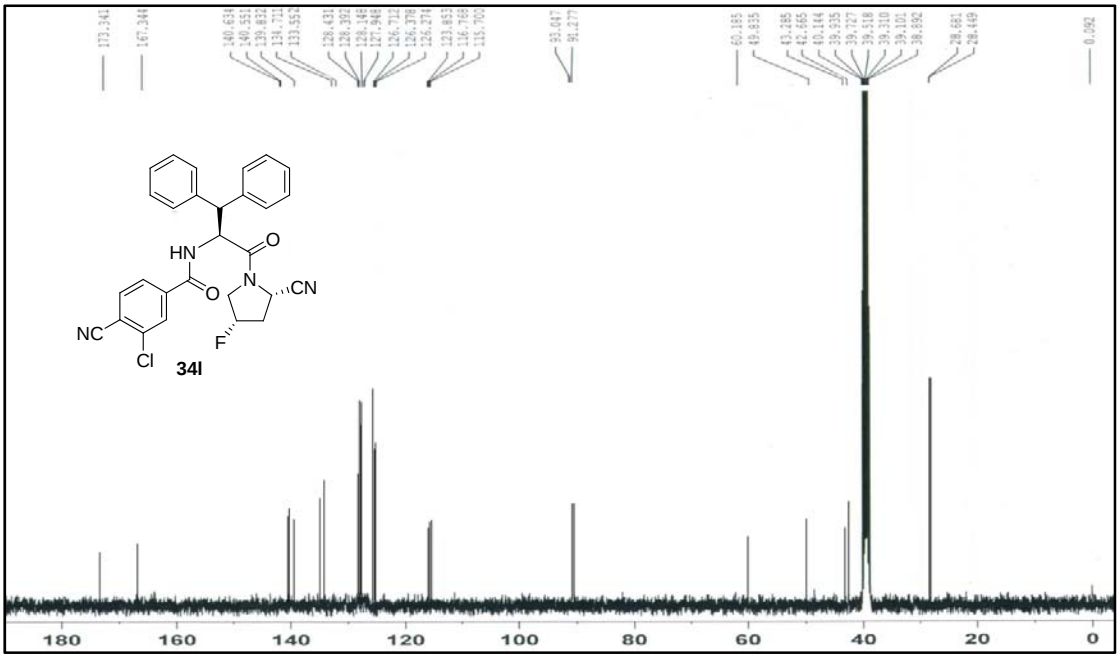
Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.153	BBA	0.1385	40.24305	4.49197	0.2983
2	18.581	BBA	0.1559	1.61317e4	1652.66687	99.3803
3	23.087	BBA	0.1572	14.15074	1.43317	0.1873
4	25.090	BBA	0.1547	21.72840	2.20956	0.1341

Totals : 1.62078e4 1660.80158

*** End of Report ***

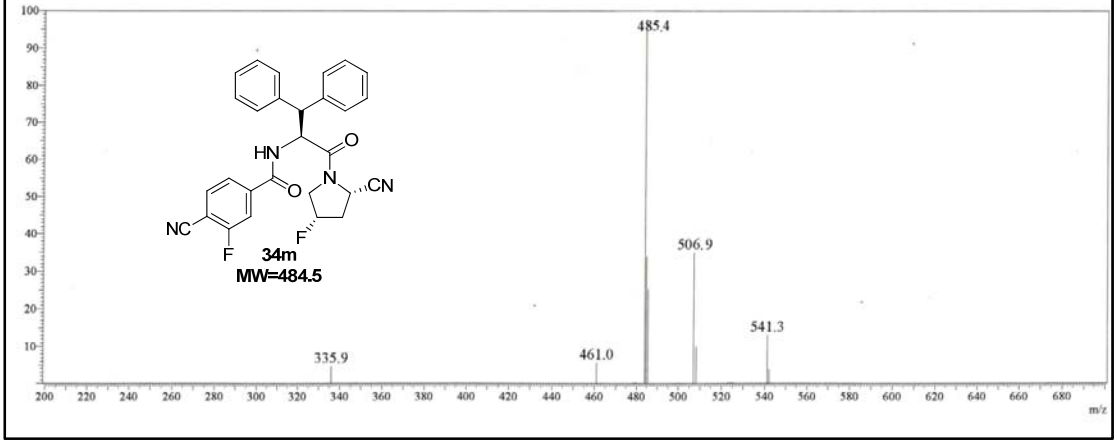

HN
O
N
CN
O
NC
Cl
F
34l
1.03
1.99
0.97
4.00
3.01
3.02
0.96
1.00
0.99
1.06
1.07
1.06
2.02
7/DMSO/Analyst ;Jigar


HN
O
N
CN
O
NC
Cl
F
34l

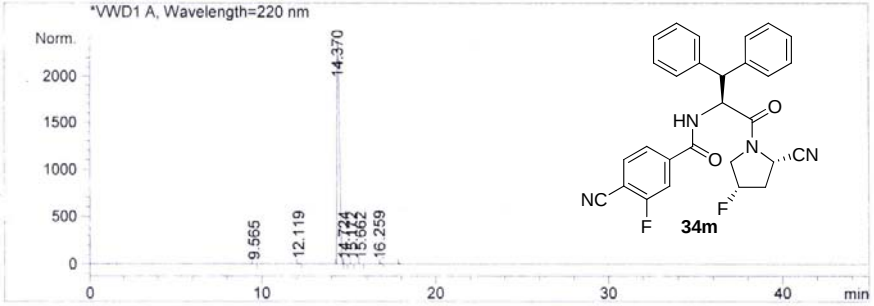
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Mass Spectrum

Positive
BasePeak:485.4



Column : YMC J'sphere C18-(150 X4.6mm)4.0 µ
Mobile Phase : 0.05 % TFA in Water : ACN (Gradient)
Flow Rate : 1.0 ml/min @ 220 nm



Area Percent Report

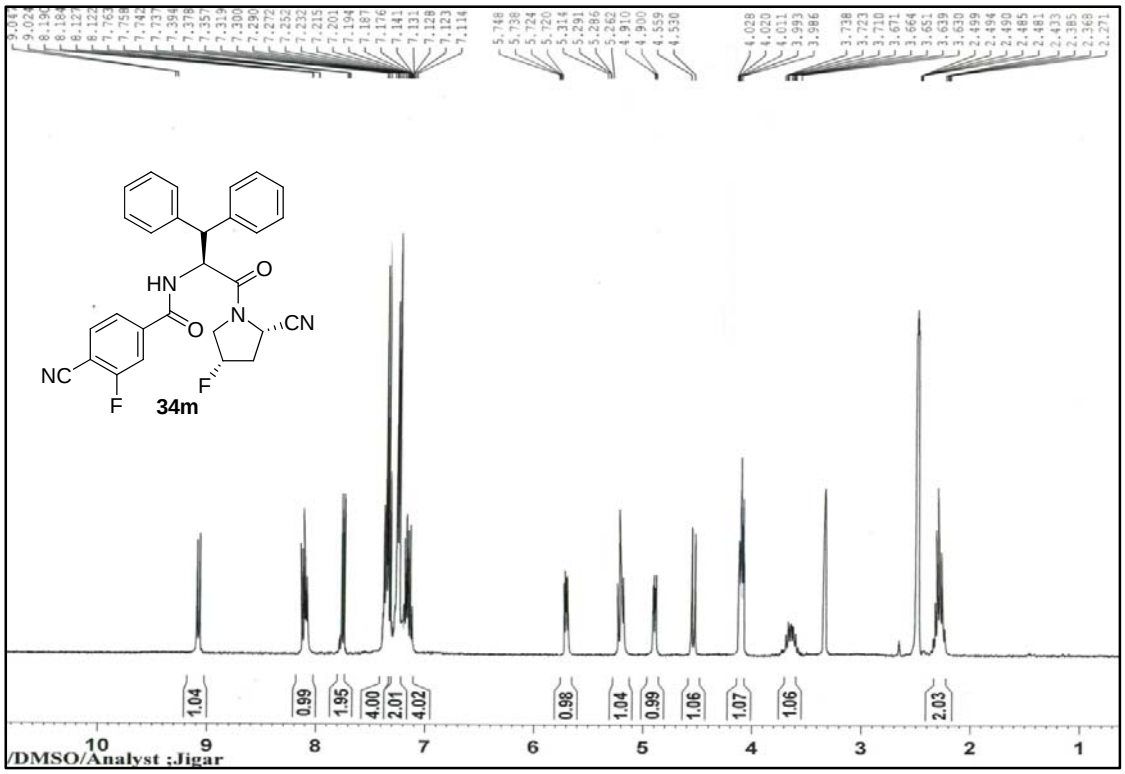
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

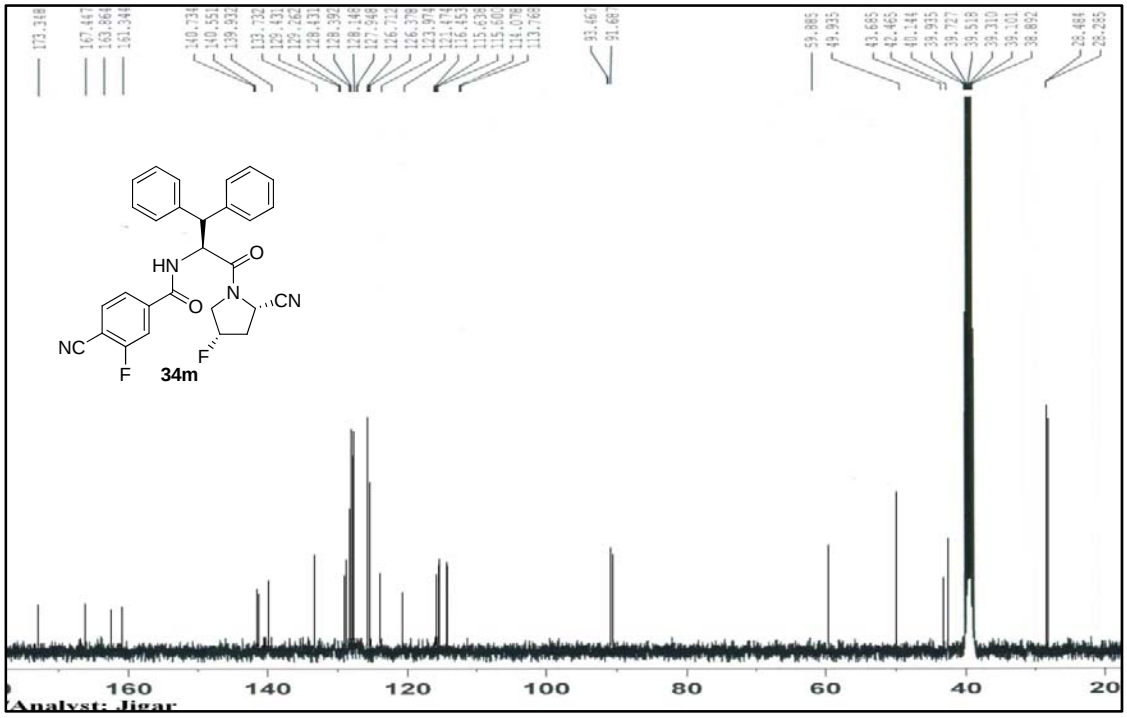
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	9.565	BBA	0.1167	2.65428	3.59458e-1	0.7537
2	12.119	BB	0.0967	38.71583	6.27446	0.1585
3	14.370	BV	0.1651	2.41367e4	2376.66919	98.7644
4	14.724	VP	0.0852	20.41733	3.59381	0.0836
5	15.172	BBA	0.0830	4.52716	8.84244e-1	0.0285
6	15.662	BBA	0.0900	5.62573	9.84399e-1	0.0230
7	16.259	PBA	0.0822	45.81187	18.64911	0.1875
Totals :				2.44337e4	2427.57190	

Results obtained with enhanced integrator!

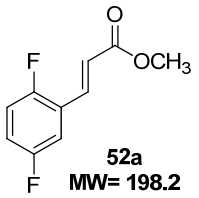
*** End of Report ***


HN
O
O
N
CN
NC
F
F
34m
/DMSO/Analyst ;Jigar


HN
O
O
N
CN
NC
F
F
34m
Analyst: Jigar

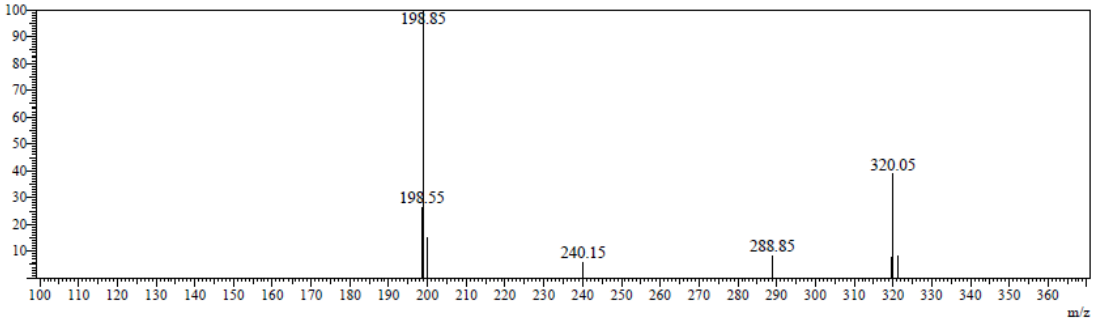
ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Sample ID : C-840-PJ-134A



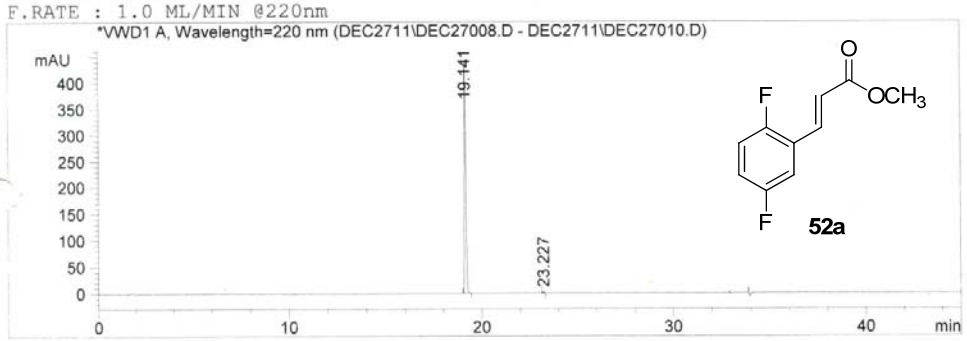
52a
MW= 198.2

Mass Spectrum



ANALYSED BY : JIGNESH CHAUHAN

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



52a

Area Percent Report

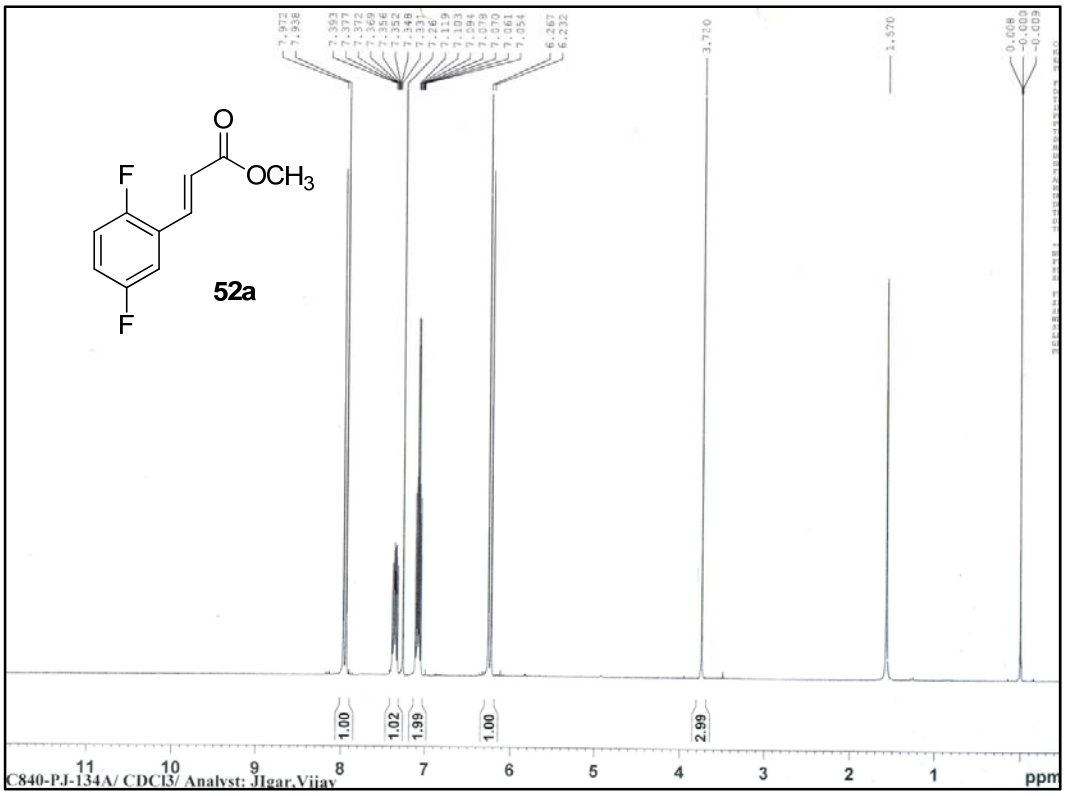
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

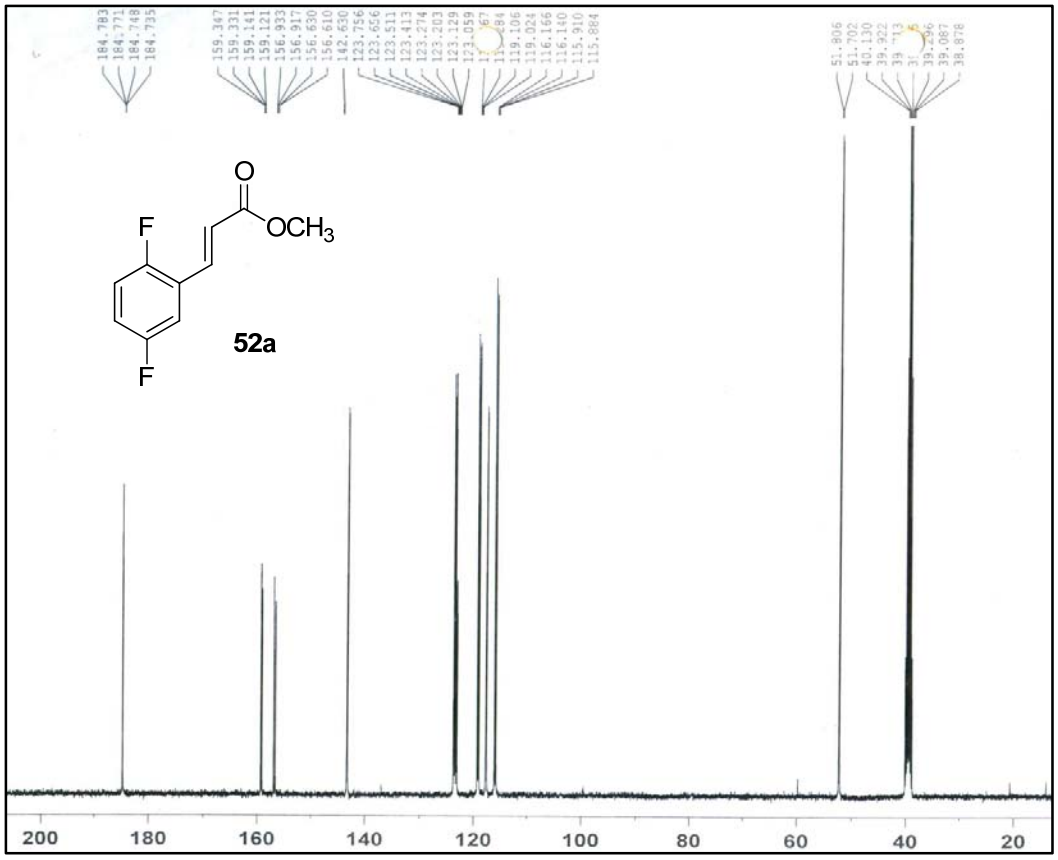
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	19.141	PBA	0.0822	2334.74951	440.43936	99.5564
2	23.227	BBA	0.0773	10.40380	2.13171	0.4436
Totals :				2345.15331	442.57107	

Results obtained with enhanced integrator!

*** End of Report ***

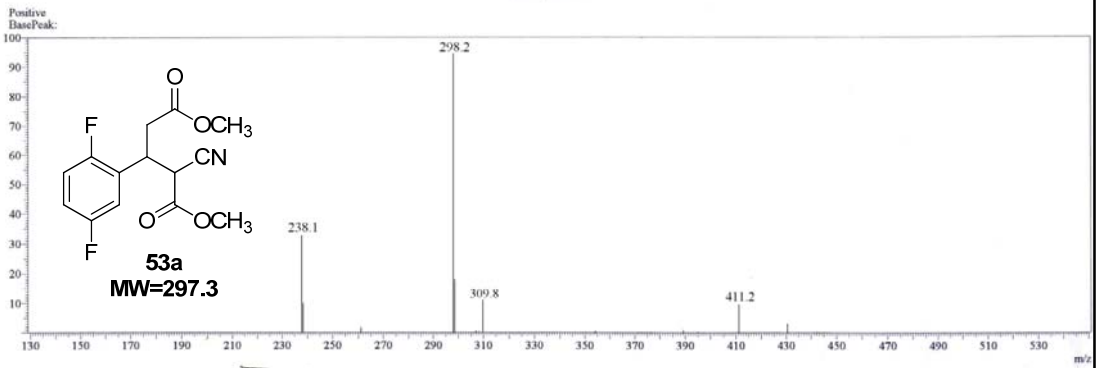

O
F
OCH3
F
52a
1.00
1.02
1.99
1.00
2.99
ppm
C840-PJ-134A/ CDCl3/ Analyst: JIgar,Vijay


O
F
OCH3
F
52a
200
180
160
140
120
100
80
60
40
20

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Sample ID : C-840-PJ-135

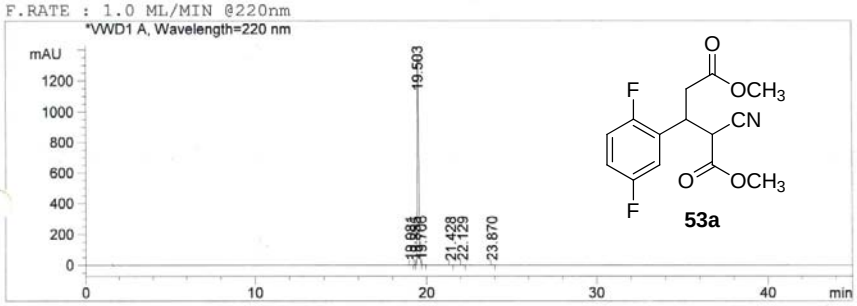
Mass Spectrum



10/03/11

ANALYSED BY : CHAUHAN JIGNESH

COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

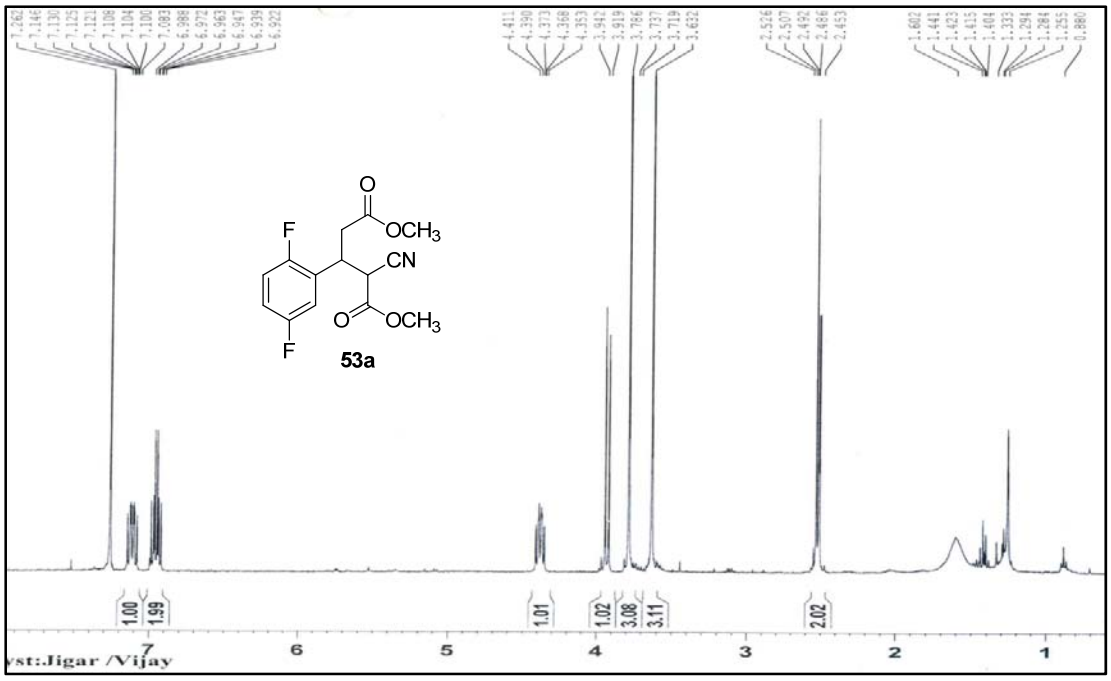
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

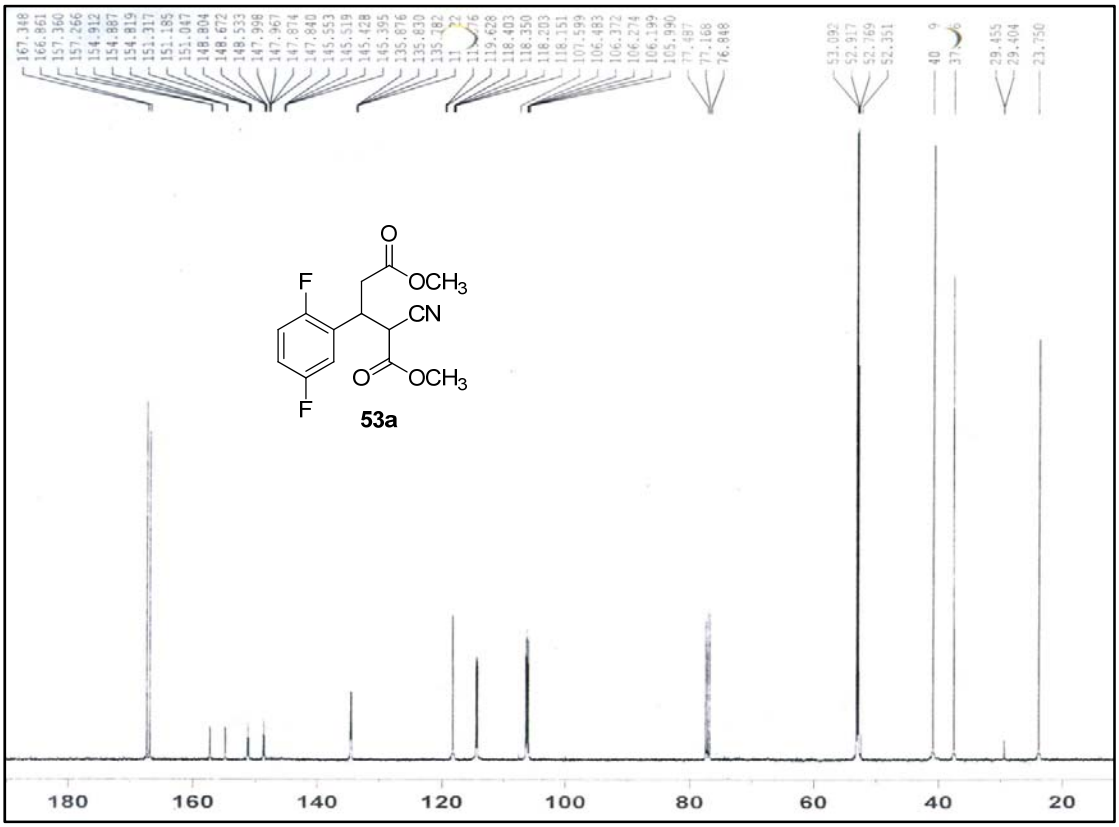
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	19.081	PBA	0.0873	10.98501	1.95878	0.1345
2	19.355	MF R	0.0546	2.76052	8.43207e-1	0.0338
3	19.503	FM R	0.0992	8105.56738	1362.13489	99.2283
4	19.706	FM R	0.0913	22.74672	4.15312	0.2785
5	21.428	BBA	0.0985	4.29100	6.79206e-1	0.0525
6	22.129	BP	0.0850	11.82558	2.13499	0.1448
7	23.870	BB	0.0821	10.42696	1.97239	0.1276
Totals :				8168.60318	1373.87657	

Results obtained with enhanced integrator!

*** End of Report ***


F
O
OCH3
CN
O
OCH3
F
53a
1.00
1.99
1.01
1.02
3.08
3.11
2.02
7
6
5
4
3
2
1
vst:Jigar /Vijay

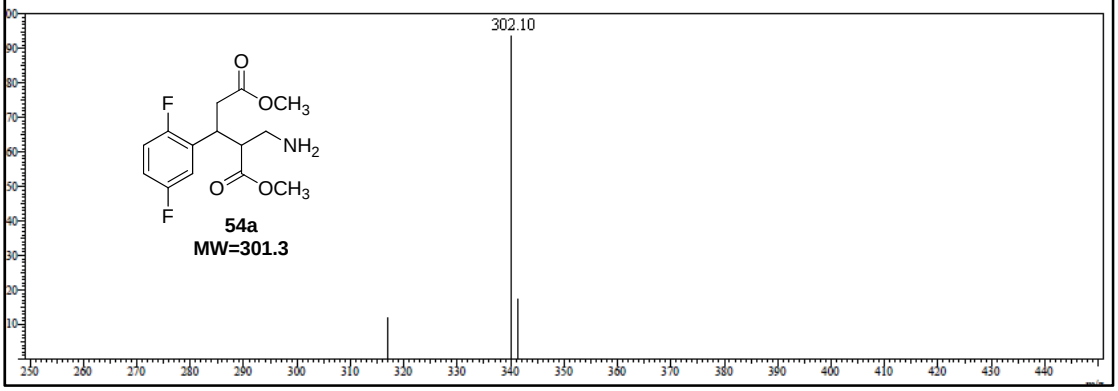

F
O
OCH3
CN
O
OCH3
F
53a
180
160
140
120
100
80
60
40
20

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

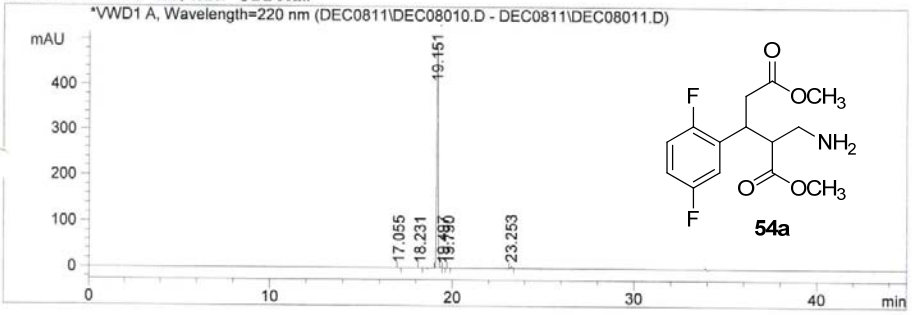
Sample ID : C-840-PJ-141

Mass Spectrum

Positive
BasePeak:340.10(6015064)



COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

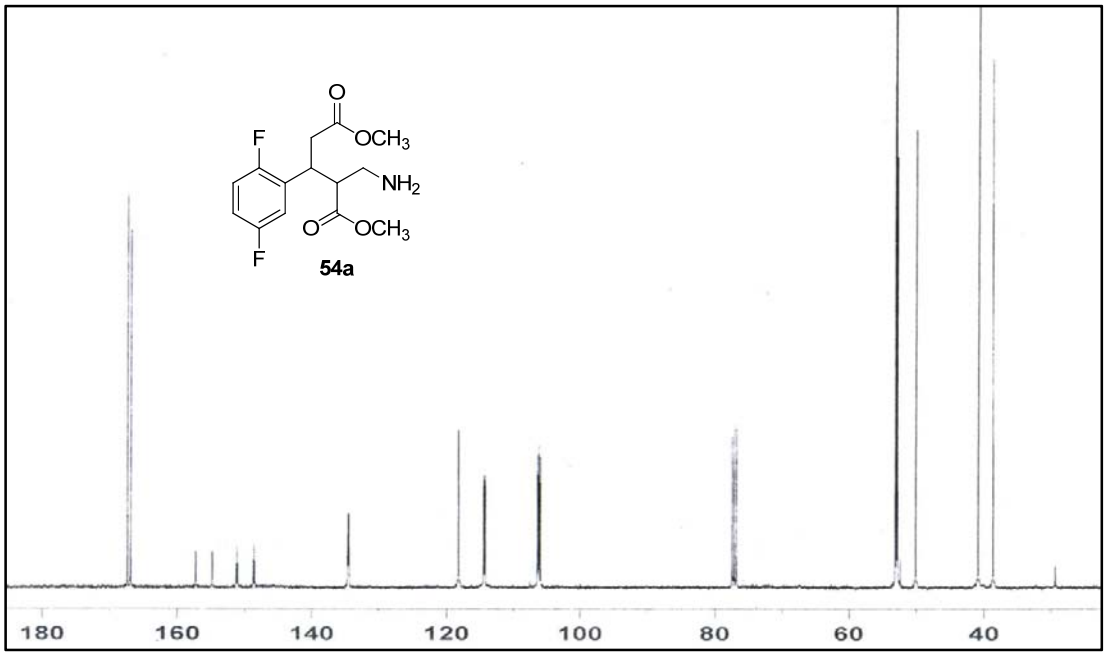
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	17.055	BBA	0.0856	8.96776e-1	1.60531e-1	0.0331
2	18.231	BBA	0.0965	1.69269	2.75301e-1	0.0625
3	19.151	VV	0.0856	2678.54150	490.06876	98.9792
4	19.497	VBA	0.0877	2.54473	4.22665e-1	0.0940
5	19.790	BBA	0.0851	7.21424e-1	1.39434e-1	0.0267
6	23.253	PBA	0.0866	21.76828	3.92207	0.8044
Totals :				2706.16540	494.98876	

Results obtained with enhanced integrator!

*** End of Report ***

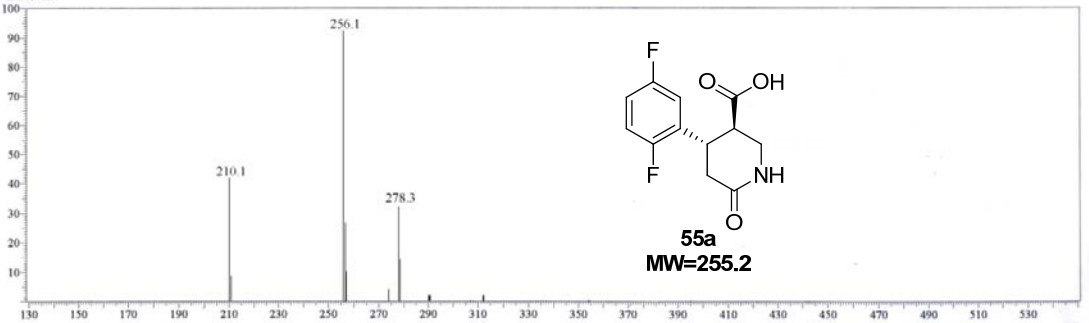

F
O
OCH3
NH2
O
OCH3
F
54a


F
O
OCH3
NH2
O
OCH3
F
54a

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

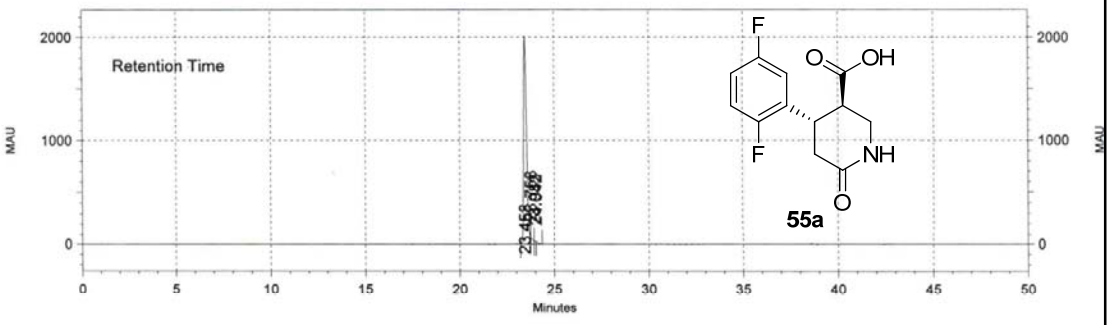
Sample ID: C840-PJ-160

Mass Spectrum



55a
MW=255.2

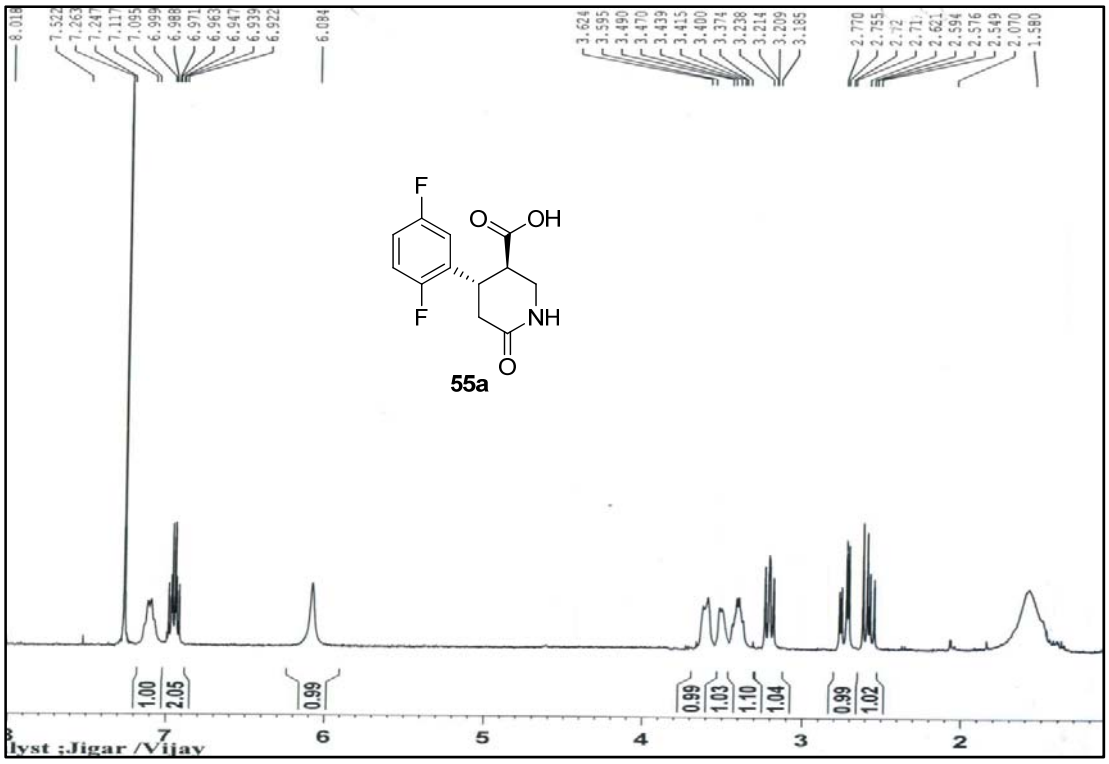
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

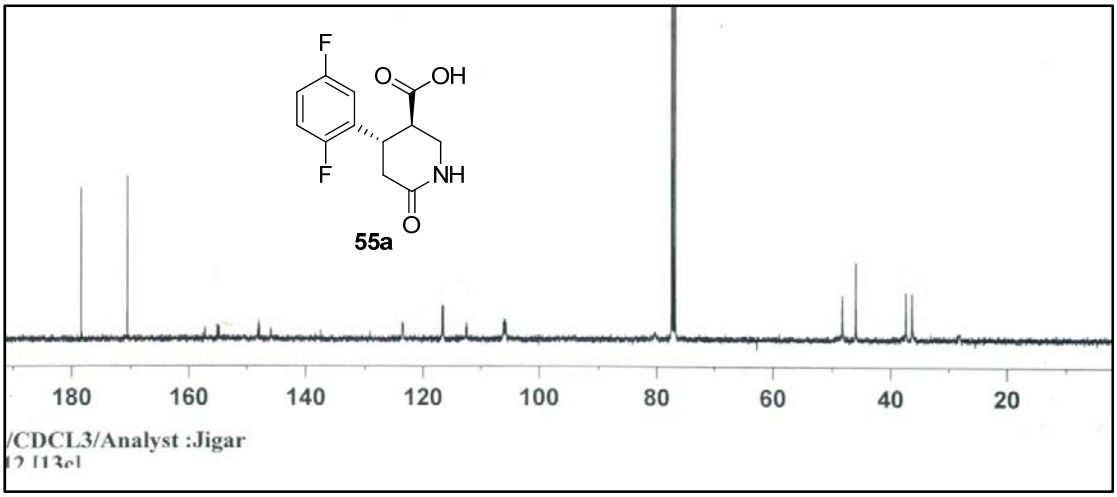


55a

Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	23.46	21972425	95.98
2	23.76	462266	2.02
3	23.98	301135	1.32
4	24.04	157746	0.69
Totals		22893572	100.00

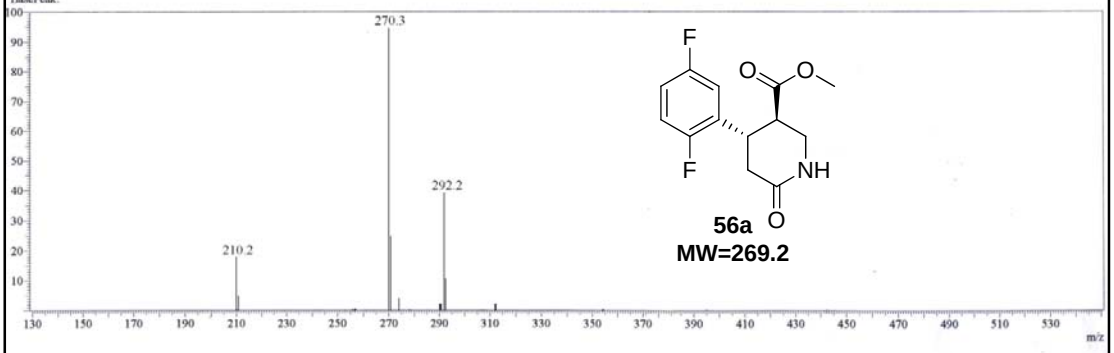

F
O
OH
F
NH
O
55a
1.00
2.05
0.99
0.99
1.03
1.10
1.04
0.99
1.02
7
6
5
4
3
2


F
O
OH
F
NH
O
55a
180
160
140
120
100
80
60
40
20
/CDCL3/Analyst :Jigar

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

Sample ID: C840-PJ-173

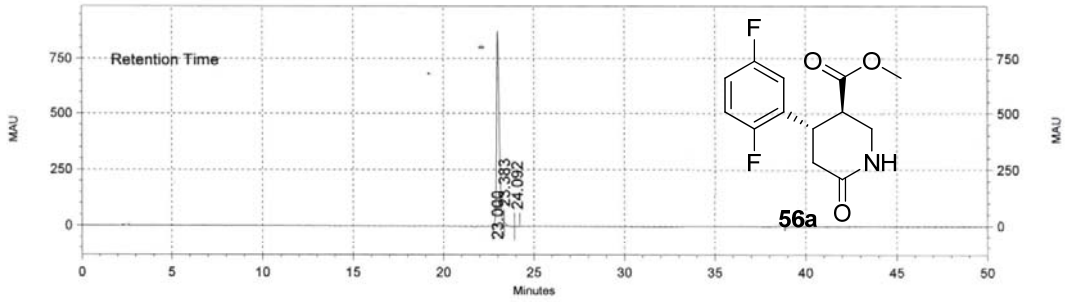
Mass Spectrum



56a
MW=269.2

ANALYSED BY : CHAUHAN JIGNESH

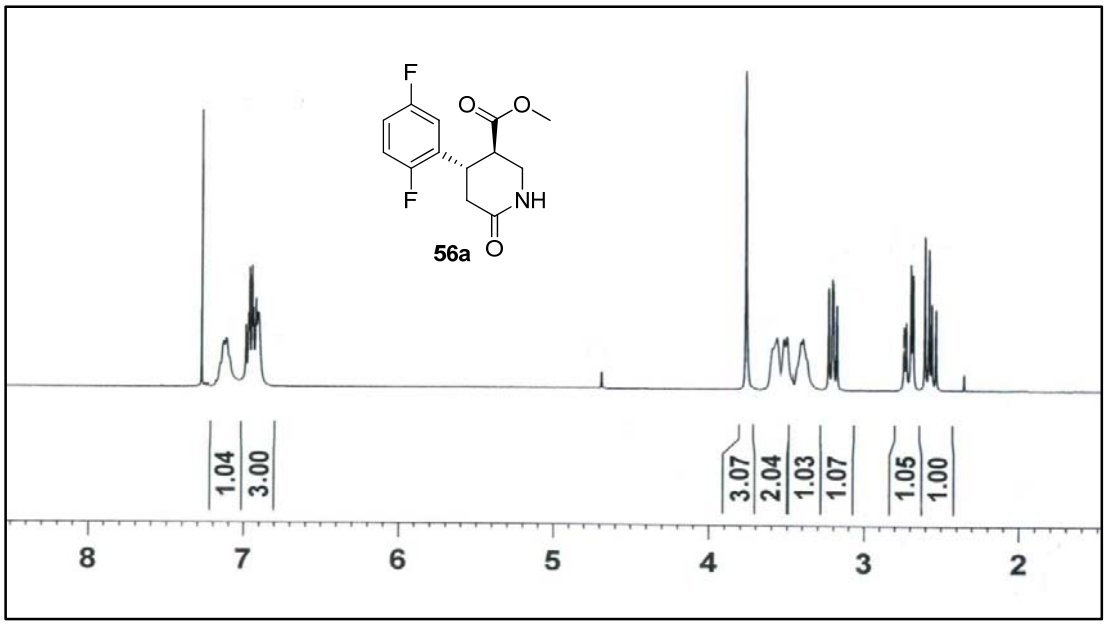
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

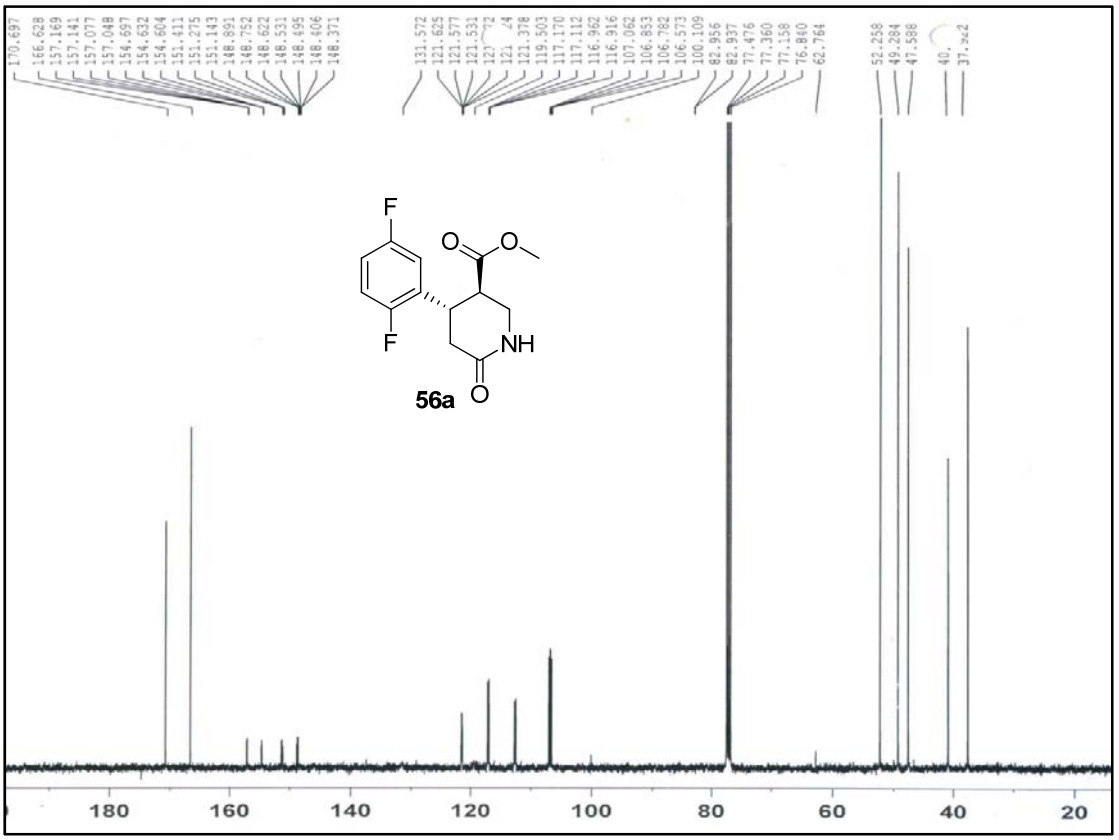


56a

Detector A - 1 (220nm)

Pk #	Retention Time	Area	Area %
1	23.00	9074073	98.40
2	23.38	125350	1.36
3	24.09	21936	0.24
Totals		9221359	100.00

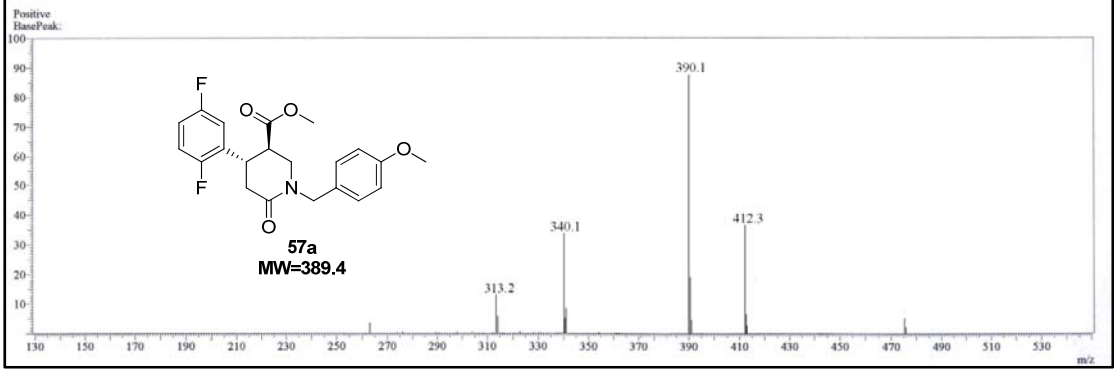

F
O
O
F
NH
O
56a
1.04
3.00
3.07
2.04
1.03
1.07
1.05
1.00
8
7
6
5
4
3
2


F
O
O
F
NH
O
56a
180
160
140
120
100
80
60
40
20

ZYDUS RESEARCH CENTRE
DEPARTMENT OF BIOPHARMACEUTICS

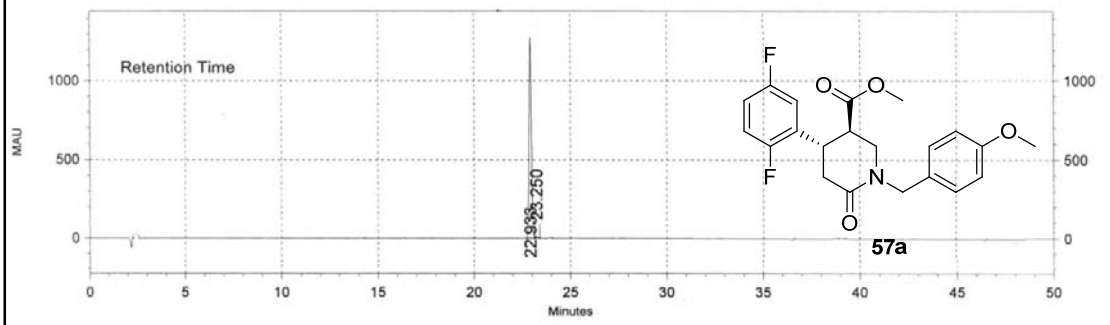
Sample ID :C840-PJ-101

Mass Spectrum



57a
MW=389.4

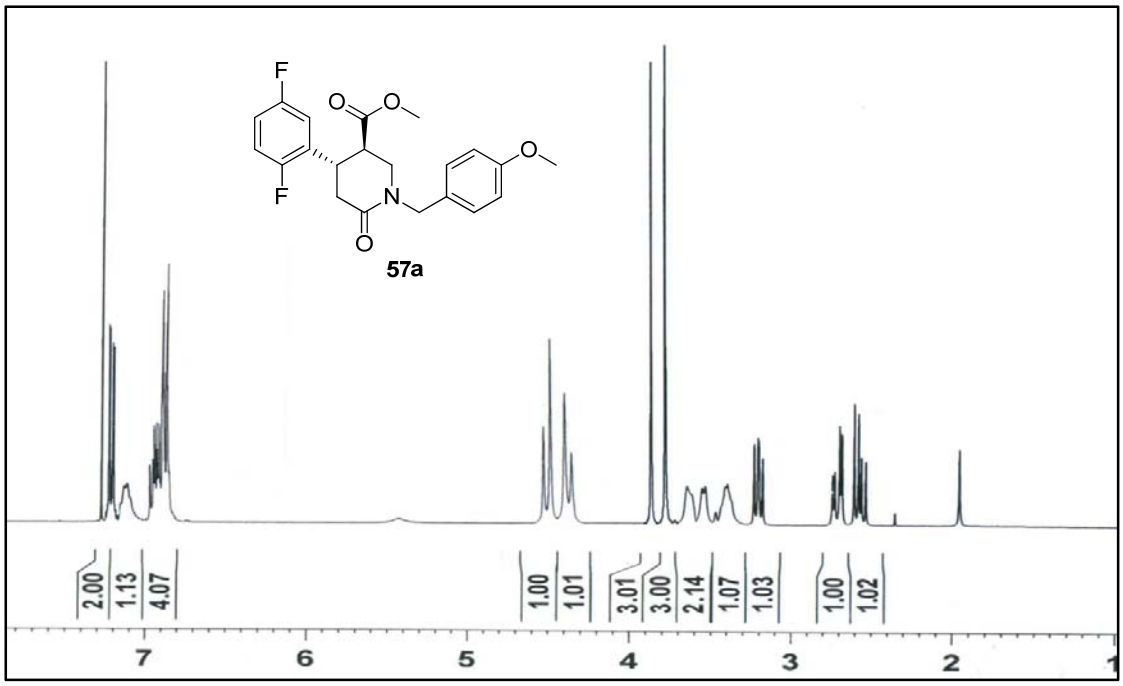
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

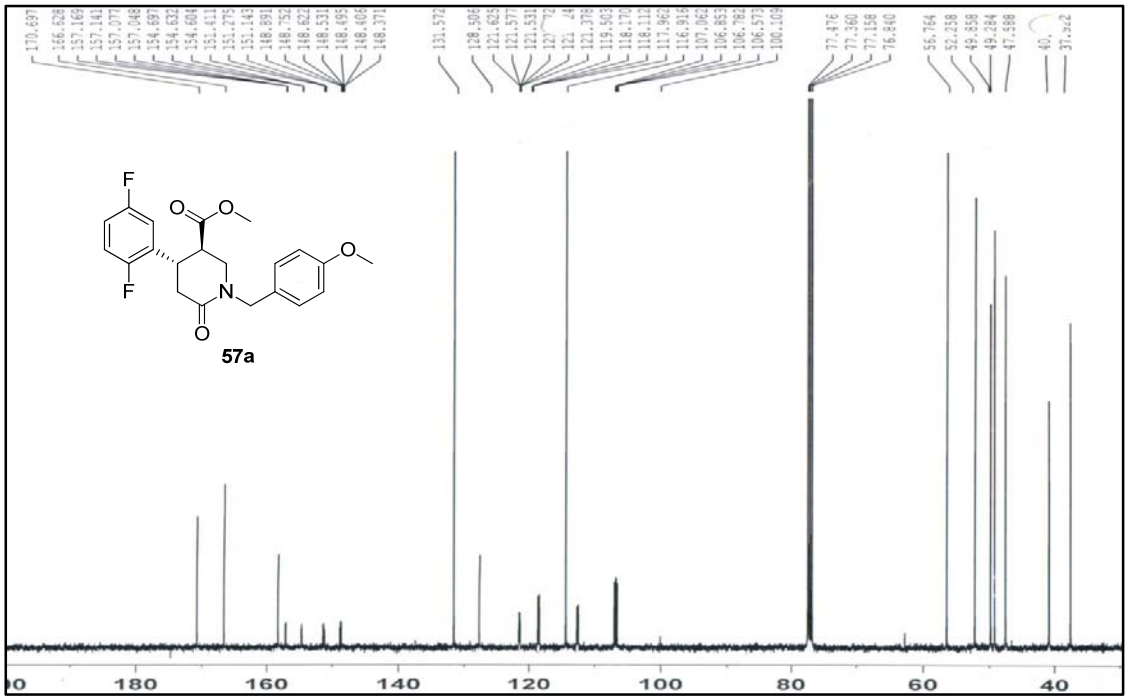


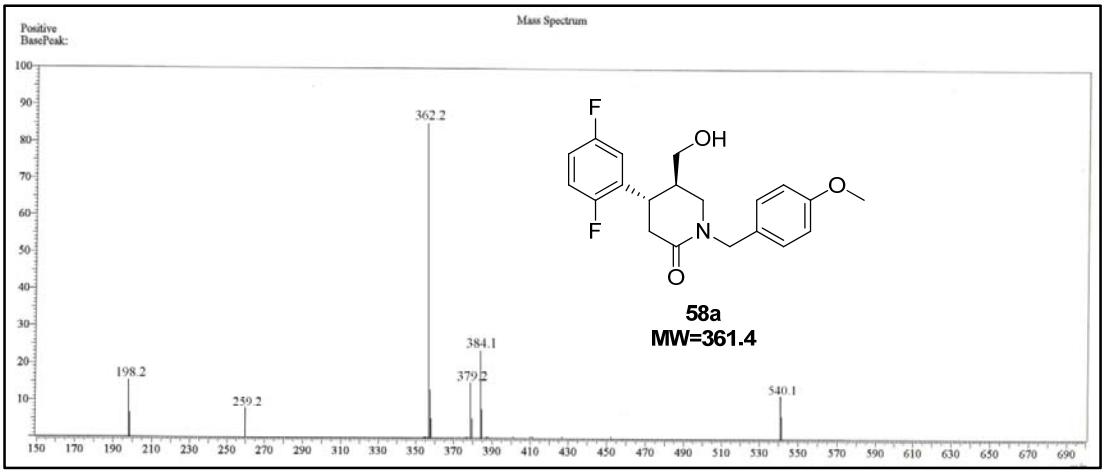
57a

Detector A - 1 (220nm)

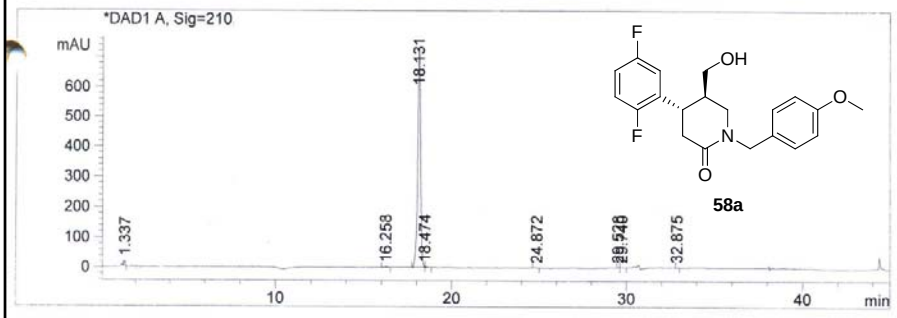
Pk #	Retention Time	Area	Area %
1	22.93	10344168	99.60
2	23.25	41245	0.40
Totals		10385413	100.00


F
F
O
O
O
N
O
57a
2.00
1.13
4.07
1.00
1.01
3.01
3.00
2.14
1.07
1.03
1.00
1.02
7
6
5
4
3
2


F
F
O
O
O
N
O
57a
180
160
140
120
100
80
60
40



Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



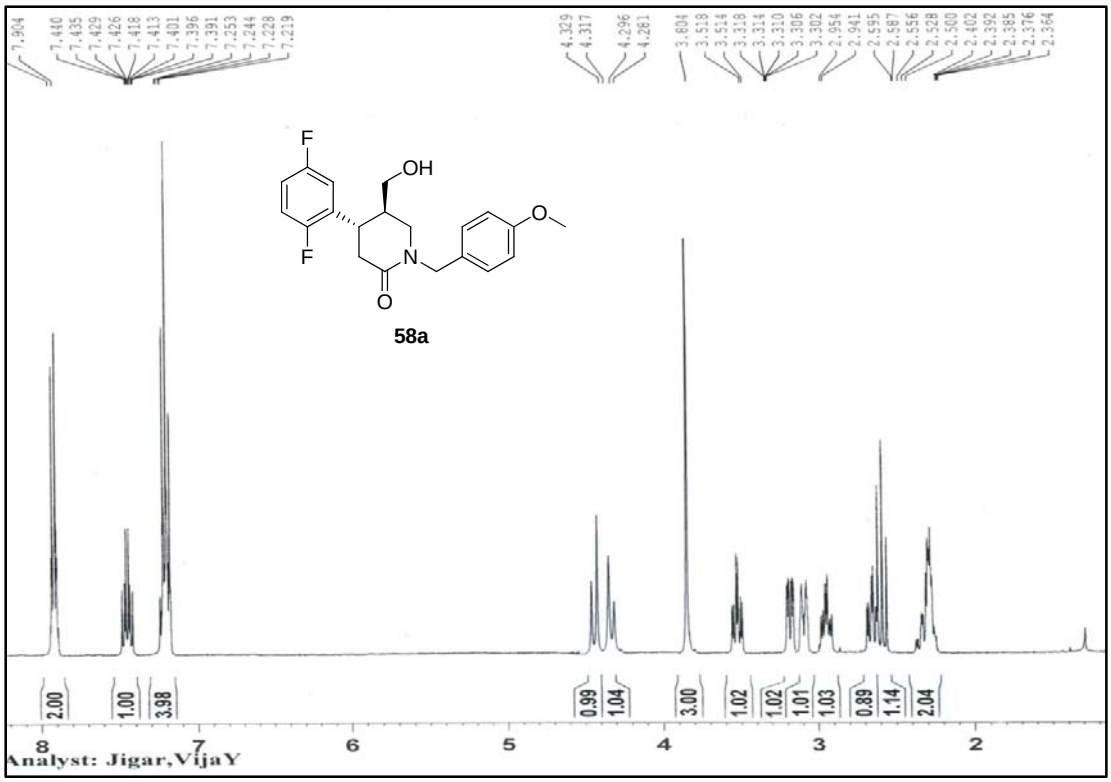
Area Percent Report

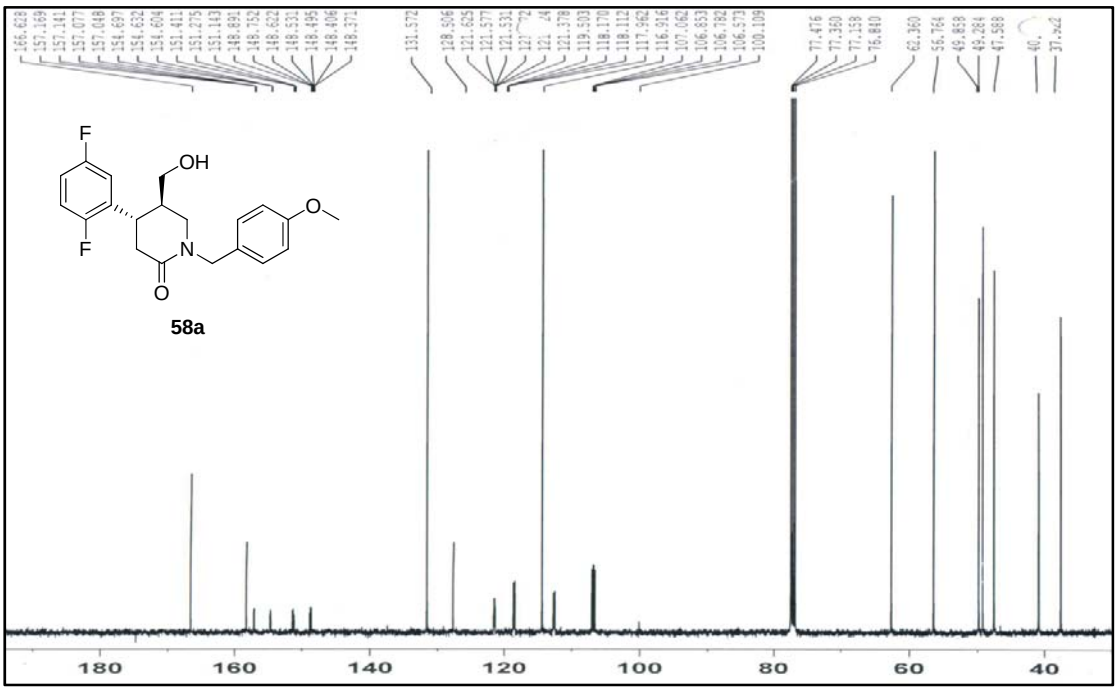
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

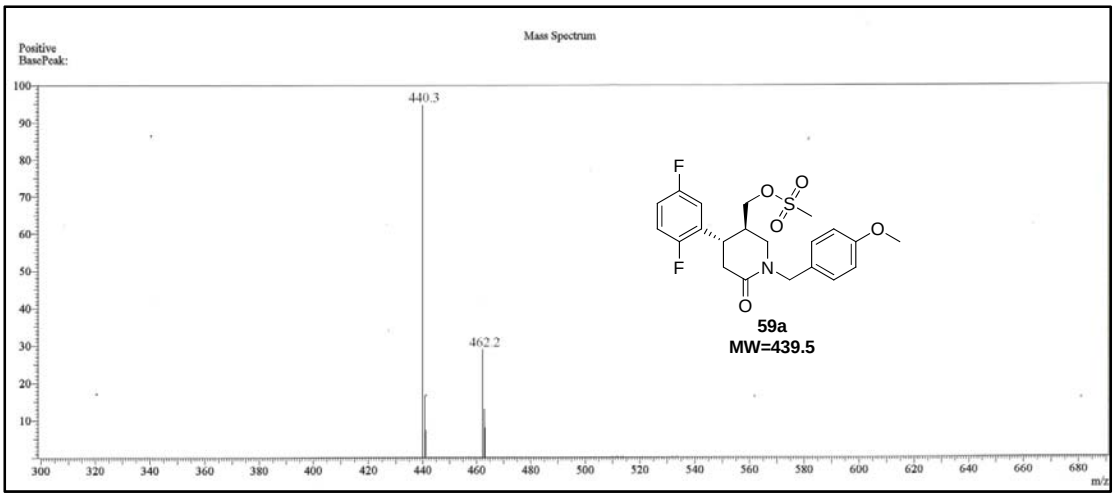
Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.337	BBA	0.0718	81.75259	17.72912	0.9780
2	16.258	BBA	0.1923	25.93850	2.15735	0.3103
3	18.131	MF R	0.1880	8223.08203	729.08527	98.3702
4	18.474	FM R	0.1136	16.33422	2.39602	0.1954
5	24.872	MM R	0.1859	4.63921	4.15861e-1	0.0555
6	29.528	BV	0.0865	1.98986	3.38825e-1	0.0238
7	29.740	VB R	0.1483	4.24995	4.18112e-1	0.0508
8	32.875	BB	0.0862	1.33533	2.28394e-1	0.0160

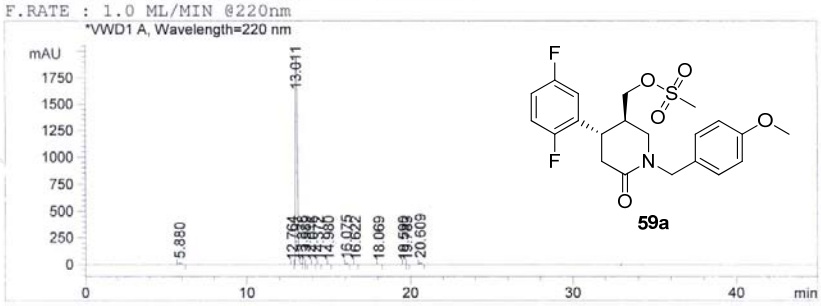
Totals : 8359.32169 752.76895


F
OH
F
N
O
O
58a
Analyst: Jigar,VijaY


F
OH
F
N
O
O
58a



COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

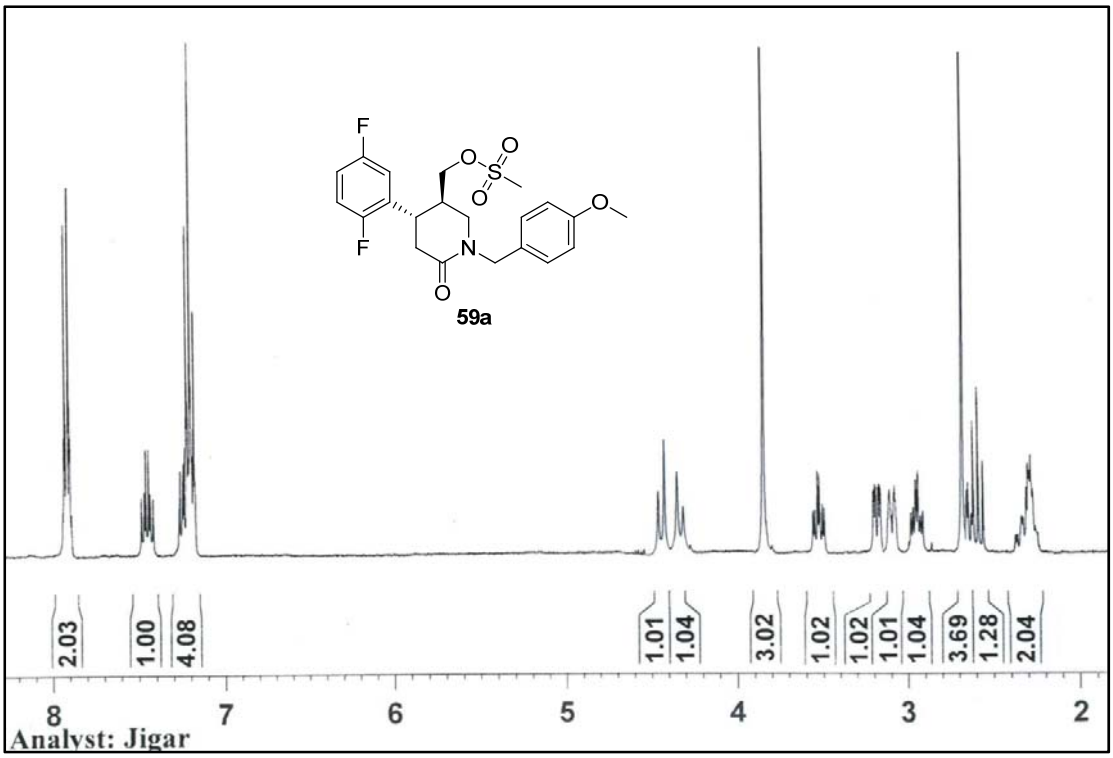
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

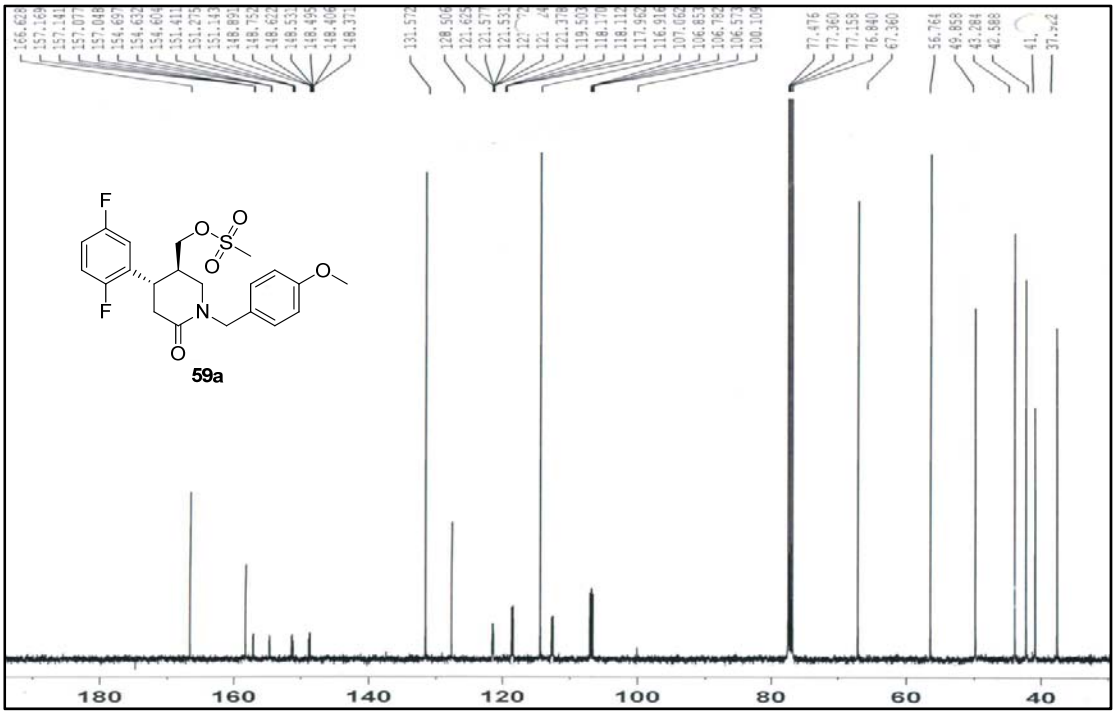
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

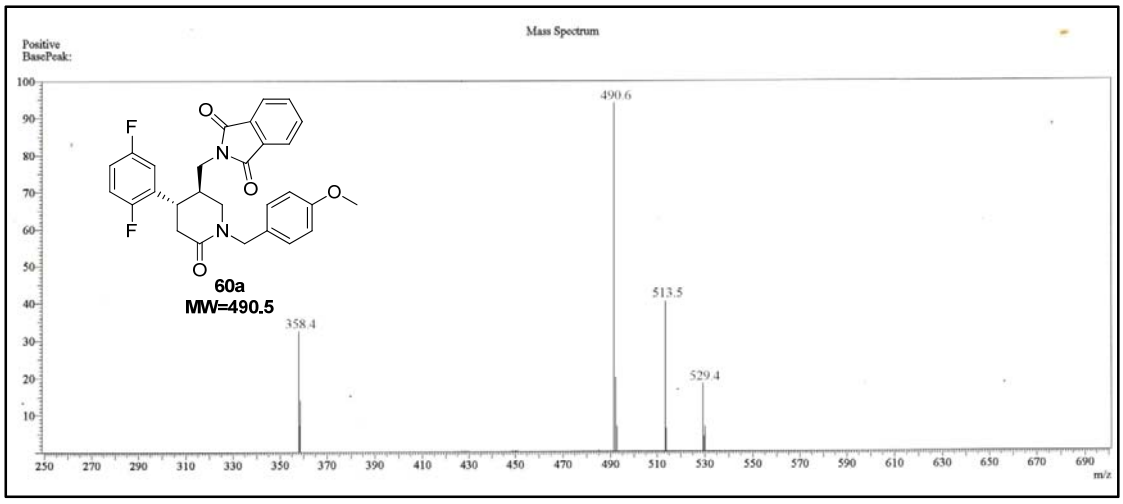
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	5.880	BBA	0.1602	177.15422	16.50138	1.0995
2	12.764	BV	0.1020	6.57375	9.74003e-1	0.0408
3	13.011	MF R	0.1331	1.56359e4	1957.34802	97.0423
4	13.378	FM R	0.0901	16.81635	3.11017	0.1044
5	13.589	VBA	0.0570	4.11689	1.09436	0.0256
6	14.016	PBA	0.0900	7.52201	1.31656	0.0467
7	14.372	PBA	0.0999	4.58343	7.25743e-1	0.0284
8	14.980	BBA	0.1010	13.87421	2.25258	0.0861
9	16.075	BBA	0.0997	73.30376	10.99073	0.4550
10	16.622	BBA	0.0980	7.73576	1.16308	0.0480
11	18.069	BBA	0.1269	3.80662	4.54415e-1	0.0236
12	19.590	BV	0.1046	17.20380	2.51256	0.1068
13	19.783	VBA	0.1032	2.90138	4.23496e-1	0.0180
14	20.609	BBA	0.1107	140.96368	19.46984	0.8749
Totals :				1.61125e4	2018.33694	

Results obtained with enhanced integrator!

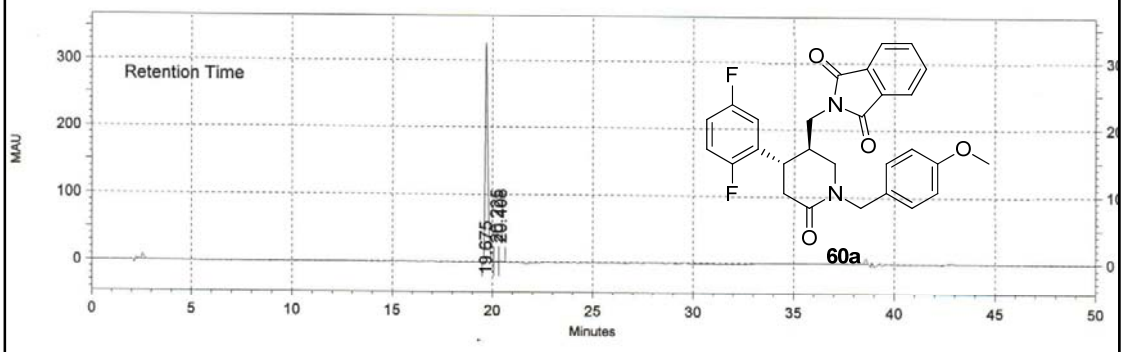
*** End of Report ***


F
F
O
O
O
S
O
O
N
O
59a
2.03
1.00
4.08
1.01
1.04
3.02
1.02
1.02
1.01
1.04
3.69
1.28
2.04
8
7
6
5
4
3
2
Analyst: Jigar


F
F
O
O
O
S
O
O
N
O
59a
180
160
140
120
100
80
60
40

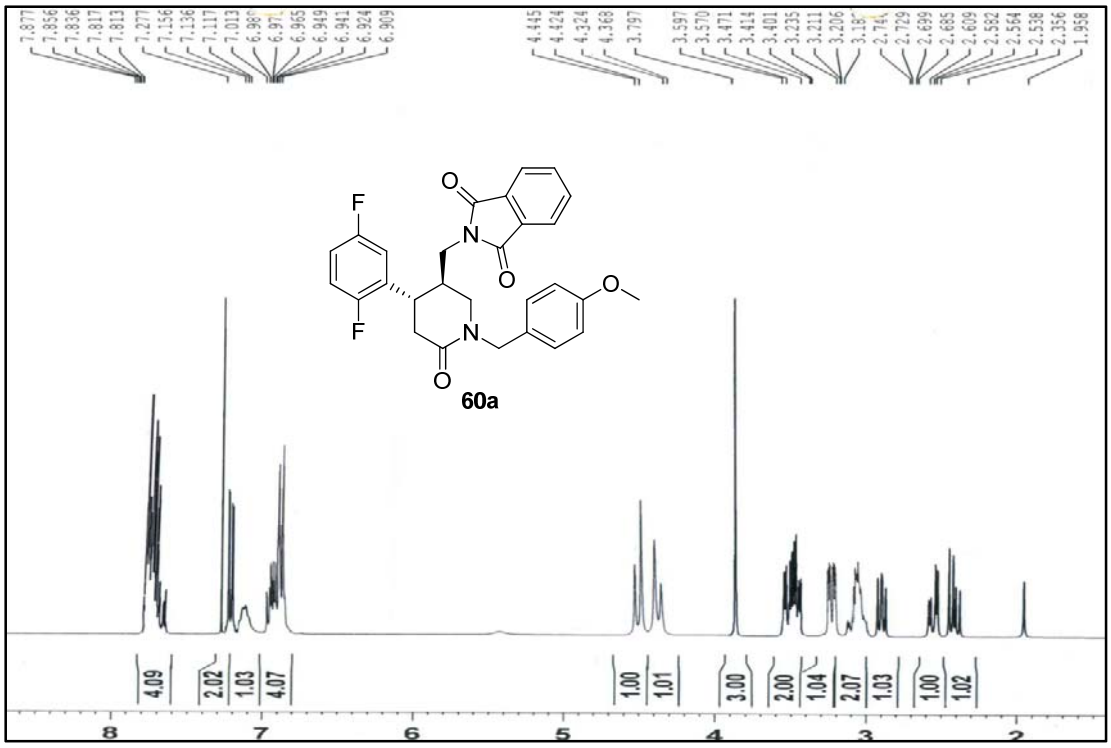


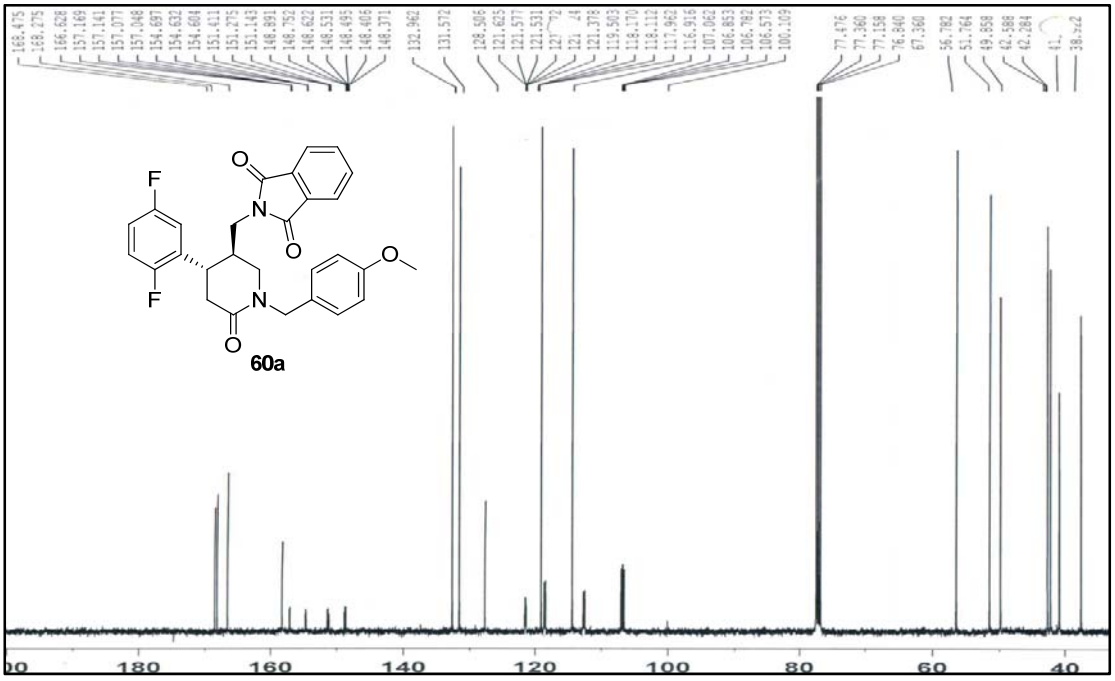
Column : YMC-ODS-AQ [150x4.6mm],5um.
M.P. : [0.1%TFA IN WATER:ACN] (GRADIENT)
Flow rate : 1.0 ml/min@220nm

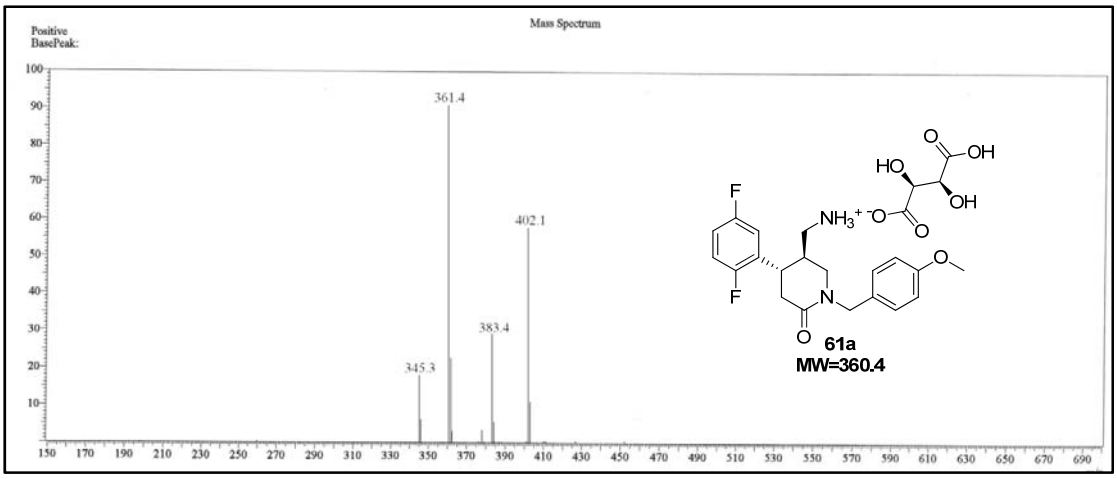


Detector A - 1 (220nm)

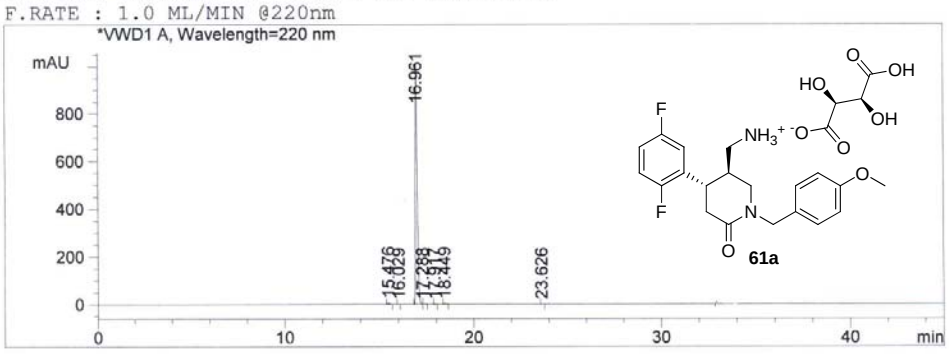
Pk #	Retention Time	Area	Area %
1	19.68	2961712	98.57
2	20.23	18824	0.63
3	20.41	24166	0.80
Totals		3004702	100.00


60a


60a



COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	15.476	BBA	0.1001	39.52889	6.00477	0.5719
2	16.029	BBA	0.0742	1.08086	1.96956e-1	0.0156
3	16.961	MF R	0.1129	6838.54736	1009.79938	98.9434
4	17.288	FM R	0.0795	6.02183	1.26204	0.0871
5	17.917	MM R	0.0990	1.15465	1.94389e-1	0.0167
6	18.449	BBA	0.0991	22.77402	3.43964	0.3295
7	23.626	BBA	0.0856	2.46693	3.89283e-1	0.0357
Totals :				6911.57454	1021.28646	

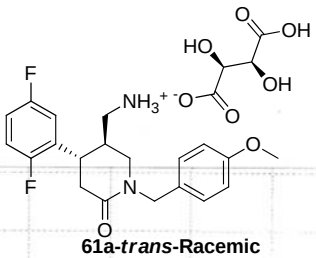
Results obtained with enhanced integrator!

*** End of Report ***

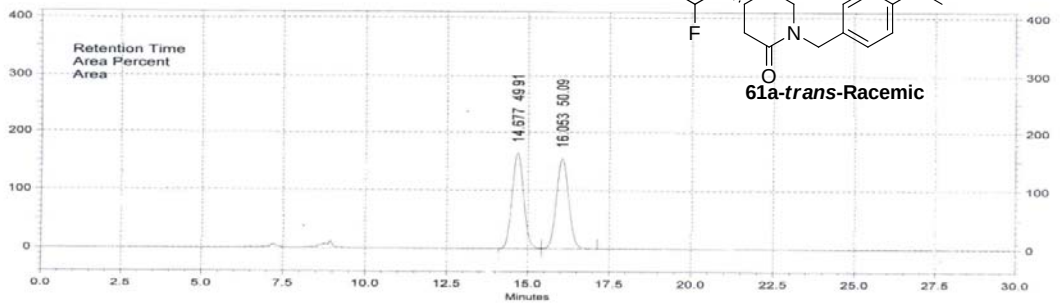
ZYDUS RESEARCH CENTRE
Analytical Research

C908-TKP-144

Sample ID : **C840-PJ-135 RACEMIC**
Method Name : **D:\LCVP-01-217\YEAR-2010\methods\ZRC-3192(99-01)B.met**
Vial No : 0
Data Name : D:\LCVP-01-217\YEAR-2012\DATA\JUNE-2012\JUNE-2012\
Inj. vol : 2 ul
User: : TIMIR PATEL
Acquired : 6/20/2012 3:53:13 PM
Flow Rate : 0.5 ml/min.
Column : CHIRALCEL OD H[250*4.6 mm] 5u
M.Phase : N-HEXANE:0.1% DEA IN ETOH (98:02)



61a-*trans*-Racemic



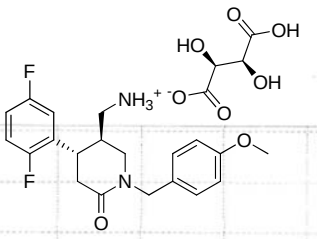
1: 220 nm, 8 nm

Pk #	Retention Time	Area	Area Percent
1	14.677	4051005	49.91
2	16.053	4065390	50.09
Totals		8116395	100.00

ZYDUS RESEARCH CENTRE
Analytical Research

C908-TKP-144

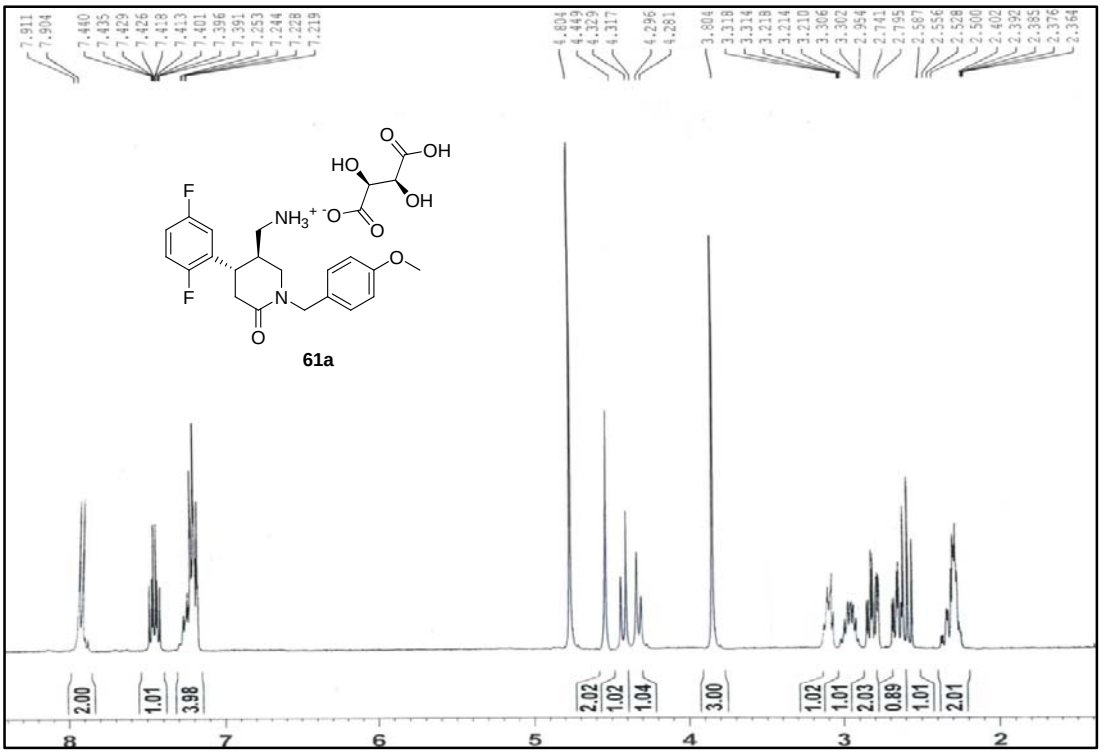
Sample ID : **C840-PJ-135**
Method Name : **D:\LCVP-01-217\YEAR-2010\methods\ZRC-3192(99-01)B.met**
Vial No : 0
Data Name : D:\LCVP-01-217\YEAR-2012\DATA\JUNE-2012\JUNE-2012\
Inj. vol : 2 ul
User: : TIMIR PATEL
Acquired : 6/20/2012 4:57:13 PM
Flow Rate : 0.5 ml/min.
Column : CHIRALCEL OD H[250*4.6 mm] 5u
M.Phase : N-HEXANE:0.1% DEA IN ETOH (98:02)

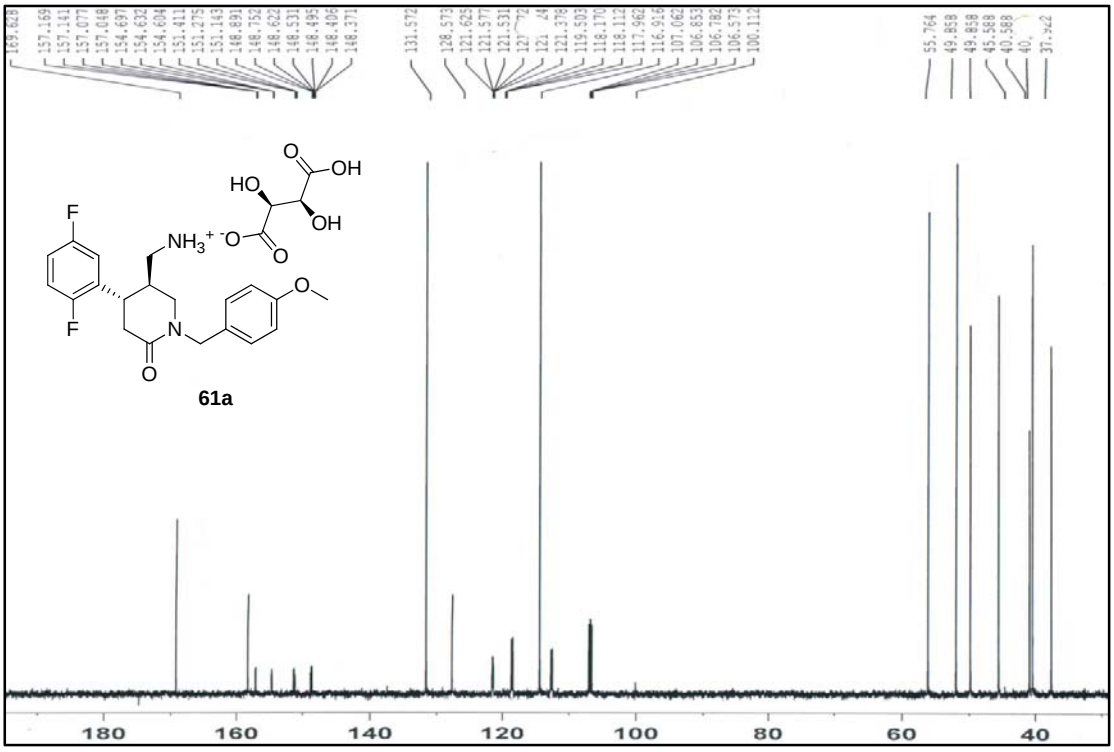


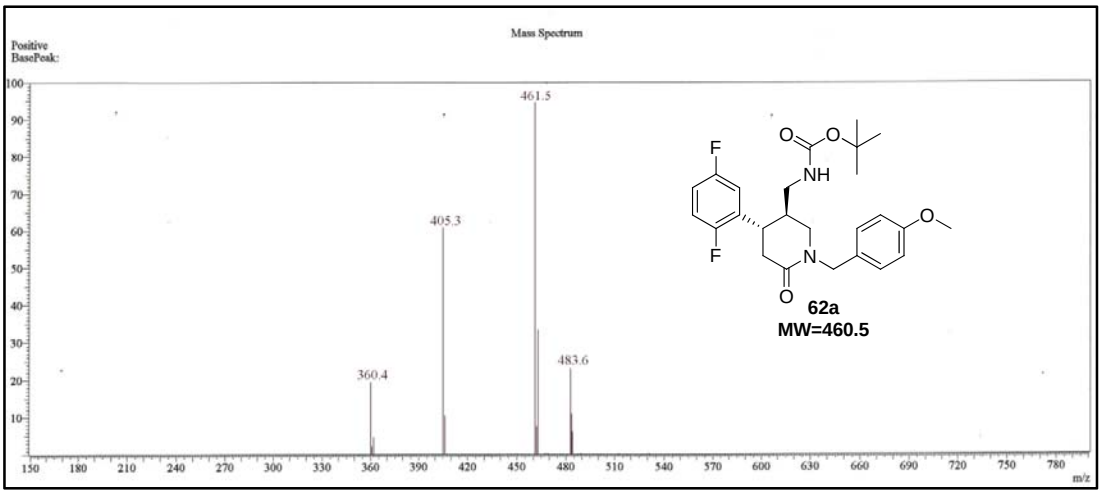
61a-Chiral Pure

1: 220 nm, 8 nm

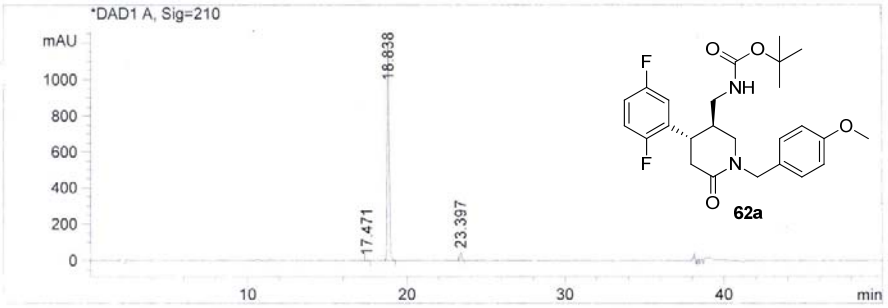
Pk #	Retention Time	Area	Area Percent
1	14.609	21005	00.91
2	16.043	8095390	99.09
Totals		8116395	100.00


61a


61a



Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



Area Percent Report

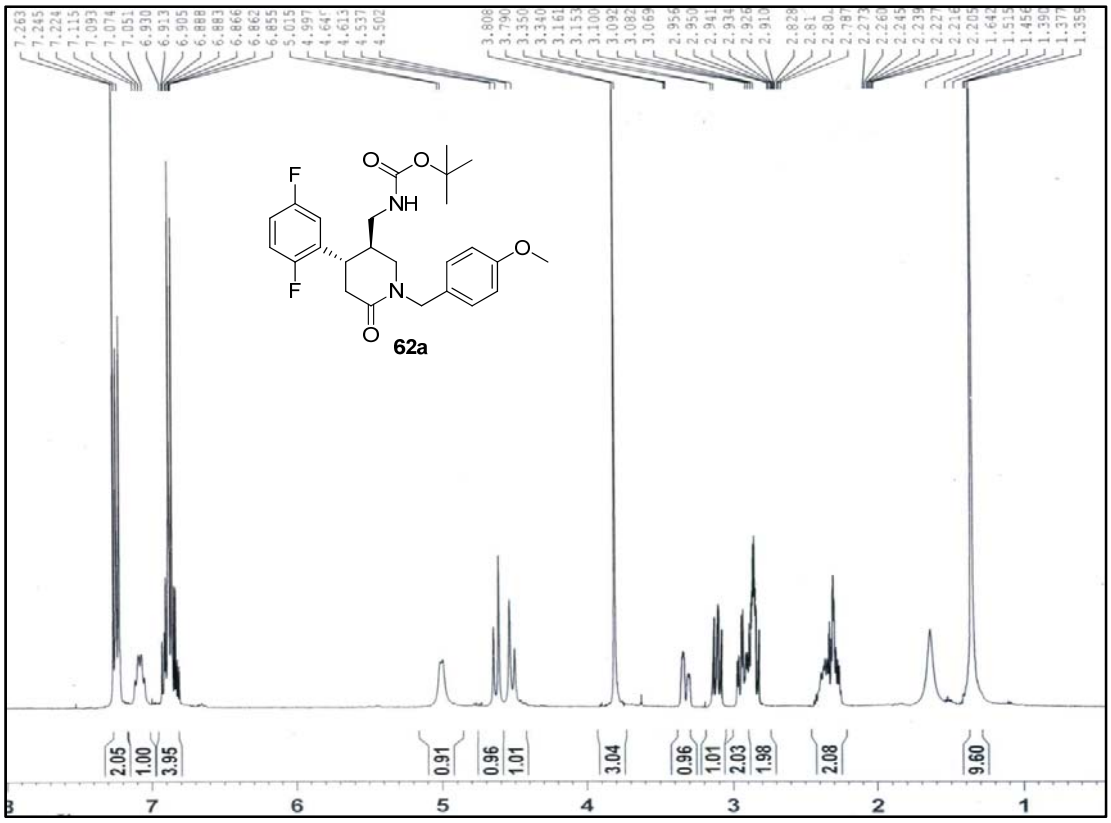
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

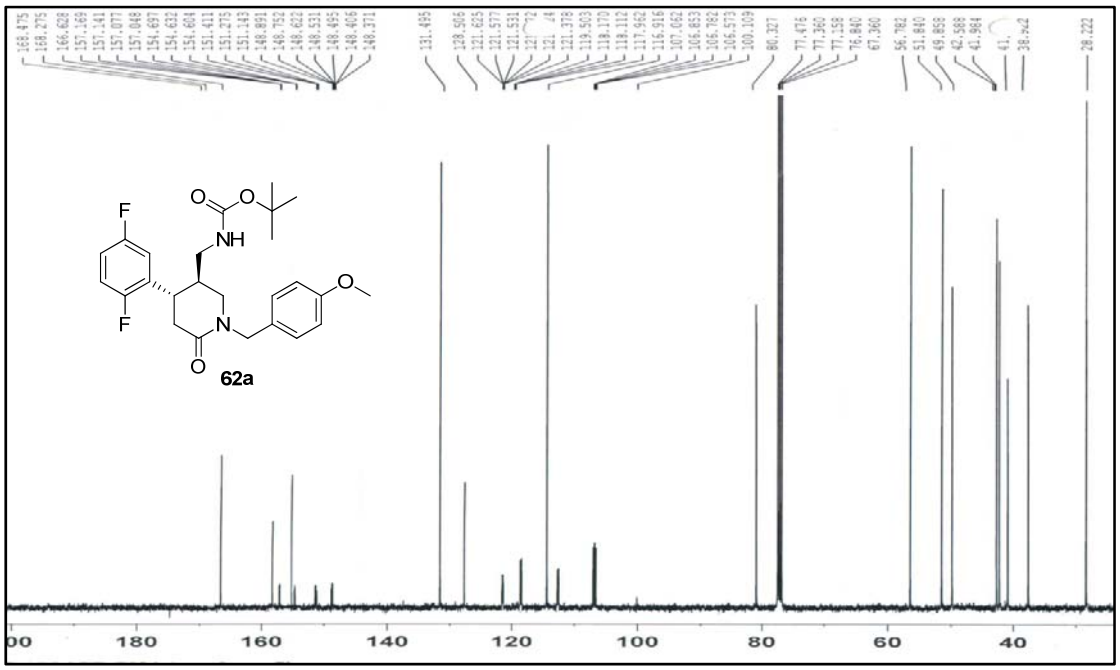
Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

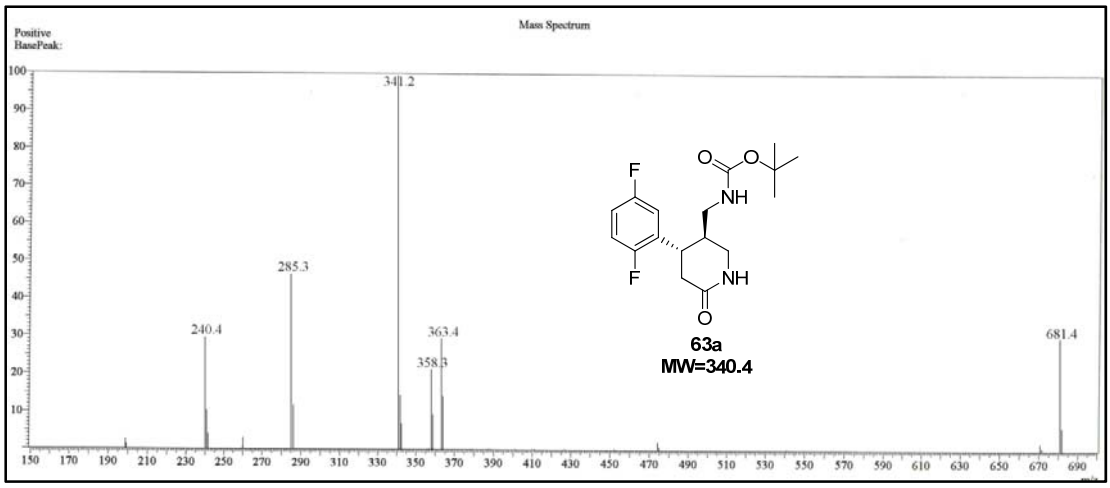
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.471	BBA	0.1447	17.08266	1.76795	0.1633
2	18.838	BBA	0.1321	1.00857e4	1199.62109	96.4143
3	23.397	BBA	0.1340	358.01276	41.77011	3.4224

Totals : 1.04608e4 1243.15916

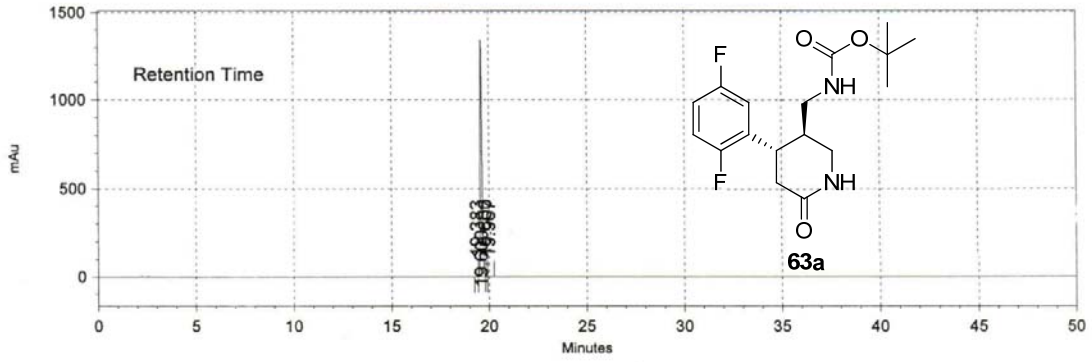
*** End of Report ***


62a


62a

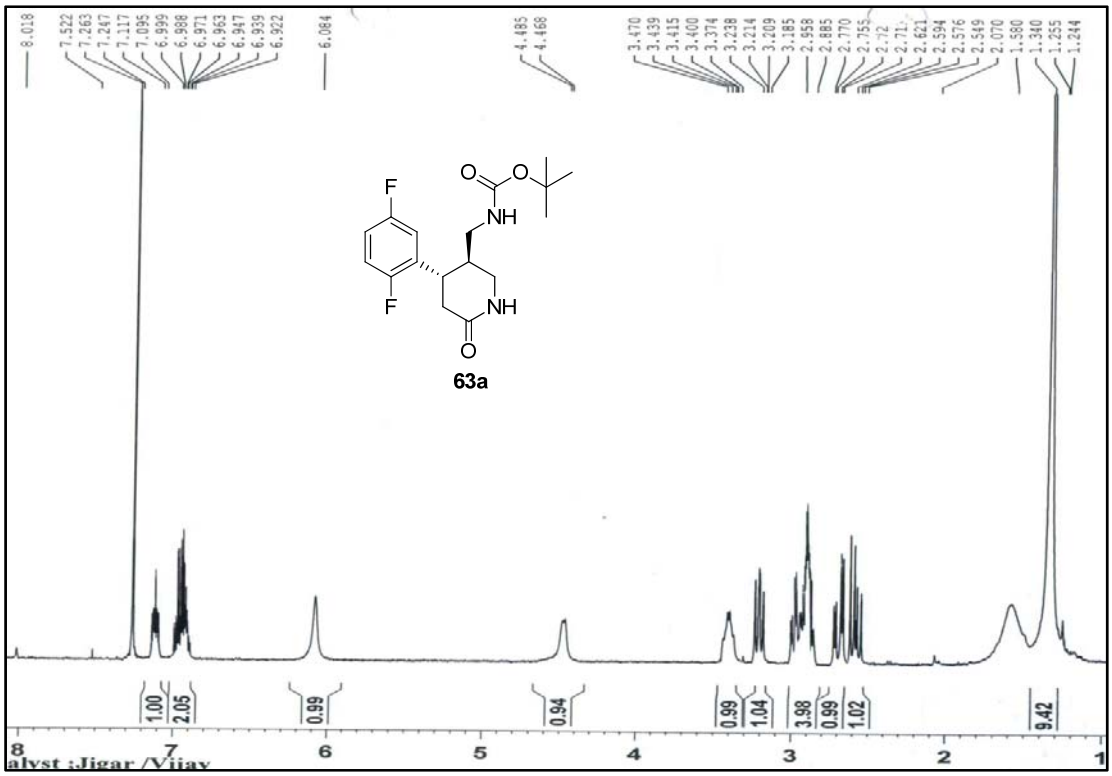


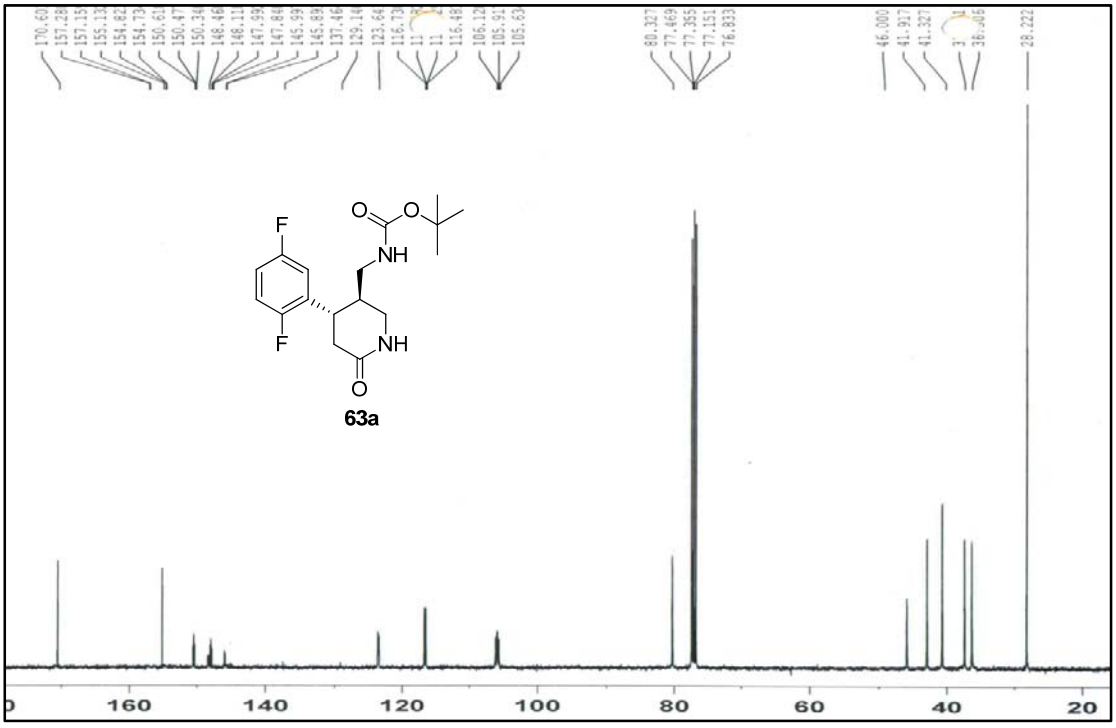
Column :YMC-ODS-AQ,[150x4.6mm],5um Injection volume:5 uL
Mobile phase :[0.1% TFA in water:ACN] (Gradient) Vial no.: 10011
Flow rate :1.0 mL/min@220nm

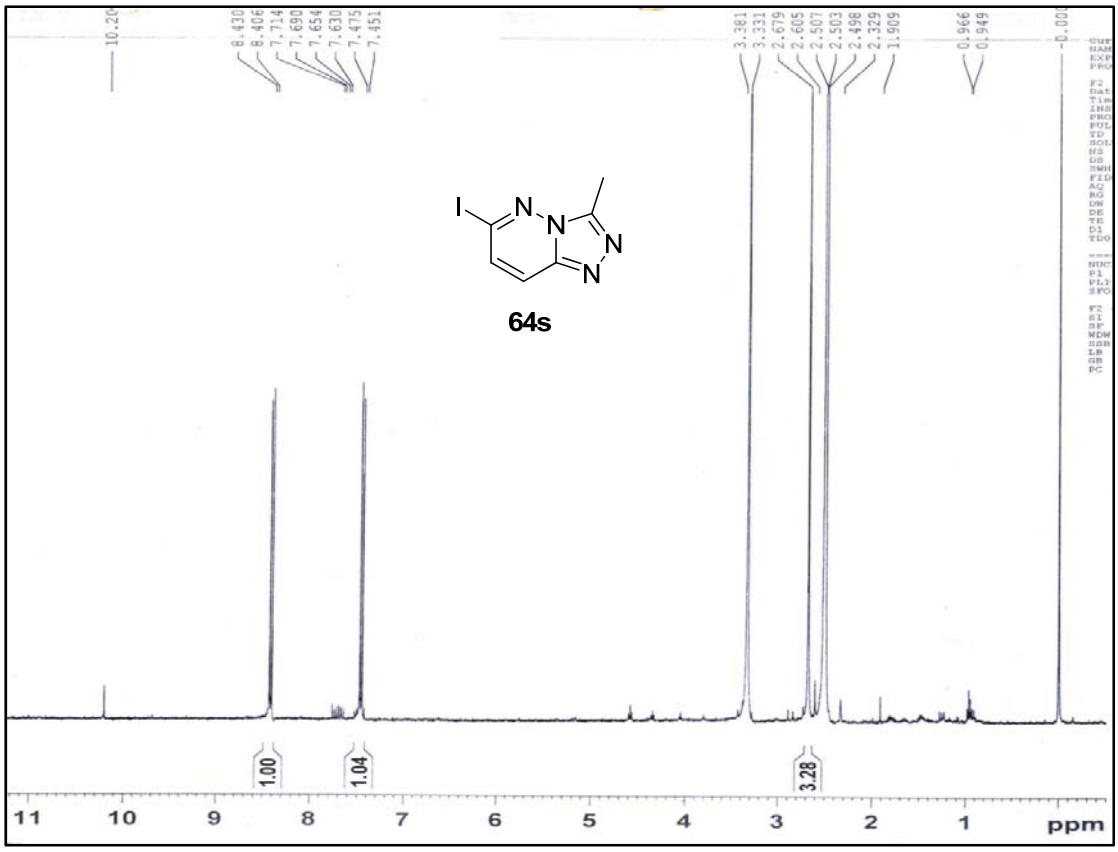


Detector A
(220nm)

Pk #	Retention Time	Area	Area Percent
1	19.383	9237	0.11
2	19.600	8633806	99.55
3	19.800	14371	0.17
4	19.967	15308	0.18
Totals		8672722	100.00


63a


63a


10.20
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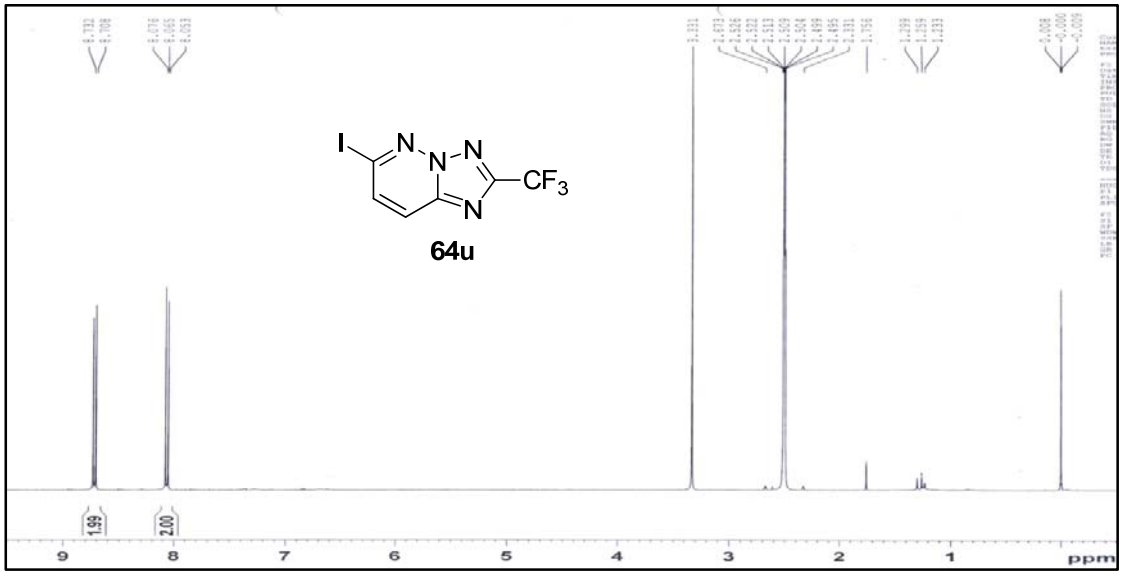
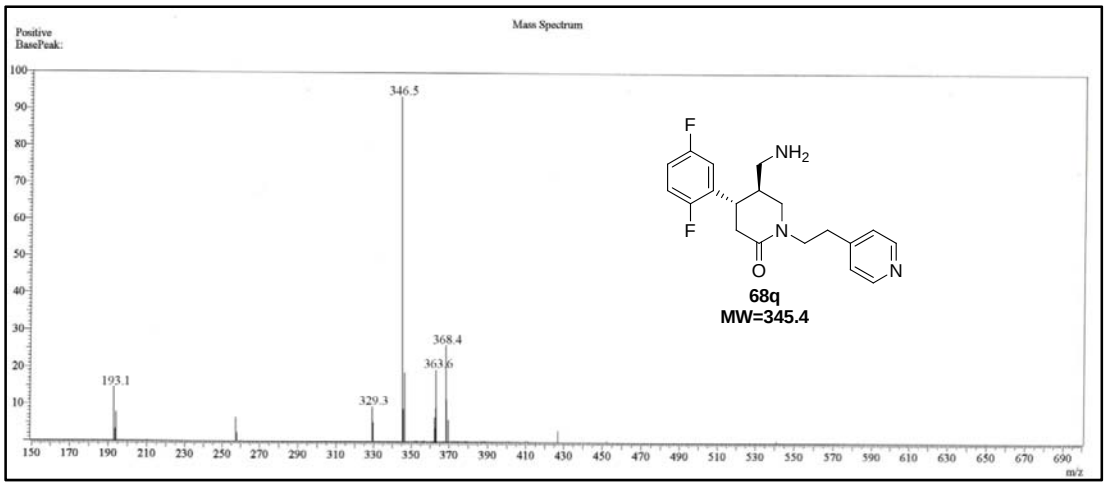
8.406
7.714
7.690
7.654
7.630
7.475
7.451
3.381
3.331
2.679
2.605
2.507
2.503
2.498
2.329
1.909
0.966
0.949
-0.000
1.00
1.04
3.28
11
10
9
8
7
6
5
4
3
2
1
ppm

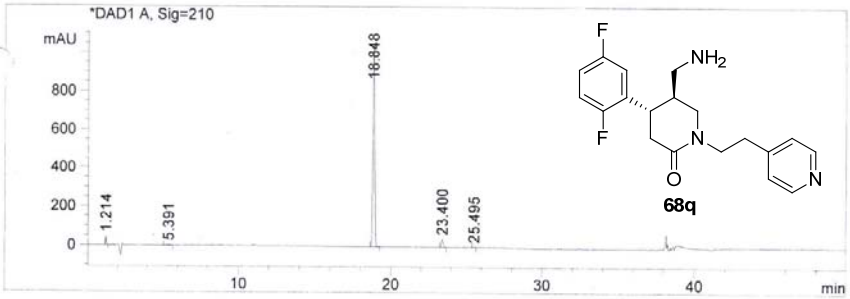
64s


1.99
2.00
9
8
7
6
5
4
3
2
1
ppm

64u



Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm



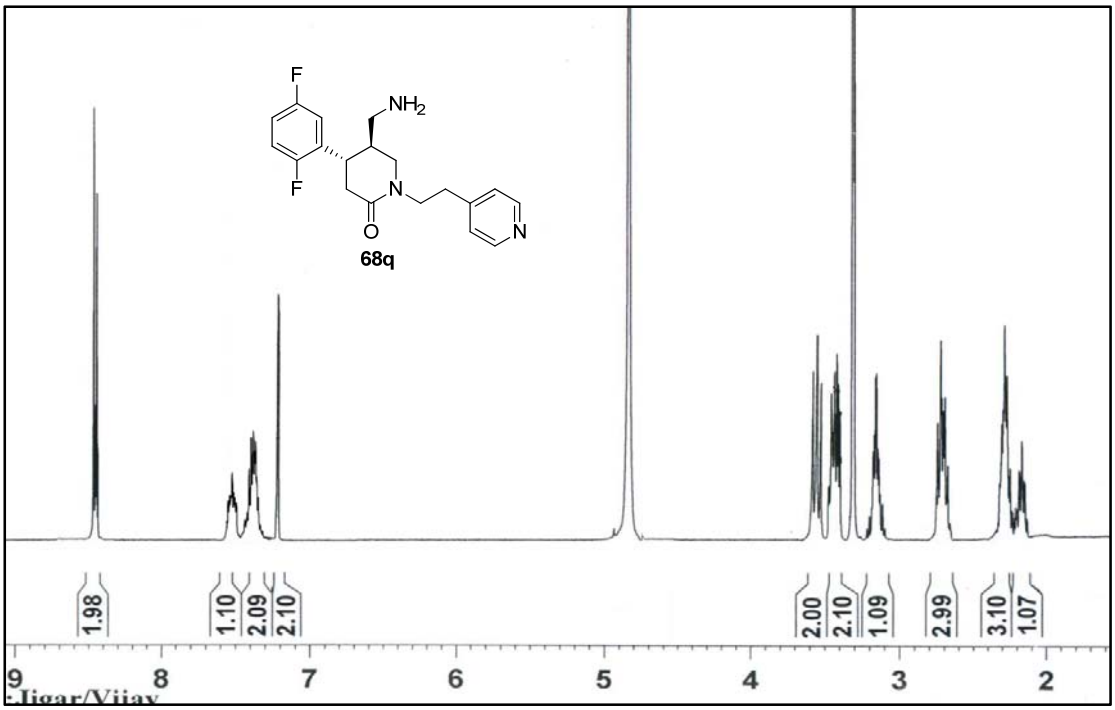
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.214	BV	0.0623	183.54565	44.37453	1.0626
2	5.391	BBA	0.2058	37.74882	2.36975	0.1136
3	18.848	BBA	0.1309	8762.86621	1055.08606	97.3294
4	23.400	BBA	0.1366	354.85754	40.36632	1.3744
5	25.495	BV	0.1228	13.09123	1.68167	0.1400
Totals :				9352.10946	1143.87833	

*** End of Report ***


F
NH2
F
N
O
N
68q
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1.10
2.09
2.10
2.00
2.10
1.09
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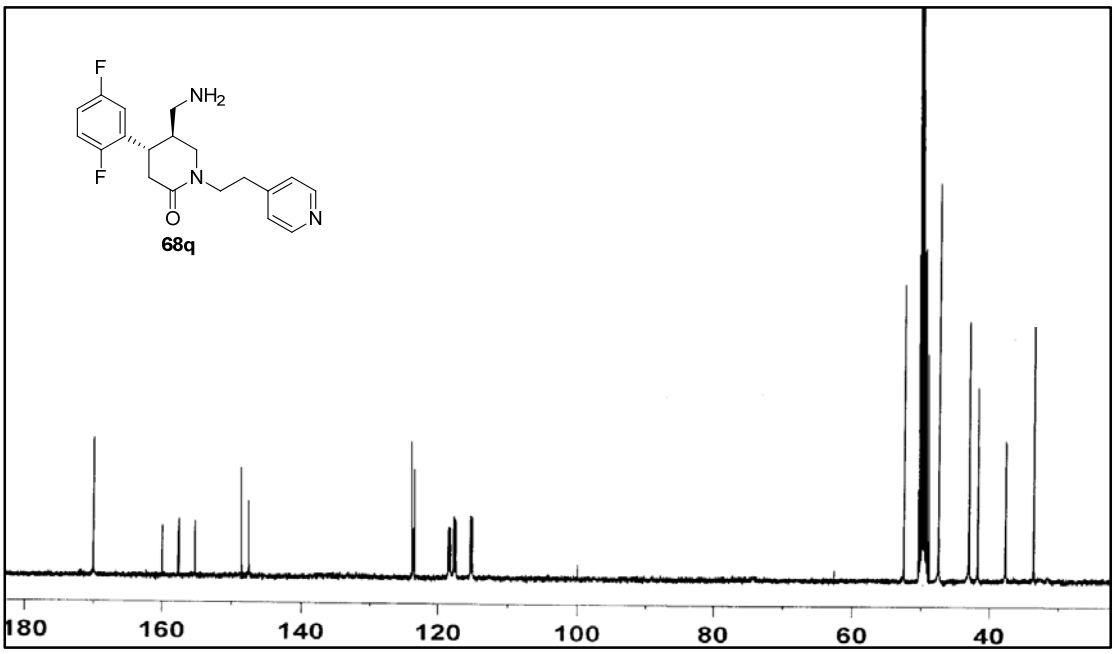
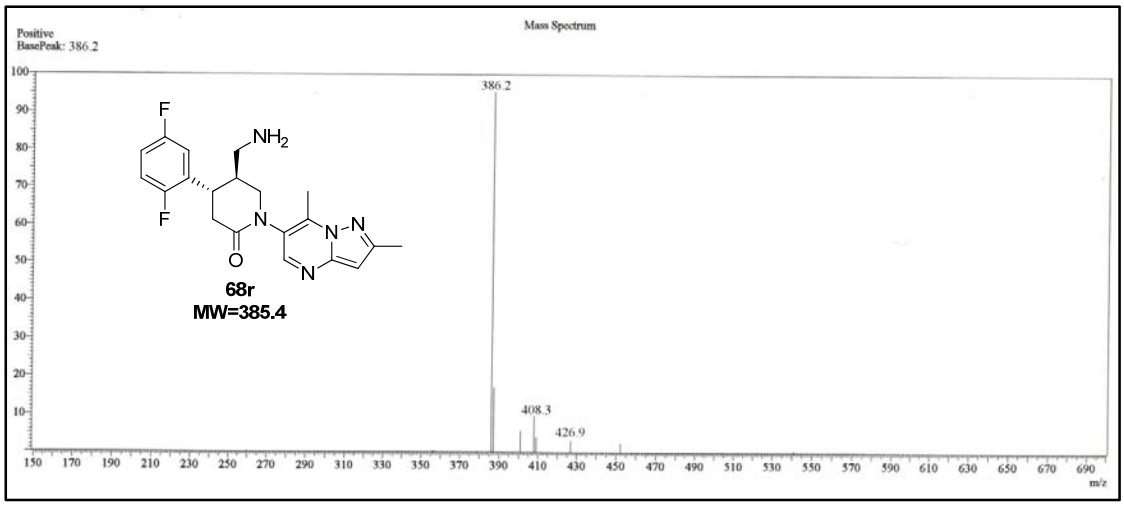
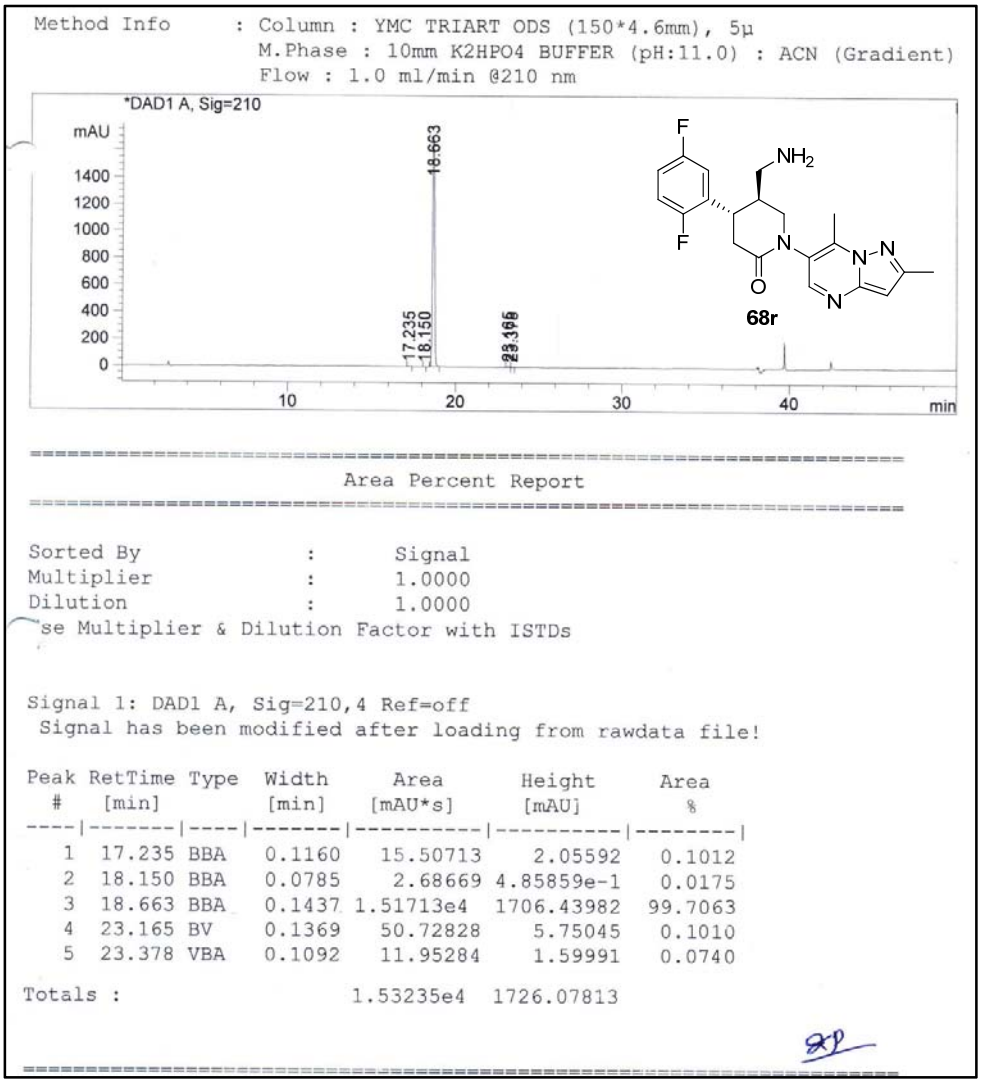
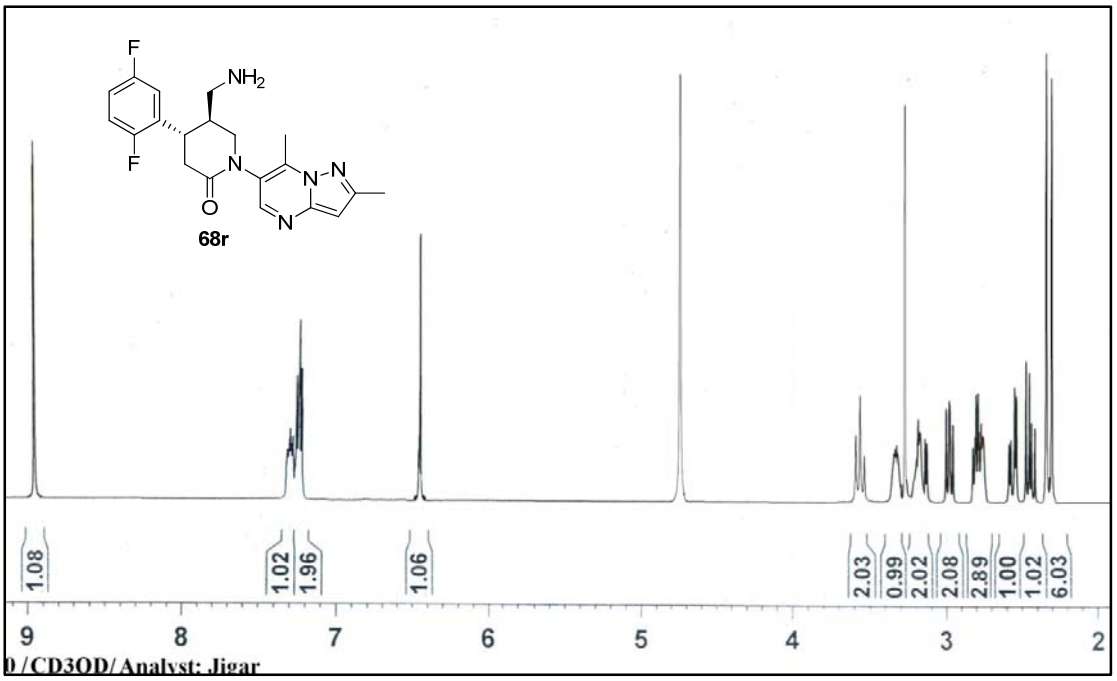
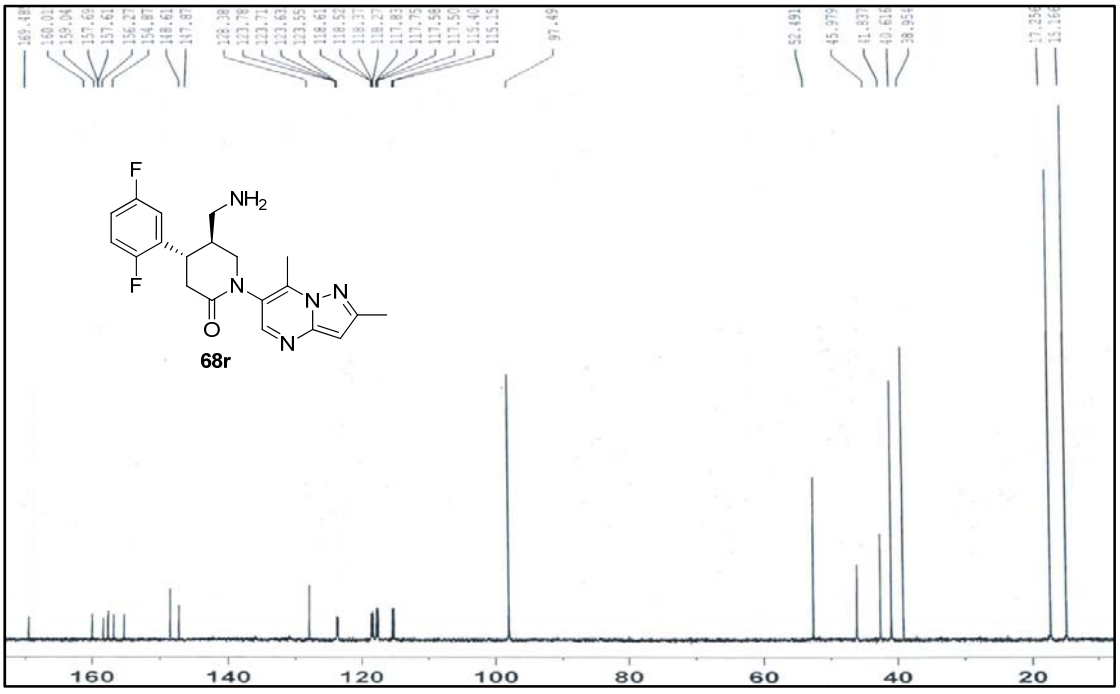
3.10
1.07
9
8
7
6
5
4
3
2

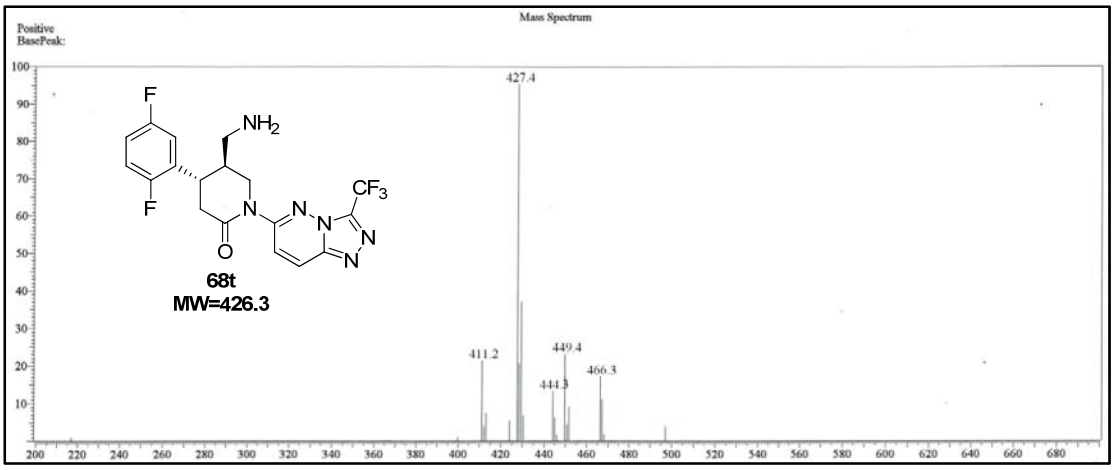

F
NH2
F
N
O
N
68q
180
160
140
120
100
80
60
40


Positive
BasePeak: 386.2
Mass Spectrum
386.2
408.3
426.9
68r
MW=385.4
m/z

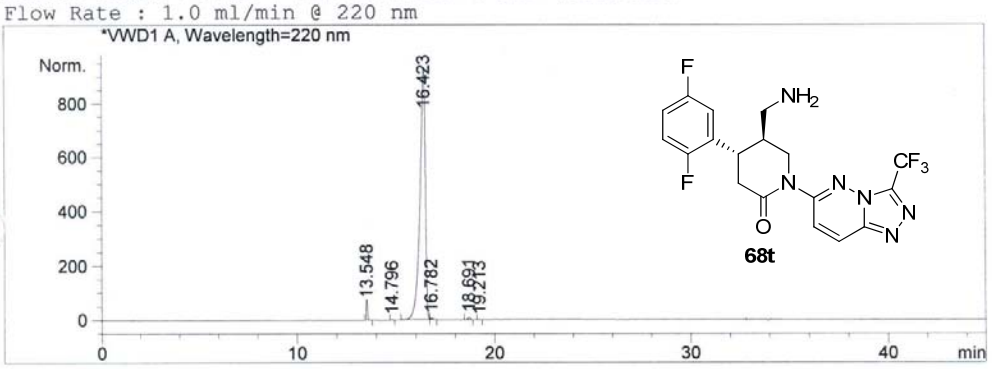

Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN (Gradient)
Flow : 1.0 ml/min @210 nm
*DAD1 A, Sig=210
mAU
18.663
17.235
18.150
68r
min
Area Percent Report
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!
Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area %
1 | 17.235 | BBA | 0.1160 | 15.50713 | 2.05592 | 0.1012
2 | 18.150 | BBA | 0.0785 | 2.68669 | 4.85859e-1 | 0.0175
3 | 18.663 | BBA | 0.1437 | 1.51713e4 | 1706.43982 | 99.7063
4 | 23.165 | BV | 0.1369 | 50.72828 | 5.75045 | 0.1010
5 | 23.378 | VBA | 0.1092 | 11.95284 | 1.59991 | 0.0740
Totals : 1.53235e4 1726.07813


F
F
NH2
N
O
N
N
N
68r
1.08
1.02
1.96
1.06
2.03
0.99
2.02
2.08
2.89
1.00
1.02
6.03
9
8
7
6
5
4
3
2
0 /CD3OD/ Analyst: Jigar


F
F
NH2
N
O
N
N
N
68r
160
140
120
100
80
60
40
20



Column : YMC J'sphere C18-(150 X4.6mm)4.0 µ
Mobile Phase : 0.05 % TFA in Water : ACN (Gradient)
Flow Rate : 1.0 ml/min @ 220 nm



Area Percent Report

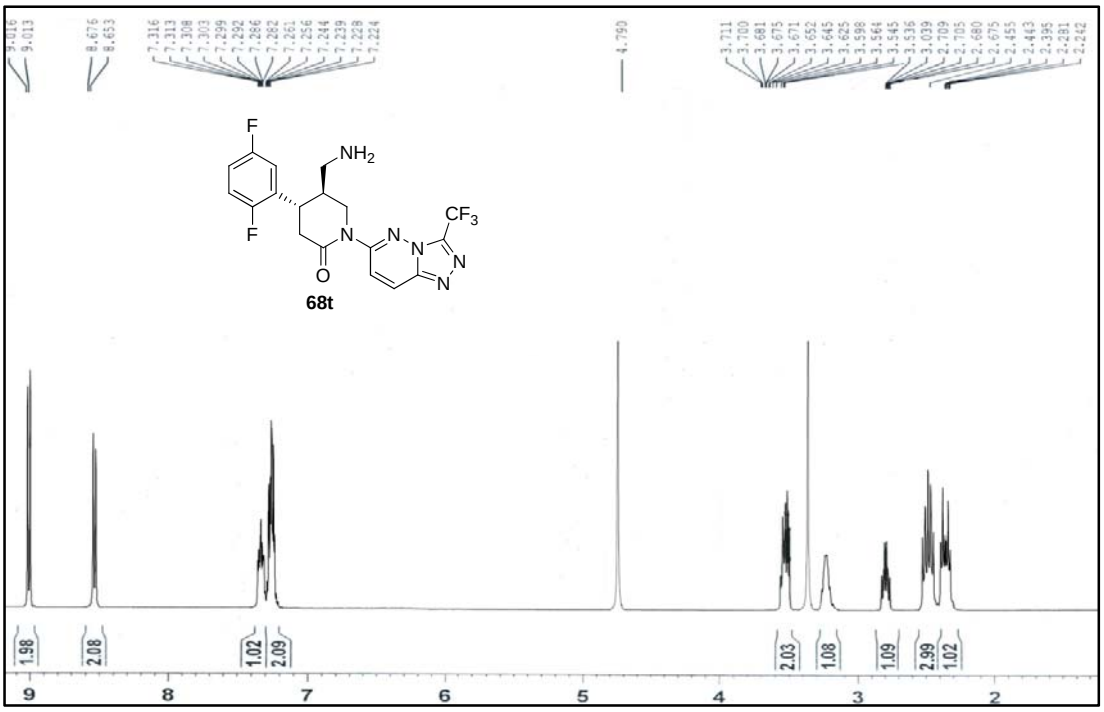
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	13.548	PBA	0.0970	780.56122	126.06636	1.2020
2	14.796	BBA	0.0945	9.28837	1.55382	0.2500
3	16.423	BV	0.2730	1.75456e4	934.05853	97.6127
4	16.782	VB	0.1181	54.77074	6.95184	0.2948
5	18.691	BBA	0.1182	77.04102	9.92417	0.4547
6	19.213	PBA	0.1027	6.99300	1.08797	0.1858
Totals :				1.85761e4	1095.38028	

Results obtained with enhanced integrator!

*** End of Report ***


F
NH2
F
CF3
N
N
N
N
N
O
68t
1.98
2.08
1.02
2.09
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1.08
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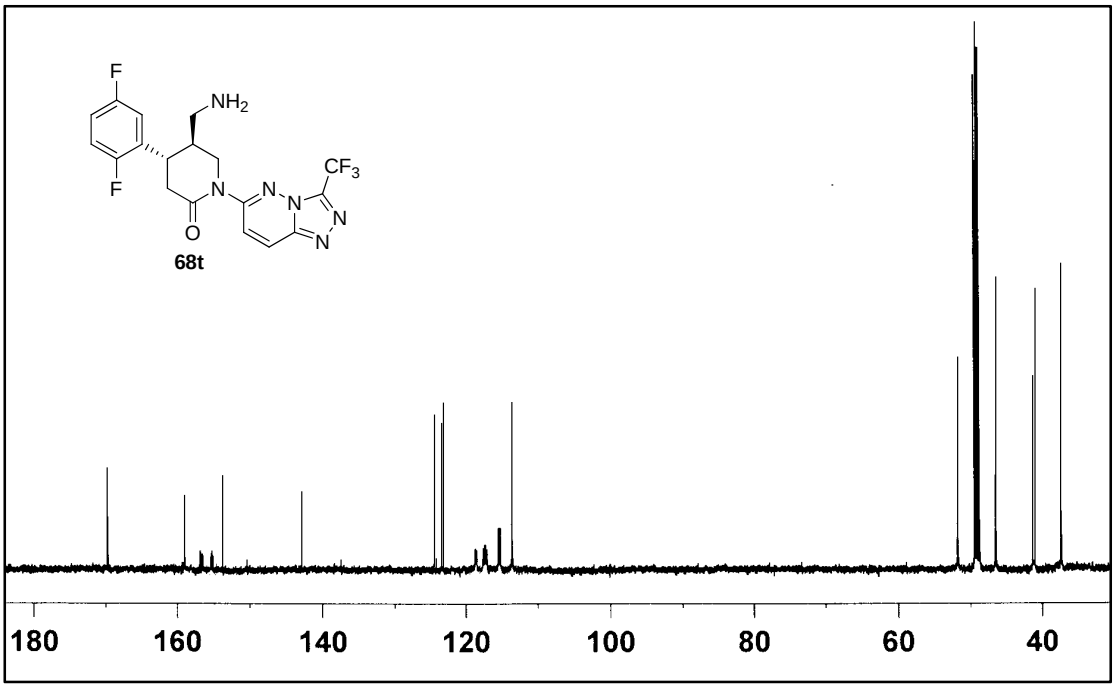
1.02
9
8
7
6
5
4
3
2


F
NH2
F
CF3
N
N
N
N
N
O
68t
180
160
140
120
100
80
60
40

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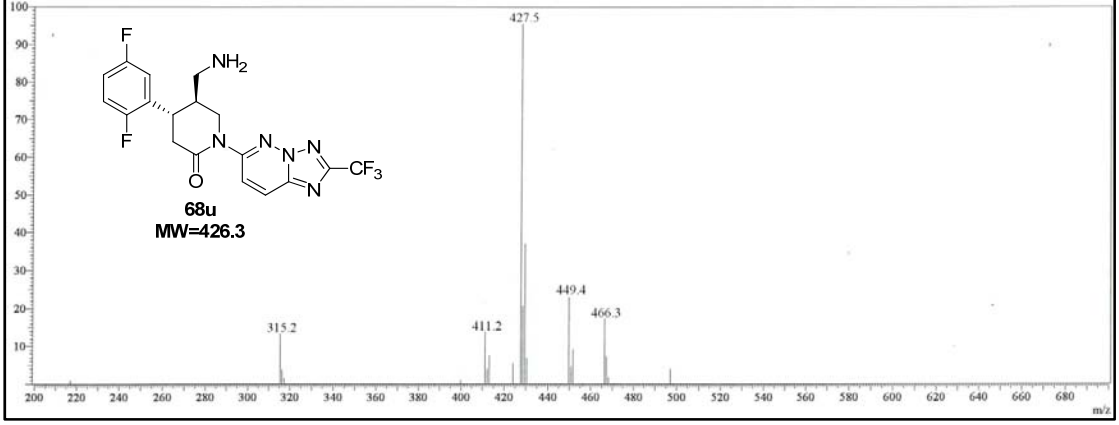
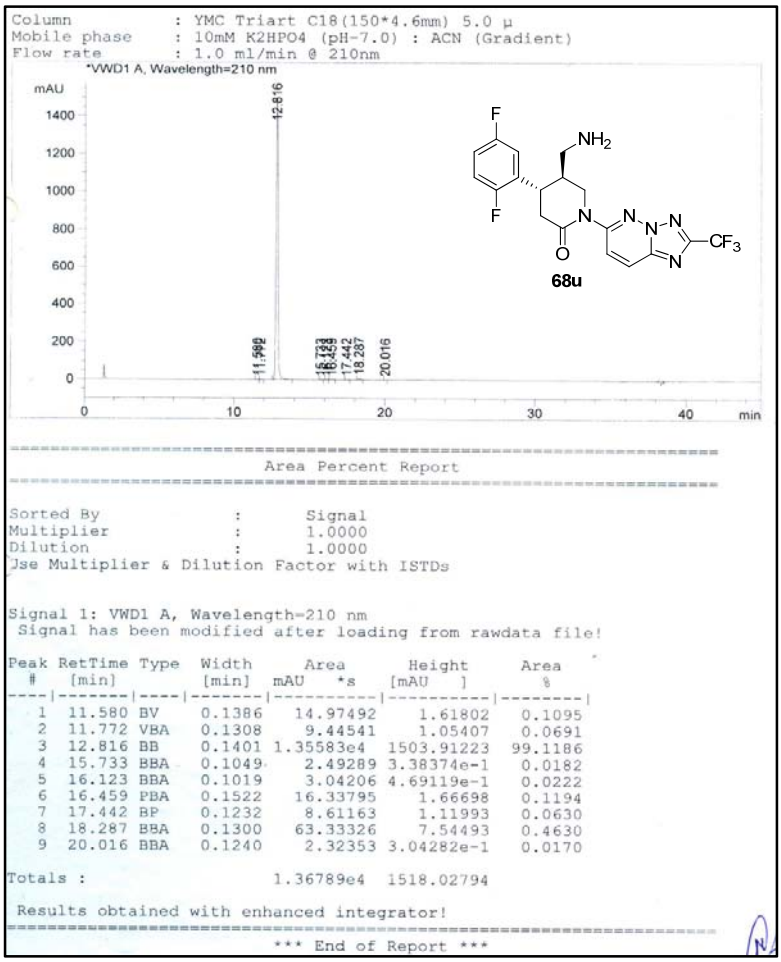
Mass Spectrum

Positive
BasePeak:



68u
MW=426.3



Area Percent Report

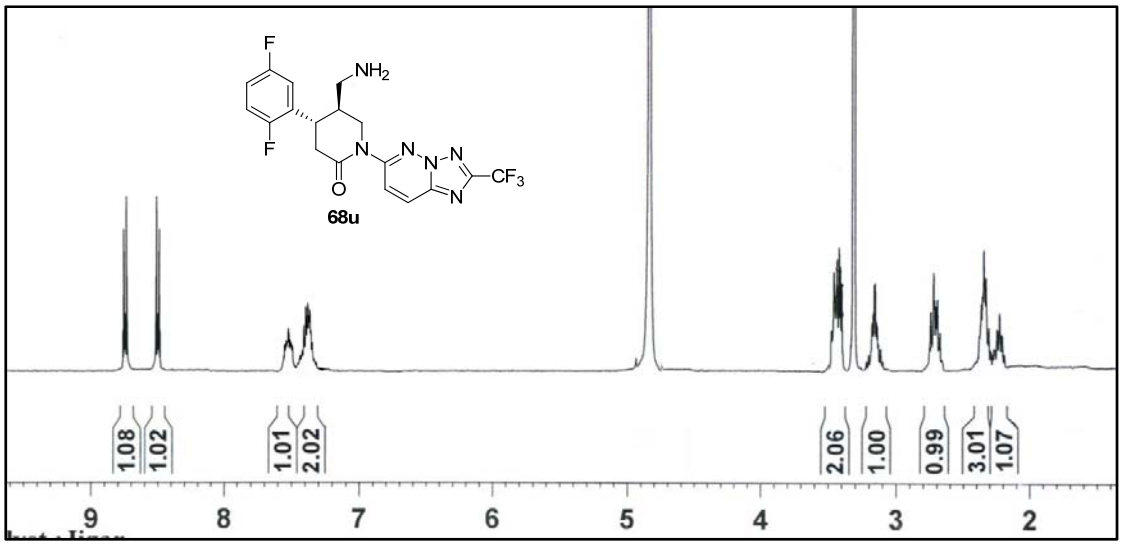
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

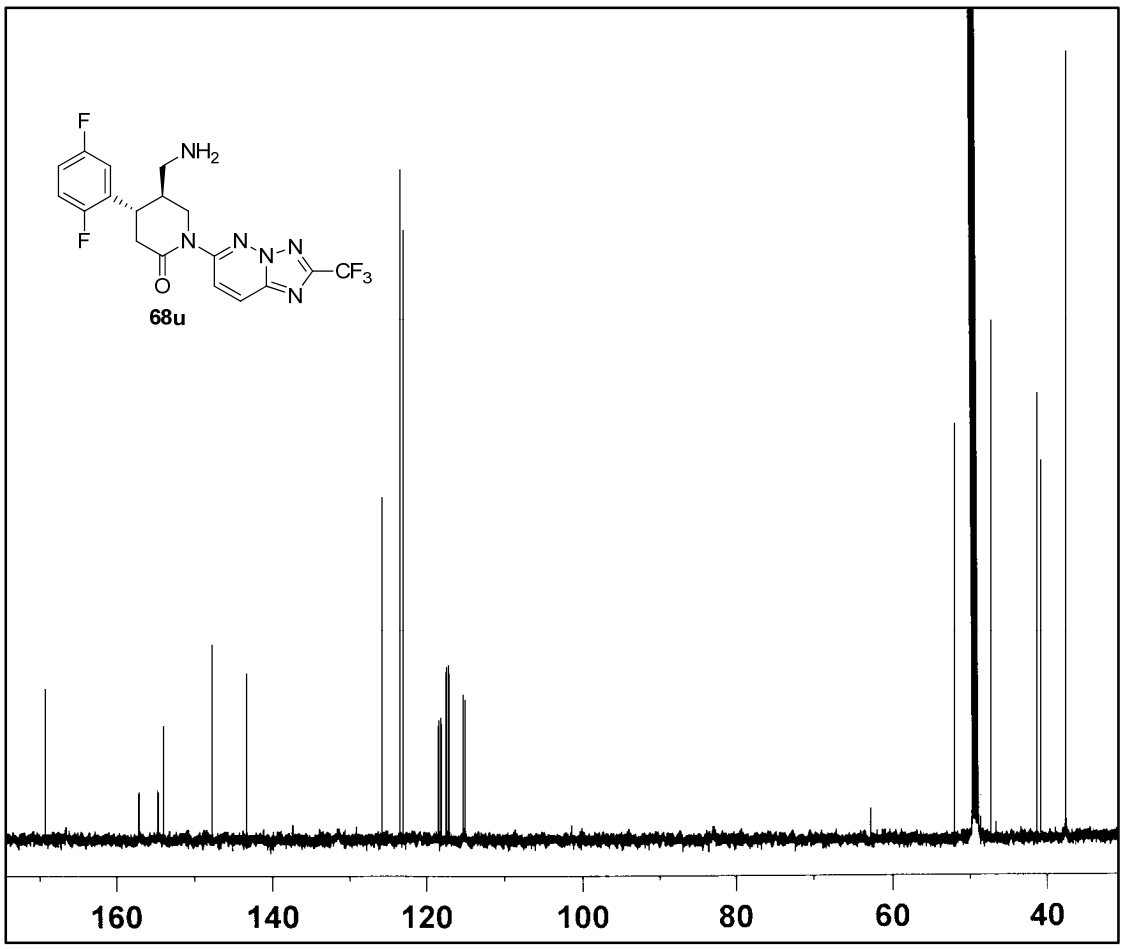
Signal 1: VWD1 A, Wavelength=210 nm
Signal has been modified after loading from rawdata file!

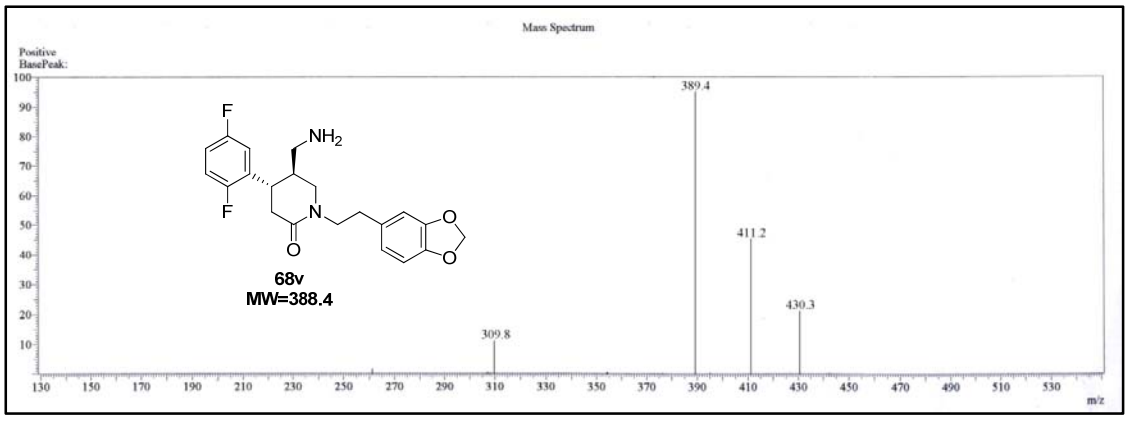
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	11.580	BV	0.1386	14.97492	1.61802	0.1095
2	11.772	VBA	0.1308	9.44541	1.05407	0.0691
3	12.816	BB	0.1401	1.35583e4	1503.91223	99.1186
4	15.733	BBA	0.1049	2.49289	3.38374e-1	0.0182
5	16.123	BBA	0.1019	3.04206	4.69119e-1	0.0222
6	16.459	PBA	0.1522	16.33795	1.66698	0.1194
7	17.442	BP	0.1232	8.61163	1.11993	0.0630
8	18.287	BBA	0.1300	63.33326	7.54493	0.4630
9	20.016	BBA	0.1240	2.32353	3.04282e-1	0.0170
Totals :				1.36789e4	1518.02794	

Results obtained with enhanced integrator!

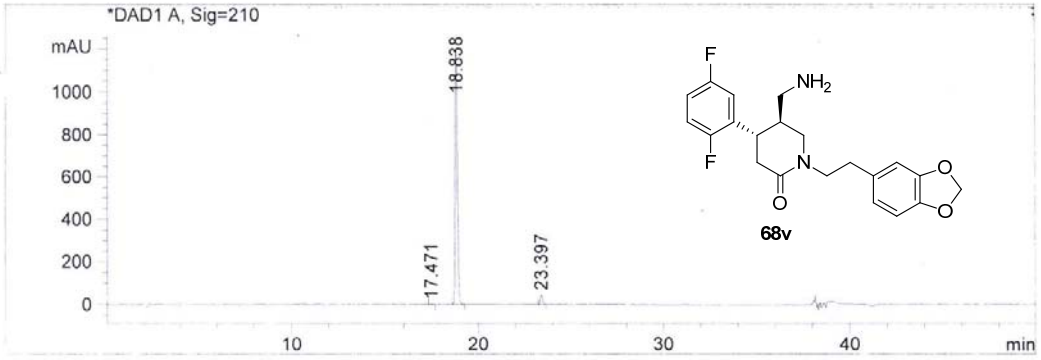
*** End of Report ***


F
NH2
F
N
N
N
N
CF3
O
N
68u
1.08
1.02
1.01
2.02
2.06
1.00
0.99
3.01
1.07
9
8
7
6
5
4
3
2


F
NH2
F
N
N
N
N
CF3
O
N
68u
160
140
120
100
80
60
40



Method Info : Column : YMC TRIART ODS (150*4.6mm), 5µ
M.Phase : 10mm K2HPO4 BUFFER (pH:11.0) : ACN
Flow : 1.0 ml/min @210 nm



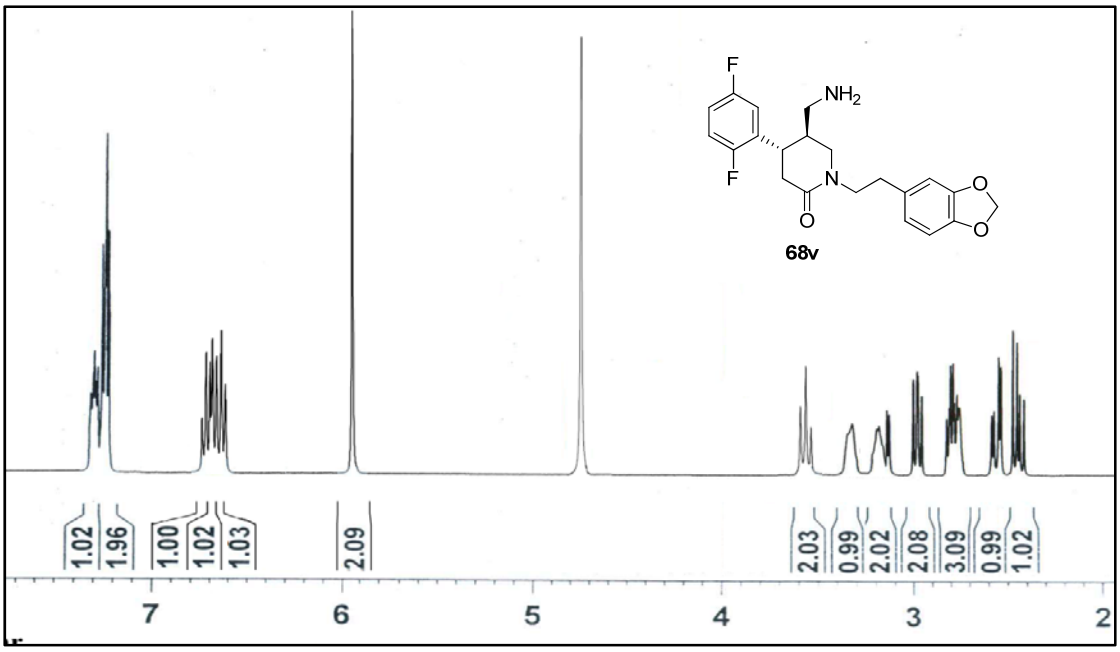
Area Percent Report

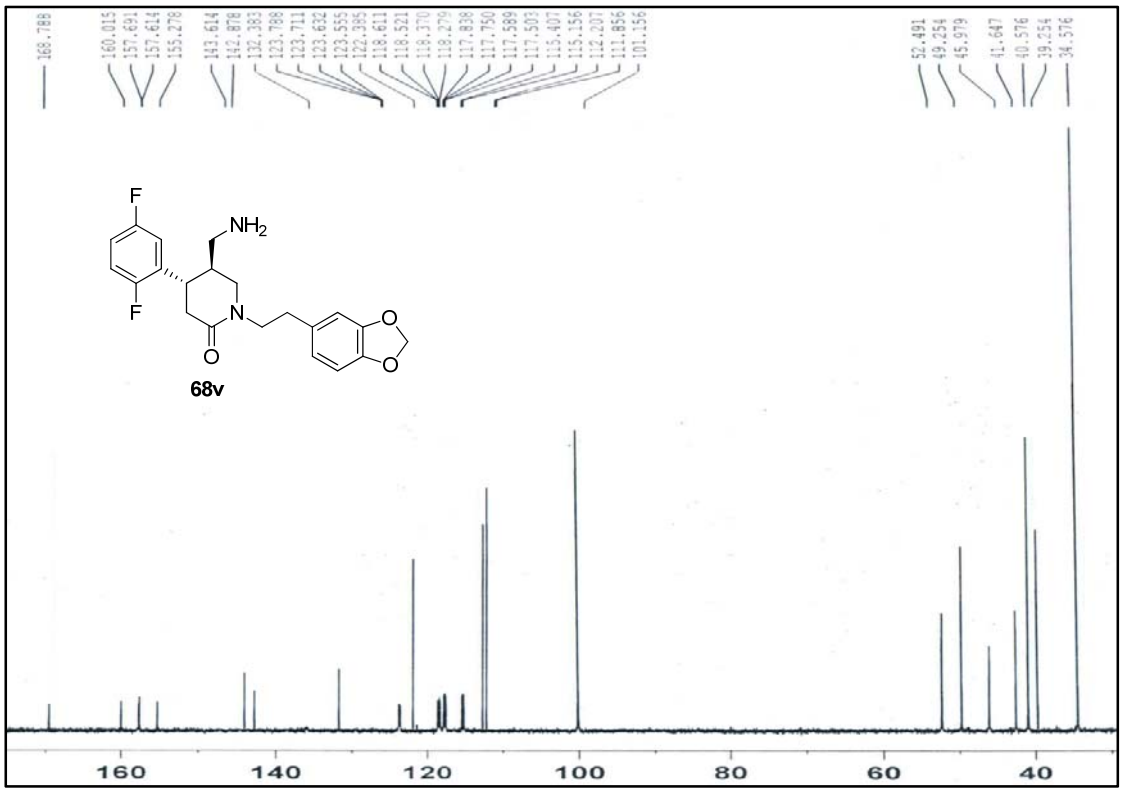
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

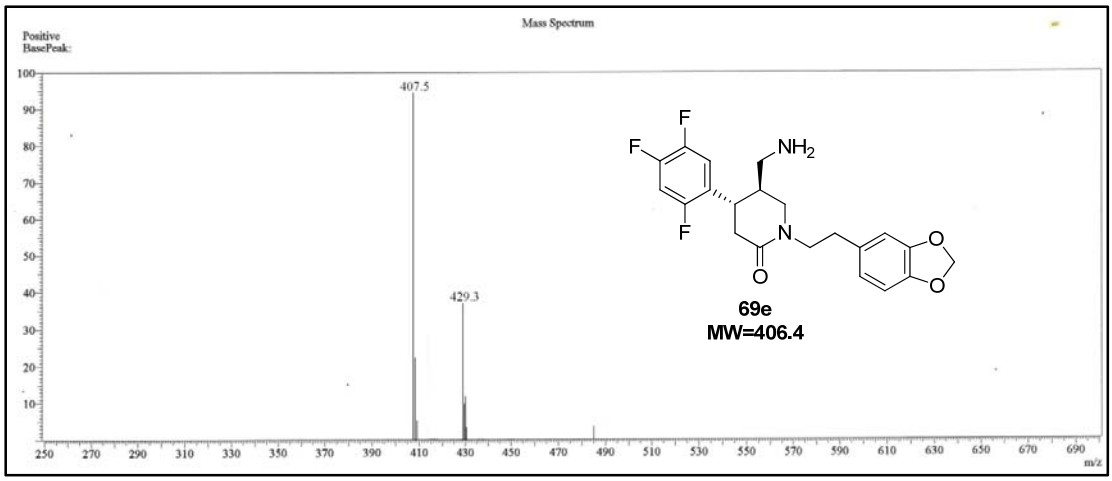
Signal 1: DAD1 A, Sig=210,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.471	BBA	0.1447	17.08266	1.76795	0.2633
2	18.838	BBA	0.1321	1.00857e4	1199.62109	99.6143
3	23.397	BBA	0.1340	358.01276	41.77011	0.1224
Totals :				1.04608e4	1243.15916	

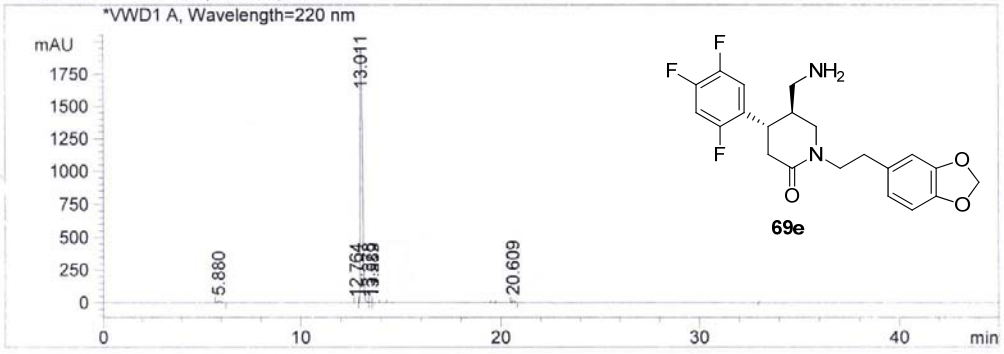
*** End of Report ***


F
NH2
F
N
O
O
O
68v
1.02
1.96
1.00
1.02
1.03
2.09
2.03
0.99
2.02
2.08
3.09
0.99
1.02
7
6
5
4
3
2


F
NH2
F
N
O
O
O
68v
160
140
120
100
80
60
40



COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	5.880	BBA	0.1602	177.15422	16.50138	1.0995
2	12.764	BV	0.1020	6.57375	9.74003e-1	0.1108
3	13.011	MF R	0.1331	1.56359e4	1957.34802	97.2023
4	13.378	FM R	0.0901	16.81635	3.11017	0.1044
5	20.609	BBA	0.1107	140.96368	19.46984	0.8749
Totals :				1.61125e4	2018.33694	

Results obtained with enhanced integrator!

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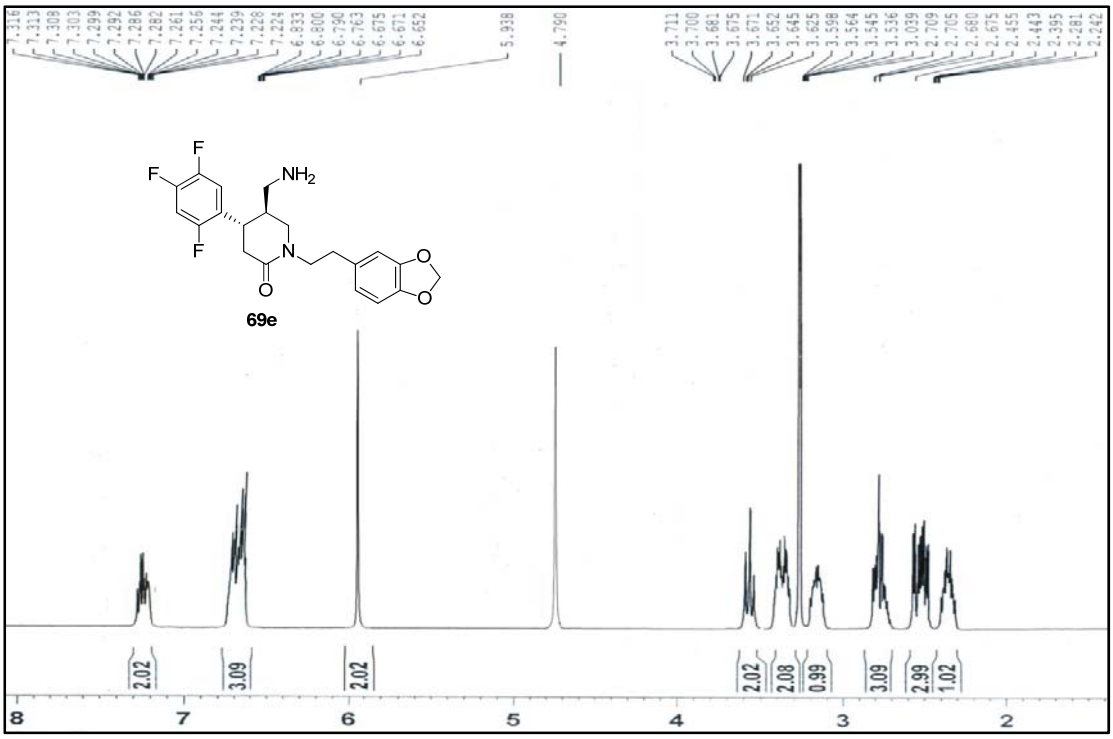
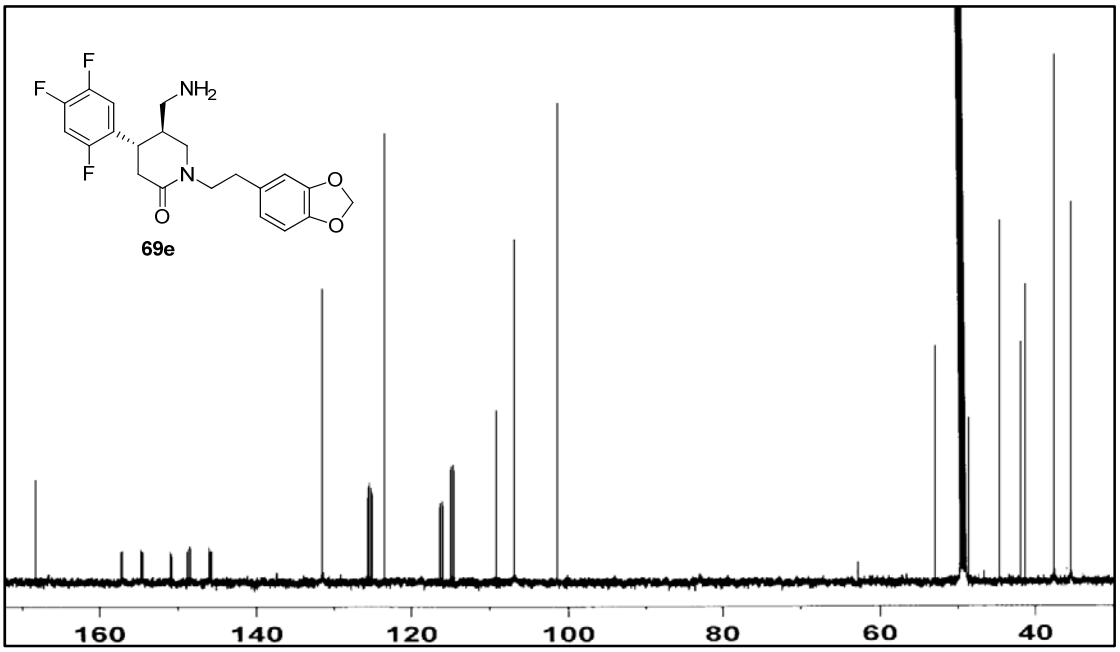
*** End of Report ***


F
F
F
NH2
N
O
O
O
69e
2.02
3.09
2.02
2.02
2.08
0.99
3.09
2.99
1.02
8
7
6
5
4
3
2


F
F
F
NH2
N
O
O
O
69e
160
140
120
100
80
60
40

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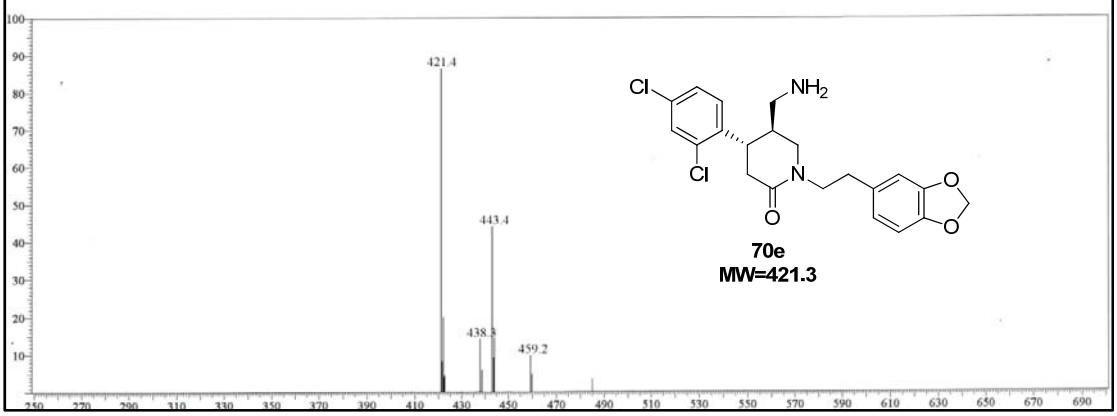
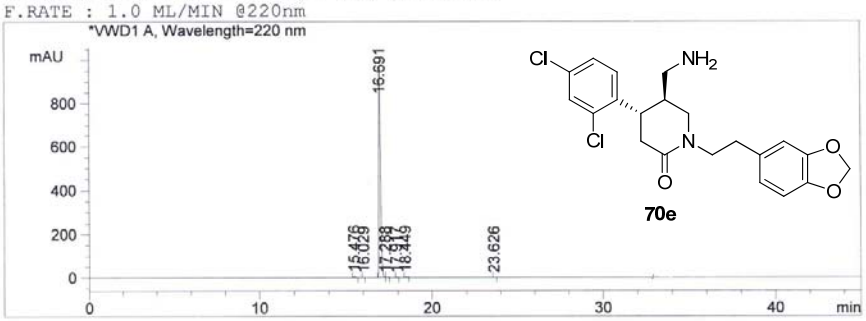
Mass Spectrum

Positive
BasePeak:



COLUMN : YMC J'SPHERE C18 (150 X4.6)4u
M.PHASE: 0.05%TFA IN WATER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN @220nm



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

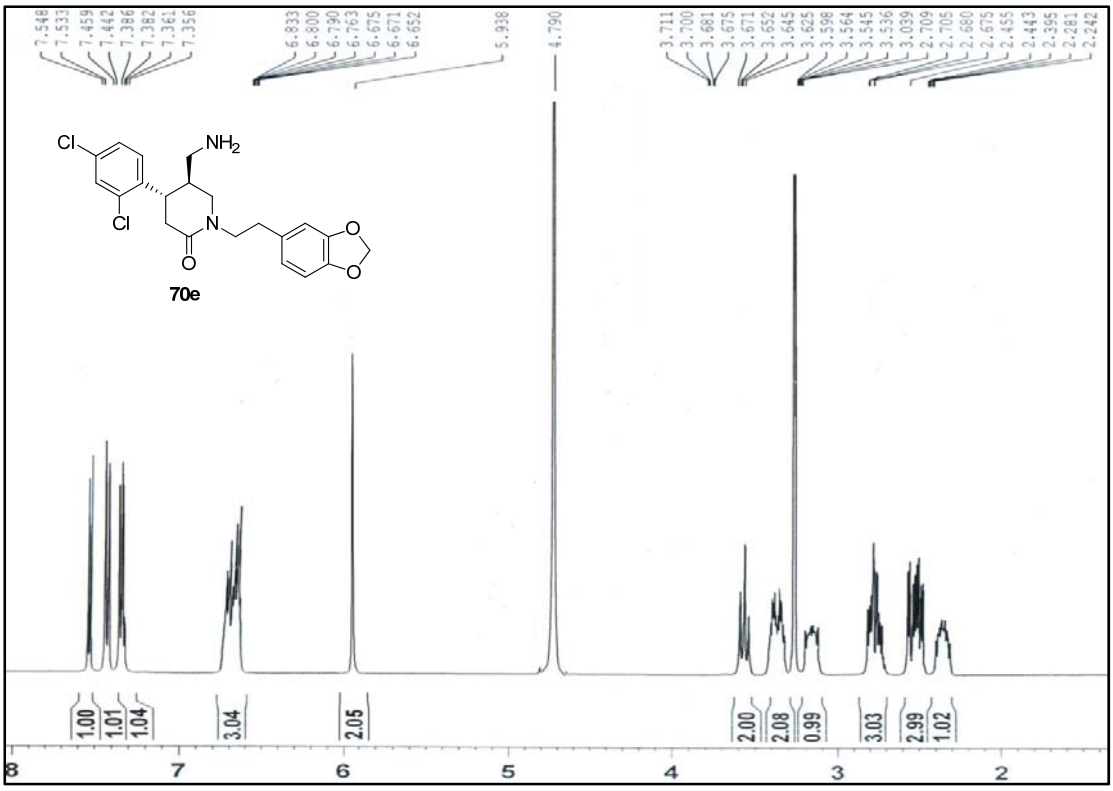
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

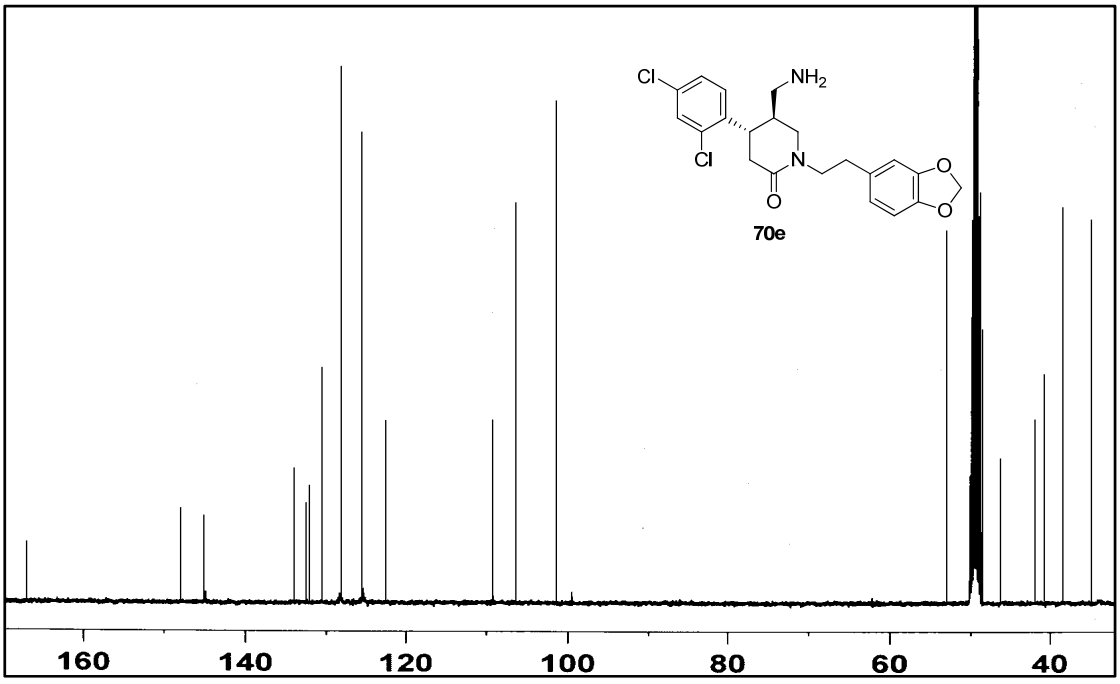
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	15.476	BBA	0.1001	39.52889	6.00477	0.9719
2	16.029	BBA	0.0742	1.08086	1.96956e-1	0.0156
3	16.691	MF R	0.1129	6838.54736	1009.79938	98.5434
4	17.288	FM R	0.0795	6.02183	1.26204	0.0871
5	17.917	MM R	0.0990	1.15465	1.94389e-1	0.0167
6	18.449	BBA	0.0991	22.77402	3.43964	0.3295
7	23.626	BBA	0.0856	2.46693	3.89283e-1	0.0357

Totals : 6911.57454 1021.28646

Results obtained with enhanced integrator!

*** End of Report ***


7.548
7.533
7.459
7.442
7.386
7.382
7.361
7.356
6.833
6.800
6.790
6.763
6.675
6.671
6.652
5.938
4.790
3.711
3.700
3.681
3.675
3.671
3.652
3.645
3.625
3.598
3.564
3.545
3.536
3.039
2.709
2.705
2.680
2.675
2.455
2.443
2.395
2.281
2.242
Cl
NH2
Cl
N
O
O
O
70e
1.00
1.01
1.04
3.04
2.05
2.00
2.08
0.99
3.03
2.99
1.02
8
7
6
5
4
3
2


Cl
NH2
Cl
N
O
O
O
70e
160
140
120
100
80
60
40

References

"I have never met a man so ignorant that I couldn't learn something from him." - Galileo Galilei

7. References

7. References

[1] James, P. T. *Clin. Dermatol.***2004**, *22*, 276.

[2] Kylin, E. *Zentralbl Inn Med.***1923**, *44*, 105.

[3] Vague, J. *Presse Medl.***1947**, *53*, 339.

[4] Avogaro, P.; Crepaldi, G. *Diabetologia***1965**, 1, 137.

[5] Reaven, G. M. *Diabetes***1988**, 37, 1595.

[6] Kaplan, N. M. *Arch. Intern. Med.***1989**, *149*, 1514.

[7] Haffner, S. M.; Valdez, R. A.; Hazuda, H. P.; Mitchell, B. D.; morales, P. A.; Stern, M. P. *Diabetes***1992**, *41*, 715.

[8] Cavanghan, M. K.; Ehrmann, D. A.; Polonsky, K. S. *J. Clin. Invest.***2000**,*160(3)*, 329.

[9] Beatrice, A-T.; Amelie, P.; Maria, P.; Julia, M.; Jieping, D.; David, M.; Stephen, H.; Bernard, M.; Lawrence, R. *Protein Expres. Purif.***2010**, *69(1)*, 1.

[10] Chehade, J.M.; Mooradian, A. D. *Drugs***2000**, *60*, 95.

[11] Burge, M. R.; Sood, V.; Sobhy, T. A.; Rassam, A. G.; Schade, D. S. *Diabetes Obes. Metab.***1999**, *1(4)*, 199.

[12] Cannon, C. P. *Am. J. Cardiol.* **2008**, *102(12)*, 5L.

[13] Cerasi, E.; Boitard, C.; Efendic, S.; Ferrannini, E.; Henquin, J. C.; Steiner, D. F. *Diabetes***2001**, *50(Suppl 1)*, S1.

[14] Butler, A. E.; Janson, J.; Bonner-Weir, S.; Ritzel, R.; Rizza, R. A.; Butler, P. C. *Diabetes***2003**, *52(1)*, 102.

[15] Thomas R Moore et al., "*Diabetes Mellitus and Pregnancy*" Med/2349 at eMedicine. Version, **2005**; Jan 27 update.

[16] Donovan et al., "*Drugs for gestational diabetes*". Australian Prescriber (33): 141.

[17] Jiang, G.; Zhang, B. B. *Am J Physiol Endocrinol Metab.***2003**, *284(4)*, E671.

[18] Tompkins, C. V.; Brandenburg, D.; Jones, R. H.; Sönksen, P. H. *Diabetologia,* **1981**, *20*, 94.

[19] Bunck, M. C.; Diamant, M.; Corner, A.; Eliasson, B.; Malloy, J. L.; Shaginian, R. M.; Deng, W.; Kendall, D. M.; Taskinen, M.-R.; Smith, U.; Yki-Jarvinen, H.; Heine, R. J. *Diabetes Care***2009**, *32(5)*, 762.

[20] Secher, A.; Jelsing, J.; Baquero, A. F.; Hecksher-Sørensen, J.; Cowley, M. A.; Dalbøge, L. S.; Hansen, G.; Grove, K. L.; Pyke, C.; Raun, K.; Schäffer, L.;

Tang-Christensen, M.; Verma, S.; Witgen, B. M.; Vrang, N.; Knudsen, L. B. *J Clin Invest.***2014**, *124*, 4473.

[21] Salle, A.; Guilloteau, G.; Ryan, M.; Bouhanick, B.; Ritz, P. *Diabetes Medicine*, **2004**, *21(12)*, 1298-1303.

[22] Ruschig, H.; Avmulle, W.; Korger, G.; Wagner, H.; Scholtz, J.; Bander, A. **US: 2976317**.

[23] Rendell, M. *MedGenMed.***2004**, *6(Suppl 3)*, 9.

[24] Maechesini, G.; Bianchi, G.; Tomassetti, S.; Zoli, M.; Melchionda, N.; *The Lancet*, **2001**, *358*, 893.

[25] Jiang, G.; Zhang, B. B. *Biochem Soc Trans.***2005**, *33*, 358.

[26] Benalla, W.; Bellahcen, S.; Bnouham, M. *Curr. Diabetes Rev.***2010**, *6(4)*, 247.

[27] Bahekar, R. H.; Lohray B. B.; Lohray V. B.; Jain, M.; Banerjee, K. M.; Patel P. R. *PCT Int. Appl.***WO: 2008/062457**.

[28] http://www.businesswire..com/news/biospace/20101130007174/en/PhaseBio-Lunches-Clinical-Program-Test-Lead-Product.

[29] http://www.novonordisk.com/science/pipeline/rd_pipeline.asp.

[30] Oxxon pipeline summary. http://www/noxxon.com/downloads/FactSheet.pdf.

[31] Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.***2005**, *48*, 141.

[32] Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.***2003**, *46*, 2774.

[33] Ristic, S.; Byiers, S.; Foley, J.; Holmes, D. *Diabetes Obes. Metab.* **2005**, *7*, 692.

[34] Ahren, B.; Gomis, R.; Standl, E.; Mills, D.; Schweizer, A. *Diabetes Care***2004**, *27*, 2874.

[35] Erhardt, W.; Bergenheim, K.; Duprat-Lomon, I.; McEwan, P. *Clinical Drug Investigation***2012**, *32(3)*, 189.

[36] Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.***2005**, *48*, 5025.

[37] AstraZeneca pipeline summary. AstraZeneca plc, Cheshire, UK, 2008.

http://www.astrazeneca.com/article/511390.aspx.

[38] Li, J.; Zhang, S.; Zhou, H.; Peng, J.; Feng, Y.; Hu, X. *Lett. Org. Chem.*, **2012**, *9(5)*, 347.

[39] Eckhardt, M.; Langkopf, E.; Mark, M.; Tadayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F. *J. Med. Chem***2007**, *50*, 6450.

[40] Schernthaner, G.; Barnett, A. H.; Emser, A.; Patel, S.; Troost, J.; Woerle, H.-J.; Eynatten, M.von. *Diabetes Obesity and Metabolism***2012**, *14(5)*, 470.

[41] Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L. *J Med Chem***2007**, *50*, 2297.

[42] Kato, N.; Oka, M.; Murase, T.; Yoshida, M.; Sakairi, M.; Yamashita, S.; Yasuda, Y.; Yoshikawa, A.; Hayashi, Y.; Makino, M.; Takeda, M.; Mirensha, Y.; Kakigami, T. *Bioorg. Med. Chem.***2011**, *19(23)*, 7221.

[43] Furuta, S.; Smart, C.; Hackett, A. et al. *Xenobiotica***2013**, *43*, 432.

[44] Kim, S, H.; Lee, S. H.; Yim, H, J. *Arch Pharm Res.***2013**, *36*, 1185.

[45] Rhee, E. J.; Lee, W. Y.; Min, K. W.; Shivane, V. K.; Sosale, A. R.; Jang, H. C.; Chung, C. H.; Nam-Goong, I. S.; Kim, J. A.; Kim, S. W.; Gemigliptin Study 006 Group. *Diabetes Obes Metab.***2013**, *15*, 523.

[46] Yoshida, T.; Akahoshi, F.; Sakashita, H.; Kitajima, H.; Nakamura, M.; Sonda, S.; Takeuchi, M.; Tanaka, Y.; Ueda, N.; Sekiguchi, S.; Ishige, T.; Shima, K.; Nabeno, M.; Abe, Y.; Anabuki, J.; Soejima, A. et al. *Bioorg. Med. Chem.***2012**, *20*, 5705.

[47] Fukuda-Tsuru, S.; Anabuki, J.; Abe, Y. et al. *Eur. J. Pharmacol*. **2012**, *696*, 194.

[48] Eto, T.; Inoue, S.; Kadowaki, T. *Diabetes Obes Metab.***2012**,*14*, 1040.

[49] Dhuppad, U.; Aravat, V.; Navhat, S.; Thomas, A.; Kadam, S. **US: 2010/0173964**.

[50] Ammirati,M. J.; Andrews, K. M.; Boyer,D. D.; Brodeur,A. M.; Danley,D. E.; Doran,S. D.; Hulin,B.; Liu,S. P.; McPherson,R. K.; Orena,S. J.; Parker,J. C.; Polivkova,J.; Qiu,X. Y.; Soglia,C. B.; Treadway,J. L.; . VanVolkenburg,M. A.; Wilder, D. C.; Piotrowski,D. W. *Bioorg. Med. Chem. Lett.,* **2009,** *19*, 1991.

[51] Sharma, R.; Sun, H.; Piotrowski, D. W.; Ryder, T. F.; Doran, S. D.; Dai, H.; Prakash, C. *Drug Metab Dispos***2012**, *40*, 2143.

[52] Dai, H.; Johanson, S. L.; Terra, S. G.; Marbury, T. C.; Smith, W. B.; Alcorn, H.; Boyd, R. A.; Wang, R.; Nguyen, T. T. *Br. J. Clin. Pharmacol.***2011**, *72(1)*, 85.

[53] http://www.furiex.com/pipeline/discoverydevelopment-pipeline/syr-472/

[54] Sanford, D. G. et al: American Diabetes Association Chicago. **2007**, Abstract Number: 604-P

[55] http://www.drugs.com/clinical_trials/arisaph-pharmaceuticals-announces-ada-potent-dpp-4-inhibitor-ari-2243-possesses-additional-4697.html

[56] Biftu, T.; Sinha-Roy, R.; Chen, P.; Qian, X.; Feng, D.; Kuethe, J. T.; Cox, J.; Hicks, J.; Lyons, K.; He, H.; Salituro, G.; Tong, S.; Patel, S.; Doss, G.; Petrov, A.; Wu, J.; Sherrie Xu, S.; Sewall, C.; Zhang, X.; Zhang, B.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.***2014**, *57*, 3205.

[57] Kim, H. J.; Kwak, W. Y.; Min, J. P.; Lee, J. Y.; Yoon, T. H.; Kim, A. D.; Shin, J. Y.; Kim, M. K.; Choi, S. H.; Kim, H. S.; Yang, E. K.; Cheong, Y. H.; Chae, Y. N.; Park, K. J.; Jang, J. M.; Choi, S. J.; Son, M. H.; Kim, S. H.; Yoo, M.; Lee, B. J. *Bioorg. Med. Chem. Lett.***2011**, *21(12)*, 3809.

[58] Cho, J. M.; Jang, H. W.; Cheon, H.; Jeong, Y. T.; Kim, D.-H.; Lim, Y.-M.; Choi, S.-H.; Yang, E.-K.; Shin, C.-Y.; Son, M. H.; Kim, S. H.; Kim, H.-J.; Lee, M.-S. *Diabetes Res Clin Pract.***2011**, *91(1)*, 72.

[59] Erion, M. D.; van Poelje, P. D.; Dang, Q.; Kasibhatla, S. R.; Potter, S. C.; Reddy, M. R.; Reddy, K. R.; Jiang, T.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U. S. A.***2005**, *102(22)*, 7970.

[60] DiaMedica pipeline summary. http://diamedica.com/sites/default/files/April-2001-Fact-Sheet.pdf.

[61] DiaMedica pipeline summary.http://diamedica.com/technology/drug-pipeline.

[62] Li, J. J.; Hamann, L. G.; Runa, Z.; Copper, C. B.; Wu, S. C.; Simpkins, L. M.; Wang, H.; Nayeem, A.; Krystek, S. R. **US: 2011/0077395**.

[63] Arbeeny, C. M.; *Obes. Res.***2004**, *12(8)*, 1191.

[64] Courtney, R.; Stewart, P. M.; Toh, M.; Ndongo, M. N.; Calle, R. A.; Hirshberg, B. *J. Clin. Endocrinol. Metab.***2008**, *93(2)*, 550.

[65] Xu, Z.; Tice, C. M.; Zhao, W.; Cacatian, S.; Ye, Y. J.; Singh, S. B.; Lindblom, P.; McKeever, B. M.; Krosky, P. M.; Kruk, B. A.; Berbaum, J.; Harrison, R. K.; Johnson, J. A>; Bukhtiyarov, Y.; Panemangalore, R.; Scott, B. B.; Zhao, Y.; Bruno, J. G.; Togias, J.; Guo, J.; Carroll, P. J.; McGeehan, G. M.; Zhuang, L.; He, W.; Claremon, D. A. *J. Med. Chem.***2011**, *54(17)*, 6050.

[66] Tiwari, A. *IDrugs.***2010**, *13(4)*, 266.

[67] Harno, E.; White, A. *Trends Endocrinol. Metab.***2010**, *21(10)*, 619.

[68] Scott, J. S.; Bowker, S. S.; Deschoolmeester, J.; Gerhardt, S.; Hargreaves, D.; Kilgour, E.; Lloyd, A.; Mayers, R. M.; McCoull,, W.; Newcombe, N. J.; Oqq, D.; Packer, M. J.; Rees, A.; Revill, J.; Schofield, P.; Selmi, N.; Swales, J.

G. Whittamore, P. R. *J. Med. Chem.***2012**, *55(12),* 5951.

[69] Veniant, M. M.; Hale, C.; Hungate, R. W.; Gahm, K.; Emery, M. G.; Jona, J.; Joseph, S.; Adams, J.; Hague, A.; Moniz, G.; Zhang, J.; Bartberger, M. D.; Li, V.; Syed, R.; Jordan, S.; Komorowski, R.; Chen, M. M.; Cupples, R.; Kim, K. W.; St Jean, D. J.; Jr. Johansson, L.; Henriksson, M. A.; Williams, M.; Vallgarda, J.; Fotsch, C.; Wang, M. *J. Med. Chem.***2010**, *53(11)*, 4481.

[70] Mitsubishi Tanabe Pharma pipeline summary.http://www.mt-pharma.co.jp/e/ir/meeting/pdf/e_presen120131_K.pdf.

[71] Meng, W.; Ellsworth, B. A.; Nirschl, A. A.; McCann, P. J.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P. M.; Morrison, E. P.; Biller, S. A.; Zahler, R.; Deshpande, P. P.; Pullockaran, A.; Hagan, D. L.; Morgan, N.; Taylor, J. R.; Obermeier, M. T.; Humphreys, W. G.; Khanna, A.; Discenza, L.; Roberston, J. G.; Wang, A.; Han, S.; Wetterau, J. R.; janovitz, E. B.; Flint, O. P.; Whaley, J. M.; Washburn, W. N. *J. Med. Chem.***2008**, *51(5)*, 1145.

[72] Weber, D.; Fiedler, T.; Filser, C.; Hamm, R.; Oplich, S.; Post, M.; Renner, S.; Wang, X.-J.; Wirth, T. *PCT Int. Appl.***WO: 2011/039108**.

[73] Wolfram, E. *PCT Int. Appl.***WO: 2010/092124**.

[74] Murakata, M.; Ikeda, T.; Kimura, N.; Kawase, A.; Nagase, M.; Yamamoto, K.; Takata, N.; Yoshizaki, S.; Takano, K. *PCT Int. Appl.***WO: 2009/154276**.

[75] Dobbins, R. L.; O'Connor-Semmes, R.; Kapur, A.; Kapitza, C.; Golor, G.; Mikoshiba, I.; Tao, W.; Hussey, E. K. *Diabetes Obes. Metab.***2012**, *14(1)*, 15.

[76] Green, J. T.; Wilkison, W. O. *PCT Int. Appl.***WO: 2012/006398**.

[77] Patterson, D. E.; Mcclure, M. S.; Powers, J. D. Crawford, C. F.; Berry, M. B. *PCT Int. Appl.***WO: 2010/127067**.

[78] Kasai, K.; Kobayashi, M.; Ozawa, T.; Nishimura, M.; Tashino, Y. **JP: 2011/201871**.

[79] Kotobuki Pharmaceutical pipeline summary. http://www.astellas.com/en/ir/library/pdf/2011_rd_en.pdf.

[80] Yamamoto, K.; Uchida, S.; Kitano, K.; Fukuhara, N.; Okumura-Kitalima, L.; Gunji, E.; Kozakai, A.; Tomoike, H.; Kojima, N.; Asami, J.; Toyoda, H.; Arai, M.; Takahashi, T.; Takahashi, K. *Br. J. Pharmacol.***2011**, *164(1)*, 181.

[81] Kakinuma, H.; Oi, T.; Hashimoto-Tsuchiya, Y.; Arai, M.; Kawakita, Y.; Fukasawa, Y.; Lida, I.; Hagima, N.; Takeuchi, H.; Chino, Y.; Asami, J.; Okumura-Kitajima, L.; Lo, F.; Yamamoto, D.; Miyata, N.; Takahashi, T.; Uchida, S.; Yamamoto, K. *J. Med. Chem.***2010**, *53(8)*, 3247.

[82] Sirona Biochem pipeline summary. http://www.sironabiochem.com/products/pipeline/; BIO 2011.

[83] Theracos pipeline summary. http://clinicaltrials.gov/ct2/show/NCT01029704.

[84] Kalgutkar, A. S.; Tugnait, M.; Zhu, T.; Kimoto, E.; Miao, Z.; Mascitti, V.; Yang, X.; Tan, B.; Walsky, R. L.; Chupka, J.; Feng, B.; Robinson, R. P. *Drug Metab. Dispos.***2011**, *39(9)*, 1609.

[85] Mascitti, V.; Maurer, T. S.; Robinson, R. P.; Bian, J.; Boustany-Kari, C. M.; Brandt, T.; Collman, B. M.; Kalgutkar, A. S.; Klenotic, M. K.; Leininger, M. T.; Lowe, A.; maguire, R. J.; Masterson, V. M.; Miao, Z.; mukaiyama, E.; Patel, J. D.; Pettersen, J. C.; Preville, C.; Samas, B.; She, L.; Sobol, Z.; Steppan, C. M.; Stevens, B. D.; Thuma, B. A.; Tugnait, M.; Zeng, D.; Zhu, T. *J. Med. Chem.***2011**, *54(8)*, 2952.

[86] Mascitti, V.; Preville, C. *Org. Lett.***2010**, *12(13)*, 2940.

[87] Theracos pipeline summary. http://clinicaltrials.gov/show/NCT01377844.

[88] Daiichi Sankyo pipeline summary. ClinicalTrials.gov, 2011, http://clinicaltrials.gov/ct2/dhow/study/NCT00408434.

[89] Lu, X.-P.; Li, Z.; Liao, C.; Shi, L.; Liu, Z.; Ma, B.; Ning, Z.; Shan, S.; Deng, T. US: 2004/0142921.

[90] Kim, J. W.; Kim, J. R.; Yi, S.; Shin, K. H.; Shin, H. S.; Yoon, S. H.; Cho, J. Y.; Kim, D. H.; Shin, S. G.; Jang, I. J.; Yu, K. S. *Clin. Ther.***2011**, *33(11)*, 1819.

[91] Lee, H. W.; Ahn, J. B.; Kang, S. W.; Ahn, S. K. Ha, D.-C. *Org. Process. Res. Dev.***2007**, *11(2)*, 190.

[92] Rikimaru, K.; Wakabayashi, T.; Abe, H.; Imoto, H.; Maekawa, T.; Ujikawa, O.; Murase, K.; Matsuo, T.; Matsumoto, M.; Nomura, C.; Tsuge, H.; Arimura, N.; Kawakami, K.; Sakamoto, J.; Funami, M.; Moi, C. D.; Snell, G. P.; Braqstad, K. A.; Sang, B. C.; Dougan, D. R.; Tanaka, T.; Katayama, N.; Horiquchi, Y.; Momose, Y. *Bioorg. Med. Chem.***2012**, *20(2)*, 714.

[93] Rikimaru, K.; Wakabayashi, T.; Abe, H.; Tawarashi, T.; Imoto, H.; Yonemori, J.; Hirose, H.; Murase, K.; Matsuo, T.; Matsumoto, M.; Nomura, C.; Tsuge, H.; Arimura, N.; Kawakami, K.; Sakamoto, J.; unami, M.; MoI, C. D.; Snell, G. P.; Braqstad, K. A.; Sang, B. C.; Dougan, D. R.; Tanaka, T.; Katayama, N.; Horiguchi, Y.; Momose, Y. *Bioorg. Med. Chem.***2012**, *20(10)*, 3332.

[94] Cho, S. Y.; Ahn, J. H.; Ha, J. D.; Kang, S. K.; Bake, J. Y.; Han, S. S.; Shin, E. Y.; Kim, S. S.; Kim, K. R.; Cheon, H. G.; Choi, J.-K. *Bull. Korean Chem. Soc.***2003**, *24*, 1455.

[95] TransTech Pharma pipeline summary.(Company Web Pages, TransTech Pharma, 4 Dec 2001 & 10 May 2007).

[96] Isis Pharmaceuticals pipeline summary.http://www.isispharm.com/Pipeline/index.htm.

[97] Basu, S.; Prasad, U. V.; Barawkar, D. A.; De, S.; Palle, V. P.; Menon, S.; Patel, M.; Thorat, S.; Singh, U. P.; Das Sarma, K.; Waman, Y.; Niranjan, S.; Pathade, V.; Gaur, A.; Reddy, S.; Ansari, S. *Bioorg. Med. Chem. Lett.***2012**, *22(8)*, 2843.

[98] McIntosh, C.; Demuth, H.; Pospisilik, J.; Pederson, R. *Regulatory Peptides***2005**, *128(2)*, 159.

[99] Behme, M. T.; Dupre, J.; McDonald, T. J. *BMC Endocr. Disord.***2003**, *3(1)*, 3.

[100] Dupre, J.; Behme, M. T.; Hramiak, I. M.; McFarlane, P.; Williamson, M. P.; Zabel, P.; McDonald, T. J. *Diabetes* **1995**, *44(6)*, 626.

[101] Kenner, K. A.; Anyanwu, E.; Olefsky, J. M.; Kusari, J. *J. Biol. Chem.***1996**, *271*, 19810.

[102] Ahmad, F.; Li, P. M.; Meyerovitch, J.; Goldstein, B. J. *J. Biol. Chem.***1995**, *270*, 20503.

[103] Elehebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C.-C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P.; *Science***1999**, *283*, 1544.

[104] Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell. Biol.***2000**, *20*, 5479.

[105] American Diabetes Association *Diabetes Care***2011**, *1*, S11.

[106] Alan, J. G. *Diabetes Care***2011**, *34(2)*, S279.

[107] Mittra, S.; Sangle, G.; Tandon, R.; Sharma, S.; Roy, S.; Khanna, V.; Gupta, A.; Sattigeri, J.; Sharma, L.; Priyadarsiny, P.; Khattar, S. K.; Bora, R. S.; Saini, K. S.; Bansal, V. S. *Br. J. Pharmacol.***2007**,*150(4)*, 480.

[108] Tannehill-Gregg, S. H.; Sanderson, T. P.; Minnema, D.; Voelker, R.; Ulland, B.; Cohen, S. M.; Arnold, L. L.; Schilling, B. E.; Waites, C. R.; Dominick, M. A. *Toxicol. Sci.***2007**, *98(1)*, 258.

[109] Raquel, G.-S.; Felix, H.; Jose, J. L.; Jesus, A. *Front. Mol. Neurosci.***2011**, *4*, 45.

[110] Elchebly, M.; Payette, P.; Michaliszyn, E.. *Science* **1999**, *283*, 1544.

[111] Bjorge, J.D.; Pang, A.; Fujita, D. J.; *J. Biol. Chem.***2000,** *275*, 41439.

[112] Deacon, C. F.; Holst, J. J. *Int.J. Biochem. Cell Biol.***2006**, *38*, 831.

[113] Reimer, M. K.; Holst, J. J.; Ahren, B. *Eur. J. Endocrin.* **2002**, *146*, 717.

[114] Drucker, D. J. *Expert. Opin. Investig. Drugs.***2003**, *12(1)*, 87.

[115] Deacon, C. F.; Ahren, B.; Holst, J. J. *Expert. Opin. Investig. Drugs.***2004**, *13(9)*, 1091.

[116] Sebokova, E.; Christ,A. D.; Boehringer, M.; Mizrahi, J. *Curr. Top.Med. Chem.***2007,** *7,*547.

[117] Augustyns, K.; Veken, P. V.; Senten, K.; Haemers. *Expert. Opin. Therp. Patents***2003**, *13(4)*, 499.

[118] Conarello, S.; Li, Z.; Ronan, J. *Proc. Natl. Acad. Sci. U.S.A.***2003**, *100*, 6825.

[119] Holst, J. J. *Gastroenterology* **1994**, *107,* 1048.

[120] Drucker, D. J. *Diabetes* **1998**, *47,* 159.

[121] Deacon, C. F.; Holst, J. J.; Carr, R. D. *Drugs of Today* **1999**. *35*, 159.

[122] Livingston, J. N.; Schoen, W. R. *Annu. Rep. Med. Chem.* **1999**, *34*,189

[123] Lambeir, A.; Durinx, C.; Scharpe, S.; De Meester, I. *Critical Reviews in Clinical Laboratory Sciences,* **2003**,*40(3)*, 209.

[124] Yaron, A.; Naider, F.*Critical Reviews in Biochemistry and Molecular Biology,***1993**, *28(1)*, 31.

[125] Cunningham, D. F.; O'Connor, B. *Biochim. Biophys. Acta***1997**, *1343*, 160.

[126] Hartel, S.; Gossrau, R.; Hanski, C.; Reutter, W. *Histochemistry***1988**, *89*, 151.

[127] McCaughan, G. W.; Wickson, J. E.; Creswick, P. F.; Gorrell, M. D. *Hepatology***1990**, *11*, 534.

[128] Gorrell,M. D.; Wickson, J.; McCaughan, G.W. *Cell. Immunol.***1991**, *134*, 205.

[129] Bu¨hling, F.; Kunz, D.; Reinhold, D.; Ulmer, A. J.; Ernst, M.; Flad, H. D.; Ansorge, S. *Nat. Immun.***1994**, *13*, 270.

[130] Iwaki-Egawa, S.; Watanabe, Y.; Kikuya, Y.; Fujimoto, Y. *J. Biochem.***1998**, *124*, 428.

[131] Kieffer, T. J.; McIntosh, C. H. S.; Pederson, T. A. *Endocrinology* **1995**, *136,* 3585.

[132] Abbott, C. A. and Gorrell, M. D. *Langner, J. and Ansorge, S., eds.***2002**, pp 171–195, Kluwer/Plenum, New York.

[133] Rosenblum, J. S.; Kozarich, J. W. *Curr.Opin.Chem.Bio*. **2003**, *7*, 496.

[134] Gorrell, M. D. *Clin. Sci.***2005**, *108*, 277.

[135] Abbott, C. A., McCaughan, G.W. and Gorrell, M. D. *FEBS Lett*. **1999***458*, 278.

[136] Wang, X. M., Yao, T.W., Nadvi, N. A., Osborne, B., McCaughan, G.W. and Gorrell, M. D. *Front. Biosci.***2008**, *13*, 3168.

[137] Ajami, K.; Abbott, C. A.; McCaughan, G.W.; Gorrell, M. D. *Biochim. Biophys. Acta***2004**, *1679*, 18.

[138] Klener, P.; Lojda, Z.; Haber, J.; Kvasnicka, J. *Neoplasma*, **1987**, *34*, 581.

[139] Bjelke, J. R.; Christensen, J.; Nielsen, P. F.; Branner, S.; Kanstrup, A. B.; Wagtmann, N.; Rasmussen, H. B. *Biochem. J*. **2006**, *396*, 391.

[140] Ajami, K.; Pitman, M. R.; Wilson, C. H.; Park, J.; Menz, R. I.; Starr, A. E.; Cox, J. H.; Abbott, C. A.; Overall, C. M.; Gorrell, M. D. *FEBS Lett*. **2008**, *582*, 819.

[141] Green, B. D.; Flatt, P. R.; Bailey, C. J. *Expert Opin. Emerging Drugs* **2006**,*11,* 525.

[142] Rummey, C.; Metz, G. *Proteins***2007**, *66*, 160.

[143] Park, J.; Knott,H.M.; Nadvi, N. A.; Collyer, C. A.; Wang, X. M.; Church, W. B.; Gorrell, M. D. *Open Enzyme Inhibition J*. **2008**, *1*, 52.

[144] Thoma, R.; Loffler, B.; Stihle, M.; Huber, W.; Ruf, A.; Hennig, M. *Structure*, **2003**, *11*, 947.

[145] "Entrez Gene: PREP prolyl endopeptidase"

[146] Chen, T.; Ajami, K.; McCaughan, G. W.; Gai, W. P.; Gorrell, M. D.; Abbott, C. A. *Biochim. Biophys. Acta***2006**, *1764*, 33.

[147] Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T.; Thornberry, N. A. *Biochem.J.*, **2003**, *371*, 525.

[148] Holst, J. J. *Diabetologia***2006**, *491*, 253.

[149] Baggio, L. L.; Drucker, D. J.*Gastroenterology***2007**, *132*, 2131.

[150] Zhu, L.; Tamvakopoulos, C.; Xie, D.; Dragovic, J.; Shen, X.; Fenyk-Melody, J. E.; Schmidt, K.; Bagchi, A.; Griffin, P. R.; Thornberry, N. A.; Roy, R. S. *J. Biol. Chem.***2003**, *278(25)*, 22418.

[151] Flatt, P. R.; Bailey, C. J.; Green, B. D. *Frontiers in Bioscience*, **2008**, *13*, 3648.

[152] Maes, M. B.; Dubois, V.; Brandt, I.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Cheng, J. D.; Chen, X.; Scharp´e, S.; De Meester, I. *J. Leukocyte Biol.***2007**, *81*, 1252.

[153] Mentlein, R.; Gallwitz, B.; Schmidt, W. E. *Eur. J. Biochem*. **1993**, *214*, 829.

[154] Brubaker, P. L., Izzo, A., Hill, M. and Drucker, D. J. *Am. J. Physiol.***1997**, *35*, E1050.

[155] Nausch, I.; Mentlein, R.; Heymann, E. *Biol. Chem. Hoppe Seyler* , **1990**, *371*, 1113.

[156] Lambeir, A. M.; Durinx, C.; Proost, P.; Van Damme, J.; Scharp´e, S.; De Meester, I. *FEBS Lett.***2001**, *507*, 327.

[157] Mentlein, R.; Dahms, P.; Grandt, D.; Kruger, R. *Regul. Pept.***1993**, *49*,133.

[158] Zhu, L.; Tamvakopoulos, C.; Xie, D. et al. *J. Biol. Chem.***2003**, *278*, 22418.

[159] Mentlein, R. *Regul. Pept.***1999**, *85*, 9.

[160] Sakurada, C.; Sakurada, S.; Hayashi, T.; Katsuyama, S.; Tan-No, K.; Sakurada, T. *Biochem. Pharmacol.***2003**, *66*, 653.

[161] Zhang, X. Y.; De Meester, I.; Lambeir, A. M. et al. *J. Mass Spec.***1999**, *34*, 255.

[162] Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. *Nat. Struct. Biol.***2003**, *10*, 19.

[163] Oefner, C.; D'Arcy, A.; Mac Sweeney, A.; Pierau, S.; Gardiner, R.; Dale, G. E. *Acta Crystallogr. D, Biol. Crystallogr.***2003**, *59*, 1206.

[164] Hiramatsu, H., Yamamoto, A., Kyono, K. et al. *Biol. Chem.***2004**, *385*, 561.

[165] Thoma, R.; Loffler, B.; Stihle, M.; Huber, W.; Ruf, A.; Hennig, M. *Structure***2003**, *11*, 947.

[166] Engel, M.; Hoffmann, T.; Wagner, L. et al. *Proc. Natl. Acad. Sci. U.S.A.***2003**,*100*, 5063.

[167] Aertgeerts, K.; Ye, S.; Tennant, M. G. et al. *Protein Sci.***2004**, *13*, 412.

[168] Weihofen, W. A.; Liu, J.; Reutter, W.; Saenger, W.; Fan, H. *J. Biol. Chem.***2004**, *41*, 43330.

[169] Tanaka, T.; Camerini, D.; Seed, B.; Torimoto, Y.; Dang, N. H.; Kameoka, J.; Dahlberg, H. N.; Schlossman, S. F.; Morimoto, C. *J. Immunol.***1992**, *149*, 481.

[170] Ollis, D. L.; Cheah, E.; Cygler, M.; Dijkstra, B.; Frolow, F.; Franken S. M.; Harel, M.; Remington, S. J.; Silman, I.; Schrag, J. *Protein Eng.***1992**, *5*, 197.

[171] Ogata, S.; Misumi, Y.; Tsuji, E.; Takami, N.; Oda, K.; Ikehara, Y. *Biochemistry***1992**, *31*, 2582.

[172] Rahfeld, J.; Schierhorn, M.; Hartrodt, B.; Neubert, K.; Heins J. *Biochim. Biophys. Acta***1991**, *1076*, 314.

[173] Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.***1968**, *32***,** 898.

[174] Brandt, W.; Ludwig, O.; Thondorf, I.; Barth, A. *Eur. J. Biochem.***1996**, *236,* 109.

[175] Heins, J.; Welker, P.; Schonlein, C.; Born, I.; Hartrodt, B.; Neubert, K.; Tsuru, D.; Barth, A. *Biochim. Biophys. Acta***1988**, *954*, 161.

[176] Yoshimoto, T.; Fischl, M.; Orlowski, R. C.; Walter, R. *J. Biol. Chem.***1978**, *253*, 3708.

[177] Abbott, C. A.; McCaughan, G. W.; Gorrell, M. D. *FEBS Lett.***1999a**, *458*, 278.

[178] http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/enzymes/GetPage.pl?ec_number=3.4.14.5

[179] Ikehara, Y.; Ogata, S.; Misumi, Y. *Method Enzymol.* **1994***, 244*, 215.

[180] Augustyns, K.; Van der Veken, P.; Haemers, A. *Expert Opin.Ther. Pat.* **2005***, 15*, 1387.

[181] Hunziker, D.; Hennig, M.; Peters, J. U. *Curr. Top.Med. Chem.* **2005***, 5*, 1623.

[182] Babine, R. E.; Bender, S. L. *Chem. Rev.***1997***, 97*, 1359.

[183] Qiao, L.; Baumann, C. A.; Crysler, C. S.; Ninan, N. S.; Abad, M. C.; Spurlino, J. C.; DesJarlais, R. L.; Kervinen, J.; Neeper, M. P.; Bayoumy, S. S.; Williams, R.; Deckman, I. C.; Dasgupta, M.; Reed, R. L.; Huebert, N. D.; Tomczuk, B. E.; Moriarty, K. J. *Bioorg. Med. Chem. Lett.* **2006***,16*, 123.

[184] Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. *Biochemistry* **1999***,38*, 11597.

[185] Feng, J.; Gwaltney II, S. L.; Stafford, J. A.; Wallace, M.; Zhang, Z.; Kassel, D. B.; Manuel, M.; Xu, R.; Prasad, G. S.; Skene, R. J.; Ye, S.; Caster, C.; Navre, M.; Shi, L.; Kaldor, S.; Webb, D. R. Poster. *Advances in Structure-Based Drug Discovery*: Philadelphia, Pa, USA, **2005**.

[186] Stockel-Maschek, A.; Stiebitz, B.; Born, I.; Faust, J.; Mogelin, W.; Neubert, K. *Adv. Exp. Med. Biol.* **2000***, 477*, 117.

[187] Hulin, B.; Cabral, S.; Lopaze, M. G.; Van Volkenburg, M. A.; Andrews, K. M.; Parker, J. C. *Bioorg. Med. Chem. Lett.* **2005***, 15*, 4770.

[188] Peters, J.-U.; Weber, S.; Kritter, S.; Weiss, P.; Wallier, A.; Boehringer, M.; Hennig, M.; Kuhn, B.; Loeffler, B.-M. *Bioorg. Med. Chem. Lett.* **2004***, 14*, 1491.

[189] Xu, J.; Ok, H. O.; Gonzalez, E. J.; Colwell, L. F.; Habulihaz, B.; He, H.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004***, 14* , 4759.

[190] Mattei, P.; Böhringer, M.; Fischer, H.; Gobbi, L.; Hennig, M.; Huwyler, J.; Kuhn, B.; Löffler, B.; Lübbers, T.; Narquizian, R.; Nielsen, J.; Wessel, H. P.; Wyss, P. Poster. *2nd Joint Italian-Swiss Meeting on Medicinal Chemistry*: Modena, Italy, **2005.**

[191] Brockunier, L. L.; He, J.; Colwell, L. F.; Habulihaz, B.; He, H.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R. A.; Teffera, Y.; Wu, J. K.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004***, 14*, 4763.

[192] Coppola, G. M.; Zhang, Y. L.; Schuster, H. F.; Russell, M. E.; Hughes, T. E. *Bioorg. Med. Chem. Lett.* **2000***, 10*, 1555.

[193] Holger Kuehne, Roche Basel, personal communication.

[194] Yasuda, N.; Nagakura, T.; Yamazaki, K.; Yoshikawa, S.; Okada, T.; Ikuta, H.; Koyanagi, M. Eur. Pat. Appl. 1258480, **2002**.

[195] Biftu, T.; Feng, D.; Qian, X.; Liang, G.-B.; Kieczykowski, G.; Eiermann, G.; He, H.; Leiting, B.; Lyons, K.; Petrov,A.; Sinha-Roy, R.; Zhang, B.; Scapin, G.; Patel, S.; Gao, Y.-D.; Singh, S.; Wu, J.; Zhang, X.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.***2007**, *17*, 49.

[196] Deacon, C. F.; Johnsen, A. H.; Holst, J. J. *J Clin. Endocrinol. Metab.* **1995**, *80*, 952.

[197] Nauck, M. A.; Wollschlager, D.; Werner, J.; Holst, J. J.; Orskov, C.; Creutzfeldt, W.; Willms, B.. *Diabetologia* **1996**, *39*, 1546.

[198] Drucker, D. J. *Gastroenterology* **2002**, *122 (2)*, 531]

[199] Juillerat-Jeanneret, L. *Curr. Chem. Biol.***2008**, *2*, 97

[200] Lankas, G. R.; Barbara Leiting, B.; Roy, R. S.; Eiermann, G. J.; Maria, G.; Beconi, M. G.; Biftu, T.; Chan, C.- C.; Scott Edmondson, S.; Feeney, P.; He, H.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X.; Reigle, L.; Woods, A.; Wu, J. K.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes***2005**, *54*, 2988.

[201] Burkey, B. F.; Hoffmann, P. K.; Hassiepen, U.; Trappe, J.; Juedes, M.; Foley, J. E. *Diabetes Obes. Metab.***2008**, *11*, 1057.

[202] Larsen, J.; Hylleberg, B.; Ng, K.; Damsbo, P. *Diabetes Care***2001**, *24*, 1416.

[203] Weber, A. E. *J. Med. Chem.* **2004,** *47,* 4135.

[204] Havale, S. H.; Pal, M. *Bioorg. Med. Chem.***2009**, *17*, 1783.

[205] Cahn, A.; Raz, I. *Expert Opin. Emerging Drugs***2013**, *18*, 245.

[206] Juillerat-Jeanneret, L. *J Med Chem.***2014**, *57*, 2197.

[207] Brigance, R. P.; Meng, W.; Fura, A.; Harrity, T.; Wang, A.; Zahler, R.; Kirby, M. S.; Hamann, L. G. *Bioorg. Med. Chem. Lett.***2010**, *20*, 4395.

[208] Meng, W.; Brigance, R. P.; Chao, H. J.; Fura, A.; Harrity, T. et al. *J. Med. Chem.***2010**, *53*, 5620.

[209] Cox, J. M.; Harper, B.; Mastracchio, A.; Leiting, B.; Sinha-Roy, R.; Patel, R. A.; Wu, J. K.; Lyons, K. A.; Huaibing He, Xu, S.; Zhu, B.; Thornberry, N. A.; Weber. A. E.; Edmondson, S. D. *Bioorg. Med. Chem. Lett.***2007**, *17*, 4579.

[210] Edmondson, S. D.; Mastracchio, A.; Cox, J. M.; Eiermann, G. J.; He, H.; Lyons, K. A.; Patel, R. A.; Patel, S, B.; Petrov, A.; Scapin, G.; Wu, J. K.; Xu, S.; Zhu, B.; Thornberry, N. A.; Sinha-Roy, R.; Weber, A. E. *Bioorg. Med. Chem. Lett.***2009**, *19*, 4097.

[211] Nordhoff, S.; Bulat, S.; Cerezo-Gálvez, S.; Hill, O.; Hoffmann-Enger, B.; López-Canet, M.; Rosenbaum, C.; Rummey, C.; Thiemann, M.; Matassa, V. G.; Edwards, P. J.; Feurer, A. *Bioorg. Med. Chem. Lett.***2009**, *19*, 6340.

[212] Miyamoto, Y,; Banno, Y.; Yamashita, T.; Fujimoto, T. et al. *J Med Chem.***2011**, *54*, 831.

[213] Hui Xie, H.; Zeng, L.; Zeng, S.; Lu, X.; Zhao, X.; Zhang, G.; Tu, Z.; Xu, H.; Yang, L.; Zhang, X.; Wang, S.; Hu, W. *Eur. J. Med. Chem.***2013**, *68*, 312.

[214] Singh, S.; Sethi, S.; Khanna, V.; Benjamin, B.; Kant, R.; Sattigeri, J.; Bansal, V. S.; Bhatnagar, P. K.; Davis, J. A. *Eur. J. Pharmacol.***2011**, *652*, 157.

[215] Kato, N.; Oka, M.; Murase, T.; Yoshida, M.; Sakairi, M.; Yakufu, M.; Yamashita, S.; Yasuda, Y.; Yoshikawa, A.; Hayashi, Y.; Shirai, M.; Mizuno, Y.; Takeuchi, M.; Makino, M.; Takeda, M.; Kakigami, T. *Org. Med. Chem. Lett.***2011**, *1*, 7.

[216] Kim, H. J.; Kwak, W. Y.; Min, J. P.; Sung, S. Y.; Kim, H. D. et al. *Bioorg. Med. Chem. Lett.***2012**, *22*, 5545.

[217] Nelson, N. R.; Nebert, D. W. "Cytochrome P450 (*CYP*) Gene Superfamily" 2011, DOI: 10.1002/9780470015902.a0005667.pub2

[218] Guengerich, F. P.; Hosea N. A.; Parikh, A.; Bell-Parikh, L. C.; Johnson, W. W.; Gillam, E.M.J.; Shimada, T. *Drug Metab Dispos.* **1998**, *26*, 1175.

[219] Baldwin, S. J.; Clark, S. E.; Chenery, R. J. *Br J Clin Pharmacol*. **1999**, *55*, 53.

[220] Niemi, M.; Backman, J. T.; Neuvonen, M.; Neuvonen, P. J. *Diabetologia,* **2003**, *46*, 347.

[221] Tanaka, Y.; Niwa, S.; Nishioka, H.; Yamanaka, T.; Torizuka, M.; Yoshinaga, K.; Kobayashi, N.; Ikeda, Y.; Arai, H. *J. Med. Chem.* **1994**, *37*(13), 2071.

[222] Li, J.; Wilk, E.; Wilk, S. *Arch. Biochem. Biophys.* **1995**, *323*(1), 148.

[223] Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D. *Bioorg. Med.Chem. Lett.***1996**, *6*, 1163.

[224] Peters, J.-U.*Curr. Top.Med. Chem.,***2007,** *7,*579.

[225] Parmee, E. R.;He, J.;Mastracchio, A.;Edmondson, S. D.; Colwell, L.;Eiermann, G.;Feeney, W. P.; Thornberry, N. A.; Weber, A. E. et al. *Bioorg. Med. Chem. Lett.***2004**, *14*, 43.

[226] Sakashita, H.; Akahoshi, F.; Kitajima, H.;Tsutsumiuchi, R.; Hayashi, Y. *Bioorg. Med. Chem.***2006**, *14*, 3662.

[227] Fukushima, H.; Hiratate, A.;Takahashi, M.; Mikami, A.; Saito-Hori, M.; Munetomo, E.;Kitano, K.; Chonan, S.; Saito, H.; Suzuki, A.; Takaoka, Y.; Yamamoto, K.*Bioorg. Med. Chem.***2008**, *16*, 4093.

[228] Olson, G.L.; Bolin, D.R.; Bonner, M.P.; Bös, M. C.; Cook, M.; Fry, D.C.; Graves, B.J.; Hatada, M.; Hill, D. E.; Kahn, M.; Rusiecki, V.K.; Sarabu, R.; Sepinwall, J.; Vincent, G.P.; Voss, M.E. *J. Med. Chem.***1993**, *36*, 3039.

[229] Senten, K.; Van der Veken, P.; De Meester, I.; Lambeir, A. –M.; Scharpe´, S.; Haemers, A.; Augustyns, K.*J. Med. Chem.* **2003,** *46,* 5005.

[230] Fukushima, H.; Hiratate, A.;Takahashi, M.; Mikami, A.; Saito-Hori, M.; Munetomo, E.;Kitano, K.; Saito, H.; Suzuki, A.; Takaoka, Y.; Yamamoto, K.*Bioorg. Med. Chem.***2004**, *12*, 6053.

[231] Nabeno, M.; Akahoshi, F.; Kishida, H.; Miyaguchi, I.; Tanaka, Y.; Ishii, S.; Kadowaki, T. *Biochem. Biophys. Res. Commun.***2013**, *434*, 191.

[232] Kang, N. S.; Ahn, J. H.; Kim, S. S.; Chae, C. H.; Yoo, S. E. *Bioorg. Med. Chem. Lett.***2007**, *17*, 3716.

[233] Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. *J. Med. Chem.***2002**, *45*, 2362.

[234] Oefner, C.; D'Arcy, A.; Mac Sweeney, A.; Pierau, S.; Gardiner, R.; Dale, G. E. *Acta Crystallografica***2003**, *D59*, 1206.

[235] Halliday, R. C.; Jones, B. C.; Park, B. K.; Smith, D. A.*Eur. J.Drug Metab. Pharmacokin*. **1997**, *22*, 291.

[236] Scapin, G.; Singh, S.; Feng, D.; Becker, J.; Doss, G.; Eiermann, G.; He, H.; Lyons, K.; Patel, S.; Petrov, A.; Sinha Roy, R.; Wu, J.; Zhang, X.; Thornberry, N. A.; Weber, A. E.; Biftu, T. *Bioorg. Med. Chem. Lett.***2007**, *17*, 3384.

[237] Sheppard, R. C.; Atherton, E. Solid Phase Peptide Synthesis- a practical approach, I.R.L. Press publication, Oxford University Press.

[238] Taber, D. F.; Berry, J. F.; Martin, T. J. *J. Org. Chem.*, **2008**, *73*, 9334.

[239] Gawande, M, B.; Branco, P. S. *Green Chem*., **2011**, *13*, 3355.

[240] Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748.

[241] Dener, J. M.; Fantauzzi, P. P.; Kshirsagar, T. A.; Kelly, D. E.; Wolfe, A. B. *OPRD***2001**, *5,* 445.

[242] Blackmon, D. L.; Watson, A. J.; Montrose, M. H. *Anal. Biochem*.,**1992**, *200*, 352.

[243] Chen, D.; Wang, M. *Diabetes Obes. Metab.* **2005**, *7(4)*, 307.

[244] Kim, J. G.; Baggio, L. L.; Bridon, D. P.; Castaigne, J. P.; Robitaille, M. F.; Jette, L.; Benquet, C.; Drucker, D. J. *Diabetes* **2003**, *52*, 751.

[245] Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.***2006**, *49*, 6177.

[246] *Schrodinger Suite 2010, Glide version 5.6, Prime version 2.2*; Schrodinger, LLC: New York, 2010.

[247] Demange, L.; Menez, A.; Dugave, C. *Tetrahedron Lett.***1998**, *39*, 1169.

[248] Chorghade, M. S.; Mohapatra, D. K.; Sahoo, G.; Gurjar, M. K.; Mandlecha, M. V.; Bhoite, N.; Moghe, S.; Raines, R. T. *J. Fluorine Chem.***2008**, *129*, 781.

[249] Zhuang, W.; Zhao, X.; Zhao, G.; Guo, L.; Lian, Y.; Zhou, J.; Fang, D. *Bioorg. Med. Chem.***2009**, *17*, 6540.

[250] Carlos Cativiela, C.;Díaz-de-Villegas, M. D.*Tetrahedron Asymm.***1998**, *9,* 3517.

[251] Storcken, R. P. M.; Panella, L.; van Delft, F. L.; Kaptein, B.; Broxterman, Q. B.; Schoemaker, H. E.; Rutjes, F. P. J. T.*Advanced Synthesis & Catalysis***2007**, *349*, 161.

[252] Lu, T.; Lin, C.*J. Org. Chem.*, **2011**, *76* (6), 1621.

[253] O'Donnell, M. J.; Wu, S. *Tetrahedron Asymm.***1992**, *3*, 591.

[254] Davis, F. A.; Lee, S.; Zhang, H.; Fanelli, D. L. *J. Org. Chem.* **2000,** *65,* 8704.

[255] Xu, P.; Li, S.; Lu, T.; Wu, C.; Botao Fan, B.; Golfis, G. *J. Org. Chem.* **2006**, *71*, 4364.

[256] Kapadia, S. R.; Spero, D. M.; Eriksson, M.*J. Org. Chem.* **2001,** *66,* 1903.

[257] Royo, S.; Jimenez, A. I.; Cativiela, C.*Tetrahedron: Asymm.,***2006**, *17*, 2393.

[258] Sibi, M. P.; Deshpande, P. K.; La Loggia, A. J.; Christen, J. W. *Tetrahedron Lett.***1995**, *36*, 8965.

[259] Koskinen, A. M. P.; Hasslla, H.; Myllymaki, V. T.; Rissanen, K. *Tetrahedron Lett.***1995**, *36(31)*, 5619.

[260] Campbell, A. D.; Raynham, T. M.; Taylor, R. J. K. *Synthesis*, **1998**, 1707.

[261] Haffner, C. D.; McDougald, D. L.; Randhawa, A. S.; Reister, S. M.; Lenhard, J. M. *PCT Int. Appl.***WO2003/002531**, January 9, 2003.

[262] Dierks, E. A.; Stams, K. R.; Lim, H. K.; Cornelius, G.; Zhang, H.; Ball, S. E. *Drug Metab. Dispos.***2001**, *29*, 23.

[263] Moos, W. H.; Gless, R. D.; Rapoport, H. *J. Org. Chem.***1981**, *46*, 5064.

[264] Klapars, A.; Xiaohua Huang, X.; Stephen L. Buchwald, S, L.*J. Am. Chem. Soc.***2002**, *124*, 7421.

[265] Cox, J. M.; Edmondson, S. D.; Harper, B.; Weber, A. E. *PCT Int. Appl.***WO 039325**, 2006.

[266] Biftu, T.; Chen, P.; Cox, J. M.; Weber, A. E. *PCT Int. Appl.***WO 056708**, 2010.

[267] Baxter, A. D.; Boyd, E, A.; Frank-Kamenetsky, M.; Jeffery, P.; Stephen, P.; Stibbard, J. H. A. **US 2005/0070578 A1**.

[268] Shawali, A. S. *ARKIVOC*, **2010**, i, 33.

[269] Guan, L. P.; Zhang, R. P.; Chang, Y.; Gan, X. X. *Asian J. Chem.*, **2013**, *25(7)*, 3660.

[270] Graceffa, R.; Kaller, M.; La, D.; Lopez, P.; Patel, V. F.; Zhong, W. *PCT Int. Appl.* **WO 2009064418 A1**, May 22, 2009.

[271] Calderwood, D. J.; Bonafoux, D. F.; Burchat, A.; Ding, P.; Frank, K. E.; Hoemann, M. Z.; Mullen, K. D.; Davis, H. M. *PCT Int. Appl.***WO 2009005675 A1**, Jan. 8, 2009.

[272] C. Avellana, C.; Gomez-Parra, V.; Madronero, R. *J. Heterocyc. Chem.*, **1977**, *14(2)*, 325.

[273] Suzuki, K.; Ohkuma, T.; Tsuchihashi, G. *J. Org. Chem.***1987**, *52(13)*, 2929.

[274] Shipps, G. W. Jr.; Rosner, K. E.; Popovici-Muller, J.; Deng, Y.; Wang, T.; Curran, P. J. *PCT Int. Appl.***WO 2003101993 A1**, Dec 11, 2003.

[275] Nam, N. L.; Grandberg, I. I.; Sorokin, V. I. *Chem. Heterocycl. Compd.***2002**, *38*, 1371.

[276] Yin, G.; Gao, M.; She, N.; Hu, S.; Wu, A.; Pan, Y. *Synthesis***2007**, *20*, 3113.

[277] Bailey, Patrick D.; Morgan, Keith M.; Smith, David I.; Vernon, John M. *Tetrahedron***2003**, *59(18)*, 3369.

[278] Shahane, S.; Louafi, F.;Moreau, J.; Hurvois, J. P.; Renaud, J. L.; van de Weghe, P.; Roisnel, T. *Eur. J. Org. Chem.,***2008**, *27*, 4622.

[279] Baetta, R.; Corsini, A. *Drugs***2011**, *71*, 1441.

[280] Scheen, A. J. *Expert Opin Pharmacother.***2012**, *13*, 81.

[281] Inzucchi, S. E.; Bergenstal, R. M.; Buse, J. B. *Diabetes Care***2012**, *35*, 1364.

[282] Villieras, J.; Rambaud, M.; Graff, M. *Tetrahedron Lett.*, **1985**, *26(1)*, 53.

[283] Kuhnel, E.; Laffan, D. D. P.; Lloyd-Jones, G. C.; Martinez del Compo, T.; Shepperson, I. R.; Slaughter, J. L. *Angewt. Chem. Int. Ed.***2007**, *46(37)*, 7075.

[284] Gabriel, S. *Ber. Dtsch. Chem. Ges.***1887**, *20*, 2224.

[285] Khan, M. N. *J. Org. Chem.***1995**, *60*, 4536.

[286] Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.***1984**, *25(20)*, 2093.

[287] Yamaura, M.; Suzuki, T.; Hashimoto, H.; Yoshimura, J.; Okamoto, T.; Shin, C. *Bull. Chem. Soc. Jpn.*, **1985**, *58*, 1413.

[288] Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.***1996,***118*, 11225.

[289] Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.***2004,***47*, 1739.

Publications

"To me, science is an expression of the human spirit, which reaches every sphere of human culture. It gives an aim and meaning to existence as well as a knowledge, understanding, love, and admiration for the world. It gives a deeper meaning to morality and another dimension to esthetics." - Isidor Isaac Rabi

8. Publication

List of Publications from the PhD work

1. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Samadhan Kshirsagar, Mukul Jain, Mubeen Shaikh, Kalapatapu V. V. M. Sairam. “Long-acting peptidomimetics based DPP-IV inhibitors”*Bioorg. Med. Chem. Lett.***2012**, *22*, 3516-3521.

2. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Kiran Shah, Shruti Bhardwaj, Kishan Patel, Kaushil Patel, Rajendra Chopade, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Mukul Jain. “Design of Peptidomimetics Based DPP-IV Inhibitors, Devoid of CYP liabilities”*Letters in Drug Design & Discovery***2012**, *9*, 867-873.

3. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Mukul Jain, Kalapatapu V. V. M. Sairam and Praveen Kumar Singh. “Design, Synthesis and Biological Evaluation of Novel Aminomethyl-piperidones based DPP-IV Inhibitors”*Bioorg. Med. Chem. Lett.***2014**, *24*, 1918-1922.

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Long-acting peptidomimetics based DPP-IV inhibitors ☆

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ABSTRACT

Pyrrolidine based peptidomimetics are reported as potent and selective DPP-IV inhibitors for the treatment of T2DM. Compounds **16c** and **16d** showed excellent in vitro potency and selectivity towards DPP-IV and the lead compound **16c** showed sustained antihyperglycemic effects, along with improved pharmacokinetic profile.

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease, which selectively cleaves first two amino acids of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1) thereby makes it inactive.[1,2] Inhibition of DPP-IV activity elevates endogenous GIP, GLP-1 and insulin levels thereby improve glucose excursion. Thus, DPP-IV inhibitors hold great potential for the treatment of type 2 diabetes mellitus (T2DM). Structurally, DPP-IV enzyme resembles with several other protease, so while designing new class of DPP-IV inhibitors, it is essential to consider selectivity of DPP-IV inhibitors over other serine protease, especially DPP-2, DPP-8 and DPP-9.[3]

DPP-IV inhibitors are classified as peptidomimetics (α- and β-series) and non-peptidomimetics. In α-series, pyrrolidine derivatives have been widely explored and depending upon nature of substituents (Z; at the C_2 position of pyrrolidine ring), it is divided into; irreversible (Z = diphenyl-phosphonate ester or *O*-acylhydroxamic acid) and reversible (Z = boronic acid/nitrile) inhibitors.[4] The NVP-DPP728, Vildagliptin, Saxagliptin and Denagliptin represents advanced molecules in cyanopyrrolidine series.[5] The β-series, such as Sitagliptin and the non-peptidomimetic DPP-IV inhibitors, including Alogliptin were developed through high-throughput screening (HTS).[6,7] The DPP-IV enzyme has three binding pockets/sites (S_1, S_2 and S_3).[8] The S_1 pocket consists of catalytic triad (Ser_{630}, Asn_{710} and His_{740}) and the S_2 pocket involves key interactions with Glu_{205} and Glu_{206} dyad. The S_3 pocket (Ser_{209}, Arg_{358} and Phe_{357}) of DPP-IV differs a lot from other protease and it govern selectivity against DPP-8 and 9.[9]

In T2DM patients, gliptins (Vildagliptin, Sitagliptin, Alogliptin and Saxagliptin; Fig. 1) do not lower post-prandial glucose to greater extent as monotherapy, but they are more effective in combination.[10] Gliptins demonstrated good oral bioavailability but due to the rapid clearance, most of them exhibit shorter half-life, thereby require repeated dosing. Thus, attempts are still underway to develop long acting DPP-IV inhibitors, which could potentially provide sustained antidiabetic effect by reducing the post-prandial glucose excursion and HbA1c (>1%), to anticipate cost and dosing frequency. One of the approaches to develop long acting DPP-IV inhibitor could be design of a cyanopyrrolidines based slow-binding inhibitors. The cyanopyrrolidines forms a reversible covalent enzyme-inhibitor complex in which inhibitor bind and dissociates slowly (two-step slow-binding inhibition). Consequently the enzyme catalytic activity can be inhibited even after the free drug has been cleared from the circulation, thereby cyanopyrrolidines inhibits DPP-IV activity for longer duration despite their short half-lives (Vildagliptin and Saxagliptin $t_{1/2}$: ~2–4 h).[11] The NVP-DPP728 represents first slow-binding cyanopyrrolidine-based DPP-IV inhibitor and it showed good antidiabetic activity in clinical trials, despite its short half-life ($t_{1/2}$ ~0.85 h).[12] Recently RBx-0597, has been reported as a potent, selective and slow-binding DPP-IV inhibitor.[13]

Considering clinical implication of long acting DPP-IV inhibitors and to overcome the limitations of existing gliptins, we report

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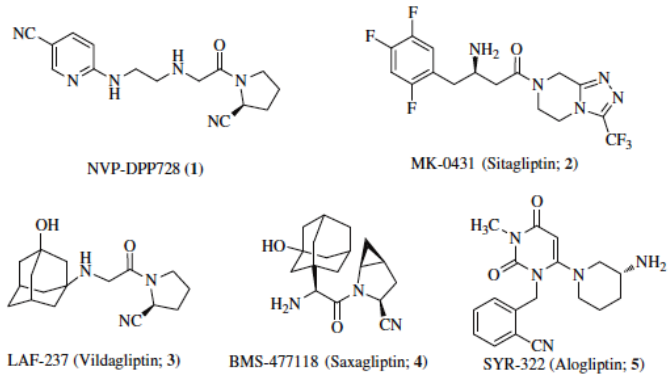


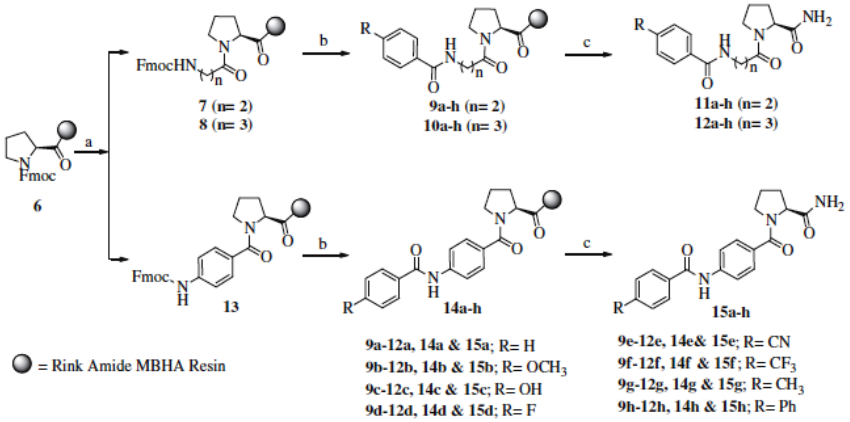
Figure 1. Structurally diverse small molecule based-DPP-IV inhibitors.

design, synthesis and biological evaluation of pyrrolidine containing peptidomimetic based DPP-IV Inhibitors. The peptidomimetics (**11a–h**, **12a–h**, **15a–h**, **16a–d** and **17a,b**) consist of substituted-pyrrolidine ring attached to sterically hinder aromatic acid, with suitable linkers/spacers.

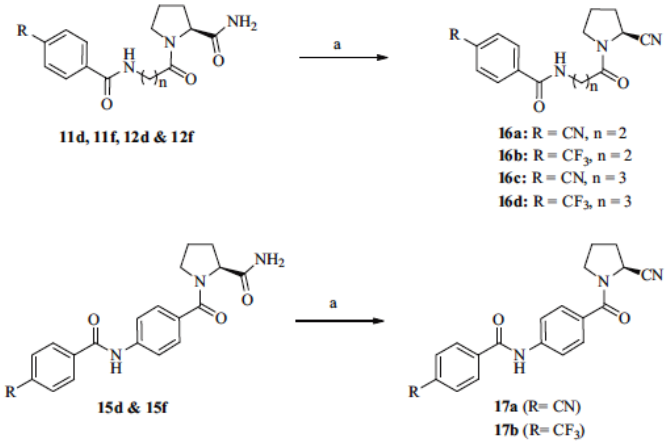
Synthesis (Schemes 1 and 2) of peptidomimetics (**11a–h**, **12a–h**, **15a–h**, **16a–d** and **17a,b**) was carried out, using Fmoc-based solid phase peptide synthesis (SPPS) approach, starting from commercially available Rink-amide MBHA resin, preloaded with Fmoc-protected proline (**6**).[14] Deprotection of **6** with piperidine (20% DMF) and 1,3-diisopropycarbodiimide (DIC) coupling with Fmoc-protected β-Ala (β-alanine), GABA (γ-amino butanoic acid) or PABA (*para*-amino benzoic acid) provided the resin-bound Fmoc-protected dipeptides (**7**, **8** or **13**). Deprotection of **7**, **8** and **13** with piperidine (20% DMF) and DIC coupling with substituted benzoic acids gives resin-bound tripeptides (**9a–h**, **10a–h** and **14a–h**). Trifluroacetic acid (TFA) mediated cleavage of resin-bound peptides (**9a–h**, **10a–h** and **14a–h**) gives pyrrolidinecarboxamides (**11a–h**, **12a–h** and **15a–h**). Trifluroacetic anhydride (TFAA) mediated dehydration of pyrrolidinecarboxamides (**11d**, **11f**, **12d**, **12f**, **15d** and **15f**) afforded title compounds as pyrrolidinecarbonitriles (**16a–d** and **17a,b**).[15] All the test compounds obtained were purified by preparative HPLC (yield 70–85%; HPLC purity >99%) and characterized by various spectroscopic technique (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined within 0.04% of theoretical values (see Supplementary data for analytical and spectral data).

The in vitro DPP-IV inhibitory activity was determined in order to establish the structure–activity relationship (SAR).[16] Two series of the peptidomimetics (**11a–h**, **12a–h**, **15a–h**, **16a–d** and **17a,b**) were prepared (Tables 1 and 2). In the first series (pyrrolidinecarboxamides), 24 compounds (**11a–h**, **12a–h**, **15a–h**) were prepared by linking proline with substituted benzoic acids, using suitable spacers (set-1: β-Ala (**11a–h**); set-2: GABA (**12a–h**) and set-3: PABA (**15a–h**)). In the second series (pyrrolidinecarbonitriles), six compounds (**16a–d** and **17a,b**) were prepared by replacing pyrrolidinecarboxamides with pyrrolidinecarbonitriles. All the test compounds showed varying degrees of DPP-IV inhibitory activity (IC_{50}) depending on the nature of the substituents.

Within the first series (**11a–h**, **12a–h**, **15a–h**), the set-1 (**11a–h**) containing β-alanine spacer attached to *para*-substituted benzamides, showed diverse DPP-IV inhibitory activity depending on the nature of substituents at the *para*-position. Compounds with electron donating groups (**11b**: –OMe and **11c**: –OH) showed weak

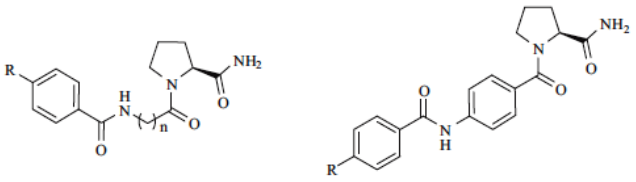


Scheme 1. Synthesis of compounds **11a–h**, **12a–h** and **15a–h**. [a]Reagents and conditions: (a) (1) 20% piperidine in DMF; (2) Fmoc–NH–$(CH_2)_n$–COOH or Fmoc–PABA, HOBt, DIC, DMF, N_2; (b) (1) 20% piperidine in DMF; (2) substituted benzoic acids, HOBt, DIC, DMF, N_2; (c) TFA/H_2O/triisopropylsilane (95:2.5:2.5), 3 h.



Scheme 2. Synthesis of compounds **16a–d** and **17a,b**. [a]Reagents and conditions: (a) TFAA, CH_2Cl_2, 25 °C, 6 h.

Table 1
In vitro DPP-IV inhibitory activity*



11a-h (n= 2); 12a-h (n= 3) **15a-h**

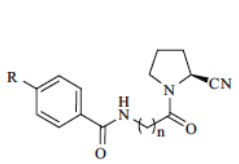
S. No.	R	DPP-IV inhibition**	S. No.	R	DPP-IV inhibition**
11a	*–H*	320 ± 29	**12e**	*–CN*	26 ± 3.1
11b	*–OCH_3*	890 ± 21	**12f**	*–CF_3*	19 ± 2.3
11c	*–OH*	863 ± 18	**12g**	*–CH_3*	694 ± 14
11d	*–F*	93 ± 9.3	**12h**	*–Ph*	104 ± 16
11e	*–CN*	31 ± 2.5	**15a**	*–H*	311 ± 24
11f	*–CF_3*	28 ± 1.7	**15b**	*–OCH_3*	879 ± 22
11g	*–CH_3*	715 ± 27	**15c**	*–OH*	863 ± 13
11h	*–Ph*	107 ± 19	**15d**	*–F*	100 ± 8.5
12a	*–H*	298 ± 19	**15e**	*–CN*	34 ± 7.6
12b	*–OCH_3*	869 ± 43	**15f**	*–CF_3*	31 ± 8.3
12c	*–OH*	843 ± 26	**15g**	*–CH_3*	723 ± 21
12d	*–F*	74 ± 11	**15h**	*–Ph*	116 ± 13

* DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ± SD (n = 3).

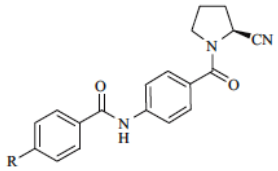
inhibitory activity relative to that of un-substituted (**11a**: –H), whereas compounds with electron withdrawing groups (**11d**; **11e** and **11f**) showed good DPP-IV inhibitory activity. Among the **11d**, **11e** and **11f** tested, **11e** and **11f** showed improved DPP-IV inhibitory activity, which could be due to increase in the electronegativity at *para*-position of benzamide. Aliphatic substitution at *para*-position (**11g**; $–CH_3$) showed weak inhibitory activity, while aromatic substitution at *para*-position (**11h**; –Ph) showed moderate DPP-IV inhibitory activity. The second and third set of compounds (**12a–h**, **15a–h**) comprising GABA and PABA spacers attached to *para*-substituted benzamides, showed similar trend in DPP-IV inhibitory activity as observed with first set of compounds, with respect to nature of *para*-substituents.

The first series was specifically designed to understand the role of spacer and effect of *para*-substituents on benzamide. The SAR study of first series reveals that the DPP-IV inhibitory activity of test compounds drastically varies with *para*-substituents, whereas alteration in spacers (aliphatic with two/three carbon chain-length versus aromatic) do not exhibit significant change. In general, neutral effect of spacers on inhibitory activity might be due to the flexibility in S_2 pocket and stapled orientation of Glu-dyad. Substituents on *para*-position of benzamide altered inhibitory activity to greater extent because in S_3 pocket, *para*-substituents play crucial role for its interaction with Ser_{209}, Arg_{358} and Phe_{357}. From first series, altogether in three different sets, **11e**, **11f**, **12e**, **12f**, **15e** and **15f** (*para*-nitrile/trifluoromethyl benzamide) were

Table 2
In vitro DPP-IV inhibitory activity and selectivity*



16a-b (n= 2); 16c-d (n= 3)



17a-b

S. No.	R	DPP-IV**	DPP2$	DPP8$	DPP9$
16a	–CN	10.3 ± 1.9	–	–	–
16b	$-CF_3$	13.2 ± 2.3	–	–	–
16c	–CN	2.3 ± 0.9	>25,000	>15,000	>15,000
16d	$-CF_3$	3.8 ± 0.5	>25,000	>15,000	>15,000
17a	–CN	11.6 ± 1.6	–	–	–
17b	$-CF_3$	14.3 ± 2.5	–	–	–
NVP-DPP728#		7.2 ± 1.3	>25,000	>15,000	>15,000
Vildagliptin#		3.2 ± 0.5	–	–	–

* DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ± SD ($n = 3$).
$ DPP2, DPP8 and DPP9 inhibitory activity represented as fold-selectivity wrt DPP-IV inhibitory activity.
# Reported literature values for NVP-DPP728 and Vildagliptin are 7 ± 1.7 and 2.7 ± 0.1 respectively. (Ref. 5b,c).

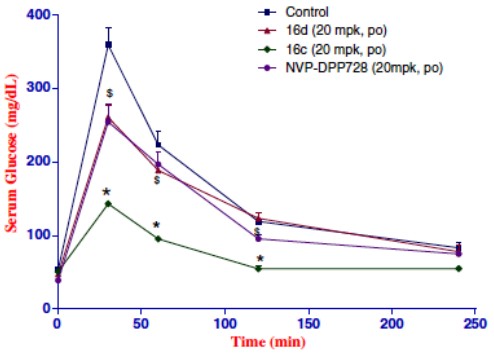
identified as primary lead compounds. Further to study effect of nitrile group on pyrrolidine ring system, a second series (**16a–d** and **17a,b**) was prepared by replacing pyrrolidinecarboxamides of first series lead compounds with pyrrolidinecarbonitriles.

As depicted in Table 2, all the six compounds (**16a–d** and **17a,b**) from second series showed potent inhibitory activity and was found to be comparable with standard compounds (NVP-DPP728 and Vildagliptin).[5] Compared to first series (pyrrolidinecarboxamides), significant improvement in the inhibitory activity was observed with second series (pyrrolidinecarbonitriles) of compounds (**16a–d** and **17a,b**), which could be due to the favorable interactions of pyrrolidinecarbonitriles with the key residues of S_1 pocket. Among six compounds tested (second series), **16c** and **16d** were found to be equipotent as Vildagliptin.

The in vitro selectivity over serine protease (Table 2), especially DPP-2, DPP-8 and DPP-9 was evaluated for most potent compounds (**16c** and **16d**).[16] Compounds **16c** and **16d** showed >25000-fold selectivity over DPP-2 and >15000-fold selectivity over DPP-8 and DPP-9, which was found to be comparable with reference standard compounds (NVP-DDP728). Among all the compounds tested, **16c** and **16d** were found most potent and selective, hence subjected for pharmacodynamic (PD) as well as pharmacokinetic (PK) profiling.

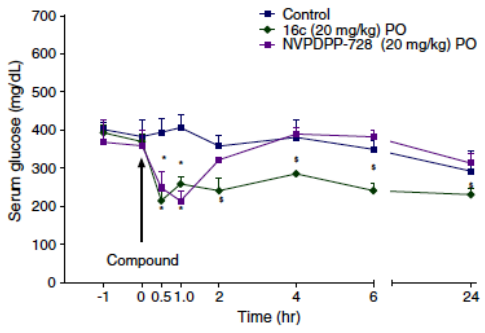
The in vivo antidiabetic activity of **16c**, **16d** and NVP-DPP728 (@ 20 mg/kg, po) was evaluated in male C57BL/6 J mice, using IPGTT (intraperitoneal glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose up to 240 min; mg/dL) is reported (Fig. 2).[17] Compound **16c** showed good oral antidiabetic activity (% decrease in AUC glucose 54.9 ± 3.86), whereas **16d** and NVP-DPP728 (positive control) showed moderate activity upon oral administration (% decrease in AUC glucose 17.4 ± 5.35 and 21.5 ± 6.1, respectively). In C57 mice (IPGTT protocol), it was interesting to observe that **16c** showed suppression in the blood glucose at all the time points (30, 60, 120 and 240 min) compared to vehicle control, while **16d** and NVP-DPP728 showed blood glucose reduction only at 30 and 60 min.

Further to understand the duration of action and effect of test compounds on post-prandial glucose excursion, single dose (@



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 2. In vivo antidiabetic activity of **16c**, **16d** and NVP-DPP728 in C57 mice (OGTT).



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 3. In vivo antidiabetic activity of **16c** and NVP-DPP728 in db/db mice.

20 mg/kg, po) antidiabetic activity of **16c** and NVP-DPP728 was evaluated in fed-db/db mice (hyperglycemic animals) for 24 h (Fig. 3). Under fed condition, compared to vehicle control, NVP-DPP728 and **16c** showed good antidiabetic activity (% decrease in AUC glucose 31.4 ± 8.7 and 33.5 ± 7.4, respectively) up to 2 h. However, **16c** showed sustained suppression in serum glucose levels for >8 h (% decrease in AUC glucose, 14.9 ± 6.3 for NVP-DPP728 and 30.8 ± 6.2 for **16c**, after 8 h).

A comparative single dose (20 mg/kg iv or po) PK profile of **16c**, **16d** and NVP-DPP728 was evaluated in male C57BL/6J mice ($n = 6$) and the various PK parameters such as T_{max}, $T_{1/2}$, C_{max}, AUC and %F were recorded (Table 3).[18] In PK study, all the test compounds showed rapid T_{max}, good C_{max} and oral bioavailability (%F ~63–72%). Compound **16c** showed higher AUC (>twofold compared to **16d** and NVP-DPP728 **1**) and extended half-life ($T_{1/2}$: >7 h compared to **16d** and NVP-DPP728). Compound **16c** showed extended half-life and higher AUC, which could be due to its low clearance compared to **16d** (elimination rate constant (kel; h^{-1}), 0.11 ± 0.03 for **16c** and 0.82 ± 0.18 for **16d**). Thus improved pharmacokinetic profile of compound **16c** justifies its potent and sustained antidiabetic activity in C57 and db/db mice.

The molecular docking analysis of **16c** and NVP-DPP728 was carried out using extra precision (XP) Glide docking method (Fig. 4).[9,19] The crystal structure of the DPP-IV enzyme (PDB ID: 2I03) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. For docking study, the ligands were minimized by applying an OPLS-AA forcefield, using ligprep module of Schrödinger.[19]

The overlay of binding poses of **16c** (Turquoise) and NVP-DPP728 (Rose) in the DPP-IV active site is shown in Fig. 4. As observed with NVP-DPP728, **16c** docks very well into all the three sites (G-scores −11.85 (9/9) and −10.16 (7/9) for **16c** and NVP-DPP728 respectively). Both NVP-DPP728 and **16c** showed covalent interaction of cyanopyrrolidine–CN with OH-group of side-chain of Ser_{630} (S_1 pocket) and H-bonding of amide-NH backbone with C=O groups of side-chains of Glu_{205} and Glu_{206} dyad (S_2 pocket), which supports excellent in vitro DPP-IV selectivity of **16c** over other protease. Especially, incorporation of GABA linkage (spacer) and *para*-nitrile benzamide in **16c** allows it to adopt new confirmation, which favors strong H-bonding of benzamide with the NH of guanidine side-chain of Arg_{358} and aromatic π–π stacking of *para*-nitrile phenyl ring with Phe_{357} in S_3 pocket (Fig. 1; Supplementary data). These additional interactions of **16c** in S_3 pocket justify its 3-fold potent DPP-IV inhibitory activity (in vitro) over NVP-DPP728.

Kinetic study of DPP-IV inhibition by radiolabeled NVP-DPP728 established its slow-binding inhibition mechanism and nitrile functionality play crucial role in the formation of the high-affinity complex, via imidate intermediate.[12] Due to unavailability of radiolabeled **16c**, inhibitory kinetic of **16c** was not evaluated. However, docking studies supports involvement of key interactions of **16c** with all the three pockets, which apparently establish slow-binding kinetic of **16c** as cyanopyrrolidine class effect.

In summary, we report SPPS approach to discover peptidomimetic based cyanopyrrolidine derivatives as potent, selective and long acting DPP-IV inhibitors for an effective treatment of T2DM. The lead compound **16c** showed sustained suppression of pre-

Table 3
Pharmacokinetic study parameters[a] of **16c**, **16d** and NVP-DPP728

Compd	T_{max} (h)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (0-∝) h μg/ml	F^* (%)
16c	0.29 ± 0.11	7.1 ± 0.83	7.99 ± 0.33	14.3 ± 1.13	72.5
16d	0.28 ± 0.10	5.9 ± 0.88	0.99 ± 0.14	6.89 ± 1.21	63.1
NVP-DPP728	0.32 ± 0.08	6.2 ± 0.91	0.88 ± 0.11	6.49 ± 1.11	65

[a] In male C57BL/6 J mice ($n = 6$), compounds were administered orally (po) at 20 mg/kg dose and plasma concentration was analyzed by LC–MS, values indicate Mean ± SD.
[*] Oral bioavailability (%F) was calculated wrt to iv AUC (**16c**: 11.02 ± 0.11; **16d**: 10.92 ± 0.12 & NVP-DPP728: 9.98 ± 0.09 h μg/ml) administered at 20 mg/kg dose, iv.

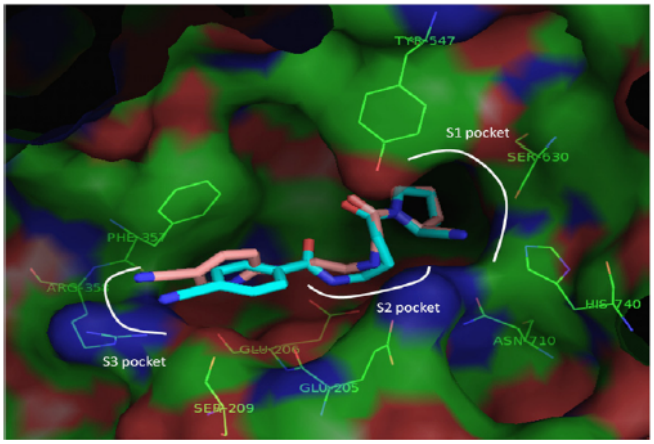


Figure 4. Key interactions of compound **16c** and NVP-DPP728 with active sites of DPP-IV enzyme. Binding pose of compound **16c** (Turquoise) and **NVP-DPP728** (Rose) in the DPP-IV active site is indicated (Surface view: Green), wherein both compounds interact closely with key residues of site S1, S2 and S3.

and post-prandial blood glucose levels (in vivo), which correlates with its extended PK profile.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.078.

References and notes

1. (a) Holst, J. J. *Diabetologia* **2006**, *491*, 253; (b) Baggio, L. L.; Drucker, D. J. *Gastroenterology* **2007**, *132*, 2131; (c) Havale, S. H.; Pal, M. *Bioorg. Med. Chem.* **2009**, *17*, 1783.
2. (a) Heins, J.; Weiker, P.; Schonlein, C.; Born, I.; Hartrodt, B.; Neubert, K.; Tsuru, D.; Barth, A. *Biochim. Biophys. Acta* **1988**, *954*, 161; (b) Hegen, M.; Niedobitek, G.; Klein, C. E.; Stein, H.; Fleischer, B. J. *Immunology* **1990**, *144*, 2908; (c) Zhu, L.; Tamvakopoulos, C.; Xie, D.; Dragovic, J.; Shen, X.; Fenyk-Melody, J. E.; Schmidt, K.; Bagchi, A.; Griffin, P. R.; Thornberry, N. A.; Roy, R. S. *J. Biol. Chem.* **2003**, *278*(25), 22418.
3. Augustyns, K.; Veken, P. V.; Senten, K.; Haemers, A. *Curr. Med. Chem.* **2005**, *12*, 971.
4. Gupta, R.; Walunj, S. S.; Tokala, R. K.; Parsa, V. L.; Singh, S.; Pal, M. *Curr. Drug Targets* **2009**, *10*, 71.
5. (a) Peters, J. *Curr. Top. Med. Chem.* **2007**, *7*, 579; (b) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. *J. Med. Chem.* **2002**, *45*, 2362; (c) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774; (d) Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025; (e) Haffner, C. D.; McDougald, D. L.; Randhawa, A. S.; Reister, S. M.; Lenhard, J. M. PCT Int. Appl. WO2003/002531, 2003.; (f) Mulakayala, M.; Reddy, C. H. U.; Iqbal, J.; Pal, M. *Tetrahedron* **2010**, *66*, 4919.
6. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
7. Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L., II *J. Med. Chem.* **2007**, *50*, 2297; (b) Parsa, K. V. L.; Pal, M. *Exp. Opin. Drug. Disc.* **2011**, *6*, 855.
8. (a) Oefner, C.; D'Arcy, A.; Sweeney, A. M.; Pierau, S.; Gardiner, R.; Dale, G. E. *Acta Crystallogr., Sect. D* **2003**, *59*, 1206; (b) Engel, M.; Hoffmann, T.; Wagner, L.; Wermann, M.; Heiser, U.; Kiefersauer, R.; Huber, R.; Bode, W.; Demuth, H.; Brandstetter, H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*(9), 5063; (c) Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. *Nat. Struct. Biol.* **2003**, *10*, 19.
9. (a) Kang, N. S.; Ahn, J. H.; Kim, S. S.; Chae, C. H.; Yoo, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3716; (b) Kuhn, B.; Hennig, M.; Mattei, P. *Curr. Top. Med. Chem.* **2007**, *7*, 609.
10. Bohannon, N. *Postgrad. Med.* **2009**, *121*, 40.
11. Deacon, C. F. *Diabetes Obes. Metab.* **2011**, *13*, 7. and referances cited therein.
12. Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. *Biochemistry* **1999**, *38*, 11597.
13. Singh, S.; Sethi, S.; Khanna, V.; Benjamin, B.; Kant, R.; Sattigeri, J.; Bansal, V. S.; Bhatnagar, P.; Davis, J. A. *Eur. J. Pharmacol.* **2011**, *652*, 157.
14. Sheppard, R. C.; Atherton, E. *Solid Phase Peptide Synthesis- a practical approach*. I.R.L. Press publication, Oxford University Press.
15. North, M.; Pattnden, G. *Tetrahedron* **1990**, *46*, 8267.
16. (a) Blackmon, D. L.; Watson, A. J.; Montrose, M. H. *Anal. Biochem* **1992**, *200*, 352; (b) In vitro enzyme (DPP-IV, DPP-2, DPP-8 and DPP-9) inhibitory activity was determined using fluorescence-based assay. The Gly-Pro-AMC was used as a substrate (which is cleaved by the enzymes to release the fluorescent AMC) and soluble human proteins (DPP-IV, DPP-2, DPP-8 and DPP-9 enzymes) produced in a baculovirus expression system (Life Technologies) was used as the enzyme source. The H-Gly-Pro-AMC (200 μM) was incubated with either DPP-IV, DPP-2, DPP-8 or DPP-9 enzymes in the presence of various concentrations of test compounds. Reaction was carried out at pH 7.8 (HEPES buffer 25 mM containing 1.0% BSA, 140 mM NaCl, 16 mM $MgCl_2$, 2.8% DMSO) in a total volume of 100 μl at 25 °C for 30 min., in the dark. Reaction was terminated with acetic acid (25 μl of 25% solution). Activity (fluorescence) was measured using Spectra Max fluorometer (Molecular Devices, Sunnyvale CA) by exciting at 380 nm and emission at 460 nm. The IC_{50} values were determined for test compounds using Graph Pad prism software.
17. (a) Chen, D.; Wang, M. *Diabetes Obes. Metab.* **2005**, *7*, 307; (b) Kim, J. G.; Baggio, L. L.; Bridon, D. P.; Castaigne, J. P.; Robitaille, M. F.; Jette, L.; Benquet, C.; Drucker, D. J. *Diabetes* **2003**, *52*, 751; (c) Study was conducted in male C57BL/6J (using IPGTT protocol) or without glucose load, in db/db mice (age 8–12 weeks). All animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'. Two days prior to the study, the animals were randomized and divided into 2 groups ($n = 6$), based upon their fed glucose levels. Animals were left for 2 days under acclimatization and maintained on a standard diet. On the day of experiment, food was withdrawn from all the cages, water was given ad-libitum and were kept for overnight fasting. Briefly, in IPGTT protocol (C57 mice) overnight fasted mice were dosed orally (po) with the test compounds (20 mg/kg), 0.5 h prior to the intraperitoneal (ip) glucose load (1.5 g/kg), while in db/db mice, fed mice were dosed orally (po) with the test compounds (20 mg/kg) and the blood samples were collected at various time points. Blood samples were centrifuged and the separated serum was immediately subjected for the glucose estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, California). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (Ver 4.0) was used to plot an area under the curve (0–240 min AUC). The AUC obtained from graphs were analyzed for two-way ANOVA, followed by Bonferroni post test, using Graph Pad prism software.
18. Briefly, for single dose PK study, test compounds were administered orally/iv on a body weight basis (20 mg/kg) to overnight fasted male C57BL/6 J mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 30 h post-dose after compounds administration. Approximately 0.3 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at −70 °C and the concentrations of compounds in plasma were determined by the LC–MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C_{18} (2.0 × 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters were calculated using a non-compartmental model of WinNonlin software version 5.2.1.
19. (a) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.* **2006**, *49*, 6177; (b) *Schrodinger Suite 2010, Glide version 5.6, Prime version 2.2*; Schrodinger, LLC: New York, 2010.

Design of Peptidomimetic Based DPP-IV Inhibitors, Devoid of CYP Liabilities#

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Abstract: A peptidomimetic based cyanopyrrolidine derivatives are reported as potent and selective DPP-IV inhibitors. Some of the test compounds (**10l** and **10m**) showed excellent potency and selectivity towards DPP-IV over various serine proteases, without CYP inhibition.

Keywords: CYP, cyanopyrrolidine, DPP-IV inhibitors, Selectivity, Peptidomimetic.

INTRODUCTION

Obesity and diabetes are emerging as the global epidemic of the 21st century and becoming major health problems worldwide.[1] Type 2 diabetes mellitus (T2DM) is characterized by elevated levels of blood glucose, resulting from impaired insulin secretion and/ or insulin resistance.[2] Currently diabetic patients are treated with various antihyperglycemic agents; however, due to the progressive nature of the disease, most of the available antihyperglycemic agents loose sustained glycemic control over a period of time.[3-4] Also, adverse events associated with the existing antihyperglycemic agents raise safety concerns.

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease, [5] which selectively cleaves the N-terminal dipeptide from the penultimate position of Glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagon-Like Peptide (GLP-1) thus makes them inactive.[6-7] Inhibition of DPP-IV activity extend the duration of action of endogenous GLP-1, thereby stimulating insulin secretion, inhibiting glucagon release and slowing gastric emptying.[8-9] Because of these multiple benefits of GLP-1 mediated glucose homeostasis, orally bioavailable DPP-IV inhibitors has been developed as promising therapeutic agents for the treatment of T2DM.

DPP-IV inhibitors offer a number of potential advantages over existing diabetes therapies, including a lowered risk of hypoglycemia and weight gain. Consequently, various DPP-IV inhibitors such as Vildagliptin (NVP-LAF237, **1**), Saxagliptin (BMS-477118, **2**) and Denagliptin (GW-823093, **3**), including Sitagliptin are in clinic for the effective treatment of T2DM Fig. (**1**) [10-14].

DPP-IV enzyme exhibits close structural analogy with several other serine proteases. So while designing new class of DPP-IV inhibitors, it is essential to consider selectivity of DPP-IV inhibitors over other serine protease, especially DPP-2, DPP-8 and DPP-9.[15-16] The X-ray crystal study of various inhibitors in complex with DPP-IV represents three major binding pockets/ sites as S_1, S_2 and S_3. The S_1 pocket consists of catalytic triad (Ser_{630}, Asn_{710} and His_{740}), S_2 pocket consists of Glu dyad (Glu_{205} and Glu_{206}), while S_3 pocket involves interactions with Ser_{209}, Arg_{358} and Phe_{357}.[17-18] Low nanomolar potency can be achieved by optimizing favorable interactions with S_1 and S_2 pockets. The S_3 pocket of DPP-IV differs a lot from DPP-8/9 and the precise interactions with Phe_{357} govern selectivity against DPP-8 and 9 [19-20].

Though several DPP-IV inhibitors are in the market, attempts are still underway to develop potent and selective DPP-IV inhibitors devoid of side effects associated with existing DPP-IV inhibitors.[21] Recently, we disclosed a series of cyanopyrrolidine based peptidomimetics as potent, selective and long acting DPP-IV inhibitors (Compound **4**, Fig. (**1**). [22] However, upon secondary profiling of compound **4**, CYP3A4 and CYP2D6 inhibitions (IC_{50}: 1.1 and 1.9 μM respectively) were observed, which halted its further preclinical development.

Cytochrome P450 (CYP450) enzymes are predominantly expressed in the liver and are essential for the detoxification and the metabolism of drugs. In addition to antidiabetic drugs diabetic patients are treated with a number of other drugs, including anti-hypertensive and lipid-lowering agents. Notably, more than 50% of these drugs are metabolized by CYP3A4 or CYP2D6 enzymes. Drugs can inhibit (decrease), induce (increase) CYP metabolism or may act as a substrate

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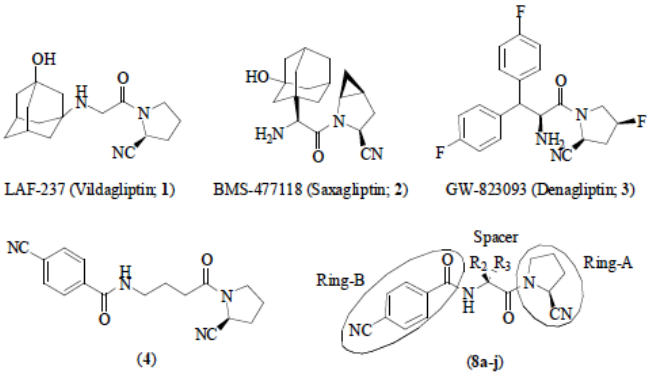


Scheme 1. Structurally diverse small molecule based-DPP-IV inhibitors.

for CYP enzymes. Inhibition of CYP metabolism will likely increase the affected drug's systemic concentrations, whereas induction of metabolism often reduces systemic concentrations [23].

CYP3A4 or CYP2D6 inhibition and induction is clinically relevant to diabetic patients, especially when treated with antidiabetic agents such as Sulfonylureas, Metformin and Meglitanides. For example, Sulfonylureas are known substrates of CYP. Thus inducers and inhibitors of CYP can affect the metabolism of Sulfonylureas. Similarly, Repaglinide is metabolized by the CYP3A4 and a serious drug-drug interactions (DDI) may occur when it is co-administered with CYP inhibitor, such as Gemfibrozil (triglyceride lowering agent), as it increases eightfold exposure of Repaglinide. Pioglitazone is a substrate for CYP3A4 and can be affected by inhibitors (Verapamil, Diltiazem) or inducers (Carbamazepine, Rifampin) of CYP3A4. Thus, CYP inhibition/ induction can have significant consequences on other antidiabetic drugs that are metabolized by these enzymes, which may result in DDI and idiosyncratic drug toxicity (IDT) [24, 25].

In general, affinity of a molecule for CYP can be attenuated by increasing / decreasing the carbon chain length.[26] So to overcome CYP liabilities, amino-alkyl spacer (-$(CH_2)_3$-; 3C) of compound **4** was specifically reduced from 3C to 2C (-$(CH_2)_2$-) and 1C (-CH_2-) and the resulting molecules were examined for CYP inhibitions. Compound **4** with 2C amino-alkyl spacer (i.e. compound **16a** reported in our previous publication,[22] with DPP-IV inhibitory IC_{50}: 10.3 nM) showed weaker CYP3A4 and CYP2D6 inhibitions (IC_{50}: 9.3 and 10.1 μM respectively), while compound **8a** Table **1** with 1C amino-alkyl spacer showed no CYP3A4 and CYP2D6 inhibitions up to 100 μM. However, **8a** showed weak DPP-IV inhibitory activity (IC_{50}: 722 nM). Thus reduction of amino-alkyl spacer attenuates CYP inhibitions but led to a significant drop in DPP-IV inhibitory activity.

Further to improve DPP-IV inhibitory activity of **8a**, two series (**8b-j** and **10a-m**) of structurally constrained cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors were designed. In the first series suitable modifications were carried out on 1C amino-alkyl spacer of **8a** and altogether nine compounds (**8b-j**) were prepared by linking ring A with ring B, using various α-substituted amino acids spacers. In the second series, thirteen compounds (**10a-m**) were prepared by modifying the best compound obtained from first series, specifically by carrying out suitable changes over ring-A and -B.

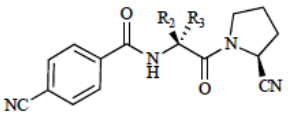
CHEMISTRY

Synthesis (Scheme **1**) of peptidomimetics (**8a-j** and **10a-m**) was carried out using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach, starting from commercially available Rink-amide MBHA resin, preloaded with Fmoc-protected prolines (**5a-b**).[27] Deprotection of **5a-b** with piperidine (20% DMF) and 1,3-diisopropycarbodiimide (DIC) coupling with Fmoc-protected amino acids provided the resin-bound Fmoc-protected dipeptides (**6a-k**). Deprotection of **6a-k** with piperidine (20% DMF) and DIC coupling with substituted benzoic acids gives resin-bound tripeptides, which upon Trifluroacetic acid (TFA) mediated cleavage gives pyrrolidinecarboxamides (**7a-j** and **9a-m**). Trifluroacetic anhydride (TFAA) mediated dehydration of pyrrolidinecarboxamides (**7a-j** and **9a-m**) afforded title compounds as pyrrolidinecarbonitriles (**8a-j** and **10a-m**).[28] All the test compounds obtained were purified by preparative HPLC (yield 70-85%; HPLC purity >97%) and characterized by various spectroscopic technique (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined and the results were within ±0.04% of theoretical values (see supplementary information for analytical and spectral data).

In Vitro DPP-IV INHIBITION STUDY

The *in vitro* DPP-IV inhibitory activity was determined using fluorescence-based enzymatic assay. The Gly-Pro-AMC was used as a substrate. The substrate was incubated with DPP-IV enzymes in the presence of various concentrations of test compounds. Activity (fluorescence) was measured using Spectra Max fluorometer (Molecular

Table 1. *In vitro* **DPP-IV inhibitory activity***



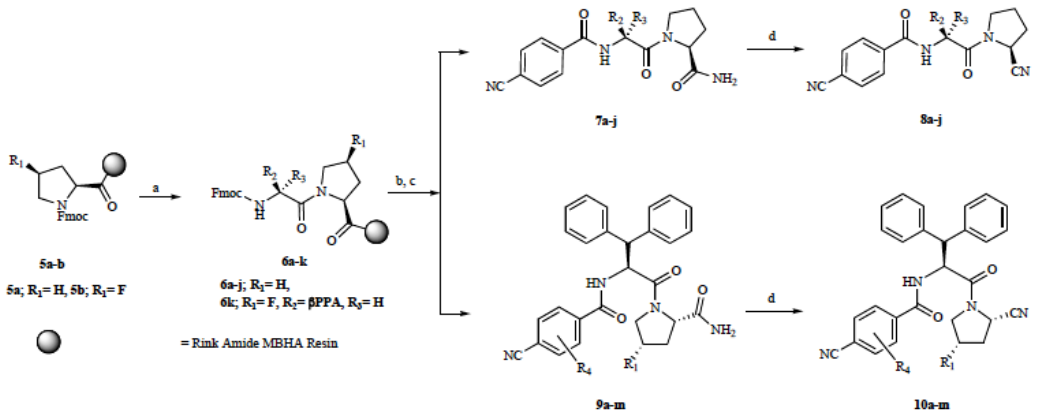
8a-j

S. No	R_2	R_3	Amino acids[$]	DPP-IV inhibition**
8a	H	-H	Gly	722 ± 3.4
8b	$-CH(CH_3)_2$	-H	Val	74 ± 2.4
8c	$-CH(CH_3)(C_2H_5)$	-H	Ile	39 ± 1.2
8d	$-CH_3$	$-CH_3$	Aib	157 ± 3.3
8e	cyclohexyl	-H	Chg	97 ± 2.7
8f	-Ph	-H	Phg	463 ± 3.8
8g	-Bz	-H	Phe	239 ± 1.9
8h	2-F Bz	-H	2-F Phe	197 ± 3.6
8i	2-F Bz	$-CH_3$	α-Me-2-F Phe	137 ± 4.9
8j	$-CH(Ph)_2$	-H	βPPA	**27 ± 1.6**
1	--	--	--	3.2 ± 0.5
4	--	--	--	2.3 ± 0.9

*DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software

** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3)

[$] R_2, R_3 together represents amino acids with absolute (S) stereo configuration.



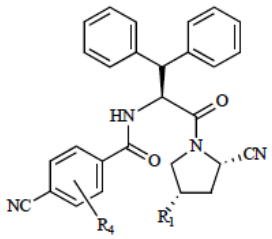
Scheme 1: Synthesis of compounds **8a-j** and **10a-m**

[a] Reagents and conditions: (a) 1. 20% Piperidine in DMF; 2. Fmoc-NH-(CHR_2R_3)-COOH, DIC, DMF, N_2; (b) 1. 20% Piperidine in DMF; 2. Substituted benzoic acids, HOBt, DIC, DMF, N_2; (c) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 3h; (d) TFAA, CH_2Cl_2, 25 °C, 6h.

Devices, Sunnyvale CA) by exciting at 380 nm and emission at 460 nm. The IC_{50} values were determined for test compounds using Graph Pad prism software.[29]

As shown in Table **1-2**, two series of peptidomimetics (**8a-j** and **10a-m**) were prepared and depending on the nature of substitutions, different degree of DPP-IV inhibitory activity was observed. In the first series, upon linking

Table 2. ***In vitro*** **DPP-IV inhibitory activity∗**



10a-m

S. No	R_1	R_4	DPP-IV inhibition∗∗	DPP2$^{\$}$	DPP8$^{\$}$	DPP9$^{\$}$
10a	-H	2-CH_3	34 ± 2.9	---	---	---
10b	-H	2-F	22 ± 1.7	---	---	---
10c	-H	3-CH_3	18 ± 1.3	---	---	---
10d	-H	3-F	9.6 ± 0.6	>25,000	>15,000	>15,000
10e	-H	2,5-di-CH_3	31 ± 2.4	---	---	---
10f	-H	2,5-di-F	19 ± 0.7	---	---	---
10g	-H	3-OH	28 ± 2.7	---	---	---
10h	-H	3-OCH_3	23 ± 1.9	---	---	---
10i	-H	3-Cl	11 ± 0.8	>25,000	>15,000	>15,000
10j	-H	3-CN	17 ± 1.3	---	---	---
10k	-H	3-CF_3	14 ± 2.1	---	---	---
10l	-F	3-Cl	**4.2 ± 0.7**	>25,000	>15,000	>15,000
10m	-F	3-F	**2.7 ± 0.3**	>25,000	>15,000	>15,000
3∗∗∗	--	--	19 ± 3.2			
4	--	--	2.3 ± 0.9	>25,000	>15,000	>15,000

∗DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
∗∗ DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3).
$^{\$}$ DPP2, DPP8 and DPP9 inhibitory activity represented as fold-selectivity wrt DPP-IV inhibitory activity.
∗∗∗ Reported literature value for Denagliptin 22 nM (Ref: 12)

cyanopyrrolidine (ring A) with *para*-cyanobenzoic acid (ring B), using α-substituted amino acid spacers (Val; **8b**, Ile; **8c** or cyclohexyl glycine (Chg); **8e**), compounds **8b**, **8c** and **8e** showed moderate DPP-IV inhibitory activities. When amino-isobutyric acid (Aib); **8d** or α-methyl-2-fluoro phenyl alanine (α-Me-2-F Phe); **8i**, were introduced as spacer, the resulting compounds however showed weak *in vitro* activities. The compounds **8f**, **8g** and **8h** containing phenyl glycine (Phg), phenyl alanine (Phe) and 2-fluoro phenyl alanine (2-F-Phe) respectively as spacers were also found to be the least potent. However compound **8j** with β-phenyl phenyl alanine (β-PPA) showed the highest DPP-IV inhibitory activity (IC_{50}: 27 nM) within the series.

The first series was specifically designed as analogs of **8a**, to understand the role of α-substituents on 1C amino-alkyl spacer so as to get the low nM DPP-IV inhibitory activity. The SAR study of first series reveals that the DPP-IV inhibitory activity of test compounds drastically varies with the nature of α-substituents and among various substituents screened, β-PPA was found to be favorable. It appears that the DPP-IV enzyme accepts changes in limited steric bulk at S_2 binding pocket, which might be due to the stapled orientation of Glu-dyad in S_2 pocket.

Compound **8j** was identified as primary hit from the first series. Further to improve DPP-IV inhibitory activity of **8j**, second series (**10a-m**) was designed, specifically by carrying out suitable changes over ring-A and -B of **8j** and in second series, five sets of compounds were prepared Table **2**. Substitutions were carried out in set-1 (**10a** and **10b**) on 2nd position, in set-2 (**10c** and **10d**) on 3rd position and in set-3

(**10e** and **10f**) on 2nd and 5th positions of cyano-benzamide (ring-A) , either with electron withdrawing (EW) or electron donating (ED) groups. In set-4 (**10g-10k**), substitutions were carried out specifically on 3rd position of cyano-benzamide (ring-A). Finally, based upon the literature presidencies (favorable substitution of 4F- pyrrolidine in Denagliptin), set-5 (**10l** and **10m**) was prepared by substituting 4th position of cyano-pyrrolidine (ring-B) with fluoro group, to improve the DPP-IV inhibitory activity [30, 31].

All the test compounds from the second series showed significant DPP-IV inhibitory activities. Set-1 and 2 showed improved but similar DPP-IV inhibitory activities, irrespective of electron withdrawing (EW) or electron donating (ED) nature of the substituents. Compare to Set-1 and 3, Set-2 showed very good DPP-IV inhibitory activities.

Based on these results, further changes were made only at 3rd position of cyano-benzamide, as set-4 (**10g-10k**). In set-4, compounds **10j and 10k** with EW groups at *meta* position of cyano-benzamide showed higher DPP-IV inhibitory activities than compounds **10g and 10h**, with ED groups. Among all the compounds tested from second series, halo substituted compounds (**10d** and **10i**) showed excellent DPP-IV inhibitory activities (IC_{50}: 9.6 and 11 nM respectively). The 4-fluoropyrrolidine-carbonitrile derivatives (**10l** and **10m**, set-5) of **10d** and **10i** showed further improvement in DPP-IV inhibitory activities (IC_{50}: 4.2 and 2.7 nM respectively, similar to compound **4**), which could be due to the favorable interactions of 4-fluoro pyrrolidine-carbonitrile with the key residues of S_1 pocket.

In Vitro DPP-IV SELECTIVITY AND CYP INHIBITION STUDIES

The *in vitro* selectivity over serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for most potent compounds **10d**, **10i**, **10l** and **10m** (fold-selectivity listed in Table **2**. [29] All the test compounds showed >25000-fold selectivity over DPP-2 and >15000-fold selectivity over DPP-8 and DPP-9, which was found to be comparable with reference standard compound **4**. Among all the compounds tested, **10l** and **10m** were found to be most potent and selective. To assess the CYP liabilities of these peptidomimetics, **10l** and **10m** were subjected for CYP3A4 and CYP2D6 inhibition studies. For CYP3A and CYP2D6 inhibition studies, Human liver microsomes (0.2 mg/ml), Testosterone (50 µM) / Dextromethorphan (5 µM) respectively, as probe substrates for CYP3A and CYP2D6 were incubated with different concentrations of test compounds at 37°C for 10 min., enzyme activity (% of control) was determined by HPLC-MS/MS and IC_{50} values were calculated. Both the test compounds were found to be devoid of CYP3A4 and CYP2D6 inhibition up to 100 µM concentrations [32].

MOLECULAR MODELING

The molecular docking analysis of **8a**, **10m** and Denagliptin was carried out using extra precision (XP) Glide docking method, to understand their critical interactions with all the three binding sites (S_1, S_2 and S_3) of DPP-IV enzyme (Fig. **2**; binding poses overlay of **8a** (Turquoise), **10m**

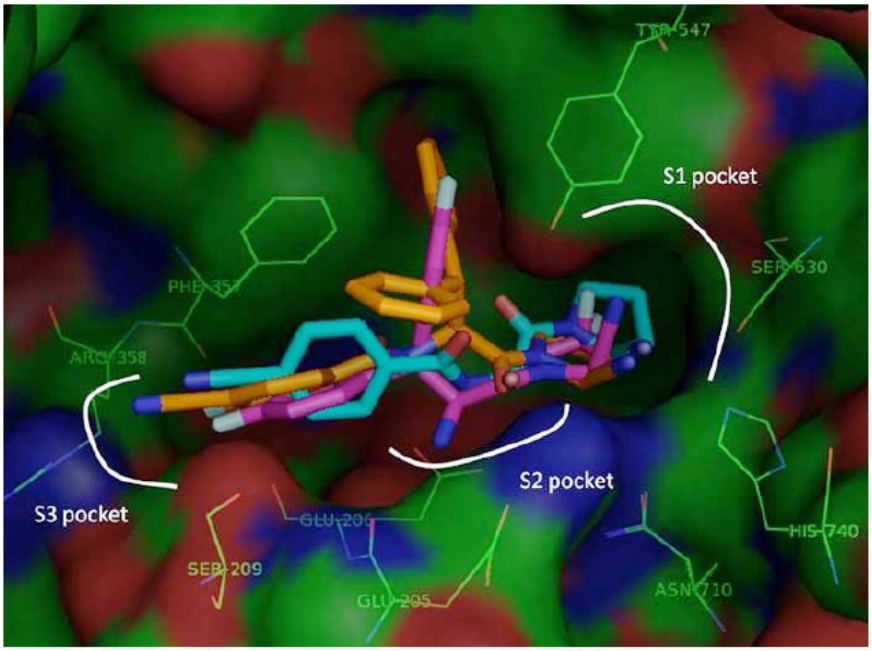


Fig. (2). Key interactions of compounds **8a**, **10m** and Denagliptin with active sites of DPP-IV enzyme.

Binding pose of compound 8a (Turquoise), 10m (Brown) and Denagliptin (Rose) in the DPP-IV active site is indicated (Surface view: Green), wherein compounds 10m and Denagliptin interacts closely with key residues of site S_1, S_2 and S_3.

(Brown) and Denagliptin (Rose)).[19-20, 33-34] The crystal structure of the DPP-IV enzyme (PDB ID: 2ajl) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box. For docking study, the ligands were minimized by applying an OPLS-AA force field, using ligprep module of Schrödinger.

The results of docking studies illustrate that all the three compounds interact closely with the key residues of S_1 pocket (as per literature presidencies, cyanopyrrolidine-CN may form covalent bond with OH-group of side-chain of Ser_{630}). In S_2 pocket, benzamide-NH of **10m** and α-amino group of Denagliptin forms H-bonding with C=O groups of side-chains of Glu_{205} and Glu_{206} dyad, while benzamide-NH of **8a** flip away from the Glu dyad. Compound **10m** interact closely in S_3 pocket (aromatic–CN forms H-bonding with the NH of guanidine side-chain of Arg_{358}), while **8a** interact weakly with the key residues of S_2 and S_3 pockets, which may justify its weak *in vitro* DPP-IV inhibitory activity.

Molecule **10m** in our docking studies has shown CH•••π, OH•••π and π•••π interactions. Diphenylmethane in **10m** has all the three aromatic interactions. One of the phenyl ring makes a CH•••π with Phe 357 and Tyr 547. At the same time the same Phe 357 and hydroxyl of Tyr 547 forms a π•••π and OH•••π stabilizing interactions with diphenylmethane. In addition to the above-mentioned aromatic interactions, we have also noticed that fluoro and cyano substituted pyrrolidine forms two CH•••π with Tyr 662 and 666. All the CH•••π interactions are with a range of 2.4 to 2.6 Å, while OH•••π is 2.5 Å and π•••π is 4.4 Å. This π•••π interaction between Phe 357 and diphenylmethane has a parallel alignment of aromatic rings and this might have resulted from the inductive effect of functionalites of respective phenyl rings.

Incorporation of β-PPA linkage (spacer) in **10m** allows it to adopt new confirmation, which may favors covalent interaction of cyanopyrrolidine ring with Ser_{630} (S_1 pocket, covalent interaction of cyanopyrrolidine ring as reported for cyanopyrrolidine derivatives), strong H-bonding of back-bone benzamide-NH with Glu dyad (S_2 pocket) and *para*-nitrile benzamide with Arg_{358}, including aromatic π- π stacking of benzamide with Phe_{357} in S_3 pocket. As observed with Denagliptin, **10m** docks very well into all the three sites (S_1, S_2 and S_3) of DPP-IV crystal structure and these favorable interactions of **10m** across all the three sites of DPP-IV enzyme support its potent *in vitro* DPP-IV inhibitory activity and excellent selectivity over other protease.

CONCLUSION

In summary, we have reported a SPPS approach to discover peptidomimetic based cyanopyrrolidines derivatives as potent and selective inhibitors of DPP-IV and devoid of CYP liabilities. Some of these novel peptidomimetics showed excellent *in vitro* potency and selectivity over other serine proteases, due to their favorable orientations across all the three binding sites.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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DISCLOSURE

The authors declare that there is no conflict of interest that would prejudice the impartiality of the research reported herein.

REFERENCES

[1] Smyth, S.; Heron, A. Diabetes and obesity: the twin epidemics. *Nat. Med.* **2006**, *12*, 75-80.

[2] Hussain, A.; Claussen, B.; Ramachandran, A.; Williams, R. Prevention of type 2 diabetes: a review. *Diabetes Res. Clin. Pract.* **2007**, *76(3)*, 317-326.

[3] Modi, P. Diabetes beyond insulin: review of new drugs for treatment of diabetes mellitus. *Curr. Drug Discov. Tech.* **2007**, *4*, 39-47.

[4] Tahrani, A. A.; Piya, M. K.; Kennedy A.; Barnett, A. H. Glycaemic control in type 2 diabetes: Targets and new therapies. *Pharmacol. Ther.* **2010**, *125*, 328-361.

[5] Lambeir, A. M.; Durinx, C; Scharpé, S; Meester, I. Dipeptidyl-peptidase IV from bench to bedside: An update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Critical Reviews in Clinical Laboratory Sciences*. **2003**, *40(3)*, 209-294.

[6] Deacon, C. F.; Johnsen, A. H.; Holst, J. J. Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. *J Clin. Endocrinol. Metab.* **1995**, *80*, 952-957.

[7] Zhu, L.; Tamvakopoulos, C.; Xie, D.; Dragovic, J.; Shen, X.; Fenyk-Melody, J. E.; Schmidt, K.; Bagchi, A.; Griffin, P. R.; Thornberry, N. A.; Roy, R. S. The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides. *J. Biol. Chem.* **2003**, *278(25)*, 22418.

[8] Nauck, M. A.; Wollschlager, D.; Werner, J.; Holst, J. J.; Orskov, C.; Creutzfeldt, W.; Willms, B. Effects of subcutaneous glucagonlike peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia* **1996**, *39*, 1546-1553.

[9] Drucker, D. J. Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* **2002**, *122 (2)*, 531-544.

[10] Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine: A potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* **2003**, *46*, 2774-2789.

[11] Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2005**, *48*, 5025-5037.

[12] Haffner, C. D.; McDougald, D. L.; Randhawa, A. S.; Reister, S. M.; Lenhard, J. M. Fluoropyrrolidines as dipeptidyl peptidase inhibitors. *PCT Int. Appl.* WO2003/002531, January 9, 2003.

[13] Havale, S. H.; Pal, M. Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. *Bioorg. Med. Chem.* **2009**, *17*, 1783-1802.

[14] Gupta, R.; Walunj, S.S.; Tokala, R.K.; Parsa, K.V.L.; Singh, S. K.; Pal, M. Emerging Drug Candidates of Dipeptidyl Peptidase IV

(DPP IV) Inhibitor Class for the Treatment of Type 2 Diabetes. *Current Drug Targets*, **2009**, *10*, 71-87.

[15] Gorrell, M. D. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clinical Science* **2005**, *108*, 277-292.

[16] Kirby, M.; Yu, D. M.T.; O'Connor, S. P.; Gorrell, M. D. Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. *Clinical Science* **2010**, *118*, 31-41.

[17] Engel M.; Hoffmann, T.; Wagner, L.; Wermann, M.; Heiser, U.; Kiefersauer, R.; Huber, R.; Bode, W.; Demuth, H.; Brandstetter, H. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. *Proc. Natl. Acad. Sci. U.S.A* **2003**, *100(9)*, 5063-5068.

[18] Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. Crystal structure of human DPP-IV/CD26 in complex with a substrate analogue. *Nat. Struct. Biol.* **2003**, *10*, 19-25.

[19] Kang, N. S.; Ahn, J. H.; Kim, S. S.; Chae, C. H.; Yoo, S. Docking-based 3D-QSAR study for selectivity of DPP4, DPP8, and DPP9 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3716-3721.

[20] Kuhn, B.; Hennig, M.; Mattei, P. Molecular Recognition of Ligands in Dipeptidyl Peptidase IV. *Curr. Top. Med. Chem.* **2007**, *7*, 609-619.

[21] Deacon, C. F. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes Obes. Metab.* **2011**, *13*, 7-18.

[22] Jadav, P.; Bahekar, R.; Shah, S. R.; Patel, D.; Joharapurkar, A.; Kshirsagar, S.; Jain, M.; Shaikh, M.; Sairam, K. V. V. M. Long-acting peptidomimetics based DPP-IV inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3516-3521.

[23] Guengerich, F. P.; Hosea N. A.; Parikh, A.; Bell-Parikh, L. C.; Johnson, W. W.; Gillam, E.M.J.; Shimada, T.; Twenty years of biochemistry of human P450s: purification, expression, mechanism and relevance to drugs. *Drug Metab Dispos.* **1998**, *26*, 1175-1178.

[24] Niemi, M.; Backman, J. T.; Neuvonen, M.; Neuvonen, P. J. Effects of Gemfibrozil, itraconazole and their combination on the pharmacokinetics and pharmacodynamics of Repaglinide: potentially hazardous interaction between Gemfibrozil and Repaglinide. *Diabetologia,* **2003**, *46*, 347-351.

[25] Baldwin, S. J.; Clark, S. E.; Chenery, R. J.; Characterization of the cytochrome P450 enzymes involved in the *in vitro* metabolism of rosiglitazone. *Br J Clin Pharmacol.* **1999**, *55*, 53-56.

[26] Halliday, R. C.; Jones, B. C.; Park, B. K.; Smith, D. A. Synthetic strategies to lower affinity for CYP2D6. *Eur. J. Drug Metab. Pharmacokin.* **1997**, *22*, 291-294.

[27] Sheppard, R. C.; Atherton, E. Solid Phase Peptide Synthesis- a practical approach, I.R.L. Press publication, Oxford University Press.

[28] North, M.; Pattnden, G. Synthetic studies towards cyclic peptides. Concise synthesis of thiazoline and thiazole containing amino acids. *Tetrahedron* **1990**, *46*, 8267-8290.

[29] Blackmon, D.L.; Watson A. J.; Montrose, M. H. Assay of apical membrane enzymes based on fluorogenic substrates. *Anal. Biochem* **1992**, *200*, 352-358.

[30] Fukushima, H.; Hiratate, A.; Takahashi, M.; Saito, M.; Munetomo, E.; Kitano, K.; Saito, H.; Takaoka, Y.; Yamamoto, K. Synthesis and structure–activity relationships of potent 3- or 4-substituted-2-cyanopyrrolidine dipeptidyl peptidase IV inhibitors. *Bioorg. Med. Chem.* **2004**, *12*, 6053-6061.

[31] Senten, K.; Van der Veken, P.; De Meester, I.; Lambeir, A. M.; Scharpe, S.; Haemers, A.; Augustyns, K. Design, synthesis, and SAR of potent and selective dipeptide-derived inhibitors for dipeptidyl peptidases. *J. Med. Chem.* **2003**, *46,* 5005-5014.

[32] Dierks, E. A.; Stams, K. R.; Lim, H. K.; Cornelius, G.; Zhang, H.; Ball, S. E. A method for the simultaneous evaluation of the activities of seven major human drug metabolizing cytocrome P450s using an *in vitro* cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectroscopy. *Drug Metab. Dispos.* **2001**, *29*, 23-29.

[33] Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Proteinâ¯Ligand Complexes. *J. Med. Chem.* **2006**, *49*, 6177-6196.

[34] *Schrodinger Suite 2010, Glide version 5.6, Prime version 2.2*; Schrodinger, LLC: New York, 2010.

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Design, synthesis and biological evaluation of novel aminomethyl-piperidones based DPP-IV inhibitors ☆

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ABSTRACT

A series of novel aminomethyl-piperidones were designed and evaluated as potential DPP-IV inhibitors. Optimized analogue **12v** ((4*S*,5*S*)-5-(aminomethyl)-1-(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)-4-(2,5-difluorophenyl)piperidin-2-one) showed excellent in vitro potency and selectivity for DPP-IV over other serine proteases. The lead compound **12v** showed potent and long acting antihyperglycemic effects (in vivo), along with improved pharmacokinetic profile.

The prevalence of Type 2 diabetes mellitus (T2DM) is rapidly increasing (~371 million diabetic patients worldwide) and there is a great need for new drug classes to ameliorate hyperglycemia, while addressing additional accompanying elements of the metabolic syndrome.[1] Dipeptidyl peptidase-IV (DPP-IV) is a serine protease,[2] responsible for the inactivation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), both of which enhance insulin secretion in a glucose-dependent manner.[3] DPP-IV inhibitors are a new class of oral medications, which have been in use for 7 years, as a second-line therapy. DPP-IV inhibitors are generally well tolerated, safe (low risk of hypoglycemia) and weight neutral. There are currently eight gliptins approved worldwide with several more on the way.[4]

In recent years, a large variety of scaffolds being discovered as a next generation DPP-IV inhibitors, particular efforts being made to develop the long acting DPP-IV inhibitors.[4,5] Two drugs (Omarigliptin and Trelagliptin) are currently under development for once-weekly dosing to improve patients compliance.[4] Structurally, DPP-IV enzyme resembles with several other protease, so while designing new class of DPP-IV inhibitors, it is essential to consider selectivity of DPP-IV inhibitors over other serine protease, especially DPP-2, DPP-8 and DPP-9.[6]

Structurally distinct and rigid analogs of Sitagliptin (**1**), such as *N*-aryl aminopiperidine (**2**), aminopiperidine-fused imidazoles (**3**) and tetrahydropyran (**4**) derivatives were identified as a novel class of DPP-IV inhibitors (Fig. 1).[7–10] These newly discovered DPP-IV inhibitors exhibit potent DPP-IV inhibitory activity, good off-target selectivity and improved pharmacokinetic profiles. Earlier, we reported peptidomimetics based long acting DPP-IV inhibitors.[11] In continuation to our ongoing research on DPP-IV inhibitors, we report herein design, synthesis and biological evaluation of novel aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**) based DPP-IV inhibitors. Title compounds are designed based on the piperidone skeleton and we anticipated that the aminomethyl and the amide groups of the piperidone ring might contribute for improved pharmacokinetic and pharmacodynamic effects, along with the potent and selective DPP-IV inhibitory activity.

As depicted in Scheme 1, synthesis of the aminomethyl-piperidones based DPP-IV inhibitors (**12a–v**, **13a–e** and **14a–e**) commenced with a Horner–Wadsworth–Emmons reaction of aldehydes (**5a–c**), followed by Michael addition, to get diester (**6a–c**). Reduction of nitrile group of **6a–c** by hydrogenation, using Adam's catalyst, followed by cyclization and ester regeneration by trimethylsilyldiazomethane yielded piperidone-carboxylate (**7a–c**), with >85% *trans* selectivity.[12] *Trans* racemic mixture [(3*R*,4*S*) and (3*S*,4*R*)] of (**7a–c**) were isolated in pure form by

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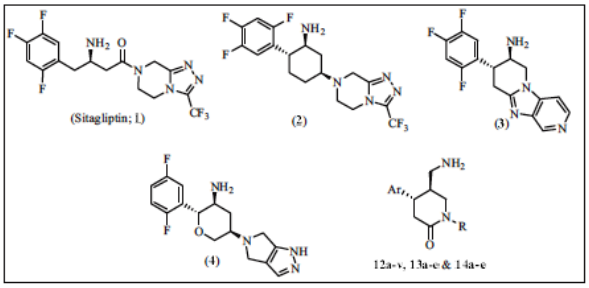
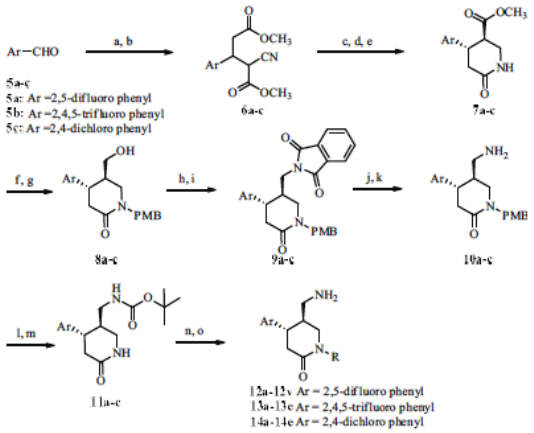


Figure 1. Structurally diverse small molecule based-DPP-IV inhibitors.



Scheme 1. Synthesis of compounds **12a–v**, **13a–e** and **14a–e**. Reagents and conditions: (a) $(Et_2O)_2POCH_2COOMe$, Na_2CO_3, EtOH; (b) $NCCH_2COOMe$, NaOMe, MeOH; (c) H_2, PtO_2, HCl, MeOH; (d) K_2CO_3, toluene/MeOH; (e) Me_3SiCHN_2, Et2O/MeOH; (f) PMB-Br, NaHMDS, THF/DMF(4:1), −78 °C; (g) $LiAlH_4$, THF, 0 °C; (h) CH_3SO_2Cl, NEt_3, DCM, 0 °C; (i) potassium phthalimide, DMF, 90 °C; (j) NH_2–NH_2, EtOH, 25 °C; (k) chiral resolution: D-tartaric acid, MeOH; (l) Boc_2O, NEt_3, THF/H_2O(3:2), 25 °C; (m) CAN, CH_3CN/H_2O(3:1), 25 °C; (n) R–X, CuI, *N,N*-dimethylethylenediamine, toluene, reflux or R–X, NaH, DMF, 0 °C to 25 °C; (o) concd HCl/EtOAc(1:3), −50 °C, 2 h, 0 °C, 1 h.

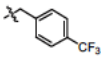
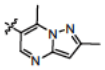
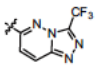
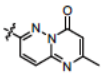
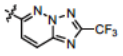
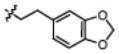
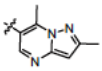
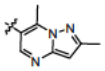
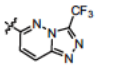
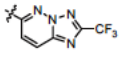
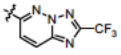
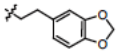
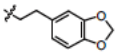
removing corresponding *cis* racemic mixture [(3*R*,4*R*) and (3*S*,4*S*)], by column chromatography (mobile phase: 0–3% methanol in DCM, using 100–200 mesh silicagel). Amide –*NH* protection of *trans* racemic **7a–c** with *para*-methoxy benzyl (PMB) group and reduction of ester with lithium aluminium hydride (LiAlH4) yielded *trans* racemic alcohol (**8a–c**). Subsequently, **8a–c** were converted to a good leaving group (methanesulfonate derivatives), which upon treatment with potassium phthalimide via Gabriel synthesis type reaction lead to the formation of *trans* racemic phthalimido-piperidones (**9a–c**).

Hydrazinolysis of phthalimido group of **9a-c** lead to the formation of *trans* racemic aminopiperidones (**10a–c**). *trans* racemic **10a–c** was subjected for chiral resolution (**10a–c** was added to a solution of D-tartaric acid (1.1 equiv D-tartaric acid, dissolved in 100 ml methanol) and the mixture was stirred for 15 h at 25 °C, solid precipitated was filtered off, washed with methanol (200 ml) and dried to get enantiomerically pure (4*S*,5*S*) desired piperidones (**10a–c**) as a tartrate salt, with >97% ee (chiral HPLC analysis conditions: CHIRALCEL OD-H column, using mobile phase as n-hexane and 0.1% diethyl amine in EtOH (98:02)). Further, protection of primary amine of **10a–c** with Boc-group and subsequent oxidative removal of PMB group gave Boc-aminopiperidones (**11a–c**). Various haloheterocycles/halo-aromatics of the interest were coupled with **11a–c**, by Goldberg reaction[13] or by nucleophilic substitution, followed by Boc-deprotection to get the chiral pure (4*S*,5*S*) aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**).[14] All the test compounds obtained were purified by preparative HPLC (yield 70–85%; HPLC purity >97% and chiral purity >97%ee) and characterized by various spectroscopic techniques (^{13}C NMR, ^1H NMR and ESI MS). Elemental analyses were determined within 0.04% of theoretical values (see Supplementary data for analytical and spectral data).

The in vitro DPP-IV inhibitory activity was determined in order to establish the structure-activity relationship (SAR).[15] Three sets of the aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**) were prepared (Table 1). In the first set (Ar = 2,5-difluoro phenyl), 22 compounds (**12a–v**) were prepared by coupling 2,5-difluoro phenyl-aminopiperidone (**11a**) with various halo-heterocycles/halo-aromatics. In the second set (Ar = 2,4,5-trifluoro phenyl), 5 compounds (**13a–e**) were prepared by replacing 2,5-difluoro phenyl with 2,4,5-trifluoro phenyl, while in third set (Ar = 2,4-dichloro phenyl), 5 compounds (**14a–e**) were prepared by replacing 2,5-difluoro phenyl with 2,4-dichloro phenyl. All the test compounds showed varying degrees of DPP-IV inhibitory activity (IC_{50}), depending on the nature of the substituents.

Within the first set (**12a–v**), test compounds showed diverse DPP-IV inhibitory activity depending on the nature of substituents on piperidone ring system. Compounds with electron withdrawing groups (**12b**: –CN, **12c**: –F and **12d**: $-CF_3$) at *para*-position of phenyl ring system showed improved DPP-IV inhibitory activity, compared to unsubstituted derivative (R = –Ph; **12a**). Compounds with electron donating groups (**12e**: –OMe and **12f**: $-SO_2$–Me) at *para*-position of phenyl ring showed further improvement in in vitro DPP-IV inhibitory activity. Replacement of phenyl ring system with 3-pyridyl (**12g**) and further substitutions with electron donating (**12h**) and withdrawing (**12i** and **12j**) groups at *para*-position showed moderate DPP-IV inhibitory activity. Replacement of phenyl ring system with quinoline (**12m**), triazolo[4,3-*a*]pyrazine (**12n**), 2-methyl-pyrimido[1,2-*b*]pyridazinone (**12o**), benzyl (**12k**) and further substitutions with electron withdrawing (**12l**) groups at *para*-position showed moderate DPP-IV inhibitory activity. Substitutions with ethylbenzene (**12p**), ethylpyridine (**12q**), dimethylpyrazolo[1,5-*a*]-pyrimidine (**12r**), 3-methyl-triazolo [4,3-*b*]pyridazine (**12s**), 3-trifluoromethyl-triazolo[4,3-*b*]pyridazine (**12t**) and 2-trifluoromethyl-triazolo[1,5-*b*]pyridazine (**12u**) showed good DPP-IV inhibitory activity, while **12v** (methylenedioxy phenethyl) showed superior DPP-IV inhibitory activity (IC_{50}: 8.5 ± 0.4 nM), compared to Sitagliptin (IC_{50}: 18±2.4 nM).

Table 1
In vitro DPP-IV inhibitory activity of aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**).*

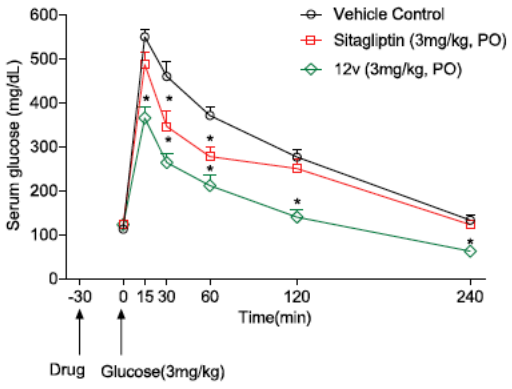
Compd	R	IC_{50}** (nM)	Compd	R	IC_{50}** (nM)	Compd	R	IC_{50}** (nM)
12a		1436 ± 12.3	**12l**		910 ± 3.1	**13a**		157 ± 4.1
12b		378 ± 1.4	**12m**		1034 ± 21.2	**13b**		119 ± 1.0
12c		382 ± 4.5	**12n**		1023 ± 3.1	**13c**		125 ± 2.7
12d		342 ± 3.3	**12o**		997 ± 13.5	**13d**		111 ± 2.1
12e		217 ± 8.6	**12p**		119 ± 4.2	**13e**		19 ± 5.1
12f		193 ± 8.4	**12q**		84 ± 2.6	**14a**		197 ± 4.2
12g		1388 ± 5.9	**12r**		77.6 ± 1.2	**14b**		148 ± 3.7
12h		452 ± 3.7	**12s**		122 ± 3.2	**14c**		134 ± 7.3
12i		443 ± 5.3	**12t**		79 ± 0.2	**14d**		137 ± 9.6
12j		404 ± 7.7	**12u**		74 ± 0.9	**14e**		43 ± 3.2
12k		885 ± 11.2	**12v**		**8.5 ± 0.4**	**Sitagliptin**	–	**18 ± 2.4**

Bold IC_{50} values of 12v and sitagliptin represents most potent compounds in Table 1.
* DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ± SD (n = 3).

In the second set (**13a–e**, Ar = 2,4,5-trifluoro phenyl), all the five compounds showed good activity, but compared to 2,5-difluoro phenyl series (Set-1 analogs, **12q**, **12r**, **12t**, **12u** and **12v**), in vitro DPP-IV inhibition were found to be bit weaker, while in set three (**14a–e**, Ar = 2,4-dichloro phenyl), in vitro DPP-IV inhibition were found to be slight weaker than Set-1 and Set-2 corresponding analogs. Thus the nature and position of halogen atom on aromatic ring system contributed significantly towards in vitro DPP-IV inhibition.

The in vitro selectivity over serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for **12V** and it showed >5000-fold selectivity over DPP-2 and >10,000-fold selectivity over DPP-8 and DPP-9.[15] To assess the CYP liabilities, **12v** was subjected for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition studies (@ 1, 10 and 100 μM concentrations) and the test compound **12v** was found to be devoid of CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition up to 100 μM concentrations.[16]

Detailed pharmacodynamic (PD) as well as pharmacokinetic (PK) profiling of **12v** was carried out. The in vivo antidiabetic activity of **12v** and Sitagliptin (@ 3 mg/kg, p.o.) was evaluated in male C57BL/6J mice, using OGTT (oral glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose up to 240 min; mg/dL) was estimated (Fig. 2).[17] Compound **12v** showed good oral antidiabetic activity (% decrease in AUC glucose 38.9 ± 5.20), which



*P<0.05, Two-Way ANOVA followed by Bonferroni posttest, Mean ± SEM

Figure 2. In vivo antidiabetic activity of **12v** and Sitagliptin in C57 mice.

was found to be better than Sitagliptin (% decrease in AUC glucose 17.9 ± 4.58). In C57 mice, it was interesting to observe that **12v** showed suppression in the blood glucose at all the time points

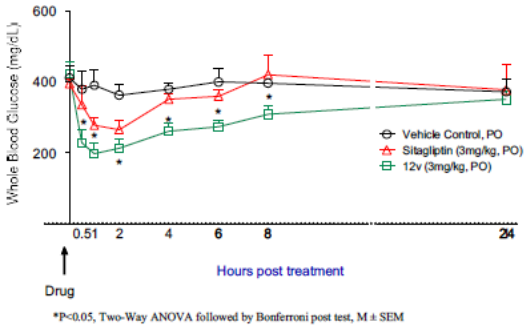


Figure 3. In vivo antidiabetic activity of **12v** and Sitagliptin in db/db mice.

(15, 30, 60, 120 and 240 min) compared to vehicle control, while Sitagliptin showed blood glucose reduction only at 30 and 60 min.

Further to understand the duration of action and effect of **12v** on post-prandial glucose excursion, single dose (@ 3 mg/kg, po) antidiabetic activity of **12v** and Sitagliptin was evaluated in fed-db/db mice (hyperglycemic animals) for 24 h (Fig. 3). Under fed condition, compared to vehicle control, **12v** and Sitagliptin showed good antidiabetic activity (% decrease in AUC glucose 38.29 ± 12.13 and 20.80 ± 11.06, respectively) up to 2 h. However, **12v** showed prolonged suppression of serum glucose levels (% decrease in AUC glucose 20.62 ± 7.05 for **12v** and 1.48 ± 11.84 for Sitagliptin, up to 24 h).

A comparative single dose (3 mg/kg iv or p.o.) PK profile of **12v** and Sitagliptin was evaluated in male C57BL/6J mice ($n = 6$) and the various PK parameters (T_{max}, $T_{1/2}$, C_{max}, AUC and %F) were recorded (Table 2).[18] In PK study, **12v** showed rapid T_{max}, higher AUC (~twofold compared to Sitagliptin), extended half-life ($T_{1/2}$: >8 h) compared to Sitagliptin and good oral bioavailability (%F: 79.5%). Compound **12v** showed extended half-life and higher AUC, which could be due to its low clearance compared to Sitagliptin (elimination rate constant (kel; h^{-1}), 0.12 ± 0.02 for **12v** and 0.84 ± 0.14 for Sitagliptin). Thus improved pharmacokinetic profile of compound **12v** justifies its potent and prolonged antidiabetic activity in C57 and db/db mice.

Interestingly, various gliptins, currently used in the clinic (Sitagliptin, Vildagliptin, Saxagliptin, Alogliptin and Linagliptin), exhibit short half-life thereby requires once or twice daily drug administration.[19] Further to regulate the pre- and post-prandial blood glucose and thereby to control HbA1c, several long-acting DPP-IV inhibitors (Omarigliptin and Trelagliptin) are under developments, as once-weekly drugs.[4] Their clinical efficacy and side effects profile appear to be comparable with other gliptins in the class, however, their infrequent dosing creates a niche and promotes patients compliance.[4] In this context, overall pre-clinical profile of **12v** demonstrated added advantages over currently practiced gliptins and appears to serve as long-acting DPP-IV inhibitors.

The molecular docking analysis of **12v** and Sitagliptin, in the binding pocket of DPP-IV was carried out using extra precision

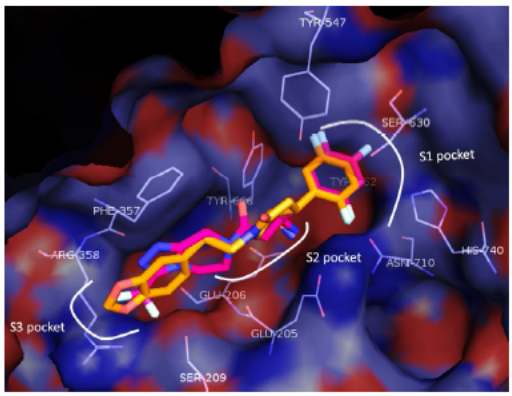


Figure 4. Key interactions of compound **12v** and Sitagliptin with active sites of DPP-IV enzyme. Binding pose of compound **12v** (Orange) and Sitagliptin (Maroon) in the DPP-IV active site is indicated (Surface view: Blue), wherein both compounds interact closely with key residues of site S_1, S_2 and S_3.

(XP) Glide docking method (Fig. 4).[20] The X-ray structure of the DPP-IV enzyme (PDB ID: 2OQI) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. For docking study, the ligands were geometrically optimized and prepared by using ligprep module of Schrödinger.[18] The overlay of binding poses of **12v** (Orange) and Sitagliptin (Maroon) in the DPP-IV active site is shown in Figure 4. As observed with Sitagliptin, **12v** docks very well into all the three sites (G-scores −11.81 (9/9) and −10.99 (9/9) for **12v** and Sitagliptin respectively). Although, G-score of **12v** and Sitagliptin are comparable, however, in vitro, DPP-IV IC_{50} of **12v** is half of that of Sitagliptin, which could be due to favorable interactions of **12v**, in all the three binding pockets. Di-fluorophenyl ring of **12v** occupies S_1 pocket. In S_2 pocket, aminomethyl groups of piperidone ring forms H-bonding with the side-chains of Glu_{205} and Glu_{206} dyad, while methylenedioxy phenyl ring of **12v** accommodates very well in S_3 pocket, which together supports excellent in vitro DPP-IV activity and selectivity of **12v** over other protease.

In summary, we report discovery of novel aminomethyl-piperidone derivatives as potent, selective and long acting DPP-IV inhibitors for the treatment of T2DM. The lead compound **12v** ((4*S*,5*S*)-5-(aminomethyl)-1-(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)-4-(2,5-difluorophenyl)-piperidin-2-one) showed prolonged suppression of pre-and post-prandial blood glucose levels (in vivo), which correlates with its extended PK profile.

Acknowledgment

We are grateful to the management of Zydus Group for encouragement and support.

Table 2
Pharmacokinetic study parameters[a] of **12v** and Sitagliptin

Compd	T_{max} (h)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (0-∞) h μg/ml	%F[b]
12v	0.28 ± 0.12	0.42 ± 0.03	8.99 ± 0.31	1.01 ± 0.09	79.5
Sitagliptin	0.22 ± 0.10	0.31 ± 0.01	1.56 ± 0.11	0.56 ± 0.02	75.7

[a] In male C57BL/6J mice ($n = 6$), compounds were administered orally (p.o.) at 3 mg/kg dose and plasma concentration was analyzed by LC–MS, values indicate mean ± SD.
[b] Oral bioavailability (%F) was calculated wrt to iv AUC (**12v**: 1.27 ± 0.08 and Sitagliptin: 0.74 ± 0.09 h μg/ml) administered at 3 mg/kg dose, iv.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.009.

References and notes

1. IDF Diabetes Atlas 6th addition update, 2013. Available from: http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures.
2. Lambeir, A. M.; Durinx, C.; Scharpé, S.; Meester, I. *Crit. Rev. Clin. Lab. Sci.* **2003**, *40*, 209.
3. Kim, W.; Egan, J. M. *Pharmacol. Rev.* **2008**, *60*, 470.
4. Cahn, A.; Raz, I. *Expert Opin. Emerg. Drugs* **2013**, *18*, 245.
5. (a) Mulakayala, M.; Reddy, C. H. U.; Iqbal, J.; Pal, M. *Tetrahedron* **2010**, *66*, 4919; (b) Mendieta, L.; Tarrago, T.; Giralt, E. *Expert Opin. Ther. Pat.* **2011**, *21*, 1693.
6. Kang, N. S.; Ahn, J. H.; Kim, S. S.; Chae, C. H.; Yoo, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3716.
7. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
8. Biftu, T.; Scapin, G.; Singh, S.; Feng, D.; Becker, J. W.; Eiermann, G.; Huaibing He, H.; Lyons, K.; Patel, S.; Petrov, A.; Sinha-Roy, R.; Zhang, B.; Wu, J.; Zhang, X.; Doss, G. A.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3384.
9. Edmondson, S. D.; Mastracchio, A.; Cox, J. M.; Eiermann, G. J.; He, H.; Lyons, K. A.; Patel, R. A.; Patel, S. B.; Petrov, A.; Scapin, G.; Wu, J. K.; Xu, S.; Zhu, B.; Thornberry, N. A.; Sinha-Roy, R.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4097.
10. Biftu, T.; Qian, X.; Chen, P.; Feng, D.; Scapin, G.; Gao, Y. D.; Cox, J.; Sinha-Roy, R.; Eiermann, G.; He, H.; Lyons, K.; Salituro, G.; Patel, S.; Petrov, A.; Xu, F.; Xu, S. S.; Zhang, B.; Caldwell, C.; Wu, J. K.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5361.
11. Jadav, P.; Bahekar, R.; Shah, S. R.; Patel, D.; Joharapurkar, A.; Kshirsagar, S.; Jain, M.; Shaikh, M.; Sairam, K. V. V. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3516.
12. Moos, W. H.; Gless, R. D.; Rapoport, H. *J. Org. Chem.* **1981**, *46*, 5064.
13. Klapars, A.; Xiaohua Huang, X.; Stephen, L.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421.
14. (a) Cox, J. M.; Edmondson, S. D.; Harper, B.; Weber, A. E. WO 039325, 2006; *PCT Int. Appl.* **2006**.; (b) Biftu, T.; Chen, P.; Cox, J. M.; Weber, A. E. WO 056708, 2010; *PCT Int. Appl.* WO 056708, **2010**.
15. (a) Blackmon, D. L.; Watson, A. J.; Montrose, M. H. *Anal. Biochem.* **1992**, *200*, 352; (b) In vitro enzyme (DPP-IV, DPP-2, DPP-8 and DPP-9) inhibitory activity was determined using fluorescence-based assay. The Gly-Pro-AMC was used as a substrate (which is cleaved by the enzymes to release the fluorescent AMC) and soluble human proteins (DPP-IV, DPP-2, DPP-8 and DPP-9 enzymes) produced in a baculovirus expression system (Life Technologies) was used as the enzyme source. The H-Gly-Pro-AMC (200 μM) was incubated with either DPP-IV, DPP-2, DPP-8 or DPP-9 enzymes in the presence of various concentrations of test compounds. Reaction was carried out at pH 7.8 (HEPES buffer 25 mM containing 1.0% BSA, 140 mM NaCl, 16 mM $MgCl_2$, 2.8% DMSO) in a total volume of 100 μl at 25 °C for 30 min., in the dark. Reaction was terminated with acetic acid (25 μl of 25% solution). Activity (fluorescence) was measured using Spectra Max fluorometer (Molecular Devices, Sunnyvale CA) by exciting at 380 nm and emission at 460 nm. The IC_{50} values were determined for test compounds using Graph Pad prism software.
16. Dierks, E. A.; Stams, K. R.; Lim, H. K.; Cornelius, G.; Zhang, H.; Ball, S. E. *Drug Metab. Dispos.* **2001**, *29*, 23.
17. (a) Chen, D.; Wang, M. *Diabetes Obes. Metab.* **2005**, *7*, 307; (b) Kim, J. G.; Baggio, L. L.; Bridon, D. P.; Castaigne, J. P.; Robitaille, M. F.; Jette, L.; Benquet, C.; Drucker, D. J. *Diabetes* **2003**, *52*, 751; (c) Study was conducted in male C57BL/6J (using OGTT protocol) or without glucose load, in db/db mice (age 8–12 weeks). All animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'. Two days prior to the study, the animals were randomized and divided into 2 groups ($n = 6$), based upon their fed glucose levels. Animals were left for 2 days under acclimatization and maintained on a standard diet. On the day of experiment, food was withdrawn from all the cages, water was given ad-libitum and were kept for overnight fasting. Briefly, in OGTT protocol (C57 mice) overnight fasted mice were dosed orally (p.o.) with the test compounds (3 mg/kg), 0.5 h prior to the oral glucose load, while in db/db mice, fed mice were dosed orally (p.o.) with the test compounds (3 mg/kg) and the blood samples were collected at various time points. Blood samples were centrifuged and the separated serum was immediately subjected for the glucose estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, California). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (Ver 4.0) was used to plot an area under the curve (0–240 min AUC). The AUC obtained from graphs were analyzed for two-way ANOVA, followed by Bonferroni post test, using Graph Pad prism software.
18. Briefly, for single dose PK study, test compounds were administered orally/iv on a body weight basis (3 mg/kg) to overnight fasted male C57BL/6 J mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 36 and 48 h post-dose after compounds administration. Approximately 0.2 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at −70 °C and the concentrations of compounds in plasma were determined by the LC–MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0 × 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters, such as T_{max}, $t_{1/2}$, C_{max}, AUC and %F were calculated using a non-compartmental model of WinNonlin software version 5.2.1.
19. (a) Baetta, R.; Corsini, A. *Drugs* **2011**, *71*, 1441; (b) Scheen, A. J. *Expert Opin. Pharmacother.* **2012**, *13*, 81.
20. Schrodinger Suite 2010, Glide version 5.8, Prime version 2.2; Schrodinger, LLC: New York, 2010.

Vitae

"Discovery consists of looking at the same thing as everyone else and thinking something different." - Albert Szent-Györgyi

9. Vitae

The author was born on 3rd July, 1979 at Rupapura, district Vadodara, Gujarat, India. He obtained S.S.C from Smt. C. R. Patel Saraswati Vidyalaya, Sarsavani & H.S.C. from H. S. Patel Highschool, Vadodara. He received B.Sc. degree in chemistry from M. B. Patel Science College, Anand in 1999 and M.Sc. degree in Organic Chemistry from Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar in 2001. He then joined Medicinal Chemistry Department at Zydus Research Centre, a division of Cadila Healthcare LTD. Ahmedabad, Gujarat, India. in May 2001 where he is currently working as a Scientist. The author contributed significantly for the development of drug **Lipaglyn™** (Saroglitazar/ZYH1) launched in India in 2013-The world's first Glitazar drug for treating diabetic dyslipidemia combines lipid and glucose lowering effects in one single molecule and is the first NCE discovered and developed indigenously by an Indian pharma company (i.e. Zydus Research Centre, Cadila Healthcare LTD.). The author is also co-inventor of several new chemical entities, which are in various stages of development for the treatment of diabetes. *viz* ZYD1 & ZYOG1- as a GLP-1 agonist, ZYDPLA1- as a Next generation long-acting DPP-IV inhibitor.

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List of Publications

1. Rajesh H. Bahekar, Mukul R. Jain, Ashish Goel, Dipam N. Patel, Vijay M. Prajapati, Arun A. Gupta, **Pradip A. Jadav** and Pankaj R. Patel. “Design, synthesis, and biological evaluation of substituted-N-(thieno[2,3-b]pyridin-3-yl)-guanidines, N-(1H-pyrrolo[2,3-b]pyridin-3-yl)-guanidines, and N-(1H-indol-3-yl)-guanidines” *Bioorg. Med. Chem.***2007**, *15*, 3248-3265.

2. Rajesh H. Bahekar, Mukul R. Jain, Arun A. Gupta, Ashish Goel, **Pradip A. Jadav**, Dipam N. Patel, Vijay M. Prajapati, and Pankaj R. Patel. “Synthesis and Antidiabetic Activity of 3,6,7-Trisubstituted-2-(1H-imidazol-2-ylsulfanyl) quinoxalines and Quinoxalin-2-ylisothioureas” *Arch. Pharm. Chem. Life Sci.***2007**, *340*, 359-366.

3. Rajesh H. Bahekar, **Pradip A. Jadav**, Dipam N. Patel, Vijay M. Prajapati, Arun A. Gupta, Mukul R. Jain and Pankaj R. Patel. “An improved synthesis of Fmoc-N-methyl serine and threonine” *Tett. Lett.***2007**, *48*, 5003-5005.

4. Rajesh H. Bahekar, Mukul R. Jain, **Pradip A. Jadav**, Ashish Goel, Dipam N. Patel, Vijay M. Prajapati, Arun A. Gupta, Honey Modi and Pankaj R. Patel. “Synthesis of 3,8,9 trisubstituted-1,7,9-triaza-fluorene-6-carboxylic acid derivatives as a new class of insulin secretagogues” *Bioorg. Med. Chem.***2007**, *15*, 5950-5964.

5. Rajesh H. Bahekar, Mukul R. Jain, **Pradip A. Jadav**, Vijay M. Prajapati, Dipam N. Patel, Arun A. Gupta, Ajay Sharma, Robby Tom, Debdutta Bandyopadhya, Honey Modi and Pankaj R. Patel. “Synthesis and antidiabetic activity of 2,5-disubstituted-3-imidazol-2-yl-pyrrolo[2,3-b]pyridines and thieno[2,3-b]pyridines” *Bioorg. Med. Chem.***2007**, *15*, 6782-6795.

6. Dipam Patel, Mukul Jain, Shailesh R. Shah, Rajesh Bahekar, **Pradip Jadav**, Brijesh Darji, Yernaidu Siriki, Debdutta Bandyopadhyay, Amit Joharapurkar, Samadhan Kshirsagar, Harilal Patel, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Pankaj Patel. “Discovery of Orally Active, Potent, and Selective Benzotriazole-Based PTP1B Inhibitors” *ChemMedChem***2011**, *6*, 1011-1016.

7. Dipam Patel, Mukul Jain, Shailesh R. Shah, Rajesh Bahekar, **Pradip Jadav**, Amit Hoharapurkar, Nirav Dhanesha, Mubeen Shaikh, Kalapatapu V. V. M. Sairam, Prashant Kapadnis. “Discovery of potent, selective and orally bioavailable triaryl-sulfonamide based PTP1B inhibitors” *Bioorg. Med. Chem. Lett.***2012**, *22*, 1111-1117.

8. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Samadhan Kshirsagar, Mukul Jai, Mubeen Shaikh, Kalapatapu V. V. M. Sairam. “Long-acting peptidomimetics based DPP-IV inhibitors”*Bioorg. Med. Chem. Lett.***2012**, *22*, 3516-3521.

9. Kiran Shah, Dipam Patel, **Pradip Jadav**, Mubeen Shaikh, Kalapatapu V. V. M. Sairam, Amit Joharapurkar, Mukul R. Jain, Rajesh Bahekar. “Discovery of liver selective non-steroidal glucocorticoid receptor antagonist as novel antidiabetic agents” *Bioorg. Med. Chem. Lett.***2012**, *22*, 5857-5862.

10. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Kiran Shah, Shruti Bhardwaj, Kishan Patel, Kaushil Patel, Rajendra Chopade, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Mukul Jain. “Design of Peptidomimetics Based DPP-IV Inhibitors, Devoid of CYP liabilities”*Letters in Drug Design & Discovery***2012**, *9*, 867-873.

11. Dipam Patel, Mukul Jain, Shailesh R. Shah, Rajesh Bahekar, **Pradip Jadav**, Kiran Shah, Amit Hoharapurkar, Mubeen Shaikh, Kalapatapu V. V. M. Sairam.“Peptidomimetic as Potent and Selective PTP1B Inhibitors” *Medicinal Chemistry***2013**, *9*, 660-671.

12. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Mukul Jai, Kalapatapu V. V. M. Sairam and Praveen Kumar Singh. “Design, Synthesis and Biological Evaluation of Novel Aminomethyl-piperidones based DPP-IV Inhibitors”*Bioorg. Med. Chem. Lett.***2014**, *24*, 1918-1922.

13. Mukul R. Jain**,** Amit A. Joharapurkar, Rajesh Bahekar, Harilal Patel, **Pradip Jadav**, Samadhan Kshirsagar, Vishal J. Patel, Kartikkumar N. Patel, Vikram K. Ramanathan, Pankaj R. Patel and Ranjit Desai. “Pharmacological characterization of ZYDPLA1, a novel long-acting DPP-4 Inhibitor.” *J. Diabetes***2014**, doi: 10.1111/1753-0407.12233.

List of Patent Publication

1. Ranjit C. Desai, Rajesh Bahekar, **Pradip Jadav**, Amitgiri Goswami, Pankaj Patel. "Title: 2-Phenyl-5-Heterocyclyl-Tetrahydro-2H-Pyrane-3-Amine Compounds for Use In the Treatment of Diabetes and Its Associated Disorders." PCT Int. Appl. **WO 2014/061031 A1.**

List of Posters

1. Bahekar R. H., Lohray V. B., **Jadav P.**, Prajapati V. and Sarkar S. S. "New drug targets for Type II Diabetes". Poster presented in 2nd RBF international Symposium, *Current Trends in Pharmaceutical Sciences: Role of Genomics and Proteomics* held at Zydus Research Centre, Ahmedabad in January 23-25, **2005**.

2. Dipam N. Patel, **Pradip A. Jadav**, Brijesh A. Darji, Yernaidu Siriki, Mukul R. Jain and Rajesh H. Bahekar. "Synthesis and Glucose Dependent Insulinotropic Activity of Substituted-Hetero-Aryl Oxazolyl Derivatives." Poster presented in 4th RBF international Symposium, *Advances in Cardiometabolic Research-Basic Science and Clinical Aspects* held at Zydus Research Centre, Ahmedabad in February 2-5, **2009**.

3. **Pradip A. Jadav**, Dipam N. Patel, Bony R. Shah, D. J. Sen, Brijesh A. Darji, Yernaidu Siriki, Mukul R. Jain, Debdutta Bandyopadhyay, Amit Joharapurkar, Harilal Patel and Rajesh H. Bahekar. "Design, Synthesis and Pharmacological Evaluation of Dipeptide Based DPP IV Inhibitors as New Class of Antidiabetic Drugs." Poster presented in 4th RBF international Symposium, *Advances in Cardiometabolic Research-Basic Science and Clinical Aspects* held at Zydus Research Centre, Ahmedabad in February 2-5, **2009**.

4. Rajendra Chopade, Vijay Prajapati, Dipam Patel, **Pradip Jadav**, Brijesh Darji, Yernaidu Siriki, Ganesh Rahane, Jiterndra Padsumbiya, Dinesh Reddy, Amit Joharapurkar, Mukul Jain and Rajesh Bahekar. "Development of Proline based DPPIV Inhibitors for the treatment of Type II Diabetes." Poster presented in 5th RBF international Symposium, *Advances in Trasnational Research and Medicine* held at Zydus Research Centre, Ahmedabad in Feb-1-4, **2011**.

5. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Kiran Shah, Rajendra Chopade, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Mukul Jain. "Design of Peptidomimetics Based DPP-IV Inhibitors, Devoid of CYP liabilities" Poster presented in 6th RBF international Symposium, *Advances in New Drug Discovery Technologies and Trasnational Research* held at Zydus Research Centre, Ahmedabad in Feb-2-4, **2013**.

6. Dipam Patel, Shailesh Shah, Rajendra Chopade, Vijay Prajapati, **Pradip Jadav**, Kiran Shah, Mubeen Shaikh, Kalapatapu V. V. M. Sairam, Amit Joharapurkar, Mukul Jain and Rajesh Bahekar. "PTP 1B inhibitors: Discovery of DFMP-substituted phosphotyrosine mimetics" Poster presented in 6th RBF international Symposium, *Advances in New Drug Discovery Technologies and Trasnational Research* held at Zydus Research Centre, Ahmedabad in Feb-2-4, **2013**.

7. Kiran Shah, Ganes Chakrabarti, Mehul Raviya, Bhavesh Sharma, Suresh Giri, Dipam Patel, **Pradip Jadav**, Mubeen Shaikh, Kalapatapu V. V. M. Sairam, Lalabhai Patel, Mukul Jain and Rajesh Bahekar. "Discovery of novel and nonsteroidal GR antagonist" Poster presented in 6th RBF international Symposium, *Advances in New Drug Discovery Technologies and Trasnational Research* held at Zydus Research Centre, Ahmedabad in Feb-2-4, **2013**.

8. Mukul R. Jain**,** Amit Arvind Joharapurkar, Rajesh Bahekar, Harilal Patel, Samadhan Kshirsagar, **Pradip Jadav**, Vishal Patel, Kartikkumar Patel, Vikram K. Ramanathan, Pankaj R. Patel and Ranjit Desai. "ZYDPLA1, a Novel Long-Acting DPP-4 Inhibitor" Poster presented in ICE/ENDO 2014:16th International Congress of Endocrinology & 96th Annual Meeting and Expo of the Endrocrine Society. Chicago,United States of America in June 21-24, **2014**.