Design and synthesis of novel compounds useful for the treatment of dyslipidemia and obesity

A Thesis submitted to

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IN

CHEMISTRY

Under the guidance of

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CERTIFICATE

This is to certify that the thesis entitled **"Design and synthesis of novel compounds useful for the treatment of dyslipidemia and obesity**" is 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 original work completed by Mr. Saurin Raval under my supervision and guidance at Zydus Research Center, Ahmedabad and work embodied in this thesis has not formed earlier the basis for award of any degree or similar title of this or any other university or examining body.

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DECLARATION

To the best of my knowledge and belief this thesis entitled **"Design and synthesis of novel compounds useful for the treatment of dyslipidemia and obesity**" is submitted to The Maharaja Sayajirao University of Baroda, Vadodara of the fulfillment for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the work carried out by me in chemistry department of Zydus Research center. This thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Date :

Place : Ahmedabad Saurin Raval

Dedicated to

Late Shri. Ramanbhai Patel

Founder of **Zydus Cadila**

PREFACE: EXPERIENCING THE PROCESS OF THESIS

This Ph.D thesis document is the outcome of my research work at Zydus Research Centre and the department of Chemistry, The Maharaja Sayajirao University of Baroda,Vadodara, India. This research work has been also a part of my job as senior scientist at Zydus Research Centre, Ahmedabad, India to which I have been affiliated since March 1997.

The thesis consists of four chapters which covers overview, design and synthesis of novel compounds and results of my research work on dyslipidemia and obesity. It begins with a review of extant literature covering the dyslipidemia and obesity. In this chapter detailed description, risk factors, existing therapies and information about the new possible targets of dyslipidemia and obesity is given. Then introduction to three selected targets (CB1 antagonists, Thyromimetics and MTP inhibitors) of my research work for the treatment of dyslipidemia and obesity is described. To work, these three targets were selected because our aim was to design tissue selective molecules which plays crucial role to avoid toxicity. The second, third and fourth chapter is about detailed research work on CB1 antagonists, Thyromimetics and MTP inhibitors respectively. In each chapter rationale for designing novel compounds is explained and the 'Results & Discussion' section describes the synthesis, biological of the novel compounds. The 'Experimental' is the section where the detailed procedures for the synthesis of the compounds as well as the characterization data are presented. The details of biological experiments were also described in this section. Finally, there is the conclusion part of research work. Copy of spectra of lead compounds and intermediates incorporated at the end of each chapter.

Working for this thesis has been a great learning experience for me and working for publication was a joy to write. Although this research work was outcome of my project but it is also for social cause because as Medicinal chemist our focus is on finding innovative therapies for diseases affecting mankind through continuous research and development.

Zydus cadila is an Integrated Global Healthcare company, dedicated to improving people's lives and our mission is to create healthier communities globally.

Saurin Raval

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A journey is easier when you travel together. Milestones in life are achieved, not by individual efforts but by blessings and guidelines of elders, nears and dears. At this juncture, I express my sincere gratitude to all those who have supported me to achieve this phenomenal mark.

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Thankful I ever remain……

Saurin Raval

CONTENTS

INTRODUCTION

1

1. Introduction

1.1 Metabolic diseases

As the world's population gets older dyslipidemia and obesity are becoming increasing burden for society and health-care resources. Dyslipidemia and obesity are major components of "Metabolic syndrome", a cluster of metabolic diseases including diabetes, obesity and dyslipidemia [1]. These abnormalities individually or in combination can lead to high risk for the development of hypercholesterolemia, hypertriglyceridemia, insulin resistance and remains the leading cause for morbidity and mortality. It is recognized that in substantial number of cases, dyslipidemia and obesity may lead to cardiovascular disease (CVD). The evolving epidemic of obesity and its consequent affect on CVD threatens to overturn the reduction in prevalence of cardiovascular disease. Although there are number of factors responsible for the epidemic in human population, but it is believed that modern lifestyle remains the main risk factor. Though lifestyle modification is considered as the first line of therapy, but still there is high unmet need as an effective drug treatment in such kind of life threatening multiple diseases is elusive. Last decade had witnessed enormous efforts to develop therapies for the treatment of dyslipidemia and obesity.

1.1.1 Dyslipidemia

Dyslipidemia is disorder of lipoprotein metabolism, which includes lipoprotein overproduction or deficiency. Dyslipidemia is characterized by the elevated levels of lipids (cholesterol and/or triglycerides) or a low high-density

lipoprotein level in the blood. The elevated levels of lipid leads to the development of atherosclerosis, a disease in which plaque builds up inside the arteries. The lipids are transported in plasma as components of lipoprotein complexes. The lipoproteins are spherical complex particles made up of many lipids and protein molecules. Lipoproteins are classified on the basis of their densities (Table1).

Table 1.1 Classification of lipoproteins

Class	Density (g/mL)	Diameter (nm)	Protein	Cholesterol	PL	TG
HDL	>1.063	5–15	33%	30%	29%	4%
LDL	**1.019–1.063**	**18–28**	**25%**	**50%**	**21%**	**8%**
IDL	1.006–1.019	25–50	18%	29%	22%	31%
VLDL	0.95–1.006	30–80	10%	22%	18%	50%
Chylomicron	<0.95	100-1000	<2%	8%	7%	84%

VLDL- Very Low Density Lipoproteins; IDL-Intermediate Density Lipoproteins; LDL-Low Density Lipoproteins; HDL-High Density Lipoproteins; TG-Triglyceride; PL-Phospholipids. (Note - The remaining composition is made up of apoproteins)

LDL cholesterol is a strong risk factor for coronary artery disease (CAD) and considered as more atherogenic to the endothelium. After it enters to the vessel wall it oxidized and trigger the atherosclerotic process. The more LDL particles a person has, higher the risk for plaque buildup thus more prone to heart attacks. Chylomicrons and VLDL are not atherogenic as they are relatively too large to penetrate into the artery. Another significant risk factor for the development of CAD is high serum triglyceride levels (hypertriglyceridemia). High serum triglyceride level, which is secondary cause of abnormal lipoprotein metabolism is commonly seen in patients having other cardiovascular risk factors like obesity, insulin resistance, diabetes mellitus, and lowered levels of high-

density lipoprotein cholesterol (HDL-C). As mentioned dyslipidemia is closely associated with atherosclerosis which is a major causal factor in the development of ischemic diseases. Ischemic cardiovascular events are leading causes of morbidity and mortality. The reason for initiation of atherosclerosis is not known, but a key initial phase in the development of atherosclerosis is the retention of cholesterol-rich lipoproteins and remnants in the subendothelial space [2,3]. The risk factors influenced by obesity also promote atherosclerosis and cardiovascular disease [4,5]. Causative factors associated with hypertriglyceridemia might be obesity. The primary dyslipidemia related to obesity is recognize by high levels of triglycerides, low HDL levels and abnormal LDL composition.

1.1.2 Obesity

Worldwide health of human being is progressively threatened by an imbalance between increased energy intake and decreased energy expenditure through physical activity resulting to obesity, a serious and chronic disease [6]. Obesity has increased at a striking rate over the three decades. It is projected by WHO (World Health Organization) is that approximately 2.3 billion adults will be overweight and more than 700 million will be obese by the year 2015 [7]. The increasing global prevalence of obesity demonstrates that neither diet and exercise nor pharmacological approaches to this health problem are well addressed till the date. This negative trend has dramatically impact on physical health and on the relative cardiovascular risk. In fact, obesity alone is strongly

associated with an increased risk of life-threatening conditions such as diabetes, arterial hypertension, dyslipidemia and cardiovascular diseases [8].

A certain amount of body fat is necessary to store energy and for protection such as from heat and shock. For men normal amount of body fat is between 18%-23% and 25%-30% in women. Women having over 30% body fat and men over 25% body fat are considered obese. Overweight and obesity often are thought to be the same but according to the WHO there are differences between both. According to WHO definition overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. The calculation of body mass index (BMI) has also been used in the definition of obesity .Overweight is defined by a body mass index (BMI) that is equal or more than 25 kg/m^2 but less than 30 kg/m^2, and obesity implies a BMI equal or more than 30 kg/m^2. Increased consumption of more energy-dense, nutrient poor foods with high levels of sugar and saturated fats, changing ways of transportation and increasingly urbanization combined with reduced physical activity, have led to obesity.

The scientists globally pursued different molecular and nuclear targets for the development of new generation of safe and more effective anti-obesity drugs. Obesity is a complex disease involving an imbalance between physical mechanisms that regulate the intake and expenditure of energy. The current treatments however are only modestly efficacious and have significant side effects. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse affect on health. The body weight is

controlled by two mechanisms, one is energy intake and the other is energy expenditure. Hypothalamic nucleus of central nervous system (CNS) is an appetite control center of the brain and responsible for energy intake and energy expenditure mechanisms [9]. Novel approaches as to treat the obesity are being explored by targeting the drugs which decrease food intake and also the drugs which increase energy expenditure.

1.2 Risk factors

The health problems associated with dyslipidemia and obesity are numerous. Insulin resistance (IR), diabetes, high cholesterol levels and high triglyceride levels (Figure 1.1) are the major manifestation of obesity and dyslipidemia.

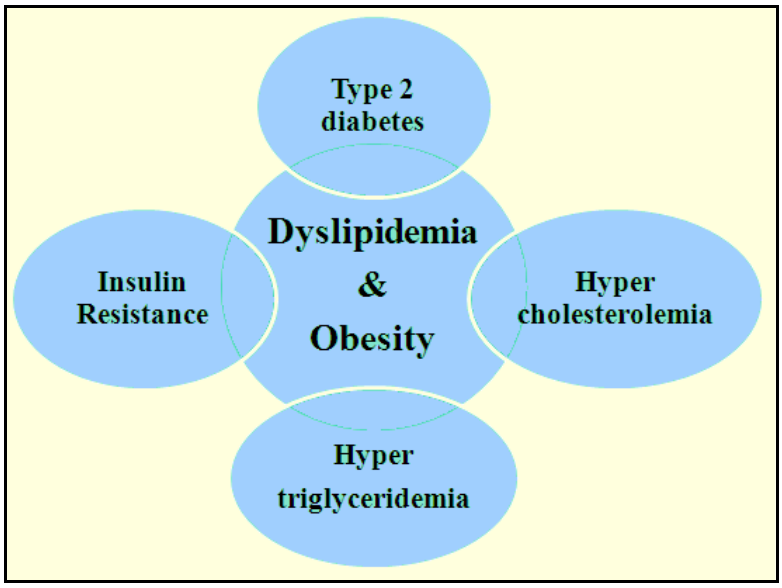


Figure 1.1 Risk factors associated with dyslipidemia and obesity

Obesity contributes to insulin resistance which generally precedes the development of type 2 diabetes, eventually it may lead to macro and micro vascular complications such as atherosclerosis, stroke, nephropathy, retinopathy [10]. The correlation between hypertriglyceridemia, hypercholesterolemia, insulin

resistance, obesity, diabetes and coronary artery disease (CAD) has been established since 1960 [11]. Following are some of the health risks associated with dyslipidemia and obesity.

1.2.1 Insulin Resistance

Insulin, a hormone produced by the pancreas, is necessary for the transport of blood glucose into the cells of muscle and fat which subsequently used for energy. By transporting glucose into cells, insulin keeps the blood glucose levels in the normal range. Insulin resistance (IR) is the condition whereby the effectiveness of insulin in transporting glucose into cells is diminished. Fat cells are more insulin resistant than muscle cells, therefore obesity is considered as important cause of IR [12] .

1.2.2 Type 2 diabetes

Type 2 diabetes is a chronic disease in which levels of blood glucose are higher than normal. Type 2 diabetes develops when body does not effectively use the insulin it produces. This ultimately increases concentration of glucose in the blood (hyperglycemia). The risk of type 2 diabetes increases with the degree and duration of obesity. Type 2 diabetes is associated with central obesity [13].

1.2.3 Hypercholesterolemia

High levels of cholesterol in the blood is termed as hypercholesterolemia. Hypercholesterolemia is not a disease, it takes place because of metabolic imbalance which can lead to many other diseases. Hypercholesterolemia can contribute to disease like atherosclerosis which is most notable cardiovascular disease [14].

1.2.4 Hypertriglyceridemia

When levels of triglyceride are elevated such condition is called as hypertriglyceridemia. Deficiency of enzyme lipoprotein lipase (LPL) in humans is the cause of hypertriglyceridemia with advancing age, obesity, and diabetes, as well as during metabolic stress [15] .

1.3 Therapeutic options

Dietary factors may have effect on CVD risk factors such as high LDL-cholesterol, triglycerides which ultimately leads to atherosclerosis [16]. Excess of food intake could be one of the causes for increase in the body weight. There is strong evidence that proper nutrition intervention can prevent progression of CVD. The first line treatment considered is to limit the food intake, which ultimately reduces excess of body fat. But these recommendations are not enough and pharmacological intervention becomes necessary. Weight loss and exercise can improve the dyslipidemia and thus reduce the risk of CVD. However, conditions such as high levels of cholesterol, triglycerides along with obesity cannot be cured by lifestyle modification alone. In such condition, patients are advised for intense lipid-lowering and or antiobesity therapy. The knowledge of pathophysiology of dyslipidemia and obesity has grown dramatically in the last few years, leading to effective treatment strategies. The risk of mortality from dyslipidemia has been reduced by a number of approaches. Following are some of the current marketed drugs for dyslipidemia and obesity (Table 1.2).

Table 1.2 Medications for dyslipidemia and obesity

DRUG - CLASS	ACTION	ADVERSE EFFECTS
5-HT reuptake inhibitor	ANOREXIANT Norepinephrine, dopamine and serotonin uptake inhibitor	Increases heart rate and blood pressure, dry mouth, insomnia, nervousness, and constipation
Lipase Inhibitor	LIPASE INHIBITOR Inhibits pancreatic lipase, decreases fat absorption	Decrease in absorption of fat-soluble vitamins, soft stools and anal leakage
Sympathomimetic agents	ANOREXIANT Sympatho-mimetic amine	Increases heart rate and blood pressure, dry mouth, insomnia, nervousness, and constipation
Statins	HMG CoA Reductase inhibitor	Rhabdomyolysis, elevation of liver enzymes and creatine kinase are some of the
Fibrates	PPAR Activators	Liver toxicity, gastrointestinal (GI) side effects, muscle toxicities
Niacin	Increase levels of HDL cholesterol	Flushing
Bile Acid sequestrant (Colesevelam.HCl)	Prevent reabsorption of bile Acid	GI adverse effects.
Cholesterol absorption inhibitor (Ezetimibe)	Inhibits cholesterol absorption	Headache and diarrhea

1.3.1 Therapeutic options (Obesity)

1.3.1.1 5-HT reuptake inhibitor

Sibutramine (Meridia-Abbott) is a weak noradrenergic and serotoninergic (5-HT) re-uptake inhibitor approved by the US FDA for long-term treatment of obesity [17]. It acts through CNS to reduce calorie intake and increase energy expenditure. The common side effects associated with Sibutramine are headache, dry mouth, anorexia, constipation, insomnia, rhinitis, and pharyngitis. Increased heart rate and blood pressure (tachycardia and hypertension respectively) with above mentioned symptoms are also observed [18-20].

1.3.1.2 Lipase Inhibitor

Orlistat is a drug used to treat obesity. It is an inhibitor of gastric and pancreatic lipases, which are the primary enzymes responsible for the hydrolysis and subsequent absorption of dietary fat in the lumen of gastrointestinal tract [21]. By inhibiting this enzyme it reduces systemic fat absorption and unabsorbed fat is excreted into the feces. The side effects like oily spotting, abdominal pain, fecal urgency and incontinence are most common [22] and it also reduces absorption of the fat-soluble vitamins A, D and E.

1.3.1.3 Sympathomimetic agents

Phentermine, Diethylpropion, Phendimetrazine, and Benzphetamine were four other drugs approved by the FDA for short-term use. These amphetamine derivates increase energy expenditure by its sympathomimetics activity [23,24]. The common adverse effects of this class of drugs are increase in heart rate and blood pressure, dry mouth, insomnia, nervousness, and constipation.

1.3.2 Therapeutic options (Dyslipidemia)

1.3.2.1 Statins

Statins inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) an enzyme involved in the rate limiting step of cholesterol synthesis [25] and mainly reduce LDL-cholesterol. Since their discovery statins remained as first-line therapy for hypercholesterolemia and at times these are prescribed in combination with other lipid lowering agents as add on therapy. Though statins are well tolerated in most of the patient population, rhabdomyolysis, elevation of liver enzymes and creatine kinase are some of the common side effects of these

agents [26,27]. There is also evidence of intolerability among very few patients to statin therapy.

1.3.2.2 PPAR agonists

Activation of Peroxisome Proliferator Activator Receptors (PPARs) is another interesting target for the treatment of metabolic disorders, which includes dyslipidemia and the efforts in this area dominated during last decade over other targets. Interestingly, fibrates are discovered as hypolipidemic agents based on rodent pharmacology and subsequently found to be PPAR *α* agonists much later. PPAR *α* is involved in oxidation of fatty acids [28] and thereby reduces triglyceride and LDL-cholesterol. Subsequently, many modifications to the structure of fibrates were carried out to achieve potent, efficacious and safe molecules. Recently dual PPAR *α/γ* agonists were explored for the treatment of dyslipidemia without success. Liver toxicity, gastrointestinal (GI) side effects, muscle toxicities are most common side effects of PPAR's [29,30], which limits their use as effective and safe treatment of metabolic disorder.

1.3.2.3 Niacin

Nicotinic acid has been in clinical use since long time and proven to increase plasma concentration of HDL, which ultimately reduces LDL-cholesterol [31-33]. These effects of niacin are found to be mediated through GPR-109. Thereafter, several compounds have been developed but most of them were dropped in clinical trials due to target related side effect flushing [34].

1.3.2.4 Bile acid sequestrants

Cholesterol is precursor of bile acid. In the intestinal lumen bile acid sequestrants (indigestible positively charged resins) that bind to negatively

charged bile acids and forms complex, which is excreted through the feces [35,36]. Main side effect of this class of drug is GI adverse effects.

1.3.2.5 Cholesterol absorption inhibitor (Ezetimibe)

Ezetimibe is a cholesterol absorption inhibitor and acts by decreasing cholesterol absorption in the intestine. Subsequently, it leads to decrease in the delivery of intestinal cholesterol to the liver and ultimately reduces hepatic cholesterol stores, and increases cholesterol clearance from the blood. Ezetimibe localises and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. Specifically, Ezetimibe inhibits cholesterol absorption in the intestine by blocking the Niemann-Pick C1-Like 1 (NPC1L1) cholesterol transporter protein, which is found at the brush border membranes of enterocytes [37,38]. It may be used alone or in combination with statins. Clinical studies with Ezetimibe demonstrated notable LDL-cholesterol lowering as monotherapy, and significant lowering in total and low density lipoprotein cholesterol in combination with statins [39]. Ezetimibe have no significant effect on the plasma concentrations of the fat-soluble vitamins A, D, and E and does not affect the absorption of triglycerides [40]. The Common side effects associated with Ezetimibe therapy are headache and diarrhea which is generally well tolerated.

1.4 Potential targets

As complex reasons are responsible for the development of dyslipidemia and obesity it is difficult to treat these diseases. In such area of high unmet clinical need, the number of approaches have been explored by virtue of the enormous

interest amongst academicians and pharmaceutical industries.Following are the potential targets for the treatment of obesity and dyslipidemia (Table 1.3 and 1.4).

1.4.1 Potential targets (Obesity)

Table 1.3 Potential targets obesity

Pathway	Target
Drugs which decrease food intake	• Dopaminergic and serotoninergic drugs • Cholecystokinin-promoting agents • Leptin-promoting agents • Agouti-related protein • Glucagon-like peptide 1 • PYY3-36 peptide • Melanocortin-4 receptor agonists • CRF receptor agonists • Neuropeptide Y receptor antagonists • Ghrelin receptor antagonists • Orexin receptor antagonists • Galanin receptor antagonists • Fatty acid synthase inhibitors • Cannabinoid receptor (CB1) antagonist • Cactus-derived P57
Drugs which increase energy expenditure	• Beta3-adrenoceptor agonists • Uncoupling proteins • Thyromimetics (TRβ agonists) • Human growth hormone fragment
Drugs which inhibit nutrient absorption	• Lipase inhibitors • α-glucosidase inhibitors • MTP inhibitors

1.4.2 Potential targets (Dyslipidemia)

Table 1.4 Potential targets dyslipidemia

Pathway	Target
Targets for HDL-C raising drugs	• Cholesteryl ester transfer protein (CETP) inhibitors
	• ATP-binding cassette transporter A1
	• Liver X Receptors
	• Nuclear retinoid X receptor
Targets for triglyceride lowering drugs	• Microsomal triglyceride transfer protein
	• Stearoyl-CoA desaturase-1 Inhibitors
	• Dual PPAR α/γ agonists
Other lipid modulators (Under development)	• HMG-CoA reductase inhibitors
	• Ileal bile acid transport inhibitors
	• Acyl-coenzyme A-cholesterol acyltransferase inhibitors
	• Lipoprotein-associated phospholipase inhibitors
	• Thyromimetics (TRβ agonists)
	• MTP inhibitors

A lack of aggressiveness in pursuing treatment, poor patient compliance and unhealthy lifestyles are all the concerns to play a part. However, both the diseases can be consequences of genetic effects that are difficult to overcome. Several drugs alone or in combination is currently in use for the treatment of dyslipidemia and obesity including the therapeutic strategies mentioned in the Table 1.3 & 1.4. However, the advancement of some of these promising drugs/targets have been suspended primarily due to clinically observed issues related to the efficacy and/or safety in some patient population. Targets belong to dyslipidemia are not effective in blocking the progression of coronary artery

disease and patients may encounter atherosclerosis. Side effects observed with current obesity targets have limited their use and both the medications have failed to capture a large market. Advancement in the pathophisiology of CVD revealed the role of several proteins in this disease and made the scientific community intrigued to explore them as targets for discovering new drugs. Among several targets mentioned, following targets could have possibility of potentially attractive therapeutic strategies, which could deliver safe and efficacious drugs.

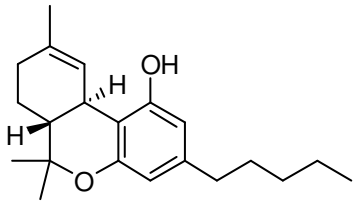
1.5 Cannabinoid receptor antagonist (CB1 Antagonists)

1.5.1 Background

Among the various pharmacological targets explored in recent years for obesity treatment the endocannabinoids system (ECS), constituted the most promising and the most intriguing. The discovery of the ECS is almost 4000 yr old, when the therapeutic and psychotropic actions of the plant cannabis sativa were used to promote feeding and to reduce vomiting in India. Since then involvement of ECS has advanced markedly with the demonstration of cannabinoid (CB) receptors and peripheral metabolic effects. Components of ECS comprising of cannabinoid receptors (the cellular receptors), endogenous ligand and the enzymes involved in ligand biosynthesis and destruction which constitutes an efficient signaling network and regulates diverse physiological response in brain and peripheral tissues [41-43]. Following are type Cannabinoids which are classified based on their occurrence.

1.5.1.1 Phytocannabinoids

They are also called as natural cannabinoids, herbal cannabinoids or classical cannabinoids, such as Δ^9-THC which occur naturally in significant quantity in the cannabis plant main psychoactive constituent of marijuana [44] . The dronabinol (Marinol) is synthetic Δ^9-THC (Figure 1.2), used as an appetite stimulant, anti-emetic and analgesic.



Tetrahydrocannabinol (9-THC)

Figure 1.2 Phytocannabinoids

1.5.1.2 Endocannabinoid

Endocannabinoids are substances produced naturally within the body (endogenous) which activate cannabinoid receptors.

1.5.1.2.1 Anandamide or AEA (Arachidonyl ethanolamide)

Anandamide (N-arachidonoylethanolamide) is important neurotransmitter which regulates appetite, memory, pain, depression and neural generation of motivation and pleasure. Direct injection of anandamide injected into the forebrain enhanced the pleasurable responses of rats to a rewarding sucrose taste, and enhances food intake as well [45].

1.5.1.2.2 2-Arachidonoyl glycerol (2AG)

Another endocannabinoid 2-Arachidonoylglycerol (2-AG) was discovered in the year 1995 [46]. 2-AG regulates the transmission signals across synapses and involved in inflammatory, immune responses and also promotes feeding

behavior. Compared to the anandamide, 2-AG is present at relatively high levels in the central nervous system.

1.5.1.2.3 2-Arachidonyl glyceryl ether (Noladin ether)

2-Arachidonyl glyceryl ether which is an endogenous agonist of the cannabinoid-1 (CB1) receptor [47]. It was discovered in the year 2000 and it is the only cannabinoid exist in ester form.

1.5.1.2.4 *N*-Arachidonyl-dopamine (NADA)

This endocannabinoid was discovered in 2002, Apart from cannabinoid receptor it also binds to transient receptor potential V1 (TRPV1) ion channel [48].

1.5.1.2.5 Virodhamine

In 2002, O-arachidonoylethanolamine named as Virodhamine was found. It is an endogenous antagonist of CB1 receptor [49].

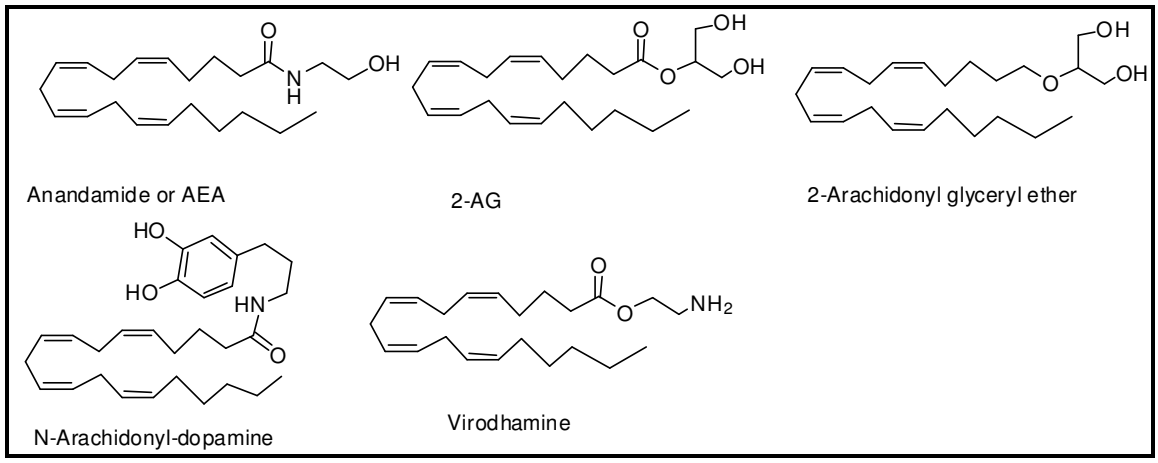


Figure 1.3: Endocannabinoids

1.5.1.3 Exogenous Cannabinoids or Synthetic cannabinoids

The role of cannabinoid in regulation of several physiological action led to the development synthetic cannabinoids. Towards optimizing the structure of Δ^9-THC and some of endocannabinoids, several cannabinoid receptor ligands have

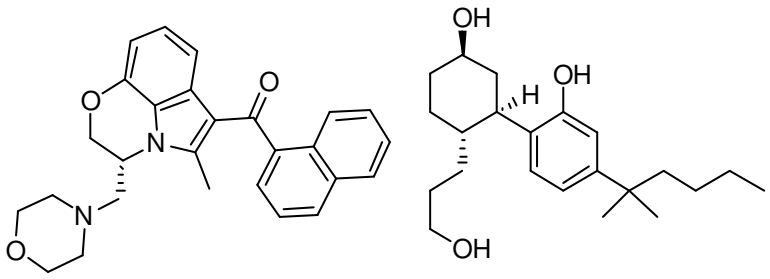
been synthesized having wide diversity in their chemical structure. Synthetic cannabinoids mainly are related to the structure of Δ^9-THC, while many others are various heterocyclic compounds.

1.5.1.3.1 WIN-55212-2

WIN 55,212-2 is a potent agonist at but has an entirely different chemical structure than Δ^9-THC [50], this compound is potent analgesic and it has played an important role in the for understanding functions of cannabinoid receptors.

1.5.1.3.2 CP-55940

It was produced in 1974, which is many times potent than THC. This compound is full agonist for both the cannabinoid receptors [51]. Structurally it is similar to naturally occurring THC.



WIN-55212-2 CP-55940

Figure 1.4 Synthetic cannabinoids

Endocannabinoids bind and activate the cannabinoid (CB1) receptors. Cannabinoid receptors send signals that play an important role in regulating body weight and glucose, and lipid metabolism. Cannabinoid receptors have agonists, antagonists and inverse agonists. Their potential or therapeutic benefits have been studied in a large variety of disorders such as obesity [52-55], metabolic syndrome, dyslipidemia and smoking addiction [56,57]. Till date two G-protein coupled type cannabinoid receptors of this system CB1 and the CB2 have been

identified. In the early 1990s, both the CB1 and CB2 receptors were cloned [58]. CB1 receptors are most abundantly expressed G-protein coupled receptor (GPCR) found primarily in brain having highest density in hippocampus, cerebellum and striatum [59]. It is also expressed in various peripheral tissues including the gastrointestinal (GI) tract, pancreas, liver, kidney, prostate, testis, uterus, eye, lungs, adipose tissue and heart [45,60], while CB2 receptors also referred to as peripheral cannabinoid receptor is completely absent in CNS and are found predominantly in immune cells and tissues [61]. Both the CB1 and CB2 receptor share approximately 48% homology in their amino acid sequences [62]. The sequence identity among human, rat and mouse for CB1 receptor is 97–99% [63] while human CB2 displays only 81 and 82% amino-acid identity with rat [64] and mouse [65] respectively.

The scientific information implicated involvement of CB1 receptor in evoking orexigenic response in rodents and humans, however in addition to their role in regulating energy uptake, recent body of evidences connect CB1 receptor mediated signaling to neuroprotection in several models of neurodegenerative diseases. It has been known for centuries that marijuana is also known as cannabis consumption stimulates appetite [66]. Cannabinoids are a group of terpenophenolic compounds present in cannabis. Substances that are structurally related to the natural active constituent ∆9-Tetrahydrocannabinol (∆9-THC) of marijuana bind to cannabinoid receptor. The exogenously administered cannabinoid agonist Δ^9 -THC is hyperphagic (excessive hunger) in rodents [67] and humans [68]. It is now fairly well established that the CB1 receptor is

implicated in the regulation of mammalian appetite. Studies in rodents have demonstrated the hyperphagic effects of peripherally administered anandamide and 2-arachidonoyl glycerol in pre-satiated rats [69]. Direct administration of anandamide into the ventromedial hypothalamus (an area rich in CB1 receptors) stimulates food intake [70]. This is also supported by data from CB1 receptor knockout mice (CB1-/-), In CB1 receptor knockout mice response to food desire is become weaker and do not have a hyperphagic response to overnight fasting [71].This finding draw attention to understand marijuana's mechanisms of action.

1.5.2 Mechanism of endocannabinoid-mediated control of food intake

CB1 receptors have been localized in neuronal circuits within the hypothalamus that are critical for the regulation of food intake and energy expenditure. CB1 receptor activation has been reported to play a significant role in protecting neurons from damage through feedback inhibition of glutametergic neuron activity by suppressing aversive memories through inhibition of signaling by GABA, and by producing central analgesia via suppression of nociceptive neurons.

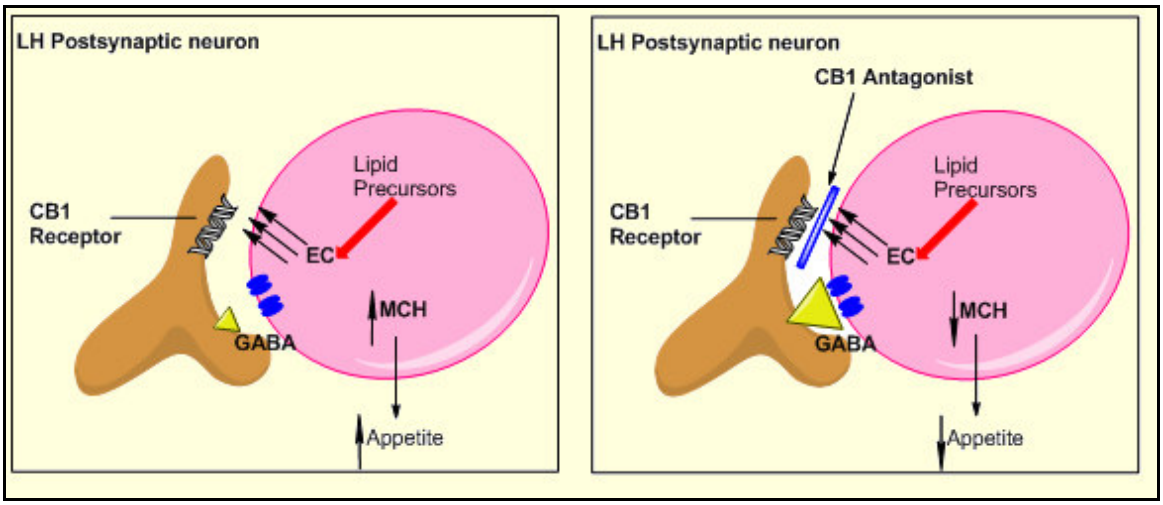


Figure 1.5 CB1 receptor signaling in appetite regulation.

The activation of CB1 receptors located on GABA terminal decreases GABA release, thereby enhancing the net excitability of perifornical LH neurons, consistent with increased feeding behavior. Antagonist would inhibit CB1 receptors, antagonizing the elevated endocannabinoids from the MCH neuron. This would potentially normalize GABA release and inhibit MCH release, leading to decreased appetite (Figure 1.5) [72].

Several lines of evidence suggest that endocannabinoids through the CB1 receptor play crucial role in controlling energy homeostasis and food intake. CB1 receptors also influence metabolic functions through effects in gastrointestinal tract, adipose tissue, liver and skeletal muscle [43]. These pharmacological investigations stimulate quest for the generation of substances acting as specific antagonists of CB1 receptors. Since the discovery of marijuana as an appetite stimulant, role of CB1 antagonist becomes an important research topic. The antagonist of CB1 receptor act both centrally and peripherally. Central effects include an anorexigenic effect, modulation of hormonal release in the hypothalamus [73,74] and decrease in motivation for palatable food in the nucleus accumbens [75] and peripheral effects reduces appetite, improves insulin resistance, and reduces hepatic lipogenesis. Drugs that antagonize CB1 receptor activation are attractive candidates for appetite suppression, however, majority of these antagonists behave as inverse agonists interfering with the constitutive activity of the receptor which invites serious malaise like anxiety, depression and mood disorder. CB1 knockout mice have been shown to have increased anxiety in several animal models of anxiety [76-78]. The wide range of

side effects generated on treatment with any of the known CB1 receptor antagonists points out to the necessity of development of neutral antagonist which would hinder ligand binding without fixing the receptor in an inactive state. Unfortunately, several CB1 receptor inverse agonists/antagonists were recently withdrawn from clinical development including the diarylpyrazole Rimonabant [79] and the acyclic amide taranabant (MK-0364) [80] due to central side-effects. Central cannabinoid-1 (CB1) receptors are distributed in the hypothalamus where they directly regulate orexigenic or anorexigenic signals is responsible for CNS side effects observed to above mentioned candidates.

1.5.3 Peripherally acting CB1 receptors antagonists

CB1 receptors are also present in peripheral tissues including the gut, [81] liver, hepatocytes, [82] and white adipose tissue [83]. There are many reports that antiobesity effects through CB1 antagonists is not limited to a central mode of action, peripheral CB1 receptors are also involved in the antiobesity effects of CB1-receptor antagonist (Figure 1.6).

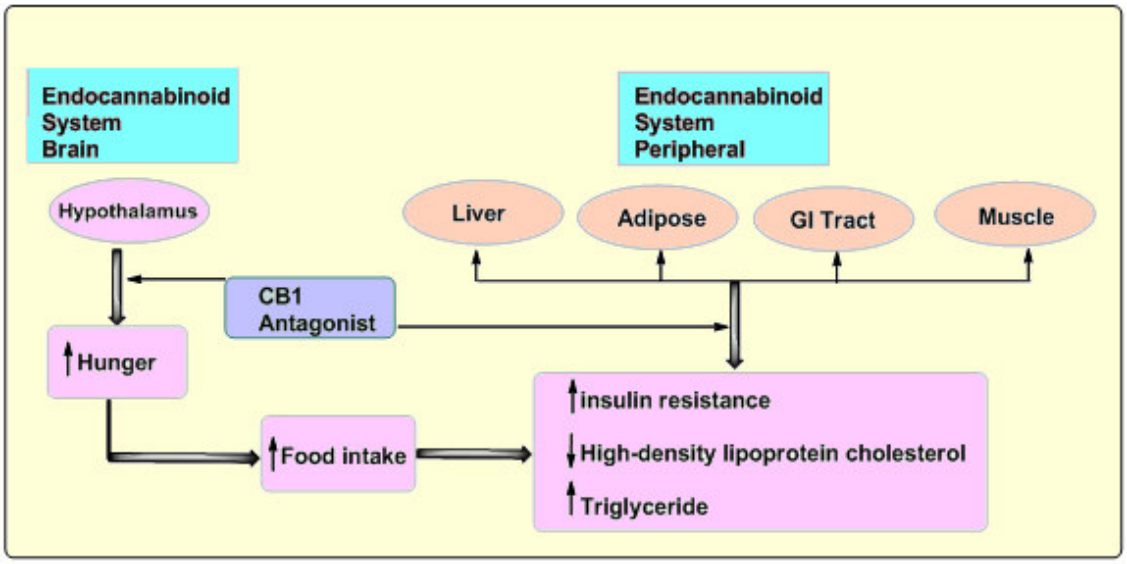


Figure 1.6 Hypothetical model for the metabolic effects of CB1 receptor antagonist

It has been presumed that in obese condition, the peripheral cannabinoid system might be up-regulated and up-regulation of the cannabinoid system by unbalanced energy intake and stress factors might cause excessive visceral fat accumulation [84]. According to a recent report, the blocker acting on peripheral CB1 receptors is alternative approach to salvage it from the demise of a drug class that was once anticipated to yield blockbusters [85]. Considering this, it is expected that modulating down this action could be an attractive target in the management of obesity and related metabolic disorders [86,87]. Peripheral effects are considered to be the stimulation of anorectic signals engaging CB1-receptor in sensory terminals in gastrointestinal tract [88]. Therefore peripherally directed CB1 receptor antagonist may provide effective therapy options for the management of metabolic disorders such as obesity.

1.6 Thyroid Hormone Receptor β (TRβ) Agonists

1.6.1 Background

Thyroid hormone are involved in lipid homeostasis and energy expenditure. Because of this unique property thyromimetics are emerged as fascinating target for the treatment of dyslipidemia and obesity. Thyroid hormone receptors (TRs) are ligand-dependent transcription factors that belong to the nuclear receptor superfamily. Thyroid hormones carryout their action by binding to thyroid hormone receptor. The thyroid gland mostly produces endogenous thyroid hormones (TH) 3,5,3'-triiodo-Lthyronine (T3) and its prohormone 3,5,3',5'tetraiodo-L-thyronine (T4). Thyroid hormones are synthesized in response to thyroid stimulating hormone (TSH), which is secreted by the pituitary

gland (Figure 1.7). Production of T4, and T3, by the thyroid gland is under negative feedback control. TSH, also known as thyrotropin, is responsible for normal thyroid gland function and thyroid hormone secretion. It is synthesized in the anterior pituitary gland, and its secretion is controlled by thyroid releasing hormone (TRH) that is synthesized in the hypothalamus.

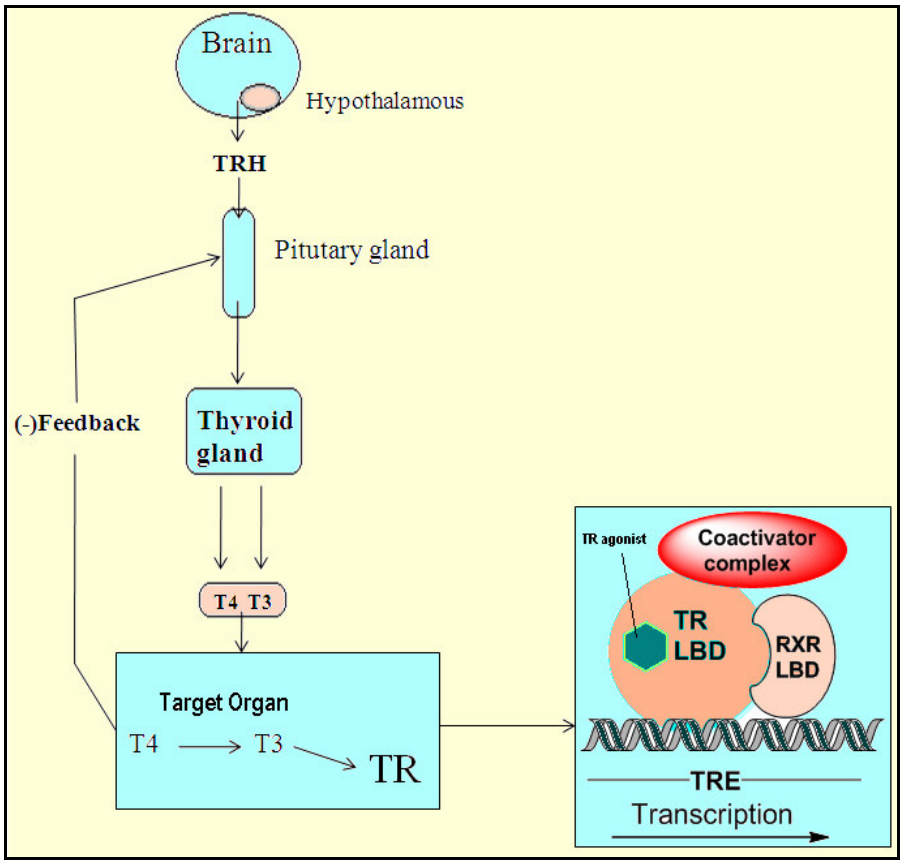


Figure 1.7 Synthesis, secretion, and actions of thyroid hormones

The natural thyroid hormones (TH) T3 and T4 are important endocrine signaling hormones. Thyroid hormones are iodinated tyrosine analogues excreted into the circulation primarily as T4. It is converted to T3 rapidly by deiodination in local tissues which is the most potent thyroid hormone. It plays important role in normal development, differentiation and maintenance of metabolic balance, control of cholesterol levels through interaction with thyroid

hormone receptors (THR) [89-92]. Natural thyroid hormone T3, exhibit its physiological effect by acting on a thyroid hormone receptor (TR). There are two different isoforms of Thyroid hormone Receptors known till date, TRα and TRβ. Further, these two isoforms are sub-classified as α 1; α 2 and β1; β2 subtypes. TRβ1 is prevalent in liver and pituitary, while TRα1 is mainly present in cardiac tissue [93]. Since there is single amino acid difference in the ligand binding pocket of TRα and TRβ (Ser277 in TRα1 and Asn331 in TRβ1) designing specific ligand is difficult.

1.6.2 Mechanism of action

Thyroid Receptors are nuclear receptors consist of different functional domains: (1) N-terminal transactivation domain (AF-1), (2) DNA binding domain (DBD), (3) Ligand binding domain (LBD) (Figure 1.7) [94]. The N-terminal domain is the less well-conserved region of the thyroid receptors. DNA binding domain is important for interaction with DNA [95,96]. The hinge region (D), located between the DBD and the LBD contains the nuclear recognition signal. Ligand binding domain which is also called as carboxyl-terminal plays an important role in dimerization, transactivation, and transrepression [97,98]. Like other nuclear receptor thyroid receptor also forms heterodimer with Retinoid X Receptor(Figure 1.7). TRs bind to TREs usually as homodimers or heterodimers along with auxiliary proteins.

At normal levels, T3 maintains body weight, metabolic rate, body temperature, mood and regulate serum cholesterol. Hypothyroidism is associated with weight gain, high levels of low-density lipoproteins (LDL)

cholesterol and depression. Hyperthyroidism leads to weight loss, hypermetabolism, lowering of serum LDL levels, cardiac arrhythmia, heart failure, muscle weakness, bone loss and anxiety. The natural thyroid hormone T3 does not show any selectivity in binding to both of the TR isoforms (TRα1 and TRβ1). Administration of T3 lowers plasma cholesterol, low-density lipoprotein (LDL) and triglyceride levels in animal models and humans.

The mechanism for LDL cholesterol lowering by thyromimetics is different from that of the HMG-CoA reductase inhibitors (3-hydroxy 3-methylglutaryl-CoA reductase inhibitors), i.e. statins, which are currently the first hand drugs for the treatment of hypercholesterolemia. Thyroid hormone are central regulator of lipid metabolism. β Selective thyromimetic and/or liver-selective thyroid hormone analogs increase hepatic LDL receptor density and also stimulate reverse cholesterol transport that partly seems to involve an increased number of hepatic high-density lipoprotein (HDL) binding receptors SR-BI and the subsequent biliary secretion of cholesterol [99,100].

1.6.3 Beta selective and/or Liver selective Thyromimetics

Synthetic ligand which produces effects similar to those of thyroid hormones (mostly thyroid receptor agonist) are called "**thyromimetics"**. Many actions of the thyroid hormone are tissue specific. The activation of TRα isoform mainly affects the heart rate and rhythm whereas, activation of TRβ isoform is known to reduce plasma cholesterol, low-density lipoprotein (LDL) and triglyceride levels [101]. Knockout animal studies as well as results with some selective ligands suggest that such cardiac side effects can be attributed to the

THR α isoform [102]. However, T3 cannot be used therapeutically to treat hypercholesterolemia due to its cardiac side effects because of its non selective actions on thyroid receptor. Several lines of evidence suggests that selective TR β agonist could be a viable therapeutic option to treat hyperlipidaemia, devoid of cardiovascular and other toxicities of native thyroid hormone. Thus, some effects of T3 may be therapeutically useful in non-thyroid disorders if adverse effects can be minimized or eliminated. These potentially usefulness influences include weight reduction, lowering of serum LDL levels, amelioration of depression and stimulation of bone formation [103]. Development of specific and selective thyroid hormone receptor ligands, particularly THR β agonist could lead to specific therapies for disorders such as obesity and hyperlipidemia, while avoiding the cardiovascular and other toxicities of native thyroid hormones. Thus, compounds mimicking only the beneficial effects of the thyroid hormone and lacking their cardiac side effects (tachycardia and arrhythmia) potentially could be used to treat a number of conditions such as obesity and dyslipidemia. In this regard, TR agonists (Thyromimetics) that interact selectively with the β isoform of the TR offer an especially attractive method for avoiding cardiotoxicity [104].

Apart from cardiac side effects another possible adverse effect is suppression of thyroid hormone axis (THA). TSH regulates thyroid hormone production and thyromimetics can negatively regulate TSH secretion which ultimately leads to suppression of TSH levels and decreased levels of endogenous T3. Knockout studies [105] have also proved that negative regulation of TSH is through activation of the thyroid receptor beta, which is

highly expressed in the pituitary. Despite of achieving TRβ selectivity possibility of TSH suppression cannot be ignored. Selective TRβ agonist may exhibit modest cardiac sparing in rodents and primates and lower lipids but it may induce the THR β mediated suppression of the THA. The liver is the target organ for thyroid hormone mediated regulation of lipid metabolism, a liver selective thyroid receptor agonist therefore may not suppress THA.

Based on all the above mentioned safety concerns, till date two strategies have been attempted for the development of novel thyromimetics. One is by making isoform selective compounds [106] and another is by making liver selective thyromimetics [107]. Liver selective compounds are expected not to suppress the THA. Thus β selective thyromimetic having with liver selectivity could be devoid of cardiac toxicity and may not suppress THA. Improved therapeutic index achieved by Selective TRβ agonists can be devoid of cardiac toxicities but it may have effects on TRβ mediated suppression of the THA in the pituitary. Therefore, in the present invention we aimed on enhancing activation of thyroid receptor in liver using selective TR β agonists.

1.7 MTP inhibitors

1.7.1 Background

Microsomal triglyceride transfer protein is one of the target area which has evidenced potential benefits to reduce triglycerides and cholesterol [108]. MTP is found in endoplasmic reticulum (ER) of enterocytes and hepatocytes. It is a heterodimeri lipid transfer protein [109,110]. MTP is 894AA protein [111] , Protein

disulfide isomerase (PDI) is a part of the microsomal triglyceride transfer protein (MTP)[112] and plays very important role in apolipoprotein B secretion [113].

1.7.2 Mechanism of action

Abetalipoproteinemia (ABL) or Bassen-Kornzweig syndrome is a hereditary disease characterized by inability to produce chylomicrons and very low-density lipoproteins in the intestine and liver respectively. Homozygous autosomal recessive mutations in the gene encoding the 97-kDa subunit of the Microsomal triglyceride transfer protein (MTP) causes this genetic disorder [114]. Since chylomicrons carry fat and cholesterol to the bloodstream, absence or reduced levels of these chylomicrons leads to low plasma cholesterol and triglyceride levels. However fat-soluble vitamins (like vitamin E and D) also require chylomicrons for their systemic transportation and the low levels of chylomicrons may lead to vitamin deficiencies. Further adverse effects of absence of chylomicrons are reported to lead to gastrointestinal, neurological, ophthalmological, and hematological abnormalities [115]. MTP is essential for assembly of triglycerides with apo-B48 producing chylomicrons in intestine and with apoB-100 in liver to synthesize and secrete VLDL. This VLDL produced by the liver are the major source of LDL in plasma. Elevated levels of LDL are associated with the development of atherosclerosis and cardiovascular disease (CVD). Increased total cholesterol and LDL cholesterol (LDL-C) both are considered primary risk factors for atherosclerosis [116].

Apo-B is glycoprotein and very important for lipid metabolism [117,118]. There are two types of apo-B known, Apo B-48 and Apo B-100, they comprises

of 2152 AA and 4536 AA respectively[119,120]. In intestine Apo B-48 is synthesized and finally forms triglyceride rich chylomicrones on the other hand in liver apo-B-100 synthesized and forms very low density lipoprotein (VLDL) (Figure 1.8). It is commonly observed that in T2DM or metabolic syndrome apo-B is over expressed [121].

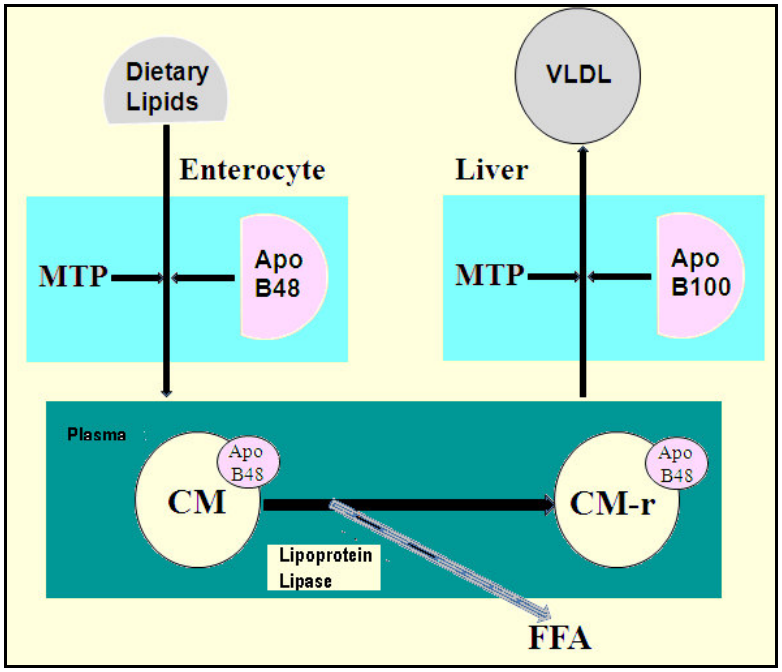


Figure 1.8 Simplified scheme of MTP in formation of chylomicron from enterocyte and VLDL production from liver cells. CM, Chylomicrone; FFA, Free Fatty acid; CM-r, Chylomicrone remant

An increase in MTP protein mass, which is associated with over secretion of intestinally derived apoB48 lipoproteins- chylomicrons, was observed in preclinical studies on fructose-fed insulin resistant hamsters [122]. Inhibition of MTP has been shown to be an effective method for reducing serum cholesterol. Moreover, diabetic rabbits and rats exhibited increased MTP expression leading to increased chylomicron production [123]. It is also reported that increased MTP expression plays a crucial role in the development of atherosclerosis[124]. In diabetic condition also there is indirect role which MTP inhibitor. Insulin

resistance might result in hyperinsulinaemia, which enhances hepatic gluconeogenesis and glucose output, to reduce the suppression of lipolysis in adipose tissue and leads to high free fatty acid (FFA) flux, ultimately this results in high hepatic very low-density lipoprotein (VLDL) secretion, causing hypertriglyceridemia .These changes are linked with increased production and secretion of apolipoprotein (apo) B-containing VLDL [125,126]. Changes in lipoprotein secretion have already been reported in the context of insulin resistance or diabetes, in animal models as well as in humans, involving enhanced activity of MTP in association with an oversecretion of apoB48 lipoproteins [122,123,127].Inhibition of MTP may reduce plasma concentrations of triglycerides along with LDL and VLDL–cholesterol. These beneficial effects could be useful for the treatment of atherosclerosis in insulin-resistant conditions [128].

As liver and intestine are target organ, it is well understood that possible side effect may be in liver and intestine. MTP inhibition in liver blocks the formation of VLDL(lipoprotein assembly) which ultimately leads to fatty liver. Enzymes like AST and ALT also increases by inhibition of MTP. Another concern because of the MTP inhibition in intestine leads to GI disturbance which ultimately results in diarrhea. There are many other side effects are also known. These side effects are because of the accumulation of triglyceride and cholesteryl esters in intestine and liver respectively as it has been not utilized.

Patients having familial hypercholesterolemia (FH) are not always able to achieve target levels for LDL cholesterol specifically because of very high levels

of low-density lipoprotein (LDL) in the blood. This could be because of mutations in the LDL-receptor gene. It is difficult to achieve target levels of LDL cholesterol with the current available LDL-C reducing therapy; even results with combination therapy are not satisfactory. In such condition patients can compromise with the adverse effects taking place because of MTP inhibitors against the threat of high CVD mortality risk. Many of the ongoing MTP inhibitors are recommended for the treatment of FH. In addition to this MTP inhibitors represent a unique class of anti-obesity agents that offer potent weight loss [129]. One of the compound dirlotepide reduces appetite and increases fecal fat, it is marketed to reduce obesity in dogs [130].

Collectively MTP has distinct function in liver as well as in the intestine. Altogether MTP plays crucial role for the generation of triglyceride rich chylomicrones in liver and for VLDL in intestine [131]. Therefore, MTP inhibitor can cause reduction in plasma triglyceride and cholesterol. It can also be used as anti-obesity agents. Several compounds are advanced up to clinical trials [132]. BMS-201038 is a 9H-fluorene-9-carboxamide derivative and was developed from its early hit BMS-200150. The BMS-201038 [108] is further developed as AGER-7323 and was taken to clinical trials [133].

1.7.3 Enterocyte specific MTP inhibitors

MTP inhibition could lead to deleterious side effects in liver. The side effects which are observed in intestine cannot be considered as chronic side effects. Because of this it was thought that MTP inhibition in intestine might be useful to avoid liver toxicity. SLX-4090, JTT-130, are some of the examples of

Enterocyte specific inhibitors of MTP. Enterocyte specific inhibition of MTP could be called as IInd generation MTP inhibitors [134].

Several targets/therapies are available for treatment of dyslipidemia and obesity. However, there is a scope for improvement in treatment with respect to safety and efficacy. Several newer targets are identified, evaluated and at the same time there is possibility of identifying newer strategy for the established targets which can overcome safety concerns. Above mentioned three targets CB1 receptor antagonists, Thyromimetics and MTP inhibitors are proven targets for the management of dyslipidemia and/or obesity. An important characteristic belongs to these targets is by generating tissue selectivity safety concerns can be addressed. Strategy to make tissue selective compound might play very important role to overcome safety concerns and develop novel therapeutic agent.

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CB1 ANTAGONISTS

2

2 CB1 antagonists

2.1 Objective

The discovery of cannabinoid-1 (CB1) receptor antagonist and understanding of their role in appetite regulation has offered a new drug target for obesity treatment. Cannabinoids act on two different (CB1 and CB2) receptors. The CB1 receptor is widely distributed in central and peripheral nervous system. In addition, CB1 receptor has been found in adrenal gland, bone marrow, heart, lungs, prostate and testicles. On the other hand CB2 receptor is less widely expressed and found mainly in the immune related organs/tissues like tonsils, spleen, thymus and bone marrow and in B-lymphocytes, monocytes / macrophages, mast cells and microglial cells. CB1 receptor antagonists act centrally as well as peripherally and reduce food intake and modulate metabolic parameters.

Several synthetic cannabinoids have been evaluated preclinically and also in clinical trials are shown in (Figure 2.1). Rimonabant (SR141716, Acomplia) is a potent and selective antagonist for the CB1 receptor. Rimonabant was approved in the European Union (EU) in June 2006 for the treatment of obesity. Later in October 2008 the European Medicines Agency (EMEA) has recommended the suspension of the marketing authorization across the EU based on the risk of serious psychiatric disorders. In humans Rimonabant showed CNS (depression, anxiety, dizziness, and insomnia) and gastrointestinal (nausea, diarrhea) side effects. Surinabant (SR147778) another compound by Sanofi could reach Phase I and was described as a potent, orally active

antagonist of the CB1 receptor for smoking cessation. However, in October 2008 Sanofi-Aventis discontinued development of Surinabant.

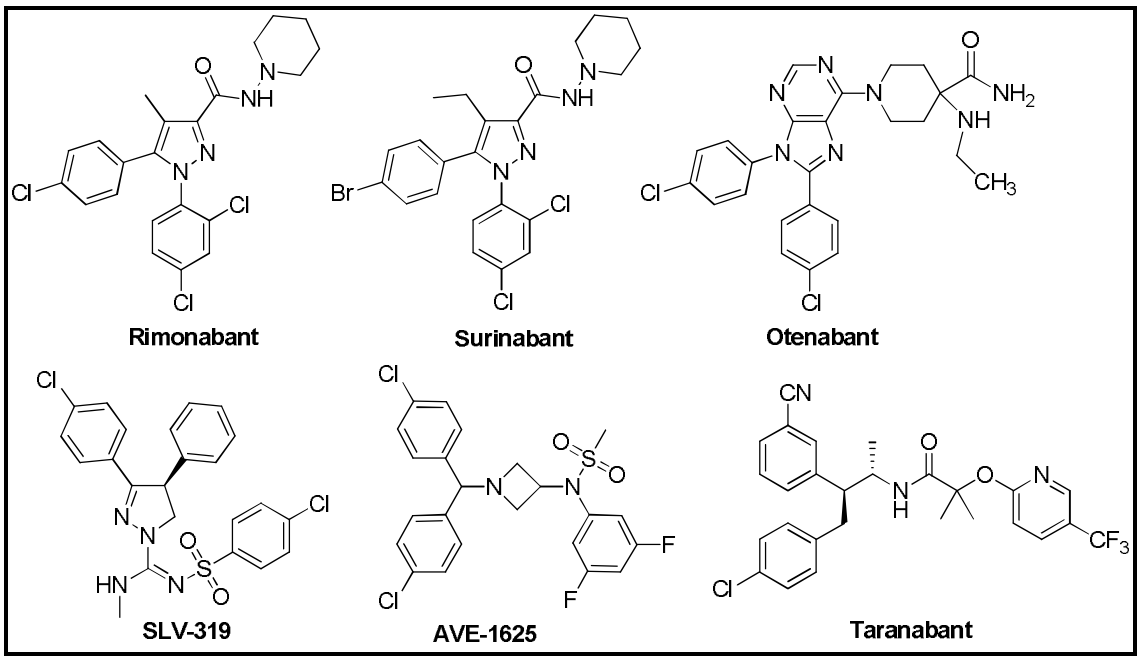


Figure 2.1: Synthetic cannabinoids

The Pfizer group began with the concept of generating constrained analogues of Rimonabant and discovered Otenabant (CP-945598), a selective antagonist of the CB1 receptor. However, the Phase III development program was terminated for the compound Otenabant. Similarly for one of the investigational compound SLV 319, solvay Pharmaceuticals announced its decision to discontinue all R&D activities [1]. Aventis Pharmaceuticals came up with AVE1625 [2] an oral selective and potent antagonist of cannabinoid receptors, which could advance up to Phase II. Taranabant (MK-0364) another compound develop by merck that did not contain cyclic central moiety was discontinued at Phase III clinical development stage [3,4].

With very few drugs in the market and the adverse effects associated with them, obesity remains an almost completely unmet challenge for the

pharmaceutical industries. Most of the CB1 antagonists have caused psychiatric side effects such as depression and subsequently these compounds were dropped from development. It is well documented that CB1 receptors are present in peripheral tissues and blocker acting on peripheral CB1 receptors can be an alternative approach to avoid safety concerns faced by centrally acting CB1 antagonists. Therefore, our aim was to target novel CB1 antagonists that would be act primarily on peripheral distribution.

2.2 Dihydropyrazole-3-methyl carboxamide derivatives

In the light of the experience with CB1antagonists, such as Rimonabant, a major paradigm shift in clinical practice might be necessary and our group at Zydus Research Center did number of attempts to address the issues pertaining to this class of compounds.

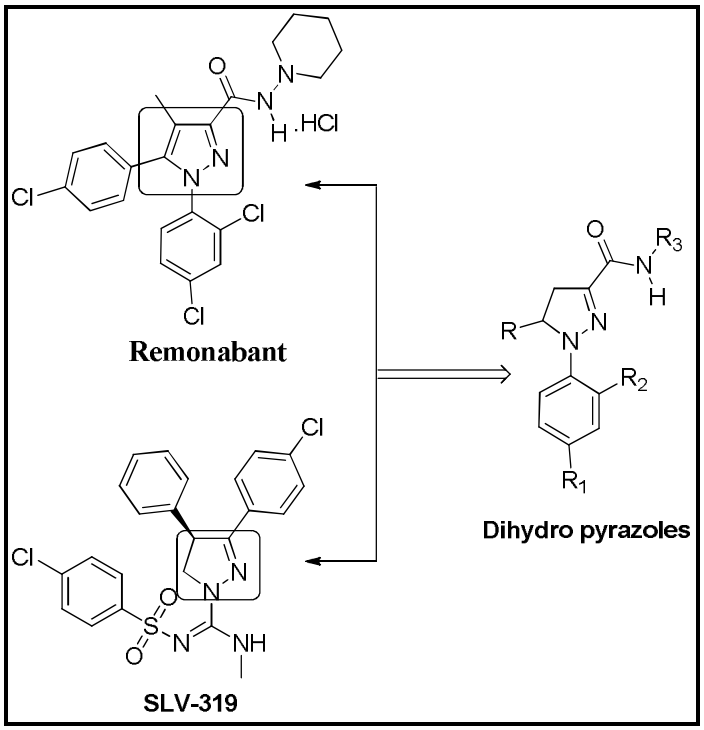


Figure 2.2: Modification to structure of Rimonabant

We have previously described optimization efforts using bioisosteric replacement in a rational approach. In conjunction with molecular modeling studies, we worked on diaryl dihydropyrazole carboxamide derivatives (Figure 2.2) as CB1 antagonists. A number of analogues of diaryl dihydropyrazole-3-carboxamides have been synthesized and evaluated for appetite suppression and body weight reduction in animal models. These compounds displayed good potency against CB1, along with oral efficacy in a number of preclinical obese rats models [5].

In continuation of our research on CB1 antagonists, we selected SLV-319 for further modification and the dihydropyrazole system of SLV-319 was replaced by isosters such as imidazole and oxazoles (Figure 2.3).

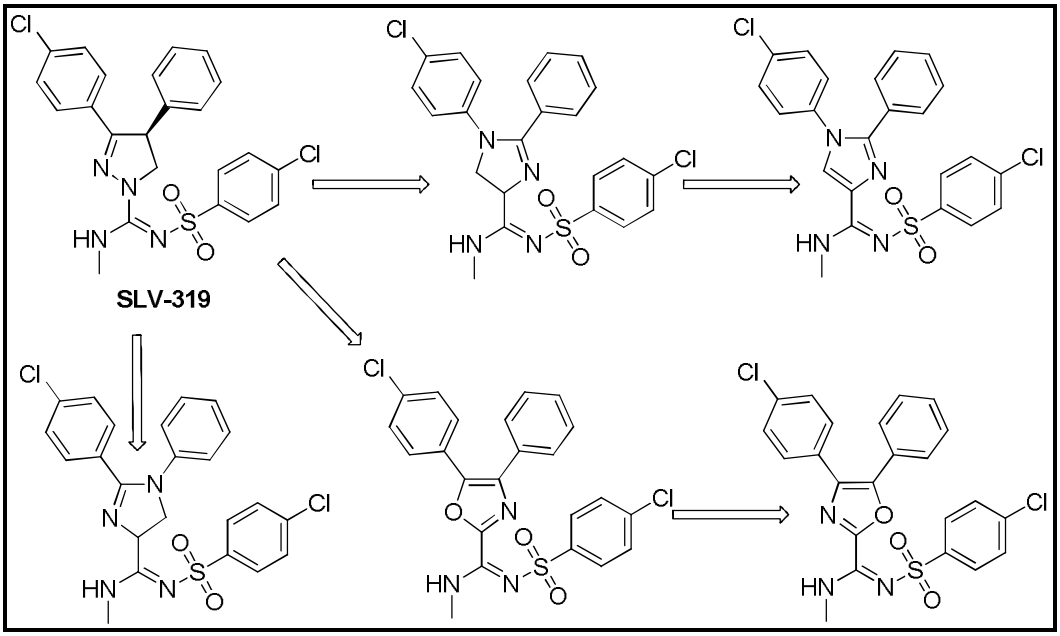


Figure 2.3: Modification to SLV-319 structure

Further the compounds imidazole and oxazoles were studied for in vitro, in vivo pharmacological evaluation in relevant CB1 antagonist models [6]. Some more structural modifications led to develop better, efficacious 3-sulfonamide

derivatives of Rimonabant [7]. Structurally similar thienyl substituted pyrazole derivatives were identified as hair growth stimulator and an antiobesity agent in animal model [8]. Despite the large number of CB1 receptor antagonists developed so far there is no clear understanding about the structural features required for access to CNS vs peripheral tissues. For very few CB1 antagonists plasma-to-brain concentration ratio is known.

URB447 (Figure 2.4) was the first peripherally restricted CB antagonist discovered. URB447 reduces food intake and bodyweight gain in mice with an efficacy comparable to that of the structurally close Rimonabant. In addition to that, URB447 also reversed the analgesic effect of (R)-methanandamide without entering the brain or antagonizing CB1 receptors in the CNS. Although URB447 is restricted to peripherally, mechanistically it was claimed to be mixed CB1/CB2 antagonist [9].

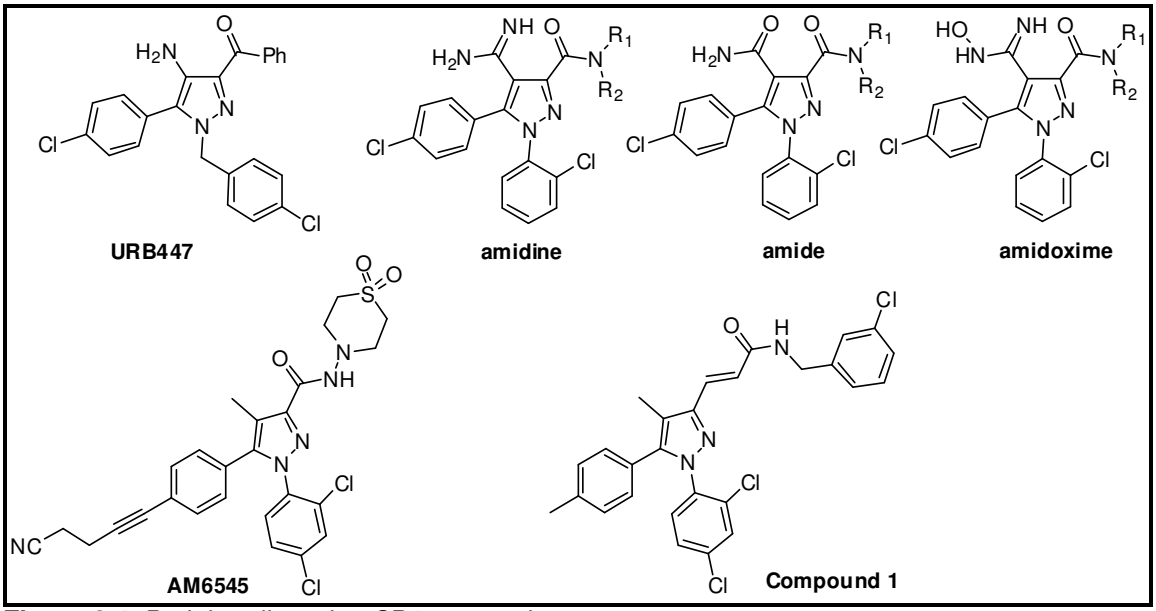


Figure 2.4: Peripherally acting CB1 antagonists

Since then many other examples are available as proof of concept, Modifications on the same scaffold amide, amidine or amidoxime (Figure 2.4) group which comprises of lower lipophilicity, higher polar surface area brought improved plasma/brain ratios compared to the centrally acting Rimonabant [10]. It is demonstrated that AM6545 (Figure 2.4) is potent peripherally restricted CB1 receptor antagonist having nanomolar potency and treatment of obese mice with AM6545 (Figure 2.4) improves glycemic control, dyslipidemia and reverses hepatic steatosis [11]. In diet-induced obesity (DIO) mice, AM6545 did reduce body weight, but it was less efficacious than the brain-penetrant CB1 receptor antagonist Rimonabant. Compound 1 (Figure 2.4) was found peripherally more selective and potent enough to dissociate the central and peripheral effects [12].

These data strongly suggest the contribution of peripheral CB1 receptor activation to these hormonal/metabolic abnormalities. Thus the aim of present work and part of thesis is to design, synthesize and characterize novel peripherally active potent CB1 receptor antagonists.

2.2.1 Rationale

Fundamentally drugs acting in the CNS tend to have lower polar surface area (PSA). Thus, the compounds having high PSA could an added advantage. Most of the claimed peripherally active CB1 antagonist belongs to pyrazole structure which is also another point for consideration. As mentioned earlier, we have previously worked on dihydropyrazoles [5] which was bioisosteric replacement of pyrazoles. However, the issue of CNS related side effect came later on and it was not addressed at that time.

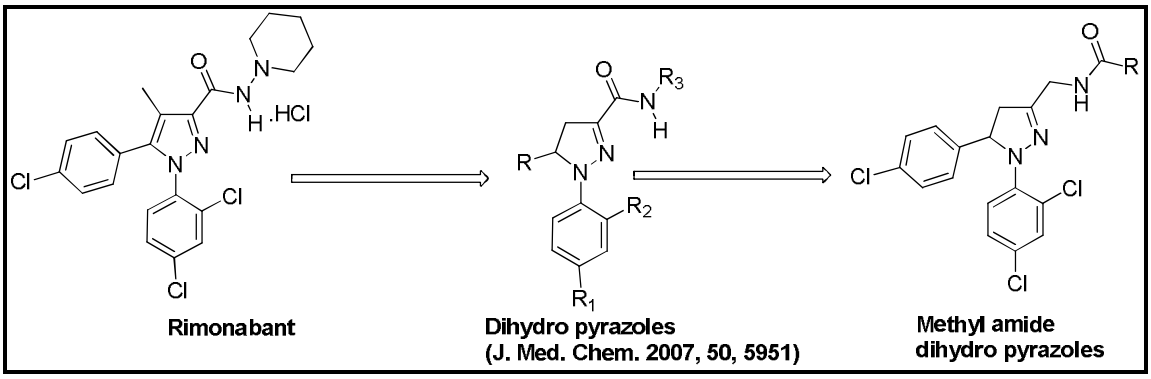


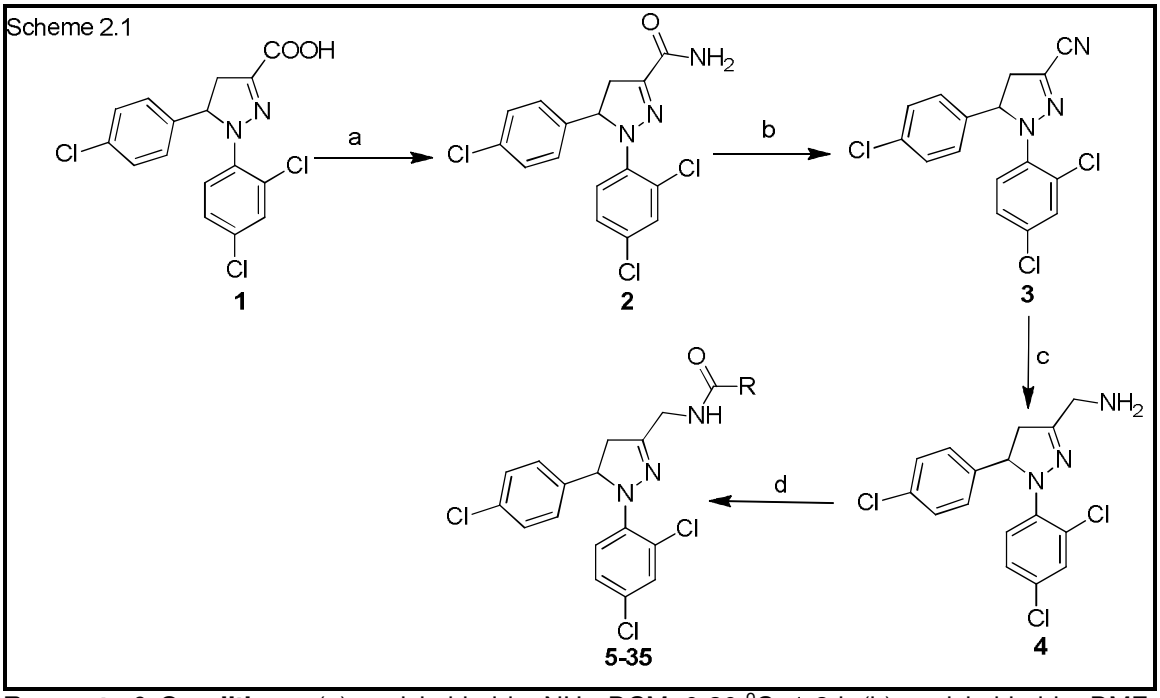
Figure 2.5: Designing novel series

The exploration started from pyrazole derivatives, as explained earlier in most cases amide portion at 3rd position is altered including recently reported by Flup et al [13]. Our prior experience suggests that dihydropyrazole turned out equally good to pyrazoles. Applying same hypothesis to subsequent compound series, we have modified further at amide position of dihydropyrazoles to methyl amide dihydropyrazoles (Figure 2.5).

In this modification, amide site was modulated to test the effect of long chain, branched and cyclic analogs. In the foresaid context, series of methyl amide dihydropyrazoles were synthesized and evaluated.

2.2.2 Synthesis

Scheme 2.1

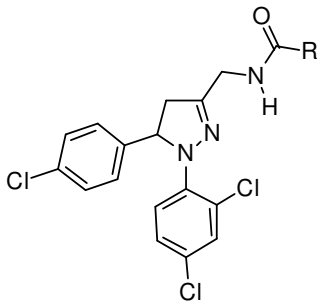


Reagents & Conditions: (a) oxalyl chloride, NH_3, DCM, 0-30 ^oC, 1-2 h (b) oxalyl chloride, DMF, C_5H_5N, 0-30 ^oC, 2-3 h (c) Et_2O, $LiAlH_4$, -30 to -35 ^oC, 2-3 h (d) RCOOH, HOBt, EDC.HCl, triethylamine, CH_2Cl_2, 26-28 ^oC, 2-4 h.

The 4,5-dihydro-1H-pyrazole-3-carbonitrile **3** was synthesized as per literature procedure [14]. The carboxamide derivatives **5-35** were synthesized as depicted in Scheme 2.1. The 4,5-dihydro-1H-pyrazole-3-carbonitrile **3** was reduced by lithium aluminum hydride in diethyl ether to afford the corresponding methylamine derivative **4**. The methylamine derivative **4** was coupled with various RCOOH under usual peptide (amide) bond formation chemistry using [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide].HCl (EDCI.HCl) and 1-hydroxy benzotriazole (HOBt) to afford carboxamide derivatives (**5-35**)**.** To build the SAR several long chain, branched and cyclic carboxylic acids were coupled with the intermediate **4.**

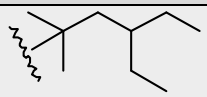
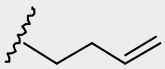
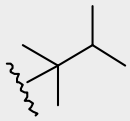
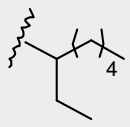
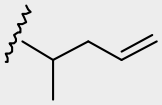
2.2.3 Result and Discussion

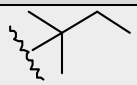
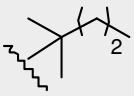
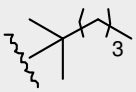
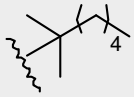
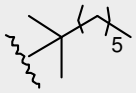
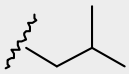
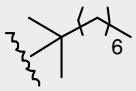
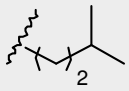
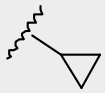
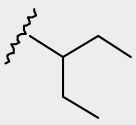
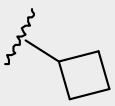
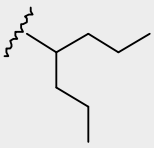
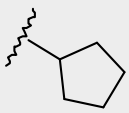
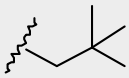
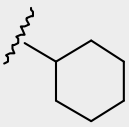
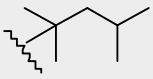
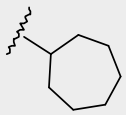
All the acyclic amides have been screened for *in vitro* hCB1 functional potency in the functional assay of cyclic AMP (cAMP) production and compared with Rimonabant.



5-35

Table 2.1: *in vitro* hCB1 functional assay for assessing cAMP activity of dihydropyrazole-3-methyl carboxamide derivatives

Compound	R	hCB1 at 10 μM [a,b]	Compound	R	hCB1 at 10 μM [a,b]
WIN552122		37.68 ± 0.03	**19**		63±2
Forskolin		100.00 ± 1.32	**20**		54 ±8
Rimonabant		106.69 ± 0.84	**21**		59±5
5		22±2	**22**		115 ±7
6		59 ±4	**23**		55±10
7		30 ±3	**24**		90 ±3

Compound	R	hCB1 at 10 μM [a,b]	Compound	R	hCB1 at 10 μM [a,b]
8		56 ±1.5	**25**		48 ±2
9		58 ±0	**26**		72±6
10		76 ±1	**27**		**135 ±2**
11		40 ±7.4	**28**		82±4
12		68 ±18	**29**		73±3
13		49 ±4	**30**		47 ±11
14		49 ±2	**31**		49 ±2
15		48 ±2	**32**		54 ±3
16		110 ±3	**33**		65±2
17		100 ±11	**34**		101 ±19
18		53±3	**35**		61 ±2

[a] Activities are presented as forskolin-stimulated cAMP production, cAMP assay was carried out in Chinese Hamster Ovarian cells stably expressing human hCB1 receptor, respectively ,

[b] Values indicate mean ± SD performed in duplicate and the results being representative of at least three independent experiments

It is evident from the Table 2.1 that most of the compounds are either equipotent or inferior than Rimonabant. Amides starting from three carbon chain length were extended up to 10 carbons which gradually increases the lipophilicity. All this liner chain compounds **5-11** were less potent than Rimonabant in cAMP production assay. Further SAR was explored with branched chain. Here also similar approach was carried out and compound **12-30** of various chain length were tested. Out of all these compounds, only **16** and **17** showed similar activity to Rimonabant and compound **27** was slightly better than Rimonabant. Further, amides formed by cyclic carboxylic acid **31-35** were also tested. From this modification, potency of cyclohexyl analog **34** was comparable to Rimonabant. Combining all the results we decided to pursue further with compound **27.** Since all the compounds tested were racemic, our next goal was to separate both the enantiomers of compound **27**. Both the enantiomer of compound **27** were synthesized (detail given in experimental section) and tested in the same assay (Table 2.2)**.**

Table 2.2: *In vitro* hCB1 functional potency of dihydropyrazole-3-methyl carboxamide derivative of enantiomers of **27**

Compound	**hCB1 at 10 μM** [a,b]
WIN552122	37.68 ± 0.03
Forskolin	100.00 ± 1.32
Rimonabant	106.69 ± 0.84
(-) Enantiomer (40)	102 ±2
(+) Enantiomer (41)	36 ±7

[a] Activities are presented as forskolin-stimulated cAMP production, cAMP assay was carried out in Chinese Hamster Ovarian cells stably expressing human hCB1 receptor, respectively**,** [b] Values indicate mean ± SD performed in duplicate and the results being representative of at least three independent experiments

The *in vitro* data suggest that (+) enantiomer **41** was found to be less active than (-) enantiomer **40** which showed comparable activity to Rimonabant. Based on this fold activation data, compound **27, 40** and Rimonabant were screened at multiple concentrations for determination of EC_{50}. Finally, the CB1 receptor functional potency (EC_{50}) of **27** and it's (-) enantiomer **40** was determined by inhibition of forskolin-stimulated intracellular cAMP increase of CB1 receptor expressing CHO cells (Table 2.3).

Table 2.3: *In vitro* EC_{50} of Rimonabant, **27** and **40**

Compound	**EC_{50}[a]**
Rimonabant	781 nM
Compound **27**	76.3 nM
Compound **40**	59.5 nM

[a]Values indicate mean ± SD of at least three independent experiments performed in duplicate. cAMP assay was carried out in Chinese Hamster Ovarian cells stably expressing human hCB1 receptor respectively

The results of EC_{50} suggest that compound **27** and it's (-) enantiomer **40** is about 10 fold potent than Rimonabant.

Table 2.4: *In vivo* efficacy of diaryl dihydro pyrazoles **27** & **40** in 5% sucrose solution intake model in female Zucker fa/fa rats at single oral dose of 10 mg/kg

Compound	**% Inhibition *vs.* Control [a]**
Rimonabant	36.7± 5.3
27	36.4 ± 10.2
40	61.7 ± 5.1

[a]Values indicate mean ± SEM for n = 6 rats in 4 h.* $p < 0.05$, when compared with the control group, one way ANOVA followed by Dunnett's multiple comparison test.

To support this results, compound **27** and **40** were evaluated for efficacy in a 5% sucrose solution intake model at single dose (Table 2.4). It was found that racemic compound **27** showed less activity compare to it's (-) enantiomer **40** in the 5% sucrose solution intake model and at the same time (-) enantiomer **40** showed better efficacy than Rimonabant. Both (-) enantiomer **40** and Rimonabant were subjected for evaluation of pharmacokinetic parameters. Both Rimonabant and (-) enantiomer **40** were tested orally at 30 mg/kg dose for it's pharmacokinetic evaluation (Table 2.5).

Table 2.5: Mean pharmacokinetic parameters of 40 and 1 in fasted female Zucker fa/fa rats.[a]

Compound	Route	dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC(0-∞) (h.μg/mL)
40	Oral	30	1.58 ± 1.10	2.04 ± 0.14	3.53 ± 6.10	9.27 ± 1.40
Rimonabant	Oral	30	2.60 ± 1.30	0.92 ± 0.31	26.55 ± 7.23	41.20 ± 3.65

[a] Values indicate mean ± SD for n=6 rats

As depicted from Table 2.5, AUC of (-) enantiomer **40** is relatively less than Rimonabant. Despite of having less AUC than Rimonabant, better efficacy of the can be explained as it is 10 fold more potent than Rimonabant.

Table 2.6: Tissue and Plasma Distribution of 40 and 1 in fasted female Zucker fa/fa rat at 3 h after oral dose of 30 mg/kg.[a]

Compound	Drug Concentration in Brain (μg/mL)	Drug Concentration in White Adipose Tissue (μg/mL)	Drug Concentration in Plasma (μg/mL)	Brain/Plasma ratio
40	0.037 ± 0.002	3.027±0.273	2.107±0.196	0.017
Rimonabant	0.231 ± 0.027	2.076 ± 0.139	0.706 ± 0.187	0.33

[a] Values indicate mean ± SEM for n=6 rats

Since selectivity of our lead compound towards peripheral tissue is very crucial, our last and important goal was to compare tissue and plasma distribution of (-) enantiomer **40** vs Rimonabant (Table 2.6). It is evident that brain/plasma ratio of compound **40** is about 20 fold less than Rimonabant, hence it is less penetrated in brain.

Several studies have demonstrated an important role of CB1 receptors in orexigenic signaling and put forward the CB1 receptor antagonists as a potential therapeutic target for obesity. In this work, Compound **27** was identified as a potent, selective and more peripherally active antagonist of CB1 receptor. The first CB1 antagonist Rimonabant was withdrawn from the market because of issues associated with induction of central nervous system (CNS) side-effects. We envisioned designing of molecules that mimics the anorexic and anti-obesity effects of the CB1 antagonist without CNS related side effects. While it is challenging to discover peripherally acting CB1 antagonists, our starting point was based on our prior work in the same field. As in our earlier work, modification from pyrazole derivative (Rimonabant) to dihydropyrazole yielded compounds of the similar profile, we expanded SAR of dihydropyrazoles further. Literature evidence suggested that modification at amide position might drive SAR towards desire profile of molecule.

Since dihyropyrazoles structurally resembles to Rimonabant a pyrazole analogue, a series of methyl amide dihydropyrazoles were synthesized and evaluated in the functional assay of cAMP production. Many compounds showed comparable activity to Rimonabant and Compound **27** showed better activity in

functional assay of cyclic AMP (cAMP) production than Rimonabant. As compound **27** is racemic, enantiomers were separated anticipating better activity of one of the enantiomer. (-) After *in vitro* evaluation. enantiomer **40** was better than it's (+) enantiomer **41** as EC_{50} of **40** was found to be lower than Rimonabant and racemic compound **27**. As Compound **40** is more potent than Rimonabant, we further examined the antiobesity activities. In vivo efficacy of **40** was evaluated using sucrose solution intake model. The results clearly suggest the translation of *in vitro* data to animal model as here also compound **40** exhibited better efficacy than Rimonabant and racemic compound **27**. Finally, pharmacokinetic parameters and tissue and plasma distribution of compound **40** were evaluated. It is interesting to see that in terms of tissue distribution 40 is less penetrated in CNS compare to Rimonabant. One of possible hypotheses for these differences is that the permeability of compound **40** across the blood–brain barrier (BBB) is significantly lower than that of Rimonabant.

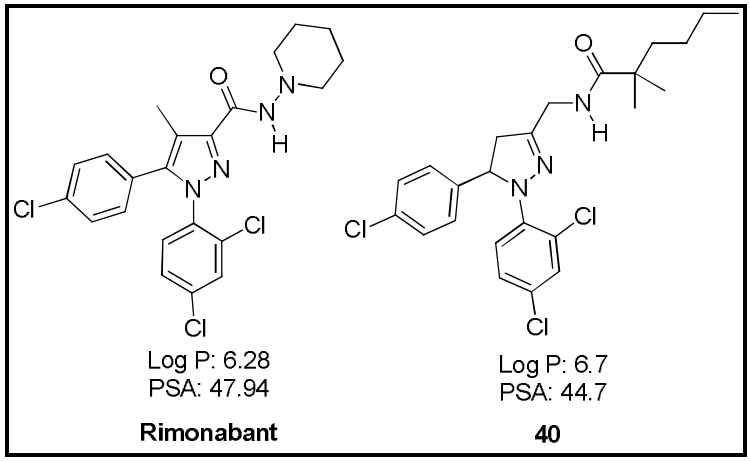


Figure 2.6: Physicochemical properties of **40** and Rimonabant.

Our hypothesis to develop peripherally acting compounds was to have lower polar surface area so as to reduce BBB penetration, But PSA of compound

40 and Rimonabant is almost same. The major difference in physicochemical property between **40** and Rimonabant is that compound **40** is more lipophilic than of Rimonabant. Lipophilicity also affects the BBB permeation and theoretically higher the lipophilicity mean better the BBB permeability. Since compound **40** is not following concept of PSA or lipophilicity, one of the explanation for reduced brain extraction of **40** might be increased non-specific binding of this compound to plasma proteins.

2.3 Conclusions

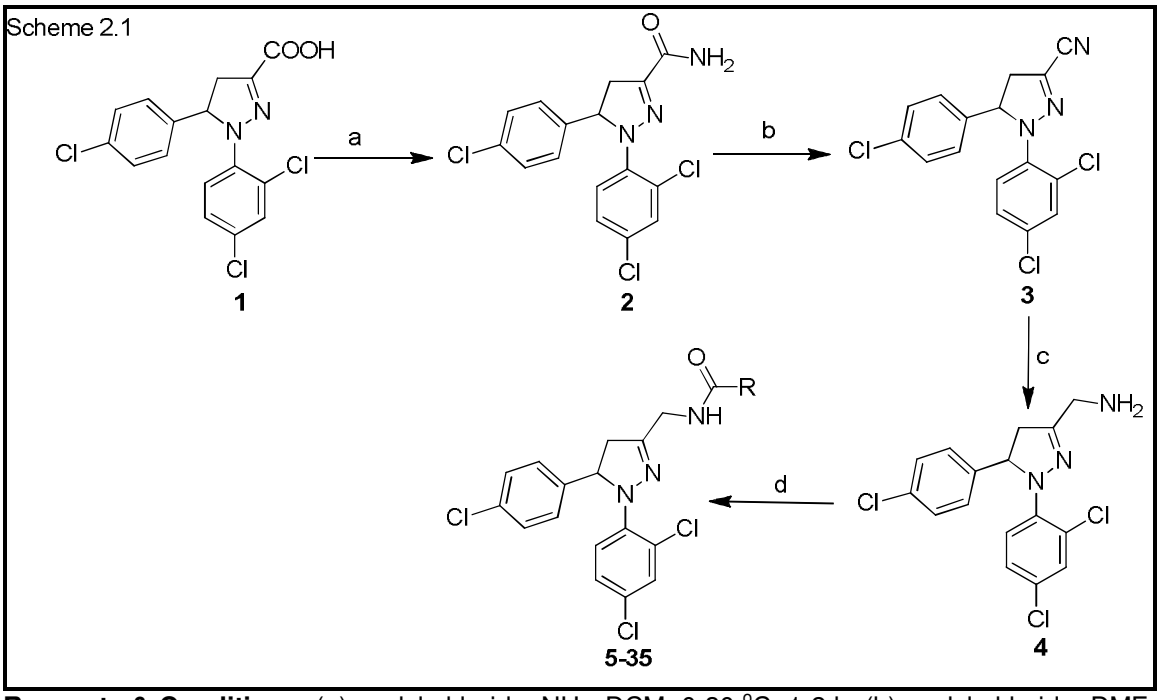
- Series of methyl amide dihydropyrazoles were synthesized and evaluated in the functional assay of cAMP production. Compound **27** showed better in fold induction than Rimonabant.
- As compound 27 which is racemic compound, its enantiomers were separated by chiral separation. The (-) enantiomer **40** was better than it's (+) enantiomer **41** and after determination of EC_{50}, **40** was found to be more potent than Rimonabant and racemic compound **27**.
- The lead compound **40** exhibited better efficacy than Rimonabant as well as racemic compound **27** in sucrose solution intake model.
- Finally pharmacokinetic parameters were evaluated and tissue and plasma distribution of compound **40** demonstrated that it is superior in terms of tissue distribution as it is less penetrated in CNS compare to Rimonabant.

2.4 Experimental

Materials and Methods

Reagents were obtained from Sigma Aldrich and 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 a scientific melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (λmax in cm-1, as film for liquids and as KBr pellets for solid compounds). The ^{1}HNMR spectra were recorded on a Bruker Avance-300 (300 MHz) or Bruker Avance-400 (400 MHz) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-d_6. Signal multiplicities are represented as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). D_2O exchange experiments were carried out to confirm the exchangeable protons when present. Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer. HPLC analyses were carried out at λmax 220 nm using column ODS C-18, 150nm * 4.6 nm * 4 m on AGILENT 1100. Progress of the reactions was monitored by TLC using precoated TLC plates (E. Merck Kieselgel 60 F254) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (230–400 mesh).

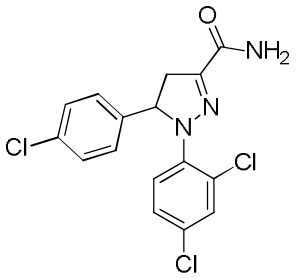
Scheme 2.1



Reagents & Conditions: (a) oxalyl chloride, NH_3, DCM, 0-30 ^oC, 1-2 h; (b) oxalyl chloride, DMF, C_5H_5N, 0-30 ^oC, 2-3 h (c) Et_2O, $LiAlH_4$, -30 to -35 ^oC, 2-3 h (d) R^3COOH, HOBt, EDC.HCl, Triethylamine, CH_2Cl_2, 26-28 ^oC, 2-4 h.

2.4.1 Dihydropyrazole-3-methyl carboxamide derivatives

2.4.1.1 5-(4-Chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-carboxylic acid amide (2)



To solution of 5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H pyrazol-3-carboxylic acid **(1)** *(Prepared as per the procedure given in J. Med. Chem. 2007, 50, 5951-5966 5951)* (9 g, 0.024 mol) in dichloromethane (DCM) (45 mL) was added dropwise oxalyl chloride (2.5 mL, 0.029 mol) at -20 ^oC. The reaction

mixture was stirred for 30 minutes at same temperature, then it was warmed up to 30 ^{o}C and stirred for 30 minutes. The reaction mixture was then cooled to -20 to -30 ^{o}C. To this, dry ammonia gas was passed over 1-1.5 hrs, the reaction mixture was diluted with water (45 mL) and dichloromethane (45 mL). The organic layer was separated and washed with water (2 x 75 mL), dried over Na_2SO_4 and solvents were evaporated reduced pressure to afford the crude product. The solid crude product was further triturated in petroleum ether (25 mL), filtered to afford 5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-carboxylic acid amide as light yellow solid (6.6 g, 74 %).

Chemical Formula: $C_{16}H_{12}Cl_3N_3O$

Molecular Weight: 368.64

IR (KBr) cm^{-1}: 3477, 3280, 1678, 1654

^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03(dd, *J* = 18.0 Hz & 5.8 Hz, 1H), 3.63(dd, *J* = 18.0 Hz & 11.9 Hz, 1H), 5.83(dd, *J* = 11.7 Hz & 5.7 Hz, 1H), 7.13(d, *J* = 8.4 Hz, 2H), 7.26 (m, 3H), 7.37(s, 1H), 7.45(m, 2H), 7.69(s, 1H)

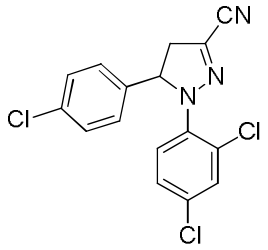
ESI-MS: 391.7 $[M+Na]^+$.

%Yield: 74 %

Purity by HPLC: 98.94 %

mp: 78-80 ^{o}C

2.4.1.2 Synthesis of 5-(4-Chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-carbonitrile (3)



To dry dimethylformamide (DMF) (20 mL) cooled to 0-10 ^{o}C, to that added oxalyl chloride (3.3 mL, 0.038 mol) dropwise during 5-10 minutes. To the resulting yellow suspension was added a solution of 5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-carboxylic acid amide **2** (6.5 g, 17.6 mmol) in dry DMF (10 mL) and the reaction mixture was stirred at same temperature for about 1-1.5 hrs. Pyridine (5 mL, 75.1 mmol) was added to the reaction mixture at 0-10 ^{o}C. The reaction mixture was warmed up to 30 ^{o}C, poured in 1N HCl (30 mL) and extracted with ethylacetate (2 x 30 mL). Organic layer was washed with water (2 x 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 and solvents were evaporated on a under reduced pressure to afford solid crude product which was further triturated in petroleum ether and di-isopropyl ether (80 mL : 20 mL). The solid obtained was filtered and dried to obtain 5-(4-chloro-phenyl)-1-(2, 4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-carbonitrile **3** as light yellow solid (3.9 g, 63.0 %).

Chemical Formula: $C_{16}H_{10}Cl_3N_3$

Molecular Weight: 350.63

IR (KBr) cm^{-1}: 3413, 2218, 1645, 1595

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.21(dd, *J* = 17.5 Hz & 5.9 Hz, 1H), 3.75(dd, *J* = 17.5 & 12.6 Hz, 1H), 5.89(dd, *J* = 12.6 Hz & 5.9 Hz, 1H), 7.25-7.28(m, 2H), 7.31-7.35(m, 4H), 7.54 (s, 1H)

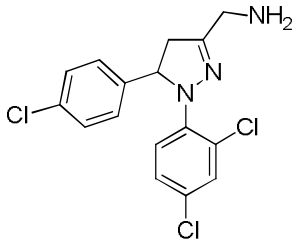
ESI-MS: 350.9 $[M]^+$

%Yield: 63 %

Purity by HPLC: 99.25 %

mp: 100-104 ^oC

2.4.1.3 Synthesis of 5-(4-Chloro-phenyl)-1-(2, 4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-yl-methyl amine (4)



Diethyl ether (200 mL) was cooled to -5 to 0 ^oC under N_2 and to that lithium aluminum hydride (LAH) (0.758 g, 19.9 mmol) was added at -10 to -15 ^oC. The suspension then was cooled to temperature -30 to -35 ^oC. To this solution of 5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-carbonitrile **3** (3.5 g, 9.98 mmol) in diethyl ether (70 mL) was added at the same temperature. The reaction mixture was stirred at same temperature for about 2-3 hrs. The reaction mixture was quenched by mixture of ice and sodium sulfate till evolution of hydrogen gas stops. The suspension was filtered through bed of hyflow, filtrate was collected and washed with water (2 x 150 mL) and brine (150 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and solvents were evaporated under reduced pressure to obtain 5-(4-chloro-phenyl)-

1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-yl-methyl amine **4** as light yellow oil (1.8 g, 51 %).

Chemical Formula: $C_{16}H_{14}Cl_3N_3$

Molecular Weight: 354.66

IR (Neat) cm^{-1}: 3413, 3020, 2399, 1566

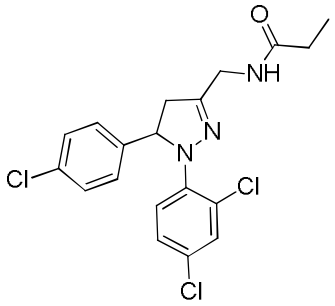
^{1}H NMR (300 MHz, DMSO-d_6): δ 2.95(dd, *J* = 17.8 Hz & 4.9 Hz, 1H), 3.57(m, 3H), 5.59 (dd, *J* = 10.9 Hz & 4.7 Hz, 1H), 7.17(m, 3H), 7.24(d, J = 8.4 Hz, 2H), 7.33 (t, *J* = 4.2 Hz, 2H)

ESI-MS: 377 $[M+Na]^+$.

%Yield: 51 %

Purity by HPLC: 98.62 %

2.4.1.4 Synthesis of N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)methyl) propionamide (5)



To a stirred solution of Propanecarboxylic acid (0.062 g, 0.84 mmol) in DCM (10 mL) was added 1-hydroxybenzotriazole (HOBt) (0.228 g, 1.69 mmol), [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] hydrochloride(EDCI.HCl) (0.242 g, 1.26 mmol) and the reaction was stirred for 5-10 minutes at ambient temperature. To this mixture, 5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-pyrazol-3-yl-methyl amine **4** (0.3 g, 0.84 mmol) was added followed

by addition of triethylamine (0.235 mL, 1.69 mmol). The reaction mixture was stirred at ambient temperature for 2-4 hrs. The reaction mixture was poured in water (25 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layer was separated washed with water (2 x 35 mL), brine (35 mL), dried over anhydrous Na_2SO_4 and solvents were evaporated under reduced pressure to get crude brown oil. The crude product was purified by column chromatography over flash silica gel (hexane : ethyl acetate 6:4) to afford pure N-((5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)methyl) propionamide **5** as off white solid (0.110 g, 32%).

Chemical Formula: $C_{19}H_{18}Cl_3N_3O$

Molecular Weight: 410.72

IR (KBr) cm^{-1}: 3271, 3066, 2976, 1645, 1253

^{1}H NMR(300 MHz, DMSO-d_6): δ 1.01(q, 3H), 2.11(q, 2H), 2.78-2.84(dd, 1H), 3.43(m, 1H), 4.06(t, 2H) , 5.57-5.61(dd, *J* = 10.8 Hz & 4.8 Hz, 1H), 7.13(d, *J* = 8.4 Hz, 2H), 7.21(m, 4H), 7.36(d, *J* = 2.0 Hz, 1H), 8.19(t, *J* = 5.6 Hz, 1H)

ESI-MS: 433.6 $[M+Na]^+$

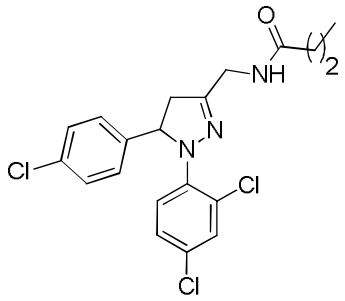
%Yield: 32 %

Purity by HPLC: 99.38 %

mp: 143-145 ^oC

Compounds **6-35** *were prepared in an analogous manner using appropriate starting materials and the process described above.*

2.4.1.5 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)butyramide (6)



Chemical Formula: $C_{20}H_{20}Cl_3N_3O$

Molecular Weight: 424.75

IR (KBr) cm^{-1}: 3448, 2958, 1647, 1544, 813

^{1}H NMR (300 MHz, DMSO-d6): δ 0.82(t, 3H), 1.48-1.53(m, 2H), 2.09(t, 2H), 2.78-2.84(dd, *J* = 17.6 Hz & 4.8 Hz, 1H), 3.32-3.43(dd, *J* = 17.6 Hz & 6.8 Hz, 1H), 4.06(d, 2H), 5.58-5.62(m, 1H), 7.14(d, *J* = 8.8 Hz , 2H), 7.18-7.21(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.23-7.29(m, 3H), 7.37(d, *J* = 2.4 Hz, 1H), 8.24(br s, 1H)

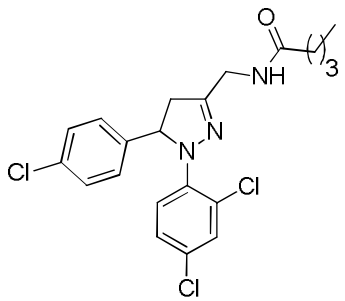
ESI-MS: 447.7 $[M+Na]^+$

%Yield: 44 %

Purity by HPLC: 99.08 %

mp: 124-126 ^oC

2.4.1.6 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl) pentanamide (7)



Chemical Formula: $C_{21}H_{22}Cl_3N_3O$

Molecular Weight: 438.78

IR (KBr) cm^{-1}: 3265, 3082, 2956, 1643, 1556, 1201

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.82(t, *J* = 7.4 Hz, 3H), 1.15-1.26(m, 2H), 1.42-1.49(m, 2H), 2.11(t, *J* = 7.2 Hz, 2H), 2.80(dd, *J* = 17.6 Hz & 4.8 Hz ,1H), 3.42(dd, *J* = 17.6 Hz & 11.2 Hz , 1H), 4.05(d, *J* = 5.6 Hz, 2H), 5.60(dd, *J* = 11.2 Hz & 4.8 Hz, 1H), 7.12-7.14(m, 2H), 7.19(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.22-7.25(m, 2H), 7.28(d, *J* = 8.8 Hz, 1H), 7.36(d, *J* = 2.4 Hz, 1H), 8.24(t, *J* = 5.8 Hz, 1H)

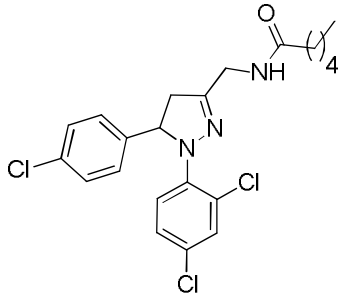
ESI-MS: 437.8 $[M]^+$

%Yield: 57 %

Purity by HPLC: 98.88 %

mp: 105-107 ^oC

2.4.1.7 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)hexanamide (8)



Chemical Formula: $C_{22}H_{24}Cl_3N_3O$

Molecular Weight: 452.80

IR (KBr) cm^{-1}: 3273, 2956, 1645, 1548, 817

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78(t, 3H), 1.16-1.23(m, 4H), 1.48(t, 2H), 2.10(t, 2H), 2.77-2.83(dd, *J* = 17.6 Hz & 4.8 Hz, 1H), 3.38-3.45(dd, *J* = 17.6 Hz &

6.4 Hz, 1H), 4.05(d, 2H), 5.58-5.61(m, 1H), 7.15(d, *J* = 6.8 Hz, 2H), 7.18-7.20(dd, *J* = 8.8 Hz & 6.4 Hz, 1H), 7.23-7.25(dd, *J* = 6.8 Hz & 4.8 Hz,, 2H), 7.28(d, *J* = 8.8 Hz, 1H), 7.36(d, *J* = 2.4 Hz, 1H), 8.22(t, 1H)

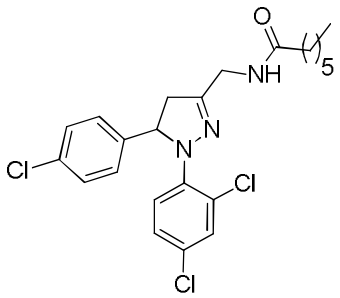
ESI-MS: 475.8 $[M+Na]^+$

%Yield: 44 %

Purity by HPLC: 98.84 %

mp: 110-112 ^oC

2.4.1.8 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)heptanamide (9)



Chemical Formula: $C_{23}H_{26}Cl_3N_3O$

Molecular Weight: 466.83

IR (KBr) cm^{-1}: 3271, 3074, 2956, 1645,1257

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79(s, 3H), 1.17(s, 5H), 1.46(s, 2H), 2.10(t, *J* = 7.5 Hz , 2H), 2.85(d, *J* = 12.0 Hz, 1H), 2.95(d, *J* = 9.0 Hz , 1H), 4.05(d, *J* = 6.0 Hz, 2H) ,5.6(d, *J* = 6.0 Hz, 1H), 7.12(s, 2H), 7.16(d, *J* = 6.0 Hz, 1H), 7.215 (d, *J* = 3.0 Hz, 2H) , 7.27(t, *J* = 7.5 Hz, 2H) , 7.35(d, *J* = 3.0 Hz, 1H), 8.24 (s,1H)

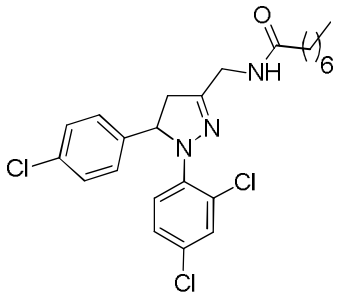
ESI-MS: 490.3 $[M+Na]^+$

%Yield: 41 %

Purity by HPLC: 96.6 %

mp: 98-102 ^{o}C

2.4.1.9 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)octanamide (10)



Chemical Formula: $C_{24}H_{28}Cl_3N_3O$

Molecular Weight: 480.86

IR (KBr) cm^{-1}: 3444, 2856, 1645, 1550, 817

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.79(t, 3H), 1.12-1.21(m, 8H), 1.46(t, 2H), 2.09(t, 2H), 2.77-2.82(dd, *J* = 17.6 Hz & 4.8 Hz, 1H), 3.38-3.45(dd, *J* = 17.6 Hz & 6.4 Hz, 1H), 4.05(d, 2H), 5.57-5.61(m, 1H), 7.13(d, *J* = 8.4 Hz, 2H), 7.17-7.20(dd, *J*=8.8 Hz & 2.0 Hz, 1H), 7.24-7.29(dd, *J* = 8.8 Hz & 2.0 Hz, 3H), 7.35(d, *J* = 2.0 Hz, 1H), 8.23 (t, 1H)

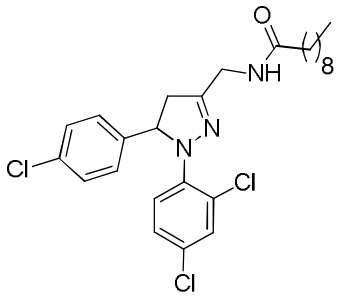
ESI-MS: 503.8 $[M+Na]^{+}$

%Yield: 20 %

Purity by HPLC: 99.23 %

mp: 108-110 ^{o}C

2.4.1.10 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)decanamide (11)



Chemical Formula: $C_{26}H_{32}Cl_3N_3O$

Molecular Weight: 508.91

IR (KBr) cm^{-1}: 3273, 2925, 1645, 1548, 817

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.82(t, 3H), 1.20(brs, 12H), 1.47(brs, 2H), 2.10(t, 2H), 2.78-2.83(dd, *J* = 17.6 Hz & 4.4 Hz, 1H), 3.38-3.45(dd, *J* = 17.6 Hz & 6.4 Hz, 1H), 4.05(d, 2H), 5.57-5.61(m, 1H), 7.14(d, *J* = 8.4 Hz, 2H), 7.17-7.20(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.23(d, *J* = 8.4 Hz, 2H), 7.28(d, *J* = 8.8 Hz, 1H), 7.36(d, *J* = 2.0 Hz, 1H), 8.23 (t, 1H)

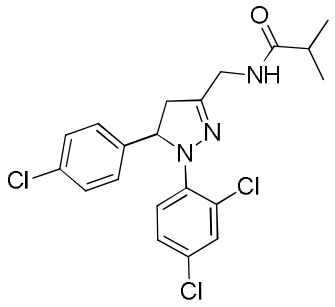
ESI-MS: 531 $[M+Na]^+$

%Yield: 41 %

Purity by HPLC: 97.93 %

mp: 74-76 ^oC

2.4.1.11 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)isobutyramide (12)



Chemical Formula: $C_{20}H_{20}Cl_3N_3O$

Molecular Weight: 424.75

IR (KBr) cm^{-1}: 3444, 2968, 1645, 1546, 815

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.01(d, 6H), 2.38-2.41(m, 1H), 2.77-2.82(dd, *J* = 17.6 Hz & 4.8 Hz, 1H), 3.37-3.42(dd, *J* = 18.0 Hz & 6.4 Hz, 1H), 4.05(t, 2H), 5.58-5.62(m, 1H), 7.12-7.14(dd, *J* = 6.8 Hz & 2.0 Hz, 2H), 7.18-7.21(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.23-7.28(m, 3H), 7.37(d, *J* = 2.4Hz, 1H), 8.21(t, 1H)

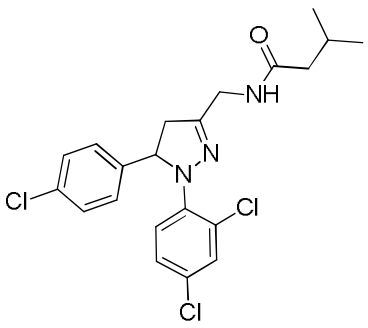
ESI-MS: 446 $[M+Na]^+$

%Yield: 53 %

Purity by HPLC: 99.57 %

mp: 122-124 ^oC

2.4.1.12 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-3-methylbutanamide (13)



Chemical Formula: $C_{21}H_{22}Cl_3N_3O$

Molecular Weight: 438.78

IR (KBr) cm^{-1}: 3276.8, 2956.7, 1643.2, 1544.2

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.84(d, *J* = 5.6 Hz, 6H), 1.94-1.99(m, 1H), 2.0(s, 2H), 2.81(dd, J = 17.6 Hz & 4.8 Hz ,1H), 3.42(dd, *J* = 17.6 Hz & 11.2 Hz , 1H), 4.06(d, *J* = 5.2 Hz, 2H), 5.61(dd, *J* = 11.2 Hz & 4.8 Hz, 1H), 7.12-7.15(m, 2H), 7.21(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.22-7.26(m, 2H), 7.28(d, *J* = 8.8 Hz, 1H), 7.36(d, *J* = 2.4 Hz, 1H), 8.25(t, *J* = 5.8 Hz, 1H)

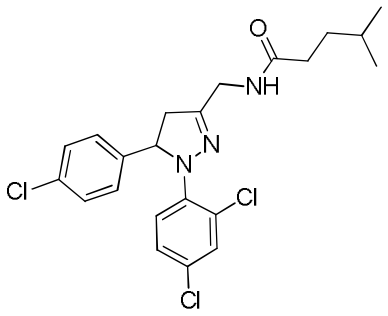
ESI-MS: 437.8 $[M]^+$

%Yield: 47 %

Purity by HPLC: 98.66 %

mp: 128-130 ^oC

2.4.1.13 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-4-methylpentanamide (14)



Chemical Formula: $C_{22}H_{24}Cl_3N_3O$

Molecular Weight: 452.80

IR (KBr) cm^{-1}: 3433, 2956, 1643.2, 1589

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.8-0.81(m, 6H), 1.37(q, *J* = 7.6 Hz, 2H), 1.42 - 1.48(m, 1H), 2.11(t, *J* = 7.6 Hz, 2H), 2.77(dd, *J* = 17.6 Hz & 4.8 Hz ,1H), 3.42(dd,

J = 17.6 Hz & 11.2 Hz, 1H), 4.05(d, *J* = 5.6 Hz, 2H), 5.61(dd, *J* = 11.2 Hz & 4.8 Hz, 1H), 7.11-7.14(m, 2H), 7.20(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.23-7.25(m, 2H), 7.29(d, *J* = 8.8 Hz, 1H), 7.36(d, *J* = 2.4 Hz, 1H), 8.26(t, *J* = 5.6 Hz, 1H)

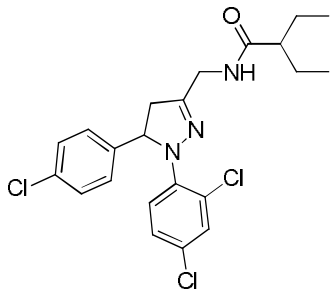
ESI-MS: 474.9 $[M+Na]^+$

%Yield: 56 %

Purity by HPLC: 97.36 %

mp: 108-110 ^oC

2.4.1.14 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2-ethylbutanamide (15)



Chemical Formula: $C_{22}H_{24}Cl_3N_3O$

Molecular Weight: 452.80

IR (KBr) cm^{-1}: 3431, 2962, 1647, 1541

^1H NMR (300 MHz, DMSO-d_6): δ 0.77-0.82(m, 6H), 1.28-1.38(m, 2H), 1.42-1.48(m, 2H), 1.96-2.03(m, 1H), 2.81(dd, *J* = 17.6 Hz & 4.8 Hz ,1H), 3.43(dd, *J* = 18.0 Hz & 11.2 Hz , 1H), 4.09(d, *J* = 5.6 Hz, 2H), 5.62(dd, *J* = 11.2 Hz & 4.8 Hz, 1H), 7.14(d, *J* = 8.4 Hz, 2H), 7.20(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.23(d, *J* = 8.4 Hz, 2H), 7.29(d, *J* = 8.8 Hz, 1H), 7.34(d, *J* = 2.4 Hz, 1H), 8.29(t, *J* = 5.6 Hz,1H)

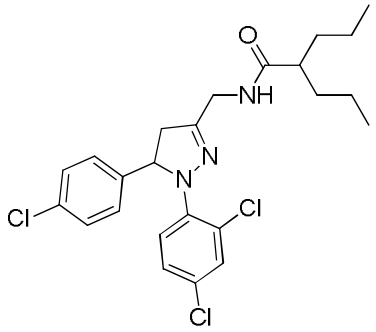
ESI-MS: 474.9 $[M+Na]^+$

%Yield: 51 %

Purity by HPLC: 98.48 %

mp: 86-88 ^{o}C

2.4.1.15 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2-propylpentanamide (16)



Chemical Formula: $C_{24}H_{28}Cl_3N_3O$

Molecular Weight: 480.86

IR (KBr) cm^{-1}: 3301, 2958, 1641, 1583, 1541

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.8(m, 7H), 1.18(m, 7H), 2.1(m, 1H), 2.79(dd, *J* = 17.6 Hz & 4.4 Hz ,1H), 3.42(dd, *J* = 17.6 Hz & 11.2 Hz , 1H), 4.0(d, *J* = 5.6 Hz, 2H), 5.63(dd, *J* = 10.8 Hz & 4.4 Hz, 1H), 7.13(d, *J* = 8.4 Hz, 2H), 7.2(m, 3H), 7.3(d, *J* = 8.8 Hz, 1H), 7.34(d, *J* = 2.4 Hz, 1H), 8.2(t, *J* = 5.6 Hz, 1H)

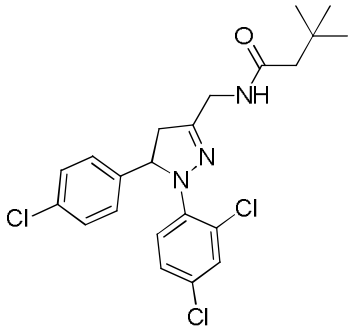
ESI-MS: 481.8 $[M+H]^{+}$

%Yield: 46 %

Purity by HPLC: 99.18 %

mp: 98-100 ^{o}C

2.4.1.16 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-3,3-dimethylbutanamide (17)



Chemical Formula: $C_{22}H_{24}Cl_3N_3O$

Molecular Weight: 452.80

IR (KBr) cm^{-1}: 3309, 2958, 1639, 1541, 819

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.93(s, 9H), 2.00(s, 2H), 2.80-2.85(dd, *J* = 17.6 Hz & 4.8 Hz, 1H), 3.39-3.44(dd, *J* = 17.4 Hz & 6.4 Hz, 1H), 4.06(d, 2H), 5.59-5.63(m, 1H), 7.13-7.15(dd, *J* = 6.8 Hz & 2 Hz, 2H), 7.18-7.25(m, 3H), 7.29(d, *J* = 8.0 Hz, 1H), 7.36(d, *J* = 2.4 Hz, 1H), 8.19(brs, 1H)

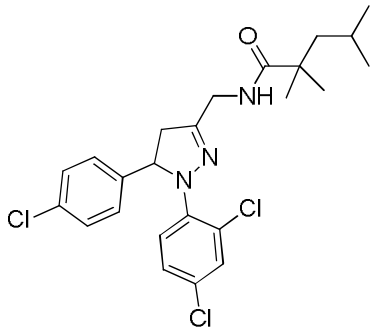
ESI-MS: 475.7 $[M+Na]^+$

%Yield: 48 %

Purity by HPLC: 99.06 %

mp: 126-130 ^oC

2.4.1.17 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2,4-trimethylpentanamide (18)



Chemical Formula: $C_{24}H_{28}Cl_3N_3O$

Molecular Weight: 480.86

IR (KBr) cm^{-1}: 3294, 2976, 1642, 1548

^{1}H NMR (**300 MHz,** DMSO-d_6): δ 0.68-0.71(d, J = 6.8 Hz, 6H), 1.06(s, 6H), 1.38(s, 2H), 1.90(m, 1H), 2.80-2.85(dd, J = 4.8 Hz & 17.6 Hz 1H), 3.39-3.44(dd, J = 6.4 Hz & 17.4 Hz, 1H), 4.06(d, J = 5.2 Hz, 2H), 5.59-5.63(m, 1H), 7.13-7.15(dd, J = 2.0 Hz & 6.8 Hz, 2H), 7.18-7.25(m, 3H), 7.29(d, J = 8.0, 1H), 7.36(d, J = 2.4 Hz, 1H), 8.12(t, J = 5.4 Hz, 1H)

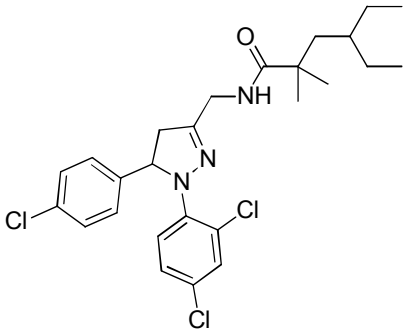
ESI-MS: 482 $[M+H]^+$

%Yield: 19 %

Purity by HPLC: 96.74 %

mp: 118-120 ^oC

2.4.1.18 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-4-ethyl-2,2-dimethylhexanamide (19)



Chemical Formula: $C_{26}H_{32}Cl_3N_3O$

Molecular Weight: 508.91

IR (KBr) cm^{-1}: 3363, 2962, 1637, 1523

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.69- 0.72(m, 6H), 1.06(s, 6H), 1.16(s, 5H), 1.38(s, 2H), 2.75-2.80(dd, *J* = 17.6 Hz & 4.4 Hz, 1H), 3.43-3.36(dd, *J* = 18 Hz & 11.2 Hz, 1H), 4.05(d, *J* = 5.2 Hz, 2H), 5.58-5.61(dd, *J* = 10.8 Hz & 4.4 Hz, 1H), 7.14(d, *J* = 8.4 Hz, 2H), 7.18-7.24(m, 3H), 7.29(d, *J* = 8.8 Hz, 1H), 7.35(d, *J* = 2.4 Hz, 1H), 7.92(t, *J* = 5.4 Hz, 1H)

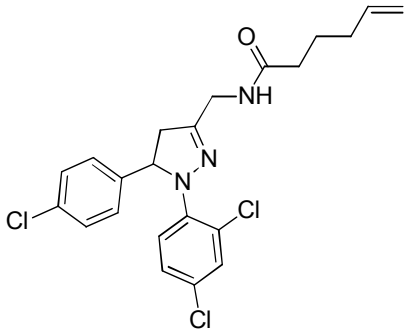
ESI-MS: 509.8 $[M+H]^+$

%Yield: 34 %

Purity by HPLC: 98.06 %

mp: 122-126 ^oC

2.4.1.19 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)pent-4-enamide (20)



Chemical Formula: $C_{22}H_{22}Cl_3N_3O$

Molecular Weight: 450.79

IR (KBr) cm^{-1}: 3290, 2929, 1645, 1541, 819

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.57(t, 2H), 1.94-1.99(m, 2H), 2.13(t, 2H), 2.78-2.83(dd, *J* = 17.6 Hz & 4.4 Hz, 1H), 3.38-3.46(dd, *J* = 17.6 Hz & 6.4 Hz, 1H), 4.06(d, 2H), 4.92(d, 2H), 5.58-5.62(m, 1H), 5.71-5.75(m, 1H), 7.12-7.15(dd, *J* = 6.8 Hz & 2.0 Hz, 2H), 7.18-7.20 (dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.23-7.29(m, 3H), 7.36(d, *J* = 2.4 Hz, 1H), 8.27(t, 1H)

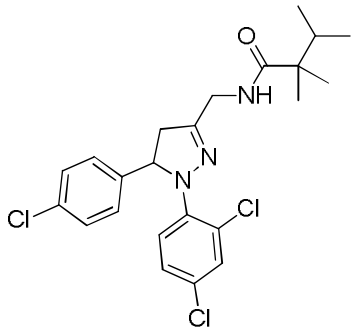
ESI-MS: 450 $[M]^+$

%Yield: 48 %

Purity by HPLC: 97.16 %

mp: 96-98 ^oC

2.4.1.20 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2,3-trimethylbutanamide (21)



Chemical Formula: $C_{23}H_{26}Cl_3N_3O$

Molecular Weight: 466.83

IR (KBr) cm^{-1}: 3431, 3292, 2925, 1751, 1629, 1490

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.74(d, *J* = 5.6 Hz, 6H), 0.97(s, 6H), 1.90(m, 1H), 2.78(d, J = 13.6Hz, 1H), 3.31-3.44(dd, *J* = 17.6 Hz & 11.2 Hz, 1H), 4.07(s, 2H), 5.6(d, *J* = 6.8 Hz, 1H), 7.13-7.29(m, 6H), 7.35(s, 1H), 7.88(s, 1H)

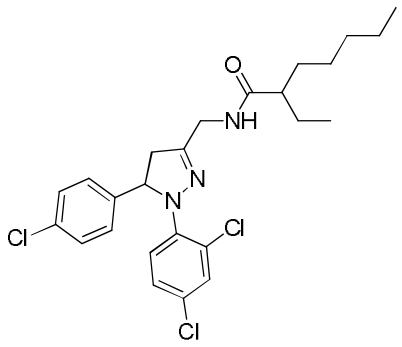
ESI-MS: 489.5 $[M+Na]^+$

%Yield: 14 %

Purity by HPLC: 96.33 %

mp: 106-108 ^oC

2.4.1.21 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2-ethylheptanamide (22)



Chemical Formula: $C_{25}H_{30}Cl_3N_3O$

Molecular Weight: 494.88

IR (KBr) cm^{-1}: 3292, 2927, 1641, 1544, 864

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 0.76(t, 3H), 0.77(t, 3H), 1.12-1.25(m, 4H), 1.27-1.34(m, 2H), 1.43(t, 2H), 2.06(t, 2H), 2. 71-2.83(dd, *J* = 17.2 Hz & 3.6 Hz, 1H), 3.31(dd, *J* = 20 Hz & 9.2 Hz, 1H), 4.05-4.10 (m, 2H), 5.60-5.64(m, 1H), 7.14(d, *J* = 8.4 Hz, 1H), 7.18-7.24(m, 3H), 7.29(d, *J* = 8.8 Hz, 2H), 7.35(d, *J* = 2.4 Hz, 2H), 8.27(t, 1H)

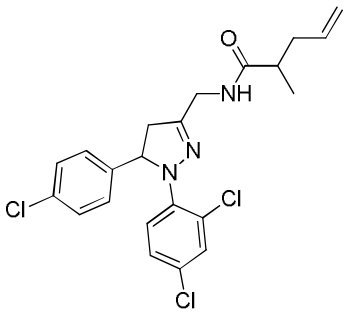
ESI-MS: 496 $[M+H]^+$

%Yield: 13 %

Purity by HPLC: 99.88 %

mp: 114-116^{o}C

2.4.1.22 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2-methylpent-4-enamide (23)



Chemical Formula: $C_{22}H_{22}Cl_3N_3O$

Molecular Weight: 450.79

IR (KBr) cm^{-1}: 3018, 2399, 1658, 1510

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.05(s, 4H), 2.19(d, *J* = 7.6 Hz, 2H), 2.78(dd, *J* = 18.0 Hz & 4.8 Hz, 1H), 3.39(dd, *J* = 17.6 Hz & 10.8 Hz, 1H), 4.06(t, 2H),

4.90(dd, *J* = 10.0 Hz & 2.4 Hz, 1H), 4.98(dd, *J* = 17.2 Hz & 5.2 Hz, 1H), 5.59(m, 1H), 5.63(m, 1H), 7.14(d, *J* = 8.4 Hz, 2H), 7.18-7.24(m, 3H), 7.27(d, *J* = 8.8 Hz, 1H), 7.34(d, *J* = 2.4 Hz, 1H), 7.97 (t, 1H)

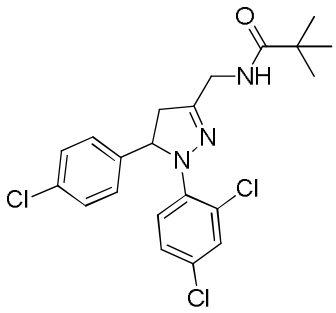
ESI-MS: 452 [M+H]+

%Yield: 15 %

Purity by HPLC: 91.07 %

mp: 105-110 ^{o}C

2.4.1.23 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)pivalamide (24)



Chemical Formula: $C_{21}H_{22}Cl_3N_3O$

Molecular Weight: 438.78

IR (KBr) cm^{-1}: 3440, 2854, 1637, 1535, 815

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.09(s, 9H), 2.74-2.80(dd, J = 18.0 Hz & 4.4 Hz, 1H), 3.38-3.43(dd, *J* = 12.4 Hz & 6 Hz, 1H), 4.05 (t, 2H), 5.57-5.61(m, 1H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.18-7.28(m, 4H), 7.35(br s, 1H), 7.96 (br s, 1H)

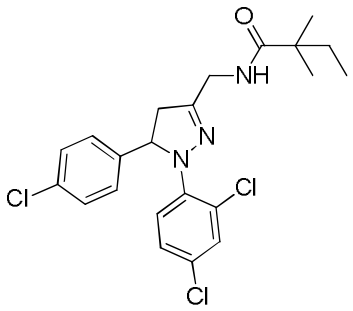
ESI-MS: 440 $[M+H]^+$

%Yield: 12 %

Purity by HPLC: 98.14 %

mp: 106-108 ^{o}C

2.4.1.24 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethylbutanamide (25)



Chemical Formula: $C_{22}H_{24}Cl_3N_3O$

Molecular Weight: 452.80

IR (KBr) cm^{-1}: 3294, 2874, 1633, 1535, 813

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.70(t, 3H), 1.04(s, 6H), 1.43-1.49(q, 2H), 2.75-2.81(dd, *J* = 17.6 Hz & 4.4 Hz, 1H), 3.36-3.40(dd, *J* = 17.6 Hz & 6.4 Hz, 1H), 4.06 (t, 2H), 5.58-5.62(m, 1H), 7.14(d, *J* = 8.4 Hz, 2H), 7.18-7.21(dd, *J* = 8.8 Hz & J = 2.4 Hz, 1H), 7.23(dd, *J* = 8.4 Hz, 2H), 7.28(d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 2.4 Hz, 1H), 7.91 (t, 1H)

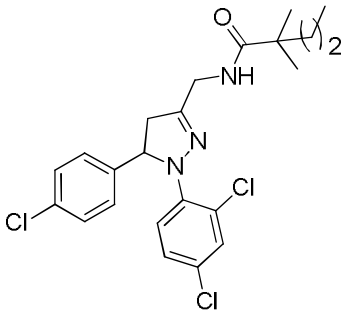
ESI-MS: 473.9 $[M+Na]^+$

%Yield: 44 %

Purity by HPLC: 99.75%

mp: 104-106 ^oC

2.4.1.25 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethylpentanamide (26)



Chemical Formula: $C_{23}H_{26}Cl_3N_3O$

Molecular Weight: 466.83

IR (KBr) cm^{-1}: 3336, 2927, 1639, 1525, 819

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78(t, 3H), 1.08(s,6H), 1.11-1.14(m, 2H), 1.37-1.41(m, 2H), 2.74-2.80(dd, *J* = 17.6 Hz & 4.8 Hz, 1H), 3.60-3.43(dd, *J* = 17.6 Hz & 6.4 Hz, 1H), 4.05 (t, 2H), 5.60-5.62(m, 1H), 7.13(d, *J* = 8.8 Hz, 2H), 7.18-7.24(m, 3H), 7.29(d, *J* = 8.8 Hz, 1H), 7.35(d, *J* = 2.0 Hz, 1H), 7.91(t, 1H)

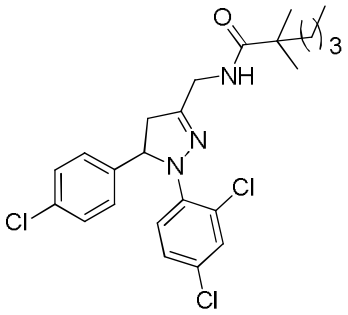
ESI-MS: 489.6 $[M+Na]^+$

%Yield: 35 %

Purity by HPLC: 98.76 %

mp: 108-110 ^oC

2.4.1.26 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethylhexanamide (27)



Chemical Formula: $C_{24}H_{28}Cl_3N_3O$

Molecular Weight: 480.86

IR (KBr) cm^{-1}: 3020, 2862, 1631, 1514, 742

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.72(t, 3H), 1.02(d,1H), 1.05(s, 6H), 1.10-1.14(m, 3H), 1.39-1.43 (m, 2H), 2.75-2.80(dd, *J* = 17.4 Hz & 4.8 Hz, 1H), 3.36-3.40(dd, *J* = 17.4 Hz & 6.4 Hz, 1H), 4.06(d, 2H), 5.58-5.62(m, 1H), 7.13-7.15(dd, *J* = 6.4 Hz & *J* = 2.0 Hz, 2H), 7.14-7.20(dd, *J* = 8.8 Hz 2.4 Hz, 1H), 7.21-7.23(dd, *J* = 6.4 Hz & *J* = 2.0 Hz, 2H), 7.29(d, *J* = 8.8 Hz, 1H), 7.34(d, *J* = 2.4 Hz, 1H), 7.89(t, 1H)

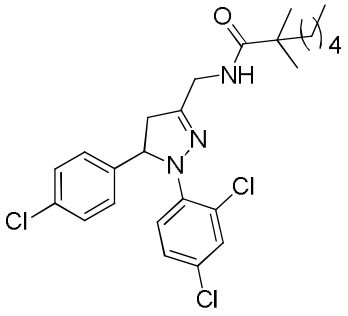
ESI-MS: 502 $[M+Na]^+$

%Yield: 56 %

Purity by HPLC: 95.87 %

mp: 95-98 ^oC

2.4.1.27 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethylheptanamide (28)



Chemical Formula: $C_{25}H_{30}Cl_3N_3O$

Molecular Weight: 494.88

IR (KBr) cm^{-1}: 3421, 2954, 1637, 1542, 813

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.04(d, 7H), 1.07(d, 5H), 1.39(br s, 2H), 2.74-2.79(dd, *J* = 17.6 Hz & 4.0 Hz, 1H), 3.38-3.43(dd, *J* = 17.6 Hz & 6.4 Hz, 1H), 4.06 (d, 2H), 5.58-5.62(m, 1H), 7.14(d, *J* = 8.4 Hz, 2H), 7.17-7.23(m, 3H), 7.29(d, *J* = 8.8 Hz, 1H), 7.35(d, *J* = 2.0 Hz, 1H), 0.73(t, 3H), 7.93(t, 1H)

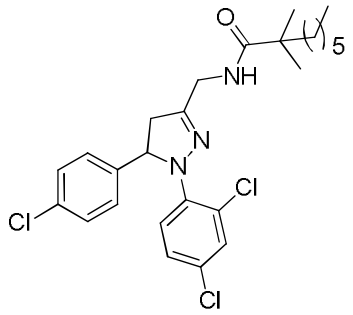
ESI-MS: 517.7 $[M+Na]^+$

%Yield: 23 %

Purity by HPLC: 98.76 %

mp: 80-82 ^oC

2.4.1.28 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethyloctanamide (29)



Chemical Formula: $C_{26}H_{32}Cl_3N_3O$

Molecular Weight: 508.91

IR (KBr) cm^{-1}: 3433, 2956, 1637, 1542, 813

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.75(t, 3H), 1.01-1.07(m, 10H), 1.10-1.15(m, 4H), 1.40(d, 2H), 2.75-2.80(dd, *J* = 17.6 Hz & 4.4 Hz, 1H), 3.32-3.40(dd, *J* = 17.6 Hz & 6.8 Hz, 1H), 4.06(d, 2H), 5.58-5.62(m, 1H), 7.13-7.15(dd, *J* = 6.8 Hz 2.4 Hz, 2H), 7.18(d, *J* = 2.4 Hz, 1H), 7.21-7.23(dd, *J* = 8.4 Hz & 1.6 Hz, 2H), 7.28(d, *J* = 8.8 Hz, 1H), 7.35(d, *J* = 2.4 Hz, 1H), 7.93(t, 1H)

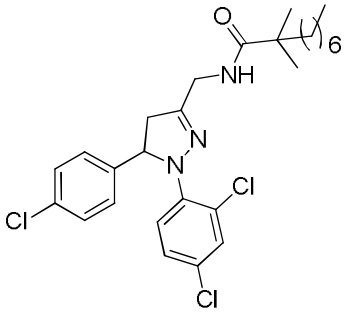
ESI-MS: 531.9 $[M+Na]^+$

%Yield: 30 %

Purity by HPLC: 99.11 %

mp: 108-110 ^{o}C

2.4.1.29 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol 3-yl) methyl)-2,2-dimethylnonanamide (30)



Chemical Formula: $C_{27}H_{34}Cl_3N_3O$

Molecular Weight: 522.94

IR (KBr) cm^{-1}: 3411, 2212, 1566, 1490, 827

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78(t, 3H), 1.05(d, 14H), 1.14(t, 2H), 1.39(br s, 2H), 2.75-2.80(dd, *J* = 17.4 Hz & 4.4 Hz, 1H), 3.39-3.40(dd, *J* = 17.2 Hz & 6.4 Hz, 1H), 4.06 (d, 2H), 5.57-5.61(m, 1H), 7.13(d, 1.6 Hz, 2H), 7.14-7.17(dd, *J* = 11.2 Hz & J = 2.0 Hz, 1H), 7.18(d, *J* = 2.4 Hz, 1H), 7.23(d, *J* = 1.6 Hz, 1H), 7.28(d, *J* = 8.8 Hz, 1H), 7.35(d, *J* = 2.4 Hz, 1H), 7.93(t, 1H)

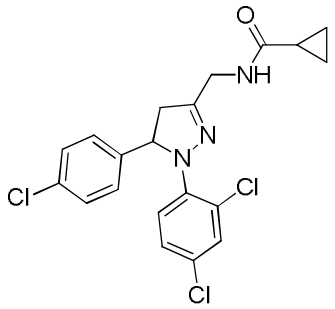
ESI-MS: 545.9 $[M+Na]^+$

%Yield: 30 %

Purity by HPLC: 99.17 %

mp: 126-128 ^{o}C

2.4.1.30 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)cyclopropanecarboxamide (31)



Chemical Formula: $C_{20}H_{18}Cl_3N_3O$

Molecular Weight: 422.74

IR (KBr) cm-1: 3424, 2872, 1612

^{1}H NMR (300 MHz, DMSO-d_6): δ 2.04(m 4H), 2.77-2.83(dd, *J* = 17.6 Hz & 4.0, 1H), 3.04(m 1H), 3.32(dd, *J* = 17.6 Hz & 8.2 Hz, 1H), 4.04(s, 2H), 5.53(dd, *J* = 8.4 Hz & 2.2 Hz, 1H), 6.72(d, *J* = 8.8 Hz, 2H), 7.02(d, *J* = 8.8 Hz, 2H), 7.14(dd, *J* = 8.2 Hz & 2.4 Hz, 1H), 7.24(d, *J* = 8.2 Hz, 1H), 7.36(d, *J* = 2.2 Hz, 1H), 8.09(t, 1H)

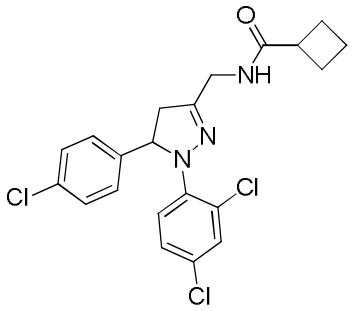
ESI-MS: 424.2 $[M+H]^+$

%Yield: 19 %

Purity by HPLC: 93.87 %

mp: 119-121 ^oC

2.4.1.31 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)cyclobutanecarboxamide (32)



Chemical Formula: $C_{21}H_{20}Cl_3N_3O$

Molecular Weight: 436.76

IR (KBr) cm^{-1}: 3428, 2881, 1640

^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.01(m, 2H), 2.04(m, 4H), 2.80(dd, J = 17.60 Hz & 4.2 Hz, 1H), 3.06(m, 1H), 3.36(dd, *J* = 17.56 Hz & 8.78 Hz, 1H), 4.06(bs, 2H), 5.53(dd, *J* = 8.4 Hz & 3.3 Hz, 1H), 6.71(d, *J* = 8.8 Hz, 2H), 7.03(d, *J* = 8.8 Hz, 2H), 7.17(dd, *J* = 8.8 Hz & 2.4 Hz, 1H), 7.26(d, *J* = 8.8 Hz, 1H), 7.34(d, *J* = 2.0 Hz, 1H), 8.09(t, 1H)

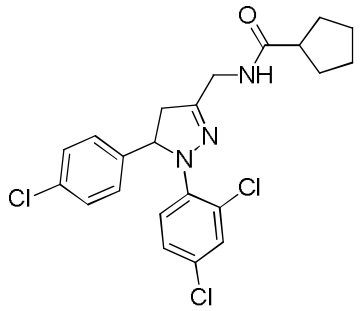
ESI-MS: 459.3$[M+Na]^+$

%Yield: 23 %

Purity by HPLC:94.96 %

mp: 112-115 ^oC

2.4.1.32 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)cyclopentanecarboxamide (33)



Chemical Formula: $C_{22}H_{22}Cl_3N_3O$

Molecular Weight: 450.79

IR (KBr) cm^{-1}: 3421,3273, 2958, 2869, 1585, 1544

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46(d, 2H) ,1.59(d, 4H),1.71(d, 2H), 2.81(d, 1H), 3.14(s, 2H), 4.04(d, 2H), 5.61(t, 1H), 7.14(d, *J* = 8.59 Hz, 2H) ,7.28(d, *J* = 9.02 Hz, 4H), 7.34(S,1H), 8.22(bs,1H)

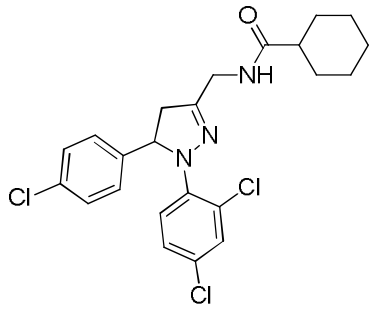
ESI-MS: 473.8 $[M+Na]^+$

%Yield: 17 %

Purity by HPLC: 91.22%

mp : 129-131 ^oC

2.4.1.33 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)cyclohexanecarboxamide (34)



Chemical Formula: $C_{23}H_{24}Cl_3N_3O$

Molecular Weight: 464.82

IR (KBr) cm^{-1}: 3482, 2842, 1638

^{1}H-NMR(300 MHz, DMSO-d_6): δ1.17-1.21(m, 2H), 1.25-1.37(t, 2H), 1.60-1.67(m, 4H), 2.13(t, 1H), 2.78(dd, *J* = 17.72 Hz & 4.75 Hz, 1H), 3.42(dd, *J* = 17.71 Hz & 11.03 Hz, 1H), 4.04(d, *J* = 5.50 Hz, 2H), 5.57(dd, *J* = 11.03 Hz & 4.76 Hz, 1H), 7.07(d, *J* = 8.36 Hz, 2H), 7.20(dd, *J* = 8.76 Hz & 2.33 Hz, 1H), 7.28(d, *J* = 8.77 Hz, 1H), 7.35-7.38(m, 3H), 8.13(t, *J* = 5.66 Hz, 1H)

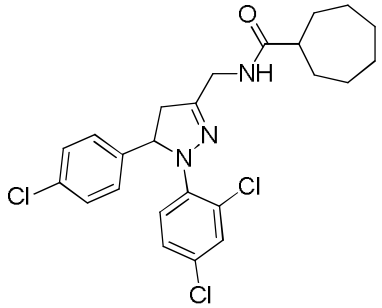
ESI-MS: 466.3 $[M+H]^+$

%Yield: 45 %

Purity by HPLC: 93.22 %

mp: 98-100 ^oC

2.4.1.34 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)cycloheptanecarboxamide (35)



Chemical Formula: $C_{24}H_{26}Cl_3N_3O$

Molecular Weight: 478.84

IR (KBr) cm^{-1}: 3658, 2858, 1661, 1512, 848

^{1}H-NMR(300 MHz, DMSO-d_6): δ 1.35-1.42(m, 2H), 1.45-1.57(m, 6H), 1.62-1.72(m, 4H), 2.30-2.31(m, 1H), 2.76-2.81(dd, *J* = 4.8 Hz, 1H), 3.37-3.41(dd, *J* = 6.4 Hz, 1H), 4.02(d, 2H), 5.57-5.61(m, 1H), 7.12-7.15(dd, *J* = 6.8 Hz & 2.0 Hz,

2H), 7.18-7.21(dd, *J* = 6.4 Hz & 2.4 Hz, 1H), 7.22-7.25(dd, *J* = 6.8 Hz & 2.0 Hz , 2H), 7.27(d, *J* = 9.2 Hz, 1H), 7.36(d, *J* = 2.4Hz, 1H), 8.12(t, 1H)

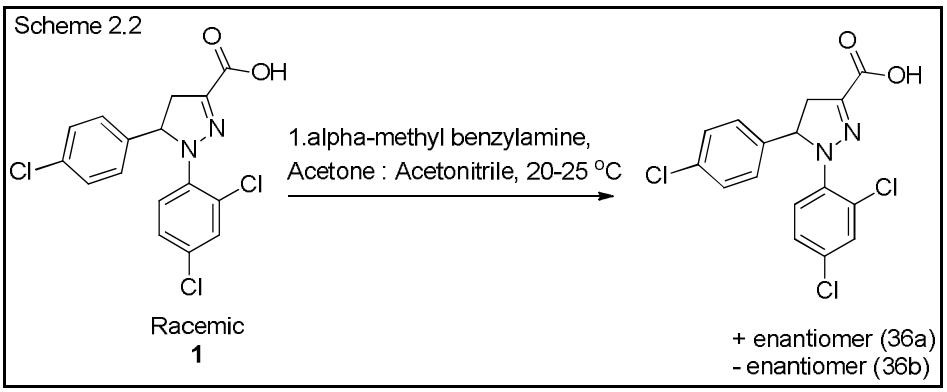
ESI-MS: 501.4 $[M+Na]^+$

%Yield: 46 %

Purity by HPLC: 95.66 %

mp: 118-120 ^oC

2.4.1.35 Procedure for the Resolution of 5-(4-Chlorophenyl)-1-(2,4-dichloro phenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic Acid



2.4.1.36 (-)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic Acid (36b).

To a solution of **1** (64 g,0.17 mole) in acetone (320 mL) was added R-(+)-α-methyl benzylamine (20.9 mL, 0.17 mole) at 26-28 °C, and the mixture was stirred for 10-15 min. To this was added acetonitrile (320 mL), and the mixture was stirred at 26-28 °C for another 2 h. The solid salt separated out was filtered on a Buchner funnel under suction and washed with chilled acetonitrile to afford the R-(+)-R-methyl benzylamine salt of the (-)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid as an off white solid salt. (34 g, 40%).

IR (KBr) cm^{-1}: 3413, 2977, 1569, 1477, 1307

^{1}H-NMR(300 MHz, DMSO-d_6): δ 1.45(d, 3H), 2.91-2.99(dd, *J* = 17.88 Hz & 5.43 Hz, 1H), 3.47-3.56(dd, *J* = 17.82 Hz & 11.82 Hz), 4.27-4.33(q, 1H), 5.65-5.71 (dd, *J* = 11.34 Hz & 6.63 Hz, 1H), 7.12(d, *J* = 8.43 Hz, 2H)), 7.19-7.21(dd, *J* = 8.76 Hz & 2.34 Hz, 1H),), 7.27 (d, *J* = 8.43 Hz, 2H), 7.31-7.35 (m, 2H), 7.37-7.40(m, 3H), 7.45 (d, *J* = 6.93 Hz, 2H)

ESI-MS: 492.7 [M + H]+.

Purity by HPLC: 99.3 %

[αD] -407°, c= 0.2, DMSO

mp: 164-165 °C

The R-(+)-R-methyl benzylamine salt of (-)-5-(4-chlorophenyl)-1-(2,4-dichloro phenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid (34.0 g) was taken in ice cold water (100 mL) and acidified to pH 3 using 10% HCl solution. The solution was extracted with dichloromethane (2 x 200 mL), and the organic layer was washed with water (3 x 200 mL). The organic layer was separated and dried over anhydrous Na_2SO_4, and the solvents were evaporated on a rotatory evaporator under reduced pressure. The residue was triturated in petroleum ether to get a solid. The solid was filtered on a Buchner funnel under suction and dried to afford **36b** as an off-white solid (20 g, 31%):

IR (KBr) cm^{-1}: 3413, 2925, 1685, 1571, 1479

^{1}HNMR (300 MHz, $CDCl_3$): δ 3.32-3.24(dd, *J* = 18.10 Hz & 6.18 Hz, 1H), 3.77-3.67(dd, *J* = 18.06 Hz & 12.14 Hz, 1H), 5.95-5.89(dd, *J* = 12.68 Hz & 6.14 Hz, 2H), 7.09 (d, *J* = 8.65 Hz, 3H), 7.19(s, 1H), 7.21(d, *J* = 1.93 Hz,1H), 7.25(m, 2H)

ESI-MS: 370.9 [M + H]+.

Purity by HPLC: 99.90%

[αD] -187°, c=0.2, DMSO

mp: 144-146 °C

2.4.1.37 (+)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic Acid (36a)

The resolution of **1** to (+)-enantiomer was done using the same procedure described for compound 36b. Compound **1** was reacted with *S*-(-)-α-methyl benzylamine to get the *S*-(-)-α-methyl benzylamine salt of (+)-5-(4-chloro phenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid as an off-white solid (18 g, 35%):

IR (KBr) cm^{-1}: 3033, 2943, 2881, 1571, 1475

^{1}H NMR (300 MHz, DMSO-d_6) : δ 2.97-2.90(dd, *J* = 17.01 Hz & 5.07 Hz, 1H), 3.55-3.45(dd, *J* = 17.19 Hz & 12.22 Hz, 1H), 4.36-4.29(q, 1H), 5.69-5.64(dd, *J* = 11.29 Hz & 4.95 Hz, 1H), 7.11(d, *J* = 8.41 Hz, 2H), 7.20-7.16(dd, *J* = 8.74 Hz & 2.33 Hz, 1H), 7.23(d, *J* = 8.38 Hz, 2H), 7.38-7.33(m, 5H), 7.45-7.41(m, 2H)

ESI-MS: 492.7 [M + H]+.

Purity by HPLC: 99.95 %

[αD]: +407°, c= 0.2, DMSO

mp: 160-161 °C

The *S*-(-)-*R*-methyl benzylamine salt of (+)-5-(4-chlorophenyl)- 1-(2,4-dichloro phenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid was converted into free acid **36a** as an off-white solid (11.5 g, 28%):

IR (KBr) cm^{-1}: 3425, 1689, 1575, 1555

^{1}H NMR (300 MHz, DMSO-d6): δ 3.08-3.01(dd, *J* = 18.03 Hz & 6.78 Hz, 1H), 3.72-3.62(dd, *J* = 17.91 Hz & 12.57 Hz, 1H), 5.90-5.84(dd, *J* = 12.15 Hz & 6.69 Hz, 2H), 7.20 (d, *J* = 8.49 Hz, 2H), 7.35-7.28(m, 4H), 7.48(d, *J* = 2.19 Hz, 1H), 13.01(br s, 1H)

ESI-MS: 370.9 [M + H]

Purity by HPLC: 99.86 %

[αD]: +187°, c = 0.2, DMSO

mp: 180-181 °C

Following synthetic procedure as per the scheme 2.1 (+) and (-) enantiomers of compound 27 were prepared from 36a and 36b respectively.

2.4.1.38 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethylhexanamide[(-) enantiomer] 40)

Purity by HPLC: 97.18 %

[αD] -155.2°, c= 0.2, DMSO

mp: 90-95 °C

2.4.1.39 N-((5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) methyl)-2,2-dimethylhexanamide[(+) enantiomer] (41)

Purity by HPLC: 94.12 %

[αD] +155.9°, c= 0.2, DMSO

mp: 65-70 °C

2.4.1.40 *In vitro* cAMP assay:

Fatty acid-free BSA, IBMX (isobutylmethyl xanthine), RO20-1724 [4-[(3-butoxy-4-methoxyphenyl) methyl]-2-imidazololidinone], forskolinand DMSO (hybrimax) were purchased from Sigma Chemical Co. cAMP detection ELISA kit was from Assay Designs, USA. Tissue culture reagents were purchased from Sigma and Hi-media. Other reagents used were all of analytical grade. The cAMP assay was carried out in Chinese Hamster Ovarian (CHO) cells (CHOK1) stably expressing human CB1 receptor following the method of Rinaldi-Carmona et al.26 Cells grown to 80% confluence were maintained in HAM'S F12 medium containing 10% heat inactivated dialyzed fetal bovine serum and 0.8 mg/mL G-418. Cells were seeded at a density of 50,000 cells/well in 24-well plate, grown for 16–18 h, washed once with PBS and incubated for 30 min at 37 ^{o}C in plain HAM'S F12 containing 0.25% free fatty acid BSA, IBMX (0.1 mM) and RO20-1724 (0.1 mM). IBMX, the pan phosphodiesterase inhibitor and RO20-1724, the specific phosphodiesterase- 4 inhibitor were added to restore cAMP up to the detection limit. After 5 min incubation with the drugs, forskolin was added at a final concentration of 10 lM and incubation was carried out for another 20 min at 37 ^{o}C. The reaction was terminated by washingonce with PBS and adding 200 lL lysis buffer comprising 0.1 N HCl and 0.1% Trition X-100. The lysates were centrifuged and aliquotes from supernatants were used for detection of cAMP by ELISA as per the manufacturer's protocol.

2.4.1.41 5% Sucrose Solution Intake in Zucker fa/fa rats

The obese Zucker fa/fa rats were housed individually and subjected to training for consuming 5% sucrose solution over 4 h, by allowing access to the 5% sucrose solution in the bottles. Food and water were withdrawn during this time. This training was given for six consecutive days, at the same time of the day. On seventh day, the animals were randomized into groups of six animals each and treated with the test compounds. After one hour of treatment, the animals were exposed to the 5% sucrose solution for 4 h as that of the training schedule. The primary pharmacokinetic measurements indicated the T1/2 of 2-3 h for all the tested compounds (Data not shown). Accordingly, this duration was selected for Sucrose consumption studies, so that the T1/2 of the compound falls exactly in between of the study duration. The amount of sucrose solution consumed by each animal was calculated. Difference between the control and treatment groups were analyzed by performing one way ANOVA followed by Dunnett's test on sucrose solution consumption using Graph pad Prism software.

2.4.1.42 Pharmacokinetic of Antiobesity compound (For CB1 antagonist)

Pharmacokinetic parameters of 1 and 40 were evaluated by administration of 30 mg/kg dose orally to female obese Zucker fa/fa rats. The oral suspension of both the compounds were prepared in 0.5% carboxymethylcellulose sodium salt in distilled water. A group of six female obese Zucker fa/fa rats were kept for an overnight fasting and then administered with a dose of 30 mg/kg orally. Serial blood samples were collected from retro-orbital of animals at various time points,

i.e., 10 min, 20 min, 40 min, 1.0, 2.0, 4.0, 6.0, 8.0, 24.0 and 48.0 h. The blood samples were subjected to centrifugation for collection of the plasma.

For tissue distribution study, a group of six female rats were administered with a single dose of 30 mg/kg orally. After 3 h, the samples of blood, brain and adipose tissues were collected from each rat of the group. The tissue samples were washed with saline and homogenized in Tris buffer (pH 7.4) using laboratory homogenizer. The plasma and tissue homogenates were extracted through liquid- liquid extraction method. The concentration of compound was determined using LC-MS. The samples were analyzed on ACE, Cyano, 100 mm x 4.6 mm x 5 μm HPLC column using the mobile phase of 0.4 mM ammonium acetate containing 0.06% trifluoroacetic acid and acetonitrile (30:70% v/v) with flow rate of 0.3 mL/min. The mass spectrometry detector parameter was put on voltage 1.8 KV, Block and CDL temperature 200 ℃ and Nebuliser gas of 1.5 L/min. The quantitation was performed using known concentration versus response curve. The calibration standards were obtained by spiking known concentration in blank matrix with internal standard, processed and analyzed it as for the samples. The pharmacokinetic parameters, Tmax, Cmax, Half-life, and AUC (0-α) were calculated using WinNolin software version 5.0.1. The brain to plasma ratio was calculated using plasma (μg/mL) concentration.

2.5 References

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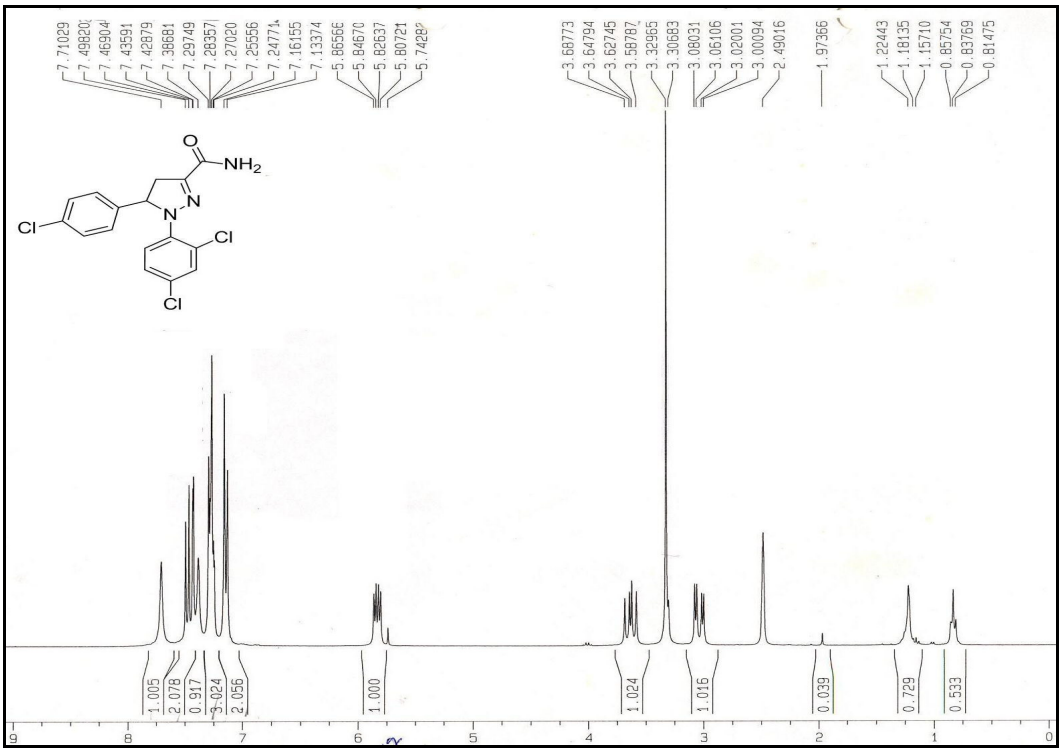
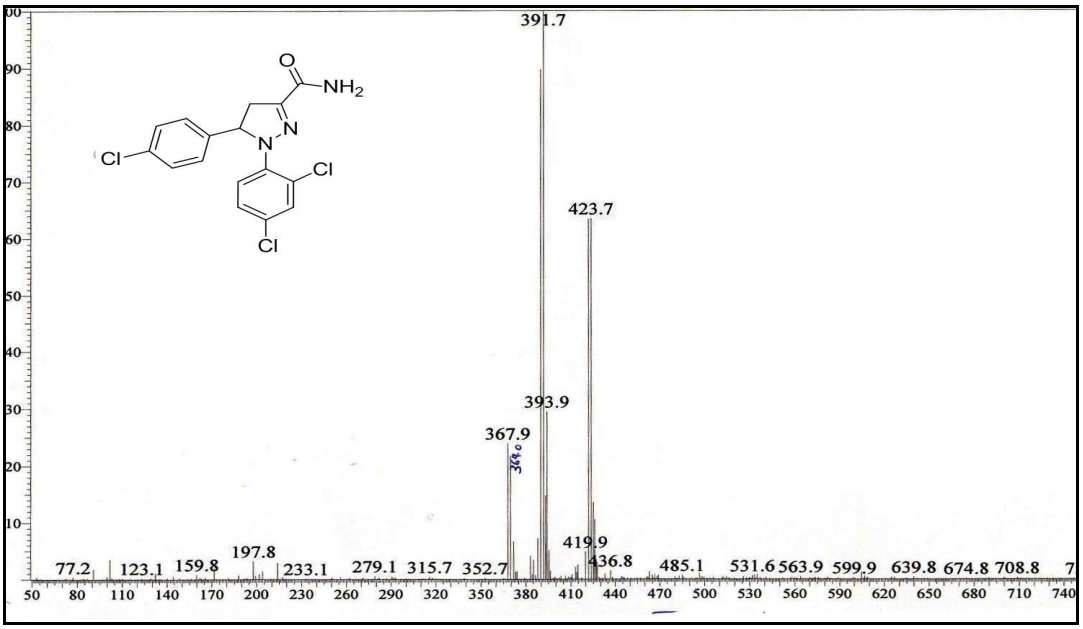
2.6 Spectra

^{1}H NMR of 2



ESI-MS of 2



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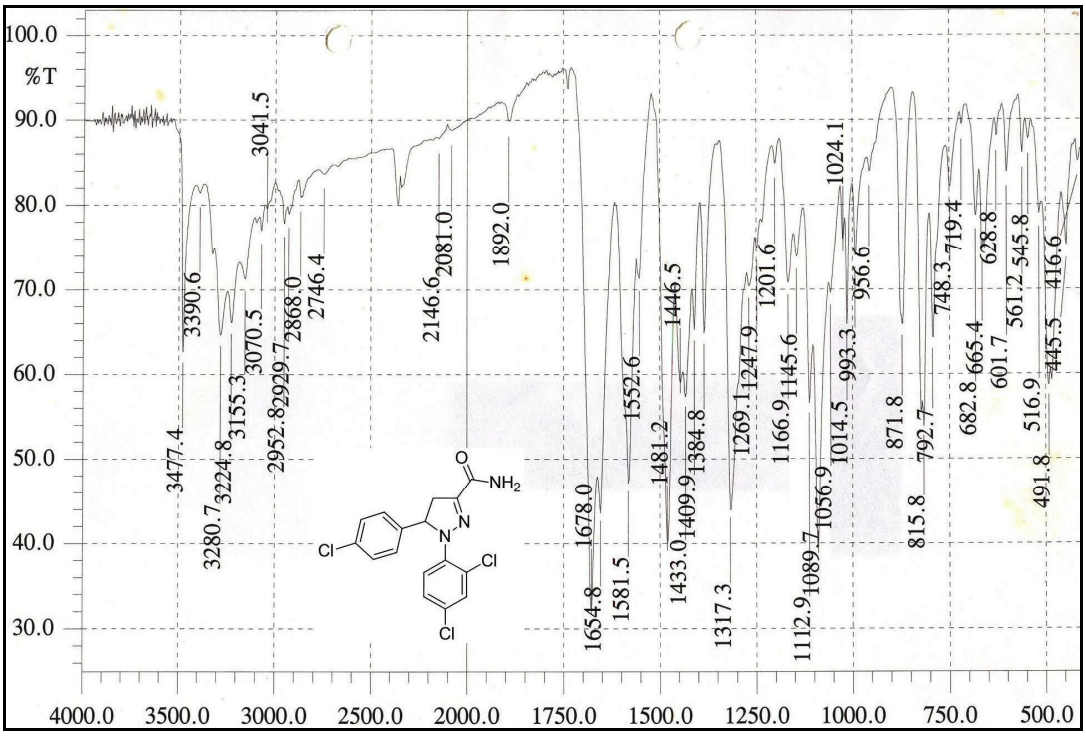

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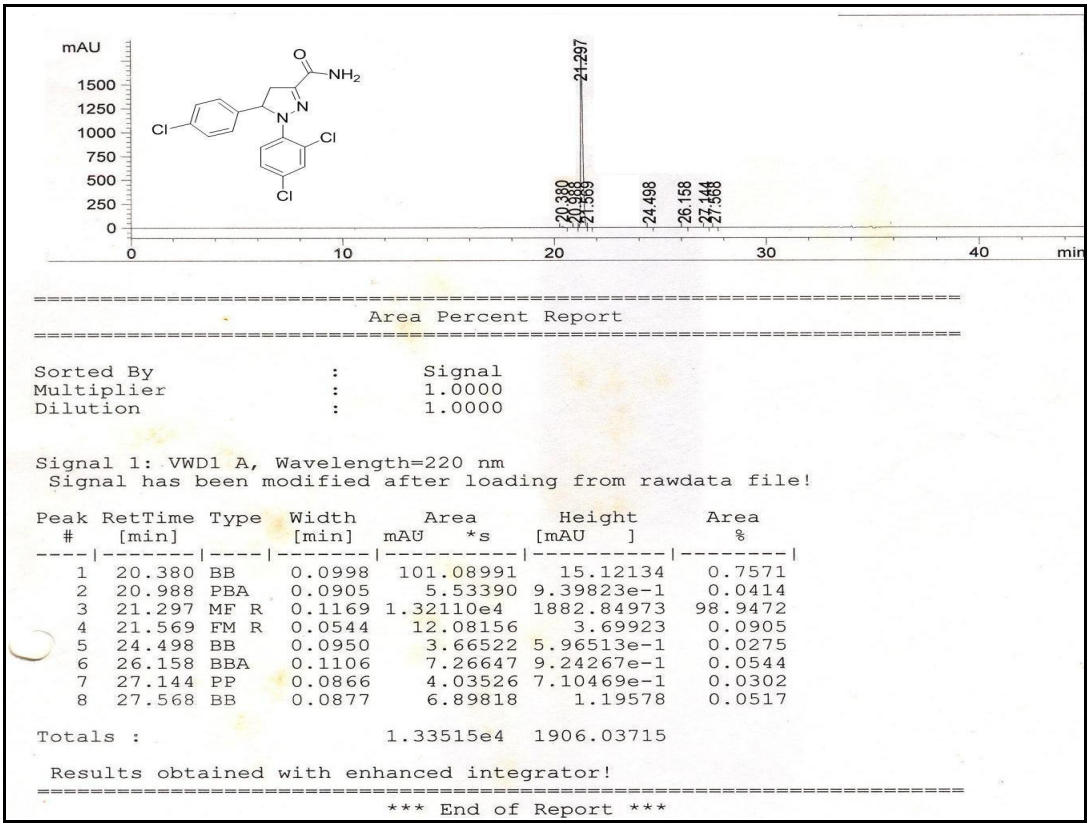


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Dilution              :      1.0000

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   2  20.988 PBA   0.0905    5.53390 9.39823e-1   0.0414
   3  21.297 MF R  0.1169 1.32110e4  1882.84973  98.9472
   4  21.569 FM R  0.0544   12.08156    3.69923   0.0905
   5  24.498 BB    0.0950    3.66522 5.96513e-1   0.0275
   6  26.158 BBA   0.1106    7.26647 9.24267e-1   0.0544
   7  27.144 PP    0.0866    4.03526 7.10469e-1   0.0302
   8  27.568 BB    0.0877    6.89818    1.19578   0.0517

Totals :                  1.33515e4  1906.03715

 Results obtained with enhanced integrator!
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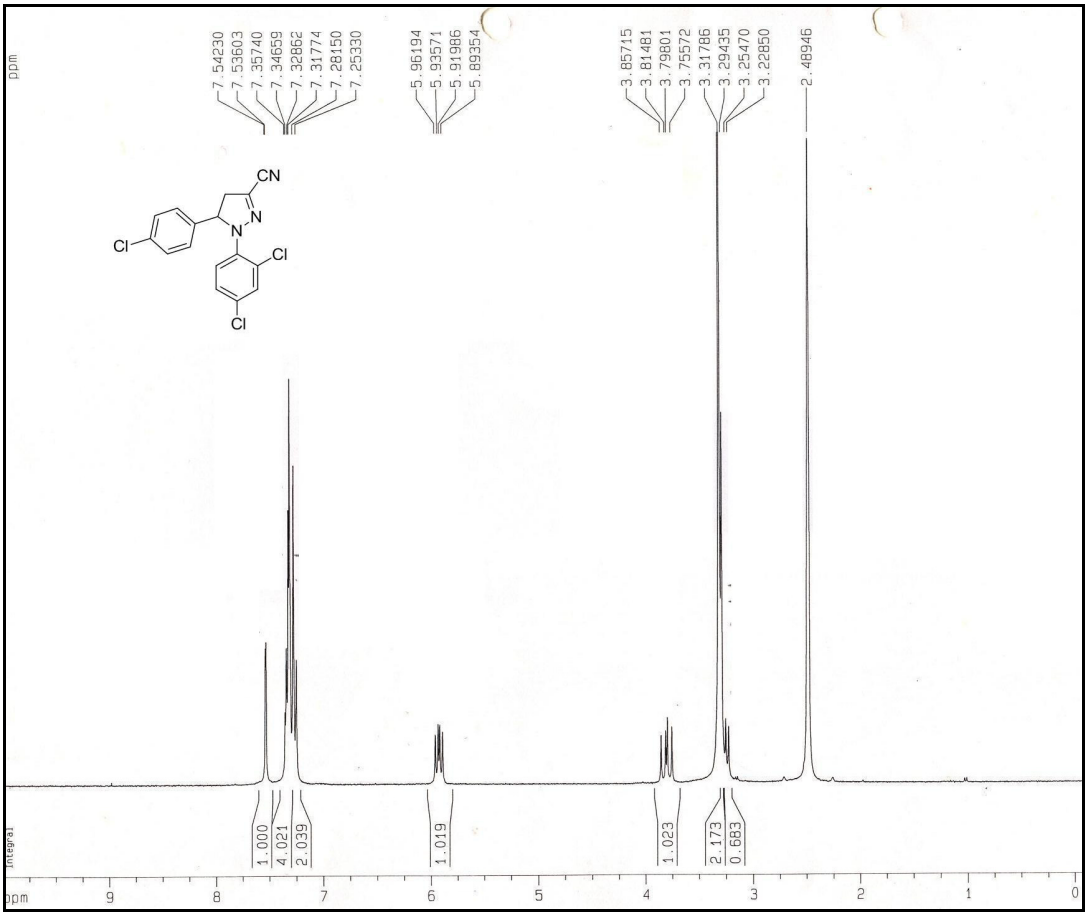
^{1}H NMR of 3



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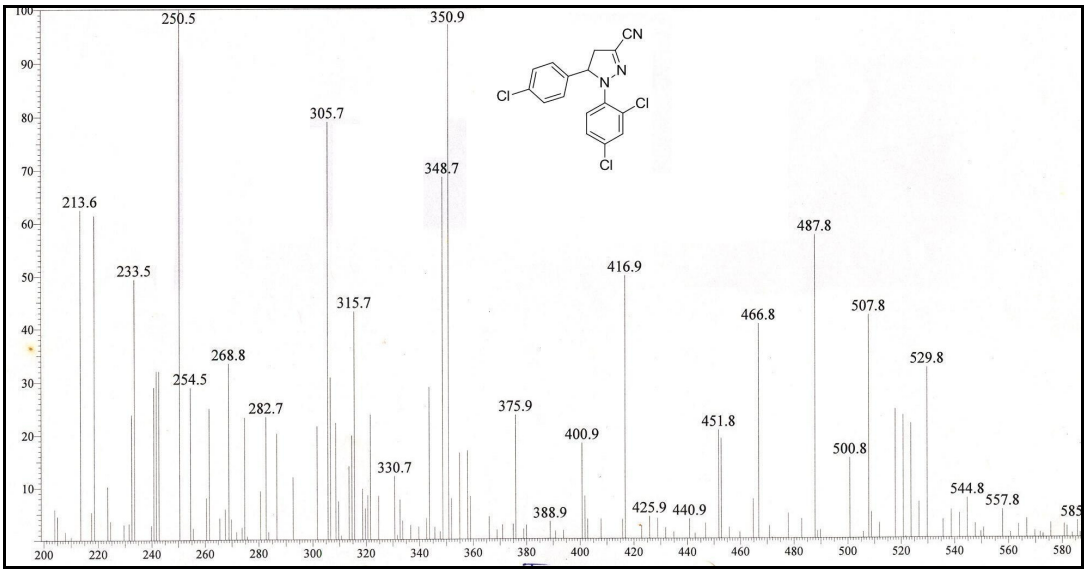

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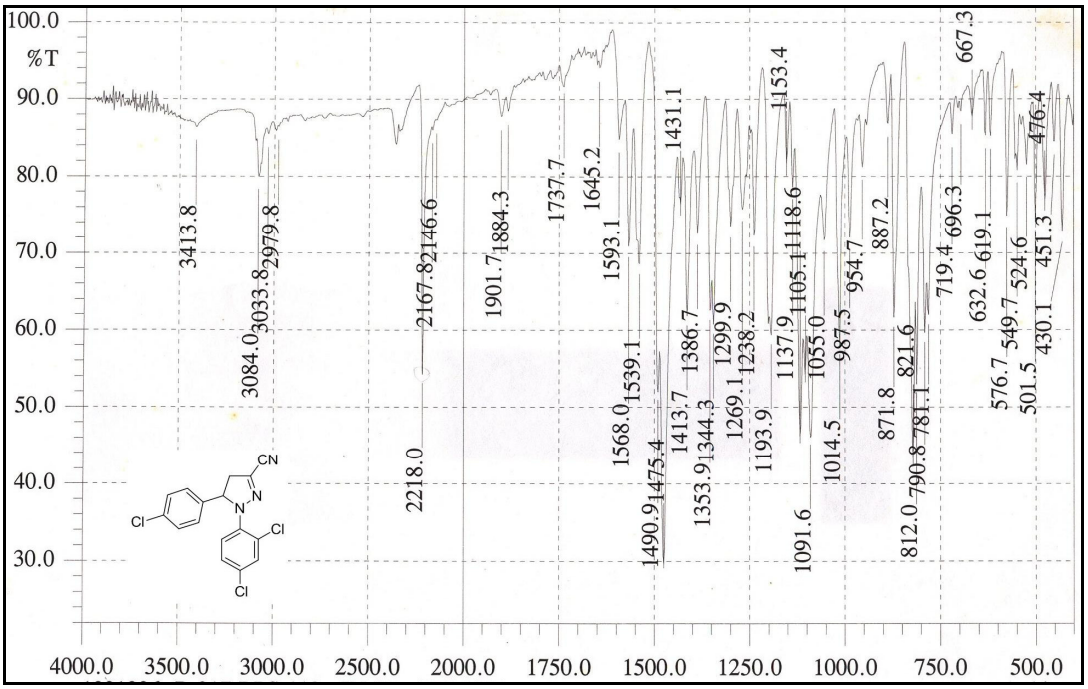
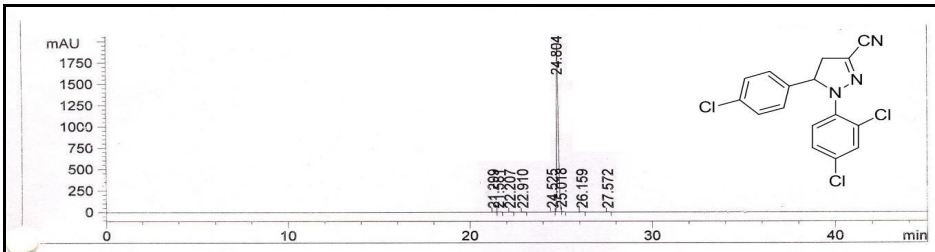


HPLC of 3

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Dilution              :      1.0000


Signal 1: VWD1 A, Wavelength=220 nm
 Signal has been modified after loading from rawdata file!

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   2  21.581 VP    0.1027   16.75897    2.46251   0.1300
   3  22.207 BP    0.1104    3.87392 5.19115e-1   0.0300
   4  22.910 BB    0.1064    2.07426 3.01623e-1   0.0161
   5  24.525 BV    0.0975   19.45762    3.00153   0.1509
   6  24.804 MF R  0.1084 1.27982e4   1968.23352  99.2527
   7  25.018 FM R  0.0464   33.68251   12.10977   0.2612
   8  26.159 BBA   0.1152    7.62033 9.23464e-1   0.0591
   9  27.572 BP    0.0884    6.42489    1.12558   0.0498

Totals :                  1.28945e4  1989.59985

 Results obtained with enhanced integrator!
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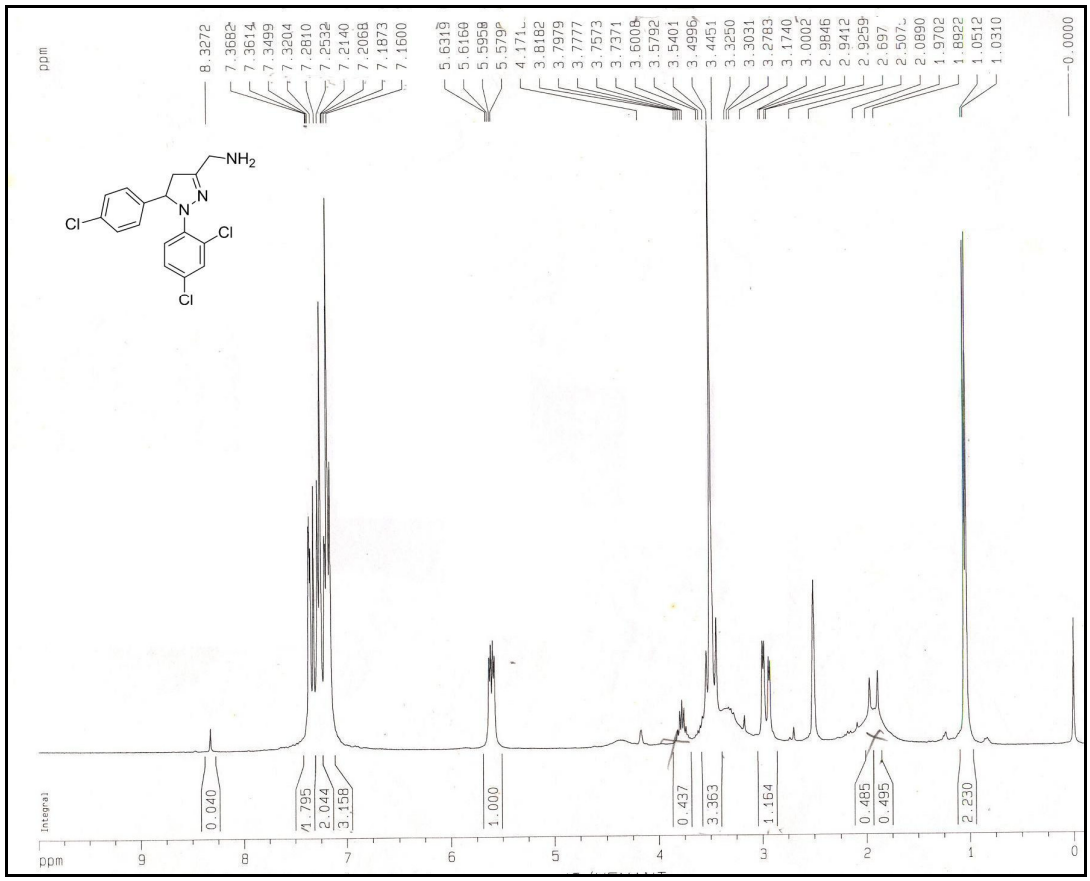
^{1}H NMR of 4



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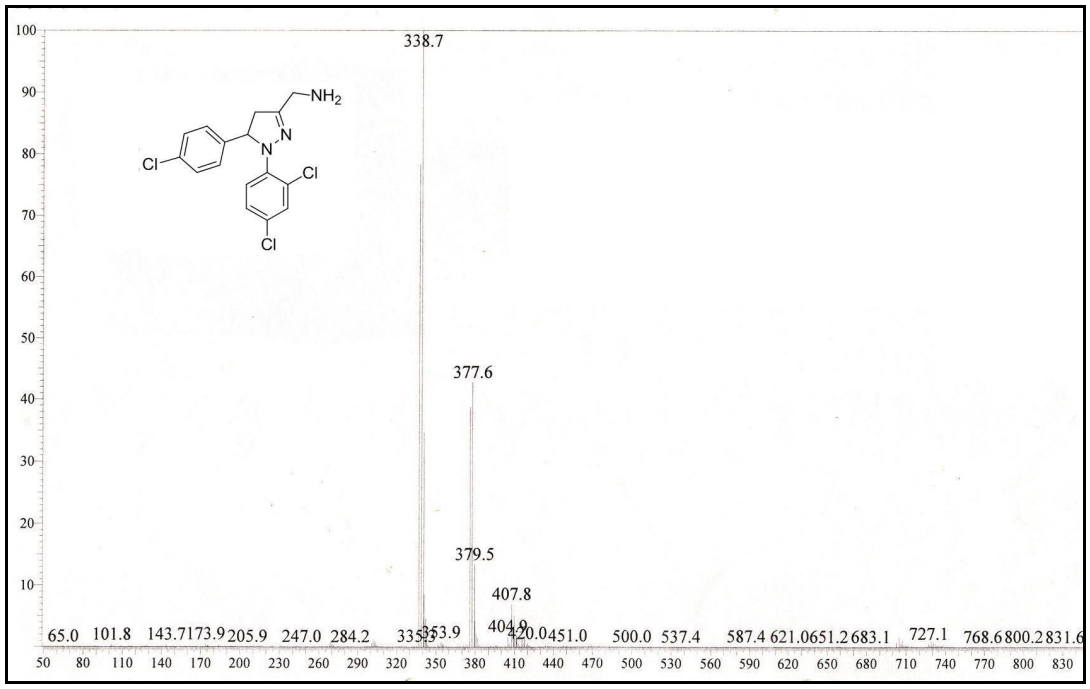
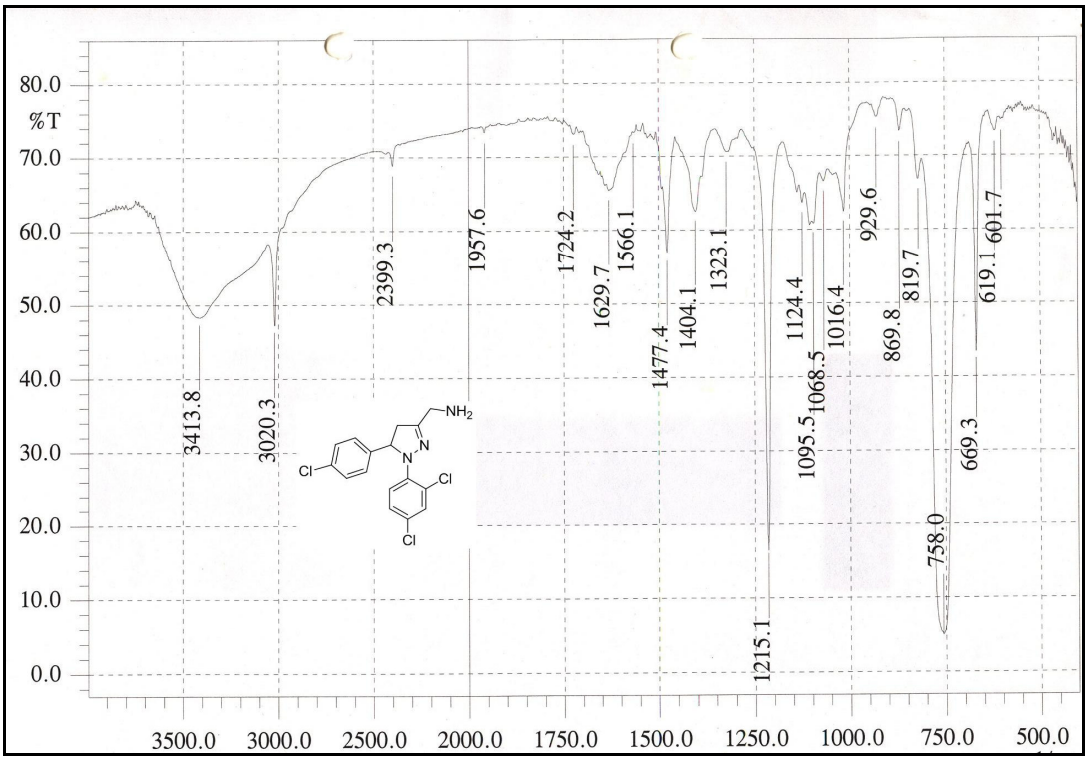


IR of 4



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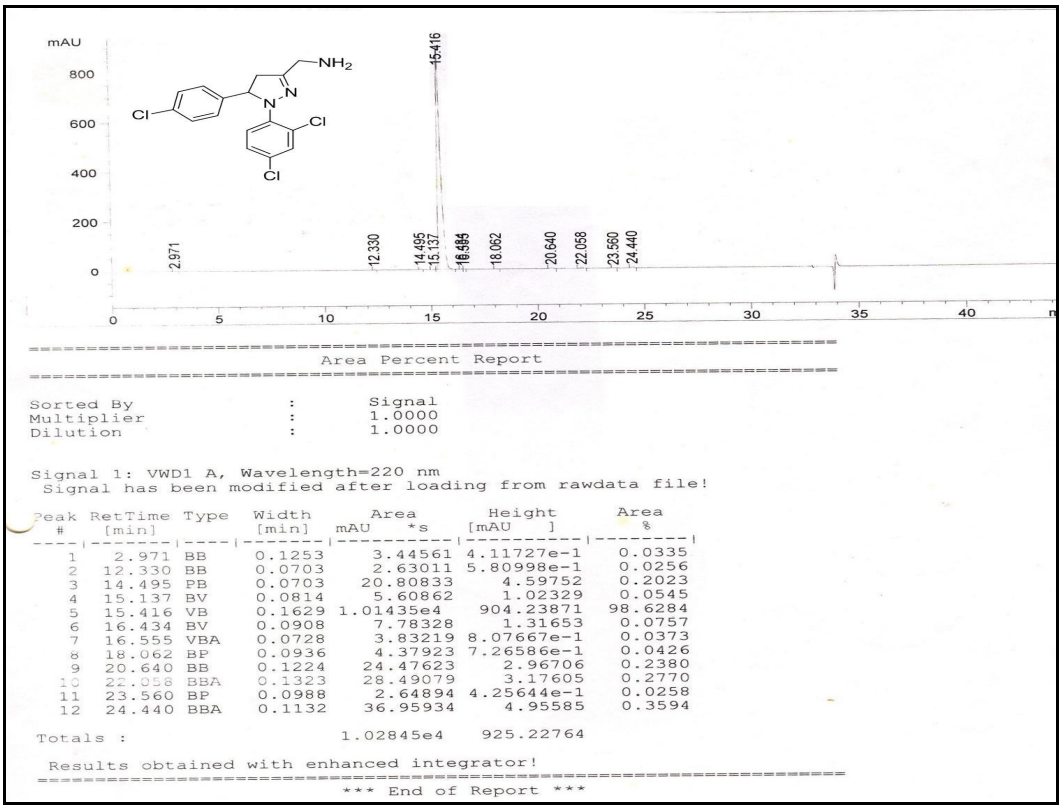

Area Percent Report

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Dilution : 1.0000

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Signal has been modified after loading from rawdata file!

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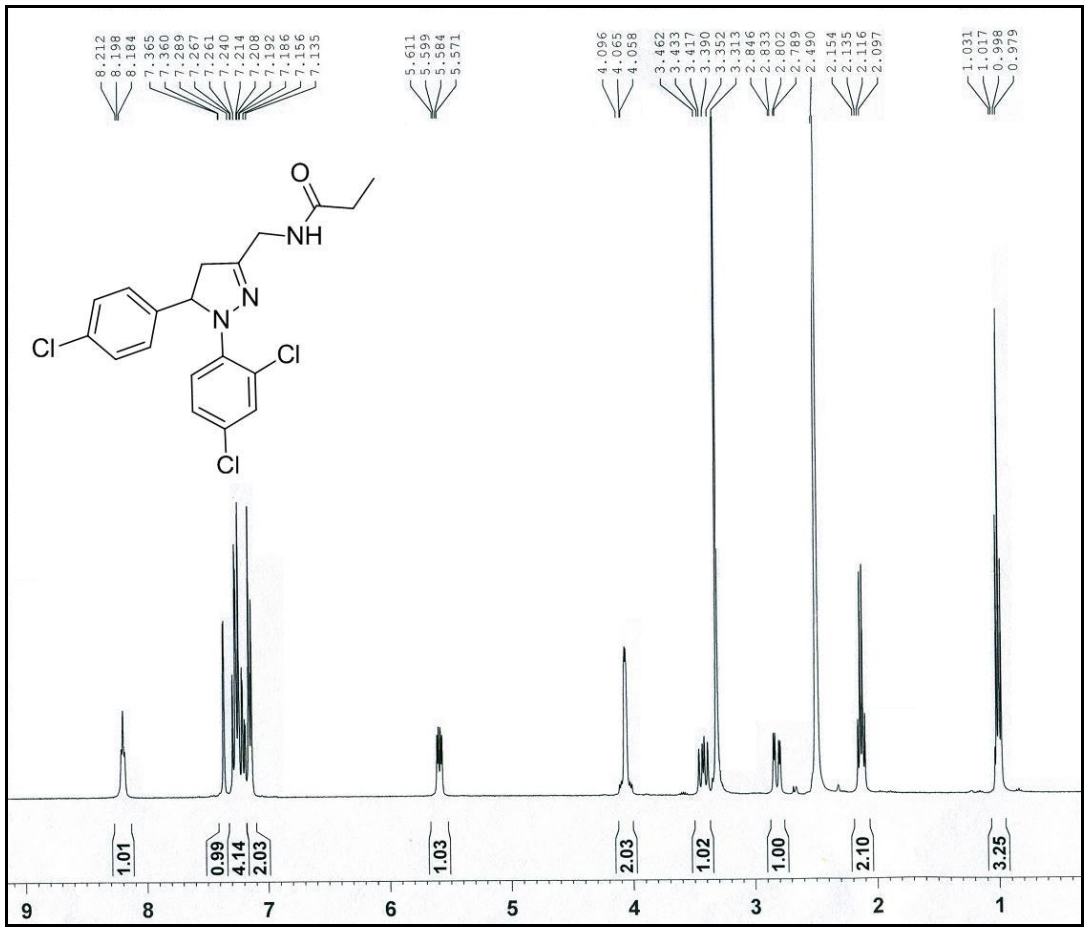
2	12.330	BB	0.0703	2.63011	5.80998e-1	0.0256
3	14.495	PB	0.0703	20.80833	4.59752	0.2023
4	15.137	BV	0.0814	5.60862	1.02329	0.0545
5	15.416	VB	0.1629	1.01435e4	904.23871	98.6284
6	16.434	BV	0.0908	7.78328	1.31653	0.0757
7	16.555	VBA	0.0728	3.83219	8.07667e-1	0.0373
8	18.062	BP	0.0936	4.37923	7.26586e-1	0.0426
9	20.640	BB	0.1224	24.47623	2.96706	0.2380
10	22.058	BBA	0.1323	28.49079	3.17605	0.2770
11	23.560	BP	0.0988	2.64894	4.25644e-1	0.0258
12	24.440	BBA	0.1132	36.95934	4.95585	0.3594
Totals :				1.02845e4	925.22764	

Results obtained with enhanced integrator!

*** End of Report ***

^{1}H NMR of 5



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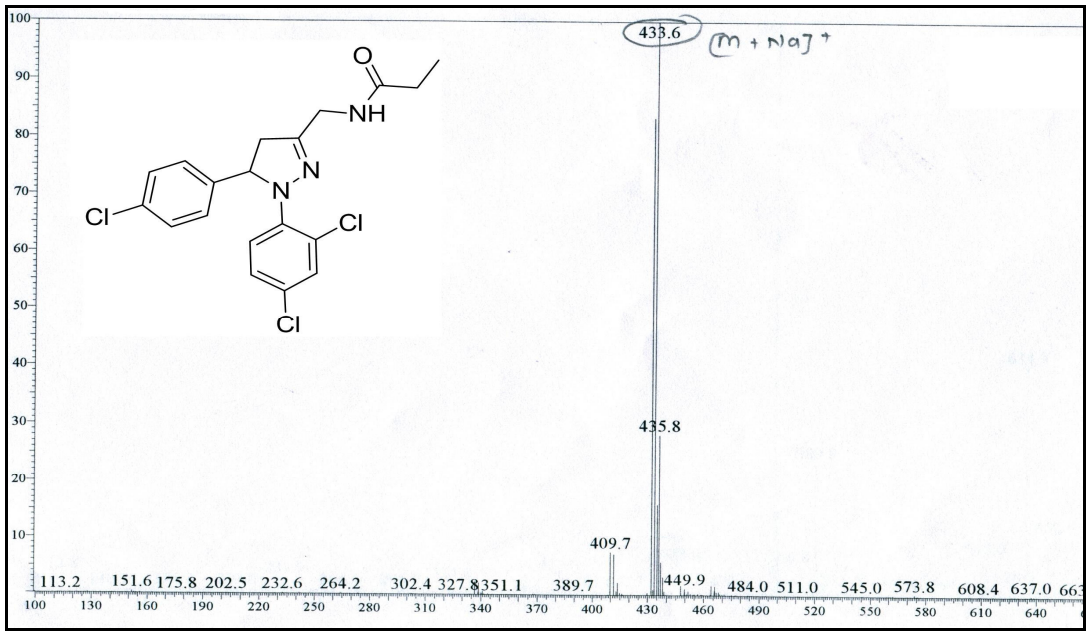

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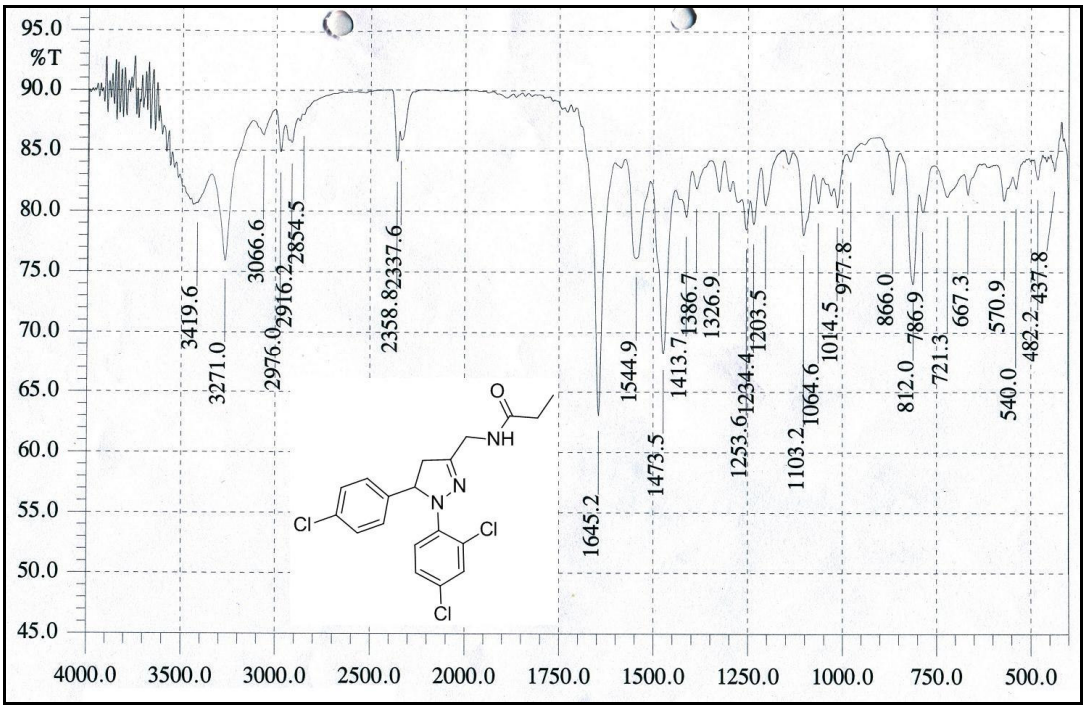

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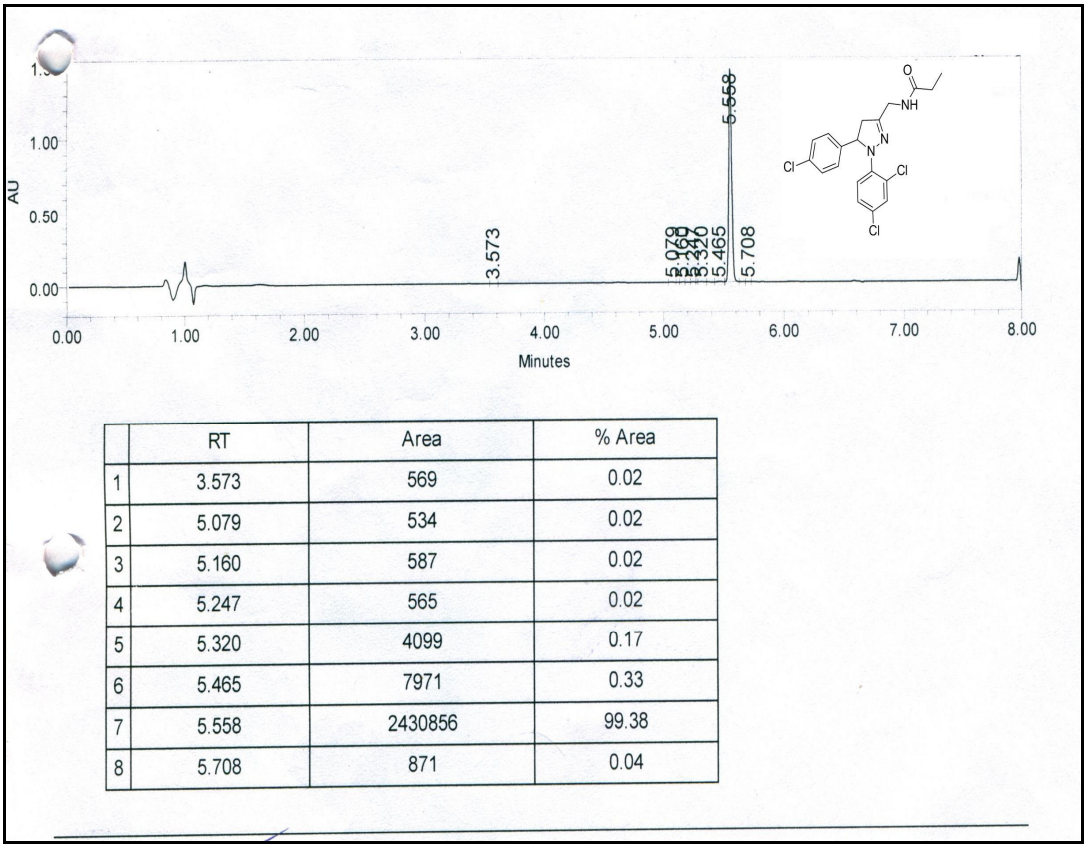

	RT	Area	% Area
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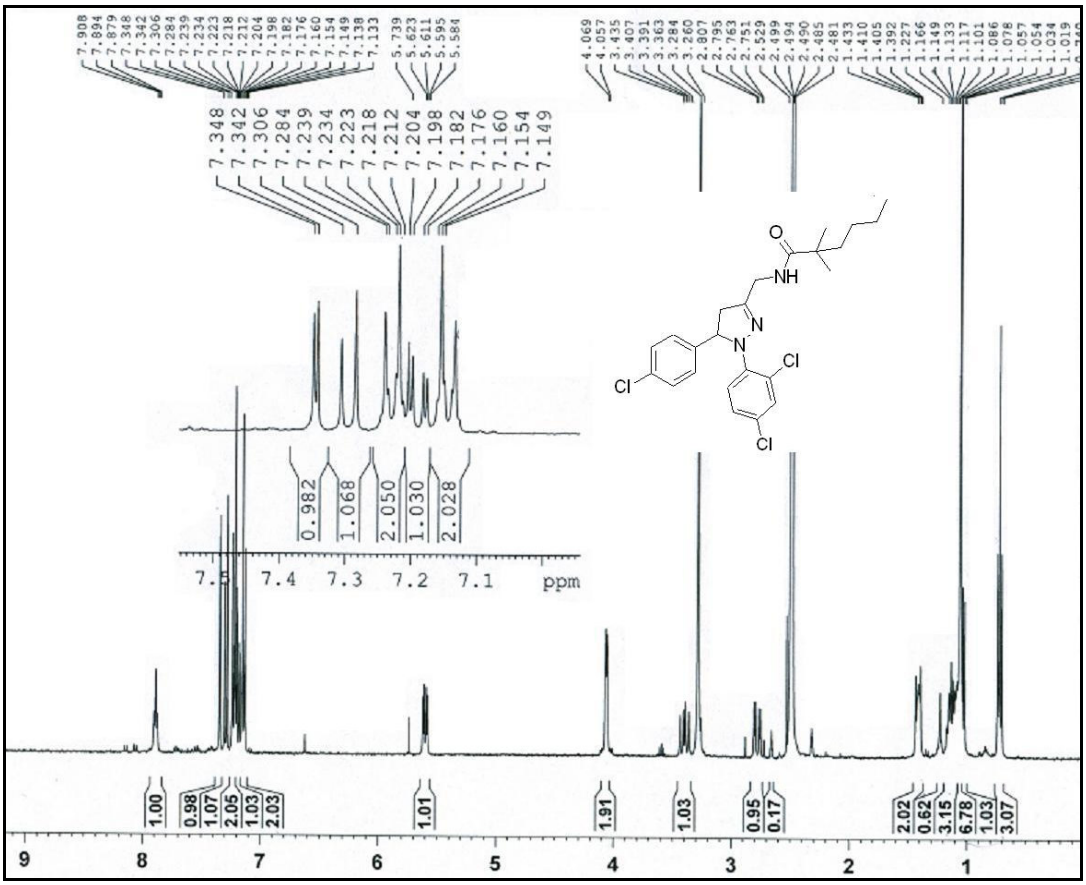
2	5.079	534	0.02
3	5.160	587	0.02
4	5.247	565	0.02
5	5.320	4099	0.17
6	5.465	7971	0.33
7	5.558	2430856	99.38
8	5.708	871	0.04

^{1}H NMR of 27



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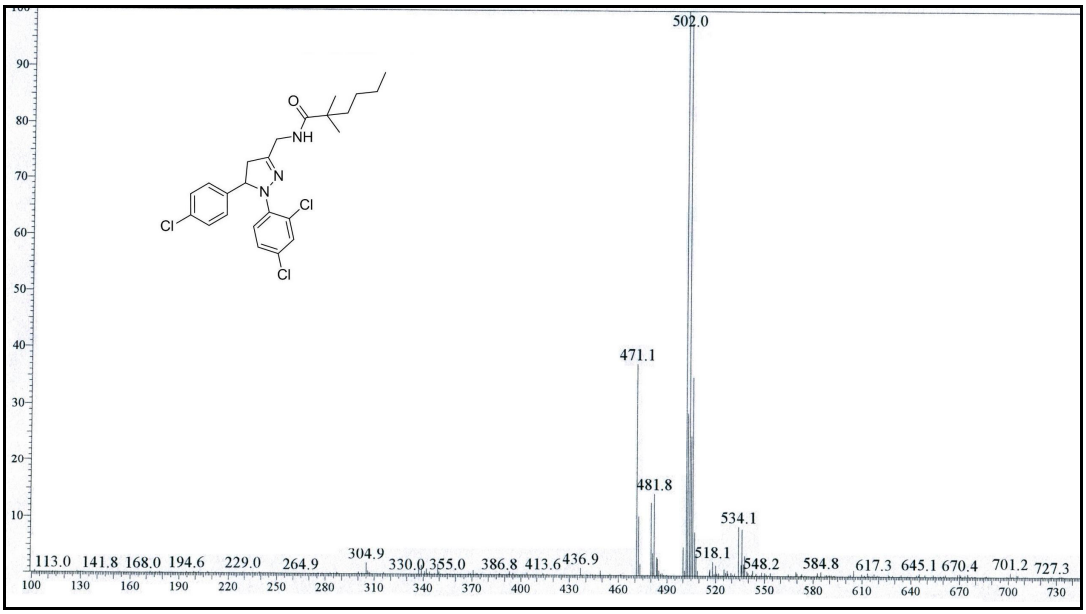

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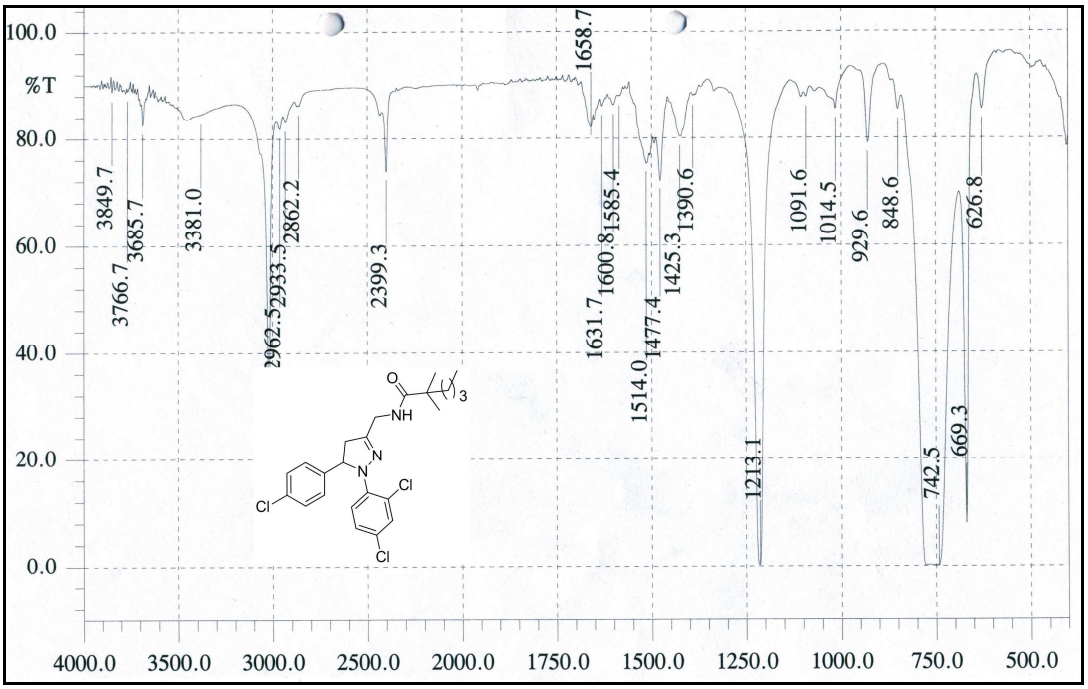
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2933.5
2862.2
2399.3
1658.7
1631.7
1600.8
1585.4
1514.0
1477.4
1425.3
1390.6
1213.1
1091.6
1014.5
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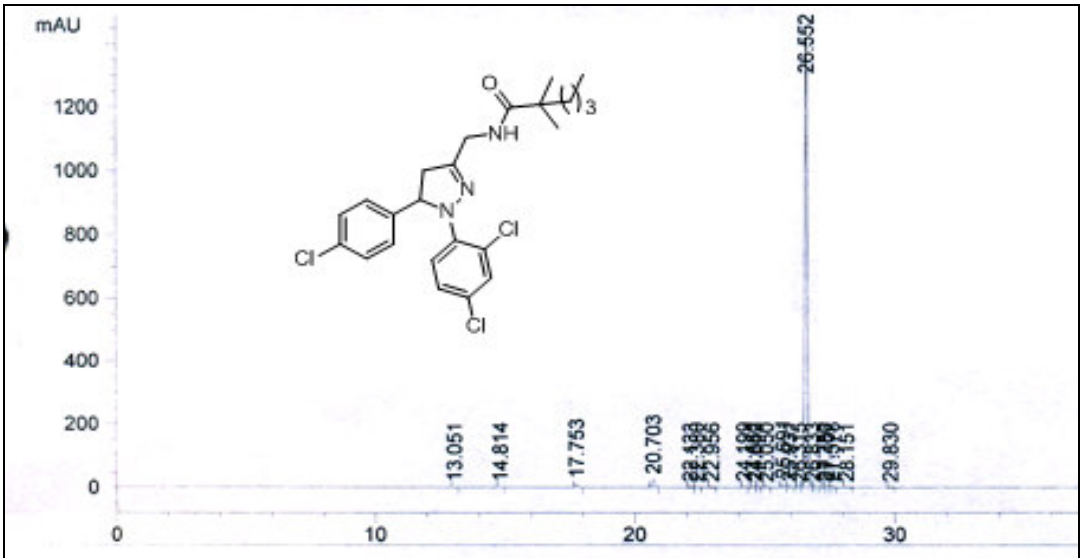
NH
N
N
Cl
Cl
Cl
3

HPLC of 27



Area Percent Report

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Dilution : 1.0000

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Signal has been modified after loading from rawdata file!

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2	14.814	BBA	0.1146	3.34030	4.71903e-1	0.0332
3	17.753	BB	0.1018	75.02438	11.58587	0.7467
4	20.703	PBA	0.1085	168.43611	24.31496	1.6764
5	22.133	BP	0.1048	2.20880	3.28060e-1	0.0220
6	22.380	VBA	0.1007	4.18943	6.55845e-1	0.0417
7	22.956	BBA	0.1094	1.76545	2.56607e-1	0.0176
8	24.199	BBA	0.1041	5.73115	8.92177e-1	0.0570
9	24.562	MF R	0.1113	13.83422	2.07217	0.1377
10	24.649	FM R	0.0922	4.09251	7.40137e-1	0.0407
11	25.050	BBA	0.1034	3.26680	4.75526e-1	0.0325
12	25.691	PV	0.1074	63.12337	9.06843	0.6282
13	25.931	VBA	0.1431	16.34624	1.83702	0.1627

Page 2

14	26.315	BV	0.0961	2.87431	4.90344e-1	0.0286
15	26.552	MF R	0.1128	9632.66992	1423.34314	95.8702
16	26.833	FM R	0.1186	6.60883	9.28405e-1	0.0658
17	27.280	PV	0.0705	2.36841	5.21311e-1	0.0236
18	27.407	VV	0.1105	18.15071	2.51075	0.1806
19	27.578	VBA	0.0995	14.71277	2.29638	0.1464
20	28.151	BBA	0.1210	3.76082	4.42452e-1	0.0374
21	29.830	BBA	0.1256	2.40139	3.04118e-1	0.0239

THYROMIMETICS

3

3 Thyromimetics

3.1 Objective

Thyroid hormones (TH) are important endocrine signaling hormones, which are involved in a number of important physiological processes such as lipid metabolism, control of energy expenditure and also in the brain development. The physiologic actions of thyroid hormones are discussed in more detail in introduction section. Natural thyroid hormone, triiodothyronine (T3) exhibits its physiological effect by acting on thyroid hormone receptors (TR). T3 is an important endocrine signaling hormone essential for normal development, differentiation and maintenance of metabolic balance in mammals. The liver represents a major target organ of TH. Microarray analysis of hepatic gene expression from livers of hypothyroid mice and mice treated with T3 showed changes in gene expression for 55 genes (14 positively regulated and 41 negatively regulated) [1]. It is also known that approximately 8% of the hepatic genes are regulated by T3 *in vivo* [2]. Many of these genes are important for synthesis and metabolism of both fatty acid and cholesterol. In addition to that T3 is also known to regulate the carbohydrates through increased glycogenosis, gluconeogenesis and decreased insulin action in liver.

Compounds which produces effects similar to those of thyroid hormones (mostly thyroid receptor agonists) are called thyromimetics. In identification of the potential benefits associated with thyromimetics, various approaches have been pursued. Initial studies with T3 and T4 in humans demonstrated their usefulness in lowering plasma cholesterol levels [3-5]. However, these benefits were offset by deleterious cardiovascular side effects (tachycardia, arrhythmia) [6,7]. Studies

have revealed that cardiac side effects of TH are mediated by their action on TR*α* present in cardiovascular system (CVS). Therefore, drug development approaches were focused on the identification of thyromimetics that bind preferentially to the thyroid hormone receptor *β* (TR*β*) [8-10].

The first TR*β* selective compound tested *in vivo* was GC-1 (Figure 3.1), which is 10 fold more selective for TR*β* vs TR*α* [11]. This compound showed great promise as it was selective for cholesterol lowering versus tachycardia that is consistent with TR*β* selectivity. It was also claimed to have preferential distribution into the liver. Another agonist KB-141(Figure 3.1), had tenfold TR*β* selectivity but did not differentially distribute into tissues as compared with T3 [12]. Prior to that Axitirome (CGS 23425) also showed preferential binding to the rat liver nuclei TR over plasma membrane TR [13] and was later found to be a TR*β* selective agonist [14]. Recently disclosed TR*β* agonist Eprotirome (KB-2115, Figure 3.1) [3,5-dibromo-4-(4′-hydroxy-3′-isopropylphenoxy)phenylamino]-3-oxo propanoic acid is being evaluated in humans [15].

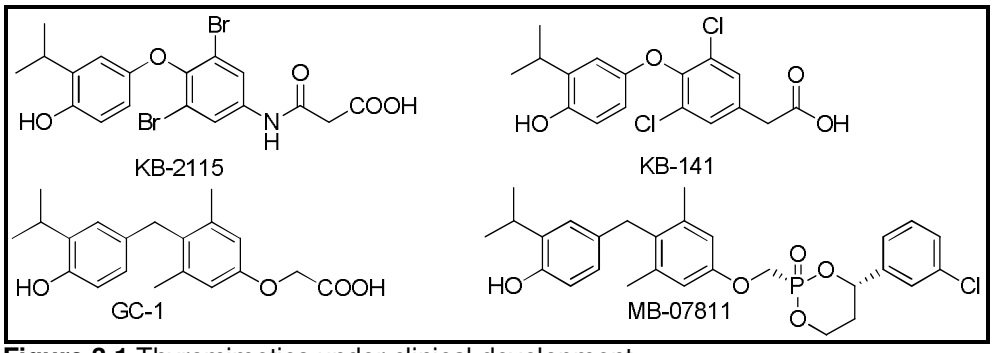


Figure 3.1 Thyromimetics under clinical development

Thyromimetics can negatively regulate TSH secretion which ultimately leads to suppression of TSH levels and decreases levels of endogenous T3, this action is mediated by TR*β* distributed in the pituitary. To avoid TSH suppression,

thyromimetics having liver selectivity could be a desirable approach. Thyromimetics that would selectively be transported into the liver will consequently avoid TR activation in the pituitary or in the heart [16], as a result liver selective compounds may not suppress the thyroid hormone axis (THA). Subsequently, Metabasis developed liver-targeted TR agonists, they were expected to retain lipid lowering effects but would have caused fewer side effects in extra hepatic tissues. MB-07811 (Figure 3.1) was developed as liver selective phosphonate prodrug which ultimately delivered targeted phosphonic acids molecule to liver after hepatocyte-specific cleavage of the prodrug [17]. Phosphonate prodrugs generates the free methyl phosphonic acid which limits the use of this prodrugs due to safety reasons. Improved therapeutic index achieved by selective TRβ agonists can be devoid of cardiac toxicities but it may have effects on TRβ mediated suppression of the THA in the pituitary. On the other hand thyromimetics having β isoform selectivity incorporated with liver selectivity could be devoid of cardiac toxicity and may not suppress THA. Therefore, our aim was enhancing activation of thyroid receptor beta in liver preferably without prodrug approach.

3.1.1 SAR with respect to the structure of T3/T4

For Thyroid receptors, T3 (Triiodothyronine) is endogenous ligand but therapeutic use of T3 is limited by its non selectivity towards thyroid receptors. Structurally T3 is a diphenyl ether derivative having alanine group at the acidic side chain position (Figure 3.2).

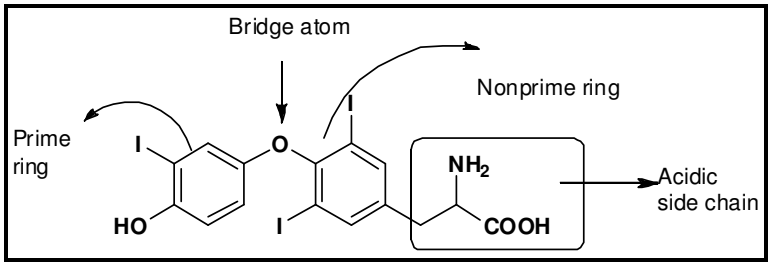


Figure 3.2 Structure of Triiodothyronine (T3)

Initially a large number of analogs were designed based on scaffold of T3. For designing of novel molecules it is very essential to know the SAR trend derived from the literature. Broadly thyroid ligands comprises of four main components.

(1) Acidic side chain

The naturally occurring hormones are biosynthesized from L-tyrosine which has acidic side chain. This acidic side chain is mainly responsible for the potency. Arg 282,316 and 320 of Ligand binding domain (LBD) forms a highly cationic environment, thus receptor structural demands acidic group. Carboxylic acids are favored as it forms H- bond with these aminoacids [18].

(2) Non-Prime ring

The phenyl ring bearing the acidic side chain is called as nonprime ring. Symmetric, lipophilic groups, not exceeding the size of iodine, like halogen and lower alkyl groups is required for activity. In general Me< Cl < Br < I substitution on prime ring increases activity [10]. Removal of groups on 3 and 5 position gives inactive compounds.

(3) Prime (Phenolic) ring

The phenolic ring, also called the prime ring is required for hormonal activity. Phenolic group at the 4-position is playing crucial role for biological

activity. Hydrogen bond between the phenolic hydroxyl group and His 435 in the receptor is important for binding as well as functional activity [18]. Replacement of the 4-hydroxyl with an amino group NH_2 results in a substantial decrease in activity, presumably because of weak hydrogen bonding ability of the NH_2 group. Substitution to ortho to phenolic ring have dramatic effects on biologic activity and the affinity for the nuclear receptor. There is direct relation to bulk and lipophilicity of the substituent, e.g., F < Cl < Br < I and Me < Et < *i*-Pr increases activity. Higher the bulk at *ortho* to phenol gives better isoform selectivity and groups with larger bulk can be accommodated to some extent without dramatic reduction in binding affinity [19].

(4) Bridging Atom

The oxygen atom between prime and nonprime ring which forms diphenyl ether is called bridging atom. The biphenyl analog of thyroxine, formed by removal of the oxygen bridge, is inactive. The bridging atom brings perpendicular orthogonal relationship between the two aromatic rings which is important for the active conformation. To the structure of T3 replacement of the bridging oxygen atom by S or by a methylene group CH_2 produces active analogs. In general two class of compounds are reported in literature as thyromimetics.

1.Thyromimetics based on T3 (diphenyl ethers)

2.Thyromimetics incorporated with heterocycle

3.1.2 Thyromimetics based on T3 (diphenyl ethers)

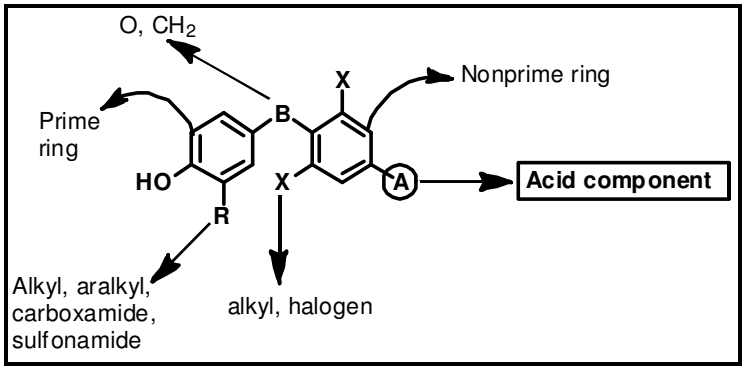


Figure 3.3 Simplified topology of typical Thyromimetics

Thyromimetics developed prior to 1990 were based on the scaffold of T3. Modifications were carried out at the *ortho* to phenol substitution, bridge atom and and at the acidic side chain (Figure 3.3). Preferred substitutions at *ortho* to phenol are alkyl, aryl, carboxamides and sulfonamides and oxy acetic acid, phenyl acetic acid at acidic side chain. Most favorable substitution on nonprime ring is methyl or halogen. Group like methylene or oxygen atom are used at bridge. Progressing compounds like Eprotirome (KB-2115) and Sobetirome (GC-1) falls in similar class of molecules (Figure 3.4) [9,15].

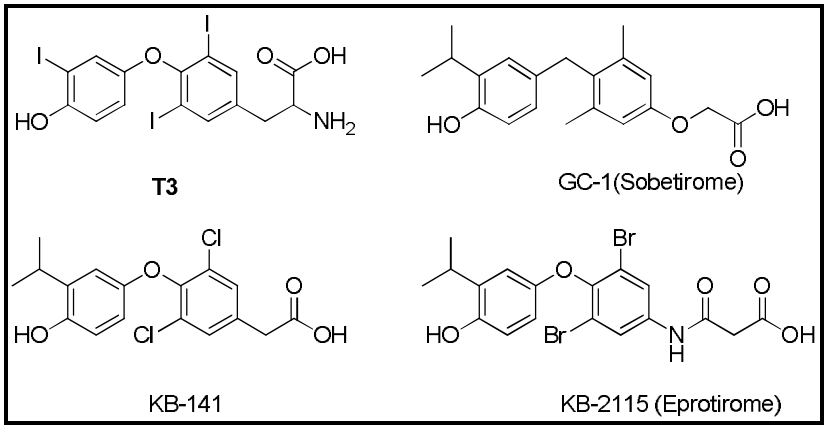


Figure 3.4 Thyromimetic optimized from structure of T3

3.1.3 Heterocyclic thyromimetics

There has been numerous examples of classical bioisosterism known in drug discovery. Replacement of Phenyl ring with heterocycles is typical example of classical bioisosterism, similar variations were also tried for the development of thyromimetics. One of the phenyl ring was replaced by heterocyclic ring like indoles, benzofuran, benzimidazole (Figure 3.5) [20-22].

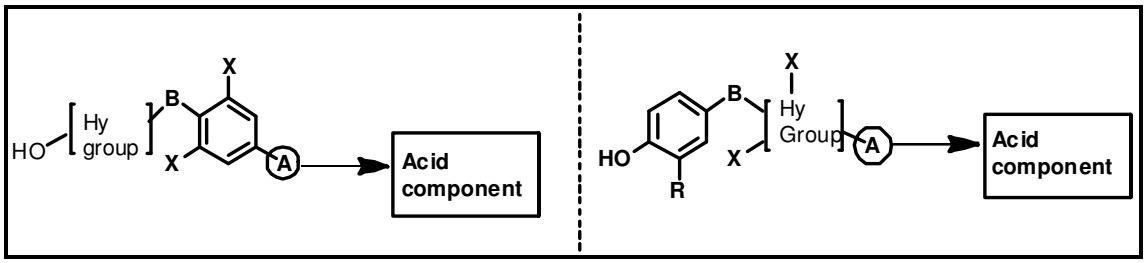


Figure 3.5 Heterocyclic thyromimetics

Like diphenyl ethers, all the possible appropriate variations were explored for heterocyclic thyromimetics. So far none of the progressing molecule belongs to this class.

3.2 Squaric acid derivatives

3.2.1 Rationale

Our goal was to target TRβ selectivity with an added advantage of liver selectivity. Structures of T3, KB-141 and GC-1 were taken as starting point. Modifications at acidic side chain were planned while keeping rest of the structural features intact.

For several class of compounds liver selectivity is desirable and structural features which drives the liver selectivity are known to a certain extent. Statin (HMG-CoA reductase inhibitors) class of compounds are well known to have liver selectivity [23]. Organic anion transporters are highly expressed in liver, thus It is

possible that highly charged molecules could be liver specific. Working on this hypothesis increase in polar substituent resulted in analogs with significantly increased cellular hepato selectivity is reported for statins [24]. To work on this concept we kept diphenyl ether part intact and altered at acidic portion. Interestingly, squaric acid is rendered as an acid part and this four membered ring system can form up to four H-Bonds. Squaric acid is an organic acid with polyfunctionality. It consists of two hydroxyl and two carbonyl groups conjugated across a double bond. Peculiar hydrogen-bonded structures in some acid derivatives have been explored as bioisosters of acid functionality [25].

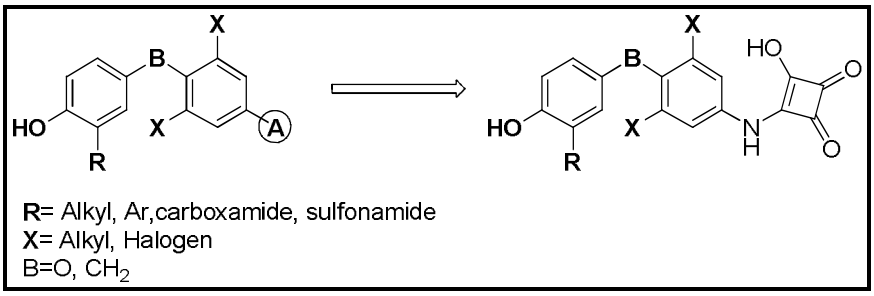


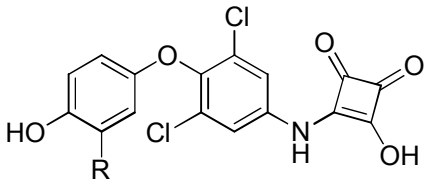
Figure 3.6 Rationale squaric acid derivatives

As it is shown in Figure 3.6 we picked out squaric acid as an alternative acidic side chain, we speculated that it will increase polarity of molecule. Proposed substitution for *ortho* to phenol were Alkyl, Aryl, carboxamide and sulfonamides, which are known substitutions of typical thyromimetic compounds. Before execution docking study of the proposed series with TRβ and TRα ligand binding domain was carried out. The molecular modeling part is covered in the later part of the chapter. Based on this novel pharmacophore, we worked on three different schemes for synthesis.

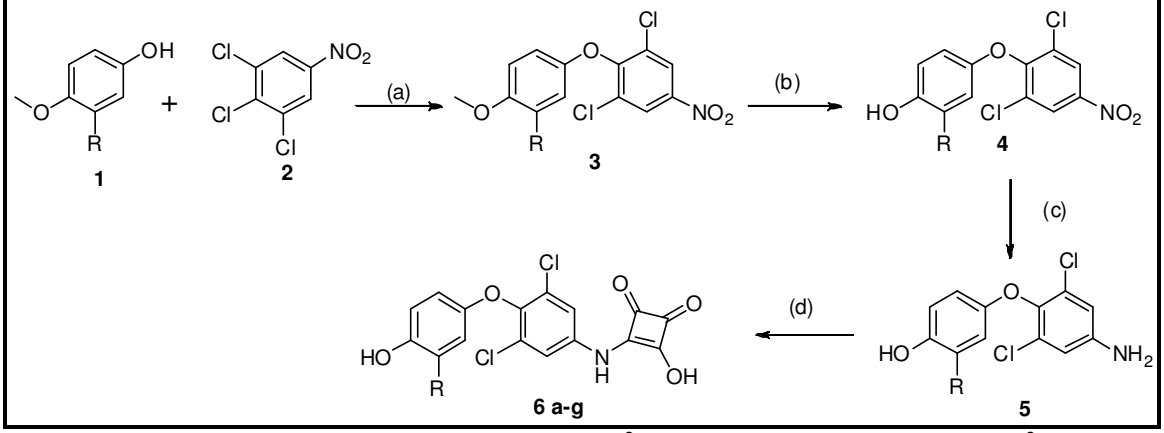
3.2.2 Synthesis of squaric acid derivatives

Scheme 3.1

R=Alkyl, Aryl



Scheme 3.1

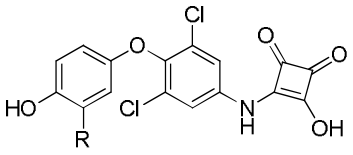


Reagents and conditions : (a) K_2CO_3, DMF, 120 ^oC, 3-4 h (b) 1M BBr_3, DCM, 25 ^oC, 3 h (c) $SnCl_2.2H_2O$, Conc.HCl, EtOH, 65-70 ^oC, 3 h (d) $C_4H_2O_4$, H_2O, 100 ^oC, 4-6 h

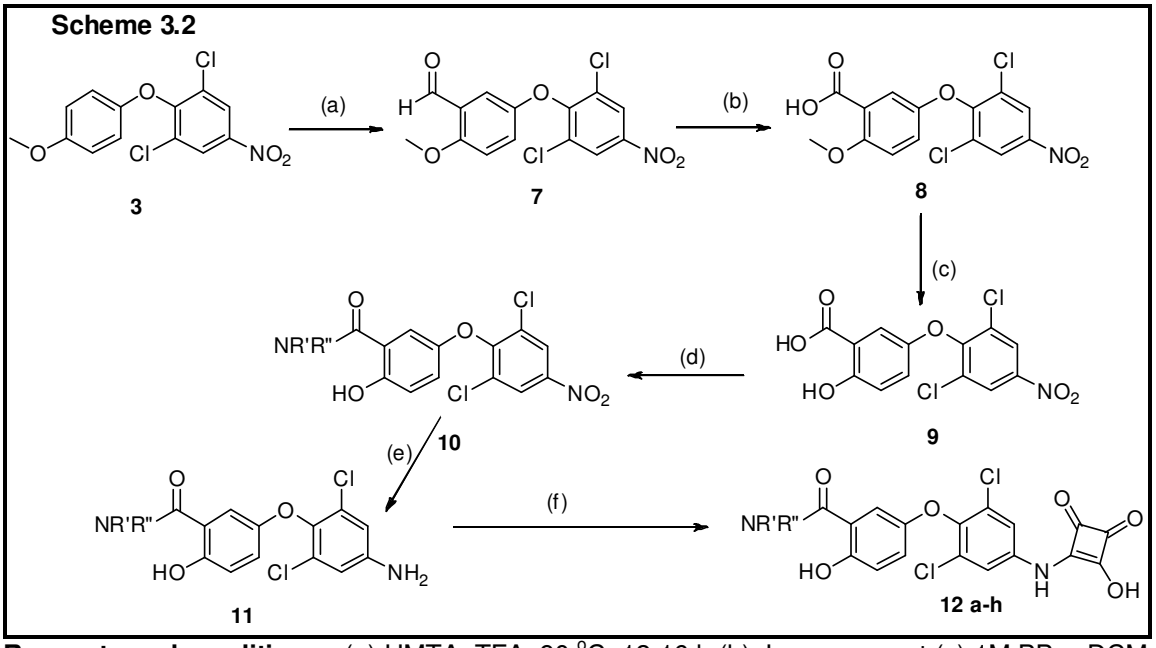
The synthesis of compounds **6** a-g (where *ortho* to phenol substitution (R=Alkyl, Aryl) has been outlined in Scheme 3.1.Phenol derivatives **1** were reacted with 1,2,3-trichloro-5-nitro benzene **2** using K_2CO_3 as base to afford nitro substituted diphenyl ether derivatives **3**. The demethylation of methoxy group from nitro-diphenyl ether derivatives **3** was achieved using solution of boron tribromide (BBr_3) to afford intermediate **4**, subsequently it was reduced using tin chloride in dil.HCl to get amino derivatives **5**. The resulting amine **5** were further reacted with 3,4-dihydroxy-3-cyclobutene 1,2-dione (squaric acid) to afford squaric acid derivatives **6 a-g**.

Scheme 3.2

R= Carboxamide



Scheme 3.2



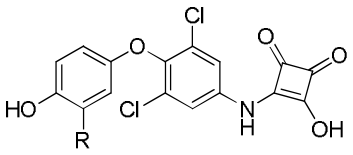
Reagents and conditions : (a) HMTA, TFA, 90 ^{o}C, 12-16 h (b) Jones reagent (c) 1M BBr_3, DCM, 25 ^{o}C, 3 h (d) R'R"NH, EDCI, HOBT, DMF, 20-30 ^{o}C, 3-5 h (e) $SnCl_2.2H_2O$, Conc.HCl,EtOH, 65-70 ^{o}C, 3 h (f) $C_4H_2O_4$, H_2O, 100 ^{o}C 4-6 h

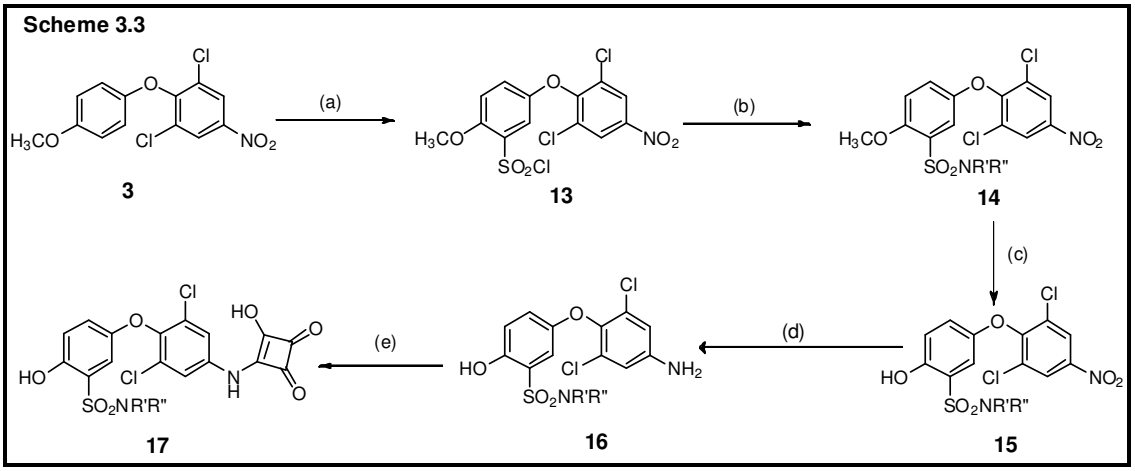
Synthesis of compounds **12** a-h (where *ortho* to phenol substitution R=carboxamide) has been outlined in Scheme 3.2. Formylation of intermediate **3** (scheme 1) was carried out by Somlet reaction using HMTA (Hexamethylene tetramine) in trifluoro aceticacid (TFA) to afford aldehyde derivative **7**, Oxidation of **7** was carried out by Jones reagent to afford acid compound **8** and methoxy group of **8** was deprotected by 1M BBr_3 to afford intermediate **9**. It was then coupled with respective amine using standard acid-base coupling reagents [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] hydrochloride (EDCI) EDCI, 1-hydroxybenzotriazole (HOBt) to afford amide **10**. Subsequently amide **10** was

reduced using tin chloride in dil.HCl to get amino derivatives **11**. Finally amine **11** were further reacted with 3,4-dihydroxy-3-cyclobutene-1,2-dione to afford squaric acid derivatives **12** a-g.

Scheme 3.3:

R= Sulfonamide



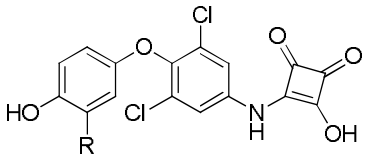


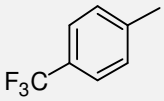
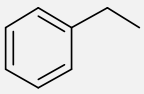
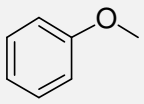
Reagents and conditions : (a) ClSO3H, 0-5 ^{o}C, 2-4 h (b) R'R"NH, DCM, triethylamine, 20-25 ^{o}C, 2-3 h (c) 1M BBr_3, DCM, 25 ^{o}C, 3h, 20-50%.(d) $SnCl_2.2H_2O$, Conc. HCl,EtOH, 65-70 ^{o}C, 3h (g) $C_4H_2O_4$, H_2O, 100 ^{o}C 4-6 h

Synthesis of compounds **17 a-c** (where *ortho* to phenol substitution R=sulfonamide) has been outlined in Scheme 3.3. In the first step Chlorosulfonation of **3 (** scheme 3.1) was carried out using chlorosulfonic acid which afforded intermediate **13**. It was then reacted with respective amine to form sulfonamide **14**. Methoxy group was deprotected from sulfonamide **14** to afford **15** which was reduced using tin chloride in dil.HCl to get amino derivatives **16**. Finally amine **16** were further reacted with 3,4-dihydroxy-3-cyclobutene- 1,2-dione to afford squaric acid derivatives **17** a-c.

3.2.3 Result and Discussion

Table 3.1 : *In vitro* activity of novel 3-hydroxy-cyclobut-3-ene-1,2-dione compounds 6a-g for TRα and TRβ with respect to T3



Compound	R	Conc. (nM)	%Activity TRα [a]	%Activity TRβ [a]
6a	H	10	6.12	14.60
		100	6.08	15.10
		1000	9.36	30.90
6b		10	22.36	30.00
		100	35.04	70.00
		1000	79.84	130.00
6c		10	15.30	14.20
		100	30.70	53.50
		1000	61.50	107.10
6d		10	08.10	10.20
		100	12.20	22.10
		1000	14.50	54.20
6e		10	07.10	12.60
		100	10.04	25.30
		1000	32.20	48.60
6f		10	9.63	08.30
		100	13.64	29.00
		1000	45.48	62.30
6g		10	7.23	8.96
		100	7.89	9.14
		1000	27.22	71.49
KB-141		10	79.20	114.58
		100	125.10	123.94
		1000	95.69	104.20

[a] The TRE-luciferase assay has been used for the *in vitro* TRα and TRβ activities and values are mean of duplicate measurements and expressed as % fold induction WRT to T3 (100 nM).

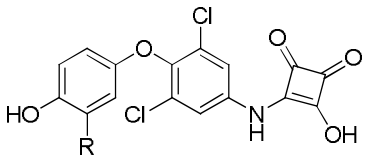
In vitro % TRα and TRβ activities of 3-hydroxy-cyclobut-3-ene-1,2-dione (squaric acid) derivatives 6a-g at different concentrations such as 10 nM, 100 nM

and 1 μM were evaluated with respect to T3 (Table 3.1) keeping KB-141 as a positive control.

First compound synthesized was without substitution at *ortho* to phenol position to confirm the significance of having substitution at *ortho* to phenol. As expected compound **6a** without substitution at *ortho* to phenol was not active. Based on literature data we assume that by increasing bulk at *ortho* to phenol increases TRα/β selectivity. Concurrently activity of various substitutions at *ortho* to phenol also depend on the acidic side chain. Following typical SAR reported in literature for *ortho* to phenol substitution, compounds having alkyl substitution like *i*-Pr **6b**, *t*-Bu **6c** were synthesized. **6b** and **6c** gave the encouraging *in vitro* activity pattern for TRα and TRβ at the concentration of 100 nM and 1μM (Table 3.1). The *t*-Bu derivative **6c** was found to be equipotent to *i*-Pr derivative **6b**. Encouraged with the *in vitro* activity of **6b** and **6c** more bulkier groups like Ph **6d** and 4-CF_3-Ph **6e** were screened and they were found to be inactive in both TRα and TRβ. Further, the benzyl derivative **6f** and phenoxy derivative **6g** were found to be inferior to the *i*-Pr derivative **6b** and the *t*-Bu **6c**.

Based on this fold activation data, potent compounds **6b** and **6c** were selected for screening at multiple concentrations to determine EC_{50} value which is the concentration of the test compound that exhibits half-maximum transactivation activity. (Table 3.2). To know the isoform selectivity EC_{50} value was calculated for both TRα and TR β.

Table 3.2 : *In vitro* EC_{50} for selected compounds 22b and c for TR*α*, TR β

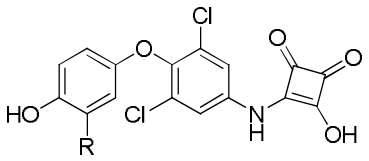


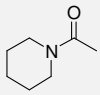
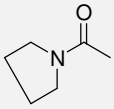
Compound	R	EC_{50} TRα(□nM□)[a]	EC_{50} TRβ□□(□nM□)[a]	EC_{50} TRα/β
6b	*i*-Pr	222.00	70.00	3.00
6c	*t*-Bu	1056.00	317.00	3.33
KB-141	----	4.5	1.14	3.8

[a]The TRE-luciferase assay has been used for the *in vitro* TR*α* and TRβ activities Both the compounds **6b** and **6c** exhibited less potency compare to KB-141. In terms of selectivity for TRα and TRβ both the compounds were comparable (3 folds) with KB-141.

It is reported by group of scientists at Pfizer [26] that carboxamides and sulfonamides at *ortho* to phenol enhanced selectivity as well as potency having azauracil ring at acidic side chain. Based on these findings it was worth looking to evaluate similar substitutions in order to improve potency and TRβ selectivity of squaric acid derivatives. Compounds having carboxamides at *ortho* to phenol were planned with squaric acid as acidic side chain. Cyclic carboxamides and noncyclic amine carboxamide derivatives (formed by primary and secondary amines) were designed to evaluate *in vitro* activity. This led us to synthesize amides **12 a-d**. The *in vitro* fold activation data of the compounds **12 a-d** are presented in Table 3.3. Overall the carboxamide derivatives **12a-d** failed to show TR*α* or TRβ activity as compared to potent TRβ selective compound KB-141 even at the concentration of 1 μM (Table 3.3).

Table 3.3 : *In vitro* activity of novel 3-hydroxy-cyclobut-3-ene-1,2-dione compounds 12a-d for TRα and TRβ with respect to T3



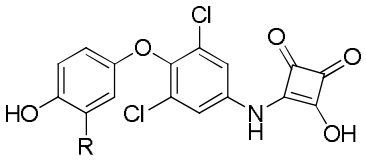
Compound	R	Conc. (nM)	% Activity TRα[a]	% Activity TRβ[a]
12a		10 100 1000	5.63 5.96 5.25	5.63 5.30 6.23
12b		10 100 1000	8.56 9.62 50.29	8.60 10.60 80.70
12c		10 100 1000	7.35 7.37 13.45	8.50 9.00 23.20
12d		10 100 1000	6.93 7.91 11.03	13.00 12.70 30.90
KB-141		10 100 1000	79.20 125.10 95.69	114.58 123.94 104.20

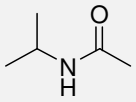
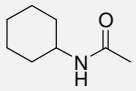
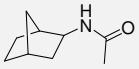
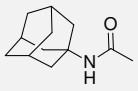
[a] The TRE-luciferase assay has been used for the *in vitro* TRα and TRβ activities and values are mean of duplicate measurements and expressed as % fold induction WRT to T3 (100 nM).

The N,N-dimethyl carboxamide derivative **12c** exhibited mild activity at 1 μM concentration for both the TR*α* and TR*β*. The pyrrolidinyl carboxamide derivative **12b** remained inactive at lower concentrations of 10 nM and 100 nM, however, the improved activity was seen at 1 μM concentration (50% for TR*α* and 80% for TR*β*), Changing the ring size of **12b** from five to six membered, piperidinyl carboxamide derivative **12a** showed diminished activities for both TRα and TRβ. The carboxamide with one more heteroatom, the morpholinyl carboxamide **12d** remained inactive against thyroid receptor luciferase assay. As reported by Pfizer scientists bulkier amine gave the highest selectivity, in

another set carboxamides **12e-h** with bulkier primary amines like norbornyl and adamentyl amine were also synthesized and screened for TR α and β activity (Table 3.4).

Table 3.4 : *In vitro* activity of novel 3-hydroxy-cyclobut-3-ene-1,2-dione compounds 12e-h for TRα and TRβ with respect to T3



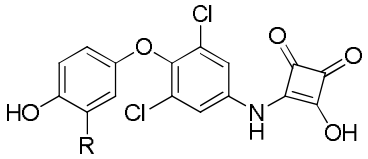
Compound	R	Conc. (nM)	% Activity TRα[a]	% Activity TRβ[a]
12e		10 100 1000	6.16 6.94 11.70	7.015 8.34 19.05
12f		10 100 1000	7.41 7.69 9.40	10.01 12.28 15.59
12g		10 100 1000	6.23 6.80 6.00	8.50 10.39 11.53
12h		10 100 1000	7.12 7.13 7.19	12.30 12.70 8.80
KB-141		10 100 1000	79.20 125.10 95.69	114.58 123.94 104.20

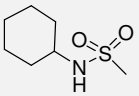
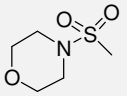
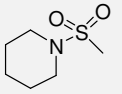
[a] The TRE-luciferase assay has been used for the *in vitro* TR-α and TR-β activities and values are mean of duplicate measurements and expressed as % fold induction WRT to T3(100 nM).

Unfortunately most of the carboxamide **12f-h** derivatives were not active. Having found carboxamides **12a-h** were detrimental to *in vitro* TRα and TRβ affinities, further we evaluated some of the sulfonamide derivatives **17a-c** in the same assay and compared with KB-141(Table 3.5). The cyclohexyl sulfonamide derivative **17a** showed similar trend of activity as that exhibited by its corresponding carboxamide derivative **12f**. Interestingly, the secondary

sulfonamide derivative **17b** showed mild activity at 1 μM concentration, however, it remained inferior to the KB-141. Similarly, the morpholinyl sulfonamide derivative **17c** showed weak activities at 1 μM concentration for both TRα and TRβ.

Table 3.5 : *In vitro* activity of novel 3-hydroxy-cyclobut-3-ene-1,2-dione compounds 17a-c for TRα and TRβ with respect to T3



Compound	R	Conc. (nM)	% Activity TRα[a]	% Activity TRβ[a]
17a		10 100 1000	6.28 6.57 16.72	12.00 13.60 48.10
17b		10 100 1000	8.96 10.55 22.86	15.70 24.70 43.30
17c		10 100 1000	8.56 9.83 32.28	12.80 32.50 63.80
KB-141		10 100 1000	79.20 125.10 95.69	114.58 123.94 104.20

[a] The TRE-luciferase assay has been used for the *in vitro* TRα and TRβ activities and values are mean of duplicate measurements and expressed in nM. The variability of the measurements is on average of 25%.

Docking studies

The X-ray crystallographic structure of the KB-141 (2) with TRα (PDB Code:1NAV) and TRβ (PDB Code: 1NAX) was selected for the docking study. Docking was performed for compounds using ArgusLab 4.030 in the presence of water molecules. Hydrogens and charges were added to the ligand and receptor using modules from DS Studio 2.0 (Accelrys, Inc.). The binding site was defined

from the coordinates of the ligand in the PDB file. Argusdock exhaustive search docking engine was used, with grid resolution of 0.20 Å. Five hundred docking runs were used for each compound. Docking precision was set to 'high precision' and 'flexible ligand docking' mode was employed for each docking run. Docked conformers for each compound were ranked and scored using Ascore31 implemented in ArgusLab. The interaction of the top ranked docked poses in the pocket were visualized using DS Visualizer 1.7 (Accelrys, Inc.). The X-ray crystallographic structures of the ligand binding domains (LBD) of TRα1 and TRβ1 determined in complex with Thyromimetics are accessible from Protein Data Bank (PDB).18,24–26,28,29.

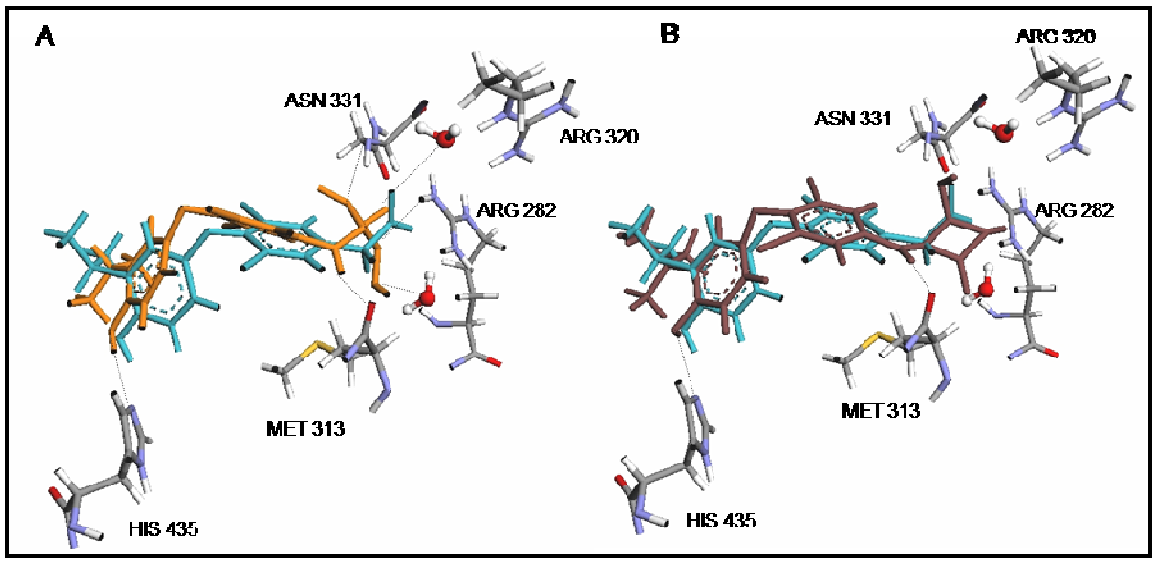


Figure 3.7 Docking of 6b and 6c in ligand binding pocket of TRβ including water molecules. Residues and ligands are shown as sticks. Hydrogen bonds are represented by dotted lines.

The hormone binding pocket is very similar in both the receptors. These receptors differ only by one single amino acid residue,Asn331 in TRβ, which is substituted by Ser277 in TRα. This results in a significantly different hydrogen

bonding patterns between the acidic group of the ligands and the LBD of the two receptors and hence accounts for selectivity (Figure 3.7).

The oxygen atoms in compound **6b** (shown in orange) makes hydrogen bonding with the side chains of residues His 435, Arg 282 and Asn 331 including water molecules. The amide linkage makes hydrogen bond with the backbone of Met 313 . Compound **6c** shown in magenta makes similar interactions as compound 6b. The docked conformers **6b** and **6c** are superimposed on 3,5-dichloro-4-[(4-hydroxy-3-isopropyl phenoxy) phenyl]acetic acid (shown in cyan as sticks). Molecular docking studies indicated that compounds **6b** and **6c** make similar interactions in the LBD of TRα and TRβ as it is reported in literature [10].

To summaries, a rational structure activity relationship for 3-hydroxy-cyclobut-3-ene-1,2-dione derivatives as thyroid hormone receptor β culminated to compounds **6b** and (**6c**) which exhibited similar selectivity for TRα and TRβ as that of shown by (KB-141). These results could demonstrate the viability of our approach towards developing novel TRβ agonists . Encouraged by *in vitro* data our next goal was to find bioavailibility as well as liver selectivity of **6b** and **6c**. Unfortunately both the compounds were not orally available when subjected to oral dose for Pharmacokinetic evaluation.

3.3 Oxo acetic acid derivatives

3.3.1 Rationale

Polar substituent can influence liver selectivity because of the involvement of organic anion transporters, keeping this hypothesis in mind squaric acid derivatives were explored. Despite the promising *in viro* profile of squaric acid

derivatives, it failed to show desirable pharmacokinetic profile. Therefore, further modification to squaric acid was needed. Acidic side chain can be varied without significant loss of affinity for the TR, which is reflected in the number of published papers. Hence we looked for some suitable modification to squaric acid.

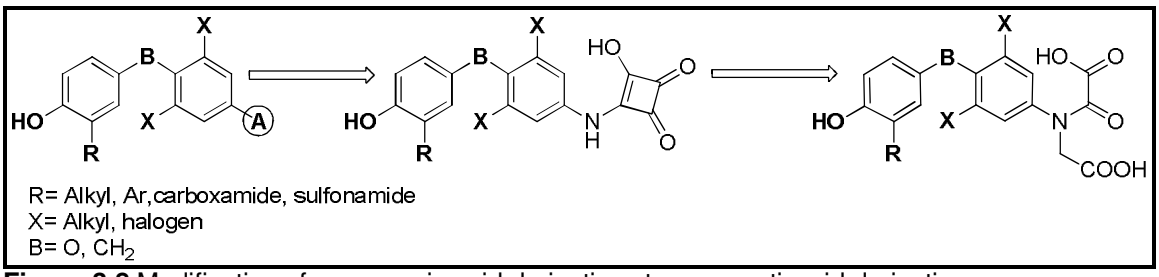
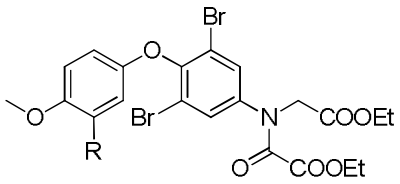


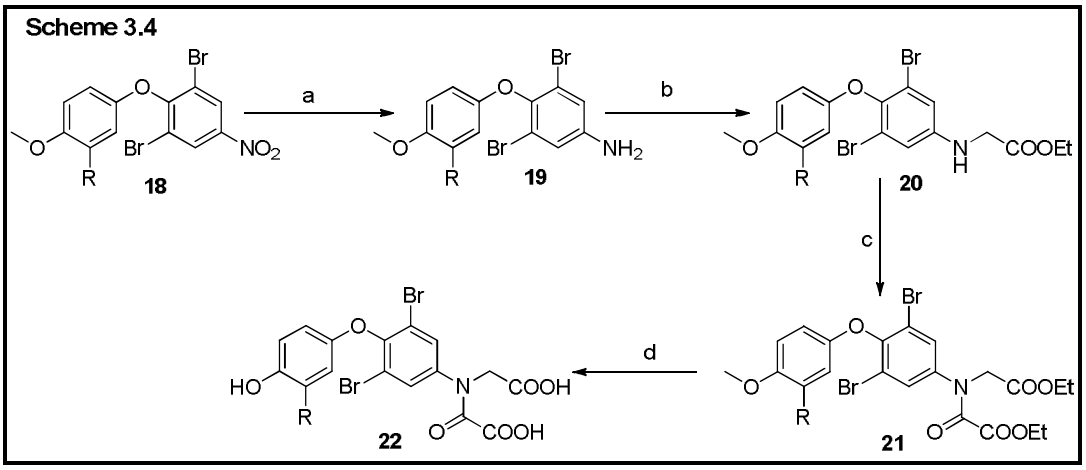
Figure 3.8 Modifications from squaric acid derivatives to oxo-aceticacid derivative

As depicted in Figure 3.8 modification from squaric acid to oxo-acetic acid derivatives was done. Literature [10] supports that dibromo compounds are more potent than dichloro substitution on non-prime ring. Eprotirome (KB-2115) which is under clinical development is having dibromo substitution on non prime ring. We applied the same theory to newly modified scaffold and decided to keep dibromo substitution to newly designed oxo-acetic acid analogs. Synthetic scheme of oxo-acetic acid derivatives is outlined in scheme 3.4.

3.3.2 Synthesis of oxo acetic acid derivatives

Scheme 3.4





Reagents and conditions : (a) $SnCl_2.2H_2O$,Conc.HCl,EtOH, 65-70 ^oC,3h(b) $BrCH_2COOEt$,DMF, DIPEA,135 ^oC, 14-16 h (c) Pyridine, ClCOCOOEt, ,DCM, 24h, 20-25 ^oC (d) 1M BBr_3, DCM, 25 ^oC, 3h

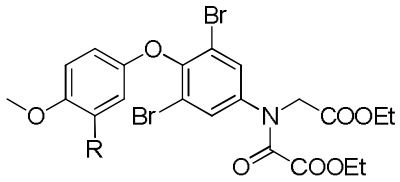
Compounds (**22a-c**) were synthesized according to the Scheme 3.4. Intermediate **18** was reduced with $SnCl_2.2H_2O$ in dil.HCl gave amine of formula **19**. The amine **19** was reacted with Ethyl bromoacetate in presence of base diisopropyl ehtylamine (*i*-Pr_2-NEt) in Dimethylformamide (DMF) afforded compound of formula **20**, Ester compound **21** were synthesized by acylation of **20** with ethyl oxalylchloride in presence of pyridine as base. Finally hydrolysis and deprotection of **21** gave the compounds **22a-c**.

3.3.3 Result and discussion

The new oxo-aceticacid derivatives **22a-c** were evaluated for their *in vitro* TRβ activity. With our experience with thyromimetics less bulkier substitution at *ortho* to Phenol were explored first to find out prospects of new acidic side chain

[27]. Selected compounds **22a-c** having less bulkier substitution like *i*-pr **22a** and **22b** *sec*-Bu were synthesized to start with and further increased bulk to Phenyl **22c**. *In vitro* activity of the said compounds is depicted in the Table 3.6.

Table 3.6 : *In vitro* activity of oxo acetic acid derivatives 22a-c for TR-α and TR-β with respect to T3.



Compound	R	Conc. (nM)	%Activity TRα[a]	%Activity TRβ[a]
22a		10	12.11	16.50
		100	25.38	43.32
		1000	60.21	75.85
22b		10	13.61	15.49
		100	30.58	57.19
		1000	91.02	110.24
22c		10	24.17	49.65
		100	17.55	39.88
		1000	27.19	90.35
KB-141		10	79.20	114.58
		100	125.10	123.94
		1000	95.69	104.20

[a] The TRE-luciferase assay has been used for the *in vitro* TRα and TRβ activities and values are mean of duplicate measurements and expressed in nM. The variability of the measurements is on average of 25%.

i-Pr **22a** and sec.Bu **22b** substituted compounds were potent as compare to Ph substituted analogue **22c**. Although **22c** was selective for TRβ as % activity for TRα was very low even at 100 nM, we moved further for EC_{50} determination with **22b** because it was more potent than **22c**(Table 3.7).

Table 3.7 *In vitro* EC_{50} for selected compounds **22b** for TR-α, TR-β

Compound	EC_{50} TRα(□nM□)[a]	EC_{50} TRβ□□(□nM□)[a]	EC_{50} TRα/β
22b	436	74.00	5.89
KB-141	4.5	1.14	3.8

[a]The TRE-luciferase assay has been used for the *in vitro* TRα and TRβ activities and values are mean of duplicate measurements and expressed in nM. The variability of the measurements is on average of 25%.

It appeared from EC_{50} results that TR isoform selectivity of **22b** is better than KB-141. Combining these results pharmacokinetic behavior of **22a-c** was studied in SD rats and the results are summarized in Table 3.8. Results indicate that modification from squaric acid to Oxo-acetic acid series has turned out to be improved towards PK profile of the **22a-c** (Table 3.8).

Table 3.8 : Pharmacokinetic results of selected compounds **22a** and **22b**

Compound	% F	Cmax[a]	AUC 0-inf[b]	Eh
22a	1.74	73.93	144.05	0.17
22b	3.03	69.95	356.93	0.18
22c	1.67	59.1	188.7	ND

[a]10mg/kg, P.O. (ng/mL), [b]10mg/kg, P.O. (hr.ng/mL) %F:Oral Bioavailibility,Eh:Hepatic extraction, ND: Not determined

All the three compounds **22a-c** showed plasma levels ranging from 150-300 ng/mL. There is indication of liver selectivity as hepatic extraction ratio (Eh) is close to 0.2. The bioavailibility of **22a-c** is less than 5 %, this can be explained as compound has trend towards liver selectivity so it could be less available in systemic circulation.

Encouraged with PK results, compound **22b** was subjected for *in vivo* efficacy as the AUC and %F of **22b** was better than **22a** and **22c**. Compound **22b** was tested for Cholesterol lowering effect in SD rats at 3 mg/kg dose and it was compared with T3. The results are presented in Table 3.9. The *in vivo* data clearly indicate that compound **22a** reduced total plasma cholesterol at 3 mg/kg in SD rats which is comparable to T3.

Table 3.9 : *in vivo* efficacy of **22a**

Compound	%Change in Cholesterol	% Change in T4	% change in Heart Wt.
22b	-42.97	-18.68	-6.92
T3 (13 µg/kg)	-51.49	-51.63	23.65

Because the compound is preferentially distributed in liver, the related adverse effect like % change in T4 and % change in Heart wt is better than T3 treated animals. These results indicate that targeting thyromimetics to the liver has the potential to lower cholesterol levels with an acceptable safety profile. It also demonstrate the viability of our approach towards developing novel liver selective thyromimetics.

The objective of this work was to design thyromimetics having novel scaffold which can be explore for liver selectivity. One of the approach to have liver selectivity is to incorporate polar groups in the structure. We thought of accommodating polar groups on the acidic side chain part and we found structural features of squaric acid which resemble carboxylic function [25] which could enhance polar groups. This remarkable four-membered ring system can form up to four hydrogen bonds. Concomitant increase in aromaticity of the ring

drives the high affinity for H-bonding. This H-bonding and aromatic switching of squaric acid have been exploited in many of the applications [28].

Novel squaric acid derivatives **6a-g** having alkyl substitution at *ortho* to phenol position were designed. The first set of molecules with these substitutions showed desire *in vitro* activity and TRβ selectivity as well. Exited by these findings further changes were made at *ortho* to phenol position without altering acidic side chain so it doesn't loses concept of having polar groups. Molecules having carboxamides and sulfonamide with azauracil ring at acidic side chain shows TRβ selectivity [26]. Anticipating similar results carboxamides **12a-h** and sulfonamide **17a-c** substitution at *ortho* to phenol position were planned. Surprisingly they turned out to be inactive. Finally, from this series **6b** and **6c** having *i*-Pr and *t*-butyl groups at ortho to phenol were further evaluated for TRα/β selectivity. The isoform selectivity (3X) was better than endogenous ligand T3 and comparable to KB-141. The squaric acid pharmacophore was therefore evaluated for Pharmacokinetic parameters. Unfortunately selected compounds were not bioavailable.

Disappointed by not having plasma levels of squaric acid derivatives, further modification were tried to the ensure that molecule should not lose activity as well as polarity. Hence oxo-acetic acid derivatives **22a-c** were synthesized and screened. Few molecules of the series were synthesized and after achieving in vitro potency and TRβ selectivity, compounds were selected for evaluation of pharmacokinetic parameters. Modifications of squaric acid derivatives to new oxo-acetic acid derivatives provided orally bioavailable

compounds and liver selectivity data (Eh) of **22a** and **22b** were encouraging. In this context, it may be contemplated that hypothesis of casting polar groups is imparting liver selectivity.

3.4 Conclusions

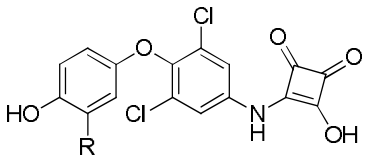
- Series of novel squaric acid derivatives were synthesized and evaluated for *in vitro* activity, Selected compounds showed better TRβ selectivity than endogenous ligand T3 and comparable selectivity to KB-141. Unfortunately compounds belongs to this series failed to show bioavailability.
- Further modified from squaric acid newly designed oxo-acetic acid derivatives were synthesized, Selected compounds showed better TRβ selectivity than KB-141. Compounds were bioavailable and showed trend towards liver selectivity.
- Finally, we have designed a novel pharmacophore as thyromimetics and synthesized compounds comprising this pharmacophore. The incorporation of this pharmacophore as liver selective has probably opened a new direction.

3.5 Experimental

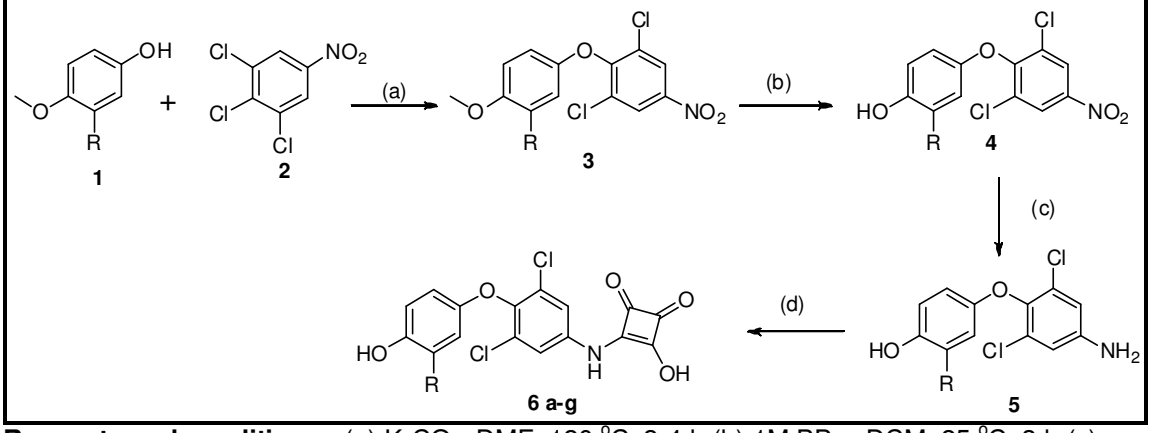
Materials and Methods

Reagents were obtained from Sigma Aldrich and 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 a scientific melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (λmax in cm-1, as film for liquids and as KBr pellets for solid compounds). The ^{1}HNMR spectra were recorded on a Bruker Avance-300 (300 MHz) or Bruker Avance-400 (400 MHz) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$, CD_3OD or DMSO-d_6. Signal multiplicities are represented as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). D_2O exchange experiments were carried out to confirm the exchangeable protons when present. Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer. HPLC analyses were carried out at λmax 220 nm using column ODS C-18, 150nm * 4.6 nm * 4 m on AGILENT 1100. Progress of the reactions was monitored by TLC using precoated TLC plates (E. Merck Kieselgel 60 F254) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (230–400 mesh).

3.5.1 Squaric acid derivatives (R=Ak, Ar)

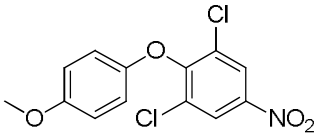


Scheme 3.1



Reagents and conditions : (a) K_2CO_3, DMF, 120 ^oC, 3-4 h (b) 1M BBr_3, DCM, 25 ^oC, 3 h (c) $SnCl_2.2H_2O$, Conc.HCl, EtOH, 65-70 ^oC, 3 h (d) $C_4H_2O_4$, H_2O, 100 ^oC, 4-6 h

3.5.1.1 Synthesis of 1,3-Dichloro-2-(4-methoxyphenoxy)-5-nitrobenzene (3a)



To a solution 4-methoxyphenol (8.0 g, 64.5 mmol) in DMF (40 mL) was added K_2CO_3 (13.35 g, 96.77 mmol) and 1,2,3 trichloro nitro benzene (14.61 g, 64.51 mmol). The reaction was stirred at 110-120 ^oC for 5 h. The reaction mixture was poured over ice. The product was extracted in ethyl acetate (2 x 80 mL), washed with water (2 x 80 mL), brine (80 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product, which was purified by column chromatography over flash silica gel (hexane : ethyl acetate, 90:10) to afford pure product.(18 g, 90 %)

Chemical Formula: $C_{13}H_9Cl_2NO_4$

Molecular Weight: 314.12

IR (KBr) cm^{-1}: 2831, 1351, 1252.

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.98(s, 3H), 6.75-6.79(m, 2H), 6.82-6.86(m, 2H), 8.29(s, 2H)

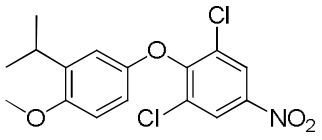
% Yield: 90 %

Purity by HPLC: 92.39 %

mp: 142-145 ^oC

Compounds 3b-g were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.1.2 1,3-Dichloro-2-(3-isopropyl-4-methoxyphenoxy)-5-nitrobenzene (3b)



Chemical Formula: $C_{16}H_{15}Cl_2NO_4$

Molecular Weight: 356.20

IR (KBr) cm^{-1} : 3413**,** 3091, 1602, 1535

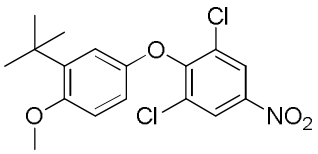
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17(d, *J* = 6.9 Hz, 6H), 3.24-3.31(m, 1H), 3.79(s, 3H), 6.43-6.47(dd, *J* = 3.0 Hz & 8.8 Hz, 1H), 6.70(d, *J* = 8.8 Hz, 1H), 6.83(d, *J* = 3.0 Hz, 1H), 8.29(s, 2H)

% Yield: 98%

Purity by HPLC: 95.72 %

mp: 128-131 ^oC

3.5.1.3 2-(3-(*t*-Butyl)-4-methoxyphenoxy)-1,3-dichloro-5-nitrobenzene (3c)



Chemical Formula: $C_{17}H_{17}Cl_2NO_4$

Molecular Weight: 370.23

IR (KBr) cm^{-1}: 3097, 2960, 1591, 1304

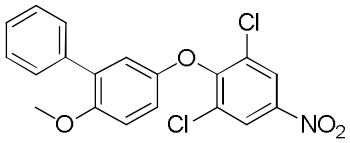
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53(s, 9H), 3.8(s, 3H), 6.42-6.46(dd, *J* = 3.0 Hz & 8.6 Hz, 1H), 6.71(d, *J* = 8.8 Hz, 1H), 6.95(s, 1H), 8.29(s, 2H)

% Yield: 95 %

Purity by HPLC: 78.5 **%**

mp: 88-92 ^oC

3.5.1.4 5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxy-1,1'-biphenyl (3d)



Chemical Formula: $C_{19}H_{13}Cl_2NO_4$

Molecular Weight: 390.22

IR (KBr) cm^{-1}: 3018, 1251, 1051

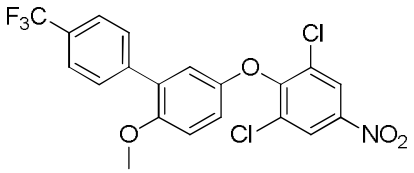
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.77(s, 3H), 6.76-6.79(dd, *J* = 3.2 Hz & 9.2 Hz, 1H), 6.82(d, *J* = 3.2 Hz, 1H), 6.90(d, J = 8.8 Hz, 1H), 7.32-7.34(m, 1H), 7.37-7.39(m, 2H), 7.47-7.49(m, 2H), 8.29(s, 2H)

% Yield: 33 %

Purity by HPLC: 89.9 **%**

mp: 98-102 ^oC

3.5.1.5 5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxy-4'-(trifluoromethyl)-1,1'-biphenyl (3e)



Chemical Formula: $C_{20}H_{12}Cl_2F_3NO_4$

Molecular Weight: 458.21

IR (KBr) cm^{-1}: 3413, 3093, 2929, 1533, 1240

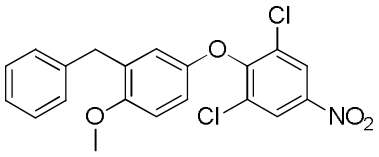
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.78(s, 3H), 6.75-6.83(m, 4H), 7.58-7.66(m, 3H), 8.29(s, 2H)

% Yield: 90 %

Purity by HPLC: 75%

mp: 85-90 ^oC

3.5.1.6 2-(3-Benzyl-4-methoxyphenoxy)-1,3-dichloro-5-nitrobenzene (3f)



Chemical Formula: $C_{20}H_{15}Cl_2NO_4$

Molecular Weight: 404.24

IR (KBr) cm^{-1}: 3085, 2831, 1598, 1535

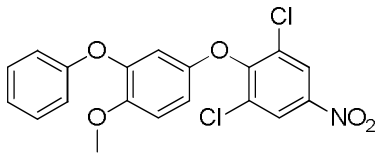
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.77(s, 3H), 3.95(s, 2H), 6.53-6.57(dd, J = 3.0 Hz & 8.7 Hz, 1H), 6.58(m, 1H), 6.64-6.65(d, J = 8.7 Hz, 1H),7.16-7.19(m, 3H), 7.24-7.29(m, 2H), 8.28(s, 2H)

% Yield: 96%

Purity by HPLC: 95.7 %

mp: 112-114 ^{o}C

3.5.1.7 1,3-Dichloro-2-(4-methoxy-3-phenoxyphenoxy)-5-nitrobenzene (3g)



Chemical Formula: $C_{19}H_{13}Cl_2NO_5$

Molecular Weight: 406.22

IR (KBr) cm $^{-1}$: 3423, 3076, 1589, 1205

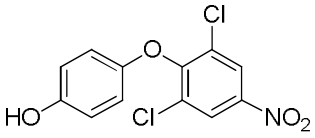
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.06(s, 3H), 6.49-6.53(dd, J = 3.0 Hz & 6.0 Hz, 1H), 6.89-6.97-7.05(m, 3H), 7.05-7.1(m, 2H), 7.26-7.34(m, 2H), 8.26(s, 2H)

% Yield: 38%

Purity by HPLC: 95.4%

mp : 122-125 ^{o}C

3.5.1.8 Synthesis of 4-(2,6-Dichloro-4-nitrophenoxy)phenol (4a)



Methoxy compound **3a** (2.5 g, 7.9 mmol) in Dichloromethane (DCM) (12.5 mL) was cooled to -60 ^{o}C under nitrogen atmosphere. To that 1M BBr_3 solution (15.92 mL) was added dropwise. The reaction mixture was allowed to warm up to 20-25 ^{o}C and stirred for 5 h. Further it was diluted with more DCM (25 mL) and quenched with water (15 mL). After stirring at 20-25 ^{o}C for 30 min, organic phase was separated, washed with water (2 x 15 mL), brine (15 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated to give crude product. The crude

product was purified by column chromatography over flash silica gel (Hexane : ethyl acetate, 90:10) to afford the pure product. (2.2 g, 93 %)

Chemical Formula: $C_{12}H_7Cl_2NO_4$

Molecular Weight: 300.09

IR (KBr) cm^{-1}: 3462, 2923, 1506

^{1}H NMR (300 MHz, CDCl$_3$): δ6.70-6.79(m, 4H), 8.29(s, 2H)

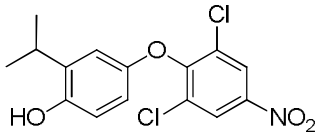
% Yield: 93%

Purity by HPLC: 94.46 %

mp: 118-120 ^oC

*Compounds 4b-g were prepared in an analogous manner using appropriate starting materials and the process described above***.**

3.5.1.9 4-(2,6-Dichloro-4-nitrophenoxy)-2-isopropylphenol (4b)



Chemical Formula: $C_{15}H_{13}Cl_2NO_4$

Molecular Weight: 342.17

IR (KBr) cm^{-1}: 3375, 3020, 1215

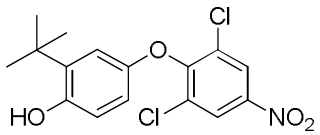
^{1}H NMR (300 MHz, CDCl$_3$): δ 1.21-1.24(d, *J* = 6.9 Hz, 6H), 3.13-3.22(m, 1H), 6.38-6.42(dd, *J* = 3 Hz & 8.7 Hz, 1H), 6.63-6.66(d, *J* = 8.7 Hz, 1H), 6.8(s, 1H), 8.29(s, 2H)

% Yield: 98%

Purity by HPLC: 95.56 %

mp: 105-110 ^oC

3.5.1.10 2-(*t*-butyl)-4-(2,6-dichloro-4-nitrophenoxy)phenol (4c)



Chemical Formula: $C_{16}H_{15}Cl_2NO_4$

Molecular Weight: 356.20

IR (KBr) cm^{-1}: 3485,3041,3960,1282

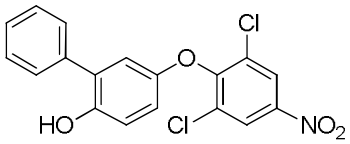
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38(s, 9H), 6.35-6.39(dd, *J* = 2.9 Hz & 8.5 Hz, 1H), 6.55(d, *J* = 8.5 Hz, 1H), 6.92(d, *J* = 3.0 Hz, 1H), 8.29(s, 2H)

% Yield: 85%

Purity by HPLC: 96.7 **%**

mp: 132-135 ^oC

3.5.1.11 5-(2,6-Dichloro-4-nitrophenoxy)-[1,1'-biphenyl]-2-ol (4d)



Chemical Formula: $C_{18}H_{11}Cl_2NO_4$

Molecular Weight: 376.19

IR (KBr) cm^{-1}: 3470, 1351, 1258

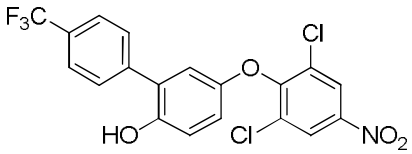
^{1}H NMR (**$CDCl_3$**, 300 MHz)**:** δ 6.70-6.78(m, 3H), 6.92-6.95(d, *J* = 6.0 Hz, 1H), 7.42-7.49(m, 4H), 8.29(s, 2H)

% Yield: 85%

Purity by HPLC: 86.72**%**

mp: 138-140 ^oC

3.5.1.12 5-(2,6-Dichloro-4-nitrophenoxy)-4'-(trifluoromethyl)-[1,1'-biphenyl]-2-ol (4e)



Chemical Formula: $C_{19}H_{10}Cl_2F_3NO_4$

Molecular Weight: 444.19

IR (KBr) cm^{-1}: 3400, 1618, 1533, 1342

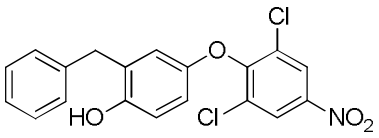
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75(m, 2H); 6.91-6.94(d, J = 9.0 Hz, 1H); 7.59-7.61(d, J = 8.1 Hz, 2H), 7.71-7.74(d, J = 8.1 Hz, 2H); 8.30(s, 2H)

% Yield: 99%

Purity by HPLC: 89.7 %

mp: 142-145 ^oC

3.5.1.13 2-Benzyl-4-(2,6-dichloro-4-nitrophenoxy)phenol (4f)



Chemical Formula: $C_{19}H_{13}Cl_2NO_4$

Molecular Weight: 390.22

IR (KBr) cm^{-1}: 3485, 3024, 1600, 1263

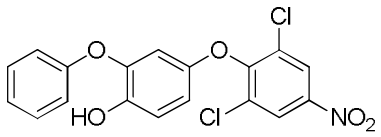
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95(s, 2H), 6.53-6.57(dd, J = 3.0 Hz & J = 8.7 Hz, 1H), 6.66-6.67(m, 1H), 6.70-6.73(m, 1H), 7.18-7.21(m, 3H), 7.23-7.33(m, 2H), 8.28(s, 2H)

% Yield: 99%

Purity by HPLC: 96.8 %

mp: 135-138 ^{o}C

3.5.1.14 4-(2,6-Dichloro-4-nitrophenoxy)-2-phenoxyphenol (4g)



Chemical Formula: $C_{18}H_{11}Cl_2NO_5$

Molecular Weight: 392.19

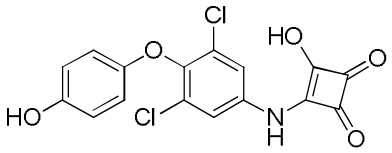
IR ($CHCl_3$) cm^{-1}: 3475, 1589, 1251

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.41-6.45(dd, J = 3.0 Hz & 6.0 Hz, 1H), 6.50(d, J = 2.7 Hz, 1H), 6.94-7.05(m, 2H), 7.13-7.17(m, 2H), 7.34-7.39(m, 2H), 8.26(s, 2H)

% Yield: 96%

Purity by HPLC: 94.5%

3.5.1.15 3-((3,5-Dichloro-4-(4-hydroxyphenoxy)phenyl)amino)-4-hydroxy cyclobut-3-ene-1,2-dione (6a)



Step I:

To a solution of stannous chloride dihydrate (5.8 g, 26 mmol) in concentrated HCl (1.0 mL) was added Nitro Compound **4a** (1.95 g, 6.5 mmol) in EtOH (30 mL).The reaction mixture was refluxed for about 2 h. The resulting mixture was brought at 20-30 ^{o}C and diluted with ethyl acetate (2 x 30 mL). The mixture was made alkaline with ammonia solution. Resulting solid was filtered through cellite. The organic phase was washed with water (2 x 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give

corresponding aniline **5a** (2 g, >100 %) which was used for further step without purification.

Step II:

To Aniline compound **5a** (470 mg, 1.74 mmol) squaric acid (178 mg, 1.56 mmol) was added in water (5 mL). The reaction mixture was stirred at reflux temperature for 2-6 hrs. The reaction mixture was then cooled to 20-30 ^{o}C and resulted solid was filtered to afford desired product which was purified by column chromatography using gradient ($CHCl_3$:MeOH) as an eluent. (0.41 g, 65 %)

Chemical Formula: $C_{16}H_9Cl_2NO_5$

Molecular Weight: 366.15

IR (KBr) cm^{-1}: 2923,1719, 1596

^{1}H NMR (300 MHz, DMSO-d6): δ 7.81(s, 2H), 9.06(s, 2H), 9.71 (s, 2H)

ESI-MS: 364.0 [M-H]

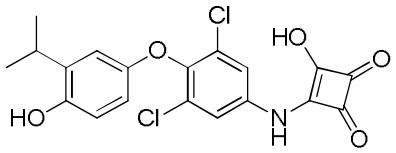
Yield: 65 %

Purity by HPLC: 98.11 %

mp: 205-208 ^{o}C

Compounds 6b-g were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.1.16 3-((3,5-Dichloro-4-(4-hydroxy-3-isopropylphenoxy)phenyl)amino)-4-hydroxy cyclobut-3-ene-1,2-dione (6b)



Chemical Formula: $C_{19}H_{15}Cl_2NO_5$

Molecular Weight: 408.23

IR (KBr) cm^{-1}: 3398,1797

^{1}H NMR (300 MHz, DMSO-d6): δ 1.10 (d, *J* = 6.9 Hz, 6H), 3.11-3.15 (m, 1H), 6.25-6.29 (dd, *J* = 8.67 Hz & 3.03 Hz, 1H), 6.62-6.65 (m, 2H), 7.90 (s, 2H).

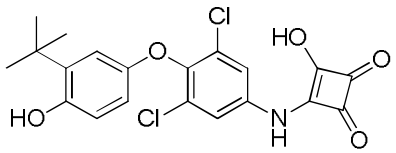
ESI-MS: 406.0 [M-H]

% Yield: 41%

Purity by HPLC: 97.25 %

mp : 210-213 ^oC

3.5.1.17 3-((4-(3-(*t*-butyl)-4-hydroxyphenoxy)-3,5-dichlorophenyl)amino)-4-hydroxycyclobut-3-ene-1,2-dione (6c)



Chemical Formula: $C_{20}H_{17}Cl_2NO_5$

Molecular Weight: 422.26

IR (KBr) cm^{-1}: 3234, 2960, 1797, 1556

^{1}H NMR (300 MHz, DMSO-d6): δ 1.29(s, 9H), 6.26-6.30(dd, *J* = 8.64 Hz & 3.03 Hz, 1H), 6.64(d, *J* = 8.7 Hz, 1H), 6.71(d, *J* = 3 Hz, 1H), 7.91(s, 2H)

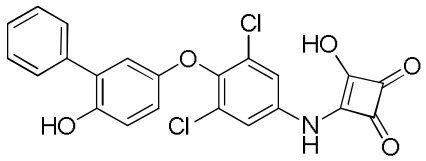
ESI-MS: 420.1 [M-H]

% Yield: 96%

Purity by HPLC: 99.1 %

mp: 215-218 ^oC

3.5.1.18 3-((3,5-Dichloro-4-((6-hydroxy-[1,1'-biphenyl]-3-yl) oxy) phenyl) amino)-4-hydroxycyclobut-3-ene-1,2-dione (6d)



Chemical Formula: $C_{22}H_{13}Cl_2NO_5$

Molecular Weight: 442.25

IR (KBr) cm^{-1}: 3358, 1780

^{1}H NMR (300 MHz, DMSO-d6): δ 6.54-6.60(dd, *J* = 3.0 Hz & 8.7 Hz, 1H), 6.65-6.70(d, *J* = 8.7 Hz, 1H), 6.79(d, *J* = 8.73 Hz, 1H), 7.10-7.26(m, 5H), 7.83(s, 2H)

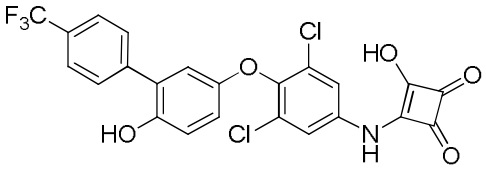
ESI-MS: 442 $[M]^+$

% Yield: 47%

Purity by HPLC: 93.18%

mp: >200 ^oC

3.5.1.19 3-((3,5-Dichloro-4-((6-hydroxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)oxy) phenyl) amino)-4-hydroxycyclobut-3-ene-1,2-dione (6e)



Chemical Formula: $C_{23}H_{12}Cl_2F_3NO_5$

Molecular Weight: 510.25

IR (KBr) cm^{-1}: 3411, 3255, 1654, 1325

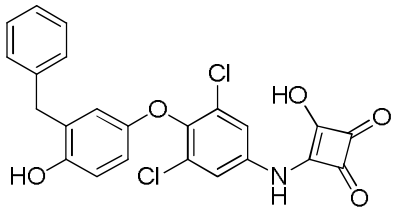
^{1}H NMR (300 MHz, DMSO-d6): δ 6.88-6.91(m, 1H), 6.64-6.68(dd, *J* = 3.0 Hz & 8.7 Hz, 1H), 6.75-6.76(d, *J* = 3 Hz, 1H), 7.72(m, 4H), 7.93(s, 2H)

ESI-MS: 508.1 [M-H]

Purity by HPLC: 90 %

mp: > 200 ^{o}C

3.5.1.20 3-((4-(3-Benzyl-4-hydroxyphenoxy)-3,5-dichlorophenyl)amino)-4-hydroxy cyclobut-3-ene-1,2-dione (6f)



Chemical Formula: $C_{23}H_{15}Cl_2NO_5$

Molecular Weight: 456.27

IR (KBr) cm^{-1}: 3388, 1787, 1726

^{1}H NMR (300 MHz, DMSO-d6): δ 3.80(s, 2H), 6.34-6.38(dd, *J* = 8.64 Hz & 3.09 Hz, 1H), 6.58(d, *J* = 3.03 Hz, 1H), 6.69(d, *J* = 8.73 Hz, 1H), 7.10-7.26(m, 5H), 7.89(s, 2H), 9.14 (s, 1H), 9.58(s, 1H)

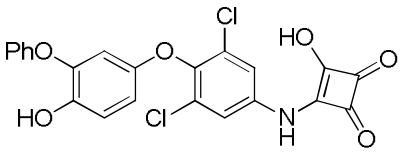
ESI-MS: 454.1 [M-H]

% Yield : 81 %

Purity by HPLC: 98.2 %

mp: 219-221 ^{o}C

3.5.1.21 3-((3,5-Dichloro-4-(4-hydroxy-3-phenoxyphenoxy)phenyl)amino)-4-hydroxycyclobut-3-ene-1,2-dione (6g)



Chemical Formula: $C_{22}H_{13}Cl_2NO_6$

Molecular Weight: 458.25

IR (KBr) cm^{-1}: 3411, 1647, 1215, 1137

^{1}H NMR (300 MHz, DMSO-d6): δ 3.80(s, 2H), 6.34-6.38(dd, *J* = 8.64 Hz & 3.09 Hz, 1H), 6.42-6.48(m, 2H), 6.81-6.88(m, 3H), 6.98-7.03(m, 1H), 7.27-7.32(m, 2H), 7.89(s, 2H)

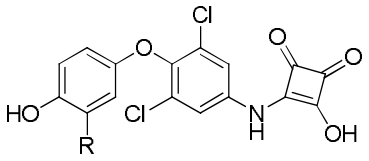
ESI-MS: 456.1 $[M-H]^+$

% Yield: 58 %

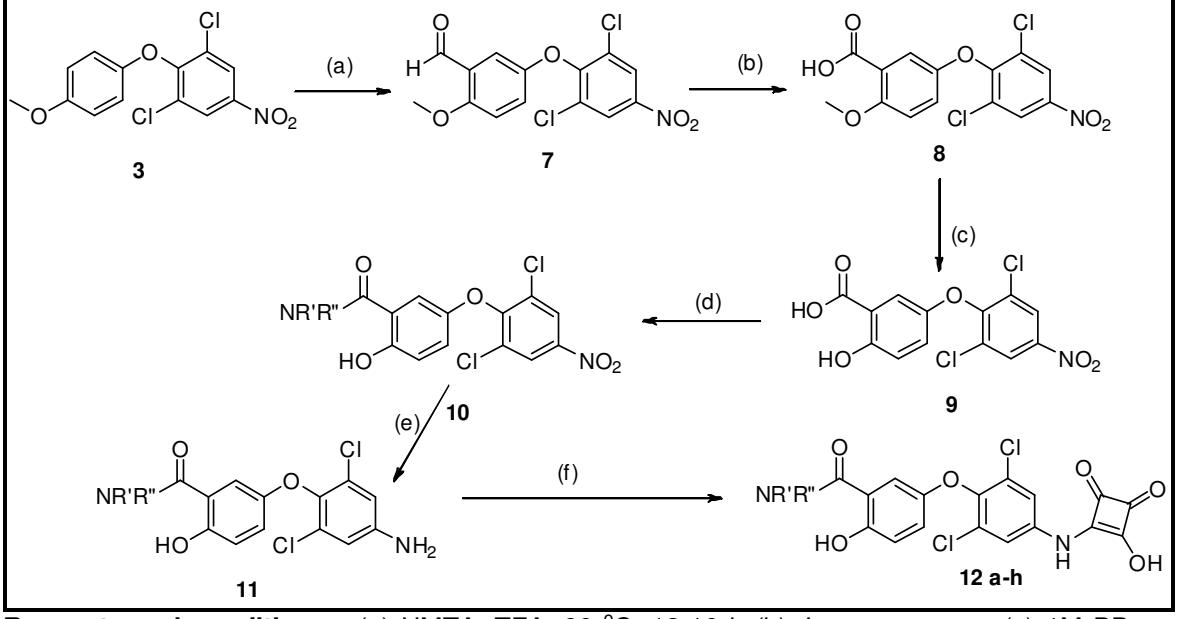
Purity by HPLC: 98.6 %

mp: > 220 °C

3.5.2 Squaric acid derivatives R= Carboxamide

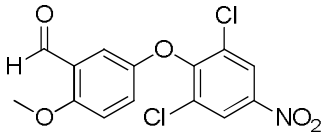


Scheme 3.2



Reagents and conditions : (a) HMTA, TFA, 90 ^{o}C, 12-16 h (b) Jones reagent (c) 1M BBr_3, DCM, 25 ^{o}C, 3 h(d) R'R"NH, EDCI, HOBT, DMF, 20-30 ^{o}C, 3-5 h (e) $SnCl_2.2H_2O$, Conc.HCl,EtOH, 65-70 ^{o}C, 3 h (f) $C_4H_2O_4$, H_2O, 100 ^{o}C 4-6 h

3.5.2.1 5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxybenzaldehyde (7)



Nitro compound **3a** (800 mg, 2.54 mmol) and hexamethylenetetramine (535 mg, 3.82 mmol) in trifluoroacetic acid (8 mL) was stirred at 70 ^{o}C for 18 h. The trifluoroacetic acid was removed under vacuum, to the residue water (30 mL) was added and the stirred for 30 min. The resulting mixture was extracted with ethyl acetate (2 x 50 mL), the organic phase washed with water (2 x 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 concentrated in vacuum and

purified by column chromatography on silica gel (35% acetone/hexanes) to afford the semisolid title compound.(900 mg, 98 %)

Chemical Formula: $C_{14}H_9Cl_2NO_5$

Molecular Weight: 342.13

IR ($CHCl_3$) cm^{-1}: 3089, 1683, 1022

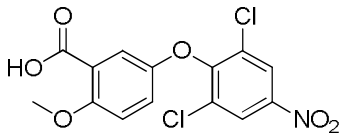
^1H NMR (300 MHz, $CDCl_3$): δ 3.94(s, 3H), 7-7.03(d, J = 9.0 Hz, 2H), 7.11-7.12(d, J = 3.0 Hz, 1H), 8.31(s, 2H), 10.40(s, 1H)

ESI-MS: 343 [M-H]

% Yield: 98 %

Purity by HPLC: 81 %

3.5.2.2 5-(2,6-Dichloro-4-nitrophenoxy)-2-hydroxybenzaldehyde (8)



To a solution of aldehyde compound **8** (800 mg, 2.33 mmol) in acetone (21.8 mL) at 20-30 ^oC was added Jones reagent (4.65 mL) with stirring until a red color persisted. The solution was stirred for 1 h, then quenched with isopropanol (3 mL). The reaction was filtered through Celite and the filtrate was concentrated under reduced pressure to afford oily residue. To that water (10 mL) was added and acidified with concentrated HCl at 10-15 ^oC to give a solid product.(450 mg, 54 %)

Note : Jones reagent was prepared by adding CrO_3 (4.2 g) in water (30 mL). It was cooled to 10-15 ^oC. To that Conc.H_2SO_4 (3.6 mL) was added. This mixture was brought at 20-25 ^oC and it was used further.

Chemical Formula: $C_{14}H_9Cl_2NO_6$

Molecular Weight: 358.13

IR (KBr) cm^{-1}: 3084, 1720, 1066

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.09(s, 3H), 7.09-7.06(d, J = 9.0 Hz, 1H), 7.20-7.21(d, J = 3.0 Hz, 1H), 7.5-7.51(d, J = 3.0 Hz, 1H), 8.32(s, 2H)

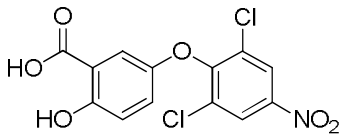
ESI-MS: 359$[M+H]^+$

% Yield: 54 %

Purity by HPLC: 98.86 %

mp: 152-155 ^oC

3.5.2.3 5-(2,6-Dichloro-4-nitrophenoxy)-2-hydroxybenzoic acid (9)



Methoxy compound **8** (400 mg, 1.11 mmol) in DCM (4 mL) was cooled to -60 ^oC under nitrogen atmosphere. To that 1M BBr_3 solution (2.25 mL) was added dropwise. The reaction mixture was allowed to warm up to 20-25 ^oC over 5 h, then diluted with more DCM (24 mL) and quenched with water (24 mL) . After stirring at 20-25 ^oC for 30 min, organic phase was separated, washed with water (2 x 24 mL), brine (24 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated to give crude product. The crude product was purified by column chromatography over flash silica gel (Hexane : ethyl acetate, 90:10) to afford the pure product. (354 mg, 92 %)

Chemical Formula: $C_{13}H_7Cl_2NO_6$

Molecular Weight: 344.10

IR (KBr) cm^{-1}: 3087, 1676, 1066

^1H NMR (300 MHz, $CDCl_3$): δ 6.99-7.02(d, J = 9.0 Hz, 1H), 7.13-7.26(m, 2H), 8.24(s, 2H)

ESI-MS: 344 $[M]^+$

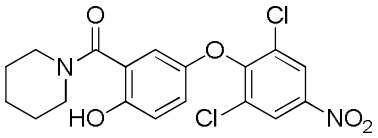
% Yield: 92 %

Purity by HPLC: 95.88 %

mp: 165-168 ^oC

Procedure for synthesis of Intermediate (10a-h)

3.5.2.4 (5-(2,6-Dichloro-4-nitrophenoxy)-2-hydroxyphenyl)(piperidin-1-yl) methanone (10a)



To a solution of 5-(2,6-dichloro-4-nitrophenoxy)-2-hydroxybenzoic acid **9** (500 mg, 1.45 mmol) in DMF (3 mL) was added [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide]hydrochloride (EDCI.HCl) (418 mg, 2.18 mmol), 1-hydroxy benzotriazole (HOBt) (294 mg, 2.18 mmol) and piperidine (185 mg, 2.18 mmol). The reaction was stirred at 20-30 ^oC for 3-5 hrs. The reaction mixture was poured over ice-water (10 mL). The product was taken up in ethyl acetate (2 x 15 mL), washed with water (2 x 15 mL), brine (15 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated to give the crude product, which was purified by column chromatography over flash silica gel (hexane : ethyl acetate, 90:10) to afford pure product. (495 mg, 83 %)

Chemical Formula: $C_{18}H_{16}Cl_2N_2O_5$

Molecular Weight: 411.24

IR (KBr) cm^{-1}: 3020, 2943, 1624, 1215

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68(m, 6H), 3.579(m, 4H), 6.63(m, 1H), 6.88-6.92(m, 2H), 8.25(s, 2H)

% Yield: 83%

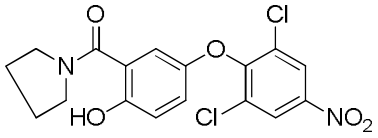
ESI-MS: 412.5 $[M+H]^+$

Purity by HPLC: 97.42 %

mp: 155-160 ^oC

Compounds 10b-h were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.2.5 (5-(2,6-Dichloro-4-nitrophenoxy)-2-hydroxyphenyl)(pyrrolidin-1-yl) methanone (10b)



Chemical Formula: $C_{17}H_{14}Cl_2N_2O_5$

Molecular Weight: 397.21

IR (KBr) cm^{-1}: 3419, 2968, 1732, 1261

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.92(m, 4H), 3.62(m, 4H), 6.84-6.88(m, 1H), 6.93-6.96(m, 2H), 8.31(s, 2H)

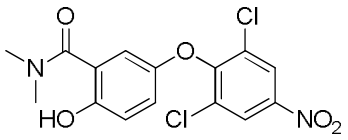
ESI-MS: 396.9 $[M]^+$

% Yield: 58%

Purity by HPLC: 98.81 %

mp: 140-142 ^oC

3.5.2.6 5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxy-N,N-dimethylbenzamide (10c)



Chemical Formula: $C_{15}H_{12}Cl_2N_2O_5$

Molecular Weight: 371.17

IR ($CHCl_3$) cm^{-1}: 3093, 1604, 1510, 1261

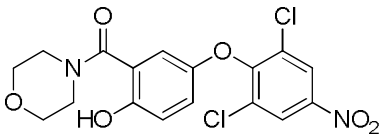
^1H NMR (300 MHz, $CDCl_3$): δ 3.11(s, 6H), 6.77(d J = 3 Hz, 1H), 6.85-6.98(m, 2H), 8.30(s, 2H),

ESI-MS: Not observed

% Yield: 45**%**

Purity by HPLC: 99.76 %

3.5.2.7 (5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxphenyl) (morpholino) methanone (10d)



Chemical Formula: $C_{17}H_{14}Cl_2N_2O_6$

Molecular Weight: 413.21

IR (KBr) CM^{-1}: 3378, 2890, 1710

^1H NMR (300 MHz, $CDCl_3$): δ 3.22-3.25(m, 4H), 3.71-3.74(m, 4H), 6.91-6.98(m, 2H), 7.34-7.36(m, 1H), 8.31(s, 2H)

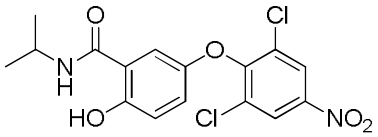
ESI-MS : 412.5 [M-H]

% Yield: 65%

Purity by HPLC: 95.62 %

mp: 110-115 ^{o}C

3.5.2.8 5-(2,6-Dichloro-4-nitrophenoxy)-N-isopropyl-2-methoxybenzamide (10e)



Chemical Formula: $C_{16}H_{14}Cl_2N_2O_5$

Molecular Weight: 385.20

IR ($CHCl_3$) cm^{-1}: 3100, 1710, 1510

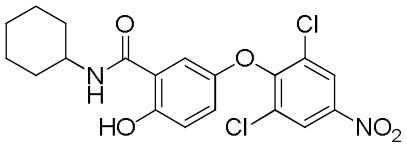
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21-1.23(d, J = 8.1 Hz, 6H), 4.23-4.34(m, 1H), 6.66-6.70 (dd, J = 2.7 Hz & 9.0 Hz, 1H), 6.88-6.91(d, J = 9.0 Hz, 1H), 7.01-7.02(d, J = 9.0 Hz, 1H), 8.25(s, 2H)

ESI-MS: 383 [M-H]

% Yield: 56%

Purity by HPLC: 89.04 %

3.5.2.9 N-Cyclohexyl-5-(2,6-dichloro-4-nitrophenoxy)-2-hydroxybenzamide (10f)



Chemical Formula: $C_{19}H_{18}Cl_2N_2O_5$

Molecular Weight: 425.26

IR (KBr) cm^{-1}: 3448, 3350, 2972

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26-1.28(m, 6H), 1.56-1.66(m, 2H), 1.70-1.81(m, 2H), 3.94(m, 1H), 6.66-6.70(dd, *J* = 2.7 Hz & 9.0 Hz, 1H), 6.88(m, 1H), 7.02(s, 1H), 8.26(s, 2H)

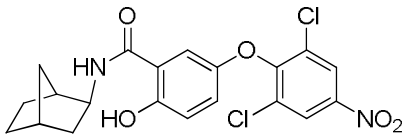
ESI-MS: 423.1[M-H]

% Yield: 63%

Purity by HPLC: 91.93%

mp: 168-170 ^oC

3.5.2.10 N-((1S,2S,4R)-Bicyclo[2.2.1]heptan-2-yl)-5-(2,6-dichloro-4-nitro phenoxy)-2-hydroxybenzamide (10g)



Chemical Formula: $C_{20}H_{18}Cl_2N_2O_5$

Molecular Weight: 437.27

IR (KBr) cm^{-1}: 3361, 2954, 1714, 1224

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19-1.92(m, 10H), 1.87-1.93(m, 1H), 6.66-6.70(m, 3H), 6.84-6.91(m, 1H), 6.99(s, 1H)

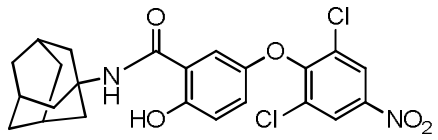
ESI-MS: 437 $[M]^+$

% Yield: 64%

Purity by HPLC: 82 %

mp: 188-190 ^oC

3.5.2.11 N-((1R,3R,5S)-Adamantan-1-yl)-5-(2,6-dichloro-4-nitrophenoxy)-2-hydroxy benzamide (10h)



Chemical Formula: $C_{23}H_{22}Cl_2N_2O_5$

Molecular Weight: 477.34

IR (KBr) cm^{-1}: 3112, 2890, 1724

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72(m, 6H), 2.11(m, 9H), 6.65-6.66(d, *J* = 2.3 Hz, 1H), 6.82-6.85(d, *J* = 9.0 Hz, 1H), 6.97(d, *J* = 2.7 Hz, 1H), 8.25(s, 2H)

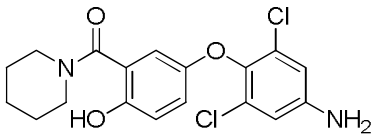
ESI-MS: Not observed

% Yield: 90**%**

Purity by HPLC: 88.37 **%**

mp: 180-185 ^oC

3.5.2.12 (5-(4-Amino-2,6-dichlorophenoxy)-2-hydroxyphenyl)(piperidin-1-yl) methanone (11a)



To a solution of stannous chloride dihydrate (437 mg, 1.9 mmol) in concentrated HCl (0.2mL) was added Nitro Compound **10a** (200 mg, 0.48 mmol) in EtOH (4.0 mL).The reaction mixture was refluxed for about 2 h. The resulting mixture was brought at 20-30 ^oC and diluted with ethyl acetate (25 mL). The mixture was made alkaline with ammonia solution. Resulting solid was filtered through cellite. The organic phase was washed with water (2 x25 mL), brine (25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give

corresponding aniline **11a** which was used for further step without purification.(160 mg, 86 %)

Chemical Formula: $C_{18}H_{18}Cl_2N_2O_3$

Molecular Weight: 381.25

IR ($CHCl_3$) cm^{-1}: 3020, 2943, 1624, 1215

^1H NMR (300 MHz, $CDCl_3$): δ 1.66(m, 6H), 3.57-3.61(m, 4H), 6.55(s, 1H), 6.60-6.68(s, 2H), 6.94(s, 2H)

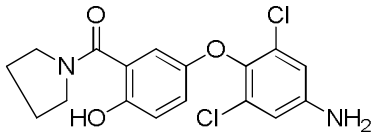
ESI-MS: 381$[M]^+$

% Yield: 86**%**

Purity by HPLC: 91.44**%**

Compounds 11b-h were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.2.13 (5-(4-Amino-2,6-dichlorophenoxy)-2-hydroxyphenyl)(pyrrolidin-1-yl)methanone (11b)



Chemical Formula: $C_{17}H_{16}Cl_2N_2O_3$

Molecular Weight: 367.23

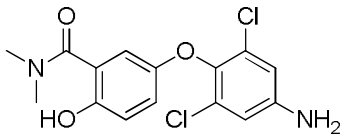
IR ($CHCl_3$) cm^{-1}: 3448, 3350, 2972, 1728, 1224

^1H NMR (300 MHz, $CDCl_3$): δ 1.92(m, 4H), 3.62(m, 4H), 6.68(m, 2H), 6.89-6.91(m, 3H)

% Yield: 99 **%**

Purity by HPLC: 91.98 %

3.5.2.14 5-(4-Amino-2,6-dichlorophenoxy)-2-hydroxy-N,N-dimethyl benzamide (11c)



Chemical Formula: $C_{15}H_{14}Cl_2N_2O_3$

Molecular Weight: 341.19

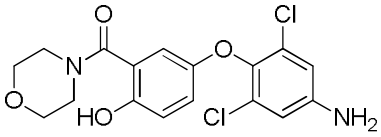
IR ($CHCl_3$) cm^{-1}: 3371, 1614, 1521

^1H NMR (300 MHz, $CDCl_3$): δ3.10(s, 6H), 6.86(m, 3H), 6.93(s, 2H),

% Yield: 45 **%**

Purity by HPLC: 90.19 %

3.5.2.15 (5-(4-Amino-2,6-dichloro phenoxy)-2-hydroxyphenyl) (morpholino) methanone (11d)



Chemical Formula: $C_{17}H_{16}Cl_2N_2O_4$

Molecular Weight: 383.23

IR ($CHCl_3$) cm^{-1}: 3450, 3280, 2982, 1742

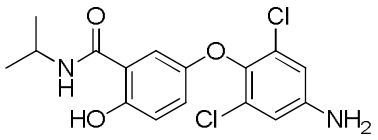
^1H NMR (300 MHz, $CDCl_3$): δ 3.02-3.05(m,4H), 3.72-3.75(m, 4H), 6.68(s, 2H), 6.85(d, J = 3.0 Hz, 1H), 7.02(d, J = 9.0 Hz, 1H), 7.09-7.13(dd, J = 3.9 Hz, 1H)

ESI-MS: 382.21[M-H]

% Yield: 92**%**

Purity by HPLC: 86.57 %

3.5.2.16 5-(4-Amino-2,6-dichlorophenoxy)-2-hydroxy-N-isopropyl benzamide (11e)



Chemical Formula: $C_{16}H_{16}Cl_2N_2O_3$

Molecular Weight: 355.22

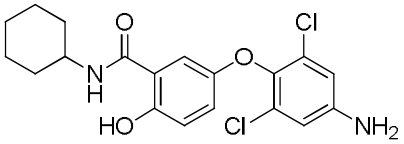
IR ($CHCl_3$) cm^{-1}: 3381, 3018, 1566, 1215

^1H NMR (300 MHz, $CDCl_3$): δ 1.24-1.26(d, *J* = 7.2 Hz, 6H), 4.24-4.31(m, 1H), 6.70(s, 2H), 6.73-6.74(d, *J* = 3.0 Hz, 1H), 6.85-6.88(d d, *J* = 9.0 Hz, 1H), 6.97-6.98(d, *J* = 3.0 Hz, 1H)

% Yield: 99 **%**

Purity by HPLC: 87.30 %

3.5.2.17 5-(4-Amino-2,6-dichlorophenoxy)-N-cyclohexyl-2-hydroxyl benzamide (11f)



Chemical Formula: $C_{19}H_{20}Cl_2N_2O_3$

Molecular Weight: 395.28

IR ($CHCl_3$) cm^{-1}: 3367, 3232, 2927, 1724, 1282

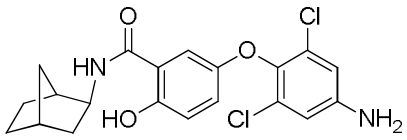
^1H NMR (300 MHz, $CDCl_3$): δ 1.23-1.28(m, 6H), 1.75(m, 2H), 2.04(m, 2H), 3.94(m, 1H), 6.72(m, 2H), 6.84(m, 1H), 6.84(m, 1H), 6.98(m, 1H)

ESI-MS: Not observed

% Yield: 99 **%**

Purity by HPLC: 80.05 %

3.5.2.18 5-(4-Amino-2,6-dichlorophenoxy)-N-((1S,2S,4R)-bicyclo [2.2.1] heptan-2-yl) -2-hydroxybenzamide (11g)



Chemical Formula: $C_{20}H_{20}Cl_2N_2O_3$

Molecular Weight: 407.29

IR ($CHCl_3$) cm^{-1}: 3361, 2954, 1714, 1224

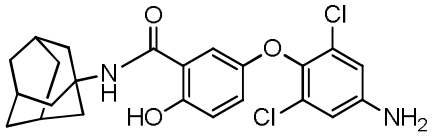
^1H NMR (300 MHz, $CDCl_3$): δ 1.19-1.92(m, 10H), 1.87-1.93(m, 1H), 6.66-6.70(m, 3H), 6.84-6.91(m, 1H), 6.99(s, 1H)

ESI-MS: 407 $[M]^+$

% Yield: 98 **%**

Purity by HPLC: 82 %

3.5.2.19 N-((1r,3R,5S)-Adamantan-1-yl)-5-(4-amino-2,6-dichlorophenoxy)-2-hydroxy benzamide (11h)



Chemical Formula: $C_{23}H_{24}Cl_2N_2O_3$

Molecular Weight: 447.35

IR ($CHCl_3$) cm^{-1}: 3398, 2908, 1589, 1047

^1H NMR (300 MHz, $CDCl_3$): δ 1.72(m, 6H), 2.11(m, 9H), 6.65-6.66(d, J = 2.7 Hz, 1H), 6.7(m, 2H), 6.82-6.85(d, J = 9.0 Hz, 1H), 6.97(d, J = 2.7 Hz, 1H)

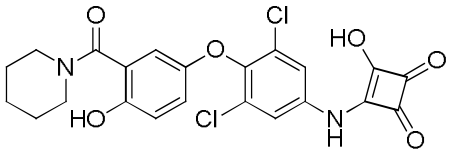
ESI-MS: 446[M-H]

% Yield: 85 **%**

Purity by HPLC: 88 %

Compounds 12b-h were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.2.20 3-((3,5-Dichloro-4-(4-hydroxy-3-(piperidine-1-carbonyl) phenoxy) phenyl) amino)-4-hydroxycyclobut-3-ene-1,2-dione (12a)



To Aniline compound **11a** (200 mg, 0.52 mmol) squaric acid (53 mg, 0.47 mmol) was added in (1 mL) H_2O. The reaction mixture was stirred at reflux temperature for 2-6 hrs. The reaction mixture was then cooled to 20-30 ^oC and resulted solid was filtered to afford desired product which was purified by column chromatography using gradient (CHCl3:MeOH) as an eluent to afford desired product(167 mg, 66 %).

Chemical Formula: $C_{22}H_{18}Cl_2N_2O_6$

Molecular Weight: 477.29

IR (KBr) cm^{-1}: 3363, 2929, 2858, 1685

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.43-1.53 (m, 6H), 3.14-3.16 (br s, 4H), 6.47 (s, 1H), 6.78 (d, J = 8.49 Hz, 2H), 8.07 (s, 2H)

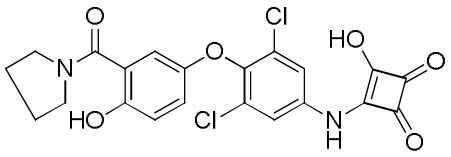
ESI-MS: 475.1 $[M-H]^+$

Yield: 66 %

Purity by HPLC: 97.1 %

mp: 186-190 ^oC

3.5.2.21 3-((3,5-Dichloro-4-(4-hydroxy-3-(pyrrolidine-1-carbonyl)phenoxy) phenyl) amino)-4-hydroxycyclobut-3-ene-1,2-dione (12b)



Chemical Formula: $C_{21}H_{16}Cl_2N_2O_6$

Molecular Weight: 463.27

IR (KBr) cm^{-1}: 3415, 2929, 1591, 1529

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77-1.79(br s, 4H), 3.18-3.20(br s, 4H), 6.51(d, *J* = 3.0 Hz,1H), 6.70-6.74(dd, *J* = 8.79 Hz & 3 Hz, 1H), 6.81(d, *J* = 8.91 Hz, 1H), 7.92(s, 2H), 9.63(s, 1H), □ 9.69 (s, 1H)

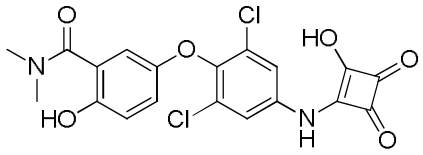
ESI-MS: 461.1 [M-H]

Yield: 61%

Purity by HPLC: 96.9 %

mp: >220 ^oC

3.5.2.22 5-(2,6-Dichloro-4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl)amino) phenoxy)-2-hydroxy-N,N-dimethylbenzamide (12c)



Chemical Formula: $C_{19}H_{14}Cl_2N_2O_6$

Molecular Weight: 437.23

IR (KBr) cm^{-1}: 3419, 2929, 1791, 1593

^{1}H NMR (300 MHz, DMSO-d6): δ 2.71-2.79(br s, 6H), 6.45(d, *J* = 3.03 Hz, 1H), 6.68-6.72(dd, *J* = 8.8 Hz & 3.06 Hz, 1H), 6.80(d, *J* = 8.91 Hz, 1H), 7.91(s, 2H)

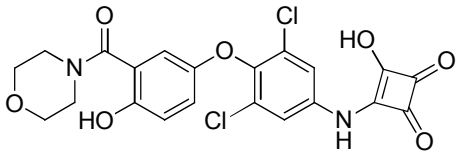
ESI-MS: 435.0 $[M-H]^+$

Yield: 51 %

Purity by HPLC: 97.1 %

mp: 217-220 ^oC

3.5.2.23 3-((3,5-Dichloro-4-(4-hydroxy-3-(morpholine-4-carbonyl) phenoxy) phenyl) amino)-4-hydroxycyclobut-3-ene-1,2-dione (12d)



Chemical Formula: $C_{21}H_{16}Cl_2N_2O_7$

Molecular Weight: 479.27

IR (KBr) cm^{-1}: 3411, 1793, 1595

^{1}H NMR (300 MHz, DMSO-d_6): δ 3.14-3.16(br s, 4H), 3.53-3.55(br s, 4H), 6.52(d, J = 2.19 Hz, 1H), 6.70(d, J = 6.21 Hz, 1H), 6.80(d, J = 8.79 Hz, 1H), 7.92 (s, 2H)

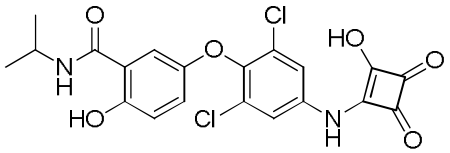
ESI-MS: 477.0 [M-H]

Yield: 64 %

Purity by HPLC: 96.3 %

mp: 210-214 ^oC

3.5.2.24 5-(2,6-Dichloro-4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl)amino) phenoxy)-2-hydroxy-N-isopropylbenzamide (12e)



Chemical Formula: $C_{20}H_{16}Cl_2N_2O_6$

Molecular Weight: 451.26

IR (KBr) cm^{-1}: 3490, 1562, 1554, 1230

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.17(d, *J* = 6.57 Hz, 6H), 4.10-4.12(m, 1H), 6.72(d, *J* = 9.03 Hz, 1H), 6.81(d, *J* = 9.0 Hz, 1H), 7.53(d, *J* = 2.76 Hz, 1H), 7.94(s, 2H),

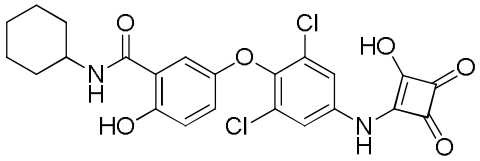
ESI-MS: 449.0 [M-H]

Yield: 70 %

Purity by HPLC: 96.1 %

mp: 203-205 °C

3.5.2.25 N-Cyclohexyl-5-(2,6-dichloro-4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl) amino) phenoxy)-2-hydroxybenzamide (12f)



Chemical Formula: $C_{23}H_{20}Cl_2N_2O_6$

Molecular Weight: 491.32

IR (KBr) cm^{-1}: 3433, 2931, 1589, 1533

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.30-1.32(br s, 4H), 1.56-1.80(m, 6H), 3.76-3.78(br s, 1H), 6.80-6.83(m, 2H), 7.52(s, 1H), 8.07 (s, 2H)

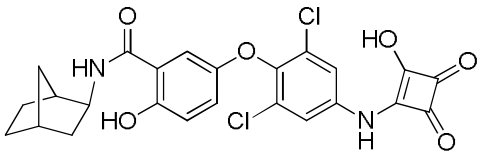
ESI-MS: 488.8 [M-H]

Yield: 60 %

Purity by HPLC: 97.7 %

mp: 206-208 °C

3.5.2.26 N-((1S,2S,4R)-Bicyclo[2.2.1]heptan-2-yl)-5-(2,6-dichloro-4-((2-hydroxy-3,4-dioxo cyclobut-1-en-1-yl)amino)phenoxy)-2-hydroxy benzamide (12g)



Chemical Formula: $C_{24}H_{20}Cl_2N_2O_6$

Molecular Weight: 503.33

IR (KBr) cm^{-1}: 3386, 2956, 2871, 1627

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.13-1.15(m, 3H), 1.45-1.47(m, 4H), 1.65-1.67(m, 1H), 2.24-2.26(br s, 2H), 3.70-3.72(br s, 1H), 6.78(d, J = 3.0 Hz, 1H), 6.84(d, J = 9.0 Hz, 1H), 7.58(d, J = 2.7 Hz, 1H), 8.08 (s, 2H)

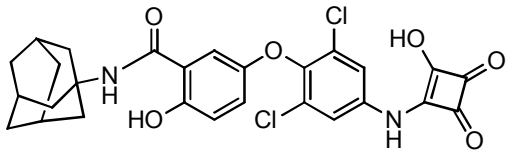
ESI-MS: 502.0 [M-H]

Yield: 62 %

Purity by HPLC: 96.5 %

mp: 198-203 ℃

3.5.2.27 N-((1R,3R,5S)-Adamantan-1-yl)-5-(2,6-dichloro-4-((2-hydroxy-3,4-dioxo cyclobut-1-en-1-yl) amino)phenoxy)-2-hydroxybenzamide (12h)



Chemical Formula: $C_{27}H_{24}Cl_2N_2O_6$

Molecular Weight: 543.40

IR (KBr) cm^{-1}: 3398, 2908, 1589, 1047

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.62-1.64(br s, 6H), 2.01-2.03(br s, 9H), 6.88(s, 2H), 7.33(s, 1H), 8.09(s, 2H), 11.49(s, 1H), 11.93 (s, 1H)

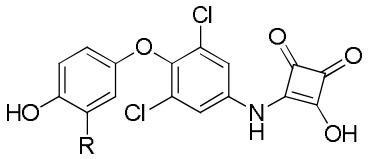
ESI-MS: 541.0 [M-H]

Yield: 50 %

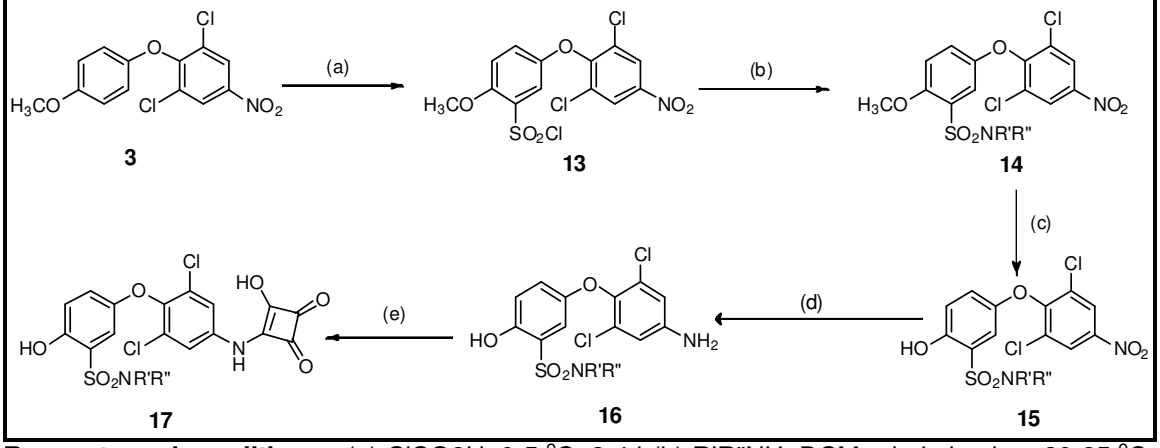
Purity by HPLC: 97.9 %;

mp: 192-196 ^oC

3.5.3 Squaric acid derivatives R= Sulfonamide

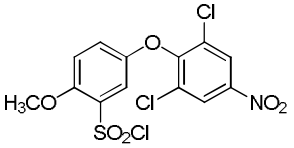


Scheme 3.3



Reagents and conditions : (a) ClSO3H, 0-5 ^oC, 2-4 h(b) R'R"NH, DCM, triethylamine, 20-25 ^oC, 2-3 h (c) 1M BBr_3, DCM, 25 ^oC, 3h, 20-50%.(d) $SnCl_2.2H_2O$, Conc. HCl,EtOH, 65-70 ^oC, 3h (g) $C_4H_2O_4$, H_2O, 100 ^oC 4-6 h

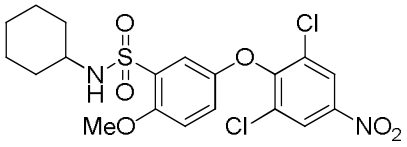
3.5.3.1 5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxybenzene-1-sulfonyl chloride (13)



To a solution of Nitro compound **3a** (1g, 3.18 mmol) was added chlorosulfonic acid (0.99 mL, 14.9 mmol) at 0-5 ^oC dropwise. The reaction was

stirred at 0-5 ^{o}C for 2-4 hrs. The reaction mixture was then diluted with ethyl acetate (25 mL) ,washed with water (25 mL), brine (25 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford sulfonyl chloride. It was used for next step without purification.(1.1 g)

3.5.3.2 N-Cyclohexyl-5-(2,6-dichloro-4-nitrophenoxy)-2-methoxy benzene sulfonamide (14a)



To a solution of sulfonyl chloride **13** (1 g, 2.4 mmol), in DCM (10 mL) was added triethylamine (360 mg, 3.6 mmol) at 25-30 ^{o}C. The reaction mixture was cooled to 0-10 ^{o}C. To that cyclohexylamine (470 mg, 4.8 mmol) was added at same temperature. The reaction mixture was stirred at 20-25 ^{o}C for 2-3 h and then poured over ice. The product was taken up in ethyl acetate (20 mL), washed with water (2 x 20 mL), brine (20 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated to give the crude product, which was purified by column chromatography over flash silica gel (hexane : ethyl acetate, 90:10) to afford pure product. (1g, 87 %)

Chemical Formula: $C_{19}H_{20}Cl_2N_2O_6S$

Molecular Weight: 475.34

IR ($CHCl_3$) cm^{-1}: 2975, 1663

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.24-1.27(m, 4H), 1.50-1.53(m, 2H), 1.59-1.64(m, 2H), 1.68-1.71(m, 2H), 3.11-3.13(m, 1H), 3.9(s, 3H), 4.84(d, J=7.6 Hz,

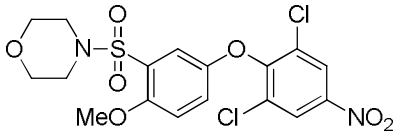
1H), 7.0(d, J = 8.8 Hz, 1H), 7.07-7.10(dd, J = 3.2 Hz & J = 9.2 Hz, 1H), 7.34(d, J = 3.2 Hz, 1H), 8.31(s, 2H)

% Yield: 87%

Purity by HPLC: 90.83%

Compounds 14b and 14c were prepared in an analogous manner using appropriate starting materials and the process described above

3.5.3.3 4-((5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxyphenyl)sulfonyl) morpholine (14b)



Chemical Formula: $C_{17}H_{16}Cl_2N_2O_7S$

Molecular Weight: 463.29

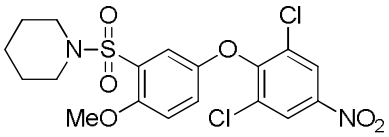
IR ($CHCl_3$) cm^{-1}: 2925, 1616, 1074

^{1}H NMR (300 MHz, DMSO-d_6): δ 3.23(t, *J* = 4.5 Hz, 4H), 3.72(t, *J* = 4.5 Hz, 4H), 3.91(s, 3H), 7.0(d, *J* = 9.0 Hz, 1H), 7.04-7.08(dd, *J* = 3.0 Hz & 9.0 Hz, 1H), 7.36(d, *J* = 3.6 Hz, 1H), 8.31(s, 2H)

% Yield: 96%

Purity by HPLC: 94%

3.5.3.4 1-((5-(2,6-Dichloro-4-nitrophenoxy)-2-methoxy phenyl) sulfonyl) piperidine (14c)



Chemical Formula: $C_{18}H_{18}Cl_2N_2O_6S$

Molecular Weight: 461.32

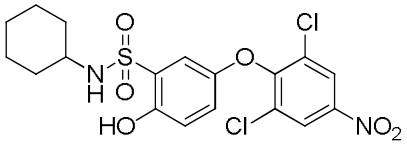
IR ($CHCl_3$) cm^{-1}: 3070, 1533, 1066

^1H NMR (300 MHz, DMSO-d_6): δ 1.51-1.61(m, 6H), 3.18-3.21(t, *J* = 5.1 Hz & 5.7 Hz, 4H), 3.90(s, 3H), 6.91-7.06(m, 2H), 7.34-7.35(d, *J* = 3.0 Hz, 1H), 8.31(s, 2H)

% Yield: 98%

Purity by HPLC: 95.63 %

3.5.3.5 N-Cyclohexyl-5-(2,6-dichloro-4-nitrophenoxy)-2-hydroxybenzene sulfonamide (15a)



Methoxy compound **14a** (1.0 g, 2.10 mmol) in DCM (10 mL) was cooled to -60 ^oC under nitrogen atmosphere. To that 1M BBr_3 solution (4.21 mL) was added dropwise. The reaction mixture was allowed to warm up to 20-25 ^oC over 5 h. then diluted with more DCM (25 mL) and quenched with water. After stirring at 20-25 ^oC for 30 min, organic phase was separated, washed with water (2 x 25 mL), brine (25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give crude product. The crude product was purified by column chromatography over flash silica gel (Hexane : ethyl acetate, 90:10) to afford the pure product. (0.99 g, 100%)

Chemical Formula: $C_{18}H_{18}Cl_2N_2O_6S$

Molecular Weight: 461.32

IR ($CHCl_3$) cm^{-1}: 3309, 1591, 1541, 1259

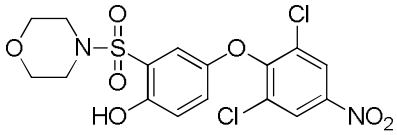
^1H NMR (300 MHz, DMSO-d6): δ 1.10-1.30(m, 6H), 1.56-1.75(m, 4H), 3.12-3.15(m, 1H), 6.91-7.12(m, 3H), 8.31(s, 2H)

% Yield: 100%

Purity by HPLC: 94.89 %

Compounds 15b and 15c were prepared in an analogous manner using appropriate starting materials and the process described above

3.5.3.6 4-(2,6-Dichloro-4-nitrophenoxy)-2-(morpholinosulfonyl)phenol (15b)



Chemical Formula: $C_{16}H_{14}Cl_2N_2O_7S$

Molecular Weight: 449.26

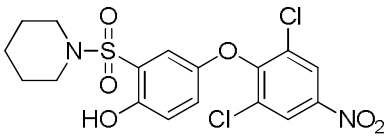
IR ($CHCl_3$) cm^{-1}: 3315, 1527, 1265

^{1}H NMR (300 MHz, DMSO-d_6): δ 3.05(t, *J* = 4.6 Hz, 4H), 3.76(t, *J* = 4.6 Hz, 4H), 6.91-6.92(m, 1H), 7.07(s, 2H), 8.32(s, 2H)

% Yield: 85 **%**

Purity by HPLC: 98.67 %

3.5.3.7 4-(2,6-Dichloro-4-nitrophenoxy)-2-(piperidin-1-ylsulfonyl)phenol (15c)



Chemical Formula: $C_{17}H_{16}Cl_2N_2O_6S$

Molecular Weight: 447.29

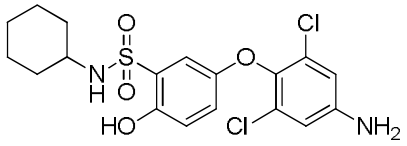
IR ($CHCl_3$) cm^{-1}: 3282, 2947, 1614, 1261

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.48-1.51(m, 2H), 1.61-1.68(m, 4H), 3.04(t, *J* = 5.41 Hz, 4H), 6.91(d, *J* = 1.80 Hz, 1H), 7.04-7.08(dd, *J* = 1.2 Hz & 9.0 Hz, 2H), 8.32 (s, 2H)

% Yield: 92 **%**

Purity by HPLC: 95.46 %

3.5.3.8 5-(4-Amino-2,6-dichlorophenoxy)-N-cyclohexyl-2-hydroxybenzene sulfonamide (16a)



To a solution of stannous chloride dihydrate (1.93 g, 8.57 mmol) in concentrated HCl (1 mL) was added Nitro Compound **15a** (0.99 g, 2.14 mmol) in EtOH (20 mL). The reaction mixture was refluxed for about 2 h. The resulting mixture was brought at 20-30 ^{o}C and diluted with ethyl acetate (40 mL). The mixture was made alkaline with ammonia solution. Resulting solid was filtered through cellite. The organic phase was washed with water (2 x 40 mL), brine (40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give corresponding aniline **16a** (920 mg, >100 %) which was used for further step without purification.

Chemical Formula: $C_{18}H_{20}Cl_2N_2O_4S$

Molecular Weight: 431.33

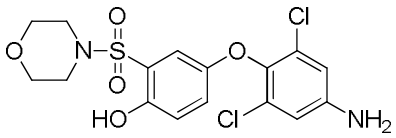
IR ($CHCl_3$) cm^{-1}: 3382, 2931, 1701, 1624

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.09-1.13(m, 6H), 1.70-1.88(m, 4H), 3.11-3.15(m, 1H), 6.67(s, 2H), 6.91-6.94(m, 2H), 7.08-7.11(m, 1H)

% Yield: 100 **%**

Purity by HPLC: 93.69 **%**

3.5.3.9 4-(4-Amino-2,6-dichlorophenoxy)-2-(morpholinosulfonyl)phenol (16b)



Chemical Formula: $C_{16}H_{16}Cl_2N_2O_5S$

Molecular Weight: 419.28

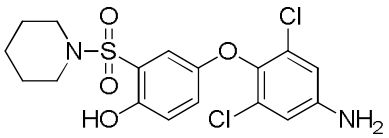
IR ($CHCl_3$) cm^{-1}: 3382, 2931, 1701, 1624

^{1}H NMR (300 MHz, DMSO-d_6): δ 3 3.03(t, *J* = 4.6 Hz, 4H), 3.73(t, *J* = 4.6 Hz, 4H), 6.68(s, 2H), 6.85(d, *J* = 3 Hz, 1H), 7.01(d, *J* = 9.0 Hz, 1H) 7.09-7.13(dd, *J* = 3 Hz & 9.0 Hz, 1H)

% Yield: 96 **%**

Purity by HPLC: 89.74 %

3.5.3.10 4-(4-Amino-2,6-dichlorophenoxy)-2-(piperidin-1-ylsulfonyl)phenol (16c)



Chemical Formula: $C_{17}H_{18}Cl_2N_2O_4S$

Molecular Weight: 417.31

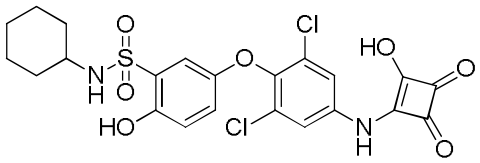
IR ($CHCl_3$) cm^{-1}: 3390, 3120, 1624, 1215

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45-1.49(m, 2H), 1.58-1.66(m, 4H), 3.02(t, *J* = 3.1 Hz, 4H), 6.67(s, 2H), 6.84(d, *J* = 3.0 Hz, 1H), 7.00(d, *J* = 9.0 Hz, 1H), 7.07-7.11(dd, *J* = 3.0 Hz & 9 Hz, 1H)

% Yield: 97 **%**

Purity by HPLC: 92.15 %

3.5.3.11 N-Cyclohexyl-5-(2,6-dichloro-4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl) amino) phenoxy)-2-hydroxybenzenesulfonamide (17a)



To Aniline compound **16a** (500 mg, 1.16 mmol), squaric acid (109 mg, 0.95 mmol) was added in (1 mL) water. The reaction mixture was stirred at reflux temperature for 2-6 hrs. The reaction mixture was then cooled to 20-30 ^{o}C and resulted solid was filtered to afford desired product which was purified by column chromatography using gradient ($CHCl_3$:MeOH) as an eluent to afford pure product (300 mg, 50 %).

Chemical Formula: $C_{22}H_{20}Cl_2N_2O7S$

Molecular Weight: 527.37

IR (KBr) cm^{-1}: 3244, 2931, 2854, 1589

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.02-1.18(m, 6H), 1.53-1.56(m, 4H), 2.78-2.80(br s, 1H), 6.93(d, *J* = 3 Hz, 1H), 7.06-7.10(dd, *J* = 8.7 Hz & 3 Hz, 1H), 7.21(d, *J* = 7.65 Hz, 1H), 8.09(s, 2H), □11.92(s, 1H)

ESI-MS: 525.9 [M-H]

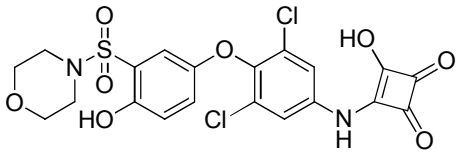
% Yield: 50 %

Purity by HPLC: 98.1 %

mp: 182-184 ^{o}C

Compounds 17b and 17c were prepared in an analogous manner using appropriate starting materials and the process described above

3.5.3.12 3-((3,5-Dichloro-4-(4-hydroxy-3-(morpholinosulfonyl) phenoxy) phenyl)amino)-4-hydroxycyclobut-3-ene-1,2-dione (17b)



Chemical Formula: $C_{20}H_{16}Cl_2N_2O_8S$

Molecular Weight: 515.32

IR (KBr) cm^{-1}: 3388, 2922, 1589

^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03-3.05(br s, 4H), 3.55-3.57(br s, 4H), 6.95-7.09(m, 3H), 8.09 (s, 2H)

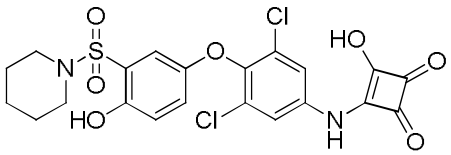
ESI-MS: 514 [M-H]

Purity by HPLC: 98.3 %

% Yield: 60%

mp: 196-199 ^oC;

3.5.3.13 3-((3,5-Dichloro-4-(4-hydroxy-3-(piperidin-1-ylsulfonyl) phenoxy) phenyl) amino)-4-hydroxycyclobut-3-ene-1,2-dione(17c)



Chemical Formula: $C_{21}H_{18}Cl_2N_2O_7S$

Molecular Weight: 513.35

IR (KBr) cm^{-1}: 3373, 2941, 1589

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45-1.47(br s, 6H), 3.03-3.05(br s, 4H), 6.94-7.07(m, 3H), 8.10 (s, 2H)

ESI-MS: 510.9 [M-H]

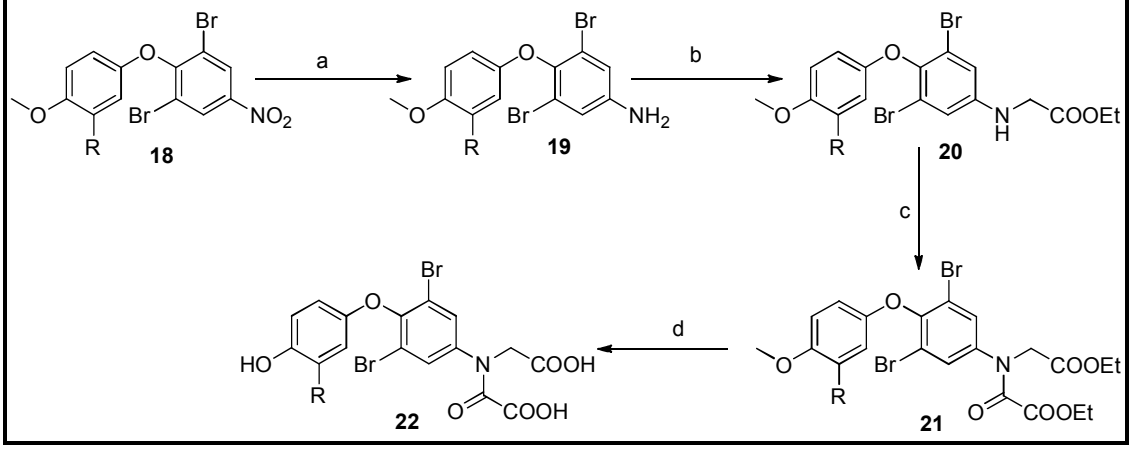
Purity by HPLC: 97.5 %

% Yield: 87 %

mp: 189-193 ^{o}C

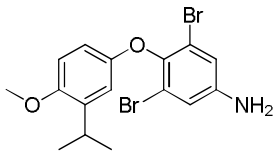
3.5.4 Oxo acetic acid derivatives

Scheme 3.4



Reagents and conditions : (a) $SnCl_2.2H_2O$,Conc.HCl,EtOH, 65-70 ^{o}C,3h, (b) $BrCH_2COOEt$,DMF, DIPEA,135 ^{o}C, 14-16h (c) Pyridine, ClCOCOOEt, ,DCM, 24h, 20-25^{o}C (d) 1M BBr_3, DCM, 25 ^{o}C, 3h

3.5.4.1 Synthesis of 3,5-Dibromo-4-(3-isopropyl-4-methoxyphenoxy)aniline (19a)



A solution of Stannous chloride dihydrate (13.17 g, 0.058 mol) in concentrated HCl (5.21 mL) was added to 3,5-dibromo-4-(4'-methoxy-3-isopropyl-phenoxy) nitro benzene **18a** (5.21 g, 0.011 mol) in EtOH (26 mL).The reaction mixture was refluxed for about 2 h. The resulting mixture was brought at 20-30 ^{o}C and diluted with ethyl acetate (50 mL). The mixture was made alkaline with ammonia solution. Resulting solid was filtered through cellite. The organic

phase was washed with water (2 x 50 mL), brine (50 mL), dried over sodium sulphate, filtered and concentrated to give desired product (4.56 g, 94 %).

Chemical Formula: $C_{16}H_{17}Br_2NO_2$

Molecular Weight: 415.

IR ($CHCl_3$) cm^{-1}: 3390, 1272, 864

^1H NMR (400 MHz, $CDCl_3$): δ 1.20(d, *J* = 6.8 Hz, 6H), 3.2-3.3(m, 1H), 3.78(s, 3H), 6.42-6.45(dd, *J* = 2.8 Hz & 8.5 Hz, 1H), 6.71(d, *J* = 8.8 Hz, 1H), 6.89(s, 2H), 6.93(d, *J* = 3.2 Hz, 1H)

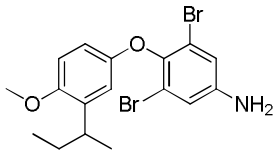
ESI-MS: 414.2 [M-H]

% Yield: 94**%**

Purity by HPLC: 88.86%

Compounds 19b and 19c were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.4.2 3,5-Dibromo-4-(3-(*sec*-butyl)-4-methoxyphenoxy)aniline (19b)



Chemical Formula: $C_{17}H_{19}Br_2NO_2$

Molecular Weight: 429.

IR ($CHCl_3$) cm^{-1}: 3414, 1280, 852

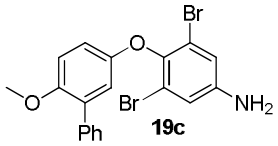
^1H NMR (400 MHz, $CDCl_3$): δ 0.78(t, *J* = 7.2 Hz, 3H), 1.16(d, *J* = 6.8 Hz, 3H), 1.49-1.54(m, 2H), 3.03-3.08(m, 1H), 3.77(s, 3H), 6.04-6.48(dd, *J* = 3.2 Hz & 8.8 Hz, 1H), 6.69(d, *J* = 8.4 Hz, 1H), 6.87(s, 2H) 6.9(d, *J* = 2.8 Hz, 1H)

ESI-MS: 428.4 [M-H]

% Yield: 92 %

Purity by HPLC: 87.41 %

3.5.4.3 3,5-Dibromo-4-((6-methoxy-[1,1'-biphenyl]-3-yl)oxy)aniline(19c)



19c

Chemical Formula: $C_{19}H_{15}Br_2NO_2$

Molecular Weight: 449.14

IR ($CHCl_3$) cm^{-1}: 3420, 1268

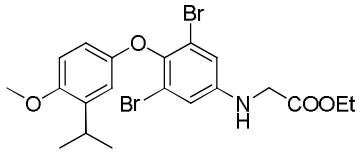
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75(s, 3H), 6.75-6.78(dd, *J* = 3.2 Hz & 9.2 Hz, 1H), 6.81(d. *J* = 2.8 Hz, 1H), 6.87-6.89(m, 3H), 7.28-7.33(m, 1H), 7.36-7.40(m, 2H), 7.49-7.52(m, 2H)

ESI-MS: 450 $[M+H]^+$

% Yield: 92 %

Purity by HPLC: 84.86 %

3.5.4.4 2-((3,5-Dibromo-4-(3-isopropyl-4-methoxy phenoxy) phenyl) amino) acetate (20a)



To 3,5-dibromo-4-(3-isopropyl-4-methoxyphenoxy)aniline **(19a)** (4.52 g, 0.01 mol) was added ethyl bromoacetate (1.81 g, 0.01 mol) and diisopropylethyl amine (1.40 g, 0.01 mol) in DMF(45 mL) & stirred at 140 ^oC for 16 h. The reaction mixture was poured in to ice-water. The product was taken up in ethyl acetate (2 x 45 mL), washed with water (2 x 45 mL), brine (45 mL), dried over sodium

sulphate, filtered and concentrated to give the crude product. The crude product was purified by column chromatography over flash silica gel (Hexane: Ethyl acetate 90:10) to afford the pure product (3.42 g, 63 %)

Chemical Formula: $C_{20}H_{23}Br_2NO_4$

Molecular Weight: 501.21

IR (KBr) cm^{-1}: 3396, 1195, 1736

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18(d, *J* = 6.8 Hz, 6H), 1.32(t, *J* = 7.2 Hz, 3H), 3.23-3.30(m, 1H), 3.77(s, 3H), 3.87(d, *J* = 5.2 Hz, 2H) 4.25-4.30(q, *J* = 7.2 Hz, 2H), 6.42-6.45(dd, *J* = 2.8 Hz & 8.8 Hz, 1H), 6.69(d, *J* = 8.8 Hz, 1H), 6.81-6.83(m, 3H)

ESI-MS: 501.5 $[M+H]^+$

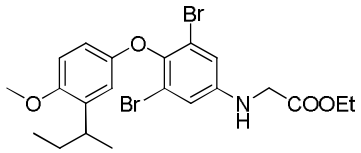
% Yield: 63 %

Purity by HPLC: 95.73 %

mp: 155-160 ^oC

Compounds 20b and 20c were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.4.5 3-Ethyl-2-((3,5-dibromo-4-(3-(sec-butyl)-4 methoxy phenoxy) phenyl)amino) acetate (20b)



Chemical Formula: $C_{21}H_{25}Br_2NO_4$

Molecular Weight: 515.24.

IR ($CHCl_3$) cm^{-1}: 3388, 1741, 1215

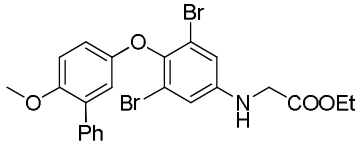
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83(t, *J* = 7.2 Hz, 3H), 1.20(d, *J* = 6.8 Hz, 3H), 1.32(t, *J* = 7.2 Hz, 3H), 1.49-1.59(m, 2H), 3.03-3.08(m, 1H), 3.76(s, 3H), 3.87(s, 2H), 4.25-4.30(q, J = 6.8 Hz, 2H), 6.46-6.49(dd, *J* = 2.8 Hz & 8.8 Hz, 1H), 6.71(d, *J* = 8.8 Hz, 1H), 6.74(d, *J* = 3.2 Hz, 1H), 6.81(s, 2H)

ESI-MS: 516.3 $[M+H]^+$

% Yield: 78 %

Purity by HPLC: 78.53 %

3.5.4.6 Ethyl 2-((3,5-dibromo-4-((6-methoxy-[1,1'-biphenyl]-3-yl)oxy) phenyl)amino) acetate (20c)



Chemical Formula: $C_{23}H_{21}Br_2NO_4$

Molecular Weight: 535.23

IR ($CHCl_3$) cm^{-1}: 3392, 1741, 1228

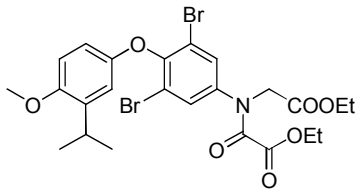
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31(t, *J* = 7.2 Hz, 3H), 3.75(s, 3H), 3.86(d, *J* = 4.8 Hz, 2H) 4.24-4.30(q, *J* = 7.2 Hz &14.4 Hz, 2H), 4.72(s, 1H), 6.74-6.77(dd, *J* = 3.2 Hz & 9.2 Hz, 1H), 6.80-6.83(m, 3H), 6.88(d, J = 9.2 Hz, 1H), 7.31(d, *J* = 7.6 Hz, 1H), 7.38(t, *J* = 7.2 Hz & 8.0 Hz, 2H), 7.51(d, *J* = 7.2 Hz, 2H)

ESI-MS: 536.5 $[M+H]^+$

% Yield: 88 %

Purity by HPLC: 83.29 %

3.5.4.7 Ethyl 2-((3,5-dibromo-4-(3-isopropyl-4-methoxyphenoxy) phenyl) (2-ethoxy-2-oxoethyl)amino)-2-oxoacetate (21a)



To a solution of ethyl 2-(3,5-dibromo-4-(3-isopropyl-4-methoxyphenoxy) phenyl amino) acetate **20a** (500 mg, 0.99 mmol) in dichloromethane (5 mL), pyridine (0.0.23 g, 2.97 mmol) was added at 0-5 ^{o}C. After 10 min. to that Ethyl oxalylchloride (0.39 g, 2.92 mmol) was added dropwise during 10-15 min. The reaction mixture was stirred at 20-25 ^{o}C for another 24h. The reaction mixture was poured in to ice-water (10 mL). The product was taken up in ethyl acetate (2 x 25 mL), washed with water (2 x 25 mL), brine, dried over sodium sulphate, filtered and concentrated to give the crude product. The crude product was purified by column chromatography over flash silica gel (Hexane : ethyl acetate, 90:10) to afford the pure product (570 mg, 95 %)

Chemical Formula: $C_{24}H_{27}Br_2NO_7$

Molecular Weight: 601.28

IR ($CHCl_3$) cm^{-1}: 3018, 1749, 1215, 756

^{1}H NMR (400 MHz, CD_3OD): δ 1.17-1.34(m, 9H), 1.29(t, *J* = 7.2 Hz, 3H), 3.31-3.27(m, 1H), 3.79(s, 3H), 4.27-4.22(q, *J* = 7.2 Hz, 2H), 4.19-4.16(q, *J* = 7.2 Hz, 2H), 4.54(s, 2H), 6.47-6.44(dd, *J* = 3.2 Hz & 8.8 Hz, 1H), 6.69(d, *J* = 3.2 Hz, 1H), 6.81(d, *J* = 8.8 Hz, 1H), 7.89(, s, 2H)

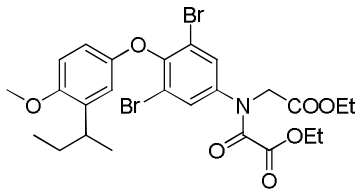
ESI-MS: 623 $[M+Na]^+$

% Yield: 95 %

Purity by HPLC: 97 %

Compounds 21b and 21c were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.4.8 Ethyl 2-((3,5-dibromo-4-(3-(sec-butyl)-4-methoxyphenoxy)phenyl)(2-ethoxy -2-oxoethyl)amino)-2-oxoacetate (21b)



Chemical Formula: $C_{25}H_{29}Br_2NO_7$

Molecular Weight: 615.31

IR ($CHCl_3$) cm^{-1}: 3018, 1749, 1685, 1215

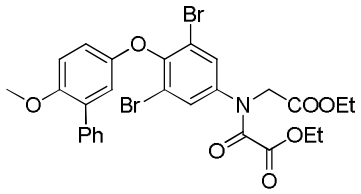
^1H NMR (400 MHz, $CDCl_3$): δ 0.83(t, J = 7.2 Hz, 3H), 1.149(d, *J* = 7.2 Hz, 3H), 1.187(t, *J* = 7.2 Hz, 3H) 1.312(t, *J* = 7.2 Hz, 3H), 1.59-1.52(m, 2H), 3.09-3.043(m, 1H), 4.21-4.153(q, *J* = 7.2 Hz, 2H), 4.29-4.23(q, *J* = 7.2 Hz, 2H), 3.78(s, 3H), 4.44(s, 2H), 6.45-6.42(dd, *J* = 3.2 Hz & 8.8 Hz, 1H) 6.73-6.71(m, 2H), 7.65(s, 2H)

ESI-MS: 616 $[M+H]^+$

% Yield: 84 %

Purity by HPLC: 95 %

3.5.4.9 Ethyl 2-((3,5-dibromo-4-((6-methoxy-[1,1'-biphenyl]-3-yl)oxy) phenyl) (2-ethoxy-2-oxoethyl)amino)-2-oxoacetate (21c)



Chemical Formula: $C_{27}H_{25}Br_2NO_7$

Molecular Weight: 635.30

IR ($CHCl_3$) cm^{-1}: 1753, 1722, 1215

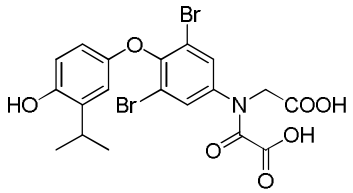
^1H NMR (400 MHz, $CDCl_3$): δ 1.07-1.16(m, 3H), 1.27-1.33(m, 3H), 3.77(s, 3H), 4.08-4.14(m, 2H), 4.22-4.27(q, *J* = 7.2 Hz, 2H), 4.44(s, 2H), 6.74-6.77(m, 2H), 6.88-6.91(m, 1H), 7.29-7.33(m, 1H), 7.37-7.40(m, 2H), 7.47-7.51(m, 2H), 7.66(s, 2H)

ESI-MS: 635.8 $[M+H]^+$

% Yield: 91 %

Purity by HPLC: 92.14 %

3.5.4.10 2-((Carboxymethyl)(3,5-dibromo-4-(4-hydroxy-3-isopropyl phenoxy)phenyl)amino)-2-oxoacetic acid (22a)



To a solution of ethyl 2-((3,5-dibromo-4-(3-isopropyl-4-methox yphenoxy) phenyl) (2-ethoxy-2-oxoethyl)amino)-2-oxoacetate **21a** (300 mg, 0.49 mmol) in DCM (12.5 mL) was cooled to -60 ^oC under nitrogen atmosphere. To that 1M BBr_3 solution (2.99 mL) was added dropwise. The reaction mixture was allowed to warm up to 20-25 ^oC over 5 h. then diluted with more DCM (30 mL) and quenched with water. After stirring at 20-25 ^oC for 30 min, organic phase was separated, washed with water (2 x 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated to give crude product. The crude product was purified by column chromatography over flash silica gel (Hexane : ethyl acetate, 90:10) to afford the pure product. (200 mg, 75 %)

Chemical Formula: $C_{19}H_{17}Br_2NO_7$

Molecular Weight: 531.15

IR (KBr) cm^{-1}: 3389, 3074, 1618, 1448

^{1}H NMR (400 MHz, CD_3OD): δ 1.17(d, *J* = 6.8 Hz, 6H), 3.21-3.56(m, 1H), 4.22(s, 2H), 6.28-6.25(m, 1H), 6.45(m, 1H), 6.67(m, 1H), 7.81(s, 2H),

ESI-MS : 529 [M-H]

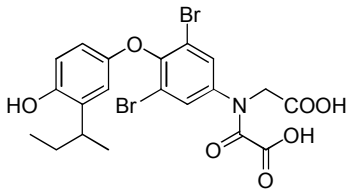
Yield: 75**%**

Purity by HPLC: 96%

mp: 146-151 ^oC

Compounds 22b and 22c were prepared in an analogous manner using appropriate starting materials and the process described above.

3.5.4.11 2-((Carboxymethyl)(3,5-dibromo-4-(3-(sec-butyl)-4-hydroxy phenoxy)phenyl) amino)-2-oxoacetic acid (22b)



Chemical Formula: $C_{20}H_{19}Br_2NO_7$

Molecular Weight: 545.18

IR (KBr) cm^{-1}: 3412, 1624, 1202, 777

^{1}H NMR (400 MHz, CD_3OD): δ 0.85(t, J = 7.2 Hz, 3H), 1.15(d, *J* = 6.8 Hz, 3H), 1.6-1.52(m, 2H), 3.05-2.99(m, 1H), 4.28(s, 2H), 6.32-6.29(dd, *J* = 2.8 Hz & 8.8 Hz, 1H), 6.62(d, *J* = 8.8 Hz, 1H), 6.482(d, *J* = 2.8 Hz, 1H), 7.797(s, 2H)

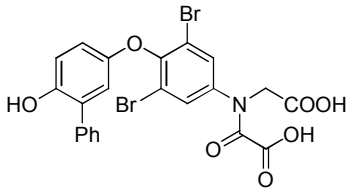
ESI-MS: 544 [M-H]

Yield: 97**%**

Purity by HPLC: 91%

mp: 85–90 ^{o}C

3.5.4.12 2-((Carboxymethyl)(3,5-dibromo-4-((6-hydroxy-[1,1'-biphenyl]-3-yl) oxy) phenyl) amino)-2-oxoacetic acid(22c)



Chemical Formula: $C_{22}H_{15}Br_2NO_7$

Molecular Weight: 565.17

IR (KBr) cm^{-1}: 3547, 1718, 1618, 1230

^{1}H NMR (300 MHz, CD_3OD): δ 4.41(s,2H), 6.52-6.55(dd, J = 2.8 Hz & 8.4 Hz, 1H,), 6.77-6.79(m, 2H), 7.25-7.29(m, 1H) , 7.34-7.38(m, 2H), 7.50-7.52(m, 2H), 7.83(s, 2H)

ESI-MS: 563 [M-H]

Yield: 61 **%**

Purity by HPLC: 92 %

mp: >200 ^{o}C

3.5.5 Thyroid receptor assay

A luciferase receptor assay has been used to find the TR α and TR β selectivities of the compounds, where luciferase gene expression is driven by a thyroid receptor binding element (TRE) upstream of the luciferase gene. Briefly 6 x 10^4 CV-1 cells were plated in each well of a 24-well cell culture plate. The cells were transfected 16 h after seeding with a plasmid bearing three copies of TRE cloned upstream of luciferase gene along with a plasmid expressing either the full length human thyroid receptor α or β isoform and a third plasmid expressing β-galactosidase. The transfection was carried out using polyfect reagent from In vitrogen, Inc. (Carlsbad, CA). The medium was replaced 6 h post transfection with fresh media having different concentrations of the agonist. The concentration of agonist is adjusted in such a way that the concentration of the solvent (DMSO) in each well was maintained at 1%. The plates were incubated at 37 ^oC for 16 h before lysing and assaying the luciferase activity using commercially available Glo-lysis kit from Promega and a standard luminometer. The β-galactosidase activity was measured by using the bgalactosidase assay kit from Promega and the absorbance was read at 415 nM.

3.5.6 Pharmacokinetics experiment

Pharmacokinetic behaviour of the test compounds was studied *via* per-oral route of administration in *SD* rats of 8 to 10 weeks of age. Animals were fasted for 18 hours and food was supplied after 4 hours of administration of the test compound. There was free access to water throughout the study. A homogenous suspension of the test substance was prepared in 0.5 % w/v CMC

in normal saline and a per-oral dose of 30 mg/kg was administered. After the administration of the test compounds, blood samples were withdrawn at various time intervals through retro-orbital plexus and collected into heparinized micro centrifuge tubes. Plasma was separated by centrifugation at 4000 rpm for 5 min at ambient temperature and analyzed immediately. Remaining samples were stored at -20 °C until analyzed.

Analysis was carried out by taking an aliquots of 180 μL plasma and 20 μL of internal standard (Atorvastatin) and was extracted with 2.5 mL of extracting solvent (ethyl acetate: acetonitrile 80:20, v/v) in glass test-tube by vortexing with spinix vortex mixture for a minute. This was then centrifuged at 2000 rpm for 2.0 min. The supernatant was transferred to another glass test-tube and the solvent was evaporated under nitrogen using Zymark evaporator at 40 °C. Finally, the tubes were reconstituted with 0.1 mL diluent (acetonitrile: MeOH: water 40:40:20, v/v/v). The reconstituted samples were analyzed on Agilent 1100 Series HPLC system with a mobile phase of 0.05 % v/v trifluoroacetic acid in water: acetonitrile (32:68, v/v); flowing at a flow rate of 1.0 mL/min through a Kromasil 250 mm x 4.6 mm x 5 μ column maintained at 30 °C. Chromatographic separation was achieved within 15 min. Agilent software version Chemstation Rev.A.09.01. (1206) was used to acquire and process all chromatographic data. Quantification was based on a series of calibrators ranging from 0.031 to 32 μg/mL, prepared by adding test compound to drug free rat plasma. Quality control samples were analyzed in parallel to verify that the system performs in control. Pharmacokinetic parameters namely; maximum plasma concentration (C_{max}), time point of

maximum plasma concentration (t_{max}), area under the plasma concentration–time curve from 0 hour to infinity ($AUC_{0-\infty}$) and half-life of drug elimination during the terminal phase ($t_{1/2}$) were calculated from plasma concentration *versus* time data, by standard non-compartmental methods, using the WinNonLin software version 4.0.1 procured from Pharsight Corporation, USA.

3.5.7 Portal-systemic cannulated rat model

Liver selectivity was determined using Portal-systemic cannulated rat model.

Method: Sprague Dawley rats of age 8-10 weeks weighing >275 g were used for the procedure. The animals were cannulated permanently at portal vein and carotid artery. From the same animal 0.3 mL blood was collected in EDTA tubes from portal venous cannula and carotid arterial cannula at the specified time-points starting from 5 min upto 24 hours simultaneously (with no cross-overs). The AUC were calculated using the portal cannula samples for hepatic and carotid cannula samples for systemic pharmacokinetics. The Eh, hepatic extraction ratio, calculated using the formula: Eh = 1-(AUC0-inf systemic/AUC0-inf portal).

3.6 References

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3.7 Spectra

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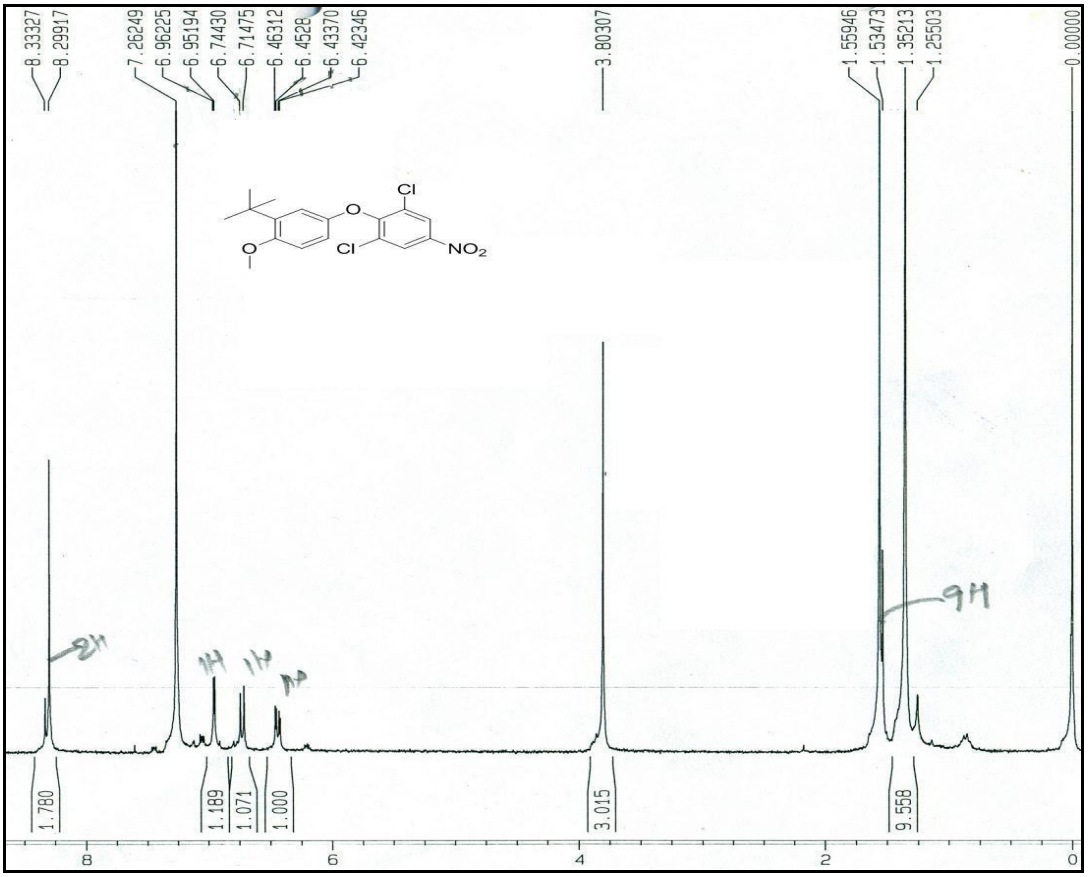

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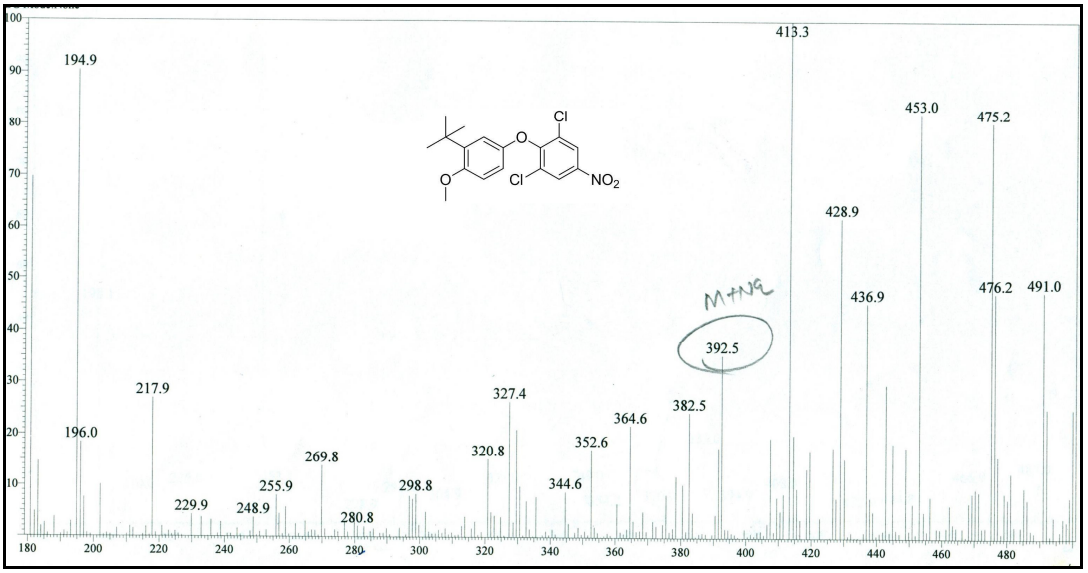

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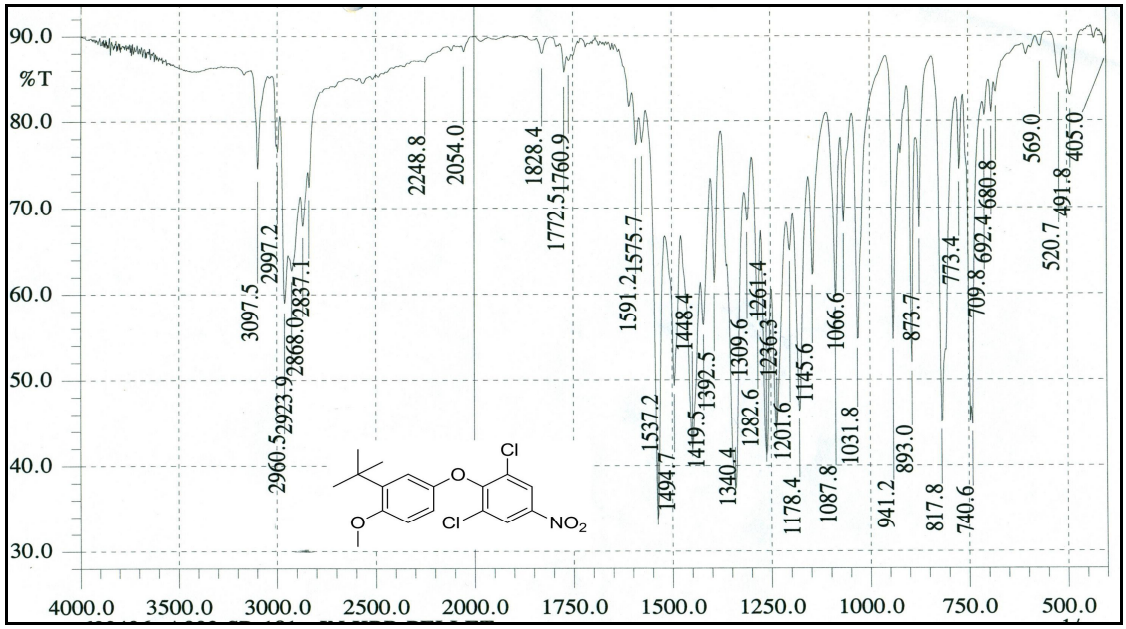

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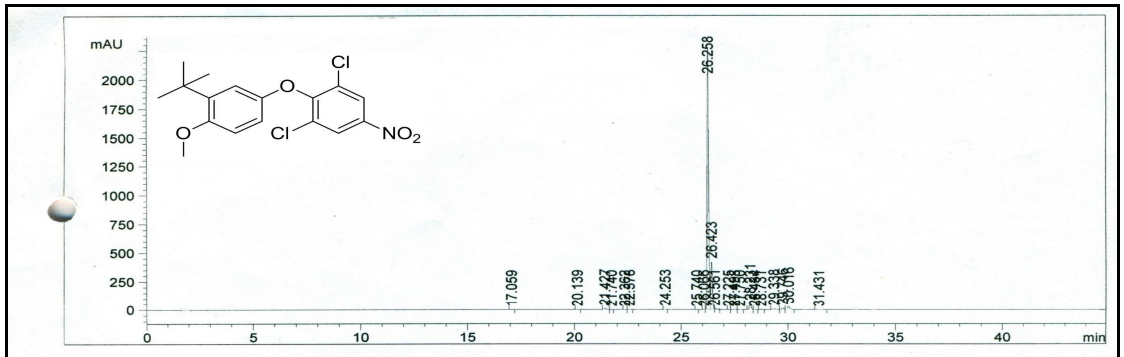

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4	21.740	BV	0.0908	3.45201	6.09476e-1	0.0241
5	22.362	BV	0.0899	5.40104	9.26221e-1	0.0378
6	22.578	VP	0.0884	17.64308	3.09073	0.1233
7	24.253	PBA	0.0860	30.02901	5.58709	0.2099
8	25.740	BBA	0.0816	3.05667	5.82630e-1	0.0214
9	26.066	BV	0.0760	7.56627	1.50905	0.0529
10	26.258	VV	0.0783	1.12358e4	2263.07202	78.5430
11	26.423	MF R	0.0775	2126.69287	421.19479	14.8665
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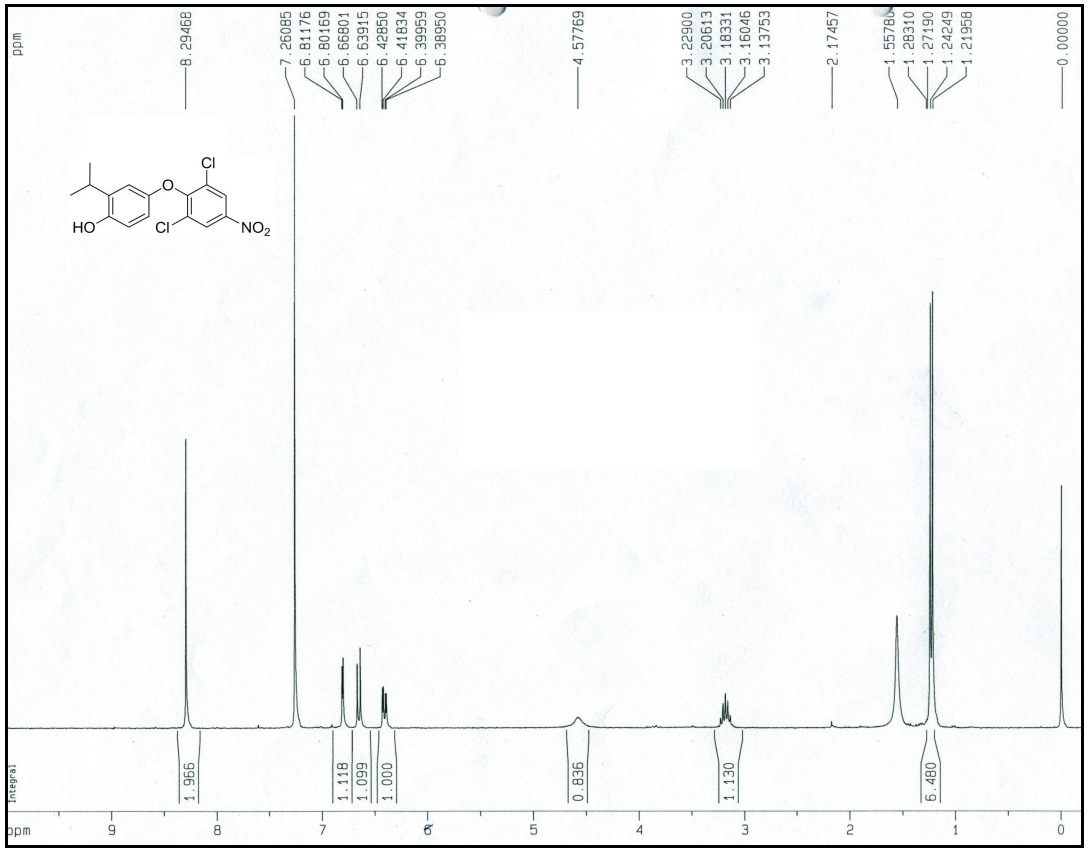
^{1}H NMR of 4b



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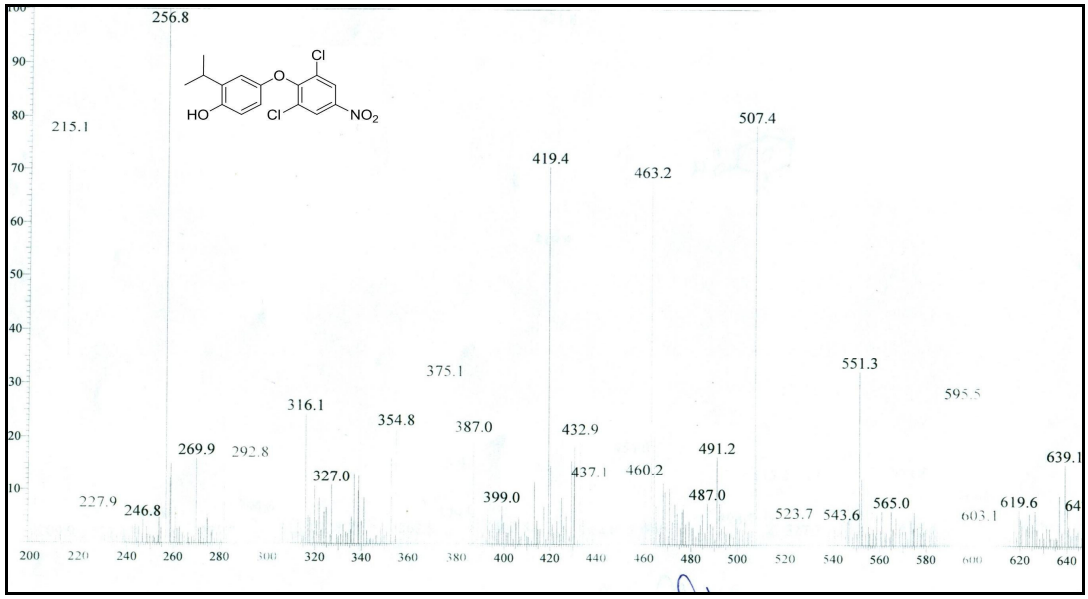

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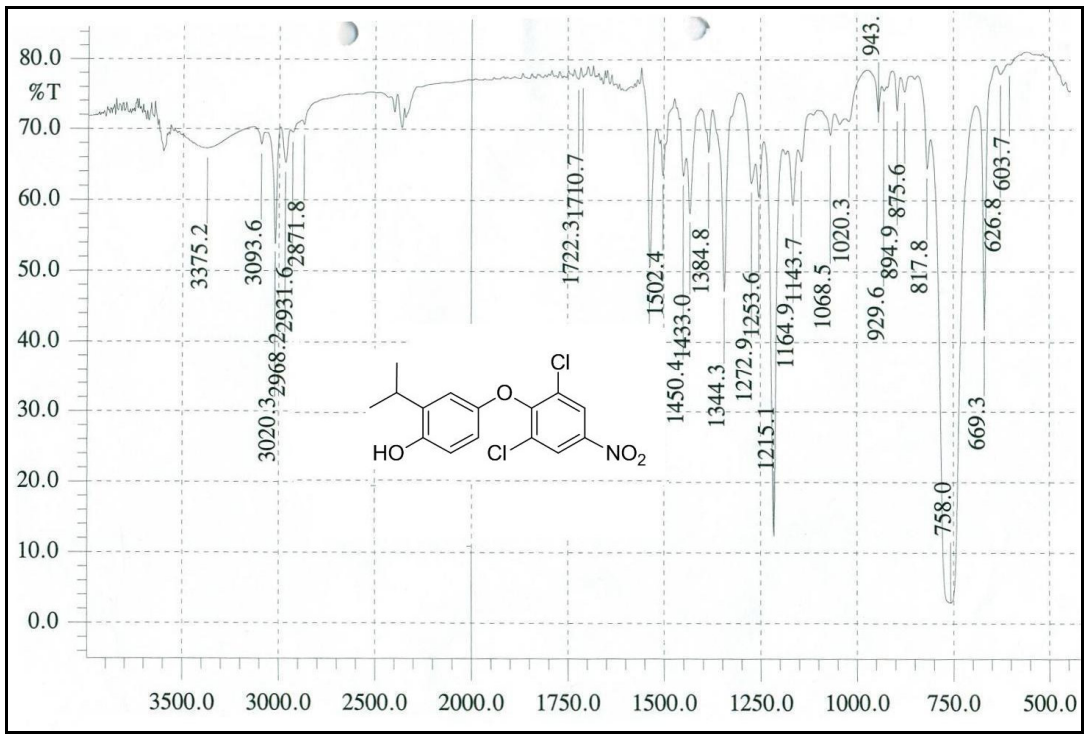
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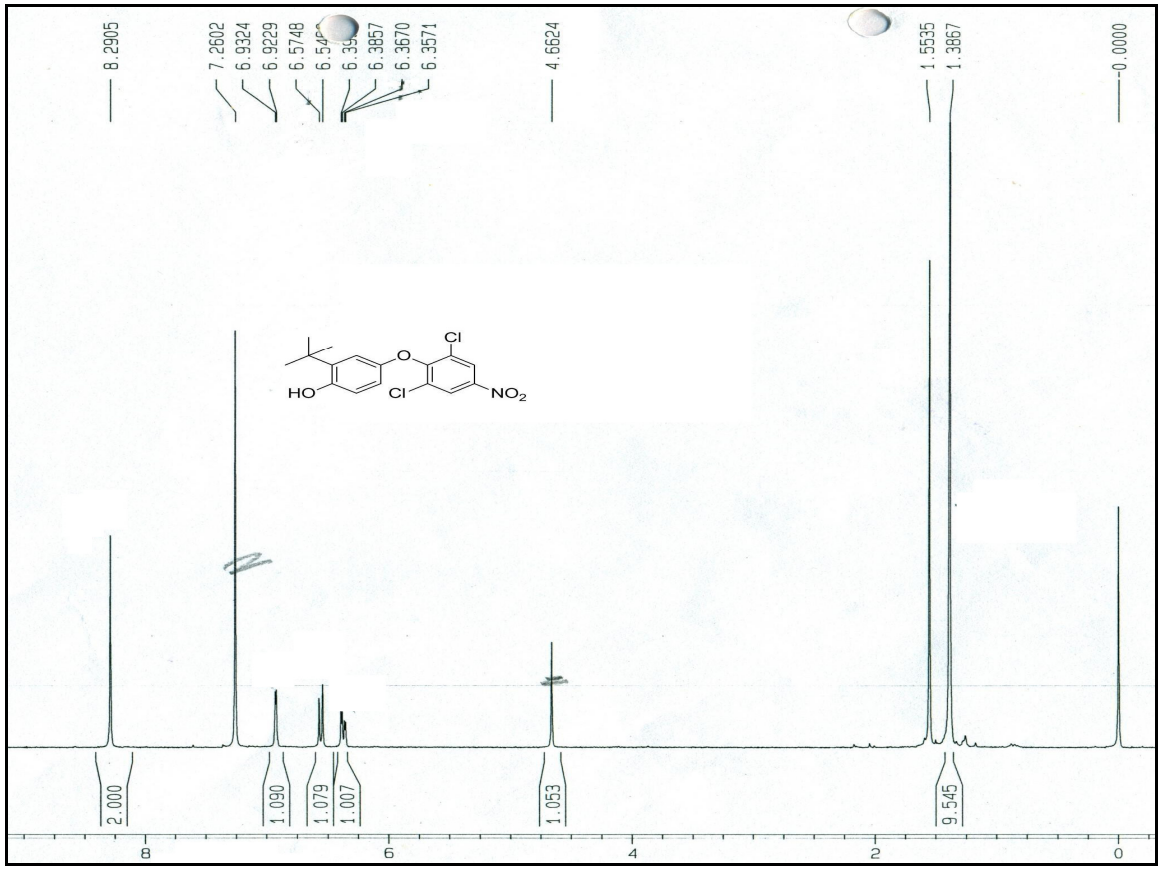
^{1}H NMR of 4c



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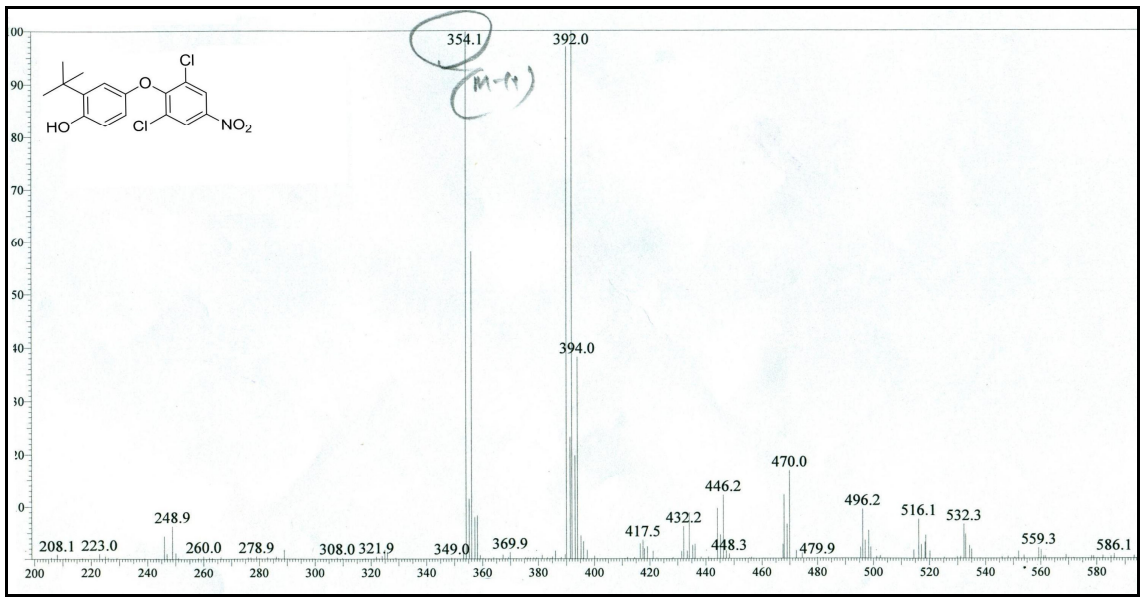

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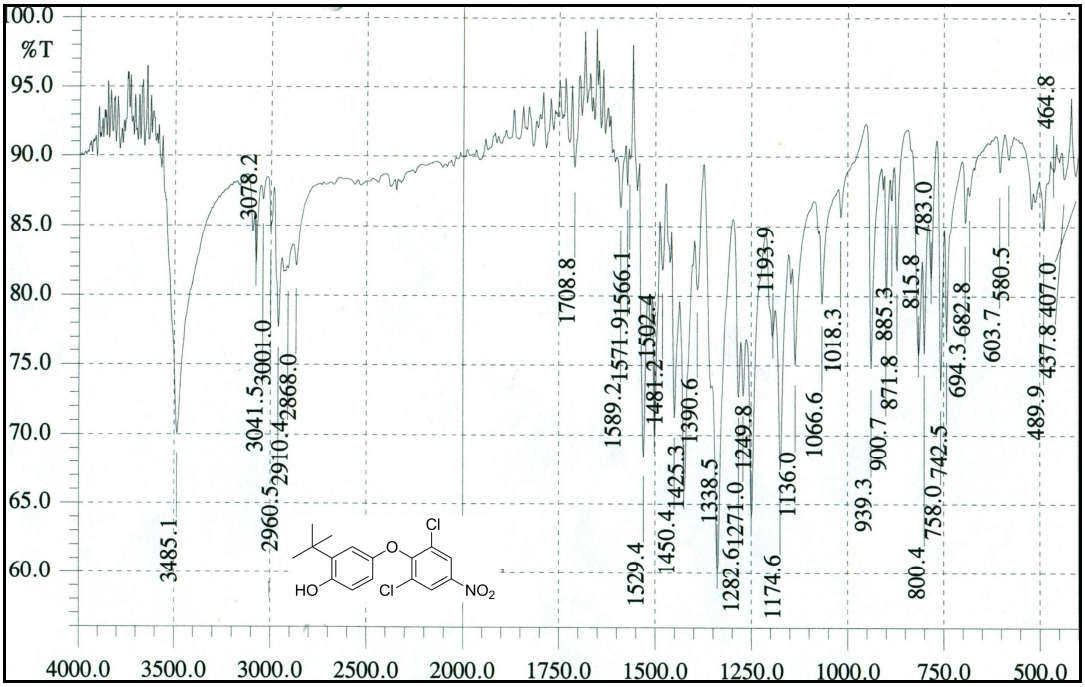
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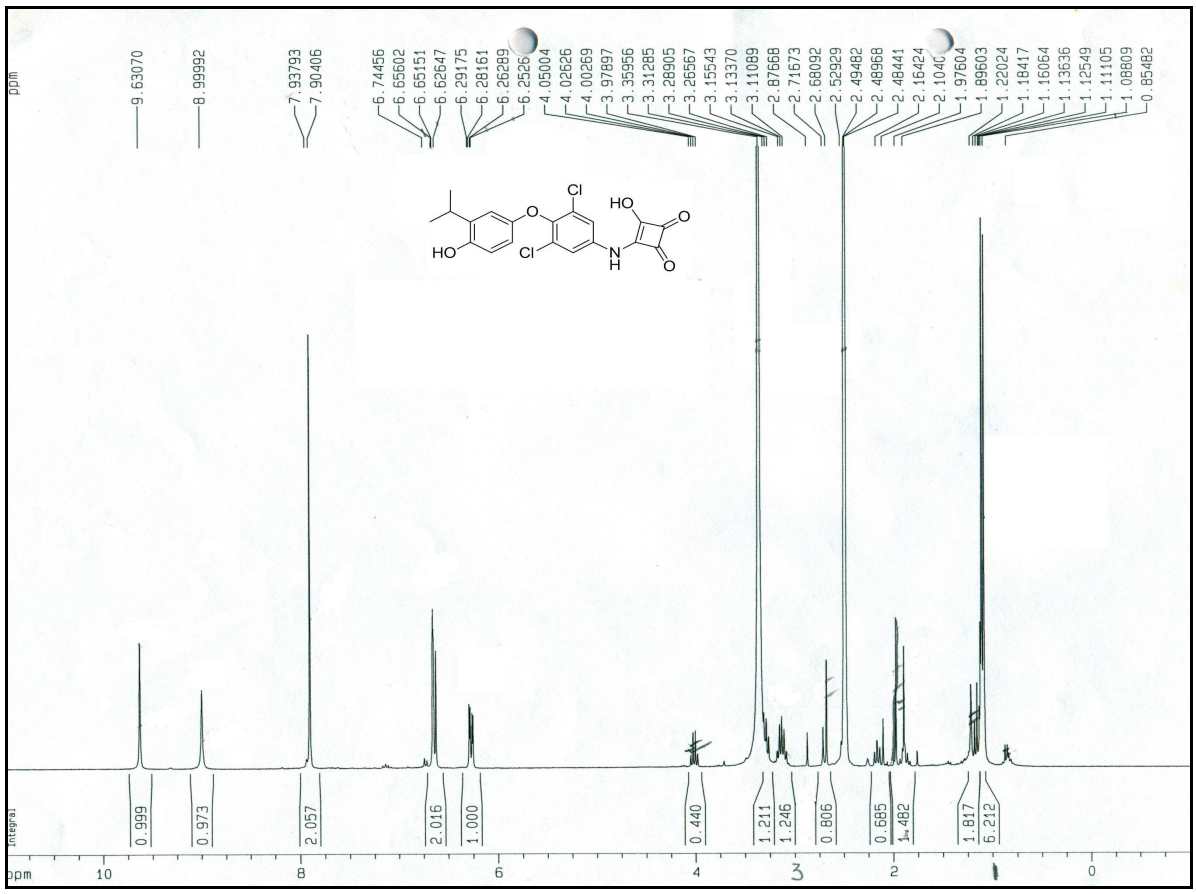
^{1}H NMR of 6b



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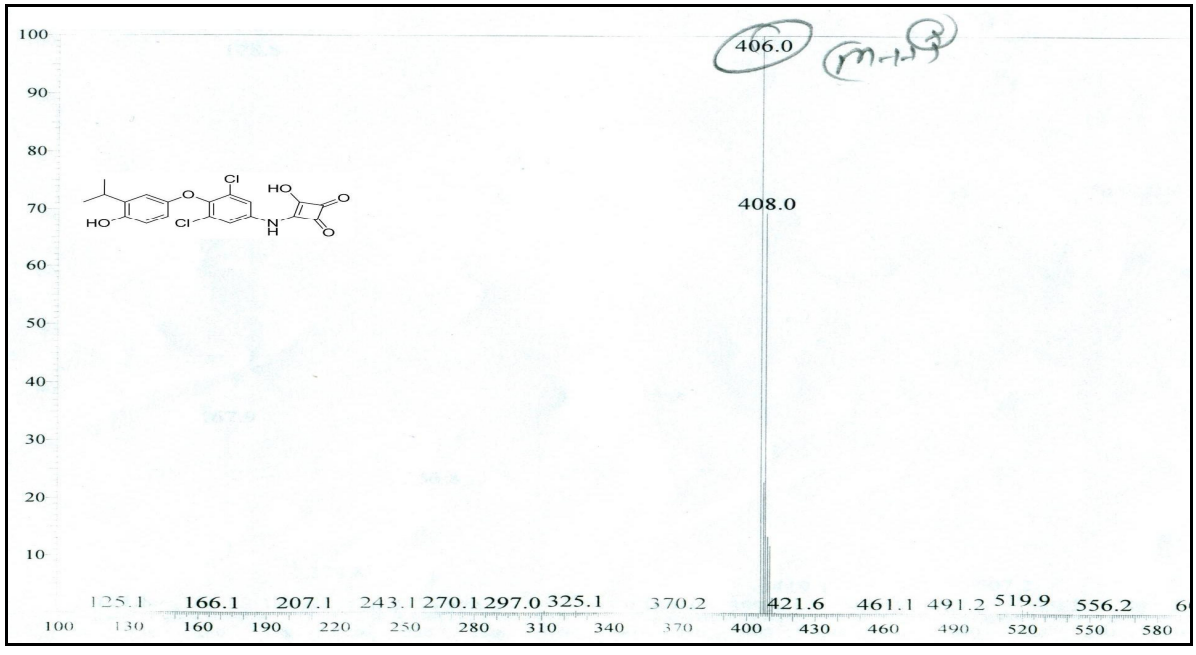

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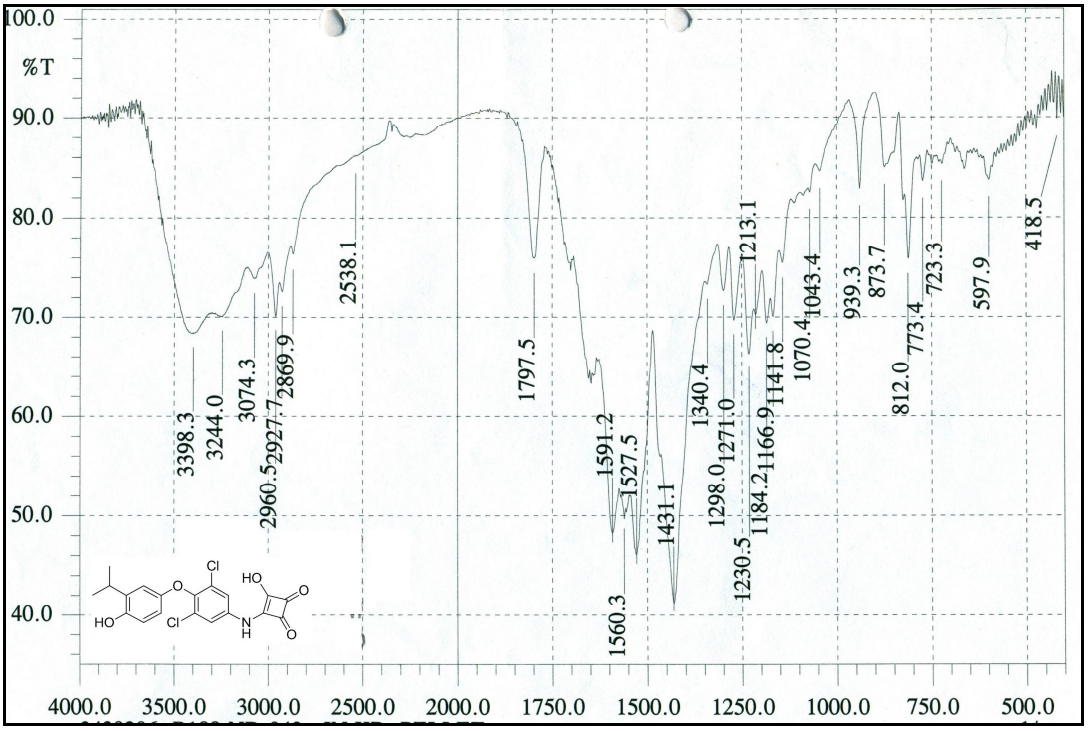
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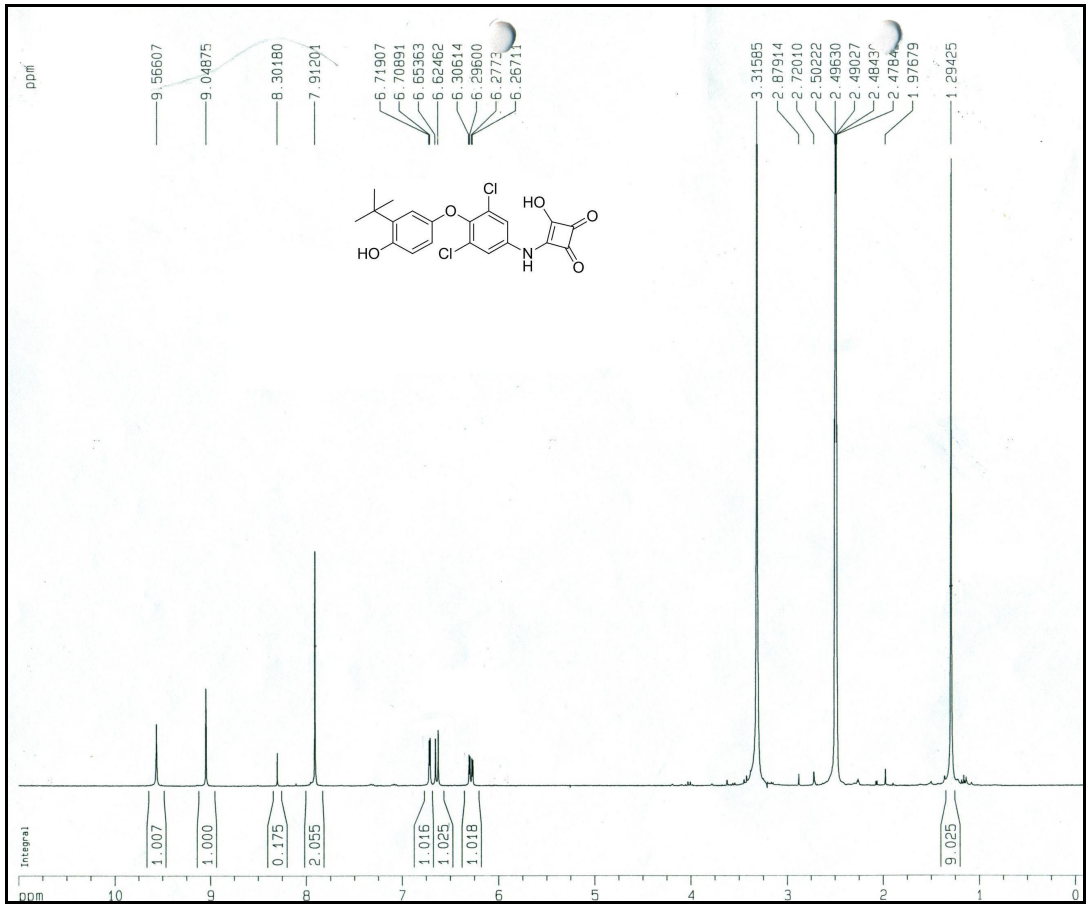
^{1}H NMR of 6c



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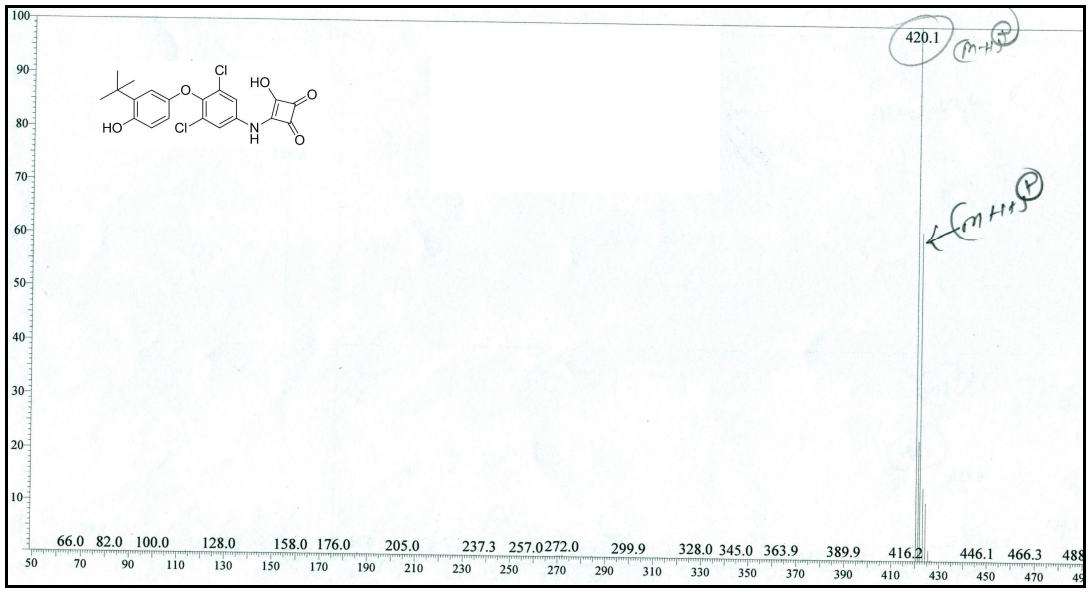

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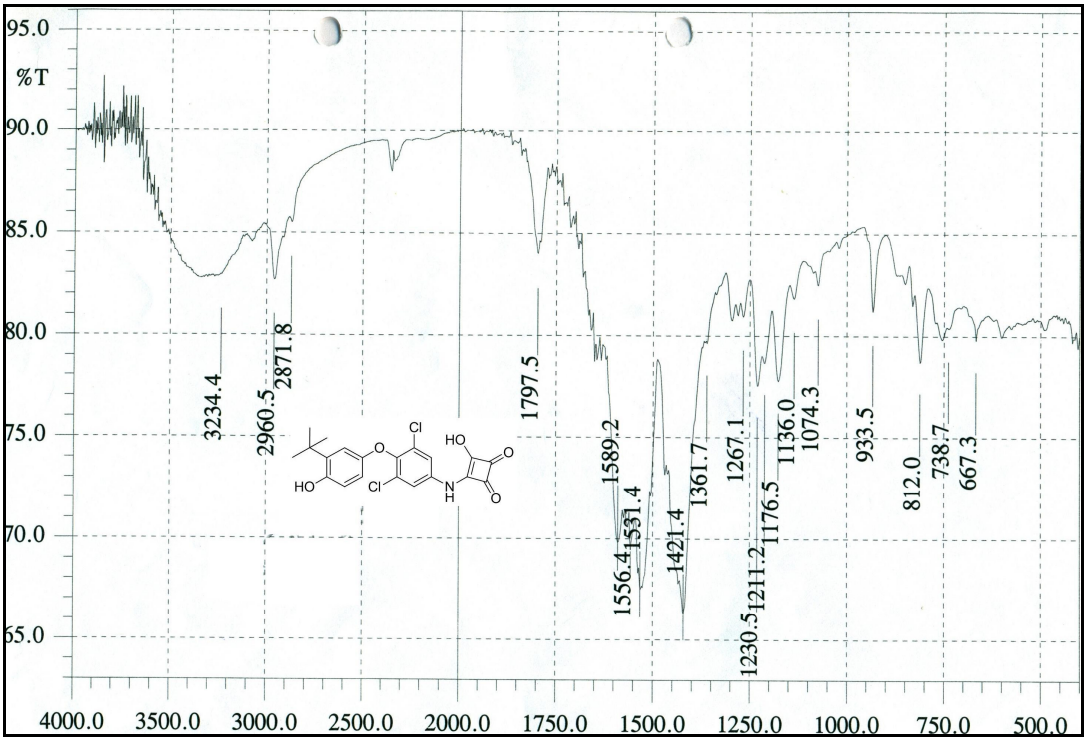
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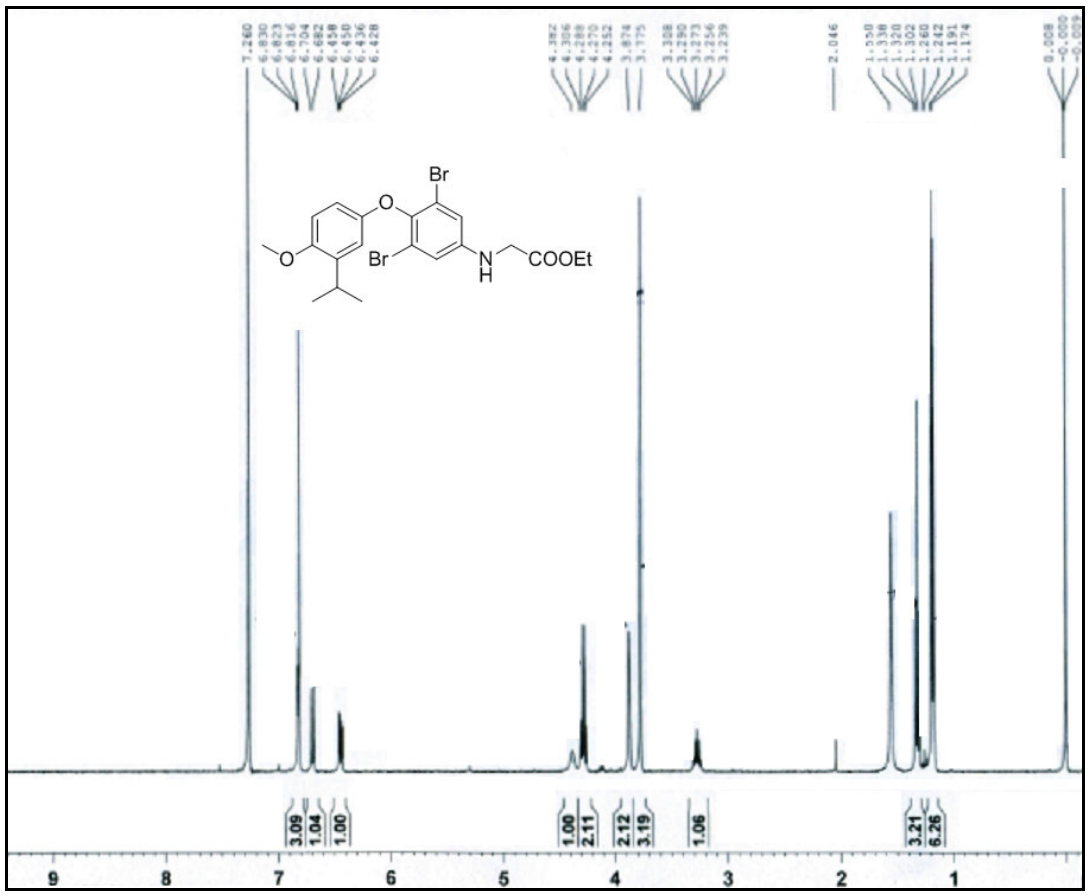
^{1}H NMR of 20a



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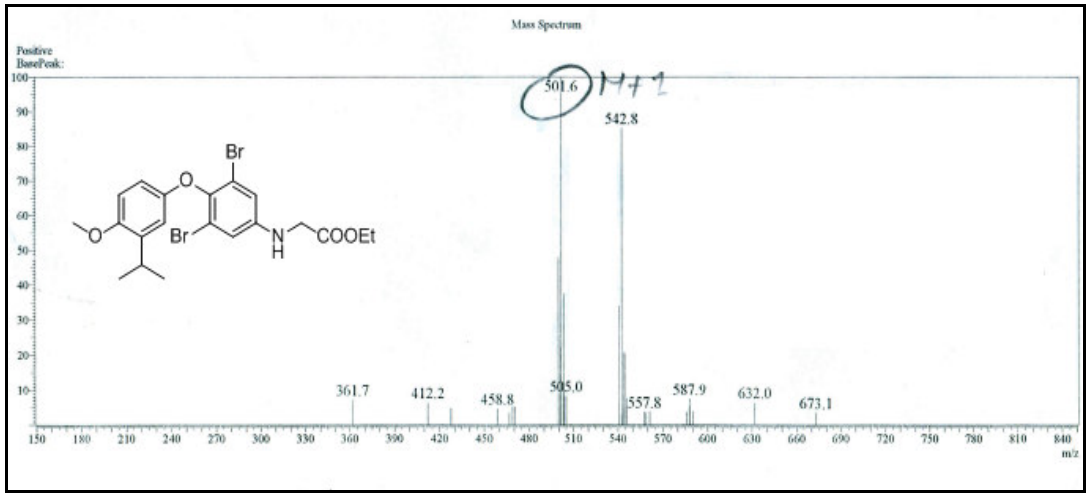

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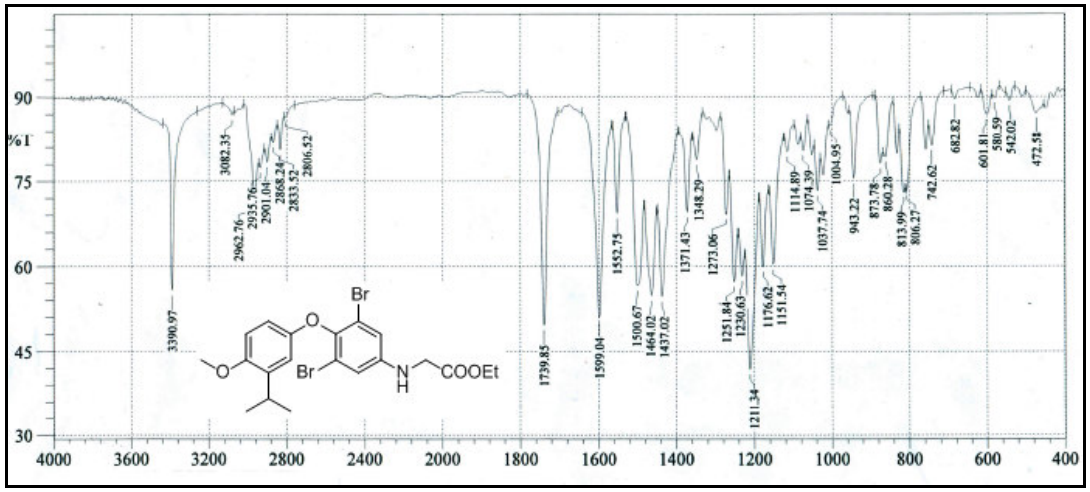

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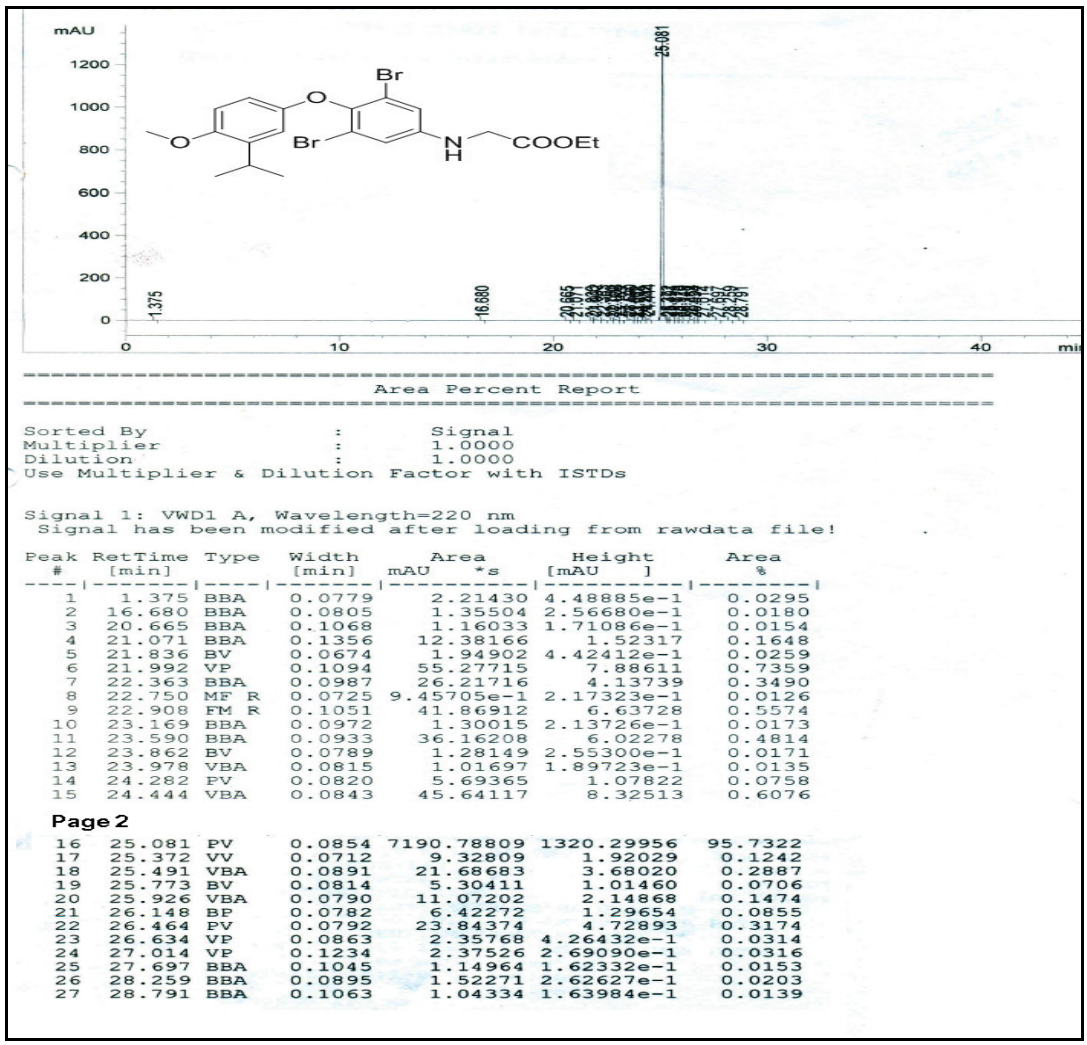

Area Percent Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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3	20.665	BBA	0.1068	1.16033	1.71086e-1	0.0154
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5	21.836	BV	0.0674	1.94902	4.42412e-1	0.0259
6	21.992	VP	0.1094	55.27715	7.88611	0.7359
7	22.363	BBA	0.0987	26.21716	4.13739	0.3490
8	22.750	MF R	0.0725	9.45705e-1	2.17323e-1	0.0126
9	22.908	FM R	0.1051	41.86912	6.63728	0.5574
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13	23.978	VBA	0.0815	1.01697	1.89723e-1	0.0135
14	24.282	PV	0.0820	5.69365	1.07822	0.0758
15	24.444	VBA	0.0843	45.64117	8.32513	0.6076

Page 2

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17	25.372	VV	0.0712	9.32809	1.92029	0.1242
18	25.491	VBA	0.0891	21.68683	3.68020	0.2887
19	25.773	BV	0.0814	5.30411	1.01460	0.0706
20	25.926	VBA	0.0790	11.07202	2.14868	0.1474
21	26.148	BP	0.0782	6.42272	1.29654	0.0855
22	26.464	PV	0.0792	23.84374	4.72893	0.3174
23	26.634	VP	0.0863	2.35768	4.26432e-1	0.0314
24	27.014	VP	0.1234	2.37526	2.69090e-1	0.0316
25	27.697	BBA	0.1045	1.14964	1.62332e-1	0.0153
26	28.259	BBA	0.0895	1.52271	2.62627e-1	0.0203
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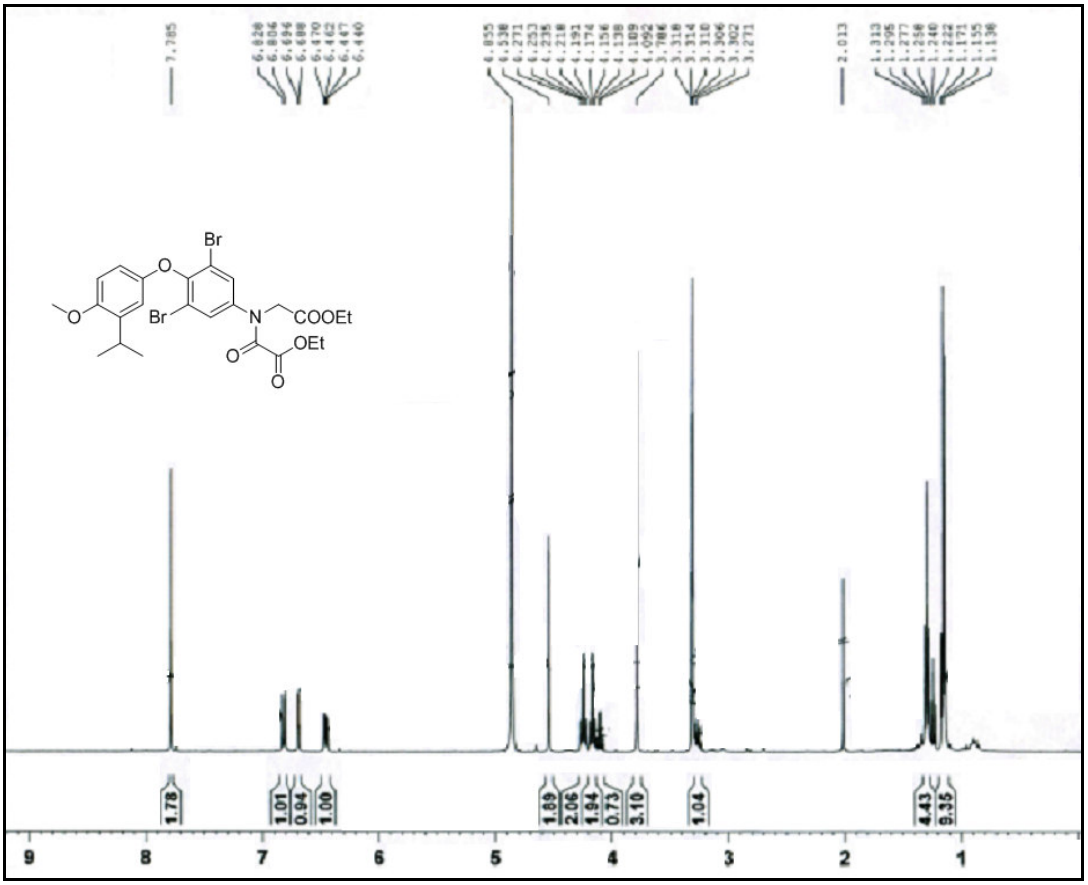
^{1}H NMR of 21a



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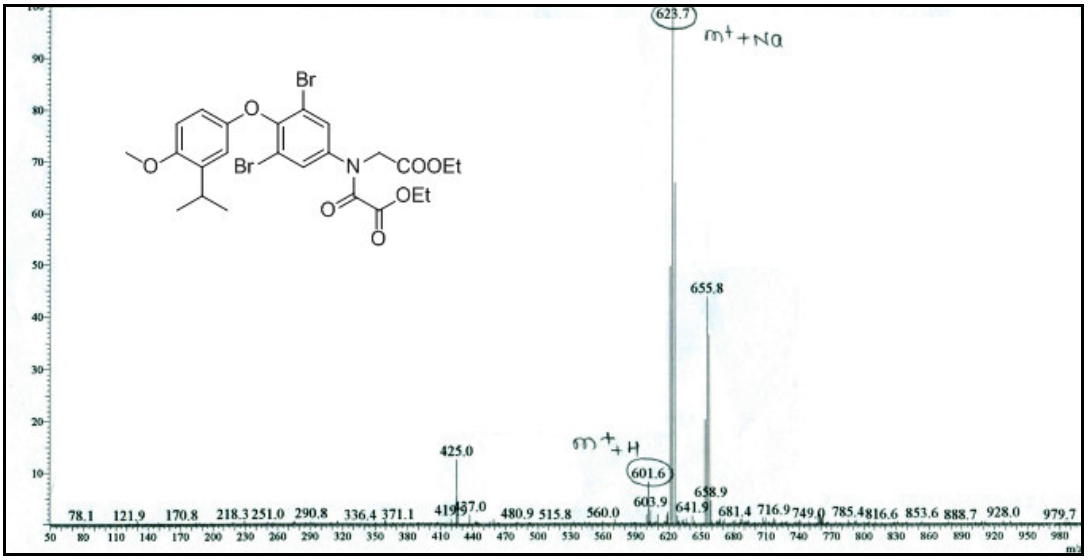
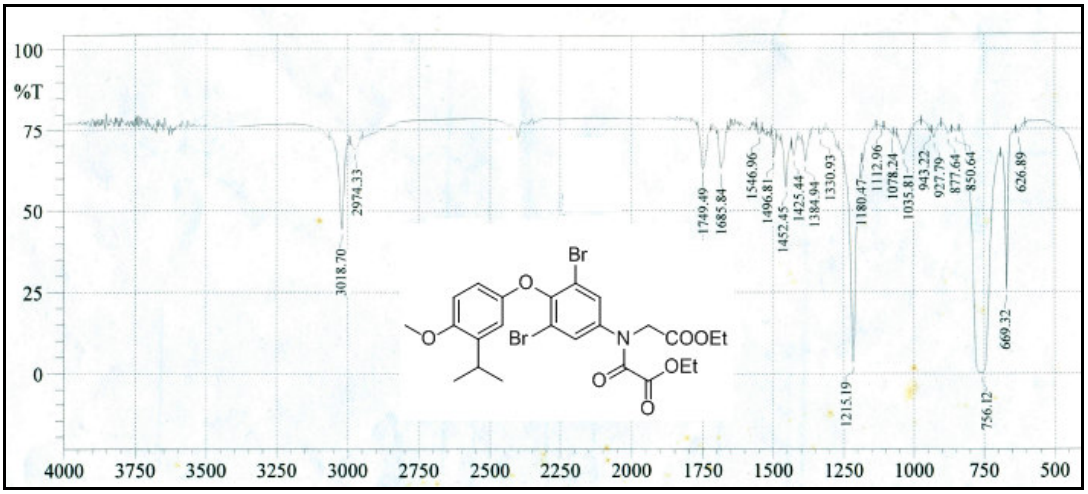


IR of 21a



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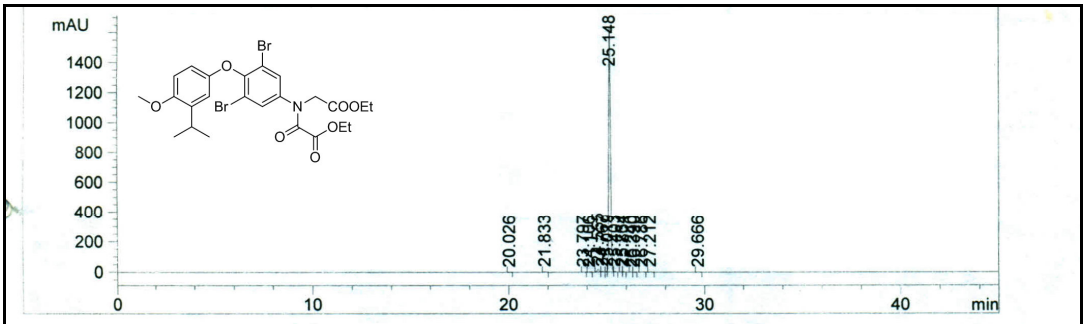


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                          Area Percent Report
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Use Multiplier & Dilution Factor with ISTDs


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  20.026 BBA   0.1176    1.45327 2.05138e-1   0.0132
   2  21.833 PBA   0.1069   20.37987    3.00002   0.1844
   3  23.797 BBA   0.1145    9.90755    1.37660   0.0897
   4  24.106 PBA   0.1052    2.86560 4.39176e-1   0.0259
   5  24.555 BV    0.1296  141.22678   17.40189   1.2780
   6  24.767 VP    0.1027   35.47479    5.30715   0.3210
   7  25.019 MF R  0.0439   16.21757    6.15853   0.1468
   8  25.148 FM R  0.1093 1.07227e4 1635.67102  97.0335
   9  25.403 VBA   0.1006   35.97558    5.42842   0.3256
  10  25.764 PV    0.0805   14.08949    2.67050   0.1275
  11  25.831 VBA   0.0876   14.48943    2.56964   0.1311
  12  26.240 BV    0.0932    1.41803 2.41726e-1   0.0128
  13  26.390 VB    0.0898    1.51726 2.60427e-1   0.0137
  14  26.786 BBA   0.1409   13.41346    1.47757   0.1214
  15  27.212 PB    0.1074   14.04774    1.91662   0.1271
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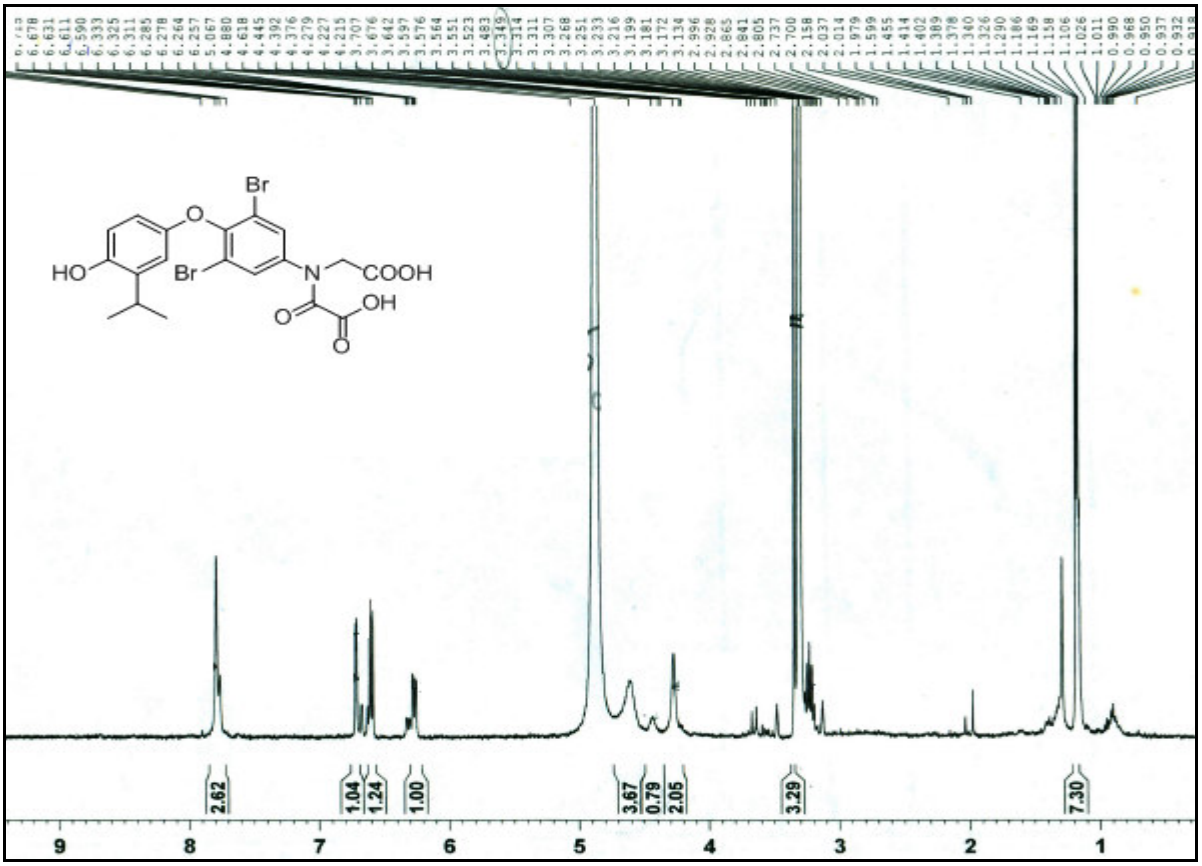
^{1}H NMR of 22a



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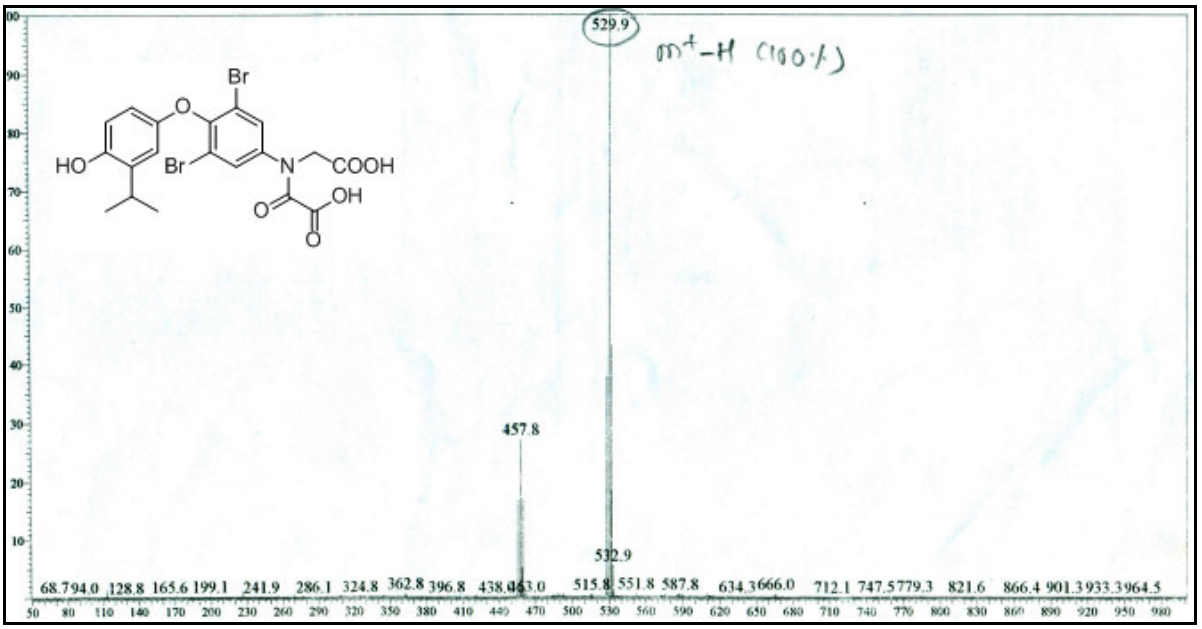
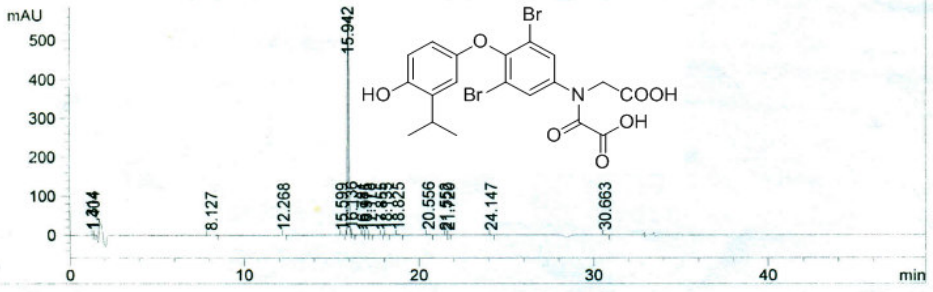


HPLC of 22a



Area Percent Report

ted By : Signal
ltiplier : 1.0000
lution : 1.0000
e 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 %
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2	1.404	VBA	0.0632	4.14389	9.37907e-1	0.1309
3	8.127	BBA	0.2597	13.00930	6.88815e-1	0.4109
4	12.268	BBA	0.0832	2.38449	4.43037e-1	0.0753
5	15.599	BBA	0.0844	3.36204	6.27194e-1	0.1062
6	15.942	BV	0.0857	3050.24927	557.42609	96.3347
7	16.136	VBA	0.0758	18.24674	3.64660	0.5763
8	16.781	BV	0.0788	9.21361e-1	1.93762e-1	0.0291
9	16.975	VV	0.1046	3.85543	5.96007e-1	0.1218
10	17.178	VBA	0.0885	2.14277	3.83353e-1	0.0677
11	17.855	BBA	0.0762	6.62581e-1	1.42126e-1	0.0209
12	18.135	BBA	0.0952	6.94538	1.12638	0.2194
13	18.825	PBA	0.1230	12.80724	1.66980	0.4045
14	20.556	BBA	0.1314	24.03908	2.86500	0.7592
15	21.552	BBA	0.0824	4.96380e-1	1.00383e-1	0.0157
16	21.720	BBA	0.0784	1.39548	2.88097e-1	0.0441
17	24.147	BBA	0.1264	1.88226	2.36265e-1	0.0594
18	30.663	PB	0.1238	6.68533	8.49478e-1	0.2111

MTP INHIBITORS

4

4 MTP Inhibitors

4.1 Objective

Microsomal triglyceride transfer protein (MTP) is one of the target which has enormous potential to reduce triglycerides (TG) and cholesterol. MTP is heterodimeric lipid transfer protein and it is found in endoplasmic reticulam of hepatocytes and enterocytes. MTP has distinct function in liver as well as in intestine. MTP is essential for assembly of triglycerides with apo-B48 producing chylomicrons in intestine and with apo-B100 in liver to synthesize and secrete very-low-density lipoprotein (VLDL). An increase in MTP protein mass, which is associated with over- secretion of intestinally derived apoB48 lipoproteins-chylomicrons, was observed in preclinical studies on fructose-fed insulin resistant hamsters. Moreover, diabetic rabbits and rats exhibited increased MTP expression leading to increased chylomicron production. It is also reported that increased MTP expression plays a crucial role in the development of atherosclerosis [1].

Therefore MTP inhibitor can cause reduction in plasma triglycerides and VLDL. This is already evidenced in clinical trials. BMS-201038 is 9H-fluorene-9-carboxamide derivative, developed as Lomitapide (AGER-733) and taken to clinical trials. There are many clinical studies reported for this compound. In preclinical and clinical studies MTP inhibitors have been known to cause fatty liver as major side effect. To overcome liver toxicity, enterocyte specific MTP–inhibitors were discovered. So far SLX-4090 is the only enterocyte specific compound in clinic (Figure 4.1B). Till date most of the enterocyte specific compounds [2,3] have been design based on Lomitapide skeleton. The efficacy

of Lomitapide is already proven in clinic. Thus, we intended to keep most of the structural features of Lomitapide while designing novel enterocyte specific MTP inhibitors.

4.2 9H-fluorene-carboxamide derivatives

4.2.1 Rationale

The Lomitapide structure has three components, 9H-fluorene-carboxamide, linker and aromatic carboxamide. In the present work by retaining some of the structural features of Lomitapide, modifications were done at linker position. Our primary goal was to generate novel compounds (Figure 4.1A) and confirm MTP inhibition, thereafter consider the newly discovered compounds for tissue selectivity (intestine specific).

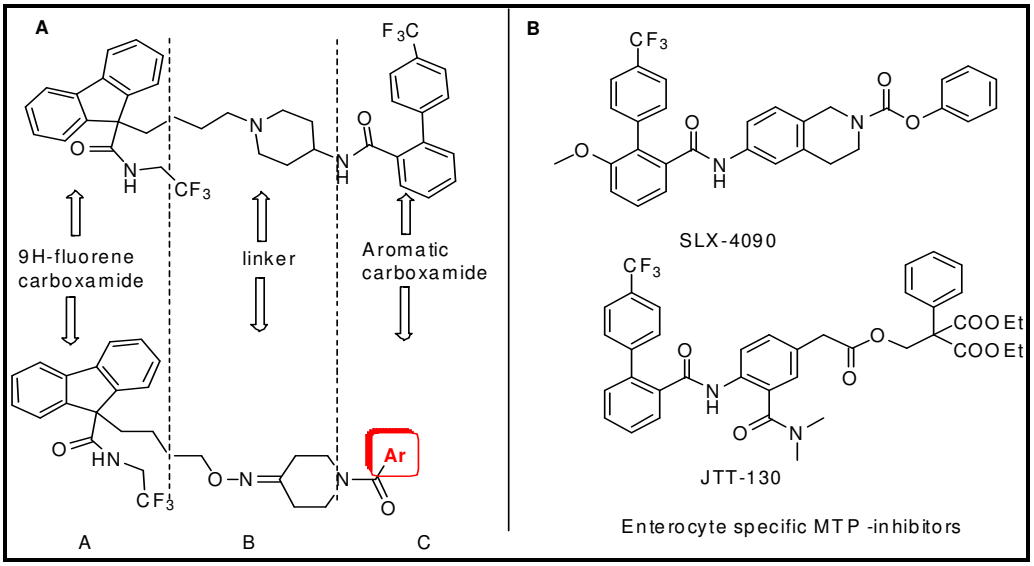


Figure 4.1 (A) Rationale, new 9H-fluorene-9-carboxamides (B) Enterocyte specific MTP inhibitors

As it is explicated in Figure 4.1A, structure of Lomitapide can be fragmented in to three components. Contribution of each part with respect to

biological activity is explained below. This understanding is required to plan / build the SAR of novel series.

(A) 9H-fluorene-carboxamide

The optimization up to Lomitapide (BMS-201038) from initial hit indicate that by introducing 9H-fluorenecomponent improved potency by (100-fold) in both the lipid transfer and HepG2 apoB secretion assays [4]. This suggests 9H- fluorine component is very essential for potency. It might be binding to MTP at a subdomain of the binding site is considered as possible reason for that. $CONHCH_2CF_3$ substitution on 9H-fluorene ring plays dramatic role for improvement in oral potency.

(B) Linker

Linker is positioned at center of molecule, one side of linker is connected to an aromatic carboxylic acid (mostly biphenyl), other side of the linker is connected to lipophilic group 9H-fluorene position. The linker chain could be of C atom ranging from C_{1-4}.

(C) Aromatic carboxamide

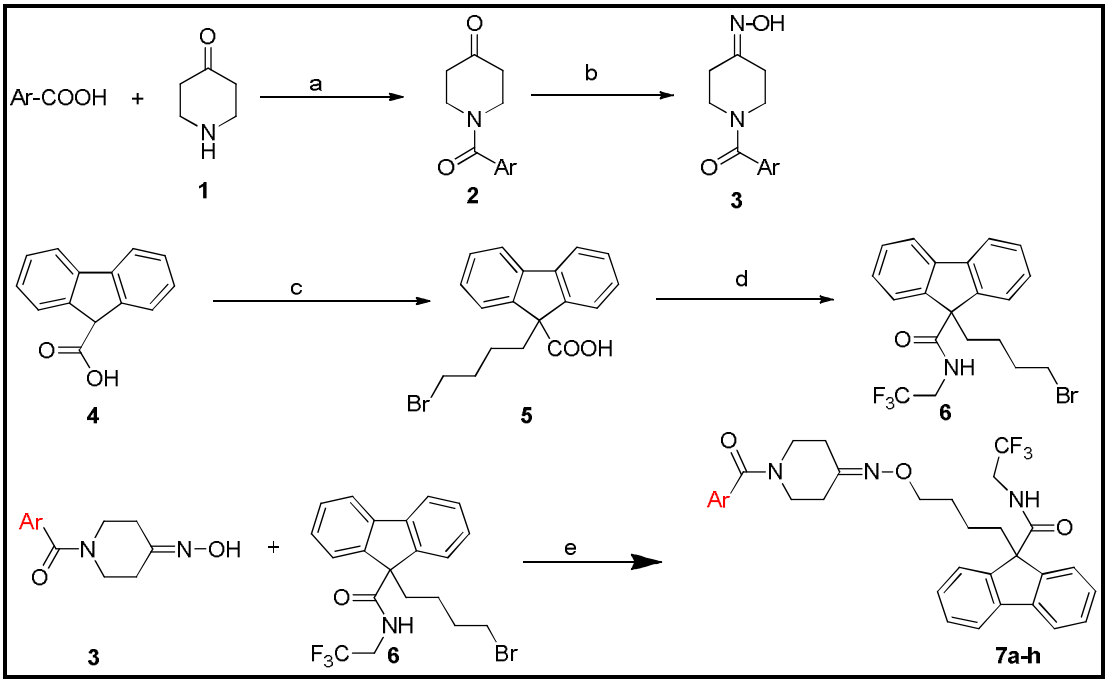
4'-CF_3-biphenyl carboxamide is considered as the best fit, it has been used extensively as one of the key components of MTP inhibitors [5].

Based on leanings from the structure of Lomitapide, it is understood that 9H-fluorene-carboxamide is responsible for potency and 4'-CF_3-biphenyl carboxamide provide the best fit. Both component are being used extensively to discover novel MTP inhibitors. Considering with the facts, first approach we adopted was to deviate at linker position. Compounds with new oximino linker have been synthesized. We kept most of the structural features of Lomitapide

(which are responsible for activity) intact. Hence, we expected that compounds having new linker will show MTP inhibitory activity. Further plan was to deviate at the aromatic acid position. As it is shown in Figure 4.1A we have replaced 4-Amino pipridine with oximino group at linker position. Initially we made amides with substituted biphenyl groups at aromatic carboxamide position followed by amides with with substituted phenyl at aromatic carboxamide position.

4.2.2 Synthesis 9H-fluorene-carboxamide derivatives

Scheme : 4.1



Reagents and conditions: (a) EDC, HOBt, triethylamine, DCM, 25 ^{o}C, 2-3 h (b) $NH_2OH.HCl$, $EtOH:H_2O$, reflux, 2 h (c) n-BuLi, THF,-78 ^{o}C, $Br(CH2)_4Br$, 3-4 h (d) OxalylChloride, DCM, $CF_3CH_2NH_2$..HCl, triethylamine, 0-5 ^{o}C, 5 h (e) KOH, DMSO, 25 ^{o}C, 2-3 h

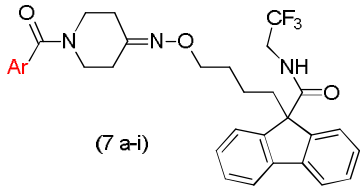
Route of synthesis of novel MTP inhibitors is outlined in scheme 4.1. Key intermediate Oxime **3** was prepared in two steps starting from coupling of Aryl carboxylic acid **1** with piperidin-4-one using standard acid base coupling method. Acid was coupled with piperidin-4-one in presence of [1-(3-dimethylaminopropyl)-

3-ethylcarbodiimide].HCl (EDCI), 1-hydroxybenzotriazole (HOBt) and dichloromethane (DCM) was used as solvent at 20-25 ^{o}C. For oxime formation of coupled product **2,** it was reacted with NH_2OH.HCl in EtOH:water as solvent at reflux temperature to obtain oxime intermediate **3**. Another Key intermediate 9H-fluorene-carboxamide was prepared as per literature procedure (WO9726240). First step was alkylation of 9H-fluorene-carboxylic acid with 1,4 dibromo butane in presence of n-Butyllithium to afford intermediate **5**. which was converted to its acid chloride by reacting it with oxalyl chloride. Further this acid chloride was coupled with 2,2,2 trifluoro ethylamine.HCl insitu in presence of triethylamine to afford amide **6**. Then finally nucleophilic substitution reaction between intermediate **3** and **6** in the presence of base KOH in dimethylsulfoxide (DMSO) gave compounds **7 a-h**.

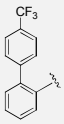
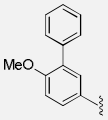
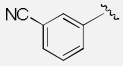
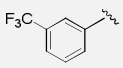
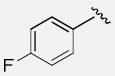
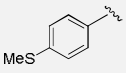
4.2.3 Result and Discussion

As described earlier our primary goal was to evaluate the linker modification. To confirm activity of molecules with new linker, remaining structural features of Lomitapide were kept constant. All the final compounds were screened for in vitro MTP inhibitory activity. Lomitapide used as positive control for comparing MTP inhibitory activity. The activities are reported for each compounds against the vehicle (DMSO) at one concentration of the test compounds as shown in Table 4.1. Procedure of in vitro experiments was described in the experimental section. The results are summarized in Table 4.1

Table 4.1 : *in vitro* evaluation for MTP inhibition activity



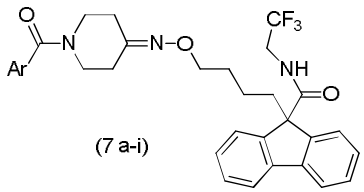
(7 a-i)

Compound	Ar	Activity @1μM[a]	% Inhibition
Control		17.58±2.36	
7a		11.92±1.70	32.55±7.25
7b		8.85±1.23	50.24±4.70
7c		9.26±2.39	47.89±4.93
7d		11.55±2.10	34.71±6.80
7e		14.54±1.25	17.48±5.70
7f		17.37±1.27	1.18±1.19
7g		14.35±2.23	18.58±1.27
7h		14.65±2.71	16.86±2.19
Lomitapide		2.79±2.27	85.09±2.77

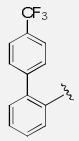
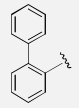
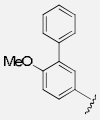
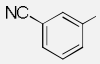
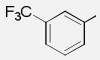
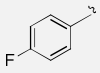
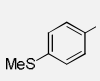
[a]In the enzyme assay, the commercially available kit from Chylos was used, The *in vitro* activity of the test compound was carried out at 1 μM conc.

The first compound tested with new linker was carboxamide **7a** having 4'-CF_3-biphenyl group at aromatic carboxamide position, it showed 32.55% inhibition against control at 1 µM. This data was encouraging for us as compound with new linker was showing MTP inhibition in our *in vitro* assay. Reducing lipophilicity of **7a** by removing CF_3 group from biphenyl ring producing compound **7b** and introducing electron donating methoxy group 2'-OMe-biphenyl **7c** also showed *in vitro* activity at 1 µM conc. This *in vitro* data proved that new linker is working but none of the compounds showed superior activity than Lomitapide. Moving further we wanted to find out significance of biphenyl core. Progressing in that direction 3-cyano phenyl substituted **7d**, 3-trifluoro phenyl substituted **7e** and 2-chloro phenyl substituted **7f** at aromatic carboxamide position were synthesized. In another set at aromatic carboxamide position 4-fluoro phenyl analogue **7g** and 4-methylthio phenyl **7h** were synthesized and screened. From this set **7d-h** only **7d** exhibited moderate activity. These results indicate that biphenyl carboxamide is certainly playing crucial role for potency. Having done this, our next goal was to confirm translation of *in vitro* activity to *in vivo* efficacy. All the compounds were further subjected for *in vivo* TG lowering activity which is given in Table 4.2. Biological studies indicated that compounds **7a-c** having biphenyl group at aromatic carboxamide position showed respectable *in vitro* MTP inhibition. These findings are reflected in their TG lowering activity

Table 4.2 : *In vivo*TG lowering activity of MTP inhibitors in Sprague Dawley rats



(7 a-i)

Compound	Ar	TGSR[a]	% Inhibition
Control		0.56±0.08	
7a		0.24±0.02	-56.33±3.86
7b		0.27±0.04	-52.22±6.79
7c		0.38±0.01	-32.65±2.16
7d		0.45±0.07	-19.68±11.65
7e		0.45±0.04	-18.92±6.91
7f		0.43±0.04	-23.98±7.32
7g		0.50±0.03	-9.77±4.80
7h		0.51±0.06	-8.37±10.8
Lomitapide		0.04±0.08	-73.5±14.80

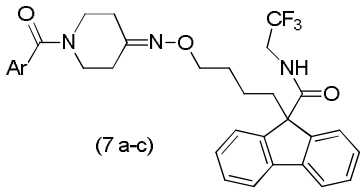
[a] Fasted animals were dosed with inhibitor orally followed after 1 h with an intravenous injection of Triton WR-1339 and Triglyceride secretion rate (TGSR, mg/min) calculated for 240 min.

The decrease in efficacy of **7c** compare to **7a** and **7b** was probably influence by electron donating effect of OMe. Another possibility is that oxidative demethylation would rapidly produce the corresponding phenol, which is susceptible for clearance [6]. *In vit*ro results of compounds **7d-f** having substituted phenyl groups showed that they are not as potent as compound **7a-c** which has biphenyl group at aromatic carboxamide position. Similar results obtained when they were tested for TG lowering activity. Further compound **7g** and **7h** also showed almost lack of efficacy. These data clearly indicate that presence of bihenyl core is very essential for MTP-inhibition. Compound **7c** is least potent with respect to **7a** and **7b** this might be because of electron donating effect of Methoxy group or clearance through demethylated metabolite, likewise an electron donating group on phenyl ring also reduce efficacy, compound **7d-f** (which has electron withdrawing groups on aromatic ring) are relatively better than **7g** and **7h** (which has electron donating group). These data suggest that in absence of biphenyl ring, electron withdrawing groups on aromatic ring can enhance the activity. Combining altogether we turned our attention to compound **7a** which is the most potent among the other derivatives. It is evident from the data presented in Table 4.1 and Table 4.2 that replacement at linker has potential to produce MTP inhibitory activity.

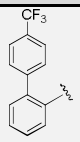
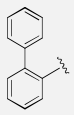
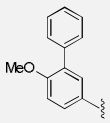
Our newly discovered linker incorporated to Lomitapide structure produce MTP inhibitory activity which was inferior to Lomitapide. To avoid liver toxicities, the IInd generations of MTP inhibitors are aspired to be enterocyte specific. The site of action in case of Lomitapide is both liver and intestine. Our next step was

to find out whether our compounds work in both the target organs (liver and intestine) or only in one of them. It is very well know that during fasting state there is little lipoprotein production by the intestine, the liver is the predominant source of nascent serum triglycerides. Hence, most of the *in vivo* experiments were carried out in fasted state. Selected compounds were also tested in fed state to compare liver vs intestinal selectivity. The data is presented in Table 4.3. The results show that the reduction in Triglyceride secretion rate (TGSR) in fasted state is higher than in fed state. This indicates that the compounds act more on the liver in comparison to intestine in terms of MTP inhibition, indicating that the intestinal selectivity in terms of MTP inhibition is lower in these compounds.

Table 4.3: *In vivo* comparisons fasted Vs fed state



(7 a-c)

Compound	Ar	% Change (TGSR) against Control (Fasted state)	% Change (TGSR) against Control (Fed state)
7a		-56.33 ±3.86	-43.25±4.12
7b		-52.22 ±6.79	-39.33±4.12
7c		-32.65 ±2.16	-22.32±9.12

4.3 Conclusions

- Series of 9H-fluorene-9-carboxamides as MTP-inhibitors have been described. Some of the title compounds have exhibited promising MTP inhibition *in vitro* as well as TG lowering in Sprague Dawley rats.
- Among the derivatives synthesized compound **7a** N-(2,2,2-trifluoroethyl)-9-(4-(((1-(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carbonyl)piperidin-4-ylidene) amino) oxy) butyl)-9H-fluorene-9-carboxamide has promising activity. Discovery of new oximino linker of the series has open new dimensions to this 9H-fluorene-9-carboxamide template.
- Interestingly SAR distinctly suggest that to bring MTP inhibitory activity biphenyl core is essential and it is driven by electron withdrawing groups. Therefore this new template could be further modified to get better and potent molecules.
- Further evolution of these compounds for enterocyte specificity to obtain a clinically useful novel class of MTP inhibitor was not successful.

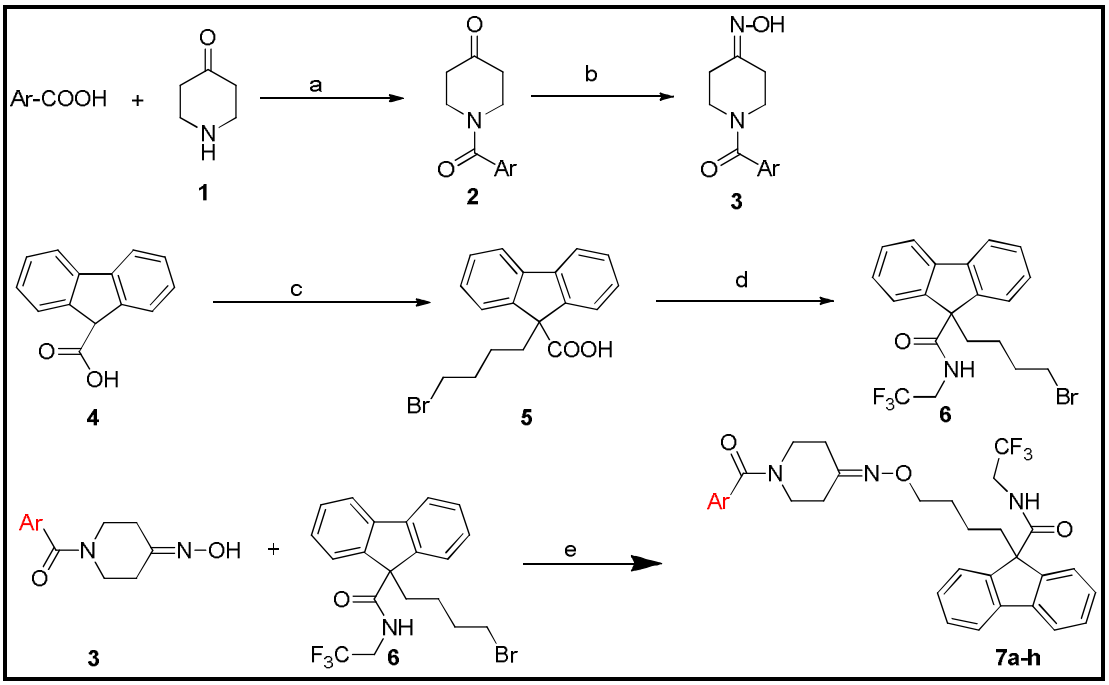
4.4 Experimental

Materials and Methods

Reagents were obtained from Sigma Aldrich and 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 a scientific melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (λmax in cm-1, as film for liquids and as KBr pellets for solid compounds). The ^{1}H NMR spectra were recorded on a Bruker Avance-300 (300 MHz) or Bruker Avance-400 (400 MHz) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-d_6. Signal multiplicities are represented as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). D_2O exchange experiments were carried out to confirm the exchangeable protons when present. Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer. HPLC analyses were carried out at λmax 220 nm using column ODS C-18, 150nm * 4.6 nm * 4 m on AGILENT 1100. Progress of the reactions was monitored by TLC using precoated TLC plates (E. Merck Kieselgel 60 F254) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (230–400 mesh).

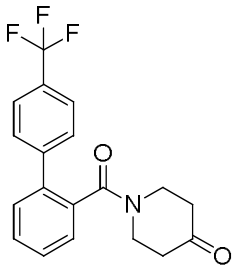
4.4.1 9H-fluorene-9-carboxamide derivatives

Scheme 4.1



Reagents and conditions: (a) EDC, HOBt, triethylamine, DCM, 25 ^{o}C, 2-3 h (b) $NH_2OH.HCl$, EtOH:H_2O, reflux, 2 h (c) n-BuLi, THF,-78 ^{o}C, $Br(CH2)_4Br$, 3-4 h (d) OxalylChloride, DCM, $CF_3CH_2NH_2$..HCl, triethylamine, 0-5 ^{o}C, 5 h (e) KOH, DMSO, 25 ^{o}C, 2-3 h

4.4.1.1 1-(4'-(Trifluoromethyl)-[1,1'-biphenyl]-2-carbonyl)piperidin-4-one (2a)



A solution of 4'-(trifluoromethyl)-[1,1'-biphenyl]-2-carboxylic acid (500 mg, 1.87 mmol) was dissolved in dichloromethane (DCM) (5 mL). To that added [1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide] hydrochloride (EDCI) (390 mg ,2.06 mmol) followed by 1-hydroxybenzotriazole (HOBt) (279 mg, 2.06 mmol)

added at 20 -25 ^{o}C.The reaction was stirred at 20-25 ^{o}C for 30 min. To this 4–Piperidone hydrochloride (288 mg, 1.87 mmol) and triethylamine (310 mL ,2.25 mmol) was added at 20-25 ^{o}C .Then reaction mix was further stirred for 2-3 hrs. Reaction mixture was quenched with sat.$NaHCO_3$ (30 mL) and product was extracted with ethylacetate (2 x 30 mL), Organic layer was washed with $NaHCO_3$ (30 mL), water (30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford crude product. It was purified by flash column chromatography over flash silica gel using 2% MeOH in chloroform as an eluent to afford pure product.(410 mg, 63 %).

Chemical Formula: $C_{19}H_{16}F_3NO_2$

Molecular Weight: 347.33

IR (KBr) cm^{-1}: 1714, 1626

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56-1.58(m, 1H), 2.04-2.11(m, 1H), 2.17-2.21(m, 1H,), 2.31-2.44(m, 1H), 3.14-3.15(m, 1H), 3.28-3.29(m, 1H), 3.82-3.87(m, 2H), 7.45-7.50(m, 2H), 7.52-7.56(m, 1H), 7.63-7.73(m, 4H)

ESI-MS: 347.9 $[M]^+$

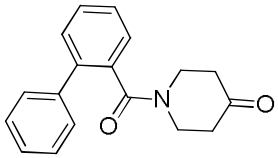
%Yield: 63 %

Purity by HPLC: 92.55 %

mp: 135-140 °C

Following compounds were prepared in an analogous manner using appropriate starting materials and the process described above.

4.4.1.2 1-([1,1'-Biphenyl]-2-carbonyl)piperidin-4-one (2b)



Chemical Formula: $C_{18}H_{17}NO_2$

Molecular Weight: 279.33

IR ($_{CHCl3}$) cm^{-1}: 1720, 1627

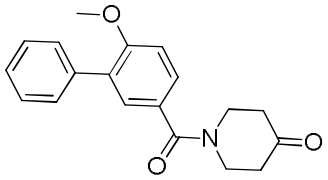
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.22-1.28(m, 1H), 1.90-1.95(m, 1H), 2.12-2.16(m, 1H), 2.30-2.35(m, 1H), 3.14-3.18(m, 2H), 3.47-3.54(m, 1H), 4.14-4.24(m, 1H), 7.39-7.41(m, 2H), 7.44-7.45(m, 3H), 7.47-7.48(m, 1H), 7.49-7.51(m, 1H), 7.52-7.55(m, 2H)

ESI-MS: 279.9 $[M]^+$

%Yield: 85 %

Purity by HPLC: 87.52 %

4.4.1.3 1-(6-Methoxy-[1,1'-biphenyl]-3-carbonyl)piperidin-4-one (2c)



Chemical Formula: $C_{19}H_{19}NO_3$

Molecular Weight: 309.36

IR ($_{CHCl3}$) cm^{-1}: 1712, 1626

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.51(s, 4H), 3.86(s, 3H), 3.89-3.91(m, 4H), 7.02(d, *J* = 8.4 Hz, 1H), 7.33- 7.39(m, 1H), 7.42-7.43(m, 2H), 7.45-7.46(m, 1H), 7.48-7.54(m, 3H)

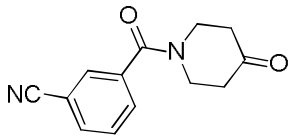
ESI-MS: 309.8 $[M]^+$

%Yield: 73 **%**

Purity by HPLC: 89.21 %

mp: 149-152 ^{0}C

4.4.1.4 3-(4-Oxopiperidine-1-carbonyl)benzonitrile (2d)



Chemical Formula: $C_{13}H_{12}N_2O_2$

Molecular Weight: 228.25

IR ($CHCl_3$) cm^{-1}: 1720, 1639

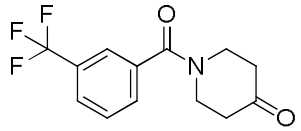
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.53-2.54(m, 4H), 3.74- 4.01(m, 4H), 7.52 -7.61(m, 1H), 7.70-7.73(m, 1H), 7.76-7.78(m, 2H)

ESI-MS: 269.9 $[M+K]^+$

%Yield: 40 %

Purity by HPLC: 99.35 %

4.4.1.5 1-(3-(Trifluoromethyl)benzoyl)piperidin-4-one (2e)



Chemical Formula: $C_{13}H_{12}F_3NO_2$

Molecular Weight: 271.24

IR ($CHCl_3$) cm^{-1}: 1720, 1637

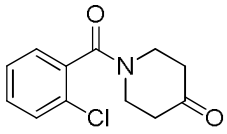
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.52(bs, 4H), 3.75-3.96(m, 4H), 7.59(t, *J* = 7.6 Hz, 1H), 7.65-7.67(m, 1H), 7.73-7.75(m, 2H)

ESI-MS: 271.89 $[M+H]^+$

%Yield: 56 %

Purity by HPLC: 95.38 %

4.4.1.6 1-(2-Chlorobenzoyl) piperidin-4-one (2f)



Chemical Formula: $C_{12}H_{12}ClNO_2$

Molecular Weight: 237.68

IR ($CHCl_3$) cm^{-1}: 1718, 1639

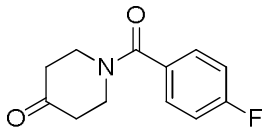
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.34-2.40(m, 1H), 2.52-2.68(m, 3H), 3.45 -3.51(m, 1H), 3.59-3.65(m, 1H), 3.91-3.98(m, 1H), 4.11-4.27(m, 1H), 7.32- 7.46 (m, 4H)

ESI-MS: Not detected.

%Yield: 54 %

Purity by HPLC: 94.76 %

4.4.1.7 1-(4-Fluorobenzoyl)piperidin-4-one (2g)



Chemical Formula: $C_{12}H_{12}FNO_2$

Molecular Weight: 221.23

IR ($CHCl_3$) cm^{-1}: 1720, 1635

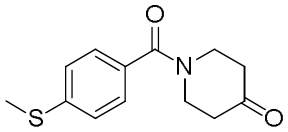
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.51(s, 4H), 3.88(s, 4H), 7.08-7.16(m, 2H), 7.47 - 7.52 (m, 2H)

ESI-MS: 221.8 $[M+H]^+$

%Yield: 63 %

Purity by HPLC: 96.93 %

4.4.1.8 1-(4-(Methylthio)benzoyl)piperidin-4-one (2h)



Chemical Formula: $C_{13}H_{15}NO_2S$

Molecular Weight: 249.33

IR ($CHCl_3$) cm^{-1}: 1716, 1627

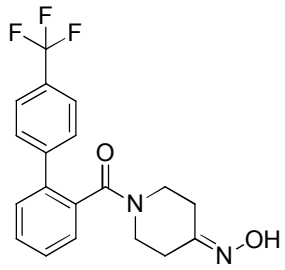
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.51(bs, 7H), 3.88(s, 4H), 7.26-7.29(d, J = 8.4 Hz, 2H), 7.40-7.42(d, J = 8.4 Hz, 2H)

ESI-MS: 249.9 $[M+H]^+$

%Yield: 67%

Purity by HPLC: 98.64 %

4.4.1.9 (4-(Hydroxyimino)piperidin-1-yl)(4'-(trifluoromethyl)-[1,1'-biphenyl]-2-yl)methanone (3a)



A Solution of 1-(4'-(trifluoromethyl)-[1,1'-biphenyl]-2-carbonyl)piperidin-4-one **2a** (410 mg, 1.18 mmol) in EtOH (2.87 mL) was added hydroxylamine hydrochloride ($NH_2OH.HCl$) (246 mg, 3.54 mol) dissolved in water (2.46 mL) at 20-25 ^oC. The Reaction mixture was heated stirred at 70 ^oC for 2 hrs. Reaction

Mixture was Cooled to 20-25 ^{o}C, product was taken in ethylacetate (20 mL) and it was washed with water (2 x 20 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated to afford solid product. Crude product purified by flash column chromatography over flash silica gel using 2% MeOH in chloroform as an eluent to afford desire product (400 mg, 93 %).

Chemical Formula: $C_{19}H_{17}F_3N_2O_2$

Molecular Weight: 362.35

IR (KBr) cm^{-1}: 3267**,** 1616

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.76-1.80(m, 1H), 2.06-2.11(m, 1H), 2.25-2.28(m, 1H), 2.35-2.36(m, 1H), 2.84-2.88, (m, 1H), 3.08-3.12 (m, 1H), 3.60-3.73 (m, 2H), 7.42-7.54(m, 4H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 7.6 Hz, 2H)

ESI-MS: 362.9 $[M]^+$

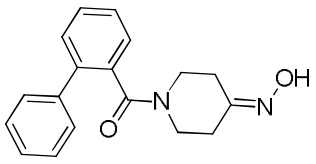
%Yield: 93 %

Purity by HPLC: 75.78 %

mp: 179-184 ℃

Following compounds were prepared in an analogous manner using appropriate starting materials and the process described above.

4.4.1.10 [1,1'-Biphenyl]-2-yl(4-(hydroxyimino)piperidin-1-yl) methanone (3b) :



Chemical Formula: $C_{18}H_{18}N_2O_2$

Molecular Weight: 294.35

IR (KBr) cm^{-1}: 3269, 1627

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.86-1.90(m, 1H), 2.04-2.12(m, 1H), 2.31-2.37(m, 1H), 2.72-2.75(m, 1H), 2.91-3.02(m, 2H), 3.40-3.44(m, 1H), 3.90-3.97(m, 1H), 7.34-7.36(m, 1H), 7.38 -7.40(m, 2H), 7.41-7.42(m, 2H), 7.44-7.47(m, 2H), 7.48-7.53(m, 2H)

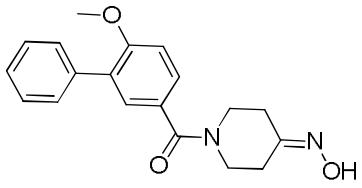
ESI-MS: 294.8 $[M]^+$

%Yield: 53%

Purity by HPLC: 83 %

mp: 100-105 ^oC

4.4.1.11 (4-(Hydroxyimino)piperidin-1-yl)(6-methoxy-[1,1'-biphenyl]-3-yl)methanone (3c)



Chemical Formula: $C_{19}H_{20}N_2O_3$

Molecular Weight: 324.37

IR ($CHCl_3$) cm^{-1}: 3408**,** 1600

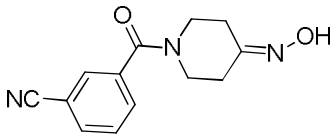
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41(s, 2H), 2.68(bs, 2H), 3.73(bs, 4H), 3.84(s, 3H), 7.00(s, 1H), 7.32-7.56(m, 8H).

ESI-MS: 324.9 $[M]^+$

%Yield: 95 %

Purity by HPLC: 96.2 %

4.4.1.12 3-(4-(Hydroxyimino)piperidine-1-carbonyl) benzonitrile (3d)



Chemical Formula: $C_{13}H_{13}N_3O_2$

Molecular Weight: 243.26

IR (KBr) cm^{-1}: 3242**,** 1604

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.36-2.40(m, 2H), 2.49-2.54(m, 2H), 3.52-3.54(m, 2H), 3.85-3.86(m, 2H), 6.89(s, 1H), 7.52-7.60(m, 1H), 7.67-7.70(m, 1H), 7.74-7.76(m, 2H).

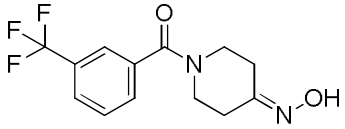
ESI-MS: 243.7 $[M]^+$

%Yield: 81%

Purity by HPLC: 99.3 %

mp: 191-194 ^oC

4.4.1.13 (4-(Hydroxyimino)piperidin-1-yl)(3-(trifluoromethyl) phenyl) methanone (3e)



Chemical Formula: $C_{13}H_{13}F_3N_2O_2$

Molecular Weight: 286.25

IR (KBr) cm^{-1}: 3232**,** 1620

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.17-2.35 (m, 2H), 2.51-2.62 (m, 2H), 3.34 (bs, 2H), 3.86 (bs, 2H), 7.19 (s, 1H), 7.52-7.80 (m, 4H).

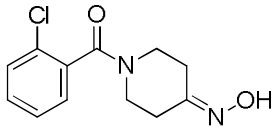
ESI-MS: 286.7 $[M]^+$

%Yield: 82 %

Purity by HPLC: 97.0 %

mp: 150-153 ^{o}C

4.4.1.14 (2-chlorophenyl)(4-(hydroxyimino)piperidin-1-yl) (3f)



Chemical Formula: $C_{12}H_{13}ClN_2O_2$

Molecular Weight: 252.70

IR (KBr) cm^{-1}: 3269**,** 1616

^{1}H NMR (400 MHz, CDCl$_3$): *δ* 2.27-2.29 (m, 1H), 2.41-2.43(m, 1H), 2.49-2.53(m, 1H), 2.61-2.67(m, 1H), 3.28-3.34(m, 1H), 3.35-3.43(m, 1H), 3.79-3.83(m, 1H), 3.96-4.04(m, 1H), 7.20(s, 1H), 7.31-7.44(m, 4H)

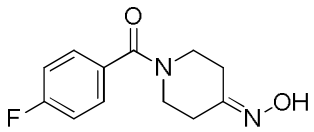
ESI-MS: Not detected.

%Yield: 71 %

Purity by HPLC: 81.5 %

mp: 175-180 ^{o}C

4.4.1.15 4-Fluorophenyl)(4-(hydroxyimino)piperidin-1-yl)methanone (3g)



Chemical Formula: $C_{12}H_{13}FN_2O_2$

Molecular Weight: 236.24

IR (KBr) cm^{-1}: 3248, 1608

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42(bs, 2H), 2.68(bs, 2H), 3.54-3.79(m, 4H), 6.99-7.14(m, 3H), 7.43-7.52(m, 2H)

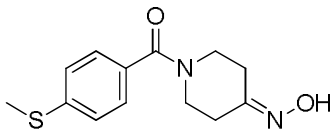
ESI-MS: 236.8 $[M]^+$

%Yield: 84 %

Purity by HPLC: 94 %

mp: 160-165 ^oC

4.4.1.16 (4-(Hydroxyimino)piperidin-1-yl)(4-methylthio) phenyl) methanone (3h)



Chemical Formula: $C_{13}H_{16}N_2O_2S$

Molecular Weight: 264.34

IR (KBr) cm^{-1}: 3277, 1612

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41(s, 2H), 2.50(s, 3H), 2.68 (s, 2H), 3.66-3.70(m, 4H), 7.20(s, 1H), 7.26-7.28(s, 2H), 7.36-7.42(s, 2H)

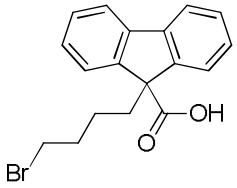
ESI-MS: 264.9 $[M]^+$

%Yield:

Purity by HPLC: 99.0 %

mp: 131-136 ^oC

4.4.1.17 9-(4-Bromobutyl)-9H-fluorene-9-carboxylic acid(5)



To a solution of 9H-fluorenecarboxylic acid **4** (15 g, 0.07 mol) in THF (330 mL) n-Butyllithium (157.03 mL, 0.15 mol) was added at 0-10 ^{o}C. The reaction mixture was then stirred at 0 ^{o}C for 1 hour. To that 1, 4 dibromobutane (16.95 g, 0.078 mol) was added dropwise to the reaction mixture at 0 ^{o}C. The reaction mixture was stirred at for 30 min at same temperature and then for 2 hrs at 20 - 25 ^{o}C. Reaction mix. was quenched with 1NHCl (15 mL). product was extracted with ethylacetate (2 x 30 mL), Organic layer was washed with $NaHCO_3$ (30 mL), water (30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford desire product. Crude product purified by flash column chromatography over flash silica gel using Hexane : Ethylacetate (9:1) as an eluent to afford pure product (16 g, 66 %).

Chemical Formula: $C_{18}H_{17}BrO_2$

Molecular Weight: 345.23

IR (KBr) cm^{-1}: 3061, 2985, 1691, 1273

^{1}H NMR (400MHz, $CDCl_3$): δ 0.87-0.94(m, 2H), 1.63-1.70(m, 2H), 2.31- 2.35(m, 2H), 3.18(t, *J* =7.00 Hz, 2H), 7.30-7.34(m, 2H), 7.38-7.42(m, 2H), 7.53(d, *J* = 7.6 Hz, 2H), 7.72(t, *J* = 7.6 Hz, 2H)

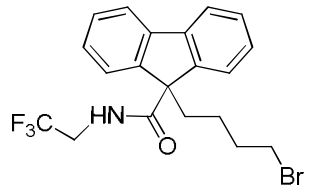
ESI-MS: 366.9 $[M+Na]^+$

%Yield: 66 %

Purity by HPLC: 97.10 **%**

mp: 120-125 ^{o}C

4.4.1.18 9-(4-Bromobutyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (6)



9-(4-bromobutyl)-9H-fluorene-9-carboxylic acid **5** (11.2 g, 0.032 mol) was dissolved in DCM (112 mL) and dimethylformamide (DMF) (1 mL). It was cooled to 0 ^{o}C and to that Oxalyl chloride (4.94 g, 0.03 mol) dissolved in DCM (35 mL) was added dropwise at 0 ^{o}C. RM was stirred at 0 ^{o}C during 15 min. The reaction mixture was stirred at 20 -25 ^{o}C for 3 hrs. Reaction mixture was concentrated to afford crude acid chloride. In a separate flask 2,2,2,-triflouro ethylamine (4.83 g ,0.03 mol) dissolved in DCM (100 mL) and triethylamine (9.83 g, 0.0352 mol) was taken and cooled to 0 ^{o}C. Then crude acid chloride dissolve in DCM (20 mL) was added to the reaction dropwise at 0 ^{o}C. After that reaction mixture was stirred at 0 ^{o}C for 2 hrs. The reaction mixture was diluted with DCM (20 mL) organic layer was washed with 1NHCl (20 mL), water (2 x 20 mL), dried over anhydrous Na_2SO_4 and evaporated to get the product (1.34, 97 %).

Chemical Formula: $C_{20}H_{19}BrF_3NO$

Molecular Weight: 426.27

IR (KBr) cm^{-1}: 3061, 2985, 2937, 1691

^{1}H NMR (400MHz, $CDCl_3$): δ 0.79-0.87(m, 2H), 1.66- 1.73(m, 2H), 2.41- 2.45(m, 2H), 3.21 (t, J = 7 Hz , 2H), 3.65 -3.73(m, 2H), 5.33 (t, 1H), 7.36-7.40(m, 2H), 7.44 -7.48(m, 2H), 7.54(t, J = 8.4 Hz, 2H), 7.78(t, J = 7.2 Hz, 2H)

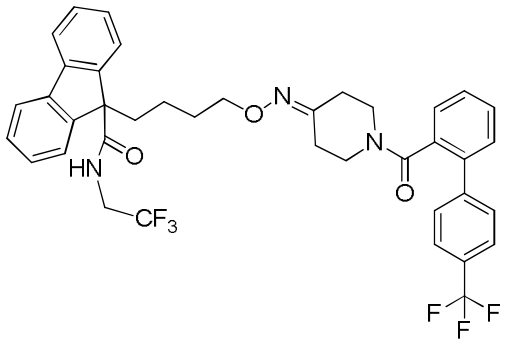
ESI-MS: 447.1 $[M+Na]^+$

%Yield: 97.10 %

Purity by HPLC: 90.62 **%**

mp: 85-88 ^oC

4.4.1.19 N-(2,2,2-Trifluoroethyl)-9-(4-(((1-(4'-(trifluoromethyl)-[1,1'-biphenyl] -2-carbonyl) piperidin -4- ylidene) amino) oxy) butyl) -9H –fluorene -9-carboxamide (7a)



To a Solution of (4-(hydroxyimino)piperidin-1-yl)(4'-(trifluoromethyl)-[1,1'-biphenyl]-2-yl) methanone **3a** (380 mg, 1.049 mmol) in DMSO (3.8 mL) was added KOH(85%) (170 mg, 2.62 mmol) at 25 ^oC. The reaction mixture was stirred at 20-25 ^oC for 30 min. To that 9-(4-bromobutyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide **6** (440 mg, 2.62 mmol) dissolved in DMSO (2.2 mL) was added slowly at 20- 25 ^oC during 10-15 min. Further reaction mixture was stirred at 20 - 25 ^oC for 2-3 hours. Product was taken in ethylacetate (2 x 15 mL), organic layer was washed with water (15 mL), brine (15 mL), dried over anhydrous Na_2SO_4 and evaporated to afford solid product. Crude product purified by flash column chromatography over flash silica gel using 40 % ethylacetate in n-hexane to afford pure product as semisolid (160 mg).

Chemical Formula: $C_{39}H_{35}F_6N_3O_3$

Molecular Weight: 707.70

IR ($CHCl_3$) cm^{-1}: 1685, 1618

^1H NMR (400 MHz, $CDCl_3$): δ 0.58– 0.66(m, 2H),1.17-1.23(m, 4H), 1.32-1.39(m, 2H), 1.97-2.36(m, 4H), 3.55-3.60(m, 2H), 3.61-3.69(m, 4H), 5.26-5.28(m, 1H), 7.23-7.27(m, 3H), 7.31- 7.40(m, 4H), 7.42-7.49(m, 4H), 7.53(d, *J* = 8.0 Hz, 2H), 7.57-7.62(m, 2H), 7.68(t, *J* = 8 Hz, 2H)

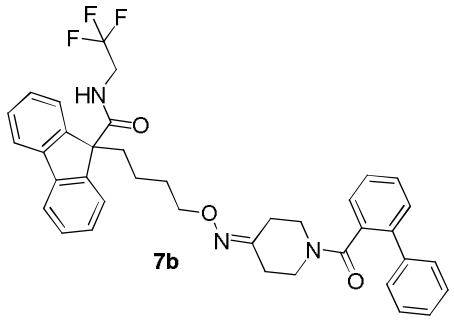
ESI-MS: 707.9 $[M]^+$.

Yield: 21 %

Purity by HPLC: 94.49 %

Following compounds were prepared in an analogous manner using appropriate starting materials and the process described above.

4.4.1.20 9-(4-(((1-([1,1'-Biphenyl]-2-carbonyl) piperidin-4- ylidene) amino) oxy) butyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9- carboxamide (7b)



7b

Chemical Formula: $C_{38}H_{36}F_3N_3O_3$

Molecular Weight: 639.71

IR (KBr) cm^{-1}: 1685, 1629

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58-0.63(m, 2H), 0.92-0.95(m, 1H), 1.17-1.18(m, 1H), 1.34-1.38(m, 2H), 1.91-1.93(m, 2H), 2.11-2.18(m, 2H), 2.32-2.38(m, 2H), 2.81-2.85(m, 1H), 3.30-3.35(m, 1H) 3.63-3.74(m, 4H), 4.59(s, 1H), 7.31-7.59(m, 15H), 7.77-7.90(m, 2H)

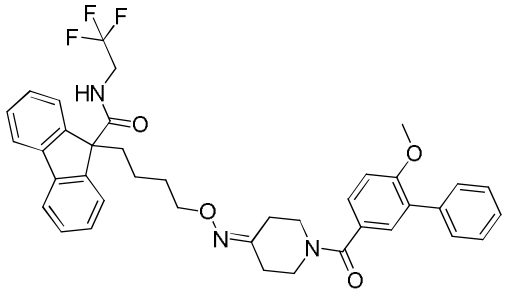
ESI-MS: 640.2 $[M]^+$

%Yield: 46 %

Purity by HPLC: 90.0 %

mp: 135-140 ^oC

4.4.1.21 9-(4-(((1-(6-Methoxy-[1,1'-biphenyl]-3-carbonyl)piperidin-4-ylidene) amino) oxy) butyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (7c)



Chemical Formula: $C_{39}H_{38}F_3N_3O_4$

Molecular Weight: 669.73

IR ($CHCl_3$) cm^{-1}: 1687, 1622

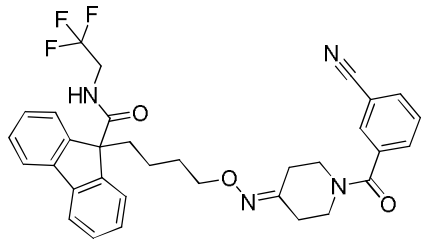
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74-0.78(m, 2H), 0.92-0.96(m, 4H), 1.40-1.48 (m, 2H), 2.31-2.39(m, 4H), 3.66-3.68(m, 4H), 3.81(m, 2H), 3.87 (s, 3H), 7.35(d, J = 6.8 Hz, 1H), 7.39-7.42(m, 4H), 7.45-7.47(m, 6H), 7.49-7.52(m, 4H), 7.73-7.75 (m, 2H)

ESI-MS: 670.1 $[M]^+$

%Yield: 38 %

Purity by HPLC: 90.0 %

4.4.1.22 9-(4-(((1-(3-Cyanobenzoyl)piperidin-4-ylidene)amino)oxy)butyl)-N-(2,2,2-trifluoro ethyl)-9H-fluorene-9-carboxamide (7d)



Chemical Formula: $C_{33}H_{31}F_3N_4O_3$

Molecular Weight: 588.62

IR (KBr) cm^{-1}: 1685, 1637

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72-0.80 (m, 2H) , 0.83-0.89(m, 4H), 1.23-1.33(m, 2H), 2.42-2.46(m, 4H), 3.66-3.70(m, 4H), 3.85(t, *J* = 6 Hz, 2H), 5.35(s, 1H), 7.36(d, *J* = 7.6 Hz, 2H), 7.44(t, *J* = 7.2 Hz, 2H), 7.53-7.55(m, 2H), 7.58(d, *J* = 7.6 Hz, 1H), 7.65-7.67(m, 1H), 7.71-7.78 (m, 4H)

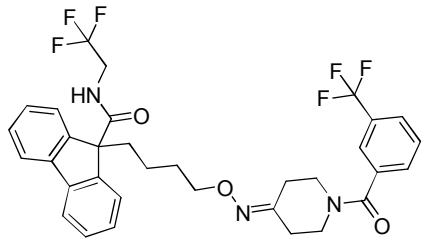
ESI-MS: 589 $[M+H]^+$

%Yield: 52 %

Purity by HPLC: 97.55 %

mp: 185-188 ^oC

4.4.1.23 N-(2,2,2-Trifluoroethyl)-9-(4-(((1-(3-(trifluoromethyl) benzoyl) piperidin-4-ylidene) amino) oxy)butyl)-9H-fluorene-9-carboxamide (7e)



Chemical Formula: $C_{33}H_{31}F_6N_3O_3$

Molecular Weight: 631.61

IR ($CHCl_3$) cm^{-1}: 1685, 1631

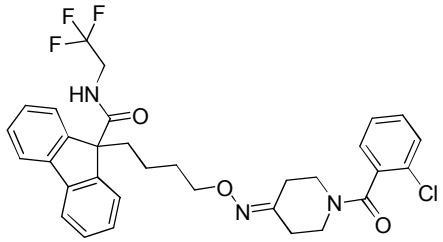
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86-0.87(m, 2H), 1.49-1.52(m, 2H), 2.03-2.44(m, 6H), 3.20-3.48(m, 2H), 3.65- 3.68(m, 4H), 3.84-3.88(m, 2H), 5.26-5.35(m, 1H), 7.36-7.43(m, 4H), 7.53-7.60(m, 4H), 7.70-7.77(m, 4H)

ESI-MS: 632 $[M+H]^+$

%Yield: 62 %

Purity by HPLC: 90.12 %

4.4.1.24 9-(4-(((1-(2-Chlorobenzoyl)piperidin-4-ylidene)amino) oxy) butyl) - N-(2,2,2-trifluoro ethyl) -9H-fluorene-9-carboxamide (7f)



Chemical Formula: $C_{32}H_{31}ClF_3N_3O_3$

Molecular Weight: 598.06

IR (N) cm^{-1}: 1680, 1633

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68-0.73(m, 2H), 1.45-1.52(m, 2H), 2.40-2.43(m, 1H), 2.45-2.51(m, 4H), 3.05-3.10(m, 1H), 3.15-3.23(m, 1H), 3.30-3.39(m, 1H), 3.65- 3.71(m, 3H), 3.81- 3.85(m, 3H), 5.34-5.36(m, 1H), 7.24-7.32(m, 1H), 7.33-7.36(m, 3H), 7.37-7.39(m, 3H), 7.41-7.46(m, 1H), 7.54(t, *J* = 8.0 Hz, 2H), 7.75(d, *J* = 7.6 Hz, 1H), 7.78(d, J = 7.5 Hz, 1H)

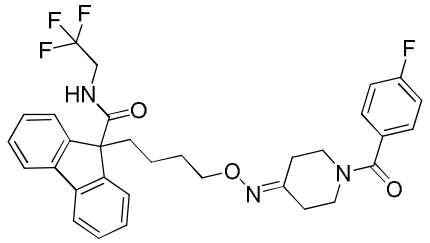
ESI-MS: 620 $[M+Na]^+$.

%Yield: 85 %

Purity by HPLC: 96.22 %

mp: 180-184 ^oC

4.4.1.25 9-(4-(((1-(4-Fluorobenzoyl)piperidin-4-ylidene)amino)oxy) butyl) -N-(2,2,2-trifluoro ethyl)-9H-fluorene-9-carboxamide (7g)



Chemical Formula: $C_{32}H_{31}F_4N_3O_3$

Molecular Weight: 581.60

IR ($CHCl_3$) cm^{-1}: 1672, 1627

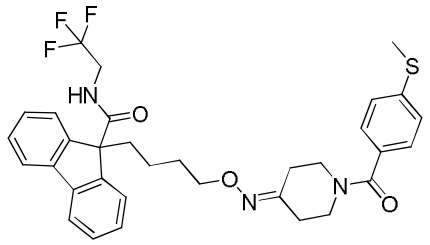
1H NMR (400 MHz, $CDCl_3$): δ 0.63-0.71(m, 2H), 0.85-0.91(m, 2H), 1.16-1.26(m, 4H), 1.29-1.47(m, 2H), 2.27-2.29(m, 4H), 3.18-3.53(m, 2H), 3.54-3.82(m, 2H), 7.21-7.25(m, 2H), 7.35-7.37(m, 4H), 7.47-7.54(m, 4H), 7.82-7.84(m, 2H) .

ESI-MS: 583.3 $[M+H]^+$

%Yield: 58 %

Purity by HPLC: 90.0 %

4.4.1.26 9-(4-(((1-(4-(Methylthio)benzoyl)piperidin-4-ylidene)amino) oxy) butyl)-N-(2,2,2-trifluoroethyl)- 9H-fluorene-9-carboxamide (7h)



Chemical Formula: $C_{33}H_{34}F_3N_3O_3S$

Molecular Weight: 609.70

IR ($CHCl_3$) cm^{-1}: 1687, 1622

^1H NMR (400 MHz, $CDCl_3$): δ 0.65-0.72(m, 2H), 0.88-0.91(m, 2H), 1.16-1.24(m, 4H), 1.47-1.50(m, 2H), 2.42-2.46(m, 4H), 2.51(s, 3H), 3.66-3.70(m, 2H), 3.82-3.85(m, 2H), 5.32(s, 1H), 7.27-7.28(m, 2H), 7.34-7.37(m, 4H); 7.42-7.44(m, 2H), 7.53-7.55(m, 2H), 7.75-7.77(m, 2H)

ESI-MS: 611.1 $[M+H]^+$

%Yield: 48 %

Purity by HPLC: 96.25 %

4.4.1.27 *In vitro* Determination of MTP activity

The *in vitro* activity of the MTP inhibitor test compounds was measured using Chylos MTP activity kit using manufacturer's instructions. Briefly, purified MTP (1 µg) was incubated with donor and acceptor vesicles for 30 min in the presence of the 1µM concentration of the test compound. Four different conditions (blank, total, positive control, and test) were used for each assay. In all assays, the reaction was started by the final addition of the MTP source. Three microliter each of acceptor and donor vesicles was put onto fluorescence microlitre (black) plates. It was added with ten microliter of 10 mM Tris, pH 7.4, containing 2mM EDTA and 10µl of 1% BSA stock in 1.5 M NaCl. In blanks, the needed amount of control buffer (which contains the MTP source in positive control and test samples) was added and the volume was made to 100 µl with water. In positive controls, MTP protein was added, whereas in tests unknown samples were added. It was incubated at 37 ^{o}C for 30 min. The readout was fluorescence units using excitation and emission wavelengths of 460–470 and 530–550 nm, respectively.

4.4.1.28 *In vivo* TG lowering activity of MTP inhibitors

For determination of triglyceride secretion, serum triglycerides were measured in fasted Sprague Dawley rats. 18 hours fasted rats were orally administered the test compound (0.3 mg/kg PO), followed one hour later by intravenous Triton WR 1339 (250 mg/Kg of body weight) administration. The test compounds were formulated in 0.1% hydroxyl ethyl cellulose, 0.5% Tween 80 in deionised water. Control group animals received vehicle by oral route of administration. The

volume of dose administration was 2 mL/Kg for all treatment groups. The animals were bled under light ether anaesthesia upto 240 min after Triton WR1339 injection and serum triglycerides were estimated.

TGSR were determined by the equation:

TGSR= [((TG1-TGo)/T1) + ((TG2 –TGo)/T2)/2] x PVE

where TGSR = triglyceride secretion rate; TGo = basal plasma TG concentration (mg/100 ml) ; TG1 = first plasma TG concentration after Triton injection; TG2 = second plasma TG concentration after Triton injection; T1 = time of first sampling in minutes after Triton injection; T2 =time of second sampling in minutes after Triton injection; PVE = estimated plasma volume (cubic centimeters) expressed by the formula: PVE = (0.016 X weight (grams)) + 4.25.

4.5 References

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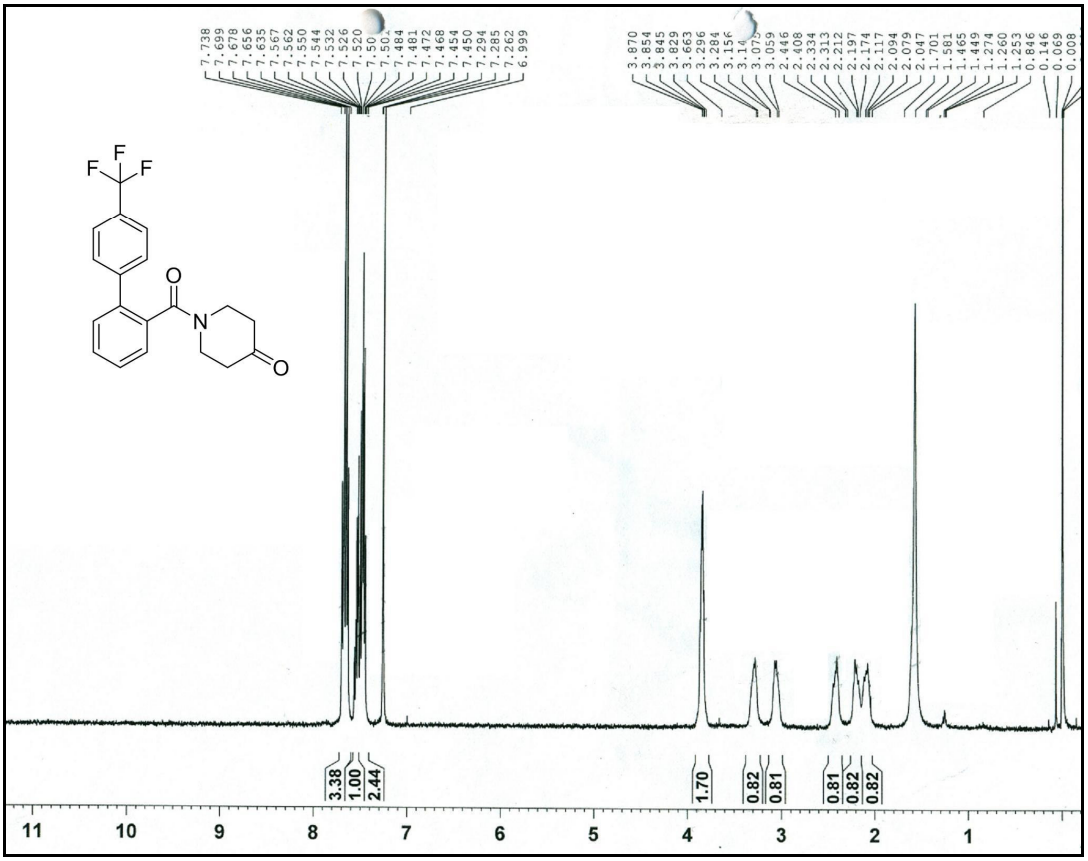
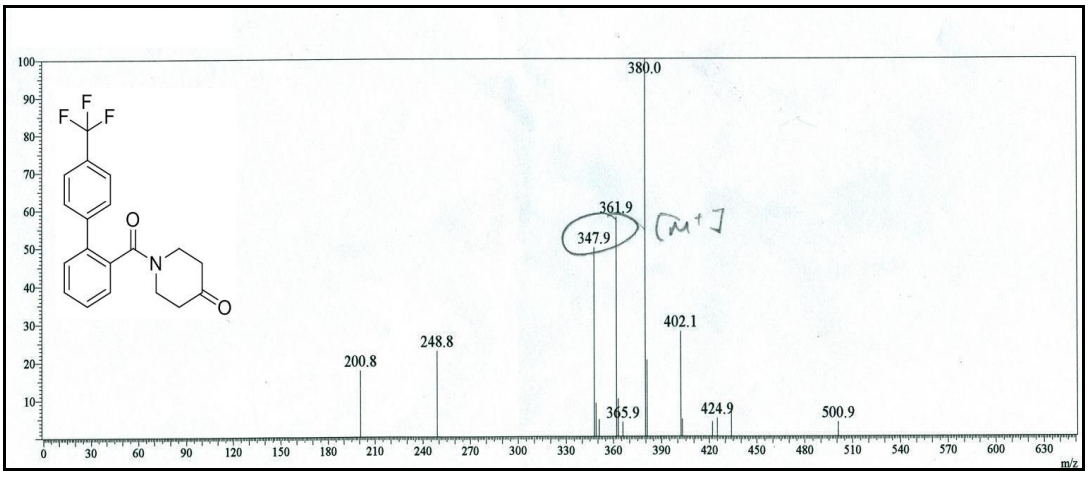
6. Robinson RP, Bartlett JA, Bertinato P, Bessire AJ, Cosgrove J, Foley PM, Manion TB, Minich ML, Ramos B, Reese MR, Schmahai TJ *et al*. **Discovery of microsomal triglyceride transfer protein (MTP) inhibitors with potential for decreased active metabolite load compared to dirlotapide.** *Bioorganic & Medicinal Chemistry letters* (**2011**) 21(14):4150-4154.

4.6 Spectra

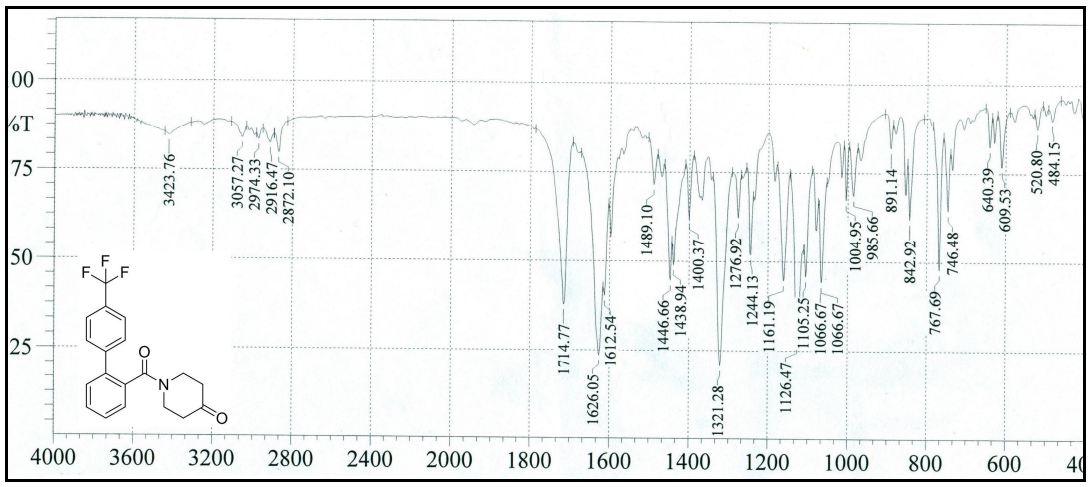
^{1}H NMR of 2a



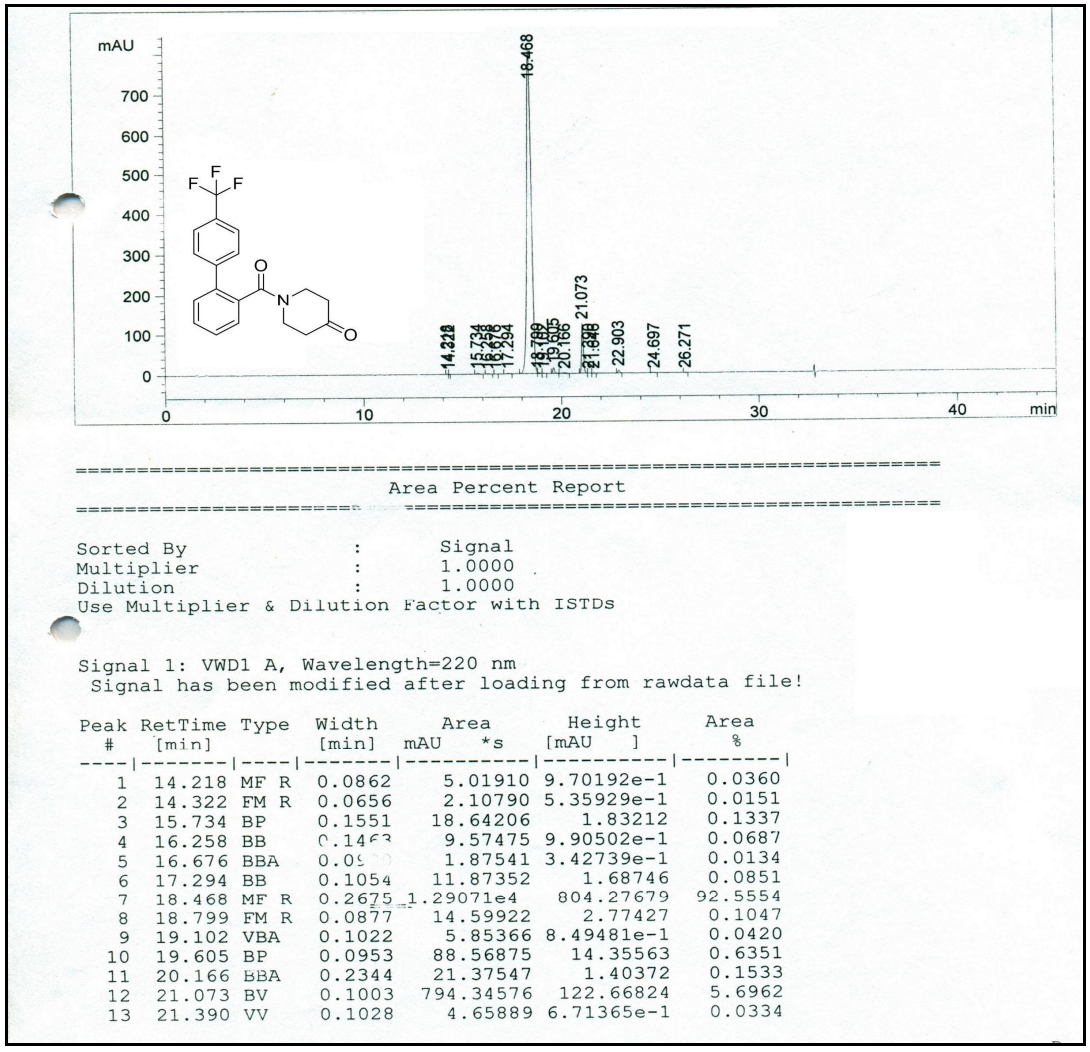
ESI-MS of 2a



IR of 2a



HPLC of 2a



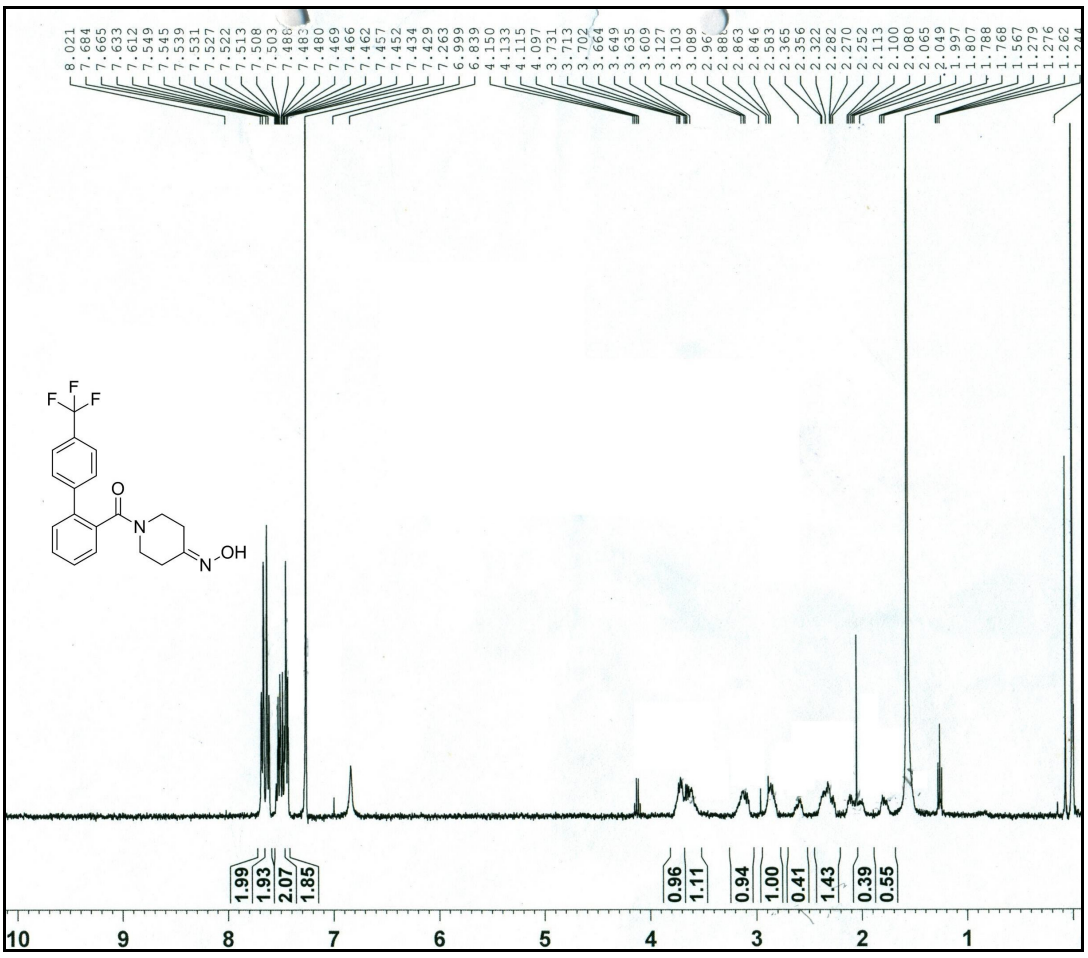
Area Percent Report

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	14.218	MF R	0.0862	5.01910	9.70192e-1	0.0360
2	14.322	FM R	0.0656	2.10790	5.35929e-1	0.0151
3	15.734	BP	0.1551	18.64206	1.83212	0.1337
4	16.258	BB	0.1463	9.57475	9.90502e-1	0.0687
5	16.676	BBA	0.0[illegible]	1.87541	3.42739e-1	0.0134
6	17.294	BB	0.1054	11.87352	1.68746	0.0851
7	18.468	MF R	0.2675	1.29071e4	804.27679	92.5554
8	18.799	FM R	0.0877	14.59922	2.77427	0.1047
9	19.102	VBA	0.1022	5.85366	8.49481e-1	0.0420
10	19.605	BP	0.0953	88.56875	14.35563	0.6351
11	20.166	BBA	0.2344	21.37547	1.40372	0.1533
12	21.073	BV	0.1003	794.34576	122.66824	5.6962
13	21.390	VV	0.1028	4.65889	6.71365e-1	0.0334

^{1}H NMR of 3a



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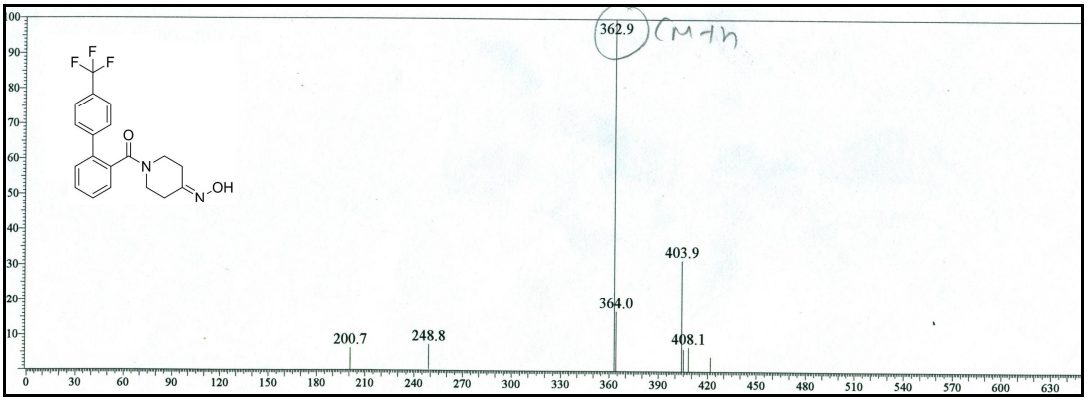

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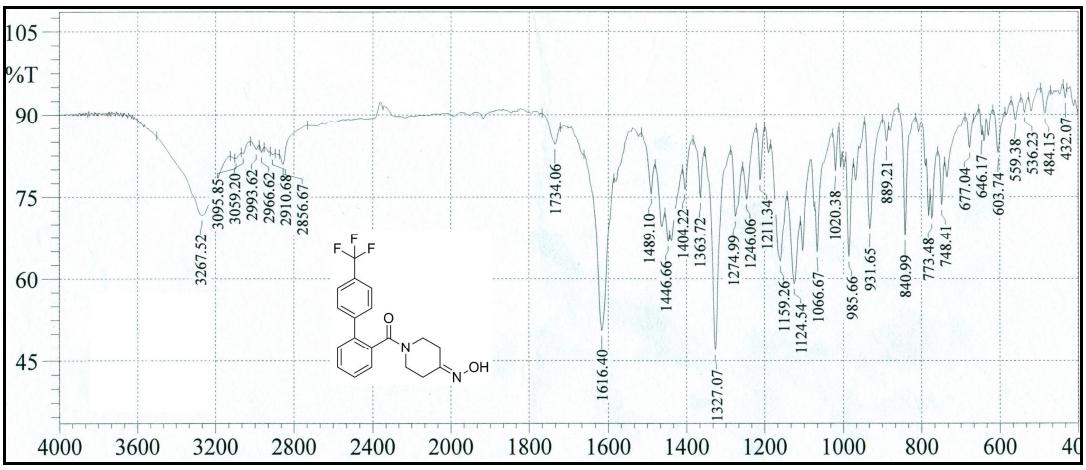

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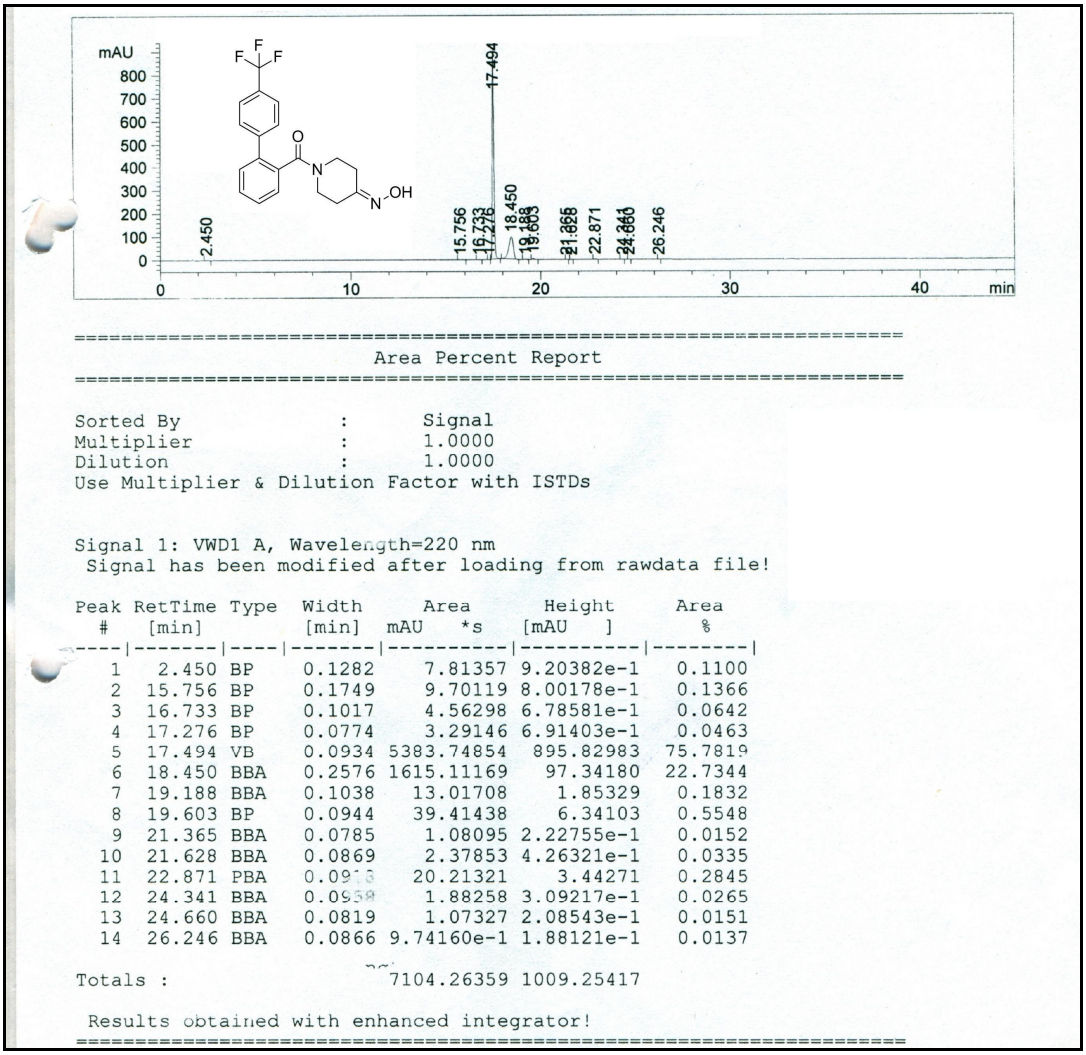

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Use Multiplier & Dilution Factor with ISTDs

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7	19.188	BBA	0.1038	13.01708	1.85329	0.1832
8	19.603	BP	0.0944	39.41438	6.34103	0.5548
9	21.365	BBA	0.0785	1.08095	2.22755e-1	0.0152
10	21.628	BBA	0.0869	2.37853	4.26321e-1	0.0335
11	22.871	PBA	0.0913	20.21321	3.44271	0.2845
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13	24.660	BBA	0.0819	1.07327	2.08543e-1	0.0151
14	26.246	BBA	0.0866	9.74160e-1	1.88121e-1	0.0137
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Results obtained with enhanced integrator!

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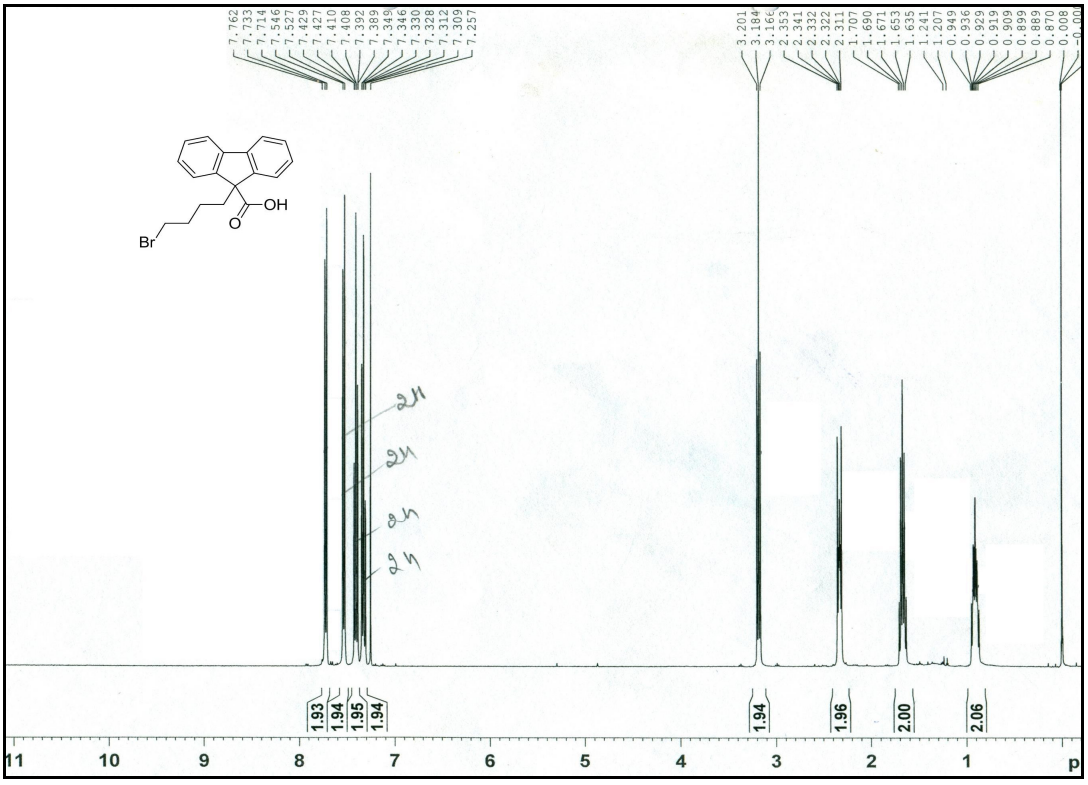

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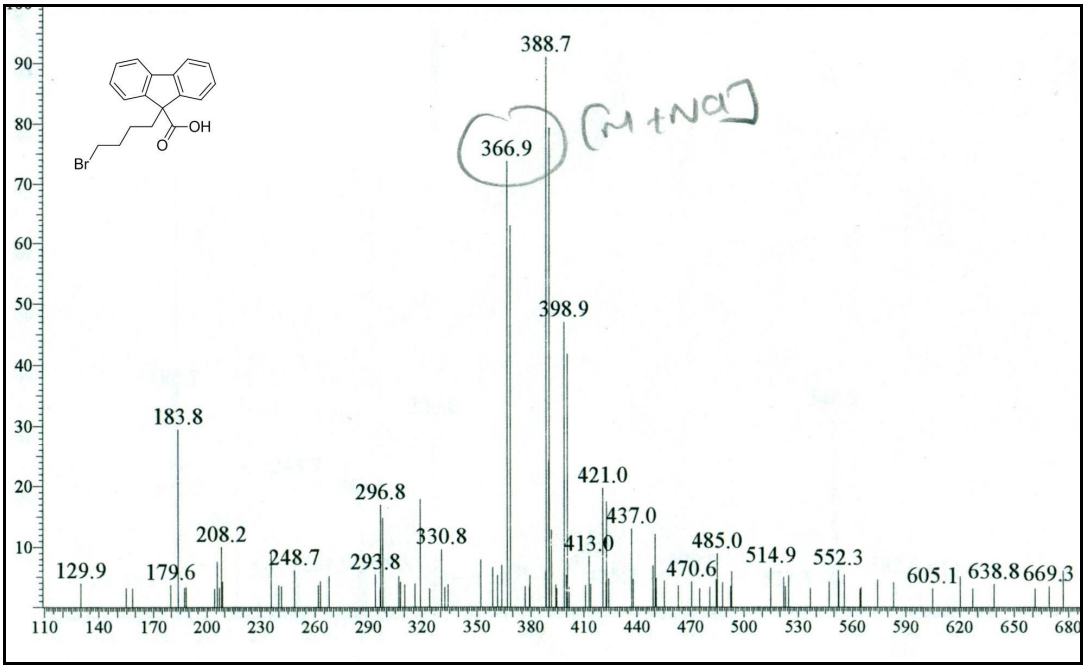

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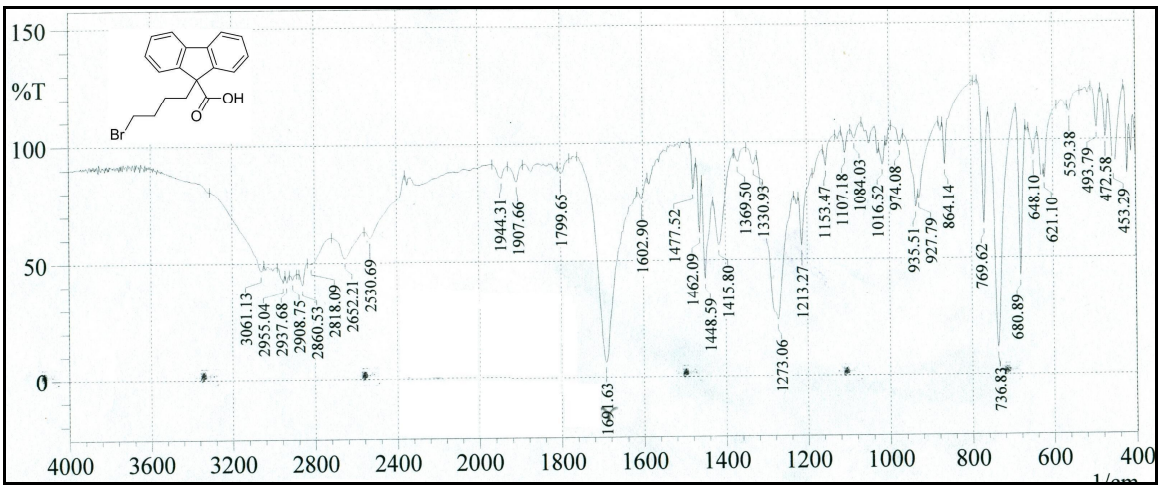

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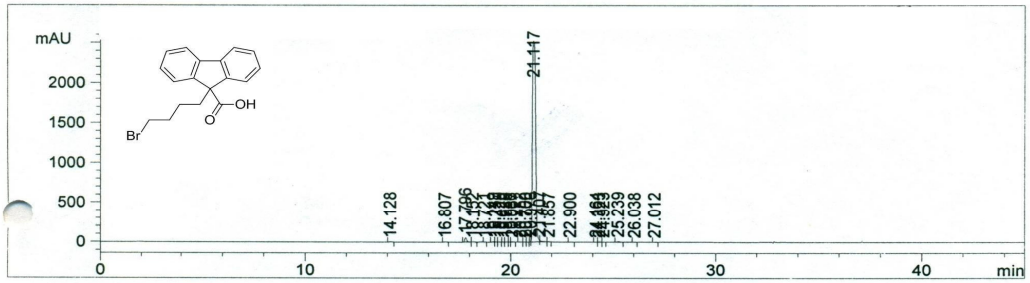


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   3  17.796 BV    0.1026   378.88394   56.75216   1.3435
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   7  19.237 VV    0.0846    16.24572    2.82240   0.0576
   8  19.429 VBA   0.0959     9.43125    1.48481   0.0334
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  11  19.986 MF R  0.0656     3.65322 9.28699e-1   0.0130
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  16  20.976 MF R  0.0273    11.99344    7.33488   0.0425
  17  21.117 FM R  0.1808 2.73838e4  2524.80054  97.1018
  18  21.407 FM R  0.1162   140.10092   20.09081   0.4968
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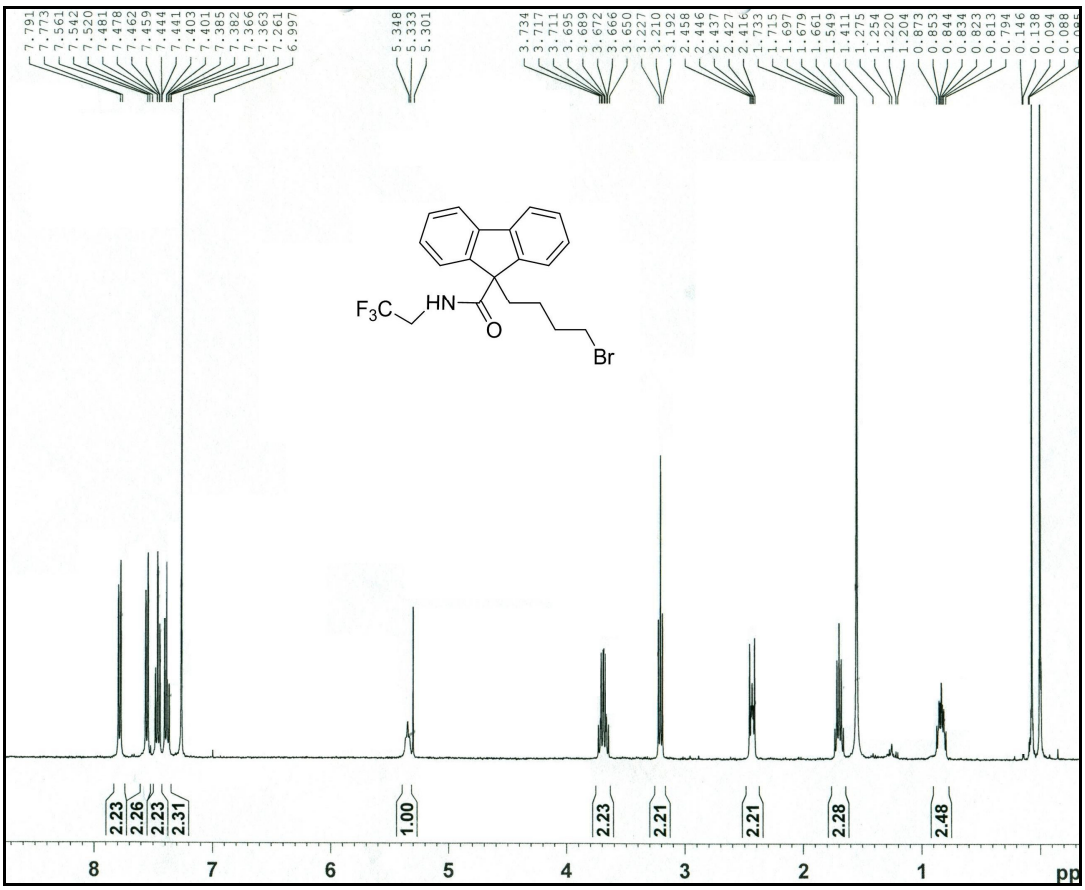

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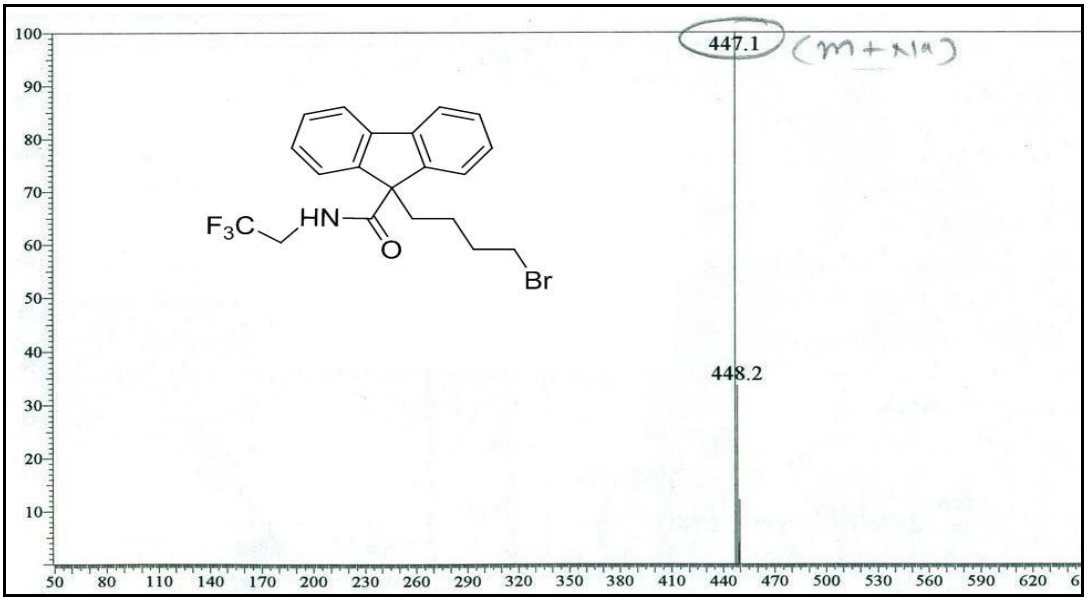

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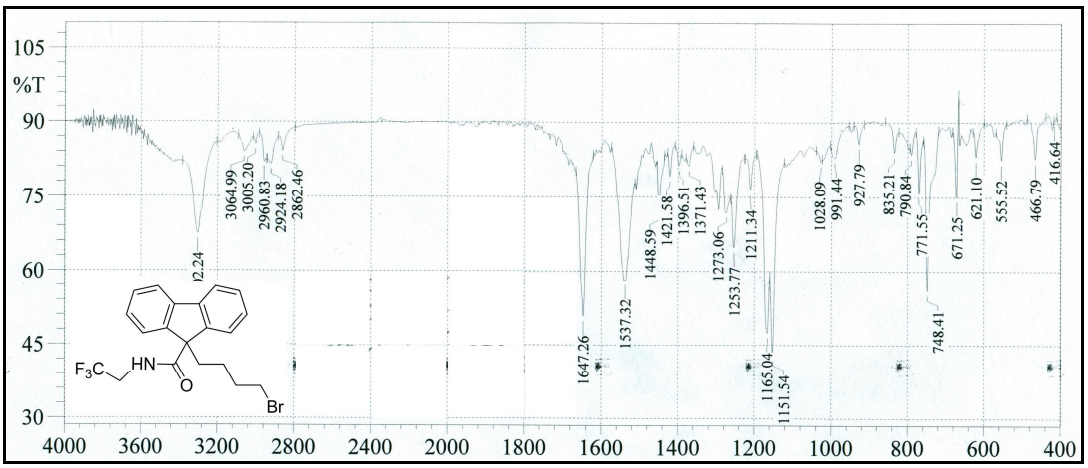
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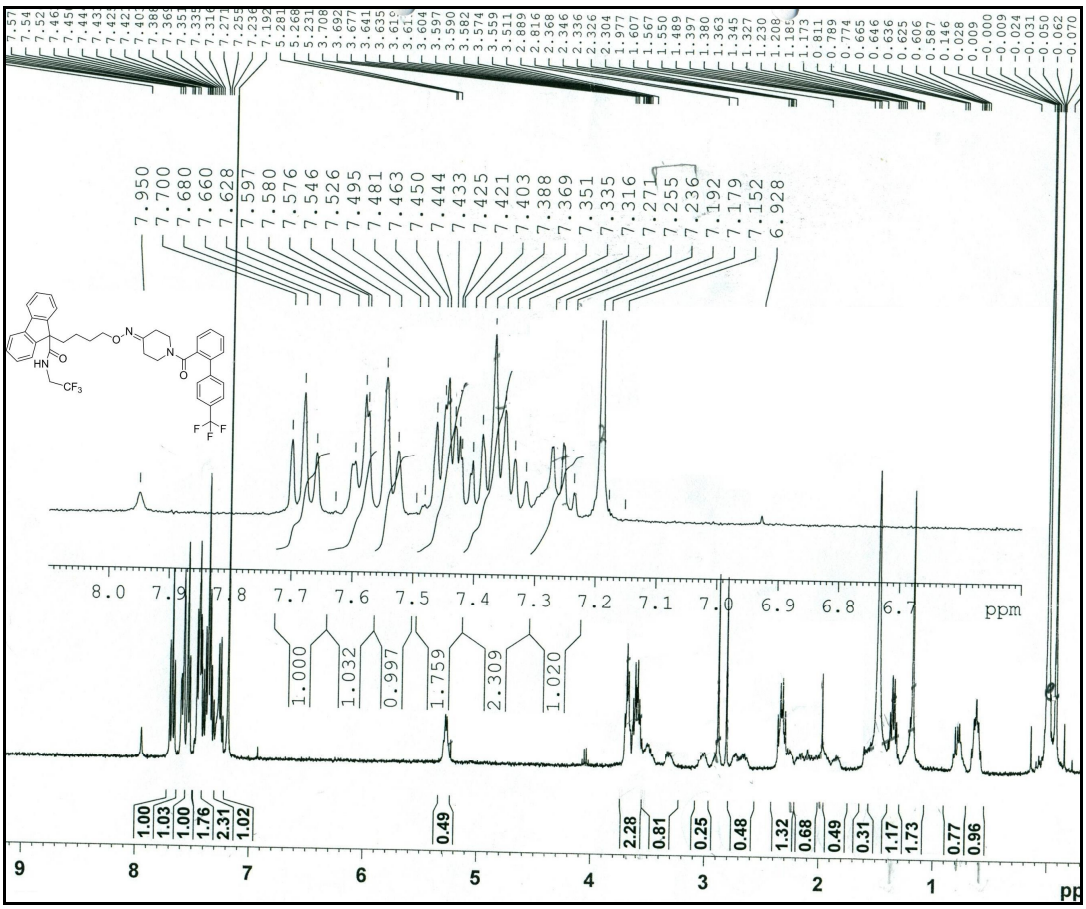
^{1}H NMR of 7a



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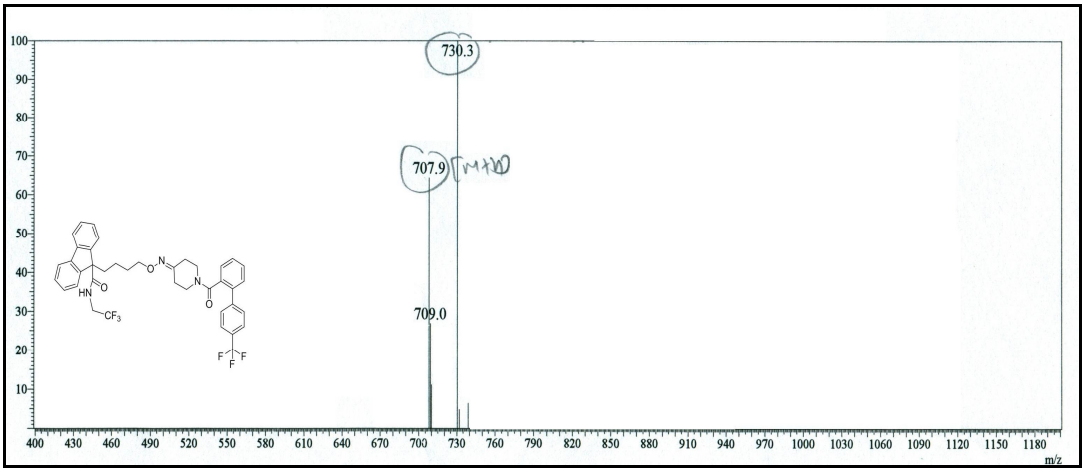

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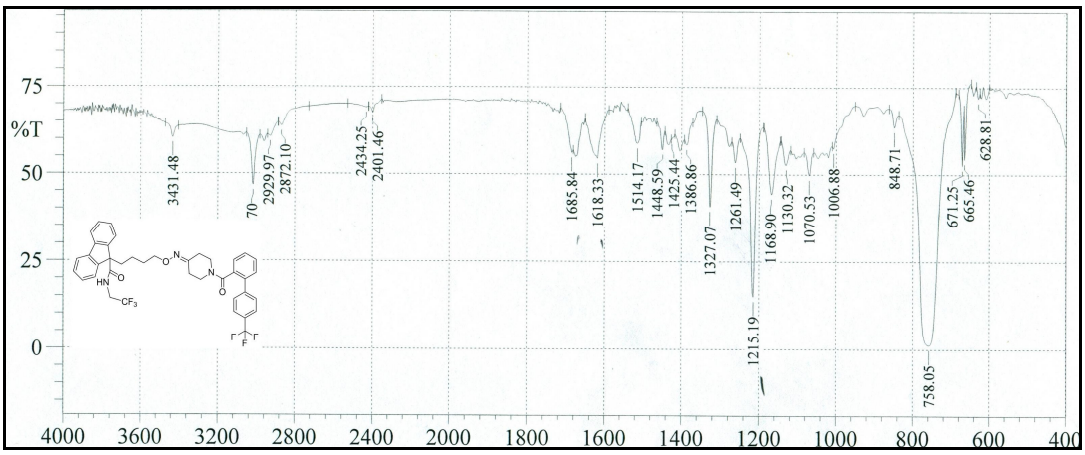

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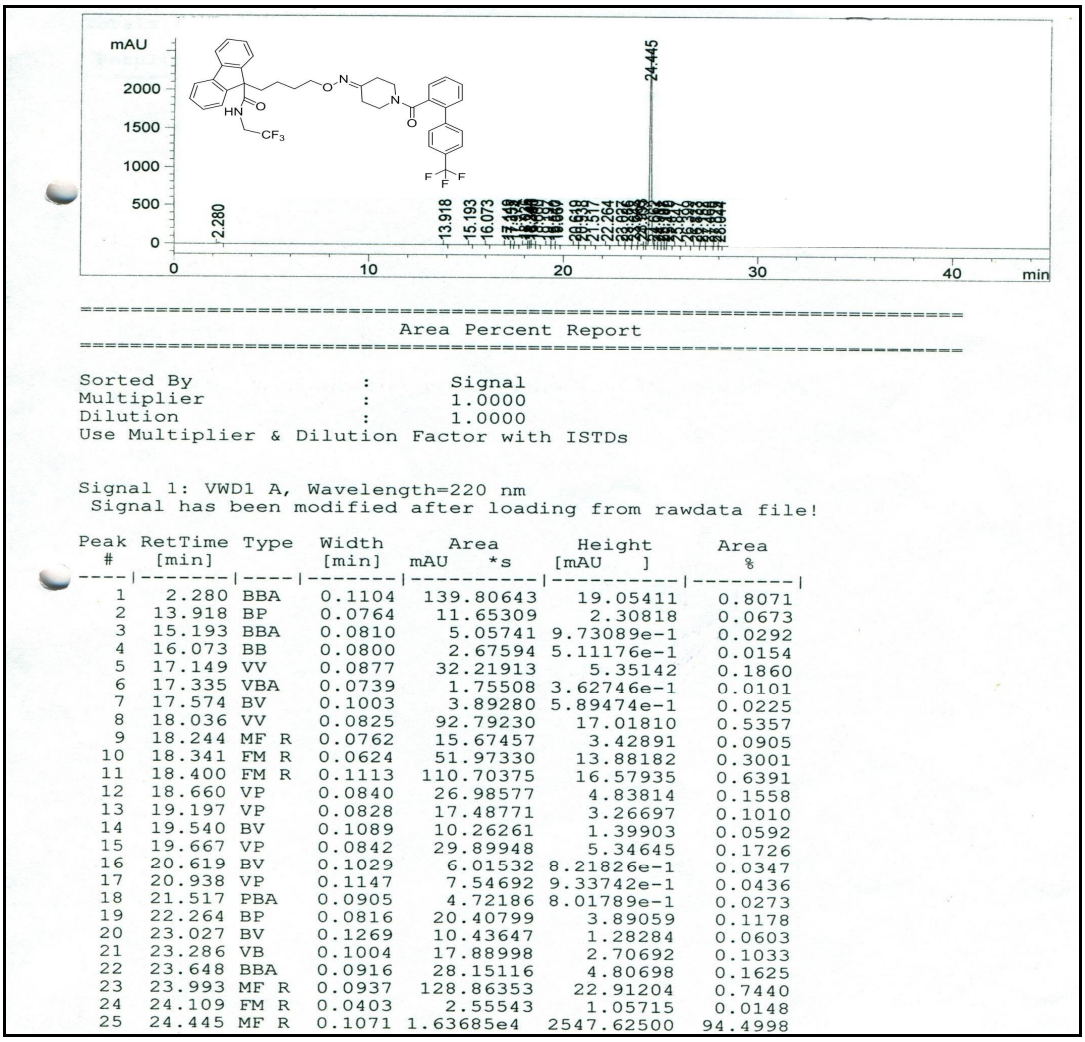

Area Percent Report

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Multiplier : 1.0000
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Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

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3	15.193	BBA	0.0810	5.05741	9.73089e-1	0.0292
4	16.073	BB	0.0800	2.67594	5.11176e-1	0.0154
5	17.149	VV	0.0877	32.21913	5.35142	0.1860
6	17.335	VBA	0.0739	1.75508	3.62746e-1	0.0101
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9	18.244	MF R	0.0762	15.67457	3.42891	0.0905
10	18.341	FM R	0.0624	51.97330	13.88182	0.3001
11	18.400	FM R	0.1113	110.70375	16.57935	0.6391
12	18.660	VP	0.0840	26.98577	4.83814	0.1558
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17	20.938	VP	0.1147	7.54692	9.33742e-1	0.0436
18	21.517	PBA	0.0905	4.72186	8.01789e-1	0.0273
19	22.264	BP	0.0816	20.40799	3.89059	0.1178
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21	23.286	VB	0.1004	17.88998	2.70692	0.1033
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SUMMARY

5

5. Summary

Identification of newer biological targets for scientifically tractable and commercially attractive indications as well as adopting new strategies to develop novel therapies involving validated targets have been long sought after in order to address the highly unmet medical need in all segments of healthcare in general and metabolic diseases in specific. Discovery of tissue selective drugs is an upcoming approach pursued by scientists to strike a balance between efficacy and adverse effects exerted by modulation of biological pathways or targets. CB1 receptor antagonists, Thyromimetics and MTP inhibitors are few among several proven targets for the management of dyslipidemia and/or obesity and as any other target these are also exert adverse effects when modulated with synthetic drugs or molecules. A plausible approach in the development of drugs for these kind of targets where the site of actions are limited to one or few organs, is to make tissue selective compounds. Medicinal chemistry tools play a significant role in this research endeavor of identifying tissue selective drugs for the above mentioned targets.

Literature precedence suggest that abdominal fat accumulation is a critical correlate of the dysregulation of the peripheral endocannabinoid system in human obesity. Thus, the peripheral endocannabinoid system may represent a primary target for the treatment of abdominal obesity and associated metabolic disorders. Further restricting the exposure of a CB1 antagonist in brain Vs peripheral tissues may offer CNS related side effects. Series of dihydropyrazole-3-methyl carboxamide derivatives were synthesized and evaluated in the

functional assay of cAMP production. One of the compound was selected for further evaluation. As it was racemic, its enantiomers were separated and (-) enantiomer emerged as peripherally active CB1 antagonist with excellent *in vitro* potency and *in vivo* efficacy**.**

Therapeutic potential of thyromimectics as lipid-lowering and anti-obesity agents need some more research work to overcome cardiac effects and effects on the thyroid hormone axis (THA). Liver targeted β selective thyromimetics (thyroid receptor agonists) is one of the strategy explored to avoid above mentioned side effects. To achieve liver selectivity, series of squaric acid derivatives were designed and screened for *in vitro* activity. Selected compounds showed better TRβ selectivity than T3 and comparable selectivity to KB-141. Unfortunately compounds belong to this series failed to show bioavailability. Modified from squaric acid derivatives new oxo-acetic acid derivatives was designed. This class of compounds were TRβ selective, bioavailable and showed trend towards liver selectivity.

For the treatment hypercholesterolemia inhibitors of MTP is considered as one of the potential target. Liver toxicity is the major safety concern for this target and by making enterocyte specific compound liver toxicity can be minimized. A series of 9H-fluorene-carboxamide derivatives were designed and evaluated for MTP inhibitory activity. Selected compounds showed *in vitro* and *in vivo* activity, further evolution of these compounds for enterocyte specificity to obtain a clinically useful novel class of MTP inhibitor was not successful.

To conclude, the major challenge was to design peripherally active CB1 antagonists, liver selective thyromimetics and enterocyte specific MTP inhibitors. Partly we have achieved our goal as an attempt to make peripherally active CB1 receptor antagonists with limited brain penetration was successful and we could identify thyromimetic molecules having trend towards liver selectivity. Successfully newly designed molecules with new linker showed MTP inhibition but enterocyte specificity was not achieved. Further research is needed to assure the future of peripherally directed CB1 antagonists, liver selective thyromimetics and enterocyte specific MTP inhibitors.

PUBLICATIONS

6

Publications

(1) Design and synthesis of novel 3-hydroxy-cyclobut-3-ene-1,2-dione derivatives as thyroid hormone receptor b (TR-β) selective ligands
Bioorganic & Medicinal Chemistry Letters 18 (2008) 3919–3924

(2) Modified 9H-fluorene-9-carboxamides as MTP-inhibitors
International Journal of Drug Design and Discovery , Volume 2 • Issue 4 • October–December 2011. 611-618

(3) Emerging Therapies for Dyslipidemia: Known Knowns and Known Unknowns of MTP Inhibitors
Recent Patents on Endocrine, Metabolic & Immune Drug Discovery 2012.6, 24-29

Bioorganic & Medicinal Chemistry Letters 18 (2008) 3919–3924

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of novel 3-hydroxy-cyclobut-3-ene-1,2-dione derivatives as thyroid hormone receptor β (TR-β) selective ligands

Saurin Raval *,†, Preeti Raval, Debdutta Bandyopadhyay, Krunal Soni, Digambar Yevale, Digvijay Jogiya, Honey Modi, Amit Joharapurkar, Neha Gandhi, Mukul R. Jain, Pankaj R. Patel

Zydus Research Centre, Medicinal Chemistry, Sarkhej-Bavla N. H.8A, Moraiya, Ahmedabad, Gujarat 382210, India

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TR-β Selective
Squaric acid
Thyroid receptor assay

ABSTRACT

Design and synthesis of a novel 3-hydroxy-cyclobut-3-ene-1,2-dione derivatives are reported and their in vitro thyroid hormone receptor selectivity has been evaluated in the thyroid luciferase receptor assay. The 3-[3,5-dichloro-4-(4-hydroxy-3-isopropylphenoxy)-phenylamino]-4-hydroxy-cyclobut-3-ene-1,2-dione **21** has shown selectivity towards thyroid hormone receptor β.

Thyroid hormones are important endocrine signalling hormones, which are involved in a number of important physiological processes such as lipid metabolism; control of energy expenditure and in the brain development.[1–4] Natural thyroid hormone, triiodothyronine (T_3) exhibits its physiological effect by acting on a thyroid hormone receptors (THR). It is an important endocrine signalling hormone essential for normal development, differentiation and maintenance of metabolic balance in mammals. The two major subtypes of THR are TR-α and TR-β. Further these two subtypes are classified as α_1, α_2 and β_1, β_2 isoforms.[5] The TR-α_1 and TR-β_1 isoforms are ubiquitously expressed, although TR-α_1 predominates in heart (70% of TRs), whereas TR-β_1 predominates in the liver (80% of TRs).[6] The activation of TR-α_1 isoform mainly affects the heart rate and rhythm whereas, activation of TR-β_1 isoform is known to affect the liver and other tissues.[7,8]

T_3 is nonselective in binding towards both of the THR isoforms (TR-α_1 and TR-β_1). Administration of T_3 lowers the plasma cholesterol, low-density lipoprotein (LDL) and triglyceride levels in animal models,[8,9] and in humans.[10–12] However, T_3 cannot be used therapeutically to treat hypercholesterolemia and obesity due to its cardiac side effects.[9,10] TR-β_1 agonist could lead to specific therapies for disorders such as obesity and hyperlipidaemia, while avoiding the cardiovascular and other toxicities of native thyroid hormone. Thus, the selectivity for the TR-β_1 over TR-α_1 remains an important criterion in the development of THR ligands.

Axitirome (±)-*N*-[4-[3-(4-fluoro-α-hydroxybenzyl)-4-hydroxyphenoxy]-3,5-dimethylphenyl] oxamic acid ethyl ester **1** (Fig. 1) has been extensively studied oxamic acid thyromimetics, which was progressed up to phase I.[13–16] However, it was discontinued in clinical trials in 1998 for unknown reasons.[17] Similarly 3,5-dichloro-4-[(4-hydroxy-3-isopropylphenoxy)phenyl]acetic acid **2** (KB-141, Fig. 1) was found to be promising due to its selectivity for TR-β subtype[18,19] and has been reviewed in detail for its biological properties.[20,21]

Replacement of biaryl ether linkage with a methylene linkage and phenoxy acetic acid led to a potent compound **3** (GC-1, Fig. 1). It has 10-fold TR-β selectivity in transactivation and lowers the serum cholesterol levels without affecting the heart rate.[22,23]

Hangeland et al. have reported compound **4** (Fig. 2) containing larger hydrophobic group replacing isopropyl group of KB-141 at position R, which demonstrated improved selectivity.[24] Similarly, substitution with aromatic hydrophobic group such as phenyl lead to more potent compound **5** (GC-24, Fig. 2) with higher affinity and stronger selectivity for the TR-β subtype.[25] Recently, compounds **6** and **7** containing 6-azauracil and 3′-carboxamide and 3′-sulfonamide linkage have been reported as potent and TR-β selective thyromimetics.[26] 4′-Amido derivatives **8** have also been described as TR-β selective (Fig. 3).[27]

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E-mail addresses: saurinraval@zyduscadila.com, saurin_raval@rediffmail.com (S. Raval).
† ZRC communication # 241.

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doi:10.1016/j.bmcl.2008.06.038

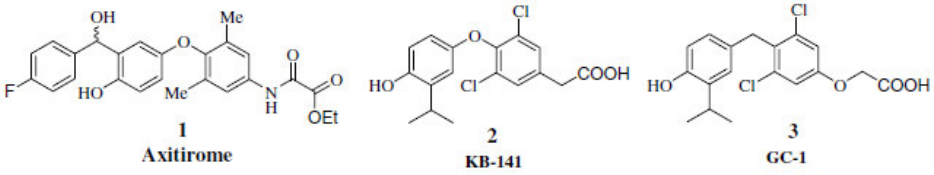


Figure 1.

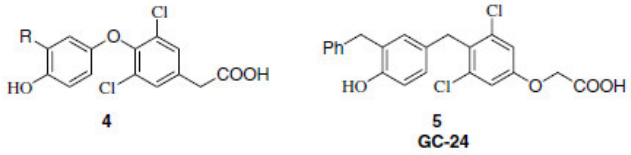


Figure 2.

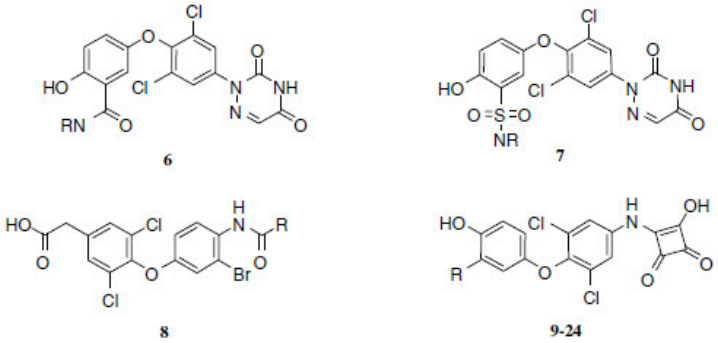


Figure 3.

The X-ray crystallographic structures of the ligand binding domains (LBD) of TR-α_1 and TR-β_1 determined in complex with thyromimetics are accessible from Protein Data Bank (PDB).[18,24–26,28,29] The hormone binding pocket is very similar in both the receptors. These receptors differ only by one single amino acid residue, Asn331 in TR-β, which is substituted by Ser277 in TR-α. This results in a significantly different hydrogen bonding patterns between the acidic group of the ligands and the LBD of the two receptors and hence accounts for selectivity. In continuation of our thyroid hormone receptor research program,[32] we focused on modification of acidic moieties in the small molecules to find TRH agonist selective for β-type. Again, the flexibility in the hydrophobic aromatic pocket in TR-β is known to accommodate bulky groups such as benzyl and retain full agonistic activity.[24,25] The hydroxy-cyclobut-3-ene-1,2-dione derivatives **9–24** were designed and validated using molecular modeling.[33]

Molecular docking studies indicated that compounds **21** and **22** make similar interactions in the LBD of TR-α and TR-β as that of exhibited by the ligand 3,5-dichloro-4-[(4-hydroxy-3-isopropylphenoxy)phenyl]acetic acid KB-141 in the crystal structures (Fig. 4).[18]

On basis of favourable orientation and similar binding of designed compound to TR-β as that of KB-141 in molecular docking studies, the derivatives **9–24** comprising 3-hydroxy-cyclobut-3-ene-1,2-dione were synthesized.[34] The in vitro activities of the compounds **9–24** for TR-α and TR-β were evaluated with respect to T_3 in a thyroid luciferase receptor assay.[35]

The synthesis of compounds **9–24** has been outlined in Scheme 1. Phenol derivatives **25** were reacted with 1,2,3-trichloro-5-nitro benzene using sodium hydride to afford nitro substituted diaryl ether derivatives **26**. The nitro-diaryl ether derivatives **26** were subsequently reduced using tin chloride in acidic medium to get amino derivatives **27**. The demethylation of methoxy derivatives **27** was achieved using solution of boron tribromide to afford amine derivatives **28**. The resulting amine derivatives **28** were further reacted with 3,4-dihydroxy-3-cyclobutene-1,2-dione(squaric acid) to afford cyclobut-3-ene-1,2-dione derivatives **9–24**.

In vitro % TR-α and TR-β activities of 3-hydroxy-cyclobut-3-ene-1,2-dione derivatives **9–24** at different concentrations such as 0.001, 0.01 and 1 μM were evaluated with respect to T_3 (Table 1) keeping KB-141 as a positive control. The primary carboxamide derivatives **9–12** failed to show TR-α or TR-β activity as compared to potent TR-β selective compound **2** even at the concentration of 1 μM (Table 1). Following the unfavourable activity of compounds **9–12**, the primary carboxamide groups were replaced by secondary carboxamides, which resulted to the derivatives **13–16**. The *N,N*-dimethyl carboxamide derivative **13** exhibited mild activity at 1 μM concentration for both the TR-α and TR-β. The cyclopentyl carboxamide derivative **14** remained inactive at lower concen-

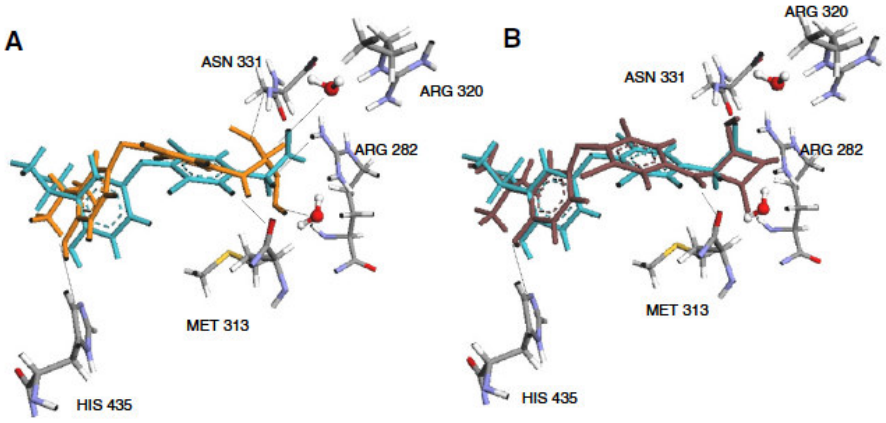
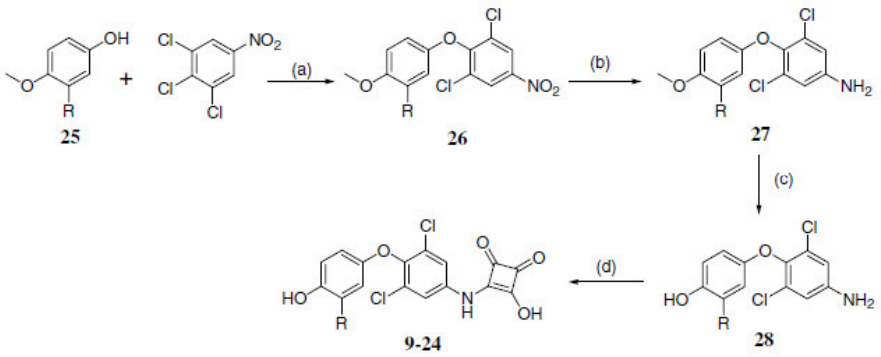


Figure 4. Docking of **21** and **22** in ligand binding pocket of TR-β including water molecules. Residues and ligands are shown as sticks. The docked conformers **21** and **22** are superimposed on compound **2** KB-141(shown in cyan as sticks). (A)The oxygen atoms in compound **21** (shown in orange) makes hydrogen bonding with the side chains of residues His435, Arg282, Asn331 and water molecules. The amide linkage makes hydrogen bond with the backbone of Met313 (B) Compound **22** shown in magenta makes similar interactions as that of shown by compound **21**.



Scheme 1. Reagents and conditions: (a) NaH, DMF, 120 °C, 3–4 h, 95%; (b) $SnCl_2{\cdot}2H_2O$, concd HCl, EtOH, 65–70 °C, 3 h, 95%; (c) 1 M BBr_3, CH_2Cl_2, 25 °C, 3 h, 20–50%; (d) $C_4H_2O_4$, H_2O, 100 °C, 4–6 h, 30–40%.

trations of 0.001 and 0.01 μM; however, the improved activity was seen at 1 μM concentration (50% for TR-α and 80% for TR-β), which is almost similar to compound **2** at the concentration of 0.001 μM. Changing the ring size of **14** from five- to six-membered, cyclohexyl carboxamide derivative **15** showed diminished activities for both TR-α and TR-β. The carboxamide with one more heteroatom, the morpholino derivative **16** remained inactive against thyroid receptor luciferase assay.

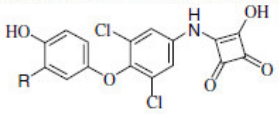
Due to the unfavourable in vitro TR-α and TR-β activities for primary and secondary carboxamide derivatives **9–16**, we evaluated some of the sulfonamide derivatives **17–19** in the same assay and compared with compound **2**. The cyclohexyl sulfonamide derivative **17** showed similar trend of inactivity as that of exhibited by its corresponding carboxamide derivative **10**. Interestingly, the secondary sulfonamide derivative **18** showed mild activity at 1 μM concentration; however, it remained inferior to the compound **2**. Similarly, the morpholino sulfonamide derivative **19** showed weak activities at 1 μM concentration for both TR-α and TR-β.

Furthermore, we tested unsubstituted analogue **20**, which did not show pronounce activities for TR-α and TR-β; however, the selectivity towards TR-β was noteworthy.

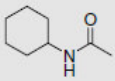
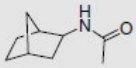
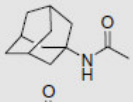
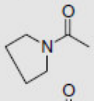
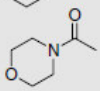
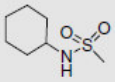
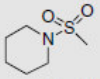
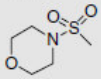
Contrary to carboxamide derivatives **9–16**, sulfonamide derivatives **17–19** and compound without amidic linkage **20**, the compounds substituted by isopropyl **21** and *tert*-butyl **22** gave the encouraging in vitro activity pattern for TR-α and TR-β at the concentration of 1 μM (Table 1). The *tert*-butyl derivative **22** was found to be equipotent to isopropyl derivative **21** (Table 1). Further, the benzyl derivative **23** and phenoxy derivative **24** were found to be inferior to the isopropyl derivative **21** and the *tert*-butyl derivatives **22**. Following the satisfactory in vitro selectivity of compounds **21** and **22**, the EC_{50} values of compounds **21** and **22** were calculated for both TR-α and TR-β (Table 2). Both the compounds **21** and **22** exhibited nearly similar selectivities for TR-α and TR-β as that of shown by the compound **2**.

In summary, a rational structure activity relationship for 3-hydroxy-cyclobut-3-ene-1,2-dione compounds **9–24** as thyroid hormone receptor β culminated to compounds **21** and **22**, which exhibited similar selectivities for TR-α and TR-β as that of shown by the compound **2** (KB-141). The more elaborative SAR, lead optimization, in vitro, in vivo studies and pharmacokinetics in rodent models is in progress at our centre and will be published in future course of time.

Table 1
In vitro % activity of novel 3-hydroxy-cyclobut-3-ene-1,2-dione compounds **9–24** for TR-α and TR-β with respect to T_3



9-24

Compound	R	Concn (μM)	% Activity for TR-α[a]	% Activity for TR-β[a]
2	KB-141	0.001	53.25	72.56
		0.01	100.00	100.00
		1	84.70	111.03
9		0.001	6.16	7.015
		0.01	6.94	8.34
		1	11.70	19.05
10		0.001	7.41	10.01
		0.01	7.69	12.28
		1	9.40	15.59
11		0.001	6.23	8.50
		0.01	6.80	10.39
		1	6.00	11.53
12		0.001	7.12	12.30
		0.01	7.13	12.70
		1	7.19	8.80
13		0.001	7.35	8.50
		0.01	7.37	9.00
		1	13.45	23.20
14		0.001	8.56	8.60
		0.01	9.62	10.60
		1	50.29	80.70
15		0.001	5.63	5.63
		0.01	5.96	5.30
		1	5.25	6.23
16		0.001	6.93	13.00
		0.01	7.91	12.70
		1	11.03	30.90
17		0.001	6.28	12.00
		0.01	6.57	13.60
		1	16.72	48.10
18		0.001	8.56	12.80
		0.01	9.83	32.50
		1	32.28	63.80
19		0.001	8.96	15.70
		0.01	10.55	24.70
		1	22.86	43.30
20	H	0.001	6.12	14.60
		0.01	6.08	15.10
		1	9.36	30.90
21		0.001	22.36	30.00
		0.01	35.04	70.00
		1	79.84	130.00
22		0.001	15.30	14.20
		0.01	30.70	53.50
		1	61.50	107.10
23		0.001	9.63	8.30
		0.01	13.64	29.00
		1	45.48	62.30
24		0.001	7.23	8.96
		0.01	7.89	9.14
		1	27.22	71.49

[a] The TRE-luciferase assay[35] has been used for the in vitro TR-α and TR-β activities and values are mean of duplicate measurements and expressed in nM. The variability of the measurements is on average of 25%.

Table 2
In vitro EC_{50} for selected compounds **21** and **22** for TR-α, TR-β

Compound	EC_{50} TR-α[a] (nM)	EC_{50} TR-β[a] (nM)	EC_{50} TR-α/β
2	4.5	1.12	4.01
21	222.00	70.00	3.00
22	1056.00	317.00	3.33

[a] EC_{50} values are expressed as mean from the duplicate measurements and are expressed in nM. The variability of the measurements is on average of 25%.

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33. The X-ray crystallographic structure of the KB-141 (**2**) with TR-α (PDB Code: 1NAV) and TR-β (PDB Code: 1NAX) was selected for the docking study.[18] Docking was performed for compounds **9–24** using ArgusLab 4.0[30] in the presence of water molecules. Hydrogens and charges were added to the ligand and receptor using modules from DS Studio 2.0 (Accelrys, Inc.). The binding site was defined from the coordinates of the ligand in the PDB file. Argusdock exhaustive search docking engine was used, with grid resolution of 0.20 Å. Five hundred docking runs were used for each compound. Docking precision was set to 'high precision' and 'flexible ligand docking' mode was employed for each docking run. Docked conformers for each compound were ranked and scored using Ascore[31] implemented in ArgusLab. The interaction of the top ranked docked poses in the pocket were visualized using DS Visualizer 1.7 (Accelrys, Inc.).
34. Spectroscopic data for compounds **9–24**:
Compound **9**: 70% Yield; 96.1% purity by HPLC; mp 203–205 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.61 (s, 1H), 7.94 (s, 2H), 7.53 (d, J = 2.76 Hz, 1H), 6.81 (d, J = 9.0 Hz, 1H), 6.72 (d, J = 9.03 Hz, 1H), 4.10–4.12 (m, 1H), 1.17 (d, J = 6.57 Hz, 6H); ESI-MS: 449.0 [M–H]$^-$.
Compound **10**: 60% Yield; 97.7% purity by HPLC; mp 206–208 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.07 (s, 1H), 11.89 (s, 1H), 8.07 (s, 2H), 7.52 (s, 1H), 6.80–6.83 (m, 2H), 3.76–3.78 (br s, 1H), 1.56–1.80 (m, 6H), 1.30–1.32 (br s, 4H); ESI-MS: 488.8 [M–H]$^-$.
Compound **11**: 62% Yield; 96.5% purity by HPLC; mp 198–203 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.96 (s, 1H), 11.92 (s, 1H), 8.08 (s, 2H), 7.58 (d, J = 2.7 Hz, 1H), 6.84 (d, J = 9 Hz, 1H), 6.78 (d, J = 3 Hz, 1H), 3.70–3.72 (br s, 1H), 2.24–2.26 (br s, 2H), 1.65–1.67 (m, 1H), 1.45–1.47 (m, 4H), 1.13–1.15 (m, 3H); ESI-MS: 500.0 [M–H]$^-$.
Compound **12**: 50% Yield; 97.9% purity by HPLC; mp 192–196 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.93 (s, 1H), 11.49 (s, 1H), 8.09 (s, 2H), 7.33 (s, 1H), 6.88 (s, 2H), 2.01–2.03 (br s, 9H), 1.62–1.64 (br s, 6H); ESI-MS: 541.0 [M–H]$^-$.
Compound **13**: 51% Yield; 97.1% purity by HPLC; mp 217–220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.61 (s, 1H), 9.53 (s, 1H), 7.91 (s, 2H), 6.80 (d, J = 8.91 Hz, 1H), 6.68–6.72 (dd, J = 8.8 and 3.06 Hz, 1H), 6.45 (d, J = 3.03 Hz, 1H), 2.71–2.79 (br s, 6H); ESI-MS: 435.0 [M–H]$^-$.
Compound **14**: 61% Yield; 96.9% purity by HPLC; mp >220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.69 (s, 1H), 9.63 (s, 1H), 7.92 (s, 2H), 6.81 (d, J = 8.91 Hz, 1H), 6.70–6.74 (dd, J = 8.79 and 3 Hz, 1H), 6.51 (d, J = 3 Hz,1H), 3.18–3.20 (br s, 4H), 1.77–1.79 (br s, 4H); ESI-MS: 461.1 [M–H]$^-$.
Compound **15**: 66% Yield; 97.1% purity by HPLC; mp 186–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.90 (s, 1H), 9.54 (s, 1H), 8.07 (s, 2H), 6.78 (d, J = 8.49 Hz, 2H), 6.47 (s, 1H), 3.14–3.16 (br s, 4H), 1.43–1.53 (m, 6H); ESI-MS: 475.1 [M–H]$^-$.
Compound **16**: 64% Yield; 96.3% purity by HPLC; mp 210–214 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ 9.65 (s, 1H), 9.63 (s, 1H), 7.92 (s, 2H), 6.80 (d, J = 8.79 Hz, 1H), 6.70 (d, J = 6.21 Hz, 1H), 6.52 (d, J = 2.19 Hz, 1H), 3.53–3.55 (br s, 4H), 3.14–3.16 (br s, 4H); ESI-MS: 477.0 [M–H]$^-$.
Compound **17**: 50% Yield; 98.1% purity by HPLC; mp 182–184 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.92 (s, 1H), 8.09 (s, 2H), 7.21 (d, J = 7.65 Hz, 1H), 7.06–7.10 (dd, J = 8.7 and 3 Hz, 1H), 6.93 (d, J = 3 Hz, 1H), 2.78–2.80 (br s, 1H), 1.53–1.56 (m, 4H), 1.02–1.18 (m, 6H); ESI-MS: 524.9 [M–H]$^-$.
Compound **18**: 87% Yield; 97.5% purity by HPLC; mp 189–193 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.94 (s, 1H), 10.43 (s, 1H), 8.10 (s, 2H), 6.94–7.07 (m, 3H), 3.03–3.05 (br s, 4H), 1.45-1.47 (br s, 6H); ESI-MS: 510.9 [M–H]$^-$.
Compound **19**: 60% Yield; 98.3% purity by HPLC; mp 196–199 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.95 (s, 1H), 10.58 (s, 1H), 8.09 (s, 2H), 6.95–7.09 (m, 3H), 3.55–3.57 (br s, 4H), 3.03–3.05 (br s, 4H); ESI-MS: 513.0 [M–H]$^-$.
Compound **20**: 65% Yield; 98.1% purity by HPLC; mp 205–208 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.71 (s, 1H), 9.06 (s, 1H), 7.81 (s, 2H), 6.53–6.62 (m, 4H); ESI-MS: 364.0 [M–H]$^-$.
Compound **21**: 41% Yield; 98.9% purity by HPLC; mp 210–213 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.63 (s, 1H), 8.99 (s, 1H), 7.90 (s, 2H), 6.62–6.65 (m, 2H), 6.25–6.29 (dd, J = 8.67 and 3.03 Hz, 1H), 3.11–3.15 (m, 1H), 1.10 (d, J = 6.9 Hz, 6H); ESI-MS: 406.0 [M–H]$^-$.
Compound **22**: 96% Yield; 99.1% purity by HPLC; mp 215–218 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.56 (s, 1H), 9.04 (s, 1H), 7.91 (s, 2H), 6.71 (d, J = 3 Hz, 1H), 6.64 (d, J = 8.7 Hz, 1H), 6.26–6.30 (dd, J = 8.64 and 3.03 Hz, 1H), 1.29 (s, 9H); ESI-MS: 420.1 [M–H]$^-$.
Compound **23**: 81% Yield; 98.2% purity by HPLC; mp 219–221 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.58 (s, 1H), 9.14 (s, 1H), 7.89 (s, 2H), 7.10–7.26 (m, 5H), 6.69 (d, J = 8.73 Hz, 1H), 6.58 (d, J = 3.03 Hz, 1H), 6.34–6.38 (dd, J = 8.64 and 3.09 Hz, 1H), 3.80 (s, 2H); ESI-MS: 454.1 [M–H]$^-$.
Compound **24**: 58% Yield;98.6% purity by HPLC; mp >222 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.61 (s, 1H), 9.27 (s, 1H), 7.89 (s, 2H), 7.27–7.32 (m, 2H), 6.98–7.03 (m, 1H), 6.81–6.88 (m, 3H), 6.42–6.48 (m, 2H); ESI-MS: 456.1 [M–H]$^-$.
35. *Thyroid receptor assay:* A luciferase receptor assay has been used to find the TR-α and TR-β selectivities of the compounds, where luciferase gene

expression is driven by a thyroid receptor binding element (TRE) upstream of the luciferase gene. Briefly 6×10^4 CV-1 cells were plated in each well of a 24-well cell culture plate. The cells were transfected 16 h after seeding with a plasmid bearing three copies of TRE cloned upstream of luciferase gene along with a plasmid expressing either the full length human thyroid receptor-α or -β isoform and a third plasmid expressing β-galactosidase. The transfection is carried out using polyfect reagent from Invitrogen, Inc. (Carlsbad, CA). The medium is replaced 6 h post transfection with fresh media having different concentrations of the agonist. The concentration of agonist is adjusted in such a way that the concentration of the solvent (DMSO) in each well is maintained at 1%. The plates are incubated at 37 °C for 16 h before lysing and assaying the luciferase activity using commercially available Glo-lysis kit from Promega and a standard luminometer. The β-galactosidase activity was measured by using the β-galactosidase assay kit from Promega and the absorbance was read at 415 nM.

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Modified 9H-fluorene-9-carboxamides as MTP-inhibitors

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Introduction

Metabolic disorder is a cluster of disorder of metabolism like hyperglycemia, dyslipidemia and obesity. Any of these alone or in combination can lead to high risk for the development of high levels of cholesterol, triglyceride and insulin resistance.

Dyslipidemia is the most common complication of insulin resistance and type-2 diabetes, which gets exacerbated by obesity[1]. Severe dyslipidemia accompanying insulin resistance is characterized by distinct changes exhibiting elevated plasma triglyceride, free fatty acids (FFA), and reduced levels of HDL. These changes are closely associated with elevated VLDL production[1] and secretion, increased levels of plasma small dense LDL levels as well as increased levels of apoB[2] Triglyceride-rich lipoproteins like VLDL and LDL are known to be atherogenic[3,4]. It has been particularly reported that increase in the concentration of apo B48 containing intestinally derived lipoproteins are significantly correlated with the progression of coronary atherosclerosis[5].

Microsomal triglyceride transfer protein (MTP) is one of the target area which has evidenced potential benefits to reduce triglycerides and cholesterol[6]. MTP is essential for assembly of triglycerides with apoB48 producing chylomicrons in intestine and with apoB100 in liver to synthesize and secrete VLDL. An increase in MTP protein mass, which is associated with over secretion of intestinally derived apoB48 lipoproteins-chylomicrons, was observed in preclinical studies on fructose-fed insulin resistant hamsters[7]. Moreover, diabetic rabbits and rats exhibited increased MTP expression leading to increased chylomicron production[8]. It is also reported that increased MTP expression plays a crucial role in the development of atherosclerosis[9]. MTP is heterodimeric lipid transfer protein and it is found in endoplasmic reticulum of hepatocytes and enterocytes[10].

Collectively MTP has distinct function in liver as well as in intestine. In liver assembly of triglyceride and apo B100 is mediated by MTP. Similarly in intestine MTP is responsible for the assembly of triglyceride and Apo B48. Altogether MTP plays crucial role for the generation of Triglyceride rich chylomicrones in liver and for VLDL in intestine[11].

Therefore MTP inhibitor can cause reduction in plasma triglyceride and cholesterol. This thought is already conceived and as a result there are compounds advanced up to clinical trials[12]. BMS-201038 is 9H-fluorene-9-carboxamide derivative and it was developed from its early hit BMS-200150[13]. BMS-201038 is further developed as AGER-733(lomitapide) and taken to clinical trials. There are many clinical studies reported for this compound[14].

In the present work, we carried out modification to lomitapide retaining some of the structural features.

As it is explicated in Figure 2, lomitapide can be fragmented into three components. (A) 9H-fluorene-carboxamide. (B) Linker and (C) Aromatic carboxylic acid. Our aim was to understand significance of 4-amino piperidine at linker position. Initially 9H-fluorene-carboxamide and aromatic acid component was kept constant, and linker position was modified. As it is shown in Figure 2, we have replaced 4-amino piperidine with oximino group at linker position. Few compounds were synthesized having biphenyl carboxylic acids at aromatic acid position and further it was replaced by substituted benzoic acids with new linker.

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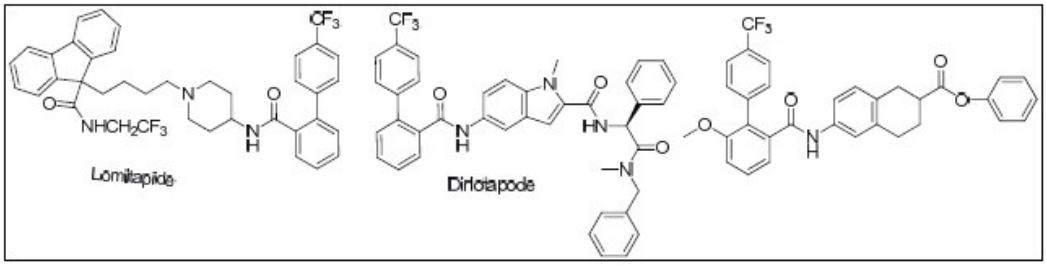


Fig. 1 Progressing Microsomal triglyceride transfer protein (MTP) inhibitors.

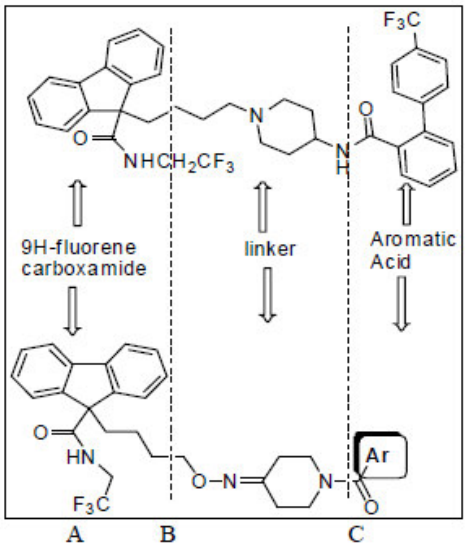
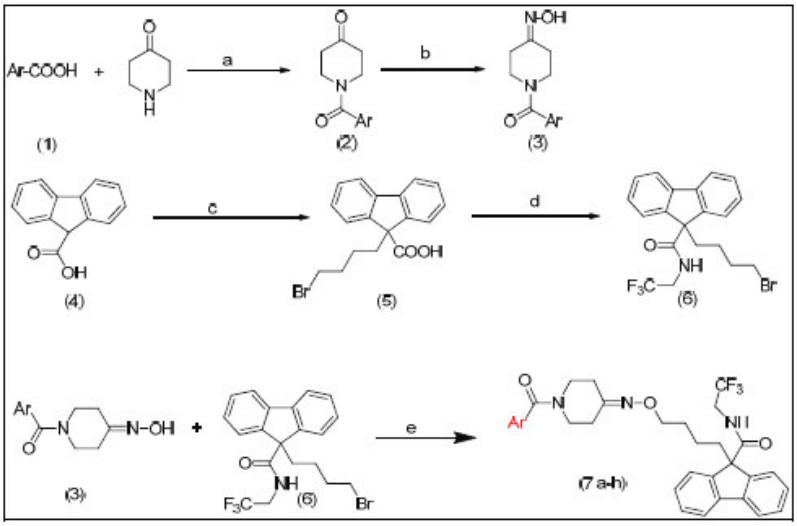


Fig. 2 Conception of new 9H-fluorene-9-carboxamides.

Chemistry :

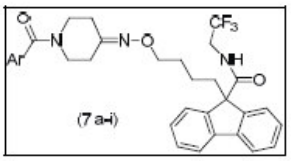


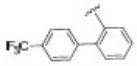
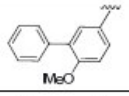
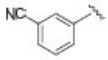
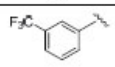
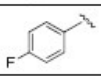
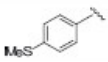
Reagents and conditions: (a) EDC, HOBT, NEt_3, DCM, 25°C (b) $NH_2OH.HCl$, $EtOH:H_2O$, Reflux
(c) n-Buli, THF,-78°C, 3-4 h, Br $(CH2)_4Br$ (d) Oxalyl Chloride, DCM, ,$CF_3CH_2NH_2$, 0°C, 1h (e) KOH, DMSO, 25 °C

Fig 1. Synthesis of 9H-fluorene-9-carboxamide derivative.

Key intermediate Oxime (**3**) was prepared in two steps starting from coupling of Aryl carboxylic acid (**1**) with piperidin-4-one using standard acid base coupling method. Acid was coupled with piperidin-4-one in presence of EDC, HOBt and DMF was used as solvent. The reaction was carried out at room temperature. The next step was oxime formation. For this coupled product (**2**) was reacted with $NH_2OH.HCl$ in $EtOH:H_2O$ as solvent at reflux temperature. Another key intermediate 9H-fluorene-carboxamide was prepared as per literature procedure. (WO9726240). First step was alkylation of 9H-fluorene-carboxylic acid, here base used was n-BuLi and it w alkylated with 1,4 dibromo butane to afford intermedi (**5**). Second step was again acid base couplir Intermediate (**5**) was converted to it's acid chloride reacting it with oxalyl chloride, *insitu this* acid chlor was coupled with 2,2,2 trifluoro ethylamine in presence triethylamine as a base to afford intermediate (**6**). Th finally both the intermediate (**3**) and (**6**) were coupled presence of KOH in DMSO to give the title compound **a-h**.

Table 1 *In vitro* evaluation for MTP inhibition activity and TG lowering activity of MTP inhibitors in Sprague Dawley rats.



Example	Ar	Activity @1μM[a]	% Change against Control	Triglyceride secretion rate[b]	% Change against Control
Control		17.58±2.36		0.56±0.08	
7a		11.92±1.70	32.55±7.25	0.24±0.02	-56.33±3.86
7b		8.85±1.23	50.24±4.70	0.27±0.04	-52.22±6.79
7c		9.26±2.39	47.89±4.93	0.38±0.01	-32.65±2.16
7d		11.55±2.10	34.71±6.80	0.45±0.07	-19.68±11.65
7e		14.54±1.25	17.48±5.70	0.45±0.04	-18.92±6.91
7f		17.37±1.27	1.18±1.19	0.43±0.04	-23.98±7.32
7g		14.35±2.23	18.58±1.27	0.50±0.03	-9.77±4.80
7h		14.65±2.71	16.86±2.19	0.51±0.06	-8.37±10.8
BMS-201038		2.79±2.27	85.09±2.77	0.04±0.08	-73.50±14.80

[a]In the enzyme assay, the commercially available kit from Chylos was used, The *in vitro* activity of the test compound was carried out at 1μM concentrated.

[b]Animals were dosed with inhibitor orally followed after 1 hour with an intravenous injection of Triton WR-1339 and triglyceride secretion rate (TGSR, mg/min) calculated for 240 minutes.

As mentioned earlier our primary goal was to evaluate the linker modification. It is evident from the data presented in Table 1 and Table 2 that replacement at linker has retains MTP inhibitory activity. Biological studies indicated that compounds **7 a-c** having biphenyl carboxylic acid at aromatic acid position showed respectable *in vitro* MTP inhibition. These findings are also reflected in TG lowering activity of **7 a-c**. Instead of Biphenyl group at aromatic acid portion, compounds **7 d-f** having sub. Phenyl groups are not as potent as compound **7 a-c.** Similar results obtained when they were tested for TG lowering activity. Further compound **7g** and **7h** also showed almost lack of efficacy. These data clearly indicate that presence of bihenyl core is very essential for MTP-inhibition. Compound **7c** is least potent WRT to **7a** and **7b**, this might be because of electron donating effect of OCH_3. Similarly an electron donating group on phenyl ring also reduce efficacy, compound **7d-f** (which has electron withdrawing groups on aromatic ring) are relatively better than **7g** and **7h.** These results suggest that in absence of biphenyl ring, electron withdrawing groups on aromatic ring can enhance the activity. Combining altogether compound **7a** is the most potent among the other derivatives. As mentioned above it is comparable to the literature compound BMS-201038.

Conclusion

In the present study, successful synthesis of 9H-fluorene-9-carboxamides as MTP-inhibitors have been described. Some of the title compounds have exhibited promising MTP inhibition *in vitro* as well as TG lowering in Sprague Dawley rats. Among the derivatives synthesized, compound **7a N-(2,2,2-trifluoroethyl)-9-(4-(((1-(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carbonyl) piperidin-4-ylidene)amino) oxy)butyl)-9H-fluorene-9-carboxamide** has promising activity. Discovery of novel oximino linker of the series has open new dimensions to this 9H-fluorene-9-carboxamide template. Interestingly SAR distinctly suggest that to bring MTP inhibitory activity biphenyl core is essential and it is driven by electron withdrawing groups. Therefore this new template could be further modify to get better and potent molecules. Further evolution of this compound for enterocyte specificity to obtain a clinically useful novel class of MTP inhibitor is under progress.

Experimental

General

The progress of the reaction was monitored on readymade silica gel plates (Merck) using chloroform-methanol (9:1) as a solvent system. Melting points were recorded on scientific melting point apparatus and are uncorrected. IR spectra were recorded as neat (for oils) or on KBr pellet (for solid) on FT-IR 8300 Shimadzu and are expressed in ν (cm^{-1}). All ^1H spectral data are recorded on a 400 MHz ^1H NMR spectrometer (M-400) using DMSO-d_6, $CDCl_3$, CD_3OD or D_2O as solvent with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ downfield form.

Synthesis of 1-(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carbonyl)piperidin-4-one (2a)

A solution of 4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylic acid (0.5 g, 1.87 mmol) was dissolved in DCM (5 ml). To that added EDC. HCl (0.39 g, 2.06 mmol) followed by HOBT (0.279 g, 2.06 mmol) at 20°C-25°C. The reaction was stirred at 20°C-25°C for 30 minutes. To this 4-piperidone hydrochloride (0.288 g, 1.87 mmol) and NEt_3 (0.31 ml, 2.25 mmol) was added at 20°C-25°C. Then reaction mixture was further stirred for 2-3 hours. Reaction mixture was quenched with saturated $NaHCO_3$ and product was extracted with ethyl acetate, Organic layer was washed with $NaHCO_3$, H_2O, brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford desire product. Crude product purified by flash column chromatography over flash silica gel using 2% methanol in chloroform as an eluent to afford pure product.(0.41g) Yield = 63 %; IR (KBr) cm^{-1}:1714,1626; ^1HNMR ($CDCl_3$) δ: 1.56-1.58 (m, 1H), 2.04-2.11 (m, 1H), 2.17-2.21 (m, 1H), 2.31-2.44 (m, 1H), 3.14-3.15 (m, 1H), 3.28-3.29 (m, 1H), 3.82-3.87 (m, 2H), 7.45-7.56 (m, 4H), 7.63-7.73 (m, 4H); MS (m/z) 347.9 (M+). Melting point =135°C-140°C

1-([1,1'-biphenyl]-2-carbonyl)piperidin-4-one (2b) : Compound **2b** was prepared in 85 % yield by the same procedure as described for **2a**

IR (KBr) cm^{-1} : 1720, 1627 ; ^1HNMR ($CDCl_3$) δ : 1.22-1.28 (m, 1H), 1.90-1.95 (m, 1H), 2.12-2.16 (m, 1H), 2.30-2.35 (m, 1H), 3.14-3.18 (m, 2H), 3.47-3.54 (m, 1H), 4.14-4.24 (m, 1H), 7.39-7.41 (m, 2H), 7.44-7.45 (m, 3H), 7.47-7.48 (m, 1H), 7.49-7.51 (m, 1H), 7.52-7.55 (m, 2H) ; MS (m/z) 279.9 (M+).

1-(6-methoxy-[1,1'-biphenyl]-3-carbonyl)piperidin-4-one (2c) : Compound **2c** was prepared in 77 % yield by the same procedure as described for **2a**

IR (KBr) cm^{-1}: 1712, 1626 ; ^1HNMR ($CDCl_3$) δ: 2.51 (s, 4H), 3.86 (s, 3H), 3.89-3.91 (m, 4H), 7.02 (d, 1H, J = 8.4 Hz), 7.33- 7.39 (m, 1H), 7.42-7.43 (m, 2H), 7.45-7.46 (m, 1H), 7.48-7.54 (m, 3H) ; MS (m/z) 309.8 (M+); Melting point = 149°C-152°C.

3-(4-oxopiperidine-1-carbonyl)benzonitrile (2d) : Compound **2d** was prepared in 40% yield by the same procedure as described for **2a**

IR (KBr) cm^{-1};1720, 1639 ; ^1HNMR ($CDCl_3$) δ: 2.53-2.54 (m, 4H), 3.74- 4.01 (m, 4H), 7.52 -7.61 (m, 1H), 7.70-7.73 (m, 1H), 7.76-7.78 (m, 2H) ; MS (m/z) 269.9 (M+K).

1-(3-(trifluoromethyl)benzoyl)piperidin-4-one (2e): Compound **2e** was prepared in 56 % yield by the same procedure as described for **2a.**

IR (KBr) cm^{-1}: 1720, 1637 ; ^{1}HNMR ($CDCl_3$) δ: 2.52 (bs, 4H), 3.75-3.96 (m, 4H), 7.59 (t, 1H, J=7.6 Hz), 7.65-7.67 (m, 1H), 7.73-7.75 (m, 2H) ; MS (m/z) 271.89 (M+).

1-(2-chlorobenzoyl) piperidin-4-one (2f): Compound **2f** was prepared in 54 % yield by the same procedure as described for **2a.**

IR (KBr) cm^{-1}: 1718, 1639 ; ^{1}HNMR ($CDCl_3$) δ: 2.34-2.40 (m, 1H), 2.52-2.68 (m, 3H), 3.45 -3.51 (m, 1H), 3.59-3.65 (m, 1H), 3.91-3.98 (m, 1H), 4.11-4.27 (m, 1H), 7.32- 7.46 (m, 4H) ; MS (m/z) 221.8 (M+H).

1-(4-fluorobenzoyl)piperidin-4-one (2g) : Compound **2g** was prepared in 63 % yield by the same procedure as described for **2a**

IR (KBr) cm^{-1}: 1720.56 , 1635.69 ; ^{1}HNMR ($CDCl_3$) δ: 2.51 (s, 4H), 3.88 (s, 4H), 7.08-7.16 (m, 2H), 7.47 -7.52 (m, 2H); MS (m/z) 221.8 (M+).

1-(4-(methylthio)benzoyl)piperidin-4-one (2h) : Compound **2h** was prepared in 67% yield by the same procedure as described for **2a**

IR (KBr) cm^{-1}: 1716.7, 1627.97 ; ^{1}HNMR ($CDCl_3$) δ: 2.51 (bs, 7H), 3.88 (s, 4H), 7.26-7.29 (d, 2H , J=8.4 Hz), 7.40-7.42 (d, 2H , J=8.4 Hz); MS (m/z) 249.9 (M+).

Synthesis of (4-(hydroxyimino)piperidin-1-yl)(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methanone (3a)

A Solution of **1-(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carbonyl)piperidin-4-one (2a)**(0.41 g, 1.18 mole) in EtOH (2.87 ml) was added hydroxylamine hydrochloride ($NH_2OH.HCl$) (0.246 g, 3.54 mole) dissolved in H_2O (2.46 ml) at 20°C-25°C. The Reaction mixture was heated at 70°C for 2 hours. Reaction Mixture was Cooled to 20°C-25°C, product was extracted with ethylacetate. Organic layer was washed with H_2O, brine, dried over Na_2SO_4 and evaporated to afford solid product. Crude product purified by flash column chromatography over flash silica gel using 2% methanol in chloroform as an eluent to afford desire product(0.40g). Yield = 93.6%, ; IR (KBr) cm^{-1}: 1616, 3267 ; ^{1}H NMR ($CDCl_3$) δ : 1.76-1.80 (m, 1H), 2.06-2.11 (m, 1H), 2.25-2.28 (m, 1H), 2.35-2.36 (m, 1H), 2.84-2.88 (m, 1H), 3.08-3.12 (m, 1H), 3.60-3.73 (m, 2H), 7.42-7.54 (m, 4H), 7.62 (d, 2H, J = 8.4 Hz), 7.67 (d, 2H, J =7.6Hz); MS (m/z) 362.9 (M+); Melting point = 179°C-184°C.

[1,1'-biphenyl]-2-yl(4-(hydroxyimino)piperidin-1- yl) methanone (3b) : Compound **3b** was prepared in 53% yield by the same procedure as described for **3a.**

IR (KBr) cm^{-1}: 1627, 3269 ; ^{1}HNMR ($CDCl_3$) δ: 1.86-1.90 (m, 1H), 2.04-2.12 (m, 1H), 2.31-2.37 (m, 1H), 2.72-2.75 (m, 1H), 2.91-3.02 (m, 2H), 3.40-3.44 (m, 1H), 3.90-3.97 (m, 1H), 7.34-7.36 (m, 1H), 7.38 -7.40 (m, 2H), 7.41-7.42 (m, 2H), 7.44-7.47 (m, 2H), 7.48-7.53 (m, 2H); MS (m/z) 294.8 (M+); Melting point = 100°C-105°C.

(4-(hydroxyimino)piperidin-1-yl)(6-methoxy-[1,1'-biphenyl]-3-yl)methanone (3c) : Compound **3c** was prepared in 95% yield by the same procedure as described for **3a.**

IR (KBr) cm^{-1}: 1600, 3408 ; ^{1}H NMR ($CDCl_3$) δ: 2.41 (s, 2H), 2.68 (bs, 2H), 3.73 (bs, 4H), 3.84 (s, 3H), 7.0 (s, 1H), 7.32-7.56 (m, 8H) ; MS (m/z) 324.9 (M+).

4-(4-(hydroxyimino)piperidine-1-carbonyl) benzonitrile (3d) : Compound **3c** was prepared in 81% yield by the same procedure as described for **3a**

IR (KBr) cm^{-1}: 1604, 3242 ; ^{1}H NMR ($CDCl_3$) δ: 2.36-2.40 (m, 2H), 2.49-2.54 (m, 2H), 3.52-3.54 (m, 2H), 3.85-3.86 (m, 2H), 6.89 (s, 1H), 7.52-7.60 (m, 1H), 7.67-7.70 (m, 1H), 7.74- 7.76 (m, 2H); MS (m/z) 243.7 (M+); Melting point = 191°C-194°C.

(4-(hydroxyimino)piperidin-1-yl)(3-(trifluoromethyl) phenyl) methanone (3e) : Compound **3e** was prepared in 82% yield by the same procedure as described for **3a.**

IR (KBr) cm^{-1}: 1620, 3232 ; ^{1}HNMR ($CDCl_3$) δ: 2.17-2.35 (m, 2H), 2.51-2.62 (m, 2H), 3.34 (bs, 2H), 3.86 (bs, 2H), 7.19 (s, 1H), 7.52-7.80 (m, 4H); MS (m/z) 286.7 (M+); Melting point = 150°C-153°C.

(2-chlorophenyl)(4-(hydroxyimino)piperidin-1-yl) (3f) : Compound **3f** was prepared in 71.26% yield by the same procedure as described for **3a.**

IR (KBr) cm^{-1}: 1616, 3269 ; ^{1}HNMR ($CDCl_3$) δ: 2.27-2.29 (m, 1H), 2.41-2.43 (m, 1H), 2.49-2.53 (m, 1H), 2.61-2.67 (m, 1H), 3.28-3.34 (m, 1H), 3.35-3.43 (m, 1H), 3.79-3.83 (m, 1H), 3.96-4.04 (m, 1H), 7.20 (s, 1H), 7.31-7.44 (m, 4H) ; MS (m/z) 254.5 (M+); Melting point = 175°C-180°C.

4-fluorophenyl)(4-(hydroxyimino)piperidin-1-yl)methanone (3g) : Compound **3f** was prepared in 84 % yield by the same procedure as described for **3a.**

IR (KBr) cm^{-1}: 3248.3, 1608.69 ; ^{1}HNMR ($CDCl_3$) δ: 2.42 (bs, 2H), 2.68 (bs, 2H), 3.54-3.79 (m, 4H), 6.99-7.14 (m, 3H), 7.43-7.52 (m, 2H); MS (m/z) 236.8 (M+); Melting point = 160°C-165°C.

(4-(hydroxyimino)piperidin-1-yl)(4-(methylthio)phenyl)methanone (3h) : Compound **3h** was prepared in 88% yield by the same procedure as described for **3a.**

IR (KBr) cm^{-1}: 3277.17, 1612.54 ; ^{1}HNMR ($CDCl_3$) δ: 2.41 (s, 2H), 2.50 (s, 3H), 2.68 (s, 2H), 3.66-3.70 (m, 4H), 7.20 (s, 1H), 7.26-7.28 (s, 2H), 7.36-7.42 (s, 2H); MS (m/z) 264.9 (M+); Melting point = 131°C-136°C.

Synthesis of N-(2,2,2-trifluoroethyl)-9-(4-(((1-(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carbonyl) piperidin-4-ylidene)amino)oxy)butyl)-9H-fluorene-9-carboxamide (7a)

To a Solution of **(4-(hydroxyimino)piperidin-1-yl)(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methanone (3a)** (0.38 g, 1.049 mmol) in DMSO (3.8 ml) was added KOH(85%) (0.17 g, 2.62 mmol) at 25°C. The reaction mixture was stirred at 20°C-25°C for 30 minutes. To that 9-(4-bromobutyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide **(6)** (0.44 g, 2.62 mmol) dissolved in DMSO (2.2 ml) was added slowly at 20°C-25°C during 10-15 minutes. Further reaction mixture was stirred at 20°C-25°C for 2-3 hours. Product was taken in ethylacetate, organic layer was washed with H_2O, brine, dried over Na_2SO_4 and evaporated to afford solid product. Crude product purified by flash column chromatography over flash silica gel using 40% ethyl acetate in n- hexane. To afford pure product (0.16 g) Yield = 21%, ; IR (KBr) cm^{-1}: 1685, 1618; ^1H NMR ($CDCl_3$) δ: 0.58– 0.66 (m, 2H), 1.17-1.23 (m, 4H), 1.32-1.39 (m, 2H), 1.97-2.36 (m, 4H), 3.55-3.60 (m, 2H), 3.61-3.69 (m, 4H), 5.26-5.28 (m, 1H), 7.23-7.27 (m, 2H), 7.31- 7.40 (m, 4H), 7.42-7.49 (m, 4H), 7.53 (d, 2H, J = 8.0 Hz), 7.57-7.62 (m, 2H), 7.68 (t, 2H, J = 8 Hz) ; MS (m/z) 707.9 (M+).

9-(4-(((1-([1,1'-biphenyl]-2-carbonyl) piperidin-4-ylidene) amino) oxy) butyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9- carboxamide (7b) : Compound **7b** was prepared in 46% yield by the same procedure as described for **7a.**

IR (KBr) cm^{-1}: 1685, 1629; ^1H NMR ($CDCl_3$) δ : 0.58-0.63 (m, 2H), 0.92-0.95 (m, 1H), 1.17-1.18 (m, 1H), 1.34-1.38 (m, 2H), 1.91-1.93 (m, 2H), 2.11-2.18 (m, 2H), 2.32-2.38 (m, 2H), 2.81-2.85 (m, 1H), 3.30-3.35 (m, 1H), 3.63-3.74 (m, 4H), 4.59 (s, 1H), 7.31-7.59 (m, 15H), 7.77-7.90 (m, 2H) ; MS (m/z) 640.2 (M+): Melting point = 135°C-140°C.

9-(4-(((1-(6-methoxy-[1,1'-biphenyl]-3-carbonyl)piperidin-4-ylidene) amino) oxy) butyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (7c) : Compound **7c** was prepared in 38 % yield by the same procedure as described for **7a**

IR (KBr) cm^{-1}: 1687 , 1622 ; ^1HNMR ($CDCl_3$) δ : 0.74-0.78 (m, 2H), 0.92-0.96 (m, 4H), 1.40 – 1.48 (m, 2H), 2.31-2.39 (m, 4H), 3.66-3.68 (m, 4H), 3.81 (m, 2H), 3.87 (s, 3H), 7.35 (d, 1H, J = 6.8 Hz), 7.39 – 7.42 (m, 4H), 7.45-7.47 (m, 6H), 7.49- 7.52 (m, 4H), 7.73- 7.75 (m, 2H); MS (m/z) 670.1 (M+).

9-(4-(((1-(3-cyanobenzoyl)piperidin-4-ylidene)amino) oxy)butyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (7d) : Compound **7d** was prepared in 52 % yield by the same procedure as described for **7a.**

IR (KBr) cm^{-1}: 1685.8, 1637.2; ^1H NMR ($CDCl_3$) δ : 0.72 – 0.80 (m, 2H), 0.83 – 0.89 (m, 4H), 1.23 -1.33 (m, 2H), 2.42 - 2.46 (m, 4H), 3.66 – 3.70 (m, 4H), 3.85 (t, 2H, J = 6 Hz), 5.35 (s, 1H), 7.36 (d, 2H, J = 7.6 Hz), 7.44 (t, 2H, J = 7.2 Hz), 7.53 – 7.55 (m, 2H), 7.58 (d, 1H, J = 7.6 Hz), 7.65 – 7.67 (m, 1H), 7.71 – 7.78 (m, 4H) ; MS (m/z) 589 (M+H) ; melting point = 185°C-188°C.

N-(2,2,2-trifluoroethyl)-9-(4-(((1-(3-(trifluoromethyl) benzoyl)piperidin-4-ylidene) amino) oxy)butyl)-9H-fluorene-9-carboxamide (7e) : Compound **7e** was prepared in 62% yield by the same procedure as described for **7a.**

IR (KBr) cm^{-1}: 1685.89, 1631.8; ^1H NMR ($CDCl_3$) δ : 0.86-0.87 (m, 2H), 1.49-1.52 (m, 2H), 2.03-2.44 (m, 6H), 3.20-3.48 (m, 2H),3.65- 3.68 (m, 4H), 3.84-3.88 (m, 2H), 5.26-5.35 (m, 1H), 7.36-7.43 (m, 4H), 7.53-7.60 (m, 4H), 7.70-7.77 (m, 4H); MS (m/z) 632 (M+H).

9-(4-(((1-(2-chlorobenzoyl)piperidin-4-ylidene)amino) oxy)butyl)- N-(2,2,2-trifluoro ethyl) -9H-fluorene-9-carboxamide (7f) : Compound **7f** was prepared in 85 % yield by the same procedure as described for **7a.**

IR (KBr) cm^{-1}: 1680.05 1633.76 ; ^1H NMR ($CDCl_3$) δ : 0.68-0.73 (m, 2H), 1.45-1.52 (m, 2H), 2.40-2.43 (m, 1H), 2.45-2.51 (m, 4H), 3.05-3.10 (m, 1H), 3.15-3.23 (m, 1H), 3.30-3.39 (m, 1H), 3.65- 3.71 (m, 3H), 3.81- 3.85 (m, 3H), 5.34-5.36 (m, 1H), 7.24-7.32 (m, 1H), 7.33-7.36 (m, 3H), 7.37-7.39 (m, 3H), 7.41-7.46 (m, 1H), 7.54 (t, 2H, J=8Hz), 7.75 (d, 1H , J = 7.6Hz), 7.78 (d, 1H , J=7.5Hz) ; MS (m/z) 620 (M+Na); Melting point = 180°C-184°C.

9-(4-(((1-(4-fluorobenzoyl)piperidin-4-ylidene)amino) oxy)butyl)-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (7g) : Compound **7g** was prepared in 58% yield by the same procedure as described for **7a.**

IR (KBr) cm^{-1}: 1672.34, 1627.97 ; ^1HNMR (CD_3OD) δ : 0.63-0.71 (m, 2H), 0.85-0.91 (m, 2H), 1.16-1.26 (m, 4H), 1.29-1.47 (m, 2H), 2.27-2.29 (m, 4H), 3.18-3.53 (m, 2H), 3.54-3.82 (m, 2H), 7.21-7.25 (m, 2H), 7.35-7.37 (m, 4H), 7.47-7.54 (m, 4H), 7.82-7.84 (m, 2H); MS (m/z) 583.3.

9-(4-(((1-(4-(methylthio)benzoyl)piperidin-4-ylidene) amino)oxy)butyl)- N-(2,2,2-trifluoroethyl)- 9H-fluorene-9-carboxamide (7h): Compound **7h** was prepared in 48% yield by the same procedure as described for **7a.**

IR (KBr) cm^{-1}: 1687.7, 1622.19; ^1HNMR ($CDCl_3$) δ: 0.65-0.72 (m, 2H), 0.88-0.91 (m, 2H), 1.16-1.24 (m, 4H), 1.47-1.50 (m, 2H), 2.42-2.46 (m, 4H), 2.51 (s, 3H), 3.66-3.70 (m, 2H), 3.82-3.85 (m, 2H), 5.32 (s, 1H), 7.27-7.28 (m, 2H), 7.34-7.37 (m, 4H), 7.42-7.44 (m, 2H), 7.53-7.55 (m, 2H), 7.75-7.77 (m, 2H); MS (m/z) 611.1.

Determination of MTP activity[15]. The *in vitro* activity of the MTP inhibitor test compounds was measured using Chylos MTP activity kit using manufacturer's instructions. Briefly, purified MTP (1µg) was incubated with donor and acceptor vesicles for 30 minutes in the presence of the 1µM concentration of the test compound. Four different conditions (blank, total, positive control, and test) were used for each assay. In all assays, the reaction was started by the final addition of the MTP source. Three microliter each of acceptor and donor vesicles was put onto fluorescence microtitre (black) plates. It was added with ten microliter of 10 mM Tris, pH 7.4, containing 2mM EDTA and 10 µl of 1% BSA stock in 1.5 M NaCl. In blanks, the needed amount of control buffer (which contains the MTP source in positive control and test samples) was added and the volume was made to 100 µl with water. In positive controls, MTP protein was added, whereas in tests unknown samples were added. It was incubated at 37°C for 30 minutes. The readout was fluorescence units using excitation and emission wavelengths of 460-470 nm and 530-550 nm, respectively.

PharmacologyDetermination of post-Triton serum triglyceride levels[16]

For determination of triglyceride secretion, serum triglycerides were measured in fasted Sprague Dawley rats. Eighteen hours fasted rats were orally administered the test compound (0.3 mg/Kg PO), followed one hour later by intravenous Triton WR 1339 (250 mg/Kg of body weight) administration. The test compounds were formulated in 0.1% hydroxyl ethyl cellulose, 0.5% Tween 80 in deionised water. Control group animals received vehicle by oral route of administration. The volume of dose administration was 2 ml/Kg for all treatment groups. The animals were bled under light ether anaesthesia upto 240 minutes after Triton WR1339 injection and serum triglycerides were estimated.

TGSR were determined by the equation:

$$TGSR = [\{(TG1-TGo)/T1\} + \{(TG2 - TGo)/T2\}/2] \times PV_E$$

where TGSR = triglyceride secretion rate; TGo = basal plasma TG concentration (mg/100 ml) ; TG1 = first plasma TG concentration after Triton injection; TG2 = second plasma TG concentration after Triton injection; T1 = time of first sampling in minutes after Triton injection; T2 = time of second sampling in minutes after Triton injection; PV_E = estimated plasma volume (cubic centimeters) expressed by the formula: PVE = {0.016 X weight (grams)} + 4.25.

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Emerging Therapies for Dyslipidemia: Known Knowns and Known Unknowns of MTP Inhibitors

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Abstract: Last decade had witnessed enormous efforts to develop therapies to treat one or more components of metabolic syndrome, a cluster of diseases including diabetes, obesity and dyslipidemia. Several newer targets are identified and evaluated to treat these metabolic disorders. Microsomal triglyceride transfer protein (MTP) has been identified as one of the promising target for the treatment of dyslipidemia. MTP plays crucial role in the assembly of triglyceride rich chylomicrones in enterocytes and VLDL in hepatocytes and several lines of evidence suggested that MTP inhibitors can be instrumental in combating familial hypercholesterolemia. Several first generation compounds are currently being evaluated in clinic and fatty liver is found to be the main adverse effect of these agents. Recently development of enterocyte specific inhibitor of MTP is emphasized in order to deal with fatty liver issue. In this review, we have dealt with important mechanistic aspects of MTP inhibition, patent scenario and clinical trial outcomes and some of the recent patents related to newly discover chemical scaffolds.

Keywords: Abetalipoproteinemia, familial hypercholesterolemia, metabolic syndrome, microsomal triglyceride transfer protein inhibitors.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause for morbidity and mortality [1] and hyperlipidemia represents the major element of this disease. Hyperlipidemia is characterized by elevated levels of cholesterol (hypercholesterolemia) and/or triglycerides. The high unmet medical need for the treatment of CVD in general and the hypercholesterolemia, the major risk factor for CVD [2] in specific draw attention of several research group's both in pharmaceutical industry and academia world over and several therapeutic options evaluated are described in brief here.

1. Statins

Statins inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) an enzyme involved in the rate limiting step of cholesterol synthesis [3]. and mainly reduce LDL-cholesterol. Since their discovery Statins remained as first-line therapy for hypercholesterolemia and at times these are prescribed in combination with other lipid lowering agents as add on therapy. Though statins are well tolerated in most of the patient population, rhabdomyolysis, elevation of liver enzymes and creatine kinase are some of the common side effects of these agents [4, 5]. There is also evidence of intolerability among very few patients to statin therapy.

2. PPAR Agonists

Activation of Peroxisome Proliferator Activator Receptors (PPARs) is another interesting target for the treatment of metabolic disorders, which includes dyslipidemia and the efforts in this area dominated during last decade over other targets. Interestingly, fibrates are discovered as hypolipidemic agents based on rodent pharmacology and subsequently found to be PPAR-α agonists much later. PPAR-α is involved in oxidation of fatty acids [6] and thereby reduces triglyceride and LDL-cholesterol. Subsequently, many modifications to the structure of fibrates were carried out to achieve potent, efficacious and safe molecules. Recently, dual PPAR-α/γ agonists were explored for the treatment of dyslipidemia without success. Liver toxicity, gastrointestinal (GI) side effects, muscle toxicities are most common side effects of PPAR's [7, 8].

3. Niacin

Nicotinic acid has been in clinical use since long time and proven to increase plasma concentration of HDL, which ultimately reduces LDL-cholesterol [9-11]. These effects of niacin are recently found to be mediated through GPR-109. Thereafter several compounds have been developed but most of them were dropped in clinical trials due to target related side effect, flushing [12].

4. Thyromimetics

Thyroid hormones are important endocrine signalling hormones, involved in a number of important physiological processes including lipid metabolism and control of energy expenditure [13-15]. Two subtypes of thyroid hormone receptors, TR- α and TR-β are known and activation of TR- α isoform mainly affects the heart rate and rhythm whereas, activation of TR-β isoform is known to reduce plasma cholesterol, low-density lipoprotein (LDL) and triglyceride lev-

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els. Triiodothyronine (T3) is non selective natural thyroid hormone that exhibits physiological effect by acting on both the subtypes. Administration of T3 lowers the plasma cholesterol, low-density lipoprotein (LDL) and triglyceride levels in animal models [16-18]. However, T3 cannot be used therapeutically to treat hypercholesterolemia due to its cardiac side effects because of its non selective actions on thyroid receptor. Several lines of evidence suggests that selective TR-β agonist could be a viable therapeutic option to treat hyperlipidaemia, devoid of cardiovascular and other toxicities of native thyroid hormone. This is one of the exiting target and compounds have reach up to clinical trials [19, 20] though there is no drug from this class is available in the market.

5. Bile Acid Sequestrants

Cholesterol is precursor of bile acid. In the intestinal lumen bile acid sequestrants (indigestible positively charged resins) that binds to negatively charged bile acids and forms complex which is excreted through the feces [21, 22]. Main side effect of this class of drug is GI adverse effects.

6. Ezetimibe

Ezetimibe is a cholesterol absorption inhibitor and acts by decreasing cholesterol absorption in the intestine. Subsequently it leads to decrease in the delivery of intestinal cholesterol to the liver and ultimately reduces hepatic cholesterol stores and increases cholesterol clearance from the blood. Ezetimibe localises and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. Specifically, ezetimibe inhibits cholesterol absorption in the intestine by blocking the Niemann-Pick C1-Like 1 (NPC1L1) cholesterol transporter protein which is found at the brush border membranes of enterocytes [23, 24]. It may be used alone or in combination with statins. Clinical studies with ezetimibe demonstrated notable LDL-cholesterol lowering as monotherapy, and significant lowering in total and low density lipoprotein cholesterol in combination with statins [25]. Ezetimibe have no significant effect on the plasma concentrations of the fat-soluble vitamins A, D, and E and does not affect the absorption of triglycerides [26]. Common side effects associated with ezetimibe therapy are headache and diarrhea and is generally well tolerated.

The therapeutic targets aimed to treat dyslipidemia including the above mentioned ones, alone or in combination have concerns related efficacy and/or safety in some patients. Ultimately, they are not effective in blocking the advancement of coronary artery disease and they may encounter against atherosclerosis. Advancement in the pathophisiology of CVD revealed the role of several proteins in this disease and made the scientific community intrigued to explore them as targets for discovering new drugs. Microsomal triglyceride transfer protein (MTP) is one among them and inhibitors of this protein may serve as future remedy for CVD.

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN (MTP) INHIBITORS

Abetalipoproteinemia (ABL) or Bassen-Kornzweig syndrome is a hereditary disease characterized by inability to produce chylomicrons and very low-density lipoproteins in the intestine and liver respectively. Homozygous autosomal recessive mutations in the gene encoding the 97-kDa subunit of the microsomal triglyceride transfer protein (MTP) causes this genetic disorder [27]. Since chylomicrons carry fat and cholesterol to the blood stream, absence or reduced levels of these chylomicrons leads to low plasma cholesterol and triglyceride levels. However, fat-soluble vitamins (like vitamin E and D) also require chylomicrons for their systemic transportation and the low levels of chylomicrons may lead to vitamin deficiencies. Further adverse effects of absence of chylomicrons are reported to lead to gastrointestinal, neurological, ophthalmological, and hematological abnormalities [28].

MTP is a heterodimeric lipid transfer protein [29], found in endoplasmic reticulum (ER) of enterocytes and hepatocytes. MTP is 894AA protein [30], protein disulfide isomerase (PDI) is a part of the MTP [31] and plays crucial role in apolipoprotein B secretion [32]. As depicted in Fig. (1) MTP is essential for assembly of triglycerides in intestine with apo-B48, a 2152 AA containing glycoprotein that plays an important role in lipid metabolism [33, 34] producing triglyceride rich chylomicrons. In liver MTP mediates the formation of very low density lipoprotein (VLDL) by the assembly of triglycerides with apoB-100 another glycoprotein containing 4536 AA [35, 36] to synthesize and secrete VLDL. It is also observed that T2DM or metabolic syndrome is associated with hypertriglyceredemia and increased expression of apo-B [37]. There is indirect role of MTP in diabetes for the treatment of atherosclerosis in insulin-resistant conditions [38].

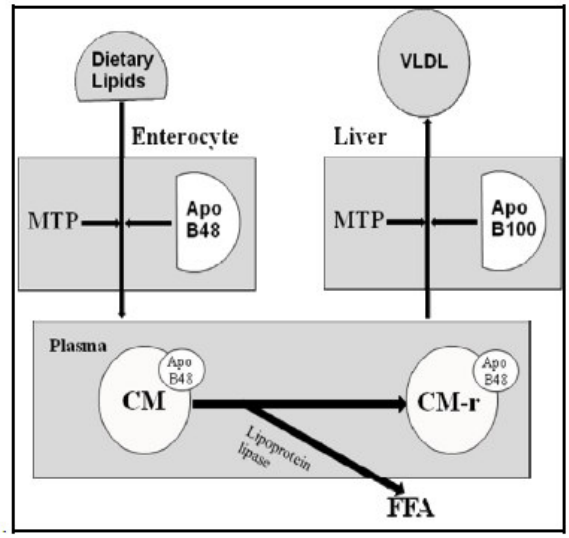


Fig. (1). Role of MTP in formation of chylomicron from enterocyte and VLDL production from liver cells.

These research findings led to a hypothesis of inhibiting MTP may play an instrumental role in the treatment of dyslipidemia and this further intrigued scientist to develop synthetic molecules to inhibit MTP.

CONCERNS AND SOLUTIONS

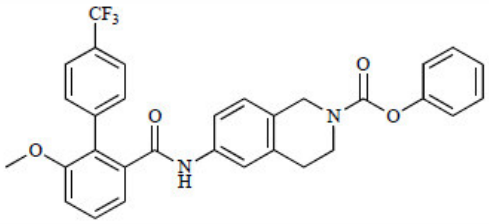
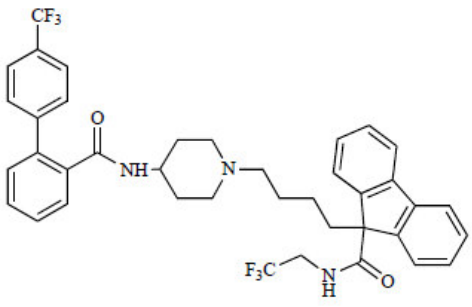
Drug discovery and development process is complex where risk of attrition is high. Safety concerns associated with every target/molecule is one of the prime reasons for failure. MTP is not exception to that. As previously discussed liver and intestine are target organs for MTP inhibitors, side effects touches on these organs more than any other. MTP inhibition in liver blocks the formation of VLDL (lipoprotein assembly) which ultimately leads to fatty liver. Enzymes like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) also increases by inhibition of MTP [39, 40]. In intestine, it leads to GI disturbance which ultimately results in diarrhea. These side effects are because of the accumulation of triglyceride and cholesteryl esters in intestine and liver respectively as it has been not utilized. The side effects which are observed in intestine cannot be considered as chronic. It was thought that MTP inhibition in intestine might be useful to avoid liver toxicity. SLX-4090 and JTT-130 [41] are some of the examples of intestine specific inhibitors of MTP. Enterocyte specific inhibitors of MTP could be called as IInd generation MTP inhibitors.

FAMILIAL HYPERCHOLESTEROLEMIA, CORONARY ARTERY DISESE AND MTP INHIBITORS

Familial hypercholesterolemia is a genetic disorder characterized by elevated plasma LDL cholesterol levels. Oxidation of these LDL molecules results in the formation of foam cells which leads to coronary artery disease (CAD), leading cause of mortality and morbidity. Mutations in the gene encoding for the LDL receptor is the main cause for this disease. Toughest part of this disease is if untreated, it is likely to propagate further in next generation through abnormal gene. Specifically patients suffering from familial hypercholesterolemia (FH) are not always able to achieve target levels of LDL cholesterol using the current available LDL-cholesterol reducing regiment. Results with combination therapy are not satisfactory. In such condition, there is unmet need for additional alternative therapies. Especially these patients can compromise with the adverse effects taking place because of MTP inhibitors against the threat of high CVD mortality risk. Many of the ongoing MTP inhibitors are recommended for the treatment of FH. There has been extensive research on MTP inhibitors and is still continue as well. There are many molecules progressed up to clinical trials. Briefly some of them are depicted below in Table 1.

(1) SLX-4090: This molecule is currently in Phase 2 clinical trials. SLX-4090 is a first-in-class inhibitor of enterocyte specific MTP inhibitor. It is designed to inhibit MTP specifically in enterocyte having IC_{50} of 8 nM. In caco-2 cells it inhibits apo-B secretion with IC_{50}~9.6 nM. In preclinical models it reduced postprandial lipids by >50% (ED_{50}~7 mg/kg). By oral administration in fasted rodents SLX-4090 was not detected in systemic or portal circulation which indicates that it is enterocyte specific inhibitor [42]. Chronic treatment with SLX-4090 resulted in reduction of LDL-cholesterol, triglyceride and bodyweight without accumulation of hepatic triglyceride and no elevation of liver enzymes. This compound is also safe at 1000 mg/Kg /day in rat toxicity. So far 3 clinical trials are reported with this compound and no more adverse effect reported with the efficacy of 60% reduction in postprandial triglyceride at 800 mg dose [43]. Structurally SLX-4090 is tetrahydroisoquinoline derivative [44]. MTP inhibitors are becoming an attractive target since enterocyte specific MTP inhibitors low-

Table 1. MTP Inhibitors Under Clinical Evaluation.

Drug	Status	Structure	Company
SLX-4090	Phase IIa		Surface Logix
Lomitapide (AEGR 733) BMS-201038	Phase III		Aegerion Pharmaceuticals Inc.

ers triglyceride without causing hepatic steatosis at least in animal studies.

(2) Lomitapide (AEGR-733): Through incremental changes BMS-201038 was identified as a lead candidate by BMS from early hit BMS-200150 [45]. Later this compound was taken by Aegerion Pharmaceuticals Inc. for further development and named as AEGR-0733. Currently it is known as Lomitapide and undergoing Phase-III clinical trial. Lomitapide is 9H-fluorene-9-carboxamide derivative [46]. In clinical trials AEGR-733 reduces 50.9% LDL-cholesterol at 1 mg/kg dose in six patients associated with FH, however treatment was also associated with increased serum transaminase levels and hepatic steatosis [47, 48]. In another study, 84 patients with moderate hypercholesterolemia were treated with ezetimibe (10 mg) alone, AEGR-733 (5 mg, 7.5 mg, and 10 mg) alone and in combination (ezetimibe 10 mg+ same dose-titration of AEGR-733). After 12 weeks, ezetimibe alone reduced LDL-cholesterol levels by 21%, AEGR-733 alone reduced LDL-cholesterol levels of 19-30% and combination of ezetimibe + AEGR-733 dose-dependent showed LDL-cholesterol reductions of 35-46%. A mild transaminase elevation was reason for discontinuations of patients receiving AEGR-733 alone [49]. In combination AEGR-733 is also used with fibrates [50]. Aegerion is conducting Phase III safety and efficacy study of lomitapide to treat homozygous familial hypercholesterolemia (FH) [51]. Total 29 patients are enrolled at the maximum tolerated dose of up to 60 mg/d. Recently, FDA has grant orphan drug designation to lomitapide, for treatment of familial chylomicronemia (FC), a genetic disorder of lipid metabolism resulting in a marked increase in chylomicrone and triglyceride levels. Main cause for FC is mutation in the lipoprotein lipase gene. Prior to Phase III total of 703 patients were treated with lomitapide in Phase I and Phase II trials.

CURRENT & FUTURE DEVELOPMENTS

Apart from above mentioned molecules, other frontrunners explored till date are Implitapide (Bay-13-9952) [52], Dirlotepide [53], Mitratepide [54] and JTT-130. MTP inhibitors are under clinical investigation as monotherapy and in combinations with various drugs. Looking at current scenario lomitapide and SLX-4090 are in advanced clinical development.

Simultaneously, there have been consistent but not aggressive efforts to discover novel chemical scaffolds. Interestingly unlike the earlier compounds, few recent scaffolds are devoid of biaryl core. 7-azaindole-based compounds were claimed by Merck recently and the representative example 1, Fig. (**2**) of this series showed 15 nM IC_{50} for Inhibition of apoB secretion and 175 nM MTP inhibitory activity [55]. Subsequent to this a series of thiazolyl piperidine derivatives [56] are also reported by the same company. In MTP inhibition assay example 2, Fig. (**2**) exhibited an IC_{50} of 51 nM while the same compound showed IC_{50} of 20 nM for Inhibition of apoB secretion. In a recent patent application [57], aryl piperidine or piperazine compounds substituted with certain 5-membered heterocycles are disclosed where apoB secretion/MTP inhibiting activity of the compound are mentioned. Another patent application claiming tetrahydronaphthalene-1-carboxylic acid derivatives [58] has also been published recently. Moving towards gut specific inhibitors, Sirtris pharma has claimed heterocyclylamides as GUT specific MTP inhibitors. Example 3, Fig. (**2**) has IC50 of 9.6 nM [59]. Even though there is limited data available for above mentioned scaffolds, experimental data related to efficacy and liver toxicity may decide the fate of this novel chemical scaffolds. Apart from this there are patents where study using MTP inhibitors in combination with anti-diabetic and lipid lowering drugs is also reported. Janseen Pharma has reported [60] combination study of MTP inhibitor with metformine in type 2 diabetic patients. In a 12 week study, 87% subjects could complete the study. There was reduction in HbA1c, body weight and beneficial effect on LDL. In another patent Aegerion Pharmaceuticals Inc. reported [61] combination of MTP inhibitor AEGR-0733 with DGAT inhibitor. In another study [62] six patients having FH received once a daily dose of 0.03, 0.1, 0.3 and 1 mg/kg lomitapide (BMS-201038) for four weeks at each dose. The drug was tolerated up to maximum given dose of 1 mg/kg with little to no stratorrhea. There was evidence of dose dependent increase in hepatic fat observed. The mean % change in total cholesterol was -30+9% and -58+8.5 %.

CONCLUDING REMARK

Results of human trials could successfully establish the proof of concept for using inhibitors of microsomal triglyceride transfer protein for LDL-cholesterol lowering. Adverse effect like steatosis and gastrointestinal side effects detected during clinical trials appear to be mechanism based. In general these effects are dose dependent and MTP inhibition induced steatosis occurs only at high doses. As discussed in earlier section in ABL patients chylomicron production is impaired completely and hence the above mentioned adverse effects are obvious to occur. Based on these facts the extent of inhibition of MTP may play a critical role in balancing

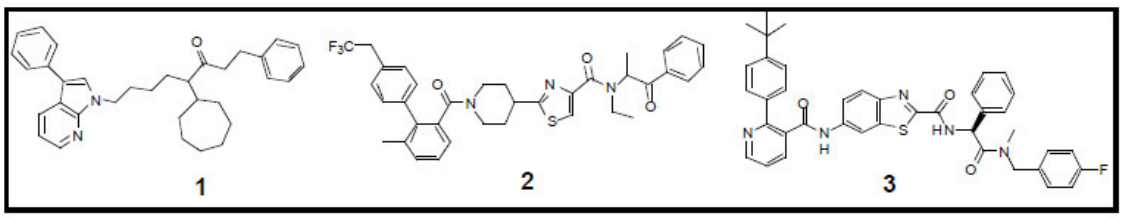


Fig. (2). Novel MTP inhibitors.

lipid lowering effects versus adverse effects. Further research is needed to develop chemical tools to modulate the MTP inhibition to such an extent where adverse effects are in acceptable range with sufficient efficacy to lower lipids. In this case, one may not expect sufficient efficacy which restricts the use of these agents as monotherapy. However such agents if developed can be better used in combination with existing agents which do not provide comprehensive benefits in dyslipidemia. Another futuristic approach could be identifying different binding sites in MTP and design compounds targeted to those sites to modulate the MTP inhibition allosterically. Further in patients with FH where the patients do not respond to existing therapies, MTP inhibitors could still serve as monotherapy considering the benefit-to-risk ratio in these patients. Over and above use of MTP inhibitors in FH as an orphan drug approach looks lucrative.

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CONFLICT OF INTEREST

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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7

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- Designing New chemical entities based on Moleculer modeling

- Designing Synthetic projects for making patentable New Chemical Entities, Execution of projects (Synthesis), Following of biological results to modify project based on structure activity correlation
- To study various targets in metabolic disorders segment to keep the track of existing targets as well as newly emerging targets
- Prior art search and Patent drafting

ACHIEVEMENTS

- Our group is successful in producing few lead molecules for diabetes and dislipidemia which are selected for further clinical evaluation. One of the NCE (ZY H1) has presently in Phase III clinical trials for dislipidemia, an other NCE (ZY H2) has completed phase I clinical trials successfully for type-2 diabetes.
- One of the NCE (ZY T1) Thyroid-beta agonist for the treatment of dyslipidemia and obesity is presently undergoing foe Phase I clinical trials. Our group has generated excellent Intellectual property by filing number of patents and few papers in international journals are on the way to get published shortly.
- Developed multistep processes for Lansoprazole, Ticlopidine, Omeprazole, Meloxicam, Carvedilol, Sildenafil Citrate and Nicorandil up to Kilogram scale which are currently being used for manufacturing.

Publications

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Patents

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2. Lohray Braj Bhushan; Lohray Vidya Bhushan; Barot Vijay Kumar; ***Raval Saurin Khimshankar;*** Raval Preeti Saurin; Basu Sujay. **Novel pyrroles having hypolipidemic hypocholesteremic activities, process for their preparation and pharmaceutical compositions containing them and their use in medicine. WO03009841, EP1414439, US6987123**

3. Lohray Braj Bhushan; Lohray Vidya Bhushan; Barot Vijay Kumar Gajubhai; ***Raval Saurin Khimshanker;*** Raval Preeti Saurin; Basu, Sujay **Novel heterocyclic compounds having hypolipidemic, hypocholesteremic activities process for their preparation and pharmaceutical compositions containing them and their use in medicine. US20030236254**

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