"Design, Synthesis and Biological Evaluation of Novel Selective TNF-α Inhibitors for the Treatment of Inflammatory Diseases''

A Thesis Submitted to

The Maharaja Sayajirao University of Baroda

For the Degree of

Doctor of Philosophy

IN

CHEMISTRY

BY

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AUGUST, 2012

CERTIFICATE

This is to certify that the thesis entitled **"Design, Synthesis and Biological Evaluation of Novel Selective TNF-α Inhibitors for the Treatment of Inflammatory Diseases''** which is being submitted to The Maharaja Sayajirao University of Baroda, Vadodara for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the original research work completed by **Mr. Anil Dattatraya Argade** under my supervision and guidance at Zydus Research Centre, Ahmedabad and the work embodied in this thesis has not formed earlier the basis for the award of any degree or similar title of this or university or examining body.

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DECLARATION

I hereby declare that the work submitted by me entitled **"Design, Synthesis and Biological Evaluation of Novel Selective TNF-α Inhibitors for the Treatment of Inflammatory Diseases''** submitted herewith to The Maharaja Sayajirao University of Baroda, Vadodara for the fulfillment of the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the work carried out by me in Medicinal Chemistry Department at Zydus Research Centre, Ahmedabad. The result of this work has not been previously submitted for any degree/fellowship to any university or institution.

Date:

Place: Ahmedabad **Anil Argade**

PREFACE

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

The thesis consists of four major sections, Introduction, Designing, Result and discussion, experimental and overall summary part covering various aspects of inflammatory diseases and development of the TACE and TNF-α inhibitors for their treatment.

In an **'Introduction'** section, pathophysiology of the disease and the current therapeutic options are discussed, followed by an introduction to TACE and TNF-α inhibitors as novel targets for the treatment of inflammatory diseases. The '**Designing**' section deals with the general information, rational for the the designing of novel TACE and TNF-α inhibitors.

The '**Results & Discussion**' section summarized discussion on synthesis, biological activities and molecular modeling studies of the novel compounds.

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

In the **'Appendix-II'** Copy of spectra (IR, NMR, ESI-MS and HPLC) of representative compounds from each series (intermediates and final compounds) are enclosed, followed by copies of our publications.

Working for this thesis has been a great learning experience for me. Understanding the physiological pathways involved in inflammatory diseases and

the biological role of TNF-α in this complex disease was very interesting and simulative. Molecular modeling experiments provided good learning and were instrumental in understanding the ligand receptor interactions and structural requirements of the compounds to be synthesized. Presenting the work in the form of publications was equally a good learning experience.

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

Anil Argade

ACKNOWLEDGEMENTS

I wish to express my whole hearted gratitude to my research supervisors **Dr. Rajesh Bahekar** for his guidance, suggestions and perceptive criticism while carrying out this work.

I owe tremendous debt to **Shri. Pankaj R. Patel**, CMD, Zydus Cadila for his encouraging and providing the necessary facilities.

It's my privilege to thank **Prof. B. V. Kamath**, Head, Department of Chemistry, M.S. University, Baroda.

My special thanks to **Prof. Shailesh R. Shah**, **Dr. Mukul R. Jain** and **Dr. Pravin Thombare** for their guidance.

No words can acknowledge Dr. Brijesh Srivastava, Dr. Jigar Desai, Dr. Harikishor Pingali, Preeti Raval, Saurin Raval, Vrajesh Pandya, Dr. Sameer Agarwal and Dr. Sairam Kalapatapu for their moral support, encouragement and guidance in the preparation of thesis, which otherwise would have been a difficult task. Dr. Sairam's guidance during *in silico* experiments is also greatly acknowledged.

Special thanks to lab colleagues Sanjay Gite, Mukunda Patekar, Pravinkumar Singh, Keval Bhambharoliya, Bhaumin Patel, Vaibhav Kalhapure, Archana Gite and Nandini Panchal for their cheerful co-operation and the help in every aspect throughout this work.

This work would have not completed without the help provided by the analytical group. My sincere thanks to Dr. R Murugan, Jigar Gajjar, Nainish Trivedi and Jignesh Chauhan for recording the NMR, IR, Mass and HPLC

spectra.

I am extremely thankful to Dr. Ganes Chakrabarti and Mehul Raviya for *in vitro* biological evaluation of the test compounds.

Extremely thankful to Dr. Abhijit Chatterjee and Dr. Jogeswar Mahapatra for *in vivo* pharmacological evaluation of test compounds and to Mr. Harilal Patel for conducting Pharmacokinetic studies.

Sincerely, thanks to Mubeen Shaikh for his help in molecular modeling studies.

I would like to extend my thanks to Kaushik Banerjee and Mrs. Clarine Pathak for his whole hearted support and encouragement throughout this work.

I take this opportunity to thank my friends and collegues, Pankaj Makadia, Dr. Kirtipalsinh Solanki, Anant Patel, Kiran Shah, Ruchir Bavadia, Pandurang Zaware, Amitgiri Goswami, Baban Thube, Prashant Deshmukh, Mayur Mukim and Pinkal Prajapati for their help and encouragement during this work.

My special thanks to Mr. Ashish Gupte (H.R. Department of ZRC) for administrative help.

Finally, no words can express the feelings towards my parents and family members, who have contributed and sacrificed a lot to reach me at this stage and will always remain a sole source of inspiration in my life.

Anil Argade

INDEX

Appendix- I: Abbreviations used in this thesis

ADAM-17	A disintegrin and metalloprotease
ACE-2	Angiotensin converting enzyme-2
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
AUC	Area under curve
AA	Arachidonic acid
BK2	Bradykinin-2
n-BuLi	n-Butyl lithium
$CHCl_3$	Chloroform
CCR4	Chemokine receptor 4
COX	Cyclo-oxygenase
CB	Cannabinoid
CYS	Cysteine rich
CYTO	Cytoplasmic
CS_2CO_3	Cesium carbonate
CH_3CN	Acetonitrile
$CaCl_2$	Calcium chloride
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	*N,N'*-Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DHFR	Dihydrofolate reductase inhibitors
DMARD	Disease modifying anti-rheumatic drugs
DIS	Disintegrin
DEAD	Diethylazodicarboxylate
DHFR	Dihydrofolate reductase
EDC.HCl	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide

	hydrochloride
EtOH	Ethanol
EtOAC	Ethyl acetate
ED_{50}	Effective dose at 50
Fab	Fragment antibody binding
Fc	Fragment constant
FPTase	Farnesyl protein transferase
GC	Glucocorticoids
GF	Growth factor
HNMR	Proton nuclear magnetic resonance
HPLC	High performance liquid chromatography
IBD	Inflammatory bowel diseases
IBS	Inflammatory bowel syndromes
IR	Infra-red
IgG	Immunoglobulin G
IL	Interleukin
iBu	Isobutyl
JAK	Janus activated kinase
KOH	Potassium hydroxide
$LiAlH_4$	Lithium aluminum hydride
LOX	Lipo-oxygenase
LBD	Ligand binding domain
LTB4	Leukotriene B4
LPS	Lipopolysaccharide
LiHMDS	Lithium hexamethyldisilazane
MeOH	Methanol
MS	Mass spectrometry
MAPK	Mitogen activated protein kinase
MMP	Matrixmetalloproteinase
mTNF	Membrane bound precursor
Me	Methyl

mp	Melting point
NSAIDs	Non-steroidal anti-inflammatory drugs
$NaIO_4$	Sodium periodide
NK	Natural killer
NF-κB	NF-Kappa B
NaOMe	Sodium methoxide
NaOH	Sodium hydroxide
$NaBH_4$	Sodium borohydride
NaO*t*Bu	Sodium tertiary butoxide
Na_2CO_3	Sodium carbonate
NaH	Sodium hydride
$NH_2OH.HCl$	Ammonium hydroxide hydrochloride
NH_2NH_2	Hydrazine hydrate
Na_2SO_4	Sodium sulfate
$NaHCO_3$	Sodium bicarbonate
OA	Osteoarthritis
OSO_4	Osmium tetraoxide
PDE-IV	Phosphodiesterase IV
PGE2	Prostaglandin E2
PK	Pharmacokinetic
PLA2	Phospholipase A2
PGH_2	Prostaglandin
PGI2	Postacyclines
PD	Pharmacodinamic
PLE	Pig liver esterase
RA	Rheumatoid arthritis
RIP	Receptor interacting protein
ROS	Reactive oxygen species
SK	Sphingosine kinase
SAR	Structure activity relationship
Syk	Spleen tyrosine kinase

sTNF-α	Soluble TNF-α
SP	Signal peptide
$SOCl_2$	Thionyl chloride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TFA	Trifluoroacetic acid
TNF-α	Tumor necrosis factor
TGF-α	Tumor growth factor
TBILI	Total bilirubin
TACE	TNF-α converting enzyme
$TiCl_4$	Titanium tetrachloride
TRADD	TNF-receptor associated death domain
TM	Transmemebrane
TIMP-3	Tissue inhibitor of metalloproteinase
TPP	Triphenylphosphine
TXA2	Thromboxane
TBILI	Total bilirubin
TEA	Trietylamine
TNFR	TNF receptor
TRAF2	TNF receptor associated factor 2
UC	Ulcerative colitis
URAT-1	Urate transporter-1
WR	Wistar rat

Chapter I: Introduction

CHAPTER I

1. Introduction

1.1. Inflammation

The word inflammation comes from the Latin term "*inflammare"* (means to set on fire). Inflammation is a complex biological process in which the body's immune cells and their secretary chemical products provide protection from injury, infection and foreign substances [**1**]. It is a protective attempt, a defense mechanism by the body to remove the injurious substances and to initiate the healing process. The conditions leading to inflammation could be mechanical damage (e.g. pressure), physical shock (e.g. temperature), chemical (e.g. toxins), internal processes (e.g. uremia) and microorganisms (e.g. bacteria, viruses, parasites). As such, inflammation is part of the regenerative process and without inflammation, wounds and infections would never heal. Similarly, progressive destruction of the tissue would compromise the survival of the organism. Inflammation is characterized by redness, swelling, pain and dysfunction of the organs involved.

Inflammation has two main components, cellular and exudative. The cellular component involves the movement of white blood cells from blood vessels into the inflamed tissue. The white blood cells or leukocytes play an important role in inflammation, they extravasate (filter out) from the capillaries into tissue and act as phagocytes, picking up bacteria and cellular debris. They may also aid by walling off an infection and preventing its spread. If inflammation of the affected site persists, released cytokines IL-1 and TNF activates

endothelial cells to upregulate receptors VCAM-1, ICAM-1, E-selectin and L-selectin for various immune cells. Receptor upregulation increases extravasation of neutrophils, monocytes, activated T-cytotoxic, memory T and B cells to the infected site.

The exudative component involves the movement of fluid, usually containing many important proteins such as fibrin and immunoglobulins (antibodies). Blood vessels are dilated upstream of an infection (causing redness and heat) and constricted downstream while capillary permeability to the affected tissue is increased, resulting in a net loss of blood plasma into the tissue giving rise to edema or swelling. The swelling distends the tissues, compresses nerve endings and thus causes pain.

1.1.1. Inflammatory pathways

Inflammation pathways are broadly classified into two categories:

(1) Macrophage mediated inflammation

(2) Arachidonic acid and cytokine mediated inflammation

1.1.1.1. Macrophage mediated inflammation

In day-to-day life one may face trivial injuries like cut in the skin or a body part gets hit or major injuries may occur while surgery or accidents. Clinically it was observed that the area surrounding these injuries become warm (hyperthermia), red (erythema), swollen (edema) and painful [**2**].

The triggering signals like injury (dead tissue, debris) or infection (bacteria, viruses etc.) causes on set of local reactions that involves stimulation of macrophages, fibroblasts, endothelial cells, neutrophils and other immune cells

(**Figure 1**). These cells present on their surfaces certain receptors named pattern recognition receptors, which recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen associated molecular patterns. These activated cells act through three broadly categorized pathways.

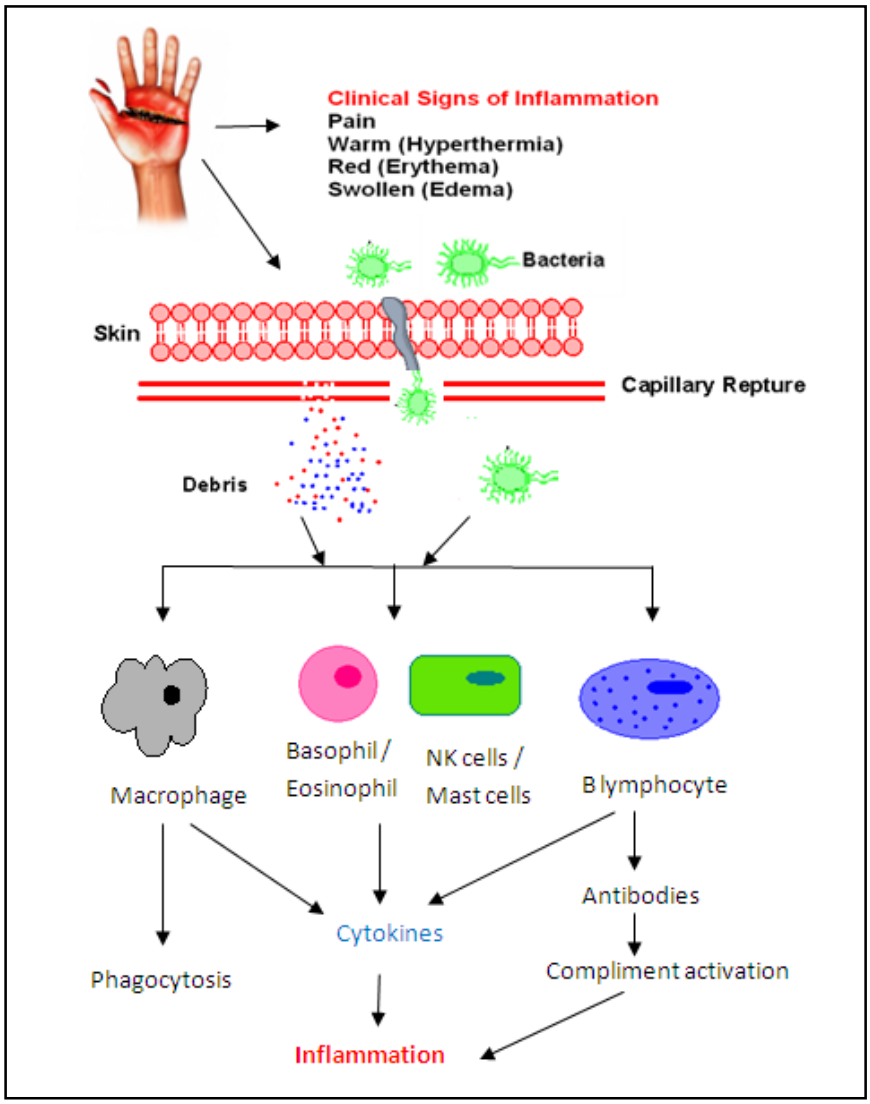


Figure 1. Macrophage mediated inflammation

In the first pathway, where macrophages engulf the recognized pathogen or debris and digest it by phagocytosis [**3**]. Activated macrophages release broad

spectrum of inflammatory mediator's, free radicals and active oxygen species (peroxides, nitric oxide), derivatives of lipids prostaglandin (PGH_2), postacyclines (PGI_2), thromboxane (TXA_2), platelet activating factor and a variety of regulatory proteins. In second pathway activated immune cells like eosinophils, basophils, natural killer cells and mast cells releases various cytokines which are inflammatory mediators [**4**]. In the third pathway, B lymphocytes start production of antibodies, which further by complement activation helps in destruction of antigen bearing pathogen and in the process lead to cytokine activation and further inflammation [**4**].

1.1.1.2. Arachidonic acid and cytokine mediated inflammation

External stimuli as injury or pathogens influence the activity of membrane phospholipids (**Figure 2**). Phospholipids are classes of lipids that are major components of cell membranes as they form lipid bilayers. Phospholipases A_2 (PLA_2) is enzymes that release fatty acids from the second carbon group of glycerol. This particular PLA_2 specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid (AA). Glucocorticoids (GCs) and PLA_2 inhibitors, inhibits this pathway of conversion of phospholipids into AA.

AA undergoes two metabolic pathways [**5**, **6**]. In the first pathway, AA get catalyzed by COX enzyme, yields the PGH_2, PGI_2 and TXA_2, which induce rapid irreversible aggregation of human platelets and are potent inductors of smooth muscle contraction and their overproduction is a major cause of inflammation [**7-11**]. In the second pathway, AA gets catalyzed by LOX enzyme, yields

leukotrienes. Leukotrienes triggers contraction of smooth muscles lining, thus overproduction of leukotrienes is a major cause of asthmatic inflammation.

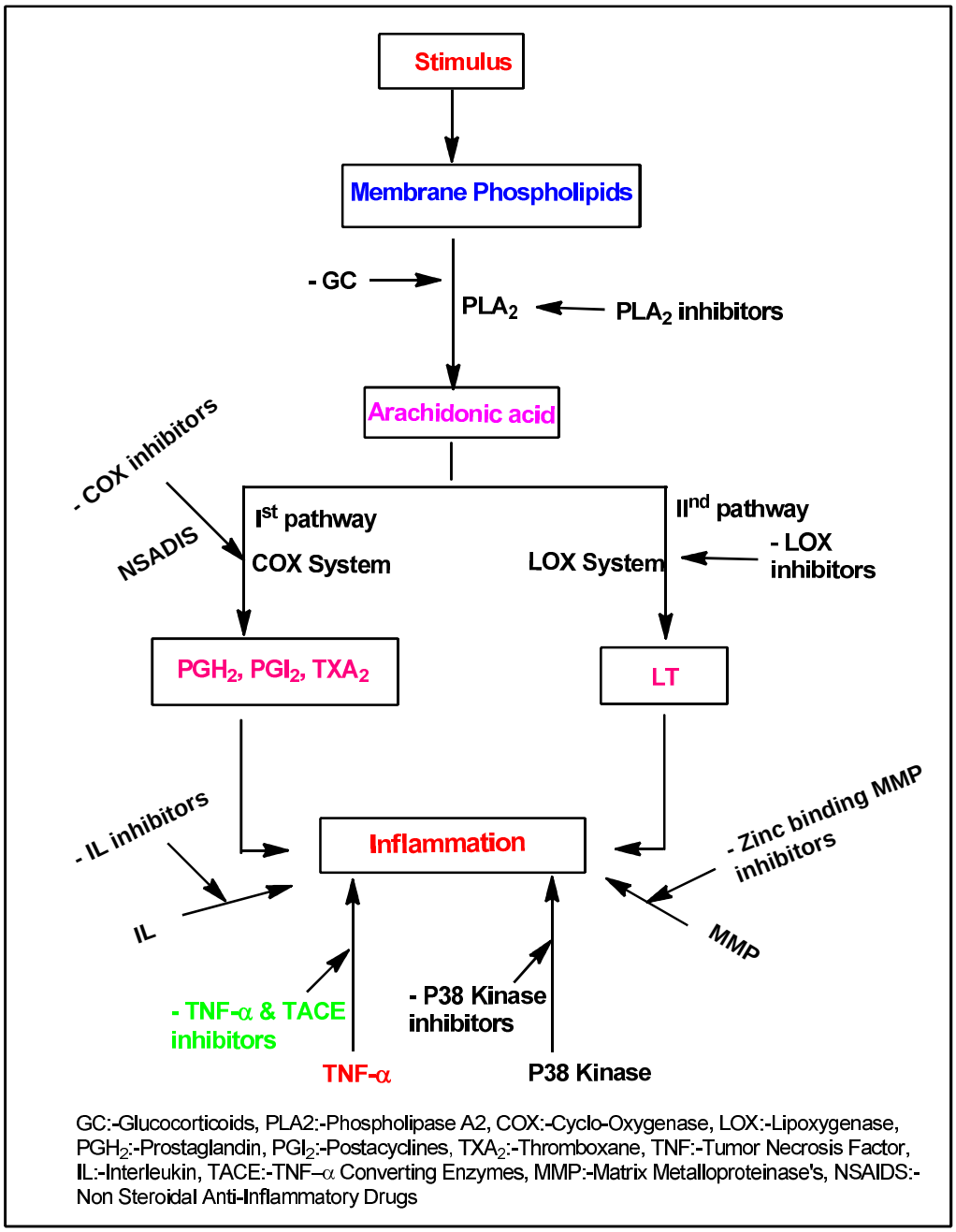


Figure 2. AA and cytokine mediated inflammation pathways

Several cytokines play key roles in mediating acute and chronic inflammatory reactions namely IL-1 to IL-15 and TNF-α [**12**]. IL-1 can trigger fever by enhancing PGE_2 synthesis by the vascular endothelium of the hypothalamus and can stimulate T cell proliferation. It can stimulate the production of Collagenase and Prostaglandin E_2 (PGE_2) by synovial cells and thus are believed to contribute to joint damage in inflammatory conditions such as rheumatoid arthritis (RA). IL-2, IL-3 and IL-5 acts as a growth factor/activator for T cells, NK cells and B cells and promotes the development of lymphokine-activated killer (LAK) cells. IL-4 acts as a B cell, T cell and mast cell growth factor, it enhances class II MHC expression on B cells [**12**].

IL-6, IL-7, IL-11, IL-12 and IL-13 stimulates the development of pre-B and pre-T cells and acts as a growth factor for B cells, T cells and early thymocytes, increases platelet production. IL-8 causes chemotactic effects on neutrophils and its ability to stimulate granulocyte activity. IL-9 promotes production of immunoglobulins by B cells and the proliferation of mast cells. IL-10 reduces antigen-specific T cell proliferation, inhibition of IL-2-induced IFN-γ production by NK cells and inhibition of IL-4 and IFN-γ induced MHC class II expression on monocytes. IL-11, IL-12 and IL-13 stimulate T cell-dependent B cell immunoglobulin secretion [**12**].

Among all these cytokines, TNF-α is extremely potent inflammatory molecule. TNF-α is secreted by activated macrophages/monocytes, fibroblasts, mast cells and some T and NK cells and it induce fever and inflammation [**12**-**13**]. TNF-α can induce fever, either directly via stimulation of PGE_2 synthesis by the

vascular endothelium of the hypothalamus or indirectly by inducing release of IL-1. TNF-α can contribute to joint damage in inflammatory conditions such as rheumatoid arthritis [**13**]. During local and systemic inflammation, membrane bound TNF-α can be cleaved extracellularly by the specific zinc-dependent metalloprotease called TACE, yielding a soluble and biologically active form of trimeric TNF-α. It is also responsible for cleavage of a variety of membrane-anchored proteins including pro inflammatory cytokine.

Matrix metalloproteinase's (MMPs) are zinc-dependent endopeptidases. The MMPs belong to a larger family of proteases known as the metzincin superfamily. The upregulation of the MMPs or the down regulation of these endogenous inhibitors affect critical physiological balance and have been associated with a number of pathological conditions, including osteoarthritis (OA) and RA [**14**].

P38 mitogen activated protein kinases (P38-map kinases) are class of mitogen activated protein kinases that are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock and osmotic shock. P38 is also involved in cell differentiation and apoptosis. P38 MAP Kinase, which participates in a signaling cascade control cellular responses to cytokines and stress. Similarly P38 MAP kinase is activated by a variety of cellular stresses including osmotic shock and inflammatory cytokines [**15**].

1.1.2. Classification of inflammation

Inflammation is broadly classified into two categories (Acute inflammation and Chronic inflammation) as shown in **Figure 3**

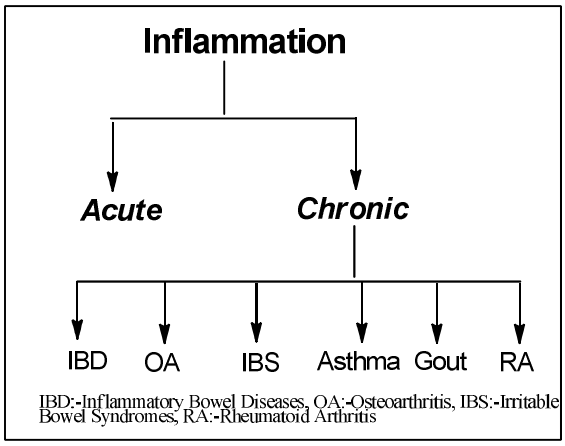


Figure 3. Classification of inflammation

1.1.2.1. Acute inflammation

Acute inflammation is characterized by rapid onset and is of short duration [**16**, **17**]. It starts by the exudation of fluids and plasma proteins and involves migration of leukocytes, most notably neutrophils into the injured area. This acute inflammatory response is believed to be a defense mechanism aimed to kill bacteria, virus and parasites while facilitating wound repairs. Same time acute inflammation may become more complex because of persisting injurious agents or their degraded products.

The different cytokines are involved in acute and chronic inflammation is shown in **Figure 4**. The IL-8, IL-6 and GCSF mediate acute inflammation, while chronic inflammation involves series of cytokines as IL-2 to IL-15, while some common cytokines are found in both types such as IL-1, IL-6, IL-11, IL-17, TNF-α, Eotaxin and GM-CSF [**12**].

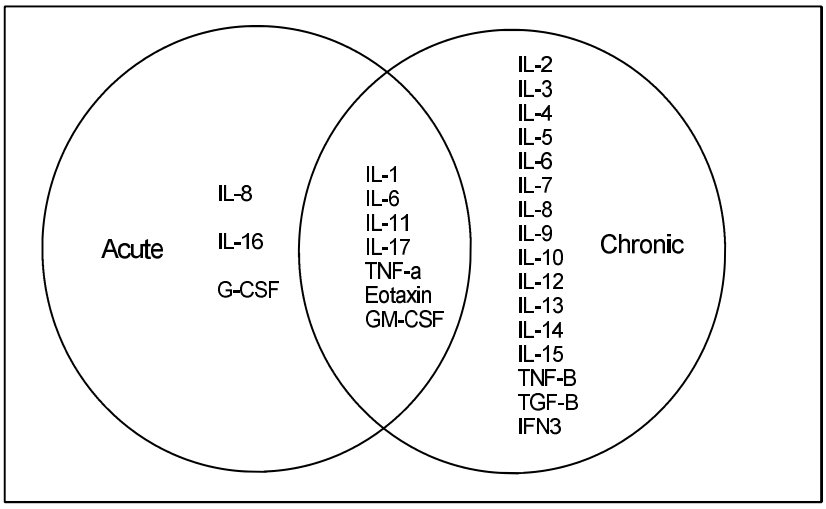
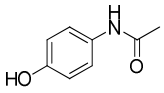
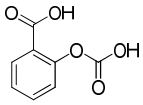
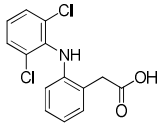
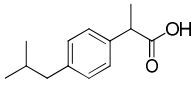
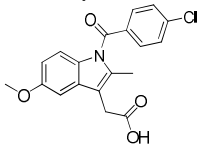
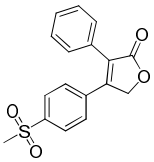
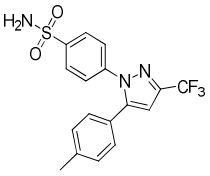
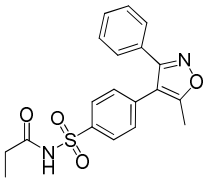
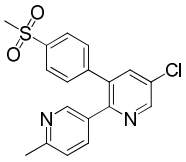
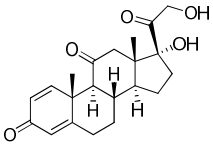
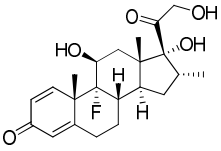


Figure 4. Cytokines involved in acute and chronic inflammatory responses

Currently acute inflammation is mainly treated with first and second generation NSAID's and steroids are listed in **Table 1**.

Table 1. Current therapies for the treatment of acute inflammation

Class	Structure/Name	MOA	Ref.
First-generation NSAID's	 Paracetamol	NCU	[**18,19**]
	 Aspirin	NCU	[**20**]
	 Diclofenac	NCU	[**21**]
	 Ibuprofen	NCU	[**22**]
	 Indomethacin	NCU	[**23,24**]

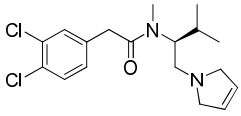
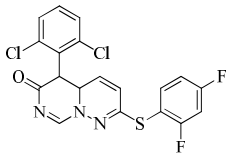
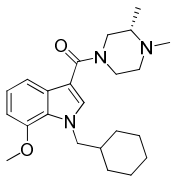
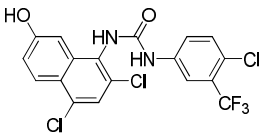
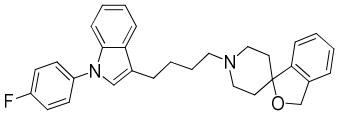
Class	Structure/Name	MOA	Ref.
Second generation NSAID'S	 Rofecoxib	COX-2 inhibitor	[**25**]
	 Celecoxib	COX-2 inhibitor	[**26**]
	 Parecoxib	COX-2 inhibitor	[**27**]
	 Etoricoxib	COX-2 inhibitor	[**28**]
Steroids	 Prednisone	GR agonist	[**29,30**]
	 Dexamethasone	GR agonist	[**31,32**]

MOA:-Mode of Action, NCU:-Not Completely Understood, GR:-Glucocorticoid Receptor.

Among various first and second generation NSAID's which are in clinical practices, most of the NSAID's exhibit serious side effects and adverse events such as ulcer, stroke, dyspepsia, perforation, bleeding, headache, sedation, confusion, nausea, allergy, renal failure, heart attack and vomiting. Steroids are mainly GR agonist, commonly used to cure acute inflammation. However its long

term use exhibit side effects such as diabetes, depression, hypertension, skin toxicity and stomach upset. To overcome the side effects and safety concern of this first and second generation NSAID's and steroids, several new therapies have been developed (**Table 2**) for the safe and effective treatment of acute inflammation.

Table 2. New therapies under clinical development for the treatment of acute inflammation

Target	Structure/Name	Company	Clinical status	Ref.
κ-Opoid agonists	 (LPK-26)	State Key	Preclinical	[**33**]
P38 MAP Kinase	 (VX-745)	Vertex	Phase II	[**34**]
CB_2 receptor full agonist	 (Org 28611)	Organon International	Phase II	[**35**]
TRPV1 inhibitors		Bayer	Preclinical	[**36**]
Sigma receptor agonist	 (Lu-28-179)	Lundbeck	Phase II	[**37**]

CB:-Cannabinoid, TRPV1:-Transient Receptor Potential Cation Channel.

As described in **Table-2**, currently several new therapies are in various stages of clinical development for the treatment of acute inflammation. Among these new therapies, κ-Opoid receptor agonists and TRPV1 inhibitors are most promising. κ-Opioid receptor activation by agonists is coupled to the G protein G_i/G_0, which increases phosphodiesterase activity, which causes break down of cAMP lead to inhibitory effect on neurons. κ-Opioid receptors also couple to inward rectifier potassium and N-type calcium ion channels result in the activation of mitogen activated protein kinases MAPK, results acute inflammation.

TRPV1 get phosphorylates in response to several algesic agents, resulting in a lower threshold of channel activation. Some substances such as bradykinin, nerve growth factor and protons have been reported to sensitize the TRPV1 receptor. Activation of TRPV1 results in the release of pronociceptive peptides and responsible causes acute inflammation, which decreases when treated with TRPV1 antagonists. Cannabinoid receptor (CB_2), is a G protein-coupled receptor (GPGR) belong to cannabinoid receptor family. CB_2 receptor closely related to the CB_1 receptor, therefore it has selectivity problem over CB_1 and thus its clinical development of CB_2 agonist has been stopped mainly due to lack of selectivity. Therefore among various new therapy listed, the TRPV1 and κ-Opioid represents most promising target for treatment of acute inflammation.

1.1.2.2. Chronic inflammation

Chronic inflammation is a prolonged duration and histologically it is characterized by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis [**12**]. The persistent chronic inflammation increases

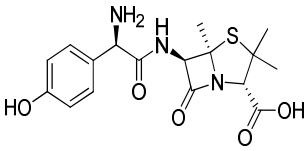
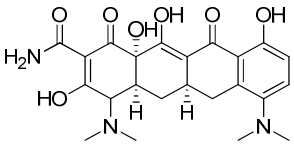
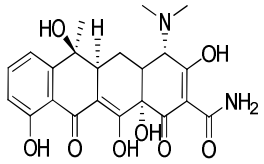
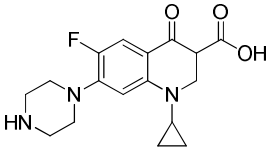
the development of the degenerative diseases such as OA, IBD, IBS, Asthma, RA and Gout, which are associated with immunopathological changes that appears to play a key role in the onset of these conditions [**16**, **17**].There are many diseases of unknown etiology which are distinguished by signs and symptoms characteristic to chronic inflammatory process. Some chronic inflammations caused by self replicating parasite like bacterium, virus or neoplasm. Such inflammation may become more complex because of persisting injurious agents or their degraded products. The chronic inflammation is implicated in following diseases.

(a) Inflammatory Bowel Disease (**IBD**)

Inflammatory bowel disease is a group of inflammatory conditions of the colon and small intestine. The major types of IBD are Crohn's disease and Ulcerative colitis (UC) [**38**-**40**]. Crohn's disease and UC are present with extra-intestinal manifestations (such as liver problems, arthritis, skin manifestations and eye problems) in different proportions. Interleukin (IL) is involved in pro-inflammatory mechanisms in RA and IBD. The treatment of IBD mainly includes antibiotics and the antibiotics which are in current use are listed in **Table 3**.

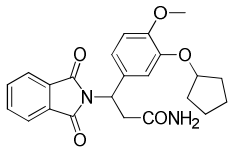
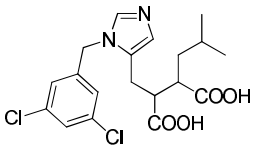
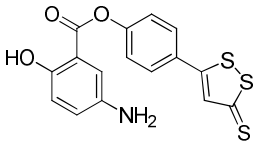
Antibiotic based therapies which are available for the treatment of IBD mainly treated bacterial and microbial infection in the GIT and thereby it prevents further progression of infection and also reduces mild to moderate pain. These therapies are associated with adverse effects such as colitis, mental changes, confusion, sensitivity to lights, unclear diarrhea, dizziness, mouth sores, headache, vomiting, acute liver failure and impaired color vision.

Table 3. Antibiotics based current therapies for the IBD treatment

Class	Structure/Name	MOA	Ref.
Antibiotics	 Amoxicillin	Bind reversibly to the small subunits of bacteria	[**41**]
	 Minocycline	Bind reversibly to the small subunits of bacteria	[**42**]
	 Tetracycline	Bind to subunit of microbial ribosome's	[**43**]
	 Ciprofloxacin	Active against both Gram-positive and Gram-negative bacteria	[**44**]

As a result, there exists unmet need to develop new therapies for the treatment of IBD. Some of the new therapies for the treatment of IBD, which are currently under clinical development, are listed in **Table 4**. Currently several new therapies are in various stages of clinical development for the treatment of IBD. Among these new therapies, PDE-IV inhibitors and ACE-2 inhibitors are most promising. In general PDE-IV inhibitors and ACE-2 enzymes are specifically located in GIT. Thus the off target side effects are likely to be less with suitable inhibitors, which could be potent and selective for these targets.

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

Target	Structure/Name	Company	Clinical status	Ref.
PDE- IV inhibitors	 (CDC-801)	Celgene	Phase I	[**45**]
IL-17 antagonist,	IL-17RC	BMS	Phase I	[**46**]
ACE-2 inhibitors	 (GL-1001)	Ore	Phase II	[**47**]
HSD of mesalazine	 (ATB-429)	Antibe	Phase I	[**48**]

PDE-IV:-Phosphodiesterase-IV, IL:-Interleukin, ACE:-Angiotensin Converting Enzyme, HSD:-Hydrogen Sulfide-based Derivative.

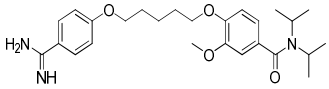
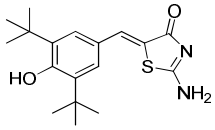
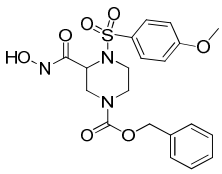
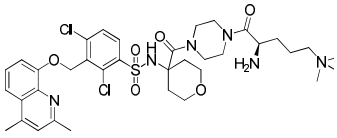
(b) Osteoarthritis (OA)

Osteoarthritis is also known as degenerative arthritis or degenerative joint disease. It's group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone [**49**]. Symptoms may include joint pain, tenderness, stiffness, locking and sometimes an effusion of joints. A variety of causes such as hereditary, developmental, metabolic and mechanical stimuli's may initiate processes leading to loss of cartilage. When bone surfaces become less well protected by cartilage, bone may be exposed and damaged [**50**].

Current therapies, for the treatment of OA mainly include analgesics and NSAID's and steroids, which relieve only mild to moderate pain. These therapies

are associated with side effects and safety concern of NSAID's and steroids limits its prolonged usage as described earlier. As a result, there exists unmet need for the development of new therapies for the safe and effective treatment of OA (**Table 5**).

Table 5. New therapies under clinical development for the treatment of OA

Target	Structure/Name	Company	Clinical status	Ref.
LTB4 antagonist	 (CGS-25019C)	Novartis	Phase III	[**51**]
Dual inhibitor of 5-LOX and COX-2	 (CI-1004)	Pfizer	Phase III	[**52**]
MMP's inhibitors	 (PGE-2946979)	P & G	Preclinical	[**53**]
Collagenase inhibitor	AZD-8955	ChondroGene	Phase II	[**54**]
BK2 antagonist	 (MEN-16132)	Menarini	Phase II	[**55**]
IL-17 antagonist	IL-17RC	BMS	Phase I	[**46**]

LTB4:-Leukotriene B4, LOX:-Lipo-Oxygenase , COX-2:-Cyclo-Oxygenase , BK2:-Bradykinin-2, MMP's:- Matrix Metalloproteinases, P&G:-Procter & Gamble

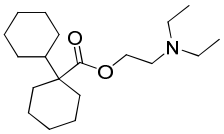
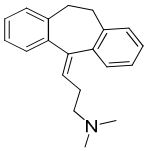
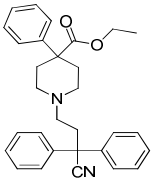
Currently several new therapies are in various stages of clinical development (**Table 5**). Among these new therapies for OA, IL-17 antagonist could be promising. Interleukin 17 (IL-17) is a cytokine that acts as a potent

mediator in delayed type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma. IL-17 is produced by T-helper cells and is induced by IL–23, which results in tissue damage in delayed-type reactions. IL-17 as a family functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. IL-17 acts synergistically with TNF and IL-1, while LTB4 and COX targets have selectivity issue. Dual inhibitors of 5-LOX and COX-2 therapies are associated with side effects and adverse effects such as ulceration, vomiting. Thus the IL-17 is most promising target for treatment of OA.

(c) Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome is a symptom-based diagnosis characterized by chronic abdominal pain, discomfort, bloating and alteration of bowel habits. As a functional bowel disorder, IBS has no known organic cause [**49**,**56**,**57**]. Several conditions may present IBS, including coeliac disease, fructose malabsorption, mild infections and parasitic infections like giardiasis. In IBS, routine clinical tests yield no abnormalities, although the bowels may be more sensitive to certain stimuli, such as balloon insufflations testing. The exact cause of IBS is unknown. The most common theory is that IBS is a disorder of the interaction between the brain and the gastrointestinal tract, although there may also be abnormalities in the gut flora or the immune system [**58**,**59**]. The treatment for IBS mainly includes Dicyclomine, Amitriptyline and Diphenoxylate, which are in current use as listed in **Table 6**.

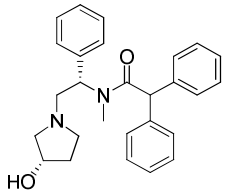
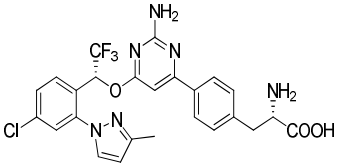
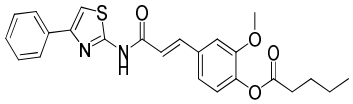
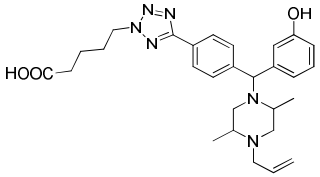
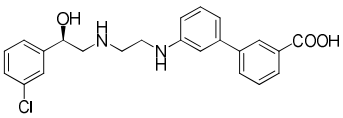
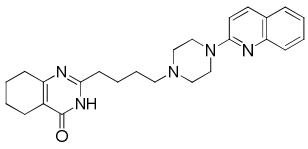
Table 6. Current therapies for the IBS treatment

Therapy	Structure/Name	MOA	Ref.
First-line therapy	 Dicyclomine	Anticholinergic that blocks muscarinic receptors	[**60**]
	 Amitriptyline	Serotonin-norepinephrine reuptake inhibitors	[**61**]
	 Diphenoxylate	Opioid agonist	[**62**]

Other than Dicyclomine, Amitriptyline, Diphenoxylate, analgesics, NSAID's and steroids are commonly used for the treatment of IBS, which relive mild to moderate pain. However, these therapies are associated with side effects and adverse effects as described earlier. To overcome side effects and safety concerns of these existing therapies, there exist profound unmet needs to develop new therapies for the safe and effective treatment for IBS. As described in **Table-7**, currently several new therapies are in various stages of clinical development for the treatment of IBS. Among these new therapies, Sphingosine Kinase (SK) inhibitors are most promising. SK has been shown to regulate diverse cellular processes. It has been characterized as a lipid signaling molecule with dual function. On one hand, it exerts its actions extracellularly by binding to the five different S1P receptors that couple to a variety of G-proteins to

regulate diverse biological functions, ranging from cell growth and survival to effectors functions, such as proinflammatory mediator synthesis.

Table 7. New therapies under clinical development for the IBS treatment

Target	Structure/Name	Company	Clinical status	Ref.
KOR agonist	 (EMD-61753)	Tioga	Phase III	[**63**,**64**]
Tryptophan hydroxylase inhibitor	 (LX-1032)	Lexicon	Phase-II	[**65**]
SK inhibitor	 (ABC-747080)	Apogee	Preclinical	[**66**]
DOR agonist	 (UK-321130)	Pfizer	Preclinical	[**67**]
ß3-Adrenoceptor agonist	 (GW-427353)	AltheRx	Phase-II	[**68**]
5-HT1A agonist and 5-HT3 antagonist	 (TZB-30878)	Aska	Preclinical	[**69**]

KOR:-Kappa Opioid Receptor, DOR:-Delta-Opioid Receptor, SK:-Sphingosine Kinase, 5-HT1A:-Hydroxytryptamine1A.

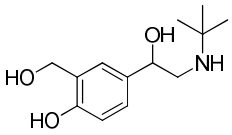
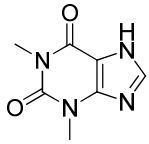
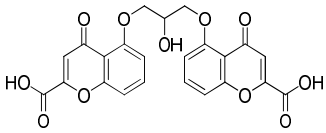
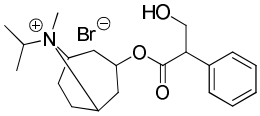
On the other hand, it appears to act as an intracellular second messenger, although the relevant molecular target to which it binds within cells remains to be discovered. In any case, a role of S1P in various functions of cells and tissues is established, including regulation of cell survival and inflammatory responses. 5-HT1A agonist and 5-HT3 antagonist showed poor bioavailability. Thus SK inhibitors represent most effective newer therapy for safe and effective treatment of IBS.

(d) Asthma

Asthma is a common chronic inflammatory disease of airways characterized by variable and recurring symptoms of reversible airflow obstruction and bronchospasm. Symptoms include wheezing, coughing, chest tightness and shortness of breath. Asthma is clinically classified according to the frequency of symptoms, forced expiratory volume per 1 second (FEV1) and peak expiratory flow rate [**70**-**72**]. Symptoms are often worse at night, in the early morning or in response to exercise or cold air. Some people with asthma experience symptoms, usually in response to triggers, such as allergens and irritants [**73**]. The treatment for asthma mainly includes Salbutamol, Theophylline, Cromolyn sodium and Ipratropium (**Table 8**).

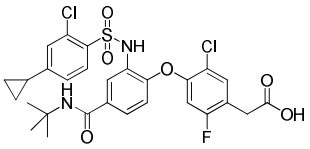
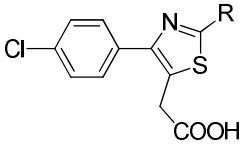
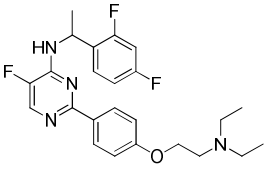
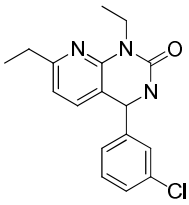
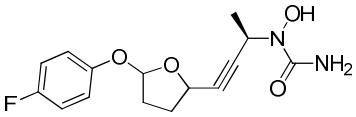
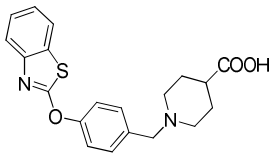
Current therapies for the treatment of asthma mainly include first generation drugs which relive mild to moderate inflammation. These therapies are associated with side effects and adverse events such as tachycardia, arrhythmia, nausea, diarrhea, CNS excitation, severe burning sensation, sinus pain and headache.

Table 8. Current therapies for the treatment of asthma

Therapy	Structure/Name	MOA	Ref.
First-line therapy	 Salbutamol	β_2-adrenergic receptor agonist	[**74**]
	 Theophylline	Xanthine derivatives	[**75**]
	 Cromolyn sodium	May inhibit release of preformed T cell cytokines	[**76**]
	 Ipratropium	Blocks the muscarinic acetylcholine receptors	[**77**]

To overcome these side effects and safety concern of these drugs, several new therapies have been developed for the safe and effective treatment for asthma (**Table 9**). Among these new therapies, IL-17 and PDE-IV inhibitors are most promising. Over expression of IL-17 gene in the airway of mice is associated with airway neutrophilia, induction of cytokines, increase in airway hyper reactivity and mucus hyper secretion. Hence, IL-17 may have a crucial role in allergic airway inflammation and have important therapeutic implications in asthma, while 5-LOX and LTA4 inhibitors are associated with side effects and adverse effects such as ulceration. Thus IL-17 inhibitors could be most effective and safe target for the treatment of asthma inflammation.

Table 9. New therapies under clinical development for the treatment of asthma

Target	Structure/Name	Company	Clinical status	Ref.
Short-acting β-agonist	 (AMG-853)	Amgen	Phase II	[**78**]
CRTH2 antagonist	 (OC000459)	Oxagen Limited	Phase II	[**79**]
CCR4	 (RS-1748)	Daiichi Sankyo	Preclinical	[**80**]
PDE-IV inhibitors	 (YM-976)	Yamanouchi	Phase III	[**81**]
IL-17 antagonist	IL-17RC	BMS	Phase I	[**46**]
5-LOX inhibitors	 (A-76745)	Abbott	Phase II	[**82**]
LTA-4 inhibitors	 (JNJ-26993135)	J & J	Preclinical	[**83**]

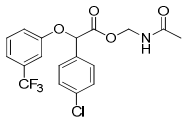
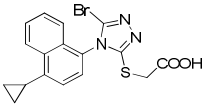
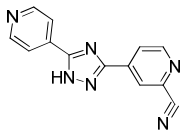
CRTH2:-Chemokine Receptor 4, CRTH2:-Chemoattractant Receptor Homologous 2, LTA:-Leukotriene.

(e) Gout

Gout is a medical condition usually characterized by recurrent attacks of acute inflammatory arthritis, red, tender, hot and swollen joints [**84**]. Treatment with NSAID's, steroids or colchicines improves symptoms. Once the acute attack has subsided, levels of uric acid are usually lowered via lifestyle changes and in those with frequent attacks, allopurinol provide long-term prevention.

Current therapies for the treatment of gout mainly include analgesics, NSAID's and steroids to reduce mild to moderate pain. However side effects and safety concerns of existing therapies urge profound unmet need to develop new therapies for the safe and effective treatment of goat. Currently several new therapies are in various stages of clinical development for the treatment of gout (**Table 10**).

Table 10. New therapies under clinical development for the treatment of gout

Target	Structure/Name	Company	Clinical status	Ref.
Uricosuric agent	 (MBX-102)	Metabolex	Phase II	[**85**]
URAT-1 inhibitor	 (RDEA-594)	Ardea	Phase III	[**86**]
XO inhibitor	 (FYX-051, SK-0910)	Fuji Yakuhin	Phase III	[**87**]

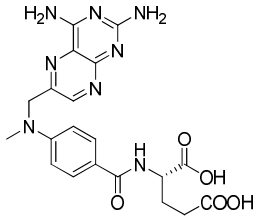
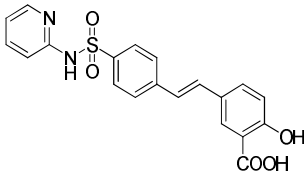
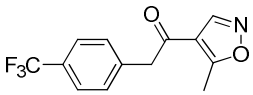
URAT:-Urate Transporte, XO:-Xanthine Oxidoreductase.

As described in **Table 10**, currently several new therapies are in various stages of clinical development for the treatment of gout. Among these new therapies, Xanthine oxidoreductase (XO) inhibitor is most promising. Xanthine oxidase is a superoxide producing enzyme found normally in serum and the lungs. XO activity increases during influenza infection. During severe liver damage, xanthinuria is a rare genetic disorder where the lack of xanthine oxidase leads to high concentration of xanthine in blood and can cause health problems such as renal failure, while uricosuric agent and URAT-1 inhibitors showed short half-lives and poor bioavailability in animal models and humans. Thus, XO inhibitors could be most effective and safe therapy for the treatment of gout.

(f) Rheumatoid Arthritis (RA)

Rheumatoid arthritis is a chronic and systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible joints. The process produces an inflammatory response the joints secondary to swelling of synovial cells, excess synovial fluid and the development of fibrous tissue in the synovium. The pathology of RA often leads to the destruction of articular cartilage of joints. RA can also produce diffuse inflammation in the lungs, membrane around the heart, white of the eye and also nodular lesions, most commonly in subcutaneous tissue. Although the cause of RA is unknown, autoimmunity plays a pivotal role in both its chronicity and progression. Thus RA is considered a systemic autoimmune disease [**88**]. Treatment of RA mainly includes Disease Modifying Anti-rheumatic Drugs (DMARD) and Biologics (**Table 11**).

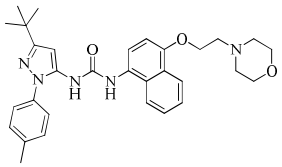
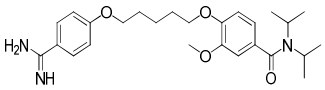
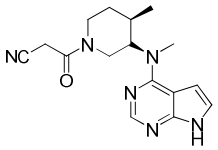
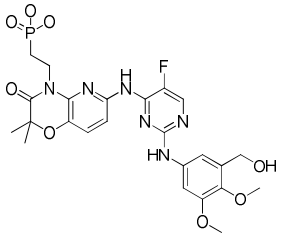
Table 11. Current therapies for RA treatment

Therapy	Structure/Name	MOA	Ref.
DMARD	 Methotrexate	DHFR inhibitors	[**89**,**90**]
	 Sulfasalazine	Its metabolite 5-ASA	[**91**]
	 Leflunomide	Inhibits mitochondrial enzyme dehydrogenase	[**92**]
Biologics	Infliximab	TNF-α inhibitors	[**93**]
	Adalimumab	TNF-α inhibitors	[**93**]
	Certolizumab	TNF-α inhibitors	[**93**]
	Natalizumab	TNF-α inhibitors	[**93**]

5-ASA:-5-Amino Salicylic Acid, DMARD:-Disease Modifying Anti-rheumatic Drugs.

Current therapies for the treatment of RA include DMARD and biologics, which relives only mild to moderate pain and current therapies are associated with side effects such as dryness of mouth, nausea, confusion, breast enlargement, blurred vision, drowsiness, headache and loss of taste. To overcome these side effects and safety concern of existing drugs, several new therapies have been developed for the safe and effective treatment of RA Currently several new therapies are in various stages of clinical development for the treatment of RA (**Table 12**). Among these new therapies, TACE and JAK inhibitors are most promising for the treatment of RA inflammation.

Table 12. New therapies under clinical development for the treatment of RA

Target	Structure/Name	Company	Clinical status	Ref.
P38 kinase	 (BIRB796)	Boehringer Ingelheim	Phase II	[**94**]
TACE inhibitors	(BMS-561392)	BMS	Phase II & Discontinue	[**95**]
TACE inhibitors	TMI-005	Wyeth	Phase II & Discontinued	[**95**]
LTB4 antagonist	 (CGS-25019C)	Novartis	Phase III	[**96**]
JAK inhibitors	 (CP690550)	Pfizer	Phase III	[**97**]
SYK inhibitors	 (R-788)	Regal	Phase III	[**98**]

JAK:-Janus Activated Kinase, SYK:-Spleen Tyrosine Kinase, LTB:-Leukotriene, TACE:-TNF-α Converting Enzyme.

Recently JAK3 and SYK inhibitors are under investigation as immunosuppressant for the treatment of RA, but lymphoma is major toxicity concern. P38 inhibitors and LTB4 antagonist were failed due to ulcer toxicity. Thus TACE inhibitors could be most effective for the treatment of RA.

NSAID's and steroids are commonly used to relieve pain and

inflammation. As described earlier, NSAD's and steroids exhibits serious side effects like stomach upset, nausea, vomiting, heartburn, headache, diarrhea, constipation and drowsiness therefore NSAD's and steroids cannot be recommended for the chronic inflammation treatment. In order to avoid side effect and toxicity of NSAID's and steroids, currently various new generations advanced therapeutic options are being researched for the safe and effective treatment of chronic inflammation. Updates on these new therapies are summarized in the next section.

1.1.3. New therapeutic options under evaluation for the treatment of chronic inflammatory diseases

In order to overcome side effects, limitations and toxicity of existing therapies available for the treatment of various inflammatory conditions, currently various new generations advanced therapeutic options are under preclinical and clinical evaluation as depicted in **Table 13**. As discussed earlier, among various inflammatory mediators, it can be concluded that TNF-α is the most dominant cause of various types of inflammations. TNF-α is a central mediator in human inflammatory diseases. Hence controlling TNF-α levels can form a promising therapy for the treatment of inflammatory diseases. Various biological protein drugs are available that blocks TNF-α but their high cost and parenteral route of administration are limiting factors. This invites research to develop small molecule based anti TNF-α therapy which could be safe and cost effective. The most suitable way to manage excess TNF-α formation is by inhibition of TACE.

Although, many small molecule based TACE inhibitors are under development at various research laboratories throughout the world, currently

none of them are in the market.

Table 13. New generation therapies for the treatment of chronic inflammatory conditions

Inflammations	Target	Company	Phase	Ref.
Acute inflammation	κ-Opoid agonists	State Key	Preclinical	[**33**]
	P38 kinase inhibitors	Vertex	Phase II	[**34**]
	CB1 and CB2 Agonist	Organon	Phase II	[**35**]
	TRPV agonist	Bayer	Phase I	[**36**]
IBD	PDE IV inhibitors	Celgene	Phase I	[**45**]
	IL-17 antagonist	BMS	Phase I, II	[**46**]
	ACE-2 inhibitor	Ore		[**47**]
OA	LTB4	Novartis	Phase III	[**51**]
	MMPs inhibitors	P& G	Preclinical	[**53**]
	Tryptophan hydroxylase inhibitor	Lexicon	Phase III	
IBS	Collagenase inhibitor	ChondroGene	Phase II	[**54**]
	KOR agonist	Tioga	Phase III	[**95**]
	SK inhibitors	Apogee	Preclinical	[**66**]
Asthma	CCR4	Diichi Sanofi	Preclinical	[**80**]
	PDE-IV inhibitors	Yamanouchi	Phase III	[**81**]
	LTA-4 inhibitors	J & J	Preclinical	[**83**]
Gout	URAT-1 inhibitor	Ardea	Phase II	[**85**]
	Xanthin inhibitors	Fuji Yakuhin	Phase II	[**87**]
RA	TACE inhibitors	BMS	Phase II & Discontinued	[**95**]
	JAK inhibitors	Pfizer	Phase III	[**97**]
	SYK inhibitors	Rigel Pharma	Phase III	[**98**]

IBD:-Inflammatory Bowel Disease, OA:-Osteoarthritis, IBS-Inflammatory Bowel Syndrome, RA:-Rheumatoid Arthritis, CB:-Cannabinoid, TRPV1:-Transient Receptor Potential Cation Channel, LTB4:-Leukotriene B4, MMP's:–Matrix Metalloproteinases, KOR:-Kappa-Opioid Receptor, SK:-Sphingosine Kinase, LTA:-Leukotriene, URAT:-Urate Transporte, TACE:-TNF-α Converting Enzyme, JAK:-Janus Activated Kinase, SYK:-Spleen Tyrosine Kinase.

The present scenario of non availability of safe, cost effective and efficacious small molecule based TNF-α and TACE inhibitors, prompted us to hunt for novel TACE and TNF-α inhibitors. The research presented in this thesis focus on the synthesis, biological evaluation of anti TNF-α molecules and TACE inhibitors. In next section an overview on TNF is presented.

1.2. Tumor Necrosis Factor (TNF)

Tumor necrosis factor is a multifunctional proinflammatory cytokine, with effects on lipid metabolism, coagulation, insulin resistance and endothelial function. It can send both cell survival and death signals to cells [**99**]. TNF play important roles in various physiological and pathological processes, including cell proliferation, differentiation, apoptosis and modulation of immune responses and induction of inflammations.

1.2.1. Types of TNF

Mainly two types of TNF (α and β) occurred naturally having molecular weight of 17 and 25 kDa respectively **Figure 5** [**88**]. TNF-α or cachectin, exists as a trimer and is one of the products of activated macrophages/monocytes, fibroblasts, mast cells and some T and NK cells [**100**]. TNF-β, also known as lymphotoxin, is produced by activated T and B lymphocytes. It binds to the same high affinity receptors (TNFR1) as TNF-α Its properties are similar to those of TNF-α which includes induction of apoptosis (programmed cell death) in many types of transformed, virally infected and tumor cells. TNF-α is expressed on the cell membrane and then hydrolyzed to release the soluble form, which forms

homotrimers. TNF-β has no cell membrane attachment domain but can form either membrane anchored hetero trimers.

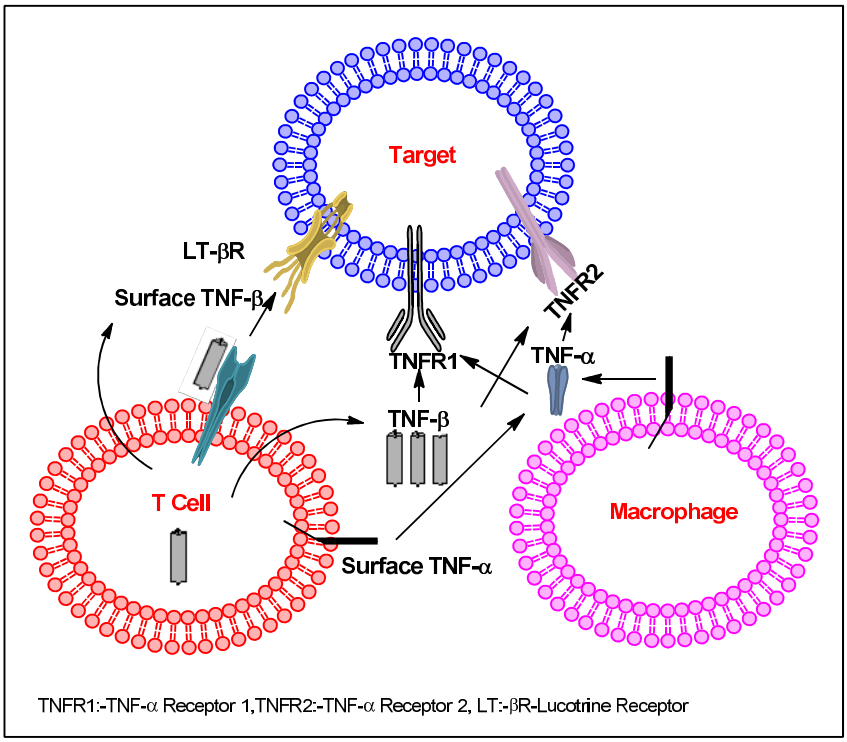


Figure 5. Types of TNF (α & β) and its receptor binding

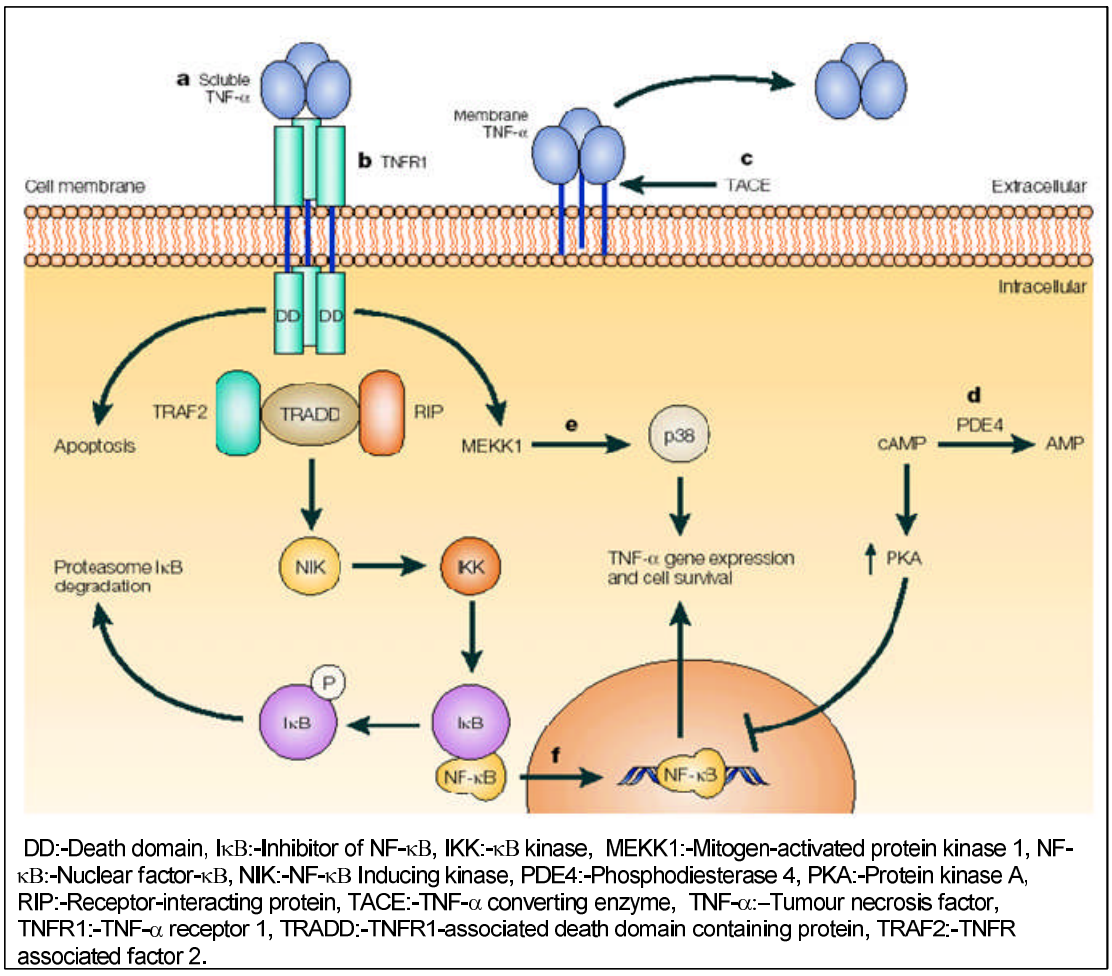
TNF acts through two receptors, TNF-α receptor 1 (TNFR1) and TNF-α receptor 2 (TNFR2) [**101**]. TNFR1 is expressed by all human tissues and is the major signaling receptor for TNF-α TNFR2 is mostly expressed in immune cells and mediates limited biological responses. TNFR2 binds both TNF-α and TNF-β [**102**]. Small quantity of TNF-α stimulates production of high amount IL-1 within a short time. TNF-β is relatively poor stimulus as high concentration of TNF-β induces very less IL-1 production. This further confirms the synergistic role of TNF-α and IL-1 in inflammatory diseases [**103**].

In summary TNF-α mediates its action through both TNFRs, covering almost all human tissues as its targets. It is also associated with key inflammatory cytokine IL-1. Hence it can be concluded that out of two types, TNF-α is a superior target for developing anti-inflammatory drug. TNF-α is a particularly powerful cytokine because it causes release of other cytokines from the body. An excessive amount of TNF-α is present in the blood and joints of patients with RA. The production of TNF-α can lead to inflammation and damage to joints. Blocking TNF-α can reduce inflammation and joint damage.

1.2.2. Signal transduction and mechanisms of TNF-α

TNF-α was identified in the mid-1970s by Lloyd Old and colleagues as an endotoxin induced serum factor that caused the necrosis of certain murine tumours *in vivo* [**104**]. TNF-α is a potent proinflammatory cytokine and it plays a central role in host defense and inflammation [**105**]. A 26 kDa, tmTNF that is cleaved by a cell membrane associated zinc-metalloprotease ADAM-17 (A Disintegrin And Metalloprotease), also known as TACE to form a 17 kDa sTNF-α (**Figure 6**). In general, TNF binds to two receptor subtypes, TNFR1 and TNFR2. Preferentially, TNFR1 get activated by sTNF-α and TNFR2 by tmTNF [**106**,**107**]. The two receptors differ significantly in their binding affinities as well as in their intracellular signaling pathways. Upon stimulation, the intracellular domain of TNFR1 binds to the TNF-receptor associated death domain (TRADD) protein, which can further activate either the apoptotic pathway via TRAF2 and RIP, resulting in the activation of NF-kB. In contrast to TNFR1, TNFR2 is unable to activate the TRADD pathway and signals only through the TRAF2 associated

pathway.



DD:-Death domain, IκB:-Inhibitor of NF-κB, IKK:-κB kinase, MEKK1:-Mitogen-activated protein kinase 1, NF-κB:-Nuclear factor-κB, NIK:-NF-κB Inducing kinase, PDE4:-Phosphodiesterase 4, PKA:-Protein kinase A, RIP:-Receptor-interacting protein, TACE:-TNF-α converting enzyme, TNF-α:–Tumour necrosis factor, TNFR1:-TNF-α receptor 1, TRADD:-TNFR1-associated death domain containing protein, TRAF2:-TNFR associated factor 2.

Potential targets for the inhibition of TNF-α related activity. Extrecelluer targets **a** and **b:**-Inhibition of binding of soluble TNF-α with TNFR1,**c:-**Prevention of conversion of mTNF-α to sTNF-α by blocking TACE, Intracelluer targets **d:-**Inhibition of PDE4, **e:-**Inhibition of P38, **f:-**Inhibition of activation of NF-kB (NIK, IKK, I kB degradation).

Figure 6. TNF-ą signaling pathways and targets for potential TNF-α inhibitors [**93**]

All nucleated cells express TNF receptors, although their distribution varies with cell types. TNFR1 is expressed constitutively on most cell types, whereas expression of TNFR2 can be induced. In addition, TNFR2 is restricted to certain cell types and can discriminate TNF-α from different species. The receptors also differ significantly in their binding affinities for homotrimeric TNF-α. Although both receptors (TNFR1and TNFR2) can be considered high-affinity, the

on-off kinetics of the two differs dramatically. Binding of homotrimeric TNF-α to TNFR1 is thought to be essentially irreversible, whereas binding to TNFR2 is associated with both rapid on and off kinetics [**108**]. This has fuelled speculation that TNFR2 might function as a 'ligand passer' in some cells transferring TNF-α to TNFR1 [**109**]. However, TNF-ą signaling through TNFR2 seems to have a dual role in T cells. In the absence of TNFR1 signaling, TNF-α promotes the proliferation of naive T cells through TNFR2 [**110**]. The shed extracellular domains of the receptors retain their ability to bind TNF-α and therefore probably function as either endogenous inhibitors or facilitators of the biological activity of TNF-α depending on their concentrations and the concentrations of the ligand [**111**]. Thus TNF-α has been shown to be an important mediator of inflammatory diseases.

1.2.3. Anti-TNF-α therapies under development

In the 1980s, RA was described as a T-cell-mediated disease. However, independent studies by Duff, Feldmann and Saxne demonstrated that the proinflammatory cytokines were present in the synovium and plasma of patients with RA [**112**-**114**]. Since these key findings were published, there have been multiple attempts to develop inhibitors of TNF-ą activity and three protein based drugs have made its way to the market: Etanercept (Enbrel; Amgen/Wyeth), Infliximab (Remicade, Centocor/Schering Plough/Tanabe Sieyaku) and Adalimumab (Humira, Abbott) approved by the US FDA [**115**] as depicted in **Table 14**. Etanercept is a fusion protein of TNFR2 and the Fc region of IgG1. The anti-TNF antibodies Infliximab and Adalimumab, bind to TNF and prevent

the molecule from interacting with its two receptors (TNFR1 and TNFR2) thereby stopping signaling events.

These large biological inhibitors prevent binding of either sTNF or proTNF to their membrane receptor targets. TACE processes proTNF from the cell membrane to yield sTNF. Inhibitors of TACE block TNFR receptor signaling by modulator the soluble TNF (sTNF), but not via the membrane bound, form of TNF (mTNF).

Table 14. Protein-based injectable anti-TNF-α therapies in clinical use [**93**]

Drugs	Status	Biological form
Etanercept	Approved	Soluble TNFR2 coupled to Fc protion of IgG
Infliximab	Approved	Mouse-human chimeric anti-human TNF-α antibody
Adalimumab	Approved	Human anti-human TNF-α antibody
Peg-STNFR1	CT	Pegylated form of soluble TNFR1
CDP-870	CT	Pegylated humanized antibody CDP-571

Fab:-Fragment Antibody Binding, Fc:-Fragment Constant, IgG:-Immunoglobulin G, TNFR1:-TNF-Receptor 1, TNFR2:-TNF Receptor 2, CT:-Under Clinical Trial.

Protein-based injectable anti-TNF-α therapies are quite successful in clinic due to their high degree of specificity reduces possibility of unwanted side effects. But these therapies has major limitations of being expensive (9000-12000 $ /patient/year) and parenteral route of drug administration is less preferred by patients. Moreover incidences of immunogenicity were observed in some patients.

To evade these limitations of biological, search for small molecule based inhibitors that can block TNF-α is still under explorations as there is an unmet medical need for orally available, small-molecule based TNF-α inhibitors. In

general it is very challenging for medicinal chemists to discover highly safe and effective anti-inflammatory drugs for the treatment of inflammatory diseases with minimum side effects. To overcome the disadvantage of currently marketed drugs novel compound search is very much needed. The most suitable target for TNF-α regulation could be TACE inhibitors. TACE has been validated in preclinical models for the treatment of RA. Clinical trials have not demonstrated whether the TACE inhibitors exhibits efficacy and minimum toxicity profile for their usage in patients with RA. However, preclinical data suggest that TACE selective inhibitors could be used in the future as viable therapeutic approach for the treatment of chronic inflammation, such as RA and OA.

1.3. TNF-α Converting Enzyme (TACE)

Tumor necrosis factor alpha converting enzyme is a membrane-bound 85 kDa Zn endopeptidase and is a member of ADAM family, adamlysin/reprolysin subfamily contained within metzincin super family of MMPs [**116**-**117**]. TACE is also a first mammalian sheddase responsible for the cleavage of a variety of membrane anchored proteins including pro inflammatory cytokine (TNF-α), pro-transforming growth factor alpha (TGF-α), TNF receptors, β-amyloid precursor protein and L-selectin [**118**]. In inflammatory conditions, TACE is responsible for modifying membrane-bound pro TNF-α (mTNF-α) to release soluble TNF-α (sTNF-α).

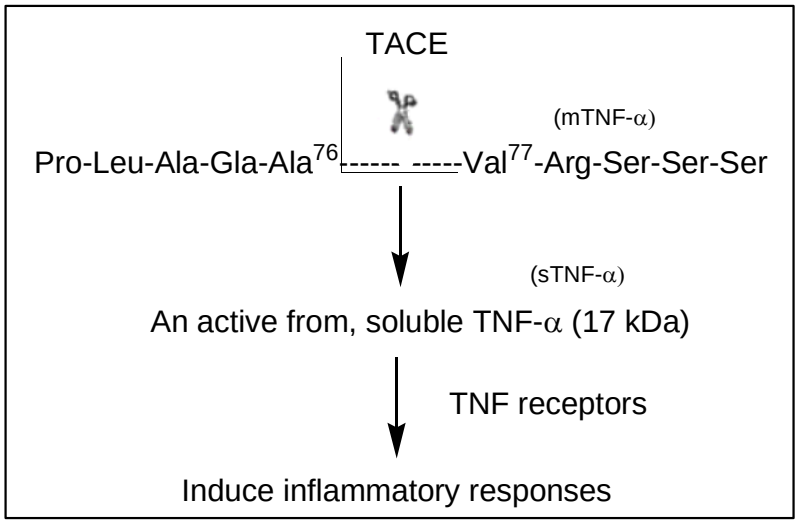


Figure 6. Proteolysis of pro TNF-α by TACE

As shown in **Figure 6**, proteolysis of Ala^{76}-Val^{77} peptide bond of 26 kDa membrane-bound pro TNF-α leads to mature and active form of cytokine (sTNF-α) being shed from the cell as homotrimer of 17 kDa terminal fragment consisting of 157 non glycosylated amino acids [**107**,**119**,**120**].

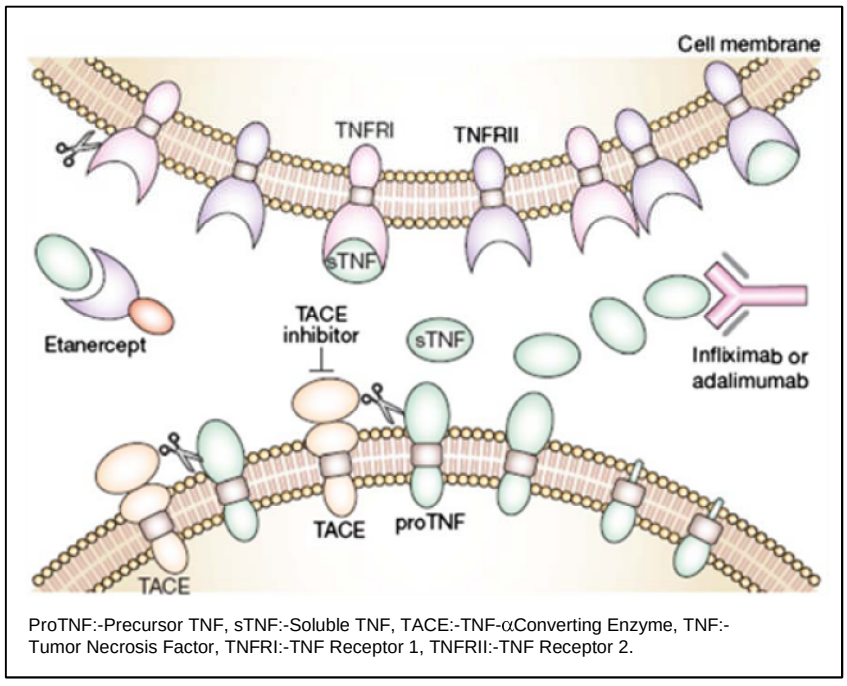


Figure 7. Mode of action of TACE inhibitors [**122**]

TACE selectively catalyzes this proteolysis and has got considerable attention as the sheddase responsible for controlling the amount of circulating TNF-α (**Figure 7**) [**121**]. Etanercept, a fusion protein of TNFR2 and Fc region of IgG1, anti TNF antibodies Infliximab and Adalimumab, bind to TNF-α and prevents interacts with its two receptors, TNFR1 and TNFR2, thereby stopping further signaling cascade.

1.3.1. Functions of TACE

The role of TACE in shedding TNF has been confirmed by in vitro experiments. Both, T-cells and monocytes derived from 'TACE $^{\Delta Zn/\Delta Zn}$' transgenic mice are deficient in releasing TNF [**107**]. Most of the 'TACE $^{\Delta Zn/\Delta Zn}$' deficient mice shows developmental defects, such as failure of eyelids to fuse and specific hair and skin defects observed in the embryos closely resembling the defects characteristics for the TGF-α deficiency. Moreover, it has been shown that the release of TGF-α from the cell membrane of 'TACE $^{\Delta Zn/\Delta Zn}$' cells is inhibited, which proves that TACE is responsible for the release of the active form of TGF-α [**123**,**124**].

1.3.2. Regulation of TACE

The regulation of TACE activity is poorly understood. It has been observed that the shedding rate of TACE increases within minutes of addition of cell activators like phorbol ester. Inhibitors of MAPK cascade blocks all increase in shedding rate in a number of cases, where TACE is primary by sheddase [**125**]. However, the mechanism of action of MAPK cascade is not clear. Interestingly, a

small protein, TIMP-3, that inhibits most MMPs also inhibits TACE, but whether TIMP-3 acts as a physiological regulator of TACE enzyme is unknown [**126**].

1.3.3. Structure of TACE

TACE contains pro-domain, catalytic domain, disintegrin and cysteine-rich region, transmembrane segment and cytoplasmic tail, **Figure 8**. TACE can be activated by nitrosation, alkylation and oxidation, resulting in the dissociation of cysteine thiol zinc linkage and thereby release of the pro domain inhibitory function [**127**].

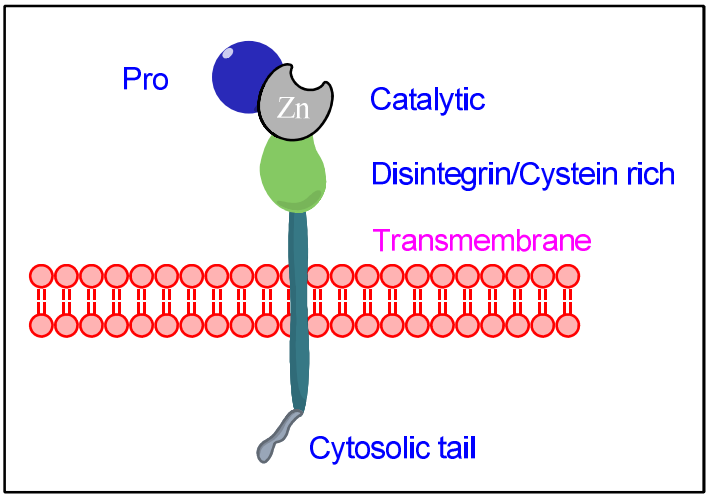


Figure 8. Domain structure of TACE

Pro domain removal mainly occurs by autocatalysis and action of furin or related pro-hormone convertases in Golgi compartment [**128**]. Pro domain catalysis is followed by catalytic domain (Asn^{223} to Val^{477}) and Disintegrin/Cysteine rich domain (Cys: Asp^{484} to Arg^{651}) cleavage, respectively. The N-terminal half of Disintegrin/Cysteine rich domain has a cysteine residue pattern resembling disintegrins of snake venoms [**129**]. To C-terminal of disintegrin module, epidermal growth factor like and crambin like motifs are

located. The role of disintegrin domain in TACE is not known but it may have an adhesion function as this domain has been shown to interact with integrins in some other ADAMs [**130**].

A single transmembrane domain (Ile^{672} to Val^{694}) defines the topology of TACE as a class-I membrane protein, with Pro catalytic and Cys domains. TACE ends in 146 residues cytoplasmic tail (Asp^{695} to Cys^{824}). Function of this tail is not defined but according to reported hypothesis it is involved in the control of TACE's intracellular trafficking. Most important, the catalytic domain of Zn metalloproteases possesses a conserved zinc ligating sequence HEbxHxbGbxHz (*b* indicates a bulky hydrophobic amino acid, *x* is variable amino acid and *z* is family specific amino acid) that forms an active site zinc, coordinately saturated by a water molecule that catalyzes the hydrolysis of amide bonds in protein substrates [**94**].

The cellular level of TACE enzyme needs to be tightly controlled because any alteration in its level may lead to various disease conditions. Therefore, TACE inhibitors can be used as potential therapeutic interventions in diseases where TNF-α is an inflammatory mediator [**122**]. TACE shares structural properties with other MMPs which make it tough for medicinal chemist to develop selective inhibitors thereby minimize side effect profile. If selectivity is achieved successfully, TACE inhibitors represents promising target for the treatment of various inflammatory diseases such as such as RA.

1.3.4. TACE and MMPs

Matrix metalloproteinase's (MMPs) are zinc-dependent endopeptidases. The MMPs belong to a larger family of proteases known as the metzincin superfamily. The upregulation of the MMPs or the down regulation of these endpeptidases affects critical physiological balance and have been associated with a number of pathological conditions, including OA and RA [**14**].

As the structure of catalytic site of TACE and MMPs are same and also both are zinc endopeptidases, some previously identified MMPs inhibitors were found to inhibit TACE as well, for example, **Marimastat**, **Prinomastat** and **CGS 27023A** (**Figure 9**). But these compounds failed in clinical trials as they showed dose-limiting musculoskeletal side effects [**131**-**133**]. Though the exact reason for this side effect is unknown, according to some researchers the efficacy of these molecules is due to their ability to inhibit TACE, but their ability to inhibit MMP-1 and/or MMP-14 could be the cause for their toxicity [**134**,**135**].

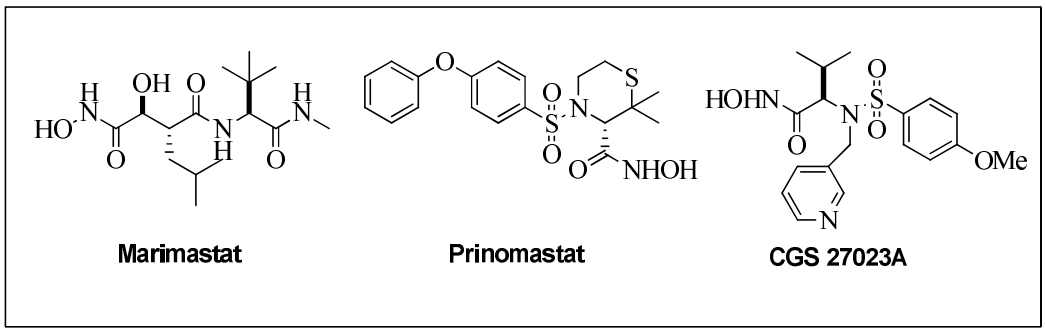


Figure 9. Structure of MMPs inhibitors

Hence, according to some researchers, it is desirable to develop selective TACE inhibitors devoid of any MMP activity [**136**,**137**]. Various research groups worldwide are engaged in developing a TACE inhibitor, which does not inhibit

MMPs [**138**-**140**]. For therapeutic use, selective inhibition of TACE over other MMPs is required. Selectivity over MMPs is essential because of distinct roles of the MMPs in various disease conditions and also to preserve normal physiological functions. Parallel inhibition of TACE and certain MMPs might occasionally be beneficial like inhibitors of MMP-9 are potentially valuable as inhibitors of tumor metastasis, while inhibition of MMP-13 may offer protection from cartilage degradation associated with osteoarthritis but inhibition of other MMPs is possible source of musculoskeletal side effects i.e. muscle degradation [**141**-**143**]. Therefore, molecules must have selectivity over MMPs. In general, achieving selectivity over MMPs is very tough because of shape similarity of binding sites of catalytic domains of TACE and MMPs, but to develop safe TACE inhibitors, MMP selectivity is essential [**144**-**145**].

1.3.5. TACE inhibitors under development

Most of the TACE inhibitors developed initially were nonspecific as they inhibit various MMPs along with TACE enzyme. GSK's GW3333 (**Compound A in Table 15**, **Figure 10**) represents first TACE inhibitors which inhibits TACE and MMPs (MMP1, MMP2, MMP3, MMP8, MMP9 and MMP13). It was under preclinical development as an orally active therapy for RA treatment [**138**-**140**]. GW3333 showed sufficient duration of action to chronically inhibit TACE and MMPs in rat. However GW333 exhibits poor oral bioavailability due to low solubility. Also because of non selective over other MMPs, it showed potential adverse events [**138**]. It exhibit potential to cause hepatotoxicity, therefore the clinical development of GW3333 was discontinued [**138**].

Table 15. Preclinical and clinical lead compounds as TACE inhibitors

Compd	Product	Company	Clinical status	MOA	Ref.
A	GW3333	GSK	Preclinical	TACE & MMP inhibitors	[**138**]
B	GW4459	GSK	Preclinical	TACE inhibitors	[**146**]
C	TMI-1	Wyeth	Preclinical	TACE & MMP inhibitors	[**147**]
D	TMI-005	Wyeth	Phase II & Discontinued	TACE & MMP inhibitors	[**148**]
E	IK-682	BMS	Preclinical	TACE inhibitors	[**120**,**150**]
F	BMS-561392 (DPC-333)	BMS	Phase II & Discontinued	TACE inhibitors	[**151**]
G	Duan et al.	BMS	Preclinical	TACE inhibitors	[**154**]
H	BMS-566394	BMS	Preclinical	TACE inhibitors	[**149**]
I	DPC-A38088	BMS	Preclinical	TACE inhibitors	[**152**]
J	DPH-067517	BMS	Preclinical	TACE inhibitors	[**153**]
K	W-3646	Wakanuga	Preclinical	TACE inhibitors	[**156**]
L	Bandarage et al.	Vertex	Preclinical	TACE inhibitors	[**157**]

BMS:-Bristol-Myers Squibb, GSK:-GlaxoSmithKline, MMP:-Matrix Metalloproteinase.

Further GSK optimized GW4459 (**Compound B in Table 15**, **Figure 10**) as potent (IC_{50} = 4.3 nM) TACE inhibitor with more than 50 fold selectivity over MMP1 and MMP3, along with improved oral bioavailability, compared to GW333. It was designed by introducing 5-methyl-thienylalanine and threonine side chains in GW4459 to get improved selectivity and oral bioavailability [**146**].

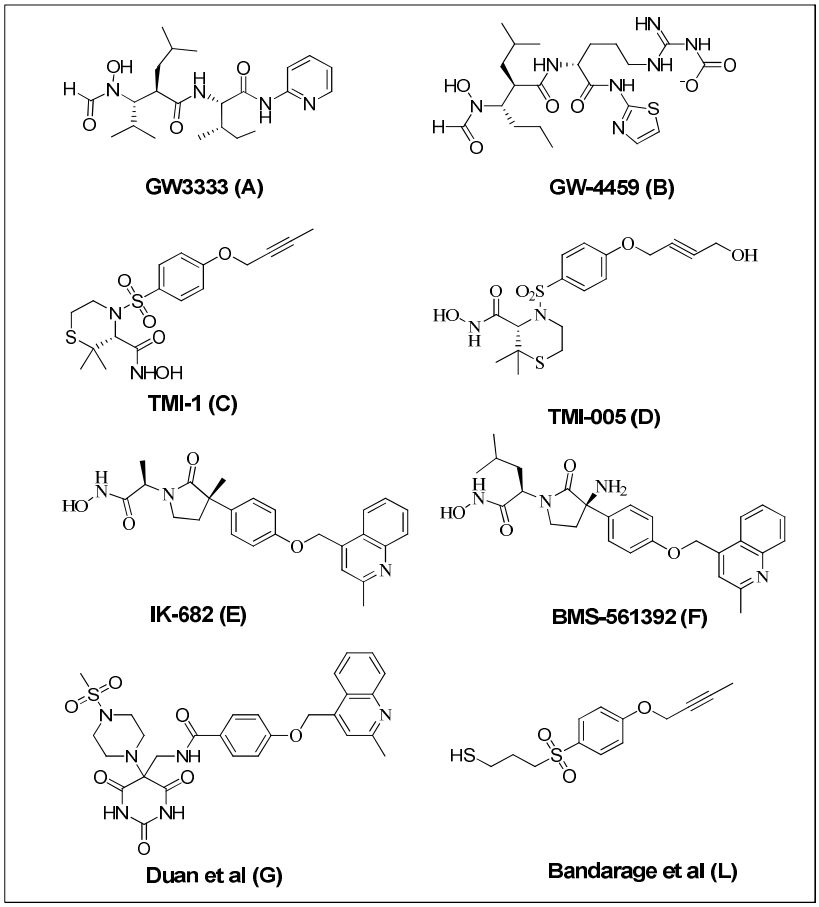


Figure 10. Structural representation TACE inhibitors

Wyeth developed orally active small-molecule based TACE inhibitors (TMI-1) for the treatment of RA. TMI-1 (**Compound C in Table 15**, **Figure 10**) is orally active small molecule based dual inhibitors of TACE and MMPs [**147**]. Several analogs of TMI-1 were prepared to understand the SAR as to get novel, potent, selective and orally bioavailable TACE inhibitors. But in clinical development, TMI-1 was discontinued because of lack of efficacy. Further, Wyeth developed partially selective TACE inhibitor, TMI‑005 (**Compound D in Table 15**, **Figure 10**).to advance into the clinic and to improve efficacy, compared to TMI-1. TMI-005 was designed by modifying side chains of TMI-005.

However, its clinical development was discontinued mainly due to lack of efficacy.

BMS-561392 (**Compound F in Table 15**, **Figure 10**) is an orally active, partially selective TACE inhibitor, developed by BMS [**151**]. It also inhibits MMP3, MMP12 and ADAMTS4 (ADAM with thrombospondin motifs). BMS-561392 is a γ-lactam based nonpeptide TACE inhibitor, mainly designed based upon IK-682 (**Compound E in Table 15**, **Figure 10**) [**53**]. It was the first partially selective TACE inhibitor to reach up to phase II clinical trials for the treatment of RA and was tested in the preclinical setting for IBD treatment. BMS-561392 showed IC_{50} of 2 nM. Despite the success of BMS in producing selective TACE inhibitors, with drug like properties, their program including the development of BMS-561392 has been halted because of concerns over mechanism based liver toxicity.

Recently BMS has synthesized series of structurally diverse TACE inhibitors as potential backup compounds to BMS-561392. At the 28th National Meeting of the American Chemical Society in 2004, BMS researchers announced the development of potent and selective TACE inhibitors from a series of β-benzamido hydroxamic acids [**152**]. The lead compound, DPC-A38088, (**Structure is not disclosed**, **Table 15**) demonstrated potent binding and good pharmacokinetic profile in rats and dogs. Another TACE inhibitor, DPH-067517, (**Structure is not disclosed**, **Table 15**) was under development by BMS for the treatment of cerebral ischemia [**153**]. Further BMS (Duan et al) has discovered a new series of TACE inhibitors as pyrimidine-2, 4, 6-trione derivatives (**Duan et al**, **Compound F in Table 15**, **Figure 10**).

Currently various pharma companies running TACE inhibitors program, such as those operated by Vertex (**Bandarage et al**, **compound L in Table 15**) thiol-based TACE inhibitors [**155**]. Bayer Schering Pharma has filed several patent applications with very broad claims that indicate their activity in this field (WO2005/121130, US2006/0178366, US2006/0252778, and US2007/0167426).

Other than selectivity issue, one of the major problem to develope small molecule based TACE inhibitors is that the low nM TACE inhibitory activity found in *in vitro* assay don't necessary translate in *in vivo* models. This discrepancy is thought to arise because inhibitors need to penetrate through or into the cell membrane to inhibit TACE.

1.4. Conclusion

Inflammation is a chronic and complex disease which involves multiple pathways. Both acute and chronic inflammations are major concerns with respect to the patient compliance. Acute is easy to treat while chronic is difficult to treat. Currently NSAID's and steroids are mainly in practice. The side effects and safety concern of NSAID's and steroids limits it's prolong usage. Therefore several new therapies are being developed among which TNF and TACE inhibitors are most promising approach for the effective treatment of acute and chronic inflammations. However to develop small molecule based TNF and TACE inhibitors, selectivity, loss of efficacy and achieving oral bioavailability are major limiting factors. To address these issues, in the next section, we have designed novel series of TNF and TACE inhibitors to develop next generation therapies for the treatment of various inflammatory conditions.

1.5. References

1. Ferrero-Miliani, Huang, M. T.; Ghai, g.; Ho, C. T. *Compr. Rev. Food Sci. Food Saf*. **2004**, *3*, 127-139.

2. Rocha e Silva M. *Agents Actions*.**1994**, *43*, 86–90.

3. Rus, H.; Cudrici, C.; Niculescu, F. *Immunol. Res.* **2005**, *33(2)*, 103-112.

4. Lotze, M. T.; Tracey, K. *J. Nat. Rev. Immunol.* **2005**, *5(4)*, 331–342.

5. Funk, C. D. *Science.* **2001**, *294*, 5548, 1871-1875.

6. Roman, R. J. *Physiol. Rev*. **2002**, *82*, 131-171.

7. Smith, W. L.; Marnett, L. J.; DeWitt, D. L. *Pharmacol. Ther*. **1991**, *49*, 153-179.

8. Smith, W. L. *Am. J. Physiol*. **1992**, *263*, 181-191.

9. Ford-Hutchinson, A. W.; Gresser, M.; Young, R. N. *Annu. Rev. Biochem*. **1994,** *63*, 383-417.

10. Imig, J. D. *Am J Physiol. Renal. Physiol*. **2000**, *279*(*6)*, 965-981.

11. Ziboh, V. A.; Cho, Y.; Mani, I.; Xi, S. *Arch. Pharm. Res*. **2002**, *25(6),* 747-758.

12. Carol, A.; Feghali, Ph. D.; Timothy, M.; Wright, M. D. *Front. Biosci.* **1997***, 2*, 12-26.

13. Papadakis, K. A.; Targan, S. R. *Annu. Rev. Med*. **2000**, *51*, 289-298.

14. Van Lint, P.; Libert, C. *J. Leukoc. Biol.* **2007**, *82(6)*, 1375–1381.

15. Han, J.; Lee, J. D.; Bibbs, L.; Ulevitch, R. J. *Science.* **1994**, *265,* 808–811.

16. Kate, L. K.; Marcela, R.; Luke, C. D.; Simon, A. J.; Philip, R. T. *Eur. J. Immunol*. **2011**, *41*, 2503-2508.

17. O'Byrne, K. J.; Dalgleish, A. G. *Br. J. Cancer*. **2001**, *85*, 473-483.

18. Beasley, R.; Clayton, T.; Crane, J.; von Mutius, E.; Lai, C.; Montefort, S.; Stewart, A. *The Lancet*. **2008***, 372*, 1039–1048.

19. Garcia Rodriguez L. A.; Hernandez-Diaz, S. *Arthritis. Res. Ther*. **2000**, *3(2)*, 98–101.

20. Sorensen, H. T.; Mellemkjaer, L.; Blot, W. J. *Am. J. Gastroenterol.* **2000**, *95(9)*, 2218–2224.

21. Solomon, D.; Avorn, J.; Stürmer, T.; Glynn, R.; Mogun, H.; Schneeweiss, S. *Arthritis Rheum.* **2006**, *54(5)*, 1378–1389.

22. Curhan, S. G.; Eavey, R.; Shargorodsky, J.; Curhan, G. C. *Am. J. Med.* **2010**, *12(3)*, 231–237.

23. Hart, F.; Boardman, P. *Br. Med. J.* **1963***, 2*, 965–970.

24. Ferreira, S.; Moncada, S.; Vane, J. *Nat. New Biol*. **1971**, *23(25)*, 237–239.

25. Sorbera, L. A.; Rabasseda, X.; Castaner, *J. Drugs Fut*. **1998**, *23(12),* 1287-1296.

26. Mukherjee, D, Nissen, S. E.; Topol, E. J. *J. Am. Med. Assoc.* **2001**, *286(8)*, 954-959.

27. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S. (2000). *J. Med. Chem.* **2000***, 43(5),* 775-777.

28. Singh, I.; Kumar, P.; Pahuja, S.; Tung, V.; Arora, S. *Acta. Pol. Pharm*. **2011**, *68(2)*, 279-284.

29. Czock, D.; Keller, F.; Rasche, F. M.; Haussler, U. *Clin. Pharmacokinet*. **2005**, *44(1),* 61-98.

30. Malipatil, B.; Ganesan, P.; Sundersingh, S.; Sagar, T. G. *Hematol. Oncol. Stem Cell Ther*. **2011**, *4(4)*, 157-160.

31. Arth, G. E.; Fried, J.; Johnston, D. B. R.; Hoff, D. R.; Sarett, L. H.; Silber, R. H.; Stoerk, H. C.; Winter, C. A. *J. Am. Chem. Soc.* **1958**, *80(12)*, 3161-3161.

32. Remy, C.; Barbaud, A.; Lebrun-Vignes, B.; Perrot, J. L.; Beyens, M. N.; Mounier, G.; Marsille, F.; Roy, M.; Mismetti, P.; Guy, C. *Ann. Dermatol. Venereol*. **2012**, *139(5)*, 350-354.

33. Tao, Y. M.; Li, Q. L.; Zhang, C. F.; Xu, X. J.; Chen, J.; Ju, Y. W.; Chi, Z. Q.; Long, Y. Q.; Liu, J. G. *Eur. J. Pharmacol.* **2008**, *584*, 306–311.

34. John P. D.; Edmund M. H.; Francesco, G.; Salituro, J. E.; Cochran, J. G.; Huai G.; Guy, W.; Bemis, G. E, Vincent, P.; Galullo, P. J.; Ford, U. A.; Germann, K. P.; Wilson, S. F.; Bellon, G. C., Paul, T.; Peter J.; Cassey, H.; S. Pazhanisamy, Yow-Ming W.; Mark, A. M.; Michael, S. S. *Med. Chem. Lett.*, **2011**, *2(10),* 758–763.

35. Adam, J. M.; Cairns, J.; Caulfield, W.; Cowley, P.; Cumming, I.; Easson, M.; Edwards, D.; Ferguson, M. *Med. Chem. Comm*. **2010**, *1*, 54-60.

36. Urbahns, k.; Yura, T.; Mogi, M.; Tajimi, M.; Fujishima, H.; Masuda, T.; Yoshida, N.; Moriwaki, T.; Lowinger, T. B.; Meier, H.; Chan, F.; Madge, D.; Gupta J. B. *Bioorg. Med. Chem. Lett*, **2011**, *21(11)*, *3354-3357.*

37. Soby, K. K, Mikkelsen, J. D.; Meier, E.; Thomsen, C. *Neuropharmacology*. **2002**, *43(1)*, 95-100.

38. Baumgart, D. C.; Carding, S. R. *The Lancet.* **2007**, 369, 1627–1640.

39. Baumgart, D. C.; Sandborn, W. J. *The Lancet.* **2007**, *369*, 1641–1657

40. Xavier, R. J.; Podolsky, D. K. *Nature.* **2007**, *448*, 427–434.

41. Cundiff, J.; Joe, S. *Amer. J. Otolaryngol*. **2007**, *28*, 28-30.

42. Cohen, P. R. *South. Med. J.* **2004**, *97(1)*, 70–73.

43. Deboyser, D.; Goethals, F.; Krack, G.; Roberfroid, M. *Toxicol. Appl. Pharmacol*. **1989**. *97(3)*, 473–479.

44. Jongen-Lavrencic, M.; Schneeberger.; Van, D. H. *Infection.* **2003**, *31(6)*: 428–429.

45. Dyke, H. J.; Montana, J. G. *Expert. Opin. Ther. Pat*. **1999**, *9*, 1101-1118.

46. Haudenschild, D.; Moseley, T.; Rose, L.; Reddi, A. H. *J. Biol. Chem.* **2002***,* 277(6), 4309–4316.

47. Byrnes, J. J.; Gross, S.; Ellard, C.; Connolly, K.; Donahue, S.; Picarella, D. *Inflamm. Res*. **2009***, 58(11)*, 819-827.

48. Distrutti, E.; Sediari, L.; Mencarelli, A.; Renga, B.; Orlandi, S.; Russo, G.; Caliendo, G.; Santagada, V.; Cirino, G.; Wallace, J. L.; Fiorucci, S. *J. Pharmacol. Exp. Ther*. **2006**, *319(1)*, 447-458.

49. Dahaghin, S.; Tehrain-Banishashemi, A.; Faezi, F.; T.; Davatchi, F. *Arth & Rheum*. **2009**, *61*, 1337-1342.

50. Davies-Tuck, M. L.; Wluka, A. E.; Wang, Y. *Osteoarthr Cartilage.* **2008**, *16(3)*, 337-342.

51. Raychaudhuri, A.; Kotyuk, B.; Pellas, T. C.; Pastor, G.; Fryer, L. R.; Morrissey, M.; Main, A. *J. Inflamm. Res*. **1995**, *44*, 141-142.

52. Inagaki, M.; Tsuri, T.; Jyoyama, H.; Ono, T.; Yamada, K.; Kobayashi, M.; Hori, Y.; Arimura, A.; Yasui, K.; Ohno, K.; Kakudo, S.; Koizumi, K.; Suzuki, R.; Kato, M.; Kawai, S.; Matsumoto, S. *J. Med. Chem.* **2000**, *43*(10), 2040–2048.

53. De, B.; Natchus, M. G.; Pikul, S.; Almstead, N. G.; Matthews, R. S.; Taiwo, Y. O.; Cheng, M. EP 0923563; EP 0925287; WO 9808825; WO 9808827.

54. Jialiang H.; Philippe, E. Van den Steen, Qing-Xiang, A. S.; Ghislain O. *Nat. Rev. Drug. Discovey*. ***2007****, 6*, 480-498.

55. Paola, C.; Stefania, M.; Alessandro, B.; Claudio, C.; Francesca, B.; Paolo, S.; Alessandro, L.; Angela, F.; Luigi, R.; Sandro, G.; Alessandro, G.; Laura, Q.; Carlo, A. M. *Eur. J. Pharmacol.* **2005**, *52, 7-16.*

56. Malagelada, J. R*. Int. J. Clin. Pract.* **2006***, 60(1)*, 57–63.

57. Cash, B. D.; Schoenfeld, P.; Chey, W. D. *Am. J. Gastroenterol.* **2002***, 9(11)*, 2812–2819.

58. Yang, C. M.; Li, Y. Q. *Zhonghua. Nei. Ke. Za. Zhi.* **2007***, 46(8)*, 641–643.

59. Stark, D.; van Hal, S.; Marriott, D.; Ellis, J.; Harkness, J. *Int. J. Parasitol.* **2007**, *37(1)*, 11–20.

60. Carlini, E. A. *Drug Alcohol Depend.* **1993***, 32(1)*, 1–7.

61. Otaka, M.; Jin, M.; Odashima, M. *Aliment. Pharmacol. Ther.* **2005**, *21*, 42–46

62. Firoozabadi, A.; Mowla, A.; Farashbandi, H.; Gorman, J. M. *J. Psychiatr. Pract*. **2007**, *13(4)*, 278-280.

63. Machelska, H.; Pflüger, M.; Weber, W.; Piranvisseh-Völk, M.; Daubert, J. D.; Dehaven, R.; Stein, C. *J. Pharmacol. Exp. Ther*. **1999**, *290(1)*, 354-361.

64. Barber, A.; Gottschlich, R. *Exp. Opin. Invest. Drugs.* **1997**, *6(10)*, 1351–1368.

65. Pappas, C.; Turnage, A.; Frazier, K. S.; Liu, Q.; Brown, P. *J Clin Oncol.* **2009**, *27*, 14555-14560.

66. Graeme, F. N. *Br. J. Pharmacol*. **2009**, *158(4)*, 982–993.

67. Maw, G. N. EP 0983251; JP 2000513024; WO 9852929.

68. Hicks, A.; McCafferty, G. P.; Riedel, E.; Aiyar, N.; Pullen, M.; Evans, C.; Luce, T.D.; Coatney, R. W.; Rivera, G. C.; Westfall, T. D.; Hieble, J. P. *J. Clin. Oncol.* **2007**, *323(1)*, 202-209.

69. Tamaoki, S.; Yamauchi, Y.; Nakano,Y.; Sakano, S.; Asagarasu, A.; Sato, M. *J. Pharmacol. Exp. Ther*. **2007**, *322*, 1315 – 1323.

70. Yawn, B. P. *Prim. CareResp. J.* **2008**, *17(3)*, 138–147.

71. Moore, W. C.; Pascual, R. M. *Am. J. Respir. Crit. Care Med.* **2010**, *181(11)*, 1181–1187.

72. Martinez, F. D. *Eur. Respi.r J.* **2007***, 29(1)*, 179–184.

73. Fanta, C. H. *N. Engl. J. Med*. **2009**, *360(10)*, 1002–1014.

74. Burggraaf, J.; Westendorp, R.; Veen, J.; Schoemaker, R.; Sterk, P.; Cohen, A.; Blauw. G. *Thorax.* **2001**. *56(7)*, 567–569.

75. Yoshikawa, H. *Acta. Neurol. Scan.* **2007***, 115*, 57–61.

76. Fanta, C. H. *N Engl. J. Med.* **2009***, 360(10),* 1002–1014.

77. Kerstjens, H. A.; Bantje, T. A.; Luursema, P. B. *Chest*. **2007**, *132(5)*, 1493–1499.

78. Jiwen, L. *Med. Chem. Lett.*, **2011**, *2(5),* 326–330.

79. Pettipher, R. *J. Pharmacol. Exp. Ther.* **2012***, 34,* 473-482*.*

80. Nakagami, Y.; Kawase, Y.; Yonekubo, K.; Nosaka, E.; Etori, M.; Takahashi, S.; Takagi, N.; Fukuda, T.; Kuribayashi, T.; Nara, F.; Yamashita, M. *Biol. Pharm. Bull*. **2010**, *33(6)*, 1067-1069.

81. Aoki, M. *J. Pharmacol. Exp. Ther.* **2000***, 295,* 255-260*.*

82. Susan, A.; Gregory, P. C.; Isakson, G. A. US: 6376528.

83. Navin, L. R. *J. Pharmacol. Exp. Ther.* **2007***, 321,* 1154-1160.

84. Eggebeen, A. T. *Am. Fam. Physician.* **2007***,* 76*(6)*, 801–808.

85. Bluestone, R.; Campion, D.; Klinenberg, J. R. *Arthritis. Rheum*. **1975**, *18*, 859-862.

86. Richette, P.; Ottaviani, S.; Bardin, T. *Presse Med*. **2011**, *40*, 844-849.

87. Okamoto, K.; Matsumoto, K.; Hille, R.; Eger, B. T.; Pai, E. F.; Nishino, T. *Proc. Natl. Acad. Sci. U.S.A*. **2004**, *101*, 7931-7936.

88. Majithia, V.; Geraci, S. A. *Am. J. Med.* **2007**, *120(11)*, 936–939.

89. Kremer, J. M.; Alarcón, G. S.; Lightfoot, R. W. Willkens, R. F.; Furst, D. E.; Williams, H. J.; Dent, P. B.; Weinblatt, M. E*. Arthritis Rheum*. **1994**, *37(3)*, 316-328.

90. Cronstein, B. N. *Pharmacol. Rev.* **2005***, 57(2)*, 163–172.

91. Cantarini, L.; Tinazzi, I.; Biasi, D.; Fioravanti, A.; Galeazzi, M. *Postgrad. Med. J. **2007**, 83(980)*, 1-2.

92. Dougados, M.; Emery, P.; Lemmel, E. M.; Zerbini, C. A.; Brin, S.; van, R. P. *Ann. rheum. dis.* **2005***,* 64*(1)*, 44–51.

93. Palladino, M. A.; Bahjat, F. R.; Theodorakis, E. A.; Lyle L.Moldawer, L. L. *Nat. Rev. Drug. Discovey*. **2003**, *2*, 736-746.

94. Pargellis, C. *Nat. Struct. Biol*. **2002**, *9*, 268-272.

95. Argade, A.; Bahekar, R.; Desai, J.; Thombare, P.; Kiran Shah, K.; Gite, S.; Sunder, R.; Ranvir, R.; Bandyopadhyay, D.; Chakrabarti, G.; Joharapurkar, A.; Mahapatra, J.; Chatterjee, A.; Patel, P.; Shaikh, M.; Sairam, K.; Jain, M.; Patel, P. *Med. Chem Comm*. **2011**, *9*, 966-972.

96. Raychaudhuri, A.; Kotyuk, B.; Pellas, T. C.; Pastor, G.; Fryer, L. R, Morrissey, M.; Main A. *J. Inflamm. Res*. **1995,** *44*, 141-142.

97. Kremer, J. M.; Bloom, B. J.; Breedveld, F. C.; Coombs, J. H.; Fletcher, M. P.; Gruben, D.; Krishnaswami, S.; Burgos-Vargas, R. N. *Arthritis. Rheum.* **2009***,* 60*(7)*, 1895–1905.

98. Braselmann, S; Taylor, V; Zhao, H; Wang, S; Sylvain, C; Baluom, M; Qu, K; Herlaar, E. *J. Pharmacol. Exp. Ther.* **2006***, 319(3)*, 998–1008.

99. Kriegler, M.; Perez, C.; DeFay, K.; Albert, I; Lu, S. D. *Cell.* **1988***, 53*, 45–53.

100. Hashimoto, Y. *Curr. Med. Chem*. **1998**, *5*, 163-178.

101. Alexopoulou, L.; Pasparakis, M.; Kollias, G. A. *Eur. J. Immunol.***1997,** *27*, 2588–2592.

102. Kawasaki, H.; Onuki, R.; Suyama, E.; Taira, K. *Nat. Biotechnol*. **2002**, *20(4)*, 376-380.

103. Locksley, R. M.; Heinzel, F. P.; Shepard, H. M.; Agosti, J.; Eessalu, T. E.; Aggarwal, B. B.; Harlan, J. M. *J. Immunol*. **1987**, *139(6)*, 1891-1895.

104. Carswell, E. A. *Proc. Natl. Acad. Sci. USA.***1975***, 72*, 3666–3670.

105. Evans, T. Moyes, D.; Carpenter, A.; Martin, R.; Loetscher, H.; Lesslauer, W.; Cohen, J. *J. Exp. Med*. **1994**, *180*, 2173-2179.

106. Ashkenazi, A.; Dixit, V. M. *Science*. **1998**, *281*, 1305–1308.

107. Black, R. A.; Rauch, C. T.; Kozlosky, C. J.; Peschon, J. J.; Slack, J. L.; Wolfson, M. F.; Castner, B. J.; Stocking, K. L.; Reddy, P.; Srinivasan, S.; N. Nelson, N.; Bolani, N.; Schooley, K. A.; Gerhart, M.; Devis, R.; Fitzner, J. N.; Johnson, R. S.; Paxton, R. J.; March, C. J.; Cerretti, D. P. *Nature.* **1997**, *385*, 729-732.

108. Grell, M.; Wajant, H.; Zimmermann, G.; Scheurich, P. *Proc. Natl. Acad. Sci. USA.* **1998***, 95*, 570–575.

109. Tartaglia, L. A.; Pennica, D.; Goeddel, D. V. *J. Biol. Chem.* **1993***, 268*, 18542–18548.

110. Grell, M.; Becke, F. M.; Wajant, H.; Mannel, D. N; Scheurich, P. *Eur. J. Immunol.* **1998***, 28*, 257–263.

111. Van Zee, K. J. *Proc. Natl. Acad. Sci. USA. **1992**, 89*, 4845–4849.

112. Di Giovine, F. S.; Nuki, G.; Duff, G. W. *Ann. Rheum. Dis.* **1998***, 47*, 768–772.

113. Buchan, G. *Clin. Exp. Immunol.***1998***, 73*, 449–455.

114. Saxne, T.; Palladino, M. A.; Jr.; Heinegard, D.; Talal, N.; Wollheim, F. A. *Arthritis. Rheum.* **1998***, 31*, 1041–1045.

115. Taylor, P. C. *Intern. Med.* **2003***, 42*, 15–20.

116. Duan, J. J. W.; Lu, Z.; Wasserman, Z. R.; Liu, R. Q.; Covington, M. B.; Decicco, C. P. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 2970–2973.

117. Cherney, R. C.; King, W. B.; Gilmore, J. L.; Liu, R. Q.; Covington, M. B.; Duan, J.J.W.; Decicco, C. P. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1028–1031.

118. Moss, M. L. *Nature.* **1997***, 385*, 733–736.

119. Moss, M. L.; Jin, S. L.; Becherer, J. D.; Bickett, D. M.; Burkhart, W.; Chen, W. J.; Hassler, D.; Leesnitzer, M. T.; McGeehan, G.; Milla, M. *J. Neuroimmunol*. **1997**, *72(2)*, 127-129.

120. Duan, J. J. W.; Chen, L.; Wasserman, Z. R.; Lu, Z.; Liu, R.; Covington, M. B.; Qian, M.; Hardman, K.; Magolda, R. L.; Newton, R. C.; Christ, D. D.; Wexler, R. R. and Decicco, C. P. *J. Med. Chem*. **2002**, *45*, 4954-4957.

121. Black, R. A. *Int. J. Biochem. Cell Biol*. **2002**, *34*, 1–5.

122. Moss, M. L.; Tavron, L. S.; Nedelman, R. *Nat. Clin. Prac. Rheum*. **2008**, *4*, 300-309.

123. Peschon, J. J.; Slack, J. L.; Reddy, P.; Stocking, K. L.; Sunnarborg, S. W.; Lee, D. C.; Russel, W. L.; Castner, B. J.; Johnson, R. S.; Fitzner, J. N.; Boyce, R. W.; Nelson, N.; Kozlosky, C. J.; Wolfson, M. W.; Rauch, C. T.; Cerretti, D. P.; Paxton, R. J.; March, C. J.; Balck, R. A. *Science*. **1998**, *282*, 1281-1284.

124. Black, R. A. *Int. J. Biochem. Cell Biol*. **2002**, *34*, 1-5.

125. Xu, K.; Zoukhri, D.; Zieske, J. D.; Dartt, D. A.; Sergheraert, C.; Loing, E.; Yu, F. X. *Am. J. Physiol. Cell. Physiol*. **2001**, *281*, 603-614.

126. Amour, A.; Slocombe, P. M.; Webster, A.; Butler, M.; Knight, C. G.; Smith, B. J.; Stephens, P. E.; Shelley, C.; Hutton, M.; Knauper, V.; Docherty, A. J. P.; Murphy, G. *FEBS. Lett*. **1998**, *435*, 39-44.

127. Zhang, Z.; Cork, J.; Ye, P.; Le, D.; Schwarzenberger, P.; Summer, W. R.; Shellito, J. E.; Nelson, S.; Kolls, J. K. *J. Leukoc. Biol*. **2000**, *67*, 856–862.

128. Marcia, L. M.; Jorg, W. B. *Biochemistry*. **2004**, *43*, 7228–7235.

129. Gould, R. J.; Polokoff, M. A.; Friedman, P. A.; Huang, T. F.; Holt, J. C.; Cook, J. J.; Niewiarowski, S. *Proc. Soc. Exp. Biol. Med*. **1990**, *195*, 168–171.

130. Schlondorff, J.; Blobel, C. P. *J. Cell. Sci*. **1999**, *112*, 3603–3617.

131. Brown, P. D. *Exp. Opin. Invest. Drugs*. **2000**, *9*, 2167-2177.

132. Shaw, T.; Nixon, J. S.; Bottomley, K. M. *Exp. Opin. Invest. Drugs.* **2000**, *9*, 1469-1478.

133. Elliot, S.; Cawston, T. *Drugs Aging*. **2001**, *18*, 87-99.

134. Aranapakam, V.; Davis, J. M.; Grosu, G. T.; Baker, J.; Ellingboe, J.; Zask, A.; Levin, J. I.; Sandanayaka, V. P.; Du, M.; Skotnicki, J. S.; DiJoseph, J. F.; Sung, A.; Sharr, M. A.; Killar, L. M.; Walter, T.; Jin, G.; Cowling, R.; Tillett, J.; Zhao, W.; McDevitt, J.; Xu, Z. B. *J. Med. Chem*. **2003**, *46*, 2376-2396.

135. Yocum, S. A.; L Lopresti-Monow, L.; Reeves, L. M.; Mitchell, P. G. In Inhibition of Matrix Metalloproteinnases: Therapeutic Applications; Greenwald, R. A., Zucker, S., Golub, L. M., Eds. *N.Y. Acad. Sci. Annals.* **1999**, *878*, 583–586.

136. Newton, R. C.; Solomon, K. A.; Covington, M. B.; Deccico, C. P.; Haley, P. J.; Friedeman, S. M.; Vaddi, K. *Ann. Rheum. Dis*. **2001**, *60*, 25- 32.

137. Kamei, N.; Tanaka, T.; Kawai, K.; Miyawaki, K.; Okuyama, A.; Murakami, Y.; Arakawa, Y.; Haino, M.; Harada, T.; Shimano, M. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 2897-900.

138. Conway, J. G.; Andrews, R. C.; Beaudet, B.; Bickett, D. M.; Bonecek, V.; Brodie, T. A.; Clark, R. L.; Crumrine, R. C.; Leenitzer, M. A.; McDougald, D. L.; Han, B.; Hedeen, K.; Lin, P.; Milla, M.; Moss, M.; Pink, H.; Rabinowitz, M. H.; Tippin, T.; Scates, P. W.; Selph, J.; Stimpson, S. A.; Warner, J.; Becherer, J. D. *J. Pharm. Exp. Ther*. **2001**, *298*, 900-908.

139. Robinson, R. P.; Laird, E. R.; Blake, J. F.; Bordner, J.; Donahue, K. M.; Lopresti-Morrow, L. L.; Mitchell, P. G.; Reese, M. R.; Reeves, L. M.; Stam, E. J.; Yocum, S. A. *J. Med. Chem*. **2000**, *43(12)*, 2293-2296.

140. Musso, D. L.; Anderson, M. W.; Andrews, R. C.; Austin, R.; Beaudet, E. J.; Becherer, J. D.; Bubacz, D. G.; Bickett, D. M.; Chan, J. H.; Conway, J. G.; Cowan, D. J.; Gaul, M. D.; Glennon, K. C.; Hedeen, K. M.; Lambert, M. H.; Leesnitzer, M. A.; McDougald, D. L.; Mitchell, J. L.; Moss, M. L.; Rabinowitz, M. H.; Rizzolio, M. C.; Schaller, L. T.; Stanford, J. B.; Tippin, T. K.; Warner, J. R.; Whitesell, L. G.;Wiethe, R. W. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2147-2151.

141. Close, D. R. *Ann. Rheum. Dis*. **2001**, *60*, 62–67.

142. Yip, D.; Ahmad, A.; Karapetis, C. S.; Hawkins, C. A.; Harper, P. G. *Invest. New. Drugs*. **1999**, *17*, 387–399.

143. Bottomley, K. M.; Johnson, W. H.; Walter, D. S. *J. Enzyme. Inhib*. **1998**, *13*, 79–101.

144. Zhao, Y.; Feng, W.; Yang, Y.; Ling, L.; Chen, R. *J. Huazhong. Univ. Sci. Technol. Med. Sci*. **2006**, *26*, 637-639.

145. Lukacova, V.; Zhang, Y.; Kroll, D. M.; Raha, S.; Comez, D.; Balaz, S. *J. Med. Chem*. **2005**, *48*, 2361-2370.

146. Rabinowitz, M. H. *J. Med. Chem.* **2001**, *44***,** 4252–4267.

147. Zhang, Y. *J. Pharmacol. Exp. Ther.* **2004***, 309*, 348–355.

148. *Thabet, M. M.; Huizinga, T. W. J. Curr. Opin. Invest. Drugs. **2006**, 7, 1014-1019.*

149. Car, B. Partnering with pharmacology to probe toxicology. Presented at Society of Toxicology 46th Annual Meeting. **2007**, March 25–29, Charlotte, N. C.

150. Horiuchi, K. *Dev. Biol*. **2005**, *283*, 459–471.

151. Grootveld, M.; McDermott, M. F. *Curr. Opin. Investig. Drug.* **2003***, 4,* 598–602.

152. Ott, G. R. Design, synthesis and evaluation of β-benzamido hydroxamic acid inhibitors of TNF-α converting enzyme (TACE). Presented at the 228 th.

153. Wang, X.; Li, X.; Schmidt, D. B.; Foley, J. J; Barone, F. C.; Ames, R. S.; Sarau, H. M. *Mol. Pharmacol.* **2004***, 65,* 890–896.

154. Duan, J. J. *Bioorg. Med. Chem. Lett.* **2007***, 17*, 266–271.

155. *Bandarage*, U. K.; Wang, T.; Come, J. H.; Perola, E.; Wei, Y.; Rao, B. G. *Bioorg. Med. Chem. Lett.* ***2008****, 18,* 44–48.

Chapter II: Designing strategy

CHAPTER II

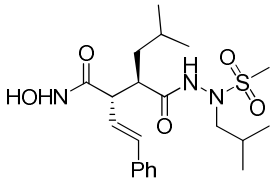
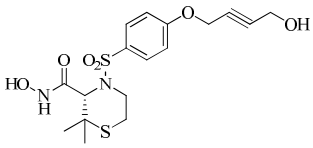
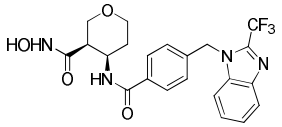
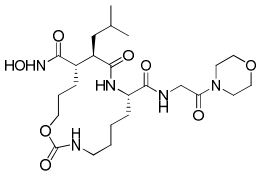
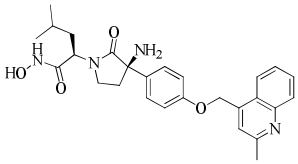
2. Designing strategy to develop TNF-α and TACE inhibitors

Brief overview on various TACE inhibitors under development

As discussed in the previous chapter, inhibitors of TNF-α and TACE appears to be the most promising therapy for the safe and effective treatment of acute and chronic inflammations. Knowing therapeutic potential of these inhibitors, we attempt to design novel series of molecules. In this section, we summarized designing of three novel series. First series: γ-Lactam hydroxamates, Second series: Thiadiazole-imidazolidinones derivatives as TACE inhibitors and Third series: Carboxy-phosphonates as novel TNF-α inhibitors.

Several attempts were made to develop selective and potent TACE and TNF-α inhibitors are shown in **Table 16**. The most advanced succinate-based TACE inhibitor, RO-32-7315, showed excellent *in vitro* potency against TACE (IC_{50} = 3 nM), but its clinical development was stopped due to poor bioavailability [**1**,**2**]. Sulfonamide hydroxamate derivatives, TMI-005 showed good *in vitro* activity (IC_{50} = 0.44 μM), however it was found to be inactive *in vivo* because of low oral bioavailability [**3**]. Although TMI-005 did not show any side effects in Phase I clinical trials, but it was withdrawn from the Phase II of clinical trials due to lack of efficacy. The β-Benzamido inhibitors showed excellent *in vitro* activity (IC_{50} = 1.4 nM) along with >10,000 fold selectivity for TACE over MMP-1, 2, 3, 7, 8, 9, 10, 12, 13, 14, 15 and 16. But like other TACE inhibitors, β-Benzamido inhibitors showed short half-lives (<5 hrs) [**4**,**5**].

Table 16. Classifications of hydroxamic acid based TACE inhibitors

Class	Structure/Name	Company	Clinical Status	Ref.
Succinate inhibitors	 (RO-32-7315)	Roche	Preclinical	[**1**,**2**]
Sulfonamide inhibitors	 (TMI-005)	Wyeth	Phase II	[**3**]
β-Benzamido inhibitors		BMS	Preclinical	[**4**,**5**]
Macrocyclic inhibitors	 (SP-057)	DuPont	Preclinical	[**6**]
γ-Lactam based inhibitors	 (BMS-561392)	BMS	Phase II & Discontinued	[**7**-**10**]

Macrocyclic inhibitors such as SP-057 showed dramatic improvement in *in vitro* (IC_{50} = 4 nM) potency. But it showed short half-lives and poor oral bioavailability in dogs [**6**].

Scientists at BMS designed and developed γ-lactam hydroxamates as selective TACE inhibitors [**7**]. In vitro BMS-561392 or DPC-333 showed excellent TACE inhibitory activity (IC_{50} = 0.20 nM), were found to be 100 fold selective for

TACE over MMPs. Upon oral administration to mice, BMS-561392 inhibited soluble TNF-α production following LPS challenge with an ED_{50} value of 6 mg/kg. This compound has shown good oral bioavailability (54%) in dogs and reasonable bioavailability in rats (16%). In Phase I clinical trials, it was observed that the compound was well tolerated in the healthy human volunteers at a dose range of 15–530 mg. It was also noted that the half-life of the molecule in humans was 3–6 h [**8**,**9**]. Though the BMS-561392 showed excellent potency and selectivity toward TACE over MMPs, it caused hepatotoxicity, hence it was withdrawn from Phase II clinical trials [**10**].

Thus among various TACE inhibitors under development, the two TACE inhibitors BMS-561392 and TMI-005 (**Table 16**) were advanced to Phase II clinical trials, but they were withdrawn because of poor bioavailability, hepatotoxicity and/or lack of efficacy [**10**,**11**]. To overcome drawbacks we focused on γ-lactam hydroxamates which showed some promising efficacy and oral bioavailability. **Figure 12** depicts the structure of BMS-561392 and its key interactions with the binding sites of TACE.

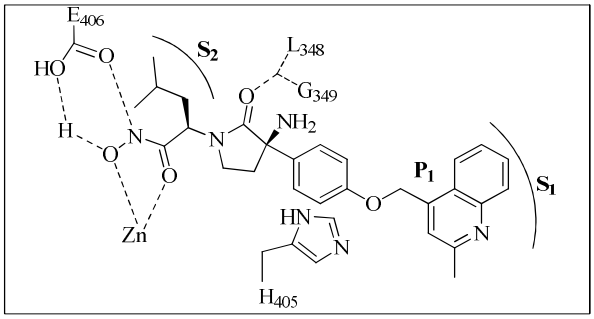


Figure 12. Pictorial representation of key interactions of BMS-561392 with corresponding binding sites of TACE

The docking studies and X-ray crystal structure of TACE co-crystallized with γ-lactam hydroxamates based TACE inhibitors revealed that the aromatic moiety (quinoline nucleus at P_1 region) occupies the S_1 site of the enzyme, oxygen atom of the pyrrolidinone ring forms hydrogen bonds with L_{348} and G_{349} and the isobutyl group occupy the small hydrophobic pocket (S_2). The hydroxamic acid interacts with the zinc atom present in the active site of TACE and coordinates with the side-chain carboxylate of E_{406}, while the phenyl ring exhibit an aromatic stacking with the imidazole side chain of H_{405} [**12**,**13**].

As the S_1 site of TACE is larger and bend-shaped, with respect to most of the MMPs and ADAM-10, substitution with bulky groups at P_1 region of TACE inhibitors (such as 2-methylquinolin-4-yl-methoxy group, in BMS-561392) known to exhibit higher potency as well as good selectivity towards TACE over MMPs [**13**,**14**]. The SAR studies of sulfonamide based TACE inhibitor scaffolds demonstrated that selectivity for TACE over MMPs and ADAM-10 were greatly enhanced due to alkynes group at P_1 position (such as 4-hydroxybutynyl group in TMI-005), which specifically get accommodated into the S_1 site of TACE, mainly due to the acquisition of its favorable bent confirmation [**15**-**17**]. Thus, the difference in the shape and size of the S_1 pocket of TACE over MMPs and ADAM-10 could be exploited to design potent and selective TACE inhibitors devoid of any MMPs and ADAM-10 activity [**18**].

2.1. γ-Lactam hydroxamate derivatives as TACE inhibitors (First series)

As a part of our ongoing research on TACE inhibitors and taking above structural features into consideration, in the present communication new series of *γ*-lactam hydroxamate based TACE inhibitors were designed mainly by introducing various substitution at 2nd position of quinoline nucleus to achieve high potency and good selectivity towards TACE over MMPs and ADAM-10.

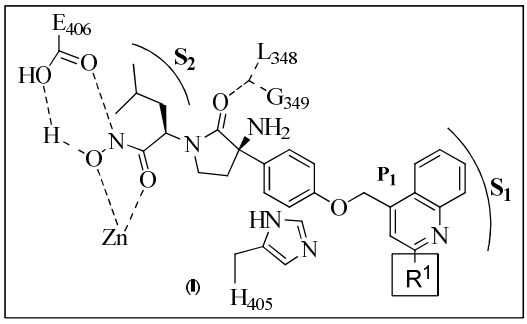


Figure 13. Pictorial representation of key interactions of modified BMS-561392 containing γ-lactam hydroxamate

The SAR studies of γ-lactam based novel TACE inhibitor scaffolds showed in **Figure 13** demonstrated that selectivity for TACE over MMPs and ADAM-10 were greatly enhanced due to various substitutions at 2nd position of quinoline nucleus at P_1 position which specifically get accommodated into the S_1 site of TACE, mainly due to the acquisition of its favorable bent confirmation [**15-17**]. Thus, the difference in the shape and size of the S_1 pocket of TACE over MMPs and ADAM-10 could be exploited to design potent and selective TACE inhibitors devoid of any MMPs and ADAM-10 activity [**18**].

Considering these facts, we decided to introduce various substitutions at 2nd position of quinoline nucleus. Altogether, we prepared four set of substitutions at 2nd position of quinoline nucleus like electron donating and electron

withdrawing, aliphatic alkyl chains, aromatic, and heterocyclic substituent's at R^1 position (**Figure 13**).

Thus in this series, all the favorable structural components of BMS-561392 essential for the key interactions with TACE were retained, except suitable changes were carried out at the 2nd position of quinoline ring to improve potency, selectivity and thereby avoid MMPs associated toxicity. In this series, we planned to prepare total twenty three compounds **15**, **16o**, **17** and **18a**-**t**, their synthetic schemes are explained in details in chemistry section 3.1.1.

2.2. Substituted thiadiazole-imidazolidinones derivatives as TACE inhibitors (Second series)

Earlier Dasgupta et al reported imidazolidinones (cyclic urea) derivatives (structure **II**) as bioisosters of amino pyrrolidine/γ-lactam ring of **BMS-561392** (structure **I**) and some of the test compound showed nM range TACE inhibitory activity shown in **Figure 14** [**19**-**21**].

Considering these facts and in search of novel scaffold, we further modified structure **II** by introducing thiadiazole ring system system as spacer between imidazole ring and phenoxy ring system (structure **III**). Thus in the second series, to understand the effect of ring size of six member aromatic ring and quinoline moiety, a substituted five-membered thiadiazole ring was incorporated.

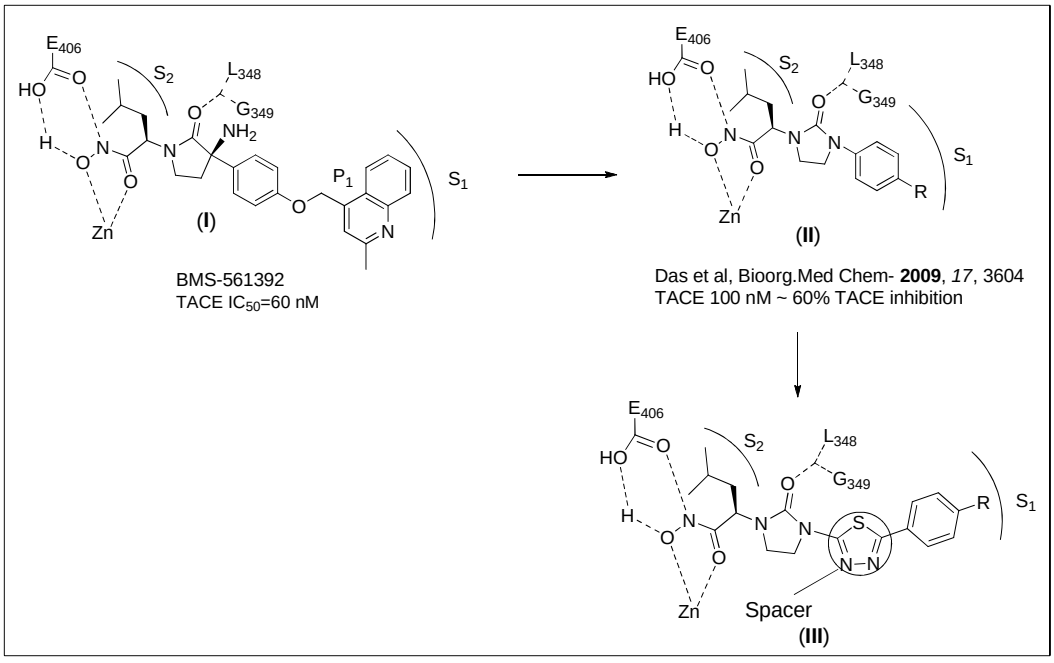


Figure 14. Design strategy of thiadiazole-imidazolidinones derivatives as TACE inhibitors

Preliminary docking studies were carried out using various hetrocycles and among various hetrocycles, thiadiazole derivatives showed favorable interactions for all TACE binding sites. Literature data also support that thiadiazole classes of compounds are known to exhibit excellent TACE inhibition activity [**22**]. Considering these facts and in continuation with our research efforts in TACE inhibition area, we envisioned thiadiazole ring as spacer between central imidazolidinone ring and phenyl ring of S_1 pocket as bioisosters replacement of phenoxy ring system of standard **BMS-561392** (structure **III**). In this series, we planned total nineteen compounds as **22g**, **23a-p**, **24** and **25**, their synthetic methodology and chemical characterization are explained in details in chemistry section 3.2.1.

2.3. Carboxy-phosphonoacetate derivatives as novel TNF-α inhibitors (Third series)

As described in the previous chapter, protein-based injectable anti-TNF-α therapies (Etanercept, Infliximab and Adalimumab) are quite successful in clinic due to their high degree of specificity which reduces possibility of unwanted side effects [**23**]. But these therapies has major limitations of being expensive (9000-12000 $ /patient/year) and parenteral route of drug administration is less preferred by patients. Moreover incidences of immunogenicity are seen with protein based anti-TNF-α therapies in some cases [**23**].

To evade these limitations of biologicals, search for small molecule based inhibitors that can block TNF-α is still continued. There is an unmet medical need for orally available, small-molecule based TNF-α inhibitors. It is very challenging for medicinal chemists to discover highly safe and effective anti-inflammatory drugs for the treatment of inflammatory diseases with minimal side effects. To overcome the disadvantages of currently marketed protein based anti-TNF-α drugs, novel compound search is very much needed for that reason in the third series, in search of small molecule based TNF-α inhibitors, we used the high throughput screening (HTS) technique for identification of safe and effective TNF-α inhibitors [**24**].

HTS method is use to discover suitable candidate compounds (primary hits) from large compound libraries [**24**]. These compounds are subsequently scored and ranked according to one or a consensus of multiple scoring functions.

It is a knowledge driven approach, ergo requires input structural information in the form of either the target site or existing ligands for the known target [**24**,**25**].

In other words, the HTS strategy was introduced mainly to increase the efficiency or hit rate of a broad screening project. However, the same strategy can also be applied into a focused screening project to increase the structural novelty of screening hits, as pointed out by Bajorath [**26**].

By applying a HTS approach, Johnson & Johnson (J&J) successfully identified a couple of novel scaffolds for JAK3 inhibition, with a quite high level of screening efficiency [**27**]. As a general strategy for chemical compound screening, this approach can be applied to any chemical databases, including virtual and nonproprietary databases. HTS greatly reduces potential acquisition and synthesis costs. It can be used in association with various computational and biological screening techniques with great flexibility, making it a practical tool for current hit to lead identification processes.

Using above HTS technique, we screened in-house compounds libraries (*ex vivo* TNF-α inhibitory activity), and compound **1** was identified as primary hits as TNF-α inhibitors [**28**]. We have a library of more than five hundred compounds of different series, general structures of which are shown in **Figure 15**.

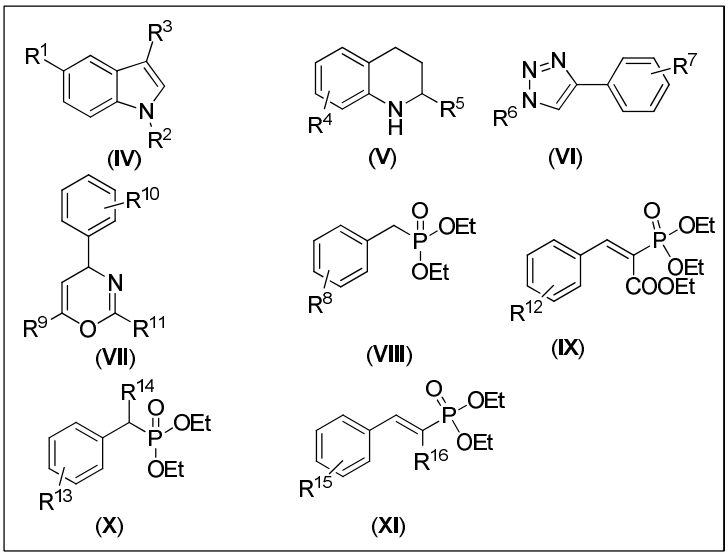


Figure 15. General structures of series used for high throughput screening (HTS)

From five series, more than five hundred compounds were screened *in vitro* for TNF-α inhibitory activity. In indole series (**IV**), we screened more than hundred compounds. In tetrahydro quinoline (**V**) series, 4-phenyl-1H-1,2,3-triazole series (**VI**) and 4-phenyl-4H-1,3-oxazine (**VII**) series, more than fifty compounds from each series were tested. In phosphate series (**VIII** to **XI**) we screened more than three hundred compounds. Among all compounds screened *in vitro*, only one molecule i.e (*E*)-ethyl 2-(diethoxyphosphoryl)-3-phenylacrylate (**1**) from carboxy-phosphate series (**XI**), the structure of which is shown in **Figure 16**, showed moderate TNF-α inhibitory activity (IC_{50} =. 987nM). Thus compound **1** was selected as primary hit for lead optimization in this series.

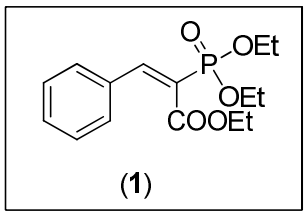


Figure 16. Initial hit from high throughput screening (HTS) approach

Further, we identify two regions for optimization in our initial hit molecule **1** (region 1 and region 2) as described in **Figure 17**.

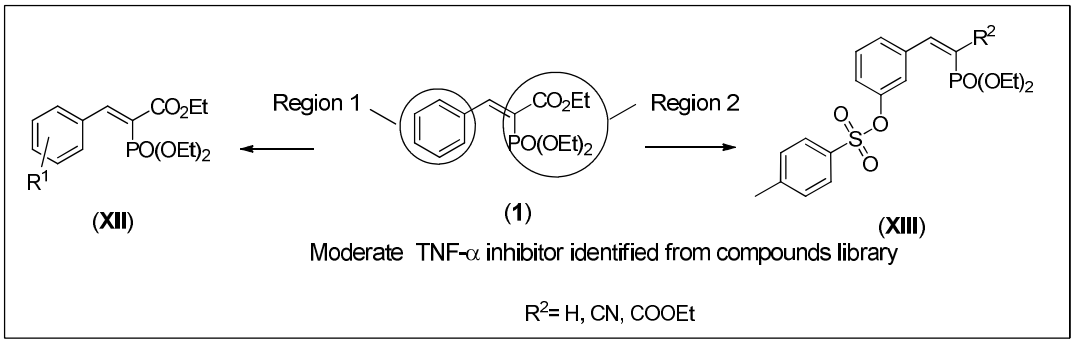


Figure 17. Further optimization of carboxy-phosphonoacetate based primary hit **1**

In the first set of compounds to optimize region 1, we substituted phenyl ring of compound **1** with selected electron donating groups (EDG), electron withdrawing groups and halo groups (**XII**).

In the next set of compounds, we explored SAR around region 2 by replacing phosponate group of compound **1** with other substitutes such as hydrogen, cyanide and carboxyl ester (**XIII**). In this series, we planned to prepare total twenty three compounds **1** and **27a**-**v**, their synthetic methodology and chemical characterization are explained in details in chemistry section 3.3.1.

2.4. Conclusion

In the present investigation, total three series (First series: γ-lactam hydroxamates, second series: thiadiazole-imidazolidinones derivatives as TACE inhibitors and Third series: carboxy-phosphonates as novel TNF-α inhibitors) were designed as potent TNF-α/TACE inhibitors for the safe and effective treatment of various inflammatory conditions. In first series, *γ*-lactam based derivatives were designed and total twenty three compounds were planned. In

the second series, thiadiazole-imidazolidinones based derivatives were designed and total nineteen compounds were planed. In the third series, carboxy-phosphonate based derivatives were designed and total twenty three compounds were planed, mainly as TNF-α inhibitors. Altogether sixty-five compounds were planed as TACE and TNF-α inhibitors. All the test compounds were synthesized and well characterized, subjected for *ex vivo*, *in vitro*, *in vivo*, PK and PD studies and results are summarized in the results and discussion section.

2.5. References

1. Watson, B. *IDrugs*. **2002**, *5*, 1151-1161.

2. Beck, G.; Bottomley, G.; Bradshaw, D.; Brewster, M.; Broadhurst, M.; Devos, R.; Hill, C.; Johnson, W.; Kim, H. J.; Kirtland, S.; Kneer, J.; Lad, N.; Mackenzie, R.; Martin, R.; Nixon, J.; Price, G.; Rodwell, A.; Rose, F.; Tang, J. P.; Walter, D. S.; Wilson, K.; Worth, E. *J. Pharmcol. Exp. Ther*. **2002**, *302*, 390-396.

3. Levin, J. I. *Curr. Topic Med. Chem*. **2004**, *4*, 1289-1310.

4. Kamei, N.; Tanaka, T.; Kawai, K.; Miyawaki, K.; Okuyama, A.; Murakami, Y.; Arakawa, Y.; Haino, M.; Harada, T.; Shimano, M. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 2897-2900.

5. Conway, J. G.; Andrews, R. C.; Beaudet, B.; Bickett, D. M.; Bonecek, V.; Brodie, T. A.; Clark, R. L.; Crumrine, R. C.; Leenitzer, M. A.; McDougald, D. L.; Han, B.; Hedeen, K.; Lin, P.; Milla, M.; Moss, M.; Pink, H.; Rabinowitz, M. H.; Tippin, T.;Scates, P. W.; Selph, J.; Stimpson, S. A.; Warner, J.; Becherer, J. D. *J. Pharm. Exp.Ther*. **2001**, *298*, 900-908.

6. Papadakis, K. A.; Targan, S. R. *Annu. Rev. Med*. **2000,** *51*, 289-298.

7. Peiretti, F.; Canault, M.; Beauclair, P. D.; Berthet, V.; Bonardo, B.; Vague, I. J.; Nalbone, G. *Exp. Cell Res*. **2003**, *285*, 278-285.

8. Grootveld, M.; McDermott, M. F. *Curr. Opin. Invest. Drugs*. **2003**, *4*, 598-602.

9. Qian, M.; Bai, S. A.; Brogdon, B.;Wu, J. T.; Liu, R. Q.; Covington, M. B.; Vaddi, K.;Newton, R. C.; Fossler, M. J.; Garner, E.; Deng, Y.; Maduskuies, T.; Trzaskos, J.; Duan, J. J. W.; Decicco, C. P.; Christ, D. D. *Drug Metab. Dispos*. **2007**, *35*, 1916-1925.

10. Moss, M. L.; Tavron, L. S.; Nedelman, R. *Nat. Clin. Prac. Rheum*. **2008**, *4,* 300-309.

11. Thabet, M. M.; Huizinga, T. W. J. *Curr. Opin. Invest. Drugs.* **2006**, *7*, 1014-1019.

12. Duan, J. J. W.; Chen, L.; Wasserman, Z. R.; Lu, Z.; Liu, R. Q.; Covington, M. B.; Qian, M.; Hardman, K. D.; Magolda, R. L.; Newton, R. C.; Christ, D. D.; Wexler, R. R.; Decicco, C. P. *J. Med. Chem*. **2002**, *45*, 4954-4957.

13. Niu, X.; Umland, S.; Ingram, R.; Beyer, B. M.; Liu, Y. H.; Sun, J.; Lundell, D.; Orth, P. *Arch. Biochem. Biophys*. **2006**, *451*, 43-50.

14. Lukacova, V.; Zhang, Y.; Kroll, D. M.; Raha, S.; Comez, D.; Balaz, S. *J. Med. Chem*. **2005**, *48*, 2361-2370.

15. Zhang, Y.; Hegen, M.; Xu, J.; Keith, J. C.; Jin, G.; Du, X.; Cummons, T.; Sheppard, B. J.; Sun, L. H.; Zhu, Y.; Rao, V. R.; Wang, Q.; Xu, W.; Cowling, R.; Nickerson-Nutter, C. L.; Gibbons, J.; Skotnicki, J.; Lin, L. L.; Levin, *J. Int. Immunopharmacol*. **2004**, *4*, 1845-1857.

16. Zhu, Z.; Mazzola, R.; Sinning, L.; McKittrick, B.; Niu, X.; Lundell, D.; Sun, J.; Orth, P.; Guo, Z.; Madison, V.; Ingram, R.; Beyer, B. M. *J. Med. Chem*. **2008**, *51*, 725-736.

17. Huang, A.; Joseph-McCarthy, D.; Lovering, F.; Sun, L.; Wang, W.; Xu, W.; Zhu, Y.; Cui, J.; Zhang, Y.; Levin, J. I. *Bioorg. Med. Chem*. **2007**, *15*, 6170–6181.

18. Wasserman, Z. R.; Duan, J. J. W.; Voss, M. E.; Xue, C. B.; Cherney, R. J.; D. J.; Hardman, K. D.; Decicco, C. P. *Chem. Biol*. **2003**, *10*, 215–223.

19. DasGupta, S.; Murumkar, P. R.; Giridhar, R.; Yadav. M. R. *Bioorg. Med. Chem.* **2009**, *17, 444*-459.

20. Duan, J. J. W.; Lu, Z.; Xue, C. B.; He, X.; Seng, J. L.; Roderick, J. J.; Wasserman, Z. R.; Liu, R. Q.; Covington, M. B.; Magolda, R. L.; Newton, R. C.; Trzaskos, J. M.; Decicco, C. P. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2035-2040.

21. DasGupta, S.; Murumkar, P. R.; Giridhar, R.; Yadav. M. R. *Bioorg. Med. Chem.* **2009**, *17, 3604*-3617.

22. Rabinowitz, M. H.; Andrews, R. C.; Becherer, J. D.; Bickett, D. M.; Bubacz, D. G.; Conway, J. G.; Cowan, D. J.; Gaul, M.; Glennon, K.; Lambert, M. H.; Leesnitzer, M. A. *J. Med. Chem*. **2001**, *44*, 4252-4267.

23. Palladino, M. A.; Bahjat, F. R.; Theodorakis, E. A.; Lyle L.Moldawer, L. L. *Nat. Rev. Drug Discovey*. **2003**, *2,* 736-746.

24. Fox, S.; Wang, H.; Sopchak, L.; Khoury, R. *J. Biomol. Screening.* **2001**, *6*, 137–140.

25. Walters, W. P.; Namchuk, M. *Nat. Rev. Drug Discovery.* **2003**, *2*, 259–266.

26. Bajorath, J. *Nat. Rev. Drug Discovery.* **2002**, *1*, 882–894.

27. Xin, C.; Lawrence, J.; Wilson, R. M.; Rochelle, L. A.; Shyh-Ming Y. *J. Med. Chem.* **2008**, *51(21),* 7015-7019.

28. Fox, S.; Wang, H.; Sopchak, L.; Khoury, R. *J. Biomol. Screening.* **2001**, *6*, 137–140.

Chapter III: Results and discussion

CHAPTER III

3. Results and discussion

As described in the previous chapter, we designed and synthesized three novel series of TACE and TNF-α inhibitors, the first series was *γ*-lactam based, second series was thiadiazole-imidazolidinones based derivatives and third series was carboxy-phosphonates based derivatives. All synthesized compounds were purified, characterized and subjected for *ex vitro* evaluation to establish SAR of individual series. Selected short listed compounds (most potent compounds) from each series were subjected for PK and PD studies.

In this section, we summarized result and discussion of:

a) γ-Lactam hydroxamates based TACE inhibitors (First series)

b) Thiadiazole-imidazolidinones derivatives as TACE inhibitors (Second series)

c) Carboxy-phosphonates as novel TNF-α inhibitors (Third series), in following sections:

1. Synthesis of three different series (Chemistry)
2. *Ex vivo* TNF-α inhibitory activity and its SAR
3. *In vitro* TACE inhibitory activity
4. Docking studies
5. *In vivo* evaluation of TACE and TNF-α inhibitors
6. PK studies of selected compounds from each series
7. Acute toxicity profile of short listed compounds from corresponding series

3.1. *γ*-Lactam hydroxamate based TACE inhibitors (First series)

3.1.1. Chemistry

For the preparation of titled compounds **15**, **16o**, **17** and **18a**-**t** (**Scheme 3**/**Table 17**), we need to first construct *γ*-lactam ring (**10**) and quinoline derivatives (**13a**-**t**).

3.1.1.1. Construction of γ-lactam ring (10)

In general contraction of γ-lactam ring is a challenge for organic synthesis. Several methods are reported in literature for the preparation of *γ*-lactam ring (**XV**, **Figure 18**). Camagna et al prepared γ–lactam ring, starting from **XVI** and after alkylation, pig liver esterase used for enzymatic resolution (**Method A**) to obtained chiraly pure amino γ-lactam ring and its enolate using base such as Lithium hexamethyldisilazane (LiHMDS) [**3**]. They used regent like ozone for preparation of aldehyde from olefin. Treatment of the aldehyde and D-amino acid with zinc, in acetic acid at elevated temperature lead to reductive amination and lactamization to give γ-lactam (**XV**) [**1**]. The disadvantage of this method was yield remain lower.

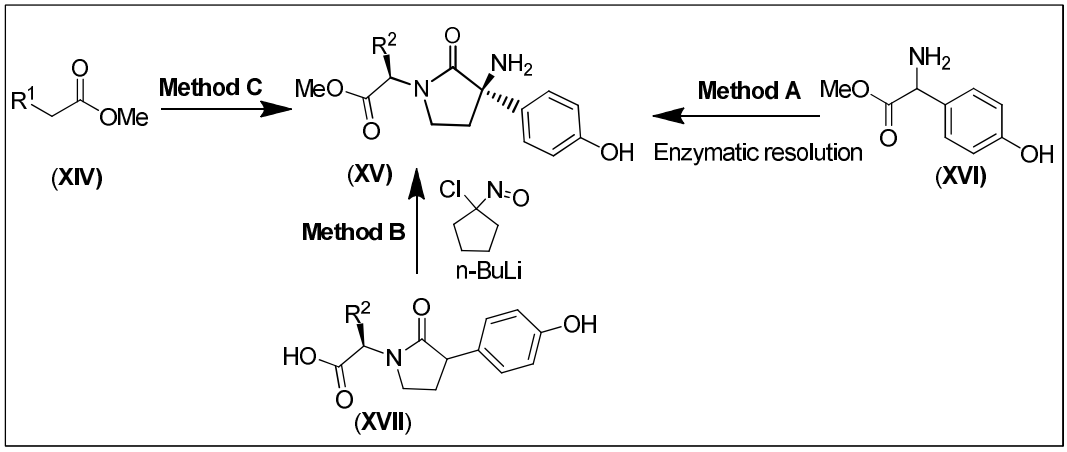
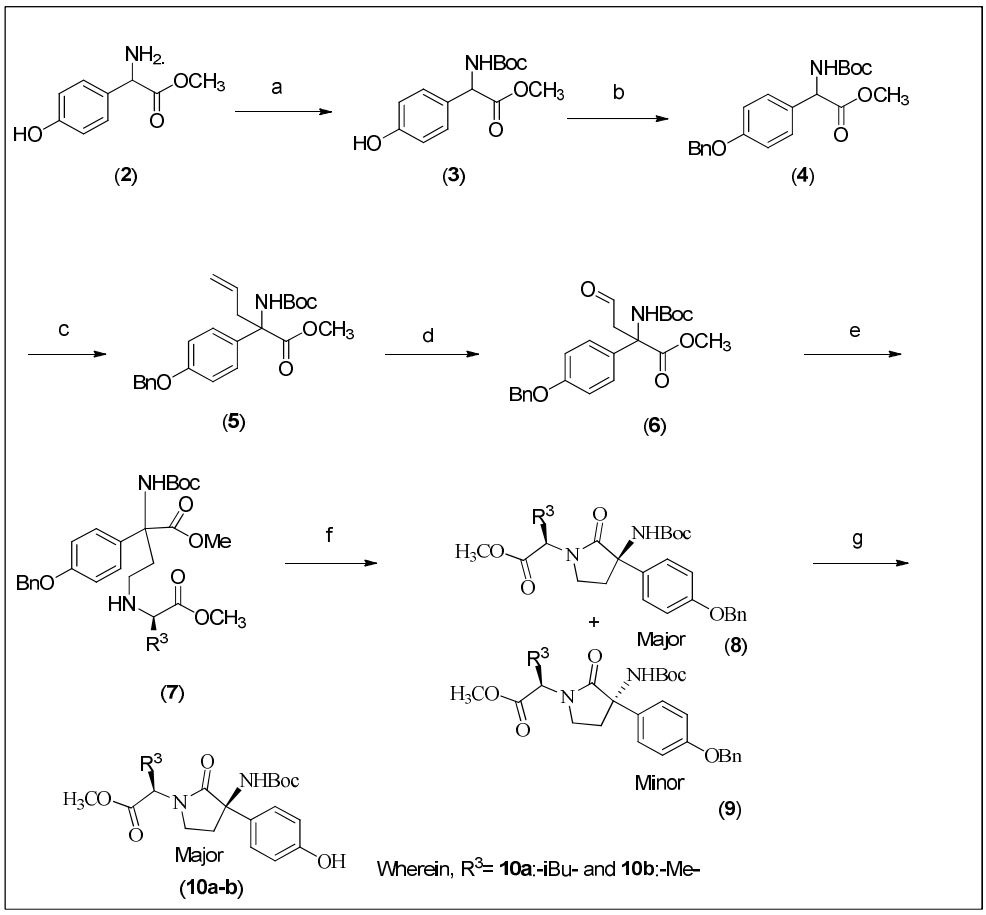


Figure 18. Different approaches for the synthesis of γ–lactam ring

In alternate method for the prepration of *γ*-lactam ring **(Method B**), Waltermire et al used chirally pure amino oxallary (**XVII**) and explosive regent like n-BuLi, which was major limitations of this method [**2**]. In another **Method C**, Duan et al used substituted methyl acetate (**XIV**) as starting material [**3**]. They used regent like ozone for the preparation of aldehyde from olefin. Treatment of the aldehyde and D-amino acid with zinc in acetic acid at elevated temperature leads to reductive amination and lactamization to give γ-lactam (**XV**). It gives mixture of two diastereomers epimerized at quaternary center. Limitation of this method was that they have not reported resolution protocol for distereomeric mixture.

Among various reported methods, we used Duan et al method (**Method A**) and we modified this method further by using Osmium tetraoxide (OSO_4) and Sodium per iodide ($NaIO_4$), instead of ozone for the preparation of aldehyde from olefin. This method was cost effective and was found to be easy for synthesis of title compound as shown in **Scheme 1**. We started construction of γ-lactam rings using commercial available **2** (methyl 2-amino-2-(4-hydroxyphenyl) acetate) as starting material. Compound **2** was treated with Boc anhydride ($(Boc)_2O$) to get *N*-Boc protected product **3**. Further compound **3** was treated with CS_2CO_3 and benzyl bromide to get benzylated product **4**, which upon treatment with allyl bromide in presence of LiHMDS lead to the formation of allylated product **5**. Oxidation of allylated product **5** was done by OSO_4, followed by treatment with $NaIO_4$ to get aldehyde **6**, which was further treated with Lucien or Alanine methyl ester and sodium triacetoxyborohydride ($NaBH(OAc)_3$) in dichloromethane

(CH_2Cl_2), to get the linear product **7**. Compound **7** was refluxed in toluene to get a mixture of disteromers, which were separated by flash column chromatography on a silica gel using 20% ethyl acetate in hexane as eluent, to get compounds **8** as desired major product and **9** as undesired minor product. Major product was debenzylated with Pd/C in ethanol to get phenol derivative (**10a**-**b**).



Reagents and conditions: a) $(Boc)_2O$, TEA, DMF; b) BnBr, CS_2CO_3, DMF; c) Allyl bromide, LiHMDS, THF; d) OSO_4, $NaIO_4$; e) Lucien or Alanine methyl ester, $NaBH(OAc)_3$, CH_2Cl_2; f) Toluene, reflux; g) Pd/C, $H_2(g)$, EtOH.

Scheme 1. Synthetic methods for the construction of γ-lactam rings (**10**)

3.1.1.2. Construction of quinoline derivatives (13a-t)

Several methods are reported for the synthesis of substituted quinoline derivatives (**Figure 19**). Aahray et al described synthesis of substituted quinoline

(**XIX**) using isatin (**XVIII**) as starting material and sodium hydroxide (NaOH) as base [**4**].

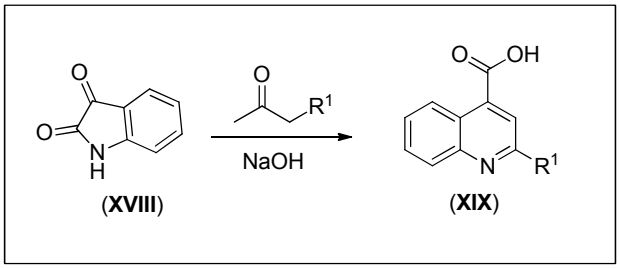
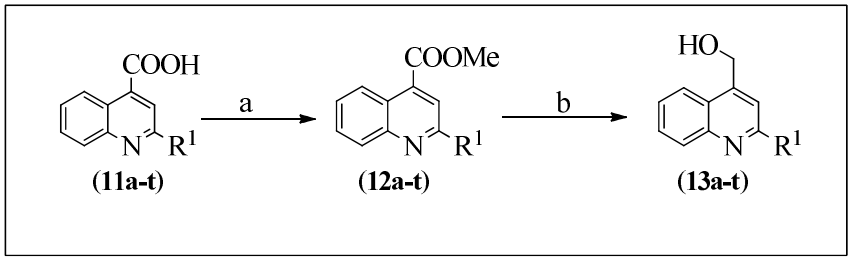


Figure 19. Literature reported method for the synthesis of quinoline derivatives

Lohray et al. and Priestley, N. D. et al also described methodology for the preparation of substituted quinoline derivatives [**5**,**6**]. But yield was limiting factor for these methods.

Among various reported methods, we used Duan et al method (**Method A**) and we modified this method further, using potassium hydroxide (KOH) as strong base and ethanol as a protic solvent for the preparation 2-substituted quinoline derivatives. This method was cost effective and easy for the synthesis of substituted quinoline derivatives.

As shown in **Scheme 2**, 2-substituted quinoline-4-yl-methanol **13a**-**t** was prepared from 2-substituted quinoline-4-carboxyl acids derivatives **11a**-**t**. These carboxyl acids derivatives were converted to corresponding methyl esters **12a**-**t** by treatment with thionyl chloride ($SOCl_2$) in CH_2Cl_2. The methyl esters **12a**-**t** was reduced to corresponding 2-substituted quinolin-4-yl-methanol **13a**-**t**, in the presence of $NaBH_4$.



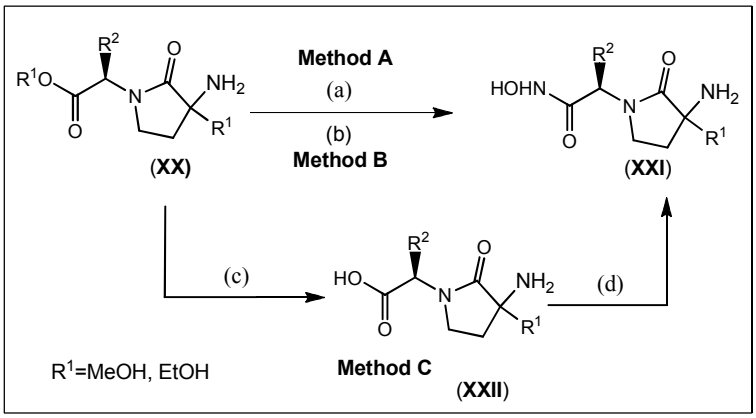
Reagents and conditions: a) $SOCl_2$, CH_2Cl_2, MeOH; b) $NaBH_4$, CH_2Cl_2. Wherein, (**13a-t**): R^1= Ph-; 3Me-Ph-; 4Me-Ph-; 4Cl-Ph-; 4F-Ph-; 4MeO-Ph-; OBzl-; Cl-; CF_3-; Et-; iPr-; Cpr-; Cbut-; MeO-; MeO-Me-; MeO-Et-; iPrO-Me- and iPrO-Et-; 2Me-Furan-.

Scheme 2. Synthetic methods for the preparation of substituted quinoline derivatives (**13a-t**)

3.1.1.3. Synthesis of titled compounds 15, 16o, 16 and 18a-t

Various synthetic routes are reported in the literature for the synthesis of hydroxamates derivatives (**Figure 20**). As depicted in **Method A**, the methyl ester of structure **XX** was converted to hydroxamic acid by treatment with NH_2OH under basic conditions (KOH or NaOMe in MeOH) [**7**]. The methyl ester can also be converted to benzyl protected hydroxamic acid with $BnONH_2$, using Weinreb's trimethylalluminium conditions (**XXI**) as described in **Method B** [**8**]. Poor yield and high cost are the limitations of **Method B**.

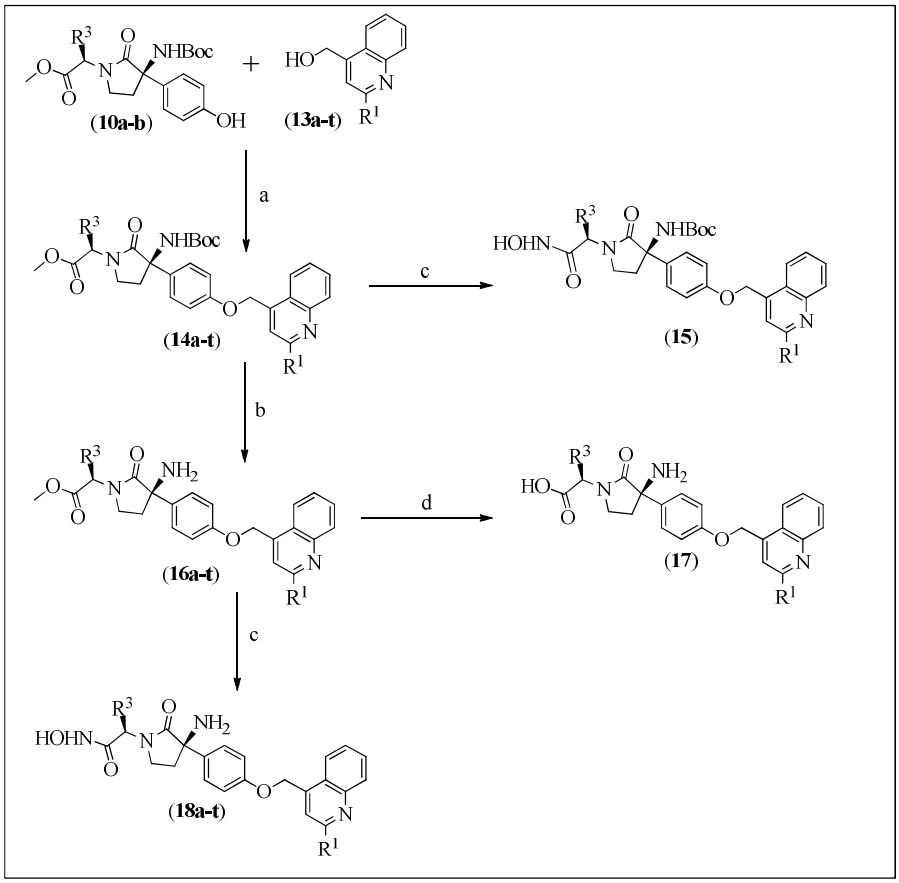
Alternatively, hydroxamic acid could be prepared through carboxylic intermediates (**XXII**) **Method C.** An intermediate **XXII** was converted in to hydroxamic acid (**XXI**) by coupling with $BnONH_2$ followed by deprotection of benzyl group. In **Method C** costly catalyst like palladium was used for deprotection of O-benzyl protecting group which attributes as limiting factors of these methods. Among all the reported methods, we followed **Method A** for the preparation of hydroxomic acid derivatives as it was easy and cost effective method.



Reagents and conditions: a) NH_2OH, KOH, MeOH; b) $BnONH_2$, $AlMe_3$, H_2, Pd $(OH)_2$/C; c) NaOH, MeOH; d) $BnONH_2$,BOP, H_2, Pd/C.

Figure 20. Different approaches for the synthesis of hydroxamic acid derivatives.

As shown in **Scheme 3**, synthesis of titled compounds **15**, **16o, 17** and **18a**-**t** were carried out by coupling phenol derivatives (**10a**-**b**) with **13a**-**t** (**Scheme 3**), under Mitsunobu condition (DEAD and TPP) to get the key intermediates **14a**-**t**. The ester group of compounds **14o** was converted into corresponding hydroxamic acid derivative **15**, using hydroxyl ammonium chloride ($NH_2OH.HCl$). Deprotection of Boc group of compounds **14a**-**t** was carried out by TFA, which lead to the formation of compounds **16a**-**t**. The hydrolysis of compound **16o** with NaOH yields compound **17**, while treatment of compound **16a**-**s**, with $NH_2OH.HCl$ in presence of base NaOH gives corresponding hydroxamic acid derivatives **18a**-**t**.



Reagents and conditions: a) DEAD, TPP, Toluene; b) TFA, CH_2Cl_2; c) $NH_2OH.HCl$, NaOH; d) NaOH, MeOH. Wherein, (**10a-b**): R^3= iBu- and Me-; (**14**, **16** & **18 a-t**): R^1= Ph-; 3Me-Ph-; 4Me-Ph-; 4Cl-Ph-; 4F-Ph-; 4MeO-Ph-; OBzl-; Cl-; CF_3-; Et-; iPr-; Cpr-; Cbut-; MeO-; MeO-Me-; MeO-Et-; iPrO-Me-; iPrO-Et-; 2Me-Furan- and R^3= iBu-; (**14**, **16,** & **18s**): R^1= MeO-Me- and R^3=Me-; (**15** & **17**): R^1= MeO-Me- and R^3= iBu-.

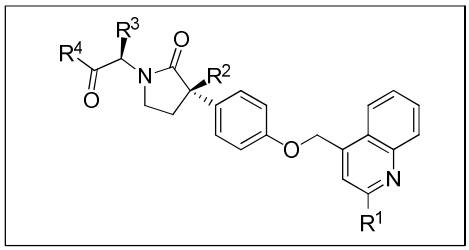
Scheme 3. Synthetic methods for the preparation of title compounds (**15**, **16o**, **17** and **18a**-**t**)

Using above synthetic routes (**Scheme 1**, **2** and **3**), altogether 23 titled compounds (**15**, **16o**, **17** and **18a**-**t**) were prepared. All the final compounds, intermediates were purified, characterized and spectral data of compounds were found to be in conformed with the structure assigned. Detail experimental procedures and chemical characterization data are described in experimental section 5.1 and the representative spectra of selected compounds are given in section 6.

3.1.2. *Ex vivo* TNF-α inhibitory activity and structure activity relationship (SAR)

Ex vivo TNF-α inhibitory activity (using human whole blood assay) was assessed (detail experimental protocol is given in experimental section 5.3), mainly to establish the SAR of new series of *γ*-lactam hydroxamate based TACE inhibitors **15**, **16o, 17** and **18a**-**t** [**9**]. As depicted in **Table 17**, depending upon the nature of substitutions, all the titled compounds showed different degree of TNF-α inhibition (IC_{50}).

Table 17. *Ex vivo* TNF-α inhibitory activity (IC_{50}) of test compounds (**15**, **16o**, **17** and **18a**-**t**)[a]



Comp. No	R^1	R^2	R^3	R^4	IC_{50} (nM)[b]
15	MeO-Me-	-NHBoc	iBu-	-NHOH	3050
16o	MeO-Me-	$-NH_2$	iBu-	-COOMe	3000
17	MeO-Me-	$-NH_2$	iBu-	-COOH	3230
18a	Ph-	$-NH_2$	iBu	-NHOH	3057
18b	3Me-Ph-	$-NH_2$	iBu	-NHOH	3876
18c	4Me-Ph-	$-NH_2$	iBu	-NHOH	2899
18d	4Cl-Ph-	$-NH_2$	iBu	-NHOH	286
18e	4F-Ph-	$-NH_2$	iBu	-NHOH	290
18f	4MeO-Ph-	$-NH_2$	iBu	-NHOH	250
18g	Obzl-	$-NH_2$	iBu	-NHOH	4000
18h	Cl-	$-NH_2$	iBu	-NHOH	450
18i	CF_3-	$-NH_2$	iBu	-NHOH	470

Comp. No	R^1	R^2	R^3	R^4	IC_{50} (nM)[b]
18j	Et-	$-NH_2$	iBu	-NHOH	111
18k	iPr-	$-NH_2$	iBu	-NHOH	118
18l	Cpr-	$-NH_2$	iBu	-NHOH	170
18m	Cbut-	$-NH_2$	iBu	-NHOH	177
18n	MeO-	$-NH_2$	iBu	-NHOH	401
18o	MeO-Me-	$-NH_2$	iBu	-NHOH	11
18p	MeO-Et-	$-NH_2$	iBu	-NHOH	13
18q	iPrO-Me-	$-NH_2$	iBu	-NHOH	123
18r	iPrO-Et-	$-NH_2$	iBu	-NHOH	98
18s	MeO-Me-	$-NH_2$	Me-	-NHOH	90
18t	2Me-Furan-	$-NH_2$	iBu-	-NHOH	805
BMS-561392					60

[a]*Ex vivo* TNF-α inhibition was carried out in human whole blood and plasma TNF-α concentration determined using ELISA kit; [b]IC_{50} values (nM) are from single determination and standard compound **BMS-561392** was used as positive std. control.

Substitution with bulky and aromatic groups at 2nd position of quinoline ring in **BMS-561392**, such as phenyl- (**18a**), 3-methyl phenyl- (**18b**), OBzl- (**18g**) and 2Me-Furan- (**18t**) analogs showed weak *ex vivo* TNF-α inhibition. Test compounds substituted with electron withdrawing groups at *para*-position of aromatic ring system, such as 4-chloro phenyl (**18d**), 4-fluro phenyl (**18e**) and 4-methoxy phenyl (**18f**) showed moderate TNF-α inhibition, while substitution with electron donating groups, such as 4-methyl phenyl (**18c**) showed weak TNF-α inhibition. Similarly, direct substitution of electron withdrawing groups such as Cl- (**18h**) and CF_3- (**18i**) at 2nd position of quinoline ring showed moderate TNF-α inhibition, while substitution with electron donating groups, such as ethyl- (**18j**), isopropyl- (**18k**), cyclopropyl- (**18l**) and cyclohexyl- (**18m**) showed good TNF-α inhibition, whereas, substitution with strong electron donating group, such as

MeO- (**18n**) showed moderate TNF-α inhibition.

Introduction of methoxy-methyl- (**18o**) and methoxy-ethyl- (**18p**) groups at 2nd position of quinoline ring exhibited extremely potent TNF-α inhibition than standard compound **BMS-561392** (IC_{50}: 60 nM), with IC_{50} values of 11 and 23 nM respectively, while isopropoxy-methyl- (**18q**) and isopropoxy-ethyl- (**18r**) showed relatively less potency than methyl analogs of BMS-561392. Interestingly, substitution of R_3- (isobutyl group) with methyl group (**18s**) showed less potency than (**18o**), while replacement of R_2- (amino group) with –NHBoc and substitution of R_4- (hydroxamic acid group) with either methyl-ester (**16o**) or carboxylic acid (**17**) resulted in complete loss of TNF-α inhibition activity, indicated that free amino group at R_2 position and hydroxamic acid at R_4 position is mandatory for TNF-α inhibition.

In general, compounds with electron donating groups showed good TNF-α inhibition, electron withdrawing substitutions showed moderate TNF-α inhibition, while bulky and aromatic substitutions showed weak TNF-α inhibition. Although, the S_1 binding pocket of TACE is larger, but the substitution with aromatic and bulky group was found to be unfavorable. On the contrary, substitution with methoxy-methyl- (**18o**) and methoxy-ethyl- (**18p**) groups at 2nd position showed excellent TNF-α inhibition, while isopropoxy-methyl- (**18q**) and isopropoxy-ethyl- (**18r**) showed relatively less potency than methyl analogs of **BMS-561392**, which could be due to the favorable bent confirmation acquisition at S_1 binding pocket [**9**]. Overall, *ex vivo* TNF-α inhibition results reveals that the potency of γ-lactam hydroxamate based TACE inhibitors can be modulated using suitable

substituents at 2nd position of quinoline ring system.

From above SAR study, out of twenty three compounds, short listed compounds **18o** and **18p** with methoxy-methyl- and methoxy-ethyl- groups at respectively 2nd position showed excellent TNF-α inhibition. These two compounds were subjected for their *in vitro* TACE inhibitory activity and selectivity.

3.1.3. *In vitro* TACE inhibitory activity and selectivity of 18o and 18p

The *in vitro* TACE inhibitory activity and selectivity over MMPs (MMP-1,-2,-3,-7,-8,-9,-13 and 14) and ADAM-10 were evaluated for most potent compounds **18o** and **18p**, using fluorescence-based FRET assay [**10**,**11**] and IC_{50} values (**Table 18**) were determined (detail experimental protocol is given in experimental **Section 5.3**). As shown in **Table 18**, both the test compounds **18o** and **18p** inhibits the recombinant TACE with IC_{50} of 2.1 and 2.3 nM respectively, compared to standard compound **BMS-561392** (IC_{50}: 12 nM).

Compounds **18o** and **18p** showed >500-fold selectivity (IC_{50}: >1000 nM) over all the tested MMPs and ADAM-10, whereas standard compound **BMS-561392** showed variable selectivity (IC_{50}: < 500 nM) over tested MMPs and it showed only 40-fold selectivity over ADAM-10.

Table 18. *In vitro* TACE inhibitory activity and selectivity over MMPs and ADAM-10 for selected test compounds (**18o**-**p** and **BMS-561392**)[a]

Enzyme[#]	BMS-561392 (std)	Compounds	
		18o	18p
TACE	12	2.1	2.3
ADAM-10	480	1211	1199
MMP-1	429	1132	1059
MMP-2	482	1167	1088
MMP-3	285	1082	1020
MMP-7	466	1242	1008
MMP-8	299	1010	1002
MMP-9	389	1156	1210
MMP-13	461	1230	1114
MMP-14	499	1067	1085

[a]*In vitro* TACE inhibitory activity and selectivity over MMPs (MMP-1,-2,-3,-7,-8,-9,-13 and 14) and ADAM-10 were evaluated for selected test compounds (**18o**, **18p)** and BMS-561392, using fluorescence-based FRET assay and IC_{50} values (nM) were determined (n=3).

Together, these *in vitro* results demonstrated improved potency and selective profile of our test compounds **18o** and **18p** over standard compound **BMS-561392**, which could be attributed mainly due to suitable substituents at 2nd position of quinoline ring system.

3.1.4. Docking studies of selected compounds

Docking study of most potent compound **18p** was carried out to understand its potency and selectivity for TACE over other MMPs, especially ADAM-10, which was not observed *in vitro* for standard compound **BMS-561392**. Docking study was carried out using Glide (version 50207) software [**12**].

As shown in **Figure 21** and **22**, the aromatic moieties (quinoline) of **18p** and **BMS-561392** occupies the S_1 site of the enzyme pocket, oxygen atom of the γ-lactam ring forms hydrogen bonds with L_{348} and G_{349}, the hydroxamic acid

interacts with the zinc atom present in the active site of TACE and coordinates with the side-chain carboxylate of E_{406}. All the five binding poses obtained for **18p** were found to be close, with an RMSD of < 0.8 Å and there were no unusual conformations. The ligand orientation and H-bonding interactions of **18p** matches closely with **BMS-561392**. These observations obtained from docking studies conformed *in vitro* potency of **18p**.

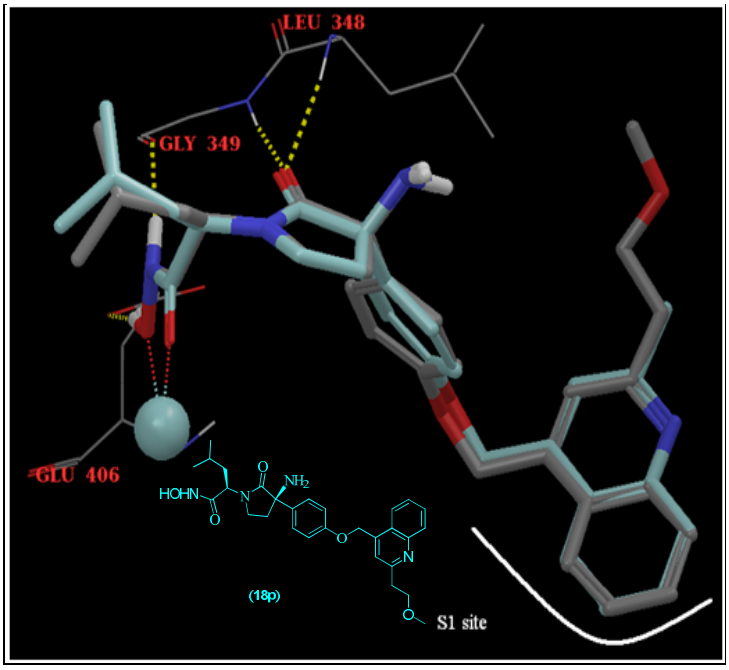


Figure 21. H-bond interactions of **18p** (elemental colour) and **BMS-561392** (turquoise colour)

The S_1 site of TACE is larger and bend-shaped, with respect to most of the MMPs, especially ADAM-10. Thus suitable substitutions on aromatic moiety are known to exhibit higher potency as well as good selectivity towards TACE over MMPs [**13**-**15**]. As shown in **Figure 22**, the methoxy-ethyl group on 2nd position of quinoline ring **18p** protrude from a groove (termed as alkoxyalkyl pocket), which was not observed with **BMS-561392**. Probably this could be the

reasons why compound **18p** was found to be more potent than **BMS-561392**.

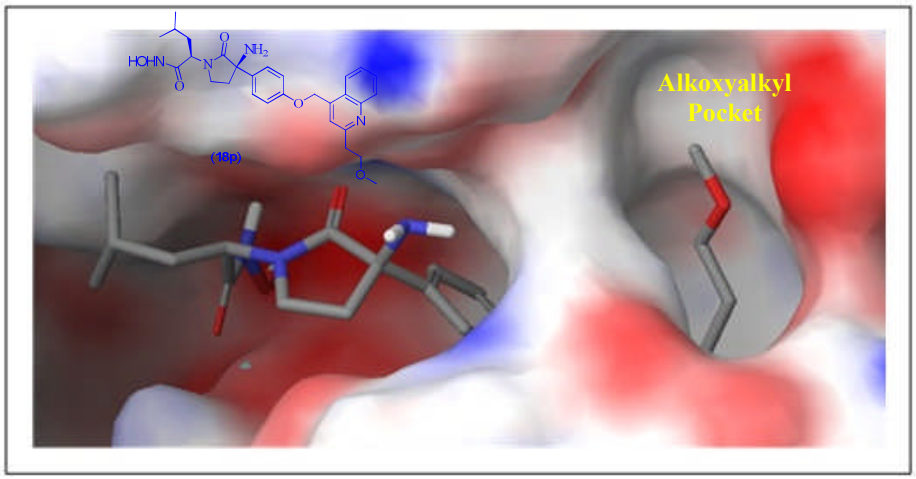


Figure 22. Compound **18p** docked pose in the active site of TACE where in alkoxyalkyl group at 2nd position of quinoline protruding from the alkoxyalkyl pocket

In conclusion, molecular docking study results indicate that the alkoxyalkyl substitutions at 2nd position of quinoline ring system are essential for potent and selective TACE inhibitory activity profile.

3.1.5. *In vivo* TNF-α inhibitory activity and PK studies of selected compounds (18o and 18p)

In vivo TNF-α inhibitory activity was carried out for most potent and selected compounds **180** and **18p**, including **BMS-561392.** These test compounds (**180** and **18p**) were subjected for *in vivo* study and ED_{50} values were determined (detail experimental protocol is given in experimental section 5.3).

Table 19. ED_{50} values of selected test compounds (**18o-p** and **BMS-561392**)[a]

Compounds	ED_{50} (mpk, po)
18o	7.2
18p	3.1
BMS-561392	6.9

[a]**ED_{50}** Values for compound **18o** and **18p** and **BMS-561392** were determined in fasted male wistar rats (n=6) and plasma concentration of compounds were measured LC-MS/MS.

As shown in **Table 19**, compound **18o** and **18p** showed ED_{50} value 7.2 and 3.1, while **BMS-561392** showed ED_{50}: 6.9 mpk, po. Compared to *ex vivo* TNF-α inhibition and *in vitro* TACE inhibitory activity results, significant difference in the *in vivo* TNF-α inhibitory activity were observed. Among the two test compounds (**18o**-**p**) screened *in vivo*, compound **18p** was found to be around 2-fold more potent (ED_{50}: 3.1 mpk, po) than (**18o**) (ED_{50}: 7.2 mpk, po) and standard compound **BMS-561392** (ED_{50}: 6.9 mpk, po). In order to understand the PK profile of test compounds, a comparative single dose (5 mpk; iv/po) PK study of our most potent compound (**18p**), along with standard compound **BMS-561392** was carried out in male wistar rats and the various PK parameters were recorded (**Table 20**).

Table 20. PK parameters comparison of compound **18p** with **BMS-561392**

	PK Parameters#	**Compd 18p**	**BMS-561392**
IV	$T_{1/2}$ (h)	1.09±0.1	2.69±0.1
	Kel (h^{-1})	1.02±0.2	0.30±0.1
	AUC (h ng/ml)	19726±188	32356±899
PO	T_{max} (h)	0.33±0.2	1.21±0.01
	$T_{1/2}$ (h)	3.81±0.4	8.67±0.26
	Kel (h^{-1})	0.44±0.01	0.08±0.05
	AUC (h ng/ml)	11776±103	5970±99
	% F	59.69	18.45

#Single dose (5 mpk; iv/po) PK study for compound **18p** and **BMS-561392** was carried out in fasted male wistar rats (n=9) and plasma concentration of compounds were determined by LC-MS/MS, data represented as M±SD.

In a single dose PK study (5 mpk, iv/po), test compound **18p** showed rapid T_{max} and clearance, good AUC and moderate half-life, while standard compound showed extended T_{max}, $t_{1/2}$ and moderate AUC and clearance (**Table 20**).

Compared to standard compound **BMS-561392**, test compound **18p** showed good plasma levels. One of the reasons why compound **18p** showed good *in vivo* TNF-α inhibitory activity could be correlated with its rapid T_{max}, good plasma exposure.

Compared to standard compound **BMS-561392**, test compound **18p** showed 3-fold higher bioavailability (F:~60%). One of the reasons why compound **18p** showed good *in vivo* TNF-α inhibitory activity could be correlated with its rapid t_{max} and good plasma exposure and higher bioavailability.

3.1.6. Safety pharmacology of 18p and BMS-561392

To assess the comparative safety profile of compound **18p** over **BMS-561392**, repeat dose acute toxicity studies (28 days) of both the compounds were carried out in male wistar rats (100 mpk, po, bid) and various parameters such as gross pathology, clinical signs, body weight, organ weights, and serum chemistry/ hematological changes were recorded (detail experimental protocol is given in experimental section 5.3) [**18**]. In general, daily oral administration of compounds **18p** and **BMS-561392** did not affect the survival of Westar rats and also no adverse changes related to gross pathology, clinical signs, body weight and feed consumption were noticed as compared to control group. However, compound **BMS-561392** showed significant changes in relative organ weights and hematological parameters.

Some of the key parameters, such as comparative hematological changes (**Table 21**), and relative organ weights (**Table 22**), which are relevant to hepatotoxicity assessment are described in detailed. Acute hepatocellular injury

markers such as, ALT and AST alone or in combination with hepatobiliary markers such as ALP and TBILI are primarily considered for the assessment of hepatotoxicity in non-clinical studies [**19**]. As shown in **Table 21**, the hematological parameters (WBC and RBC) of **BMS-561392** and **18p** were found to be comparable to that of control animals. Similarly, compound **18p** showed no significant changes in serum ALP, AST, ALT and TBILI as compared to control group.

Table 21. Comparison hematological parameters and serum chemistry of compound **18p** with **BMS-561392** [a]

Parameters	Control	Compounds	
		18p	BMS-561392
WBC (10^3/µl)	8.20±0.33	8.41±0.21	8.99±0.41
RBC (10^6/µl)	7.38±0.11	8.01±0.23	7.69±0.99
ALP (U/L)	134.66±5.3	121.2±12.1	**523.43±7.1**[b]
AST (U/L)	147.16±11.75	140.2±9.32	**457.21±8.3**[b]
ALT (U/L)	20.78±1.31	21.04±8.36	**39.22±1.99**[b]
TBILI (mg/dL)	0.14±0.01	0.17±0.08	**0.79±0.02**[b]

[a]Values expressed as M±SD; n=9, Male WR, dose 100 mpk, po (bid), 28 days repeated dose toxicity study; [b]represent significant elevation of serum liver enzymes at P<0.01, compared to control

However, compound **BMS-561392** treated group showed significantly elevated levels of all the serum liver enzymes (ALP, AST, ALT and TBILI), which infer its hepatotoxic effects in animals. Also, treated group elicit hepatocellular hypertrophy, marked by significant increased in liver weight as compared to control and **18p** treated groups (**Table 22**), while other key organs (heart, kidney, spleen and brain) remained un-changed.

Table 22. Comparison of relative organ weights (%) after 28 days repeat dose treatment with compound **18p** with **BMS-561392**[a]

Organs	Control (Vehicle)	Compounds	
		18p	BMS-561392
Heart	0.345±0.007	0.358±0.008	0.351±0.021
Liver	3.659±0.001	**3.801±0.069**	**4.993±0.127**[b]
Kidney	0.821±0.003	0.878±0.024	0.831±0.04
Spleen	0.201±0.007	0.203±0.001	0.211±0.026
Brain	0.727±0.024	0.703±0.028	0.714±0.015

[a]Values expressed as M±SD; n=9, Male WR, dose 100 mpk, po, 28 days repeated dose toxicity study; [b]Significant from control @ 5% level (p<0.05)

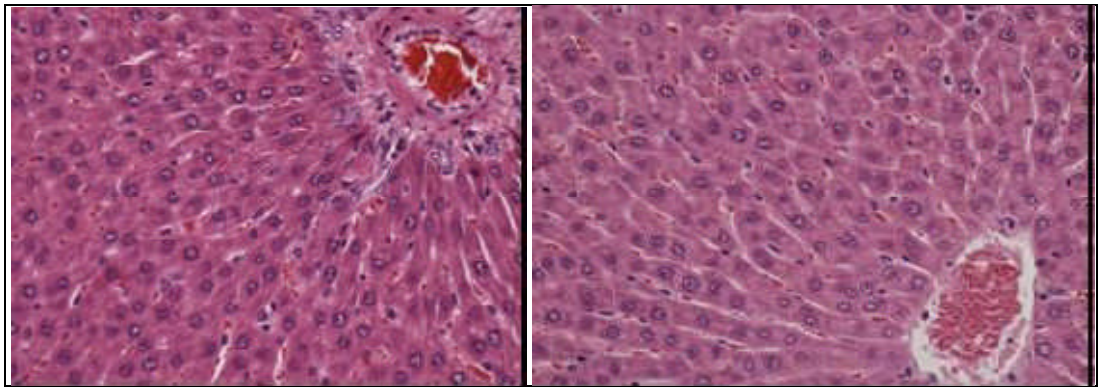
Finally to confirm these observations, detailed liver histopathological studies were conducted and test compounds associated histopathological changes were recorded (**Figure 24** and **Table 23**).

Table 23. Comparison of relative liver histopathological changes after 28 days repeats dose treatment with compound **18p** with **BMS-561392**[a]

Parameters	Control (Vehicle)	Compounds	
		18p	BMS-561392
Bile duct proliferation	0	0	4[b]
Sinusoidal dilatation	0	1	4[b]
Focal mononuclear infiltration	0	0	o
Focal degeneration	0	0	2
Eosinophilic droplets	0	1	2
Connective tissue proliferation	0	0	2
Hepatic congestion	0	0	0
Focal area of necrosis	0	0	0

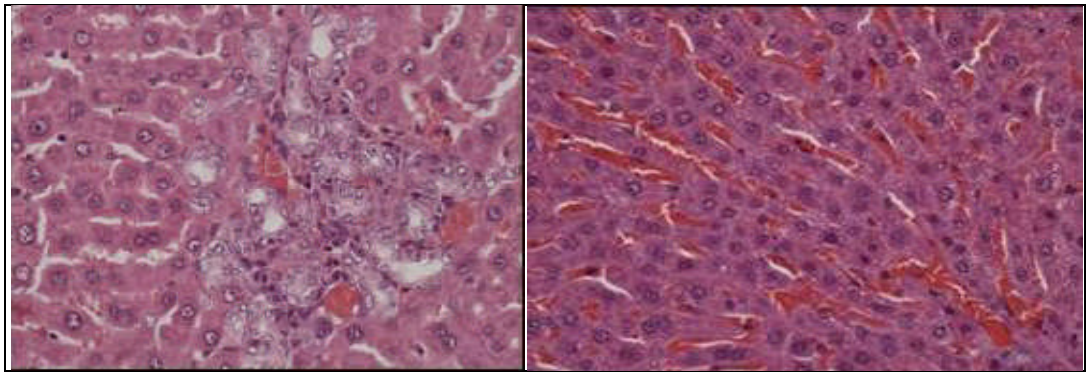
[a]Values expressed as M±SD; n=9, Male WR, dose 100 mpk, po, 28 days repeated dose toxicity study; [b]Significant from control @ 5% level (p<0.05)

Animals treated with compound **18p** showed normal liver histology without any changes in hepatic cords, portal artery and bile duct as observed in the vehicle treated group. While, animals treated with **BMS-561392** showed severe bile duct proliferation and sinusoidal dilation, indicates periportal fibrosis (**Figure 23**).



A **B**

A: Control (vehicle treatment); **B**: treatment with compound **18p** (100 mpk, po, bid). Both groups showed normal hepatic cords, portal artery and bile duct



A **B**

Treatment with standard **BMS-561392** (100 mpk, po, bid); **A**: showing bile duct proliferation; **B** showing sinusoidal congestion.
Male WR, dosed 100 mpk, po, for 28 days repeatedly (bid) and the livers of sacrificed rats were dissected, sectioned into 4-5 μm thick sections, stained with hematoxylin-eosin and observed with a photomicroscope (Model N-400ME) at H& E 40X magnification.

Figure 23: Histopathological examination of rat livers after 28 days repeat dose treatment with compounds **18p** and **BMS-561392**

Also, epithelial cells lining bile duct showed mild focal degeneration and eosinophilic/ hyaline droplets, while other key parameters (focal mononuclear cell

infiltration, hepatic vascular congestion and focal area of necrosis) remained un-changed

Together, hematological and histopathological examination results revealed that **BMS-561392** induced significant bile duct proliferation and sinusoidal congestion, which is indicative of acute hepatocellular injury, while test compound **18p** showed no adverse effects and also in histopathological examination, no apparent sign of liver toxicity was observed with **18p**, which confirms its adequate safety profile (safety index ~66X, compared to ED_{50} dose) over standard **BMS-561392**, in preliminary animal models. In conclusion, **18p** was found to be safer in toxicological evaluation than standard compound **BMS-561392**.

3.1.7. Conclusion

Ex vivo and *in vitro* TNF-α inhibitory activity and molecular docking studies results clearly demonstrated that the potency and selectivity of *γ*-lactam hydroxamate based TACE inhibitors can be modulated using suitable substituents at 2^{nd} position of quinoline ring system. Furthermore, it was observed that suitable substituent's at 2^{nd} position contributed significantly towards improvement in the *in vivo* TNF-α inhibitory activity, which could be correlated with its improved oral bioavailability. Finally, in repeat dose acute toxicity study, most potent and selective test compound **18p** showed no adverse changes related to gross pathology, clinical signs and liver toxicity, indicating that the good selectivity profile of new class *γ*-lactam hydroxamate based TACE inhibitors over MMPs and ADAM-10 is essential to overcome hepatotoxicity

concerns associated with similar class of TACE inhibitors. Compound **18p** is considered as the promising candidate for safe and effective treatment of acute and chronic inflammations and need to subject for further pre-clinical evaluation.

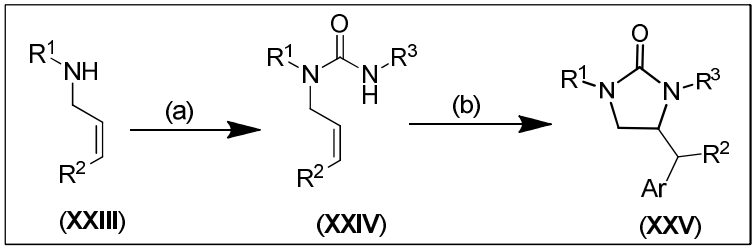
3.2. Substituted thiadiazole-imidazolidinones derivatives as TACE inhibitors (Second series)

3.2.1. Chemistry

Imidazolidinone derivatives are useful for various biological activities. Billot et al claimed 1, 5-disubstituted imidazolidin-2-one derivatives as prostaglandin receptor agonist for the treatment of eye and bone diseases [**20**]. Lee et al and Commons et al reported substituted imidazolidinone-2-one derivatives as, androgen receptor (AR) modulators and progesterone receptor (PR) modulators respectively [**21**-**22**]. Recently Das et al reported substituted imidazolidinones as potential TACE inhibitors [**23**].

3.2.1.1. Construction of imidazolidinone moiety

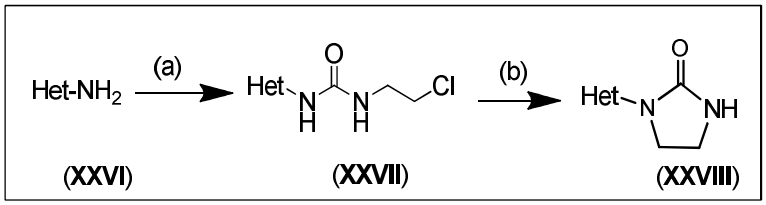
For the synthesis of title compounds **22g**, **23a-p**, **24** and **25**, we need to first construct imidazolidinone ring. As described above, imidazolidione moiety exhibits various biological activity and various routes are depicted in literature for the efficient synthesis of substituted imidazolidinone derivatives. Fritz et al reported a new strategy for the preparation of substituted imidazolidinone in two steps (**Figure 24**) [**24**]. Addition of the amine (**XXIII**) to isocyanates affords N-allylureas (**XXIV**), which can be converted to imidazolidinone (**XXV**), upon treatment with aryl bromides, using $Pd_2(dba)_3$/Xantphos catalyst. Disadvantage of this method was usage of costly catalyst like Pd_2 $(dba)_3$/Xantphos and poor yield.



Regents: (a) R^3-NCO, RT; (b) ArBr, $Pd_2(dba)_3$/Xantphos, NaO_tBu.

Figure 24.Literature synthetic methods for the preparation of imidazolidinone derivatives

Robert et al and Dasgupta et al, prepared substituted imidazolidinones using 2-chloroethylisocynante (**Figure 25**) [**23**-**25**].



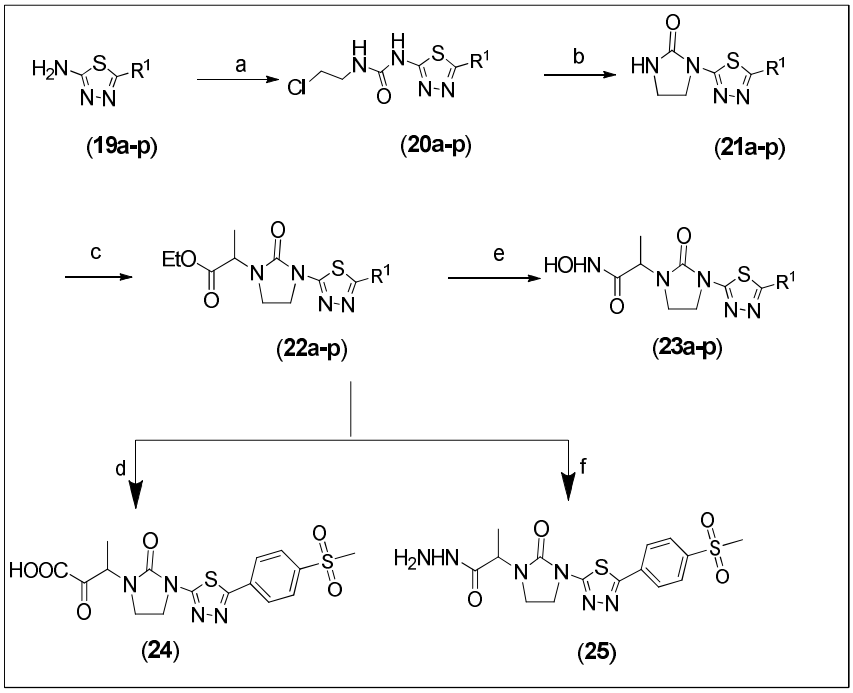
Reagents and conditions: (a) 2-chloroethylisocynante, $CHCl_3$, reflux; (b) CH_3CN, Na_2CO_3, reflux, 16 h.

Figure 25. Literature synthetic methods for the preparation of imidazolidinone derivatives

Condensation of appropriate hetroaryl amine (**XXVI**) with 2-chloroethyl isocynante affords corresponding urea (**XXVII**). The intermolecular cyclisation of **XXVII**, using sodium bicarbonate gives mono substituted imidazolidinones (**XXVIII**). The major disadvantage of this method was yields remained lower and slow rate of reaction. To overcome the drawbacks of literature methods reported for the synthesis of imidazolidinones derivatives, we modified this method further using Sodium hydride (NaH) as base, instead of sodium bicarbonate and we found that sodium hydride improves the rate of reaction (**Schemes 4**).

As shown in **Scheme-4**, synthesis of titled compounds **22g**, **23a-p**, **24** and **25** was carried out using starting from commercial available substituted phenyl

thiadiazole-2-amine (**19a**-**p**), which reacted with 2-chloroethyl isocyanate at 25^0 C to get intermediate **20a**-**p**. Further treatment with NaH at room temperature gives **21a**-**p**. Treated of **21a**-**p** with ethyl 2-bromopropanoate and NaH in DMF resulted in **22a**-**p**, which further reacted with $NH_2OH.HCl$, in presence of base (NaOH), to get substituted hydroxamic acid derivatives **23a**-**p**. The compound **22a** upon treatment with NaOH to provide **24** and upon reaction with hydrazine hydrate (NH_2-NH_2), gives acid derivative **25**.



Reagents and conditions: a) 2-Chloroethyl isocyanate, CH_2Cl_2, 25-30^0C; b) NaH, DMF, 25-3^0C; c) Ethyl 2-bromopropanoate, NaH, DMF, 25-3^0C; d) NaOH, MeOH, 25-30^0C; e) $NH_2OH.HCl$, NaOH, MeOH, 25-30^0C; f) NH_2-NH_2, MeOH, 25-30^0C. Wherein, (**23a-p**): R^1= 4OMe-; 4Me-; 4MeS-; $4NO_2$-; $4MeSO_2$-; 4F-; 3,5Di-*t*but-; 3,5Di-*t*but 4-OH-; 4Ph-; 4Py-; 4ipr-: 4Cpr-:

Scheme 4. Synthetic route for the preparation of imidazolidinone derivatives (**22g**, **23a**-**p**, **24** and **25**)

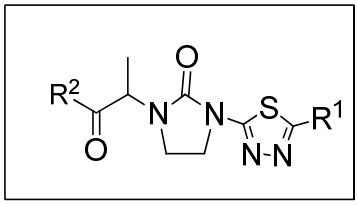
Using above synthetic routes (**Scheme 4**), altogether nineteen titled compounds (**22g**, **23a-p**, **24** and **25**) were prepared. All the final compounds, intermediates were purified, characterized and the spectral data of compounds

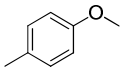
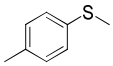
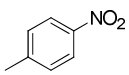
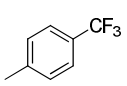
were found to be in conformity with the structure assigned. Detailed experimental procedures are described in experimental section 5.1 and the representative spectra's of selected compounds are given in section 6.

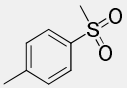
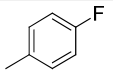
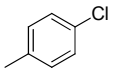
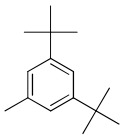
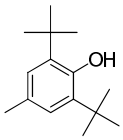
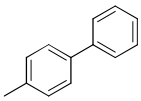
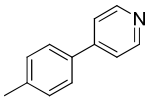
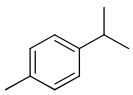
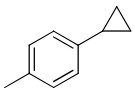
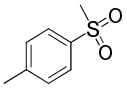
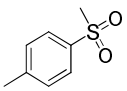
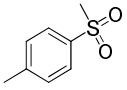
3.2.3. *Ex vivo* TNF-α inhibitory activity and SAR

Ex vivo TNF-α inhibitory activity (using human whole blood assay) was assessed (detail experimental protocol is given in experimental **Section 5.3**), mainly to establish the SAR of new series of substituted thiadiazole-imidazolidinones based TACE inhibitors **22g**, **23a**-**p**, **24** and **25** [**9**]. As depicted in **Table 24**, depending upon the nature of substitutions, all the titled compounds showed different degree of TNF-α inhibition (IC_{50}).

Table 24. *Ex vivo* TNF-α inhibitory (IC_{50}) activity of test compounds (**22g**, **23a**-**p, 24** and **25**)[a]



Compd. No.	R^1	R^2	IC_{50} (nM)[b]
23a		-NHOH	495
23b		-NHOH	856
23c		-NHOH	923
23d		-NHOH	810
23e		-NHOH	712
23f		-NHOH	634

Compd. No.	R^1	R^2	IC_{50} (nM)[b]
23g		-NHOH	427
23h		-NHOH	967
23i		-NHOH	989
23j		-NHOH	IA
23k		-NHOH	IA
23l		-NHOH	IA
23m		-NHOH	IA
23n		-NHOH	696
23o		-NHOH	688
23p		-NHOH	997
22a		-COOMe	IA
24		-COOH	IA
25		-$NHNH_2$	IA
BMS-561392			60

[a]*Ex vivo* TNF-α inhibition was carried out in human whole blood and plasma TNF-α concentration determined using ELISA kit; [b]IC_{50} values (nM) are from single determination and standard compound **BMS-561392** was used as positive std. control. IA denotes IC_{50} >10uM concentration.

Substituting phenyl ring as R^1 at thiadiazole ring (**23a**) showed good *ex vivo* TNF-α inhibition. Further to improve TNF-α inhibitory activity, we introduced various groups on phenyl ring such as electron donating, electron withdrawing, halo, bulky groups, aromatic and aliphatic ring system. The electron donating groups at *para* position of aromatic ring system such as 4-methoxy (**23b**), methyl (**23c**) and methylthio (**23d**) analogs does not improved TNF-α inhibition. The electron withdrawing groups at *para* position of aromatic ring system such as 4-nitro (**23e**) and 4-trifluro (**23f**) showed good TNF-α inhibitory activity. But substitution of 4-methylsulfonyl (**23g**) analogs showed improved *ex vivo* TNF-α inhibitory activity. However, substitution with halo analogs such as 4-fluro (**23h**) and 4-chloro (**23i**) showed weak TNF-α inhibitory activity. Similarly substitution with bulky groups (**23j** and **23k**) showed weak *ex vivo* TNF-α inhibitory activity. However, substitution with biphenyl (**23l**) and 4-phenyl pyridine (**23m**) showed weak TNF-α inhibitory activity. Substitution with aliphatic groups such as 4-isopropyl (**23n**) and 4-cyclopropyl (**23o**) on benzene ring, showed good *ex vivo* TNF-α inhibitory activity. Replacement of Phenyl ring by hetrocycles like pyridine (**23p**) showed weak TNF-α inhibitory activity. Intestinally replacing hydrozomic acid of compound **23a** by methyl ester (**22a**), carboxylic acid (**24**) and hydrazine (**25**), showed complete loss of TNF-α inhibitory activity, indicated that hydazomic acid is essential for TNF-α inhibition.

In conclusion, compounds substituted with electron donating groups, showed weak TNF-α inhibitory activity. However electron withdrawing groups on benzene ring at thiadiazole showed good TNF-α inhibitory activity, while bulky

substitutions showed weak TNF-α inhibitory activity. Although, the S_1 binding pocket of TACE is larger, but the substitution with bulky group was found to be unfavorable. On the contrary, substitution with non bulky groups on benzene ring showed good TNF-α inhibition, which could be due to its favorable bent confirmation acquisition at S_1 binding pocket [**13**].

From above SAR study, out of nineteen compounds, short listed compounds (**23a** and **23g**) showed satisfactory TNF-α inhibitory activity. Compared to **BMS-561392**, compound **23a** and **23g** showed weak potency but still these two compounds were subjected for their *in vitro* TACE inhibitory activity, selectivity against MMPs and ADAM-10, *in vivo* TNF-α inhibitory activity and PK evaluations.

3.2.3. *In vitro* TACE inhibitory activity and selectivity of 23a and 23g

The *in vitro* TACE inhibitory activity and selectivity over MMPs (MMP-1,-2,-3,-7,-8,-9,-13 and 14) and ADAM-10 were evaluated for most potent compounds **23a** and **23g**, using fluorescence-based FRET assay [**10**,**11**] and IC_{50} values (**Table 25**) were determined (detail experimental protocol is given in experimental section 5.3). As shown in **Table 25**, both the test compounds **23a** and **23g** inhibits the recombinant TACE with IC_{50} of 445 and 402 nM respectively, compared to standard compound **BMS-561392** (IC_{50}: 12 nM). Unlike TNF-α inhibitory activity, both compounds (**23a** and **23g**) showed weak *in vitro* TACE inhibitory activity as compared to **BMS-561392**.

Table 25. *In vitro* TACE inhibitory activity for selected test compounds **23a** and **23g**[a]

Enzyme[#]	Compounds		
	BMS-561392	**23a**	**23g**
TACE	12	445	402
ADAM-10	480	23523	22425
MMP-1	429	22145	23156
MMP-2	482	IA	22287
MMP-3	285	IA	IA
MMP-7	466	23276	22481
MMP-8	299	IA	IA
MMP-9	389	22876	23456
MMP-13	461	IA	IA
MMP-14	499	22324	23235

[a]*In vitro* TACE inhibitory activity and selectivity over MMPs (MMP-1,-2,-3,-7,-8,-9,-13 and 14) and ADAM-10 were evaluated for selected test compounds **23a, 23g** and **BMS-561392**, using fluorescence-based FRET assay and IC_{50} values (nM) were determined (n=3). IA denotes IC_{50} >10uM concentration.

Compounds **23a** and **23g** showed >50-fold selectivity (IC_{50}: >20000 nM) over all the tested MMPs and ADAM-10, whereas standard compound **BMS-561392** showed variable selectivity (IC_{50}: <480 nM) over tested MMPs and it showed only 40-fold selectivity over ADAM-10. Together, these *in vitro* results demonstrated moderate TACE inhibitory activity but improved selective profile of our test compounds **23a** and **23g** over standard compound **BMS-561392**, which indicates that bioisosteric replacement of thiadiazole mimetic with phenoxy ring of **BMS-561392**, although it need further optimization with respect to potency improvement but as per selectivity is considered, thiadiazole ring offers remarkable selectivity over tested MMPs and ADAM-10.

3.2.4. Docking studies of 23g and BMS-561392

Docking study of most potent compound **23g** was carried out to understand its potency and selectivity for TACE over other MMPs, especially ADAM-10, which was not observed *in vitro* for standard compound **BMS-561392**. Docking study was carried out using Glide (version 50207) software [**12**].

As shown in **Figure 26**, the heterocyclic ring of **23g** and **BMS-561392** occupies the S_1 site of the TACE enzymes pocket.

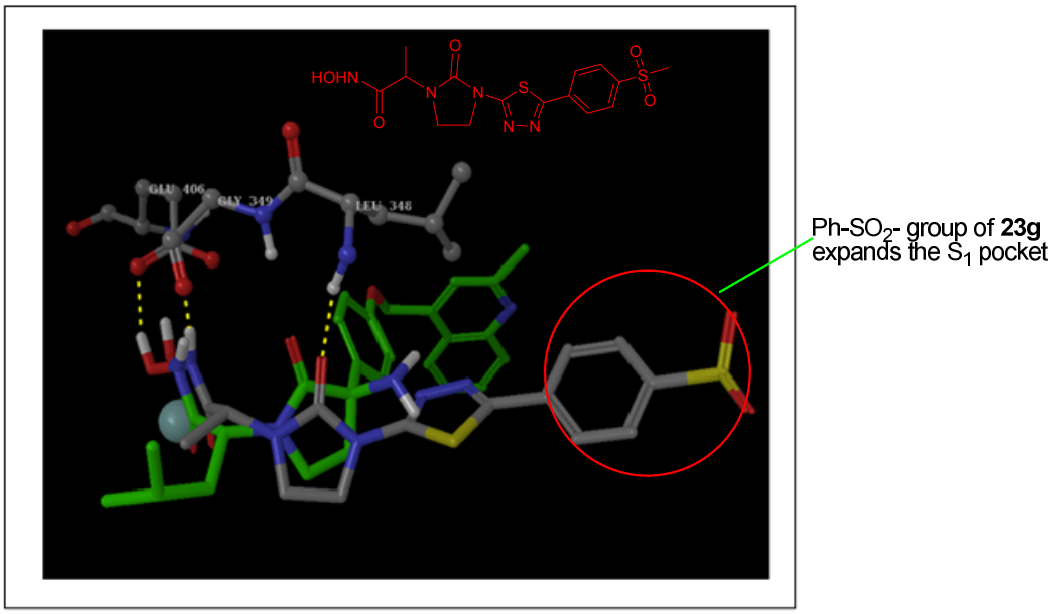


Figure 26. H-bond interaction compound **23g** (green) and **BMS-561392** (gray)

The substituted thiadiazole moiety of compounds **23g** occupies the S_1 site the oxygen atom of the imidazolidinone ring forms hydrogen bonds with L_{348}, the hydroxamic acid interacts with the zinc atom present in the active site of TACE and coordinates with the side-chain carboxylate of E_{406}. All the five binding poses obtained for **23g** were found to be close, with an RMSD of < 0.9 Å and there were no unusual conformations [**13**-**15**]. The ligand orientation and H-bonding

interactions of **23g** matches closely with **BMS-561392**, except Ph-SO_2- group of **23g** expands the S_1 pocket of TACE enzymes due to thiadiazole spacer, which contributes towards its selectivity for TACE against MMPs and ADAM-10. Loss of H-bonding with G_{349} and long distance H-bonding with Glu-dyed might be responsible for comparative weak TACE inhibitory activity as compared to **BMS-561392**. The Ph-SO_2- group of compound **23g** expands the S_1 pockets, which was not observed with **BMS-561392**. Probably this could be the reasons why **23g** was found to be more selective against MMPs than **BMS-561392**.

3.2.5. *In vivo* TNF-α inhibitory activity and PK studies of selected compounds

In vivo TNF-α inhibitory activity was carried out for most potent and selected compounds **23a** and **23g** from this series, including **BMS-561392.** These test compounds **23a** and **23g** were subjected for *in vivo* study and ED_{50} values were determined (detail experimental protocol is given in experimental **Section 5.3**).

Table 26. ED_{50} values of selected test compounds (**23a**, **23g** and **BMS-561392**)[a]

Compounds	ED_{50} (mpk, po)
23a	523
23g	298
BMS-561392	6.9

[a]**ED_{50}** Values for compound **23a** and **23g** and **BMS-561392** were evaluated in fasted male wistar rats (n=6) and plasma concentration of compounds were determined by LC-MS/MS.

As shown in **Table 26**, compound **23a** and **23g** showed ED_{50} values of 523 and 298 (mpk, po), while **BMS-561392** showed ED_{50}: of 6.9 mpk, po. Compared to *ex vivo* TNF-α inhibition and *in vitro* TACE inhibitory activity results, significant difference in the *in vivo* TNF-α inhibitory activity were observed.

Among the two test compounds (**23a** and **23g**) screened *in vivo*, compound (**23g**) was found to be around 2-fold more potent (ED_{50}: 298 mpk, po) than **23a** (ED_{50}: 523 mpk, po). While compared to standard compound, **BMS-561392** (ED_{50}=6.9), **23g** was found to be around 40 fold less potent.

In order to understand the PK profile of **23g**, a comparative single dose (100 mpk; po and 1 mpk; iv) PK study of **23g**, along with standard compound **BMS-561392** was carried out in male wistar rats and the various PK parameters (detail experimental protocol is given in experimental section 5.3) were recorded in **Table 27** [**16**].

Table 27. PK parameters comparison of compound **23g** with **BMS-561392**

	PK parameters#	**Compounds**	
		23g	**BMS-561392**
IV	$T_{1/2}$ (h)	0.7±0.1	3.23±0.3
	Kel (h^{-1})	0.6±0.1	0.28±0.2
	AUC (h ng/ml)	941±50	31213±765
PO	T_{max} (h)	0.35±0.2	1.32±0.02
	$T_{1/2}$ (h)	1.02±0.2	7.83±0.04
	Kel (h^{-1})	0.14±0.01	0.07±0.13
	AUC (h ng/ml)	234±50	2177±92
	% F	4	

#Single dose PK study for compound **23g** (100 mpk; po and 1 mpk; iv) and **BMS-561392** (10 mpk; po and 1 mpk; iv) was carried out in fasted male wistar rats (n=7) and plasma concentration of compounds were determined by LC-MS/MS, data represented as M±SD.

In a single dose PK study, test compound **23g** showed rapid T_{max} and clearance, poor AUC and half-life, compared to standard compound **BMS-561392**. **23g** showed poor plasma levels, probably this could be reasons why **23g** was found to be less potent in *in vivo* studies. Based upon above observation, we decided not to subject this compound (**23g**) further for

toxicological evaluation.

Compared to standard compound **BMS-561392**, test compound **23g** showed poor bioavailability (F:~4%). One of the reasons why compound **23g** showed weak *in vivo* TNF-α inhibitory activity could be correlated with its rapid t_{max} and poor plasma exposure and lower bioavailability.

3.2.6. Conclusion

In conclusion, *ex vivo*, *in vitro* and molecular docking study results clearly demonstrated that our attempt to bioisosterically replace γ-lactam ring of **BMS-561392** with imidazolidinone ring, followed by introducing of thiadiazole based spacer as replacement of phenoxy group of **BMS-561392**, lead to developed novel and structurally diverse scaffold as TNF-α and TACE inhibitors. Most potent compound (**23g**) from this series showed nM potency (TNF-α and TACE inhibitory activity), which was found to be comparative weaker than standard **BMS-561392**. However, it showed improvement in the fold selectivity for TACE against MMPs and ADAM-10 tested. Thus preliminary PK/PD studies results reveals that further modification on **23g** may lead to the identification of a potent compound from this series, with improved PK/PD profile for the efficient treatment of various inflammatory conditions.

3.3. Discovery and preliminary SAR studies of carboxy-phosphono acetate derivatives as novel TNF-α inhibitors (Third series)

As described in designed section, we screened in-house compounds libraries and in HTS screening (*ex vivo* TNF-α inhibitory assay), compound **1** was identified as primary hits with moderate TNF-α inhibitory activity (IC_{50} activity in μM range). We further modified the primary hit (compound **1**) using rational designed strategy. In this section we depicted synthesis, *in vitro* TNF-α inhibitory activity of various derivatives of compound **1**, which we prepared to establish optimize lead from preliminary hit (compound **1**).

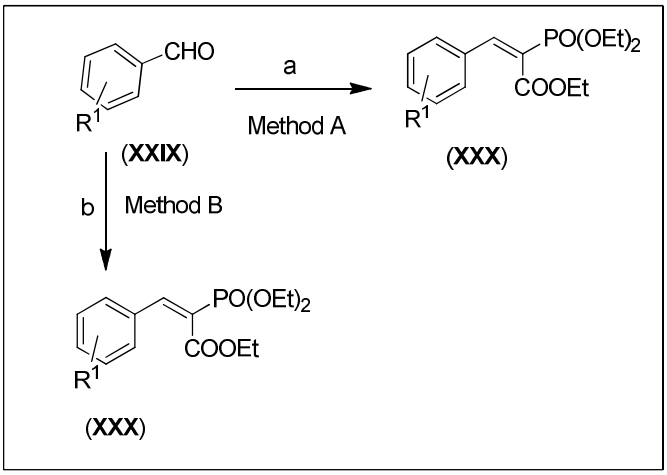
Brief overview on phosphonates

Over the past few decades, phosphonates have emerged as valuable compounds, possessing various biological properties. Phosphonates are widely used in the treatment of bone diseases, also reported as an anti-inflammatory and hypolipidemic agents [**26**]. Phosphonates are used for the treatment of atherosclerosis and hyperlipidemia [**27**-**28**]. These classes of compounds are also reported as PPAR-γ antagonist with anti-obesity and anti-diabetic activities [**29**]. Recently, phosphonamide derivatives are also documented as inhibitors of MMP and TACE [**30**].

3.3.1. Chemistry

Very few methods have been reported for the synthesis of carboxy-phosphonoacetate derivatives (**Figure 27**). Carboxy-phosphonoacetate (**XXX**), was prepared by condensation of triethylphosphonoacetate with aromatic

aldehyde (**XXIX**) by refluxing in benzene with azeotropic removal of water, **Method A** [**31**].

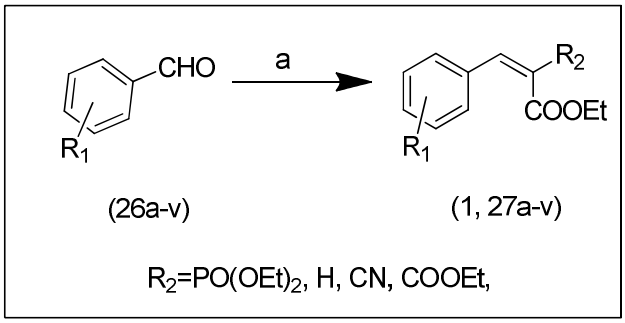


Reagents and conditions: a) Triethylphosphonoacetate, benzane, refluxe, 3 to 6 days. b) $TiCl_4$, *N*-methyl morpholine, triethylphosphonoacetate, 5 ^{0}C

Figure 27. Literature synthetic methods for the preparation of carboxy-phosphonoacetate derivatives

The major disadvantage of this method includes its long reaction time (3 to 6 days). Lehnert et al carried out the same reaction in the presence of $TiCl_4$ and *N*-methyl morpholine as a base to yield desired phosphonoacetate (**Method B**) [**32**]. However as a disadvantage this method leads to poor yield.

Among various reported methods, we used modified Lehnert et al method (**Method B**), wherein condensation reaction was carried out at 5 ^{0}C, in order to improve overall yield (**Scheme 5**). Title compounds **1** and **27a**-**v** were prepared by the condensation of commercially available benzaldehydes (**26a**-**v**), with triethyl phosphonoacetate, in the presence $TiCl_4$ and *N*-methyl morpholine to get desired α, β-unsaturated phosphonoacetate derivatives **1** and **27a**-**v**.



Reagents and conditions: a) $TiCl_4$, Ethyl 2-(diethoxyphosphoryl) acetate, CH_2Cl_2, 25-30^{0}C. Wherein, (**1** and **27a-s**): R^1= H-; 2OMe-; 2Me-; 3OMe-; 3Me-; 4OMe-; 4Me-; $3CF_3$-; $3NO_2$-; 3Cl-; 3F-; 3Ph-; 3Py-; 3OH-; $3OSO_2Ph$-; $3OSO_2Ph4CF_3$-; $3OSO_2Ph4Cl$-; $3OSO_2Ph4OCF_3$-; $3OSO_2Ph4Me$-; $3OSO_2Ph2,4,6Me$-: (**27t-v**): R^1= $3OSO_2Ph4Me$-; R^2= $PO(OEt)_2$-

Scheme 5. Synthetic route for the preparation of carboxy-phosphonoacetate derivatives (**1** and **27a**-**v**)

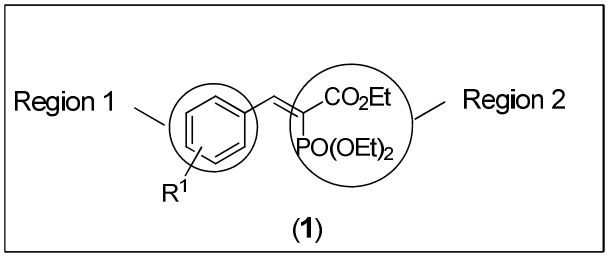
During the condensation between aromatic aldehyde and triethyl phosphonoacetate, formation of only Trans or E α,β-unsaturated phosphonoacetate was observed which was confirmed by its ^{1}H NMR spectra. In literature, for *E*-configuration, reported coupling constants of olefinic protons and α, β-unsaturated phosphonates are in the range of J_{cis}=10-30 Hz and J_{trans}= 30-50 Hz [**33**]. The ^{1}H NMR of our compounds showed J_{cis}=23-25 Hz, which confirms the *E* configuration of all the titled compounds (**1** and **27a-v**).

Using above synthetic route (**Scheme 5**), altogether twenty three titled compounds (**1** and **27a**-**v**) were prepared. All the final compounds, intermediates were purified, characterized and spectral data of compounds were found to be in conformity with the structure assigned. Detailed experimental procedures and chemical characterization data are described in the experimental section 5.1 and the representative spectra's of selected compounds are given in section 6.

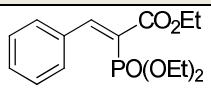
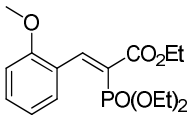
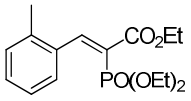
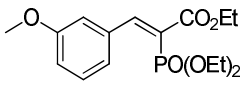
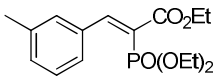
3.3.2. *Ex vivo* TNF-α inhibitory activity and SAR

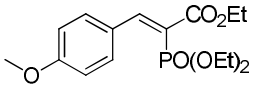
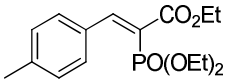
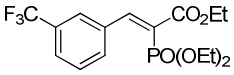
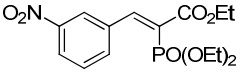
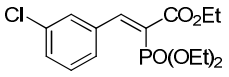
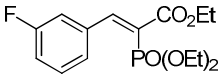
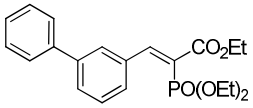
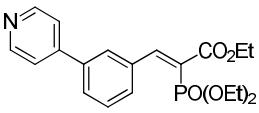
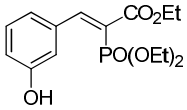
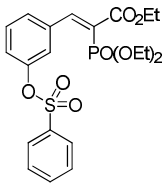
Ex vivo TNF-α inhibitory activity (using human whole blood assay) was assessed (detail experimental protocol is given in experimental section 5.3), mainly to establish the SAR of new series of carboxy-phosphonoacetate based TNF-α inhibitors (**1** and **27a**-**v**) [**9**]. As depicted in **Table 28**, depending upon the nature of substitutions, all the titled compounds showed different degree of TNF-α inhibition (IC_{50}).

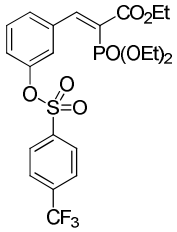
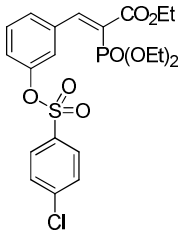
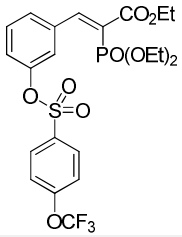
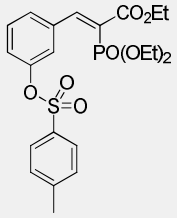
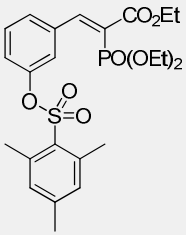
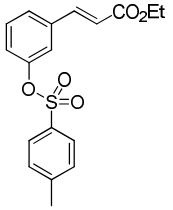
Table 28. *Ex vivo* TNF-α inhibitory (IC_{50}) activity of test compounds (**1** and **27a**-**v**)[a]

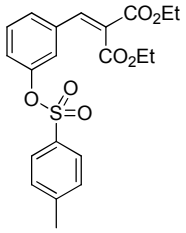
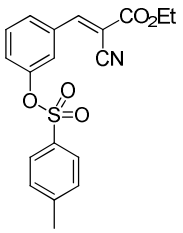


(**1**)

Compd No.	Structure	IC_{50} (nM)[b]
1		987
27a		IA
27b		IA
27c		775
27d		894

Compd No.	Structure	IC_{50} (nM)[b]
27e		1102
27f		1205
27g		1123
27h		IA
27i		1324
27j		1345
27k		IA
27l		IA
27m		698
27n		602

Compd No.	Structure	IC_{50} (nM)[b]
27o		708
27p		742
27q		587
27r		502
27s		525
27t		IA

Compd No.	Structure	IC_{50} (nM)[b]
27u		IA
27v		IA
BMS-561392		60

[a]*Ex vivo* TNF-α inhibition was carried out in human whole blood and plasmTNF-α concentration determined using ELISA kit; [b]IC_{50} values (nM) are from single determination and compound **BMS-561392** was used as positive std. control. IA denotes IC_{50} >10uM concentration.

Our primary hit, compound **1** showed moderate inhibition of TNF-α. To improve activity, we identified two regions of compound **1**, which could be responsible for its TNF-α inhibitory activity. First we started exploring SAR around the phenyl ring (region 1) by keeping phosponate group (region 2) intact. To identify optimize position of substituent's (*ortho*, *meta* and *para*), we selected electron donating group like methoxy and methyl and introduced them on phenyl ring sequensely.

In region 1, firstly we introduced electron donating group at *ortho* position of phenyl ring, however electron donating methoxy (**27a**) and methyl (**27b**) did not show any TNF-α inhibitory activity. Further, we switched from *ortho* position to *meta* position. Interestingly electron donating group methoxy (**27c**) and methyl group (**27d**) showed dramatic improvement in TNF-α inhibitory activity. Electron donating group like methoxy (**27e**) and methyl group (**27f**) were also introduced at *para* position of phenyl ring, however it did not showed improvement in TNF-α

inhibitory activity. Thus *meta* substitution on phenyl ring was identified as an optimized portion in region 1 and hence further SAR was explored at *meta* position of phenyl ring in region 1.

The most potent compound (**27c**), optimized lead from region 1 modifications was replaced with electron withdrawing group like triflurомethyl (**27g**) and nitro (**27h**) on phenyl ring, but these changes did not show any improvement in TNF-α inhibitory activity. Substitution with halo group like chloro (**27i**) or fluro (**27j**) on phenyl ring, lead to complete loss of activity. However, substitution with aromatic (**27k**) and basic pyridyl group (**27l**) found to be inactive. Demethylated derivatives of compound (**27c**) i.e. compound (**27m**) showed improved activity.

To derive rational for further optimization, we introduced benzene sulfonyl oxy group (**27n**) on phenolic OH of **27m** and **27n** showed improved activity. Literature data also support that substituted sulfonyl class of compounds are known to exhibit excellent TNF-α inhibitory activity [**34**]. The most potent compound **27n** (optimized lead) was substituted with electron withdrawing group like triflurомethyl (**27o**) or 4-chloro groups (**27p**) on benzene sulfonyl oxy group but these changes does not improve TNF-α inhibitory activity. However, electron donating groups like 4-OCF_3 (**27q**) and 4-Me (**27r**) showed improved TNF-α inhibitory activity. Substitution with 2,4,6-trimethyl (**27s**) on benzene ring showed similar TNF-α inhibitory activity as **27r**.

Then we started evaluating SAR at region 2, by keeping *meta* position substituted with 4-methyl sulfonyloxy group as optimized region 1. We replaced

phosphonate group of our most potent compound (**27r**) by hydrogen (**27t**), carboxy ethyl ester (**27u**) or cyano (**27v**) groups and these changes showed complete loss of TNF-α inhibitory activity, which indicates that phosphate group is essential for TNF-α inhibitory activity.

From above SAR study, out of twenty-three compounds prepared, short listed compounds **27r** and **27s** showed satisfactory TNF-α inhibitory activity. Out of these two compounds (**27r** and **27s**), compound **27r** was subjected for TNF-α inhibitory activity, (*in vivo)* and single dose PK studies.

3.3.4 In vivo TNF-α inhibitory activity and PK studies of selected 27r

In vivo TNF-α inhibitory activity was carried out for **27r** as a representative compound from this series. Initially *in vivo* TNF-α inhibitory activity of **27r** was assessed at 10 and 30 mpk orally and at these doses, **27r** showed no TNF-α inhibitory activity. Further to conformed *in vivo* activity, **27r** was administered parentally (subcutaneous (sc) route of administration) at 10 mpk dose and it showed moderate *in vivo* activity (~35% TNF-α inhibitory activity with **27r**).

In order to understand difference in the oral vs sc *in-vivo* TNF-α inhibitory activity, a single dose (10 mpk) PK study of **27r** was carried out both by oral and sc route of administration in male WR and various PK parameters (detail experimental protocol is given in experimental section 5.3) were recorded, **Table 29** [**16**].

Table 29. PK parameters of **27r**

ROA	PK Parameters[#]	Compd 27r
Sc route	T_{max} (h)	0.23±0.05
	$T_{1/2}$ (h)	1.0 ±0.04
	Kel (h^{-1})	0.3±0.07
	AUC (h ng/ml)	1234±19
PO	No levels	

#Single dose (10 mpk; sc/po route) PK study of **27r** was carried out in fasted male wistar rats (n=6) and plasma concentrations were determined by LC-MS/MS, data represented as M±SD, ROA:-Route of administration.

As shown in **Table 29**, **27r** showed rapid T_{max}, short $T_{1/2}$, rapid clearance and moderate AUC, when administered via subcutaneous route of administration. Whereas orally no levels was found with compound **27r**, which indicates that compound **27r** exhibits poor metabolic stability or there could be concern with its oral absorption.

In order to confirm these facts, *ex vivo* stability of **27r** was carried out in simulated gastric fluid (SGF) and stimulated intestinal fluid (SIF), blood and plasma. In general, compound **27r** was incubated in SGF, SIF, blood and plasma for 24 hrs at RT and MS of parent's peak and metabolites were monitored by LC-MS. In *ex vivo* stability studies **27r** showed formation of 2 metabolites in SIF and blood, with in 30 min (>90% metabolism).

As shown in **Figure 28**, in the first path (path A), phosponate ester group and carboxy ester group of compound **27r** may get hydrolyzed within 30 min and produce the acid metabolite **A**. In the second path (path B), *O*-tosylate group of compound **27r** may get cleaved and produce the hydroxyl metabolite **B**. Using LC-MS method, all the 2 possible metabolites (**A** and **B**) of compound **27r** were

confirmed.

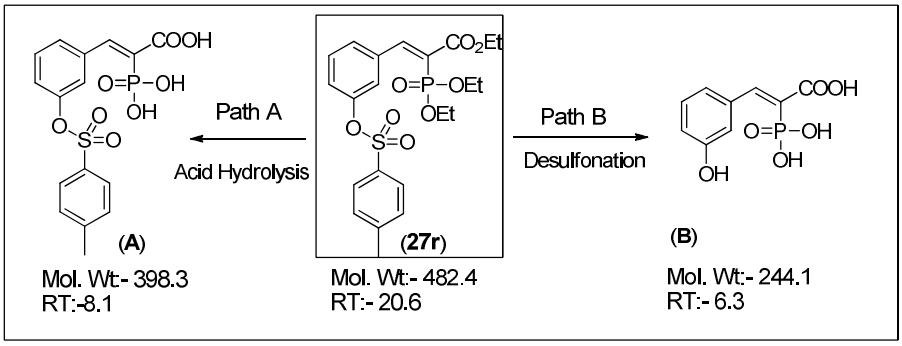
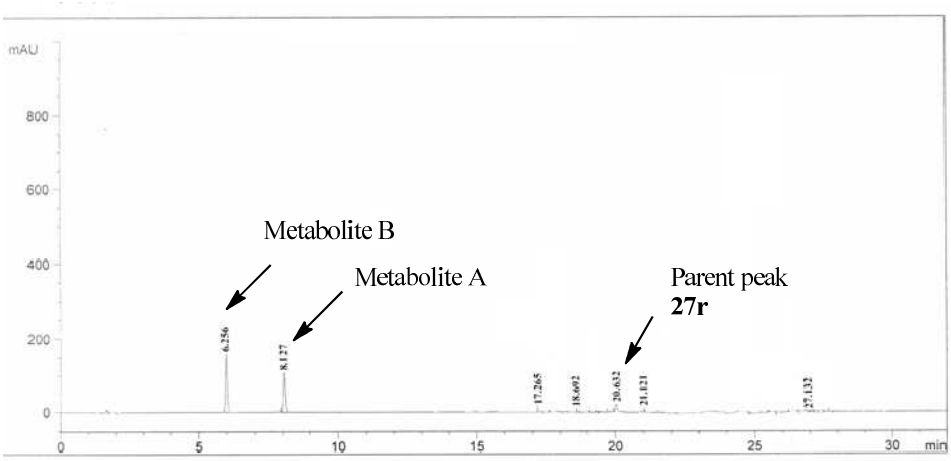


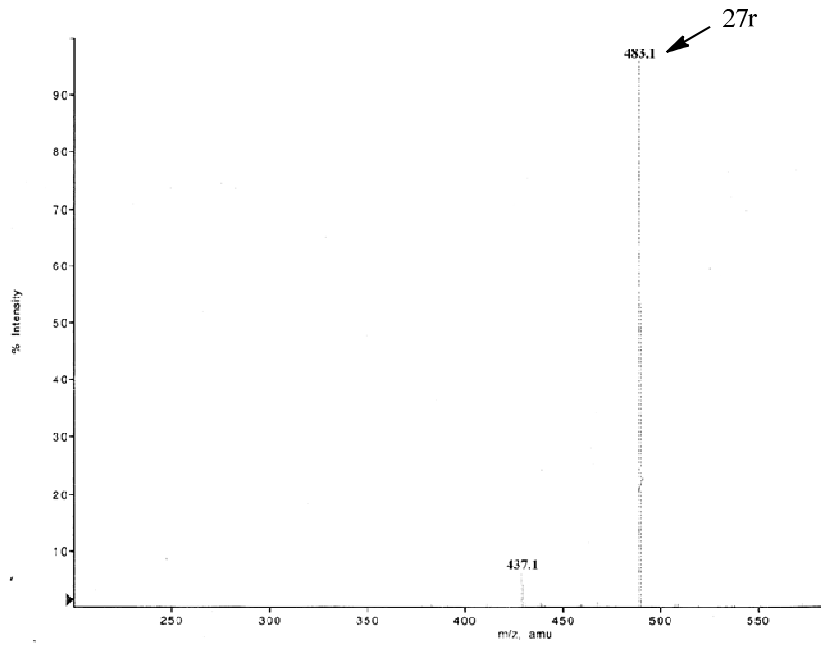
Figure 28. Various possible metabolite paths of compound **27r**

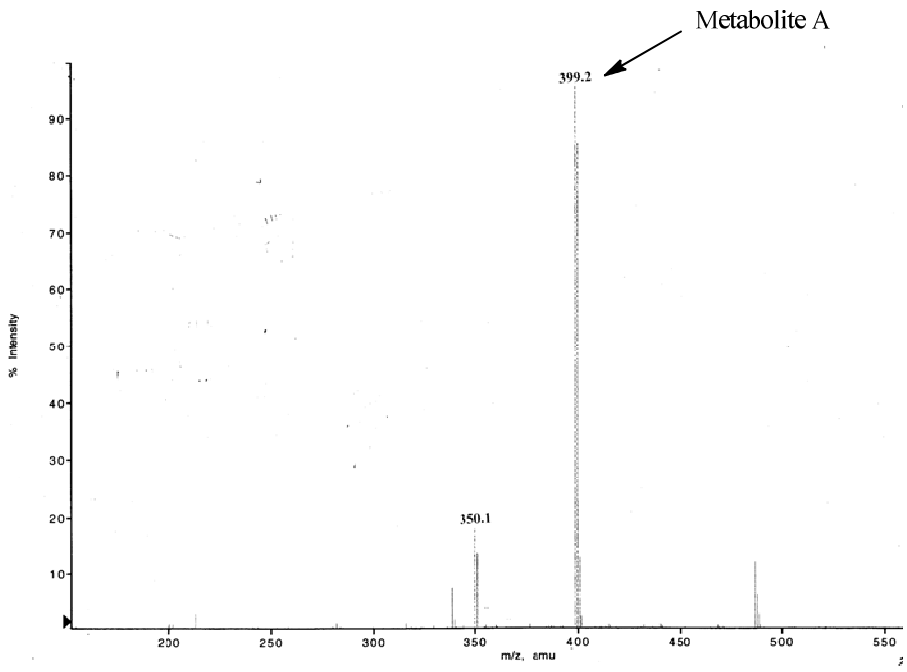


RP-HPLC conditions: Beckman Gold Nouveau System equipped with a 168 photodiode array detector. Column; Vydac 218TP53 (C_{18}, 300 ^{0}A, 5 min 3.2 mm i.d. x 250 mm). 0.5 mL min^{-1}, Buffer A = 0.1% TFA in water. Buffer B = 90 % ACN (acetonitrile) containing 10 % buffer A. Linear gradient from 0% B to 90% B over 30 min, total run time 50 min. Retention time (Rt) for parent peak 20.6 min, metabolites A and B Rt = 8.1 and 6.3 min respectively.

Figure 29. Analytical reverse phase HPLC of compound **27r** and its metabolites.

In LC-MS, parent compound **27r** showed peak at 20.6 min (**Figure 29**) corresponds with its mol. Wt 482.4, while metabolite **A** and **B**, elute at RT 8.1, 6.3 respectively (much polar than parent compound **27r**) with MS (399.2 and 245.1) which corresponds with calculated masses (observed masses, **Figure 30**) and were found to be in with the hydrolysis product of compound **27r**, which confirms the formation of metabolite **A** and **B** respectively.


27r
485.1
437.1
% Intensity
m/z, amu


Metabolite A
399.2
350.1
% Intensity
m/z, amu

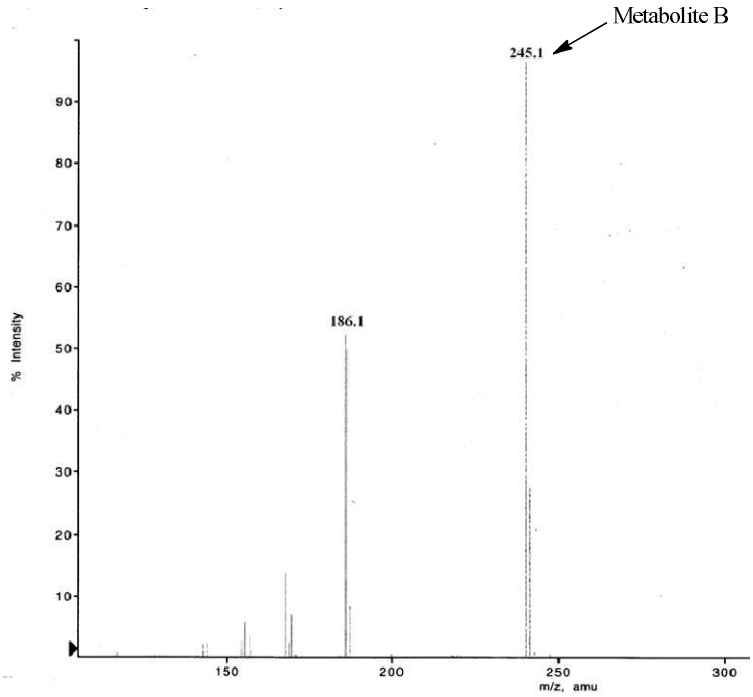


Figure 30. LC-MS of compound **27r** and its metabolites (A and B). Expected molecular weight of compound **27r** [483.1, M+H], metabolites A and B [399.2 and 245.1, M+H]

Thus *in vivo* after oral administration compound **27r** did not show TNF-α inhibitory activity and PK due to its poor metabolic stability in SIF and blood. Further modification is required in compound **27r** to address its metabolic stability and oral PK/PD.

3.3.5 Conclusion

In conclusion, in the third series, we reported discovery and preliminary SAR of carboxy-phosphonoacetate derivatives as novel TNF-α inhibitors. As described earlier carboxy-phosphonoacetate derivatives (compound **1**, preliminary hit) was identified through HTS screening and further by carrying out suitable changes at R1 and R2 regions of compound **1**, we identified progressive lead, compound **27r**, which showed TNF-α inhibitory activity in nM range (*ex vivo*) and moderate TNF-α inhibition (*in vivo)*, although via sc route of

administration.

Compared to previous two series, compound **27r** was not subjected for further detailed pharmacological evaluation, due to its metabolic instability and oral PK issue. However compound **27r** can be considered as primary lead from this series, which can be converted into clinical candidate by improving its metabolic stability and oral PK, thereby these series expands further scope for suitable structural modifications to treat various inflammatory conditions.

3.3.6 References

1. Campaga, S.; Savage, S.; Bordawekar, S.; Maduskuie, T. P.; Waltermire, R. E.; Desikan, S.; Anderson, S. R. WO:2004/002956.
2. Waltermire, R. E.; Savage, S. A.; Camagna, S.; Magnus, N. A.; Confalone, P. N.; Yates, M.; Maloni, D. J. WO:2003/104220.
3. Duan, J.; Decicco, C. P.; Wasserman, Z. R.; Maduskuie, T. P. WO:1999/018074
4. Ashray, E. S. H. El. Ramadana, H. A. Hamida, M. Hagara, *Synth.Commun.*, **2005**, *35*, 2243-2250.
5. Lohray, B. B.; Lohray, V. B.; Jain, M. R.; Thombare, P. S. WO 2005077937.
6. Priestley, N. D. et al, *Bioorg. Med. Chem. Lett*. **1996**, *4*, 1135-1147.
7. Levin, J. L.; Turos, E.; Weinreb, S. M. *Synth. Commun*. **1982**, *12*, 989-993.
8. Wang, W. B.; Roskamp, E. J. *J. Org. Chem*. **1992**, *57*, 6101-6103.
9. Newton, R. C.; Solomon, K. A.; Covington, M. B.; Decicco, C. P.; Haley, P. J.; Friedman, S. M.; Vaddi, K. *Ann. Rheum. Dis*. **2001**, *60*, 25-32.
10. Jin, G.; Huang, X.; Black, R.; Wolfson, M.; Rauch, C.; McGregor, H.; Ellestad, G.; Cowling, R. *Anal. Biochem*. **2002**, *302*, 269-275.
11. Park, K.; Gopalsamy, A.; Aplasca, A.; Ellingboe, J. W.; Xu, W.; Zhang, Y.; Levin, J. I. *Bioorg. Med. Chem*. **2009**, *17*, 3857-3865.
12. Glide 5.0; Schrodinger, LLC: New York, NY, **2008**.
13. Niu, X.; Umland, S.; Ingram, R.; Beyer, B. M.; Liu, Y. H.; Sun, J.; Lundell, D.; Orth, P. *Arch. Biochem. Biophys*. **2006**, *451*, 43-50.
14. Lukacova, V.; Zhang, Y.; Kroll, D. M.; Raha, S.; Comez, D.; Balaz, S. *J. Med. Chem*. **2005**, *48*, 2361-2370.
15. Wasserman, Z. R.; Duan, J. J. W.; Voss, M. E.; Xue, C. B.; Cherney, R. J.; D. J.; Hardman, K. D.; Decicco, C. P. *Chem. Biol*. **2003**, *10*, 215–223.
16. Mingxin, Q.; Stephen, A. B.; Bernice, B.; Jing-Tao, W.; Rui-Qin, L. *Drug Metab. Dispos*. **2007**, *35*, 1916-1925.
17. Levin, J. I.; Chen, J. M.; Cheung, K.; Cole, D.; Crago, C.; Santos, E. D.; Du, X.; Khafizova, G.; MacEwan, G.; Niu, C.; Salaski, E. J.; Zask, A.; Cummons, T.; Sung, A.; Xu, J. Zhang, Y.; Xu, W.; Ayral-Kaloustian, S.; Jin, G.; Cowling, R.; Barone, D.; Mohler, K. M.; Black, R. A.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2799-2803.
18. Yamazaki, K.; Aiso, S.; Matsumoto, M.; Arito, H.; Nagano, K.; Yamamoto, S.;

Matsushima, T. *Industrial Health*. **2005**, *43*, 597-610.

19. Boone, L.; Meyer, D.; Cusick, P.; Ennulat, D.; Bolliger, A. P.; Everds, N.; Meador, V.; Elliott, G.; Honor, D.; Bounous, D.; Jordan, H. *Vet. Clin. Pathol*. **2005**, *34*, 182–188.
20. Billot, X.; Young, R.; N. WO: 2003/103664.
21. Lee Y.; Eum, H.; Kim, S.; Baek, A.; Son, M.; Lee, K. W.; Whan. S. *Bull. Korean Chem. Soc*. **2010**, *31*(3), 611-614.
22. Commons, T. J.; Fensome, A.; Heffernan, G. D.; Mccomas, C. C.; Woodworth, R. P.; Webb, M. B.; Marella, M. A.; Melenski, E. G. WO: 2008/021309.
23. DasGupta, S.; Murumkar, P. R.; Giridhar, R.; Yadav. M. R. *Bioorg. Med. Chem.* **2009**, *17, 3606*-3617.
24. Fritz, J. A.; Nakhla, J. S.; Wolfe, J. P. *Org Lett*. **2006**, *8(12),* 2531-2534.
25. Robert J.-M. H.; Sabourin, C.; Alvarez, N.; Robert-Piessard, S.; Le Baut, G.; Le Pape, P. *Eur. J. Med. Chem.* **2003**, *38,* 711-718.
26. Nguyen, L.; Niesor, E.; Phan, H.; Machler, T. P.; Ouates, P.; Bogis-Bossey, B. C. USP: 5,043,330, Filed: March 14, **1989**.
27. Ngayen, L.; Niesor, E.; Bentzen, G. *J. Med. Chem.* **1987**, *30*, 1426-1433.
28. Bentzen,C.; Morg,L.; Nyon Niesor, E.; US P: 4309364.
29. Rieusset, J.; Touri, F.; Michalik, L.; Escher, P.; Desuergne, B.; Niesor, E.; Wahli, W. *Mol. Endocrinol*. **2002**, *16(11)*, 2628-2644.
30. Sawa, M.; Kurokawa, K.; Inoue, Y.; Kondo, H.; Yoshino, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2021-2024.
31. Robinson C. N.; Addison, J. F. *J. Org. Chem.* **1966**, *31*, 4325-4326.
32. Lehnert, W. *Tetrahedron.* **1974**, *30(2)*, 301-305.
33. Xu, Y.; Flavin, M. T.; Xu, Z. *J. Org. Chem.* **1996**, *61*, 7697-7701.
34. Matsui, T.; Kondo, T.; Nishita, Y.; Itadani, S.; Nakatani, S.; Omawari, N.; Sakai, N. M.; Ogata, A.; Mori, A. B.; Terai, K.; Kamoshima, W.; Ohno, H.; Obata, T.; Nakai, H.; Todab, M. *Bioorg. Med. Chem.***2002***, 10*, 3757–3786.

Chapter IV: Overall summary and Future plans

CHAPTER IV

4. Overall summary and Future plans

4.1. Overall summary of current investigation

Acute and chronic inflammatory conditions, such as RA and Crohn's disease affects >1% of the adult population worldwide, which occurs mainly due to the excessive production of TNF-α and the selective TNF-α inhibitors, which control the excessive production of TNF-α offers safe and effective mean for treating such inflammatory diseases. From last two decades, several orally active small molecules based TNF-α inhibitors are reported in the literature, however, no TNF-α inhibitor reached to the market, mainly because of toxicity and/or lack of efficacy.

In the present investigation, altogether three series of TNF-α and TACE inhibitors were designed. In first the series (γ-lactam hydroxamates based TACE inhibitors), total twenty three compounds were prepared. In the second series (thiadiazole-imidazolidinones derivatives as TACE inhibitors), total nineteen compounds were prepared. In the third series (carboxy-phosphonate derivatives as TNF-α inhibitors), total twenty three compounds were prepared. Altogether sixty-five compounds were synthesized, purified, characterized and subjected for *ex vivo*, *in vitro*, and *in vivo* TNF-α/TACE inhibitory activity, including PK studies of representative compounds from each series. All the three series were found to be active as TNF-α and TACE inhibitors, with different degree of potency and selectivity.

In the first series, test compounds **18o** and **18p** showed excellent TNF-α inhibition (*in vitro*), along with selectivity over MMPs and AMDM 10, therefore **18o** and **18p** was considered as optimize lead from this series.

Ex vivo and *in vitro* TNF-α inhibitory activity and molecular docking studies results clearly demonstrated that the potency and selectivity of *γ*-lactam hydroxamate based TACE inhibitors can be modulated using suitable substituents at 2nd position of quinoline ring system. Furthermore, it was observed that suitable substituent's at 2nd position contributed significantly towards improvement in the *in vivo* TNF-α inhibitory activity, which could be correlated with its improved oral bioavailability. Finally, in repeat dose acute toxicity study, most potent and selective test compound **18p** showed no adverse changes related to gross pathology, clinical signs and liver toxicity, indicating that the good selectivity profile of new class *γ*-lactam hydroxamate based TACE inhibitors over MMPs and ADAM-10 is essential to overcome hepatotoxicity concerns associated with similar class of TACE inhibitors. Compound **18p** is considered as the promising candidate for safe and effective treatment of acute and chronic inflammations and need to subject for further pre-clinical evaluation.

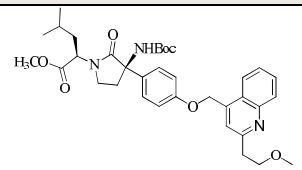
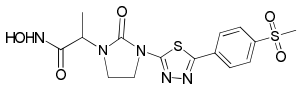
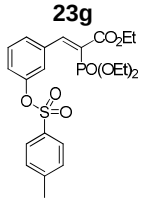
In the second series, our attempt to bioisosterically replace γ-lactam ring of **BMS-561392** with imidazolidinone ring, followed by introducing of thiadiazole based spacer as a replacement of pheoxy group of **BMS-561392**, lead to the developed novel and structurally diverse scaffold as TNF-α and TACE inhibitors. Most potent compound (**23g**) from this series showed nM potency (TNF-α and TACE inhibitory activity), which was found to be comparative weaker than

standard **BMS-561392**. However, it showed improvement in the fold selectivity for TACE against MMPs and ADAM-10 tested. Thus preliminary PK/PD studies results reveals that further modifications in **23g** may lead to the identification of a potent compound from this series, with improved PK /PD profile for the efficient treatment of various inflammatory conditions.

In the third series, the carboxy-phosphonoacetate derivatives (compound **1**, preliminary hit) was identified through HTS screening and further by carrying out suitable changes at R_1 and R_2 region of compound **1**, we identified progress lead compound **27r**, which showed TNF-α inhibitory activity in nM range (*ex vivo*) and moderate TNF-α inhibition (*in vivo)*, although via sc route of administration.

Compared to previous two series, compound **27r** was not subjected further for detailed pharmacological evaluation, due to its metabolic instability and oral PK issue. However compound **27r** can be considered as primary lead from this series, which can be converted into clinical candidate by improving its metabolic stability and oral PK, thereby these series opens further scope for doing suitable structural modifications to treat various inflammatory conditions. The key compounds from each series are listed in **Table 30**.

Table 30. Short listed key compounds from 3 different series

Series	Structure	*Ex vivo* IC_{50} *nM*	*In vitro* IC_{50} *nM*	*In vivo* ED_{50} *(mpk,po)*	PK *(h ng/ml)* AUC
1	 **18o**	13	2.3	3.1	11776
2	 **23g**	427	402	298	234
3	 **27r**	502	-	In active	No levels

4.2. Future plans

Compound **18p** from first series, showed excellent TNF-α and TACE inhibitory activities (*in vitro*, *ex vivo and in vivo)*, The PK and safety profile of **18p** was found to be satisfactory with respect standard compound and therefore **18p** represents a promising candidate for further exploration. Future work includes some additional pre-clinical studies before it has been subjected for clinical development. Compound **18p** should be subjected for chronic efficacy studies and for long term toxicological evaluation, along with its PK profiling in higher animals such as dog or monkey.

In the second series, **23g** showed less *in vitro* TNF-α and TACE inhibitory activities, due to loss of H-bonding with G_{349} and long distance H-bonding with Glu-dyed, as compared to **BMS-561392**. Ph-SO_2- group of compound **23g** expands the S_1 pockets, which was not observed with **BMS-561392**. Probably

this could be the reasons why compound **23g** was found to be more selective against MMPs than **BMS-561392**, which demands further structural modification in P_1, S_1 region of compound **23g**. We propose following future modifications in **23g** (**Figure 31**) to improve its *ex vivo, in vivo* TNF-α and TACE inhibitory activities, PK and PD profiles.

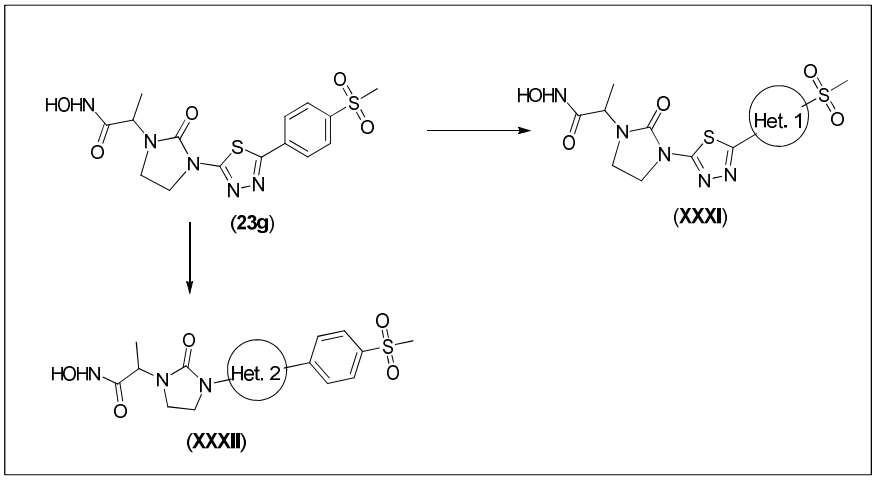


Figure 31. Future plans for series 2 modifications

We suggest, replacing thiadiazole ring of **23g** with suitable hetrocycles such as pyridine, pyrimidine, thiazole, oxadiazole and triazole as spacers (**XXXII**). We also suggest replacing benzene ring of **23g** with suitable hetrocycles such as thiazole, pyridine, triazole, quinoline etc (**XXXI**), which may likely to improve TNF-α inhibitory activity, *in vivo* potency and PK profile.

In case of third series, compound **27r** which was found to be active in *in vitro* TNF-α inhibitory assay but showed poor oral bioavailability. Thus we may need to improve metabolic stability of third series to achieve good oral bioavailability. We propose following future modifications in **27r** (**Figure 32**) to improve it's (*ex vivo and in vivo)* TNF-α and TACE inhibitory activities and PK

and PD profile.

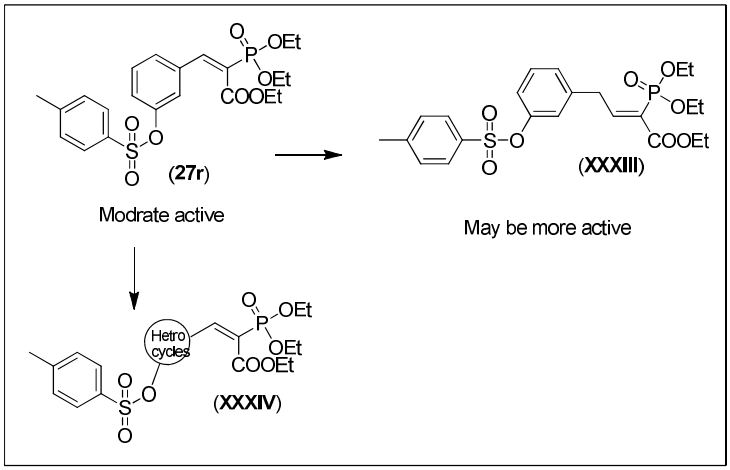


Figure 32. Future plans for series 3 modifications

For further modifications, we suggest, to increase the spacer length with one carbon chain in compound **27r** (**XXXIII**) and also to replace benzene ring with suitable hetrocycles such as pyridine, quinoline, thiazole, tetrazole, oxadiazole etc (**XXXIV**). Thus above modifications may likely to improve overall TNF-α inhibitory activity profile along with improved oral pharmacokinetics.

Chapter V: Experimental

CHAPTER V

5. Experimental

5.1. Chemistry

5.1.1. Materials and Methods

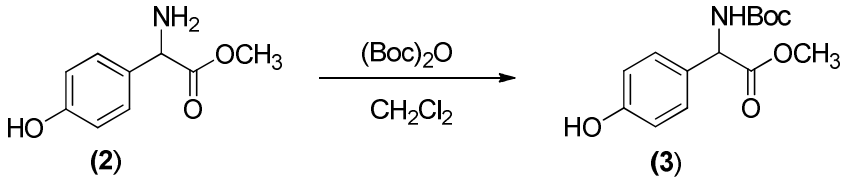
All reagents used were obtained from Sigma Aldrich and were used without further purification. Solvents were procured from commercial source and used after distilling or drying according to the known methods. All the air and/or moisture sensitive reactions were carried out in dry solvents, under nitrogen atmosphere. Melting points were recorded in open glass capillaries, using a scientific melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (νmax 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). ^{13}CNMR spectra were recorded on Bruker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-d_6. Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer. Elemental analyses were carried out using a Perkin-Elmer 2400 CHN analyzer. HPLC analyses were carried out at λ_{max} 220 nm using column ODS C-18, 150nm * 4.6 nm * 4 μ 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). Few compounds directly used for next step without purification and analysis. Detailed synthetic procedures and characterization data of all the final compounds and intermediates are described in the next section.

Experimental details

5.1.2. Methyl2-((*tert*-butoxycarbonyl) amino)-2-(4-hydroxyphenyl)acetate (3)

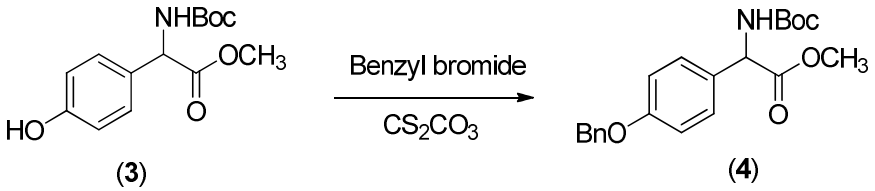


(2) **(3)**

To a solution of methyl 2-amino-2-(4-hydroxyphenyl) acetate (**2**, 25g, 115 mmol) in CH_2Cl_2 (200 mL) was added TEA (36 mL, 253 mmol), followed by $(Boc)_2O$ portion wise, (30g, 138 mmol) with constant stirring. The reaction mixture was stirred at room temperature (25 ^{0}C) for 2h and quenched with water (200 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 100 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the title compound **3** as white solid.

Yield = 90 %; mp 120-122 °C; IR (KBr,cm^{-1}): 3431, 3369, 2956, 1733, 1674, 1506, 1321; ESI (*m*/*z*) 282.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H), 3.60 (s, 3H), 5.01 (d, 1H, *J* = 7.82 Hz), 6.67 (d, 2H, *J* = 8.51 Hz), 7.13 (d, 2H, *J* = 8.54 Hz), 7.59 (d, 1H, *J* = 7.45 Hz), 9.48 (s, 1H).

5.1.3. Methyl2-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)acetate (4)

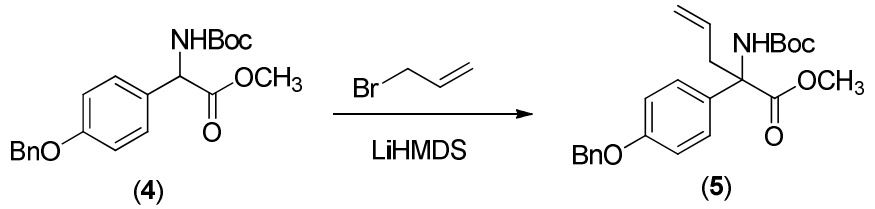


(**3**) (**4**)

To a solution of **3**, (28 g, 99.6 mmol), in toluene (200 mL), was added benzyl bromide (18.7 g, 109.6 mmol) and CS_2CO_3 (49 g, 149.6 mmol) with constant stirring. The reaction mixture was stirred for 3 h at 30 ^{0}C and quenched with water (200 mL). The aqueous layer was extracted with EtOAC (3 x 100 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the title compound **4** as an oil.

Yield = 80 %; IR (KBr,cm^{-1}): 3371, 2976, 2868, 1745, 1666, 1708, 1514; ESI (*m*/*z*) 372.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H), 3.70 (s, 3H), 5.04 (s, 2H), 5.26 (d, 1H, *J* = 7.23 Hz), 5.48 (m, 1H), 6.93 (d, 2H, *J* = 8.72 Hz), 7.25 (d, 2H, *J* = 8.82 Hz), 7.32-7.74 (m, 5H).

5.1.4. Methyl2-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)pent-4-enoate (5)



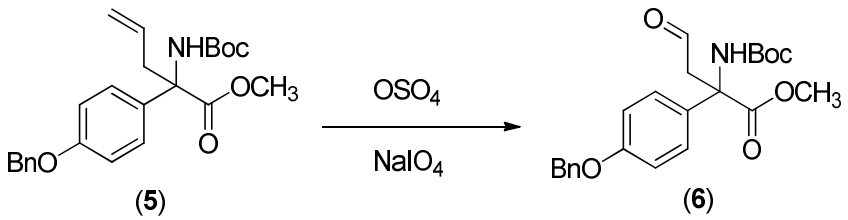
(**4**) (**5**)

To an ice cold solution of **4**, (25 g, 67.3 mmol) in dry THF (200 mL), was added LiHMDS (16.8 g, 100 mmol) drop wise at -60 ^{0}C, with constant stirring. The reaction mixture was stirred at -60 ^{0}C for 1h. Allyl bromide (8.96 g, 74.1 mmol)

was added drop wise at -60 ^{0}C, with constant stirring and further stirred for 1h and quenched with water (100 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 100 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the title compound **5** as white solid.

Yield = 72 %; mp 170-172 °C; Purity: 96 % by HPLC; IR (KBr,cm^{-1}): 3354, 2981, 1730, 1745, 1502, 1245; ESI (*m*/*z*) 412.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 9H), 3.13 (m, 1H), 3.66 (s, 3H), 5.04 (s, 2H), 5.17 (m, 2H), 5.68 (m, 1H), 5.95 (s, 1H), 6.92 (d, 2H, *J* = 8.91 Hz), 7.29-7.50 (m, 5H).

5.1.5. Methyl2-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)-4 oxobutanoate (6)

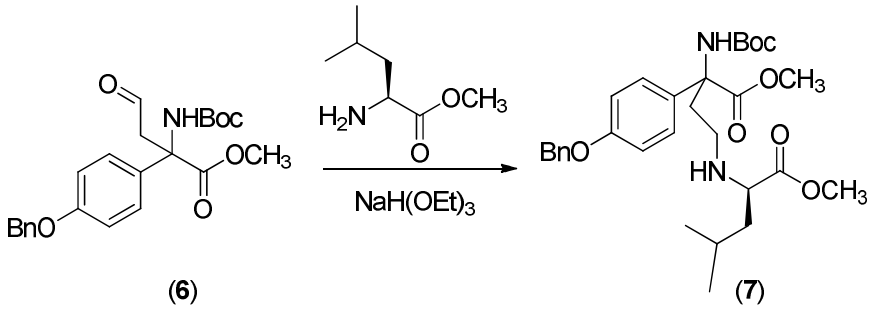


(**5**) (**6**)

To a solution of **5**, (23 g, 56 mmol), in dry THF (200 mL), was added OSO_4 (17g, 67.1 mmol) portion wise at -60 ^{0}C, with constant stirring. The reaction mixture was stirred at 25 ^{0}C for 2h. $NaIO_4$ (18 g, 84 mmol) was added portion wise at 25 ^{0}C, with constant stirring and stirred for 3h and quenched with water (200 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 100 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the title compound **6** as an oil.

Yield = 60 %; ESI (*m*/*z*) 414.1 (M+H). Product was used for next step without purification.

5.1.6. (2R)-Methyl 2-((4-(4-(benzyloxy) phenyl)-4-((tert-butoxycarbonyl) amino)-3-methoxybutyl) amino)-4-methylpentanoate (7)

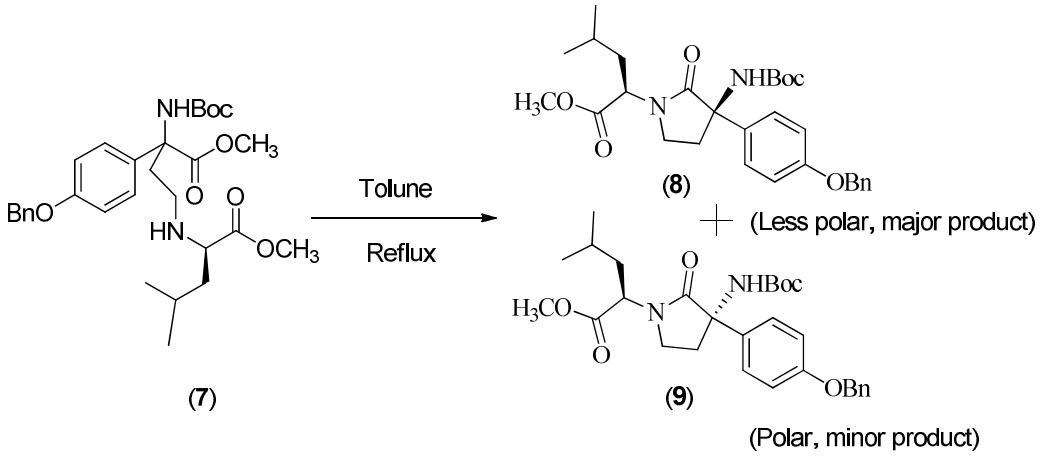


(**6**) (**7**)

To an ice cold solution of **6**, (20 g, 48.4 mmol), in CH_2Cl_2 (150 mL), was added (R)-1-methoxy-4-methylpentan-2-amine (7.7 g, 53.2 mmol) at 25 ^{0}C, with constant stirring. The reaction mixture was stirred at 25 ^{0}C for 2h. $NaH(OEt)_3$ (15.4 g, 72.6 mmol), was added portion wise at 25 ^{0}C, with constant stirring and stirred for 1h and quenched with water (200 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the title compound **7** as an oil.

Yield = 68 %; ESI (*m*/*z*) 543.3 (M+H). Product was used for next step without purification.

5.1.7. ((R)-methyl 2-((R)-3-(4-(benzyloxy) phenyl)-3-((tert-butoxycarbonyl) amino)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (8 and 9)



To a solution of **7**, (15g, 27.6 mmol), in toluene (150 mL), the reaction mixture was refluxed for 3h. The aqueous layer was extracted with EtOAC (3 x 100 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get mixture of disteromers, which were separated by means of flash column chromatography on a silica gel using 20% EtOAC in hexane as eluent to yield the title compounds **8**, (9 g) and **9**, (2 g) as white solid. The physicochemical properties and spectral data of **8** and **9** were found to be in confirmity with the reported data [**1**,**2**] and are listed below.

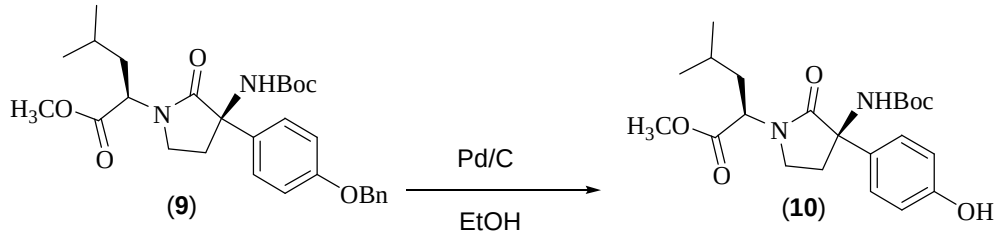
5.1.8. (R)-Methyl 2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (Less polar, major product) (8)

Yield = 63 %; mp 90-92 °C; Purity: 99% by HPLC; IR (KBr,cm^{-1}): 3383, 2929, 1720, 1697, 1514, 1452; ESI (*m*/*z*) 511.4 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.94 (m, 6H), 1.40 (s, 9H), 1.66 (s, 4H), 1.73 (m, 2H), 2.77 (m, 2H), 3.35 (d, 2H, *J* = 5.31 Hz), 3.54 (s, 3H), 4.91 (m, 1H), 5.05 (s, 2H), 5.59 (s, 1H), 6.92 (d, 2H, *J* = 8.86 Hz), 7.28-7.42 (m, 7H).

5.1.9. (R)-Methyl2-((S)-3-(4-(benzyloxy)phenyl)-3-((tert-butoxycarbonyl)amino)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (polar, minor product) (9)

Yield = 14 %; IR (KBr,cm^{-1}): 3382, 2960, 1737, 1697, 1490, 1242; ESI (*m*/*z*) 511.3 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.74 (m, 6H), 1.28 (m, 2H), 1.37 (s, 9H), 2.60 (m, 1H), 3.21 (m, 2H), 3.58 (t, 1H, *J* = 8.55 Hz), 3.70 (s, 3H), 4.76 (t, 1H, *J* = 8.13 Hz), 5.05 (s, 2H), 5.75 (s, 1H), 6.90 (d, 2H, *J* = 6.75 Hz), 7.26-7.44 (m, 7H).

5.1.10. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (10)

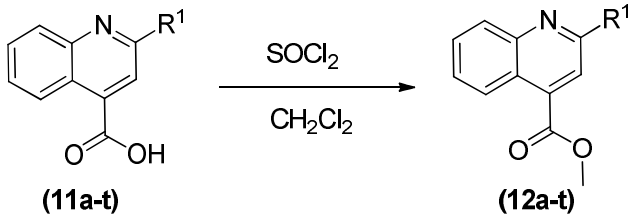


(**9**) (Less polar, major product) (**10**)

To a solution of **8**, (9 g, 17.6 mmol), was dissolved in dry EtOH (50 mL), Pd/C (500 mg) was added portion wise at 25 ^{0}C, (12.8 mmol), with constant stirring. The reaction mixture was hydrogenated at 60 psi pressure for 8h, filtered through hyflow and washed with EtOH (100 mL). The combined organic layer was evaporated under reduced pressure to get the title compound **10** as white solid.

Yield = 72 %; mp 95-97 °C; Purity: 99 % by HPLC; IR (KBr,cm^{-1}): 3367, 3018, 1693, 1515, 1369; ESI (*m*/*z*) 421 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.94 (m, 6H), 1.40 (s, 9H), 1.71 (m, 2H), 3.39 (d, 2H, *J* = 6.24 Hz), 3.49 (s, 3H), 4.91 (m, 1H), 5.61 (s, 1H), 5.82 (s, 1H), 6.68 (d, 2H, *J* = 8.73 Hz), 7.28 (d, 2H, *J* = 8.72 Hz).

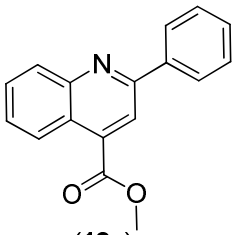
5.1.11. General procedure for the synthesis of methyl 2-substiuted quinoline-4-carboxylate (11a-t)



(11a-t) **(12a-t)**

To an ice cold solution of **11a**-**t**, (1 mole equiv) [**1**,**2**], in CH_2Cl_2 (10 mL), was added $SOCl_2$ (1.2 mole equiv) drop wise, with constant stirring. The reaction mixture was stirred at room temperature (25 ^{0}C) for 30 min, refluxed for 2 h and quenched with MeOH (2 mL). Excess solvents were removed under reduced pressure and the residue obtained was dissolved in 10 % solution of $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the compounds **12a**-**t**. Using above procedure, derivatives of methyl 2-substiuted quinoline-4-carboxylate (**12a**-**t**) were prepared and physicochemical properties and spectral data of some of the representative compounds are listed below.

5.1.11.1. Methyl 2-phenyl quinoline-4-carboxylate (12a)

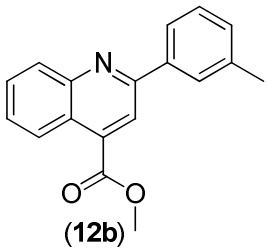


(**12a**)

The title compound was obtained as a white solid, with 82% yield; mp 52-54° C; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 1720, 1591, 1342; ESI (*m*/*z*) 264.1 (M+H);

^{1}H NMR ($CDCl_3$): δ 4.08 (s, 3H), 7.46-7.57 (m, 3H), 7.60 (m, 1H), 7.78 (m, 1H), 8.19 (m, 3H, m), 8.41 (s, 1H), 8.73 (d, 1H, *J* = 11.32 Hz).

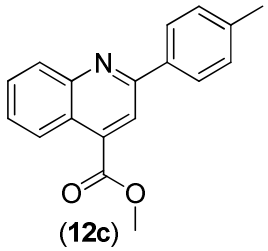
5.1.11.2. Methyl 2-(m-tolyl) quinoline-4-carboxylate (12b)



(**12b**)

The title compound was obtained as an oil, with 83% yield; ^{1}H NMR ($CDCl_3$): δ 2.70 (s, 3H), 4.08 (s, 3H), 7.38 (m, 3H), 7.65 (m, 2H), 8.21 (d, 1H, *J* = 7.55 Hz), 8.44 (d, 1H, *J* = 8.80 Hz), 8.74 (d, 1H, *J* = 7.46 Hz), 9.08 (s, 1H).

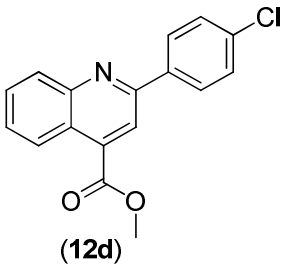
5.1.11.3. Methyl 2-(p-tolyl) quinoline-4-carboxylate (12c)



(**12c**)

The title compound was obtained as an oil, with 82% yield; mp 113° C; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 2943, 1720, 1591, 1504, 1440; ESI (*m*/*z*) 278.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 3H), 4.07 (s, 3H), 7.35 (d, 3H, *J* = 7.98 Hz), 7.63 (t, 1H, *J* = 7.11 Hz), 7.76 (t, 1H, *J* = 7.08 Hz), 8.12 (d, 1H, *J* = 8.10 Hz), 8.20 (d, 1H, *J* = 8.37 Hz), 8.39 (s, 1H), 8.72 (d, 1H, *J* = 8.37 Hz).

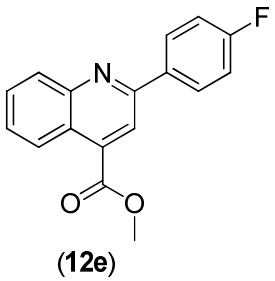
5.1.11.4. Methyl 2-(4-chlorophenyl) quinoline-4-carboxylate (12d)



(**12d**)

The title compound was obtained as an oil, with 77% yield; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 2950, 1724, 1591, 1490, 1342; ESI (*m*/*z*) 282.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 4.08 (s, 3H), 7.50 (d, 2H, *J* = 8.49 Hz), 7.66 (t, 2H, *J* = 7.91 Hz), 7.78 (t, 1H, *J* = 7.08 Hz), 8.18 (m, 2H), 8.37 (s, 1H), 8.75 (d, 1H, *J* = 8.49 Hz).

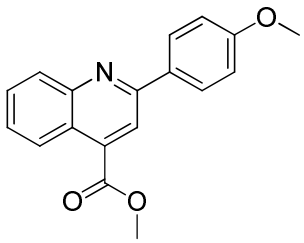
5.1.11.5. Methyl 2-(4-fluorophenyl) quinoline-4-carboxylate (12e)



(**12e**)

The title compound was obtained as an oil, with 86% yield; IR (KBr, cm^{-1}): 3433, 1728, 1608, 1502, 1350; ESI (*m*/*z*) 282.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 4.07 (s, 3H), 7.20 (d, 2H, *J* = 8.64 Hz), 7.63 (t, 1H, *J* = 7.26 Hz), 7.77 (t, 1H, *J* = 7.08 Hz), 8.21 (m, 3H), 8.36 (s, 1H), 7.74 (d, 1H, *J* = 8.55 Hz).

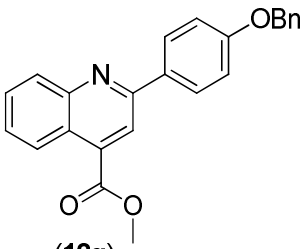
5.1.11.6. Methyl 2-(4-methoxyphenyl) quinoline-4-carboxylate (12f)



(**12f**)

The title compound was obtained as a white solid, with 53% yield; mp 86-88° C; Purity: 89.5 % by HPLC; IR (KBr, cm^{-1}): 3423, 2933, 1726, 1591, 1504, 1346; ESI (*m*/*z*) 294.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H), 4.07 (s, 3H), 7.05 (d, 2H, *J* = 8.68 Hz), 7.57 (t, 1H, *J* = 7.84 Hz), 7.76 (t, 1H, *J* = 9.52 Hz), 8.17 (d, 3H, *J* = 8.35 Hz), 8.36 (s, 1H), 8.72 (d, 1H, *J* = 7.36 Hz).

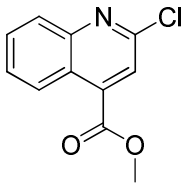
5.1.11.7. Methyl 2-(4-(benzyloxy) phenyl) quinoline-4-carboxylate (12g)



(**12g**)

The title compound was obtained as a white solid, with 80% yield; mp 102-103° C; Purity: 97 % by HPLC; IR (KBr, cm^{-1}): 3433, 1728, 1680, 1502, 1350; ESI (*m*/*z*) 370.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 4.07 (s, 3H), 5.17 (s, 2H), 7.13 (d, 2H, *J* = 7.28 Hz), 7.26-7.43 (m, 5H), 7.60 (t, 1H, *J* = 7.56 Hz), 7.76 (t, 1H, *J* = 7.00 Hz), 8.18 (d, 3H, *J* = 8.49 Hz), 8.36 (s, 1H), 8.72 (d, 1H, *J* = 8.25 Hz).

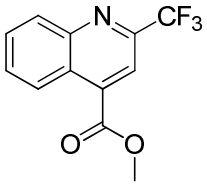
5.1.11.8. Methyl 2-chloro quinoline-4-carboxylate (12h)



(**12h**)

The title compound was obtained as an oil, with 54% yield; Purity: 92 % by HPLC; IR (KBr, cm^{-1}): 3425, 1725, 1594, 1247; ESI (*m*/*z*) 222.03 (M+H).

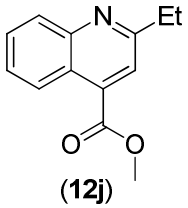
5.1.11.9. Methyl 2-(trifluoromethyl) quinoline-4-carboxylate (12i)



(**12i**)

The title compound was obtained as an oil, with 50% yield; Purity: 90 % by HPLC; IR (KBr, cm^{-1}): 3421, 1724, 1594, 1246; ESI (*m*/*z*) 255.1 (M+H).

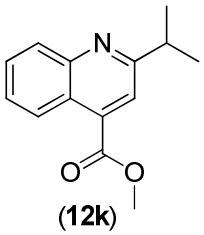
5.1.11.10. Methyl 2-ethyl quinoline-4-carboxylate (12j)



(**12j**)

The title compound was obtained as an oil, with 59% yield; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3429, 1728, 1594, 1243; ESI (*m*/*z*) 215.1 (M+H).

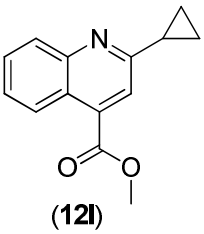
5.1.11.11. Methyl 2-isopropyl quinoline-4-carboxylate (12k)



(**12k**)

The title compound was obtained as an oil, with 45% yield; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 3422, 1724, 1596, 1243; ESI (*m*/*z*) 230.1 (M+H).

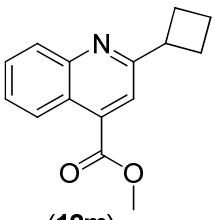
5.1.11.12. Methyl 2-cyclopropyl quinoline-4-carboxylate (12l)



(**12l**)

The title compound was obtained as a white solid, with 76% yield; mp 180-181° C; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 3446, 1728, 1668, 1502,; ESI (*m*/*z*) 228.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.10 (m, 4H), 2.38 (t, 2H, *J* = 5.37 Hz), 3.41 (s, 3H), 7.54 (t, 1H, *J* = 6.92 Hz), 7.72 (t, 1H, *J* = 8.27 Hz), 7.82 (s, 1H), 7.89 (d, 3H, *J* = 8.49 Hz), 8.54 (d, 1H, *J* = 8.45 Hz).

5.1.11.13. Methyl 2-cyclobutyl quinoline-4-carboxylate (12m)

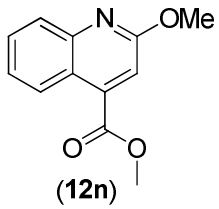


(**12m**)

The title compound was obtained as a white solid, with 35% yield; mp 108-110° C; Purity: 96 % by HPLC; IR (NEAT, cm^{-1}): 2949, 1728, 1593, 1506; ESI (*m*/*z*)

242.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 1.98 (m, 1H), 2.09 (m, 1H), 2.46 (m, 4H), 3.87 (m, 1H), 4.04 (s, 1H), 7.57 (t, 1H, *J* = 7.35 Hz), 7.12 (t, 1H, *J* = 7.05 Hz), 7.83 (s, 1H), 8.10 (d, 1H, *J* = 8.34 Hz), 8.65 (d, 1H, *J* = 8.46 Hz).

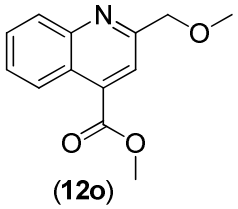
5.1.11.14. Methyl 2-methoxy quinoline-4-carboxylate (12n)



(**12n**)

The title compound was obtained as an oil, with 57% yield; Purity: 92 % by HPLC; IR (NEAT, cm^{-1}): 2949, 1728, 1593, 1506; ESI (*m*/*z*) 218.3 (M+H); ^{1}H NMR ($CDCl_3$): δ 3.57 (s, 3H), 3.82 (s, 3H), 7.50 (t, 1H, *J* = 7.35 Hz), 7.19 (t, 1H, *J* = 7.12 Hz), 7.86 (s, 1H), 8.10 (d, 1H, *J* = 8.34 Hz), 8.62 (d, 1H, *J* = 8.41 Hz).

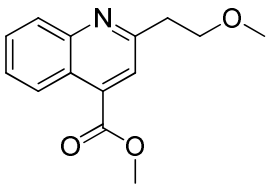
5.1.11.15. Methyl 2-(methoxymethyl) quinoline-4-carboxylate (12o)



(**12o**)

The title compound was obtained as an oil, with 52% yield; Purity: 90 % by HPLC; IR (KBr, cm^{-1}): 3421, 1720, 1593, 1247; ESI (*m*/*z*) 232.3 (M+H).

5.1.11.16. Methyl 2-(2-methoxyethyl) quinoline-4-carboxylate (12p)

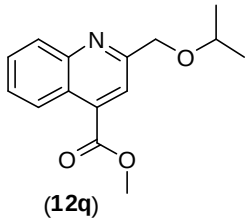


(**12p**)

The title compound was obtained as an oily, in 49% yield; Purity: 91 % by HPLC;

IR (KBr, cm^{-1}): 3423, 1720, 1585, 1245; ESI (*m*/*z*) 246.3 (M+H).

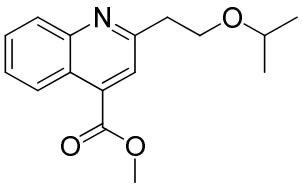
5.1.11.17. Methyl 2-(isopropoxymethyl) quinoline-4-carboxylate (12q)



(**12q**)

The title compound was obtained as an oil, with 32% yield; Purity: 93 % by HPLC; IR (KBr, cm^{-1}): 3413, 1716, 1589, 1257; ESI (*m*/*z*) 260.3 (M+H).

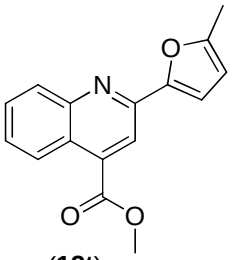
5.1.11.18. Methyl 2-(2-isopropoxyethyl) quinoline-4-carboxylate (12r)



(**12r**)

The title compound was obtained as an oil, with 82% yield; Purity: 92 % by HPLC; IR (KBr, cm^{-1}): 3424, 1721, 1582, 1248; ESI (*m*/*z*) 374.3 (M+H).

5.1.11.19. Methyl 2-(5-methylfuran-2-yl) quinoline-4-carboxylate (12t)

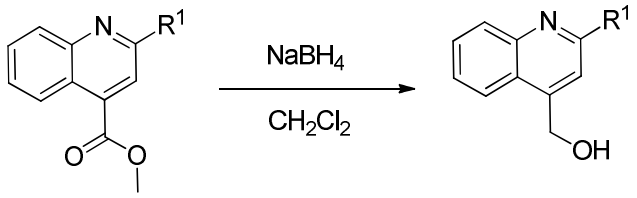


(**12t**)

The title compound was obtained as a white solid, with 90% yield; mp 108-110° C; Purity: 99 % by HPLC; IR ($CHCl_3$, cm^{-1}): 3425, 1722, 1602, 1500; ESI (*m*/*z*) 268.9 (M+H); ^1H NMR ($CDCl_3$): δ 2.47 (s, 3H), 4.07 (d, 1H, *J* = 6.20 Hz), 7.17 (d, 1H, *J* = 11.32 Hz), 7.56 (t, 1H, *J* = 7.29 Hz), 7.70 (d, 1H, *J* = 11.32 Hz), 8.13 (d,

1H, *J* = 8.46 Hz), 8.26 (s, 1H), 8.65 (d, 1H, *J* = 8.66 Hz).

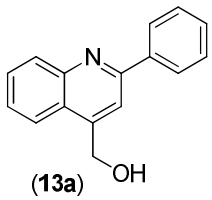
5.1.12. General procedure for the synthesis of methyl 2-substiuted quinoline-4-yl methanol (13a-t)



(**12a-t**) (**13a-t**)

To an ice cold solution of **12a**-**t**, (1 mole equiv), in CH_2Cl_2 (10 mL), $NaBH_4$ (1.2 mole equiv) was added portion wise, with constant stirring. The reaction mixture was stirred at room temperature (25 ^{0}C) for 4h and quenched with water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get compounds **13a**-**t**. Using above procedure, derivatives of methyl 2-substiuted quinoline-4-yl methanol (**13a**-**t**) were prepared and the physicochemical properties and spectral data of some of the representative compounds are listed below.

5.1.12.1. (2-Phenylquinolin-4-yl) methanol (13a)

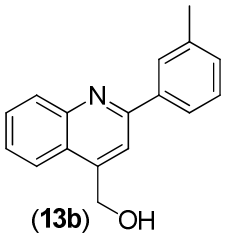


(**13a**)

The title compound was obtained as a white solid, with 78% yield; mp 94-96 °C; Purity: 96 %; IR (KBr, cm^{-1}): 3398, 1602, 1444, 1352, 1093, 1006; ESI (*m*/*z*) 236. (M+H); ^{1}H NMR ($CDCl_3$): δ 5.27 (d, 2H, *J* = 4.92 Hz), 7.44-7.57 (m, 4H), 7.73 (t,

1H, *J* = 7.14 Hz), 7.94 (d, 1H, *J* = 8.37 Hz), 8.01 (s,1H), 8.16-8.22 (m, 3H).

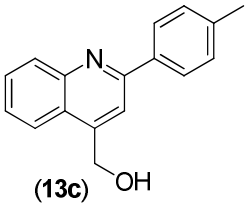
5.1.12.2. (2-(m-Tolyl) quinolin-4-yl) methanol (13b)



(**13b**)

The title compound was obtained as an oil, with 90% yield; Yield = 66 %; ^{1}H NMR ($CDCl_3$): δ 2.21 (s, 3H), 5.25 (s, 2H), 7.18 (s, 2H), 7.55 (s, 1H), 7.70 (m, 1H), 7.82 (m, 3H), 8.26 (s, 1H), 8.98 (s, 1H).

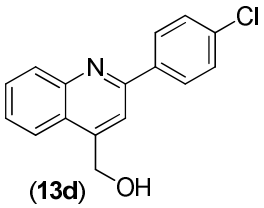
5.1.12.3. (2-(p-Tolyl) quinolin-4-yl) methanol (13c)



(**13c**)

The title compound was obtained as an oil, with 75% yield; Purity: 96 % by HPLC; IR (KBr, cm^{-1}): 3348, 3018, 1728, 1602, 1552, 1506, 1446; ESI (*m*/*z*) 250.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 3H), 5.16 (s, 2H), 7.27 (d, 2H, *J* = 7.92 Hz), 7.55 (t, 1H, *J* = 7.35 Hz), 7.75 (t, 1H, *J* = 7.2 Hz), 7.86 (d, 2H, *J* = 9.48 Hz), 7.90 (d, 2H, *J* = 8.04 Hz), 8.17 (d, 1H, *J* = 8.43 Hz).

5.1.12.4. (2-(4-Chlorophenyl) quinolin-4-yl) methanol (13d)

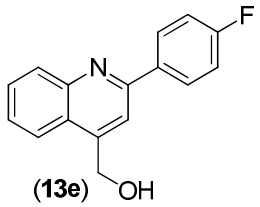


(**13d**)

The title compound was obtained as an oil, with 52% yield; IR (KBr, cm^{-1}): 3246,

3037, 2835, 2345, 1718, 1600, 1492, 1425, 1348; ESI (*m/z*) 270.2 (M+H); ^{1}H NMR ($CDCl_3$): δ 5.26 (s, 2H), 7.26 (m, 2H), 7.55 (m, 1H), 7.76 (m, 1H), 7.90 (m, 2H), 8.10 (d, 2H, *J* = 8.52 Hz), 8.21 (d, 1H, *J* = 6.15 Hz).

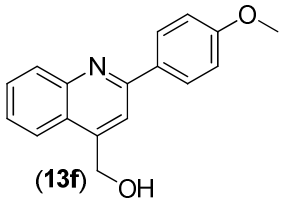
5.1.12.5. (2-(4-Fluorophenyl) quinolin-4-yl) methanol (13e)



(**13e**)

The title compound was obtained as an oil, with 60% yield; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 3433, 3066, 2933, 1595, 1504, 1425, 1350; ^{1}H NMR ($CDCl_3$): δ 5.06 (s, 2H), 7.18 (m, 2H), 7.64 (m, 1H), 7.78 (m, 1H), 7.89 (s, 1H), 8.14 (d, 2H, *J* = 8.52 Hz), 8.18 (m, 3H).

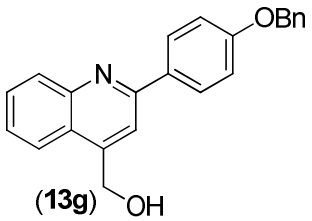
5.1.12.6. (2-(4-Methoxyphenyl) quinolin-4-yl) methanol (13f)



(**13f**)

The title compound was obtained as a white solid, with 92% yield; mp 102-103°C; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 3377, 2999, 1598, 1577, 1548, 1506, 1251, 1172; ESI (*m/z*) 266.1 (M+H); ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 5.18 (s, 2H), 6.99 (d, 1H, *J* = 8.71 Hz), 7.49 (t, 1H, *J* = 7.22 Hz), 7.69 (t, 1H, *J* = 7.13 Hz), 7.86 (d, 2H, *J* = 8.15 Hz), 8.07 (d, 2H, *J* = 8.73 Hz), 8.15 (d, 1H, *J* = 8.40 Hz).

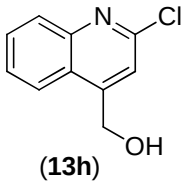
5.1.12.7. (2-(4-(Benzyloxy) phenyl) quinolin-4-yl) methanol (13g)



(**13g**)

The title compound was obtained as a white solid, with 90% yield; mp 98-99 °C; IR (KBr, cm^{-1}): 3448, 3340, 1641, 1604, 1581, 1552, 1421, 1355, 1244 ; ESI (*m*/*z*) 342.2 (M+H); ^{1}H NMR ($CDCl_3$): δ 5.15 (s, 2H), 5.2 (s, 2H), 7.49 (m, 6H), 7.70 (t, 1H, *J* = 8.0 Hz), 7.93 (t, 2H, *J* = 8.48 Hz), 8.16 (t, 3H, *J* = 6.2 Hz).

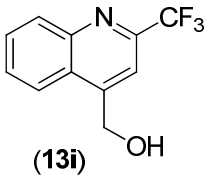
5.1.12.8. (2-Chloroquinolin-4-yl) methanol (13h)



(**13h**)

The title compound was obtained as an oily, with 45% yield; Purity: 90 % by HPLC; IR (KBr, cm^{-1}): 3433, 3066, 2933, 1595, 1504, 1425, 1350; ESI (*m*/*z*) 194.1 (M+H).

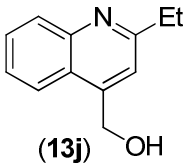
5.1.12.9. (2-(Trifluoromethyl) quinolin-4-yl) methanol (13i)



(**13i**)

The title compound was obtained as an oily, with 56% yield; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3434, 3065, 2936, 1595, 1502, 1427, 1350; ESI (*m*/*z*) 228.1 (M+H).

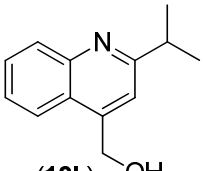
5.1.12.10. (2-Ethylquinolin-4-yl) methanol (13j)



(**13j**)

The title compound was obtained as an oil, with 51% yield; Purity: 92 % by HPLC; IR (KBr, cm^{-1}): 3435, 3069, 2931, 1594, 1506, 1427, 1355; ESI (*m*/*z*) 188.1 (M+H).

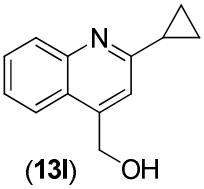
5.1.12.11. (2-Isopropylquinolin-4-yl) methanol (13k)



(**13k**)

The title compound was obtained as an oil, with 45% yield; Purity: 90 % by HPLC; IR (KBr, cm^{-1}): 3437, 3065, 2931, 1597, 1503, 1422, 1358; ESI (*m*/*z*) 202.1 (M+H).

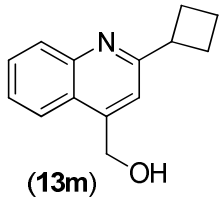
5.1.12.12. (2-Cyclopropylquinolin-4-yl) methanol (13l)



(**13l**)

The title compound was obtained as a white solid, with 89% yield; mp 85-86 °C; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 3446, 3087, 1670, 1610, 1433, 1244, 812; ESI (*m*/*z*) 200.1. (M+H); ^{1}H NMR (DMSO-d_6): δ 1.05 (m, 4H), 2.21 (m, 1H), 2.24 (m, 1H), 5.15 (s, 2H), 7.45 (t, 2H, *J* = 7.21 Hz), 7.63 (t, 1H, *J* = 7.29 Hz), 7.84 (d, 1H, *J* = 8.31 Hz), 7.97 (d, 1H, *J* = 8.55 Hz).

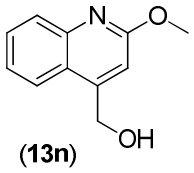
5.1.12.13. (2-Cyclobutylquinolin-4-yl) methanol (13m)



(**13m**)

The title compound was obtained as a white solid, with 85% yield; mp 82-83 °C; Purity: 99 %; IR (KBr, cm^{-1}): 3062, 2935, 1602, 1562; ESI (*m*/*z*) 214.0. (M+H); ^{1}H NMR ($CDCl_3$): δ 1.96 (m, 2H), 2.11 (m, 1H), 2.48 (m, 4H), 3.87 (m, 1H), 5.20 (s, 2H), 7.50 (s, 2H), 7.68 (t, 2H, *J* = 7.08 Hz), 7.89 (d, 1H, *J* = 8.26 Hz), 8.08 (d, 1H, *J* = 8.40 Hz).

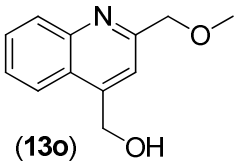
5.1.12.14. (2-Methoxyquinolin-4-yl) methanol (13n)



(**13n**)

The title compound was obtained as an oily, with 78% yield; Purity: 92 %; IR (KBr, cm^{-1}): 3018, 2931, 1600, 1506; ESI (*m*/*z*) 189.08 (M+H).

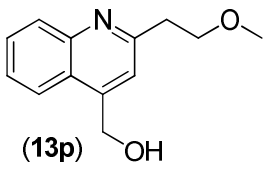
5.1.12.15. (2-(Methoxymethyl) quinolin-4-yl) methanol (13o)



(**13o**)

The title compound was obtained as an oil, with 42% yield; IR (KBr, cm^{-1}): 3143, 2825, 1604, 1568, 1510, 1446, 1346; ESI (*m*/*z*) 203.9 (M+H); ^{1}H NMR (DMSO-d_6) : δ 3.39 (s, 3H), 4.64 (m, 2H), 5.02 (d, 2H, *J* = 7.04 Hz), 5.59 (m, 1H), 7.54 (t, 1H, *J* = 9.83 Hz), 7.70 (m, 2H), 7.98 (t, 2H, *J* = 11.68 Hz).

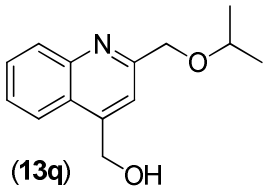
5.1.12.16. (2-(2-Methoxyethyl) quinolin-4-yl) methanol (13p)



(**13p**)

The title compound was obtained as an oil, with 36% yield; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3421, 3018, 1606, 1413, 1089; ESI (*m*/*z*) 218.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 3.15 (t, 2H, *J* = 9.45 Hz), 3.35 (s, 3H), 3.78 (t, 2H, *J* = 7.72 Hz), 5.00 (d, 2H, *J* = 7.21 Hz), 5.55 (d, 1H, *J* = 7.34 Hz), 7.52 (m, 2H), 7.71 (d, 1H, *J* = 9.64 Hz), 7.95 (m, 2H).

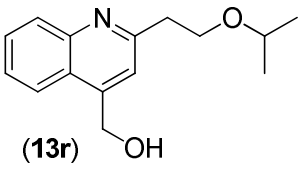
5.1.12.17. (2-(Isopropoxymethyl) quinolin-4-yl) methanol (13q)



(**13q**)

The title compound was obtained as an oil, with 89% yield; IR (KBr, cm^{-1}): 3400, 3192, 1647, 1608; ESI (*m*/*z*) 232.2. (M+H); ^{1}H NMR (DMSO-d_6): δ 1.18 (d, 6H, *J* = 6.07 Hz), 3.67 (m, 1H), 4.74 (s, 2H), 4.98 (d, 2H, *J* = 5.43 Hz), 5.56 (t, 1H, *J* = 5.42 Hz), 7.53 (t, 1H, *J* = 7.34 Hz), 7.69 (m, 2H), 7.94 (d, 1H, *J* = 8.53 Hz), 7.98 (d, 1H, *J*= 8.35 Hz).

5.1.12.18. (2-(2-Isopropoxyethyl) quinolin-4-yl) methanol (13r)

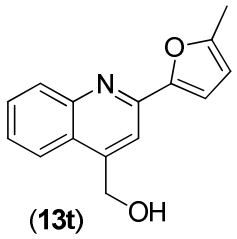


(**13r**)

The title compound was obtained as an oil, with 62% yield; Purity: 93 %; IR (KBr,

cm^{-1}): 3199, 1670, 1606, 1510; ESI (*m*/*z*) 246.2 (M+H).

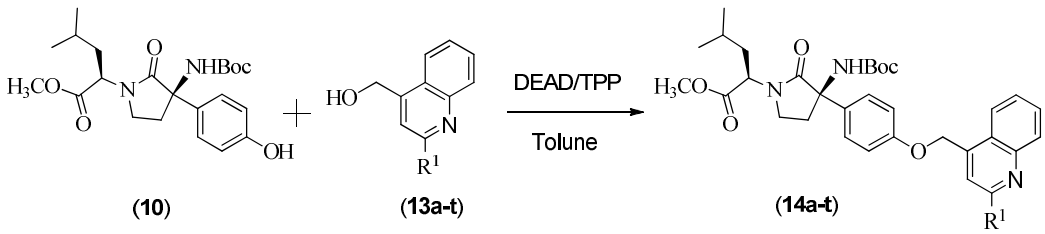
5.1.12.19. (2-(5-Methylfuran-2-yl) quinolin-4-yl) methanol (13t)



(**13t**)

The title compound was obtained as a white solid, with 78% yield; mp 138-140 °C; Purity: 98 %; IR (KBr, cm^{-1}): 3244, 1606, 1532, 1311; ESI (*m*/*z*) 240.2. (M+H); ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H), 5.02 (d, 2H, *J* = 5.37 Hz), 5.61 (t, 1H, *J* = 5.52 Hz), 6.33 (d, 2H, *J* = 3.18 Hz), 7.52 (t, 1H, *J* = 7.23 Hz), 7.15 (t, 1H, *J* = 7.44 Hz), 7.95 (m, 3H).

5.1.13. General procedure for the synthesis of ester derivatives (14a-t)

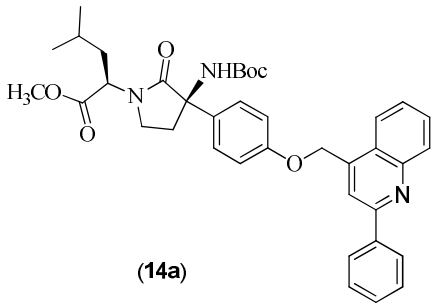


(**10**) (**13a-t**) (**14a-t**)

To a stirred solution of phenol derivative of **10**, (1 mole equiv) and alcohol derivatives **13a**-**t**, (1 mole equiv), in dry toluene (15 mL), TPP (1 mole equiv) and DEAD, (1.2 mole equiv) was added at 0-5 ^{0}C under N_2. The reaction mixture was stirred at room temperature (25 ^{0}C) for 16 h, quenched with ice cold-water (50 mL) and extracted with EtOAC (3 x 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get oily compound. The crude product was purified by column chromatography, using a mixture of EtOAC and hexane (1:3) as an eluant to get ester derivatives **14a**-**t**.

Using above procedure, compounds **14a**-**t** were prepared and the physicochemical properties and spectral data of some of the representative compounds are listed below.

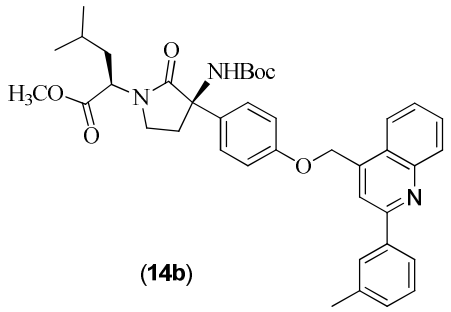
5.1.13.1. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-2-oxo-3-(4-((2-phenylquinolin-4-yl)methoxy)phenyl)pyrrolidin-1-yl)-4-methylpentanoate (14a)



(**14a**)

The title compound was obtained as an oil, with 85% yield; IR (KBr, cm^{-1}): 3411, 2960, 11751, 1699, 1604; ESI (*m*/*z*) 638.4 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.96 (m, 6H), 1.50 (s, 9H), 1.78 (m, 2H), 2.75 (m, 1H), 2.88 (m, 1H), 2.37 (m, 1H), 3.28 (m, 1H), 3.58 (s, 3H), 4.91 (m, 1H), 5.58 (s, 2H), 5.60 (s, 2H), 7.03 (d, 2H, *J* = 7.0 Hz), 7.45-7.55 (m, 4H), 7.73 (t, 1H, *J* = 7.02 Hz), 7.97 (d, 1H, *J* = 7.05 Hz), 8.14 (d, 2H, *J* = 7.08 Hz), 8.22 (d, 1H, *J* = 8.4 Hz).

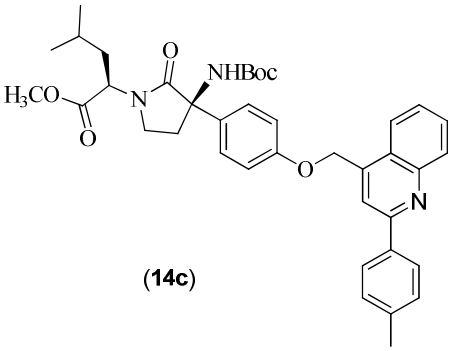
5.1.13.2. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-2-oxo-3-(4-((2-(m-tolyl)quinolin-4-yl)methoxy)phenyl)pyrrolidin-1-yl)-4-methylpentanoate (14b)



(**14b**)

The title compound was obtained as a white solid, with 57% yield; mp 83-85 °C; Purity: 97 % by HPLC; IR (KBr, cm^{-1}): 3421, 2925, 1751, 1710, 1654, 1163, 1018; ESI (*m*/*z*) 652.4 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.96 (m, 6H), 1.41 (s, 3H), 1.41 (s, 9H), 1.72 (m, 2H), 2.48 (s, 3H), 3.54 (s, 3H), 5.62 (s, 1H), 7.03 (d, 2H, *J* = 8.82 Hz), 7.43 (m, 2H), 7.48 (m, 2H), 7.75 (t, 1H, *J* = 7.91 Hz), 7.89 (t, 2H, *J* = 7.76 Hz), 7.99 (d, 2H, *J* = 5.29 Hz), 8.24 (d, 1H, *J* = 8.32 Hz).

5.1.13.3. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-2-oxo-3-(4-((2-(p-tolyl)quinolin-4-yl)methoxy)phenyl)pyrrolidin-1-yl)-4-methylpentanoate (14c)

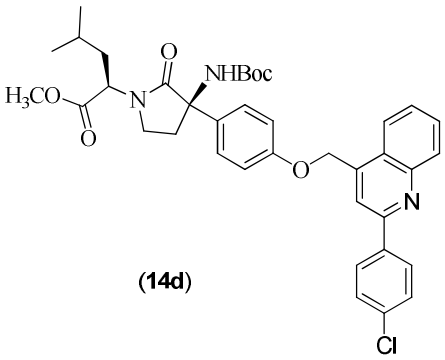


(**14c**)

The title compound was obtained as an oil, with 49% yield;^{1}H NMR (DMSO-d_6): δ

0.93 (m, 6H), 1.12 (d, 2H, *J* = 8.12 Hz), 1.76 (s, 9H), 2.42 (s, 3H), 3.34 (m, 2H), 3.36 (s, 3H), 4.90 (t, 1H, *J* = 8.25 Hz), 5.56 (s, 2H), 7.07 (d, 2H, *J* = 8.76 Hz), 7.32 (d, 2H, *J* = 7.8 Hz), 7.53 (d, 2H, *J* = 8.79 Hz), 7.60 (m, 1H), 7.78 (t, 1H, *J* = 7.23 Hz), 7.76 (d, 1H, *J* = 8.13 Hz), 8.06 (m, 3H), 8.31 (d, 1H, *J* = 8.4 Hz).

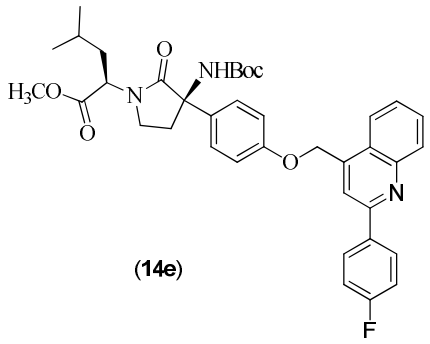
5.1.13.4. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(4-chlorophenyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14d)



(**14d**)

The title compound was obtained as an oil, with 67% yield; IR (KBr, cm^{-1}): 3419, 2956, 1743, 1701, 1604, 1490, 1431; ESI (*m/z*) 672 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 6H), 1.40 (s, 9H), 1.75 (m, 2H), 2.26 (m, 1H), 7.79 (m, 1H), 2.89 (m, 1H), 3.4 (d, 2H, *J* = 7.89 Hz), 3.55 (s, 3H), 4.91 (m, 1H), 5.57 (s, 2H), 5.62 (s, 1H), 7.03 (d, 2H, *J* = 8.76 Hz), 7.48 (m, 4H), 7.58 (t, 1H, *J* = 7.14 Hz), 7.76 (t, 1H, *J* = 7.35 Hz), 8.12 (d, 2H, *J* = 8.46 Hz), 8.20 (d, 1H, *J* = 8.31 Hz).

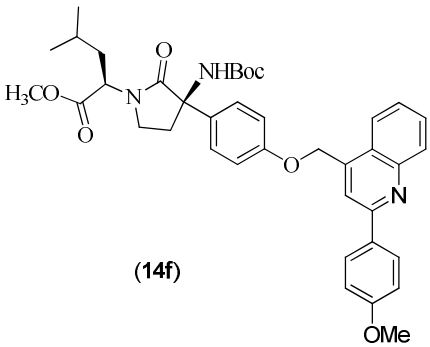
5.1.13.5. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(4-fluorophenyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14e)



(14e)

The title compound was obtained as an oill, with 46% yield; IR (KBr, cm^{-1}): 2956, 2869, 2927, 1741, 1701, 1604, 1488; ESI (*m*/*z*) 656 (MH^+); ^1H NMR ($CDCl_3$): δ 0.88 (d, 6H, *J* = 6.24 Hz), 1.28 (s, 3H), 1.40 (s, 9H), 1.73 (m, 2H), 3.38 (m, 1H), 3.38 (m, 1H), 3.67 (s, 3H), 4.91 (s, 1H), 5.57 (s, 2H), 5.62 (s, 1H), 7.03 (d, 2H, *J* = 8.7 Hz), 7.20 (t, 1H, *J* = 7.47 Hz), 7.76 (t, 1H, *J* = 7.20 Hz), 7.96 (d, 2H, *J* = 9.75 Hz), 8.16 (m, 2H), 8.18 (m, 3H).

5.1.13.6. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(4-methoxyphenyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14f)

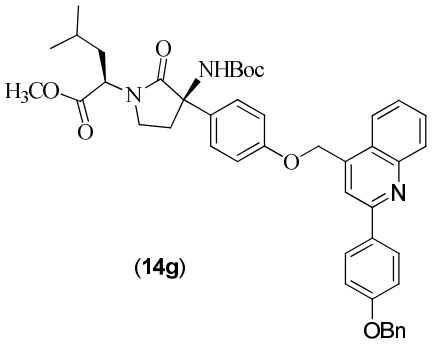


(14f)

The title compound was obtained as an oil, with 74% yield; IR (KBr, cm^{-1}): 3413,

3018, 1751, 1697, 1604, 1583, 1552; ESI (*m*/*z*) 668.7 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.94 (m, 6H), 1.15 (s, 3H), 1.42 (s, 9H), 1.75 (m, 2H), 3.39 (m, 1H), 3.48 (s, 2H), 3.79 (t, 1H, *J* = 4.02 Hz), 3.88 (s, 3H), 4.95 (s, 1H), 5.56 (s, 2H), 5.65 (s, 2H), 7.47 (d, 2H, *J* = 8.3 Hz), 7.55 (t, 1H, *J* = 7.29 Hz), 7.75 (t, 1H, *J* = 7.23 Hz), 7.97 (t, 2H, *J* = 6.18 Hz), 8.13 (d, 2H, *J* = 8.9 Hz), 8.19 (d, 1H, *J* = 8.28 Hz).

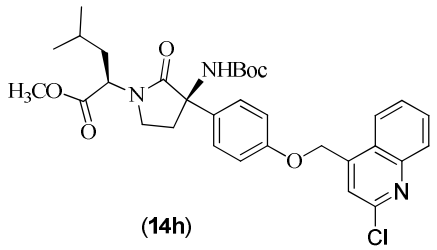
5.1.13.7. (R)-Methyl2-((R)-3-(4-((2-(4-(benzyloxy)phenyl)quinolin-4-yl) methoxy)phenyl)-3-((tert-butoxycarbonyl)amino)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14g)



(**14g**)

The title compound was obtained as an oil, with 63% yield; IR ($CHCl_3$, cm^{-1}): 3413, 2954, 1741, 1699, 1602, 1581, 1550, 1488; ESI (*m*/*z*) 744.0 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.97 (m, 6H), 1.17 (m, 2H), 1.25 (s, 9H), 1.75 (m, 2H), 2.79 (m, 2H), 3.37 (d, 2H, *J* = 7.72 Hz), 3.54 (s, 3H), 3.88 (s, 3H), 5.29 (s, 1H), 5.56 (s, 2H), 5.62 (s, 1H), 7.03 (d, 2H, *J* = 8.71 Hz), 7.11 (d, 1H, *J* = 8.65 Hz), 7.32 (d, 1H, *J* = 6.93 Hz), 7.41 (d, 2H, *J* = 6.96 Hz), 7.47 (d, 3H, *J* = 8.45 Hz), 7.55 (t, 1H, *J* = 7.36 Hz), 7.40 (t, 1H, *J* = 7.21 Hz), 7.96 (t, 2H, *J* = 8.33 Hz), 8.18 (m, 3H).

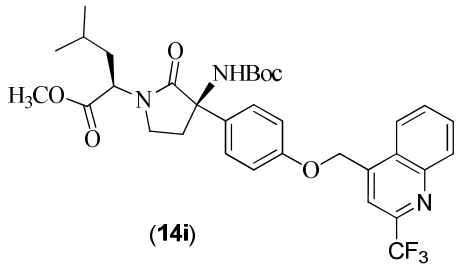
5.1.13.8. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-chloroquinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14h)



(**14h**)

The title compound was obtained as an oil, with 45% yield; ESI (*m*/*z*) 596.2 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.91 (m, 6H), 1.12 (m, 2H), 1.41 (s, 9H), 2.20 (m, 2H), 3.38 (m, 1H), 3.52 (s, 3H), 5.04 (m, 3H), 5.43 (m, 1H), 6.42 (s, 2H), 6.85 (d, 2H, *J* = 8.24 Hz), 7.43 (s, 1H), 7.65 (d, 2H, *J* = 8.15 Hz), 7.86 (m, 1H), 7.89 (d, 1H, *J* = 8.23 Hz), 8.09 (m, 1H).

5.1.13.9. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-2-oxo-3-(4-((2-(trifluoromethyl)quinolin-4-yl)methoxy)phenyl)pyrrolidin-1-yl)-4-methyl pentanoate (14i)

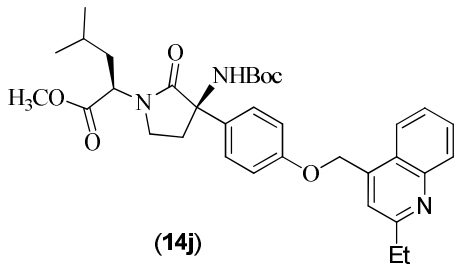


(**14i**)

The title compound was obtained as an oil, with 58% yield; ESI (*m*/*z*) 630.2 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.98 (m, 6H), 1.15 (m, 2H), 1.44 (s, 9H), 2.26 (m, 2H), 3.31 (m, 1H), 3.46 (s, 3H), 5.01 (m, 3H), 5.40 (m, 1H), 6.32 (s, 2H), 6.75 (d, 2H, *J* = 8.24 Hz), 7.41 (s, 1H), 7.62 (d, 2H, *J* = 8.12 Hz), 7.83 (m, 1H), 7.84 (d, 1H, *J* =

8.23 Hz), 8.03 (m, 1H).

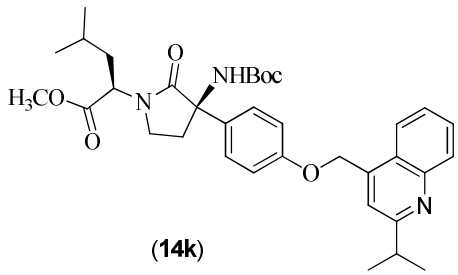
5.1.13.10. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-ethyl quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (14j)



(**14j**)

The title compound was obtained as an oil, with 35% yield; ESI (*m*/*z*) 590.2 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.97 (m, 6H), 1.35 (t, *J* = 7.22 Hz), 1.15 (m, 2H), 1.45 (s, 9H), 2.21 (m, 2H), 2.25 (q, 2H, *J* = 6.98 Hz), 3.40 (m, 1H), 3.41 (s, 3H), 5.05 (m, 3H), 5.45 (m, 1H), 6.42 (s, 2H), 6.65 (d, 2H, *J* = 8.24 Hz), 7.47 (s, 1H), 7.60 (d, 2H, *J* = 8.12 Hz), 7.83 (m, 1H), 7.84 (d, 1H, *J* = 8.23 Hz), 8.05 (m, 1H).

5.1.13.11. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-isopropylquinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14k)

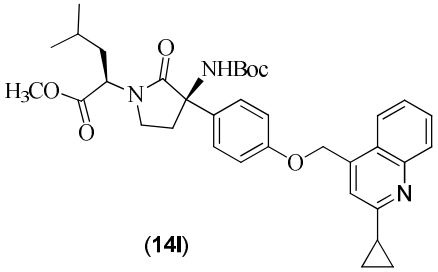


(**14k**)

The title compound was obtained as an oil, with 39% yield; ESI (*m*/*z*) 604.3 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.98 (m, 6H), 1.05 (m, 3H), 1.35 (m, 6H), 1.42 (s, 9H), 2.20 (m, 2H), 2.21 (m, 2H), 3.45 (m, 1H), 3.38 (m, 1H), 3.54 (s, 2H), 5.02 (m,

3H), 5.46 (m, 1H), 6.32 (s, 2H), 6.99 (d, *J* = 8.25 Hz), 7.32 (s, 1H), 7.65 (d, 2H, *J* = 8.15 Hz), 7.86 (m, 1H), 7.89 (d, 1H, *J* = 8.24 Hz), 8.04 (m, 1H).

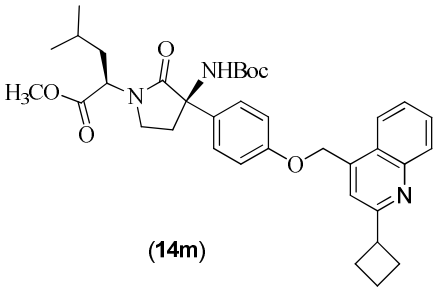
5.1.13.12. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-cyclopropylquinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14l)



(**14l**)

The title compound was obtained as an oil, with 50% yield; ESI (*m*/*z*) 602.3 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.97 (m, 6H), 1.09 (m, 3H), 1.41 (s, 9H), 2.20 (m, 2H), 2.21 (m, 2H), 3.38 (m, 1H), 3.57 (s, 2H), 5.00 (m, 3H), 5.47 (m, 1H), 6.31(s, 2H), 6.99 (d, *J* = 8.23 Hz), 7.32 (s, 1H), 7.66 (d, 2H, *J* = 8.14 Hz), 7.86 (m, 1H), 7.88 (d, 1H, *J* = 8.28 Hz), 8.02 (m, 1H).

5.1.13.13. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-cyclobutylquinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (14m)

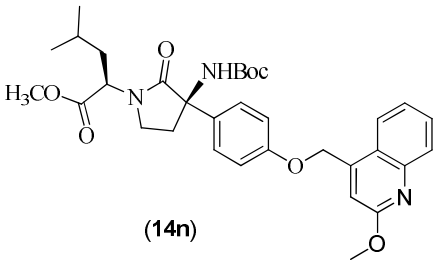


(**14m**)

The title compound was obtained as an oily, in 53% yield; IR ($CHCl_3$, cm^{-1}): 3412, 2951, 1741, 1693, 1608, 1581, 1552, 1488; ESI (*m*/*z*) 616.8 (M+H); ^{1}H NMR

($CDCl_3$): δ 0.92 (m, 6H), 1.06 (m, 3H), 1.43 (s, 9H), 2.24 (m, 2H), 2.22 (m, 2H), 3.39 (m, 1H), 3.56 (s, 2H), 5.02 (m, 3H), 5.48 (m, 1H), 6.33 (s, 2H), 6.99 (d, *J* = 8.82 Hz), 7.31 (s, 1H), 7.66 (d, 2H, *J* = 8.04 Hz), 7.85 (m, 1H), 7.82 (d, 1H, *J* = 8.23 Hz), 8.05 (m, 1H).

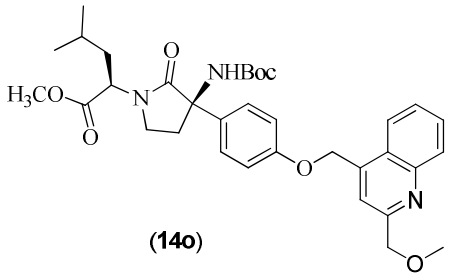
5.1.13.14. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-methoxyquinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14n)



(**14n**)

The title compound was obtained as an oil, with 58% yield; IR ($CHCl_3$, cm^{-1}): 3415, 2956, 1742, 1693, 1609, 1585, 1558, 1487; ESI (*m*/*z*) 592.7 (M+H); ^1H NMR ($CDCl_3$): δ 0.93 (m, 6H), 1.08 (m, 3H), 1.42 (s, 9H), 2.28 (m, 2H), 3.32 (m, 1H), 3.59 (s, 2H), 3.61 (s, 3H), 5.01 (m, 3H), 5.46 (m, 1H), 6.32 (s, 2H), 6.97 (d, *J* = 8.85 Hz), 7.32 (s, 1H), 7.62 (d, 2H, *J* = 8.07 Hz), 7.81 (m, 1H), 7.85 (d, 1H, *J* = 8.26 Hz), 8.06 (m, 1H).

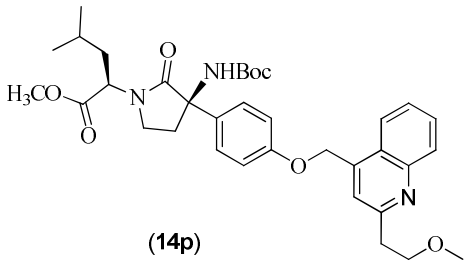
5.1.13.15. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(methoxymethyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (14o)



(**14o**)

The title compound was obtained as an oil, with 53% yield; IR (KBr, cm^{-1}): 3417, 3325, 2956, 2929, 2871, 1174, 1701, 1606; ESI (*m/z*) 605.9 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 6H), 1.25 (m, 2H), 1.40 (s, 9H), 1.62 (m, 2H), 1.72 (m, 2H), 2.78 (m, 2H), 3.35 (m, 2H), 3.41 (s, 3H), 4.73 (s, 2H), 4.91 (m, 1H), 5.41 (s, 2H), 5.62 (s, 1H), 7.05 (d, 2H, *J* = 8.73 Hz), 7.44 (d, 2H, *J* = 8.73 Hz), 7.54 (t, 1H, *J* = 7.17 Hz), 7.76 (m, 2H), 7.98 (d, 1H, *J* = 8.25 Hz), 8.11 (d, 1H, *J* = 8.49 Hz).

5.1.13.16. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(methoxyethyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (14p)

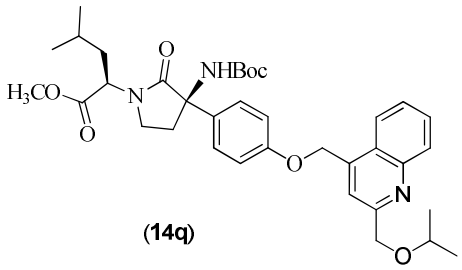


(**14p**)

The title compound was obtained as an oil, with 68% yield; Purity: 94 % by HPLC; IR ($CHCl_3$, cm^{-1}): 3018, 1697, 1608, 1215; ESI (*m/z*) 619.9 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.95 (m, 6H), 1.41 (s, 9H), 1.65 (m, 5H), 1.75 (m, 2H), 3.25

(t, 2H, *J* = 6.66 Hz), 3.58 (s, 3H), 3.85 (t, 2H, *J* = 6.63 Hz), 4.95 (m, 1H), 5.49 (s, 2H), 5.62 (s, 1H), 7.03 (d, 2H, *J* = 8.76 Hz), 7.45 (m, 2H), 7.74 (m, 2H), 7.95 (d, 2H, *J* = 8.25 Hz), 8.08 (d, 2H, *J* = 8.37 Hz).

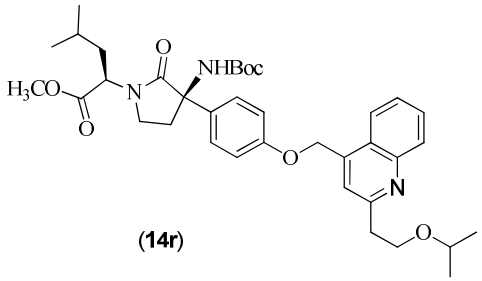
5.1.13.17. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(isopropoxymethyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (14q)



(**14q**)

The title compound was obtained as an oil, with 43% yield; IR (KBr, cm^{-1}): 3414, 3326, 2952, 2920, 2871, 1173, 1705, 1606; ESI (*m*/*z*) 634.8 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H), 1.32 (s, 3H), 137 (s, 3H), 1.52 (s, 9H), 1.76 (m, 2H), 3.32 (m, 2H), 3.62 (s, 2H), 5.45 (s, 2H), 5.63 (s, 1H), 7.02 (d, 2H, *J* = 8.71 Hz), 7.48 (m, 4H), 7.64 (t, 1H, *J* = 7.18 Hz), 7.87 (d, 1H, *J* = 8. 13 Hz), 8.06 (d, 1H, *J* = 8.32 Hz).

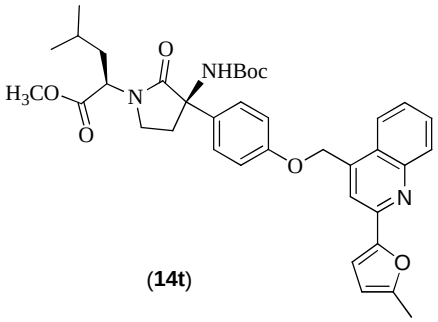
5.1.13.18. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(2-isopropoxyethyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (14r)



(**14r**)

The title compound was obtained as an oil, with 44% yield; Purity: 97 % by HPLC; IR ($CHCl_3$, cm^{-1}): 3018, 1697, 1609, 1215; ESI (*m*/*z*) 648.8 (M+H); ^1H NMR (DMSO-d_6): δ 0.88 (m, 6H), 1.33 (s, 3H), 139 (s, 3H), 1.57 (s, 9H), 1.74 (m, 2H), 3.38 (m, 2H), 3.64 (s, 2H), 5.47 (s, 2H), 5.62 (s, 1H), 7.00 (d, 2H, *J* = 8.79 Hz), 7.48 (m, 4H), 7.64 (t, 1H, *J* = 7.17 Hz), 7.87 (d, 1H, *J* = 8.37 Hz), 8.04 (d, 1H, *J* = 8.37 Hz).

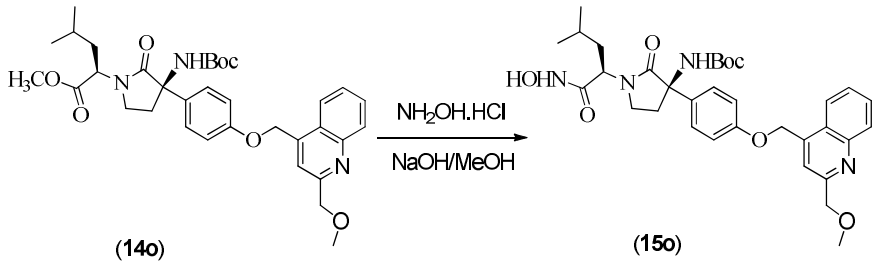
5.1.13.19. (R)-Methyl2-((R)-3-((tert-butoxycarbonyl)amino)-3-(4-((2-(5-methylfuran-2-yl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methyl pentanoate (14t)



(**14t**)

The title compound was obtained as an oil, with 52% yield; Purity: 99 % by HPLC; IR (KBr, cm^{-1}): 3421, 2927, 1712, 1697, 1477; ESI (*m*/*z*) 642.4 (M+H); ^1H NMR ($CDCl_3$): δ 0.97 (m, 6H), 1.25 (s, 2H), 1.40 (s, 9H), 2.46 (s, 3H), 2.75 (m, 1H), 2.88 (m, 1H), 2.39 (m, 2H), 3.55 (s, 3H), 4.92 (m, 1H), 5.51 (s, 2H), 5.62 (s, 1H), 6.19 (d, 1H, *J* = 7.08 Hz), 7.05 (d, 2H, *J* = 8.19 Hz), 7.13 (d, 1H, *J* = 7.12 Hz), 7.46 (m, 3H), 7.71 (t, 1H, *J* = 7.23 Hz), 7.91 (m, 2H), 8.14 (d, 1H, *J* = 8.31 Hz).

5.1.14. General procedure for the synthesis of tert-butyl((R)-1-((R)-1-(hydroxyamino)-4-methyl-1-oxopentan-2-yl)-3-(4-((2-(methoxymethyl) quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-3-yl)carbamate (15o)

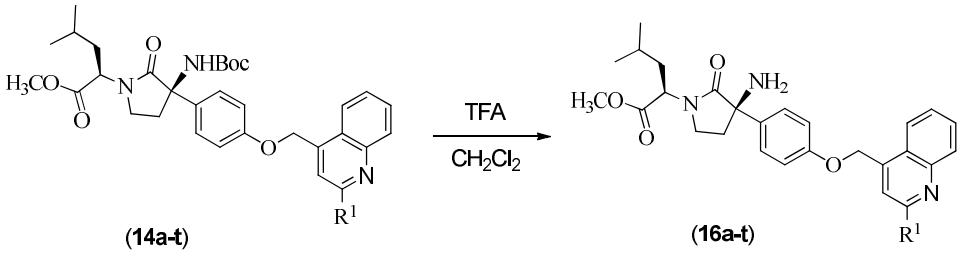


(**14o**) (**15o**)

To a solution of **14o**, (1 mole equiv) in MeOH (20 mL), NH_2OH solution in MeOH (20 mL) was added and the reaction mixture was stirred at 0-5 ^{0}C. Mixture was further stirred for 4 h at room temperature (25 ^{0}C) and quenched with ice-cold water (50 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude hydroxamic acid derivative **15o**, which was purified by column chromatography, using a mixture of $CHCl_3$ and MeOH (1:2) as an eluant to obtain title compound **15o** as an oil.

Yield = 81%; Purity: 94 % by HPLC ; IR (KBr, cm^{-1}): 3246,2929, 1676, 1490,1388; ESI (*m*/*z*) 607.4 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.85 (m, 6H), 1.15 (m, 4H), 1.46 (s, 9H), 1.71 (m, 2H), 3.37 (s, 3H), 4.46 (m, 1H), 4.62 (s, 2H), 5.58 (s, 2H), 7.06 (d, 2H, *J* = 8.67 Hz), 7.35 (d, 2H, *J* = 8.68 Hz), 7.66 (t, 1H, *J* = 8.68 Hz), 7.72 (s, 1H), 7.78 (t, 1H, *J* = 8.66 Hz), 8.03 (d, 1H, *J* = 8.28 Hz), 8.16 (d, 1H, *J* = 8.26 Hz), 8.85 (s, 1H), 10.78 (s, 1H).

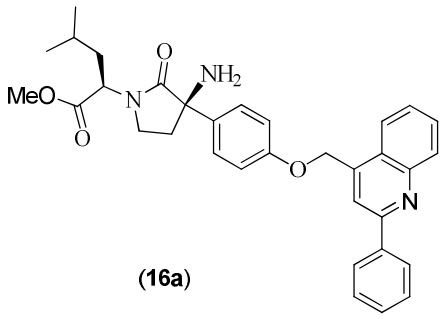
5.1.15. General procedure for the synthesis of compounds (16a-t)



(**14a-t**) (**16a-t**)

Boc protected ester derivatives of **14a**-**t**, (1 mole equiv) were dissolved in CH_2Cl_2 (25 mL), to this, TFA (3 mole equiv) was added at 0-5 ^{0}C under N_2. The reaction mixture was stirred at room temperature for 2h. Organic volatiles were evaporated under reduced pressure to get the deprotected ester derivatives **16a**-**t**. Using above procedure, **16a**-**t** were prepared and the physicochemical properties and spectral data of some of the representative compounds are listed below. Compounds **16h**-**m** and **16q**-**r** were directly used for next step without purification.

5.1.15.1. (R)-Methyl2-((R)-3-amino-2-oxo-3-(4-((2-phenylquinolin-4-yl) methoxy)phenyl)pyrrolidin-1-yl)-4-methylpentanoate (16a)

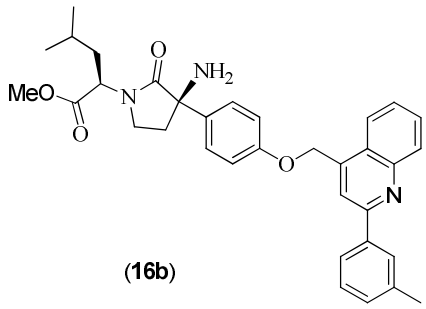


(**16a**)

The title compound was obtained as an oil, with yield = 85%; IR ($CHCl_3$): 3018, 1739, 1691, 1510, 1045; ESI (*m*/*z*) 506.5 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.97 (d, 6H, *J* = 6.96 Hz), 1.22 (m, 3H), 1.78 (m, 2H), 1.81 (m, 1H), 2.15 (m, 1H), 2.34 (m, 1H), 3.33 (m, 2H), 4.76 (s, 3H), 3.70 (s, 3H), 4.76 4.76 (s, 2H), 4.99 (t, 1H, *J* =

7.95 Hz), 5.52 (s, 2H), 7.01 (d, 2H, *J* = 8.73 Hz), 7.47 (d, 2H, *J* = 8.22 Hz), 7.58 (t, 1H, *J* = 7.65 Hz), 7.73 (m, 2H), 7.96 (d, 1H, *J* = 8.22 Hz), 8.11 (d, 1H, *J* = 8.34 Hz).

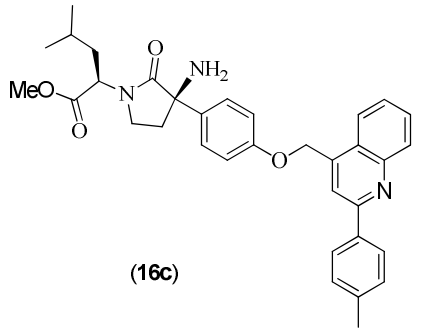
5.1.15.2. (R)-Methyl2-((R)-3-amino-2-oxo-3-(4-((2-(m-tolyl)quinolin-4-yl) methoxy)phenyl)pyrrolidin-1-yl)-4-methylpentanoate (16b)



(**16b**)

The title compound was obtained as a white solid, with 87% yield; mp 68-70 °C; IR (KBr, cm^{-1}): 3367, 2952, 2869, 1741, 1691, 1602, 1510; ESI (*m*/*z*) 552.3 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.97 (m, 6H), 1.52 (m, 1H), 1.75 (2H, m), 1.80 (m, 4H), 2.13 (m, 1H), 2.47 (s, 3H), 3.67 (s, 3H), 4.96 (t, 1H, *J* = 7.83 Hz), 5.57 (s, 2H), 7.03 (d, 2H, *J* = 8.46), 7.29 (m, 2H), 7.53 (m, 3H), 7.89 (m, 1H), 7.90 (m, 3H), 8.24 (d, 1H, *J* = 8.4 Hz).

5.1.15.3. (R)-Methyl2-((R)-3-amino-2-oxo-3-(4-((2-(p-tolyl)quinolin-4-yl) methoxy)phenyl)pyrrolidin-1-yl)-4-methylpentanoate (16c)

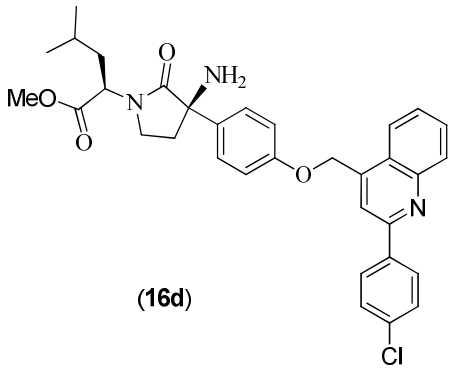


(**16c**)

The title compound was obtained as an oil, with 65% yield; Purity: 96 % by HPLC

; IR (KBr, cm^{-1}): 3433, 1739, 1670, 1515, 1259; ESI (*m*/*z*) 535.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.93 (m, 6H), 1.14 (d, 2H, *J* = 8.12 Hz), 1.51 (m, 2H), 2.17 (m, 1H), 2.32 (s, 3H), 2.42 (m, 2H), 2.71 (m, 2H), 3.36 (m, 2H), 3.62 (s, 3H), 4.90 (t, 1H, *J* = 8.25 Hz), 5.56 (s, 2H), 7.07 (d, 2H, *J* = 8.76 Hz), 7.32 (d, 2H, *J* = 7.8 Hz), 7.53 (d, 2H, *J* = 8.79 Hz), 7.60 (m, 1H), 7.78 (t, 1H, *J* = 7.23 Hz), 7.76 (d, 1H, *J* = 8.13 Hz), 8.05 (m, 3H), 8.31 (d, 1H, *J* = 8.4 Hz).

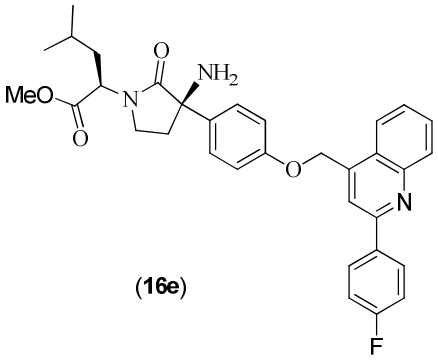
5.1.15.4. (R)-Methyl2-((R)-3-amino-3-(4-((2-(4-chlorophenyl)quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16d)



(**16d**)

The title compound was obtained as an oil, with 62% yield; Purity: 92 % by HPLC ; IR (KBr, cm^{-1}): 3435, 2956, 2869, 1741, 1689, 1602, 1492, 1425; ESI (*m*/*z*) 572.3 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.98 (d, 6H, *J* = 6.39 Hz), 1.78 (d, 2H, *J* = 7.38 Hz), 2.14 (m, 1H), 2.46 (m, 1H), 3.34 (m, 2H), 3.69 (s, 3H), 4.99 (t, 1H, *J* = 8.4 Hz), 5.75 (s, 2H), 7.03 (d, 2H, *J* = 8.73 Hz), 7.5 (d, 2H, *J* = 7.98 Hz), 7.58 (t, 1H, *J* = 7.20 Hz), 7.76 (t, 1H, *J* = 7.23 Hz), 7.90 (d, 1H, *J* = 8.49 Hz), 8.01 (s, 1H), 8.12 (d, 2H, *J* = 8.49 Hz), 8.21 (d, 1H, *J* = 8.19 Hz).

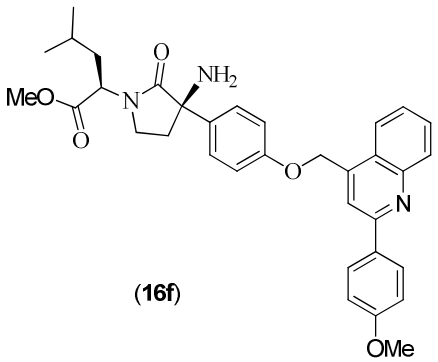
5.1.15.5. (R)-Methyl2-((R)-3-amino-3-(4-((2-(4-fluorophenyl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16e)



(**16e**)

The title compound was obtained as an oil, with 78% yield; Purity: 91 % by HPLC ; IR (KBr, cm^{-1}): 3369, 3018, 2958, 2873, 1739, 1693, 1604, 1556; ESI (*m*/*z*) 556.2 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.98 (d, 6H, *J* = 6.61 Hz), 1.78 (m, 2H), 2.12 (m, 2H), 2.45 (m, 1H), 3.35 (m, 2H), 3.69 (s, 3H), 4.99 (t, 1H, *J* = 7.98 Hz), 5.57 (s, 2H), 7.03 (d, 2H, *J* = 8.93 Hz), 7.20 (t, 1H, *J* = 8.64 Hz), 7.55 (d, 2H, *J* = 8.73 Hz), 7.57 (t, 1H, *J* = 7.47 Hz), 7.56 (t, 1H, *J* = 7.17 Hz), 7.98 (m, 2H), 8.98 (m, 2H), 8.19 (m, 3H).

5.1.15.6. (R)-Methyl2-((R)-3-amino-3-(4-((2-(4-methoxyphenyl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16f)

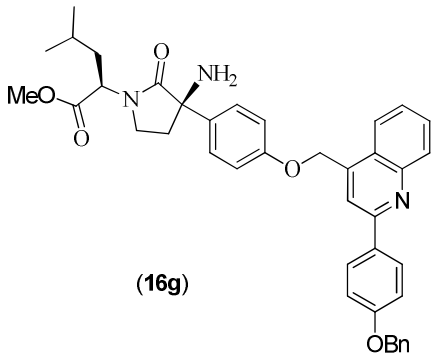


(**16f**)

The title compound was obtained as a white solid, with 60% yield; mp 165-167

°C; IR ($CHCl_3$, cm^{-1}): 3379, 3004, 2960, 1739, 1674, 12604, 1583, 1552, 1427; ESI (*m*/*z*) 568.3 (M+H); ^1H NMR ($CDCl_3$): δ 0.98 (d, 6H, *J* = 6.45 Hz), 3.33 (m, 6H), 3.68 (s, 1H), 3.88 (s, 3H), 5.99 (t, 1H, *J* = 8.9 Hz), 5.52 (s, 2H), 5.52 (s, 2H), 7.04 (d, 4H, *J* = 7.92 Hz), 7.53 (d, 2H, *J* = 8.7 Hz), 7.55 (d, 1H, *J* = 7.5 Hz), 7.74 (t, 1H, *J* = 7.47 Hz), 7.95 (d, 2H, *J* = 8.25 Hz), 7.99 (s, 1H), 8.13 (d, 2H, *J* = 8.7 Hz), 8.19 (d, 1H, *J* = 8.37 Hz).

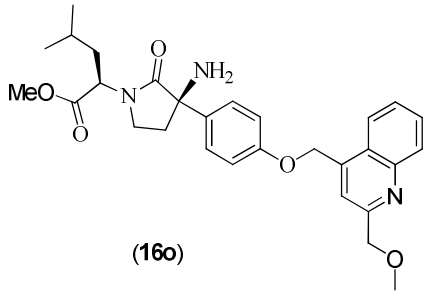
5.1.15.7. (R)-Methyl2-((R)-3-amino-3-(4-((2-(4-(benzyloxy)phenyl) quinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16g)



(**16g**)

The title compound was obtained as an oil, with 96% yield; Purity: 96 % by HPLC; IR ($CHCl_3$, cm^{-1}): 3367, 3008, 2958, 1739, 1674, 1604, 1427, 1406; ESI (*m*/*z*) 644.4 (M+H); ^1H NMR ($CDCl_3$): δ 0.99 (m, 2H), 1.99 (m, 2H), 2.15 (m, 4H), 3.35 (m, 5H), 3.68 (s, 3H), 4.88 (t, 1H), 5.01 (s, 2H), 5.15 (s, 2H), 7.04 (d, 4H, *J* = 6.6 Hz), 7.1 (d, 2H, *J* = 8.27 Hz), 7.38 (m, 4H), 7.52 (m, 2H), 7.59 (d, 1H, *J* = 8.28 Hz), 7.95 (t, 1H, *J* = 8.0 Hz), 8.13 (d, 2H, *J* = 8.7 Hz), 8.19 (d, 6H, *J* = 8.3 Hz).

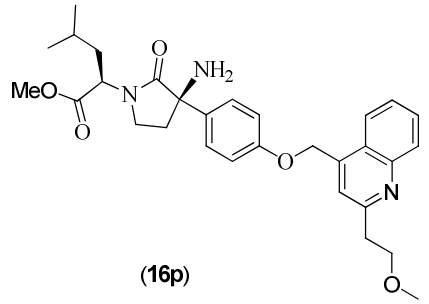
5.1.15.8. (2R)-Methyl2-(3-amino-3-(4-((2-(methoxymethyl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16o)



(16o)

The title compound was obtained as an oil, with 67% yield; IR (KBr, cm^{-1}) : 3367, 3292, 3065, 2823, 1732, 1681, 1606, 1568; ESI (m/z) 506.3 ($M+H^+$); ^1H NMR (DMSO-d_6): δ 0.97 (d, 6H, *J* = 6.45 Hz), 1.52 (m, 1H), 2.15 (m, 1H), 2.45 (m, 1H), 2.47 (m, 1H), 3.31 (m, 2H), 3.36 (s, 3H), 3.70 (s, 3H), 4.76 (s, 2H), 4.99 (m, 1H), 5.52 (s, 2H), 7.01 (d, 2H, *J* = 8.73 Hz), 7.50 (d, 2H, *J* = 8.73 Hz), 7.60 (m, 1H), 7.76 (m, 2H), 7.99 (d, 2H, *J* = 6.64 Hz), 8.14 (d, 1H, J = 8.45 Hz).

5.1.15.9. (2R)-Methyl2-(3-amino-3-(4-((2-(2-methoxyethyl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16p)

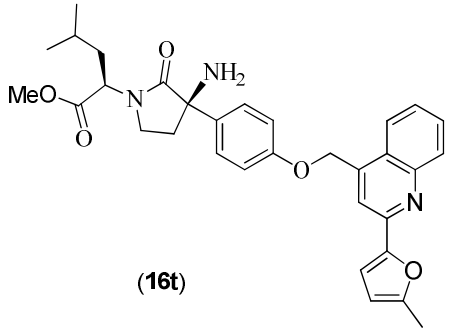


(16p)

The title compound was obtained as an oil, with 49% yield; Purity: 96 % by HPLC; IR ($CHCl_3$, cm^{-1}): 3469, 3018, 2929, 1739, 1691, 1608, 1510, 1215; ESI (m/z) 521.2 (M+H); ^1H NMR (DMSO-d_6): δ 0.82 (m, 6H), 1.15 (m, 2H), 1.17 (m, 4H), 1.61 (m, 2H), 1.74 (m, 2H), 1.87 (m, 2H), 2.12 (m, 2H), 2.24 (m, 2H), 3.14

(m, 3H), 1.15 (m, 2H), 3.18 (s, 3H), 3.75 (s, 3H), 4.74 (m, 1H), 5.58 (s, 2H), 7.41 (d, 2H, *J* = 8.67 Hz), 7.56 (s, 2H), 7.74 (t, 1H, *J* = 8.74 Hz), 7.76 (d, 1H, *J* = 8.29 Hz), 8.09 (d, 1H, *J* = 8.18 Hz).

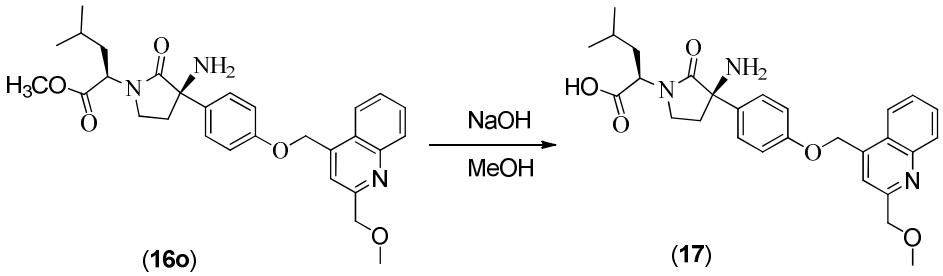
5.1.15.10. (R)-Methyl2-((R)-3-amino-3-(4-((2-(5-methylfuran-2-yl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate (16t)



(**16t**)

The title compound was obtained as an oil, with 85% yield; Purity: 95 % by HPLC; IR (KBr, cm^{-1}): 3377, 2954, 1741, 1606, 1431; ESI (*m*/*z*) 542.4 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.97 (m, 6H), 1.52 (m, 2H), 1.78 (m, 2H), 2.46 (s, 3H), 2.95 (m, 2H), 2.67 (s, 3H), 4.97 (t, 1H, *J* = 7.02 Hz), 6.19 (d, 2H, *J* = 7.06 Hz), 7.07 (d, 1H, *J* = 8.70 Hz), 7.16 (d, 1H, *J* = 8.70 Hz), 7.50 (d, 3H, *J* = 7.02 Hz), 7.71 (t, 1H, *J* = 7.98 Hz), 7.90 (s, 2H), 8.17 (d, 1H, *J* = 8.46 Hz).

5.1.16. General procedure for the synthesis of compound (17)



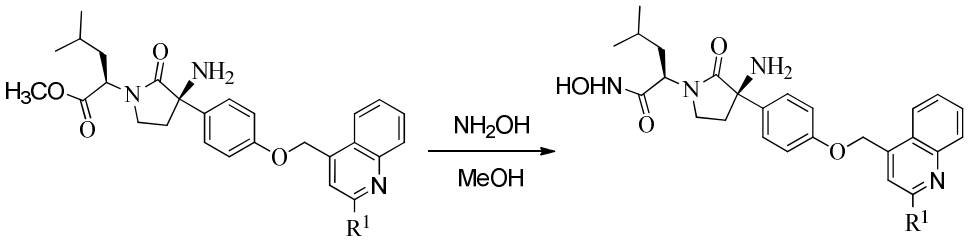
(**16o**) (**17**)

To a solution of **16o**, (1 mole equiv) in MeOH (20 mL), was added NaOH solution (4 mole equiv) to the reaction mixture at 0-5 ^{0}C. The reaction mixture was stirred

at room temperature for 4 h, quenched with ice-cold water (50 mL) and acidify with acetic acid (pH ~ 5).The aqueous layer was extracted with EtOAC (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude compound, which was purified by column chromatography, using a mixture of $CHCl_3$ and MeOH (1:2) as an eluant to get pure compound **17** as a white solid,

Yield = 70%; Purity: 93 % by HPLC ; IR (KBr, cm^{-1}): 3413, 2927, 1649, 1517; ESI (*m*/*z*) 495.6 ^{1}H NMR (DMSO-d_6): δ 0.91 (m, 6H), 1.03 (d, 3H, *J* = 6.06 Hz), 1.80 (m, 2H), 2.41 (m, 2H), 2.70 (m, 2H), 3.44 (m, 1H), 3.47 (m, 2H), 4.64 (m, 1H), 5.71 (s, 2H), 7.24 (d, 2H, *J* = 8.74 Hz), 7.51 (t, 1H, *J* = 6.45 Hz), 7.85 (t, 1H, *J* = 6.48 Hz), 8.05 (d, 1H, *J* = 8.33 Hz), 8.18 (d, 1H, *J* = 8.33 Hz), 8.99 (s, 1H).

5.1.17. General procedure for the synthesis of compound (18a-t)

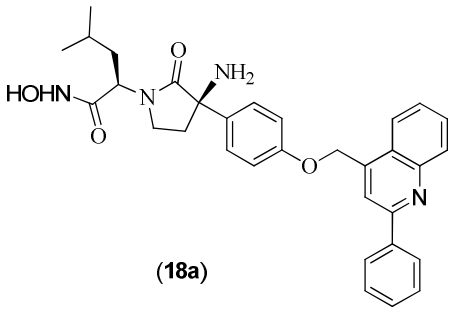


(**16a-t**) (**18a-t**)

The deprotected ester derivatives of **16a**-**t**, (1 mole equiv) was dissolved in MeOH (20 mL) and NH_2OH solution in MeOH (20 mL) was added to the reaction mixture at 0-5 ^{0}C. Mixture was stirred for 4 h at room temperature (25 ^{0}C) and quenched with ice-cold water (50 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude hydroxamic acid derivatives, which was purified by column chromatography, using a mixture of

$CHCl_3$ and MeOH (1:2) as an eluant to obtain pure hydroxamic acid derivatives **18a**-**t**. Using above procedure, compounds **18a**-**t** were prepared and the physicochemical properties and spectral data of representative compounds are listed below.

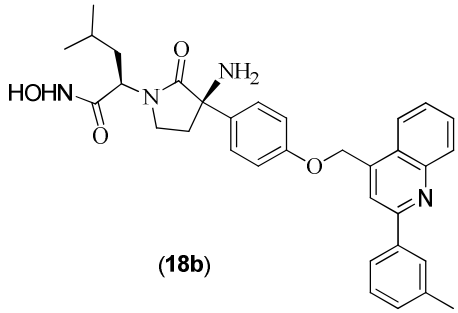
5.1.17.1. (R)-2-((R)-3-Amino-2-oxo-3-(4-((2-phenyl quinolin-4-yl) methoxy) phenyl) pyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18a)



(**18a**)

The title compound was obtained as a white solid, with 66% yield; mp 88-90 °C; Purity: 96 % by HPLC; IR (KBr, cm^{-1}): 3466, 2925, 2856, 1670, 1604, 1510, 1431, 1242; ESI (*m*/*z*) 539.4 (M+H); ^{1}H NMR ($CDCl_3$): δ 0.85 (m, 6H), 1.49 (m, 4H), 1.48 (m, 2H), 1.64 (m, 2H), 2.11 (m, 2H), 4.55 (m, 1H), 5.67 (s, 2H), 7.11 (d, 2H, *J* = 8.64 Hz), 7.35 (d, 2H, *J* = 8.52 Hz), 7.45 (m, 3H), 7.64 (t, 1H, *J* = 7.35 Hz), 7.81 (t, 1H, *J* = 7.14 Hz), 8.16 (dd, 2H, *J* = 8.07 & 8.16 Hz), 8.27 (m, 3H), 8.91 (s 1H), 10.86 (s 1H).

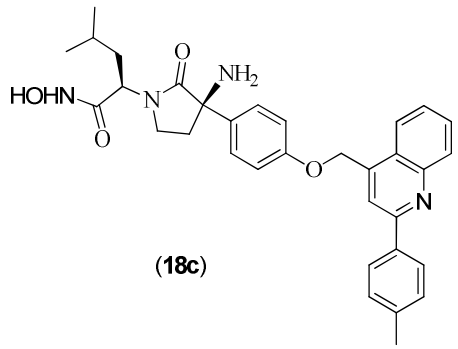
5.1.17.2. (R)-2-((R)-3-Amino-2-oxo-3-(4-((2-(m-tolyl) quinolin-4-yl) methoxy) phenyl) pyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18b)



(**18b**)

The title compound was obtained as a white solid, with 88% yield; mp 120-122 °C; Purity: 92 % by HPLC; IR (KBr, cm^{-1}): 3219, 2954, 2925, 1670, 1604; ESI (*m*/*z*) 553.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.89 (6H, m), 1.50 (2H, m), 1.63 (1H, m), 2.03 (2H, m), 2.43 (3H, s), 4.52 (1H, m), 5.66 (2H, s), 7.10 (2H, d, *J* = 8.46 Hz), 7.33 (3H, t, *J* = 7.75 Hz), 7.44 (1H, t, *J* = 7.82 Hz), 7.63 (1H, t, *J* = 8.46 Hz), 7.80 (1H, t, *J* = 7.92 Hz), 8.02 (1H, d, *J* = 8.46 Hz), 8.12 (3H, m), 8.25 (1H, s), 8.90 (1H, s), 10.85 (1H, s).

5.1.17.3. (R)-2-((R)-3-Amino-2-oxo-3-(4-((2-(p-tolyl) quinolin-4-yl) methoxy) phenyl) pyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18c)

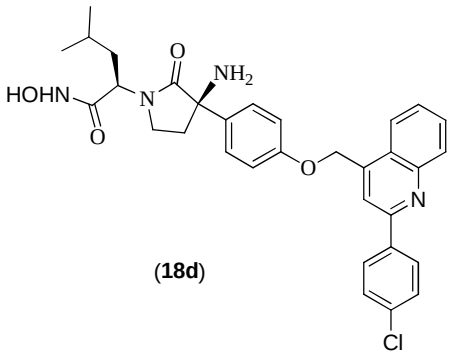


(**18c**)

The title compound was obtained as a white solid, with 34% yield; mp 131-132 °C; Purity: 96 % by HPLC; IR (KBr, cm^1): 3423, 3267, 2956, 2923, 2869,

1654,1550, 1510, 1452, 1442; ESI (*m*/*z*) 553.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.94 (m, 6H), 1.22 (s, 2H), 2.05 (m, 2H), 2.25 (m, 1H), 2.26 (s, 3H), 4.52 (t, 1H, *J* = 7.8 Hz), 5.66 (s, 2H), 7.08 (d, 1H, *J* = 7.5 Hz), 7.35 (m, 4H), 7.76 (m, 1H), 8.08 (m, 1H), 8.30 (m, 4H), 8.90 (s, 1H), 10.85 (s, 1H).

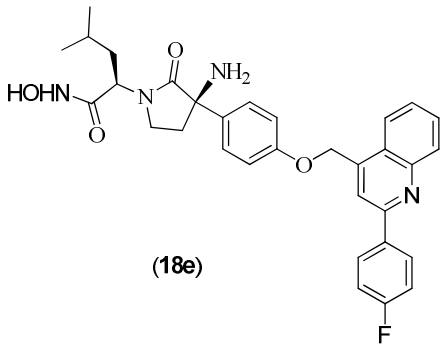
5.1.17.4. (R)-2-((R)-3-Amino-3-(4-((2-(4-chlorophenyl) quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18d)



(**18d**)

The title compound was obtained as a white solid, with 77% yield; mp 102-104 °C; Purity: 96 % by HPLC; IR (KBr, cm^{-1}): 3197, 2923, 2868, 1666, 1602, 1510, 1423, 1242, 19091; ESI (*m*/*z*) 557.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.91 (d, 6H, *J* = 6.03 Hz), 1.17 (m 2H), 1.5 (m, 1H), 2.12 (m, 2H), 3.37 (s, 2H), 4.52 (m, 1H), 5.67 (s, 2H), 7.1 (d, 1H, *J* = 8.67 Hz), 7.35 (d, 2H, *J* = 8.64 Hz), 7.65 (m, 3H), 7.81 (t, 1H, *J* = 7.29, 15.15 Hz), 8.12 (d, 1H, *J* = 8.34 Hz), 8.20 (d, 1H, *J* = 8.28 Hz), 8.30 (m, 3H), 8.90 (s, 1H), 10.58 (s, 1H).

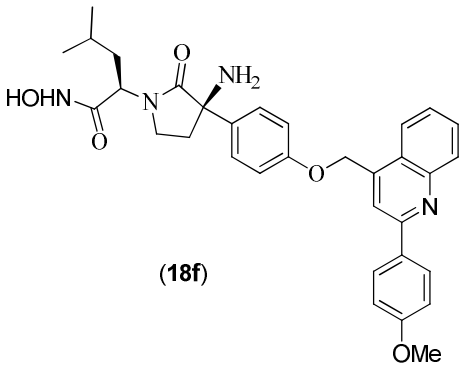
5.1.17.5. (R)-2-((R)-3-Amino-3-(4-((2-(4-fluorophenyl) quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18e)



(**18e**)

The title compound was obtained as a white solid, with 56% yield; mp 100-102 °C; Purity: 95 % by HPLC; IR (KBr, cm^{-1}): 3230, 2869,1664, 1604, 1554, 1508, 1423, 1234; ESI (*m*/*z*) 573.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.90 (d, 6H, *J* = 6.00 Hz), 1.50 (s, 2H), 1.62 (m, 2H), 2.05 (m, 2H), 4.52 (m, 2H), 4.52 (m, 1H), 5.66 (s, 2H), 7.11 (d, 1H, *J* = 8.73 Hz), 7.37 (m, 4H), 7.64 (t, 1H, *J* = 7.44 Hz), 7.81 (t, 1H, *J* = 7.26 Hz), 8.10 (d, 1H, *J*= 8.43 Hz), 8.17 (d, 1H, *J*= 8.10 Hz), 8.32 (s, 4H), 8.91 (s, 1H), 10.86 (s, 1H).

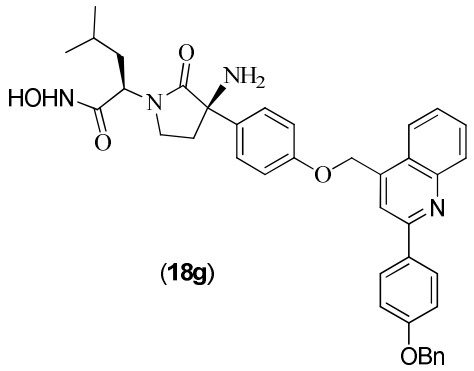
5.1.17.6. (R)-2-((R)-3-Amino-3-(4-((2-(4-methoxy phenyl) quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18f)



(**18f**)

The title compound was obtained as a white solid, with 60% yield; mp 165-167 °C; Purity: 91 % by HPLC; IR (KBr, cm^{1}): 3311, 2927, 1670, 1604, 1510, 1427; ESI (*m*/*z*) 591.4 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.90 (m, 6H), 1.6 (m, 4H), 2.13 (m, 3H), 2.6 (s, 1H), 3.84 (s, 3H), 4.52 (t, 1H, *J* = 6.21 Hz), 5.65 (s, 2H), 7.12 (t, 3 H, *J* = 4.83 Hz), 7.37 (d, 2H, *J* = 8.65 Hz), 7.61 (t, 1H, *J* = 7.56 Hz), 7.76 (t, 1H, *J* = 7.23 Hz), 8.13 (d, 1H, *J* = 11.2 Hz), 8.27 (d, 1H, *J* = 7.47 Hz), 8.92 (d, 3H, *J* = 7.12 Hz), 10.16 (s, 1H), 10.87 (s, 1H).

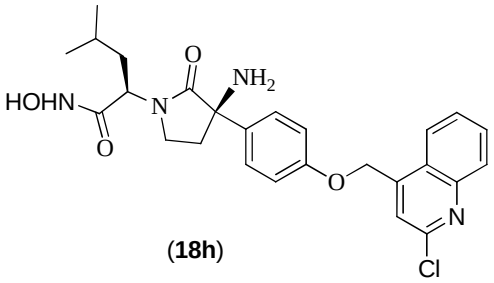
5.1.17.7. (R)-2-((R)-3-Amino-3-(4-((2-(4-(benzyloxy) phenyl) quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18g)



(**18g**)

The title compound was obtained as a white solid, with 57% yield; mp 106-108 °C; Purity: 97 % by HPLC; IR (KBr, cm^{-1}): 3207, 2954, 1664, 1602, 1581, 1508, 1427, 1384, 1350, 1282, 1174; ESI (*m*/*z*) 645.4 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.90 (m, 6H), 1.32 (m, 2H), 1.64 (m, 1H), 2.08 (m, 6H), 3.84 (s, 3H), 4.52 (m, 1H), 5.20 (s, 2H), 5.64 (s, 2H), 7.09 (d, 2H, *J* = 8.32), 7.18 (d, 2 H, *J* = 4.83 Hz), 7.36 (m, 3H), 7.41(t, 2H, *J* = 6.92 Hz), 7.49 (d, 2H, *J* = 7.12 Hz), 7.59 (t, 1H, *J* = 7.32 Hz), 7.77 (t, 1H, *J* =7.0 Hz), 8.06 (d, 1H, *J* = 8.3 Hz), 8.14 (d, 1H, *J* = 8.21 Hz), 8.22 (d, 3H, *J* = 8.12 Hz), 8.89 (s, 1H), 10.84 (s, 1H).

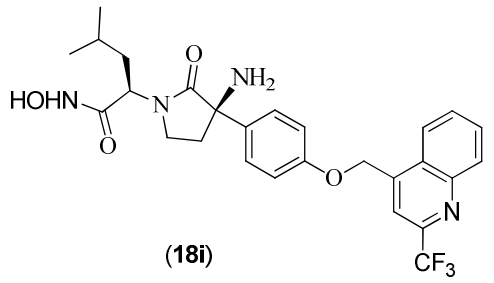
5.1.17.8. (R)-2-((R)-3-Amino-3-(4-((2-chloroquinolin-4-yl)methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18h)



(**18h**)

The title compound was obtained as a white solid, with 61% yield; mp 94-96 °C; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3217, 2953, 1672, 1605, 1240, 832, 759; ESI (*m*/*z*) 497.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H, *J* = 5.95 Hz), 1.15 (m, 1H), 1.46 (m, 2H), 2.05 (m, 2H), 2.42 (m, 2H), 4.55 (q, 1H, *J* = 6.26 Hz), 5.54 (s, 2H), 7.06 (d, 2H, *J* = 8.75 Hz), 7.35 (d, 2H, *J* = 8.79 Hz), 7.42 (m, 2H, *J* = 7.05 Hz), 7.67 (d, 1H *J* = 8.05 Hz), 7.81 (d, 1H, *J* = 8.15 Hz), 8.01 (d, 1H, *J* = 8.15 Hz), 8.94 (s, 1H), 10.85 (s, 1H).

5.1.17.9. (R)-2-((R)-3-Amino-2-oxo-3-(4-((2-(trifluoromethyl) quinolin-4-yl) methoxy) phenyl) pyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18i)

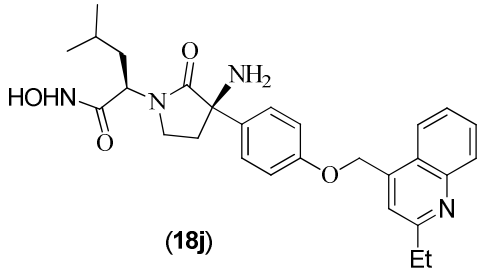


(**18i**)

The title compound was obtained as a white solid, with 54% yield; mp 81-83 °C; Purity: 90 % by HPLC; IR (KBr, cm^{-1}): 3214, 2955, 1672, 1605, 1245, 836, 756; ESI (*m*/*z*) 531.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 6H, *J* = 5.92 Hz), 1.16 (m, 1H), 1.43 (m, 2H), 2.15 (m, 2H), 2.45 (m, 2H), 4.56 (q, 1H, *J* = 6.25 Hz), 5.53

(s, 2H), 7.02 (d, 2H, *J* = 8.72 Hz), 7.36 (d, 2H, *J* = 8.72 Hz), 7.42 (m, 2H, *J* = 7.16 Hz), 7.64 (d, 1H *J* = 8.02 Hz), 7.81 (d, 1H, *J* = 8.58 Hz), 8.01 (d, 1H, *J* = 8.16 Hz), 8.95 (s, 1H), 10.88 (s, 1H).

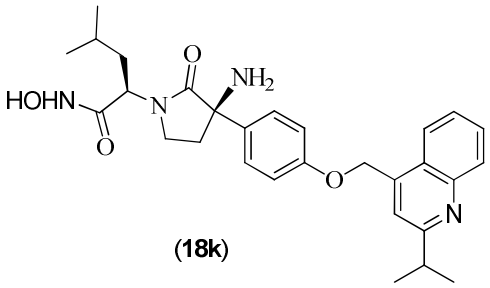
5.1.17.10. (R)-2-((R)-3-Amino-3-(4-((2-ethylquinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18j)



(**18j**)

The title compound was obtained as a white solid, with 45% yield; mp 97-99 °C; Purity: 93 % by HPLC; IR (KBr, cm^{-1}): 3210, 2957, 1672, 1605, 1242, 832, 758; ESI (*m*/*z*) 491.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.81 (m, 6H, *J* = 5.91 Hz), 1.01 (t, 3H, *J* = 6.92 Hz), 1.05 (m, 2H), 1.13 (m, 1H), 1.45 (m, 2H), 2.01 (q, 2H, *J* = 6.95 Hz), 2.05 (m, 2H), 2.48 (m, 2H), 4.56 (q, 1H, *J* = 6.25 Hz), 5.53 (s, 2H), 7.03 (d, 2H, *J* = 8.76 Hz), 7.35 (d, 2H, *J* = 8.73 Hz), 7.45 (m, 2H, *J* = 7.08 Hz), 7.66 (d, 1H *J* = 8.13 Hz), 7.82 (d, 1H, *J* = 8.15 Hz), 8.01 (d, 1H, *J* = 8.12 Hz), 8.92 (s, 1H), 10.85 (s, 1H).

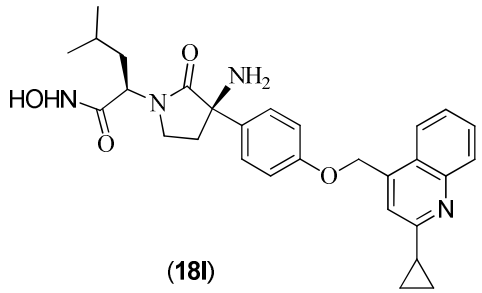
5.1.17.11. (R)-2-((R)-3-Amino-3-(4-((2-isopropyl quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18k)



(**18k**)

The title compound was obtained as a white solid, with 35% yield; mp 92-93 °C; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3215, 2956, 1673, 1606, 1242, 832, 756; ESI (*m*/*z*) 505.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 6H, *J* = 5.94 Hz), 1.05 (m, 6H), 1.09 (m, 2H), 1.15 (m, 1H), 1.43 (m, 2H), 2.01 (m, 1H), 2.03 (m, 2H), 2.45 (m, 2H), 4.56 (q, 1H, *J* = 6.23 Hz), 5.53 (s, 2H), 7.06 (d, 2H, *J* = 8.76 Hz), 7.35 (d, 2H, *J* = 8.73 Hz), 7.45 (m, 2H, *J* = 7.06 Hz), 7.65 (d, 1H *J* = 8.17 Hz), 7.86 (d, 1H, *J* = 8.14 Hz), 8.04 (d, 1H, *J* = 8.13 Hz), 8.95 (s, 1H), 10.87 (s, 1H).

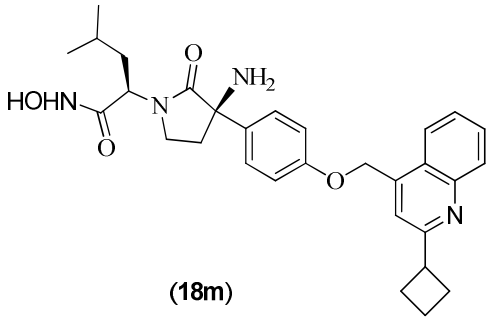
5.1.17.12. (R)-2-((R)-3-Amino-3-(4-((2-cyclopropylquinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18l)



(18l)

The title compound was obtained as a white solid, with 67% yield; mp 90-91 °C; Purity: 90 % by HPLC; IR (KBr, cm^{-1}): 3219.0, 2956.7, 1670, 1608, 1244, 833, 759; ESI (*m*/*z*) 503.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H, *J* = 5.94 Hz), 1.01 (m, 4H), 1.15 (m, 1H), 1.45 (m, 2H), 2.07 (m, 2H), 2.48 (m, 2H), 4.53 (q, 1H, *J* = 6.24 Hz), 5.56 (s, 2H), 7.03 (d, 2H, *J* = 8.73 Hz), 7.33 (d, 2H, *J* = 8.73 Hz), 7.45 (m, 2H, *J* = 7.08 Hz), 7.67 (d, 1H *J* = 8.13 Hz), 7.85 (d, 1H, *J* = 8.16 Hz), 8.01 (d, 1H, *J* = 8.16 Hz), 8.91 (s, 1H), 10.86 (s, 1H).

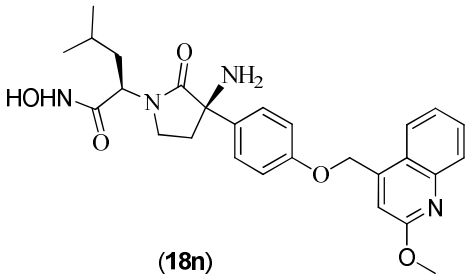
5.1.17.13. (R)-2-((R)-3-Amino-3-(4-((2-cyclobutylquinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18m)



(**18m**)

The title compound was obtained as a white solid, with 45% yield; mp 108-109 °C; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3219, 2956, 1670, 1606, 1510; ESI (*m*/*z*) 517.0 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.90 (m, 6H), 1.22 (m, 2H), 1.48 (m, 1H), 2.09 (m, 2H), 2.12 (m, 4H), 3.83 (m, 2H), 4.52 (m, 1H), 5.57 (s, 2H), 7.04 (d, 2H, *J* = 8.64 Hz), 7.33 (d, 2H, *J* = 8.70 Hz), 7.57 (m, 2H), 7.73 (t, 1H, *J* = 7.47 Hz), 8.01 (d, 1H, *J* = 8.26 Hz), 8.07 (d, 1H, *J* = 7.98 Hz), 8.91 (s, 1H), 10.86 (s, 1H).

5.1.17.14. (R)-2-((R)-3-amino-3-(4-((2-methoxyquinolin-4-yl)methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18n)

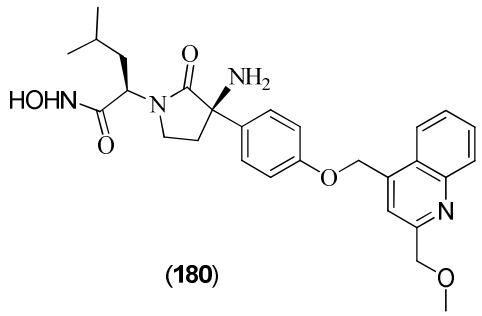


(**18n**)

The title compound was obtained as a white solid, with 64% yield; Purity: 90% by HPLC; IR (KBr, cm^{-1}): 2870, 1670, 1583, 1510; ESI (*m*/*z*) 493.6 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H), 1.48 (m, 2H), 1.46 (m, 1H), 2.05 (m, 2H), 2.11 (m,

3H), 3.36 (s, 3H), 4.50 (m, 1H), 4.66 (s, 2H), 7.04 (d, 2H, *J* = 8.72 Hz), 7.32 (d, 2H, *J* = 8.82 Hz), 7.75 (m, 2H), 7.77 (t, 2H, *J* = 7.42 Hz), 8.03 (d, 1H, *J* = 8.56 Hz), 8.30 (d, 1H, *J* = 7.93 Hz), 8.90 (s, 1H), 10.86 (s, 1H).

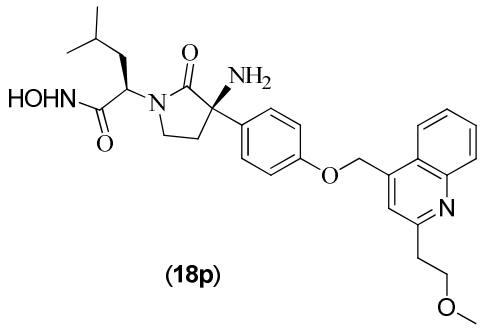
5.1.17.15. (R)-2-((R)-3-Amino-3-(4-((2-(methoxymethyl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methylpentanamide (18o)



(**180**)

The title compound was obtained as a white solid, with 64% yield; mp 85-87 °C; Purity: 97 % by HPLC; IR (KBr, cm^{-1}): 3431, 3232, 2927, 1664, 1606, 1510; ESI (*m*/*z*) 507.3 ($M+H^+$); ^1H NMR (DMSO-d_6) : δ 0.89 (m, 6H), 1.48 (m, 2H), 1.48 (m, 1H), 1.52 (2, 1H), 1.65 (s, 2H), 3.36 (s, 3H), 4.52 (q, 1H, J = 6.21 Hz), 5.64 (s, 2H), 7.05 (d, 2H, *J* = 8.64 Hz), 7.32 (d, 2H, *J* = 8.67 Hz), 7.63 (m, 1H), 7.71 (s, 1H), 7.80 (m, 1H), 8.03 (d, 1H, *J* = 8.25 Hz), 8.16 (d, 1H, *J* = 8.25 Hz), 8.91 (s,1H), 10.87 (s,1H).

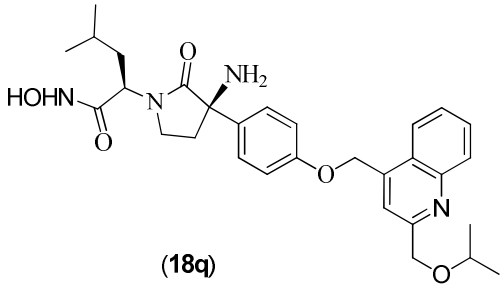
5.1.17.16. (R)-2-((R)-3-Amino-3-(4-((2-(2-methoxyethyl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methylpentanamide (18p)



(**18p**)

The title compound was obtained as a white solid, with 66% yield; mp 70-72 °C; Purity: 92 % by HPLC; IR (KBr, cm^{-1}): 3234, 2927, 2869, 2958, 1606, 1583, 1510, 1429; ESI (*m*/*z*) 521.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H), 1.48 (m, 2H), 1.52 (m, 2H), 2.08 (m, 2H), 2.25 (m, 2H), 3.15 (t, 2H, *J* = 6.54 Hz), 3.25 (s, 3H), 3.35 (m, 2H), 3.75 (t, 2H, *J* = 6.57 Hz), 4.55 (m, 1H), 5.57 (s, 2H), 7.05 (d, 2H, *J* = 8.67 Hz), 7.32 (d, 2H, *J* = 8.64 Hz), 7.65 (m, 2H), 7.75 (m, 2H), 7.95 (d, 2H, *J* = 8.25 Hz), 8.05 (d, 2H, *J* = 8.22 Hz), 8.90 (s, 1H), 10.86 (s, 1H).

5.1.17.17. (R)-2-((R)-3-Amino-3-(4-((2-(isopropoxymethyl) quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanamide (18q)

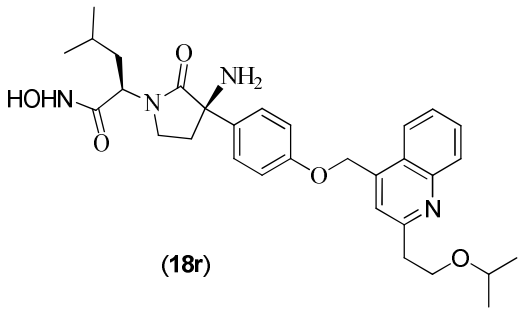


(**18q**)

The title compound was obtained as a white solid, with 52% yield; Purity: 96 %

by HPLC; IR (KBr, cm^{-1}): 3469, 3018, 2929, 1739, 1691, 1608, 1510, 1215; ESI (m/z) 521.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H), 1.10 (d, 2H, *J* = 6.06 Hz), 1.48 (m, 2H), 1.48 (m, 2H), 2.0 (m, 2H), 2.1 (m, 2H), 2.12 (m, 2H), 2.24 (m, 2H), 4.50 (m, 1H), 4.66 (s, 2H), 5.64 (s, 1H), 7.04 (d, 2H, *J* = 8.70 Hz), 7.35 (d, 2H, *J* = 8.67 Hz), 7.63 (t, 1H, *J* = 8.29 Hz), 8.00 (d, 1H, *J* = 8.22 Hz), 7.63 (t, 1H, *J* = 8.29 Hz), 8.13 (t, 1H, *J* = 8.29 Hz), 8.89 (s, 1H), 10.84 (s, 1H).

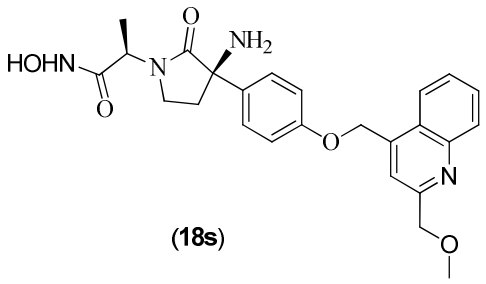
5.1.17.18. (R)-Methyl 2-((R)-3-amino-3-(4-((2-(2-isopropoxyethyl) quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methyl pentanoate (18r)



(**18r**)

The title compound was obtained as a white solid, with 34% yield; Purity: 91 % by HPLC; IR (KBr, cm^{-1}): 3419, 2958, 1670, 1512, 1247; ESI (*m*/*z*) 548.6 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H), 1.52 (m, 2H), 1.78 (m, 2H), 2.09 (m, 2H), 3.12 (m, 2H), 3.77 (t, 1H, *J* = 7.02 Hz), 4.54 (m, 1H), 6.19 (d, 2H, *J* = 7.06 Hz), 7.04 (d, 1H, *J* = 8.72 Hz), 7.33 (d, 1H, *J* = 8.60 Hz), 7.71 (s, 2H), 7.74 (t, 1H, *J* = 7.82 Hz), 7.97 (d, 1H, *J* = 7.88 Hz), 8.08 (d, 1H, *J* = 8.46 Hz), 8.93 (s, 1H), 10.88 (s, 1H).

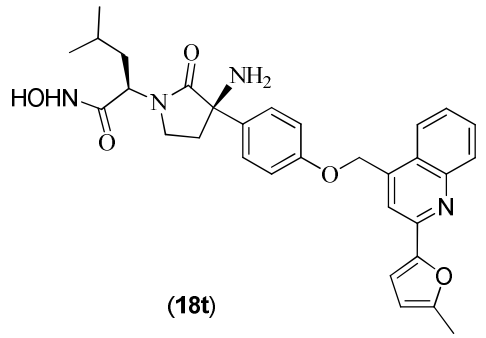
5.1.17.19. (R)-2-((R)-3-Amino-3-(4-((2-(methoxymethyl)quinolin-4-yl) methoxy) phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxypropanamide (18s)



(**18s**)

The title compound was obtained as a white solid, with 63% yield; mp 81-82 °C; Purity: 98 % by HPLC; IR (KBr, cm^{-1}): 3419, 2980, 2970, 1695, 1608, 1514, 1384, 1249, 1190; ESI (m/z) 492.5 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 1H, *J* = 7.11 Hz), 1.28 (m, 3H), 1.21 (m, 1H), 1.45 (m, 1H), 1.35 (m, 2H), 4.55 (q, 1H, *J* = 6.27 Hz), 5.61 (s, 2H), 7.05 (d, 2H, *J* = 8.7 Hz), 7.32 (d, 2H, *J* = 8.67 Hz), 7.61 (m, 1H), 7.73 (m, 1H), 8.02 (d, 2H, *J* = 8.22 Hz), 8.15 (d, 2H, *J* = 8.37 Hz), 8.89 (s, 1H), 10.02 (s, 1H).

5.1.17.20. (R)-2-((R)-3-Amino-3-(4-((2-(5-methylfuran-2-yl)quinolin-4-yl) methoxy)phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methylpentanamide (18t)

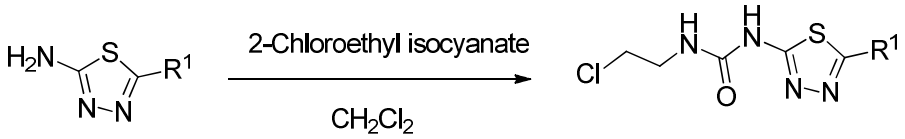


(**18t**)

The title compound was obtained as a white solid, with 66% yield; mp 100-102 °C; Purity: 98 % by HPLC; IR (KBr, cm^{-1}): 3205, 2956, 1647, 1670, 1608, 1244;

ESI (*m*/*z*) 543.4 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H), 1.49 (m, 2H), 1.64 (m, 1H), 1.03 (m, 2H), 2.43 (s, 3H), 3.38 (s, 3H), 4.51 (m, 1H), 5.63 (s, 2H), 5.35 (d, 1H, *J* = 8.64 Hz), 7.09 (d, 2H, *J* = 8.52 Hz), 7.27 (m, 1H), 7.36 (d, 2H, *J* = 8.64 Hz), 7.59 (t, 1H, *J* = 7.41 Hz), 7.77 (t, 1H, *J* = 7.29 Hz), 8.02 (m, 2H), 8.12 (d, 1H, *J* = 8.12 Hz), 8.92 (s 1H), 10.87 (s 1H).

5.1.18. General procedure for the synthesis of compound (20a-p)



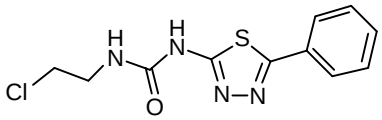
(**19a-p**)

A= Ar, Hetrocycles.

(**20a-p**)

To an ice cold solution of **19a**-**p**, (1 mole equiv) in CH_2Cl_2 (100 mL), was added 2-chloroethyl isocyanate (1.1 mole equiv) drop wise, with constant stirring and the reaction mixture was stirred at room temperature (25 ^{0}C) for 1h and quenched with water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 150 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain pure derivative **20a**-**p**. The physicochemical properties and spectral data of some of the representative compounds are listed below.

5.1.18.1. 1-(2-Chloroethyl)-3-(5-phenyl-1, 3, 4-thiadiazol-2-yl)urea (20a)

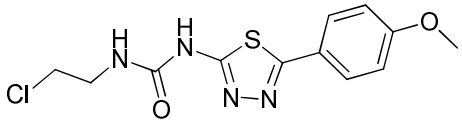


(**20a**)

The title compound was obtained as an oil, with 73% yield; Purity: 96% by HPLC; IR (KBr, cm^{-1}): 3390, 1720, 1535, 1436, 1261; ESI (*m*/*z*) 283.0 (M+H); ^{1}H NMR

(DMSO-d_6): δ 3.52 (t, 2H, *J* = 5.82 Hz), 3.69 (t, 2H, *J* = 6.00 Hz), 6.90 (s, 1H), 7.52 (m, 1H), 7.87 (m, 2H), 7.87 (s, 1H), 11.20 (s, 1H).

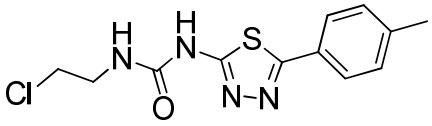
5.1.18.2. 1-(2-Chloroethyl)-3-(5-(4-methoxyphenyl)-1, 3, 4-thiadiazol-2-yl) urea (20b)



(**20b**)

The title compound was obtained as an oil, with 85% yield; Purity: 97.46 % by HPLC; ESI (*m*/*z*) 313.1 (M+H); IR (KBr, cm^{-1}): 3379, 2837, 1701, 1606; ^{1}H NMR (DMSO-d_6): δ 3.49 (t, 2H, *J* = 5.82 Hz), 3.66 (t, 2H, *J* = 6.18 Hz), 3.83 (s, 3H), 6.87 (s, 1H), 7.04 (d, 2H, *J* = 6.92 Hz), 7.81 (d, 2H, *J* = 8.76 Hz), 11.08 (s, 1H).

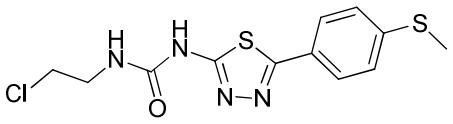
5.1.18.3. 1-(2-Chloroethyl)-3-(5-(*p*-tolyl)-1, 3, 4-thiadiazol-2-yl)urea (20c)



(**20c**)

The title compound was obtained as an oily, in 78% yield; Purity: 91.42 % by HPLC; ESI (*m*/*z*) 297.1 (M+H); IR (KBr, cm^{-1}): 3377, 2836, 1703, 1607; ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H), 3.42 (t, 2H, *J* = 5.86 Hz), 3.65 (t, 2H, *J* = 6.13 Hz), 6.86 (s, 1H), 7.05 (d, 2H, *J* = 6.96 Hz), 7.82 (d, 2H, *J* = 8.76 Hz), 11.07 (s, 1H).

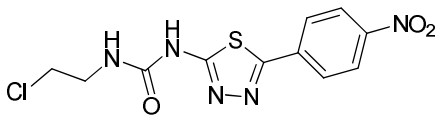
5.1.18.4. 1-(2-Chloroethyl)-3-(5-(4-(methylthio)phenyl)-1,3,4-thiadiazol-2-yl) urea (20d)



(**20d**)

The title compound was obtained as an oil, with 58% yield; Purity: 90.71 % by HPLC; ESI (*m*/*z*) 315.7 (M+H); IR (KBr, cm^{-1}): 3388, 2856, 1720, 1442; ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H), 3.53 (t, 2H, *J* = 8.37 Hz), 4.10 (t, 2H, *J* = 6.52 Hz), 6.89 (s, 1H), 7.34 (d, 2H, *J* = 8.41 Hz), 7.80 (d, 2H, *J* = 8.43 Hz).

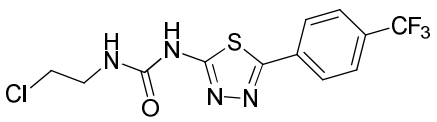
5.1.18.5. 1-(2-Chloroethyl)-3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)urea (20e)



(**20e**)

The title compound was obtained as an oily, in 91% yield; Purity: 93.1 % by HPLC; IR (KBr, cm^{-1}): 3612, 3395, 2956, 1712, 1532; ESI (*m*/*z*) 328.8 (M+H); ^{1}H NMR (DMSO-d_6): 3.58 (t, 2H, *J* = 8.33 Hz), 4.15 (t, 2H, *J* = 6.43 Hz), 7.84 (d, 2H, *J* = 8.47 Hz), 7.73 (d, 2H, *J* = 8.85 Hz).

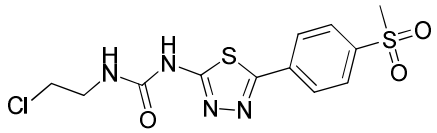
5.1.18.6. 1-(2-Chloroethyl)-3-(5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)urea (20f)



(**20f**)

The title compound was obtained as an oil, with 43% yield; Purity: 91.5 % by HPLC; IR (KBr, cm^{-1}): 3615, 3397, 2952, 1719, 1533; ESI (*m*/*z*) 351.2 (M+H); ^{1}H NMR (DMSO-d_6): 3.65 (t, 2H, *J* = 8.33 Hz), 4.25 (t, 2H, *J* = 6.43 Hz), 7.85 (m, 2H), 7.75 (m, 2H).

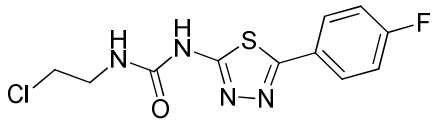
5.1.18.7. 1-(2-Chloroethyl)-3-(5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)urea (20g)



(**20g**)

The title compound was obtained as an oil, with 45% yield; Purity: 94.86 % by HPLC; ESI (*m*/*z*) 361.8 (M+H); IR (KBr, cm^{-1}): 3383, 2854, 1723, 1446; ^{1}H NMR (DMSO-d_6): δ 3.43 (s, 3H), 3.67 (t, 2H, *J* = 8.32 Hz), 4.15 (t, 2H, *J* = 6.55 Hz), 6.83 (s, 1H), 7.38 (d, 2H, *J* = 8.45 Hz), 7.85 (d, 2H, *J* = 8.48 Hz).

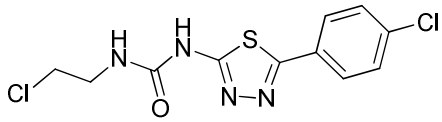
5.1.18.8. 1-(2-Chloroethyl)-3-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)urea (20h)



(**20h**)

The title compound was obtained as an oil, with 76% yield; Purity: 95.5 % by HPLC; ESI (*m*/*z*) 301.1 (M+H); IR (KBr, cm^{-1}): 3345, 1647, 1583, 1449; ^{1}H NMR (DMSO-d_6): δ 3.45 (t, 2H, *J* = 6.35 Hz), 3.68 (t, 2H, *J* = 6.27 Hz), 6.85 (s, 1H), 7.34 (m, 2H), 7.82 (m, 2H).

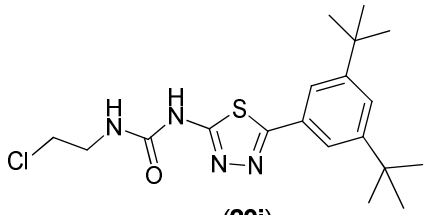
5.1.18.9. 1-(2-Chloroethyl)-3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)urea (20i)



(**20i**)

The title compound was obtained as an oil, with 75% yield; Purity: 95.2 % by HPLC; ESI (*m*/*z*) 317.1 (M+H); IR (KBr, cm^{-1}): 3343, 1648, 1584, 1445; ^{1}H NMR (DMSO-d_6): δ 3.42 (t, 2H, *J* = 6.36 Hz), 3.64 (t, 2H, *J* = 6.25 Hz), 6.72 (s, 1H), 7.55(d, 2H, *J* = 8.44 Hz), 7.92 (d, 2H, *J* = 8.45 Hz).

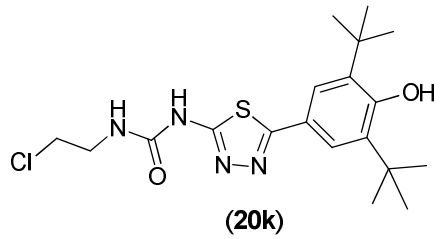
5.1.18.10. 1-(2-Chloroethyl)-3-(5-(3,5-di-tert-butylphenyl)-1,3,4-thiadiazol-2-yl)urea (20j)



(**20j**)

The title compound was obtained as an oil, with 67% yield; Purity: 92.1 % by HPLC; IR (KBr, cm^{-1}): 3612, 3395, 2958, 1713, 1536; ESI (*m*/*z*) 395.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 18H), 3.45 (m, 2H), 3.67 (t, 2H, *J* = 6.94 Hz), 7.65 (s, 1H), 6.85 (s, 1H), 7.57 (s, 1H), 11.05 (s, 1H).

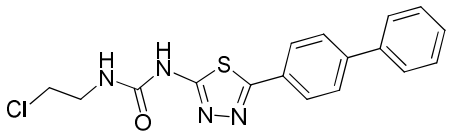
5.1.18.11. 1-(2-Chloroethyl)-3-(5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)urea (20k)



(**20k**)

The title compound was obtained as an oil, with 93% yield; Purity: 93.1 % by HPLC; IR (KBr, cm^{-1}): 3618, 3394, 2958, 1710, 1539; ESI (*m*/*z*) 411.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.41 (s, 18H), 3.47 (m, 2H), 3.68 (t, 2H, *J* = 5.97 Hz), 6.84 (s, 1H), 7.57 (s, 2H), 11.08 (s, 1H).

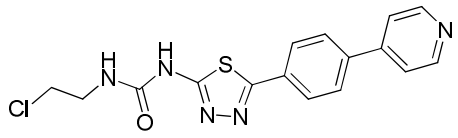
5.1.18.12. 1-(5-([1,1'-Biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)-3-(2-chloroethyl) urea (20l)



(**20l**)

The title compound was obtained as an oil, with 87% yield; Purity: 95.5 % by HPLC; ESI (*m*/*z*) 359.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 2.75 (s, 3H), 3.43 (t, 2H, *J* = 7.95 Hz), 3.64 (t, 2H, *J* = 6.13 Hz), 5.75 (s, 2H), 6.97 (s, 1H), 7.25 (s, 1H), 7.55 (m, 5H), 7.95 (d, 1H, *J* = 8.45 Hz), 8.03 (d, 1H, *J* = 8.24 Hz), 11.25 (s, 1H).

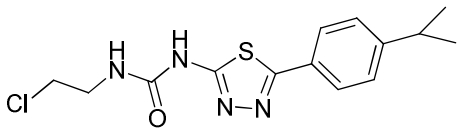
5.1.18.13. 1-(2-Chloroethyl)-3-(5-(4-(pyridin-4-yl)phenyl)-1,3,4-thiadiazol-2-yl)urea (20m)



(**20m**)

The title compound was obtained as an oil, with 88% yield; Purity: 92.3 % by HPLC; ESI (*m*/*z*) 360.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 2.73 (s, 3H), 3.42 (t, 2H, *J* = 7.94 Hz), 3.65 (t, 2H, *J* = 6.18 Hz), 5.72 (s, 2H), 6.95 (s, 1H), 7.22 (s, 1H), 7.55 (m, 5H), 7.65 (m, 1H), 7.95 (d, 1H, *J* = 8.45 Hz), 8.03 (d, 1H, *J* = 8.24 Hz), 8.85 (m, 1H), 8.92 (m, 1H), 11.25 (s, 1H).

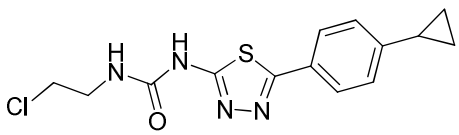
5.1.18.14. 1-(2-Chloroethyl)-3-(5-(4-isopropylphenyl)-1,3,4-thiadiazol-2-yl) urea (20n)



(**20n**)

The title compound was obtained as an oily, in 53% yield; ESI (*m*/*z*) 325.1 (M+H); IR (KBr, cm^{-1}): 3242, 2874, 1692, 1565.

5.1.18.15. 1-(2-Chloroethyl)-3-(5-(4-cyclopropylphenyl)-1,3,4-thiadiazol-2-yl) urea (20o)

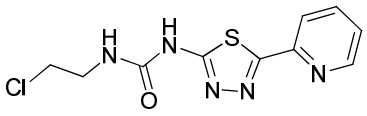


(**20o**)

The title compound was obtained as an oil, with 53% yield; Purity: 92.58 % by

HPLC; ESI (*m*/*z*) 323.0 (M+H); IR (KBr, cm^{-1}): 3243, 2875, 1692, 1563; ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 2H), 1.03 (m, 2H), 2.25 (m, 1H), 3.42 (t, 2H, *J* = 8.54 Hz), 3.62 (t, 2H, *J* = 6.13 Hz), 6.80 (s, 1H), 7.25(d, 2H, *J* = 8.44 Hz), 7.32 (d, 2H, *J* = 8.45 Hz),10.86 (s, 1H).

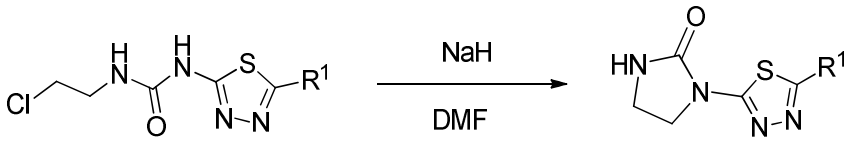
5.1.18.16. 1-(2-Chloroethyl)-3-(5-(pyridin-2-yl)-1,3,4-thiadiazol-2-yl)urea (20p)



(20p)

The title compound was obtained as an oil, with 53% yield; Purity: 98.76 % by HPLC; ESI (*m*/*z*) 384.7 (M+H); ^{1}H NMR (DMSO-d_6): δ 3.51 (t, 2H, *J* = 5.94 Hz), 3.66 (t, 2H, *J* = 6.13 Hz), 6.91 (m, 1H), 7.29 (d, 1H, *J* = 8.38 Hz), 7.66 (m, 1H), 8.16 (m, 1H), 8.51 (s, 1H), 9.32 (s, 1H).

5.1.19. General procedure for the synthesis of compound (21a-p)

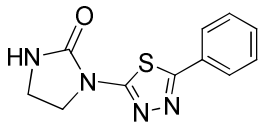


(20a-p) **(21a-p)**

Under nitrogen atmosphere 50 mL of dry DMF was placed in a 100 mL reaction flask and was cooled to 0^0C. NaH (1.5 equiv) was added followed by **20a**-**p**, (1 mole equiv) in reaction mixture and mixture was stirred for 1h at room temperature. The reaction mixture was partitioned between EtOAC (150 mL) and water (150 mL), and the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain pure derivatives **21a**-

p. The physicochemical properties and spectral data of some of the representative compounds are listed below.

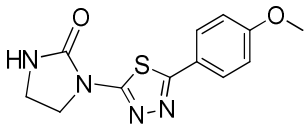
5.1.19.1. 1-(5-Phenyl-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21a)



(**21a**)

The title compound was obtained as an oil, with 55% yield; Purity: 97 % by HPLC; IR (KBr, cm^{-1}): 3427, 1724, 1500, 1342; ESI (*m*/*z*) 247.0 (M+H); ^{1}H NMR (DMSO-d_6): δ 3.54 (t, 2H, *J* = 8.42 Hz), 4.12 (t, 2H, *J* = 7.62 Hz), 7.50 (m, 3H), 7.88 (m, 2H),.7.91 (s, 1H), 7.96 (s, 1H).

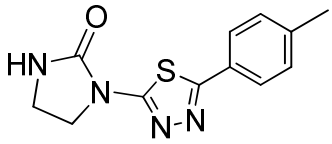
5.1.19.2. 1-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21b)



(**21b**)

The title compound was obtained as an oil, with 73% yield; Purity: 97.55 % by HPLC; ESI (*m*/*z*) 277.1 (M+H); IR (KBr, cm^{-1}): 3093, 1741, 1579, 1411; ^{1}H NMR (DMSO-d_6): δ 3.55 (t, 2H, *J* = 7.73 Hz), 3.81 (s, 3H), 4.10 (t, 2H, *J* = 7.47 Hz), 7.03 (d, 2H, *J* = 8.77 Hz), 7.81 (d, 2H, *J* = 8.72 Hz).

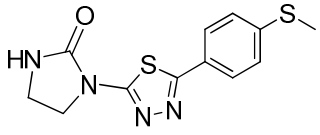
5.1.19.3. 1-(5-(p-Tolyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21c)



(**21c**)

The title compound was obtained as an oil, with 72% yield; ESI (*m*/*z*) 261.1 (M+H); IR (KBr, cm^{-1}): 3095, 1742, 1573, 1415; ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H), 3.55 (t, 2H, *J* = 5.83 Hz), 4.15 (t, 2H, *J* = 6.15 Hz), 6.86 (s, 1H), 7.12 (d, 2H, *J* = 6.92 Hz), 7.85 (d, 2H, *J* = 8.75 Hz).

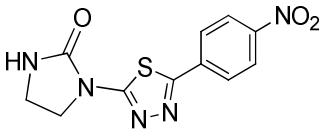
5.1.19.4. 1-(5-(4-(Methylthio)phenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21d)



(**21d**)

The title compound was obtained as an oil, with 53% yield; Purity: 92.71 % by HPLC; ESI (*m*/*z*) 293.0 (M+H); IR (KBr, cm^{-1}): 3427, 3109, 1726, 1477; ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H), 3.56 (t, 2H, *J* = 8.35 Hz), 4.10 (t, 2H, *J* = 6.58 Hz), 7.37 (d, 2H, *J* = 8.49 Hz), 7.83 (d, 2H, *J* = 8.46 Hz).

5.1.19.5. 1-(5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21e)

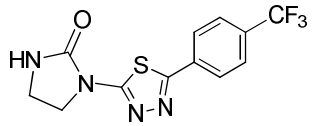


(**21e**)

The title compound was obtained as an oil, with 55% yield; Purity: 93.72 % by

HPLC; ESI (*m*/*z*) 292.3 (M+H); IR (KBr, cm^{-1}): 3428, 3103, 1724, 1530, 1477; ^1H NMR (DMSO-d_6): δ 3.52 (t, 2H, *J* = 8.45 Hz), 4.15 (t, 2H, *J* = 6.55 Hz), 7.84 (d, 2H, *J* = 8.53 Hz), 7.89 (d, 2H, *J* = 8.42 Hz).

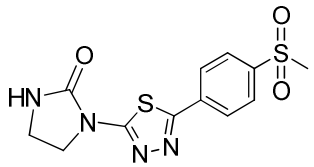
5.1.19.6. 1-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21f)



(**21f**)

The title compound was obtained as an oil, with 43% yield; ESI (*m*/*z*) 265.1 (M+H); ^1H NMR (DMSO-d_6): 3.42 (t, 2H, *J* = 8.36 Hz), 4.12 (t, 2H, *J* = 6.43 Hz), 7.55 (m, 2H), 7.67 (m, 2H).

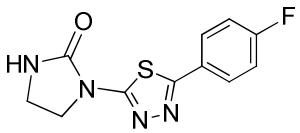
5.1.19.7. 1-(5-(4-(Methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21g)



(**21g**)

The title compound was obtained as an oil, with 55% yield; Purity: 95.91 % by HPLC; ESI (*m*/*z*) 324.38 (M+H); IR (KBr, cm^{-1}): 3422, 3101 1725, 1474; ^1H NMR (DMSO-d_6): δ 3.45 (s, 3H), 3.52 (t, 2H, *J* = 8.36 Hz), 4.16 (t, 2H, *J* = 6.45 Hz), 7.32 (d, 2H, *J* = 8.51 Hz), 7.85 (d, 2H, *J* = 8.47 Hz).

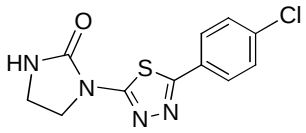
5.1.19.8. 1-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21h)



(**21h**)

The title compound was obtained as an oil, with 76% yield; Purity: 95.5 % by HPLC; ESI (*m*/*z*) 301.1 (M+H); IR (KBr, cm^{-1}): 3345, 1647, 1583, 1449; ^{1}H NMR (DMSO-d_6): δ 3.45 (t, 2H, *J* = 6.35 Hz), 3.68 (t, 2H, *J* = 6.27 Hz), 6.85 (s, 1H), 7.34 (m, 2H), 7.82 (m, 2H).

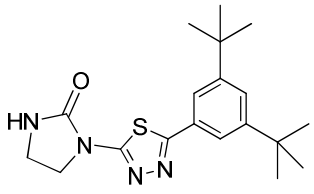
5.1.19.9. 1-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21i)



(**21i**)

The title compound was obtained as an oil, with 65% yield; ESI (*m*/*z*) 281.1 (M+H); IR (KBr, cm^{-1}): 3343, 1644, 1588, 1444; ^{1}H NMR (DMSO-d_6): δ 3.52 (t, 2H, *J* = 6.36 Hz), 4.14 (t, 2H, *J* = 6.25 Hz), 6.72 (s, 1H), 7.52 (d, 2H, *J* = 8.42 Hz), 7.95 (d, 2H, *J* = 8.43 Hz).

5.1.19.10. 1-(5-([1,1'-Biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (20j)

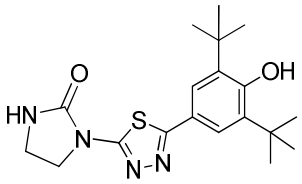


(**21j**)

The title compound was obtained as an oil, with 52% yield; Purity: 89.1 % by

HPLC; IR (KBr, cm^{-1}): 3613, 3393, 2955, 1712, 1535; ESI (*m*/*z*) 359.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.47 (s, 18H), 3.53 (m, 2H), 4.12 (t, 2H, *J* = 6.95 Hz), 7.62 (s, 1H), 6.83 (s, 1H), 7.57 (s, 1H).

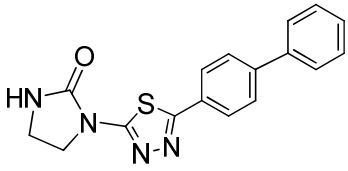
5.1.19.11. 1-(5-(3,5-Di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl) imidazolidin-2-one (21k)



(**21k**)

The title compound was obtained as an oil, with 91% yield; Purity: 95.23 % by HPLC; IR (KBr, cm^{-1}): 3228, 2954, 1728, 1500, 1407; ESI (*m*/*z*) 375.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.41 (s, 18H), 3.55 (t, 2H, *J* = 7.95 Hz), 4.09 (t, 2H, *J* = 7.29 Hz), 7.58 (s, 2H), 7.88 (s, 1H).

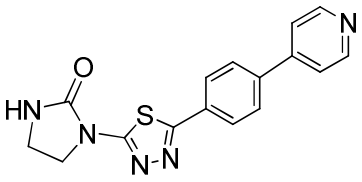
5.1.19.12. 1-(5-([1,1'-Biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21l)



(**21l**)

The title compound was obtained as an oil, with 45% yield; ESI (*m*/*z*) 323.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 3.43 (t, 2H, *J* = 7.95 Hz), 3.14 (t, 2H, *J* = 6.12 Hz), 5.75 (s, 2H), 6.95 (s, 1H), 7.25 (s, 1H), 7.52 (m, 5H), 7.94 (d, 1H, *J* = 8.45 Hz), 8.04 (d, 1H, *J* = 8.28 Hz).

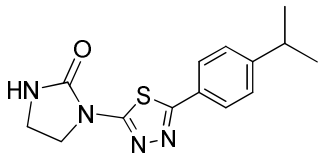
5.1.19.13. 1-(5-(4-(Pyridin-4-yl)phenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21m)



(**21m**)

The title compound was obtained as an oil, with 48% yield; ESI (*m*/*z*) 323.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 3.42 (t, 2H, *J* = 7.94 Hz), 3.15 (t, 2H, *J* = 6.15 Hz), 5.73 (s, 2H), 6.92 (s, 1H), 7.24 (s, 1H), 7.89 (m, 1H), 7.93 (m, 1H), 7.95 (d, 1H, *J* = 8.45 Hz), 8.07 (d, 1H, *J* = 8.24 Hz), 8.25 (m, 5H), 8.45 (m, 1H).

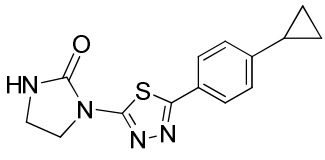
5.1.19.14. 1-(5-(4-Isopropylphenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21n)



(**21n**)

The title compound was obtained as an oil, with 53% yield; ESI (*m*/*z*) 289.1 (M+H); IR (KBr, cm^{-1}): 3241, 2874, 1693, 1564.

5.1.19.15. 1-(5-(4-Cyclopropylphenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21o)

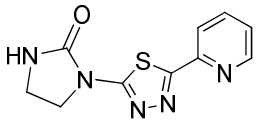


(**21o**)

The title compound was obtained as an oil, with 53% yield; Purity: 92.58 % by

HPLC; ESI (*m*/*z*) 287.1 (M+H); IR (KBr, cm^{-1}): 3242, 2874, 1692, 1567; ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 2H), 1.04 (m, 2H), 2.27 (m, 1H), 3.42 (t, 2H, *J* = 8.54 Hz), 4.12 (t, 2H, *J* = 6.13 Hz), 6.80 (s, 1H), 7.22 (d, 2H, *J* = 8.45 Hz), 7.34 (d, 2H, *J* = 8.45 Hz).

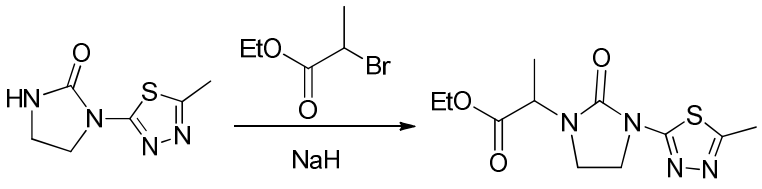
5.1.19.16. 1-(5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-yl)imidazolidin-2-one (21p)



(**21p**)

The title compound was obtained as an oil, with 63% yield; Purity: 97.71 % by HPLC; ESI (*m*/*z*) 247.2 (M+H); IR (KBr, cm^{-1}): 3236, 2922, 1700, 1473; ^{1}H NMR (DMSO-d_6): δ 3.38 (t, 2H, *J* = 5.54 Hz), 3.97 (t, 2H, *J* = 6.18 Hz), 6.94 (m, 1H), 7.16 (s, 1H), 7.67 (m, 1H), 8.13 (d, 1H, *J* = 8.54 Hz), 8.26 (m, 1H).

5.1.20. General procedure for the synthesis of compound (22a-p)

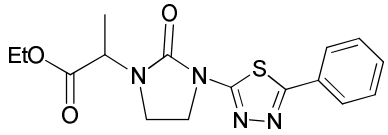


(**21a-p**) (**22a-p**)

Under nitrogen atmosphere, 50 mL of dry DMF was placed in a 250 mL reaction flask and was cooled to 0^0C. NaH (1.5 mole equiv) was added followed by **21a**-**p**, (1 mole equiv). The reaction mixture was stirred for 1h at room temperature and halo compound was added (1.1 mole equiv), reaction mixture was stirred for 30 min. The reaction mixture was partitioned between EtOAC (100 mL) and water (100 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and

evaporated under reduced pressure to obtain pure derivative. Using above procedure, compounds **22a**-**p** were prepared, the physicochemical properties and spectral data of some of the representative compounds are listed below.

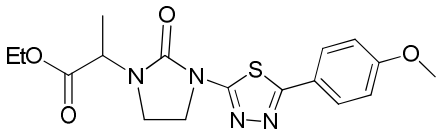
5.1.20.1. Ethyl2-(2-oxo-3-(5-phenyl-1,3,4-thiadiazol-2-yl)imidazolidin-1-yl) propanoate (22a)



(**22a**)

The title compound was obtained as an oil, with 71% yield; Purity: 93% by HPLC; IR (KBr, cm^{-1}): 3427, 1724, 1500, 1400, 1267; ESI (*m*/*z*) 247.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 3H), 1.27 (m, 3H), 1.42 (m, 4H), 3.71 (m, 1H), 4.24 (m, 2H), 7.44 (m, 3H), 7.91 (m, 1H).

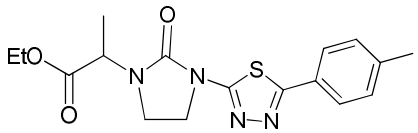
5.1.20.2. Ethyl2-(3-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22b)



(**22b**)

The title compound was obtained as an oil, with 86% yield; Purity: 95.13 % by HPLC; ESI (*m*/*z*) 377.2 (M+H); IR (KBr, cm^{-1}): 3454, 1739, 1602, 1402; ^{1}H NMR (DMSO-d_6): δ 1.19 (t, 3H, *J* = 7.14 Hz), 1.44 (d, 3H, *J* = 7.40 Hz), 3.71 (m, 2H), 3.81 (s, 3H), 4.10 (m, 2H), 4.56 (q, 1H, *J* = 7.34 Hz), 7.04 (d, 2H, *J* = 8.77 Hz), 7.81 (d, 2H, *J* = 8.72 Hz).

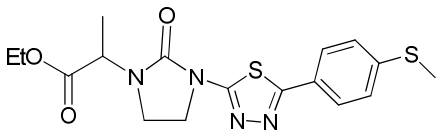
5.1.20.3. Ethyl2-(2-oxo-3-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)imidazolidin-1yl) propanoate (22c)



(**22c**)

The title compound was obtained as an oil, with 82% yield; Purity: 94.1 % by HPLC; ESI (*m*/*z*) 360.2 (M+H); IR (KBr, cm^{-1}): 3452, 1735, 1608, 1405; ^{1}H NMR (DMSO-d_6): δ 1.19 (t, 3H, *J* = 7.11 Hz), 1.44 (d, 3H, *J* = 7.45 Hz), 2.31 (s, 3H), 3.73 (m, 2H), 4.15 (m, 2H), 4.65 (q, 1H, *J* = 7.35Hz), 7.14 (d, 2H, *J* = 8.75 Hz), 7.81 (d, 2H, *J* = 8.77 Hz).

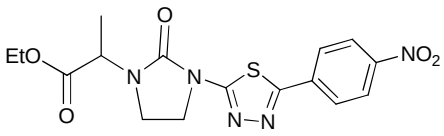
5.1.20.4. Ethyl2-(3-(5-(4-(methylthio)phenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22d)



(**22d**)

The title compound was obtained as an oil, with 62% yield; Purity: 93.31 % by HPLC; ESI (*m*/*z*) 393.0 (M+H); IR (KBr, cm^{-1}): 2916, 1712, 1502, 1431; ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 3H, *J* = 7.55 Hz), 1.44 (d, 3H, *J* = 7.16 Hz), 2.55 (s, 3H), 3.67 (m, 2H), 4.09 (m, 4H), 4.16 (q, 1H, *J* = 7.24 Hz), 7.35 (d, 2H, *J* = 8.40 Hz), 7.80 (d, 2H, *J* = 8.37 Hz).

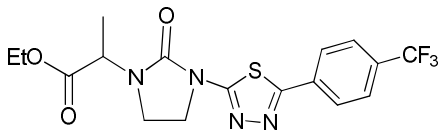
5.1.20.5. Ethyl2-(3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22e)



(**22e**)

The title compound was obtained as an oil, with 42% yield; Purity: 95.42 % by HPLC; ESI (*m*/*z*) 393. (M+H); IR (KBr, cm^{-1}): 2913, 1715, 1528, 1430; ^{1}H NMR (DMSO-d_6): δ 1.12 (t, 3H, *J* = 7.56 Hz), 1.48 (d, 3H, *J* = 7.12 Hz), 3.62 (m, 2H), 4.03 (m, 4H), 4.26 (q, 1H, *J* = 7.23 Hz), 7.44 (d, 2H, *J* = 8.45 Hz), 7.85 (d, 2H, *J* = 8.34 Hz).

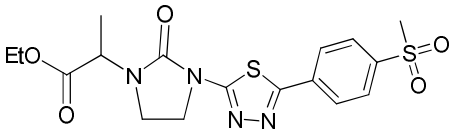
5.1.20.6. Ethyl2-(2-oxo-3-(5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl) imidazolidin-1-yl)propanoate (22f)



(**22f**)

The title compound was obtained as an oil, with 43% yield; Purity: 94.31 % by HPLC; ESI (*m*/*z*) 415.1 (M+H); IR (KBr, cm^{-1}): 2912, 1714, 1525, 1432; ^{1}H NMR (DMSO-d_6): δ 1.22 (t, 3H, *J* = 7.55 Hz), 1.45 (d, 3H, *J* = 7.15 Hz), 3.63 (m, 2H), 4.05 (m, 4H), 4.22 (q, 1H, *J* = 7.28 Hz), 7.44 (d, 2H, *J* = 8.43 Hz), 7.87 (d, 2H, *J* = 8.35 Hz).

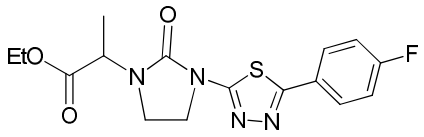
5.1.20.7. Ethyl2-(3-(5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22g)



(**22g**)

The title compound was obtained as an oil, with 57% yield; Purity: 97.9 % by HPLC; ESI (*m*/*z*) 425.1 (M+H); IR (KBr, cm^{-1}): 1735, 1508, 1431; ^1H NMR (DMSO-d_6): δ 1.19 (t, 3H, *J* = 7.08 Hz), 1.42 (d, 3H, *J* = 7.38 Hz), 3.32 (s, 3H), 3.72 (m, 2H), 4.12 (m, 4H), 4.17 (q, 1H, *J* = 7.33 Hz), 8.03 (d, 2H, *J* = 8.37 Hz), 7.16 (d, 2H, *J* = 8.45 Hz).

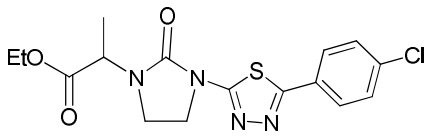
5.1.20.8. Ethyl2-(3-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22h)



(**22h**)

The title compound was obtained as an oil, with 73% yield; Purity: 93.2 % by HPLC; ESI (*m*/*z*) 365.1 (M+H); IR (KBr, cm^{-1}): 1735, 1501, 1437; ^1H NMR (DMSO-d_6): δ 1.12 (t, 3H, *J* = 7.05 Hz), 1.57 (d, 3H, *J* = 7.33 Hz), 3.82 (m, 2H), 4.28 (m, 4H), 4.25 (q, 1H, *J* = 7.32 Hz), 8.15 (d, 2H, *J* = 8.39 Hz), 7.25 (d, 2H, *J* = 8.45 Hz).

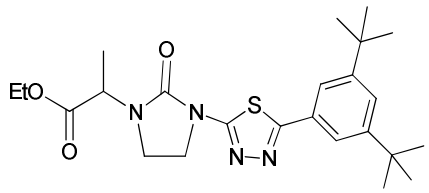
5.1.20.9. Ethyl2-(3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22i)



(**22i**)

The title compound was obtained as an oil, with 78% yield; Purity: 94.5% by HPLC; ESI (*m*/*z*) 381.1 (M+H); IR (KBr, cm^{-1}): 1732, 1505, 1433; ^1H NMR (DMSO-d_6): δ 1.15 (t, 3H, *J* = 7.09 Hz), 1.54 (d, 3H, *J* = 7.33 Hz), 3.87 (m, 2H), 4.22 (m, 4H), 4.24 (q, 1H, *J* = 7.35 Hz), 8.15 (d, 2H, *J* = 8.49 Hz), 7.26 (d, 2H, *J* = 8.35 Hz).

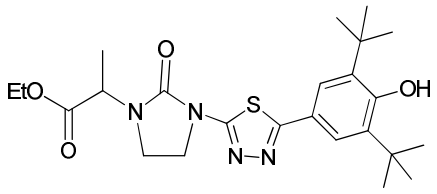
5.1.20.10. Ethyl2-(3-(5-(3,5-di-tert-butylphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22j)



(**22j**)

The title compound was obtained as an oil, with 62% yield; IR (KBr, cm^{-1}): 3567, 2953, 1737, 1503, 1265; ESI (*m*/*z*) 459.1 (M+H);

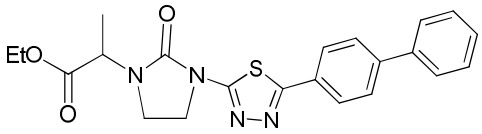
5.1.20.11. Ethyl2-(3-(5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22k)



(**22k**)

The title compound was obtained as an oil, with 63% yield; Purity: 96.59% by HPLC; IR (KBr, cm^{-1}): 3568, 2954, 1733, 1508, 1267; ESI (*m*/*z*) 475.3 (M+H); ^1H NMR (DMSO-d_6): δ 1.27 (t, 2H, *J* = 7.02 Hz), 1.47 (s, 18H), 1.57 (d, 3H, *J* = 6.02 Hz), 3.71 (m, 1H), 3.82 (m, 1H), 4.23 (m, 3H), 4.72 (m, 1H), 5.50 (s, 1H), 7.74 (s, 1H).

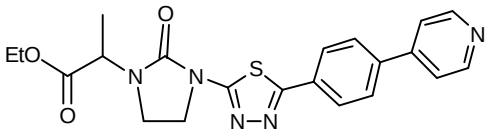
5.1.20.12. Ethyl2-(3-(5-([1, 1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22l)



(**22l**)

The title compound was obtained as an oil, with 72% yield; ESI (*m*/*z*) 423.1 (M+H); IR (KBr, cm^{-1}): 1735, 1507, 1433.

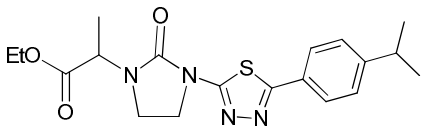
5.1.20.13. Ethyl2-(2-oxo-3-(5-(4-(pyridin-4-yl)phenyl)-1,3,4-thiadiazol-2-yl) imidazolidin-1-yl)propanoate (22m)



(**22m**)

The title compound was obtained as an oil, with 72% yield; ESI (*m*/*z*) 424.1 (M+H); IR (KBr, cm^{-1}): 1739, 1502, 1436.

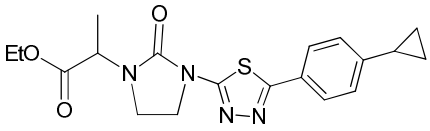
5.1.20.14. Ethyl2-(3-(5-(4-isopropylphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22n)



(**22o**)

The title compound was obtained as an oil, with 48% yield; ESI (*m*/*z*) 389.2 (M+H); IR (KBr, cm^{-1}): 3019, 1712, 1675, 1482.

5.1.20.15. Ethyl2-(3-(5-(4-cyclopropylphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoate (22o)

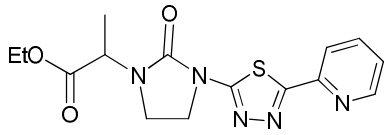


(**22o**)

The title compound was obtained as an oil, with 43% yield; Purity: 90.13 % by HPLC; ESI (*m*/*z*) 387.2 (M+H); IR (KBr, cm^{-1}): 3018, 1716, 1672, 1481; ^{1}H NMR (DMSO-d_6): δ 0.92 (m, 2H), 1.09 (m, 2H), 1.39 (d, 3H, *J* = 7.41 Hz), 2.35 (m, 1H),

3.55 (m, 2H), 3.63 (s, 3H), 4.00 (m, 2H), 4.58 (q, 1H, *J* = 7.39 Hz), 3.90 (s, 3H), 4.05 (m, 2H), 4.55 (m, 1H), 8.12 (d, 2H, *J* = 8.51 Hz), 7.25 (d, 2H, *J* = 8.36 Hz).

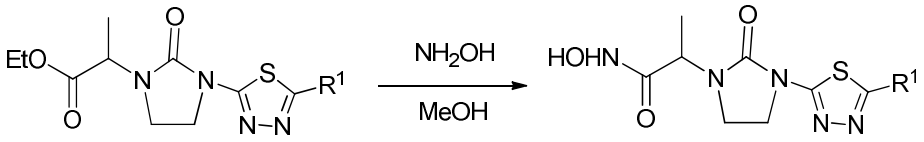
5.1.20.16. Ethyl2-(2-oxo-3-(5-(pyridin-2-yl)-1,3,4-thiadiazol-2-yl)imidazolidin-1-yl)propanoate (22p)



(**22p**)

The title compound was obtained as an oil, with 43% yield; Purity: 94.74 % by HPLC; ESI (*m*/*z*) 348.4 (M+H); IR (KBr, cm^{-1}): 3018, 1735, 1591, 1425; ^{1}H NMR (DMSO-d_6): δ 1.28 (t, 2H, *J* = 6.54 Hz), 1.50 (d, 3H, *J* = 6.28 Hz), 3.54 (m, 1H), 3.66 (m, 1H), 4.10 (m, 2H), 4.18 (m, 2H), 4.73 (q, 1H, *J* = 7.54 Hz), 6.91 (m, 1H), 7.61 (m, 2H), 8.27 (m, 2H).

5.1.21. General procedure for the synthesis of compound (23a-p)

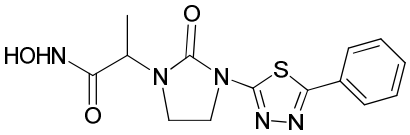


(**22a-p**) (**23a-p**)

The ester derivatives of **22a**-**p**, (1 mole equiv) was dissolved in MeOH (30 mL) and NH_2OH solution in MeOH (30 mL) was added to the reaction mixture at 0-5 ^{0}C. Mixture was stirred for 4 h at room temperature (25 ^{0}C) and quenched with ice-cold water (50 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude hydroxamic acid derivative, which was purified by column chromatography, using a mixture of $CHCl_3$ and MeOH (1:2) as

an eluant to obtain pure hydroxamic acid derivatives **23a**-**p**. Using above procedure, compounds **23a**-**p** were prepared, the physicochemical properties and spectral data of some of the representative compounds are listed below.

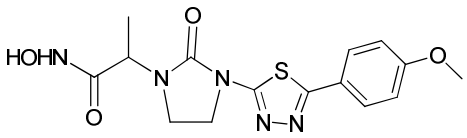
5.1.21.1. *N*-Hydroxy-2-(2-oxo-3-(5-phenyl-1,3,4-thiadiazol-2-yl)imidazolidin-1-yl)propanamide (23a)



(**23a**)

The title compound was obtained as a white solid, with 50% yield; mp 180-181°C; Purity: 95.20 % by HPLC; IR (KBr, cm^1): 3427, 1718, 1431, 1023; ESI (*m*/*z*) 334.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.35 (d, 3H, *J* = 7.38 Hz), 2.49 (m, 4H), 3.68 (m, 2H), 4.10 (m, 2H), 4.36 (q, 1H, *J* = 7.26 Hz), 7.51 (m, 3H), 7.88 (m, 2H), 8.93 (s, 1H), 10.13 (s, 1H), 10.81 (s, 1H).

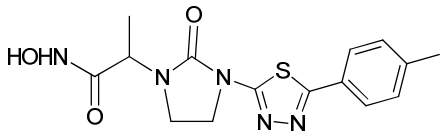
5.1.21.2. N**-hydroxy-2-(3-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanamide (23b)**



(**23b**)

The title compound was obtained as a white solid, with 63% yield; mp 180-184°C; Purity: 93.32 % by HPLC; IR (KBr, cm^1): 3423, 2854, 1710, 1452; ESI (*m*/*z*) 363.9 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.33 (d, 3H, *J* = 9.64 Hz), 3.67 (m, 2H), 3.79 (s, 3H), 4.07 (m, 2H), 4.36 (m, 1H), 7.04 (d, 2H, *J* = 7.38 Hz), 7.81 (d, 2H, *J* = 8.42 Hz), 10.78 (s, 1H).

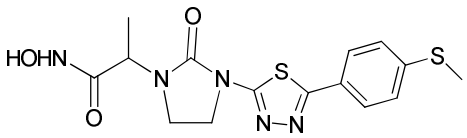
5.1.21.3. *N*-Hydroxy-2-(2-oxo-3-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)imidazolidin-1-yl)propanamide (23c)



(**23c**)

The title compound was obtained as a white solid, with 61% yield; Purity: 94.51 % by HPLC; IR (KBr, cm^1): 3422, 2854, 1715, 1458; ESI (*m*/*z*) 340.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.35 (d, 3H, *J* = 7.54 Hz), 2.35 (s, 3H), 3.65 (m, 2H), 4.15 (m, 2H), 4.46 (m, 1H), 7.05 (d, 2H, *J* = 7.35 Hz), 7.85 (d, 2H, *J* = 8.48 Hz), 10.72 (s, 1H).

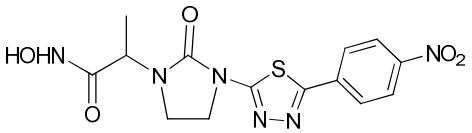
5.1.21.4. *N*-Hydroxy-2-(3-(5-(4-(methylthio)phenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanamide (23d)



(**23d**)

The title compound was obtained as a white solid, with 63% yield; mp 170-172°C; Purity: 94.57 % by HPLC; IR (KBr, cm^1): 3423, 2854, 1710, 1452; ESI (*m*/*z*) 380.0 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.31 (d, 3H, *J* = 9.64 Hz), 2.48 (s, 3H), 3.70 (m, 2H), 4.10 (m, 2H), 4.36 (m, 1H), 7.35 (d, 2H, *J* = 7.38 Hz), 7.80 (d, 2H, *J* = 8.42 Hz), 8.92 (s, 1H), 10.80 (s, 1H).

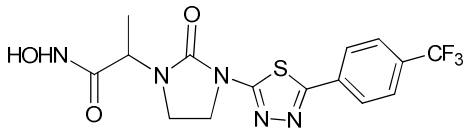
5.1.21.5. *N*-Hydroxy-2-(3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanamide (23e)



(**23e**)

The title compound was obtained as a white solid, with 60% yield; mp 205-210°C; Purity: 94.36% by HPLC; IR (KBr, cm^{1}): 3365, 1701, 1598, 1475; ESI (*m*/*z*) 379.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.32 (d, 3H, *J* = 7.29 Hz), 3.89 (m, 2H), 3.94 (m, 2H), 4.37 (q, 1H, *J* = 7.27 Hz), 7.77 (d, 1H, *J* = 9.33 Hz), 8.19 (d, 1H, *J* = 9.27 Hz), 8.85 (s, 1H), 10.72 (s, 1H).

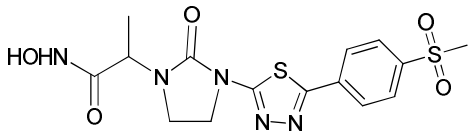
5.1.21.6. *N*-Hydroxy-2-(2-oxo-3-(5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)imidazolidin-1-yl)propanamide (23f)



(**23f**)

The title compound was obtained as a white solid, with 63% yield; Purity: 92.40% by HPLC; IR (KBr, cm^{1}): 3362, 1704, 1596, 1479; ESI (*m*/*z*) 402.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.42 (d, 3H, *J* = 7.28 Hz), 3.92 (m, 2H), 3.95 (m, 2H), 4.45 (q, 1H, *J* = 7.25 Hz), 7.75 (d, 1H, *J* = 9.35 Hz), 8.25 (d, 1H, *J* = 9.25 Hz), 8.87 (s, 1H), 10.79 (s, 1H).

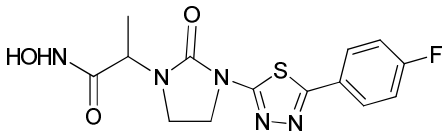
5.1.21.7. *N*-Hydroxy-2-(3-(5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanamide (23g)



(**23g**)

The title compound was obtained as a white solid, with 68% yield; mp 210-212°C; Purity: 98 % by HPLC; IR (KBr, cm^1): 3431, 2852, 1431, 1685, 1436, 1313; ESI (*m*/*z*) 412.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.34 (d, 3H, *J* = 7.64 Hz), 3.26 (s, 3H), 3.65 (m, 2H), 4.12 (m, 2H), 3.40 (m, 1H), 8.00 (d, 2H, *J* = 8.00 Hz), 8.19 (d, 2H, *J* = 8.12 Hz), 8.94 (s, 1H), 10.82 (s, 1H).

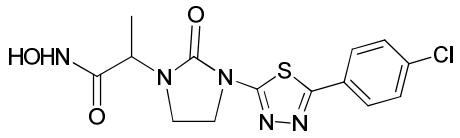
5.1.21.8. 2-(3-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)-*N*-hydroxypropanamide (23h)



(**23h**)

The title compound was obtained as a white solid, with 71% yield; Purity: 93.67 % by HPLC; IR (KBr, cm^1): 3427, 2857, 1718, 1454; ESI (*m*/*z*) 352.0 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.25 (d, 3H, *J* = 7.65 Hz), 2.52 (s, 3H), 3.81 (m, 2H), 4.15 (m, 2H), 4.39 (m, 1H), 7.45 (d, 2H, *J* = 7.48 Hz), 7.85 (d, 2H, *J* = 8.51 Hz), 8.95 (s, 1H), 10.82 (s, 1H).

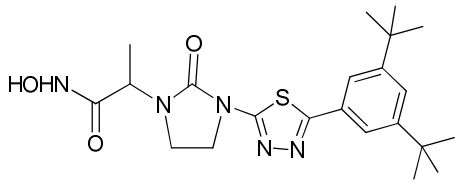
5.1.21.9. 2-(3-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)-*N*-hydroxypropanamide (23i)



(**23i**)

The title compound was obtained as a white solid, with 75% yield; Purity: 94.23 % by HPLC; IR (KBr, cm^1): 3421, 2853, 1715, 1453; ESI (*m*/*z*) 367.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.27 (d, 3H, *J* = 7.63 Hz), 2.58 (s, 3H), 3.89 (m, 2H), 4.12 (m, 2H), 4.35 (m, 1H), 7.48 (d, 2H, *J* = 7.42 Hz), 7.88 (d, 2H, *J* = 8.54 Hz), 8.98 (s, 1H), 10.83 (s, 1H).

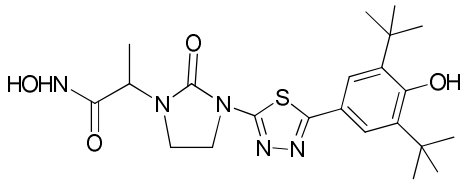
5.1.21.10. 2-(3-(5-(3,5-Di-tert-butylphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)-*N*-hydroxypropanamide (23j)



(**23j**)

The title compound was obtained as a white solid, with 58% yield; Purity: 92.45 % by HPLC; ESI (*m*/*z*) 445.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.35 (d, 3H, *J* = 6.43 Hz), 2.65 (s, 3H), 3.62 (t, 2H, *J* = 7.76 Hz), 4.05 (t, 2H, *J* = 7.25 Hz), 4.32 (q, 1H, *J* = 7.22 Hz), 7.68 (s, 2H), 7.04 (s, 1H), 7.42 (t, 1H, *J* = 7.47 Hz), 7.62 (t, 1H, *J* = 7.16 Hz), 7.76 (d, 1H, *J* = 8.25 Hz), 8.01 (d, 1H, *J* = 8.23 Hz), 8.94 (s, 1H), 10.71 (s, 1H).

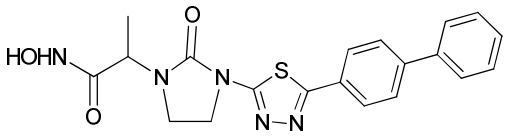
5.1.21.11. 2-(3-(5-(3,5-Di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)-*N*-hydroxypropanamide (23k)



(**23k**)

The title compound was obtained as a white solid, with 51% yield; Purity: 91.60 % by HPLC; ESI (*m*/*z*) 429.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.33 (d, 3H, *J* = 6.35 Hz), 2.61 (s, 3H), 3.68 (t, 2H, *J* = 7.98 Hz), 4.06 (t, 2H, *J* = 7.22 Hz), 4.33 (q, 1H, *J* = 7.22 Hz),7.75 (s, 2H), 7.17 (s, 1H), 7.52 (t, 1H, *J* = 7.50 Hz), 7.72 (t, 1H, *J* = 7.02 Hz), 7.86 (d, 1H, *J* = 8.37 Hz), 8.02 (d, 1H, *J* = 8.04 Hz), 8.90 (s, 1H), 10.78 (s, 1H).

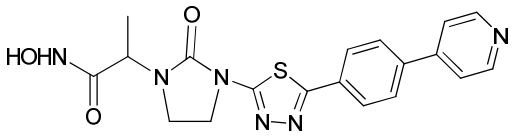
5.1.21.12. 2-(3-(5-([1,1-Biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)-*N*-hydroxypropanamide (23l)



(**23l**)

The title compound was obtained as a white solid, with 45% yield; Purity: 93.27 % by HPLC; IR (KBr, cm^1): 3425, 2863, 1735, 1423; ESI (*m*/*z*) 501.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.22 (d, 3H, *J* = 7.63 Hz), 2.54 (s, 3H), 3.86 (m, 2H), 4.12 (m, 2H), 4.37 (m, 1H), 7.35 (m, 5H), 7.43 (d, 2H, *J* = 7.13 Hz), 7.82 (d, 2H, *J* = 8.12 Hz), 8.96 (s, 1H), 10.85 (s, 1H).

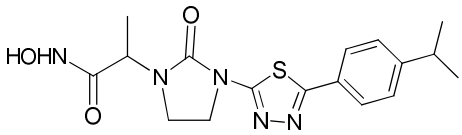
5.1.21.13. *N*-Hydroxy-2-(2-oxo-3-(5-(4-(pyridin-4-yl)phenyl)-1,3,4-thiadiazol-2-yl) imidazolidin-1-yl)propanamide (23m)



(**23m**)

The title compound was obtained as a white solid, with 49% yield; Purity: 92.42 % by HPLC; IR (KBr, cm^1): 3415, 2872, 1756, 1413; ESI (*m*/*z*) 511.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.23 (d, 3H, *J* = 7.23 Hz), 2.45 (s, 3H), 3.79 (m, 2H), 4.14 (m, 2H), 4.35 (m, 1H), 7.43 (d, 2H, *J* = 7.13 Hz), 7.82 (d, 2H, *J* = 8.12 Hz), 8.85 (m, 2H), 8.86 (m, 2H), 8.96 (s, 1H), 10.85 (s, 1H).

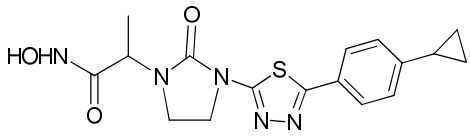
5.1.21.14. *N*-Hydroxy-2-(3-(5-(4-isopropylphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanamide (23n)



(**23n**)

The title compound was obtained as a white solid, with 56% yield; Purity: 94.23 % by HPLC; IR (KBr, cm^1): 3143, 1672, 1425, 1272; ESI (*m*/*z*) 376.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.95 (m, 2H), 1.06 (m, 2H), 1.35 (d, 3H, *J* = 7.73 Hz), 2.35 (m, 1H), 3.67 (m, 2H), 3.92 (m, 2H), 4.37 (q, 1H, *J* = 7.14 Hz), 7.45 (d, 2H, *J* = 7.13 Hz), 7.87 (d, 2H, *J* = 8.12 Hz), 8.92 (s, 1H), 10.75 (s, 1H).

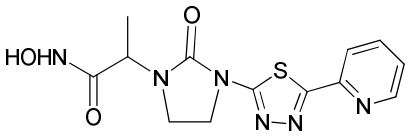
5.1.21.15. 2-(3-(5-(4-Cyclopropylphenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)*N*-hydroxypropanamide (23o)



(**23o**)

The title compound was obtained as a white solid, with 67% yield; mp 180-182°C; Purity: 98.66 % by HPLC; IR (KBr, cm^{1}): 3149, 1670, 1429, 1272; ESI (*m*/*z*) 298.2 (M+H); ^{1}H NMR (DMSO-d_6): δ 0.91 (m, 2H), 1.08 (m, 2H), 1.30 (d, 3H, *J* = 7.73 Hz), 2.34 (m, 1H), 3.65 (m, 2H), 3.97 (m, 2H), 4.32 (q, 1H, *J* = 7.26 Hz), 7.42 (d, 2H, *J* = 7.43 Hz), 7.86 (d, 2H, *J* = 8.25 Hz), 8.90 (s, 1H), 10.77 (s, 1H).

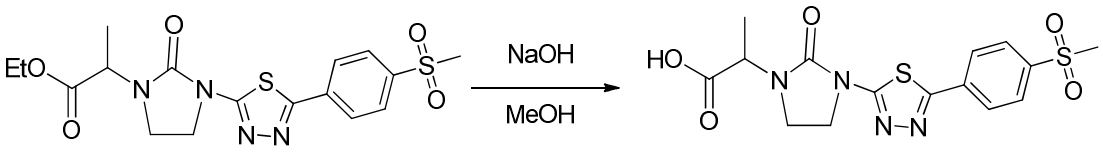
5.1.21.16. *N*-Hydroxy-2-(2-oxo-3-(5-(pyridin-2-yl)-1,3,4-thiadiazol-2-yl) imidazolidin-1-yl)propanamide (23p)



(**23p**)

The title compound was obtained as a white solid, with 53% yield; Purity: 96.93 % by HPLC; IR (KBr, cm^{-1}): 3184, 2979, 1706, 1641; ESI (*m*/*z*) 335.3 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.28 (t, 2H, *J* = 7.23 Hz), 3.92 (m, 2H), 3.93 (m, 2H), 4.36 (q, 1H, *J* = 7.26 Hz), 6.97 (m, 1H), 7.70 (m, 1H), 8.13 (d, 1H, *J* = 8.49 Hz), 8.27 (m, 1H), 8.85 (s, 1H), 10.73 (s, 1H).

5.1.22. General procedure for the synthesis of 2-(3-(5-(4-(methylsulfonyl) phenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanoicacid (24)

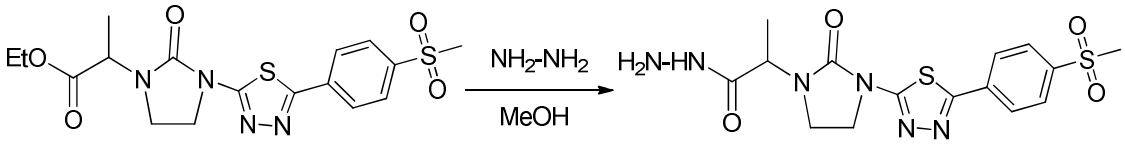


(**22a**) (**24**)

The ester derivatives of (**22a**, 1 mole equiv) was dissolved in MeOH (30 mL) and NaOH (4 mole equiv) was added to the reaction mixture at 0-5 ^{0}C. Mixture was stirred for 4 h at room temperature (25 ^{0}C) and quenched with ice-cold water (50 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude as title compound, which was purified by column chromatography, using a mixture of $CHCl_3$ and MeOH (1:2) as an eluant to obtain pure title compound **24** as a white solid.

Yield = 54%; mp 240-242 °C; Purity: 98.8. % by HPLC; IR (KBr, cm^{-1}): 2925, 1676, 1436, 1280; ESI (*m*/*z*) 396.9 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.43 (d, 3H, *J* = 7.44 Hz), 2.56 (s, 3H), 3.64 (m, 2H), 4.10 (m, 2H), 4.49 (q, 2H, *J* = 7.25 Hz), 8.03 (d, 2H, *J* = 8.22 Hz), 8.16 (d, 2H, *J* = 7.34 Hz).

5.1.23. General procedure for the synthesis of 2-(3-(5-(4-(methylsulfonyl) phenyl)-1,3,4-thiadiazol-2-yl)-2-oxoimidazolidin-1-yl)propanehydrazide (25)

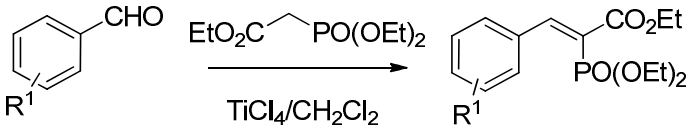


(**22a**) (**25**)

The ester derivatives of (**22a**, 1 mole equiv) was dissolved in MeOH (30 mL) and NH_2-NH_2 (1.1 mole equiv) was added to the reaction mixture at 0-5 ^{0}C. Mixture was stirred for 4 h at room temperature (25 ^{0}C) and quenched with ice-cold water (50 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 20 mL), the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude as title compound, which was purified by column chromatography, using a mixture of $CHCl_3$ and MeOH (1:2) as an eluant to obtain pure title compound **25** as a white solid.

Yield = 52%; Purity: 94.5% by HPLC; IR (KBr, cm^{-1}): 3302, 3234, 1719, 1670, 1517; ESI (*m*/*z*) 411.1 (M+H); ^{1}H NMR (DMSO-d_6): δ 1.43 (d, 3H, *J* = 7.44 Hz), 2.55 (s, 3H), 3.69 (t, 2H, *J* = 9.24 Hz), 4.09 (m, 2H), 4.25 (s, 2H), 4.41 (q, 1H, *J* = 7.26 Hz), 7.50 (m, 3H), 7.88 (m, 2H), 9.32 (s, 1H).

5.1.24. General procedure for the synthesis of compound (27a-w)

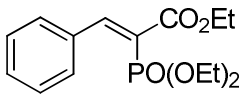


(**26a-w**) (**27a-w**)

Under nitrogen atmosphere 60 mL of dry CH_2Cl_2 was placed in a 250 mL reaction flask and was cooled to 0^{0}C. $TiCl_4$ (1.2 mole equiv) was added drop wise, followed by addition of substituted benzaldehyde (**26a**-**w**, 1 mole equiv). Ethyl 2-(diethoxyphosphoryl) acetate (1.1 mole equiv) was added followed by 4-methyl morpholine (1.2 mole equiv). The mixture was stirred for 3h at room temperature. The reaction mixture was partitioned between EtOAC (90 mL) and water (100 mL). The EtOAC and evaporated to dryness to get crude compounds. Crude compounds were subjected to column chromatography over silica gel with

10% acetonitrile in CH_2Cl_2 as mobile phase, to get the pure compounds **27a**-**w** as oil. Using above procedure, compounds **27a**-**w** were prepared, the physicochemical properties and spectral data of some of the representative compounds are listed below.

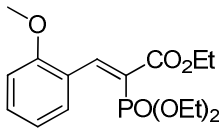
5.1.24.1. (E)-Ethyl2-(diethoxyphosphoryl)-3-phenylacrylate (1)



(**1**)

The title compound was obtained as an oil, with 85% yield; Purity: 96% by HPLC; IR (KBr, cm^{-1}) : 2987, 1722, 1614, 1249; ESI (*m*/*z*) 313.2 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.26 (t, 3H, *J* = 7.14 Hz), 1.32 (t, 6H, *J* = 7.65 Hz), 4.19 (m, 4H), 4.27 (q, 2H, *J* = 7.14 Hz), 7.38 (m, 5H), 7.61 (d, 1H, *J* = 24.18 Hz).

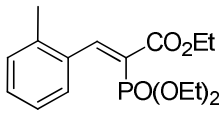
5.1.24.2. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(2-methoxyphenyl)acrylate (27a)



(**27a**)

The title compound was obtained as an oil, with 62% yield; Purity: 93.42 % by HPLC; IR (KBr, cm^{-1}) : 2982, 1721, 1605, 1518; ESI (*m*/*z*) 343.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.23 (t, 3H, *J* = 7.15 Hz), 1.32 (t, 6H, *J* = 6.98 Hz), 3.83 (s, 3H), 4.15 (q, 4H, *J* = 6.35 Hz), 4.32 (q, 2H, *J* = 7.25 Hz), 6.95 (d, 1H, *J* = 8.83 Hz), 6.97 (m, 1H), 7.22 (1H, m), 7.66 (d, 1H, *J* = 8.83 Hz), 7.53 (d, 1H, *J* = 24.7 Hz).

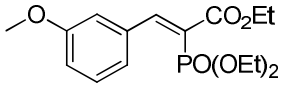
5.1.24.3. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(o-tolyl)acrylate (27b)



(**27b**)

The title compound was obtained as an oil, with 71% yield; Purity: 94.43% by HPLC; IR (KBr, cm^{-1}) : 2983, 1724, 1602, 1515; ESI (*m*/*z*) 327.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.25 (t, 3H, *J* = 7.12 Hz), 1.31 (t, 6H, *J* = 6.95 Hz), 2.48 (s, 3H), 4.15 (q, 4H, *J* = 6.33 Hz), 4.32 (q, 2H, *J* = 7.23 Hz), 7.01 (d, 1H, *J* = 8.82 Hz), 7.21 (1H, m), 7.21 (m, 1H), 7.25 (d, 1H, *J* = 8.85 Hz), 7.53 (d, 1H, *J* = 24.2 Hz).

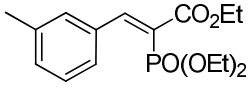
5.1.24.4. (E)-ethyl 2-(diethoxyphosphoryl)-3-(3-methoxyphenyl)acrylate (27c)



(**27c**)

The title compound was obtained as an oil, with 67% yield; Purity: 97.97 % by HPLC; IR (KBr, cm^{-1}) : 3001, 1718, 1599, 1246; ESI (*m*/*z*) 343.0 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.09 (t, 3H, *J* = 7.23 Hz), 1.33 (t, 6H, *J* = 6.93 Hz), 3.75 (s, 3H), 4.19 (q, 4H, *J* = 6.33 Hz), 4.25 (q, 2H, *J* = 7.22 Hz), 6.92 (m, 1H), 6.97 (d, 1H, *J* = 7.21 Hz), 7.26 (1H, s), 7.57 (d, 1H, *J* = 24.5 Hz).

5.1.24.5. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(m-tolyl)acrylate (27d)

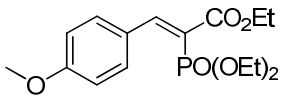


(**27d**)

The title compound was obtained as an oil, with 62% yield; Purity: 96.52 % by

HPLC; IR (KBr, cm^{-1}) : 2982, 1724, 1607, 1512; ESI (*m*/*z*) 327.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.15 (t, 3H, *J* = 7.22 Hz), 1.38 (t, 6H, *J* = 6.92 Hz), 2.34 (s, 3H), 4.19 (q, 4H, *J* = 6.31 Hz), 4.27 (q, 2H, *J* = 7.25 Hz), 6.94 (m, 1H), 7.05 (d, 1H, *J* = 7.22 Hz), 7.28 (1H, s), 7.59 (d, 1H, *J* = 24.6 Hz).

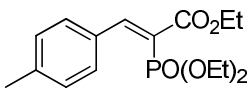
5.1.24.6. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(4-methoxyphenyl)acrylate (27e)



(**27e**)

The title compound was obtained as an oil, with 69% yield; Purity: 97.43 % by HPLC; IR (KBr, cm^{-1}) : 2987, 1720, 1604, 1512; ESI (*m*/*z*) 343.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.25 (t, 3H, *J* = 7.17 Hz), 1.35 (t, 6H, *J* = 6.99 Hz), 3.84 (s, 3H), 4.16 (q, 4H, *J* = 6.36 Hz), 4.31 (q, 2H, *J* = 7.20 Hz), 6.87 (d, 2H, *J* = 8.85 Hz), 7.40 (d, 2H, *J* = 8.81 Hz), 7.61 (d, 1H, *J* = 24.14 Hz).

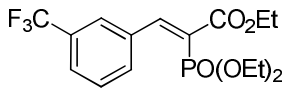
5.1.24.7. (E)-ethyl 2-(diethoxyphosphoryl)-3-(p-tolyl)acrylate (27f)



(**27f**)

The title compound was obtained as an oil, with 69% yield; Purity: 92.45 % by HPLC; IR (KBr, cm^{-1}) : 2982, 1725, 1602, 1518; ESI (*m*/*z*) 327.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.22 (t, 3H, *J* = 7.15 Hz), 1.33 (t, 6H, *J* = 6.92 Hz), 2.34 (s, 3H), 4.15 (q, 4H, *J* = 6.38 Hz), 4.35 (q, 2H, *J* = 7.22 Hz), 6.85 (d, 2H, *J* = 8.82 Hz), 7.47 (d, 2H, *J* = 8.83 Hz), 7.65 (d, 1H, *J* = 24.18 Hz).

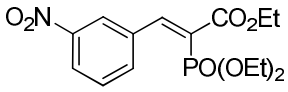
5.1.24.8. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-(trifluoromethyl)phenyl) acrylate (27g)



(**27g**)

The title compound was obtained as an oil, with 62% yield; Purity: 94.46 % by HPLC; IR (KBr, cm^{-1}) : 3012, 1724, 1625, 1472; ESI (*m*/*z*) 381.2 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.25 (t, 3H, *J* = 7.14 Hz), 1.32 (t, 6H, *J* = 7.16 Hz), 4.25 (m, 4H), 4.26 (q, 2H, *J* = 7.21 Hz), 7.19 (m, 1H), 7.48 (s, 1H), 7.50 (d, 1H, *J* = 8.32 Hz), 7.80 (d, 1H, *J* = 8.32 Hz), 7.62 (d, 1H, *J* = 23.72 Hz).

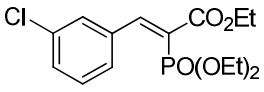
5.1.24.9. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-nitrophenyl)acrylate (27h)



(**27h**)

The title compound was obtained as an oil, with 58% yield; Purity: 93.86 % by HPLC; IR (KBr, cm^{-1}) : 3015, 1727, 1622, 1474; ESI (*m*/*z*) 358.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.22 (t, 3H, *J* = 7.23 Hz), 1.37 (t, 6H, *J* = 7.27 Hz), 4.23 (m, 4H), 4.37 (q, 2H, *J* = 7.31 Hz), 7.25 (m, 1H), 7.95 (d, 1H, *J* = 8.13 Hz), 8.14 (d, 1H, *J* = 8.12 Hz), 8.31 (s, 1H), 7.62 (d, 1H, *J* = 24.12 Hz).

5.1.24.10. (E)-Ethyl 3-(3-chlorophenyl)-2-(diethoxyphosphoryl)acrylate (27i)

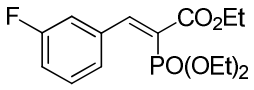


(**27i**)

The title compound was obtained as an oil, with 68% yield; Purity: 95.85 % by HPLC; IR (KBr, cm^{-1}) : 2993, 1720, 1490, 1251; ESI (*m*/*z*) 347.1 ($M+H^+$); ^1H NMR

($CDCl_3$) : δ 1.24 (t, 3H, *J* = 7.26 Hz), 1.32 (t, 6H, *J* = 6.91 Hz), 4.18 (m, 4H), 4.24 (q, 2H, *J* = 7.26 Hz), 7.30 (s, 1H), 7.34 (m, 1H), 7.37 (d, 1H, *J* = 7.26 Hz), 7.47 (d, 1H, *J* = 7.16 Hz), 7.37 (d, 1H, *J* = 24.06 Hz).

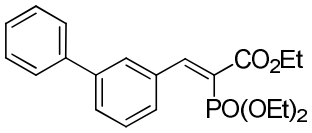
5.1.24.11. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-fluorophenyl)acrylate (27j)



(**27j**)

The title compound was obtained as an oil, with 61% yield; Purity: 92.87 % by HPLC; IR (KBr, cm^{-1}) : 2992, 1723, 1491, 1255; ESI (*m*/*z*) 331.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.25 (t, 3H, *J* = 7.25 Hz), 1.31 (t, 6H, *J* = 6.94 Hz), 4.15 (m, 4H), 4.22 (q, 2H, *J* = 7.25 Hz), 6.16 (m, 1H), 7.12 (m, 1H), 7.25 (m, 1H), 7.32 (m, 1H), 7.12 (d, 1H, *J* = 24.12 Hz).

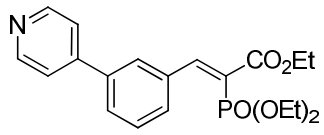
5.1.24.12. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(m-tolyl)acrylate (27k)



(**27k**)

The title compound was obtained as an oil, with 64% yield; Purity: 96.41 % by HPLC; IR (KBr, cm^{-1}) : 2981, 1724, 1602, 1514; ESI (*m*/*z*) 388.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.11 (t, 3H, *J* = 7.23 Hz), 1.32 (t, 6H, *J* = 6.91 Hz), 4.15 (q, 4H, *J* = 6.32 Hz), 4.22 (q, 2H, *J* = 7.21 Hz), 7.40 (d, 2H, *J* = 7.21 Hz), 7.41 (m, 1H), 7.46 (m, 1H), 7.51 (m, 2H), 7.52 (m, 2H), 7.56 (d, 1H, *J* = 7.24 Hz), 7.95 (s, 1H), 7.59 (d, 1H, *J* = 24.52 Hz).

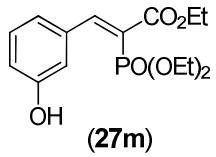
5.1.24.13. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-(pyridin-4-yl)phenyl) acrylate (27l)



(**27l**)

The title compound was obtained as an oil, with 61% yield; Purity: 94.21 % by HPLC; IR (KBr, cm^{-1}) : 2982, 1723, 1605, 1518; ESI (*m*/*z*) 390.1 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.10 (t, 3H, *J* = 7.25 Hz), 1.35 (t, 6H, *J* = 6.92 Hz), 4.15 (q, 4H, *J* = 6.31 Hz), 4.25 (q, 2H, *J* = 7.27 Hz), 7.46 (m, 1H), 7.56 (d, 2H, *J* = 7.25 Hz), 7.40 (d, 1H, *J* = 7.31 Hz), 7.95 (s, 1H), 7.99 (m, 2H), 8.76 (m, 2H), 7.23 (d, 1H, *J* = 24.51 Hz).

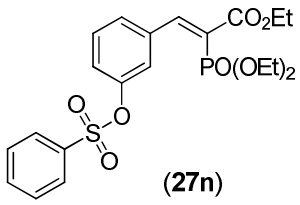
5.1.24.14. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-hydroxyphenyl)acrylate (27m)



(**27m**)

The title compound was obtained as an oil, with 87% yield; Purity: 91.41 % by HPLC; IR (KBr, cm^{-1}) : 3018, 1720, 1593, 1452, 1392; ESI (*m*/*z*) 328.9 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.23 (t, 3H, *J* = 7.15 Hz), 1.34 (t, 6H, *J* = 7.07 Hz), 4.16 (m, 4H), 4.25 (q, 2H, *J* = 7.51 Hz), 6.89 (m, 2H), 7.09 (s, 1H), 7.20 (m, 1H), 7.60 (d, 1H, *J* = 24.54 Hz), 8.75 (s, 1H).

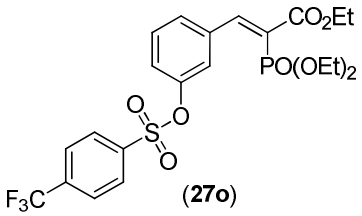
5.1.24.15. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-((phenylsulfonyl)oxy) phenyl)acrylate (27n)



(**27n**)

The title compound was obtained as an oil, with 55% yield; Purity: 99.74 % by HPLC; IR (KBr, cm^{-1}): 3018, 1720, 1620, 1577, 1479, 1450, 1382, 1190; ESI (*m*/*z*) 468.9 ($M+H^+$); ^1H NMR ($CDCl_3$): δ 1.25 (t, 3H, *J* = 7.07 Hz), 1.36 (t, 6H, *J* = 7.10 Hz), 4.15 (m, 4H), 4.25 (q, 2H, *J* = 7.14 Hz), 6.95 (m, 1H), 7.14 (m, 1H), 7.22 (m, 2H), 7.55 (m, 3H), 7.65 (m, 1H), 7.82 (d, 2H, *J* = 7.22 Hz).

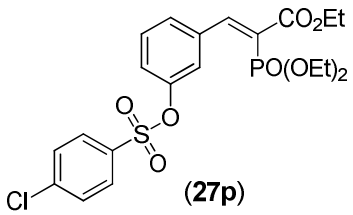
5.1.24.16. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-(((4-(trifluoromethyl) phenyl)sulfonyl)oxy)phenyl) acrylate (27o)



(**27o**)

The title compound was obtained as an oil, with 53% yield; Purity: 99.96 % by HPLC; IR (KBr, cm^{-1}): 3018, 1720, 1620, 1577, 1407 ,1384, 1323; ESI (*m*/*z*) 537.0 ($M+H^+$); ^1H NMR ($CDCl_3$): δ 1.11 (t, 3H, *J* = 7.14 Hz), 1.25 (t, 6H, *J* = 7.08 Hz), 4.15 (m, 4H), 4.24 (q, 2H, *J* = 7.11 Hz), 6.95 (m, 1H), 7.32 (d, 2H, *J* = 8.41 Hz), 7.49 (d, 1H, *J* = 24.42 Hz), 7.80 (d, 2H, *J* = 8.42 Hz), 7.97 (d, 3H, *J* = 8.22 Hz).

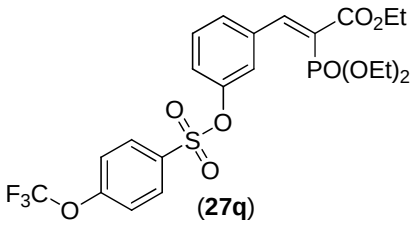
5.1.24.17. (E)-Ethyl 3-(3-(((4-chlorophenyl)sulfonyl)oxy)phenyl)-2-(diethoxyphosphoryl)acrylate (27p)



(**27p**)

The title compound was obtained as an oil, with 45% yield; Purity: 99.94 % by HPLC; IR (KBr, cm^{-1}): 3018, 1720, 1384, 1215; ESI (*m*/*z*) 502.9 ($M+H^+$); ^1H NMR ($CDCl_3$): δ 1.11 (t, 3H, *J* = 7.08 Hz), 1.34 (t, 6H, *J* = 7.08 Hz), 4.14 (m, 4H), 4.24 (q, 2H, *J* = 7.14 Hz), 6.95 (m, 1H), 7.32 (d, 2H, *J* = 8.41 Hz), 7.52 (d, 3H, *J* = 6.92 Hz), 7.77 (d, 2H, *J* = 6.81 Hz).

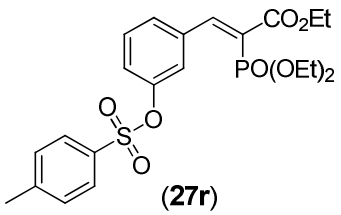
5.1.24.18. (E)-Ethyl2-(diethoxyphosphoryl)-3-(3-(((4(trifluoromethoxy) phenyl) sulfonyl)oxy)phenyl)acrylate (27q)



(**27q**)

The title compound was obtained as an oil, with 56% yield; Purity: 99.96 % by HPLC; IR (KBr, cm^{-1}): 3018, 1720, 1591, 1384, 1261, 1190; ESI (*m*/*z*) 552.9 ($M+H^+$); ^1H NMR ($CDCl_3$): δ 1.11 (t, 3H, *J* = 7.17 Hz), 1.34 (t, 6H, *J* = 7.05 Hz), 4.17(m, 4H), 4.25 (m, 2H), 6.96 (m, 1H), 7.35 (m, 4H), 7.88 (d, 2H, *J* = 24.03 Hz), 8.11 (d, 2H, *J* = 6.92 Hz).

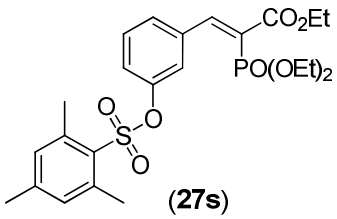
5.1.24.19. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-(tosyloxy) phenyl)acrylate (27r)



(**27r**)

The title compound was obtained as an oil, with 42% yield; Purity: 99.64 % by HPLC; IR (KBr, cm^{-1}): 3128, 3020, 1726, 1631, 1380; ESI (*m*/*z*) 483.1 ($M+H^+$);^{1}H NMR ($CDCl_3$): δ 1.27(3H, t, *J* = 7.15 Hz), 1.36 (6H, t, *J* = 7.03 Hz), 2.45 (3H, s), 4.15 (4H, q, *J* = 7.15 Hz), 4.24 (2H, q, *J* = 7.11 Hz), 6.96 (1H, m), 7.12 (1H, s), 7.28 (5H, m), 7.47 (1H, d, *J* = 8.33 Hz), 7.68 (2H, d, *J* = 8.33 Hz).

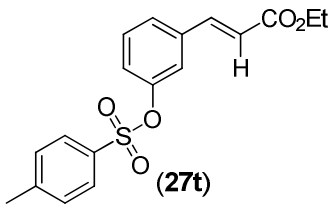
5.1.24.20. (E)-Ethyl 2-(diethoxyphosphoryl)-3-(3-((mesitylsulfonyl)oxy) phenyl) acrylate (27s)



(**27s**)

The title compound was obtained as an oil, with 64% yield; Purity: 93.89 % by HPLC; IR (KBr, cm^{-1}): 2991, 1722, 1602, 1577, 1371; ESI (*m*/*z*) 510.5 ($M+H^+$); ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, *J* = 7.11 Hz), 1.26 (t, 6H, *J* = 7.08 Hz), 2.29 (s, 3H), 2.55 (s, 6H), 4.15 (m, 4H), 4.25 (q, 2H, *J* = 7.11 Hz), 6.97 (s, 1H), 7.10 (s, 1H), 7.26 (s, 2H), 7.46 (d, 2H, *J* = 24.03 Hz).

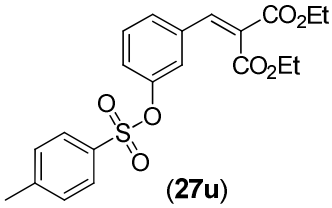
5.1.24.21. (E)-Ethyl 3-(3-(tosyloxy)phenyl)acrylate (27t)



(**27t**)

The title compound was obtained as an oil, with 55% yield; Purity: 96.86 % by HPLC; IR (KBr, cm^{-1}): 3038, 2029, 1708, 1641, 1380; ESI (*m*/*z*) 346.9 ($M+H^+$); ^1H NMR ($CDCl_3$): δ 1.33 (t, 3H, *J* = 6.42 Hz), 2.46 (s, 3H), 4.25 (q, 2H, *J* = 4.85 Hz), 6.28 (d, 1H, *J* = 16 Hz), 7.01 (m, 1H), 7.01 (s, 1H), 7.32 (m, 3H), 7.53 (d, 1H, *J* = 16 Hz), 7.72 (d, 2H, *J* = 8.42 Hz).

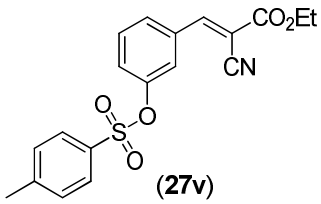
5.1.24.22. Diethyl 2-(3-(tosyloxy)benzylidene)malonate (27u)



(**27u**)

The title compound was obtained as an oil, with 82% yield; Purity: 99.64 % by HPLC; IR (KBr, cm^{-1}): 3020, 1720, 1379; ESI (*m*/*z*) 419.0 ($M+H^+$); ^1H NMR ($CDCl_3$) : δ 1.12 (t, 3H, *J* = 7.10 Hz), 1.36 (t, 6H, *J* = 7.01 Hz), 2.45 (s, 3H), 4.13 (m, 4H), 4.27 (q, 2H, *J* = 7.15 Hz), 6.96 (m, 1H), 7.12 (s, 1H), 7.30 (m, 4H), 7.47 (d, 2H, *J* = 23.98 Hz), 7.68 (d, 2H, *J* =8.23 Hz).

5.1.24.23. 2-Cyano-3-[3-(toluene-4-sulfonyloxy)phenyl]-acrylicacidethyl ester (27v)



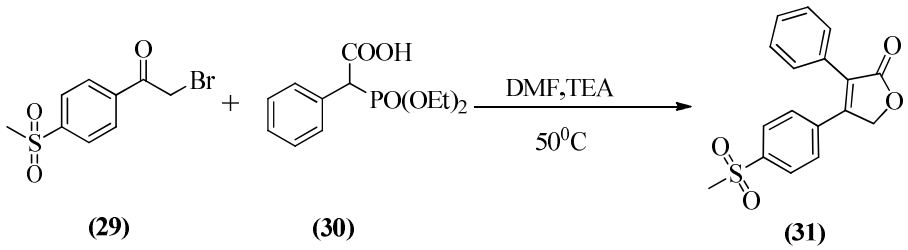
(**27v**)

The title compound was obtained as an oil, with 55% yield; IR (KBr, cm^{-1}): 3020, 1957, 1708, 1641, 1380; ESI (*m*/*z*) 346.9 ($M+H^+$); ^1H NMR ($CDCl_3$): δ 1.35 (t, 3H, *J* = 6.8Hz), 2.46 (s, 3H), 4.27 (q, 4H, *J* = 4.8 Hz), 6.28 (d, 1H, *J* = 16 Hz), 7.05 (m, 1H), 7.21 (s, 1H), 7.35 (m, 4H), 7.55 (d, 1H, *J* = 16 Hz), 7.76 (d, 2H, *J* = 8.4 Hz).

5.1.25. Efficient synthesis of Rofecoxib and related furanones class of compounds (31)

For various *in vitro* and *in vivo* studies, we needed standard compound Rofecoxib. It is an antiinflammatory drug from class of furanone [**3**]. In the past two decades, several methods were disclosed for its synthesis. We attempted few of these methods, but yield and purity was not satisfactory. So we developed mild, efficient and one-pot method for preparation of Rofecoxib and substituted 2(5*H*)-furanones, starting with commercially available (dialkoxy-phosphonyl)-phenyl-acetic acid and 2-Bromo-1-(4-methanesulfonyl-phenyl)-ethanone, which involved formation of phosphonate ester (*in situ*) and subsequent intramolecular Horner-Emmons-type cyclization in less than 2 h, lead to the formation of Rofecoxib with good yield and purity (>90%). The synthesis was carried out up to 10 g scale using following **Scheme 6** brief experimental procedure and

charactrization data is given below.



(29) (30) (31)

Reagents and conditions: a) TEA, DMF, 50^0C.

Scheme 6: Synthesis of Rofecoxib

2-(Diethoxyphosphino)-2-phenylacetic acid (**30**, 5g, 18.3 mmol) was added to a mixture of 2-bromo-1-(4-(methylsulfonyl) phenyl) ethanone (**29**, 5.09g, 18.3 mmol) in DMF (50 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0°C and TEA (7.94 mL, 55.0 mmol) was added. Further, mixture was stirred at 50°C for 2 h and quenched in ice-cold water (200 mL). The precipitated compound was filtered, washed by cold acetone and dried under reduced pressure to afford the title compound **31** as yellow solid.

Yield = 92 %; mp 203-204 °C, Lit mp 206-207°C, Purity: 95.88 % by HPLC; IR (KBr, cm^{-1}): 3481, 3018,1745, 1747, 1446, 1340; ESI (*m*/*z*) 315.06 (M+H); ^{1}H NMR (DMSO-d_6): δ = 3.24 (s, 3H), 5.40 (s, 2H), 7.32 (m, 2H), 7.41 (m, 3H), 7.59 (d, 2H, *J* = 8.46 Hz), 7.92 (d, 3H, *J* = 8.47 Hz); ^{13}C (DMSO-d_6): δ = 38.89, 40.14, 43.14, 70.80, 80.20, 126.90, 127.59, 128.92, 129.77,135.69, 141.95, 155.94, 172.56 ppm.

This work was publication in journal of *Synth. Commun*. **2012**, *42*, 3140-3149 as One-pot Synthesis of 3, 4-di aryl Substituted 2(5*H*)-Furanones and its Commercial Application.

5.2. Docking study

The test compounds were geometrically optimized using Ligprep module of Schrodinger. The crystal structure 2FV5 [**4**], using Glide (version 50207) [**5**] of Schrodinger was obtained from RCSB protein data bank [**6**] and the protein was prepared using protein preparation wizard of Schrodinger. The receptor grid files were generated using gridreceptor generation program. The grid box was generated at the centroid of the IK-682 ligand of the receptor. The ligands were docked using "xtra precision" Glide algorithm [**7**].

5.3. Biology

5.3.1. Inhibition of TNF-α in human whole blood assay (*Ex vivo*)

Fresh human blood (500 ml) drawn aseptically in the presence of heparin from healthy adult volunteers was incubated with the test compounds at various concentrations, for 1 h, at 37 ^{0}C. At the end of the incubation period, lipopolysaccharide (LPS, Sigma; 100 ng/ml) was added to the blood and the samples were further incubated for 5h, at 37 ^{0}C, with constant rotation. The reactions were terminated by placing the samples over ice for 10 min. Finally, the plasma was separated by centrifugation at 3000 rpm, for 10 min, at 4 ^{0}C and stored at -70^{0}C until further analysis. Concentrations of TNF-α in the plasma were determined by an ELISA kit according to the manufacturer's instruction (BD Biosciences, USA) and concentration required for 50% TNF-α inhibition (IC_{50} values) were calculated and reported [**8**].

5.3.2. TACE and MMP enzymatic assays (*in vitro*)

TACE FRET assay

Test compounds were assessed for their ability to inhibit the cleavage of the substrate by the purified enzyme in a fluorescence-based FRET assay as per modified literature procedure [**9**,**10**]. Briefly, the human catalytic domain of TACE (1 µg/ml) was pretreated with the test compounds at various concentration for 10 min at room temperature. The reaction was initiated by the addition of pro-TNF-α peptide (50 µM final concentration) to the TACE protein and the increase in fluorescence was monitored at excitation of 320 nm and emission of 420 nm, over a period of 10 min and the IC_{50} values (nM) were determined (n=3) and reported.

MMP FRET assays

The MMP-1,-2,-3,-7,-8,-9,-13 and -14 assays were carried as per modified literature procedure [**11**]. Briefly, the assays were carried out at room temperature in a buffer containing Hepes (50 mM; pH 7.4), NaCl (100 mM), $CaCl_2$ (5 mM) and Brij-35 (0.005%). The substrate used was Mca-PQGL-(3-[2, 4-dinitrophenyl]-l-2, 3-diaminopropionyl)-AR-OH (synthesized in-house) at a final concentration of 10 µM. The enzymatic reactions were initiated by adding the substrate to a final concentration of 20 µM. The initial rate of increase in fluorescence by the cleavage reaction was determined immediately after substrate addition and the IC_{50} values (nM) were determined (n=3) and reported.

ADAM-10 assay

ADAM-10 activity was assayed by a FRET assay in a buffer containing Tris-HCl (50 mM, pH 9 at 37 °C), $ZnCl_2$ (2.5 mM) Brij-35 (0.005%) [**12**]. The final concentration

of ADAM-10 enzyme (R&D Systems) was 1 ng/ml. The fluorescent substrate used was Mca-PQGL-(3-[2,4-dinitrophenyl]-l-2,3-diaminopropionyl)-AR-OH (synthesized in-house) at a final concentration of 10 µM. Test compounds were dissolved in DMSO and assayed at various concentrations following a 15-min pretreatment of the enzyme with inhibitors at room temperature. The reaction was monitored by a flourimeter (GeminiXS from Molecular Devices) for 15 min at 37 °C at excitation of 320 nm and emission of 420 nm. IC_{50} values (nM) were determined (n=3) by plotting % inhibition against inhibitor concentration and equation was fit to a sigmoidal curve with a Hill slope (B to 100) using the LSW software package on Excel (Microsoft) and the IC_{50} values (nM) are reported.

5.3.3. LPS induced acute TNF-α production in mouse (*in vivo*)

In vivo TNF-α inhibition was assessed using LPS induced acute TNF-α production in mouse [**12**]. Inhibition of the TNF-α production was taken as an indicator for inhibition of TACE activity. This study was conducted on Swiss Albino Mice (SAM) of either sex (age 8-12 weeks), weighing between 20 to 25g. The animals were divided into two groups (control and standard) and each experimental group consisted of six animals. All the animals were left for 2 days under laboratory conditions for acclimatisation and maintained on a standard pellet diet and water ad libitum before the day of the experiment. On the last day food was withdrawn and they were given water only. A 12 hours dark: light cycle was also maintained. All the animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'.

Vehicle (normal saline)/test/standard compounds were administered orally on a body weight basis, 30-min prior to the LPS (50 mg/kg/iv) injection. Blood samples were collected from each animal 60 min after the LPS injection, via retro-orbital plexus. Blood samples were centrifuged (3000 rpm, 15 min at 40 °C) and the separated serum was immediately subjected for TNF estimation. Concentrations of TNF-α in the serum were determined by an ELISA kit according to the manufacturer's instruction (BD Biosciences, USA). Mean values of duplicate samples were calculated using Microsoft excel and % TNF- α inhibition values (ED_{50}) values of test compounds are reported.

5.3.4. Pharmacokinetic study in wistar rats

The pharmacokinetic parameters of test compounds were determined in male wistar rats (n=6) [**13**]. Briefly, test compounds were administered orally / iv / sc on a body weight basis to overnight fasted rats. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 30 h post-dose after compounds administration. Approximately 0.3 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at -70 °C and the concentrations of compounds in plasma were determined by the LC-MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C_{18} (2.0 x 50 mm, 3 µm) column (YMC Inc., USA). The pharmacokinetic parameters, such as Tmax, $t_{1/2}$, Kel, AUC and %F were calculated using a non-compartmental model of WinNonlin software version 5.2.1.

5.3.5. Repeated dose toxicity study (28 days) of test compounds in wistar Rats (po)

Repeated dose toxicity studies (28 days) of test compounds were carried out in male wistar rats (WR). Briefly, animals were divided into three groups (n=10), a control group and separate groups for test compounds. To each of the test groups, daily oral dose of 100 mpk test compounds was administered, twice a day (bid), under fasted conditions for 28 days. After completion of treatment period (28 days), animals were sacrificed and subjected for complete necropsy examination and also changes in toxicological parameters, such as gross pathology, clinical signs, body weight, organ weights and serum chemistry/ hematological changes were recorded.

Measurement of markers

Rats were anesthetized 24h post treatment and blood samples were collected. The whole blood was centrifuged at 3000 rpm using a centrifuge at 37 ^{o}C for 15 min and serum ALT, AST and ALP were assayed using diagnostic kit (Boehringer Mannheim) [**14**].

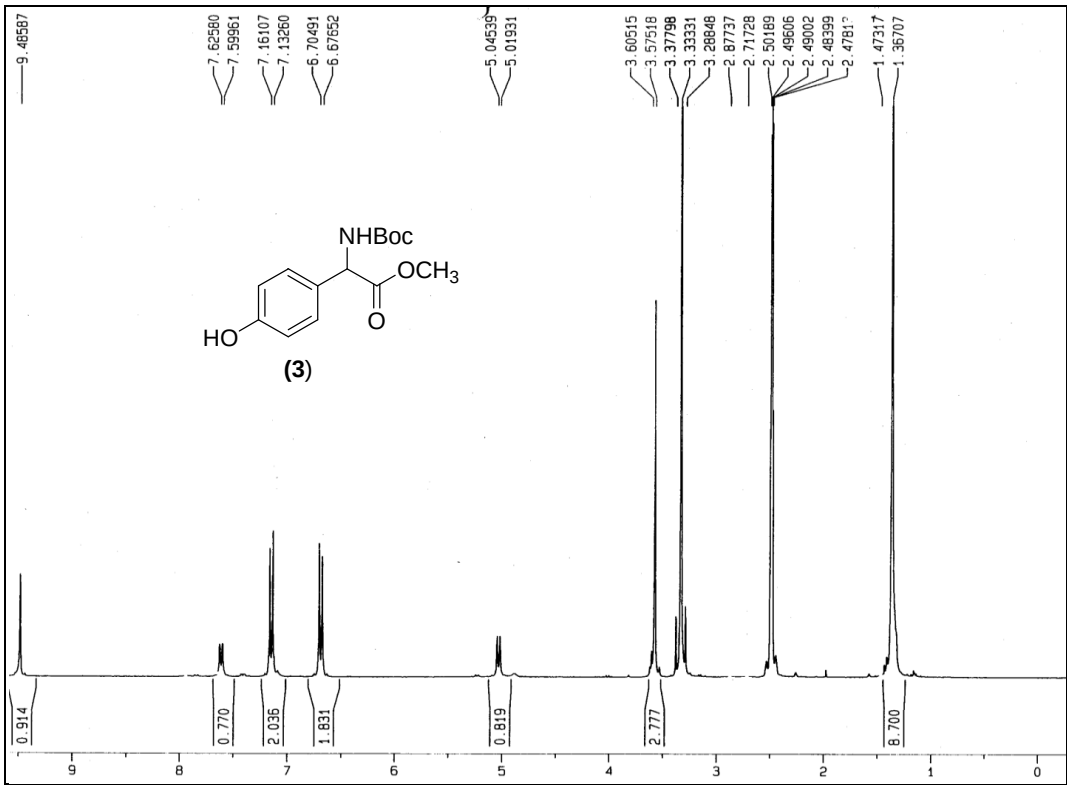
5.4. Refrannces

1. Lohray, B. B.; Lohray, V. B.; Jain, M. R.; Thombare, P. S. WO 2005077937.
2. El Ashray, E. S. H.; Ramadana, E. S.; Hamida, H. A.; Hagara, M. *Synth.Commun.* **2005**, *35*, 2243-2250.
3. Sindhuramachandran, C.V.; Sreekumar, P.K.; *Int. J. Pharm. Pharm. Sci.* **2011**, *3,* 225-228.
4. Xiaoda, N.; Shelby, U.; Richard, I.; Brian, M. B.; Yan, H. L.; Jing, S.; Daniel L.; Peter, O. *Arch. Biochem. Biophys*. **2006**, *451*, 43–50.
5. Glide 5.0; Schrodinger, LLC: New York, NY, **2008**.
6. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic. Acids. Res*. **2000**, *28*, 235–242.
7. Xin, H.; Stefan, B.; William, H. S. *J. Mol. Graph*. **2004,** *22*, 293-307.
8. Newton, R. C.; Solomon, K. A.; Covington, M. B.; Decicco, C. P.; Haley, P. J.; Friedman, S. M.; Vaddi, K. *Ann. Rheum. Dis*., **2001**, *60*, 25-32.
9. Jin, G.; Huang, X.; Black, R.; Wolfson, M.; Rauch, C.; McGregor, H.; Ellestad, G.; Cowling, R. *Anal. Biochem*. **2002**, *302*, 269-275.
10. Park, K.; Gopalsamy, A.; Aplasca, A.; Ellingboe, J. W.; Xu, W.; Zhang, Y.; Levin, J. I. *Bioorg Med. Chem*. **2009**, *17*, 3857-3865.
11. Zhang, Y.; Xu, J.; Levin, J.; Hegen, M.; Li, G.; Robertshaw, H. *J. Pharmacol. Exp. Ther*. **2004**, *309*, 348-355.
12. Levin, J. I.; Chen, J. M.; Cheung, K.; Cole, D.; Crago, C.; Santos, E. D.; Du, X.; Khafizova, G.; MacEwan, G.; Niu, C.; Salaski, E. J.; Zask, A.; Cummons, T.; Sung, A.; Xu, J. Zhang, Y.; Xu, W.; Ayral-Kaloustian, S.; Jin, G.; Cowling, R.; Barone, D.; Mohler, K. M.; Black, R. A.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2799-2803.
13. Mingxin, Q.; Stephen, A. B.; Bernice, B.; Jing-Tao, W.; Rui-Qin, L. *Drug. Metab. Dispos*. **2007**, *35*, 1916-1925.
14. Yamazaki, K.; Aiso, S.; Matsumoto, M.; Arito, H.; Nagano, K.; Yamamoto, S.; Matsushima, T. *Ind. Health*. **2005**, *43*, 597-610.

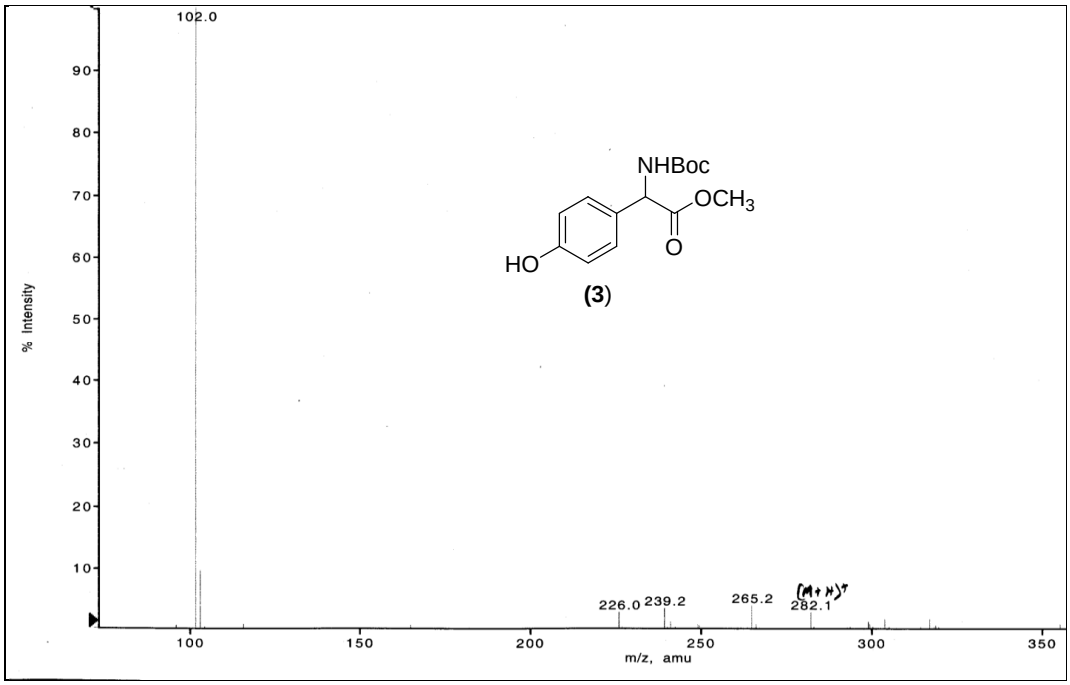
Appendix- II: Spectra

6. Appendix-II: Spectra

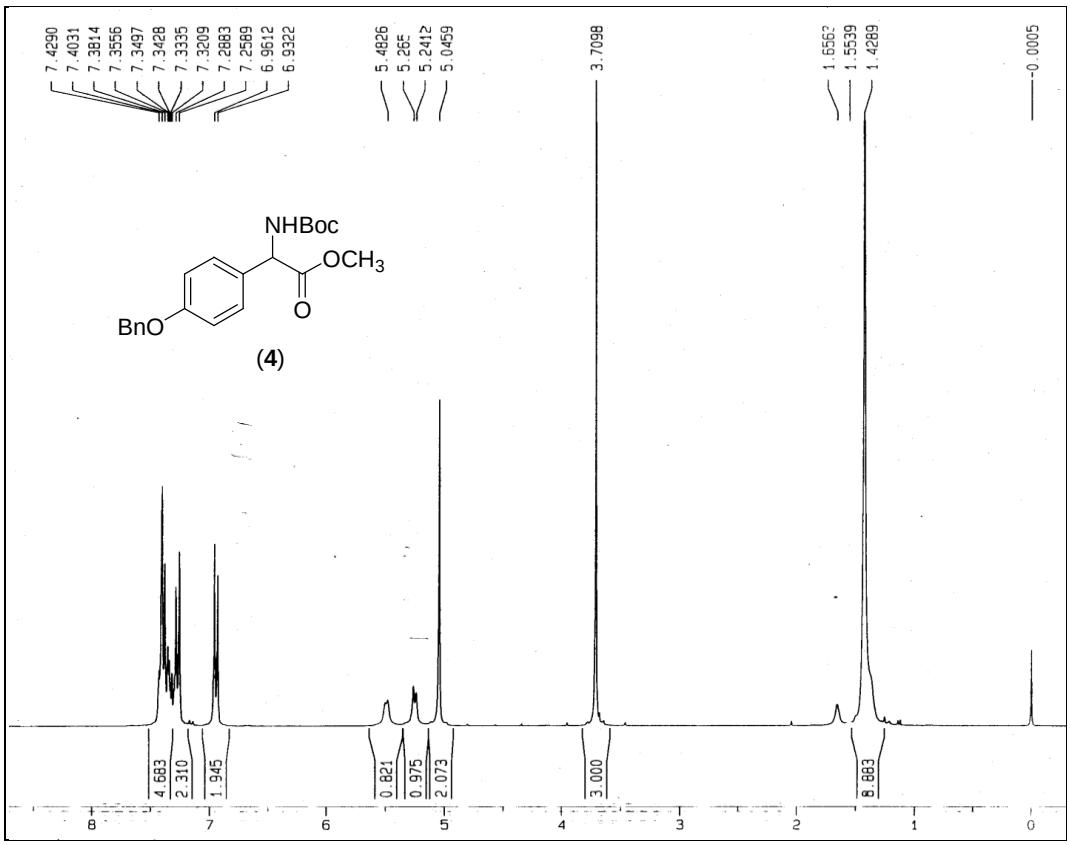
^{1}H NMR of compound **3**



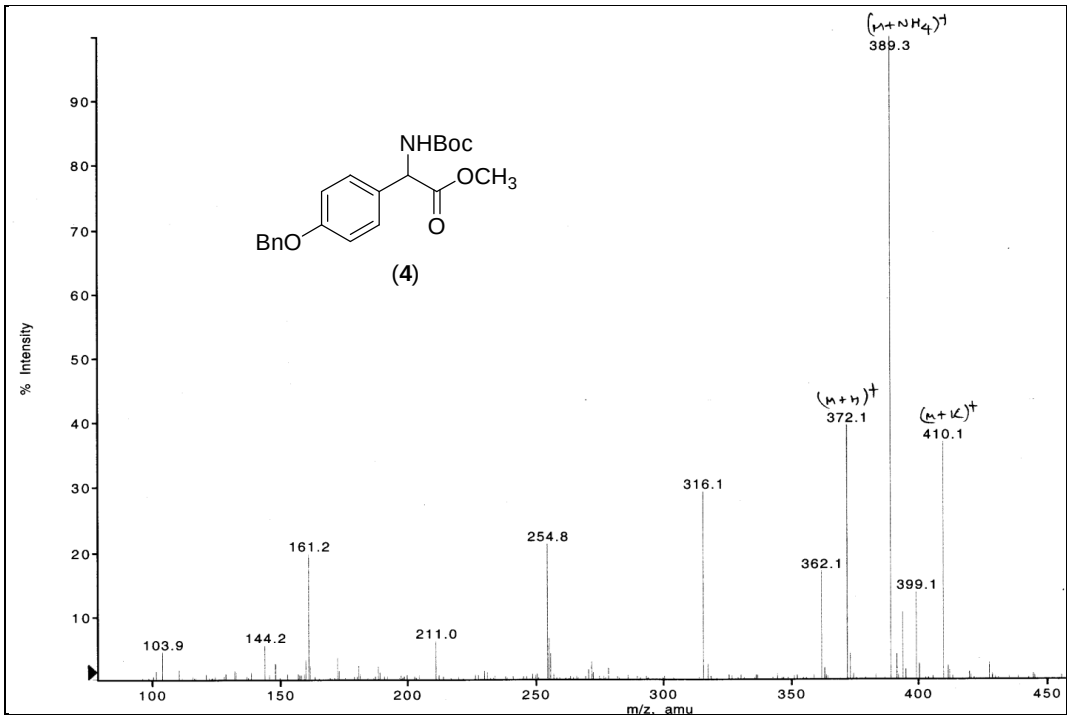
ESI-MS of compound **3**



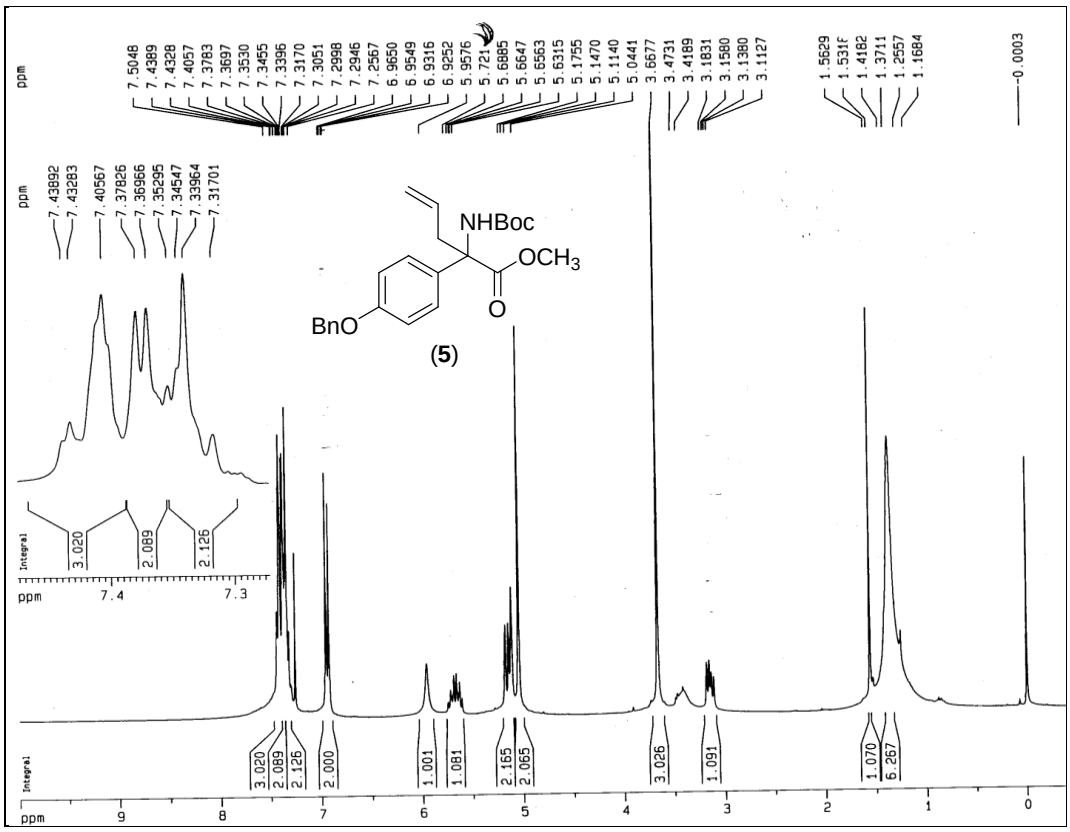
^{1}H NMR of compound **4**



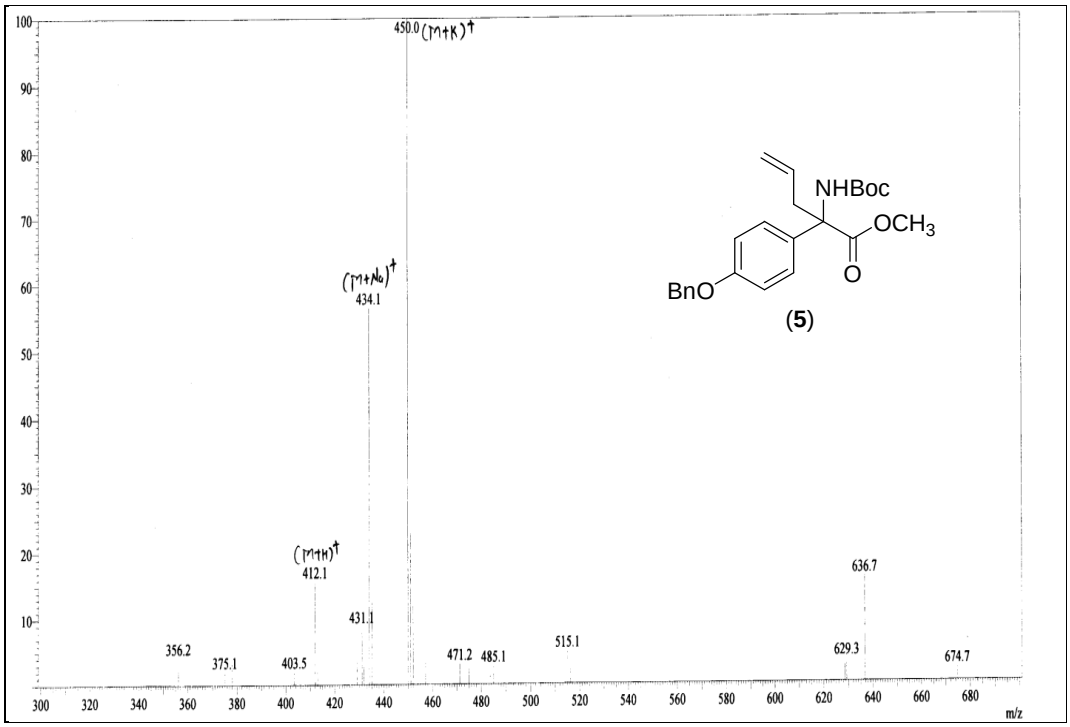
ESI-MS of compound **4**



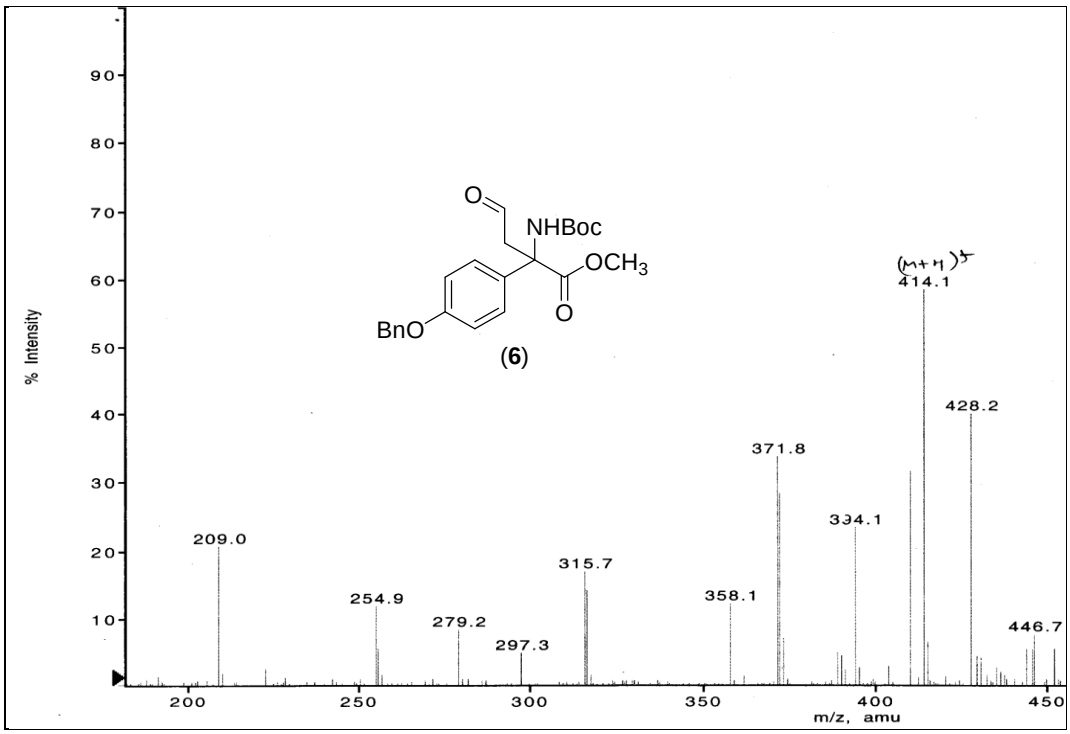
^{1}H NMR of compound **5**



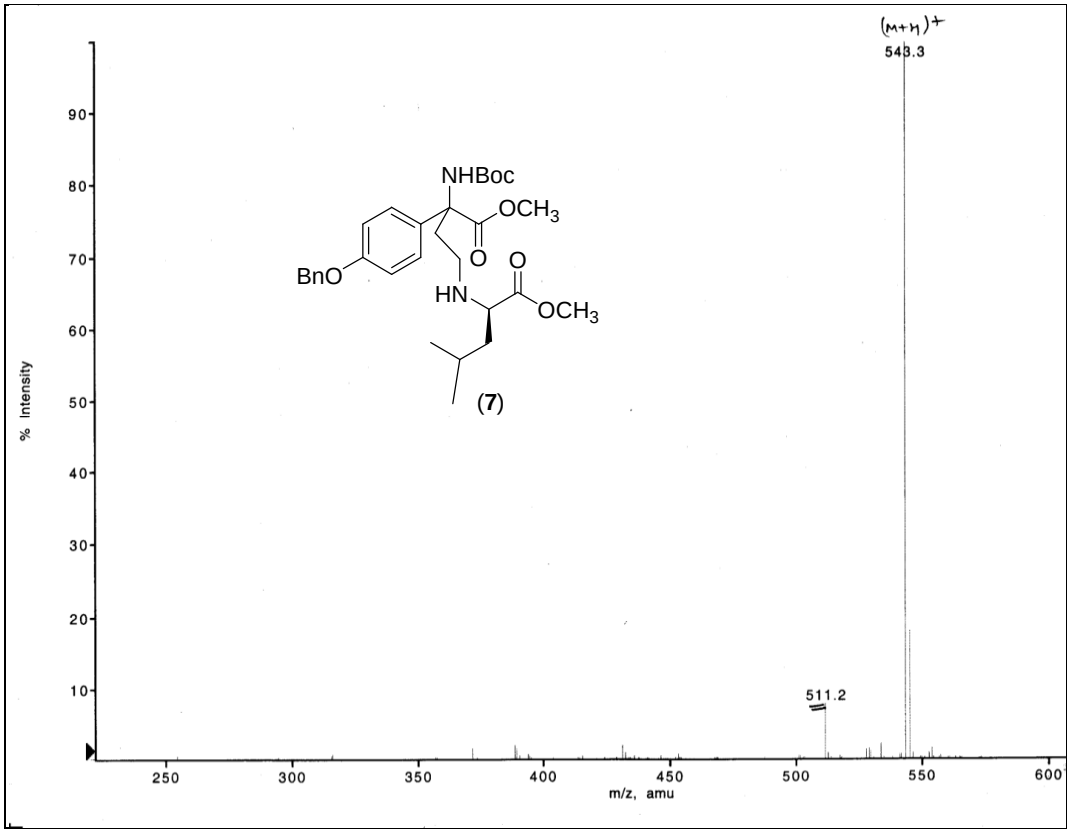
ESI-MS of compound **5**



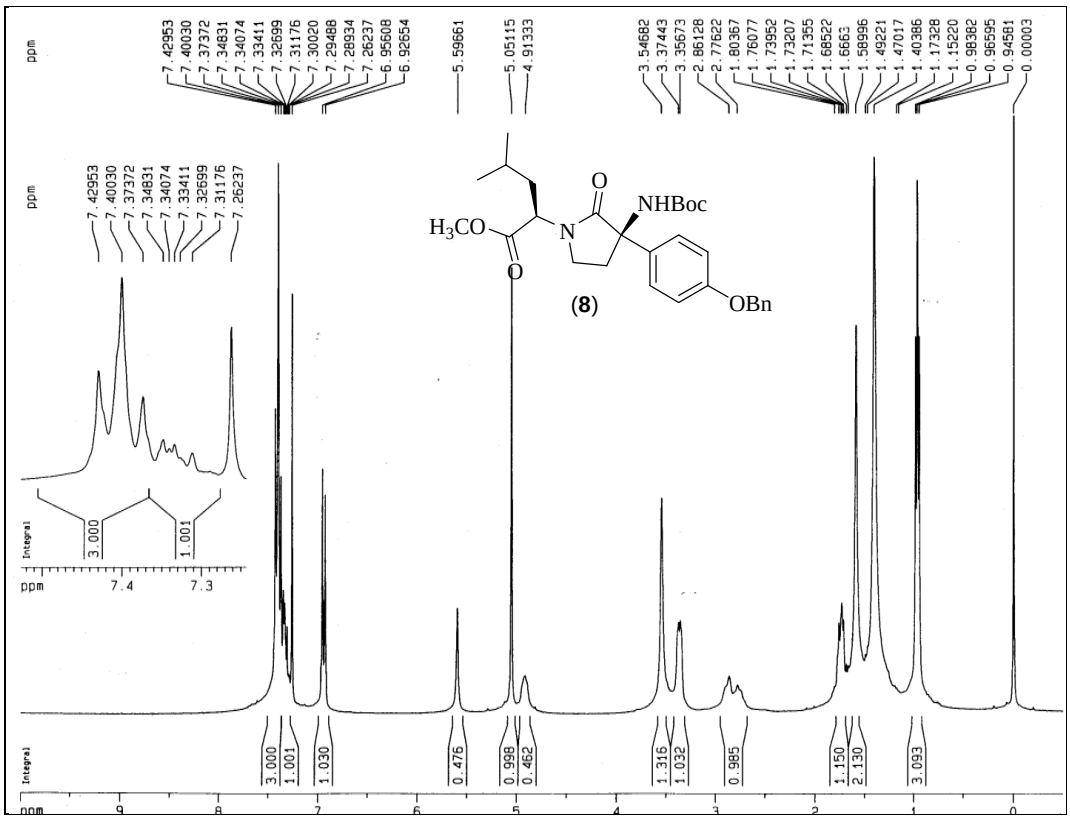
ESI-MS of compound **6**



ESI-MS of compound **7**



^{1}H NMR of compound **8**



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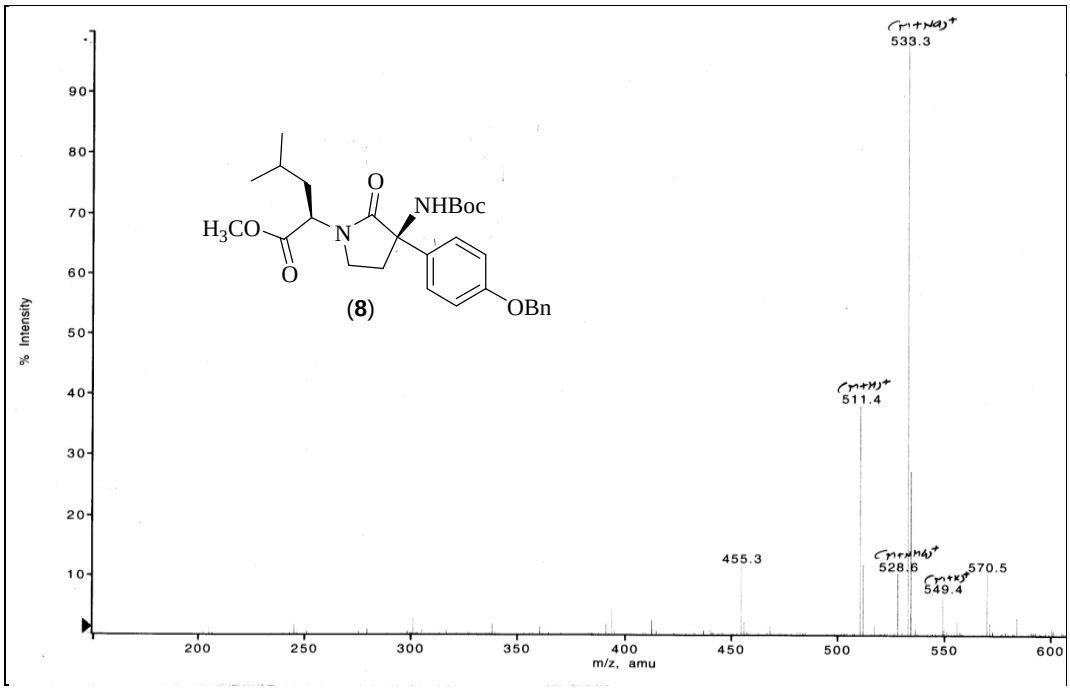
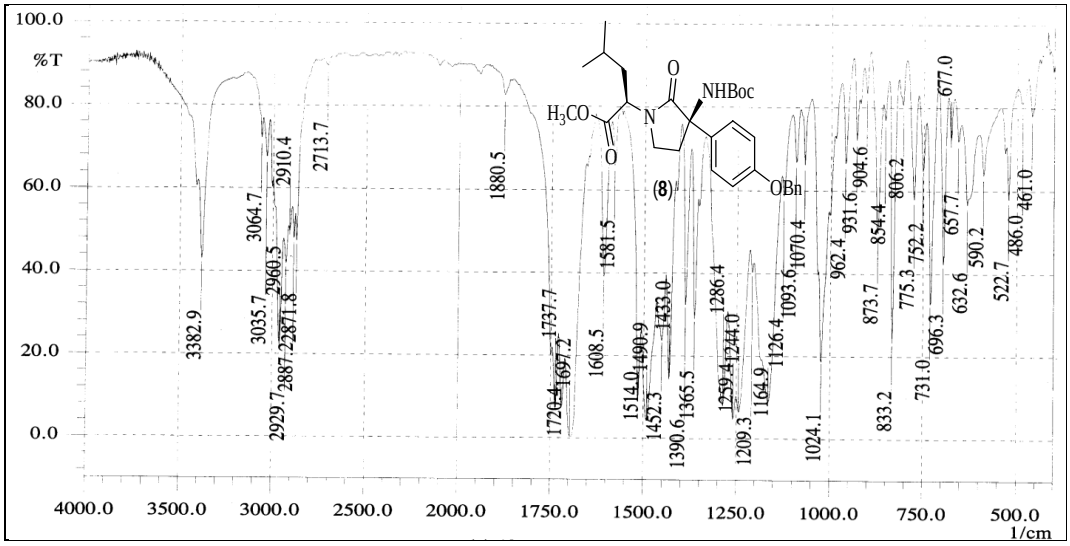
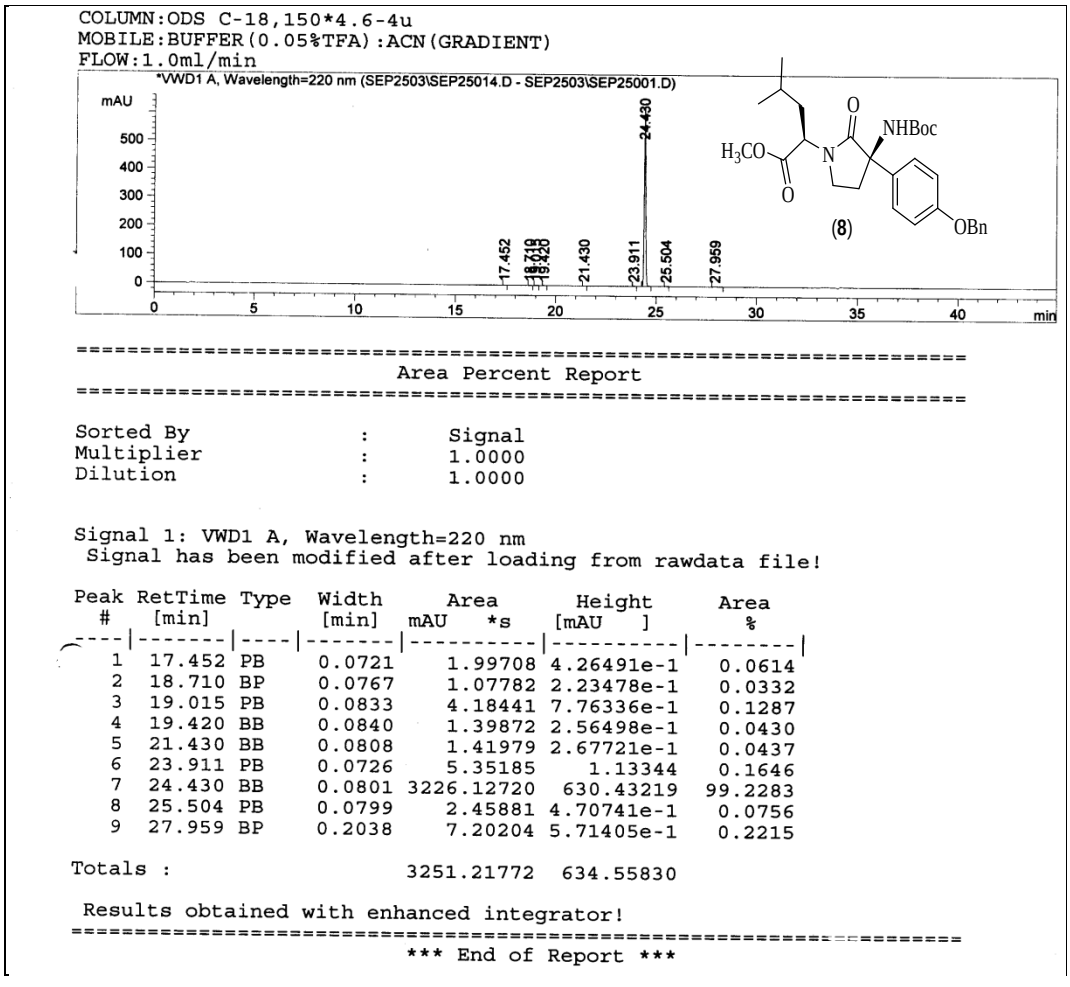


IR of compound **8**



HPLC of compound **8**

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                          Area Percent Report
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Multiplier            :      1.0000
Dilution              :      1.0000


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 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
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   2  18.710 BP    0.0767    1.07782 2.23478e-1   0.0332
   3  19.015 PB    0.0833    4.18441 7.76336e-1   0.1287
   4  19.420 BB    0.0840    1.39872 2.56498e-1   0.0430
   5  21.430 BB    0.0808    1.41979 2.67721e-1   0.0437
   6  23.911 PB    0.0726    5.35185    1.13344   0.1646
   7  24.430 BB    0.0801 3226.12720  630.43219  99.2283
   8  25.504 PB    0.0799    2.45881 4.70741e-1   0.0756
   9  27.959 BP    0.2038    7.20204 5.71405e-1   0.2215

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 Results obtained with enhanced integrator!
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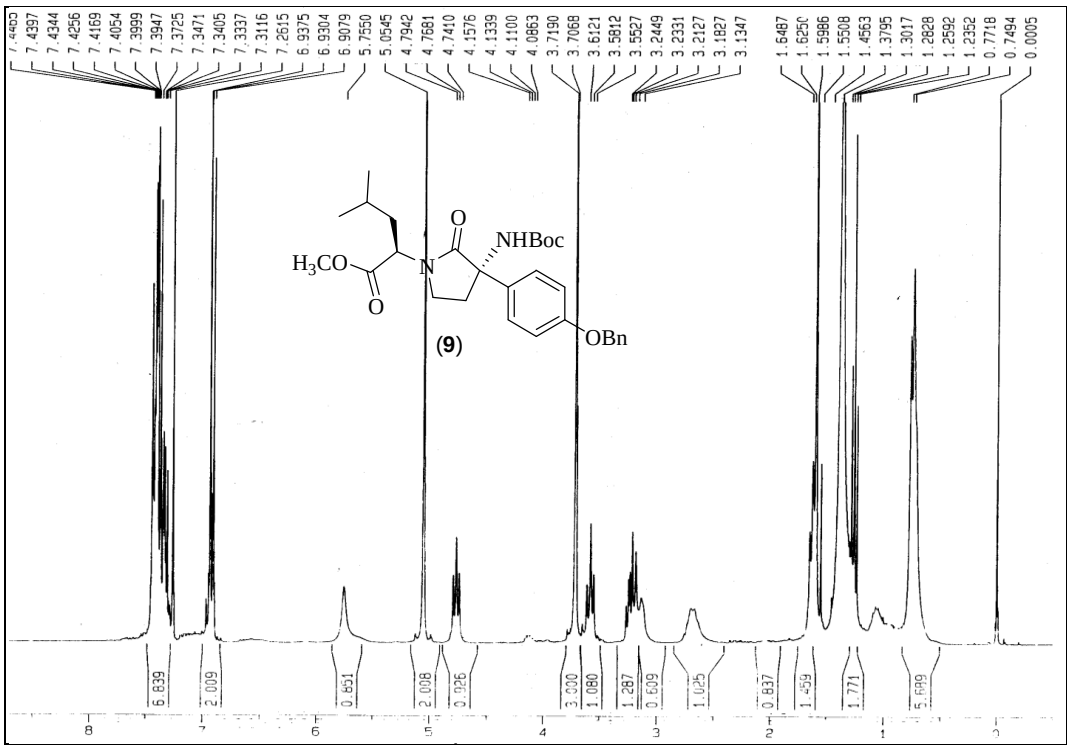
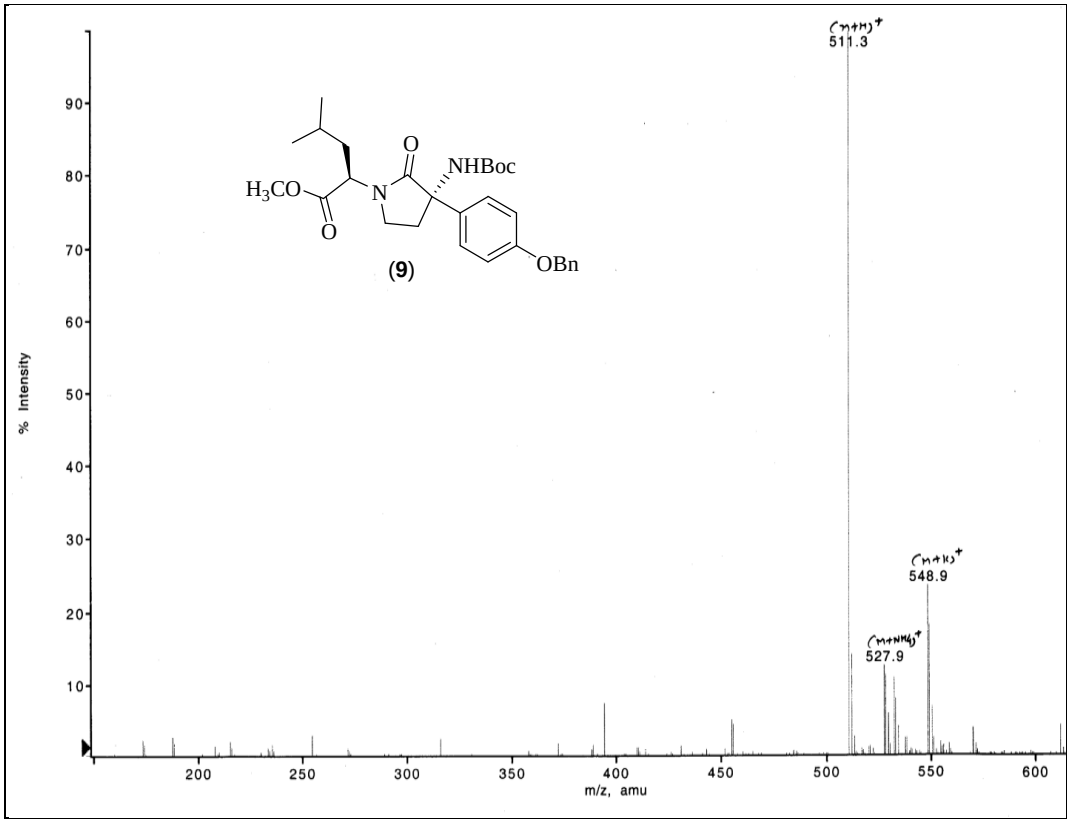
^{1}H NMR of compound **9**



ESI-MS of compound **9**



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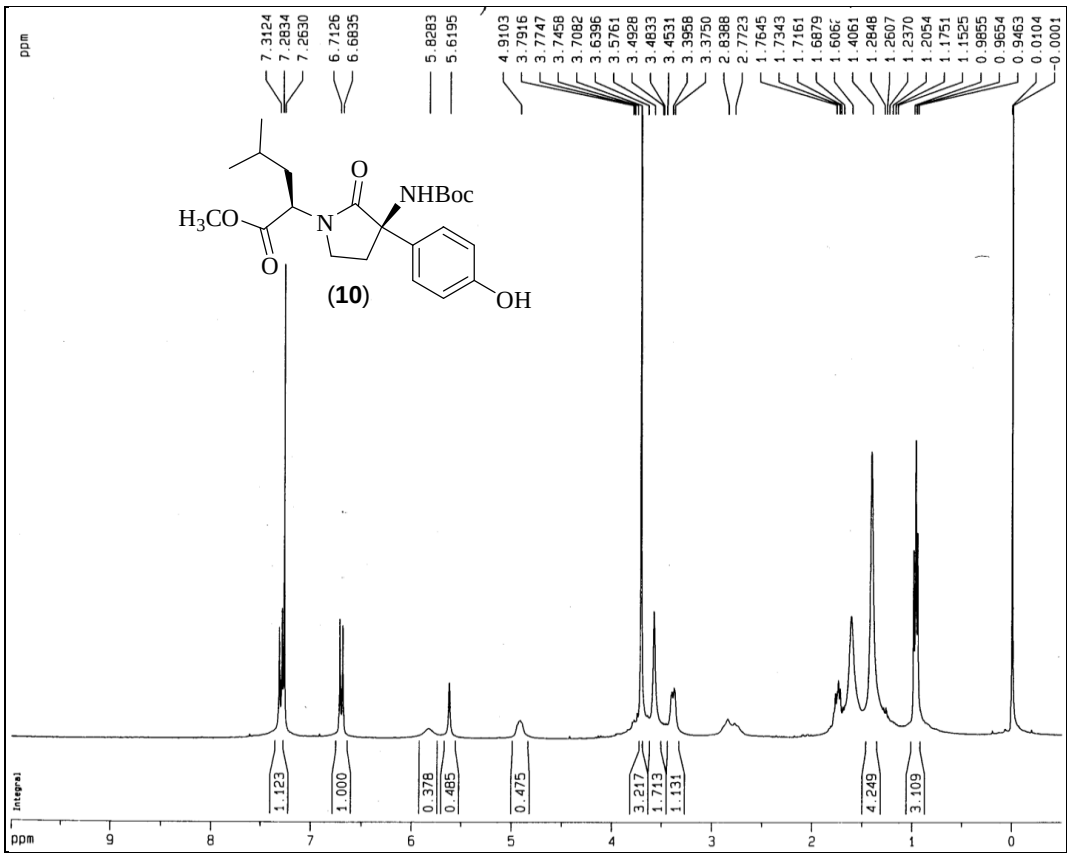
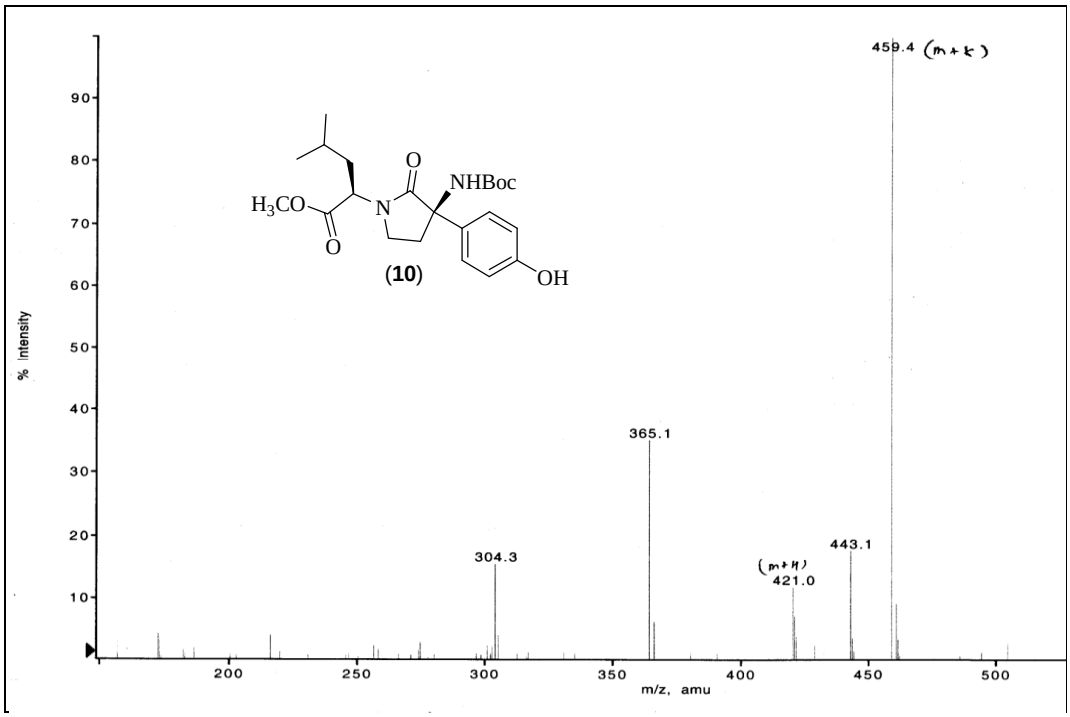


ESI-MS of compound **10**



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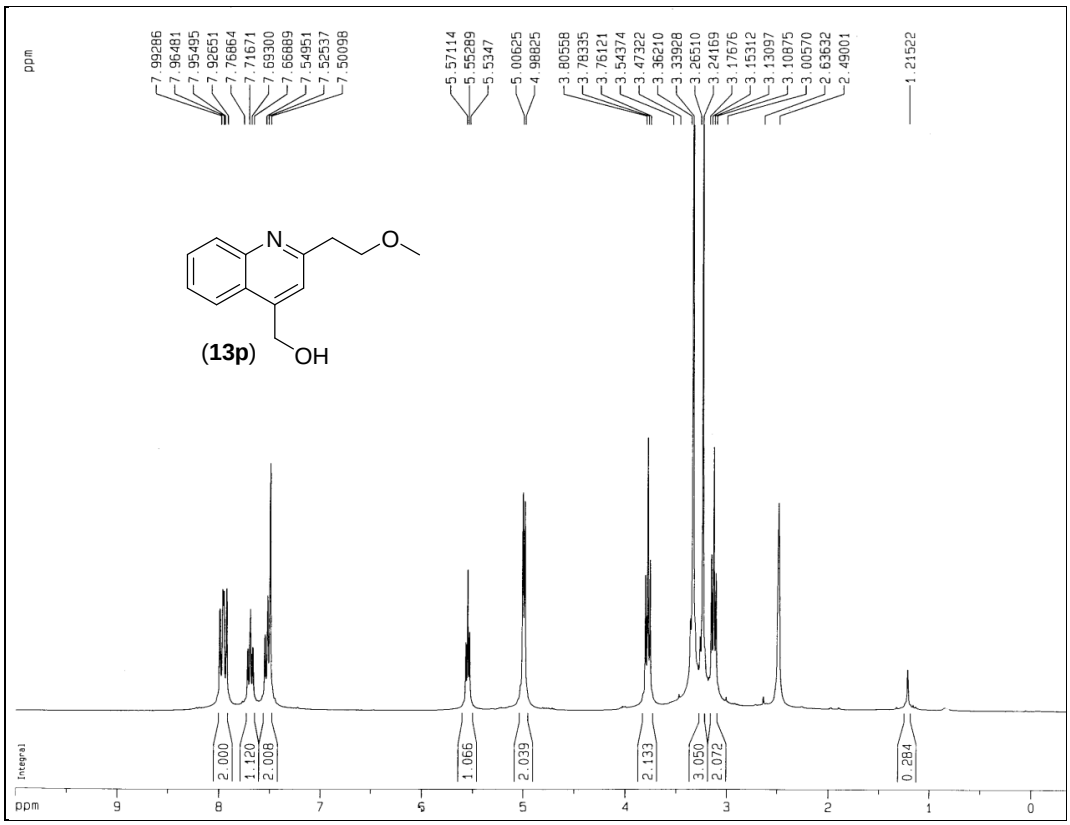

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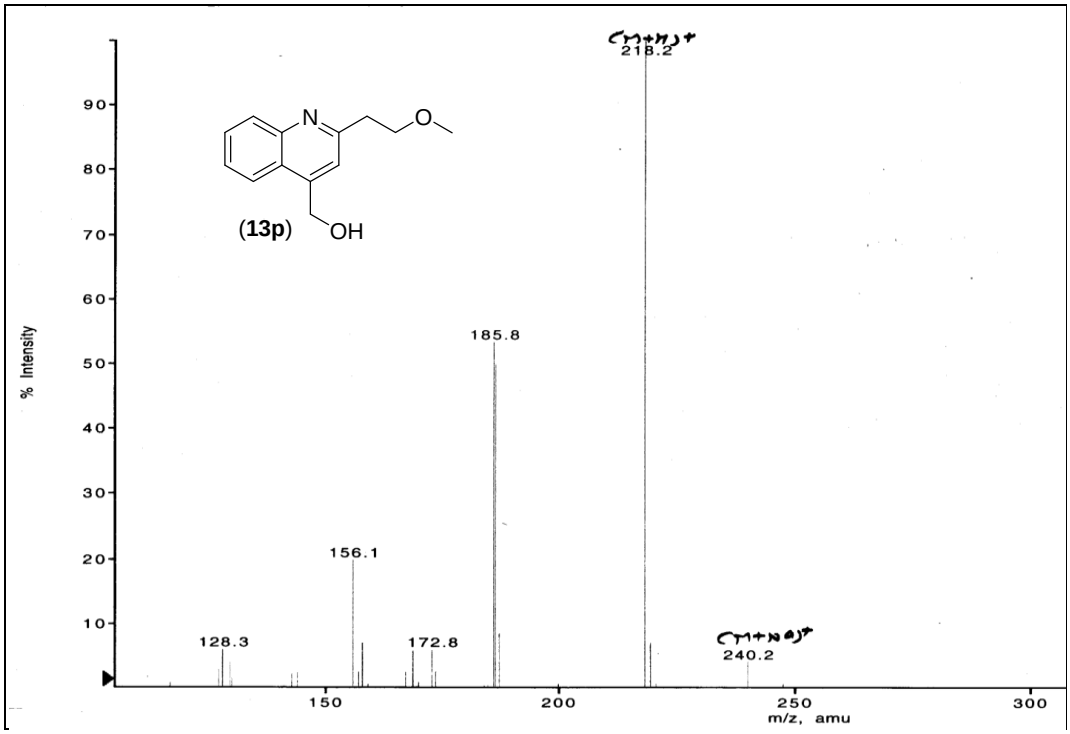
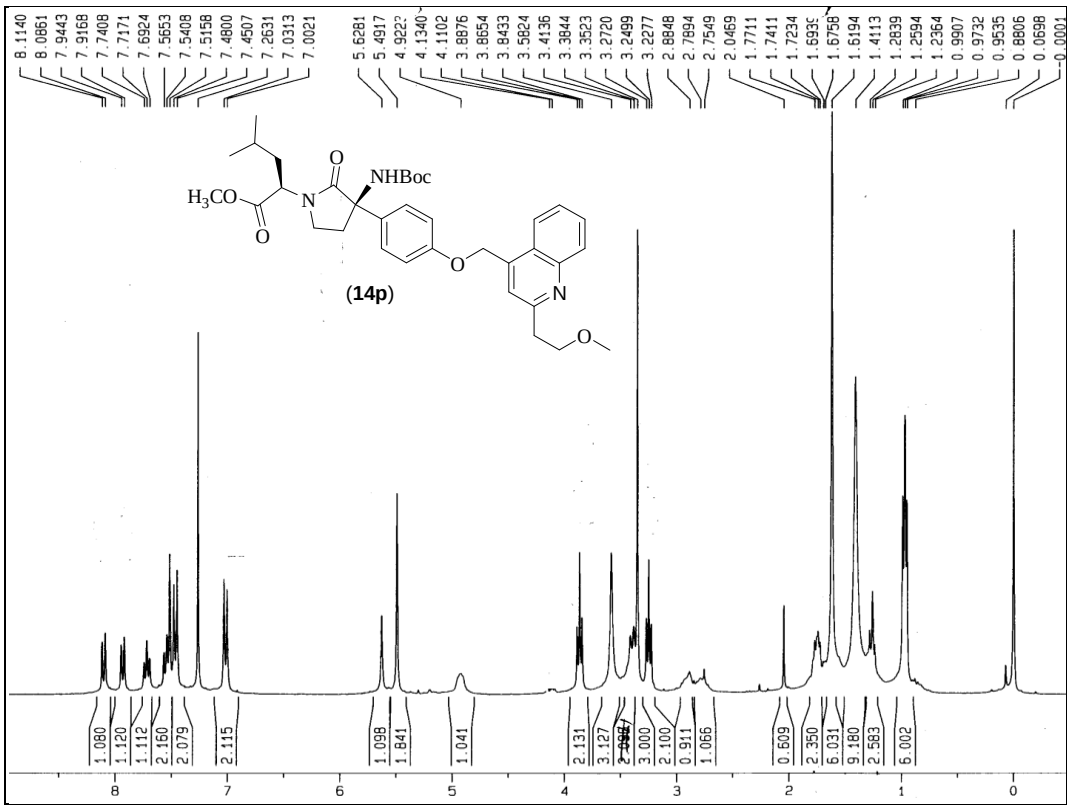
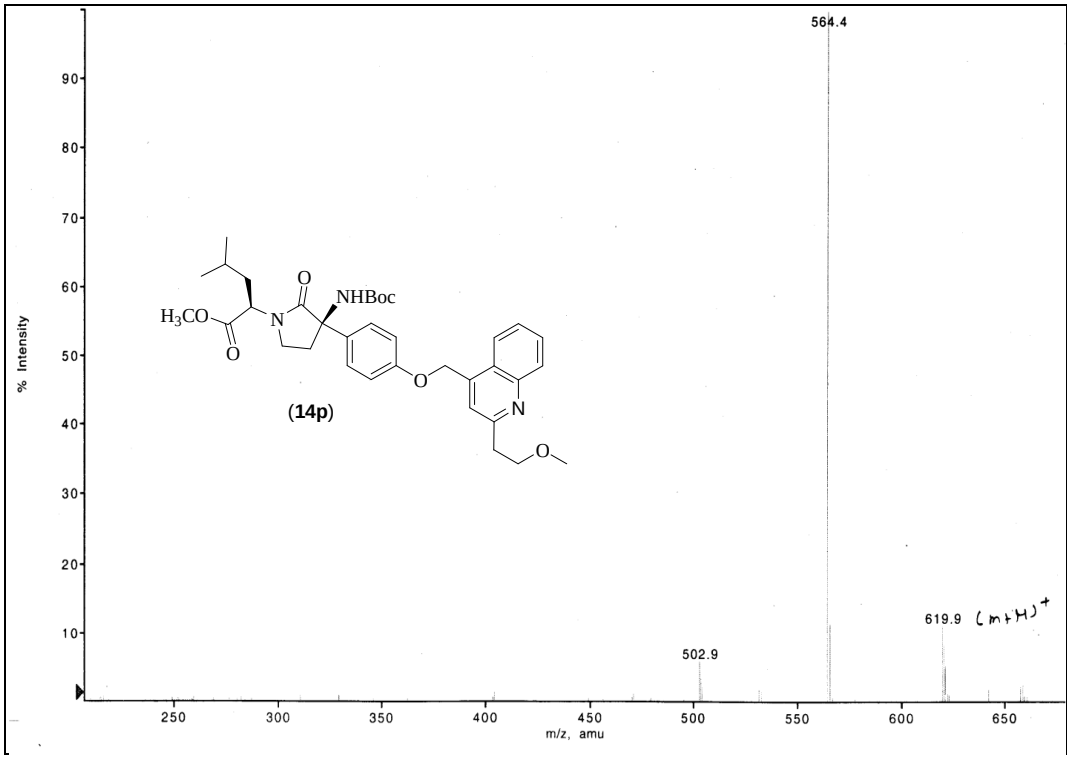


^{1}H NMR of compound **14p**



ESI-MS of compound **14p**



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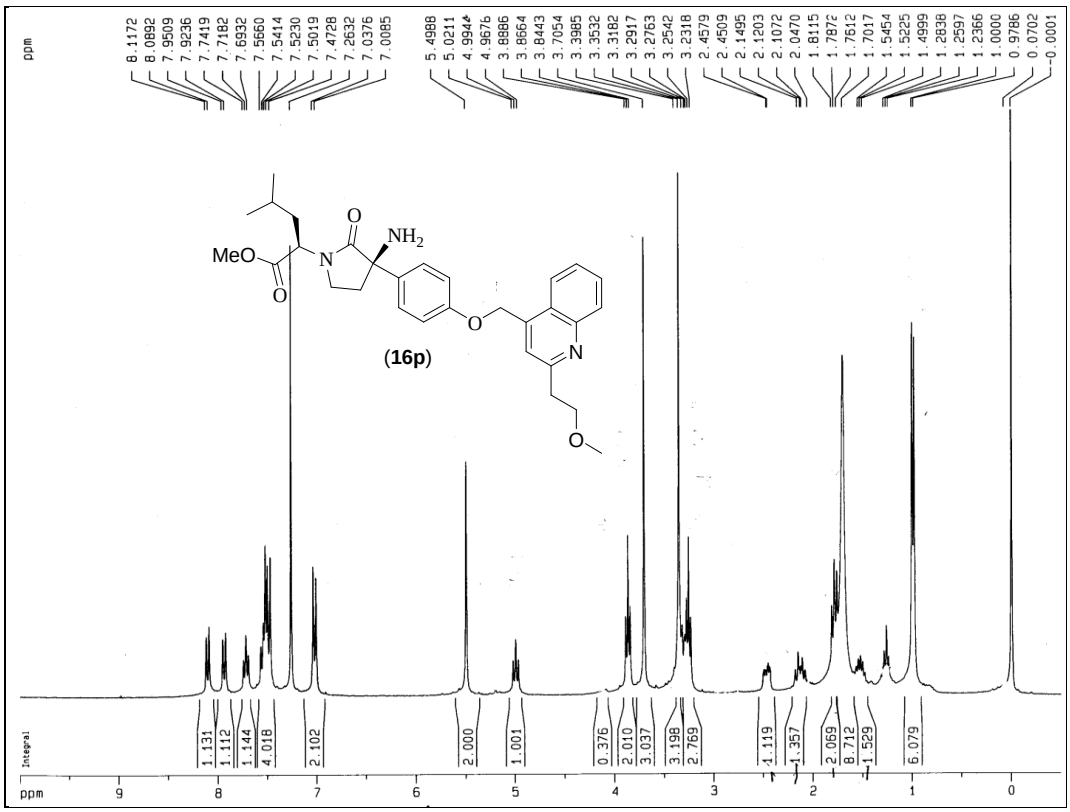
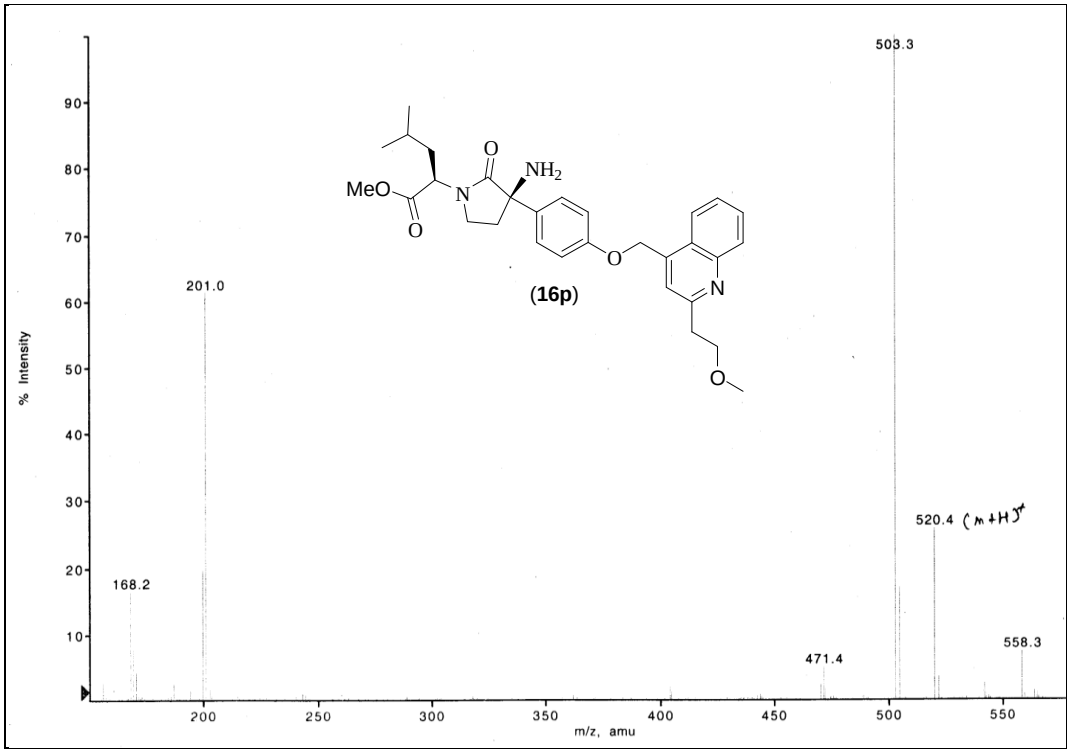
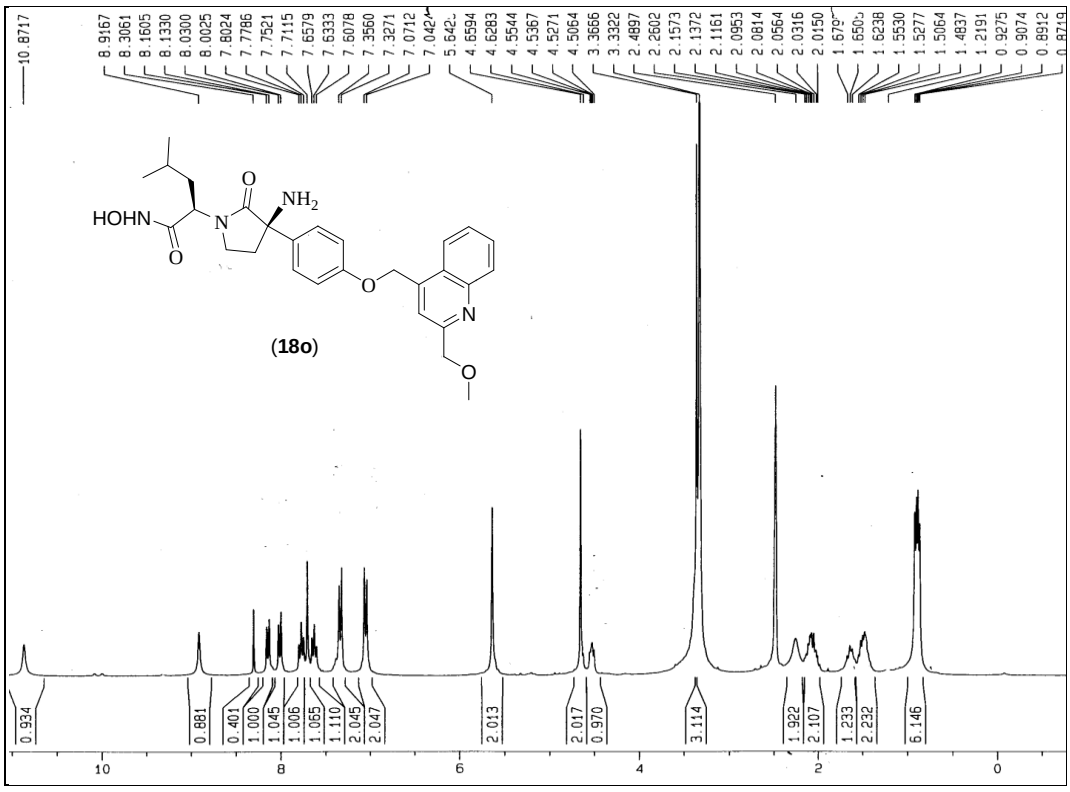


ESI-MS of compound **16p**



^{1}H NMR of compound **18o**



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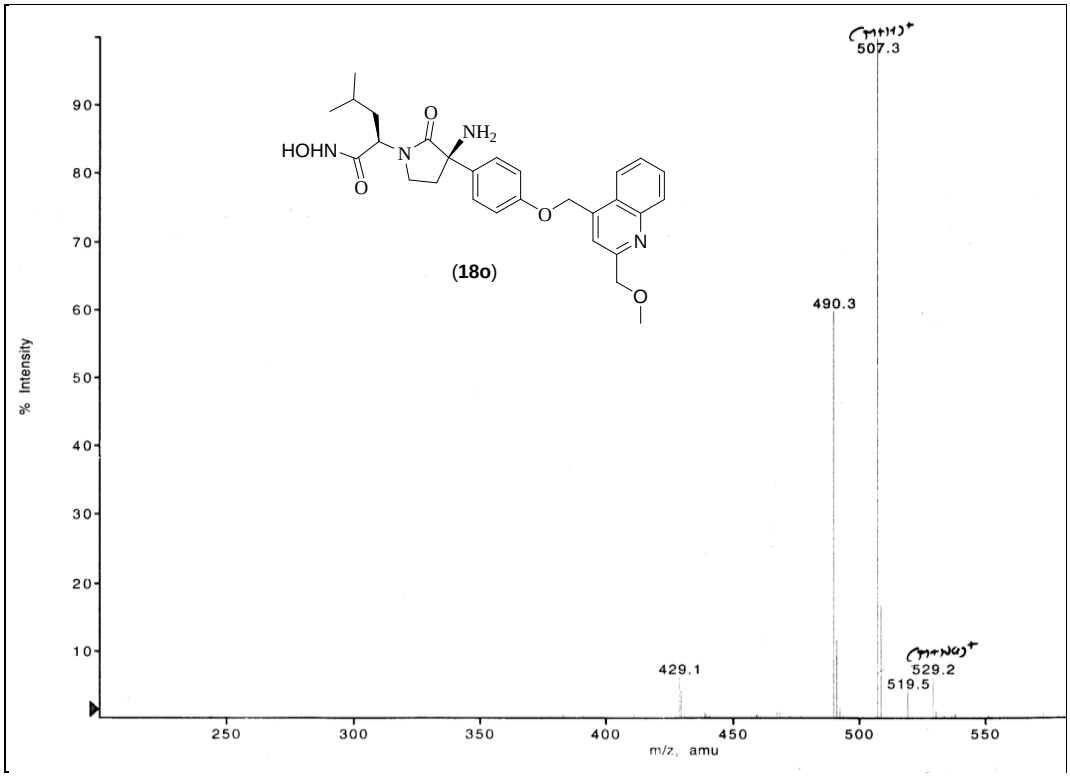
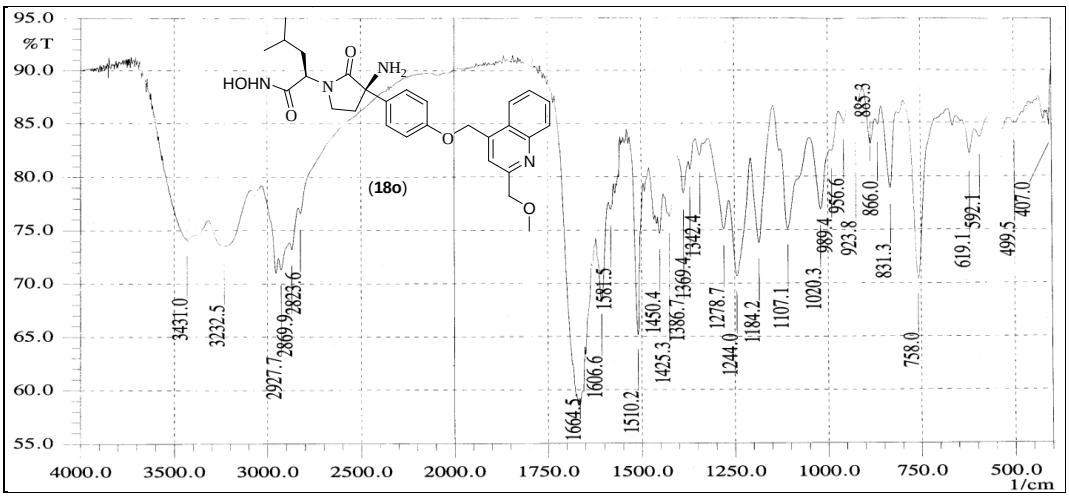


IR of compound **18o**



HPLC of compound **18o**

COLUMN : ODS-150 X4.6-4u, C-18
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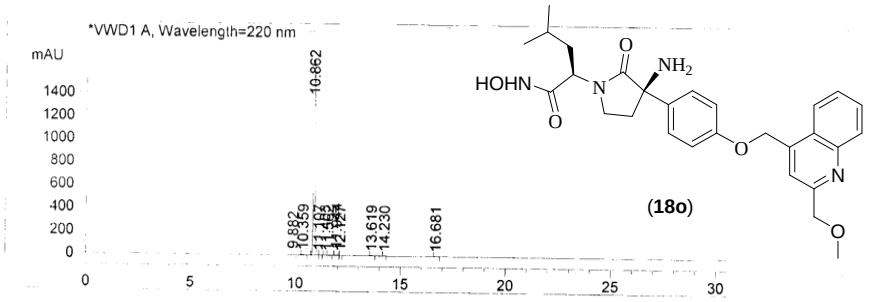
F.RATE : 1.0 ML/MIN



Area Percent Report

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Multiplier : 1.0000
Dilution : 1.0000

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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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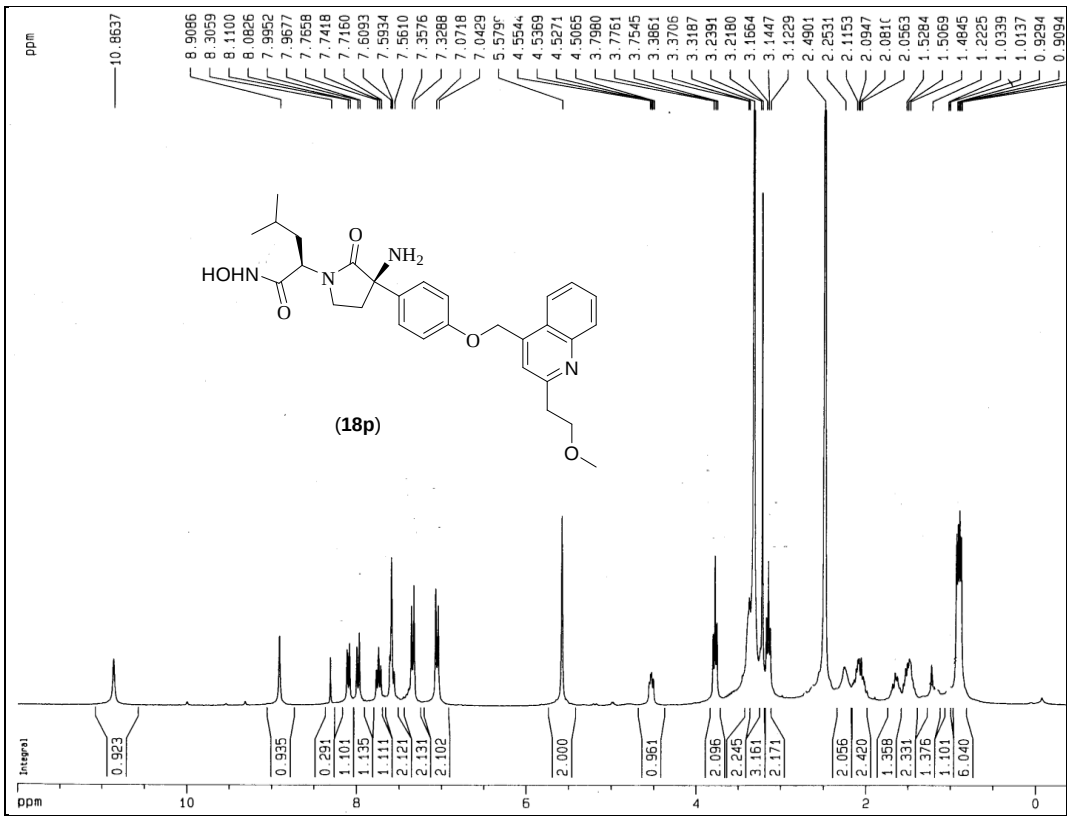
2	10.359	PB	0.0865	36.12045	6.51478	0.3792
3	10.862	MF R	0.0898	9247.99414	1708.62085	97.0948
4	11.107	FM R	0.0703	11.11835	2.63638	0.1167
5	11.403	BP	0.0819	5.88172	1.17183	0.0618
6	11.562	VB	0.1005	2.29877	3.41112e-1	0.0241
7	11.944	MF R	0.0991	111.15721	18.68830	1.1670
8	12.127	FM R	0.0943	5.62972	9.95194e-1	0.0591
9	13.619	BP	0.0888	17.53715	3.12576	0.1841
10	14.230	MM R	0.0886	2.86408	5.38841e-1	0.0301
11	16.681	BB	0.1044	3.21147	4.88101e-1	0.0337
Totals :				9524.70274	1759.11773	

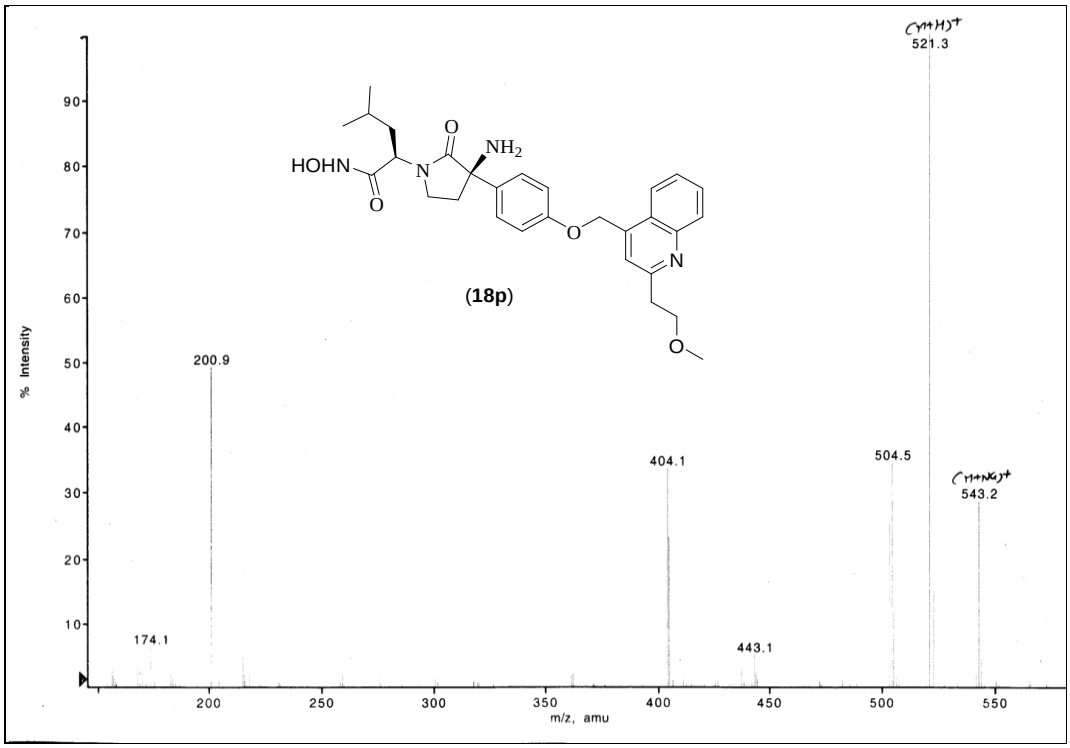
Results obtained with enhanced integrator!

*** End of Report ***

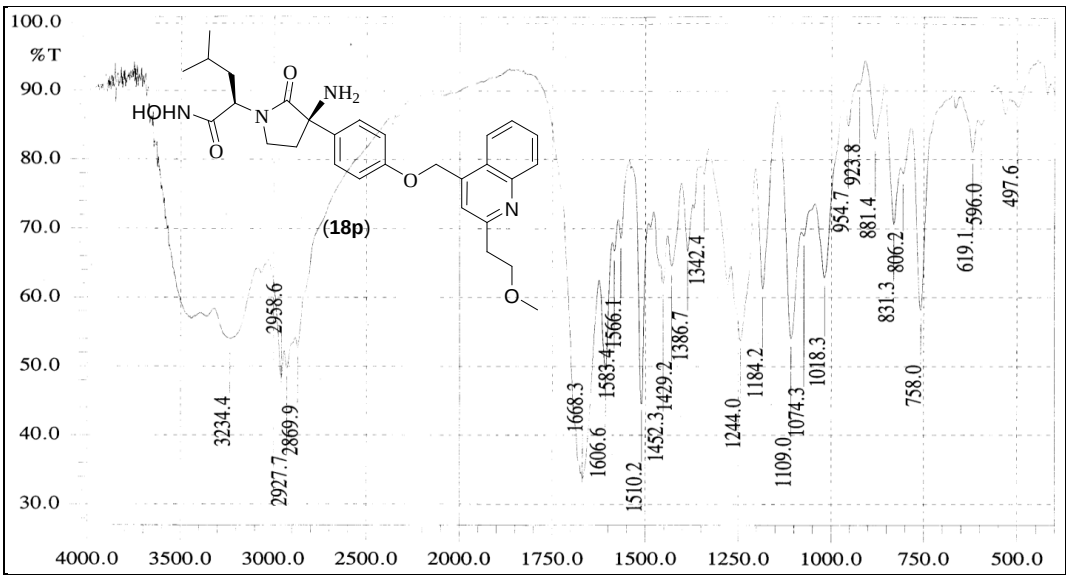
^{1}H NMR of compound **18p**



ESI-MS of compound **18p**

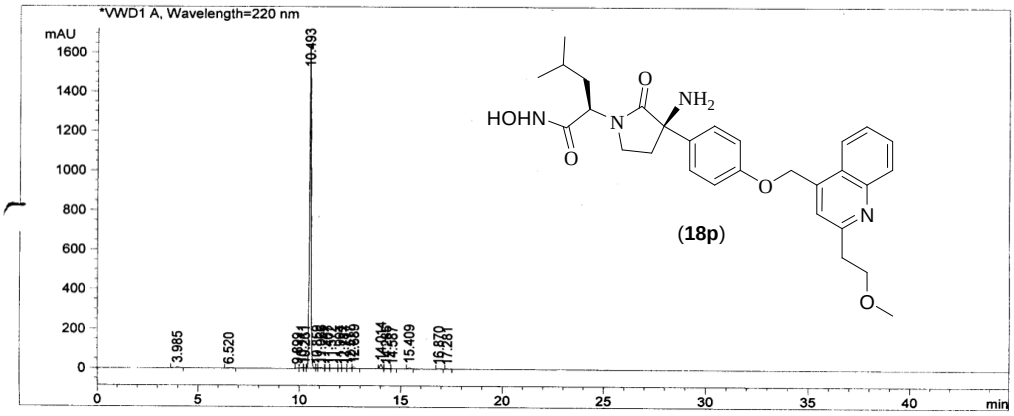


IR of compound **18p**



HPLC of compound **18p**

COLUMN : ODS-150 X4.6-4u, C-18
M.PHASE: 0.05%TFA BUFFER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN

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                          Area Percent Report
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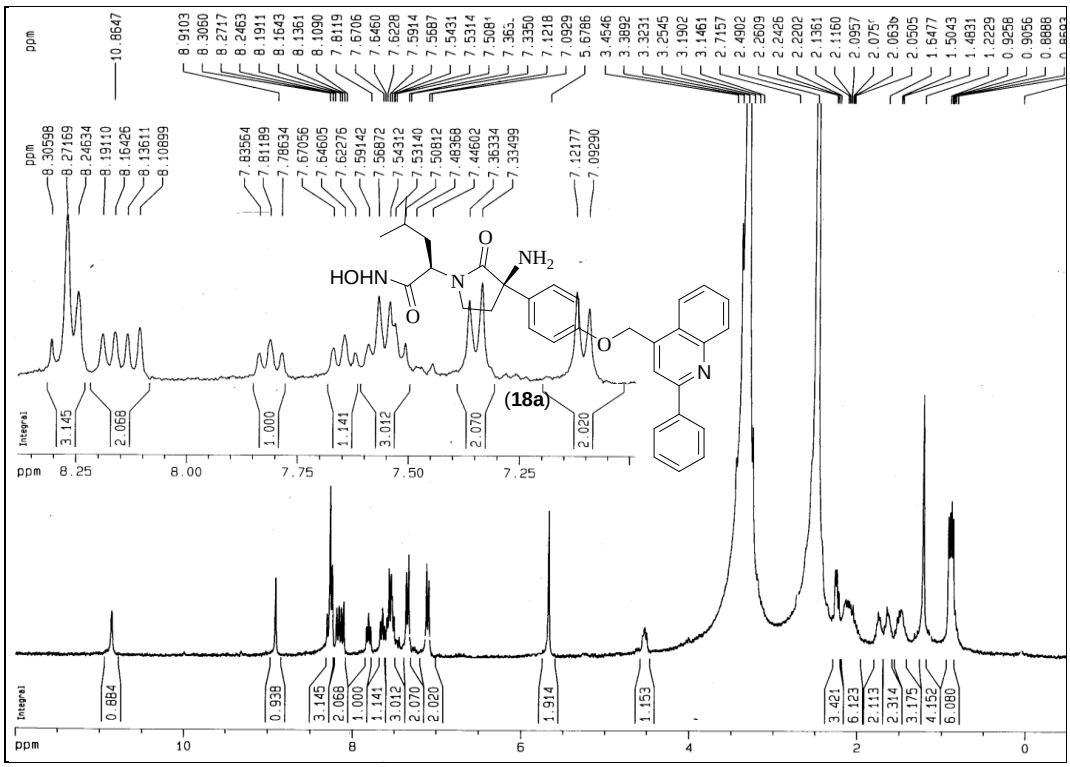
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

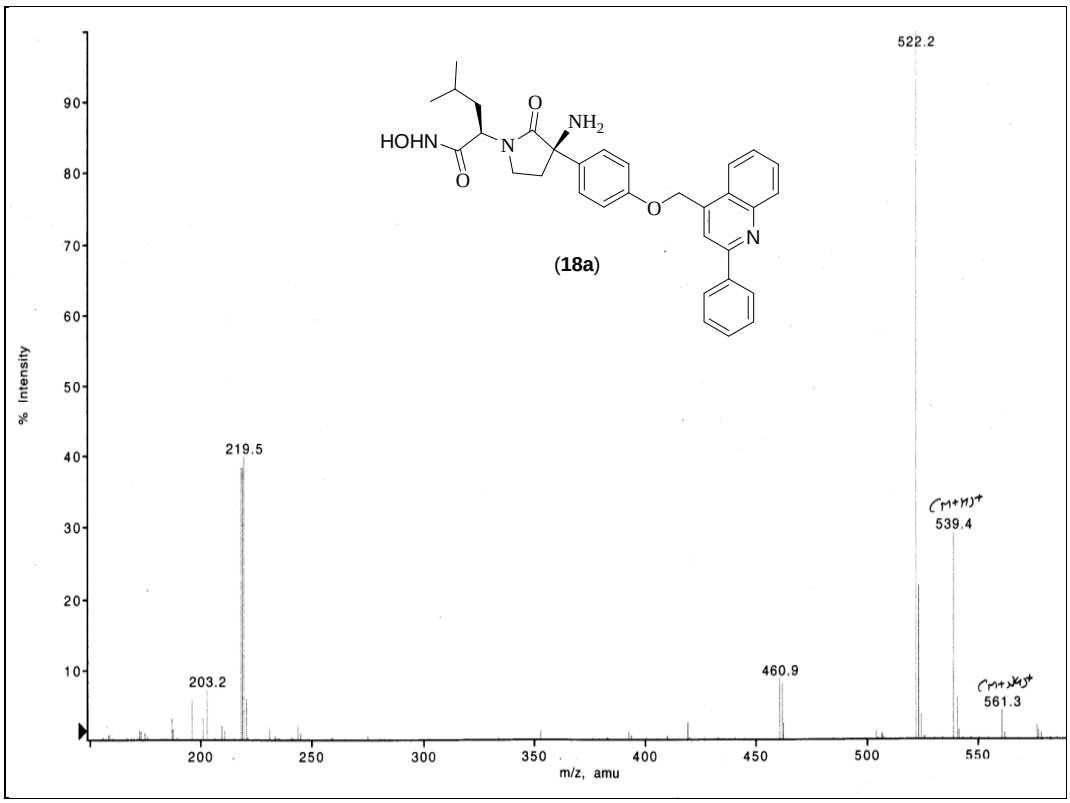
Signal 1: VWD1 A, Wavelength=220 nm
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   3.985 BB    0.1564   81.26367    7.80654   0.8059
   2   6.520 BP    0.1881   23.49431    1.99905   0.2330
   3   9.899 BV    0.0924   14.90393    2.57087   0.1478
   4  10.111 VV    0.1068   43.18470    6.24773   0.4283
   5  10.261 VV    0.0977   19.78181    3.10388   0.1962
   6  10.493 VV    0.0881 9342.66602 1644.44910  92.6573
   7  10.859 VV    0.0897   21.24337    3.43152   0.2107
   8  11.066 VV    0.1757   65.40516    5.15588   0.6487
   9  11.407 VV    0.1484   24.17115    2.33608   0.2397
  10  11.572 VV    0.2042   45.60789    2.91728   0.4523
  11  11.994 VV    0.1180   20.78712    2.64158   0.2062
  12  12.187 VV    0.1522   19.06235    1.80618   0.1891
  13  12.517 VV    0.1337   26.78694    2.98789   0.2657
```

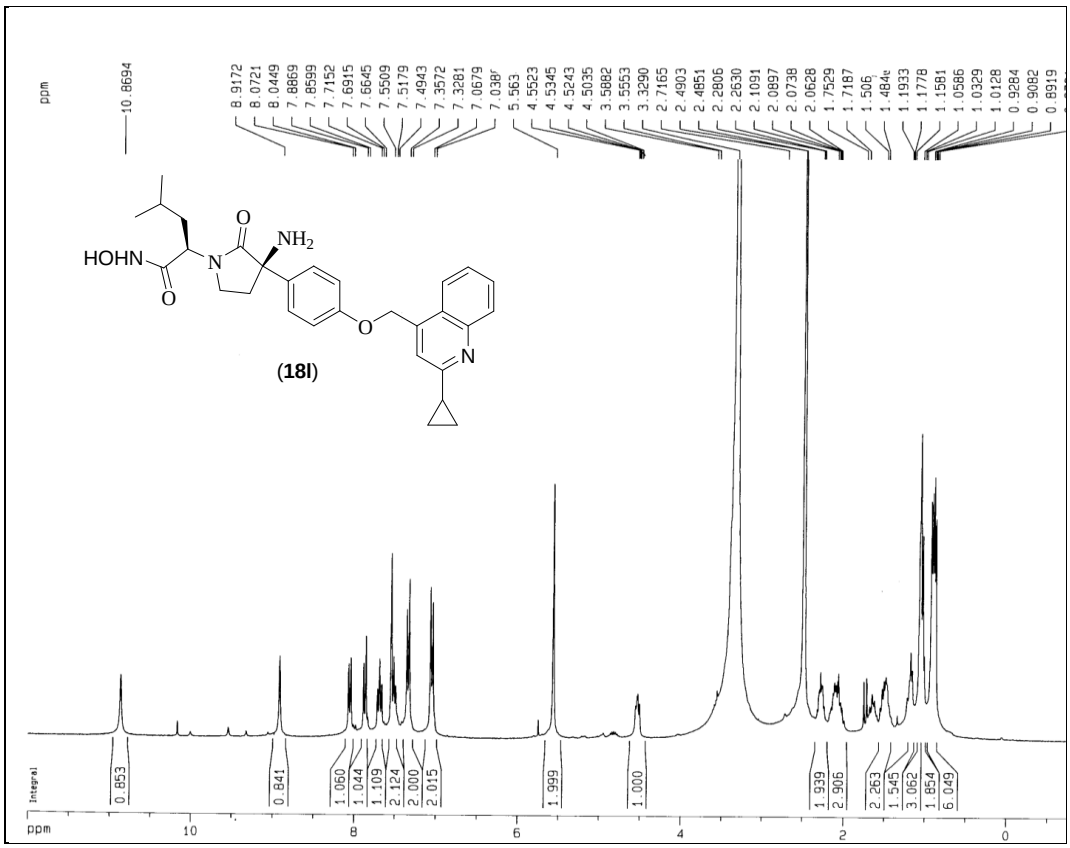
^{1}H NMR of compound **18a**



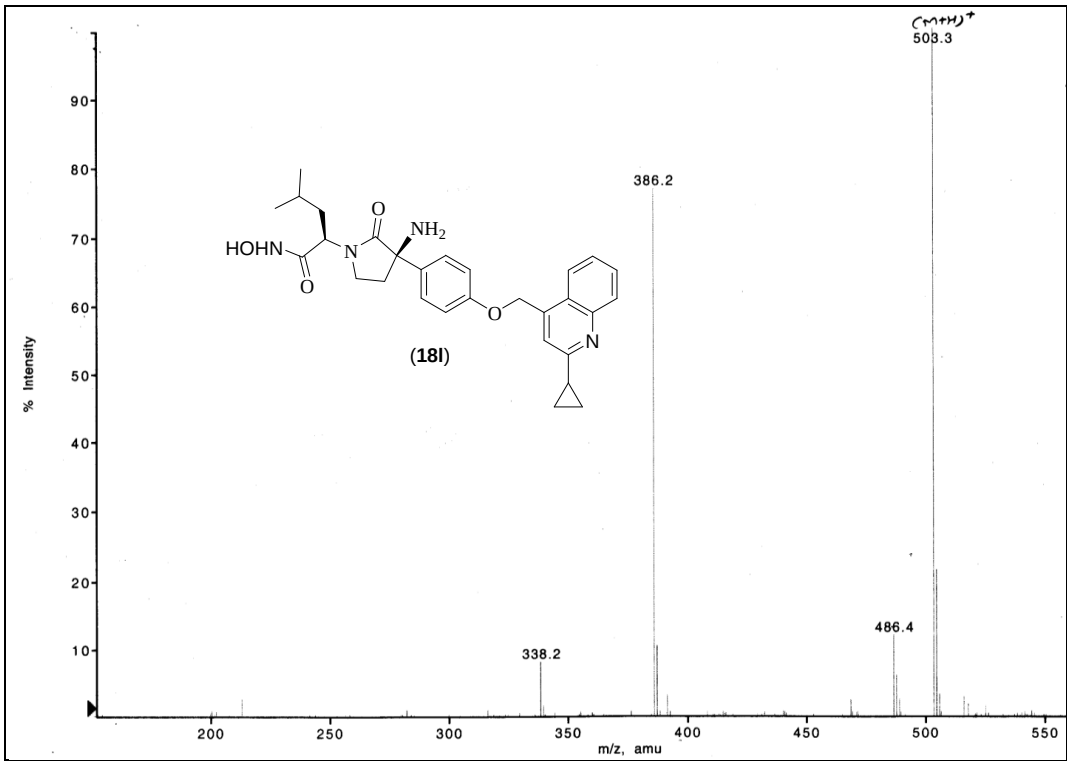
ESI-MS of compound **18a**



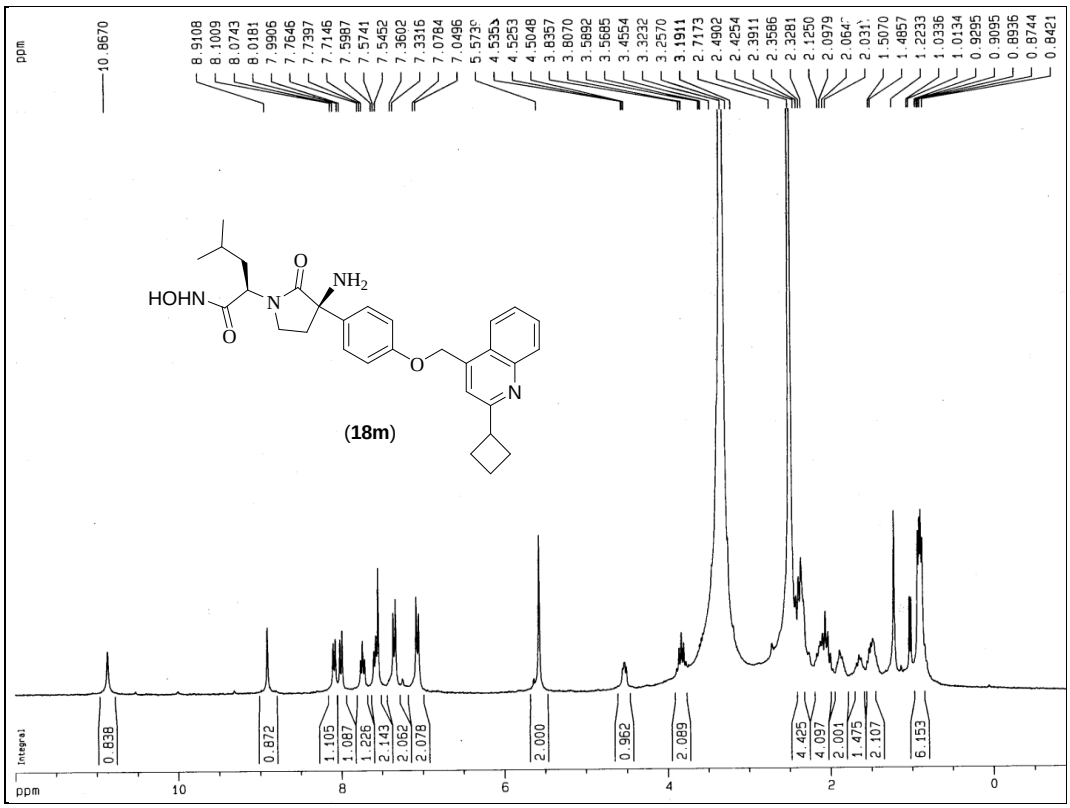
^{1}H NMR of compound **18l**



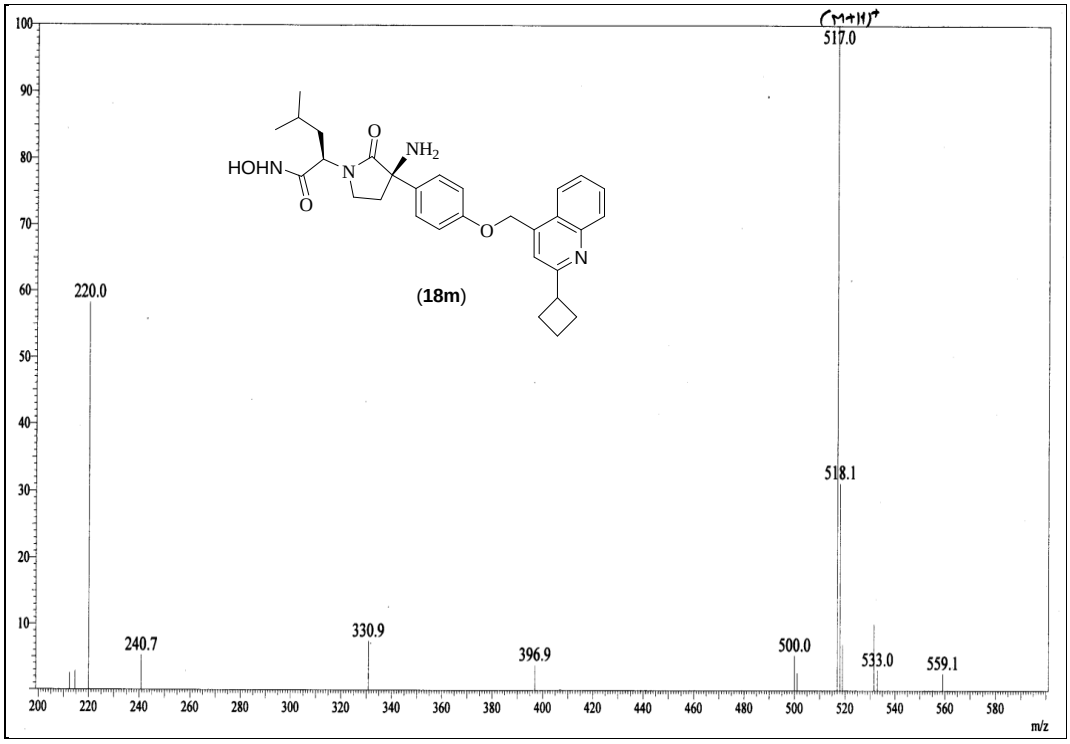
ESI-MS of compound **18l**



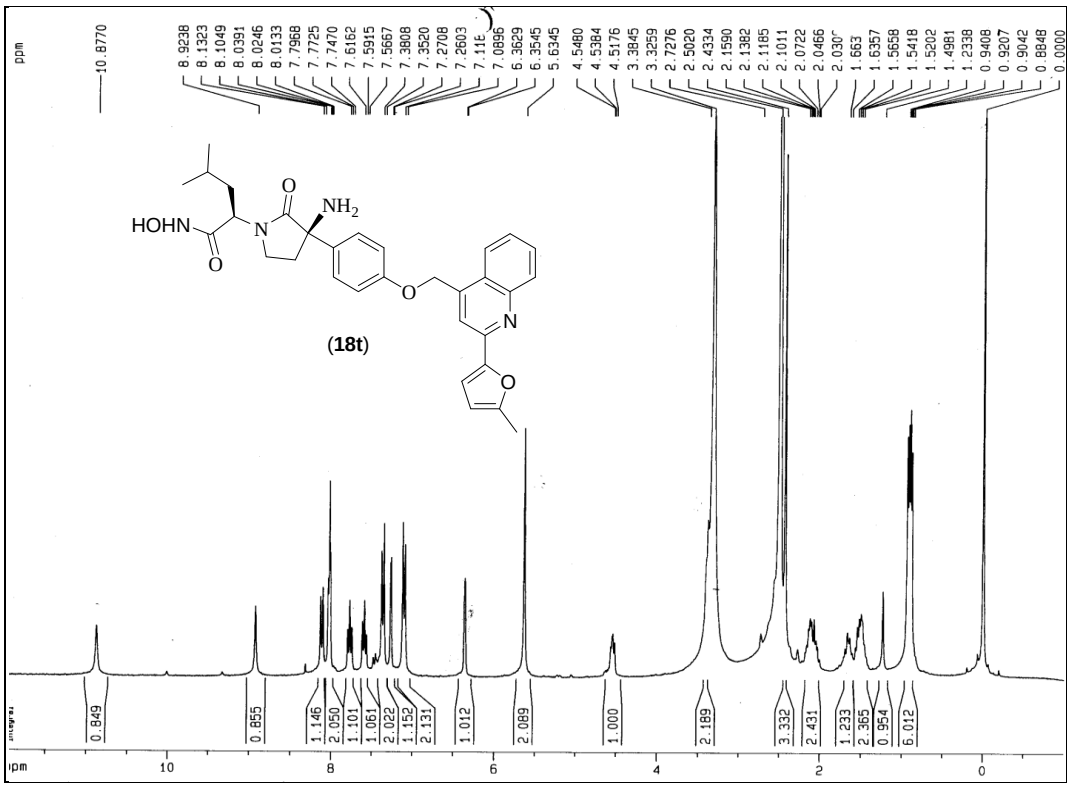
^{1}H NMR of compound **18m**



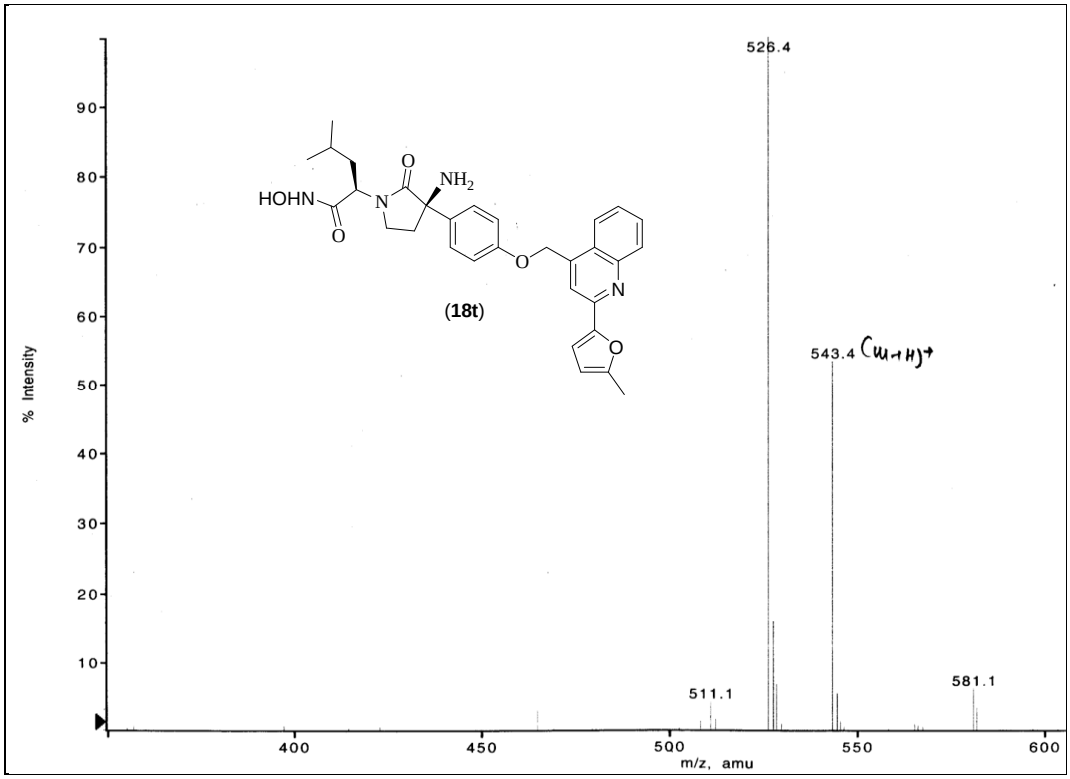
ESI-MS of compound **18m**



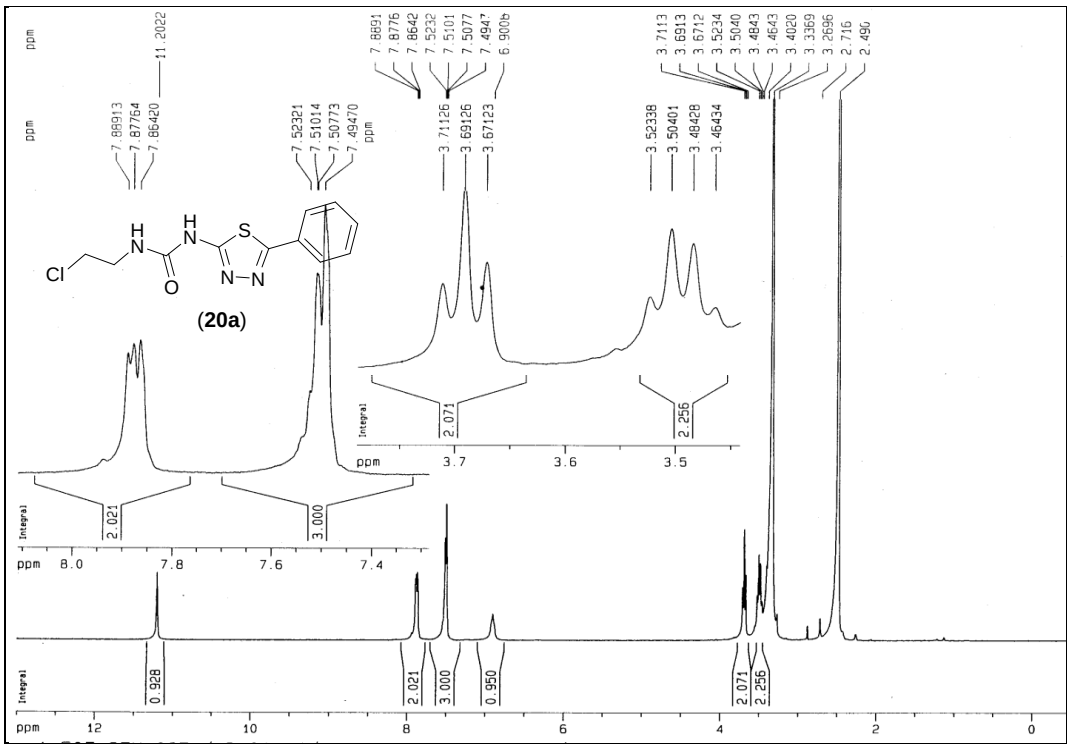
^{1}H NMR of compound **18t**



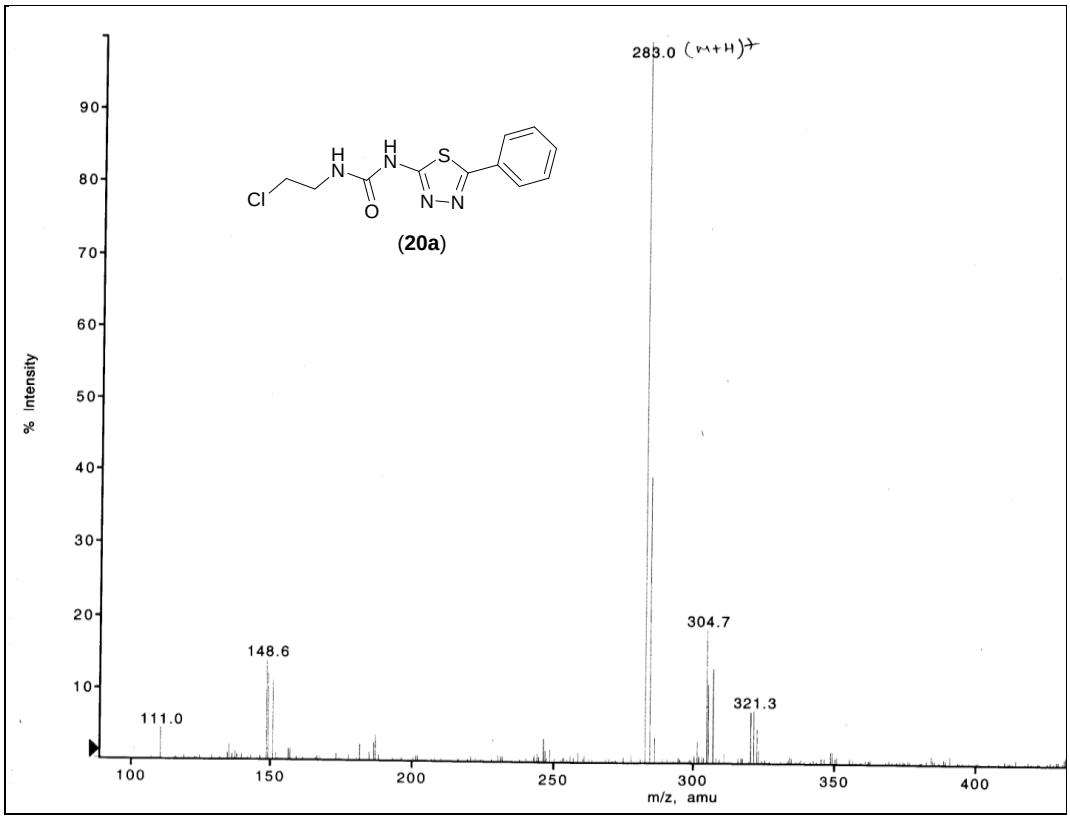
ESI-MS of compound **18t**



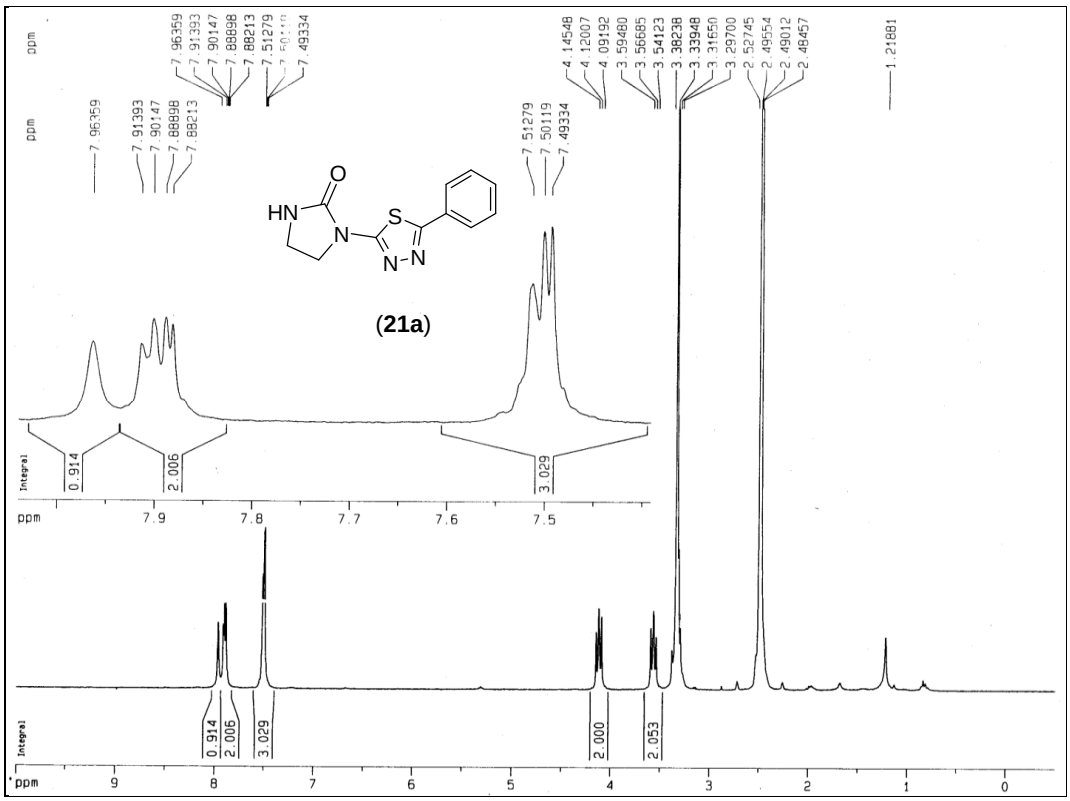
^{1}H NMR of compound **20a**



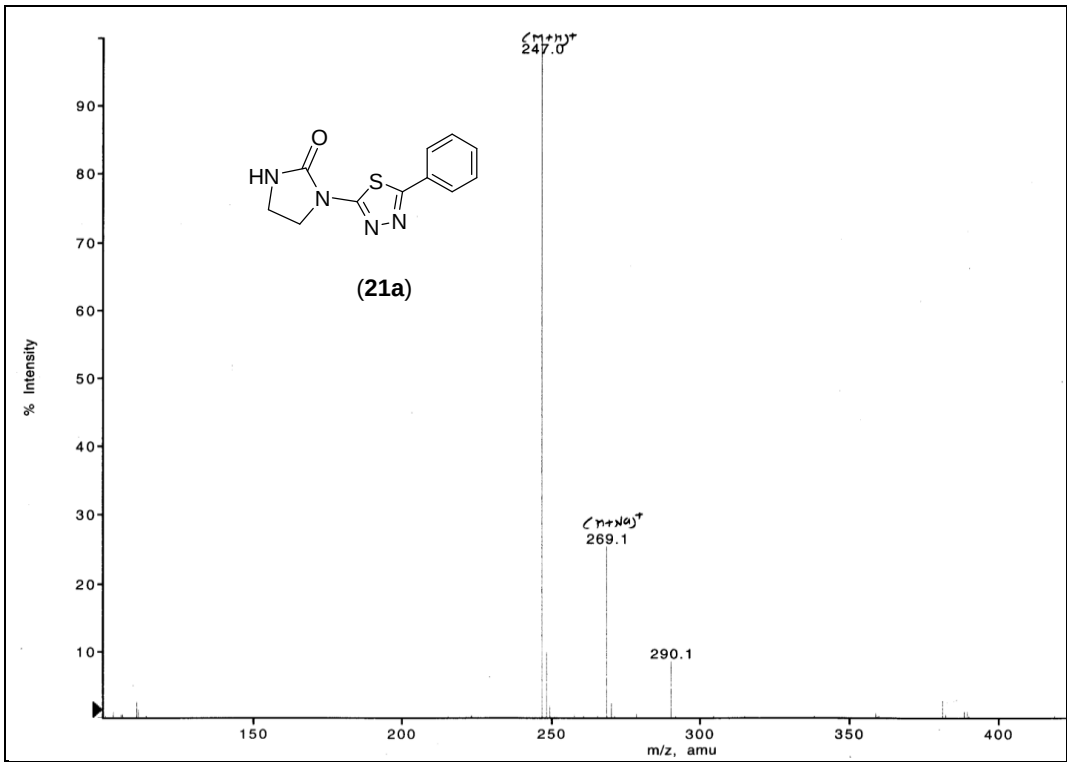
ESI-MS of compound **20a**



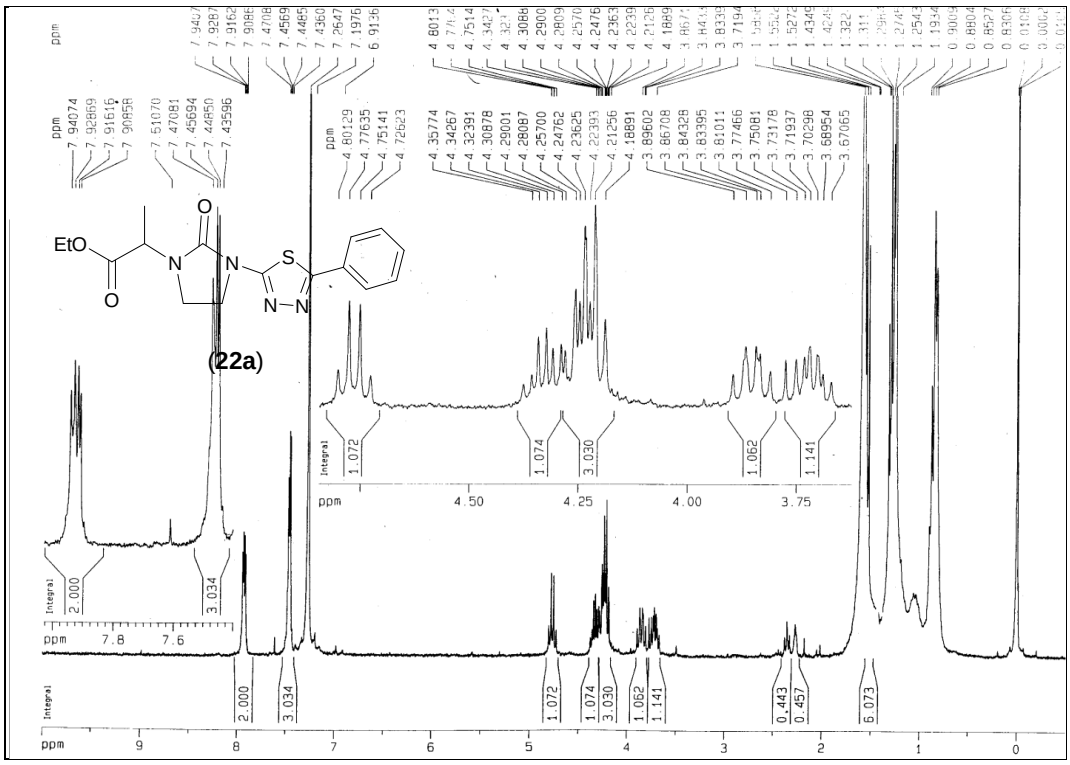
^{1}H NMR of compound **21a**



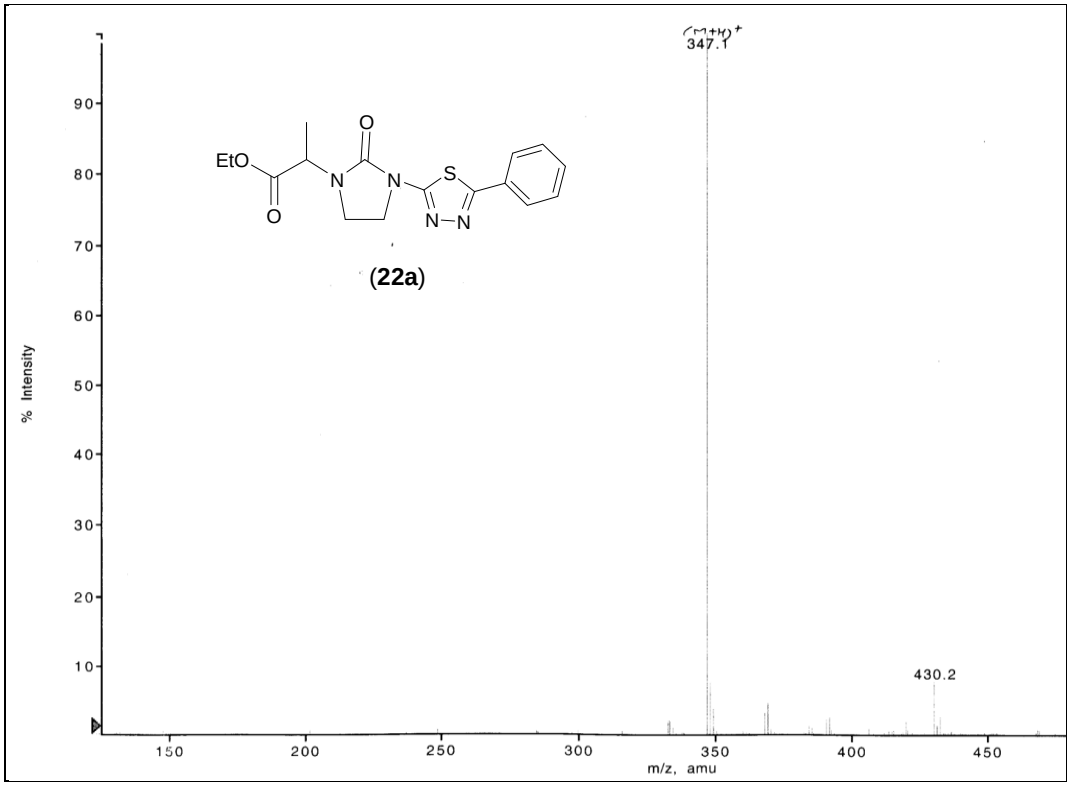
ESI-MS of compound **21a**



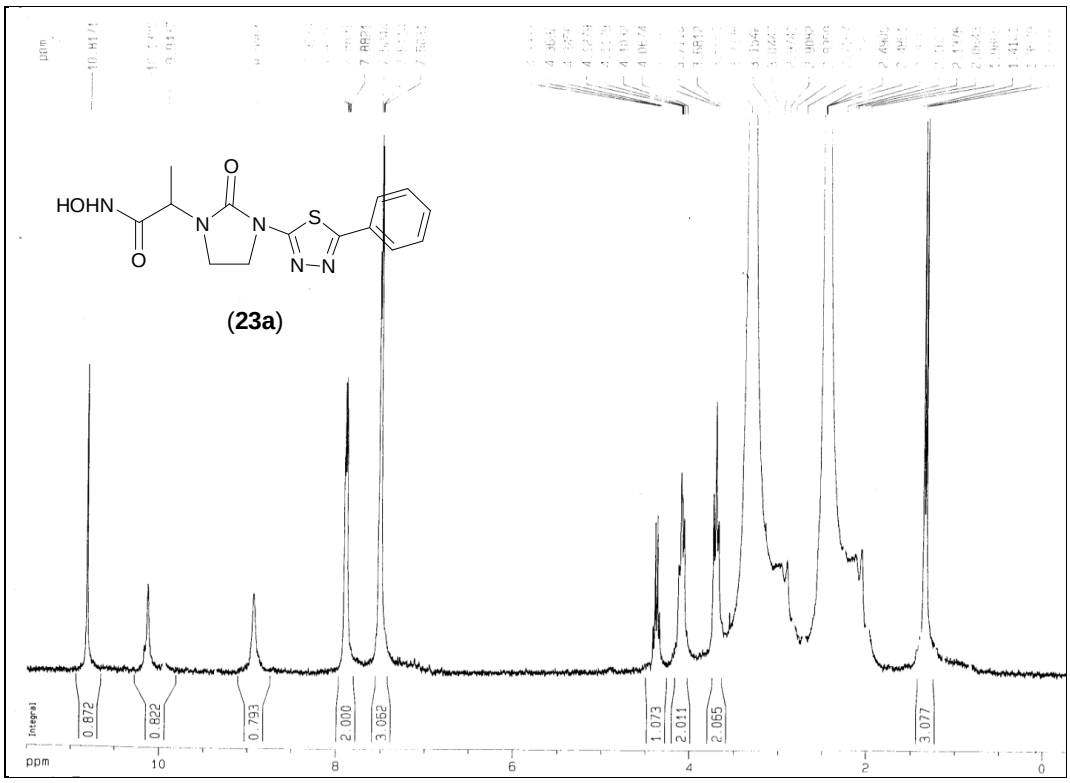
^{1}H NMR of compound **22a**



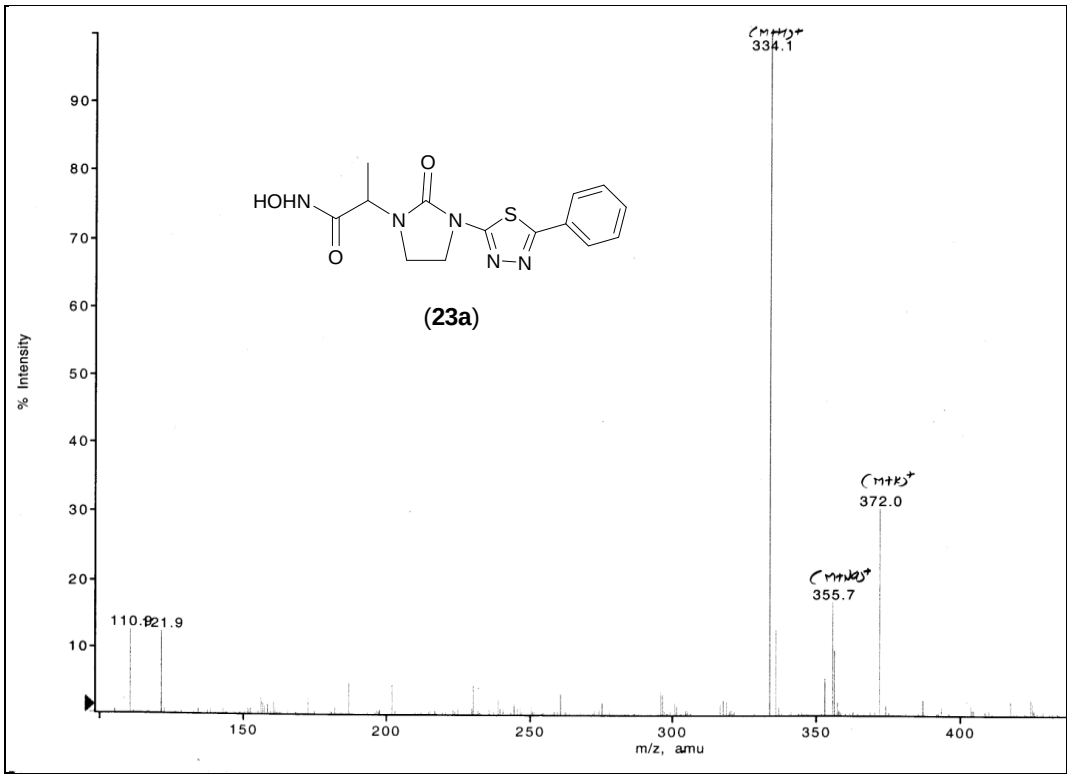
ESI-MS of compound **22a**



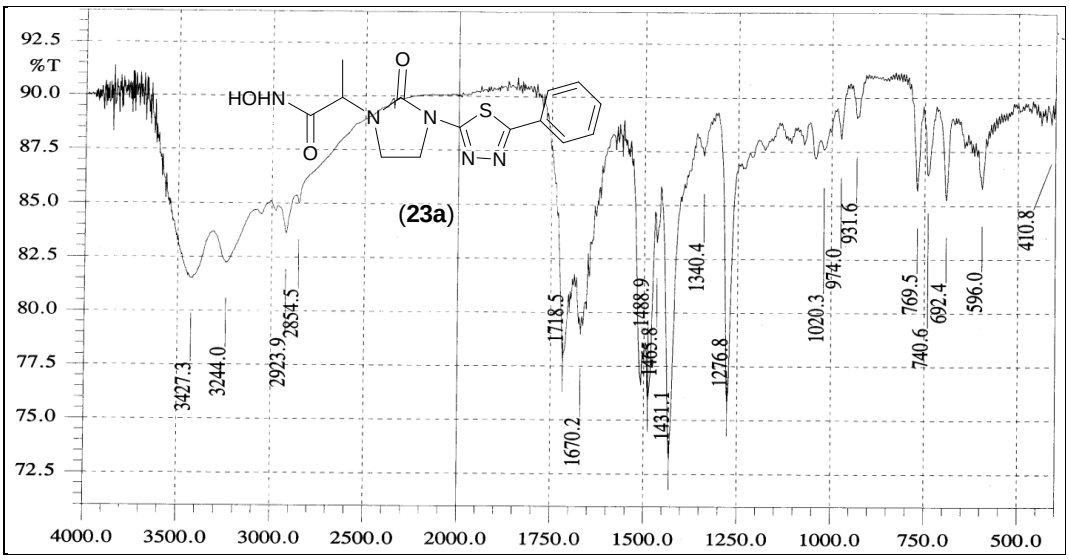
^{1}H NMR of compound **23a**



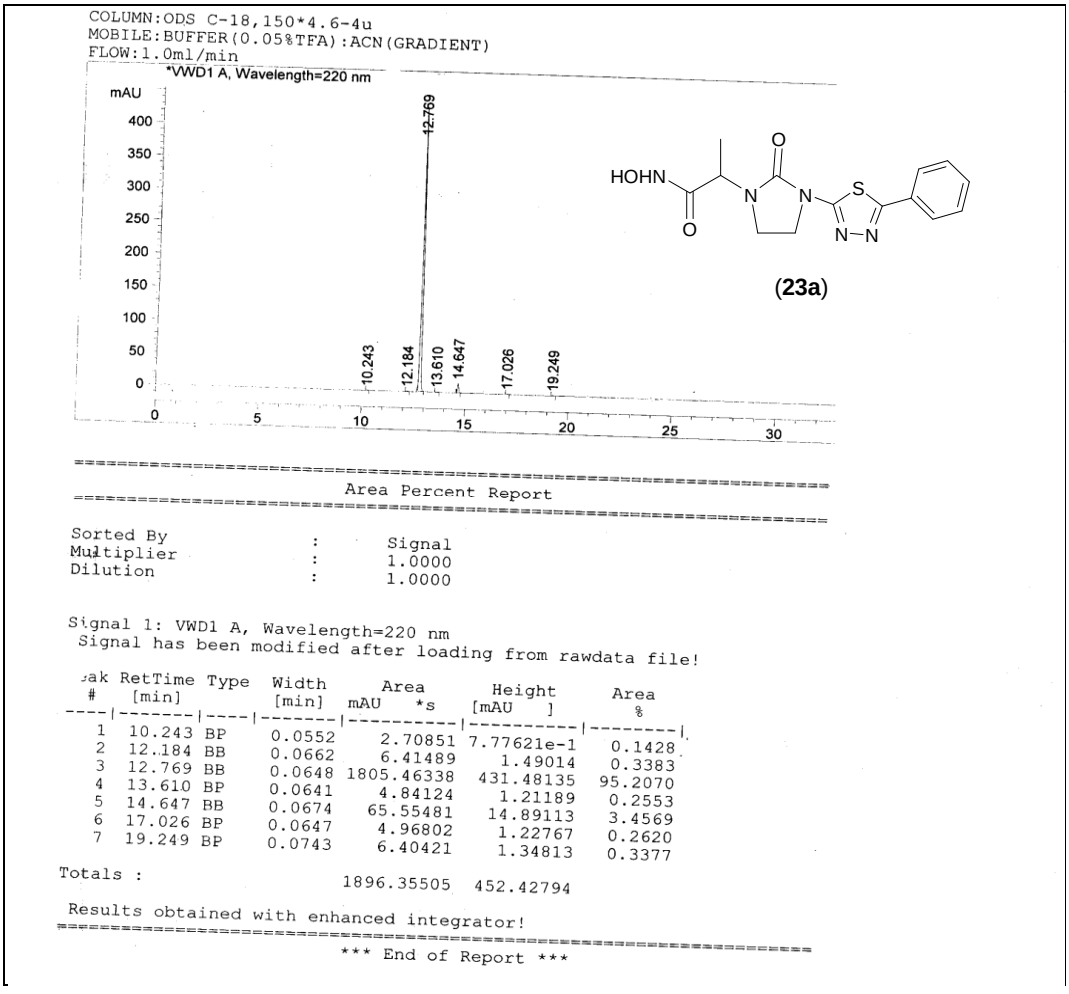
ESI-MS of compound **23a**



IR of compound **23a**



HPLC of compound **23a**



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

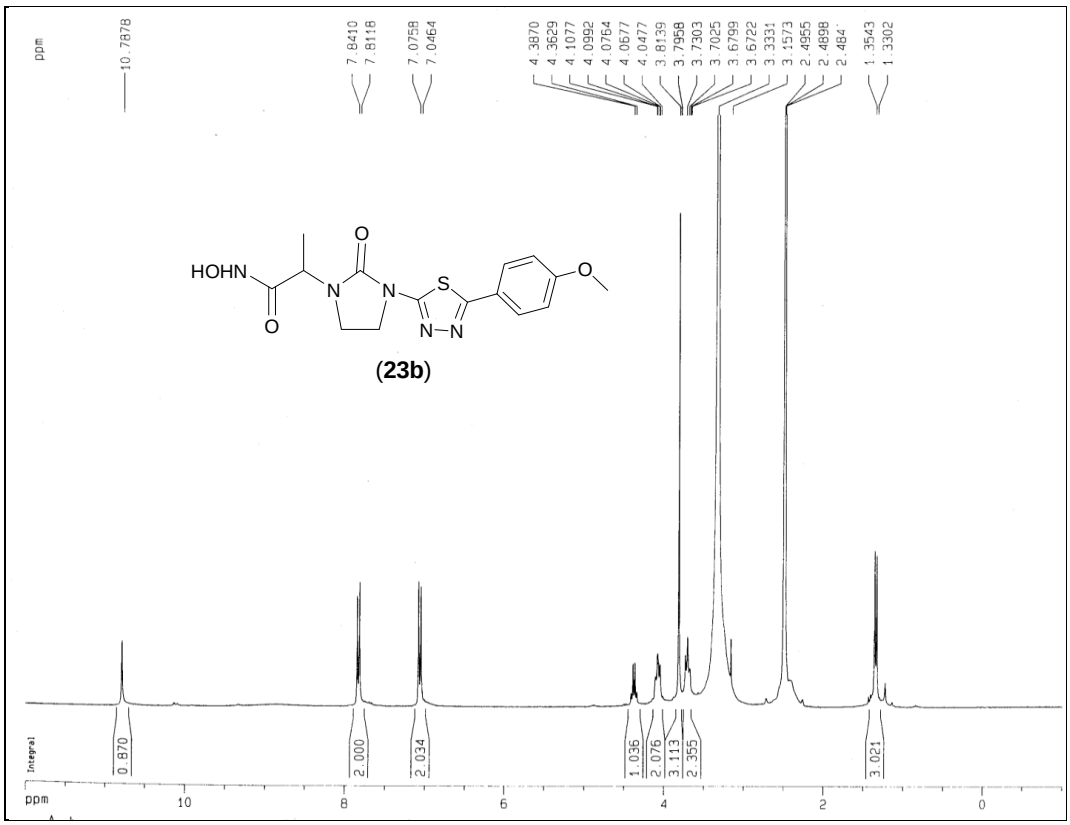
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	10.243	BP	0.0552	2.70851	7.77621e-1	0.1428
2	12.184	BB	0.0662	6.41489	1.49014	0.3383
3	12.769	BB	0.0648	1805.46338	431.48135	95.2070
4	13.610	BP	0.0641	4.84124	1.21189	0.2553
5	14.647	BB	0.0674	65.55481	14.89113	3.4569
6	17.026	BP	0.0647	4.96802	1.22767	0.2620
7	19.249	BP	0.0743	6.40421	1.34813	0.3377
Totals :				1896.35505	452.42794	

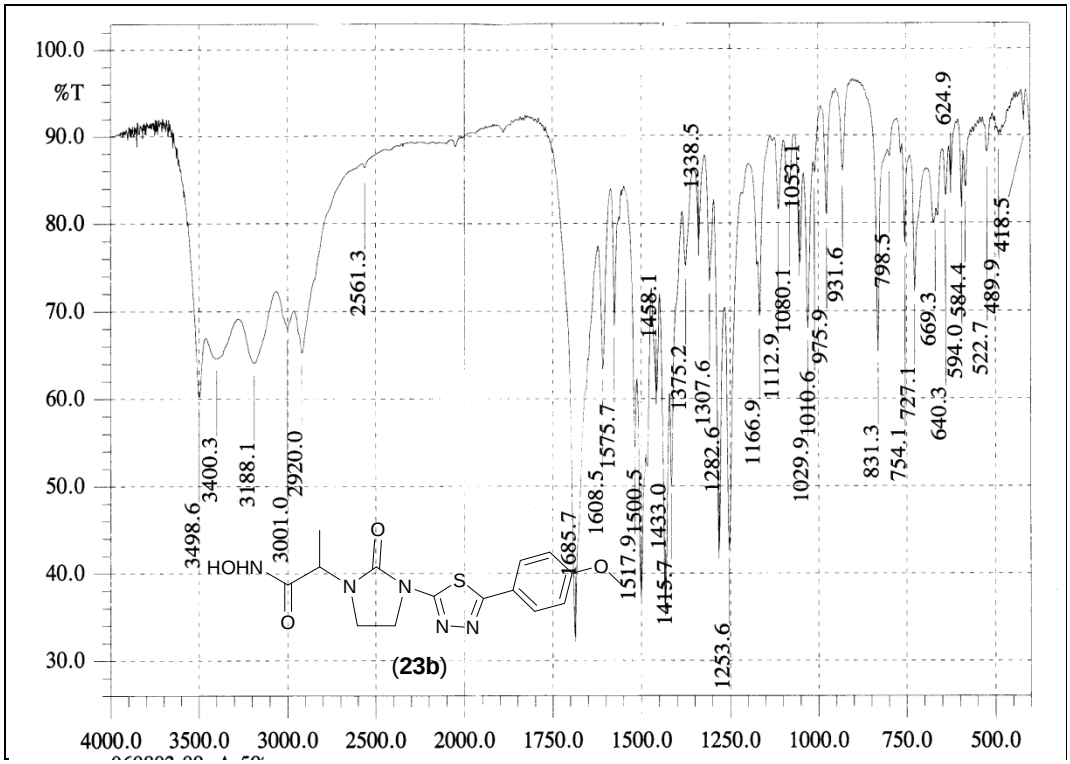
Results obtained with enhanced integrator!

*** End of Report ***

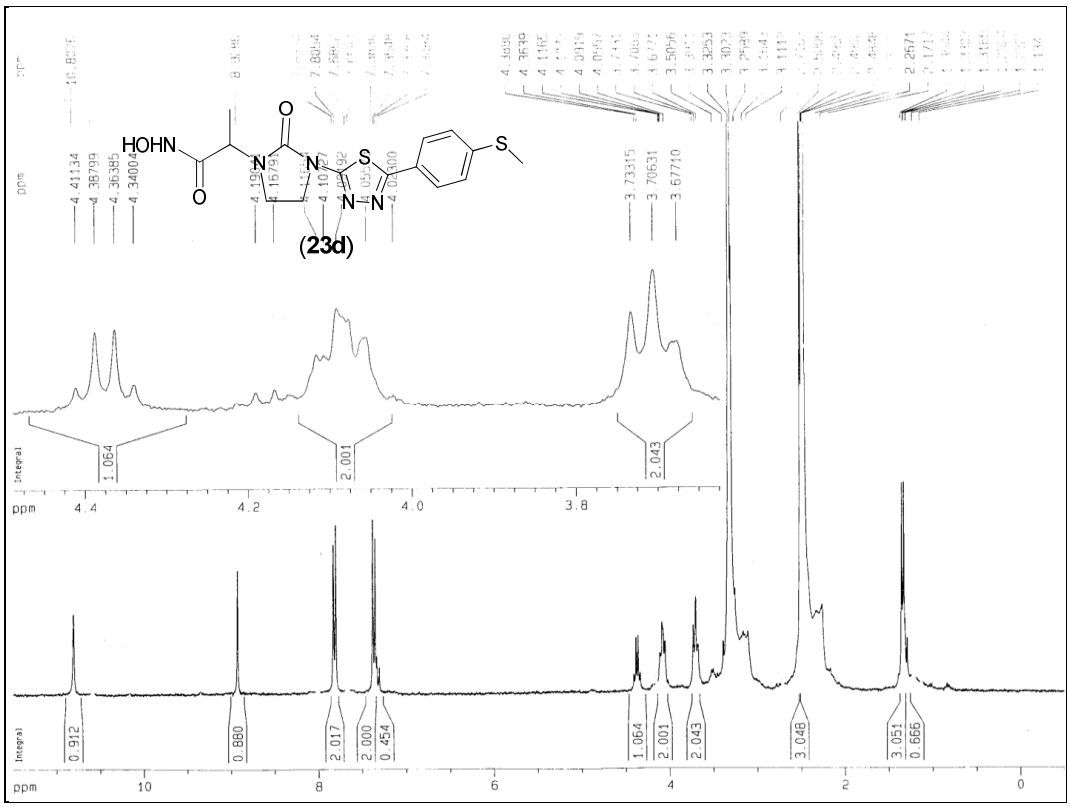
^{1}H NMR of compound **23b**



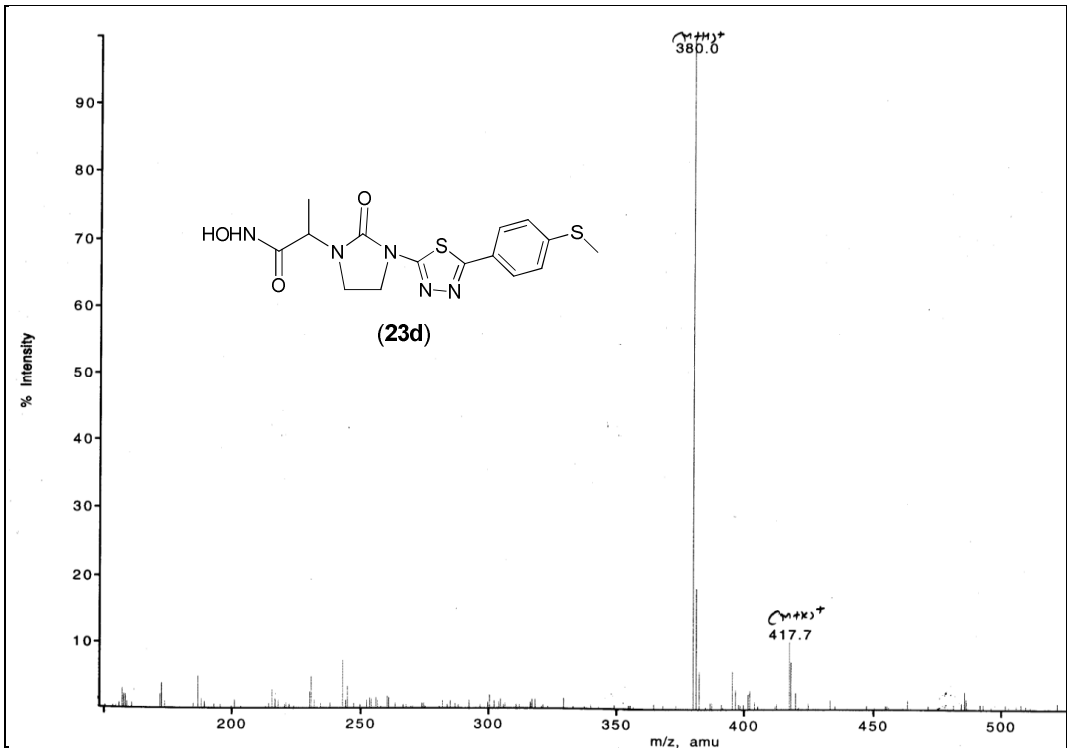
IR of compound **23b**



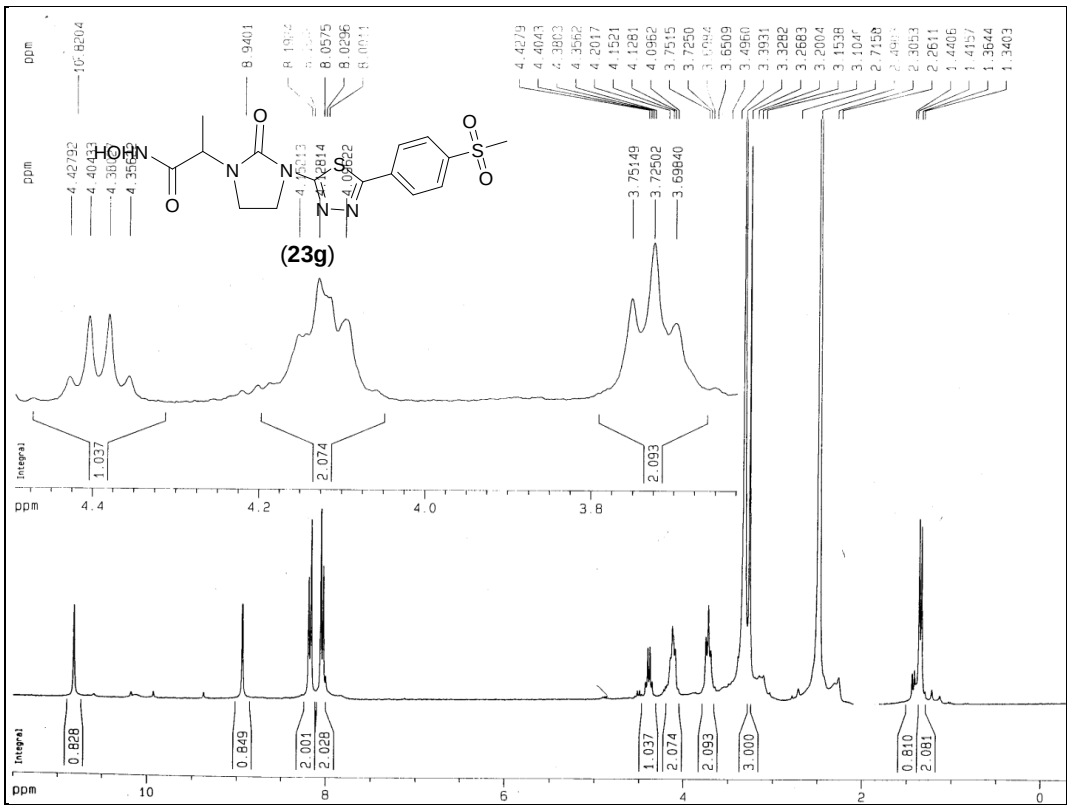
^{1}H NMR of compound **23d**



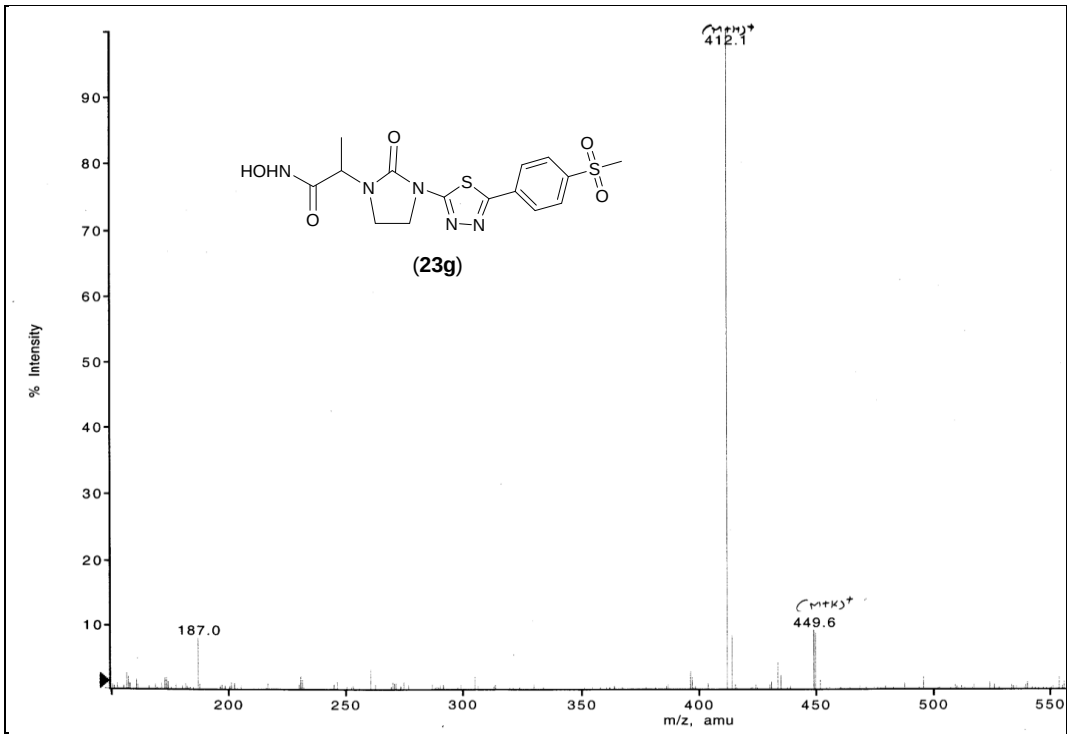
ESI-MS of compound **23d**



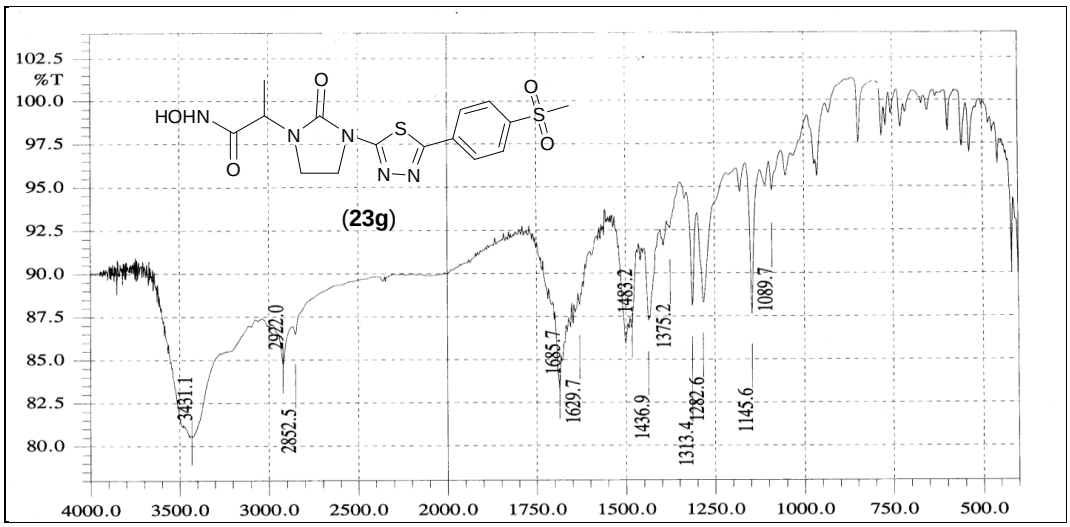
^{1}H NMR of compound **23g**



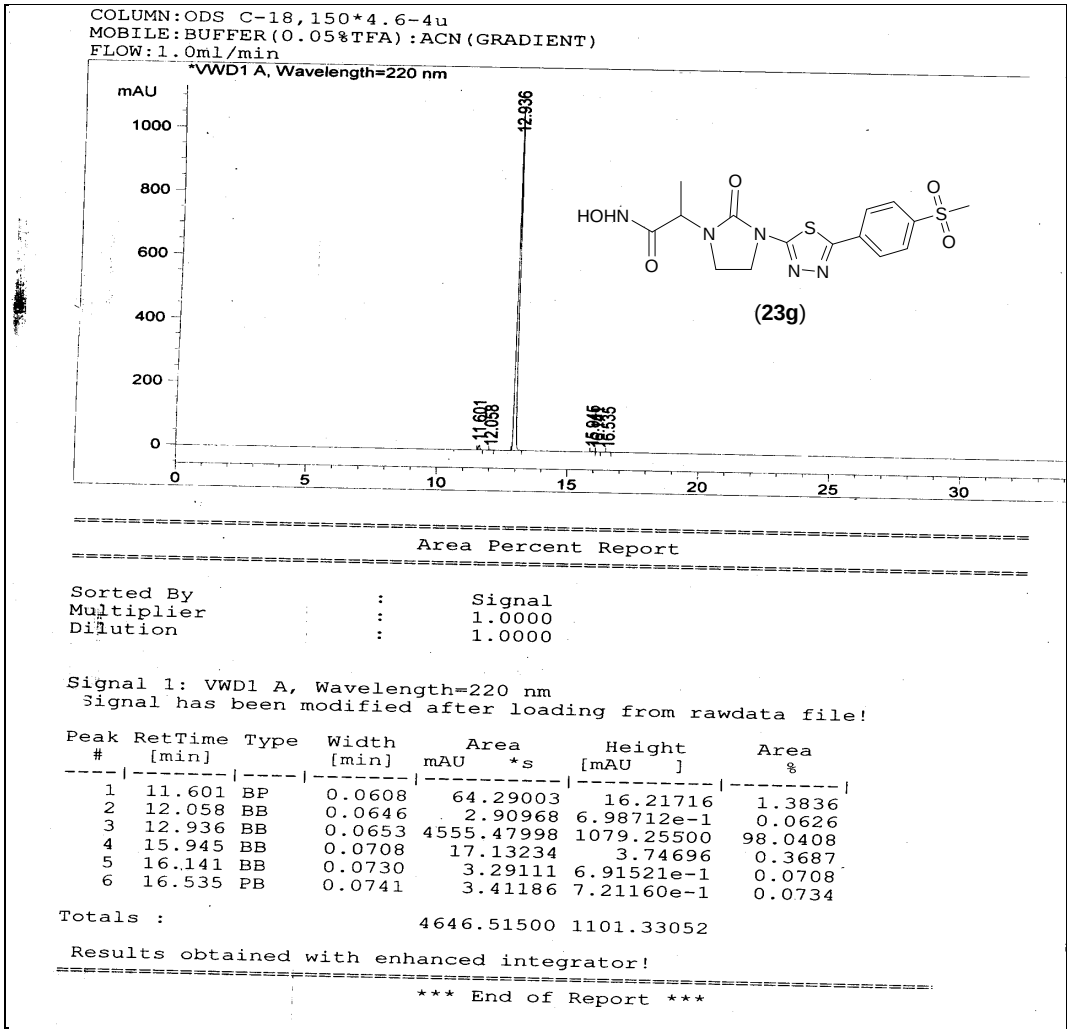
ESI-MS of compound **23g**



^{1}H NMR of compound **23g**



HPLC of compound **23g**



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

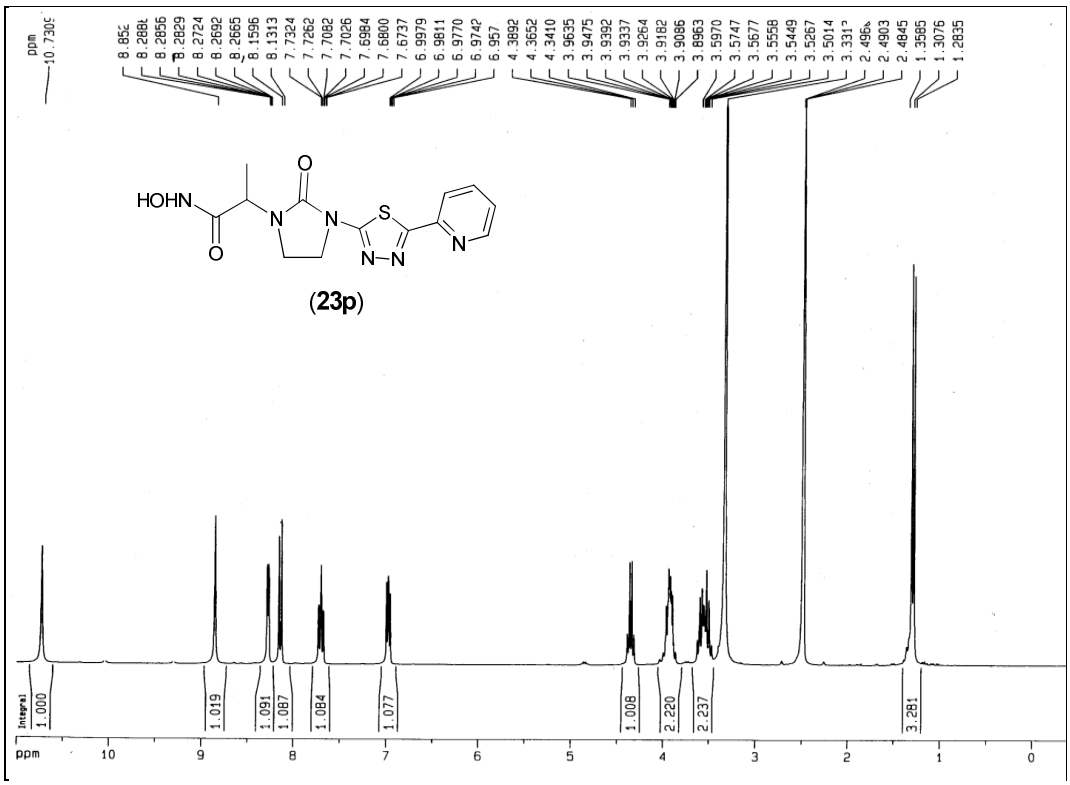
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	11.601	BP	0.0608	64.29003	16.21716	1.3836
2	12.058	BB	0.0646	2.90968	6.98712e-1	0.0626
3	12.936	BB	0.0653	4555.47998	1079.25500	98.0408
4	15.945	BB	0.0708	17.13234	3.74696	0.3687
5	16.141	BB	0.0730	3.29111	6.91521e-1	0.0708
6	16.535	PB	0.0741	3.41186	7.21160e-1	0.0734
Totals :				4646.51500	1101.33052	

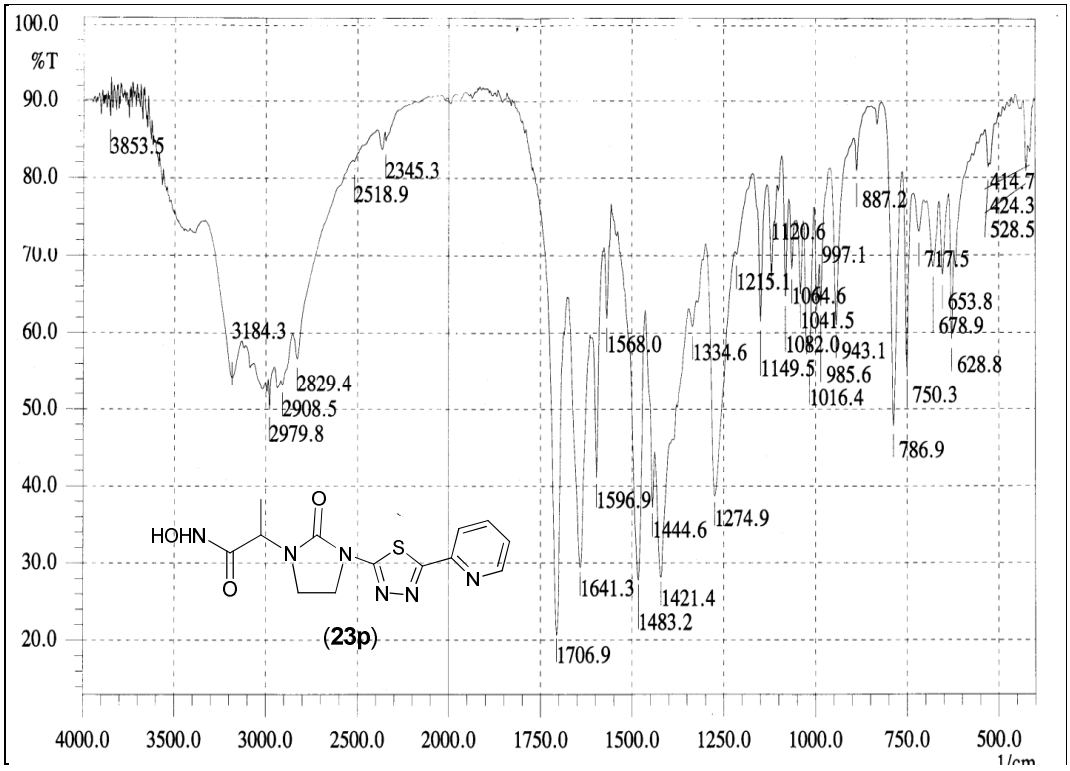
Results obtained with enhanced integrator!

*** End of Report ***

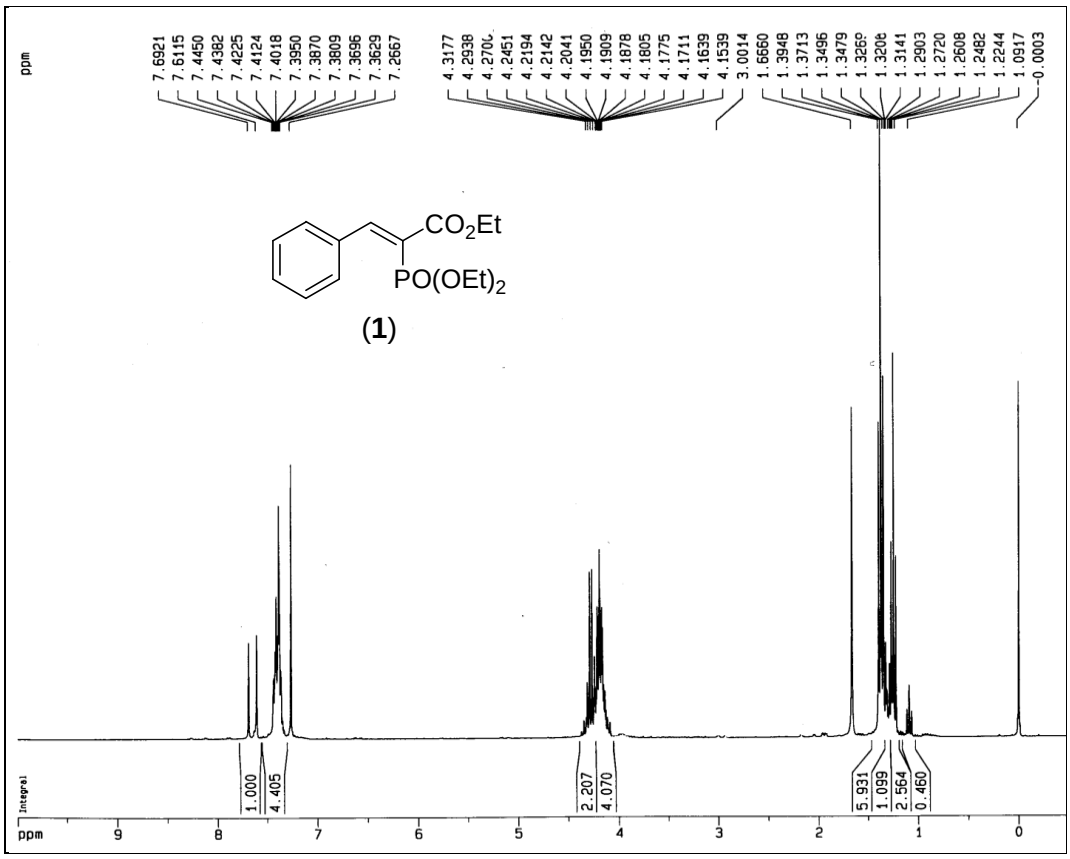
^{1}H NMR of compound **23p**



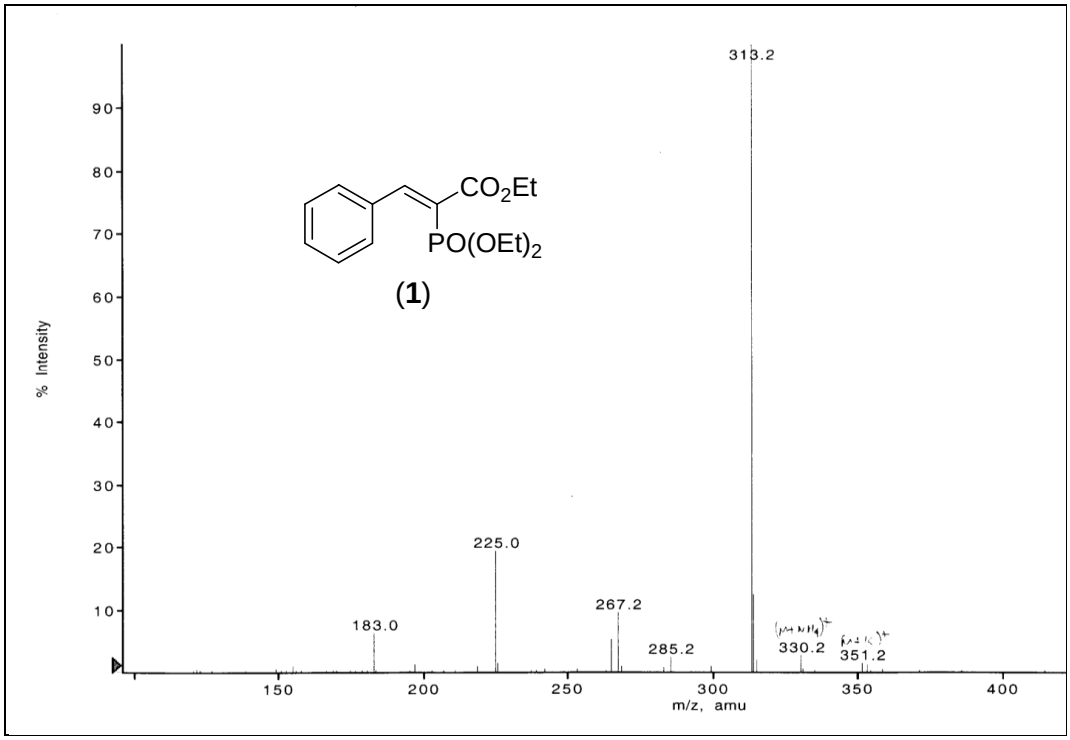
IR of compound **23p**



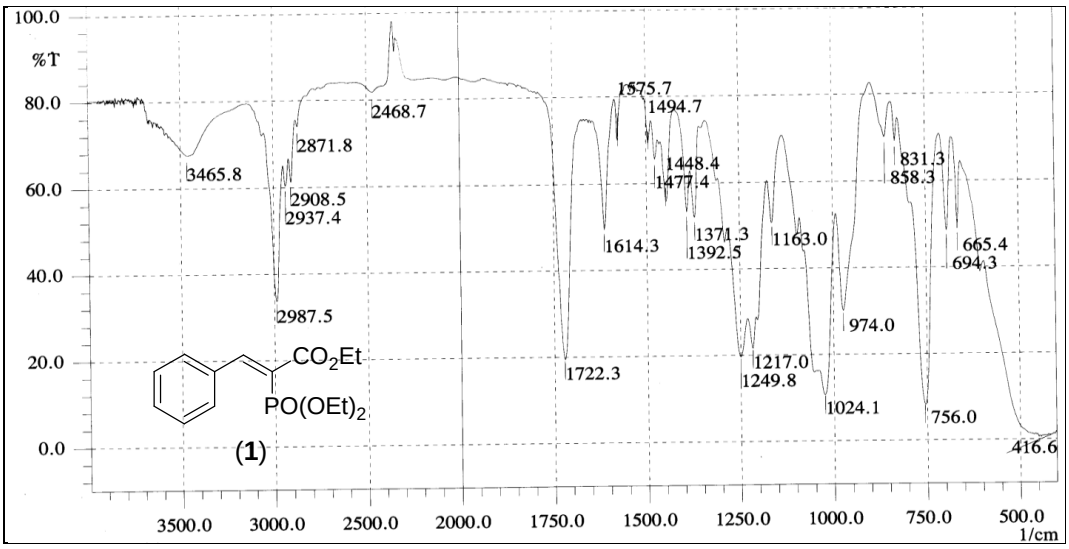
^{1}H NMR of compound **1**



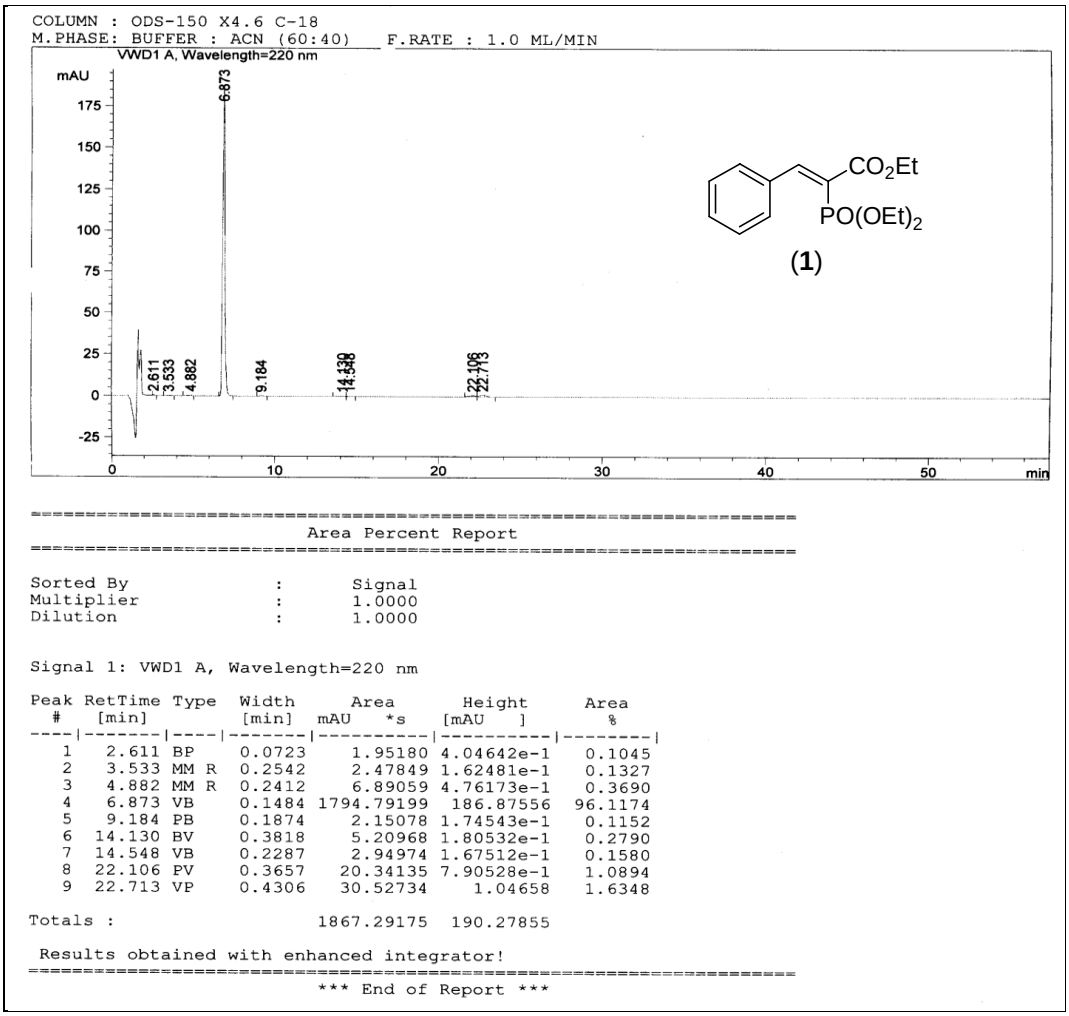
ESI-MS of compound **1**



IR of compound **1**



HPLC of compound **1**

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================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: VWD1 A, Wavelength=220 nm

Peak RetTime Type  Width     Area      Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   2.611 BP    0.0723    1.95180 4.04642e-1   0.1045
   2   3.533 MM R  0.2542    2.47849 1.62481e-1   0.1327
   3   4.882 MM R  0.2412    6.89059 4.76173e-1   0.3690
   4   6.873 VB    0.1484 1794.79199  186.87556  96.1174
   5   9.184 PB    0.1874    2.15078 1.74543e-1   0.1152
   6  14.130 BV    0.3818    5.20968 1.80532e-1   0.2790
   7  14.548 VB    0.2287    2.94974 1.67512e-1   0.1580
   8  22.106 PV    0.3657   20.34135 7.90528e-1   1.0894
   9  22.713 VP    0.4306   30.52734    1.04658   1.6348

Totals :                  1867.29175  190.27855

 Results obtained with enhanced integrator!
================================================================================
                          *** End of Report ***
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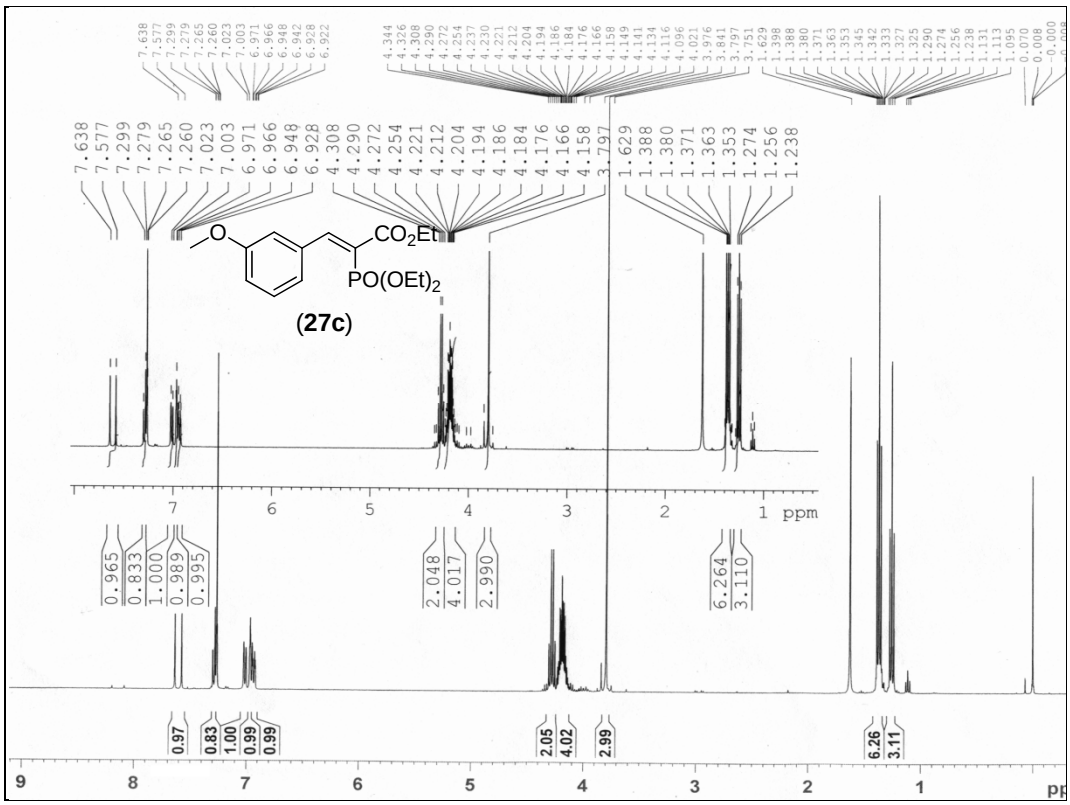
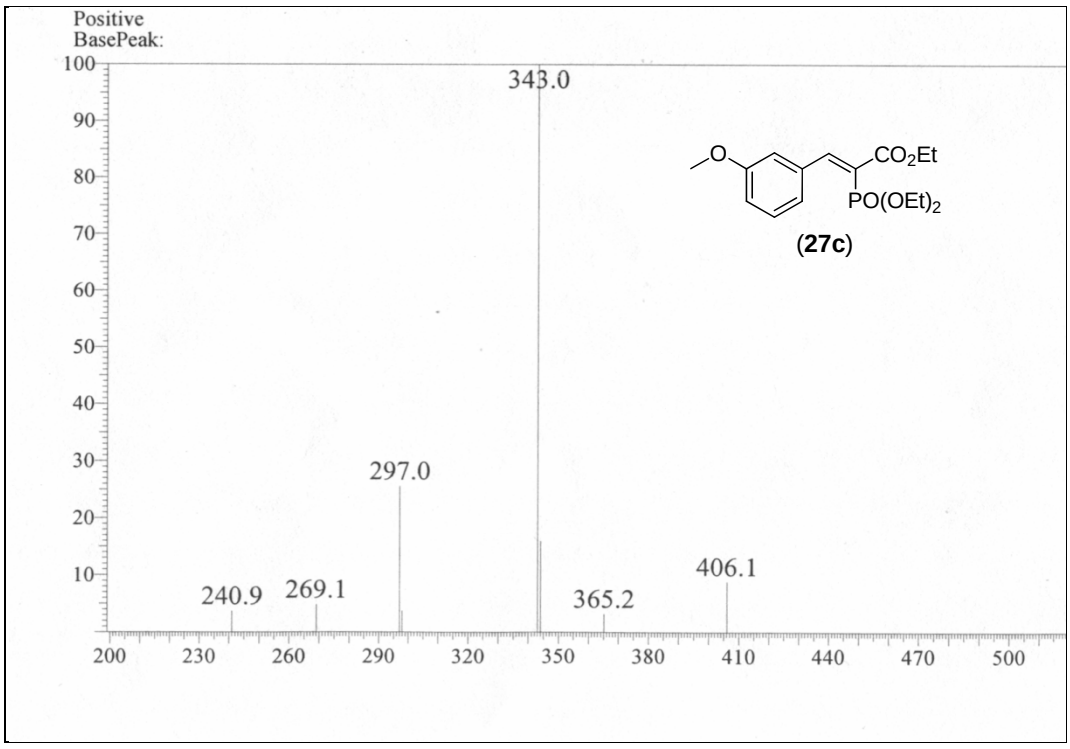
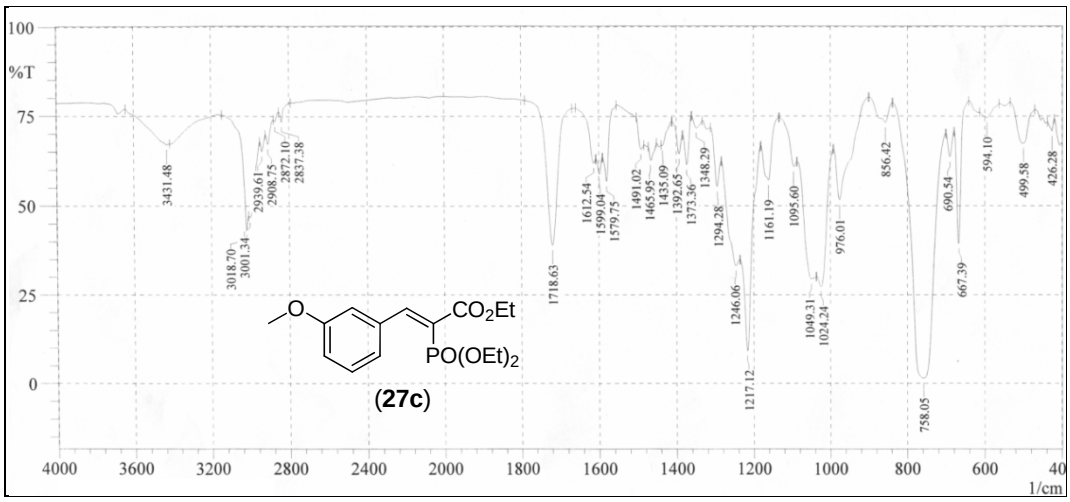
^{1}H NMR of compound **27c**



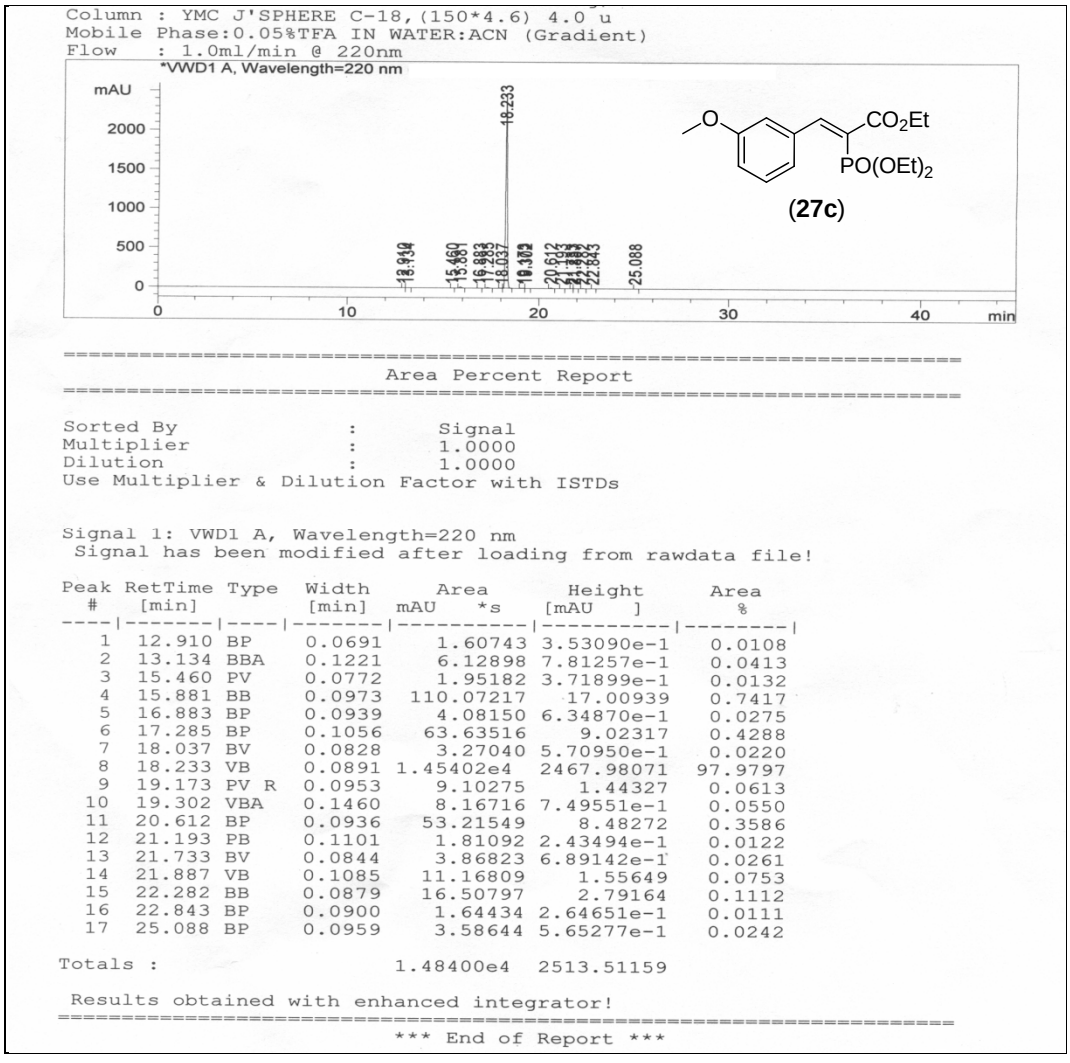
ESI-MS of compound **27c**



IR of compound **27c**



HPLC of compound **27c**



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

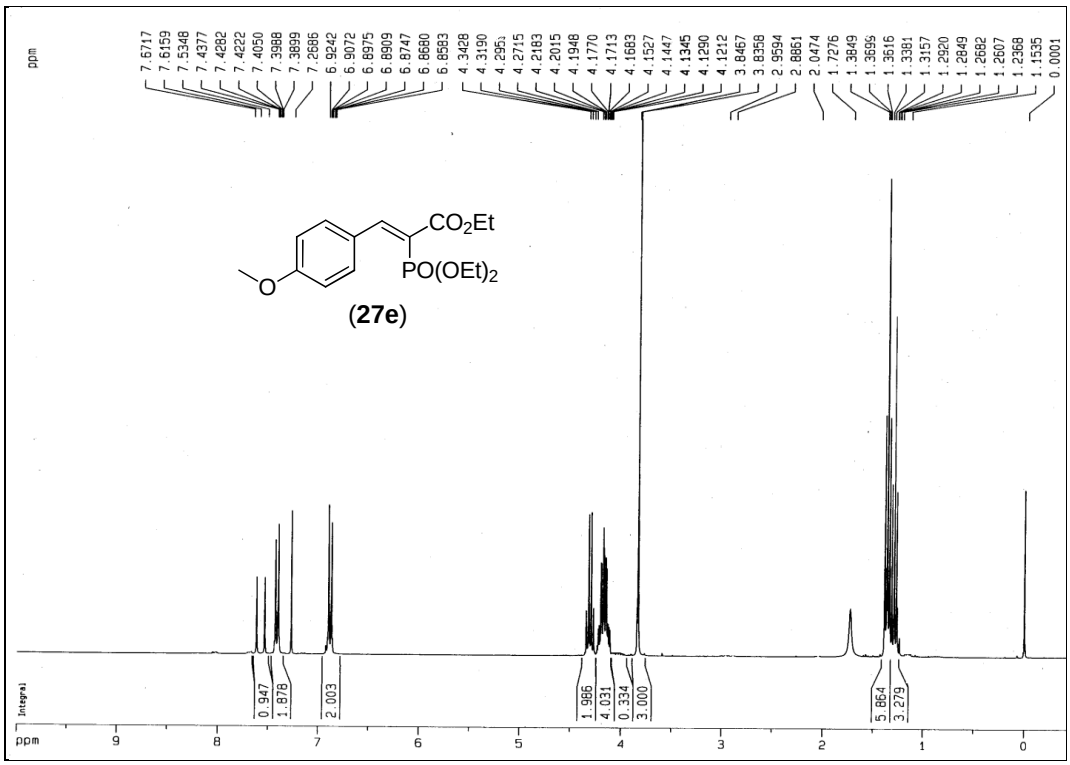
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	12.910	BP	0.0691	1.60743	3.53090e-1	0.0108
2	13.134	BBA	0.1221	6.12898	7.81257e-1	0.0413
3	15.460	PV	0.0772	1.95182	3.71899e-1	0.0132
4	15.881	BB	0.0973	110.07217	17.00939	0.7417
5	16.883	BP	0.0939	4.08150	6.34870e-1	0.0275
6	17.285	BP	0.1056	63.63516	9.02317	0.4288
7	18.037	BV	0.0828	3.27040	5.70950e-1	0.0220
8	18.233	VB	0.0891	1.45402e4	2467.98071	97.9797
9	19.173	PV R	0.0953	9.10275	1.44327	0.0613
10	19.302	VBA	0.1460	8.16716	7.49551e-1	0.0550
11	20.612	BP	0.0936	53.21549	8.48272	0.3586
12	21.193	PB	0.1101	1.81092	2.43494e-1	0.0122
13	21.733	BV	0.0844	3.86823	6.89142e-1	0.0261
14	21.887	VB	0.1085	11.16809	1.55649	0.0753
15	22.282	BB	0.0879	16.50797	2.79164	0.1112
16	22.843	BP	0.0900	1.64434	2.64651e-1	0.0111
17	25.088	BP	0.0959	3.58644	5.65277e-1	0.0242
Totals :				1.48400e4	2513.51159	

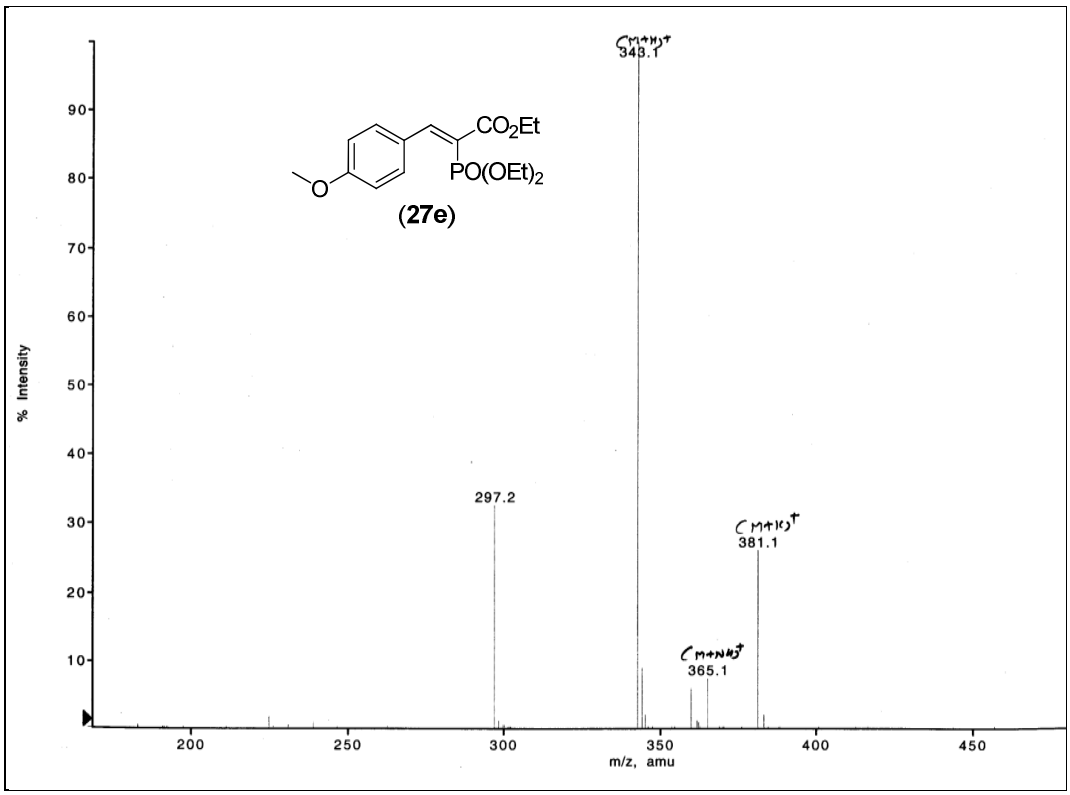
Results obtained with enhanced integrator!

*** End of Report ***

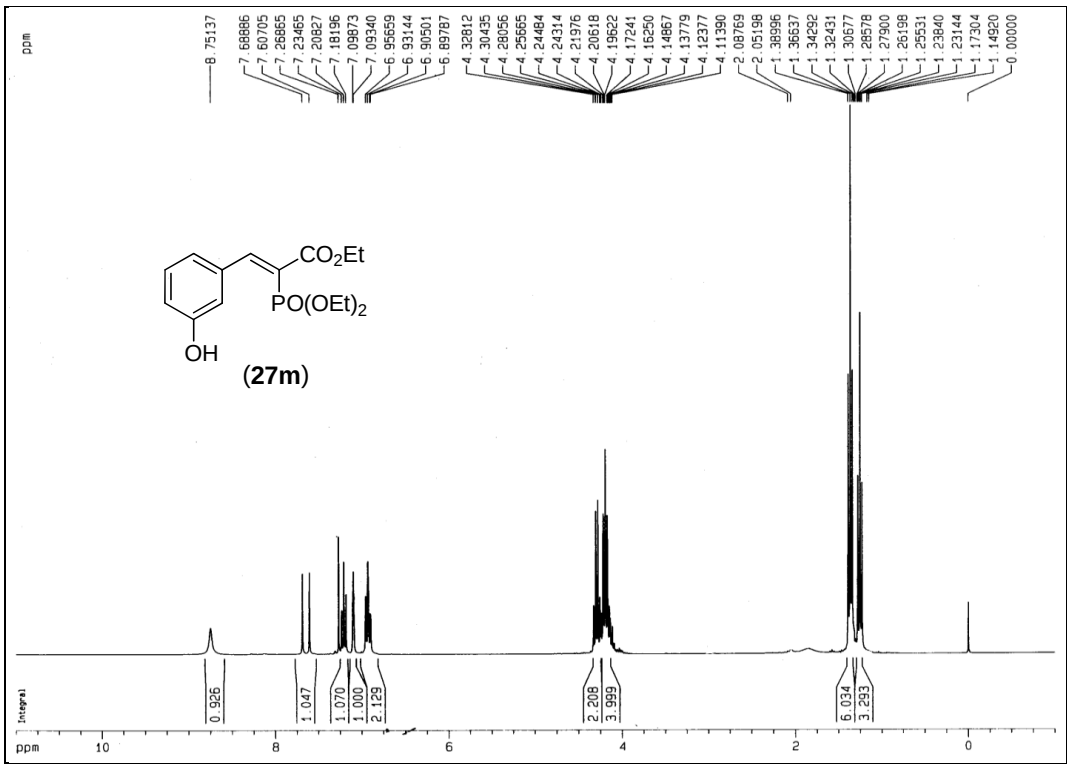
^{1}H NMR of compound **27e**



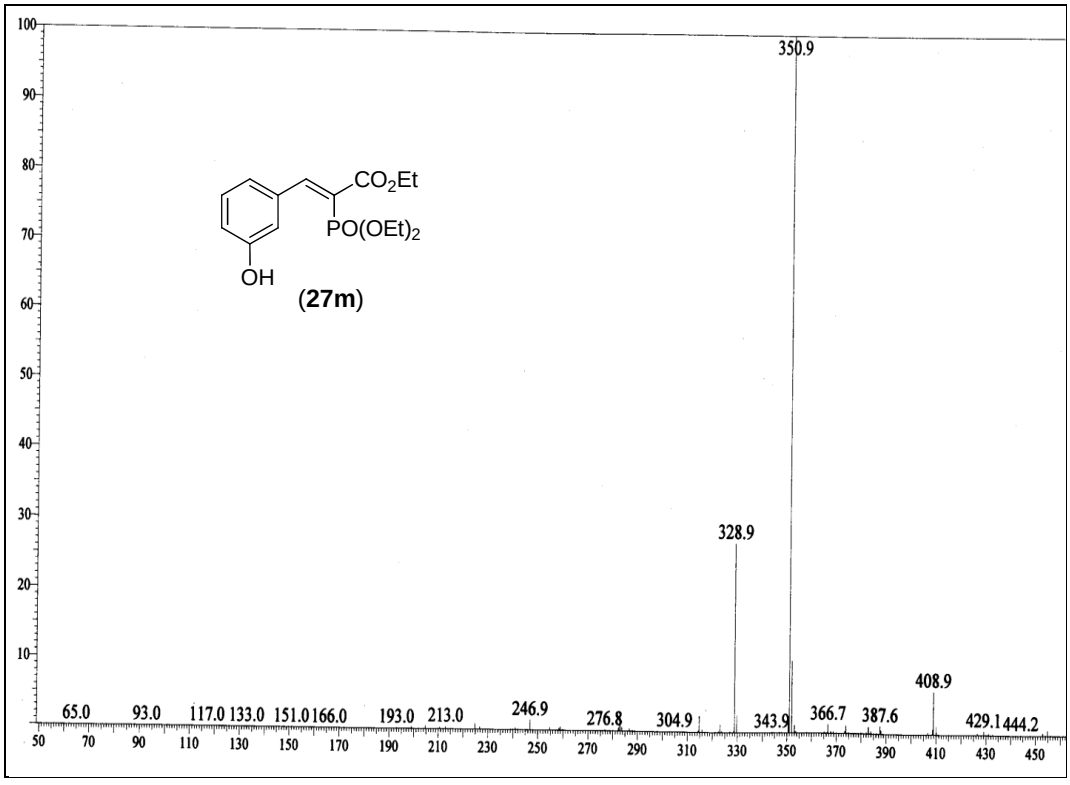
ESI-MS of compound **27e**



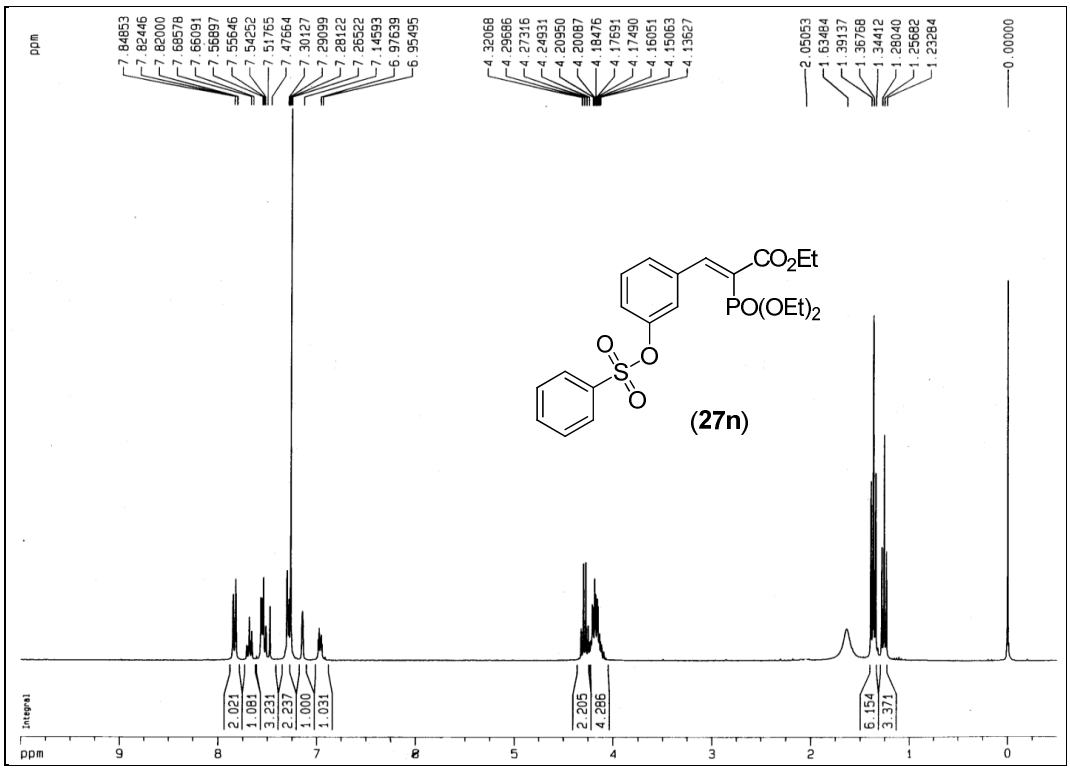
^{1}H NMR of compound **27m**



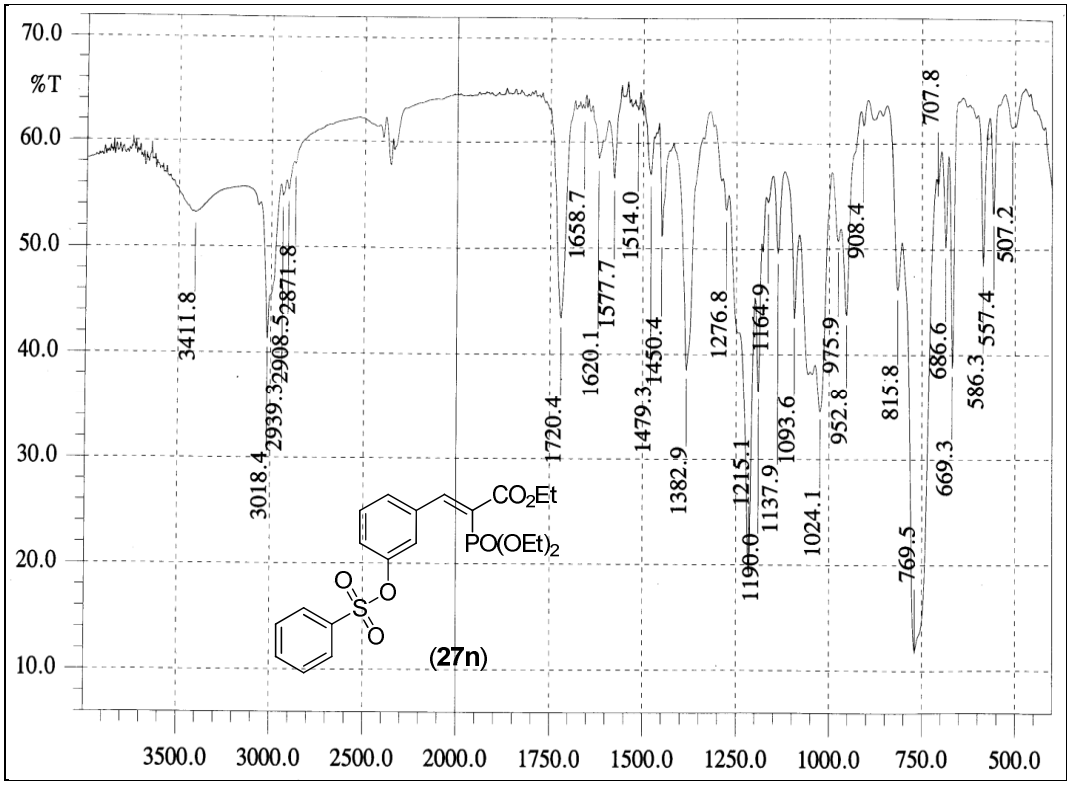
ESI-MS of compound **27m**



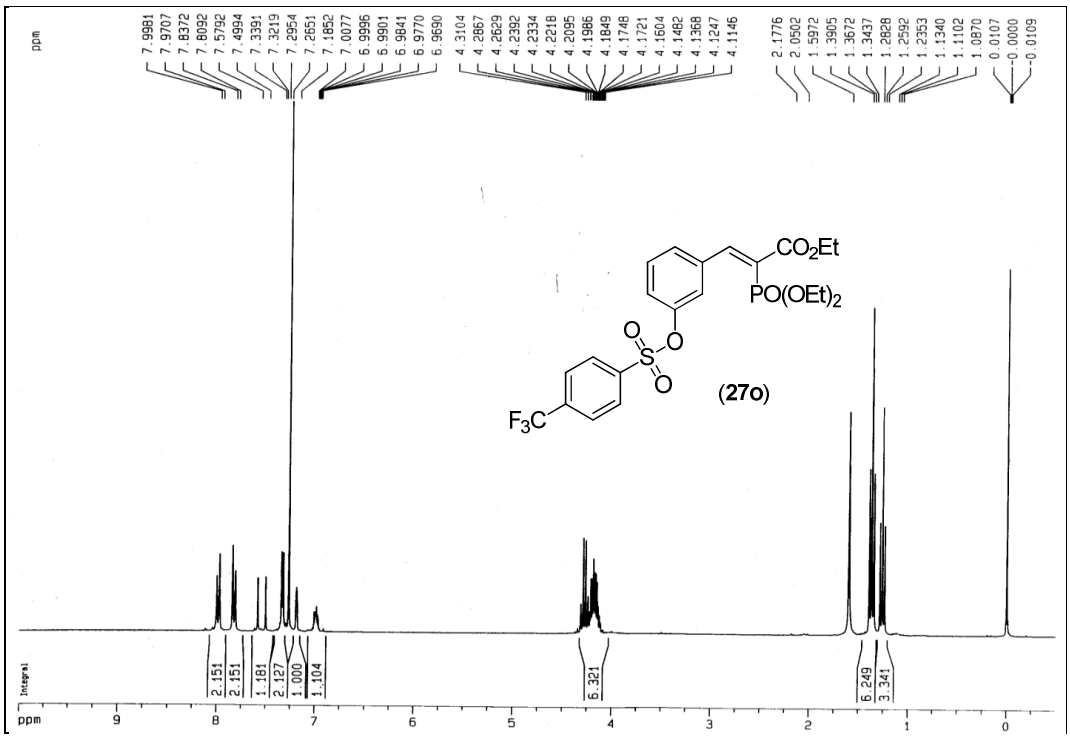
^{1}H NMR of compound **27n**



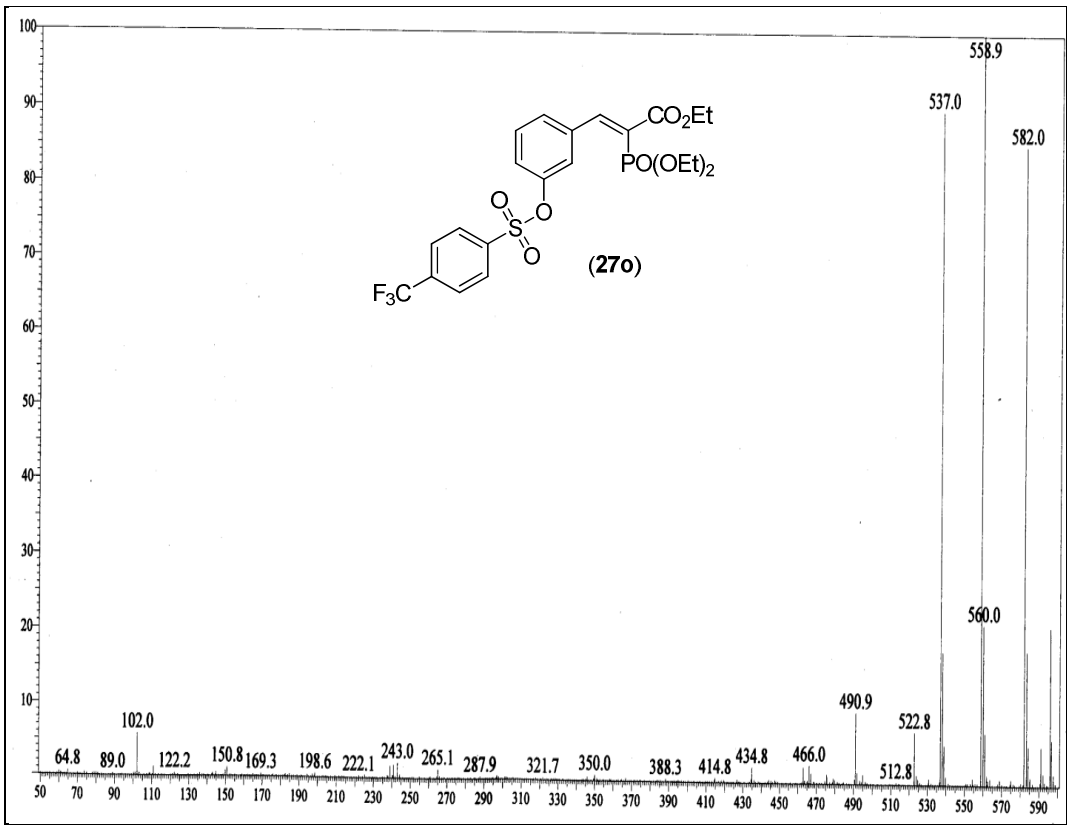
IR of compound **27n**



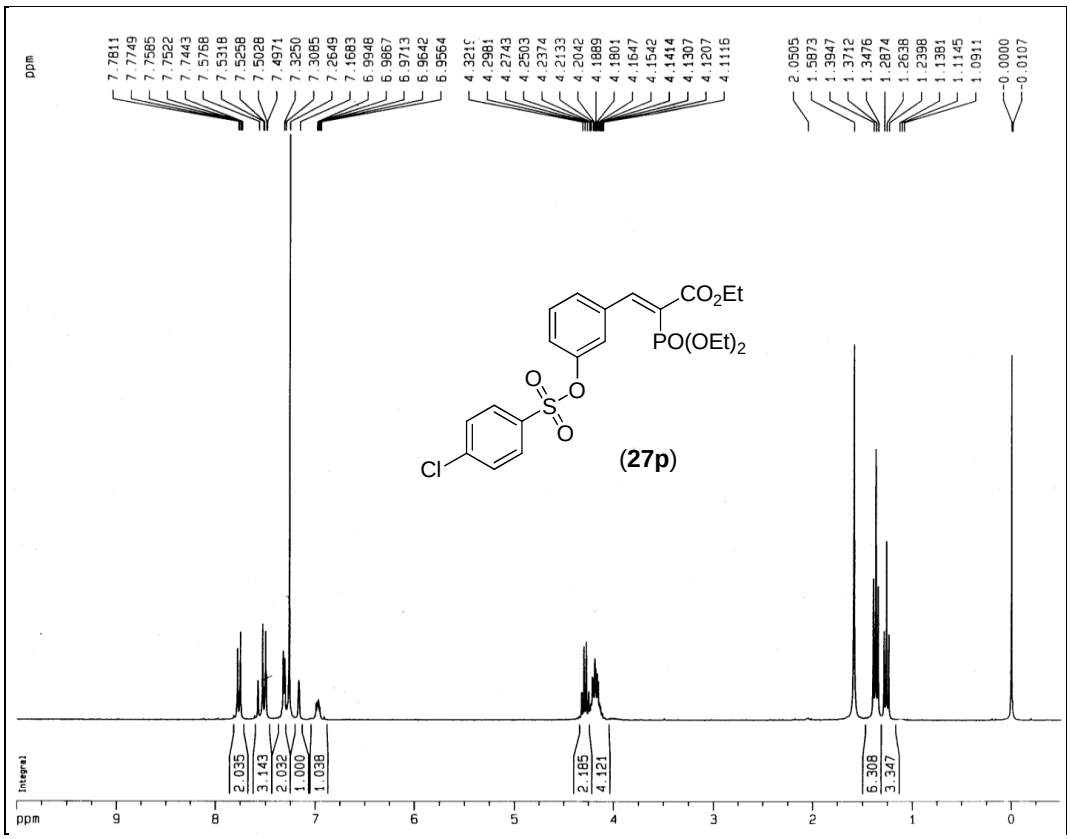
^{1}H NMR of compound **27o**



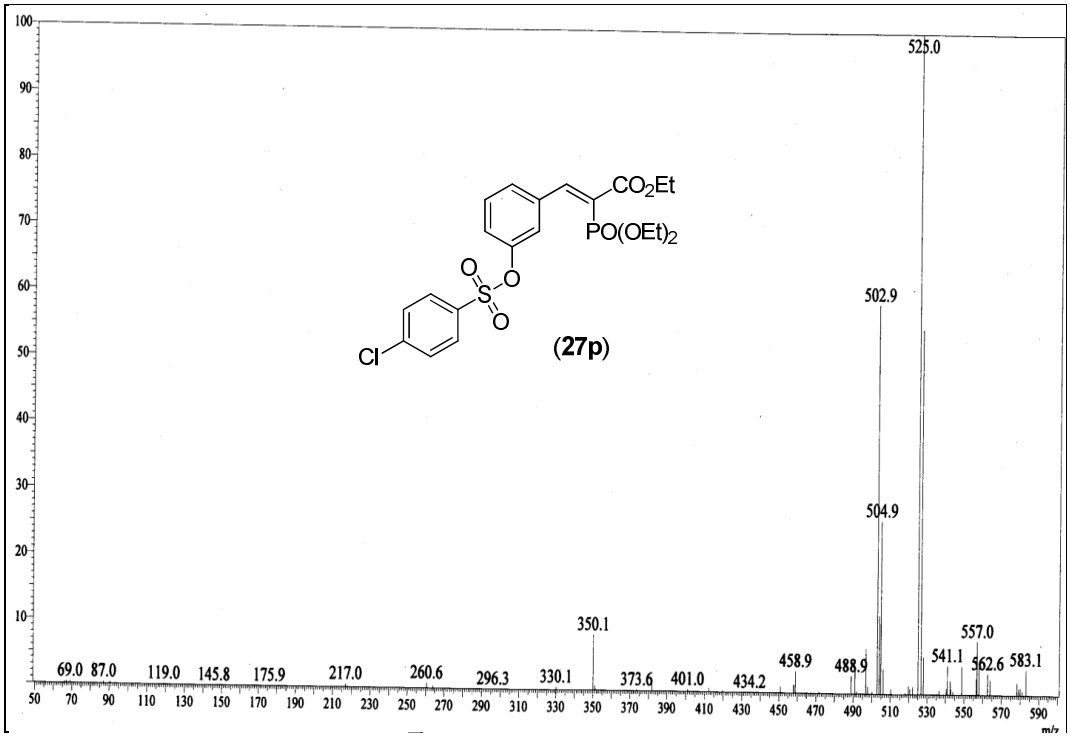
ESI-MS of compound **27o**



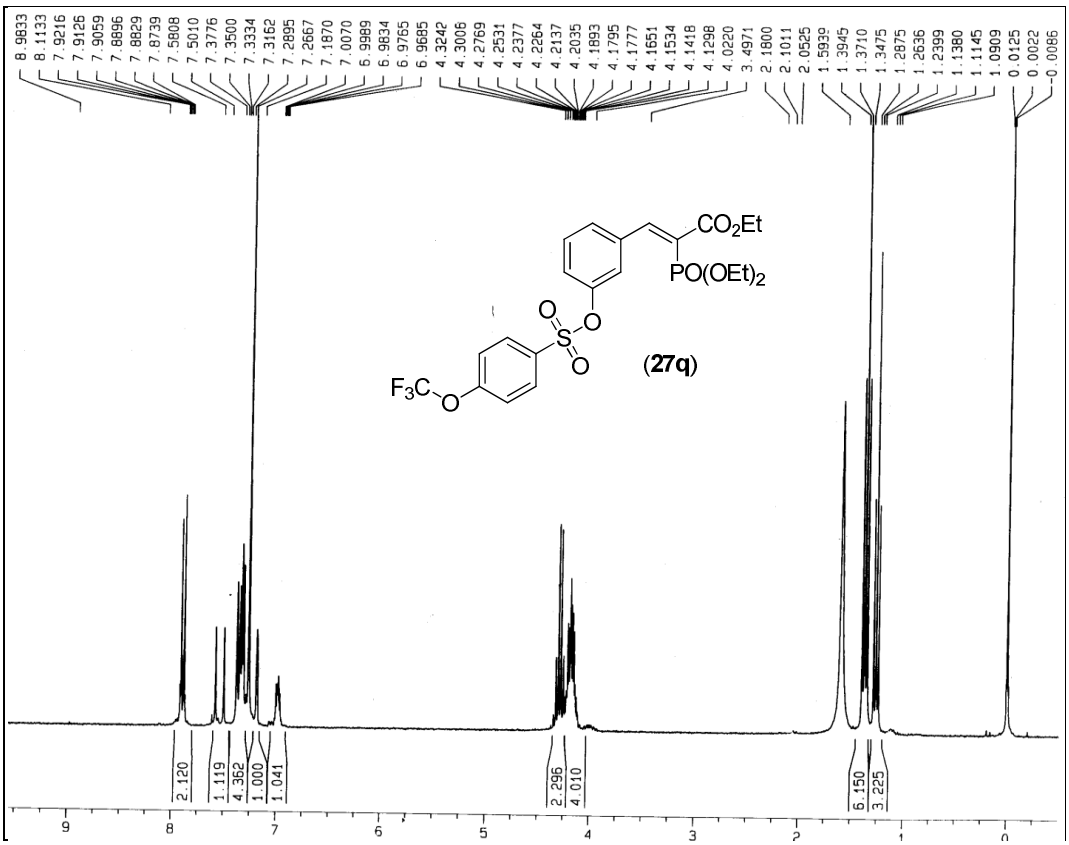
^{1}H NMR of compound **27p**



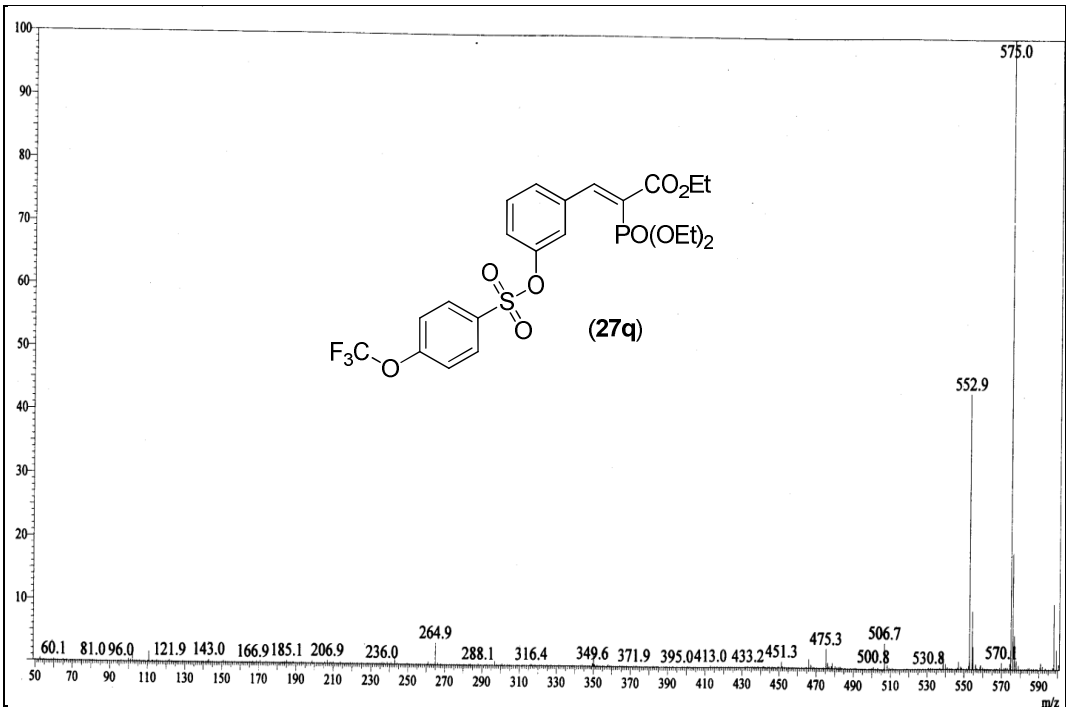
ESI-MS of compound **27p**



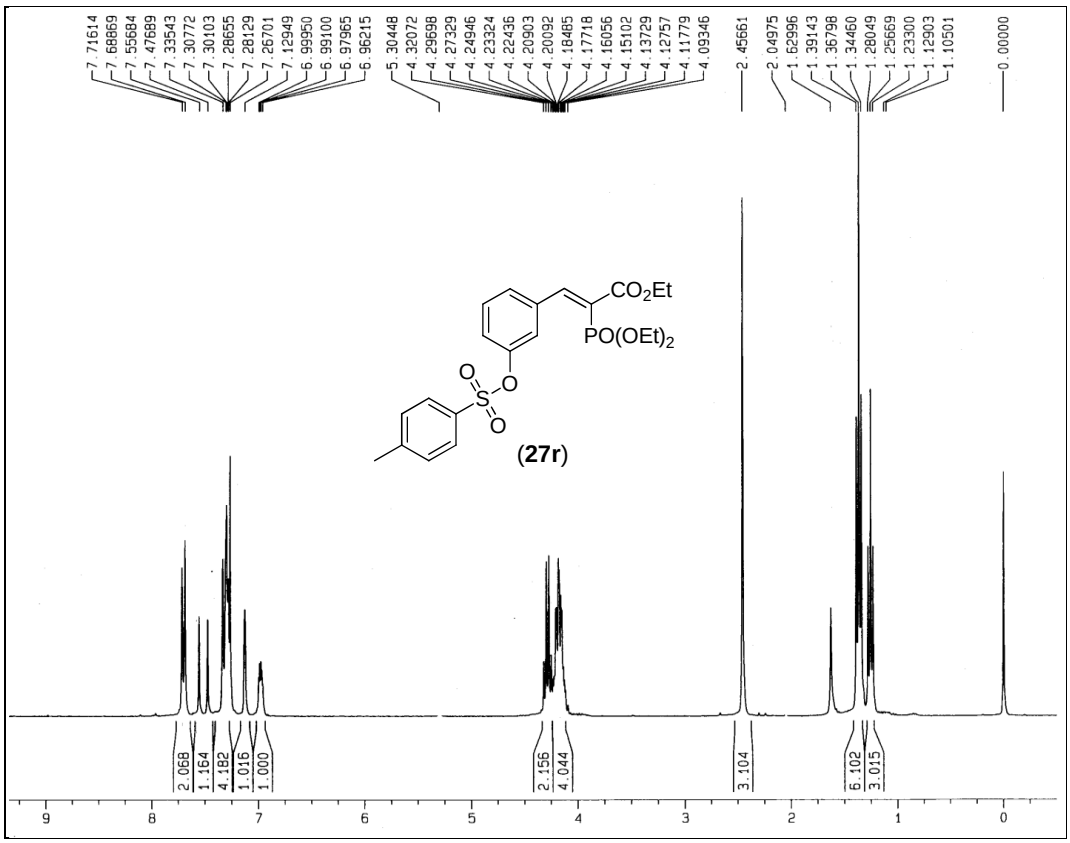
H[1] NMR of compound **27q**



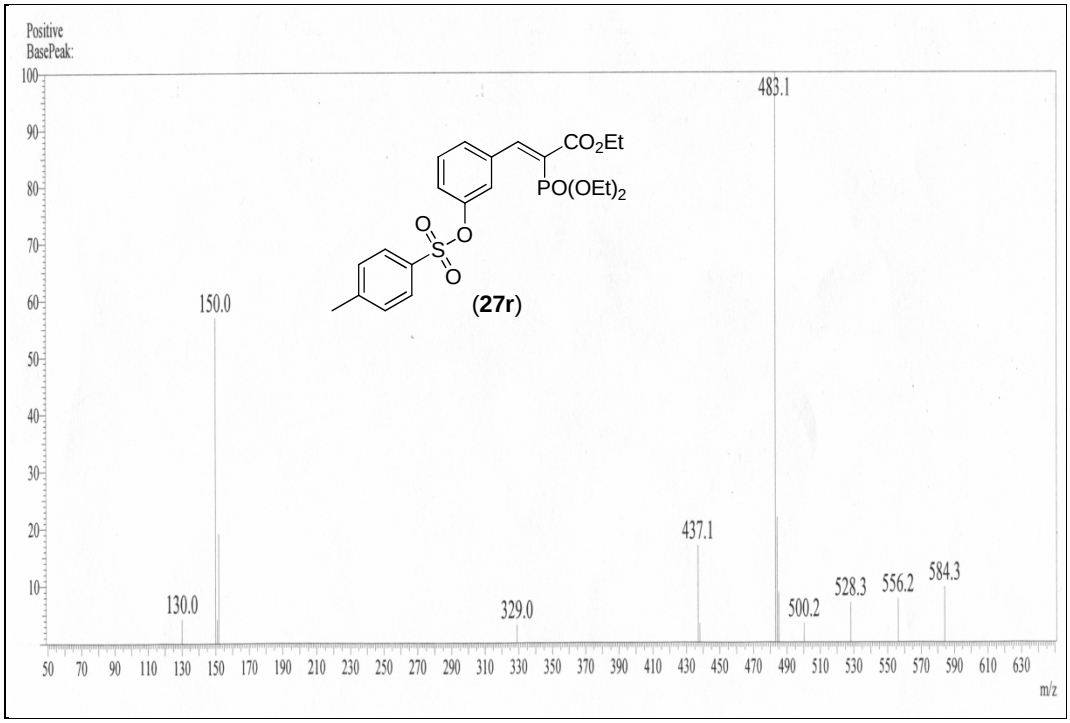
ESI-MS of compound **27q**



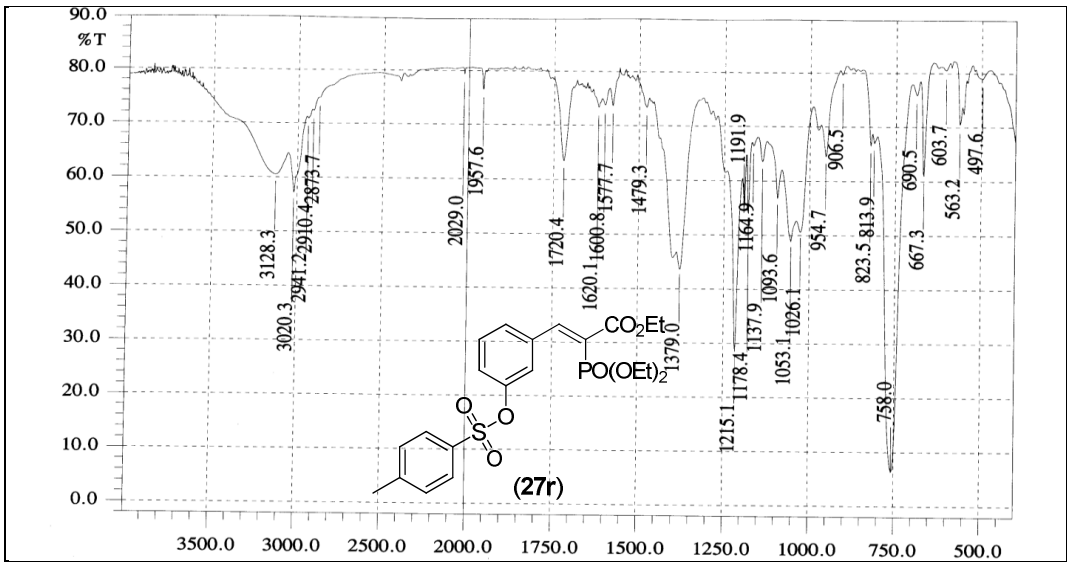
^{1}H NMR of compound **27r**



ESI-MS of compound **27r**

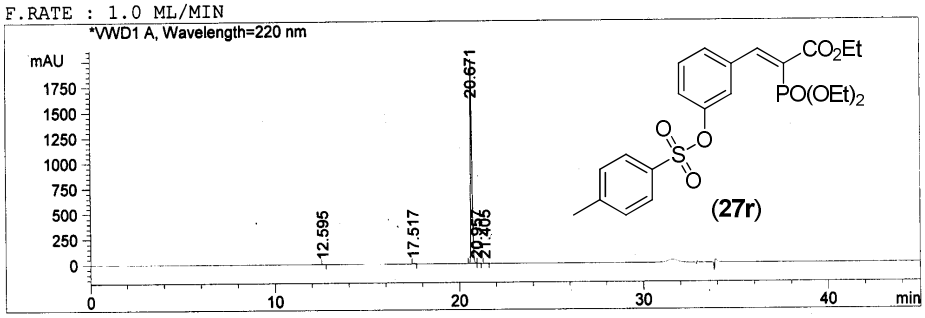


^{1}H NMR of compound **27r**



HPLC of compound **27r**

COLUMN : ODS-150 X4.6 C-18
M.PHASE: 0.05%TFA BUFFER : ACN (GRADIENT)
F.RATE : 1.0 ML/MIN



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

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

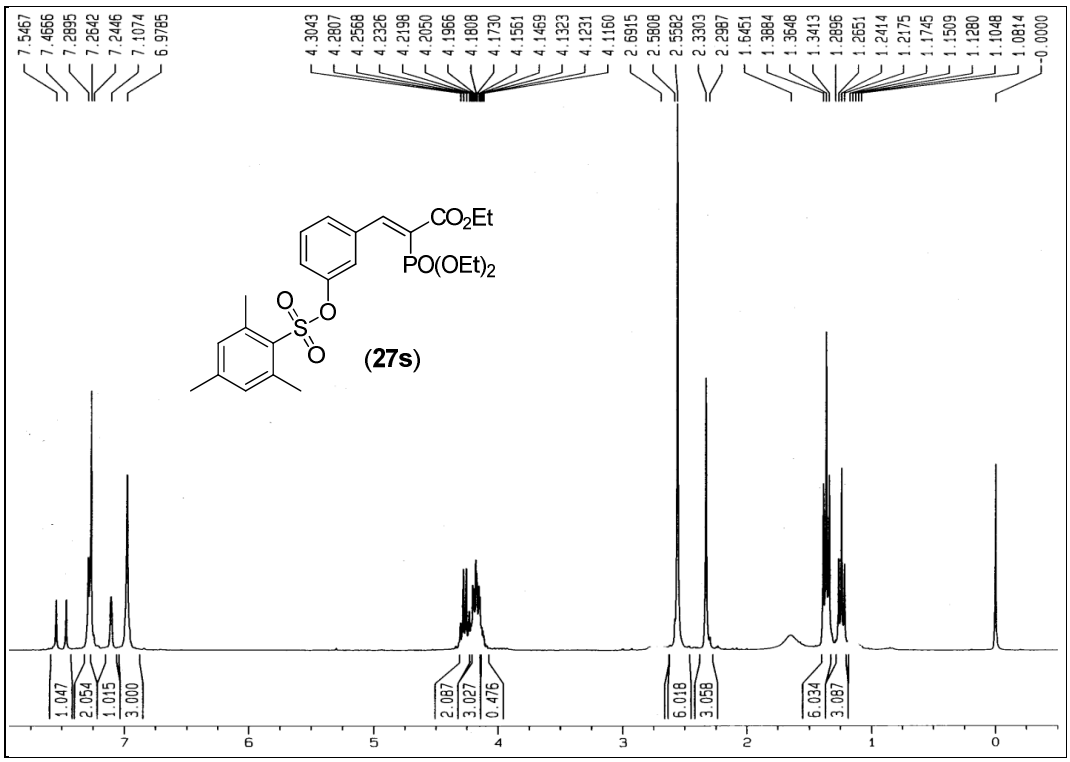
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	12.595	PB	0.0771	1.48880	2.84503e-1	0.0135
2	17.517	BB	0.0775	29.68228	5.91229	0.2693
3	20.671	MF R	0.0909	1.09831e4	2014.07056	99.6439
4	20.957	FM R	0.0575	5.54894	1.60954	0.0503
5	21.405	BB	0.1012	2.53525	4.18541e-1	0.0230

Totals : 1.10224e4 2022.29544

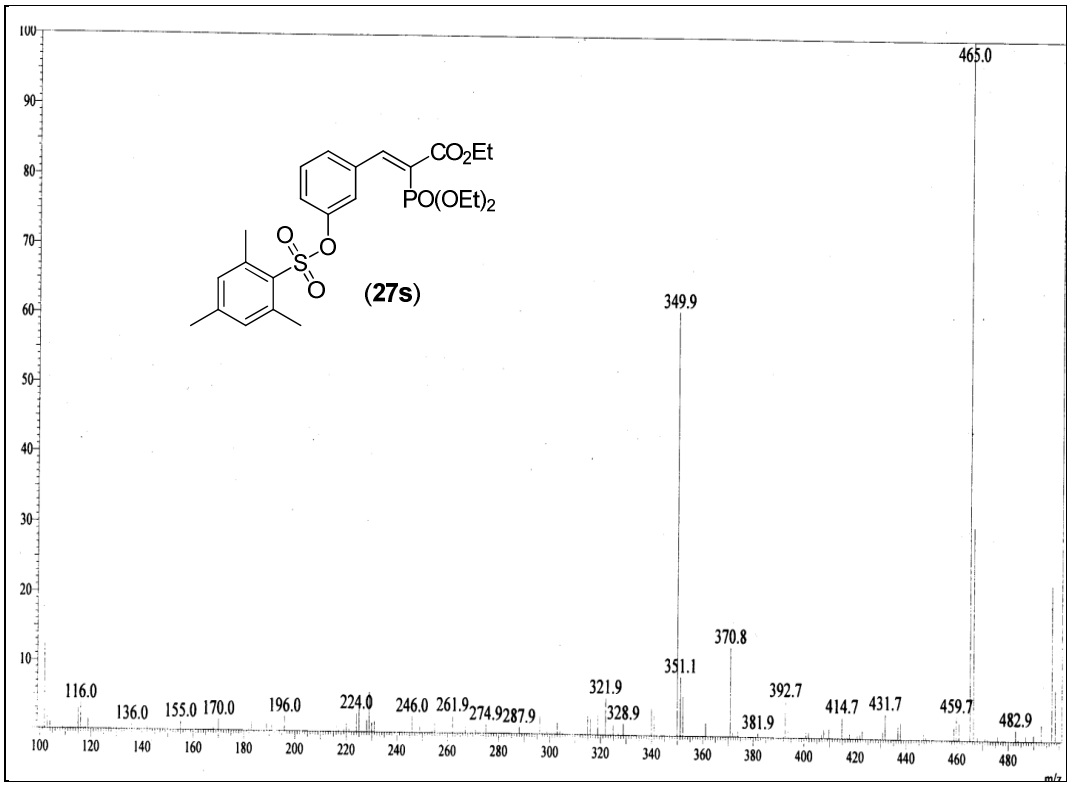
Results obtained with enhanced integrator!

*** End of Report ***

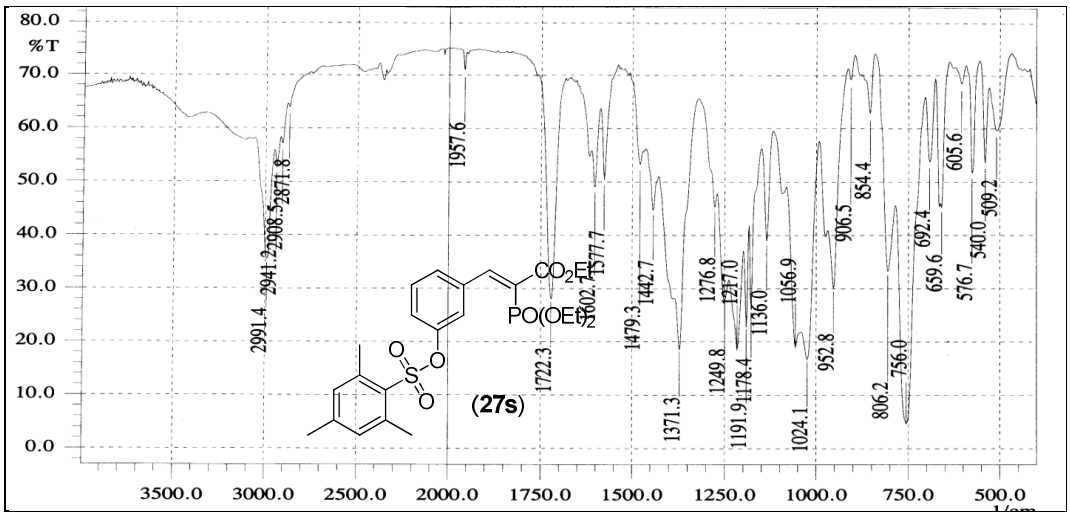
^{1}H NMR of compound **27s**



ESI-MS of compound **27s**

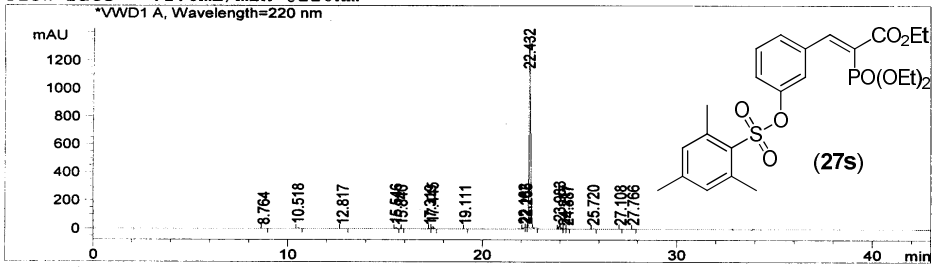


IR of compound **27s**



HPLC of compound **27s**

Column :ODS-J Sphere,150*4.6-4u
Mobile phase:0.05%TFA BUFFER:ACN (Gradient)
Flow rate :1.0mL/min @220nm



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

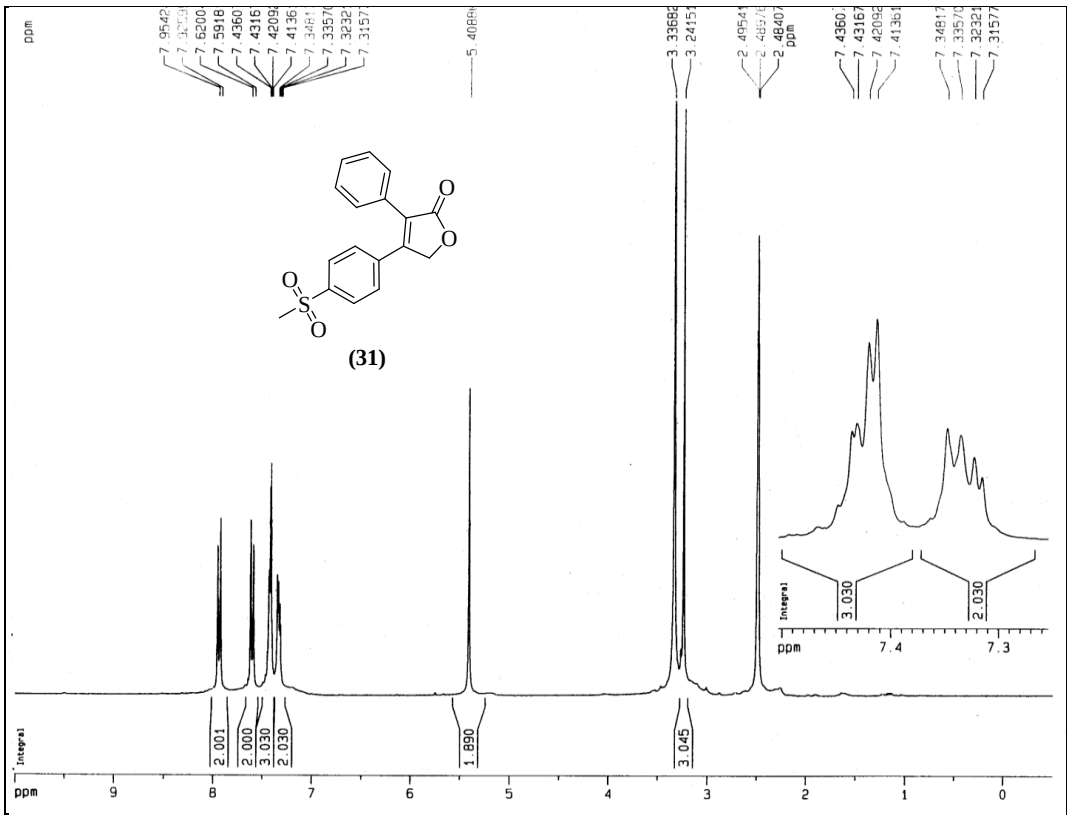
Signal 1: VWD1 A, Wavelength=220 nm
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	8.764	BP	0.1201	3.19454	4.03105e-1	0.0385
2	10.518	BB	0.0796	42.06518	8.09393	0.5069
3	12.817	BP	0.1455	3.52761	3.17934e-1	0.0425
4	15.546	BB	0.0725	39.51075	8.37738	0.4761
5	15.840	BB	0.0656	1.27655	3.09623e-1	0.0154
6	17.319	BV	0.0626	2.54176	6.36398e-1	0.0306
7	17.445	VB	0.0763	88.77635	17.60247	1.0697
8	19.111	BP	0.0765	3.29685	6.67686e-1	0.0397
9	22.162	PV	0.0807	51.58447	9.73741	0.6216
10	22.265	VV	0.0794	65.90494	12.42012	0.7941
11	22.432	VB	0.0885	7792.52002	1363.66907	93.8961
12	23.983	BV	0.0877	162.50165	28.17101	1.9581
13	24.217	VV	0.0880	5.85376	1.03202	0.0705
14	24.387	VBA	0.0807	6.22125	1.20395	0.0750
15	25.720	MM R	0.0961	14.26960	2.47448	0.1719
16	27.108	MM R	0.0770	6.49730	1.40695	0.0783
17	27.766	MM R	0.0918	9.54687	1.73406	0.1150
Totals :				8299.08945	1458.25760	

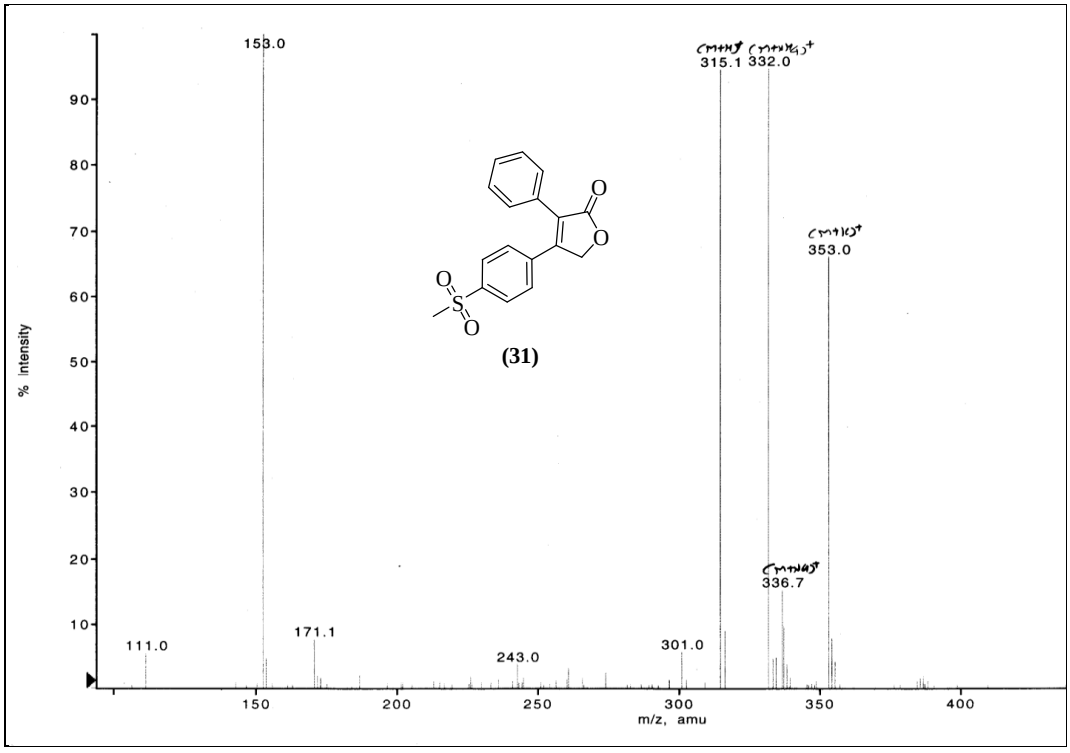
Results obtained with enhanced integrator!

*** End of Report ***

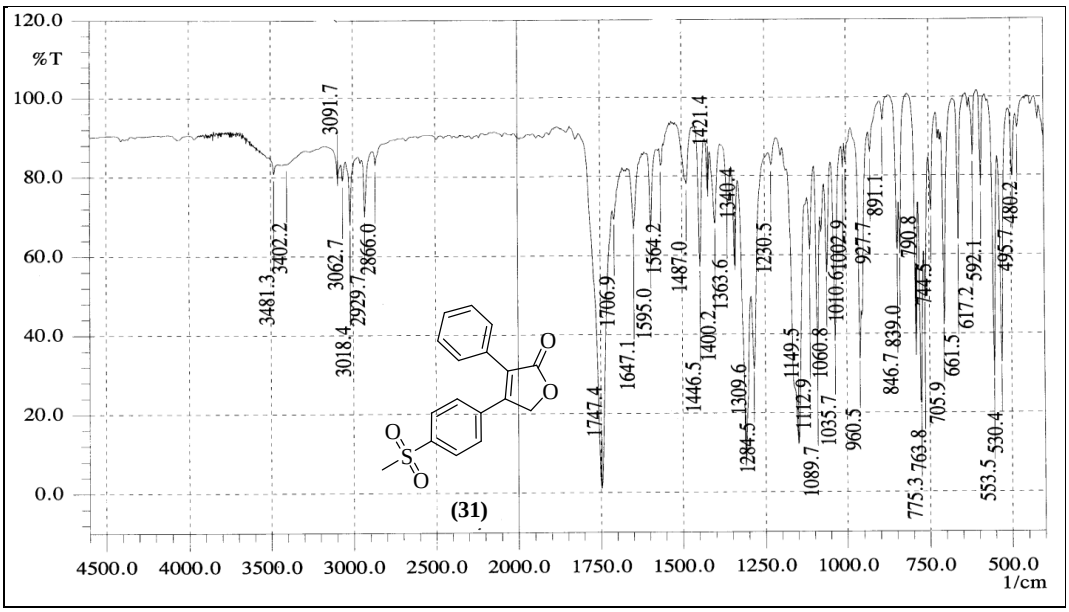
^{1}H NMR of compound **31**



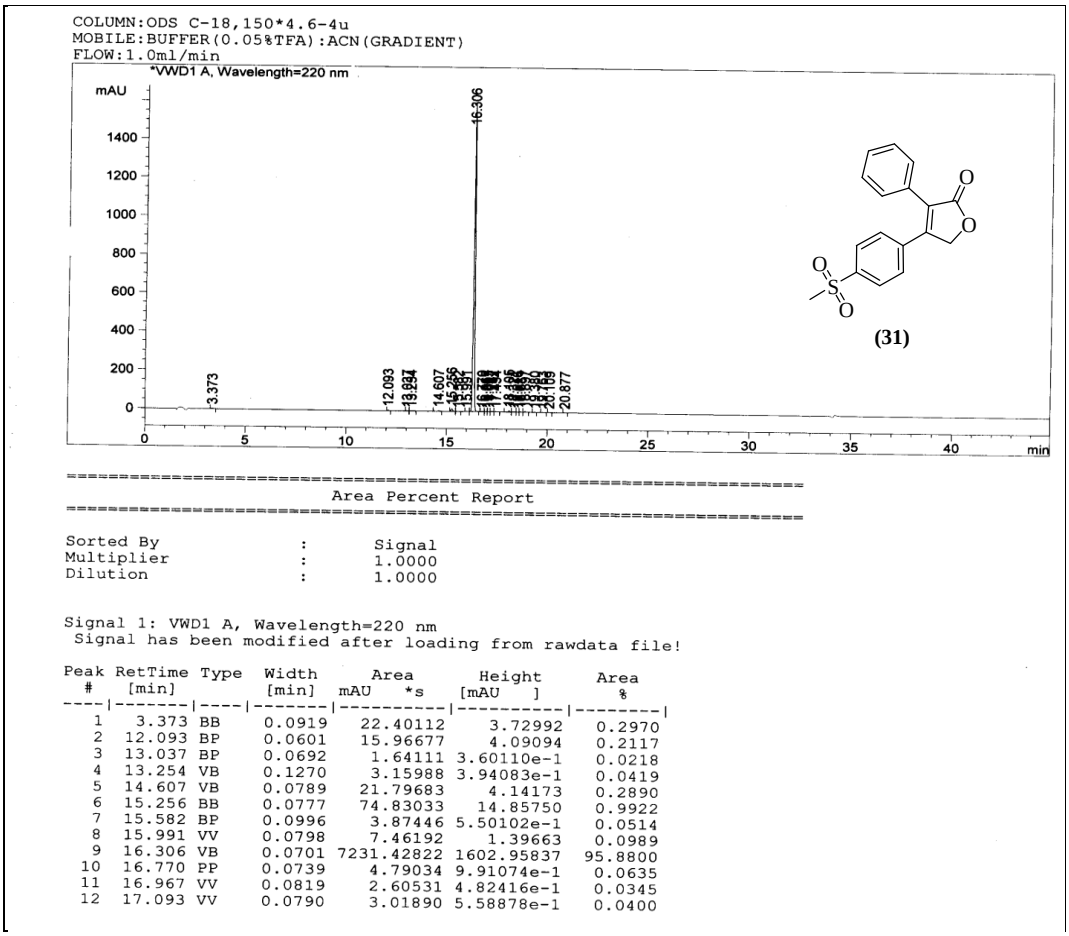
ESI-MS of compound **31**



IR of compound **31**



HPLC of compound **31**



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

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	3.373	BB	0.0919	22.40112	3.72992	0.2970
2	12.093	BP	0.0601	15.96677	4.09094	0.2117
3	13.037	BP	0.0692	1.64111	3.60110e-1	0.0218
4	13.254	VB	0.1270	3.15988	3.94083e-1	0.0419
5	14.607	VB	0.0789	21.79683	4.14173	0.2890
6	15.256	BB	0.0777	74.83033	14.85750	0.9922
7	15.582	BP	0.0996	3.87446	5.50102e-1	0.0514
8	15.991	VV	0.0798	7.46192	1.39663	0.0989
9	16.306	VB	0.0701	7231.42822	1602.95837	95.8800
10	16.770	PP	0.0739	4.79034	9.91074e-1	0.0635
11	16.967	VV	0.0819	2.60531	4.82416e-1	0.0345
12	17.093	VV	0.0790	3.01890	5.58878e-1	0.0400

Publications and Posters

7. Publications and Posters

List of Publications

1. **Anil Argade**, Rajesh Bahekar, Jigar Desai, Pravin Thombare, Kiran Shah, Sanjay Gite, Rajesh Sunder, Ramchandra Ranvir, Debdutta Bandyopadhyay, Ganes Chakrabarti, Amit Joharapurkar, Jogeswar Mahapatra, Abhijit Chatterjee, Harilal Patel, Mubeen Shaikh, Kalapatapu V.V.M. Sairam, Mukul Jain and Pankaj Patel. Design, synthesis and biological evaluation of *γ*-lactam hydroxamate based TACE inhibitors. *Med. Chem. Commun*. **2011**, *9*, 966-972.

2. **Anil Argade**, Jigar Desai, Pravin Thombare, Kiran Shah, Sanjay Gite, Mukul Jain, Pankaj Patel and Rajesh Bahekar. One-pot Synthesis of 3, 4-di aryl Substituted 2(5*H*)-Furanones and its Commercial Application. *Synth. Commun*. **2012**, *42*, 3140-3149.

List of Posters

1. **Anil Argade**, Kiran Shah, Sanjay Gite, Archana Gite, Mukunda Pateker, Gaurang Trivedi, Bhaumin Patel, Praveen kumar singh, Keval Bhambharoliya, Jogeshwar Mohapatra, Rajesh Bahekar, Jigar Desai, Pravin Thombare, Mukul Jain. γ-Lactum hydroxamate based TACE inhibitors. Poster presented in 5th RBF international Symposium, *Advances in Traditional Research and Medicine* held at Zydus Research Centre, Ahmedabad in Feb-1-4, **2011**.

8. Vitae

The author was born on 22nd November, 1976 at Dearde Chandwad, district Ahmednagar, Maharashtra. After obtaining S.S.C. from G. R. Autade High School, Phoegon and H.S.C. from Junior College, Kolpewadi, he joined S.S.G.M College, Kopargaon and obtained B.Sc degree in 1998 and M.Sc degree in 2000 from Pune University. He then joined Medicinal Chemistry Department at Zydus Research Centre in January 2001 where he is currently working as a scientist. The author is co-inventor of the molecule ZYI1 for the treatment of inflammatory for which phase-II clinical trials have been completed successfully.

Email: anilargade2007@rediffmail.com

List of Publications

1. **Anil Argade**, Rajesh Bahekar, Jigar Desai, Pravin Thombare, Kiran Shah, Sanjay Gite, Rajesh Sunder, Ramchandra Ranvir, Debdutta Bandyopadhyay, Ganes Chakrabarti, Amit Joharapurkar, Jogeswar Mahapatra, Abhijit Chatterjee, Harilal Patel, Mubeen Shaikh, Kalapatapu V.V.M. Sairam, Mukul Jain and Pankaj Patel. Design, synthesis and biological evaluation of *γ*-lactam hydroxamate based TACE inhibitors. *Med. Chem. Commun*. **2011**, *9*, 966-972.

2. **Anil Argade**, Jigar Desai, Pravin Thombare, Kiran Shah, Sanjay Gite, Mukul Jain, Pankaj Patel and Rajesh Bahekar. One-pot Synthesis of 3,4-di aryl Substituted 2(5*H*)-Furanones and its Commercial Application. *Synth. Commun*. **2012**, *42*, 3140-3149.

3. Jigar Desai, **Anil Argad**e, Sanjay Gite, Kiran Shah, Laxmikant Pavase, Pravin Thombare & Pankaj Patel. Novel and Efficient Synthesis of *tert*-Butyl-2-(4-(2-aminoethyl) phenylthio)-2-methylpropanoate, a Key Intermediate in the Synthesis of Ureido Thioisobutyric Acid. *Synth. Commun.* **2011**, *41*, 748-753.

4. Pravin Thombare, Jigar Desai, **Anil Argade**, Sanjay Gite, Kiran Shah, Laxmikant Pavase & Pankaj Patel. Novel and Efficient Route for the Synthesis of 4-Aryl-Substituted 2(5H)-Furanones. *Synth.commun*. **2009,** *39*, 2423-2429.

List of Posters

1. **Anil Argade**, Kiran Shah, Sanjay Gite, Archana Gite, Mukunda Pateker, Gaurang Trivedi, Bhaumin Patel, Praveen kumar singh, Keval Bhambharoliya, Jogeshwar Mohapatra, Rajesh Bahekar, Jigar Desai, Pravin Thombare, Mukul Jain. γ-Lactum hydroxamate based TACE inhibitors. Poster presented in 5th RBF international Symposium, *Advances in Traditional Research and Medicine* held at Zydus Research Centre, Ahmedabad in Feb-1-4, **2011**.

2. Jigar Desai, **Anil Argade**, Kiran Shah, Sanjay Gite, Laxmikant Pavase, Pravin Thombare, Prasenjit Mitra, Mukul R. Jain and Pankaj R. Patel N-(4-*Tert*-butyl benzyl)-N-phenylethyl amine, A Novel Class of CETP Inhibitors. Poster presented in 4th RBF international Symposium, *Advances in Cardiometabolic Research-Basic Science and Clinical Aspects* held at Zydus Research Centre, Ahmedabad in Feb-2-5, **2009**.

3. Sanjay Gite, Jigar Desai, **Anil Argade**, Kiran Shah, Jogeswar Mahapatra Mukul Jain, Pravin Thombare. Synthesis and Activity of 5-Substituted Thiadiazole Class of Compounds as TACE and MMP Inhibitors. Poster presented in 3rd RBF international Symposium, *Advances In Diabetes Therapy-Basic Science and Clinical Aspects* held at Zydus Research Centre, Ahmedabad in Feb-1-4, **2007**.

4. Kiran Shah, Jigar Desai, **Anil Argade**, Sanjay Gite, Laxmikant Pavase, Jogeswar Mahapatra, Mukul R Jain and Pravin Thombar. Synthesis and activity of 4, 5-

Diaryl Thiazole Class of Compounds as TACE and MMP Inhibitors. Poster presented in 3rd RBF international Symposium, *In Diabetes Therapy-Basic Science and Clinical Aspects* held at Zydus Research Centre, Ahmedabad in Feb-1-4, **2007**.

5. **A. D. Argade**, Pravin Thombare, V. B. Lohray. Novel Synthesis of 2(5H)-furanone. Poster presented in 2nd RBF international Symposium, *Genomics and proteomics* held at Zydus Research Centre, Ahmedabad in Jan-23-25, **2005**.

6. P. S. Thombare, **A. D. Argade**, K. Y. Shah, V. B. Lohray, J. Mohapatra, V. M. Kanoje, B. R. Patel and M. R. Jain. Novel alkoxy-phosphonate and carboxy-phosphonate derivatives as anti-inflammatory agents. Poster presented at *RBF 1st international symposium on recent trends in Pharmaceutical Sciences* held at Zydus Research Centre, Ahmedabad in Jan 23-24, **2003**.

7. P. S. Thombare, **A. D. Argade**, K. Y. Shah, V. B. Lohray, J. Mohapatra, V. M. Kanoje, B. R. Patel and M. R. Jain. Novel alkoxy-phosphonate and carboxy-phosphonate derivatives as anti-inflammatory agents. Poster presented at *RBF 1st international symposium on Recent Trends in Pharmaceutical Sciences* held at Zydus Research Centre, Ahmedabad in Jan 23-24, **2003**.

8. Srinivas Kone, H. K. Jajoo, Manish Jain, **Anil Argade**, S. K. Shah. Development of chiral HPLC methods for examining enzymatic resolution of racemic methyl 2-hydroxy-3-(4-benzyloxyphenyl) propionate. Poster presented at *RBF 1st international symposium on Recent trends in Pharmaceutical Sciences* held at Zydus Research Centre, Ahmedabad in Jan 23-24, **2003**.