DESIGN AND SYNTHESIS OF NOVEL COMPOUNDS FOR THE TREATMENT OF THROMBOTIC AND RELATED CARDIOVASCULAR DISORDERS

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BY

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CERTIFICATE

This is to certify that the thesis entitled "**DESIGN AND SYNTHESIS OF NOVEL COMPOUNDS FOR THE TREATMENT OF THROMBOTIC AND RELATED CARDIOVASCULAR DISORDERS**" 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. Vrajeshkumar B. Pandya** under my supervision and guidance at Zydus Research Centre, Ahmedabad and the work embodied in this thesis has not formed earlier the basis for award of any degree or similar title of this or any other university or examining body.

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DECLARATION

I hereby declare that the thesis entitled "**DESIGN AND SYNTHESIS OF NOVEL COMPOUNDS FOR THE TREATMENT OF THROMBOTIC AND RELATED CARDIOVASCULAR DISORDERS**" submitted herewith to The Maharaja Sayajirao University of Baroda, Vadodara of the fulfillment for the award of the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY** is the result of the work carried out by me in Chemistry Department of Zydus Research Centre, Ahmedabad. The result of this work has not been previously submitted for any degree/fellowship to any university or institution.

Date:

Place: Ahmedabad **Vrajesh Pandya**

Dedicated to

Late Shri. Ramanbhai B. Patel

Founder of Zydus Cadila

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 contains research work in the area of thrombotic disorders which includes design and synthesis of novel compounds and their evaluation for its usefulness in the treatment and prevention of this diseases. The whole research work of this thesis is divided in four major sections. The work has been shared to the scientific community by publishing three papers in international journals.

The **'Introduction'** section deals with the general information about thrombotic disorders, pathophysiology and etiology of thrombosis, current therapeutic options and unmet needs. It also describes theoretical evaluation of various therapeutic targets and finally selection of PAI-1 and coagulation Factor Xa as a targets for my research endeavor.

The **'Plasminogen Activator Inhibitor-1 (PAI-1) inhibitors'** section contains designing strategy, chemistry and results & discussion of novel PAI-1 inhibitors.

The **'Coagulation Factor Xa (FXa) inhibitors'** section describes designing strategy, chemistry and results & discussion of novel FXa inhibitors. It also describes new and efficient synthesis of Dibenzothiophenes, a sulfur containing heterocycle found in many natural products and also used in synthesis of novel FXa inhibitors in this thesis.

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.

Copy of spectra of selected compounds and their intermediates are incorporated at the end of the thesis.

Working for this thesis has been a great learning experience for me. Literature search helped me to strengthen my knowledge about thrombosis and its life threatening complications. It gave me clear idea of what type of treatments are available for patients with thrombotic disorders and how I can contribute in improving current treatment options.

Designing and synthesizing novel inhibitors of two distinct therapeutic targets, but for one cause was very interesting and at the same time very challenging. Recognition of the work by the scientific community in the form of publications was highly motivating.

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 thrombotic disorders and treatment options are good but not adequate enough and hence every endeavor in the direction of developing novel therapies in this area would be a significant contribution towards alleviating human suffering.

Vrajesh Pandya

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Last but not the least, my family for their unconditional love and support, which has made this journey smoother, exciting and enjoyable.

Vrajesh Pandya

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AF	Atrial fibrillation
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
aPC	Activated protein C
aPL	Anionic phospholipids
aPTT	Activated partial thromboplastin time
AT	Antithrombin
ATE	Arterial thromboembolism
CCL-2	Chemokine (C-C motif) ligand 2
CVD	Cardiovascular disease
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CYP3A4	Cytochrome P-450 3A4
DIC	Disseminated intravascular coagulation
DVT	Deep vein thrombosis
ECM	Extracellular matrix
EGF	Epidermal growth factor
FII	Factor II
FV	Factor V
FVII	Factor VII
FVIII	Factor VIII
FIX	Factor IX
FX	Factor X
FXa	Activated Factor X
FXI	Factor XI
FXIII	Factor XIII
HIT	Heparin induced thrombocytopenia

IL	Interleukin
LMWH	Low molecular weight heparin
MCP-1	Monocyte chemotactic protein-1
MMP	Matrix metalloproteinase
NF-κB	Nuclear factor kappa-B
PA	Plasminogen activator
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease-activated receptor
PCI	Percutaneous coronary intervention
PDE-3	Phosphodiesterase-3
PE	Pulmonary embolism
PTCT2	Concentration required to double prothrombin time
RCL	Reactive center loop
SK	Streptokinase
STEMI	ST-elevated myocardial infarction
TAFI	Thrombin-activatable fibrinolysis inhibitor
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TM	Thrombomodulin
tPA	Tissue plasminogen activator
TxA2	Thromboxane A2
UFH	Unfractionated heparin
UK	Urokinase
uPA	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor
VKA	Vitamin K antagonist
VTE	Venous thromboembolism
vWF	Von Willebrand factor
ZPI	Z-dependent protease inhibitor

Introduction

1. INTRODUCTION

1.1. Thrombosis

The blood coagulation or haemostasis is a physiological process by which body prevents blood loss by forming stable clot at the site of injury. The clot which forms at unwanted place and blocks the blood flow is known as thrombus and the phenomenon is known as thrombosis.

1.1.1. Haemostasis

Historical perspectives

Curiosity to understand blood clotting among ancient researchers led to many theories. Greek philosophers and physicians were the first to observe and debate about the possible mechanisms of blood clotting. Plato in *Omnia divini Platonis opera*, Hippocrates in *De Carnibus* and Aristotle in *Meteorology* mentioned that this effect was resulting from the drop in temperature when blood leaves the body. In the 18th century, physicians became aware that blood clotting is a natural mechanism to prevent the blood loss from an injury. In the 1730s, a French surgeon named Jean-Louis Petit first recognized that bleeding after amputation was controlled by clotting **[1]**. In 1790, John Hunter suggested that exposure to air was the underlying mechanism.

Current understanding of haemostasis

The process of haemostasis occurs in two stages: primary and secondary. Primary haemostasis involves blood vessel constriction through smooth muscle contractions to reduce the blood flow surrounding the site of injury. This reduction

in flow rate allows circulating platelets to adhere at the site of injury, creating an unstable platelet plug that temporarily blocks the cut in the vessel wall restricting the blood loss. In secondary haemostasis, this platelet plug is stabilized by initiation of the coagulation cascade, a series of enzymatic activations of coagulation factors, that ultimately result in the formation of a stable fibrin blood clot at the site of injury **[2**, **3]**.

The modern concept of coagulation that explains this secondary haemostasis was presented in 1964 as the Waterfall/Cascade model **[4]**, which involves two separate pathways known as intrinsic pathway and extrinsic pathway. Both pathways initiated independently with different blood coagulation factors converging at the point of coagulation Factor Xa (FXa), whose role is to initiate common pathway leading to the fibrin (clot) formation. However, it has become clear that these pathways do not function in the body as independent systems. An increasing understanding of the role of different factors and cells involved in blood coagulation has led to a cell surface-based model, which suggested that intrinsic and extrinsic pathways are not independent but occur simultaneously on platelet surface. It involves three overlapping phases: Initiation, amplification, and propagation (**Figure 1**) **[5]**.

The initiation phase is localized to TF-bearing cells (TF = Tissue factor, a transmembrane glycoprotein with MW ~ 47 kDa) that are exposed from the subendothelial tissue along with anionic phospholipids (aPL) to flowing blood upon vascular injury. Tissue factor, being a cellular receptor for the plasma factor VII/VIIa (FVII/FVIIa) forms a proteolytic TF/FVIIa complex to activate small

amounts of factor IX (FIX) to FIXa and FX to FXa **[6]**. FXa then activates factor V (FV) on the TF-bearing cells and then associates with FVa to form the prothrombinase complex comprising also a calcium and aPL. FVa derives from several sources, including activated platelets adhering at injury sites, as well as from plasma, where FV can be activated by FXa. The prothrombinase complex then cleaves prothrombin (FII) to generate small amounts of thrombin (FIIa), the final coagulation enzyme responsible for clot formation. FXa can cleave prothrombin in the absence of FVa, aPL and calcium, however within the prothrombinase complex, FXa mediated activation of prothrombin to thrombin is accelerated approximately 300,000-fold **[7]**. FIXa then moves in the fluid phase from TF-bearing cells to nearby platelets at the injury site.

In the amplification phase, this small amount of thrombin activates platelets, leading to release of factor VIIIa (FVIIIa) from factor VIII-von Willebrand factor (vWF, a large multimeric glycoprotein **[8]**) complexes on the platelet surfaces and also generation of FVa and FXIa. Thrombin also activates FXI bound to platelets whose role can be considered as a booster of FIXa production on the platelet surface and thus increases thrombin generation.

In the final propagation phase, the phospholipid surface of activated platelets acts as a cofactor for the activation of the FVIIIa-FIXa complex (termed ‘Xase”) and of the FVa-FXa complex (‘prothrombinase’), which accelerates the generation of FXa and thrombin, respectively. In addition, FXIa bound to the platelet surface activates FIX to form more Xase. FXa, thus produced, associates rapidly with FVa on the platelet surface, resulting in a burst of thrombin.

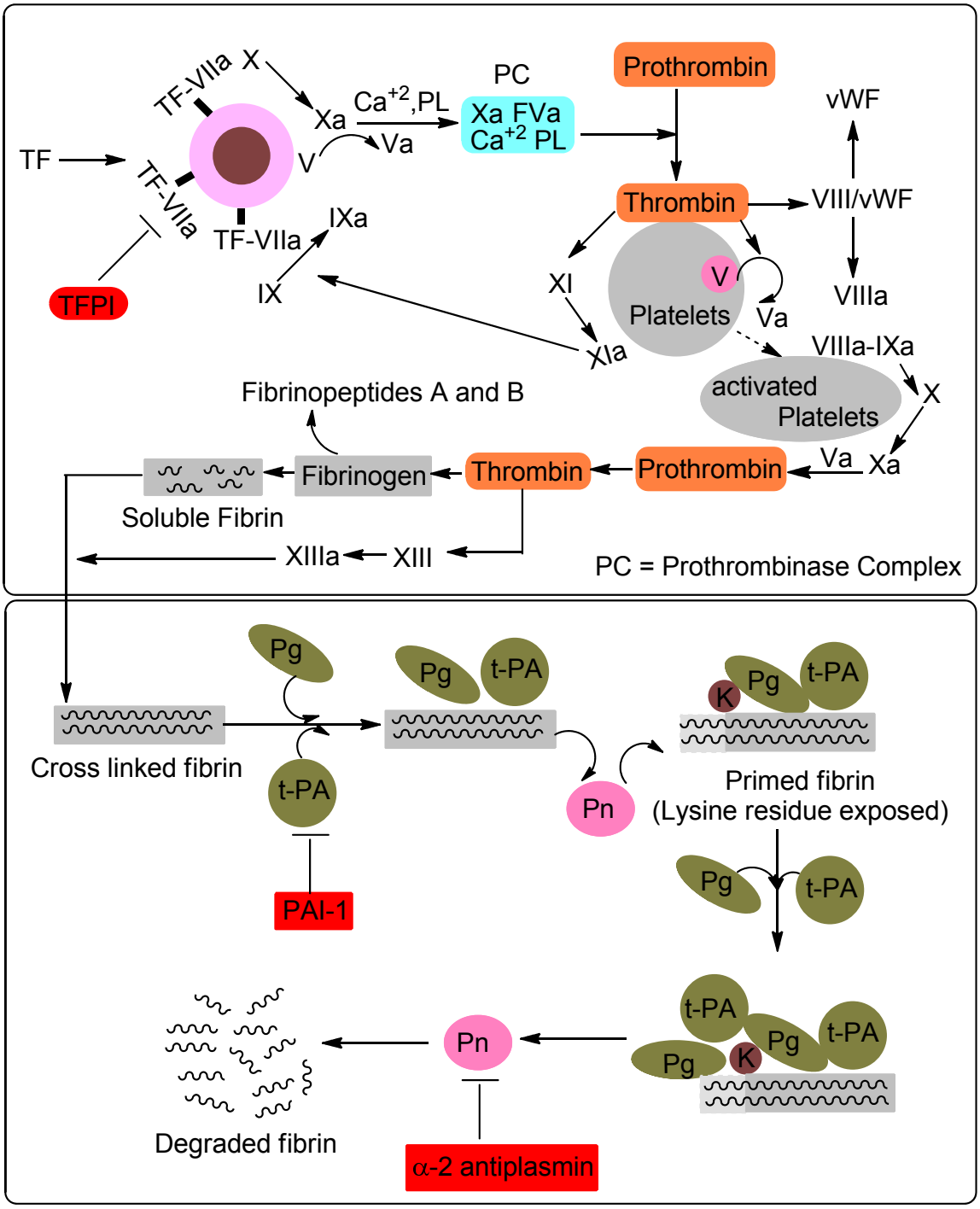

Figure 1. Cell surface-based model for coagulation (Upper half) and fibrinolysis pathway (Lower half)

Thrombin thus generated converts circulating fibrinogen monomers (produced by the liver) to soluble fibrin by removing the fibrinopeptides A and B

from fibrinogen. This soluble fibrin then polymerizes to form a cross linked fibrin by the cross-linking enzyme factor XIIIa (FXIIIa) activated by thrombin **[9]**.

All blood coagulation factors are serine proteases except FV (glycoprotein), FVIII (glycoprotein) and FXIII (transglutaminase). They all are secreted as inactive zymogen and sequentially activated by other serine proteases. The serine proteases form a class of proteolytic enzymes characterized by the presence of an active site, the so-called “catalytic triad” consisting of serine195, aspartic acid102 and histidine57, which is involved in peptide bond cleavage of other proteins or substrates **[10]**.

Regulation of haemostasis

Regulation of the coagulation cascade occurs at different stages. Antithrombin (AT, a 432aa glycoprotein produced by the liver) is the most important physiological inhibitor of the coagulation cascade. Its anticoagulant activity is focused on the regulation of thrombin, FXa and FIXa **[11]**. The principle inhibitor of TF-initiated coagulation is tissue factor pathway inhibitor (TFPI, a 276aa Kunitz-type protease inhibitor with MW = ~42 kDa), which binds and inhibits TF-factor VIIa complex **[12]**. A third inhibitor of the coagulation cascade is activated protein C (aPC), which is activated by thrombomodulin (TM, a transmembrane molecule expressed on endothelial cells)-thrombin complex and its role is to catalyze the inactivation of coagulation FV(a) and FVIII(a) in the presence of its cofactor protein S **[13]**.

1.1.2. Fibrinolysis

Fibrinolysis is the physiological breakdown of fibrin to limit and resolve blood clot formed due to activation of haemostasis pathway, thus restoring normal blood flow to that area. There is a delicate balance between the coagulation cascade, favoring clot formation, and the fibrinolytic system, favoring clot lysis and these processes occur simultaneously.

The fibrinolytic system consists of a proenzyme plasminogen (Pg, an enzyme secreted by the liver as ~92 kDa glycoprotein) that can be converted into the active enzyme plasmin (**Figure 1**). Plasmin is responsible for dissolution of the fibrin network in stable blood clots resulting in soluble fibrin degradation products and therefore plays a very important role in restoring normal blood flow **[14]**. Besides the degradation of fibrin, plasmin is also responsible for the conversion of latent metalloproteases (pro-MMPs) into active MMPs, which in turn degrade the extracellular matrix (ECM) **[15]**.

In the first phase of fibrinolysis, fibrin acts as a cofactor for the enzyme tissue-type plasminogen activator (t-PA, a serine protease secreted by endothelial cells as 530aa protein) by co-localizing small amounts of t-PA and its substrate, plasminogen, to the clot surface through weak interactions with intact fibrin **[16]**. Small quantities of plasminogen are converted by t-PA to the key enzyme plasmin (Pn), which proteolytically cleaves the fibrin mesh by exposing C-terminal lysine (K) residues. In the second phase of fibrinolyis, these exposed lysine residues on this altered fibrin act as additional receptors for both t-PA and

plasminogen, thereby increasing plasmin generation to form fibrin degradation products and finally dissolve the clot **[17]**.

Another physiological activator of plasminogen is urokinase-type plasminogen activator (u-PA). However, u-PA does not specifically bind to fibrin. t-PA is the main plasminogen activator in fibrinolysis pathway, however exact role of u-PA in vascular fibrinolysis is less clear **[18]**. Instead, u-PA is majorly involved in cell migration and tissue remodeling through extravascular activation of plasminogen **[19]**.

Regulation of fibrinolysis

The amplification of the fibrinolytic process is blocked by the presence of the primary physiological inhibitor of plasmin, α2-antiplasmin (70 kDa glycoprotein from serpin family) **[20]**. This circulating serine protease inhibitor not only binds to free plasmin to irreversibly inhibit its fibrinolytic activity, it also binds circulating plasminogen to prevent interaction with fibrin, and hence delays the initiation of fibrinolysis **[21**, **22]**. Fibrinolysis is also regulated by a physiological inhibitor of t-PA, plasminogen activator inhibitor 1 (PAI-1, a single-chain glycoprotein with a molecular weight of ~50 kDa) **[23]**. PAI-1 is a serpin (a protein family which acquired this name due to their ability to inhibit serine proteases) which circulates in four-fold excess over t-PA in blood to prevent systemic fibrinolysis by forming a stable covalent complex with t-PA.

Thrombin-activatable fibrinolysis inhibitor (TAFI), a pancreatic carboxypeptidase B-like enzyme, which is activated to TAFIa by thrombin in presence of TM inhibits fibrinolysis by removing carboxyl terminal lysine/arginine

residues from fibrin molecules. The terminal basic residues are important for plasminogen binding to fibrin during the activation of plasminogen **[24]**.

1.1.3. Pathophysiology of thrombosis

Thrombosis is a pathological consequence of haemostasis and it occurs when the haemostatic response exceeds the normal regulatory counterbalance by anticoagulant factors or due to failure of fibrinolytic pathway, which are supposed to limit and localize thrombus formation to the injured area. Localized thrombus which travels within the body is known as embolus and the phenomenon is known as embolism. There are two types of thrombosis: (1) Arterial thrombosis (2) Venous thrombosis (**Figure 2**) **[25]**.

In the arterial circulation, soluble clotting factors washed out due to higher flow rates and thus reduces fibrin formation. The primary trigger of arterial thrombosis is rupture of an atherosclerotic plaque **[26]**. This involves disruption of the endothelium and release of constituents of the plaque into the lumen of the blood vessel. Haemostasis in the arterial circulation requires mainly platelets that accelerate thrombin formation, forms a physical barrier and provides a base upon which fibrin can accumulate **[27]**. Haemostatic plugs and thrombus that form in the arterial circulation are therefore enriched in platelets as well as fibrin and known as white clot due to its physical appearance.

In the venous system, low flow rates and stasis allow the accumulation of activated coagulation factors and hence fibrin formation, mostly without the involvement of platelets. Venous thrombosis can be triggered by three major factors: abnormal blood flow (such as the absence of blood flow or stasis);

altered properties of the blood itself (Thrombophilia); and alterations in the endothelium, collectively known as Virchow's triad **[28]**. Although venous thrombi contain platelets, the dominant cellular components are trapped erythrocytes (RBC) and therefore are known as red clot based on their physical appearance.

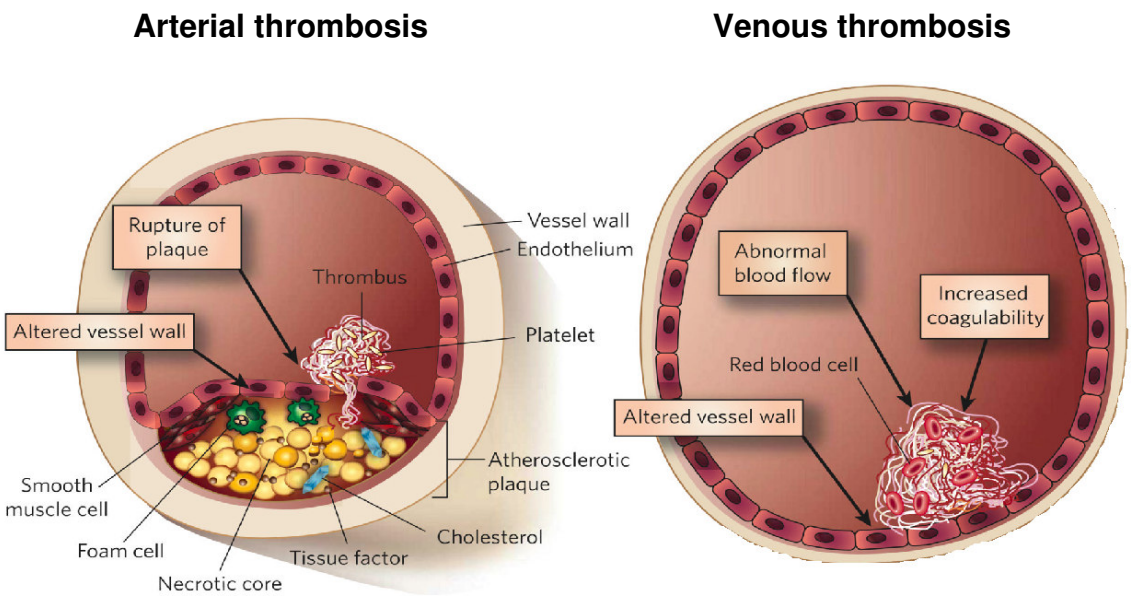

Figure 2. Pathophysiology of thrombosis

Arterial thrombus

- Occur in areas of rapid flow (arteries).
- In response to an injured or abnormal vessel wall.
- Composed of primarily of platelets, fibrin & leukocytes.
- Appears as white clot.

Venous thrombus

- Occur in areas of low flow rates (veins).
- In response to venous stasis or vascular injury.
- Composed almost entirely of fibrin & erythrocyte.
- Appears as red clot.

1.1.4. Etiology of thrombosis

In human body, there is a balance between procoagulant factors (allows clot formation) and anticoagulant factors (resists clot formation). A disrupted balance between this two forces will lead to the thrombosis related morbidity and mortality. These conditions may develop due to aging, hereditary causes, acquired disease conditions (e.g., atheromatous disease, cancer, antiphospholipid syndrome) and use of drugs (e.g., oral contraceptive) **[29**, **30]**.

Exponential increase in the incidence of venous and arterial thrombosis has been found with increase in age, which is explained by advanced atheromatous disease, cancer, various surgical procedures, and immobility **[31**, **32]**. In cancer patients, TF and other cytokines (e.g., interleukin-1β), may initiate coagulation and down regulate the anticoagulant factors, increasing the incidence of venous thromboembolism **[33]**. A number of chemotherapeutic drugs are also associated with increased thrombosis (l-Asparaginase, Lenalidomide, Tamoxifen, etc.) **[33]**.

Deficiencies of AT, protein C, and protein S result in a reduced ability to regulate thrombin generation, and thus, predispose affected individuals to deep venous thrombosis and pulmonary embolism **[34]**. A single nucleotide polymorphism in the Factor V gene (commonly Arg5063Gln; Factor V Leiden) and polymorphisms of prothrombin (G20210A variant) is often associated with increased risk of VTE **[34**, **35]**. Various risk factors for venous and arterial thrombosis are listed in **Table 1**.

Table 1. Risk factors for venous thrombosis and arterial thrombosis

Venous thrombosis	Arterial thrombosis
Age	Age
Major surgery or Trauma	Atrial fibrillation
Cancer	Hypercholesterolemia, Hypertension, Diabetes, Smoking
Antiphospholipid syndrome	Thrombotic thrombocytopenic purpura
Heparin induced thrombocytopenia	Heparin induced thrombocytopenia (less frequent)
Medication (e.g., Lenalidomide)	Medication (e.g., COX-2 inhibitors)
Devices (e.g., central venous catheter)	Devices (e.g., mechanical valve, drug-eluting stent)
Virchow's triad	Hereditary factors
Obesity	Hyperhomocystinemia
Pregnancy, contraceptive use or hormone replacement	Fibrinogen β-chain polymorphism (inconsistent)
Factor V Leiden, Prothrombin G20210A, AT deficiency, Protein C deficiency, Protein S deficiency	PAI-1 polymorphism (inconsistent) TAFI polymorphism (inconsistent)

1.2. Thrombotic and related cardiovascular disorders

Thrombotic disorders are the set of diseases, which occurs due to abnormal thrombus formation in arteries and veins in the body, leading to broad range of cardiovascular diseases. Cardiovascular disease is a term that refers to more than one disease of the circulatory system that includes the heart and blood vessels, whether the blood vessels are affecting the lungs, the brain, kidneys or other parts of the body. Thrombotic disorders are further classified in two categories: (**1**) Venous thromboembolism (**2**) Arterial thromboembolism.

1.2.1. Venous thromboembolism (VTE)

It is a term used for phenomenon of thrombus formation in veins and its complications. VTE is associated with high mortality and morbidity. The disease comprises deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT occurs in calf, knee or pelvis area and it leads to PE, if part of the thrombus detaches itself and travels into lungs and blocks pulmonary arteries leading to serious breathing problem and death (**Figure 3**) **[36**, **37]**.

1.2.1.1. VTE epidemiology

VTE is the third leading cause of cardiovascular-associated death, after myocardial infarction (MI) and stroke. Annual VTE incidence in the European Union and US is estimated at over one million and 600000 respectively, which results in mortality of more than 500,000 deaths in Europe **[38]** and 300,000 deaths in US per year **[39]**. The risk of recurrent events is a major concern, given that the cumulative incidence after an unprovoked DVT is nearly 18% at 2 years and this increase to 30% at 8 years **[40]**. VTE-related morbidity is further worsened by long term complications. It is estimated that after a DVT event, 20–50% of patients develop post-thrombotic syndrome (PTS) within 1–2 years **[41]**, and after a PE, 5% of individuals evolve chronic thromboembolic pulmonary hypertension **[42]**. Patients with PTS experience aching pain, heaviness, swelling, cramps, itching, or tingling in the affected limb **[43]**. Approximately 30-40% patients with VTE will present with symptomatic PE **[44]**.

1.2.2. Arterial thromboembolism (ATE)

In arterial emboli, blood flow is blocked at the junction of major arteries, usually at the groin, knee, or thigh. Arterial emboli are generally a complication of heart disease such as MI and atrial fibrillation (AF), one form of cardiac arrhythmia **[45]**. There is a significant association between myocardial ischemia commonly known as acute coronary syndrome (ACS) and AF with an estimated relative risk for AF of 2.8 for angina and 3.6 for MI **[46]**. ACS is usually the outcome of atherosclerosis and thrombosis in the coronary arteries. Atherosclerosis and thrombosis shares common mechanism as build up of lipids in the inner walls of blood vessels induces narrowing of blood vessels leading to restricted blood flow (Atherosclerosis), which in turn further blocked by thrombus formation and stops the blood flow (Thrombosis). Most acute myocardial infarctions are caused by thrombosis developed on a culprit coronary atherosclerotic plaque. Thrombosis is also the major initiating factor in unstable angina. An arterial thrombus that is rich in fibrin is often fully occlusive and results in ST-elevated myocardial infarction (STEMI), whereas a platelet-rich arterial thrombus is often partially occlusive, resulting in unstable angina and non-ST-elevated myocardial infarction.

An arterial embolism in the brain (cerebral embolism) causes stroke with symptoms such as an inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or an inability to see one side of the visual field. Stroke can cause permanent neurological damage and death. Patients with AF are at higher risk of cerebral stroke if small thrombi developed in

the atria of the heart transported into a cerebral blood vessel due to the patient's heartbeat (**Figure 3**). An estimated 5-14% of all strokes are caused by cerebral emboli **[47**, **48]**. Arterial emboli and hence oxygen unavailability can lead to tissue death and amputation of the affected limb if not treated effectively within hours. Intestines and kidneys can also suffer damage from emboli.

1.2.2.1. ATE epidemiology

The most common cause of death in the developed world is acute myocardial infarction (AMI), which is caused by coronary artery thrombosis. Recent World Health Organization (WHO) statistics have highlighted the true global impact of this disease with ~80% of the world's deaths from atherothrombosis occurring in low and middle-income countries. In US, it is estimated that over 850000 AMI occur annually, with an 18% and 35% recurrence rate within 6 years for men and women respectively.

Ischemic stroke is the third leading cause of death in developed countries, affecting 12 million people each year and causing 5.5 million deaths and permanent disability in an additional 5 million people **[49]**. Approximately 2.5 million people in the US are affected by AF, which increases relative risk of ischemic stroke by 4-5 fold (**Figure 3**) **[48**, **50]**. AF affects 1% of general population and up to 10% of those >80 years of age. Approximately 2.3 million individuals in the North America and 4.5 million in Europe are affected by AF **[51]**. These numbers are projected to increase to 15 million in North America alone by 2050 due to aging of the population **[51]**. As per WHO report in 2009, the incidence of stroke in India is around 130 per 100,000 people every year.

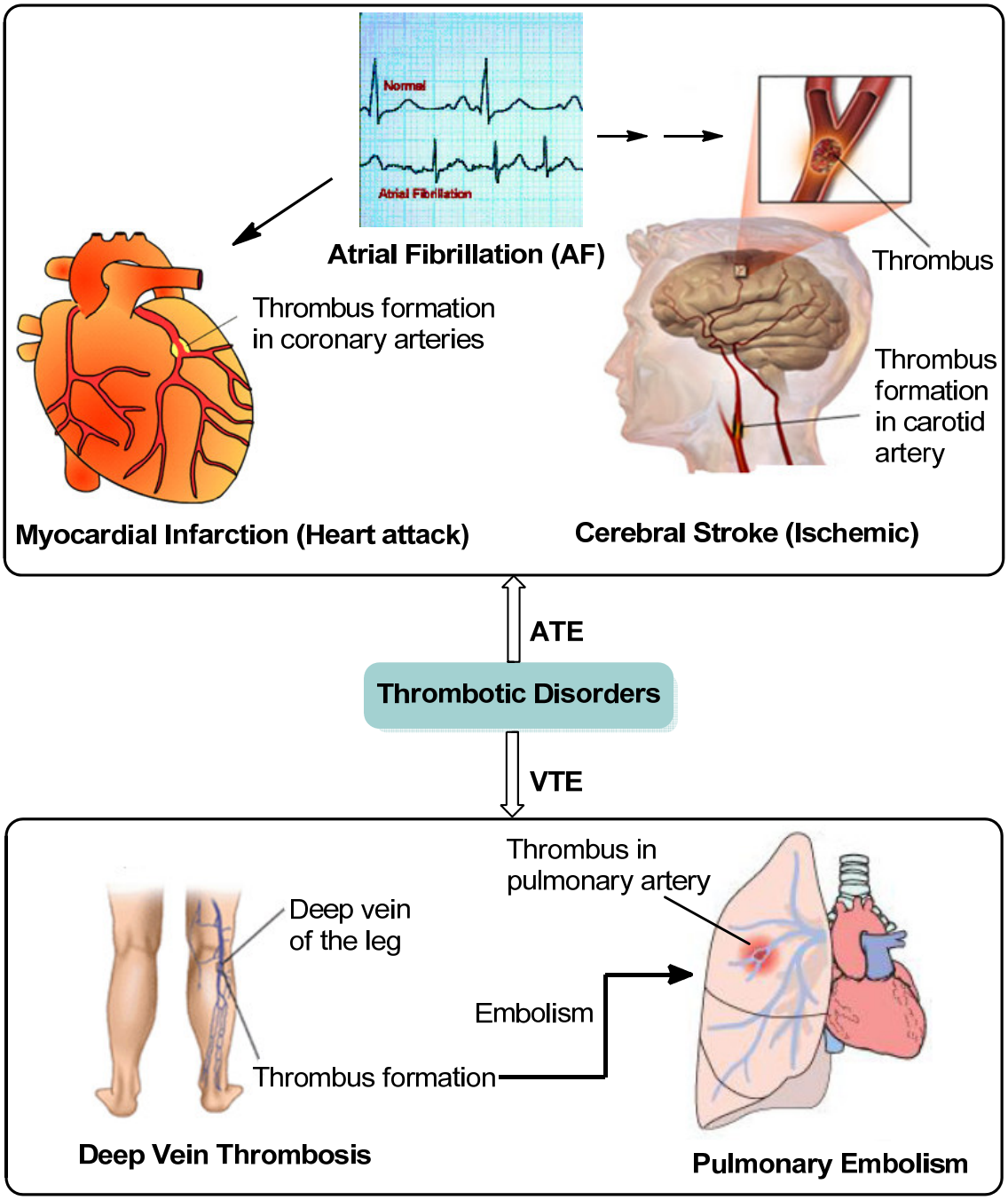

Figure 3. Schematic representation of thrombotic disorders

1.3. Current therapeutic options for treatment of thrombotic and related cardiovascular disorders and unmet needs

The pharmacological interventions to treat cardiovascular disorders are either prevention of thrombosis or targeting the individual risk factors. Treatment of thrombosis can be achieved by either preventing thrombus formation or dissolving existing thrombus. This is achieved by current antithrombotic agents based on three strategies as described in **Figure 4**.

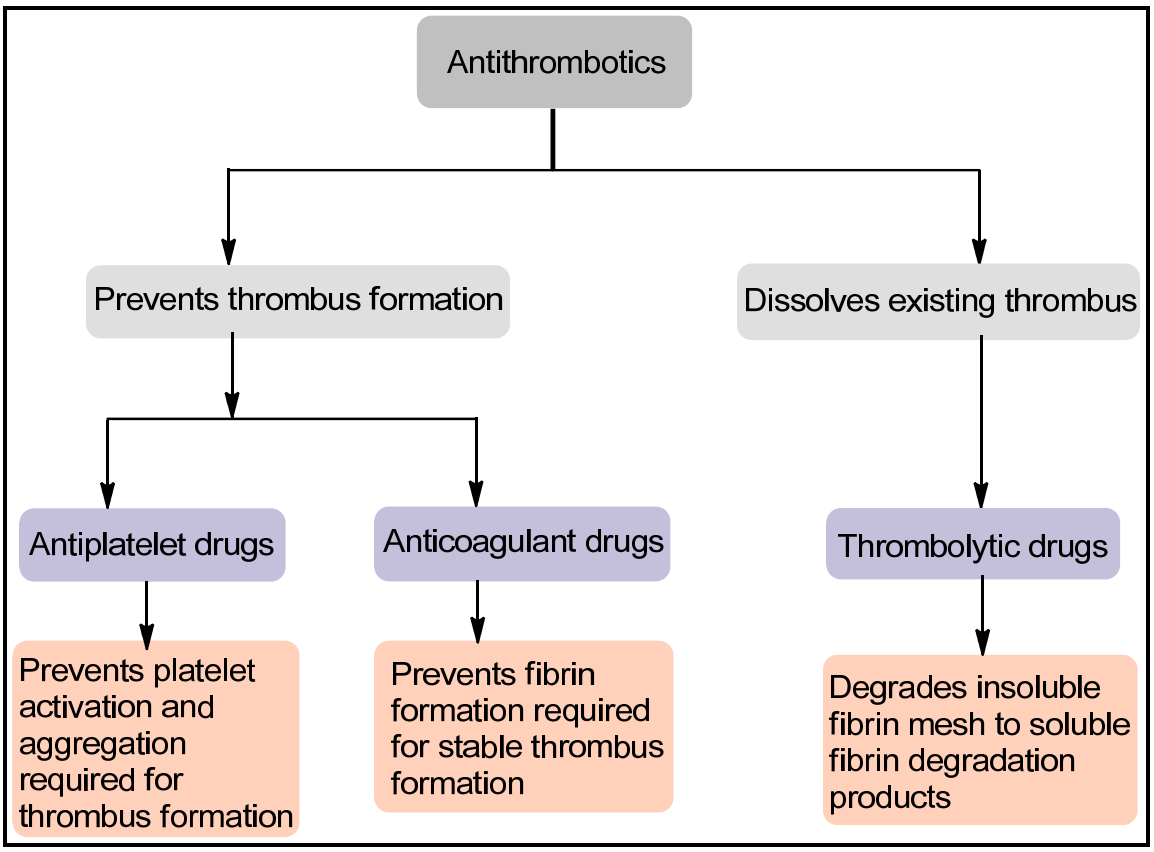

Figure 4. Current antithrombotic strategies

1.3.1. Antiplatelet agents

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality globally. An estimated 17.5 million people died from CVDs in 2005,

representing 30% of all global deaths. Of these deaths, an estimated 7.6 million were due to coronary heart disease and 5.7 million were due to stroke. Platelets play a major role in atherothrombosis, which is the final event complicating cardiovascular diseases as well as peripheral vascular diseases. Antiplatelet therapy improves survival of patients with these disorders **[52-54]**. Platelets are fragments of cytoplasm derived from precursor cells in the bone marrow and have a half life of 7-10 days. They are a critical physical component of clot. During clot formation, platelets undergo adhesion, activation, and then aggregation. As a result, therapies targeting key pathways of platelet activation and aggregation have established role in the treatment of ATE **[55]**.

1.3.1.1. History of development of antiplatelet agents

Historically, development of antiplatelet agents started from the discovery of a wonder drug “Aspirin” which has long been established as useful analgesic, antipyretic and anti-inflammatory drug **[56]**. Aspirin's effects on blood clotting (as an antiplatelet agent) were first noticed in 1950 by Lawrence Craven **[57]**. Craven, a family doctor in California, who had been directing tonsillectomy patients to chew Aspergum, an aspirin containing chewing gum accidently found that an unusual number of patients using very high amounts of Aspergum had to be hospitalized due to severe bleeding. Craven then started recommending daily aspirin to all his patients, and claimed that the patients who followed the aspirin regimen had no signs of thrombosis. However, Craven's studies were not taken into count by the medical community, because he had not done a placebo-controlled study.

The idea of using Aspirin to prevent clotting diseases (such as heart attacks and strokes) was revived in the 1960s, when medical researcher Harvey Weiss found that Aspirin had an anti-adhesive effect on blood platelets **[58, 59]**. Mechanistic investigation of aspirin's antiplatelet potential was thereafter investigated by several scientists such as Armand Quick **[60]**, Priscilla Piper, Sir John Vane **[61]**, Smith and Willis **[62]**, at different interval of time to conclude that aspirin inhibits platelet activation by irreversible inhibition of COX-1 (cyclooxygenase-1).

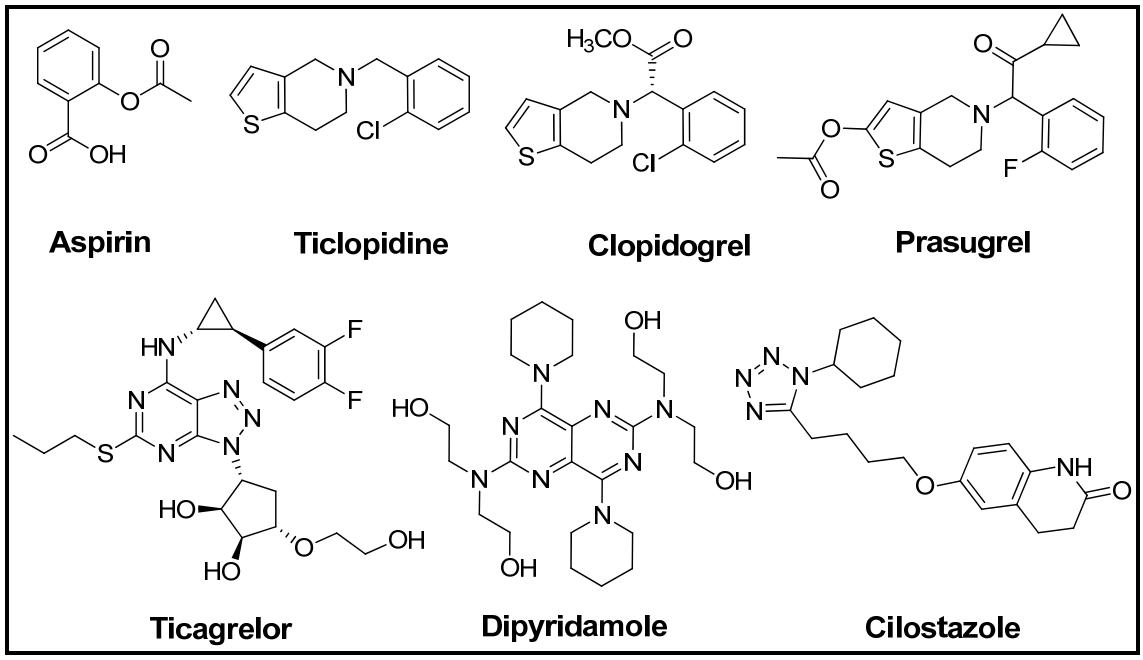

Figure 5. Approved antiplatelet agents

Aspirin, the first antiplatelet medication discovered remains the most widely used and the most studied; however, many others have been developed thereafter which are shown in **Figure 5**. Clopidogrel (P2Y12 inhibitor) together with Aspirin covers most of the oral antiplatelet regime as on today.

List of approved antiplatelet agents and their clinical usefulness and drawbacks are listed in **Table 3**.

1.3.2. Anticoagulants

Anticoagulants can inhibit the initiation or propagation of coagulation, or by targeting thrombin, they can attenuate fibrin formation. Thus by preventing fibrin formation anticoagulants stops stable clot formation initiated on platelet surface. Venous thrombus is rich in fibrin and contains less platelets, hence VTE can be effectively prevented by anticoagulants **[63]**. However, ATE can also be prevented by use of anticoagulants as fibrin is always required to form stable clot.

1.3.2.1. History of development of anticoagulants [64]

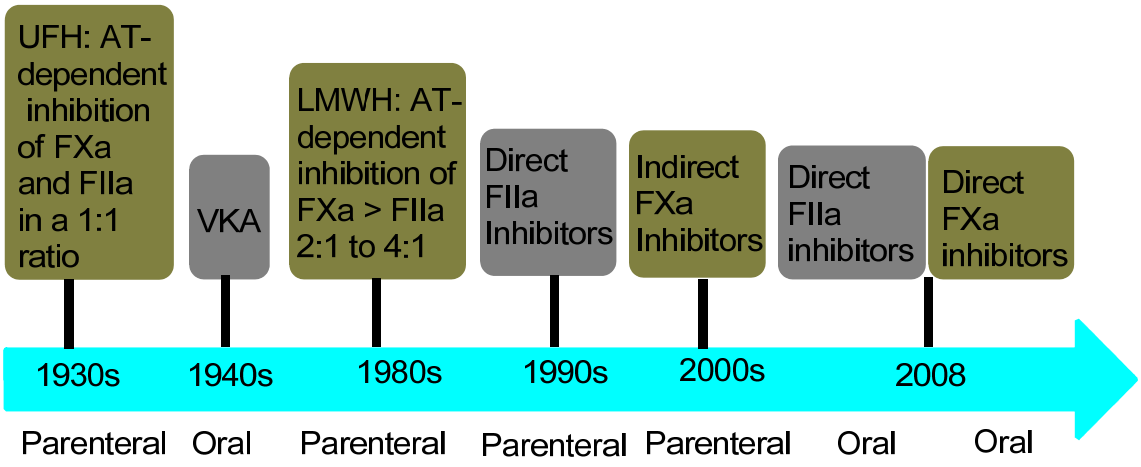

Figure 6. Development of anticoagulants over the past century

The developmental history of anticoagulants started with identification of Heparin which inhibits both FXa and thrombin. The journey from this nonselective

parenteral anticoagulant to selective parenteral FXa and thrombin inhibitors and finally to selective and direct oral anticoagulants is depicted in **Figure 6**.

Heparin [65]

The original anticoagulant discovery story dates back to the 1920s. Heparin, the major anticoagulant for parenteral use acquired its name because it is abundant in liver tissues. It was discovered in 1922 in the laboratory of Howell during their studies on the release of an anticoagulant from dog's livers during an anaphylactic shock. Heparin is composed of a heterogeneous mixture of straight chain anionic mucopolysaccharides spanning 20-100 monosaccharides and also known as unfractionated heparin (UFH) for which the first trial in humans was reported in 1937.

Coumarins [65]

Livestock farmers in the prairies of North Dakota in the US, and in Alberta, Canada, had been amazed by severe bleeding in cattle, which remained unexplained until scientists linked the disease to feeding on spoiled hay or silage made from the sweet clover grown in these regions as a substitute for corn. In 1939, Campbell and Link in Wisconsin identified the dicoumarol (coumarin derivative) as the responsible agent, and the first clinical experiences with dicoumarol as an anticoagulant were published in 1942 **[66]**. Subsequently, several coumarin variants were synthesized in Link's laboratory, including Warfarin. Its initial use was as a rodenticide, but after a man attempted suicide by taking a large dose of a Warfarin-based rodenticide, and yet survived **[67]**, clinical trials of warfarin as an anticoagulant were initiated, for which the first

results were published in 1953 **[68]**. Warfarin is now the leading coumarin and oral anticoagulant, which works by antagonizing Vitamin K dependent synthesis of multiple coagulation factors and thus known as Vitamin K antagonist (VKA).

Low Molecular Weight Heparin (LMWH)

Subsequent clinical advance has been the use of LMWHs derived from unfractionated heparin using different degradation methods (**Table 2**).

Table 2. Basic characteristics of LMWHs applied in clinical practice

Name	Degradation method	Mean MW (kDa)	aXa/alla ratio
Ardeparin-sodium	Peroxidation at elevated temperature	4.0-6.0	1.7-2.4
Bemiparin-sodium	Basic degradation in a nonaqueous media and fractionation	3.6	8.1
Certoparin-sodium	Hydrolysis with isoamylnitrite	4.2-6.2	1.5-2.5
Dalteparin-sodium	Hydrolysis with HNO_2	5.6-6.4	1.9-3.2
Enoxaparin-sodium	Benzylation and alkaline β elimination	3.5-5.5	3.3-5.3
Nadroparin-calcium	Hydrolysis with HNO_2 and fractionation	3.6-5.0	2.5-4.0
Parnaparin-sodium	Radical-catalyzed degradation with H_2O_2 and Cu-salt	4.0-6.0	1.5-3.0
Reviparin-sodium	Hydrolysis with HNO_2	3.5-4.5	3.6-6.3
Tinzaparin-sodium	Heparinase digestion	5.6-7.5	1.5-2.5

LMWHs were first clinically reported in 1982 **[69]** and they have mean molecular mass of 3-8 kDa, compared with a 3-30 kDa for unfractionated heparin and inhibits coagulation FXa and FIIa in variable ratio (anti FXa (aFXa)/anti FIIa (aFIIa)) **[70]**.

Selective anticoagulants

The search of an ideal anticoagulant became more precise and specific with an identification of selective and direct thrombin inhibitors like recombinant Hirudines (Desirudin, Lepirudin, Bivalirudin) in 1990s **[71-73]**. Argatroban (**Figure 7**) is a small molecule thrombin inhibitor which was approved as an anticoagulant in 2000 **[74]**. Selective indirect inhibitor of FXa, Fondaparinux was approved in 2001 as parenteral anticoagulant **[75]**.

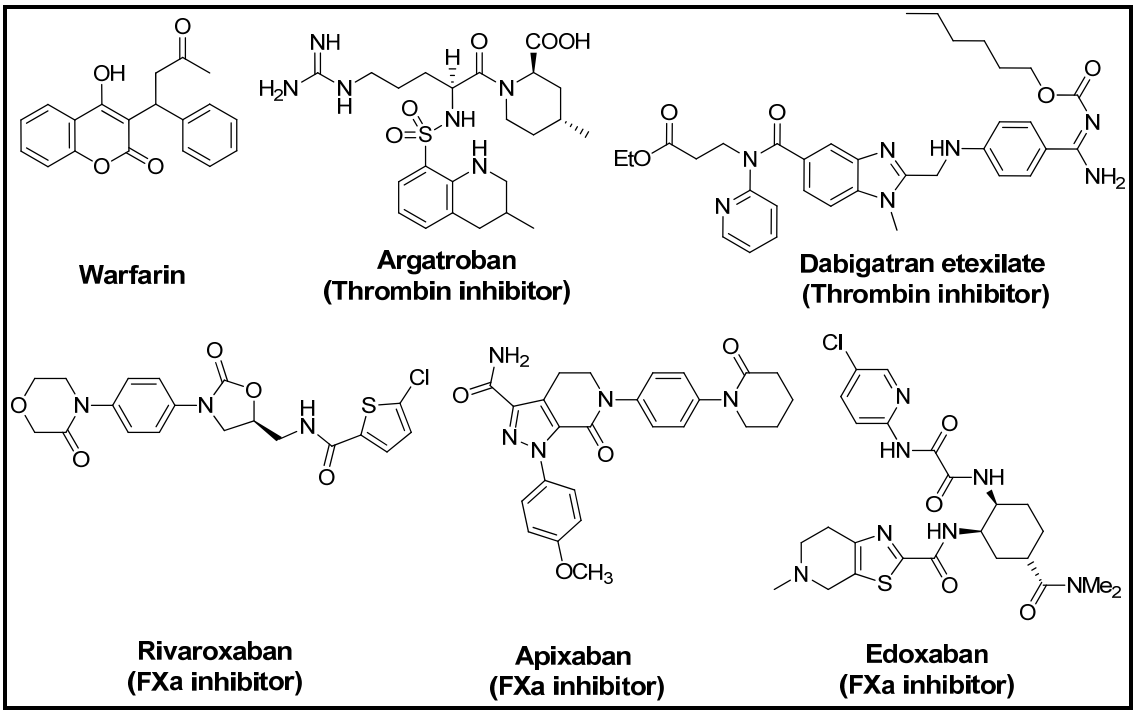

Figure 7. Small molecule anticoagulants approved in market

The search for an oral anticoagulant resulted in several small molecule FXa inhibitors (Rivaroxaban, Apixaban and Edoxaban) and thrombin inhibitor (Dabigatran etexilate), which are recently approved as oral anticoagulants for specific therapeutic indications (**Figure 7**) **[76]**. List of approved anticoagulants and their clinical usefulness and drawbacks are listed in **Table 4**.

1.3.3. Thrombolytic agents

Thrombolytic drugs dissolve blood clots by activating plasminogen, which forms a cleaved product called plasmin. Plasmin is a proteolytic enzyme that is capable of breaking cross-links between fibrin molecules, which provide the structural integrity of blood clots. Because of these actions, thrombolytic drugs are also called "plasminogen activators" or "fibrinolytic drugs" or “clot bursting drugs”.

1.3.3.1. History of development of thrombolytic agents [77, 78]

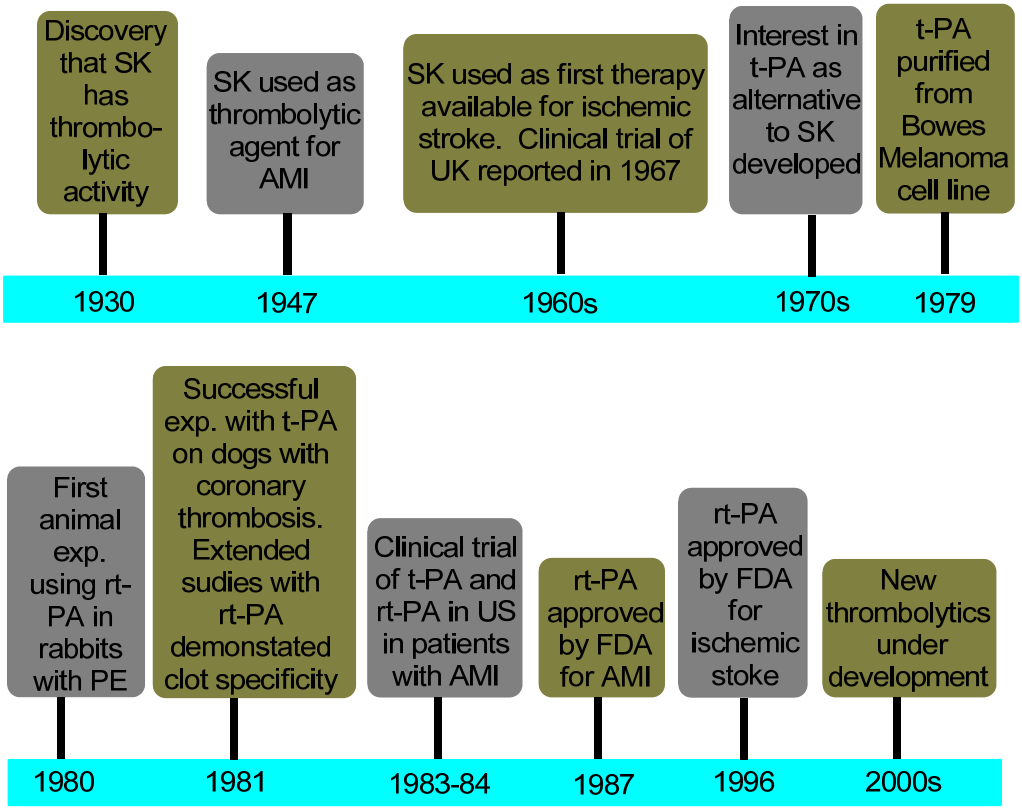

Figure 8. Development of thrombolytic agents over the past century

The journey of development of thrombolytic agents is depicted in **Figure 8**. Streptokinase (SK) was the first thrombolytic agent to be identified. Looking at

the historical perspectives however, proteolytic activity of Urokinase (UK) was noticed much before (1861) in form of human urine, which is further extended for fibrin in 1886. Further development in this area led to an identification rt-PA (recombinant t-PA) which was approved for AMI in 1987 and for ischemic stroke in 1996.

Urokinase (UK)

The history of thrombolytic agents begins in 1861, when von Brucke noticed proteolytic activity of human urine. In 1886, Sahli noted urinary proteolytic activity with some specificity for fibrin. Purification and isolation of the fibrinolytic enzyme in urine was achieved in 1947 by MacFarlane and Pilling **[79]** and in 1951 by Williams **[80]**. Sobel *et al*., gave the name "urokinase" in 1952. He and others demonstrated that UK is not a direct fibrin-degrading enzyme but rather an activator of endogenous plasminogen, thereby generating plasmin, which then degrades fibrin, fibrinogen, and other coagulation proteins. This mechanism was found to be shared by all current thrombolytic agents. Fletcher *et al*., used UK to maintain a thrombolytic condition in human volunteers by intravenous infusion of UK in 1965 **[81]**, later on use in AMI was reported by others.

The first studies of intravenous UK in PE began in 1967 followed by large randomized, controlled trials demonstrated the efficacy of thrombolytics in pulmonary embolism **[82]**.

Streptokinase (SK)

Dr. Sol Sherry in 1930s, working at Washington University in St. Louis with the Drs. Anthony Fletcher and Norma Alkjaersig, using knowledge of bacterial

enzymology demonstrated that blood clots could be dissolved *in vivo* with SK, a single-chain protein isolated from the broth of Lancefield group C β-hemolytic streptococci **[83]**. Sherry and his team became the first to employed crude SK to humans in early 1947 **[84]**. The usefulness of both intravenous SK and UK in VTE was shown in several studies in the 1960s and 1970s, leading to FDA approval of both agents for intravenous use in 1977. SK was initially approved for DVT, PE, thrombosed dialysis fistulas, and arterial thrombosis; UK was initially approved only for PE and thrombosed intravenous catheters. Subsequently, FDA approvals for other indications came in 1982 for use of both SK and UK in treatment of AMI and in 1987 for intravenous use of SK in AMI.

Tissue plasminogen activator (t-PA)

In 1947, researchers reported finding of an naturally occurring anti-clotting agent that human cells uses to clear up blood clotting, later called tissue plasminogen activator or t-PA **[85]**. In 1979, Dr. Matsuo and colleagues received a line of aggressive melanoma cells derived from a patient named Bowes. These melanoma cells showed high amount of fibrinolytic activity, and they worked to purify the fibrin-digesting agent. Within the year, they demonstrated that the agent was in fact a t-PA. In 1980s, they used t-PA to dissolve clots in rabbits with pulmonary embolus **[86]**. After large randomized multicentre human clinical trial in 1984, rt-PA had received FDA approval for treating acute myocardial infarction in 1987. List of approved thrombolytic agents and their clinical usefulness and drawbacks are listed in **Table 5**.

1.3.4. Unmet needs

Current treatment for thrombotic disorders offers several drugs based on multiple targets and various antithrombotic strategies, which are listed in **Tables 3**, **4** and **5**.

Antiplatelet agents (**Table 3**), currently approved inhibit platelet activation for e.g., Aspirin, Clopidogrel, Dipyridamol etc., and platelet aggregation for e.g., Abciximab, Eptifibatide and Tirofiban. Aspirin **[87]**, by inhibiting COX-1, inhibits the generation of prostaglandin H_2 and, subsequently, thromboxane A_2 (TxA2), which in turn blocks platelet activation through the thromboxane receptor.

Ticlopidine **[88]** and Clopidogrel **[89, 90]** irreversibly inhibits P2Y12 receptor for adenosine diphosphate (ADP), an important platelet agonist, and thus blocks platelet activation. Prasugrel **[91]** is an improved version of Clopidogrel that has been recently approved in Canada, Mexico and the US. Ticagrelor **[92]** works through reversible inhibition of P2Y12 receptor and is recently approved.

GPIIb-IIIa inhibitors **[93]** Abciximab, Eptifibatide, and Tirofiban bocks platelet aggregation but requires parenteral administration. Dipyridamole **[94]** and Cilostazol **[95]** are oral cyclic nucleotide phosphodiesterase 3 (PDE3) inhibitor with antiplatelet, vasodilatory and antimitogenic effects. The limitation of antiplatelet agents is that they have less influence on the prevention of fibrin rich thrombus as observed in DVT and PE **[96]**.

Table 3. Antiplatelet drugs available in market

Drug	Mechanism of action	Therapeutic indication	Disadvantages
Aspirin	Irreversible acetylation of COX-1, inhibiting generation of TxA2	Acute and chronic coronary, cerebral and peripheral vascular disease	Bleeding, GI toxicity, nausea, vomiting, weak agent
Ticlopidine	Irreversible P2Y12 inhibitor	Stroke prevention, intracoronary stenting	Bleeding, GI toxicity, nausea, Thrombocyto-penic purpura, Neutropaenia
Clopidogrel	Irreversible P2Y12 inhibitor	ACS, Peripheral artery disease, stroke prevention, intracoronary stenting	Bleeding, Rash, Thrombocytope-nic purpura, Neutropaenia, patient variability
Prasugrel	Irreversible P2Y12 inhibitor	ACS, stroke prevention, intracoronary stenting	Bleeding more frequent than Clopidogrel
Ticagrelor	Reversible P2Y12 inhibitor	ACS, stroke prevention, intracoronary stenting (in combination with aspirin)	Bleeding, increased dyspnoea and ventricular pauses
Abciximab Eptifibatide Tirofiban	GPIIb-IIIa inhibition	ACS, Percutaneous coronary intervention (PCI)	Bleeding, Thrombocytope-nia, IV administration
Dipyridamole Cilostazol	PDE3 inhibitors	Stroke prevention, prosthetic cardiac valves, Peripheral vascular disease	Headache, Dizziness, GI toxicity, nausea, vomiting, Rash, two or three times daily dosing

Anticoagulants (**Table 4**) such as heparin **[97]** and LMWHs **[70]** (Enoxaparin, Dalteparin, Tinzaparin, Bemiparin etc.), which offers parenteral treatment for ACS, suffer from several drawbacks particularly, bleeding and heparin induced thrombocytopenia (HIT).

Table 4. Anticoagulant drugs available in market

Drug	Mechanism of action	Therapeutic indication	Disadvantages
UFH	AT-III dependent inhibitor of FXa and thrombin	ACS, clot prevention during haemodialysis	HIT, unpredictable response, requires monitoring and IV dosing
LMWH: Enoxaparin, Dalteparin, etc.	AT-III dependent inhibitor of FXa and thrombin	VTE prophylaxis	HIT, IV administration,
Warfarin	VKA Inhibits coagulation factors synthesis	VTE treatment and prophylaxis, stoke prevention in patients with AF	Slow onset-offset action, unpredictable response, narrow therapeutic window, multiple food and drug interaction.
Fondaparinux	AT-III dependent selective inhibitor of FXa	VTE treatment and prophylaxis	IV administration, Bleeding complications in patients with renal insufficiency.
Desirudin Lepirudin, Bivalirudin	Thrombin inhibitors	Treatment of HIT complicated by thrombosis	IV administration Bleeding in patients with renal insufficiency.
Argatroban	Thrombin inhibitor	Prophylaxis of VTE in patients with HIT and during PCI	IV administration
Dabigatran	Thrombin inhibitor	Prophylaxis of VTE, Stroke prevention in patients with AF	NR
Rivaroxaban	FXa inhibitor	Prophylaxis of VTE, Stroke prevention in patients with AF	NR
Apixaban Edoxaban	FXa inhibitor	VTE prevention in patients after orthopedic surgery	NR

NR = Not reported.

Fondaparinux is an analogue of the pentasaccharide sequence required to promote the binding of AT to FXa and found to be safer in terms of thrombocytopenia but requires parenteral administration **[98]**.

A VKA Warfarin, an oral anticoagulant possesses anticoagulant property by inducing the synthesis of several defective coagulation factors by inhibiting the essential vitamin-K-dependent posttranslational modifications of this coagulation factors. It suffers from several drawbacks such as patient variability, delay onset-offset of action and bleeding complications **[99, 100]**.

Hirudin derivatives Desirudin, Lepirudin, and Bivalirudin **[101]** are inhibitors of thrombin and used for treatment of thrombosis in patients with HIT as parenteral treatment. Argatroban **[74]** is a small molecule thrombin inhibitor used for prophylaxis or treatment of VTE in patients with HIT and during percutaneous coronary intervention (PCI), but requires parenteral administration.

Several novel oral anticoagulants are now in clinical development. FXa inhibitors (Rivaroxaban, Apixaban and Edoxaban) and Thrombin inhibitor (Dabigatran) are approved for the prophylaxis of VTE. Rivaroxaban and Dabigatran are also approved for stroke prevention in patients with AF, while clinical trials are in progress for the treatment and prophylaxis of ACS **[76]**.

A number of thrombolytic agents (**Table 5**) based on physiological activators of plasminogen are currently available for the pharmacological treatment of a variety of thrombosis-related conditions. SK **[102]** by forming a complex with circulatory plasminogen activates plasminogen to plasmin in a fibrin-independent manner, making it a very effective but non-specific fibrinolytic

agent. There is a significant risk of life-threatening systemic bleeding associated with SK treatment, especially in elderly patients **[103]**. Anistreplase **[104]** is a complex of SK and plasminogen and does not require circulating plasminogen for activation. UK **[105]** directly cleaves plasminogen to activate it to plasmin.

Table 5. Thrombolytic drugs available in market

Drug	Mechanism of action	Therapeutic indication	Disadvantages
Streptokinase	Plasminogen activation by forming complex with it	Acute treatment of STEMI, acute treatment of VTE	Severe bleeding and requires parenteral administration, antigenic
Anistreplase	Plasminogen-streptokinase complex	Acute coronary arterial thrombosis	Severe bleeding and requires parenteral administration
Urokinase	Plasminogen activator	Acute coronary arterial thrombosis, acute PE	Severe bleeding, parenteral administration
Alteplase	Recombinant t-PA	Acute treatment of STEMI, acute ischemic stroke, acute massive PE	Severe bleeding, parenteral administration
Reteplase	Recombinant t-PA	For treatment of AMI	Bleeding (but lesser than Alteplase), parenteral administration
Tenecteplase	Recombinant t-PA	For treatment of AMI	Bleeding, parenteral administration

Recombinant t-PA (generic name: Alteplase) **[106]** represents a significant improvement over treatment with SK primarily due to its fibrin specificity. Attempts to improve both the safety and efficacy of t-PA have produced a number of variant recombinant t-PA preparations, including Reteplase **[107]** and

Tenecteplase **[108]**. However, they failed to yield significant improvements in benefit-to-risk ratios over Alteplase clinically **[109]**. A narrow administration window for t-PA therapy restricts the number of patients eligible for treatment. Beside this, there is no oral thrombolytic therapy available in the market. Thus, to offer safe oral thrombolytic treatment to the patients is highly desirable.

Further, there are limited options available as an antidote for current antithrombotic treatments **[110]**. To reverse the action of Warfarin, intravenous vitamin K or rVIIa can be given. Protamin sulfate is commonly used as an antidote for Heparin **[111]**. Reversal of antiplatelet agents requires administration of desmopressin and transfusing platelets. Cryoprecipitate may be utilized to replenish fibrogen stores to reverse action of fibrinolytic agents. There is no specific antidote for novel antithrombotic agents which are recently approved, such as Rivaroxaban, Apixaban and Dabigatran **[112]**.

In summary, thrombolytic agents that dissolve existing clot seems to be the treatment of choice as they take care of both venous and arterial thrombus. Anticoagulants which targets both arterial and venous thrombosis are another attractive option to prevent fibrin rich clot formation. There is a great unmet need in the area of oral thrombolytic agents and oral anticoagulants to treat several life threatening disease.

1.4. Strategies for development of novel antithrombotics:Selection of biological target

In continuation of our research interest and also based on the unmet needs in antithrombotic therapies, several investigational therapeutic strategies were evaluated for developing novel anticoagulants and thrombolytic agents (**Figure 9**).

The strategies for the development of novel anticoagulants mainly involve targeting several coagulation factors in coagulation cascade, which is divided in three groups:

1. Inhibition of the initiation of coagulation

2. Inhibition of the propagation step of coagulation

3. Inhibition of the thrombin (FIIa)

Drugs that target the factor VIIa/TF complex inhibit the initiation of coagulation. Only parenteral agents in this category have reached in clinical trials which includes Tifacogin (Recombinant form of TFPI expressed in *Saccharomyces cerevisae*), rNAPc2 (Recombinant nematode anticoagulant peptide) and PCI-27483 (Recombinant factor VIIa without active site for TF binding).

Tifacogin was evaluated in phase III clinical trial in severe sepsis patients, but further development is not reported for it **[113]**. PCI-27483 (Pharmacyclics) is a small molecule inhibitor of FVIIa/TF complex. It was evaluated in a Phase Ib/II trial in patients with pancreatic cancer receiving treatment with Gemcitabine **[114]**. rNAPc2 was initially evaluated for VTE prophylaxis **[115]** then for ACS **[116]**, but later on its development was discontinued.

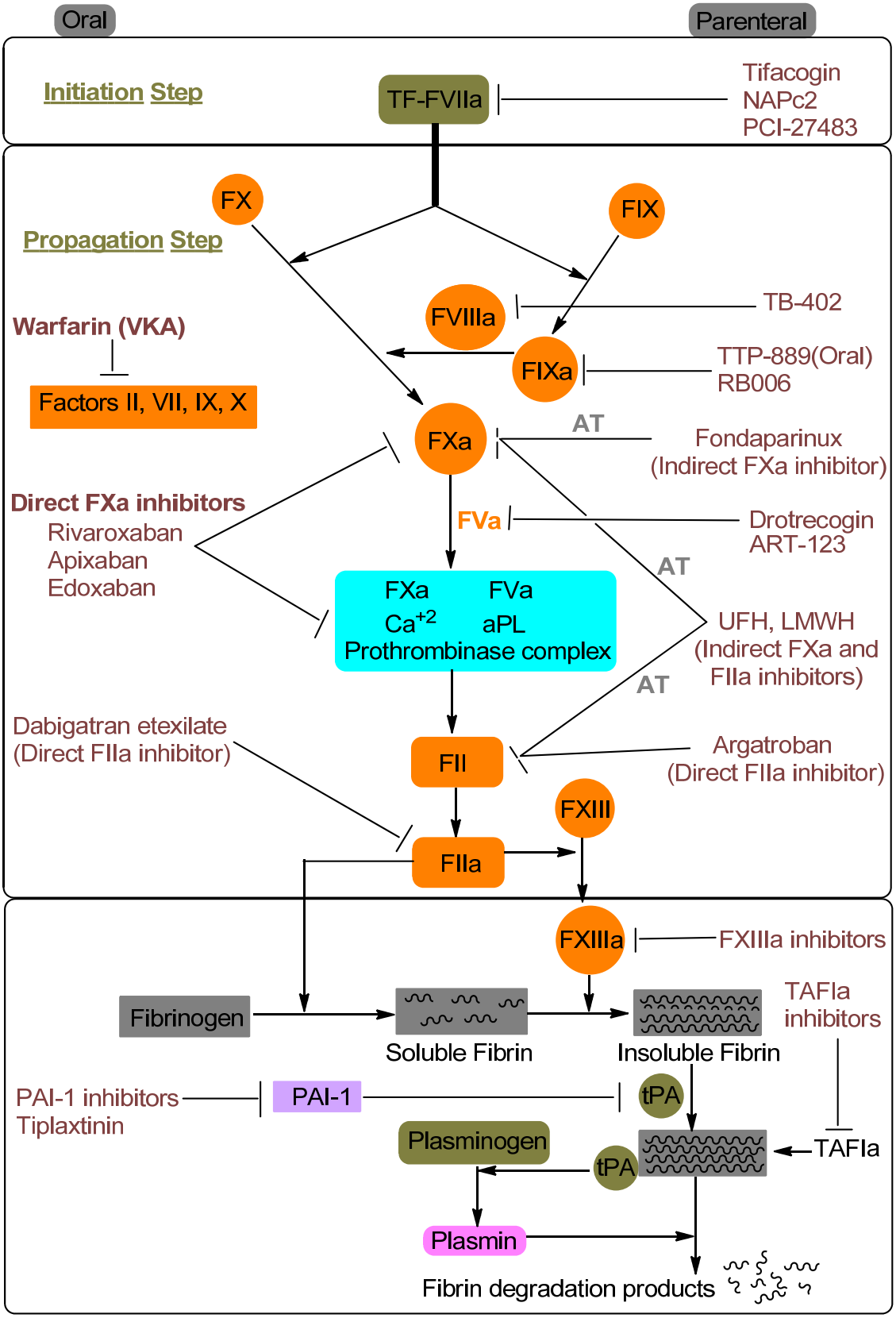

Figure 9. Therapeutic strategies for developing novel antithrombotics

Drugs that targets FIXa or FXa or that inactivate their respective cofactors, FVIIIa and FVa, respectively inhibits propagation of coagulation. Both parenteral (RB006) and oral (TTP889) FIXa inhibitors were evaluated in clinical trials. RB006 is an RNA aptamer that binds FIXa with high affinity **[117]** and it is being developed with its complementary oligonucleotide antidote (RB007) in Phase IIa clinical trial in elective PCI **[118]**. TTP889 is an oral agent which could reached up to phase II, but due to lack of efficacy its development work was stopped **[119]**.

FVa, a cofactor of FXa is proteolytically degraded by aPC. Drotrecogin, a recombinant form of aPC is licensed for severe sepsis but later on withdrawn from the market **[120]**. ART-123 is a recombinant human soluble thrombomodulin. ART-123 binds to thrombin to inactivate coagulation, and the thrombin-ART-123 complex activates aPC, which in turn inactivated FVa. Currently, ART-123 is in Phase III trial to assess the safety and efficacy on subjects with sepsis and disseminated intravascular coagulation (DIC) **[121]**. TB-402, a FVIII inhibitor was in Phase IIb for the prophylaxis of VTE after total hip surgery but due to excessive bleeding its development work was stopped **[122]**.

As depicted in **Figure 9**, coagulation FXa is located at center of the coagulation cascade whose role as discussed previously is to convert prothrombin to thrombin, a final coagulation factor required for fibrin formation. Several oral direct FXa inhibitors are now in clinical trials. Rivaroxaban, Apixaban, and Edoxaban are approved for prophylaxis of VTE and they can inhibit both free FXa and FXa bound in prothrombinase complex.

Thrombin, being the last coagulation factor before fibrin formation represents potential target to develop anticoagulants along with FXa. Several thrombin inhibitors are in clinical trial. Argatroban (parenteral agent) and Dabigatran etexilate (Oral agent) are examples of direct thrombin inhibitors, which are approved as anticoagulants **[76]**. Based on clinical data and success rate for novel anticoagulants, it is evident that FXa and Thrombin represents a potential targets to identify an efficacious and safe oral anticoagulant. FXa inhibition approach has always been considered as preferred over thrombin inhibition due to several reasons. First, due to the amplified nature of the coagulation cascade smaller dose of an anticoagulant is required to inhibit coagulation when targeted against the more upstream factor. For e.g., one molecule of FXa is thought to produce 1000 molecules of thrombin **[123]**. Second, FXa seems progressively inhibited over a wider concentration range than thrombin, due to which FXa inhibitors may have a wider therapeutic window than thrombin inhibitors. Third, direct thrombin inhibitors seems to be associated with a rebound hypercoagulable state that does not appear to be associated with FXa inhibitors **[124]**. Thrombin has also a substantial role in platelet activation pathway through Protease Activated Receptor-1 (PAR-1) activation **[125]**. All together, this data suggest FXa as a viable and potential target to treat thrombotic disorders.

The strategies for the development of novel thrombolytic agents include inhibitors of PAI-1, TAFIa or FXIIIa (**Figure 9**). Strategies involving t-PA and

plasminogen analogous or agents targeting directly fibrin are also explored and are successful.

Existing fibrinolytic agents as described previously are plasminogen activators that act by converting plasminogen to plasmin. Several t-PA analogues are approved for the treatment of thrombotic disorders but they require parenteral administration and also suffer from severe bleeding **[109]**. Other novel strategies under evaluation are degradation of fibrin directly (Alfimeprase) and using plasminogen variant (BB10153), which can be activated by thrombin instead of t-PA. Alfimeprase in a truncated form of fibrolase that directly degrades the α-chain of fibrin and fibrinogen **[126]**. Development of Alfimeprase has been discontinued, while BB10153 is still in clinical trials as parenteral agent **[127]**.

PAI-1 is the major physiological inhibitor of t-PA and u-PA. Thus blocking PAI-1 activity prevents degradation of t-PA and subsequent retaining fibrinolytic activity of plasmin. Role of PAI-1 inhibitors for treatment of thrombotic disorders have been proven in animal model of thrombosis. Several small molecule PAI-1 inhibitors were evaluated preclinically among which Tiplaxtinin is the most studied PAI-1 inhibitor till date, which could reach up to Phase I human clinical trial **[128]**. These PAI-1 inhibitor are also orally bioavailable and thus fulfills unmet needs in the area of oral fibrinolytic agents, however none of the molecules were evaluated in Phase II clinical trial.

TAFIa, by cleaving carboxy-terminal lysine residues from fibrin and subsequent preventing binding of plasminogen or plasmin to fibrin negatively controls fibrinolysis. Studies in dogs and rabbits demonstrated that a potato-

derived TAFIa inhibitor increases plasminogen activator-induced thrombolysis **[129]**. Several small molecule TAFIa inhibitors are identified, however none of could reach in clinical trial **[130]**.

Factor XIIIa has a substantial role in cross linking soluble fibrin to insoluble fibrin mesh. This cross linking makes clot more refractory to degradation by plasmin. Tridegin, a peptide isolated from natural source is a specific inhibitor of FXIIIa and enhances fibrinolysis *in vitro* **[131]**. There are no FXIIIa inhibitors which are studied clinically.

The extensive research work in PAI-1 inhibition approach and preclinical animal data suggests that PAI-1 inhibitors could become future oral thrombolytic therapy.

In summary, PAI-1 inhibition to discover novel oral thrombolytic agent and FXa inhibition to discover novel oral anticoagulant are potential targets for further research in antithrombotic area.

1.5. Plasminogen Activator Inhibitor-1 (PAI-1)

1.5.1. Discovery of PAI-1

PAI-1 was initially identified as a fibrinolytic inhibitor in the culture medium of bovine aortic endothelial cells **[132]**, but later its production was found by numerous tissues and cells. At the time of its discovery, the circulating PAI was termed PAI-1. Two closely related family members of PAI-1 are termed as PAI-2, the intracellular PAI, contained mainly in leukocytes, the placenta, and the plasma of pregnant women **[133]**, and PAI-3, which is synthesized in liver and is

present in plasma and urine inhibits u-PA, t-PA and aPC and thus known as protein C inhibitor **[134]**.

New nomenclature has been proposed on the basis of their phylogenetic relationships **[135]**. PAI-1 is now called Serpin E1, PAI-2 Serpin B2, and PAI-3 Serpin A5. Serpins (Serine Protease Inhibitors) are a superfamily of proteins classified into 16 clades (A-P). The systematic name of each serpin is, SERPINXy where X is the clade and y is the number within the clade **[136]**.

1.5.2. Structure of PAI-1

PAI-1 is a single-chain glycoprotein with a molecular weight of ~50 kDa. The mature, secreted form of PAI-1 consists of 379 amino acids and possesses ~13% carbohydrate. It lacks cysteine residues but contains multiple methionine residues, which could be the reason for its susceptibility to irreversible inactivation by oxidizing agents. The lack of cysteine residues and hence disulfide bonds may in turn account for its poor stability in solution. The reactive center of the inhibitor (Arg^{346}-Met^{347}) resides within the exposed "strained loop region" at the carboxy terminus of the molecule and which serves as a pseudo substrate for the target serine protease (**Figure 10**). The isoelectric point of PAI-1 is 4.5-5.0 **[137]**. PAI-1 possesses three potential glycosylation sites i.e. Asn209, Asn265 and Asn329, where Asn329 is not glycosylated **[138]**.

PAI-1 is synthesized as an active molecule with very short half-life in plasma ($t_{1/2}$ = 2 h at 37°C) **[139]**. Active PAI-1 spontaneously converts to an inactive latent conformation that can be partially reactivated by denaturing agents **[140]**. In plasma, active PAI-1 can be stabilized by binding to vitronectin (75 kDa

glycoprotein) **[141]**. A third structural conformation of PAI-1, which acts as a non-inhibitory substrate toward its target proteinases has also been reported **[142]**. PAI-1 is the only serpin that can reversibly switch between the active and latent conformational states.

Three dimensional structure of the active form of a stable PAI-1 mutant has shown that the N-terminal side of the reactive site loop is exposed and accessible to the target proteinase **[143]**. The C-terminal side of the reactive site loop (P4'-P13') forms strand s1C in β-sheet C. PAI-1 inhibits plasminogen activators (PA) by the formation of a covalent complex, thus blocking PA for further interaction with its substrate **[144]**.

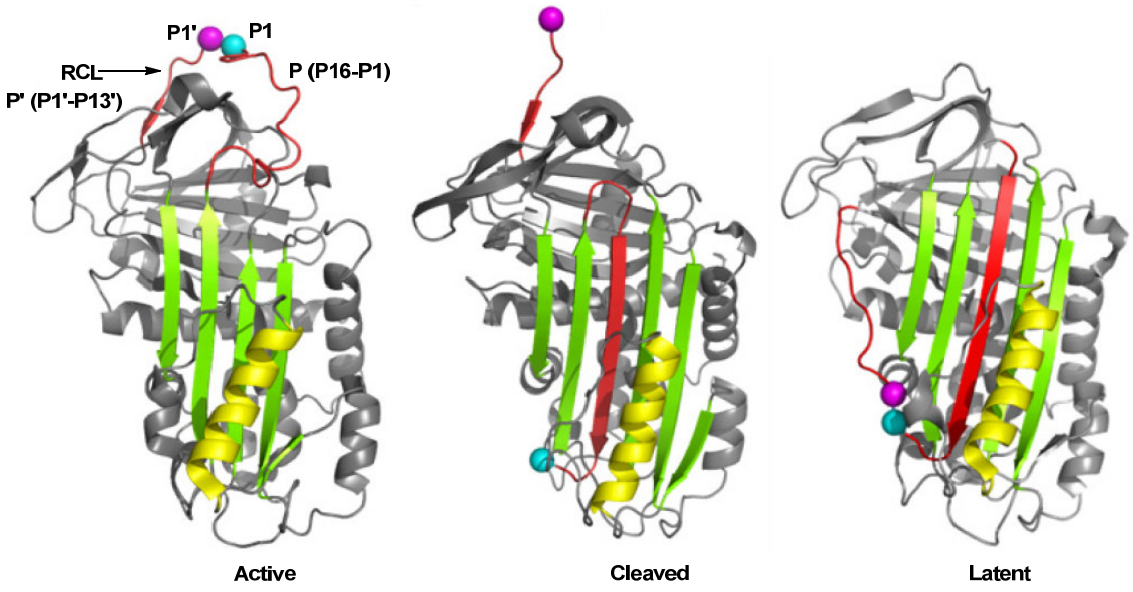

Figure 10. The structure of PAI-1 in the active, cleaved and latent conformation. β-sheet A is indicated in green, α-helix F in yellow, the reactive site loop (RCL) is indicated in red and the reactive site residue Arg^{346}-Met^{347} are represented as blue and purple spheres, respectively

The inhibitory process by the serpin involves the formation of a noncovalent reversible Michaelis-like complex, followed by an acyl intermediate

and finally the formation of an ester bond between the carboxyl group of the P1 residue and the hydroxyl group of the serine residue of protease **[145]**. Once the initial complex is formed, the P1-P1' bond in the RCL is cleaved followed by an insertion of the P1-PA complex from the initial interaction site into the opposite side of the PAI-1. This distortion of the protease inhibits its catalytic activity. In this covalent complex, the PAI-1 is cleaved at the P1-P1' site, which limits inhibitory activity of PAI-1 up to a single encounter with its target protease **[146]**. Active form of PAI-1 get converted into latent state through insertion of the N-terminal side of the reactive site loop into β-sheet A forming the new β-strand 4A. The loss of strand s1C from β-sheet C and the formation of an unusual extended loop by the C-terminal side of the reactive site loop results in the distortion of the P1-P1' peptide bond **[147]**. The three-dimensional structure of a cleaved form (non-inhibitory substrate form) of PAI-1 revealed the insertion of the P1-P16 portion of the RCL into β-sheet A **[148]**.

1.5.3. PAI-1 gene

The human PAI-1 gene is located on chromosome 7 and contains nine exons and eight introns (**Figure 11**) **[149]**. There are reports on polymorphisms within the gene, which includes a cytosine–adenine $(CA)_n$ dinucleotide repeat, a *Hin*dIII restriction-fragment–length polymorphism, and a common single-base-pair polymorphism (four or five guanine bases) in the promoter region of the gene, 675 bp upstream of the transcriptional start site (4G/5G). **[150]**. Subjects who are homozygous for the 4G allele (4G/4G genotype) possesses 25 percent

higher plasma PAI-1 concentrations when compared to those who are homozygous for the 5G allele (5G/5G genotype).

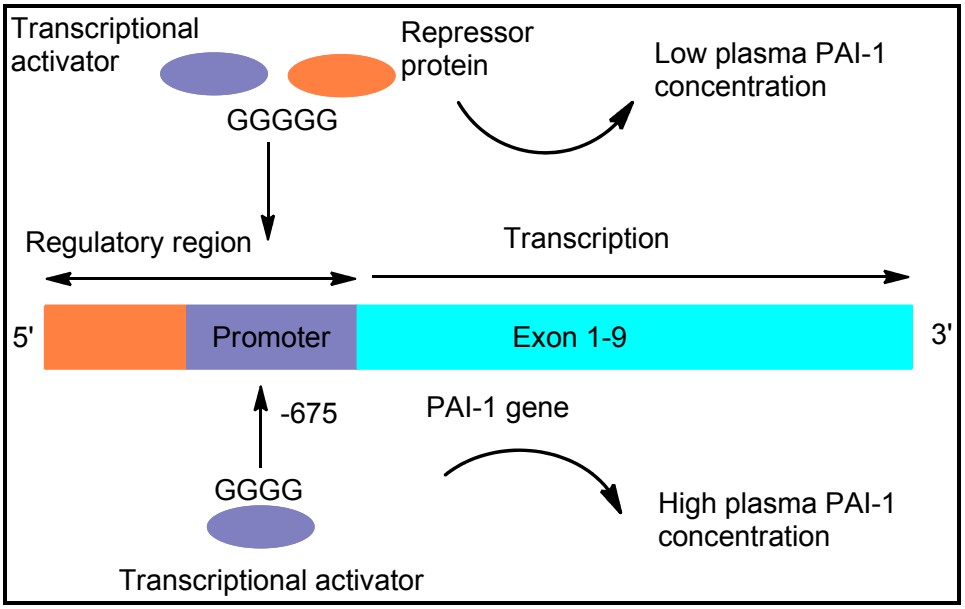

Figure 11. Structure of the gene for PAI-1 and the site of the 4G/5G polymorphism in the promoter region

In vitro studies have identified differential binding of transcription-regulating proteins at this site. Increased gene transcription is associated with four guanine bases (the 4G allele), and results in the binding of a transcriptional activator alone, whereas with five guanine bases (the 5G allele), there is also binding of a repressor protein that decreases the binding of the activator (**Figure 11**) **[151, 152]**.

1.5.4. PAI-1 expression

PAI-1 production is observed in endothelial cells, megakaryocytes, smooth muscle cells, fibroblasts, monocytes/macrophages, adypocytes, endometrium, peritoneum, liver cells, mesothelial cells, and cardiac myocytes **[153]**. Once

produced, PAI-1 is mainly stored in platelets, but only about 10% of this is in the active form. The number of PAI-1 molecules per platelet was reported to be 4000-8000 **[154]**. After release into the bloodstream, PAI-1 is present either in an active form or complexed with either its target protease t-PA or its cofactor vitronectin.

Physiological concentration of PAI-1 in mouse and human plasma ranges from 10-20 ng/ml **[140]**. Under pathological conditions the plasma concentration of PAI-1 rises several folds depending on disease severity. There is a circadian variation in plasma PAI-1 levels, and hence variable PAI-1 activity resulting in diurnal variation in net fibrinolytic activity **[155]**. PAI-1 levels peak in the early morning and hence decrease in fibrinolytic activity, whereas the afternoon fall in plasma PAI-1 increases endogenous fibrinolysis. High PAI-1 concentration in the morning, which corresponds to low fibrinolytic activity results in the high incidence of AMI **[156]**.

1.5.5. Regulation of PAI-1

PAI-1 is an acute phase reactant, and its expression is up regulated by inflammatory cytokines such as interleukin-6 (IL-6) and tissue necrosis factor, as well as TGF-β **[157-159]**. Circadian clock proteins such as CLOCK, BMAL and CRY regulate PAI-1 expression, leading to diurnal variation in circulating PAI-1 concentrations **[160]**. Nitric oxide **[161]**, angiotensin-converting enzyme inhibitors and mineralocorticoid receptor antagonists decrease PAI-1 expression or PAI-1 activity **[162]**. PAI-1 expression is up regulated by angiotensin II and aldosterone in a variety of cell types **[163]**.

1.5.6. PAI-1 target proteins

PAI-1 is the major physiological inhibitor of t-PA, within the intravascular space. t-PA, when bound to fibrin in a clot, activates plasminogen to its active form of plasmin, which subsequently degrades fibrin **[17]**. The primary role of PAI-1 in the extravascular space is to regulate matrix remodeling via inhibition of the u-PA. PAI-1 inactivates both t-PA and u-PA by forming 1:1 complex. (**Figure 12**).

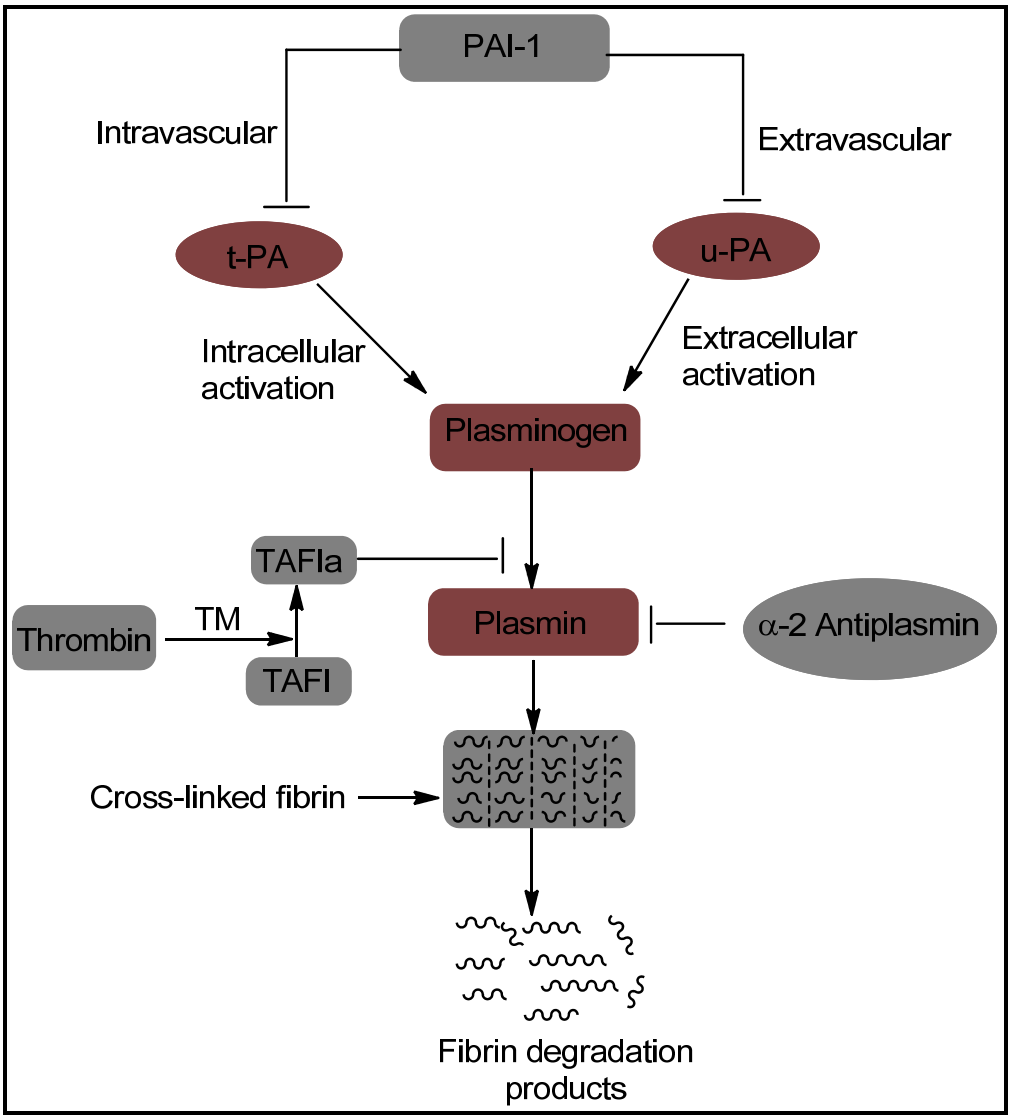

Figure 12. Physiological role of PAI-1

t-PA is secreted by endothelial cells as a single polypeptide chain, resulting in plasma concentrations of about 5 ng/ml. This 68 kDa serine protease has a fast clearance from the circulation with a plasma half-life of only 5 min **[164]**. u-PA was originally isolated from urine **[80]** but later it was found to be secreted by a variety of cells.

The other target protein includes Vitronectin, Heparin and Lipoprotein receptor-related protein (LRP). PAI-1 is normally bound to vitronectin in the circulation, which leads to increased stability of PAI-1 **[165]**. Vitronectin is a 75 kDa multifunctional glycoprotein which is deposited at sites of injury, where it binds multiple ligands such as collagens, fibrin, uPAR, integrins, and PAI-1. Vitronectin is also found to be present in plasma, platelets, and the ECM.

Heparin is the analog of sulfated polysaccharide which binds to PAI-1, and activates serpins such as AT-III, heparin cofactor II, protease nexin 1 and protein C inhibitor. In contrast to vitronectin, heparin does not stabilize the active conformation of PAI-1 **[166]**.

PAI-1 not only binds to free u-PA but also to uPAR–bound u-PA **[167]**. u-PA/PAI-1 complex interact with the transmembrane α2-macroglobulin receptor low-density lipoprotein (LDL) receptor-related protein (LRP), an endocytic receptor. The u-PA/PAI-1 complex is internalized by the combined action of uPAR and LRP, leading to degradation of complex in lysosomes. uPAR is then recycled back to the cell surface **[168]**.

1.5.7. PAI-1 and diseases

1.5.7.1. PAI-1 in thrombosis

As an inhibitor of plasminogen activation and fibrin degradation, it is logical that elevated levels of PAI-1 in serum would lead to thrombosis. PAI-1 knockout mice showed increased tendency toward spontaneous fibrinolysis **[169]**. In an experimental model, transgenic mice expressing a stable form of human PAI-1 develop spontaneously coronary thrombus **[170]**. Elevated levels of PAI-1, especially in the elderly, are thought to be associated with both venous and arterial thrombosis **[171]**. PAI-1 deficient patients have bleeding problems after surgery or trauma which confirms its role in controlling of fibrinolysis. On the other hand, increased levels of PAI-1 in patients are associated with thrombotic disorders such as MI **[172]**, unstable angina **[173]**, stroke **[174]**, DVT **[175]**, PE **[176]**, and peripheral artery disease **[177]**. The correlation between clinical cardiovascular events and high plasma PAI-1 levels was also observed in survivors of a first MI **[178]**.

1.5.7.2. PAI-1 in atherosclerosis

Atherosclerosis is a chronic inflammatory condition initiated in the endothelium and maintained by interactions between lipoproteins, macrophages and the arterial wall. Several experimental findings indicate that PAI-1 may also contribute to the development of atherosclerosis. For e.g., Schneiderman *et al.,* studied the expression of PAI-1 mRNA in aortic segments from patients with atherosclerotic diseases **[179]**. Further experiments confirmed this finding by measuring PAI-1 protein in atherosclerotic lesions or stenosed vein grafts **[180]**.

Together, these data suggest that inhibition of fibrinolytic activity by PAI-1 in the vessel wall may accelerate atherosclerosis possibly by facilitating fibrin deposition within the lesions.

1.5.7.3. PAI-1 in metabolic syndrome

The metabolic syndrome consists of a cluster of metabolic abnormalities which include obesity, impaired glucose tolerance, hyperinsulinemia, dyslipidemia with elevated triglyceride level, low high-density lipoprotein cholesterol concentration, and hypertension, all well-documented risk factors for cardiovascular disease **[181]**. Increased circulating PAI-1 concentrations and activity are a hallmark of insulin resistance and type 2 diabetes **[182]**. PAI-1 is synthesized in adipose tissue, and circulating PAI-1 concentrations correlate with body mass index and markers of insulin resistance in clinical studies. Studies in PAI-1-deficient mice suggest that PAI-1 contributes to the development of obesity and diabetes **[183]**.

1.5.7.4. PAI-1 in cellular migration

Cell migration is the locomotion of a cell over an ECM substratum. u-PA stimulates cell migration by catalyzing plasminogen activation for proteolysis of substratum and thus releasing cells from the substratum **[184]**. PAI-1 activation would be expected to inhibit plasminogen activation-dependent cell migration due to negative regulation of plasminogen by it. However, it has been shown that PAI-1 can directly block cell attachment and migration independent of its anti-proteolytic activity **[185]**. PAI-1 can play a role in cell migration in view of its dual roles in regulating the cell adhesion. Pro-migratory or anti-migratory role of PAI-1

depends on its location at the leading or trailing edge of the cell and also on its concentration **[186]**.

1.5.7.5. PAI-1 in cancer

The role of PAI-1 in angiogenesis has not been established even after extensive studies **[153]**. It has been discussed that PAI-1 could be a therapeutic target in cancer, as high levels of PAI-1 in extracts of human primary malignant tumors is used as informative biochemical marker of poor prognosis in several cancer types **[187]**.

1.5.7.6. PAI-1 in diabetic nephropathy

PAI-1-deficient mice are found to be protected against the fibrogenic response to ureteral obstruction, diabetic nephropathy, and aldosterone/salt-induced glomerular injury **[188-190]**. In an experimental model of glomerulonephritis, intravenous administration of a mutant PAI-1 that lacks its protease inhibitory activity restores plasmin generation and reduces matrix accumulation of collagen I, collagen III, fibronectin and laminin **[191]**. Studies of Gonzalez *et al*., have suggest that PAI-1 inhibitors may be useful in preventing progression of chronic kidney injury and also in preventing acute kidney injury **[192]**.

1.5.7.7. PAI-1 in tissue fibrosis

Fibrosis can be defined as a fibroproliferative or abnormal fibroblast activation–related disease. Deregulation of wound healing leads to hyperactivation of fibroblasts and increased accumulation of ECM proteins in the

wound area, the pathological expression of fibrosis. During wound healing, elevated levels of PAI-1 inhibit u-PA/t-PA/plasmin and plasmin-dependent MMP activities, promoting the process of wound healing. However, under pathologic conditions, excessive PAI-1 contributes to excessive accumulation of collagen and other ECM protein in the wound area and thus preserves scarring. Thus PAI-1 is implicated in the pathology of fibrosis in different organs including lungs **[193]**, kidneys **[194]**, liver **[195]**, skin **[196]** and heart **[197]**.

1.6. Coagulation Factor Xa (FXa)

1.6.1. Discovery of FXa

FXa was first identified in 1956 by Telfer and colleagues who described a bleeding tendency in a patient (called Miss Prower) which was not assignable to a deficiency in any known coagulation factor at the time. In 1957, Hougie and co-workers described abnormal coagulation parameters in a 36-year old man named Mr. Stuart who similarly was deficient for FX. Coagulation FX then given a name of Stuart-Prower factor derived from the name of patients. The International Committee for the Standardization of the Nomenclature of Blood Coagulation Factors officially adopted the Roman numeral designation in 1958 and Stuart-Prower factor was renamed FXa.

It took almost seven years to recognize the role of FX in the conversion of prothrombin to thrombin and it was given a unique position in the coagulation cascade at the point of convergence of the intrinsic and extrinsic coagulation pathways **[4]**.

1.6.2. Structure of FXa

The inactive precursor to FXa is a 27 kb F10 gene and is located on chromosome 13q34, adjacent to the *F7* gene and contains 8 exons and 7 introns. Exon I codes for the signal peptide; exon II encodes the propeptide sequence and the Gla domain; exon III codes for a short aromatic stack; exons IV and V encode epidermal growth factor-like (EGF) domains 1 and 2, respectively; exon VI codes for the activation peptide; and exons VII and VIII encode the serine protease domain (**Figure 13A**) **[198]**.

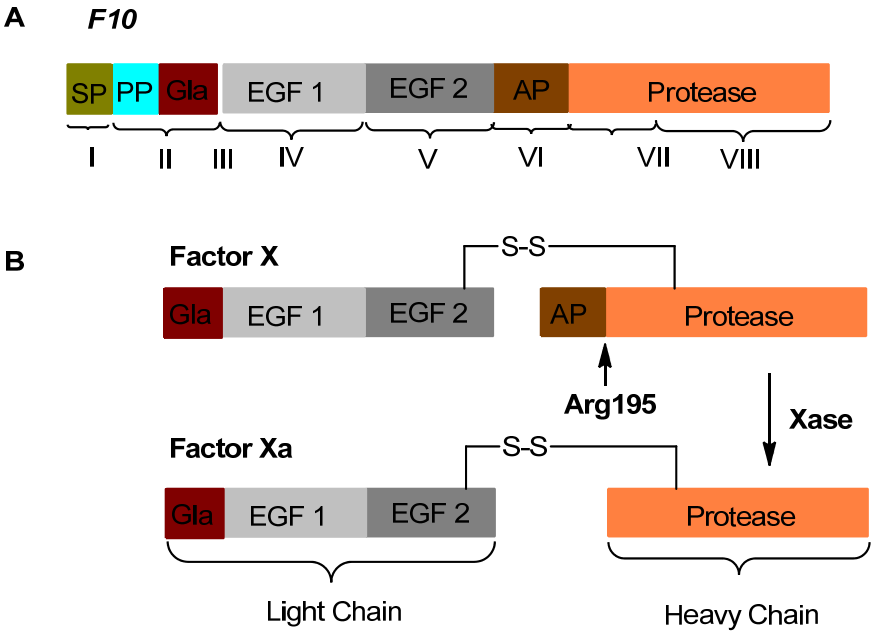

Figure 13. Structure of *F*10 gene and activation of FX to FXa by the Xase complexes **(A)** *F*10 gene **(B)** FX structure and activation: FX circulates as a two-chain disulfide-linked zymogen. During activation, either a intrinsic or extrinsic Xase complex cleaves after Arg195 to release the activation peptide from the N-terminus of the heavy chain

The production of FX is in the liver as a single-chain 59 kDa precursor and it circulates in the bloodstream as a two-chain zymogen (inactive precursor) at a concentration of approximately 170 nM **[199]**. The intracellular excision of an

Arg-Lys-Arg tripeptide between the second EGF domain and the activation peptide converts single chain FX to two-chain FX. The heavy chain of this mature FX is 303 residues in length and contains the activation peptide and the serine protease domain (**Figure 13B**). The light chain having 139 residues is connected to the heavy chain by a single disulfide link at Cys132-Cys302 and contains the Gla domain, the aromatic stack and both EGF domains.

FX is subjected to extensive post-translational modifications which include glycosylation, γ-glutamyl carboxylation and β-hydroxylation. During processing, the activation peptide undergoes glycosylation at several sites such as, Thr159 (O-linked), Thr171 (O-linked), Asn181 (N-linked), and Asn191 (N-linked) **[200]**. The 11 glutamic acid residues in the N-terminal Gla domain are modified to γ-carboxyl glutamic acids by the vitamin K-dependent enzyme γ-glutamyl carboxylase which is important for proper enzymatic function as the Gla domain is responsible for calcium-dependent aPL binding.

The first crystal structure of human FXa describing a Gla-domainless form of FXa was published in 1993 **[201]**, (**Figure 14**). The first EGF domain was found to be flexibly disordered while the second EGF domain was oriented to make a number of interactions with residues on the surface of the catalytic domain. Authors during crystallization of FXa observed heterogeneous cleavage of a basic surface exposed region known as the autolysis loop within the catalytic domain. This loop (Arg326-Arg336) contains four basic residues and is susceptible to both proteolytic and autoproteolytic cleavage and also plays a crucial role in inhibitor recognition and interaction **[202]**.

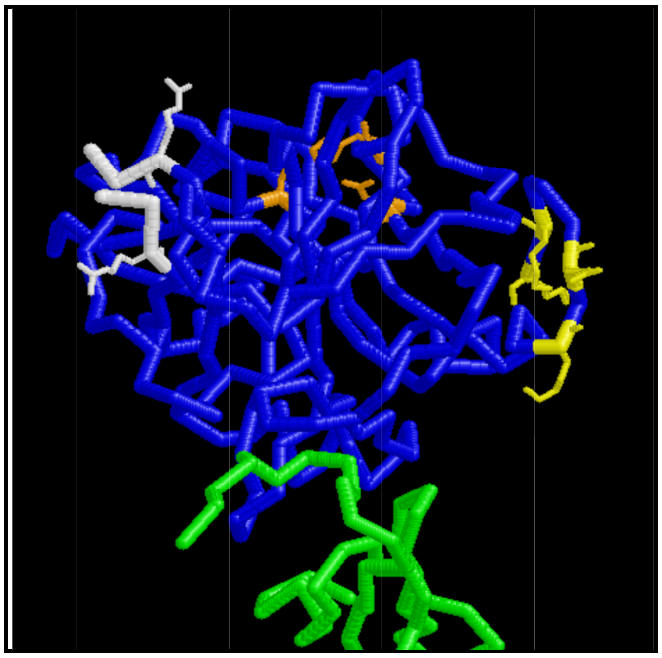

Figure 14. FXa crystal structure (PDB accession code *1HCG*) highlighting the autolysis loop and β-peptide. The FXa heavy chain (blue) and a portion of the light chain (green) are shown. The autolysis loop, comprised of residues Arg326-Arg336, is colored white with basic residues displayed as sticks. The β- peptide basic residues are colored yellow. The catalytic triad is highlighted in orange.

1.6.3. Expression of FX

FX (inactive precursor of FXa) is synthesized in liver and circulates in blood vessels to effect intravascular blood coagulation. Ectopic expression of FX occurs in a variety of tissues, including microglia, neurons, epithelial cells of the nose, bronchus, duodenum, kidney, lung, heart and macrophages **[203, 204]**. Ectopic expression of FX by cancer cells was recently reported in ovarian cancer cells **[205]**. Additionally, ectopic expression of FX has been observed in a murine model of Parkinson's disease **[206]**, during experimental glomerulonephritis **[207]** and in a murine model of asthma **[208]**.

1.6.4. Regulation of FXa activity

There are three direct plasma inhibitors of FXa, which includes TFPI, protein Z-dependent protease inhibitor (ZPI), and AT. TFPI circulates in plasma at a concentration of approximately 8 nM. It downregulates the extrinsic pathway and thus FXa by two-fold **[209]**. FXa in the prothrombinase complex is protected from TFPI-mediated inhibition.

Another plasma inhibitor of FXa is ZPI, a serine protease inhibitor (serpin) which circulates in plasma as a tight complex with its cofactor, protein Z (PZ). ZPI:PZ binds to phospholipid membrane-bound FXa, at which time PZ dissociates from the newly formed ZPI:FXa complex **[210]**. The ZPI:FXa complex is reversible unlike most serpins which forms stable inhibitory complexes with their target proteases **[202]**. Incorporation of FXa into the prothrombinase complex does not appear to protect the enzyme from ZPI-mediated inhibition **[211]**.

The third physiological FXa inhibitor, AT, is also a serpin and circulates at a relatively high plasma concentration of 2.3 μM **[212]**. AT forms an irreversible inhibitory complex with FXa and its interaction with FXa is enhanced approximately 1000-fold in the presence of heparin. There is extensive evidence that incorporation of FXa into the prothrombinase complex protects FXa from inhibition by AT both in the presence and absence of heparin **[213]**.

1.6.5. FXa target proteins and cofactors

The primary role of FXa in coagulation is activation of prothrombin to thrombin in the prothrombinase complex with non-enzymatic cofactor FVa,

calcium and aPL. FV, the single-chain inactive procofactor form of FVa, is a 330 kDa protein that circulates in human plasma at a concentration of 20 nM **[214]**.

FXa first cleaves prothrombin at Arg320, which separates the A and B chains, generating the intermediate known as meizothrombin (**Figure 15**). A second FXa-mediated cleavage then occurs at Arg271 to release the Gla domain and two Kringle domains, collectively known as fragment 1.2, from the N-terminus of the A chain to yield the mature thrombin enzyme **[215]**.

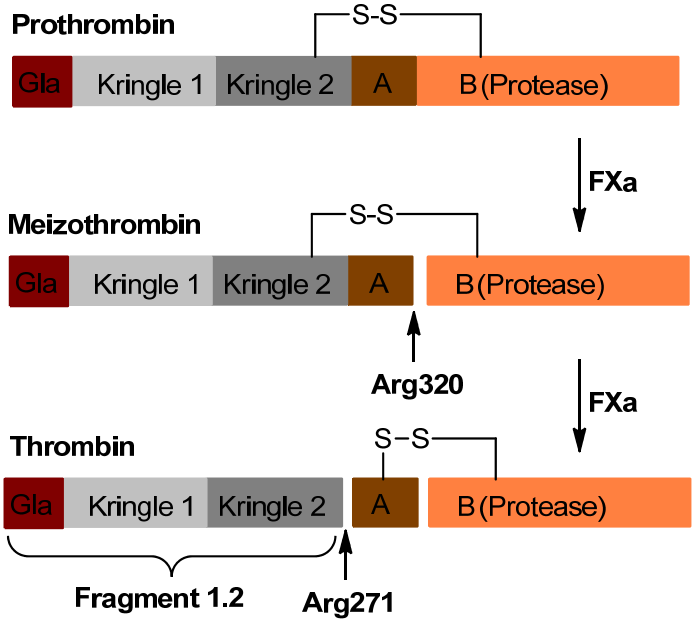

Figure 15. Structure of prothrombin and activation to thrombin by FXa

FXa target receptors outside the coagulation cascade

Recent studies suggest that FXa mediates intracellular signaling via activation of either proteinase-activated receptor-1 (PAR-1) or PAR-2 **[216]**. The PAR family represents seven transmembrane domain G-coupled protein comprising four members, PAR1 to PAR4 out of which PAR1 and PAR2 are the major receptors for FXa signaling and both this receptors are expressed in a

variety of tissues and cells such as the airways, the cardiovascular system, the epidermis, osteoblasts, the immune system, the kidney, nervous system, and in all regions of the gastrointestinal tract.

1.6.6. FXa and diseases

1.6.6.1. FXa in thrombosis

Patients deficient in FXa suffer from severe bleeding tendencies **[217]** suggesting important role in maintaining haemostasis. Alternatively, several preclinical and clinical data of FXa inhibitors have demonstrated potential of FXa inhibition in antithrombotic therapy. Recently, direct FXa inhibitors have been developed for the treatment of thromboembolic diseases **[76]**.

1.6.6.2. FXa in inflammation

The interplay between coagulation and inflammation has a pathological importance in the context of a wide variety of inflammatory conditions, including sepsis, MI, stroke, acute lung injury and glomerulonephritis **[218]**. FXa is involved in the secretion of a host of pro-inflammatory cytokines by numerous cell types, including IL-1, IL-6, IL-8 and MCP-1/CCL2 by fibroblasts **[219, 220]**, IL-2 by lymphocytes **[221]**, as well as IL-6 and IL-8 by endothelial cells **[222]**. These proinflammatory effects of FXa may be mediated via the activation of the PARs.

1.6.6.3. FXa in tissue remodeling and fibrosis

Tissue remodeling and fibrosis share common features such as enhanced fibroblast migration, proliferation and abnormal ECM synthesis. A fibroblast is a type of cell that synthesizes the ECM and collagen, the structural framework for

animal tissues. The accumulation of myofibroblasts (a cell which is in between a fibroblast and a smooth muscle cell in differentiation), associated with excessive ECM biosynthesis and organ destructive remodeling, also actively participates in the progression of fibrotic lesions **[223]**. Various experimental findings support an important role of FXa-dependent signal transduction (PAR-1 or PAR-2) in tissue remodeling and fibrosis **[224, 225]**.

1.6.6.4. FXa in angiogenesis

Beside role in tissue remodeling, myofibroblasts also control angiogenesis either during fibrosis or tumor growth **[226]**. During pathological angiogenesis, activation of the coagulation cascade is frequently observed. FXa-stimulated fibroblasts secrete vascular endothelial growth factor (VEGF) and latent MMPs. FXa also activates these MMPs, which might enhance tissue remodeling during angiogenesis **[227]**. Taking all this facts together, it appears that FXa-dependent signaling in fibroblasts not only affects fibrosis but also be crucial for angiogenesis in case of both fibrotic disease and tumor progression.

1.7. Project rationale

As described, thromboembolic diseases are associated with high mortality and morbidity. Current therapeutic options to treat thrombotic disorders are effective but not adequate enough to meet the patient's requirements. The physiological roles of PAI-1 and FXa in thrombosis have been very well established. In fact, few FXa inhibitors are already approved as oral anticoagulants, which provides a rationale to develop inhibitors of PAI-1 as a thrombolytic agent and new and improved FXa inhibitors as an anticoagulants.

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PAI-1 inhibitors

2. PAI-1 INHIBITORS

2.1. Background

In view of well proven role of PAI-1 inhibition in various thrombotic disorders, several polyclonal and monoclonal antibodies, antisense oligonucleotides against PAI-1 have been developed. Orally bioavailable small molecule inhibitors of PAI-1 have also been identified with significant antithrombotic efficacy in various preclinical animal models. A detailed literature search of known PAI-1 inhibitors was carried out to derive a synthetic rationale for developing novel PAI-1 inhibitor.

2.1.1. Natural products as PAI-1 inhibitors

The use of *Panax notoginseng* as a tonic to promote normal blood circulation is well known in Chinese traditional medicines. Zhang *et al.,* examined effects of 20 ('S)-protopaxatrol notoginsenoside R1 (NR1), a major constituent of *Panax notoginseng*, on t-PA and PAI-1 synthesis in endothelial cells **[1**, **2]** and found that in cell culture, NR1 stimulated t-PA production by human umbilical vein endothelial cells in a dose-dependent manner.

2.1.2. Monoclonal antibodies and peptides as PAI-1 inhibitors

Monoclonal antibodies that inhibit the inhibitory activity of PAI-1 can be classified in to three different categories: (1) monoclonal antibodies that inhibits PAI-1 by preventing the initial formation of the Michaelis complex between PAI-1 and its target protease **[3]**, (2) monoclonal antibodies that induce substrate behavior in PAI-1, resulting in the formation of cleaved PAI-1 upon the addition of

the proteinase and the regeneration of the active proteinase **[4]**, (3) monoclonal antibodies that accelerate the latency conversion of PAI-1 **[5]**. Various monoclonal antibodies have been identified that neutralize PAI-1 activity [e.g., MAb2 **[6]**, MA-8H9D4, MA-33B8, MA-33H1F7, MA-35A5, MA-55F4C12 and MA-56A7C10 **[4]**]. Eitzman *et al*., designed a 14-amino acid peptide corresponding to the PAI-1 reactive center loop which causes a transition of PAI-1 into a non-reactive form **[7]**. A 17-mer peptide, based on domain 5 of high molecular weight kininogen (which constitutes another vitronectin ligand) prevented the binding of PAI-1 to vitronectin and thus causes destabilization **[8]**.

2.1.3. Small molecule PAI-1 inhibitors

Inhibition of PAI-1 production

Several currently marketed drugs have been reported to inhibit PAI-1 secretion or production by endothelial cells. Fibrates (e.g., Gemfibrozil), a class of antihyperlipidemic agents have shown concentration dependent inhibition of PAI-1 production **[9**-**11]**.

A small molecule compound (3E, 4E)-3-benzylidene-4-(3,4,5-trimethoxy-benzylidene)-pyrrolidine-2,5-dione (T-686) reported by Vinogradsky *et al*., (**Figure 16**) at 10 μM reduced PAI-1 production by 32% in cultured human umbilical vein endothelial cells **[12]**. To improve the physiochemical property of T-686, Hiroshi Miyazaki *et al*., have reported SAR of butadiene derivatives and subsequent identification of T-2639 as orally active inhibitor of PAI-1 production (**Figure 16**). T-2639 showed 49% oral bioavailability in dog and good antithrombotic effect in rat venous thrombosis model **[13**, **14]**.

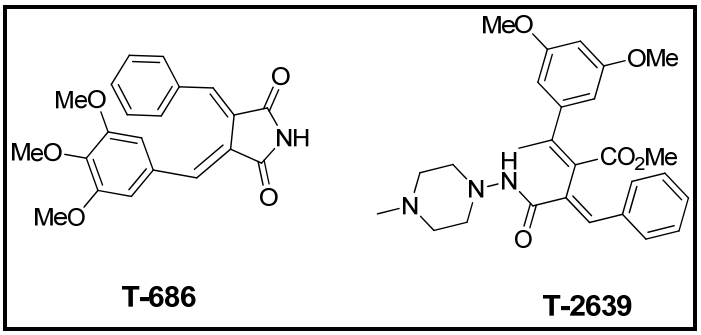


Figure 16. Inhibitors of PAI-1 production

Inhibition of PAI-1 activity

A number of small molecule inhibitors of PAI-1 activity have been developed and tested in preclinical models. The first compounds those falls in this category (i.e. XR330 and XR334) were two diketopiperazines (DKP), produced by and purified from *Streptomyces* species (**Figure 17**) **[15]**. Based on the DKP template, more potent PAI-1 inhibitors have been developed i.e. XR5118 **[16]** and XR11211 **[17]** with IC_{50} values of 15 ± 1 μM and 0.2 ± 0.015 μM, respectively (as measured by a plasmin generation assay) (**Figure 17**). In rabbit jugular vein thrombosis model, two fold increases in endogeneous thrombolysis was found upon administration of XR5118. Tetramic acid based and isoquinoline-based PAI-1 inhibitors were reported by Adrian Folkes *et al*., **[18]** and Shouming Wang *et al*., **[19]** respectively.

In addition to diketopiperazines, AR-H029953XX (**Figure 17**), an anthranilic acid derivative derived from flufenamic acid **[20**, **21]** (IC_{50} of 54 ± 8 μM), fendosal with an IC_{50} value of 36 ± 4 μM have been identified **[21]**. Rupin *et al*., have reported benzothiophene derivative S35225 (**Figure 17**). In a direct chromogenic assay S35225 has an IC_{50} value of 44 ± 0.9 μM for PAI-1 inhibition

[22]. Liang *et al*., have reported menthol-based PAI-1 inhibitors among which **ZK4044** (**Figure 17**) showed very good PAI-1 inhibitory activity in chromogenic assay (IC_{50} = 0.38 μM) and also in clot lysis assay (IC_{50} = 0.01 μM) **[23]**. To improve the oral bioavailability of menthol-based compounds same authors have come up with piperazine-based PAI-1 inhibitors. The most potent compound (**I**) (**Figure 17**) (IC_{50} = 0.5 μM) showed 43% oral bioavailability in rat **[24]**.

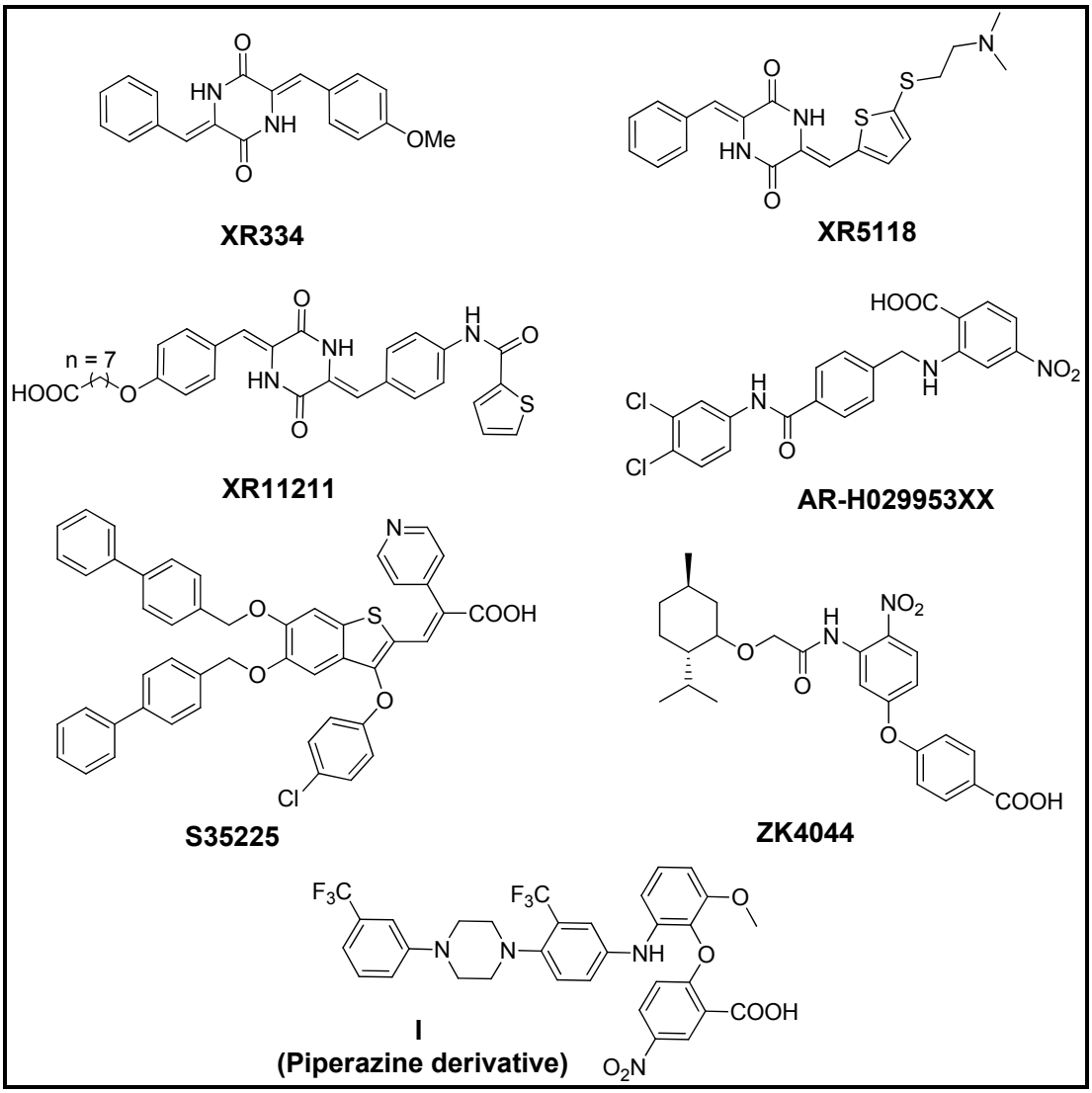


Figure 17. Small molecule PAI-1 inhibitors

Scientists at Wyeth worked extensively on PAI-1 inhibitors. Ariamala Gopalsamy *et al*., have reported oxadiazolidinediones-based PAI-1 inhibitors (**II**, **Figure 18**) (IC_{50} = 5.29 μM) **[25]**. Baihua Hu *et al*., have reported SAR of indole-2-carboxylic acid as PAI-1 inhibitors (**III**, **Figure 18**) (IC_{50} = 8.3 μM) **[26]**. Benzofuran derivative WAY140312 (**Figure 18**) (IC_{50} = 11.7 μM) upon administration to animals, followed by an acute vascular insult, resulted in resistance to arterial and venous thrombosis indicating efficacy in prevention of thrombosis with no effect on platelet aggregation **[27]**.

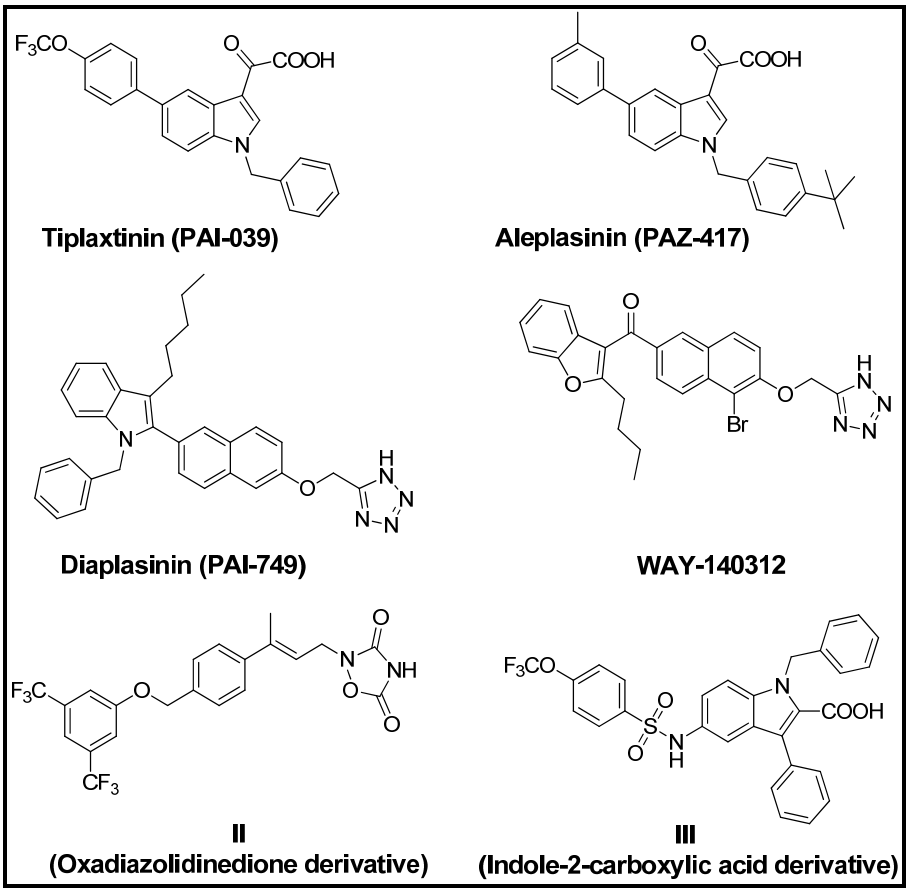


Figure 18. Small molecule inhibitors of PAI-1 reported by Wyeth Pharma

Indole derivative Diaplasinin (PAI-749, **Figure 18**) preserved t-PA and u-PA activity with an IC_{50} equal to 157 ± 9 nM and 87 ± 3 nM respectively **[28]**. However, in clinical model of fibrinolysis, Diaplasinin failed to show any efficacy **[29]**. Indole oxoacetic acid derivatives Tiplaxtinin (PAI-039, **Figure 18**) **[30]** and Aleplasinin (PAZ-417, **Figure 18**) **[31]** were also reported by Wyeth.

Tiplaxtinin is the most studied PAI-1 inhibitor so far which could reach up to Phase I human clinical trial stage. Tiplaxtinin (PAI-039) inactivated human PAI-1 *in vitro* with an IC_{50} of 2.7 µM **[30]**. Acute oral administration of PAI-039 decreased thrombosis in a canine coronary artery model with subsequent thrombus weight reduction in the rat carotid or abdominal vena cava after $FeCl_3$-induced injury **[32]**. PAI-039 does not inhibit other related serpins such as AT-III, α1-antitrypsin, or α2-antiplasmin. In a model of diet-induced obesity, pair-fed C57 Bl/6 mice administered PAI-039 in a high-fat diet exhibited a dose-dependent reduction in body weight, epididymal adipose tissue weight, and circulating plasma active PAI-1 suggesting role of PAI-1 inhibitors in metabolic syndrome. Plasma glucose, triglycerides, and leptin were also significantly reduced in drug-treated mice **[33]**.

Recently, Hiroshi Miyazaki *et al*., have reported pyrrolin-2-one based PAI-1 inhibitors. One of the pyrroline-2-one derivative T-1776 (**Figure 19**) inhibited PAI-1 with IC_{50} = 9.6 µM **[34]**. Further refining in activity produced compound **IV** with more potent PAI-1 inhibitory activity (IC_{50} = 0.65 µM) **[35]**. Izuhara and colleagues identified orally active compounds TM5001 and TM5007 (**Figure 19**) **[36]**, which prevented the formation of PAI-1/t-PA complexes and inhibited PAI-1

activity with an IC_{50} of 28.6 ± 7.3 μM and 29.2 ± 4.2 μM respectively. *In vivo*, TM5007 (300 mg/kg) decreased thrombus formation in a rat arteriovenous shunt model and prolonged time to occlusion in $FeCl_3$-treated mouse artery. TM5007 also significantly reduced bleomycin induced pulmonary fibrosis in mice suggesting the role of PAI-1 inhibitors in tissue fibrosis.

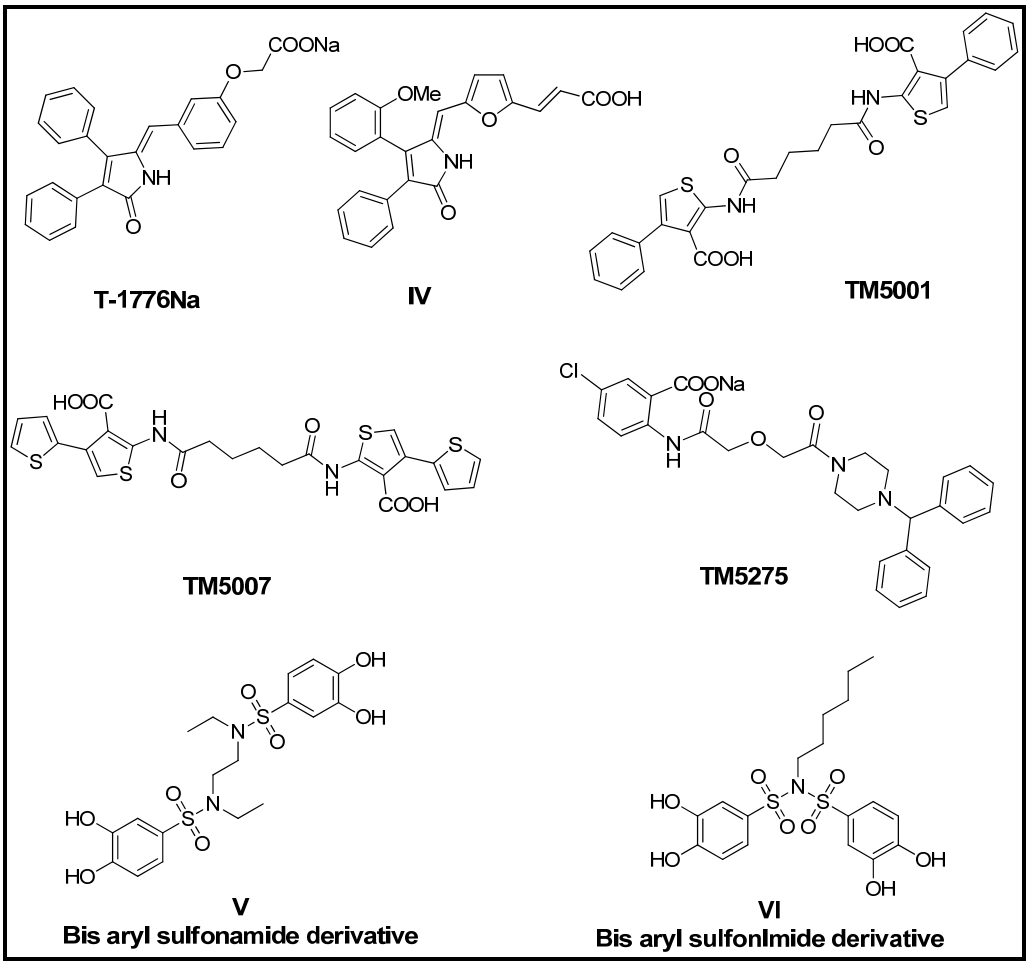


Figure 19. Recently reported small molecule inhibitors of PAI-1

Based on a study of the structure-activity relationship for TM5007, the same group developed a compound TM5275 (**Figure 19**) **[37, 38]**. The authors compared this orally active inhibitor with Ticlopidine in a rat arteriovenous shunt

model, as well as with Clopidogrel in a $FeCl_3$-treated carotid artery thrombosis model in rat and in a photochemical-induced arterial thrombosis model in non-human primate. At the doses tested, TM5275 was similar to Ticlopidine and Clopidogrel in its antithrombotic effects but devoid of bleeding time prolongation to the same extent **[39]**.

El-Ayache *et al.*, described a novel series of compounds based on bis-aryl sulfonamide (**V**, IC_{50} = 9.32 μM) and aryl sulfonimide (**VI**, IC_{50} = 0.284 μM) chemotypes as potent and specific PAI-1 inhibitors (**Figure 19**) **[40]**. Cale and colleagues recently described second generation polyphenolic PAI-1 inhibitors which had IC_{50} values for PAI-1 between 10 and 200 nM with reduction in PAI-1 activity *in vivo* in PAI-1-overexpressing mice **[41]**. However, no PAI-1 inhibitor is currently in clinical use despite the extensive research in PAI-1 inhibition area.

In summary, orally bioavailable small molecule PAI-1 inhibitors have already shown promise in preclinical animal models for thrombosis. Diversified scaffolds have been reported by many research groups. These literature data provides a strong rationale for developing novel oral PAI-1 inhibitor to treat various thrombotic and related cardiovascular disorders.

2.2. Oxalamide derivatives as PAI-1 Inhibitors

2.2.1. Designing strategy

In previous section, we described several small molecule PAI-1 inhibitors reported in literature and their antithrombotic efficacy in preclinical animal models. As a part of our strategy to discover novel PAI-1 inhibitors, we derived common structural features of some advanced or extensively studied PAI-1

inhibitors shown in **Figures 17**, **18** and **19**. It can be seen that the majority of PAI-1 inhibitors contain acid or acid equivalent group as tail. They contain central core to which lipophilic groups containing ring systems are attached (**Figure 20**).

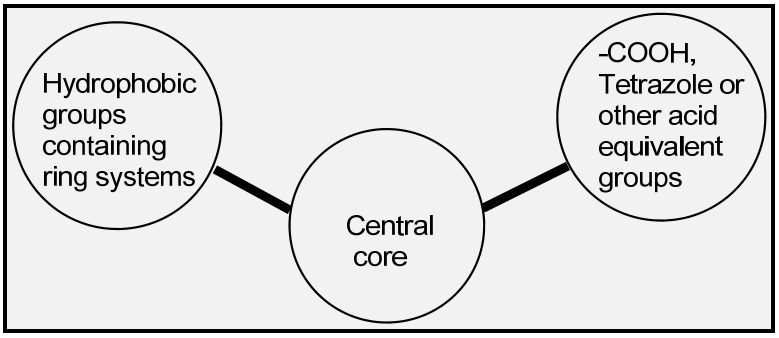


Figure 20. Common structural features of PAI-1 inhibitors

Keeping these structure features in mind, we adopted high throughput screening (HTS) technique to screen compounds containing acid or acid equivalent groups from our compound library and identified compound **1** as our initial lead. In an *in vitro* chromogenic assay for PAI-1 inhibition, **1** inhibited PAI-1 with IC_{50} = 96 μM (**Figure 21**). We then identified two regions for optimization in **1** as described in **Figure 21**. In a first set of compounds to optimize region 2, we replaced phenyl ring of **1** with selected aryl or heteroaryl ring systems possessing hydrophilic and hydrophobic groups represented by general structure **VII**. *In vitro* PAI-1 inhibitory activity data of compounds synthesized based on **VII** revealed 3,5-Bis trifluoromethyl benzyl group as optimized region 2.

In a next set of compounds, we introduced a spacer $–SO_2NH-$ group between region 1 and central phenyl ring and synthesized compounds with general formula **VIII**. Various aryl sulfonyl chlorides were tried to optimize PAI-1

inhibitory activity. Additionally, -NH- of sulfonamide group was substituted with alkyl groups to further optimize the PAI-1 inhibitory activity (**Figure 21)**.

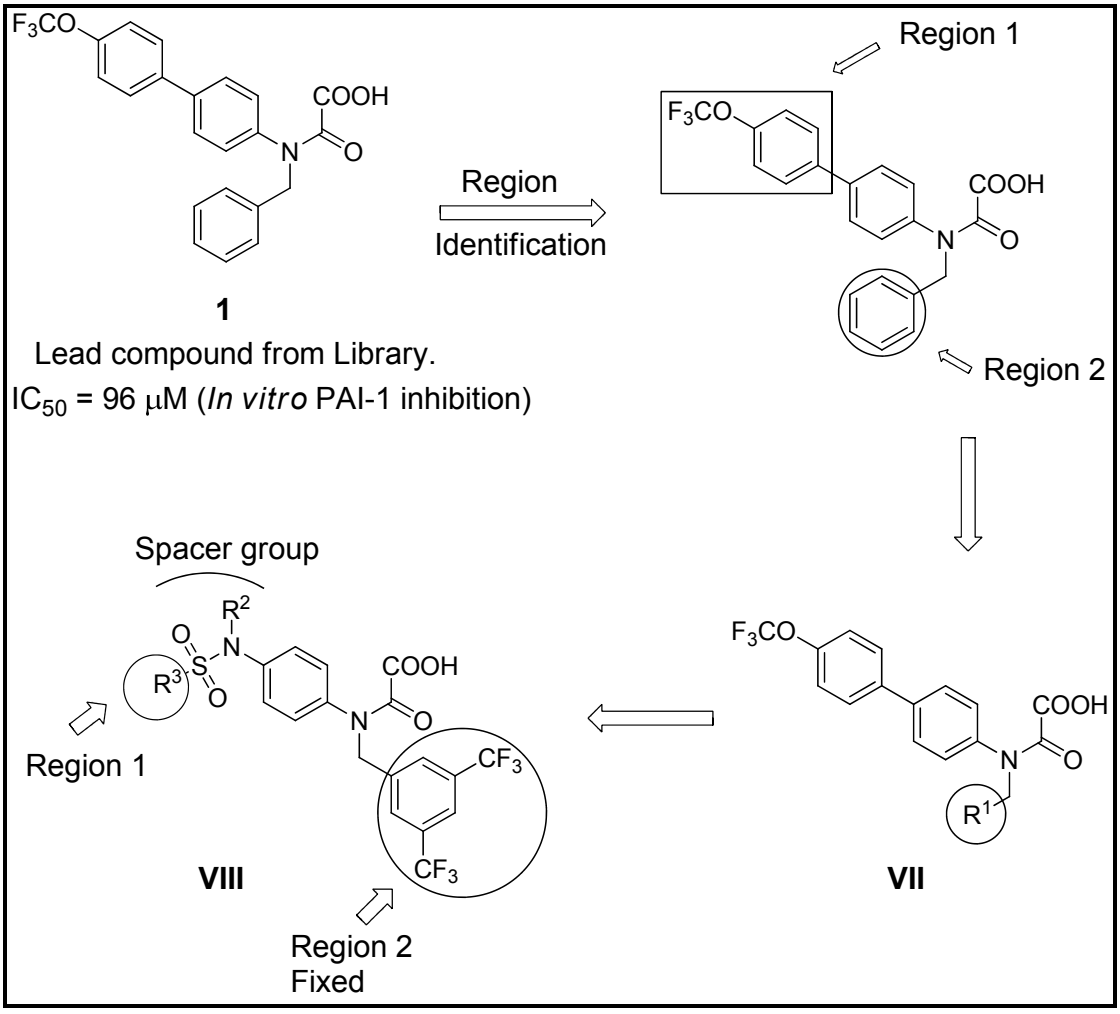


Figure 21. Oxalamide derivatives as PAI-1 inhibitors

2.2.2. Results and discussion

2.2.2.1. Chemistry

Synthetic methodology was designed for general structures **VII** and **VIII** derived from **1** based on the reterosynthetic analysis (**Figure 22**) and the schemes described below.

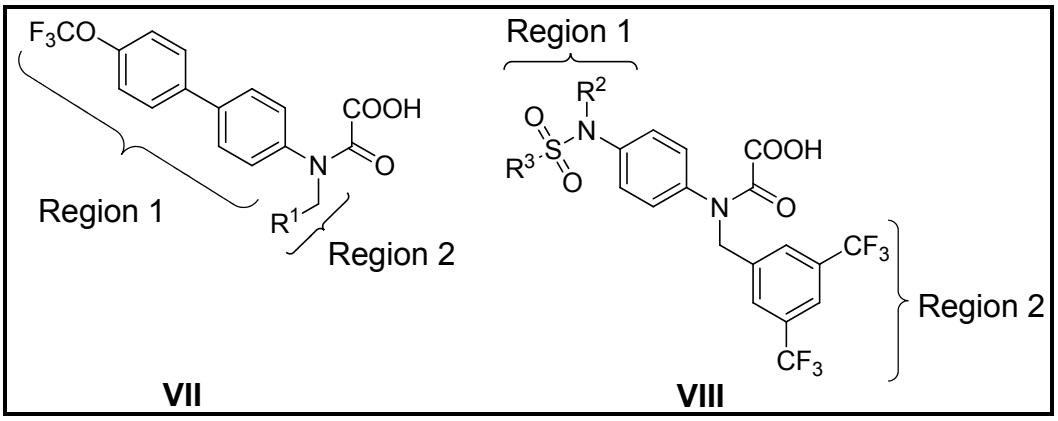


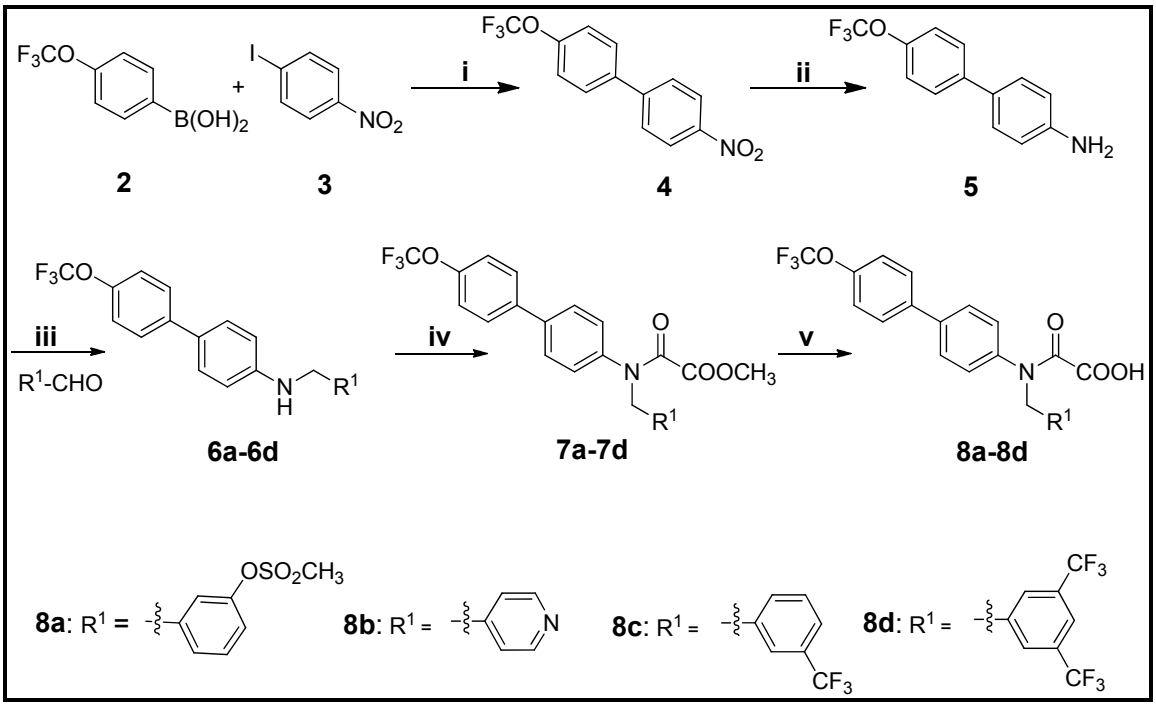
Figure 22. Reterosynthetic analysis of general structures **VII** and **VIII**

General structure **VII** contains biaryl aniline in region 1 (**Figure 22**), which can be synthesized using various procedures reported for biaryl ring system generation. The most common and efficient technique is known to be Suzuki coupling **[42]**, which was successfully used to generate biaryl ring system in our compounds. Aralkyl groups (R^1, region 2) were easily attached to biaryl anilines using reductive amination of corresponding aromatic aldehydes **[43]**. Oxoacetic acid part was introduced with commercially available methyl chlorooxoacetate using simple acid chloride coupling to prepare amides.

General structure **VIII** was prepared similarly as **VII**, since they differ in sulfonamide linker. Various aryl sulfonyl chlorides were reacted with *p*-phenelene diamine in controlled reaction conditions to get desired aryl aniline derivative (**Figure 22**, region 1). Aniline derivative was then reacted using similar synthetic strategies as described for **VII**. Detailed schemes to synthesize analogues of **VII** and **VIII** are described below.

The first set of oxalamide derivatives **8a**-**8d** derived from general structure **VII** were prepared as shown in **Scheme 1**.

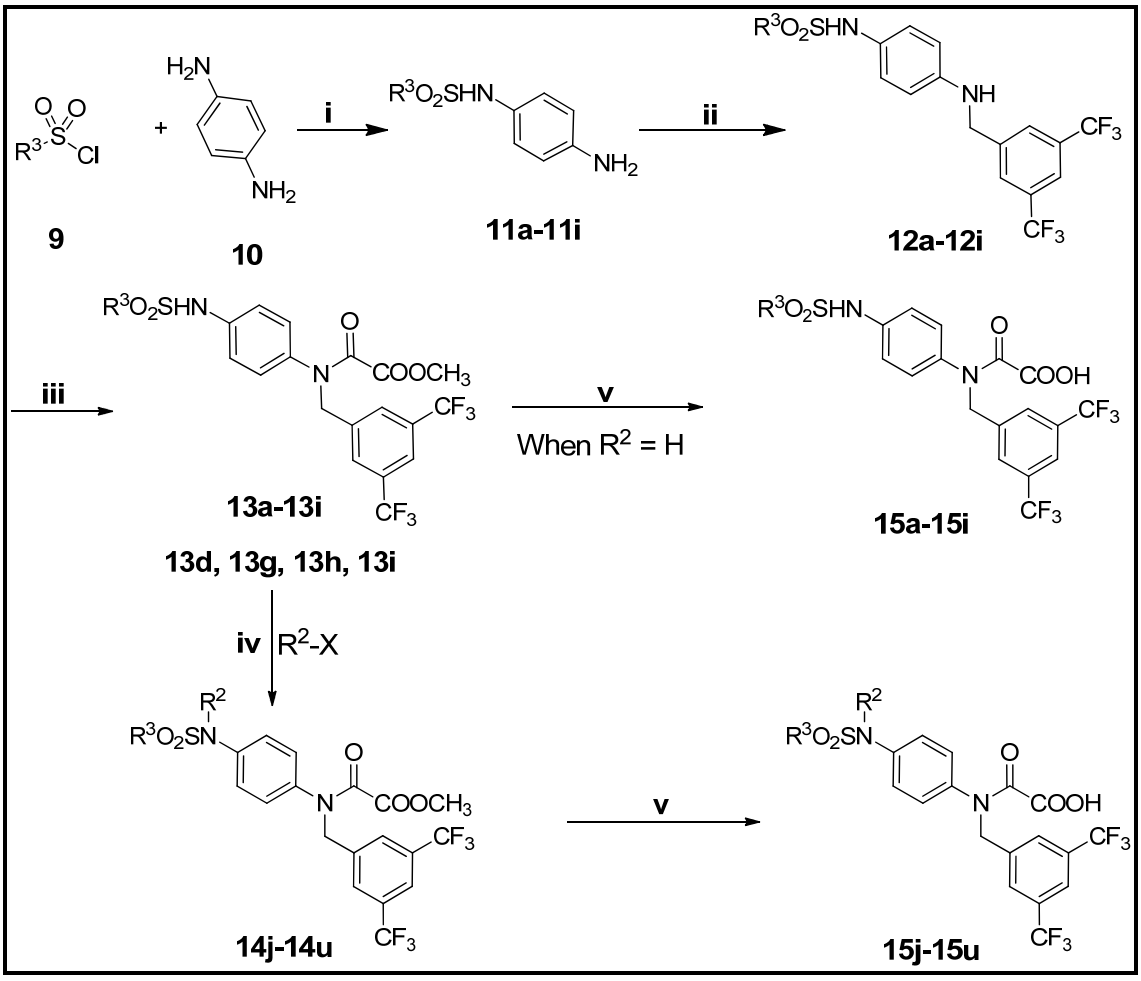
Scheme 1. Synthesis of compounds **8a-8d**



Reagents and conditions: (i) $Pd(OAc)_2$, K_3PO_4, $(C_4H_9)_4N^+Br^-$, DMF, 45-50 ^oC, 16 h, 65%; (ii) Pd-C, H_2 (60 psi), MeOH, 25-28 ^oC, 3 h; (iii) $NaBH_4$, EtOH, 40-45 ^oC, 4-5 h; (iv) ClCOCOOMe, Pyridine, CH_2Cl_2, 5-10 ^oC, 3 h; (v) KOH, THF, water, 25-28 ^oC.

The Suzuki coupling of 4-trifluoromethoxyphenyl boronic acid (**2**) with 4-nitro-iodobenzene (**3**) using palladium acetate ($Pd(OAc)_2$) as a catalyst and tripotassium phosphate (K_3PO_4) as a base gave biaryl nitro derivative (**4**). The catalytic hydrogenation using hydrogen gas and Pd-C produced key intermediate biarylaniline (**5**). The reductive amination of **5** with various aromatic aldehydes was accomplished by first forming Schiff base in ethanol followed by reduction with sodium borohydride ($NaBH_4$) to get reductive aminated products **6a**-**6d**. Reaction of **6a**-**6d** with methyl chlorooxoacetate (ClCOCOOMe) using pyridine as base furnished ester derivative of desired compounds (**7a**-**7d**). The conventional alkaline hydrolysis of compound **7a**-**7d** provided first set of oxalamide derivatives **8a**-**8d**.

Scheme 2. Synthesis of compounds **15a-15u**



Reagents and conditions: (i) DIPEA, CH_2Cl_2, 25-28 ^oC, 15-30 min; (ii) 3,5-bistrifluoromethyl benzaldehyde, $NaBH_4$, EtOH, 40-45 ^oC, 4-5 h; (iii) ClCOCOOMe, Pyridine, CH_2Cl_2 , 5-10 ^oC, 3 h; (iv) K_2CO_3, Acetone , 25-28 ^oC, 2-10 h; (v) KOH, THF, water, 25-28 ^oC.

The second set of oxalamide derivatives **15a**-**15u** derived from **VIII** were prepared as shown in **Scheme 2**. Controlled reaction of substituted aryl sulfonyl chlorides (**9**) with *p*-phenylenediamine (**10**) in presence of diisopropyl ethyl amine (DIPEA) gave sulfonamide derivative **11a**-**11i**. Small amount of dimer, formed when aryl sulfonylchloride reacts with both amino groups of *p*-phenelene diamine was removed by acid-base work up, where only monomer will form salt with HCl. The reductive amination of sulfonamide derivatives **11a**-**11i** with 3,5-

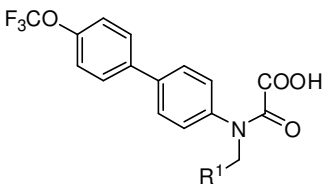
bistrifluoromethyl benzaldehyde gave compounds **12a**-**12i**. The reaction of **12a**-**12i** with methyl chlorooxoacetate yielded ester derivatives **13a**-**13i**, which upon alkaline hydrolysis gave desired acid derivatives **15a**-**15i**. Additionally, the alkylation of **13a**-**13i** with substituted alkyl halide provided compounds **14j**-**14u**, which upon alkaline hydrolysis furnished desired compounds **15j**-**15u**.

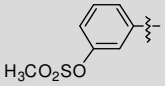
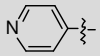
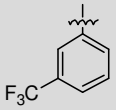
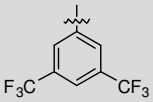
2.2.2.2. Structure-activity relationship discussion of oxalamide derivatives

Oxalamide derivatives synthesized based on general structure **VII** and **VIII** were evaluated for their *in vitro* PAI-1 inhibitory activity using chromogenic assay for human PAI-1 inhibition. Tiplaxtinin was used as positive standard in this assay to rank biological activity of our compounds. The PAI-1 inhibitory activity of oxalamide derivatives **8a**-**8d** (General structure **VII**) and **15a**-**15u** (General structure **VIII**) is summarized in **Tables 6** and **7**.

The first synthesized compounds with polar groups i.e. 3-methanesulfonate phenyl derivative **8a** and pyridyl derivative **8b** did not show PAI-1 inhibitory activity in the chromogenic assay. Introduction of an electron-withdrawing trifluoromethyl group on phenyl ring (**8c**) showed considerable improvement in the activity with an IC_{50} of 23 µM. Additional increase in bulk by introducing trifluoromethyl group on **8c** gave 3,5-bistrifluoromethyl phenyl derivative **8d**, which displayed even better PAI-1 inhibitory activity with an IC_{50} of 14.4 µM (**Table 6**). Based on these results, 3,5-bistrifluoromethyl-substituted phenyl ring in region 2 was considered optimum for PAI-1 inhibition.

Table 6. PAI-1 inhibitory activity of oxalamide derivatives **8a**-**8d**



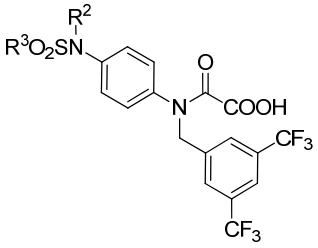
Compound	R^1	IC_{50} (μM)[a]
1		96
8a		No inhibition
8b		No inhibition
8c		23
8d		14.4
Tiplaxtinin	-	10

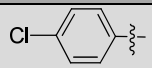
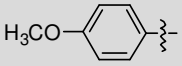
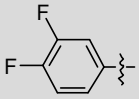
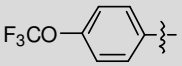
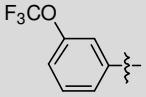
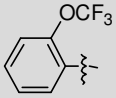
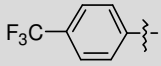
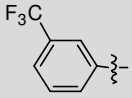
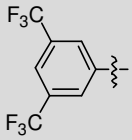
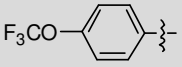
[a]Values determined using *in vitro* PAI-1 inhibitory chromogenic assay.

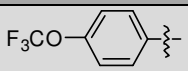
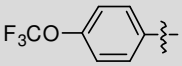
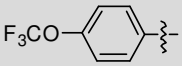
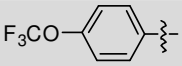
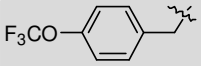
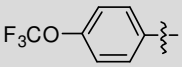
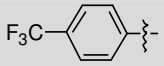
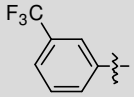
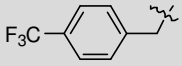
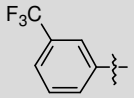
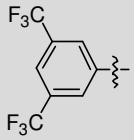
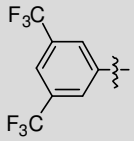
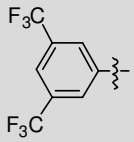
Furthermore, for the modification of region 1, we introduced -SO_2NR^2- as a spacer group between two phenyl rings (**Table 7**). Substitution of electron-releasing groups at the *para* position of the phenyl ring (R^3 of region 1) such as chloro (**15a**) and methoxy (**15b**) produced compounds with low PAI-1 inhibitory activity. Introduction of a less bulky electron-withdrawing fluoro substituent on phenyl ring produced compound **15c** with improved PAI-1 inhibitory activity (IC_{50} = 29.9 μM). Literature reports suggest that the compounds containing electron-withdrawing groups such as trifluoromethyl and trifluoromethoxy groups on phenyl ring displayed good PAI-1 inhibitory activity **[24, 30]**. Taking clue from the literature, first trifluoromethoxy group was introduced on the phenyl ring, which

resulted in compound **15d** and it inhibited PAI-1 activity with IC_{50} = 9.3 μM. However, both positional isomers **15e** (meta, IC_{50} = 15.4 μM) and **15f** (ortho, IC_{50} = 114 μM) displayed inferior inhibitory activity than **15d** (para).

Table 7. PAI-1 inhibitory activity of oxalamide derivatives **15a**-**15u**



Compound	R^2	R^3	IC_{50} (μM)[a]
15a	H		75
15b	H		>80
15c	H		29.9
15d	H		9.3
15e	H		15.4
15f	H		114
15g	H		86
15h	H		4.5
15i	H		6.2
15j	CH_3		25

Compound	R^2	R^3	IC_{50} (μM)[a]
15k	C_3H_7		21
15l	C_5H_{11}		14.9
15m			13.3
15n	Bn		43
15o			8.5
15p	CH_3		61
15q			11
15r			5.4
15s	CH_3		5
15t	C_3H_7		8.4
15u			12.8
Tiplaxtinin	-	-	10

[a]Values determined using *in vitro* PAI-1 inhibitory chromogenic assay.

Introduction of 4-CF_3 group on phenyl ring (R^3 of region 1) produced very less potent compound **15g** with IC_{50} of 86 μM. Further changing the position of -CF_3 group from *para* to *meta*, **15h** showed dramatic improvement in potency (IC_{50} = 4.5 μM). Compound **15i** with 3,5-bistrifluoromethyl substituent showed

comparable PAI-1 inhibitory activity with an IC_{50} of 6.2 μM. In order to see the effect of substituent at free H of sulfonamide group (R^2 of region 1), various compounds were synthesized replacing H atom with methyl in **15j**, propyl in **15k**, pentyl in **15l** and allyl in **15m**. Results of PAI-1 chromogenic assay indicate that bulky alkyl groups helps in improving activity, as **15j** (IC_{50} = 25 μM) is less active than **15k** (IC_{50} = 21 μM), which in turn is less active than **15l** (IC_{50} = 14.9 μM). Compound **15m** (IC_{50} = 13.3 μM) showed similar activity to that of **15l**. However, benzyl substituted compound **15n** showed inferior activity with an IC_{50} = 43 μM. Introduction of 4-OCF_3 group on benzyl ring of **15n** produced compound **15o** with improved PAI-1 inhibition (IC_{50} = 8.5 μM), which may be due to combined interaction of two trifluoromethoxy groups. Substitution of methyl group on sulfonamide linker of **15g** gave compound **15p** with marginally improved activity (IC_{50} = 61 μM). Substitution on sulfonamide linker of **15h** to get **15q** (allyl, IC_{50} = 11 μM) and **15r** (4-CF_3-benzyl, IC_{50} = 5.4 μM) also gave potent PAI-1 inhibitors. In fact, compound **15r** was found to be as potent as **15h** in PAI-1 inhibitory activity. Substituting methyl group in compound **15i** (R^2 of region 1) to get **15s** showed a very promising PAI-1 inhibitory activity with IC_{50} of 5 μM. However, changing methyl group from sulfonamide linker (R^2 of region 1) of **15s** with bulky groups propyl (**15t**, IC_{50} = 8.4 μM) and allyl (**15u**, IC_{50} = 12.8 μM) led to compounds with mediocre *in vitro* activity. This is in contrast to the observation found in compounds **15j**-**15l**, which may be due to crowding of alkyl groups with *meta*-CF_3 group.

Based on *in vitro* PAI-1 inhibitory activity data, few compounds were evaluated for their pharmacokinetic parameters in rats. Compounds (**8d**, **15d**, **15h**, **15i**, **15o**, **15r**, **15s** and **15t**) were dosed orally in rats at 30 mg/kg, and plasma levels were determined. Unfortunately, plasma levels of all the compounds were found to be poor, which precluded further evaluation of these compounds.

In summary, novel oxalamide derivatives as potent PAI-1 inhibitors have been identified. Oral bioavailability was found to be poor for these compounds but they provide a novel template for developing next generation of PAI-1 inhibitors with good oral bioavailability.

2.3. 5-Nitro-2-phenoxy benzoic acid derivatives as PAI-1 inhibitors

2.3.1. Designing strategy

In a next strategy, to discover novel oral PAI-1 inhibitors, we opted two well known and successful strategies which are used to optimize existing molecules and also enable identification of novel scaffold. (**1**) Hybridization of two chemo types (**2**) Conformational restriction.

In order to achieve this objective, we selected Tiplaxtinin (IC_{50} **=** 2.7 μM, Lit. value) **[30]**, the most studied oral PAI-1 inhibitor which could reached upto Phase I human clinical trial and piperazine derivative (**I**), with potent PAI-1 inhibitory activity (IC_{50} = 0.5 μM, Lit. value) (**Figure 23**) **[24]** but with no further progress from preclinical to clinical front. Tiplaxtinin contains indole oxoacetic acid scaffold and published structure-activity relationship (SAR) data suggests importance of 4-trifluoromethoxyphenyl group (lipophilic part) and also its postion

on indole. Piperazine derivative (**I**) contain 5-nitro-2-phenoxybenzoic acid part as an acid group, which was found to be optimum after employing various acid groups.

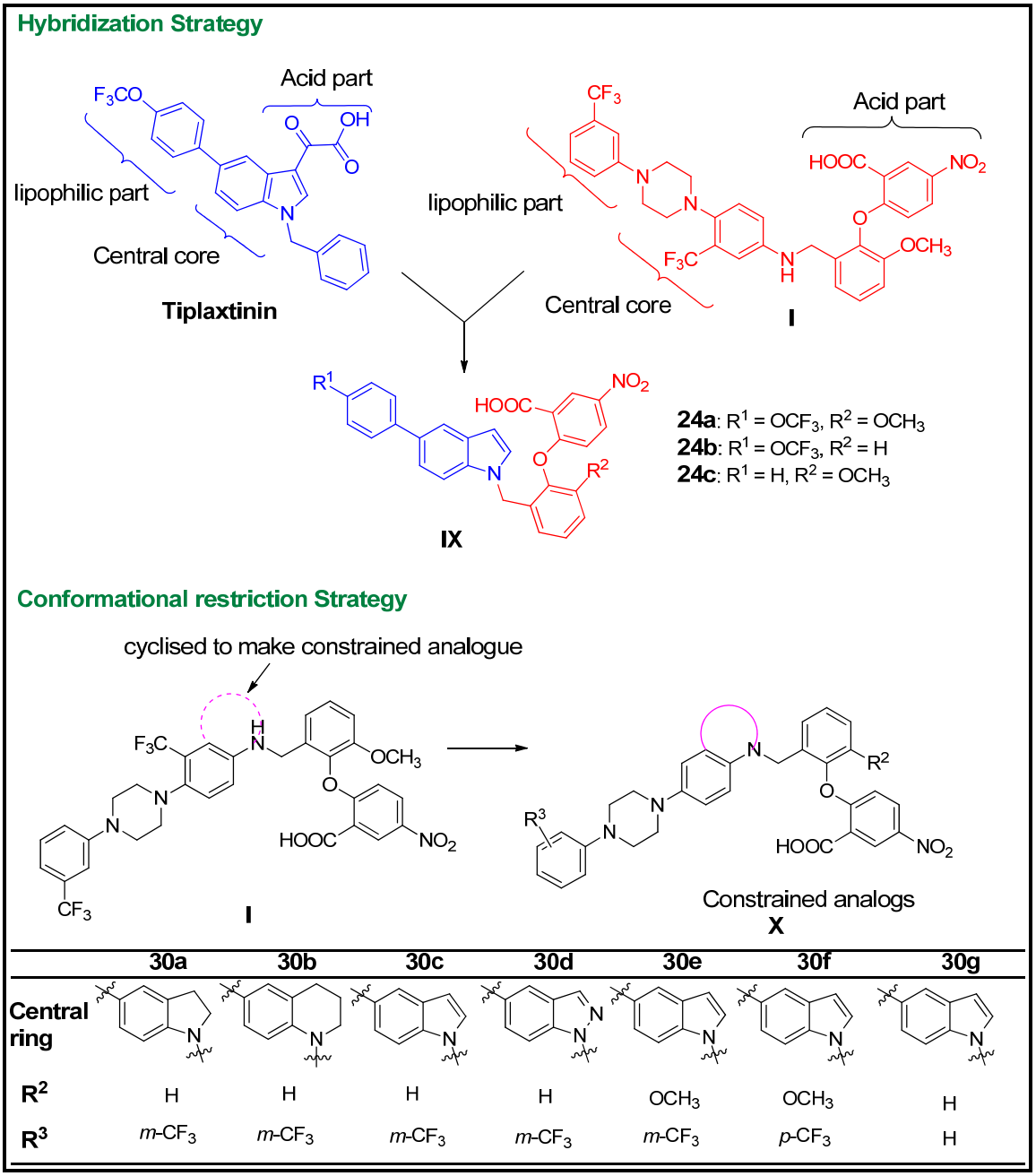


Figure 23. 5-Nitro-2-phenoxybenzoic acid derivatives as PAI-1 inhibitors

We thus proceeded and designed the compounds by incorporating the acid part of (**I)** in the Tiplaxtinin as a probable replacement of oxoacetic acid group of Tiplaxtinin, to get the hybridized molecules **IX** (**24a**-**24c**). Further, rational has been derived from docking study of **24a** and Tiplaxtinin, which revealed that both the compounds possess similar orientation in the ligand binding pocket of PAI-1. The H-bond interaction of carboxylic group of **24a** with Arg118 was found to be the key interaction (**Figure 24**). As an alternative strategy, we intended to make the constrained analogues of (**I)** and subsequntely few analogue **X** (**30a**-**30g**) were synthesized.

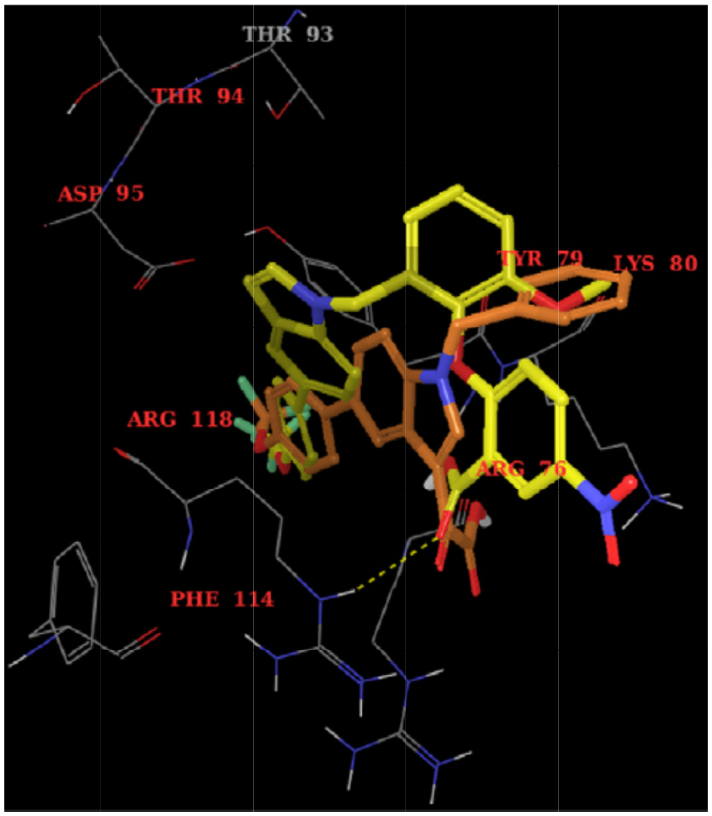


Figure 24. Overlay of docking images of Tiplaxtinin (Orange) and **24a** (Yellow) into active site of PAI-1: H bond interaction of **24a** with Arg118 is shown in dashed line

2.3.2. Results and discussion

2.3.2.1. Chemistry

Synthetic methodology was designed for general structures **IX** and **X** based on the reterosynthetic analysis (**Figure 25**) and the schemes described below. Reterosynthetic analysis of compounds **IX** revealed two regions (**Figure 25**). Region 1 contains biaryl system, which was easily prepared by Suzuki coupling reaction using 5-bromoindole and appropriate aryl boronic acids as discussed in previous section. Region 2 contains biaryl ether part, which was prepared by nucleophilic substitution of activated halogen by substituted phenol in ring containing electron-withdrawing nitro group. Region 1 and 2 were then combined by reacting –NH- group of indole (region 1) with leaving group substituted biaryl ether system (region 2) using normal aliphatic nucleophilic substitution as shown in **Scheme 3**.

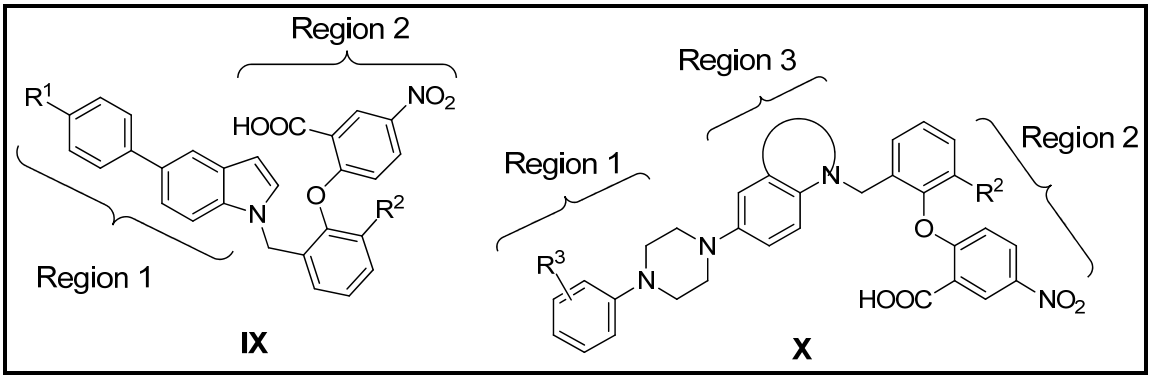
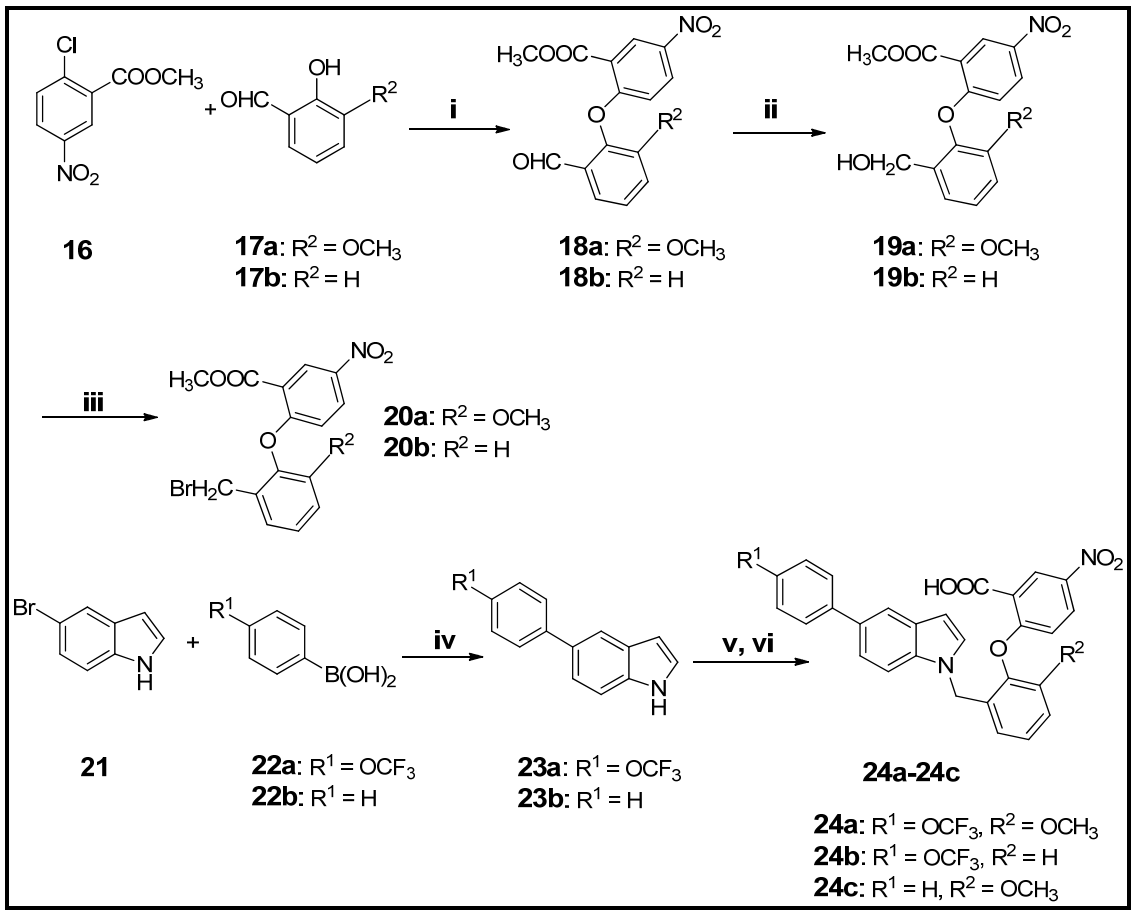


Figure 25. Reterosynthetic analysis of general structures **IX** and **X**

Reterosynthetic analysis of compounds **X** revealed three regions (**Figure 25**). First region contains arylpiperazine functionality which are commercially available. Synthesis of region 2 was similarly carried out as **IX**. Region 3 contains

various heterocycles which are either commercially available or synthesized as described in **Scheme 4**. Region 1 and region 3 were combined using general N-arylation techniques such as Buchwald coupling and palladium based coupling methods **[44, 45]**. Final coupling of region 2 with central core was achieved by general nucleophlic substitution reaction conditions. The compounds **24a**-**24c** derived from **IX** were prepared as shown in the **Scheme 3**.

Scheme 3. Synthesis of compounds **24a-24c**



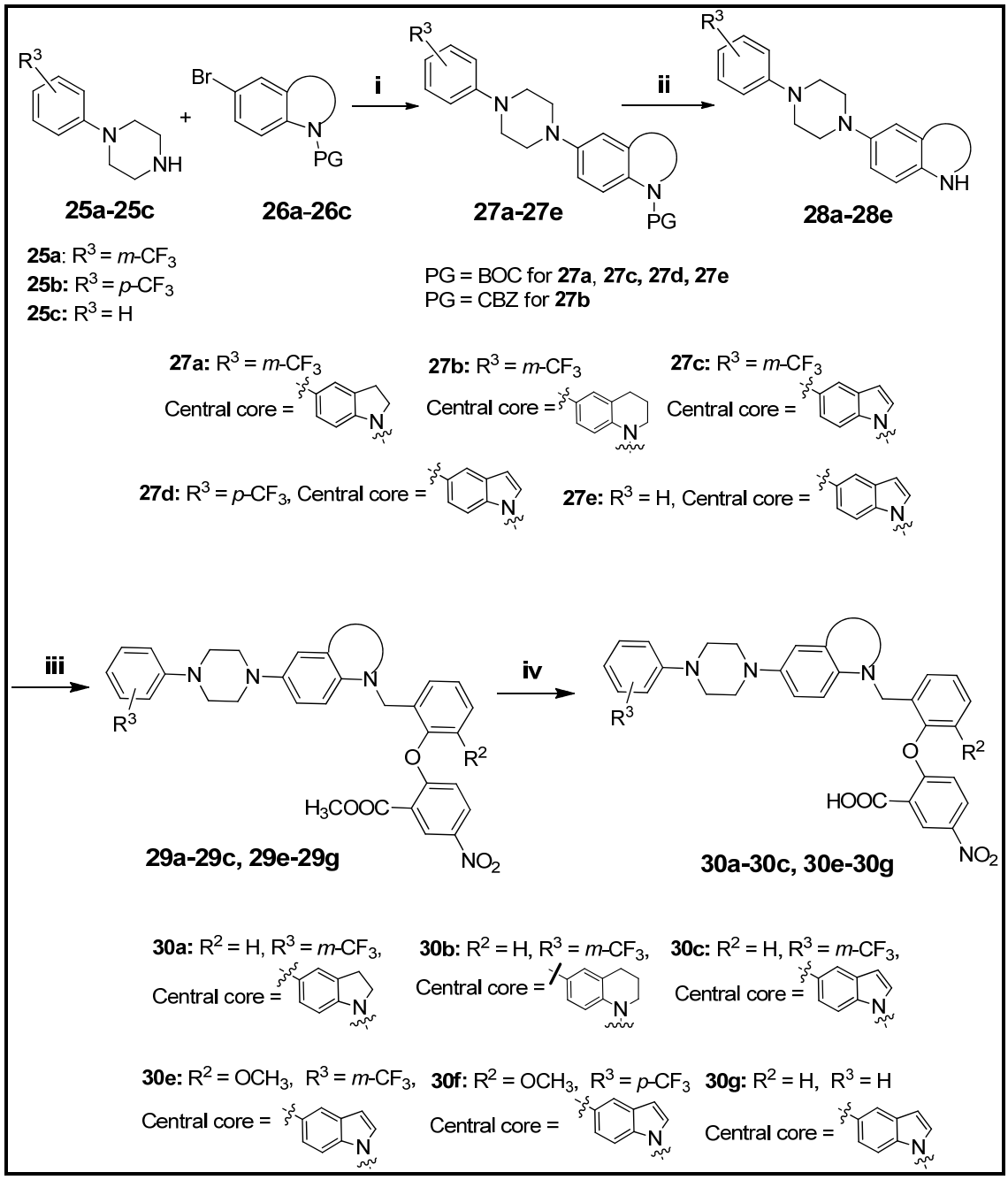
Reagents and conditions: (i) NaH, DMSO, 0 ^{o}C to 25 ^{o}C; (ii) $NaBH_4$, MeOH, 10 ^{o}C to 25 ^{o}C; (iii) PBr_3, CH_2Cl_2, 10 ^{o}C to 25 ^{o}C; (iv) $Pd(OAc)_2$, Ph_3P, Na_2CO_3, n-propanol, water, reflux; (v) **20a**-**20b**, *t*-BuOK, DMF, 10 ^{o}C to 25 ^{o}C; (vi) KOH, THF, water, 25 ^{o}C.

The salicylaldehyde derivatives **17a**-**17b** were reacted with methyl 2-chloro-5-nitrobenzoate (**16**) using sodium hydride (NaH) as a base to get diphenyl ether derivatives **18a**-**18b**. Reduction of **18a**-**18b** with $NaBH_4$ gave corresponding alcohol derivatives **19a**-**19b**, which were then brominated with PBr_3 to afford bromo derivatives **20a**-**20b**. The 5-bromoindole (**21**) was reacted with boronic acids **22a**-**22b** using $Pd(OAc)_2$ as a catalyst and triphenyl phosphine (Ph_3P) as a ligand to afford the indole derivatives **23a**-**23b**. The coupling of indole derivatives **23a**-**23b** with the appropriate halides **20a**-**20b** in presence of potassium *tert*-butoxide (*t*-BuOK), followed by basic hydrolysis with KOH afforded the target compounds **24a**-**24c**.

The compounds **30a**-**30g** derived from **X** were prepared as depicted in the **Schemes 4** and **5**. The piperazine derivatives **25a**-**25c** were coupled with N-protected heterocycles **26a**-**26c** using $Pd(OAc)_2$ as a catalyst and 2-(Di-tert-butylphosphino)biphenyl as a ligand to to get compounds of general formula **27a**-**27e**, which were subsequently deprotected using either trifluoro acetic acid (TFA) or concd. sulfuric acid to furnish **28a**-**28e** (**Scheme 4**).

The coupling of intermediates **28a**-**28e** with the halogen derivative **20a**-**20b** (Synthesized according to scheme 3) in presence of *t*-BuOK or K_2CO_3 as a base provided the ester derivatives **29a**-**29c** and **29e**-**29g**. The alkaline hydrolysis of derivatives (**29**) with KOH afforded the desired compounds **30a**-**30c** and **30e**-**30g**.

Scheme 4. Synthesis of compounds **30a-30c** and **30e-30g**

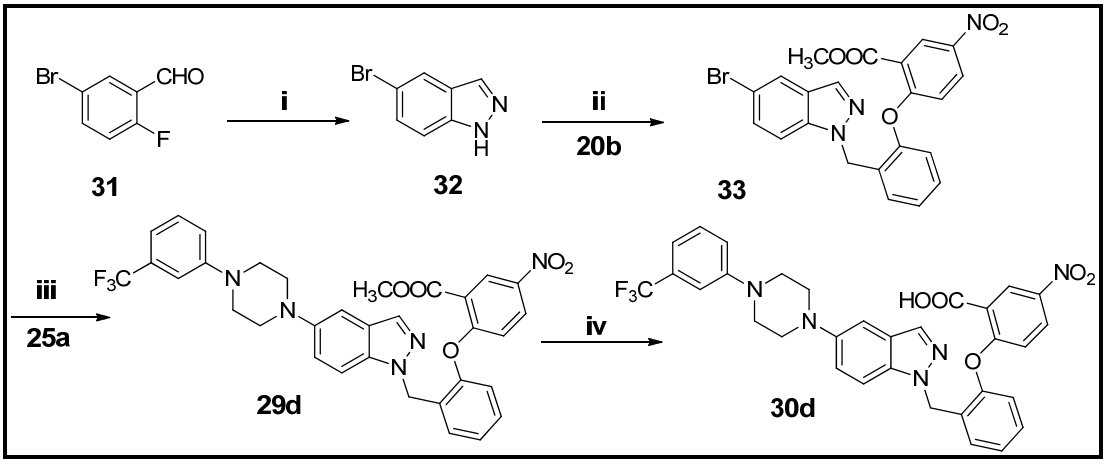


Reagents and conditions: (i) $Pd(OAc)_2$, 2-(Di-tert-butylphosphino)biphenyl, K_3PO_4, DME, reflux; (ii) TFA, CH_2Cl_2 or neat H_2SO_4, 0 °C to 25 °C; (iii) **20a**-**20b**, *t*-BuOK, DMF or K_2CO_3, CH_3CN, 25 °C to 30 °C; (iv) KOH, THF, water, 25 °C.

The compound **30d** was prepared as shown in **Scheme 5**. 5-bromo-2-fluoro benzaldehyde (**31**) was reacted with hydrazine hydrate (NH_2NH_2:H_2O) to

get central core 5-bromoindazole (**32**), which was then coupled with **20b** using K_2CO_3 as a base to get coupled derivative **33**. The coupling of **33** with aryl piperazine derivative **25a** using $Pd(OAc)_2$ as a catalyst and 2-(Di-tert-butylphosphino)biphenyl as a ligand gave ester derivative **29d**, which on subsequent hydrolysis gave desired compound **30d**.

Scheme 5. Synthesis of compound **30d**



Reagents and conditions: (i) NH_2NH_2:H_2O, reflux; (ii) **20b**, K_2CO_3, CH_3CN, 10 °C to 25 °C; (iii) $Pd(OAc)_2$, 2-(Di-tert-butylphosphino)-biphenyl, K_3PO_4, DME, reflux; (iv) KOH, THF, water, 25 °C.

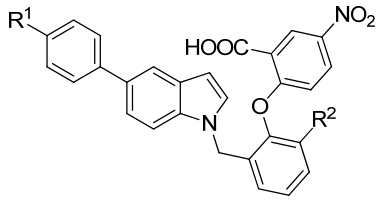
2.3.2.2. Structure-activity relationship discussion of 5-Nitro-2-phenoxy benzoic acid derivatives

5-Nitro-2-phenoxybenzoic acid derivatives synthesized based on general structure **IX** and **X** were evaluated for their *in vitro* PAI-1 inhibitory activity using chromogenic assay for human PAI-1 inhibition. The PAI-1 inhibitory activity of 5-Nitro-2-phenoxybenzoic acid derivatives **24a**-**24c** (General structure **IX**) and **30a**-**30g** (General structure **X**) is summarized in **Tables 8** and **9**.

The hybridized derivative **24a** synthesized as a part of strategy 1, inhibited PAI-1 with an IC_{50} of 3.4 μM in the chromogenic assay (**Table 8**). The removal of

methoxy group from **24a** gave **24b**, which exhibited slightly lower potency (IC_{50} = 4.9 μM) than **24a**. The removal of trifluoromethoxy group is found to be detrimental for activity as evident from the compound **24c** (IC_{50} = 98 μM).

Table 8. PAI-1 inhibitory activity of compounds **24a**-**24c**



Compound	R^1	R^2	IC_{50} (μM)[a]
24a	OCF_3	OCH_3	3.4
24b	OCF_3	H	4.9
24c	H	OCH_3	98
Tiplaxtinin			14.8

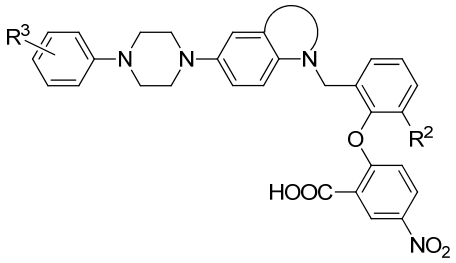
[a]Values determined using *in vitro* PAI-1 inhibitory chromogenic assay.

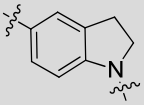
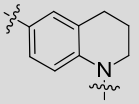
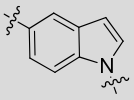
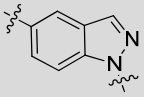
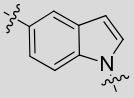
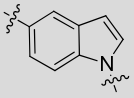
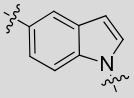
PAI-1 inhibitory activity data of conformationally restricted derivatives **30a**-**30g** synthesized as part of strategy 2 are shown in **Table 9**. The conformational restriction of (**I**) with two carbon atoms produced indoline derivative **30a**, which showed less potency (IC_{50} = 29 μM) compared to the Tiplaxtinin (IC_{50} = 14.8 μM). The ring expansion in the **30a** to get tetrahydroquinoline derivative **30b** found to be detrimental to PAI-1 inhibitory activity (IC_{50} = 63.8 μM), probably due to conformational misfit of the molecule (six membered ring of **30b** vs five membered ring of **30a**).

The introduction of the double bond in the indoline derivative **30a** to get indole derivative **30c** inhibited the PAI-1 activity with impressive IC_{50} of 3.2 μM,

(**Table 9**). The incorporation of an extra N atom in the ring of **30c** gave indazole derivative **30d**, which showed inferior potency with an IC_{50} of 14.6 μM.

Table 9. PAI-1 inhibitory activity of compounds **30a**-**30g**



Compound	R^2	R^3	Central Ring	IC_{50} (μM)[a]
30a	H	*m*-CF_3		29
30b	H	*m*-CF_3		63.8
30c	H	*m*-CF_3		3.2
30d	H	*m*-CF_3		14.6
30e	OCH_3	*m*-CF_3		2.4
30f	OCH_3	*p*-CF_3		22
30g	H	H		No inhibition
Tiplaxtinin	-	-	-	14.8

[a]Values determined using *in vitro* PAI-1 inhibitory chromogenic assay.

Further, a methoxy group was introduced in the most potent compound **30c** to get the compound **30e**, interestingly the compound **30e** exhibited slightly

higher potency (IC_{50} = 2.4 μM) compared to **30c** (IC_{50} = 3.2 μM). The translocation of *m*-CF_3 group of **30e** (IC_{50} = 2.4 μM) at para position was found to be detrimental in terms of potency as witnessed from IC_{50} value of **30f**, (IC_{50} = 22 μM). The removal of CF_3 group from **30c** to get the compound **30g** resulted in the deterioration of the PAI-1 inhibition (**Table 9**), which further supported the importance of the CF_3 group.

The compounds with potent PAI-1 inhibitory activity, **24a**, **30c** and **30e** were evaluated for their pharmacokinetic parameters in rats (**Table 10**). The compound **24a** showed good plasma levels (C_{max} = 2.4 μg/mL) and a half life ($t_{1/2}$ = 3.27 h) when dosed orally at 30 mg/kg in wistar rats (**Table 10**). The compound **30c** showed impressive plasma levels (C_{max} = 6.8 μg/mL) and a long half life ($t_{1/2}$ = 9.86 h), which is favorable for this class of compounds. However, plasma concentration of the methoxy derivative **30e** was found to be modest when compared to **30c**.

Table 10. Pharmacokinetic parameters for compounds[a] **24a**, **30c**, **30e**

Compound	C_{max} (μg/mL)	tmax (h)	$t_{1/2}$ (h)	AUC(0-24) (h μg/mL)
24a	2.4	1	3.27	10.38
30c	6.8	6	9.86	80.18
30e	1.78	4	6.88	5.39

[a]Compounds were dosed in fasted male wistar rats at 30 mg/kg po formulated with a Tween-80: PEG: CMC (5:5: 90 % v/v).

The significant plasma concentration of the compounds **24a**, **30c** and **30e** prompted us to study the compounds for their *in vivo* efficacy in rats using $FeCl_3$-induced arterial thrombosis model using Clopidogrel, a well known antiplatelet

agent as a positive control. However, only compound **30c** exhibited antithrombotic efficacy while compounds **24a** and **30e** failed to show any *in vivo* efficacy, in spite of their impressive *in vitro* PAI-1 inhibitory activity and favorable pharmacokinetic parameters (**Figure 26**). The plasma levels of **30c** were highest compare to other two compounds, which could be the reason for antithrombotic efficacy displayed by **30c**.

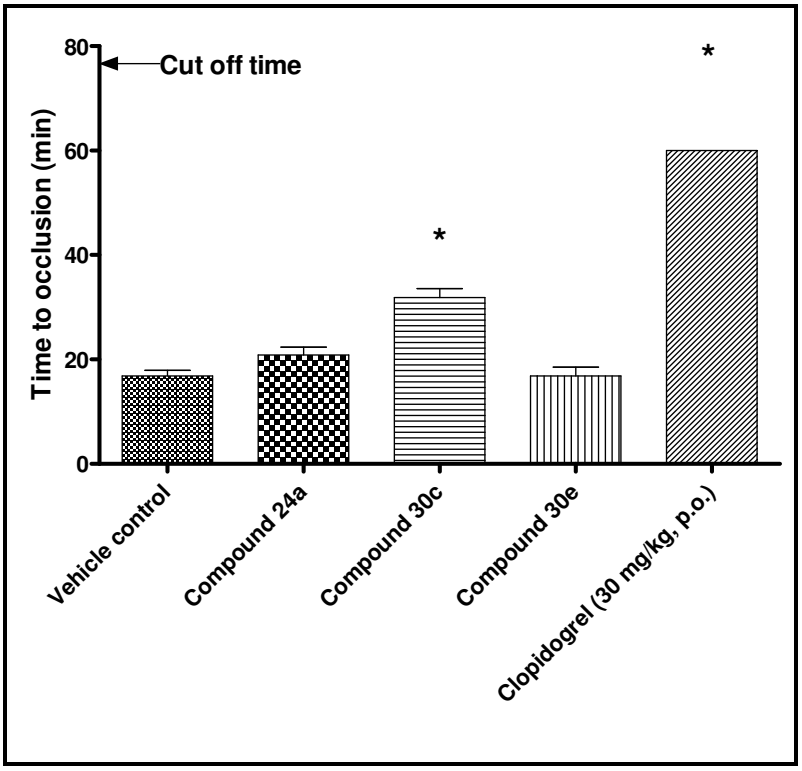


Figure 26. Effects of the compound **24a**, **30c** and **30e** on time to thrombus formation in $FeCl_3$-induced arterial thrombosis in rats at 30 mg/kg. Each value represents mean ± SEM (n = 6). * indicates $p < 0.05$ vs vehicle control. Clopidogrel was used as positive control and administered orally 2 h before application of $FeCl_3$ paper on the carotid artery

2.4. Conclusions

In summary, two novel series of PAI-1 inhibitors were identified. The first series i.e. Oxalamide derivatives were derived from initial lead **1** obtained from compound library. Systematic SAR evaluation around **1** produced several *in vitro* potent PAI-1 inhibitors, which unfortunately could not be evaluated further due to their poor oral bioavailability. Oxalamide derivatives certainly provides a novel template for future research in developing PAI-1 inhibitors as novel antithrombotics.

Second novel series, the 5-Nitro-2-phenoxybenzoic acid derivatives derived using hybridization and conformational restriction strategies of known chemotypes exhibited potent PAI-1 inhibitory activity and favorable pharmacokinetic parameters. Oxoacetic acid part of Tiplaxtinin was effectively replaced with 5-Nitro-2-phenoxybenzoic acid part of (**I)** producing potent PAI inhibitor **24a**. The docking study confirmed the similar orientation of **24a** and Tiplaxtinin in PAI-1 ligand binding site. Conformational restriction of (**I)** with indole as a central core (**30c**) showed potent PAI-1 inhibitory activity and excellent pharmacokinetic profile with antithrombotic efficacy in rats using $FeCl_3$-induced arterial thrombosis model.

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Coagulation Factor Xa inhibitors

3. COAGULATION FACTOR Xa INHIBITORS

3.1. Background

Factor Xa is a serine protease which has a key role in coagulation cascade and by regulating thrombin formation and subsequent fibrin production controls haemostasis. Alternatively, FXa inhibition is a well proven approach to treat thrombotic disorders. Several small molecule FXa inhibitors are now in clinical trials and few of them are already approved. A detailed literature search of known FXa inhibitors was carried out to derive a synthetic rationale for developing novel FXa inhibitor.

3.1.1. Naturally occurring inhibitors of FXa

In 1987, Tuszynski *et al*., discovered antistasin as an anticoagulant, which they isolated from extracts of the Mexican leech, *Haementeria officinalis*. Antistasin is a selective inhibitor of FXa with a Ki of 0.3-0.6 nM **[1**, **2]**. At the same time, Waxman *et al*., discovered tick anticoagulant peptide (TAP), a single chain 60 amino acid peptide that was isolated from the extracts of the tick *Ornithodoros moubata* **[3]**. TAP is a reversible inhibitor of FXa with an estimated Ki of 0.5 nM.

3.1.2. Indirect, AT-dependent FXa inhibitors

Fondaparinux is a synthetic pentasaccharide, which inhibits FXa indirectly through interaction with AT. Fondaparinux was synthesized in 1983 and has demonstrated improved or similar clinical benefit over LMWHs in venous thrombotic indications **[4]** and also found to be superior in ACS patients with

unstable angina/non- ST-segment elevation MI for reducing risk of death or recurrent heart attack **[5**, **6]**. The safety and efficacy of Fondaparinux provided the first clinical proof of principle that provoked extensive research activities in developing FXa inhibitors as anticoagulants.

Sanofi-Aventis identified Idraparinux, a hypermethylated derivative of Fondaparinux with a high affinity for AT-III, in which the amino functional groups were replaced with hydroxyl or methoxy groups **[7]**. In a phase III trial, long-term treatment with Idraparinux (once weekly) for the prevention of stroke and systemic embolism in patients with AF was found to be noninferior to Warfarin but caused significantly more bleeding **[8]**. Subsequently development of Idraparinux was discontinued.

3.1.3. Direct FXa inhibitors (**Parenteral**)

DX-9065a (**Figure 27**, FXa Ki = 41 nM) was one of the first potent and selective FXa inhibitors identified, with good anticoagulant activity ($PTCT_2$ = 0.52 μM) **[9]**. Due to low human oral bioavailability (F = 2-3%), DX-9065a was advanced clinically as a parenteral agent **[10]**.

Otamixaban (**Figure 27**) is a 2,3-disubstituted β-aminoester derivative which displayed potent FXa inhibitory activity (Ki = 0.5 nM) and good *in vitro* anticoagulant activity ($PTCT_2$ = 1.1 μM) **[11]**. In phase I/II studies, Otamixaban was administered intravenously and was found to be well tolerated in healthy human volunteers and patients with coronary artery disease **[12]**.

Fidexaban (**Figure 27**) is a third parenteral agent that was advanced to human clinical trials which contains two amidine groups and a polar carboxylic

acid moiety. The dihydrochloride salt of Fidexaban is a potent inhibitor of FXa (Ki = 0.10 nM) and has been shown to be efficacious in several *in vivo* animal models of thrombosis **[13-15]**.

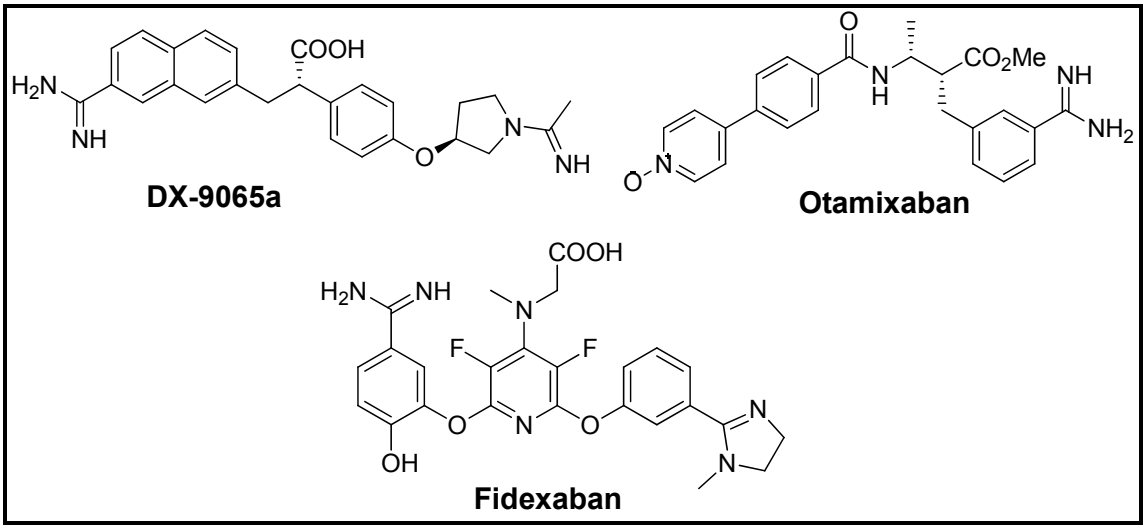


Figure 27. Parenteral FXa inhibitors advanced into clinical trials

3.1.4. Oral direct FXa inhibitors

Astellas Pharma discovered dibasic benzamidine analogue YM-60828 (FXa Ki = 1.3 nM, $PTCT_2$ = 0.21 μM, **Figure 28**), which showed oral activity in monkeys (F = 20%) and found to be efficacious in several animal models of thrombosis. YM-75466, the methanesulfonate salt of YM-60828, had entered into clinical development, but no further development reported for it **[16**, **17]**.

To improve oral bioavailability of the FXa inhibitors, several groups have come up with strategy of replacement of highly basic amidine group, traditionally used in majority of FXa inhibitors and that fits in both S1 and S4 binding pocket of FXa **[18]**. Pyrazole-2-carboxamide derivatives DPC-423 (FXa, Ki = 0.15 nM)

[19] and Razaxaban (FXa, Ki = 0.19 nM) **[20]** were initially forwarded in clinical development but later on discontinued (**Figure 28**).

Rivaroxaban (**Figure 28**) is the first oral direct FXa inhibitor to be approved which contains neutral chloro-thiophene ring (S1 ligand) and morpholone ring (S4 ligand) as replacement of classical amidine groups. It has shown very potent FXa inhibitory activity (IC_{50} = 0.7 nM) and anticoagulant activity ($PTCT_2$ = 0.23 μM using human plasma) **[21]**. In a rabbit AV shunt model, it reduced thrombus formation in a dose-dependent manner with an ED_{50} value of 0.6 mg/kg (po), with no significant increase in bleeding time **[22]**. The oral bioavailability was high (F = 60-86% and 57-66% in dogs and rats, respectively) and displayed short half-life ($t_{1/2}$ = 0.9 and 1.2-2.3 h, respectively) **[23]**. Rivaroxaban was found to be superior over Enoxaparin in various Phase III trials leading to several approvals in many countries for indications shown in **Table 11**.

Apixaban (**Figure 28**) is the second compound to be approved and have potent FXa inhibitory and anticoagulant activity (FXa, Ki = 0.08 nM, $PTCT_2$ = 3.8 μM using human plasma). It has shown high oral bioavailability (F > 50%) in dogs but poor in rabbits **[24]**. In rabbits, Apixaban was highly efficacious in AV shunt model (ED_{50} = 0.27 mg/kg/h) **[25]**.

Edoxaban (**Figure 28**) is a potent inhibitor of human FXa *in vitro* (FXa, Ki = 0.56 nM), with > 10000-fold selectivity against relevant serine proteases, and demonstrated very good anticoagulant activity ($PTCT_2$ = 0.26 μM using human plasma). It displayed *in vivo* efficacy in various animal models of thrombosis, with minimal bleeding **[26**, **27]**. It is approved in Japan for VTE prevention.

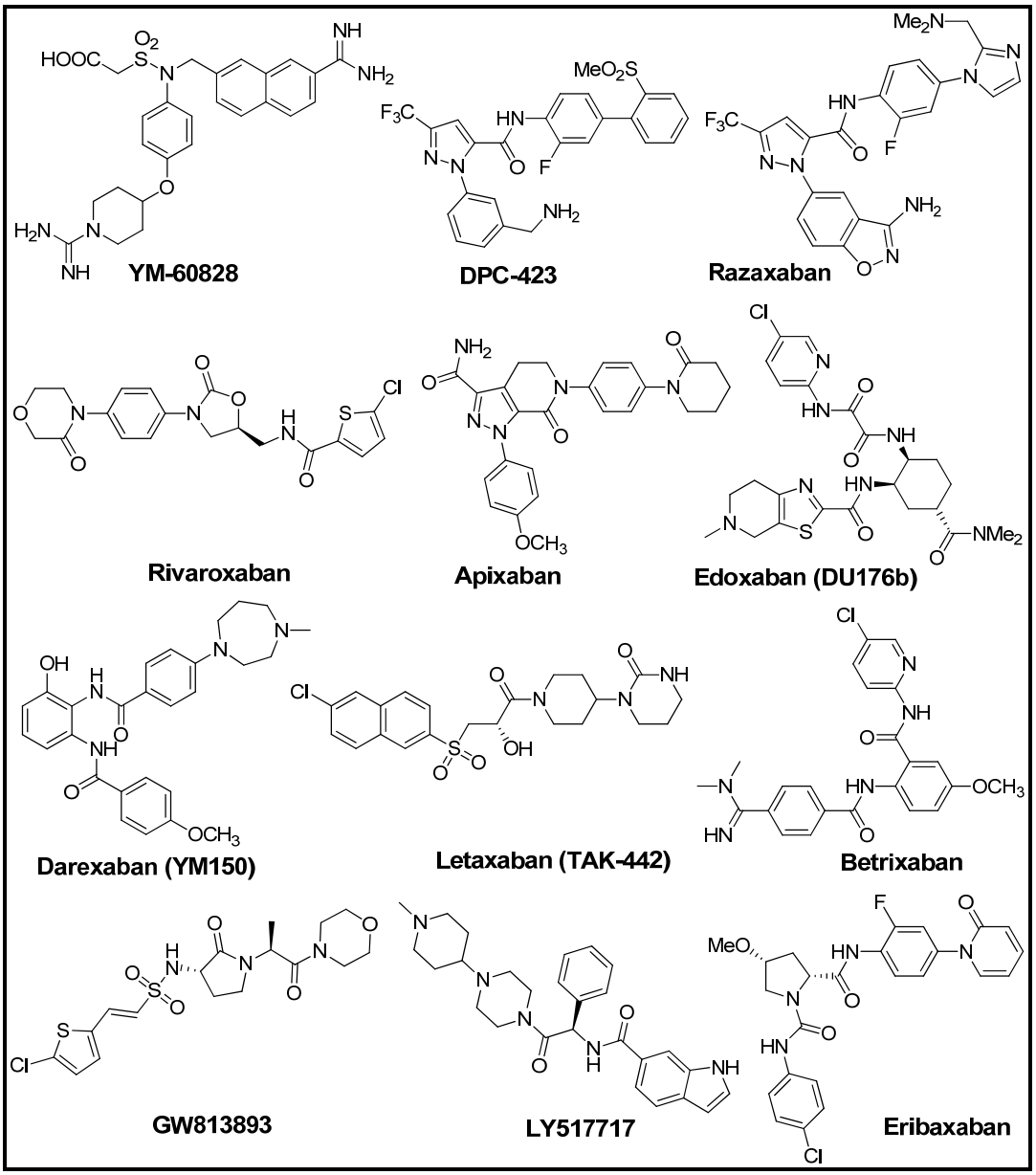


Figure 28. Oral FXa inhibitors advanced into clinical trials

Darexaban (**Figure 28**) is orthophenelene diamine derivative developed by Astellas. Darexaban (IC_{50} = 49 nM, $PTCT_2$ = 1.2 μM) and its glucuronide metabolite (IC_{50} = 29 nM, $PTCT_2$ = 0.95 μM) has shown dose-dependent reduction in rat thrombosis model **[28**, **29]**. Currently the development of Darexaban was stopped.

Table 11. Oral FXa inhibitors in clinical development and their status

Drug Name	Company	Clinical stage	Indications	Current status
Rivaroxaban	Bayer	Approved	• VTE prophylaxis after total hip or knee replacement • Stroke prophylaxis in patients with non- valvular AF • Secondary prevention in ACS, treatment of VTE	Approved in US and EU for first indication, approved in US for second indication, for other indications Phase III trials are ongoing.
Apixaban	BMS-Pfizer	Approved	• VTE prophylaxis after total hip or knee replacement • Stroke prophylaxis in patients with non- valvular AF • Secondary prevention in ACS, treatment of VTE	Approved in Europe for first indication, while for other indications Phase III trials are ongoing.
Edoxaban	Daiichi Sankyo	Approved	• Prevention of VTE after major orthopedic surgery • Stroke prophylaxis in patients with non- valvular AF	Approved in Japan for first indication. For other indication Phase III trial are ongoing
Darexaban	Astellas	Phase III	• Prevention of VTE after major orthopedic surgery.	Discontinued
Betrixaban	Portola	Phase II	• Prevention of VTE • Stroke prevention in non-valvular AF	Clinical trials are ongoing
Letaxaban	Takeda	Phase II	• Prevention of VTE in patients who have had knee replacement surgery	Discontinued
Eribaxaban	Pfizer	Phase II	• Prevention of VTE in patients who have had knee replacement surgery	Discontinued
LY517717	Lilly	Phase II	• Prevention of VTE after major orthopedic surgery.	Discontinued
GW813893	GSK	Phase II	• Prevention of VTE in patients who have had knee replacement surgery	Discontinued

Betrixaban (**Figure 28**) is a potent inhibitor of FXa (Ki = 0.12 nM) and also displayed potency in a thrombin generation assay (TG2x = 0.33 μM) and has a lower affinity for the hERG channel (patch clamp hERGIC_{50} = 8.9 μM) **[30]**. It has shown good oral bioavailability in rat, dog, monkey (F = 23.8%, 51.6%, and 58.7%, respectively).

Letaxaban (**Figure 28**) displayed potent FXa inhibitory and anticoagulant activity (FXa, IC_{50} = 3.5 nM, $PTCT_2$ = 0.58 μM using human plasma) **[31]**. It is orally bioavailable in monkeys (F = 52.5%). The compound was efficacious in an intravenous rabbit venous thrombosis model with minimal effect on bleeding time. Development of this molecule was discontinued from Phase II.

Eribaxaban (**Figure 28**) is a potent and selective inhibitor of FXa (IC_{50} = 0.32 nM) with appreciable *in vitro* clotting activity ($PTCT_2$ = 0.58 μM using human plasma) **[32]**. Development of Eribaxaban is ceased.

LY517717 (**Figure 28**) is potent FXa inhibitor (FXa Ki = 4.6-6.6 nM, aPTT1.5x = 0.46 μM using human plasma) with good oral bioavailability in rats and dogs (F = 25-82%) and also long plasma half-life of 7-10 h. In a rat AV shunt model, it had an ED_{50} value of 5-10 mg/kg po. Clinical development of LY517717 was discontinued **[33]**.

GW813893 (**Figure 28**) is a potent FXa inhibitor developed by GSK (FXa Ki = 4 nM, aPTT1.5x = 1.2 μM using human plasma) **[34]** with good oral bioavailability in rats and dogs (F = 75% and 53%, respectively). This compound was efficacious in preclinical models of thrombosis **[35]**. Development of this compound was discontinued.

In summary, FXa inhibition is a validated approach to correct haemostasis and subsequent thrombosis. FXa inhibitors have already shown promise in various thormboembolic disorders and also approved in market (**Table 11**). These data thus provides a strong rationale to develop novel small molecule FXa inhibitor.

3.2. Sulfoximine-substituted anthranilamide derivatives as FXa inhibitors

3.2.1. Designing strategy

The FXa binding site for its inhibitors is defined by the S1 and S4 subsites and surrounding residues. S1 binding pocket of FXa is a deep, largely hydrophobic cleft at the bottom of which lies the Asp189 and Tyr228 side chains. S4 is a strongly hydrophobic pocket characterized by the side chains of Tyr99, Phe174, and Trp215. Potent ligands reported in the literature occupy both sites. Other features include the catalytic triad consisting of His57, Asp102, and Ser195.

X-ray crystal structure of Rivaroxaban (FXa inhibitor) in complex with human FXa is shown in **Figure 29**. H-bond interaction of Rivaroxaban with FXa is shown as dotted line. In the S1 pocket of FXa, neutral chloro atom of thiophene ring interacts with aromatic ring of Tyr228, a novel interaction that excludes use of highly basic amidine groups earlier used as S1 binding ligand. The morpholinone moiety of rivaroxaban is lying in between Tyr99 and Phe174. Rivaroxaban forms two H-bond interactions with Gly219 of FXa, first is formed by carbonyl oxygen of the oxazolidone core and second interaction is formed by amino group of the chlorothiophene carboxamide moiety. All FXa inhibitors

reported are designed using a knowledge of amino acids that forms S1 and S4 pocket of FXa.

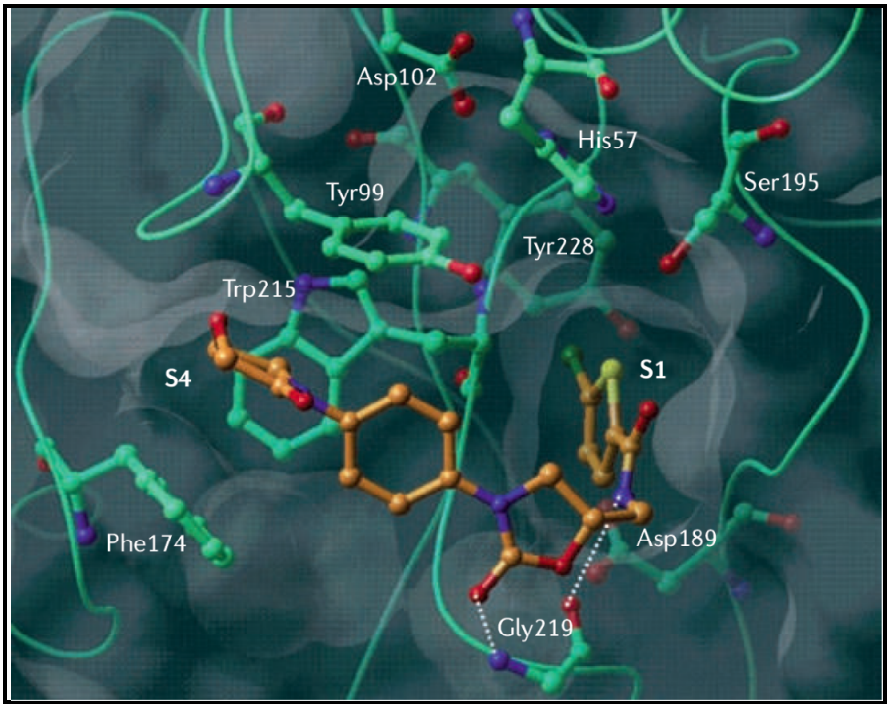


Figure 29. X-ray crystal structure of Rivaroxaban in complex with human FXa **[21]**

Various S1 and S4 ligands were reported by many research groups **[18]** which provides a starting point to discover novel and efficacious FXa inhibitor. These newly discovered FXa inhibitors are devoid of highly basic amidine group, a known culprit for poor oral bioavailibility and was earlier used successfully as both S1 and S4 ligand. Vicinal diamide based FXa inhibitors (Anthranilamide and cis diamine based inhibitors) have been explored by many research groups as they provides U or V shape to the molecule, which is ideal for FXa binding **[36-41]**. Three molecules are already in clinical trials, which contains vicinal diamide scaffold. Edoxaban, which is recently approved in Japan utilized the 5-Chloro-2-aminopyridine group as S1 ligand and the methyltetrahydrothiazolo[5,4-c]pyridine

group as S4 ligand **[26]**. Betrixaban has common S1 ligand with Edoxaban but utilized classical amidine group as S4 ligand **[30]**. Darexaban possesses 4-methoxyphenyl group as S1 ligand and 4-methylhomopiperazine group as S4 ligand **[28]**.

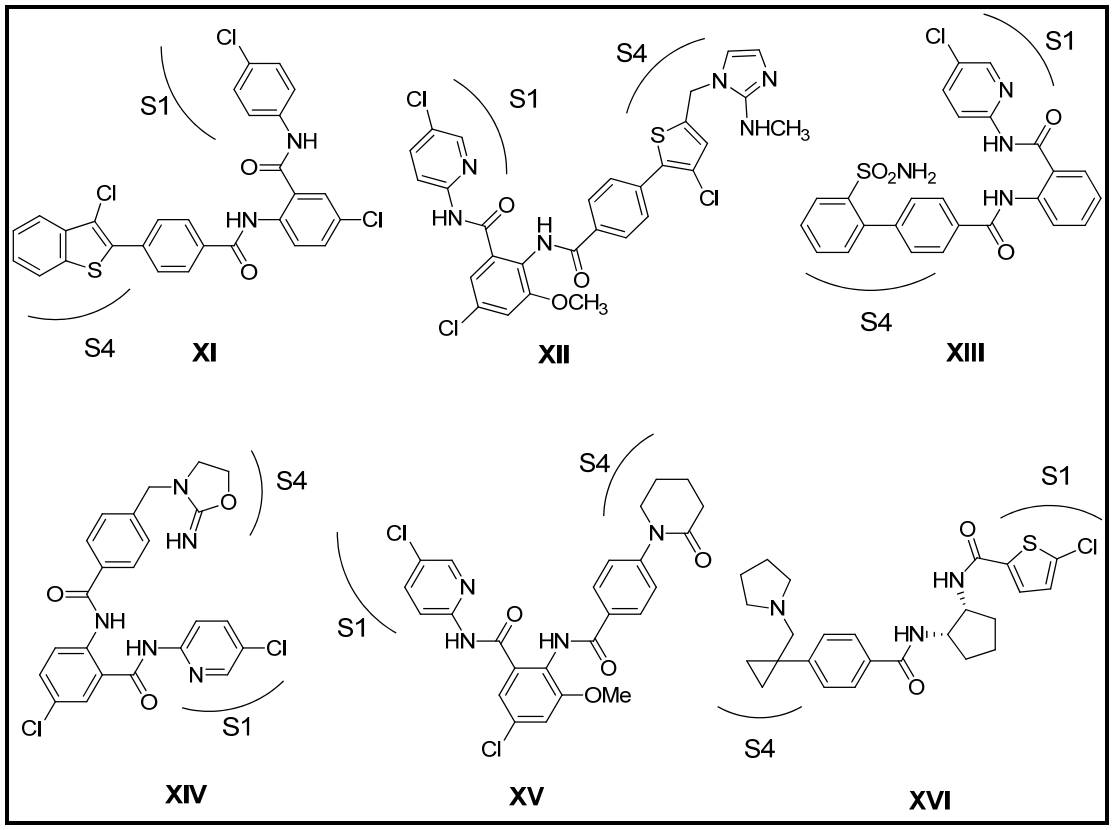


Figure 30. Vicinal diamide based FXa inhibitors

Several similar FXa inhibitors with diversified S4 ligands were reported by many research groups as described in **Figure 30**. Researchers at Berlex identified compound **XI** possessing 3-chlorobenzothiophene as S4 ligand **[36]**, which was then modified to improve anticoagulant activity by introducing polar imidazole ring (**XII**) **[42]**. Research group at Portola employed biaryl group **[41]** and 2-iminooxazolidine group **[43]** as S4 ligands as shown in compounds **XIII**

and **XIV** respectively. The use of lactum ring (**XV**) and α-substituted phenylcyclopropyl group (**XVI**) as S4 ligand was repoted by BMS **[39]**.

As a part of our strategy to discover potent and orally efficacious FXa inhibitors, we decided to replace highly basic amidine P4 group of Betrixaban with less basic sulfoximine group **(Figure 31)**. Sulfoximine group also provides an additional two sites for analogue making and thus further finetuning of FXa inhibitory potency. Both Betrixaban and Edoxaban shares common S1 ligand that is 5-chloro-2-pyridylamine part which was kept unchanged in our designed compounds **XVII**.

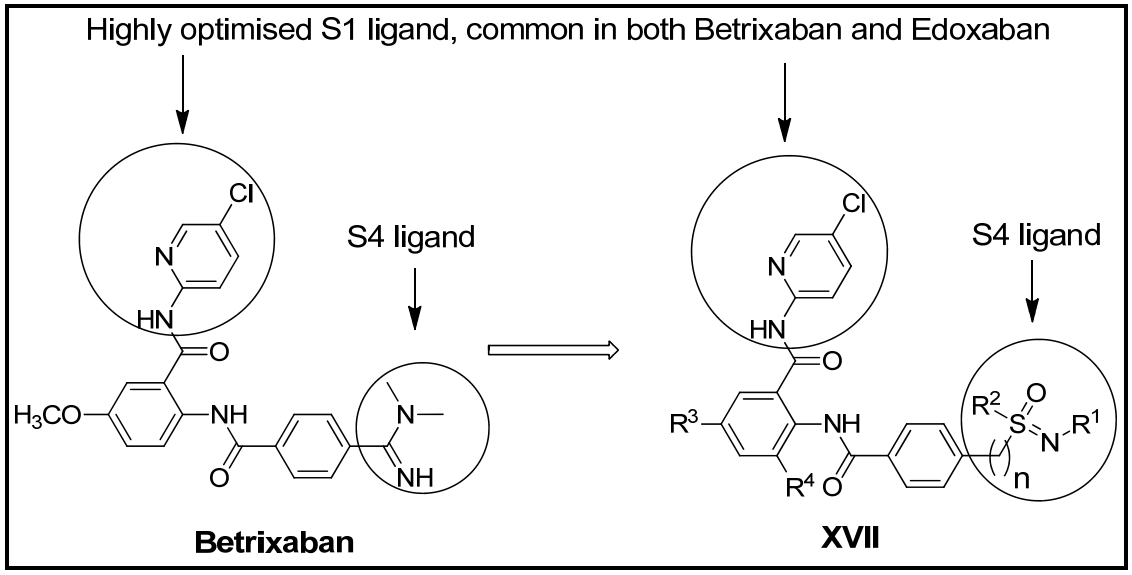


Figure 31. Sulfoximine-substituted anthranilamide derivatives as FXa inhibitors

3.2.2. Results and discussion

3.2.2.1. Chemistry

Synthetic methodology was designed for general structure **XVII** based on the reterosynthetic analysis (**Figure 32**) and the schemes described below.

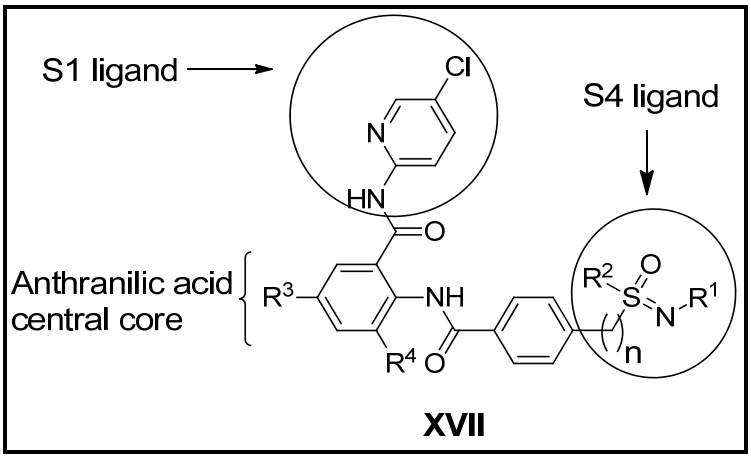
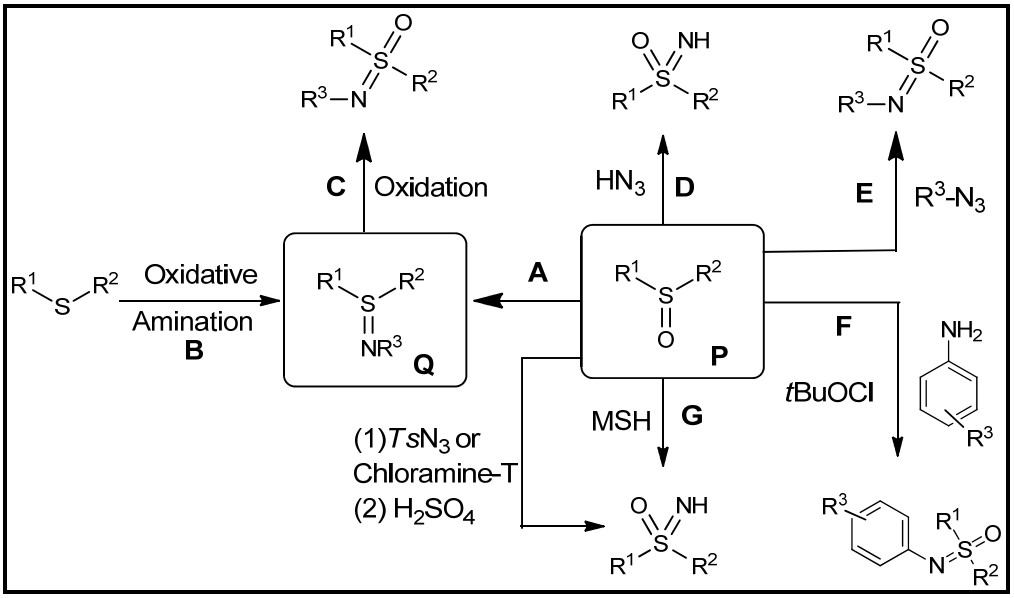


Figure 32. Reterosynthetic analysis of compounds of general formula **XVII**

General structure **XVII** contains anthranilic acid as central core and 5-chloro-2-aminopyridine as S1 ligand (**Figure 32**). Both are commercially available and coupled using amide bond formation technique reported in literature **[44]**. Sulfoximine-substituted phenyl ring represents our novel S4 ligand. Several literature methods are available for generation of sulfoximine group which involves sulfoxides and sulfilimines as starting materials as described in **Scheme 6 [45]**. The oldest method for the oxidative imination of sulfoxides (**P**) using hydrazoic acid generated *in situ* by reaction of sodium azide (NaN_3) with conc. sulfuric acid is one of the most used technique so far. Alternatively, sulfoxides is converted into sulfilimines (**Q**), which after oxidation forms sulfoximine group. Sulfilimines can also be prepared from sulfides through

oxidative imination, which in turn converted into sulfoximine through oxidation (**Scheme 6**). Hydrazoic acid method for generation of sulfoximine group was used for synthesis of target compounds since it requires cheap starting materials and also widely used and optimized.

Scheme 6. Synthesis of sulfoximines from sulfoxides **P** and sulfilimines **Q [45]**

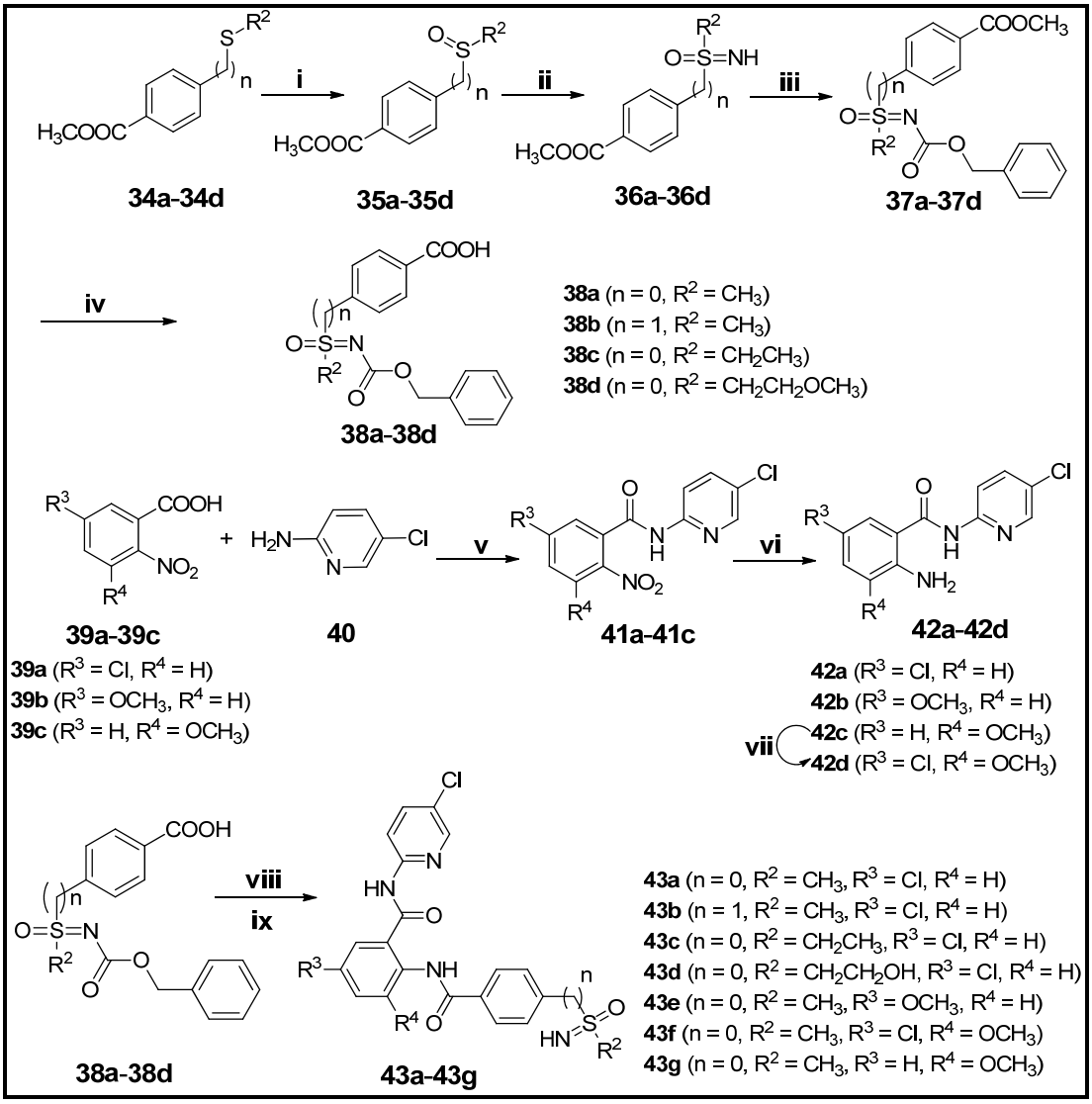


Reagents and conditions: **A**: *Ts*N=S=N*Ts; Ts*N=S=O; $ArSO_2NH_2$, P_4O_{10}, Et_3N; **B**: *t*BuOCl, (-)-*Menth*-ONa, H_2NR^3; **C**: $KMnO_4$; MCPBA; $NaIO_4$, RuO_2; H_2O_2, NaOH; **D**: NaN_3, H_2SO_4; **E**: hμ or heat or *Raney*-Cu, heat; **F**: Et_3N; **G**: 2 h, rt, then 10 % NaOH.

The synthesis of first set of compounds **43a**-**43g** derived from **XVII** is outlined in **Scheme 7**. We chose the ester derivatives **34a**-**34d** as starting materials to generate sulfoximine group. Controlled oxidation of compounds **34a**-**34d** using H_2O_2 and catalytic amount of vanadium pentoxide (V_2O_5) gave sulfoxides **35a**-**35d**, which upon treatment with NaN_3 and sulfuric acid converted into sulfoximine derivatives **36a**-**36d**. Sulfoximine group was then protected by reacting it with benzyloxy carbonyl chloride (ClCOOBn) using pyridine as base to

get intermediates **37a**-**37d**. Alkaline hydrolysis of **37a**-**37d** gave key acid intermediates **38a**-**38d**.

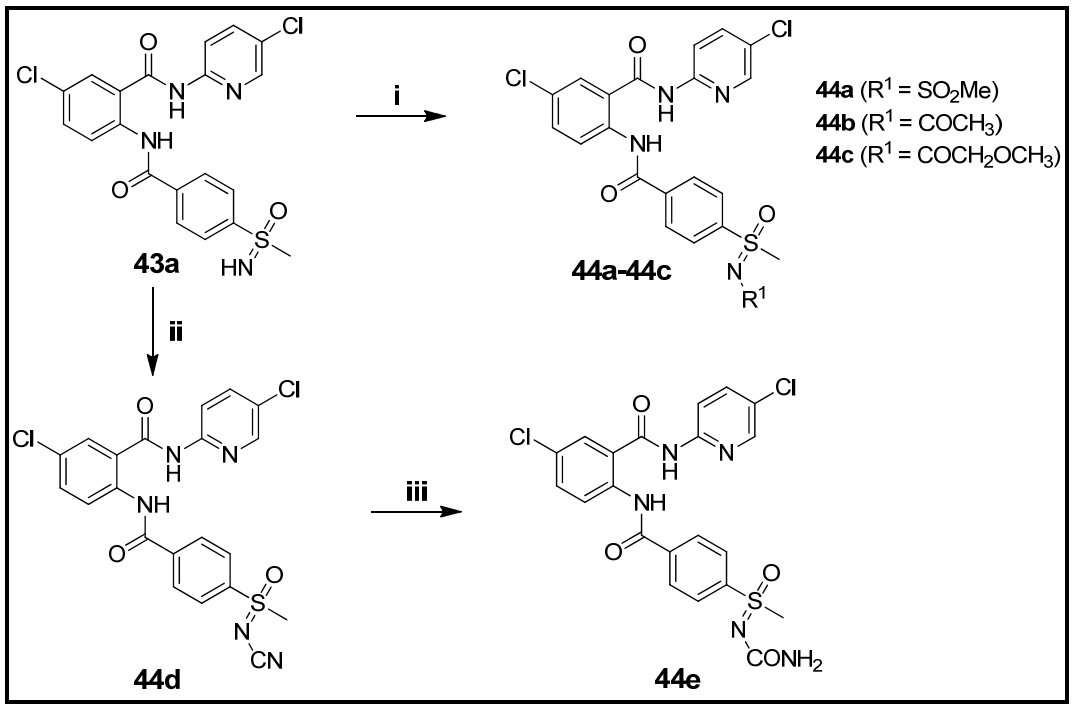
Scheme 7. Synthesis of compounds **43a-43g**



Reagents and conditions: (i) H_2O_2, cat. V_2O_5, CH_3CN, 0 °C to 25 °C; (ii) NaN_3, H_2SO_4, $CHCl_3$, -20 °C to 45 °C; (iii) ClCOOBn, CH_2Cl_2, Pyridine, 0 °C to 25 °C; (iv) NaOH, THF, water, 25 °C; (v) $POCl_3$, Pyridine, CH_3CN, 25 °C; (vi) $SnCl_2$, Ethyl acetate, 25 °C; (vii) NCS, Benzene, 60-65 °C; (viii) Oxalyl chloride, CH_2Cl_2, 25-30 °C, then **42a**-**42d**, THF, 25-30 °C, 2 h; (ix) Sulfuric acid, 0 °C to 25 °C, or BBr_3, CH_2Cl_2, -30 °C to 25 °C, (for **43d**).

Commercially available 2-nitrobenzoic acid derivatives **39a**-**39c** were condensed with 5-chloro-2-amino pyridine (**40**) using $POCl_3$-pyridine coupling method to get intermediates **41a**-**41c**, which upon reduction using $SnCl_2$ furnished anilines **42a**-**42c**. Synthesis of **42d** was accomplished by chlorination of **42c** using N-chloro succinimide (NCS). The compounds **43a**-**43g** were obtained by converting **38a**-**38d** into acid chlorides using oxalyl chloride followed by condensation with appropriate anilines (**42a**-**42d**) and subsequent deprotection of Cbz (Benzyloxycarbonyl) group using sulfuric acid (H_2SO_4) or boron tribromide (BBr_3).

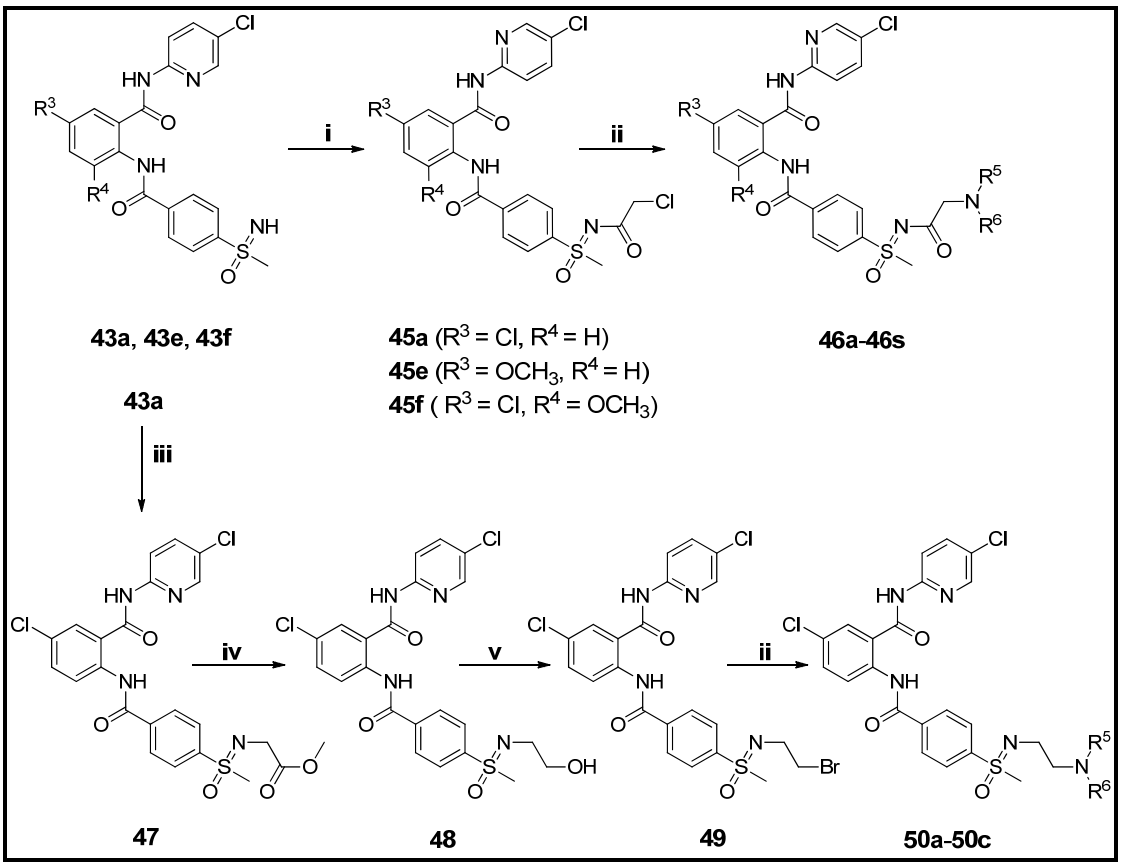
Scheme 8. Synthesis of compounds **44a-44e**



Reagents and conditions: (i) $MeSO_2Cl$, Pyridine, CH_2Cl_2, 0 °C to 25 °C (for **44a**); CH_3COCl, Pyridine, CH_2Cl_2, 0 °C to 25 °C (for **44b**); CH_3OCH_2COCl, Pyridine, CH_2Cl_2, 0 °C to 25 °C (for **44c**); (ii) BrCN, CH_2Cl_2, 25 °C to 30 °C; (iii) Sufuric acid, 0 °C to 30 °C.

The compounds **44a**-**44e** derived from **XVII** were synthesized as shown in **Scheme 8**. Compound **43a** was reacted with methanesulfonyl chloride ($MeSO_2Cl$), acetyl chloride (CH_3COCl) and methoxyacetyl chloride (CH_3OCH_2COCl) to obtained **44a**, **44b** and **44c** respectively. Reaction of **43a** with cyanogen bromide (BrCN) gave **44d**, which after treatment with sulfuric acid produced amide derivative **44e**.

Scheme 9. Synthesis of compounds **46a-46s** and **50a-50c**



Reagents and conditions: (i) chloroacetyl chloride, TEA, THF, 25 ^oC to 30 ^oC; (ii) NHR^5R^6, DMF, 25 ^oC to 30 ^oC; (iii) NaH, Methyl bromo acetate, DMF, 40 ^oC; (iv) $NaBH_4$, DMSO, 60 ^oC; (v) TPP, CBr_4, CH_2Cl_2, 25 ^oC to 30 ^oC.

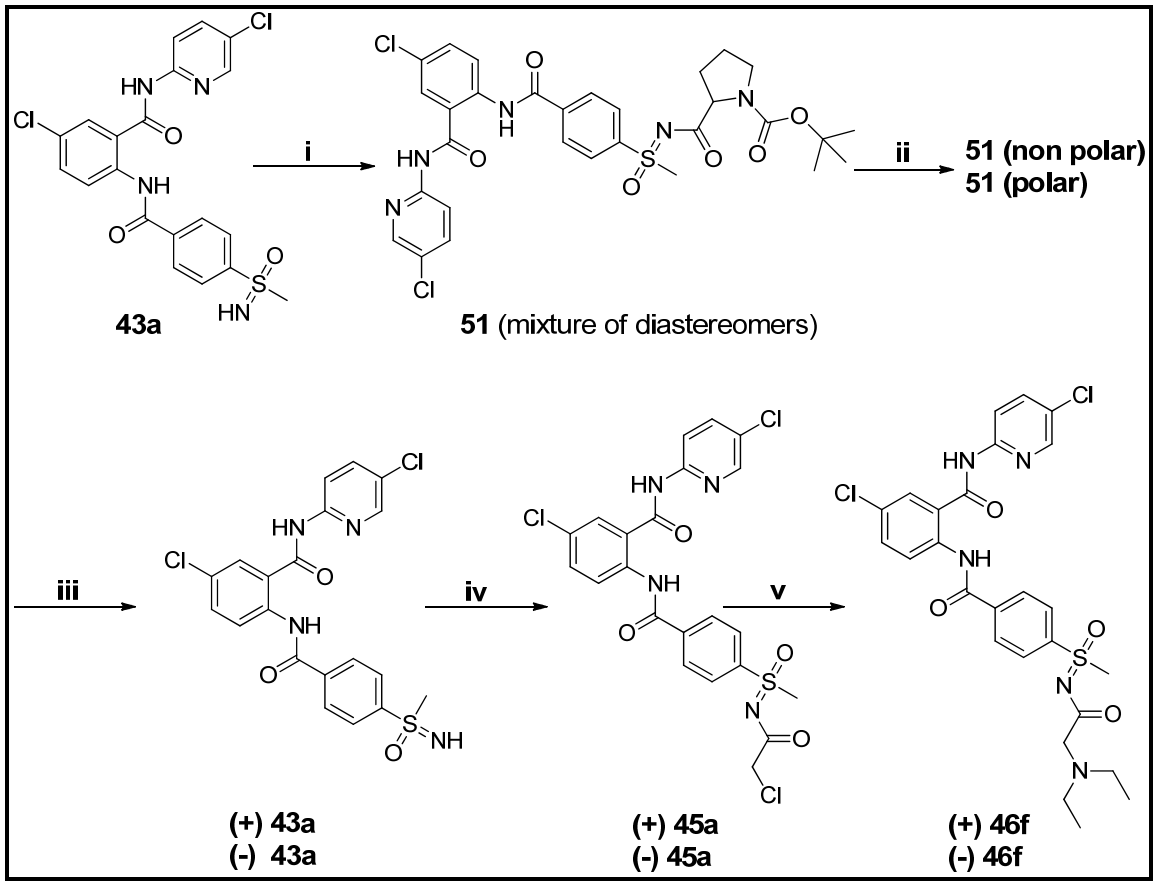
The aminoacyl derivatives (**46a**-**46s**) and aminoalkyl derivatives (**50a**-**50c**) derived from **XVII** were synthesized as shown in **Scheme 9**. Anthranilamide

derivatives **43a**, **43e**, and **43f** were reacted with the chloroacetyl chloride in presence of triethyl amine (TEA) to get key intermediates **45a**, **45e**, and **45f** respectively. The displacement of chloro from **45a**, **45e**, and **45f** with appropriately substituted amines gave desired compounds **46a**-**46s**.

Alternatively, **43a** was reacted with methyl bromo acetate using NaH to get **47**. Ester group of **47** was reduced using $NaBH_4$ to get alcohol derivative **48**. The hydroxyl group of **48** was then converted into leaving group using triphenyl phosphine (TPP) and carbon tetrabromide (CBr_4) as brominating agent to get bromo derivative **49**. The compounds **50a**-**50c** were obtained by displacement of bromo group from **49** with appropriate amines.

All these compounds possesses chirality at S atom of sulfoximine group. Both the stereoisomers of compound **46f** (Selected on the basis of biological data) were separated using chemical resolution technique as shown in **Scheme 10**. The compound **43a** was coupled with *tert*-butyl oxycarbonyl protected L-proline (N-BOC-L-Proline) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and catalytic amount of dimethyl amino pyridine (DMAP) to get diastereomeric mixture (**+** and **-**) **51**. Both the diastereomers were separated using 230-400 mesh silica gel as stationary phase and 30-35% ethyl acetate in hexane as mobile phase and then individually reacted with sulfuric acid in methanol to get deprotected derivatives (**+**) **43a** and (**-**) **43a**. Compounds (**+**) **43a** and (**-**) **43a** were reacted with chloroacetyl chloride to get intermediates (**+**) **45a** and (**-**) **45a** respectively, which upon treatment with diethylamine gave desired stereoisomers (**+**) **46f** and (**-**) **46f**.

Scheme 10. Chiral seperation of stereoisomers of **46f**



Reagents and conditions: (i) N-BOC-L-Proline, EDCI, DMAP, DMF, 25 °C to 30 °C; (ii) Column purification using 230-400 mesh silica gel as stationary phase and 30-35% ethyl acetate in hexane as mobile phase (iii) Sulfuric acid, MeOH, 25 °C to 30 °C; (iv) Chloroacetyl chloride, TEA, THF, 25 °C to 30 °C; (v) Diethylamine, DMF, 25 °C to 30 °C.

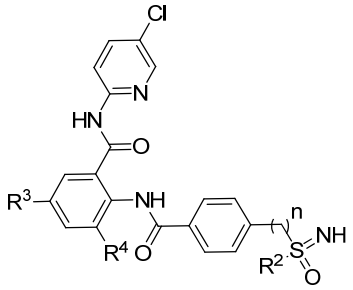
3.2.2.2. Structure-activity relationship discussion of sulfoximine-substituted anthranilamide derivatives

The compounds thus synthesized were evaluated for their *in vitro* inhibitory activity of human FXa, expressed as IC_{50} or Ki values or % inhibition at 0.1 μM and their anticoagulant activity in human and rat plasma was measured as prolongation of prothrombin time (PT), expressed as the concentration of the compound required to double the clotting time ($PTCT_2$) in the PT assay.

Prediction of oral bioavailability and efficacy of the compounds have been achieved simultaneously by measuring *ex vivo* PT prolonging activity in rats. PT prolongation was measured at 2 h after oral administration to rat at a dose of 30 mg/kg and expressed as fold prolongation with respect to control group.

Results of an initial SAR study at sulfoximine P4 group, where we studied the effect of distance of sulfoximine group from the phenyl ring of its attachment, effect of alkyl substituent at S atom, and effect of hydrophilicity in central phenyl ring are shown in **Table 12**.

Table 12. Effect of distance of sulfoximine group from the phenyl ring, effect of alkyl chain at S atom and effect of hydrophilicity in central phenyl ring



Compound	n	R^2	R^3	R^4	% inhibition[a] at 0.1 μM
43a	0	CH_3	Cl	H	76
43b	1	CH_3	Cl	H	No inhibition
43c	0	C_2H_5	Cl	H	No inhibition
43d	0	CH_2CH_2OH	Cl	H	No inhibition
43e	0	CH_3	OCH_3	H	42
43f	0	CH_3	Cl	OCH_3	69
43g	0	CH_3	H	OCH_3	No inhibition

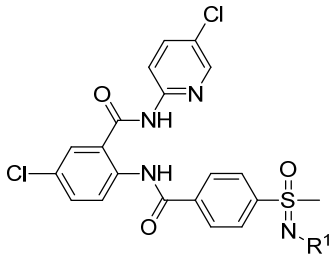
[a]Inhibitory activity against human FXa. Values shown are the mean of duplicate measurements.

The simplest sulfoximine group with methyl group at S atom and chloro substituent at central phenyl ring (**43a**) displayed 76% inhibition of FXa activity at 0.1 μM. Insertion of methylene substituent between phenyl ring and sulfoximine group in **43a** was not tolerated and led to the inactive compound **43b**. Increasing alkyl chain at S atom was also not tolerated as reflected from the data of **43c** (S-ethyl) and **43d** (S-hydroxyethyl). We then evaluated SAR at central phenyl ring of compound **43a** (**Table 12**). Replacement of neutral chloro substituent in **43a** with polar methoxy substituent produced compound **43e** with diminished potency (42% inhibition at 0.1 μM). Additional methoxy substituent in central ring of **43a** had no advantage in improving FXa inhibitory activity, which can be seen from the data of compound **43f**. Removal of chloro substituent from **43f** produced inactive compound (**43g**) suggesting an importance of chloro substituent on central phenyl ring.

Next, SAR at N atom of sulfoximine group was evaluated. Initially, simple substituents were introduced at N atom to get compounds **44a**-**44e** (**Table 13**). Compound **44a** with methanesulfonyl group inhibited FXa with slightly lower potency compare to unsubstituted sulfoximine derivative **43a**. However, anticoagulant activity of **44a** using human plasma was found to be much better than **43a** ($PTCT_2$ = 7.2 μM for **43a** vs 2.6 μM for **44a**), which in line with the fact that anticoagulant activity of FXa inhibitors has been observed to be a function not only of potency, but also of lipophilicity and plasma protein binding. Compound **44b** with acetyl substituent showed 61% inhibition of FXa activity at 0.1 μM, but displayed poor anticoagulant activity ($PTCT_2$ = 7 μM). Methoxyacetyl

derivative **44c** with increased polarity showed improvement in anticoagulant activity ($PTCT_2$ = 3 μM). Compound **44d** with cyano substituent showed improved inhibition of FXa (76% inhibition) and also appreciable anticoagulant activity ($PTCT_2$ = 2.3 μM). Conversion of cyano group of **44d** to amide group (**44e**) found to be detrimental for both FXa inhibitory activity (41% inhibition) and anticoagulant activity ($PTCT_2$ = 4.1 μM).

Table 13. Effect of simple substituents at N atom of sulfoximine group



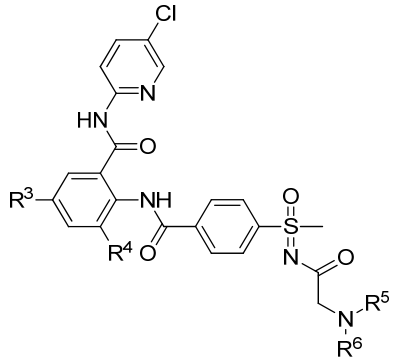
Compound	R^1	% inhibition[a] at 0.1 μM	$PTCT_2$[b] in human plasma (μM)
43a	H	76	7.2
44a	SO_2Me	55	2.6
44b	$COCH_3$	61	7
44c	$COCH_2OMe$	48	3
44d	CN	76	2.3
44e	$CONH_2$	41	4.1

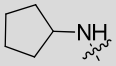
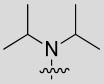
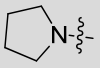
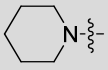
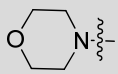
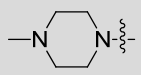
[a]Inhibitory activity against human FXa. Values shown are the mean of duplicate measurements. [b]Concentration of the compound required to double the clotting time in the PT assay using human plasma. $PTCT_2$ values shown are the mean of duplicated measurements.

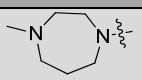
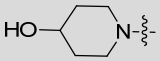
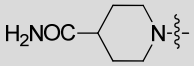
Taking clue from the difference observed for compound **44b** and **44c** in anticoagulant activity, we decided to replace polar methoxy group of **44c** with several alkylamino substituents to see its effect on anticoagulant potential and subsequently several amioacyl derivatives **46a**-**46s** were synthesized. *In vitro*

FXa inhibitory activity and anticoagulant activity in human plasma of **46a**-**46s** are listed in **Table 14**.

Table 14. SAR of aminoacyl derivatives at sulfoximine N atom



Compound	NR^5R^6	R^3	R^4	%inhibition[a] at 0.1 μM	$PTCT_2$[b] using human plasma (μM)
46a		Cl	H	93	0.77
46b		Cl	H	100	0.87
46c		Cl	H	97	0.95
46d		Cl	H	100	2.26[c]
46e		Cl	H	85	1.2
46f		Cl	H	100	0.68
46g		Cl	H	79	1.45
46h		Cl	H	95	0.46
46i		Cl	H	97	1.73[c]
46j		Cl	H	45	ND
46k		Cl	H	88	2.03[c]

Compound	NR^5R^6	R^3	R^4	%inhibition[a] at 0.1 μM	$PTCT_2$[b] using human plasma (μM)
46l		Cl	H	75	1.82[c]
46m		Cl	H	69	2.16[c]
46n		Cl	H	49	2.54[c]
46o		OCH_3	H	56	1.44
46p		Cl	OCH_3	92	0.58
46q		Cl	OCH_3	100	0.74
46r		Cl	OCH_3	97	0.92
46s		Cl	OCH_3	90	0.53

[a]Inhibitory activity against human FXa. Values shown are the mean of duplicate measurements. [b]Concentration of the compound required to double the clotting time in the PT assay using human plasma. $PTCT_2$ values shown are the mean of duplicated measurements unless otherwise indicated. [c]Single determination.

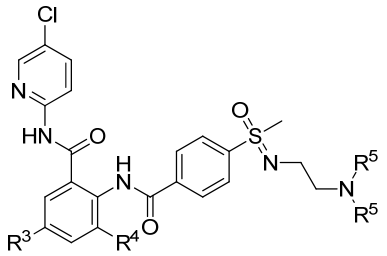
We investigated several aliphatic and cyclic amines. Compound **46a** with methylamino substituent showed 93% inhibition at 0.1 μM with improved anticoagulant activity in human plasma ($PTCT_2$ = 0.77 μM). Increasing alkyl chain from methyl (**46a**) to ethyl (**46b**) and to isopropyl (**46c**) was well tolerated for both FXa inhibitory and anticoagulant activity as reflected in their data (**Table 14**). Further increase in bulk with cyclopentylamino substituent (**46d**) was found to be detrimental for anticoagulant activity even though FXa inhibitory potency was retained ($PTCT_2$ = 2.26 μM). Dialkyl amino substituents were also well tolerated with comparable anticoagulant activity as evident from data of **46e** (dimethylamino, $PTCT_2$ = 1.2 μM) and **46f** (diethylamino, $PTCT_2$ = 0.68 μM).

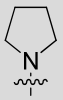
However, compound **46g** with diisopropylamino substituent showed decreased anticoagulant activity ($PTCT_2$ = 1.45 μM). We then examined the effect of cyclic amines. Compound **46h** with pyrrolidinyl substituent showed potent FXa inhibitory activity and excellent anticoagulant activity ($PTCT_2$ = 0.46 μM). Ring expansion in **46h** (5 membered pyrrolidine) to **46i** (6 membered piperidine) retained its FXa inhibitory activity, but showed significant reduction in anticoagulant activity ($PTCT_2$ = 0.46 μM of **46h** vs 1.73 μM of **46i**). Substitution with morpholine group (**46j**) resulted in a diminished activity of only 45% FXa inhibition at 0.1 μM. Compound **46k** with 4-methylpiperazine substituent showed 88% inhibition of FXa activity, but failed to show improvement in anticoagulant activity ($PTCT_2$ = 2.03 μM). Increasing ring size from 4-methypiperazine (6 membered, **46k**) to 4-methylhomopiperazine (7 membered, **46l**) produced compound with slightly inferior FXa inhibitory activity and almost comparable anticoagulant activity ($PTCT_2$ = 1.82 μM). Introduction of additional hydrophilic substituent in **46i** afforded compounds **46m** (4-hydroxypiperidine) and **46n** (piperidine-4-carboxamide), with inferior FXa inhibitory and anticoagulant activity (**46m**: $PTCT_2$ = 2.16 μM, **46n**: $PTCT_2$ = 2.54 μM).

Further refining of FXa inhibition and anticoagulant effect had been achieved by studying effect of chloro and methoxy substituents on central phenyl ring, which represents neutral and hydrophilic substituents respectively and also frequently used in anthranilamide class of compounds. Replacement of chloro substituent in **46f** with methoxy substituent produced compound **46o** with reduced potency (56% inhibition at 0.1 μM), which matches with our earlier

observation for compound **43e**. Incorporating additional methoxy substituent along with chloro group produced compounds with almost similar FXa inhibitory and anticoagulant activity, when compared with only chloro substituted compounds as evident from compounds **46p**-**46s** (**46p** vs **46a**, **46q** vs **46b**, **46r** vs **46f**, and **46s** vs **46h**).

Table 15. SAR of aminoalkyl derivatives at sulfoximine N atom



Compound	NR^5R^6	%inhibition[a] at 0.1 μM	$PTCT_2$[b] using human plasma (μM)
50a		82	1.14
50b		77	1.78
50c		74	2.16

[a]Inhibitory activity against human FXa. Values shown are the mean of duplicate measurements. [b]Concentration of the compound required to double the clotting time in the PT assay using human plasma. $PTCT_2$ values shown are the mean of duplicated measurements.

We then replaced aminoacyl group of derivatives described in **Table 14** with aminoalkyl group and subsequently few selected compounds **50a**-**50c** were synthesized (**Table 15**). Compound **50a** with dimethyl amino substituent showed 82% FXa inhibition at 0.1 μM and moderate anticoagulant activity ($PTCT_2$ = 1.14 μM). Compounds with diethylamino substituent (**50b**) and pyrrolidinyl substituent (**50c**) showed similar FXa inhibition (77% and 74% respectively) but their

anticoagulant potency decreased significantly ($PTCT_2$ = 1.78 μM and 2.16 μM respectively).

The compounds selected on the basis of FXa inhibition and anticoagulant activity from **Tables 14** and **15** were evaluated for their *ex vivo* PT prolonging activity in rats, *in vitro* anticoagulant activity in rat plasma, and IC_{50} value determination. Results are summarized in **Table 16**.

Table 16. *In vitro* and *ex vivo* anticoagulant activities of potent FXa inhibitors

Compound	IC_{50} (nM)[a]	Human $PTCT_2$[b]	Rat $PTCT_2$[b]	*Ex vivo* rat PT ratio[c]
46a	5.4	0.77	1.7	1.1
46b	2.7	0.81	2.1	1.3
46c	4	0.95	1.9	1.5
46e	9	1.2	1.4	1.6
46f	2.1	0.68	0.8	2.2
46h	3.2	0.46	1.57	1.4
46p	5.6	0.58	0.9	1.1
46q	2.4	0.74	1.15	1.1
46r	3.4	0.92	1.3	1.1
46s	6.4	0.53	1.1	1.1
50a	11.6	1.14	2.01	1.0
Rivaroxaban	1.6	0.39	1.5	ND[d]

[a]Inhibitory activity against human FXa. IC_{50} values shown are the mean of duplicate measurements. [b]Concentration of the compound required to double the clotting time in the PT assay using human and rat plasma. $PTCT_2$ values shown are the mean of duplicated measurements. [c]The *ex vivo* PT prolonging activity was determined 2 h after oral administration to rat (n = 4)at a dose of 30 mg/kg. [d]Not determined.

All tested compounds have their IC_{50} value for human FXa inhibition less than 12 nM. They all displayed relatively low anticoagulant activity in rat plasma compare to human plasma as reflected in their $PTCT_2$ values. However,

compound **46f** (IC_{50} = 2.1 nM) showed balanced anticoagulant activity in both rat and human plasma ($PTCT_2$ = 0.8 μM for rat vs 0.68 μM for human). Compound **46e** (IC_{50} = 9 nM) is another compound with similar anticoagulant activity in both species ($PTCT_2$ = 1.4 μM for rat vs 1.2 μM for human). The *ex vivo* PT prolonging activity in rats was determined 2 h after oral administration to rat at a dose of 30 mg/kg and data are summarized in **Table 16**. Compounds **46f**, **46e**, and **46c** have shown PT prolongation 2.2, 1.6 and 1.5 fold respectively, while remaining compounds failed to show significant PT prolongation which could be due to their poor oral bioavailability. Compound **46f** possesses chiral centre at S atom, hence both the enantiomers were separated using chemical resolution technique (**Scheme 10**) and checked individually for their *in vitro* FXa inhibitory activity and anticoagulant activity in human plasma. Both enantiomers showed similar activity in both assay ($PTCT_2$ using human plasma: 0.65 for (**+**) **46f** and 0.67 for (**-**) **46f**).

Based on its *ex vivo* anticoagulant effect, racemic **46f** was selected for pharmacokinetics (PK) evaluation in rats. However, to the contrary hydrochloride salt of **46f** showed low plasma levels (less than 100 ng/mL), when dosed orally at 30 mg/kg in wistar rats. The lack of correlation between plasma concentration and *ex vivo* anticoagulant activity prompted us to search the possibility for an active metabolite. From thorough evaluation of metabolism study data, it was found that mono de-ethylated compound is generating from **46f**, which is in fact a compound **46b** (**Tables 14** and **16**), further confirmed by matching UPLC retention time. In a separate study, plasma levels of compounds **46f** and its

metabolite **46b** were estimated by dosing hydrochloride salt of **46f** orally in rats at 30 mg/kg (**Table 17**).

Table 17. Pharmacokinetic parameters[a] for compound **46f**[b] and its metabolite **46b**

Compound	C_{max} (µg/mL)	tmax (h)	$t_{1/2}$ (h)	AUC(0-24) (h µg/mL)
46f (46b)[c]	3.28 ± 0.26	2.75 ± 0.47	4.79 ± 0.2	43.51 ± 2.81

[a]Data are expressed as the mean ± SEM. [b]Monohydrochloride salt was used. [c]Compound **46f** was dosed in fasted male wistar rats at 30 mg/kg po formulated with a Tween-80: PEG: CMC (5:5: 90 % v/v) and plasma levels of metabolite **46b** were determined (n = 4).

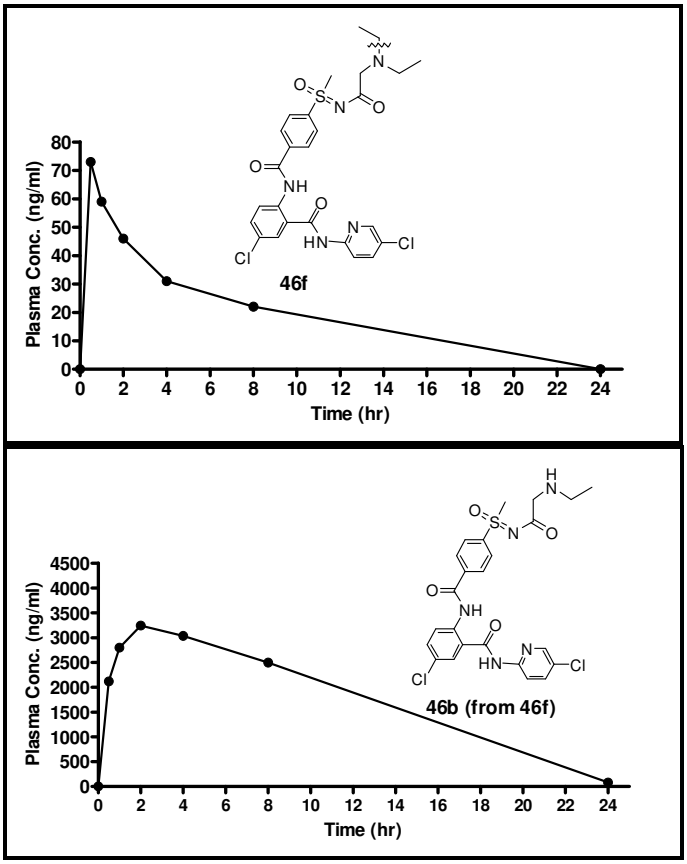


Figure 33. Plasma concentration pattern of **46f** and its metabolite **46b** in fasted male wistar rats at 30 mg/kg po.

The plasma levels of **46b** was found to be excellent (*C*max = 3.28 µg/mL) in comparison with parent compound **46f**. The plasma concentration pattern of **46f** along with metabolite **46b** is shown in **Figure 33**. The half life of **46b** was found to be 4.79 h which is fairly good predicator for a drug to be dosed once a daily clinically. The low peak vs trough ratio in PK profile of **46b** is desirable for this therapeutic class to reduce the bleeding liability.

Selectivity against related serine proteases is a decisive criteria for developing FXa inhibitors and hence, we then examined the effect of compounds **46f** and its metabolite **46b** against related serine proteases.

Table 18. Selectivity profile for compounds **46f** and **46b** against related serine proteases[a] and effect on CYP3A4

Compound	Ki (nM)						
	FXa	**thrombin**	**plasmin**	**trypsin**	**t-PA**	**aPC**	**CYP3A4**
46f	1.1	>20 µm[b]	>20 µm[b]	>20 µm[b]	>20 µm[b]	>20 µm[b]	18% inhibition at 10 µM
46b	1.5	>20 µm[b]	>20 µm[b]	>20 µm[b]	>20 µm[b]	>20 µm[b]	27% inhibition at 10 µM

[a]Inhibitory constant for human enzymes. Ki values shown are the mean of duplicate measurements. [b]Single determination.

Both the compounds **46f** and **46b** were found to be more than 10^4 fold selective against thrombin, plasmin, trypsin, t-PA and aPC (**Table 18**). Additionally, compounds **46f** and **46b** were checked for their effect on human cytochrome P450 enzyme (CYP3A4), which is known to be a primary factor

responsible of metabolism of most drugs. Both the compounds showed insignificant inhibition of CYP3A4 at 10 μM (18% for **46f** and 27% for **46b**).

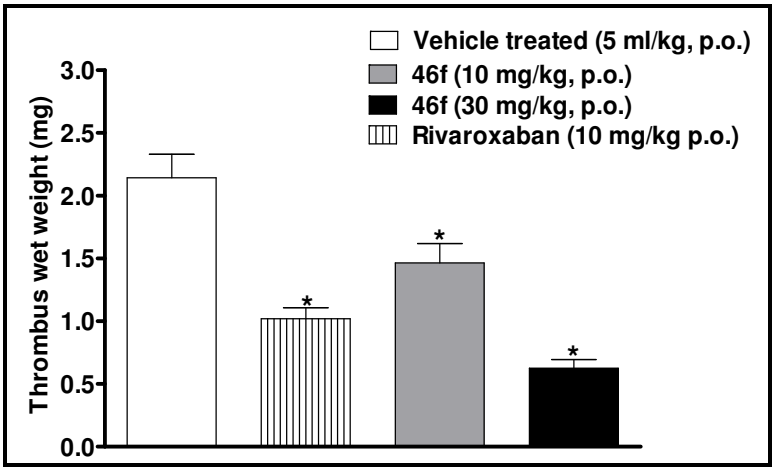


Figure 34. Effect of **46f** (monohydrochloride) and Rivaroxaban on $FeCl_3$-induced carotid artery thrombus weight after 2 hours of oral administration in male wistar rats (n=10). * $p < 0.01$ vs Vehicle control, ANOVA followed by Dunnett's test

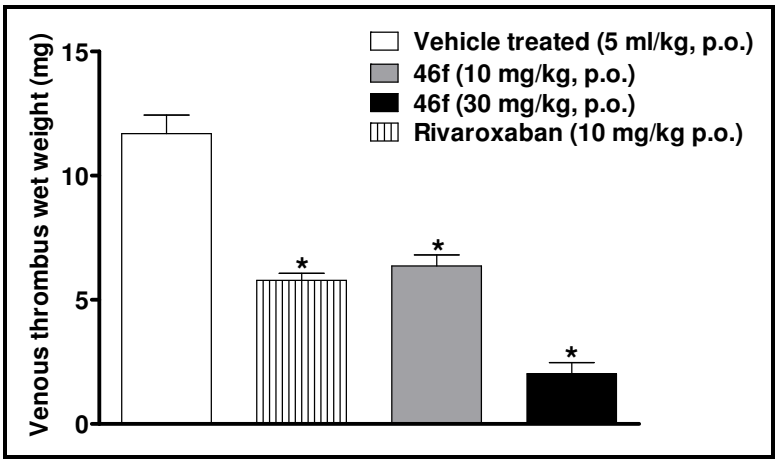


Figure 35. Effect of **46f** (monohydrochloride) and Rivaroxaban on partial stasis combined with $FeCl_3$-induced venous thrombus weight after 2 hours of oral administration in male wistar rats (n = 10). * $p < 0.01$ vs Vehicle control, ANOVA followed by Dunnett's test

Further, the *in vivo* antithrombotic efficacy of compound **46f** was evaluated in rats using $FeCl_3$-induced arterial and venous thrombosis model. Dose

dependent thrombus weight reduction was found in both arterial and venous thrombosis model, when **46f** was dosed orally at 10 mg/kg and 30 mg/kg in rats (**Figure 34** and **Figure 35**). In an arterial thrombosis model (**Figure 34**), **46f** reduced thrombus weight by 32% and 71% at 10 mg/kg and 30 mg/kg respectively. In venous thrombosis model (**Figure 35**), reduction in thrombus weight was 45% and 81% at 10 mg/kg and 30 mg/kg respectively. Rivaroxaban was used as positive standard at single dose of 10 mg/kg. In an arterial thrombosis model, Rivaroxaban reduced thrombus weight by 50% and in venous thrombosis model, reduction in thrombus weight was 63%.

To predict the binding mode of compound **46f** and its active metabolite **46b**, a molecular modeling study was carried out. **Figure 36** show inhibitors **46f** (**A**) and **46b** (**B**) docked in the active site of FXa. The proposed binding model indicated that the 5-chloro-2-aminopyridyl moiety deeply occupied the S1 pocket with chlorine atom pointing towards the center of the Tyr228 aromatic ring, while the substituted sulfoximine group fits in the S4 pocket formed by the residues Phe174, Trp215, and Tyr99. We have also observed that there are several π-π and CH-π interactions in **46f** and **46b**. These two molecules forms a CH-π interaction with Tyr99 with a distance of 3.6 Å between aromatic CH and centroid of Tyr99, at the same time an inclined π-π interaction between Trp215 and phenyl ring attached with sulfoximine group with a distance of 5.4 Å. The –NH- of ethylamino group attached to sulfoximine group of **46b** showed H-bond interaction with Glu97, which is at the periphery of the S4 site. The model further suggested that the –NH- group of the amide bond linked to the 5-chloro-2-

aminopyridyl moiety and carbonyl group of the amide bond connected to the sulfoximine-substituted benzene forms H-bond with Gly218 and Gly216 respectively. Additionally, carbonyl group of amide bond linked to the sulfoximine group substituted benzene forms a H-bond with Gln192.

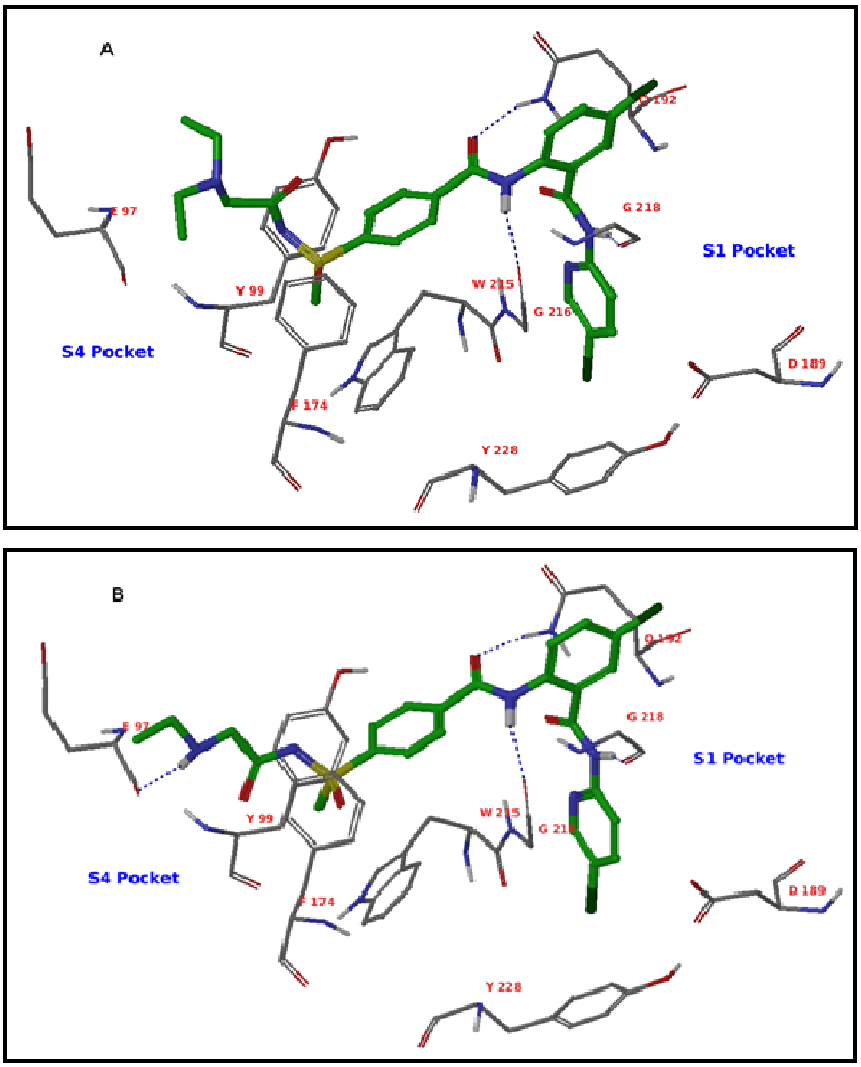


Figure 36. Docking study of **46f** (A) and **46b** (B) in the active site of FXa

In summary, application of sulfoximine group as novel S4 ligand in anthranilamide chemotype resulted in identification of lead molecule **43a**. Further optimization was achieved by synthesizing several derivatives with substituents

at both S and N atom of sulfoximine group. Biological evaluation of synthesized compounds led to identification of compound **46f** with potent FXa inhibitory activity and anticoagulant activities in both rat and human plasma. Oral dosing of this compound in rats produced active metabolite **46b**, which was responsible for potent *ex vivo* anticoagulant activity of compound **46f**. *In vivo* antithrombotic efficacy was determined using $FeCl_3$-induced arterial and venous thrombosis model in rats in which **46f** displayed dose dependent thrombus weight reduction at tested two doses. Both the compounds (**46f** and its metabolite **46b**) were found to be more than 10^4 fold selective against related serine proteases. Compound **46f** has insignificant effect on CYP3A4. The PK profile of **46f** along with its metabolite showed fairly good half life and low peak vs trough ratio, which may be an advantage for this compound clinically in terms of bleeding.

3.3. Dibenzothiophene-substituted anthranilamide derivatives as FXa inhibitors

3.3.1. Designing strategy

In continuation of our research efforts in anthranilamide-based FXa inhibitors, we then investigated dibenzothiophene group as novel S4 ligand which is derived from 3-chlorobenzothiophene derivative reported in the literature **[36]**. 3-Chlorobenzothiophene derivatives, even though having very high FXa inhibitory activity *in vitro*, suffer from poor anticoagulant activity due to their high lipophilicitiy.

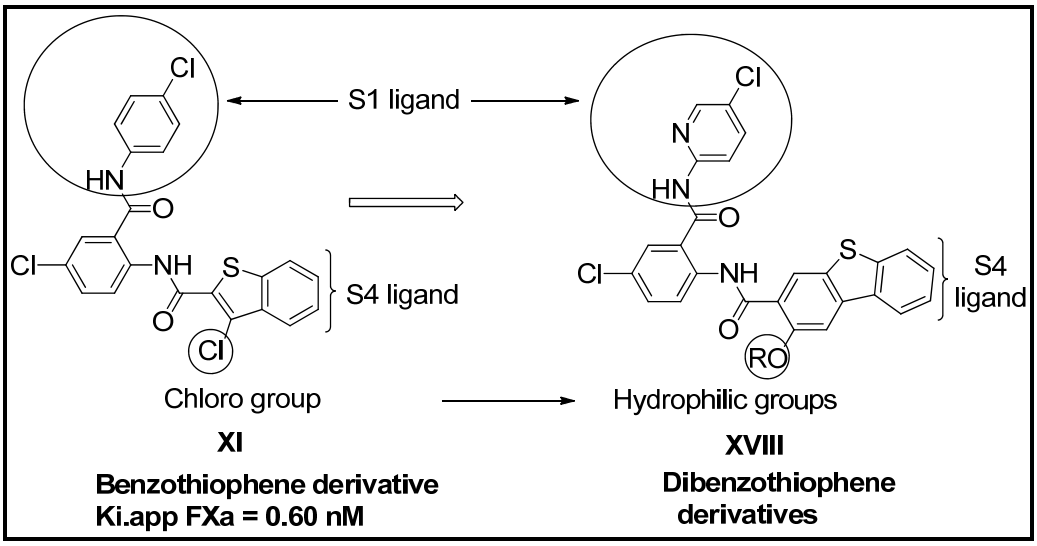


Figure 37. Dibenzothiophene substituted anthranilamide derivatives

Taking 3-chlorobenzothiophene derivative **XI** (Ki.app FXa = 0.60 nM) as our starting point, we derived novel derivatives **XVIII**, in which 4-chloroaniline group (S1 ligand) of **XI** was replaced with the highly optimized S1 ligand 5-chloro-2-pyridylamine. We then replaced chloro group with hydrophilic substituents like alkoxy, hydroxyl etc. to study its effect in both *in vitro* FXa inhibition and anticoagulant potential. As a part of this strategy, three selected compounds represented by general formula **XVIII** were synthesized as shown in **Figure 37**.

3.3.2. Results and discussion

3.3.2.1. Chemistry

Synthetic methodology was designed for general structure **XVIII** based on the reterosynthetic analysis (**Figure 38**) and the schemes described below. Synthetic strategies for S1 ligand and central core is similar to previously described anthranilamide derivatives. Synthesis of dibenzothiophene nucleous

was achieved by using new and efficient method which involves acid-mediated intramolecular cyclisation of biaryl sulfoxide which was discussed in next section. Coupling of these S1 ligand and S4 ligand with central core was accomplished by using general amide bond formation techniques.

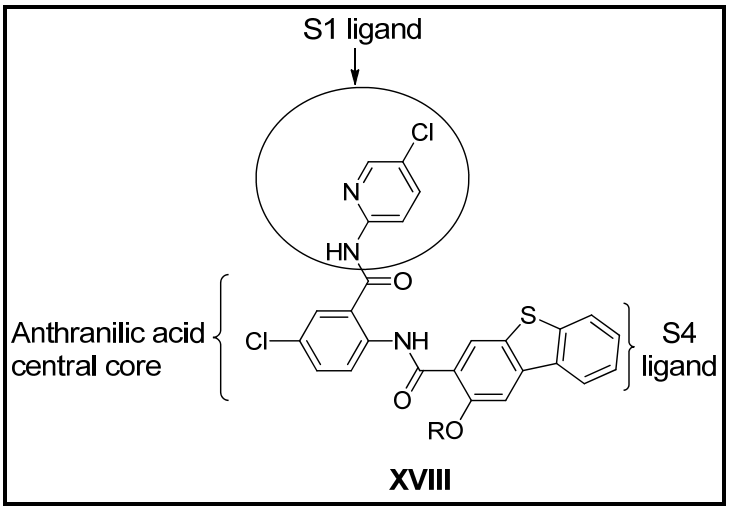
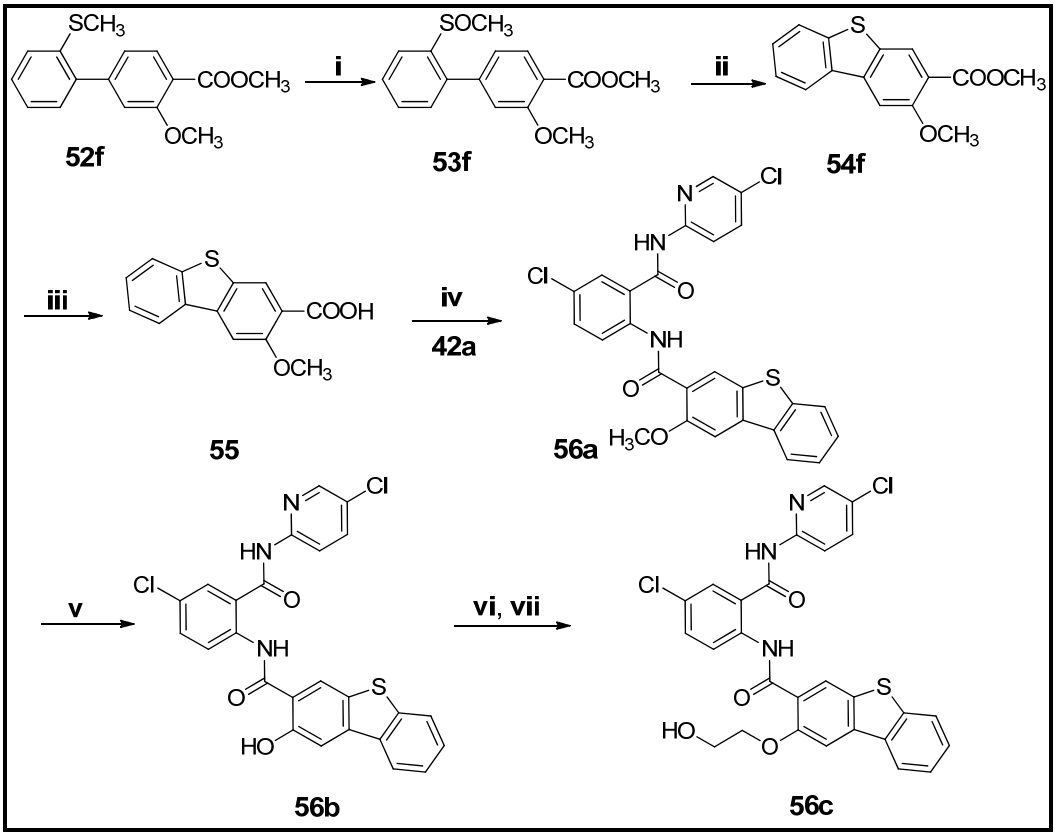


Figure 38. Reterosynthetic analysis of dibenzothiophene-substituted anthranilamide derivatives as FXa inhibitors

As a part of general structure **XVIII**, we designed three molecules and their synthesis is described in **Scheme 11**. Methylthio group of biaryl compound **52f** was oxidized to sulfoxide derivative **53f** using H_2O_2. Cyclisation using sulfuric acid gave Methyl 2-methoxydibenzo[b,d] thiophene-3-carboxylate (**54f**), which was then converted into its carboxylic acid derivative (**55**) using *t*-BuOK and water (2 mol eq.) in DMSO. Acid derivative **55** was then coupled with **42a** (Synthesized according to scheme 7) by first converting acid group of **55** into acid chloride using oxalyl chloride and then reacting with aniline derivative **42a** to get desired compound **56a**. Methoxy group of **56a** was then deprotected using BBr_3 in CH_2Cl_2 to get phenolic derivative **56b**. Reaction of **56b** with ethyl

bromoacetate and subsequent reduction with $NaBH_4$ gave desired compound **56c**.

Scheme 11. Synthesis of compounds **56a-56c**



Reagents and conditions: (i) H_2O_2 (50%), Cat.V_2O_5, CH_3CN; (ii) Conc.H_2SO_4, 0-75 ^oC, then aq. K_2CO_3, 25-30 ^0C; (iii) *t*-BuOK, DMSO, water, 25-30 ^0C; (iv) Oxalyl chloride, CH_2Cl_2, 25-30 ^0C, then **42a**, CH_2Cl_2, 25-30 ^0C; (v) BBr_3, CH_2Cl_2, -50 ^0C to 0 ^oC then 25-30 ^0C; (vi) Ethyl bromoacetate, NaH, DMF, 55 ^0C; (vii) $NaBH_4$, DMSO, Ethanol, 60-70 ^0C.

3.3.2.1.1. Efficient synthesis of unsymmetrical dibenzothiophenes by acid-mediated intramolecular cyclization of biaryl methyl sulfoxides

For synthesis of Dibenzothiophenes, which was used as novel S4 binding element in our designed compounds, we adopted a new and efficient synthesis which was due to its synthetic applicability studied in details by synthesizing few

more substituted dibenzothiophene derivatives **54a**-**54j**. The synthetic plan which involves acid catalyzed intramolecular cyclization of biaryl methyl sulfoxides is described in **Figure 40**.

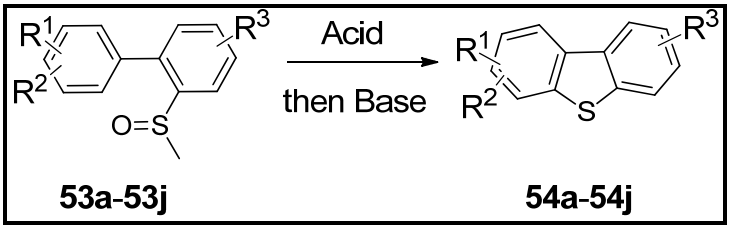


Figure 40. Acid-mediated cyclisation of biaryl sulfoxides

Literature methods

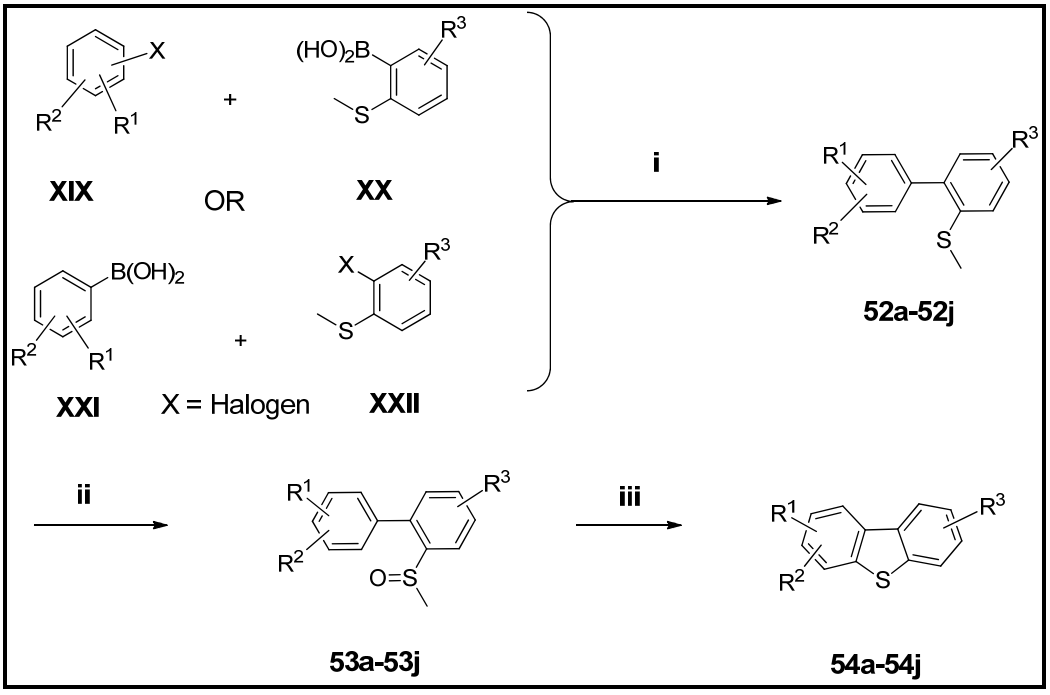
Literature discloses different approaches for preparation of substituted dibenzothiophenes (**54**), such as disulfide ring closure **[46]**, photochemical cyclization of 2-(2'-methylthio)biphenyl radical **[47]**, sulfur insertion in biphenyl using alluminium trichloride ($AlCl_3$) **[48]**, fusion of 2,2'-dihydroxybiphenyl with phosphorous pentasulfide (P_2S_5) **[49]**, ring contraction of thianthrene using copper bronze **[50]** and cyclization of biphenyl-2-sulfonyl chloride using $AlCl_3$ gives dibenzothiophene dioxide **[51]** which upon deoxygenation gives dibenzothiophene **[52]**. Sirringhaus *et al.* have reported synthesis of dibenzothienobisbenzothiophene by acid-mediated intramolecular cyclization of sulfoxide derivative **[53]**. This literature report involved the use of expensive and hazardous chemicals like trifluoromethanesulfonic acid and pyridine and also requires long reaction time and high temperature. It does not have wider applicability in terms of functional group sensitivity. Recently, Sanz *et al.* have reported the synthesis of regioselectively functionalized dibenzothiophenes

through anionic cyclization of benyne-tethered aryllithiums **[54]**.

The aforementioned literature methods involve high temperature reactions using sulfur, sulfur containing reagents or organolithium reagents. Also, some of these methods suffer from poor yields. Direct introduction of functional groups in dibenzothiophene core has limitations of regioselectivity. Literature methods for synthesis of 4-substituted dibenzothiophene ring involve metallation reactions in dibenzothiophene ring at 4th position followed by the addition of appropriate electrophile **[55]**. Electrophilic substitution reaction on dibenzothiophene ring goes at 2- position. Hence the synthesis of 1- and 3-substituted dibenzothiophene derivative is relatively difficult and involves multiple steps.

New synthetic method

Scheme 12. Synthetic scheme for dibenzothiophenes

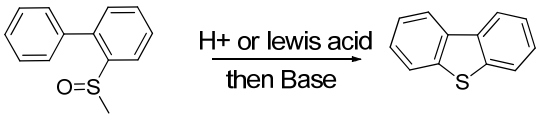


Reagents and conditions: (i) $Pd(OAc)_2$, Na_2CO_3, MeOH; (ii) H_2O_2 (50%), Cat.V_2O_5, CH_3CN; (iii) Conc.H_2SO_4, 0-75 ^oC, then aq. K_2CO_3, rt.

Biaryl sulfoxides are the penultimate intermediates which were used for synthesis of dibenzothiophenes and to access biaryl sulfoxides **53a**-**53j** (**Figure 40**), synthetic strategy depicted in **Scheme 12** is adapted. Several methods are reported to make the biphenyl ring system. For the sake of simplicity and easy availability of substituted boronic acids, we adapted Suzuki coupling reaction **[56]**. Thus, the coupling of substituted phenylboronic acid with substituted halobenzene using $Pd(OAc)_2$ as a catalyst gave the biphenyl ring which upon oxidation with H_2O_2 **[57]** produced desired sulfoxides **53a**-**53j** in excellent yield.

Once sulfoxides **53a**-**53j** were in hand, we set forth to screen the best acidic reagent to achieve ring closure and thereby producing dibenzothiophene. To achieve this goal we took unsubstituted sulfoxide **53a** as a tool compound (**Table 19**).

Table 19. Screening of acidic reagents for cyclization

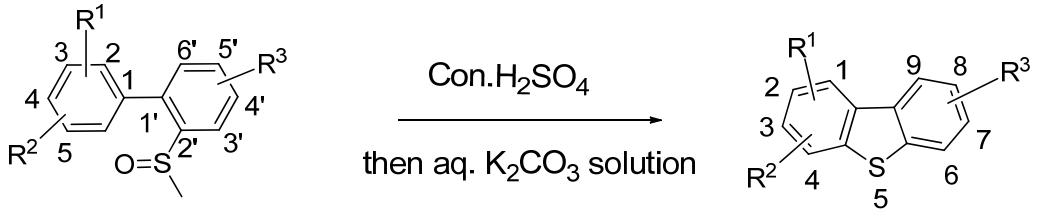


53a **54a**

Sr.No.	Cyclization reagent	Solvent	Reaction time (h)	Temperature °C	Yield (%)
1	H_2SO_4 (3 v/w)	Neat	0.25	0-25	94
2	I_2 (2 eq.)	Toluene	20	70	No reaction
3	$AlCl_3$ (2 eq.)	Toluene	20	70	No reaction
4	TFA (3 v/w)	Neat	20	0-25	10
5	H_2SO_4 (3 v/w)	Chloroform	15	0-25	50

Sulfuric acid was found to give the best result in terms of yield and purity of dibenzothiophene **54a** as compared to other lewis or mineral acids screened. It was also found that, the cyclization worked excellent when neat sulfuric acid was used. Sulfuric acid in solvent took longer time to bring desired cyclization and also isolated yield was low.

Table 20. Synthesis of substituted dibenzothiophenes



53a-53j **54a-54j**

Comp. no.	Substituent on 53	Substituent on 54	Temp. (°C)	Time (h)	Yield (%)
54a	$R^1 = R^2 = R^3 = H$	$R^1 = R^2 = R^3 = H$	0-25	0.5	94
54b	$R^1 = R^3 = H$, R^2 = 4-F	$R^1 = R^3 = H$, R^2 = 3-F	0-25	0.5	92
54c	$R^1 = R^3 = H$, R^2 = 2-CH_3	$R^1 = R^3 = H$, R^2 = 1-CH_3	0-25	0.5	97
54d	$R^1 = R^3 = H$, R^2 = 4-$COOCH_3$	$R^1 = R^3 = H$, R^2 = 3-$COOCH_3$	0-25	1	75
54e	$R^1 = R^3 = H$, R^2 = 4-NO_2	$R^1 = R^3 = H$, R^2 = 3-NO_2	0-75	2	49
54f	R^1 = 3-OCH_3, R^2 = 4-$COOCH_3$, $R^3 = H$	R^1 = 2-OCH_3, R^2 = 3-$COOCH_3$ $R^3 = H$	0-25	1	74

Comp. no.	Substituent on 53	Substituent on 54	Temp. (°C)	Time (h)	Yield (%)
54g	R^1 = 2-CH_3, R^2 = H, R^3 = 5'-$COCH_3$	R^1 = 1-CH_3, R^2 = H, R^3 = 8-$COCH_3$	0-25	0.25	82
54h	R^1 = 2-CH_3, R^2 = H, R^3 = 5'-CH_2CH_3	R^1 = 1-CH_3, R^2 = H, R^3 = 8-CH_2CH_3	0-25	0.25	85
54i	R^1 = 3-$COCH_3$, R^2 = R^3 = H	R^1 = 2-$COCH_3$, R^2 = R^3 = H	0-25	0.25	70
54j	R^1 = 2-$COOCH_3$, R^2 = R^3 = H	R^1 = 1-$COOCH_3$, R^2 = R^3 = H	0-25	1	55

To extend the application of above reaction for the synthesis of substituted dibenzothiophenes, sulfoxides **53a**-**53j** with various substituents have been cyclized in neat sulfuric acid (**Table 20**). It was observed that, sulfoxides **53a**-**53j** containing electron-releasing groups produced higher yield of corresponding dibenzothiophenes (**54a**-**54c, 54g** and **54h**). An electron-withdrawing group like ester (**53d**) and nitro (**53e**) resist cyclization and hence gave relatively lower yields of corresponding dibenzothiophenes **54d** and **54e** respectively. Most of the reactions were completed at 25 ^oC within 30 min., except reactions with ring system bearing electron-withdrawing groups (**54d**, **54e** and **54f**). Compound **54f**, our target intermediate was synthesized in 74% yield. 1-substituted dibenzothiophenes were effectively synthesized as can be seen from examples **54c** and **54j**. Substituents on both the rings are possible through this methodology (**54g** and **54h**).

The proposed mechanism of the reaction is shown in **Figure 41**. The first step involves protonation of sulfoxide followed by nucleophilic attack of neighboring aromatic ring. Dehydration eventually led to aromatization furnishing salt **57**. The demethylation of this salt using base gives desired dibenzothiophene derivatives.

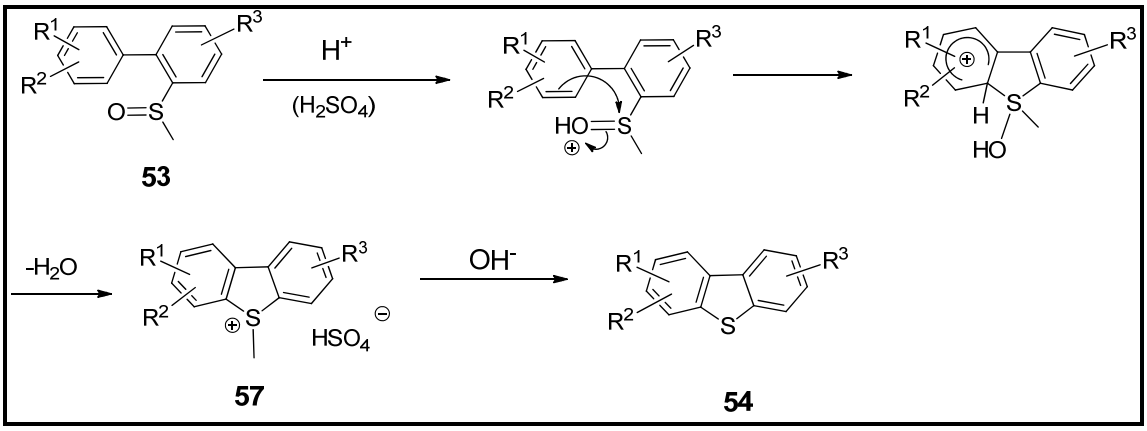


Figure 41. Proposed mechanism for cyclization

Mechanism correlates with our experimental observation of low yield and more reaction time for electron-withdrawing group substituted sulfoxide as it destabilizes positively charged transition state. Nenaidenko *et al*. have reviewed synthetic capabilities of sulfonium salts with mechanistic aspects **[58]**. Nucleofugality of group attached to *S* atom is one of the main factors that determines direction of nucleophilic attack on sulfonium salt. In our case biaryl sulfide is good leaving group compare to methyl in sulfonium salt **57** and also methyl group provides electrophilic centre for nucleophile that is hydroxide ion generated from aqueous K_2CO_3.

In summary, a convenient and efficient method for the synthesis of unsymmetrical dibenzothiophene derivatives has been reported. The advantage of the described methodology over the reported ones is incorporation of substituents in dibenzothiophene core with ease and good yields. The present method utilizes inexpensive and environment friendly reagents. Further the duration of reaction is very short and also reactions are accomplished at low temperature. This method gives access to substituent at every position of dibenzothiophene. 1-subsituted dibenzothiophene, which is difficult to make by direct substitution reaction on dibenzothiophene can be easily prepared by this method. This methodology has a potential application for the synthesis of sulfur containing fused heterocycles.

3.3.2.2. Structure-activity data of dibenzothiophene-substituted anthranilnamide derivatives

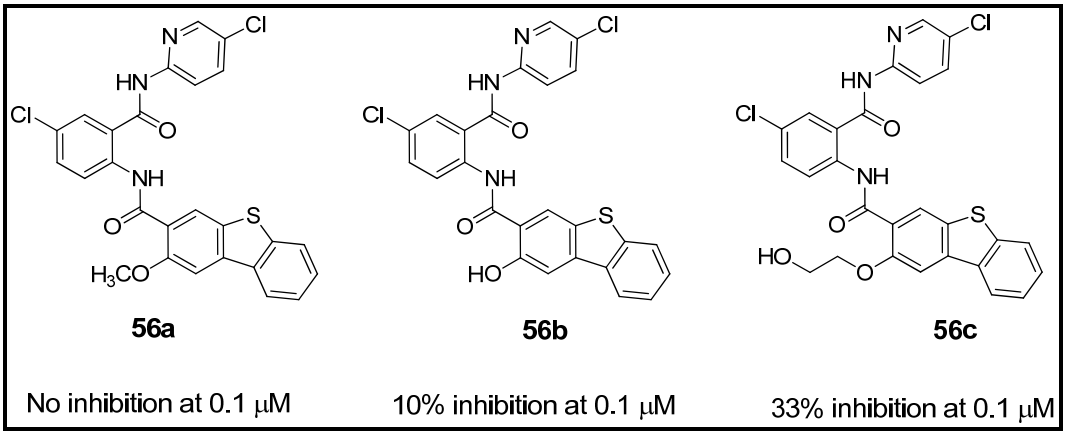


Figure 39. Structure-activity data of dibenzothiophene-substituted anthranilamide derivatives

Compounds synthesized using **Scheme 11** were evaluated for their *in vitro* FXa inhibitory activity (**Figure 39**). However, none of the compounds

showed appreciable FXa inhibition. Compound **56c** showed 33% FXa inhibition at 0.1µM.

3.4. Conclusions

In summary, two novel S4 ligands were identified and applied in anthranilamide chemotype to discover potent FXa inhibitors. Biological evaluation of sulfoximine-substituted anthranilamide derivatives led to identification of compound **46f** and its active metabolite **46b**, which has shown strong human FXa inhibitory activity and anticoagulant activities in both rat and human plasma. Compound **46f** displayed dose dependent thrombus weight reduction in $FeCl_3$-induced arterial and venous thrombosis model in rats. Both the compounds (**46f** and its metabolite **46b**) were found to be more than 10^4 fold selective against related serine proteases and also showed insignificant effect on CYP3A4. The PK profile of **46f** along with its metabolite showed fairly long half life and low peak vs trough ratio, which may be an advantage for this compound clinically in terms of reduced bleeding complications.

Second series utilized dibenzothiophene as S4 ligand in same anthranilamide chemotype. Although the designed three compounds did not showed appreciable FXa inhibitory activity, the synthesis of this novel dibenzothiophene ligand was achieved by new and efficient method which involved acid-mediated cyclization of biaryl sulfoxides. This method has a potential to be useful for synthesis of several sulfure containing heterocycles.

3.5. References

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Summary and future directions

4. SUMMARY AND FUTURE DIRECTIONS

4.1. Plasminogen Activator Iihibitor-1 (PAI-1) inhibitors

Thrombotic disorders are associated with high mortality and morbidity. Fibrinolysis pathway provides an useful strategy to develop novel antithrombotic agent which dissolves existing thrombus and restores normal blood flow by removing thrombotic occlusions. The role of PAI-1 has been evaluated in thrombosis and it provided a strong rationale to explore PAI-1 inhibitors for the treatment of thrombotic disorders.

The first step to identify potential PAI-1 inhibitors started with HT screening of compound library, which resulted in the discovery of our initial lead **1**, an oxalamide derivative. Systematic SAR evaluation around **1** produced several *in vitro* potent PAI-1 inhibitors such as **8d**, **15d**, **15h**, **15i**, **15o**, **15r**, **15s** and **15t**, which unfortunately could not be evaluated further due to their poor oral bioavailability.

To identify a new series with improved oral bioavailability, we then designed 5-Nitro-2-phenoxybenzoic acid derivatives derived using hybridization and conformational restriction strategies of two known chemotypes. As a part of hybridization strategy, oxoacetic acid part of Tiplaxtinin (First chemotype) was effectively replaced with 5-nitro-2-phenoxybenzoic acid part of Piperazine derivative (**I**, second chemotype) producing potent PAI-1 inhibitor **24a**. The docking study confirmed the similar orientation of **24a** and Tiplaxtinin in PAI-1 ligand binding site and hence provided a rationale for its synthesis.

As a part of conformational restriction strategy, several cyclized derivatives of (**I**) were prepared. Conformational restriction of (**I**) with indole as a central core (**30c**) showed potent PAI-1 inhibitory activity and excellent pharmacokinetic profile with long half life. Compound (**30c**) displayed antithrombotic efficacy in rats using $FeCl_3$-induced arterial thrombosis model and hence can be useful for the treatment of thrombotic disorders especially MI and stroke.

4.2. Coagulation Factor Xa inhibitors

Prevention of thrombus formation is another attractive approach to treat thrombotic disorders. Anticoagulants prevents fibrin (clot) formation by targeting coagulation cascade and have established role as antithrombotic agents. We evaluated several coagulation factors as theoretical possibility to identify new anticoagulant drug. FXa being a central enzyme in coagulation cascade has a key role in blood coagulation and also FXa inhibitors have a clinical proof of concept thus provides a strong rationale to identify an improved FXa inhibitors.

Anthranilamide-based FXa inhibitors were explored by many research group among them three are already in clinic. First series involve application of sulfoximine group as novel S4 ligand in anthranilamide chemotype. Biological evaluation of synthesized compounds led to identification of compound **46f** and its active metabolite **46b**, which has shown strong human FXa inhibitory activity and anticoagulant activities in both rat and human plasma. Compound **46f** displayed excellent antithrombotic efficacy in both venous and arterial thrombosis model in rats. It has shown high selectivity against related serine proteases and

also has insignificant effect on CYP3A4 inhibition. The PK profile of **46f** along with its metabolite showed fairly long half life and low peak vs trough ratio, which may be an advantage for this compound clinically in terms of reduced bleeding complications and hence can be useful for the treatment of thrombotic disorders especially VTE and stroke prevention in patients with AF.

In continuation of research efforts in anthranilamide-based FXa inhibitors, second series which utilizes dibenzothiophene as novel S4 ligand was identified. Designing strategy was derived from literature compound **XI** (3-chlorobenzothiophene derivative), which showed very good *in vitro* FXa inhibitory activity but failed to show anticoagulant activity in human plasma due to high lipophilicity. Three selected polar analogues were designed as a part of this new strategy and evaluated for *in vitro* FXa inhibition. Unfortunately, they failed to show expected FXa inhibitory activity.

Synthesis of this novel dibenzothiophene ligand was achieved by new and efficient method which involved acid-mediated cyclization of biaryl sulfoxides. Several substituted dibenzothiophene derivatives were prepared in good yield and purity. This method has a potential to be useful for synthesis of several sulfur containing heterocycles.

4.3. Future directions

Research efforts in PAI-1 inhibition approach revealed compound (**30c**) which has displayed very good *in vitro* PAI-1 inhibitory activity and also *in vivo* antithrombotic efficacy in rat arterial thrombosis model. Preclinical toxicity study needs to be initiated for this compound for developing it as a clinical candidate.

Although, PAI-1 inhibitors have shown ray of hope at preclinical stage, there are no active oral PAI-1 inhibitors in clinic as of now. It will be great achievement for if compound **30c** can fulfill the unmet needs in the area of oral thrombolytic agents.

Using FXa inhibition approach, compound **46f** and its active metabolite **46b** have been identified as potent and efficacious FXa inhibitors. The plasma concentration profile of **46b** suggested that it has low peak vs trough ratio, which is ideal for any anticoagulant drug to be safer in terms of bleeding complications clinically. The preclinical toxicity study needs to be initiated for this compound to derive differentiating parameters or possible advantages for this drug over approved FXa inhibitors such as Rivaroxaban, Apixaban and Edoxaban. I fore-see an optimistic scenario which would eventually display additional benefits over approved FXa inhibitors and hence may fulfill the high unmet needs in area of safe oral anticoagulants.

Experimental

5. EXPERIMENTAL

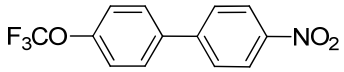
5.1. Chemistry

5.1.1. General

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using commercial silica gel (100-200 or 230-400 mesh). Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (Vmax in cm^{-1}, using KBr pellets, $CHCl_3$ or CCl_4). The ^1H NMR spectra were recorded on a Brucker Avance-300 spectrometer (300 MHz) and Brucker Avance-400 spectrometer (400 MHz). The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, in either $CDCl_3$ or DMSO-d_6 solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), bd (broad doublet), and m (multiplet). ^{13}CNMR spectra were recorded on Brucker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-d_6 solution. Mass spectra (ESI-MS) were obtained on Shimadzu LC-MS 2010-A spectrometer. Elemental analyses were carried out using a Perkin-Elmer 2400 CHN analyzer. Purity of compounds were determined by Ultra Performance Liquid Chromatography (UPLC) (Column, BEH C-18, 2.1x100 mm; UV detection, 220 nm; eluent, 0.05% TFA buffer:ACN (gradient); flow rate, 0.4 mL/min) or by HPLC analysis (column ODS C-18, 150nm * 4.6 nm * 4 μ on AGILENT 1100 series; UV detection, 220 nm; eluent, 0.05% TFA buffer:ACN (gradient); flow rate, 1 mL/min).

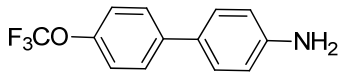
➢ PAI-1 inhibitors

5.1.2. 4-Nitro-4'-(trifluoromethoxy)-1,1'-biphenyl (4)



To a stirring solution of 4-trifluoromethoxy phenylboronic acid (**2**) (1 g, 0.0048 mol), tripotassium phosphate (2.05 g, 0.0097 mol), tetrabutyl ammonium bromide (TBAB) (0.31 g, 0.00096 mol) and 4-iodo nitrobenzene (**3**) (1.2 g, 0.0048 mol) in DMF (5 mL), a suspension of palladium acetate (5.4 mg, 0.000024 mol) in DMF (2 mL) was added at 25-30 ^{0}C under N_2 atmosphere. Reaction mixture was then stirred at 45-50 ^{o}C for 16 h. The reaction mixture was then diluted with 25 mL water and extracted with 50 mL ethyl acetate. The organic layer was washed with water & brine solution, dried over Na_2SO_4 and evaporated on a Rota vapor. The crude product was purified by column chromatography using 1% ethyl acetate in hexane as eluent and 100-200 silica gel to furnish title compound **4** as a white solid; Yield: 80%; mp: 71-74 ^{0}C; Purity by HPLC: 96.5%; IR (KBr) 1602, 1515, 1346, 1261, 842 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.53 (d, J = 8.2 Hz, 2H), 7.90-7.99 (m, 4H), 8.32 (d, J = 8.8 Hz, 2H); ESI/MS m/z No $(M+H)^+$ observed.

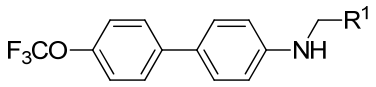
5.1.3. 4'-(Trifluoromethoxy)-[1,1'-biphenyl]-4-amine (5)



To a solution of **4** (3.0 g, 0.01060 mol) in MeOH (30 mL) was added Pd/C (10% w/w) (300 mg) in MeOH (30 mL) at 25-30 ^{o}C. The reaction mixture was then subjected for hydrogenation by applying 60 psi hydrogen pressure. After 3 h, reaction mixture was filtered through hyflow bed. Filtrate was evaporated on a

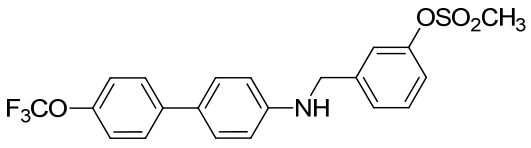
rotavapor to get crude material which was column purified using 100-200 silica gel and 10% ethyl acetate in hexane as mobile phase to get title compound **5** as off-white solid; Yield: 93%; mp: 94-97 ^{0}C; Purity by UPLC: 93.87%; IR (KBr) 3512, 3419, 1624, 1502, 1211, 823 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.31 (s, 2H), 6.62-6.66 (m, 2H), 7.32-7.38 (m, 4H), 7.61-7.65 (m, 2H); ESI/MS m/z 253.9 $(M+H)^+$.

5.1.4. General procedure for the synthesis of compounds of general formula (6a-6d)



To a solution of **5** (1 mole eq.) in EtOH (10 v/w) was added R^1-CHO (1 mol eq.) at 25 ^{0}C and refluxed for 4-5 h. The reaction mixture was cooled up to 40 ^{o}C. To this was added $NaBH_4$ (1 mole eq.) lot wise and stirred for 2 h. Reaction mixture was then diluted with water (30 v/w) and extracted with ethyl acetate (50 v/w). The organic layer was washed with water & brine solution, dried over Na_2SO_4 and evaporated under reduced pressure to get desired product in fairly good purity.

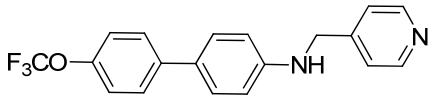
5.1.4.1. 3-(((4'-(Trifluoromethoxy)-[1,1'-biphenyl]-4-yl)amino)methyl)phenyl methane sulfonate (6a)



Compound **6a** was prepared by reacting 3-methanesulfonyloxy benzaldehyde with **5** using general procedure described above as off-white solid; Yield: 80%;

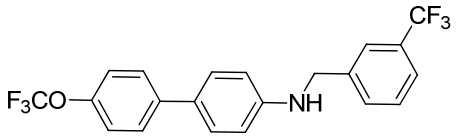
mp: 120-123 ^{0}C; Purity by UPLC: 90%; IR (KBr) 3396, 1610, 1500, 1356, 1180, 808 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.35 (s, 3H), 4.38 (d, J = 6 Hz, 2H), 6.59 (t, J = 6 Hz, 1H), 6.67 (d, J = 8.8 Hz, 2H), 7.20-7.22 (dd, J = 1.2 and 8 Hz, 1H), 7.32-7.37 (m, 3H), 7.39-7.46 (m, 4H), 7.61-7.65 (m, 2H); ESI/MS m/z 437.8 $(M+H)^+$.

5.1.4.2. Pyridin-4-ylmethyl-(4'-trifluoromethoxy-biphenyl-4-yl)-amine (6b)



Compound **6b** was obtained by reacting pyridine-4-carboxaldehye with **5** using general procedure described above as brownish solid; Yield: 55%; mp: 110-115 ^{0}C; Purity by HPLC: 94.5%; IR (KBr) 3311, 2923, 1610, 1502, 1253, 804 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.36 (d, J = 6.1 Hz, 2H), 6.63 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 6 Hz, 1H), 7.31-7.35 (m, 4H), 7.40 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.7 Hz, 2H), 8.49 (d, J = 5.7 Hz, 2H); ESI/MS m/z 345.1 $(M+H)^+$.

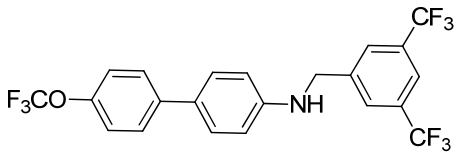
5.1.4.3. (4'-Trifluoromethoxy-biphenyl-4-yl)-(3-trifluoromethyl-benzyl)-amine (6c)



Compound **6c** was prepared by reacting 3-trifluoromethyl benzaldehyde with **5** using general procedure described above as off-white solid; Yield: 85%; mp: 78-81 ^{0}C; Purity by HPLC: 97.5%; IR (KBr) 3435, 1612, 1504, 1309, 1272, 1176, 821, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.45 (s, 2H), 6.67-6.70 (dd, J = 1.86

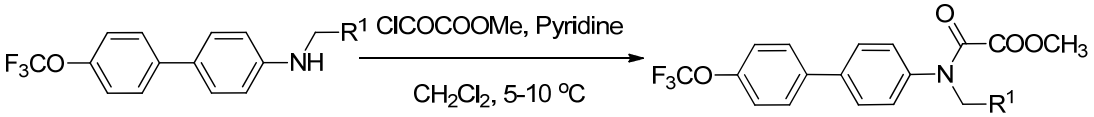
and 6.75 Hz, 2H), 7.24 (d, *J* = 2.7 Hz, 2H), 7.41 (d, *J* = 2 Hz, 2H), 7.50-7.59 (m, 6H), 7.65 (s, 1H); ESI/MS m/z 412.3 $(M+H)^+$.

5.1.4.4. (3,5-Bis-trifluoromethyl-benzyl)-(4'-trifluoromethoxy-biphenyl-4-yl)-amine (6d)



Compound **6d** was prepared by reacting 3-5-bistrifluoromethyl benzaldehyde with **5** using general procedure described above as off-white solid; Yield: 75%; mp: 76-80 ^{0}C; Purity by UPLC: 97.93%; IR (KBr) 3439, 2856, 1614, 1535, 1504, 1386, 1274, 1141, 806, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.54 (d, *J* = 6.4 Hz, 2H), 6.68-6.72 (m, 3H), 7.34 (d, *J* = 8 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.62-7.66 (m, 2H), 7.98 (s, 1H), 8.08 (s, 2H); ESI/MS m/z 478 (M-H).

5.1.5. General procedure for the synthesis of compounds of general formula (7a-7d)

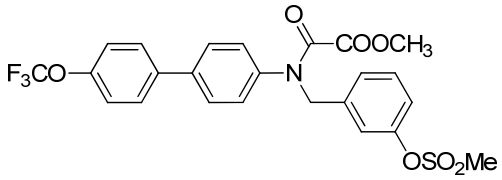


6a-6d **7a-7d**

To a solution of **6a**-**6d** (1 mole eq.) in CH_2Cl_2 (10 v/w) was added Pyridine (1.5 mole eq.) at 25 ^{0}C. Reaction mixture was cooled to 5-10 ^{o}C under N_2 atmosphere. A solution of methyl chloro oxoacetate (ClCOCOOMe, 1.1 mol eq.) in CH_2Cl_2 (2 v/w) was added to it in 5 min. The reaction mixture was stirred at same temperature for 3-5 hr. Reaction mixture was then diluted with water (10 v/w) and CH_2Cl_2 (10 v/w). The organic layer was washed with water, dried over

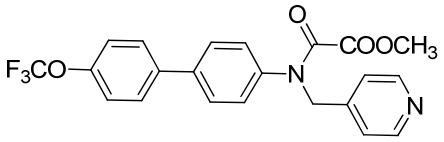
Na_2SO_4 and evaporated under reduced pressure to get desired product in fairly good purity.

5.1.5.1. Methyl2-((3-((methylsulfonyl)oxy)benzyl)(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)amino)-2-oxoacetate (7a)



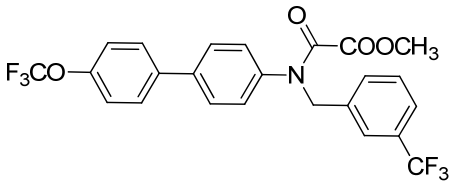
Prepared using general procedure as described above with **6a** as a starting material. Crude material was directly used for next step.

5.1.5.2. Methyl 2-oxo-2-((pyridin-4-ylmethyl)(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)amino)acetate (7b)



Prepared using general procedure as described above with **6b** as a starting material. Crude material was directly used for next step.

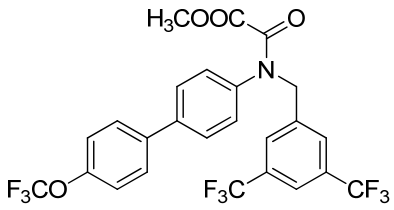
5.1.5.3. N-(4'-Trifluoromethoxy-biphenyl-4-yl)-N-(3-trifluoromethyl-benzyl)-oxalamic acid methyl ester (7c)



Compound **7c** was prepared from **6c** using general procedure described above as oily material; Yield: 76%; Purity by HPLC: 97.6%; IR (CCl_4) 1751, 1678, 1496, 1261, 1170, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.60 (s, 3H), 5.03 (s, 2H),

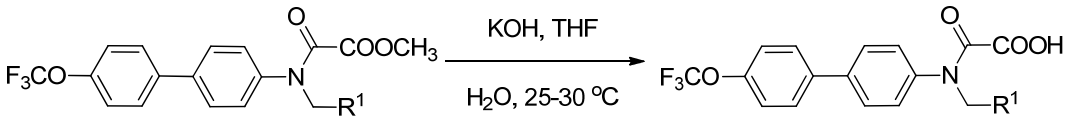
7.15 (d, *J* = 8.49 Hz, 2H), 7.27-7.30 (m, 2H), 7.53-7.57 (m, 4H), 7.45-7.51 (m, 4H); ESI/MS m/z 498.3 $(M+H)^+$.

5.1.5.4. N-(3,5-Bis-trifluoromethyl-benzyl)-N-(4'-trifluoromethoxy-biphenyl-4-yl)- oxalamic acid methyl ester (7d)



Compound **7d** was prepared from **6d** using general procedure described above as oily material; Yield: 87%; Purity by HPLC: 95.50%; IR (CCl_4) 1753, 1679, 1498, 1278, 1261, 1178, 761 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.62 (s, 3H), 5.08 (s, 2H), 7.16 (d, *J* = 8.49 Hz, 2H), 7.31 (d, *J* = 8.07 Hz, 2H), 7.53-7.58 (m, 4H), 7.70 (s, 2H), 7.82 (s, 1H); ESI/MS m/z 588.0 $(M+Na)^+$.

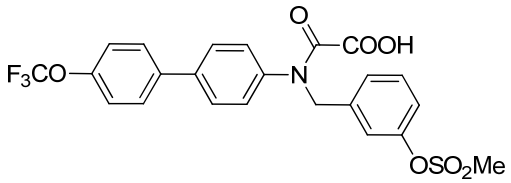
5.1.6. General procedure for the synthesis of compounds of general formula (8a-8d)



7a-7d **8a-8d**

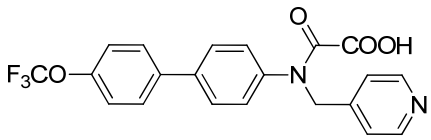
To a solution of **7a**-**7d** (1 mole eq.) in THF (5 v/w) was added solution of KOH (1.5 mole eq.) in water (5 v/w) at 25 ^0C and stirred for 1-2 h. Reaction mixture was then diluted with water (5 v/w) and washed with diisopropyl ether (DIPE) (20 v/w). Aqueous layer was acidified using diluted HCl and extracted with ethyl acetate (25 v/w).The organic layer was washed with water, dried over Na_2SO_4 and evaporated to get desired product in fairly good purity.

5.1.6.1. 2-((3-((Methylsulfonyl)oxy)benzyl)(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)amino)-2-oxoacetic acid (8a)



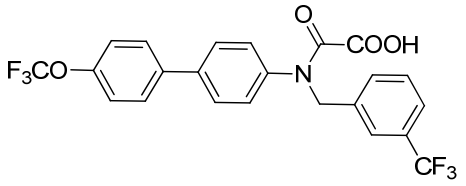
Compound **8a** was prepared from **7a** using general procedure described above as off-white solid; Yield: 47%; mp: 70-72 ^{0}C; Purity by UPLC: 94.79%; IR (KBr) 3433, 3022, 1654, 1219, 773 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.33 (s, 3H), 5.03 (s, 2H), 7.26-7.56 (bt, 8H), 7.68-8.0 (bd, 4H); ESI/MS m/z 508 (M-H).

5.1.6.2. 2-Oxo-2-((pyridin-4-ylmethyl)(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)amino) acetic acid (8b)



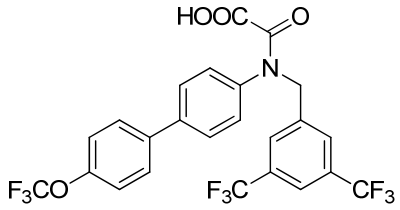
Compound **8b** was prepared from **7b** using general procedure described above as light yellow solid; Yield: 40%; mp: 151-154 ^{0}C; Purity by HPLC: 96.8%; IR (KBr) 3435, 3074, 1643, 1498, 1267, 831 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.00 (s, 2H), 7.27 (d, J = 8 Hz, 2H), 7.27-7.43 (m, 4H), 7.68 (d, J = 8 Hz, 2H), 7.77 (d, J = 8.5 Hz, 2H), 8.51 (d, J = 4.1 Hz, 2H); ESI/MS m/z 417.1 $(M+H)^+$.

5.1.6.3. 2-Oxo-2-((4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)(3-(trifluoromethyl) benzyl) amino)acetic acid (8c)



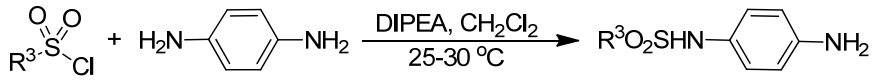
Compound **8c** was prepared from **7c** using general procedure described above as semi solid; Yield: 41%; Purity by HPLC: 96.4%; IR (CCl_4) 1745, 1664, 1498, 1261, 786 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 5.10 (s, 2H), 7.30 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.56-7.66 (m, 4H), 7.71-7.79 (m, 4H); ESI/MS m/z 482 (M-H).

5.1.6.4. 2-((3,5-Bis(trifluoromethyl)benzyl)(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)amino)-2-oxoacetic acid (8d)



Compound **8d** was prepared from **7d** using general procedure described above as oily material; Yield: 70%; Purity by HPLC: 96.5%; IR (CCl_4) 3446, 3020, 1670, 1498, 1280, 1215, 759 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 5.19 (s, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.71-7.78 (m, 4H), 7.90 (s, 2H), 8.04 (s, 1H); ESI/MS m/z 550.2 (M-H).

5.1.7. General procedure for the synthesis of compounds of general formula (11a-11i)

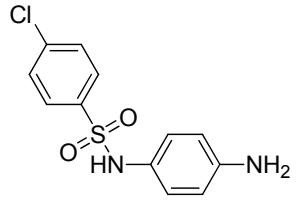


9 **10** **11a-11i**

To a solution of **10** (1 mole eq.) in CH_2Cl_2 (10 v/w) was added diisopropyl ethyl amine (DIPEA) (2 mole eq.) at 25-30 ^0C. Substituted aryl sulfonyl chlorides with general formula **9** (1 mole eq.) dissolved in CH_2Cl_2 (4-6 v/w) was added to it

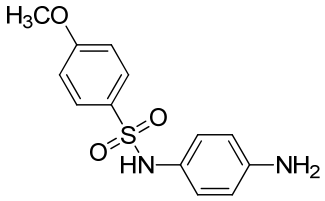
slowly at 25-30 ^{0}C. Stirring continued for another 30 min. CH_2Cl_2 from reaction mixture was distilled out. Reaction mixture was then diluted with water (20 v/w). Aqueous layer was acidified using diluted HCl and extracted with DIPE (2 x 10 v/w). Aqueous layer was then basified using aq. Na_2CO_3 solution till pH 8-9. Aqueous layer was extracted with ethyl acetate (50 v/w). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to get desired product in fairly good purity.

5.1.7.1. N-(4-Aminophenyl)-4-chlorobenzenesulfonamide (11a)



Compound **11a** was prepared by reacting 4-chlorobenzene sulfonyl chloride with **10** using general procedure described above as brown solid; Yield: 25%; mp: 150 ^{0}C (decomp.); Purity by HPLC: 98.9%; IR (KBr) 3392, 3062, 1514, 1323, 1153, 821, 646 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.68 (broad peak, 2H), 6.1 (s, 1H), 6.53-6.56 (dd, *J* = 2.0 and 6.6 Hz, 2H), 6.79-6.82 (dd, *J* = 2.0 and 6.6 Hz, 2H), 7.37-7.41 (m, 2H), 7.57-7.61 (m, 2H); ESI/MS m/z 283 $(M+H)^+$.

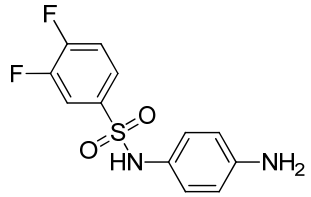
5.1.7.2. N-(4-Aminophenyl)-4-methoxybenzenesulfonamide (11b)



Compound **11b** was prepared by reacting 4-methoxybenzene sulfonyl chloride with **10** using general procedure described above as brown solid; Yield: 80%;

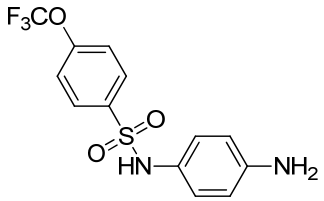
mp: 150 ^{0}C (decomp.); Purity by HPLC: 98.4%; IR (KBr) 3417, 1647, 1512, 1328, 1163, 833 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.62 (broad peak, 2H), 3.83 (s, 3H), 6.0 (s, 1H), 6.55 (d, J = 8.6 Hz, 2H), 6.79-6.9 (bd, 4H), 7.61 (d, J = 8.8 Hz, 2H); ESI/MS m/z 279 $(M+H)^+$.

5.1.7.3. N-(4-Aminophenyl)-3,4-difluorobenzenesulfonamide (11c)



Compound **11c** was prepared by reacting 3,4-difluorobenzene sulfonyl chloride with **10** using general procedure described above as light brown solid; Yield: 73%; mp: 183-185 ^{0}C; Purity by UPLC: 99.10%; IR (KBr) 3423, 3344, 1610, 1514, 1348, 1116, 846 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.69 (s, 2H), 6.0 (s, 1H), 6.54-6.57 (m, 2H), 6.79-6.83 (m, 2H), 7.18-7.24 (m, 1H), 7.41-7.49 (m, 1H), 7.50-7.54 (m, 1H); ESI/MS m/z 282.8 (M-H).

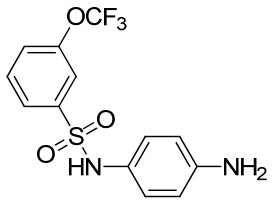
5.1.7.4. N-(4-Aminophenyl)-4-(trifluoromethoxy) benzenesulfonamide (11d)



Compound **11d** was prepared by reacting 4-trifluoromethoxybenzene sulfonyl chloride with **10** using general procedure described above as light brown solid; Yield: 63%; mp: 158^{0}C (decomp.); Purity by HPLC: 99.19%; IR (KBr) 3411, 1596, 1336, 1271, 1168, 740 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.99 (s, 2H), 6.38

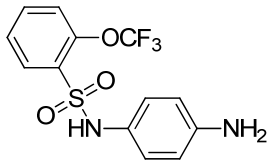
(d, *J* = 8.6 Hz, 2H), 6.65 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.7 Hz, 2H), 9.57 (s, 1H); ESI/MS m/z 354.8 (M+Na).

5.1.7.5. N-(4-Aminophenyl)-3-(trifluoromethoxy) benzenesulfonamide (11e)



Compound **11e** was prepared by reacting 3-trifluoromethoxybenzene sulfonyl chloride with **10** using general procedure described above as light brown solid; Yield: 74%; mp: 75-80 ^{0}C; Purity by HPLC: 95.92%; IR (KBr) 3435, 3377, 3074, 1627, 1510, 1338, 1261, 839, 740 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.0 (s, 2H), 6.35-6.38 (dd, *J* = 1.9 and 6.7 Hz, 2H), 6.64 (d, *J* = 9 Hz, 2H), 7.51 (s, 1H), 7.60-7.69 (m, 3H), 9.63 (s, 1H); ESI/MS m/z No $(M+H)^+$ observed.

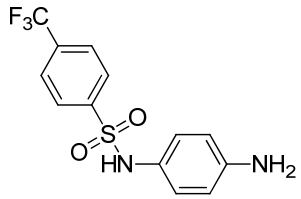
5.1.7.6. N-(4-Aminophenyl)-2-(trifluoromethoxy) benzenesulfonamide (11f)



Compound **11f** was prepared by reacting 2-trifluoromethoxybenzene sulfonyl chloride with **10** using general procedure described above as light brown solid; Yield: 76%; mp: 118-122 ^{0}C; Purity by HPLC: 98.55%; IR (KBr) 3369, 3294, 1616, 1591, 1515, 1332, 1207, 835, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.61 (s, 2H), 6.42 (s, 1H), 6.48-6.51 (dd, *J* = 2.0 and 6.6 Hz, 2H), 6.83-6.86 (dd, *J* = 2.0 and 6.6 Hz, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.55-7.60

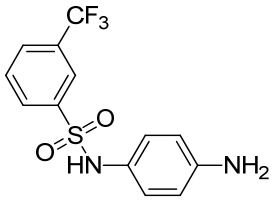
(m, 1H), 7.80-7.84 (dd, *J* = 1.6 and 7.8 Hz, 1H); ESI/MS m/z No $(M+H)^+$ observed.

5.1.7.7. N-(4-Aminophenyl)-4-(trifluoromethyl)benzenesulfonamide (11g)



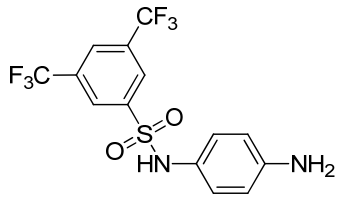
Compound **11g** was prepared by reacting 4-trifluoromethylbenzene sulfonyl chloride with **10** using general procedure described above as off-white solid; Yield: 71%; mp: 165-168 ^{0}C; Purity by UPLC: 99.13%; IR (KBr) 3421, 3257, 1643, 1512, 1325, 1170, 839, 719 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.0 (s, 2H), 6.36-6.39 (m, 2H), 6.63-6.66 (m, 2H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H), 9.69 (s, 1H); ESI/MS m/z 316.8 $(M+H)^+$.

5.1.7.8. N-(4-Aminophenyl)-3-(trifluoromethyl)benzenesulfonamide (11h)



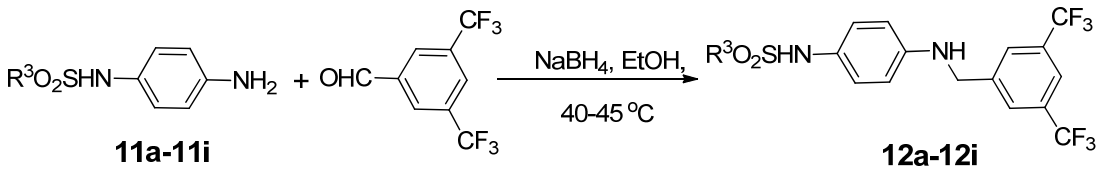
Compound **11h** was prepared by reacting 3-trifluoromethylbenzene sulfonyl chloride with **10** using general procedure described above as off-white solid; Yield: 70%; mp: 85-88 ^{0}C; Purity by HPLC: 94.34%; IR (KBr) 3438, 3276, 1625, 1510, 1332, 1161, 840 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.69 (s, 2H), 6.17 (s, 1H), 6.53-6.57 (m, 2H), 6.78-6.81 (dd, *J* = 2.0 and 6.7 Hz, 2H), 7.54-7.60 (bt, 1H), 7.83 (t, *J* = 8 Hz, 2H), 7.95 (s, 1H); ESI/MS m/z No $(M+H)^+$ observed.

5.1.7.9. N-(4-Aminophenyl)-3,5-bis(trifluoromethyl) benzene sulfonamide (11i)



Compound **11i** was prepared by reacting 3,5-bistrifluoromethylbenzene sulfonyl chloride with **10** using general procedure described above as off-white solid; Yield: 73%; mp: 183-185 ^{0}C; Purity by HPLC: 98.84%; IR (KBr) 3436, 3357, 3247, 1625, 1512, 1317, 1109, 842, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.04 (s, 2H), 6.33-6.36 (dd, J = 3 and 4.9 Hz, 2H), 6.57 (d, J = 8.6 Hz, 2H), 8.04 (s, 2H), 8.42 (s, 1H), 9.73 (s, 1H); ESI/MS m/z 383 (M-H).

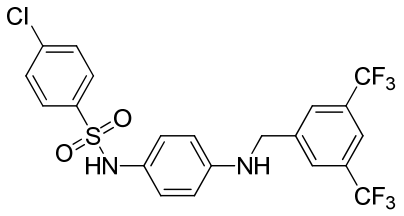
5.1.8. General procedure for the synthesis of compounds of general formula (12a-12i)



11a-11i **12a-12i**

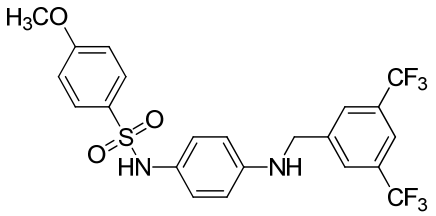
To a solution of **11a**-**11i** (1 mole eq.) in EtOH (10 v/w) was added 3,5-bistrifluoromethyl benzaldehyde (1.05 mol eq.) at 25 ^{0}C and refluxed for 4-5 h. The reaction mixture was cooled up to 40^{o}C. To this was added $NaBH_4$ (1 mole eq.) lot wise and stirred for 2 h. Reaction mixture was then diluted with water (30 v/w) and extracted with ethyl acetate (50 v/w). The organic layer was washed with water & brine solution, dried over Na_2SO_4 and evaporated under reduced pressure to get desired product in fairly good purity.

5.1.8.1. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-4-Chloro-benzene sulfonamide (12a)



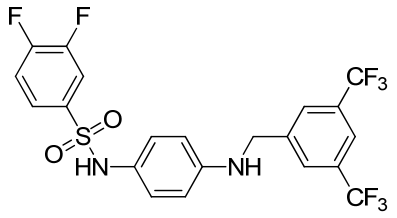
Compound **12a** was prepared by reacting **11a** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as light brown solid; Yield: 33%; mp: 185-190 ^{0}C; Purity by HPLC: 96.34%; IR (KBr) 3388, 3261, 1616, 1512, 1326, 1124, 831, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.39 (d, J = 6 Hz, 2H), 6.40-6.45 (bt, 3H), 6.71 (d, J = 8.6 Hz, 2H), 7.53-7.59 (bt, 4H), 7.97 (bd, 3H), 9.6 (s, 1H); ESI/MS m/z 509 $(M+H)^+$.

5.1.8.2. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-4-Methoxy benzene sulfonamide (12b)



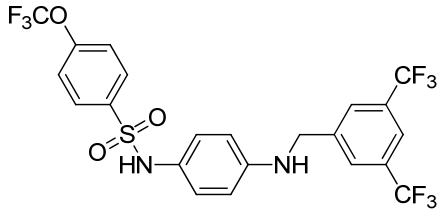
Compound **12b** was prepared by reacting **11b** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as light brown solid; Yield: 81%; mp: 221-225 ^{0}C; Purity by HPLC: 93.11%; IR (KBr) 3386, 3261, 1612, 1517, 1323, 1280, 1126, 829 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.83 (s, 3H), 4.22 (bs, 1H), 4.45 (d, J = 5.5 Hz, 2H), 6.0 (s, 1H), 6.46 (d, J = 8.7 Hz, 2H), 6.82-6.91 (m, 4H), 7.59 (d, J = 8.9 Hz, 2H), 7.78 (s, 3H); ESI/MS m/z 527 $(M+Na)^+$.

5.1.8.3. N-(4-((3,5-Bis(trifluoromethyl)benzyl)amino)phenyl)-3,4-difluoro benzene sulfonamide (12c)



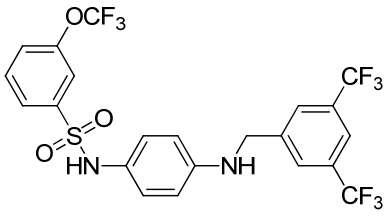
Compound **12c** was prepared by reacting **11c** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as greenish solid; Yield: 59%; Purity by UPLC: 97.92%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.40 (d, J = 6.4 Hz, 2H), 6.40-6.43 (m, 3H), 6.73 (d, J = 8.8 Hz, 2H), 7.44-7.47 (m, 1H), 7.53-7.60 (m, 2H), 7.93 (s, 1H), 7.96 (s, 2H), 9.63 (s, 1H); ESI/MS m/z 509 (M-H).

5.1.8.4. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-4-trifluoromethoxy-benzenesulfonamide (12d)



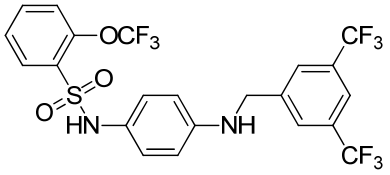
Compound **12d** was prepared by reacting **11d** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as off-white solid; Yield: 90%; mp: 150-153 ^{0}C; Purity by HPLC: 98.97%; IR (KBr) 3387, 3282, 1612, 1519, 1386, 1284, 1126, 842, 705 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.40 (d, J = 6.2 Hz, 2H), 6.37-6.45 (m, 3H), 6.72 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 8.1 Hz, 2H), 7.68-7.72 (bd, 2H), 7.96 (bd, 3H), 9.64 (s, 1H); ESI/MS m/z 557.1 (M-H).

5.1.8.5. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-3-trifluoromethoxy-benzenesulfonamide (12e)



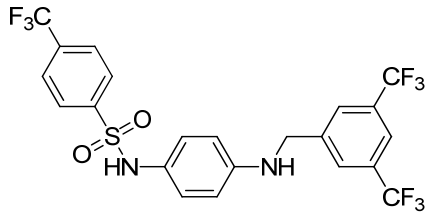
Compound **12e** was prepared by reacting **11e** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as off-white solid; Yield: 75%; mp: 140-143 ^{0}C; Purity by HPLC: 95.38%; IR (KBr) 3388, 3269, 1614, 1519, 1330, 1168, 802 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.39 (d, J = 6.1 Hz, 2H), 6.41-6.47 (bt, 3H), 6.70 (d, J = 8.7 Hz, 2H), 7.4 (s, 1H), 7.58-7.65 (m, 3H), 7.97-7.99 (bd, 3H), 9.69 (s, 1H); ESI/MS m/z No $(M+H)^+$ was observed.

5.1.8.6. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-2-trifluoromethoxy-benzenesulfonamide (12f)



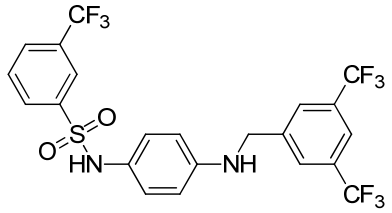
Compound **12f** was prepared by reacting **11f** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as off-white solid; Yield: 73%; mp: 115-118 ^{0}C; Purity by UPLC: 97.97%; IR (KBr) 3444, 3292, 1614, 1525, 1326, 1120, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.2 (bs, 1H), 4.41 (d, J = 5.1 Hz, 2H), 6.42 (bd, 3H), 6.89 (d, J = 8.6 Hz, 2H), 7.30 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 8.3 Hz, 1H), 7.54-7.60 (m, 1H), 7.77 (d, J = 9.7 Hz, 2H), 7.79-7.84 (m, 2H); ESI/MS m/z No $(M+H)^+$ was observed.

5.1.8.7. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-4-trifluoromethyl -benzenesulfonamide (12g)



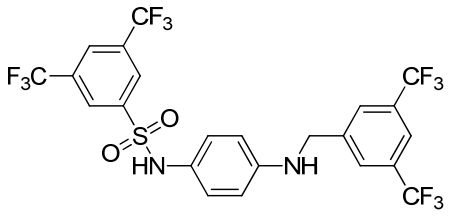
Compound **12g** was prepared by reacting **11g** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as off-white solid; Yield: 62%; mp: 160-163 ^{0}C; Purity by HPLC: 97.61%; IR (KBr) 3390, 3269, 1614, 1521, 1328, 1328, 1134, 719 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.39 (d, J = 6.1 Hz, 2H), 6.45 (bd, 3H), 6.72 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 8.3 Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H), 7.95 (bd, 3H), 9.78 (s, 1H); ESI/MS m/z 565.2 $(M+Na)^+$.

5.1.8.8. N-[4-(3,5-Bis-trifluoromethyl-benzylamino)-phenyl]-3- trifluoromethyl-benzenesulfonamide (12h)



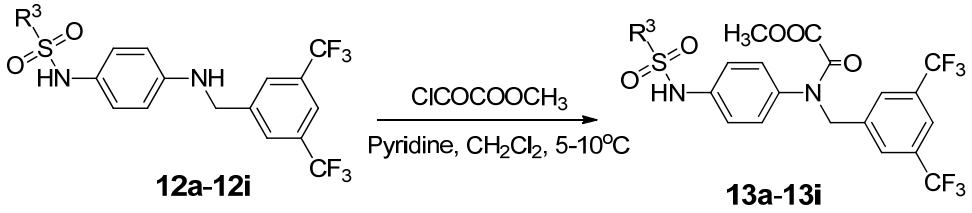
Compound **12h** was prepared by reacting **11h** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as off-white solid; Yield: 80%; mp: 165-168 ^{0}C; Purity by HPLC: 97.50%; IR (KBr) 3373, 2877, 1633, 1569, 1488, 1315, 1103, 850 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.39 (d, J = 6.1 Hz, 2H), 6.45 (bt, 3H), 6.69 (d, J = 8.7 Hz, 2H), 7.73 (bt, 2H), 7.88 (d, J = 8.4 Hz, 1H), 7.96 (bd, 4H), 9.70 (s, 1H); ESI/MS m/z 543.4 $(M+H)^+$.

5.1.8.9. N-(4-((3,5-Bis(trifluoromethyl)benzyl)amino)phenyl)-3,5-bis (trifluoromethyl) benzenesulfonamide (12i)



Compound **12i** was prepared by reacting **11i** with 3,5-bistrifluoromethyl benzaldehyde using general procedure described above as off-white solid; Yield: 70%; mp: 125-128 ^{0}C; Purity by HPLC: 99.81%; IR (KBr) 3417, 3396, 1614, 1527, 1334, 1274, 844, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.41 (d, J = 6.2 Hz, 2H), 6.47 (d, J = 8.8 Hz, 2H), 6.53 (t, 1H), 6.68 (d, J = 8.7 Hz, 2H), 7.91-8.01 (bt, 5H), 8.40 (s, 1H), 9.80 (s, 1H); ESI/MS m/z 610.8 $(M+H)^+$.

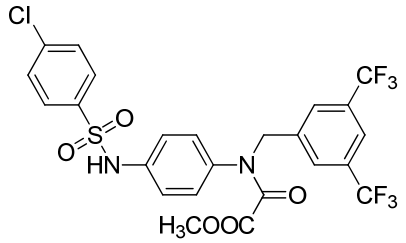
5.1.9. General procedure for the synthesis of compounds of general formula (13a-13i)



To a solution of **12a**-**12i** (1 mole eq.) in CH_2Cl_2 (10 v/w) was added pyridine (1.5 mole eq.) at 25 ^{0}C. Reaction mixture was cooled to 5-10 ^{o}C under N_2 atmosphere. A solution of methyl chloro oxoacetate (1.2 mol eq.) in CH_2Cl_2 (2 v/w) was added to it in 5 min. The reaction mixture was stirred at same temperature for 3-5 h. Reaction mixture was then diluted with water (10 v/w) and CH_2Cl_2 (10 v/w). The organic layer was washed with water, dried over Na_2SO_4

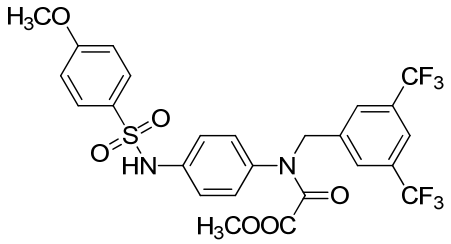
and evaporated under reduced pressure to get desired product in fairly good purity.

5.1.9.1. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(4-chlorophenyl sulfonamido) phenyl)amino)-2-oxoacetate (13a)



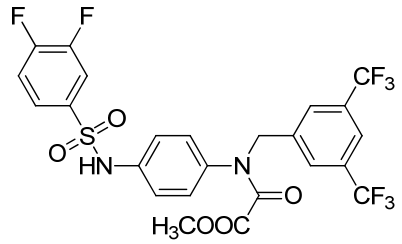
Compound **13a** was prepared from **12a** using general procedure described above as oily material; Yield: 55%; Purity by HPLC: 93.37%; IR (KBr) 3203, 1747, 1652, 1512, 1350, 1280, 1164, 754, 682 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.38 (s, 3H), 5.07 (s, 2H), 7.0 (s, 4H), 7.59 (d, J = 8.5 Hz, 2H), 7.7 (d, J = 8.6 Hz, 2H), 7.78 (s, 2H), 8.0 (s, 1H), 10.56 (s, 1H); ESI/MS m/z 595.1 $(M+H)^+$.

5.1.9.2. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(4-methoxyphenyl sulfonamido) phenyl)amino)-2-oxoacetate (13b)



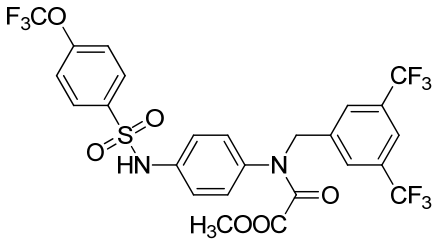
Compound **13b** was prepared from **12b** using general procedure described above as oily material and directly used for next step.

5.1.9.3. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(3,4-difluorophenyl sulfonamido) phenyl)amino)-2-oxoacetate (13c)



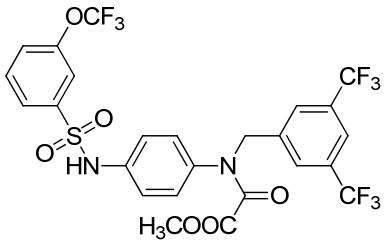
Compound **13c** was prepared from **12c** using general procedure described above as oily material and directly used for next step.

5.1.9.4. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(4-(trifluoromethoxy) phenyl sulfonamido)phenyl)amino)-2-oxoacetate (13d)



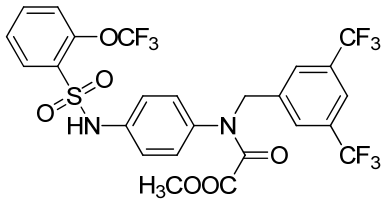
Compound **13d** was prepared from **12d** using general procedure described above as off-white solid; Yield: 85%; mp: 127-130 ^{0}C; Purity by HPLC: 97.77%; IR (KBr) 3201, 1760, 1643, 1512, 1346, 1205, 1137, 842 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.36 (s, 3H), 5.07 (s, 2H), 7.06 (s, 4H), 7.51 (d, J = 8.2 Hz, 2H), 7.77-7.85 (bt, 4H), 7.99 (s, 1H), 10.63 (s, 1H); ESI/MS m/z 643.1 (M-H).

5.1.9.5. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(3-(trifluoromethoxy) phenyl sulfonamido)phenyl)amino)-2-oxoacetate (13e)



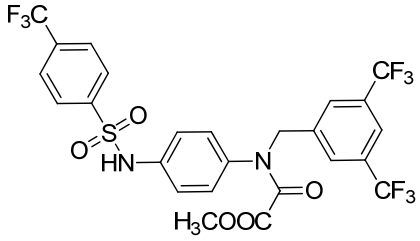
Compound **13e** was prepared from **12e** using general procedure described above as oily material and directly used for next step.

5.1.9.6. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(2-(trifluoromethoxy) phenyl sulfonamido)phenyl)amino)-2-oxoacetate (13f)



Compound **13f** was prepared from **12f** using general procedure described above as oily material and directly used for next step.

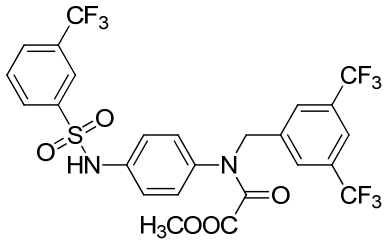
5.1.9.7. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(4-(trifluoromethyl) phenyl sulfonamido)phenyl)amino)-2-oxoacetate (13g)



Compound **13g** was prepared from **12g** using general procedure described above as semi solid; Yield: 67%; Purity by HPLC: 98.35%; IR (KBr) 1705, 1598, 1500, 1367, 1149, 794, 696 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.35 (s, 3H),

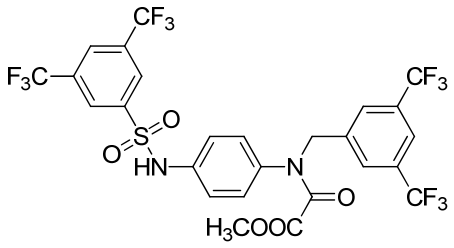
5.06 (s, 2H), 7.06 (s, 4H), 7.76 (s, 2H), 7.90 (s, 5H), 10.74 (s, 1H); ESI/MS m/z 651.2 $(M+Na)^+$.

5.1.9.8. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(3-(trifluoromethyl) phenyl sulfonamido)phenyl)amino)-2-oxoacetate (13h)



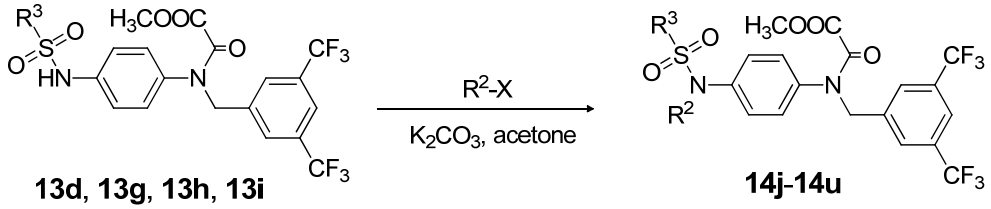
Compound **13h** was prepared from **12h** using general procedure described above as off-white solid; Yield: 85%; mp: 143-146 ^{0}C; Purity by UPLC: 99.28%; IR (KBr) 3151, 1743, 1653, 1506, 1350, 1278, 1166, 798, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.38 (s, 3H), 5.08 (s, 2H), 7.08 (s, 4H), 7.78 (bt, 3H), 7.96-8.0 (m, 4H), 10.66 (s, 1H); ESI/MS m/z 627 (M-H).

5.1.9.9. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(3,5-bis (trifluoromethyl) phenyl sulfonamido)phenyl)amino)-2-oxoacetate (13i)



Compound **13i** was prepared from **12i** using general procedure described above as off-white solid; Yield: 80%; mp: 136-139 ^{0}C; Purity by HPLC: 98.04%; IR (KBr) 3166, 1759, 1651, 1512, 1382, 1278, 1132, 821, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.40 (s, 3H), 5.09 (s, 2H), 7.11 (s, 4H), 7.79 (s, 2H), 7.97 (s, 1H), 8.24 (s, 2H), 8.48 (s, 1H), 10.79 (s, 1H); ESI/MS m/z 694.9 (M-H).

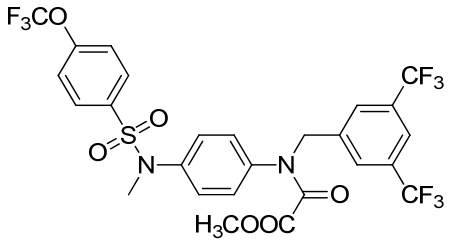
5.1.10. General procedure for the synthesis of compounds of general formula (14j-14u)



13d, **13g**, **13h**, **13i** **14j-14u**

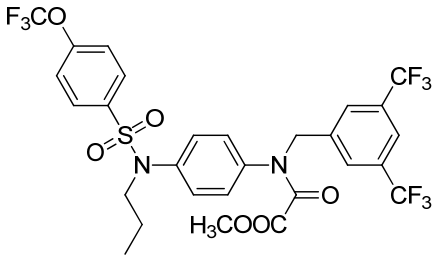
To a solution of **13** (**13d**, **13g**, **13h**, **13i**, 1 mole eq.) in acetone (15 v/w) was added K_2CO_3 (2 mole eq.) at 25-30 ^{0}C. R^2-X (1-2 mole eq) was added to it slowly at 25-30 ^{0}C, (X = halide or mesyl group). Stirring continued for 2-18 h till TLC showed completion of reaction. Reaction mixture was then diluted with water (20 v/w). Aqueous layer was extracted with ethyl acetate (2 x 25 v/w). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to get desired product in fairly good purity.

5.1.10.1. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(N-methyl-4-(trifluoromethoxy) phenylsulfonamido)phenyl)amino)-2-oxoacetate (14j)



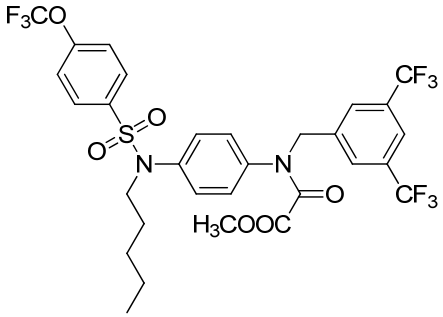
Compound **14j** was obtained by reacting **13d** with 1.5 eq. methyl iodide using general procedure described above and directly used for next step.

5.1.10.2. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(N-propyl-4-(trifluoromethoxy) phenylsulfonamido)phenyl)amino)-2-oxoacetate (14k)



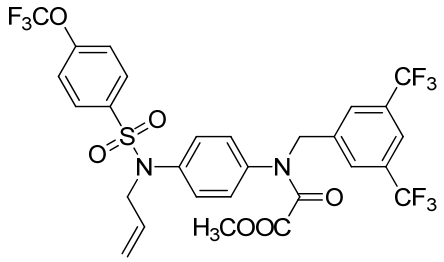
Compound **14k** was obtained by reacting **13d** with 1.5 eq. 1-iodopropane using general procedure described above and directly used for next step.

5.1.10.3. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(N-pentyl-4-(trifluoromethoxy) phenylsulfonamido)phenyl)amino)-2-oxoacetate (14l)



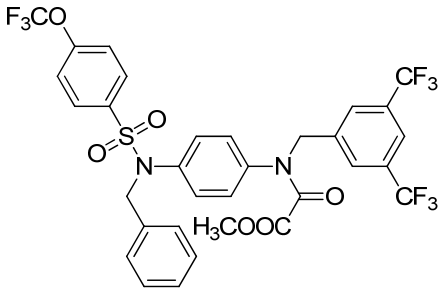
Compound **14l** was obtained by reacting **13d** with 2 eq. pentyl methane sulfonate using general procedure described above as oily material; Yield: 75%; Purity by UPLC: 99.68%; IR (KBr) 1753, 1674, 1506, 1352, 1284, 1170, 904, 682 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.73 (t, 3H), 1.19 (bt, 6H), 3.47 (s, 3H), 3.53 (bd, 2H), 5.18 (s, 2H), 7.11 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.84 (s, 2H), 8.03 (s, 1H); ESI/MS m/z 715.1 $(M+H)^+$.

5.1.10.4. Methyl 2-((4-(N-allyl-4-(trifluoromethoxy)phenylsulfonamido) phenyl) (3,5-bis(trifluoromethyl)benzyl)amino)-2-oxoacetate (14m)



Compound **14m** was obtained by reacting **13d** with 1.2 eq. allyl iodide using general procedure described above as off-white solid; Yield: 84%; mp: 92-96 ^{0}C; Purity by HPLC: 99.17%; IR (KBr) 1757, 1668, 1512, 1386, 1134, 844, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.44 (s, 3H), 4.22 (d, J = 6.0 Hz, 2H), 4.97-5.08 (m, 2H), 5.16 (s, 2H), 5.54-5.65 (s, 1H), 7.09-7.16 (m, 4H), 7.50 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.8 Hz, 2H), 7.84 (s, 2H), 8.05 (s, 1H); ESI/MS m/z 707 $(M+Na)^+$.

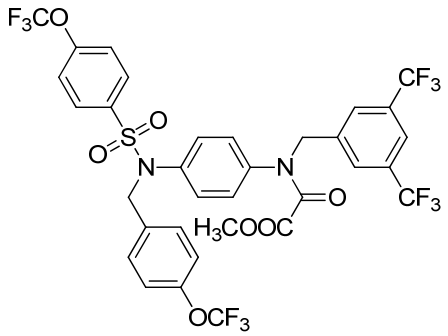
5.1.10.5. Methyl 2-((4-(N-benzyl-4-(trifluoromethoxy)phenylsulfonamido) phenyl)(3,5-bis(trifluoromethyl)benzyl)amino)-2-oxoacetate (14n)



Compound **14n** was obtained by reacting **13d** with 1.5 eq. benzylbromide using general procedure described above as oily material; Yield: 72%; Purity by UPLC: 98.24%; IR (KBr) 1743, 1681, 1508, 1359, 1280, 1166, 731, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.23 (s, 3H), 4.78 (s, 2H), 5.11 (s, 2H), 7.05-7.21 (m,

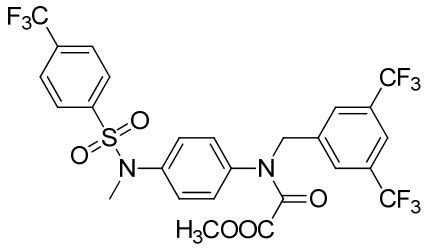
9H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.81 (s, 2H), 8.03 (s, 1H); ESI/MS m/z 735.1 $(M+H)^+$.

5.1.10.6. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(4-(trifluoromethoxy)-N-(4-(trifluoromethoxy)benzyl)phenylsulfonamido)phenyl)amino)-2-oxoacetate (14o)



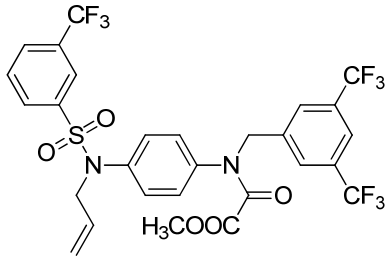
Compound **14o** was obtained by reacting **13d** with 1.3 eq. 4-trifluoromethoxy benzylbromide using general procedure described above as white solid; Yield: 70%; mp: 90-94 ^{0}C; Purity by UPLC: 98.97%; IR (KBr) 1755, 1676, 1597, 1508, 1383, 1356, 1276, 1166, 895, 684 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.26 (s, 3H), 4.84 (s, 2H), 5.13 (s, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.16 (d, *J* = 8.8 Hz, 2H), 7.21 (d, *J* = 8 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.81 (s, 2H), 8.02 (s, 1H); ESI/MS m/z 835.9 $(M+NH_4)^+$.

5.1.10.7. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(N-methyl-4-(trifluoromethyl) phenylsulfonamido)phenyl)amino)-2-oxoacetate (14p)



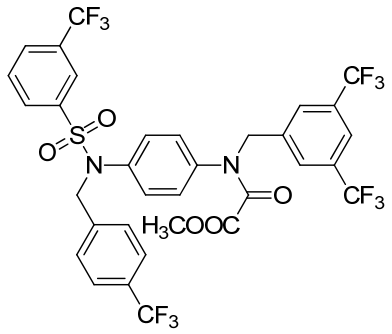
Compound **14p** was obtained by reacting **13g** with 1.5 eq. methyl iodide using general procedure described above as white solid; Yield: 86%; mp: 101-105 ^{0}C; Purity by HPLC: 97.02%; IR (KBr) 3431, 1749, 1678, 1510, 1348, 1280, 1130, 842, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.13 (s, 3H), 3.78 (s, 3H), 5.17 (s, 2H), 7.18 (s, 4H), 7.59 (d, J = 8.1 Hz, 2H), 7.88 (bd, 4H), 8.07 (s, 1H); ESI/MS m/z 665.2 $(M+Na)^+$.

5.1.10.8. Methyl 2-((4-(N-allyl-3-(trifluoromethyl)phenylsulfonamido)phenyl) (3,5-bis(trifluoromethyl)benzyl)amino)-2-oxoacetate (14q)



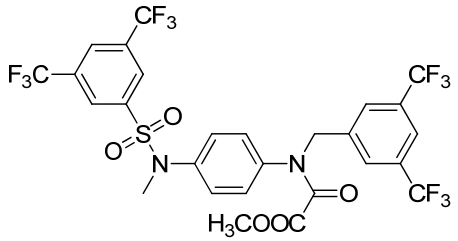
Compound **14q** was obtained by reacting **13h** with 1.5 eq. allyl iodide using general procedure described above and directly used for next step.

5.1.10.9. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(3-(trifluoromethyl)-N-(4-(trifluoromethyl)benzyl)phenylsulfonamido)phenyl)amino)-2-oxoacetate (14r)



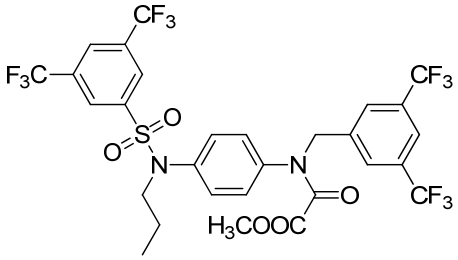
Compound **14r** was obtained by reacting **13h** with 1.2 eq. 4-trifluoromethyl benzyl bromide using general procedure described above and directly used for next step.

5.1.10.10. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(N-methyl-3,5-bis (trifluoromethyl) phenylsulfonamido)phenyl)amino)-2-oxoacetate (14s)



Compound **14s** was obtained by reacting **13i** with 1.5 eq. methyl iodide using general procedure described above as white solid; Yield: 69%; mp: 97-99 ^{0}C; Purity by UPLC: 99.17%; IR (KBr) 1749, 1658, 1508, 1371, 1280, 1128, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.13 (s, 3H), 3.52 (s, 3H), 5.20 (s, 2H), 7.23 (s, 4H), 7.82-7.84 (bd, 4H), 8.03 (s, 1H), 8.54 (s, 1H); ESI/MS m/z 710.9 $(M+H)^+$.

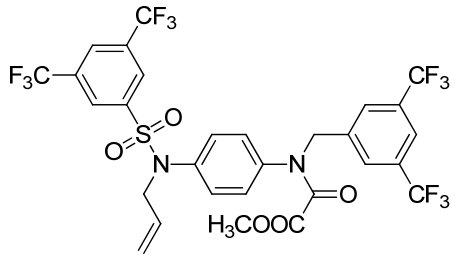
5.1.10.11. Methyl 2-((3,5-bis(trifluoromethyl)benzyl)(4-(N-propyl-3,5-bis (trifluoromethyl) phenylsulfonamido)phenyl)amino)-2-oxoacetate (14t)



Compound **14t** was obtained by reacting **13i** with 1.5 eq. 1-iodo propane using general procedure described above as white solid; Yield: 84%; mp: 134-137 ^{0}C; Purity by UPLC: 99.16%; IR (KBr) 1735, 1693, 1510, 1363, 1070, 808 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.78 (t, 3H), 1.16-1.25 (m, 2H), 3.47 (s, 3H), 3.52

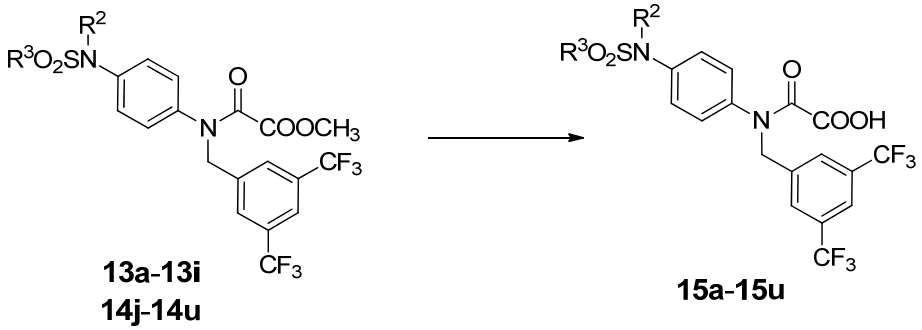
(t, 2H), 5.20 (s, 2H), 7.16-7.22 (broad quartet, 4H), 7.82-7.89 (bd, 4H), 8.01 (s, 1H), 8.52 (s, 1H); ESI/MS m/z No $(M+H)^+$ observed.

5.1.10.12. Methyl 2-((4-(N-allyl-3,5-bis(trifluoromethyl)phenylsulfonamido) phenyl)(3,5-bis(trifluoromethyl)benzyl)amino)-2-oxoacetate (14u)



Compound **14u** was obtained by reacting **13i** with 1.5 eq. allyl iodide using general procedure described above as off-white solid; Yield: 79%; mp: 132-136 ^{0}C; Purity by HPLC: 97.85%; IR (KBr) 1737, 1693, 1508, 1363, 1282, 1109, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.45 (s, 3H), 4.24 (d, J = 6.0 Hz, 2H), 5.0 (t, 2H), 5.21 (s, 2H), 5.55-5.63 (m, 1H), 7.15-7.18 (bd, 4H), 7.84-8.03 (bt, 5H), 8.56 (s, 1H); ESI/MS m/z 759 $(M+Na)^+$.

5.1.11. General procedure for the synthesis of compounds (15a-15u)



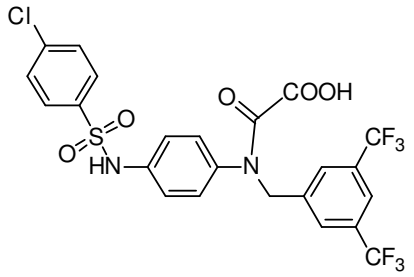
13a-13i
14j-14u

15a-15u

To a solution of ester derivatives **13a**-**13i** and **14j**-**14u** (1 mole eq.) in THF (3 v/w) was added KOH (1.5 mole eq.) dissolved in water (3 v/w) at 25-30 ^{o}C. Reaction mixture was stirred at same temperature for 0.5-2 h and then acidified with dil.

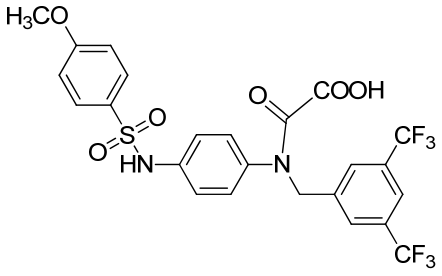
HCl. Product was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to get desired product in fairly good purity.

5.1.11.1. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[4-(4-chloro-benzenesulfonyl amino)-phenyl]-oxalamic acid (15a)



Compound **15a** was obtained from **13a** using general procedure described above as off-white solid; Yield: 78%; mp: 130-135 ^{0}C; Purity by HPLC: 95.78%; IR (KBr) 3234, 1757, 1678, 1512, 1340, 1276, 1134, 835, 704, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.04 (s, 2H), 7.0-7.09 (m, 4H), 7.59 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.6 Hz, 2H), 7.78 (s, 2H), 8.00 (s, 1H), 10.56 (s, 1H); ESI/MS m/z 581.1 $(M+H)^+$.

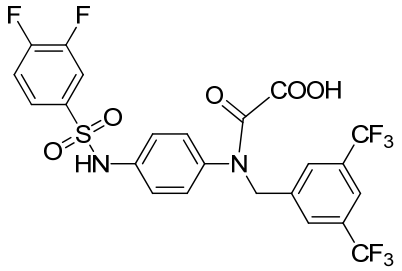
5.1.11.2. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[4-(4-methoxy-benzene sulfonyl amino)-phenyl]-oxalamic acid (15b)



Compound **15b** was obtained from **13b** using general procedure described above as off-white solid; Yield: 65%; mp: 138-142 ^{0}C; Purity by HPLC: 96.94%;

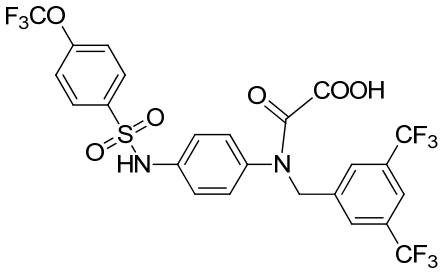
IR (KBr) 3230, 1755, 1678, 1502, 1338, 1278, 1132, 831, 705 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.78 (s, 3H), 5.03 (s, 2H), 7.0-7.07 (m, 6H), 7.66 (d, J = 8.8 Hz, 2H), 7.78 (s, 2H), 8.0 (s, 1H), 10.36 (s, 1H); ESI/MS m/z 575 (M-H).

5.1.11.3. 2-((3,5-Bis(trifluoromethyl)benzyl)(4-(3,4-difluoro phenyl sulfonamido) phenyl) amino)-2-oxoacetic acid (15c)



Compound **15c** was obtained from **13c** using general procedure described above as off-white solid; Yield: 55%; mp: 142-145 ^0C; Purity by UPLC: 99.10%; IR (KBr) 3417, 3225, 1755, 1676, 1512, 1344, 1138, 705 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 5.05 (s, 2H), 7.03-7.09 (m, 4H), 7.57-7.63 (m, 2H), 7.72-7.76 (m, 3H), 7.98 (s, 1H), 10.60 (s, 1H); ESI/MS m/z 580.9 (M-H).

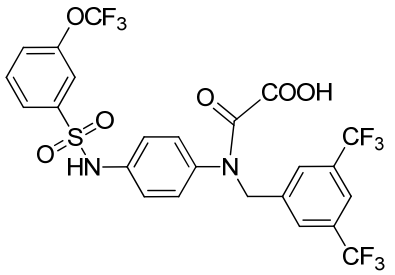
5.1.11.4. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[4-(4-trifluoromethoxy-benzene sulfonyl amino)-phenyl]-oxalamic acid (15d)



Compound **15d** was obtained from **13d** using general procedure described above as off-white solid; Yield: 68%; mp: 120-125 ^0C; Purity by HPLC: 99.13%; IR (KBr) 3411, 3224, 1753, 1674, 1514, 1342, 1280, 1132, 839, 705 cm^{-1}; ^1H

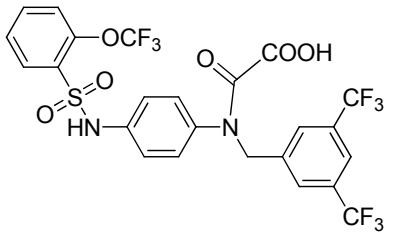
NMR (DMSO-d_6, 400 MHz) δ: 5.06 (s, 2H), 7.04-7.10 (m, 4H), 7.51 (d, J = 8.4 Hz, 2H), 7.78 (s, 2H), 7.82-7.86 (m, 2H), 7.99 (s, 1H), 10.62 (s, 1H); ESI/MS m/z 629.1 (M-H).

5.1.11.5. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[3-(4-trifluoromethoxy-benzene sulfonyl amino)-phenyl]- oxalamic acid (15e)



Compound **15e** was obtained from **13e** using general procedure described above as off-white solid; Yield: 88%; mp: 122-125 ^{0}C; Purity by HPLC: 95.77%; IR (KBr) 3217, 1751, 1672, 1508, 1280, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.0 (s, 2H), 7.0-7.11 (q, 4H), 7.58-7.76 (m, 6H), 7.99 (s, 1H), 10.64 (s, 1H); ESI/MS m/z 628.8 (M-H).

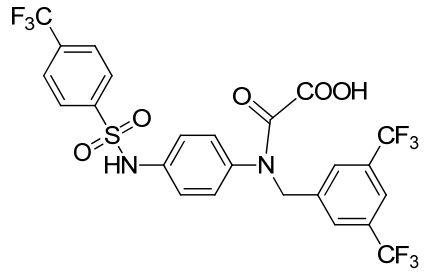
5.1.11.6. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[4-(2-trifluoromethoxy-benzene sulfonyl amino)-phenyl]-oxalamic acid (15f)



Compound **15f** was obtained from **13f** using general procedure described above as off-white solid; Yield: 85%; mp: 135-140 ^{0}C; Purity by HPLC: 98.85%; IR (KBr) 3265, 1772, 1662, 1515, 1340, 1276, 1176, 704, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6,

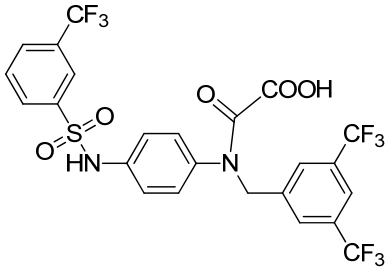
300 MHz) δ: 5.04 (s, 2H), 6.99-7.08 (q, 4H), 7.50 (t, J = 8.8 Hz, 2H), 7.73 (bd, 3H), 7.95-8.00 (bt, 2H), 10.79 (s, 1H); ESI/MS m/z 629 (M-H).

5.1.11.7. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[4-(4-trifluoromethyl-benzene sulfonyl amino)-phenyl]-oxalamic acid (15g)



Compound **15g** was obtained from **13g** using general procedure described above as light pink solid; Yield: 61%; mp: 135-138 ^{0}C; Purity by HPLC: 98.00%; IR (KBr) 3440, 3257, 1755, 1658, 1514, 1325, 1278, 1134, 715, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.04 (s, 2H), 7.06 (m, 4H), 7.75 (s, 2H), 7.84 (bs, 4H), 7.97 (s, 1H), 10.7 (s, 1H); ESI/MS m/z 615 $(M+H)^+$.

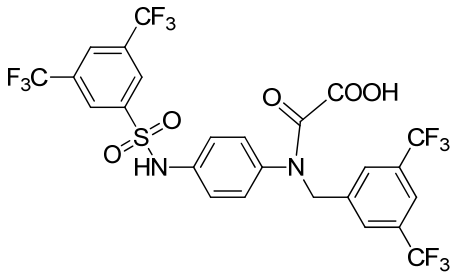
5.1.11.8. N-(3,5-Bis-trifluoromethyl-benzyl)-N-[4-(3-trifluoromethyl-benzene sulfonyl amino)- phenyl]-oxalamic acid (15h)



Compound **15h** was obtained from **13h** using general procedure described above as light pink solid; Yield: 80%; mp: 86-89 ^{0}C; Purity by HPLC: 98.02%; IR (KBr) 2929, 1730, 1651, 1512, 1328, 1280, 1132, 731, 682 cm^{-1}; ^{1}H NMR

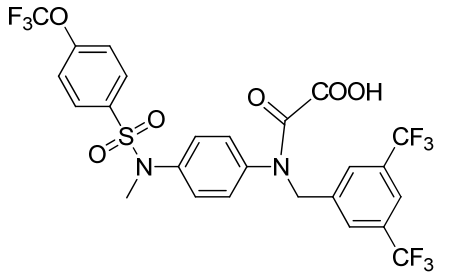
(DMSO-d_6, 300 MHz) δ: 5.01 (s, 2H), 6.98-7.11 (bd, 4H), 7.75 (broad peak, 3H), 7.97 (broad peak, 4H), 10.58 (s, 1H); ESI/MS m/z 613.1 (M-H).

5.1.11.9. 2-((3,5-Bis(trifluoromethyl)benzyl)(4-(3,5-bis(trifluoromethyl) phenyl sulfonamido)phenyl)amino)-2-oxoacetic acid (15i)



Compound **15i** was obtained from **13i** using general procedure described above as off-white solid; Yield: 87%; mp: 126-129 ^{0}C; Purity by UPLC: 97.93%; IR (KBr) 3165, 1741, 1641, 1514, 1359, 1284, 1128, 702, 680 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.07 (s, 2H), 7.10 (d, J = 8.8 Hz, 2H), 7.15 (d, J = 8.8 Hz, 2H), 7.73 (s, 2H), 7.85 (s, 1H), 8.22 (s, 2H), 8.46 (s, 1H), 10.77 (s, 1H), 14.25 (bs, 1H); ESI/MS m/z 681.1 (M-H).

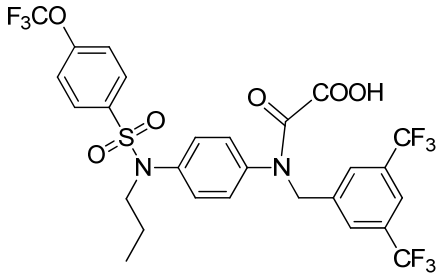
5.1.11.10. N-(3,5-Bis-trifluoromethyl-benzyl)-N-{4-[methyl-(4-trifluoromethoxy-benzene sulfonyl)-amino]- phenyl}-oxalamic acid (15j)



Compound **15j** was obtained from **14j** using general procedure described above as off-white solid; Yield: 42%; mp: 100-102 ^{0}C; Purity by HPLC: 97.32%; IR (KBr) 3047, 2935, 1751, 1670, 1510, 1348, 1280, 1134, 877, 704 cm^{-1}; ^{1}H NMR

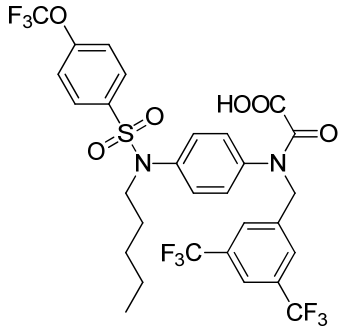
($CDCl_3$, 300 MHz) δ: 3.16 (s, 3H), 5.05 (s, 2H), 7.01 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 7.9 Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H), 7.66 (s, 2H), 7.84 (s, 1H); ESI/MS m/z 643.1 (M-H).

5.1.11.11. N-(3,5-Bis-trifluoromethyl-benzyl)-N-{4-[propyl-(4-trifluoromethoxy-benzene sulfonyl)-amino]-phenyl}-oxalamic acid (15k)



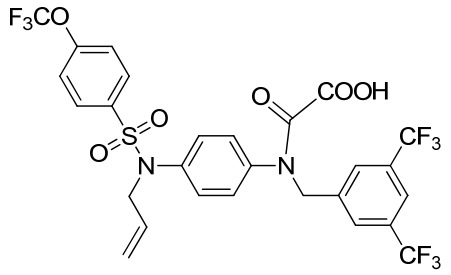
Compound **15k** was obtained from **14k** using general procedure described above as off-white solid; Yield: 51%; mp: 94-98 ^{0}C; Purity by HPLC: 99.06%; IR (KBr) 3423, 2945, 1722, 1664, 1508, 1382, 1265, 1170, 704, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.77 (t, 3H), 1.24 (q, 2H), 3.47 (t, 2H), 5.15 (s, 2H), 7.08-7.19 (bd, 4H), 7.44-7.55 (bd, 4H), 7.84 (s, 2H), 8.03 (s, 1H); ESI/MS m/z 671.2 (M-H).

5.1.11.12. N-{4-[Pentyl-(4-trifluoromethoxy-benzenesulfonyl)-amino]-phenyl}-N-(3,5-bis-trifluoromethyl- benzyl)-oxalamic acid (15l)



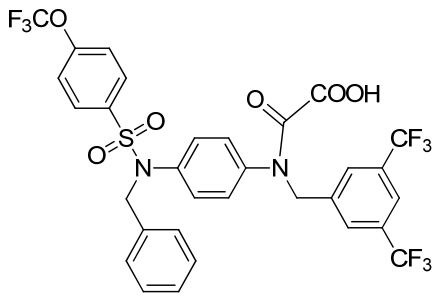
Compound **15l** was obtained from **14l** using general procedure described above as semi solid; Yield: 50%; Purity by HPLC: 95.45%; IR (CCl_4) 3303, 1651, 1508, 1357, 1278, 1178, 761 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.83 (broad peak, 3H), 1.16-1.21 (broad peak, 6H), 2.48 (m, 2H), 5.1 (s, 2H), 7.01 (bd, 2H), 7.19 (bd, 2H), 7.44-7.56 (bd, 4H), 7.83-7.98 (bd, 3H); ESI/MS m/z 698.9 (M-H).

5.1.11.13. N-{4-[Allyl-(4-trifluoromethoxy-benzenesulfonyl)-amino]-phenyl}-N-(3,5-bis-trifluoromethyl- benzyl)-oxalamic acid (15m)



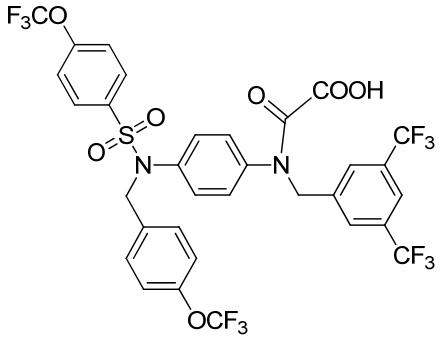
Compound **15m** was obtained from **14m** using general procedure described above as off-white solid; Yield: 55%; mp: 117-120 ^0C; Purity by HPLC: 97.07%; IR (KBr) 3433, 1629, 1508, 1359, 1280, 1136, 707, 682 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 4.17 (d, J = 5.8 Hz, 2H), 4.98-5.12 (broad peak, 4H), 5.57-5.66 (m, 1H), 6.98 (d, J = 8.6 Hz, 2H), 7.14 (bs, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.8 Hz, 2H), 7.96 (broad peak, 3H); ESI/MS m/z 669.1 (M-H).

5.1.11.14. N-{4-[Benzyl-(4-trifluoromethoxy-benzenesulfonyl)-amino]-phenyl}-N-(3,5- benzyl)-oxalamic acid (15n)



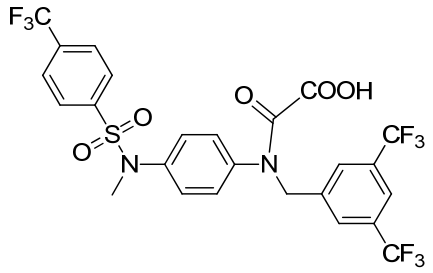
Compound **15n** was obtained from **14n** using general procedure described above as off-white solid; Yield: 30%; mp: 70-72 ^{0}C; Purity by UPLC: 96.69%; IR (KBr) 3433, 1627, 1508, 1355, 1280, 1170, 700 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.76 (s, 2H), 5.03 (s, 2H), 7.10 (s, 4H), 7.15-7.22 (m, 5H), 7.50 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.8 Hz, 2H), 7.79 (s, 2H), 8.01 (s, 1H); ESI/MS m/z 718.8 (M-H).

5.1.11.15. N-{4-[4-Trifloromethoxybenzyl-(4-trifluoromethoxy-benzene sulfonyl)-amino]-phenyl}- N-(3,5-bis-trifluoromethyl-benzyl)-oxalamic acid (15o)



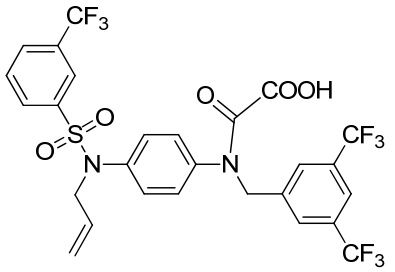
Compound **15o** was obtained from **14o** using general procedure described above as off-white solid; Yield: 50%; mp: 72-75 ^{0}C; Purity by HPLC: 98.09%; IR (KBr) 3433, 1637, 1508, 1359, 1280, 1136, 705, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.79 (s, 2H), 5.02 (s, 2H), 7.00 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 6.8 Hz, 2H), 7.19 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 4.4 Hz, 2H), 7.63-7.65 (dd, J = 2 and 6.8 Hz, 2H), 7.92 (bs, 3H); ESI/MS m/z 803.2 (M-H).

5.1.11.16. N-(3,5-Bis-trifluoromethyl-benzyl)-N-{4-[methyl-(4-trifluoromethyl-benzene sulfonyl)- amino]-phenyl}-oxalamic acid (15p)



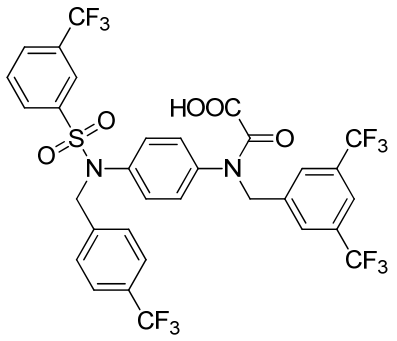
Compound **15p** was obtained from **14p** using general procedure described above as off-white solid; Yield: 72%; mp: 50-53 ^{0}C; Purity by HPLC: 94.33%; IR (KBr) 3435, 1747, 1668, 1508, 1325, 1280, 1176, 705, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.10 (s, 3H), 5.13 (s, 2H), 7.15-7.20 (broad peak, 4H), 7.56 (d, J = 8.1 Hz, 2H), 7.86 (bd, 4H), 8.04 (s, 1H); ESI/MS m/z 627.1 (M-H).

5.1.11.17. N-{4-[Allyl-(3-trifluoromethyl-benzenesulfonyl)-amino]-phenyl}-N-(3,5-bis-trifluoromethyl-benzyl)-oxalamic acid (15q)



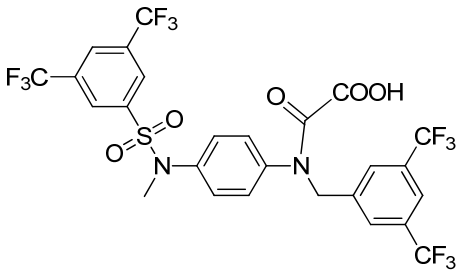
Compound **15q** was obtained from **14q** using general procedure described above as off-white solid; Yield: 44%; mp: 72-75 ^{0}C; Purity by HPLC: 91.80%; IR (KBr) 3433, 1654, 1508, 1326, 1280, 1132, 806, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.18 (bd, 2H), 4.99 (bd, 1H), 5.10 (broad peak, 3H), 5.63 (m, 1H), 7.16 (broad peak, 4H), 7.54 (bs, 1H), 7.80 (broad peak, 3H), 8.06 (broad peak, 3H); ESI/MS m/z 653.2 (M-H).

5.1.11.18. N-(3,5-Bis-trifluoromethyl-benzyl)-N-{4-[(3-trifluoromethyl-benzene sulfonyl)-(4-trifluoromethyl-benzyl)-amino]-phenyl}-oxalamic acid (15r)



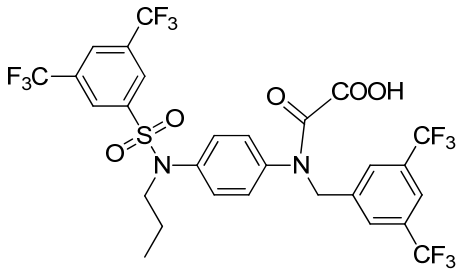
Compound **15r** was obtained from **14r** using general procedure described above as off-white solid; Yield: 47%; mp: 103-107 ^{0}C; Purity by HPLC: 98.57%; IR (KBr) 3433, 1624, 1508, 1326, 1280, 1168, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.87 (s, 2H), 5.06 (s, 2H), 7.14 (bd, 4H), 7.44 (d, J = 8.1 Hz, 2H), 7.60 (bd, 3H), 7.84 (broad peak, 4H), 8.09 (broad peak, 2H); ESI/MS m/z 771.3 (M-H).

5.1.11.19. N-{4-[(3,5-Bis-trifluoromethyl-benzenesulfonyl)-methyl-amino]-phenyl}-N-(3,5-bis-trifluoromethyl-benzyl)-oxalamic acid (15s)



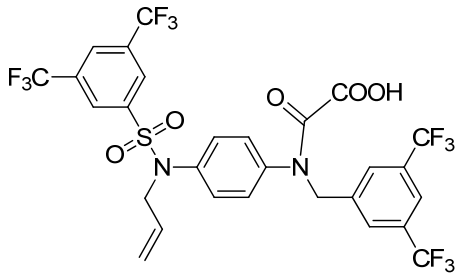
Compound **15s** was obtained from **14s** using general procedure described above as off-white solid; Yield: 68%; mp: 92-95 ^{0}C; Purity by UPLC: 96.37%; IR (KBr) 3423, 3020, 1639, 1508, 1381, 1278, 1141, 771, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.1 (s, 3H), 5.09 (s, 2H), 7.09 (d, J = 8.8 Hz, 2H), 7.22 (broad peak, 2H), 7.75-8.02 (broad peak, 5H), 8.49 (s, 1H); ESI/MS m/z 694.7 (M-H).

5.1.11.20. N-{4-[(3,5-Bis-trifluoromethyl-benzenesulfonyl)-propyl-amino]-phenyl}-N-(3,5-bis-trifluoromethyl-benzyl)-oxalamic acid (15t)



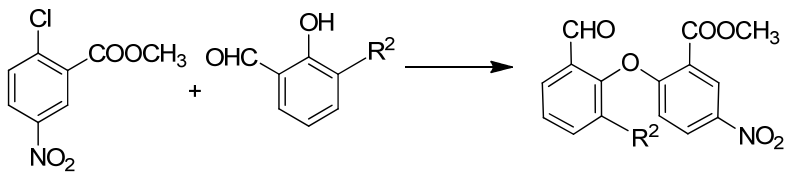
Compound **15t** was obtained from **14t** using general procedure described above as off-white semi solid; Yield: 40%; mp: 43-46 ^{0}C; Purity by HPLC: 98.30%; IR ($CHCl_3$) 3022, 1743, 1672, 1506, 1382, 1280, 1143, 758, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.80 (t, 3H), 1.22 (q, 2H), 3.50 (t, 2H), 5.18 (s, 2H), 7.15-7.24 (q, 4H), 7.85 (bd, 4H), 8.02 (s, 1H), 8.51 (s, 1H); ESI/MS m/z 723.1 (M-H).

5.1.11.21. N-{4-[(3,5-Bis-trifluoromethyl-benzenesulfonyl)-allyl-amino]-phenyl}-N-(3,5-bis-trifluoromethyl-benzyl)-oxalamic acid (15u)



Compound **15u** was obtained from **14u** using general procedure described above as off-white solid; Yield: 60%; mp: 50-53 ^{0}C; Purity by HPLC: 98.62%; IR (KBr) 3020, 1670, 1508, 1280, 1215, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.22 (d, J = 5.6 Hz, 2H), 5.04 (t, 2H), 5.14 (s, 2H), 5.58-5.63 (m, 1H), 7.15-7.23 (m, 4H), 7.82-7.87 (bd, 4H), 7.99 (s, 1H), 8.51 (s, 1H); ESI/MS m/z 721.1 (M-H).

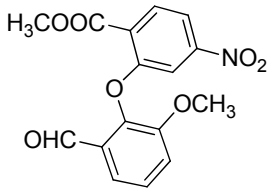
5.1.12. General procedure for the synthesis of compounds (18a, 18b)



16 **17a, 17b** **18a, 18b**

To a stirring solution of salicylaldehyde derivatives (**17a**, **17b**) (1.2 mol eq.) in DMSO (4 v/w) cooled at 0-5 ^{o}C was added 60% NaH (1.4 mol eq.) in 10 min. Methyl 2-chloro-5-nitro benzoate (**16**) (1 mol eq.) was then added to it at same temperature. Reaction mixture was then stirred at 25-30 ^{o}C for 18 h. The reaction mixture was then diluted with water (25 v/w) and extracted with diethyl ether (20 v/w). The organic layer was washed with water & brine solution, dried over Na_2SO_4 and evaporated on a Rota vapor. The product was then precipitated by adding hexane (5 v/w) and filtered to get title compounds **18a** and **18b** with fairly good purity.

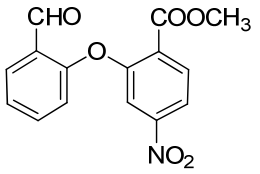
5.1.12.1. Methyl 2-(2-formyl-6-methoxyphenoxy)-4-nitrobenzoate (18a)



Compound **18a** was obtained by reacting **16** with 2-hydroxy-3-methoxybenzaldehyde (**17a**) using general procedure described above as off-white solid; Yield: 60%; mp: 171-176 ^{0}C; Purity by HPLC: 97.17%; IR (KBr) 1739, 1691, 1589, 1517, 1350, 1265, 746, 783 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.76 (s, 3H), 3.89 (s, 3H), 6.83 (d, J = 9.2 Hz, 1H), 7.51-7.62 (m, 3H), 8.26-8.30

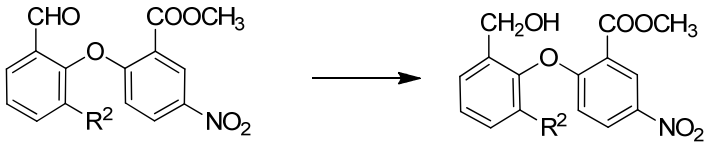
(dd, *J* = 2.9 and 9.2 Hz, 1H), 8.62 (d, *J* = 2.9 Hz, 1H), 10.11 (s, 1H); ESI/MS m/z 332 $(M+H)^+$.

5.1.12.2. Methyl 2-(2-formylphenoxy)-4-nitrobenzoate (18b)



Compound **18b** was obtained by reacting **16** with 2-hydroxy benzaldehyde (**17b**) using general procedure described above as off-white solid; Yield: 55%; mp: 118-123 ^{0}C; Purity by HPLC: 98.54%; IR (KBr) 1728, 1687, 1529, 1573, 1352, 765 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.90 (s, 3H), 6.98 (d, *J* = 8.46 Hz, 1H), 7.05 (d, *J* = 9 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.60-7.63 (m, 1H), 8.00-8.03 (dd, *J* = 1.68 and 7.7 Hz, 1H), 8.31-8.35 (dd, *J* = 2.8 and 9 Hz, 1H), 8.87 (d, *J* = 2.8 Hz, 1H), 10.41 (s, 1H); ESI/MS m/z 302 $(M+H)^+$.

5.1.13. General procedure for the synthesis of compounds (19a, 19b)

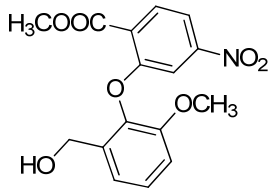


18a, 18b

19a: $R^2 = OCH_3$
19b: $R^2 = H$

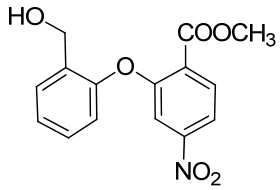
To a solution of **18a** and **18b** (1 mol eq.) in MeOH (6 v/w) cooled at 15-20 ^{o}C was added solution of $NaBH_4$ (0.4 mol eq.) in water (1 v/w). Reaction mixture was then stirred at 15-20 ^{o}C for 30 min. Reaction mixture was then diluted with water (20 v/w) and filtered to get title compounds **19a** and **19b** with fairly good purity.

5.1.13.1. Methyl 2-(2-(hydroxymethyl)-6-methoxyphenoxy)-5-nitrobenzoate (19a)



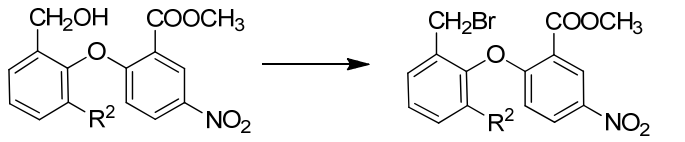
Compound **19a** was obtained by reducing **18a** using general procedure described above as off-white solid; Yield: 95%; mp: 158-163 ^{0}C; Purity by HPLC: 92.83%; IR (KBr) 3514, 1724, 1614, 1514, 1346, 1247, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.69 (s, 3H), 3.89 (s, 3H), 4.40 (d, J = 5.3 Hz, 2H), 5.16 (t, J = 5.5 Hz, 1H), 6.68 (d, J = 9.2 Hz, 1H), 7.15 (bt, 2H), 7.33 (bt, 1H), 8.26-8.30 (dd, J = 2.9 and 9.2 Hz, 1H), 8.58 (d, J = 2.9 Hz, 1H); ESI/MS m/z 356.1 $(M+Na)^+$.

5.1.13.2. Methyl 2-(2-(hydroxymethyl)phenoxy)-5-nitrobenzoate (19b)



Compound **19b** was obtained by reducing **18b** using general procedure described above as off-white solid; Yield: 92%; mp: 140-145 ^{0}C; Purity by HPLC: 93.21%; IR (KBr) 3398, 3118, 3080, 1739, 1616, 1577, 1344, 1247, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.86 (s, 3H), 4.45 (d, J = 5.5 Hz, 2H), 5.21 (t, J = 5.5 Hz, 1H), 6.89 (d, J = 9.2 Hz, 1H), 7.06 (bt, 1H), 7.30-7.39 (m, 2H), 7.59 (bt, 1H), 8.32-8.36 (dd, J = 2.9 and 9.1 Hz, 1H), 8.61 (d, J = 2.8 Hz, 1H); ESI/MS m/z 326 $(M+Na)^+$.

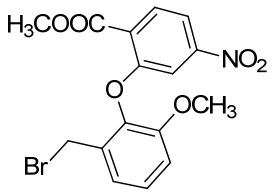
5.1.14. General procedure for the synthesis of compounds (20a, 20b)



19a, **19b** **20a**: $R^2 = OCH_3$ **20b**: $R^2 = H$

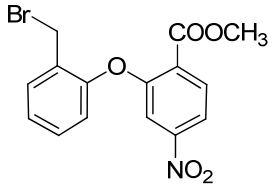
To a solution of **19a** and **19b** (1 mol eq.) in CH_2Cl_2 (5 v/w) cooled at 10-15 ^oC was added solution of PBr_3 (0.5 mol eq.) in CH_2Cl_2 (5 v/w). Reaction mixture was then stirred at 20-25 ^oC for 1 h. Reaction mixture was then diluted with CH_2Cl_2 (10 v/w) and water (10 v/w). Reaction mixture was basified using aq. $NaHCO_3$ till pH 9. CH_2Cl_2 layer was separated, dried over sodium sulfate and evaporated to get title compounds **20a** and **20b** with fairly good purity.

5.1.14.1. Methyl 2-(2-(bromomethyl)-6-methoxyphenoxy)-4-nitrobenzoate (20a)



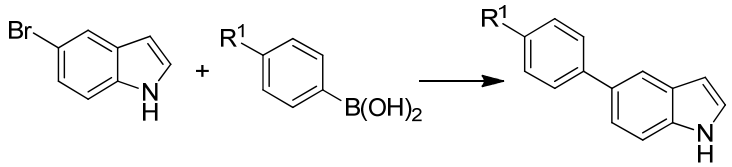
Compound **20a** was obtained by brominating **19a** using general procedure described above as white solid; Yield: 80%; mp: 140-144 ^0C; Purity by HPLC: 95.17%; IR (KBr) 3053, 1743, 1720, 1614, 1577, 1514, 1438, 1348, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.73 (s, 3H), 3.99 (s, 3H), 4.54 (bs, 2H), 6.70 (d, J = 9.2 Hz, 1H), 6.97-7.00 (dd, J = 1.3 and 8.2 Hz, 1H), 7.09-7.12 (dd, J = 1.5 and 7.8 Hz, 1H), 7.24-7.29 (m, 1H), 8.17-8.21 (dd, J = 2.9 and 9.25 Hz, 1H), 8.83 (d, J = 2.8 Hz, 1H); ESI/MS m/z 396 and 398 $(M+H)^+$.

5.1.14.2. Methyl 2-(2-(bromomethyl)phenoxy)-4-nitrobenzoate (20b)



Compound **20b** was obtained by brominating **19b** using general procedure described above as white solid; Yield: 75%; mp: 130-134 ^{0}C; Purity by HPLC: 90.75%; IR (KBr) 3118, 2997, 1712, 1616, 1579, 1515, 1440, 1342, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.86 (s, 3H), 4.67 (s, 2H), 7.04 (d, J = 9.1 Hz, 1H), 7.08 (d, J = 0.58 Hz, 1H), 7.29-7.32 (m, 1H), 7.44 (bt, 1H), 7.63-7.66 (dd, J = 1.5 and 7.5 Hz, 1H), 8.36-8.40 (dd, J = 2.9 and 9.1 Hz, 1H), 8.63 (d, J = 2.9 Hz, 1H); ESI/MS m/z No $(M+H)^+$ observed.

5.1.15. General procedure for the synthesis of compounds (23a, 23b)

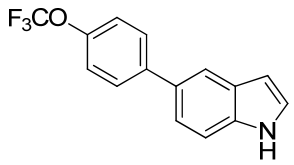


21 **22a, 22b** **23a**: $R^1 = OCF_3$ **23b**: $R^1 = H$

To a stirring solution of 5-bromoindole (**21**) (1 mol eq.) in n-propanol (7 v/w) was added aryl boronic acids (**22a** and **22b**) (1.3 mol eq.) at 25-30 ^{0}C. A solution of Na_2CO_3 (2.5 mol eq.) in water (3 v/w) was then added to it. Reaction flask was then flushed with nitrogen. $Pd(OAc)_2$ (0.07 mol eq.) and TPP (0.24 mol eq.) was added to it under nitrogen atmosphere. Reaction mixture was then heated at 90-95 ^{o}C for 5 h. Excess n-propanol was removed under vacuum and ethyl acetate (20 v/w) was added to it. Reaction mixture was filtered through hyflow bed. The

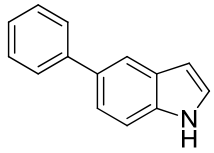
organic layer was washed with water and brine solution, dried over Na_2SO_4 and evaporated on a Rota vapor. The product was then purified by column chromatography using 100-200 silica gel and 5-10% ethyl acetate in hexane as mobile phase to get title compounds.

5.1.15.1. 5-(4-(Trifluoromethoxy)phenyl)-1H-indole (23a)



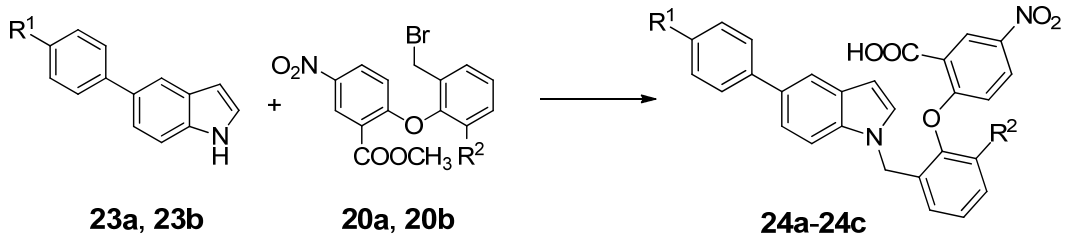
Compound **23a** was obtained by Suzuki coupling of **21** with 4-trifluoromethoxyphenyl boronic acid (**22a**) using general procedure described above as white solid; Yield: 35%; mp: 77-82 ^{0}C; Purity by HPLC: 99.62%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.62 (s, 1H), 7.28-7.32 (broad peak, 2H), 7.39-7.49 (m, 3H), 7.63-7.66 (bd, 2H), 7.82 (s, 1H), 8.22 (s, 1H); ESI/MS m/z 278 $(M+H)^+$.

5.1.15.2. 5-Phenyl-1H-indole (23b)



Compound **23b** was obtained by Suzuki coupling of **21** with phenyl boronic acid (**22b**) using general procedure described above as white solid; Yield: 38%; mp: 57-60 ^{0}C; Purity by HPLC: 96.21%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.61 (t, J = 2.8 Hz, 1H), 7.24-7.33 (broad peak, 2H), 7.41-7.46 (m, 4H), 7.64-7.67 (bd, 2H), 7.86 (s, 1H), 8.18 (s, 1H); ESI/MS m/z 192 (M-H).

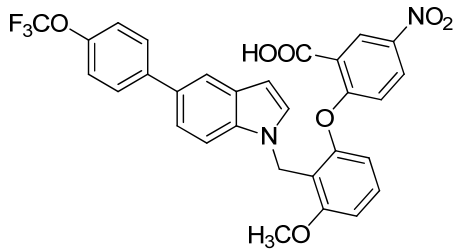
5.1.16. General procedure for the synthesis of compounds (24a-24c)



23a, **23b** **20a**, **20b** **24a-24c**

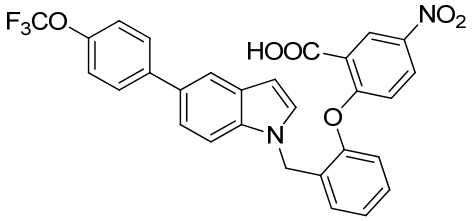
To a solution of indole derivatives (**23a** and **23b**) (1 mole eq.) in DMF (4 v/w) was added NaH (1.5 mole eq.) at 10-15 ^{0}C under nitrogen atmosphere. Reaction mixture was then stirred at 25-30 ^{o}C for another 15 min. To this was added bromo derivatives (**20a** and **20b**) (1 mole eq.) Reaction mixture was stirred for 5 h at 25-30 ^{o}C. Reaction mixture was then diluted with water (10 v/w) and extracted with ethyl acetate (50 v/w). The organic layer was evaporated under reduced pressure to get oily compound which was immediately dissolved in THF (5 v/w). 10% aq. KOH solution (5 v/w) was added to it. Reaction mixture was stirred at 25-30 ^{o}C for 1 h. Reaction mixture was diluted with 3 v/w water and washed with DIPE (2 x 5 v/w) to remove unreacted starting materials and impurities. Aqueous layer was acidified using dil. HCl and product was extracted with ethyl acetate (50 v/w). The organic layer was washed with water and brine solution, dried over Na_2SO_4 and evaporated on a Rota vapor to get desired product in fairly good purity.

5.1.16.1. 2-(3-Methoxy-2-((5-(4-(trifluoromethoxy)phenyl)-1H-indol-1-yl)methyl) phenoxy)-5-nitrobenzoic acid (24a)



Compound **24a** was obtained from **23a** and **20a** using general procedure described above as pale yellow solid; Yield: 50%; mp: 225-230 ^{0}C; Purity by UPLC: 97.44%; IR (KBr) 3423, 3090, 1701, 1681, 1614, 1519, 1479, 1356, 1165, 1076, 798, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.68 (s, 3H), 5.34 (s, 2H), 6.41 (d, J = 2.8 Hz, 1H), 6.49 (d, J = 9.2 Hz, 1H), 6.83 (d, J = 7.2 Hz, 1H), 7.16-7.18 (dd, J = 1.2 and 8.4 Hz, 1H), 7.26 (t, J = 8.4 Hz, 1H), 7.31-7.34 (dd, J = 1.4 and 8.8 Hz, 1H), 7.40 (d, J = 8 Hz, 2H), 7.44 (d, J = 3.2 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.69-7.71 (m, 3H), 8.07-8.10 (dd, J = 2.8 and 9.2 Hz, 1H), 8.50 (d, J = 2.8 Hz, 1H); ESI/MS m/z 578.9 $(M+H)^+$.

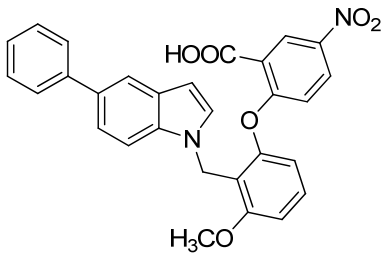
5.1.16.2. 5-Nitro-2-(2-((5-(4-(trifluoromethoxy)phenyl)-1H-indol-1-yl)methyl) phenoxy) benzoic acid (24b)



Compound **24b** was obtained from **23a** and **20b** using general procedure described above as pale yellow solid; Yield: 45%; mp: 205-210 ^{0}C; Purity by UPLC: 96.35%; IR (KBr) 3423, 3082, 1706, 1618, 1521, 1477, 1346, 1255, 1012,

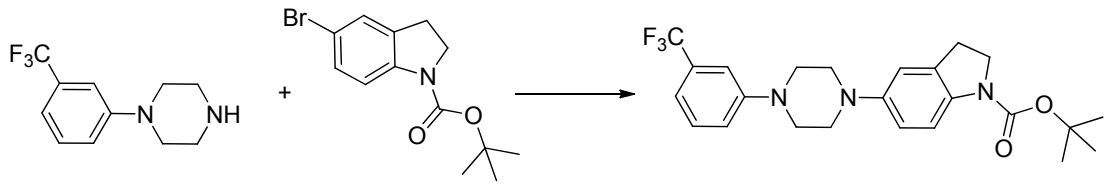
800, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 5.48 (s, 2H), 6.49 (d, J = 3.2 Hz, 1H), 6.85 (d, J = 9.2 Hz, 1H), 6.96 (d, J = 8 Hz, 1H), 7.02 (d, J = 7.2 Hz, 1H), 7.11 (t, J = 7.2 Hz, 1H), 7.28-7.32 (m, 1H), 7.36-7.41 (m, 3H), 7.60-7.62 (bd, 2H), 7.73-7.76 (m, 2H), 7.79 (d, J = 1.6 Hz, 1H), 8.09-8.12 (dd, J = 2.8 and 8.8 Hz, 1H), 8.42 (bs, 1H); ESI/MS m/z 547.1 (M-H).

5.1.16.3. 2-(3-Methoxy-2-((5-phenyl-1H-indol-1-yl)methyl)phenoxy)-5-nitro benzoic acid (24c)



Compound **24c** was obtained from **23b** and **20a** using general procedure described above as off-white solid; Yield: 54%; mp: 237-240 ^0C; Purity by HPLC: 93.95%; IR (KBr) 3433, 3084, 1701, 1681, 1519, 1346, 1076, 754, 698 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.70 (s, 3H), 5.35 (s, 2H), 6.42 (d, J = 3.2 Hz, 1H), 6.50 (d, J = 9.2 Hz, 1H), 6.80-6.82 (dd, J = 1.2 and 8 Hz, 1H), 7.19 (d, J = 1.2 Hz, 1H), 7.24-7.28 (m, 2H), 7.31-7.34 (dd, J = 2.4 and 8.4 Hz, 1H), 7.40-7.43 (m, 2H), 7.46-7.48 (m, 2H), 7.58-7.61 (dd, J = 1.6 and 8.4 Hz, 2H), 7.69 (d, J = 1.2 Hz, 1H), 8.07-8.10 (dd, J = 3.4 and 9.2 Hz, 1H), 8.49 (d, J = 2.8 Hz, 1H); ESI/MS m/z 493.3 (M-H).

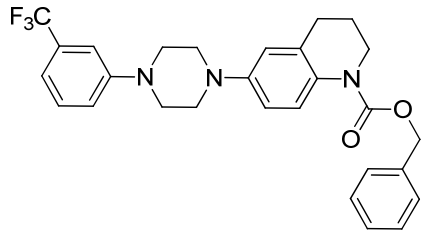
5.1.17. tert-Butyl 5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)indoline-1-carboxylate (27a)



25a **26a** **27a**

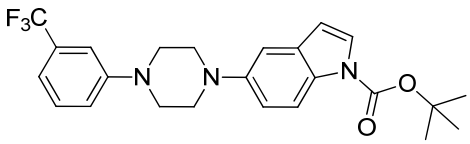
To a solution of 2-(Di-tert-butylphosphino)biphenyl (126 mg, 0.00042 mol) in dimethoxy ethane (DME) (3 mL) was added $Pd(OAc)_2$ (95 mg, 0.00042 mol) at 25 ^{0}C and stirred for 1 h. 10 mL DME was added follwed by **26a** (5 g, 0.01689 mol) and **25a** (4.7 g, 0.0202 mol). K_3PO_4 (8.3 g, 0.0253 mol) was added to the reaction mixture in one lot. Reaction mixture was heated to reflux for 8 h. Reaction mixture was then diluted with water (50 mL) and ethyl acetate (100 mL). The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product. Column purification using 100-200 silica gel and 5-10% ethyl acetate in hexane mobile phase gave titled compound as off-white solid; Yield: 60%; mp: 137-139 ^{0}C; Purity by HPLC: 96.68%; IR (KBr) 2976, 1689, 1606, 1589, 1498, 1448, 1396, 1329, 1170, 1130, 960, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.49 (s, 9H), 3.02 (t, 2H), 3.18 (t, 4H), 3.36 (t, 4H), 3.86 (t, 2H), 6.77-6.80 (dd, J = 2.4 and 8.8 Hz, 1H), 6.92 (d, J = 2.4 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.22 (s, 1H), 7.27-7.30 (dd, J = 2 and 8.4 Hz, 1H), 7.44 (t, J = 8 Hz, 1H), 7.57(bs, 1H); ESI/MS m/z 448.1 $(M+H)^+$.

5.1.18. Benzyl 6-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-3,4-dihydro quinoline-1(2H)-carboxylate (27b)



Compound **27b** was prepared from **25a** and **26b** by means of a procedure similar to that reported for **27a** as off-white solid; Yield: 40%; mp: 118-120 ^{0}C; Purity by UPLC: 98.39%; IR (KBr) 2956, 1691, 1608, 1504, 1448, 1404, 1340, 1311, 1232, 1159, 1116, 966, 694 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.80-1.86 (m, 2H), 2.7 (t, 2H), 3.22 (t, 4H), 3.35 (t, 4H), 3.68 (t, 2H), 5.16 (s, 2H), 6.75 (dd, *J* = 2.8 Hz, 1H), 6.79-6.82 (dd, *J* = 2.8 and 8.8 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 7.22 (s, 1H), 7.27-7.30 (dd, *J* = 2 and 9.2 Hz, 1H), 7.31-7.41 (m, 5H), 7.44 (t, *J* = 8 Hz, 1H), 7.51 (bd, *J* = 8 Hz,1H); ESI/MS m/z 496.3 $(M+H)^+$.

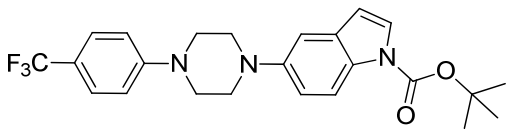
5.1.19. tert-Butyl 5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indole-1-carboxylate (27c)



To a solution of 2-(Di-tert-butylphosphino)biphenyl (126 mg, 0.00042 mol) in toluene (3 mL) was added $Pd(OAc)_2$ (95 mg, 0.00042 mol) at 25 ^{0}C and stirred for 5 h. 30 mL toluene was added followed by **26c** (5 g, 0.01689 mol) and **25a** (4.7 g, 0.0202 mol). Cesium carbonate (8.3 g, 0.0253 mol) and TEA (0.852 g, 0.084 mol) were added to the reaction mixture in one lot. Reaction mixture was heated at 110-115 ^{o}C for 8 h. Reaction mixture was then diluted with water (50

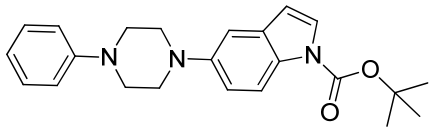
mL) and ethyl acetate (100 mL). The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product. Column purification using 100-200 silica gel and 2-5% ethyl acetate in hexane as mobile phase gave titled compound as off-white solid; Yield: 35%; mp: 125-128 ^{0}C; Purity by HPLC: 97.05%; IR (KBr) 3435, 2831, 1726, 1610, 1473, 1448, 1377, 1259, 727, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.66 (s, 9H), 3.33 (t, J = 3.5 Hz, 4H), 3.43 (t, J = 3.8 Hz, 4H), 6.51 (d, J = 3.6 Hz, 1H), 7.05-7.15 (m, 4H), 7.18 (s, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.56 (d, J = 3.4 Hz, 1H), 8.05 (d, J = 8.2 Hz, 1H); ESI/MS m/z 446.2 $(M+H)^+$.

5.1.20. tert-Butyl 5-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indole-1-carboxylate (27d)



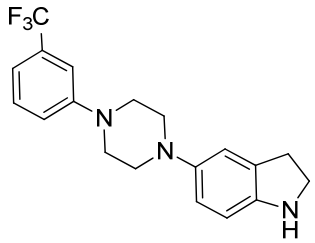
Compound **27d** was prepared from **25b** and **26c** by means of a procedure similar to that reported for **27c**. Crude product was column purified using 100-200 silica gel and 1-3% ethyl acetate in hexane as mobile phase to get off-white solid; Yield: 21%; mp: 162-164 ^{0}C; Purity by HPLC: 99.73%; IR (KBr) 3433, 3093, 2833, 1726, 1616, 1581, 1521, 1477, 1369, 1226, 835, 721 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.60 (s, 9H), 3.27 (broad peak, 4H), 3.45 (broad peak, 4H), 6.60 (d, J = 3.6 Hz, 1H), 7.09-7.15 (m, 4H), 7.51-7.58 (m, 3H), 7.92 (d, J = 8.9 Hz, 1H); ESI/MS m/z 446.1 $(M+H)^+$.

5.1.21. tert-Butyl 5-(4-phenylpiperazin-1-yl)-1H-indole-1-carboxylate (27e)



Compound **27e** was prepared from **25c** and **26c** by means of a procedure similar to that reported for **27c**. Crude product was column purified using 100-200 silica gel and 1-3% ethyl acetate in hexane as mobile phase to get off-white solid; Yield: 24%; mp: 174-178 ^{0}C; Purity by HPLC: 99.44%; IR (KBr) 3062, 2817, 1733, 1598, 1577, 1477, 1371, 1238, 804, 754, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.66 (s, 9H), 3.37 (bd, 8H), 6.50 (d, J = 3.4 Hz, 1H), 6.90 (t, J = 7.2 Hz , 1H), 7.00-7.13 (m, 4H), 7.28-7.33 (m, 2H), 7.55 (s, 1H), 8.02 (bs, 1H); ESI/MS m/z 378 $(M+H)^+$.

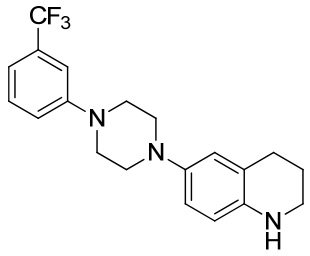
5.1.22. 5-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)indoline (28a)



To a solution of **27a** (4 g, 0.0089 mol) in CH_2Cl_2 (28 mL) was added TFA (trifloroacetic acid, 8 mL) at 25 ^{0}C and stirred for 4 h. Reaction mixture was then diluted with CH_2Cl_2 (15 mL) and saturated $NaHCO_3$ solution (20 mL). The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product which was solidified using 25 mL hexane. Solid obtained was filtered and dried to get desired product as brown solid; Yield: 45%; mp: 120-123 ^{0}C; Purity by UPLC: 93.23%; IR (KBr) 3373, 1602,

1587, 1492, 1442, 1379, 1356, 1228, 1145, 962, 806, 729 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.86 (t, J = 8.4 Hz, 2H), 3.04-3.07 (t, 4H), 3.30-3.37 (m, 6H), 5.08 (s, 1H), 6.44 (d, J = 8.4 Hz, 1H), 6.59-6.62 (dd, J = 2.4 and 8.4 Hz, 1H), 6.82 (d, J = 2 Hz, 1H), 7.09 (d, J = 7.6 Hz, 1H), 7.21 (s, 1H), 7.26-7.29 (dd, J = 2 and 8.4 Hz, 1H), 7.43 (t, J = 8 Hz, 1H); ESI/MS m/z 348.1 $(M+H)^+$.

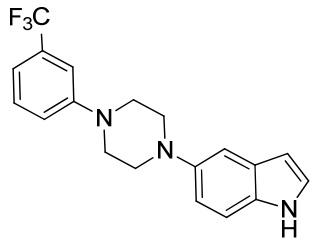
5.1.23. 6-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)-1,2,3,4-tetrahydro quinoline (28b)



To a H_2SO_4 cooled at 0-5 ^0C (3 v/w) was added **27b** (2.5 g, 0.0050 mol) portion wise and stirred for 0.5 h at 5-10 ^0C. Reaction mixture was then diluted with water and basified using dil NaOH solution till pH = 9-10. The aqueous layer was extracted with ethyl acetate (25 v/w). The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product which was solidified using 25 mL hexane. Solid obtained was filtered and dried to get desired product as brown solid; Yield: 44%; mp: 88-89 ^0C; Purity by UPLC: 96.86%; IR (KBr) 3423, 2947, 1614, 1506, 1450, 1356, 1303, 1273, 1234, 1161, 1109, 960, 785, 692 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.73-1.79 (m, 2H), 2.66 (t, 2H), 3.03 (t, 4H), 3.11 (t, 2H), 3.32 (t, 4H), 5.18 (s, 1H), 6.38 (d, J = 8.4 Hz, 1H), 6.55 (d, J = 2.8 Hz, 1H), 6.59-6.62 (dd, J =

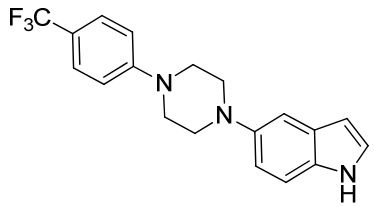
2.8 and 8.8 Hz, 1H), 7.09 (d, *J* = 8 Hz, 1H), 7.20 (s, 1H), 7.26-7.28 (dd, *J* = 2.4 and 9.2 Hz, 1H), 7.43 (t, *J* = 8 Hz, 1H); ESI/MS m/z 362.2 $(M+H)^+$.

5.1.24. 5-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)-1H-indole (28c)



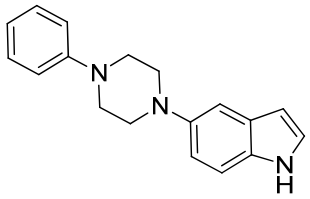
To a solution of **27c** (2 g, 0.00449 mol) in THF (10 mL) and MeOH (10 mL) was added sodium methoxide (1.24 g, 0.02245 mol) at 25 ^{0}C Reaction mixture was heated at 45-50 ^{o}C for 4 h. Reaction mixture was then diluted with water (60 mL). The pH of the reaction mixture was adjusted to 6-7 using dil. HCl. The product was extracted with diethyl ether (50 mL). The organic layer was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product. Column purification using 100-200 silica gel and 10% ethyl acetate in hexane as mobile phase gave titled compound as off-white solid; Yield: 89%; mp: 106-110 ^{0}C; Purity by UPLC: 99.19%; IR (KBr) 3398, 2808, 1604, 1475, 1448, 1315, 1228, 729, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.18 (t, 4H), 3.40 (t, 4H), 6.31 (t, *J* = 2 Hz, 1H), 6.91-6.94 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.08 (d, *J* = 2 Hz , 1H), 7.10 (s, 1H), 7.23-7.25 (m, 2H), 7.28-7.31 (m, 2H), 7.45 (t, *J* = 8.0 Hz, 1H), 10.85 (s, 1H); ESI/MS m/z 346 $(M+H)^+$.

5.1.25. 5-(4-(4-(Trifluoromethyl)phenyl)piperazin-1-yl)-1H-indole (28d)



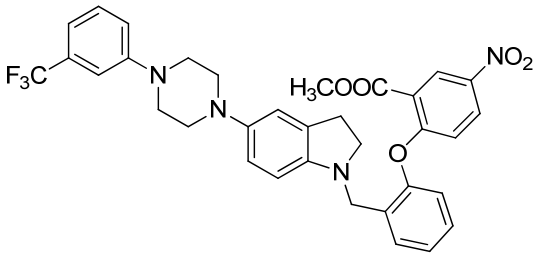
Compound **28d** was prepared from **27d** by means of a procedure similar to that reported for **28c** as off-white solid; Yield: 49%; mp: 206-210 ^{0}C; Purity by HPLC: 98.88%; IR (KBr) 3462, 2833, 1614, 1475, 1450, 1328, 1228, 829, 725, 665 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.16 (t, J = 5.0 Hz, 4H), 3.43 (t, J = 4.5 Hz, 4H), 6.30 (s, 1H), 6.89-6.93 (dd, J = 2 and 8.7 Hz, 1H), 7.07 (d, J = 1.8 Hz, 1H), 7.14 (d, J = 8.7 Hz, 2H), 7.23-7.29 (m, 2H), 7.53 (d, J = 8.7 Hz, 2H), 10.85 (s, 1H); ESI/MS m/z 345.9 $(M+H)^+$.

5.1.26. 5-(4-Phenylpiperazin-1-yl)-1H-indole (28e)



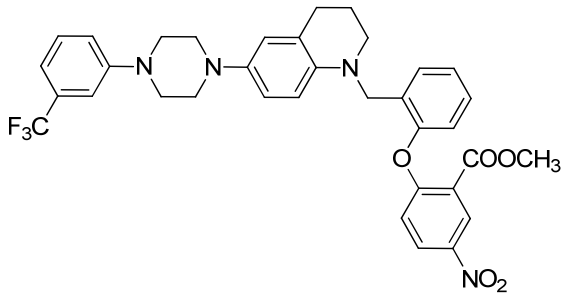
Compound **28e** was prepared from **27e** by means of a procedure similar to that reported for **28c** as off-white solid; Yield: 80%; mp: 170-176 ^{0}C; Purity by HPLC: 99.57%; IR (KBr) 3382, 2825, 1596, 1575, 1496, 1473, 1323, 1228, 769, 700 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.17 (q, 4H), 3.28 (q, 4H), 6.30 (s, 1H), 6.79 (t, J = 7.1 Hz, 1H), 6.89-6.93 (dd, J = 2.1 and 8.7 Hz, 1H), 7.00 (d, J = 8.2 Hz, 2H), 7.06 (d, J = 1.8 Hz, 1H), 7.20-7.29 (m, 4H), 10.85 (s, 1H); ESI/MS m/z 277.8 $(M+H)^+$.

5.1.27. Methyl 5-nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)indolin-1-yl)methyl)phenoxy)benzoate (29a)



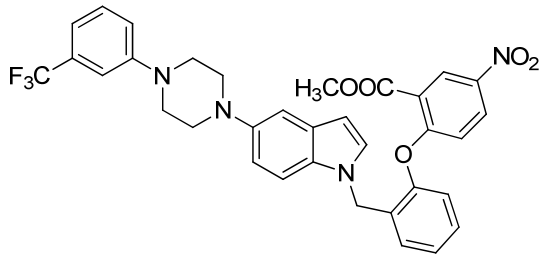
To a solution of **28a** (1 g, 0.00288 mol) in CH_3CN (10 mL) was added K_2CO_3 (0.6 g, 0.00432 mol) at 25 ^{0}C. To this was added **20b** (1.16 g, 0.00317 mol) in one lot and reaction mixture was stirred at 25-30 ^{o}C for 16 h. Reaction mixture was then diluted with water (50 mL) The product was extracted with ethyl acetate (50 mL). The organic layer was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product, which was directely used for next step.

5.1.28. Methyl 5-nitro-2-(2-((6-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-3,4-dihydroquinolin-1(2H)-yl)methyl)phenoxy)benzoate (29b)



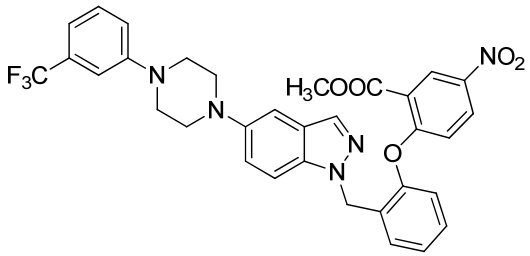
Compound **29b** was prepared from **28b** and **20b** by means of a procedure similar to that reported for **29a** and directely used for next step.

5.1.29. Methyl 5-nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indol-1-yl)methyl)phenoxy)benzoate (29c)

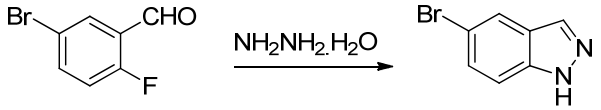


To a suspension of *t*-BuOK (180 mg, 0.00159 mol) in DMF (5 mL) was added **27c** (500 mg, 0.00145 mol) at 10-15 ^{0}C under nitrogen atmosphere. Reaction mixture was stirred at 25-30 ^{o}C for 10 min. Compound **20b** (580 mg, 0.00159 mol) was added to it in one lot. Reaction mixture was stirred at 25-30 ^{o}C for 2 h. Reaction mixture was then diluted with cold water (30 mL) and pH of the reaction mixture was adjusted to 4-5. Ethyl acetate (50 mL) was added to it. The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get crude product. Column purification using 100-200 silica gel and 10% ethyl acetate in hexane as mobile phase gave titled compound as brown solid; Yield: 25%; Purity by HPLC: 99.32%; IR (KBr) 2821, 1737, 1714, 1616, 1579, 1519, 1479, 1446, 1321, 1164, 1072, 746, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.24 (t, 4H), 3.41 (t, 4H), 3.96 (s, 3H), 5.30 (s, 2H), 6.31 (d, J = 3 Hz, 1H), 6.48 (d, J = 9 Hz, 1H), 6.92 (d, J = 2.1 Hz, 1H), 6.98-7.22 (m, 9H), 7.33-7.38 (m, 2H), 7.96-8.00 (d, J = 2.8 and 9.1 Hz, 1H), 6.31 (d, 1H); ESI/MS m/z 631.23 $(M+H)^+$.

5.1.30. Methyl 5-nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indazol-1-yl)methyl)phenoxy)benzoate (29d)



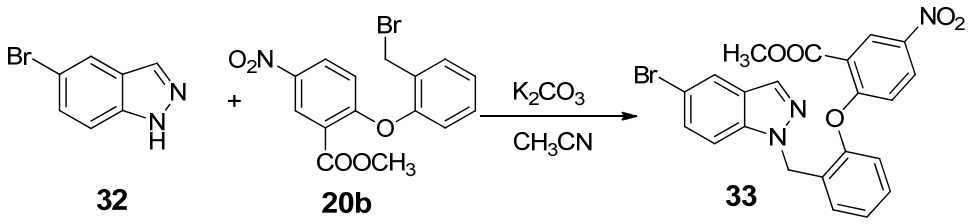
Step 1: 5-Bromo-1H-indazole **(32)**



31 **32**

To a solution of NH_2NH_2:H_2O (6 mL) was added **31** (2 g, 0.00985 mol) at 0-5 ^{0}C. The reaction mixture was refluxed for 20 h. Reaction mixture was then diluted with water (30 mL). Precipitated solid was filtered and dried to get titled compound as off-white solid; Yield: 53%; mp: 122-125 ^{0}C; Purity by UPLC: 98.61%; IR (KBr) 3174, 1749, 1618, 1487, 1384, 1334, 1278, 1188, 950, 877, 781 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.42 (d, J = 8.8 Hz, 1H), 7.48-7.53 (m, 1H), 7.92-7.93 (m, 1H), 8.04 (s, 1H), 10.17 (bs, 1H); ESI/MS m/z 197.8 and 198.8 $(M+H)^+$.

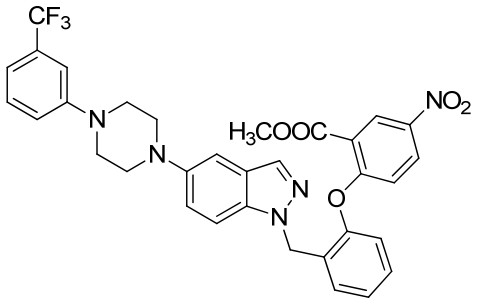
Step 2: Methyl 2-(2-((5-bromo-1H-indazol-1-yl)methyl)phenoxy)-5-nitrobenzoate **(33)**



32 **20b** **33**

To a solution of **32** (0. 484 g, 0.002459 mol) in CH_3CN (5 mL) was added **20b** (1 g, 0.002732 mol) at 25-30 ^{0}C. K_2CO_3 (0.565 g, 0.004098 mol) was added to the reaction mixture in one lot. The reaction mixture was stirred at 25-30 ^{o}C for 5 h. Reaction mixture was then diluted with water (30 mL) and CH_2Cl_2 (50 mL). CH_2Cl_2 layer was separated and distilled out to get crude product. Column purification using 100-200 silica gel and 15-20% ethyl acetate in hexane as mobile phase gave title compound as pale yellow solid; Yield: 30%; Purity by UPLC: 97.33%; IR (KBr) 3437, 1735, 1614, 1519, 1481, 1346, 1251, 1180, 1070, 904, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.84 (s, 3H), 5.62 (s, 2H), 6.57 (d, J = 9.2 Hz, 1H), 7.07-7.10 (dd, J = 0.8 and 8 Hz, 1H), 7.27-7.31 (m, 1H), 7.35-7.38 (m, 2H), 7.38-7.45 (m, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.84-7.85 (dd, J = 0.8 and 2 Hz, 1H), 7.94 (d, J = 0.8 Hz, 1H), 8.13-8.16 (dd, J = 2.8 and 9.2 Hz, 1H), 8.48 (d, J = 3.2 Hz, 1H); ESI/MS m/z 482 and 484 $(M+H)^+$.

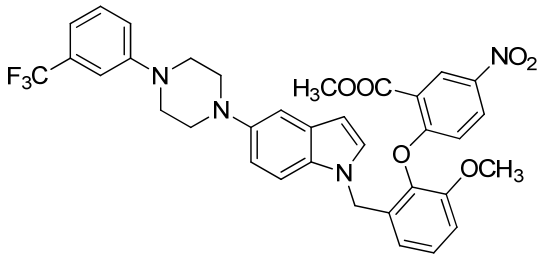
Step 3: Methyl 5-nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indazol-1-yl)methyl)phenoxy)benzoate **(29d)**



Compound **29d** was prepared from **33** and **25a** by means of a procedure similar to that reported for **27a** using 2-(Di-tert-butylphosphino)-biphenyl as ligand. Product obtained was column purified using 30% ethyl acetate in hexane to get desired product as pale yellow solid; Yield: 20%; Purity by UPLC: 96.13%; IR

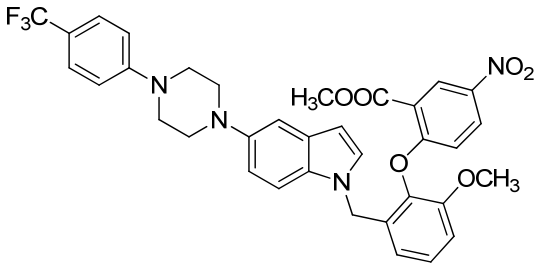
(KBr) 3450, 3086, 2953, 2827, 1732, 1614, 1579, 1514, 1494, 1450, 1344, 1251, 1120, 1072, 792, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.16 (t, J = 3.8 Hz, 4H), 3.40 (t, J = 4.4 Hz, 4H), 3.89 (s, 3H), 5.55 (s, 2H), 6.47 (d, J = 9.2 Hz, 1H), 6.96 (d, J = 2 Hz, 1H), 7.12 (d, J = 8 Hz, 2H), 7.14-7.16 (dd, J = 2 and 9.2 Hz, 1H), 7.25 (s, 1H), 7.30-7.37 (m, 3H), 7.41-7.45 (m, 3H), 7.78 (d, J = 0.4 Hz, 1H), 8.07-8.10 (dd, J = 2.8 and 9.2 Hz, 1H), 8.44 (d, J = 3.2 Hz, 1H); ESI/MS m/z 632.2 $(M+H)^+$.

5.1.31. Methyl 2-(2-methoxy-6-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indol-1-yl)methyl)phenoxy)-5-nitrobenzoate (29e)



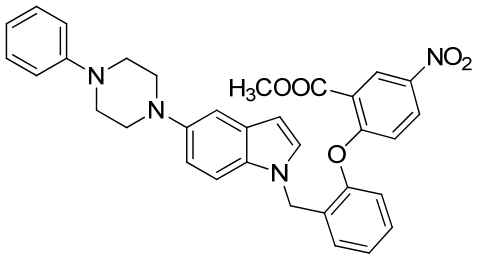
Compound **29e** was prepared from **28c** and **20a** by means of a procedure similar to that reported for **29c** as pale yellow solid; Yield: 52%; mp: 180-185 ^0C; Purity by HPLC: 98.51%; IR (KBr) 3419, 2950, 2817, 1739, 1714, 1616, 1589, 1519, 1446, 1344, 1230, 1166, 1070, 783, 727 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.23 (t, J = 5.2 Hz, 4H), 3.41 (t, J = 4.5 Hz, 4H), 3.71 (s, 3H), 4.00 (s, 3H), 5.26 (s, 2H), 6.23 (t, J = 3.3 Hz, 2H), 6.79-6.89 (dd, 2H), 6.96-7.00 (m, 3H), 7.12-7.24 (m, 5H), 7.37(t, 1H) 7.85-7.89 (dd, J = 3 and 9.3 Hz, 1H), 8.66 (d, J = 2.8 Hz, 1H); ESI/MS m/z 661.3 $(M+H)^+$.

5.1.32. Methyl 2-(2-methoxy-6-((5-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indol-1-yl)methyl)phenoxy)-5-nitrobenzoate (29f)



Compound **29f** was prepared from **28d** and **20a** by means of a procedure similar to that reported for **29c** as pale yellow solid; Yield: 59%; mp: 160-164 ^{0}C; Purity by HPLC: 97.43%; IR (KBr) 2925, 2823, 1733, 1720, 1614, 1589, 1521, 1483, 1438, 1344, 1234, 1110, 1072, 823, 754 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.23 (bs, 4H), 3.41 (bs, 4H), 3.67 (s, 3H), 3.93 (s, 3H), 5.25 (s, 2H), 6.20 (d, J = 3 Hz, 1H), 6.41 (d, J = 9.2 Hz, 1H), 6.84 (t, J = 3.4 Hz, 2H), 6.89 (d, J = 8.8 Hz, 1H), 7.14 (d, J = 8.7 Hz, 2H), 7.16-7.21 (m, 3H), 7.28 (t, J = 8.0 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 8.04-8.08 (dd, J = 2.8 and 9.2 Hz, 1H), 8.49 (d, J = 2.8 Hz, 1H); ESI/MS m/z 661.1 (M+H)$^+$.

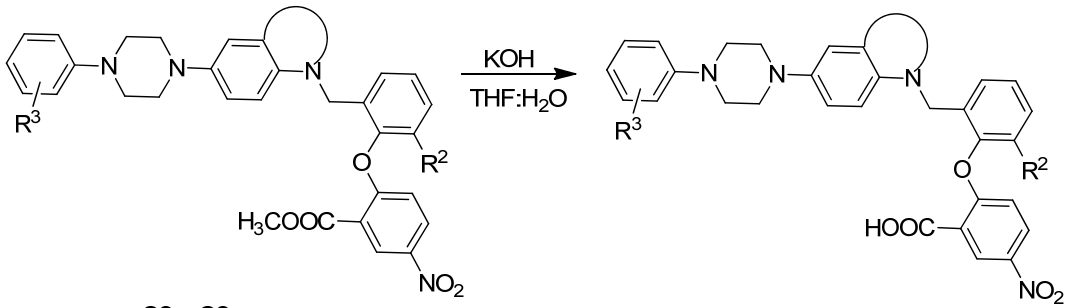
5.1.33. Methyl 5-nitro-2-(2-((5-(4-phenylpiperazin-1-yl)-1H-indol-1-yl)methyl) phenoxy) benzoate (29g)



Compound **29g** was prepared from **28e** and **20b** by means of a procedure similar to that reported for **29c** as pale yellow solid; Yield: 43%; mp: 146-150 ^{0}C; Purity by HPLC: 97.48%; IR (KBr) 2950, 2821, 1733, 1714, 1616, 1596, 1579, 1523,

1477, 1448, 1346, 1228, 1130, 1072, 756, 696 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.14 (bd, 4H), 3.27 (bd, 4H), 3.89 (s, 3H), 5.31 (s, 2H), 6.26 (d, J = 3 Hz, 1H), 6.70 (d, J = 9.1 Hz, 1H), 6.81 (d, J = 7.2 Hz, 1H), 6.88 (d, J = 8.9 Hz, 1H), 6.97 (bt, 3H), 7.11 (d, J = 8 Hz, 1H), 7.20-7.26 (m, 6H), 7.39 (bt, 1H), 8.17-8.21 (dd, J = 2.1 and 9.1 Hz, 1H), 8.54 (d, J = 2.8 Hz, 1H); ESI/MS m/z 563.1 $(M+H)^+$.

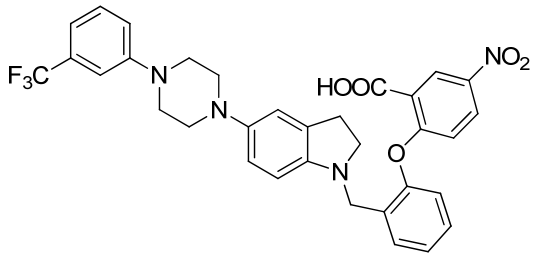
5.1.34. General procedure for the synthesis of compounds of general formula (30a-30g)



29a-29g **30a-30g**

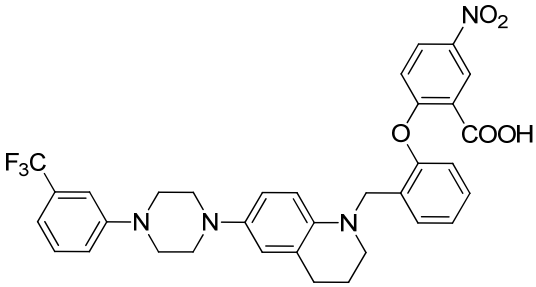
To a solution of **29a**-**29g** (1 mol eq.) in THF (5 v/w) was added solution of KOH (1.5 mol eq.) in water (5 v/w) at 25-30 ^{0}C. Reaction mixture was stirred at 25-30 ^{o}C for 1 h. Reaction mixture was then diluted with water (10 v/w) and extracted with diethyl ether (25 v/w). Aquous layer was acidified using dil. HCl and precipitated product was extracted with ethyl acetate (30 v/w). The organic layer was separated, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to get desired product.

5.1.34.1. 5-Nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)indolin-1-yl)methyl)phenoxy)benzoic acid (30a)



Compound **30a** was prepared from **29a** using general procedure as described above as brownish solid; Yield: 45%; mp: 151-153 ^{0}C; Purity by UPLC: 95.49%; IR (KBr) 3458, 1687, 1614, 1581, 1496, 1475, 1450, 1344, 1244, 1165, 1122, 945, 868, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.74 (t, 2H), 3.04 (t, 4H), 3.16 (t, 2H), 3.33 (t, 4H), 4.29 (s, 2H), 6.39 (d, J = 8 Hz, 1H), 6.58 (bs, 1H), 6.78 (bs, 1H), 6.88 (d, J = 9.2 Hz, 1H), 7.09 (d, J = 7.2 Hz, 1H), 7.14 (d, J = 8 Hz, 1H), 7.20 (s, 1H), 7.26-7.34 (m, 2H), 7.40-7.45 (m, 2H), 7.58 (d, J = 6.8 Hz, 1H), 8.25-8.28 (dd, J = 2.4 and 9.2 Hz, 1H), 8.55 (d, J = 2.8 Hz, 1H), 13.6 (broad peak, 1H); ESI/MS m/z 619.1 $(M+H)^+$.

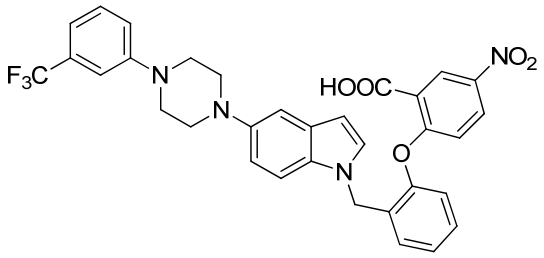
5.1.34.2. 5-Nitro-2-(2-((6-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-3,4-dihydroquinolin -1(2H)-yl)methyl)phenoxy)benzoic acid (30b)



Compound **30b** was prepared from **29b** using general procedure as described above as brownish solid; Yield: 65%; mp: 125-127 ^{0}C; Purity by UPLC: 92.81%;

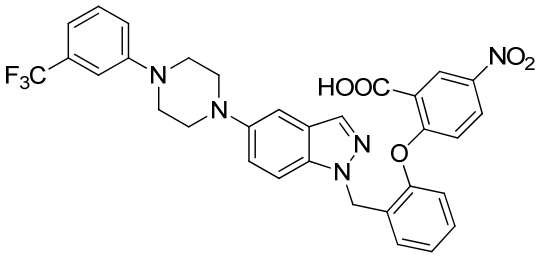
IR (KBr) 3416, 2926, 1710, 1612, 1516, 1450, 1344, 1236, 1165, 1120, 945, 860, 748, 696 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.84-1.86 (m, 2H), 2.66 (t, 2H), 3.03 (t, 4H), 3.25 (t, 2H), 3.32 (t, 4H), 4.33 (s, 2H), 6.34 (d, J = 8.4 Hz, 1H), 6.56-6.62 (m, 2H), 6.96 (d, J = 9.2 Hz, 1H), 7.09 (d, J = 8 Hz, 2H), 7.20 (s, 1H), 7.24-7.27 (m, 2H), 7.30-7.34 (m, 2H), 7.43 (t, J = 8 Hz, 1H), 8.32-8.36 (dd, J = 3.2 and 9.2 Hz, 1H), 8.58 (d, J = 2.8 Hz, 1H), 13.6 (broad peak, 1H); ESI/MS m/z 633.5 $(M+H)^+$.

5.1.34.3. 5-Nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indol-1-yl)methyl)phenoxy)benzoic acid (30c)



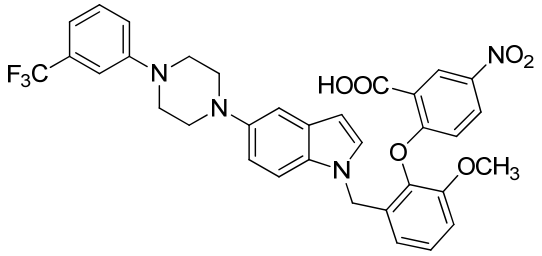
Compound **30c** was prepared from **29c** using general procedure as described above as pale yellow solid; Yield: 88%; mp: 180-185 ^0C; Purity by HPLC: 97.07%; IR (KBr) 3431, 2923, 2850, 1718, 1616, 1579, 1521, 1479, 1450, 1346, 1232, 1164, 1122, 788, 727 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.16 (bs, 4H), 3.34 (bs, 4H), 5.34 (s, 2H), 6.30 (d, J = 3 Hz, 1H), 6.78 (d, J = 9.1 Hz, 1H), 7.00-7.06 (m, 2H), 7.08-7.12 (m, 3H), 7.24 (d, J = 7.5 Hz, 1H), 7.30-7.45 (m, 6H), 8.20-8.24 (dd, J = 3 and 9.1 Hz, 1H), 8.57 (d, J = 3 Hz, 1H), 13.71 (bs, 1H); ESI/MS m/z 617.2 $(M+H)^+$.

5.1.34.4. 5-Nitro-2-(2-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indazol-1-yl)methyl)phenoxy)benzoic acid (30d)



Compound **30d** was prepared from **29d** using general procedure as described above as pale yellow solid; Yield: 70%; mp: 110-114 ^{0}C; Purity by UPLC: 95.82%; IR (KBr) 3435, 2831, 1718, 1616, 1579, 1508, 1477, 1450, 1346, 1244, 1165, 1122, 1074, 792, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.16 (t, J = 4.4 Hz, 4H), 3.40 (t, J = 4 Hz, 4H), 5.58 (s, 2H), 6.56 (d, J = 9.2 Hz, 1H), 7.02 (d, J = 1.6 Hz, 1H), 7.07 (d, J = 8 Hz, 1H), 7.11 (d, J = 7.6 Hz, 1H), 7.14-7.16 (dd, J = 2 and 9.2 Hz, 1H), 7.20 (d, J = 7.6 Hz, 1H), 7.25 (d, J = 6 Hz, 2H), 7.31 (d, J = 8.4 Hz, 1H), 7.37-7.41 (m, 1H), 7.43-7.49 (m, 2H), 7.84 (s, 1H), 8.06-8.09 (dd, J = 2.8 and 9.2 Hz, 1H), 8.45 (d, J = 2.8 Hz, 1H); ESI/MS m/z 618.2 $(M+H)^+$.

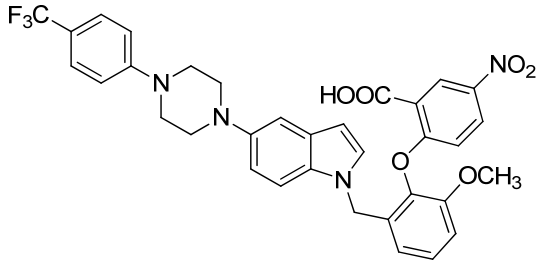
5.1.34.5. 2-(2-Methoxy-6-((5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indol-1-yl)methyl)phenoxy)-5-nitrobenzoic acid (30e)



Compound **30e** was prepared from **29e** using general procedure as described above as pale yellow solid; Yield: 90%; mp: 120-125 ^{0}C; Purity by UPLC: 97.49%; IR (KBr) 3435, 2926, 2841, 1710, 1612, 1518, 1481, 1450, 1344, 1232,

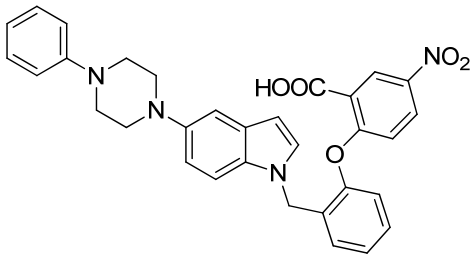
1166, 1122, 786, 727 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.14 (t, J = 4.8 Hz, 4H), 3.38 (t, J = 4 Hz, 4H), 3.69 (s, 3H), 5.26 (s, 2H), 6.24 (d, J = 3.2 Hz, 1H), 6.45 (d, J = 9.2 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 6.85-6.87 (dd, J = 2 and 8.8 Hz, 1H), 6.95 (d, J = 2 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.16-7.18 (dd, J = 0.8 and 8 Hz, 1H), 7.23-7.26 (m, 2H), 7.27-7.30 (m, 3H), 7.44 (t, J = 8.4 Hz, 1H), 8.06-8.09 (dd, J = 2.8 and 9.2 Hz, 1H), 8.51 (d, J = 2.8 Hz, 1H); ESI/MS m/z 647 $(M+H)^+$.

5.1.34.6. 2-(2-Methoxy-6-((5-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-1H-indol-1-yl)methyl)phenoxy)-5-nitrobenzoic acid (30f)



Compound **30f** was prepared from **29f** using general procedure as described above as pale yellow solid; Yield: 89%; mp: 200 ^0C (decomp.); Purity by HPLC: 97.55%; IR (KBr) 3085, 2933, 2835, 1701, 1681, 1616, 1521, 1481, 1452, 1334, 1282, 1230, 1164, 1072, 825, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.12 (bs, 4H), 3.41 (bs, 4H), 3.68 (s, 3H), 5.25 (s, 2H), 6.22 (d, J = 2.7 Hz, 1H), 6.44 (d, J = 9.2 Hz, 1H), 6.77-6.93 (m, 3H), 7.11-7.17 (bt, 3H), 7.23-7.27 (bt, 3H), 7.53 (d, J = 8.4 Hz, 2H), 8.05-8.09 (dd, J = 2.9 and 9.2 Hz, 1H), 8.51 (d, J = 2.8 Hz, 1H); ESI/MS m/z 645.2 (M-H).

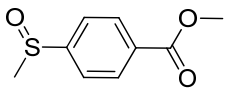
5.1.34.7. 5-Nitro-2-(2-((5-(4-phenylpiperazin-1-yl)-1H-indol-1-yl)methyl) phenoxy)benzoic acid (30g)



Compound **30g** was prepared from **29g** using general procedure as described above as pale yellow solid; Yield: 86%; mp: 110-114 ^{0}C; Purity by HPLC: 99.59%; IR (KBr) 3419, 2923, 2823, 1732, 1714, 1614, 1598, 1519, 1479, 1452, 1344, 1232, 1172, 1122, 754 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.15 (bs, 4H), 3.27 (bs, 4H), 5.33 (s, 2H), 6.28 (d, J = 3 Hz, 1H), 6.74-6.79 (m, 2H), 6.89 (d, J = 8.8 Hz, 1H), 6.98-7.10 (m, 5H), 7.20-7.35 (m, 6H), 8.18-8.22 (dd, J = 2.9 and 9.2 Hz, 1H), 8.55 (d, J = 2.8 Hz, 1H); ESI/MS m/z 549.1 (M+H)$^+$.

➢ Factor Xa inhibitors

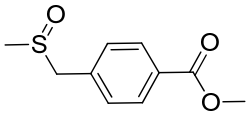
5.1.35. Methyl 4-(methylsulfinyl)benzoate (35a)



To a stirring solution of methyl 4-(methylthio)benzoate (**34a**) (10 g, 0.0548 mol) and V_2O_5 (100 mg, 0.0004 mol) in CH_3CN (10 v/w) cooled at -20 to -25 ^{0}C was added 50% H_2O2 (2.05 g, 0.0604 mol) drop wise in 20-30 min. under nitrogen atmosphere. The reaction mixture was stirred at 25-30 ^{0}C for 5 h. Reaction mixture was diluted with water (30 v/w) and extracted with ethyl acetate (30 v/w). Evaporation and drying over sodium sulfate afforded title compound **35a** (9.5 g,

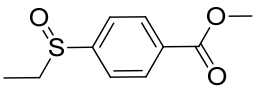
88%) as white solid: mp: 120-122 ^{0}C; Purity by HPLC: 94.19%; IR (KBr) 2991, 2952, 1718, 1595, 1429, 1400, 1373, 1274, 1045, 860, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.78 (s, 3H), 3.87 (s, 3H), 7.83 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 8.4 Hz, 2H); ESI/MS m/z 198.9 (M+H)$^+$.

5.1.36. Methyl 4-((methylsulfinyl)methyl)benzoate (35b)



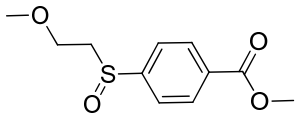
This compound was prepared from **34b** by means of a procedure similar to that reported for **35a**. Off-white solid; Yield: 52%; mp: 80-83 ^{o}C; Purity by HPLC: 95.35%; IR (KBr) 2999, 2949, 1718, 1610, 1575, 1508, 1434, 1280, 1182, 1031, 862, 709 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.48 (s, 3H), 3.84 (s, 3H), 4.03 (d, J = 12.8 Hz, 1H), 4.24 (d, J = 12.4 Hz, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.94-7.96 (dd, J = 1.6 and 6.4 Hz, 2H); ESI/MS m/z 212.8 (M+H)$^+$.

5.1.37. Methyl 4-(ethylsulfinyl)benzoate (35c)



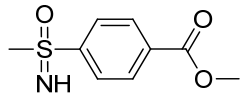
This compound was prepared from **34c** by means of a procedure similar to that reported for **35a**. Off-white solid; Yield: 80%; mp: 72-74 ^{o}C; Purity by HPLC: 91.35%; IR (KBr) 2952, 2933, 1718, 1595, 1488, 1436, 1398, 1278, 1045, 860, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.00 (t, 3H), 2.74-2.83 (m, 1H), 3.04-3.13 (m, 1H), 3.87 (s, 3H), 7.78 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 8.4 Hz, 2H); ESI/MS m/z 212.9 (M+H)$^+$.

5.1.38. Methyl 4-((2-methoxyethyl)sulfinyl)benzoate (35d)



This compound was prepared from **34d** by means of a procedure similar to that reported for **35a**. Off-white solid; Yield: 72%; mp: 60-63 ^{o}C; Purity by UPLC: 94.50%; IR (KBr) 2842, 1724, 1595, 1571, 1442, 1396, 1280, 1195, 1172, 1047, 860, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.94-3.0 (m, 1H), 3.19-3.22 (m, 1H), 3.24 (s, 3H), 3.53-3.58 (m, 1H), 3.68-3.74 (m, 1H), 3.87 (s, 3H), 7.82 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 8.4 Hz, 2H); ESI/MS m/z 242.9 $(M+H)^{+}$.

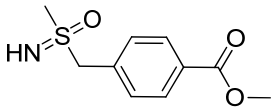
5.1.39. Methyl 4-(S-methylsulfonimidoyl)benzoate (36a)



To a stirring solution of **35a** (6.8 g, 0.034 mol) in $CHCl_3$ (10 v/w) was added NaN_3 (6.63 g, 0.102 mol) at 25 ^{0}C. To this was added drop wise H_2SO_4 (20 g, 0.204 mol) at -20 to -25 ^{0}C under N_2 atmosphere in 20-30 min. The reaction mixture was stirred at 25-30 ^{0}C for 12 h and then at 45-50 ^{0}C for 3 h. $CHCl_3$ was removed from reaction mixture and remaining residue was made alkaline by using aqueous K_2CO_3 solution. Ethyl acetate was added to it and organic layer was separated out. Drying over sodium sulfate and evaporation afforded title compound **36a** (6.75 g, 92%) as white solid: mp: 113-115 ^{0}C; Purity by HPLC: 97.48%; IR (KBr) 3280, 2999, 2923, 1703, 1595, 1573, 1438, 1398, 1298, 1220, 1097, 1008, 856, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.10 (s, 3H), 3.89

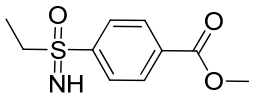
(s, 3H), 4.42 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H); ESI/MS m/z 213.9 $(M+H)^+$.

5.1.40. Methyl 4-((S-methylsulfonimidoyl)methyl)benzoate (36b)



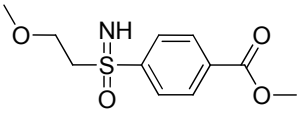
This compound was prepared from **35b** by means of a procedure similar to that reported for **36a**. Off-white solid; Yield: 65%; mp: 131-133 °C; Purity by HPLC: 91.68%; IR (KBr) 3255, 2956, 2914, 1718, 1612, 1573, 1510, 1436, 1417, 1280, 864, 705 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.77 (s, 3H), 3.72 (s, 1H), 3.84 (s, 3H), 4.42-4.50 (q, *J* = 12.8 Hz, 2H), 7.57 (d, *J* = 8 Hz, 2H), 7.94-7.96 (dd, *J* = 2.0 and 6.8 Hz, 2H); ESI/MS m/z 227.7 $(M+H)^+$.

5.1.41. Methyl 4-(S-ethylsulfonimidoyl)benzoate (36c)



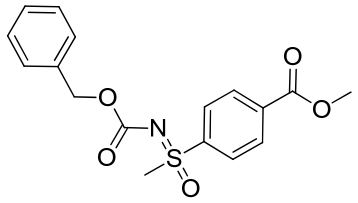
This compound was prepared from **35c** by means of a procedure similar to that reported for **36a**. Off-white solid; Yield: 80%; mp: 77-80 °C; Purity by HPLC: 93.33%; IR (KBr) 3267, 2956, 2916, 1720, 1593, 1571, 1434, 1396, 1296, 1217, 972, 866, 731 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.04 (t, *J* = 7.2 Hz, 3H), 3.13-3.18 (q, *J* = 7.2 Hz, 2H), 3.88 (s, 3H), 4.40 (s, 1H), 8.01(d, *J* = 8.4 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H); ESI/MS m/z 228 $(M+H)^+$.

5.1.42. Methyl 4-(S-2-methoxyethylsulfonimidoyl)benzoate (HCl salt) (36d)



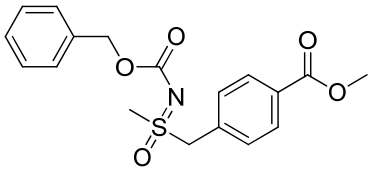
This compound was prepared from **35d** by means of a procedure similar to that reported for **36a**. Crude product was stirred with HCl:diethylether to obtained hydrochloride salt of title compound as off-white solid; Yield: 67%; mp: 143-145 ^{o}C; Purity by UPLC: 95.16%; IR (KBr) 3441, 1732, 1573, 1550, 1438, 1282, 1114, 864, 734 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.99 (s, 3H), 3.65-3.69 (m, 2H), 3.90 (s, 3H), 4.06-4.13 (m, 2H), 8.13-8.26 (m, 4H); ESI/MS m/z 257.9 $(M+H)^{+}$.

5.1.43. Methyl 4-(N-((benzyloxy)carbonyl)-S-methylsulfonimidoyl)benzoate (37a)



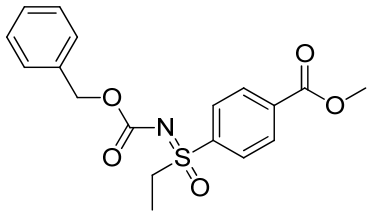
To a stirring solution of **36a** (2 g, 0.0093 mol) in CH_2Cl_2 (5 v/w) was added pyridine (1.1 g, 0.0139 mol). To this was added benzyloxy carbonyl chloride (50% in toluene) (1.9 g, 0.0116 mol) at 15-20 ^{0}C under nitrogen atmosphere. The reaction mixture was stirred at 25-30 ^{0}C for 3 h and then diluted with CH_2Cl_2 (10 v/w). Organic layer was washed with water and evaporated after drying over Na_2SO_4 to get title compound **37a** (3.1 g, 95%) as off-white solid: mp: 141-143 ^{0}C; Purity by UPLC: 98.13%; IR (KBr) 1718, 1668, 1573, 1496, 1286, 1255, 1219, 1085, 896, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.52 (s, 3H), 3.90 (s, 3H), 4.88-4.97 (q, J = 12 Hz, 2H), 7.19-7.21 (m, 2H), 7.27-7.33 (m, 3H), 8.06-8.09 (dd, J = 2 and 6.8 Hz, 2H), 8.16-8.18 (dd, J = 2 and 6.8 Hz, 2H); ESI/MS m/z 369.9 $(M+Na)^{+}$.

5.1.44. Methyl 4-((N-((benzyloxy)carbonyl)-S-methylsulfonimidoyl) methyl) benzoate (37b)



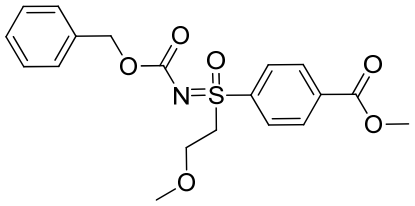
This compound was prepared from **36b** by means of a procedure similar to that reported for **37a**; Yield: 85%; mp: 116-118 ^{o}C; Purity by HPLC: 96.13%; IR (KBr) 1720, 1666, 1610, 1573, 1500, 1288, 1209, 1114, 869, 785 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.19 (s, 3H), 3.85 (s, 3H), 4.97-5.06 (m, 4H), 7.30-7.38 (m, 5H), 7.54 (d, J = 8 Hz, 2H), 7.95 (d, J = 8.4 Hz, 2H); ESI/MS m/z 384 $(M+Na)^{+}$.

5.1.45. Methyl 4-(N-((benzyloxy)carbonyl)-S-ethylsulfonimidoyl)benzoate (37c)



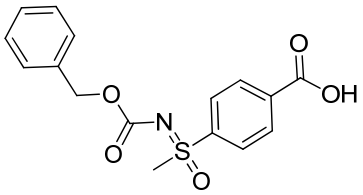
This compound was prepared from **36c** by means of a procedure similar to that reported for **37a**; Yield: 82%; Purity by HPLC: 94.98%; IR (KBr) 2976, 2943, 1720, 1658, 1575, 1498, 1382, 1271, 1087, 860, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.09 (t, J = 7.2 Hz, 3H), 3.58-3.69 (m, 2H), 3.91 (s, 3H), 4.87-4.97 (q, J = 12.4 Hz, 2H), 7.18-7.20 (m, 2H), 7.28-7.37 (m, 3H), 8.03 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H); ESI/MS m/z 362 $(M+H)^{+}$.

5.1.46. Methyl 4-(N-((benzyloxy)carbonyl)-S-2-methoxyethylsulfonimidoyl) benzoate (37d)



This compound was prepared from **36d** by means of a procedure similar to that reported for **37a**; Yield: 88%; mp: 95-97 ^{o}C; Purity by UPLC: 92.66%; IR (KBr) 3020, 1728, 1678, 1517, 1400, 1215, 1116, 759, 669 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.98 (s, 3H), 3.60-3.67 (m, 2H), 3.91 (s, 3H), 3.94-3.99 (m, 2H), 4.89-4.98 (q, J = 12.4 Hz, 2H), 7.19-7.21 (m, 2H), 7.27-7.30 (m, 3H), 8.02-8.04 (dd, J = 2.0 and 6.8 Hz, 2H), 8.13-8.15 (dd, J = 2.0 and 6.8 Hz, 2H); ESI/MS m/z 391.8 $(M+H)^{+}$.

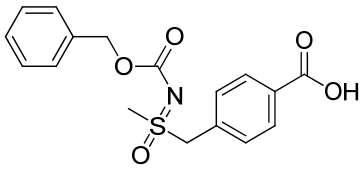
5.1.47. 4-(N-((Benzyloxy)carbonyl)-S-methylsulfonimidoyl)benzoic acid (38a)



To a stirring solution of NaOH (0.518 g, 0.01296 mol) in the solvent mixture of water (15 ml) and THF (15 ml) was added **37a** (3 g, 0.00864 mol). Reaction mixture was stirred at 25-30 ^{0}C for 3 h and then diluted with water (10 v/w). Aqueous layer was washed with methyl tert-butyl ether (10 v/w). Aqueous layer was then cooled to 0 ^{0}C and acidified with diluted HCl. Extracted with ethyl acetate, which on drying over sodium sulfate and evaporation afforded title

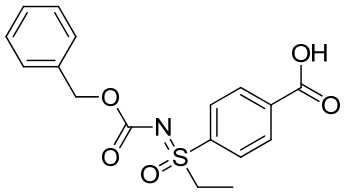
compound (2.6 g, 90%) as off-white solid: mp: 172-174 ^{0}C; Purity by HPLC: 95.20%; IR (KBr) 3435, 1693, 1664, 1602, 1575, 1375, 1272, 1228, 1087, 746, 698 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.51 (s, 3H), 4.89-4.97 (q, J = 12.4 Hz, 2H), 7.19-7.21 (m, 2H), 7.25-7.33 (m, 3H), 8.06 (d, J = 8.8 Hz, 2H), 8.16 (d, J = 8.4 Hz, 2H), 13.56 (bs, 1H); ESI/MS m/z 333.8 $(M+H)^+$.

5.1.48. 4-((N-((Benzyloxy)carbonyl)-S-methylsulfonimidoyl)methyl)benzoic acid (38b)



This compound was prepared from **37b** by means of a procedure similar to that reported for **38a**; Yield: 82%; mp: 180-183 ^{o}C; Purity by HPLC: 95.55%; IR (KBr) 3444, 1676, 1641, 1427, 1382, 1259, 1103, 790, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.19 (s, 3H), 4.96 (s, 2H), 4.98-5.06 (q, J = 12.4 Hz, 2H), 7.30-7.38 (m, 5H), 7.50 (d, J = 8 Hz, 2H), 7.93 (d, J = 8 Hz, 2H); ESI/MS m/z 370 $(M+Na)^+$.

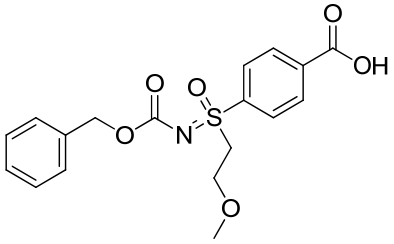
5.1.49. 4-(N-((Benzyloxy)carbonyl)-S-ethylsulfonimidoyl)benzoic acid (38c)



This compound was prepared from **37c** by means of a procedure similar to that reported for **38a**; Yield: 84%; Purity by HPLC: 97.91%; IR (KBr) 3435, 1720, 1633, 1500, 1456, 1392, 1294, 1228, 1085, 902, 734 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.07 (t, J = 7.6 Hz, 3H), 3.58-3.67 (m, 2H), 4.88-4.98 (q, J = 12.4

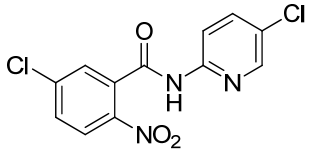
Hz, 2H), 7.18-7.20 (dd, *J* = 2.0 and 7.6 Hz, 2H), 7.25-7.32 (m, 3H), 7.98 (d, *J* = 8.4 Hz, 2H), 8.15 (d, *J* = 8.8 Hz, 2H); ESI/MS m/z 347.9 $(M+H)^+$.

5.1.50. 4-(N-((Benzyloxy)carbonyl)-S-2-methoxyethylsulfonimidoyl)benzoic acid (38d)



This compound was prepared from **37d** by means of a procedure similar to that reported for **38a** as oily compound; Yield: 73%; Purity by UPLC: 97.37%; IR (KBr) 3433, 1718, 1637, 1500, 1388, 1286, 1230, 1105, 1082, 898, 796, 731 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.00 (s, 3H), 3.57-3.68 (m, 2H), 3.90-4.01 (m, 2H), 4.90-4.98 (q, *J* = 12.4 Hz, 2H), 7.20-7.32 (m, 5H), 8.02 (d, *J* = 8.4 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H); ESI/MS m/z 377.8 $(M+H)^+$.

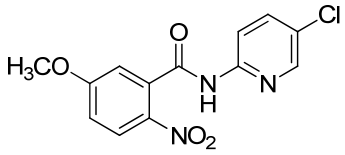
5.1.51. 5-Chloro-N-(5-chloropyridin-2-yl)-2-nitrobenzamide (41a)



To a stirring solution of **39a** (5 g, 0.02487 mol) and 2-amino-5-chloro pyridine (**40**) (3.2 g, 0.02487 mol) in CH_3CN (20 mL) was added pyridine (5.9 g, 0.0746 mol) at 25-30 ^0C. The reaction mixture was then cooled to 0-10 ^0C under N_2 atmosphere. To this was added $POCl_3$ (4.57 g, 0.0298 mol) drop wise by maintaining exothermicity. After stirring at 25-30 ^0C for 1 h, reaction mixture was poured in cooled water and filtered. Solid obtained was stirred in saturated solution of $NaHCO_3$ for 10 min. Filtration and drying afforded title compound **41a**

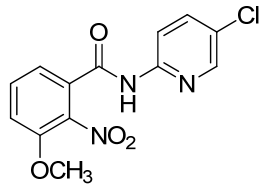
(7 g, 90%) as pale yellow solid: mp: 182-183 ^{0}C; Purity by HPLC: 96.28%; IR (KBr) 1691, 1575, 1525, 1461, 1375, 1299, 1114, 1016, 914, 842, 761, 678 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.80-7.83 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (s, 1H), 7.96-7.99 (dd, J = 2.4 and 8.8 Hz, 1H), 8.16-8.19 (dd, J = 4.0 and 8.8 Hz, 2H), 8.41 (s, 1H), 11.43 (s, 1H); ESI/MS m/z 309.8 (M-H).

5.1.52. N-(5-Chloropyridin-2-yl)-5-methoxy-2-nitrobenzamide (41b)



This compound was prepared from **39b** by means of a procedure similar to that reported for **41a** as off-white solid; Yield: 94%; mp: 155-158 ^{0}C; Purity by UPLC: 97.65%; IR (KBr) 1691, 1579, 1517, 1461, 1377, 1234, 1110, 1070, 835, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.91 (s, 3H), 7.19-7.24 (m, 2H), 7.96-7.99 (dd, J = 2.4 and 8.8 Hz, 1H), 8.16-8.22 (m, 2H), 8.40 (d, J = 1.6 Hz, 1H), 11.30 (s, 1H); ESI/MS m/z 329.8 (M+Na)$^+$.

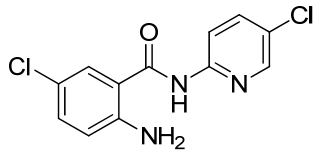
5.1.53. N-(5-Chloropyridin-2-yl)-3-methoxy-2-nitrobenzamide (41c)



This compound was prepared from **39c** by means of a procedure similar to that reported for **41a** as off white solid; Yield: 97%; mp: 198-200 ^{0}C; Purity by UPLC: 99.22%; IR (KBr) 1691, 1577, 1537, 1473, 1375, 1307, 1276, 1058, 852, 792 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.92 (s, 3H), 7.44 (d, J = 7.6 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.66 (t, J = 8 Hz, 1H), 7.94-7.96 (dd, J = 2.4 and 8.8 Hz, 1H),

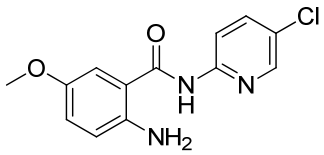
8.07 (d, *J* = 8.8 Hz, 1H), 8.44 (d, *J* = 2.4 Hz, 1H), 11.47 (s, 1H); ESI/MS m/z 306.7 (M-H).

5.1.54. 2-Amino-5-chloro-N-(5-chloropyridin-2-yl)benzamide (42a)



To a stirring solution of **41a** (5 g, 0.016 mol) in ethyl acetate (50 ml) was added stannous chloride dihydrate ($SnCl_2$:H_2O, 18 g, 0.08 mol) at 25-30 ^{0}C. After stirring at same temperature for 2 h, reaction mixture was diluted with ethyl acetate (10 v/w) and made alkaline with aqueous ammonia solution. Reaction mixture was then filtered through hyflow bed and organic layer was dried over Na_2SO_4. Evaporation of solvent afforded **42a** (3.2 g, 71%) as pale yellow solid; mp: 182-184 ^{0}C; Purity by UPLC: 99.22%; IR (KBr) 3489, 3377, 1658, 1614, 1571, 1373, 1294, 1087, 850, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.54 (s, 2H), 6.78 (d, *J* = 8.8 Hz, 1H), 7.20-7.23 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.76 (d, *J* = 2.4 Hz, 1H), 7.90-7.93 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.08-8.10 (dd, *J* = 0.4 and 8.8 Hz, 1H), 8.412-8.419 (dd, *J* = 0.4 and 2.8 Hz, 1H), 10.78 (s, 1H); ESI/MS m/z 303.7 $(M+Na)^+$.

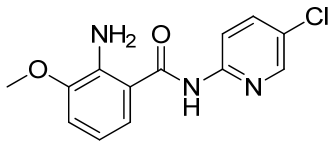
5.1.55. 2-Amino-N-(5-chloropyridin-2-yl)-5-methoxybenzamide (42b)



This compound was prepared from **41b** by means of a procedure similar to that reported for **42a** as off-white solid; Yield: 75%; mp: 69-71 ^{0}C; Purity by UPLC:

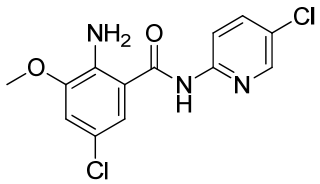
99.16%; IR (KBr) 3462, 3367, 1658, 1593, 1571, 1514, 1375, 1298, 1159, 1091, 856, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.72 (s, 3H), 6.01 (s, 2H), 6.73 (d, *J* = 8.8 Hz, 1H), 6.87-6.90 (dd, *J* = 2.8 and 8.8 Hz, 1H), 7.20 (d, *J* = 2.8 Hz, 1H), 7.90-7.93 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.11-8.14 (dd, *J* = 0.4 and 8.8 Hz, 1H), 8.40-8.41 (dd, *J* = 0.8 and 2.8 Hz, 1H), 10.73 (s, 1H); ESI/MS m/z 277.8 $(M+H)^+$.

5.1.56. 2-Amino-N-(5-chloropyridin-2-yl)-3-methoxybenzamide (42c)



This compound was prepared from **41c** by means of a procedure similar to that reported for **42a** as off-white solid; Yield: 60%; mp: 137-139 ^0C; Purity by UPLC: 98.34%; IR (KBr) 3493, 3367, 1658, 1612, 1587, 1552, 1500, 1373, 1226, 1080, 846, 740 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.80 (s, 3H), 6.14 (s, 2H), 6.55 (t, *J* = 8 Hz, 1H), 6.96 (t, *J* = 6.8 Hz, 1H), 7.37-7.40 (dd, *J* = 1.2 and 8.4 Hz, 1H), 7.90-7.93 (dd, *J* = 2.8 and 8.8 Hz, 1H), 8.10-8.12 (dd, *J* = 0.4 and 9.2 Hz, 1H), 8.40-8.41 (dd, *J* = 0.8 and 2.8 Hz, 1H), 10.54 (s, 1H); ESI/MS m/z 277.9 $(M+H)^+$.

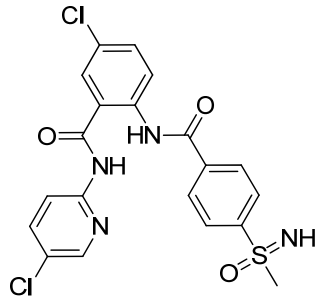
5.1.57. 2-Amino-5-chloro-N-(5-chloropyridin-2-yl)-3-methoxybenzamide (42d)



To a stirring solution of **42c** (15.5 g, 0.0558 mol) in benzene (155 mL) was added NCS (8.17 g, 0.0614 mol) at 25-30 ^0C. Reaction mixture was then heated at 60-

65 ^{0}C for 24 h. Excess solvent was removed under vacuum. Water was added to it and product was extracted with ethyl acetate. Ethyl acetate was distilled out under vacuum to get **42d** (12.3 g, 71%) as off-white solid: mp: 116-118 ^{0}C; Purity by UPLC: 98.15%; IR (KBr) 3485, 3367, 1712, 1666, 1593, 1573, 1519, 1375, 1303, 1238, 1051, 833, 740 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.83 (s, 3H), 6.24 (s, 2H), 6.99 (d, J = 2 Hz, 1H), 7.47 (d, J = 2 Hz, 1H), 7.91-7.94 (dd, J = 2.4 and 8.8 Hz, 1H), 8.07-8.09 (dd, J = 0.8 and 9.2 Hz, 1H), 8.41-8.42 (dd, J = 0.8 and 2.8 Hz, 1H), 10.73 (s, 1H); ESI/MS m/z 312 $(M+H)^+$.

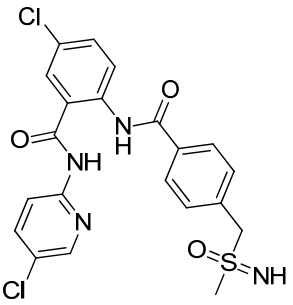
5.1.58. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methylsulfonimidoyl) benzamido) benzamide (43a)



To a stirring solution of **38a** (2 g, 0.0060 mol) and DMF (one drop) in CH_2Cl_2 (10 v/w) was added oxalyl chloride (0.91 g, 0.0072 mol) at 10-15 ^{0}C under N_2 atmosphere. Reaction mixture was stirred at 25-30 ^{0}C for 3 h and then evaporated to dryness. Acid chloride obtained was dissolved in dry THF (8 mL) and was added to a solution containing **42a** (1.52 g, 0.0054 mol) in THF (6 mL) at 10-15 ^{0}C. After stirring at 25-30 ^{0}C for 2 h, reaction mixture was quenched with water (10 v/w). Product was extracted with $CHCl_3$ which on drying over Na_2SO_4, evaporation gave solid product.

The crude product obtained above was added to H_2SO_4 (5 v/w) cooled to 0-5 ^{0}C. The reaction mixture was stirred at same temperature for 15-20 min. Above reaction mixture was slowly poured in chilled water and basified using aqueous K_2CO_3 solution. Precipitated product was filtered and washed with water. Drying afforded **43a** (1 g, 64%) as off-white solid: mp: 239-242 ^{0}C; Purity by UPLC: 98.19%; IR (KBr) 3315, 1685, 1651, 1602, 1573, 1519, 1460, 1371, 1292, 1217, 1030, 1006, 833, 746, 677 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.11 (s, 3H), 4.40 (s, 1H), 7.66-7.69 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.92 (d, *J* = 2.4 Hz, 1H), 7.94-7.97 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.07 (bs, 4H), 8.12 (d, *J* = 4.4 Hz, 1H), 8.14 (d, *J* = 4.4 Hz, 1H), 8.44 (d, *J* = 2.4 Hz, 1H), 11.19 (s, 1H), 11.27 (s, 1H); ESI/MS m/z: 462.6 $(M+H)^+$.

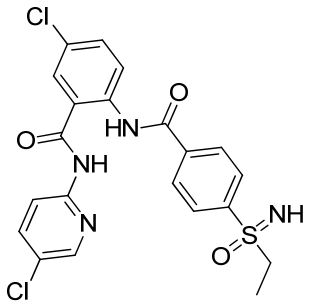
5.1.59. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-((S-methylsulfonimidoyl) methyl) benzamido) benzamide (43b)



This compound was prepared from **38b** and **42a** by means of a procedure similar to that reported for **43a** as off-white solid; Yield: 63%; mp: 217-219 ^{0}C; Purity by HPLC: 95.07%; IR (KBr) 3178, 1674, 1652, 1602, 1575, 1521, 1460, 1375, 1296, 1209, 1035, 831, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.78 (s, 3H), 3.69 (s, 1H), 4.41-4.50 (q, *J* = 13.6 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.64-7.67 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.89 (d, *J* = 8 Hz, 2H), 7.91-7.95 (m, 2H), 8.11 (d, *J* = 8

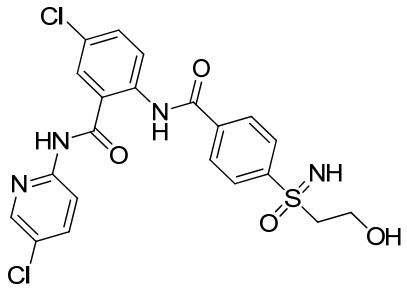
Hz, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 8.43 (d, *J* = 2.4 Hz, 1H), 11.18 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 499 $(M+Na)^+$.

5.1.60. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-ethylsulfonimidoyl) benzamido) benzamide (43c)



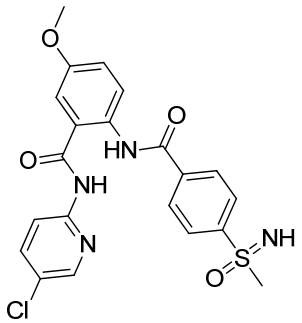
This compound was prepared from **38c** and **42a** by means of a procedure similar to that reported for **43a** as off-white solid; Yield: 67%; mp: 213-215 ^{0}C; Purity by HPLC: 97.47%; IR (KBr) 3259, 1735, 1666, 1602, 1583, 1571, 1517, 1438, 1373, 1218, 1002, 829, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.05 (t, *J* = 7.2 Hz, 3H), 3.14-3.19 (q, *J* = 7.2 Hz, 2H), 4.37 (s, 1H), 7.65-7.68 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.92-7.95 (dd, *J* = 2.8 and 8.8 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 2H), 8.07 (d, *J* = 8.4 Hz, 2H), 8.09-8.12 (dd, *J* = 2.4 and 8.8 Hz, 2H), 8.43 (d, *J* = 2.8 Hz, 1H), 11.17 (s, 1H), 11.25 (s, 1H); ESI/MS m/z 477 $(M+H)^+$.

5.1.61. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-2-hydroxyethyl sulfonimidoyl) benzamido) benzamide (43d)



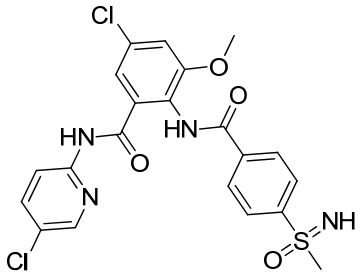
This compound was prepared from **38d** and **42a** by means of a procedure similar to that reported for **43a**. Deprotection of Cbz group and methoxy group was achieved as follows: To a stirring solution of 2-(4-(N-benzyloxycarbonyl-2-methoxyethylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl) benzamide (300 mg, 0.00046 mol) in CH_2Cl_2 (3 mL) was added BBr_3 (0.577 g. 0.0023 mol) under N_2 atmosphere at -30 ^{0}C. Reaction mixture was stirred at 25-30 ^{0}C for 3 h. Reaction mixture was diluted with water and basified with aq. Na_2CO_3 solution. Organic layer was separated, dried and distilled out to get crude product, which was column purified using 100-200 silica gel and 2% MeOH in $CHCl_3$ as mobile phase producing title compound as off-white solid; Yield: 28%; mp: 207-210 ^{0}C; Purity by UPLC: 93.94%; IR (KBr) 3019, 1731, 1672, 1601, 1584, 1513, 1403, 1375, 1215, 1024, 757, 699 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.33 (t, J = 6.4 Hz, 2H), 3.62-3.67 (m, 2H), 4.47 (s, 1H), 4.79 (t, 1H), 7.65-7.68 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93-7.95 (dd, J = 2.4 and 8.8 Hz, 1H), 8.01-8.04 (m, 4H), 8.10-8.12 (dd, J = 2.8 and 8.8 Hz, 2H), 8.43 (d, J = 2.4 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 492.6 $(M+H)^+$.

5.1.62. N-(5-Chloropyridin-2-yl)-5-methoxy-2-(4-(S-methylsulfonimidoyl) benzamido) benzamide (43e)



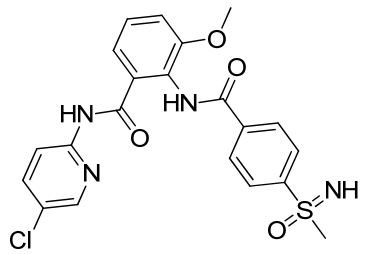
This compound was prepared from **38a** and **42b** by means of a procedure similar to that reported for **43a** as off-white solid; Yield: 61%; mp: 247-250 ^{0}C; Purity by UPLC: 95.78%; IR (KBr) 3435, 3288, 1676, 1651, 1608, 1523, 1458, 1373, 1298, 1218, 1072, 835, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.10 (s, 3H), 3.84 (s, 3H), 7.16-7.19 (dd, J = 2.8 and 9.2 Hz, 1H), 7.40 (d, J = 2.8 Hz, 1H), 7.92-7.97 (m, 2H), 8.04 (s, 4H), 8.14 (d, J = 8.8 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 10.97 (s, 1H), 11.11 (s, 1H); ESI/MS m/z 458.9 $(M+H)^+$.

5.1.63. 5-Chloro-N-(5-chloropyridin-2-yl)-3-methoxy-2-(4-(S methyl sulfonimidoyl) benzamido)-benzamide (43f)



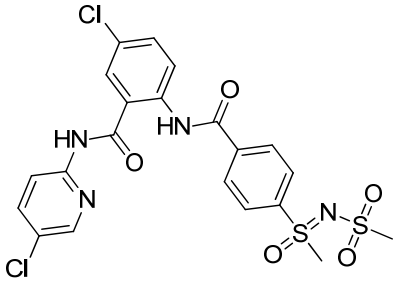
This compound was prepared from **38a** and **42d** by means of a procedure similar to that reported for **43a** as off-white solid; Yield: 75%; mp: 188-190 ^{0}C; Purity by UPLC: 97.66%; IR (KBr) 3269, 1664, 1577, 1458, 1375, 1307, 1228, 1064, 839, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.08 (s, 3H), 3.84 (s, 3H), 4.36 (s, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.85-7.88 (dd, J = 2.4 and 8.8 Hz, 1H), 7.98-8.06 (m, 5H), 8.36 (d, J = 2.4 Hz, 1H), 9.98 (s, 1H), 10.87 (s, 1H); ESI/MS m/z 492.6 $(M+H)^+$.

5.1.64. N-(5-Chloropyridin-2-yl)-3-methoxy-2-(4-(S-methylsulfonimidoyl) benzamido) benzamide (43g)



This compound was prepared from **38a** and **42c** by means of a procedure similar to that reported for **43a** as off-white solid; Yield: 65%; mp: 185-187 ^{0}C; Purity by UPLC: 98.82%; IR (KBr) 3277, 1662, 1583, 1518, 1469, 1375, 1266, 1062, 1004, 833, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.08 (s, 3H), 3.82 (s, 3H), 4.36 (s, 1H), 7.26 (d, J = 8 Hz, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 8 Hz, 1H), 7.85-7.88 (dd, J = 2.8 and 9.2 Hz, 1H), 7.99-8.10 (m, 5H), 8.35 (d, J = 2.8 Hz, 1H), 9.92 (s, 1H), 10.62 (s, 1H); ESI/MS m/z 458.8 $(M+H)^+$.

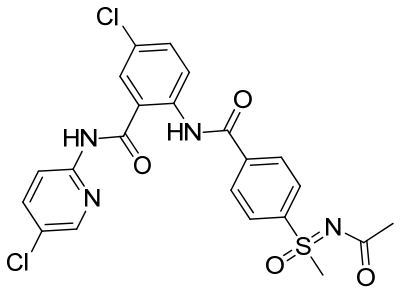
5.1.65. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(methylsulfonyl) sulfonimidoyl) benzamido)benzamide (44a)



To a stirring solution of **43a** (0.5 g, 0.0010 mol) and pyridine (0.12 g, 0.0015 mol) in CH_2Cl_2 (3 mL) was added CH_3SO_2Cl (0.137 g, 0.0012 mol) at 10-15 ^{0}C under N_2 atmosphere and stirred at 25-30 ^{0}C for 3 h. Water (10 v/w) was added to it and product was extracted with CH_2Cl_2, which on drying over Na_2SO_4 and

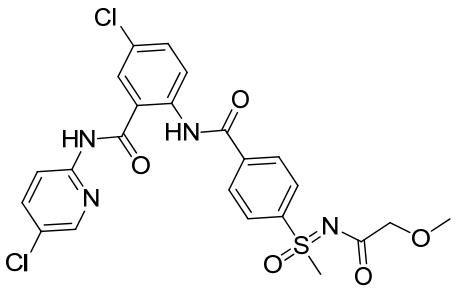
evaporation gave solid product. Product obtained was column purified using ethyl acetate:hexane mobile phase (0-60%) and 100-200 silica gel to get title compound as off-white solid; Yield: 51%; mp: 220-222 ^{0}C; Purity by UPLC: 98.96%; IR (KBr) 3342, 1676, 1653, 1599, 1502, 1460, 1371, 1307, 1072, 848, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.04 (s, 3H), 3.66 (s, 3H), 7.67-7.70 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.8 and 8.8 Hz, 1H), 8.08-8.19 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.21 (s, 1H), 11.27 (s, 1H); ESI/MS m/z: 540.9 $(M+H)^+$.

5.1.66. 2-(4-(N-Acetyl-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl) benzamide (44b)



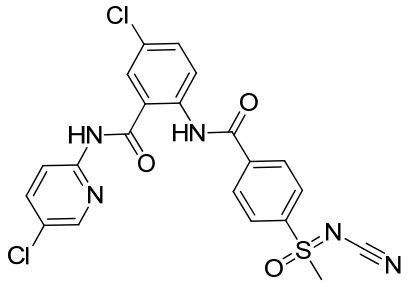
This compound was prepared from **43a** and acetyl chloride by means of a procedure similar to that reported for **44a** as off-white solid; Yield: 61%; mp: 215-217 ^{0}C; Purity by UPLC: 96.00%; IR (KBr) 3431, 1637, 1602, 1575, 1512, 1460, 1373, 1296, 1217, 1033, 831, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.97 (s, 3H), 3.46 (s, 3H), 7.67-7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.4 and 8 Hz, 1H), 8.09-8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.19 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 503.1 (M-H).

5.1.67. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-methoxyacetyl)-S-methylsulfonimidoyl) benzamido)benzamide (44c)



This compound was prepared from **43a** and methoxyacetyl chloride by means of a procedure similar to that reported for **44a** as off-white solid; Yield: 69%; mp: 206-208 ^{0}C; Purity by UPLC: 94.98%; IR (KBr) 2926, 2823, 1666, 1604, 1573, 1516, 1462, 1373, 1296, 1217, 1116, 922, 837, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.26 (s, 3H), 3.52 (s, 3H), 3.94 (d, J = 2.8 Hz, 2H), 7.66-7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.0 Hz, 1H), 7.93-7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.09-8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.20 (s, 1H), 11.25 (s, 1H); ESI/MS m/z 532.9 (M-H).

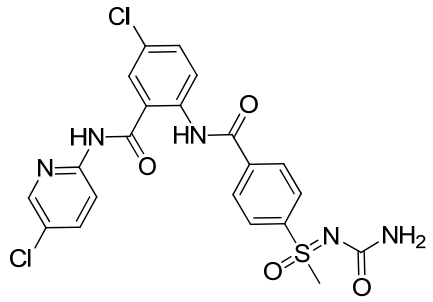
5.1.68. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-cyano-S-methyl sulfonimidoyl) benzamido) benzamide (44d)



To a stirring solution of **43a** (200 mg, 0.000432 mol) in CH_2Cl_2 (3 mL) was added catalytic amount of DMAP followed by BrCN (58 mg, 0.000476 mol) under N_2 atmosphere. Reaction mixture was stirred at 25-30 ^{0}C for 6 h and then quenched

with water (10 v/w). Product was extracted with CH_2Cl_2 which on drying over Na_2SO_4, evaporation gave solid product. Product obtained was column purified using ethyl acetate:hexane mobile phase (0-40%) and 100-200 silica gel to get off-white solid; Yield: 57%; mp: 166-168 ^{0}C; Purity by UPLC: 96.44%; IR (KBr) 2195, 1710, 1676, 1654, 1602, 1516, 1460, 1375, 1246, 1114, 827, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.80 (s, 3H), 7.67-7.70 (dd, J = 2.4 and 8.4 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.8 and 8.8 Hz, 1H), 8.08 (d, J = 8.8 Hz, 1H), 8.14 (d, J = 8.8 Hz, 1H), 8.19-8.24 (m, 4H), 8.44 (d, J =2.4 Hz 1H), 11.21 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 487.8 $(M+H)^+$.

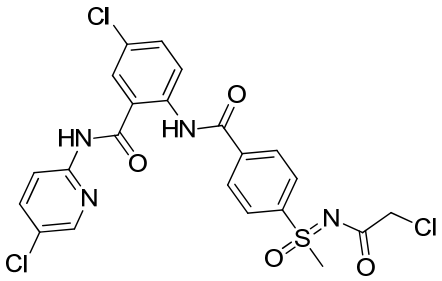
5.1.69. 2-(4-(N-Carbamoyl-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl)benzamide (44e)



44d (100 mg, 0.000205 mol) was added to H_2SO_4 (2 ml) at 10-15 ^{o}C. Reaction mixture was stirred at 25-30 ^{0}C for 6 h and then quenched with water (10 v/w). Solid obtained was filtered, washed with water and dried to get off-white solid; Yield: 45%; mp: 205-208 ^{0}C; Purity by HPLC: 96.78%; IR (KBr) 3423, 1654, 1600, 1577, 1510, 1460, 1375, 1298, 1226, 1118, 835, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.37 (s, 3H), 6.05 (bs, 1H), 6.45 (bs, 1H), 7.65-7.68 (dd, J = 2.4 and 8.4 Hz, 1H), 7.91 (d, J = 2.8 Hz, 1H), 7.92-7.95 (dd, J = 2.4 and 8.8

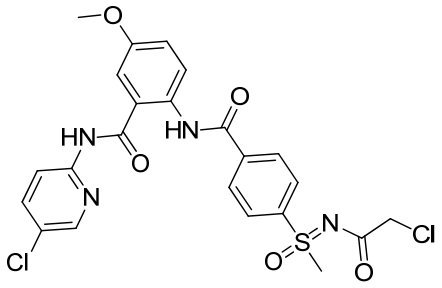
Hz, 1H), 8.03-8.11 (m, 5H), 8.13 (d, *J* = 4.4 Hz, 1H), 8.43 (d, *J* = 0.4 Hz, 1H), 11.19 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 527.8 $(M+Na)^+$.

5.1.70. 5-Chloro-2-(4-(N-(2-chloroacetyl)-S-methylsulfonimidoyl) benzamido)-N-(5-chloropyridin-2-yl)benzamide (45a)



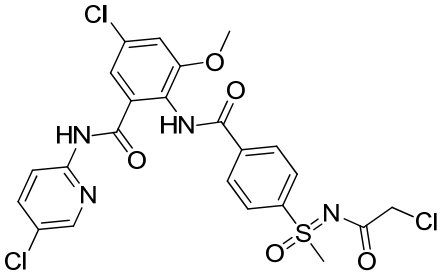
To a stirring solution of **43a** (2 g, 0.0042 mol) and TEA (1.06 g, 0.0105 mol) in THF (20 ml) was added chloroacetyl chloride (0.71 g, 0.0063 mol) at 0 ^{0}C. Reaction mixture was stirred at 25-30 ^{o}C for 2 h. Reaction mixture was diluted with water (10 v/w). Precipitated product was filtered and purified by refluxing in solvent mixture of 6 v/w hexane and 3 v/w ethyl acetate. Filtration at 25-30 ^{o}C afforded **45a** (1.86 g, 80%) as off-white solid; mp: 190-193 ^{0}C; Purity by UPLC: 95.83%; IR (KBr) 1687, 1660, 1602, 1573, 1516, 1460, 1404, 1375, 1298, 1209, 1008, 839, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.57 (s, 3H), 4.27 (s, 2H), 7.67-7.69 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.94-7.96 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.08-8.13 (m, 6H), 8.44 (d, *J* = 2.8 Hz, 1H), 11.19 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 538.9 $(M+H)^+$.

5.1.71. 2-(4-(N-(2-Chloroacetyl)-S-methylsulfonimidoyl)benzamido)-N-(5-chloropyridin-2-yl)-5-methoxybenzamide (45e)



This compound was prepared from **43e** by means of a procedure similar to that reported for **45a** as off-white solid; Yield: 80%; mp: 178-180 ^{0}C; Purity by UPLC: 98.74%; IR (KBr) 1681, 1652, 1610, 1573, 1515, 1461, 1373, 1207, 1028, 839, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.56 (s, 3H), 3.84 (s, 3H), 4.26 (s, 2H), 7.16-7.19 (dd, J = 2.8 and 9.2 Hz, 1H), 7.39 (d, J = 2.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.93-7.95 (dd, J = 2.8 and 5.6 Hz, 1H), 8.10-8.14 (m, 5H), 8.42 (d, J = 2.4 Hz, 1H), 10.97 (s, 1H), 11.11 (s, 1H); ESI/MS m/z 557.1 $(M+Na)^+$.

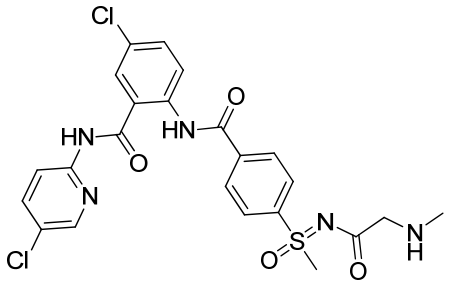
5.1.72. 5-Chloro-2-(4-(N-(2-chloroacetyl)-S-methylsulfonimidoyl) benzamido)-N-(5-chloropyridin-2-yl)-3-methoxybenzamide (45f)



This compound was prepared from **43f** by means of a procedure similar to that reported for **45a** as off-white solid; Yield: 82%; mp: 143-145 ^{0}C; Purity by UPLC: 90%; IR (KBr) 1656, 1573, 1521, 14601, 1375, 1315, 1226, 1064, 842, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.54 (s, 3H), 3.85 (s, 3H), 4.26 (s, 2H), 7.29 (d,

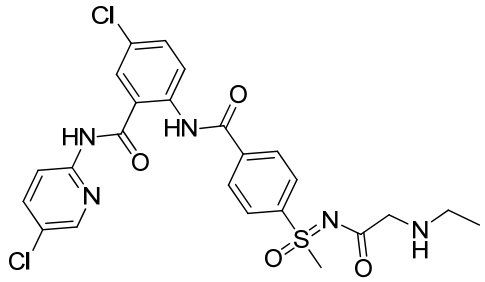
J = 2 Hz, 1H), 7.38 (d, *J* = 2 Hz, 1H), 7.85-7.88 (dd, *J* = 2.8 and 8.8 Hz, 1H), 8.05-8.10 (m, 5H), 8.36 (d, *J* = 2.8 Hz, 1H), 10.09 (s, 1H), 10.89 (s, 1H); ESI/MS m/z 592.6 $(M+Na)^+$.

5.1.73. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(methylamino) acetyl) sulfonimidoyl) benzamido)benzamide (46a)



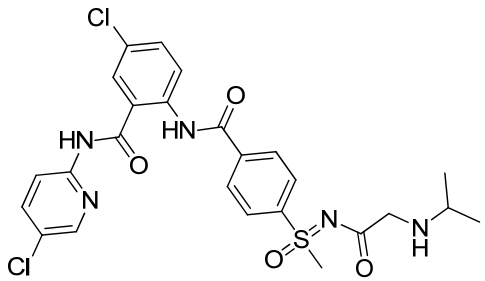
To a stirring solution of **45a** (1 g, 0.0018 mol) in DMF (5 ml) was added 40% aq. Methylamine solution (0.576 g, 0.0185 mol) followed by catalytic amount of KI. The reaction mixture was stirred at 25-30 ^{0}C for 12 h and then diluted with water. Filtration and drying afforded title compound, which was column purified using 230-400 silica gel and MeOH: CH_2Cl_2 mobile phase (0-3%); Yield: 42%; mp: 174-176 ^{0}C; Purity by UPLC: 98.72%; IR (KBr) 3392, 1683, 1649, 1602, 1573, 1525, 1460, 1373, 1296, 1217, 1114, 833, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.38 (s, 3H), 3.47 (bs, 2H), 3.57 (s, 3H), 7.65 (d, *J* = 6.8 Hz, 1H), 7.93-7.96 (m, 2H), 8.12-8.20 (m, 6H), 8.45 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 33.64, 42.87, 53.56, 116.17, 124.65, 125.68, 126.41, 126.87, 127.58, 128.51, 129.15, 131.52, 137.72, 140.42, 140.75, 146.41, 151.01, 164.01, 166.01, 175.67; ESI/MS m/z 533.9 $(M+H)^+$.

5.1.74. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(ethylamino)acetyl)-S-methyl sulfonimidoyl) benzamido)benzamide (46b)



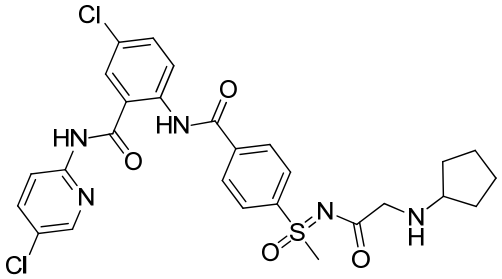
This compound was prepared from **45a** and 10 mol eq. 70% ethylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 48%; mp: 158-160 ^{0}C; Purity by UPLC: 99.38%; IR (KBr) 3433, 1681, 1639, 1602, 1523, 1458, 1373, 1296, 1224, 1116, 922, 835, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.0 (t, J = 7.2 Hz, 3H), 2.57-2.66 (m, 2H), 3.42 (d, J = 9.6 Hz, 2H), 3.54 (s, 3H), 7.60 (bd, J = 9.2 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93-7.95 (dd, J = 2.8 and 8.0 Hz, 1H), 8.10 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 1H), 8.25 (bs, 3H), 8.45 (d, J = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.92, 42.67, 42.95, 53.30, 116.17, 124.60, 125.35, 126.40, 126.78, 127.37, 128.65, 129.11, 131.39, 137.65, 140.40, 140.77, 146.44, 151.25, 164.37, 165.97, 178.05; ESI/MS m/z 547.8 (M+H)$^+$.

5.1.75. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(isopropylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (46c)



This compound was prepared from **45a** and 2 mol eq. isopropylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 57%; mp: 176-178 ^{0}C; Purity by UPLC: 99.24%; IR (KBr) 3313, 1681, 1637, 1600, 1523, 1458, 1373, 1296, 1226, 1093, 833, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.97-1.00 (q, 6H), 2.78-2.84 (m, 1H), 3.42 (s, 2H), 3.54 (s, 3H), 7.62 (bd, 1H), 7.91-7.95 (m, 2H), 8.11 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.8 Hz, 1H), 8.31 (bs, 3H), 8.45 (d, J = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 21.71, 42.91, 48.09, 51.35, 116.16, 124.66, 125.43, 126.32, 126.61, 127.40, 128.62, 129.13, 131.42, 137.69, 140.40, 140.72, 146.44, 151.10, 164.33, 165.96, 178.23; ESI/MS m/z 561.9 $(M+H)^+$.

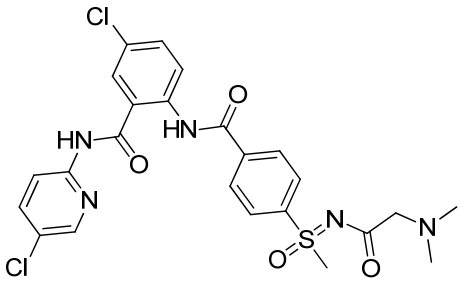
5.1.76. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(cyclopentylamino) acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (46d)



This compound was prepared from **45a** and 2 mol eq. cyclopentylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 40%; mp: 141-143 ^{0}C; Purity by UPLC: 97.52%; IR (KBr) 3416, 1681, 1651, 1602, 1510, 1458, 1373, 1296, 1219, 1114, 920, 833, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.31-1.35 (m, 2H), 1.43-1.46 (m, 2H), 1.54-1.59 (m, 2H), 1.67-1.71 (m, 2H), 3.05-3.08 (m, 1H), 3.41 (s, 2H), 3.54 (s, 3H), 7.64 (bd, 1H), 7.92-

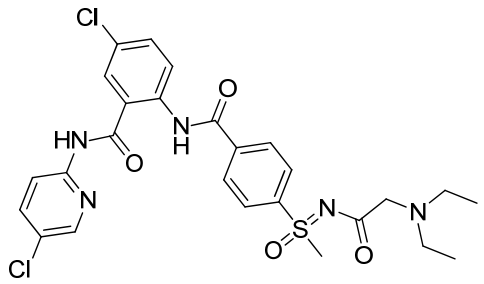
7.94 (m, 2H), 8.09-8.19 (m, 6H), 8.45 (d, *J* = 2.8 Hz, 1H); ESI/MS m/z 587.8 and 589.9 $(M+H)^+$.

5.1.77. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(dimethylamino)acetyl)-S-methyl sulfonimidoyl)benzamido)benzamide (46e)



This compound was prepared from **45a** and 10 mol eq. 50%. dimethylamine solution by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 66%; mp: 134-136 ^{0}C; Purity by UPLC: 99.19%; IR (KBr) 1683, 1647, 1602, 1518, 1458, 1373, 1294, 1217, 1116, 922, 839, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.20 (s, 6H), 3.09 (s, 2H), 3.48 (s, 3H), 7.64-7.66 (dd, *J* = 2 and 8.8 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.92-7.94 (dd, *J* = 2.8 and 8.8 Hz, 1H), 8.06-8.13 (m, 6H), 8.43 (d, *J* = 2.8 Hz, 1H), 11.23 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 42.94, 44.70, 63.86, 116.18, 124.47, 125.80, 126.70, 127.47, 127.87, 128.39, 129.17, 131.63, 137.76, 139.01, 141.58, 146.35, 150.58, 163.97, 166.06, 177.91; ESI/MS m/z 548 $(M+H)^+$.

5.1.78. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methyl sulfonimidoyl)benzamido)benzamide (46f)

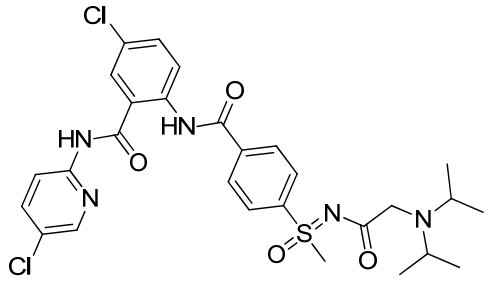


To a stirring solution of **45a** (1 g, 0.0018 mol) in DMF (5 ml) was added diethylamine (0.263 g, 0.0036 mol) followed by catalytic amount of KI. The reaction mixture was stirred at 25-30 ^{0}C for 16 h and then diluted with water. Filtration and drying afforded title compound, which was column purified using 230-400 silica gel and 0-3% MeOH in $CHCl_3$ as mobile phase to get **46f** (0.710 g, 66%) as off-white solid; mp: 170-172 ^{0}C; Purity by UPLC: 98.91%; IR (KBr) 1687, 1666, 1600, 1573, 1510, 1464, 1398, 1375, 1294, 1217, 1114, 920, 831, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.94 (t, J = 7.2 Hz, 6H), 2.54-2.59 (q, J = 7.2 Hz, 4H), 3.26 (s, 2H), 3.49 (s, 3H), 7.65-7.68 (dd, J = 2.0 and 8.4 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.92-7.95 (dd, J = 2.8 and 8.8 Hz, 1H), 8.07-8.14 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.24 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 12.16, 42.95, 46.87, 57.70, 116.19, 124.52, 125.80, 126.79, 127.48, 127.85, 128.40, 129.19, 131.63, 137.77, 139.07, 141.66, 146.37, 150.64, 164.02, 166.07, 178.89; ESI/MS m/z 576 and 577.6 $(M+H)^+$.

To a stirring solution of **46f** (0.5 g, 0.000869 mol) in CH_2Cl_2 (5 ml) was added HCl: diethylether (12 % w/w, 0.2 mL) at 10-15 ^{0}C. The reaction mixture was stirred at 25-30 ^{0}C for 1 h and then diluted with diethyl ether. Precipitated solid

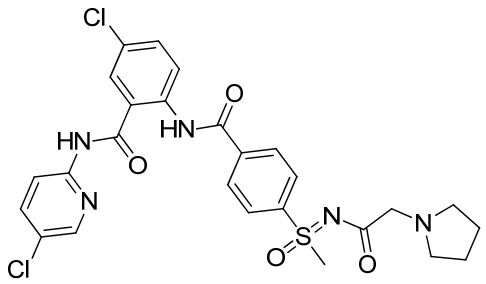
was filtered and dried to get mono hydrochloride salt of **46f** (0.490 g, 92%) as off-white solid; Purity by UPLC: 98.14%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.15-1.23 (m, 6H), 3.06-3.17 (m, 4H), 3.66 (s, 3H), 4.08 (d, J = 5.2 Hz, 2H), 7.67-7.70 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.94-7.97 (dd, J = 2.8 and 8.8 Hz, 1H), 8.09 (d, J = 4.8 Hz, 1H), 8.11 (d, J = 4.8 Hz, 1H), 8.15-8.21 (m, 4H), 8.45 (d, J = 2.4 Hz, 1H), 9.43 (bs, 1H), 11.28 (s, 2H); ESI/MS m/z 575.9 $(M+H)^+$.

5.1.79. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diisopropylamino) acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (46g)



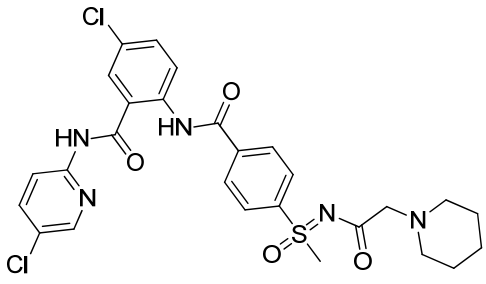
This compound was prepared from **45a** and 2 mol eq. diisopropylamine by means of a procedure similar to that reported for **46a** as off-white solid Yield: 86%; mp: 151-153 ^{0}C; Purity by UPLC: 97.94%; IR (KBr) 1680, 1656, 1602, 1518, 1460, 1375, 1296, 1213, 1116, 920, 831, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.92 (d, J = 4.8 Hz, 12H), 2.95-2.98 (m, 2H), 3.17 (s, 2H), 3.46 (s, 3H), 7.67 (d, J = 8 Hz, 1H), 7.92-7.95 (dd, J = 2.4 and 8.8 Hz, 2H), 8.06-8.14 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.25 (bs, 2H); ESI/MS m/z 604 $(M+H)^+$.

5.1.80. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(pyrrolidin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (46h)



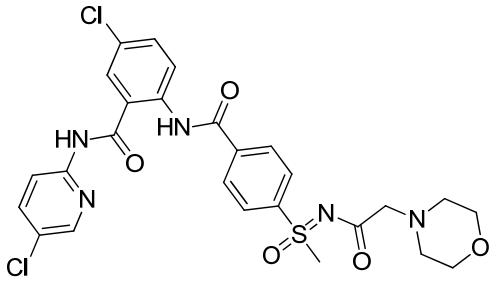
This compound was prepared from **45a** and 2 mol eq. pyrrolidine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 69%; mp: 133-135 ^{0}C; Purity by UPLC: 99.47%; IR (KBr) 1685, 1656, 1602, 1573, 1518, 1458, 1373, 1296, 1203, 1116, 922, 839, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.67 (bs, 4H), 2.60-2.61 (bd, 4H), 3.16 (s, 2H), 3.51 (s, 3H), 7.68 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.93-7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.08-8.14 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.25 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 23.26, 42.94, 53.25, 60.20, 116.20, 124.60, 125.80, 126.95, 127.51, 127.93, 128.43, 129.20, 131.63, 137.79, 139.06, 141.49, 146.39, 150.61, 164.01, 166.06, 177.39; ESI/MS m/z 574 and 575.7 $(M+H)^{+}$.

5.1.81. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(piperidin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (46i)



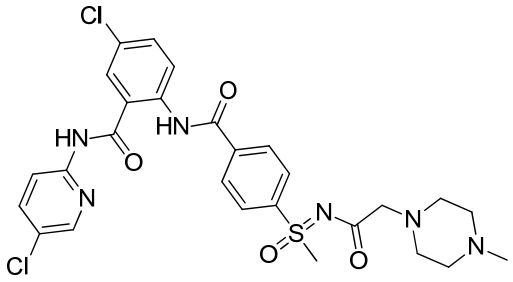
This compound was prepared from **45a** and 2 mol eq. piperidine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 91%; mp: 125-127 ^{0}C; Purity by UPLC: 98.90%; IR (KBr) 1681, 1654, 1600, 1510, 1458, 1374, 1296, 1217, 1112, 920, 833, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.29 (bs, 2H), 1.34-1.46 (bs, 4H), 2.42-2.50 (bs, 4H), 3.15 (bs, 2H), 3.50 (s, 3H), 7.68 (d, J = 7.2 Hz, 1H), 7.91-7.96 (m, 2H), 8.07-8.13 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.27 (bd, 2H); ESI/MS m/z 587.9 and 589.9 $(M+H)^+$.

5.1.82. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-morpholino acetyl) sulfonimidoyl) benzamido)benzamide (46j)



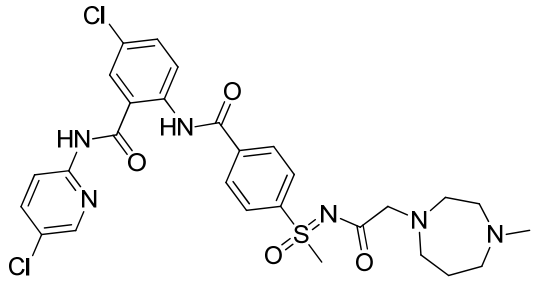
This compound was prepared from **45a** and 2 mol eq. morpholine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 60%; mp: 135-137 ^{0}C; Purity by UPLC: 98.71%; IR (KBr) 1681, 1656, 1600, 1575, 1510, 1458, 1373, 1296, 1215, 1112, 918, 833, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.45 (t, J = 4.4 Hz, 4H), 3.12 (s, 2H), 3.50 (s, 3H), 3.53 (t, J = 4.8 Hz, 4H), 7.66-7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.07-8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.19 (s, 1H), 11.27(s, 1H); ESI/MS m/z 590.1 $(M+H)^+$.

5.1.83. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(4-methyl piperazin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (46k)



This compound was prepared from **45a** and 2 mol eq. N-methylpiperazine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 60%; mp: 178-180 ^{0}C; Purity by UPLC: 98.83%; IR (KBr) 1689, 1653, 1602, 1516, 1460, 1375, 1296, 1224, 1010, 920, 823, 742 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.14 (s, 3H), 2.33 (bs, 4H), 2.45 (bs, 4H), 3.10 (s, 2H), 3.49 (s, 3H), 7.67 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.92-7.95 (dd, J = 2.8 and 9.2 Hz, 1H), 8.07-8.15 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.25 (bs, 2H); ESI/MS m/z 603.2 $(M+H)^+$.

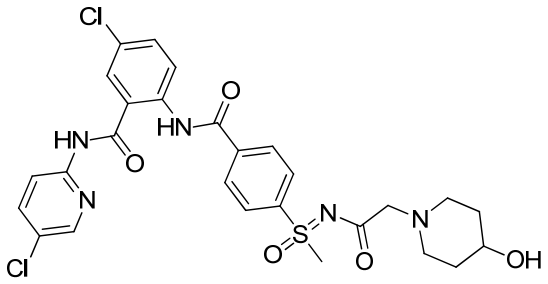
5.1.84. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(4-methyl-1,4-diazepan-1-yl)acetyl)sulfonimidoyl)benzamido)benzamide (46l)



This compound was prepared from **45a** and 2 mol eq. N-methylhomopiperazine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 69%; mp: 177-180 ^{0}C; Purity by UPLC: 95.57%; IR (KBr) 1654, 1600, 1575,

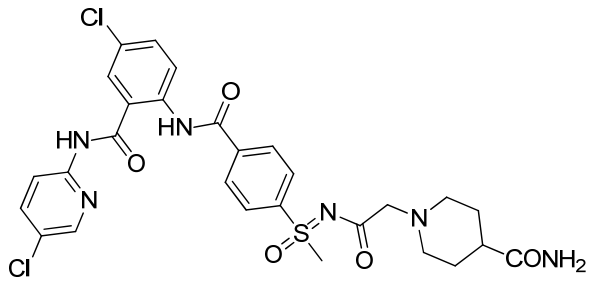
1510, 1460, 1373, 1298, 1215, 1112, 920, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.86 (bs, 2H), 2.68 (s, 3H), 2.78 (bs, 2H), 2.91 (bs, 2H), 2.99-3.15 (m, 4H), 3.50 (s, 2H), 3.65 (s, 3H), 7.69 (d, J = 7.6 Hz, 1H), 7.92-7.96 (bd, 2H), 8.10-8.12 (bd, 6H), 8.45 (s, 1H), 11.27 (bs, 2H); ESI/MS m/z 616.8 and 618.8 $(M+H)^+$.

5.1.85. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(4-hydroxypiperidin-1-yl)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (46m)



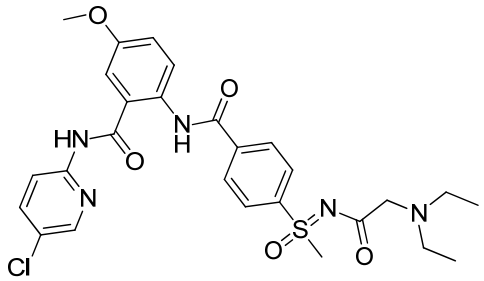
This compound was prepared from **45a** and 2 mol eq. 4-hydroxyopiperidine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 75%; mp: 204-206 ^0C; Purity by UPLC: 94.75%; IR (KBr) 3417, 1681, 1653, 1604, 1573, 1521, 1460, 1373, 1294, 1215, 1114, 920, 837, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.37 (bs, 2H), 1.66 (bs, 2H), 2.20 (bs, 2H), 2.72 (bs, 2H), 3.12 (bs, 2H), 3.35-3.40 (m, 1H), 3.50 (s, 3H), 4.55 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.08-8.14 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.20 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 604.1 and 605.8 $(M+H)^+$.

5.1.86. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(4-aminocarbonyl-1-piperidinyl) acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (46n)



This compound was prepared from **45a** and 2 mol eq. piperidine-4-carboxamide by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 42%; mp: 246-248 ^{0}C; Purity by UPLC: 98.55%; IR (KBr) 3408, 3155, 1689, 1662, 1602, 1521, 1460, 1373, 1294, 1215, 1120, 922, 839, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.49-1.55 (m, 2H), 1.59-1.62 (m, 2H), 1.96-2.08 (m, 3H), 2.82 (bs, 2H), 3.11 (bs, 2H), 3.49 (s, 3H), 6.7 (s, 1H), 7.19 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.8 and 8.8 Hz, 1H), 8.07-8.14 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.19 (s, 1H), 11.25 (s, 1H); ESI/MS m/z 630.8 (M+H)$^+$.

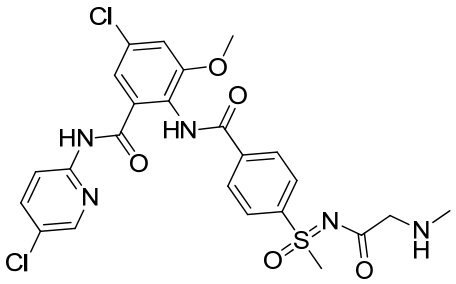
5.1.87. N-(5-Chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methyl sulfonimidoyl) benzamido)-5-methoxybenzamide (46o)



This compound was prepared from **45e** and 2 mol eq. diethylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 41%; mp:

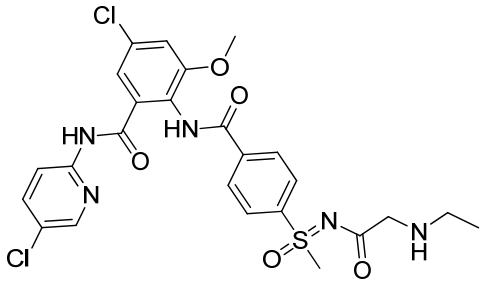
113-115 ^{0}C; Purity by UPLC: 99.58%; IR (KBr) 1656, 1610, 1573, 1529, 1460, 1375, 1299, 1220, 1074, 837, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.92 (t, J = 7.2 Hz, 6H), 2.49-2.54 (m, 4H), 3.21 (s, 2H), 3.47 (s, 3H), 3.84 (s, 3H), 7.18 (d, J = 8 Hz, 1H), 7.39 (s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 8.05-8.14 (m, 5H), 8.41 (s, 1H), 10.96 (s, 1H), 11.09 (s, 1H); ESI/MS m/z 572 $(M+H)^+$.

5.1.88. 5-Chloro-N-(5-chloropyridin-2-yl)-3-methoxy-2-(4-(S-methyl-N-(2-(methylamino) acetyl) sulfonimidoyl)benzamido)benzamide (46p)



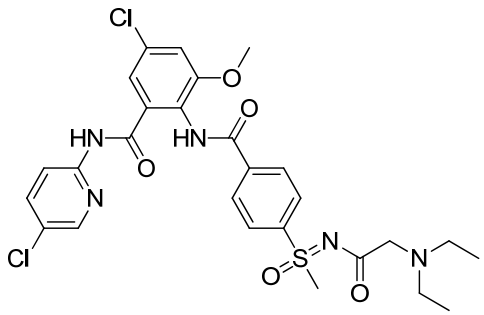
This compound was prepared from **45f** and 10 mol eq. 40% aq. methylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 64%; mp: 157-160 ^{0}C; Purity by UPLC: 97.68%; IR (KBr) 3263, 1662, 1573, 1529, 1458, 1375, 1307, 1222, 1064, 840, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.22 (s, 3H), 3.20 (d, 2H), 3.47 (s, 3H), 3.84 (s, 3H), 7.30 (d, J = 2.4 Hz, 1H), 7.36 (s, 1H), 7.85-7.88 (dd, J = 2.4 and 8.8 Hz, 1H), 8.02-8.09 (m, 5H), 8.36 (d, J = 2.4 Hz, 1H), 10.10 (bs, 1H), 10.90 (bs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 34.63, 42.92, 54.96, 56.63, 114.31, 115.24, 120.14, 123.03, 125.47, 127.32, 128.65, 131.40, 135.17, 137.83, 138.61, 141.03, 146.34, 150.60, 155.35, 164.77, 177.61; ESI/MS m/z 563.7 $(M+H)^+$.

5.1.89. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(ethylamino)acetyl)-S-methyl sulfonimidoyl) benzamido)-3-methoxybenzamide (46q)



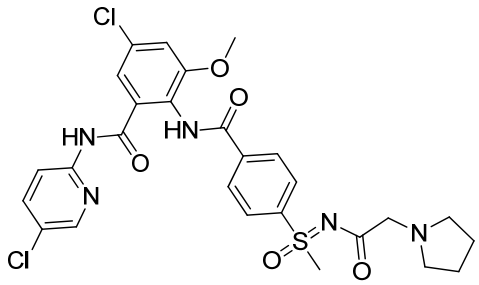
This compound was prepared from **45f** and 10 mol eq. 70% ethylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 53%; mp: 178-180 ^{0}C; Purity by UPLC: 95.77%; IR (KBr) 3433, 1664, 1573, 1529, 1460, 1375, 1307, 1224, 1114, 1064, 840, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.10 (t, J = 7.2 Hz, 3H), 2.79 (t, J = 7.2 Hz, 2H), 3.58 (s, 3H), 3.71 (s, 2H), 3.86 (s, 3H), 7.31 (s, 1H), 7.39 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H), 8.12 (s, 4H), 8.38 (s, 1H), 10.10 (bs, 1H), 10.81 (bs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 11.83, 42.05, 42.85, 51.04, 56.66, 114.35, 115.28, 120.19, 123.07, 125.51, 127.44, 128.70, 131.47, 135.22, 137.83, 138.74, 140.59, 146.38, 150.62, 155.37, 164.82, 177.63; ESI/MS m/z 578.0 $(M+H)^+$.

5.1.90. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methyl sulfonimidoyl) benzamido)-3-methoxybenzamide (46r)



This compound was prepared from **45f** and 2 mol eq. diethylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 64%; mp: 168-170 ^{0}C; Purity by UPLC: 98.31%; IR (KBr) 1666, 1573, 1521, 1458, 1375, 1307, 1220, 1114, 1066, 839, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.95 (t, J = 3.2 Hz, 6H), 2.49-2.52 (m, 4H), 3.26 (s, 2H), 3.47 (s, 3H), 3.86 (s, 3H), 7.30 (d, J = 2.0 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 7.86-7.89 (dd, J = 2.4 and 8.8 Hz, 1H), 8.01-8.10 (m, 5H), 8.37 (d, J = 2.4 Hz, 1H), 10.07 (s, 1H), 10.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 12.14, 42.98, 46.88, 56.63, 57.64, 114.29, 115.33, 120.45, 122.95, 125.46, 127.20, 128.62, 131.44, 135.24, 137.85, 138.43, 141.46, 146.31, 150.59, 155.36, 164.75, 177.83; ESI/MS m/z 605.9 $(M+H)^+$.

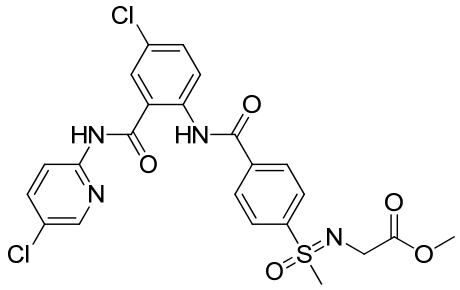
5.1.91. 5-Chloro-N-(5-chloropyridin-2-yl)-3-methoxy-2-(4-(S-methyl-N-(2-(pyrrolidin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (46s)



This compound was prepared from **45f** and 2 mol eq. pyrrolidine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 74%; mp: 154-156 ^{0}C; Purity by UPLC: 97.66%; IR (KBr) 1687, 1656, 1575, 1521, 1460, 1377, 1309, 1215, 1066, 842, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.67 (bs, 4H), 2.58 (bs, 4H), 3.27 (s, 2H), 3.48 (s, 3H), 3.86 (s, 3H), 7.30 (s, 1H), 7.38 (s, 1H), 7.86-7.89 (dd, J = 2.4 and 8.8 Hz, 1H), 8.02-8.10 (m, 5H), 8.37 (d, J = 1.6 Hz, 1H), 10.07 (s, 1H), 10.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 23.28, 42.97,

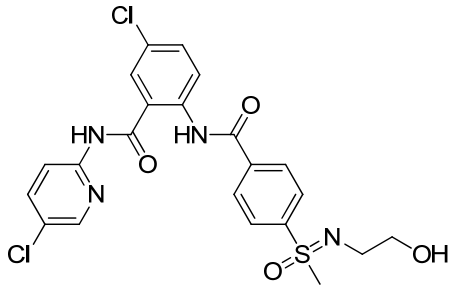
53.19, 56.62, 60.29, 114.29, 115.23, 120.12, 123.10, 125.45, 127.20, 128.62, 131.41, 135.20, 137.84, 138.45, 140.97, 146.31, 150.58, 155.35, 164.74, 177.43; ESI/MS m/z 604.0 $(M+H)^+$.

5.1.92. 5-chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-Methoxycarbonylmethyl-S-methylsulfonimidoyl)benzamido)-benzamide (47)



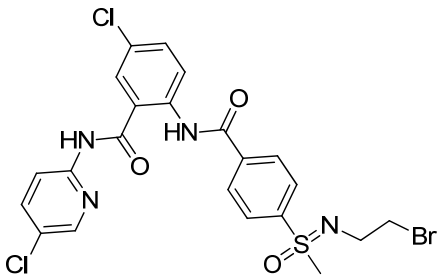
To a stirring solution of **43a** (5 g, 0.0108 mol) in DMF (15 ml) was added NaH (1.76 g, 0.0367 mol) at 10-15 ^{0}C under N_2 atmosphere. To this was added methyl bromoacetate (2.48 g, 0.0162 mol) in one lot. Reaction mixture was stirred at 40 ^{0}C for 3 h and cooled to 20 ^{o}C. Product was diluted with water and extracted with CH_2Cl_2. Crude product was column purified using 100-200 silical gel and 0-50% ethyl acetate in hexane as mobile phase to get **47** (1.1 g, 20 %) as off-white solid; mp: 211-213 ^{0}C; Purity by UPLC: 94.47%; IR (KBr) 1747, 1678, 1656, 1600, 1572, 1512, 1460, 1375, 1296, 1153, 837, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.26 (s, 3H), 3.49-3.66 (q, J = 16.8 Hz, 2H), 3.55 (s, 3H), 7.66-7.69 (dd, J = 2.8 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.94-7.97 (dd, J = 2.8 and 8.8 Hz, 1H), 8.01-8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.17 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 532.9 (M-H).

5.1.93. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-hydroxyethyl)-S-methyl sulfonimidoyl) benzamido)benzamide (48)



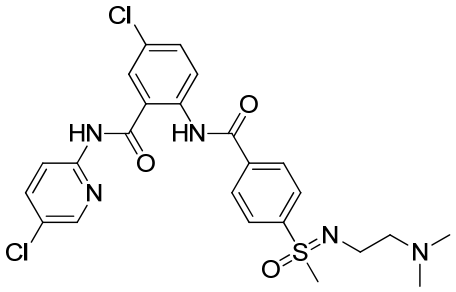
To a stirring solution of **47** (2 g, 0.00374 mol) in DMSO (10 mL) was added $NaBH_4$ (0.71 g, 0.0149 mol) at 20-25 ^{0}C. Reaction mixture was stirred at 60 ^{0}C for 3 h and cooled to 25 ^{o}C. Product was diluted with water and extracted with CH_2Cl_2, which on drying over Na_2SO_4, evaporation gave crude product. Product obtained was column purified using 100-200 silica gel and 0-2% MeOH in CH_2Cl_2 to get **48** (1.02 g, 54%) as off-white solid; mp: 210-212 ^{0}C; Purity by UPLC: 96.52%; IR (KBr) 3416, 1680, 1656, 1602, 1514, 1460, 1373, 1294, 1220, 1139, 1070, 920, 831, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.70-2.75 (m, 1H), 2.83-2.89 (m, 1H), 3.18 (s, 3H), 3.37-3.42 (m, 2H), 4.42 (t, J = 6 Hz, 1H), 7.66-7.69 (dd, J = 2.8 and 8.8 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.94-7.97 (dd, J = 2.4 and 8.8 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 8.13 (d, J = 8.8 Hz, 2H), 8.44 (d, J = 2.0 Hz, 1 H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 506.9 and 508.6 $(M+H)^+$.

5.1.94. 2-(4-(N-(2-Bromoethyl)-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl) benzamide (49)



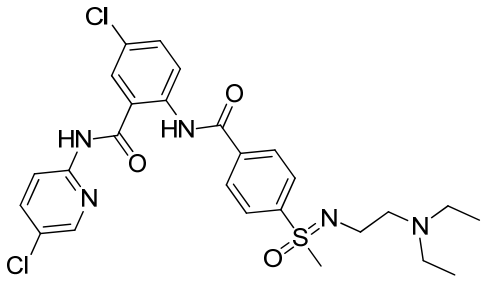
To a stirring solution of **48** (1.2 g, 0.0023 mol) in CH_2Cl_2 (15 ml) was added TPP (0.92 g, 0.0035 mol) and CBr_4 (1.1 g, 0.0035 mol) at 25-30 ^{0}C. Reaction mixture was stirred at 30 ^{0}C for 3 h and cooled to 20 ^{o}C. Product was diluted with water and extracted with CH_2Cl_2 which on drying over Na_2SO_4, evaporation gave crude product. Product obtained was column purified using 100-200 silica gel and 0-40% ethyl acetate in hexane to get **49** (0.270 g, 20%) as off- white solid; mp: 198-200 ^{0}C; Purity by HPLC: 96.15%; IR (KBr) 1676, 1600, 1573, 1514, 1458, 1373, 1294, 1130, 920, 833, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.02-3.09 (m, 1H), 3.16-3.22 (m, 1H), 3.24 (s, 3H), 3.48 (t, J = 6.8 Hz, 2H), 7.66-7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93-7.96 (dd, J = 2.8 and 9.2 Hz, 1H), 8.02-8.04 (bd, 2H), 8.09-8.13 (m, 4H), 8.44 (d, J =2.4 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 570.6 $(M+H)^+$.

5.1.95. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(dimethylamino)ethyl)-S-methyl sulfonimidoyl)benzamido)benzamide (50a)



This compound was prepared from **49** and 10 mol eq. 50% dimethylamine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 26%; mp: 131-133 ^{0}C; Purity by UPLC: 99.06%; IR (KBr) 1684, 1635, 1575, 1507, 1459, 1374, 1296, 1229, 835, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.11 (s, 6H), 2.31-2.38 (bd, 2H), 2.72-2.78 (m, 1H), 2.87-2.93 (m, 1H), 3.17 (s, 3H), 7.64 (d, J = 8.8 Hz, 1H), 7.91 (bs, 2H), 7.99 (d, J = 8.4 Hz, 2H), 8.12-8.14 (bd, 4H), 8.42 (d, J = 2.0 Hz, 1H), 11.93 (bs, 2H); ESI/MS m/z 533.9 $(M+H)^+$.

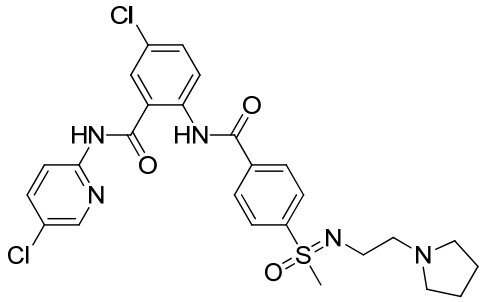
5.1.96. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)ethyl)-S-methyl sulfonimidoyl) benzamido)benzamide (50b)



This compound was prepared from **49** and 2 mol eq. diethylamine by means of a procedure similar to that reported for **46a** as off white solid; Yield: 60%; mp: 63-65 ^{0}C; Purity by UPLC: 98.34%; IR (KBr) 1654, 1577, 1506, 1458, 1373, 1294, 1072, 831, 744 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.98 (t, J = 7.2 Hz, 6H), 2.46-

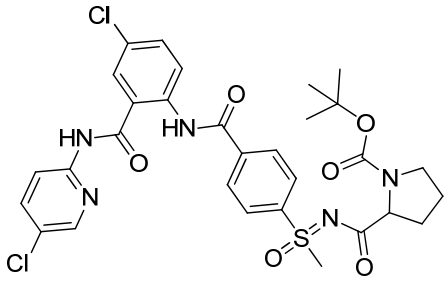
2.53 (m, 4H), 2.64 (t, *J* = 4.0 Hz, 2H), 2.87-2.95 (m, 1H), 3.04-3.09 (m, 1H), 3.12 (s, 3H), 7.58-7.61 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.76-7.79 (dd, *J* = 2.8 and 8.8 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 8.18 (d, *J* = 8.4 Hz, 2H), 8.28-8.38 (m, 2H), 8.87 (d, *J* = 8.8 Hz, 1H), 11.93 (bs, 2H); ESI/MS m/z 561.6 $(M+H)^+$.

5.1.97. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(pyrrolidin-1-yl)ethyl)sulfonimidoyl) benzamido)benzamide (50c)



This compound was prepared from **49** and 2 mol eq. pyrrolidine by means of a procedure similar to that reported for **46a** as off-white solid; Yield: 64%; mp: 88-90 ^{0}C; Purity by UPLC: 99.03%; IR (KBr) 1656, 1573, 1521, 1460, 1375, 1315, 1226, 1064, 842, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.72 (bs, 4H), 2.47 (bs, 4H), 2.59-2.74 (m, 2H), 2.93-3.00 (m, 1H), 3.06-3.18 (m, 1H), 3.13 (s, 3H), 7.58-7.61 (dd, *J* = 2.4 and 9.2 Hz, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 7.77-7.80 (dd, *J* = 2.8 and 9.2 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 8.17 (d, *J* = 8.4 Hz, 2H), 8.19-8.32 (m, 2H), 8.87 (d, *J* = 8.8 Hz, 1H), 11.95 (bs, 2H); ESI/MS m/z 559.6 and 561.4 $(M+H)^+$.

5.1.98. S-[4-(N-{2-[N-(5-Chloro(2-pyridyl))carbamoyl]-4 chlorophenyl} carbamoyl) phenyl]-S-methyl-N-(1-tert-butoxycarbonyl-pyrrolidin-2-carbonyl) Sulfoximide (51)

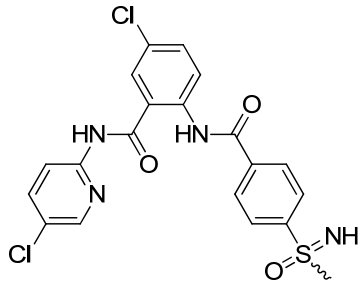


To a stirring solution of **43a** (4 g, 0.00868mol) and Boc protected (L)-Proline (2.8 g, 0.01302 mol) in DMF (10 mL) was added EDCI (2.5 g, 0.01302 mol) followed by catalytic amount of DMAP. The reaction mixture was stirred at 25-30 ^{0}C for 4 h and then diluted with water (50 mL). Product was filtered to get titled compound. Diastereomers were separated by column chromatography (230-400 mesh silica gel, 30-35% ethyl acetate in hexane) to get 1.2 g nonpolar diastereomer and 1.3 g polar diastereomer. Product formation was confirmed by mass analysis.

Non polar isomer: ESI/MS m/z 658 (M-H); Purity by UPLC: 99.59%.

Polar isomer: ESI/MS m/z 658 (M-H); Purity by UPLC: 99.56%.

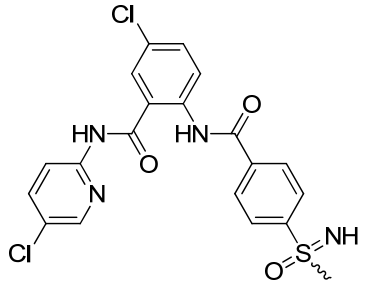
5.1.99. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methylsulfonimidoyl) benzamido) benzamide ((-) 43a)



(-) 43a

To a stirring solution of **51** (nonpolar isomer) (1.2 g, 0.00182 mol) in MeOH (4 mL) was added conc. H_2SO_4 (0.7 g, 0.00728 mol) at 5-10 ^oC. The reaction mixture was stirred at 25-30 ^0C for 4 h and then diluted with water (50 mL) and product was extracted in $CHCl_3$ (50 mL x 2) at 8-9 pH. Organic layer was dried and concentrated to get desire product as off-white solid; Yield: 63%; mp: 248-250 ^0C; Purity by UPLC: 96.85%; SOR = -21.84^o at 25 ^oC (c = 0.05% in DMSO); ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.10 (s, 3H), 4.39 (s, 1H), 7.65-7.68 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.93-7.96 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.06 (bs, 4H), 8.11 (d, *J* = 4.4 Hz, 1H), 8.13 (d, *J* = 4.4 Hz, 1H), 8.43 (d, *J* = 2.4 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z: 484.8 $(M+Na)^+$.

5.1.100. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methylsulfonimidoyl) benzamido) benzamide ((+) 43a)

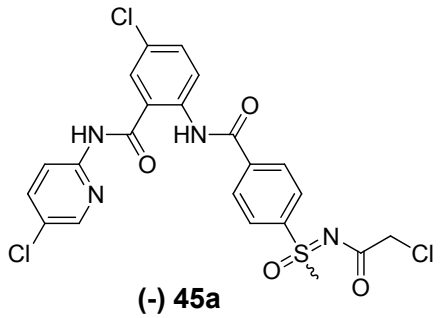


(+) 43a

This compound was prepared from **51** (polar isomer) by means of a procedure similar to that reported for **(-) 43a** as off-white solid; Yield: 71%; mp: 247-249 ^0C; Purity by UPLC: 95.78%; SOR = +19.52^o at 25 ^oC (c = 0.05% in DMSO); ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.10 (s, 3H), 4.39 (s, 1H), 7.65-7.68 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.93-7.96 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.05

(bs, 4H), 8.11 (d, *J* = 4.4 Hz, 1H), 8.13 (d, *J* = 4.4 Hz, 1H), 8.43 (d, *J* = 2.4 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z: 484.7 $(M+Na)^+$.

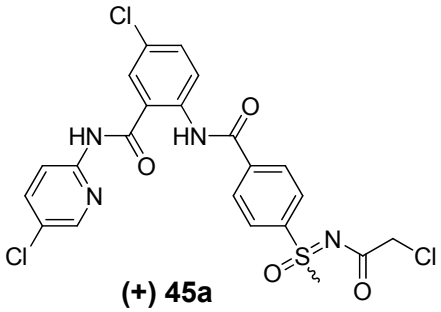
5.1.101. 5-Chloro-2-(4-(N-(2-chloroacetyl)-S-methylsulfonimidoyl) benzamido)-N-(5-chloropyridin-2-yl)benzamide ((-) 45a)



(-) 45a

This compound was prepared from **(-) 43a** by means of a procedure similar to that reported for **45a** as off-white solid; Purity by UPLC: 87.57%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.56 (s, 3H), 4.26 (s, 2H), 7.65-7.68 (dd, *J* = 2.4 and 8.8 Hz, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.92-7.95 (dd, *J* = 2.4 and 8.8 Hz, 1H), 8.06-8.11 (m, 6H), 8.43 (d, *J* = 2.8 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 562.6 $(M+Na)^+$.

5.1.102. 5-Chloro-2-(4-(N-(2-chloroacetyl)-S-methylsulfonimidoyl) benzamido)-N-(5-chloropyridin-2-yl)benzamide ((+) 45a)

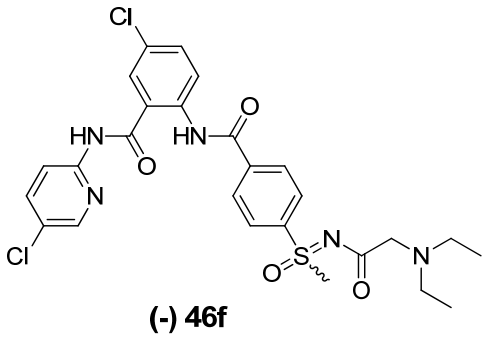


(+) 45a

This compound was prepared from **(+) 43a** by means of a procedure similar to that reported for **45a** as off-white solid; Purity by UPLC: 92.47%; ^{1}H NMR

(DMSO-d_6, 400 MHz) δ: 3.56 (s, 3H), 4.26 (s, 2H), 7.66-7.68 (dd, J = 2.4 and 8.8 Hz, 1H), 7.89 (d, J = 2.4 Hz, 1H), 7.92-7.95 (dd, J = 2.4 and 8.8 Hz, 1H), 8.06-8.11 (m, 6H), 8.43 (d, J = 2.8 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 560.9 $(M+Na)^+$.

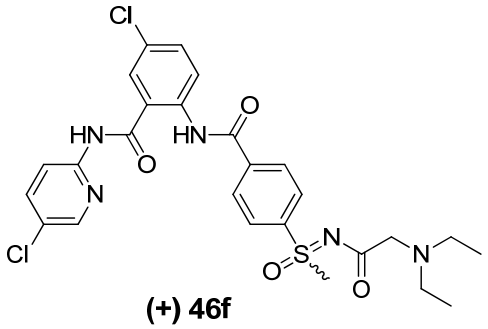
5.1.103. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide ((-) 46f)



(-) 46f

This compound was prepared from **(-) 45a** by means of a procedure similar to that reported for **46f** as off-white solid; mp: 187-190 ^{0}C; SOR = -42.01^o at 25 ^{o}C (c = 0.2% in DMSO); Purity by UPLC: 99.37%; IR (KBr) 3433, 1685, 1654, 1637, 1508, 1294, 1203, 1114, 921, 839, 742 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.92 (t, J = 7.2 Hz, 6H), 2.48-2.55 (q, J = 7.2 Hz, 4H), 3.24 (s, 2H), 3.48 (s, 3H), 7.66 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.91-7.94 (dd, J = 2.8 and 8.8 Hz, 1H), 8.06-8.13 (m, 6H), 8.43 (d, J = 2.8 Hz, 1H), 11.23 (bs, 2H); ESI/MS m/z 575.9 $(M+H)^+$.

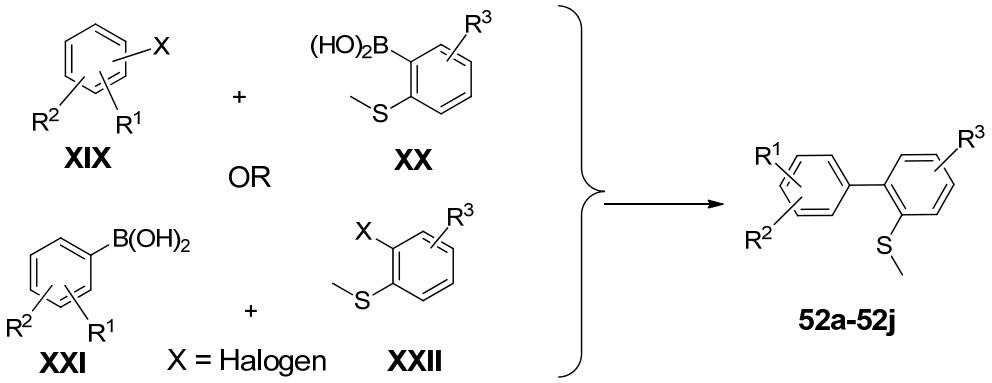
5.1.104. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methyl sulfonimidoyl)benzamido)benzamide ((+) 46f)



(+) 46f

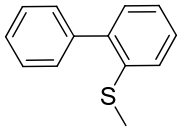
This compound was prepared from **(+) 45a** by means of a procedure similar to that reported for **46f** as off-white solid; mp: 186-190 ^{0}C; SOR = +39.35^o at 25 ^{o}C (c = 0.2% in DMSO); Purity by UPLC: 98.79%; IR (KBr) 3433, 1654, 1666, 1512, 1458, 1373, 1296, 1215, 1116, 921, 833, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.93 (t, J = 7.2 Hz, 6H), 2.49-2.56 (q, J = 7.2 Hz, 4H), 3.24 (s, 2H), 3.48 (s, 3H), 7.66 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.91-7.94 (dd, J = 2.8 and 8.8 Hz, 1H), 8.06-8.13 (m, 6H), 8.43 (d, J = 2.8 Hz, 1H), 11.24 (bs, 2H); ESI/MS m/z 575.9 $(M+H)^+$.

5.1.105. General procedure for the synthesis of compounds (52a-52j)



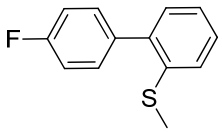
To a mixture of substituted halobenzene (**XIX** or **XXII**) (1 mol eq.) in dry MeOH (10 v/w) placed in round bottomed flask attached with condenser was added Na_2CO_3 (2 mol eq.) followed by $Pd(OAc)_2$ (2 mol %). To this was added substituted phenylboronic acid (**XX** or **XXI**) (1 mol eq.) in one lot and reaction mixture was refluxed for 5-16 h. Reaction mixture was cooled to 25-30 ^oC. MeOH (3 v/w) was added and the mixture was vacuum filtered through a sintered glass funnel using celite as a filter-aid to furnish residue. The residue obtained was purified by flash chromatography using 100-200 mesh silica gel as a stationary phase and ethyl acetate:*n*-hexane as a mobile phase.

5.1.105.1. [1,1'-Biphenyl]-2-yl(methyl)sulfane (52a)



Compound **52a** was obtained by reacting Bromobenzene with 2-methylthio phenylboronic acid using general procedure described above as semi solid; Yield: 70%; Purity by UPLC: 99.82%; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.35 (s, 3H), 7.15-7.23 (m, 2H), 7.31-7.34 (m, 2H), 7.34-7.39 (m, 3H), 7.40-7.44 (m, 2H); ESI/MS m/z No $(M+H)^+$ observed.

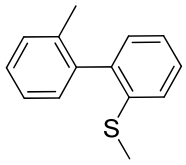
5.1.105.2. (4'-Fluoro-[1,1'-biphenyl]-2-yl)(methyl)sulfane (52b)



Compound **52b** was obtained by reacting 4-fluoro-bromobenzene with 2-methylthio phenylboronic acid using general procedure described above as off-white solid; Yield: 72%; mp: 59-60 ^0C; Purity by UPLC: 99.30%; ^1H NMR (DMSO-

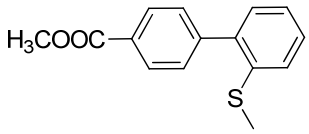
d_6, 400 MHz) δ: 2.37 (s, 3H), 7.17-7.20 (m, 1H), 7.22-7.29 (m, 3H), 7.32-7.34 (m, 1H), 7.36-7.42 (m, 3H); ESI/MS m/z No $(M+H)^+$ observed.

5.1.105.3. Methyl (2'-methyl-[1,1'-biphenyl]-2-yl)sulfane (52c)



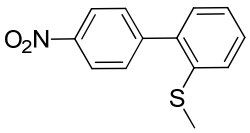
Compound **52c** was obtained by reacting 2-methyl-iodobenzene with 2-methylthio phenylboronic acid using general procedure described above as off-white solid; Yield: 80%; mp: 65-67 ^{0}C; Purity by UPLC: 99.72%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.00 (s, 3H), 2.33 (s, 3H), 7.03-7.05 (m, 2H), 7.17-7.19 (m, 1H), 7.20-7.25 (m, 1H), 7.26-7.28 (bd, 2H), 7.30 (bd, 1H), 7.35-7.40 (m, 1H); No $(M+H)^+$ observed.

5.1.105.4. Methyl 2'-(methylthio)-[1,1'-biphenyl]-4-carboxylate (52d)



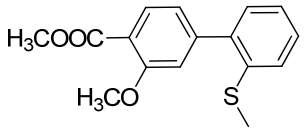
Compound **52d** was obtained by reacting Methyl 4-iodo benzoate with 2-methylthio phenyl boronic acid using general procedure described above as off-white solid; Yield: 88%; mp: 91-93 ^{0}C; Purity by UPLC: 99.37%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.35 (s, 3H), 3.86 (s, 3H), 7.18-7.24 (m, 2H), 7.34-7.41 (m, 2H), 7.51 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H); ESI/MS m/z 259.29 $(M+H)^+$.

5.1.105.5. Methyl (4'-nitro-[1,1'-biphenyl]-2-yl)sulfane (52e)



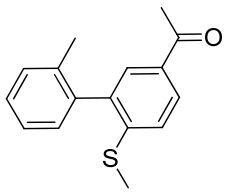
Compound **52e** was obtained by reacting 4-nitro iodobenzene with 2-methylthio phenyl boronic acid using general procedure described above as pale yellow solid; Yield: 95%; mp: 75-77 ^{0}C; Purity by UPLC: 99.71%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.39 (s, 3H), 7.25-7.27 (m, 2H), 7.41-7.45 (m, 2H), 7.65-7.67 (dd, J = 2 and 7.2 Hz, 2H), 8.27-8.29 (dd, J = 2 and 7.6 Hz, 2H); ESI/MS m/z No $(M+H)^+$ observed.

5.1.105.6. Methyl 3-methoxy-2'-(methylthio)-[1,1'-biphenyl]-4-carboxylate (52f)



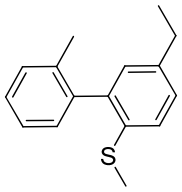
Compound **52f** was obtained by reacting Methyl 2-methoxy-4-iodobenzoate with 2-methylthio phenyl boronic acid using general procedure described above as off-white solid; Yield: 89%; mp: 70-73 ^{0}C; Purity by UPLC: 98.92%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.39 (s, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 7.00-7.03 (dd, J = 1.6 and 8.0 Hz, 1H), 7.11 (d, J = 1.2 Hz, 1H), 7.22-7.28 (m, 2H), 7.37 (d, J = 7.6 Hz, 1H), 7.39-7.44 (m, 1H), 7.71 (d, J = 8 Hz, 1H); ESI/MS m/z 288.8 $(M+H)^+$.

5.1.105.7. 1-(2'-Methyl-6-(methylthio)-[1,1'-biphenyl]-3-yl)ethanone (52g)



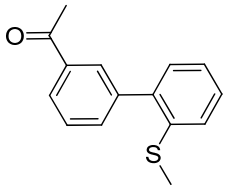
Compound **52g** was obtained by reacting 1-(3-iodo-4-(methylthio)phenyl) ethanone with 2-methyl phenyl boronic acid using general procedure described above and directely used for next step.

5.1.105.8. (5-Ethyl-2'-methyl-[1,1'-biphenyl]-2-yl)(methyl)sulfane (52h).



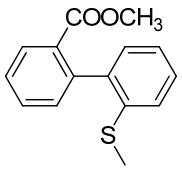
Compound **52h** was obtained by reacting (4-ethyl-2-iodophenyl)(methyl)sulfane with 2-methyl phenyl boronic acid using general procedure described above and directely used for next step.

5.1.105.9. 1-(2'-(Methylthio)-[1,1'-biphenyl]-3-yl)ethanone (52i)



Compound **52i** was obtained by reacting 3-bromoacetophenone with 2-methylthio phenyl boronic acid using general procedure described above as off-white solid; Yield: 70%; mp: 75-77 ^{0}C; Purity by UPLC: 90.38%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.38 (s, 3H), 2.61 (s, 3H), 7.23-7.28 (m, 2H), 7.36-7.44 (m, 2H), 7.58-7.65 (m, 2H), 7.92-7.96 (m, 1H), 7.96-7.99 (m, 1H); ESI/MS m/z No $(M+H)^+$ observed.

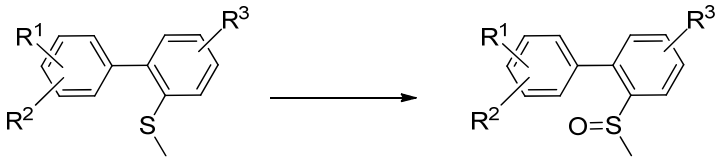
5.1.105.10. Methyl 2'-(methylthio)-[1,1'-biphenyl]-2-carboxylate (52j)



Compound **52j** was obtained by reacting Methyl 2-iodobenzoate with 2-methylthio phenyl boronic acid using general procedure described above as off-white solid; Yield: 90%; mp: 60-62 ^{0}C; Purity by UPLC: 99.61%; ^{1}H NMR (DMSO-

d_6, 400 MHz) δ: 2.28 (s, 3H), 3.52 (s, 3H), 7.03-7.05 (dd, J = 1.2 and 7.6 Hz, 1H), 7.16-7.20 (m, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.30 (d, J = 7.2 Hz, 1H), 7.32-7.34 (m, 1H), 7.48-7.52 (m, 1H), 7.60-7.62 (m, 1H), 7.86-7.88 (dd, J = 0.8 and 7.6 Hz, 1H); ESI/MS m/z 281 $(M+Na)^+$.

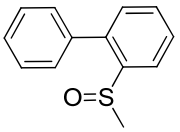
5.1.106. General procedure for the synthesis of compounds (53a-53j)



52a-52j **53a-53j**

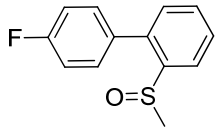
A solution of biphenyl compound (**52a-52j**) (1 mol eq.), V_2O_5 (1 mol %) in CH_3CN (5 v/w) was cooled at 0 ^oC under N_2 environment. Aqueous H_2O_2 (1.2 mol eq., 50 %) was added to reaction mixture and stirred at 10 ^oC for 1 h. After 1 h reaction mixture was diluted with water (3 v/w) and extracted with ethyl acetate (5 v/w). Organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to offer corresponding sulfoxides.

5.1.106.1. 2-(Methylsulfinyl)-1,1'-biphenyl (53a)



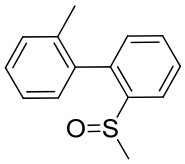
Compound **53a** was obtained from **52a** using general procedure described above as off-white solid; Yield: 90%; mp: 88-89 ^0C; Purity by UPLC: 99.31%; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.40 (s, 3H), 7.28-7.39 (m, 3H), 7.42-7.48 (m, 3H), 7.60 (t, J = 7.2 Hz, 1H), 7.67 (t, J = 7.6 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H); ESI/MS m/z 217 $(M+H)^+$.

5.1.106.2. 4'-Fluoro-2-(methylsulfinyl)-1,1'-biphenyl (53b)



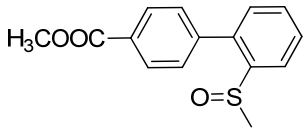
Compound **53b** was obtained from **52b** using general procedure described above as off-white solid; Yield: 80%; mp: 88-90 ^{0}C; Purity by UPLC: 92.31%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.42 (s, 3H), 7.30 (t, J = 8.8 Hz, 2H), 7.37 (d, J = 7.6 Hz, 1H), 7.43-7.46 (m, 2H), 7.58-7.62 (m, 1H), 7.68 (t, J = 7.6 Hz, 1H), 8.00 (d, J = 7.6 Hz, 1H); ESI/MS m/z 235 $(M+H)^+$.

5.1.106.3. 2-Methyl-2'-(methylsulfinyl)-1,1'-biphenyl (53c)



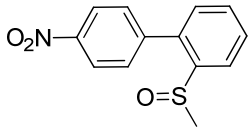
Compound **53c** was obtained from **52c** using general procedure described above as off-white solid; Yield: 82%; m/z 231.46 $(M+H)^+$.

5.1.106.4. Methyl 2'-(methylsulfinyl)-[1,1'-biphenyl]-4-carboxylate (53d)



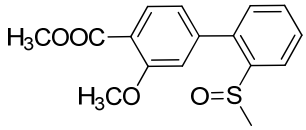
Compound **53d** was obtained from **52d** using general procedure described above as off-white solid; Yield: 85%; mp: 132-134 ^{0}C; Purity by UPLC: 99.27%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.42 (s, 3H), 3.87 (s, 3H), 7.39-7.41 (dd, J = 0.8 and 7.6 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.61-7.66 (m, 1H), 7.69-7.74 (m, 1H), 8.04 (bd, 3H); ESI/MS m/z 275.25 $(M+H)^+$.

5.1.106.5. 2-(Methylsulfinyl)-4'-nitro-1,1'-biphenyl (53e)



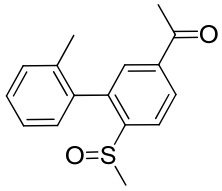
Compound **53e** was obtained from **52e** using general procedure described above as pale yellow solid; Yield: 94%; mp: 167-169 ^{0}C; Purity by UPLC: 98.63%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.35 (s, 3H), 6.88-6.94 (m, 3H), 7.17-7.22 (m, 2H), 7.29-7.38 (m, 3H); ESI/MS m/z 262.21 $(M+H)^+$.

5.1.106.6. Methyl 3-methoxy-2'-(methylsulfinyl)-[1,1'-biphenyl]-4-carboxylate (53f)



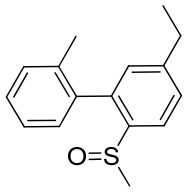
Compound **53f** was obtained from **52f** using general procedure described above as off-white solid; Yield: 80%; mp: 102-104 ^{0}C; Purity by UPLC: 98.86%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.47 (s, 3H), 3.81 (s, 3H), 3.86 (s, 3H), 7.04-7.06 (dd, J = 1.2 and 7.6 Hz, 1H), 7.19 (d, J = 1.2 Hz, 1H), 7.45-7.47 (dd, J = 1.2 and 7.6 Hz, 1H), 7.63-7.67 (m, 1H), 7.70-7.75 (m, 2H), 8.02-8.04 (dd, J = 0.8 and 7.6 Hz, 1H); ESI/MS m/z 304.7 $(M+H)^+$.

5.1.106.7. 1-(2'-Methyl-6-(methylsulfinyl)-[1,1'-biphenyl]-3-yl)ethanone (53g)



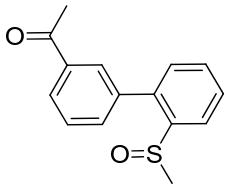
Compound **53g** was obtained from **52g** using general procedure described above as off-white solid; Yield: 83%; ESI/MS m/z 273.1 $(M+H)^+$.

5.1.106.8. 5-Ethyl-2'-methyl-2-(methylsulfinyl)-1,1'-biphenyl (52h)



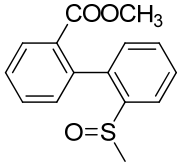
Compound **53h** was obtained from **52h** using general procedure described above as off-white solid; Yield: 75%; ESI/MS m/z 259.0 $(M+H)^+$.

5.1.106.9. 1-(2'-(Methylsulfinyl)-[1,1'-biphenyl]-3-yl)ethanone (52i)



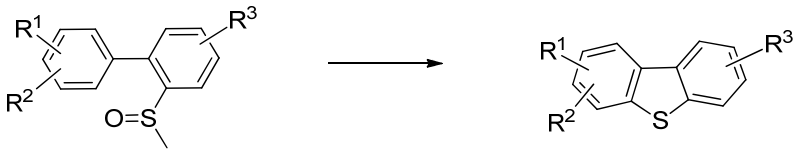
Compound **53i** was obtained from **52i** using general procedure described above as off-white solid; Yield: 79%; mp: 89-91 ^{0}C; Purity by UPLC: 97.58%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.45 (s, 3H), 2.62 (s, 3H), 7.42-7.45 (dd, J = 1.2 and 7.6 Hz, 1H), 7.63 (bd, J = 7.6 Hz, 1H), 7.65-7.69 (m, 2H), 7.70-7.74 (m, 1H), 7.95 (t, J = 1.2 Hz, 1H), 7.99-8.04 (m, 2H); ESI/MS m/z 258.8 $(M+H)^+$.

5.1.106.10. Methyl 2'-(methylsulfinyl)-[1,1'-biphenyl]-2-carboxylate (53j)



Compound **53j** was obtained from **52j** using general procedure described above as off-white solid; Yield: 81%; ESI/MS m/z 275.17 $(M+H)^+$.

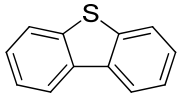
5.1.107. General procedure for the synthesis of compounds (54a-54j)



53a-53j **54a-54j**

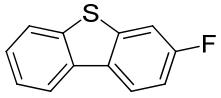
To a stirred conc. H_2SO_4 (3 v/w) in one necked round bottomed flask containing guard tube was added sulfoxides (**53a-53j**) (0.5 g) in portions at 0-5 ^oC. Reaction mixture was stirred at 25 ^oC for 0.5-2 h. Reaction mixture was poured on ice cold water (10 v/w) and then it was made basic with aqueous K_2CO_3 solution (pH 8), aqueous layer was extracted with ethyl acetate (15 v/w). Organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to get titled compounds.

5.1.107.1. Dibenzo[*b,d*]thiophene (54a)



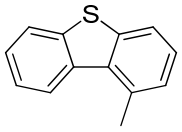
Compound **54a** was obtained from **53a** using general procedure described above as white solid; Yield: 94%; mp: 97-98 ^oC; Purity by UPLC: 99.74%; IR (KBr) 3051, 1583, 1415, 1307, 1230, 1130, 1066, 929, 734, 495 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.47-7.53 (m, 4H), 7.99-8.04 (m, 2H), 8.34-8.38 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 122.0, 123.0, 124.7, 127.0, 135.0, 138.5; CHNS: Calculated for $C_{12}H_8S$: C, 78.22; H, 4.38; S, 17.40; Found: C, 78.32; H, 4.34; S,17.20.

5.1.107.2. 3-Fluorodibenzo[*b,d*]thiophene (54b)



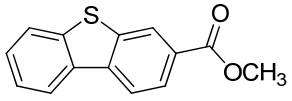
Compound **54b** was obtained from **53b** using general procedure described above as white solid; Yield: 92%; mp: 100-101 ^{o}C; Purity by UPLC: 98.89%; IR (KBr) 3387, 1604, 1440, 1396, 1315, 1240, 891, 840, 758, 732 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.32-7.37 (m, 1H), 7.46-7.51 (m, 2H), 7.92-7.95 (m, 1H), 7.98-8.02 (m, 1H), 8.30-8.32 (m, 1H), 8.33- 8.38 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 109.3, 112.8, 121.8, 122.9, 123.4, 124.9, 126.6, 131.7, 134.2, 138.50, 140.0; ESI-MS m/z 225.4 $(M+Na)^{+}$; CHNS: Calculated for $C_{12}H_7FS$: C, 71.26; H, 3.49; S, 15.85; Found: C, 70.90; H, 3.36; S, 15.98.

5.1.107.3. 1-Methyldibenzo[*b,d*]thiophene (54c)



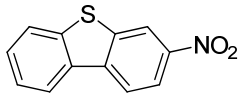
Compound **54c** was obtained from **53c** using general procedure described above as white solid; Yield: 97%; mp: 73-74 ^{o}C; Purity by UPLC: 99.47%; IR (KBr) 3387, 3059, 2949, 1905, 1438, 1307, 1028, 773, 738, 729, 707 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.87 (s, 3H), 7.30 (d, J = 8 Hz, 1H), 7.38-7.42 (m, 1H), 7.49-7.54 (m, 2H), 7.87 (d, J = 8 Hz, 1H), 8.02-8.06 (m, 1H), 8.37-8.41 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 22.1, 120.6, 122.9, 124.6, 125.0, 126.1, 126.4, 127.1, 133.0, 134.6, 135.8, 138.7, 138.9; CHNS: Calculated for $C_{13}H_{10}S$: C, 78.75; H, 5.08; S, 16.17; Found: C, 78.30; H, 5.05; S, 16.11.

5.1.107.4. Methyl dibenzo[*b,d*]thiophene-3-carboxylate (54d)



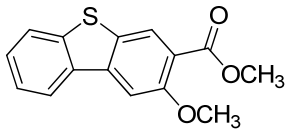
Compound **54d** was obtained from **53d** using general procedure described above as white solid; Yield: 75%; mp: 137-138 ^{o}C; Purity by UPLC: 98.91%; IR (KBr) 2939, 1712, 1597, 1442, 1388, 1288, 1253, 1112, 974, 848, 754 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.90 (s, 3H), 7.55-7.60 (m, 2H), 8.04-8.10 (m, 2H), 8.44-8.47 (m, 1H), 8.51 (d, J = 8.4 Hz, 1H), 8.67 (d, J = 1.6 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 52.3, 122.0, 122.9, 123.0, 124.0, 125.0, 125.1, 127.9, 128.1, 134.0, 138.1, 138.7, 140.2, 165.9; CHNS: Calculated for $C_{14}H_{10}O_2S$: C, 69.40; H, 4.16; S, 13.23; Found: C, 69.48; H, 4.17; S, 13.41.

5.1.107.5. 3-Nitrodibenzo[*b,d*]thiophene (54e)



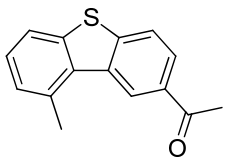
Compound **54e** was obtained from **53e** using general procedure described above as yellow solid; Yield: 49%; mp: 148-150 ^{o}C; Purity by UPLC: 99.76%; IR (KBr) 2962, 1604, 1518, 1448, 1330, 1261, 1103, 1022, 879, 771, 738 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.61-7.66 (m, 2H), 8.14 (d, J = 8 Hz, 1H), 8.32 (dd, J = 2 and 8.8 Hz, 1H), 8.52 (d, J = 8 Hz, 1H), 8.60 (d, J = 8.8 Hz, 1H), 9.08 (d, J = 2 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 118.5, 119.6, 122.5, 123.3, 123.5, 125.3, 128.8, 133.2, 139.1, 140.0, 141.3, 145.8; ESI-MS: m/z: 230 $(M+H)^{+}$; CHNS: Calculated for $C_{12}H_7NO_2S$: C, 62.87; H, 3.08; N, 6.11; S, 13.99; Found: C, 62.86; H, 3.10; N, 6.10; S, 13.95.

5.1.107.6. Methyl 2-methoxydibenzo[*b,d*]thiophene-3-carboxylate (54f)



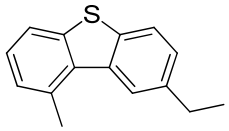
Compound **54f** was obtained from **53f** using general procedure described above as white solid; Yield: 74%; mp: 127-128 ^{o}C; Purity by UPLC: 99.70%; IR (KBr) 1718, 1602, 1546, 1465, 1433, 1396, 1238, 1082, 840, 765 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.82 (s, 3H), 3.96 (s, 3H), 7.51-7.57 (m, 2H), 8.03 (d, J = 8.4 Hz, 1H), 8.10 (s, 1H), 8.29 (s, 1H), 8.49 (d, J = 8.4 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 52.0, 56.3, 105.3, 120, 123.0, 123.1, 124.6, 125.2, 127.9, 129.5, 134.4, 139.0, 140.8, 156.1, 165.9; ESI-MS: m/z: 273 $(M+H)^+$; CHNS: Calculated for $C_{15}H_{12}O_3S$: C, 66.16; H, 4.44; S, 11.77; Found: C, 66.15; H, 4.51; S, 11.65.

5.1.107.7. 1-(9-Methyldibenzo[b,d]thiophen-2-yl) ethanone (54g)



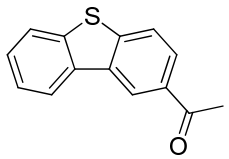
Compound **54g** was obtained from **53g** using general procedure described above as white solid; Yield: 82%; mp: 123-125 ^{o}C; Purity by UPLC: 99.62%; IR (KBr) 1678, 1356, 1313, 1244, 879, 840, 767 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.72 (s, 3H), 2.94 (s, 3H), 7.38 (d, J = 7.2 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.95 (d, J = 8 Hz, 1H), 8.11 (dd, J = 1.6 and 8.4 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 8.90 (d, J = 1.6 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 21.8, 26.7, 120.8, 121.9, 124.6, 125.5, 127, 127.5, 132.5, 133.3, 134.9, 135.6, 139.3, 143.8, 197.3; ESI-MS: m/z: 240.8 $(M+H)^+$; CHNS: Calculated for $C_{15}H_{12}OS$: C, 74.97; H, 5.03; S, 13.34; Found: C, 74.90; H, 4.99; S, 13.10.

5.1.107.8. 8-Ethyl-1-methyldibenzo[b,d]thiophene (54h)



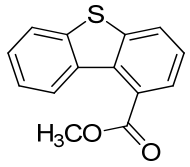
Compound **54h** was obtained from **53h** using general procedure described above as semi solid; Yield: 85%; Purity by UPLC: 99.21%; IR (KBr) 2999, 1460, 1217, 1033, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.30 (t, 3H), 2.83 (q, 2H), 2.88 (s, 3H),7.29 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 0.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 16.0, 22.2, 28.4, 120.6, 122.6, 124.0, 126.2, 126.4, 127.0, 133.0, 134.6, 135.9, 136.0, 139.2, 140.2; CHNS: Calculated for $C_{15}H_{14}S$: C, 79.60; H, 6.23; S, 14.17; Found: C, 79.37; H, 6.33; S, 14.32.

5.1.107.9. 1-(Dibenzo[b,d]thiophen-2-yl)ethanone (54i)



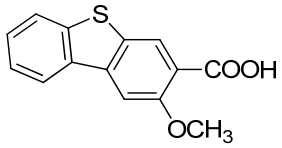
Compound **54i** was obtained from **53i** using general procedure described above as white solid; Yield: 70%; mp: 123-125 ^oC; Purity by UPLC: 99.16%; IR (KBr) 1664, 1446, 1354, 1269, 964, 754, 603 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.77 (s, 3H), 7.51-7.58 (m, 2H), 7.74 (t, 1H), 8.05-8.09 (m, 1H), 8.36 (d, J = 8 Hz, 1H), 8.42-8.46 (m, 1H), 8.70 (d, J = 8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 26.5, 122.0, 122.8, 124.8, 124.8, 126.7, 127.4, 130.1, 130.3, 133.4, 136.6, 137.5, 141.1, 197.7; CHNS: Calculated for $C_{14}H_{10}OS$: C, 74.31; H, 4.45; S, 14.17; Found: C, 74.38; H, 4.43; S, 14.27.

5.1.107.10. Methyl dibenzo[b,d]thiophene-1-carboxylate (54j)



Compound **54j** was obtained from **53j** using general procedure described above as white solid; Yield: 55%; mp: 87-89 ^{o}C; Purity by UPLC: 99.06%; IR (KBr) 1664, 1446, 1354, 1269, 964, 754, 603 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.02 (s, 3H), 7.47-7.51 (m, 1H), 7.54-7.57 (m, 1H), 7.59-7.61 (m, 1H), 7.72 (dd, *J* = 4 and 8 Hz, 1H), 8.10 (d, *J* = 8 Hz, 1H), 8.21 (d, *J* = 8 Hz, 1H), 8.26 (dd, *J* = 4 and 8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 52.8, 123.2, 124.6, 124.7, 125.5, 126.1, 126.2, 127.5, 128.4, 131.0, 132.9, 139.2, 139.9, 168.7; ESI-MS: m/z 265.16 $(M+Na)^{+}$; CHNS: Calculated for $C_{14}H_{10}O_2S$: C, 69.40; H, 4.16; S, 13.21 ; Found: C, 69.47; H, 4.28; S, 13.15.

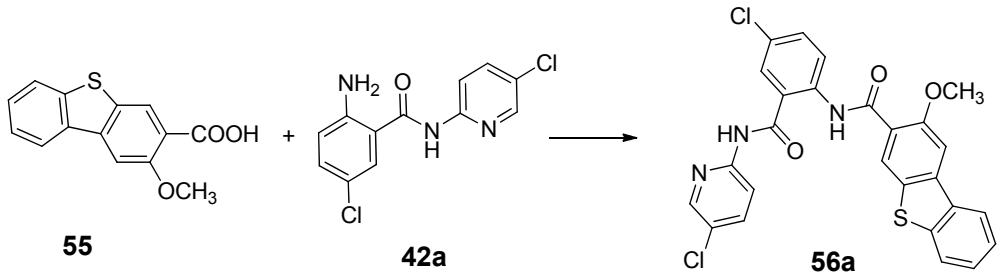
5.1.108. 2-Methoxydibenzo[b,d]thiophene-3-carboxylic acid (55)



To a stirring solution of 2-methoxydibenzo[b,d]thiophene-3-carboxylic acid methyl ester (**54f**, synthesis shown in next section of experimental) (2.2 g, 0.0081 mol) in DMSO (18 ml) was added *t*-BuOK (0.91 g, 0.0081 mol) and water (0.3 g, 0.0162 mol) at 25 ^{0}C. Reaction mixture was stirred at 25-30 ^{0}C for 1 h and then quenched with water (10 v/w) and acidified using dil. HCl. Precipitated solid was filtered and dried to get 1.8 g of title compound as off-white solid; Yield: 86%; mp: 260-263 ^{0}C; Purity by UPLC: 98.86%; IR (KBr) 3228, 1728, 1608, 1467, 1417,

1220, 1010, 854, 725 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.97 (s, 3H), 7.51-7.58 (m, 2H), 8.02-8.04 (dd, J = 1.6 and 6.4 Hz, 1H), 8.08 (s, 1H), 8.27 (s, 1H), 8.48-8.50 (m, 1H), 12.81 (s, 1H); ESI/MS m/z 258.9 $(M+H)^+$.

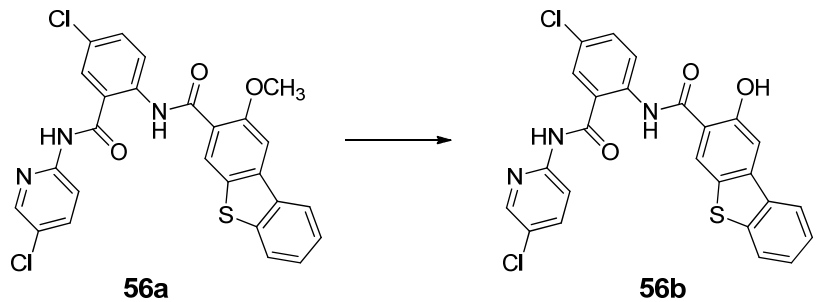
5.1.109. N-(4-Chloro-2-((5-chloropyridin-2-yl)carbamoyl)phenyl)-2-methoxy dibenzo[b,d] thiophene-3-carboxamide (56a)



55 **42a** **56a**

This compound was prepared from **55** and **42a** by means of a procedure similar to that reported for **43a** as off-white solid; Yield: 42%; mp: 284 ^0C; Purity by HPLC: 98.15%; IR (KBr) 3225, 1681, 1639, 1599, 1572, 1498, 1460, 1400, 1371, 1292, 1217, 1114, 1028, 914, 825, 759 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.14 (s, 3H), 7.53-7.60 (m, 2H), 7.65-7.68 (dd, J = 2.4 and 8.8 Hz, 1H), 7.87 (d, J = 2.4 Hz, 1H), 8.02-8.06 (m, 2H), 8.18 (s, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.48 (d, J = 2.4 Hz, 1H), 8.53 (d, J = 7.2 Hz, 1H), 8.59 (d, J = 8.8 Hz, 1H), 8.63 (s, 1H), 11.40 (s, 1H), 11.61 (s, 1H); ESI/MS m/z 522 $(M+H)^+$.

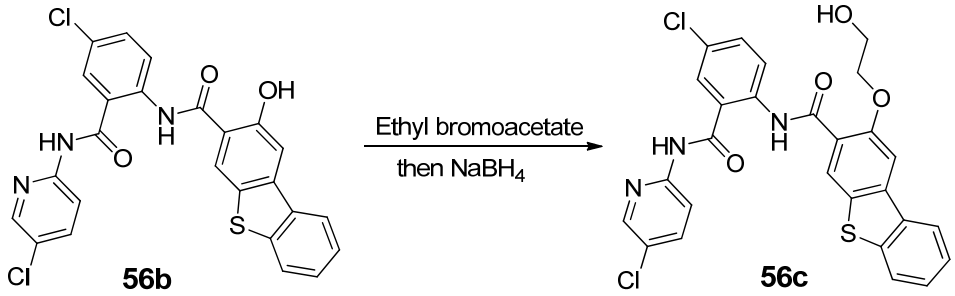
5.1.110. N-(4-Chloro-2-((5-chloropyridin-2-yl)carbamoyl)phenyl)-2-hydroxy dibenzo [b,d] thiophene-3-carboxamide (56b)



56a **56b**

To a stirring solution of **56a** (1.3 g, 0.0025 mol) in CH_2Cl_2 (10 mL) was added BBr_3 (10 v/w 1 M solution in CH_2Cl_2) at -50 ^{0}C under N_2 atmosphere. Reaction mixture was stirred at 25-30 ^{0}C for 3 h. CH_2Cl_2 was distilled out and then diluted with aq. $NaHCO_3$ solution. Precipitated solid was filtered and dried to get yellow solid; Yield: 80%; mp: 254-256 ^{0}C; Purity by UPLC: 98.00%; IR (KBr) 3336, 1662, 1600, 1518, 1458, 1402, 1373, 1294, 1192, 1114, 918, 829, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.49-7.53 (m, 1H), 7.54-7.58 (m, 1H), 7.64-7.67 (dd, J = 2.4 and 8.8 Hz, 1H), 7.82 (t, 2H), 7.99-8.03 (m, 2H), 8.18 (d, J = 8.8 Hz 1H), 8.24 (d, J = 7.6 Hz, 1H), 8.41 (d, J = 9.2 Hz, 1H), 8.45 (d, J = 2.4 Hz 1H), 8.55 (s, 1H), 11.33 (s, 1H), 11.57 (s, 1H), 11.72 (s, 1H); ESI/MS m/z 506.0 (M-H).

5.1.111. N-(4-Chloro-2-((5-chloropyridin-2-yl)carbamoyl)phenyl)-2-(2-hydroxyethoxy) dibenzo[b,d]thiophene-3-carboxamide (56c)



To a stirring solution of **56b** (1 g, 0.00195 mol) in DMF (5 v/w) was added 60% NaH (95 mg, 0.00234 mol) at 10-15 ^{0}C To this was added ethyl bromoacetate (0.39 g, 0.00234 mol) in one lot. Reaction mixture was stirred at 55 ^{0}C for 3 h and then cooled and quenched with water (10 v/w). Precipitated product was filtered and dissolved in DMSO (4 v/w) and EtOH (4 v/w). $NaBH_4$ (0.25 g, 0.25 w/w) was added to it and reaction mixture was heated at 55-60 ^{0}C for 4 h. Reaction mixture was cooled and diluted with water. Precipitated product was filtered, dried and

then column purified using 100-200 silica gel and 0.5% MeOH in CH_2Cl_2 as mobile phase to get title compound as off-white solid; Yield: 15%; mp: 252-254 ^{0}C; Purity by UPLC: 99.30%; IR (KBr) 3493, 1649, 1600, 1573, 1512, 1456, 1375, 1301, 1211, 1212, 837, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.85 (d, J = 4.4 Hz, 2H), 4.45 (t, J = 4.8 Hz, 2H), 4.92 (s, 1H), 7.52-7.59 (m, 2H), 7.65-7.68 (dd, J = 2.4 and 8.8 Hz, 1H), 7.86 (d, J = 2.0 Hz, 1H), 7.96-7.99 (dd, J = 2.4 and 8.8 Hz, 1H), 8.05 (d, J = 7.2 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 8.23 (s, 1H), 8.36 (d, J = 9.2 Hz, 1H), 8.44 (d, J = 2.0 Hz, 1H), 8.49-8.50 (m, 2H), 11.30 (s, 2H); ESI/MS m/z 551.9 (M-H).

5.2. Biology

5.2.1. Plasminogen Activator Inhibitor-1 (PAI-1)

5.2.1.1. *In vitro* PAI-1 inhibition using chromogenic assay

In vitro PAI-1 inhibitory activity of compounds was determined using chromogenic assay that was based upon the interaction between tPA and active PAI-1. tPA coated assay plates obtained from Trinity Biotech., NY, USA were kept at 4 ^{o}C. Required quantity of phosphate buffer containing EDTA and tween 20 was added in each well and incubated for 2 min at 27-28 ^{o}C with gentle shaking in order to dissolve tPA. Compounds were dissolved in DMSO and diluted to a range of concentration between 1 and 100 µM. Varying concentrations of the compounds were then incubated with human PAI-1 (50 nM, Molecular Innovations, MI, USA) for 30 min at 25 ^{o}C. An aliquot of this solution along with a monoclonal antibody against human PAI-1 conjugated with HRP (Trinity Biotech., NY, USA) was added to the t-PA-coated plate. The Plate was then incubated for 30 min at 27-28

^{o}C with gentle shaking. The solution was aspirated from the plate, which was then washed thrice with a buffer consisting of 0.05% tween 20 and 0.1% BSA in PBS. Aliquot of 100 µL of HRP substrate solution was added and incubated for 5 min at 25 ^{o}C. Reaction was terminated with the addition of 50 µL of 1.6 M H_2SO_4 followed by the determination of absorbance at 490 nm. This assay detects only active inhibitory PAI-1 bound to the plate. The quantization of residual active PAI-1 bound to t-PA at varying concentrations of compounds was used to determine the IC_{50} by fitting the results to a logistic dose-response program (Graphpad Prism, CA, USA). IC_{50} was defined as the concentration of compound required to achieve 50% inhibition of PAI-1 activity. The assay sensitivity was 5 ng/mL of human PAI-1 as determined from a standard curve ranging from 0-100 ng/mL of human PAI-1.

5.2.1.2. *In vivo* efficacy study in rats

In this study $FeCl_3$-induced chemical injury was used as a model of arterial thrombosis in rat model. Rats (n = 6) were anaesthetized with urethane (1.25 g/kg, ip) and secured in supine position. A midline cervical incision was made on the ventral side of the neck, and left carotid artery was isolated by blunt dissection. Compounds (each 30 mg/kg) were formulated in polyethylene glycol (PEG) and 0.5% sodium carboxymethyl cellulose (CMC) (1:10) and administered by oral gavage. Exactly after 2 h of administration, a 2 x 3 mm strip of Whatman # 1 filter paper saturated with 35% (w/v) $FeCl_3$ was placed on the carotid artery for 5 min. A temperature probe (Thermalert-TH8, Physitemp Instruments Inc., Clifton, N.J., USA) was placed distal to filter paper to monitor the temperature of

carotid artery. A sudden fall in temperature (about 1–2 ^{o}C) was taken as an indication of cessation of blood flow as a result of thrombus formation. Time to occlusion (TTO) was defined as the time from $FeCl_3$ application to time of thrombus formation (indicated by sudden fall in carotid temperature). In case, no thrombus formation was seen in drug-treated animals, a cutoff time was fixed at 1 h.

5.2.2. Coagulation Factor Xa

5.2.2.1. *In vitro* FXa inhibition assay

The inhibitory activity of different compounds against purified serine proteases was measured using chromogenic substrates in 96-well microtiter plates at RT. The enzymes were incubated with test compound or its solvent, dimethyl sulfoxide (DMSO). The plate was incubated at 37 ^{0}C for 45 min. At the end of incubation the plate was read at 405 nm using a Spectra Max. Following buffer (final concentrations) was used in the Assay: human FXa (10 ng), 50mM Tris–HCl buffer pH 7.5, 150 µM NaCl, and 1mM calcium chloride and 500 µM substrate (S-2765) (Hyphen Biomed), 2.5 % DMSO with varying concentrations of test compound. The % Inhibition was based on comparing values from wells without any inhibitor [only substrate, enzyme and buffer (Test Well)] and wells without any enzyme [only substrate and buffer (served as substrate blank wells)]. The IC_{50} was the amount of inhibitor required to inhibit 50% enzymatic activity compared to control.

5.2.2.2. *In vitro* prothrombin time prolongation in human and rat plasma

Blood was obtained from healthy human volunteers or rat in 3.8% sodium citrate. Plasma was obtained after centrifugation at 2000 g for 10 min. An initial stock solution of the inhibitor was prepared in DMSO. Subsequent dilutions were done in plasma. Clotting time was determined on control plasma and plasma containing five to seven different concentrations of inhibitor. Prothrombin time (PT) measurement was performed in a temperature-controlled automated coagulation device (Sysmex CA50, Dade-Behring) using Thromborel-S (Dade Behring) kit according to the reagent instructions. Determinations at each plasma concentration were done in duplicate. Anticoagulant activity was defined as the concentration required to double the prothrombin time [PTCT2].

5.2.2.3. *Ex vivo* PT prolongation in rat

Male rats weighing 230–280 g was used in these studies. The test drug was formulated in polyethyleneglycol (PEG)-400:0.5% sodium carboxymethylcellulose (1:9 v/v) and administered to rats orally at 30 mg/kg/5 ml using a gastric tube. Blood was collected from retro orbital plexus 2 hours after oral administration and citrated. Platelet-poor plasma was prepared by centrifugation for measurement of PT. All data were expressed as relative fold values, compared with the baseline value of the vehicle group in rat.

5.2.2.4. Enzyme selectivity assay

Reaction mixtures were prepared in 96-well plates containing the chromogenic substrate and test compound. The reaction was initiated by the addition of enzyme, and the color was continuously monitored at 405 nm using a microplate

reader SpectraMax 340PC (Molecular Devices, CA, U.S.) at 37 ℃. Each enzyme was used at final concentration as follows: 0.024 U/mL FXa, 0.080 U/mL thrombin, 0.040 μg/mL trypsin, 0.040 U/mL plasmin, 4000 U/mL t-PA and 0.12 μg/mL aPC. The enzymatic activities were assessed by the amidolysis of the following chromogenic substrates for the corresponding protease: S-2765 (FXa), S-2238 (thrombin), S-2222 (trypsin), S-2302 (plasmin), S-2288 (t-PA) and S-2366 (aPC). The rate of substrate hydrolysis (mOD min^{-1}) was measured at 37 ℃. The mode of inhibition was estimated from a Lineweaver–Burk plot. The K_i was determined from a Dixon plot by plotting the reciprocal of the initial reaction velocities at different substrate concentrations against different inhibitor concentrations.

5.2.2.5. $FeCl_3$-induced arterial thrombosis in rat

Male rats (n = 10) were treated orally with compound at 10 and 30 mg/kg/5 ml using a gastric tube and then subjected to $FeCl_3$-induced arterial thrombosis after 2 hours of administration. Rats were anaesthetized with urethane (1.25 g/kg, intraperitoneally). A midline cervical incision was made on the ventral side of the neck, and left carotid artery was isolated. A 2×3 mm strip of Whatman filter paper no. #1 saturated with 35% (w/v) $FeCl_3$ was kept on the carotid artery for 5 min. One hour after removal of the filter paper, arterial thrombus was excised, blotted of excess blood and immediately weighed.

5.2.2.6. Partial stasis combined with $FeCl_3$-induced venous thrombosis in rat

Male Wistar rats (180-250 g, n = 10) were treated orally with compound at 10 and 30 mg/kg/5 ml using a gastric tube and then subjected to $FeCl_3$-induced arterial

thrombosis after 2 h of administration. Rats were anesthetized with urethane (1.25 g/kg, intraperitoneally) after 2 hours of compound administration. The abdomen was opened by making an incision along the linea alba towards the sternum, followed by exposition of the posterior vena cava. Partial stasis was induced in the posterior vena cava by tying a cotton thread together with a blunt needle (21 G, BD) just caudally of the junction of the posterior vena cava and left renal vein. The needle was then removed. A round piece of Wattmann #1 filter paper saturated with 7 µl of 6% w/v $FeCl_3$ solution was then applied to the external surface of the posterior vena cava for 5 min and then removed. Warm saline was sprayed over tissues, and muscle layer and skin were provisionally closed. One hour after removal of the filter paper, ligatures were applied near the bifurcation of the posterior vena cava and around all side branches of the ligated posterior vena cava segment. The ligated venous segment was excised, the thrombus removed, blotted of excess blood and immediately weighed.

5.3. Pharmacokinetic study in rats

Compounds were formulated with a Tween-80:polyethylene glycol (PEG):0.5% carboxymethyl cellulose in water (CMC) (5:5:90% v/v), A graduated dose volume (5 ml/kg) of suspension was administered to fasted male Wistar rats at 30 mg/kg po. The animals were anesthetized for blood sample collection from retro-orbital plexus. Serial blood samples were collected into heparinised containers at various time points and blood centrifuged to yield plasma. Plasma concentration was determined by using LC–MS/MS method.

5.4. In silico study

5.4.1. Plasminogen Activator Inhibitor-1 (PAI-1)

X-ray crystal structure of PAI-1 (PDB code: 3Q03) was obtained from PDB database. Protein crystal structure of PAI-1 was prepared using the Schrödinger's protein preparation wizard module. Docking study was carried out by using the induced fit docking (Schrödinger Suite 2010 Induced Fit Docking protocol; Glide version 5.6, Schrödinger, LLC, New York, NY, 2010; Prime version 2.2, Schrödinger, LLC, New York, NY, 2010).

5.4.2. Coagulation Factor Xa

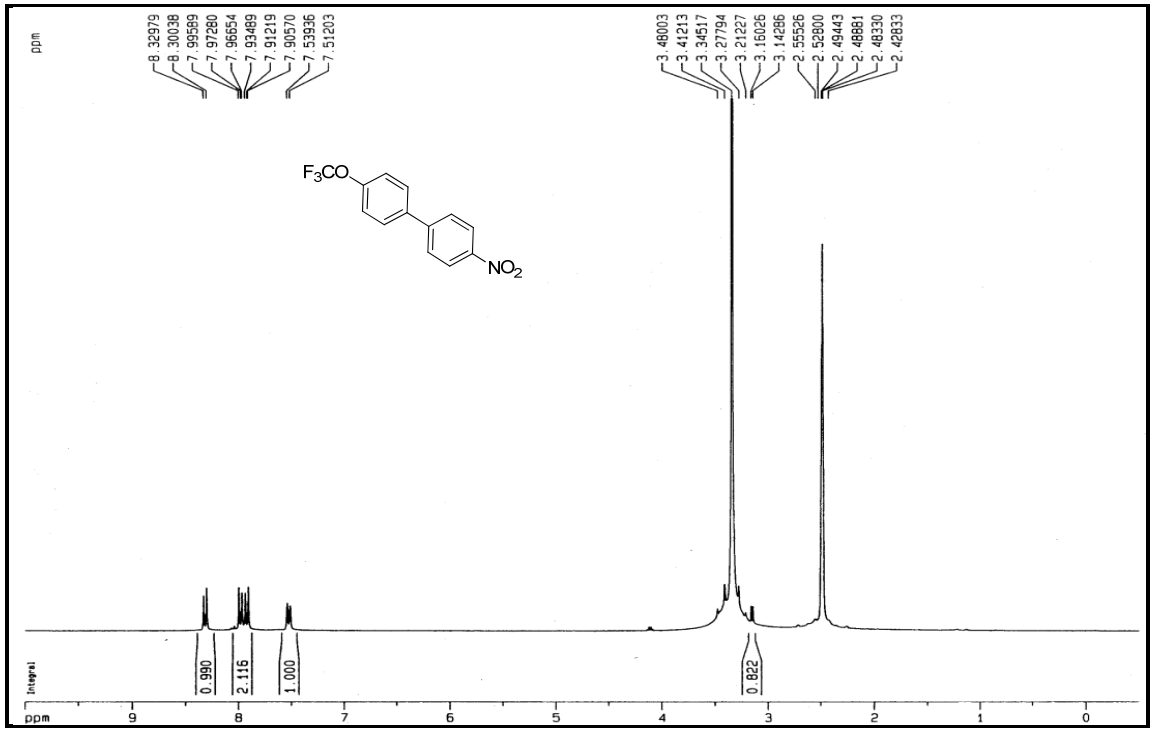
Docking studies of the molecules **46f** and **46b** were carried out to understand the binding mode and intermolecular hydrogen bonding interactions of the molecules with FXa. Glide version 5.7 (GLIDE 5.7, Schrodinger, LLC, New York, NY, 2011) with default parameter and OPLS force-field was used. Molecules were minimized with *LigPrep version 2.5* (LigPrep 2.5, Schrodinger, LLC, New York, NY, 2011) and coordinates for the docking study was conducted using 1MQ6 obtained from RCSB protein data bank.

Spectra

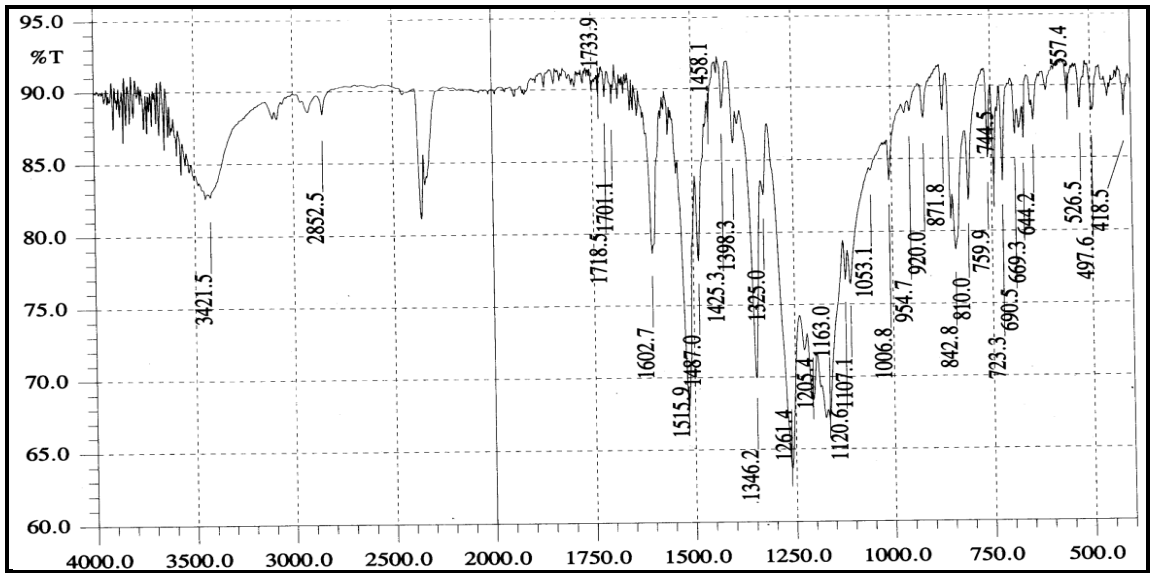
6. SPECTRA

6.1. Plasminogen Activator Inhibitor-1 (PAI-1) Inhibitors

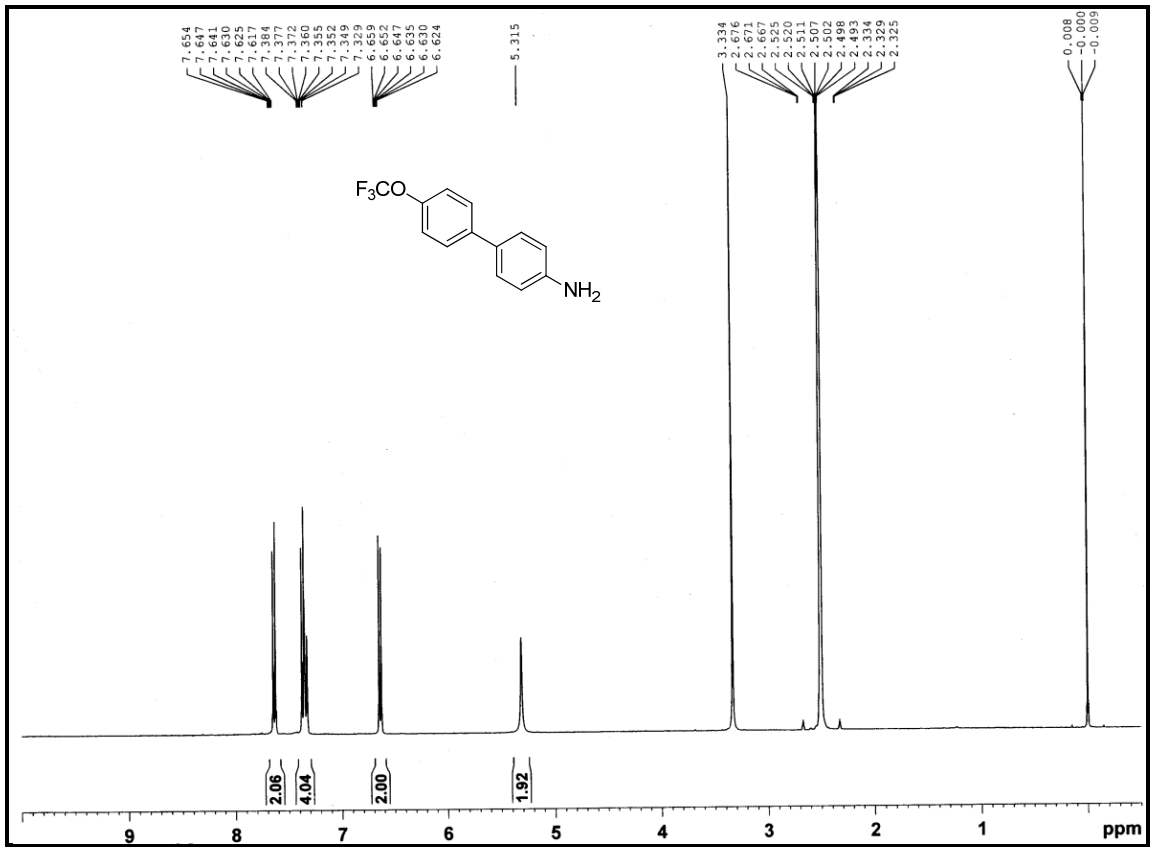
^{1}H NMR of 4



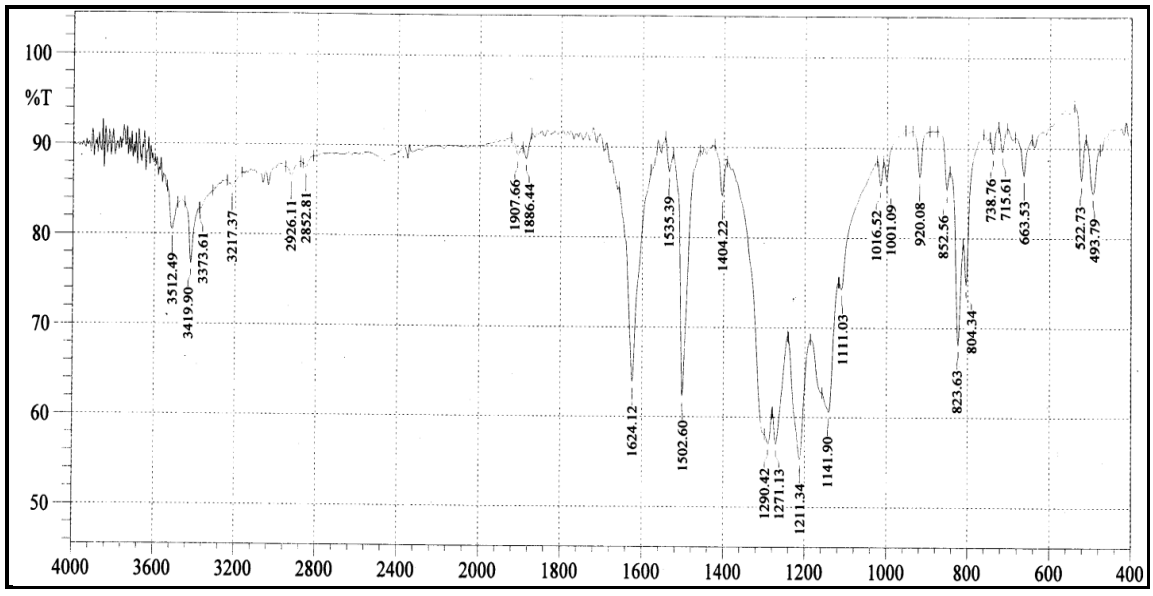
IR of 4



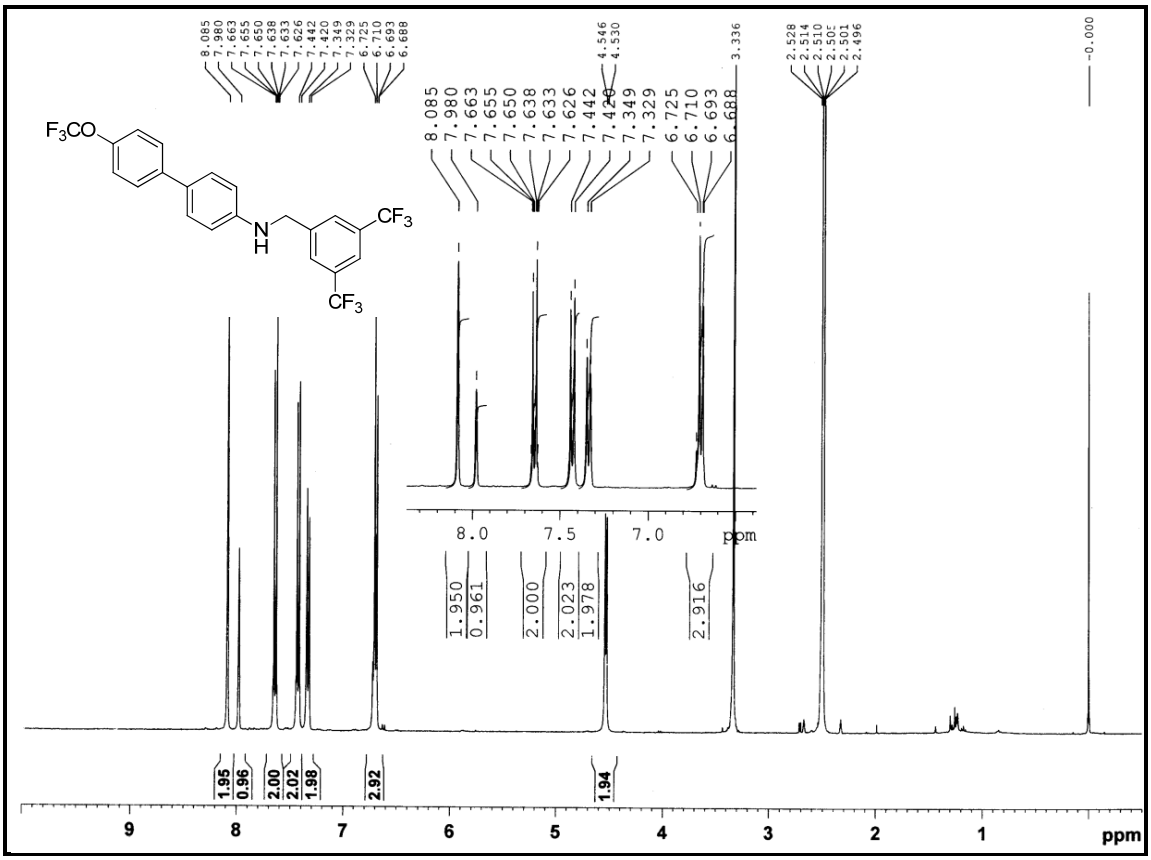
^{1}H NMR of 5



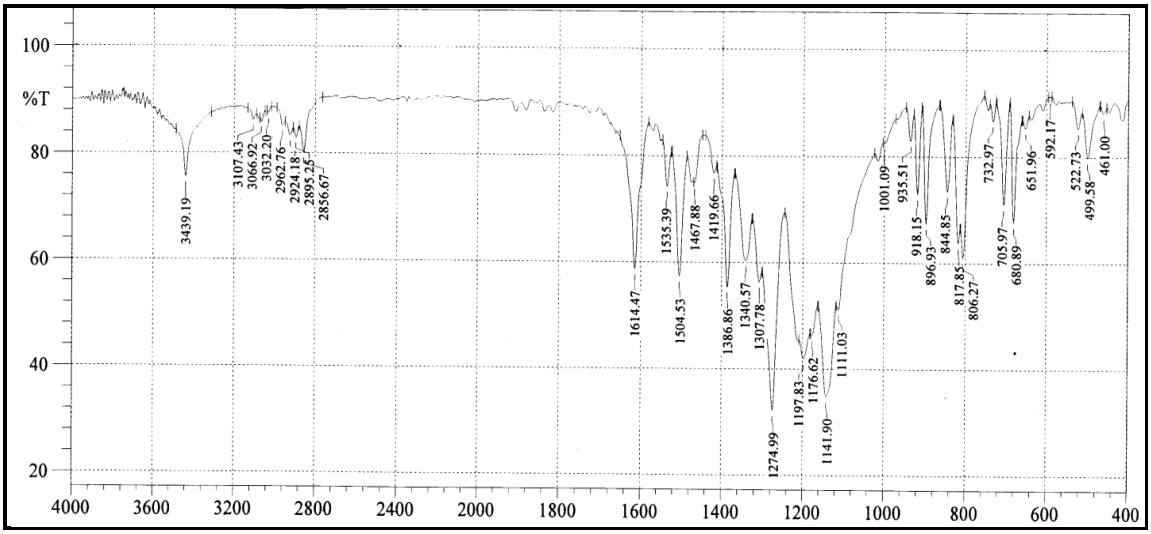
IR of 5



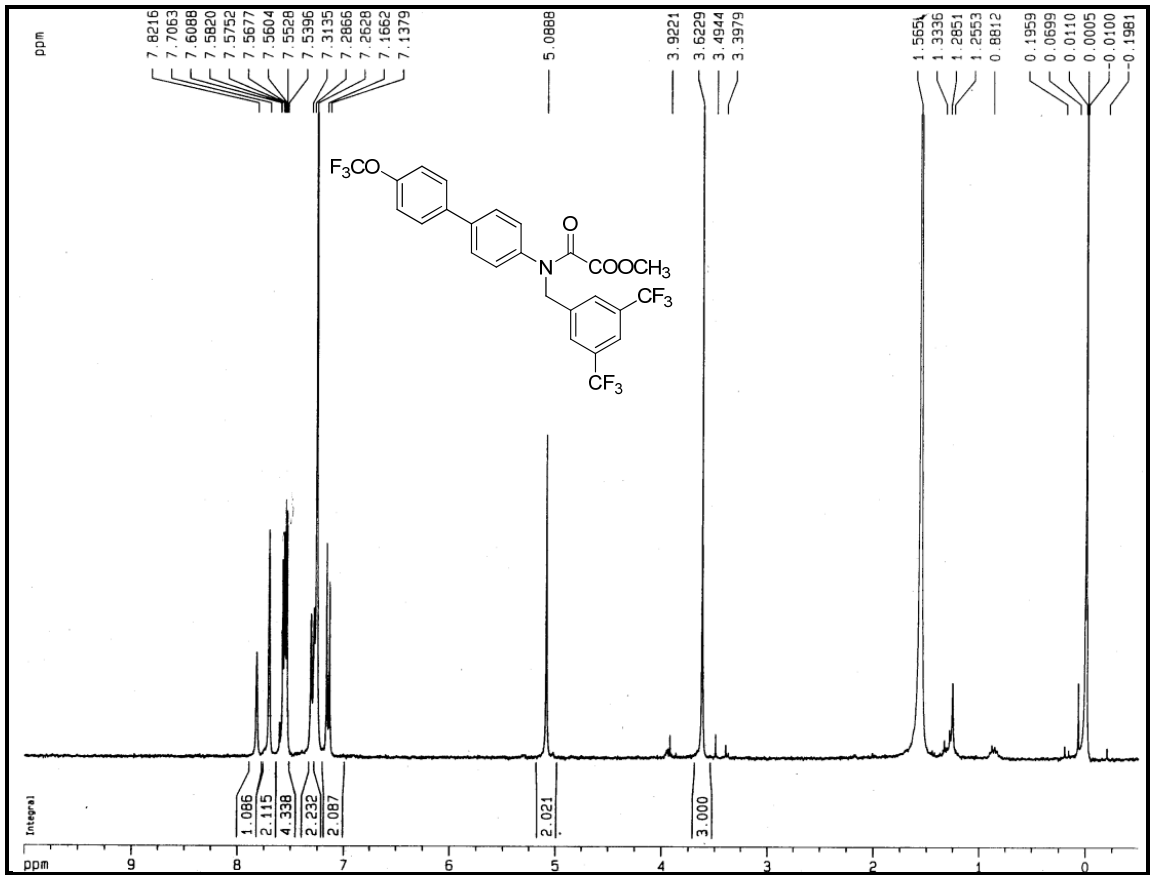
^{1}H NMR of 6d



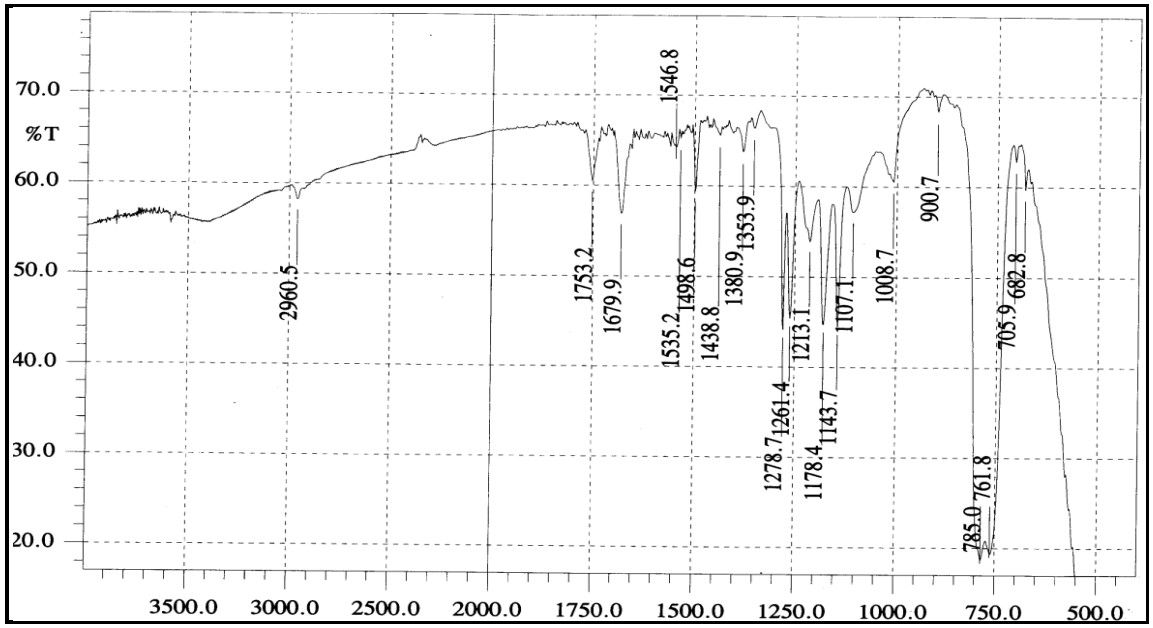
IR of 6d



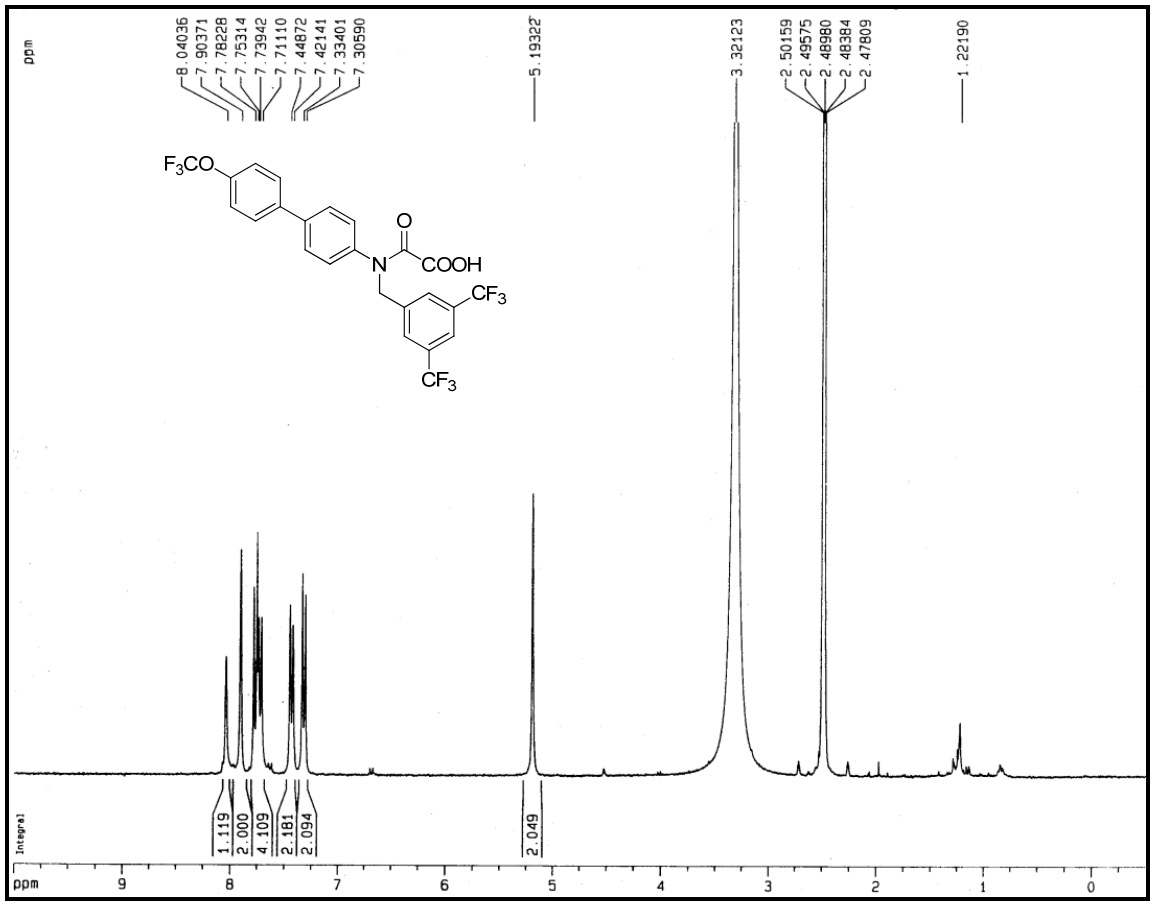
^{1}H NMR of 7d



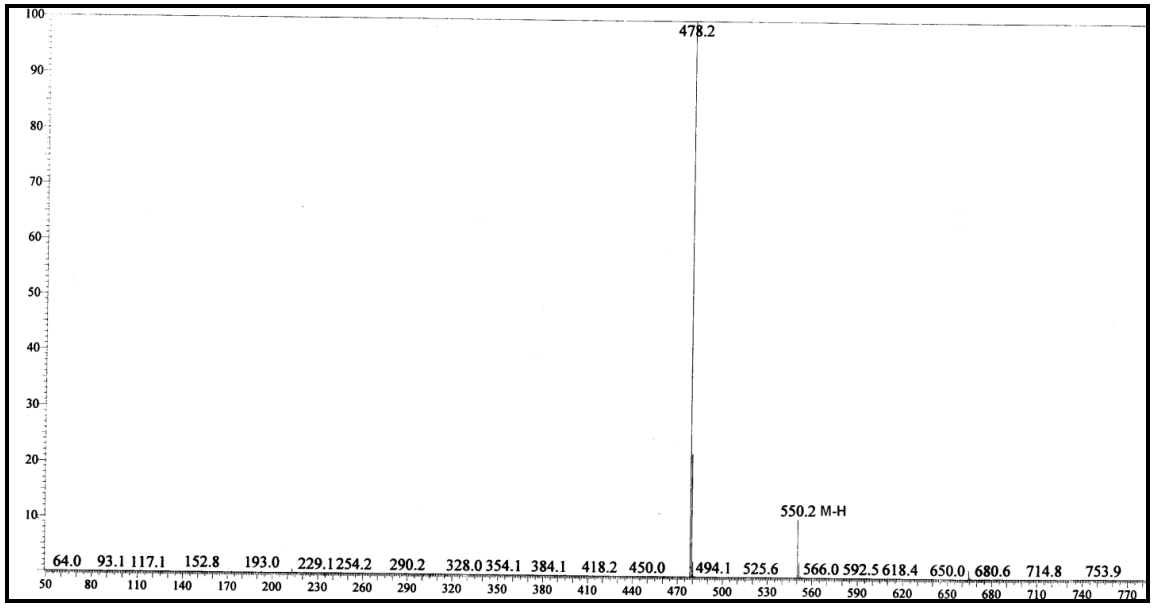
IR of 7d



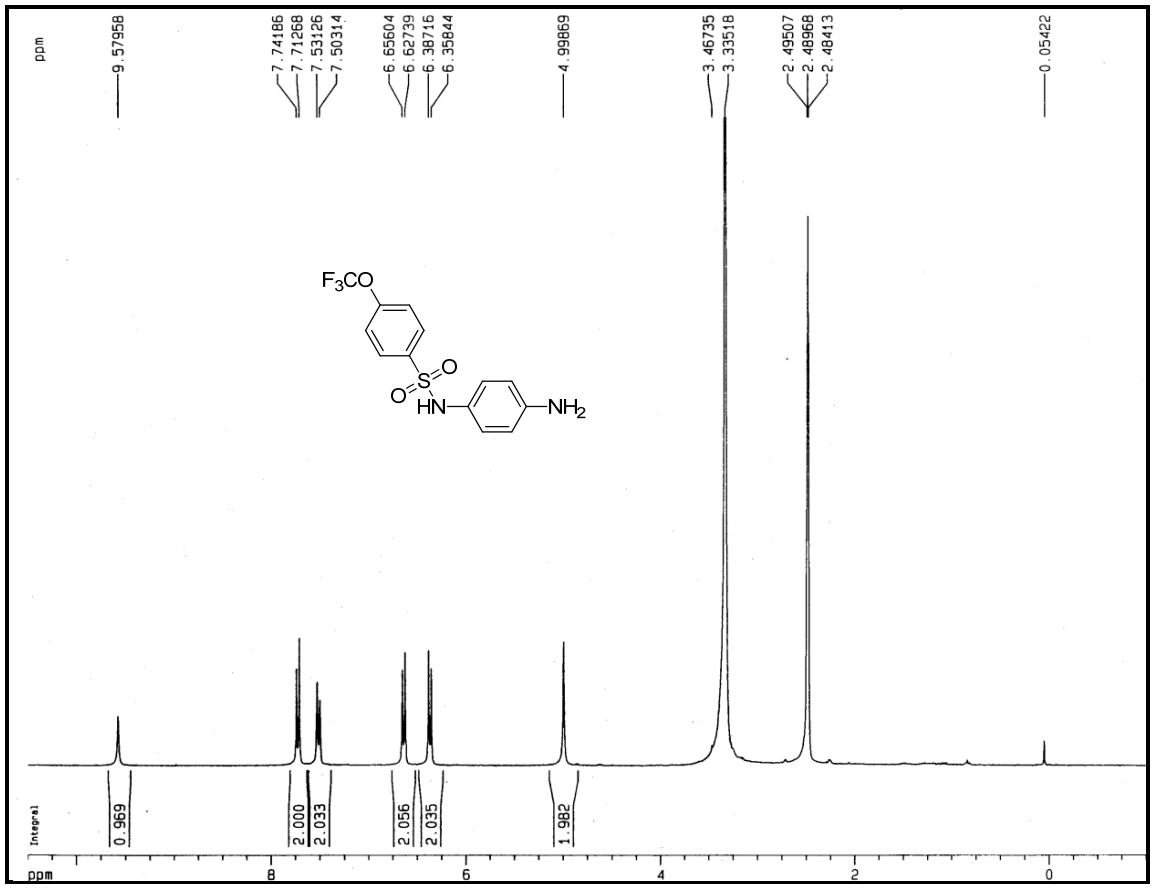
^{1}H NMR of 8d



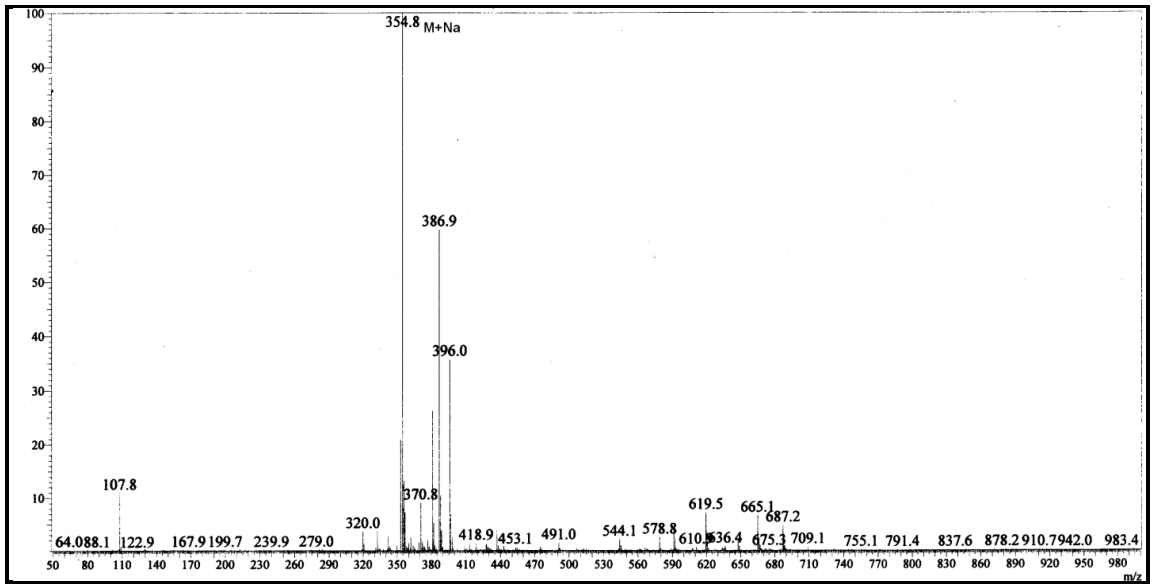
ESI-Mass of 8d



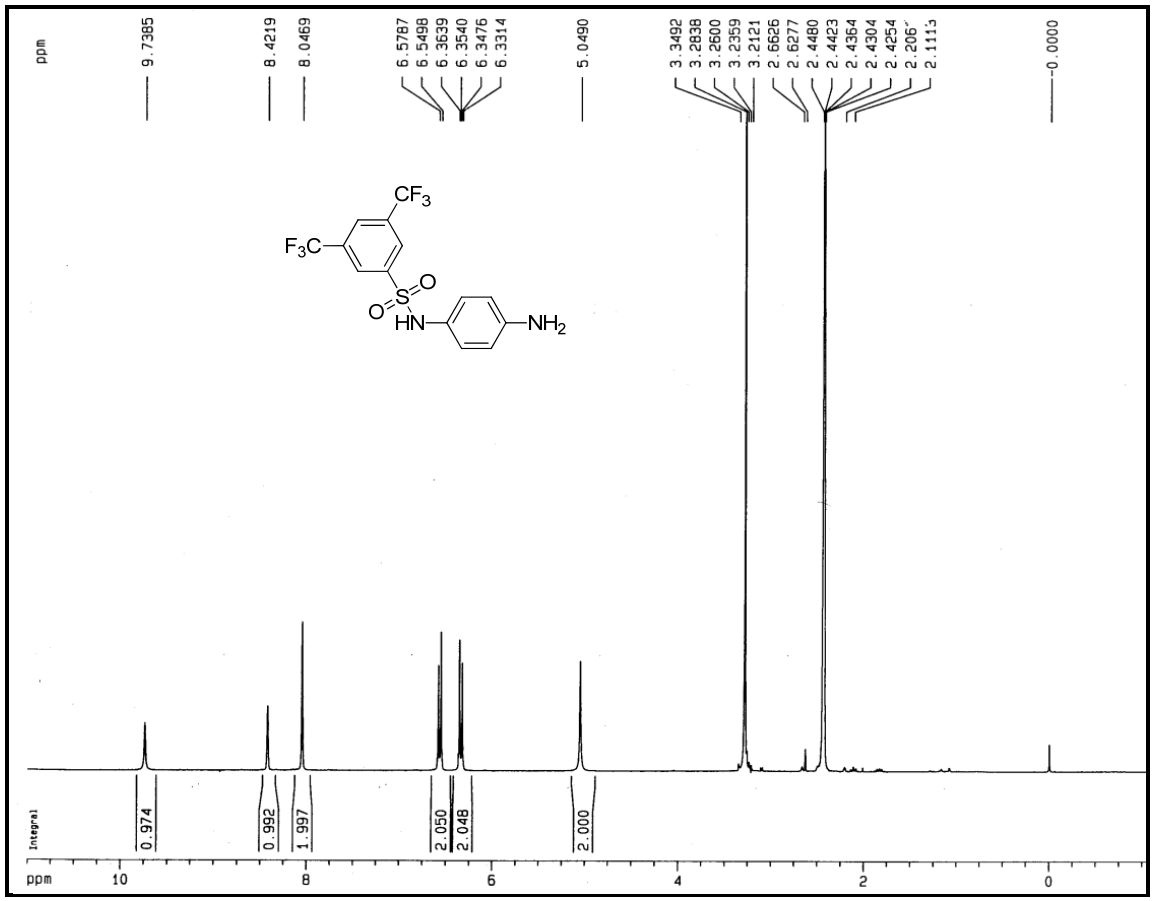
^{1}H NMR of 11d



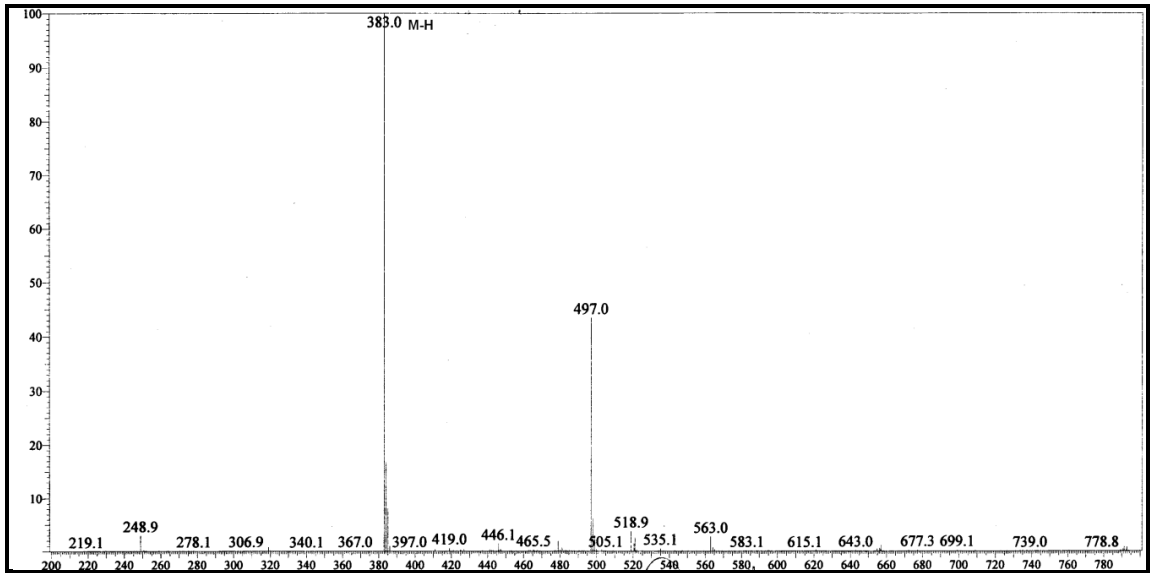
ESI-Mass of 11d



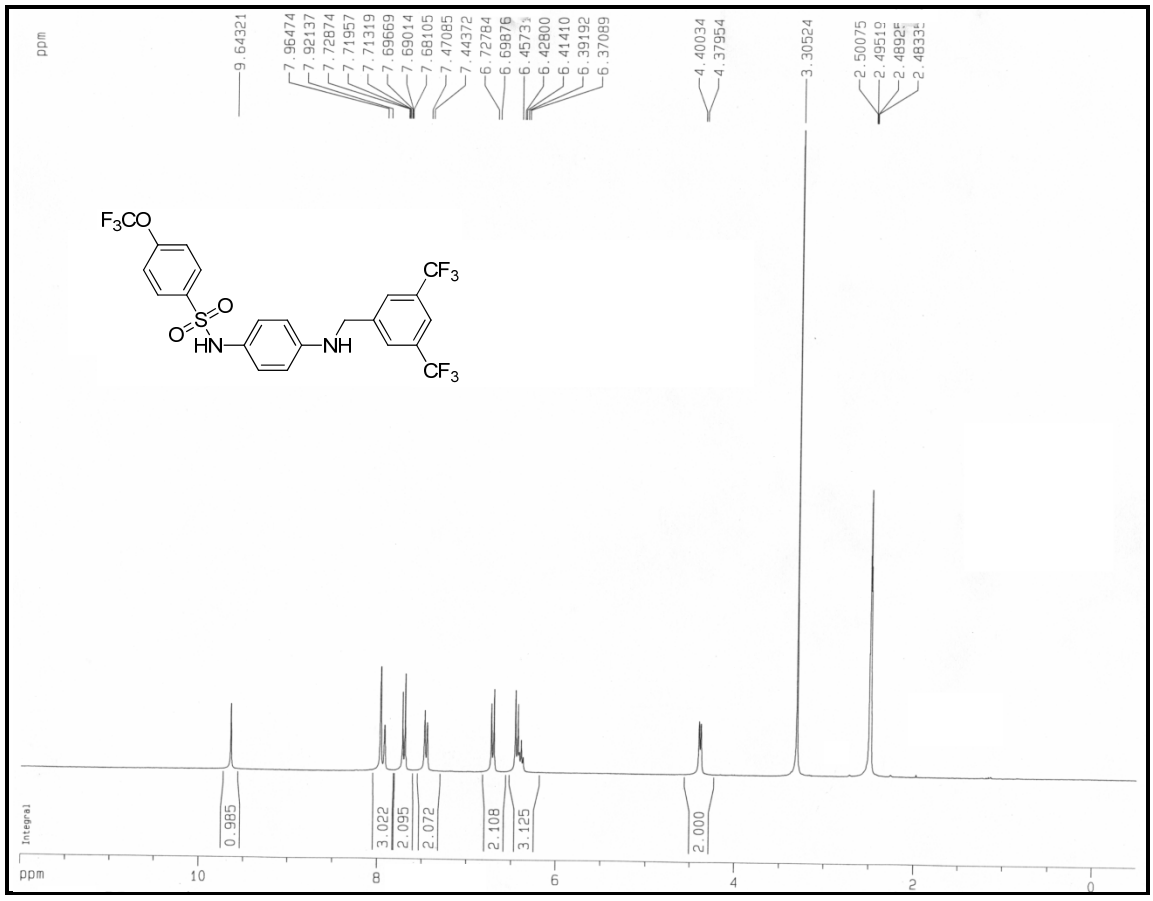
^{1}H NMR of 11i



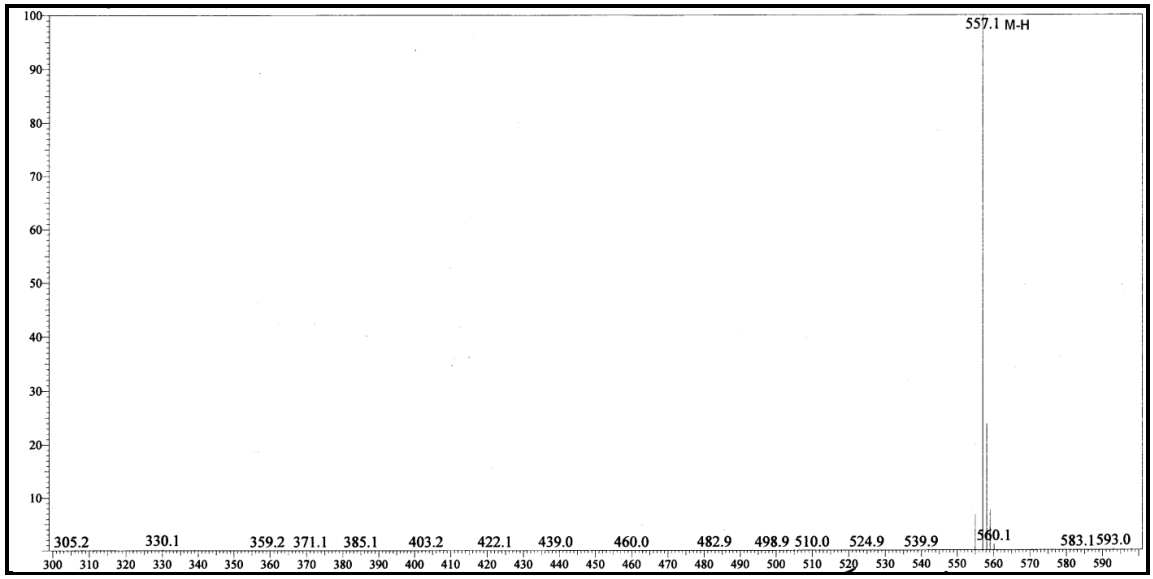
ESI-Mass of 11i



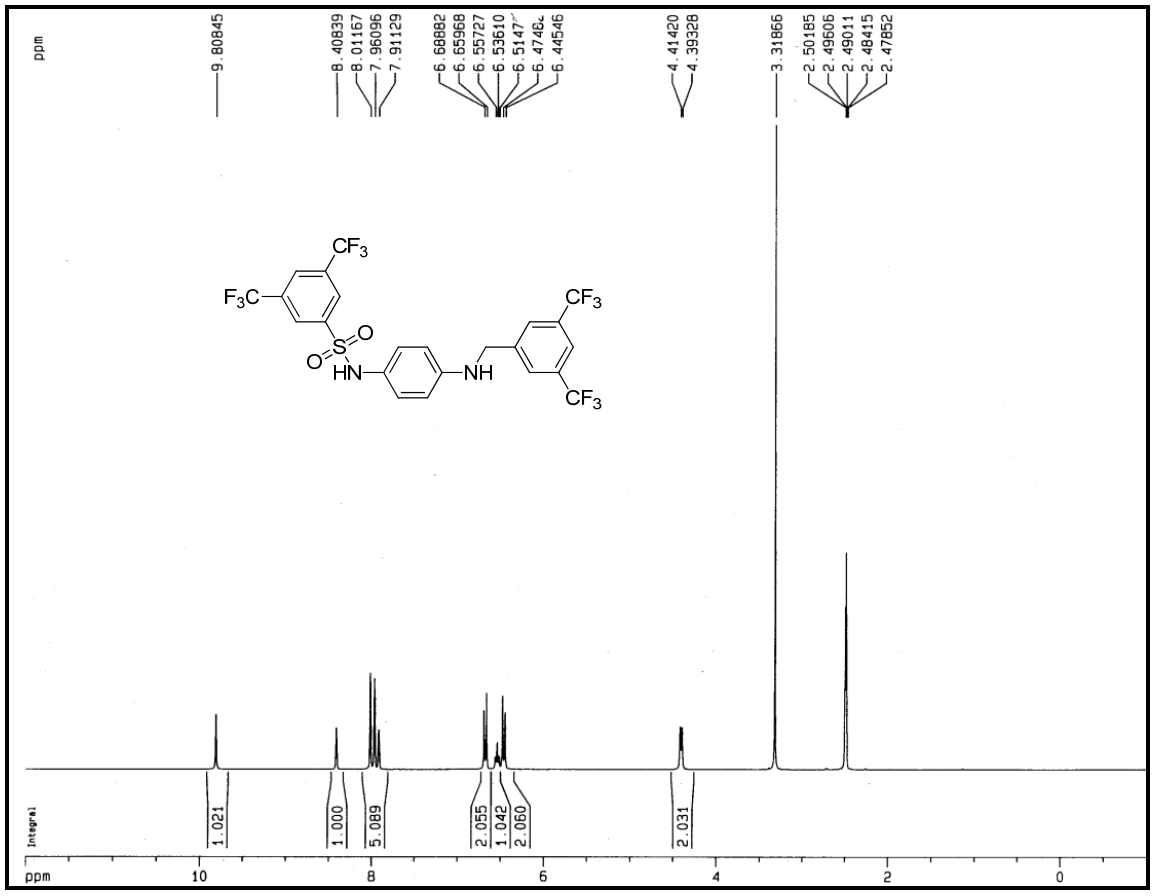
^{1}H NMR of 12d



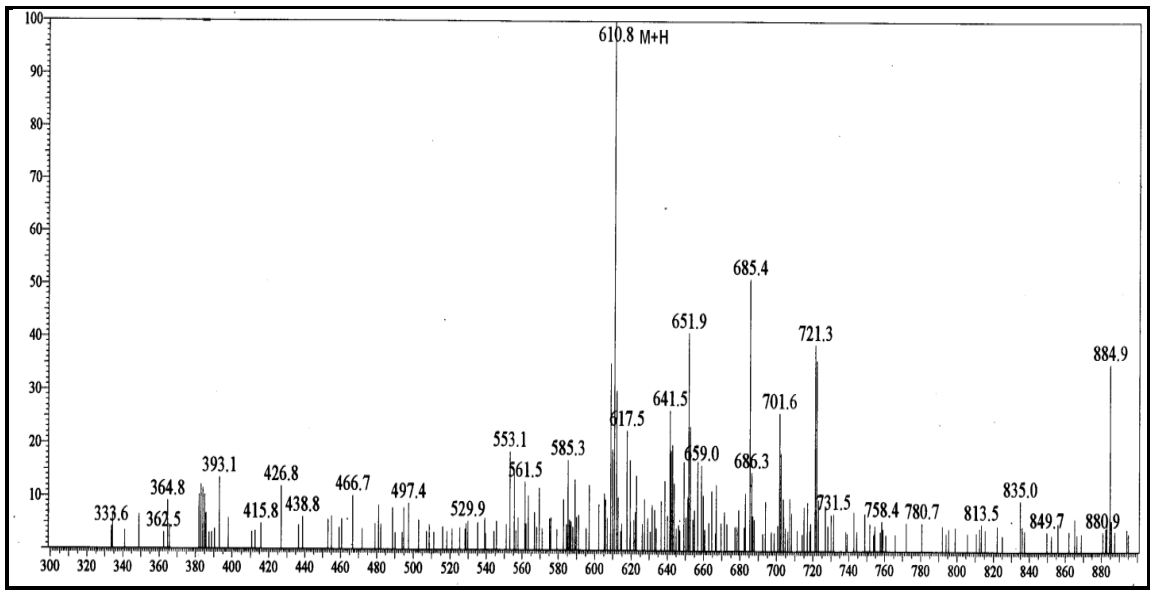
ESI-Mass of 12d



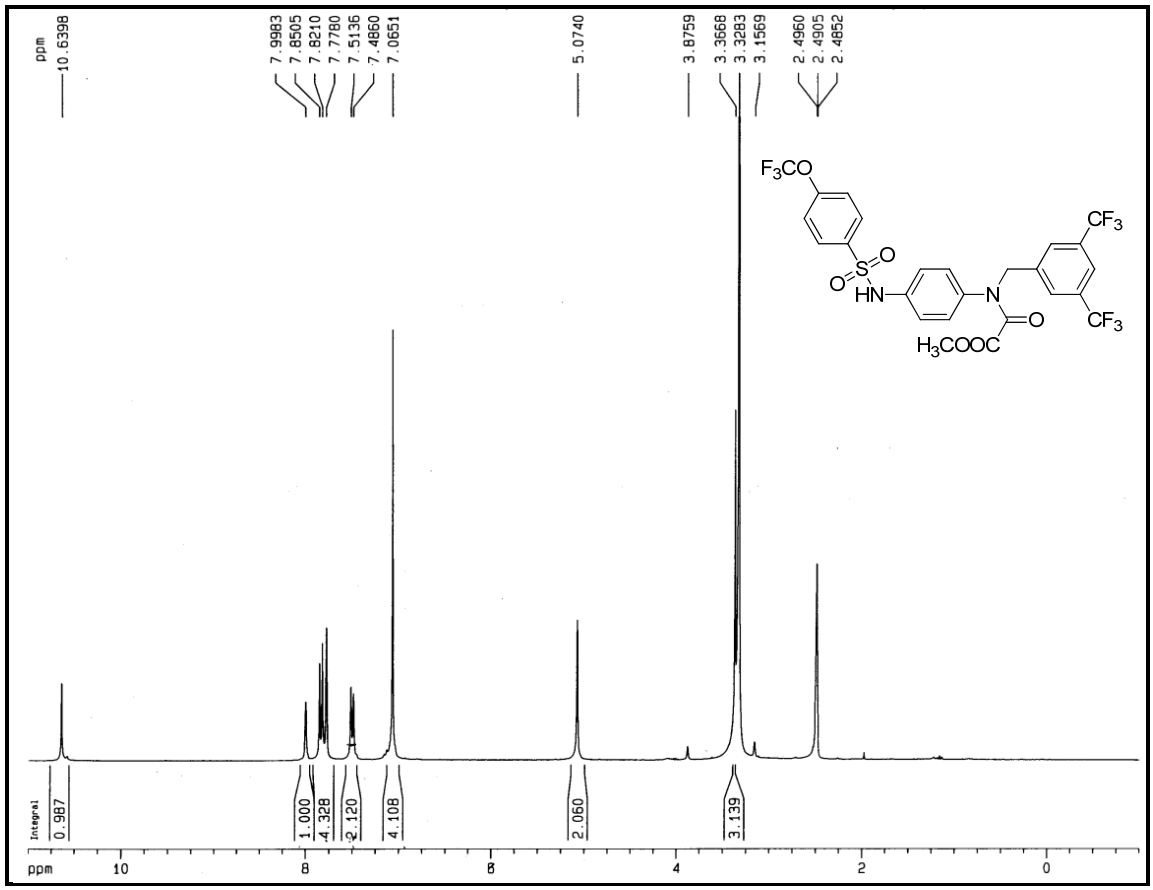
^{1}H NMR of 12i



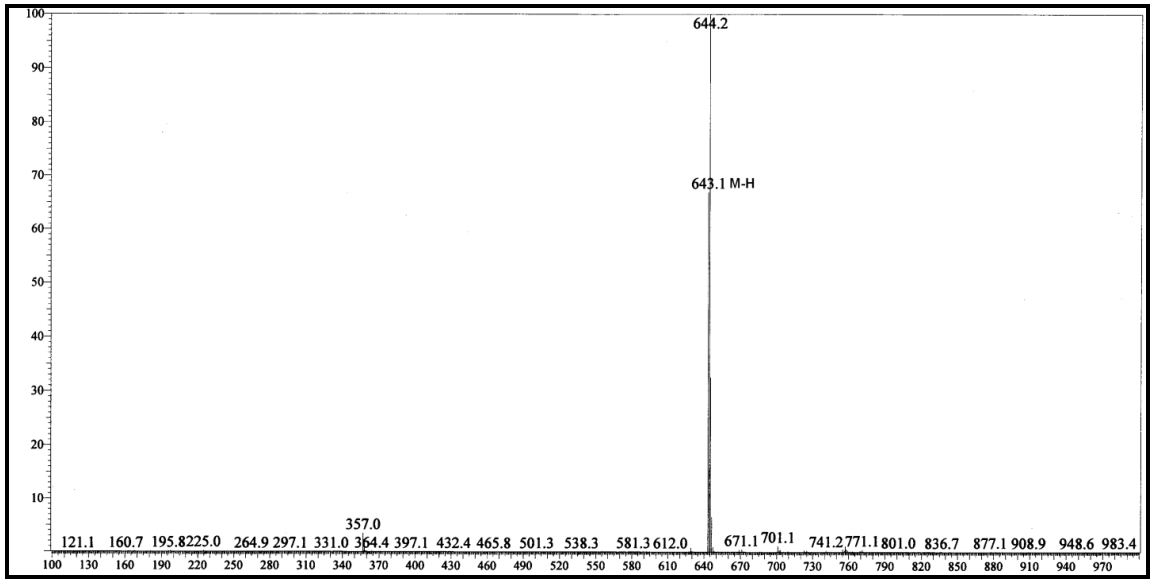
ESI-Mass of 12i



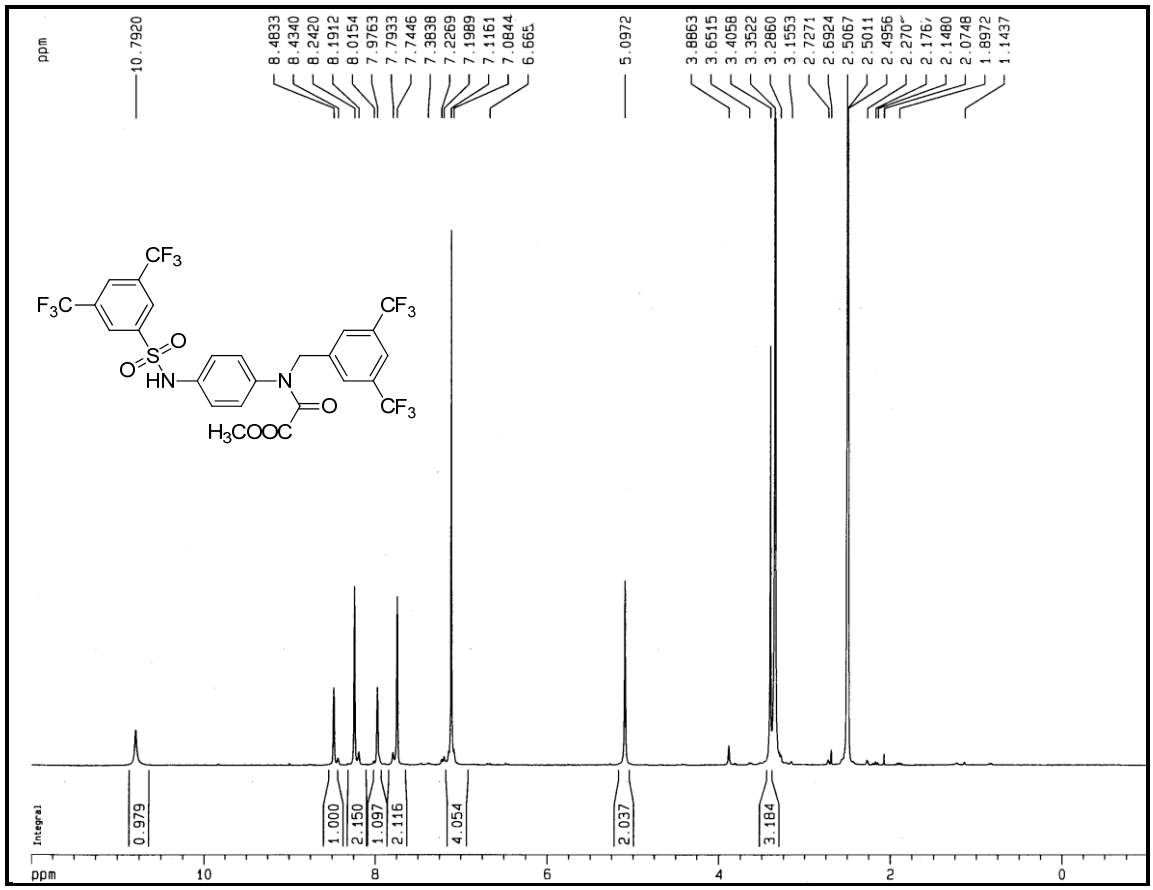
^{1}H NMR of 13d



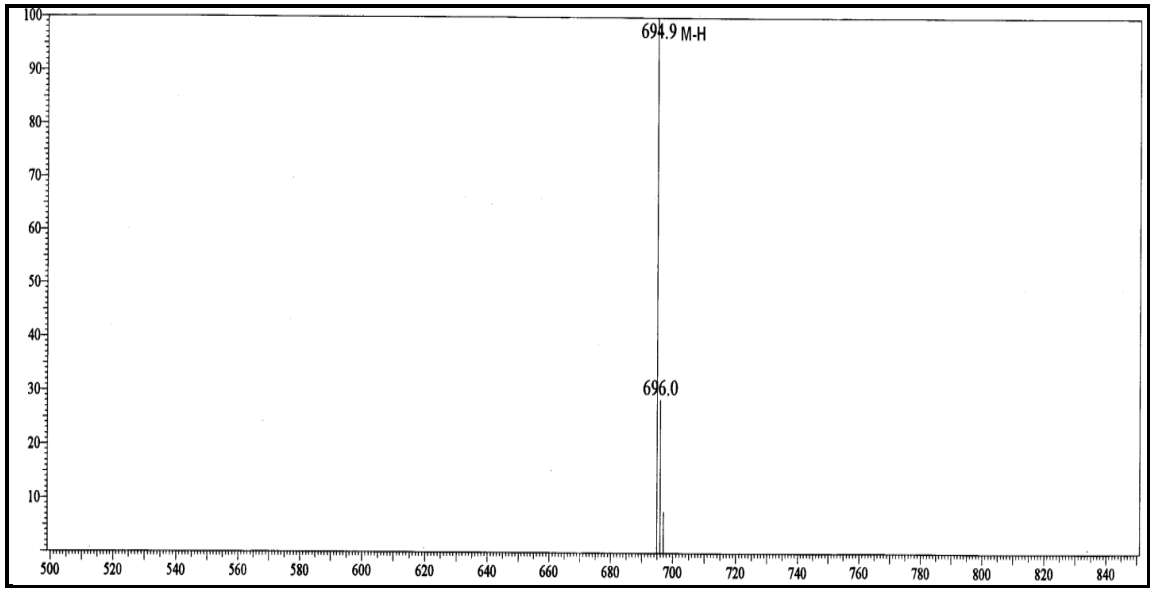
ESI-Mass of 13d



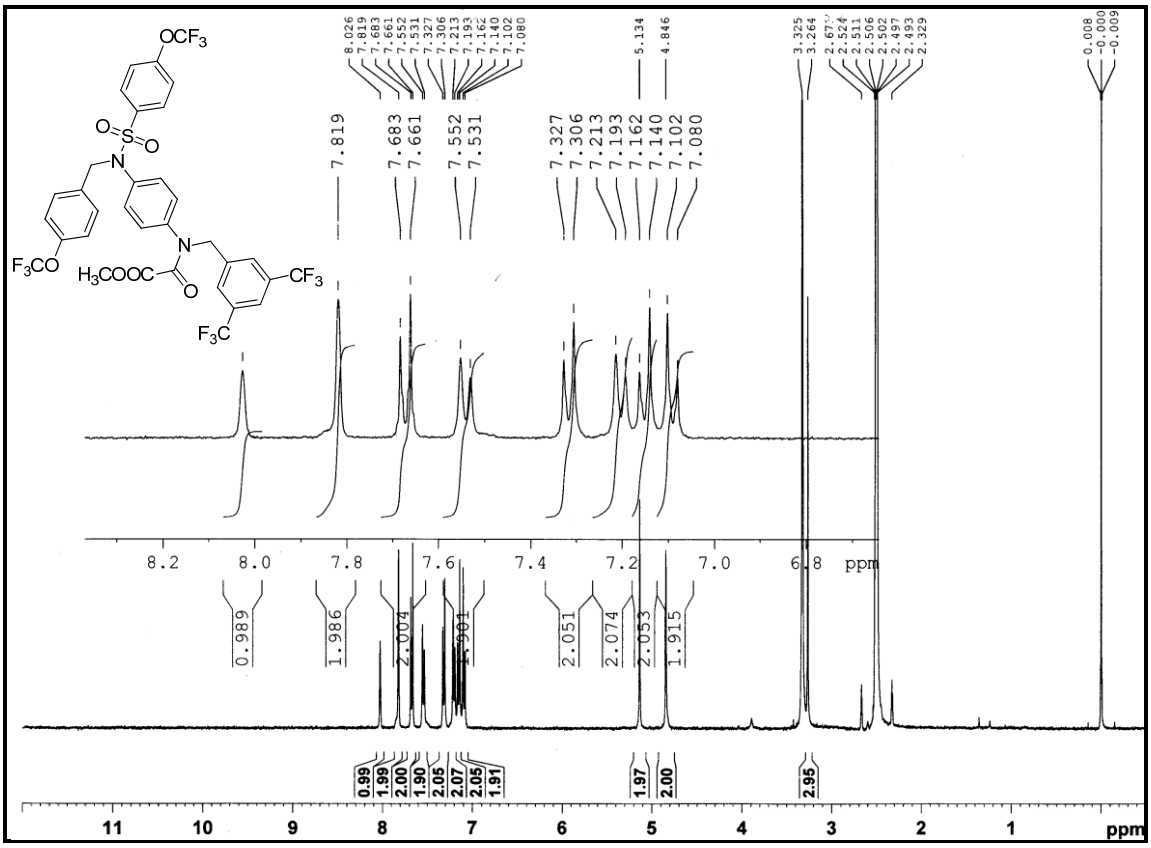
^{1}H NMR of 13i



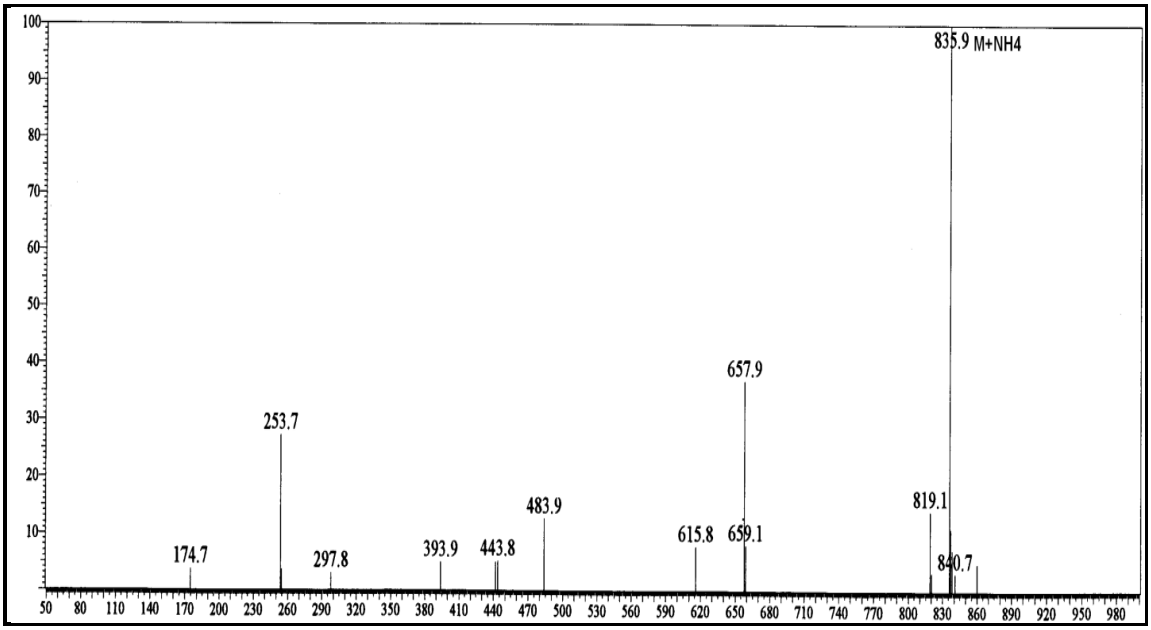
ESI-Mass of 13i



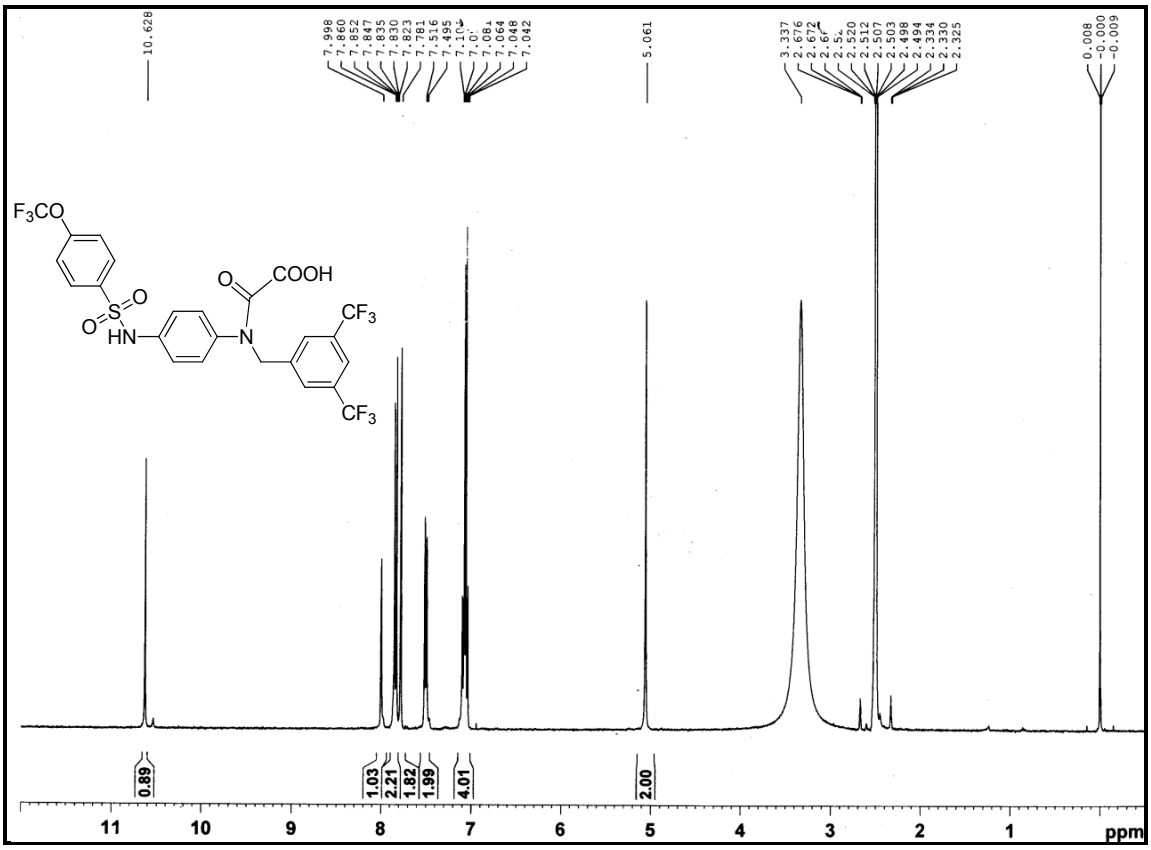
^{1}H NMR of 14o



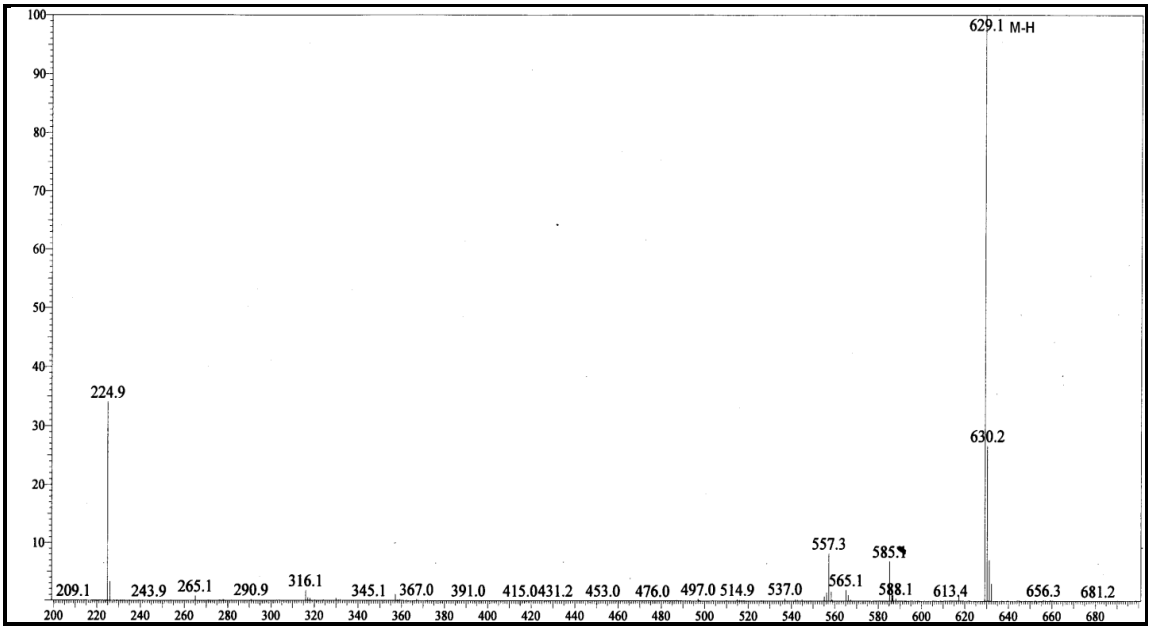
ESI-Mass of 14o



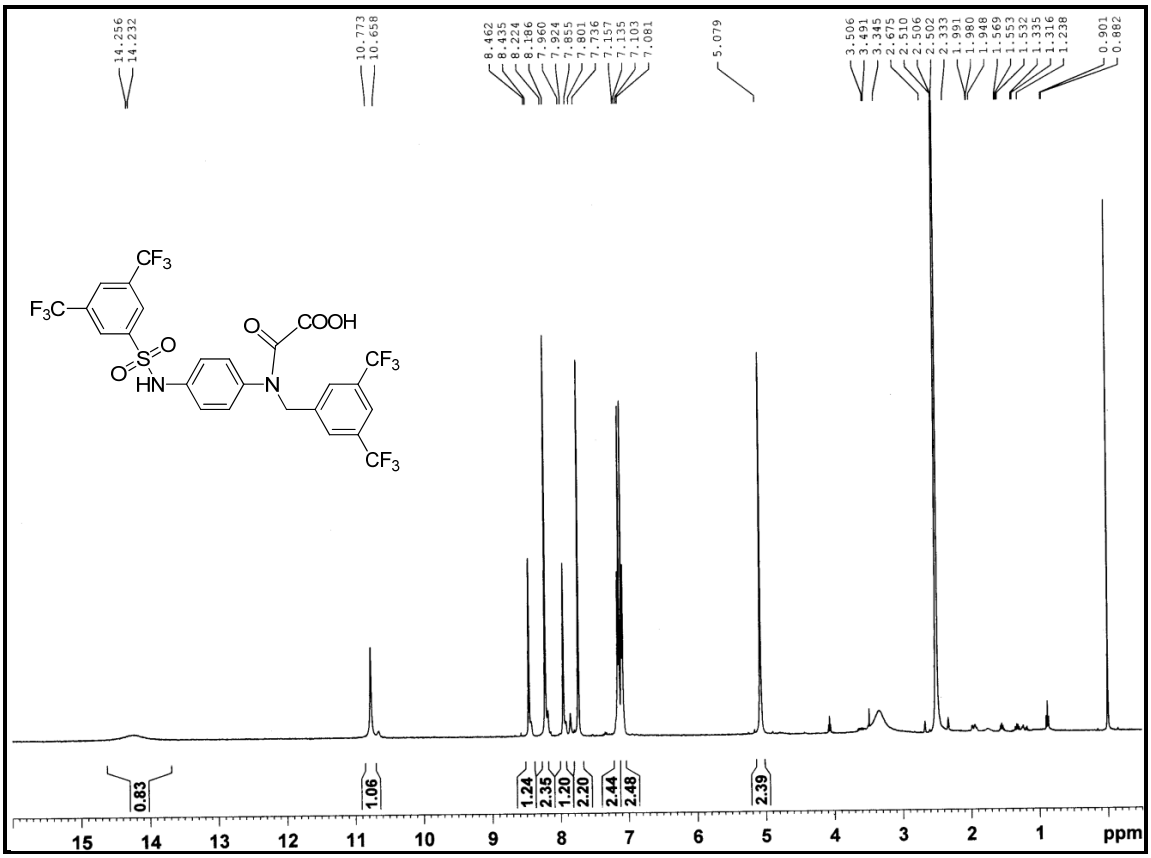
^{1}H NMR of 15d



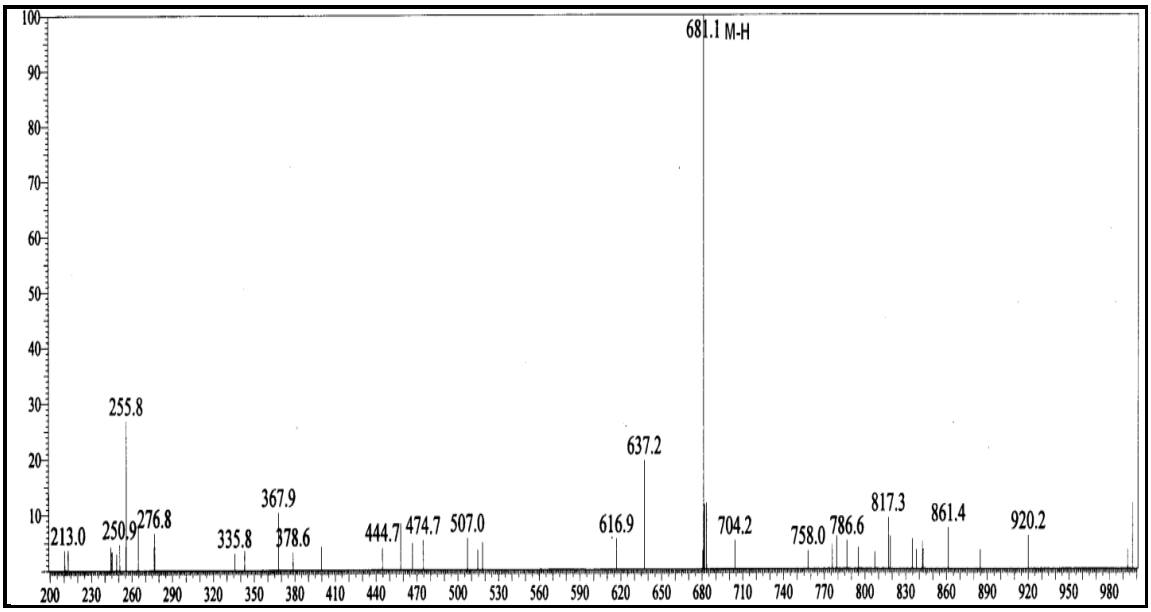
ESI-Mass of 15d



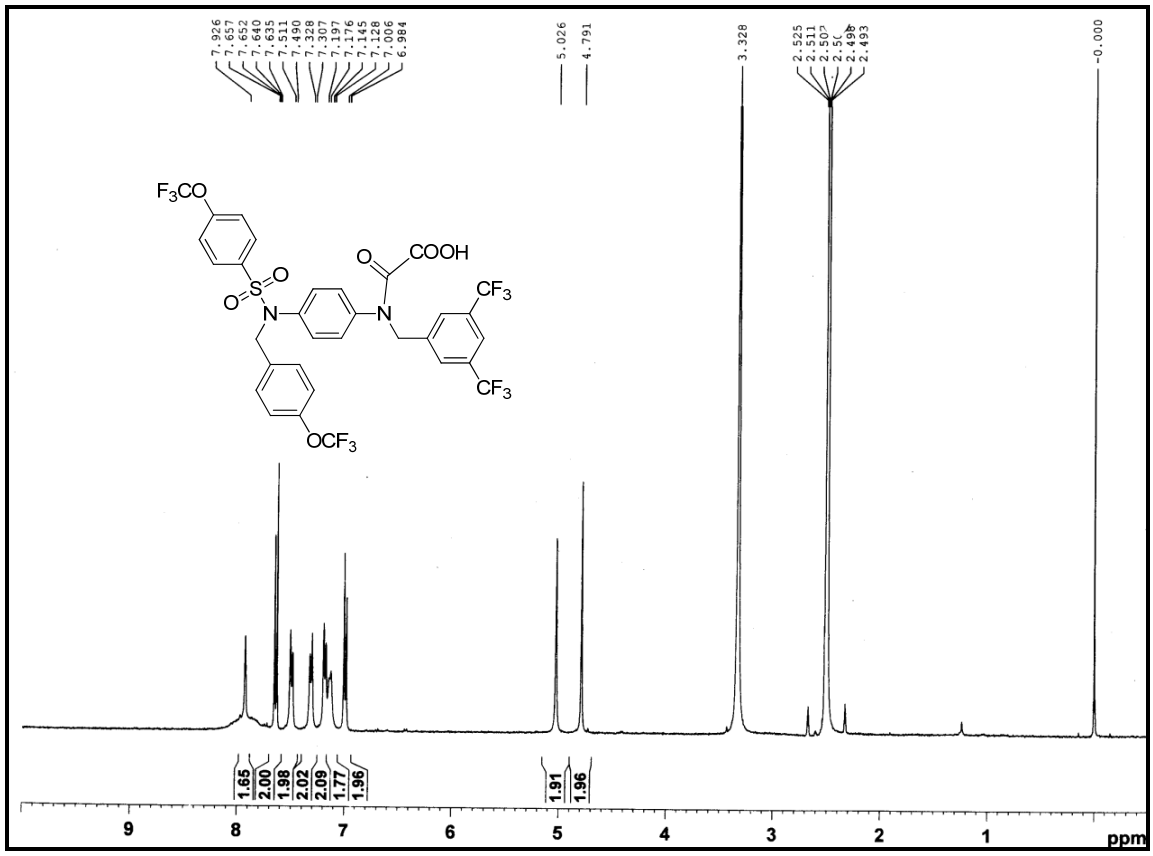
^{1}H NMR of 15i



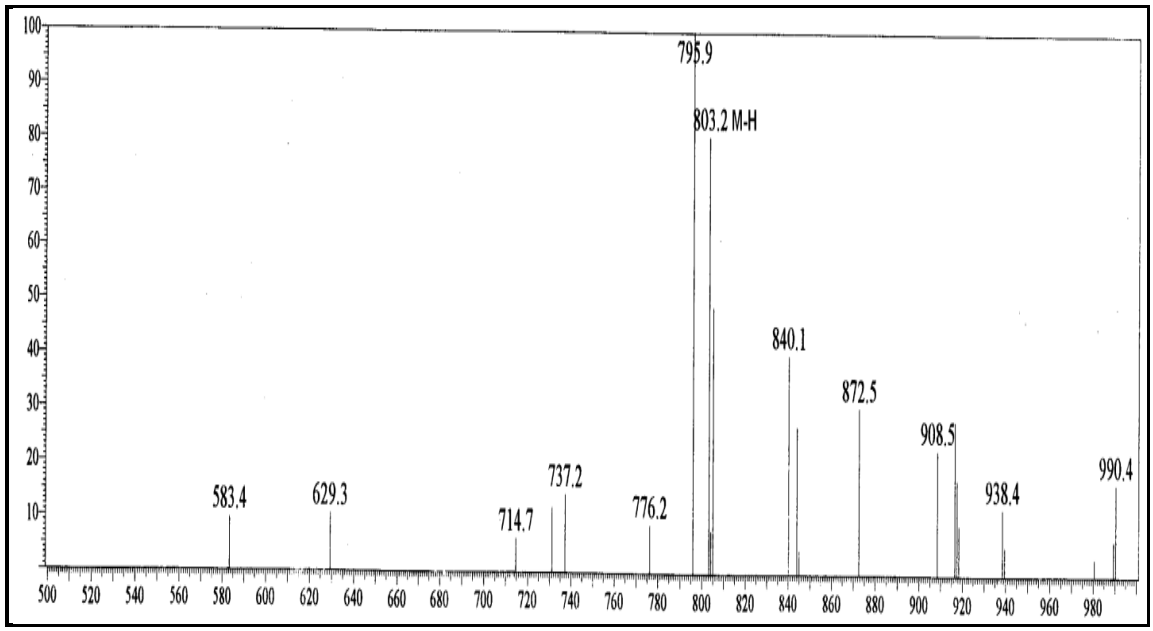
ESI-Mass of 15i



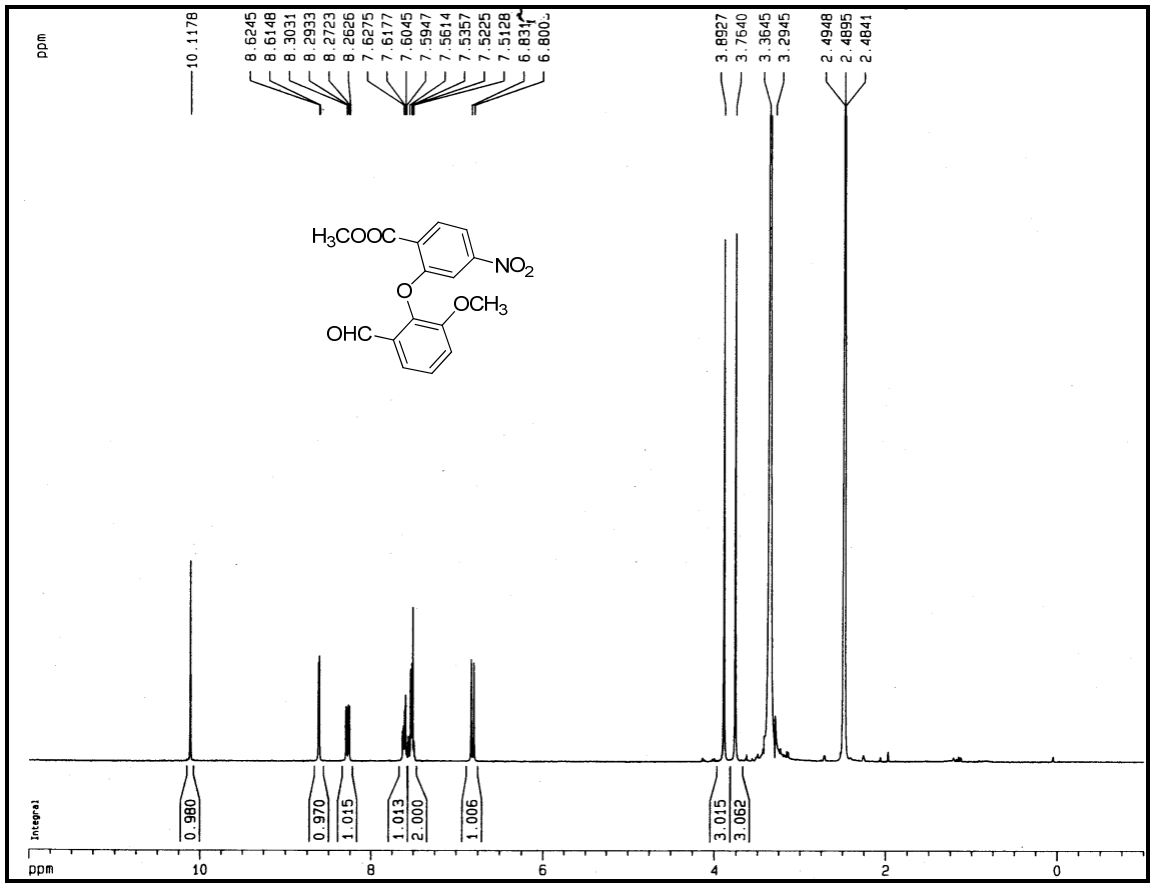
^{1}H NMR of 15o



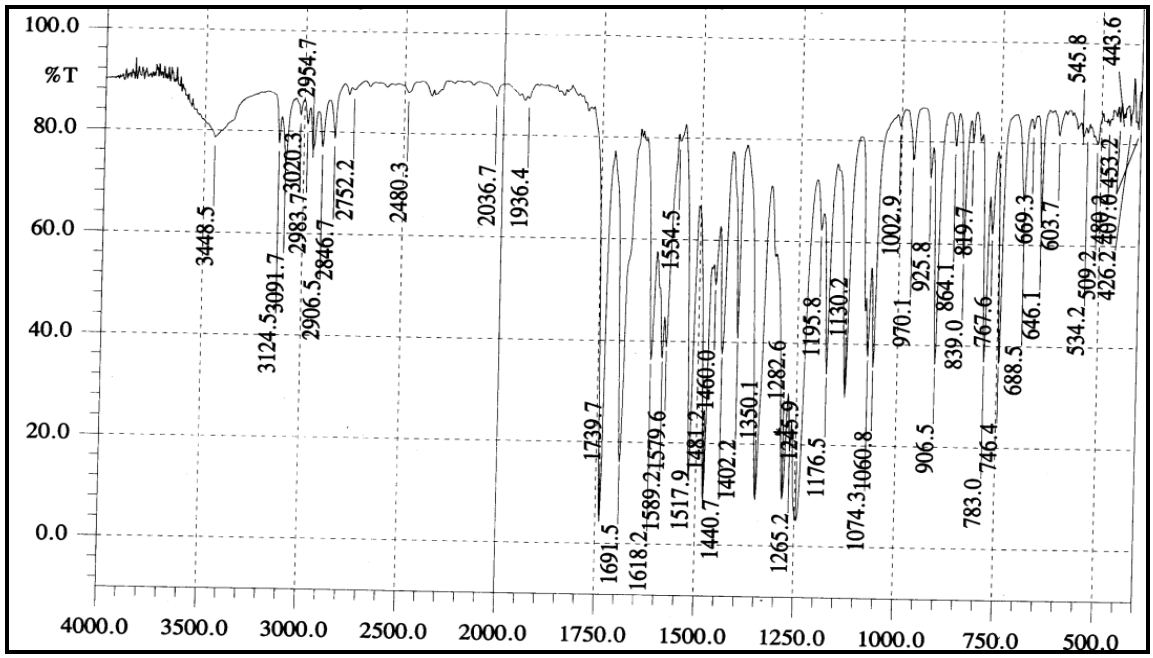
ESI-Mass of 15o



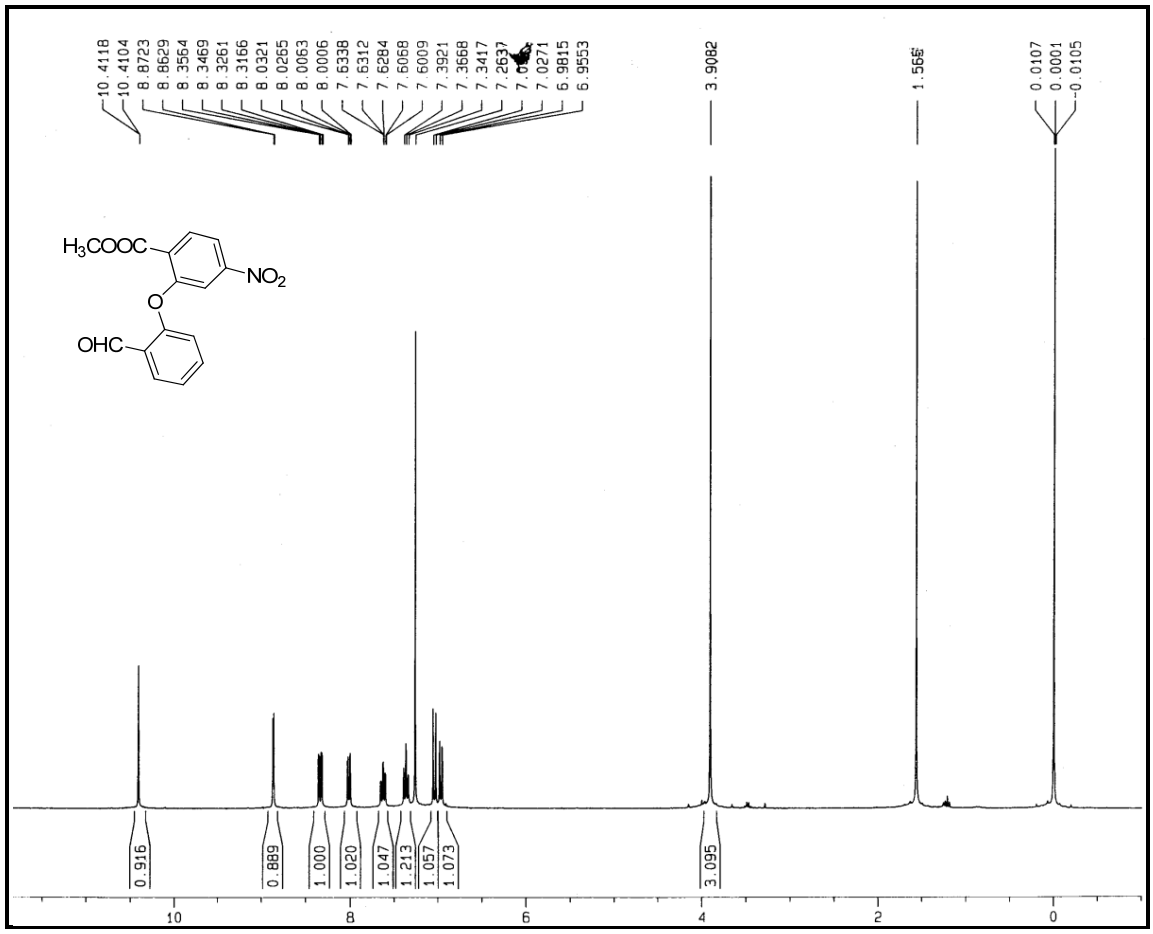
^{1}H NMR of 18a



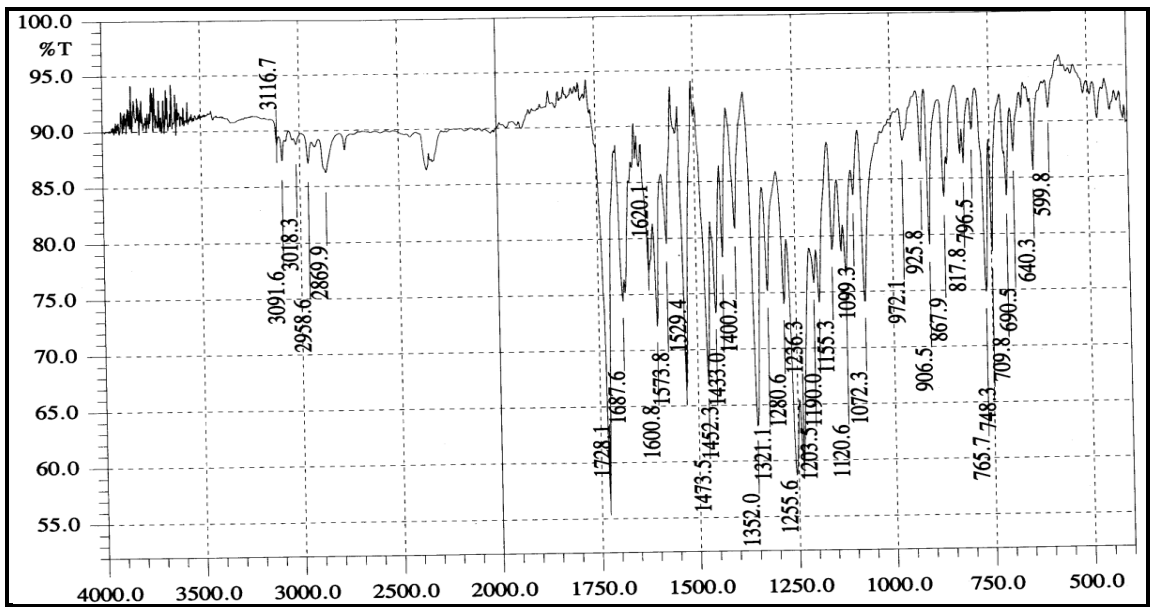
IR of 18a



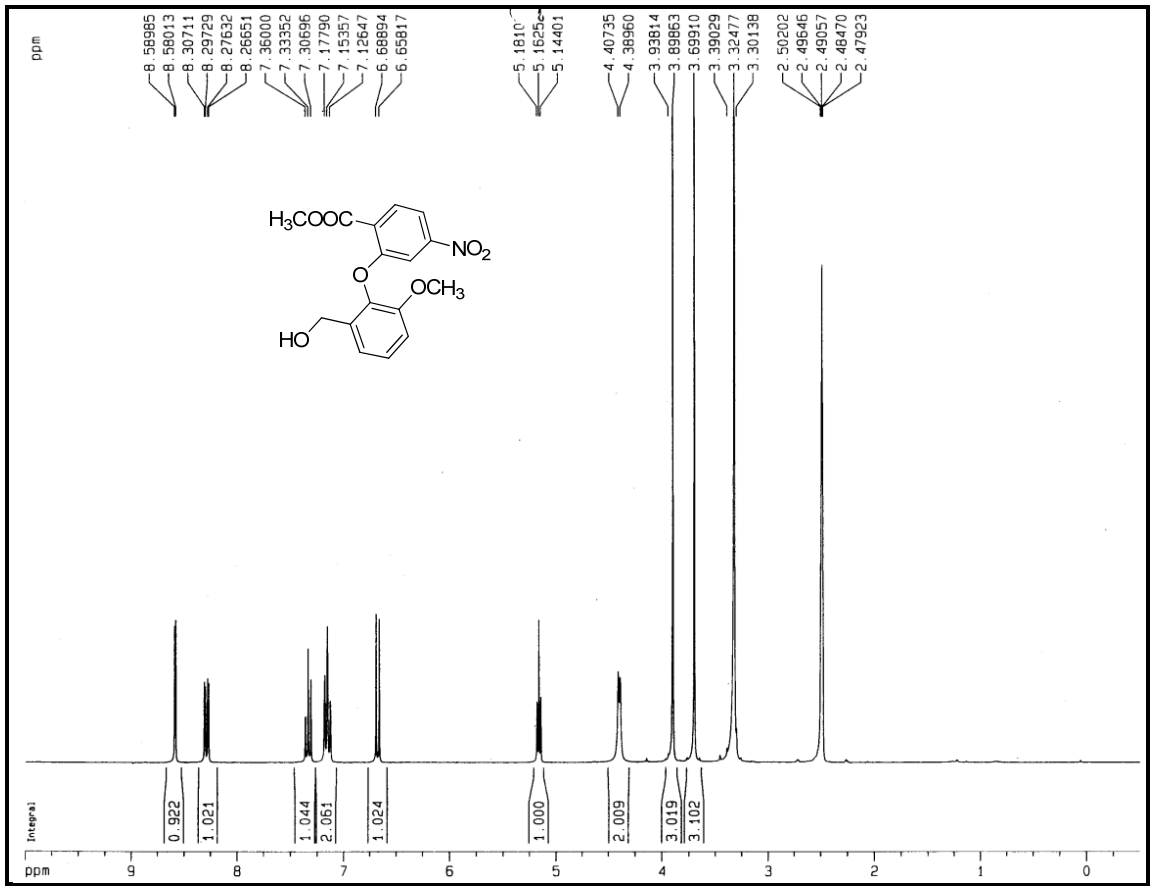
^{1}H NMR of 18b



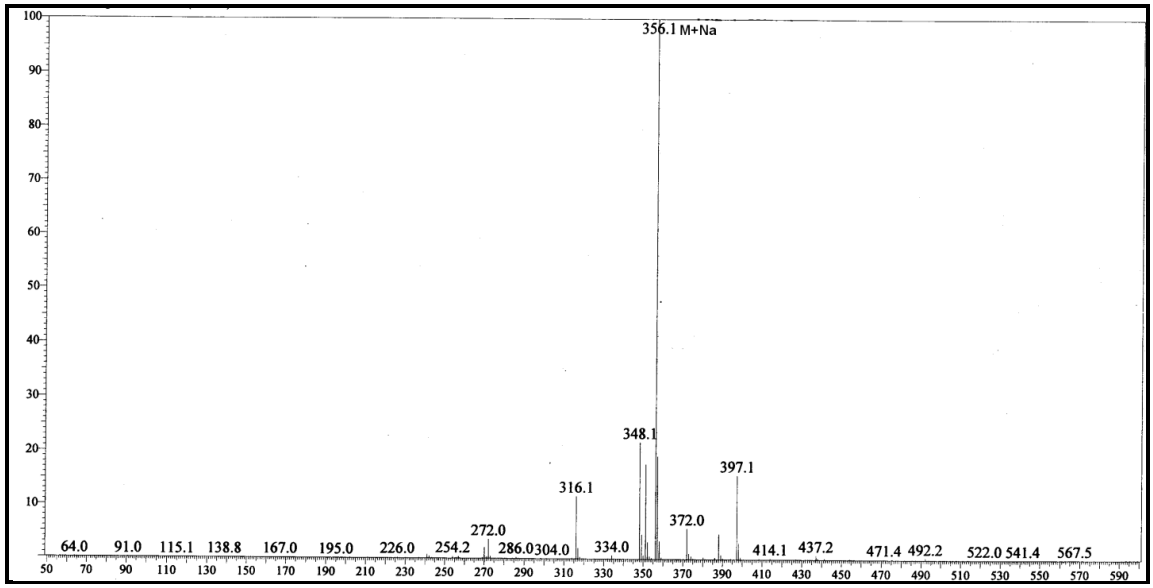
IR of 18b



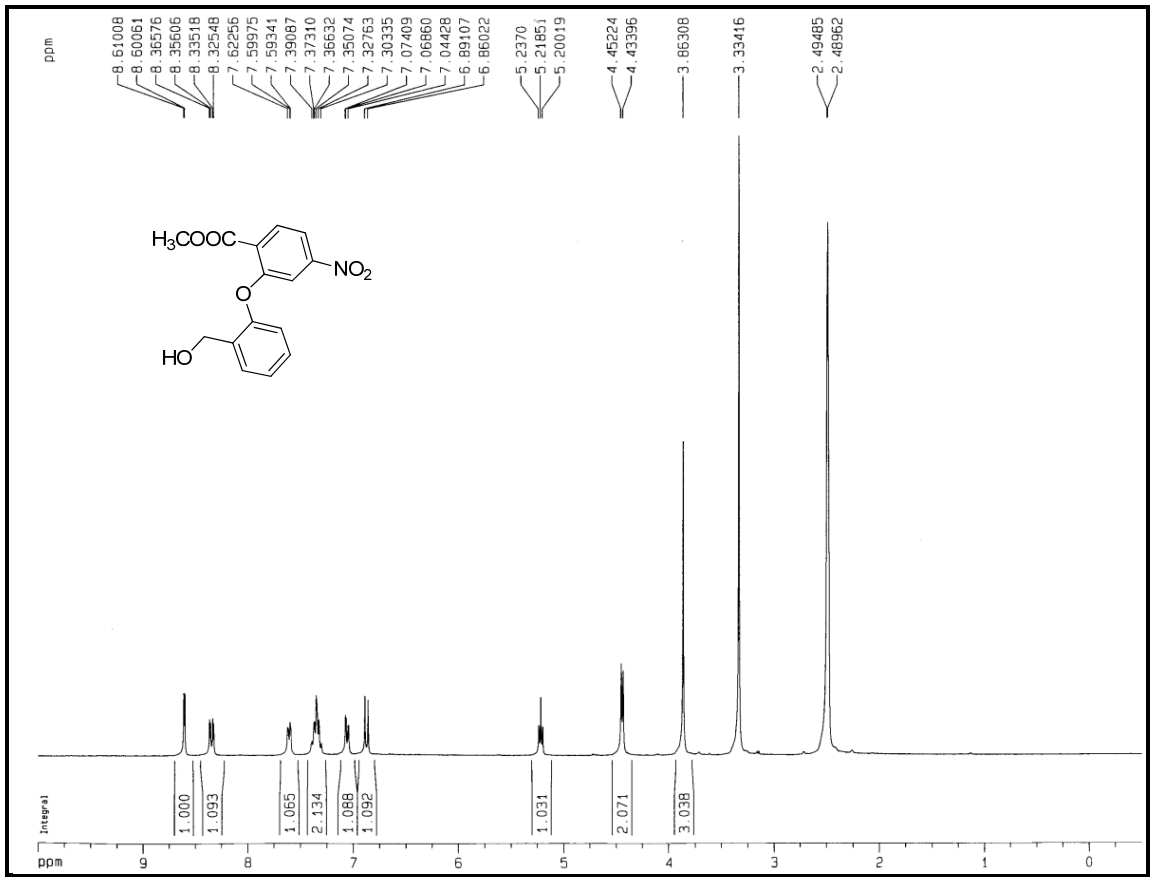
^{1}H NMR of 19a



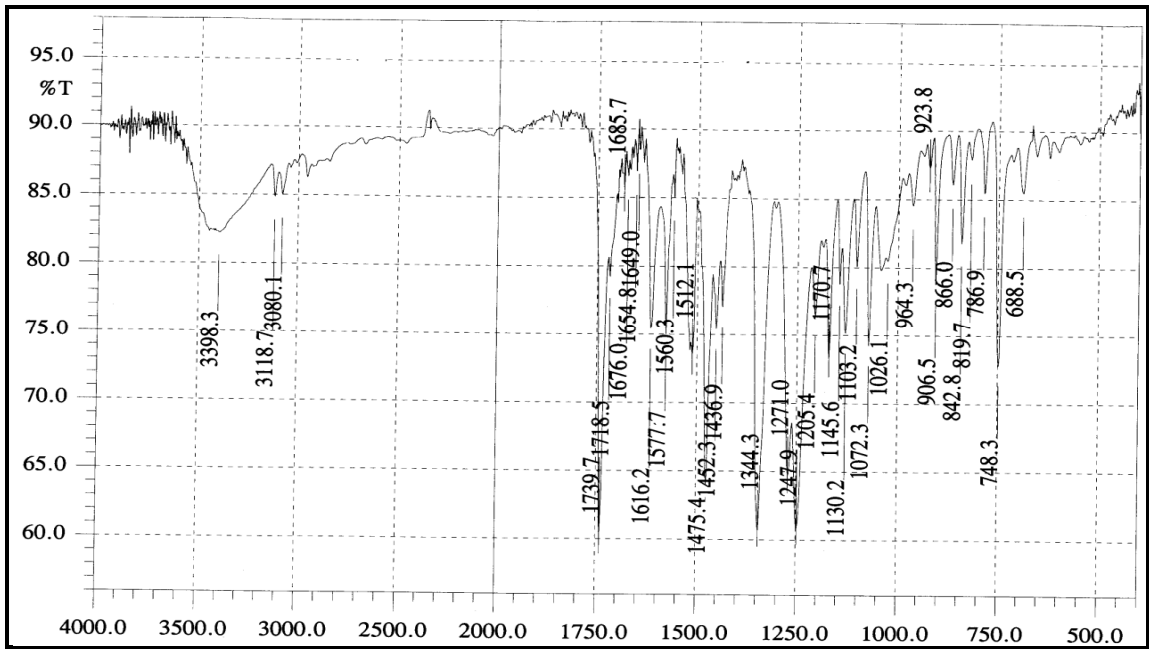
ESI-Mass of 19a



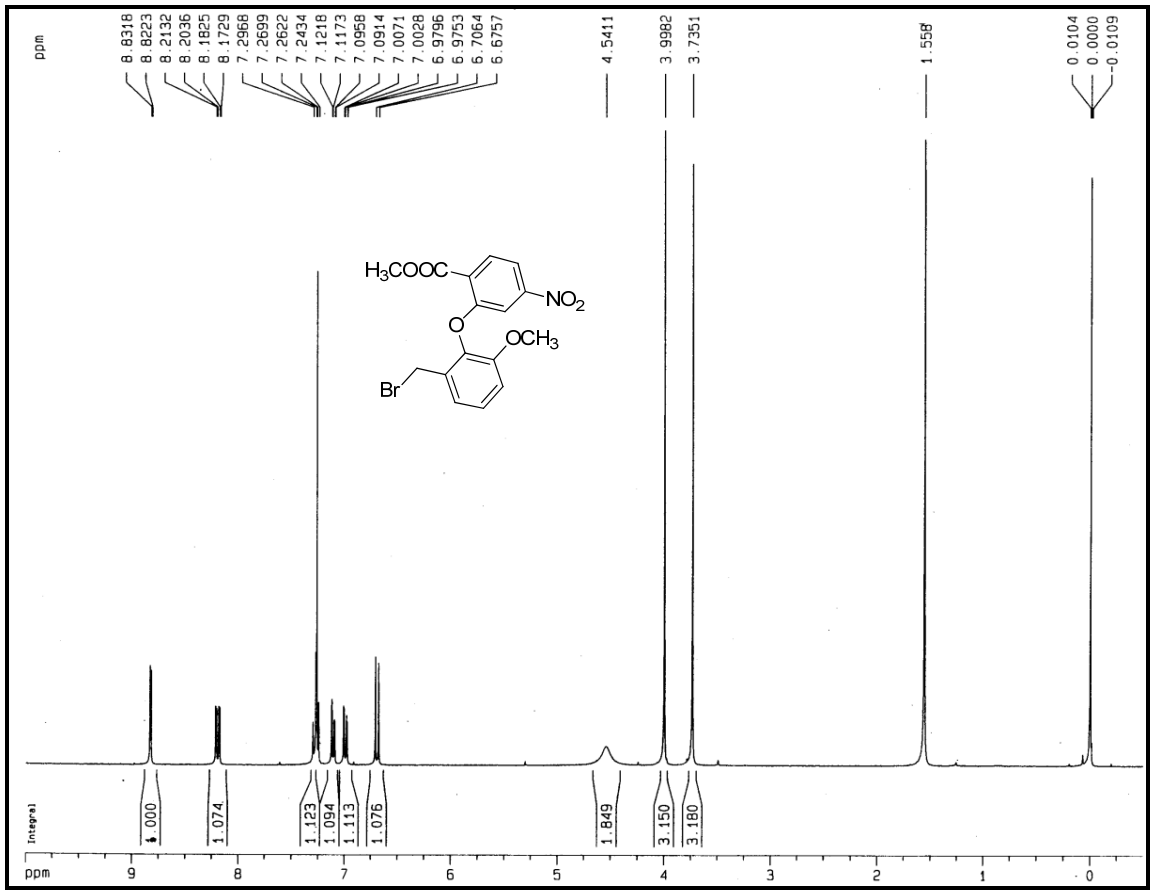
^{1}H NMR of 19b



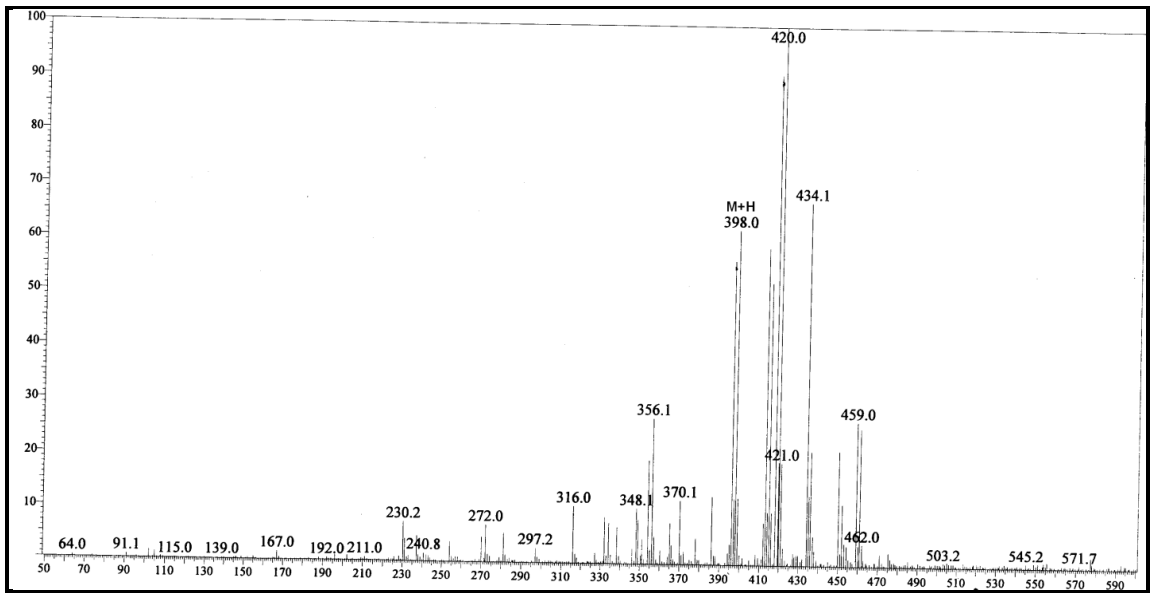
IR of 19b



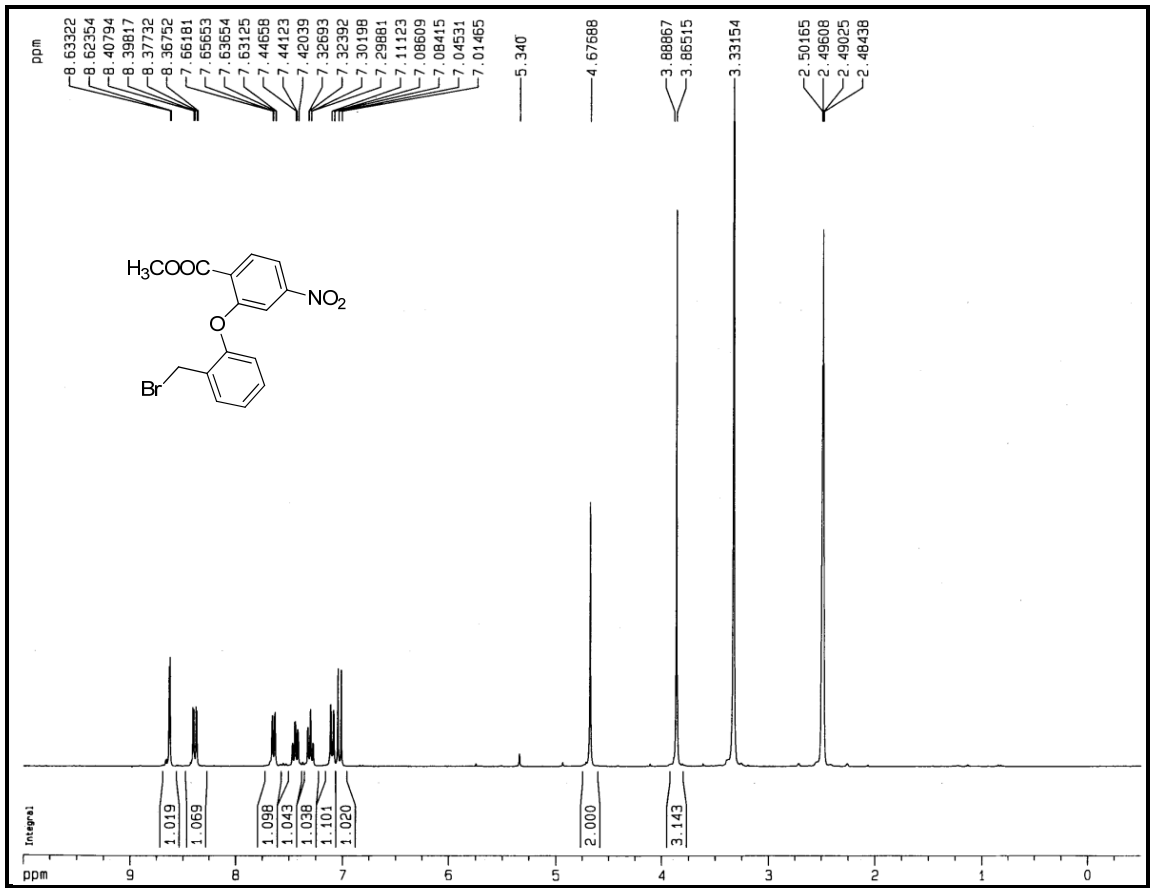
^{1}H NMR of 20a



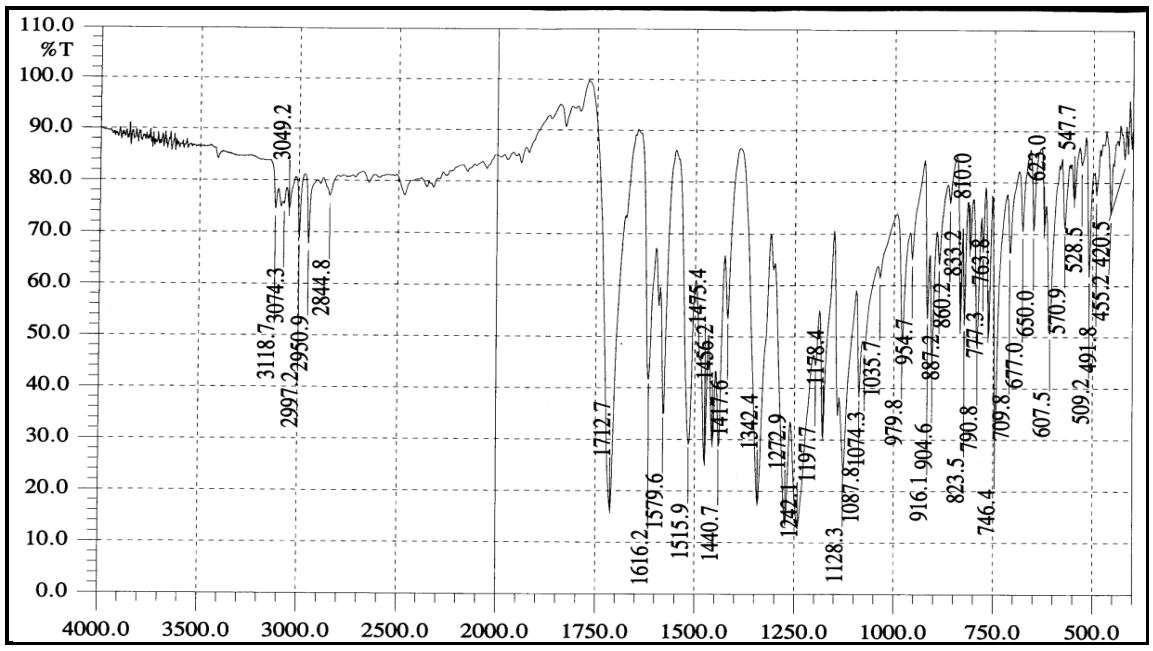
ESI-Mass of 20a



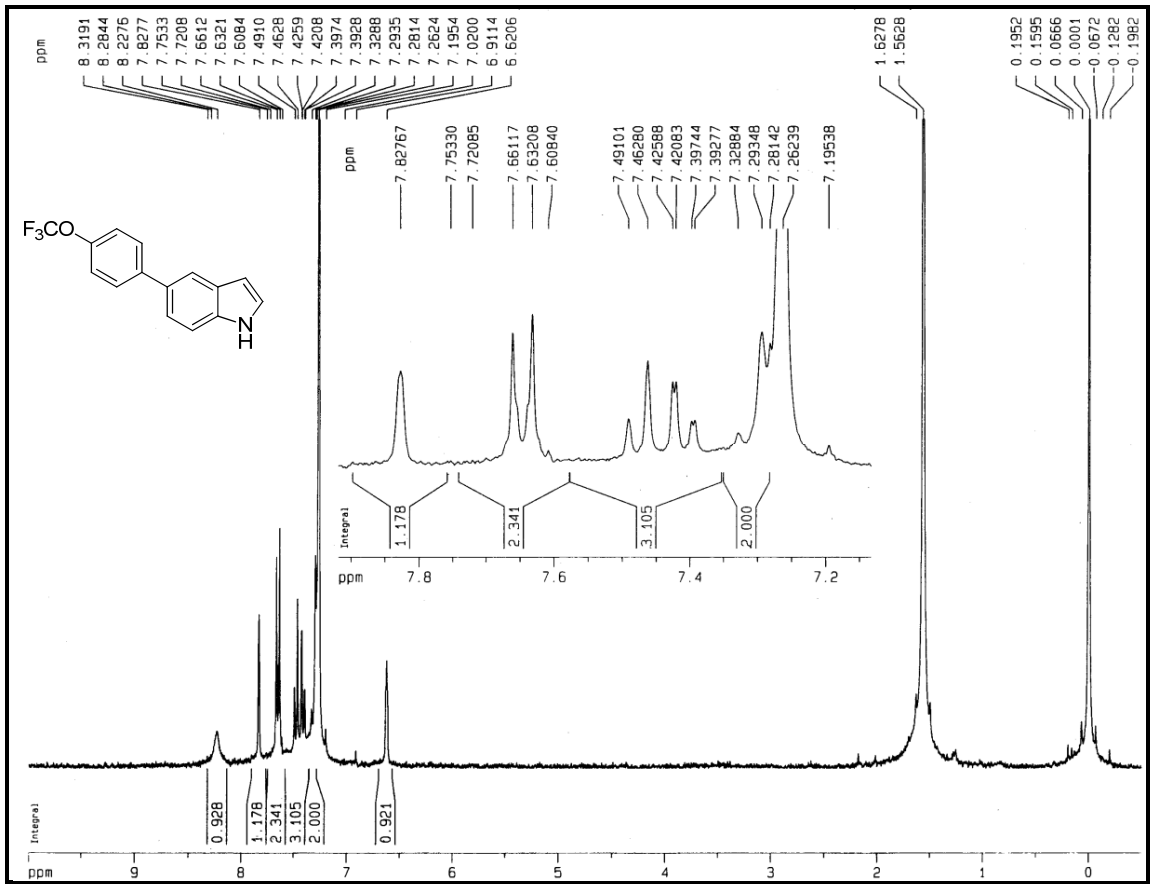
^{1}H NMR of 20b



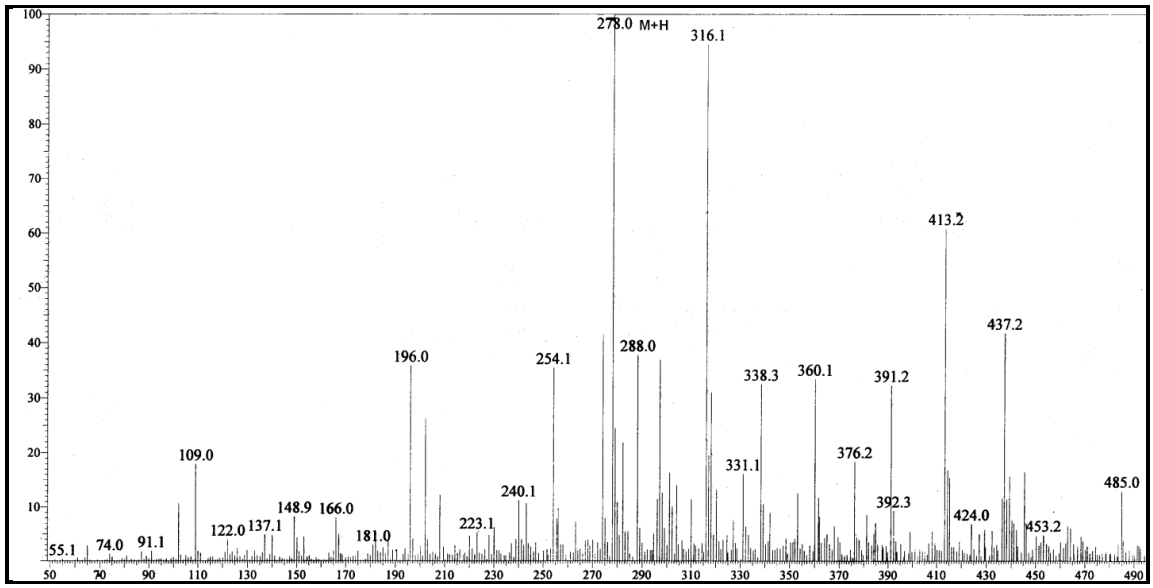
IR of 20b



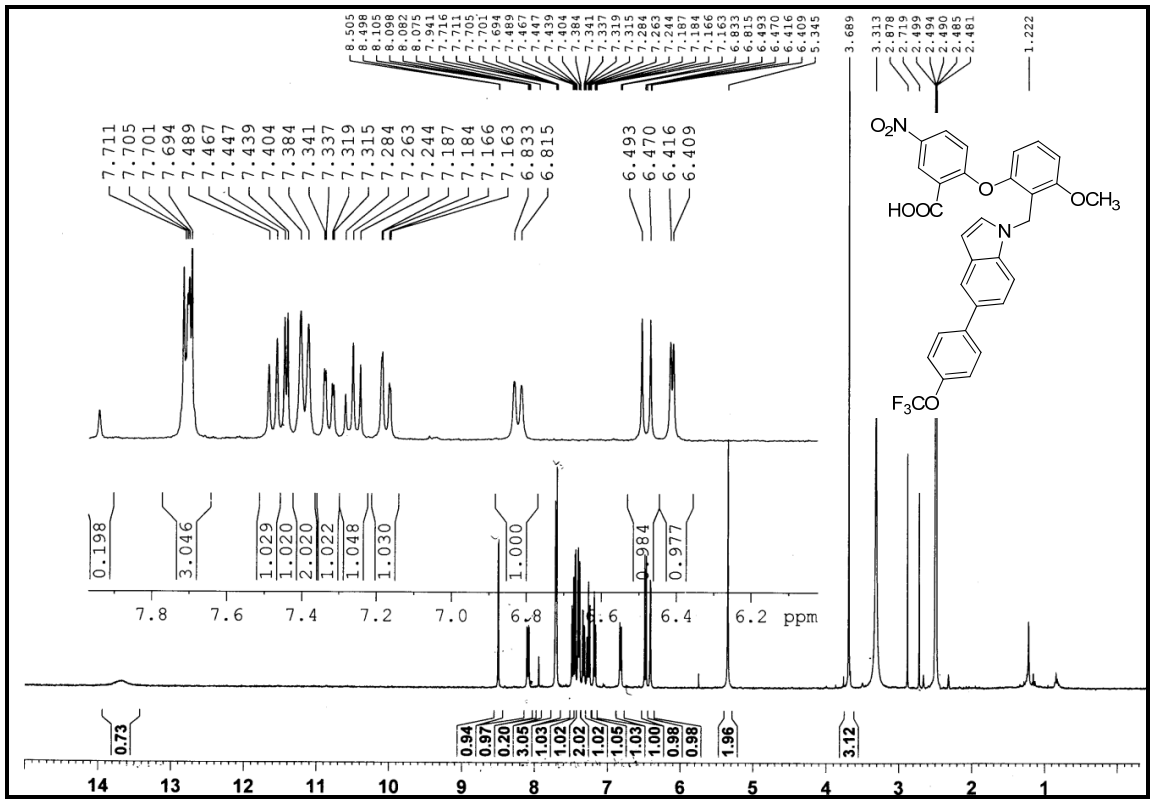
^{1}H NMR of 23a



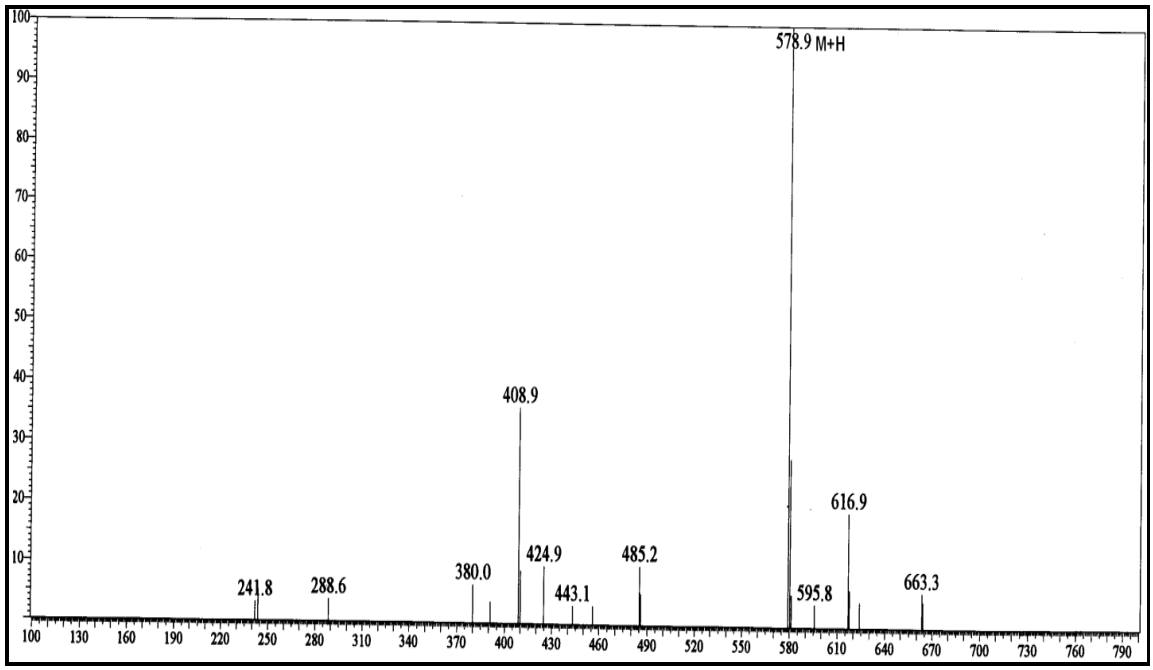
ESI-Mass of 23a



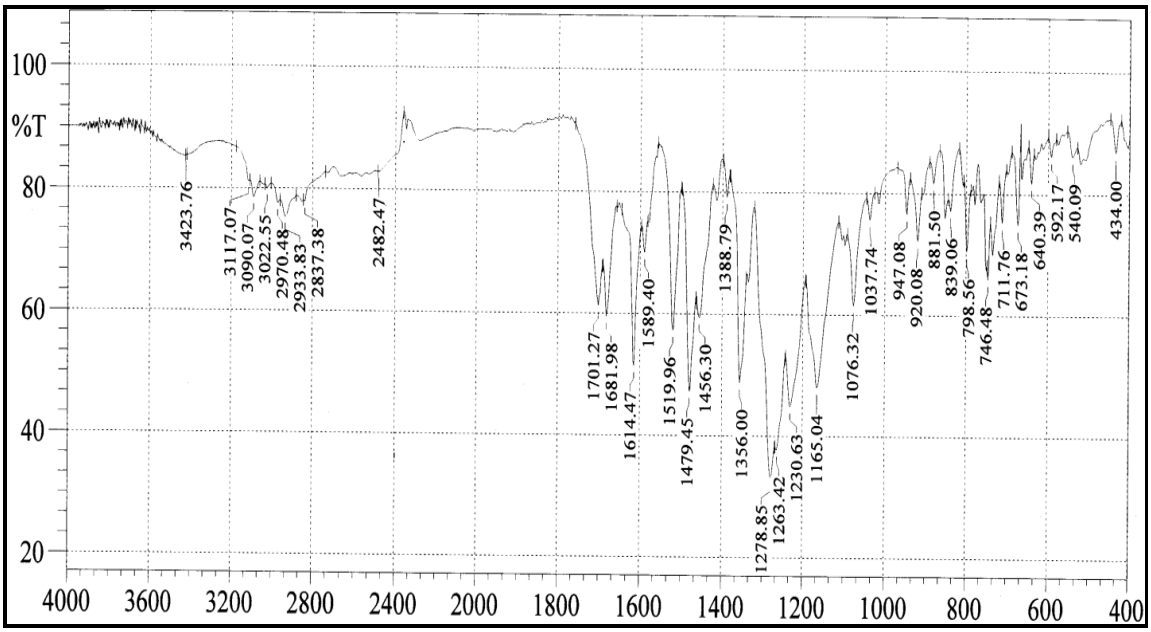
^{1}H NMR of 24a



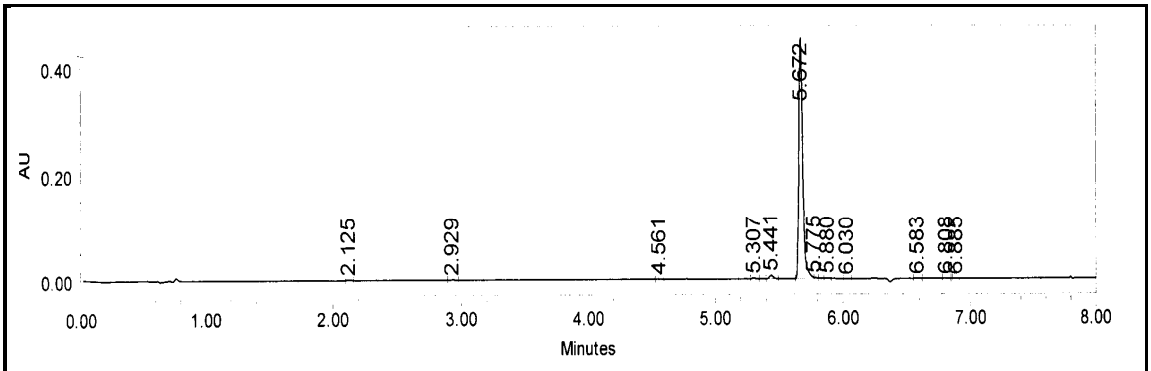
ESI-Mass of 24a



IR of 24a

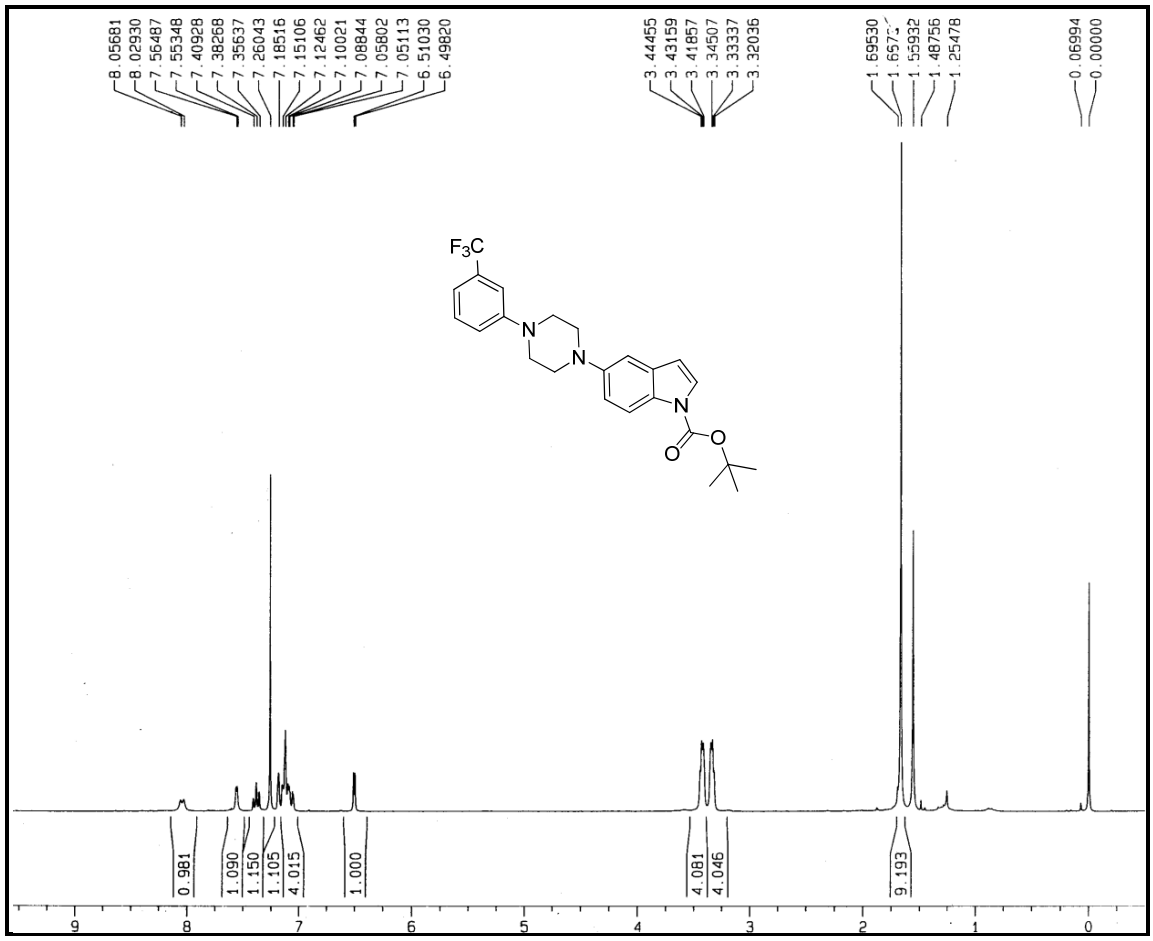


UPLC of 24a

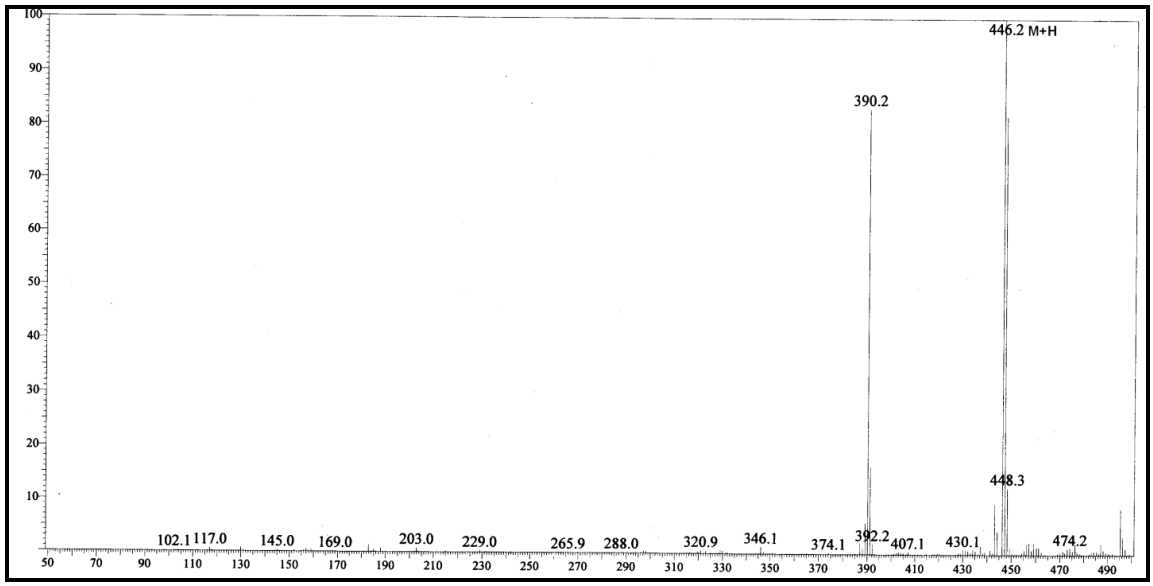


	RT	Area	% Area
1	2.125	1873	0.19
2	2.929	849	0.09
3	4.561	1266	0.13
4	5.307	2759	0.28
5	5.441	12715	1.30
6	5.672	952402	97.44
7	5.775	1583	0.16
8	5.880	1328	0.14

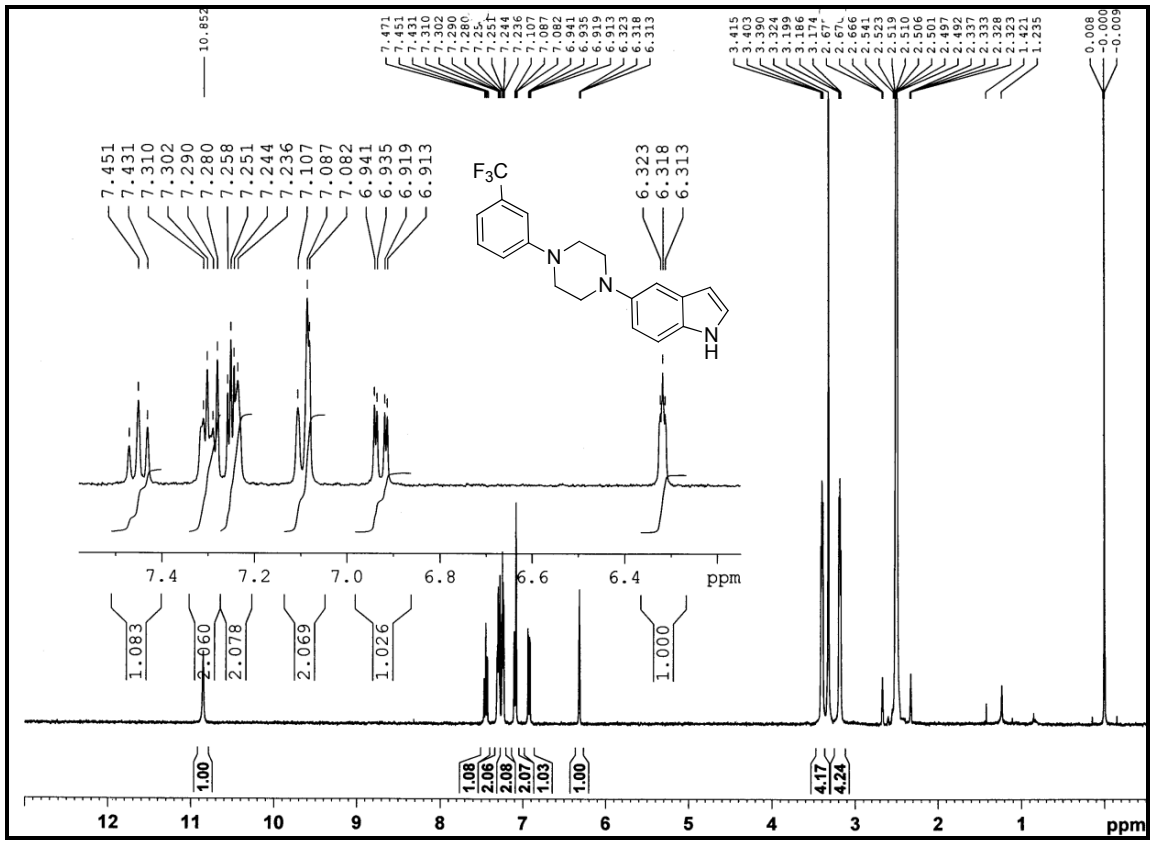
^{1}H NMR of 27c



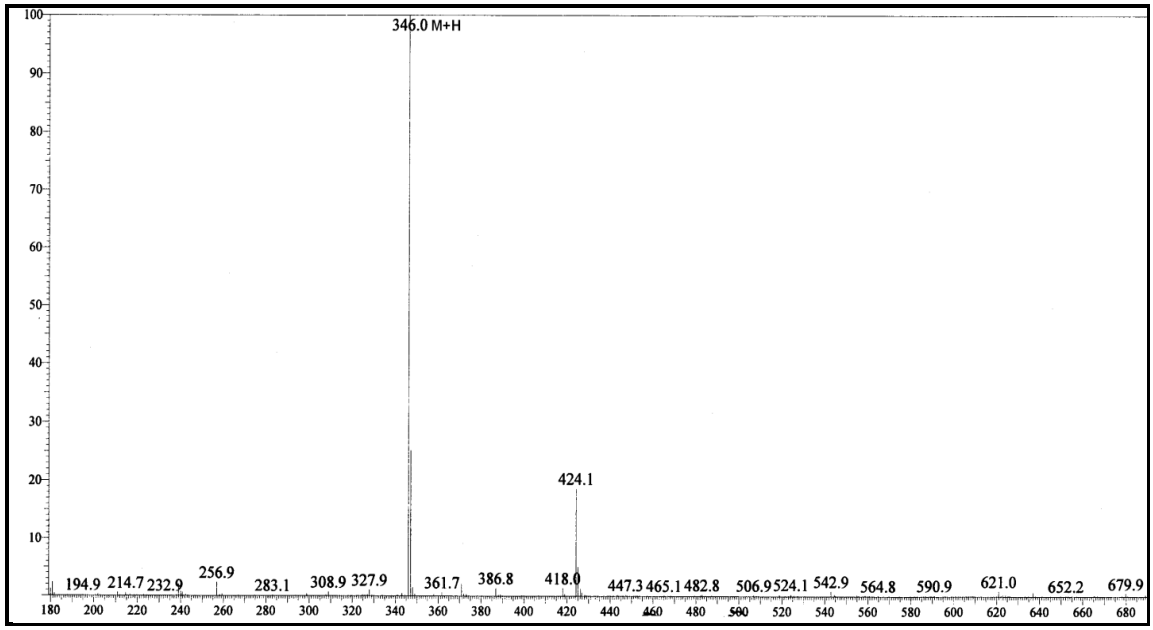
ESI-Mass of 27c



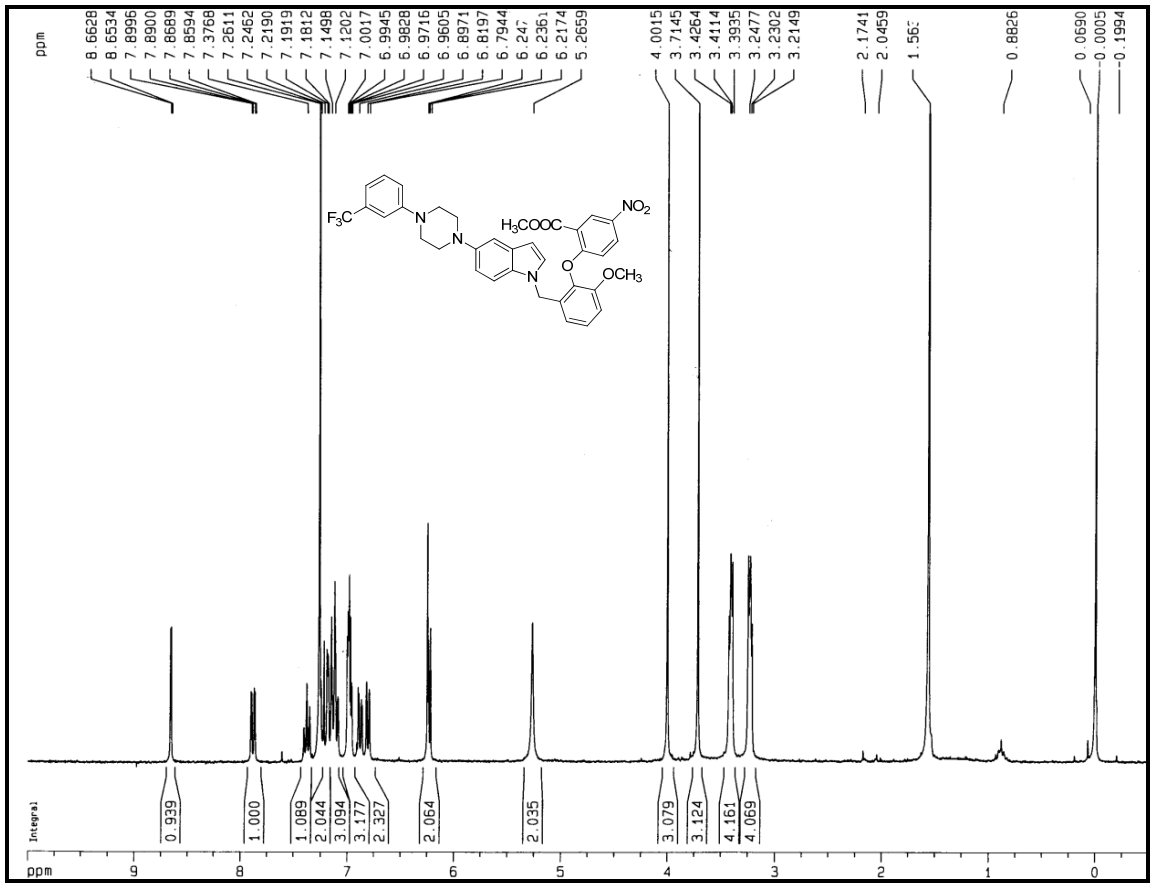
^{1}H NMR of 28c



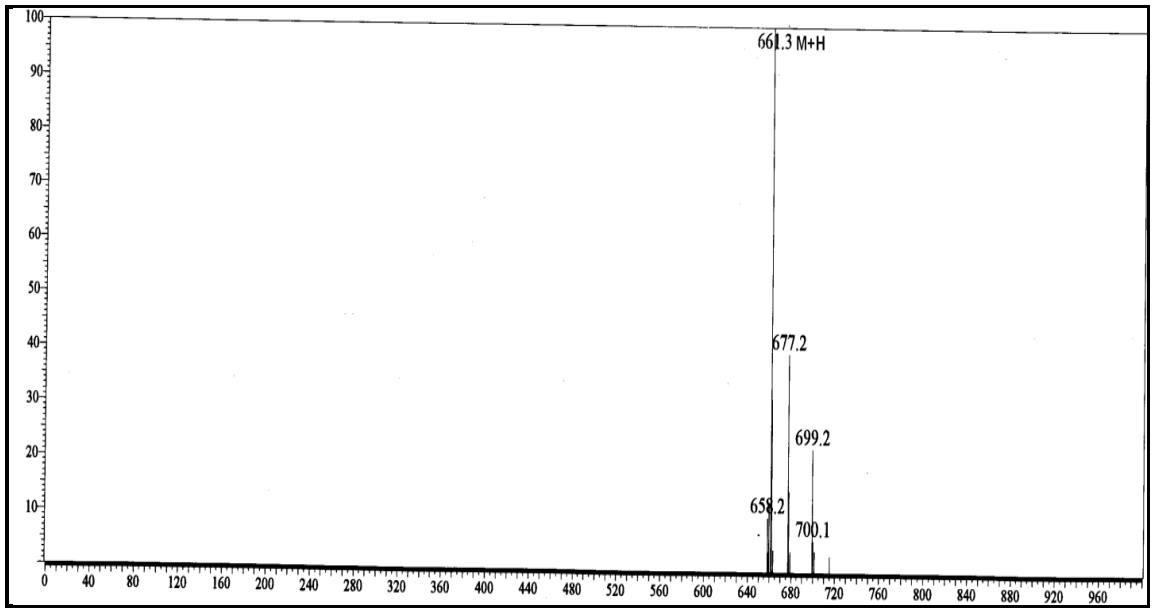
ESI-Mass of 28c



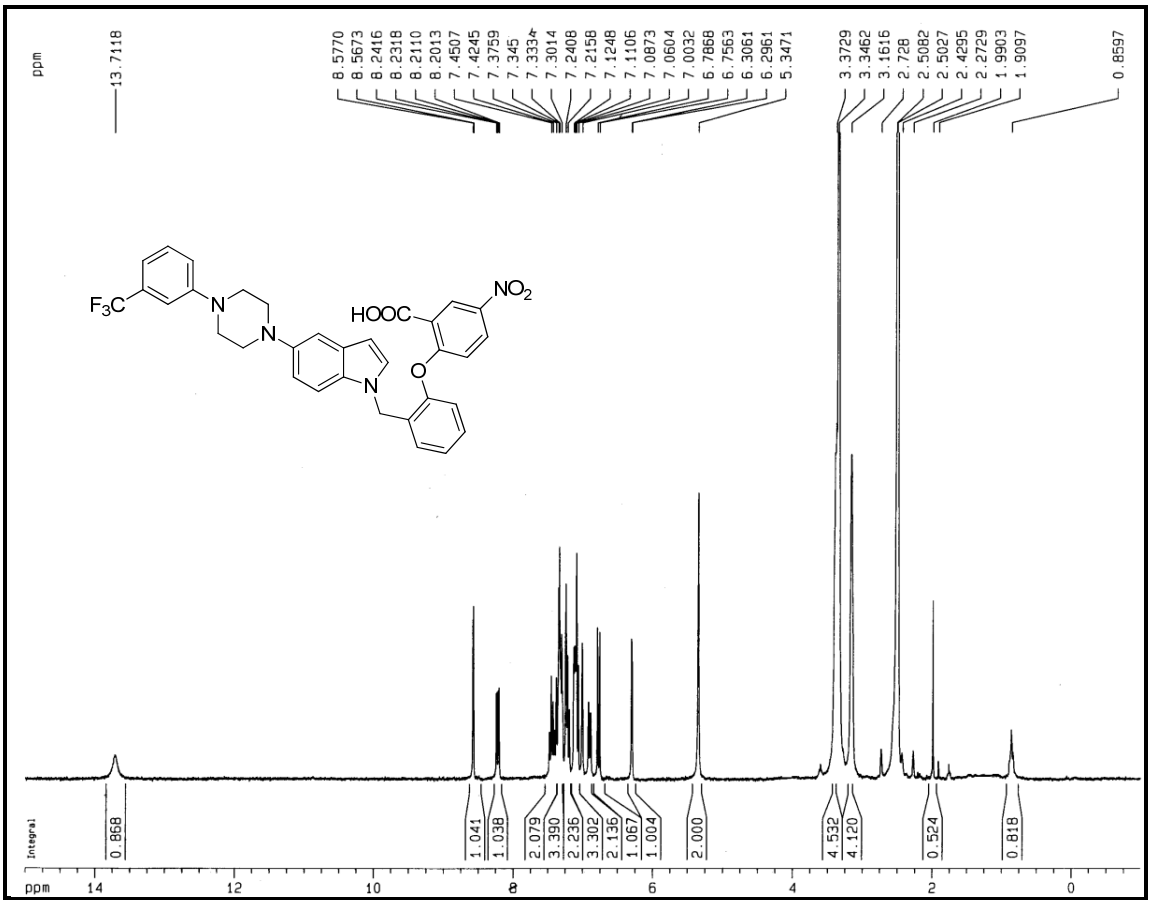
^{1}H NMR of 29e



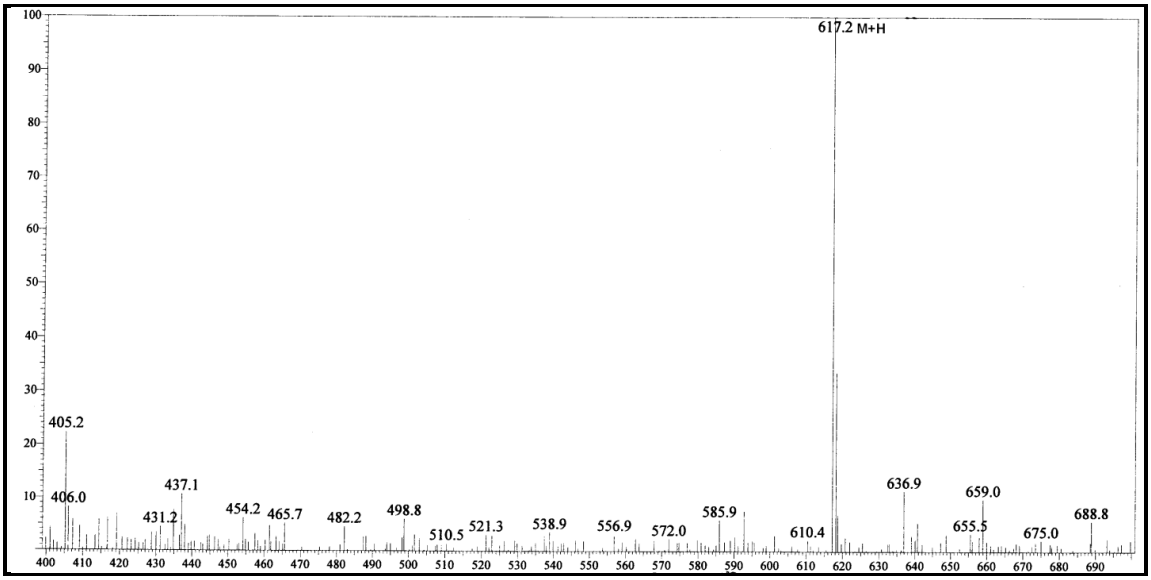
ESI-Mass of 29e



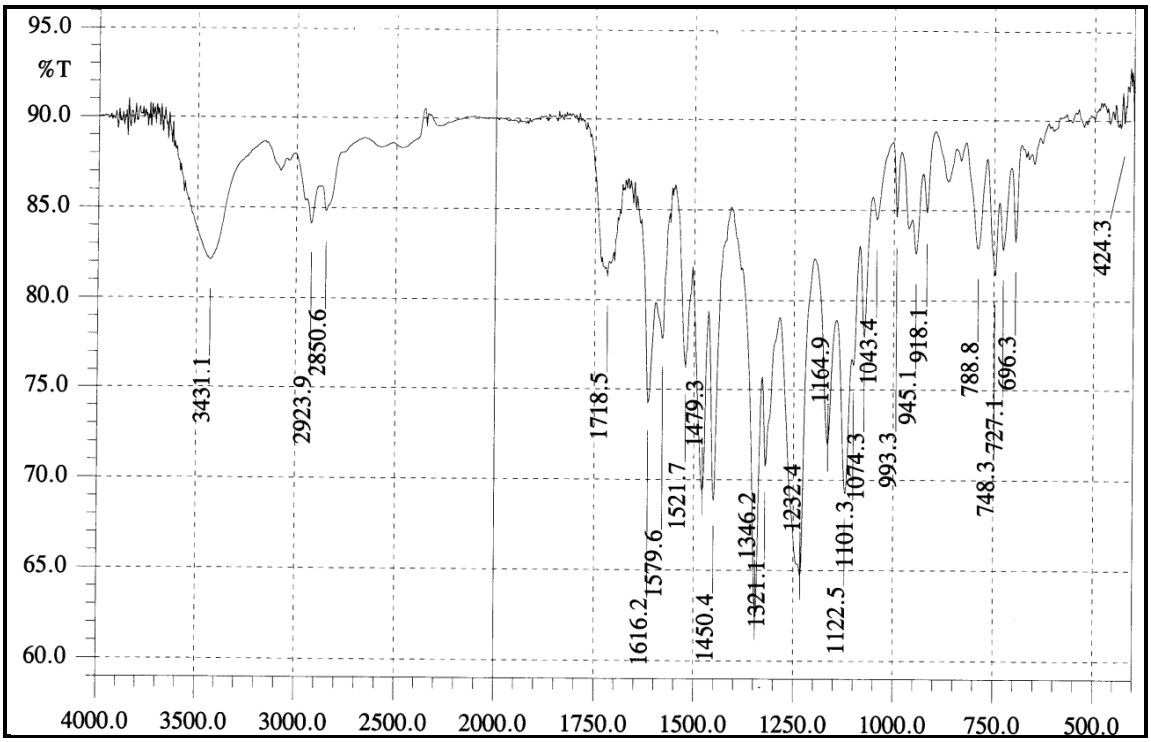
^{1}H NMR of 30c



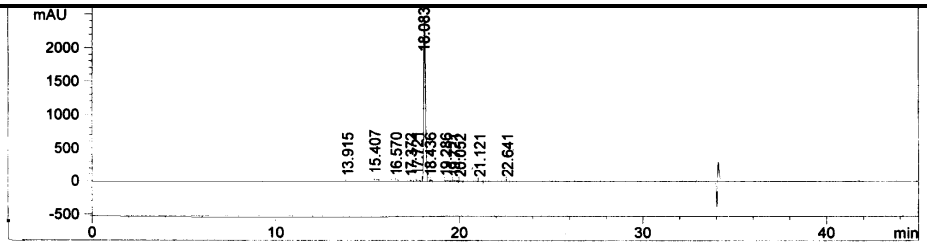
ESI-Mass of 30c



IR of 30c



HPLC of 30c

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================================================================================
                            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 Type  Width      Area       Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
   1  13.915 PV    0.0992   30.94856    4.75783   0.1720
   2  15.407 BB    0.0741  167.59048   34.52605   0.9313
   3  16.570 BBA   0.0726   11.50965    2.50082   0.0640
   4  17.372 BBA   0.0883    4.45702 7.81903e-1   0.0248
   5  17.721 BP    0.0731  137.63637   28.85466   0.7648
   6  18.083 VV    0.1090 1.74681e4  2462.45776  97.0706
   7  18.436 VBA   0.0839   56.97889   10.01177   0.3166
   8  19.286 BB    0.0874   60.55747   10.53612   0.3365
   9  19.721 PP    0.0811   29.49340    5.66398   0.1639
  10  20.052 BP    0.0746   14.66257    3.07154   0.0815
  11  21.121 BB    0.0897    9.20028    1.58283   0.0511
  12  22.641 BBA   0.0849    4.11343 7.43511e-1   0.0229
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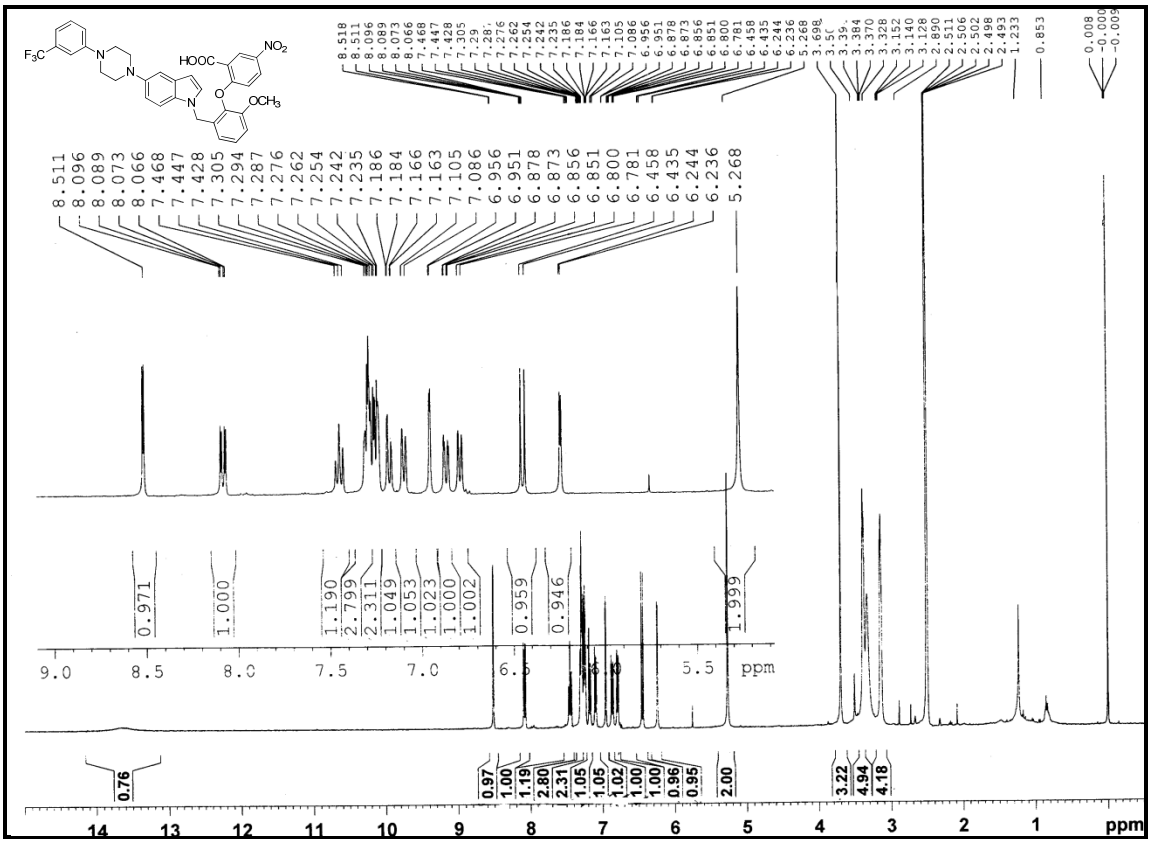
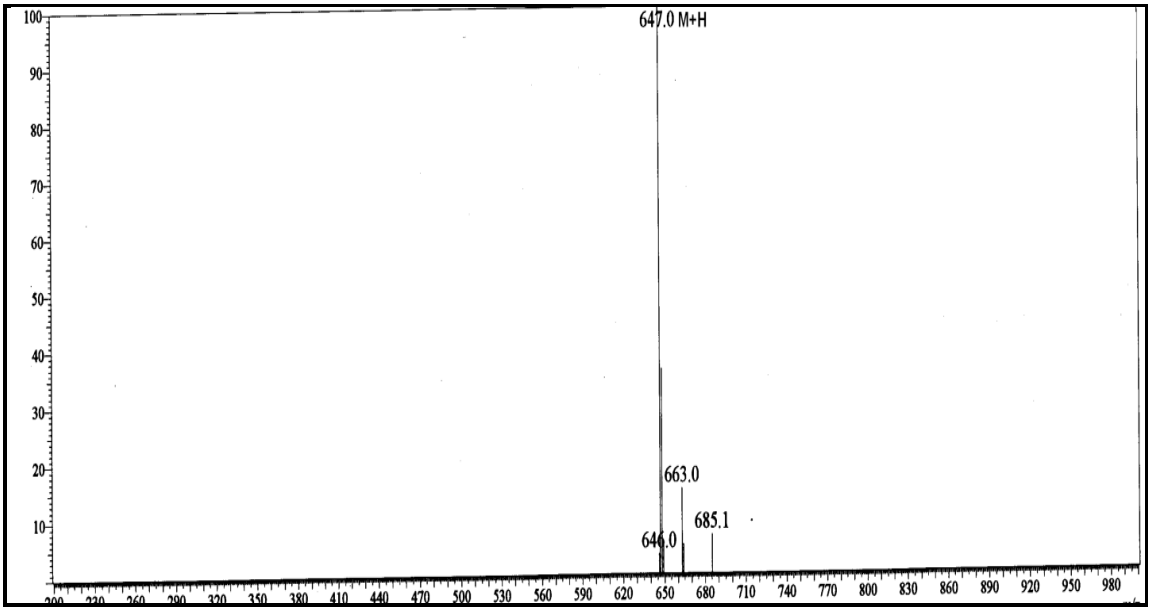
^{1}H NMR of 30e

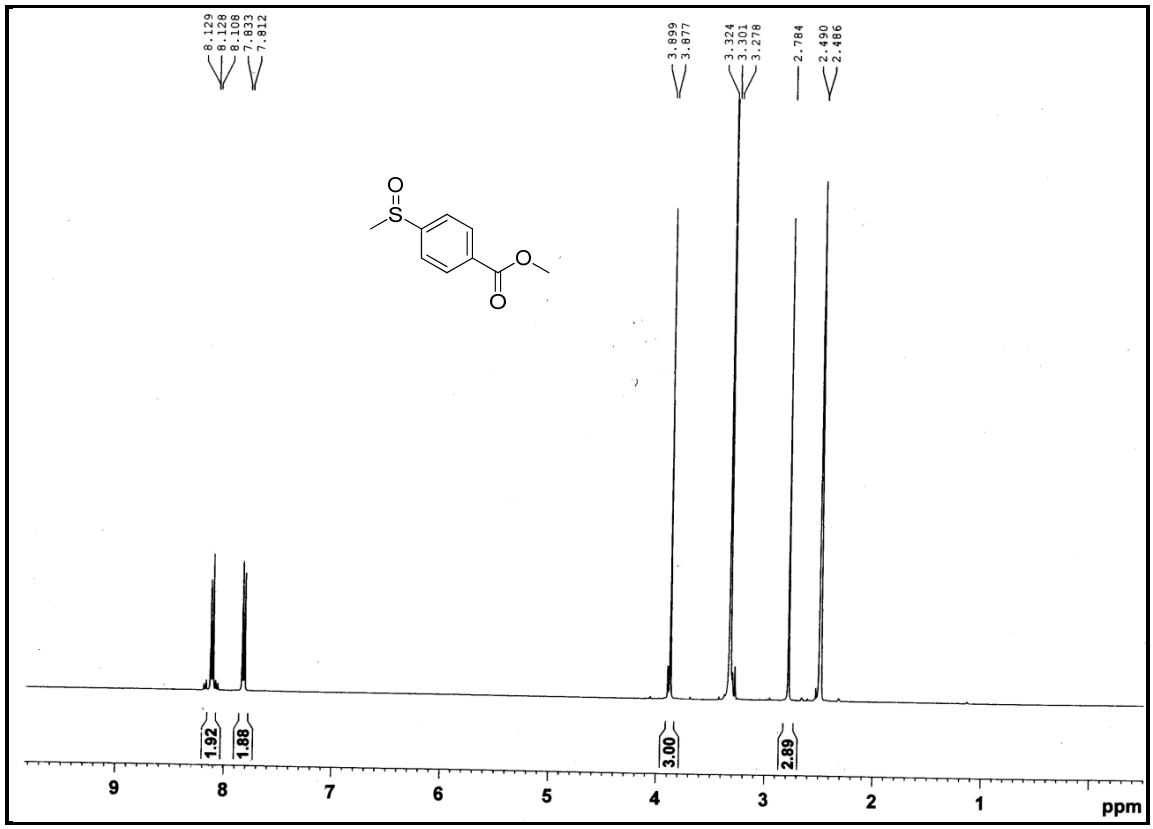


ESI-Mass of 30e

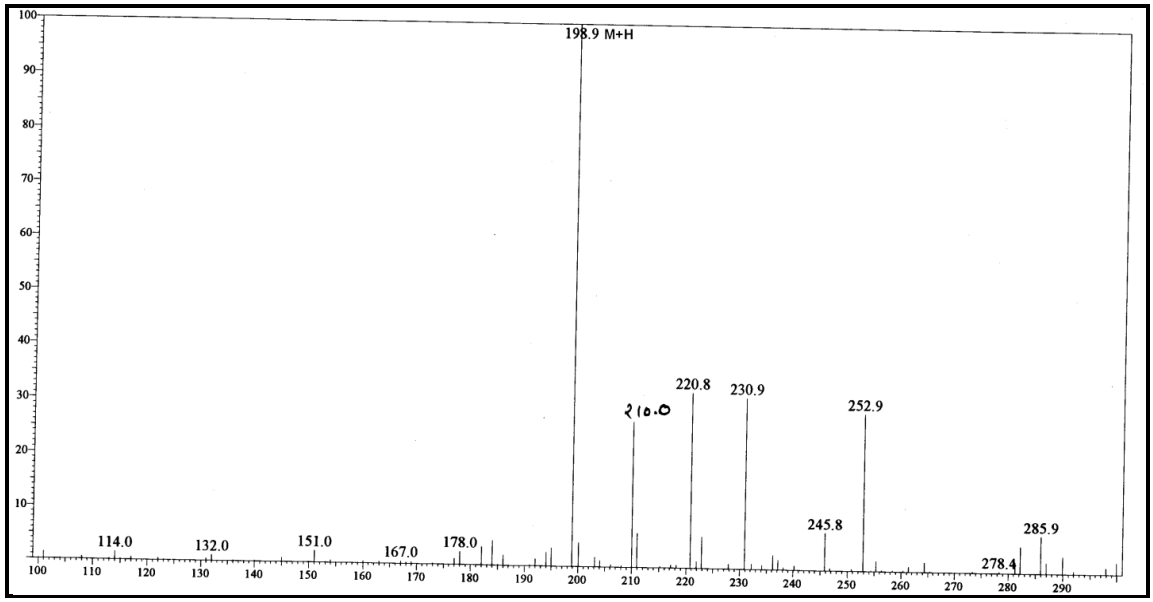


6.2. Coagulation Factor Xa Inhibitors

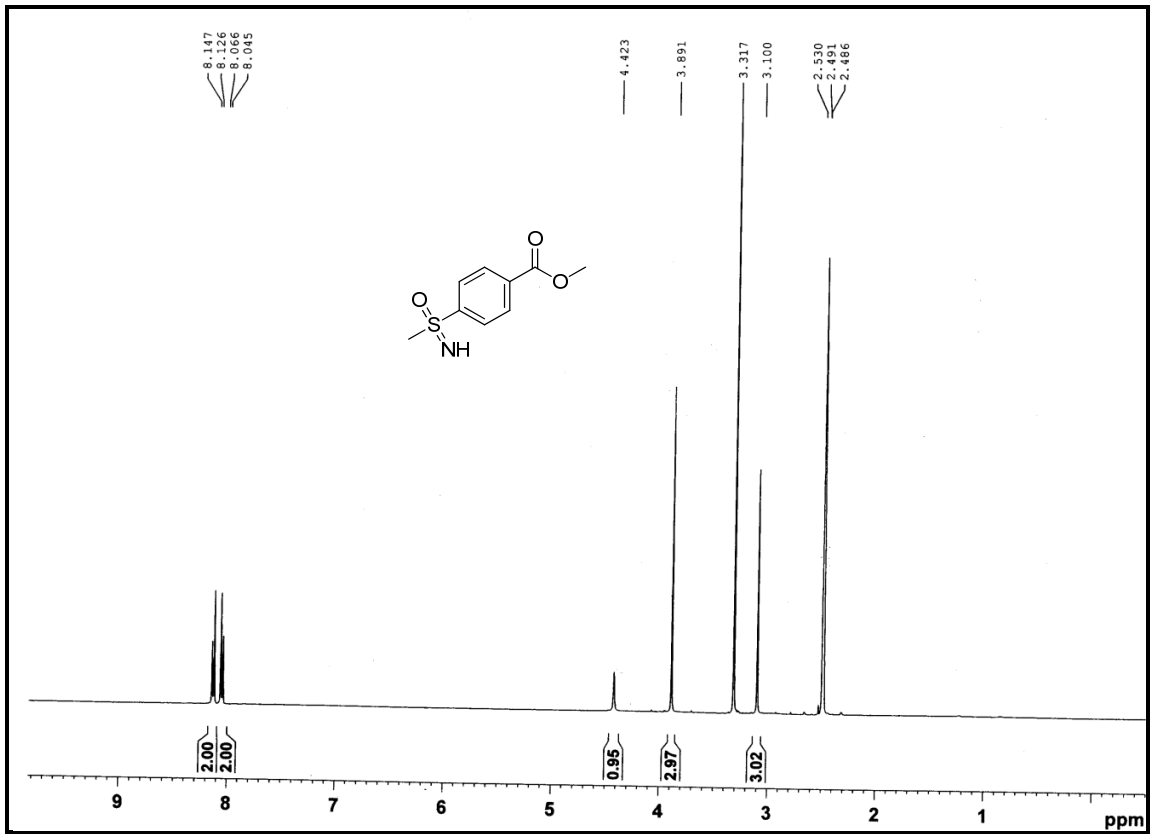
^{1}H NMR of 35a



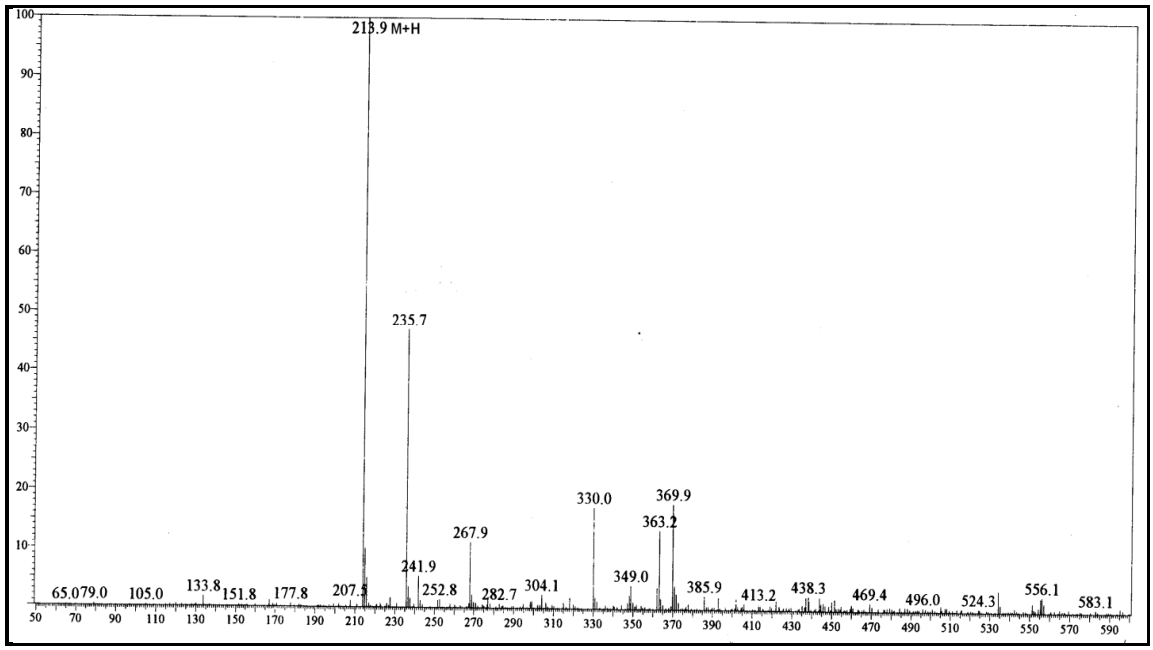
ESI-Mass of 35a



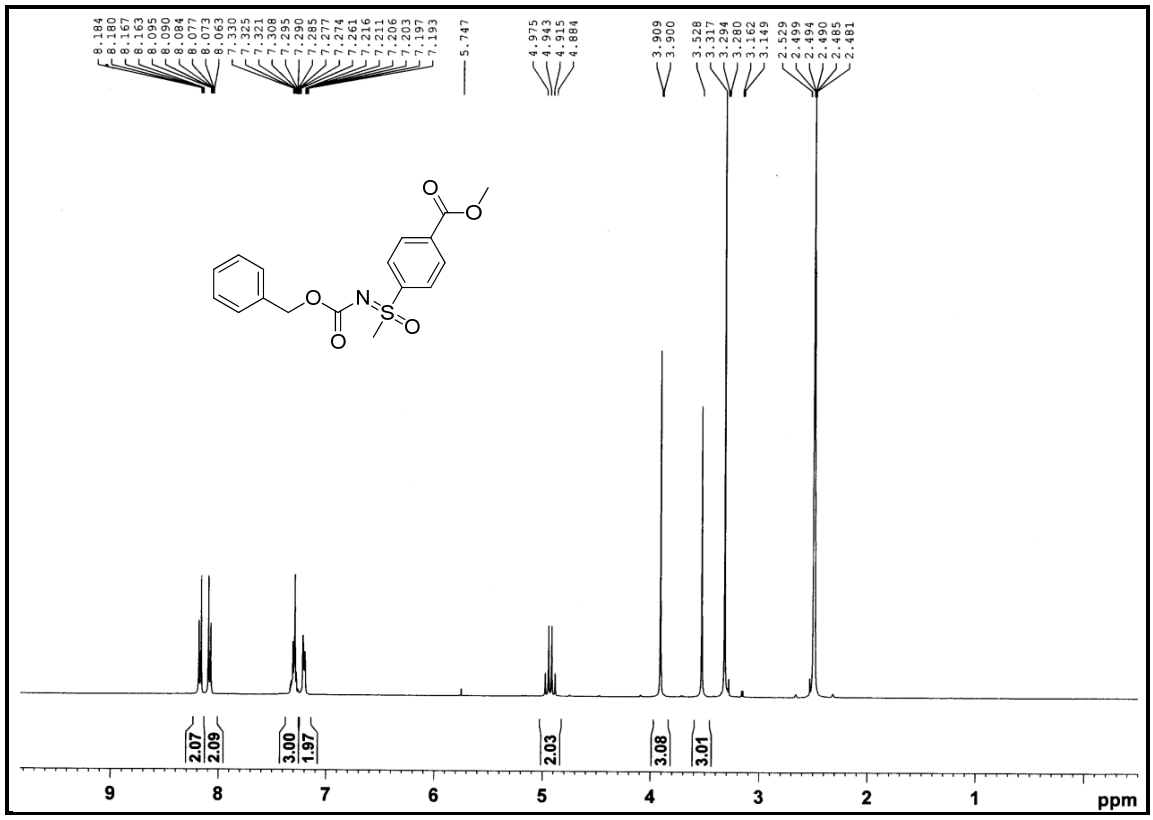
^{1}H NMR of 36a



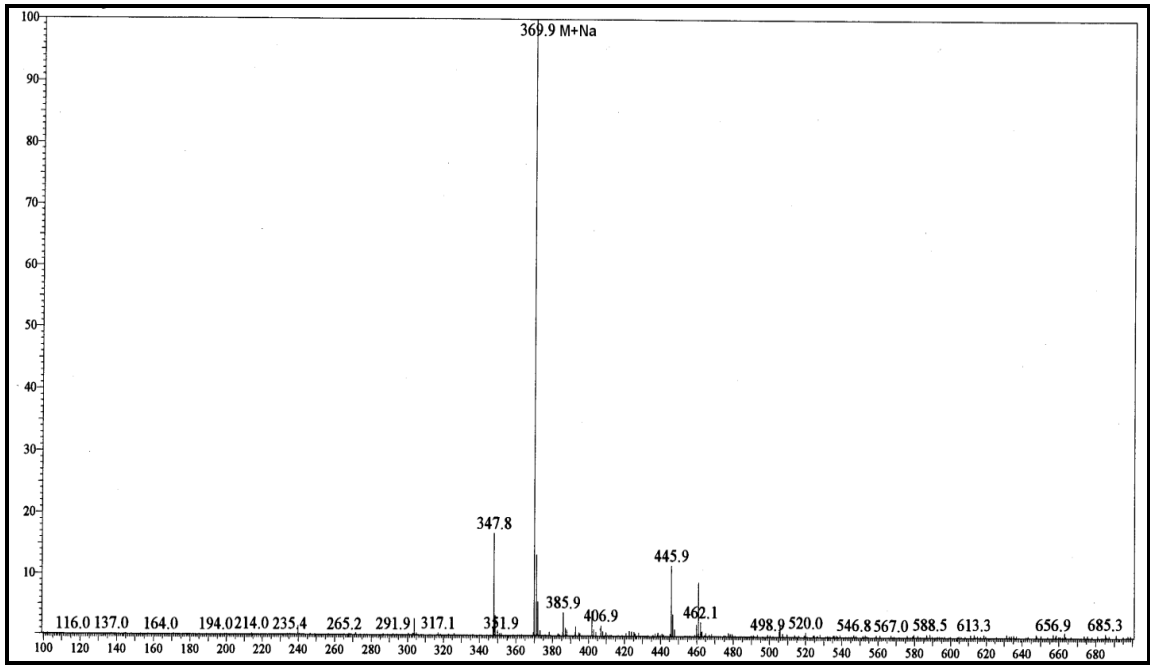
ESI-Mass of 36a



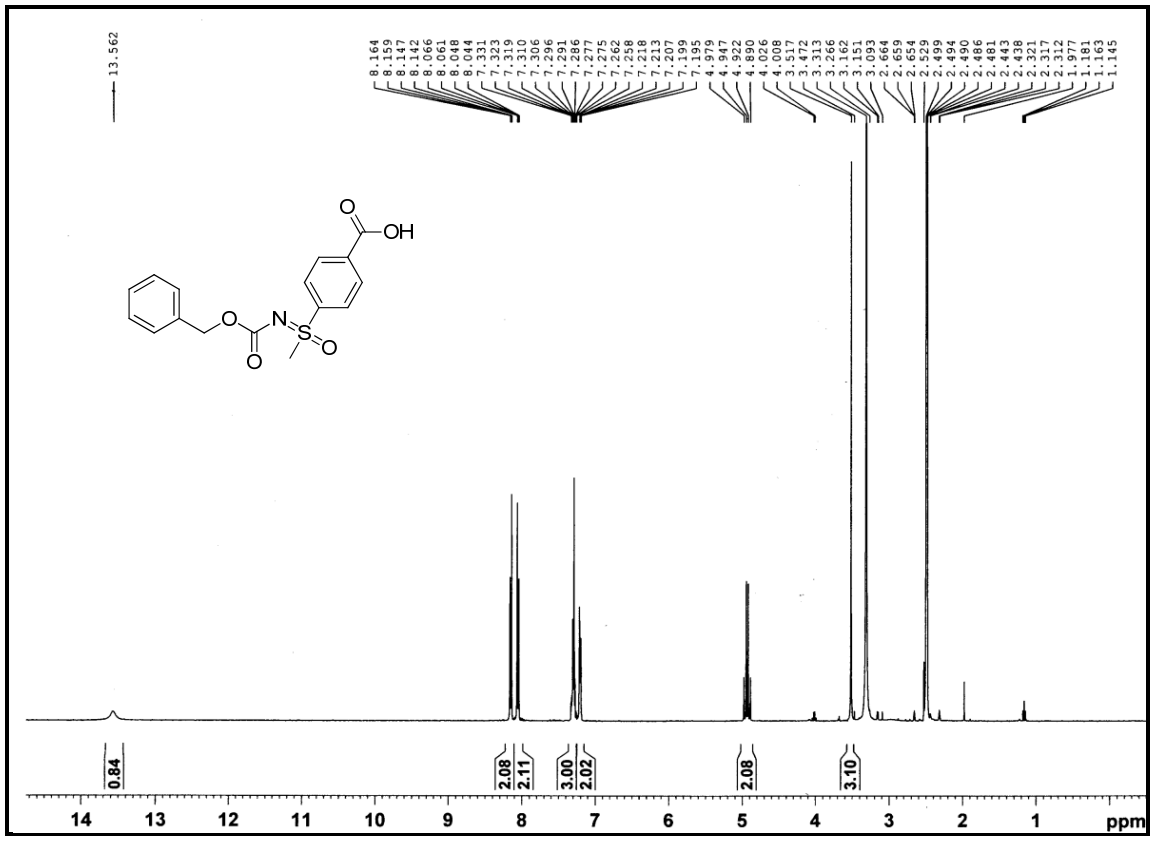
^{1}H NMR of 37a



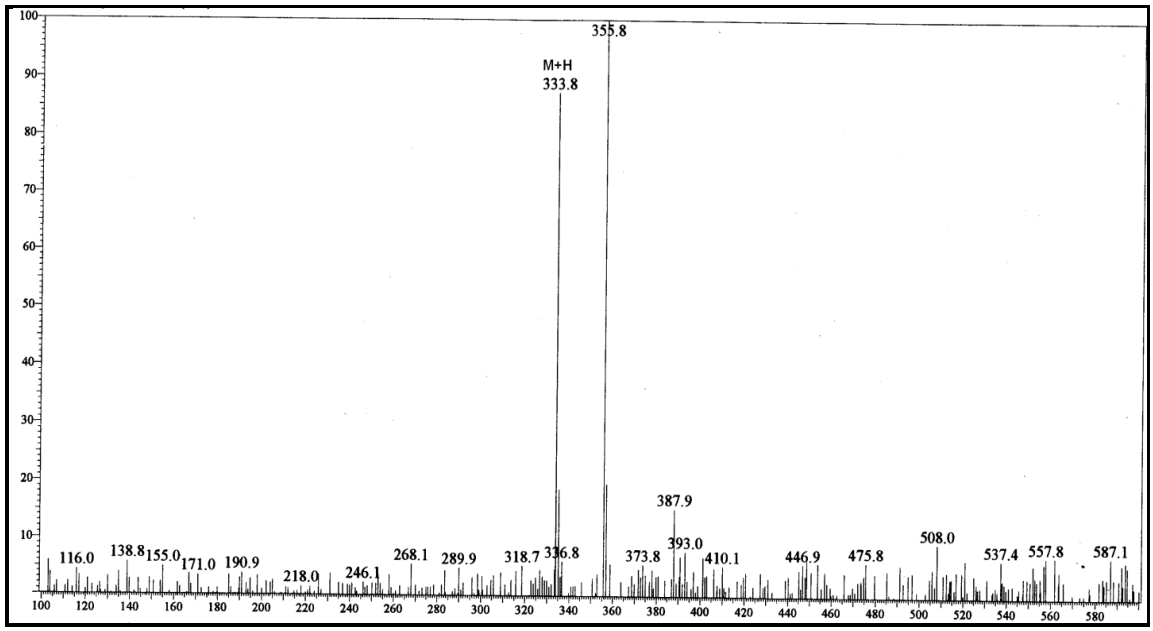
ESI-Mass of 37a



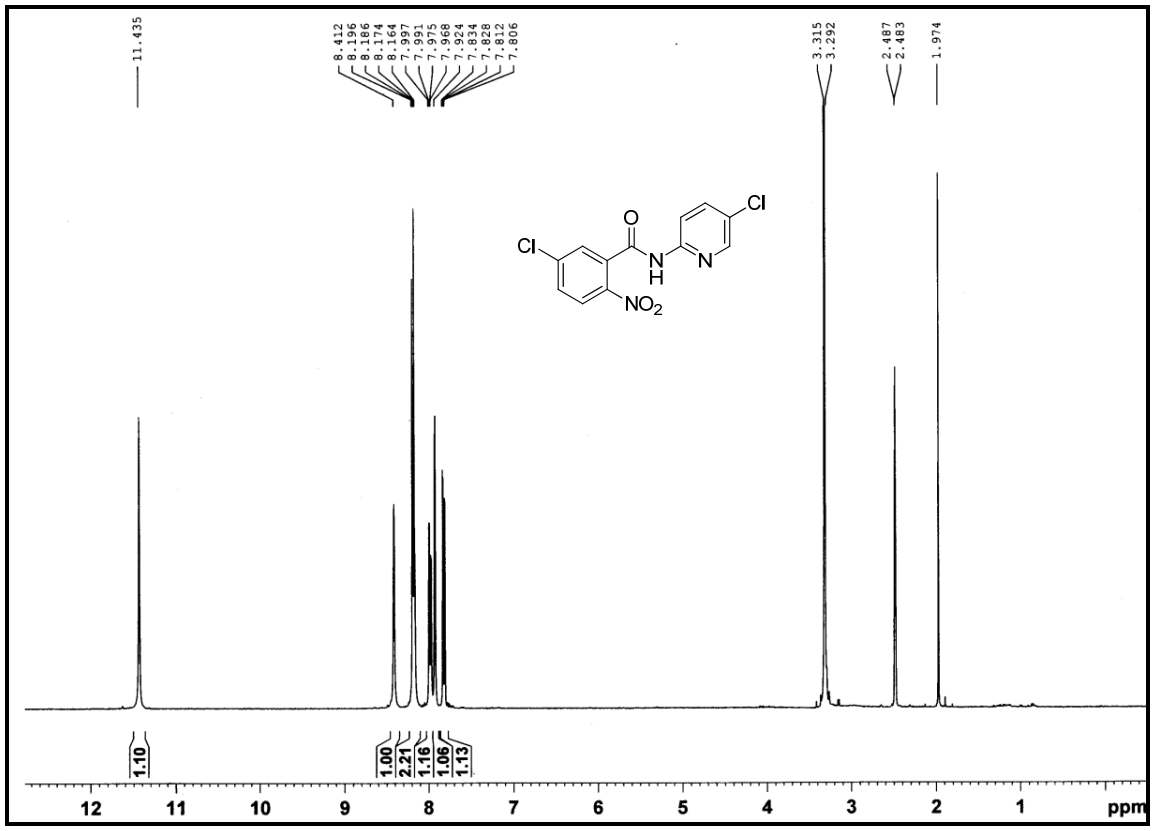
^{1}H NMR of 38a



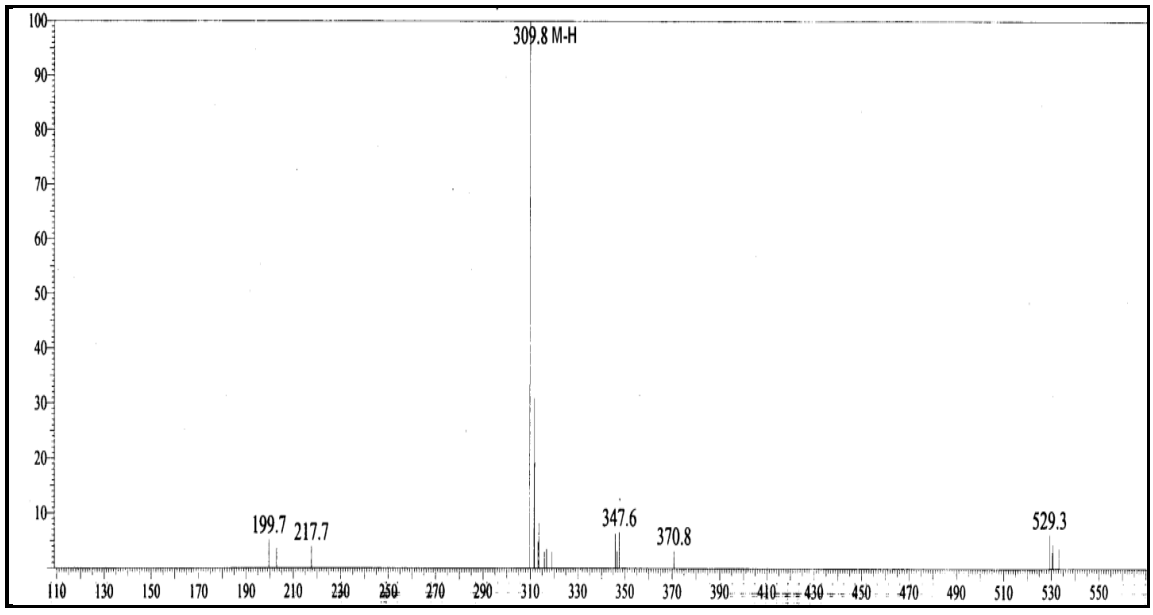
ESI-Mass of 38a



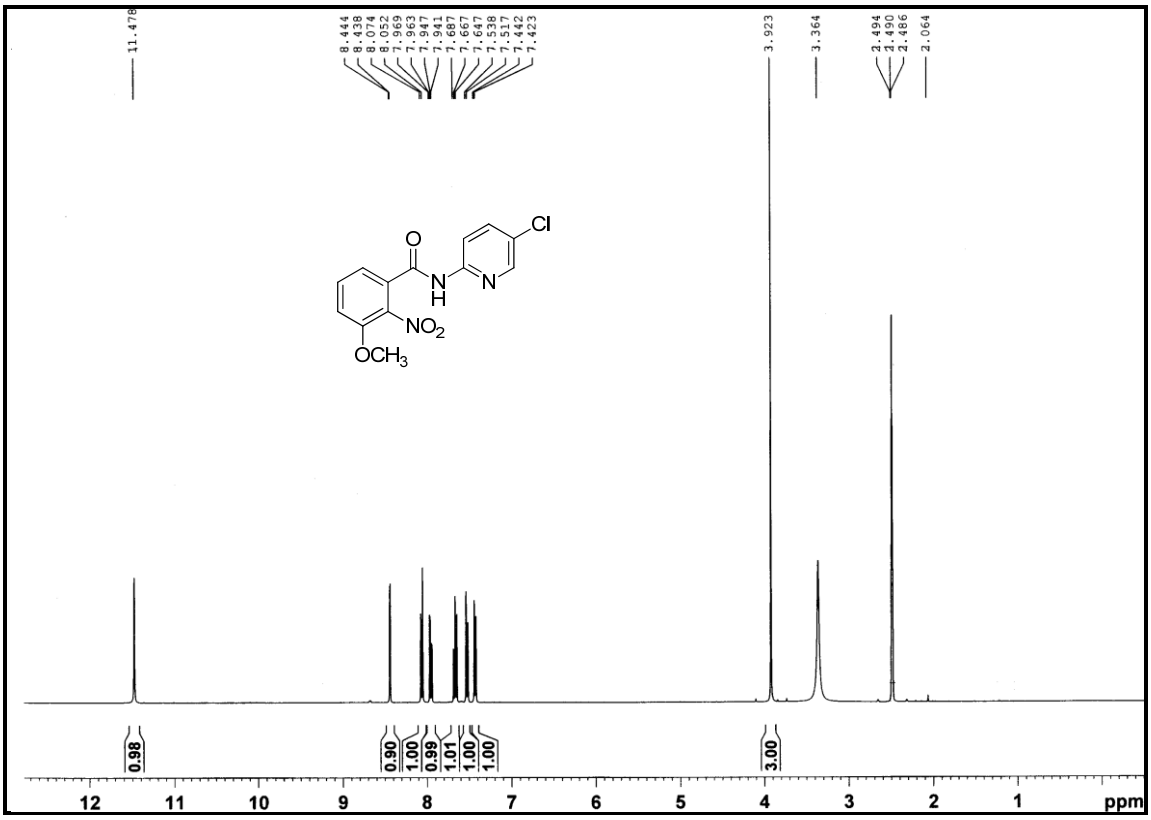
^{1}H NMR of 41a



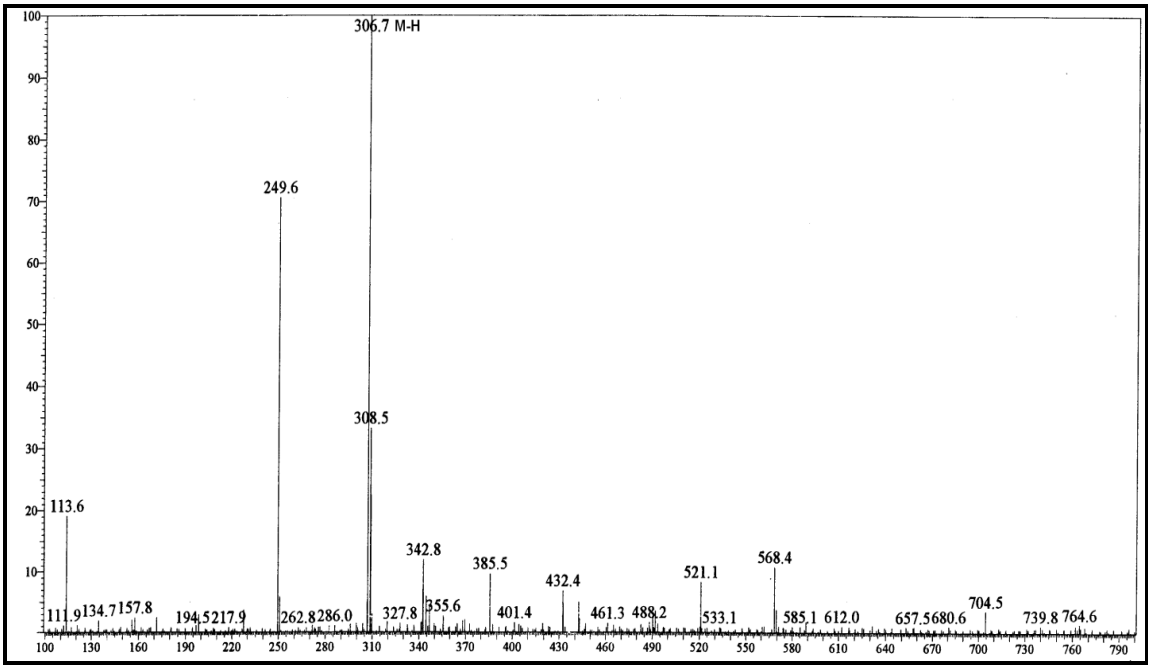
ESI-Mass of 41a



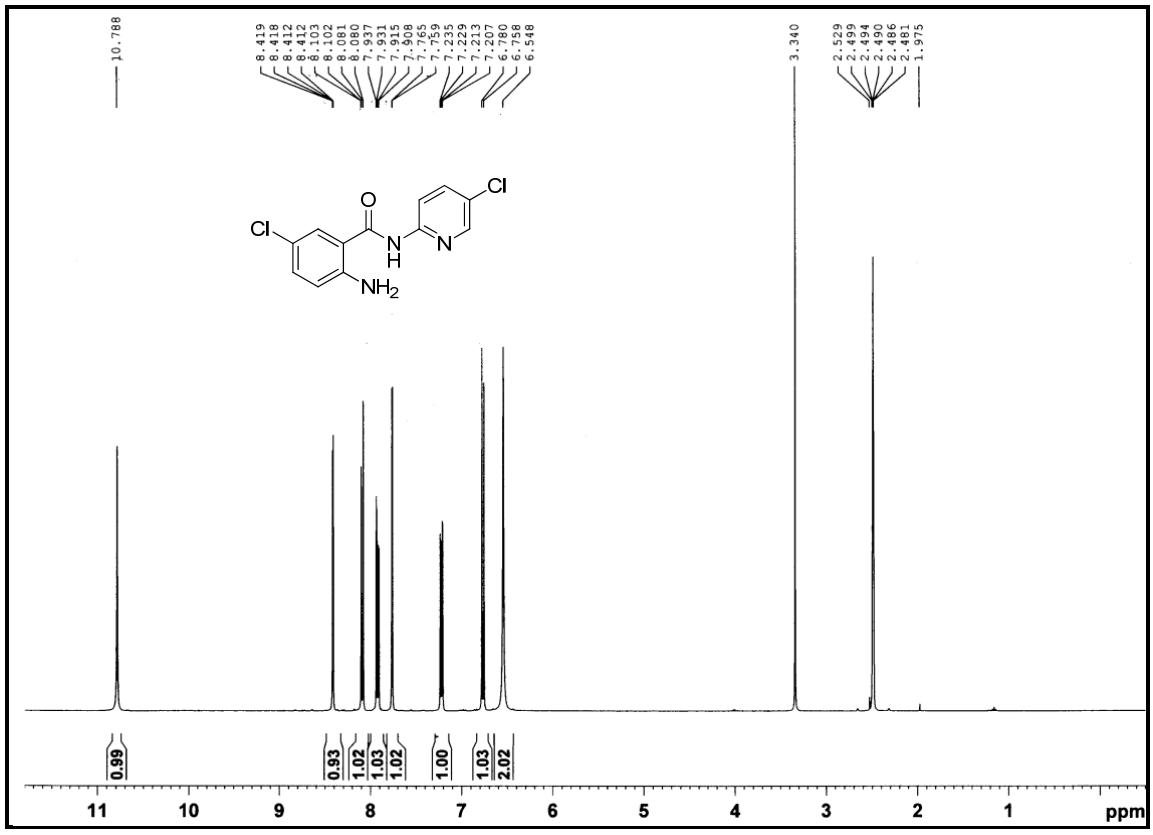
^{1}H NMR of 41c



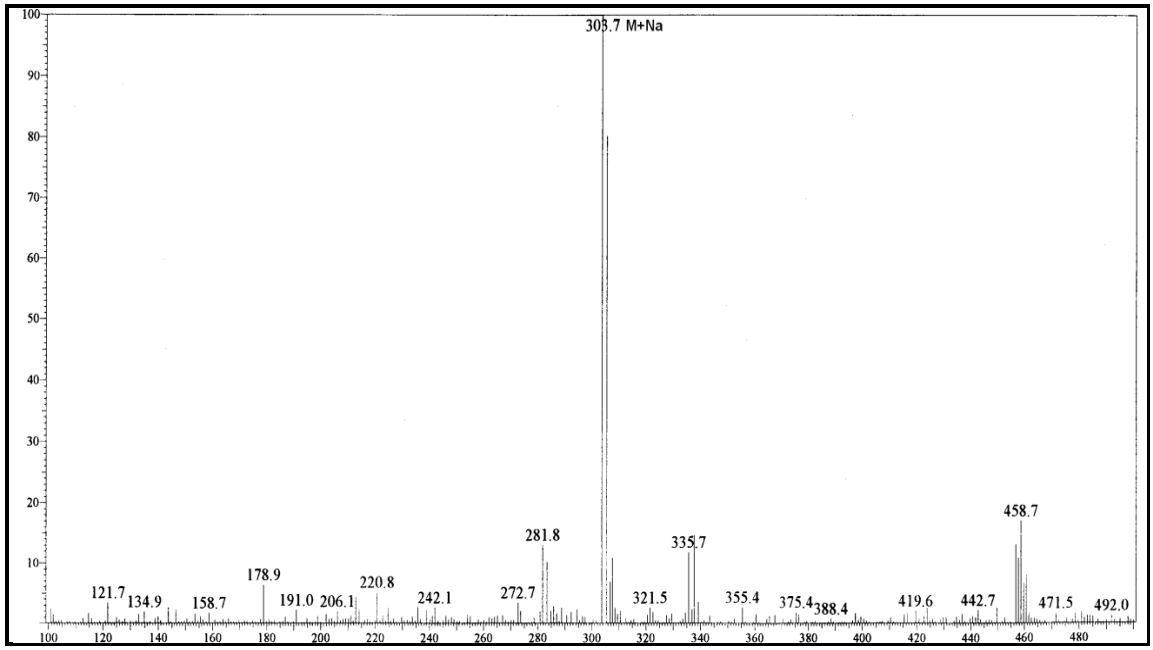
ESI-Mass of 41c



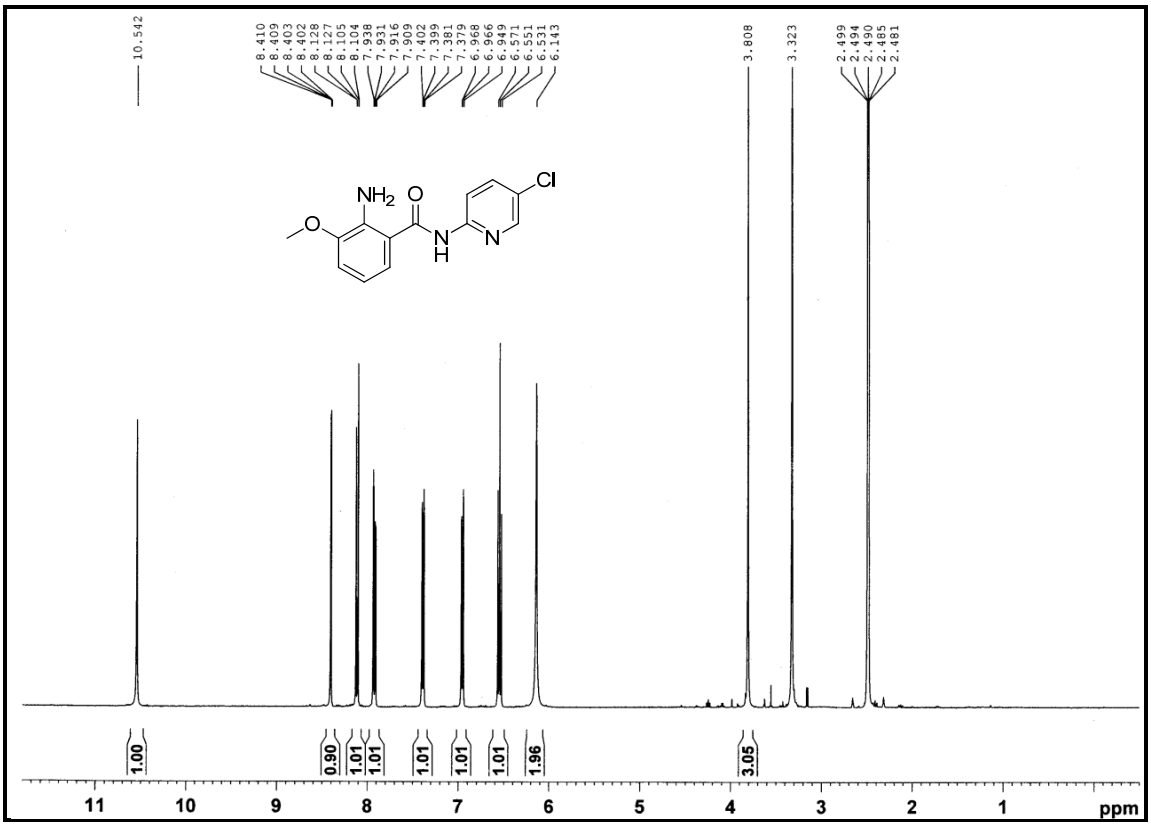
^{1}H NMR of 42a



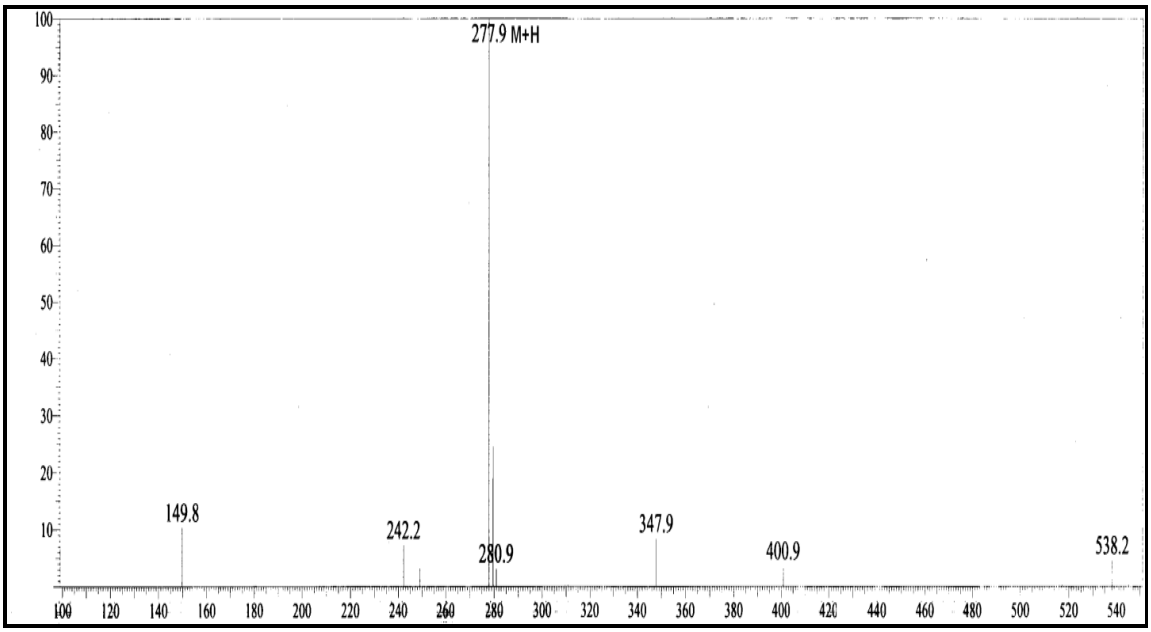
ESI-Mass of 42a



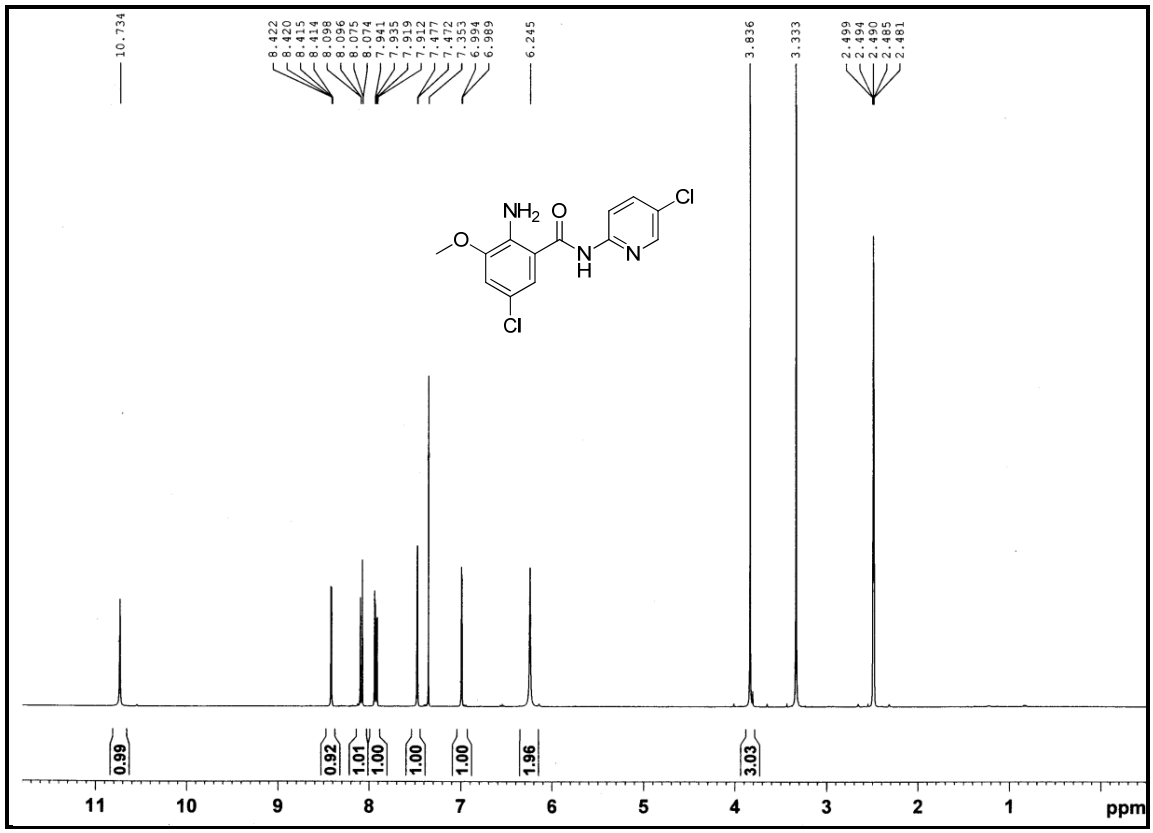
^{1}H NMR of 42c



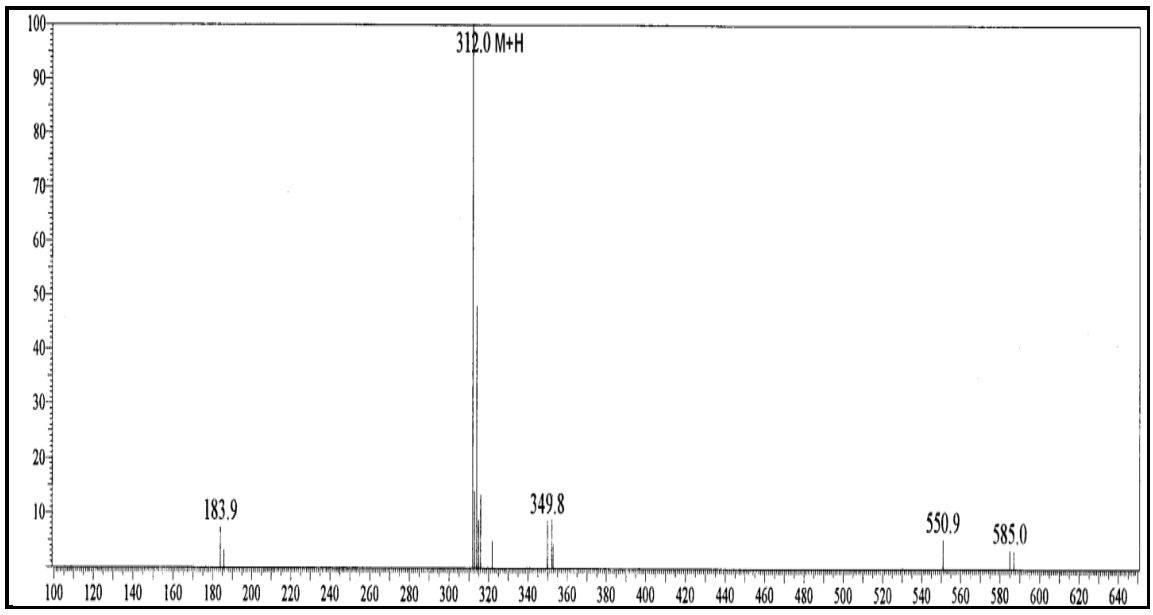
ESI-Mass of 42c



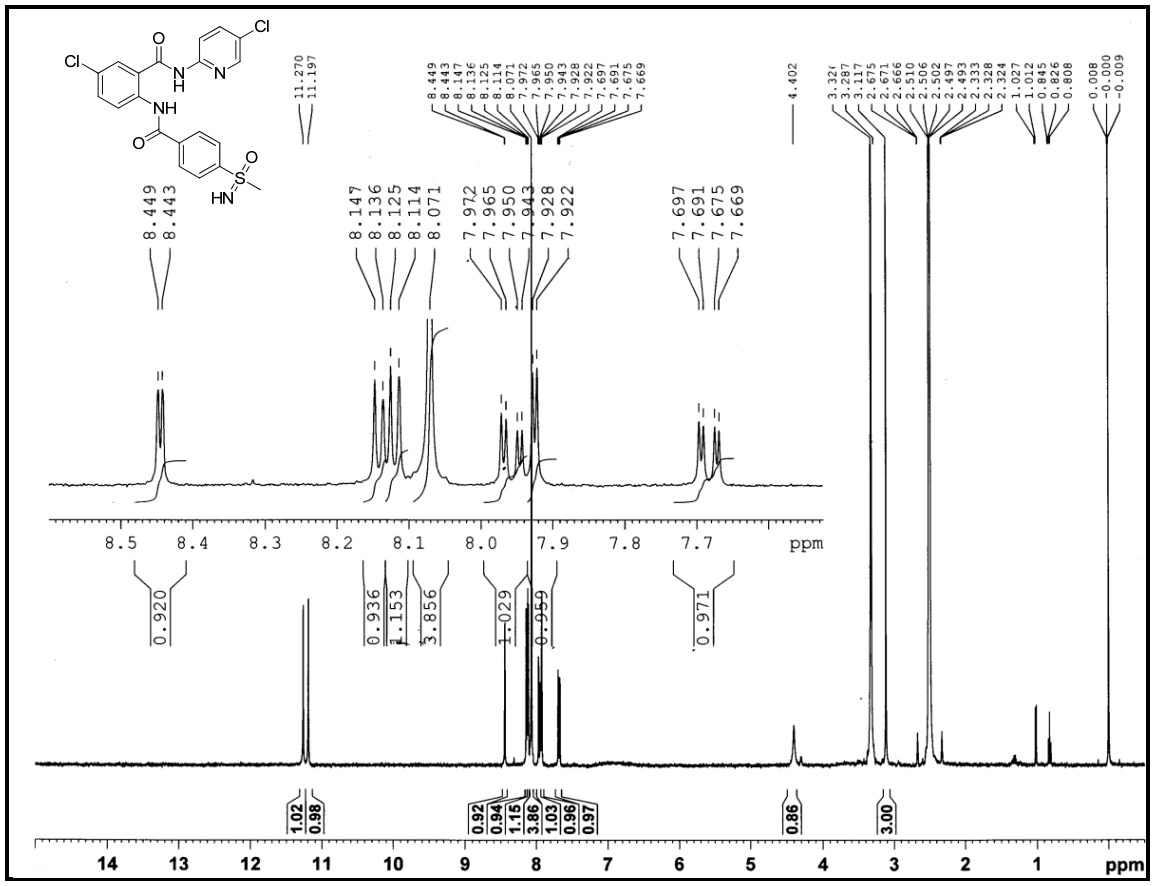
^{1}H NMR of 42d



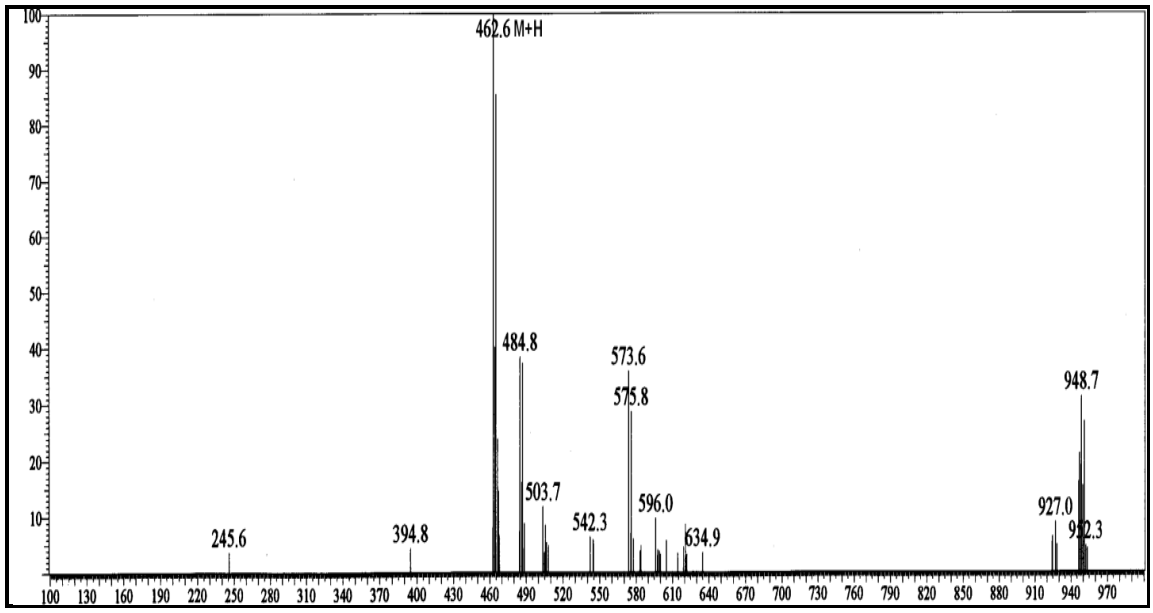
ESI-Mass of 42d



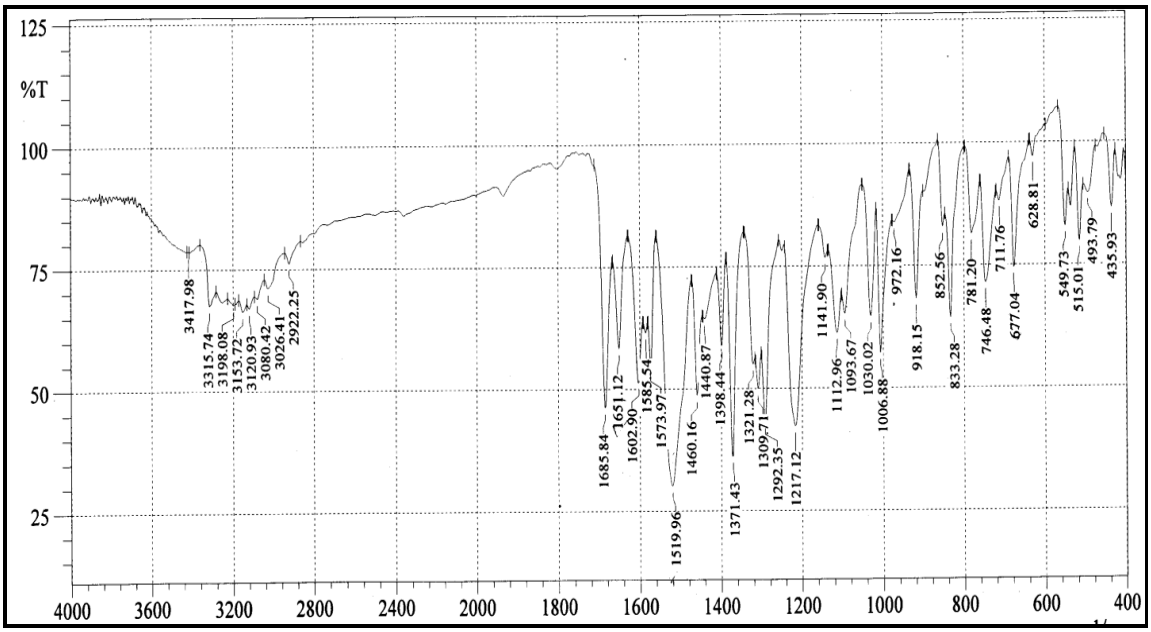
^{1}H NMR of 43a



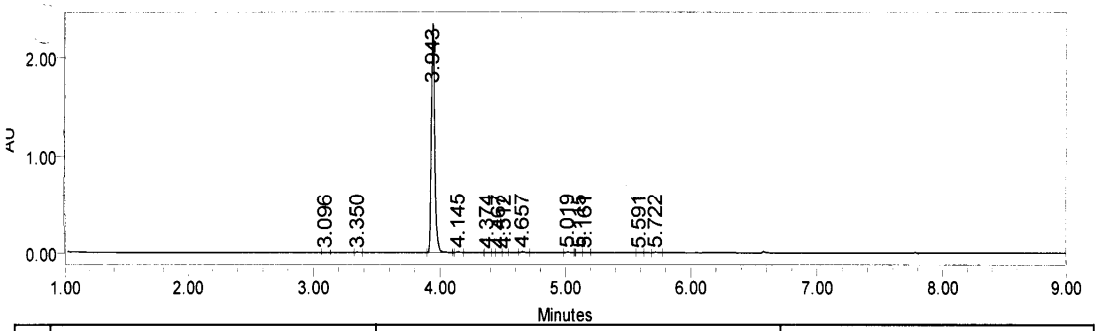
ESI-Mass of 43a



IR of 43a

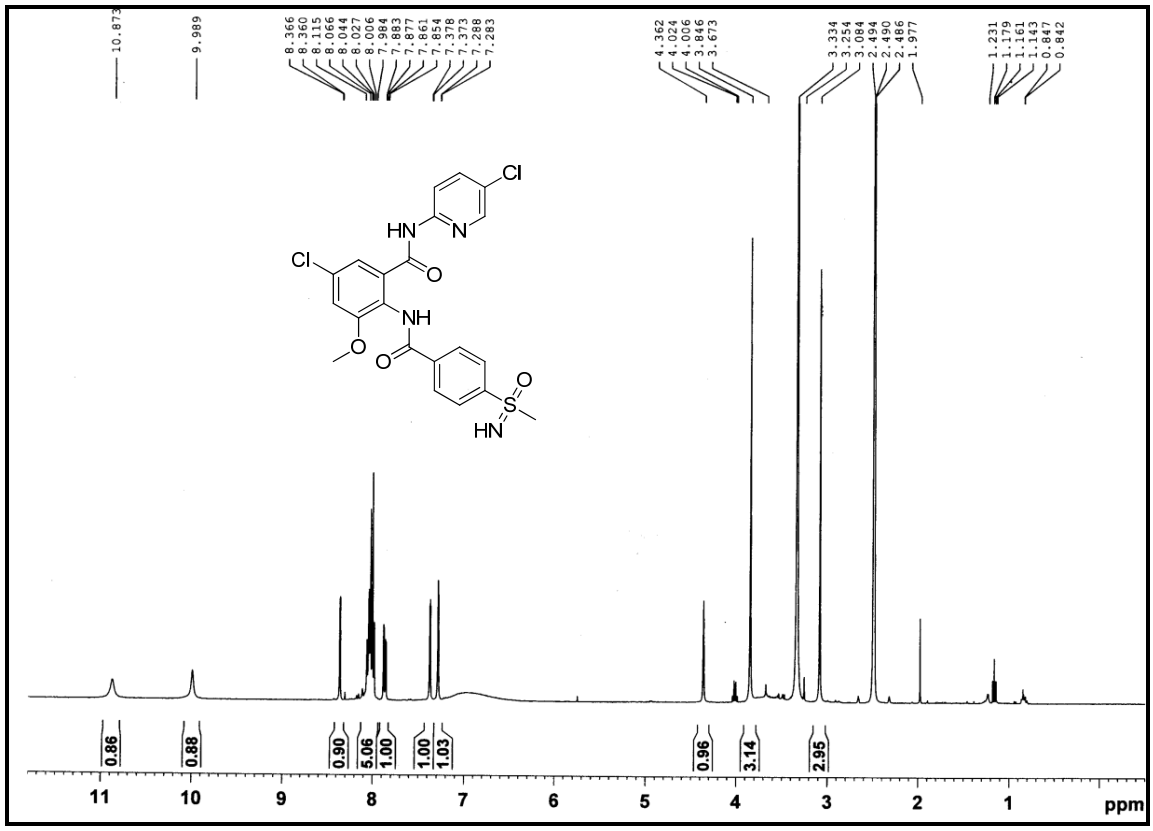


UPLC of 43a

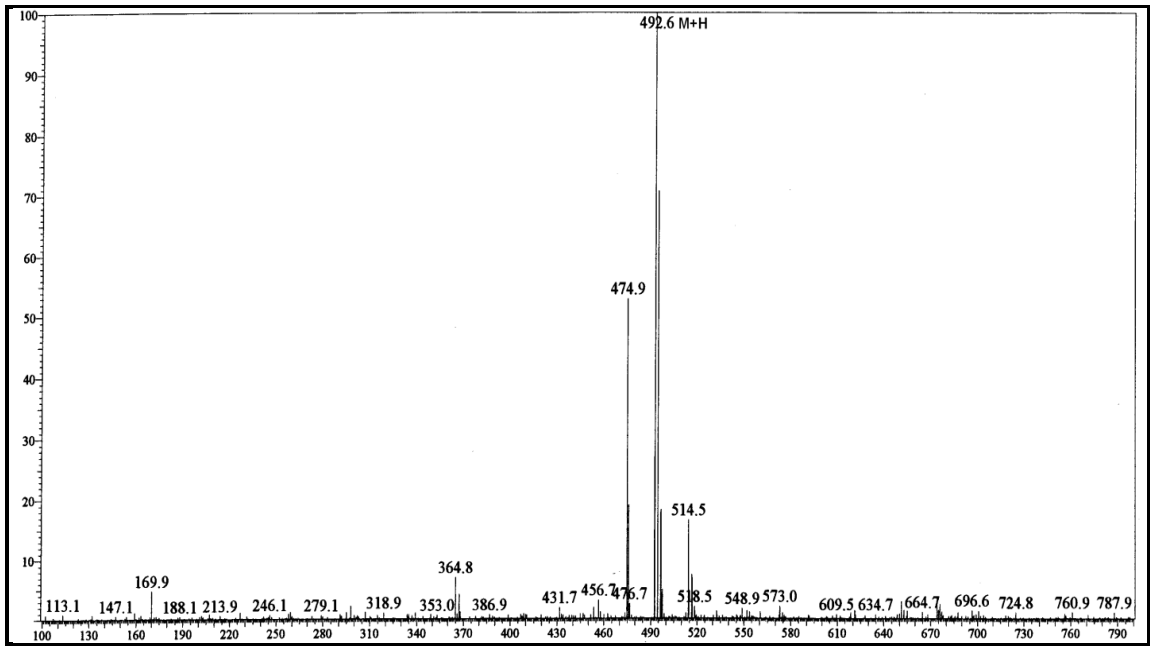


	RT	Area	% Area
1	3.096	3592	0.08
2	3.350	6215	0.14
3	3.943	4378891	98.19
4	4.145	12857	0.29
5	4.374	1032	0.02
6	4.467	1523	0.03
7	4.512	1236	0.03
8	4.657	18591	0.42
9	5.019	12791	0.29
10	5.115	3169	0.07

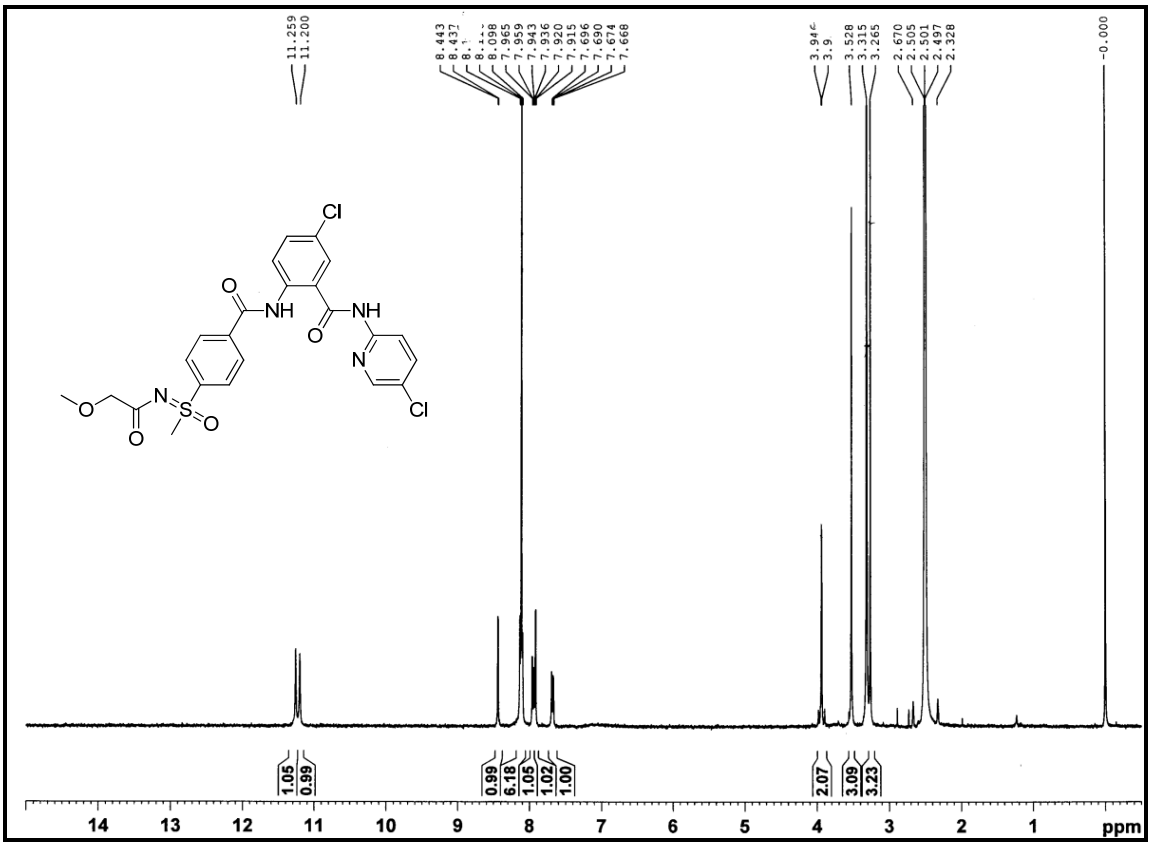
^{1}H NMR of 43f



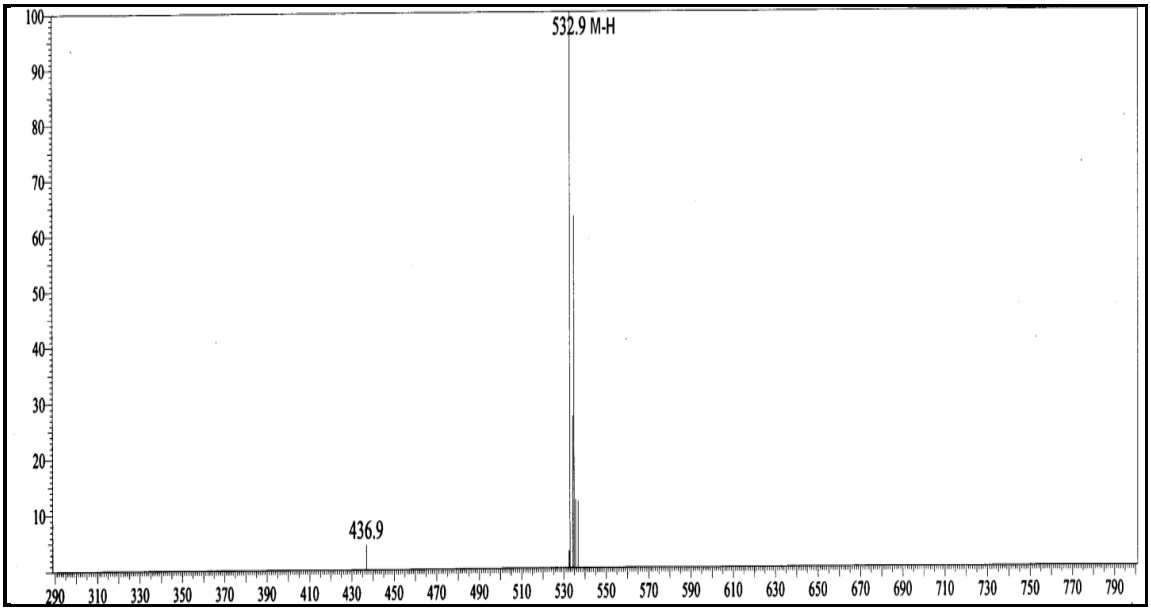
ESI-Mass of 43f



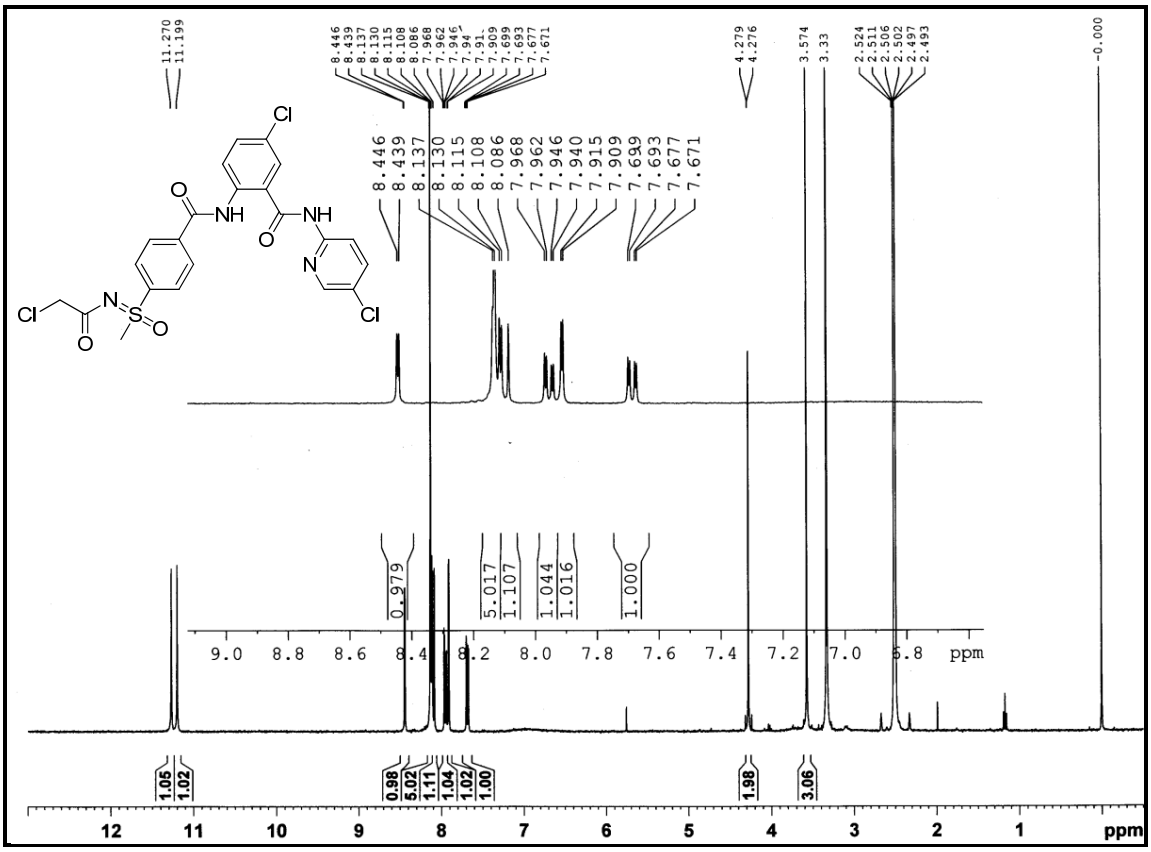
^{1}H NMR of 44c



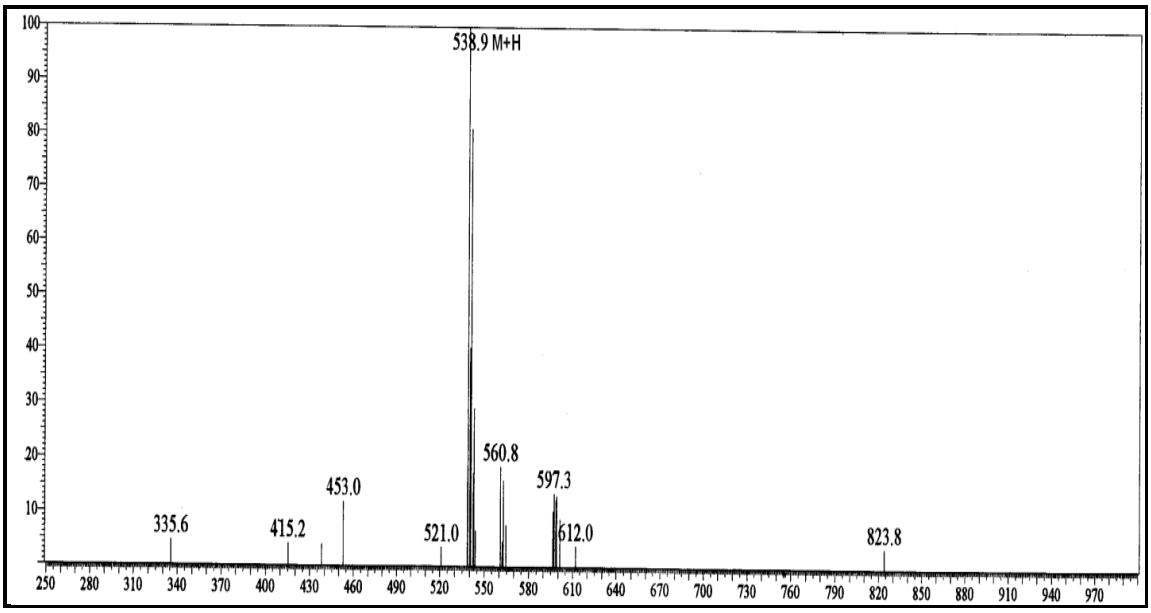
ESI-Mass of 44c



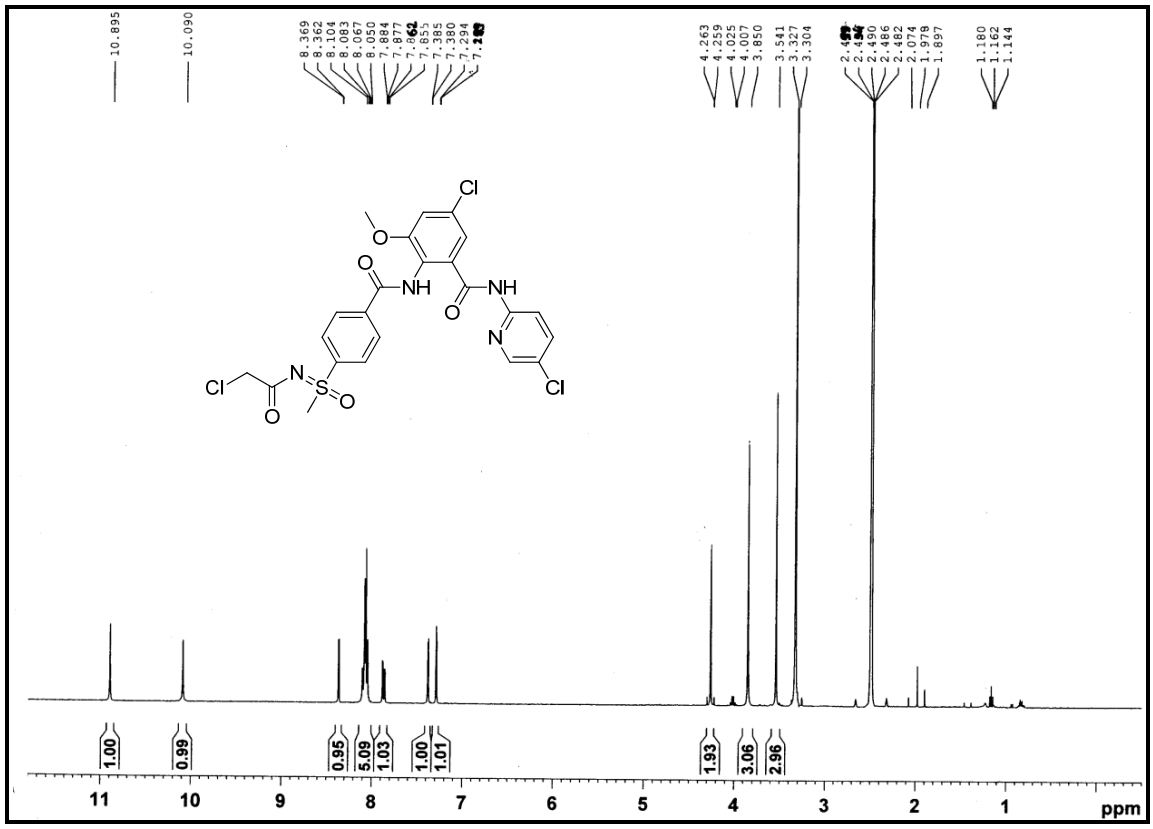
^{1}H NMR of 45a



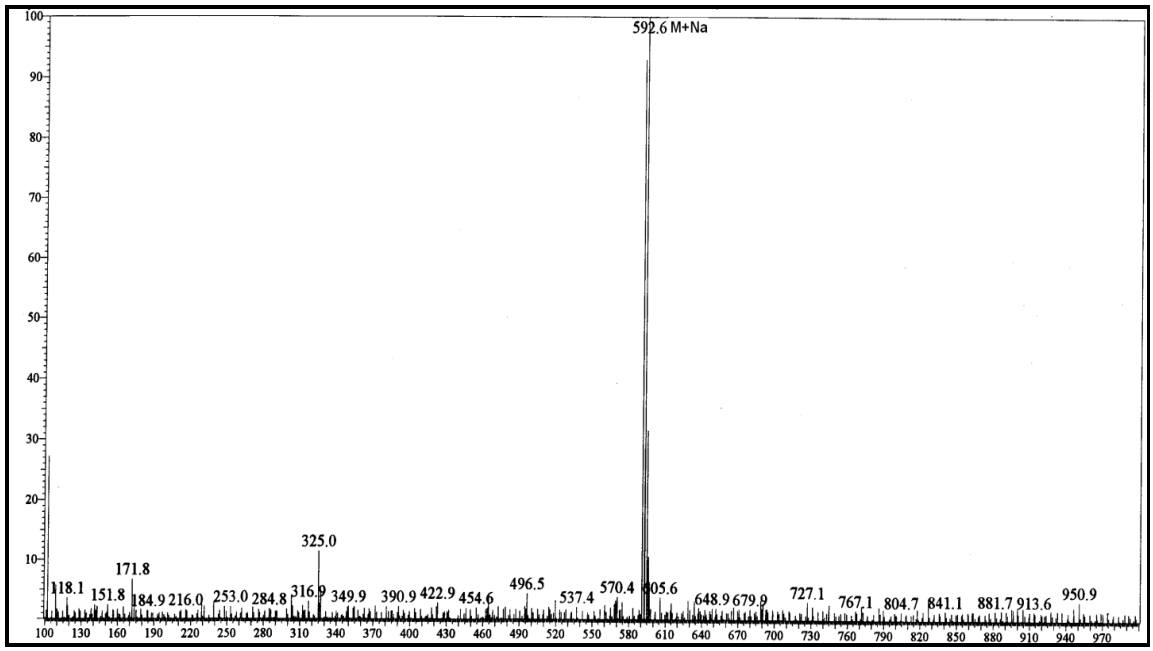
ESI-Mass of 45a



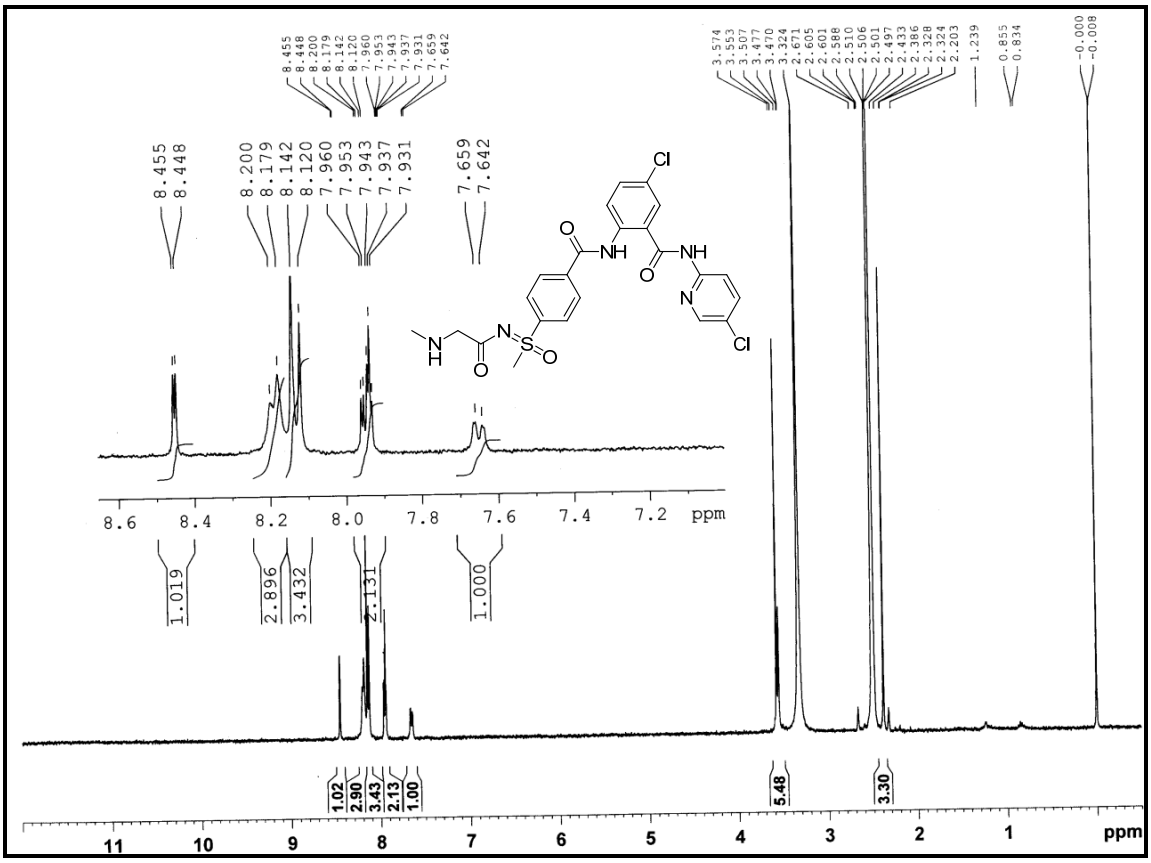
^{1}H NMR of 45f



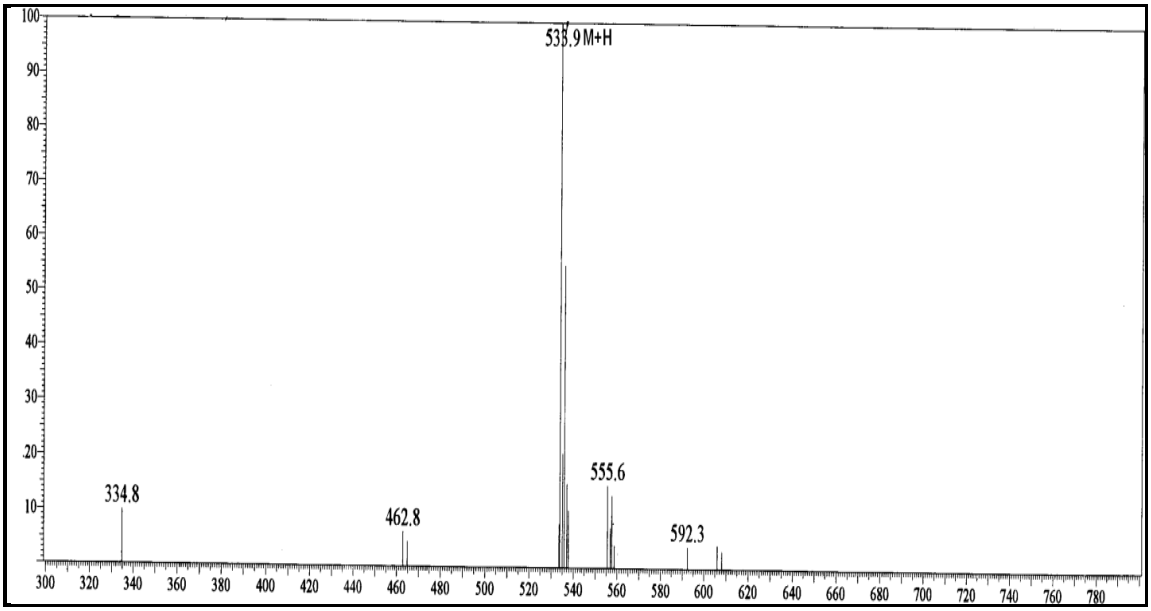
ESI-Mass of 45f



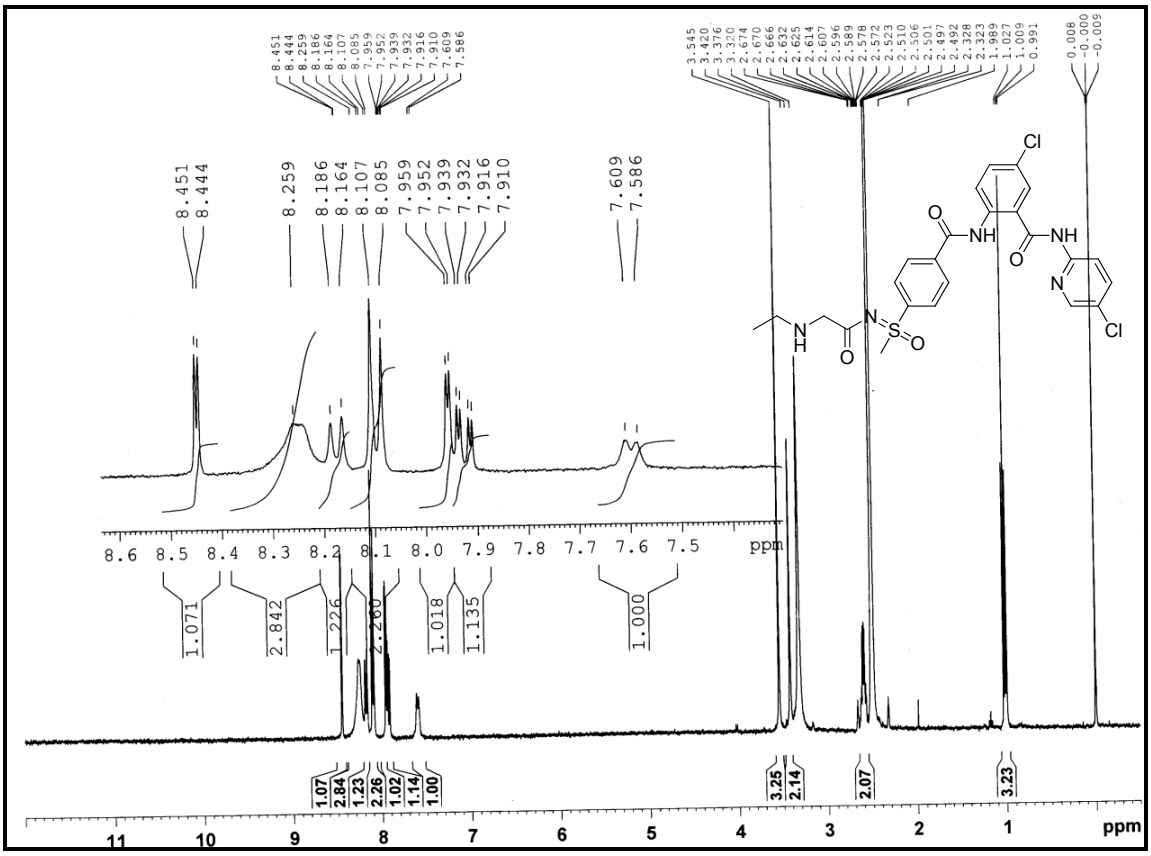
^{1}H NMR of 46a



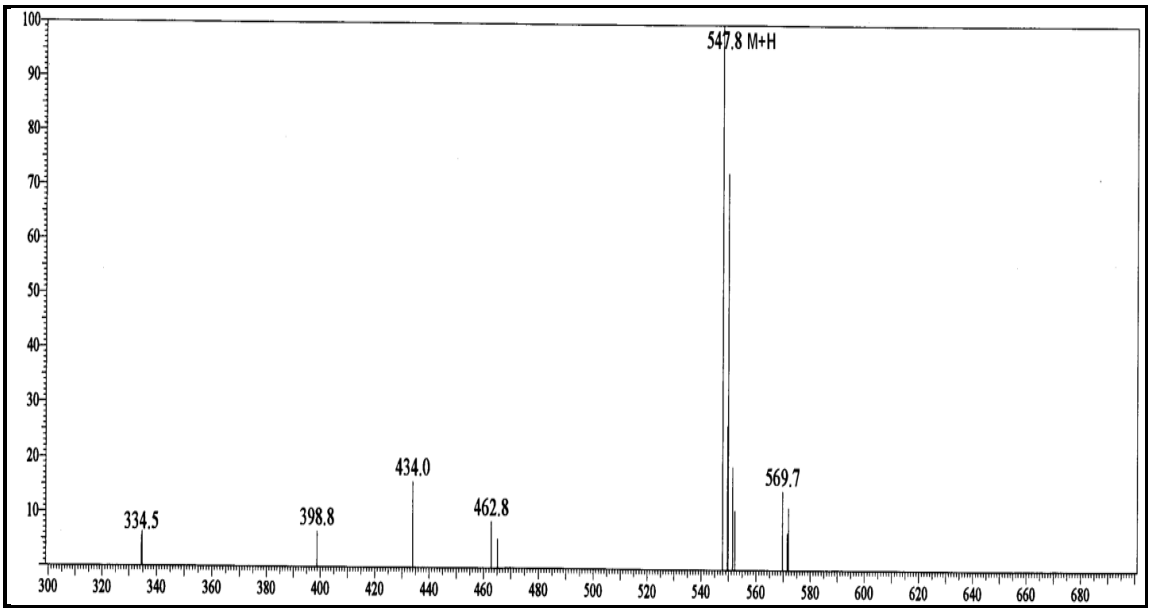
ESI-Mass of 46a



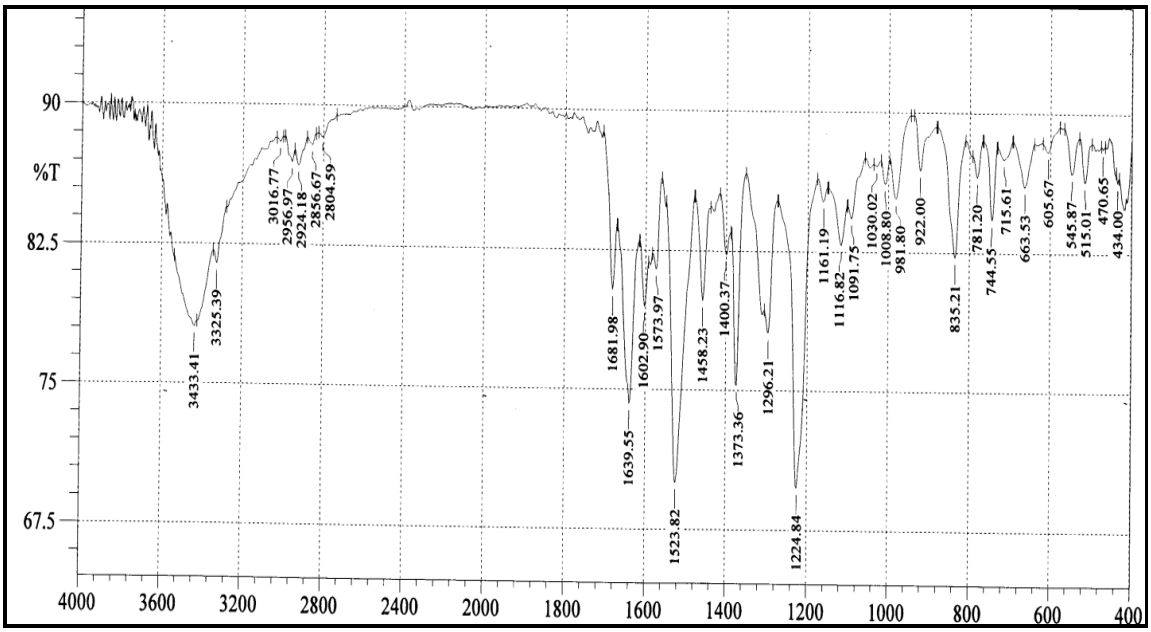
^{1}H NMR of 46b



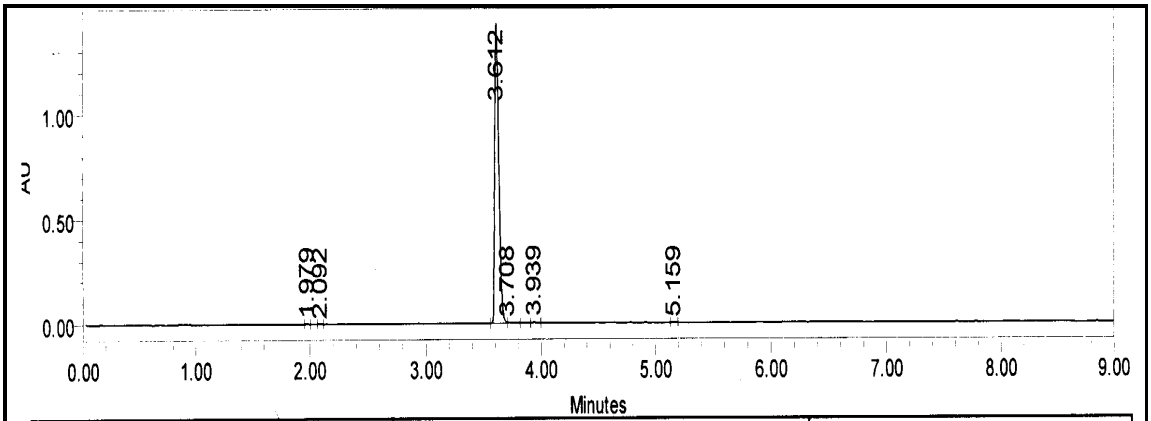
ESI-Mass of 46b



IR of 46b

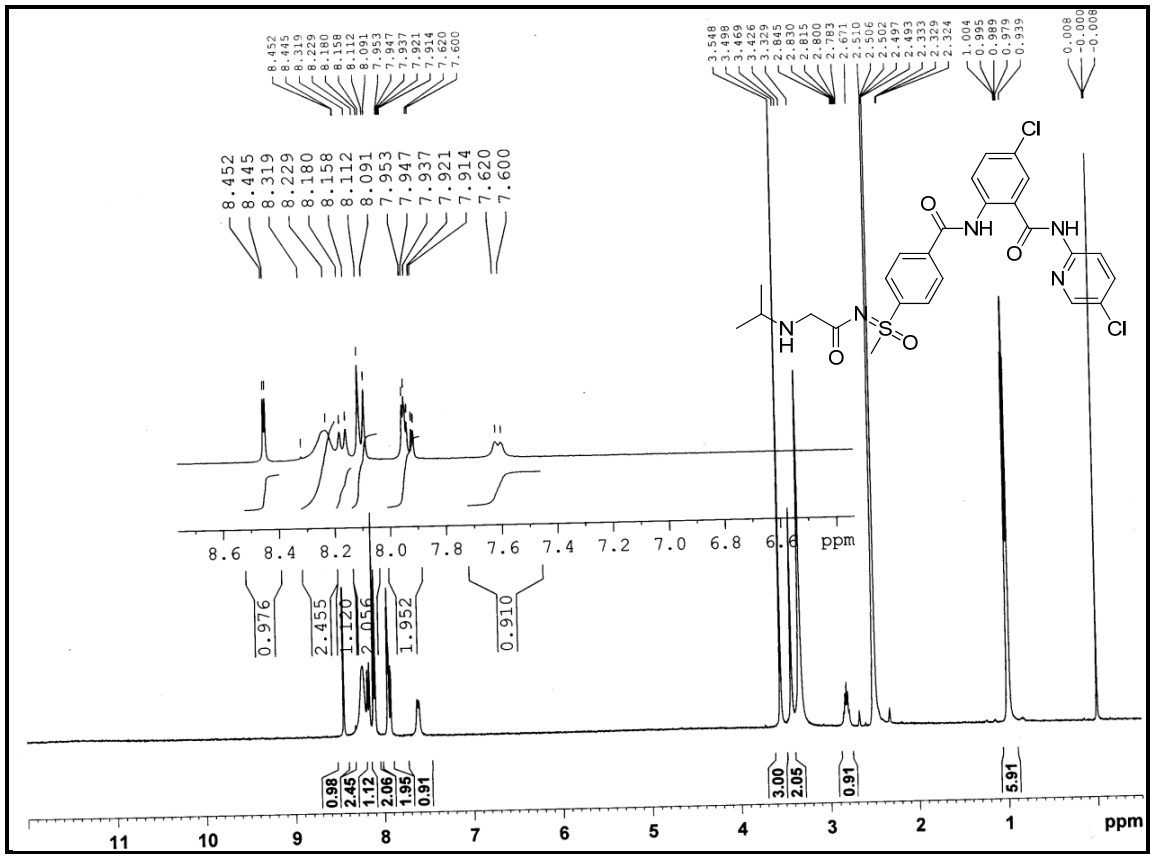


UPLC of 46b

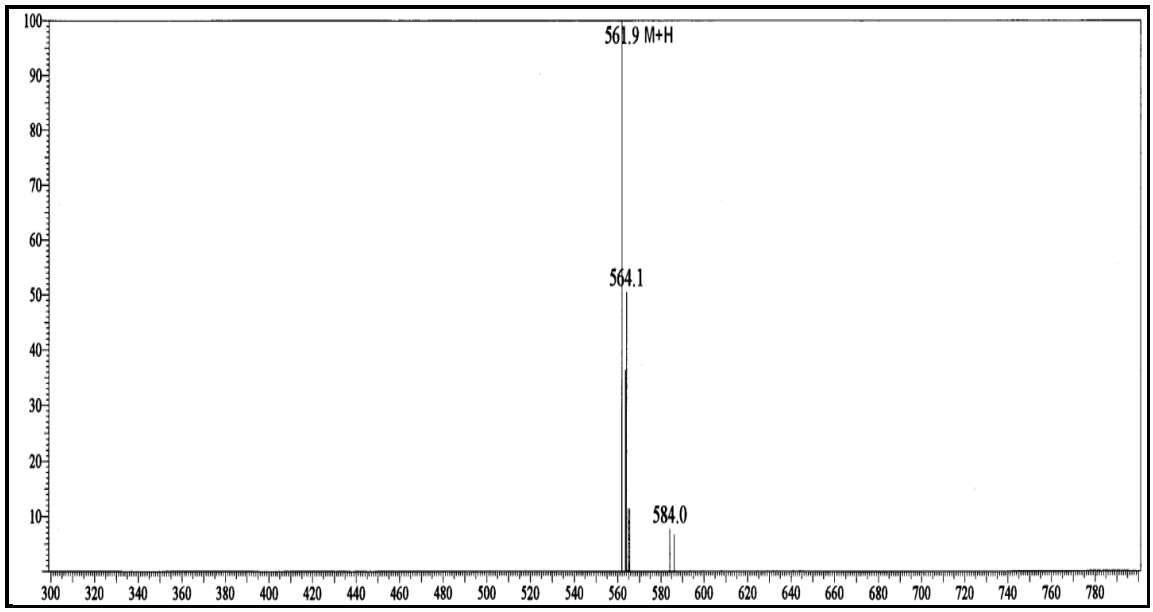


	RT	Area	% Area
1	1.979	562	0.02
2	2.092	581	0.02
3	3.612	3577016	99.38
4	3.708	9681	0.27
5	3.939	10541	0.29
6	5.159	942	0.03

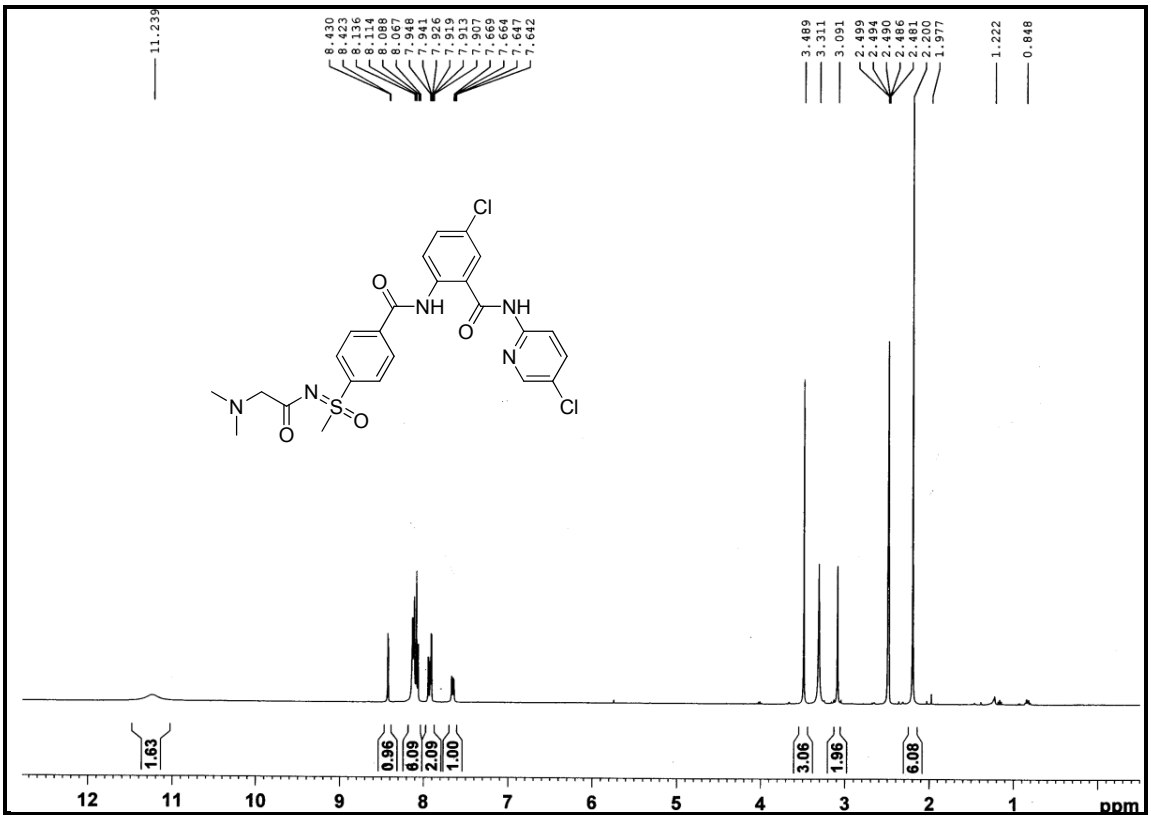
^{1}H NMR of 46c



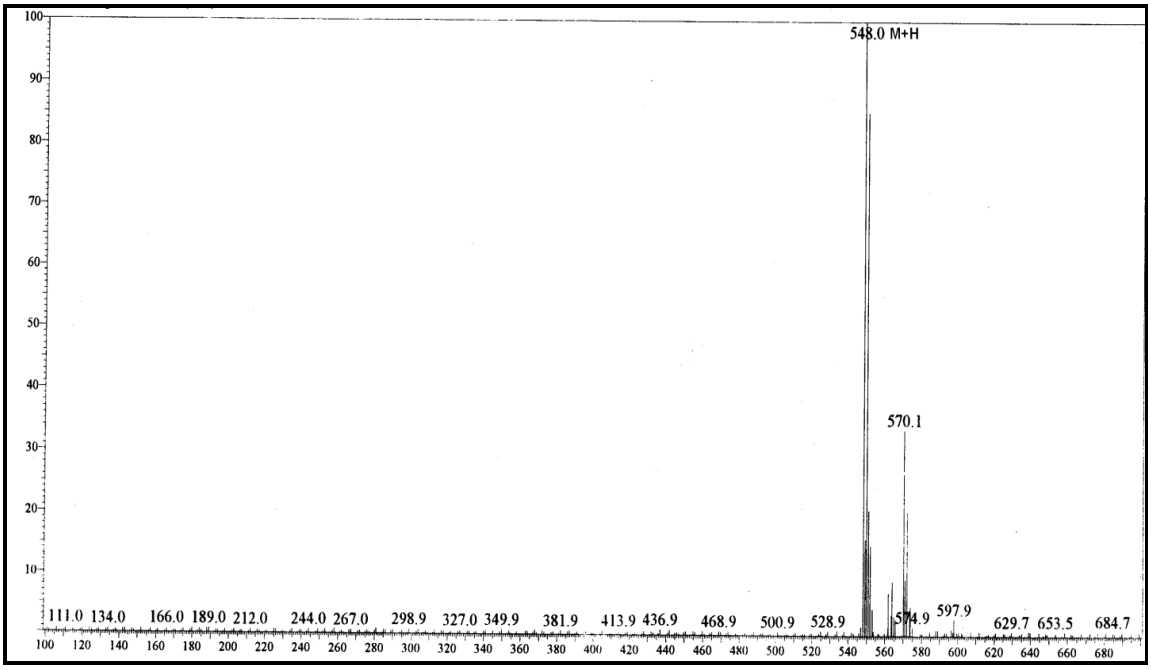
ESI-Mass of 46c



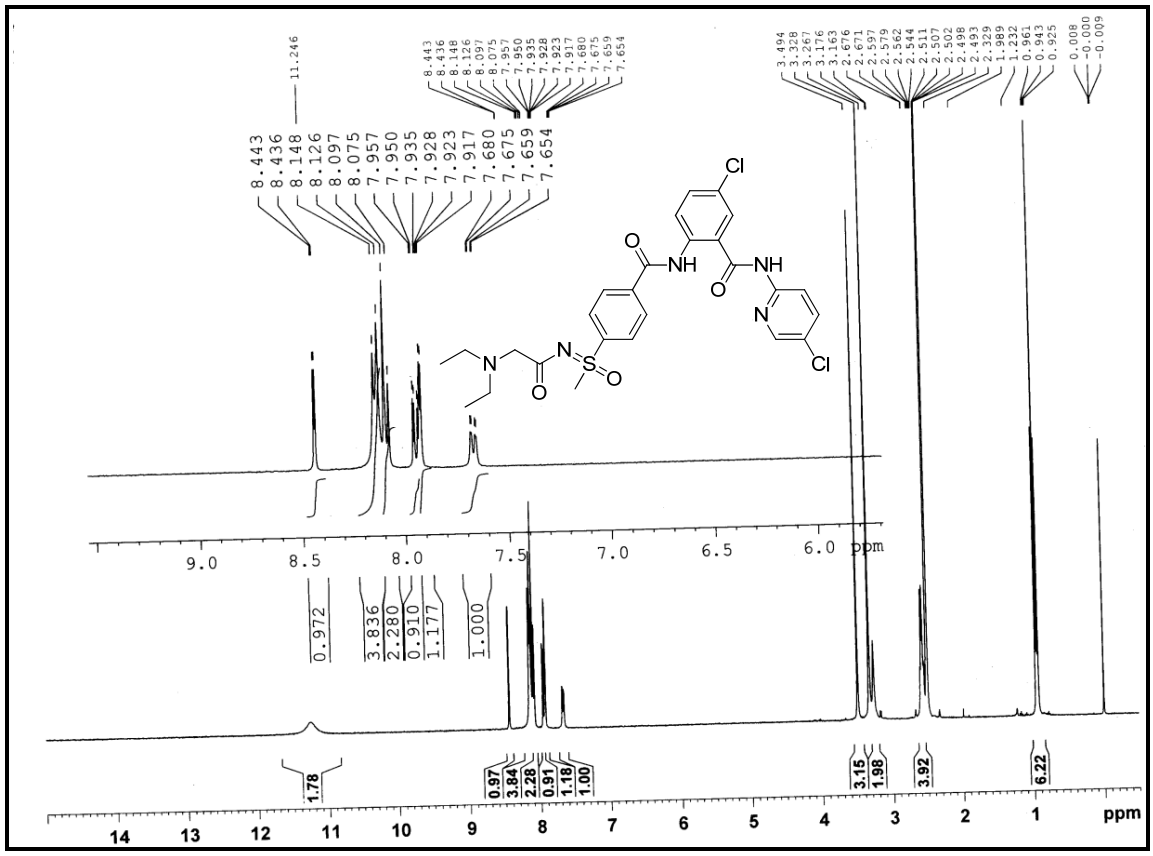
^{1}H NMR of 46e



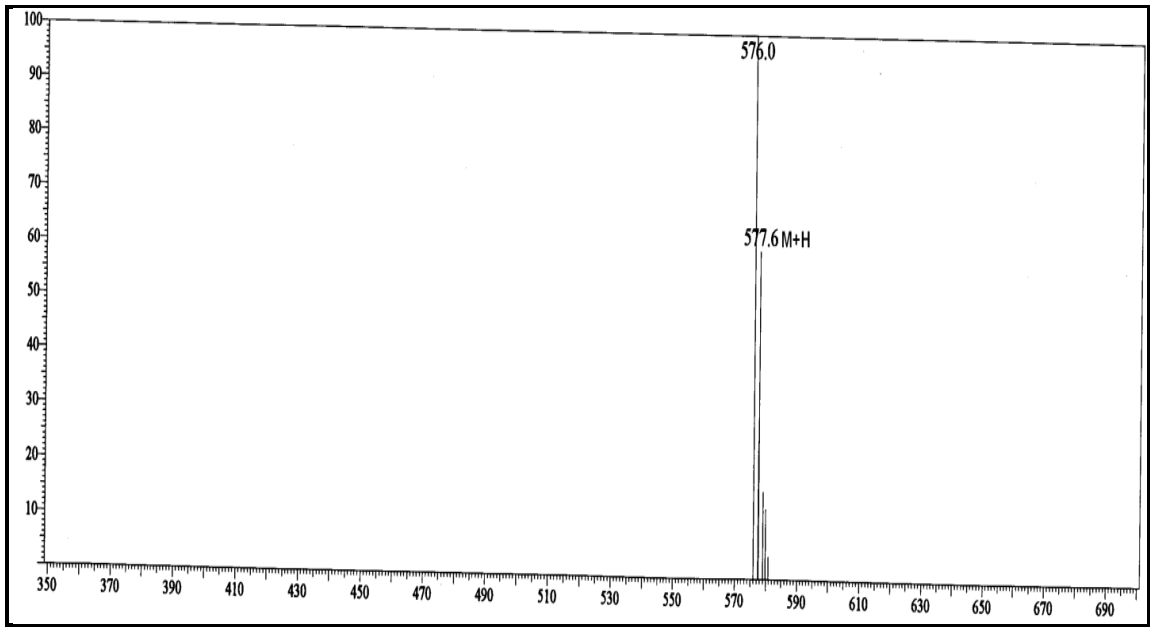
ESI-Mass of 46e



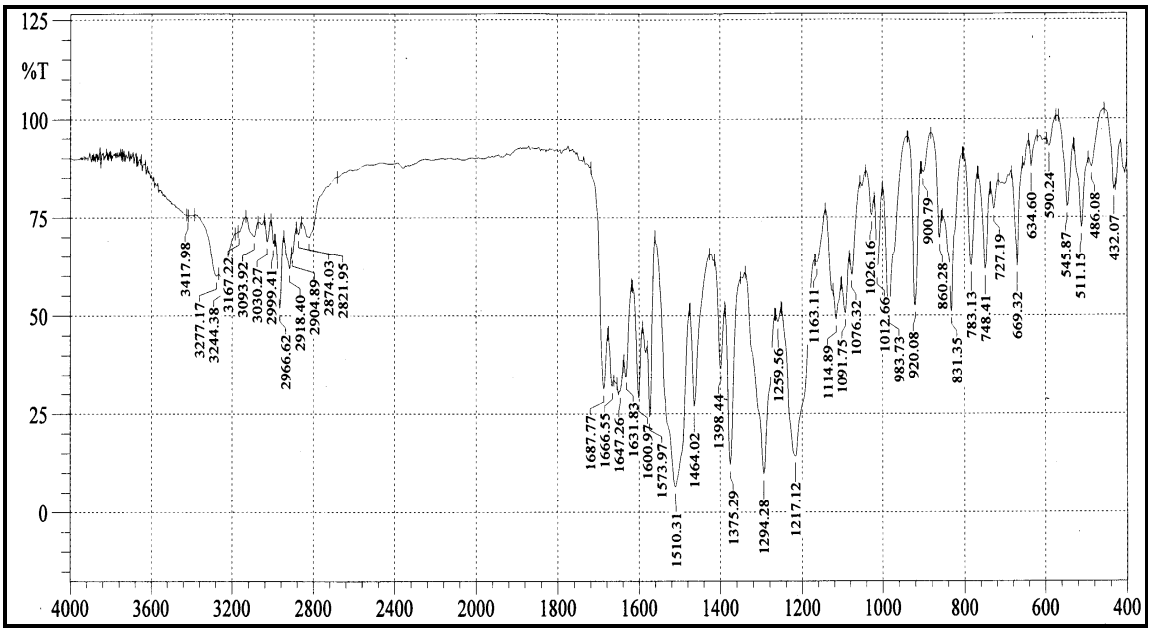
^{1}H NMR of 46f



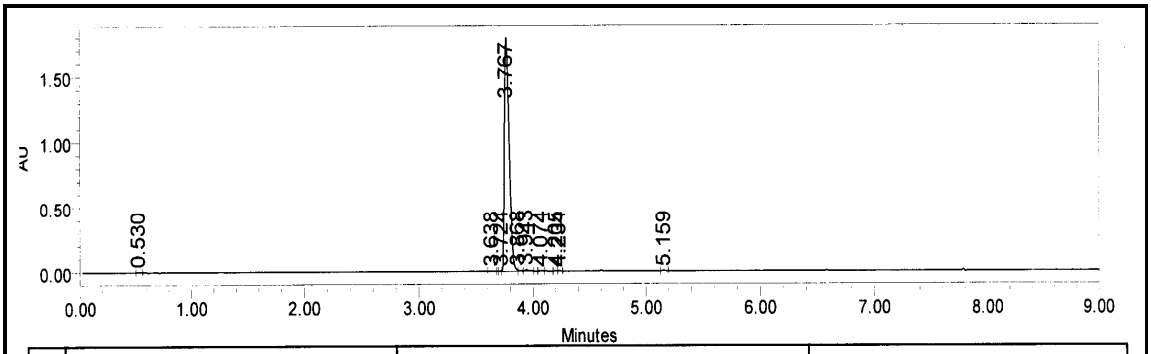
ESI-Mass of 46f



IR of 46f



UPLC of 46f



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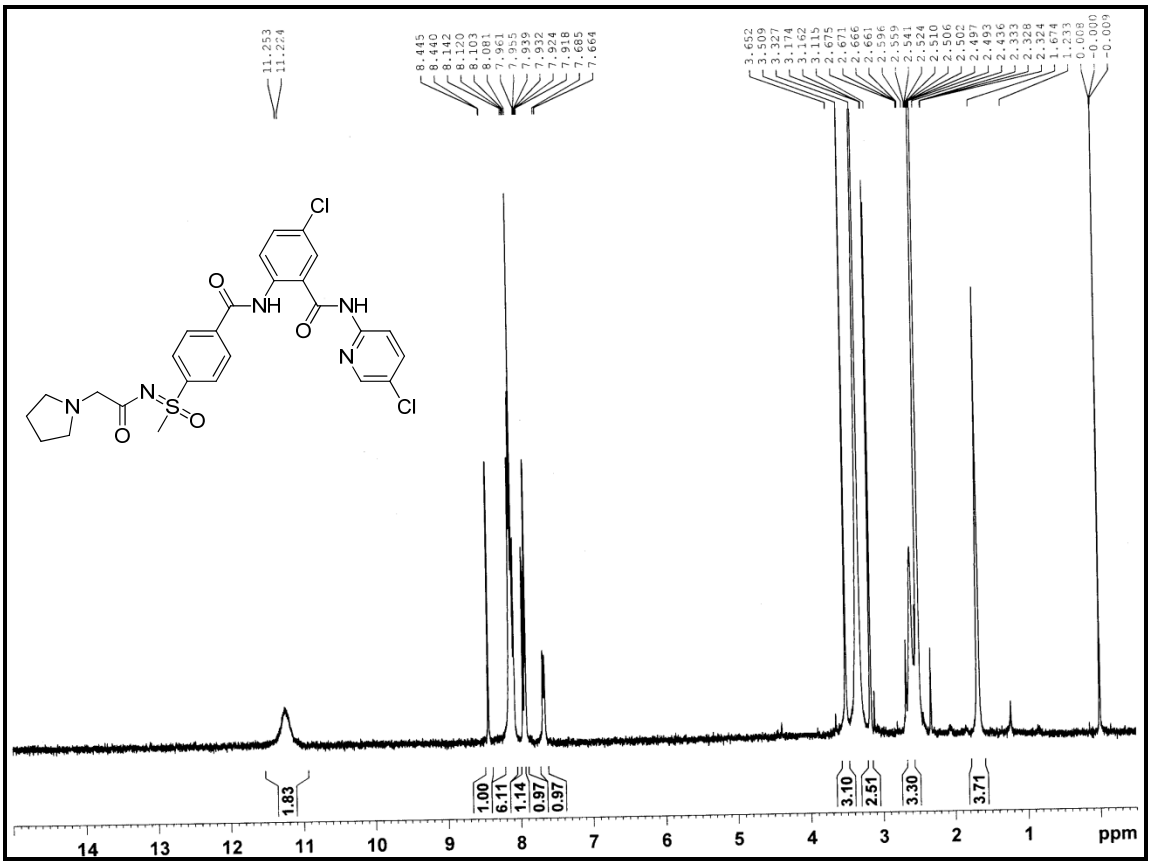
2	3.638	3971	0.07
3	3.724	3923	0.07
4	3.767	5253926	98.91
5	3.868	11670	0.22
6	3.943	23831	0.45
7	4.074	3614	0.07
8	4.205	1810	0.03
9	4.234	1587	0.03
10	5.159	2314	0.04

^{1}H NMR of 46h



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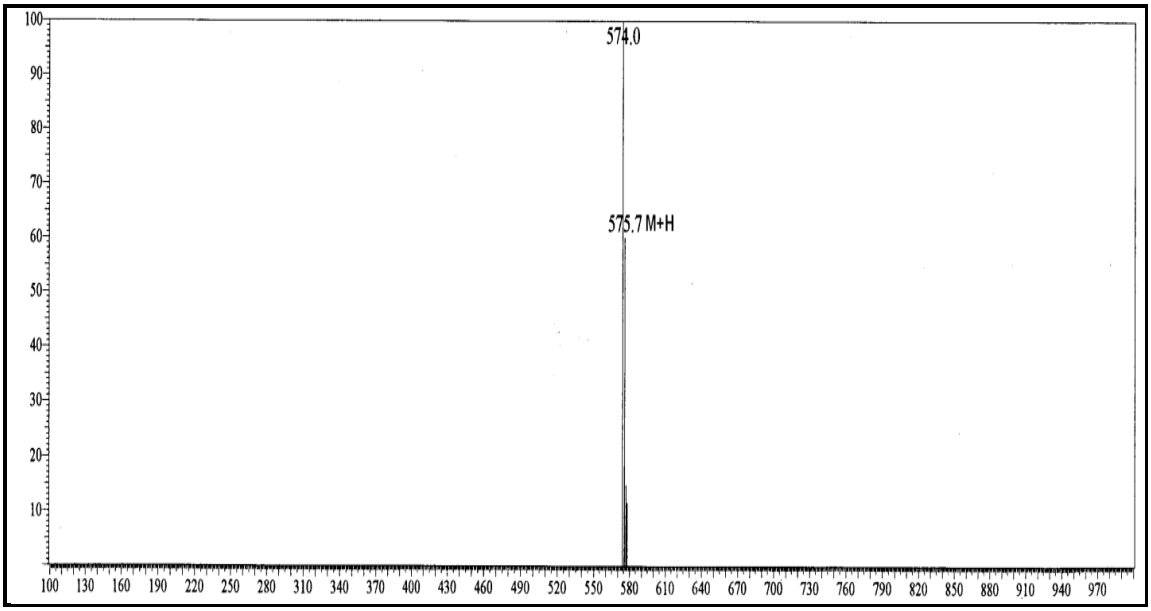

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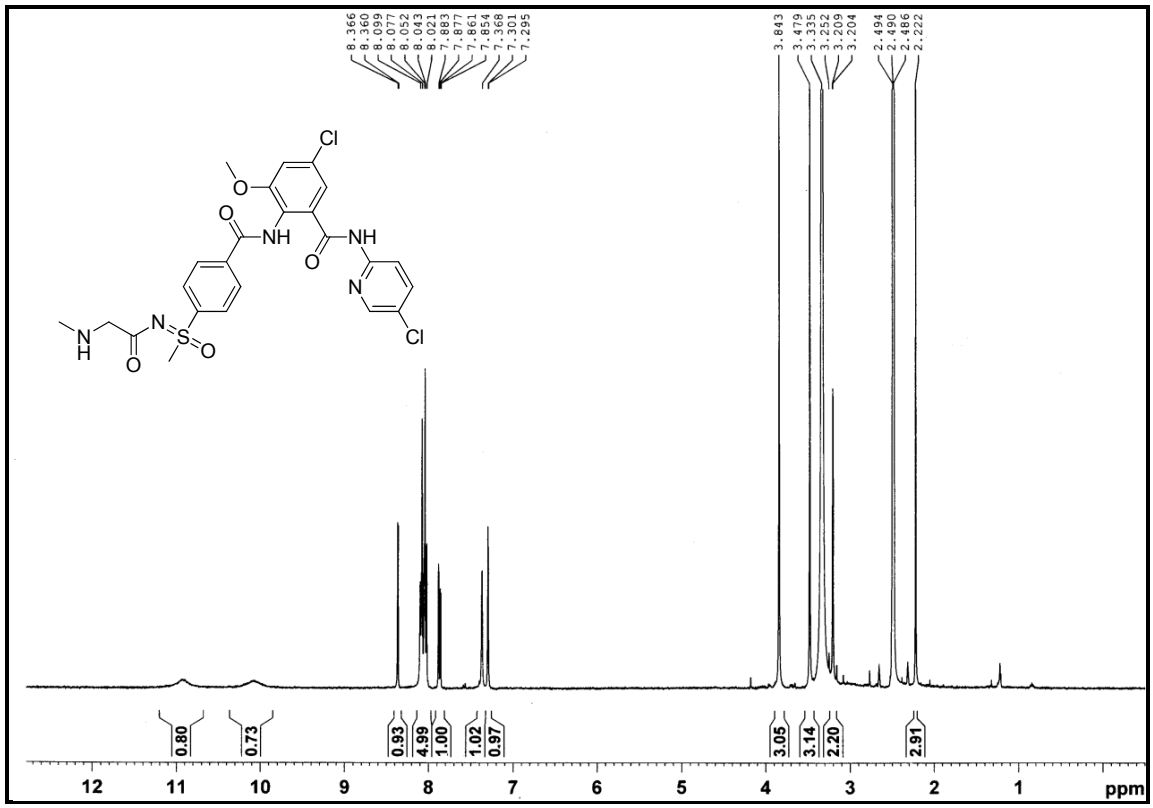
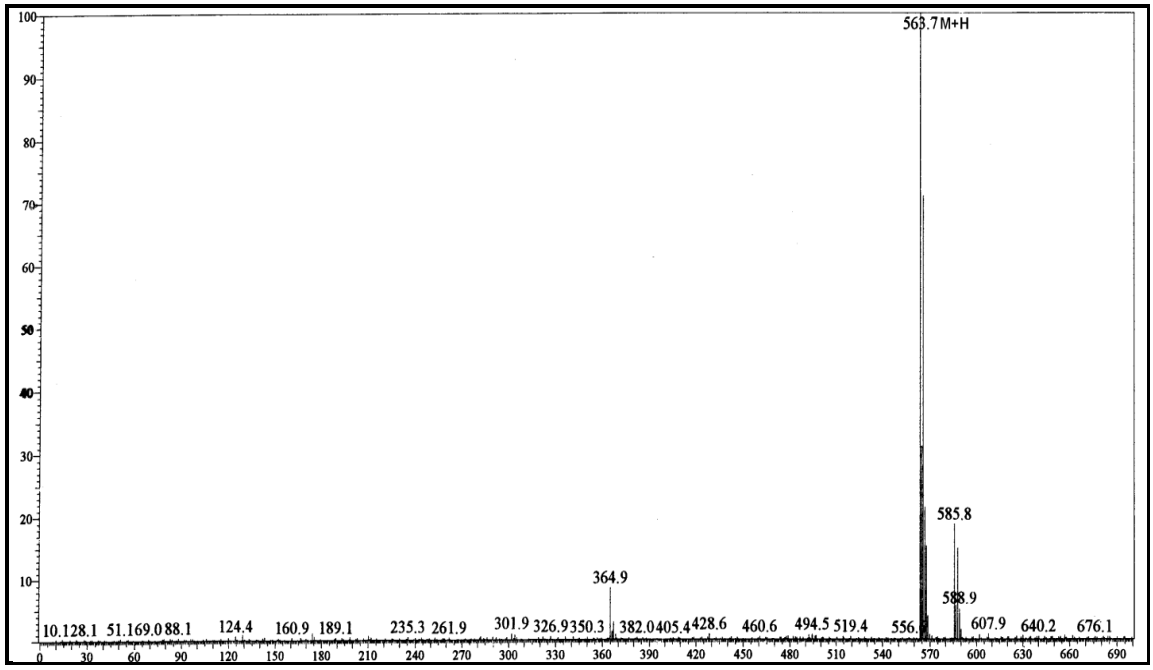
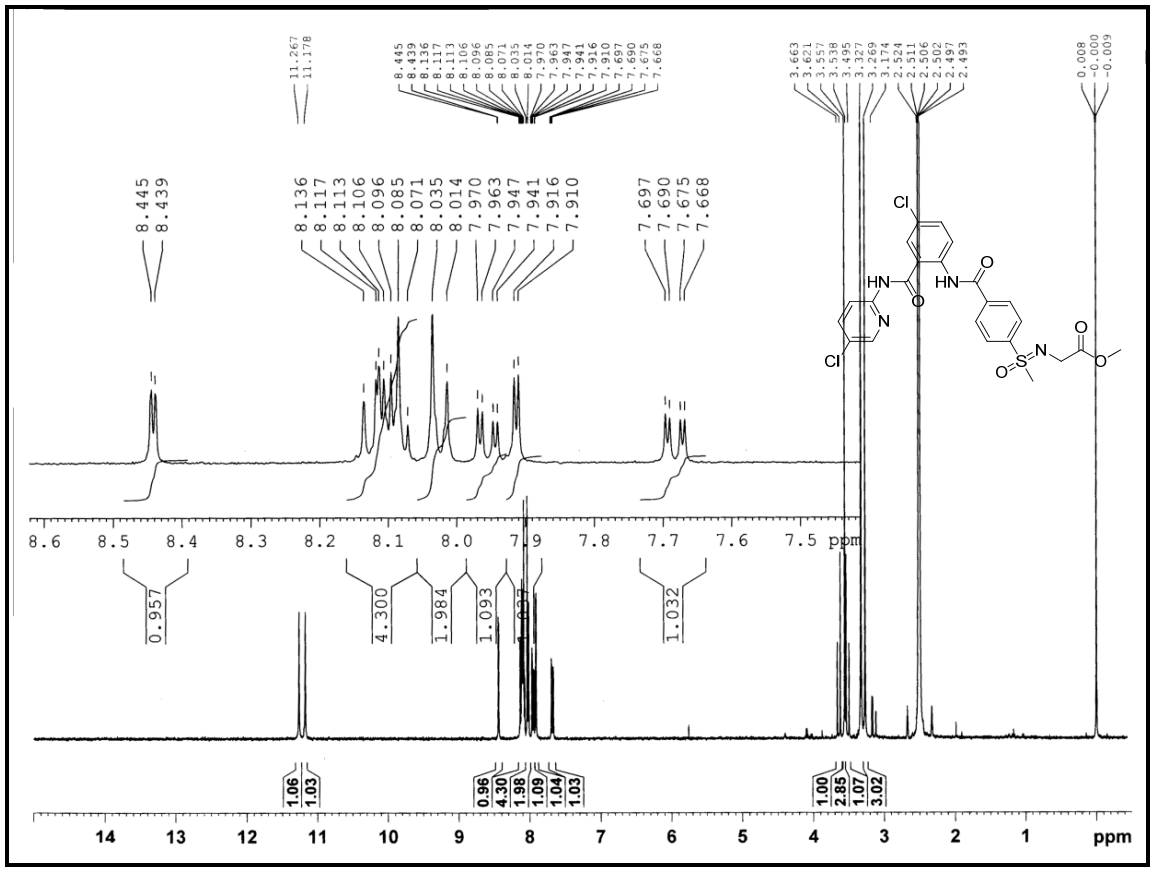


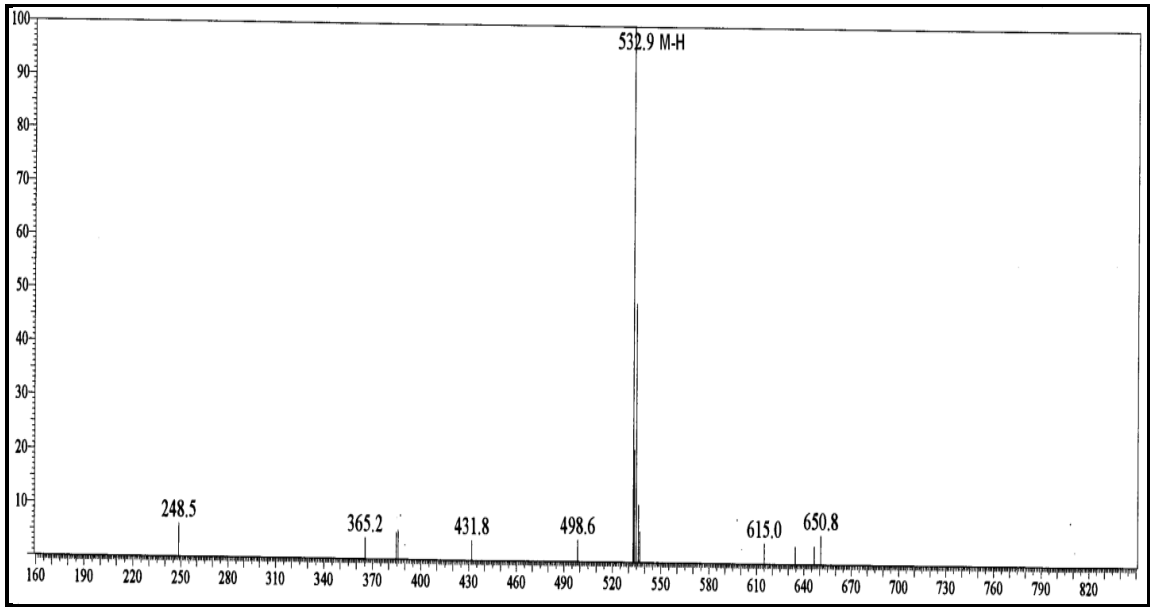
ESI-Mass of 46p



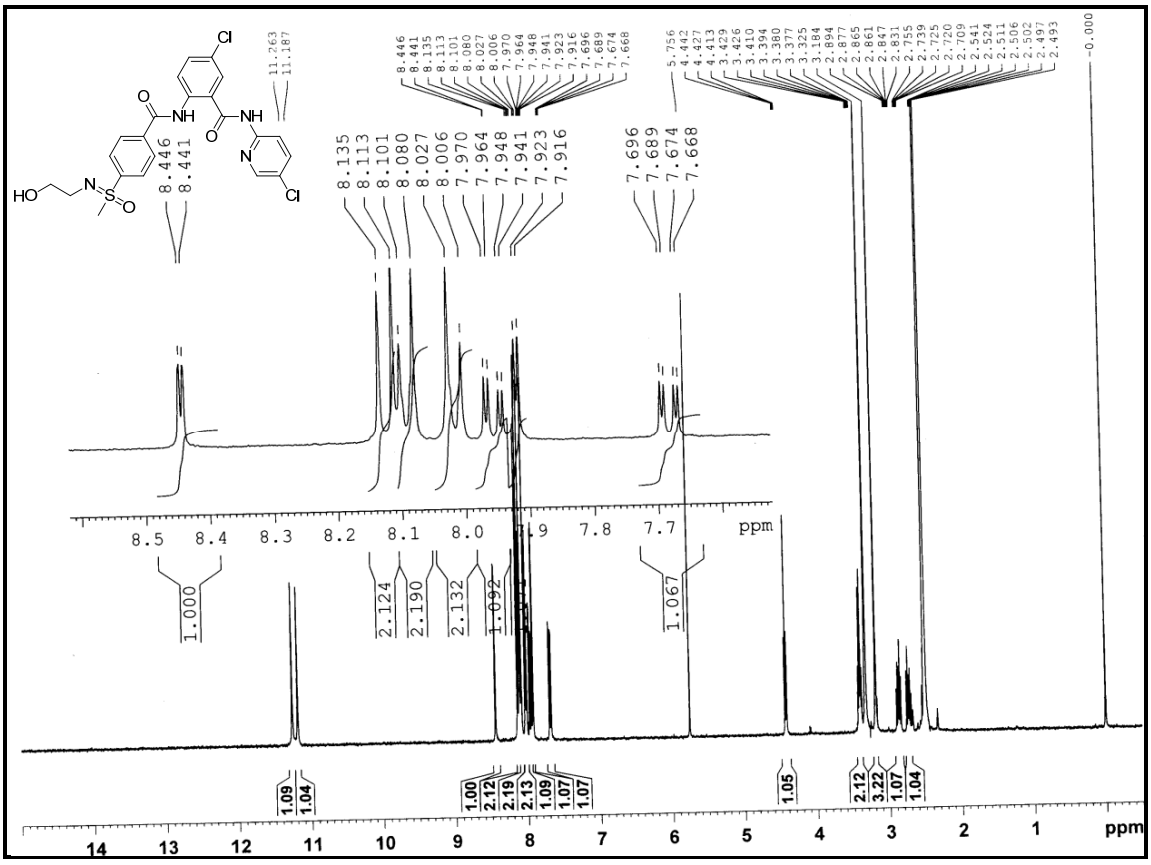
^{1}H NMR of 47



ESI-Mass of 47



^{1}H NMR of 48



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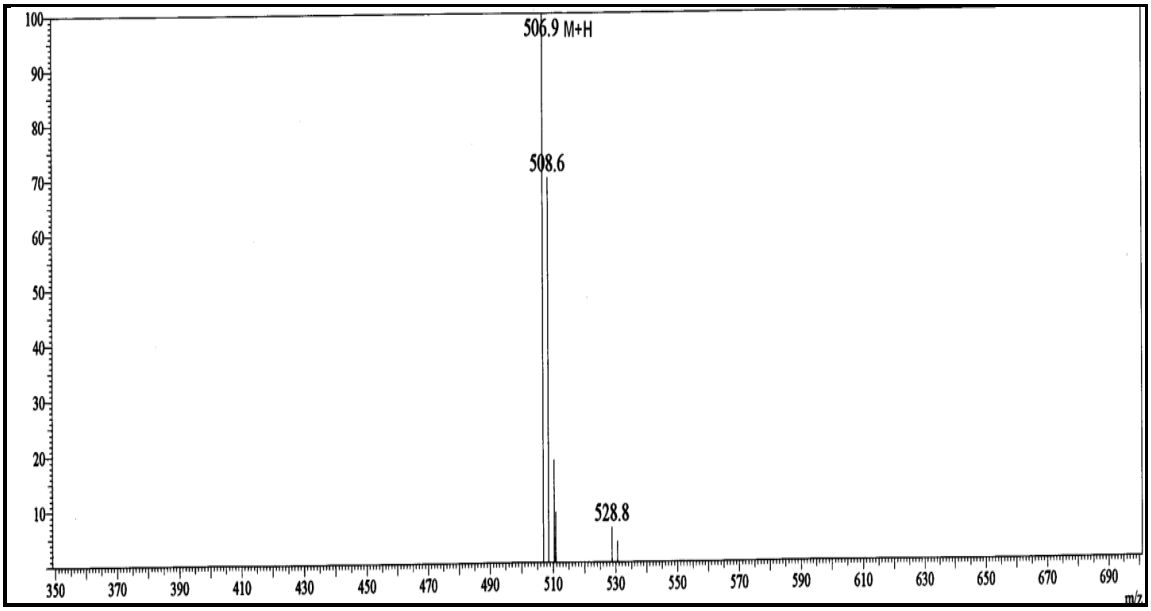

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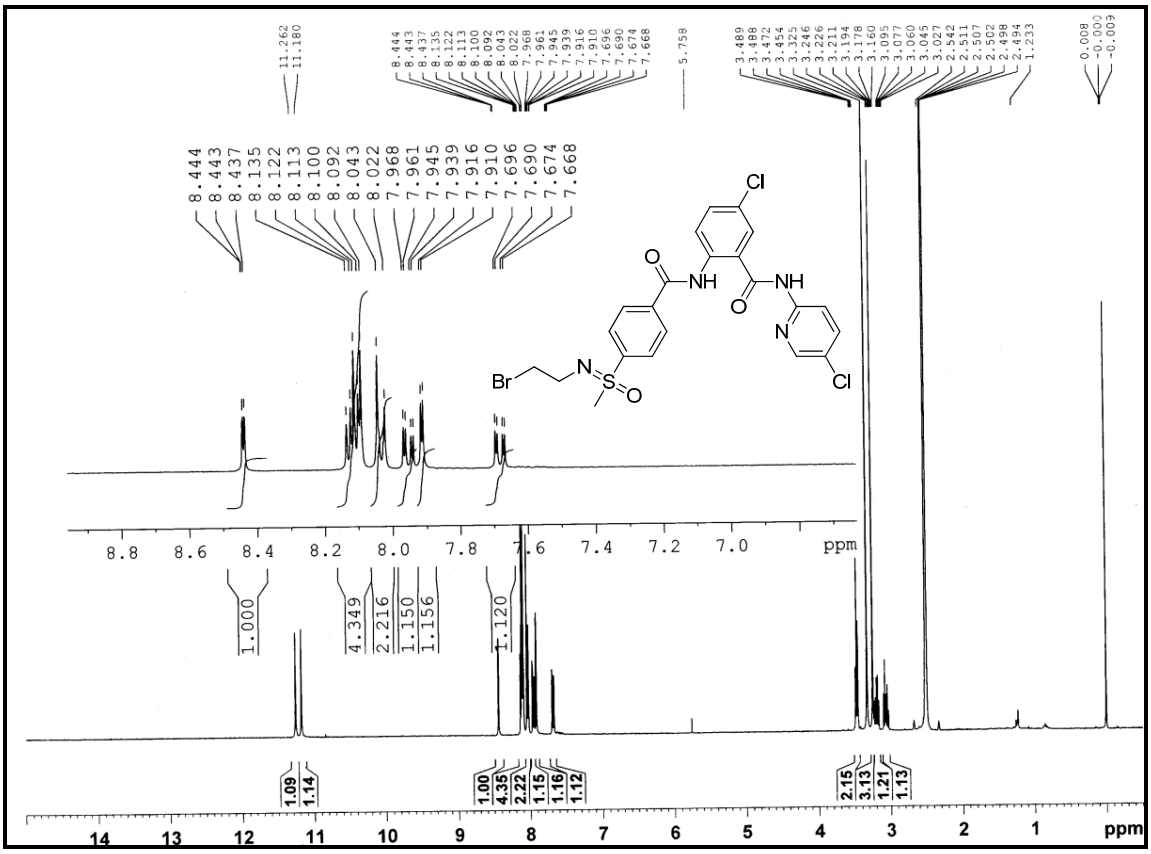
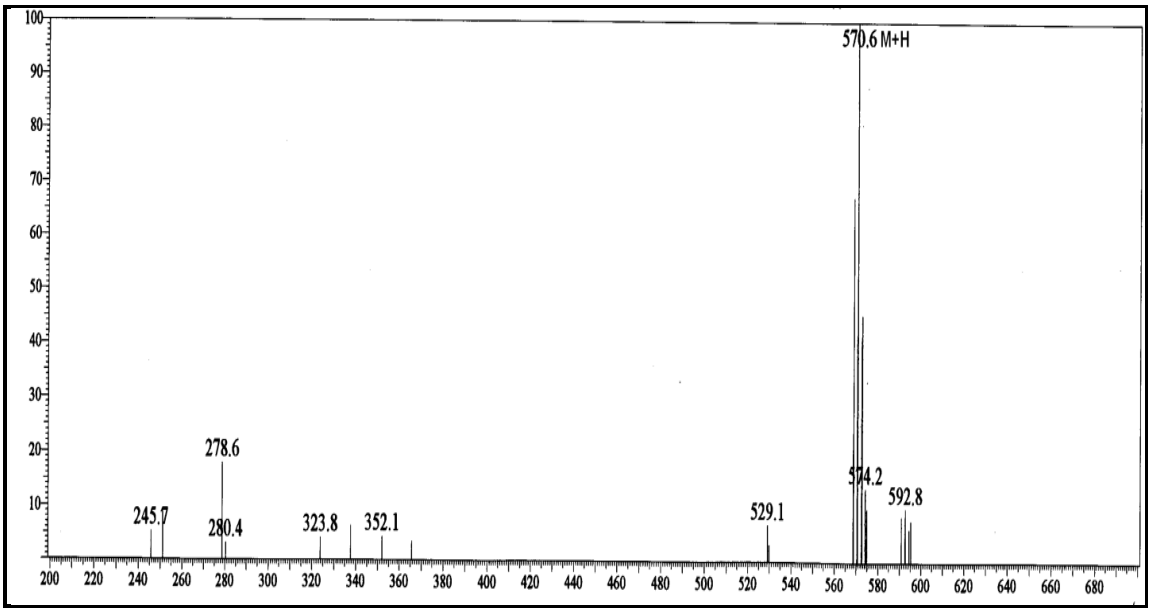
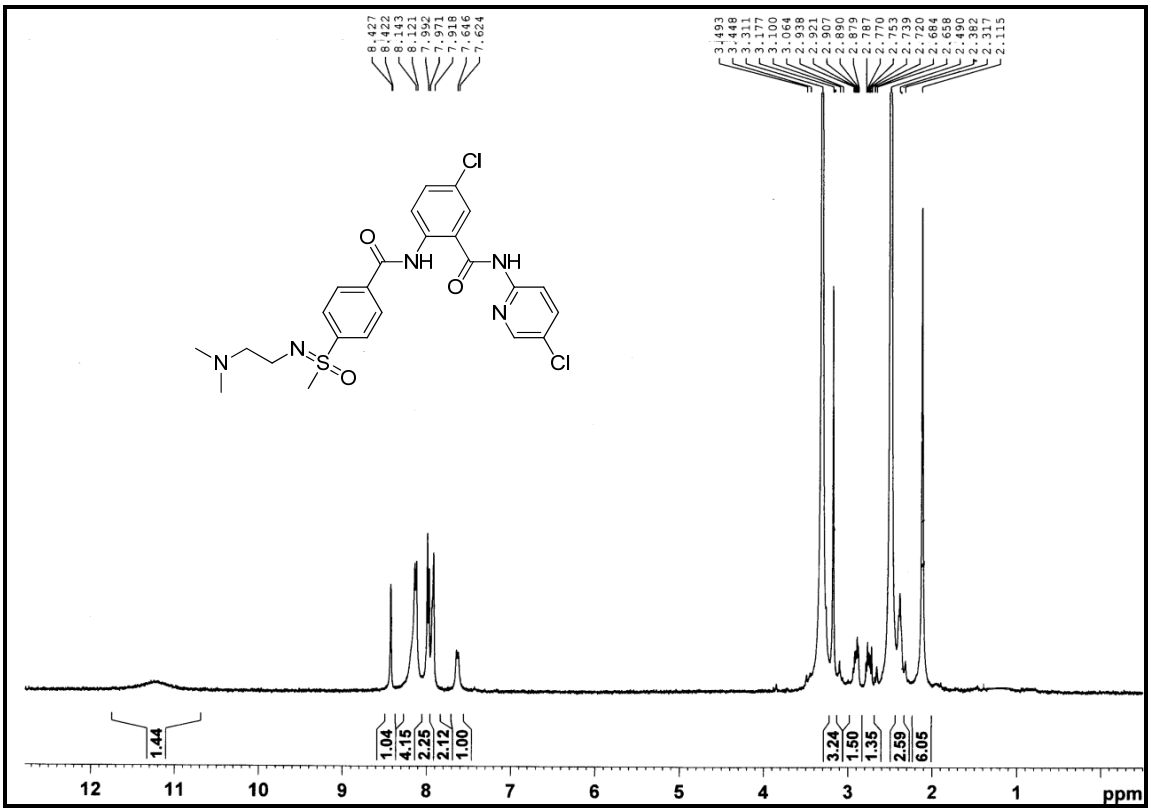


ESI-Mass of 49



^{1}H NMR of 50a



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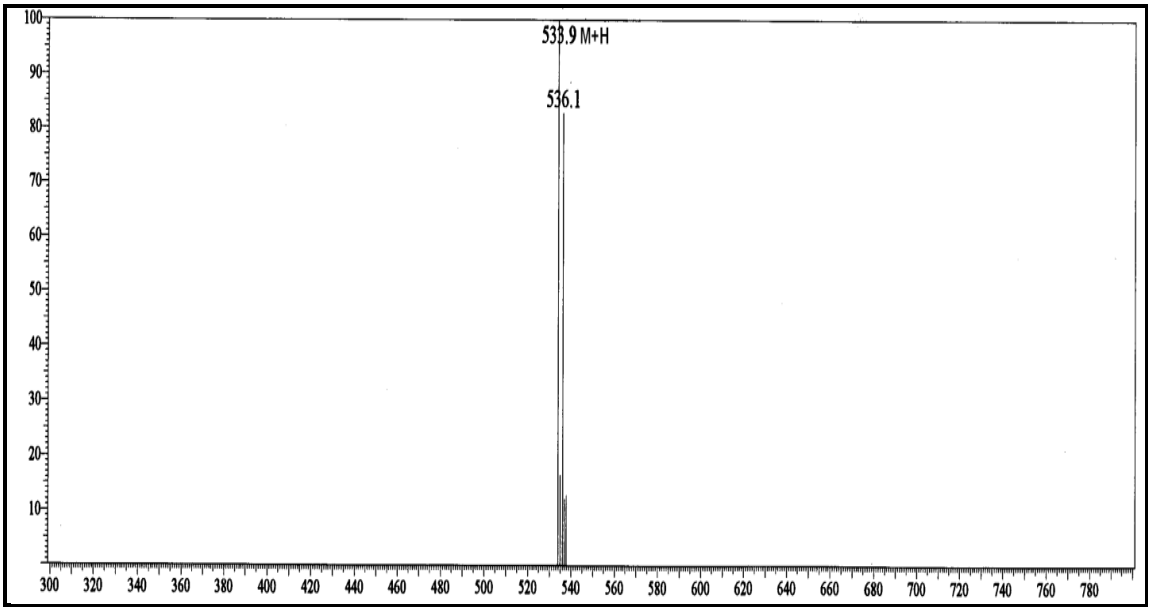
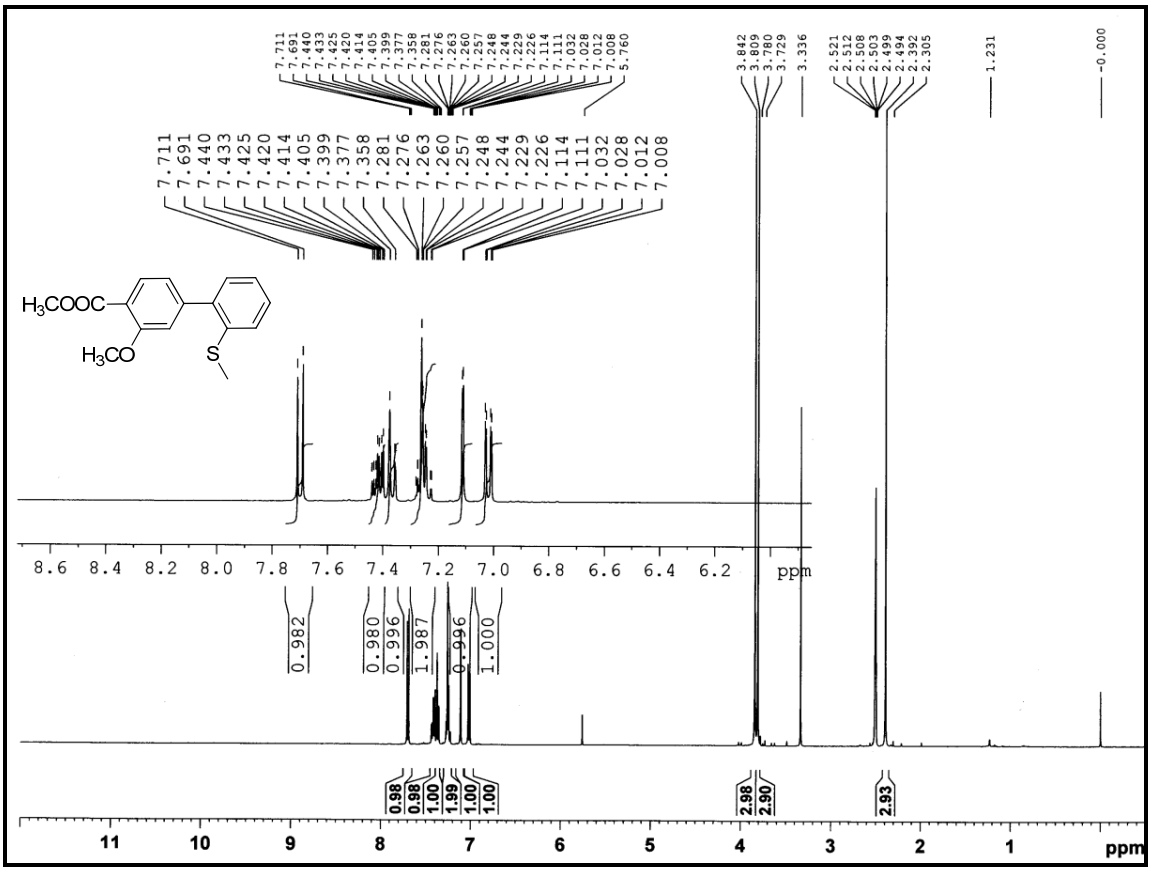
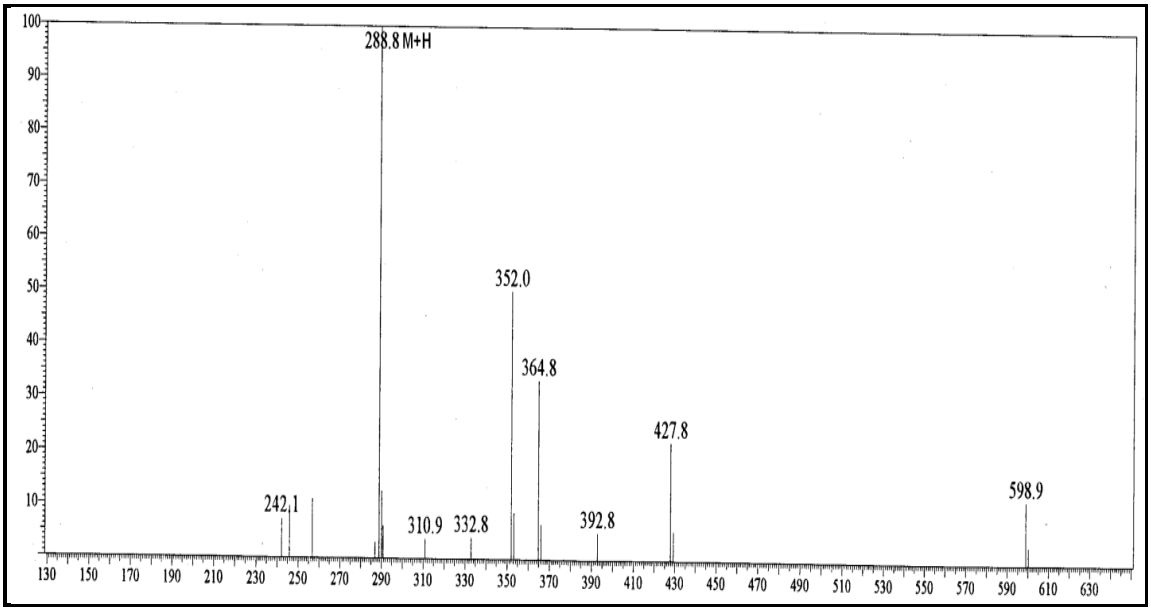


^{1}H NMR of 52f



ESI-Mass of 52f



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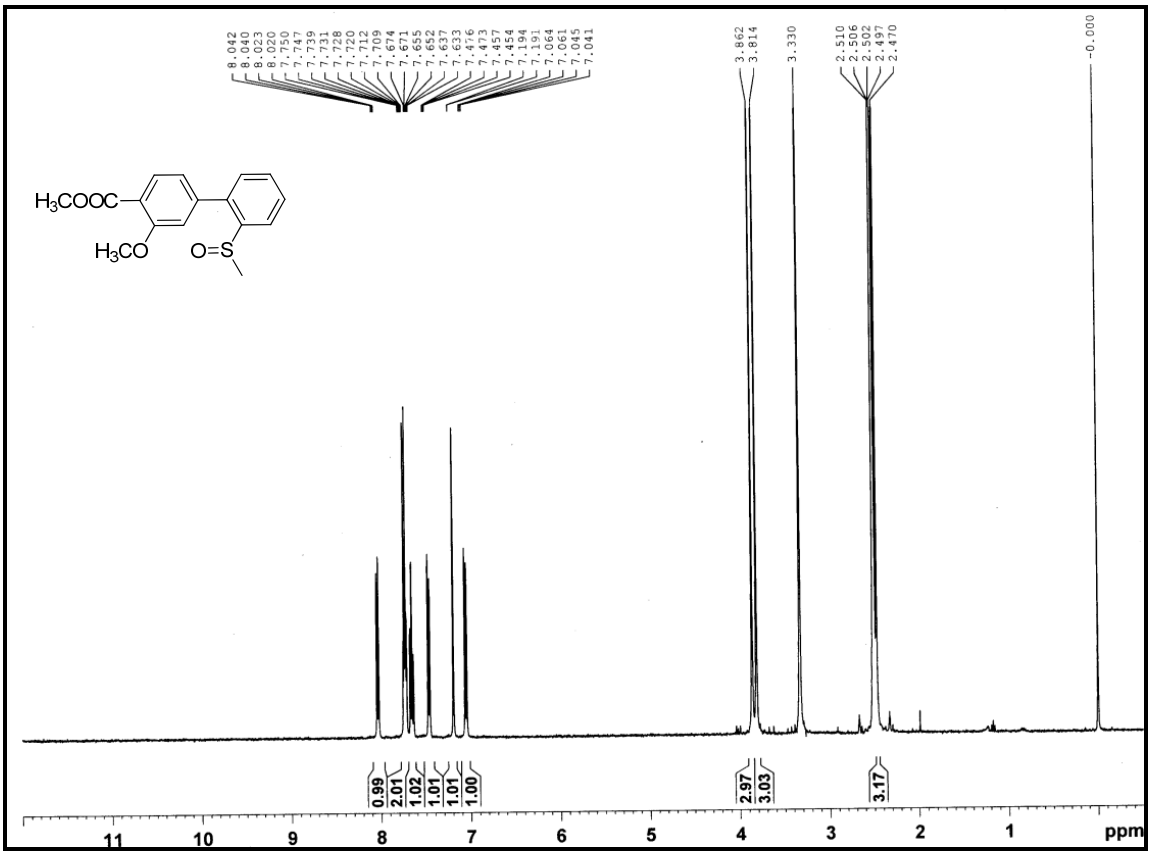
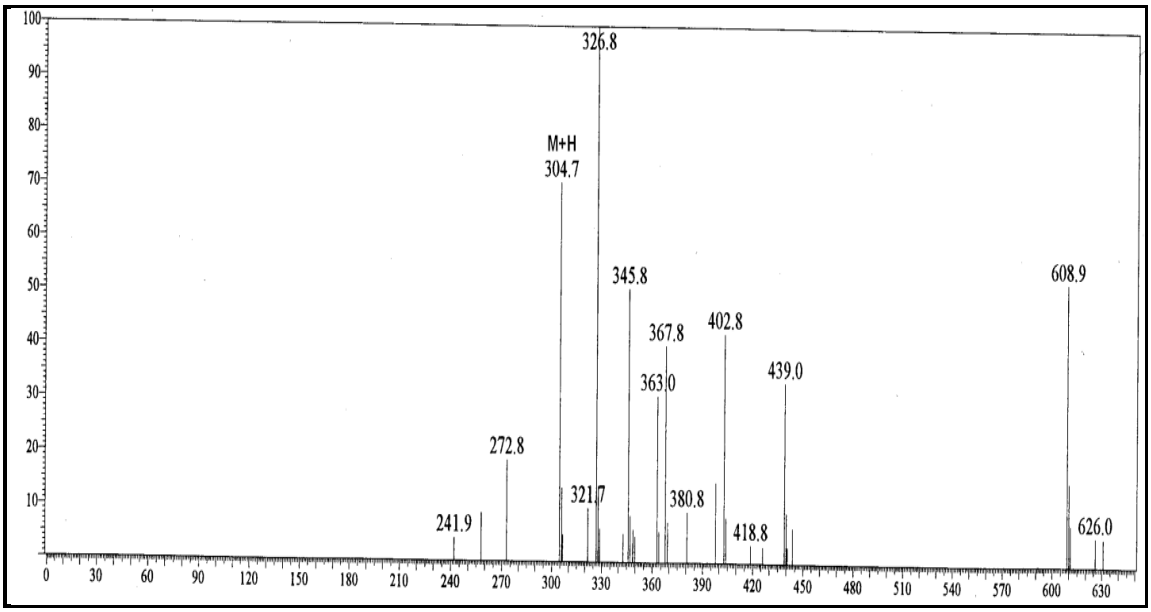
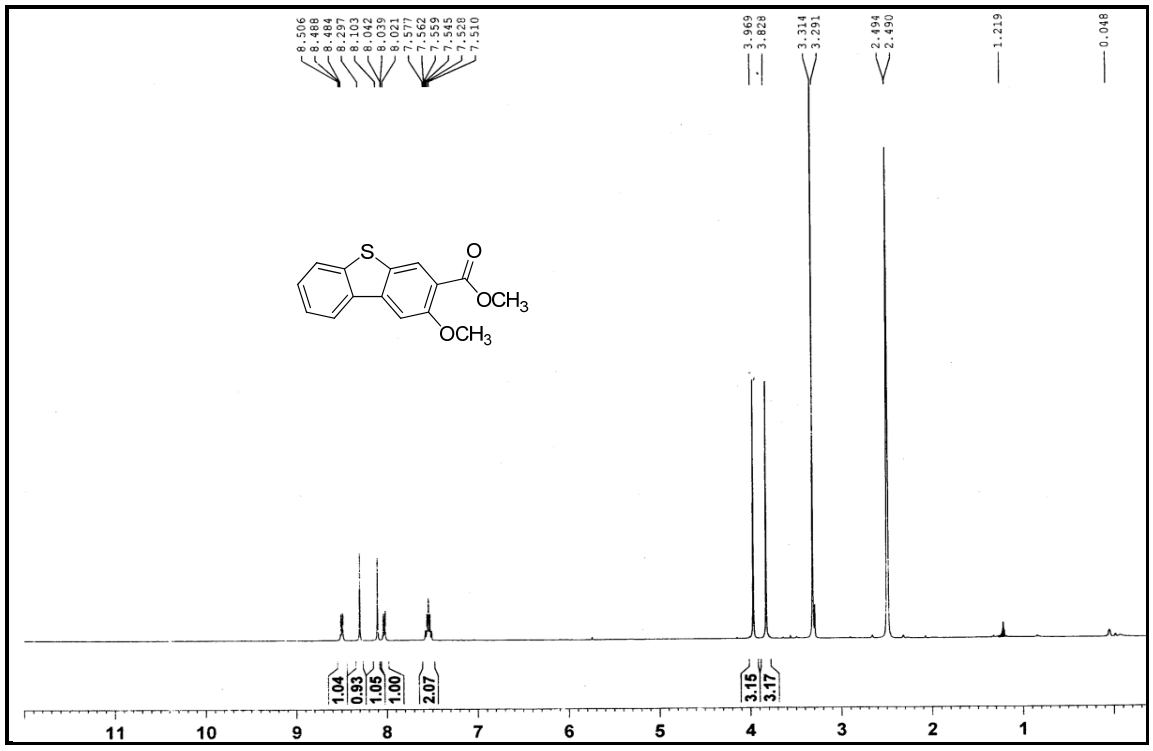


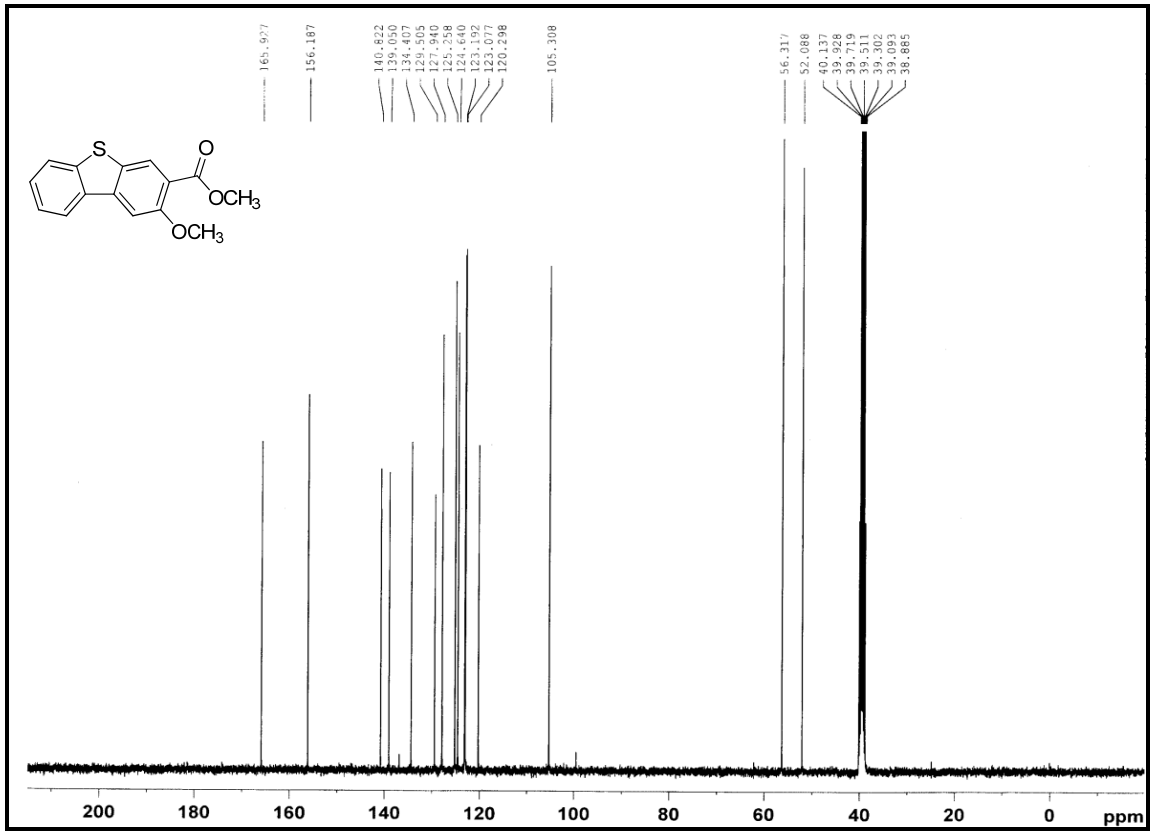
ESI-Mass of 53f



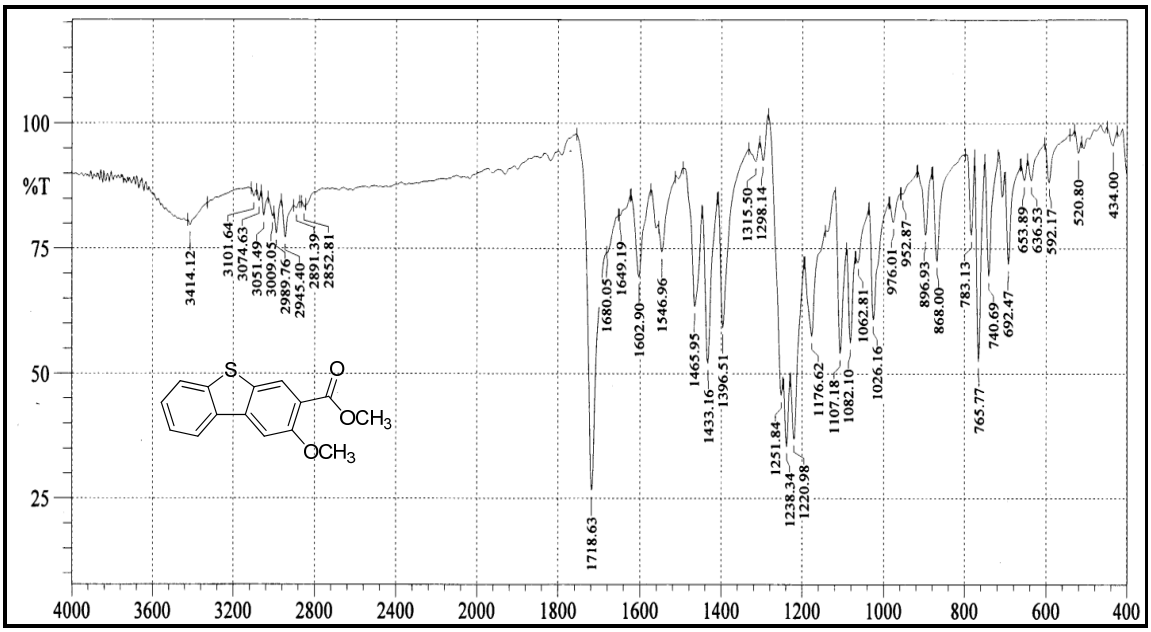
^{1}H NMR of 54f



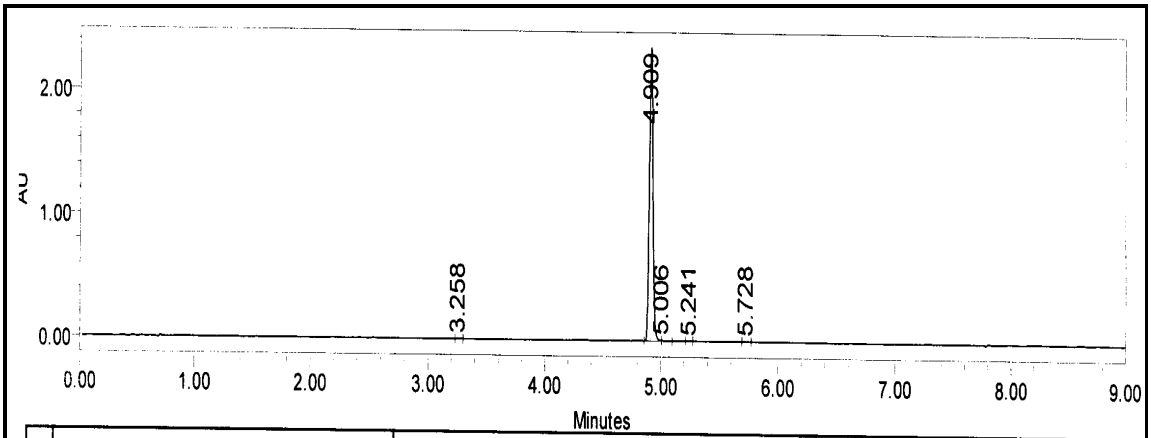
^{13}C NMR of 54f



IR of 54f



UPLC of 54f



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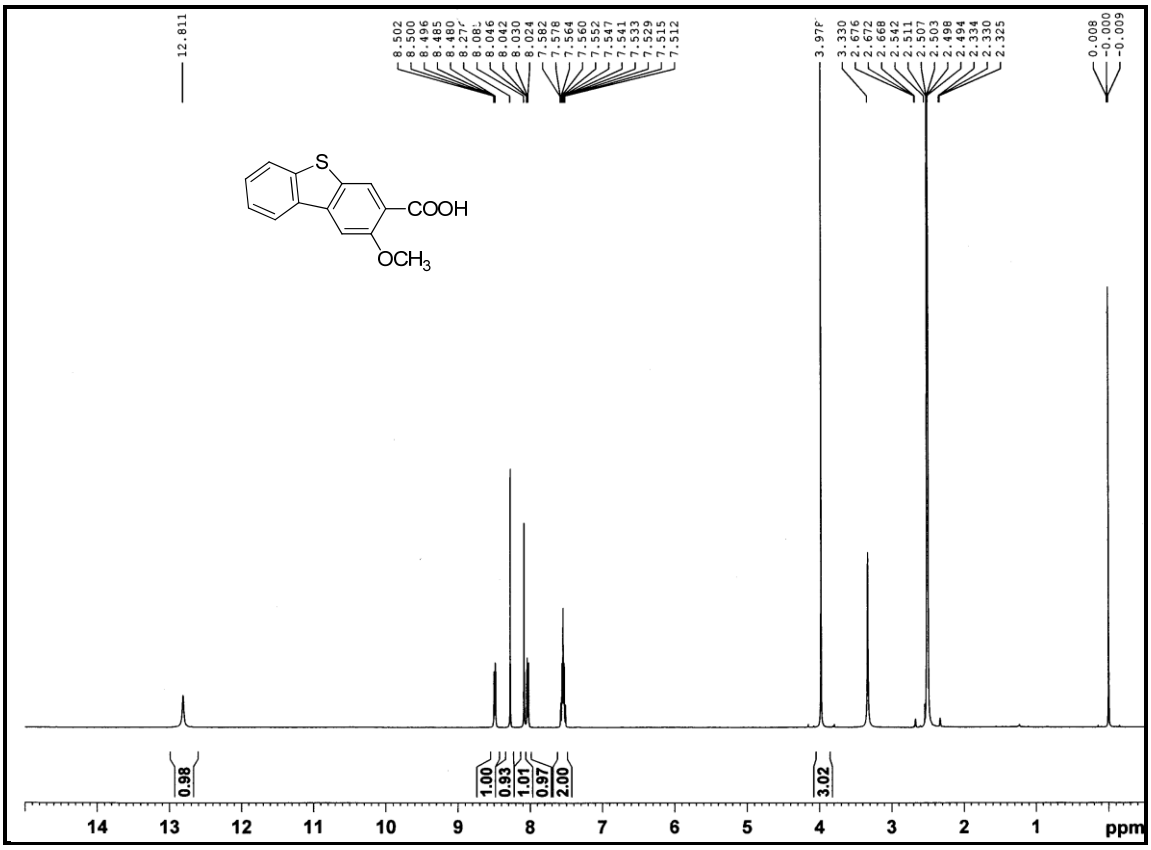
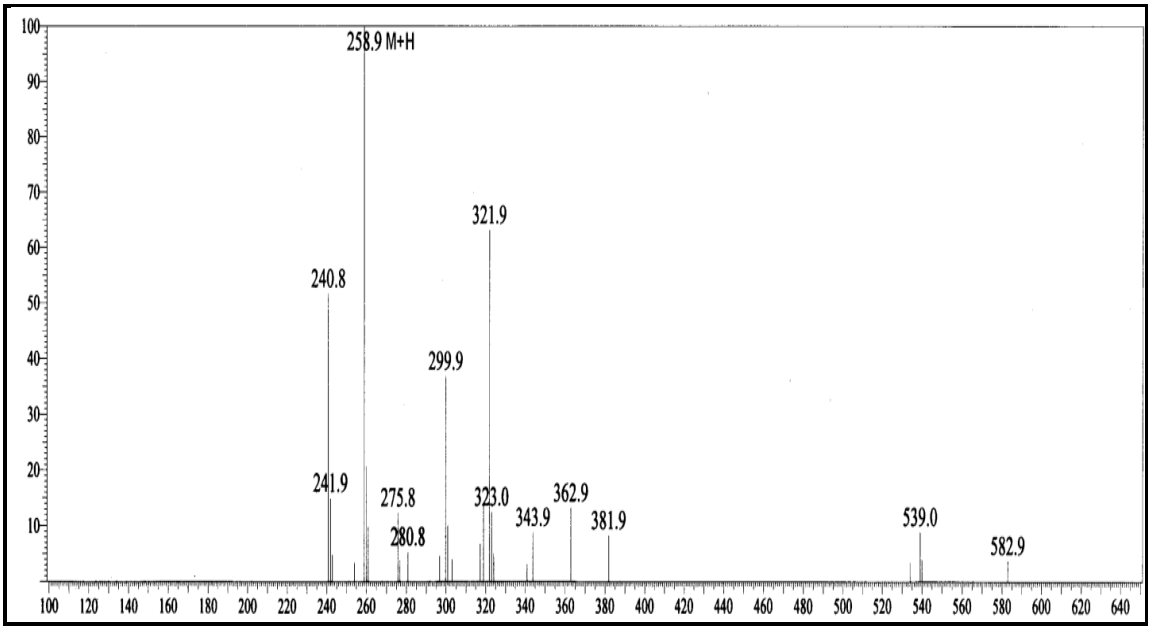
4	5.241	1634	0.03
	5.728	4496	0.09

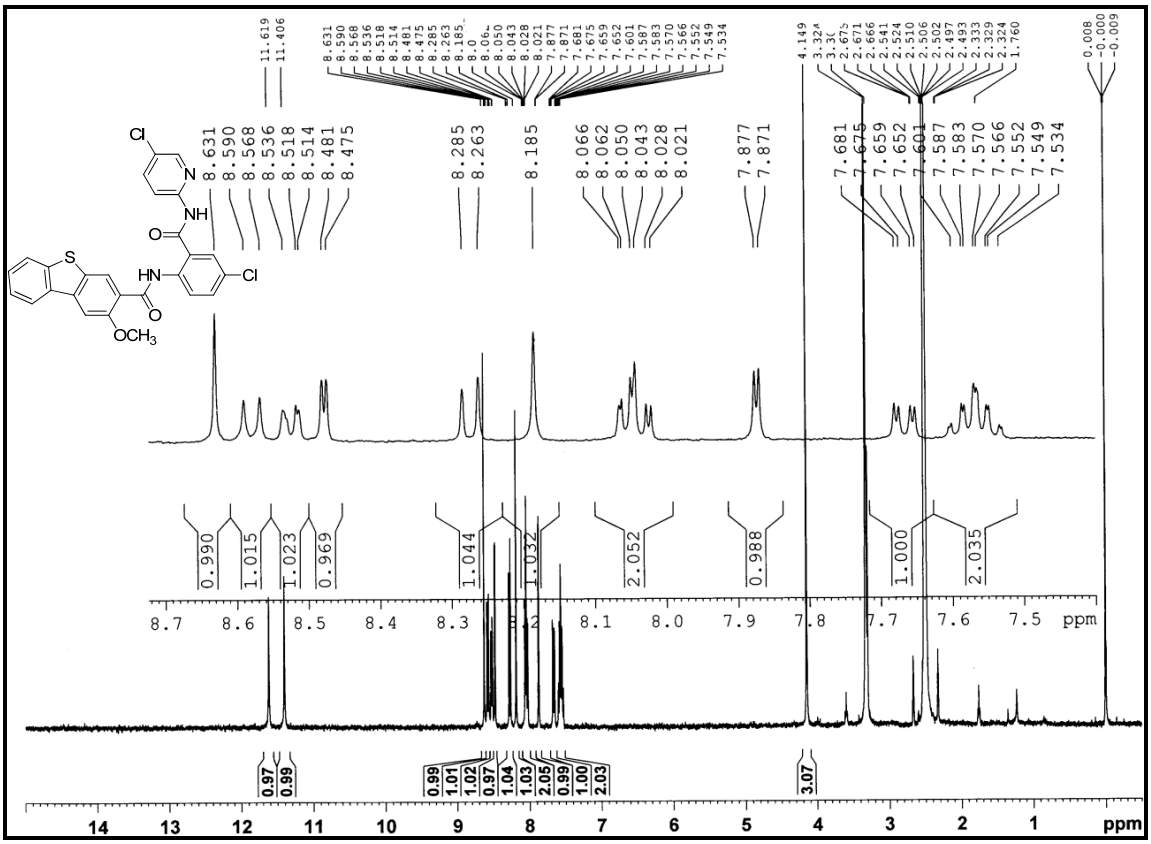
^{1}H NMR of 55



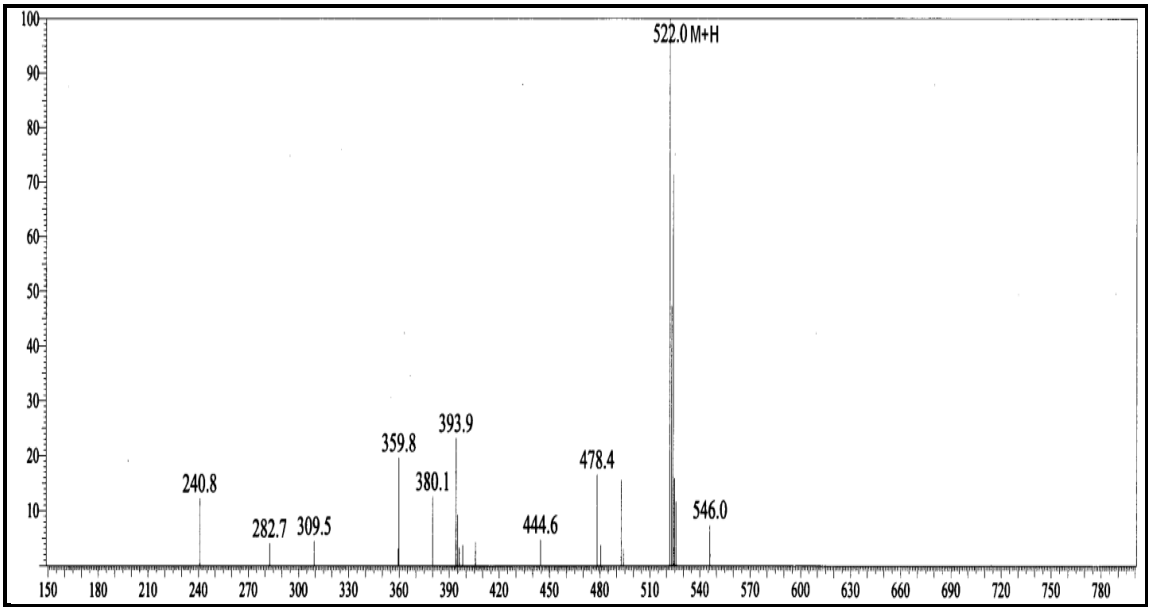
ESI-Mass of 55



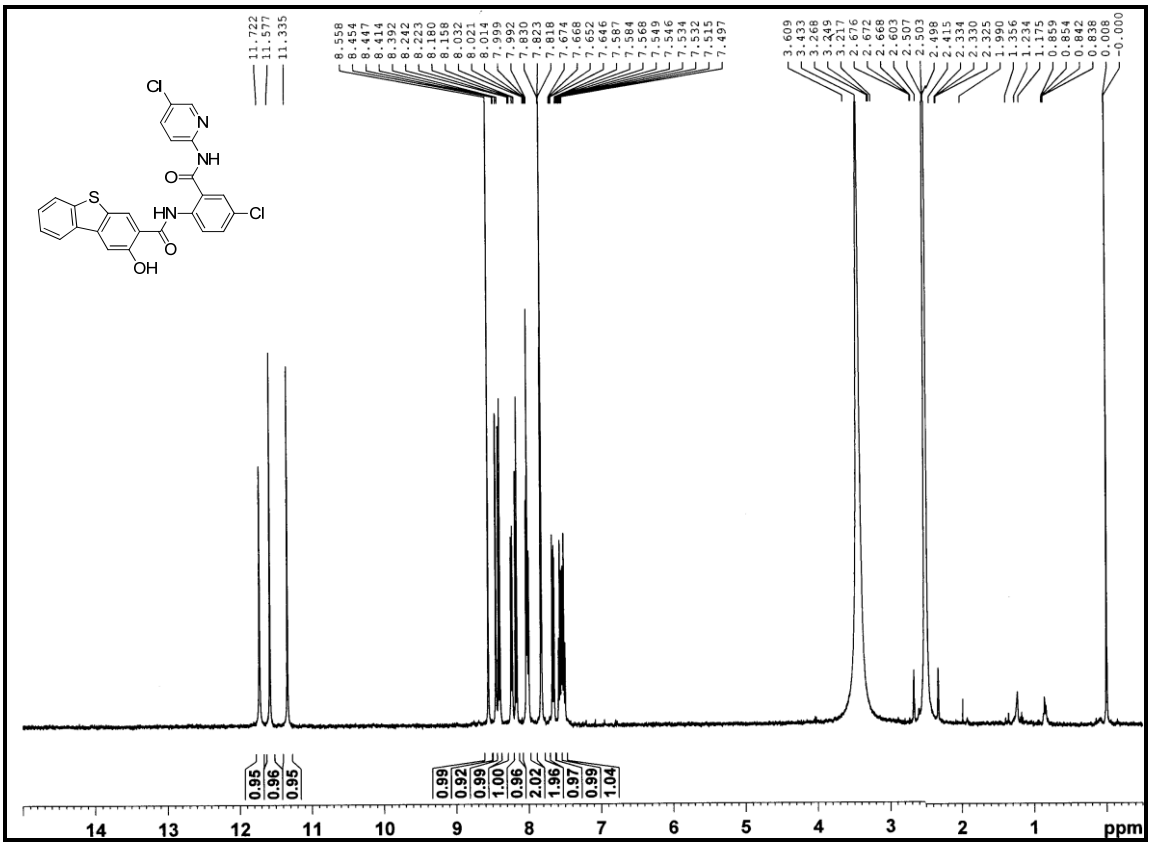
^{1}H NMR of 56a



ESI-Mass of 56a



^{1}H NMR of 56b



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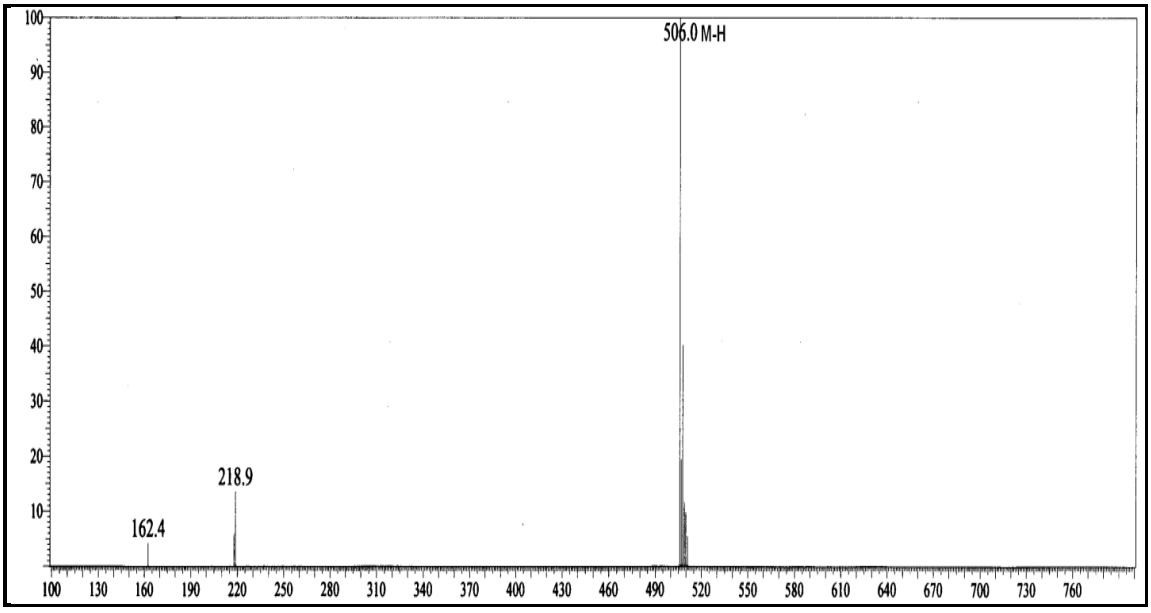

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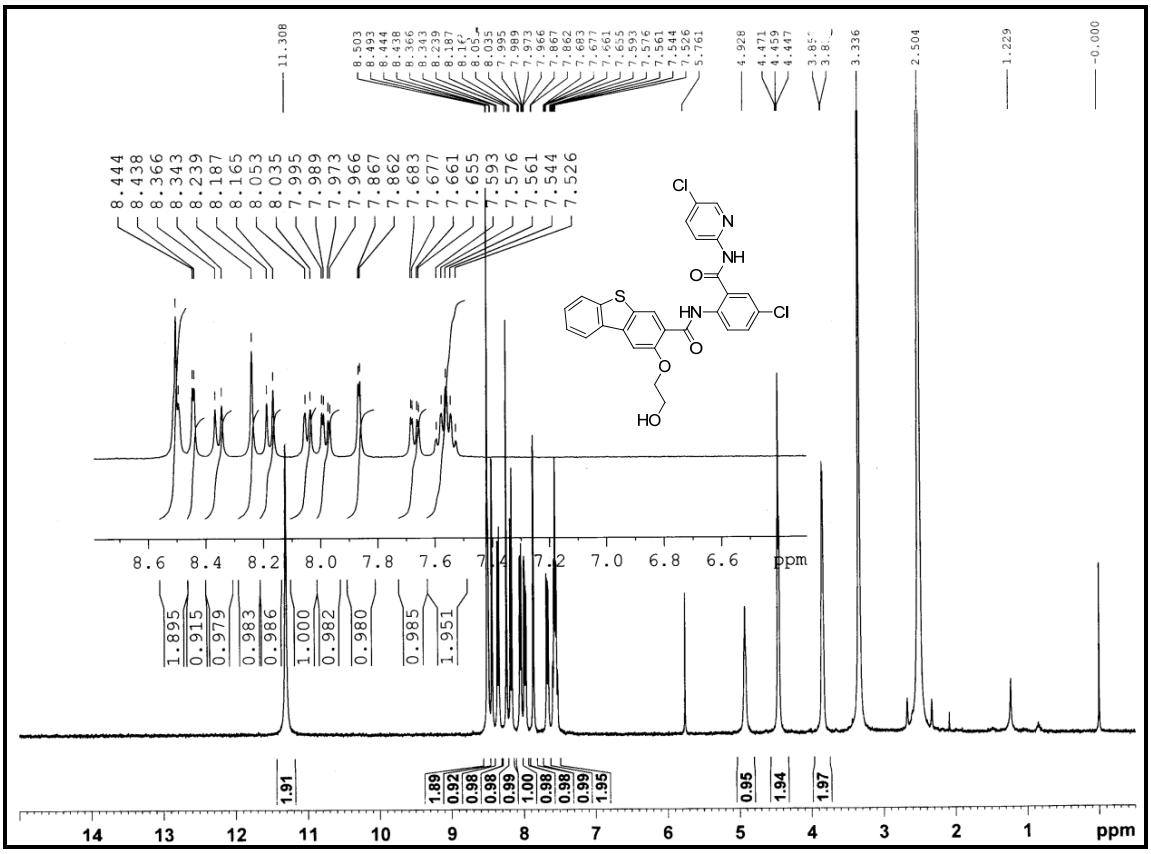
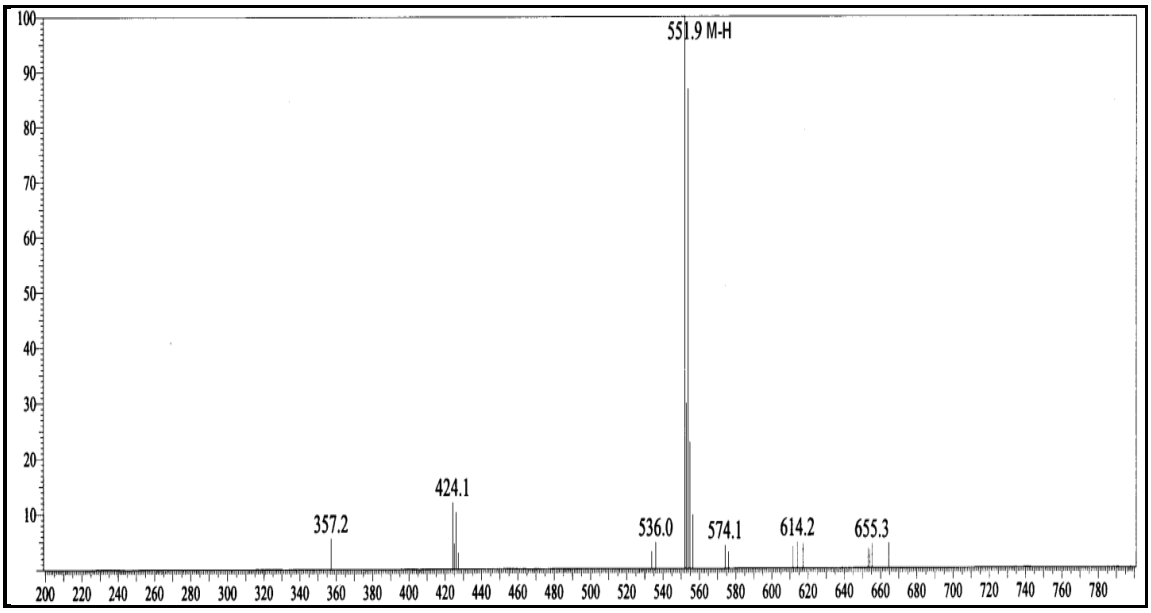


ESI-Mass of 56c



Publications

7. PUBLICATIONS

List of Publications

1. ***In vitro* PAI-1 inhibitory activity of oxalamide derivatives**. Mukul R. Jain, Shankar Shetty, Ganes Chakrabarti, Vrajesh Pandya, Ajay Sharma, Bhavesh Parmar, Soma Srivastava, Mehul Raviya, Hitesh Soni and Pankaj Patel. *European Journal of Medicinal Chemistry*, **2008**, *43,* 880-884.

2. **Discovery of inhibitors of plasminogen activator inhibitor-1: Structure-activity study of 5-nitro-2-phenoxybenzoic acid derivatives**. Vrajesh Pandya, Mukul Jain, Ganes Chakrabarti, Hitesh Soni, Bhavesh Parmar, Balaji Chaugule, Jigar Patel, Jignesh Joshi, Nirav Joshi, Akshyaya Rath, Mehul Raviya, Mubeen Shaikh, Kalapatapu V.V.M. Sairam, Harilal Patel and Pankaj Patel. *Bioorganic & Medicinal Chemistry Letters*, **2011**, *21*, 5701-5706.

3. **Efficient Synthesis of Unsymmetrical Dibenzothiophenes by Acid-Mediated Intramolecular Cyclization of Biaryl Methyl Sulfoxides**. Vrajesh B. Pandya, Mukul R. Jain, Balaji V. Chaugule, Jigar S. Patel, Bhavesh M. Parmar, Jignesh K. Joshi and Pankaj R. Patel. *Synthetic Communications*, **2012**, *42*, 497-505.

4. **Synthesis and structure-activity relationship of potent, selective, and orally active anthranilamide-based factor Xa inhibitors: Application of weakly basic sulfoximine group as novel S4 binding element**. Vrajesh Pandya, Mukul Jain, Ganes Chakrabarti, Hitesh Soni, Bhavesh Parmar, Balaji Chaugule, Jigar Patel, Tushar Jarag, Jignesh Joshi, Nirav Joshi, Akshyaya Rath, Vishal Unadkat, Bhavesh Sharma, Haresh Ajani, Jeevan Kumar, Kalapatapu V.V.M. Sairam, Harilal Patel and Pankaj Patel. *European Journal of Medicinal Chemistry*, **2012**, *58*, 136-152.

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European Journal of Medicinal Chemistry 43 (2008) 880–884
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Short communication

In vitro PAI-1 inhibitory activity of oxalamide derivatives☆

Mukul R. Jain*, Shankar Shetty, Ganes Chakrabarti, Vrajesh Pandya, Ajay Sharma, Bhavesh Parmar, Soma Srivastava, Mehul Raviya, Hitesh Soni, Pankaj R. Patel

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Abstract

A number of oxalamide derivatives have been synthesized and evaluated for PAI-1 inhibitory activity. *In vitro* PAI-1 inhibitory activities of oxalamide derivatives are evaluated by chromogenic assay. Few compounds have shown significant PAI-1 inhibitory activity.

Keywords: PAI-1; Oxalamide; Thrombosis; tPA; uPA

1. Introduction

The activity of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) is negatively regulated by a serine protease inhibitor (SERPIN) named plasminogen activator inhibitor-1 (PAI-1) [1]. Platelets contain high amount of PAI-1 [2,3] and release of PAI-1 from activated platelets may lead to its high local concentration, which may be responsible for higher incidence of tPA resistant thrombus formation [4–6]. PAI levels are reported to be elevated in various thrombotic disorders including deep vein thrombosis (DVT) [7,8] and other diseases like diabetes [9,10], obesity [11,12], and syndrome 'X' [11,13]. All of these disorders are associated with an increased risk of systemic thrombosis; therefore, inhibition of PAI-1 may represent a useful strategy for treating thrombotic diseases. This hypothesis is supported by studies suggesting that transgenic mice expressing high levels of PAI-1 develops spontaneous thrombosis [14,15], whereas PAI-1 knockout mice are resistant to venous or arterial thrombosis [16,17].

Recently, several classes of small molecule PAI-1 inhibitors have been reported [18], such as menthol based inhibitors **1** [19], piperazine analogues **2** [20], and indole oxoacetic acid **3** [21] (Fig. 1).

These inhibitors are found to show good in vitro PAI-1 inhibitory activity and are under different stages of preclinical/clinical development [20]. The process of finding a better inhibitor by high throughput screening of various compound libraries having carboxylic acid functionality culminated in the identification of oxalamide derivative **4** (Fig. 2) that showed an *in vitro* IC_{50} value of 96 μM in PAI-1 inhibitory assay.

Taking oxalamide derivative **4** as the suitable candidate for modification, several compounds **9** and **15** having carboxylic acid functionality were synthesized [22] and evaluated for their PAI-1 inhibitory activity in chromogenic assay [23] (Fig. 3). The PAI-1 protein and ligand interaction was also studied by Native PAGE (Poly Acrylamide Gel Electrophoresis) experiment.

2. Chemistry

The oxalamide derivatives **4**, **9** and **15** were prepared as shown in Schemes 1 and 2. The aminobiphenyl intermediate **6** was prepared by Suzuki coupling of 4-trifluoromethoxyphenyl boronic acid with 4-nitro-iodobenzene followed by reduction of $-NO_2$ group, which upon reductive amination with substituted aldehyde produced **7**. The reaction of **7** with methyl chlorooxoacetate furnished compound **8**. The conventional

☆ ZRC communication # 184.

* Corresponding author. Tel.: +91 2717 250801; fax: +91 2717 250606.
E-mail address: mukul.jain@zyduscadila.com (M.R. Jain).

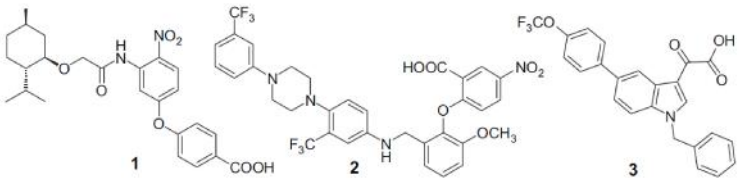


Fig. 1. PAI-1 inhibitors.

alkaline hydrolysis of compound **8** provided **9** (Scheme 1). Controlled reaction of substituted sulfonyl chloride **10** with *p*-phenylenediamine gave sulfonamide derivative **11**. The reductive amination of sulfonamide derivative **11** with 3,5-bistrifluoromethyl benzaldehyde gave **12**. The reaction of **12** with methyl chlorooxoacetate yielded oxalamide ester **13**. The alkylation of **13** with substituted alkyl halide provided compound **14** and subsequent alkaline hydrolysis of compound **14** gave **15** (Scheme 2).

3. Results and discussion

The PAI-1 inhibitory activity of oxalamide derivatives **9a**–**9d** and **15a**–**15t** is summarized in Tables 1 and 2.

The compound **4** inhibited PAI-1 activity with an IC_{50} of 96 μM in chromogenic assay [23], hence compound **4** was selected for further modification in region 1 and region 2 (Fig. 2), which gave compounds **15a**–**15t** and **9a**–**9d** respectively (Fig. 3).

The first synthesized compounds *i.e.* 3-methanesulfonate phenyl derivative **9a** and pyridyl derivative **9b** did not show PAI-1 inhibitory activity in the chromogenic assay. Introduction of an electron-withdrawing and bulky trifluoromethyl group on phenyl ring **9c** showed considerable improvement in the activity with an IC_{50} of 23 μM. Additional increase in bulk by introducing trifluoromethyl group on **9c** gave 3,5-bistrifluoromethyl phenyl derivative **9d**, which displayed even better PAI-1 inhibitory activity with an IC_{50} 14.4 μM (Table 1). Based on these results, 3,5-bistrifluoromethyl-substituted phenyl ring in region 2 was finalized.

Furthermore, the modification of region 1, we introduced –SO_2NR^2– as a spacer group between two phenyl rings (Table 2). Substitution of electron releasing groups at the *para* position of the phenyl ring (R^3 of region 1) such as chloro (**15a**) and methoxy (**15b**) produced compounds with low PAI-1 inhibitory activity. The compounds containing electron-withdrawing groups such as trifluoromethyl and trifluoromethoxy groups on phenyl ring demonstrated good PAI-1 inhibitory activity [20,21]. Taking clue from the literature, first trifluoromethoxy group was introduced on the phenyl ring, which resulted in compound **15d** and it inhibited PAI-1 activity with IC_{50} 9.3 μM. However, both positional isomers **15e** (*meta*) and **15f** (*ortho*) displayed inferior inhibitory activity than **15d** (*para*). Introduction of a less bulky electron-withdrawing fluoro substituent on phenyl ring produced compound **15c** with low PAI-1 inhibitory activity.

In order to see the effect of substituents at free H of sulfonamide group (R^2 of region 1), various compounds were synthesized in which H atom was replaced by methyl in **15g**, propyl in **15h**, pentyl in **15i** and allyl in **15j**. Results of PAI-1 assay indicate that bulky alkyl groups help in improving activity, as **15g** is less active than **15h**, which in turn is less active than **15i**. Compound **15j** showed similar activity to that of **15i**. However, benzyl substituted compound **15k** showed inferior activity. Introduction of 4-OCF_3 group on benzyl ring of **15k** produced compound **15l** with very good activity (IC_{50} 8.5 μM), which may be due to combined interaction of two trifluoromethoxy groups.

Next, compounds containing trifluoromethyl group on phenyl ring were synthesized. Introduction of 4-CF_3 group on phenyl ring (R^3 of region 1) produced very less potent compound **15m** with IC_{50} of 86 μM. Substitution of methyl group on sulfonamide linker of **15m** gave compound **15n** with marginally improved activity (IC_{50} 61 μM). Further changing the position of –CF_3 group from *para* to *meta*, **15o** showed dramatic improvement in potency (IC_{50} 4.5 μM). Substitution on sulfonamide linker of **15o** to get **15p** and **15q** also gave good compounds. The compound **15q** was found to be as

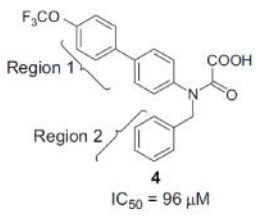


Fig. 2. Lead compound from library.

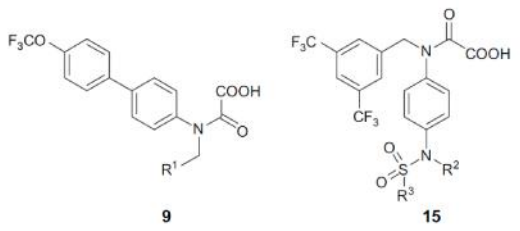
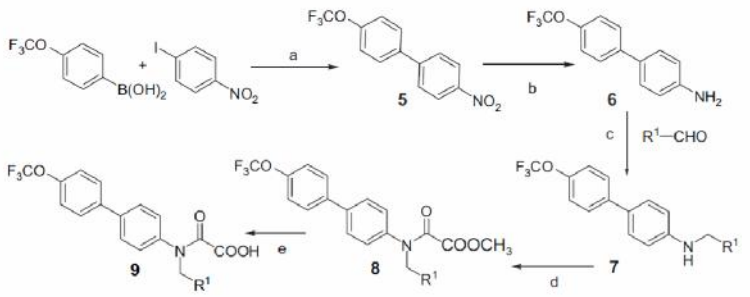
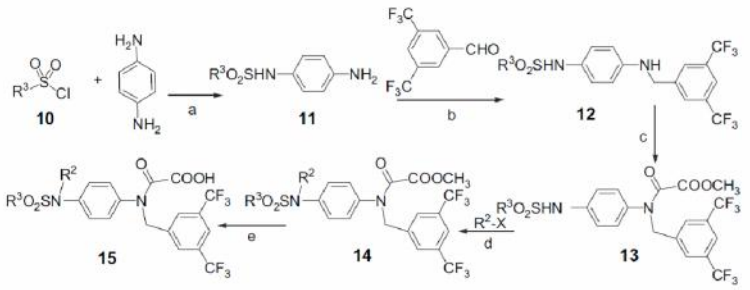


Fig. 3. General structure derived from **4**.



Scheme 1. Reagents and conditions: (a) $Pd(OAc)_2$, K_3PO_4, $(C_4H_9)_4N^+Br^-$, DMF, 45–50 °C, 16 h, 65%; (b) Pd–C, H_2 (60 psi), MeOH, 25–28 °C, 3 h, 90%; (c)$NaBH_4$, EtOH, 40–45 °C, 4–5 h, 80%; (d) ClCOCOOMe, pyridine, CH_2Cl_2, 5–10 °C, 3 h, 85% and (e) KOH, THF, H_2O, 25–28 °C, 80%.



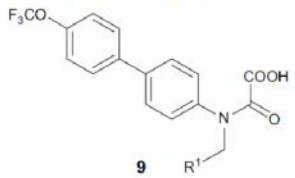
Scheme 2. Reagents and conditions: (a) DIPEA, CH_2Cl_2, 25–28 °C, 15–30 min, 75%; (b) $NaBH_4$, EtOH, 40–45 °C, 4–5 h, 80%; (c) ClCOCOOMe, pyridine, CH_2Cl_2, 5–10 °C, 3 h, 85%; (d) K_2CO_3, acetone, 25–28 °C, 2–10 h, 80%; (e) KOH, THF, H_2O, 25–28 °C, 80%.

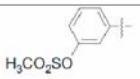
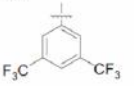
potent as **15o** in PAI-1 inhibitory activity. Compound **15r** with 3,5-bistrifluoromethyl-substituted ring (R^2 of region 1) showed a very promising PAI-1 inhibitory activity with an IC_{50} of 5 μM. However, changing methyl group from sulfonamide linker (R^2 of region 1) of **15r** with bulky groups allyl (**15t**) and propyl (**15s**) lead to compounds with mediocre *in vitro* activity. This is in contrast to the observation in compounds **15g–15l**, which may be due to crowding of alkyl groups with *meta*-CF_3 group. In Native PAGE (Poly Acrylamide Gel Electrophoresis) experiment, we observed that PAI-1-ligand complex stays above the free PAI-1 (gel picture is not given). The intensity of the PAI-1-ligand complex in Native PAGE and unavailability of free PAI-1 after ligand interaction clearly demonstrates that high concentration of the ligand does not induce any PAI-1 inactivation.

4. Conclusion

In summary, we have explored the structure–activity relationships around the PAI-1 inhibitor **4** by modification of the regions 1 and 2. It was observed that regions 1 and 2 accommodate electron-withdrawing bulky groups on phenyl ring with enhancement of potency, but electron releasing groups in region 1 and polar groups in region 2 were not tolerated. Introduction of sulfonamide spacer retained its inhibitory

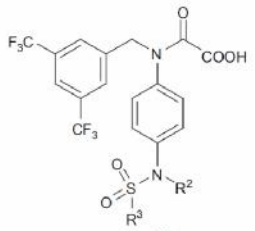
Table 1
PAI-1 inhibitory activity of oxalamide derivatives **9a–9d**



Compound	R^1	IC_{50} (μM)[a]
9a		No inhibition
9b		No inhibition
9c		23
9d		14.4
3 Tiplaxtinin	–	10

[a] Values determined using *in vitro* chromogenic assay.

Table 2
PAI-1 inhibitory activity of oxalamide derivatives **15a–15t** with sulfonamide spacer



15

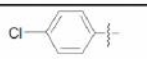
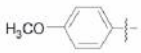
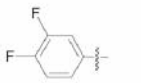
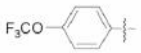
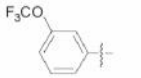
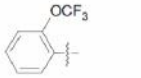
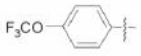
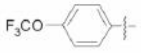
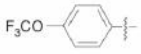
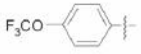
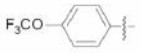
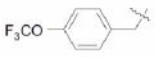
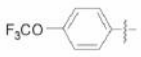
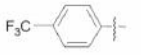
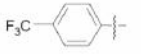
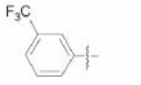
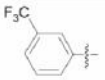
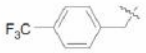
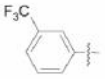
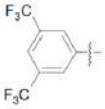
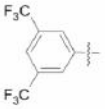
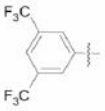
Compound	R^2	R^3	IC_{50} (μM)[a]
15a	H		75
15b	H		>80
15c	H		29.9
15d	H		9.3
15e	H		15.4
15f	H		114
15g	CH_3		25
15h	C_3H_7		21
15i	C_5H_{11}		14.9
15j			13.3
15k	Bn		43
15l			8.5
15m	H		86
15n	CH_3		61
15o	H		4.5

Table 2 (*continued*)

Compound	R^2	R^3	IC_{50} (μM)[a]
15p			11
15q			5.4
15r	CH_3		5
15s	C_3H_7		8.4
15t			12.8
3 Tiplaxtinin	–	–	10

[a] Values determined using *in vitro* chromogenic assay.

activity. Substitution at sulfonamide group with bulky alkyl groups and benzyl group substituted with electron-withdrawing groups resulted some good compounds **15l**, **15o**, **15q**, **15r** and **15s** with good potency.

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Discovery of inhibitors of plasminogen activator inhibitor-1: Structure–activity study of 5-nitro-2-phenoxybenzoic acid derivatives ☆

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ABSTRACT

Two novel series of 5-nitro-2-phenoxybenzoic acid derivatives are designed as potent PAI-1 inhibitors using hybridization and conformational restriction strategy in the tiplaxtinin and piperazine chemo types. The lead compounds **5a**, **6c**, and **6e** exhibited potent PAI-1 inhibitory activity and favorable oral bioavailability in the rodents.

Plasminogen activator inhibitor-1 (PAI-1), a member of serpin (serine protease inhibitor) superfamily, prevents the formation of plasmin by inhibiting the activity of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), the key enzymes

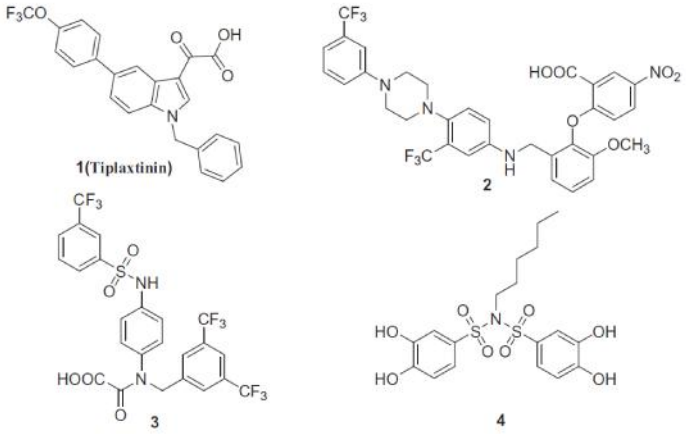

Figure 1. Structures of known PAI-1 inhibitors.

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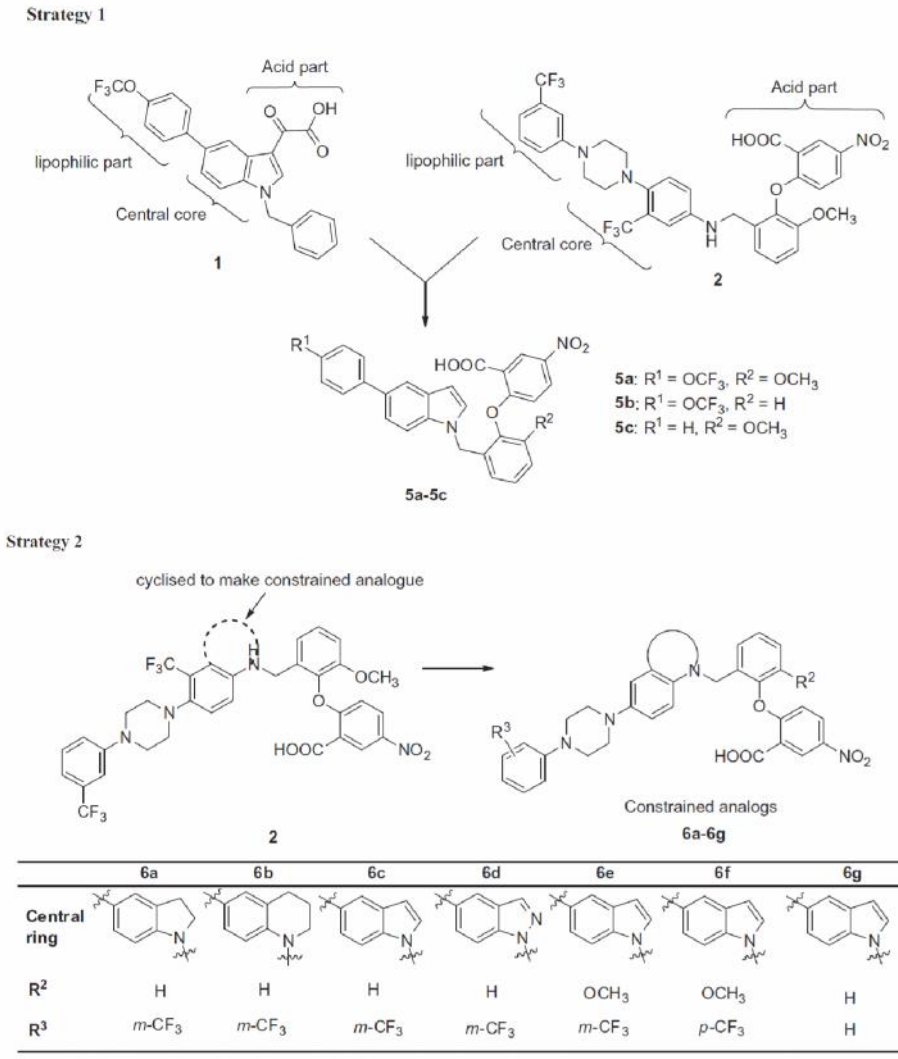
whose proteolytic action converts plasminogen to plasmin.[1] Plasmin dissolves fibrin clots by degrading insoluble fibrin molecules to small soluble fragments. Deficiency of PAI-1 in humans results in a hyperfibrinolytic state suggesting its important role in the fibrosis.[2–4] PAI-1 has been reported to have its implications in various patho-physiological processes, such as cancer,[5] diabetic nephropathy,[6] obesity,[7] metabolic syndrome,[8] venous thrombosis,[9] and atherosclerosis.[10,11] The therapeutic potential of PAI-1 inhibitors has been reviewed recently.[12] Several small molecules have been identified using the concept of the high throughput screening or virtual screening.[13] The most studied PAI-1 inhibitor tiplaxtinin **1** could reach up to Phase I.[14] Consistent research efforts resulted in the development of inhibitors with improved potency over tiplaxtinin exemplified by piperazine derivative **2**,[15] and aryl sulfonamide derivative **4**[16] (Fig. 1). There are few PAI-1 inhibitors which, demonstrated good antithrombotic efficacy in the various preclinical models however, none of them could advance to clinic.[17–26]

As a part of our research endeavor to develop viable therapies for the treatment of thrombotic diseases, we have previously reported the structure-activity relationship of a series of oxalamide derivatives **3**[27] (Fig. 1) identified by highthroughput screening of our compound library. However, none of those oxalamide derivatives could be further evaluated due to their poor oral bioavailibility. In continuation of our efforts to identify potent and orally bioavailable PAI-1 inhibitors, we further opted hybridization and conformational restriction strategies using known chemotypes. In order to achieve this objective, we selected tiplaxtinin **1**(IC_{50} = 2.7 μM, Lit. value),[14] and piperazine derivative **2**, with potent PAI-1 inhibitory activity (IC_{50} = 0.5 μM, Lit. value).[15] The known PAI-1 inhibitors reported mostly possess a carboxylic aci-



	6a	6b	6c	6d	6e	6f	6g
Central ring							
R^2	H	H	H	H	OCH_3	OCH_3	H
R^3	*m*-CF_3	*m*-CF_3	*m*-CF_3	*m*-CF_3	*m*-CF_3	*p*-CF_3	H

Figure 2. Strategies for the synthesis of PAI-1 inhibitors.

or an acid equivalent group attached to a lipophilic aromatic ring as a key structural feature. Tiplaxtinin **1** contains indole oxoacetic acid scaffold and published SAR[14] suggests importance of 4-trifloromethoxyphenyl group (lipophilic part) and also its position on indole. Piperazine derivative **2** contain 5-nitro-2-phenoxybenzoic acid part as an acid group, which was found to be optimum after employing various acid groups.[15] We thus proceeded and designed the compounds by incorporating the acid part of **2** in the **1** as a probable replacement of oxoacetic acid group of **1**, to get the hybridized molecules **5a–5c** (Fig. 2, strategy 1). Further, rational has been derived from docking study[28] of **5a** and tiplaxtinin, which reveled that both compounds possess similar orientation in the ligand binding pocket of PAI-1. The H bond interaction of carboxylic group of **5a** with Arg118 was found to be the key interaction (Fig. 3). As an alternative strategy, we intended to make the constrained analogues of **2** and subsequently few analogue **6a–6g** (Fig. 2, strategy 2) were synthesized. The compounds **5a–5c** and **6a–6g** were evaluated for their PAI-1 inhibitory activity (Tables 1 and 2). The pharmacokinetics parameters[29] of the potent compounds **5a**, **6c**, and **6e** were studied in the male wistar rats (Table 3).

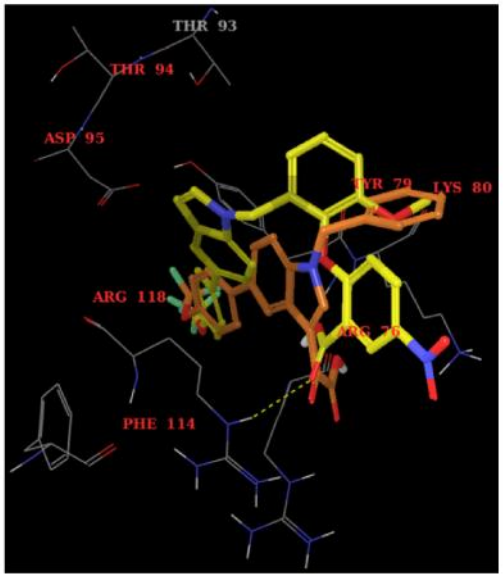
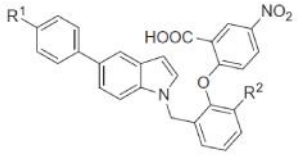


Figure 3. Overlay of docking images of tiplaxtinin (orange) and **5a** (yellow) into active site of PAI-1: H bond interaction of **5a** with Arg118 is shown in dashed line.

Table 1
PAI-1 inhibitory activity of compounds **5a–5c**



Compound	R^1	R^2	IC_{50}[a] (μM)
5a	OCF_3	OCH_3	3.4
5b	OCF_3	H	4.9
5c	H	OCH_3	98
Tiplaxtinin (**1**)			14.8

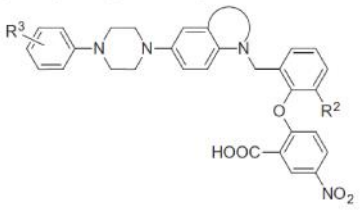
[a] Values determined using in vitro chromogenic assay.

The compounds **5a–5c** were prepared as shown in the Scheme 1. The salicylaldehyde derivative **8** was reacted with methyl 2-chloro-5-nitrobenzoate **7** using NaH as a base to give diphenyl ether derivative **9**, which upon reduction with sodium borohydride followed by bromination with PBr_3 afforded the bromo derivative **10**. The BOC protected 5-bromoindole **11** was reacted with the appropriate boronic acids **12** under Suzuki coupling[30] reaction conditions to afford the indole derivative **13** after the BOC deprotection using the TFA. The coupling of indole derivative **13** with the **10** in presence of *t*-BuOK provided the ester derivative **14**, which upon basic hydrolysis with KOH afforded the target compounds **5a–5c**.

The compounds **6a–6g** were prepared as depicted in the Scheme 2. The piperazine derivative **15** was coupled with the BOC protected heterocycle **16** using $Pd(OAc)_2$ as a catalyst and BINAP as a ligand to give compound **17**,[31] which was subsequently deprotected using either TFA or concd sulfuric acid to furnish **18**. The coupling of **18** with the halogen derivative **10** in presence of *t*-BuOK or K_2CO_3 as a base provided the ester derivative **19**. The basic hydrolysis of derivative **19** with KOH afforded the compounds **6a–6g**.

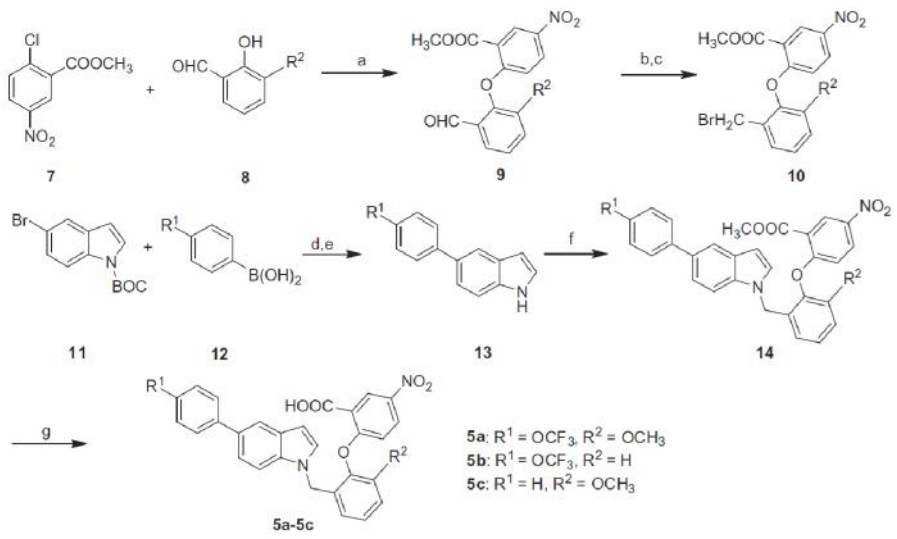
All the compounds **5a–5c** and **6a–6g** synthesized[32] were evaluated for their in vitro PAI-1 inhibitory activities[33] (Tables 1 and 2). The hybridized derivative **5a** synthesized as a part of strategy 1,

Table 2
PAI-1 inhibitory activity of compounds **6a–6g**

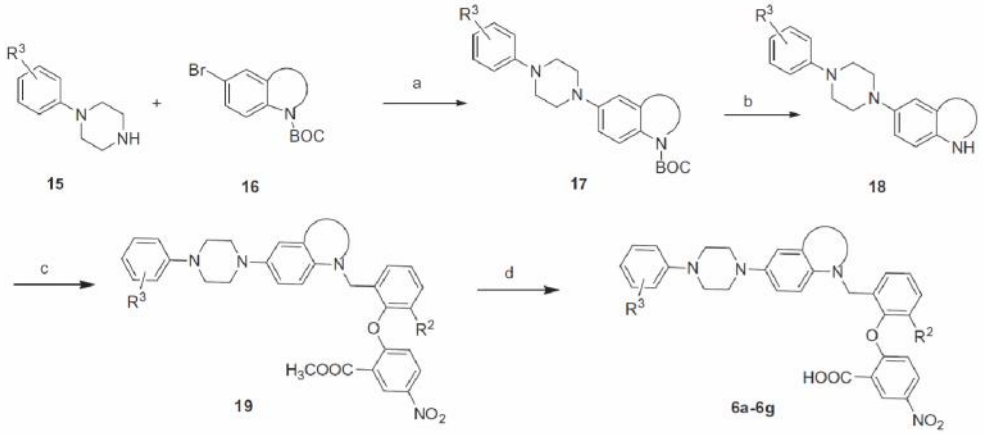


Compound	R^2	R^3	Central ring	IC_{50}[a] (μM)
6a	H	*m*-CF_3		29
6b	H	*m*-CF_3		63.8
6c	H	*m*-CF_3		3.2
6d	H	*m*-CF_3		14.6
6e	OCH_3	*m*-CF_3		2.4
6f	OCH_3	*p*-CF_3		22
6g	H	H		No inhibition
Tiplaxtinin (**1**)	–	–	–	14.8

[a] Values determined using in vitro chromogenic assay.



Scheme 1. Reagents and conditions: (a) NaH, DMSO, 0–25 °C, 60–70%; (b) $NaBH_4$, MeOH, 10–25 °C, 90–95%; (c) PBr_3, CH_2Cl_2, 10–25 °C, 75–80%; (d) $Pd(OAc)_2$, MeOH, reflux, 70–80%; (e) TFA, CH_2Cl_2, 25 °C, 80–90%; (f) **10**, *t*-BuOK, DMF, 10–25 °C, 60–75%; (g) KOH, MeOH, H_2O, 25 °C, 85–95%.



Scheme 2. Reagents and conditions: (a) $Pd(OAc)_2$, BINAP, K_3PO_4, DME, reflux, 20–50%; (b)TFA, CH_2Cl_2 or neat H_2SO_4, 0–25 °C, 80–90%; (c) **10**, *t*-BuOK, DMF or K_2CO_3, CH_3CN, 10–25 °C, 70–80%; (g) KOH, MeOH, H_2O, 25 °C, 85–95%.

Table 3
Pharmacokinetic parameters for compounds[a] **5a**, **6c**, **6e**

Compound	C_{max} (μg/mL)	T_{max}(h)	$T_{1/2}$ (h)	AUC(0–24) (h μg/mL)
5a	2.4	1	3.27	10.38
6c	6.8	6	9.86	80.18
6e	1.78	4	6.88	5.39

[a] Compounds were dosed in fasted male wistar rats at 30 mpk po formulated with a Tween-80: PEG: CMC(5:5: 90% v/v).

inhibited PAI-1 with an IC_{50} of 3.4 μM in the chromogenic assay. The removal of methoxy group from **5a** gave **5b**, which exhibited slightly lower potency (IC_{50} = 4.9 μM) than **5a**. The removal of triflorometh-oxy group is found to be detrimental for activity as evident from the compound **5c** (IC_{50} = 98 μM). Further more the compounds **6a–6g** were synthesized (Table 2) as part of strategy 2 (Fig. 2). The confor-mational restriction of **2** with two carbon atoms produced indoline derivative **6a**, which showed less potency (IC_{50} = 29 μM) compared to the tiplaxtinin (IC_{50} = 14.8 μM). The ring expansion in the **6a** to get tetrahydroquinoline derivative **6b** found to be detrimental to PAI-1 inhibitory activity (IC_{50} = 63.8 μM), probably due to confor-mational misfit of the molecule (six-membered ring of **6b** vs five-membered ring of **6a**). The introduction of the double bond in the indoline derivative **6a** to get indole derivative **6c** inhibited the PAI-1 activity with impressive IC_{50} of 3.2 μM, (Table 2). The incorpora-tion of an extra N atom in the ring gave indazole derivative **6d**, which showed inferior potency with an IC_{50} of 14.6 μM. Further, a methoxy group was introduced in the most potent compound **6c** to get the compound **6e**, interestingly the compound **6e** exhibited slightly

higher potency (IC_{50} = 2.4 μM) compared to **6c** (IC_{50} = 3.2 μM). The translocation of *m*-CF_3 group of **6e** (IC_{50} = 2.4 μM) at para position was found to be detrimental in terms of potency as witnessed from IC_{50} value of **6f**, (IC_{50} = 22 μM). The removal of CF_3 group from **6c** to get the compound **6g** resulted in the deterioration of the PAI-1 inhibition (Table 2), which further supported the importance of the of the CF_3 group. The compounds with potent PAI-1 inhibitory activity, **5a**, **6c**, and **6e** were evaluated for their pharmacokinetic parameters in rats (Table 3).

The compound **5a** showed good plasma levels (C_{max} = 2.4 μg/mL) and a half life ($T_{1/2}$ = 3.27 h) when dosed orally at 30 mg/kg in wistar rats (Table 3). The compound **6c** showed impressive plasma levels (C_{max} = 6.8 μg/mL) and a long ($T_{1/2}$ = 9.86 h), which is favorable for this class of compounds. However, plasma concentration of the methoxy derivative **6e** was found to be modest when compared to **6c**. The significant plasma concentration and long half life of the compounds **5a**, **6c**, and **6e** prompted us to study the compounds for their in vivo efficacy in rats using $FeCl_3$ induced arterial thrombosis model using Clopidogrel, a well known antiplatelet agent as a positive control.[34] However, compound **6c** exhibited moderate antithrombotic efficacy while compounds **5a** and **6e** failed to show any in vivo efficacy, inspite of their impressive in vitro PAI-1 inhibitory activity and favorable pharmacokinetic parameters (Fig. 4). The further optimization efforts for this class of compounds to get the appropriate pharamacodynamics and pharmacokinetics correlation are in progress.

In summary, the novel 5-nitro-2-phenoxybenzoic acid derivatives derived using hybridization and conformational restriction strategies display potent PAI-1 inhibitory activity and favorable pharmacokinetic parameters. Oxoacetic acid part of Tiplaxtinin **1** has been effectively replaced with 5-nitro-2-phenoxybenzoic acid part of **2** producing potent PAI inhibitor **5a**. The docking study confirmed the similar orientation of **5a** and tiplaxtinin in PAI-1 ligand binding site. Conformational restriction of **2** with indole as a central core (**6c**) showed potent PAI-1 inhibitory activity and excellent pharmacokinetic profile with moderate efficacy in rats using $FeCl_3$ induced arterial thrombosis model. These findings provided the impetus for further studies on the refinement of these templates which will be reported in due course.

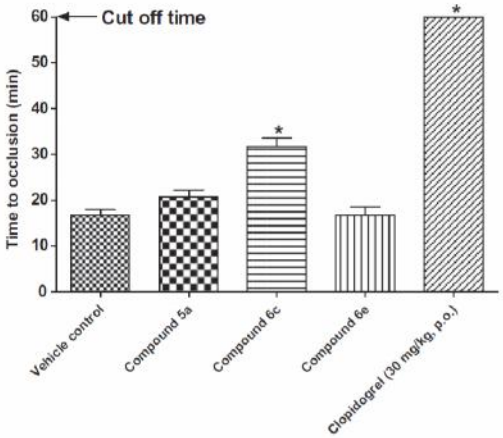


Figure 4. Effects of the compound **5a** and **6c** and **6e** on time to thrombus formation in $FeCl_3$-induced arterial thrombosis in rats at 30 mpk. Each value represents mean ± SEM (n = 6). * indicates $p < 0.05$ versus vehicle control. Clopidogrel was used as positive control and administered orally 2 h before application of $FeCl_3$ paper on the carotid artery.

Acknowledgments

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References and notes

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28. *Protocol for docking study*: (a) X-ray crystal structure of PAI-1 (PDB code: 3Q03) was obtained from PDB database. Protein crystal structure of PAI-1 was prepared using the Schrödinger's protein preparation wizard module. The binding site of the protein structure was identified using the sitemap module of the Schrödinger software. The probable site according to the literature was used as the centroid to generate the grid files for the docking. Docking study was carried out by using the induced fit docking: (b) Schrödinger Suite 2010 Induced Fit Docking protocol; Glide version 5.6, Schrödinger, LLC, New York, NY, 2010; Prime version 2.2, Schrödinger, LLC, New York, NY, 2010; (c) Docking study of Tiplaxtinin using the Glide software in PAI-1 protein crystal structure (PDB code: 1B3K) obtained from PDB did not gave a reported binding mode,[22] hence PAI-1 crystal structure recently reported by Jensen et al. was used for docking study of compounds along with tiplaxtinin; (d) Jensen, J. K.; Thompson, L. C.; Bucci, J. C.; Nissen, P.; Gettins, P. G. W.; Peterson, C. B.; Andreason, P. A.; Morth, J. P. *J. Biol. Chem.* **2011**, in press. doi:10.1074/jbc.M111.236554.
29. *Pharmacokinetic study*: Compounds were formulated with a Tween-80:PEG:CMC (5:5:90% v/v). A graduated dose volume (5 ml/kg) of suspension was administered to fasted male Wistar rats at 30 mg/kg po. The animals were anesthetized for blood sample collection from retro-orbital plexus. Serial blood samples were collected into heparinised containers at various time points and blood centrifuged to yield plasma. Plasma concentration was determined by using LC–MS/MS method.
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32. *Spectroscopic characterization of selected compounds*: **5a**: 97.8% by HPLC; mp 225–228 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 8.5 (d, J = 2.8 Hz, 1H), 8.08 (dd, J = 2.8 and 9.4 Hz, 1H), 7.7 (m, 3H), 7.48 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 3.2 Hz, 1H), 7.40 (d, J = 8 Hz, 2H), 7.33 (dd, J = 1.6 and 8.8 Hz, 1H), 7.26 (m, 1H), 7.16 (dd, J = 1.2 and 8.4 Hz, 1H), 6.83 (d, J = 7.2 Hz, 1H), 6.49 (d, J = 9.2 Hz, 1H), 6.41 (d, J = 2.8 Hz, 1H), 5.34 (s, 2H), 3.68 (s, 3H); ESI-MS: 578.9 (M+H)$^+$, **6c**: 97% by HPLC; mp 180–185 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 8.56 (d, J = 3.2 Hz, 1H), 8.23 (dd, J = 2.9 and 9.1 Hz, 1H), 7.42 (m, 2H), 7.33 (m, 3H), 7.21 (m, 2H), 7.21 (m, 2H), 7.12 (m, 3H), 7.0 (m, 2H), 6.78 (d, J = 9.1 Hz, 1H), 6.3 (d, J = 3 Hz, 1H), 5.34 (s, 2H), 3.37 (t, J = 4.2 Hz, 4H), 3.16 (t, J = 4.4 Hz, 4H); ESI-MS: 617.2 (M+H)$^+$; 6e: 97.5% by HPLC; mp 120–125 °C: ^{1}H NMR (400 MHz, DMSO-d_6): 8.51 (d, J = 2.8 Hz, 1H), 8.07 (dd, J = 2.8 and 9.2 Hz, 1H), 7.44 (m, 1H), 7.28 (m, 3H), 7.24 (m, 2H), 7.18 (dd, J = 0.8 and 8 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 2 Hz, 1H), 6.87 (dd, J = 2 and 8.8 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 6.45 (d, J = 9.2 Hz, 1H), 6.24 (d, J = 3.2 Hz, 1H), 5.26 (s, 2H), 3.69 (s, 3H), 3.38 (t, J = 4 Hz, 4H), 3.14 (t, J = 4.8 Hz, 4H); ESI-MS: 647 (M+H)$^+$.

33. *Chromogenic assay*: The chromogenic assay was based upon the interaction between tPA and active PAI-1. tPA coated assay plates obtained from Trinity Biotech., NY, USA were kept at 4 °C overnight. phenoxybenzoic acid derivatives were dissolved in DMSO and diluted to a range of concentration between 1 and100 μM. Varying concentrations of phenoxybenzoic acid were then incubated with human PAI-1 (50 nM, Molecular Innovations, MI, USA) for 30 min at 25 °C. An aliquot of this solution along with a monoclonal antibody against human PAI-1 conjugated with HRP (Trinity Biotech., NY, USA) was added to the t-PA-coated plate. The Plate was then incubated for 30 min at room temperature with gentle shaking. The solution was aspirated from the plate, which was then washed thrice with a buffer consisting of 0.05% Tween 20 and 0.1% BSA in PBS. This assay detects only active inhibitory PAI-1 (not latent or substrate) bound to the plate. 100 μl aliquot of HRP substrate solution was added and incubated for 5 min at 25 °C. Reaction was terminated with the addition of 50 μl of 1.6 (M) H_2SO_4 followed by the determination of absorbance at 490 nm. The quantization of residual active PAI-1 bound to t-PA at varying concentrations of phenoxybenzoic acid was used to determine the IC_{50} by fitting the results to a logistic dose–response program (Graphpad Prism, CA, USA). IC_{50} was defined as the concentration of compound required to achieve 50% inhibition of PAI-1 activity. The assay sensitivity was 5 ng/ml of human PAI-1 as determined from a standard curve ranging from 0–100 ng/ml of human PAI-1.

34. *Protocol for in vivo study*: In this study $FeCl_3$ induced chemical injury was used as a model of arterial thrombosis in rat model. Rats (n = 6) were anaesthetized with urethane (1.25 g/kg, ip) and secured in supine position. A midline cervical incision was made on the ventral side of the neck, and left carotid artery was isolated by blunt dissection. Compound **5a**, 6c and 6e (each 30 mg/kg) were formulated in polyethylene glycol (PEG) and 0.5% sodium carboxymethyl cellulose (1:10) and administered by oral gavage. Exactly after 2 h of administration, a 2 × 3 mm strip of Whatman # 1 filter paper saturated with 35% (w/v) $FeCl_3$ was placed on the carotid artery for 5 min. A temperature probe (Thermalert-TH8, Physitemp Instruments Inc., Clifton, N.J., USA) was placed distal to filter paper to monitor the temperature of carotid artery. A sudden fall in temperature (about 1–2 °C) was taken as an indication of cessation of blood flow as a result of thrombus formation. Time to occlusion (TTO) was defined as the time from $FeCl_3$ application to time of thrombus formation (indicated by sudden fall in carotid temperature). In case if no thrombus formation was seen in drug-treated animals, a cutoff time was fixed at 1 h.

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EFFICIENT SYNTHESIS OF UNSYMMETRICAL DIBENZOTHIOPHENES BY ACID-MEDIATED INTRAMOLECULAR CYCLIZATION OF BIARYL METHYL SULFOXIDES

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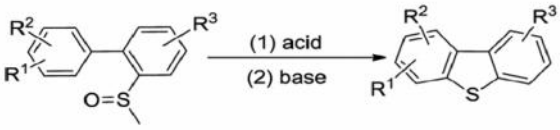
GRAPHICAL ABSTRACT



Abstract *A convenient and high-yielding synthesis of unsymmetrical dibenzothiophenes has been achieved by an acid-mediated ring closure of the biphenyl ring having a sulfoxide substituent at the* ortho *position. Various functional groups are well tolerated in this methodology.*

Keywords Cyclization; dibenzothiophene; intramolecular; sulfoxide

INTRODUCTION

Dibenzothiophenes **1** (Fig. 1) are biologically important because of their occurrence in a wide variety of natural products possessing useful biological activities.[1] Also, different substituted dibenzothiophene derivatives are reported to be anti-angiogenic agents,[2] antiobesity agents,[3] antiviral agents,[4] and agents for the treatment of *Pneucystis carinii* pneumonia.[5] The literature discloses different approaches for preparation of substituted dibenzothiophenes, such as disulfide ring closure,[6] photochemical cyclization of 2-(2′-methylthio)biphenyl radical,[7] sulfur insertion in biphenyl using $AlCl_3$,[8] fusion of 2,2′-dihydroxybiphenyl with P_2S_5,[9] ring contraction of thianthrene using copper bronze[10] and cyclization of biphenyl-2-sulfonyl chloride using $AlCl_3$ gives dibenzothiophene dioxide,[11] which upon deoxygenation gives dibenzothiophene.[12]

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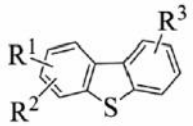

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Figure 1. General structure of dibenzothiophenes.

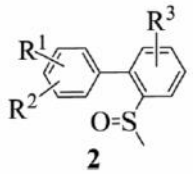

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Figure 2. General structure of sulfoxide.

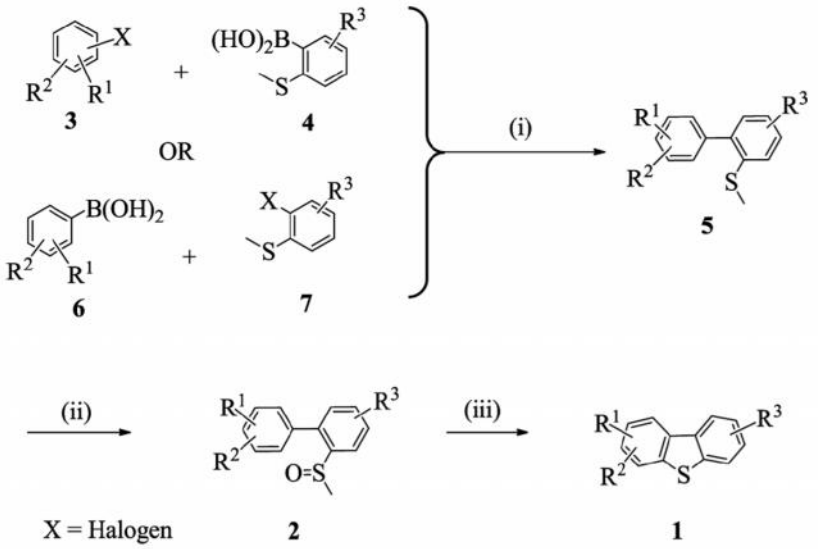
Sirringhaus et al. have reported synthesis of dibenzothienobisbenzothiophene by acid-mediated intermolecular cyclization of a sulfoxide derivative.[13] This literature report involved the use of expensive and hazardous chemicals such as trifluoromethanesulfonic acid and pyridine, and also the reaction took a long time and involved high temperature. The method does not have wider applicability in terms of functional group sensitivity. Recently, Sanz et al. have reported the synthesis of regioselectively functionalized dibenzothiophenes through anionic cyclization of benyne-tethered aryllithiums.[14]

The aforementioned literature methods involve high-temperature reactions using sulfur or sulfur-containing reagents or organolithium reagents. Also, some of these methods suffer from poor yields. Direct introduction of functional groups in dibenzothiophene nucleus also has limitations of regioselectivity. Literature methods for synthesis of 4-substituted dibenzothiophene ring involve metallation reactions in dibenzothiophene ring at the 4-position followed by the addition of appropriate electrophile.[15] Electrophilic substitution reaction on dibenzothiophene ring goes at the 2-position. Hence the synthesis of 1- and 3-substituted dibenzothiophene derivatives is relatively difficult and involves multiple steps.

In continuation of our interest in synthesizing dibenzothiophenes with a variety of substituents, herein we report a simple, cost-effective, and industrially applicable synthesis of unsymmetrical dibenzothiophenes by acidic ring closure of sulfoxide **2** (Fig. 2) using inorganic reagents, sulfuric acid, and potassium carbonate.

RESULTS AND DISCUSSION

To access sulfoxide **2**, the synthetic strategy depicted in Scheme 1 is adapted. Several methods are reported to make the biphenyl ring system. For the sake of simplicity and easy availability of substituted boronic acids, we adapted the Suzuki coupling[16] reaction. Thus, the coupling of substituted phenylboronic acid with substituted halobenzene gave the biphenyl ring, which upon oxidation with hydrogen peroxide[17] produced desired sulfoxide **2** in excellent yield.

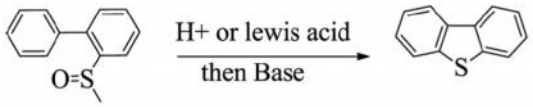


Scheme 1. Reagents and conditions: (i) $Pd(OAc)_2$, Na_2CO_3, MeOH, 70–95%; (ii) H_2O_2 (50%), Cat. V_2O_5, MeCN, 80–90%; (iii) conc.H_2SO_4, 0–75 °C, then aq. K_2CO_3, rt.

Once sulfoxide **2** was in hand, we set forth to screen the best acidic reagent to achieve ring closure and thereby produce dibenzothiophene. Sulfuric acid was found to give the best result in terms of yield and purity of dibenzothiophene as compared to the other Lewis or mineral acids screened. It was also found that the cyclization worked excellently when neat sulfuric acid was used. Sulfuric acid in solvent took a longer time to bring the desired cyclization and also the isolated yield was less (Table 1).

To extend the application of this reaction for the synthesis of substituted dibenzothiophenes, sulfoxide **2** with various substituents has been synthesized and subjected to cyclization in neat sulfuric acid (Table 2). It was observed that sulfoxide **2** containing electron-releasing groups produced greater yield of the corresponding dibenzothiophenes (**2a, 2b**, and **2c**). Electron withdrawing group such as nitro (**2e**)

Table 1. Screening of acidic reagents for cyclization



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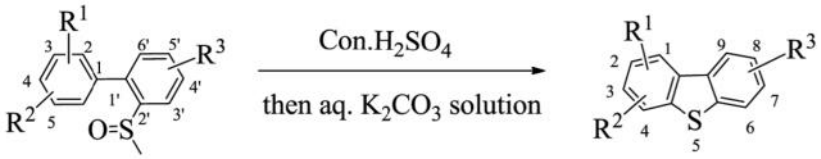
No.	Cyclization reagent	Solvent	Reaction time (h)	Temperature (°C)	Yield (%)
1	H_2SO_4 (3v/w)	Neat	0.25	0–25	94
2	I_2 (2 equiv.)	Toluene	20	70	No reaction
3	$AlCl_3$ (2 equiv.)	Toluene	20	70	No reaction
4	TFA (3v/w)	Neat	20	0–25	10
5	H_2SO_4 (3v/w)	Chloroform	15	0–25	50

Table 2. Synthesis of substituted dibenzothiophenes

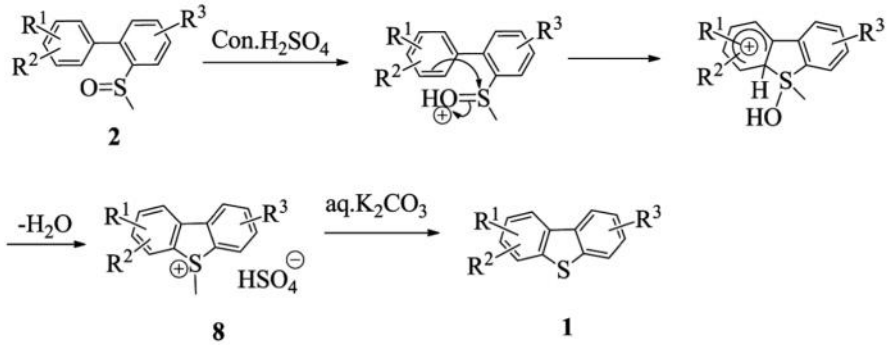


2 **1**

No.	Substituent on 2	Substituent on 1	Temperature (°C)	Time (h)	Yield (%)
a	$R^1=R^2=R^3=H$	$R^1=R^2=R^3=H$	0–25	0.5	94
b	$R^1=R^3=H$, R^2=4-F	$R^1=R^3=H$, R^2=3-F	0–25	0.5	92
c	$R^1=R^3=H$, R^2=2-CH_3	$R^1=R^3=H$, R^2=1-CH_3	0–25	0.5	97
d	$R^1=R^3=H$, R^2=4-$COOCH_3$	$R^1=R^3=H$, R^2=3-$COOCH_3$	0–25	1	75
e	$R^1=R^3=H$, R^2=4-NO_2	$R^1=R^3=H$, R^2=3-NO_2	0–75	2	49
f	R^1=3-OCH_3, R^2=4-$COOCH_3$, $R^3=H$	R^1=2-OCH_3, R^2=3-$COOCH_3$ $R^3=H$	0–25	1	74
g	R^1=2-CH_3, $R^2=H$, R^3=5′-$COCH_3$	R^1=1-CH_3, $R^2=H$, R^3=8-$COCH_3$	0–25	0.25	82
h	R^1=2-CH_3, $R^2=H$, R^3=5′-CH_2CH_3	R^1=1-CH_3, $R^2=H$, R^3=8-CH_2CH_3	0–25	0.25	85
i	R^1=3-$COCH_3$, $R^2=R^3=H$	R^1=2-$COCH_3$, $R^2=R^3=H$	0–25	0.25	70
j	R^1=2-$COOCH_3$, $R^2=R^3=H$	R^1=1-$COOCH_3$, $R^2=R^3=H$	0–25	1	55

and ester (**2d**) resist cyclization and hence gave relatively lesser yields. Most of the reactions were completed at 25 °C within 30 min, except for reactions with ring systems bearing electron-withdrawing groups (**2d, 2e**, and **2f**). The 1-substituted dibenzothiophenes have been effectively synthesized, as can be seen from examples **2c** and **2j**. Substituents on both rings are possible through this methodology (**2 h** and **2 g**).

The proposed mechanism of the reaction is shown in Scheme 2. The first step involves protonation of sulfoxide followed by nucleophilic attack of neighboring aromatic ring. Dehydration eventually led to aromatization, furnishing salt **8**. The demethylation of salt using base gives the desired dibenzothiophene derivatives. This mechanism correlates with our experimental observation of poor yield and more reaction time for electron-withdrawing group substituted sulfoxide as it destabilizes the positively charged transition state. Nenaidenko et al. have reviewed the synthetic capabilities of sulfonium salts with mechanistic aspects.[18] Nucleofugality of the group attached to the *S* atom is one of the main factors that determines direction of nucleophilic attack on sulfonium salt. In our case, biaryl sulfide is a good leaving group compared to methyl in sulfonium salt **8** and also methyl group provides an



Scheme 2. Proposed mechanism for cyclization.

electrophilic center for the nucleophile, that is, hydroxide ion generated from aqueous potassium carbonate.

CONCLUSION

In summary, a convenient and efficient method for the synthesis of unsymmetrical dibenzothiophene derivatives has been reported. The advantage of the described methodology over the reported ones is incorporation of substituents in the dibenzothiophene core with ease and good yields. The present method utilizes inexpensive and environmentally friendly reagents. Further, the duration of the reaction is very short and also reactions are accomplished at low temperature. This method gives access to a substituent at every position of dibenzothiophene. 1-Subsituted dibenzothiophene, which is difficult to make by direct substitution reaction on dibenzothiophene, can be easily prepared by this method. This methodology has potential applications for the synthesis of sulfur-containing fused heterocycles.

EXPERIMENTAL

Melting points were recorded with a Thomas–Hoover capillary melting-point apparatus and are uncorrected. Infrared (IR) spectra were recorded with an Fourier transform (FT-IR) instrument as a thin film or using KBr pellets, and are expressed in centimeters^{-1}. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded using dimethylsulfoxide (DMSO) as a solvent. Ultra performance liquid chromatography (UPLC) purity were recorded in 0.05% TFA in water–ACN as a mobile phase and BEH C18, 2.1 × 100 mm column as a stationary phase. Elemental analyses were carried out with a C, Hanalyzer. Thin-layer chromatography (TLC) was performed on precoated plates (0.25 mm, silica gel 60 F254). Column chromatography was carried out with silica gel (100–200 mesh). The reactions were carried out in oven-dried glassware under dry N_2. MeOH and ACN were purified and dried before use. Distilled n-hexane and EtOAc were used for column chromatography.

General Procedure for Suzuki Coupling

Sodium carbonate (2 mmol) was added to a mixture of substituted halobenzene (**3** or **7**) (1 mmol) in dry methanol (10 v/w) placed in a round-bottomed flask

attached with condenser followed by palladium acetate (2 mol%). Substituted phenylboronic acid (**4** or **6**) (1 mmol) was added in one lot, and the reaction mixture was subjected to reflux. After 5 h, the reaction mixture was cooled to 25–30 °C. Methanol (3 v/w) was added, and the mixture was vacuum filtered through a sintered glass funnel using celite as a filter aid to furnish a residue. The residue obtained was purified by flash chromatography using 100 to 200-mesh silica gel as a stationary phase and *n*-hexane–ethyl acetate as a mobile phase (yield 70–90%).

General Procedure for Oxidation

A solution of biphenyl compound (**5**) (1 mmol) and V_2O_5 (0.1 mmol) in acetonitrile (5 mL) was cooled at 0 °C by keeping a round-bottomed flask in an ice bath under a nitrogen environment. Aqueous hydrogen peroxide (1.2 mmol, 50%) was added to the reaction mixture and it was stirred at 10 °C for 1 h. After 1 h, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to offer the corresponding sulfoxide (yield = 80–90%).

General Procedure for Cyclization

Sulfoxide compound (**2**) (0.5 g) was added in portions to stirred concentrated H_2SO_4 (1.5 mL, 3 v/w) in a one-necked, round-bottomed flask containing a guard tube at 0–5 °C. The reaction mixture was stirred at 25 °C for 0.5–2 h. The reaction mixture was poured on ice-cold water (10 mL) and then made basic with aqueous potassium carbonate solution (pH 8). The aqueous layer was extracted with ethyl acetate (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to give the titled compound (yield = 49–97%).

Spectral Data of Synthesized Compounds

Dibenzo[*b,d*]thiophene (1a). White solid, mp: 98 °C, IR (KBr): ν_{max} 3051, 1583, 1415, 1307, 1230, 1130, 1066, 929, 734, 495 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.47–7.53 (m, 4H), 7.99–8.04 (m, 2H), 8.34–8.38 (m, 2H); ^1H NMR (400 MHz, $CDCl_3$): δ 7.43–7.47 (m, 4H), 7.83–7.87 (m, 2H), 8.13–8.18 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 122.0, 123.0, 124.7, 127.0, 135.0, 138.5. CHNS: Calculated for $C_{12}H_8S$: C, 78.22; H, 4.38; S, 17.40. Found: C, 78.32; H, 4.34; S,17.20.

3-Fluorodibenzo[*b,d*]thiophene (1b). White solid, mp: 101 °C; IR (KBr): ν_{max} 3387, 1604, 1440, 1396, 1315, 1240, 891, 840, 758, 732 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.32–7.37 (m, 1H), 7.46–7.51 (m, 2H), 7.92–7.95 (m, 1H), 7.98–8.02 (m, 1H), 8.30–8.32 (m, 1H), 8.33– 8.38 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 109.3, 112.8, 121.8, 122.9, 123.4, 124.9, 126.6, 131.7, 134.2, 138.50, 140.0; ESI-MS: m/z: 225.4 $(M + Na)^+$. CHNS: Calculated for $C_{12}H_7FS$: C, 71.26; H, 3.49; S, 15.85. Found: C, 70.90; H, 3.36; S, 15.98.

1-Methyldibenzo[*b,d*]thiophene (1c). White solid; mp: 74 °C; IR (KBr): ν_{max} 3387, 3059, 2949, 1905, 1438, 1307, 1028, 773, 738, 729, 707 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.87 (s, 3H), 7.30 (d, J = 8 Hz, 1H), 7.38–7.42 (m, 1H),

7.49–7.54 (m, 2H), 7.87 (d, $J = 8$ Hz, 1H), 8.02–8.06 (m, 1H), 8.37–8.41 (m, 1H);^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 120.6, 122.9, 124.6, 125.0, 126.1, 126.4, 127.1, 133.0, 134.6, 135.8, 138.7, 138.9. CHNS: Calculated for $C_{13}H_{10}S$: C, 78.75; H, 5.08; S, 16.17. Found: C, 78.30; H, 5.05; S, 16.11.

Methyldibenzo[*b,d*]thiophene-3-carboxylate (1d). White solid, mp: 138 °C; IR (KBr): ν_{max} 2939, 1712, 1597, 1442, 1388, 1288, 1253, 1112, 974, 848, 754 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.90 (s, 3H), 7.55–7.60 (m, 2H), 8.04–8.10 (m, 2H), 8.44–8.47 (m, 1H), 8.51 (d, $J = 8.4$ Hz, 1H), 8.67 (d, $J = 1.6$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.3, 122.0, 122.9, 123.0, 124.0, 125.0, 125.1, 127.9, 128.1, 134.0, 138.1, 138.7, 140.2, 165.9. CHNS: Calculated for $C_{14}H_{10}O_2S$: C, 69.40; H, 4.16; S, 13.23. Found: C, 69.48; H, 4.17; S, 13.41.

3-Nitrodibenzo[*b,d*]thiophene (1e). Yellow solid; mp: 150 °C; IR (KBr): ν_{max} 2962, 1604, 1518, 1448, 1330, 1261, 1103, 1022, 879, 771, 738 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61–7.66 (m, 2H), 8.14 (d, $J = 8$ Hz, 1H), 8.32 (dd, $J = 8.8$ Hz, 2 Hz, 1H), 8.52 (d, $J = 8$ Hz, 1H), 8.60 (d, $J = 8.8$ Hz, 1H), 9.08 (d, $J = 2$ Hz, 1H);^{13}C NMR (100 MHz, DMSO-d_6): δ 118.5, 119.6, 122.5, 123.3, 123.5, 125.3, 128.8, 133.2, 139.1, 140.0, 141.3, 145.8; ESI-MS: m/z: 230 $(M + H)^+$, CHNS: Calculated for $C_{12}H_7NO_2S$: C, 62.87; H, 3.08; N, 6.11; S, 13.99. Found: C, 62.86; H, 3.10; N, 6.10; S, 13.95.

Methyl 2-methoxydibenzo[*b,d*]thiophene-3-carboxylate (1f). White solid, mp: 128 °C; IR (KBr): ν_{max} 3347, 1610, 1450, 1366, 1345, 1250, 891, 840, 760, 740 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 3.82 (s, 3H), 3.96 (s, 3H), 7.51–7.57 (m, 2H), 8.03 (d, $J = 8.4$ Hz, 1H), 8.10 (s, 1H), 8.29 (s, 1H), 8.49 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.0, 56.3, 105.3, 120, 123.0, 23.1, 124.6, 125.2, 127.9, 129.5, 134.4, 139.0, 140.8, 156.1, 165.9; ESI-MS: m/z: 273 $(M + H)^+$. CHNS: Calculated for $C_{15}H_{12}O_3S$: C, 66.16; H, 4.44; S, 11.77. Found: C, 66.15; H, 4.51; S, 11.65.

1-(9-Methyldibenzo[b,d]thiophen-2-yl)ethanone (1g). White solid, mp: 125 °C; IR (KBr): ν_{max} 1678, 1356, 1313, 1244, 879, 840, 767 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.72 (s, 3H), 2.94 (s, 3H), 7.38 (d, $J = 7.2$ Hz, 1H), 7.49 (t, $J = 7.6$ Hz, 1H), 7.95 (d, $J = 8$ Hz, 1H), 8.11 (dd, $J = 8.4$, 1.6 Hz, 1H), 8.21 (d, $J = 8.4$ Hz, 1H), 8.90 (d, $J = 1.6$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.8, 26.7, 120.8, 121.9, 124.6, 125.5, 127, 127.5, 132.5, 133.3, 134.9, 135.6, 139.3, 143.8, 197.3; ESI-MS: m/z: 240.8 $(M + H)^+$; CHNS. Calculated for $C_{15}H_{12}OS$: C, 74.97; H, 5.03; S, 13.34. Found: C, 74.90; H, 4.99; S, 13.10.

8-Ethyl-1-methyldibenzo[b,d]thiophene (1h). Semisolid, IR (KBr): ν_{max} 2999, 1460, 1217, 1033, 758 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.30 (t, 3H), 2.83 (q, 2H), 7.29 (d, $J = 7.6$ Hz, 1H), 7.38 (d, $J = 7.6$ Hz, 1H), 7.40 (d, $J = 8.4$ Hz, 1H), 7.85 (d, $J = 7.6$ Hz, 1H), 7.94 (d, $J = 8.4$ Hz, 1H), 8.20 (d, $J = 0.8$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 16.0, 22.2, 28.4, 120.6, 122.6, 124.0, 126.2, 126.4, 127.0, 133.0, 134.6, 135.9, 136.0, 139.2, 140.2. CHNS: Calculated for $C_{15}H_{14}S$: C, 79.60; H, 6.23; S, 14.17; Found. C, 79.37; H, 6.33; S, 14.32.

1-(Dibenzo[b,d]thiophen-2-yl)ethanone (1i). White solid, mp: 125 °C; IR (KBr): ν_{max} 1664, 1446, 1354, 1269, 964, 754, 603 cm^{-1}; ^{1}H NMR (400 MHz,

DMSO-d_6): δ 2.77 (s, 3H), 7.51–7.58 (m, 2H), 7.74 (t, 1H), 8.05–8.09 (m, 1H), 8.36 (d, $J=8$ Hz, 1H), 8.42–8.46 (m, 1H), 8.70 (d, $J=8$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.5, 122.0, 122.8, 124.8, 124.8, 126.7, 127.4, 130.1, 130.3, 133.4, 136.6, 137.5, 141.1, 197.7. CHNS: Calculated for $C_{14}H_{10}OS$: C, 74.31; H, 4.45; S, 14.17. Found: C, 74.38; H, 4.43; S, 14.27.

Methyldibenzo[b,d]thiophene-1-carboxylate (1j). White solid, mp: 88 °C; IR (KBr): ν_{max} 1664, 1446, 1354, 1269, 964, 754, 603 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.02 (s, 3H), 7.47–7.51 (m, 1H), 7.54–7.57 (m, 1H), 7.59–7.61 (m, 1H), 7.72 (dd, $J=8$, 4 Hz, 1H), 8.10 (d, $J=8$ Hz, 1H), 8.21 (d, $J=8$ Hz, 1H), 8.26 (dd, $J=8$, 4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.8, 123.2, 124.6, 124.7, 125.5, 126.1, 126.2, 127.5, 128.4, 131.0, 132.9, 139.2, 139.9, 168.7; ESI-MS: m/z: 265.16 $(M+Na)^+$. CHNS: Calculated for $C_{14}H_{10}O_2S$: C, 69.40; H, 4.16; S, 13.21. Found: C, 69.47; H, 4.28; S, 13.15.

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8. VITAE

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