Novel STAT3 Small-Molecule Inhibitors as Potential Anticancer Agents

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This thesis describes research conducted in the School of Pharmacy, University of London between 2007 and 2011 under the supervision of Dr. Giovanna Zinzalla and Professor David. E. Thurston. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any parts of this dissertation that has already appeared in publication.

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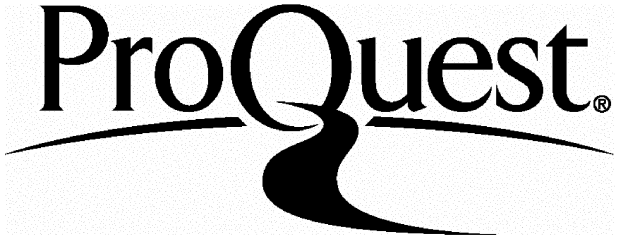

To My Parents

ACKNOWLEDGEMENT

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ABBREVIATIONS

ADME – Absorption, distribution, metabolism and excretion

AIDS – Acquired immune deficiency syndrome

ALK – Anaplastic lymphoma kinases

ALL – Acute lymphocytic leukemia

AML – Acute myelogenous leukemia

APRF – Acute-phase response factor

ARNT – Aryl hydrocarbon receptor nuclear translocator

ASA – Accessible surface areas

Bad – Bcl-2-associated agonist of cell death

BAF – BRG1/brm-associated factor

Bak – Bcl-2 homologous antagonist/killer

Bax – Bcl-2–associated X protein

Bcl-X_L – B cell leukemia/lymphoma xL

BCR – breakpoint cluster region

ABL – V-abl Abelson murine leukemia viral oncogene homolog 1

BH3 – Bcl-2 homology domain-3

bHLH-LZ – Basic helix–loop–helix leucine zipper

bHLH-PAS – Basic helix-loop-helix/Per-Arnt-Sim homology

BRAF – v-raf murine sarcoma viral oncogene homolog B1

BRCA1 – Breast cancer type 1 susceptibility protein

CAMK – Ca^{2+}/Calmodulin-Dependent Protein Kinase

cAMP – Cyclic adenosine monophosphate

CAS – Cellular apoptosis susceptibility

CBF – Core-binding factor

CBP – CREB binding protein

CCAAT – Cytidine-cytidine-adenosine-adenosine-thymidine

CD4 – Cluster of differentiation 4

cdc25A – Cell division cycle 25 homolog A

CDK – Cyclin dependent kinase

CFP – Cyan fluorescent protein

CID – Chemical inducer of dimerization

CIITA– Class-II transactivator

CIS – Cytokine-inducible SH2-containing protein

CLL – Chronic lymphocytic leukemia

CML – Chronic myelogenous leukemia

CNTF – Ciliary neurotrophic factor

CPE – Cytopathic effect

CREB – Cyclic AMP response element binding protein

ABBREVIATIONS

CRM – Chromosome region maintenance

DCM – Dichloromethane

DCoH – Dimerisation cofactor of hepatocyte nuclear factor

DHFR– Dihydrofolate reductase

DMAP – 4-Dimethylaminopyridine

DMF – Dimethylformamide

DMSO – Dimethylsulfoxide

EBP – Enhancer-binding proteins

EBPS – Cell surface elastin binding protein

EBV – Epstein Barr virus

EC_{50} – Half maximal effective concentration

ECM – Extracellular matrix

EDC.HCl – 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride

EGFR – Epidermal growth factor receptor

ELISA – Enzyme-linked immunosorbent assay

EPO – Erythropoietin

EPOR – Erythropoietin receptor

Eq. – Equivalents

ES – Embryonic stem

EtOH – Ethanol

FKBP12 – FK506 binding protein

FP – Fluorescence polarization

FRAP – Fluorescence recovery after photobleaching

FRB – FKBP12-Rapamycin Binding

FRET – Fluorescence resonance energy transfer

FT-IR – Fourier transform infrared spectroscopy

GAS – Gamma Interferon Activated Site

G-CSF – Granulocyte colony stimulating factor

GFAP – Glial fibrillary acidic protein

GPCR – G-protein coupled receptor

GR – Glucocorticoid receptor

GRIM 19 – Gene associated with retinoic and interferon-induced mortality 19 protein

GTP – Guanosine triphosphate

HAART – Highly active antiretroviral therapy

HATs – Histone Acetyl Transferases

HATU – 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HDM – Human homologue of MDM2 (murine double minute)

HER – Human Epidermal growth factor Receptor 2

hGH – Human growth hormone

HIF – Hypoxia-inducible factors

HIV – Human immunodeficiency viruses

HMBC – Heteronuclear Multiple Bond Correlation

HRMS – High resolution mass spectroscopy

HRP – Horseradish peroxidase

HSQC – Heteronuclear Single Quantum Coherence

HSV – Herpes simplex viruses

HTS – High-throughput screening

Hz – Hertz

IC_{50} – Inhibitory concentration

ICAMs – Intercellular adhesion molecules

IFN – Interferon

IL – Interleukin

IRF – Interferon regulatory transcription factor

ISG – Interferon-stimulated-gene

ISRE – Interferon Stimulated Response Element

J – Coupling constant

JAB – JAK binding protein

JAKs – Janus kinases

K_a – Association constant

K_d – Dissociation constant

LCMS – Liquid chromatography mass spectrometry

LEF – Lymphoid enhancer factor

LFA-1 – Lymphocyte function associated antigen-1

LGL – Large granular lymphocyte

LH-RH – Luteinizing hormone releasing hormone

LPS – Lipopolysaccharide

LRMS – Low resolution mass spectroscopy

MAPK – Mitogen-activated protein kinase

Max – Myc-associated factor X

MCF-7 – Michigan Cancer Foundation - 7

MCM – Mini Chromosome Maintenance

MDM – Murine double minute

ME-1 – Malic enzyme 1

MEKK– Mitogen-activated protein kinase kinase

MeOH – Methanol

MHC – Major histocompatibility complex

MIDAS – Metal ion-dependent adhesion site

MIP – Macrophage Inflammatory Proteins

MMPs – Matrix metalloproteinases

mp – Melting point

mTOR – Mammalian target of Rapamycin

Myb – Myeloblastosis oncogene

Myc – Myelocytomatosis viral oncogene homolog

NCoA1– Nuclear receptor coactivator 1

NES – Nuclear export signal

NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells

NGF – Nerve growth factor

NKC – Natural Killer Cells

NLS – Nuclear localization signal

NMR – Nuclear magnetic resonance

NOESY – Nuclear overhauser effect spectroscopy

NPCs – Nuclear pore complex

PBD – Pyrrolobenzodiazepine

PCNA – Proliferating cell nuclear antigen

PDGFR – Platelet derived growth factor receptor

PDT – Photodynamic therapy

PIAS – Protein inhibitor of activated STAT

PKA – Protein kinase A

PNAs – Peptide nucleic acids

PPI – Protein-protein interaction

PTP – Protein tyrosine phosphatases

pVHL – Von Hippel–Lindau tumor suppressor

Ran – Ras-related nuclear protein

RANTES – Regulated upon Activation, Normal T-cell Expressed, and Secreted

Ras– Rat sarcoma

R_f – Retention Factor

RISC – RNA-Inducing Silencing Complex

RMSD – Root mean square deviation

RT – Room temperature

Runx – Runt-related transcription factor

SAR – Structure activity relationship

SCCHN – Squamous cell carcinoma of the head and neck

SH2 – Src homology 2

SHP – SH2-domain-containing PTP

siRNA – Small-interfering Ribonucleic acid

SMLCK – Smooth Muscle Myosin Light Chain Kinase

SMMHC – Smooth muscle myosin heavy-chain

SMRT – Silencing mediator for retinoid or thyroid-hormone receptors

SOCS – Suppressor of cytokine signaling

SPR – Surface plasmon resonance

SSI – STAT-induced STAT inhibitor

STAT – Signal transducers and activators of transcription

SUMO – Small Ubiquitin-like Modifier

TAD – Transactivation domain

TBAI – Tetrabutylammonium iodide

TCF – T-cell factor

Tc-PTP – T cell protein tyrosine phosphatase

TCR – T-cell receptor

TEA – Triethylamine

TF – Transcription factors

THF – Tetrahydrofuran

TK – Tyrosine kinase

TLC – Thin layer chromatography

TOF – Time-of-flight

TPPII – Tripeptidyl-peptidase II

Ub – Ubiquitin

Ubn – Ubinuclein

VEGF – Vascular endothelial growth factor

VEGFR – Vascular endothelial growth factor receptor

YFP – Yellow fluorescent protein

ABSTRACT

The STAT3 transcription factor plays a key role in a wide range of biological responses for cell survival and growth. Furthermore, the STAT3 signalling pathway has been found to be up-regulated in more than 70% of human tumours. To date STAT3 is a particularly promising molecular target for chemotherapeutic intervention, and a number of strategies are under investigation to selectively down-regulate STAT3 signalling in cancer cells to inhibit cell proliferation and promote cell death. In the molecularly-targeted drug discovery era, protein-protein interactions (PPIs) are emerging as an attractive class of novel targets. Proteins are associated with unique recognition patterns, thus targeting PPIs has the potential to develop highly selective drugs. In this context, blockade of STAT3 signalling through the modulation or inhibition of key protein-protein interactions is a valuable approach to inhibit STAT3 transcriptional activity.

This research project has focused on the design and synthesis of small-molecule inhibitors of the STAT3:STAT3 protein interaction as a potential means to discover novel therapeutic agents using computational approaches such as virtual screening and structure-based design. In particular, a medicinal chemistry approach has been used to produce a novel library of potential PPI inhibitors based on a "hit" from an *in silico* screen. This library was screened using a primary PPI binding assay based on fluorescence polarisation (FP). Hits from this assay were screened in a MTS cell viability assay, a Trypan blue exclusion assay and a Luciferase reporter assay in STAT3-dependent (MDA-MB-231) and STAT3-null (A4) cell lines. Compounds with interesting activity in these assays were further studied in cellular assays to assess the extent of activity and specificity towards unphosphorylated STAT3, phosphorylated STAT3, phosphorylated STAT1, unphosphorylated STAT1 and the downstream mediators (*i.e.*, Survivin, Bcl-X_L and Cyclin D1) of STAT3 signalling. One compound identified, **60**, has the ability to down-regulate IL-6 signalling at EC_{50} of 15 μM.

CONTENTS

1 Introduction

1.1 Cancer

Cancer is the abnormal, uncontrolled growth of previously normal cells. The transformation of a cell results from alterations to its DNA that accumulates over time. The change in genetic or epigenetic information causes a cell to no longer carry out its functions properly. Characteristically cancer cells are able to divide rapidly, and tumours result from accumulation of cancer cells. A tumour can be benign (grows and does not invade the surrounding tissues), malignant (spreads to nearby or distant tissues) or metastatic (cancer cells break out from the original primary tumour and migrate to either local or distant locations in the body where they will divide and form secondary tumours). Cell division is a complex and tightly regulated process carried out by a large number of proteins. The proteins produced from protooncogenes promote cell division while those from tumour suppressor genes inhibit cells from dividing. Multiple gain- and loss-of-function mutations in these genes cause cells not only to lose their ability to strictly regulate progression through the cell cycle but also to perform other normal tasks.[1] Tumour development involves a sequence of genetic modifications, each providing a different type of growth advantage, leading to the progressive change (Figure 1.1) of healthy cells into tumour cells.[2]

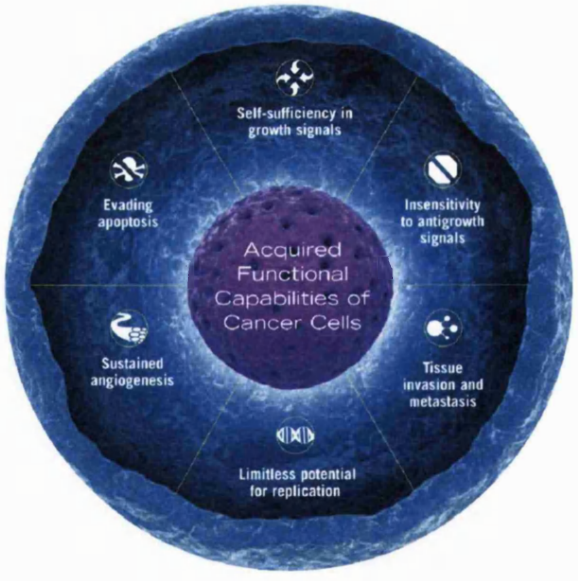


Figure 1.1: Six essential modifications to cell physiology that collectively induce tumorigenesis.[3]

1.1.1 Treatment of Cancer

The treatment of cancer is usually achieved through more than one strategy, and the choice of strategy depends largely on the nature and extent of the cancer. The main treatments at present are surgery, radiotherapy, and chemotherapy. Other approaches such as photodynamic therapy (PDT), antibody- and vaccine related approaches, and gene therapy are in the developmental stage.[2] There are a number of factors to be considered to decide the form of treatment to use,

such as the type of cancer, its stage, whether or not there is evidence of metastasis, the age and health of the patient and, in some cases, the presence of certain genetic mutations. Complete removal of the cancer without damage to the rest of the body is the goal of treatment. Surgery and radiation are most often used in the treatment of tumours that are confined to specific locations, while chemotherapy is a systemic method. A combination of one or more treatment methods is often used to eliminate as much of the cancer as possible and to reduce the chance of a future recurrence.

1.1.2 Cancer Chemotherapy

Chemotherapy involves the use of low-molecular-weight drugs to selectively destroy a tumour or at least limit its growth. The first agents to be used clinically were the nitrogen mustards, which resulted from the accidental discovery of their antileukemic properties during World War II. Later, the serendipitous discovery of cisplatin produced advancement in the treatment of testicular and ovarian carcinomas. The more recent discovery of imatinib (Gleevec™) has shown high response rates in chronic phase Philadelphia chromosome-positive CML patients.[2] Currently more than 70 different drugs are used in cancer therapy, and more than 300 drugs are in pre-clinical development or clinical trials.[4] The advantage of chemotherapy is that, after intravenous administration, the drugs distribute throughout most tissues of the body and kill tumour cells in protected areas (*e.g.*, the brain) or metastatic cancers. For older generations of anticancer drugs the disadvantages include unpleasant side effects (*e.g.*, bone marrow suppression, GI tract lesions, hair loss, nausea) and the development of clinical resistance.[2] The side effects occur because cytotoxic agents (*e.g.*, DNA-interactive drugs, tubulin inhibitors, antimetabolites) act on both tumour cells (often triggering apoptosis) and healthy cells. The various classes of chemotherapeutic agents used in current clinical are shown in Table 1.1.[2]

Among the chemotherapeutic agents, the antimetabolites, DNA-alkylating, cross-linking, intercalating and cleaving agents and topoisomerase inhibitors target DNA processing and cell division. The antitubulin agents, on the other hand, target the spindle apparatus crucial for cell division. All of these agents generally produce severe cytotoxic side effects due to a lack of selectivity for tumour cells versus normal cells. Therefore, molecularly targeted agents are being developed that target a unique biochemical pathway or protein uniquely up-regulated in cancer cells. Such agents are less toxic and can be given orally. Due to up-regulation and dependency on these pathways within tumour cells inhibition can provide an anticancer effect. The development of imatinib (Gleevec™), the first molecularly targeted anticancer drug, has strengthened the concept of "molecular targeting" as one of the fastest growing research areas in cancer chemotherapy. Further proof-of-principle has been obtained with spectacular clinical results for the agents PLX-4032[5], a BRAF kinase inhibitor in the treatment of metastatic

melanoma, and Crizotinib, an inhibitor of the tyrosine kinases ALK and MET in the treatment of non-small cell lung cancer.[6]

Table 1.1: Classification of chemotherapeutic agents

Classes	Subclasses	Examples
Anti-metabolites	DHFR Inhibitors (Antifolates)	Methotrexate
	Purine Antimetabolites	Fludarabine, cladribine
	Pyrimidine Antimetabolites	Cytarabine, 5-fluorouracil
	Thymidylate Synthase Inhibitors	Raltitrexed
	Adenosine Deaminase Inhibitors	Pentostatin
	Ribonucleotide Reductase Inhibitors	Hydroxycarbamide
DNA-Interactive Agents	Alkylating Agents	Methylating Agents (Dacarbazine), Ecteinascidin-743, Pyrrolobenzodiazepine (PBD) Monomers (Tomaymycin)
	Cross-Linking Agents	Nitrogen Mustards, Aziridines, Epoxides, Methanesulfonates, Nitrosoureas (Lomustine), Platinum Complexes (Cisplatin), Carbinolamines Cyclopropanes, Mitomycin-C, Sequence-Selective DNA Cross-Linking Agents (PBD Dimers (SJG-136), Cyclopropanepyrroloindole (CPI) Dimer (Bizelesin))
	Intercalating Agents	Anthracyclines (Doxorubicin), Anthracenes (Mitoxantrone), Phenoxazines (Dactinomycin)
	Topoisomerase Inhibitors	Topoisomerase I Inhibitors (Topotecan), Topoisomerase II Inhibitors (Etoposide)
	DNA-Cleaving Agents	Bleomycins, Enediynes
Anti-tubulin Agents	Vinca Alkaloids	Vinblastine, Vincristine, Vindesine
	Taxanes	Paclitaxel, Docetaxel
Molecularly Targeted Agents	Kinase Inhibitors	BCR-ABL Inhibitor (Imatinib), HER2/neu Inhibitor (Trastuzumab), EGFR Inhibitor (Gefitinib), VEGFR Inhibitor (Semaxanib), PDGFR Inhibitor (SU-6668), Multiple Target Inhibitors (ARRY-334543)
	Inhibitors of *Ras* Pathway Signalling	Inhibitors of Farnesyl transferase (Tipifarnib), BMS-214662, Inhibitors of MEK (AZD6244)
	Cell Cycle Inhibitors	Flavopiridol, olomoucine
	Proteasome Inhibitors	Bortezomib
	mTOR Inhibitors	Rapamycin
Hormonal Agents	Anti-Estrogens	Tamoxifen, Toremifene, Selective ER Modulators (SCH 57068)
	Aromatase Inhibitors	Anastrozole, Letrozole
	LH-RH Receptor Antagonists	Cetrorelix
	Anti-Androgens	Cyproterone Acetate, Flutamide
	Estrogenic Agents	Diethylstilbestrol, Ethinylestradiol
	Progestins	Gestonorone Caproate, Megestrol Acetate, Norethisterone

1.1.3 Protein-Protein Interactions (PPIs) as a Target for Anticancer Therapy

The target specific enzymatic (*e.g.*, protein kinase and phosphatase) activities derives predominately from Protein-Protein interactions (PPIs) rather than enzyme substrate specific binding. Likewise, PPIs also mediate the signal transduction through interaction of biological effectors (*e.g.*, hormones and cytokines) with their receptors. In the molecularly-targeted drug discovery era, Protein-Protein Interactions (PPIs) have emerged as an attractive class of novel targets. With the advancement of molecular biology and genomics a notable number of PPIs have been identified which can be potential intervention points for the treatment of cancer. Many proteins possess interactive surfaces with unique recognition patterns, thus allowing them to selectively recognize and bind to other proteins. Therefore, targeting PPIs has the potential to generate highly selective drugs for use in many therapeutic areas including cancer.[7]

1.2 Protein-Protein Interactions (PPIs)

Proteins modulate a wide variety of functions in cellular system. They have an irrefutable role in a number of functions including cell-signalling, catalysis of reactions, transportation, formation of the building blocks of viral capsids, establishment of regulated channels, transmitting information from DNA to the RNA. Proteins are also crucial for the immune response and the prevention of viral entry into cells. Due to their involvement in cellular processes, researchers have focused on understanding their functions based on their sequences and 3-dimensional structures. Identification of the binding partners of a particular protein can lead to an understanding of its function. From this it is possible to assign its functional and signalling pathway. Therefore, the identification of protein-protein interactions (PPI) is at the core of functional genomics.[8]

Proteins interact through their interfaces, and protein-protein interactions (PPIs) can result in complexes of two or more proteins. Some protein-protein interactions are obligatory and some are transient, continuously forming and dissociating.[9-15] The protein binding results from non-covalent hydrophobic[16-18] and electrostatic[19-22] (van der Waals, H-bond, salt bridge) interactions and is opposed by the transitional and rotational freedom[23] in the binding residues of interacting proteins. Therefore, proteins interact with each other to acquire favourable thermodynamic conditions which are usually achieved through joining their hydrophobic surfaces while in a polar protic environment. Shape complementarity of protein interfaces also help strong binding through excluding water effectively. The affinity of two protein molecules to associate depends on the change of entropic (ΔS) and enthalpic (ΔH) energy of the corresponding system. A negative ΔH favours association and a negative ΔS disfavours association. The Gibbs free energy upon complex formation (*i.e* binding free energy) can be calculated from the association (K_a) and dissociation (K_d) constants of the thermodynamic equilibrium reaction. The K_d can vary

between micromolar and picomolar in PPIs, and free energy changes (ΔG_a) can vary between –6 to –19 kcal mol^{-1}.[24]

Protein interfaces typically comprise of the amino acid residues from two different chains and the residues which are spatially close to each other. Therefore, a well-defined fragment of amino acid chains and a few isolated functional groups are responsible for the interactions. Figure 1.2 illustrates some examples of protein-protein interfaces.

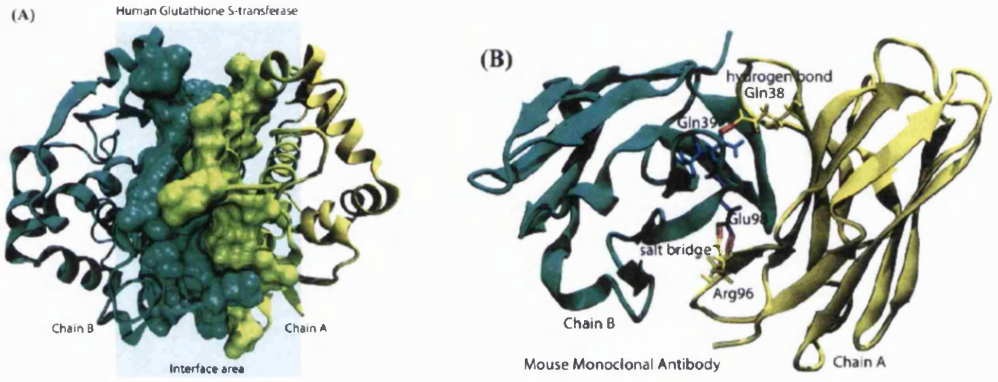


Figure 1.2: (A) Two interacting proteins (human glutathione S transferase, PDB ID: 10gs, chains A and B); (B) The interface of mouse monoclonal antibody D1:3 (PDB ID: 1Kir, Chains A and B) showing H-bond and salt bridge between two chains.[8]

To investigate a particular PPI it is essential to know not only the responsible residues and atoms groups on the interacting interface but also the residues in their vicinity to understand the chemical effects on the supporting matrix.[25-30] By calculating the solvent accessible surface area of the protein surface it is possible to identify the residue and atoms that line-up on the surface.[25,27,31,32] To understand this pattern, the intermolecular interactions involved at the protein-protein interface have to be thoroughly considered. These properties include the surface area that is buried by the interacting molecules, the non-polar fraction, the hydrogen bonds across the interface, the salt bridges, buried water molecules, the composition of the interface, residue conservation, the strength of the interaction, residues that contribute significantly to the free energy of binding, the shape of the binding interface, and the types of secondary structures.[14,28,33-37]

For the analysis of protein-protein binding interfaces, it is possible to obtain useful information from available crystal structures. However, the conformation of a protein in such a complex might not be the same as in solution. Also, depending on its binding state, *i.e.*, whether it is already bound to another protein (or ligand), or different conformational states may exist.[38-41] For example, the protein importin has different conformations in different complexes and this conformational variability is essential for this protein to function as a nucleo-cytoplasmic transporter. The bound/unbound conformational states are coupled with the importin functions of cargo binding and release by RanGTP binding. The importin conformations in the three

available crystal structures differ significantly in their binding sites with an overall root mean square deviation (RMSD) around 3.5 Å (Figure 1.3), where as in solution much larger conformational variations are observed.[42]

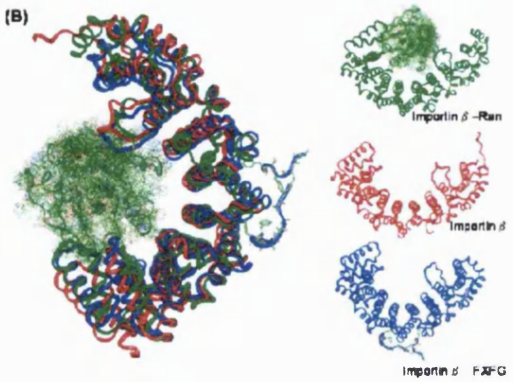


Figure 1.3: The superimposition (left panel) of three crystal structures of importin in the free state (red ribbon, left panel, PDB ID: 1gcj), bound to RanGTP (green ribbon, left panel, PDB ID: 1ibr; RanGTP is represented by ribbon and surface dots), and bound to nucleoporin (blue ribbon, left panel, PDB ID: 1f59).[8]

Some comparisons of proteins between their bound, complexed and free (apo) states are shown in Figure 1.4, where K-binding protein and Glutathione S-transferase-I show the conformational changes between the free form and when bound to the ligand. The ligand introduces a conformational change in the loop.

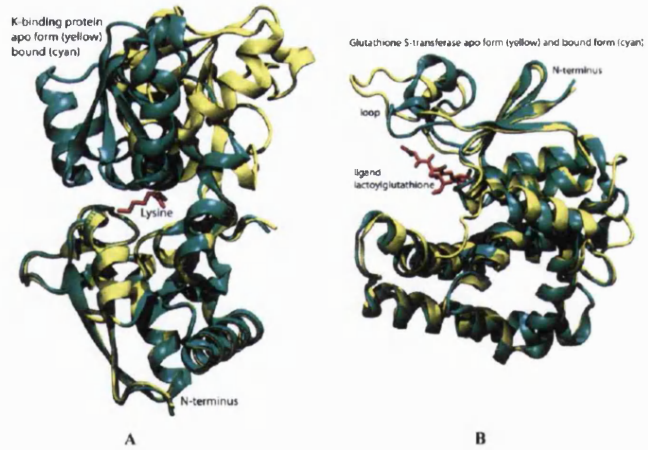


Figure 1.4: (A) K-binding protein in free and bound forms (PDB IDs: 2lao (yellow) and 1lst (cyan)), respectively; (B) Glutathione S-transferase-I in free and bound forms (PDB IDs: 1aw9 (shown in cyan) and 1axd (yellow), respectively). The ligand, shown in red, belongs to the cyan structure.[8]

1.2.1 "Hot Spots"

Most of the binding energy of protein interfaces is usually localised to a small region.[43] Some residues at the protein interface contribute dominantly to the binding free energy of the protein-protein complex compared to the rest of the residues. Those critical side chain residues assemble cooperatively to locales near the centre of a protein-protein interaction. These locales are known as binding hot spots.[44,45] The concept of critical contacts on protein interfaces can be illustrated by the example of proliferating cell nuclear antigen (PCNA). Forming stable trimeric complexes is essential for the function of PCNA in assembling the cellular replication machinery on DNA strands[46] and to act as a DNA replication accessory factor.[47] Mutation of Tyr^{114}, central at the subunit interface, to Ala, causes complete loss of ability of PCNA to trimerise[47] (Figure 1.5).

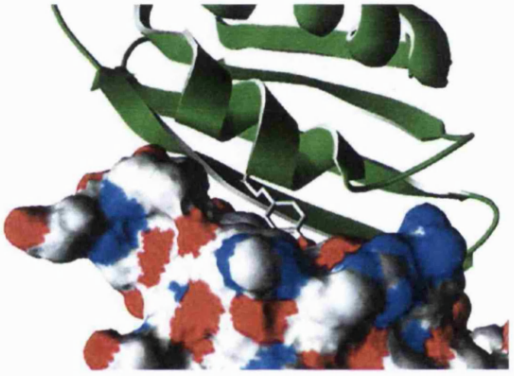


Figure 1.5: Interface between two PCNA subunits; one as a molecular surface (CPK) and the other as a ribbon model (green). The side chain of Tyr^{114}, whose mutation to Ala abolishes PCNA monomer association, is showed in CPK colouring.[48]

The existence of a hot spot was unveiled by Wells and colleagues through carrying out alanine scanning,[49] where residues at the interface were systematically replaced by alanine, and the difference in the binding free energy ($\Delta\Delta G$) between the wild type and each mutant was measured. A residue is considered as a hot spot when substitution by alanine leads to a significant ($\Delta\Delta G \geq 2$ kcal mol^{-1}) drop in the binding free energy.[44] Usually hot spots have an ASA of around 600 Å^2 or less.[50] They are rich in both aromatic (His, Tyr, Phe, and Trp) and aliphatic residues (Leu, Ile, Val, and Met), but lack charged residues (Glu, Asp, and Lys), with the exception of the highly represented Arg.[51] Hot spots are structurally conserved in a hydrophobic environment[52] and located within densely packed areas[25] to stabilise the complex. Hot spot residues couple across the interface to a great extent, but hydrogen bonds and electrostatic interactions dominate over charge-charge coupling. However, charged and polar residues may interact through water exclusion mechanisms. Hot spots usually appear in clusters, where they are packed tightly in contact with each other and form a network of interactions (Figure 1.6), known as hot regions.[25,53] Furthermore, hot spots take part

cooperatively to stabilise individual hot regions, and hot regions participate additively to stabilise a particular protein-protein association.

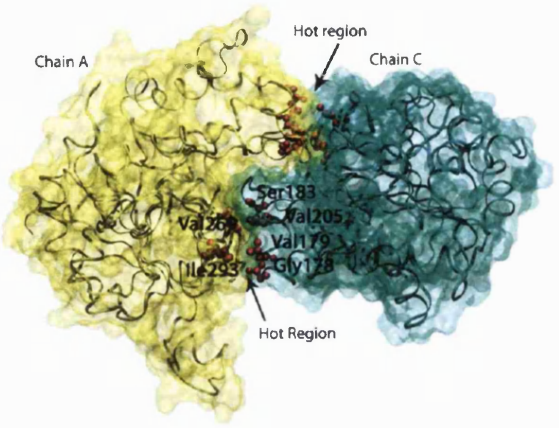


Figure 1.6: Crystal structure of a complex displaying the hot regions between two M chains (yellow and cyan) of the human muscle L-lactate dehydrogenase (PDB ID: 1i10). The figure illustrates that hot spot residues (red) are in contact with each other and form a network of interactions constituting two hot regions at the interface of the homodimer.[8]

Due to the tendency to find tightly packing hot spots, most are located in complemented pockets (pockets that disappear upon protein binding) rather than in unfilled pockets (pockets remain unfilled by protein partner).[27] As the key residues have preferred states to organise, these pockets are usually pre-organized in the unbound state[54] before protein complex formation (Figure 1.7).

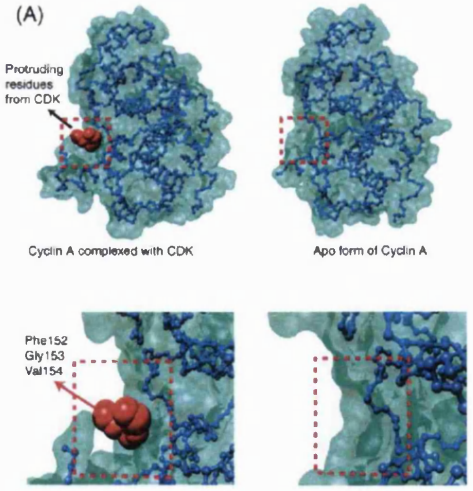


Figure 1.7: (A) The Cyclin A protein in free form (right, PDB ID: 1vin) and bound form (left, PDB ID: 1fin) with Cyclin dependent kinase (CDK). (B) The red residues (part of CDK) protrude into the pocket (left) and the same pocket exists in the free form (right).[8]

1.2.2 Allosteric Site

From X-ray crystal and macromolecular NMR structures it appears that proteins are not rigid,[55] rather, they exist as conformational ensembles. For many proteins a dynamic equilibrium exists between different conformations, and so binding sites unique to individual or subgroups of receptor conformational states also exist.[48] There is usually only one conformational state in which a protein is bioactive, and trapping any of the inactive conformations is useful to understand protein interaction. Gibbs energy of stabilisation is not equally distributed in the protein structure, and hence theoretical and experimental considerations suggest that binding at one site can affect the conformational and dynamic changes at other sites. Allostery correlates the conformational and dynamic changes between two nearby or widely separated protein binding sites. Structural disruption at any site leads to a redistribution of the protein conformational populations. Structural disruption can be caused by the binding of inhibitors (or effectors), mutations, binding to sister molecules, binding to nucleic acids or to small molecules, changes in pH, ionic strength, temperature, and covalent modifications such as phosphorylation and acetylation.[8] The disturbance at one site does not yield a homogeneous distribution; therefore allosteric activation does not generate an alternative rigid binding site, but instead produces a less stable complex.

The p53 protein presents an example of protein allostery. The last 30 residues of the C-terminal domain are responsible for negative regulation of DNA binding by an allosteric mechanism.[56] The interaction of p53 with a short oligonucleotide containing a consensus p53-binding site is greatly enhanced either by the deletion of the C-terminal basic region (30 residues) or by binding of the antibody PAb421 (which activates p53 transcriptional activity) to the same region.[57] Another example includes tumour suppressors that serve as hub proteins, such as pVHL or suppressors of the cytokine signalling (SOCS) family.[58] Binding of pVHL to the elongin B-elongin C complex leads to a conformational change that allows it to bind to HIF. In contrast, without pVHL binding to elongin C/elongin B, the pVHL has not been observed to bind to HIF.[59]

1.2.3 Induced Proximity

Sometimes a pharmacological receptor may only exert an effect partly or not at all related to the response mediated by the natural agonist through activation of the receptor. In this case, the first binding event involves a different recognition site from the second binding event. Here the second binding site is a composite of the ligand-monomer complex from the first binding.[60]

For example, the receptors for effectors of the cytokine type are different from ligand-triggered receptors, *e.g.*, insulin receptors. Cytokine receptors are monomeric and do not have inherent activity. The activating ligand promotes homo- or heterodimerisation of receptor monomers in

the fluid cell membrane, leading to dimerisation and productive juxtaposition of cytoplasmic tails.[61]

The example of EPO receptor agonism can clarify the concept which is described in Figure 1.8 of EPO receptor dimerisation. In preformed EPOR dimers in the absence of ligand, the juxtaposition of the EPOR monomers is very different from that in the active EPO-liganded complex[62] (Figure 1.8b). Two non-identical interfaces between EPO and the two extracellular ligand-binding domain EPOR monomers orient the membrane proximal part of the complex (position of C-terminal) to bring the loops into contact for receptor dimerisation[63,64] (Figure 1.8a). Structural analysis of an EPOR agonist peptide selected from a phage display peptide library revealed that the peptide itself dimerises and forms a nearly symmetrical 2:2 complex with EPOR monomers. The relative orientation of the membrane proximal parts of the monomers is significantly different to that in the EPO complex[65] (Figure 1.8c).

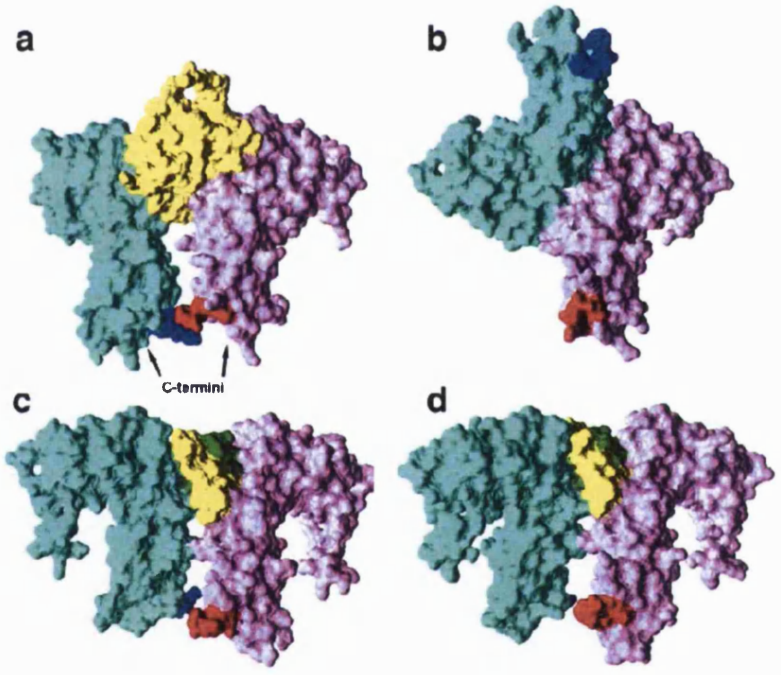


Figure 1.8: (a) Interfaces of EPO (yellow) and ligand binding domain EPOR monomers (red and blue) with the loops (darker shade of red and blue) (PDB # 1EER); (b) EPOR dimers in the absence of ligand (PDB # 1ERN); (c) Self dimerisation of the peptide (yellow and green) (PDB # 1EBP); (d) Inactive EPOR dimer (PDB # 1EBA).[48]

1.2.4 Favourable Organizations

Protein-protein interfaces have favoured architectures.[8] The molecular architecture of protein binding sites is known as secondary structural organisations.[33] Though the interfaces are not homogenous considering their size, shape, chemical composition and amino acid sequence, a small set of groups with similar architectures can be identified.[66] Different structural interfaces may share similar scaffolds but belong to functionally different protein families.[67] In Figure 1.9(A), the interfaces and global protein architectures are similar, but in figure 1.9(B) the three-dimensional structures of the monomers are different, although their interfaces have similar architectures.

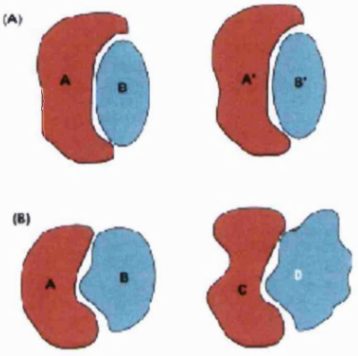


Figure 1.9: (A) Both the interfaces and the global architectures are similar; Protein A is homologous to Protein A` and B is homologous to B`; (B) The three-dimensional structures of the monomers are different, Proteins A and C are non-homologous, as are proteins B and D.[8]

For example, two complexes, cytochrome C and the neuropeptide/membrane protein, are not related evolutionarily, yet their interface architectures are similar. Global features of the architectural motifs that are present in monomers reoccur at the interfaces in spite of the absence of chain connections. However, the details of the architectural motifs may change. The number of secondary structural organizations is limited due to the restriction upon secondary structure formation.[68] So, secondary structure motifs can play a key role in protein association through limiting the conformational space.

1.2.5 Shape Similarity & Binding Site

Due to the characteristic feature of favourable organizations, different protein partners can occupy common binding sites.[10] Some proteins are centrally connected, where as some are on the edge. Some proteins may have numerous connections. Some binding sites are distinct, where as some can bind different molecules with different affinities. An example of multiple proteins binding at the same site on the protein interface is the dimerization cofactor of hepatocyte nuclear factor (DCoH). DCoH serves as an enzyme and a transcription co-activator (Figure 1.10).

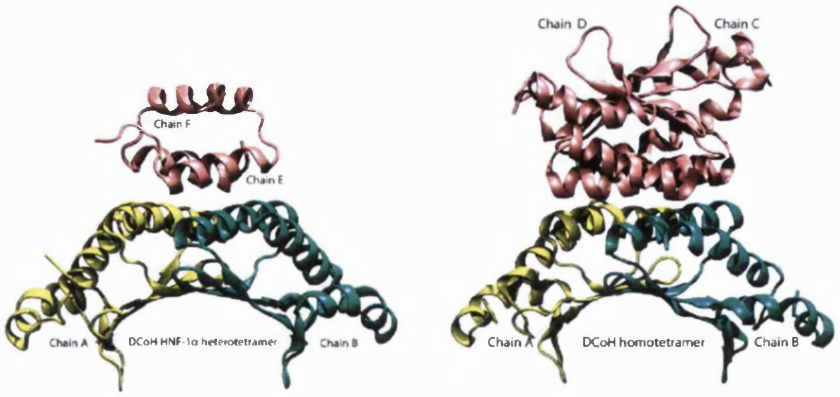


Figure 1.10: (left) The crystal structure of the hepatocyte nuclear factor dimerization domain, HNF-1α, bound to a DCoH dimer (PDB ID: 1F93, Chains A, B of DCoH, and Chains E, F of HNF-1R); (right) To act as a coactivator, DCoH binds to HNF-1α. And forming dimers of dimers (PDB ID: 1DCH).[69]

Similar interactions are responsible when multiple protein partners interact with the same binding site. Therefore, the patterns of the local interactions may be similar in multipartners and single partners. Since, hot spots are the conserved residues of protein interface, in multipartners these residues pre-organize for optimum stability.[8] Figure 1.11 shows the conserved interactions of a binding site interacting with multiple partners.

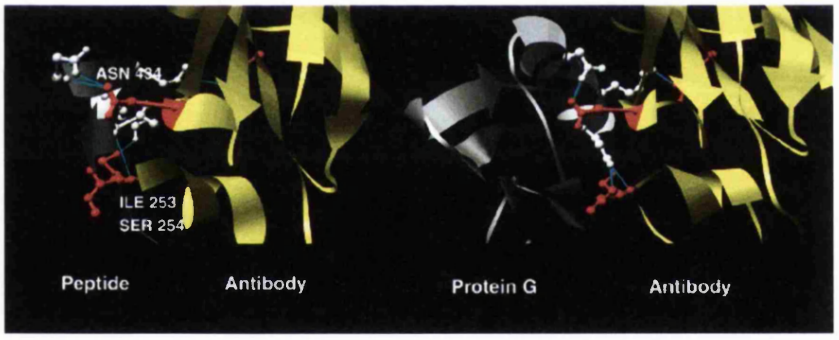


Figure 1.11: The yellow protein is the antibody interacting with a peptide and protein G (PDB IDs: 1dn2 and 1fcc). The residues shown in red belong to the antibody.[8]

1.3 PPIs as Molecular Targets for Drug Discovery

To date, most molecular targets for drugs have been enzymes and cell surface receptors.[48] For these molecular targets the ligand binding sites can be blocked by small molecules with favourable physiological absorption, distribution, metabolism and excretion (ADME) properties, unlike macromolecules which can have unattractive properties from this perspective. It is also relatively easy to devise a biochemical assay for the natural ligands, and to design inhibitors based on the structure of the natural ligands through molecular modelling approaches guided by NMR and crystallographic data from the receptor. Many GPCR and enzyme inhibitors have been developed as drugs. However, great academic and pharma interest is currently focusing on the discovery of inhibitors of PPIs to develop more effective and selective therapeutic agent. Whereas most PPIs are unique, recruitment of enzymes into signalling pathways often involves scaffolds, anchoring and adaptor proteins, each containing specialized modules that bind different domains or motifs. The specificity in the protein-binding is affected either through structural modification or specific temporal recruitment through post-translational modifications.

The use of therapeutic antibodies is an increasingly popular approach to target PPIs. These types of molecules have excellent properties, are highly specific for their targets and are highly stable in human serum. Nevertheless, antibodies are difficult to manufacture and cannot cross cell readily. They are also very expensive, suffer from poor bioavailability and thus can only be administered parenterally. In this context, drug-like small molecules directed against PPIs have progressed significantly over the past few years. Although finding potent and lead/drug-like small molecule PPI antagonists is a challenging task, targeting hot spots and utilising the concept of allosteric inhibition have proved to be productive strategies.

It is important to distinguish between lead compounds and existing drugs. Many existing drugs contain structural and physicochemical features that do not have lead-like characteristics. This is because such molecules were chosen and developed classically, *i.e.*, based purely on pharmacological data. Modern drug design is based predominantly on biochemical assays. Furthermore, it is feasible to eliminate potentially non-selective, poorly absorbed, and toxic compounds at an early stage of development. Thus, the identification of high quality PPI leads for medicinal chemistry optimisation remains challenging.

1.4 PPIs Modulators

1.4.1 PPI Modulation through Targeting "Hot Spots"

"Hot spots" can be defined as a small subset of amino acids at protein interfaces where most of the PPI binding energy is localised.[7] Therefore, hot-spots are the "active sites" of protein-protein interactions. A ligand competitively bound to a hot-spot thus prevents interaction of the original protein partner of the PPI and thus modulates function. (Figure 1.12)

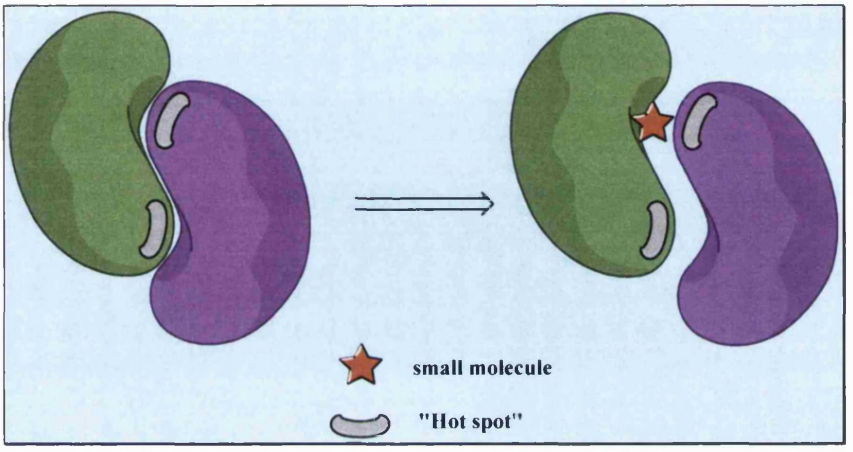
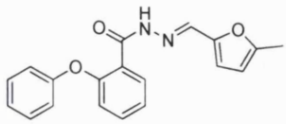


Figure 1.12: Inhibition of protein interactions through the hot-spot approach.[7]

Due to a limited number of protein interfaces that have been analyzed for hot-spot identification, it has been estimated that only 9.5% of the interfacial residues in PPIs are within hot-spots.[44] In the past five years, extensive efforts have been made to develop computational strategies, that can be used to screen protein interfaces to identify hot-spots.[70-74]

1.4.1.1 β-catenin:TCF4 Interaction Inhibitor

The interaction between the β-catenin transcription factor and lymphoid enhancer factor/T-cell factor (LEF/TCF) proteins is a recognized potential therapeutic target due to their presence in constitutively active signalling pathways of several cancer types.[75,76] The interface of β-catenin with TCF3 or TCF4 is large and shallow with a binding constant of K_d = 10 nM.[77,78] Trosset and co-workers have targeted hot-spots on the protein surface and successfully found an inhibitor of this PPI.[79] At the beginning, they identify six cavities as potential hot-spots from the crystal structure of the β-catenin:TCF3 complex (having similar structural data to the TCF4 complex) using computational studies. Then virtual screening was been carried out with a library of 17,700 drug-like compounds against the most promising hot-spot. Later, combined with biophysical assays (*e.g.*, NMR and isothermal titration calorimetry [ITC]), the furan containing molecule **1** (PNU-74654; Figure 1.13) was identified by this approach as a potent inhibitor (K_d = 450 nM) of the β-catenin:TCF4 PPI interaction.



1 (PNU 74654)

Figure 1.13: A β-catenin:TCF4 inhibitor, PNU 74654 (**1**), identified by targeting a hot-spot combined with *in silico* and biophysical studies.[7]

1.4.2 PPI Modulators from the Allosteric Approach

Allosteric sites may be defined as a small subset of amino acids localized distant from the protein-protein interface where the binding of a ligand can induce conformational change and result in disruption of the packing or overall secondary structure of a protein, thus affecting the integrity of the main PP interface and its ability to interface with other proteins.[7] The ligand bound to the allosteric site does not compete with the binding protein partner, but can block protein interaction through changing the main PP interface (Figure 1.14). Allosteric regulation allows effective control of PPI modulation as well as better specificity. In comparison to the hot-spot approach, the allosteric approach is less challenging due to the availability of greater numbers of amenable allosteric binding sites (*e.g.*, "grooves"). High-throughput screening (HTS), x-ray crystallography, phase display combined with crystallography and tethering are all techniques that can be applied to optimize allosteric site identification.[70]

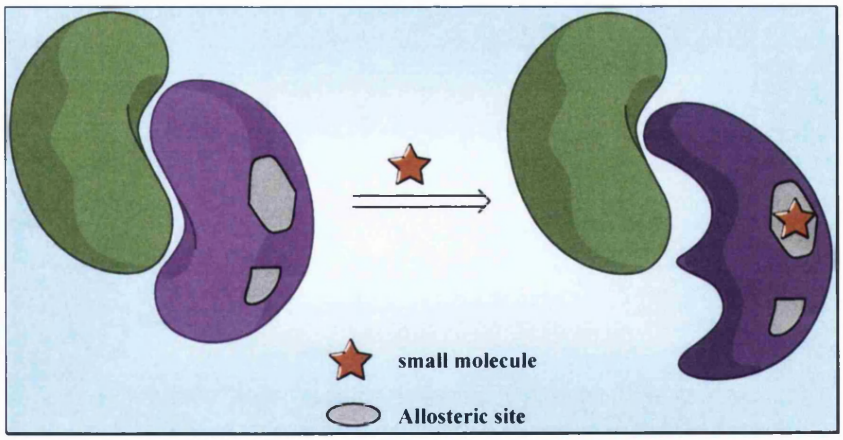
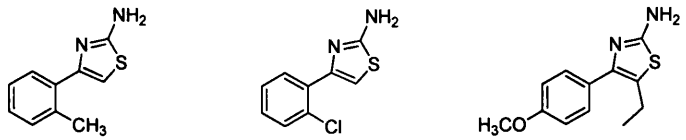


Figure 1.14: Inhibition of protein interaction through allosteric regulation.[7]

1.4.2.1 Runx1:CBFβ Inhibitors

The transcription factor, corebinding factor (CBF) is an essential regulator of normal hematopoiesis, and the gene encoding this protein is the focus of chromosomal translocations found in a large percentage of human leukaemias (*e.g.*, acute myeloid leukaemia [AML]).[80] The interaction of the CBFβ smooth muscle myosin heavy-chain (SMMHC) subunit and Runx1 results in formation of a heterodimeric complex, which is essential for CBF function and leukemogenesis.[81]

Bushweller and co-workers have studied the binding interface of CBFβ with Runx1 by NMR (chemical shift perturbation) based on the NMR 3D-structure of CBFβ and alanine-mutagenesis studies.[82] The heterodimerisation interface was then used for a virtual screen against small molecule libraries to identify PPI inhibitors.[83] The 35 potential ligands identified through the virtual screening of 70,000 commercially-available drug-like compounds were then validated by physical screening using NMR (*i.e.*, chemical shift changes in the 2D ^{15}N-^{1}H or ^{13}C-^{1}H HSQC spectra of proteins in the presence of the compounds), followed by FRET-based and ELISA screening. NMR chemical shift data proved that the ligands bound to an allosteric site rather than a hot spot. Further investigations identified an allosteric site and three effective small-molecule inhibitors of the Runx1:CBFβ interaction (**2**, **3** & **4**, Figure 1.15). These molecules were shown to inhibit the proliferation of ME-1 cells (a leukemic cell line expressing CBFβ-SMMHC) with IC_{50} values in the low micromolar region.



2 3 4

Figure 1.15: The most active Runx1:CBFβ inhibitors identified through an allosteric approach.[7]

1.5 Strategies to Identify Small-Molecule PPI Modulators

The feasibility of using small organic molecules to target protein–protein interactions has been verified through the activity of a number of natural products. For example, the taxane agents such as paclitaxel (Taxol),[84] a diterpenoid isolated from the bark of the Pacific yew tree (Taxus brevifolia), and its semi-synthetic derivative docetaxel (Taxotère),[85] are approved for the treatment of a number of human cancers. Their mode of action involves a PPI with the structural protein, tubulin. Further microtubule-stabilizing natural products[86,87] include laulimalide,[88-91] the epothilones A and B,[92-94] eleutherobin,[92] and discodermolide.[95,96] An analogue of epothilone B is currently in clinical trials.[97,98] The protein-binding ability of certain natural products was exploited by Schreiber and Crabtree in their pioneering design of CID (chemical inducer of dimerization) systems. FK1012[96] (a dimeric form of the naturally occurring small molecule FK506), a fusion molecule comprised of FK506 and cyclosporin A,[99] rapamycin and dimeric cyclosporin A[100] were shown to induce dimerization of genetically engineered receptors that lacked their extracellular dimerization domains, thus inducing signal transduction and specific target-gene activation.[101,102]

For the discovery of small molecule inhibitors of protein-protein interactions, three major challenges have to be considered: first, structural features of protein-protein interacting surfaces; second, physical binding as well as chemical properties and protein complex organisation state; and third, similarity of binding sites (*e.g.*, domain homology). However, there are PPI features

that are amenable for the design of small molecules as PPI inhibitors (*i.e.*, "hot spot" and "allostery"). As described in detail in paragraph **1.2.1**, targeting hot spots with small molecules is a potential approach to disrupt the interaction rather than extensive coverage of the protein interface with relatively large molecules. Examples of such inhibitors have been discussed previously in the context of β-catenin:TCF4 interaction inhibitor. An alternative approach is the development of allosteric inhibitors of protein-protein interactions where the molecules bind at a site offset from the protein-protein interface and do not compete with binding partners. Examples of such inhibitors have been successfully developed to target include Runx1 and CBFβ,[83] betalactamase, LFA-1,[103] and nitric oxide synthase,[104] and one of these have been previously discussed in Section **1.4.2**.

A number of small organic modulators of large protein–protein interactions have been identified to date. The strategies employed for this identification can be divided into three classes. The first involves the identification of peptides, derived from the interface between proteins, that are able to inhibit the interaction between the proteins by binding to one of the protein interfaces in a competitive fashion. These peptides are subsequently optimized by the incorporation of non-natural amino acids or other chemical modifications. The second approach focuses on the *in vitro* or cell-based screening of chemical libraries for modulators of protein-protein interactions. The third approach is based on computational approaches such as the virtual screening of chemical databases for molecules that are likely to bind to a hot spot at the protein–protein interface, structure-based design and NMR.

1.5.1 Peptide- and Peptidomimetic-Based Approaches

Peptides that are constructed on the basis of the amino acid sequences found at PP interfaces are potential leads for the development of PPI modulators. Though they are not promising candidate for drug development considering their mass, conformational flexibility, proteolytic lability, polarity and cell permeability, a number of small synthetic drug-like molecules mimicking natural peptidic substrate have been developed through innovative medicinal chemistry design. Replacement of amide bonds with bioisosteric subunits and incorporation of conformationally-constrained monocyclic and bicyclic unnatural amino acids are two common design strategies. This approach has led to the identification of non-peptide scaffolds, that have been employed for the discovery of PPI inhibitors of MDM2:p53[105], Bak BH3:Bcl2/Bcl-X_L[106,107] and smMLCK:calmodulin.[105]

1.5.1.1 HIV-1 Protease Inhibitors

HIV-1 protease is an important target for anti-AIDS drugs. Some of the most promising inhibitors are based on peptidomimetics.[108] HIV-1 protease inhibitors inhibit budding and virion maturation resulting in non-infectious viruses. HIV-1 protease is a homodimeric enzyme, and its

catalytic activity is retained only in its dimeric form. Therefore, inhibition of dimerization is an effective mechanism to block enzyme activity. Structural data relating to the dimeric form of the enzyme lead to the identification of two amino acid sequences at the interface containing 75% of the localized free energy of binding. These two amino acid sequences are located in the N- and C-terminal monomer regions, respectively, and form a four-stranded anti-parallel β-sheet upon dimerization. A number of peptides and peptidic derivatives of progressively smaller size have been identified[109-114] which possess low nanomolar inhibitory activity against HIV-1 protease.

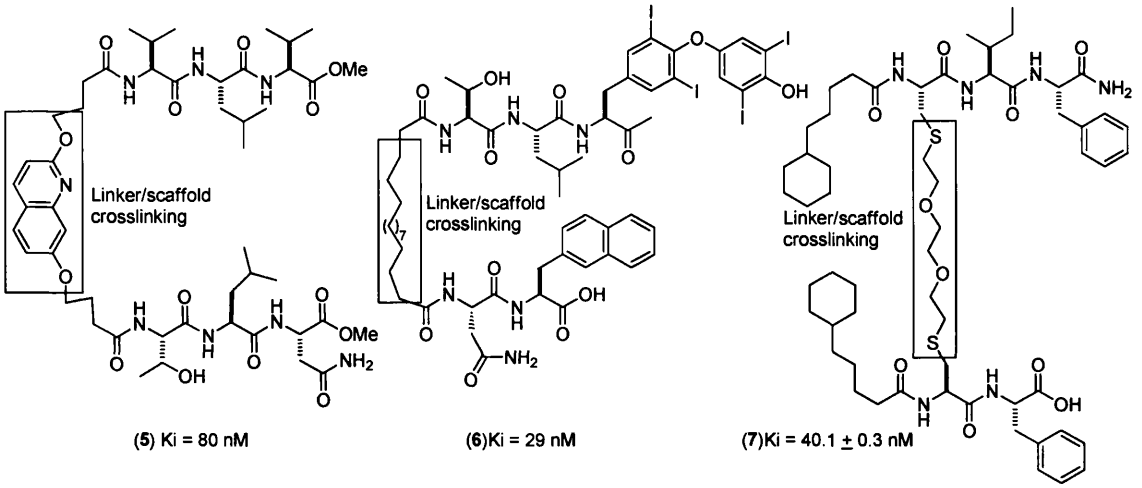


Figure 1.16: Novel HIV-1 protease inhibitors: an example of the potential of peptide-based analogues (Linkers highlighted with boxes).[109-114]

The core structure of these molecules was designed by joining two non-contiguous peptides (crosslinked or side chain-linked), derived from the dimerization interfaces, with suitable linkers/scaffolds. The three derivatives, **5**, **6** & **7**, shown in Figure 1.16 are the most potent HIV-1 protease inhibitors described to date.

1.5.1.2 Peptide Inhibitors of the p53:MDM2 Interaction

D-α-peptides (L-α-peptide enantiomers with reversed chirality), L-α-retro-peptides (with the opposite order of residues) and "retro-inverso" or *D*-α-retro-peptides (with both chirality and sequence reversal) have been identified with improved cell permeability and enhanced proteolytic and metabolic stability. Bioisosteric modifications of these peptide classes have led to diversified conformational preferences with distinct molecular topologies.[115] This strategy to prepare a small library of retro- and retro-inverso peptides has been used to investigate the effect of backbone modifications on the interaction of a p53 α-helical segment with MDM2.[116] The retro-inverso peptide of the natural L-α-peptide (*i.e.*, residues 15–29 of p53) (**8**, Figure 1.17) had an equivalent binding affinity and comparable potency in the PPI primary binding ELISA assay. Such molecules also provide a feasible starting point for *in silico* studies and structure-based design.

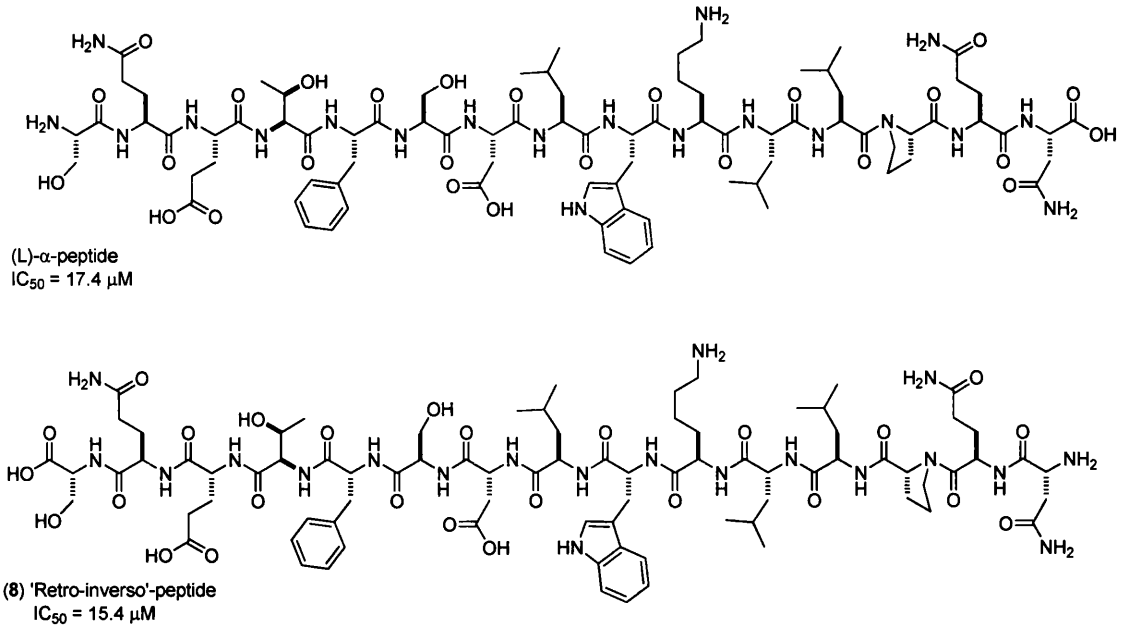


Figure 1.17: Lead peptide inhibitors of the p53:MDM2 interaction: an example (**8**) of the use of "retroinverso" peptides as valuable scaffolds for the design of small molecule PPI modulators.[116]

1.5.2 HTS Approaches

The high-throughput screening of natural and synthetic compounds is an efficient approach for the discovery of selective "hit" or lead PPI modulators.[104,117-119] When insufficient structural information is available for *in* silico studies or biophysical assays it is the only possible approach. To obtain a potential lead through HTS, two key features must be considered. *First*, to maximize the chances of identifying a suitable protein-binding ligand (with high binding affinity and selectivity), compound libraries must have a high degree of molecular diversity (*i.e.*, structural features and 3-D geometries) and complexity rather than size of the molecules.[120,121] For this reason, either new synthetic methodologies to prepare structurally and conformationally diverse libraries[122-129] need to be developed, or natural product-based libraries should be considered due to the richness in structural diversity of molecules produced by plants, bacteria and fungi.

Second, to identify ligands that target a PPI of interest, a primary PPI binding assay can be employed. In a competitive binding assay one (or both) of the protein partners, or the peptide surrogate for one of the protein partners, is provided with a fluorescence tag. In the FRET (Fluorescence Resonance Energy Transfer) assay, the proximity of two suitably labelled protein partners can be exploited based on the quenching phenomenon. In FP (Fluorescence Polarisation) based assays, the binding affinity of a PPI inhibitor is measured *via* a fluorescent probe which can be displaced by the ligand.[130] Generally, fluorimetry-based techniques are very sensitive and advantageous as the protein system is in solution, freed from physical/chemical constraints.[131-133] FP assays are the most feasible for HTS compared to other more-complex assays (*e.g.*, ELISA) due to their simplicity (*e.g.*, fewer washing steps required) and lower cost

(*e.g.*, no antibodies required). Phenotypic cellular assays in HTS are used to select for ligands that are cell-permeable and produce the desired effect in living cells with sufficient potency.[134] For PPI targets associated with transcription factors (TFs), the cellular screening approach can be employed utilising cell lines transfected with plasmids that contain the relevant TF binding site in the promoter region of a gene providing a read-out such as a luciferase insert.[135,136] With suitable controls (*e.g.*, plasmids without the relevant TF recognition sequence), it is possible to screen for molecules that are cell permeable and selectively inhibit the TF-promoted read-out.[137,138] Biophysical assays based on NMR spectroscopy[139,140] and surface plasmon resonance (SPR)[141,142] have also been recently developed for HTS purposes.

1.5.2.1 Discovery c-Myc/Max Dimerization Inhibitors through HTS of Synthetic Compound Libraries

Myc-family genes are over-expressed in more than 70% of human cancers, and are associated with aggressive, poorly differentiated and metastatic types of solid tumours.[143-145] De-regulated expression of c-Myc can initiate cell growth and vasculogenesis, reduce cell adhesion and promote metastasis and genomic instability. The c-Myc and Max proteins belong to the basic helix-loop-helix leucine zipper (bHLH-LZ) family. The crystal structure of the related c-Myc:Max heterodimeric complexes reveals that dimerization occurs *via* α-helical domains containing leucine zipper motifs.[146] c-Myc first dimerises with Max in order to exert its biological effect, and so inhibition of this interaction regulates the c-Myc activity.

The amino acids responsible for alteration of the specificity of dimerisation have been identified within the α-helical LZ-motif through mutagenesis studies.[147] However, the structural features of this domain were not sufficiently available for *in silico* studies to identify potential binding sites for inhibitory ligands on a completely rational basis. A HTS approach using a primary PPI binding assay allowed the identification of potential "hits" with drug-like properties. Screening of commercially available compound libraries employing fluorescence based assays (FRET and FP), a yeast two-hybrid-based system for c-Myc:MAX dimerisation and DNA-binding inhibition, and ELISA assays for PPI inhibitory activity validation, identified a number of "hit" compounds with IC_{50} values in the 0.5-60 μm range (three best "hits" shown in Figure 1.18).[138,148-154] To date, *in silico* approaches to the design of modulators of this c-Myc:MAX interaction have proved challenging due to the complex molecular dynamics of the interaction. For example, while the c-Myc bHLHZip domain is predominantly α-helical in its dimeric form, the monomeric form is disordered. 10058-F4 (**10**) disrupts the c-Myc:Max heterodimerisation process by specifically binding to c-Myc and stabilising the intrinsically disordered (ID) monomeric domain, thus inducing a global destabilizing conformational disordering that affects the protein-protein interaction.

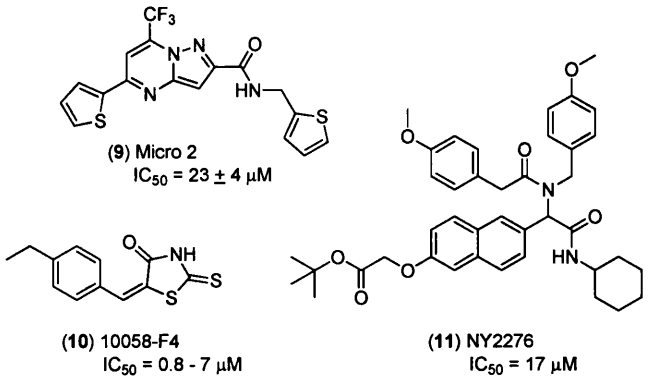


Figure 1.18: The three best inhibitors of the Myc:MAX interaction described to date in the literature.[7]

1.5.2.2 Discovery of a HIF-1 Pathway Inhibitor through HTS of Natural Product-based Compounds

Twenty four novel natural products have been developed into approved drugs globally between 1981 and 2006.[155,156] As natural products are the richest source of novel and diverse molecular frameworks, they are the ideal starting points for the preparation of compound libraries for a HTS approach. Synthetic modification of natural product chemotypes can provide libraries with a high degree of molecular diversity.[122,157-159] Rolitetracycline (**12**, Figure 1.19) is a PPI inhibitor of the HIF-1 pathway from natural product sources.

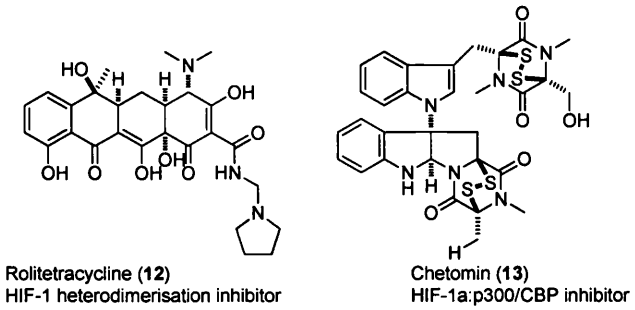


Figure 1.19: Structures of the potent and selective natural product PPI inhibitors of the HIF-1 signalling pathway, rolitetracycline and chetomin.[7]

HIF-1 is a key regulator of angiogenic and glucose metabolic processes utilised by tumour cells for both survival and growth. The presence of hypoxia in solid tumours triggers HIF-1 activity.[154,160-162] HIF-1, a heterodimeric protein complex, is a member of the bHLH-PAS family and is comprised of HIF-1α and HIF-1β (or ARNT) subunits.[163,164] The active heterodimer of HIF-1 is formed through interaction of the PAS-A and PAS-B domains of the two subunits. Following heterodimerisation, HIF-1 binds to the hypoxic response element (HRE), and activates the expression of hypoxia response genes. Inhibition of the PAS domain interactions of the HIF-1α and β subunits should suppress HIF-1 activity, and could be an attractive strategy for small-molecule intervention.[165] To date, the natural product rolitetracycline is the only known selective inhibitor of this interaction. It was identified through an ELISA screen[166] but was not active in cell-based assays, presumably due to a lack of cell permeability.

The complex of HIF-1α with the co-activator p300/CBP binding protein (cAMP-response element or CREB), also enhances HIF-1 transcriptional activity. The natural product chetomin (**13**, Figure 1.19) was identified to selectively inhibit this PPI through binding to the CH1 domain p300. The identification of chetomin was achieved by HTS of more than 600,000 compounds (natural products and commercially available small molecule libraries) using a time-resolved fluorescence assay followed by an *in vitro* "interaction screen" and cell-based luciferase assay.[167]

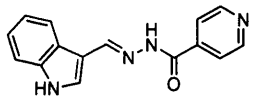
1.5.3 Computational Approaches

The importance of computational approaches at various stages of the drug discovery process has been confirmed through the discovery of PPI modulators *via in silico* studies along with medicinal chemistry approaches.[48,104,105,117,118,168-172] Virtual screening (*e.g.*, using compound databases such as Zinc™) based on X-ray or NMR structures can be used not only to reduce the size of the physical compound libraries required for "hit" identification, but also to identify a suitable ligand binding site (*i.e.*, hot-spot and allosteric sites).[70,170,173] To date, computational approach are less efficient and successful than HTS and peptidomimetic approaches due to the limited quality and quantity of structural information available for PPI targets. Molecular dynamics simulations are essential for understanding the dynamic equilibria of protein complexes whereas *in silico* studies are generally based only X-ray crystallographic data which represent PP complexes in the static state. However, for large protein complexes these simulations are limited by computational capacity and the predictive power of the software. Better understanding of surface mobility and functionality as well as accurate computational analysis methodologies can be used to improve the potency and selectivity of PPI modulators.

1.5.3.1 Discovery of Inhibitors of CD4/MHC Class II Interactions

$CD4^+$ T cells participate in the pathogenesis of a number of clinical states including various autoimmune diseases,[174-176] allogenic organ transplant rejection,[177] and graft-versus-host disease following bone-marrow transplantation.[178] The interaction between CD4 and the major histocompatibility complex class II (MHC class II) on the surface of antigen presenting cells is crucial for the activation of $CD4^+$ T cells.[179,180] However, small-molecule inhibitors of the CD4/MHC class II interaction could act as immunosuppressive agents. CD4 is a glycoprotein expressed on the surface of helper T cells consisting of four immunoglobulin(Ig)-like extracellular domains termed D1–D4.[181,182] The MHC class II complex expressed on the surface of antigen-presenting cells is a heterodimeric glycoprotein. Antigenic peptides taken up from the local environment become associated with the MHC class II dimer prior to their display on the cell surface, and the MHC class II–peptide complex is recognized by the T-cell receptor (TCR). This interaction is stabilized by a second association between the MHC class II dimer itself and CD4.[183,184]

To identify potential small-molecule binding pockets within the CD4 protein, surface ligand-binding site-searching algorithms,[185,186] solvent-accessible-surface-area calculations,[187] and analyses of the electrostatic properties of CD4 surface structures were performed on the D1 domain of CD4.[188] Through these approaches, binding pocket in the D1 domain of CD4 was identified.[189,190] A cyclic heptapeptide mimicking a surface loop, which is part of the proposed binding pocket, was shown to inhibit stable CD4/MHC class II interactions and CD4-mediated immune responses *in vitro* and *in vivo*.[191,192] A X-ray structural analysis of the human CD4 D1 domain was then used as a receptor for molecular docking.[193] A virtual library consisting of approximately 150,000 commercially available compounds was analysed using the criteria of shape complementarity and force-field energy through a molecular-docking program.[186] The 1000 best-scoring compounds were visually examined in terms of distinctive chemical structures, receptor binding modes, and electrostatic and shape complementarity. Among the 41 compounds chosen for testing in a CD4/MHC class II cell-adhesion assay, eight showed inhibitory activity (31–74% inhibition at 100 μM). The compound TJU103 (**14,** Figure 1.20, $IC_{50} \approx 90$ μM) was found to be non-toxic to lymphocytes *in vivo* and appeared to specifically inhibit $CD4^+$ T-cell-mediated responses.[194,195]



(**14**) TJU103

Figure 1.20: Structure of TJU103, an inhibitor of CD4/MHC class II interactions.

1.5.4 Innovative Approaches

1.5.4.1 Miniature Proteins

A new class of PPI modulators generated by a two-step grafting process whereby a selected cut-down protein fragment (well-folded, but not active) of a defined secondary structure of a protein (*e.g.*, an α-helix or β-strand) acts as a scaffold upon which a PPI epitope can be installed and then varied chemically (*i.e.*, amino acid modification) for library generation.[7] Miniature proteins provide novel protein-interacting sites to act as scaffolds to prepare libraries for ligand identification (Figure 1.21). Miniature proteins have an advantage in PPI modulation is that they preserve part of the protein interface but with significantly lower molecular weight, and have a higher proteolytic stability compared to synthetic peptides.

For example, Schepartz and co-workers have utilised the small, folded avian pancreatic polypeptide (aPP) as a scaffold to apply this approach to protein targets including Bcl-2,[196,197] p53:MDM2,[198] CBP,[199,200] ActA,[201,202] the Src family kinases[203] and cAMP dependent protein kinase (PKA).[204] Vita and co-workers have used charybdotoxin (37 amino acid residues) as a scaffold[205] comprising of an anti-parallel triple-stranded β-sheet on one face and a short α-helix

on the opposite face, stabilised by three disulfide bonds in the core. This was employed to prepare miniature protein CD4-mimics to target the conserved surfaces of the HIV-1 envelope.[206,207]

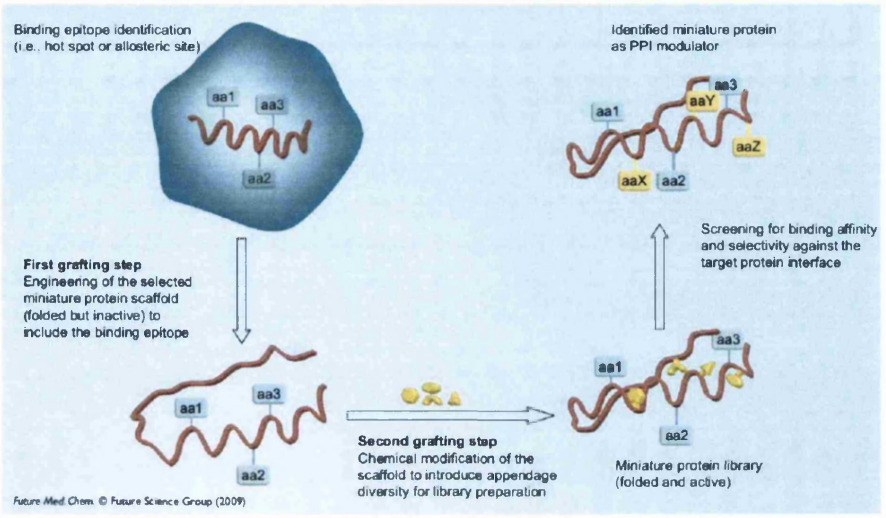


Figure 1.21: The miniature protein approach.[208,209]

1.5.4.2 Hydrocarbon-Stapled Peptides

The α-helix protein scaffold is a very common secondary structure responsible for intracellular PPIs. In solution, synthetic peptides remain free and tend to form an α-helical conformation resulting in increased binding affinity to a protein partner. Stabilisation of the helical form also enhances cell permeability and resistance to protease cleavage through a reduction in exposure of the polar amide backbone to the solvent environment. Therefore, a number of covalent helix-stabilizing methods have been developed based on cross-linking (*e.g.*, using disulfide and lactam bridges).

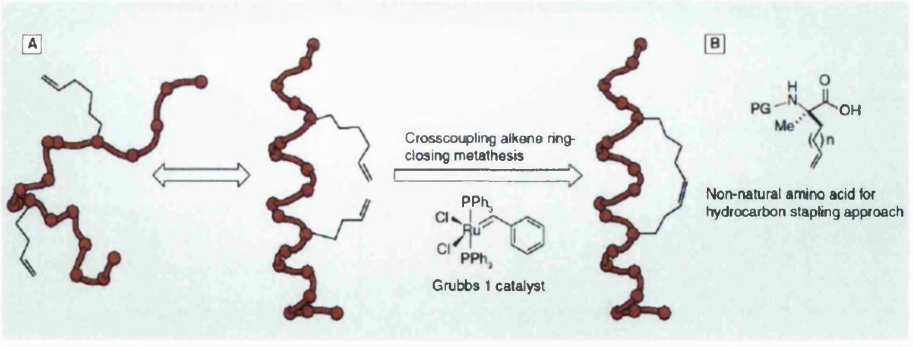


Figure 1.22: Hydrocarbon-stapling of α-helices.[7]

Verdine and co-workers have introduced a novel technique known as "peptide stapling",[210] where a ring-closing metathesis is used to identify hydrocarbon cross-linkers of the correct configuration (*i.e.*, position of attachment, stereochemistry and length) to stabilise the helix (Figure 1.22A).[211] Then the terminal alkenes attached to the α-carbon of the selected amino

acids units are cross-coupled ("bridged") *via* ruthenium-catalysed ring-closing alkene metathesis, followed by reduction of the double bond (Figure 1.22A and B). This approach has been applied to the two PPIs, p53:HDM2[212] and Bcl-2:Bax/Bad.[213,214]

1.5.4.3 PPI Stabilisation

The stabilisation of PPIs could be an alternative approach to modulating signalling pathways, and a number of strategies have been formulated for this purpose.[173] If modulation of a signalling pathway can be achieved by stabilisation of PPIs, this could have advantages over inhibition of a PPI. This is due to the fact that stabilisation (*i.e.*, entropy and enthalpy) is more thermodynamically favourable than inhibitor. The principles and methodologies developed for structure-based drug design are applicable to the theory of stabilisation. PPIs are known to exist in normal physiological processes and as part of the mode of action of some drug molecules. For example, stabilisation of a PPI that triggers the proteolytic degradation of a transcription factor could down-regulate the associated signalling pathway in tumours cells where transcription factors are often over-expressed.

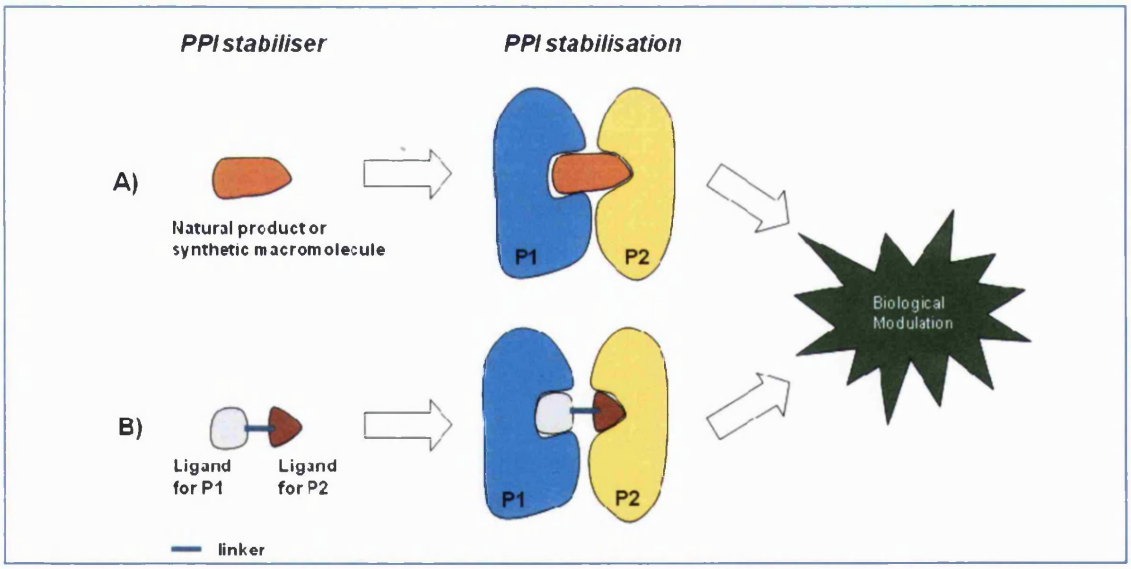


Figure 1.23: PPI stabilisation by a direct mechanism: (A) Bifunctional compounds that recognize and bind to pockets on the interfaces of both protein partners, and (B) Two moieties joined *via* a suitable flexible linker and each moiety recognises the epitope of its respective interacting protein.[7]

Identification of small molecules that fit into gaps on the protein-protein interfaces can result in the binding of both macromolecules together more tightly. Allosteric stabilisation could also be valuable where a small molecule binds to one of the protein partners at a site distant from the PP-interface and enhances stabilisation by altering conformation of the protein at its interface. For the direct stabilisation approach, two classes of molecules are possible considered. First, bifunctional compounds having domains that can recognize and bind to pockets on the interfaces of both protein partners, thus helping them to "lock" together (Figure 1.23A). The

immunosuppressive macrolide rapamycin , is an example of the first type of stabiliser. It binds to both the receptor protein FKBP12 and to the FKBP12-Rapamycin Binding (FRB) domain of mTOR (mammalian target of Rapamycin), thus fastening the two proteins together.[215] The second type of stabiliser consists of two moieties (*i.e.*, two small molecules) joined *via* a flexible linker, where by each moiety recognizes the epitope of its respective interacting protein (Figure 1.23B).

1.6 Concluding Remarks

Protein–protein interactions are clearly more challenging as drug targets than receptors and enzymes that bind small molecules. While a large number of protein–protein interactions are emerging as potential intervention points for developing therapeutic agents for cancer therapy, many of these do not appear to be suitable at the molecular level. Developing small molecules that modulate protein–protein interactions is difficult due mainly to the lack of well-defined binding pockets. Nevertheless, there has been important progress in recent years. For example, for the p53–MDM2 interaction it has been demonstrated that development of a potent and pharmacologically active small molecule can be achieved.

Most PPI modulators known to date have been identified through screening chemical libraries or from structure-based rational design. With the help of combinatorial synthetic methods, libraries containing thousands of compounds can be prepared and screened within a short period of time.[216,217] By applying techniques such as mass spectrometry, NMR spectroscopy and nanotechnology in high-throughput screens it is possible to successfully identify small molecules that disrupt protein–protein interactions.[218,219] Once "hit" molecules have been identified, it is then possible to obtain one or more molecules with higher potency and binding affinity, and with improved bioavailability through structure–activity relationship (SAR) studies followed by further synthesis. Computer-aided rational design is also a promising approach in the search for synthetic agents that target protein–protein interfaces. In addition to issues relating to surface mobility and functionality, accurate computational analysis can help to design molecules with improved potency and selectivity.

1.7 The Signal Transducers and Activators of Transcriptions (STATs)

1.7.1 STATs – Structure & Isoforms

First discovered in the early 1990s,[220] signal transducers and activators of transcription (STAT) proteins are latent cytoplasmic proteins that transduce extracellular signals from the cell-surface to the nucleus and modulate transcription through binding to promoter region of the genome. The STAT family consists of seven mammalian proteins, containing STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6, which are activated upon binding of various extracellular signalling polypeptides (>35)[221] with specific cell surface receptors. They are activated by Tyr phosphorylation which is a post-translational modification critical for dimerisation, nuclear importation, DNA binding and thus transcriptional activation. Therefore, the activated STAT proteins accumulate in the nucleus to initiate transcription. The duration and degree of gene activation initiated by STAT signalling are strictly regulated by a series of negatively acting proteins and pathways.

1.7.1.1 Structures of STAT Proteins

X-ray crystal structures of several partial STATs (*e.g.*, N-domain of STAT4,[222] phosphorylated dimers of STAT1[223] and STAT3β[224]) were first reported in 1998. STAT proteins consist of 700–850 amino acids and share several functional domains (Figure 1.24a).

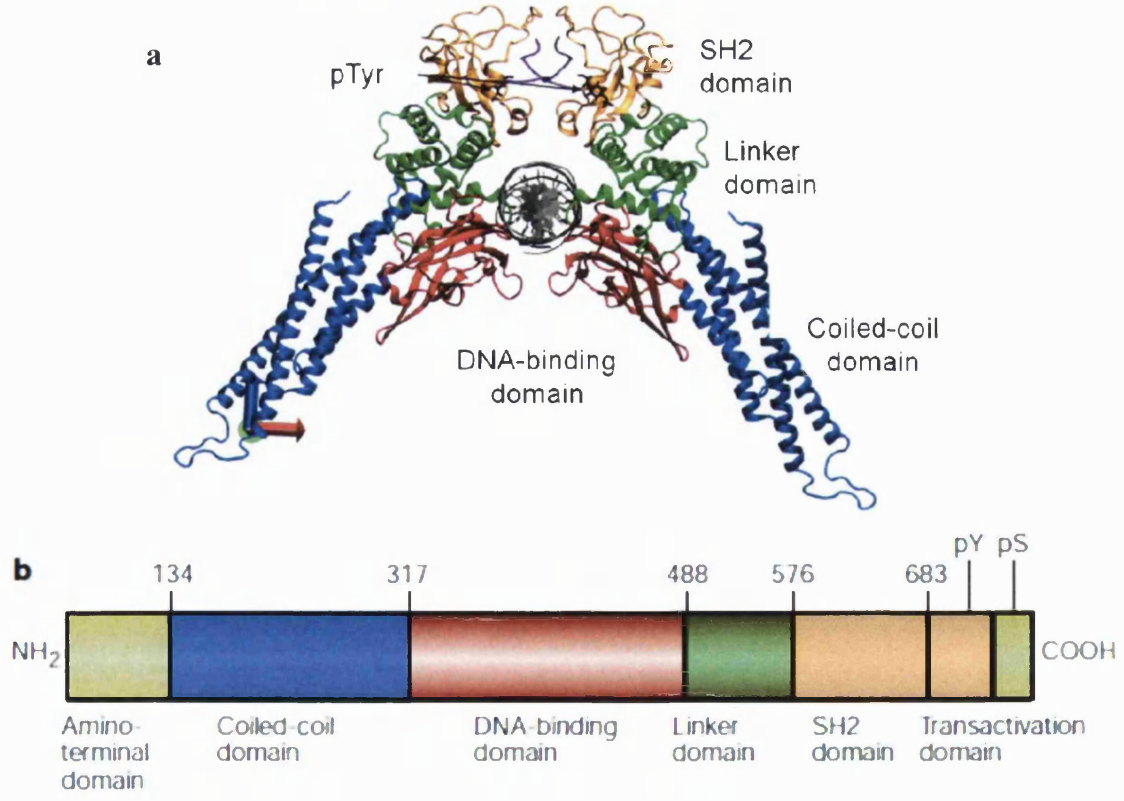


Figure 1.24: a) Ribbon diagram of the mouse STAT3βTC dimer bound to DNA (centre) based on a crystal structure from the protein data bank (PDB;1BG1) and created using Chimera™ software. b) Different domains of STATs.[225]

STAT proteins consist of an N-domain/STAT protein interaction domain, coiled-coil domain/STAT all-alpha domain, DNA-binding domain, linker domain, SH2 domain and transactivation domain. The four domains that comprise the coiled-coil, DNA binding, linker and SH2 domains have extensive and intimate interdomain interactions to form a contiguous hydrophobic core. The dimer interface exhibits two kinds of contacts, of which 75% exhibits SH2 interaction with its cognate phosphopeptide region, and with the other 25% due to SH2–SH2 dimer contacts. STATs contain a conserved Tyr residue at the C-terminus that undergoes phosphorylation upon activation, and forms intermolecular interaction with the SH2 domain of the dimer partner. Most STATs, except for STAT2 and STAT6, contain a second conserved phospho-amino acid residue at the C-terminus, a phospho-Ser, at the transactivation domain (Figure 1.24b).

1.7.1.2 Functions of Domains of STAT

From extensive studies on the functions of various domains of STATs (Table 1.2), it has been revealed that the N-domain is involved in dimerization and tetramerization[222] and protein–protein interaction, the coiled-coil domain of STAT3 is essential for receptor binding[226] and interacts with other proteins including IRF-9/p48 (STAT1)[227], c-Jun,[228] StIP1,[229]and GRIM-19[230] (STAT3) and SMRT (STAT5A & STAT5B)[231]. The DNA binding domain binds to DNA as a dimer and is involved in nuclear translocation through maintaining an appropriate conformation for importin binding.[232] The linker domain is involved in transcriptional activation[233] and protein–protein interaction.[230] The most conserved SH2 domain is crucial for receptor association and phospho-dimer formation (*e.g.*, homodimers and heterodimers between STAT1 and STAT3, and STAT5A and STAT5B).[234] The least conserved transcriptional activation domain is also involved in protein–protein interactions with the CREB-binding protein (CBP)/p300,[235-239] MCM5,[240] BRCA1,[241] and NCoA-1.[242]

Table 1.2: Function of the STAT domains.[220]

Functional Domains	N	CC	DNA	LK	SH2	Y	TA
Receptor binding	-	+	-	-	+	-	-
Dimerisation	+	-	-	-	+	+	+
Tetramerisation	+	-	-	-	-	-	-
Nuclear import	+	+	+	-	-	-	-
Nuclear export	-	+	+	+	+	-	+
DNA binding	-	-	+	+	-	-	-
Transcriptional activity	-	-	-	+	-	-	+
Protein-protein interaction	+	+	+	+	+	-	+

Several domains are involved in nuclear export and import. For STAT1, the N-domain,[243] K410/K413[244,245] or L407[246] in the DNA binding domain, for STAT2, R409/K415 in the DNA

binding domain[244], for STAT3, R214/R215[247] in the coiled-coil domain and R414/R417[247] in the DNA binding domain and for STAT5B, the N-domain and coiled-coil domain are involved in cytokine-induced import.[248] The coiled-coil domain,[249] the DNA binding domain,[250] the linker and SH2 domains,[248] or the C-terminus tail.[251] are involved in CRM1/exportin1-dependent nuclear export.

1.7.1.3 Isoforms of STAT

The full length STATs have been termed the α-isoforms, whereas shorter isoforms, due to either alternative mRNA splicing or post-translational proteolytic processing, are named as β, γ or δ isoforms. β isoforms with truncated C-terminal transactivation domains have been generated through alternative splicing at the 3'-end gene transcripts of STAT1, 3, 4, 5A and 5B. STAT1β isoforms lack the C-terminal 38 amino acids of STAT1α,[252] STAT3β isoforms lacks the 55 C-terminal amino acids of STAT3α while gaining a unique 7 amino acids,[253] and the STAT4β isoforms are 44 amino acids shorter than STAT4α at the C-terminus.[254] STAT5Aβ and STAT5Bβ isoforms with C-terminal truncations of 77 and 80 kDa proteins, respectively, are generated due to incomplete transcript splicing.[255] The β isoforms do not retain the phospho-Ser residue, but contain the critical phospho-Tyr residue and, like the α-isoforms, the β-isoforms upon Tyr phosphorylation form homo- or heterodimers with the α-isoforms thus binding DNA. Truncated isoforms of STATs formed through proteolytic processing at the C-terminus are found mainly in cells involved in hematopoiesis and in leukemia.[220] They are short of the transactivation domain and assumed to act as dominant-negative factors.

1.7.1.4 Unphosphorylated STATs

Crystal structures of unphosphorylated STAT1 and STAT5A were published in 2005[256,257] and show two dimer interfaces: one between two N-domains, and the other between two core fragments, consisting of a coiled-coil domain for the Tyr-phosphorylated tail segment. It has also been revealed that the unphosphorylated STAT1α in its dimeric[256] form between core fragment pairs exists in a boat-like arrangement. Based on the position (the opposite or same end of the dimer) of the SH2 domain, two possible unphosphorylated dimers, "antiparallel" and "parallel" can be observed (Figure 1.25).

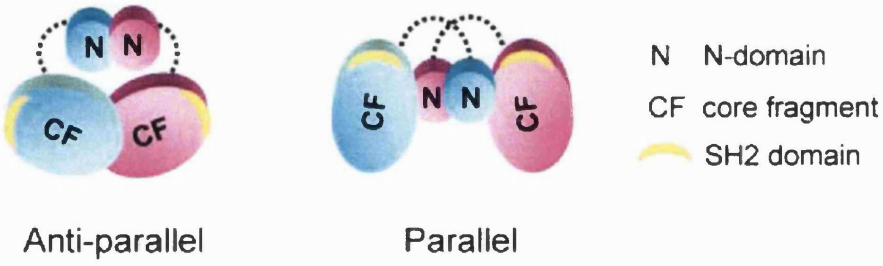


Figure 1.25: Schematics of antiparallel and parallel unphosphorylated STAT dimer.[220]

Both of the unphosphorylated forms are relatively weak dimers compared to the phosphorylated dimers. As the SH2 domains and C-terminal tails lie in close proximity in a "parallel" conformation, this can facilitates an easier transition to the parallel phospho-dimer after Tyr phosphorylation. Therefore, "parallel" conformation is the most likely structure of STAT when bound to its receptor, and the "antiparallel" conformation is the most likely the predominant structure in the latent state prior to cytokine stimulation. Unphosphorylated dimer docks onto the receptor for Tyr phosphorylation and several intermolecular interactions of reciprocal pTyr and SH2 domain between the two dimers take place to transform the previous into the stable DNA-bound phosphorylated STAT dimers.

1.7.2 STAT Activation and Inactivation

STATs are activated by over 40 different polypeptide-binding cytokine receptors, G protein-coupled receptors, receptor tyrosine kinases, epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), and by non-receptor tyrosine kinases (*e.g.*, Src and Abl) or protein adaptors such as MEKK1-C. Several modes of activation have been described for STATs (Figure 1.26).

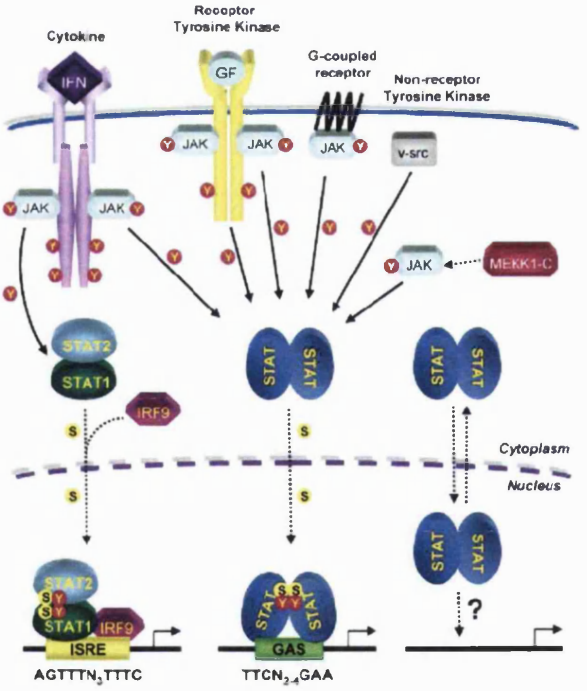


Figure 1.26: A schematic diagram showing the different modes of STAT activation and inactivation.[220]

Cytokine signalling through the JAK-STAT pathway is the classical signalling route. Binding of cytokine to its receptor results in dimerization/oligomerization of the receptor, and this subsequently activates the receptor-associated tyrosine kinases known as Janus kinases (JAKs). JAKs trans-phosphorylate the intracellular domain of the receptor, and the resulting phospho-Tyr residues[258] act as docking sites for latent cytoplasmic STATs *via* their SH2 domain. JAKs phosphorylate STATs on a specific Tyr residue on their cytoplasmic tail. Stable homo- or

hetero-dimers of STATs are formed *via* reciprocal binding of this critical pTyr of one monomer and the SH2 domain of the partner protein. These STAT dimers are released from the receptor, move to the nucleus *via* association with importins, then binding to specific DNA-binding elements (ISRE and GAS response elements) of cytokine responsive genes and activate transcription.

Receptors with intrinsic tyrosine kinase activities, including the EGF receptor, PDGF receptor, and FGF receptor, can activate STATs directly, or indirectly by JAKs.[259] Non-receptor tyrosine kinases, including viral oncoproteins such as v-src, v-Fps, v-Sis, polyoma virus middle T antigen, and v-abl can also cause STAT activation.[260] G-protein-coupled receptors including chemokines receptors such as receptors for MIP-1 and RANTES, activate STAT1 and STAT3 in T-cells upon chemokine binding.[261] Proteins serving as adaptors (*e.g.*, MEKK1-C and islet-1) activate STATs by bringing JAKs within close proximity to STATs to activate them.[262,263] Various extracellular cytokines, growth factors, hormones and intracellular signalling molecules can also activate cytoplasmic STAT homo- or heterodimers which then translocate to the nucleus and activate ISRE and GAS-containing target genes. It is also reported that unphosphorylated STATs participate in gene transcription independent of phosphorylation, but the mechanism is yet to be revealed.[264,265]

Inactivation of this signalling pathway is regulated by negative regulators of STATs which include cytokine-inducible SH2-containing protein (CIS)/suppressor of cytokine signaling (SOCS)/JAK binding protein (JAB)/STAT-induced STAT inhibitor (SSI), protein inhibitor of activated STAT (PIAS) and phosphatases, or by protein degradation *via* the ubiquitin–proteosome pathway.[266] Two phosphatases, SH2-domain-containing PTP-2 (SHP-2) and a nuclear T-cell protein tyrosine phosphatase (Tc-PTP), named TC45, have been reported to dephosphorylate and inactivate STATs.[220]

1.7.3 Regulation of STAT Proteins

Post-translational modifications of STAT proteins are regulated by Tyr phosphorylation, Ser phosphorylation, dephosphorylation by phosphatases, Arg methylation, acetylation/deacetylation, isomerization, ubiquitination, SUMOylation, ISGylation and proteolysis to modulate activities of the STATs (Table 1.3).

Table 1.3: Post-translational modifications of STATs.

Post-translational modifications of STATs	Biological function
Tyrosine phosphorylation	Activation of STATs
Serine phosphorylation	Enhances activation of STATs
Acetylation	Activation of STAT6
ISGylation	Positive feedback loop of STAT1
Arginine methylation	Increased DNA binding of STAT1
Glycosylation	Activation of STAT5
Ubiquitylation	Degradation of STATs
Sumoylation	No clear function

1.7.3.1 Phosphorylation and Dephosphorylation

Tyr phosphorylation is crucial for STAT activation.[267] STATs are phosphorylated by the prominent JAK family kinases (JAK1, JAK2, JAK3 and Tyk2),[268] Src family Tyr kinases[260] and receptor Tyr kinases such as EGF receptor.[269] Serine phosphorylation enhances transcriptional activity of STAT1, STAT3 and STAT4,[270] but has no effect on nuclear translocation of STAT1/STAT3 and STAT5a/STAT5b.[266] Different kinases have been reported to participate in serine phosphorylation of the STATs (*e.g.*, CAMK2, MEKK1).[271]

1.7.3.2 Arg Methylation and Lys Acetylation

Methylation leads to a weaker interaction of STAT1 with Protein Inhibitor of Activated STATs (PIAS1), resulting in increased DNA binding of STAT1. Methylation of arginine 31 in the N-terminal domain is independent of tyrosine or serine phosphorylation and does not require cytokine stimulation.[272] Acetylation of STAT6 by histone acetyltransferase CREB-binding protein (CBP)/p300 is required for transcriptional activation of the 15-lipoxygenase-1 (15-LOX-1) by IL-4.[273]

1.7.3.3 Glycosylation

Glycosylation is observed on STATs, which might be essential for interaction with co-activators following cytokine stimulation.[274] The formation of O-linked N-acetylglucosamine (O- GlcNAc) on threonine 92 is essential for the transcriptional activity of STAT5. O-linked glycosylation has also been observed on nuclear STAT5 following cytokine stimulation, but glycosylation is not essential for nuclear translocation of STAT5.

1.7.3.4 Ubiquitylation

Ubiquitination is a process through which target proteins are degraded *via* the proteosome pathway. The ubiquitin-proteosome process involves three enzymes; ubiquitin-activating enzyme E1 (to activate ubiquitin), ubiquitin-conjugating enzyme E2, and ubiquitin ligase E3

(that catalyzes the transfer of ubiquitin to the ε-amino group of lysine residues in the substrate protein).

1.7.3.5 Nuclear Import and Export of STAT Proteins

STAT proteins accumulate in the nucleus upon Tyr phosphorylation and dimerization, and translocate back into the cytoplasm after dephosphorylation. Both processes involve active transport through the nuclear pore complex (NPC) due to the large size (> 50 kD) of the STAT dimer. Expression of a nuclear localization signal (NLS) is essential for this active transport to occur. Members of the importin α (impα) family bind to the NLS signal of target proteins. Importin β then binds to impα and carries impα and its cargo protein, *via* binding to nucleoporins in the NPC, through the NPC and into the nucleus where it associates with Ran-GTP, which leads to the release of impα and its load. Exportin CAS is important for the export of impα, while general exportin CRM1 (chromosome region maintenance 1) is associated with nuclear export signal (NES) consisting of a short amino acid sequence rich in leucine which binds the NES-carrying cargo together with Ran-GTP to form a stable ternary complex. The complex travels through the NPC and dissociates in the cytoplasm after the hydrolysis of Ran-GTP (Figure 1.27).[275]

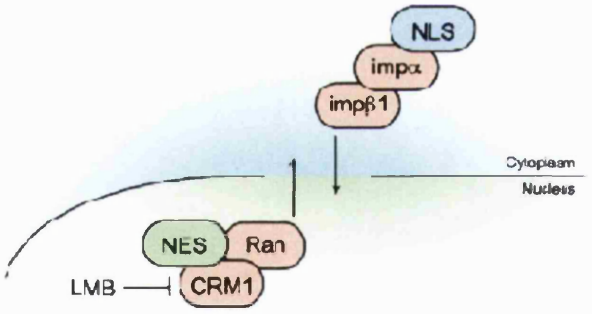


Figure 1.27: Depiction of nuclear trafficking.[275]

FRAP (Fluorescence Recovery After Photobleaching) approaches have revealed that constitutive import of non-phosphorylated STAT1 through the nuclear pores is independent of the karyopherin (importin) mechanisms.[276] The nuclear import mechanisms applicable for the respective STAT proteins can be different for the non-phosphorylated and phosphorylated species.[277,278]

1.7.3.6 Targeted Degradation

Ubiquitylation, a process involving the conjugation of proteins with ubiquitin (Ub), is essential for the degradation of proteins whose levels have to be regulated either constitutively, or in response to extracellular stimuli and changes in the cellular environment. Ubiquitin-proteosomal

degradation is important for down-regulation of the JAK/STAT signalling pathways. It is a multistep process involving at least three types of enzymes (Figure 1.28).

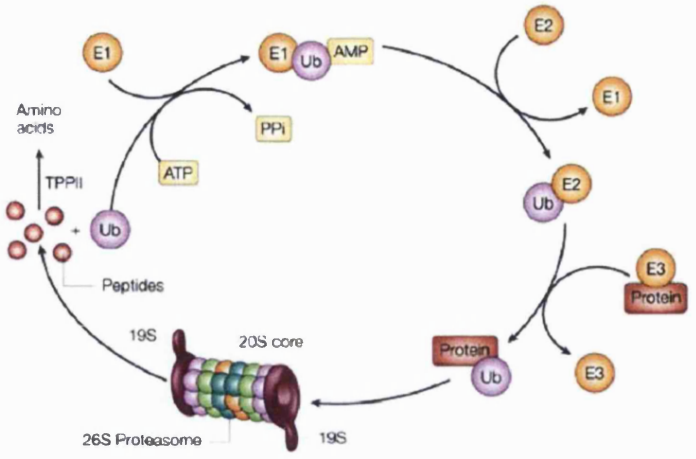


Figure 1.28: The ubiquitin pathway.[279]

Free ubiquitin is activated in an ATP-dependent manner by an ubiquitin activation enzyme (E1), leading to formation of a thiol-ester linkage between E1 and the carboxyl terminus of ubiquitin (glycine 76). Subsequently, the ubiquitin group is transferred to one of many distinct ubiquitin-conjugating enzymes (E2). Finally, a ubiquitin protein ligase (E3) catalyses the transfer of Ub from the E2 enzyme to the target protein. Degradation of ubiquitylated substrates is then performed by the 26S proteasome, which comprises the two terminal 19S regulatory sub-complexes bound to the proteolytic 20S core. Substrates destined for degradation are labeled with polyubiquitin (Ubn), which is recognized by the 19S complex. Short oligopeptides are released from the 26S proteasome after degradation and are further degraded into tripeptides by the protease tripeptidyl peptidase (TPPII).

1.7.3.7 STAT Interacting Proteins (Co-activators)

The transcriptional activity of all STATs depends mainly on the carboxy-terminal TAD that binds co-activators. However, other regions (such as the amino terminus) might also bind such co-activators. The TADs of STAT2 and STAT6 have at least a ten-fold greater stimulatory activity than the TADs of other STAT proteins. The underlying cause of the enhanced transcriptional activity of these serine-phosphorylated STAT proteins is the greater selective recruitment of co-activators.[225]

1.7.3.8 STATs and HATs

The TADs of STATs all interact with the co-activator Histone Acetyl Transferases (HATs), especially p300/CBP (CREB binding protein).[237,280] STAT2 can stimulate transcription through a complex TATA-binding protein, which allows STAT2-mediated transcription to continue under conditions in which general host-cell transcription is inhibited.

1.7.3.9 STATs and Other DNA-Binding Proteins

STAT proteins interact together with other site-specific DNA-binding proteins for transcriptional activation. For example, interactions between STAT1, Sp1 and upstream stimulatory factor;[281,282] STAT5 and glucocorticoid receptor (GR);[283] STAT6 and CCAAT/enhancer binding protein (C/EBP);[284] several STAT proteins with N-Myc interactor (Nmi);[285] and STAT3 with c-Jun, GR, androgen receptor and SMAD.[253,286-288]

1.7.3.10 STAT Proteins Side-by-Side

STAT proteins also interact with each other on tandem DNA sites to achieve maximum transcriptional stimulation.[289,290] The amino terminus is required for these dimer-dimer interaction. It has been shown that the STATs are physically present in chromatin at the time of transcriptional activation. For example, the IFN-dependent presence of STAT2 on the ISG54 (interferon-stimulated-gene 54) promoter,[291] of STAT1 on the IRF1 (interferon-regulatory-factor 1) promoter and the class-II transactivator (CIITA) promoter,[292] and of STAT3 on the α2-macroglobulin promoter.[293]

1.8 Biological Function of STATs

The biological functions of the seven mammalian STAT proteins known to date, have been studied through their distribution in tissue, specific cell responses *in vitro* and gene-targeted removal in mice of each of their seven genes. Table 1.4 summarizes the main phenotypes originally discovered by these procedures.

Table 1.4: Role of STAT proteins as revealed by gene-targeting in mice.[225,280,294-296]

STAT protein	Phenotype of null mice
STAT1	Impaired responses to interferons; increased susceptibility to tumours; impaired growth control
STAT2	Impaired responses to interferons
STAT3	Embryonic lethality; multiple defects in adult tissues including impaired cell survival (both positive and negative) and impaired response to pathogens
STAT4	Impaired T_H1 differentiation owing to loss of IL-12 responsiveness
STAT5A	Impaired mammary gland development owing to loss of prolactin responsiveness
STAT5B	Impaired growth owing to loss of growth hormone responsiveness
STAT6	Impaired T_H1 differentiation owing to loss of IL-4 responsiveness

IL, interleukin; T_H1, T helper 1 cell.

1.8.1 Role in Growth Control

Signal transduction from the cell surface and movement of transcriptional proteins to the nucleus can be dysfunctional in cancer cells.[297] Lack of STAT1 in mice increases susceptibility to both chemically-induced primary tumours and transplantable tumours;[298-300] and in human

cancer cells often enforce growth restraint.[301] Constitutively active STAT3 occurs in a wide variety of human tumours, and can be converted into an oncogene by experimental mutation.[302]

1.8.2 Role in Infection

There are a number of STAT proteins that play important roles in the defence mechanism (Table 1.5). STAT1 and STAT2 mediate the effects of IFNs; STAT4 and STAT6 mediate the effects of IL-12 and IL-4, respectively; and STAT3 mediates the effects of IL-6 and other gp130 ligands. The absence of STAT1 or STAT2 makes the host vulnerable to microbial infections,[303-305] and STAT1-mutation results in decreased resistance to mycobacteial infection.[306]

Table 1.5: Tissue-specific roles of STAT3 as revealed by conditional gene targeting in mice.[225,307-310]

Target tissue	Phenotype
Skin	Impaired second hair cycle, wound repair and keratinocyte migration
Thymic epithelium	Age-dependent thymic hypoplasia, hypersensitivity to stress
T lymphocytes	Impaired Il-6 dependent survival and Il-2rα expression
Monocytes/ neutrophils	Enhanced inflammatory responses and T_H1 differentiation, chronic colitis
Granulocytes	Enhanced proliferation owing to impaired negative feedback
Mammary epithelium	Defective apoptosis, delayed mammary involution
Liver	Impaired acute phase response
Neurons	Impaired cell survival

Il-2rα, interleukin-2 receptor-α; T_H1, T helper 1 cell.

1.9 STATs and Cancer

STATs are critical mediators of oncogenic signalling, and constitutive activation of STAT proteins participate in development and progression of human tumors[271] because:

- STATs are selectively activated by oncogenic tyrosine kinase signaling pathways;
- Dominant negative STAT mutants block STAT-dependent transcription and transformation induced by activated TKs;
- Constitutively activated mutants of STATs can induce some aspects of cell transformation;
- Inappropriate activation of STATs in oncogenesis leads to induction of genes involved in controlling cell proliferation and survival.

The role of STATs in oncogenesis is summerised in Table 1.6, where an elevated STAT DNA-binding activity has been detected in both tumour cell lines and primary tumour specimens in different human tumours. Though STAT1 is found activated in some tumours, STAT3 and STAT5 are involved in promoting oncogenesis.

Table 1.6: Activation of STATs in Human Cancers.[271]

Tumor type		Activated STATs
Breast cancer	Cell lines	STAT3
	Tumors	STAT1, STAT3
Multiple myeloma	Cell lines and tumors	STAT1, STAT3
Head and neck cancer	Cell lines and tumors	STAT1, STAT3
Leukemia (tumors and cell lines)	HTLV-I-dependent	STAT3, STAT5
	Erythroleukemia	STAT1, STAT5
	Acute lymphocytic leukemia (ALL)	STAT1, STAT5
	Chronic lymphocytic leukemia (CLL)	STAT1, STAT3
	Acute myelogenous leukemia (AML)	STAT1,STAT3, STAT5
	Chronic myelogenous leukemia (CML)	STAT5
	Megakaryotic leukemia	STAT5
	Large granular lymphocyte (LGL) leukemia	STAT3
Lymphoma (tumors and cell lines)	EBV-related/Burkitt's	STAT3
	Mycosis fungoides	STAT3
	HSV saimiri-dependent (T cell)	STAT3
	Cutaneous T cell lymphoma	STAT3
Lung cancer	Cell lines	STAT3
Other cancers (tumors and cell lines)	Renal cell carcinoma	STAT3
	Prostate carcinoma	STAT3
	Melanoma	STAT3
	Pancreatic adenocarcinoma	STAT3
	Ovarian carcinoma	STAT3

1.10 STAT3 and its Target Genes

STAT3 was initially identified as the acute-phase response factor (APRF) activated by IL-6.[311] STAT3 activation has been observed in the cytoplasm when phosphorylation is essential. STAT3 binds to IL-6 response elements of various acute-phase protein genes (*e.g.*, the alpha 2-macroglobulin, fibrinogen, and alpha 1-acid glycoprotein genes),[311] and can be activated by many different cytokines, growth factors and oncogenes. The IL-6 family of cytokines has many biological functions, and STAT3 plays a major role in these processes.[312] IL-6 activation in mouse myeloid leukemia M1 cells has been shown to lead to growth arrest and terminal differentiation into macrophages. Overexpression of STAT3DN abolished the IL-6-induced effects and resulted in inhibition of IL-6-induced repression of c-myb and c-Myc.[313,314] Therefore, STAT3 activation is essential for IL-6 mediated growth arrest. Conversely, Fukada et al. have found that STAT3 is involved in anti-apoptosis, proliferation and upregulation of Bcl-2 by overexpression of STAT3DN in mouse pro-B (BAF/B03) cells.[315] It was also confirmed that STAT3 activation is not only essential for cell survival but also required for cell cycle transition *via* STAT3-mediated up-regulation of Cyclins D2, D3 and A, and cdc25A, and the associated down-regulation of p21 and p27.[316] Expression of GFAP was severely reduced in the brain of $gp130^{-/-}$ mice as well as in mice expressing a mutant gp130, which is defective in STAT3 signalling but not in SHP2 signalling.[287,317] The CD40 receptor, which is capable of inducing B cell differentiation and lacks intrinsic tyrosine kinase activity, is still able to induce tyrosine phosphorylation and activation of constitutively associated JAK3, as well as STAT3. Mutation

of the JAK3 binding domain inhibits B cell differentiation.[318] Therefore, STAT3 is involved in both astrocyte and B cell differentiation.

STAT3 is able to inhibit cell differentiation upon its activation with IL-6 or LIF. IL-6 induces differentiation of PC12 cells pre-treated with nerve growth factor (NGF). Stimulation of the MAPK pathways is important for neurite outgrowth, and over-expression of mutant gp130, which is defective in STAT3 signalling, stimulates neurite outgrowth. IL-6-induced activation of STAT3 is inhibited if cells are pre-treated with NGF, and over-expression of STAT3DN does not require NGF pre-treatment for neurite outgrowth, which confirms that STAT3 is involved in PC12 differentiation.[319] STAT3 is essential for self-renewal of embryonic stem (ES) cells.[320-322]

Activated STAT3 has various functions in different cell types due to the expression of different STAT3 target genes. Genes that are regulated by STAT3 are very important since they trigger signalling cascades that lead to the final observed biological effects (Table 1.7).

Table 1.7: STAT3 target genes.

Target Genes, Up-regulated by STAT3	
Target genes	**Cells**
Angiotensinogen II	HepG2[323]
Bcl-X_L	STAT3C transformed NIH3T3, U266 myeloma cells, head and neck squamous cell carcinomas[302,324,325]
cdc25A	BAF/B03 pro-B cells[316]
C/EBP δ	MCF-7[326]
c-Myc	STAT3C transformed NIH3T3, BAF/B03, murine pro-B cells, MCF-7,HepG2 [302,326-328]
Cyclin A	BAF/B03 pro-B cells[316]
Cyclin D1	STAT3C transformed NIH3T3, MCF-7, v-src transformed, NIH3T3 and BALB/c 3T3[302,326,329]
Cyclin D2	BAF/B03 pro-B cell[316]
Cyclin D3	BAF/B03 pro-B cells[316]
Cyclin E	v-src transformed NIH3T3 and BALB/c 3T3[329]
Fibronectin	MCF-7, T47D[326]
mcl-1	Large granular lymphocyte (LGL) leukemia cells[330]
p21	v-src transformed NIH3T3 and BALB/c 3T3[329]
p27	A375[331]
p53	MCF-7[326]
pim-1 and pim-2	BAF/B03 pro-B cell[332]
VEGF	NIH3T3, B16 tumor cells, human pancreatic cancer cell lines[333,334]
Target Genes, Down-regulated by STAT3	
Cyclin D1	fetal hepatocytes[335]
Cyclin D2	fetal hepatocytes[335]
p21	BAF/B03 pro-B cells[316]
p27	BAF/B03 pro-B cells[316]

STAT3 induces its effects on malignant transformation through gene regulation. STAT3 up-regulates the antiapoptotic genes such as Bcl-X_L, and Pim, the genes that are important for cell cycle transition, for example, Cyclin D1 and c-Myc, and activates pro-angiogenic factors, such as VEGF (Figure 1.29). Though STAT3 up-regulates p27 and C/EBPδ, genes that are involved in STAT3-mediated growth inhibition in cancer cells, p27 is down-regulated by STAT3 in BAF/B03 pro-B cells.[331]

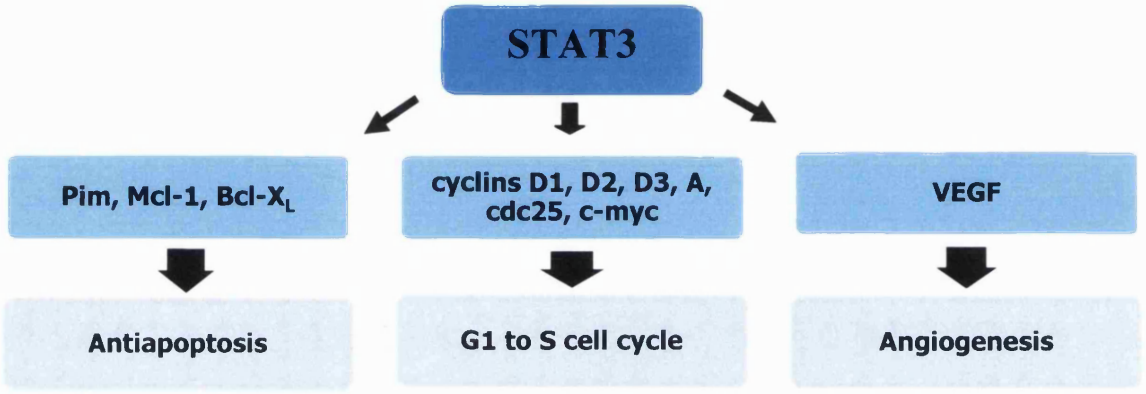


Figure 1.29: STAT3 regulated genes important for cancer progression.

1.11 Targeting STAT3 for Cancer Therapy

STAT3 is a novel molecular target for anti-cancer drug discovery[271,336,337] because it acts as a critical mediator of oncogenic signalling. STAT3 is constitutively activated in fibroblasts transformed by oncoproteins such as v-Src,[260,338] and is essential for v-Src-mediated transformation.[339,340] Over-expression of a constitutively active form of STAT3 in immortalized rat or mouse fibroblasts induces their transformation and the formation of tumours in nude mice.[302] STAT3 is activated in many human cancers, including 82% of prostate cancers,[341] 70% of breast cancers,[342] more than 82% of squamous cell carcinoma of the head and neck,[343] and 71% of nasopharyngeal carcinomas.[344] STAT3 participates in oncogenesis through up-regulation of genes encoding apoptosis inhibitors (Bcl-X_L, Mcl-1 and Survivin), cell-cycle regulators (Cyclin D1 and c-Myc) and inducers of angiogenesis [vascular endothelial growth factor (VEGF)].[337]

Bcl-X_L is an anti-apoptotic protein within the Bcl-2 family that inhibits apoptosis by binding pro-apoptotic proteins and preventing cytochrome C release.[345-347] High levels of Bcl-X_L expression are observed in several tumour types.[348] Mcl-1 is a survival factor for human cancer cells[330,349] as well. Suppression of the expression of Bcl-X_L and Mcl-1 induce cell apoptosis and this could be useful in cancer therapy. Survivin is a protein that regulates both the cell cycle and apoptosis, and is over-expressed in a number of human cancers.[350] Over-expression of Cyclin D1 helps to generate oncogenesis by regulating cell-cycle progression.[351] The c-Myc protooncogene is over-expressed in Burkett's lymphoma, breast and colon carcinomas resulting in increased cellular proliferation and inhibition of differentiation.[352-354] STAT3 also induces

increased expression of VEGF which plays a crucial role in invasion and metastasis in ovarian carcinoma.[333,355] Therefore, interrupting constitutive STAT3 signalling in tumour cells should down-regulate expression of several important classes of oncogenic proteins.

1.12 Strategies to Regulate STAT3 Transcriptional Activity

There are a number of strategies that can be followed to obtain inhibitors that disrupt STAT signalling. Strategies to regulate STAT3 transcriptional activity can be grouped by form (*e.g.*, oligonucleotide, aromatic polycyclic compound, etc.) or by function (*e.g.*, inhibition of expression, of activation, or of DNA-binding activity). The indirect approach involves the identification of upstream STAT3 activators (*e.g.*, cytokines, growth factors, TKs and serine kinases), followed by the design and synthesis of pharmacologic inhibitors that specifically disrupt their function. A more direct approach involves the rational design of small molecules that directly target the STAT3 proteins and disrupt its function. The major categories of STAT3 inhibitors can be grouped by the mechanisms of inhibition followed by the type of substances affecting the specific mechanism. A summary is given in Table 1.8.

Table 1.8: List of current STAT3 Inhibitors.[356]

Class	Target
Antisense	mRNA
13410 and 13411	STAT3:DNA binding (binds to genomic site)
Decoys	STAT3:DNA binding (binds to genomic site)
G-quartets	STAT3:DNA binding (binds to STAT3)
siRNA	mRNA
Aptamers	STAT3:DNA binding
c-Src inhibitor	STAT3 activation
Phosphotyrosyl peptides	STAT3:DNA binding (binds to STAT3)
Novel cisplatin analogs	STAT3:DNA binding (binds to STAT3)
Curcumin	STAT3 activation
Cucurbitacins	STAT3 activation
Tkip	STAT3 activation
Tyrphostins, Piceatannol	STAT3 activation
Indirubin	STAT3:DNA binding (binds to STAT3)

1.12.1 Inhibition of the STAT3 Signalling Pathway

Inhibition of the enzymatic activities of the tyrosine kinases that activate STAT3 on the conserved tyrosine residue between the SH2 domain and the transactivation domain can result in inhibition of STAT3 signalling.

1.12.1.1 Small-Molecule Inhibitors

The alkylated indirubin oxime E804 (**15**; Figure 1.30) inhibits STAT3 signalling in breast cancer cells by inhibiting upstream kinase activity, probably c-Src, with an IC_{50} of 430 nM *in vitro*.[357] Indirubin (**16**) known as an inhibitor of Cyclin-dependent kinases[358] is used for treatment of chronic myelogenous leukemia.[359] Indirubin derivatives induce apoptosis through inhibition of the expression of the STAT3-regulated anti-apoptotic proteins, Mcl-1 and survivin.[357] Another natural product Resveratrol (**17**) has a similar mechanism of action to Indirubin.[360] Cucurbitacin I (JSI-124; **18**)[361] and other cucurbitacin family members (*e.g.*, withacnistin; **19**)[362,363] have been shown to inhibit signal transduction *via* STAT3. Curcumin (**20**) has been identified as an inhibitor of STAT3 signalling[364] and additional signalling pathways.[365] Magnolol (**21**) inhibits signalling not only *via* STAT3, but also *via* NF-κB.[366,367]

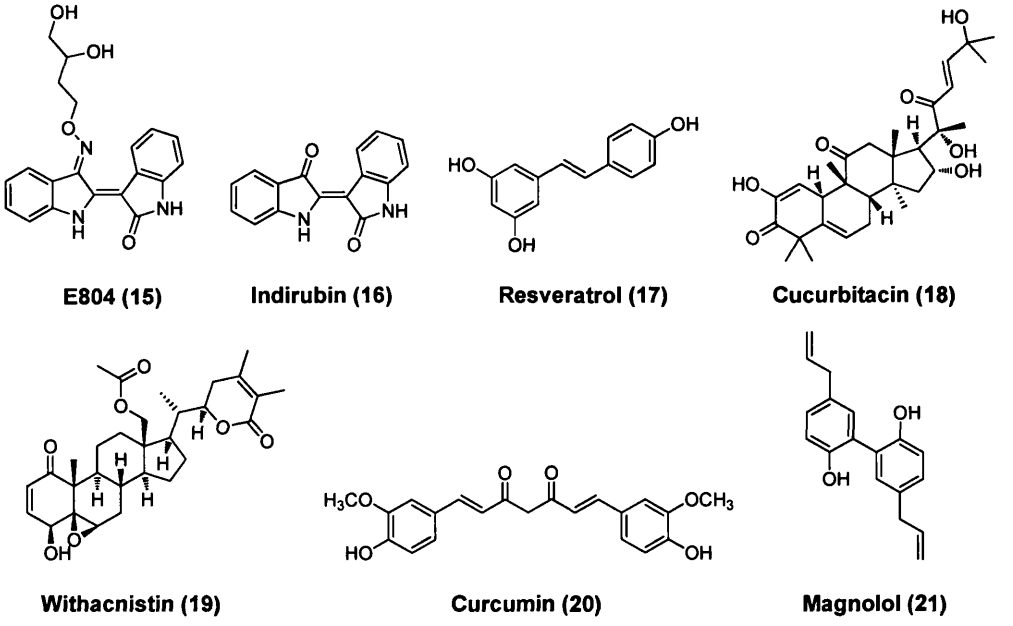


Figure 1.30: Examples of indirect inhibitors of STAT3.[368]

1.12.1.2 Oligonucleotide-Based Inhibitors

1.12.1.2.1 Antisense Oligonucleotides

Among the oligonucleotide-based STAT3 inhibitors, the antisense oligonucleotides have had some success. Antisense oligonucleotides are typically 15- to 20-mer oligonucleotides designed to inhibit transcriptional gene expression.[356] *In vitro* studies have revealed that antisense oligonucleotides against STAT3 can slow cell proliferation and induce apoptosis in the DU145 prostate cancer cell line.[341]

1.12.1.2.2 Novel STAT3-Inhibiting Oligonucleotides and Peptide Nucleic Acids

As STAT3 has to enter the nucleus and bind to DNA for gene expression to occur, inhibition of STAT3:DNA binding could affect the survival of cancer cells. Each complementary strand of

the STAT3 hSIE binding sequence has been synthesised as a phosphorothiorated and 2'-O-methoxylated 24-mer and then transfected into DU145 cells. The hSIE sequences, known as 13410 and 13411, prevent STAT3:DNA binding presumably through annealing to DNA.[369] A PNA form of 13410 (PNA-13410) has shown significantly improved efficacy compared to 13410.[356]

1.12.1.2.3 Double-Stranded Decoys

Double-stranded *cis*-promoter element decoys have been designed to bind to phosphorylated and dimerized STAT3, thus physically blocking the genome docking site on STAT3. *Cis*-element double-stranded decoys have been tested for activity in several biological systems, including helper T cell activation, breast cancer cells, and myocardial infarction.[370,371] A double-stranded STAT3 DNA decoy has also been developed for the treatment of head and neck squamous cell carcinoma.[372] Although they inhibit STAT3:DNA binding, further studies are needed to confirm *in vivo* efficacy of anti-STAT3 decoys for cancer therapy.

1.12.1.2.4 G-Quartet Oligonucleotides

G-quartets are structures formed in DNA strands containing series of guanines (G). Therefore, four-stranded structures, also known as G-quadruplexes. They are found in the transcriptional regulatory regions in several oncogenes.[373] STAT3- inhibiting G-quartets form H-bonds with STAT3 proteins and exhibit preferential binding to STAT3 over STAT1.[374] Although G-quartets inhibit STAT3-mediated gene transcription *in vitro*, they are only effective in slowing the growth of breast and prostate xenografts.[375]

1.12.1.2.5 siRNA

Silencing of STAT3 gene expression by small-interfering (si)RNA has been reported in astrocytoma cell lines it induced apoptosis. siRNA has been found to reduce the amount of STAT3 bound to DNA, and to induce apoptosis in DU145 and LN17 human prostate cancer cell lines.[376]

1.12.1.2.6 Aptamers

Aptamers are nucleic acid ligands that can control the release of nanoparticle polymer particles in a cell- or tissue-specific manner. Novel conjugates composed of nanoparticle polymers and aptamers have been found to be effective in prostate cancer cells.[377] Short peptides that specifically interact with defined functional domains of STAT3 have been selected to design STAT3-specific aptamers. Peptide aptamers interact with the STAT3 dimerization domain or with the STAT3 DNA-binding domain and inhibit STAT3:DNA binding, suppressing STAT3 transactivation, inducing apoptosis, and inhibiting expression of Bcl-X_L in EGF-responsive cell lines, and in melanoma and myeloma cell lines.[378]

1.12.1.3 Platinum-Based Inhibitors

A number of platinum-containing compounds are known to disrupt STAT3 signalling and suppress its biological functions.[379] Figure 1.31 shows some representative platinum complexes (**22** – **32**). Compounds **22**, **23** and **24** block STAT3 DNA-binding activity *in vitro* at low micromolar concentrations (*e.g.*, 1.5 – 5.8 μM). The novel platinum complex **23** has been identified as an inhibitor of STAT3 signalling in an *in vitro* nuclear extraction-based DNA binding assay.[136] The platinum complex IS3 295 (NSC 295558; **26**) blocks DNA binding of STAT3 by binding to the protein, inhibits STAT3 functions in tumor cells, and thereby induces cell cycle arrest and apoptosis.[136] In human and mouse tumour cell lines with constitutively-active STAT3, the complex **26** was shown to attenuate selectively STAT3 signalling. **26** also repressed expression of Cyclin D1 and Bcl-X_L, two of the known STAT3-regulated genes that are over-expressed in malignant cells.[380]

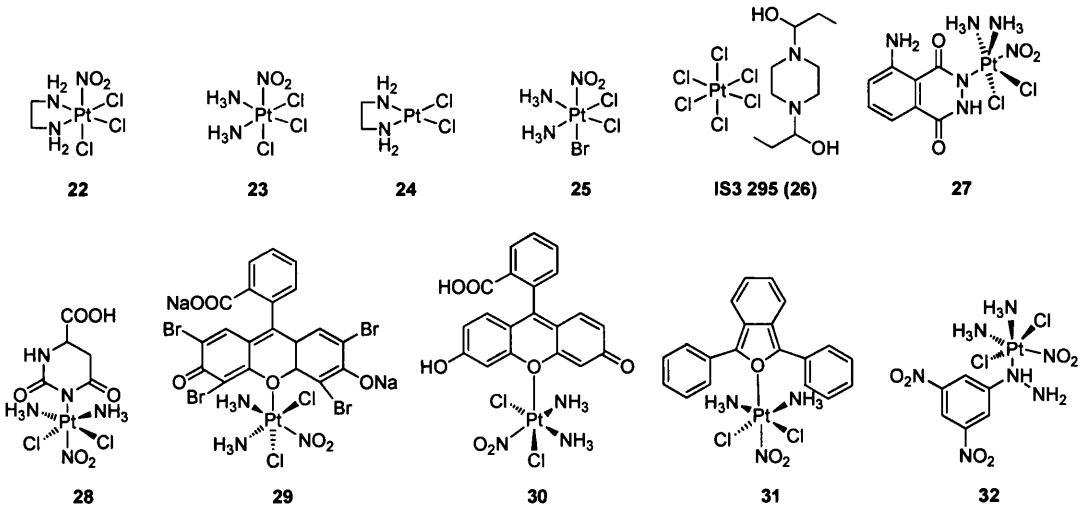


Figure 1.31: Structures of representative platinum complexes. [380]

1.12.2 Inhibition through the DNA-Binding Domain

The natural product galiellalactone (**33**, Figure 1.32) has been found to inhibit interleukin (IL)-6-mediated STAT3 signalling.[381] As galiellalactone inhibited DNA binding of activated STAT3 without affecting STAT3 tyrosine phosphorylation, the compound was assumed to bind to the DNA-binding domain of dimeric STAT3, possibly by covalently modifying a cysteine residue in the STAT3 DNA binding domain.

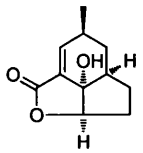


Figure 1.32: Inhibitors of STAT3 DNA binding.[368]

1.12.3 Inhibition of the STAT3 Dimerization Event

The SH2 domain is essential for both tyrosine-phosphorylation and dimerization of STAT3. The inhibition of the function of its SH2 domain could result in not only the inhibition of STAT3 activation,[382] but also the prevention of dimerization of STAT3 molecules which escape inhibition of activation. A number of inhibitors have been identified, and these are described below.

1.12.3.1 Peptide-Based Inhibitors

A fusion peptide comprised of the hexapeptide PpYLKTK (the motif which mediates STAT3 dimerization) and a membrane translocating sequence has been found to inhibit STAT3 tyrosine phosphorylation, STAT3-dependent gene transcription, and oncogenic transformation.[383] The tripeptide motif A/PpYL (**34**, Figure 1.33) was found to be an inhibitor of STAT3 dimerization *in vitro*, and was considered as the starting point for the design of peptide mimetics. The peptide mimetic ISS 610 (**35**) inhibits DNA binding of activated STAT3 but has reduced peptidic character.[384] From an analysis of the binding interactions between ISS610 and the STAT3 SH2 domain,[224] the oxazole-based peptide mimetic S3I-M2001 (**36**) with minimal peptide character was designed using computational modelling.[385] S3I-M2001 has strong STAT3-dependent inhibitory activity in cellular assays at 30–100 μM, disrupts tyrosine phosphorylated STAT3, and inhibits STAT3-mediated gene transcription, malignant transformation, survival and migration. However, the introduction of a *m*-methoxyaniline group at the carboxy terminus of ISS610 results in a change of specificity from STAT1 to STAT3. The compound ISS840 (**37**) has a 20-fold preference for disruption of activated STAT1 dimers compared to STAT3 dimers.[386]

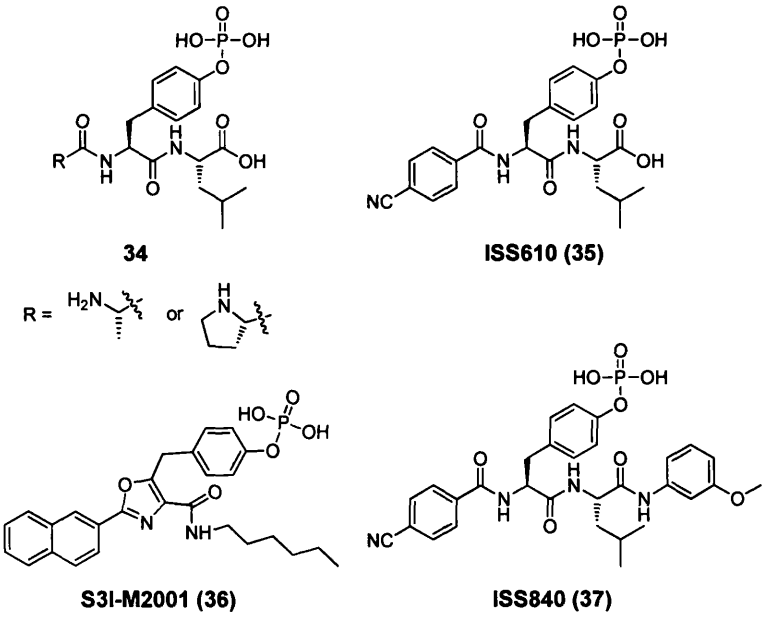


Figure 1.33: Peptide-based inhibitors of STAT SH2 domains.[368]

1.12.3.2 STAT3:STAT3 Small-Molecule Inhibitors

The availability of the crystal structure of DNA-bound, tyrosine phosphorylated STAT3 permitted two independent studies which used the virtual screening of chemical databases for the identification of non-peptidic inhibitors of the STAT3 SH2 domain.[387,388] Molecules which could be docked in the STAT3 SH2 domain were subsequently evaluated in biochemical assays. The first compound discovered by this route, STA-21 (NSC 628869; **38**; Figure 1.34),[387] was shown to inhibit the DNA-binding of pre-phosphorylated STAT3, and to display STAT3-dependent inhibitory cellular effects. The STA-21 derivative **39** which has similar activity has also been reported.[389] Using structure-based drug design, a novel STAT3 inhibitor LLL12 (**40**) has been identified.[390] Computer models with docking simulation confirmed that LLL12 binds directly to the phosphoryl tyrosine 705 (pTyr705) binding site of the STAT3 monomer. Investigation of inhibitory effects of the LLL12 in cancer cells has revealed that LLL12 inhibits STAT3 phosphorylation (Tyr705) and its consequent functions, down-regulates STAT3 downstream targets, inhibits proliferation, colony formation and cell migration, and induces apoptosis in various human breast and pancreatic cancer cells as well as in glioblastoma cells.

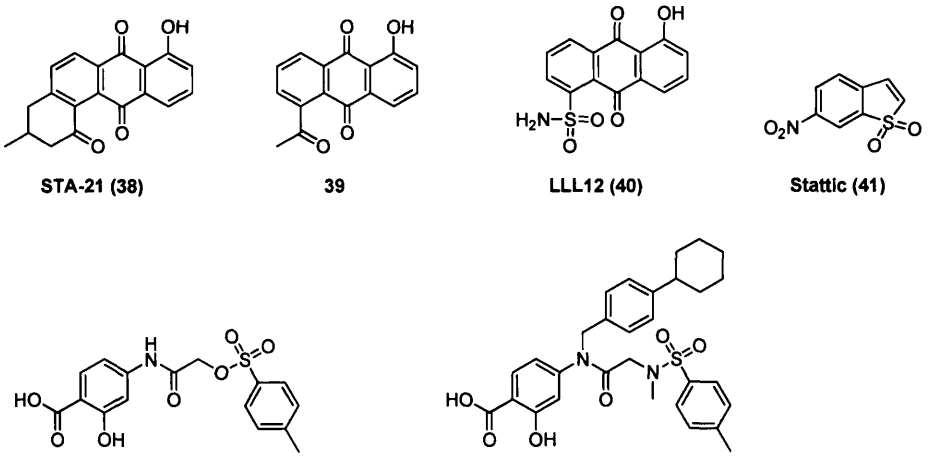


Figure 1.34: Non-peptidsic inhibitors of STAT SH2 domains.

Stattic (**41**) was discovered in an *in vitro* fluorescence polarization assay which measured the effect of test compounds on the function of the STAT3 SH2 domain.[391] Stattic was found to inhibit the function of the SH2 domain of both unphosphorylated and phosphorylated STAT3, and displayed a preference for STAT3 over the family members STAT1 and STAT5b *in vitro*. Furthermore, Stattic was shown to inhibit nuclear translocation of STAT3 with good selectivity over STAT1 in a hepatocellular carcinoma cell line, and selectively increased the apoptotic rate of breast cancer cell lines.[368]

The compound S3I-201 (NSC 74859; **42**) was designed by modelling the STAT3 SH2 domain, and was shown to inhibit STAT3 dimerization.[388] This compound inhibited STAT3 DNA-binding activity with an IC_{50} value of 86 μM, inhibited STAT3-mediated gene expression,

induced apoptosis in cells with constitutively activated STAT3 and inhibited tumor growth in a mouse xenograft model. From the key structural information from the computational modeling of S3I-201 bound to the STAT3 SH2 domain, a novel analog S3I-201.1066 (**43**) was designed which had improved STAT3-inhibitory activity. S3I-201.1066 inhibited STAT3 DNA-binding activity with an IC_{50} value of 35 μM. Recent studies has proved that S3I-201.1066 directly interacts with the STAT3 protein *in vitro*, disrupting STAT3 binding to cognate pTyr peptide motifs of receptors and thereby inhibiting STAT3 phosphorylation and activation, and STAT3 nuclear localization.[392]

2 Aim of the project

The main objective of this research project was to identify a novel series of small-molecule inhibitors of STAT3:STAT3 dimerisation for the down-regulation of this signalling pathway as a possible cancer therapy. Proof-of-concept studies by others in cell culture and animal models have validated the use of small-molecule inhibitors of STAT3 signalling as promising anticancer drugs.[337,343,393] Numbers of strategies were initially considered for the identification of inhibitors with different points of intervention either up- or down-stream.[343] Approaches considered included the discovery of both inhibitors and stabilizers of PPIs through peptidomimicry, computational studies, and the physical screening of chemical libraries.[368,380] In particular, the protein-protein interaction between two STAT monomers (the dimerisation event in the signalling cascade) was identified as a valid target to prevent DNA-binding and transcriptional activation. To date, the most successful approach to target this PPI by others has involved the use of peptides[382,394] containing the Pro-pTyr-Leu-Lys-Thr-Lys sequence (either in full, or in part) of the STAT3 monomer responsible for binding of the SH2 domain of the other monomer (*i.e.*, the "hot spot"), and peptidomimetics that mimic the chemical features of this protein interface.[395] However, peptides are not amenable candidates for drug development. To date, five small molecules have been identified as potential STAT3:STAT3 inhibitors: STA21,[387] S31-201,[388] S3I-M2001,[385] S3I-201.1066[392] and LLL12 (Figure 2.1).[390] These were discovered using the structural information provided by the X-ray crystal structure of the truncated mouse STAT3βTC dimer[224] (human STAT3β has an identical amino acid sequence) bound to DNA, and a combination of approaches including (a) computational modeling and automated docking using both natural compounds and synthetic agents (*e.g.*, NCI database, Sigma-Aldrich, Maybridge, Merck Index, Ryan Scientific), and (b) structure-based design of small molecules in the SH2 domain of the STAT3 monomer relative to the bound native pTyr peptide. Their activity and STAT3 selectivity have been evaluated in *in vitro* cell-based assays and against specific cancer cell lines, (*e.g.*, breast cancer MDA-MB-231 and -435), showing promising activity (*i.e.*, IC_{50} between 15 to 86 μM). Some of those exhibit antitumor effects *in vivo* in mouse xenografts with MDA-MB-231 breast cancer cells (*i.e.*, 3-5 mg/kg every 2-3 days).

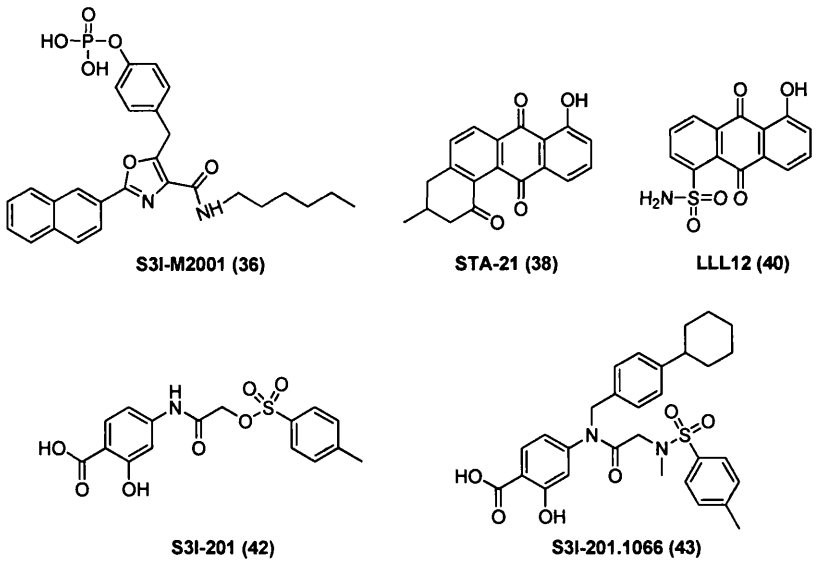


Figure 2.1. Small molecule inhibitors of the STAT3:STAT3 interaction described in literature.

Our aim was to initially carry out an *in silico* study to identify and design small molecules as potential inhibitors of STAT3:STAT3 interaction using X-ray crystal structure coordinates of the STAT3βTC protein. This model would then be employed for a structure-based virtual screening of small molecules followed by molecular dynamics simulations of promising "hits". The aim was to use the drugable subset of the Zinc database[396] for the initial screen to identify a number of potential "hit" compounds as ligands of the STAT SH2 domain. Structure-based design would also be used to design novel small molecules as STAT3:STAT3 inhibitors (see Section **3.2**).

3 Results and Discussion

3.1 *In Silico* Study: Virtual Screening

The *in Silico* studies (Section **3.1**, **3.2**, **3.3.5.1** and **3.5**) have been carried out by Dr Shozeb Haider of the CRUK Biomolecular Structure Group at The School of Pharmacy, University of London.

The X-ray crystal structure coordinates of the mouse STAT3βTC protein (PBD ID: 1BG1) have been used to carry out the molecular modelling studies. It is to be noted that the human and murine STAT3 protein differ of only one amino acid residue in the terminal part of the C-TAD region, which is constitutively unstructured and is not responsible for the dimerisation event to form the active STAT3 dimer. The downloaded coordinates were incomplete due to a lack of suitable electron density being observed in 2 loop regions of the structure in the binding region of the PPI. Before beginning of the studies, these missing regions were constructed. Homology modelling was employed in order to build the missing regions along with the N and C termini (Figure 3.1). This was carried out using the Modeller 9v2 software. Stereochemical checks on the modeled structures were carried out using the program PROCHECK. This model was then employed in structure-based virtual screening of small molecules and molecular dynamics simulations.

Figure 3.1: Amino acid sequences of the STAT3 monomeric protein

The Zinc database[396] "drug-like" subset libraries (87 subsets) were used for initial screening and contained >2m small compounds. Zinc is a free database of commercially-available compounds

for virtual screening. It contains over 13 million purchasable compounds in 3D formats belonging to the catalogs of compounds from vendors (*i.e.*, ChemBridge, ChemDiv, Ryan, Asinex, Sigma-Aldrich, Maybridge, Specs, Comgenex and Otava). The "drug-like" subset libraries are among other subset (*e.g.*, lead-like, fragment-like) libraries of Zinc database that has mean molecular weight between 150 and 500, clogP $\leq$ 5, H-bond acceptors $\leq$ 10, polar surface area $\leq$ 150 $Å^2$, number of rotatable bonds $\leq$ 8. This database has been chosen due to molecular diversity and commercial accessibility.

Virtual screening was performed using the DOCK6 program. DOCK program suite is a composite of SPHGEN, GRID and DOCK program itself. Prerequisites to the DOCK program include preparation of the structure by generating a molecular surface of protein and identifying cavities on that molecular surface that could behave as potential docking sites. The program SPHGEN helps to identify the active site (cavities) of interest by producing sphere centres which fill the site. Six potential binding sites, identified in the SH2 domain were considered suitable for docking because SH2 domain interaction with its cognate phosphopeptide region is responsible for stable STAT3 dimer formation.[220]

Figure 3.2b shows the binding interface pY^{705} containing peptide PpYLKTKFI and SH2 domain. One of the identified binding sites resulted to be the binding pocket in the SH2 domain, where the natural ligand PpY^{705}LKTKFI binds (interacting residues Arg^{609}, Ser^{611}, Glu^{612}, Ser^{613}, Ser^{636} and Glu^{638}). Moreover, this identified site overlaps with the proposed binding site of the small molecule STA-21 (interacting residues $Arg^{609}$and Ile^{634})[387] and S3I-201 (interacting residues Ser^{611}, Ser^{613}, and Arg^{609}).[388]

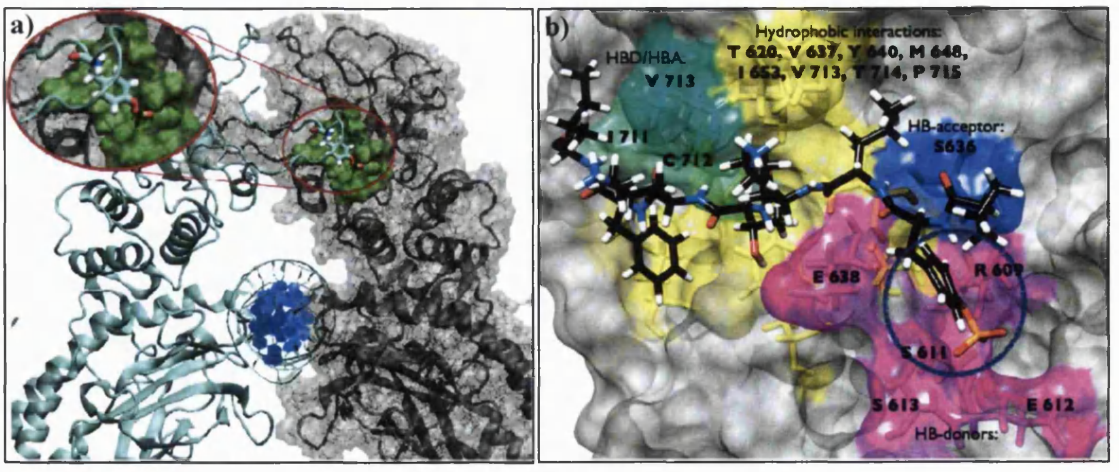


Figure 3.2: a) STAT3 dimer bound to DNA and pY^{705} bound to the pocket of SH2domains; **b)** The PpY^{705}LKTKFI residues (black stick) bound to the partner SH2 domain (surface representation). The pY^{705} itself is marked in a circle and interacting residues on SH2 domain are marked.

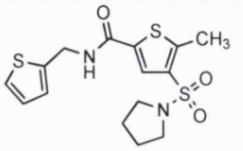
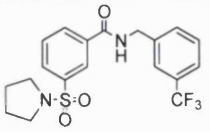
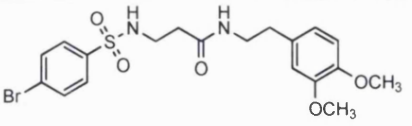
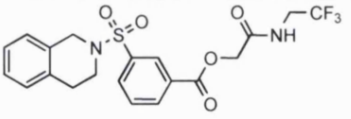
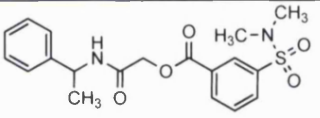
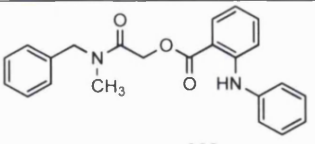
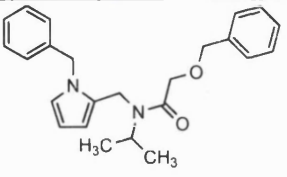
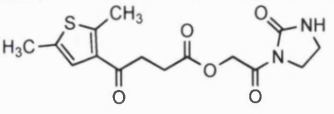
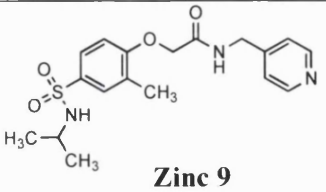
The program GRID generates scoring grids around this site which was large enough to accommodate the volume of the ligand STA-21 for quick dock evaluation. Approximate sum of the van der Waals attractive, van der Waals dispersive and coulombic electrostatic potentials

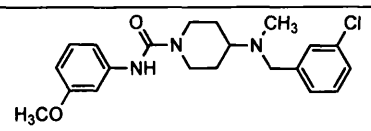
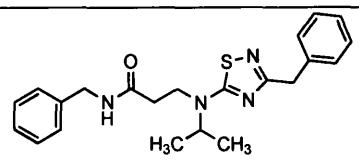
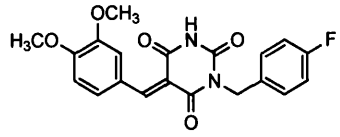
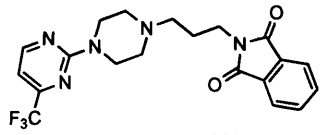
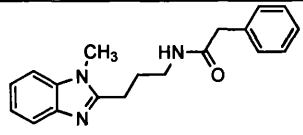
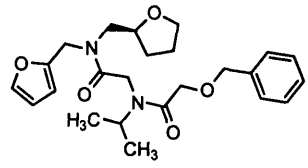
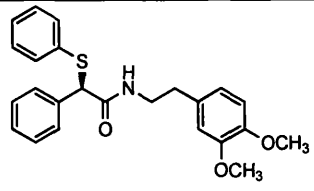
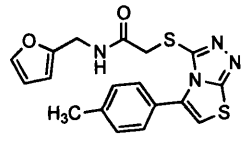
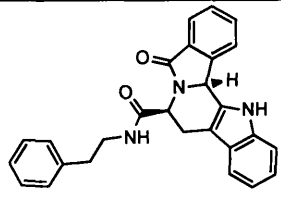
were then calculated for the active site enclosed within the grid. The program DOCK correlates the generated sphere centres on a protein surface with the atoms of the binding partners or ligands. Through utilising the scoring grids the optimum orientation of the ligand was determined with minimisation of energy based score.

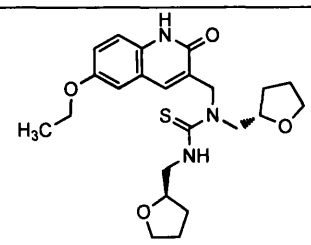
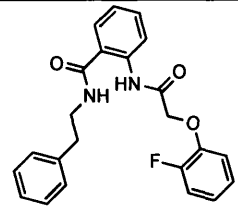
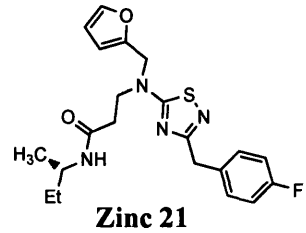
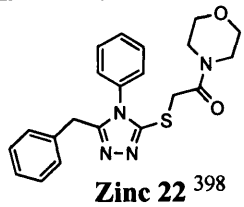
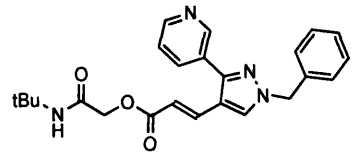
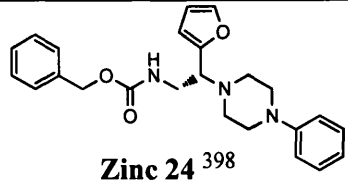
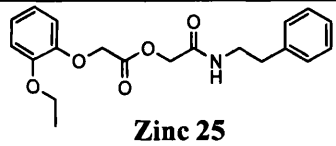
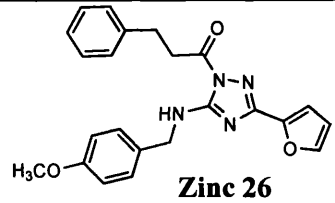
The top 50 hits from each of the subsets (4350 in total) were then selected by the AMBER program[397] based on an intermolecular energy score. The top 4350 hits from the first round of screening were then subjected to 3000 steps of molecular dynamics in AMBER to energy minimise the docked molecules while the ligand and the active site(s) remain flexible for small structural rearrangements. The new calculated energies (Amber Score) were then used to re-rank the compounds. Amber score corresponds to the binding energy involved during a ligand/active site interaction.

The top 100 hits were selected and analysed manually. Stringent criteria were set which the hits were required to meet in order to be chosen for further analysis. These included (i) the number of hydrogen bonds formed between the compound and active site and (ii) their occupancy of both the STA-21 and S3I-201 binding sites. On this basis, only 27 compounds (listed in Table 3.1) were chosen as promising candidates for synthesis, analysis in the primary biochemical assays and for the synthesis of related compound libraries.

Table 3.1: List of the "hit" compounds.

Hit rank	Zinc ID	Amber Score	No. H-bonds	Structure
1	6679729	-32.168854	1	 **Zinc 1**
2	6647321	-31.441601	1	 Zinc 2
3	3418389	-27.995914	0	 **Zinc 3**
4	3433724	-27.580748	2	 **Zinc 4**
5	6384749	-26.777020	3	 **Zinc 5**
6	5559299	-20.922855	0	 **Zinc 6** [398]
7	4913398	-20.169907	1	 **Zinc 7**
8	4883310	-18.451633	4	 **Zinc 8**
9	6703950	-17.638332	2	 **Zinc 9**

10	6750539	-17.084114	1	 **Zinc 10**
11	4796360	-16.946575	0	 **Zinc 11**
12	4823291	-16.716057	1	 **Zinc 12**
13	4299616	-16.503889		 **Zinc 13** [399]
14	2721473	-16.177917	2	 **Zinc 14**
15	4922146	-15.587399	3	 **Zinc 15**
16	2739191	-15.465598	3	 **Zinc 16**
17	937506	-15.394800		 **Zinc 17** [398]
18	4085246	-14.432161	0	 **Zinc 18**

19	879485	-12.735105	1	 **Zinc 19**
20	3426034	-12.592486	1	 **Zinc 20**
21	4526519	-12.574412	0	 **Zinc 21**
22	868706	-12.521400	0	 **Zinc 22** [398]
23	5806086	-10.972743	1	 **Zinc 23**
24	4272913	-7.511615	3	 **Zinc 24** [398]
25	3288369	-7.343826	0	 **Zinc 25**
26	5452189	-6.184393	2	 **Zinc 26**
27	3302749	-3.658094	0	 **Zinc 27**

3.1.1 Synthesis of the Potential "Hits"

The 27 molecules identified by the virtual screening were known compounds (the majority of them belonging to compound libraries owned by pharmaceutical companies). In summary, 14 compounds could be purchased commercially, 11 had to be synthesised in the laboratory, and 2 (**Zinc 18 & Zinc 19**) were not commercially available and were also considered sufficiently low ranking in the screen to not synthesise. Therefore, initially the chemistry research work was focused on the preparation of the "hit" compounds illustrated in Figure 3.3.

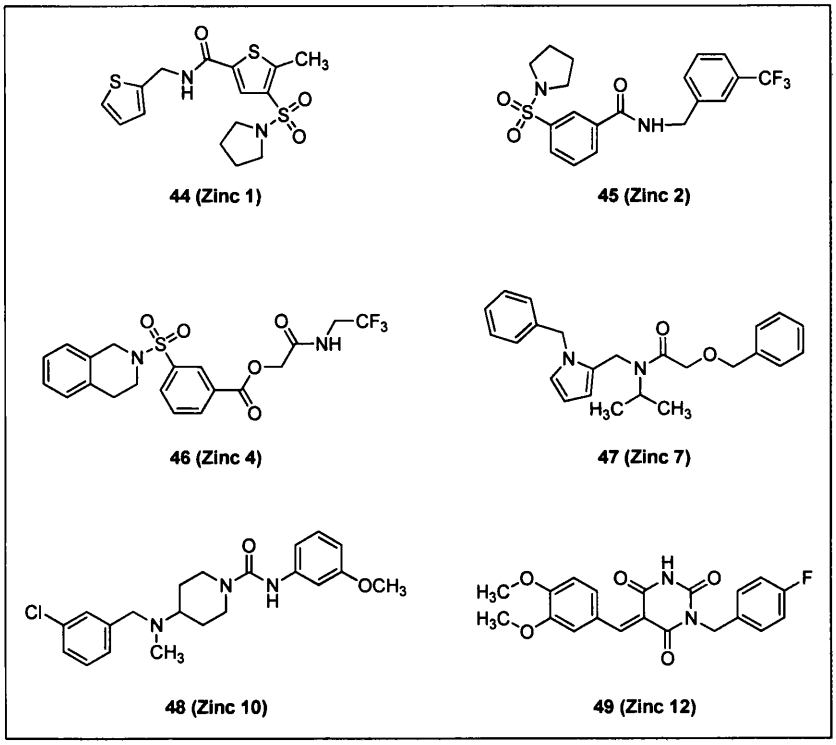
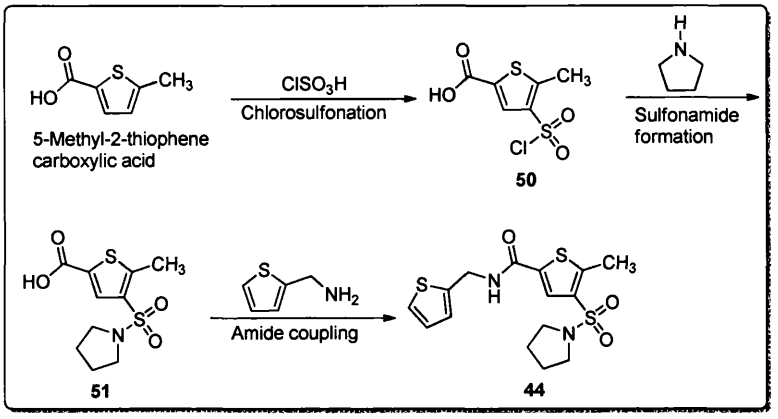


Figure 3.3: A selection of the synthesised "hit" compounds.

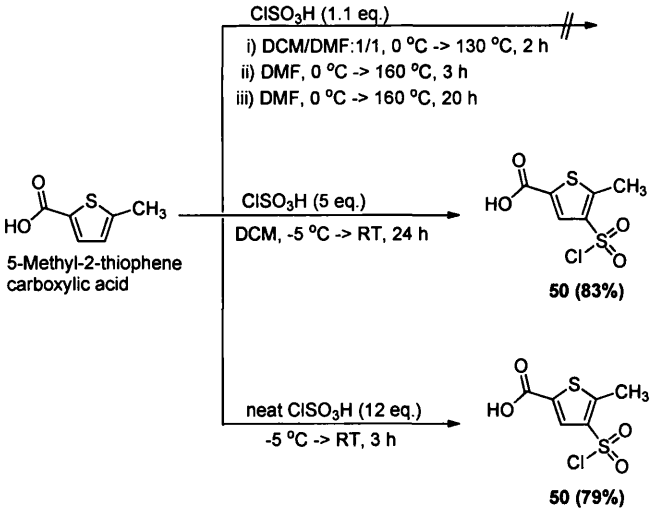
3.1.1.1 Synthesis of Compound 44 (Zinc 1)

Compound **44** was prepared in three steps according to the general synthetic route illustrated in Scheme 1.



Scheme 1: General scheme for synthesis of **44**.

Initially, chlorosulfonation of 5-methyl-2-thiophene carboxylic acid was attempted with 0.1 eq. excess of chlorosulfonic acid (99%) in different solvent systems and at different temperatures (Scheme 2). Although the starting material was totally consumed, formation of the desired product was not observed (LC-MS monitoring). The use, instead, of 5eq. of chlorosulfonic acid gave the chlorosulfonylated product **50** after 24 h at room temperature. The desired product **50** (≥ 98% pure, based on NMR and LC-MS analysis) was isolated in 83% yield as a single regioisomer.

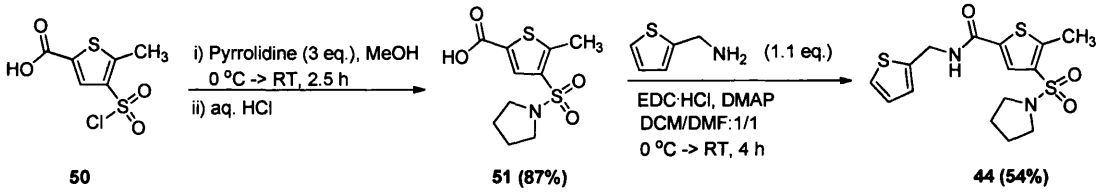


Scheme 2: Chlorosulfonation of 5-methyl-2-thiophene carboxylic acid.

With a larger excess of acid (*i.e.*, 12 eq.) and in neat conditions, cholorosulfonation was achieved within only 3 h and with a 79% yield (Scheme 2). In all cases, it was important to add the chlorosulfonic acid to the reaction mixture at low temperature (-5 °C) as the reaction is exothermic. The structure of the product was elucidated by 1D and 2D NMR, and mass spectral

analysis. The presence of a single regioisomer was confirmed through HMBC (Heteronuclear Multiple Bond Coherence) and ^{1}H NMR analysis.

The sulfonamide **51** of the chlorosulfonyl thiophene derivative **50** was formed *via* addition-elimination reaction[400] of **50** with excess pyrrolidine (3 eq.) in methanol. The excess of pyrrolidine allowed completion of the reaction within 2.5 h at room temperature in good yield (87%). The amide coupling between thiophene-2-methyl amine and **51** was achieved with the coupling reagents EDC·HCl and DMAP. The mechanism of the amide coupling is shown in Figure 3.4. The 0.1 eq. excess of the amine facilitated completion of the reaction (LC-MS and TLC monitoring) within 4 h at room temperature (Scheme 3). Column chromatography was employed to isolate the amide product (**44**) in 54% yield with a purity of ≥ 98% (based on NMR and LC-MS analysis). It was fully characterised by NMR, mass and elemental analysis. The reaction was not optimised, although modification of reaction conditions (*e.g.*, higher equivalents of amine) or employing different coupling reagents (*e.g.*, HATU) were considered as possible means to achieve this.



Scheme 3: Sulfonamide formation and amide coupling.

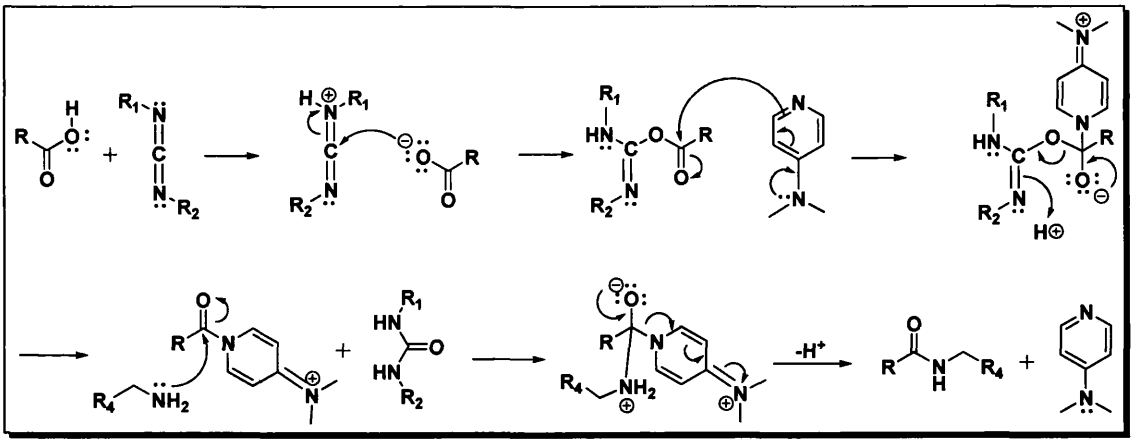
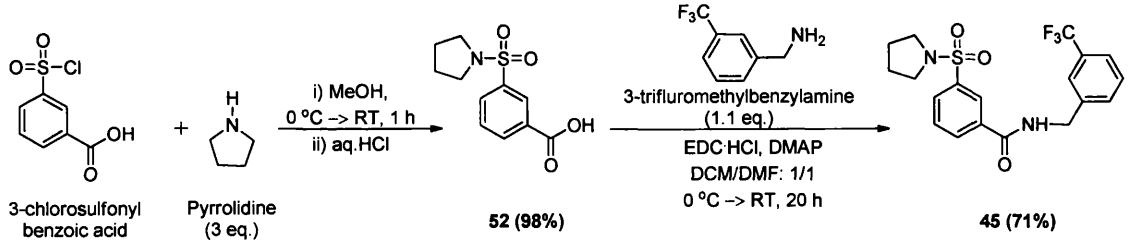


Figure 3.4: Mechanism of amide coupling using EDC·HCl and DMAP.

3.1.1.2 Synthesis of Compound 45 (Zinc 2)

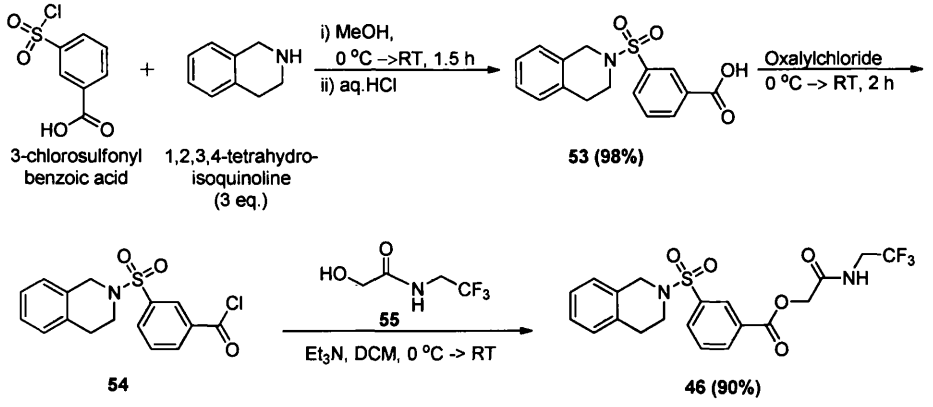
Compound **45** was prepared in two steps as illustrated in Scheme 4. Reaction of 3-chlorosulfonylbenzoic acid and an excess of pyrrolidine (3 eq.) in methanol at room temperature afforded the sulfonamide **52** in an addition-elimination mechanism.[400] The excess of pyrrolidine drove the reaction to completion within 3 h in good yield (98%). Amide coupling between **52** and 3-trifluoromethylbenzylamine was achieved using the coupling reagents EDC·HCl and DMAP. A 10% excess of the amine was employed, and starting material had disappeared after 20 h at room temperature according to LC-MS and TLC (Scheme 4). The resulting amide (**45**) was isolated in 71% yield and high purity (≥ 98%, based on NMR and LC-MS analysis) using gravity column chromatography with silica gel. The final product was fully characterised by 1D and 2D NMR, and mass spectroscopy.



Scheme 4: Synthetic route for the preparation of **45**.

3.1.1.3 Synthesis of Compound 46 (Zinc 4)

Compound **46** was prepared in three steps according to the route shown in Scheme 5. The sulfonamide **53** was obtained by coupling 3-chlorosulfonylbenzoic acid with an excess of tetrahydroisoquinoline (3 eq.) in methanol at room temperature for 1.5 h. The desired product **53** was isolated in 98% yield (Scheme 5).



Scheme 5: Synthetic route for preparation of **46**.

Next, oxalyl chloride was used to prepare the acylchloride **54** using an excess of oxalyl chloride (2.5 eq.), and a drop of DMF. The mechanism of the catalytic activity of DMF is shown in Figure 3.5. The final step involved reaction of the acylchloride **54** with alcohol **55** at room

temperature in DCM, employing TEA as organic base (Scheme 5). Reaction was completed within four hours, and the desired product **46** was isolated by column chromatography in good yield (90%) and high purity (≥ 98%, based on NMR and LC-MS analysis), and was fully characterised by NMR, mass spectroscopy and elemental analysis.

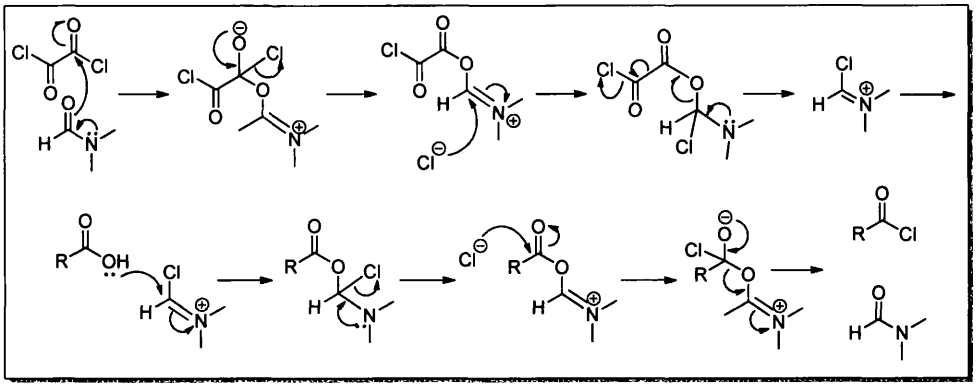
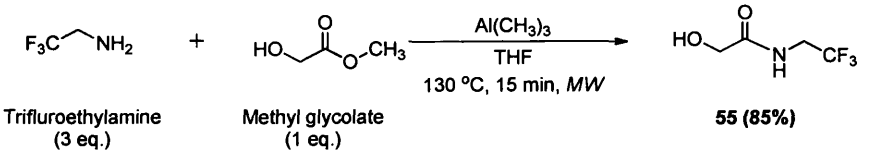


Figure 3.5: Mechanism of the formation of acyl chlorides from the corresponding carboxylic acids employing oxalyl chloride and DMF.

The non-commercially available alcohol **55** was prepared according to the method of Evans *et al*[401] by treating a mixture of trifluoroethylamine and methyl glycolate with trimethylaluminium in THF. Flash column chromatography on silica gel afforded the alcohol **55** in 85% yield with purity of ≥ 98% (based on NMR and LC-MS analysis) (Scheme 6).

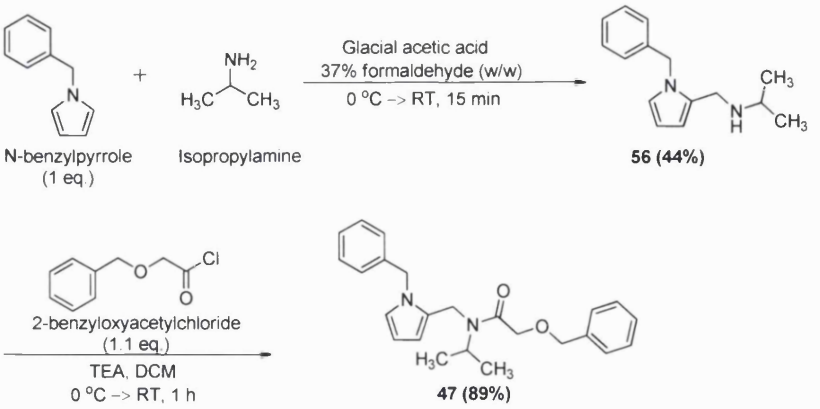


Scheme 6: Synthesis of the non-commercially available alcohol **55**.

3.1.1.4 Preparation of Compound 47 (Zinc 7)

Compound **47** was prepared in two steps *via* a Mannich reaction to form the intermediate **56** followed by amide formation as outlined in Scheme 7. Condensation of a CH-activated compound (N-benzylpyrrole) with a primary or secondary amine (isopropylamine) and a non-enolizable aldehyde (formaldehyde) to afford aminoalkylated derivatives such as **56** is known as a Mannich reaction.

The formation of the Mannich base **56** was accomplished by condensing N-benzylpyrrole with isopropylamine and formaldehyde using the catalytic activity of glacial acetic acid. Intermediate **56** was isolated with 44% yield with good purity (≥ 99%, based on NMR and LC-MS analysis) by employing flash chromatography on silica gel (Scheme 7).



Scheme 7: Synthesis of **47** using the Mannich reaction.

The presence of a single regioisomer was confirmed by HMBC as well as NOESY. In the NOESY 2D spectra, the -CH_2- protons, H^7 have spatial connectivity to the benzylic proton H^6 and the pyrrolic proton H^5 has also shown connectivity to the benzylic proton H^6 (Figure 3.6A). The absence of spatial connectivity between H^7 and H^2 (or H^4) (Figure 3.6B) confirmed the product to be a single regioisomer. The mechanism of the Mannich reaction is illustrated in Figure 3.7.

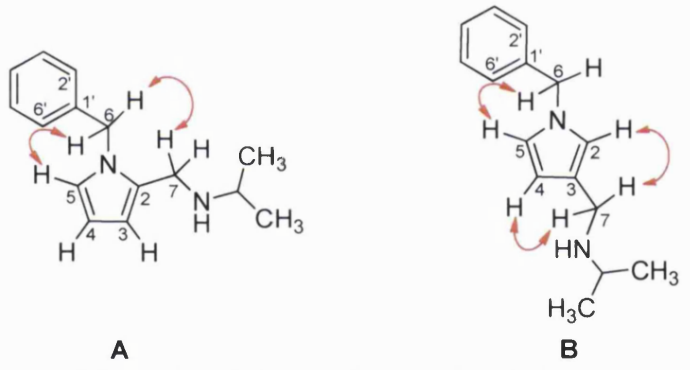


Figure 3.6: Spatial connectivity confirmed the formation of a single regioisomer in the Mannich reaction.

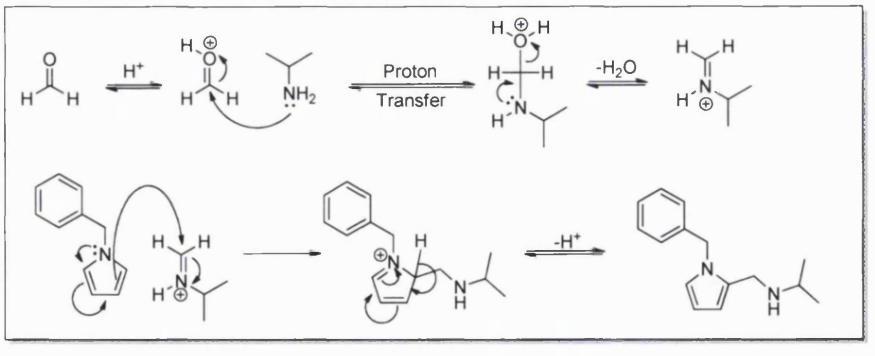


Figure 3.7: Mechanism of the Mannich reaction.

It may be highlighted that the yields of the Mannich bases obtained employing primary amines with formaldehyde on similar substrates described in literature lie between 15% and 40%.[402,403] Nevertheless, different reaction conditions (*e.g.*, solvent system, acid) have been investigated in the attempt to optimise the formation of the Mannich base **56** (Table 3.2). The reaction was carried out with the stoichiometry mentioned in entries 1-3, based on the reaction protocol

outlined on a similar substrate in the literature (Table 3.2).[404] The reaction was conducted using 27 equivalent of glacial acetic acid, 2.7 equivalent of formaldehyde solution and 3 equivalent of isopropylamine for variable reaction times ranging from 10 minutes to 5 h. It was observed that prolonged reaction time (5 h) resulted in no product formation, whereas 30 minutes gave better a yield (12%) compared to a 10 min reaction (6%). Based on these initial results it was noticed that time was a key factor. Following this observation, the reaction was then tried for 15 minutes varying the amount of acid. It was found that 6 equivalents of glacial acetic acid provided the desired product in 39% yield (entry 5), and 13 equivalent of the acid afforded 44% yield (entry 4). Then, the nature of the acid was taken into consideration. Conducting the reaction using mineral acid (HCl) (entry 6) instead of glacial acetic acid with identical stoichiometry for entries 1-3 resulted in no product. The use of a Lewis acid (i.e. yttrium triflate) was also explored. Using the protocol for a similar substrate described in the literature,[405] the stoichiometry for the reagents was kept the same (entry 7) and the type of acid was varied (entries 8 and 9). None of the reactions led to formation of the desired product. Varying the stoichiometry of the reagents was also investigated and in some cases the salt of isopropylamine was employed (entry 10). However, formation of the desired product was not observed.

Table 3.2: Reaction conditions investigated for Mannich reaction. All the reactions have been conducted at room temperature.

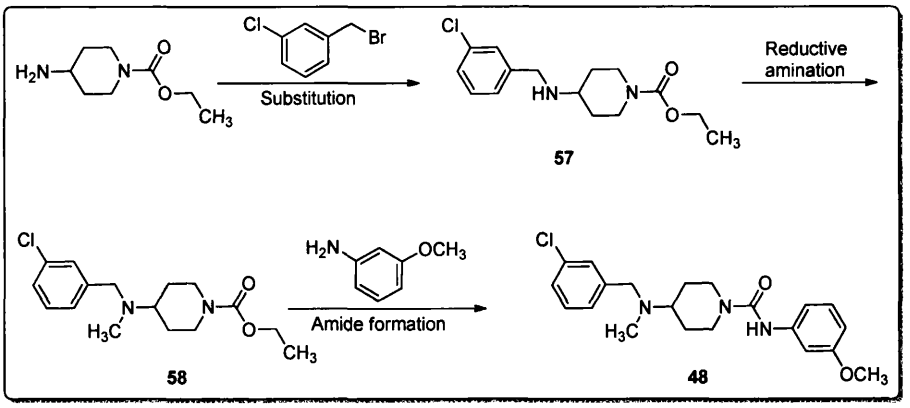
Entry	Reagent (Equivalent)				Solvent	Time	Yield (%)
	Acid	HCHO 37% (w/w)	NH_2	$(CF_3SO_3)_3Y$			
1	MeCOOH (27)	2.7	3	-	-	10 min	6
2	MeCOOH (27)	2.7	3	-	-	30 min	12
3	MeCOOH (27)	2.7	3	-	-	5 h	-
4	MeCOOH (13)	2.7	3	-	-	15 min	44
5	MeCOOH (6)	2.7	3	-	-	15 min	39
6	HCl (27)	2.7	3	-	-	2 h	-
7	-	90	1.33*	0.133	H_2O:THF = 10:1	20 h	-
8	MeCOOH (2)	90	1.33	0.133	H_2O:THF = 10:1	3 h	-
9	HCl (2)	90	1.33	0.133	H_2O:THF = 10:1	3 h	-
10	-	1	1*	-	H_2O	24 h	-

* as hydrochloride salt

In the second and final synthetic steps the condensation of Mannich base **56** and 2-benzyloxyacetyl chloride was conducted in DCM using TEA. An excess of highly reactive acetyl chloride (1.1 eq.) drove the condensation to completion within 1h at room temperature (Scheme 7). The desired amide **47** was isolated in 89% yield (≥ 98% pure, based on NMR and LC-MS analysis), and was fully characterised by NMR and mass spectroscopy.

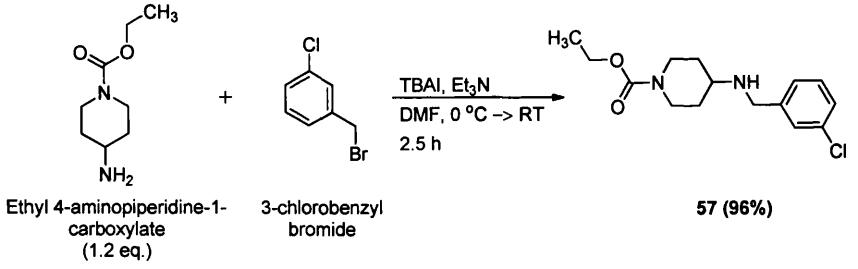
3.1.1.5 Preparation of Compound 48 (Zinc 10)

The compound **48** was prepared in 3 steps according to the synthetic pathway outlined in Scheme 8.



Scheme 8: General scheme for synthesis of compound **48**.

The condensation of the ethyl 4-aminopiperidine-1-carboxylate and 1-bromomethyl-3-chlorobenzene was performed in DMF at room temperature within 2.5 h (Scheme 9). Catalytic amount of TBAI (0.1 eq.) and a small excess of TEA (1.2 eq.) was sufficient for the reaction to be completed (monitored by LC-MS). The secondary amine **57** was isolated in good yield (96%) and high purity (≥ 99%, based on NMR and LC-MS analysis) by flash column chromatography on silica gel and was fully characterised.

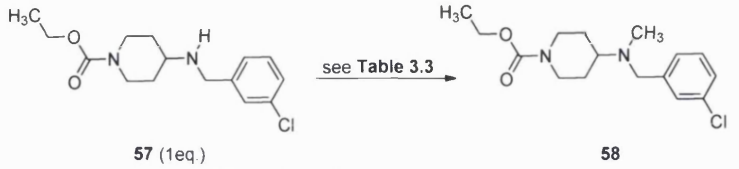


Scheme 9: Formation of intermediate **57**.

The methylation of the N-atom to form tertiary amine **58** was performed *via* reductive amination employing paraformaldehyde, zinc dust and glacial acetic acid. The desired product **58** was isolated by column chromatography in 50% yield (Scheme 10). An attempt to optimise the yield using different solvent systems and reaction conditions were investigated (Table 3.3). Each time the product was purified by column chromatography (≥ 98% pure, based on NMR and LC-MS analysis).

Initially the reductive amination was conducted using three different reducing agents in an organic solvent system, with the reaction time ranging from 18 h to 48 h (entries 1-4). Using both zinc chloride and sodium triacetoxyborohydride the first three reactions (entries 1-3) were considered at three different reaction times which afforded the yields between 30% to 43%.

Based on these initial results it appeared that the yield of the reaction was dependent on time although this could be simply due to normal experimental variation. Sodium cyanoborohydride as reducing agent did not give the product. Then, acidic aqueous media and the use of zinc as reducing agent were used. On similar a substrate, described in the literature[406] with Zn dust employed in acidic aqueous medium (entries 5-6) for the reductive amination. This resulted in a comparable yield of 50%.



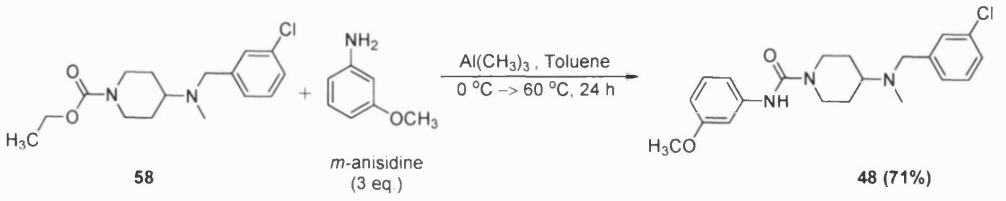
Scheme 10. Synthesis of the compound **58** *via* reductive amination.

Table 3.3: Reaction conditions investigated for reductive amination. All the reactions have been conducted at room temperature.

Entry	Reagent (Equivalent)			Solvent	Time (h)	Yield (%)
	Reducing agent	HCHO*	MeCOOH			
1	$ZnCl_2$ (2) $NaBH(CH_3COO)_3$ (2)	2	1 drop	DCM:MeOH = 20:1	24	43
2	$ZnCl_2$ (2) $NaBH(CH_3COO)_3$ (2)	2	1 drop	DCM:EtOH = 2:1	48	37
3	$ZnCl_2$ (2) $NaBH(CH_3COO)_3$ (2)	2.5	1 drop	DCM:EtOH = 2:1	18	30
4	$ZnCl_2$ (2) $NaBH_3CN$ (2)	2	1 drop	DCM:EtOH = 2:1	24	-
5	Zn dust (2)	1.5	4	H_2O	24	50
6	Zn dust (4)	3	6	H_2O	24	49

* Paraformaldehyde and foramaldehyde 37% solution (w/w) was used for entries 1-4 and entries 5-6 respectively.

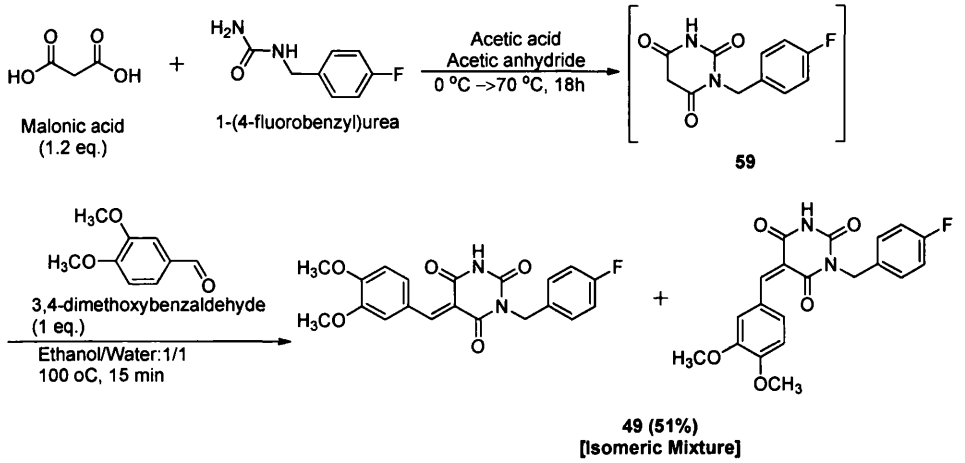
In the third and final steps of the synthetic route, formation of the amide from *m*-anisidine and ester **58** was conducted using the strong Lewis acid, trimethylaluminium in toluene (Scheme 11). An excess of amine (3 eq.) and heating (up to 60 °C) helped to complete the reaction within 24 h. The addition of trimethylaluminium (2.5 eq.) to the reaction mixture was strictly maintained at 0 °C. The desired product **48** isolated by column chromatography in 71% yield (≥ 99% pure, based on NMR and LC-MS analysis) was fully characterised by 1D and 2D NMR and mass spectroscopy.



Scheme 11: Synthesis of the amide **48**.

3.1.1.6 Preparation of Compound 49 (Zinc 12)

The intermediate **49** was formed by condensing malonic acid with 1-(4-fluorobenzyl) urea. An excess of acetic anhydride (3 eq.) and acetic acid (20 eq.) yielded the condensation product **59** within 18 h (Scheme 12). An excess of malonic acid (1.2 eq.) and heating (up to 70 °C) ensured the total conversion of urea to the barbiturate derivative **59** (Monitoring by LC-MS).



Scheme 12: Two-steps synthesis of compound **49**.

The mixture of intermediate **59** and the 3,4-dimethoxybenzaldehyde in EtOH/H_2O (1/1) was heated under reflux for 3 h to afford the aldol type condensation product **49**. Compound **49** (observed by LC-MS) was isolated (51 % yield) as an isomeric mixture (2:1) and was characterised by ^1H NMR and ^{13}C NMR. An attempt to separate the diastereomers failed due to poor solubility in appropriate organic solvents during purification (*e.g.*, column chromatography, crystallisation). The mechanism of barbiturate derivative formation is illustrated in Figure 3.8.

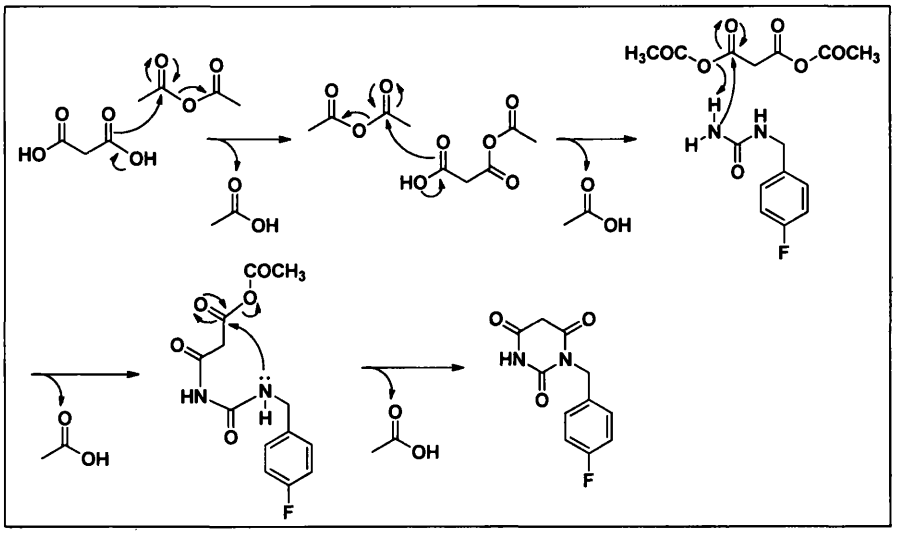


Figure 3.8: Mechanism for the formation of the intermediate **59**.

3.2 Design of Novel Small-Molecule Inhibitors of the STAT3:STAT3 Interactions

Further *in silico* studies and a structure-based design approach was carried out by modifying the top "hit" of the virtual screening list to potentially improve its binding profile. Once the docked conformation and orientation of ligands were identified by automated docking, further modifications were carried out on the top hit molecule (**Zinc 1**). An electrostatic map was generated for the protein using the program APBS.[407] This was analysed in detail around the ligand binding site with each atom being assigned partial charges. This helped us to position chemical groups on the ligand with complementary charges. The ligand-protein complex with assigned charges was then imported to the program InsightII for further analysis.

The next step was to add functional groups to the chemical skeleton of the ligand based on charge and shape, complementary to the binding site on the receptor. The substituents at the C2 and C4 positions of the thiophene ring of the top hit compound were found to dock into the two targeted pockets forming possible H-bonds with $Ser^{611}$and Glu^{612} (identical interacting residues like pY^{705} described in Figure 3.2b of section **3.1**) and with Phe^{610}. The carbonyl oxygen of amide bond forms H-bond with Arg^{609} (identical interacting residue like pY^{705} described in Figure 3.2b of section **3.1**). Thus, to enhance the binding affinity of the top hit from the virtual screen, the methyl group at the C5 position was replaced by a tolyl group to explore the hydrophobic environment which was identified within 6Å of the Tyr phopshorylated residue of the STAT3 SH2 domain. Figure 3.9 shows the docking of the modified hit that can maintain the H-bonding with the important interacting residues (Arg^{609}, Ser^{611}, Glu^{612} and Ser^{613}) as well as can access the hydrophobic pocket near Ser^{636} residue. The binding energies for the new ligand (**60,** Figure 3.10) were calculated and improved to –45.7 Kcal mol^{-1} from –39.6 Kcal mol^{-1}.

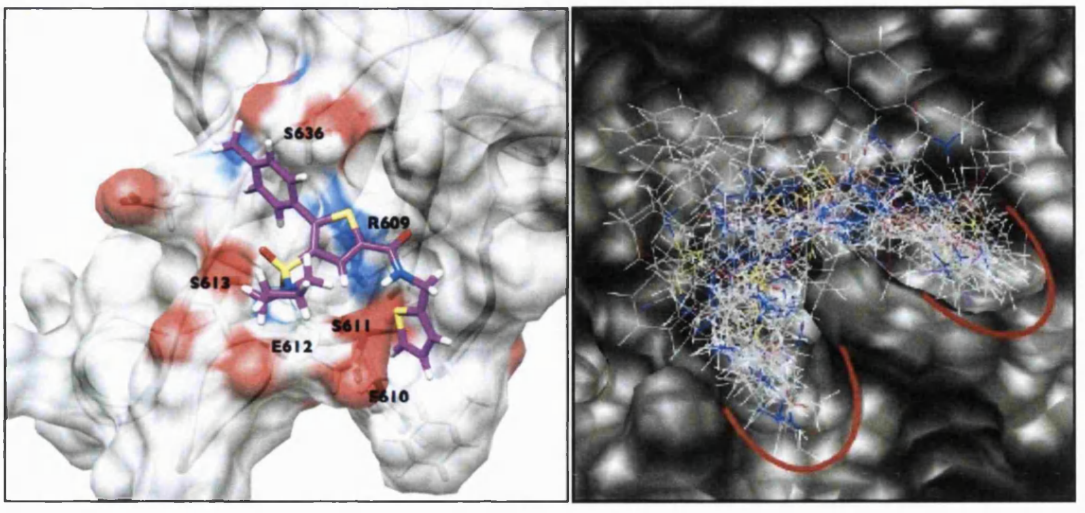


Figure 3.9: The docking of ligand **60** on the STAT3 SH2 domain with possible interactions (left); the docking of members of the library of compounds based on molecular modifications (right).

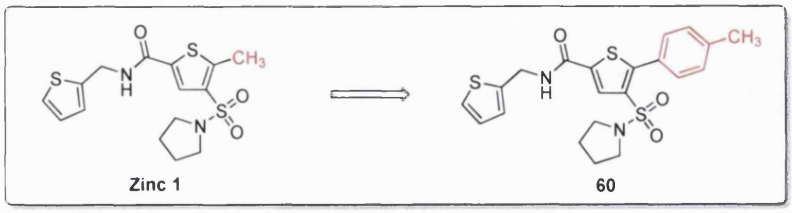


Figure 3.10: The compound **60** identified through *in silico* design.

This ligand was chosen as the top hit and taken for further medicinal chemistry studies for the preparation of focused libraries to identify drug-like small molecules as potential STAT3:STAT3 inhibitors. The focused libraries (**A** - **G**) have been designed and synthesised following modifications of compound **60** (Figure 3.11):

Library **A**: Substitution on the phenyl ring of position 5 of thiophene ring **A**.

Library **B**: Substitution of aryl moiety on position 5 of thiophene ring **A**.

Library **C**: Alteration of sulfonamide moiety.

Library **D**: Substitution on the thiophene ring **B**.

Library **E**: Alteration of the thiophene ring **B** system.

Library **F**: Alteration of the hetero atom of thiophene ring **A**.

Library **G**: Alteration of position of sulfonyl moiety.

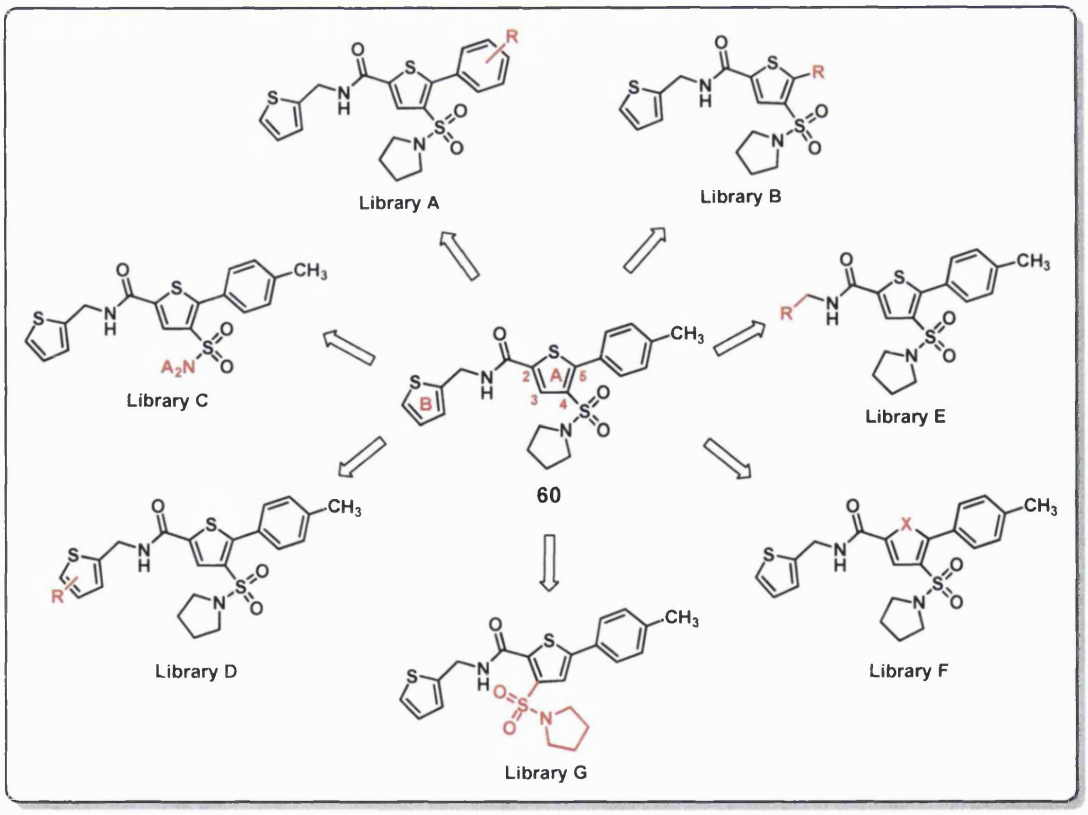


Figure 3.11: Structural modifications of **60** for the preparation of the focused libraries (**A** - **G**).

The focused libraries (**H**, **I** & **J**) have been designed and synthesised following modifications of second top hit compound **45** (Figure 3.12):

Library **H**: Alteration of substituents and their position on the aminobenzyl ring.

Library **I**: Alteration of ring system of sulfonamide moiety.

Library **J**: Alteration of position of the substitution on benzene ring.

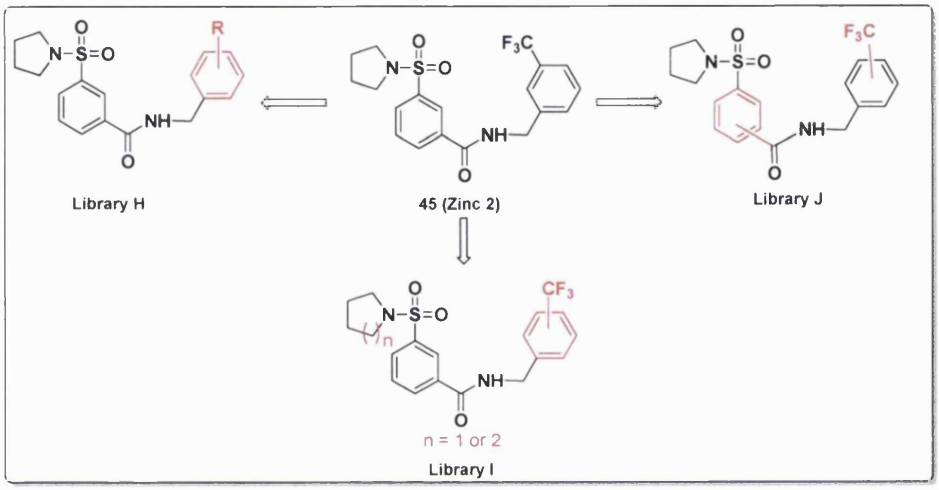
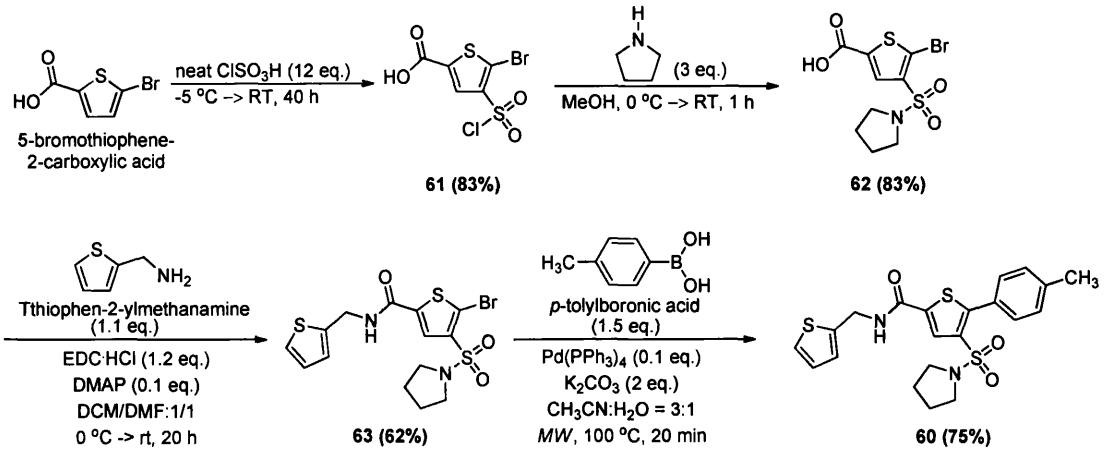


Figure 3.12: Structural modifications of second top "hit" molecule **45** for the preparation of the focused libraries (**H**, **I** & **J**).

3.3 Synthesis of the Novel Small-Molecule Ligands

3.3.1 Synthesis of the Molecule (60) Designed by an *In Silico* & Structure-Based Approach

The compound **60** was prepared in 4 steps as illustrated in Scheme 13.



Scheme 13: Synthesis of compound **60**.

Chlorosulfonation of 5-bromo-2-thiophene carboxylic acid was accomplished with an excess of chlorosulfonic acid (99%, 12 eq.) in neat conditions (Scheme 13). The starting material was totally consumed and formation of the desired product was observed (LC-MS monitoring) within 40 h. The product (≥ 98% pure, based on NMR and LC-MS analysis) was crystallized (83% yield) as a single regioisomer. The addition of chlorosulfonic acid to the reaction mixture was carried out at low temperature (-5 °C) as the reaction was exothermic. The structure of the product was elucidated by extensive 1D, 2D NMR and mass spectroscopy. The presence of a single regioisomer was confirmed by HMBC and ^{1}H NMR analysis as well as by NOESY.

The sulphonamide intermediate **62** could be formed *via* addition-elimination reaction[400] of **61** and excess pyrrolidine (3 eq.) in methanol. The excess of pyrrolidine allowed the reaction to be completed within 1 h at room temperature in good yield (83%) (Scheme 13). The next amide coupling step between thiophene-2-methylamine and the carboxylic acid derivative **62** was conducted using the coupling reagents EDC·HCl and DMAP. A slight excess of the amine (1.1eq) forced the reaction to completion (LC-MS and TLC monitoring) within 20 h at room temperature (Scheme 13). Column chromatography was employed to isolate the amide **63** in 62% yield with a purity of ≥ 98% (based on NMR and LC-MS analysis). The addition of the amine to the reaction mixture was carried out at 0 °C due to the exothermic nature of the reaction. The yield was not optimised, although it is envisaged that modifying the reaction conditions (*e.g.*, solvent system, stoichiometry) or employing a different coupling reagent (*e.g.*, HATU) could improve the yield.

The palladium-catalyzed cross coupling reaction between organoboron compounds and organic halides or triflates is a powerful and general method of forming carbon-carbon bonds and is known as Suzuki cross coupling. The next step involved Suzuki cross coupling reaction between **63** and *p*-tolylboronic acid, employing tetrakis(triphenylphosphine)palladium (0.1 eq.) and K_2CO_3 (2 eq.) as Pd-catalyst and base, respectively. Five equivalents of the boronic acid drove the reaction to completion within 20 min at 100 °C using microwave irradiation (Scheme 13). The complete consumption of starting material was confirmed by LC-MS. The desired product **60** in isolated with 75% yield and high purity (≥ 98%, based on NMR and LC-MS analysis) by column chromatography, and was fully characterised by NMR, mass spectral analysis and elemental analysis. The general mechanism of the Suzuki cross coupling is illustrated in Figure 3.13.

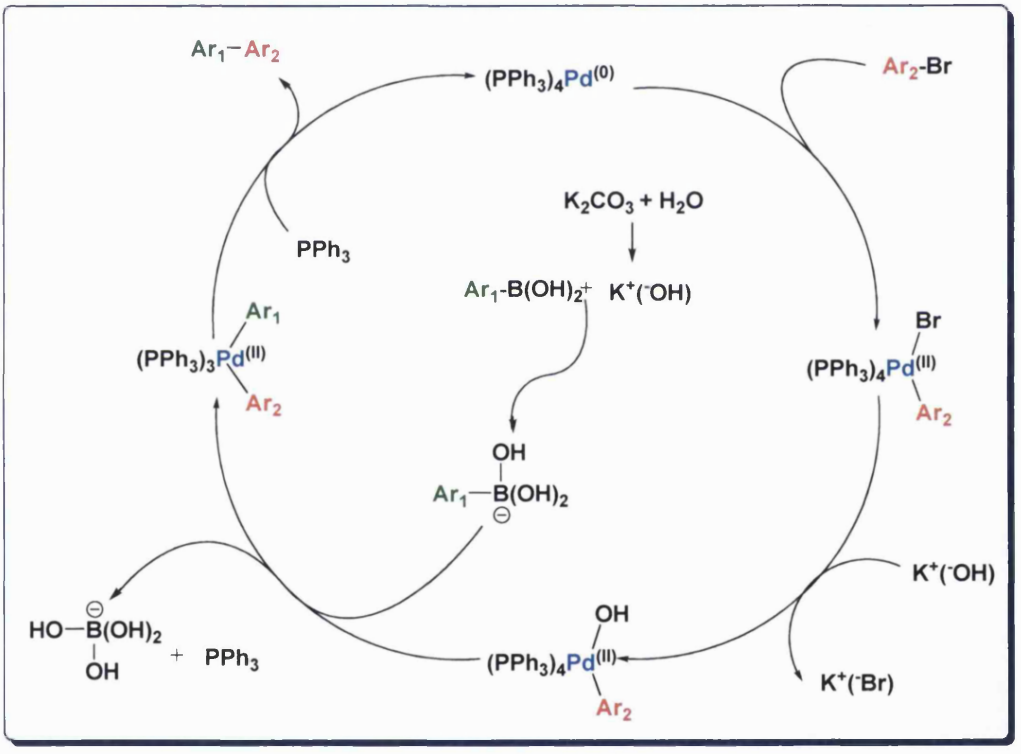
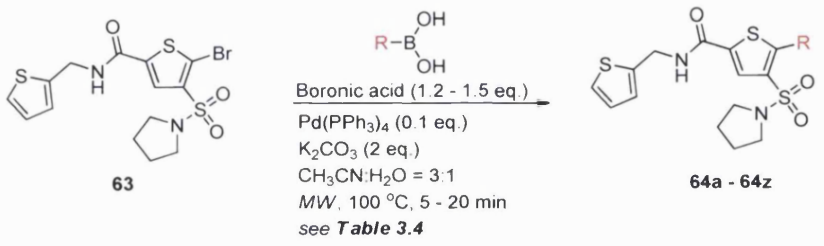


Figure 3.13: Mechanism of the Suzuki cross-coupling reaction.

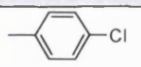
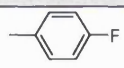
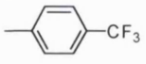
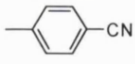
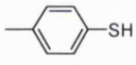
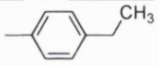
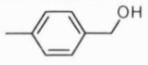
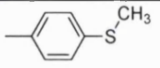
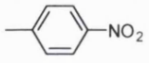
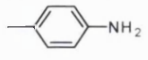
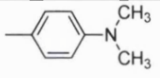
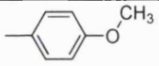
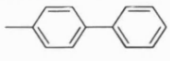
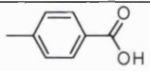
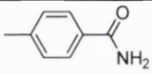
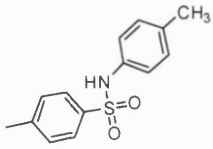
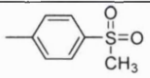
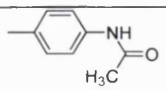
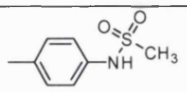
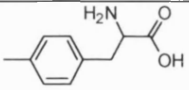
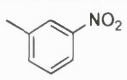
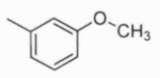
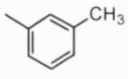
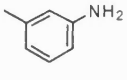
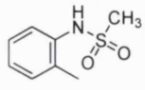
3.3.2 Synthesis of Focused Library "A"

Compounds **64a – 64z** were prepared *via* Suzuki cross-coupling of three different substituted phenyl boronic acid with the common intermediate **63** (Scheme 14). Similar procedures and stoichiometries of reagents were employed for the synthesis of compounds **64a – 64z** as were utilised for the synthesis of compound **60** (section **3.3**). In each case, the consumption of starting material was confirmed by LC-MS. The library compounds were isolated in overall good yield and high purity (≥ 98%, based on NMR and LC-MS analysis) by column chromatography and were fully characterised by NMR, mass spectral analysis and elemental analysis. The yield of these compounds and their corresponding boronic acids are listed in Table 3.4. The intermediate **63** was synthesised as outlined in Scheme 13 (section **3.3**).



Scheme 14: Generalised synthetic route for compounds **64a – 64z.**

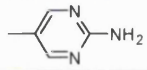
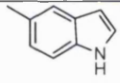
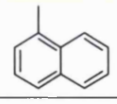
Table 3.4: Yields (Y) of compounds **64a – 64z** using different boronic acid in the Suzuki cross coupling reaction. (*as pinacol ester; [‡]Reaction carried out at 130 °C)

Ligand	R	Y (%)	Ligand	R	Y (%)	Ligand	R	Y (%)
64a		88	**64b**		86	**64c**		44
64d		95	**64e**		91	**64f**		88
64g		90	**64h**		88	**64i**		59
64j		82	**64k***		65	**64l**		75
64m		29	**64n**		63	**64o**		64
64p		85	**64q***		72	**64r**		93
64s*		66				**64t***		41
64u[‡]		78	**64v**		86	**64w**		85
64x		75	**64y**		59	**64z***		21

3.3.3 Synthesis of Focused Library "B"

Compounds **65a – 65i** were prepared *via* Suzuki cross-coupling of three different aromatic and heteroaromatic boronic acids with the common intermediate **63** (Scheme 14). Similar procedures and stoichiometries of reagents were employed to those used for the synthesis of compound **60** (section **3.3**). The consumption of starting material was monitored by LC-MS. The library compounds were isolated in several good yield and high purity (≥ 98%, based on NMR and LC-MS analysis) by column chromatography and were fully characterised by NMR, mass spectral analysis and elemental analysis. The corresponding boronic acids and the yields of products are listed in Table 3.5. The intermediate **63** was synthesised as outlined in Scheme 13 (section **3.3**).

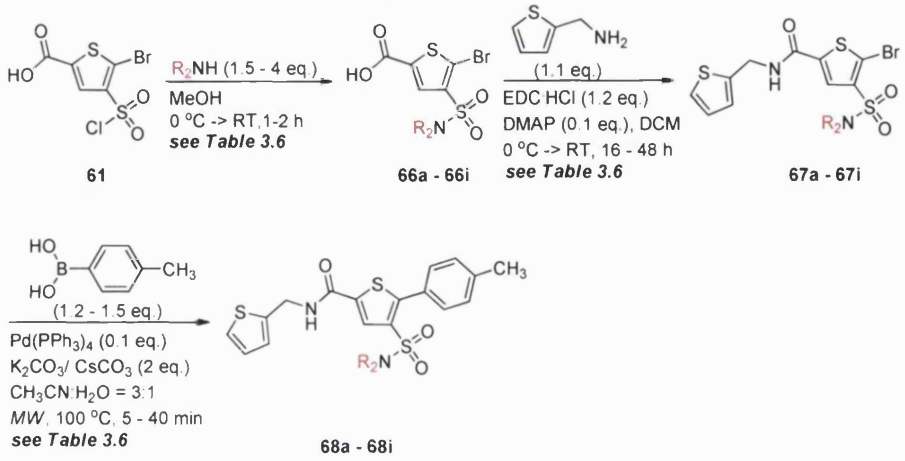
Table 3.5: Yields of compounds **65a – 65i** using different boronic acid in the Suzuki cross coupling reaction.

Ligand	R	Yield (%)	Ligand	R	Yield (%)	Ligand	R	Yield (%)
65a		70	**65b**		49	**65c***		74
65d		75	**65e**		87	**65f**		55
65g		98	**65h**		83	**65i**		87

(*as pinacol ester)

3.3.4 Synthesis of Focused Library "C"

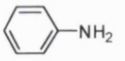
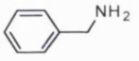
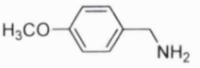
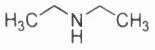
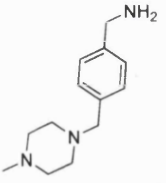
A library of sulfonamides **66a – 66i** was prepared *via* addition-elimination reaction[400] of the common intermediate **61** with different secondary amines (Scheme 15). An excess of the amine (1.5 – 4.0 equiv.) in each case allowed the reactions to be completed the reaction within 1 – 2 h at room temperature (Scheme 15).



Scheme 15: Generalised synthetic route for the compounds **68a – 68i**.

Similar procedures and stoichiometries of reagents were employed for the amide coupling and Suzuki cross-coupling steps in the synthesis of compounds **68a – 68i** from the intermediate amides **66a – 66i** based on the synthesis of intermediate amide **63** and compound **60** (section **3.3**). The consumption of starting material was monitored by TLC and LC-MS. The library compounds and their corresponding starting amides were isolated in overall good yield and high purity (≥ 98%, based on NMR and LC-MS analysis) by column chromatography and were fully characterised by NMR, mass spectral analysis and elemental analysis. The yield of these compounds and the corresponding intermediate and secondary amines are listed in Table 3.6. The intermediate **61** was synthesised as outlined in Scheme 13 (section **3.3**).

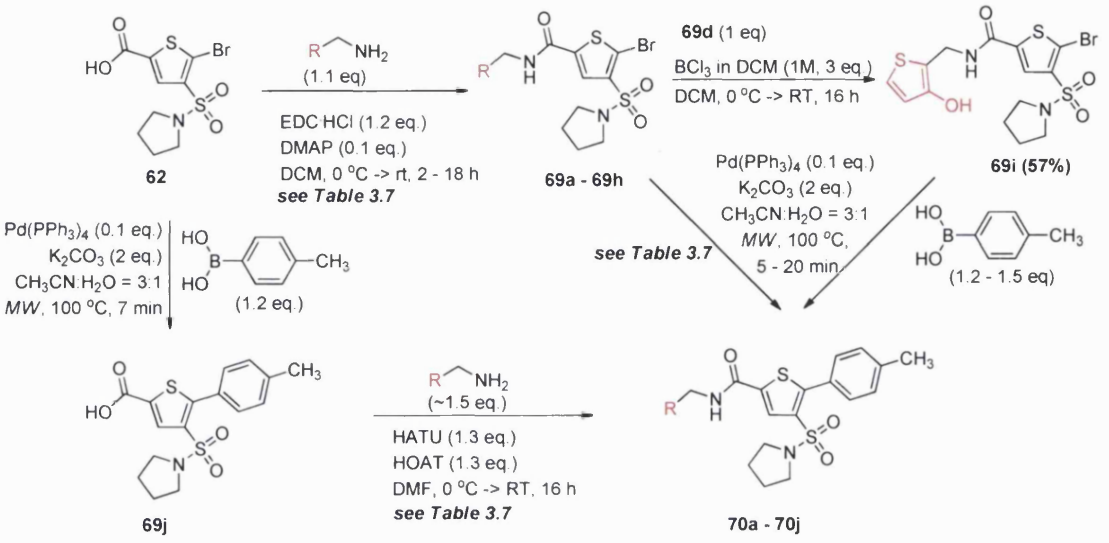
Table 3.6: Yields (Y) of compounds **68a – 68i** using different secondary amines.

R_2NH	Sulfon-amide (Y %)	Amide (Y %)	Ligand (Y %)	R_2NH	Sulfon-amide (Y %)	Amide (Y %)	Ligand (Y %)
	**66a** (68%)	**67a** (65%)	**68a** (75%)		**66f** (-%)	**67f** (-%)	**68f*** (29%)
	**66b** (83%)	**67b** (54%)	**68b** (72%)		**66g** (-%)	**67g** (-%)	**68g*** (7%)
	**66c** (68%)	**67c** (-%)	**68c** (55%)		**66h** (-%)	**67h** (-%)	**68h*** (6%)
	**66d** (-%)	**67d** (-%)	**68d*** (55%)		**66i** (-%)	**67i** (-%)	**68i*** (13%)
	**66e** (-%)	**67e** (-%)	**68e*** (13%)				

(-%) denotes the intermediates were not isolated before moving to the next steps.

3.3.5 Synthesis of Focused Library "D"

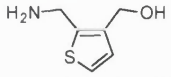
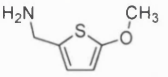
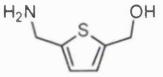
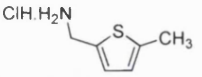
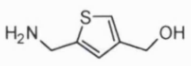
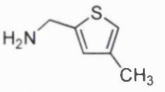
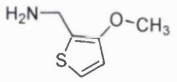
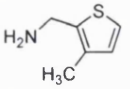
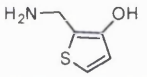
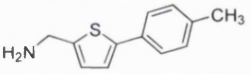
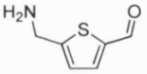
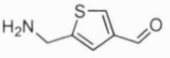
A library of intermediate amides **69a – 69h** were prepared *via* amide coupling of common intermediate **62** with different primary amines (Scheme 16). Using a slight excess of EDC·HCl and a catalytic amount of DMAP as coupling reagent helped to complete the reaction at room temperature within 2 – 18 h.



Scheme 16: Generalised synthetic route for compounds **70a – 70j**.

A similar procedure and stoichiometry of reagents as used for compound **60** (section **3.3**) were employed for the Suzuki cross-coupling for the synthesis of compounds **70a – 70h** from intermediates **69a – 69h**. The complete consumption of starting material was confirmed by TLC and LC-MS. The library compounds and their corresponding starting amides were isolated with high purity (≥ 98%, based on NMR and LC-MS analysis) by column chromatography and were fully characterised by NMR, mass spectral analysis and elemental analysis. The yields of these compounds and their corresponding intermediates and primary amines are listed in Table 3.7. The intermediate **62** was synthesised as outlined in Scheme 13 (section **3.3**).

Table 3.7: Yields of compounds **70a** – **70j** using different substituted thiophen-2-ylmethanamines.

H_2N R	Inter-mediate (Yield %)	Ligand (Yield %)	H_2N R	Inter-mediate (Yield %)	Ligand (Yield %)
	**69a** (43%)	**70a** (93%)		**69e** (86%)	**70e** (90%)
	**69b** (40%)	**70b** (33%)		**69f** (62%)	**70f** (80%)
	**69c** (46%)	**70c** (56%)		**69g** (68%)	**70g** (88%)
	**69d** (68%)	**70d** (90%)		**69h** (77%)	**70h** (21%)
	**69i** (57%)	**70i** (38%)		**69j** (65%)	**70j** (30%)
	-	**70k** (33%)		-	**70l** (26%)

The intermediate **69i** was obtained through ether cleavage of **69d** with the help of Lewis acid. BCl_3 in DCM (1M) was used in excess (3 eq) to complete the conversion of the ether to its corresponding alcohol (**69i**) within 16 h at room temperature. The compound **70i** and intermediate **69j** were synthesised from their corresponding starting materials using a similar procedure and stoichiometry of reagents to those employed for the Suzuki cross-coupling for the synthesis of **60** (scheme 16). Compound **70j** was obtained through amide coupling between (5-(p-tolyl)thiophen-2-yl)methanamine and carboxylic acid derivative **69j** using the coupling reagents HATU and HOAT. Excess amine (~1.5 eq) forced the reaction to completion (LC-MS and TLC monitoring) within 16 h at room temperature (Scheme 16). Column chromatography was employed to isolate the amide **70j** in 30% yield with a purity of ≥ 98% (based on NMR and LC-MS analysis). Compounds **70k** and **70l** were obtained as by-products of the Suzuki cross coupling reaction during in the synthesis of compounds **70b** and **70c**, respectively, when air has been passed through the reaction mixture for 30 min prior to microwave irradiation.

3.3.5.1 Synthesis of Primary Amines

The amines (**71a – e**) used for the preparation of focused library **D** were designed (Figure 3.14b) to improve the binding affinity of the ligand towards the SH2 domain of STAT3. From the docking study of lignad **60**, in to the SH2 binding site, it has been observed that binding pockets for thiophene ring-**B** (arrow-pointed region in figure 3.14a) could be explored more by introducing H-bond donor /acceptor to the ring A molecular dynamics simulation was employed to identify the most relevant functional group on the thiophene ring-**B**.

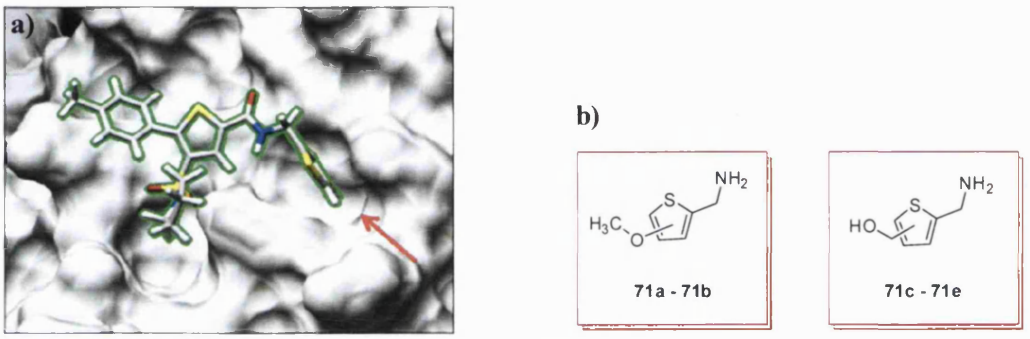


Figure 3.14: a) The docking study of ligand **60** to improve the binding affinity; (**b**) General structure of the substituted thiophen-2-yl methanamines designed through a medicinal chemistry approach.

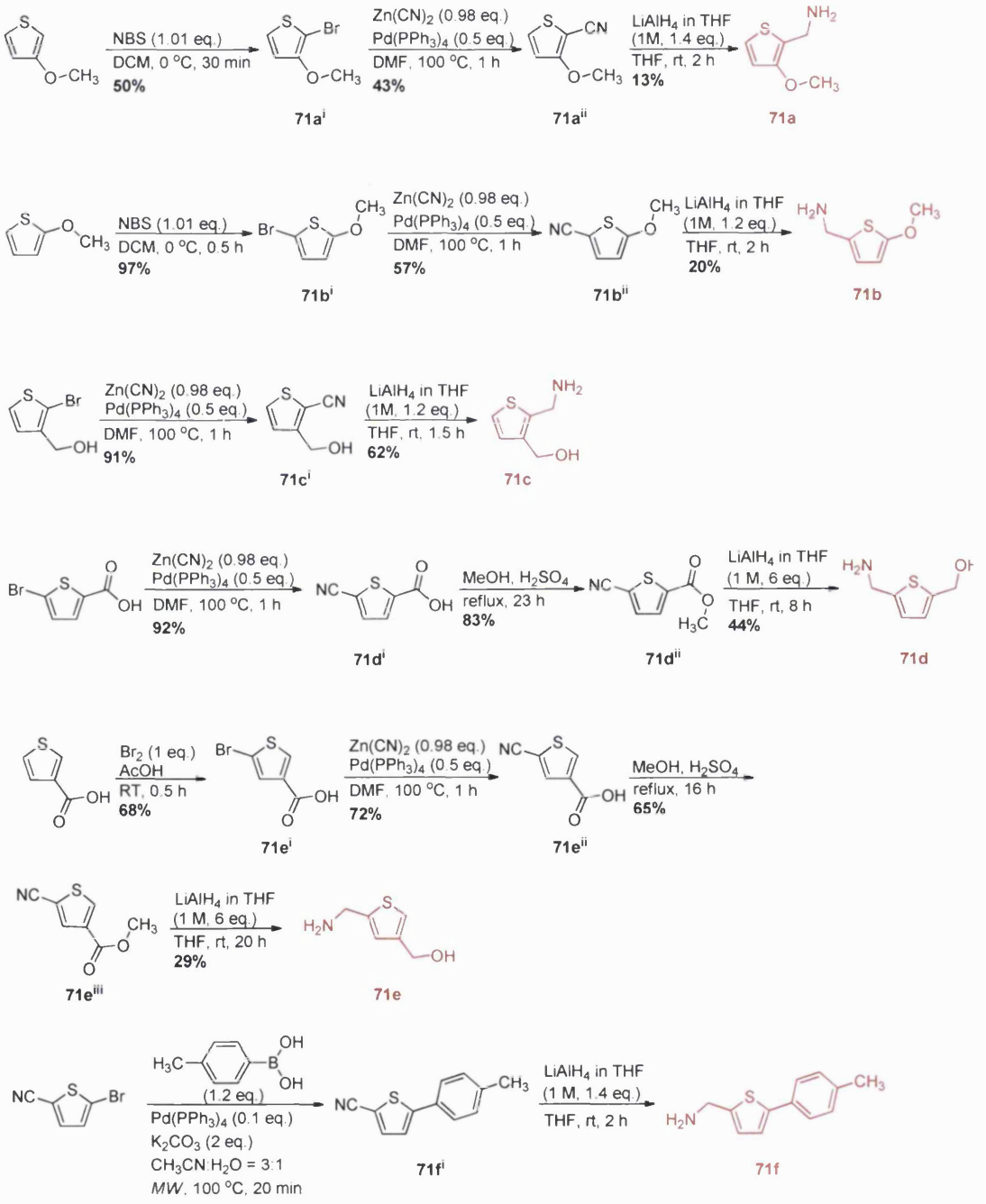
3.3.5.2 Synthesis of Substituted Thiophen-2-yl methanamines

Not all of the primary amines used in the synthesis of library **D** compounds are commercially available. Some of them were synthesised from commercially available building blocks following the synthetic route illustrated in Scheme 17.

The synthesis of amines **71a – 71b** was carried out through bromination of the commercially available methoxythiophene, followed by cyanation and subsequent reduction. The bromination of methoxythiophene was performed using N-bromosuccinimide in DCM at 0 °C for 0.5 h, and was monitored by TLC and LCMS. The formation of a single regioisomer was confirmed by NOESY when the signal multiplicity of ^{1}H NMR would not conclusive. The cyanation of bromothiophene resulted from nucleophilic substitution of –Br with the –CN group[408]. The reaction was carried out by heating a mixture of the bromo compound, tetrakis palladium (0) and zinc cyanide in DMF at 100 °C. Subsequent reduction of the –CN group to the primary amine was achieved using $LiAlH_4$ in THF (1M). Excess of reducing agent completed the reduction within 2 h at room temperature.

The amines **71c – 71e** were synthesised from their corresponding bromo derivatives by cyanation and subsequent reduction. For amines **71d** and **71e**, the –COOH group of the cyano derivatives were esterified before reduction was carried out. The esterified intermediates resulted from overnight reflux of the corresponding acids in methanol using catalytic amount of concentrated H_2SO_4.

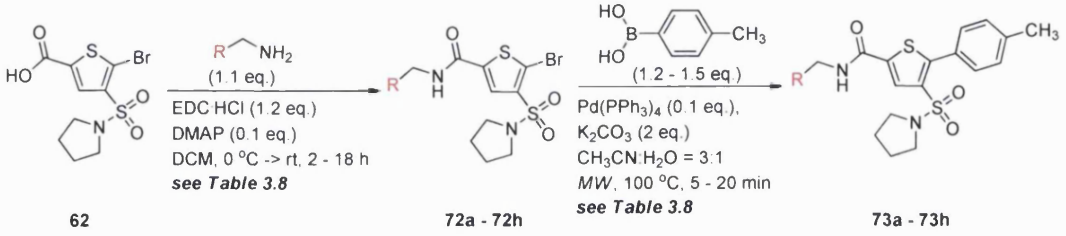
The (5-(p-tolyl)thiophen-2-yl)methanamine (**71f**) was synthesised by reduction of its corresponding cyano derivative 5-(*p*-tolyl)thiophene-2-carbonitrile. The cyano derivative was obtained through Suzuki cross-coupling of commercially available 5-bromothiophene-2-carbonitrile and *p*-tolylboronic acid, where K_2CO_3 and tetrakis palladium (0) were used as base and catalyst, respectively. Microwave irradiation at 100 °C led to complete reaction within 5 min and in 80% yield.



Scheme 17: Synthetic pathway for the primary amine library.

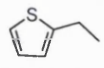
3.3.6 Synthesis of Focused Library "E"

A library of intermediate amides **72a – 72h** were prepared *via* amide coupling of the common intermediate **62** with the different primary amines (Scheme 18). A similar procedure and stoichiometry of reagents were employed for the Suzuki cross-coupling for the synthesis of compounds **73a – 73h** from intermediates **72a – 72h** as was used for the synthesis of compound **60** (Section **3.3**). The total consumption of starting material was confirmed by TLC and LC-MS. The library compounds and their corresponding starting amides were isolated in a good yield and high purity (≥ 98%, based on NMR and LC-MS analysis) by column chromatography and were fully characterised by NMR, mass spectral analysis and elemental analysis. The yields of these compounds and the corresponding intermediates and primary amines are listed in Table 3.8. The intermediate **62** was synthesised as outlined in Scheme 13 (Section **3.3**).



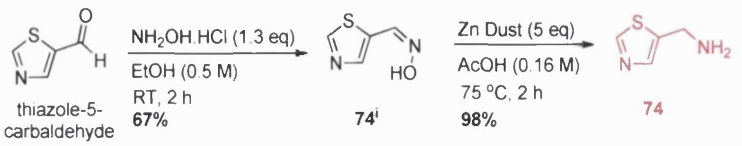
Scheme 18: Generalised synthetic route for compounds **73a – 73h**.

Table 3.8: Yields of compounds **73a – 73h** using different primary amines.

R	Amide (Yield %)	Ligand (Yield %)	R	Amide (Yield %)	Ligand (Yield %)
	**72a** (69%)	**73a** (75%)		**72e** (51%)	**73e** (75%)
	**72b** (79%)	**73b** (79%)		**72f** (77%)	**73f** (73%)
	**72c** (62%)	**73c** (82%)		**72g** (-%)	**73g** (42%)
	**72d** (50%)	**73d** (75%)		**72h** (-%)	**73h** (21%)

Thiazol-5-yl methanamine (**74**) was synthesised from the commercially available building block thiazole-5-carbaldehyde illustrated in Scheme 19. The aldehyde was converted to the aldoxime, which was in turn reduced to primary amine **74**. Aldoxime formation was carried out through reaction of the aldehyde and excess of hydroxylamine hydrochloride salt in ethanol. Complete conversion to the aldoxime took 2 hours at room temperature, and the oxime was purified by filtration followed by washing with water several times. Reduction of the oxime to the primary amine was performed with the aid of a large excess (5 eq.) of Zn dust in acetic acid (97%). Heating the reaction mixture at 75 °C led to completion of reaction within 2 h. The amine was

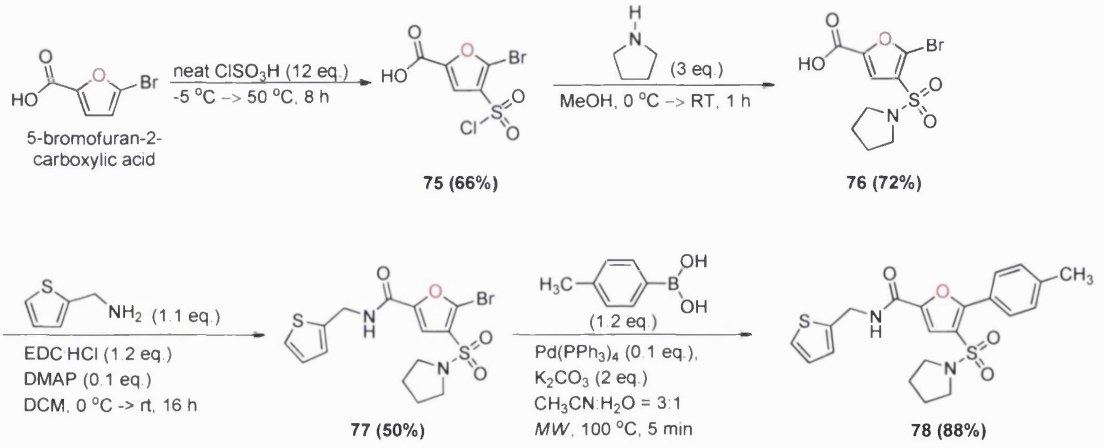
purified using Isolute® SCX-2 column, and was characterised by LC-MS before going to the next step.



Scheme 19: Synthetic pathway for the Thiazol-5-yl methanamine, **74**.

3.3.7 Synthesis of Focused Library "F"

The only member **78** of Library F was synthesised according to route shown in Scheme 20.

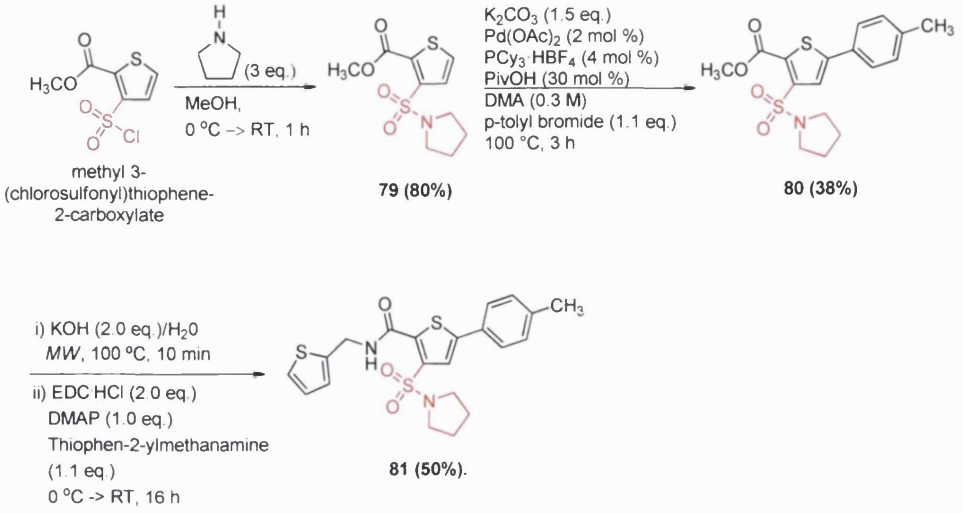


Scheme 20: Synthesis of compound **78**.

Compound **78** was synthesised following a similar synthetic pathway to that used for compound **60** (see Section **3.3**). Commercially available 5-bromofuran-2-carboxylic acid was used as starting material. The chlorosulfonation was accomplished using an excess of chlorosulfonic acid (99%, 12 eq.) in neat conditions (Scheme 20). The starting material was completely consumed and the desired product was observed (LC-MS monitoring) within 8 h. The product **75** (≥ 98% pure, based on NMR and LC-MS analysis) was crystallized (66% yield) as a single regioisomer and its structure was confirmed by HMBC. The sulfonamide (**76**) of the chlorosulfonyl furan derivative **75** could be formed through addition-elimination reaction[400] between **75** and excess pyrrolidine (3 eq.) in methanol. The amide coupling between thiophene-2-methylamine and carboxylic acid derivative **76** was conducted using the coupling reagents EDC.HCl and DMAP. The amide **77** was obtained by precipitation in 50% yield using different solvents with a purity of ≥ 98% (based on NMR and LC-MS analysis). The Suzuki cross coupling reaction between **77** and *p*-tolylboronic acid was performed using tetrakis(triphenylphosphine)palladium and K_2CO_3 as Pd-catalyst and base, respectively. The desired product **78** was isolated in 88% yield and high purity (≥ 98%, based on NMR and LC-MS analysis) using column chromatography and was fully characterised by NMR, mass spectral analysis and elemental analysis.

3.3.8 Synthesis of Focused Library "G"

Compound **81**, a regioisomer of **60**, was synthesised following the synthetic pathway shown in Scheme 21.

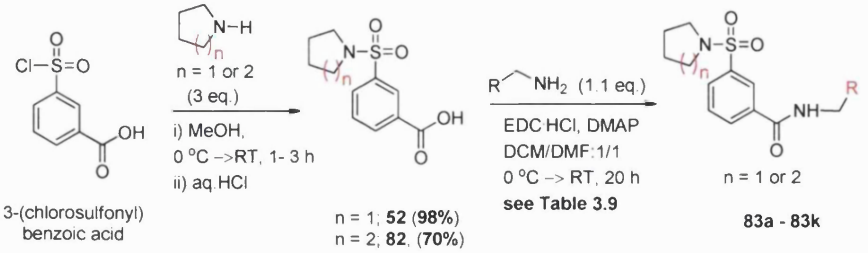


Scheme 21: Synthesis of the regioisomer **81**.

The compound **81** was synthesised *via* a 3 step reaction starting with the commercially available methyl 3-(chlorosulfonyl)thiophene-2-carboxylate. Addition-elimination reaction[400] of the sulfonyl moiety with pyrrolidine in methanol yielded the sulfonamide **79** at room temperature within 1 h. The intermediate **80** was obtained through a coupling between the aromatic halide and sulfonamide using tetrakis(triphenylphosphine)palladium, $PCy_3 \cdot HBF_4$ and PivOH in DMA. A high temperature (100 °C) helped the reaction to reach completion after 3 h. Hydrolysis and subsequent amide coupling of **80** resulted in the formation of **81**. KOH and microwave irradiation were used to hydrolyse the ester, and EDC·HCl and DMAP were used as amide coupling reagents (see Scheme 21).

3.3.9 Synthesis of Focused Libraries "H", "I" & "J"

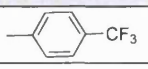
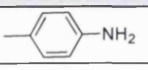
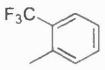
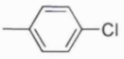
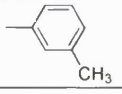
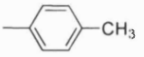
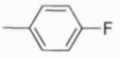
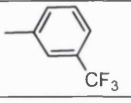
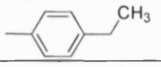
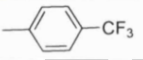
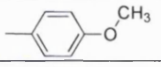
Compounds belonging to the focused libraries **H** and **I** were synthesised from commercially available 3-(chlorosulfonyl) benzoic acid. Compounds **83a – 83i** were synthesised from the common intermediate **52**, and compounds **83j – 83k** were prepared from the corresponding sulphonamide (**82**) by amide coupling with five different commercially available amines as outlined in Scheme 22.



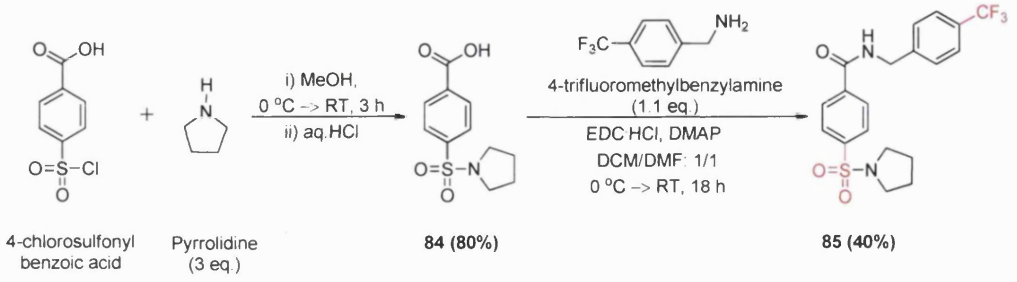
Scheme 22: Generalised synthetic route for compounds **83a – 83k**.

1.2 eq. of EDC.HCl and 0.1 eq. of DMAP were sufficient to drive complete consumption of starting material. A slightly excess of amine (1.1 eq.) afforded the products within 20 h at room temperature. Purification was achieved *via* column chromatography. The yields of these compounds and their corresponding amine substrates are listed in Table 3.9. The synthesis of intermediate **52** is shown in section **3.1.1**.

Table 3.9: Yields of compounds **83a** – **83k**.

Ligand	n	R	Yield (%)	Ligand	n	R	Yield (%)
83a	1		88	**83g**	1		57
83b	1		64	**83h**	1		94
83c	1		78	**83i**	1		86
83d	1		55	**83j**	2		83
83e	1		61	**83k**	2		81
83f	1		65				

Compound **85** of Library **J** was prepared in two steps as shown in Scheme 22. 4-chlorosulfonylbenzoic acid was converted into sulfonamide **84** by addition-elimination reaction[400] of the acid and excess pyrrolidine (3 eq.) in methanol. The excess pyrrolidine allowed the reaction to complete within 3 h at room temperature in good yield (80%). The amide coupling between *p*-trifluoromethyl benzylamine and sulfonamide **84** was carried out using the coupling reagents EDC.HCl and DMAP. 0.1 eq. excess of the amine drove the reaction to completion (no starting material observed by LC-MS and TLC monitoring) at room temperature after 18 h (Scheme 22). The amide **85** was isolated in 40% yield and high purity (≥ 98%, based on NMR and LC-MS analysis) using column chromatography. The addition of amine to the reaction mixture was carried out at 0 °C due to the exothermic nature of the reaction. Compound **85** was fully characterised by NMR and MS. To date, the yield has not been optimised, but it is envisaged that modifying the reaction conditions (*e.g.*, solvent system, stoichiometry) or employing a different coupling reagent (*e.g.*, HATU) should improve the yield.



Scheme 22: Synthesis of compound **85**.

3.4 Biological Investigation of the Ligands

Biological investigation of the synthesised ligands involved an initial *in vitro* screen in a Fluorescent Polarisation (FP)-based primary PPI binding assay (Section **3.4.1**). This was followed by a cell-based screen in a MTS assay (Section **3.4.2.1**) for cell viability in STAT3-dependent MDA MB231 breast cancer cells and STAT3-null A4 cells. Compounds that showed a good binding affinity (% relative inhibition > 20%), and were STAT3 specific in the MTS assay, were progressed into a STAT3-Luciferase assay to evaluate the ability of the compound to inhibit STAT3 transcriptional activity. A control luciferase SV40-Luc assay was used in parallel to assess STAT3 specificity (Section **3.4.2.3**). A cytostatic profile of active compounds was also evaluated in a Trypan Blue exclusion assay (Section **3.4.2.2**). Compounds that were active and specific in these luciferase assays were further investigated in molecular biology based studies to validate their mode of action and elucidate the ability of the compounds to control the regulation of STAT3 signalling. These studies involved checking the level of inhibition of un-phosphorylated STAT3, phosphorylated STAT3, phosphorylated STAT1, un-phosphorylated STAT1, Bcl-X_L, Cyclin D1 and survivin (Sections **3.4.2.4** and **3.4.2.5**).

3.4.1.1 Biochemical Primary PPI Binding Assay

Fluorescence Polarisation (FP) assays has been carried out by Dr Khondaker M. Rahman of CRUK Protein–Protein Interactions Drug Discovery Research Group, The School of Pharmacy, University of London.

In vitro screening of the focused libraries was carried out in a PPI primary binding assay employing Fluorescence Polarisation (FP) (Figure 3.15). This homogeneous assay is generally applicable for the analysis of protein–protein interactions with higher molecular weight protein and can be adapted to a high-throughput format. The degree of interaction between the fluorescent-labeled phosphotyrosine peptide and the synthetic ligands was assessed by analyzing the polarization of the emitted fluorescence upon excitation with polarized light. The focused libraries of novel compounds were screened using the pYLKTKFI peptide to measure the inhibitory activity of the small molecules. This peptide is a known inhibitor and its sequence is the same as the loop in the SH2 domain responsible for the formation of the STAT3 dimer (*i.e.* it is the natural ligand).

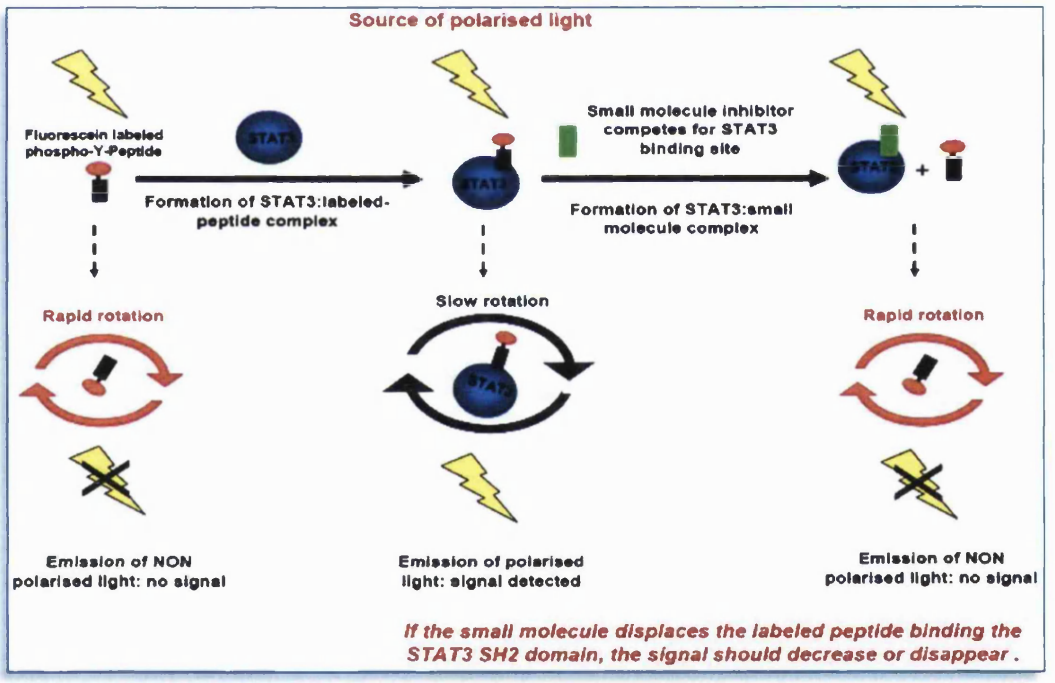


Figure 3.15: Schematic diagram of the Fluorescence Polarisation (FP) PPI assay.

The novel ligands **73d**, **78**, **64i**, **70f**, **70g**, **60** and **68b** were shown to have significant binding affinity for the SH2 domain with relative inhibitions of 55-70%, based on 100% for the natural ligand YLTKTKFI. Ligands **64y, 64n, 68g**, **64p**, **64v**, **73h**, **68h**, **68a**, **73g**, **64d**, **64g**, **64j**, **68f**, **68d**, **65h**, **73a**, **64a**, **73c**, **70h**, **65f**, **64e**, **64b**, **64x**, **64w**, **68c**, **73b**, **64k**, **64m** and **83k** were shown to have competitive binding affinities in comparison to **S3I-201** (41%), a small-molecule inhibitor described in the literature[388] (Figure 3.16). Among these ligands, **60** showed both the greatest binding affinity and the best selectivity towards MDA-MB-231 (STAT3-dependent) cells in the MTS cell-viability assay (Section **3.4.2.1**).

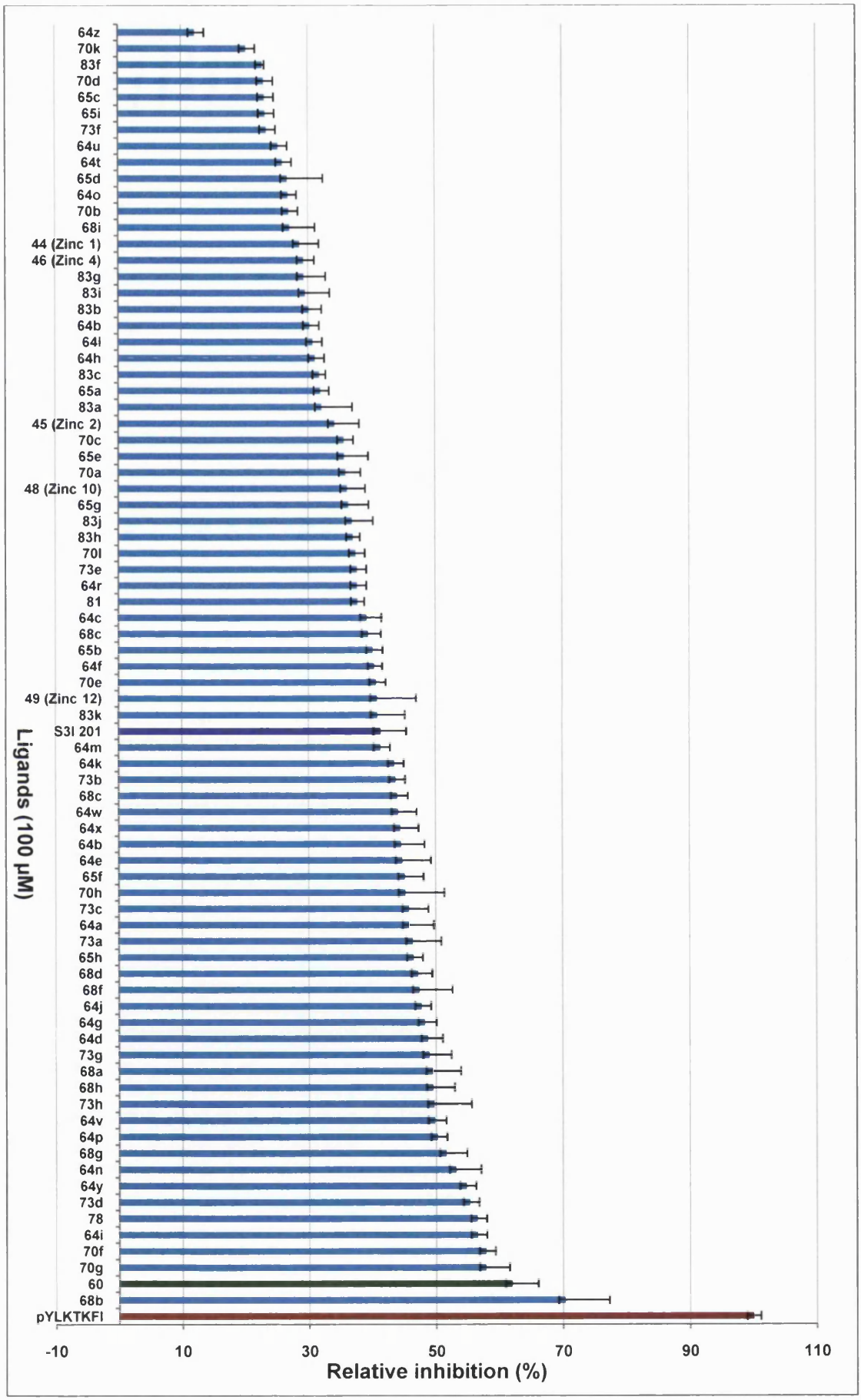
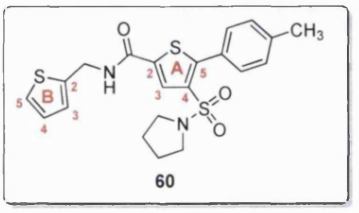


Figure 3.16: Relative inhibitions of ligands towards the STAT3 SH2 domain at 100 µM ligand concentration in the FP assay considering the inhibition of natural ligand pYLKTKFI as 100%.

Based on the results of the screening in FP-based assay, some SAR features were identified.



60

Replacing the sulfur atom in thiophene ring **A** with an oxygen atom (**78**) has no effect on the binding affinity whereas replacing the sulfur atom in thiophene ring **B** with an oxygen (**73a**) or a nitrogen atom (**73b**) reduces the binding affinity. Whether thiophene ring **B** is connected to the thiophene ring **A** methylamide moiety at position 2 (**60**) or 3 (**73d**) does not affect the binding affinity. Moving from the methyl- (**60**) to ethylamide (**73c**) linker reduces the ligand affinity.

Methyl substitution on thiophene ring **B** at positions 4 (**70g**) and 5 (**70f**) did not affect the binding affinity but the methyl substituent at position 3 (**70h**) decreases the ligand affinity. Hydroxymethyl substitution at positions 3, 4 and 5 on thiophene ring **B** (**70a-c**), or methoxy substitution at positions 3 and 5 (**70d** and **70e**), or formyl substitution at positions 4 and 5 (*i.e.*, **70l** and **70k**) results in reduction of binding affinity. Replacement of thiophene ring **B** with a phenyl ring (**73h**) or a thiazol-2-yl ring (**73f**) or a thiazol-5-yl system (**73e**) does not have any significant on effect binding affinity. Replacement of the aromatic ring system with an aliphatic amine like ethyl amine (**73g**) also has no effect.

Changing the pyrrolidine substituent of the sulfonyl moiety with a piperidine (**68a**) or a thiomorpholine ring (**68c**) has no effect. However, when the pyrrolidine N-substituent is changed to a morpholine ring (**68b**), this increases the binding affinity. The benzylamine (**68g**) and aniline (**68f**) substituents of the sulfonyl moiety result in similar or comparatively less binding affinity than the pyrrolidine ring (**60**). Bulky substituent groups such as (4-((4-methylpiperazin-1-yl)methyl)phenyl)methanamine (**68i**) decrease the affinity significantly. Decreasing the bulkiness of the sulfonamide moiety (**68d** and **68c**) did not improve the affinity.

The effect of different groups in the *para* position of the phenyl ring has been investigated, which included CF_3, CH_2CH_3, OCH_3, CN, SH, Cl, F, OH, CH_2OH, SCH_3, NH_2, $N(CH_3)_2$, NO_2, COOH, $CONH_2$, $NHCOCH_3$, SO_2CH_3, Ph, alanine, or even bulkier groups. The replacement of the methyl group with any of these substituents was not found to improve the binding affinity. Use of different aromatic (*e.g.*, napthalyl) or heteroaromatic ring systems (*e.g.*, thiophenyl, furyl, indolyl, pymidinyl, pyridinyl) also does not have any great effect on the binding affinity. Regioisomerisation (**81**) results in a decrease of binding affinity compared to compound **60**.

3.4.2 Cell-Based Assays

All the experiments (section **3.4.2.1**, **3.4.2.2**, **3.4.2.3** and **3.4.2.4**) in cell based assays have been carried out by Dr B. Piku Basu at UCL Institute of Child Health, 30 Guilford Street, London.

3.4.2.1 MTS Assay

The MTS assay is a non-radioactive, colorimetric assay for measuring the number of viable cells. It uses the soluble tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and the electron coupling reagent, phenazine methosulfate (PMS). MTS is chemically reduced by viable cells into formazan, which is soluble in the tissue culture medium. Measurement of the absorbance of the formazan is carried out in 96 well microplates at 492 nm. In effect, the assay measures dehydrogenase enzyme activity in metabolically active cells (Figure 3.17). Since the production of formazan is proportional to the number of living cells, the intensity of the colour produced reflects the viability of the cells. Typically, inhibition ability of a particular test molecule is evaluated using the dose-response curve to determine the IC_{50} (the concentration of the test substance required to reduce the light absorbance capacity of exposed cell cultures by 50%).

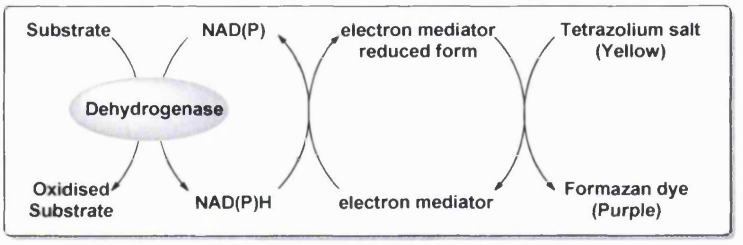


Figure 3.17: Flowchart of formation of the Formazan dye by viable cell in the MTS assay.

Members of the focused libraries were evaluated in the MTS cell viability assay using both MDA-MB-231 breast cells (STAT3-dependent) in which STAT3 signalling had been stimulated with IL-6, and A4 (STAT3-null) cancer cells.[387,409] Both cell lines were treated with up to 125 μM of each library member, and then monitored for 24 h. Compound **60** emerged as a potential STAT3-specific inhibitor, leading to a reduction of ~40% of viable cells differentially at low concentration in the MDA-MB-231 line compared to A4 (Figure 3.18). All other library members showed either no reduction in both cell lines (Ligand **70i**), or a reduction of viable cells in both the MDA-MB-231 and A4 cell lines (ligand **65e**). Therefore, compound **60** was the only member of which is STAT3-specific activity.

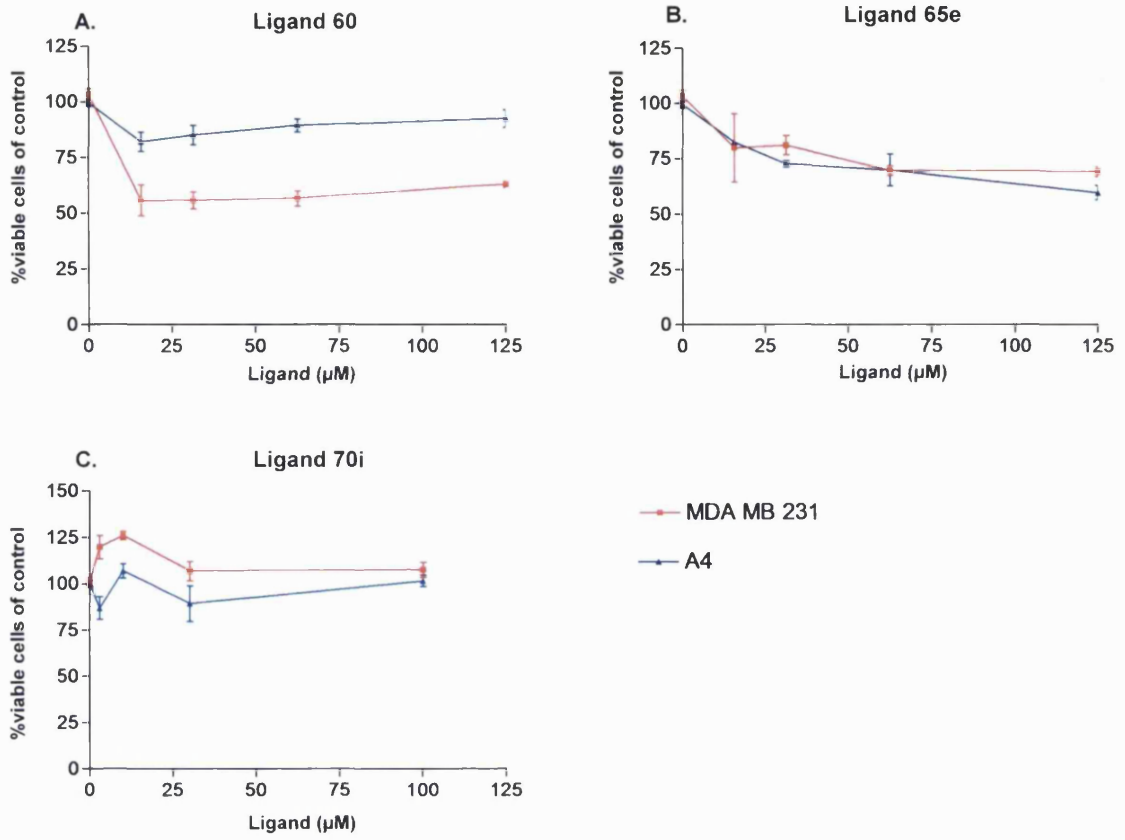


Figure 3.18: MTS cell-viability assay in MDA-MB-231 (STAT3-dependent) and A4 (STAT3-null) cells treated for 24 hours. Graphs for: **A.** ligand **60**; **B. 65e** as an example of compound that is active in the STAT3-activated cells, and also in STAT3-non-activated cells; **C. 70i** as an example of compound that shows no activity in both cell lines.

3.4.2.2 Trypan Blue Exclusion Assay

This exclusion assay is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that can exclude certain dyes such as trypan blue, eosin, or propidium, whereas dead cells cannot. In this assay, a cell suspension is mixed with dye and then visually examined to determine whether the cells take up or exclude the dye. A viable cell will show a clear cytoplasm whereas the cytoplasm of a non-viable cell will become blue. This is a simple and rapid (5 to 10 min) technique to measure cell viability but is limited in that viability is determined indirectly from cell membrane integrity.

In the cell proliferation assay for **60** in the MDA-MB-231 breast cancer cells for 24 h, it arrested proliferation by more than 50% compared to control. Ligands such as **Zinc 5**, **64m**, **Zinc 13**, **Zinc 28, Zinc 14**, **Zinc 1** also resulted in 10 – 40% reduction of cell proliferation, whereas other ligands such as **Zinc 3**, **Zinc 6** and **Zinc 8** showed no inhibitory effects (Figure 3.19). In the case of ligand **60**, the trypan blue exclusion assay confirmed that it is a cytostatic agent, as high percentage (95%) of viable cells has been detected by Countess® Automated Cell Counter (Invitrogen™).

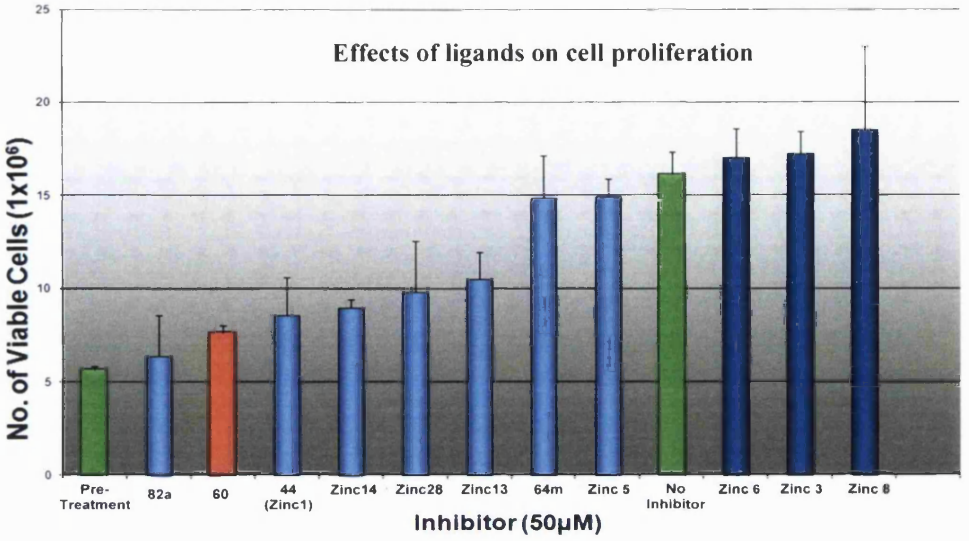


Figure 3.19: The cell proliferation assay in the MDA-MB-231 breast cancer cells for 24 h, which shows ligand **60** (red bar) arrested cell proliferation >50% compared to control (green bar).

3.4.2.3 Luciferase Reporter Assay

Luciferase is commonly used as a reporter to assess the transcriptional activity of cells that have been transfected with a genetic construct containing the luciferase gene under the control of a promoter of interest (*e.g.*, the STAT3 promoter).[410] Firefly Luciferase is an oxidative enzyme from the firefly *Photinus pyralis* and produces bioluminescence in this species. The laboratory reagent "Firefly luciferase" usually refers to *P. pyralis* luciferase, although recombinant luciferases from several other species of fireflies are also commercially available. Luciferase can be biosynthesised in the lab through genetic engineering and then inserted into organisms or luciferase gene constructs are transfected into cells. In the luciferase reaction, light is emitted when luciferase acts on the appropriate luciferin substrate (Figure 3.20). To observe these biological processes, photon emission is detected by a light sensitive apparatus (*i.e.*, a luminometer).

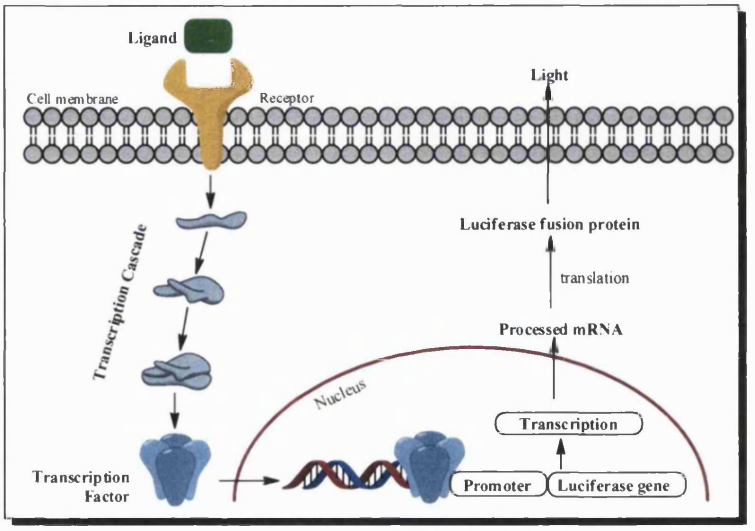


Figure 3.20: Schematic of Luciferase reporter assay.[411]

In this study, a luciferase reporter assay based on HeLa cells containing plasmids constructed with either a STAT3 minimal promoter (stable line) or a SV40 control promoter (transient transfection) upstream from luciferase was used. The cells were treated with Oncostatin M to activate STAT3 signalling *via* the IL-6/gp130 receptor prior to addition of ligand **60** which was shown to selectively inhibit STAT3 transcriptional activity with an EC_{50} of 15 μM (Figure 3.21). There was negligible effect on the cell line containing the SV40 promoter. Interestingly, although some molecules of similar structure to ligand **60** had a range of activities in the MTS assay in the MDA-MB-231 cell line (*i.e.*, 5 – 100 μM) and modest activity in the STAT3-reporter assay, ligand **60** was the only molecule in the library with STAT3-selective properties.

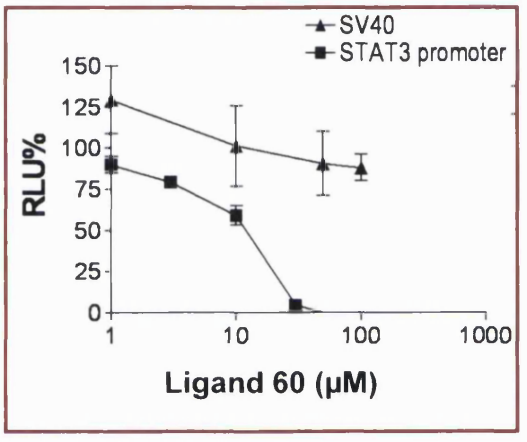


Figure 3.21: Effect of ligand **60** in a luciferase reporter assay in HeLa cells containing either STAT3 or SV40 promoters upstream of luciferase for 24 hours. Error bars indicate mean ± SEM from three independent experiments.

3.4.2.4 mRNA Q-PCR Analysis of STAT3 Down-Regulation

A PCR-based method was used to quantitate changes in mRNA levels of genes including STAT3. In this approach, total mRNA are converted to cDNA and then PCR carried out on a number of family members simultaneously, using primers derived from conserved regions. This was followed by gel electrophoresis and blotting of the products onto filters. The level of expression of individual family members was determined by separate hybridizations using probes unique for each member and derived from sequences between the PCR primers.

Q-PCR analysis on the STAT3 downstream targets Survivin, Bcl-X_L and Cyclin D1 showed that they were not significantly affected by **60** (Figure 3.22). It had no effect at all on Bcl-X_L and had only a mild suppressive effect on Survivin which was not concentration dependent. The effect of ligand **60** on Cyclin D1 was not conclusive.

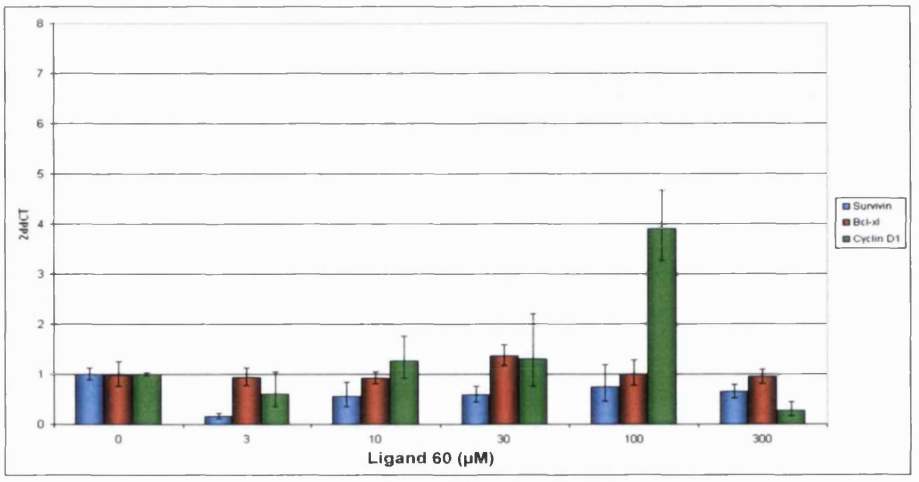


Figure 3.22: Q-PCR-mRNA analysis of Survivin, Bcl-X_L and Cyclin D1 in MDA-MB-231 cells over 24 hours after treatment with Ligand **60**.

This analysis led to the conclusion that although ligand **60** showed a good binding profile in the FP assay (Section **3.4.1**), good cytostatic activity in the cell-based MTS assay (Sections **3.4.2.1** & **3.4.2.2**) and excellent STAT3 selectivity in the luciferase assay (Section **3.4.2.3**), it does not work by targeting the STAT3 SH2 domain. As IL-6 is the specific cytokine responsible for the activation and dimerisation of STAT3, it is more likely that the molecule is acting on some component of the signalling pathway of IL-6-stimulated STAT3 activation. In particular, IL-6 initially complexes with its membrane-bound receptor, gp130, inducing dimerisation and activating bound JAK (Janus Associated Kinase) protein which then autophosphorylates and in-turn phosphorylates STAT3 on its tyrosine 705 residue, which triggers homodimerisation of the STAT3 protein (Figure 3.23). To explore the specificity of ligand **60** on the STAT3 activation pathway, western blotting analysis was performed on IL-6 stimulated MDA-MB-231 cells to investigate the effect on phosphorylated and un-phosphorylated STAT3 and STAT1.

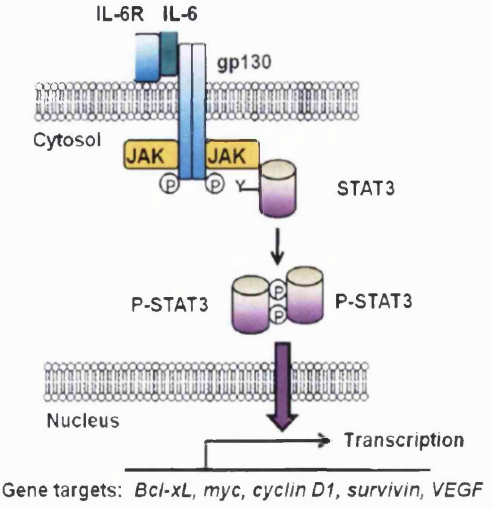


Figure 3.23: The IL-6 stimulated STAT3 activation pathway.[412]

3.4.2.5 Western Blotting Analysis

Protein blotting is an analytical method that involves the immobilization of proteins on membranes followed by detection using labelled monoclonal or polyclonal antibodies. Among different blotting protocols (*e.g.*, dot blot, 2D blot), Western blotting is the most powerful. In Western blotting, prior to protein immobilization on PVDF or nitrocellulose membranes, sample proteins are separated using SDS polyacrylamide gel electrophoresis (SDS-PAGE) which provides information about the existence of different isoforms of the proteins.

In these studies, the effect of ligand **60** on phosphorylation of the tyrosine 705 residue within the STAT3 protein in serum-starved MDA-MB-231 and HeLa cells was investigated. Ligand **60** was found to inhibit STAT3 phosphorylation downstream of IL-6 twenty minutes after the addition of IL-6 in both HeLa and MDA-MB-231 cells at concentrations of 10-30 and 30-100 μM, respectively (Figure 3.24).

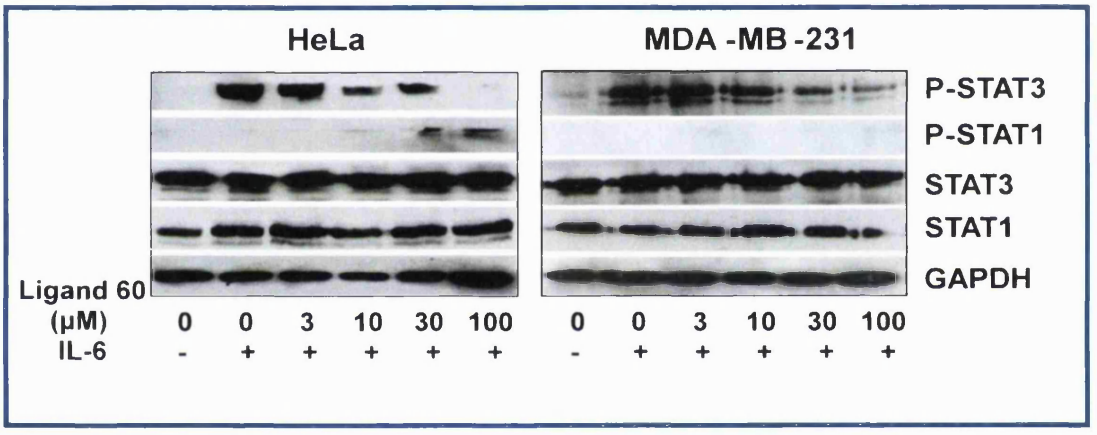


Figure 3.24: Western Blots showing the effect of ligand **60** on the expression of STAT3, P-STAT3, STAT1, P-STAT1 and GAPDH in IL-6-stimulated HeLa and MDA-MB-231 cells.

IL-6 did not induce phosphorylation of STAT1 in either cell line, although some phosphorylation was observed in HeLa cells in the presence of higher concentrations (30 and 100 μM) of **60**. This could have been caused by diversion of IL-6 signalling through STAT1 due to STAT3 signalling blockade at these higher concentrations of ligand **60**, a phenomenon that has been previously described.[413] The effect of ligand **60** on endogenous STAT3 was also investigated in MDA-MB-231 cells, where it showed no inhibition of p-STAT3 (Figure 3.25). Therefore, ligand **60** had an effect only on IL-6-stimulated STAT3, implying that it interferes with STAT3 signalling pathway *via* the IL-6/α-receptor/gp130 complex.

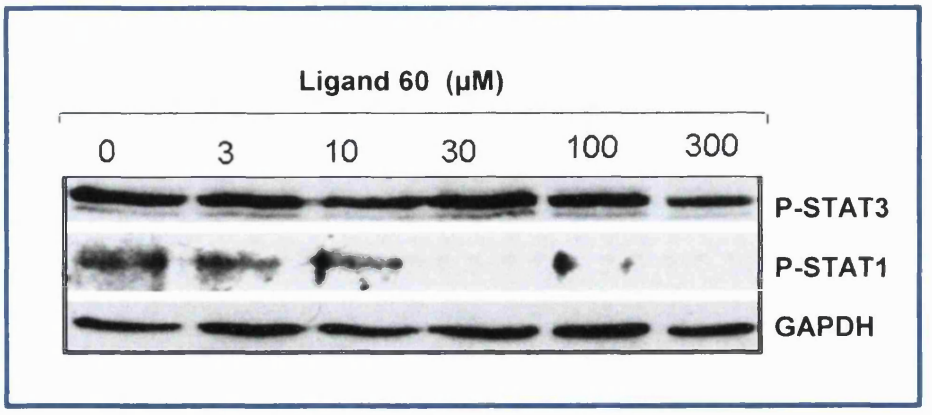


Figure 3.25: Western Blots showing the effect of ligand **60** on the expression of P-STAT3 and P-STAT1, and GAPDH in MDA-MB-231 cells where STAT3 is stimulated endogenously.

3.5 Ligand 60 as an IL-6 Inhibitor

There is no information available as yet on a likely binding site for ligand **60**, although some modelling studies were carried out based on the available crystal structure (PDB 1P9M) of part of the IL-6/α-receptor/gp130 complex.[414] This was used as a starting point for an *in silico* modelling study, which suggested two possible low-energy sites. In one site (labelled B in Figure 3.26), compound **60** is shown binding in a cavity on the surface of the IL-6/α-receptor interface. This could potentially block interaction of IL-6 with its receptors. In the other site (labelled A), compound **60** is shown buried in a cavity at the IL-6/gp130 interface, which is less likely to interfere with receptor binding. The interaction energies for the dockings were calculated as –8.82 kcalmol^{-1} and –8.25 kcalmol^{-1} for sites A and B, respectively.

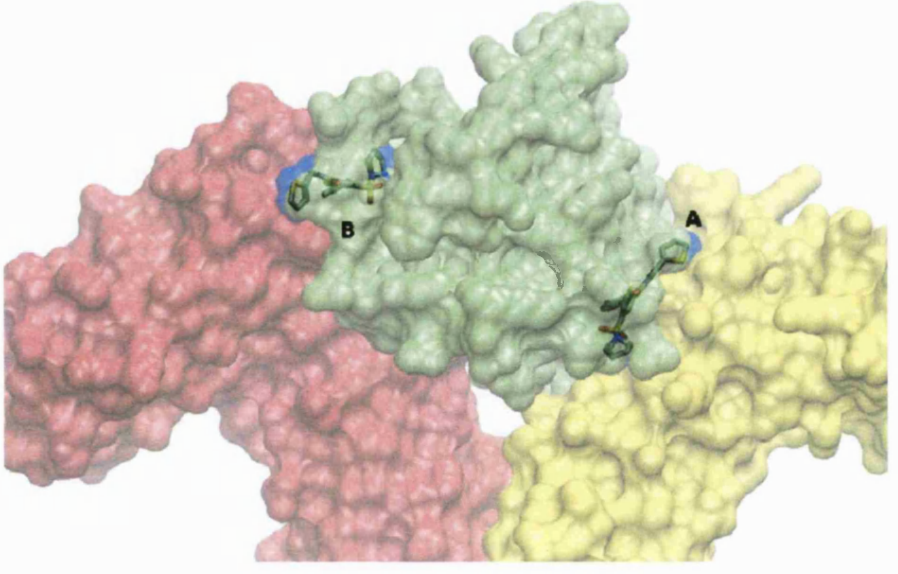


Figure 3.26: Potential binding sites of ligand **60** at the protein–protein interfaces of the IL-6/ gp130 complex. Best docks of ligand **60** (represented as sticks) are shown at positions **A** and **B**. The model (PDB 1P9M) of the IL-6 a-receptor (red)/gp130 (yellow)/IL-6 (green) complex is coloured distinctly to highlight the protein–protein interfaces.

4 Summary and Conclusions

With the objective of discovering potential inhibitors of the STAT3:STAT3 interaction, the initial *in silico* virtual screen was performed on a STAT3 SH2 molecular model based on X-ray data and homology modelling reported in the literature. Structure-based design was then used to improve the *in silico* binding affinity of the top "hit" molecule. To validate the proposed STAT3 SH2 molecular model and to identify potential SARs, focused libraries around the two top "hits" were designed. In particular, the binding affinity of library members to the SH2 domain was investigated employing a FP-based primary PPI binding assay developed in our laboratory. Their potency and selectivity for down-regulation of the STAT3 signalling pathway were assessed in MTS and luciferase reporter assays both in STAT3-dependent and STAT3-null cell lines. Compound **60** resulted in the most promising activity in terms of binding affinity (% relative inhibition ~ 60%) and selective inhibition of the STAT3-dependent MDA-MB-231 cell lines (EC_{50} = 15 μM). However, it did not show any significant effect on the downstream targets of STAT3 (*i.e.*, Survivin, Cyclin D1 and Bcl-X_L). However, these studies on downstream targets indicated that **60** was in fact selectively inhibiting the IL-6 signalling pathway that regulates upstream activation of STAT3 *via* the IL-6 cytokine. A preliminary docking study indicated that **60** could potentially bind in a cavity either on the surface of the IL-6/α-receptor interface or at the IL-6/gp130 interface.

In conclusion, although the *in silico* screening approach did not yet lead to molecules that selectively inhibit down-stream protein products of STAT3 signalling, none-the-less an effective IL-6 inhibitor (**60**) has been identified. Molecular dynamic simulations have helped to improve the molecular model to support a second campaign of virtual screening redefining the docking site in the SH2 domain. Therefore, further computer-aided rational designing is currently under investigation.

5 Future Objectives

Compound **60** could be further investigated as an IL-6 inhibitor with a view to developing a novel therapeutic agent. Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates hematopoiesis, inflammation and the immune system. It is implicated in the pathogenesis of various inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, psoriasis, endotoxemia and toxic shock syndrome.[415,416] It also plays a key role in the pathophysiology of several cancers, and its over-expression has been implicated in the tumourigenesis of multiple myeloma, ovarian, renal cell, prostate, cervical and breast carcinomas.[415,417-419] Therefore, the identification of inhibitory agents to target IL-6 signalling is currently of interest in both anti-inflammatory and anticancer drug discovery. Although to date no selective small-molecule IL-6 inhibitors have been reported, there are examples of molecules in the patent literature that inhibit IL-6 signalling in combination with a number of other signalling pathways. Thus, further cell-based assays could be carried out to confirm the selectivity of compound **60** for IL-6 inhibition. Further biochemical and biophysical analysis could also be employed to assess the binding affinity of the molecule for the of IL-6 cytokine. These studies could validate the *in silico* molecular modelling and lead to the design of more focused libraries for IL-6 inhibitor.

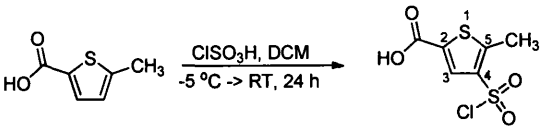
6 General Experimental Procedure

All reagents and solvents used were supplied from commercial sources mainly Sigma-Aldrich and Fluka. Reactions requiring anhydrous conditions were conducted in glassware, which had been oven-dried overnight and used the following day. All reactions were monitored by analytical thin-layer chromatography (TLC) performed using indicated solvent on E. Merck silica gel 60 F_{254} plates (0.25 mm). TLC plates were visualized using UV light (254 or 360 nm) and /or staining the plates with a cerium sulphate-ammonium molybdate solution or basic $KMnO_4$ followed by heating. An LCMS machine, liquid chromatography (Waters 2695 HPLC) coupled with mass spectrophotometer (Waters Micromass ZQ instrument with a Waters 2996 Photodiode Array Detector), was also used to monitor the progress of the reactions. Waters Micromass ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; Desolvation flow rate (L/h), 250. All microwave irradiation was performed in 5 ml and 20 ml sealed pyrex vials in an Emry's Optimizer Personal Chemistry Instrument (Biotage AG). Solvents were removed by rotary evaporator at or below 40 °C and the compounds further dried using low pressure vacuum pumps. The purification of the compounds was achieved by column chromatography using Merck Flash Silica Gel 60 (230-400 mesh). ^1H and ^{13}C NMR were recorded on a Bruker Avance 400 MHz spectrophotometer and Bruker Avance 500 MHz spectrophotometer. Chemical shifts (δ H) are quoted in ppm (parts per million) and referenced to $CDCl_3$ residual chloroform signal ^1H δ = 7.26, ^{13}C δ = 77.16 or d_6-DMSO residual dimethyl-sulfoxide signal ^1H δ = 2.50, ^{13}C δ = 39.52 or CD_3OD residual methanol signal ^1H δ = 3.31, ^{13}C δ = 49.00. Multiplicities in ^1H NMR spectra are quoted as: s = singlet, d = doublet, t = triplet q = quartet, m = multiplet, dd = double doublet, ddd = double double doublet, dt = double triplet, td = triple doublet. High resolution mass spectra (HRMS) were obtained on a Thermo Navigator mass spectrometer coupled with LC using electrospray ionisation (ES) and time-of-flight (TOF) mass spectrometry. Infrared spectra were recorded using neat conditions on Specac ATR in a Perkin Elmer FT-IR Spectrum 1000.

6.1 Synthesis of Potential "Hit" Compounds from Zinc Database

6.1.1 Synthesis of Compound 44

6.1.1.1 Procedure for Synthesis of Compound 50



50
$C_6H_5ClO_4S_2$
Mol. Wt.: 240.68

Chlorosulfonic acid (0.47 mL, 7.03 mmol, 5 equiv.) was added dropwise to the vigorously stirred solution of 5-methyl-2-thiophene carboxylic acid (200 mg, 1.41 mmol, 1 equiv.) in anhydrous DCM (10 mL per 1 mmol of 5-methyl-2-thiophene carboxylic acid) at -5 °C and under N_2. The solution was stirred for 24 h at room temperature, poured into the ice-water (20 g per 1 mmol of acid) and extracted with DCM (10 mL per 1 mmol of acid). The organic phases were dried on $MgSO_4$ and the solvent was evaporated *in vacuo* to afford compound **50**.

Compound **50** (281 mg, 83% yield) was obtained as a light yellow solid.

R_f = 0.21 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 141.5 – 142.6 °C.

IR (ν_{max}/cm^{-1}): 756, 837, 1158, 1374, 1455, 1530, 1683 and 2863.

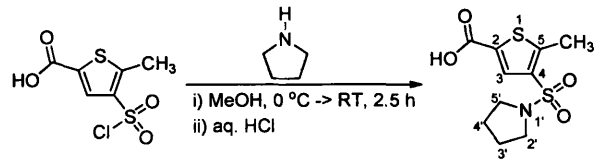
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (1H, br s, COOH), 8.20 (1H, s, H^3), 2.94 (3H, s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2 (COOH), 155.7 (C^5), 139.5 (C^4), 134.1 (C^3), 129.7 (C^2), 15.4 (CH_3).

LRMS (ESI): *m/z* = 239.15 $[C_6H_5{}^{35}ClO_4S_2\text{-}H]^-$ (75%), 240.92 $[C_6H_5{}^{37}ClO_4S_2\text{-}H]^-$ (25%).

HRMS (TOF MS ES): Found 238.9249, calculated for $C_6H_4{}^{35}ClO_4S_2$ 238.9245 $[M\text{-}H]^-$.

6.1.1.2 Procedure for Synthesis of Sulfonamide 51



50 **51**

$C_{10}H_{13}NO_4S_2$
Mol. Wt.: 275.34

Pyrrolidine (0.13 mL, 1.56 mmol, 3 equiv.) was added dropwise to a vigorously stirred solution of **50** (125 mg, 0.52 mmol, 1 equiv.) at 0 °C in anhydrous methanol (6.5 mL per 1 mmol of **50**) and under N_2. The solution was stirred for 2.5 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1mL of 1 M (HCl) was added to the aqueous phase that was extracted with DCM (x 3). The combined organic phases were dried on $MgSO_4$ and evaporated *in vacuo* to afford compound **51**.

Compound **51** (125 mg, 87% yield) was obtained as a light yellow powder.

R_f = 0.262 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 152.0 – 153.0 °C.

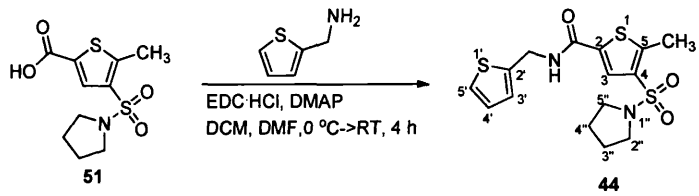
IR (ν_{max}/cm^{-1}): 1146, 1325, 1453, 1532, 1682 and 2922.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (1H, s, H^3), 3.38 (4H, t, J = 6.8 Hz, $H^{2'}$ & $H^{5'}$), 2.86 (3H, s, CH_3), 1.95 (4H, dt, J = 6.8, 3.6 Hz, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9 (COOH), 153.1 (C^5), 135.3 (C^3), 134.7 (C^4), 128.9 (C^2), 47.8 ($C^{2'}$ & $C^{5'}$), 25.5 ($C^{3'}$ & $C^{4'}$), 15.7 (CH_3).

LRMS (ESI): *m/z* = 276.35 $[M+H]^+$, 100%.

6.1.1.3 Procedure for Synthesis of Amide 44



51

44
$C_{15}H_{18}N_2O_3S_3$
Mol. Wt.: 370.51

Thiophene-2-methylamine (0.045 mL, 0.44 mmol, 1.1 equiv.) was added dropwise to a vigorously stirred solution of **51** (110 mg, 0.4 mmol, 1 equiv.), EDC hydrochloride (91.9 mg, 0.48 mmol, 1.2 equiv.) & DMAP (4.88mg, 0.04 mmol, 0.1 equiv.) in anhydrous DCM (5 mL per 1 mmol of **51**) and anhydrous DMF (2.5 mL per 1 mmol of **51**) at 0 °C and under N_2. The mixture was stirred at room temperature for 4 h, quenched by the addition of water and extracted with ethyl acetate. The combined extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane: 1/1) on silica gel 60 to afford compound **44**.

Compound **44** (89 mg, 54% yield) was obtained as a yellow crystal.

$\mathbf{R_f}$ = 0.448 (Silica gel, Ethyl acetate/Hexane: 7/3).

mp = 163.0 – 164.0 °C.

IR (ν_{max}/cm^{-1}): 834, 1145, 1324, 1457, 1549, 1629, 2852, 2923 and 3331.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (1H, s, H^3), 7.32 (1H, dd, J = 3.6, 1.2 Hz, $H^{3'}$), 7.12 (1H, d, J = 3.6 Hz, $H^{5'}$), 7.04 (1H, dd, J = 5.2, 3.6 Hz, $H^{4'}$), 6.29 (1H, t, J = 5.6 Hz, NH), 4.82 (2H, d, J = 5.6 Hz, CH_2NH), 3.32 (4H, t, J = 6.8 Hz, $H^{2''}$ & $H^{5''}$), 2.80 (3H, s, CH_3), 1.90 (4H, dt, J = 6.8, 3.6 Hz, $H^{3''}$ & $H^{4''}$).

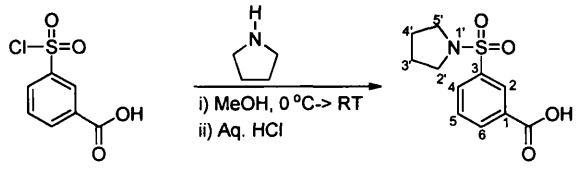
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4 (COOH), 149.8 (C^5), 140.2 ($C^{2'}$), 135.6 (C^3), 133.3 (C^4), 127.9 ($C^{4'}$), 127.1 ($C^{5'}$), 126.8 ($C^{3'}$), 125.7 (C^2), 47.8 ($C^{2''}$ & $C^{5''}$), 38.7 (CH_2NH), 25.4 ($C^{3''}$ & $C^{4''}$), 15.4 (CH_3).

LRMS (ESI): *m/z* = 369.34 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 371.0546, calculated for $C_{15}H_{19}N_2O_3S_3$ 371.0552 $[M+H]^+$.

6.1.2 Synthesis of Compound 45

6.1.2.1 Procedure for Synthesis of Sulfonamide 52



52
$C_{11}H_{13}NO_4S$
Mol. Wt.: 255.29

Pyrrolidine (0.57 mL, 6.8 mmol, 3 equiv.) was added dropwise to a vigorously stirred solution of 3-chlorosulfonylbenzoic acid (525 mg, 2.27 mmol, 1 equiv.) in anhydrous methanol (5mL per 1 mmol of 3-chlorosulfonyl benzoic acid) at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1 mL of 1 M HCl was added to the aqueous phase that was extracted with DCM (x 3). The combined organic phases were dried on $MgSO_4$ and evaporated *in vacuo* to afford compound **52**.

Compound **52** (568 mg, 98% yield) was obtained as a white powder.

R_f = 0.23 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 163.8 – 164.5 °C.

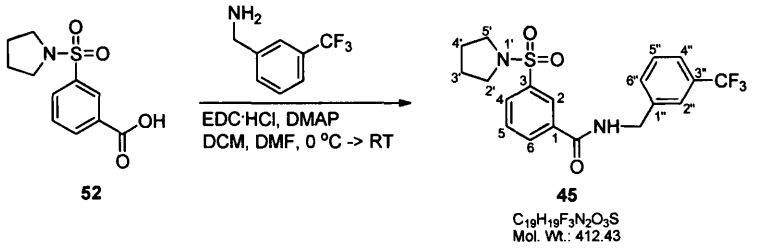
IR (ν_{max}/cm^{-1}): 750, 1164, 1342, 1601, 1680 and 2949.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (1H, t, *J* = 1.6 Hz, H^2), 8.33 (1H, dt, *J* = 7.6, 1.6 Hz, H^6), 8.09 (1H, dt, *J* = 7.6, 1.6 Hz, H^4), 7.83 (1H, t, *J* = 7.6 Hz, H^5), 3.30 (4H, t, *J* = 6.8 Hz, $H^{2'}$ & $H^{5'}$), 1.80 (4H, dt, *J* = 6.8, 3.2 Hz, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2 (COOH), 138.4 (C^3), 134.1 (C^4), 132.5 (C^6), 130.5 (C^1), 129.6 (C^5), 129.1 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 256.06 $[M+H]^+$, 100%.

6.1.2.2 Procedure for Synthesis of Amide 45



52

45

$C_{19}H_{19}F_3N_2O_3S$
Mol. Wt.: 412.43

3-trifluoromethyl benzylamine (0.04 mL, 0.26 mmol, 1.1 equiv.) was added dropwise to a stirred solution of **52** (60mg, 0.24 mmol, 1 equiv.), EDC hydrochloride (54 mg, 0.28 mmol, 1.2 equiv.) & DMAP (2.9 mg, 0.03 mmol, 0.1 equiv.) in anhydrous DCM (2 mL per 1 mmol of **52**) and anhydrous DMF (2 mL per 1 mmol of **52**) at 0 °C and under N_2. The mixture was stirred at room temperature for 20 h, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane: 7/3) on silica gel 60 to afford compound **45**.

Compound **45** (70 mg, 71% yield) was obtained as a white powder.

R_f = 0.57 (Silica gel, Ethyl acetate).

mp = 146.5 – 147.3 °C.

IR (ν_{max}/cm^{-1}): 790, 1017, 1072, 1114, 1154, 1331, 1541, 1632 and 3268.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (1H, t, *J* = 1.6 Hz, H^2), 8.12 (1H, d, *J* = 8.0 Hz, H^6), 7.95 (1H, d, *J* = 8.0 Hz, H^4), 7.63 (1H, t, *J* = 8.0 Hz, H^5), 7.60-7.55 (3H, m, $H^{6"}$, $H^{4"}$& $H^{2"}$), 7.48 (1H, t, *J* = 8.0 Hz, $H^{5"}$), 6.98 (1H, t, *J* = 6.0 Hz, NH), 4.72 (2H, d, *J* = 6.0 Hz, CH_2NH), 3.23 (4H, t, *J* = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.75 (4H, dt, *J* = 6.4, 3.6 Hz, $H^{3'}$ & $H^{4'}$).

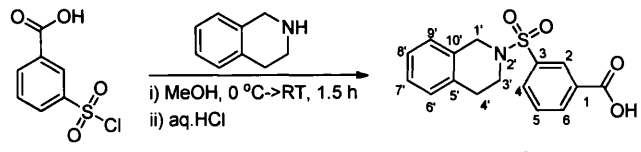
^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1 (COOH), 139.1 ($C^{1"}$), 137.8 (C^3), 136.3 ($C^{5"}$), 135.3 (C^1), 132.0 (C^6), 131.6 ($C^{6"}$), 130.3 (C^4), 129.4 ($C^{5"}$), 125.5 (C^2), 124.86 (CF_3), 124.82 ($C^{4"}$), 124.71 ($C^{2"}$), 124.67 (C^5), 48.1 ($C^{2'}$ & $C^{5'}$), 43.8 (CH_2NH), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 412.90 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 413.1128, calculated for $C_{19}H_{20}F_3N_2O_3S$ 413.1147 $[M+H]^+$.

6.1.3 Synthesis of Compound 46

6.1.3.1 Procedure for Synthesis of Sulfonamide 53



53
$C_{16}H_{15}NO_4S$
Mol. Wt.: 317.36

1,2,3,4 - tetrahydroisoquinoline (0.34 mL, 2.72 mmol, 3 equiv.) was added dropwise to a stirred solution of 3-chlorosulfonyl benzoic acid (200 mg, 0.91 mmol, 1 equiv.) in anhydrous methanol (5mL per 1 mmol of 3-chlorosulfonyl benzoic acid) at 0 °C and under N_2. The solution was stirred for 1.5 h at room temperature. 3 mL NaOH (1N) was then added and extracted with DCM (x 3). 5 mL of 1 M HCl was added to the aqueous phase that was extracted with DCM (x 3). The combined organic phases were dried on $MgSO_4$ and evaporated at reduced pressure to afford compound **53**.

Compound **53** (283 mg, 98% yield) was obtained as a white powder.

$\mathbf{R_f}$ = 0.18 (Silica gel, Methanol/Ethyl acetate: 1/9).

mp = 219 – 220 °C.

IR (ν_{max}/cm^{-1}): 740, 920, 1068, 1165, 1313, 1421, 1681 and 2838.

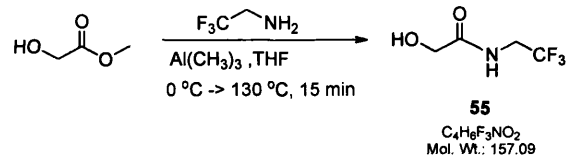
^{1}H NMR (400 MHz, DMSO): δ 8.14 (1H, t, J = 1.6 Hz, H^2), 8.22 (1H, dt, J = 8.0, 1.6 Hz, H^6), 8.07 (1H, dt, J = 8.0, 1.6 Hz, H^4), 7.77 (1H, t, J = 8.0 Hz, H^5), 7.16 – 7.13 (4H, m, $H^{6',7',8',9'}$), 4.25 (2H, s, $H^{1'}$), 3.36 (2H, t, J = 6.0 Hz, $H^{3'}$), 2.85 (2H, t, J = 6.0 Hz, $H^{4'}$).

^{13}C NMR (100 MHz, DMSO): δ 164.1 (COOH), 136.73 (C^3), 134.8 (C^4), 131.7 (C^6), 130.1 (C^5), 131.0 (C^1), 129.54 ($C^{5'}$ or $C^{10'}$), 129.44 ($C^{5'}$ or $C^{10'}$), 128.2 ($C^{2'}$), 127.7 ($C^{6'}$), 126.7 ($C^{7'}$), 126.4 ($C^{9'}$), 126.1 ($C^{8'}$), 47.1 ($C^{1'}$), 43.5 ($C^{3'}$), 27.8 ($C^{4'}$).

LRMS (ESI): *m/z* = 315.95 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 318.0792, calculated for $C_{16}H_{16}NO_4S$ 318.0800 $[M+H]^+$.

6.1.3.2 Procedure for Synthesis of Amide 55



55

$C_4H_6F_3NO_2$
Mol. Wt.: 157.09

Trimethylaluminium (2M in toluene, 3.33 mL, 6.67 mmol, 3 equiv.) was added slowly into a sealed microwave reaction vessel (10-20 mL) containing the stirred solution of methyl glycolate (0.17 mL, 2.22 mmol, 1 equiv.) and 2,2,2-trifluoroethylamine (0.53 mL, 6.66 mmol, 3 equiv.) in anhydrous THF (2.25 mL per 1 mmol of methyl glycolate) at 0 °C and under N_2. The mixture was irradiated in microwave reactor at 130 °C for 15 minutes, quenched by ice-cooled 2% HCl, treated with saturated $NaHCO_3$ solution (upto pH~9) and extracted with ethyl acetate. The combined organic phase was dried on $MgSO_4$ and evaporated *in vacuo* to yield a crude oil, which was purified by column chromatography under pressure on silica gel 60 using ethyl acetate and *n*-hexane as eluent (9:1→7:3) to afford compound **55**.

Compound **55** (296 mg, 85% yield) was obtained as a yellow oil.

$\mathbf{R_f}$ = 0.39 (Silica gel, Ethyl acetate).

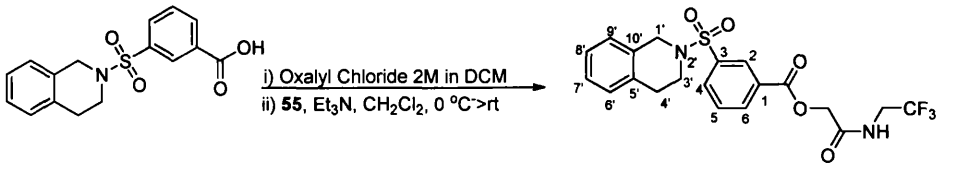
IR (ν_{max}/cm^{-1}): 986, 1148, 1250, 1537, 1659, 2919 and 3316.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.02 (1H, br. s, NH), 4.19 (2H, s, CH_2OH), 3.96 (2H, m, CH_2CF_3), 2.99 (1H, s, OH).

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6 (C=O), 125.1 (CF_3), 62.1 (CH_2OH), 40.1 (CH_2CF_3).

HRMS (TOF MS ES): Found 158.0429, calculated for $C_4H_7F_3NO_2$ 158.0351 $[M+H]^+$.

6.1.3.3 Procedure for Synthesis of Ester 46



53

46
$C_{20}H_{19}F_3N_2O_5S$
Mol. Wt.: 456.44

i) To a stirred solution of **53** (150 mg, 0.47 mmol, 1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **53**) and anhydrous DMF (1 drop), was added oxalyl chloride (0.1 mL, 1.18 mmol, 2.5 equiv.) dropwise at 0 °C and under N_2 for 2 h. Once CO_2 release had ended (15 min), the mixture was evaporated *in vacuo* to afford acylchloride (**54**) in a quantitative yield.
ii) The acyl chloride was then diluted in anhydrous DCM (6 mL per 1 mmol of **53**), the solution of **55** in DCM (1 mL) was put in the mixture and the solution cooled with an ice bath. Under vigorous stirring, triethylamine (0.07 mL, 0.49 mmol, 1 equiv.) was added dropwise. The resulting solution was stirred at room temperature for 4 h, quenched with water and extracted with DCM (x 3). The organic phases were evaporated *in vacuo* to yield a crude solid, which was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate as eluent (4:1→1:1) to afford compound **46**.

Compound **46** (193 mg, 90% yield) was obtained as a white powder.

R_f = 0.65 (Silica gel, Ethyl acetate).

mp = 157.1 – 158.2 °C.

IR (ν_{max}/cm^{-1}): 728, 832, 955, 1159, 1237, 1338, 1423, 1553, 1682 and 2330.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (1H, t, *J* = 1.6 Hz, H^2), 8.26 (1H, dt, *J* = 8.0, 1.2 Hz, H^4), 8.06 (1H, dt, *J* = 8.0. 1.2 Hz, H^6), 7.67 (1H, t, *J* = 8.0 Hz, H^5), 7.15 - 7.05 (4H, m, $H^{6',7',8',9'}$), 6.39 (1H, br. s, NH), 4.91 (2H, s, CH_2O), 4.33 (2H, s, $H^{1'}$), 4.01 (2H, m, CH_2CF_3), 3.44 (2H, t, *J* = 6.0 Hz, $H^{3'}$), 2.92 (2H, t, *J* = 6.0 Hz, $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0 (CONH), 164.0 (COO), 138.3 (C^3), 133.9 (C^4), 133.0 ($C^{5'}$), 132.5 (C^6), 131.2 ($C^{10'}$), 130.1 (C^1), 129.9 (C^5), 129.0 ($C^{6'}$), 128.9 (C^2), 127.1 (C^9), 126.6 ($C^{7'}$), 126.4 ($C^{8'}$), 124.3 (CF_3), 63.7 (CH_2O), 47.6 ($C^{1'}$), 43.8 ($C^{3'}$), 40.3 (CH_2CF_3), 28.7 ($C^{4'}$).

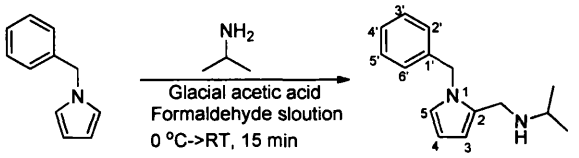
LRMS (ESI): *m/z* = 457.09 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 457.1030, calculated for $C_{20}H_{20}F_3N_2O_5S$, 457.1045 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{19}F_3N_2O_5S$: C, 52.63; H, 4.20; N, 6.14 % Found: C, 52.57; H, 4.11; N, 6.04 %.

6.1.4 Synthesis of Compound 47

6.1.4.1 Synthesis of the Mannich Base 56



56
$C_{15}H_{20}N_2$
Mol. Wt.: 228.33

To a vigorously stirred solution of N-benzylpyrrole (0.2 mL, 1.23 mmol, 1.0 equiv.) and glacial acetic acid (0.92 mL, 16 mmol, 13 equiv.) was added dropwise isopropylamine (0.31 mL, 3.7 mmol, 3.0 equiv.) at 0 °C and under N_2 followed by the addition of 37% formaldehyde solution (0.27 mL, 3.33 mmol, 2.7 equiv.). The mixture was stirred at room temperature for 15 minutes. The reaction was quenched with ice (5 g), extracted with ethyl acetate (5 x 20 mL). The organic phase was washed by $NaHCO_3$ solution (pH ~ 8), dried on $MgSO_4$ and evaporated *in vacuo* to yield oily residue which was purified by column chromatography under pressure on silica gel 60 using *n*-hexane and ethyl acetate (19:1→4:1) as eluent to afford compound **56**.

Compound **56** (124 mg, 44% yield) was obtained as a yellow oil.

$\mathbf{R_f}$ = 0.18 (Silica gel, Ethyl acetate).

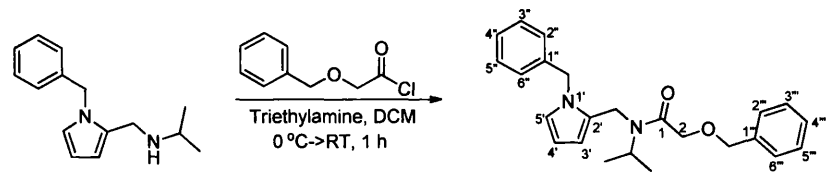
IR (ν_{max}/cm^{-1}): 705, 1023, 1067, 1170, 1294, 1453, 1491 and 2989.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (2H, m, $H^{3'}$ & $H^{5'}$), 7.22 (1H, m, $H^{4'}$), 7.03 (2H, d, J = 7.6 Hz, $H^{2'}$ & $H^{6'}$), 6.64 (1H, t, J = 1.6 Hz, H^5), 6.09 (1H, dd, J = 3.2, 2.8 Hz, H^4), 6.06 (1H, s, H^3), 5.17 (2H, s, CH_2(*N*-pyrrole)), 3.60 (2H, s, CH_2NH), 2.70 (1H, dq, J = 6.4, 6.0 Hz, $CH(CH_3)_2$), 0.93 (6H, d, J = 6.4 Hz, $CH(CH_3)_2$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 138.9 (C^2), 131.5 ($C^{1'}$), 128.8 ($C^{3'}$ & $C^{5'}$), 126.5 ($C^{2'}$ & $C^{6'}$), 126.4 ($C^{4'}$), 122.3 (C^5), 108.3 (C^3), 107.0 (C^4), 50.5 (CH_2(*N*-pyrrole)), 48.0 ($CH(CH_3)_2$), 43.2 (CH_2NH), 22.9 ($CH(CH_3)_2$).

LRMS (ESI): *m/z* = 229.21 $[M+H]^+$, 100%.

6.1.4.2 Synthesis of Amide 47



56

47
$C_{24}H_{28}N_2O_2$
Mol. Wt.: 376.49

2-(benzyloxy) acetyl chloride (0.09 mL, 0.55 mmol, 1.1 equiv.) was added slowly to the stirred mixture of **56** (114 mg, 0.5 mmol, 1 equiv.), triethylamine (0.1 mL, 0.75 mmol, 1.5 equiv.) and DCM (5 mL) at 0 °C under N_2. The mixture was stirred for 1 h at room temperature. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined extracts were dried on $MgSO_4$ and evaporated *in vacuo* to give crude oil, which was purified by column chromatography under pressure on silica gel 60 using *n*-hexane and ethyl acetate (19:1 → 4:1) as eluent to afford **47**.

Compound **47** (168 mg, 89% yield) was obtained as a light yellow oil.

R_f = 0.33 (Silica gel, *n*-Hexane/Ethyl acetate: 1/1).

IR (ν_{max}/cm^{-1}): 698, 731, 806, 1023, 1126, 1196, 1292, 1453, 1645 and 1726.

^{1}H NMR (400 MHz, DMSO): δ 7.36 – 7.25 (8H, m, $H^{3'',4'',5'',2''',3''',4''',5''',6'''}$), 7.08 (2H, d, J = 7.6 $H^{2''}$& $H^{6''}$), 6.75 (1H, s, $H^{5'}$), 6.00 (1H, dd, J = 3.2, 2.8 Hz, $H^{4'}$), 5.88 (1H, d, J = 1.2 Hz, $H^{3'}$), 5.15 (2H, s, CH_2(*N*-pyrrole)), 4.48 (2H, s, CH_2(OBn)), 4.31 (2H, s, CH_2(-NC=O)), 4.26 (1H, br. s, *CH*(CH_3)$_2$), 4.08 (2H, s, H^2), 1.02 (6H, d, J = 6.8 Hz, CH(*CH_3*)$_2$).

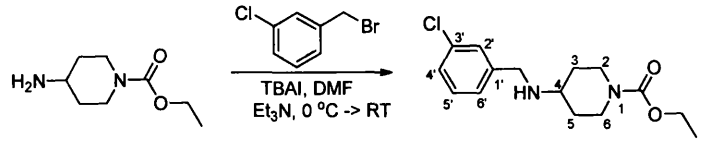
^{13}C NMR (100 MHz, DMSO): δ 168.4 (CONH), 138.53 ($C^{2'}$ or $C^{1'''}$), 138.03 ($C^{2'}$ or $C^{1'''}$), 129.4 ($C^{1''}$), 128.4 ($C^{3''}$ $C^{5''}$), 128.1 ($C^{3'''}$ & $C^{5'''}$), 127.5 ($C^{2'''}$ & $C^{6'''}$), 127.3 ($C^{4'''}$), 127.1 ($C^{4''}$), 126.6 ($C^{2''}$ & $C^{6''}$), 121.9 ($C^{5'}$), 107.4 ($C^{3'}$), 106.8 ($C^{4'}$), 72.2 (CH_2(OBn)), 68.9 (C^2), 49.7 (CH_2(*N*-pyrrole)), 46.5 (*CH*(CH_3)$_2$), 37.3 (CH_2(-NC=O))*, 20.1 (CH(*CH_3*)$_2$).

LRMS (ESI): *m/z* = 376.68 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 377.2231, calculated for $C_{24}H_{29}N_2O_2$ 377.2229 $[M+H]^+$.

6.1.5 Synthesis of Compound 48

6.1.5.1 Synthesis of Secondary Amine 57



57
$C_{15}H_{21}ClN_2O_2$
Mol Wt.: 296.79

1-bromomethyl-3-chlorobenzene (0.16 mL, 1.21 mmol, 1 equiv.) was added dropwise to a vigorously stirred solution of ethyl 4-aminopiperidine-1-carboxylate (0.25 mL, 1.45 mmol, 1.2 equiv.) and triethylamine (0.2 mL, 1.45 mmol, 1.2 equiv.) in anhydrous DMF (2.5 mL per 1 mmol of 1-bromomethyl-3-chlorobenzene) at 0 °C and under N_2. Then TBAI (4.5 mg, 0.12 mmol, 0.1 equiv.) was added to the mixture and was stirred for 3 h at room temperature. The mixture was quenched by addition of water and extracted with ethyl acetate (x 3). The combined extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and evaporated *in vacuo* to give a crude oil. The crude mixture was purified by column chromatography by gravity (ethyl acetate/methanol: 19/1) on silica gel 60 to afford compound **57**.

Compound **57** (344 mg, 96% yield) was obtained as a yellow oil.

$\mathbf{R_f}$ = 0.47 (Silica gel, Methanol/Ethyl acetate: 1/4).

IR (ν_{max}/cm^{-1}): 1137, 1225, 1428, 1686 and 2919.

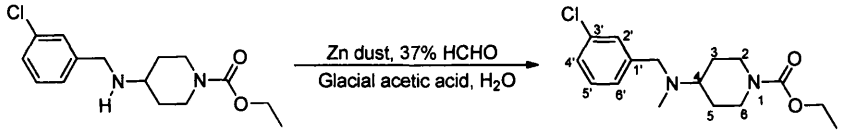
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (1H, s, $H^{2'}$), 7.25 (1H, s, $H^{4'}$), 7.22 (1H, m, $H^{5'}$), 7.20 (1H, s, $H^{6'}$), 4.71 (1H, s, NH), 4.11 (2H, q, J = 7.2 Hz, CH_2CH_3), 4.06 (2H, br. S, H^2 & H^6), 3.79 (2H, s, CH_2NH), 2.86 (2H, t, J = 12.4 Hz, H^2 & H^6), 2.66 (1H, m, H^4), 1.86 (2H, d, J = 12.4 Hz, H^3 & H^5), 1.30 (2H, d, J = 12.4 Hz, H^3 & H^5), 1.25 (3H, t, J = 7.2 Hz, CH_2CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5 (C=O), 142.6 ($C^{1'}$), 134.2 ($C^{3'}$), 129.6 ($C^{5'}$), 128.0 ($C^{2'}$), 127.0 ($C^{4'}$), 126.0 ($C^{6'}$), 61.1 (CH_2CH_3), 54.0 (C_4), 50.2 (CH_2NH), 42.4 (C^2 & C^6), 32.3 (C^3 & C^5), 14.6 (CH_2CH_3).

LRMS (ESI): *m/z* = 298.21 $[C_{15}H_{21}{}^{35}ClN_2O_2+2H]^+$ (75%), 300.06 $[C_{15}H_{21}{}^{37}ClN_2O_2+2H]^+$ (25%).

HRMS (TOF MS ES): Found 297.1364, calculated for $C_{15}H_{22}{}^{35}ClN_2O_2$ 297.1370 $[M+H]^+$.

6.1.5.2 Procedure for Synthesis of Compound 58



57

58
$C_{16}H_{23}ClN_2O_2$
Mol. Wt.: 310.82

Zinc dust (51 mg, 0.78 mmol, 2 equiv.) was added to a stirred solution of **57** (115mg, 0.4 mmol, 1 equiv.), glacial acetic acid (0.09 mL, 1.76 mmol, 4 equiv.) & 37% formaldehyde sloution (0.05 mL, 0.09 mmol, 1.5 equiv.) in water (2.25 mL per 1 mmol of **57**) at 0 °C and under N_2. The mixture was stirred for 24 h at room temperature, quenched by the addition of saturated NH_4Cl solution and extracted with and chloroform (x 3). The combined organic fractions were dried on $MgSO_4$ and solvent was evaporated at reduced pressure to yield crude oil, which was purified by column chromatography on silica gel 60 using ethyl acetate as eluent to afford compound **58**.

Compound **58** (62 mg, 50% yield) was obtained as a light yellow oil.

$\mathbf{R_f}$ = 0.62 (Silica gel, Methanol/Ethyl acetate: 1/4).

IR (ν_{max}/cm^{-1}): 734, 1110, 1236, 1430, 1597, 1690 and 1763.

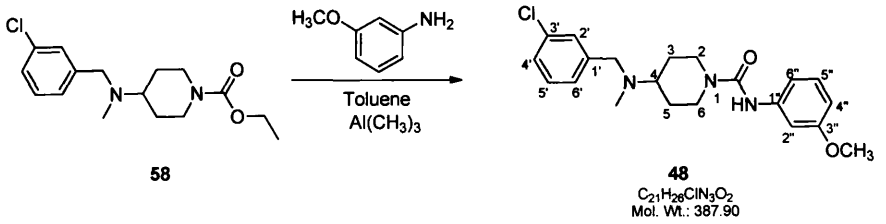
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (1H, s, $H^{2'}$), 7.23 (1H, s, $H^{4'}$), 7.21 (1H, m, $H^{5'}$), 7.19 (1H, s, $H^{6'}$), 4.19 (2H, br. S, H^2 & H^6), 4.13 (2H, q, J = 7.2 Hz, CH_2CH_3), 3.54 (2H, s, CH_2N), 2.74 (2H, t, J = 12.4 Hz, H^2 & H^6), 2.59 (1H, tt, J = 11.6, 3.6 Hz, H^4), 2.19 (3H, s, NCH_3), 1.80 (2H, d, J = 12.4 Hz, H^3 & H^5), 1.49 (2H, dq, J = 12.4, 4.4 Hz, H^3 & H^5), 1.26 (3H, t, J = 7.2 Hz, CH_2CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.6 (C=O), 142.3 ($C^{1'}$), 134.3 ($C^{3'}$), 129.6 ($C^{2'}$), 128.7 ($C^{4'}$), 127.2 ($C^{6'}$), 126.7 ($C^{5'}$), 61.4 (CH_2CH_3), 61.0 (C^4), 57.6 (CH_2N), 43.6 (C^2 & C^6), 37.7 (NCH_3), 28.0 (C^3 & C^5), 14.8 (CH_2CH_3).

LRMS (ESI): *m/z* = 313.21 $[C_{16}H_{23}{}^{35}ClN_2O_2+2H]^+$ (75%), 314.03 $[C_{16}H_{23}{}^{37}ClN_2O_2+2H]^+$ (25%).

HRMS (TOF MS ES): Found 311.1532, calculated for $C_{16}H_{24}{}^{35}ClN_2O_2$ 311.1526 $[M+H]^+$.

6.1.5.3 Procedure for Synthesis of Amide 48



58

48
$C_{21}H_{26}ClN_3O_2$
Mol. Wt.: 387.90

Trimethylaluminium (2.0M in toluene, 0.22 mL, 0.44 mmol, 2.5 equiv.) was slowly added to a solution of *m*-anisidine (0.06 mL, 0.53 mmol, 3 equiv.) in anhydrous toluene (5 mL per 1 mmol **58**) at 0 °C and under N_2. After stirring the solution for 1 h at room temperature the mixture was added dropwise to a stirred solution of **58** (60 mg, 0.18 mmol, 1 equiv.) in toluene (3 mL per 1 mmol **58**) at 0 °C and under N_2. The resulting solution was heated at 60 °C for 24 h, quenched by THF (10 mL) & H_2O (3 mL) for 20 minutes and extracted with ether (x 3). The ether phases were dried with $MgSO_4$ and evaporated *in vacuo* to give crude oil. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and *n*-hexane as eluent (1:1→7:3) to afford compound **48**.

Compound **48** (50 mg, 71% yield) was obtained as a brown powder.

R_f = 0.59 (Silica gel, Methanol/Ethyl acetate 1:4).

mp = 104.1 – 105.3 °C.

IR (ν_{max}/cm^{-1}): 776, 1040, 1158, 1237, 1429, 1538, 1599, 1636 and 3313.

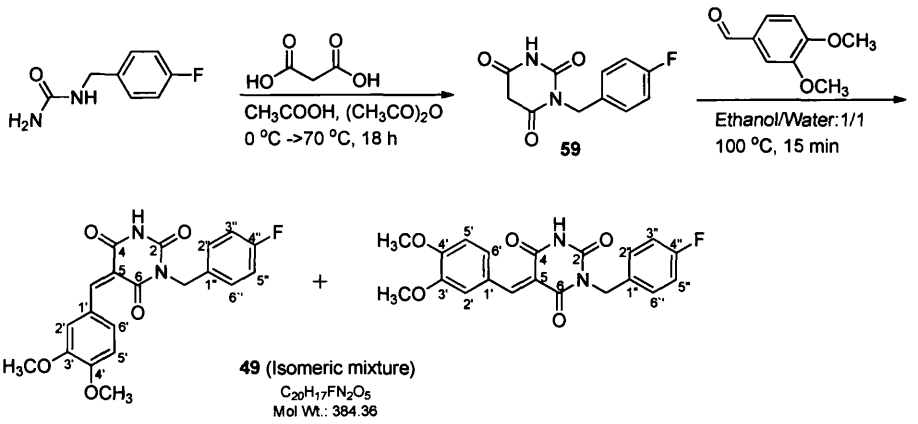
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (1H, s, $H^{2'}$), 7.24 (1H, s, $H^{4'}$), 7.22 (1H, t, *J* = 2.4 Hz, $H^{5'}$), 7.18 (1H, s, $H^{6'}$), 7.16 (1H, s, $H^{2''}$), 7.14 (1H, t, *J* = 2.4 Hz, $H^{5''}$), 6.82 (1H, dd, *J* = 8.4, 2.4 Hz, $H^{6''}$), 6.58 (1H, dd, *J* = 8.4, 2.4 Hz, $H^{4''}$), 6.46 (1H, br. S, NH), 4.13 (2H, t, *J* = 7.2 Hz, H^2 & H^6), 3.79 (3H, s, OCH_3), 3.56 (2H, s, CH_2N), 2.88 (2H, t, *J* = 12.0 Hz, H^2 & H^6), 2.64 (1H, tt, *J* = 11.2, 3.6 Hz, H^4), 2.20 (3H, s, NCH_3), 1.87 (2H, d, *J* = 11.2 Hz, H^3 & H^5), 1.59 (2H, qd, *J* = 12.0, 4.0 Hz, H^3 & H^5).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.3 ($C^{3''}$), 155.6 (C=O), 140.6 ($C^{1''}$), 134.4 ($C^{1'}$), 129.7 ($C^{3'}$), 129.6 ($C^{4'}$ & $C^{2'''}$), 128.7 ($C^{2'}$), 127.2 ($C^{5'}$), 126.8 ($C^{6'}$), 112.0 ($C^{6''}$), 109.1 ($C^{4''}$), 105.5 ($C^{5''}$), 60.8 (C^4), 57.6 (CH_2N), 55.4 (OCH_3), 44.1 (C^2 & C^6), 37.7 (NCH_3), 28.0 (C^3 & C^5).

LRMS (ESI): *m/z* = 388.81 $[C_{21}H_{26}{}^{35}ClN_3O_2+H]^+$, (75%), 391.05 $[C_{21}H_{26}{}^{37}ClN_3O_2+H]^+$ (25%).

HRMS (TOF MS ES): Found 388.1774, calculated for $C_{21}H_{27}{}^{35}ClN_3O_2$ 388.1792 $[M+H]^+$.

6.1.6 Synthesis of the Compound 49



A solution of N-(4-fluorobenzyl) urea (91 mg, 0.54 mmol, 1 equiv.) and malonic acid (65 mg, 0.63 mmol, 1.2 equiv.) in glacial acetic acid (0.62 mL, 10.8 mmol, 20 equiv.) was stirred for 5 h at 70 °C. Then 0.15 mL of acetic anhydride (1.6 mmol, 3 equiv.) was added to the mixture at room temperature and heated at 70 °C for 18 h. The mixture was evaporated *in vacuo* to afford intermediate **59** in a quantitative yield. The intermediate was diluted in 30 mL ethanol/water (1/1) mixture and 3,4-dimethoxybenzaldehyde (84 mg, 0.51 mmol, 1 equiv.) was added, the solution was heated under reflux for 15 min and stirred at room temperature for 3 h. The formed precipitate was washed with ice-cold ethanol and diethyl ether to afford bright yellow powder **49**.

Compound **49** (105 mg, 51% yield) was obtained as a yellow solid.

R_f = 0.169 (Silica gel, Ethyl acetate).

mp = 230.8 – 232.6 °C.

IR (ν_{max}/cm^{-1}): 790, 1017, 1150, 1273, 1427, 1505, 1547, 1659, 2358 and 3347.

^{1}H NMR (400 MHz, DMSO): δ 11.46 (1H, s, NH (minor isomer)), 11.34 (1H, s, NH (major isomer)), 8.36 (1H, s, CH (major isomer)), 8.35 (1H, s, $H^{2'}$ (major isomer)), 8.34 (1H, s, $H^{2'}$ (minor isomer)), 8.14 (1H, s, CH (minor isomer)), 7.91 (1H, t, J = 8.2 Hz, $H^{6'}$), 7.41 (2H, t, J = 5.6 Hz, $H^{2''}$ & $H^{6''}$), 7.10 – 7.15 (3H, m, $H^{5'}$, $H^{3''}$ & $H^{5''}$), 5.00 (2H, s, CH_2), 3.90 (3H, s, *o*-OCH_3), 3.82 (3H, s, *m*-OCH_3).

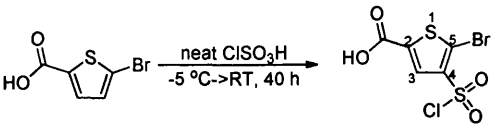
^{13}C NMR (100 MHz, DMSO): δ 156.6 (CH (major isomer)), 156.0 (CH (minor isomer)), 131.8 ($C^{6'}$ (major isomer)), 131.2 ($C^{6'}$ (minor isomer)), 129.6 – 129.7 ($C^{2'}$ & $C^{6'}$), 117.0 ($C^{2'}$ (major isomer)), 116.9 ($C^{2'}$ (minor isomer)), 114.9 – 115.1 ($C^{2''}$ & $C^{6''}$), 111.1 ($C^{5'}$), 55.9 (*o*-OCH_3), 55.5 (*m*-OCH_3), 43.3(CH_2 (major isomer)), 42.7 (CH_2 (minor isomer)).

LRMS (ESI): *m/z* = 386.48 $[M+2H]^+$, 100%.

HRMS (TOF MS ES): Found 385.1167, calculated for $C_{20}H_{18}FN_2O_5$ 385.1200 $[M+H]^+$.

6.2 Synthesis of compound 60

6.2.1 Procedure for the Chlorosulfonylation to Synthesise 61



61

$C_5H_2BrClO_4S_2$
Mol. Wt.: 305.55

Chlorosulfonic acid (7.84 mL, 116 mmol, 12 equiv.) was slowly added to stirred solution of 5-bromo-2-thiophene carboxylic acid (2 gm, 9.66 mmol, 1 equiv.) at -5 °C and under N_2. The solution was stirred for 40 h at room temperature and quenched by pouring into 500 gm of ice very slowly with a constant N_2 flow. The resulting precipitated **61** was collected by filtration and was dried overnight over $CaCl_2$.

Compound **61** (2.45 gm, 83% yield) was obtained as a white solid.

R_f = 0.34 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 142.2 –143.4 °C.

IR (ν_{max}/cm^{-1}): 751, 869, 1143, 1171, 1265, 1375, 1519, 1673 and 2560.

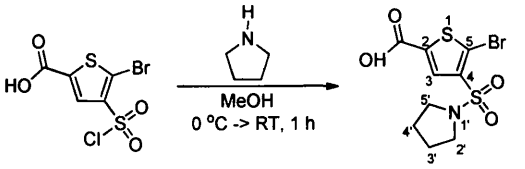
^{1}H NMR (400 MHz, $CDCl_3$): δ 9.02 (1H, br s, COOH), 8.22 (1H, s, H^3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (COOH), 142.5 (C^2), 134.0 (C^3), 133.6 (C^4), 127.7 (C^5).

LRMS (ESI): *m/z* = 258.86 $[C_5H_2{}^{79}Br^{35}ClO_4S_2\text{-}COOH]^-$ (70%), 260.86 $[C_5H_2{}^{81}Br^{35}ClO_4S_2\text{-}COOH]^-$ (100%), 262.83 $[C_5H_2{}^{81}Br^{37}ClO_4S_2\text{-}COOH]^-$ (30%).

HRMS (TOF MS ES): Found 302.8190, calculated for $C_5H^{79}Br^{35}ClO_4S_2$ 302.8188 $[M-H]^-$.

6.2.2 Synthesis of Sulfonamide 62



61

62
$C_9H_{10}BrNO_4S_2$
Mol. Wt.: 340.21

Pyrrolidine (0.62 mL, 7.36 mmol, 3 equiv.) was added dropwise to stirred solution of **61** (750 mg, 2.46 mmol, 1 equiv.) in anhydrous methanol (6.5mL per 1 mmol of **61**) at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). The aqueous phase was cooled in a ice bath and 10 mL of 1 M HCl was added slowly with continuous stirring. The product **62** was precipitated out, collected by filtration and was dried overnight over $CaCl_2$.

Compound **62** (695 mg, 83% yield) was obtained light yellow powder.

R_f = 0.4 (Silica gel, Methanol/Ethyl acetate: 2/3).

mp = 214.8 – 215.6 °C.

IR (ν_{max}/cm^{-1}): 863, 1011, 1167, 1294, 1347, 1522, 1685 and 2543.

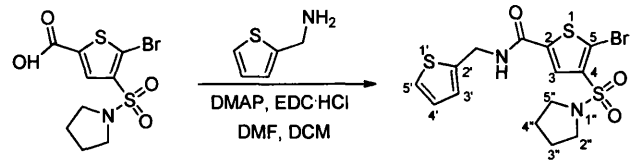
^{1}H NMR (400 MHz, MeOD): δ 7.80 (1H, s, H^3), 3.41 (4H, t, J = 6.4 Hz, $H^{2'}$& $H^{5'}$), 1.87 (4H, dt, J = 6.4, 3.6 Hz, $H^{3'}$& $H^{4'}$).

^{13}C NMR (100 MHz, MeOD): δ 162.7 (COOH), 139.7 (C^2), 136.9 (C^4), 134.6 (C^3), 123.0 (C^5), 48.3 ($C^{2'}$& $C^{5'}$), 26.4 ($C^{3'}$& $C^{4'}$).

LRMS (ESI): *m/z* = 339.89 $[C_9H_{10}{}^{79}BrNO_4S_2+H]^+$ (95%), 341.82 $[C_9H_{10}{}^{81}BrNO_4S_2+H]^+$ (100%).

HRMS (TOF MS ES): Found 339.9315, calculated for $C_9H_{11}{}^{79}BrNO_4S_2$ 339.9313 $[M+H]^+$.

6.2.3 Synthesis of Amide 63



62 **63**
$C_{14}H_{15}BrN_2O_3S_3$
Mol. Wt.: 435.38

Thiophene-2-methylamine (0.22 mL, 2.1 mmol, 1.1 equiv.) was added dropwise to stirred solution of **62** (650 mg, 1.91 mmol, 1 equiv.), EDC hydrochloride (440 mg, 2.29 mmol, 1.2 equiv.) and DMAP (23 mg, 0.19 mmol, 0.1 equiv.) in anhydrous DCM (1.5 mL per 1 mmol of **62**) and anhydrous DMF (1.5 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 20 h, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity on silica gel 60 (ethyl acetate/*n*-hexane: 7/3) to afford compound **63**.

Compound **63** (515 mg, 62% yield) was obtained as a light yellow solid.

R_f = 0.7 (Silica gel, Methanol/Ethyl acetate: 1/9).

mp = 166.5 – 167.8 °C.

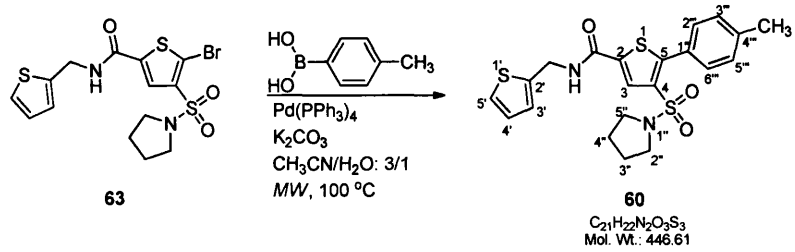
IR (ν_{max}/cm^{-1}): 864, 1016, 1137, 1314, 1404, 1548, 1652, 2925 and 3358.

^{1}H NMR (400 MHz, DMSO): δ 9.45 (1H, t, *J* = 5.6 Hz, NH), 8.04 (1H, s, H^3), 7.43 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.05 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 4.60 (2H, d, *J* = 5.6 Hz, CH_2NH),3.28 (4H, t, *J* = 6.4 Hz, $H^{2''}$& $H^{5''}$), 1.78 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, DMSO): δ 159.1 (CONH), 141.3 ($C^{2'}$), 140.7 (C^2), 137.0 (C^4), 128.7 (C^3), 126.7 ($C^{4'}$), 126.1 ($C^{3'}$), 125.4 ($C^{5'}$), 120.6 (C^5), 47.8 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 435.15 $[C_{14}H_{15}{}^{79}BrN_2O_3S_3+H]^+$ (90%), 437.17 $[C_{14}H_{15}{}^{81}BrN_2O_3S_3+H]^+$ (100%).

6.2.4 Synthesis of Compound 60



63

60

$C_{21}H_{22}N_2O_3S_3$
Mol. Wt.: 446.61

A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **63** (70 mg, 0.16 mmol, 1 equiv.), *p*-tolylboronic acid (33 mg, 0.24 mmol, 1.5 equiv.) and K_2CO_3 (45 mg, 0.32 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (19 mg, 0.02 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (19:1→4:1) as eluent to afford compound **60**.

Compound **60** (54 mg, 75% yield) was obtained as a yellow solid.

R_f = 0.26 (Silica gel, Ethyl acetate/Hexane: 2/3).

mp = 209.7 – 210.5 °C.

IR (ν_{max}/cm^{-1}): 873, 1126, 1298, 1447, 1547, 1656 and 3360.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (1H, s, H^3), 7.48 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.25 (1H, d, J = 1.2 Hz, $H^{5'}$), 7.24 (2H, d, J = 8.8 Hz, $H^{3'''}$& $H^{5'''}$), 7.06 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.96 (1H, dd, J = 5.2, 4.8 Hz, $H^{4'}$), 6.75 (1H, t, J = 5.6 Hz, NH), 4.77 (2H, d, J = 5.6 Hz, CH_2NH), 2.95 (4H, t, J = 6.4 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.58 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (CONH), 151.6 (C^5), 140.3 ($C^{2'}$), 140.2 ($C^{4'''}$), 137.2 (C^2), 134.6 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.4 (C^3), 129.1 ($C^{5'}$), 128.4 ($C^{1'''}$), 127.1 ($C^{4'}$), 126.7 ($C^{3'}$), 125.6 ($C^{3'''}$& $C^{5'''}$), 47.5 ($C^{2''}$& $C^{5''}$), 38.8 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

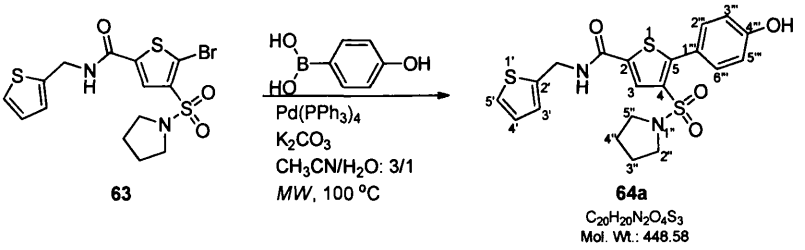
LRMS (ESI): *m/z* = 447.23 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 447.0854, calculated for $C_{21}H_{23}N_2O_3S_3$ 447.0865 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_3S_3$: C, 56.48; H, 4.97; N, 6.27% Found: C, 56.52; H, 4.89; N, 6.20%.

6.3 Preparation of Focused Library "A"

6.3.1 Synthesis of Compound 64a



A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), *p*-hydroxyphenylboronic acid (71 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **64a**.

Compound **64a** (138 mg, 88% yield) was obtained as a yellow solid.

R_f = 0.63 (Silica gel, Ethyl acetate).

mp = 206.0 – 206.2 °C.

IR (ν_{max}/cm^{-1}): 824, 1111, 1227, 1281, 1365, 1433, 1550, 1737, 2970 and 3193.

^{1}H NMR (400 MHz, DMSO): δ 9.42 (1H, t, *J* = 6.0 Hz, NH), 8.10 (1H, s, H^3), 7.418 (2H, d, *J* = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.42 (1H, d, *J* = 3.2 Hz, $H^{5'}$), 7.05 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'}$), 6.84 (2H, d, *J* = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 4.62 (2H, d, *J* = 6.0 Hz, CH_2NH), 2.96 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

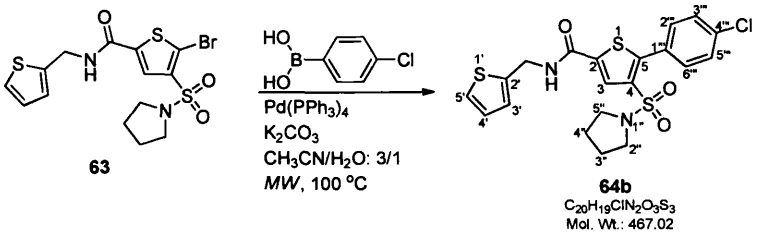
^{13}C NMR (100 MHz, DMSO): δ159.8 (CONH),158.8 ($C^{4'''}$), 151.0 (C^5), 141.8 ($C^{2'}$), 137.2 (C^2), 133.3 (C^4), 131.5 ($C^{2'''}$& $C^{6'''}$), 129.3 (C^3), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 121.4 ($C^{1'''}$), 115.0 ($C^{3'''}$& $C^{5'''}$), 47.2 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 357.46 $[M - PhOH + H]^+$, 100%.

HRMS (TOF MS ES): Found 449.0656, calculated for $C_{20}H_{21}N_2O_4S_3$ 449.0663 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{20}N_2O_4S_3$: C, 53.55; H, 4.49; N, 6.24% Found: C, 53.28; H, 4.41; N, 6.03%.

6.3.2 Synthesis of Compound 64b



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), *p*-cyanophenylboronic acid (81 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:1) as eluent to afford compound **64b**.

Compound **64b** (140 mg, 86% yield) was obtained as a white solid.

R_f = 0.71 (Silica gel, Ethyl acetate).

mp = 191.6 – 191.9 °C.

IR (ν_{max}/cm^{-1}): 709, 827, 1135, 1216, 1290, 1365, 1543, 1652, 1737, 2971 and 3364.

^{1}H NMR (400 MHz, DMSO): δ 9.48 (1H, t, *J* = 5.6 Hz, NH), 8.15 (1H, s, H^3), 7.60 (2H, d, *J* = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.54 (2H, d, *J* = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.42 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.05 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 1.6 Hz, $H^{4'}$), 4.63 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.00 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.64 (4H, m, $H^{3''}$& $H^{4''}$).

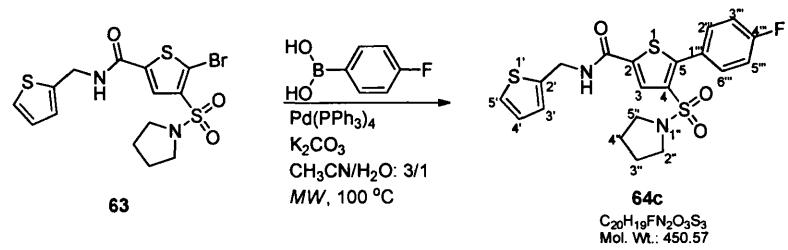
^{13}C NMR (100 MHz, DMSO): δ 159.6 (CONH), 148.6 (C^5), 141.6 ($C^{2'}$), 139.0 (C^2), 134.6 ($C^{1'''}$), 134.2 (C^4), 132.0 ($C^{2'''}$& $C^{6'''}$), 129.9 ($C^{4'''}$), 128.7 (C^3), 128.2 ($C^{3'''}$& $C^{5'''}$), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.4 ($C^{5'}$), 47.3 ($C^{2''}$& $C^{5''}$), 37.7 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 467.19 $[C_{20}H_{19}{}^{35}ClN_2O_3S_3+H]^+$, (75%), 469.15 $[C_{20}H_{19}{}^{37}ClN_2O_3S_3+H]^+$ (25%).

HRMS (TOF MS ES): Found 467.0313, calculated for $C_{20}H_{20}{}^{35}ClN_2O_3S_3$ 467.0325 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{19}ClN_2O_3S_3$: C, 51.43; H, 4.10; N, 6.00% Found: C, 51.30; H, 4.03; N, 5.95%.

6.3.3 Synthesis of Compound 64c



A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **63** (100 mg, 0.23 mmol, 1 equiv.), 4-fluorophenylboronic acid (48 mg, 0.35 mmol, 1.5 equiv.) and K_2CO_3 (63 mg, 0.46 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (27 mg, 0.02 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (19:1→7:3) as eluent to afford compound **64c**.

Compound **64c** (46 mg, 44% yield) was obtained as a light yellow powder.

R_f = 0.67 (Silica gel, Ethyl acetate).

mp = 197.4 – 198.2 °C.

IR (ν_{max}/cm^{-1}): 833, 1132, 1222, 1290, 1403, 1543, 1599, 1651 and 3375.

^{1}H NMR (400 MHz, $CDCl_3$): δ7.78 (1H, s, H^3), 7.60 (2H, dd, J = 8.8, 3.6 Hz, $H^{2'''}$ & $H^{6'''}$), 7.26 (1H, d, J = 3.6 Hz, $H^{5'}$), 7.13 (2H, t, J = 8.8 Hz, $H^{3'''}$ & $H^{5'''}$), 7.06 (1H, d, J = 3.4 Hz, $H^{3'}$), 6.98 (1H, dd, J = 3.6, 3.4 Hz, $H^{4'}$), 6.66 (1H, t, J = 5.6 Hz, NH), 4.79 (2H, d, J = 5.6 Hz, CH_2NH), 2.96 (4H, t, J = 6.4 Hz, $H^{2''}$ & $H^{5''}$), 1.63 (4H, dt, J = 6.4, 3.6 Hz, $H^{3''}$ & $H^{4''}$).

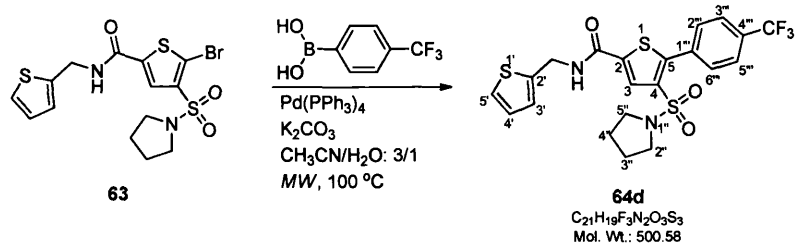
^{13}C NMR (100 MHz, $CDCl_3$): δ162.6 ($C^{4'''}$), 160.4 (CONH), 150.0 (C^5), 140.0 ($C^{2'}$), 137.6 (C^2), 135.1 (C^4), 132.4 ($C^{2'''}$ or $C^{6'''}$), 132.3 ($C^{2'''}$ or $C^{6'''}$), 129.2 (C^3), 127.3 ($C^{1'''}$), 127.2 ($C^{3'}$), 126.8 ($C^{4'}$), 125.8 ($C^{5'}$), 115.7 ($C^{3'''}$ or $C^{5'''}$), 115.5 ($C^{3'''}$ or $C^{5'''}$), 47.5 ($C^{2''}$ & $C^{5''}$), 38.8 (CH_2NH), 25.6 ($C^{3''}$ & $C^{4''}$).

LRMS (ESI): *m/z* = 450.71 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 451.0598, calculated for $C_{20}H_{20}FN_2O_3S_3$ 451.0620 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{19}FN_2O_3S_3$: C, 53.31; H, 4.25; N, 6.22% Found: C, 55.03; H, 4.14; N, 6.20%.

6.3.4 Synthesis of Compound 64d



63

64d
$C_{21}H_{19}F_3N_2O_3S_3$
Mol. Wt.: 500.58

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.). Then, K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.), tetrakis(triphenylphospine) palladium (40 mg, 0.03 mmol, 0.1 equiv.) and 4-(trifluoromethyl)phenylboronic acid (79 mg, 0.41 mmol, 1.2 equiv.) were added sequentially to the mixture. The vial was sealed and irradiated in microwave reactor at 100 °C for 5 minutes. The mixture was filtered and the filtrate was partitioned between DCM and water. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture was purified by column chromatography on silica gel, using DCM and ethyl acetate (99:1→93:7) as eluent to obtain **64d**.

64d (161 mg, 95% yield) was obtained as a yellow powder.

R_f = 0.41 (Silica gel, Ethyl acetate).

mp = 198.5 – 198.7 °C.

IR (ν_{max}/cm^{-1}): 840, 1067, 1121, 1322, 1407, 1547, 1654, 3106 and 3361.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, s, H^3), 7.74 (2H, d, J = 8.0 Hz, $H^{3'''}$ & $H^{5'''}$), 7.70 (2H, d, J = 8.0 Hz, $H^{2'''}$ & $H^{6'''}$), 7.27 (1H, d, J = 5.2, $H^{5'}$), 7.05 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, J = 5.2, 3.6 Hz, $H^{4'}$), 6.61 (1H, s, NH), 4.80 (2H, d, J = 5.6 Hz, CH_2NH), 2.98 (4H, t, J = 6.8 Hz, $H^{2''}$ & $H^{5''}$), 1.64 (4H, m, $H^{3''}$ & $H^{4''}$).

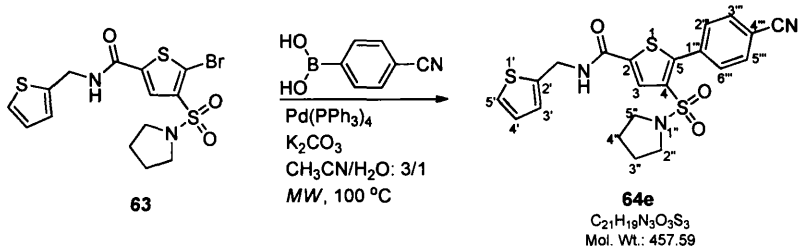
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.1 (CONH), 150.1 (C^5), 139.9 ($C^{2'}$), 138.5 (C^4), 135.7 (C^2), 134.8 ($C^{1'''}$), 132.0 ($C^{4'''}$), 130.8 ($C^{3'''}$ & $C^{5'''}$), 129.0 (C^3), 127.2 ($C^{4'}$), 126.9 ($C^{3'}$), 125.8 ($C^{5'}$), 125.3 ($C^{2'''}$ & $C^{6'''}$), 125.2 (CF_3), 47.5 ($C^{2''}$ & $C^{5''}$), 38.9 (CH_2NH), 25.5 ($C^{3''}$ & $C^{4''}$).

LCMS: *m/z* =501.64 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 501.0597, calculated for $C_{21}H_{20}F_3N_2O_3S_3$ 501.0588 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{19}F_3N_2O_3S_3$: C, 50.39; H, 3.83; N, 5.60% Found: C, 50.09; H, 3.78; N, 5.38%.

6.3.5 Synthesis of Compound 64e



63

64e
$C_{21}H_{19}N_3O_3S_3$
Mol. Wt.: 457.59

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), *p*-cyanophenylboronic acid (76 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **64e**.

Compound **64e** (146 mg, 91% yield) was obtained as a light brown solid.

R_f = 0.69 (Silica gel, Ethyl acetate).

mp = 206.1 – 206.3 °C.

IR (ν_{max}/cm^{-1}): 833, 997, 1142, 1291, 1435, 1544, 1651, 2227 and 3080.

^{1}H NMR (400 MHz, DMSO): δ 9.52 (1H, t, *J* = 5.6 Hz, NH), 8.18 (1H, s, H^3), 7.78 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.44 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.05 (1H, s, $H^{3'}$), 7.00 (1H, t, *J* = 4.4 Hz, $H^{4'}$), 7.96 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 4.65 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.02 (4H, t, *J* = 6.4 Hz, $H^{2''}$& $H^{5''}$), 1.66 (4H, m, $H^{3''}$& $H^{4''}$).

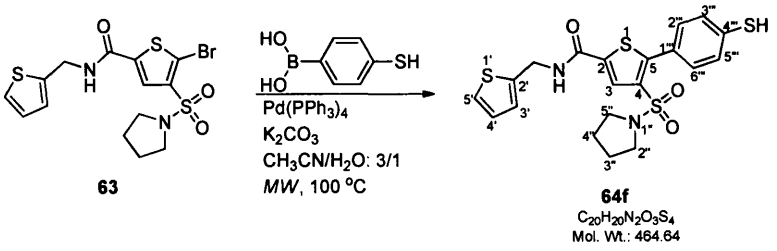
^{13}C NMR (100 MHz, DMSO): δ 159.5 (CONH), 147.8 (C^5), 141.6 ($C^{2'}$), 139.8 (C^2), 135.8 ($C^{1'''}$), 134.6 (C^4), 131.9 ($C^{3'''}$& $C^{5'''}$), 131.2 ($C^{2'''}$& $C^{6'''}$), 128.4 (C^3), 126.7 ($C^{4'}$), 126.0 ($C^{3'}$), 125.4 ($C^{5'}$), 112.2 ($C^{4'''}$), 47.3 ($C^{2''}$& $C^{5''}$), 37.7 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 458.44 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 458.0642, calculated for $C_{21}H_{20}N_3O_3S_3$ 458.0667 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{19}N_3O_3S_3$: C, 55.12; H, 4.19; N, 9.18% Found: C, 55.20; H, 4.17; N, 9.07%.

6.3.6 Synthesis of Compound 64f



63

64f
$C_{20}H_{20}N_2O_3S_4$
Mol. Wt.: 464.64

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), *p*-sulfanylphenylboronic acid (80 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and methanol (9:1→3:2) as eluent to afford compound **64f**.

Compound **64f** (143 mg, 88% yield) was obtained as a brown solid.

R_f = 0.63 (Silica gel, Ethyl acetate/Methanol: 1/1).

mp = 159.6 –160.0 °C.

IR (ν_{max}/cm^{-1}): 862, 1139, 1314, 1392, 1547, 1627, 1739, 2915 and 3369.

^{1}H NMR (400 MHz, MeOD): δ 7.84 (1H, s, H^3), 7.74 (2H, d, J = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.62 (2H, br. d, J = 7.6 Hz, $H^{2'''}$& $H^{6'''}$), 7.27 (1H, dd, J = 5.2, 1.2 Hz, $H^{5'}$), 6.99 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.92 (1H, dd, J = 5.2, 1.6 Hz, $H^{4'}$), 4.61 (2H, s, CH_2NH), 3.39 (4H, m, $H^{2''}$& $H^{5''}$), 1.86 (4H, m, $H^{3''}$& $H^{4''}$).

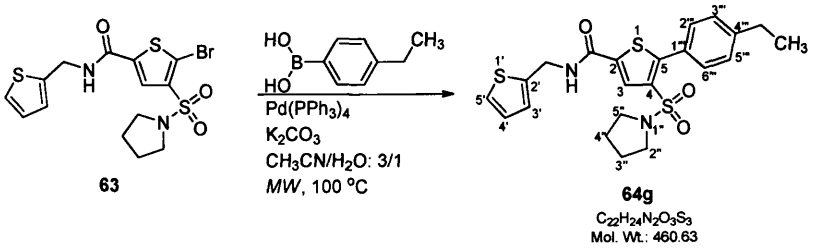
^{13}C NMR (100 MHz, MeOD): δ 162.0 (CONH), 151.1 (C^5), 142.2 ($C^{2'}$), 136.4 (C^2), 136.2 ($C^{3'''}$& $C^{5'''}$), 134.3 ($C^{2'''}$& $C^{6'''}$), 132.7 ($C^{4'''}$), 132.5 (C^4), 130.1 (C^3), 127.6 ($C^{4'}$), 127.0 ($C^{3'}$), 126.8 ($C^{1'''}$), 126.0 ($C^{5'}$), 49.2 ($C^{2''}$& $C^{5''}$), 39.0 (CH_2NH), 26.3 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 509.68 $[M + 2Na + H]^+$, 100%.

HRMS (TOF MS ES): Found 466.9652, calculated for $C_{20}H_{21}N_2O_3S_4$ 465.0435 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{20}N_2O_3S_4$: C, 51.70; H, 4.34; N, 6.03% Found: C, 51.63; H, 4.26; N, 6.08%.

6.3.7 Synthesis of Compound 64g



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 4-ethylphenylboronic acid (78 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **64g**.

Compound **64g** (145 mg, 90% yield) was obtained as a yellow solid.

R_f = 0.69 (Silica gel, Ethyl acetate).

mp = 189.9 – 190.1 °C.

IR (ν_{max}/cm^{-1}): 708, 874, 1125, 1286, 1449, 1545, 1650, 1737, 2968 and 3364.

^{1}H NMR (400 MHz, DMSO): δ 9.46 (1H, t, *J* = 5.6 Hz, NH), 8.13 (1H, s, H^3), 7.50 (2H, d, *J* = 8.0 Hz, $H^{2'''}$ & $H^{6'''}$), 7.43 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.06 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 7.32 (2H, d, *J* = 8.0 Hz, $H^{3'''}$ & $H^{5'''}$), 4.63 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.96 (4H, t, *J* = 6.4 Hz, $H^{2''}$ & $H^{5''}$), 2.67 (2H, q, *J* = 7.6 Hz, CH_2CH_3), 1.60 (4H, m, $H^{3''}$ & $H^{4''}$), 1.22 (3H, t, *J* = 7.6 Hz, CH_2CH_3).

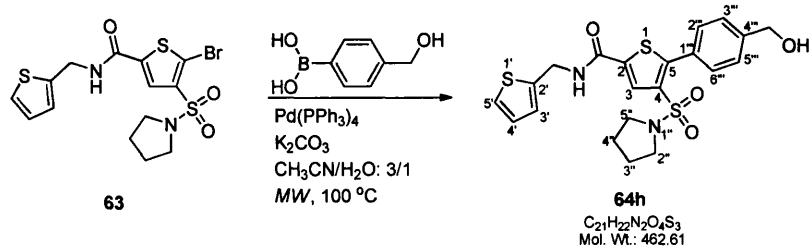
^{13}C NMR (100 MHz, DMSO): δ 159.7 (CONH), 150.4 (C^5), 141.7 ($C^{2'}$), 138.1 (C^2), 128.3 ($C^{1'''}$), 133.8 (C^4), 127.5 ($C^{3'''}$ & $C^{5'''}$), 130.0 ($C^{2'''}$ & $C^{6'''}$), 129.0 (C^3), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 145.6 ($C^{4'''}$), 47.2 ($C^{2''}$ & $C^{5''}$), 37.6 (CH_2NH), 27.9 (CH_2CH_3), 24.8 ($C^{3''}$ & $C^{4''}$), 15.3 (CH_2CH_3).

LRMS (ESI): *m/z* = 461.76 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 461.1007, calculated for $C_{22}H_{25}N_2O_3S_3$ 461.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_3S_3$: C, 57.36; H, 5.25; N, 6.08% Found: C, 57.45; H, 5.55; N, 6.07%.

6.3.8 Synthesis of Compound 64h



63

64h

$C_{21}H_{22}N_2O_4S_3$
Mol. Wt.: 462.61

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 4-(hydroxymethyl)phenylboronic acid (63 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (9:1 →3:1) as eluent to afford compound **64h**.

Compound **64h** (142 mg, 88% yield) was obtained as a white solid.

R_f = 0.21 (Silica gel, Ethyl acetate/DCM: 1/3).

mp = 60.1 – 60.4 °C.

IR (ν_{max}/cm^{-1}): 878, 1013, 1137, 1316, 1547, 1628, 2873 and 3299.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (1H, s, H^3), 7.60 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.44 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.27 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.07 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.98 (1H, dd, J = 5.2, 3.2 Hz, $H^{4'}$), 6.61 (1H, t, J = 5.6 Hz, NH), 4.79 (2H, d, J = 5.6 Hz, CH_2NH), 4.76 (2H, d, J = 5.6 Hz, CH_2OH), 2.97 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.85 (1H, t, J = 5.6 Hz, *OH*), 1.54 (4H, m, $H^{3''}$& $H^{4''}$).

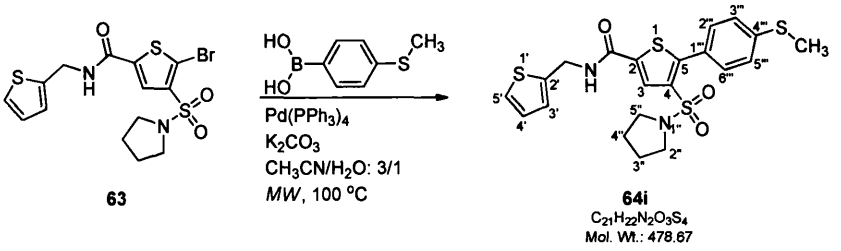
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4 (CONH),151.0 (C^5), 142.9 ($C^{4'''}$), 140.0 ($C^{2'}$), 137.4 (C^2), 134.9 (C^4), 130.59 ($C^{2'''}$&$C^{6'''}$), 130.50 ($C^{1'''}$), 129.3 (C^3), 127.2 ($C^{4'}$), 126.8 ($C^{3'}$), 126.6 ($C^{3'''}$& $C^{5'''}$), 125.8 ($C^{5'}$), 67.8 (CH_2OH), 47.5 ($C^{2''}$& $C^{5''}$), 38.8 (CH_2NH), 24.5 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 463.54 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 463.0811 calculated for $C_{21}H_{23}N_2O_4S_3$ 463.0820 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_3$: C, 54.52; H, 4.79; N, 6.06% Found:C, 54.69; H, 4.68; N, 6.15%.

6.3.9 Synthesis of Compound 64i



63

64i
$C_{21}H_{22}N_2O_3S_4$
Mol. Wt.: 478.67

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.). Then, K_2CO_3 (95.23 mg, 0.69 mmol, 2 equiv.), tetrakis(triphenylphospine) palladium (39.81 mg, 0.03 mmol, 0.1 equiv.) and 4-(methylthio)phenylboronic acid (69.46 mg, 0.41 mmol, 1.2 equiv.) were added sequentially to the mixture. The vial was sealed and irradiated in microwave reactor at 100 °C for 5 minutes. The mixture was filtered and the filtrate was partitioned between DCM and water. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture was purified by column chromatography on silica gel, using DCM and ethyl acetate (99:1→19:1) as eluent to obtain **64i**.

64i (97 mg, 59% yield) was obtained as a yellow powder.

R_f = 0.55 (Silica gel, DCM/Ethyl acetate: 9/1).

mp = 175.5 – 175.8 °C.

IR (ν_{max}/cm^{-1}): 752, 831, 1134, 1318, 1437, 1545, 3085 and 3354.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (1H, s, H^3), 7.53 (2H, d, J = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.27 (2H, d, J = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.25 (1H, s, $H^{5'}$), 7.06 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.97 (1H, dd, J = 4.8, 3.6 Hz, $H^{4'}$), 6.69 (1H, t, J = 5.6 Hz, NH), 4.78 (2H, d, J = 5.6 Hz, CH_2NH), 2.97 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.52 (3H, s, SCH_3), 1.61 (4H, m, $H^{3''}$& $H^{4''}$).

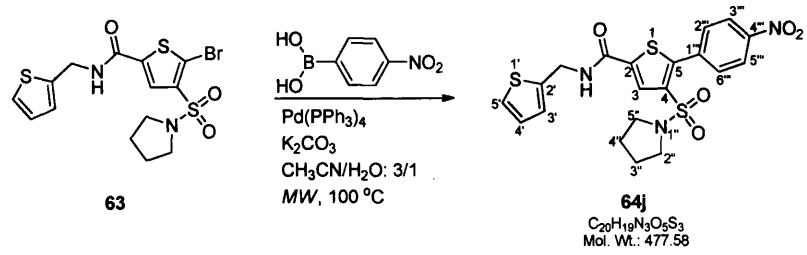
^{13}C NMR (100 MHZ, $CDCl_3$): δ 160.4 (CONH), 150.9 (C^5), 141.7 ($C^{4'''}$), 140.1 ($C^{2'}$), 137.2 (C^2), 134.6 (C^4), 130.6 ($C^{2'''}$& $C^{6'''}$), 129.5 (C^3), 127.5 ($C^{1'''}$), 127.1 ($C^{4'}$), 126.7 ($C^{3'}$), 125.7 ($C^{5'}$), 125.5 ($C^{3'''}$ & $C^{5'''}$), 47.6 (CH_2NH), 38.8 ($C^{2''}$ & $C^{5''}$), 25.5 ($C^{3''}$ & $C^{4''}$), 15.3 (SCH_3).

LRMS (ESI): *m/z* = 479.75 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 479.0597, calculated for $C_{21}H_{23}N_2O_3S_4$ 479.0591 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_3S_4$: C, 52.69; H, 4.63; N, 5.85% Found: C, 52.90; H, 4.53; N, 5.65%.

6.3.10 Synthesis of Compound 64j



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 4-nitrophenylboronic acid (58 mg, 0.35 mmol, 1 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→24:1) as eluent to afford compound **64j**.

Compound **64j** (137 mg, 82% yield) was obtained as a yellow solid.

R_f = 0.31 (Silica gel, Ethyl acetate/DCM: 1/19).

mp = 205.2 – 205.6 °C.

IR (ν_{max}/cm^{-1}): 852, 1119, 1289, 1345, 1518, 1654, 3111 and 3355.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (2H, d, J = 8.8 Hz, $H^{2'''}$&$H^{6'''}$), 7.80 (2H, d, J = 8.8 Hz, $H^{3'''}$& $H^{5'''}$), 7.78 (1H, s, H^3), 7.28 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.07 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.99 (1H, dd, J = 5.2, 3.2 Hz, $H^{4'}$), 6.63 (1H, t, J = 5.6 Hz, NH), 4.80 (2H, d, J = 5.6 Hz, CH_2NH), 3.01 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.69 (4H, m, $H^{3''}$& $H^{4''}$).

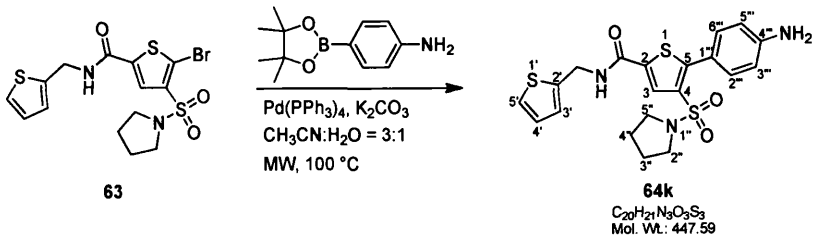
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.0 (CONH), 148.66 ($C^{4'''}$), 148.61 (C^5), 139.8 ($C^{2'}$), 139.3 (C^2), 137.7 ($C^{1'''}$), 136.1 (C^4), 131.5 ($C^{2'''}$&$C^{6'''}$), 128.9 (C^3), 127.2 ($C^{4'}$), 127.0 ($C^{3'}$), 125.9 ($C^{5'}$), 123.5 ($C^{3'''}$& $C^{5'''}$), 47.7 ($C^{2''}$& $C^{5''}$), 38.9 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 478.57 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 478.0571 calculated for $C_{20}H_{20}N_3O_5S_3$ 478.0565 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{19}N_3O_5S_3$: C, 50.30; H, 4.01; N, 8.80% Found: C, 51.06; H, 3.79; N, 8.06%.

6.3.11 Synthesis of Compound 64k



63

64k

$C_{20}H_{21}N_3O_3S_3$

Mol. Wt.: 447.59

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 4-(aminophenyl) boronic acid pinacol ester (83 mg, 0.38 mmol, 1.1 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol, 0.1 equiv.) was the added under N_2 and irradiated in microwave reactor at 100 °C for five minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:49 → 3:17) as eluent to afford compound **64k**.

Compound **64k** (102 mg, 65% yield) was obtained as a yellow solid.

R_f = 0.47 (silica gel, Ethyl acetate/DCM: 3/7).

mp = 209.2 - 210.6 °C.

IR (ν_{max}/cm^{-1}): 824, 1171, 1334, 1646, 3085, 3366 and 3476.

^{1}H NMR (400 MHz, DMSO): δ 9.37 (1H, t, *J* = 6.0 Hz, CONH), 8.06 (1H, s, H^3), 7.42 (1H, dd, *J* = 5.2, 5.2 Hz, $H^{5'}$), 7.29 (2H, d, *J* = 6.8 Hz, $H^{2'''}$& $H^{6'''}$), 7.04 (1H, d, *J* = 4.8 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 4.8, 5.2 Hz, $H^{4'}$), 6.60 (2H, d, *J* = 6.8 Hz, $H^{3'''}$& $H^{5'''}$), 5.59 (2H, s, NH_2), 4.61 (2H, d, *J* = 6.0 Hz, CH_2NH), 2.96 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.58 (4H, m, $H^{2''}$& $H^{4''}$).

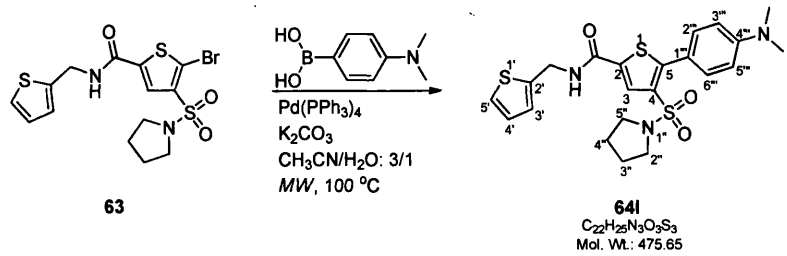
^{13}C NMR (100 MHz, DMSO): δ 159.9 (CONH), 152.3 (C^5), 150.4 ($C^{4'''}$), 141.9 ($C^{2'}$), 135.9 (C^2), 132.3 ($C^{1'''}$), 130.9 ($C^{2'''}$& $C^{6'''}$), 129.7 (C^3), 126.7 ($C^{4'}$), 125.8 ($C^{3'}$), 125.2 ($C^{5'}$), 117.5 (C^4), 112.9 ($C^{3'''}$& $C^{5'''}$), 47.2 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 448.46 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 448.0802, calculated for $C_{20}H_{22}N_3O_3S_3$ 448.0823 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{21}N_3O3S_3$: C, 53.67; H, 4.73; N, 9.39% Found: C, 53.75; H, 4.69; N, 9.19%.

6.3.12 Synthesis of Compound 64l



63

64l
$C_{22}H_{25}N_3O_3S_3$
Mol. Wt.: 475.65

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (350 mg, 0.80 mmol, 1 equiv). Then, K_2CO_3 (222 mg, 1.60 mmol, 2 equiv.), tetrakis(triphenylphospine) palladium (93 mg, 0.04 mmol, 0.1 equiv.) and 4-(dimethylamino)phenylboronic acid (158 mg, 0.96 mmol, 1.2 equiv.) were added sequentially to the mixture. The vial was sealed and irradiated in microwave reactor at 100 °C for 5 minutes. The mixture was filtered and the filtrate was partitioned between DCM and water. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture was purified by column chromatography on silica gel, using DCM and ethyl acetate (99:1→19:1) as eluent to obtain **64l**.

64l (285 mg, 75% yield) was obtained as a yellow powder.

R_f = 0.49 (Silica, DCM/Ethyl acetate: 9/1).

mp = 173.2 – 173.8 °C.

IR (ν_{max}/cm^{-1}): 750, 1135, 1313, 1506, 1607, 1648, 2920 and 3351.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, s, H^3), 7.51 (2H, d, *J* = 8.8 Hz, $H^{2'''}$& $H^{6'''}$), 7.25 (1H, d, *J* = 4.8, $H^{5'}$), 7.06 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 4.8, 3.6 Hz, $H^{4'}$), 6.48 (1H, s, NH), 6.37 (2H, d, *J* = 8.8 Hz, $H^{3'''}$& $H^{5'''}$), 4.78 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.01 (6H, s, $N(CH_3)_2$), 2.99 (4H, m, $H^{2''}$& $H^{5''}$), 1.59 (4H, m, $H^{3''}$& $H^{4''}$).

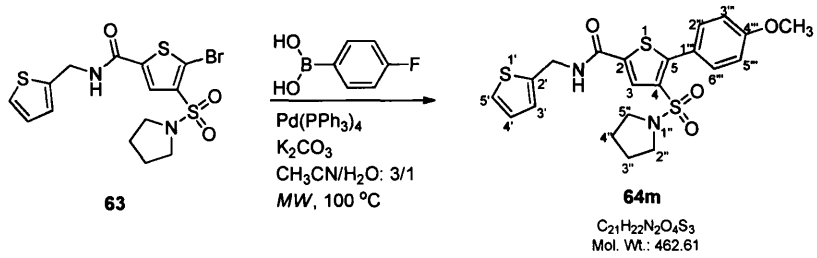
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.7 (CONH), 153.3 (C^2), 151.4 ($C^{4'''}$), 140.2 ($C^{2'}$), 135.3 (C^4), 133.2 (C^5), 131.3 ($C^{2'''}$& $C^{6'''}$), 130.0 (C^3), 127.1 ($C^{4'}$), 126.7 ($C^{3'}$), 125.7 ($C^{5'}$), 118.5 ($C^{1'''}$), 111.4 ($C^{3'''}$ & $C^{5'''}$), 47.5 ($C^{2''}$ & $C^{5''}$), 40.3 ($N(CH_3)_2$), 38.8 (CH_2NH), 25.6 ($C^{3''}$ & $C^{4''}$).

LRMS (ESI): *m/z* = 476.62 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 476.1138, calculated for $C_{22}H_{26}N_3O_3S_3$ 476.1136 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{25}N_3O_3S_3$: C, 55.55; H, 5.30; N, 8.83% Found: C, 56.20; H, 5.24; N, 7.91%.

6.3.13 Synthesis of Compound 64m



63 **64m**

$C_{21}H_{22}N_2O_4S_3$
Mol. Wt.: 462.61

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (100 mg, 0.23 mmol, 1 equiv.), 4-methoxyphenylboronic acid (52 mg, 0.35 mmol, 1.5 equiv.) and K_2CO_3 (63 mg, 0.46 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (27 mg, 0.02 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (19:1→7:3) as eluent to afford compound **64m**.

Compound **64m** (31 mg, 29% yield) was obtained as a yellow solid.

R_f = 0.65 (Silica gel, Ethyl acetate).

mp = 173.9 – 174.8 °C.

IR (ν_{max}/cm^{-1}): 874, 1027, 1174, 1320, 1448, 1546, 1650, 2934 and 3361.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (1H, s, H^3), 7.56 (2H, d, J = 8.8 Hz, $H^{2'''}$ & $H^{6'''}$), 7.25 (1H, d, J = 3.6 Hz, $H^{5'}$), 7.06 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.98 (1H, dd, J = 5.2, 3.6 Hz, $H^{4'}$), 6.95 (2H, d, J = 8.8 Hz, $H^{3'''}$ & $H^{5'''}$), 6.60 (1H, t, J = 5.6 Hz, NH), 4.79 (2H, d, J = 5.6 Hz, CH_2NH), 3.85 (3H, s, OCH_3), 2.96 (4H, t, J = 6.4 Hz, $H^{2''}$ & $H^{5''}$), 1.60 (4H, dt, J = 6.4, 3.6 Hz, $H^{3''}$ & $H^{4''}$).

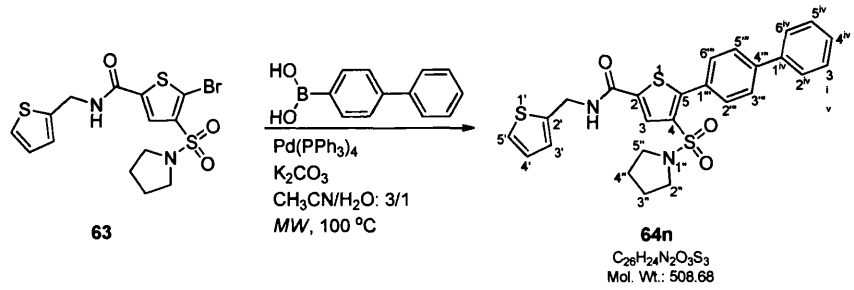
^{13}C NMR (100 MHz, $CDCl_3$): δ 161.0 ($C^{4'''}$), 160.5 (CONH), 151.5 (C^5), 140.1 ($C^{2'}$), 136.7 (C^2), 136.3 (C^4), 131.7 ($C^{2'''}$ & $C^{6'''}$), 129.6 (C^3), 127.1 ($C^{4'}$), 126.8 ($C^{3'}$), 125.7 ($C^{5'}$), 123.5 ($C^{1'''}$), 123.5 ($C^{1'''}$), 55.5 (OCH_3), 47.5 ($C^{2''}$ & $C^{5''}$), 38.8 (CH_2NH), 25.6 ($C^{3''}$ & $C^{4''}$).

LRMS (ESI): *m/z* = 463.74 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 463.0798, calculated for $C_{21}H_{23}N_2O_4S_3$ 463.0820 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_3$: C, 54.52; H, 4.79; N, 6.06% Found: C, 54.58; H, 4.73; N, 6.01%.

6.3.14 Synthesis of Compound 64n



63

64n

$C_{26}H_{24}N_2O_3S_3$

Mol. Wt.: 508.68

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (92 mg, 0.21 mmol, 1 equiv.), biphenyl-4-ylboronic acid (50 mg, 0.25 mmol, 1.2 equiv.), K_2CO_3 (58 mg, 0.42 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.02 mmol, 0.1 equiv.). The mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0 → 47:3) as eluent to afford compound **64n**.

Compound **64n** (67 mg, 63% yield) was obtained as a light yellow solid.

R_f = 0.65 (silica gel, Ethyl acetate/DCM: 1/1).

mp = 200.4 – 200.9 °C.

IR (ν_{max}/cm^{-1}): 757, 878, 1136, 1286, 1443, 1546, 1644 and 3342.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (1H, s, H^3), 7.71 – 7.65 (4H, m, $H^{2'''}$,$H^{3'''}$, $H^{5'''}$& $H^{6'''}$), 7.63 (2H, d, J = 8.0 Hz, H^{2iv} & H^{6iv}), 7.47 (2H, d, J = 7.2 Hz, H^{3iv} & H^{5iv}), 7.39 (1H, t, J = 7.2 Hz, H^{4iv}), 7.27 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.07 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, J = 5.2, 3.6 Hz, $H^{4'}$), 6.58 (1H, t, J =5.6 Hz, CONH), 4.80 (2H, d, J = 5.6 Hz, CH_2NH), 3.01 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

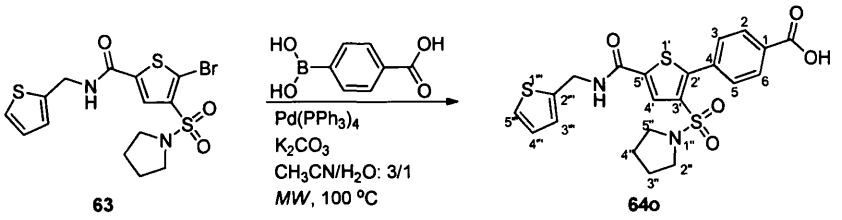
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4 (CONH), 151.0 (C^5), 142.8 ($C^{4'''}$), 140.1 ($C^{2'}$ & C^{1iv}), 137.4 (C^2), 135.0 (C^4), 130.4 & 127.0 ($C^{2'''}$,$C^{3'''}$,$C^{5'''}$& $C^{6'''}$), 130.2 ($C^{1'''}$), 129.4 (C^3), 129.1 (C^{3iv} & C^{5iv}), 128.1 (C^{4iv}), 127.2 (C^{2iv} & C^{6iv}), 127.16 ($C^{4'}$), 126.8 ($C^{3'}$), 125.8 ($C^{5'}$), 47.5 ($C^{2''}$& $C^{5''}$), 38.8 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 509.45 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 509.1043, calculated for $C_{26}H_{25}N_2O_3S_3$ 509.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{26}H_{24}N_2O_3S_3$: C, 61.39; H, 4.76; N, 5.51% Found: C, 61.21; H, 4.61; N, 5.53%.

6.3.15 Synthesis of Compound 64o



63 64o

$C_{21}H_{20}N_2O_5S_3$
Mol. Wt.: 476.59

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.). Then, K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.), tetrakis(triphenylphospine) palladium (40 mg, 0.03 mmol, 0.1 equiv.) and 4-boronobenzoic acid (69 mg, 0.41 mmol, 1.2 equiv.) were added sequentially to the mixture. The vial was sealed and irradiated in microwave reactor at 100 °C for 5 minutes. The mixture was filtered and the filtrate was partitioned between DCM and water. The aqueous phase was cooled to 0 °C to facilitate precipitation of the product **64o**, which was collected by filtration.

64o (107 mg, 64% yield) was obtained as a white solid.

R_f = 0.31 (Silica gel, DCM/Ethyl acetate: 9/1).

mp = 86.1 – 86.3 °C.

IR (ν_{max}/cm^{-1}): 843, 1142, 1313, 1389, 1540, 1618, 3090 and 3250.

^{1}H NMR (400 MHz, DMSO): δ 9.57 (1H, s, NH), 8.16 (1H, s, $H^{4'}$), 7.91 (2H, d, J = 8.0 Hz, H^2 & H^6), 7.50 (2H, d, *J* = 8.0 Hz, H^3 & H^5), 7.42 (1H, s, $H^{5'''}$), 7.05 (1H, s, $H^{3'''}$), 6.98 (1H, s, $H^{4'''}$), 4.63 (2H, s, CH_2NH), 2.95 (4H, d, *J* = 4.0 Hz, $H^{2''}$& $H^{5''}$), 1.58 (4H, d, *J* = 4.0 Hz, $H^{3''}$& $H^{4''}$).

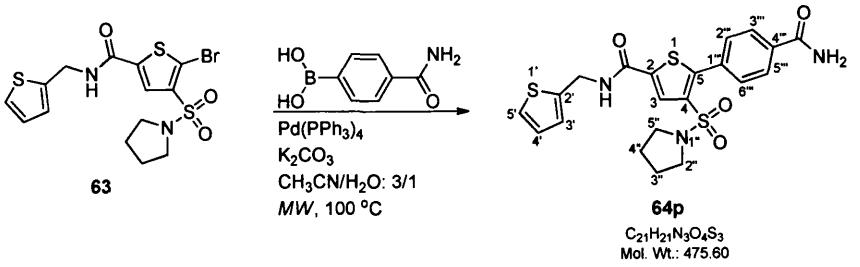
^{13}C NMR (100 MHz, DMSO): δ 168.8 (COOH), 159.7 (*C*ONH), 150.5 ($C^{2'}$), 141.8 ($C^{2'''}$), 141.7 (C^4), 138.2 ($C^{5'}$), 134.0 ($C^{3'}$), 131.0 (C^1), 129.2 ($C^{4'}$), 129.1 (C^2 & C^6), 128.6 (C^3 & C^5), 126.7 ($C^{4'''}$), 125.9 ($C^{3'''}$), 125.3 ($C^{5'''}$), 47.2 ($C^{2''}$ & $C^{5''}$), 37.6 (CH_2NH), 24.9 ($C^{3''}$ & $C^{4''}$).

LRMS (ESI): *m/z* = 477.67[M+H]$^+$, 100%.

HRMS (TOF MS ES): Found 477.0621, calculated for $C_{21}H_{21}N_2O_5S_3$ 477.0612 [M+H]$^+$.

Elem. Anal. Calculated for $C_{21}H_{20}N_2O_5S_3$: C, 52.92; H, 4.23; N, 5.88% Found: C, 53.56; H, 4.20; N, 4.47%.

6.3.16 Synthesis of Compound 64p



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (200 mg, 0.46 mmol, 1 equiv.), 4-carbamoylphenylboronic acid (114 mg, 0.69 mmol, 1.5 equiv.) and K_2CO_3 (127 mg, 0.92 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (53 mg, 0.046 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The product (**64p**) precipitated out which was collected by filtration. **64p** was further washed with DCM.

Compound **64p** (186 mg, 85% yield) was obtained as a white solid.

R_f = 0.82 (Silica gel, Ethyl acetate/Methanol: 1/1).

mp = 209.7 – 210.5 °C.

IR (ν_{max}/cm^{-1}): 766, 1014, 1138, 1324, 1501, 1651 and 3441.

^{1}H NMR (400 MHz, DMSO): δ 9.49 (1H, t, *J* = 6.0 Hz, CH_2*NH*), 8.16 (1H, s, H^3), 8.09 (1H, s, $CONH_2$), 7.94 (2H, d, *J* = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.66 (2H, d, *J* = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.48 (1H, s, $CONH_2$), 7.43 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.07 (1H, s, $H^{3'}$), 7.00 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'}$), 4.64 (2H, d, *J* = 6.0 Hz, *CH_2*NH), 2.99 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.63 (4H, m, $H^{3''}$& $H^{4''}$).

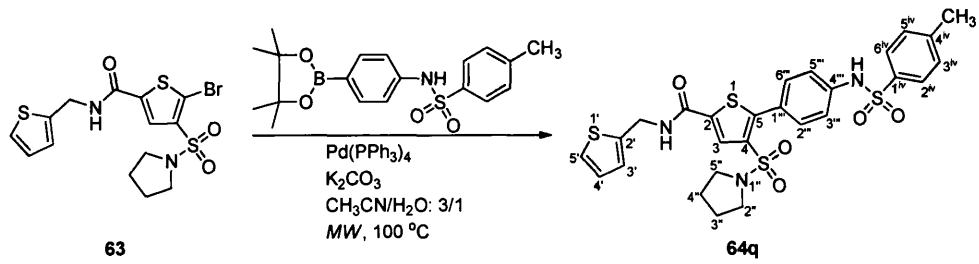
^{13}C NMR (100 MHz, DMSO): δ 167.2 ($CONH_2$), 159.6 (CONH), 149.2 (C^5), 141.6 ($C^{2'}$), 139.0 (C^2), 135.1 ($C^{1'''}$), 134.3 (C^4), 127.11 ($C^{3'''}$& $C^{5'''}$), 130.08 ($C^{2'''}$& $C^{6'''}$), 128.8 (C^3), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.4 ($C^{5'}$), 133.7 ($C^{4'''}$), 47.3 ($C^{2''}$& $C^{5''}$), 37.7 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 476.77 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 476.0793, calculated for $C_{21}H_{22}N_3O_4S_3$ 476.0772 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{21}N_3O_4S_3$: C, 53.03; H, 4.45; N, 8.84% Found: C, 53.06; H, 4.35; N, 8.64%.

6.3.17 Synthesis of Compound 64q



63

64q

$C_{27}H_{27}N_3O_5S_4$
Mol. Wt.: 601.78

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (5 mL) followed by the addition of **63** (96 mg, 0.22 mmol, 1 equiv.), 4-(*p*-Toluenesulfonylamino) phenylboronic acid pinacol ester (99 mg, 0.27 mmol, 1.2 equiv.), K_2CO_3 (61 mg, 0.44 mmol, 2 equiv.) and tetrakis- (triphenylphosphine)palladium(0) (25 mg, 0.022 mmol, 0.1 equiv.). The reaction mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→23:2) as eluent to afford compound **64q**.

Compound **64q** (95 mg, 72% yield) was obtained as a light yellow solid.

R_f = 0.55 (silica gel, Ethyl acetate/DCM: 1/1).

mp = 164.4 – 164.6 °C.

IR (ν_{max}/cm^{-1}): 816, 910, 1021, 1160, 1297, 1446, 1545, 1631 and 3176.

^{1}H NMR (400 MHz, DMSO): δ 10.57 (1H, br. s, NH), 9.43 (1H, t, *J* =5.6 Hz, CONH), 8.09 (1H, s, H^3), 7.70 (2H, d, *J* = 8.4 Hz, H^{2iv} & H^{6iv}), 7.43 (2H, d, *J* = 8.4 Hz, $H^{2'''}$ & $H^{6'''}$), 7.41 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.36 (2H, d, *J* = 8.4 Hz, H^{3iv} & H^{5iv}), 7.16 (2H, d, *J* = 8.4 Hz, $H^{3'''}$ & $H^{5'''}$), 7.04 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.97 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'}$), 4.60 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.81 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.36 (3H, s, CH_3), 1.47 (4H, m, $H^{3''}$& $H^{4''}$).

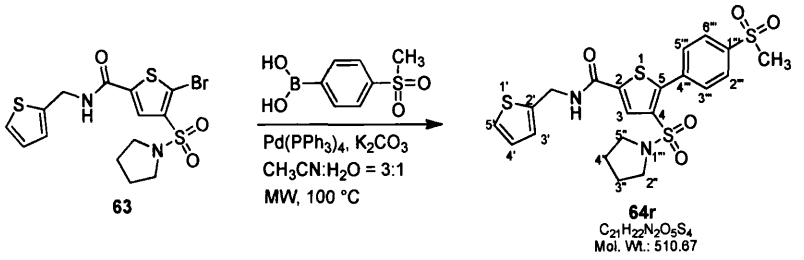
^{13}C NMR (100 MHz, DMSO): δ 159.7 (CONH), 149.5 (C^5), 143.5 (C^{4iv}), 141.7 ($C^{2'}$), 137.9 (C^2), 137.0 (C^{1iv}), 136.7 ($C^{4'''}$), 134.1 (C^4), 131.1 ($C^{2'''}$ & $C^{6'''}$), 129.8 (C^{3iv} & C^{5iv}), 129.2 (C^3), 127.0 (C^{2iv} & C^{6iv}), 126.7 ($C^{4'}$), 126.6 ($C^{1'''}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 118.4 ($C^{3'''}$& $C^{5'''}$), 47.1 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$), 20.9 (CH_3).

LRMS (ESI): *m/z* = 602.98 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 602.0894, calculated for $C_{27}H_{28}N_3O_5S_4$ 602.0912 $[M+H]^+$.

Elem. Anal. Calculated for $C_{27}H_{27}N_3O_5S_4$: C, 53.89; H, 4.52; N, 6.98% Found: C, 54.01; H, 4.41; N, 7.14%.

6.3.18 Synthesis of Compound 64r



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 4-(methanesulfonyl) phenyl boronic acid (76 mg, 0.38 mmol, 1.1 equiv.), K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.). The reaction mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:49 → 3:17) as eluent to afford compound **64r**.

Compound **64r** (166 mg, 93% yield) was obtained as a brown solid.

R_f = 0.56 (silica gel, Ethyl acetate/DCM: 2/3).

mp = 191.1 - 192.5 °C.

IR (ν_{max}/cm^{-1}): 774, 1307, 1350, 1558, 1617 and 3256.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (2H, d, J = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.82 (2H, d, J = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.78 (1H, s, H^3), 7.27 (1H, dd, J = 5.2, 5.2 Hz, $H^{5'}$), 7.07 (1H, d, J = 4.8 Hz, $H^{3'}$), 6.99 (1H, dd, J = 4.8, 5.2 Hz, $H^{4'}$), 6.61 (1H, t, J = 5.6 Hz, CONH), 4.80 (2H, d, J = 5.6 Hz, CH_2NH), 3.09 (3H, s, CH_3), 3.01 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.68 (4H, m, $H^{3''}$& $H^{4''}$).

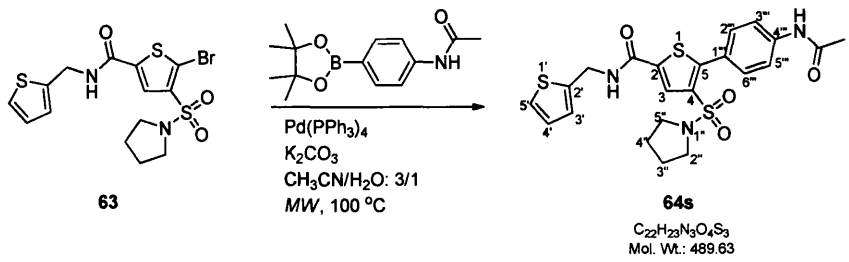
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.0 (CONH), 148.1 (C^5), 141.7 ($C^{1'''}$), 139.8 ($C^{2'}$), 139.0 (C^2), 136.0 (C^4), 131.4 ($C^{2'''}$& $C^{6'''}$), 128.9 (C^3), 127.5 ($C^{3'''}$& $C^{5'''}$), 127.2 ($C^{3'}$), 126.9 ($C^{4'}$), 125.8 ($C^{5'}$), 47.6 ($C^{2''}$& $C^{5''}$), 44.5 (CH_3), 38.9 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 511.33 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 511.0510, calculated for $C_{21}H_{23}N_2O_5S_4$ 511.0490 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_5S_4$: C, 49.39; H, 4.34; N, 5.49% Found: C, 49.52; H, 4.30; N, 5.41%.

6.3.19 Synthesis of Compound 64s



63

64s

$C_{22}H_{23}N_3O_4S_3$

Mol. Wt.: 489.63

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 4-Acetamidophenylboronic acid pinacol ester (135 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (1:1→1:4) as eluent to afford compound **64s**.

Compound **64s** (113 mg, 66% yield) was obtained as a yellow solid.

R_f = 0.313 (Silica gel, Ethyl acetate).

mp = 222.5 – 222.8 °C.

IR (ν_{max}/cm^{-1}): 729, 1118, 1282, 1514, 1635, 1692 and 3328.

^{1}H NMR (400 MHz, DMSO): δ 10.15 (1H, s, *NH*COCH$_3$), 9.45 (1H, t, *J* = 6.0 Hz, NHCO), 8.12 (1H, s, H^3), 7.67 (2H, d, *J* = 8.4 Hz, H$^{3'''}$& H$^{5'''}$), 7.52 (2H, d, *J* = 8.4 Hz, H$^{2'''}$& H$^{6'''}$), 7.43 (1H, d, *J* = 4.8 Hz, H$^{5'}$), 7.06 (1H, d, *J* = 3.2 Hz, H$^{3'}$), 6.99 (1H, dd, *J* = 4.8, 3.2 Hz, H$^{4'}$), 4.63 (2H, d, *J* = 6.0 Hz, *CH*$_2$NH), 2.97 (4H, t, *J* = 6.8 Hz, H$^{2''}$& H$^{5''}$), 2.08 (3H, s, CO*CH*$_3$), 1.61 (4H, m, H$^{3''}$& H$^{4''}$).

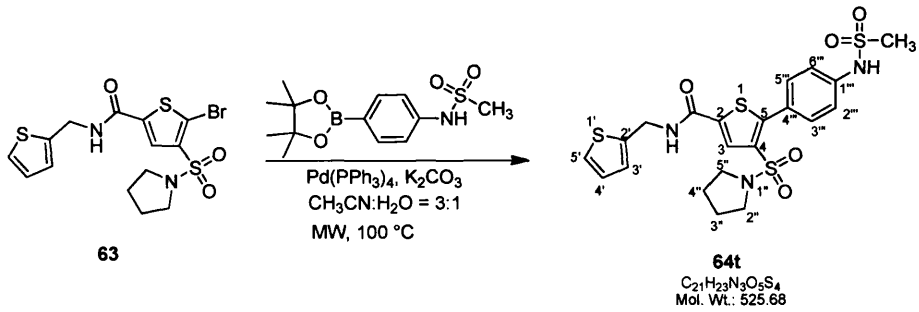
^{13}C NMR (100 MHz, DMSO): δ168.6 (*CO*CH$_3$), 159.7 (CONH), 150.3 (C^5), 141.4 (C$^{2'}$), 140.6 (C$^{4'''}$), 137.8 (C^2), 133.6 (C^4), 130.6 (C$^{2'''}$& C$^{6'''}$), 129.1 (C^3), 126.7 (C$^{4'}$), 125.9 (C$^{3'}$), 125.3 (C$^{5'}$), 125.2 (C$^{1'''}$), 118.2 (C$^{3'''}$& C$^{5'''}$), 47.3 (C$^{2''}$& C$^{5''}$), 37.6 (CH_2NH), 24.9 (C$^{3''}$& C$^{4''}$), 24.1 (CO*CH*$_3$).

LRMS (ESI): *m/z* = 490.69 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 490.0909, calculated for $C_{22}H_{24}N_3O_4S_3$ 490.0929 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{23}N_3O_4S_3$: C, 53.97; H, 4.73; N, 8.58% Found: C, 53.90; H, 4.67; N, 8.48%.

6.3.20 Synthesis of Compound 64t



63

64t
$C_{21}H_{23}N_3O_5S_4$
Mol. Wt.: 525.68

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 4-(methanesulfonylamino) phenyl boronic acid pinacol ester (113 mg, 0.38 mmol, 1.1 equiv.), K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol, 0.1 equiv.). The reaction mixtue was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:49→3:17) as eluent to afford compound **64t**.

Compound **64t** (75 mg, 41% yield) was obtained as a white solid.

R_f = 0.55 (silica gel, Ethyl acetate/DCM :1/1).

mp = 209.2 - 210.7 °C.

IR (ν_{max}/cm^{-1}): 753, 1182, 1326, 1450, 2934 and 3418.

^{1}H NMR (400 MHz, DMSO): δ 10.09 (1H, s, $NHSO_2$), 9.46 (1H, t, *J* = 5.6 Hz, CONH), 8.13 (1H, s, H^3), 7.56 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.43 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.27 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.05 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 7.00 (1H, t, *J*= 5.2, 3.6 Hz, $H^{4'}$), 4.63 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.08 (3H, s, CH_3), 2.98 (4H, t, *J* = 6.0 Hz, $H^{2''}$& $H^{5''}$), 1.62 (4H, d, *J* = 6.0 Hz, $H^{3''}$& $H^{4''}$).

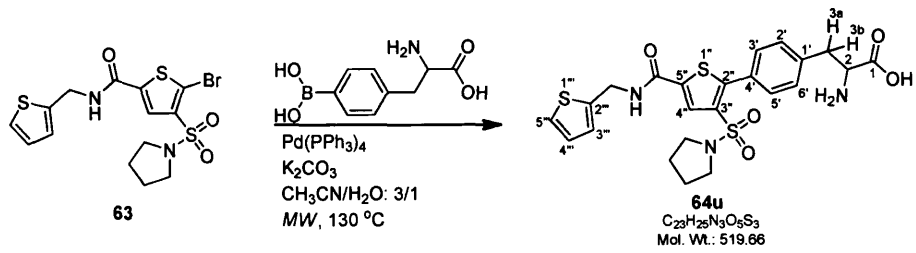
^{13}C NMR (100 MHz, DMSO): δ 159.7 (CONH), 149.8 (C^5), 141.7 ($C^{2'}$), 139.8 ($C^{1'''}$), 138.1 (C^2), 133.8 ($C^{4'''}$), 131.2 ($C^{3'''}$& $C^{5'''}$), 129.0 (C^3), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.8 (C^4), 125.3 ($C^{5'}$), 118.2 ($C^{2'''}$& $C^{6'''}$), 47.2 ($C^{2''}$& $C^{5''}$), 39.7 (CH_3), 37.6 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 526.52 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 526.0609, calculated for $C_{21}H_{24}N_3O_5S_4$ 526.0599 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{23}N_3O_5S_4$: C, 47.98; H, 4.41; N, 7.99% Found: C, 47.99; H, 4.41; N, 7.88%.

6.3.21 Synthesis of Compound 64u



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 2-amino-3-(4-boronophenyl)propanoic acid (86 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 130 °C for 15 minutes. The reaction mixture was evaporated *in vacuo*, passed through the Isolute® SCX-2 column using ammonia (2M) in methanol as eluent. The combined organic extract evaporated *in vacuo* to yield crude solid which was dried over P_2O_5 to afford compound **64u**.

Compound **64u** (142 mg, 78% yield) was obtained as a yellow solid.

R_f = 0.13 (Silica gel, Methanol/Ethyl acetate: 1/1).

mp = 216.5 – 216.9 °C.

IR (ν_{max}/cm^{-1}): 881, 1137, 1316, 1546, 1608 and 2962.

^{1}H NMR (400 MHz, DMSO): δ 9.48 (1H, t, *J* = 5.6 Hz, CONH), 8.13 (1H, s, $H^{4''}$), 7.49 (2H, d, *J* = 8.4 Hz, $H^{3'}$ & $H^{5'}$), 7.42 (1H, d, *J* = 5.2 Hz, $H^{5'''}$), 7.36 (2H, d, *J* = 8.4 Hz, $H^{2'}$& $H^{6'}$), 7.05 (1H, d, *J* = 3.6 Hz, $H^{3'''}$), 6.98 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'''}$), 4.62 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.46 (1H, dd, *J* = 8.0, 4.8 Hz, H^2), 3.21 (1H, d, *J* = 4.8 Hz, $H^{3a/3b}$), 3.18 (1H, d, *J* = 4.8 Hz, $H^{3a/3b}$), 2.97 (4H, t, *J* = 6.8 Hz, $(CH_2CH_2)_2NSO_2$), 2.91 (2H, dd, *J* = 8.0, 6.4 Hz, NH_2), 1.61 (4H, m, $(CH_2CH_2)_2NSO_2$).

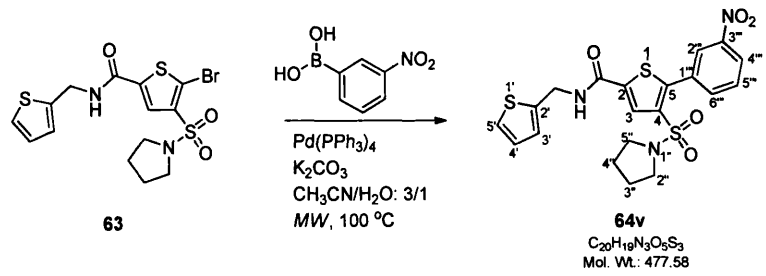
^{13}C NMR (100 MHz, DMSO): δ 169.2 (C^1), 159.7 (*C*ONH), 150.5 ($C^{2''}$), 141.7 ($C^{2'''}$), 139.5 ($C^{1'}$), 138.3 ($C^{5''}$), 133.7 ($C^{3''}$), 130.2 ($C^{4'}$), 130.0 ($C^{3'}$ & $C^{5'}$), 129.1 ($C^{2'}$ & $C^{6'}$), 129.0 ($C^{4''}$), 126.7 ($C^{4'''}$), 125.4 ($C^{3'''}$), 125.3 ($C^{5'''}$), 55.1 (C^2), 47.3 ($(CH_2CH_2)_2NSO_2$), 37.6 (CH_2NH), 36.8 (C^3), 24.9 ($(CH_2CH_2)_2NSO_2$).

LRMS (ESI): *m/z* = 520.68 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 520.1025 calculated for $C_{23}H_{26}N_3O_5S_3$ 520.1035 $[M+H]^+$.

Elem. Anal. Calculated for $C_{23}H_{25}N_3O_5S_3$: C, 53.16; H, 4.85; N, 8.09% Found: C, 53.24; H, 4.93; N, 7.95%.

6.3.22 Synthesis of Compound 64v



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 3-nitrophenylboronic acid (86 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **64v**.

Compound **64v** (144 mg, 86% yield) was obtained as a light yellow solid.

R_f = 0.65 (Silica gel, Ethyl acetate).

mp = 175.8 – 176.1 °C.

IR (ν_{max}/cm^{-1}): 710, 1141, 1346, 1548, 1736, 2360 and 3359.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (1H, t, *J* = 2.0 Hz, $H^{2'''}$), 8.32 (1H, d, J = 8.0 Hz, $H^{4'''}$), 7.96 (1H, d, J = 7.6 Hz, $H^{6'''}$), 7.80 (1H, s, H^3), 7.64 (1H, t, *J* = 8.0 Hz, $H^{5'''}$), 7.27 (1H, d, *J* = 3.6 Hz, $H^{5'}$), 7.07 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 6.73 (1H, t, *J* = 5.6 Hz, NH), 4.80 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.05 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.72 (4H, m, $H^{3''}$& $H^{4''}$).

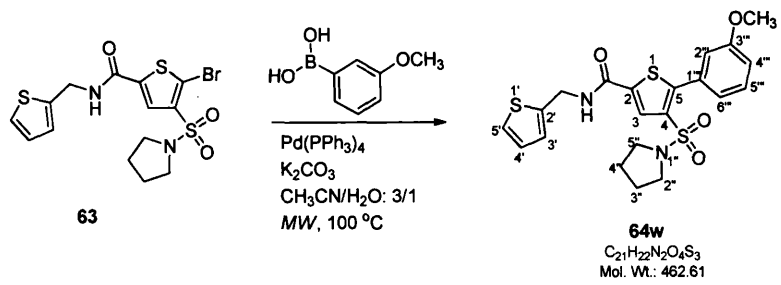
^{13}C NMR (100 MHz, $CDCl_3$): δ 161.7 (CONH), 149.8 ($C^{3'''}$), 149.5 (C^5), 141.9 ($C^{2'}$), 140.5 (C^2), 138.5 ($C^{1'''}$), 138.45 ($C^{6'''}$), 138.2 (C^4), 131.5 ($C^{5'''}$), 130.8 (C^3), 129.2 ($C^{4'}$), 128.9 ($C^{3'}$), 127.8 ($C^{5'}$), 127.2 ($C^{2'''}$), 126.6 ($C^{4'''}$), 49.6 ($C^{2''}$& $C^{5''}$), 40.8 (CH_2NH), 27.5 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 478.35 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 478.0587, calculated for $C_{20}H_{20}N_3O_5S_3$ 478.0565 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{19}N_3O_5S_3$: C, 50.30; H, 4.01; N, 8.80% Found: C, 50.45; H, 3.96; N, 8.71%.

6.3.23 Synthesis of Compound 64w



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 3-methoxyphenylboronic acid (79 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **64w**.

Compound **64w** (138 mg, 85% yield) was obtained as a light yellow solid.

R_f = 0.63 (Silica gel, Ethyl acetate).

mp = 117.9 – 118.2 °C.

IR (ν_{max}/cm^{-1}): 875, 1043, 1135, 1319, 1545, 1650, 1737, 2961 and 3359.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (1H, s, H^3), 7.33 (1H, d, J = 8.0 Hz, $H^{5'''}$), 7.25 (1H, dd, J = 5.2, 1.2 Hz, $H^{5'}$), 7.21 (1H, t, J = 2.0 Hz, $H^{2'''}$), 7.15 (1H, d, J = 7.6 Hz, $H^{6'''}$), 7.06 (1H, d, J = 3.2 Hz, $H^{3'}$), 7.01 - 6.96 (2H, m, $H^{4'}$& $H^{4'''}$), 6.74 (1H, t, J = 5.6 Hz, NH), 4.79 (2H, d, J = 5.6 Hz, CH_2NH), 3.85 (3H, s, OCH_3), 2.95 (4H, t, J = 6.4 Hz, $H^{2''}$& $H^{5''}$), 1.59 (4H, m, $H^{3''}$& $H^{4''}$).

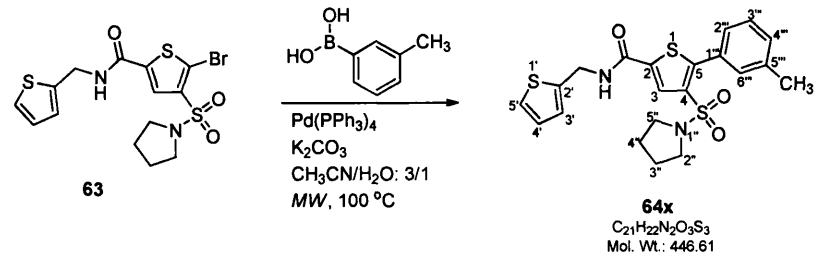
^{13}C NMR (100 MHz, $CDCl_3$): δ160.5 (CONH), 159.3 ($C^{3'''}$), 150.9 (C^5), 140.2 ($C^{2'}$), 137.4 (C^2), 135.0 (C^4), 132.5 ($C^{1'''}$), 129.4 (C^3 & $C^{5'''}$), 127.1 ($C^{4'}$), 126.7 ($C^{3'}$), 125.7 ($C^{5'}$), 122.6 ($C^{6'''}$), 116.1 ($C^{4'''}$), 115.7 ($C^{2'''}$), 55.6 (OCH_3), 47.5 ($C^{2''}$& $C^{5''}$), 38.8 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 463.64 $[M+H]^+$, 64%.

HRMS (TOF MS ES): Found 463.0802, calculated for $C_{21}H_{23}N_2O_4S_3$ 463.0820 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_3$: C, 54.52; H, 4.79; N, 6.06% Found: C, 54.52; H, 4.48; N, 5.98%.

6.3.24 Synthesis of Compound 64x



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (5 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), *m*-tolylboronic acid (56 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **64x**.

Compound **64x** (117 mg, 75% yield) was obtained as a white solid.

R_f = 0.7 (Silica gel, Ethyl acetate).

mp = 61.9 – 62.2 °C.

IR (ν_{max}/cm^{-1}): 869, 1138, 1310, 1545, 1627, 2950 and 3307.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, s, H^3), 7.41 (1H, s, $H^{2'''}$or $H^{6'''}$), 7.38 (1H, s, $H^{2'''}$or $H^{6'''}$), 7.31 (1H, t, *J* = 7.6 Hz, $H^{5'''}$), 7.26 (1H, m, $H^{4'''}$), 7.25 (1H, d, *J* = 1.2 Hz, $H^{5'}$), 7.06 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 5.2, 1.6 Hz, $H^{4'}$), 6.61 (1H, t, *J* = 5.6 Hz, NH), 4.78 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.94 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.58 (4H, m, $H^{3''}$ & $H^{4''}$).

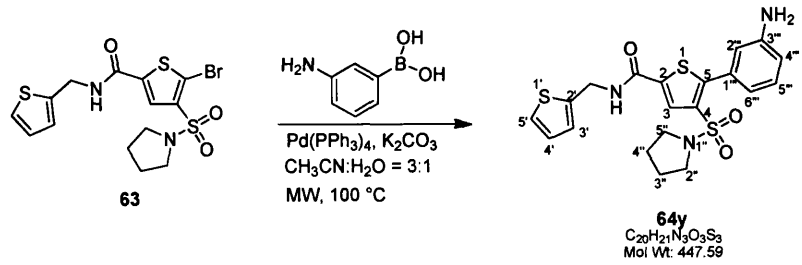
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (CONH), 151.5 (C^5), 140.1 ($C^{2'}$), 138.1 ($C^{3'''}$), 137.2 (C^2), 135.0 (C^4), 131.26 ($C^{1'''}$), 131.0 ($C^{6'''}$), 130.7 ($C^{4'''}$), 129.3 (C^3), 128.3 ($C^{5'''}$), 127.4 ($C^{2'''}$), 127.1 ($C^{4'}$), 126.7 ($C^{3'}$), 125.7 ($C^{5'}$), 47.5 ($C^{2''}$& $C^{5''}$), 38.8 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.4 (CH_3).

LRMS (ESI): *m/z* = 447.47 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 447.0895, calculated for $C_{21}H_{23}N_2O_3S_3$ 447.0871 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_3S_3$: C, 56.48; H, 4.97; N, 6.27% Found: C, 56.04; H, 4.78; N, 6.25%.

6.3.25 Synthesis of Compound 64y



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 3-(aminophenyl) boronic acid monohydrate (59 mg, 0.38 mmol, 1.1 equiv.), K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol, 0.1 equiv.). The reaction mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:49 → 3:17) as eluent to afford compound **64y**.

Compound **64y** (92 mg, 59% yield) was obtained as a yellow solid.

R_f = 0.35 (silica gel, Ethyl acetate/DCM: 3/7).

mp = 72.8 – 74.1 °C.

IR (ν_{max}/cm^{-1}): 852, 1139, 1302, 1624, 2879 and 3358.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (1H, s, H^3), 7.25 (1H, m, $H^{5'}$), 7.18 (1H, t, J = 7.6, 8.0 Hz, $H^{5'''}$), 7.05 (1H, s, $H^{2'''}$), 6.95 (3H, m, $H^{3',4'\&\ 6'''}$), 6.74 (2H, d, J = 8.0 Hz, $H^{4'''}$& CONH), 4.85 (2H, d, J = 5.2 Hz, CH_2NH), 3.81 (2H, s, NH_2), 2.97 (4H, m, $H^{2''}$& $H^{5''}$), 1.59 (4H, d, J = 3.2 Hz, $H^{3''}$& $H^{4''}$).

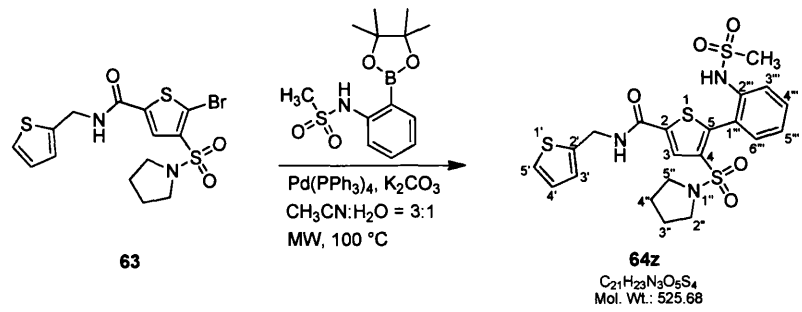
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (CONH), 146.4 (C^5), 140.2 ($C^{2'}$), 137.0 (C^2), 134.9 ($C^{3'''}$), 132.2 ($C^{1'''}$), 129.5 (C^3), 129.3 ($C^{5'''}$), 127.1 ($C^{4'}$), 126.7 ($C^{2'''}$), 125.6 ($C^{5'''}$), 120.2 ($C^{3'}$), 116.7 ($C^{6'''}$), 116.4 ($C^{4'''}$), 47.6 ($C^{2''}$& $C^{5''}$), 38.8 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 448.32 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 448.0832, calculated for $C_{20}H_{22}N_3O_3S_3$ 448.0823 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{21}N_3O_3S_3$: C, 53.67; H, 4.73; N, 9.39% Found: C, 53.57; H, 4.88; N, 9.20%.

6.3.26 Synthesis of Compound 64z



63

64z
$C_{21}H_{23}N_3O_5S_4$
Mol. Wt.: 525.68

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 2-(methylsulfonamido)-phenyl boronic acid pinacol ester (89 mg, 0.41 mmol, 1.2 equiv.), K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol, 0.1 equiv.).The reaction mixture was irradiated in microwave reactor at 100 °C for five minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:49→2:23) as eluent to afford compound **64z**.

Compound **64z** (39 mg, 21% yield) was obtained as a white solid.

R_f = 0.78 (silica gel, Ethyl acetate/DCM: 1/4).

mp = 92.9 – 94.3 °C.

IR (ν_{max}/cm^{-1}): 752, 1140, 1319, 1632, 2966 and 3276.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (1H, s, H^3), 7.72 (1H, d, J = 8.8 Hz, $H^{6'''}$), 7.50 (1H, dd, J = 8.4, 8.8 Hz, $H^{4'''}$), 7.26 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.24 (1H, d, J = 8.8 Hz, $H^{5'''}$), 7.09 (1H, s, $H^{3'''}$), 7.06 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.96 (1H, dd, J = 3.2, 5.2 Hz, $H^{4'}$), 6.91 (1H, t, J = 5.6 Hz, CONH), 4.778 (2H, d, J = 5.6 Hz, CH_2NH), 2.95 (4H, s, $H^{2''}$& $H^{5''}$), 2.94 (3H, s, CH_3), 1.70 (4H, t, J = 6.4 Hz, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.1 (NH), 145.2 (C^5), 140.0 ($C^{2'''}$), 139.9 (C^2), 138.0 (C^4), 135.9 ($C^{2'}$), 132.0 ($C^{5'''}$), 131.7 ($C^{4'''}$), 128.6 (C^3), 127.1 ($C^{4'}$), 126.8 ($C^{3'}$), 125.7 ($C^{3'''}$), 124.9 ($C^{5'}$), 124.0 ($C^{1'''}$), 122.9 ($C^{6'''}$), 47.5 ($C^{2''}$& $C^{5''}$), 40.5 (CH_3), 38.8 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

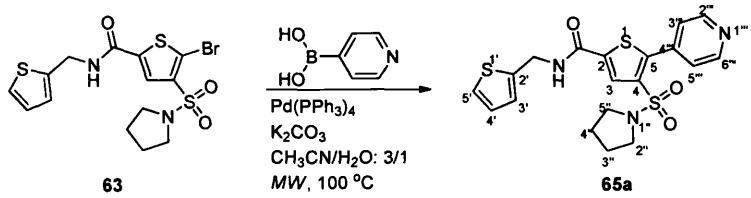
LRMS (ESI): *m/z* = 526.68 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 526.0596, calculated for $C_{21}H_{24}N_3O_5S_4$ 526.0599 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{23}N_3O_5S_4$: C, 47.98; H, 4.41; N, 7.99% Found: C, 47.81; H, 4.48; N, 7.90%.

6.4 Preparation of Focused Library "B"

6.4.1 Synthesis of Compound 65a



63

65a

$C_{19}H_{19}N_3O_3S_3$
Mol. Wt.: 433.57

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (5 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 4-pyridinylboronic acid (64 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.7 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (19:1→3:7) as eluent to afford compound **65a**.

Compound **65a** (106 mg, 70% yield) was obtained as a light yellow powder.

R_f = 0.35 (Silica gel, Ethyl acetate).

mp = 75.1 – 75.9 °C.

IR (ν_{max}/cm^{-1}): 883, 1142, 1316, 1545, 1648 and 3248.

^{1}H NMR (400 MHz,$CDCl_3$): δ 8.70 (2H, d, *J* = 4.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.81 (1H, s, H^3), 7.54 (2H, d, *J* = 4.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.26 (1H, d, *J* = 3.6 Hz, $H^{5'}$), 7.06 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 6.79 (1H, t, *J* = 5.6 Hz, NH), 4.79 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.99 (4H, t, *J* = 6.4 Hz, $H^{2''}$& $H^{5''}$), 1.66 (4H, dt, *J* = 6.4, 3.4 Hz, $H^{3''}$& $H^{4''}$).

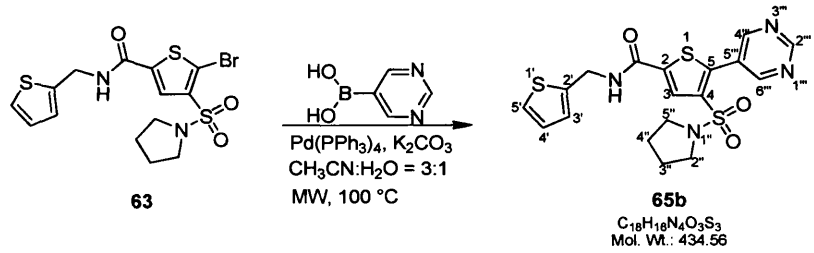
^{13}C NMR (100 MHz,$CDCl_3$): δ 150.0 (CONH), 150.1 ($C^{2'''}$& $C^{6'''}$), 147.2 ($C^{4'''}$), 139.9 ($C^{2'}$), 139.3 (C^5), 136.1 (C^2), 129.1 (C^3), 128.7 (C^4), 127.2 ($C^{4'}$), 126.9 ($C^{3'}$), 125.8 ($C^{5'}$), 124.6 ($C^{3'''}$& $C^{5'''}$), 47.5 ($C^{2''}$& $C^{5''}$), 38.9 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 433.94 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 434.0650, calculated for $C_{19}H_{20}N_3O_3S_3$ 434.0667 $[M+H]^+$.

Elem. Anal. Calculated for $C_{19}H_{19}N_3O_3S_3$: C, 52.63; H, 4.42; N, 9.69% Found: C, 52.61; H, 4.37; N, 8.73%.

6.4.2 Synthesis of Compound 65b



63

65b

$C_{18}H_{18}N_4O_3S_3$
Mol. Wt.: 434.56

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 5-pyrimidinyl boronic acid (48 mg, 0.38 mmol, 1.1 equiv.), K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol, 0.1 equiv.). The reaction mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:9 → 3:7) as eluent to afford compound **65b**.

Compound **65b** (74 mg, 49% yield) was obtained as a green solid.

R_f = 0.32 (silica gel, Ethyl acetate/DCM: 1/1).

mp = 213.1 – 214.4 °C.

IR (ν_{max}/cm^{-1}): 705, 1139, 1404, 1650 and 3346.

^{1}H NMR (400 MHz, DMSO): δ 9.54 (1H, t, *J* = 5.6 Hz, NH), 9.27 (1H, s, $H^{2'''}$), 8.99 (2H, s, $H^{4'''}$& $H^{6'''}$), 8.24 (1H, s, H^3), 7.44 (1H, dd, *J* = 5.2 Hz, $H^{5'}$), 7.07 (1H, dd, *J* = 3.6 Hz, $H^{3'}$), 7.00 (1H, dd, *J* = 3.6, 5.2 Hz, $H^{4'}$), 4.66 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.05 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.71 (4H, m, $H^{3''}$& $H^{4''}$).

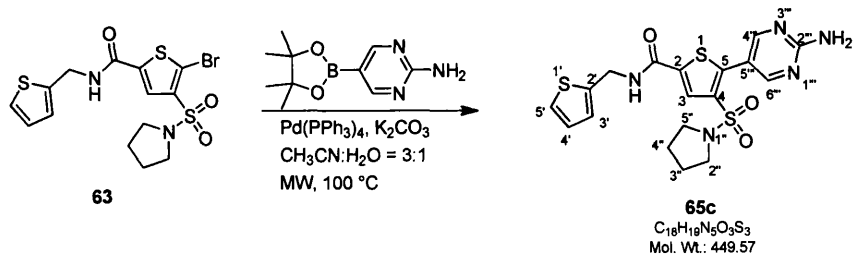
^{13}C NMR (100 MHz, DMSO): δ 159.4 (CONH), 158.6 ($C^{2'''}$), 157.1 ($C^{4'''}$& $C^{6'''}$), 142.5 ($C^{5'''}$), 141.5 (C^2), 140.9 (C^5), 135.3 (C^4), 128.0 (C^3), 126.8 ($C^{4'}$), 126.2 ($C^{2'}$), 126.0 ($C^{3'}$), 125.4 ($C^{5'}$), 47.4 ($C^{2''}$& $C^{5''}$), 47.4 ($C^{2''}$& $C^{5''}$), 37.7 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 435.37 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 435.0601, calculated for $C_{18}H_{19}N_4O_3S_3$ 435.0619 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{18}N_4O_3S_3$: C, 49.75; H, 4.18; N, 12.89% Found: C, 48.89; H, 4.33; N, 12.23%.

6.4.3 Synthesis of Compound 65c



63

65c
$C_{18}H_{19}N_5O_3S_3$
Mol. Wt.: 449.57

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.34 mmol, 1 equiv.), 2-aminopyrimidine-5-boronic acid pinacol ester (86 mg, 0.38 mmol, 1.1 equiv.), K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol, 0.1 equiv.). The reaction mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using ethyl acetate and DCM (1:19 → 1:3) as eluent to afford compound **65c**.

Compound **65c** (116 mg, 74% yield) was obtained as a white solid.

R_f = 0.36 (silica gel, Ethyl acetate).

mp = 235.9 – 237.4 °C.

IR (ν_{max}/cm^{-1}): 802, 1125, 1650, 3145 and 3343.

^{1}H NMR (400 MHz, DMSO): δ 9.45 (1H, t, *J* = 6.0 Hz, CONH), 8.39 (2H, s, $H^{4'''}$& $H^{6'''}$), 8.14 (1H, s, H^3), 7.43 (1H, dd, *J* = 5.2, 5.2 Hz, $H^{5'}$), 7.12 (2H, s, NH_2) 7.05 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 3.2, 5.2 Hz, $H^{4'}$), 4.63 (2H, d, *J* = 6.0 Hz, CH_2NH), 3.05 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.68 (4H, m, $H^{2''}$& $H^{4''}$).

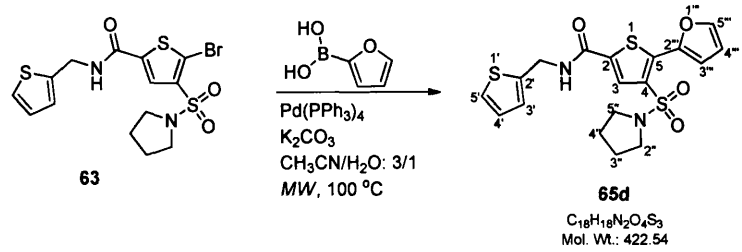
^{13}C NMR (100 MHz, DMSO): δ 163.3 ($C^{2'''}$), 159.7 (CONH), 158.2 ($C^{4'''}$& $C^{6'''}$), 145.6 (C^5), 141.7 ($C^{2'}$), 138.4 (C^2), 133.6 (C^4), 128.7 (C^3), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 113.8 ($C^{5'''}$), 47.3 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 450.52 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 450.0723, calculated for $C_{18}H_{20}N_5O_3S_3$ 450.0728 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{19}N_5O_3S_3$: C, 48.09; H, 4.26; N, 15.58% Found: C, 48.07; H, 4.35; N, 15.80%.

6.4.4 Synthesis of Compound 65d



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 2-furylboronic acid (58 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **65d**.

Compound **65d** (111 mg, 75% yield) was obtained as a yellow solid.

R_f = 0.67 (Silica gel, Ethyl acetate).

mp = 189.1 – 189.4 °C.

IR (ν_{max}/cm^{-1}): 750, 877, 1013, 1138, 1316, 1547, 1651, 2927 and 3369.

^{1}H NMR (400 MHz, DMSO): δ 9.47 (1H, t, *J* = 6.0 Hz, NH), 8.10 (1H, s, H^3), 7.91 (1H, s, $H^{5'''}$), 7.43 (1H, d, *J* = 4.8 Hz, $H^{5'}$), 7.33 (1H, d, *J* = 3.2 Hz, $H^{3'''}$), 7.06 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.99 (1H, m, $H^{4'}$), 6.72 (1H, m, $H^{4'''}$), 4.63 (2H, d, *J* = 6.0 Hz, CH_2NH), 3.18 (4H, m, $H^{2''}$& $H^{5''}$), 1.74 (4H, m, $H^{3''}$& $H^{4''}$).

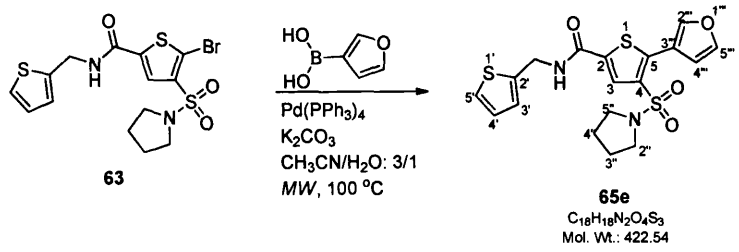
^{13}C NMR (100 MHz, DMSO): δ 159.6 (CONH), 145.2 ($C^{5'''}$), 144.8 ($C^{2'''}$), 138.3 (C^5), 141.6 ($C^{2'}$), 137.2 (C^2), 130.4 (C^4), 129.8 (C^3), 126.7 ($C^{4'}$), 126.0 ($C^{3'}$), 125.4 ($C^{5'}$), 113.7 ($C^{3'''}$), 113.0 ($C^{4'''}$), 47.7 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 423.53 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 423.0493, calculated for $C_{18}H_{19}N_2O_4S_3$ 423.0507 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{18}N_2O_4S_3$: C, 51.16; H, 4.29; N, 6.63% Found: C, 51.41; H, 4.21; N, 6.61%.

6.4.5 Synthesis of Compound 65e



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 3-furylboronic acid (58 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **65e**.

Compound **65e** (129 mg, 87% yield) was obtained as a yellow solid.

R_f = 0.69 (Silica gel, Ethyl acetate).

mp = 151.0 – 151.2 °C.

IR (ν_{max}/cm^{-1}): 705, 868, 1011, 1140, 1337, 1522, 1643, 2925 and 3355.

^{1}H NMR (400 MHz, DMSO): δ 9.20 (1H, t, *J*= 5.6 Hz, NH), 7.95 (1H, s, $H^{2'''}$), 7.87 (1H, s, H^3), 7.58 (1H, s, $H^{5'''}$), 7.18 (1H, d, *J* = 4.8 Hz, $H^{5'}$), 6.81 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.74 (1H, dd, *J* = 4.8, 3.6 Hz, $H^{4'}$), 6.64 (1H, s, $H^{4'''}$), 4.38 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.85 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.45 (4H, m, $H^{3''}$& $H^{4''}$).

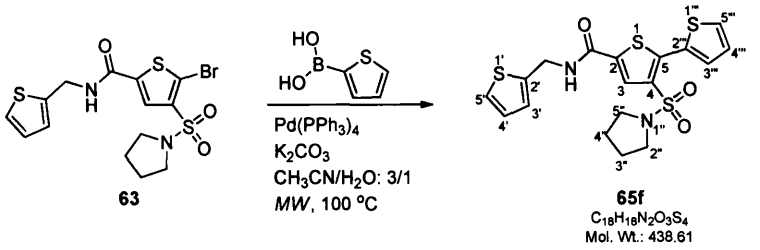
^{13}C NMR (100 MHz, DMSO): δ 159.6 (CONH), 144.1 ($C^{5'''}$), 143.1 ($C^{2'''}$), 141.7 ($C^{2'}$), 141.1 (C^5), 137.3 (C^2), 132.8 (C^4), 129.5 (C^3), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 116.3 ($C^{3'''}$), 112.0 ($C^{4'''}$), 47.5 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 423.60 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 423.0518, calculated for $C_{18}H_{19}N_2O_4S_3$ 423.0507 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{18}N_2O_4S_3$: C, 51.16; H, 4.29; N, 6.63% Found: C, 51.12; H, 4.33; N, 6.58%.

6.4.6 Synthesis of Compound 65f



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 2-thienylboronic acid (66 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **65f**.

Compound **65f** (84 mg, 55% yield) was obtained as a yellow solid.

$\mathbf{R_f}$ = 0.69 (Silica gel, Ethyl acetate).

mp = 197.2 – 197.5 °C.

IR (ν_{max}/cm^{-1}): 744, 845, 1013, 1135, 1283, 1546, 1643, 1736, 3074 and 3365.

^{1}H NMR (400 MHz, DMSO): δ 9.48 (1H, t, *J* = 6.0 Hz, NH), 8.13 (1H, s, H^3),7.82 (1H, d, *J* = 5.2 Hz, $H^{3'''}$), 7.56 (1H, d, *J* = 3.2 Hz, $H^{5'''}$), 7.43 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.20 (1H, t, *J* = 3.6 Hz, $H^{4'''}$), 7.06 (1H, s, $H^{3'}$), 6.99 (1H, t, *J* = 5.2 Hz, $H^{4'}$), 4.63 (2H, d, *J* = 6.0 Hz, CH_2NH), 3.07 (4H, t, *J* = 6.4 Hz, $H^{2''}$& $H^{5''}$), 1.67 (4H, m, $H^{3''}$& $H^{4''}$).

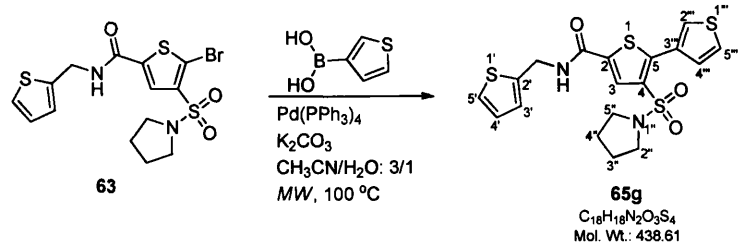
^{13}C NMR (100 MHz, DMSO): δ 159.5 (CONH), 142.3 (C^5), 141.6 ($C^{2'}$), 138.2 (C^2), 133.7 (C^4), 131.07 ($C^{5'''}$), 130.8 ($C^{2'''}$), 130.4 ($C^{3'''}$), 129.7 (C^3), 127.99 ($C^{4'''}$), 126.7 ($C^{4'}$), 126.0 ($C^{3'}$), 125.4 ($C^{5'}$), 47.4 ($C^{2''}$& $C^{5''}$), 37.7 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 439.61 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 439.0278, calculated for $C_{18}H_{19}N_2O_3S_4$ 439.0279 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{18}N_2O_3S_4$: C, 49.29; H, 4.14; N, 6.39% Found: C, 49.47; H, 4.02; N, 6.34%.

6.4.7 Synthesis of Compound 65g



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 3-thienylboronic acid (66 mg, 0.52 mmol, 1.5 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **65g**.

Compound **65g** (150 mg, 98% yield) was obtained as a yellow solid.

R_f = 0.69 (Silica gel, Ethyl acetate).

mp = 160.3 – 160.5 °C.

IR (ν_{max}/cm^{-1}): 852, 1013, 1120, 1278, 1545, 1644, 1736 and 3365.

^{1}H NMR (400 MHz, DMSO): δ 9.46 (1H, t, *J*= 5.6 Hz, NH), 8.12 (1H, s, H^3), 7.97 (1H, dd, *J* = 3.2, 1.2 Hz, $H^{2'''}$), 7.69 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'''}$),7.42 (2H, m, $H^{5'''}$&$H^{5'}$), 7.06 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'}$), 4.63 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.01 (4H, t, *J* = 6.4 Hz, $H^{2''}$& $H^{5''}$), 1.64 (4H, m, $H^{3''}$& $H^{4''}$).

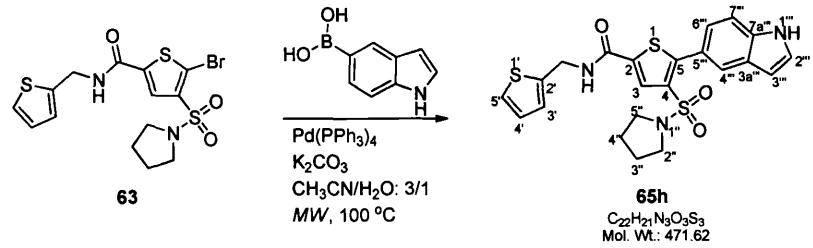
^{13}C NMR (100 MHz, DMSO): δ 159.7 (CONH), 144.7 (C^5), 141.6 ($C^{2'}$), 137.5 (C^2), 133.5 (C^4), 129.2 ($C^{5'''}$), 127.9 ($C^{2'''}$), 130.7 ($C^{3'''}$), 129.4 (C^3), 126.7 ($C^{4'''}$), 126.8 ($C^{4'}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 47.3 ($C^{2''}$& $C^{5''}$), 37.6 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 439.61 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 439.0274, calculated for $C_{18}H_{19}N_2O_3S_4$ 439.0279 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{18}N_2O_3S_4$: C, 49.29; H, 4.14; N, 6.39% Found: C, 49.31; H, 4.01; N, 6.34%.

6.4.8 Synthesis of Compound 65h



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), 1*H*-indol-5-ylboronic acid (67 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (49:1→37:3) as eluent to afford compound **65h**.

Compound **65h** (137 mg, 83% yield) was obtained as a yellow solid.

R_f = 0.17 (Silica gel, Ethyl acetate/DCM: 1/9).

mp = 183.4 – 183.7 °C.

IR (ν_{max}/cm^{-1}): 1006, 1131, 1300, 1428, 1550, 1637 and 3315.

^{1}H NMR (400 MHz, DMSO): δ 11.38 (1H, s, NH), 9.46 (1H, t, *J* = 6.0 Hz, CONH), 8.13 (1H, s, H^3), 7.69 (1H, s, $H^{4'''}$), 7.61 (1H, d, *J* = 8.0 Hz, $H^{6'''}$), 7.48 (1H, t, *J* = 2.4 Hz, $H^{2'''}$), 7.43 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.19 (1H, d, *J* = 8.0 Hz, $H^{7'''}$), 7.06 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'}$), 6.50 (1H, t, *J* = 2.4 Hz, $H^{3'''}$), 4.63 (2H, d, *J* = 6.0 Hz, CH_2NH), 2.93 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.52 (4H, m, $H^{3''}$& $H^{4''}$).

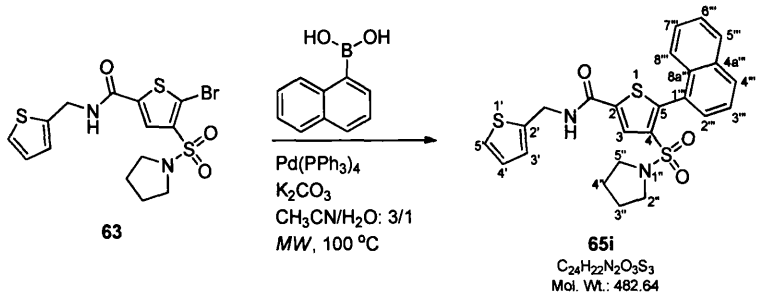
^{13}C NMR (100 MHz, DMSO): δ 159.9 (CONH), 151.9 (C^5), 141.8 ($C^{2'}$), 137.4 (C^2), 135.1 ($C^{7a'''}$), 133.3 (C^4), 129.4 (C^3), 128.5 ($C^{3a'''}$), 127.6 ($C^{2'''}$), 127.6 ($C^{5'''}$), 126.7 ($C^{4'}$), 125.9 ($C^{3'}$), 125.3 ($C^{5'}$), 120.92 ($C^{7'''}$), 119.7 ($C^{6'''}$), 113.4 ($C^{4'''}$), 101.2 ($C^{3'''}$), 47.2 ($C^{2''}$ & $C^{5''}$), 37.6 (CH_2NH), 24.8 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 472.36 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 472.0826 calculated for $C_{22}H_{22}N_3O_3S_3$ 472.0823 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{21}N_3O_3S_3$: C, 56.03; H, 4.49; N, 8.91% Found: C, 56.04; H, 4.51; N, 8.81%.

6.4.9 Synthesis of Compound 65i



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **63** (150 mg, 0.35 mmol, 1 equiv.), naphthalen-1-ylboronic acid (71 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.035 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→19:1) as eluent to afford compound **65i**.

Compound **65i** (146 mg, 87% yield) was obtained as a light yellow solid.

R_f = 0.23 (Silica gel, Ethyl acetate/DCM: 1/19).

mp = 87.3 – 87.6 °C.

IR (ν_{max}/cm^{-1}): 878, 1013, 1139, 1316, 1554, 1631 and 3307.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (1H, d, *J* = 8.4 Hz, $H^{8'''}$), 7.90 (1H, d, *J* = 8.4 Hz, $H^{5'''}$), 7.89 (1H, s, H^3), 7.66 (1H, s, $H^{4'''}$), 7.62 (1H, dd, *J* = 6.8, 5.6 Hz, $H^{2'''}$), 7.55 (1H, d, *J* = 8.4 Hz, $H^{3'''}$), 7.53 – 7.45 (2H, m, $H^{6'''}$ & $H^{7'''}$), 7.27 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.07 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.98 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 6.69 (1H, t, *J* = 5.2 Hz, CONH), 4.81 (2H, s, CH_2NH), 2.62 (4H, m, $H^{2''}$& $H^{5''}$), 1.28 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (CONH), 148.3 (C^5), 140.1 ($C^{2'}$), 138.56 (C^2), 138.52 (C^4), 133.3 ($C^{4a'''}$), 132.7 ($C^{1'''}$), 130.3 ($C^{8'''}$), 129.5 ($C^{2'''}$), 128.4 (C^3), 128.2 ($C^{5'''}$), 128.1 ($C^{8a'''}$), 126.9 ($C^{4'}$), 126.8 ($C^{3'}$), 126.5 ($C^{6'''}$ & $C^{7'''}$), 125.6 ($C^{4'''}$), 125.4 ($C^{5'}$), 125.0 ($C^{3'''}$), 47.3 ($C^{2''}$ & $C^{5''}$), 38.8 (CH_2NH), 25.3 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 483.43 $[M+H]^+$, 100%.

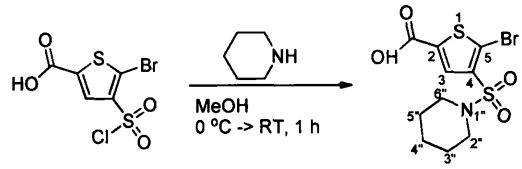
HRMS (TOF MS ES): Found 483.0861 calculated for $C_{24}H_{23}N_2O_3S_3$ 483.0871 $[M+H]^+$.

Elem. Anal. Calculated for $C_{24}H_{22}N_2O_3S_3$: C, 59.73; H, 4.59; N, 5.80% Found: C, 59.90; H, 4.61; N, 5.58%.

6.5 Preparation of Focused Library "C"

6.5.1 Synthesis of Compound 68a

6.5.1.1 Synthesis of Sulfonamide 66a



61

66a

$C_{10}H_{12}BrNO_4S_2$
Mol. Wt.: 354.24

Piperidine (0.58 mL, 5.79 mmol, 3 equiv.) was added dropwise to stirred solution of **61** (590 mg, 1.93 mmol, 1 equiv.) in anhydrous methanol (5 mL) at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1 M Hydrochloric acid (HCl) was added dropwise to the aqueous phase at 0 °C to reduce the pH down to ~1. The resulting precipitate **66a** was collected through filtration and dried over $CaCl_2$ under vacuum.

Compound **66a** (465 mg, 68% yield) was obtained as a white powder.

R_f = 0.18 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 206.2 – 207.1 °C.

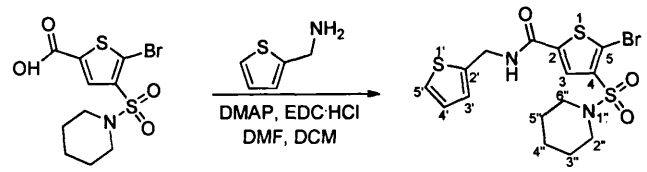
IR (ν_{max}/cm^{-1}): 866, 930, 1164, 1344, 1528 and 1670.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (1H, s, H^3), 3.22 (4H, t, J = 5.6 Hz, $H^{2'}$& $H^{6'}$), 1.67 (4H, m, $H^{3'}$& $H^{5'}$), 1.52 (2H, m, $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8 (COOH), 138.5 (C^2), 132.9 (C^4), 135.8 (C^3), 124.1 (C^5), 47.1 ($C^{2'}$& $C^{6'}$), 25.4 ($C^{3'}$& $C^{5'}$), 23.7 ($C^{4'}$).

LRMS (ESI): *m/z* = 354.09 $[C_{10}H_{12}{}^{79}BrNO_4S_2+H]^+$ (90%), 356.04 $[C_{10}H_{12}{}^{81}BrNO_4S_2+H]^+$ (100%).

6.5.1.2 Synthesis of Amide 67a



66a

67a
$C_{15}H_{17}BrN_2O_3S_3$
Mol. Wt.: 449.41

Thiophene-2-methylamine (0.052 mL, 0.51 mmol, 1.2 equiv.) was added dropwise to stirred solution of **66a** (150 mg, 0.42 mmol, 1 equiv.), EDC hydrochloride (106 mg, 0.55 mmol, 1.3 equiv.) & DMAP (5.17 mg, 0.042 mmol, 0.1 equiv.) in anhydrous DCM (2 mL) and anhydrous DMF (1 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 20 h, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity on silica gel 60 (ethyl acetate/*n*-hexane) to afford compound **67a**.

Compound **67a** (123 mg, 65% yield) was obtained as a white solid.

R_f = 0.65 (Silica gel, Ethyl acetate).

mp = 149.3 – 150.1 °C.

IR (ν_{max}/cm^{-1}): 716, 866, 929, 1044, 1143, 1344, 1542 and 1633.

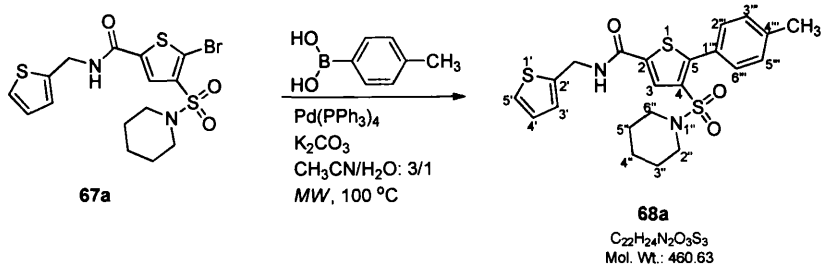
^{1}H NMR (400 MHz, DMSO): δ 9.46 (1H, t, *J* = 5.6 Hz, NH), 7.99 (1H, s, H^3), 7.43 (1H, dd, *J* = 5.2, 1.2 Hz, $H^{5'}$), 7.05 (1H, dd, *J* = 3.6, 0.8 Hz, $H^{3'}$), 6.99 (1H, t, *J* = 3.6 Hz, $H^{4'}$), 4.61 (2H, d, *J* = 5.6 Hz, *CH*$_2$NH),3.09 (4H, t, *J* = 5.2 Hz, $H^{2"}$& $H^{6"}$), 1.56 (4H, m, $H^{3"}$& $H^{5"}$), 1.45 (2H, m, $H^{4"}$).

^{13}C NMR (100 MHz, DMSO): δ 159.0 (CONH), 141.3 ($C^{2'}$), 140.6 (C^2), 136.1 (C^4), 128.6 (C^3), 126.7 ($C^{4'}$), 126.1 ($C^{3'}$), 125.5 ($C^{5'}$), 120.9 (C^5), 46.4 ($C^{2"}$& $C^{6"}$), 37.6 (CH_2NH), 24.8 ($C^{3"}$& $C^{5"}$), 22.6 ($C^{4"}$).

LRMS (ESI): *m/z* = 449.05 $[C_{15}H_{17}{}^{79}BrN_2O_3S_3+H]^+$ (90%), 451.11 $[C_{15}H_{17}{}^{81}BrN_2O_3S_3+H]^+$ (100%).

HRMS (TOF MS ES): Found 448.9663, calculated for $C_{15}H_{18}{}^{79}BrN_2O_3S_3$ 448.9641$[M+H]^+$.

6.5.1.3 Synthesis of Compound 68a



67a

68a

$C_{22}H_{24}N_2O_3S_3$

Mol. Wt.: 460.63

A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **67a** (117 mg, 0.26 mmol, 1 equiv.), *p*-tolylboronic acid (42 mg, 0.31 mmol, 1.2 equiv.) and K_2CO_3 (72 mg, 0.52 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (30 mg, 0.026 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **68a**.

Compound **68a** (90 mg, 75% yield) was obtained as a white solid.

R_f = 0.74 (Silica gel, Ethyl acetate).

mp = 157.0 – 157.2 °C.

IR (ν_{max}/cm^{-1}): 875, 933, 1139, 1319, 1442, 1545, 1653, 2935 and 3359.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, s, H^3), 7.49 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.26 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.22 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.06 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.98 (1H, dd, J = 5.2, 1.6 Hz, $H^{4'}$), 6.51 (1H, t, J = 5.6 Hz, NH), 4.79 (2H, d, J = 6.0 Hz, CH_2NH), 2.80 (4H, s, $H^{2''}$& $H^{6''}$), 2.40 (3H, s, CH_3), 1.35 (6H, s, $H^{3'',4'',5''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (CONH), 152.0 (C^5), 140.1 ($C^{2'}$), 140.0 ($C^{4'''}$), 136.9 (C^2), 133.5 (C^4), 130.4 ($C^{2'''}$& $C^{6'''}$), 129.4 (C^3), 125.7 ($C^{5'}$), 128.3 ($C^{1'''}$), 127.1 ($C^{4'}$), 126.8 ($C^{3'}$), 129.0 ($C^{3'''}$& $C^{5'''}$), 46.4 ($C^{2''}$& $C^{6''}$), 38.8 (CH_2NH), 25.2 ($C^{3''}$& $C^{5''}$), 23.6 ($C^{4''}$), 21.5 (CH_3).

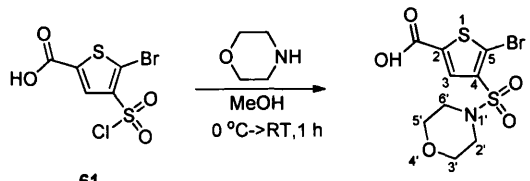
LRMS (ESI): *m/z* = 461.21 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 461.1023, calculated for $C_{22}H_{25}N_2O_3S_3$ 461.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_3S_3$: C, 57.36; H, 5.25; N, 6.08% Found: C, 57.28; H, 5.04; N, 6.23%.

6.5.2 Synthesis of Compound 68b

6.5.2.1 Synthesis of Sulfonamide 66b



61

66b
$C_9H_{10}BrNO_5S_2$
Mol. Wt.: 356.21

Morpholine (0.43 mL, 4.91 mmol, 3 equiv.) was added dropwise to a stirred solution of **61** (500 mg, 1.64 mmol, 1 equiv.) in anhydrous methanol (5 mL) at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1 M Hydrochloric acid (HCl) was added dropwise to the aqueous phase at 0 °C to reduce the pH down to ~1. The resulting precipitate **66b** was collected through filtration and dried over $CaCl_2$ under vacuum.

Compound **66b** (485 mg, 83% yield) was obtained as a white solid.

R_f = 0.1 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 247.8– 248.1 °C.

IR (ν_{max}/cm^{-1}): 836, 943, 1055, 1101, 1144, 1263, 1354, 1421, 1524, 1704 and 2923.

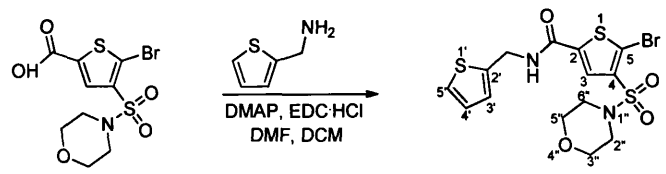
^{1}H NMR (400 MHz, DMSO): δ 14.01 (1H, br. s, COOH), 7.66 (1H, s, H^3), 3.65 (4H, t, J = 4.8 Hz, $H^{3'}$& $H^{5'}$), 3.10 (4H, t, J = 4.8 Hz, $H^{2'}$& $H^{6'}$).

^{13}C NMR (100 MHz, DMSO): δ 162.9 (COOH), 136.0 (C^2), 135.4 (C^4), 132.7 (C^3), 123.0 (C^5), 65.3 ($C^{3'}$& $C^{5'}$), 45.8 ($C^{2'}$& $C^{6'}$).

LRMS (ESI): *m/z* = 356.21 $[C_9H_{10}{}^{79}BrNO_5S_2+H]^+$ (95%), 358.41 $[C_9H_{10}{}^{81}BrNO_5S_2+H]^+$ (100%).

HRMS (TOF MS ES): Found 355.9274, calculated for $C_9H_{11}{}^{79}BrNO_5S_2$ 355.9262 $[M+H]^+$.

6.5.2.2 Synthesis of Amide 67b



66b

67b

$C_{14}H_{15}BrN_2O_4S_3$

Mol. Wt.: 451.38

Thiophene-2-methylamine (0.14 mL, 1.35 mmol, 1.2 equiv.) was added dropwise to stirred solution of **66b** (400 mg, 1.123 mmol, 1 equiv.), EDC hydrochloride (258 mg, 1.35 mmol, 1.2 equiv.) & DMAP (14 mg, 0.112 mmol, 0.1 equiv.) in anhydrous DCM (5 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 20 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash step. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The product **67b** was precipitated out from the crude by using different solvent. The solid was filtered off and dried overnight over $CaCl_2$.

Compound **67b** (274 mg, 54% yield) was obtained as a white solid.

R_f = 0.5 (Silica gel, Ethyl acetate/DCM : 1/1).

mp = 138.2 – 138.5 °C.

IR (ν_{max}/cm^{-1}): 724, 864, 946, 1073, 1113, 1353, 1543, 1631 and 3347.

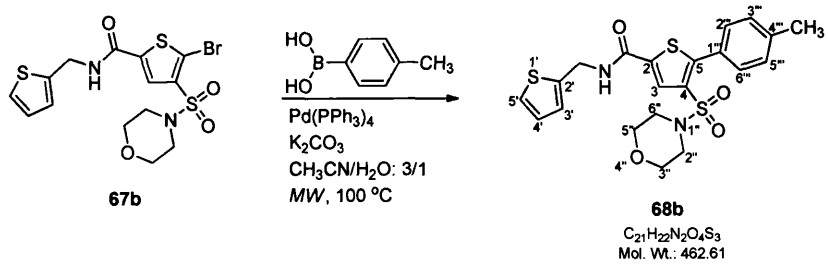
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (1H, s, H^3), 7.27 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.05 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.98 (1H, dd, J = 5.2, 3.2 Hz, $H^{4'}$), 6.53 (1H, t, J = 5.6 Hz, NH), 4.76 (2H, d, J = 5.6 Hz, CH_2NH), 3.74 (4H, m, $H^{3''}$& $H^{5''}$), 3.20 (4H, t, J = 4.8 Hz, $H^{2''}$& $H^{6''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4 (CONH), 140.3 (C^2), 139.6 ($C^{2'}$), 136.1 (C^4), 128.0 (C^3), 127.2 ($C^{4'}$), 127.0 ($C^{3'}$), 125.9 ($C^{5'}$), 121.7 (C^5), 66.2 ($C^{3''}$& $C^{5''}$) 46.1 ($C^{2''}$& $C^{6''}$), 38.9 (CH_2NH).

LRMS (ESI): *m/z* = 448.84 $[C_{14}H_{15}{}^{79}BrN_2O_4S_3\text{-}H]^-$ (85%), 450.70 $[C_{14}H_{15}{}^{81}BrN_2O_4S_3\text{-}H]^-$ (100%).

HRMS (TOF MS ES): Found 450.9434, calculated for $C_{14}H_{16}{}^{79}BrN_2O_4S_3$ 450.9456 $[M+H]^+$.

6.5.2.3 Synthesis of Compound 68b



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **67b** (150 mg, 0.33 mmol, 1 equiv.), *p*-tolylboronic acid (54 mg, 0.40 mmol, 1.2 equiv.) and K_2CO_3 (92 mg, 0.67 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (38 mg, 0.03 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (9:1 →1:1) as eluent to afford compound **68b**.

Compound **68b** (110 mg, 72% yield) was obtained as a white solid.

R_f = 0.26 (Silica gel, Ethyl acetate/DCM : 1/1).

mp = 95.5 – 95.8 °C.

IR (ν_{max}/cm^{-1}): 719, 943, 1111, 1142, 1294, 1444, 1545, 1629 and 2856.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (1H, s, H^3), 7.50 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.27 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.24 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.06 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 6.43 (1H, t, *J* = 5.6 Hz, NH), 4.79 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.43 (4H, t, *J* = 4.8 Hz, $H^{3''}$& $H^{5''}$), 2.82 (4H, t, *J* = 4.8 Hz, $H^{2''}$& $H^{6''}$), 2.40 (3H, s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.3 (*C*ONH), 152.6 (C^5), 140.5 ($C^{2'}$), 139.9 ($C^{4'''}$), 137.2 (C^2), 132.6 (C^4), 130.4 ($C^{2'''}$& $C^{6'''}$), 129.4 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 128.1 ($C^{1'''}$), 127.2 ($C^{4'}$), 126.8 ($C^{3'}$), 125.8 ($C^{5'}$), 45.5 ($C^{2''}$& $C^{6''}$), 38.9 (CH_2NH), 66.1 ($C^{3''}$& $C^{5''}$), 21.4 (CH_3).

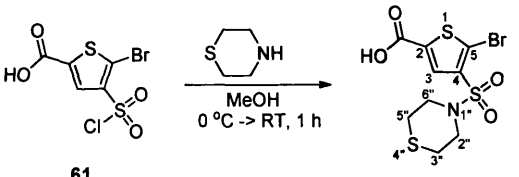
LRMS (ESI): *m/z* = 463.45 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 463.0842, calculated for $C_{21}H_{23}N_2O_4S_3$ 463.0820 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_3$: C, 54.52; H, 4.79; N, 6.06% Found: C, 54.61; H, 4.61; N, 5.85%.

6.5.3 Synthesis of Compound 68c

6.5.3.1 Synthesis of Sulfonamide 66c



61

66c
$C_9H_{10}BrNO_4S_3$
Mol. Wt.: 372.28

Thiomorpholine (0.49 mL, 4.91 mmol, 3 equiv.) was added dropwise to stirred solution of **61** (500 mg, 1.64 mmol, 1 equiv.) in anhydrous methanol (5 mL) at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1 M Hydrochloric acid (HCl) was added dropwise to the aqueous phase to reduce the pH down to ~1. The acidic mixture was partitioned between ethyl acetate and the organic phase was dried over $MgSO_4$ to afford the crude product. The crude mixture was purified by column chromatography on silica gel 60 using methanol and ethyl acetate (0:10→3:7) as eluent to afford compound **66c**.

Compound **66c** (390 mg, 68% yield) was obtained as a white powder.

R_f = 0.14 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 226.2 – 226.8 °C.

IR (ν_{max}/cm^{-1}): 773, 901, 1062, 1138, 1411 and 1573.

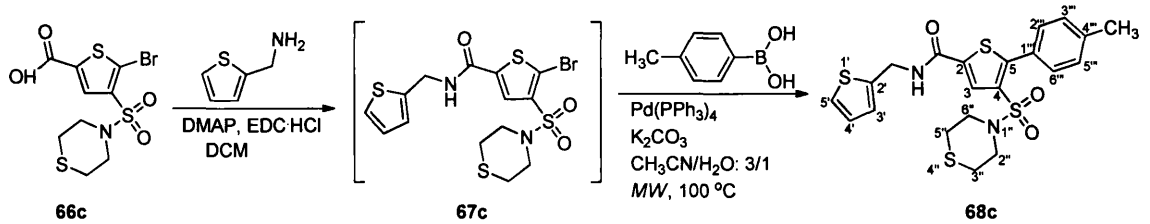
^{1}H NMR (400 MHz, DMSO): δ 7.31 (1H, s, H^3), 3.38 (4H, d, J = 4.8 Hz, $H^{2'}$& $H^{6'}$), 2.68 (4H, d, J = 4.8 Hz, $H^{3'}$& $H^{5'}$).

^{13}C NMR (100 MHz, DMSO): δ 161.42 (COOH), 149.4 (C^2), 135.6 (C^4), 128.2 (C^3), 117.6 (C^5), 47.6 ($C^{2'}$& $C^{6'}$), 26.4 ($C^{3'}$& $C^{5'}$).

LRMS (ESI): *m/z* = 369.65 $[C_9H_{10}{}^{79}BrNO_4S_3\text{-}H]^-$ (95%), 371.78 $[C_9H_{10}{}^{81}BrNO_4S_3\text{-}H]^-$ (100%).

HRMS (TOF MS ES): Found 371.9032, calculated for $C_9H_{11}{}^{79}BrNO_4S_3$ 371.9034 $[M+H]^+$.

6.5.3.2 Synthesis of Compound 68c



66c

67c
$C_{14}H_{15}BrN_2O_3S_4$
Mol. Wt.: 467.44

68c
$C_{21}H_{22}N_2O_3S_4$
Mol. Wt.: 478.67

Thiophen-2-ylmethanamine (0.061 mL, 0.6 mmol, 1.5 equiv.) was added dropwise to stirred solution of **66c** (150 mg, 0.4 mmol, 1 equiv.), EDC hydrochloride (93 mg, 0.48 mmol, 1.2 equiv.) & DMAP (4.92 mg, 0.04 mmol, 0.1 equiv.) in anhydrous DCM (4 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h. The precipitated product **67c** was collected by filtration followed by rinse with cold DCM. The filtrate was partitioned between 1 M citric acid, saturated sodium bicarbonate solution and brine in three separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give the product further **67c**. The product **67c** was combined and dried overnight over $CaCl_2$.

A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **67c** (50 mg, 0.11 mmol, 1 equiv.), *p*-tolylboronic acid (22 mg, 0.16 mmol, 1.5 equiv.) and K_2CO_3 (30 mg, 0.21 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (12 mg, 0.011 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM as eluent to afford compound **68c**.

Compound **68c** (28 mg, 55% yield) was obtained as a light yellow solid.

R_f = 0.57 (Silica gel, Ethyl acetate/DCM: 1/9).

mp = 87.9 – 88.2 °C.

IR (ν_{max}/cm^{-1}): 813, 902, 1061, 1138, 1284, 1444, 1543, 1631, 2913 and 3322.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, s, H^3), 7.48 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.28 (1H, d, *J* = 5.2 Hz, $H^{5'}$), 7.25 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.07 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, *J* = 5.2, 3.6 Hz, $H^{4'}$), 6.39 (1H, t, *J* = 5.6 Hz, NH), 4.80 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.09 (4H, m, $H^{2''}$& $H^{6''}$), 2.41 (3H, s, CH_3), 2.40 (4H, m, $H^{3''}$& $H^{5''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.3 (CONH), 152.1 (C^5), 140.5 ($C^{4'''}$), 139.9 ($C^{2'}$), 138.5 (C^2), 133.8 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.3 (C^3), 129.2 ($C^{3'''}$& $C^{5'''}$), 128.0 ($C^{1'''}$), 127.2 ($C^{4'}$), 126.9 ($C^{3'}$), 125.9 ($C^{5'}$), 47.3 ($C^{2''}$& $C^{6''}$), 38.9 (CH_2NH), 27.2 ($C^{3''}$& $C^{5''}$), 21.5 (CH_3).

LRMS (ESI): *m/z* = 479.4113 $[M+H]^+$, 100%.

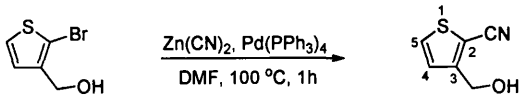
HRMS (TOF MS ES): Found 479.0591, calculated for $C_{21}H_{23}N_2O_3S_4$ 479.0604 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_3S_4$: C, 52.69; H, 4.63; N, 5.85% Found: C, 52.79; H, 4.47; N, 5.61%.

6.6 Preparation of Focused Library "D"

6.6.1 Preparation of Compound 70a

6.6.1.1 Synthesis of Cynano Derivative 71c[i]



71c[i]
C_6H_5NOS
Mol. Wt.: 139.17

Zinc cyanide (337 mg, 2.87 mmol, 0.98 equiv.) was added to the solution of 2-bromothiophene 3-yl methanol (565 mg, 2.93 mmol, 1 equiv.) in anhydrous *N*,*N*-dimethylformamide (17 mL). Tetrakis(triphenylphosphine)palladium (1.7 g, 1.5 mmol, 0.5 equiv.) was added to the mixture. The reaction mixture was heated to 100 °C for 1 h, cooled to ambient temperature, subjected to addition of 5% $Na_2S_2O_3$ aqueous solution, 10% K_2CO_3 aqueous solution and ethyl acetate. The insoluble solids were filtered off, and the solution was washed with water and brine. The organic fraction was dried over $MgSO_4$ and evaporated, and purified by column chromatography using gravity on silica gel 60 using *n*-hexane and ethyl acetate (1:1) as eluent to afford compound **71c[i]**.

Compound **71c[i]** (371 mg, 91% yield) was obtained as a yellow oil.

R_f = 0.28 (Silica gel, Ethyl acetate/*n*-hexane: 1/1).

IR (ν_{max}/cm^{-1}): 722, 1040, 1379, 1500, 2214 and 3275.

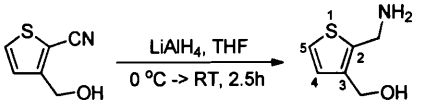
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (1H, d, J = 5.2 Hz, H^5), 7.21 (1H, d, J = 5.2 Hz, H_4), 4.85 (2H, d, J = 5.6 Hz, CH_2), 2.11 (1H, t, J = 5.6 Hz, OH).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.7 (C^3), 132.5 (C^5), 127.9 (C^4), 113.6 (CN), 106.1 (C^2), 59.5 (CH_2).

LRMS (ESI): *m/z* = 141.26 $[M+2H]^+$, 100%.

HRMS (TOF MS ES): Found 140.0175, calculated for C_6H_6NOS 140.0170 $[M+H]^+$.

6.6.1.2 Synthesis of Primary Amine 71c



71c[i] **71c**
C_6H_9NOS
Mol. Wt.: 143.21

To a solution of **71c[i]** (110 mg, 0.79 mmol, 1 equiv.) in anhydrous THF (8 mL) was added portionwise lithium aluminium hydride (1 M) in THF (0.95 mL, 0.95 mmol, 1.2 equiv.) at ambient temperature under N_2. The reaction mixture was stirred at the same temperature for 1.5

h. To the mixture water (1 mL) was added dropwise in an ice/water bath. The precipitate was removed by vacuum filtration through celite, which was then washed with ethyl acetate. The filtrate and washings were combined, and the organic layer was separated, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel 60 using ethyl acetate and methanol (10:0 → 1:1) as eluent to afford **71c**.

Compound **71c** (70 mg, 62% yield) was obtained as a light yellow oil.

R_f = 0.20 (Silica gel, Ethyl acetate/Methanol: 1/1).

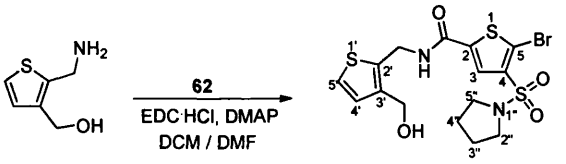
IR (ν_{max}/cm^{-1}): 831, 1000, 1244, 1433, 1621, 2857 and 3279.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07 (1H, dd, *J* = 5.2, 4.8 Hz, H^5), 6.96 (1H, dd, *J* = 5.2, 4.8 Hz, H_4), 4.59 (2H, d, *J* = 4.8 Hz, CH_2OH), 4.02 (2H, d, J = 5.2 Hz, CH_2NH_2), 3.22 (2H, s, NH_2), 3.18 (1H, s, OH).

^{13}C NMR (100 MHz, $CDCl_3$): δ 140.2 (C^3), 140.0 (C^2), 129.5 (C^4), 122.4 (C^5), 58.6 (CH_2OH), 38.9 (CH_2NH_2).

LRMS (ESI): *m/z* = 208.41[M+ ACN + Na +H]$^+$, 100%.

6.6.1.3 Synthesis of Amide 69a



71c **69a**

$C_{15}H_{17}BrN_2O_4S_3$

Mol. Wt.: 465.41

The solution of **71c** (0.70 mg, 0.49 mmol, 1.1 equiv.) in DCM (1 mL) was added dropwise to a vigorously stirred solution of **62** (151 mg, 0.44 mmol, 1 equiv.), EDC hydrochloride (102 mg, 0.53 mmol, 1.2 equiv.) & DMAP (5.43 mg, 0.044 mmol, 0.1 equiv.) in anhydrous DCM (2 mL) and anhydrous DMF (1 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 3.5 h, quenched by the addition of water and extracted with ethyl acetate. The combined extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by flash (ethyl acetate/*n*-hexane, 3:7 → 1:1) to afford compound **69a**.

Compound **69a** (88 mg, 43% yield) was obtained as a pale yellow solid.

R_f = 0.4 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 145.2 – 145.5 °C.

IR (ν_{max}/cm^{-1}): 865, 1008, 1145, 1320, 1545, 1630 and 3348.

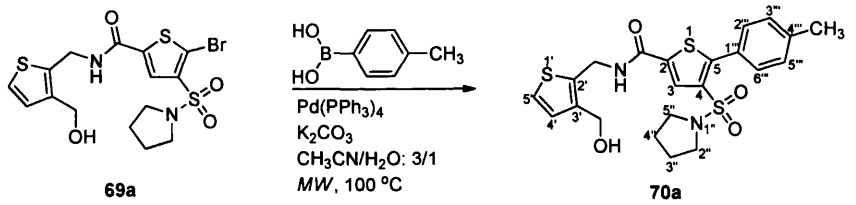
^{1}H NMR (400 MHz, MeOD): δ 7.85 (1H, s, H^3), 7.25 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.02 (1H, d, J = 5.2, 3.6 Hz, $H^{4'}$), 4.70 (2H, s, CH_2NH), 4.66 (2H, s, CH_2OH), 3.37 (4H, m, $H^{2"}$& $H^{5"}$), 1.86 (4H, m, $H^{3"}$& $H^{4"}$).

^{13}C NMR (100 MHz, MeOD): δ 160.0 (CONH), 119.8 (C^5), 137.9 ($C^{2'}$), 130.1 (C^3), 137.5 (C^4), 129.6 ($C^{4'}$), 125.2 ($C^{5'}$), 139.3 ($C^{3'}$), 140.4 (C^2), 58.2 (CH_2OH), 49.0 ($C^{2"}$& $C^{5"}$), 36.9 (CH_2NH), 26.3 ($C^{3"}$& $C^{4"}$).

LRMS (ESI): *m/z* = 463.28 [$C_{15}H_{17}{}^{79}BrN_2O_4S_3$-H]$^-$ (90%), 465.28 [$C_{15}H_{17}{}^{81}BrN_2O_4S_3$-H]$^-$ (100%).

HRMS (TOF MS ES): Found 464.9617, calculated for $C_{15}H_{18}{}^{79}BrN_2O_4S_3$ 464.9612 [M+H]$^+$.

6.6.1.4 Synthesis of Compound 70a



A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **69a** (77 mg, 0.17 mmol, 1 equiv.), *p*-tolylboronic acid (27 mg, 0.2 mmol, 1.2 equiv.) and K_2CO_3 (46 mg, 0.33 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (19 mg, 0.017 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **70a**.

Compound **70a** (75 mg, 93% yield) was obtained as a light yellow solid.

R_f = 0.47 (Silica gel, Ethyl acetate).

mp = 199.2 – 199.6 °C.

IR (ν_{max}/cm^{-1}): 875, 1114, 1183, 1288, 1542, 1658, 1739, 2883, 3374 and 3545.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (1H, s, H^3), 7.48 (2H, d, J = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.18 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.22 (2H, d, J = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 6.98 (1H, d, J = 5.2 Hz, $H^{4'}$), 7.38 (1H, t, J = 5.6 Hz, NH), 4.78 (2H, d, J = 5.6 Hz, CH_2NH), 4.73 (2H, s, CH_2OH), 2.95 (4H, m, $H^{2"}$& $H^{5"}$), 2.40 (3H, s, CH_3), 1.59 (4H, m, $H^{3"}$& $H^{4"}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9 (CONH), 151.8 (C^5), 140.2 ($C^{4'''}$), 139.7 ($C^{2'}$), 137.1 ($C^{3'}$), 136.8 (C^2), 134.4 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.6 (C^3), 129.13 ($C^{4'}$), 129.07 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$),124.4 ($C^{5'}$), 58.3 (CH_2OH), 47.5 ($C^{2''}$& $C^{5''}$), 36.5 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

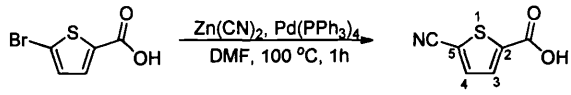
LRMS (ESI): *m/z* = 475.60 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 477.0959, calculated for $C_{22}H_{25}N_2O_4S_3$ 477.0977 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_4S_3$: C, 55.44; H, 5.08; N, 5.88% Found: C, 55.61; H, 5.32; N, 5.76%.

6.6.2 Preparation of Compound 70b

6.6.2.1 Synthesis of Derivative 71d^i



71d^i
$C_6H_3NO_2S$
Mol. Wt.: 153.16

Zinc cyanide (278 mg, 2.37 mmol, 0.98 equiv.) was added to the solution of 2-bromo thiophene-5-carboxylic acid (500 mg, 2.42 mmol, 1 equiv.) in anhydrous *N,N*-dimethylformamide (14 mL). Tetrakis(triphenylphosphine)palladium (1.4 g, 1.21 mmol, 0.5 equiv.) was added to the mixture. The reaction mixture was heated to 100 °C for 1 h, cooled to ambient temperature, subjected to addition of 5% $Na_2S_2O_3$ aqueous solution, 10% K_2CO_3 aqueous solution and ethyl acetate. The insoluble solids were filtered off, and the solution was partitioned between water. The pH of the aqueous phase was adjusted to 1 by addition of 1 M HCl and partitioned between ethyl acetate. The organic fraction was dried over $MgSO_4$ and evaporated *in vacuo* to afford compound **71d^i**.

Compound **71d^i** (341 mg, 92% yield) was obtained as a light yellow solid.

R_f = 0.32 (Silica gel, Ethyl acetate/Methanol: 4/1).

mp = 201.8 – 202.1 °C.

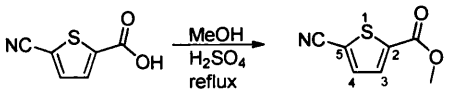
IR (ν_{max}/cm^{-1}): 749, 834, 888, 1297, 1457, 1656, 1737, 2229 and 2969.

^{1}H NMR (400 MHz, MeOD): δ 7.77 (1H, d, *J* = 4.0 Hz, H^3), 7.75 (1H, d, *J* = 4.0 Hz, H^4).

^{13}C NMR (100 MHz, MeOD): δ 163.1 (COOH), 142.6 (C^2), 139.3 (C^4), 133.9 (C^3), 115.6 (C^5), 114.1 (CN).

LRMS (ESI): *m/z* = 154.23 $[M+H]^+$, 100%.

6.6.2.2 Synthesis of Ester 71d[ii]



71d[i] **71d[ii]**

$C_7H_5NO_2S$

Mol. Wt.: 167.19

15 drops of concentrated sulphuric acid was added dropwise to the solution of **71d[i]** (300 mg, 1.99 mmol, 1 equiv.) in anhydrous methanol (30 mL) and the reaction mixture was heated under reflux at 90 °C. After 23 h sodium bicarbonate was added portion wise very slowly to quench the acid, as long as gas evolved. The solvent was evaporated and the residue was partitioned between ethyl acetate and water. The organic fraction was dried by $MgSO_4$ and evaporated *in vacuo* to afford the compound **71d[ii]**.

Compound **71d[ii]** (276 mg, 83% yield) was obtained as a yellow solid.

R_f = 0.62 (Silica gel, Ethyl acetate).

mp = 69.9 – 70.1 °C.

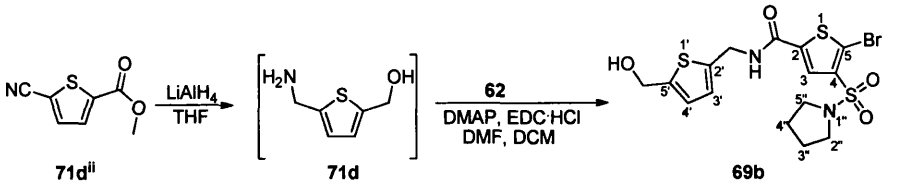
IR (ν_{max}/cm^{-1}): 743, 828, 1092, 1243, 1429, 1714, 2228 and 3100.

^{1}H NMR (400 MHz, MeOD): δ 7.82 (1H, d, *J* = 4.0 Hz, H^3), 7.78 (1H, d, *J* = 4.0 Hz, H^4), 3.92 (3H, s, CH_3).

^{13}C NMR (100 MHz, MeOD): δ 162.3 (CO), 141.0 (C^2), 139.4 (C^4), 134.2 (C^3), 116.1 (C^5), 114.0 (CN), 53.4 (CH_3).

LRMS (ESI): *m/z* = 168.46 $[M+H]^+$, 90%.

6.6.2.3 Synthesis of Amide 69b



71d[ii] **71d** **69b**

$C_{15}H_{17}BrN_2O_4S_3$

Mol. Wt.: 465.41

$LiAlH_4$ (1 M) in THF (37 mL, 37 mmol, 3 equiv) was added dropwise to the solution of **71d[ii]** (1.03 g, 6.17 mmol, 1 equiv.) in THF (60 mL) at 0 °C under N_2. After 8 h the reaction mixture was cooled to 0 °C then quenched by the very slow addition of water (25 mL). The quenched reaction mixture was filtered through the pad of celite followed by rinsing with ethyl acetate. The filtrate and the washings were combined together, dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture (**71d**) was partially purified by passing through the Isolute® SCX-2 column and its solution in 15 mL DCM/DMF (2:1) was added dropwise to stirred solution of **62**

(1.26 g, 3.69 mmol, 1 equiv.), EDC hydrochloride (848 mg, 4.43 mmol, 1.2 equiv.) & DMAP (45 mg, 0.37 mmol, 0.1 equiv.) in anhydrous DCM (20 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography on silica gel 60 (ethyl acetate/DCM 1:9 → 2:3) to afford compound **69b**.

Compound **69b** (687 mg, 40% yield) was obtained as a white solid.

R_f = 0.6 (Silica gel, Ethyl acetate).

mp = 147.1 – 147.5 °C.

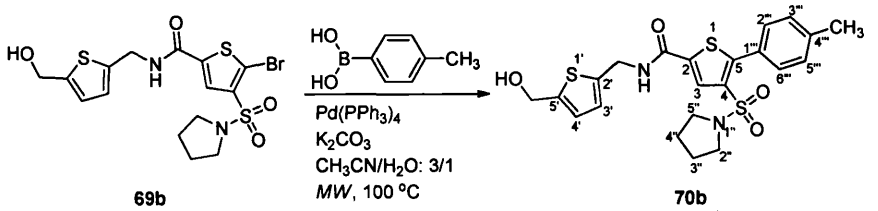
IR (ν_{max}/cm^{-1}): 811, 1022, 1140, 1312, 1406, 1546, 1649 and 3379.

^{1}H NMR (400 MHz, MeOD): δ7.86 (1H, s, H^3), 6.88 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.83 (1H, d, J = 3.6 Hz, $H^{4'}$), 4.67 (2H, s, CH_2OH), 4.62 (2H, s, CH_2NH), 3.37 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.85 (4H, m,$H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, MeOD): δ 161.7 (CONH), 146.1 ($C^{5'}$), 142.1 ($C^{2'}$), 141.6 (C^2), 139.4 (C^4), 130.2 (C^3), 126.9 ($C^{4'}$), 125.8 ($C^{3'}$), 121.9 (C^5), 49.3 ($C^{2''}$& $C^{5''}$), 39.5 (CH_2NH), 26.4 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 466.30 $[M+H]^+$, 100%.

6.6.2.4 Synthesis of Compound 70b



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **69b** (150 mg, 0.32 mmol, 1 equiv.), *p*-tolylboronic acid (53 mg, 0.39 mmol, 1.2 equiv.) and K_2CO_3 (89 mg, 0.65 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (37 mg, 0.032 mmol, 0.1 equiv.) was then added. N_2 was passed through the reaction mixture for 15 minutes and the reaction mixture was irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (20:1→3:2) as eluent to afford compound **70b**.

Compound **70b** (95 mg, 62% yield) was obtained as a light yellow solid.

R_f = 0.6 (Silica gel, Ethyl acetate).

mp = 67.9 – 68.1 °C.

IR (ν_{max}/cm^{-1}): 811, 1011, 1139, 1202, 1308, 1440, 1543, 1630, 1738, 2969 and 3297.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, s, H^3), 7.49 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 6.91 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.86 (1H, d, *J* = 3.6 Hz, $H^{4'}$), 6.67 (1H, t, *J* = 5.6 Hz, NH), 4.78 (2H, s CH_2OH), 4.73 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.96 (4H, t,*J* =6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.59 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9 (CONH), 151.6 (C^5), 144.0 ($C^{5'}$), 140.1 ($C^{4'''}$), 140.2 ($C^{2'}$), 136.5 (C^2), 134.4 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$),130.1 ($C^{1'''}$), 129.5 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 126.5 ($C^{4'}$), 125.4 ($C^{3'}$), 60.3 (CH_2OH), 47.5 ($C^{2''}$& $C^{5''}$), 39.1 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

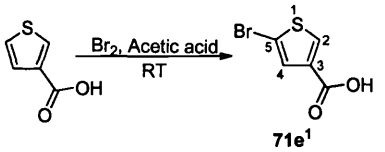
LRMS (ESI): *m/z* = 475.78 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 475.0829, calculated for $C_{22}H_{23}N_2O_4S_3$ 475.0820 $[M-H]^-$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_4S_3$: C, 55.44; H, 5.08; N, 5.88% Found: C, 55.30; H, 4.98; N, 5.69%.

6.6.3 Preparation of Compound 70c

6.6.3.1 Synthesis of Bromothiophene Derivative 71e^i



71e^1
$C_5H_3BrO_2S$
Mol. Wt.: 207.05

A solution of Br_2 (0.804 mL, 15.61 mmol, 1 equiv.) in 10 mL glacial acetic acid was added slowly to a stirred solution of 3-thionic acid (2 g, 15.61 mmol, 1 equiv.) in 20 mL of glacial acetic acid at room temperature. The reaction mixture was stirred for 0.5 h before pouring into 100 mL of ice-cold water. The compound precipitated out **71e^i** was obtained by filtration.

Compound **71e^i** (2.2 gm, 68% yield) was obtained as a white solid.

R_f = 0.38 (Silica gel, Ethyl acetate/Methanol: 4/1).

mp = 128.5 – 128.8 °C.

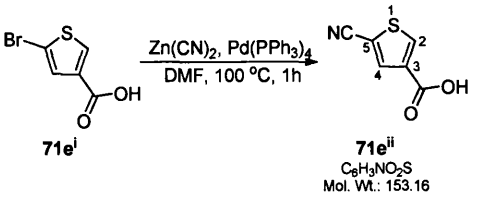
IR (ν_{max}/cm^{-1}): 744, 848, 902, 1179, 1256, 1432, 1526, 1672, 2599 and 3097.

^{1}H NMR (400 MHz, MeOD): δ 8.13 (1H, d, J = 1.6 Hz, H^2), 7.45 (1H, d, J = 1.6 Hz, H^4).

^{13}C NMR (100 MHz, MeOD): δ 164.6 (COOH), 136.0 (C^3), 135.7 (C^2), 131.5 (C^4) 113.6 (C^5).

LRMS (ESI): *m/z* = 205.25 [$C_5H_3{}^{79}BrO_2S$-H]$^-$ (95%), 207.33 [$C_5H_3{}^{81}BrO_2S$-H]$^-$ (100%).

6.6.3.2 Synthesis of Derivaive 71e^{ii}



71e^i **71e^{ii}**
$C_6H_3NO_2S$
Mol. Wt.: 153.16

Zinc cyanide (278 mg, 2.37 mmol, 0.98 equiv.) was added to the solution of 2-bromo thiophene-5-carboxylic acid (500 mg, 2.42 mmol, 1 equiv.) in anhydrous *N,N*-dimethylformamide (14 mL). Tetrakis(triphenylphosphine)palladium (1.4 g, 1.21 mmol, 0.5 equiv.) was added to the mixture. The reaction mixture was heated to 100 °C for 1 h, cooled to ambient temperature, subjected to addition of 5% $Na_2S_2O_3$ aqueous solution, 10% K_2CO_3 aqueous solution and ethyl acetate. The insoluble solids were filtered off, and the solution was partitioned between water. The pH of the aqueous phase was adjusted to 1 by addition of 1 M HCl and partitioned between ethyl acetate. The organic fraction was dried over $MgSO_4$ and evaporated *in vacuo* to afford compound **71e^{ii}**.

Compound **71e^{ii}** (267 mg, 72% yield) was obtained as a light yellow solid.

R_f = 0.18 (Silica gel, Ethyl acetate/Methanol: 4/1).

mp = 212.3 – 212.9 °C.

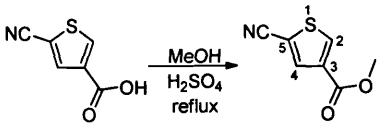
IR (ν_{max}/cm^{-1}): 881, 1047, 1274, 1441, 1529, 1666, 2222, 2526, 3093 and 3377.

^{1}H NMR (400 MHz, MeOD): δ 8.52 (1H, d, J = 1.2 Hz, H^2), 8.09 (1H, d, J = 1.2 Hz, H^4).

^{13}C NMR (100 MHz, MeOD): δ 162.1 (COOH), 140.3 (C^2), 139.4 (C^4), 134.7 (C^3), 114.1 (CN), 111.7 (C^5).

LRMS (ESI): *m/z* = 154.15 [M+H]$^+$, 100%.

6.6.3.3 Synthesis of Ester 71e[iii]



71e[ii] **71e[iii]**
$C_7H_5NO_2S$
Mol. Wt.: 167.19

20 drops of concentrated sulphuric acid was added dropwise to the solution of **71e[ii]** (380 mg, 2.48 mmol) in anhydrous methanol (30 mL) and the reaction mixture was heated under reflux at 90 °C. After 16 h sodium bicarbonate was added portion wise very slowly to quench the acid, as long as gas evolved. The solvent was evaporated and the residue was partitioned between ethyl acetate and water. The organic fraction was dried by $MgSO_4$ and evaporated *in vacuo*. The compound **71e[iii]** was purifiedby column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→4:1) as eluent.

Compound **71ei[ii]** (249 mg, 60% yield) was obtained as a light yellow solid.

R_f = 0.62 (Silica gel, Ethyl acetate).

mp = 70.2 – 70.5 °C.

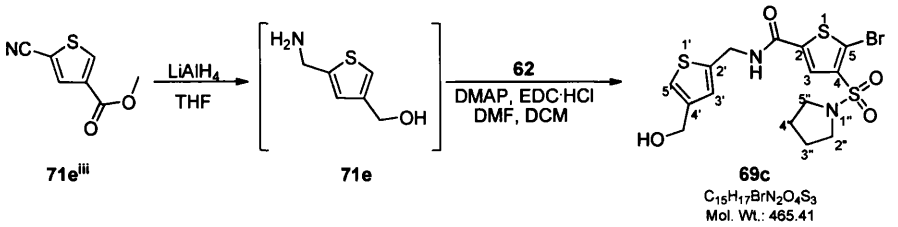
IR (ν_{max}/cm^{-1}): 744, 864, 980, 1103, 1235, 1439, 1535, 1706, 2225 and 3103.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (1H, d, J = 1.2 Hz, H^2), 8.02 (1H, d, J = 1.2 Hz, H^4), 3.91 (3H, s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 162.2 (COOH), 138.1 (C^2), 137.9 (C^4), 134.6 (C^3), 113.7 (CN), 110.1 (C^5), 52.4 (CH_3).

LRMS (ESI): *m/z* = 168.30 $[M+H]^+$, 100%.

6.6.3.4 Synthesis of Amide 69c



$LiAlH_4$ (1 M) in THF (29.4 mL, 19.4 mmol, 3 equiv.) was added dropwise to the solution of **71e**^{iii} (819 mg, 4.9 mmol, 1 equiv.) in THF (49 mL) at 0 °C under N_2. After 8 h the reaction mixture was cooled to 0 °C then quenched by the very slow addition of water (25 mL). The quenched reaction mixture was filtered through the pad of celite and which was washed by ethyl acetate. The filtrate and the washings were combined together, dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture (**71e**) was partially purified by passing through the Isolute® SCX-2 column and its solution in 15 mL DCM/DMF (2:1) was added dropwise to stirred solution of **62** (935 mg, 2.75 mmol, 1 equiv.), EDC hydrochloride (632 mg, 3.30 mmol, 1.2 equiv.) & DMAP (34 mg, 0.28 mmol, 0.1 equiv.) in anhydrous DCM (20 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography on silica gel 60 (ethyl acetate/DCM, 1:9 → 2:3) to afford compound **69c**.

Compound **69c** (589 mg, 46% yield) was obtained as a light yellow solid.

R_f = 0.42 (Silica gel, Ethyl acetate).

mp = 159.9 – 160.3 °C.

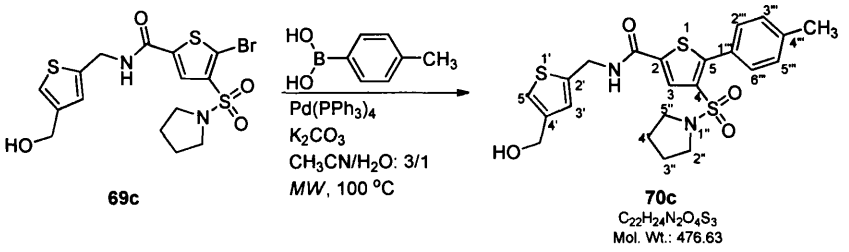
IR (ν_{max}/cm^{-1}): 740, 866, 1012, 1145, 1315, 1424, 1526, 1644, 2923 and 3291.

^{1}H NMR (400 MHz, MeOD): δ 7.86 (1H, s, H^3), 7.14 (1H, s, $H^{5'}$), 7.02 (1H, s, $H^{3'}$), 4.64 (2H, s, CH_2NH), 4.53 (2H, s, CH_2OH), 3.37 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.86 (4H, m,$H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, MeOD): δ 160.1 (CONH), 142.3 ($C^{2'}$), 142.1 ($C^{4'}$), 139.8 (C^2), 138.2 (C^4), 130.2 (C^3), 127.2 ($C^{3'}$), 122.4 ($C^{5'}$), 120.7 (C^5), 49.3 ($C^{2''}$& $C^{5''}$), 39.3 (CH_2NH), 26.4 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 462.83 [$C_{15}H_{17}{}^{79}BrN_2O_4S_3$-H]$^-$ (90%), 464.56 [$C_{15}H_{17}{}^{81}BrN_2O_4S_3$-H]$^-$ (100%).

6.6.3.5 Synthesis of Compound 70c



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (5 mL) followed by the addition of **69c** (97 mg, 0.21 mmol, 1 equiv.), *p*-tolylboronic acid (34 mg, 0.25 mmol, 1.2 equiv.) and K_2CO_3 (58 mg, 0.42 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (24 mg, 0.21 mmol, 0.1 equiv.) was then added. N_2 was passed through the reaction mixture for 15 minutes and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (9:1→7:3) as eluent to afford compound **70c**.

Compound **70c** (70 mg, 70% yield) was obtained as a light yellow solid.

R_f = 0.33 (Silica gel, Ethyl acetate/DCM : 1/1).

mp = 68.4 – 68.9 °C.

IR (ν_{max}/cm^{-1}): 721, 1015, 1117, 1321, 1436, 1545, 1647, 2873 and 3240.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, s, H^3), 7.49 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.13 (1H, s, $H^{5'}$), 7.05 (1H, s, $H^{3'}$), 6.66 (1H, t, J = 5.6 Hz, NH), 4.63 (2H, s, CH_2OH), 4.73 (2H, d, J = 5.6 Hz, CH_2NH), 2.96 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.59 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (CONH), 151.7 (C^5), 142.4 ($C^{4'}$), 141.2 ($C^{2'}$), 140.2 ($C^{4'''}$), 137.0 (C^2), 134.7 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.5 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$), 126.5 ($C^{3'}$), 122.1 ($C^{5'}$), 60.9 (CH_2OH), 47.5 ($C^{2''}$& $C^{5''}$), 38.9 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

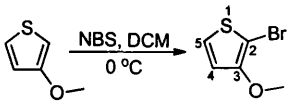
LRMS (ESI): *m/z* = 475.80 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 475.0821, calculated for $C_{22}H_{23}N_2O_4S_3$ 475.0820 $[M-H]^-$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_4S_3$: C, 55.44; H, 5.08; N, 5.88% Found: C, 55.52; H, 4.78; N, 5.87%.

6.6.4 Preparation of Compound 70d

6.6.4.1 Procedure for Synthesis of 71a[i]



71a[i]
C_5H_5BrOS
Mol. Wt.: 193.06

N-bromosuccinimide (2.39 g, 13.4 mmol, 1.01 equiv.) was added to a solution of 3-methoxy thiophene (1.52 g, 13.3 mmol, 1equiv.) in anhydrous DCM (61 mL) at 0 °C and was stirred at 0 °C for 0.5 h. The resulting solution was washed five times with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the compound **71a[i]** was obtained.

Compound **71a[i]** (2.5 gm, 96% yield) was obtained as a light yellow oil.

R_f = 0.56 (Silica gel, Ethyl acetate/*n*-hexane: 3/7).

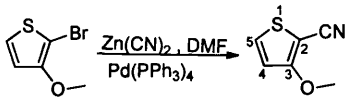
IR (ν_{max}/cm^{-1}): 703, 828, 1067, 1247, 1376, 1550 and 2933.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (1H, d, J = 5.6 Hz, H^5), 6.77 (1H, d, J = 5.6 Hz, H^4), 3.89 (3H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3 (C^3), 124.4 (C^5), 116.5 (C^4), 90.8 (C^2), 59.3 (OCH_3).

LRMS (ESI): *m/z* = 192.48 $[C_5H_5{}^{79}BrOS+H]^+$ (90%), 194.38 $[C_5H_5{}^{81}BrOS+H]^+$ (100%).

6.6.4.2 Synthesis of Cyano Derivative 71a[ii]



71a[i] **71a[ii]**

C_6H_5NOS

Mol. Wt.: 139.17

Zinc cyanide (1.49 g, 12.7 mmol, 0.98 equiv.) was added to the solution of **71a[i]** (2.5 g, 12.95 mmol, 1 equiv.) in anhydrous DMF (75 mL). Tetrakis(triphenylphosphine)palladium (7.5 g, 6.5 mmol, 0.5 equiv.) was added to the mixture. The reaction mixture was heated to 100 °C for 1.5 h, cooled to ambient temperature, subjected to addition of 5% $Na_2S_2O_3$ aqueous solution, 10% K_2CO_3 aqueous solution and ethyl acetate. The insoluble solids were filtered off, and the solution was washed with water and brine. The organic fraction was dried over $MgSO_4$ and evaporated *in vacuo*, and purified by column chromatography using gravity on silica gel 60 using *n*-hexane and ethyl acetate (9:1 →4:1) as eluent to afford compound **71a[ii]**.

Compound **71a[ii]** (1.4 gm, 78% yield) was obtained as a yellow crystal.

R_f = 0.28 (Silica gel, Ethyl acetate/*n*-hexane: 3/7).

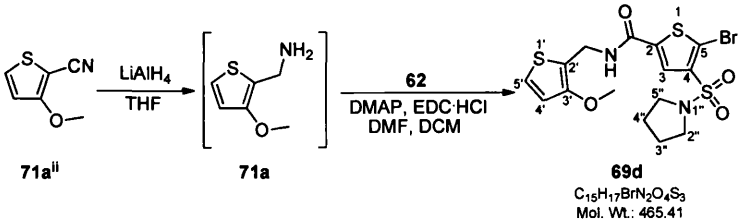
IR (ν_{max}/cm^{-1}): 842, 1075, 1268, 1391, 1462, 1539, 1774, 2202, 2946 and 3102.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (1H, d, *J* = 5.2 Hz, H^5), 6.79 (1H, d, *J* = 5.2 Hz, H^4), 4.03 (3H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7 (C^3), 131.7 (C^5), 116.3 (C^4), 113.6 (*CN*), 87.1 (C^2), 59.2 (OCH_3).

LRMS (ESI): *m/z* = 140.35 $[M+H]^+$, 100%.

6.6.4.3 Synthesis of Amide 69d



71a[ii] **71a** **69d**
$C_{15}H_{17}BrN_2O_4S_3$
Mol. Wt.: 465.41

$LiAlH_4$ (1 M) in THF (11.6 mL, 11.6 mmol, 1.2 equiv.) was added dropwise to the solution of **71a**[ii] (1.34 g, 9.63 mmol, 1 equiv.) in THF (95 mL) at 0 °C under N_2. After 2 h the reaction mixture was cooled to 0 °C then quenched by the very slow addition of water (12 mL). The quenched reaction mixture was filtered through the pad of celite and which was washed by ethyl acetate. The filtrate and the washings were combined together, dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture was partially purified by passing through the Isolute® SCX-2 column and its solution in 10 mL DCM was added dropwise to stirred solution of **62** (2.11 g, 6.2 mmol, 1 equiv.), EDC hydrochloride (1.43 g, 7.4 mmol, 1.2 equiv.) & DMAP (76 mg, 0.62 mmol, 0.1 equiv.) in anhydrous DCM (20mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of water and extracted with DCM. The organic fraction was washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography on silica gel 60 (ethyl acetate/DCM 1:99 →3:47) to afford compound **69d**.

Compound **69d** (2 gm, 68% yield) was obtained as a light yellow solid.

R_f = 0.72 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 136.2 – 136.5 °C.

IR (ν_{max}/cm^{-1}): 739, 854, 1066, 1147, 1345, 1547, 1614, 2961 and 3229.

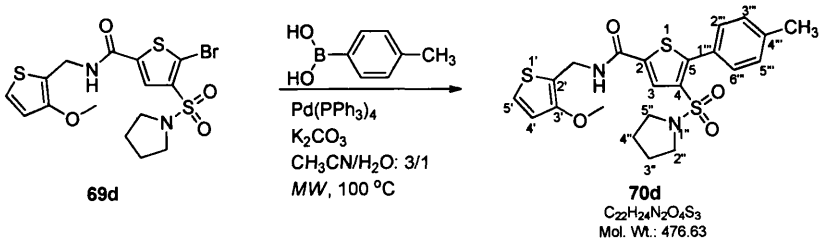
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (1H, s, H^3), 7.13 (1H, d, J = 5.6 Hz, $H^{5'}$), 6.82 (1H, d, J = 5.6 Hz, $H^{4'}$), 4.62 (2H, d, J = 5.6 Hz, CH_2NH), 3.86 (3H, s, OCH_3), 3.37 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.86 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7 (CONH), 155.6 ($C^{3'}$), 140.4 (C^2), 138.0 (C^4), 128.1 (C^3), 123.4 ($C^{5'}$), 120.5 (C^5), 116.1 ($C^{4'}$), 115.1 ($C^{2'}$), 58.8 (OCH_3), 48.1 ($C^{2''}$& $C^{5''}$), 35.0 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 463.43 $[C_{15}H_{17}{}^{79}BrN_2O_4S_3\text{-}H]^-$ (95%), 465.50 $[C_{15}H_{17}{}^{81}BrN_2O_4S_3\text{-}H]^-$ (100%).

HRMS (TOF MS ES): Found 464.9613, calculated for $C_{15}H_{18}{}^{79}BrN_2O_4S_3$ 464.9612 $[M+H]^+$.

6.6.4.4 Synthesis of Compound 70d



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (16 mL) followed by the addition of **69d** (300 mg, 0.65 mmol, 1 equiv.), *p*-tolylboronic acid (105 mg, 0.77 mmol, 1.2 equiv.) and K_2CO_3 (178 mg, 1.29 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (75 mg, 0.064 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→19:1) as eluent to afford compound **70d**.

Compound **70d** (279 mg, 90% yield) was obtained as a light yellow solid.

R_f = 0.72 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 170.9 – 171.3 °C.

IR (ν_{max}/cm^{-1}): 716, 873, 1118, 1305, 1540, 1656, 2945 and 3385.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, s, H^3), 7.49 (2H, d, *J* = 8.4 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, *J* = 8.4 Hz, $H^{3'''}$& $H^{5'''}$), 7.15 (1H, d, *J* = 5.6 Hz, $H^{5'}$), 6.84 (1H, d, *J* = 5.6 Hz, $H^{4'}$), 6.44 (1H, t, *J* = 5.6 Hz, NH), 4.66 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.89 (3H, s, OCH_3), 2.95 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.59 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (CONH), 155.6 ($C^{3'}$), 151.4 (C^5), 140.1 ($C^{4'''}$), 137.2 (C^2), 134.5 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.2 (C^3), 129.0 ($C^{3'''}$& $C^{5'''}$), 128.5 ($C^{1'''}$), 123.3 ($C^{5'}$), 116.1 ($C^{4'}$), 115.2 ($C^{2'}$), 58.9 (OCH_3), 47.5 ($C^{2''}$& $C^{5''}$), 35.1 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

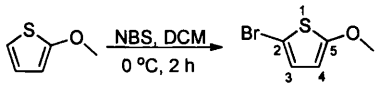
LRMS (ESI): *m/z* = 475.80 [M-H]$^-$, 100%.

HRMS (TOF MS ES): Found 475.0840, calculated for $C_{22}H_{23}N_2O_4S_3$ 475.0820 [M-H]$^-$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_4S_3$: C, 55.44; H, 5.08; N, 5.88% Found: C, 55.31; H, 5.18; N, 5.68%.

6.6.5 Preparation of Compound 70e

6.6.5.1 Synthesis of Bromothiophene Derivative 71b[i]



71b[i]
C_5H_5BrOS
Mol. Wt.: 193.06

N-bromosuccinimide (3.15 g, 17.7 mmol, 1.01 equiv.) was added to a solution of 2-methoxy thiophene (2 g, 17.5 mmol, 1 equiv.) in anhydrous DCM (81 mL) at 0 °C and was stirred at 0 °C for 0.5 h. The resulting solution was washed five times with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the compound **71b[i]** was obtained.

Compound **71b[i]** (3.28 gm, 97% yield) was obtained as a light yellow volatile oil.

R_f = 0.58 (Silica gel, Ethyl acetate/*n*-hexane: 3/7).

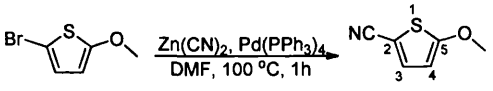
IR (ν_{max}/cm^{-1}): 757, 954, 1057, 1199, 1423, 1548 and 2931.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.69 (1H, d, J = 4.0 Hz, H^3), 5.97 (1H, d, J = 4.0 Hz, H^4), 3.85 (3H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2 (C^5), 127.3 (C^3), 104.4 (C^4), 97.4 (C^2), 60.5 (OCH_3).

LRMS (ESI): *m/z* = 192.79 $[C_5H_5{}^{79}BrOS+H]^+$ (92%), 194.79 $[C_5H_5{}^{81}BrOS+H]^+$ (100%).

6.6.5.2 Procedure for Synthesis of 71b[ii]



71b[i] **71b[ii]**
C_6H_5NOS
Mol. Wt.: 139.17

Zinc cyanide (1.9 g, 16.6 mmol, 0.98 equiv.) was added to the solution of **71b[i]** (3.27 g, 16.94 mmol, 1 equiv.) in anhydrous DMF (98 mL). Tetrakis(triphenylphosphine)palladium (9.8 g, 8.5 mmol, 0.5 equiv.) was added to the mixture. The reaction mixture was heated to 100 °C for 1.5 h, cooled to ambient temperature, subjected to addition of 5% $Na_2S_2O_3$ aqueous solution, 10% K_2CO_3 aqueous solution and ethyl acetate. The insoluble solids were filtered off, and the solution was washed with water and brine. The organic fraction was dried over $MgSO_4$ and evaporated *in vacuo*, and purified by column chromatography using gravity on silica gel 60 using *n*-hexane and ethyl acetate (9:1 → 4:1) as eluent to afford compound **71b[ii]**.

Compound **71b[ii]** (1.34 gm, 57% yield) was obtained as a yellow oil.

R_f = 0.39 (Silica gel, Ethyl acetate/*n*-hexane: 3/7).

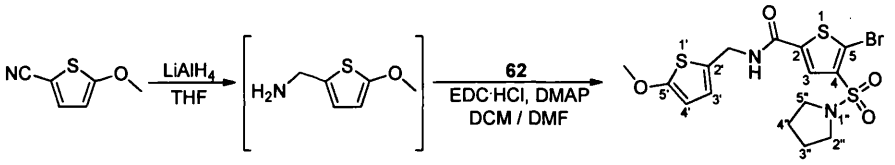
IR (ν_{max}/cm^{-1}): 776, 983, 1046, 1207, 1413, 1473, 1537, 2210 and 2934.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (1H, d, J = 4.4 Hz, H^3), 6.21 (1H, d, J = 4.4 Hz, H^4), 3.96 (3H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8 (C^5), 137.4 (C^3), 115.1 (CN), 105.0 (C^4), 95.5 (C^2), 60.9 (OCH_3).

LRMS (ESI): m/z = 140.23 $[M+H]^+$, 100%.

6.6.5.3 Synthesis of Amide 69e



$LiAlH_4$ (1 M) in THF (7.8 mL, 7.8 mmol, 1.2 equiv.) was added dropwise to the solution of **71b^{ii}** (0.9 g, 6.5 mmol, 1 equiv.) in THF (64 mL) at 0 °C under N_2. After 2 h the reaction mixture was cooled to 0 °C then quenched by the very slow addition of water (8 mL). The quenched reaction mixture was filtered through the pad of celite and which was washed by ethyl acetate. The filtrate and the washings were combined together, dried over $MgSO_4$ and evaporated *in vacuo*. The crude mixture (**71b**) was partially purified by passing through the Isolute® SCX-2 column and its solution in 10 mL DCM was added dropwise to stirred solution of **62** (1.6 g, 4.7 mmol, 1 equiv.), EDC hydrochloride (1.08 g, 5.6 mmol, 1.2 equiv.) & DMAP (57 mg, 0.47 mmol, 0.1 equiv.) in anhydrous DCM (20 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of water and extracted with DCM. The organic fraction was washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography on silica gel 60 (ethyl acetate/DCM 1:49 → 3:47) to afford compound **69e**.

Compound **69e** (2.19 gm, 86% yield) was obtained as a yellow solid.

R_f = 0.68 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 130.9 – 131.3 °C.

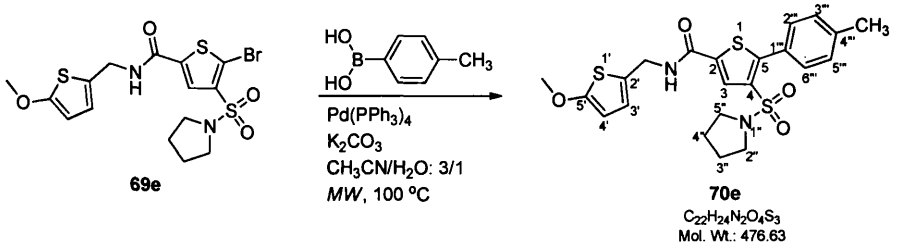
IR (ν_{max}/cm^{-1}): 873, 1045, 1144, 1351, 1508, 1551, 1625, 2888, 3074 and 3291.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (1H, s, H^3), 6.66 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.61 (1H, t, J = 5.6 Hz, NH), 6.03 (1H, d, J = 3.6 Hz, $H^{5'}$), 4.58 (2H, d, J = 5.6 Hz, CH_2NH), 3.86 (3H, s, OCH_3), 3.37 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.87 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1 ($C^{5'}$), 159.6 (CONH), 125.7 ($C^{2'}$), 103.4 ($C^{4'}$), 140.2 (C^{2}), 138.1 (C^{4}), 129.2 (C^{3}), 124.7 ($C^{3'}$), 120.7 (C^{5}), 60.4 (OCH_3), 48.1 ($C^{2"}$& $C^{5"}$), 39.6 (CH_2NH), 25.6 ($C^{3"}$& $C^{4"}$).

LRMS (ESI): *m/z* = 463.51 $[C_{15}H_{17}{}^{79}BrN_2O_4S_3\text{-}H]^-$ (90%), 465.53 $[C_{15}H_{17}{}^{81}BrN_2O_4S_3\text{-}H]^-$ (100%).

6.6.5.4 Synthesis of Compound 70e



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (16 mL) followed by the addition of **69e** (300 mg, 0.65 mmol, 1 equiv.), *p*-tolylboronic acid (105 mg, 0.77 mmol, 1.2 equiv.) and K_2CO_3 (178 mg, 1.29 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (75 mg, 0.064 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0 →19:1) as eluent to afford compound **70e**.

Compound **70e** (289 mg, 90% yield) was obtained as a yellow solid.

R_f = 0.74 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 64.5 – 65.1 °C.

IR (ν_{max}/cm^{-1}): 750, 876, 1009, 1137, 1319, 1430, 1542, 1625, 2945 and 3307.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (1H, s, H^3), 7.50 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.04 (1H, d, *J* = 4.0 Hz, $H^{4'}$), 6.66 (1H, d, *J* = 4.0 Hz, $H^{3'}$), 6.44 (1H, t, *J* = 5.6 Hz, NH), 4.61 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.87 (3H, s, OCH_3), 2.96 (4H, t, *J* = 6.8 Hz, $H^{2"}$& $H^{5"}$), 2.40 (3H, s, CH_3), 1.60 (4H, m, $H^{3"}$& $H^{4"}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7 ($C^{5'}$), 160.5 (CONH), 151.6 (C^{5}), 140.2 ($C^{4'''}$), 137.1 (C^{2}), 134.7 (C^{4}), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.4 (C^{3}), 129.1 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$), 126.0 ($C^{2'}$), 124.5 ($C^{3'}$), 103.4 ($C^{4'}$), 60.5 (OCH_3), 47.5 ($C^{2"}$& $C^{5"}$), 39.6 (CH_2NH), 25.5 ($C^{3"}$& $C^{4"}$), 21.5 (CH_3).

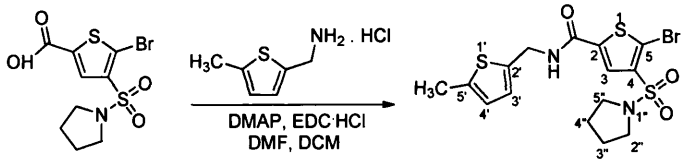
LRMS (ESI): *m/z* = 475.87 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 475.0836, calculated for $C_{22}H_{23}N_2O_4S_3$ 475.0820 $[M-H]^-$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_4S_3$: C, 55.44; H, 5.08; N, 5.88% Found: C, 55.65; H, 5.13; N, 5.79%.

6.6.6 Preparation of Compound 70f

6.6.6.1 Synthesis of Amide 69f



A solution of (5-methylthiophen-2-yl)methanamine hydrochloride (132 mg, 0.81 mmol, 1.1 equiv.) in DMF (2 mL) was added dropwise to stirred solution of **62** (250 mg, 0.74 mmol, 1 equiv.), EDC hydrochloride (169 mg, 0.88 mmol, 1.2 equiv.) & DMAP (9 mg, 0.07 mmol, 0.1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **62**) and DMF (10 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 20 h, concentrated *in vacuo* and quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **69f** was filtered off and dried overnight over $CaCl_2$.

Compound **69f** (206 mg, 62% yield) was obtained as a brown solid.

R_f = 0.68 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 185.6 – 186.1 °C.

IR (ν_{max}/cm^{-1}): 803, 1019, 1137, 1312, 1546, 1651, 2918 and 3354.

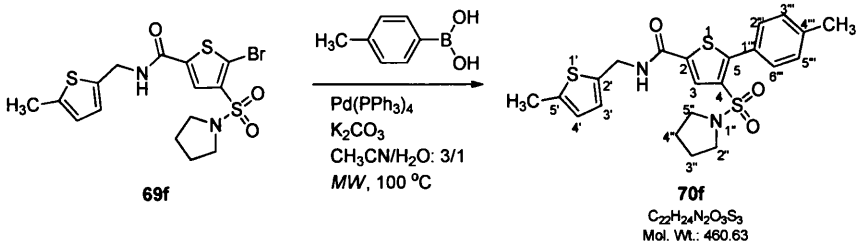
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (1H, s, H^3), 6.82 (1H, d, *J* = 3.6 Hz, $H^{3'}$), 6.61 (1H, d, *J* = 3.6 Hz, $H^{4'}$), 6.45 (1H, t, *J* = 5.2 Hz, NH), 4.66 (2H, d, *J* = 5.2 Hz, CH_2NH), 3.38 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.45 (3H, s, -CH_3), 1.88 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5 (CONH), 140.6 ($C^{5'}$), 140.1 (C^2), 138.2 (C^4), 137.2 ($C^{2'}$), 128.2 (C^3), 126.9 ($C^{3'}$), 125.1 ($C^{4'}$), 120.7 (C^5), 48.1 ($C^{2''}$& $C^{5''}$), 39.1 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$), 15.4 (CH_3).

LRMS (ESI): *m/z* = 447.39 $[C_{15}H_{17}{}^{79}BrN_2O_3S_3\text{-}H]^-$ (90%), 449.33 $[C_{15}H_{17}{}^{81}BrN_2O_3S_3\text{-}H]^-$ (100%).

HRMS (TOF MS ES): Found 448.9672, calculated for $C_{15}H_{18}{}^{79}BrN_2O_3S_3$ 448.9663 $[M+H]^+$.

6.6.6.2 Synthesis of Compound 70f



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **69f** (150 mg, 0.33 mmol, 1 equiv.), *p*-tolylboronic acid (54 mg, 0.40 mmol, 1.2 equiv.) and K_2CO_3 (92 mg, 0.67 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (39 mg, 0.03 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→97:3) as eluent to afford compound **70f**.

Compound **70f** (122 mg, 80% yield) was obtained as a light yellow solid.

R_f = 0.511 (Silica gel, Ethyl acetate/DCM: 1/9).

mp = 133.5 – 133.9 °C.

IR (ν_{max}/cm^{-1}): 809, 1012, 1137, 1305, 1542, 1626, 2877 and 3354.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, s, H^3), 7.50 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 6.82 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.61 (1H, d, *J* = 3.2 Hz, $H^{4'}$), 6.40 (1H, t, *J* = 5.6 Hz, NH), 4.69 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.96 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.46 (3H, s, thiophene-CH_3), 2.40 (3H, s, Ph-CH_3), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4 (CONH), 151.5 (C^5), 140.5 ($C^{5'}$), 140.2 ($C^{4'''}$), 137.5 ($C^{2'}$), 137.2 (C^2), 134.7 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.3 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$), 126.7 ($C^{3'}$), 125.1 ($C^{4'}$), 47.5 ($C^{2''}$& $C^{5''}$), 39.1 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (Ph-CH_3), 15.4 (thiophene-CH_3).

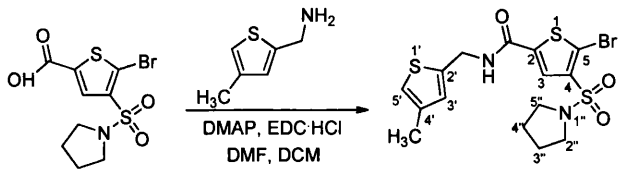
LRMS (ESI): *m/z* = 461.48 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 461.1014, calculated for $C_{22}H_{25}N_2O_3S_3$ 461.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_3S_3$: C, 57.36; H, 5.25; N, 6.08% Found: C, 57.49; H, 4.99; N, 5.96%.

6.6.7 Preparation of Compound 70g

6.6.7.1 Synthesis of Amide 69g



62

69g

$C_{15}H_{17}BrN_2O_3S_3$
Mol. Wt.: 449.41

A solution of (4-methylthiophen-2-yl)methanamine (93 mg, 0.73 mmol, 1.1 equiv.) in DCM (1 mL) was added dropwise to stirred solution of **62** (226 mg, 0.66 mmol, 1 equiv.), EDC hydrochloride (153 mg, 0.80 mmol, 1.2 equiv.) & DMAP (8.1 mg, 0.07 mmol, 0.1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **69g** was filtered off and dried overnight over $CaCl_2$.

Compound **69g** (202 mg, 68% yield) was obtained as a white solid.

R_f = 0.68 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 169.4 – 169.8 °C.

IR (ν_{max}/cm^{-1}): 740, 848, 1012, 1139, 1313, 1407, 1543, 1657, 2920 and 3368.

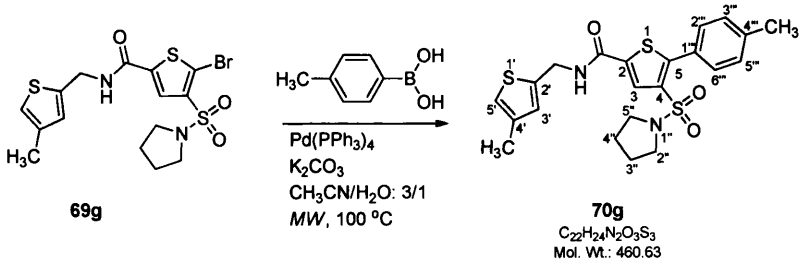
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (1H, s, H^3), 6.86 (1H, s, $H^{3'}$), 6.82 (1H, s, $H^{5'}$), 6.53 (1H, t, *J* = 5.2 Hz, NH), 4.69 (2H, d, *J* = 5.2 Hz,CH_2NH), 3.38 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.23 (3H, s, -CH_3), 1.87 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5 (CONH), 140.1 (C^2), 139.4 ($C^{2'}$), 138.2 ($C^{4'}$), 137.9 (C^4), 129.3 ($C^{5'}$), 128.2 (C^3), 121.0 ($C^{3'}$), 120.8 (C^5), 48.1 ($C^{2''}$& $C^{5''}$), 39.0 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$), 15.7 (CH_3).

LRMS (ESI): *m/z* = 446.96 [$C_{15}H_{17}{}^{79}BrN_2O_3S_3$-H]$^-$ (85%.), 448.96 [$C_{15}H_{17}{}^{81}BrN_2O_3S_3$-H]$^-$ (100%.)

HRMS (TOF MS ES): Found 448.9659, calculated for $C_{15}H_{18}{}^{79}BrN_2O_3S_3$ 448.9663 [M+H]$^+$.

6.6.7.2 Synthesis of Compound 70g



69g

70g
$C_{22}H_{24}N_2O_3S_3$
Mol. Wt.: 460.63

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **69g** (150 mg, 0.33 mmol, 1 equiv.), *p*-tolylboronic acid (54 mg, 0.40 mmol, 1.2 equiv.) and K_2CO_3 (92 mg, 0.67 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (39 mg, 0.03 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→24:1) as eluent to afford compound **70g**.

Compound **70g** (134 mg, 88% yield) was obtained as a light yellow solid.

R_f = 0.474 (Silica gel, Ethyl acetate/DCM: 1/9).

mp = 142.2 – 142.7 °C.

IR (ν_{max}/cm^{-1}): 749, 813, 1015, 1147, 1305, 1446, 1545, 1659, 2922 and 3364.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, s, H^3), 7.50 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 6.87 (1H, s, $H^{3'}$), 6.82 (1H, s, $H^{5'}$), 6.44 (1H, t, *J* = 5.6 Hz, NH), 4.72 (2H, d, *J* = 5.6 Hz, CH_2NH), 2.96 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, Ph-CH_3), 2.24 (3H, s, thiophene-CH_3), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (CONH), 151.6 (C^5), 140.2 ($C^{4'''}$), 139.7 ($C^{2'}$), 137.9 ($C^{4'}$), 137.1 (C^2), 134.7 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.4 ($C^{3'}$), 129.16 (C^3), 129.12 ($C^{3'''}$& $C^{5'''}$), 128.1 ($C^{1'''}$), 120.8 ($C^{5'}$), 47.5 ($C^{2''}$& $C^{5''}$), 39.0 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (Ph-CH_3), 15.8 (thiophene-CH_3).

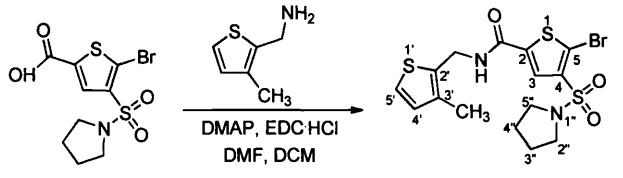
LRMS (ESI): *m/z* = 461.48 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 461.1022, calculated for $C_{22}H_{25}N_2O_3S_3$ 461.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_3S_3$: C, 57.36; H, 5.25; N, 6.08% Found: C, 57.29; H, 4.99; N, 6.01%.

6.6.8 Preparation of Compound 70h

6.6.8.1 Synthesis of Amide 69h



62

69h

$C_{15}H_{17}BrN_2O_3S_3$

Mol. Wt.: 449.41

A solution of (3-methylthiophen-2-yl)methanamine (95 mg, 0.74 mmol, 1.1 equiv.) in DCM (1 mL) was added dropwise to stirred solution of **62** (230 mg, 0.68 mmol, 1 equiv.), EDC hydrochloride (156 mg, 0.81 mmol, 1.2 equiv.) & DMAP (8.3 mg, 0.07 mmol, 0.1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **69h** was filtered off and dried overnight over $CaCl_2$.

Compound **69h** (235 mg, 77% yield) was obtained as a yellow solid.

R_f = 0.5 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 173.8 – 174.2 °C.

IR (ν_{max}/cm^{-1}): 798, 863, 1018, 1140, 1315, 1403, 1547, 1649, 2920 and 3353.

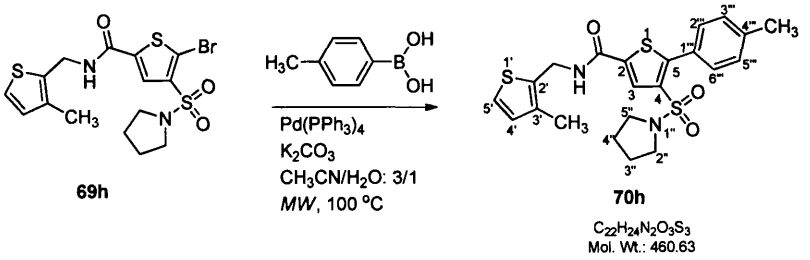
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (1H, s, H^3), 7.17 (1H, d, J = 5.2 Hz, $H^{5'}$), 6.84 (1H, d, J = 5.2 Hz, $H^{4'}$), 6.32 (1H, s, NH), 4.67 (2H, d, J = 5.2 Hz, CH_2NH), 3.38 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.27 (3H, s, CH_3), 1.89 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5 (CONH), 140.1 (C^2), 138.3 (C^4), 136.1 ($C^{2'}$), 132.4 ($C^{3'}$), 130.4 ($C^{5'}$), 128.1 (C^3), 124.1 ($C^{4'}$), 120.7 (C^5), 48.1 ($C^{2''}$& $C^{5''}$), 36.9 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$), 13.8 (CH_3).

LRMS (ESI): *m/z* = 446.97 $[C_{15}H_{17}{}^{79}BrN_2O_3S_3\text{-}H]^-$ (95%.), 448.98 $[C_{15}H_{17}{}^{81}BrN_2O_3S_3\text{-}H]^-$ (100%).

HRMS (TOF MS ES): Found 448.9673, calculated for $C_{15}H_{18}{}^{79}BrN_2O_3S_3$ 448.9663 $[M+H]^+$.

6.6.8.2 Synthesis of Compound 70h



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **69h** (150 mg, 0.33 mmol, 1 equiv.), *p*-tolylboronic acid (54 mg, 0.40 mmol, 1.2 equiv.) and K_2CO_3 (92 mg, 0.67 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (39 mg, 0.03 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→24:1) as eluent to afford compound **70h**.

Compound **70h** (32 mg, 21% yield) was obtained as a light yellow solid.

R_f = 0.43 (Silica gel, Ethyl acetate/DCM: 1/9).

mp = 231.3 – 231.8 °C.

IR (ν_{max}/cm^{-1}): 727, 867, 1017, 1039, 1305, 1447, 1543, 1657, 2879 and 3362.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (1H, s, H^3), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$ & $H^{6'''}$), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$ & $H^{5'''}$), 7.18 (1H, d, J = 5.2 Hz, $H^{5'}$), 6.85 (1H, d, J = 5.2 Hz, $H^{4'}$), 6.19 (1H, t, J = 5.2 Hz, NH), 4.71 (2H, d, J = 5.2 Hz, CH_2NH), 2.96 (4H, t, J = 6.8 Hz, $H^{2''}$ & $H^{5''}$), 2.40 (3H, s, Ph-CH_3), 2.27 (3H, s, thiophene-CH_3), 1.60 (4H, m, $H^{3''}$ & $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9 (CONH), 151.7 (C^5), 139.8 ($C^{4'''}$), 136.1 ($C^{2'}$), 130.5 ($C^{4'}$), 136.8 (C^2), 130.26 (C^4), 130.23 ($C^{2'''}$ & $C^{6'''}$), 132.7 ($C^{3'}$), 129.3 (C^3), 129.1 ($C^{3'''}$ & $C^{5'''}$), 128.5 ($C^{1'''}$), 124.0 ($C^{5'}$), 47.5 ($C^{2''}$ & $C^{5''}$), 36.9 (CH_2NH), 25.5 ($C^{3''}$ & $C^{4''}$), 21.5 (Ph-CH_3), 13.8 (thiophene-CH_3).

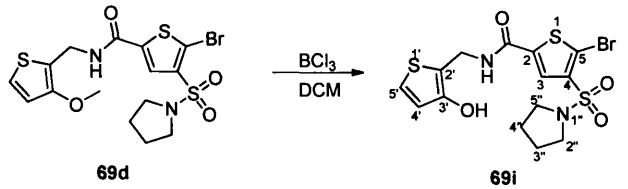
LRMS (ESI): *m/z* = 460.95 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 461.1046, calculated for $C_{22}H_{25}N_2O_3S_3$ 461.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_3S_3$: C, 57.36; H, 5.25; N, 6.08% Found: C, 57.56; H, 5.17; N, 5.92%.

6.6.9 Preparation of Compound 70i

6.6.9.1 Synthesis of Alcohol 69i



69d

69i
$C_{14}H_{15}BrN_2O_4S_3$
Mol. Wt.: 451.38

Boron trichloride solution (1 M) in DCM (0.645 mL, 0.645 mmol, 3 equiv.) was added to a solution of **69d** (100 mg, 0.22 mmol, 1 equiv.) in anhydrous DCM (2 mL) at 0 °C under N_2. The reaction mixture was stirred for 16 h at RT and was poured into 1 mL of ice water. The organic fraction was separated, dried over $MgSO_4$ and evaporated *in vacuo*. The compound **69i** was purified by column chromatography on silica gel using DCM and ethyl acetate (10:0 → 49:1) as eluent.

Compound **69i** (57 mg, 57% yield) was obtained as a yellow solid.

R_f = 0.264 (Silica gel, Ethyl acetate/DCM: 1:19).

mp = 202.5 – 202.8 °C.

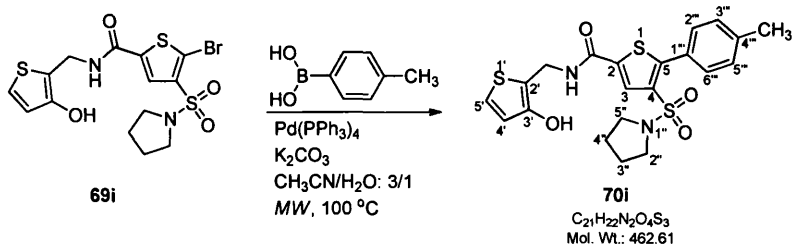
IR (ν_{max}/cm^{-1}): 763, 858, 1139, 1303, 1557, 1614, 1739, 2956, 3167 and 3336.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (1H, s, OH), 7.71 (1H, s, H^3), 7.42 (1H, t, J = 6.0 Hz, NH), 7.03 (1H, d, J = 5.6 Hz $H^{4'}$), 6.70 (1H, d, J = 5.6 Hz, $H^{5'}$), 4.48 (2H, d, J = 6.0 Hz,CH_2NH), 3.40 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.89 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3 (CONH), 153.7 ($C^{3'}$), 138.8 (C^2), 129.3 (C^3), 123.0 ($C^{4'}$), 121.5 (C^4), 121.4 ($C^{5'}$), 118.2 (C^5), 111.7 ($C^{2'}$), 48.2 ($C^{2''}$& $C^{5''}$), 35.3 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 449.51 $[C_{14}H_{15}{}^{79}BrN_2O_4S_3\text{-}H]^-$ (98%), 451.65 $[C_{14}H_{15}{}^{81}BrN_2O_4S_3\text{-}H]^-$ (100%).

6.6.9.2 Synthesis of Compound 70i



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (16 mL) followed by the addition of **69i** (256 mg, 0.567 mmol, 1 equiv.), *p*-tolylboronic acid (93 mg, 0.68 mmol, 1.2 equiv.) and K_2CO_3 (157 mg, 1.13 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (66 mg, 0.057 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (19:1→7:3) as eluent to afford compound **70i**.

Compound **70i** (100 mg, 38% yield) was obtained as a white solid.

R_f = 0.42 (Silica gel, Ethyl acetate/*n*-hexane: 1/1).

mp = 99.2 – 99.6 °C.

IR (ν_{max}/cm^{-1}): 759, 869, 1013, 1134, 1304, 1444, 1542, 1621, 2971 and 3342.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.06 (1H, s, OH), 7.94 (1H, s, H^3), 7.60 (1H, t, J = 6.8 Hz, NH), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.25 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.02 (1H, d, J = 5.6 Hz, $H^{5'}$), 6.70 (1H, d, J = 5.6 Hz, $H^{4'}$), 4.49 (2H, d, J = 6.8 Hz, CH_2NH), 2.97 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.41 (3H, s, CH_3), 1.61 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 163.3 (CONH), 153.7 ($C^{3'}$), 152.5 (C^5), 140.5 ($C^{4'''}$), 135.8 (C^2), 134.6 (C^4), 130.6 (C^3), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.2 ($C^{3'''}$& $C^{5'''}$), 128.2 ($C^{1'''}$), 122.8 ($C^{5'}$), 121.4 ($C^{4'}$), 112.0 ($C^{2'}$), 47.6 ($C^{2''}$& $C^{5''}$), 35.2 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

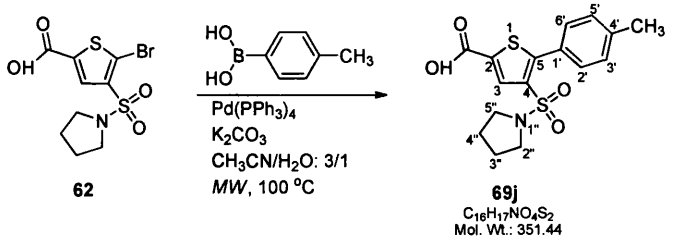
LRMS (ESI): *m/z* = 461.68 [M-H]$^-$, 100%.

HRMS (TOF MS ES): Found 461.0680, calculated for $C_{21}H_{23}N_2O_4S_3$ 461.0663 [M+H]$^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_3$: C, 54.52; H, 4.79; N, 6.06% Found: C, 54.60; H, 4.78; N, 5.95%.

6.6.10 Preparation of Compound 70j

6.6.10.1 Synthesis of Carboxylic Acid Derivative 69j



62

69j
$C_{16}H_{17}NO_4S_2$
Mol. Wt.: 351.44

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (17 mL) followed by the addition of **62** (320 mg, 0.94 mmol, 1 equiv.), *p*-tolylboronic acid (158 mg, 1.13 mmol, 1.2 equiv.) and K_2CO_3 (260 mg, 1.88 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (109 mg, 0.094 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 7 minutes. The reaction mixture was evaporated *in vacuo*, quenched with water and extracted with DCM. 1 M HCl solution was added dropwise to the ice cold aqueous phase. The resulting precipitate **69j** was filtered off and dried over $CaCl_2$ overnight.

Compound **69j** (215 mg, 65% yield) was obtained as a white solid.

R_f = 0.12 (Silica gel, Ethyl acetate/methanol: 4/1).

mp = 153.9 – 154.4 °C.

IR (ν_{max}/cm^{-1}): 749, 810, 877, 1012, 1066, 1137, 1347, 1446, 1535, 1699 and 2961.

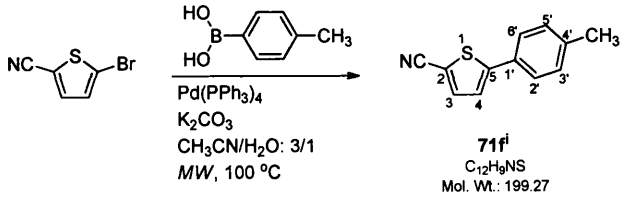
^{1}H NMR (400 MHz, DMSO): δ 13.81 (1H, br. s, COOH), 7.82 (1H, s, H^3), 7.51 (2H, d, J = 8.0 Hz, $H^{2'}$& $H^{6'}$), 7.30 (2H, d, J = 8.0 Hz, $H^{3'}$& $H^{5'}$), 2.97 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.38 (3H, s, CH_3), 1.62 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, DMSO): δ 161.7 (CONH), 152.0 (C^5), 139.7 ($C^{4'}$), 134.3 (C^2), 133.3 (C^3), 133.2 (C^4), 129.9 ($C^{2'}$& $C^{6'}$), 128.8 ($C^{3'}$& $C^{5'}$), 127.9 ($C^{1'}$), 47.3 ($C^{2''}$& $C^{5''}$), 24.9 ($C^{3''}$& $C^{4''}$), 20.9 (CH_3).

LRMS (ESI): *m/z* = 350.95 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 352.0669, calculated for $C_{16}H_{18}NO_4S_2$ 352.0677 $[M+H]^+$.

6.6.10.2 Synthesis of Derivative 71f[i]



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (14 mL) followed by the addition of 5-bromothiophene-2-carbonitrile (360 mg, 1.91 mmol, 1 equiv.), *p*-tolylboronic acid (322 mg, 2.3 mmol, 1.2 equiv.) and K_2CO_3 (529 mg, 3.83 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (221 mg, 0.19 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was cooled to 0 °C and the resulting precipitate **71f[i]** collected by filtration dried overnight over $CaCl_2$.

Compound **71f[i]** (304 mg, 80% yield) was obtained as a light yellow solid.

R_f = 0.73 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 105.8 – 106.1 °C.

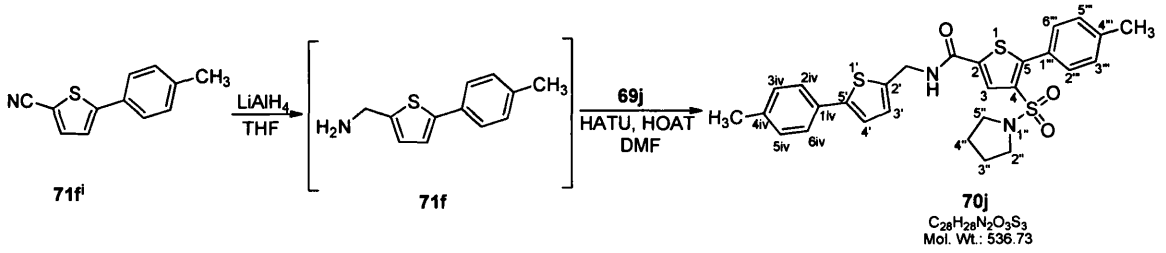
IR (ν_{max}/cm^{-1}): 801, 1053, 1250, 1313, 1442, 1503 and 2219.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 4.0 Hz, H^3), 7.51 (2H, d, J = 8.4 Hz, $H^{2'}$& $H^{6'}$), 7.29 – 7.26 (3H, m, H^4, $H^{3'}$& $H^{5'}$), 2.42 (3H, s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.2 (C^5), 139.8 ($C^{4'}$), 138.5 (C^3), 130.1 ($C^{2'}$& $C^{6'}$), 129.7 ($C^{1'}$), 126.4 ($C^{3'}$& $C^{5'}$), 122.9 (C^4), 114.6 (CN), 107.8 (C^2), 21.4 (CH_3).

LRMS (ESI): *m/z* = 200.11 $[M+H]^+$, 100%.

6.6.10.3 Synthesis of Compound 70j



$LiAlH_4$ (1 M) in THF (5.04 mL, 5.04 mmol, 1.4 equiv) was added dropwise to the solution of **71f[i]** (718 mg, 3.6 mmol, 1 equiv.) in THF (36 mL) at 0 °C under N_2. After 2 h the reaction mixture was cooled to 0 °C then quenched by the very slow addition of $Na_2SO_4.10\ H_2O$ (5 gm). The quenched reaction mixture was filtered through the pad of celite and which was washed by ethyl acetate. The filtrate and the washings were combined together, dried over $MgSO_4$ and

evaporated *in vacuo*. The crude mixture (**71f**) was partially purified by column chromatography using ethyl acetate and methanol (10:0→4:1) as eluent and its solution in (136 mg in 2 mL of DMF) was added dropwise to stirred solution of **69j** (196 mg, 0.56 mmol, 1 equiv.), HATU (276 mg, 0.73 mmol, 1.3 equiv.) & HOAT (99 mg, 0.73 mmol, 1.3 equiv.) in anhydrous DMF (5 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, evaporated *in vacuo* and quenched by the addition of water and extracted with ethyl acetate. The combined extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography on silica gel 60 (ethyl acetate/*n*-Hexane 1:9→1:1) to afford compound **70j**.

Compound **70j** (90 mg, 30% yield) was obtained as a light yellow solid.

R_f = 0.55 (Silica gel, Ethyl acetate/DCM: 1/9).

mp = 227.8 – 228.2 °C.

IR (ν_{max}/cm^{-1}): 881, 1014, 1077, 1137, 1176, 1282, 1448, 1538, 1651, 2919 and 3347.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (1H, s, H^3), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.46 (2H, d, J = 8.0 Hz, H^{2iv} & H^{6iv}), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.17 (2H, d, J = 8.0 Hz, H^{3iv} & H^{5iv}), 7.12 (1H, d, J = 3.6 Hz, $H^{4'}$), 7.00 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.62 (1H, t, J =5.6 Hz, NH), 4.77 (2H, d, J = 5.6 Hz, CH_2NH), 2.95 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, $C^{4'''}$-CH_3), 2.36 (3H, s, C^{4iv}-CH_3), 1.57 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (CONH), 151.7 (C^5), 145.0 ($C^{5'}$), 140.2 ($C^{4'''}$), 139.0 ($C^{2'}$), 131.5 (C^{1iv}), 137.0 (C^2), 134.6 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 129.1 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$), 129.5 (C^3), 129.7 (C^{3iv} & C^{5iv}), 137.7 (C^{4iv}), 125.8 (C^{2iv} & C^{6iv}), 122.4 ($C^{4'}$), 127.7 ($C^{3'}$), 47.5 ($C^{2''}$& $C^{5''}$), 39.1 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (C^{4iv}-CH_3), 21.3 ($C^{4'''}$-CH_3).

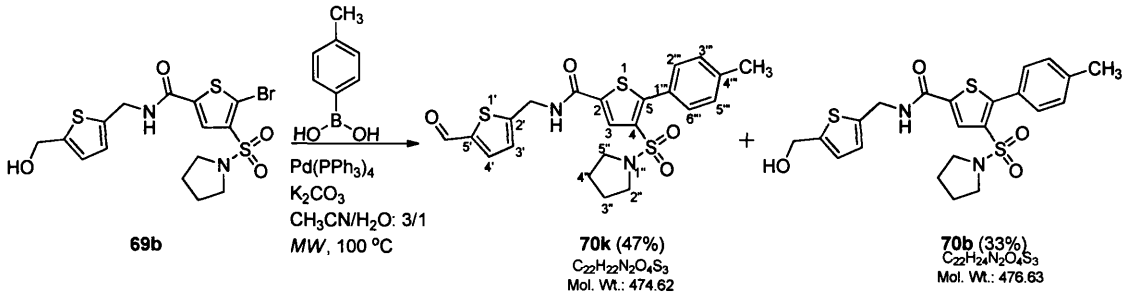
LRMS (ESI): *m/z* = 535.67 $[M-H]^-$, 100%.

HRMS (TOF MS ES): Found 537.1340, calculated for $C_{28}H_{29}N_2O_3S_3$ 537.1353 $[M+H]^+$.

Elem. Anal. Calculated for $C_{28}H_{28}N_2O_3S_3$: C, 62.66; H, 5.26; N, 5.22% Found: C, 62.78; H, 5.18; N, 5.09%.

6.6.11 Preparation of Compound 70k

6.6.11.1 Synthesis of Compound 70k



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **69b** (150 mg, 0.32 mmol, 1 equiv.), *p*-tolylboronic acid (53 mg, 0.39 mmol, 1.2 equiv.) and K_2CO_3 (89 mg, 0.65 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (37 mg, 0.032 mmol, 0.1 equiv.) was then added. Air was passed through the reaction mixture for 30 minutes and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (20:1→7:3) as eluent to afford compound **70k**.

Compound **70k** (94 mg, 62% yield) was obtained as a light yellow solid.

R_f = 0.56 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 71.2 – 71.4 °C.

IR (ν_{max}/cm^{-1}): 812, 1137, 1320, 1445, 1542, 1650, 2960 and 3312.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.85 (1H, s, CHO), 7.92 (1H, s, H^3), 7.49 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.64 (1H, d, J = 4.0 Hz, $H^{4'}$), 7.29 (1H, t, J = 6.0 Hz, NH), 7.24 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.07 (1H, d, J = 4.0 Hz, $H^{3'}$), 4.82 (2H, d, J = 6.0 Hz, CH_2NH), 2.94 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.41 (3H, s, CH_3), 1.59 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 182.9 (CHO), 160.9 (CONH), 152.1 (C^5),152.0 ($C^{2'}$), 143.3 ($C^{5'}$), 140.4 ($C^{4'''}$), 136.7 ($C^{4'}$), 136.4 (C^2), 134.2 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 128.2 ($C^{1'''}$), 129.8 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 127.1 ($C^{3'}$), 47.6 ($C^{2''}$& $C^{5''}$), 39.1 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

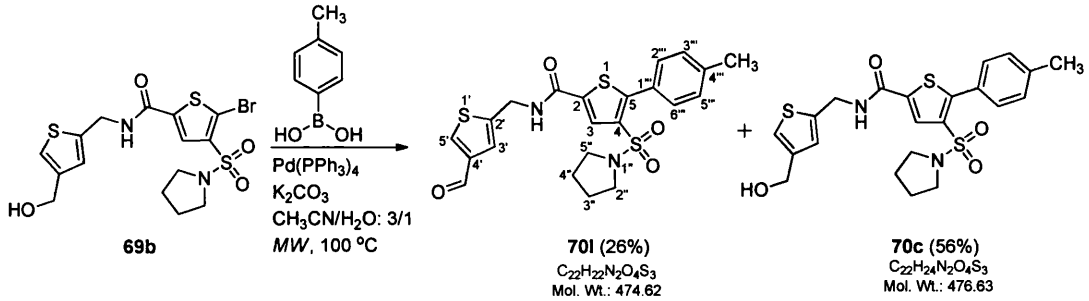
LRMS (ESI): *m/z* = 475.54 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 473.0667, calculated for $C_{22}H_{21}N_2O_4S_3$ 473.0663 $[M-H]^-$.

Elem. Anal. Calculated for $C_{22}H_{22}N_2O_4S_3$: C, 55.67; H, 4.67; N, 5.90% Found: C, 55.72; H, 4.55; N, 5.95%.

6.6.12 Preparation of Compound 70l

6.6.12.1 Synthesis of Compound 70l



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (5 mL) followed by the addition of **69b** (97 mg, 0.21 mmol, 1 equiv.), *p*-tolylboronic acid (34 mg, 0.25 mmol, 1.2 equiv.) and K_2CO_3 (58 mg, 0.42 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (24 mg, 0.21 mmol, 0.1 equiv.) was then added. Air was passed through the reaction mixture for 30 minutes and the reaction mixture was irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (9:1→7:3) as eluent to afford compound **70l**.

Compound **70l** (19 mg, 20% yield) was obtained as a white solid.

R_f = 0.6 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 66.4 – 66.7 °C.

IR (ν_{max}/cm^{-1}): 868, 1010, 1145, 1307, 1444, 1536, 1679, 1793, 2966 and 3322.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.83 (1H, s, CHO), 8.02 (1H, s, $H^{5'}$), 7.87 (1H, s, H^3), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.47 (1H, s, $H^{3'}$), 7.24 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.09 (1H, t, J = 6.0 Hz, NH), 4.77 (2H, d, J = 6.0 Hz, CH_2NH), 2.95 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.41 (3H, s, CH_3), 1.58 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 185.0 (CHO), 160.8 (CONH), 152.0 (C^5), 143.4 ($C^{2'}$), 142.8 ($C^{4'}$), 140.3 ($C^{4'''}$), 137.2 ($C^{5'}$), 136.6 (C^2), 134.1 (C^4), 130.2 ($C^{2'''}$& $C^{6'''}$), 130.1 ($C^{1'''}$), 129.7 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 124.0 ($C^{3'}$), 47.6 ($C^{2''}$& $C^{5''}$), 38.6 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

LRMS (ESI): *m/z* = 475.58 $[M+H]^+$, 100%.

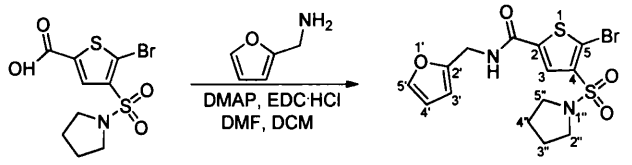
HRMS (TOF MS ES): Found 473.0657, calculated for $C_{22}H_{21}N_2O_4S_3$ 473.0663 $[M-H]^-$.

Elem. Anal. Calculated for $C_{22}H_{22}N_2O_4S_3$: C, 55.67; H, 4.67; N, 5.90% Found:C, 55.55; H, 4.68; N, 5.99%.

6.7 Preparation of Focused Library "E"

6.7.1 Synthesis of Compound 73a

6.7.1.1 Synthesis of Amide 72a



62

72a
$C_{14}H_{15}BrN_2O_4S_2$
Mol. Wt.: 419.31

Furfurylamine (0.043 mL, 0.49 mmol, 1.1 equiv.) was added dropwise to stirred solution of **62** (150 mg, 0.44 mmol, 1 equiv.), EDC hydrochloride (101 mg, 0.53 mmol, 1.2 equiv.) & DMAP (5.39 mg, 0.044 mmol, 0.1 equiv.) in anhydrous DCM (5 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 4 h, quenched by the addition of water and extracted with DCM. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (4:1→7:3) as eluent to afford compound **72a**.

Compound **72a** (127 mg, 69% yield) was obtained as a light yellow solid.

R_f = 0.61 (Silica gel, Ethyl acetate).

mp = 181.1 – 181.8 °C.

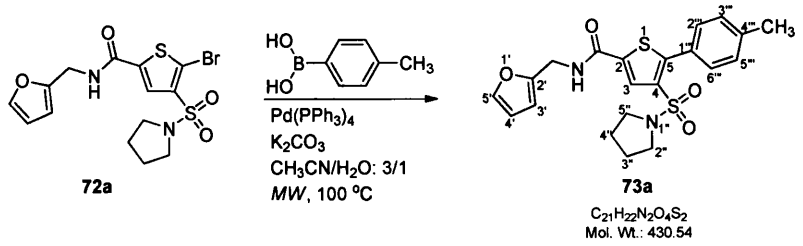
IR (ν_{max}/cm^{-1}): 739, 865, 1014, 1140, 1314, 1406, 1548 and 1625.

^{1}H NMR (400 MHz, DMSO): δ 9.31 (1H, t, *J* = 5.6 Hz, NH), 8.07 (1H, s, H^3), 7.61 (1H, d, *J* = 2.0 Hz, $H^{5'}$), 6.42 (1H, dd, *J* = 3.2, 2.0 Hz, $H^{4'}$), 6.34 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 4.44 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.28 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.78 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, DMSO): δ 159.1 (CONH), 151.3 ($C^{2'}$), 142.4 ($C^{5'}$), 140.7 (C^2), 136.9 (C^4), 128.7 (C^3), 120.6 (C^5), 110.5 ($C^{4'}$), 107.6 ($C^{3'}$), 47.8 ($C^{2''}$& $C^{5''}$), 35.9 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 419.01 $[C_{14}H_{15}{}^{79}BrN_2O_4S_2+H]^+$ (90%), 421.04 $[C_{14}H_{15}{}^{81}BrN_2O_4S_2+H]^+$ (100%).

6.7.1.2 Synthesis of Compound 73a



A 5 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (4 mL) followed by the addition of **72a** (102 mg, 0.24 mmol, 1 equiv.), *p*-tolylboronic acid (40 mg, 0.29 mmol, 1.2 equiv.) and K_2CO_3 (67 mg, 0.49 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (28 mg, 0.024 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **73a**.

Compound **73a** (77 mg, 75% yield) was obtained as a light yellow solid.

R_f = 0.72 (Silica gel, Ethyl acetate).

mp = 167.1 – 167.3 °C.

IR (ν_{max}/cm^{-1}): 815, 874, 1120, 1182, 1306, 1448, 1553, 1661, 1736 and 3359.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (1H, s, H^3), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$ & $H^{6'''}$), 7.39 (1H, d, J = 2.0 Hz, $H^{5'}$), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$ & $H^{5'''}$), 6.32 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.35 (1H, dd, J = 3.2, 2.0 Hz, $H^{4'}$), 6.47 (1H, t, J = 5.6 Hz, NH), 4.51 (2H, d, J = 5.6 Hz, CH_2NH), 2.96 (4H, t, J = 4.0 Hz, $H^{2''}$ & $H^{5''}$), 2.40 (3H, s, CH_3), 1.60 (4H, m, $H^{3''}$ & $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.7 (CONH), 151.6 (C^5), 150.6 ($C^{2'}$), 142.6 ($C^{5'}$), 140.2 ($C^{4'''}$), 137.0 (C^2), 134.7 (C^4), 130.2 ($C^{2'''}$ & $C^{6'''}$), 129.4 (C^3), 129.1 ($C^{3'''}$ & $C^{5'''}$), 128.4 ($C^{1'''}$), 110.7 ($C^{4'}$), 108.2 ($C^{3'}$), 47.5 ($C^{2''}$ & $C^{5''}$), 37.0 (CH_2NH), 25.5 ($C^{3''}$ & $C^{4''}$), 21.5 (CH_3).

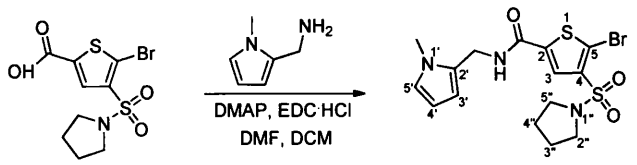
LRMS (ESI): *m/z* = 431.28 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 431.1112, calculated for $C_{21}H_{23}N_2O_4S_2$ 431.1099 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_2$: C, 58.58; H, 5.15; N, 6.51% Found:C, 58.46; H, 5.15; N, 6.40%.

6.7.2 Synthesis of Compound 73b

6.7.2.1 Synthesis of Amide 72b



Cold solution of (1-methyl-1H-pyrrol-2-yl) methylamine (65 mg, 0.57 mmol, 1.1 equiv.) in DCM (1 mL) was added dropwise to stirred solution of **62** (177 mg, 0.52 mmol, 1 equiv.), EDC hydrochloride (120 mg, 0.62 mmol, 1.2 equiv.) & DMAP (6.36 mg, 0.052 mmol, 0.1 equiv.) in anhydrous DCM (5 mL) at 0 °C and under N_2. The mixture was stirred at room temperature for 2 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was separated, dried on $MgSO_4$ and evaporated *in vacuo* to give compound **72b**.

Compound **72b** (178 mg, 79% yield) was obtained as a brown solid.

R_f = 0.36 (Silica gel, *n*-hexane/Ethyl acetate: 1/1).

mp = 162.4 – 162.6 °C.

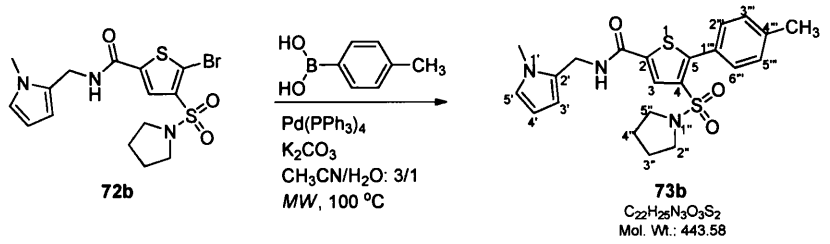
IR (ν_{max}/cm^{-1}): 866, 1014, 1148, 1216, 1351, 1548, 1618, 1737, 2969 and 3240.

^{1}H NMR (400 MHz, MeOD): δ 7.88 (1H, s, H^3), 6.61 (1H, t, J = 2.4 Hz, $H^{5'}$), 6.06 (1H, m, $H^{3'}$), 5.96 (1H, t, J = 3.2 Hz, $H^{4'}$), 4.50 (2H, s, CH_2NH), 3.69 (3H, s, NCH_3), 3.36 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.85 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, MeOD): δ 161.5 (CONH), 141.7 (C^2), 139.2 (C^4), 130.0 (C^3), 129.2 ($C^{2'}$), 123.8 ($C^{5'}$), 121.6 (C^5), 110.0 ($C^{3'}$), 107.7 ($C^{4'}$), 49.0 ($C^{2''}$& $C^{5''}$), 36.3 (CH_2NH), 33.8 (NCH_3), 26.3 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 430.41 [$C_{15}H_{18}{}^{79}BrN_3O_3S_2$-H]$^-$ (90%), 432.43 [$C_{15}H_{18}{}^{81}BrN_3O_3S_2$-H]$^-$ (100%).

6.7.2.2 Synthesis of Compound 73b



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (6 mL) followed by the addition of **72b** (164 mg, 0.38 mmol, 1 equiv.), *p*-tolylboronic acid (77 mg, 0.57 mmol, 1.5 equiv.) and K_2CO_3 (105 mg, 0.76 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (44 mg, 0.038 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 20 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on Na_2SO_4 and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1→3:2) as eluent to afford compound **73b**.

Compound **73b** (133 mg, 79% yield) was obtained as a brown solid.

R_f = 0.36 (Silica gel, *n*-hexane/Ethyl acetate: 1/1).

mp = 207.2 – 207.4 °C.

IR (ν_{max}/cm^{-1}): 723, 878, 1116, 1293, 1447, 1552, 1663, 1733, 2909 and 3373.

^{1}H NMR (400 MHz, DMSO): δ 9.09 (1H, t, *J* = 5.6 Hz, NH), 8.18 (1H, s, H^3), 7.46 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.28 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.00 (1H, d, *J* = 2.0 Hz, $H^{5'}$), 6.01 (1H, dd, *J* = 3.2, 2.0 Hz, $H^{3'}$), 5.93 (1H, t, *J* = 3.2 Hz, $H^{4'}$), 4.44 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.59 (3H, s, NCH_3), 2.96 (4H, t, *J* = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.37 (3H, s, CH_3), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, DMSO): δ 159.5 (CONH), 150.2 (C^5), 139.3 ($C^{4'''}$), 138.4 (C^2), 133.6 (C^4), 130.0 ($C^{2'''}$& $C^{6'''}$), 129.0 (C^3), 128.7 ($C^{3'''}$& $C^{5'''}$), 128.6 ($C^{1'''}$), 128.2 ($C^{2'}$), 122.5 ($C^{5'}$), 106.2 ($C^{4'}$), 108.5 ($C^{3'}$), 47.2 ($C^{2''}$& $C^{5''}$), 34.8 (CH_2NH), 33.8 (NCH_3), 24.8 ($C^{3''}$& $C^{4''}$), 20.8 (CH_3).

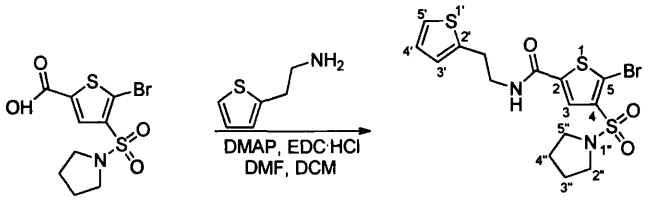
LRMS (ESI): *m/z* = 442.60 [M-H]$^-$, 100%.

HRMS (TOF MS ES): Found 444.1412, calculated for $C_{22}H_{26}N_3O_3S_2$ 444.1416 [M+H]$^+$.

Elem. Anal. Calculated for $C_{22}H_{25}N_3O_3S_2$: C, 59.57; H, 5.68; N, 9.47% Found: C, 59.51; H, 5.61; N, 9.40%.

6.7.3 Synthesis of Compound 73c

6.7.3.1 Synthesis of Amide 72c



62 **72c**
$C_{15}H_{17}BrN_2O_3S_3$
Mol. Wt.: 449.41

2-(thiophen-2-yl)ethanamine (0.103 mL, 0.88 mmol, 1.2 equiv.) was added dropwise to stirred solution of **62** (250 mg, 0.74 mmol, 1 equiv.), EDC hydrochloride (169 mg, 0.88 mmol, 1.2 equiv.) & DMAP (9 mg, 0.07 mmol, 0.1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **72c** was filtered off and dried overnight over $CaCl_2$.

Compound **72c** (206 mg, 62% yield) was obtained as a white solid.

R_f = 0.414 (Silica gel, Ethyl acetate/DCM: 1:19).

mp = 151.9 – 152.4 °C.

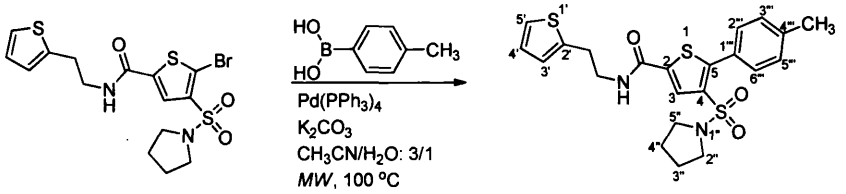
IR (ν_{max}/cm^{-1}): 848, 1010, 1138, 1318, 1405, 1548, 1659, 2916 and 3369.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, s, H^3), 7.18 (1H, d, J = 5.2 Hz, $H^{5'}$), 6.96 (1H, dd, J = 5.2, 3.6 Hz, $H^{4'}$), 6.87 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.34 (1H, t, J = 6.8 Hz, NH), 3.69 (2H, d, J = 6.8 Hz, $CH_2\mathit{CH_2}NH$), 3.39 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 3.14 (2H, d, J = 6.8 Hz, $\mathit{CH_2}CH_2NH$), 1.89 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9 (CONH), 140.7 ($C^{2'}$), 140.3 (C^2), 138.1 (C^4), 128.0 (C^3), 127.3 ($C^{4'}$), 125.7 ($C^{3'}$), 124.3 ($C^{5'}$), 120.5 (C^5), 48.1 ($C^{2''}$& $C^{5''}$), 41.5 ($CH_2\mathit{CH_2}NH$), 29.8 ($\mathit{CH_2}CH_2NH$), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 448.77 $[C_{15}H_{17}{}^{79}BrN_2O_3S_3+H]^+$ (90%), 450.80 $[C_{15}H_{17}{}^{81}BrN_2O_3S_3+H]^+$ (100%).

6.7.3.2 Synthesis of Compound 73c



72c

73c

$C_{22}H_{24}N_2O_3S_3$
Mol. Wt.: 460.63

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **72c** (150 mg, 0.33 mmol, 1 equiv.), *p*-tolylboronic acid (54 mg, 0.40 mmol, 1.2 equiv.) and K_2CO_3 (92 mg, 0.67 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (39 mg, 0.033 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0 →23:2) as eluent to afford compound **73c**.

Compound **73c** (125 mg, 82% yield) was obtained as a light brown solid.

R_f = 0.63 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 176.4 – 176.9 °C.

IR (ν_{max}/cm^{-1}): 812, 1011, 1139, 1320, 1443, 1546, 1629 and 3329.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, s, H^3), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.19 (1H, d, J = 5.2 Hz, $H^{5'}$), 6.97 (1H, dd, J = 5.2, 3.2 Hz, $H^{4'}$), 6.88 (1H, d, J = 3.2 Hz, $H^{3'}$), 6.30 (1H, t, J = 6.4 Hz, NH), 3.72 (2H, q, J = 6.4 Hz, CH_2CH_2NH), 3.16 (2H, t, J = 6.4 Hz, CH_2CH_2NH), 2.97 (4H, t, J = 6.4 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9 (CONH), 151.4 (C^5), 137.3 (C^2), 140.2 ($C^{4'''}$), 140.9 ($C^{2'}$), 129.1 ($C^{3'''}$& $C^{5'''}$), 130.2 ($C^{2'''}$& $C^{6'''}$), 127.3 ($C^{4'}$), 125.7 ($C^{3'}$), 124.3 ($C^{5'}$), 129.6 ($C^{1'''}$), 134.6 (C^4), 129.1 (C^3), 47.5 ($C^{2''}$& $C^{5''}$), 41.5 (CH_2CH_2NH), 30.0 (CH_2CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.6 (CH_3).

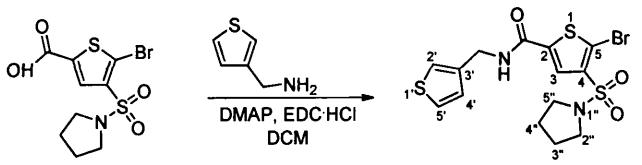
LRMS (ESI): *m/z* = 461.48 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 461.1005, calculated for $C_{22}H_{25}N_2O_3S_3$ 461.1027 $[M+H]^+$.

Elem. Anal. Calculated for $C_{22}H_{24}N_2O_3S_3$: C, 57.36; H, 5.25; N, 6.08% Found: C, 57.63; H, 5.20; N, 5.86%.

6.7.4 Synthesis of Compound 73d

6.7.4.1 Synthesis of Amide 72d



62

72d
$C_{14}H_{15}BrN_2O_3S_3$
Mol. Wt.: 435.38

A solution of thiophen-3-ylmethanamine (206 mg, 1.76 mmol, 1.2 equiv.) in DCM (1 mL) was added dropwise to stirred solution of **62** (500 mg, 1.47 mmol, 1 equiv.), EDC hydrochloride (338 mg, 1.76 mmol, 1.2 equiv.) & DMAP (18 mg, 0.15 mmol, 0.1 equiv.) in anhydrous DCM (4 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 18 h. The precipitated product **72d** was collected by filtration followed by rinse with cold DCM. The filtrate was partitioned between 1 M citric acid, saturated sodium bicarbonate solution and brine in three separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to afford the further **72d**. The product **72d** was combined and dried overnight over $CaCl_2$.

Compound **72d** (320 mg, 50% yield) was obtained as a white solid.

R_f = 0.6 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 192.8 – 193.3 °C.

IR (ν_{max}/cm^{-1}): 748, 865, 1015, 1139, 1404, 1549, 1649 and 3364.

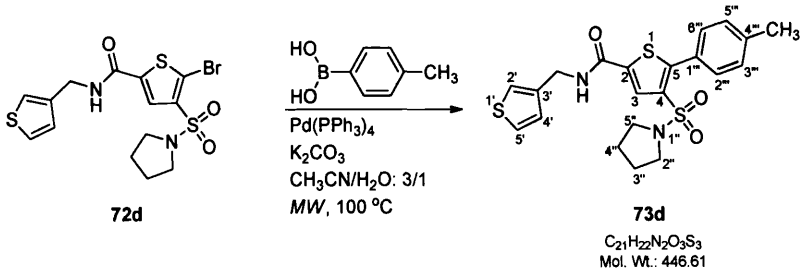
^{1}H NMR (400 MHz, DMSO): δ 9.33 (1H, t, J = 5.6 Hz, NH), 8.07 (1H, s, H^3), 7.50 (1H, dd, J = 4.8, 3.2 Hz, $H^{5'}$), 7.37 (1H, d, J = 3.2 Hz, $H^{2'}$), 7.08 (1H, d, J = 4.8 Hz, $H^{4'}$), 4.42 (2H, d, J = 5.6 Hz, CH_2NH), 3.28 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.77 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, DMSO): δ159.1 (CONH), 139.3 ($C^{3'}$), 141.0 (C^2), 136.9 (C^4), 128.5 (C^3), 127.6 ($C^{4'}$), 122.4 ($C^{2'}$), 126.4 ($C^{5'}$), 120.4 (C^5), 47.8 ($C^{2''}$& $C^{5''}$), 38.1 (CH_2NH), 24.9 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 435.09 $[C_{14}H_{15}{}^{79}BrN_2O_3S_3+H]^+$ (90%), 437.14 $[C_{14}H_{15}{}^{81}BrN_2O_3S_3+H]^+$ (100%).

HRMS (TOF MS ES): Found 434.9505, calculated for $C_{14}H_{16}{}^{79}BrN_2O_3S_3$ 434.9507 $[M+H]^+$.

6.7.4.2 Synthesis of Compound 73d



72d

73d

$C_{21}H_{22}N_2O_3S_3$

Mol. Wt.: 446.61

A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (5 mL) followed by the addition of **72d** (150 mg, 0.35 mmol, 1 equiv.), *p*-tolylboronic acid (56 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.03 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using *n*-hexane and ethyl acetate (9:1 →7:3) as eluent to afford compound **73d**.

Compound **73d** (117 mg, 75% yield) was obtained as a light yellow solid.

R_f = 0.32 (Silica gel, Ethyl acetate/Hexane: 2/3).

mp = 189.2 – 189.7 °C.

IR (ν_{max}/cm^{-1}): 872, 1015, 1139, 1300, 1546, 1648, 2922 and 3371.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (1H, s, H^3), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$ & $H^{6'''}$), 7.32 (1H, dd, J = 4.8, 3.2 Hz, $H^{5'}$), 7.25 (1H, d, J = 3.2 Hz, $H^{2'}$), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$ & $H^{5'''}$), 7.10 (1H, d, J = 4.8 Hz, $H^{4'}$), 6.54 (1H, t, J = 5.6 Hz, NH), 4.62 (2H, d, J = 5.6 Hz, CH_2NH), 2.95 (4H, t, J = 6.8 Hz, $H^{2''}$ & $H^{5''}$), 2.40 (3H, s, CH_3), 1.59 (4H, m, $H^{3''}$ & $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (CONH), 151.5 (C^5), 140.2 ($C^{4'''}$), 138.4 ($C^{3'}$), 137.3 (C^2), 134.6 (C^4), 130.2 ($C^{2'''}$ & $C^{6'''}$), 129.2 (C^3), 129.1 ($C^{3'''}$ & $C^{5'''}$), 128.4 ($C^{1'''}$), 127.6 ($C^{4'}$), 126.7 ($C^{5'}$), 123.1 ($C^{2'}$), 47.5 ($C^{2''}$ & $C^{5''}$), 39.2 (CH_2NH), 25.5 ($C^{3''}$ & $C^{4''}$), 21.5 (CH_3).

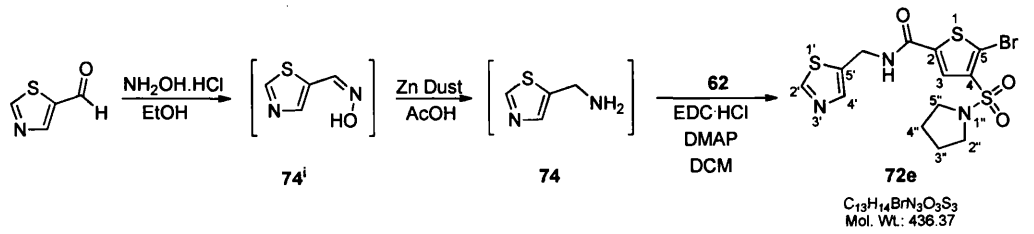
LRMS (ESI): *m/z* = 447.48 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 447.0858, calculated for $C_{21}H_{23}N_2O_3S_3$ 447.0871 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_3S_3$: C, 56.48; H, 4.97; N, 6.27% Found: C, 56.58; H, 5.01; N, 6.30%.

6.7.5 Synthesis of Compound 73e

6.7.5.1 Synthesis of Amide 72e



Hydroxylamine hydrochloride (599 mg, 8.62 mmol, 1.3 equiv.) was added to the solution of thiazole-5-carbaldehyde (750 mg, 6.63 mmol, 1 equiv.) in ethanol (13 mL) at room temperature. After 2 h the solvent was evaporated in *vacuo*. The solid **74^{i}**, isolated by filtration followed by washing with water was characterised by LC-MS. Then **74^{i}** (561 mg, 4.38 mmol, 1 equiv.) was dissolved in acetic acid (27 mL). Zn dust (1.432 g, 21.9 mmol, 5 equiv.) was added at room temperature and heated at 75 °C. After 2 h the reaction mixture was cooled down, filtered and evaporated *in vacuo*, passed through Isolute® SCX-2 column to afford **74**. The solution of **74** (185 mg, 1.62 mmol, 1.1 equiv.) in DCM (1 mL) and DMF (0.5 mL) was added dropwise to stirred solution of **62** (500 mg, 1.47 mmol, 1 equiv.), EDC hydrochloride (338 mg, 1.76 mmol, 1.2 equiv.) & DMAP (18 mg, 0.15 mmol, 0.1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature. After 16 h the precipitation formed, was filtered off and dried overnight over $CaCl_2$ to afford compound **72e**.

Compound **72e** (327 mg, 51% yield) was obtained as a light yellow solid.

R_f = 0.125 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 191.2 – 191.6 °C.

IR (ν_{max}/cm^{-1}): 864, 1017, 1137, 1314, 1406, 1549, 1654, 2927 and 3364.

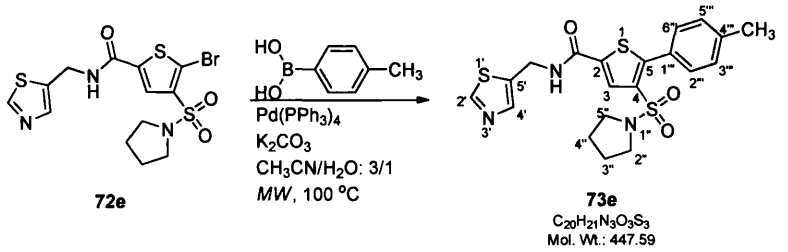
^{1}H NMR (400 MHz, DMSO): δ 9.48 (1H, t, *J* = 5.6 Hz, NH), 9.00 (1H, s, H$^{2'}$), 8.01 (1H, s, H^{3}), 7.03 (1H, s, H$^{4'}$), 4.66 (2H, d, *J* = 5.6 Hz, *CH*$_2$NH), 3.27 (4H, t, *J* = 6.8 Hz, H$^{2"}$& H$^{5"}$), 1.77 (4H, m, H$^{3"}$& H$^{4"}$).

^{13}C NMR (100 MHz, DMSO): δ 159.2 (CONH), 154.3 (C$^{2'}$), 141.9 (C$^{4'}$), 140.3 (C^{2}), 137.0 (C^{4}), 135.8 (C$^{5'}$), 128.8 (C^{3}), 120.8 (C^{5}), 47.8 (C$^{2"}$& C$^{5"}$), 35.0 (CH$_2$NH), 24.8 (C$^{3"}$& C$^{4"}$).

LRMS (ESI): *m/z* = 435.60 [$C_{13}H_{14}{}^{79}BrN_3O_3S_3$+H]$^+$ (90%), 437.61 [$C_{13}H_{14}{}^{81}BrN_3O_3S_3$+H]$^+$ (100%).

HRMS (TOF MS ES): Found 435.9454 calculated for $C_{13}H_{15}{}^{79}BrN_3O_3S_3$ 435.9459 [M+H]$^+$.

6.7.5.2 Synthesis of Compound 73e



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **72e** (150 mg, 0.34 mmol, 1 equiv.), *p*-tolylboronic acid (56 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (9:1→3:2) as eluent to afford compound **73e**.

Compound **73e** (134 mg, 88% yield) was obtained as a white solid.

R_f = 0.184 (Silica gel, Ethyl acetate/DCM: 1/1).

mp = 119.5 – 120.1 °C.

IR (ν_{max}/cm^{-1}): 720, 872, 1012, 1139, 1184, 1317, 1544, 1651 and 3342.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (1H, s, $H^{2'}$), 7.94 (1H, s, H^3), 7.92 (1H, s, $H^{4'}$), 7.56 (2H, d, J = 8.0 Hz, $H^{2'''}$&$H^{6'''}$), 7.31 (2H, d, J = 8.0 Hz, $H^{3'''}$&$H^{5'''}$), 4.92 (2H, d, J = 6.0 Hz, CH_2NH), 3.00 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.48 (3H, s, CH_3), 1.65 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.1 (CONH), 153.9 ($C^{2'}$), 152.1 (C^5), 142.6 ($C^{4'}$), 140.4 ($C^{4'''}$), 137.1 (C^2), 135.4 ($C^{5'}$), 134.5 (C^4), 130.3 ($C^{2'''}$& $C^{6'''}$), 129.8 (C^3), 129.2 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$), 47.7 ($C^{2''}$& $C^{5''}$), 35.9 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$), 21.6 (CH_3).

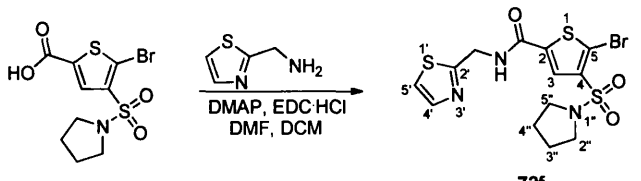
LRMS (ESI): *m/z* = 448.38 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 448.0823 calculated for $C_{20}H_{22}N_3O_3S_3$ 448.0823 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{21}N_3O_3S_3$: C, 53.67; H, 4.73; N, 9.39% Found: C, 53.67; H, 4.68; N, 9.20%.

6.7.6 Synthesis of Compound 73f

6.7.6.1 Synthesis of Amide 72f



62

72f
$C_{13}H_{14}BrN_3O_3S_3$
Mol. Wt.: 436.37

A solution of thiazol-2-yl methanamine (111 mg, 0.97 mmol, 1.1 equiv.) in DCM (1 mL) was added dropwise to stirred solution of **62** (300 mg, 0.88 mmol, 1 equiv.), EDC hydrochloride (203 mg, 1.06 mmol, 1.2 equiv.) & DMAP (10.8 mg, 0.09 mmol, 0.1 equiv.) in anhydrous DCM (6 mL per 1 mmol of **62**) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **72f** was filtered off and dried overnight over $CaCl_2$.

Compound **72f** (296 mg, 77% yield) was obtained as a yellow solid.

R_f = 0.363 (Silica gel, Ethyl acetate).

mp = 172.5 – 172.8 °C.

IR (ν_{max}/cm^{-1}): 748, 860, 1061, 1144, 1299, 1410, 1555, 1649, 2961 and 3193.

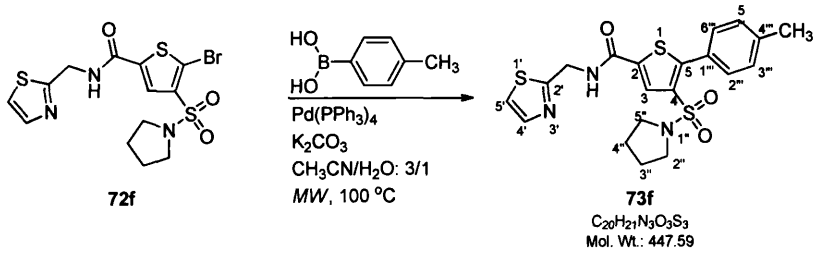
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (1H, s, H^3), 7.75 (1H, d, J = 3.2 Hz, $H^{4'}$), 7.34 (1H, d, J = 3.2 Hz, $H^{5'}$), 7.29 (1H, t, J = 5.6 Hz, NH), 4.90 (2H, d, J = 5.6 Hz, CH_2NH), 3.39 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.88(4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9 ($C^{2'}$), 159.9 (*C*ONH), 142.5 ($C^{4'}$), 139.5 (C^2), 138.4 (C^4), 128.7 (C^3), 121.1 (C^5), 120.1 ($C^{5'}$), 48.1 ($C^{2''}$& $C^{5''}$), 41.3 (CH_2NH), 25.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 434.37 $[C_{13}H_{14}{}^{79}BrN_3O_3S_3\text{-}H]^-$ (90%), 436.39 $[C_{13}H_{14}{}^{81}BrN_3O_3S_3\text{-}H]^-$ (100%).

HRMS (TOF MS ES): Found 435.9457 calculated for $C_{13}H_{15}{}^{79}BrN_3O_3S_3$ 435.9459 $[M+H]^+$.

6.7.6.2 Synthesis of Compound 73f



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **72f** (150 mg, 0.34 mmol, 1 equiv.), *p*-tolylboronic acid (56 mg, 0.41 mmol, 1.2 equiv.) and K_2CO_3 (95 mg, 0.69 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (40 mg, 0.034 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (9:1→1:1) as eluent to afford compound **73f**.

Compound **73f** (111 mg, 73% yield) was obtained as a light yellow solid.

R_f = 0.34 (Silica gel, Ethyl acetate/DCM: 7/3).

mp = 179.2 – 179.5 °C.

IR (ν_{max}/cm^{-1}): 744, 813, 873, 1013, 1139, 1306, 1544, 1657, 2878 and 3342.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (1H, s, H^3), 7.74 (1H, d, J = 3.2 Hz, $H^{4'}$), 7.50 (2H, d, J = 8.0 Hz, $H^{2'''}$&$H^{6'''}$), 7.33 (1H, d, J = 3.2 Hz, $H^{5'}$), 7.30 (1H, t, J = 5.6 Hz, NH), 7.23 (2H, d, J = 8.0 Hz, $H^{3'''}$&$H^{5'''}$), 4.94 (2H, d, J = 5.6 Hz, CH_2NH), 2.97 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.60 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1 ($C^{2'}$), 160.1 (CONH), 151.8 (C^5), 142.6 ($C^{4'}$), 140.2 ($C^{4'''}$), 136.5 (C^2), 134.8 (C^4), 130.2 ($C^{2'''}$&$C^{6'''}$), 129.9 (C^3), 129.1 ($C^{3'''}$& $C^{5'''}$), 128.4 ($C^{1'''}$), 119.9 ($C^{5'}$), 47.5 ($C^{2''}$& $C^{5''}$), 41.4 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.5 (CH_3).

LRMS (ESI): *m/z* = 448.70 $[M+H]^+$, 100%.

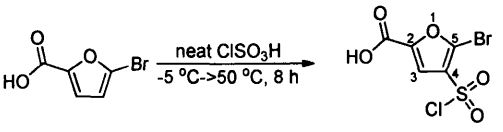
HRMS (TOF MS ES): Found 448.0830 calculated for $C_{20}H_{22}N_3O_3S_3$ 448.0823 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{21}N_3O_3S_3$: C, 53.67; H, 4.73; N, 9.39% Found: C, 53.67; H, 4.68; N, 9.41%.

6.8 Preparation of Focused Library "F"

6.8.1 Synthesis of Compound 78

6.8.1.1 Synthesis of Chlorosulfonated Derivative 75



75
$C_5H_2BrClO_5S$
Mol. Wt.: 289.49

Chlorosulfonic acid (4.21 mL, 62.8 mmol, 12 equiv.) was slowly added with stirring to 5-bromofuran-2-carboxylic acid (1 g, 5.24 mmol, 1 equiv.) at -5 °C and under N_2. The solution was stirred for 8 h at 50 °C and quenched by slowly pouring the reaction mixture into the ice with a constant N_2 flow. The mixture was extracted with DCM (x 3) and dried on $MgSO_4$. The evaporation of solvent *in vacuo* afforded solid compound **75**.

Compound **75** (1 gm, 66% yield) was obtained as a white solid.

R_f = 0.38 (Silica gel, Methanol/Ethyl acetate: 3/7).

mp = 127.4 –128.1 °C.

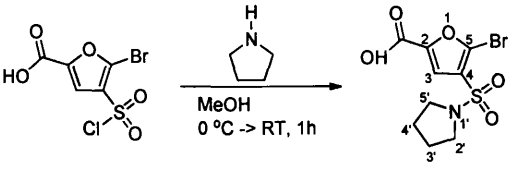
IR (ν_{max}/cm^{-1}): 755, 852, 1177, 1273, 1380, 1467, 1582, 1698 and 3125.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (1H, s, H^3).

^{13}C NMR (100 MHz, $CDCl_3$): δ159.5 (COOH), 145.4 (C^2), 133.8 (C^4), 132.6 (C^5), 119.2 (C^3).

LRMS (ESI): *m/z* = 287.48 $[C_5H_2{}^{79}Br^{35}ClO_5S\text{-}H]^-$ (70%), 289.42 $[C_5H_2{}^{81}Br^{35}ClO_5S\text{-}H]^-$ (100%), 291.44 $[C_5H_2{}^{81}Br^{37}ClO_5S\text{-}H]^-$ (30%).

6.8.1.2 Synthesis of Sulfonamide 76



75 **76**
$C_9H_{10}BrNO_5S$
Mol. Wt.: 324.15

Pyrrolidine (0.81 mL, 9.80 mmol, 3 equiv.) was added dropwise to stirred solution of **75** (946 mg, 3.27 mmol, 1 equiv.) in anhydrous methanol (6.5mL per 1 mmol of **75**) at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was partitioned between water and DCM. 1 M Hydrochloric acid (HCl) was added dropwise to the ice-cold aqueous phase. The resulting precipitate **76** was isolated by filtration and dried over $CaCl_2$ overnight.

Compound **76** (763 mg, 72% yield) was obtained white powder.

$\mathbf{R_f}$ = 0.4 (Silica gel, Methanol/Ethyl acetate: 2/3).

mp = 218.8 – 219.4 °C.

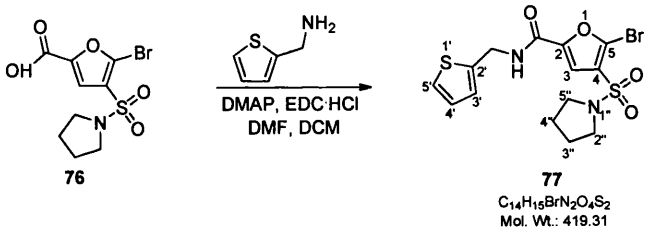
IR (ν_{max}/cm^{-1}): 760, 1008, 1187, 1336, 1466, 1567, 1688 and 3102.

^{1}H NMR (400 MHz, DMSO): δ 13.91 (1H, br. s, COOH), 7.50 (1H, s, H^3), 3.25 (4H, t, J = 6.8 Hz, $H^{2'}$& $H^{5'}$), 1.76 (4H, m, $H^{3'}$& $H^{4'}$).

^{13}C NMR (100 MHz, DMSO): δ 157.7 (COOH), 146.9 (C^2), 130.7 (C^4), 124.6 (C^5), 117.8 (C^3), 47.8 ($C^{2'}$& $C^{5'}$), 24.8 ($C^{3'}$& $C^{4'}$).

LRMS (ESI): *m/z* = 324.17 $[C_9H_{10}{}^{79}BrNO_5S+H]^+$ (88%), 326.19 $[C_9H_{10}{}^{81}BrNO_5S+H]^+$ (100%).

6.8.1.3 Ssynthesis of Amide 77



Thiophene-2-methylamine (0.25 mL, 2.40 mmol, 1.1 equiv.) was added dropwise to stirred solution of **76** (707 mg, 2.18 mmol, 1 equiv.), EDC hydrochloride (502 mg, 2.62 mmol, 1.2 equiv.) & DMAP (27 mg, 0.22 mmol, 0.1 equiv.) in anhydrous DCM (4 mL per 1 mmol of **76**) at 0 °C and under N_2. The mixture was stirred at room temperature for 16 h, quenched by the addition of 1 M citric acid. The organic phase was separated and partitioned between saturated sodium bicarbonate solution and brine in two separate wash steps. The organic phase was collected, dried with $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **77** was filtered off and dried overnight over $CaCl_2$.

Compound **77** (457 mg, 50% yield) was obtained as a light yellow solid.

$\mathbf{R_f}$ = 0.65 (Silica gel, Ethyl acetate).

mp = 176.2 – 176.5 °C.

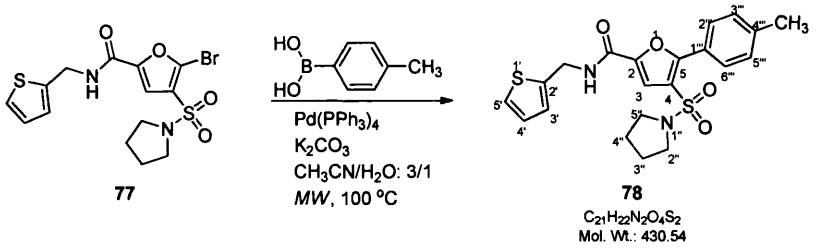
IR (ν_{max}/cm^{-1}): 849, 1152, 1294, 1351, 1475, 1523, 1593, 1650 and 3351 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (1H, s, H^3), 7.27 (1H, d, J = 5.2 Hz, $H^{5'}$), 7.06 (1H, d, J = 3.6 Hz, $H^{3'}$), 6.99 (1H, dd, J = 5.2, 3.6 Hz, $H^{4'}$), 6.64 (1H, br. s, NH), 4.78 (2H, d, J = 6.0 Hz, CH_2NH), 3.35 (4H, t, J = 6.8 Hz, $H^{2''}$& $H^{5''}$), 1.87 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9 (CONH), 148.5 (C^2), 139.7 ($C^{2'}$), 127.5 (C^4), 127.2 ($C^{4'}$), 126.9 ($C^{3'}$), 126.6 (C^5), 125.9 ($C^{5'}$), 116.0 (C^3), 48.2 ($C^{2''}$& $C^{5''}$), 38.1 (CH_2NH), 24.6 ($C^{3''}$& $C^{4''}$).

LRMS (ESI): *m/z* = 419.19 $[C_{14}H_{15}{}^{79}BrN_2O_4S_2+H]^+$ (87%), 421.14 $[C_{14}H_{15}{}^{81}BrN_2O_4S_2+H]^+$ (100%).

6.8.1.4 Synthesis of Compound 78



A 20 mL Pyrex microwave reactor vial was charged with 3:1 acetonitrile:water (8 mL) followed by the addition of **77** (150 mg, 0.36 mmol, 1 equiv.), *p*-tolylboronic acid (58 mg, 0.43 mmol, 1.2 equiv.) and K_2CO_3 (99 mg, 0.72 mmol, 2 equiv.). Tetrakis(triphenylphosphine)palladium (41 mg, 0.036 mmol, 0.1 equiv.) was then added under N_2 and irradiated in microwave reactor at 100 °C for 5 minutes. The reaction mixture was quenched with water and extracted with DCM (x 3). The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo* to yield crude solid. The crude mixture was purified by column chromatography on silica gel 60 using DCM and ethyl acetate (10:0→47:3) as eluent to afford compound **78**.

Compound **78** (136 mg, 88% yield) was obtained as a white solid.

R_f = 0.15 (Silica gel, Ethyl acetate/DCM: 1/19).

mp = 183.8 – 184.1 °C.

IR (ν_{max}/cm^{-1}): 820, 1008, 1160, 1210, 1348, 1484, 1546, 1645 and 3268.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (2H, d, *J* = 8.0 Hz, $H^{2'''}$& $H^{6'''}$), 7.43 (1H, s, H^3), 7.258 (1H, s, $H^{5'}$), 7.26 (2H, d, *J* = 8.0 Hz, $H^{3'''}$& $H^{5'''}$), 7.06 (1H, d, *J* = 3.2 Hz, $H^{3'}$), 6.66 (1H, t, *J* = 5.2 Hz, N-H), 6. 26 (1H, dd, *J* = 5.2, 3.2 Hz, $H^{4'}$), 4.80 (2H, d, *J* = 5.2 Hz, CH_2NH), 3.19 (4H, t, *J* = 6.4 Hz, $H^{2''}$& $H^{5''}$), 2.40 (3H, s, CH_3), 1.75 (4H, m, $H^{3''}$& $H^{4''}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.0 (CONH), 155.7 (C^5), 145.4 (C^2), 141.2 ($C^{4'''}$), 140.1 ($C^{2'}$), 129.2 ($C^{3'''}$& $C^{5'''}$), 129.1 ($C^{2'''}$& $C^{6'''}$), 127.2 ($C^{4'}$), 126.7 ($C^{3'}$), 125.8 ($C^{5'}$), 125.2 ($C^{1'''}$), 122.6 (C^4), 116.8 (C^3), 48.0 ($C^{2''}$& $C^{5''}$), 38.1 (CH_2NH), 25.5 ($C^{3''}$& $C^{4''}$), 21.6 (CH_3).

LRMS (ESI): *m/z* = 431.46 $[M+H]^+$, 100%.

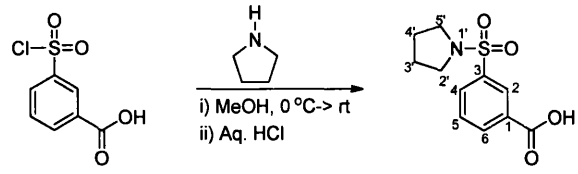
HRMS (TOF MS ES): Found 431.1096, calculated for $C_{21}H_{23}N_2O_4S_2$ 431.1099 $[M+H]^+$.

Elem. Anal. Calculated for $C_{21}H_{22}N_2O_4S_2$: C, 58.58; H, 5.15; N, 6.51% Found: C, 58.59; H, 5.05; N, 6.60%.

6.9 Preparation of Focused library "H", "I" & "J"

6.9.1 Synthesis of Compound 83a

6.9.1.1 Synthesis of Sulfonamide 52



52
$C_{11}H_{13}NO_4S$
Mol. Wt.: 255.29

Pyrrolidine (0.57 mL, 6.8 mmol, 3 equiv.) was added dropwise to a vigorously stirred solution of 3-chlorosulfonylbenzoic acid (525 mg, 2.27 mmol, 1 equiv.) in anhydrous methanol (5mL per 1 mmol of 3-chlorosulfonyl benzoic acid at 0 °C and under N_2. The solution was stirred for 1 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1 mL of 1 M HCl was added to the aqueous phase that was extracted with DCM (x 3). The combined organic phases were dried on $MgSO_4$ and evaporated *in vacuo* to afford compound **52**.

Compound **52** (568 mg, 98% yield) was obtained as a white powder.

R_f = 0.23 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 163.8 – 164.5 °C.

IR (ν_{max}/cm^{-1}): 750, 1164, 1342, 1601, 1680 and 2949.

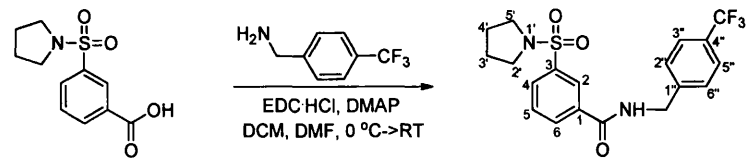
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (1H, t, J = 1.6 Hz, H^2), 8.33 (1H, dt, J = 7.6, 1.6 Hz, H^6), 8.09 (1H, dt, J = 7.6, 1.6 Hz, H^4), 7.83 (1H, t, J = 7.6 Hz, H^5), 3.30 (4H, t, J = 6.8 Hz, $H^{2'}$ & $H^{5'}$), 1.80 (4H, dt, J = 6.8, 3.2 Hz, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2 (COOH), 138.4 (C^3), 134.0 (C^4), 132.5 (C^6), 130.5 (C^1), 129.6 (C^5), 129.1 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): m/z = 256.06 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 256.0657, calculated for $C_{11}H_{14}NO_4S$ 256.0644 $[M+H]^+$.

6.9.1.2 Synthesis of Compound 83a



52 **83a**

$C_{19}H_{19}F_3N_2O_3S$
Mol. Wt.: 412.43

4-Trifluoromethyl benzylamine (0.06 mL, 0.43 mmol, 1.1 equiv.) was added dropwise to a stirred solution of **52** (100mg, 0.39 mmol, 1 equiv.), EDC hydrochloride (90 mg, 0.47 mmol, 1.2 equiv.) & DMAP (4.8 mg, 0.04 mmol, 0.1 equiv.) in anhydrous DCM (2 mL per 1 mmol of **52**) and anhydrous DMF (2 mL per 1 mmol of **52**) at 0 °C and under N_2. The mixture was stirred for 20 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 7:3) to afford compound **83a**.

Compound **83a** (141 mg, 88% yield) was obtained as a white powder.

R_f = 0.53 (Silica gel, Ethyl acetate).

mp = 145 – 146 °C.

IR (ν_{max}/cm^{-1}): 817, 1064, 1108, 1145, 1322, 1547, 1633 and 3253.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (1H, s, H^2), 8.13 (1H, d, J = 6.8 Hz, H^6), 7.93 (1H, d, J = 6.8 Hz, H^4), 7.62 (1H, t, J = 7.6 Hz, H^5), 7.59 (2H, d, J = 8.0 Hz, $H^{3"}$ & $H^{5"}$), 7.50 (2H, d, J = 8.0 Hz, $H^{2"}$ & $H^{6"}$), 7.18 (1H, t, J = 5.2 Hz, NH), 4.68 (2H, d, J = 6.0 Hz, CH_2NH), 3.21 (4H, t, J = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.74 (4H, dt, J = 6.4, 3.6 Hz, $H^{3'}$ & $H^{4'}$).

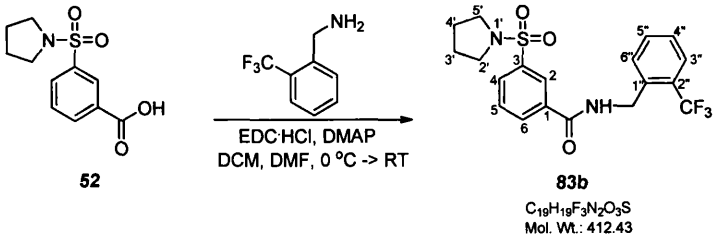
^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0 (CONH), 142.3 ($C^{1"}$), 137.6 (C^3), 135.3 (C^1), 132.1 (C^6), 130.3 (C^4), 130.1 ($C^{4"}$), 129.8 (C^5), 128.3 ($C^{2"}$ & $C^{6"}$), 125.8 ($C^{3"}$ & $C^{5"}$), 125.8 (CF_3), 125.5 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 43.8 (CH_2NH), 25.3 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 413.00 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 413.1127, calculated for $C_{19}H_{20}F_3N_2O_3S$ 413.1147 $[M+H]^+$.

Elem. Anal. Calculated for $C_{19}H_{19}F_3N_2O_3S$: C, 55.33; H, 4.64; N, 6.79 % Found: C, 55.40; H, 4.99; N, 7.00 %.

6.9.2 Synthesis of Compound 83b



52

83b

$C_{19}H_{19}F_3N_2O_3S$
Mol. Wt.: 412.43

2-Trifluoromethylbenzylamine (0.04 mL, 0.26 mmol, 1.1 equiv.) was added dropwise to a stirred solution of **52** (60 mg, 0.24 mmol, 1 equiv.), EDC hydrochloride (54 mg, 0.28 mmol, 1.2 equiv.) & DMAP (2.9 mg, 0.03 mmol, 0.1 equiv.) in anhydrous DCM (2 mL per 1 mmol of **52**) and anhydrous DMF (2 mL per 1 mmol of **52**) at 0 °C and under N_2. The mixture was stirred for 20 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 7:3) to afford compound **83b**.

Compound **83b** (63 mg, 64% yield) was obtained as a white solid.

R_f = 0.6 (Silica gel, Ethyl acetate).

mp = 119.0 – 119.8 °C.

IR (ν_{max}/cm^{-1}): 764, 1106, 1146, 1314, 1543, 1636 and 3301.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (1H, s, H^2), 8.02 (1H, d, J = 7.6 Hz, H^6), 7.95 (1H, d, J = 7.6 Hz, H^4), 7.69 (1H, d, J = $H^{3"}$), 7.63 (1H, d, J = 7.6 Hz, $H^{6"}$), 7.62 (1H, t, J = 7.6 Hz, H^5), 7.56 (1H, t, J = 7.6 Hz, $H^{5"}$), 7.43 (1H, t, J = 7.6 Hz, $H^{4"}$), 6.67 (1H, s, NH), 4.84 (2H, d, J = 6.0 Hz, CH_2NH), 3.25 (4H, t, J = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.77 (4H, dt, J = 6.4, 3.6 Hz, $H^{3'}$ & $H^{4'}$).

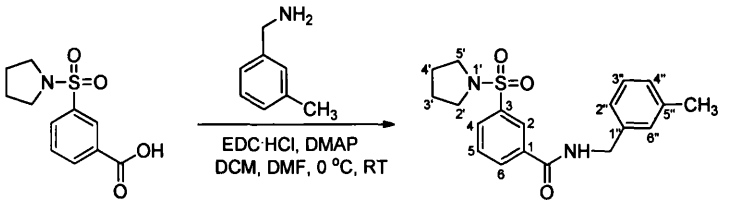
^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9 (CONH), 138.1 (C^3), 136.3 (C^1), 136.1 ($C^{1"}$), 135.4 ($C^{2"}$), 132.6 ($C^{5"}$), 131.4 ($C^{3"}$), 131.4 ($C^{3"}$), 130.3 (C^4), 129.7 (C^5), 128.1 ($C^{4"}$), 126.4 (CF_3), 126.3 ($C^{6"}$), 125.7 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 41.2 (CH_2NH), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 414.08 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 413.1137, calculated for $C_{19}H_{20}F_3N_2O_3S$ 413.1147 $[M+H]^+$.

Elem. Anal. Calculated for $C_{19}H_{19}F_3N_2O_3S$: C, 55.33; H, 4.64; N, 6.79 % Found: C, 55.49; H, 4.67; N, 7.01 %.

6.9.3 Synthesis of Compound 83c



52

83c
$C_{19}H_{22}N_2O_3S$
Mol. Wt.: 358.45

3-methylbenzylamine (0.081 mL, 0.65 mmol, 1.1 equiv.) was added dropwise to a vigorously stirred solution of **52** (150 mg, 0.59 mmol, 1 equiv.), EDC hydrochloride (135 mg, 0.71 mmol, 1.2 equiv.) & DMAP (7.1 mg, 0.059 mmol, 0.1 equiv.) in anhydrous DCM (4 mL) at 0 °C and under N_2. The mixture was stirred for 16 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 2:8 → 4:6) to afford compound **83c**.

Compound **83c** (165 mg, 78% yield) was obtained as a white solid.

R_f = 0.55 (Silica gel, Ethyl acetate).

mp = 138.9 – 139.2 °C.

IR (ν_{max}/cm^{-1}): 748, 1155, 1305, 1537, 1666 and 3399.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (1H, s, H^2), 8.07 (1H, d, J = 8.0 Hz, H^4), 7.95 (1H, d, J = 8.0 Hz, H^6), 7.61 (1H, t, J = 8.0 Hz, H^5), 7.25 (1H, t, J = 7.6 Hz, $H^{5''}$), 7.18 (1H, s, $H^{2''}$), 7.13 (2H, t, J =7.6 Hz , $H^{4''}$ & $H^{6''}$), 6.57 (1H, s, NH), 4.62 (2H, d, J = 5.6 Hz, CH_2NH), 3.25 (4H, t, J = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.77 (4H, m, $H^{3'}$ & $H^{4'}$).

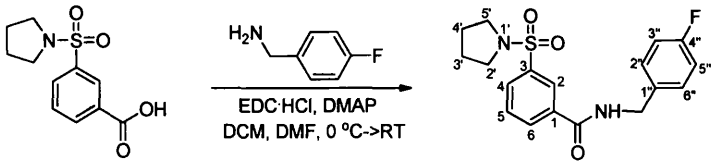
^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8 (CONH), 138.7 ($C^{3''}$), 137.9 (C^3), 137.7 ($C^{1''}$), 135.7 (C^1), 131.6 (C^4), 130.2 (C^6), 129.7 (C^5), 129.0 ($C^{6''}$), 128.9 ($C^{5''}$), 128.7 ($C^{4''}$), 125.6 ($C^{2''}$), 125.2(C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 44.5 (CH_2NH), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 359.52 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 359.1411, calculated for $C_{19}H_{23}N_2O_3S$ 359.1429 $[M+H]^+$.

Elem. Anal. Calculated for $C_{19}H_{22}N_2O_3S$: C, 63.66; H, 6.19; N, 7.82 % Found: C, 63.97; H, 6.17; N, 7.73%.

6.9.4 Synthesis of Compound 83d



52

83d

$C_{18}H_{19}FN_2O_3S$

Mol. Wt.: 362.42

4-Fluorobenzylamine (0.03 mL, 0.26 mmol, 1.1 equiv.) was added dropwise to a stirred solution of **52** (60 mg, 0.24 mmol, 1 equiv.), EDC hydrochloride (54 mg, 0.28 mmol, 1.2 equiv.) & DMAP (2.9 mg, 0.03 mmol, 0.1 equiv.) in anhydrous DCM (2 mL per 1 mmol of **52**) and anhydrous DMF (2 mL per 1 mmol of **52**) at 0 °C and under N_2. The mixture was stirred for 20 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 7:3) to afford compound **83d**.

Compound **83d** (48 mg, 55% yield) was obtained as a white solid.

R_f = 0.57 (Silica gel, Ethyl acetate).

mp = 137.6 – 138.4 °C.

IR (ν_{max}/cm^{-1}): 808, 1015, 1144, 1218, 1340, 1509, 1540, 1633 and 3262.

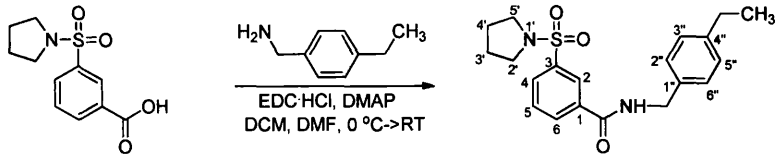
^{1}H NMR (400 MHz, MeOD): δ 8.29 (1H, t, J = 2 Hz, H^2), 8.12 (1H, d, J = 8.0 Hz, H^6), 8.00 (1H, d, J = 8.0 Hz, H^4), 7.71 (1H, t, J = 8.0 Hz, H^5), 7.39 (2H, dd, J = 8.8, 5.6 Hz, $H^{2"}$ & $H^{6"}$), 7.06 (2H, t, J = 8.8 Hz, $H^{3"}$ & $H^{5"}$), 4.57 (2H, s, CH_2NH), 3.26 (4H, t, J = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.75 (4H, dt, J = 6.4, 3.6 Hz, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, MeOD): δ 168.2 (CONH), 163.4 ($C^{4"}$), 138.9 (C^3), 136.7 (C^1), 136.0 ($C^{1"}$), 132.4 (C^6), 131.3 (C^4), 130.7 (C^5), 130.6 ($C^{2"}$ or $C^{6"}$), 130.5 ($C^{2"}$ & $C^{6"}$), 127.4 (C^2), 116.2 ($C^{3"}$ or $C^{5"}$), 116.0 ($C^{3"}$ or $C^{5"}$), 49.1 ($C^{2'}$ & $C^{5'}$), 44.0 (CH_2NH), 25.2 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 363.78 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 363.1162, calculated for $C_{18}H_{20}FN_2O_3S$ 363.1179 $[M+H]^+$.

6.9.5 Synthesis of Compound 83e



52

83e
$C_{20}H_{24}N_2O_3S$
Mol. Wt.: 372.48

4-Ethylbenzylamine (41 mg, 0.3 mmol, 1.1 equiv.) was added to a stirred solution of **52** (70 mg, 0.27 mmol, 1 equiv.), EDC hydrochloride (63 mg, 0.33 mmol, 1.2 equiv.) & DMAP (3.4 mg, 0.03 mmol, 0.1 equiv.) in anhydrous DCM (2 mL per 1 mmol of **52**) and anhydrous DMF (2 mL per 1 mmol of **52**) at 0 °C and under N_2. The mixture was stirred for 20 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 1:1) to afford compound **83e**.

Compound **83e** (61 mg, 61% yield) was obtained as a white solid.

R_f = 0.62 (Silica gel, Ethyl acetate).

mp = 115 – 116 °C.

IR (ν_{max}/cm^{-1}): 810, 1014, 1144, 1169, 1544, 1632 and 3295.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (1H, t, *J* = 1.6 Hz, H^2), 8.06 (1H, d, *J* = 8.0 Hz, H^6), 7.93 (1H, d, *J* = 8.0 Hz, H^4), 7.61 (1H, t, *J* = 8.0 Hz, H^5), 7.26 (2H, d, *J* = 8.0 Hz, $H^{2"}$ & $H^{6"}$), 7.20 (2H, d, *J* = 8.0 Hz, $H^{3"}$ & $H^{5"}$), 6.52 (1H, s, NH), 4.62 (2H, d, *J* = 6.0 Hz, CH_2NH), 3.25 (4H, t, *J* = 4.0 Hz, $H^{2'}$ & $H^{5'}$), 2.65 (2H, q, *J* = 7.6 Hz, CH_2CH_3), 1.77 (4H, dt, *J* = 6.4, 4.0 Hz, $H^{3'}$ & $H^{4'}$), 1.24 (3H, t, *J* = 7.6 Hz, CH_2CH_3).

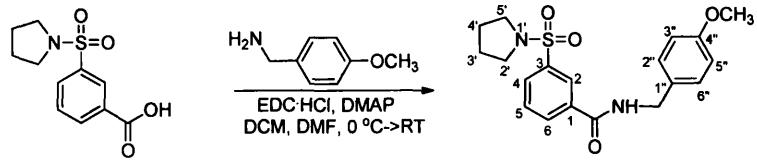
^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7 (CONH), 137.9 (C^3), 144.2 ($C^{4"}$), 135.7 (C^1), 134.9 ($C^{1"}$), 131.6 (C^6), 130.2 (C^4), 129.7 (C^5), 128.5 ($C^{2"}$ & $C^{6"}$), 128.3 ($C^{3"}$ & $C^{5"}$), 125.6 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 44.3 (CH_2NH), 28.7 (CH_2CH_3), 25.4 ($C^{3'}$ & $C^{4'}$), 15.7 (CH_2CH_3).

LRMS (ESI): *m/z* = 372.84 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 373.1572, calculated for $C_{20}H_{25}N_2O_3S$ 373.1586 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{24}N_2O_3S$: C, 64.49; H, 6.49; N, 7.52 % Found: C, 64.38; H, 6.29; N, 7.21 %.

6.9.6 Synthesis of Compound 83f



52

83f
$C_{19}H_{22}N_2O_4S$
Mol. Wt.: 374.45

4-Methoxybenzylamine (0.034 mL, 0.26 mmol, 1.1 equiv.) was added dropwise to a stirred solution of **52** (60 mg, 0.24 mmol, 1 equiv.), EDC hydrochloride (54 mg, 0.28 mmol, 1.2 equiv.) & DMAP (2.9 mg, 0.03 mmol, 0.1 equiv.) in anhydrous DCM (2 mL per 1 mmol of **52**) and anhydrous DMF (2 mL per 1 mmol of **52**) at 0 °C and under N_2. The mixture was stirred for 20 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 7:3) to afford compound **83f**.

Compound **83f** (58 mg, 65% yield) was obtained as a white solid.

R_f = 0.58 (Silica gel, Ethyl acetate).

mp = 93.1 – 93.9 °C.

IR (ν_{max}/cm^{-1}): 811, 1014, 1144, 1169, 1243, 1340, 1509, 1542, 1630 and 3291.

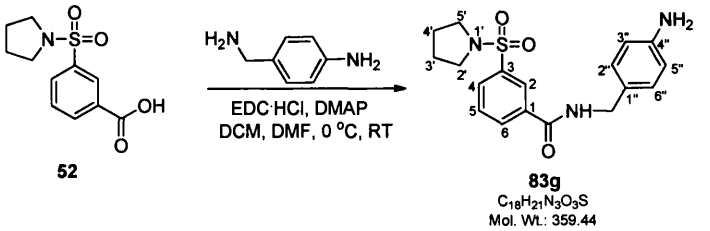
^{1}H NMR (400 MHz, MeOD): δ 8.28 (1H, t, *J* = 2.0, H^2), 8.10 (1H, dt, *J* = 7.6, 1.6 Hz, H^6), 7.98 (1H, dt, *J* = 7.6, 1.2 Hz, H^4), 7.70 (1H, t, *J* = 7.6 Hz, H^5), 7.29 (2H, d, *J* = 8.4 Hz, $H^{2"}$ & $H^{6"}$), 6.89 (2H, d, *J* = 8.4 Hz, $H^{3"}$ & $H^{5"}$), 4.52 (2H, s, CH_2NH), 3.77 (3H, s, OCH_3), 3.26 (4H, t, *J* = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.75 (4H, dt, *J* = 6.4, 4.0 Hz, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, MeOD): δ 168.1 (CONH), 160.4 ($C^{4"}$), 138.9 (C^3), 136.8 (C^1), 132.4 (C^6), 131.9 ($C^{1"}$), 131.2 (C^4), 130.6 (C^5), 130.0 ($C^{2"}$ & $C^{6"}$), 127.4 (C^2), 114.9 ($C^{3"}$ & $C^{5"}$), 55.6 (OCH_3), 49.1 ($C^{2'}$ & $C^{5'}$), 44.2 (CH_2NH), 26.1 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 374.88 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 375.1360, calculated for $C_{19}H_{23}N_2O_4S$ 375.1378 $[M+H]^+$.

6.9.7 Synthesis of Compound 83g



52

83g
$C_{18}H_{21}N_3O_3S$
Mol. Wt.: 359.44

4-aminobenzylamine (0.09 mL, 0.79 mmol, 1 equiv.) was added dropwise to a vigorously stirred solution of **52** (202 mg, 0.79 mmol, 1 equiv.), EDC hydrochloride (182 mg, 0.95 mmol, 1.2 equiv.) & DMAP (9.6 mg, 0.079 mmol, 0.1 equiv.) in anhydrous DCM (5 mL) at 0 °C and under N_2. The mixture was stirred for 17 h at room temperature, quenched by the addition of water and extracted with DCM. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 1:9 → 1:1) to afford compound **83g**.

Compound **83g** (162 mg, 57% yield) was obtained as a light yellow solid.

R_f = 0.76 (Silica gel, Methanol/Ethyl acetate: 1/1).

mp = 172.2 – 172.4 °C.

IR (ν_{max}/cm^{-1}): 834, 1144, 1332, 1518, 1627, 1737, 2971 and 3297.

^{1}H NMR (400 MHz, MeOD): δ 8.27 (1H, t, *J* = 1.6, H^2), 8.10 (1H, dt, *J* = 7.6, 1.6 Hz, H^4), 7.98 (1H, dt, *J* = 7.6, 1.6 Hz, H^6), 7.69 (1H, t, *J* = 7.6 Hz, H^5), 7.12 (2H, d, *J* = 8.4 Hz, $H^{2"}$ & $H^{6"}$), 6.70 (2H, d, *J* = 8.4 Hz, $H^{3"}$ & $H^{5"}$), 4.45 (2H, s, CH_2NH), 3.26 (4H, t, *J* = 6.8 Hz, $H^{2'}$ & $H^{5'}$), 1.75 (4H, m, $H^{3'}$ & $H^{4'}$).

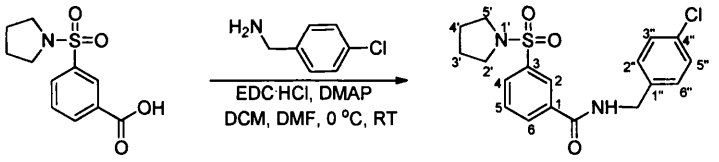
^{13}C NMR (100 MHz, MeOD): δ 166.6 (CONH), 146.4 ($C^{4"}$), 132.4 (C^4), 131.2 (C^6), 130.7 (C^3), 130.6 (C^5), 129.8 ($C^{2"}$ & $C^{6"}$), 129.4 (C^1), 128.4 ($C^{1"}$), 127.4 (C^2), 116.6 ($C^{3"}$ & $C^{5"}$), 49.1 ($C^{2'}$ & $C^{5'}$), 44.4 (CH_2NH), 26.2 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 360.43 $[M+H]^+$, 78%.

HRMS (TOF MS ES): Found 360.1371, calculated for $C_{18}H_{22}N_3O_3S$ 360.1382 $[M+H]^+$.

Elem. Anal. Calculated for $C_{18}H_{21}N_3O_3S$: C, 60.15; H, 5.89; N, 11.69 % Found: C, 60.13; H, 5.76; N, 11.51%.

6.9.8 Synthesis of Compound 83h



52

83h
$C_{18}H_{19}ClN_2O_3S$
Mol. Wt.: 378.87

4-chlorobenzylamine (0.08 mL, 0.65 mmol, 1.1 equiv.) was added dropwise to a vigorously stirred solution of **52** (150 mg, 0.59 mmol, 1 equiv.), EDC hydrochloride (135 mg, 0.71 mmol, 1.2 equiv.) & DMAP (7.1 mg, 0.059 mmol, 0.1 equiv.) in anhydrous DCM (4 mL) and anhydrous *N,N*- Dimethylformamide (1 mL) at 0 °C and under N_2. The mixture was stirred for 16 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 1:4 → 2:3) to afford compound **83h**.

Compound **83h** (210 mg, 94% yield) was obtained as a white powder.

R_f = 0.58 (Silica gel, Ethyl acetate).

mp = 169.8 – 170.1 °C.

IR (ν_{max}/cm^{-1}): 798, 1149, 1343, 1541, 1636 and 3317.

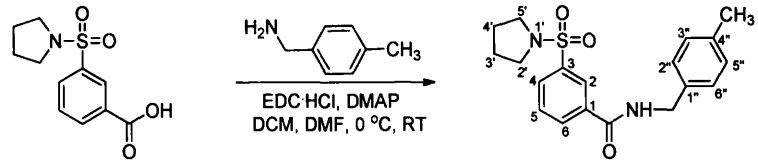
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (1H, t, *J* = 2.0 Hz, H^2), 8.08 (1H, d, *J* = 8.0 Hz, H^4), 7.95 (1H, d, *J* = 8.0 Hz, H^6), 7.63 (1H, t, *J* = 8.0 Hz, H^5), 7.34 – 7.30 (4H, m, $H^{2",3",5",6"}$), 6.68 (1H, s, *-NH-*), 4.53 (2H, d, *J* = 6.0 Hz, *CH_2*NH), 3.24 (4H, t, *J* = 6.4 Hz, $H^{2'}$ & $H^{5'}$), 1.77 (4H, m, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9 (CONH), 138.0 (C^3), 136.4 ($C^{1"}$), 135.4 (C^1), 133.8 ($C^{4"}$), 131.7 (C^4), 130.1 (C^6), 129.8 (C^5), 129.5 ($C^{2"}$ & $C^{6"}$), 129.1 ($C^{3"}$ or $C^{5"}$), 125.5 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 43.7 (CH_2NH), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 379.43 $[C_{18}H_{19}{}^{35}ClN_2O_3S+H]^+$ (75%), 381.39 $[C_{18}H_{19}{}^{37}ClN_2O_3S+H]^+$ (25%).

HRMS (TOF MS ES): Found 379.0877, calculated for $C_{18}H_{20}{}^{35}ClN_2O_3S$ 379.0883 $[M+H]^+$.

6.9.9 Synthesis of Compound 83i



52

83i
$C_{19}H_{22}N_2O_3S$
Mol. Wt.: 358.45

4-methylbenzylamine (0.082 mL, 0.65 mmol, 1.1 equiv.) was added dropwise to a vigorously stirred solution of **52** (150 mg, 0.59 mmol, 1 equiv.), EDC hydrochloride (135 mg, 0..71 mmol, 1.2 equiv.) & DMAP (7.1 mg, 0.059 mmol, 0.1 equiv.) in anhydrous DCM (4 mL) and anhydrous *N,N*- Dimethylformamide (0.2 mL) at 0 °C and under N_2. The mixture was stirred for 16 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 1:4 → 2:3) to afford compound **83i**.

Compound **83i** (182 mg, 86% yield) was obtained as a white solid.

R_f = 0.55 (Silica gel, Ethyl acetate).

mp = 162.5 – 162.8 °C.

IR (ν_{max}/cm^{-1}): 758, 801, 1148, 1343, 1637 and 3335.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (1H, t, *J* = 2.0 Hz, H^2), 8.06 (1H, d, *J* = 7.6 Hz, H^4), 7.94 (1H, d, *J* = 7.6 Hz, H^6), 7.61 (1H, t, *J* = 7.6 Hz, H^5), 7.26 (2H, d, *J* = 8.0 Hz, $H^{2"}$ & $H^{6"}$), 7.17 (2H, d, *J* =8.0 Hz, $H^{3"}$ & $H^{5"}$), 6.52 (1H, s, NH), 4.61 (2H, d, *J* = 5.6 Hz, CH_2NH), 3.25 (4H, t, *J* = 4.0 Hz, $H^{2'}$ & $H^{5'}$), 1.77 (4H, m, $H^{3'}$ & $H^{4'}$).

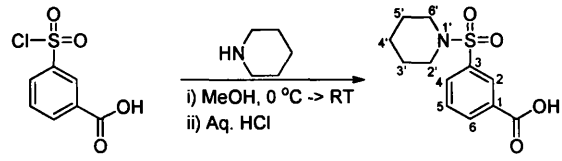
^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8 (CONH), 137.9 ($C^{4"}$), 137.7 (C^3), 135.7 (C^1), 134.7 ($C^{1"}$), 131.6 (C^4), 130.2 (C^6), 129.7 ($C^{3"}$ or $C^{5"}$), 129.6 (C^5), 128.2 ($C^{2"}$ & $C^{6"}$), 125.6 (C^2), 48.1 ($C^{2'}$ & $C^{5'}$), 44.3 (CH_2NH), 25.4 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 359.52 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 359.1446, calculated for $C_{19}H_{23}N_2O_3S$ 359.1429 $[M+H]^+$.

6.9.10 Synthesis of Compound 83j

6.9.10.1 Synthesis of Sulfonamide 82



82
$C_{12}H_{15}NO_4S$
Mol. Wt.: 269.32

Piperidine (1.34 mL, 13.6 mmol, 3 equiv.) was added dropwise to a vigorously stirred solution of 3-chlorosulfonyl benzoic acid (1 g, 4.53 mmol, 1 equiv.) in anhydrous methanol (5mL per 1 mmol of 3-chlorosulfonyl benzoic acid at 0 °C and under N_2. The solution was stirred for 3 h at room temperature. 1 mL brine was then added. The mixture was extracted with DCM (x 3). 1 mL of 1 M HCl was added to the aqueous phase that was extracted with DCM (x 3). The combined organic phases were dried on $MgSO_4$ and evaporated *in vacuo* to afford compound **82**.

Compound **82** (854 mg, 70% yield) was obtained as a white powder.

R_f = 0.14 (Silica gel, Ethyl acetate).

mp = 180.2 – 180.5 °C.

IR (ν_{max}/cm^{-1}): 744, 922, 1167, 1339, 1678 and 2934.

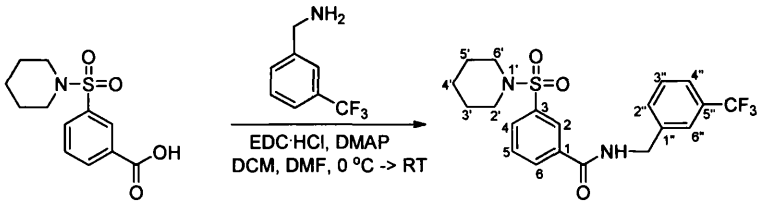
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (1H, t, J = 1.6 Hz, H^2), 8.33 (1H, dt, J = 8.0, 1.2 Hz, H^4), 8.02 (1H, dt, J = 8.0, 1.2 Hz, H^6), 7.68 (1H, t, J = 8.0 Hz, H^5), 3.05 (4H, t, J = 5.6 Hz, $H^{2'}$ & $H^{6'}$), 1.66 (4H, m, $H^{3'}$ & $H^{5'}$), 1.45 (2H, m, $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1 (COOH), 137.8 (C^3), 134.0 (C^4), 132.6 (C^6), 130.4 (C^1), 129.5 (C^5), 129.3 (C^2), 47.1 ($C^{2'}$ & $C^{6'}$), 25.3 ($C^{3'}$ & $C^{5'}$), 23.6 ($C^{4'}$).

LRMS (ESI): *m/z* = 270.26 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 270.0814, calculated for $C_{12}H_{16}NO_4S$ 270.0800 $[M+H]^+$.

6.9.10.2 Synthesis of Compound 83j



82

83j
$C_{20}H_{21}F_3N_2O_3S$
Mol. Wt.: 426.45

3-Trifluoromethyl benzylamine (0.09 mL, 0.61 mmol, 1.1 equiv.) was added dropwise to a vigorously stirred solution of **82** (150 mg, 0.56 mmol, 1 equiv.), EDC hydrochloride (128 mg, 0.67 mmol, 1.2 equiv.) & DMAP (6.8 mg, 0.06 mmol, 0.1 equiv.) in anhydrous DCM (5 mL) at 0 °C and under N_2. The mixture was stirred for 18 h at room temperature, quenched by the addition of water and extracted with DCM. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **83j** was filtered of and dried overnight over $CaCl_2$.

Compound **83j** (198 mg, 83% yield) was obtained as a white solid.

R_f = 0.64 (Silica gel, Ethyl acetate).

mp = 160.8 – 171.0 °C.

IR (ν_{max}/cm^{-1}): 793, 932, 1144, 1318, 1543, 1633, 2935 and 3250.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (1H, t, *J* = 1.6 Hz, H^2), 8.09 (1H, dt, *J* = 8.0, 1.6 Hz, H^4), 7.88 (1H, dt, *J* = 8.0, 1.6 Hz, H^6), 7.63 (1H, t, *J* = 8.0 Hz, H^5), 7.62 (1H, s, $H^{2"}$), 7.57 (2H, t, *J* = 7.6 Hz , $H^{4"}$ & $H^{6"}$), 7.48 (1H, t, *J* = 7.6 Hz, $H^{5"}$), 6.84 (1H, t, *J* = 5.2 Hz, NH), 4.72 (2H, d, *J* = 6.0 Hz, CH_2NH), 3.00 (4H, t, *J* = 5.6 Hz, $H^{2'}$ & $H^{6'}$), 1.62 (4H, m, $H^{3'}$ & $H^{5'}$), 1.44 (2H, m, $H^{4'}$).

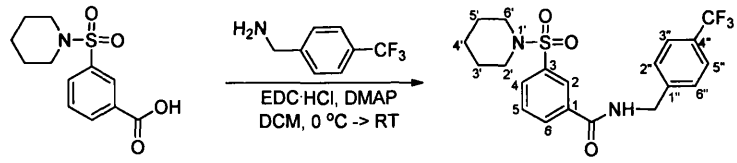
^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0 (CONH), 124.8 ($C^{3"}$), 137.3 (C^3), 139.1 ($C^{1"}$), 135.3 (C^1), 131.8 (C^4), 131.5 ($C^{4"}$ & $C^{6"}$), 130.6 (C^6), 129.7 (C^5), 129.4 ($C^{5"}$), 125.9 (CF_3), 124.7 ($C^{2"}$), 125.7 (C^2), 47.1 ($C^{2'}$ & $C^{6'}$), 43.9 (CH_2NH), 25.2 ($C^{3'}$ & $C^{5'}$), 23.5 ($C^{4'}$).

LRMS (ESI): *m/z* = 427.39 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 427.1322, calculated for $C_{20}H_{22}F_3N_2O_3S$ 427.1303 $[M+H]^+$.

6.9.11 Synthesis of Compound 83k

6.9.11.1 Synthesis of Compound 83k



82

83k

$C_{20}H_{21}F_3N_2O_3S$

Mol. Wt.: 426.45

4-Trifluoromethyl benzylamine (0.09 mL, 0.61 mmol, 1.1 equiv.) was added dropwise to a stirred solution of **82** (140 mg, 0.56 mmol, 1 equiv.), EDC hydrochloride (128 mg, 0.67 mmol, 1.2 equiv.) & DMAP (6.8 mg, 0.06 mmol, 0.1 equiv.) in anhydrous DCM (5 mL) at 0 °C and under N_2. The mixture was stirred for 18 h at room temperature, quenched by the addition of water and extracted with DCM. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and evaporated *in vacuo* to give a crude solid. The crude was redissolved in minimum amount of DCM and cooled to 0 °C. *n*-hexane was added dropwise along with stirring by a glass rod. The resulting precipitate **83k** was filtered of and dried overnight over $CaCl_2$.

Compound **83k** (193 mg, 81% yield) was obtained as a white powder.

R_f = 0.62 (Silica gel, Ethyl acetate).

mp = 183.8 – 184.4 °C.

IR (ν_{max}/cm^{-1}): 709, 809, 930, 1145, 1328, 1545, 1630, 1736, 2930 and 3255.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (1H, t, *J* = 1.6 Hz, H^2), 8.09 (1H, d, *J* = 8.0 Hz, H^4), 7.88 (1H, d, *J* = 8.0 Hz, H^4), 7.63 (1H, t, *J* = 8.0 Hz, H^5), 7.61 (2H, d, *J* = 8.4 Hz, $H^{3"}$ & $H^{5"}$), 7.50 (2H, d, *J* = 8.4 Hz, $H^{2"}$ & $H^{6"}$), 6.87 (1H, t, *J* = 6.0 Hz, NH), 4.72 (2H, d, *J* = 6.0 Hz, CH_2NH), 2.99 (4H, t, *J* = 5.6 Hz, $H^{2'}$ & $H^{6'}$), 1.62 (4H, m, $H^{3'}$ & $H^{5'}$), 1.42 (2H, m, $H^{4'}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0 (CONH), 142.1 ($C^{1"}$), 137.3 (C^3), 135.3 (C^1), 131.8 (C^4), 130.5 (C^6), 129.7 (C^5), 129.0 ($C^{4"}$), 128.3 ($C^{2"}$ & $C^{6"}$), 125.9 (CF_3), 125.8 ($C^{3"}$ & $C^{5"}$), 125.7 (C^2), 47.1 ($C^{2'}$ & $C^{6'}$), 43.9 (CH_2NH), 25.2 ($C^{3'}$ & $C^{5'}$), 23.5 ($C^{4'}$).

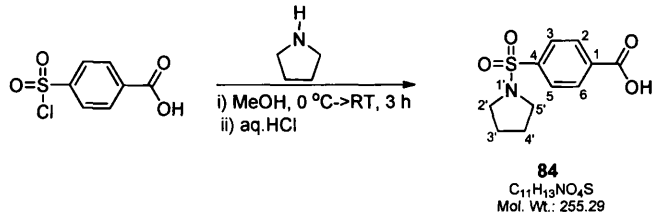
LRMS (ESI): *m/z* = 427.36 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 427.1301, calculated for $C_{20}H_{22}F_3N_2O_3S$ 427.1303 $[M+H]^+$.

Elem. Anal. Calculated for $C_{20}H_{21}F_3N_2O_3S$: C, 56.33; H, 4.96; N, 6.57% Found: C, 56.04; H, 5.09; N, 6.41 %.

6.9.12 Synthesis of Compound 85

6.9.12.1 Synthesis of Sulfonamide 84



84
$C_{11}H_{13}NO_4S$
Mol. Wt.: 255.29

Pyrrolidine (0.26 mL, 3.18 mmol, 3 equiv.) was added dropwise to a vigorously stirred solution of 4-chlorosulfonyl benzoic acid (200 mg, 1.06 mmol, 1 equiv.) in anhydrous methanol (5mL per 1 mmol of 4-chlorosulfonyl benzoic acid) at 0 °C and under N_2. The solution was stirred for 3 h at room temperature. 1 ML brine was then added. The mixture was extracted with DCM (x 3). 1 mL of 1 M HCl was added to the aqueous phase that was extracted with DCM (x 3). The combined organic phases were dried on $MgSO_4$ and evaporated *in vacuo* to afford compound **84**.

Compound **84** (216 mg, 80% yield) was obtained as a white powder.

R_f = 0.16 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 247.5 – 248.7 °C.

IR (ν_{max}/cm^{-1}): 1388, 1654, 2931 and 3429.

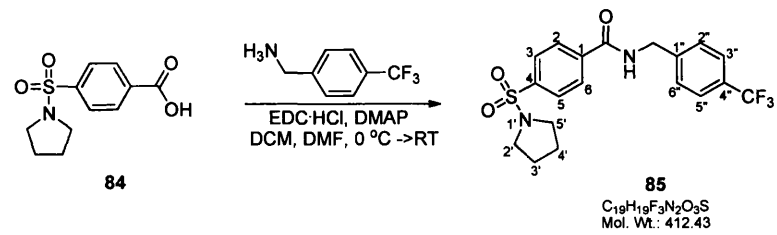
^{1}H NMR (400 MHz, DMSO): δ 13.44 (1H, s, COOH), 8.14 (2H, d, J = 8.4 Hz, H^2 & H^6), 7.91 (2H, d, J = 8.4 Hz, H^3 & H^5), 3.16 (4H, t, J = 6.8 Hz, $H^{2'}$ & $H^{5'}$), 1.65 (4H, dt, J = 6.8, 3.6 Hz, $H^{3'}$& $H^{4'}$).

^{13}C NMR (100 MHz, DMSO): δ 166.2 (COOH), 139.9 (C^1), 134.6 (C^4), 130.2 (C^2 & C^6), 127.5 (C^3 & C^5), 47.8 ($C^{2'}$ & $C^{5'}$), 24.7 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 256.26 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 256.0634, calculated for $C_{11}H_{14}NO_4S$ 256.0644 $[M+H]^+$.

6.9.12.2 Synthesis of Compound 85



84

85
$C_{19}H_{19}F_3N_2O_3S$
Mol. Wt.: 412.43

4-Trifluoromethyl benzylamine (0.14 mL, 0.99 mmol, 1.1 equiv.) was added dropwise to a vigorously stirred solution of **84** (200 mg, 0.9 mmol, 1 equiv.), EDC hydrochloride (206.1 mg, 1.075 mmol, 1.2 equiv.) & DMAP (10.9 mg, 0.09 mmol, 0.1 equiv.) in anhydrous DCM (1.7 mL per 1 mmol of **84**) and anhydrous DMF (1.7 mL per 1 mmol of **84**) at 0 °C and under N_2. The mixture was stirred for 18 h at room temperature, quenched by the addition of water and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of NaCl, dried on $MgSO_4$ and concentrated *in vacuo* to give a crude solid. The crude mixture was purified in column chromatography by gravity (ethyl acetate/*n*-hexane 7:3) to afford compound **85**.

Compound **85** (148 mg, 40% yield) was obtained as a white powder.

$\mathbf{R_f}$ = 0.73 (Silica gel, Methanol/Ethyl acetate: 1/4).

mp = 211.0 – 212.2 °C.

IR (ν_{max}/cm^{-1}): 1163, 1332, 1538 and 1646.

^{1}H NMR (400 MHz, DMSO): δ 9.38 (1H, t, *J* = 6.0 Hz, NH), 8.11 (2H, dt, *J* = 8.5, 1.8 Hz, H^2 & H^6), 7.91 (2H, dt, *J* = 8.4, 1.8 Hz, H^3 & H^5), 7.71 (2H, d, *J* = 8.0 Hz, $H^{3"}$ & $H^{5"}$), 7.54 (2H, d, *J* = 8.0 Hz, $H^{2"}$ & $H^{6"}$), 4.57 (2H, d, *J* = 5.8 Hz, CH_2NH), 3.17 (4H, t, *J* = 6.8 Hz, $H^{2'}$ & $H^{5'}$), 1.65 (4H, dt, *J* = 6.2, 3.6 Hz, $H^{3'}$ & $H^{4'}$).

^{13}C NMR (100 MHz, DMSO): δ 165.1 (COOH), 144.1 (C^1), 138.6 (C^4), 137.8 ($C^{1"}$), 128.3(C^2 & C^6), 127.9 ($C^{2"}$ & $C^{6"}$), 127.7 ($C^{3"}$ & $C^{5"}$), 127.4 (C^3 & C^5), 125.7 ($C^{4"}$), 125.2 (CF_3), 48.4 ($C^{2'}$ & $C^{5'}$), 42.4 (CH_2NH), 24.7 ($C^{3'}$ & $C^{4'}$).

LRMS (ESI): *m/z* = 413.38 $[M+H]^+$, 100%.

HRMS (TOF MS ES): Found 413.1160, calculated for $C_{19}H_{20}F_3N_2O_3S$ 413.1141 $[M+H]^+$.

Elem. Anal. Calculated for $C_{19}H_{19}F_3N_2O_3S$: C, 55.33; H, 4.64; N, 6.79 % Found: C, 55.46; H, 4.47; N, 6.75%.

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