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**Design and Synthesis of Small
Molecule Inhibitors of Coagulation
FXIIa**

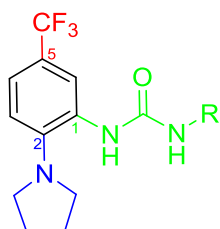
Xi Chen, MSc

Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy

May 2018

Abstract

Blood coagulation factors, factor XII (FXII), plasma kallikrein (PK), factor XI (FXI), and high-molecular-weight kininogen (HMWK), are four proteins that are involved in the plasma contact activation system (CAS) and kallikrein-kinin system (KKS). FXII is the initiation point of the intrinsic pathway of blood coagulation and is also the link between procoagulant and proinflammatory reactions. A number of inhibitors for blood coagulation factors have been approved for clinical usage. These inhibitors not only show inhibitory activities against thrombosis, but also disrupt haemostasis causing excessive bleeding. Contrasted with other coagulation proteases, the deficiency in FXII, instead of causing excessive bleeding, only reduces thrombus formation in blood coagulation. This unique property has made FXII a potential target for the treatments of thrombosis. FXII inhibitors such as aptamers, antibodies and peptides have been reported. Due to their limitations, some inhibitors have poor selectivity or weak binding affinity. Some inhibitors (biopolymers) have pharmaceutical problems. A chemically synthesised FXII inhibitor, with high selectivity is of great importance. The current lack of small molecule FXII inhibitors is limiting efforts to fully understand and validate FXII as a drug target. This project aims to design and synthesise a novel class of 1, 2, 5-tri-substituted benzene urea compounds as potent FXIIa inhibitors.



General structure of urea lead compounds

Using Lossen rearrangement, a total of 134 urea compounds were chemically synthesised in this project. All compounds were biologically tested against activated factor XII (FXIIa). Detailed structure-activity relationships (SAR) information was obtained by functional enzyme assays (FXIIa). The range of activity of urea lead compounds in series is between 5.7 μ M and 182.0 μ M. The urea compound **(127)** bearing a benzamidine functional group (4-((2-(3-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)ethyl)sulfonamido)benzamidine) had the most potent inhibition against FXIIa (α -FXIIa: $5.7 \pm 0.1 \mu\text{M}$; β -FXIIa: $5.9 \pm 0.2 \mu\text{M}$). The top 25 most potent inhibitors were selected and were biologically tested for their selectivity between FXIIa and activated factor X (FXa). There are four out of 25 compounds showed their activities against FXa in a range between $1.9 \pm 0.6 \mu\text{M}$ and $141.7 \pm 16.5 \mu\text{M}$. The selectivity between FXa and FXIIa was rationalised by serine protease-ligand crystal structure (FXa-rivaroxaban complex) and computational modelling simulation (FXIIa-hybrid model). In conclusion, potent FXIIa inhibitors were identified. The present study provides a rationale for further development of FXIIa inhibitors for the treatment of thrombosis and the investigation of selectivity between FXIIa and factor Xa (FXa).

Acknowledgement

I would like to express my greatest thanks to my supervisor, Prof. Peter Fischer, for his patience, tireless support, understanding and invaluable advice throughout my PhD. I would also like to thank Prof. Jonas Emsley for the support of FXIIa homology model. Meanwhile, I am also grateful to associated Prof. Lodewijk Dekker, Dr. James Wilson and James Awford. They gave me generous help in biological assay studies.

I also want to thank the staff and students in C35 lab, for their generous advice in lab work and thesis writing. Further thanks to Lee Hibbett for his technical support. I wish to thank other members in C floor for help and support.

The University of Nottingham and School of Pharmacy are gratefully acknowledged for supporting my PhD study.

In the end, special thanks to my parents, Guangrong Chen and Yanping He, for providing financial support throughout my PhD study. Their understanding and encouragement give me a great deal of help. I appreciate it very much.

List of Abbreviations

A

7-AMC	7-Amino-4-methylcoumarin
AT	Antithrombin III
APC	Activated protein C
aPTT	Activated partial thromboplastin time

B

BK	Bradykinin
(Boc) ₂ O	Di- <i>tert</i> -butyl dicarbonate
B2R	G protein-coupled bradykinin B2 receptor

C

CAS	Contact activation system
CDCl ₃	Chloroform- <i>d</i>
C1NH	C1-esterase inhibitor
CTI	Corn trypsin inhibitor

D

DCM	Dichloromethane
DIPEA	N, N-Diisopropylethylamine
DMAP	N, N-Dimethylpyridin-4-amine
DMSO- <i>d</i> ₆	Dimethyl Sulfoxide- <i>d</i> ₆

E

ECMO	Extracorporeal membrane oxygenation
EDC	1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide
EDG	Electron donating groups
EGF	Epidermal growth factor

ERH	Extended right hand
EtOAc	Ethyl acetate
EWG	Electron withdrawing groups
F	
FII	Factor II
FVa	Activated factor V
FVII	Factor VII
FVIIa	Activated factor VIIa
FIX	Factor IX
FXa	Activated factor X
FXI	Factor XI
FXII	Factor XII
FXIIa	Activated factor XII
FXIII	Factor XIII
H	
HMWK	High-molecular-weight kininogen
HPLC-MS	Liquid chromatography-mass spectrometry
HTS	High throughput screening
HUVEC	Human umbilical vein endothelial cell
I	
IC ₅₀	Half maximal inhibitory concentration
K	
KKS	Kallikrein-kinin system
L	
LiAlH ₄	Lithium aluminium hydride

LR	Lossen rearrangement
M	
MCCC	Managed chemical compound collection
N	
4-NBsOXY	Ethyl- 2 cyano-2-(4-nitrophenylsulfonyloxyimino)acetate
NETs	Neutrophil extracellular traps
NMM	4-Methylmorpholine
O	
OSCS	Over-sulphated chondroitin sulphate
Oxyma	Ethyl 2-cyano-2-(hydroxyimino)acetate
P	
PAMAM	Polyamidoamine dendrimers
PAR1	Protease-activated receptor 1
PBS	Phosphate-buffered saline
PCK	H-D-Pro-Phe-Arg-chloromethylketone
Pd/C	Palladium on carbon
Pd(PPh ₃) ₄	Tetrakis(triphenylphosphine)palladium (0)
PK	Plasma kallikrein
PMA	Phosphomolybdic acid
PPACK	D-Phenylalanyl-prolyl-arginyl Chloromethyl Ketone
S	
SAR	Structure-activity relationships
T	
TF	Tissue factor
THF	Tetrahydrofuran

TLC	Thin layer chromatography
TosCl	4-Toluenesulfonyl chloride
tPa	Tissue plasminogen activator
T3P	1-Propanephosphonic acid cyclic anhydride
TXA2	Thromboxane A2
U	
UHRAs	Universal heparin reversal agents
uPA	Urokinase-type plasminogen
V	
VKAs	Vitamin K antagonists
VKER	Vitamin K epoxide reductase

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1. Introduction

1.1. Haemostasis

Haemostasis is a complex mechanism which is initiated after tissue damage.¹ It is used to protect the organism from life-threatening blood loss. Two pathways are involved and activated in haemostasis. Firstly, there is the release of Von Willebrand factor in primary (cellular) haemostasis following arterial vessel wall damage. The damaged arterial vessel wall can also have arteriosclerotic problems. Von Willebrand factor binds to platelets, which initiates the aggregation of platelets and the formation of a blood clot. Secondly, the blood-clotting cascade is triggered by blood coagulation, resulting in stabilising platelet aggregates by fibrin cross-linking. When surgery, immobilization or atrial fibrillation occurs, the blood flow is reduced and then the blood coagulation cascade is initiated. As a result of shear forces and the predominance of hypoxic conditions, the tissue factor (TF, a membrane glycoprotein) is formed, which leads to clot formation in blood coagulation.

1.1.1. Blood coagulation cascade

Blood coagulation is the sequential activation of factors and results in amplification of each step by proteolytic cleavage. Blood coagulation can be subdivided into three pathways, namely extrinsic pathway, intrinsic pathway and common pathway (**Figure 1.1**).¹

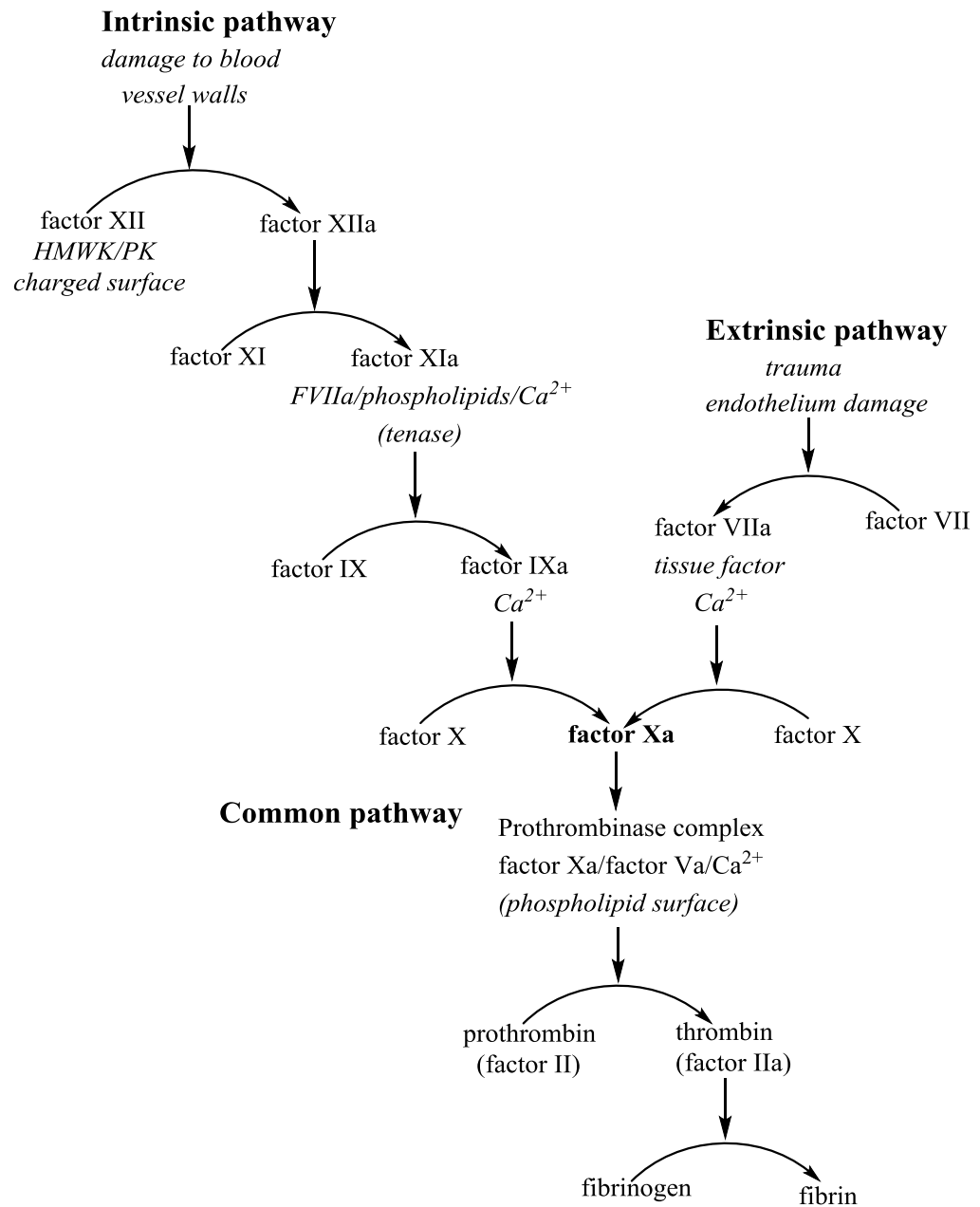


Figure 1.1: The blood coagulation biochemical cascade¹

1.1.1.1. The extrinsic pathway

The extrinsic pathway is initiated by TF and factor VIIa (FVIIa).² After exposure to the circulating blood at a disrupted vessel wall, TF binds to factor VII (FVII) and forms the TF-FVII complex. In the presence of calcium ions, the conversion of FVII to FVIIa is accelerated by TF-FVII complex proteolysis. This TF-FVIIa complex cleaves a peptide bond (Arg⁵²-Ile⁵³) in FX and converts FX into FXa.

1.1.1.2. The intrinsic pathway

1.1.1.2.1. Contact activation system

The intrinsic pathway is initiated by the contact activation system, involved with four proteins, FXII, PK, HMWK and FXI.³⁻⁴

1.1.1.2.1.1. FXII

As a serine protease enzyme, FXII is produced by the liver and is circulated in the plasma.⁵ FXII consists of two chains which are linked through a disulfide bond. One is a heavy chain which has 353 amino acids (residues 1-353), and another light chain composes of 243 amino acids (residues 354-596).⁶ Both chains are made up of several domains: fibronectin type II domain, epidermal growth factor (EGF) like domains, fibronectin type I domain, kringle domain, proline-rich region and catalytic domain or light chain (**Figure 1.2**).⁷ All of these domains have been proposed to be associated with artificial surface binding sites.⁸ In other serine proteases, all of these domains, except for proline-rich domain, can be observed. The proline-rich domain in FXII does not share sequence homology with other proline-rich proteins. Thus, proline domain in FXII may be unique to distinguish FXII from other plasma proteins. The significance of this region in FXII is still undetermined.

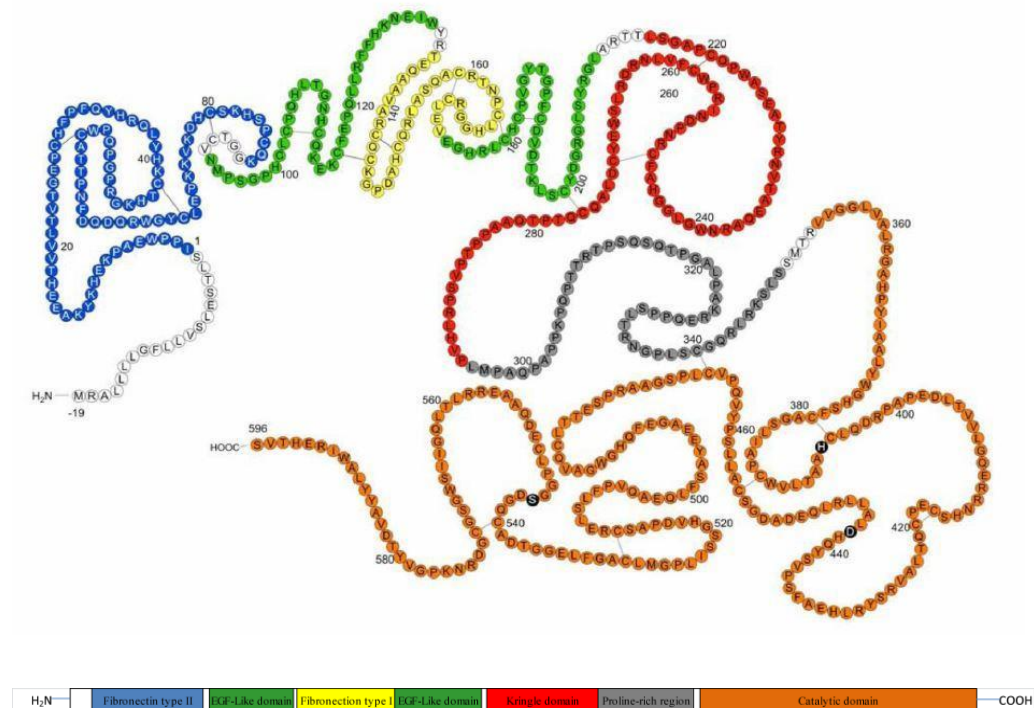


Figure1.2: Structural basis of the domains of FXII.⁷ Each region of amino acid sequence is presented by using different colour coding. Leader peptide (amino acid -19-1, white), fibronectin type II domain (1-88, blue), EGF-like domain (94-131, green), fibronectin type I domain (133-173, yellow), EGF-like domain (174-210, green), kringle domain (215-295, red), proline-rich region (296-349, grey), catalytic domain or light chain (354-596, orange).

1.1.1.2.1.1.1. Catalytic domain

The catalytic domain is the largest domain in FXII. It is formed by 243 amino acids (residues 354 to 596).⁷ FXII contains 3 amino acids at the catalytic trial (H394, D442, S544). Zymogen FXII (80 kDa) is proteolytically cleaved at its R353-V354 site and is then converted to α -FXIIa. α -FXIIa has two chains and both chains are connected by a disulfide bond between two cysteines. When the disulfide bond is reduced, a light chain (approximately 30 kDa) is liberated by the cleavage of PK. This light chain retains activity of proteolysis towards

protein substrate. It can not bind to a negatively charged surface and promote clotting.

1.1.1.2.1.2. Plasma kallikrein

PK is a single-chain glycoprotein, which is coded by the *KLKB1* gene on chromosome 4. The peptidase domain of PK is located in the light chain of PK. FXII, plasminogen, HMWK, and urokinase-type plasminogen activator (uPA) are established substrates for PK. When FXIIa cleaves PK into kallikrein, this protease kallikrein reciprocally activates additional FXIIa.⁹⁻¹⁰

1.1.1.2.1.3. High-molecular-weight kininogen

HMWK is a single-chain glycoprotein, which is coded by a single gene on chromosome 3q35.⁶ Normally, HMWK is not enzymatically active until it adheres to binding proteins beneath an endothelium exposed by injury, which further leads to the initiation of blood coagulation. As a nonenzymatic cofactor, HMWK serves for the activation of FXII and PK in the CAS. It is also important for the activation of FXI by FXIIa.¹¹⁻¹²

1.1.1.2.1.4. FXI

FXI has two chains, which are a N-terminal heavy chain with four apple domains and a C-terminal light chain with activity of a trypsin-like protease. FXI is the substrate of FXIIa. When FXIIa activates FXI, blood coagulation is promoted in the intrinsic pathway. Activated FXI itself can activate FXI (autoactivation). Factor IX (FIX) is the substrate of FXI. Via Ca^{2+} -dependent activation of FIX, FXI promotes coagulation in the intrinsic coagulation pathway. Similar to PK, FXI and HMWK both circulate in noncovalent complex. HMWK is a functional docking protein. This function of HMWK enhances FXI binding to platelets and the activation of zymogen FXI.

So far, FXI is perhaps the best understood in terms of inhibitor design for all the proteases in CAS. Various FXI inhibitors are developed, such as small-molecule inhibitors,¹³⁻¹⁵ antibodies,¹⁶ antisense oligonucleotides¹⁷⁻¹⁸ and allosteric inhibitors.¹⁹⁻²⁰ Without exception, these FXI inhibitors all have roles in thromboprotection in rodent thrombosis models using ferric chloride or vena cava ligation. These developments allow FXI to become a promising target for thrombosis.

1.1.1.2.2. Overview of FXII in innate immunity

Two distinct pathologies, coagulation and inflammation, are considered to interact closely at multiple levels. A link between procoagulant and proinflammatory reactions is provided by FXII-driven CAS. For example, proinflammatory reactions are triggered by initiation of the KKS by FXIIa (**Figure 1.3**).²¹ Through FXIIa-activated PK, HMWK is cleaved and liberates the vasoactive peptide hormone bradykinin (BK). Then BK binds to the G protein-coupled bradykinin B2 receptor (B2R). This increases intracellular calcium levels, which activates nitric oxide- and prostaglandin-mediated signaling pathways. These pathways then culminate in actin cytoskeleton rearrangement at interendothelial junctions.²²⁻²³ BK signaling pathway leads to vasodilation and increases endothelial permeability.²⁴ Tissue plasminogen activator (tPa) that is released from endothelial cells, is triggered by B2R signaling. In turn, the fibrinolytic system is activated by tPa, which leads to clot lysis.²⁵ Also, FXIIa is capable of directly converting plasminogen to plasmin.²⁶ In addition, via C1r activation *in vitro*, FXIIa has the ability to activate the classical pathway of complement system; whether FXIIa has an influence on complement activation *in vivo* needs to be determined.²⁷

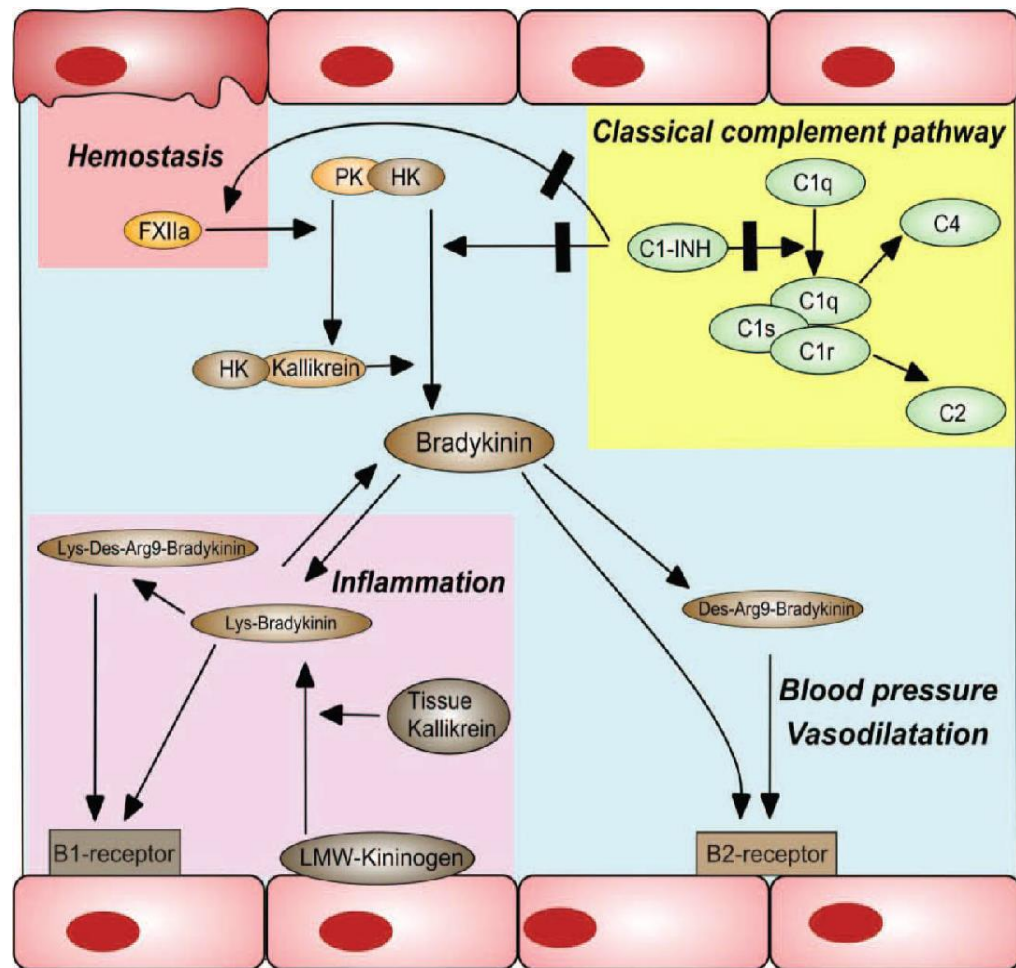


Figure 1.3:²¹ The contact system in health and disease. The FXII-driven CAS is activated, which leads to the liberation of BK from HK by kallikrein. BK and its derivatives play important roles in blood pressure regulation, vasodilation and inflammation. C1-esterase inhibitor (C1INH), as the major inhibitor of the classical complement pathway, is also an important regulator of kallikrein and FXIIa activity.

Since FXIIa-driven BK formation leads to proinflammatory reactions, it is involved in sepsis with disseminated intravascular coagulation and angioplasty-induced vessel occlusion. The formation of BK by FXIIa-driven CAS causes hypotonic shock in sepsis, anaphylactic reaction and vasculitis, as well as elevated vascular leak in hereditary angioedema. This suggests the inhibition of

FXII in CAS, not only can be useful for developing a next-generation antithrombotic therapeutic strategy without having bleeding risk, but also can have implications for inflammatory diseases.

1.1.1.3. The common pathway

The common pathway includes three components (FXa, activated factor V (FVa) and thrombin). This pathway is used for generating thrombin and fibrin (**Figure 1.1**). Firstly, the extrinsic and intrinsic pathways merge at the level of FXa. After the transformation of FXa, the FXa-FVa complex (sometimes referred to prothrombinase) is formed by binding FXa to FVa in the presence of calcium ions and phospholipids on thrombocytes. By this route, a small quantity of FXa is produced. The FXa-FVa complex then converts prothrombin to thrombin. Finally, fibrin monomers are generated from the proteolysis of fibrinogen and then are cross-linked by factor XIII (FXIII).

1.2. Thrombosis

Haemostasis maintains the integrity of a closed, high pressured circulatory system. When pathological processes occur, thrombosis is initiated. It can occur in the veins (venous thrombosis) and arteries (arterial thrombosis). Acute arterial thrombosis is the cause of most myocardial infarctions (heart attack) and strokes in the developed countries. Venous thrombosis is the third leading cause of human death related to cardiovascular disease.² In 2012, there were 7.4 million deaths in developed countries caused by ischaemic heart disease and 6.7 million deaths due to strokes. Nearly three in ten human deaths worldwide can be attributed to those diseases alone. Cardiovascular disease is a crucial target for scientists and intensive research has been carried out over the last decade in the search for treatments of cardiovascular disease.

1.2.1. Occurrence of thrombosis

1.2.1.1. Arterial thrombosis

At vascular injury sites, an atherosclerotic plaque is ruptured, resulting in arterial thrombosis. When arterial thrombosis occurs, lipid deposits and lipid-laden macrophages (foam cells) accumulate in the arterial wall, causing a hardening and narrowing of the artery (**Figure 1.4a**).² The ruptured plaque is surrounded by platelets. Platelets are disc-shaped anucleate cells and are produced by megakaryocytes in the bone marrow.²⁸⁻²⁹

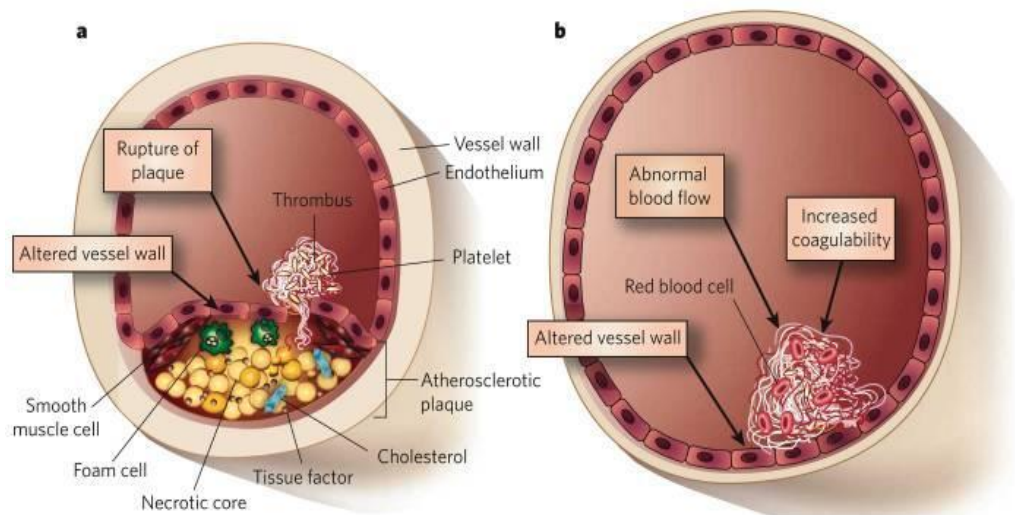


Figure 1.4:² Triggers of arterial and venous thrombosis. **a**, Artery. Rupture of an atherosclerotic plaque is the primary trigger of arterial thrombosis. Disruption of the endothelium and release of constituents of the plaque into the lumen of the blood vessel are involved. **b**, Vein. In venous thrombosis, endothelium by contrast, remains intact but can be converted from a surface with anticoagulant properties to one with procoagulant properties. Several factors triggered venous thrombosis: abnormal blood flow (for example, the absence of blood flow); alterations in the endothelium; and thrombophilia.

Platelets circulate in the blood and adhere to the altered vessel walls rapidly, through platelet cell-surface receptors with collagen and von Willebrand factor (adhesion, **Figure 1.5**).³⁰⁻³¹ Then, platelets aggregate to form a primary haemostatic plug rapidly via receptor-mediated interaction. Platelets are formed at ruptured plaques and contain blood clots. Their aggregation leads to the growth of the blood clots. Additional platelets become activated at this stage. Also, platelet receptors (protease-activated receptor 1, PAR1) are activated by factor II (protease thrombin, FII).³² Activated platelets release the contents of granules and contribute to recruiting more platelets, resulting in more promotion of adhesion, aggregation and activation of platelets.

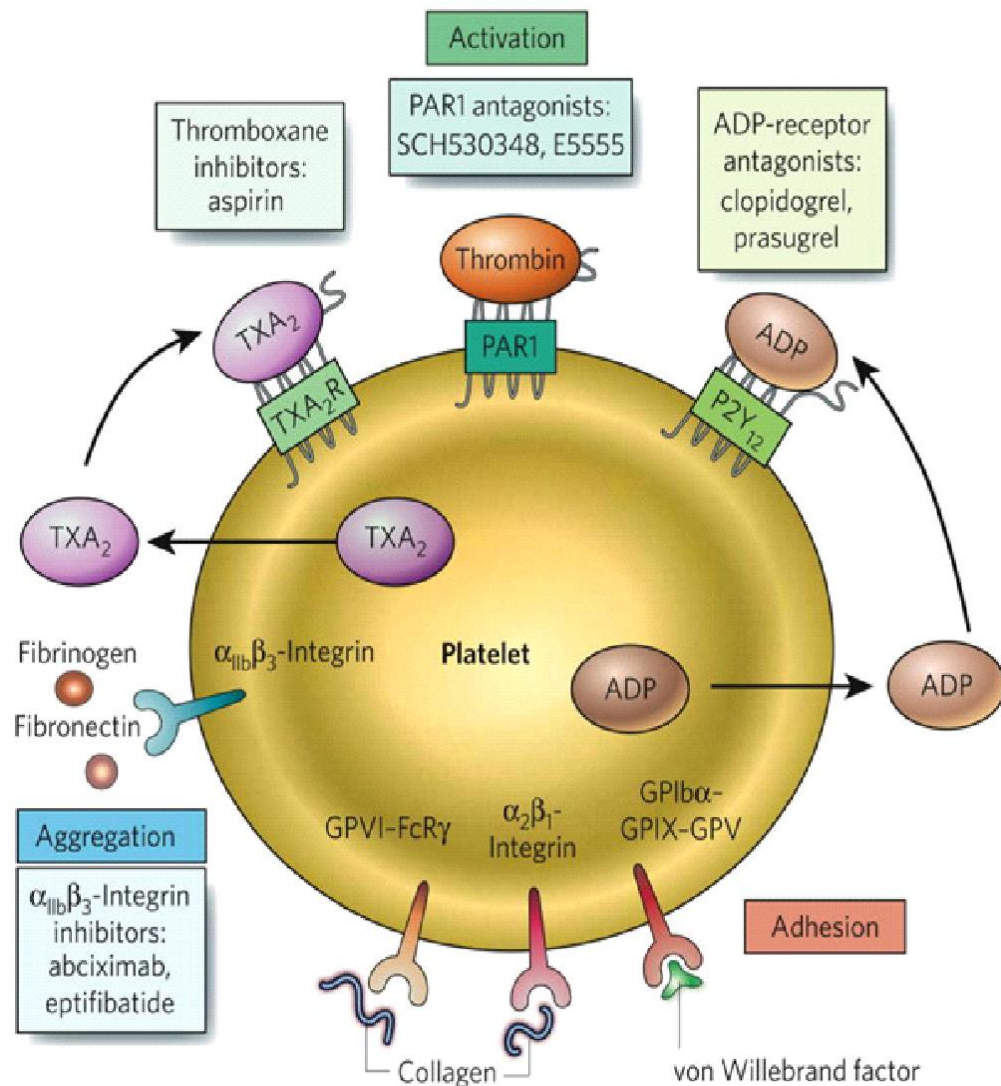


Figure 1.5:³¹ Targets of antiplatelet drugs. Platelets activation (green shading), their adhesion to the blood vessel wall (red) and their aggregation with each other (blue) are mediated by a variety of cell-surface receptors on platelets. A variety of ligands with their targeted receptors are indicated; Targeted receptors include $\alpha_{IIb}\beta_3$ -integrin, the ADP receptor P2Y₁₂, protease-activated receptor 1 (PAR1) and thromboxane A₂ (TXA₂).

Blood coagulation occurs concomitantly with platelet activation. This coagulation involves TF. When blood is exposed to TF, the blood coagulation cascade is initiated.³³⁻³⁴ This sequential process activates thrombin receptor

(PAR1) by thrombin and generates fibrin, the main protein component of the thrombus.

1.2.1.2. Venous thrombosis

There are two types of venous thrombosis, namely deep vein thrombosis and pulmonary embolism. Deep vein thrombosis often occurs in the large veins of legs. When thrombus partially breaks away and travels into a pulmonary artery of the lungs, the blood flow gets disrupted and pulmonary embolism can occur. Venous thrombi are rich in fibrin. They trap red blood cells and thus are called red clots. When the composition of the blood is changed, or blood flow is reduced, or vessel walls altered, deep vein thrombosis would mainly occur (**Figure 1.4b**). The risk of venous thrombosis can be increased when venous thrombosis is caused by genetics or environmental factors.³⁵⁻³⁶ Genetic factor V can also decrease proteins that inhibit coagulation. For instance, FV is an essential human coagulation protein in pro- and anticoagulant pathways. When expression of FV or controlling function of FV fails, bleeding or thromboembolic disease will be caused. Also, the activated protein C (APC) cleavage site is located in FV and is activated by anticoagulant protease. Since the first description of APC resistance, a mutated FV is so-called Factor V_{Leiden}. Patients with mutated FV are resistant to the activation of protein C and lead to the increase of blood clots.³⁷ Environmental factors, such as cancer and obesity, as well as major surgery, are the main risk factors for venous thrombosis.³⁵⁻³⁶

1.2.2. Treatments of thrombosis

Arterial thrombosis and venous thrombosis have different pathophysiologies, which are reflected by their different treatments. Anti-arterial thrombosis drugs

normally target platelets and anti-venous thrombosis drugs aim to target proteins of the coagulation cascade.²

1.2.2.1. Antiplatelet agents

Antiplatelet agents are anti-arterial thrombosis drugs that aim to reduce the risk of arterial thrombosis in patients with cardiovascular disease.³⁸ Activation and aggregation of platelets are the early stage of arterial thrombus formation. Thus, this early stage becomes the main target of antiplatelet inhibitors. Platelets also plays a primary role in haemostasis (normal blood clotting). Inhibition of this early step may lead to an increase in the chances of excessive bleeding in arteries. Cyclooxygenase inhibitors (cyclooxygenase 1 inhibitor aspirin,³⁹ cyclooxygenase 2 inhibitors rofecoxib and valdecoxib⁴⁰⁻⁴¹), ADP-receptor P2Y₁₂ antagonists (clopidogrel⁴²⁻⁴³ and prasugrel⁴⁴), protease-activated-receptor-1 inhibitors and $\alpha_{IIb}\beta_3$ -Integrin inhibitors (abciximab and eptifibatide⁴⁵) are the four major types of antiplatelet drugs.

1.2.2.2. Degradation of fibrin

Degradation of fibrin is an alternative method used for the treatment of thrombosis.⁴⁶ This degradation uses activators of the fibrinolytic system (also called “clot busters”) to oppose thrombus stability. Clot busters include tissue plasminogen activator and streptokinase. This type of treatment is critical because its success is crucially dependent on the timing of the treatment. Earlier treatment normally has a better result. For example, treatment of a stroke can be beneficial only when intervention of fibrinolytic therapy is within 3 h.⁴⁷ This therapy can induce the side effect of brain haemorrhage, therefore, strategies for reducing the incidence of brain haemorrhage are the main concern where fibrinolytic therapy is being used to protect the vasculature.

1.2.2.3. Anticoagulants

Anticoagulants are one of the major treatments for thrombotic diseases. They are widely used to prevent ischaemic strokes in patients with atrial fibrillation and to prevent venous thromboembolism. Atrial fibrillation commonly forms an atrial appendage clot. Patient groups with atrial fibrillation are thus at heightened risk of stroke.

Clinically, the major anticoagulant drugs are vitamin K antagonists and heparins, which act by reducing the activity of multiple proteases in the coagulation cascades.

1.2.2.3.1. Vitamin K antagonists

Vitamin K antagonists (VKAs) are the only anticoagulants used for long-term therapy of thrombosis. Warfarin is the classical VKA and is commonly used for inhibiting vitamin K epoxide reductase (VKER), several coagulation proteins (FVII, FIX, FX and prothrombin) are post-translationally modified. Following biosynthesis, enzymatic modifications of proteins are occurred. For example, glutamic acid residue on prothrombin has to be carboxylated by vitamin K. The inhibition of vitamin K by VKAs will stop the enzymatic conversion from prothrombin to thrombin. Warfarin is a racemic mixture of the R and S stereoisomers in the administration of usage. Despite careful administration, 1-3% of patients with warfarin treatment still had major bleeding problems every year.⁴⁸ Also, diet and genetic factors both have an effect on the activity of warfarin. For example, polymorphisms exist in the gene encoding vitamin K epoxide reductase and in the cytochrome P450 gene *CYP2C9*. *CYP2C9* mainly metabolises the (S)-warfarin. They are different in each individual and account

for up to 50% of the variable warfarin dosing. Thus, the accuracy of warfarin dosing can be improved by tests for polymorphisms in these two genes.

1.2.2.3.2. Heparin

In 1916, it was described that unfractionated heparin showed the anticoagulant properties. Heparin binds to the enzyme inhibitor antithrombin III (AT), resulting in a conformational change of AT and the activation of AT. In order to prevent venous thromboembolism, heparin is commonly used for cardiovascular surgery. Unfractionated heparin is currently widely used in this circumstance. Administration of unfractionated heparin showed that heparin induces thrombocytopenia syndrome.² Specifically, heparin generates antibodies for heparin-platelet-factor-4 complexes. In order to reduce the risk of heparin-induced thrombocytopenia, low-molecular-weight fractionated heparins (synthetic pentasaccharides fondaparinux and idraparinux) were introduced.⁴⁹ These new types of heparins, only inhibit FXa and not thrombin. The administration of pentasaccharides also showed that the rate of bleeding is only 1.4%, which is lower than the 2.3% for unfractionated heparin.⁵⁰

1.2.2.3.3. Direct inhibitors of FXa and thrombin

In comparison with heparin (an indirect coagulation inhibitor) potentiates the inactivation of FXa and other serine protease, a few inhibitors have been developed and can directly inhibit the active sites of thrombin and FXa. Lepirudin and desirudin are both direct thrombin inhibitors.² They can avoid heparin-induced syndrome of thrombocytopenia. They can thus replace unfractionated heparin and have been widely used for anticoagulant therapy. Dabigatran is an orally administrated thrombin inhibitor and reduces the risk of

venous thromboembolism effectively after hip-replacement.⁵¹ It shows a similar potency and safety profile of low-molecular-weight heparin enoxaparin.

Rivaroxaban, an orally active FXa inhibitor, was developed by Bayer and came on to the market in 2008. Rivaroxaban has been in clinical use for preventing venous thromboembolism by inhibition of FXa in orthopaedic surgery without increasing the bleeding.⁵² There is no requirement for the dose usage of rivaroxaban administration by hospitals.

1.3. Gene-knockout experiments of coagulation proteases in mice

From existing evidence, all of the components in blood coagulation are essential for haemostasis.⁵³ By using gene-knockout experiments in mice, it was demonstrated that disruption of individual genes in the procoagulant, anticoagulant and fibrinolytic pathways can affect haemostasis and thrombosis.⁵³ The phenotypes of mice that have defects in TF, FVIIa, FXa and prothrombin, lead to excessive bleeding and indicate that these components of haemostasis indeed have regulation in a tissue-specific manner. In addition, via using various mutations in mice, haemostasis is restored and the generation of fibrin is rebalanced. This leads to the conclusion that components of TF, FVIIa, FXa and prothrombin are indeed essential for haemostasis.

FXI knockout mice was first described in 1997.⁵⁴ It has shown that the FXI-deficient mice are protected against arterial and venous thrombosis (artificially induced) without having bleeding risk.

Similar to FXII-deficient humans, F12^{-/-} mice have a completely normal capacity of haemostasis and do not have other obvious abnormalities under basal

conditions despite having a prolonged activated partial thromboplastin time (aPTT, details in **1.4.2.1.1**). F12^{-/-} mice is investigated in a variety of thrombosis models in order to study the enigma of a pathological aPTT which apparently lacks an equivalent phenotype *in vivo*. In line with observations from FXII-deficient mice, there is a severe defect in thrombus formation in arterial and venous vascular injuries induced by different methods (mechanical and chemical vessel injuries). After FXII null mice are reconstituted with human FXII protein, they have prolonged aPTT and fully restored thrombosis. The experiment also revealed that ischaemic stroke and pulmonary embolism can be prevented by both F12^{-/-} mice and FXI-deficient mice. Via using the FXII inhibitor infestin-4, FXII-deficient mice are protected against FeCl₃-induced thrombosis. Also, pretreatment with infestin-4-based inhibitor, inhibitor-treated mice attenuated a number of cerebral microinfarcts. This did not compromise the haemostatic ability. This implied infestin-4 affects the coagulation of FXII. Peptide-based inhibitors PCK (H-D-Pro-Phe-Arg-chloromethylketone) can inhibit the activation of FXII and PK and thus protected mice from lethal pulmonary embolism. These results suggested that FXII-driven fibrin formation has great importance for pathological thrombosis but does not affect fibrin formation during normal haemostasis.

1.4. New Target: FXII

1.4.1. Overview

All clotting factors in blood coagulation interact and are activated in a sequential process. Ideally, targeting upstream components (such as FXII, TF), might be the right choice and more effective than targeting downstream components (such

as FXa and thrombin). According to gene-knockout mice experiments (in section 1.3), the components (TF, FVIIa, FXa and prothrombin) are essential for haemostasis and targeting these components leads to excess bleeding. The clinically available antithrombotic drugs effectively prevent formation of a blood clot. As in the case of antiplatelet drugs, anticoagulant drugs VKA, heparin, direct thrombin inhibitors and direct FXa inhibitors, all have the same problem of excessive bleeding.

The review of blood coagulation suggested that mice deficient in FXII of the intrinsic pathway can survive without having bleeding risk. FXII becomes a promising target for the thrombotic therapy.

1.4.2. The activation of FXII

When FXII binds to anionic and hydrophilic surfaces, there is a conformational change. Zymogen-like FXII is activated and results in the active protease FXIIa.⁵⁵ The precise mechanism for inducing contact activation and the surface binding site in FXII have remained unclear.⁵⁶⁻⁵⁷

1.4.2.1. FXII contact-activation on non-physiological surfaces

There are some well-known non-physiological negatively charged surfaces, such as kaolin, glass or polymers.⁵⁸ They have the capacity for inducing FXII contact activation. By using the most common coagulation assay aPTT, it has been shown that FXII is auto-activated upon binding to kaolin (white clay). Ellagic acid is another contact-activator for triggering aPTT assays. It contains insoluble aggregates of ellagic acid-metal-ion complexes. Some immobilized transition metals on the surface of synthetic phospholipid micelles, such as Ni²⁺, Cu²⁺, Co²⁺ or Zn²⁺, consistently stimulate FXII-driven coagulation.⁵⁹

During a variety of clinical procedures, blood unavoidably comes into contact with medical devices. At the beginning, abundant plasma proteins (fibrinogen) rapidly adsorb on the surface. Other plasma proteins (including FXII) adhere to the surface, resulting in the active protease FXIIa.⁶⁰ Indeed, it has been known that some clinically used surfaces are associated with prothrombotic risk,⁶¹⁻⁶² such as polyvinylchloride tubing or membranes used for haemodialysis and extracorporeal circulation. Patients with cardiopulmonary bypass have an increase of plasma levels of FXIIa and BK.⁶³ This supported a role of FXII activation upon contact with those surfaces. Also, dextran sulphate was used as a contact system activator in experimental settings.⁶⁴ Dextran sulphate is polysaccharide with high molecular weight. When pigs are intravenously injected with dextran sulphate, hypotension was caused and it is completely blocked by pretreatment with the B2R antagonist Icatbant.⁶⁴ Specifically, the contact activator Dextran sulphate triggers the kallikrein-kinin system, instead of initiating fibrin formation via the intrinsic pathway of coagulation *in vivo*.⁶⁵

1.4.2.1.1. Activated partial thromboplastin time (aPTT)

The aPTT assay is used to measure the overall speed of the formation of blood clots in the intrinsic pathway and the common pathway in the blood coagulation. It is one of the most commonly used diagnostic clotting assays clinically.²⁴ The assay is termed with 'partial' due to the absence of tissue factor in reaction mixture. Normal aPTT requires the presence of the following coagulation proteases: I, II, V, VIII, IX, X, XI and XII. The deficiency of FVIII, IX, XI and XII may lead to a prolonged aPTT.

It has been estimated that currently more than 5 billion of aPTT assays are being performed annually.⁶⁰ aPTT assays have been used to diagnose lupus

anticoagulants (immunoglobulins which bind to phospholipids and proteins associated with the cell membrane), to monitor heparin infusions or in preoperative screenings.

In preoperatively performed coagulation aPTT screening, FXII-deficient patients are mostly discovered and diagnosed. Via aPTT assays, it is believed that FXII is important for thrombin formation in artificial surface. The role of FXII *in vivo* has still remained unclear for decades.

1.4.2.2. FXII contact-activation on natural FXII activators

In order to further understand the mechanism of FXII activation, the discovery that inhibition of FXII activity interferes with thrombosis without risks of excessive bleeding has initiated screens for naturally occurring FXII contact activators.

1.4.2.2.1. Activated mast cell: Heparin

It has been known that mast cell have associated with FXII activation (**Figure 1.6**).⁶⁶ Heparin, as a linear and highly sulphated polysaccharide, is exclusively found in mast cells and initiates the contact-activation of FXII which results in the formation of BK via the kallikrein-kinin system. When mast cell mediators are released into the circulation suddenly, it drives anaphylactic attack. During anaphylactic reactions in patients, the levels of heparin are largely elevated and FXII is activated. There is a correlation between the intensity of FXII activation and BK formation and severity of clinical symptoms (grade of anaphylaxis).⁶⁶ Furthermore, when patients have anaphylactic attacks, plasma samples are taken and tested. It was found that FXII, PK and HK are activated. While in basal conditions, there is no significant activation of contact system detected.

1.4.2.2.2. Contaminated heparin

Heparin is a heterogeneous agent. Different manufacturers offer various heparins in both chain lengths and modifications of the polysaccharide backbone.⁶⁷ In 2008, some patients received intravenous heparin from a single manufacturer,⁶⁸ it dramatically increased lethal acute hypersensitivity reactions.

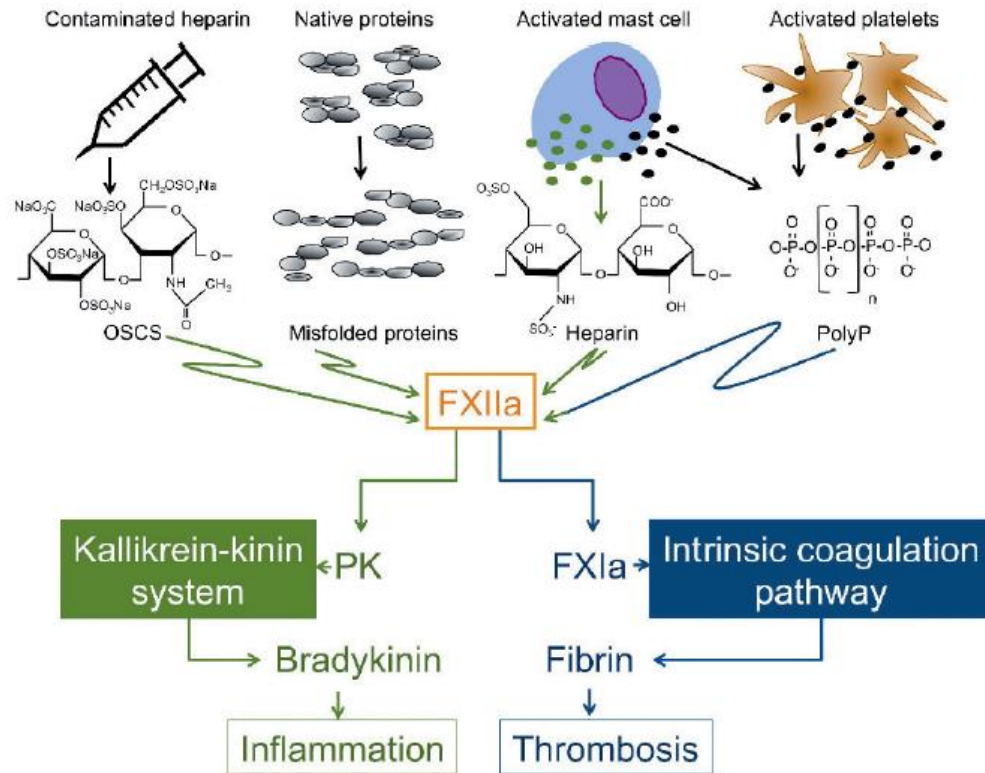


Figure 1.6⁶⁶ The zymogen form of FXII is activated upon binding to contact activators. There are several established FXII contact activators, such as heparin (released from activated mast cells), misfolded proteins, polyphosphates (polyP, come from activated platelets and mast cells) and over-sulfated chondroitin sulfate (OSCS, a heparin contaminant). Heparin, misfolded proteins and OSCS activate the kallikrein-kinin system pathway selectively. While polyP not only trigger the KKS, but also activate the intrinsic coagulation pathway.

After an investigation, it was found that the heparin product was contaminated. A non-naturally occurring over-sulfated chondroitin sulfate (OSCS, **Figure 1.6**) was identified.⁶⁹ Similar to dextran sulphate, the charge density of OSCS contributed to the potency for FXII activation of the contact activator. In comparison with mast cell heparin, OSCS has a higher negative charge and therefore contact activation is more potently triggered.⁷⁰ Also, there are some possibilities for activating FXII when sulphur-containing group (sulfatides and amylose sulphates) molecules are involved, but these totally depend on the clustering of charges. In brain, kidney and erythrocyte cell membranes, there are sulfatides that have procoagulant activity and trigger PK-like amidolytic activities in plasma.⁷¹

1.4.2.2.3. Misfolded protein

Misfolded protein aggregates is another activator, which leads to the kallikrein-kinin system activation instead of inducing coagulation. When insoluble amyloid proteins deposit in organs, FXIIa and PK were detected in the blood from patients with systemic amyloidosis.⁷² The level of FXIIa is elevated by the aggregation of amyloid proteins. This amyloid protein is also detected in the cerebrospinal fluid of Alzheimer's disease. Recent studies showed that the FXII is activated in Alzheimer's disease patient and a mouse model. It suggests that the activation of FXIIa is associated with the Alzheimer's disease. Furthermore, FXIIa seems to take part in neuronal function disruption and may provide a new drug target for interfering with diseases that cause the degeneration of brain cells.

1.4.2.2.4. Polyphosphate PolyP

Some independent studies have demonstrated that plasma clotting is promoted by activated platelets and procoagulant activity of activated platelets strictly relies on blood coagulation FXII.⁷³⁻⁷⁵ Also, the formation of FXII-driven BK is initiated by activated platelets.⁷⁴ How procoagulant platelets activate FXII have remained somehow unclear.⁷⁶

A large amount of polyP are secreted in human activated platelets and mast cells. This polyP initiates the contact activation of FXII in plasma and also triggers plasma coagulation in a FXII-dependent manner.⁷⁷ Thrombosis and oedema models in mice and a human disease model are used in studies of polyP as the activator of FXII *in vivo*. These experiments have demonstrated that the activation of polyP-driven FXII is a critical response for platelet-driven thrombosis and inflammation.⁷⁸

By using genetic and pharmacologic methods, recent studies demonstrated a critical role of platelet polyP for FXII-driven thrombosis. For example, genetically modified mice are deficient of IP6K1. Similarly to Hermansky-Pudlak syndrome patients, these IP6K1 deficient mice have the decreased levels of platelet polyP which are associated with a prolonged FXII-driven clotting time. This supports platelet polyP playing a critical role in thrombosis. Similarly to FXII deficient mice,⁷⁸⁻⁷⁹ IP6K1 deficient mice are protected from lethal pulmonary thromboembolism.⁸⁰ Moreover, specific poly interfering agents have been generated. Platelet driven thrombus formation is targeted by inhibition of polyP in arterial and venous thrombosis model *in vivo*. In conclusion, platelet polyP is critical for thrombus formation is supported by the new findings.⁸¹

1.4.2.3. Molecular mechanisms: surface binding leads to shape shifting

According to the biochemical studies of FXII, it has shown that the interaction between FXII and an activating surface is not on one site but several sites. All of the results are summarized in **Table 1.1**. Several forms of (truncated) FXII are compared individually in a complementary study.⁸² Unsurprisingly, superior binding properties was shown in wild-type FXII, because wild-type FXII contains all surface-binding domains. For truncated mutants, they only contain some of the surface-binding domains. Only some truncated mutants retained significant binding capacity, which means multiple binding sites exist in FXII.

Table 1.1 Surface binding domains/regions in FXII⁸³

Domain	Binds to:	Mediates coagulation?	Mediates activation?	PPK
N-terminus/fibronectin type II (1–28)	Kaolin	Yes	Not determined	
Fibronectin type II (39–47)	Kaolin/ dextran sulphate /HUVECs	Yes (kaolin)	Not determined	
Fibronectin type I	Kaolin/protein aggregates	Yes (kaolin)	Not determined	
EGF-like 2/kringle	Kaolin/glass/dextran sulphate	No	Yes	
Proline-rich region	Kaolin/glass/dextran sulphate	No	No	

EGF, epidermal growth factor; HUVEC, human umbilical vein endothelial cell; PK, plasma prekallikrein.

Each individual binding site has a different contribution to the functions of FXII. This indicated that FXII binding mode is specific and important for molecular function.

A hypothetical molecular model which may help to explain in details how clotting is caused by FXII activation on particulate surfaces, but not on polymeric activators.⁸⁴ This hypothetical model is outlined: a minimal requirement for activation of PPK is that, FXII has to bind to a surface by using

its second EGF-like domain and kringle domain. FXIIa triggered coagulation require more N-terminally located domains. This means an extended conformation is needed for functionality of FXII. When FXII partially binds to a surface, only PK can be activated. Furthermore, even though all materials can activate FXII, coagulation is only triggered by some of them.⁸⁵ This suggests that all binding domains have to be involved when those materials provoke FXII to trigger coagulation via FXI. Also, it leads to factor II full extension and the FXI-binding site exposure.

FXII activity has conformation-dependent regulation. FXII binding to a surface actually changed its conformation.⁸⁶ PK is susceptible to cleave and activate FXII. Similarly, binding an antibody against the FXII kringle domain can lead to the same effect.⁸⁷ This conceptual model is very helpful to understand the relationship between the structure and function of FXII during activation on surface.

1.4.2.4. Cleavage of FXII upon activation

There are three cleavage sites in FXII, which are sensitive to PK. All these cleavage sites have an arginine at their P1 position (R334, R343 and R353),⁸⁵ cleavage by PK are critical for activation of FXII. When patients have the deficiency of congenital PK, clotting times are prolonged *in vitro*. *In vivo*, PK continuously mediated FXII. When small interfering RNA treatment knocked down PK, the plasma levels of FXII rose.⁸⁸ Continuous low-grade production of bradykinin is contributed by this continuously ongoing process.⁸⁹ There is increasing evidence that indicates that the activation of FXII has a stepwise cleavage mechanism and it is reminiscent of the activation of prothrombin.⁹⁰ The

enzyme is firstly activated and then its function is directed by further fragmentation.

FXII cleavage at R353 is regarded as a molecular on/off switch. The mechanism for FXII cleavage is very reminiscent of the other serine protease activation such as trypsin.⁹¹⁻⁹² This first cleavage will become an essential connecting step in the contact system activation. When a second cleavage of α -FXIIa occurred at R334, an active fragment is generated (β -FXIIa). The surface-binding domain can be separated from the protease domain by the cleavage at R334. For the cleavage at R343, it can not trigger FXII activation independently. In other words, the site of R343 does not have the function of an auxiliary on/off switch that can bypass R353.⁹¹⁻⁹²

1.5. Strategies to target FXII

There are several strategies to target FXII, including (a) FXII directed Antisense oligonucleotides (ASO) which are used for reducing the clotting proteins' hepatic synthesis;⁹³ (b) monoclonal antibodies that block activation of FXII or their activities by binding to FXII;⁹³ (c) FXII directed aptamers⁹⁴ and active site directed inhibitors of FXIIa. Each strategy has unique characteristics and a different mechanism of action.⁹⁵

1.5.1. Targeting PolyP

Thrombosis is interfered by targeting FXII and FXIIa. Therefore, blocking the endogenous FXII contact activator polyP is believed to be a tempting novel antithrombotic strategy. Phosphate bonds in polyP can be hydrolyzed by enzyme phosphatases, which leads to degrade polyP.⁹⁶ PdSP15a/b is an odorant-binding protein and found in insects. Via binding to polyp, PdSP15a/b inhibits the

activation of polyP-driven FXII, the FXI cleavage by FXIIa or thrombin and the leakage of polyP-induced plasma in mouse models.⁹⁷

Polyamidoamine dendrimers (PAMAM), as polycationic polymers, bind with high affinity to polyP and RNA. PolyP-driven clotting and RNA are inhibited by PAMAM in plasma and whole blood *in vitro*. There is very little evidence to believe that dendrimeric polar charged polymers will make useful drugs in our opinion. Further research of dendrimeric polymers drugs are required. Also, it was discovered that PAMAM has significant toxicities *in vivo*, especially multiple toxic primary amines are found in PAMAM dendrimers.⁹⁸⁻⁹⁹ Therefore, universal heparin reversal agents (UHRAs) becomes the target for the inhibition of polyP. UHRAs interfere with thrombosis caused by chemical-induced injury in carotid arteries and laser-induced injury in cremaster arterioles.

1.5.2. Targeting FXII and FXIIa

PolyP-FXII have an essential role for thrombosis based on multiple animal models. Additionally, people with FXII deficiency have no abnormal bleeding diathesis. This raises the interesting prospect that targeting FXII or the active form FXIIa by using their inhibitors would have effects on thrombosis without interrupting haemostasis. In a ferric chloride-induced mouse thrombosis model and a graft-induced baboon thrombosis model, an antibody (15H8) blocked zymogen FXII and fully inhibited FXII prothrombotic function. Fibrin and platelet accumulation is reduced by 15H8, which means FXII indeed plays a role in the formation of thrombus in primates.¹⁰⁰ ASO, as an inhibitor used to selective knockdown FXII in mice, attenuated the formation of thrombus in both arterial and venous thrombosis models. ASO did not have an effect on haemostasis apparently.⁸⁹ Moreover, when catheters were implanted in the

jugular vein of rabbits, via ASO treatment, catheter thrombosis is reduced in rabbits.⁹³ The time to occlusion of such catheters is prolonged by over 2-fold, whereas the knockdown of factor VII had only little effect.⁹³ For ASO treatments, the disadvantage is ASO drugs have to be injected for a few weeks at a high concentration in order to suppress FXII levels sufficiently. Therefore, acute antithrombotic treatment cannot use this ASO-mediated knockdown FXII inhibitors.

Corn trypsin inhibitor (CTI) as a potent FXIIa inhibitor, is used for reducing the prothrombotic activity of catheters.¹⁰¹ CTI with coated catheters has longer potency than uncoated catheters CTI or catheters coated with albumin when inserted in rabbit's jugular veins.¹⁰¹ Furthermore, in comparison with catheters with albumin or uncoated catheters, CTI coating endowed catheters with properties of anticoagulation. It reversibly interacts with FXIIa active site.

Infestin-4 is another biological FXIIa inhibitor (selectivity: a single target FXIIa) and is derived from the midgut of the kissing bug (*Triatoma infestans*). As a serine protease, Infestin-4 is used to prevent ingested blood coagulation.¹⁰² After fused Infestin-4 to human albumin, its bioavailability is enhanced in experimental mice thrombosis models.¹⁰³ Furthermore, using Infestin-4 also reduces the silent brain ischemia morbidity in mice.¹⁰⁴ When Infestin-4 is at a high concentration *in vivo*, it has a limitation of this molecule and brings to bear off-target effects on FXIIa.¹⁰⁵

Monoclonal antibody 3F7 was produced and specifically used for binding to the enzymatic site of FXIIa.¹⁰⁶ As a fully humanized recombinant antibody, 3F7 interfered with the activity of FXIIa and the coagulation mediated by FXIIa in human plasma. Utilising 3F7, the formation of thrombus is completely abolished

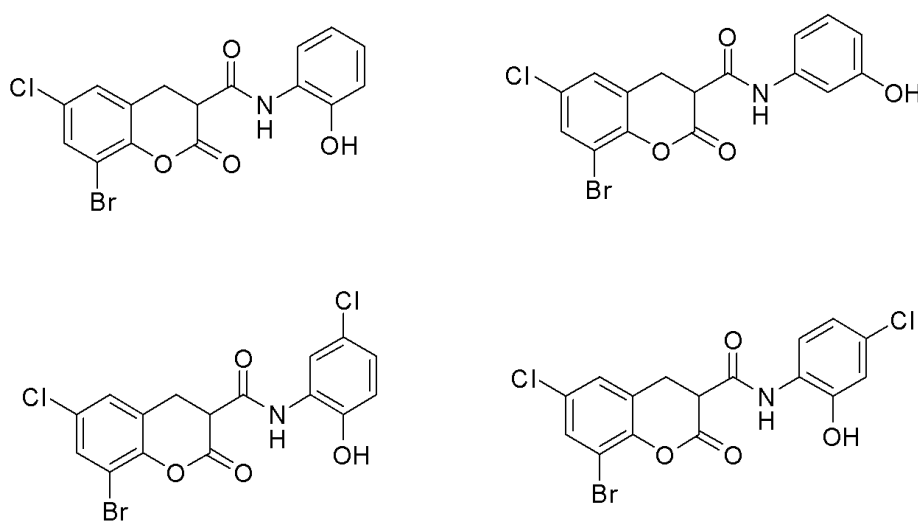
in human and mouse blood. In mouse models, FeCl_3 triggered thrombosis in arteries. This anti-FXIIa antibody can also block FeCl_3 in this circumstance. Furthermore, extracorporeal membrane oxygenation (ECMO) cardiopulmonary bypass system is offered thromboprotection by 3F7 in rabbits. Compared to heparin, the treatment of ECMO by 3F7 is confirmed to be as efficient as heparin; 3F7 did not affect haemostasis and thus did not increase excessive bleeding.¹⁰⁷ Based on the data obtained with 3F7, it suggests that there is a new safe approach of anticoagulation by targeting FXIIa. Moreover, it will not cause excessive bleeding and therefore it would be useful to test the antibody in other scenarios of thrombosis.

1.5.3. Assessments of current FXII inhibitors

The biggest indication for FXII inhibitors is prevention of stroke in atrial fibrillation patients without causing bleeding risk. For sub-chronic or acute cases (knee or hip replacement surgery, catheter thrombosis and cardiopulmonary bypass), peptides, antibodies or proteins are suitable for these applications and can be administrated parenterally. Existing FXII inhibitors have the limitation which is the relatively low selectivity over paralogous proteases. For example, infestin 4 ($K_i = 0.128 \text{ nM}$) is one of the most selective FXII inhibitors and inhibits four out of the five tested proteases, including the plasmin ($K_i = 2.1 \text{ nM}$, only 16-fold selectivity over FXII). Because over 70 trypsin-like proteases in humans have a large structural similarity, highly selective serine protease inhibitors have been challenging.

Also, there are no small molecules of FXII inhibitor undergoing clinical trials up to date. As far as can be ascertained, carboxamide-coumarins are probably the only small molecular weight FXII inhibitors undergoing experimental

development (**Figure 1.8**).¹⁰⁸ *In vivo* model of thrombosis, carboxamide-coumarins did not display any activity. Probably, these compounds have weak potency or poor pharmacokinetics. Another concern is that these coumarins are originally designed and based on some original coumarins which were used for inhibition of FXa. In this circumstance, they probably have the similar physiological properties and cause selectivity problems.



Carboxamide-coumarins

Figure 1.8: Structures of carboxamide-coumarins¹⁵⁴

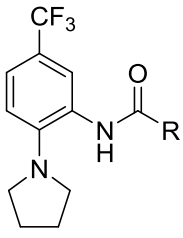
1.5.4. Problems of FXII inhibitor usage

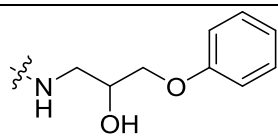
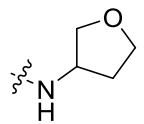
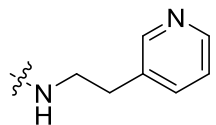
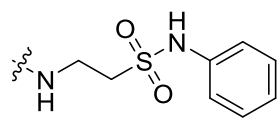
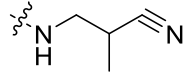
When FXII inhibitors are used as a sole therapy, there is an unanswered question that, whether FXII inhibition will be sufficient for treatment of established venous or arterial thrombosis.⁹⁵ This question has to be addressed by more studies. So far, the important drivers of thrombus growth at sites of venous or arterial injury are associated with FXa and clot-bound thrombin. Via promoting contact activation, neutrophil extracellular traps (NETs) can amplify thrombus formation.¹⁰⁹⁻¹¹⁰ Therefore, identification of the optimal indications for FXII directed anticoagulant strategies needs considerably more work.

1.6. High throughput screening (HTS)

The Centre of Biomolecular Sciences (The University of Nottingham) owns fully automated and integrated Managed Chemical Compound Collection (MCCC), containing approximately 83,000 compounds. The collection of *ca.* 80k compounds was screened against FXIIa (α -FXIIa and β -FXIIa) by Dr Dekker group (the University of Nottingham). All biological assays of compounds were replicated three times. The standard deviation was a measure which was used to quantify the amount of variation in biological assay. Potential active compounds (more than 40% inhibition at 100 μ M) were selected. The total amount of active compound was 312. After excluded signal quenchers and auto-fluorescence, 112 active compounds were remained. By analysis of 112 active compounds, two main series have been developed and that one series of active compounds were the phenylureas that form the starting point of my research.

Lead compound-Urea derivatives: By analysing structures in one of series, they all have the common substructure of the lead cluster, turning out the phenylureas.

Figure 1.9: Active lead compounds discovered in MCCC


Compd	R	IC ₅₀ (μM) ^{a,b}	
		α-FXIIa	β-FXIIa
1		13.2 ± 2.4	14.3 ± 3.5
2		8.0 ± 1.4	8.5 ± 1.2
3		6.9 ± 1.0	8.3 ± 0.7
4		10.3 ± 0.8	14.2 ± 1.3
5		6.7 ± 1.9	5.9 ± 2.1

^a The data given are mean values derived from three replicates.^b Results are expressed in mean ± SD

1.7. Aim of this research project

Due to the limitations of current FXII inhibitors, trying to find and chemically synthesise a small molecular inhibitor will be important for the study of FXII enzyme in the intrinsic coagulation pathway. Urea compounds have been found to have good inhibition against FXII enzymes. The activities of urea are not potent enough and also the correlation between inhibitors and FXII is still unclear. Therefore, our project will try to synthesise urea inhibitors chemically

and promote the activities of urea compounds against FXII by the modifications of inhibitors. During the experiment, improving the efficiency of process would also be considered.

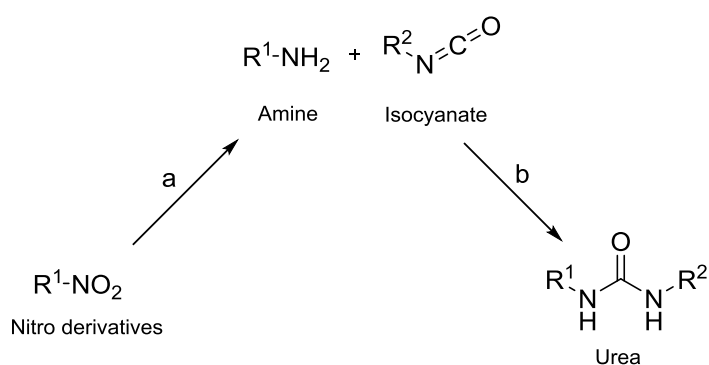
2. Methodology

2.1. Chemistry

2.1.1. Overview of the urea synthesis

Many urea-linkage based compounds have interesting applications in medicinal chemistry.¹¹¹ For example, unsymmetrically substituted ureas are potential inhibitors of microbial alkaline protease,¹¹¹ HIV-1 protease¹¹² and several other classes of enzymes.¹¹³ Also, they are importantly used in dye chemistry.

Many synthetic methods for the synthesis of ureas have been published. Through azide, isocyanate, or carbamate intermediates, urea and its derivatives were synthesised.¹¹¹ The most commonly used method is reaction of isocyanate with amines (**Scheme 2.1**). Isocyanates themselves are important commercial precursors and are often used for the synthesis of polyurethanes and polyureas. Moreover, the synthesis of biologically active compounds use isocyanates as intermediates.¹¹⁴



Scheme 2.1: General procedure for the synthesis of urea compounds. Reagents and conditions: (a) Hydrogenation (10 wt % Pd/C, H_2); (b) Dichloromethane, 0 °C.

2.1.2. Synthesis of amine

Polysubstituted aromatic anilines, as an important class of compounds, show various interesting properties. They are active agents and are used in a variety of areas, including pharmaceutical¹¹⁵ and agrochemical industries,¹¹⁶ as well as in materials science.¹¹⁷ Even though free anilines themselves may cause some problems, such as carcinogen and mutagen, they are still commonly used in the preparation of numerous aromatic carbon-nitrogen bond containing molecules.¹¹⁸ There are various methods for synthesis of anilines, including electrophilic nitration,¹¹⁹ hydrogenation of nitro derivatives,¹²⁰ nucleophilic aromatic amination,¹²¹ as well as palladium copper-catalysed amination.¹²² Curtius rearrangement of acyl azides is also very popular to access anilines, especially in the preparation of pharmacologically active compounds.¹¹⁷ The most widely used reagent in this case is diphenylphosphorazidate.¹²³ It directly transform carboxylic acids into carbamates. There is a difficulty in purifying the desired product from the phosphorus residues, which becomes a serious drawback associated with the use of this reagent.¹²⁴

In my project, the main method used for the synthesis of amine is hydrogenation of nitro derivatives. The yield of hydrogenation reaction is high and the work-up process of reaction is simple.

2.1.3. Synthesis of isocyanate

2.1.3.1. Curtius rearrangement

A well-known reaction for the synthesis of isocyanates is the classical Curtius rearrangement of acyl azides.¹²⁵ When carboxylic acids are activated in the form of acid chlorides or mixed anhydrides, they can be subjected to azidolysis; alternatively, by employing azide-transfer reagents, the carboxylic acids are

directly converted into acyl azides, which are then rearranged under thermal conditions.¹²⁵⁻¹²⁸ The Curtius rearrangement can lead to great yields, but the cost of some of the reagents, multistep sequences, and the potentially explosive nature of acid azides limited this method. It can be mainly used for bench-scale preparations. In my project, Curtius rearrangement is used for synthesis of some urea compounds.

2.1.3.2. Lossen rearrangement

The Lossen rearrangement (LR) is an alternative method to produce isocyanate.¹²⁵ It transforms activated hydroxamic acids into isocyanates. There are some reagents that have been applied in this circumstance and lead to this transformation, such as *p*-toluenesulfonyl chloride, N,N-disuccinimidocarbonate,¹²⁹ and EDC (1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide)/DMAP (N,N-dimethylpyridin-4-amine).¹³⁰ Up to date, a common drawback of most of the coupling reagents is the generation of undesired by-products and chemical waste as well as racemization.¹¹¹ Via Lossen rearrangement, two methods are used for the synthesis of isocyanates in my project. The synthesised isocyanates are further used for reactions between isocyanates and amines, which lead to formation of the urea compounds. They are described separately in section 2.1.4.1 and 2.1.4.2.

2.1.4. Synthesis of urea compounds by coupling reagents

2.1.4.1. Method B: Ethyl- 2 cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (4-NBsOXY)

A method for synthesizing ureas is developed at ambient conditions.¹¹¹ This method will not use any acid chlorides or strong bases. Ethyl- 2 cyano-2-(4-

nitrophenylsulfonyloxyimino)acetate (4-NBsOXY, **Figure 2.1**) is the coupling reagent which is normally used for synthesis hydroxamic acid. The coupling reagent 4-NBsOXY is synthesised by myself and the preparation of 4-NBsOXY is described in a reported protocol.¹³¹ In the presence of N, N-diisopropylethylamine (DIPEA), Oxyma (ethyl 2-cyano-2-(hydroxyimino)acetate) reacted with sulfonyl chloride and formed 4-NBsOXY. This coupling reagent is stable and safe to store for a long period of time at the room temperature.

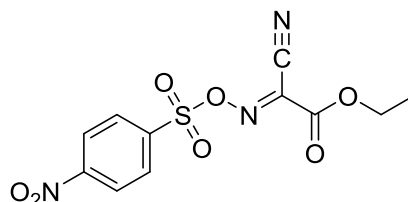
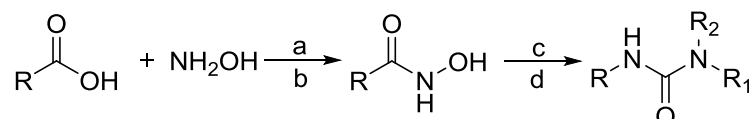


Figure 2.1: Ethyl- 2 cyano-2-(4-nitrophenylsulfonyloxyimino) acetate (4-NBsOXY)

During the experiment, carboxylic acid was converted into hydroxamic acid in the first step by using the coupling reagent 4-NBsOXY. Via Lossen rearrangement, synthesised hydroxamic acid was converted into isocyanates by using the same reagent 4-NBsOXY and further reacted with amines to form urea compounds, resulting in formation of urea compounds. The whole conversion is in a one-pot protocol and is described in details (**Scheme 2.2**).¹¹¹ Good yields without racemization were achieved under milder and simpler reaction conditions. This method is friendly to the environment and cost-effective. By-products Oxyma and 4-nitrobenzenesulfonic acid can be recycled and re-used for the preparation of the reagent 4-NBsOXY again.



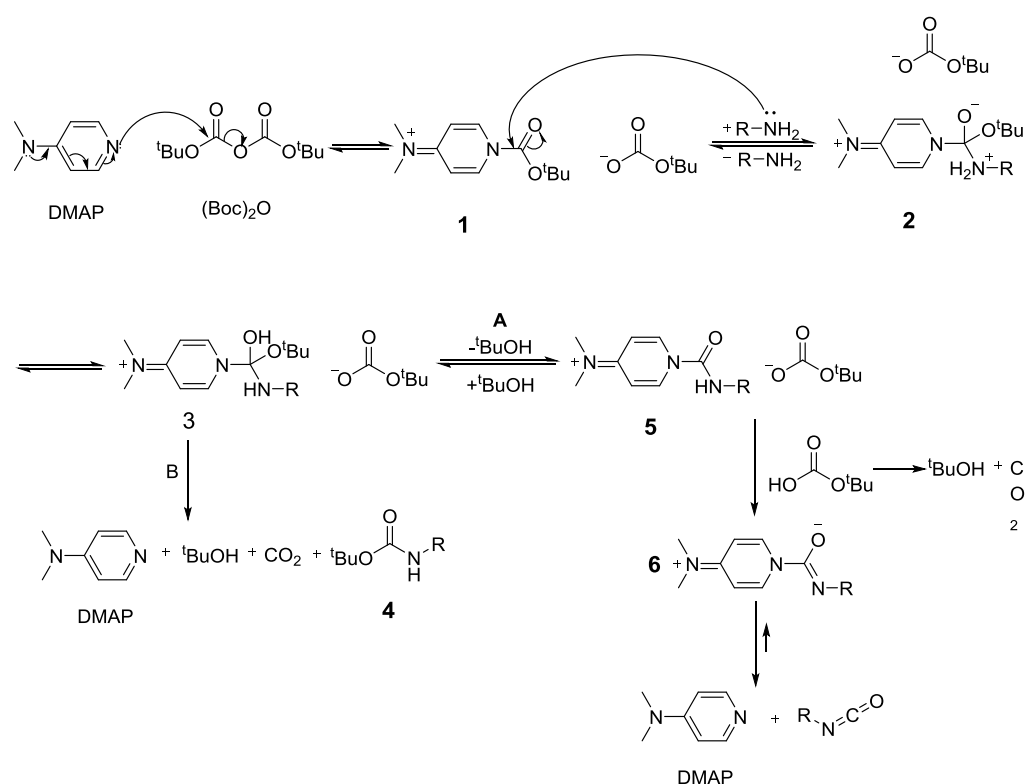
Scheme 2.2:¹¹¹ Synthesis of urea compounds by using coupling reagent 4-NBsOXY. Reagents and conditions: (a) 4-NBsOXY, THF, r.t.; (b) DIPEA/DMAP; (c) 4-NBsOXY, DIPEA; (d) R₁R₂NH (amines)

2.1.4.2. Method C: Di-*tert*-butyl dicarbonate (Boc)₂O and 4-dimethylaminopyridine (DMAP)

A novel method was developed to convert alkyl- and aryl amines into urea derivatives in high yields. In the presence of a catalytic amount of a nucleophilic nitrogen base, alkyl- and arylamines react with activated carbonates (for example, di-*tert*-butyl dicarbonate) at room temperature.¹³² It resulted in the formation of isocyanates. Di-*tert*-butyl dicarbonate, (Boc)₂O, as a protecting group, is commonly utilized in peptide synthesis. In the presence of a stoichiometric amount of 4-dimethylaminopyridine (DMAP),¹³³ substituted anilines react with (Boc)₂O in an inert solvent (dichloromethane, acetonitrile, tetrahydrofuran, ethyl acetate, toluene) at room temperature, which lead to aryl isocyanates in almost quantitative yields.

Acylation reactions can have high efficiency when 4-dimethylaminopyridine (DMAP) is used as a catalyst¹⁵⁴. N-acylpyridinium ions are the reactive intermediates of these acylation reactions. During Steglich's experiments, acetic anhydride reacted with DMAP and showed that 1-acetyl-4-dimethylaminopyridinium acetate is formed spontaneously in equilibrium.¹³³ Therefore, based on spectroscopic studies and additional experiments, it was suggested that the novel isocyanate synthesis and its reaction mechanism described in **Scheme 2.4**. There is an equilibrium formed initially between the two starting reagents, DMAP and (Boc)₂O, and 1-*tert*-butoxycarbonyl-4-dimethylaminopyridinium-*tert*-butyl carbonate (**1**). At the *tert*-butoxycarbonyl

group of the pyridinium system **1**, nucleophilic attack of the amine presumably leads to the tetrahedral intermediate **2**. Via a transprotonation, this intermediate is then converted into **3**. In theory, the carbonyl group could be regenerated by loss of the pyridinium species (pathway B), which form *tert*-butanol, carbon dioxide, DMAP, and urethane **4** (N-Boc derivative of the amine). The experiments indicate that intermediate **3** decomposes into *tert*-butanol and **5** (pathway A). Intermediate **6** is formed by deprotonation of **5** by *tert*-butyl carbonate. *Tert*-butyl monoester of carbonic acid is decarboxylated rapidly and irreversibly, which generates *tert*-butanol. This shifts the equilibrium towards the intermediate **6**. Finally, isocyanate is formed and DMAP is regenerated by the loss of the pyridinium leaving group in **6**.



Scheme 2.4: The mechanism for the synthesis of urea compounds by using $(\text{Boc})_2\text{O}$ and DMAP.

In conclusion, a convenient laboratory method for the phosgene-free isocyanation of amines is constituted with activated carbonates (Boc)₂O and catalytic amount of DMAP. Therefore, we used this method for the synthesis of symmetrical and unsymmetrical ureas.

2.1.4.3. Method D: 1-propanephosphonic acid cyclic anhydride (T3P)

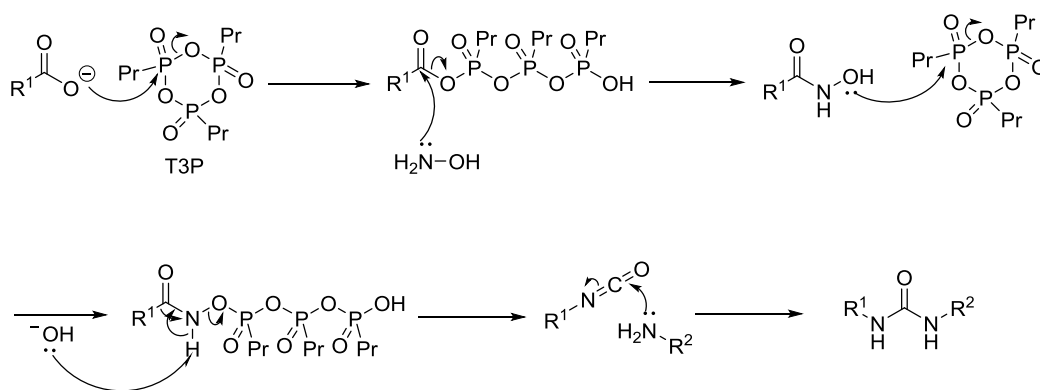
Recently, 1-propanephosphonic acid cyclic anhydride (T3P), as a coupling reagent, has been reported and applied in Lossen rearrangement.¹²⁵ This reagent is economic and efficient. There are some advantages of T3P in chemistry, such as broad functional group tolerance, high-yielding reactions, low epimerization and easy workup procedures. All of these benefits make the T3P reagent meritorious over several contemporaries. In the reaction, the by-products are water soluble and eliminate the step of chromatographic purification. Currently, T3P are well described and used in the synthesis of peptides,¹³⁴⁻¹³⁵ Weinreb amides and nitrile.¹³⁶⁻¹³⁹

T3P, as a carboxyl activator, can be used to react with carboxylic acid and form the corresponding hydroxamates;¹²⁵ T3P is a dehydrating agent and can become a promoter for the Lossen rearrangement suitably via O-activation. They reported a meticulous study on the conversion of hydroxamates into isocyanates by T3P mediated Lossen rearrangement and conversion of the latter into urea, carbamate and thiocarbamate derivatives. Hydroxamates are derived from aromatic acids.

T3P is an environmentally benign promoter for N-acylation. It is superior and traceless. There was a protocol reported for the T3P-mediated synthesis of hydroxamic acids.¹⁴⁰ By following this protocol, the completion of the synthesis

of the urea compounds is approximately 12 h at room temperature. It was suggested that reactions can be accelerated by using ultrasonic waves based on their previous experience and published work.¹⁴¹ Hence, they applied it into experiments and rendered the formation of hydroxamic acids from the corresponding carboxylic acids in a shorter period of time. They demonstrated the reaction time can be reduced from 12 h to 60 mins by using ultrasonication. Following the procedure described previously, all of the experiments were tested and repeated at several times. Instead of a high yielding reaction, the completion of T3P mediated Lossen rearrangement is less than 40% in assistance with ultrasonication.

After analysis of the reactions, it was discovered that the limitation for completing reactions is due to poor solubility of hydroxamic acid hydrochloride in organic solvents (E.g. acetonitrile). Even though the dissolution of hydroxamic acid hydrochloride was improved by using ultrasonication, there was still a large amount of hydroxamic acid hydrochloride undissolved. Therefore, this method was modified. Instead of using ultrasonication to obtain better yield of reaction, hydroxamic acid hydrochloride was dissolved with excessive DIPEA before added into starting material mixture. It turned out the yields of reactions are improved from 40% to 85% (minimum).



Scheme 2.3: The mechanism of T3P methods utilised for the synthesis of urea compounds

2.2. Biology

2.2.1. Overview of serine protease assays

A variety of peptidyl-nitroanilides have been introduced that suit a specificity requirement of proteinases.¹⁴² Thence, they are used as specific substrates for blood coagulation factors,¹⁴³ urokinase¹⁴² and kallikrein. These substrates are now available in commercial markets. Several specific assays have been used for substrates, such as assays of prekallikrein,¹⁴⁴ prothrombin,¹⁴⁵ FXa,¹⁴² antiplasmin,¹⁴⁶ and antithrombin III.¹⁴² 7-amino-4-methylcoumarin (7-AMC) is a peptide amide and originally developed for the sensitive assay of chymotrypsin.¹⁴⁷ It has been demonstrated that 7-AMC is also useful for proteases assays including aminopeptidase,¹⁴⁸ elastase,¹⁴⁹ and X-prolyl dipeptidyl-aminopeptidase.¹⁴² In comparison with the chromogenic assay, this assay method (7-AMC used for proteases assay) seems more sensitive. Because 7-amino-4-methylcoumarin, as the reaction product, is highly fluorescent.¹⁴⁷ The availability of fluorogenic substrates was extended further and 20 peptide-AMCs was generated. As specific substrates, these peptide-AMCs were all tested for their potential use on assays of proteolytic enzymes with limited

specificity. The results showed that the peptide-AMC substrates are very useful for specific enzyme assays. They preferentially fit the specificity requirements of FXa, urokinase, α -thrombin and kallikrein. Of these peptides, the following were discovered to be specific substrates for each enzymes: Boc-Ser-Gly-Arg-MCA and Boc-Ile-Glu-Gly-Arg-MCA for FXa, Boc-Val-Pro-Arg-MCA for α -thrombin, Pro-Phe-Arg-MCA for pancreatic and urinary kallikrein, Z-Phe-Arg-MCA for plasma kallikrein and glutaryl-Gly-Arg-MCA for urokinase. Furthermore, these peptide-MCA substrates showed resistance to plasmin. All of these fluorogenic peptides contained arginine-MCA (carboxyl-terminus). By releasing 7-amino-4-methylcoumarin, assay was determined fluorometrically.

2.2.2. Procedure of biological assays

2.2.2.1. FXIIa biological assay

Based on the mechanism of peptide-MCA in section 2.2.1, the biological assays of proteolytic enzymes are designed by Dr Lodewijk Dekker group (the University of Nottingham). FXII enzyme assay was utilized for all the synthesized compounds at this project. The FXII inhibitory potency of each compound was investigated by myself and measured by the half maximal inhibitory concentration (IC_{50}). This measurement IC_{50} indicates the quantity of an inhibitor needed to inhibit enzymes by half. Molar concentration is the typical value expressed in IC_{50} enzyme assay. The general procedure of FXII enzyme assay is detailed in experimental chapter 5.

3. Results and Discussion

3.1. Structure-activity relationships analysis

3.1.1. Overview

In order to understand the structure-activity relationship (SAR) between phenylureas and FXIIa, a series of urea compounds were designed and synthesized based on the results of high throughput screening (Chapter 1). Analysis of urea structure, resulted in five different functional groups that could be modified and are highlighted in **Figure 3.1**.

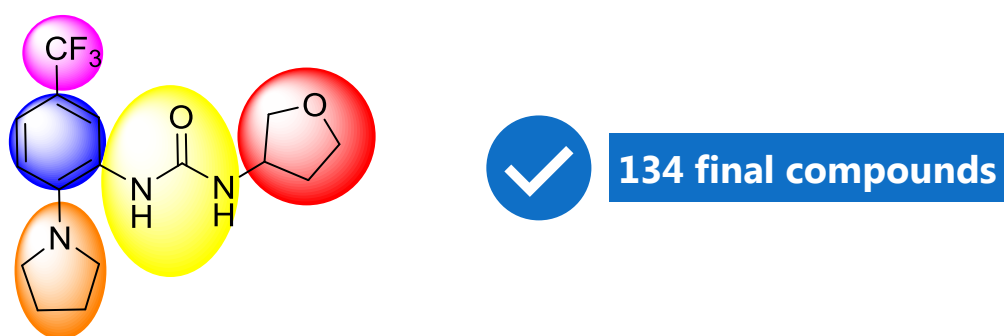


Figure 3.1: Scaffold of the urea analogues. A total number of 134 urea derivatives were chemically synthesised. Trifluoromethyl group (purple), pyrrolidine ring (orange), tetrahydrofuran group (extended right hand; red), urea moiety (yellow) and positions between trifluoromethyl group and pyrrolidine ring (blue).

The approaches used for synthesis of urea compounds are fully described in chapter 2. Once final urea compounds were obtained, all the final compounds and amines were screened against both FXIIa subtypes (α -FXIIa and β -FXIIa).

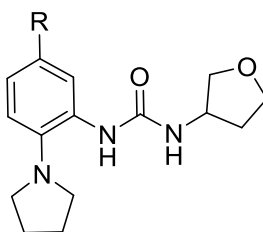
The screening procedure is described in details in the experimental section of chapter 5.

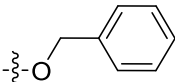
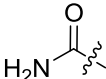
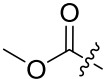
In total, more than 200 compounds have been synthesized and characterised, including 134 final compounds, 7 intermediates, 36 amines and 23 nitrobenzene. The top 25 of the most active compounds were also screened against FXa for the exploration of FXIIa inhibitors selectivity in section 3.5.2.

3.1.2. The SARs of trifluoromethyl substitution

As a strong electron withdrawing group (EWG), trifluoromethyl group can change the electronic properties of compounds, either electronically or sterically and may also affect the interaction between compounds (ligands) and enzymes (receptors) when it is attached to benzene molecule.¹⁵⁰⁻¹⁵¹

In order to understand if there is a relationship between physical properties and activity at the site of trifluoromethyl position, a series of replacements of trifluoromethyl functional group were undertaken. Based on the magnitude of the electron withdrawing effect, several EWGs were selected from strong effect to weak effect: trifluoromethyl (-CF₃) > nitrile (-CN) > nitro (-NO₂) > methyl ester (-COOCH₃) > amide (-CO-NH₂) > fluorine (-F)/chlorine (-Cl). There are also various electron donating groups (EDG), which are used for experiments and are ordered based on the magnitude of the electron donating effect (from strong effect to weak effect): benzyloxy (-OCH₂Ph) > methoxy (-OCH₃) > *tert*-butyl (-C(CH₃)₃) > methyl (-CH₃). All compounds synthesized are presented in **Table 3.1**.

Table 3.1 Potency of 5-substituted-1-(2-(pyrrolidin-1-yl) phenyl)-3-(tetrahydrofuran-3-yl) ureas

Compd	R	IC ₅₀ (μM) ^{a,b}	
		α-FXIIa	β-FXIIa
6	- ^c	NA ^d	NA
7		NA	NA
8		NA	NA
9		NA	NA
10		NA	NA
11		NA	NA
12		NA	NA
13		NA	NA
14		NA	NA
15		20.5 ± 2.2	21.3 ± 1.5
16		NA	NA
2		8.0 ± 1.4	8.5 ± 1.2

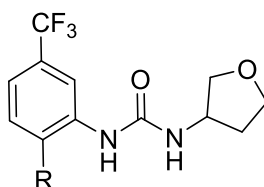
^a The data given are mean values derived from three replicates.^b Results are expressed in mean ± SD^c "-": no substitution^d NA = not active: inhibition <10% at 100 μM

When the trifluoromethyl group was replaced by electron donating groups, compounds **7-10** did not inhibit against FXIIa. Compound **15** showed some inhibitory potency (α-FXIIa: 20.5 ± 2.2 μM, β-FXIIa: 8.5 ± 1.2 μM) when a nitro moiety replaced the trifluoromethyl functional group. The rest of the electron withdrawing substituents, such as chlorine, fluorine, amide, methyl ester and

nitrile, were not active against FXIIa. In conclusion, it was observed that only replacement of nitro functional group shows activity against FXIIa, indicating that a strong electron-withdraw group is essential for inhibitory potency of urea compounds. Therefore, the CF₃ group was retained during exploration of the SARs at other positions in the molecule.

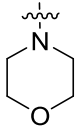
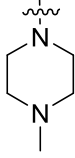
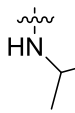
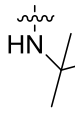
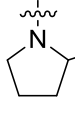
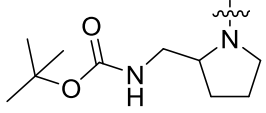
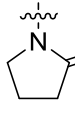
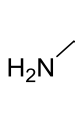
3.1.3. The SARs of a pyrrolidine ring substitution

In order to investigate the substitution of the pyrrolidine ring, the physical properties of pyrrolidine ring, such as the size of ring, saturation of ring, the replacement of pyrrolidine ring with aliphatic chains and hetero-atoms on the ring were explored. Pyrrolidine was replaced with different rings to see the effect of the size on potency. Pyrrolidine was initially replaced with either four-membered (azetidine **18**) or six-membered saturated ring (piperidine **19**). Considering that the pyrrolidine ring is saturated, unsaturated heterocycles, such as 1H-pyrrole **20**, 1H-imidazole **21**, 1H-1,2,3-triazole **22**, 1H-pyrazole **23**, 1H-1,2,4-triazole **24** and aniline **25** were used to replace saturated rings. Addition of more hetero-atoms to pyrrolidine ring could demonstrate if more hetero-atoms (morpholine **26** or 1-methylpiperazine **27**) have an effect on inhibition of compounds. Pyrrolidine was also replaced with aliphatic amines, for example, dimethylamine **28**, diethylamine **29**, isopropylamine **30** and *tert*-butylamine **31**. Pyrrolidine derivatives, such as 2-methylpyrrolidine **32**, 2-N-Boc-aminomethylpyrrolidine **33**, 2-pyrrolidone **34** and 2-(aminomethyl)pyrrolidine **35** were initial points for investigation. As a result, a series of derived pyrrolidine ring compounds were synthesized and are listed below (**Table 3.2**).

Table 3.2 Potency of 1-(2-substituted-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea

Compd	Physical properties	R	IC ₅₀ (μM) ^{a,b}	
			α-FXIIa	β-FXIIa
17		- ^c	NA ^d	NA
2			8.0 ± 1.4	8.5 ± 1.2
18			20.2 ± 2.2	21.3 ± 1.8
19	Size		NA	NA
20			X ^e	X
21			NA	NA
22			NA	NA
23	Saturation		NA	NA
24			NA	NA
25			NA	NA

Continued **Table 3.2**

Compd	Physical properties	R	IC ₅₀ (μM) ^{a,b}	
			α-FXIIa	β-FXIIa
26	Hetero-atoms		NA	NA
27			NA	NA
28	Aliphatic chains		NA	NA
29			NA	NA
30			NA	NA
31			NA	NA
32	Pyrrolidine derivatives	 (racemic mixture)	29.1 ± 6.9	32.2 ± 5.4
33			NA	NA
34			NA	NA
35			NA	NA

^a The data given are mean values derived from three replicates.^b Results are expressed in mean ± SD^c “-”: no substitution^d NA = not active: inhibition <10% at 100 μM^e “X” = unsuccessful synthesis

From the point of view of exploring the ring size, when the five-membered pyrrolidine ring was replaced by a four-membered azetidine, the inhibitory

potency of compound **18** was slightly decreased in comparison with compound **2**. The replacement of the pyrrolidine ring with a six-membered piperidine led to a completely inactive compound **19**. This indicated that the size of ring was limited and was required to be either 5-membered or less.

For the saturation of ring, saturated pyrrolidine ring was replaced by unsaturated rings, such as 1H-pyrrole **20**, 1H-imidazole (**21**), 1H-1,2,3-triazole (**22**), 1H-pyrazole (**23**), 1H-1,2,4-triazole (**24**) and aniline (**25**). The chemical synthesis of compound **20** was not successful after several attempts. All the other compounds (**21-25**) showed complete loss of inhibitory activity against FXIIa. It was therefore indicated that the saturated heterocycle is more likely to be essential for the potency of the compound.

Under the investigation of hetero-atoms on the ring, morpholine **26** and 1-methylpiperazine **27** were used for the replacement of pyrrolidine. The results showed both compounds (**26** and **27**) being inactive against FXIIa. Compared to compound **19**, addition of heteroatoms on the rings seems have no effect on the change of inhibitory potency.

The replacements of cyclic structures with aliphatic chains (dimethylamine **28**, diethyl amine **29**, isopropylamine **30** and *tert*-butylamine **31**), give compounds which were inactive against FXIIa, which indicated that the heterocyclic pyrrolidine ring is critical for inhibition.

Furthermore, several pyrrolidine ring derivatives, such as 2-methylpyrrolidine (**32**), 2-N-Boc-aminomethylpyrrolidine (**33**), 2-pyrrolidone (**34**) and 2-(aminomethyl) pyrrolidine (**35**), were investigated. With the addition of a methyl functional group on a pyrrolidine ring, the activity of the compound **32** against FXIIa was decreased slightly. This demonstrated that the pyrrolidine ring is

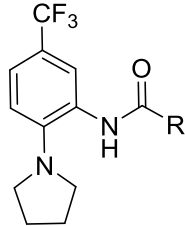
highly significant for the inhibitory potency of the compounds, and hence, there can be limited modifications at the site of pyrrolidine ring.

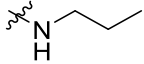
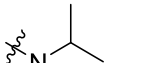
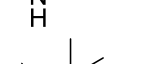
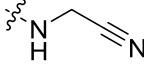
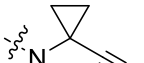
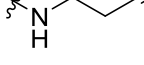
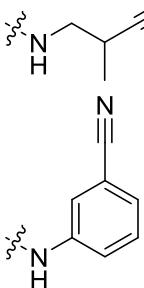
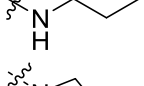
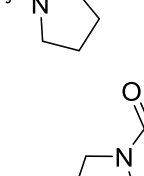
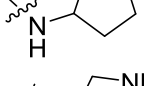
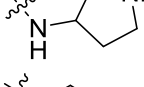
In conclusion, the best compound in this series so far was the tetrahydrofuran urea substituted by a 5-trifluoromethyl-2-pyrrolidin-1-yl group with an IC_{50} for FXIIa (α - FXIIa $8.0 \pm 1.4 \mu M$ and β - FXIIa $8.5 \pm 1.2 \mu M$). Considering their potency, substitution of pyrrolidine ring was retained for further SAR optimization.

3.1.4. The SARs of extended right hand (ERH) substitution

According to the results of HTS screening in section 1.11, a variety of ERH, such as saturated heterocycle, aliphatic sides chains containing nitrile group and side chains attached to aromatic rings, have been discovered and showed some inhibitory potency against FXIIa.

In order to explore the relationship between ERH and activity of the compounds, more ERH substituents were investigated. A library of compounds containing various ERH substitutions was generated (**Table 3.3**). They included aliphatic chains, side chains containing nitrile functional group, aliphatic heterocycle, aromatic ring, combination of aliphatic side chains with aromatic rings and functional groups containing pyrazol-amine.

Table 3.3 Potency of 1-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-substituted urea


Compd	Physical properties	R	IC ₅₀ (μM) ^{a,b}	
			α-FXIIa	β-FXIIa
36	Aliphatic chain		NA ^c	NA
37			NA	NA
38			NA	NA
39	Nitrile		17.6 ± 4.6	18.6 ± 3.4
40			NA	NA
41			7.8 ± 1.6	8.1 ± 1.4
5			6.7 ± 1.9	5.9 ± 2.1
42			NA	NA
43	Aliphatic Heterocycle		NA	NA
44			101.6 ± 24.7	103.2 ± 21.7
45			19.0 ± 3.4	21.1 ± 4.3
46			20.6 ± 4.0	23.1 ± 5.7
47			111.8 ± 27.6	132 ± 29.1

Continued **Table 3.3**

Compd	Physical properties	R	IC ₅₀ (μM) ^{a,b}	
			α-FXIIa	β-FXIIa
48	Aliphatic Heterocycle		NA ^c	NA
49			70.9 ± 10.3	74.5 ± 11.3
50			66.4 ± 21.1	69 ± 14.5
51			35.4 ± 4.4	39.2 ± 3.4
52			NA	NA
53			NA	NA
54	Aromatic ring		NA	NA
55			NA	NA
56			NA	NA
57			34.1 ± 9.6	39.8 ± 6.4
58	Side chain-aromatic ring		NA	NA

Continued **Table 3.3**

Compd	Physical properties	R	IC ₅₀ (μM) ^{a,b}	
			α-FXIIa	β-FXIIa
3			6.9 ± 1.0	8.3 ± 0.7
59			15.6 ± 6.5	19.1 ± 5.2
1	Side chain-aromatic ring		13.2 ± 2.4	14.3 ± 3.5
4			10.3 ± 0.8	14.2 ± 1.3
60			NA	NA
61	Pyrazol-amine		8.4 ± 2.9	9.4 ± 3.5

^aThe data given are mean values derived from three replicates.^bResults are expressed in mean ± SD^cNA = not active: inhibition <10% at 100 μM

For the aliphatic chains, when the tetrahydrofuran functional group was replaced by propan-amine substituents, compounds **36-38** were completely inactive compared to compound **2**. This indicated that the saturated heterocycle is a better choice for ERH functional group and replacements with simple aliphatic side chains would lose the potency of the compounds.

For the side chains containing nitrile functional group, the replacements of tetrahydrofuran with 2-aminoacetonitrile (**39**), 1-aminocyclopropane-1-carbonitrile (**40**), 3-aminopropanenitrile (**41**), 3-amino-2-methylpropanenitrile (**5**) and 3-aminobenzonitrile (**42**) were also investigated. In contrast to aliphatic

side chains compounds **36-38**, compounds **39, 41** and **5** showed some inhibitory potency against FXIIa, only when a nitrile functional group was adjacent to the aliphatic side chain. When the aliphatic side chains in compound **39** and **41** were sterically inserted with bulky cyclic rings, both compounds **40** and **42** showed a complete loss of inhibitory activity against FXIIa. There is probably a limitation of flexibility at the binding site of ERH.

Considering tetrahydrofuran is a five-membered heterocycle, a series of compounds containing heterocyclic rings, such as cyclohexanamine (**43**), pyrrolidine (**44**), tert-butyl 3-aminopyrrolidine-1-carboxylate (**45**), pyrrolidin-3-amine (**46**), piperidine (**47**), piperidin-1-amine (**48**), morpholine (**49**), morpholin-4-amine (**50**), 1-methylpiperazine (**51**), benzyl piperazine-1-carboxylate (**52**) and piperazine (**53**), were synthesised. Compounds **49-51** showed IC₅₀ in a range of 33 μ M to 81 μ M against FXIIa. Similar to the structure of tetrahydrofuran, pyrrolidin-3-amine is involved with a secondary amine group on the ring instead of the oxygen atom. When tetrahydrofuran was replaced by pyrrolidin-3-amine or its derivatives, compounds **45** and **46** have slightly decreased inhibitory potency against FXIIa compared to the compound **2**. The other substituents of aliphatic heterocyclic rings (**44, 44, 47, 48, 52, 53**), were all inactive against FXIIa. The lack of heteroatoms in the ERH group is more likely to be the reason for the loss of activity of inhibitors.

The replacements of tetrahydrofuran with aromatic rings (aniline, 4-fluoroaniline and benzene-1, 4-diamine), led to neither compounds **54, 55** and **56**, but these did not have any inhibition against FXIIa. Only when a primary amine was attached to the aromatic ring at a *para* position, compound **57** showed little potency (α -FXIIa: $34.1 \pm 9.6 \mu\text{M}$, β -FXIIa: $39.8 \pm 6.4 \mu\text{M}$). This is again

indicated that the loss of activity of inhibitors may be caused by the lack of heteroatoms in the ERH group.

Furthermore, when ERH was replaced by aliphatic side chains and aromatic rings, such as 2-phenylethan-1-amine (**58**), 2-(pyridin-3-yl)ethan-1-amine (**3**), 2-(pyridin-4-yl)ethan-1-amine (**59**), 1-amino-3-phenoxypropan-2-ol (**1**), 2-amino-N-phenylethane-1-sulfonamide (**4**), a series of investigations was carried out. Except for the replacement of tetrahydrofuran with 2-phenylethan-1-amine (**58**), compounds (**1**, **3** and **59**) showed inhibitory potency against FXIIa. This suggested that more interactions (hydrogen binding by heteroatoms and van der waals interactions) were required at the end of ERH group.

When the tetrahydrofuran was replaced by pyrazol-amine or its derivatives, only compound **61** (pyrazol-amine derivatives) showed some potency with an IC₅₀ of $8.4 \pm 2.9 \mu\text{M}$ (α -FXIIa) and $9.4 \pm 3.5 \mu\text{M}$ (β -FXIIa). This result also suggested that the inhibition of compounds required more interactions (van der waals interactions and hydrogen binding) at the site of ERH group.

In conclusion, based on the potency results, the best compounds in this series so far were tetrahydrofuran urea (**2**) or 3-aminopropanenitrile urea (**41**) substituted by a 5-trifluoromethyl-2-pyrrolidin-1-yl group.

3.1.5. The SARs of urea moiety substitution at two –NH groups

Urea moiety is a carbamide, with two NH groups joined by a carbonyl (C=O) functional group. Urea and its derivatives have been investigated for their biological activities including antiatherosclerotic, antibiotic, hypoglycaemic effect and antitumour activities. As an important role in the metabolism of nitrogen-containing compounds, both NH groups (hydrogen binding interaction) are potentially important for the inhibitions of compounds against enzymes.

Therefore, a series of modifications of the urea moiety was undertaken, to exploit the SAR around it.

The two NH groups of the urea moiety may have hydrogen bonding interactions with the binding sites of enzymes. Both NH functional groups in urea moiety were replaced by carbon atom or oxygen atom individually or together. Also, instead of replacement of nitrogen, methylation of each NH group was carried out. Furthermore, the distance between the aromatic ring and the NH group was investigated and thus the addition of a CH₂ group was introduced for the extension of distance between NH group and aromatic ring.

A schematic procedure of modification of urea moiety at the NH groups is presented below (**Scheme 3.1**). Due to the limitation of commercially available chemicals, the modification of compound **2** was restricted. Instead of synthesizing tetrahydrofuran urea derivatives, compounds **39** and **41** were chosen as lead compounds for the investigation of urea moiety modification since they all had a similar inhibitory potency against FXIIa as compared to the compound **2**. The replacement of NH groups is highlighted in red in **Scheme 3.1**. The results for compounds inhibition against FXIIa are listed in **Table 3.4**.

Scheme 3.1 Schematic representation of the modification of urea moiety at NH groups

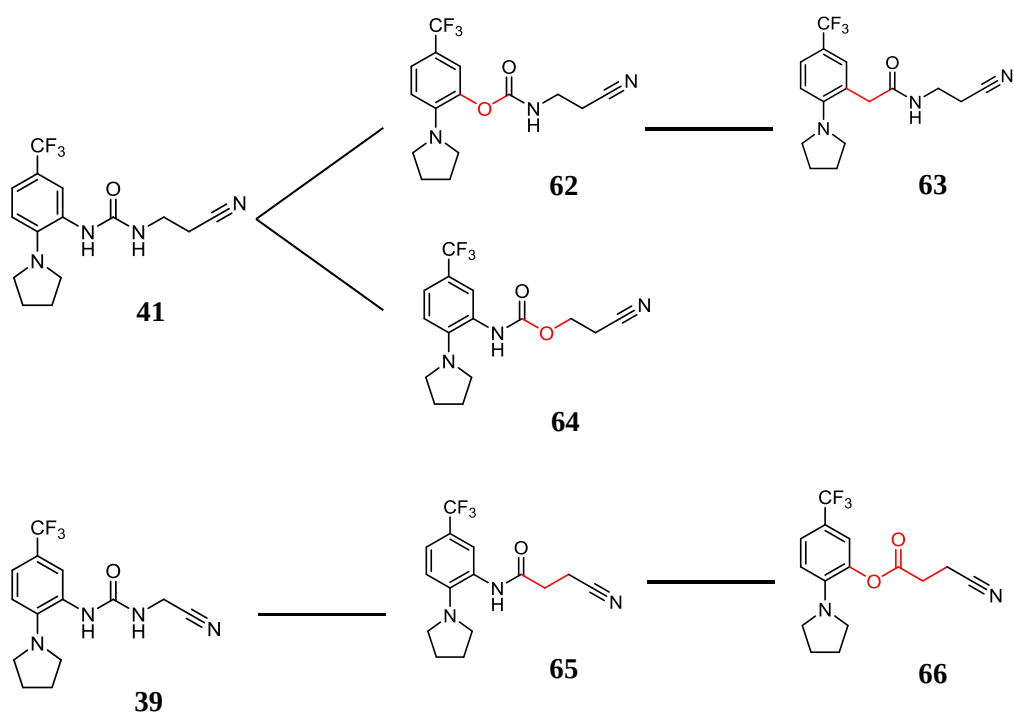


Table 3.4 Potency of urea derivatives based on modification of urea moiety

Compd	Modification	IC ₅₀ (μM) ^{a, b}	
		α-FXIIa	β-FXIIa
41		7.8 ± 1.6	8.1 ± 1.4
62	Replace NH group with O atom or C atom	NA	NA
63		82.7 ± 16.2	82.7 ± 16.2
64		14.1 ± 3.8	16.2 ± 3.2
39		17.6 ± 4.6	18.6 ± 3.4
65	Replace NH group with O atom or C atom.	55.7 ± 9.6	59.3 ± 11.2
66		167.1 ± 21.2	172.3 ± 28.1
67	Methylation of NH groups	82.5 ± 12.1	87.9 ± 9.3
68		NA ^c	NA
2	Extension of distance between aromatic ring and adjacent NH group	8.0 ± 1.4	8.5 ± 1.2
69		15.0 ± 10.6	17.9 ± 8.8

^a The data given are mean values derived from three replicates.

^b Results are expressed in mean ± SD

^c NA = not active: inhibition <10% at 100 μM

The replacement of two NH groups in urea moiety was performed with one adjacent to the aromatic ring and the other adjacent to the ERH functional group, reformed with oxygen atom or carbon atoms. When the NH group (adjacent to aromatic ring) was replaced by an oxygen atom (**62**) or carbon atom (**63**), the latter completely lost its activity; the inhibitory potency of the former was decreased as compared to compound **41**. When the NH group (adjacent to ERH group) was replaced by an oxygen atom (**64**) or carbon atom (**65**), the inhibitory potency of the former was only slightly decreased in comparison with compound **41**. The potency of the compound **65** was reduced by approximately 3-fold as compared to **39**. Finally, when the NH group (adjacent to the aromatic ring) was replaced by an oxygen atom and the NH group (adjacent to the ERH group) was replaced by methylene group (CH₂), compound **66** has the least activity (α-FXIIa: 152.1 ± 46.1 μM and β-FXIIa: 172.3 ± 28.1 μM) as compared to the compounds **63-65**. As a result, both NH groups in urea moiety were shown to

be highly significant for the activities of compounds against FXIIa, especially the NH group adjacent to aromatic ring.

When the NH group adjacent to ERH in compound **41** was methylated (**Figure 3.2**), leading to the formation of the compound **67**. Similar to compound **63** (the NH adjacent to ERH was replaced by a carbon atom), this new compound showed inhibitory potency of $82.5 \pm 12.1 \mu\text{M}$ (α -FXIIa) and $87.9 \pm 9.3 \mu\text{M}$ (β -FXIIa) (**Table 3.4**). The methylation of the NH group adjacent to the aromatic ring in compound **2**, resulted in compound **68**, which was inactive toward FXIIa. It was thus concluded that both of the NH groups in urea moiety were important functional group for compounds inhibition.

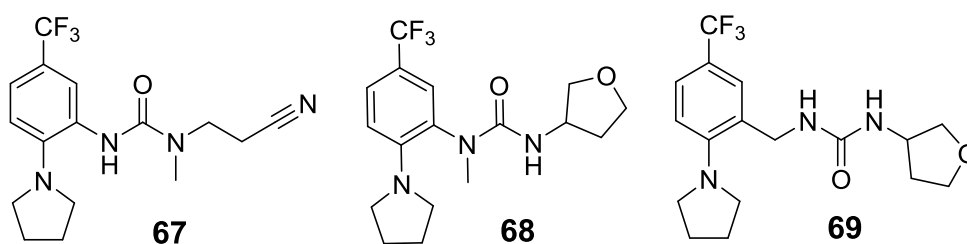


Figure 3.2 Structure of compounds **67-69**

Finally, the distance between the aromatic ring and urea moiety was taken into account. By inserting a CH_2 group, the inhibitory potency of compound **69** was of only 2-fold difference as compared to compound **2**. This means NH group (adjacent to aromatic ring) may be not necessary next to aromatic ring but it is essential for the inhibition of the compound against FXIIa. The potency of inhibitors was retained, as far as both NH groups in urea moiety maintained.

3.1.6. The SARs of urea moiety substitutions at carbonyl (C=O) group

Urea derivatives, for example, thiourea, have been found to exhibit a potent inhibiting effect on HIV-1 protease enzyme and also showed some properties of anticancer, anticonvulsive and sedative-hypnotic activities.

Previously, it was demonstrated that NH groups in the urea moiety are important for the inhibitory potency (section 3.1.5). Instead of the substitution of NH groups, an investigation of carbonyl (C=O) group was undertaken, which led to the formation of thiourea and cyclised urea (**Figure 3.3**). The results for compounds inhibition against FXIIa have been listed in **Table 3.5**.

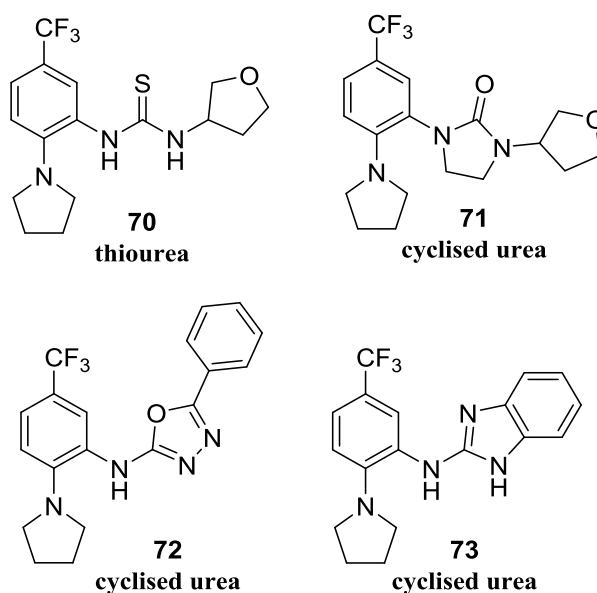


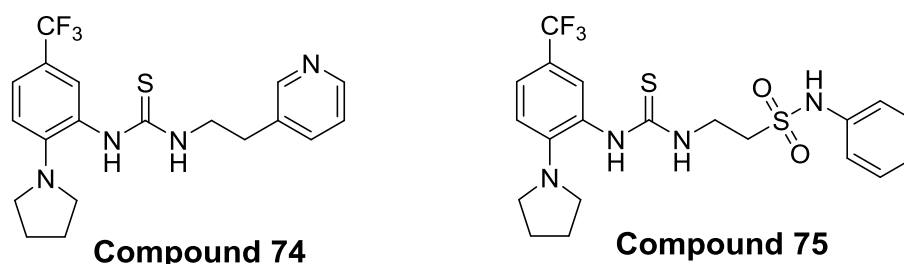
Figure 3.3 structures of thiourea **70** and cyclic ureas **71-73**

Table 3.5 Potency of urea derivatives based on modification of urea moiety

Compd	Types of urea	IC ₅₀ (μM) ^{a, b}	
		α-FXIIa	β-FXIIa
2	Urea	8.0 ± 1.4	8.5 ± 1.6
70	Thiourea	29.7 ± 7.8	31.2 ± 8.3
71		X ^c	X
72	Cyclised urea	NA ^d	NA
73		NA	NA

^a The data given are mean values derived from three replicates.^b Results are expressed in mean ± SD^c “X” = unsuccessful synthesis^d NA = not active: inhibition <10% at 100 μM

With the replacement of urea with thiourea, the inhibitory potency of compound **70** (thiourea) was decreased as compared to the compound **2**. As oxygen is more electronegative than sulphur, an electron-withdrawing carbonyl functional group is likely to be more active and have stronger hydrogen bonding interactions with the active site of FXIIa enzyme.

**Figure 3.4** Structures of thiourea **74** and **75****Table 3.6** Potency of thioureas **74** and **75**

Compd	Types of urea	ERH substitution	IC ₅₀ (μM) ^{a, b}	
			α-FXIIa	β-FXIIa
3	Urea	2-(pyridin-3-yl)ethan-1-amine	6.9 ± 1.0	8.3 ± 0.7
4		2-amino-N-phenylethane-1-sulfonamide	10.3 ± 0.8	14.2 ± 1.3
74	Thiourea	2-(pyridin-3-yl)ethan-1-amine	NA ^c	NA
75		2-amino-N-phenylethane-1-sulfonamide	NA	NA

^aThe data given are mean values derived from three replicates.^b Results are expressed in mean ± SD^c NA = not active: inhibition <10% at 100 μM

Two other thiourea compounds **74** and **75** were used for further thiourea exploration (**Figure 3.4** and **Table 3.6**). Compared to their corresponding urea compounds **3** and **4**, it was observed that both newly synthesised thiourea compounds were not active against FXIIa. This indicated that carbonyl group of urea moiety is highly important for urea compounds inhibition.

The replacement of urea moiety by a cyclised urea, resulted in compounds **71-73**. The chemical synthesis of compound **71** was not successful after several attempts. Compounds **72** and **73** were synthesised and screened against FXIIa. Both compounds (**72** and **73**) were completely inactive against FXIIa.

Considering that the cyclised urea to some extent is less flexible in comparison with chained urea in compound **2**, indicated that the compounds require a certain flexibility at the site of urea moiety in order to obtain better interactions between the inhibitor and enzymes.

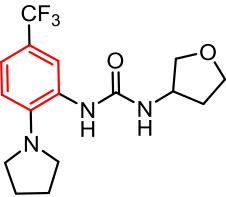
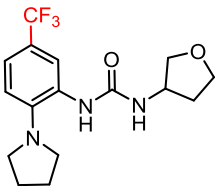
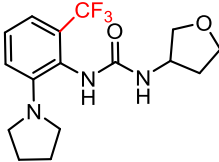
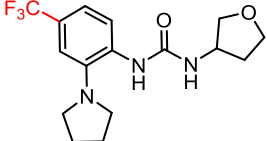
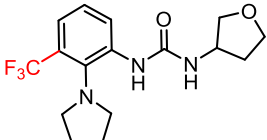
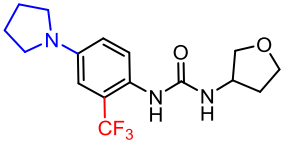
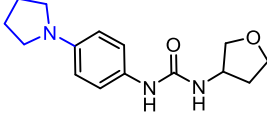
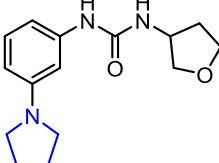
Therefore, based on compounds inhibitory potency, chained urea was retained during exploration of the SARs at other positions in the molecule.

3.1.7. The SARs of position between trifluoromethyl group and pyrrolidine ring

Trifluoromethyl group was at a *para* position to pyrrolidine and was at a *meta* position to urea moiety in lead compound **2**. A series of the Compounds **76-81** was synthesized and is presented in **Table 3.7**.

Table 3.7 Potency of urea derivatives based on the trifluoromethyl group, the pyrrolidine ring and the urea moiety at different positions

Compd	Functional groups involved	IC ₅₀ (μM) ^{a, b}	
		α-FXIIa	β-FXIIa

			
2		8.0 ± 1.4	8.5 ± 1.6
76		NA ^c	NA
77		NA	NA
78		NA	NA
79		NA	NA
80		NA	NA
81		NA	NA

^a The data given are mean values derived from three replicates.

^b Results are expressed in mean ± SD

^c NA = not active: inhibition <10% at 100 μM

It was observed that the modification of the trifluoromethyl group, pyrrolidine ring, urea moiety and ERH functional group, the compounds **76-79** were all inactive against FXIIa. These results indicated that the positioning of the trifluoromethyl functional group and the pyrrolidine ring is very restricted. It was observed that the trifluoromethyl group has to be at a *para* position to the pyrrolidine ring and at a *meta* position to urea moiety, otherwise, the inhibitory potency of compounds would be completely lost. The trifluoromethyl functional group is electron-withdrawing. Compared to compound **2** (the trifluoromethyl group at a *para* position to the pyrrolidine ring), compound **78** (the trifluoromethyl group at a *meta* position to the pyrrolidine ring) was totally inactive against FXIIa. This indicated that steric effect of the trifluoromethyl functional group may be the key for the loss of the inhibition of the inhibitors. In the absence of the trifluoromethyl functional group, compounds **80-81** were also inactive against FXIIa while the pyrrolidine ring is at *para*- or *meta*-positions to urea moiety separately. It again demonstrated that the trifluoromethyl group was important for compounds inhibition (referred in section 3.1.2). In conclusion, it was found that only when the trifluoromethyl group is at a *para* position to the pyrrolidine ring, it showed some inhibitory potency.

3.1.8. The SARs of trifluoromethyl group, pyrrolidine ring and ERH functional group simultaneously

The lead compound **2** was mainly used in the previous studies of SARs modification. In order to further demonstrate the importance of trifluoromethyl and pyrrolidine group, active compounds **1**, **3**, **4**, **41** and **44** were explored. The procedure of modification was described in **Figure 3.5**.

Figure 3.5 Schematic procedure of modifications between the trifluoromethyl group (R^1), the pyrrolidine ring (R^2) and the ERH functional group (R^3).

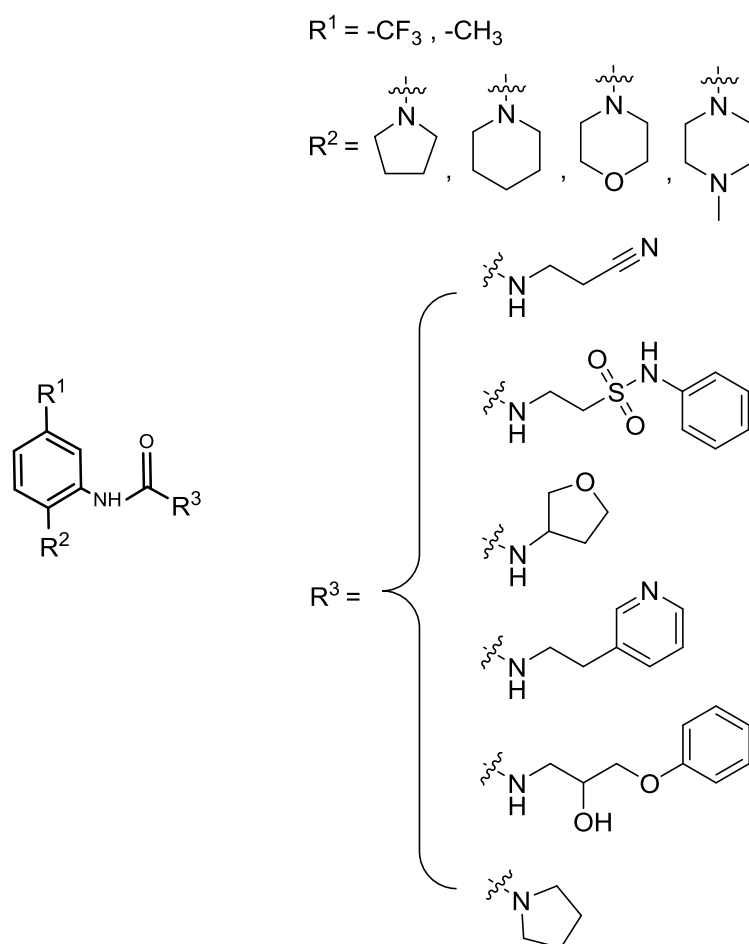
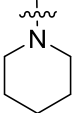
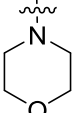
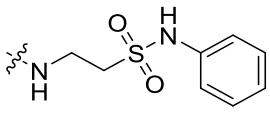
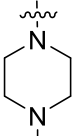
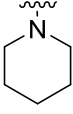
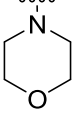
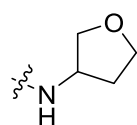
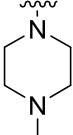
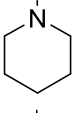
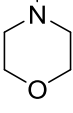
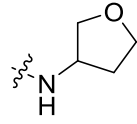
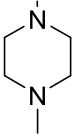


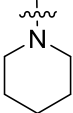
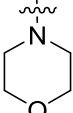
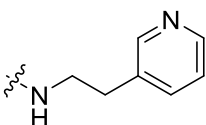
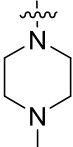
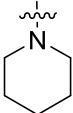
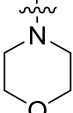
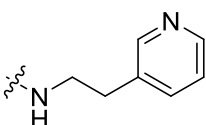
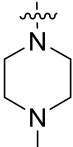
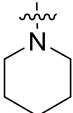
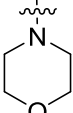
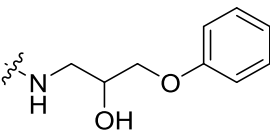
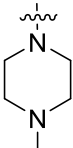
Table 3.8 Potency of urea derivatives based on modification in Figure 3.5

Compd	R ¹	R ²	R ³	IC ₅₀ (μM) ^{a,b}	
				α-FXIIa	β-FXIIa
41				7.8 ± 1.6	8.1 ± 1.4
82				NA ^c	NA
83	-CF ₃			NA	NA
84				NA	NA
85				NA	NA
86				NA	NA
87	-CH ₃			NA	NA
88				NA	NA
4				10.3 ± 0.8	14.2 ± 1.3
89				NA	NA
90	-CF ₃			NA	NA
91				NA	NA

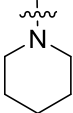
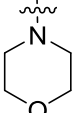
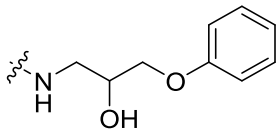
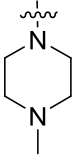
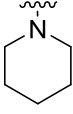
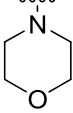
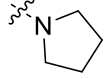
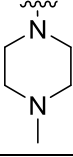
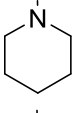
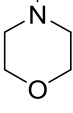
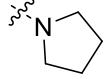
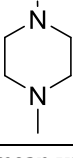
Continue Table 3.8

Compd	R ¹	R ²	R ³	IC ₅₀ (μM) ^{a, b}	
				α-FXIIa	β-FXIIa
92				NA	NA
93				NA	NA
94	-CH ₃			NA	NA
95				NA	NA
2				8.0 ± 1.4	8.5 ± 1.2
19				NA	NA
26	-CF ₃			NA	NA
27				NA	NA
10				NA	NA
96				NA	NA
97	-CH ₃			NA	NA
98				NA	NA

Continue Table 3.8

Compd	R ¹	R ²	R ³	IC ₅₀ (μM) ^{a, b}	
				α-FXIIa	β-FXIIa
3				6.9 ± 1.0	8.3 ± 0.7
99				NA	NA
100	-CF ₃			NA	NA
101				NA	NA
102				NA	NA
103				NA	NA
104	-CH ₃			NA	NA
105				NA	NA
1				13.2 ± 2.4	14.3 ± 3.5
106				NA	NA
107	-CF ₃			NA	NA
108				NA	NA

Continue Table 3.8

Compd	R ¹	R ²	R ³	IC ₅₀ (μM) ^{a, b}	
				α-FXIIa	β-FXIIa
109				NA	NA
110				NA	NA
111	-CH ₃			NA	NA
112				NA	NA
44				101.6 ± 24.7	103.2 ± 21.7
113				NA	NA
114	-CF ₃			NA	NA
115				NA	NA
116				NA	NA
117				NA	NA
118	-CH ₃			NA	NA
119				NA	NA

^a The data given are mean values derived from three replicates.

^b Results are expressed in mean $\pm$ SD

^c NA = not active: inhibition <10% at 100 μ M

When the replacement of the trifluoromethyl group with methyl group and the substitution of pyrrolidine ring with piperidine morpholine, 1-methylpiperazine happened individually or together, it was observed that all the compounds (**84-98** and **100-119**) had no inhibitory potency against FXIIa compared to corresponding active compounds (**1, 3, 4, 41** and **44**) (**Table 3.8**).

This again demonstrated that trifluoromethyl and pyrrolidine both were highly important for the urea compounds potency. Limited modifications are at the site of pyrrolidine ring and trifluoromethyl functional group. Considering the relationship between inhibitor activity and ERH structure, it suggested that modification of ERH functional group is more flexible. Further research will mainly focus on ERH group for the exploration of the SARs of urea compounds in section 3.4.2.

3.2. Conclusion

In order to explore the SAR of the starting urea compound, a variety of substituents were tried on all the different positions. It was observed that the replacement of trifluoromethyl group has to be with a strong electron withdrawing group to show some inhibitory potential. The nitro moiety is the only available functional group for the trifluoromethyl group substitution. In the absence of the trifluoromethyl group, the inhibition of the inhibitors against FXIIa was completely inactive. The replacement of the pyrrolidine ring has its limitations and only a smaller ring size (azetidine) or slightly modified pyrrolidine derivative (2-methylpyrrolidine) showed their activity against

FXIIa. This could occur due to limited interaction between the site of pyrrolidine and enzyme receptor.

For the ERH functional group, a diversity of substitutions was available and unfortunately the IC_{50} for the inhibition of compounds were all in the micromolar range. The modifications at the site of ERH seem have no much influence on the activities of urea compounds against FXIIa. At the end of the ERH functional group, stronger interactions are required for the increase of compound inhibition.

The NH groups in the urea moiety are critical for inhibitory activity of compounds. The replacement of either NH groups led to a complete loss of the inhibition. Urea derivatives such as, thiourea and cyclised urea also demonstrated that non-cyclised urea is the better choice for the inhibitory potency of compounds. Some flexibilities are needed for compounds binding to enzymes at the site of urea moiety.

Finally, the position between the trifluoromethyl group, the pyrrolidine ring and the urea moiety seems strict. The trifluoromethyl functional group has to be *para*-position to the pyrrolidine ring and to be *meta*-position to the urea moiety. These results potentially indicated that the active site of FXIIa has limited binding area for inhibitors indirectly.

3.3. Docking analysis

3.3.1. Molecular modelling

In order to understand the interactions between the lead compounds and FXIIa and to further explore SARs, molecular modelling studies were carried out. A hybrid 3D model of FXIIa was used to perform docking studies.

3.3.1.1. Hybrid FXIIa model

Coagulation protein FXII has been known for fifty years, but the crystal structure of the catalytic side chain has only recently been obtained and published (by Jonas Emsley group. at Nottingham University).¹⁵² By using two different recombinant FXII proteins, two structures of FXII (FXIIac and FXIIc) were crystallised and both were reported by Emsley group. Both crystal structures showed an unexpected zymogen-like conformation. Recently, the Emsley group successfully obtained the β -FXIIa crystal structure. (Data has been used with permission)

Unfortunately, this β -FXIIa is only at medium solution (4 Å) and increases the difficulties to get better understanding of the binding site with inhibitors. Thus, the Emsley group created a homology model for the activated conformation of the FXIIa protease. This model is built using a similar approach by previous authors (Charlotte group)¹⁵³ and is based on all of the useful data collected in previous crystallography work.¹⁵²

3.3.1.2. Docking studies

The docking study by the Emsley group predicts an ARG34 side chain at the site of the CTI central inhibitory loop P1 forms a salt bridge with ASP189 in the FXIIa S1 pocket (**Figure 3.6**). CTI helix α 1 has TRP22 and interacts with the FXII S3 pocket. ASP60A from the FXIIa forms a salt bridge with the CTI helix α 2 ARG43. Then Emsley and coworkers expressed and purified recombinant variants of CTI. They substituted key residues predicted to interact with FXIIa. Except for CTI amino acid substitutions W37A, W39A, R27A and R42A had no effects, substitution R34A negated all activities of inhibitors. Also, CTI amino acid substitutions L35A, R42A/R43A, G32W and L35A reduced

inhibitory activity by 41-fold, 100-fold, 108-fold and 158-fold respectively. Short-length CTI is synthesised by using peptides spanning CIT residues 20-44. Its inhibition is 3-fold to 4000-fold less than that of full-length CTI. All of this data validates the interaction of this canonical model of the FXIIa-CTI. TRP22 from CTI helix α 1, ARG 43 from CTI helix α 2, and ARG34 from CTI central inhibitory loop P1 showed effectively binding to S3 and S1 pockets respectively.

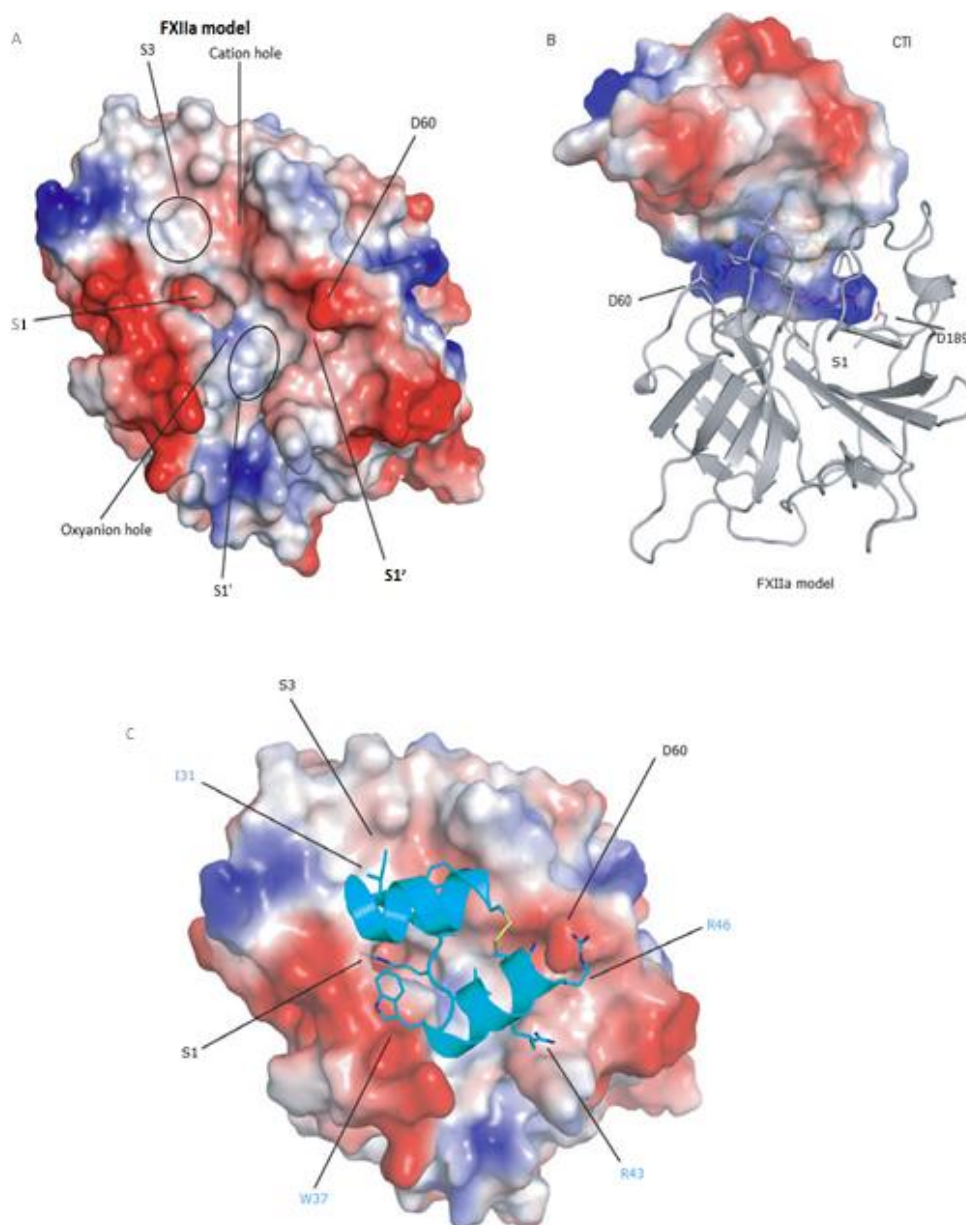


Figure 3.6.¹⁵² Charge surface representations of the activated FXII (FXIIa)–corn trypsin inhibitor (CTI) interaction (blue = positive, red = negative). (A) The FXIIa model is shown with the key surface pockets labeled. (B) Docked complex of FXIIa (shown as a cartoon in gray) and CTI shown as a transparent charged surface illustrating the basic region interacting with the negatively charged S1 (D189) residue shown as a stick. (C) FXIIa protease as viewed in (A) but with CTI residues 20–44 shown as a cartoon in cyan, with key residues labelled as sticks in cyan.

3.3.2. SARs study based on hybrid model FXIIa

The docking study was based on the hybrid model FXIIa. The hybrid model FXIIa is created by the Emsley group.¹⁵⁴ This hybrid model is a structure for the

activated conformation of the FXIIa protease. Therefore, binding modes could not be determined unambiguously. For the numbering of amino acid sequences, it is based on the mature sequence of FXII (chymotrypsin numbering). A series of urea derivatives are assessed by Schrödinger (Glide) with default parameters in docking simulation. All of ligands are minimized with lowest binding energy in the active site. For each ligand, 20 solutions were generated. They are all ranked based on glide scoring function. For each compound, the best pose was retained. After analysis of docking solutions, two binding modes of urea lead compounds are suggested.

3.3.3. First binding mode

3.3.3.1. Overview of first binding mode

The first binding mode was associated with a series of urea compounds containing specific ERH groups, which are aliphatic side chains with nitrile functional group and aliphatic heterocycle. The best binding mode of compound **2** (based on the Glide score = -5.955) was selected and illustrated for first binding mode below (**Figure 3.7**)

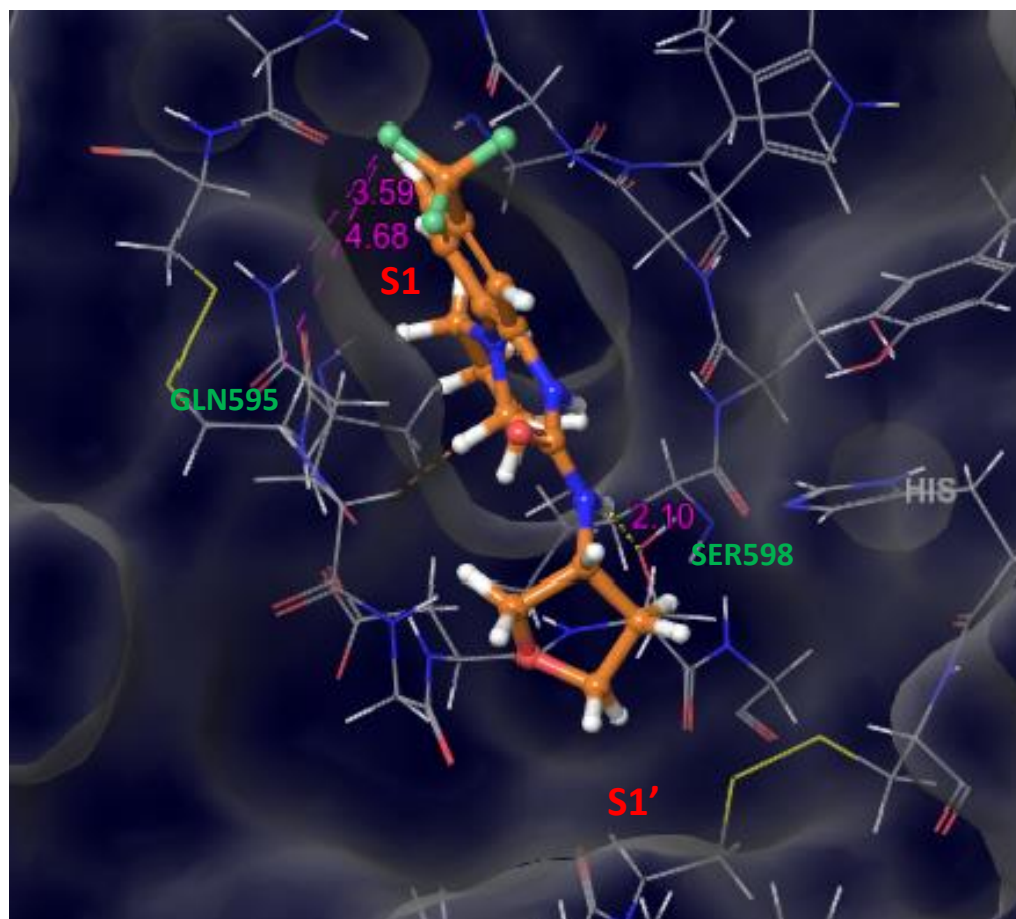
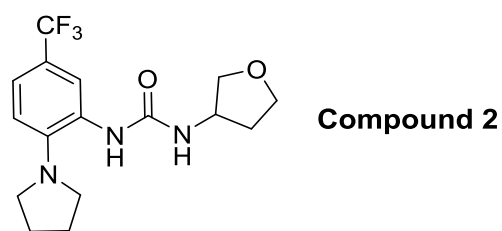


Figure 3.7: The best binding pose of compound **2** in the active site of the FXIIa hybrid model. The FXIIa model is shown with the key surface pockets labeled. Compound **2** is presented in brown CPK and amino acid residues around ligands are depicted in grey CPK. (Glide score = -5.955)

As depicted in **Figure 3.7**, fluorine atoms of the trifluoromethyl functional group in compound **2** are pointing to GLN595 in the S1 pocket. After the measurements of distance between fluorine atoms and carbon/nitrogen of GLN595 ($\text{F} \cdots \text{C}_{\text{GLN595}}$, distance = 4.7 Å; $\text{F} \cdots \text{NH}_{\text{GLN595}}$, distance = 3.6 Å), it indicated that the trifluoromethyl group is not close enough to have a van der Waals (halogen bond) interaction between trifluoromethyl and amino acid

GLN595. The binding site of the trifluoromethyl functional group in the first binding mode is still unclear. Methoxyltrifluoromethyl group ($-\text{OCF}_3$) was then introduced for further investigation of the interaction between the trifluoromethyl group and amino acid GLN595 in section **3.4.1**.

The pyrrolidine ring has a carbon-hydrogen backbone. This means there is probably a strong van der waals interaction between the pyrrolidine ring and the enzyme. Based on the best binding pose in **Figure 3.7**, it clearly showed the pyrrolidine ring geometrically fitted well inside the FXIIa S1 pocket, which leads to a strong van der waals interaction between pyrrolidine rings and amino acids in the S1 pocket.

Similar to the pyrrolidine ring, tetrahydrofuran (saturated heterocyclic ring), as an ERH functional group, also has a strong van der waals interaction with the enzyme. Based on the binding pose, it indicated the ERH group seems to have an interaction with the S1' pocket of the enzyme. In order to have a better understanding of binding conformation, a binding pose of ligand is presented within surrounding amino acids of FXIIa (**Figure 3.8**). It clearly showed that tetrahydrofuran was close to the S1' pocket in FXIIa enzyme, indicating a strong interaction of van der waals between the tetrahydrofuran and the S1' pocket.

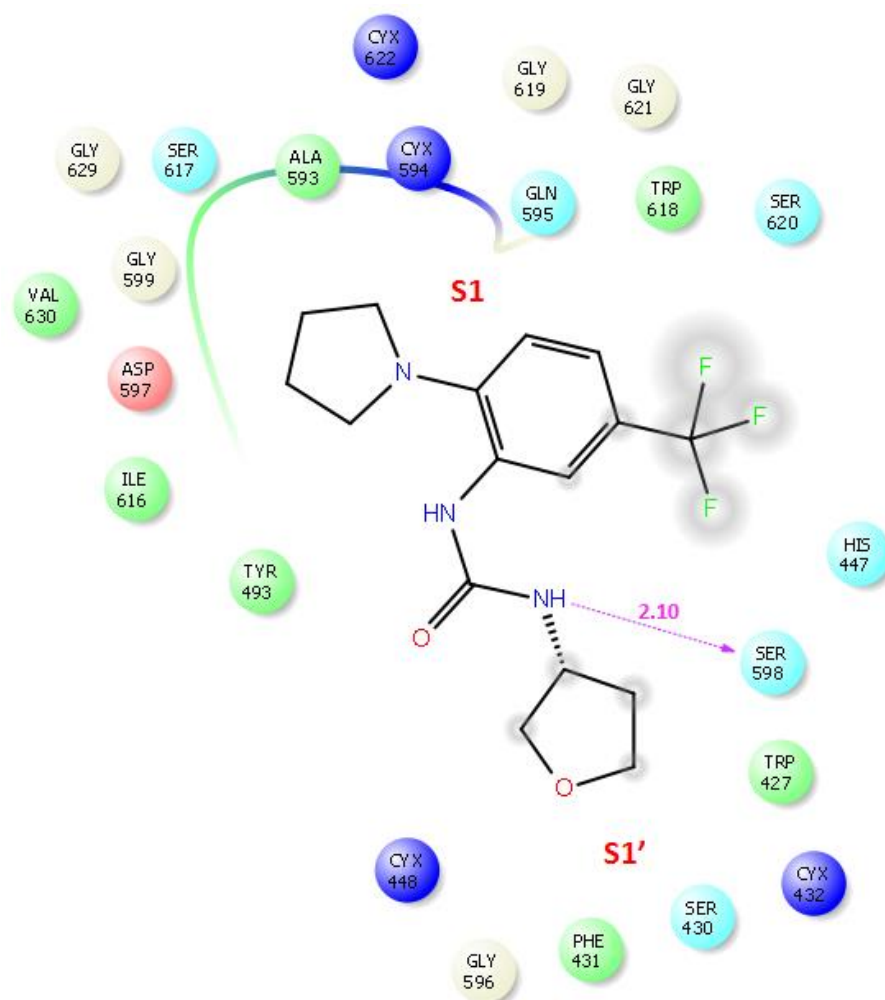


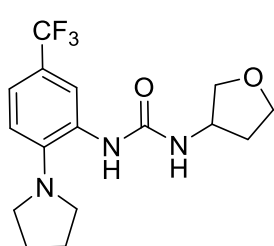
Figure 3.8: Interactions between FXIIa active site and compound 2. The Figure is generated by schrödinger (Glide) Ligand interaction diagram. The hydrogen bonding interaction (N-H---O_{ser598}, distance = 2.6 Å) between the NH group of compound 2 and SER598 is displayed by dotted lines (purple).

For the urea moiety, according to compound 2-FXIIa interaction (**Figure 3.8**), the hydrogen atom of the NH group adjacent to the ERH functional group has hydrogen bonding with the oxygen atom of SER598 (N-H---O_{ser598}, distance = 2.6 Å). As mentioned in section 3.1.5, the NH groups in urea moiety have important influence on the FXIIa inhibitors activities. This means the interaction between the NH group in urea moiety and SER598 (hydrogen bonding) is the key for the inhibition of the FXIIa inhibitor (compound 2).

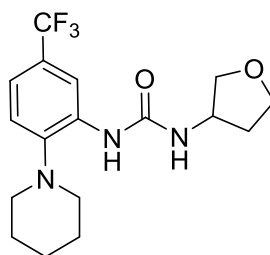
The Emsley group stated that the S1' pocket is a distinctive feature of FXIIa. Regarding the stabilization in pockets, compound **2** has interaction at the site of the S1' pocket through van der Waals interaction between the tetrahydrofuran ERH group and S1'pocket. This could be an explanation for the activity of compound **2** towards FXIIa. According to first binding mode, it is still difficult to explain the relationship between trifluoromethyl group and inhibitory activity of FXIIa. Furthermore, because compound **2** only showed limited binding interactions with enzyme FXIIa, it could explain the potency of inhibitor in the micromolar range.

3.3.3.2. Discrimination of active compound and inactive compounds in first binding mode

The first binding mode was established, following up with discriminating active compounds and inactive compounds based on the relationship of structure and activities (SARs). Initially, active compound **2** and its inactive derivative compound **19** are selected and represented to distinguish the different binding pose between active- and inactive- compounds (**Figure 3.9**).



Active compound 2



Inactive compound 19

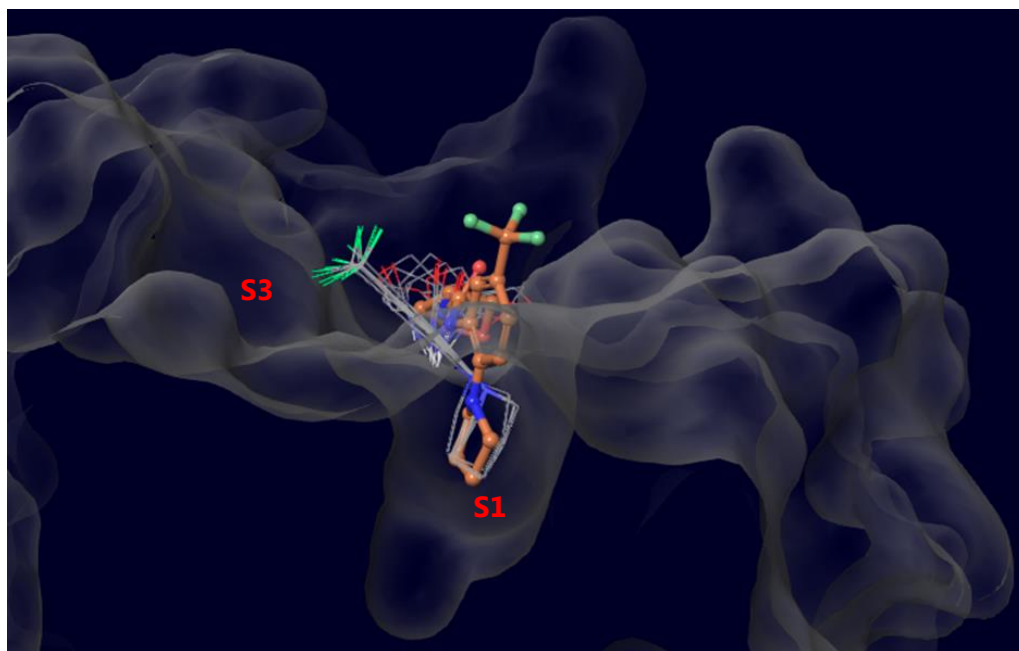


Figure 3.9: Binding poses of active compound **2** and inactive compound **19**. The FXIIa model is shown with the key surface pockets labeled. Compound **2** is presented in brown CPK (Glide score = -5.955) and Compound **19** is depicted in grey CPK. Over 20 poses of compound **19** are also presented.

Similar to compound **2**, the piperidine ring of inactive compound **19** was located in the S1 pocket and showed the same interaction at the site of the pyrrolidine ring in compound **2**. The urea moiety and ERH functional group in compound **19** were pointing towards the S1' pocket. Furthermore, according to **Figure 3.9**, the orientation of the trifluoromethyl group in both compounds showed significant difference. Instead of the trifluoromethyl group pointing out of the S1 pocket, the ligand can not geometrically fit in S1 pocket between the aromatic ring (adjacent to trifluoromethyl group) and the piperidine ring. The bending pose to some extent forced the trifluoromethyl group to orientate towards the S3 pocket of FXIIa. The explanation for this case is, ligand (compound **19**) with larger size of piperidine ring attempted to fit all of the functional groups at the bind site of FXIIa, which lead to the change of compound binding conformation. As a result, it leads to inactive compound **19** toward FXIIa. Moreover, all the

poses of inactive compound **19** were superposed and they all consistently presented in the same binding way as shown in the first pose of compound **19** (**Figure 3.9**).

We also analysed other inactive compounds associated with compound **2** and they all showed the same results as compound **19**. By repeating the same analysing protocol of compound **2**, we also investigated other active compounds mentioned in section 3.3.1.1., the docking studies showed the results similar to compound **2**.

Taken together, these results indicate that this binding mode (orientation of the trifluoromethyl group) may be considered as discriminating between active and inactive compounds within the series presented in this study.

3.3.4. Second binding mode

3.3.4.1. Overview of second binding mode

The second binding mode is associated with a series of urea compounds, containing different ERH groups (combination of aliphatic side chains with aromatic rings), such as 2-(pyridin-3-yl)ethan-1-amine, 2-(pyridin-4-yl)ethan-1-amine, 1-amino-3-phenoxypropan-2-ol, and 2-amino-N-phenylethane-1-sulfonamide. 2-(pyridin-3-yl)ethan-1-amine urea compound **3** is presented under this circumstance. The best binding mode of compound **3** was selected and illustrated for the second binding mode below (**Figure 3.10**).

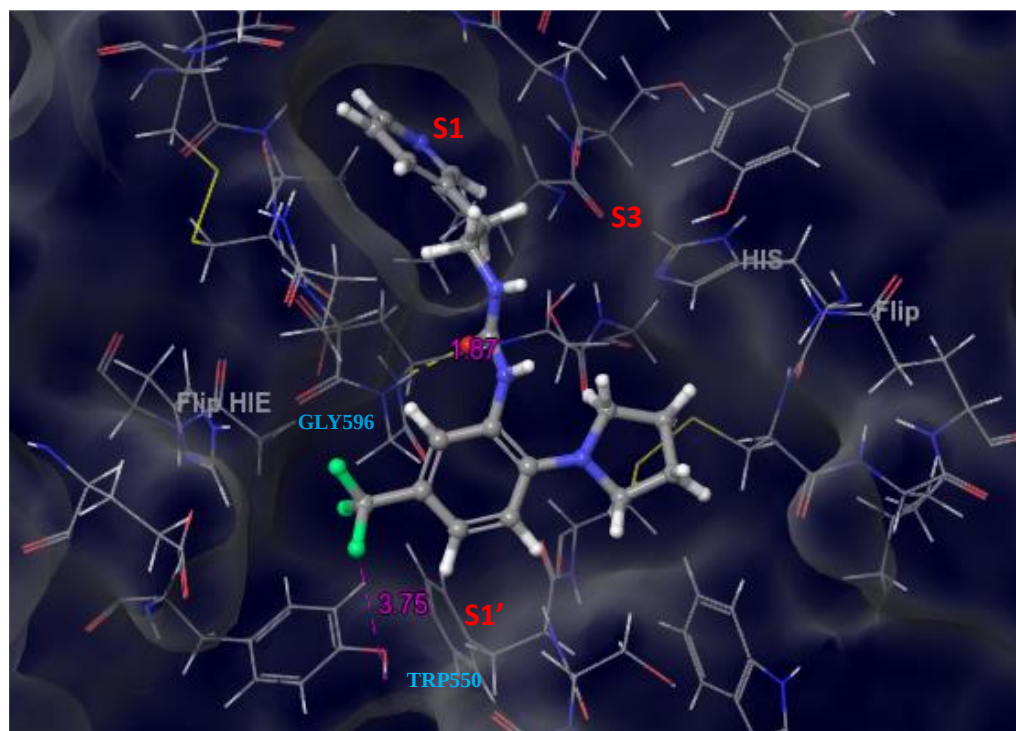
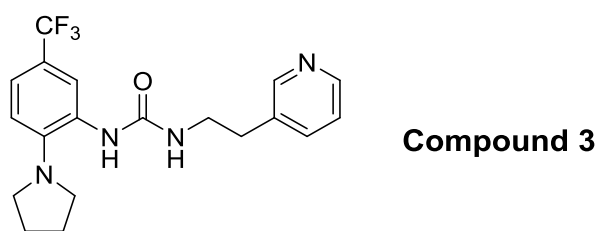


Figure 3.10: The best binding pose of compound **3** in the active site of the FXIIa hybrid model. The FXIIa model is shown with the key surface pockets labeled. Compound **3** is presented in grey CPK. (Glide score = -7.671)

As depicted in **Figure 3.10**, the fluorine atoms of the trifluoromethyl functional group in compound **3** are pointing towards the S1' pocket instead of interacting with GLN598 (S1 pocket) in compound **2** (first binding mode in section 3.3.3.1). Two amino acids are involved with the interaction of the trifluoromethyl group, which are TRP550 and HIS542. Via the measurements of distance between fluorine atoms and hydrogen of TRP550 (H---C_{TRP550}, distance = 3.8 Å), this indicated that the trifluoromethyl group is close to amino acid TRP550 and therefore there will have strong hydrogen bonding interaction.

For the pyrrolidine ring, according to the binding pose of compound **3**, it was found that the pyrrolidine ring is located in the S1' pocket of FXIIa. As an aliphatic five-membered saturated heterocycle, the pyrrolidine ring interacts with amino acids by van der waals at the binding site of the S1' pocket.

2-(pyridin-3-yl)ethan-1-amine is unsaturated heterocyclic ring in the ERH functional group of compound **3**. Similar to the pyrrolidine ring, it could have strong van der waals interaction with the enzyme. Based on the binding pose, the ERH group was found to interact with the S1 pocket of enzyme FXIIa. The details of binding information between the ERH group and the S1 pocket is illustrated in **Figure 3.11**. It is clearly showed that 2-(pyridin-3-yl)ethan-1-amine is surrounded with amino acids in the S1 pocket and the ERH group indeed has strong interaction of van der vaals with enzyme FXIIa. Compared to compound **2** in section 3.3.3.1, the ERH functional group of compound **2** in the first binding mode pointed towards the S1' pocket of FXIIa and may be likely to have some interactions (van der waals) between ERH functional group and S1' pocket.

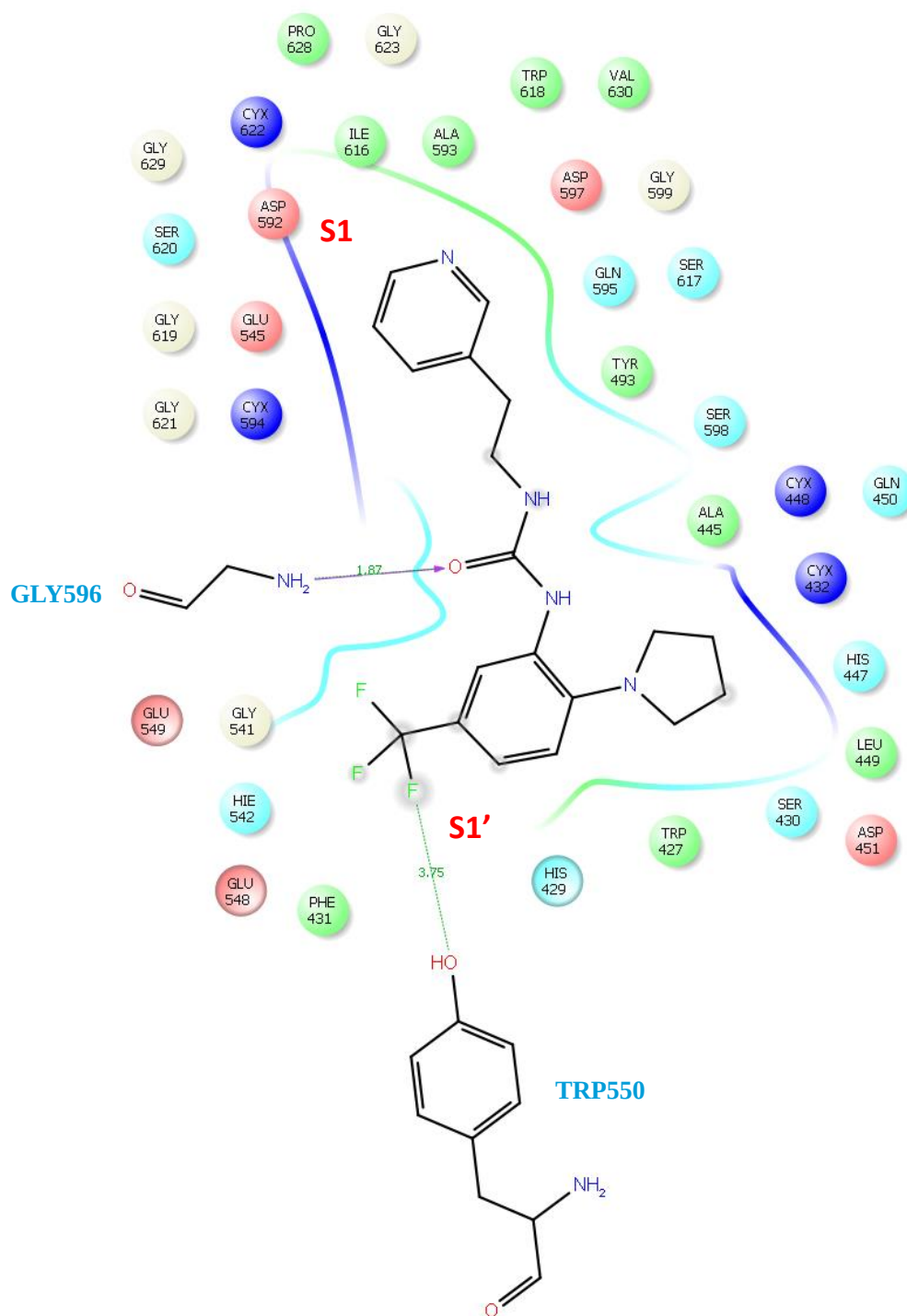


Figure 3.11: Interaction between FXIIa active site and compound **3**. The Figure is generated by schrödinger (Glide) Ligand interaction diagram. The dotted lines (green) represented the interactions between the halogen fluorine (-F) in the trifluoromethyl group of compound **3** and hydrogen atom in residue TRP550. (F---H-O_{TRP550}, distance = 3.8 Å). Also, there is a hydrogen interaction between the carbonyl group in the urea moiety and the hydrogen atom in the NH group of residue GLY596 (C=O---H-N_{TRP550}, distance = 1.9 Å).

For the urea moiety, according to compound **3**-FXIIa interaction (**Figure 3.11**), the oxygen atom of the carbonyl group (C=O) has hydrogen bonding with the hydrogen atom of GLY596 (C=O---H-N_{GLY596}, distance = 1.9 Å).

The distinctive features of FXIIa is the S1' pocket. Considering the stabilization of ligand in pockets, compound **3** interacted with amino acids at the site of the S1' pocket through van der waals contacts. Similar to the trifluoromethyl group, the pyrrolidine ring fitted in the S1' pocket and has the van der waals interaction at the site of the FXIIa S1' pocket. Furthermore, in comparison with first binding mode, the second binding mode is more convincing in terms of the relationship between the trifluoromethyl group and the inhibitory activity against FXIIa. Compound **3** has the potency of inhibitor only in the micromolar range. Further research of increasing binding site between the compound and the enzyme is required in this circumstance (section 3.4.2).

3.3.4.2. Discrimination of active compound and inactive compounds in second binding mode

After establishing the second binding mode, the follow up step is to discriminate active compounds and inactive compounds based on SARs. Active compound **3** and its inactive derivative compound **99** are selected for distinguishing the different binding pose between active- and inactive- compounds in second binding mode (**Figure 3.12**).

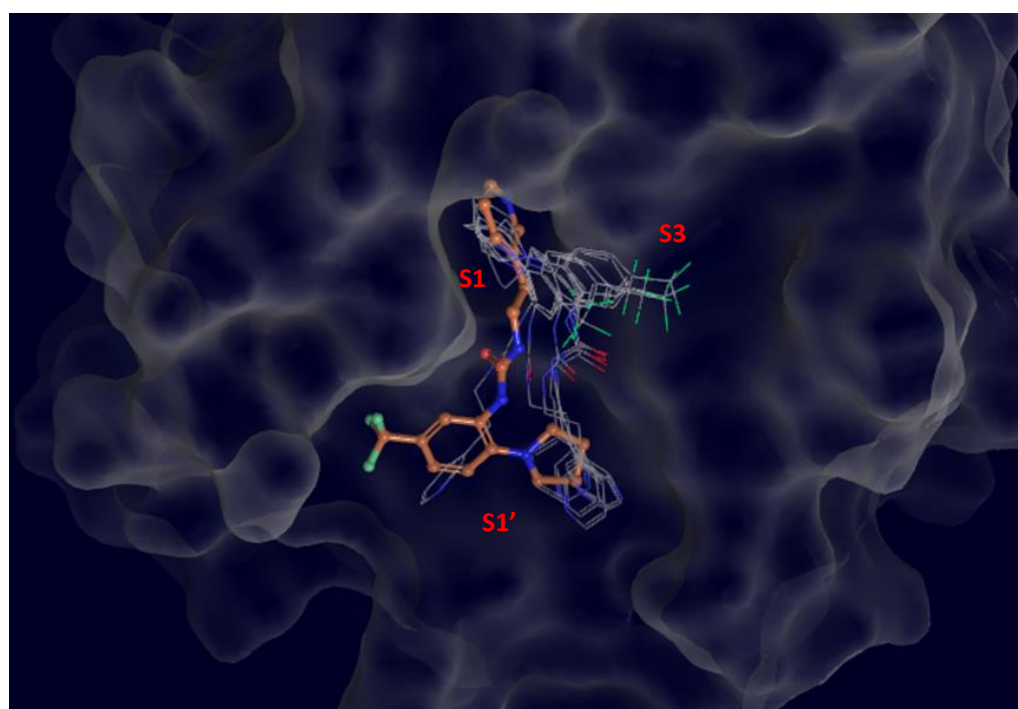
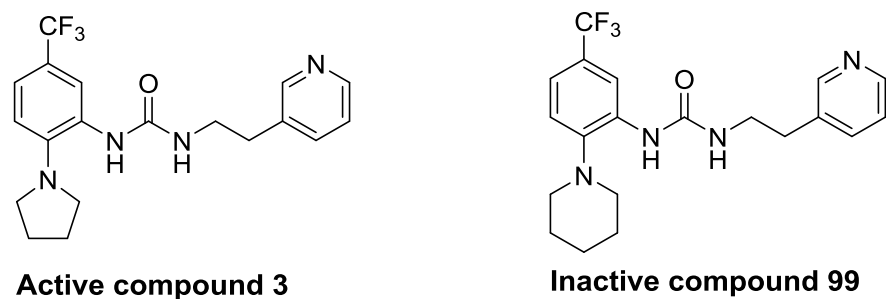


Figure 3.12: binding poses of active compound **3** and inactive compound **99**. The FXIIa model is shown with the key surface pockets labeled. Compound **3** is presented with brown CPK. Compound **99** is depicted with grey CPK. Over 20 poses of compound **99** are also presented.

In comparison with active compound **3**, the piperidine ring of inactive compound **99** was located in the S1 pocket instead of the S1' pocket. Its urea moiety and the ERH functional group were pointing towards the S1' pocket and

showed some interaction between the ERH group and binding site of the S1' pocket of FXIIa.

Furthermore, according to **Figure 3.12**, the trifluoromethyl group in both compounds showed completely different binding areas. Instead of interacting with the distinctive S1' pocket in compound **3**, the trifluoromethyl group of inactive compound **99** was located and orientated towards the S3 pocket of FXIIa. It was discovered that the ligand did not geometrically fit to active sites of FXIIa in compound **99** between the aromatic ring (adjacent to the trifluoromethyl group) and the piperidine ring. In the second binding mode, the binding pose of inactive compound **99** was the same to the cases of inactive compound **19** in the first binding mode. Moreover, all inactive compound **99** poses were superposed and almost every pose showed the same binding way, which is the first pose of compound **99** (**Figure 3.12**). Other inactive compounds associated with compound **3** were analysed and all results are the same as compound **99**.

The binding position of compound **99** was changed during the interaction between ligand and enzyme. In order to explain this case, the size of the piperidine ring was taken into account. Because of this, the inactive compound **99** must have difficulties fitting the binding site of the S1' pocket, which leads to the change of compound **99** conformation. The interaction was disturbed between compound **99** and enzyme FXIIa at the site of the S1' pocket. Taken together, these results indicated that the S1' pocket must have limited binding space for interaction. As a result, it lead to inactive compound **99** toward FXIIa. For other active compounds and their inactive derivatives mentioned in section 3.3.2.1, they were all investigated by using the same analysing protocol of

compound **3** and **99**. Similarly, the same agreement with poses of active compounds and inactive compounds came out.

In conclusion, the second binding mode presented that the trifluoromethyl group was orientated towards the S1' pocket; the pyrrolidine ring was next to the trifluoromethyl group and located in the S1' pocket. This binding mode is more likely to be the better binding pose of ligand-receptor. Also, it was considered as discriminating between active and inactive compounds within the series presented in this study.

3.4. New molecules chosen for two binding modes

Two binding modes have been established and described in section 3.3.2. In order to evaluate both modes and justify which binding mode is more likely to be reliable, additional molecules were suggested for demonstrating these two binding modes (**Figure 3.13**).

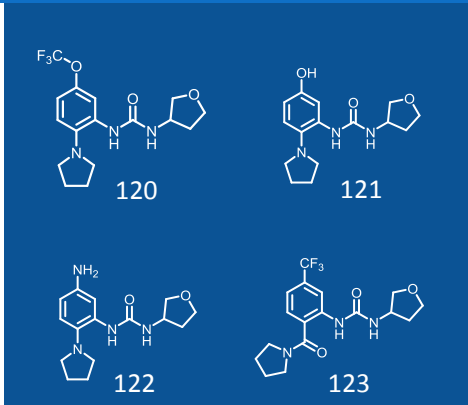
3.4.1. Molecules chosen to validate first binding mode

For the first binding mode, the trifluoromethyl functional group is associated with the inhibitory potency of compounds. In the previous work (section 3.1.2), the majority of the trifluoromethyl replacement of the inhibitors were completely inactive against FXIIa. The binding mechanism of the trifluoromethyl group still remained unknown. Therefore, further modification of the trifluoromethyl functional group was carried out in tetrahydrofuran urea compounds.

According to the magnitude of the electron donating effect, two electron-donating functional groups are used for the replacement of the trifluoromethyl group and they are ordered from strong effect to weak effect: primary amine (-

NH₂) > alcohol (-OH). Moreover, the trifluoromethoxy group (-OCF₃) is a related functional group of the trifluoromethyl group. As pharmaceuticals, there are some relevance between these two functional groups. Therefore, the trifluoromethoxy group replaced the trifluoromethyl functional group and demonstrated the change of compound inhibitory potency in this case.

For the pyrrolidine ring, it was shown that there are strong van der Waals interactions between heterocycle and the FXIIa S1 pocket. Via analysis of the binding pose (section 3.1.3), it was found that there should have more binding sites deeper in the S1 pocket. Therefore, extension of the length of pyrrolidine was suggested. Based on the inhibitory potency of the compound, it was then used for demonstrating whether the pyrrolidine ring was actually located in the S1 pocket or not.

1 st Binding Mode			
			
Compd		IC ₅₀ (μM) ^{a, b}	
		α-FXIIa	β-FXIIa
2		8.0 ± 1.4	8.5 ± 1.2
120	CF ₃	NA ^c	NA
121		NA	NA
122		NA	NA
123	Pyrrolidine	X	X

^a The data given are mean values derived from three replicates.
^b Results are expressed in mean ± SD
^c NA = not active: inhibition <10% at 100 μM

Figure 3.13 New molecules chosen for first binding mode. They were designed based on the results of molecular docking in section 3.3.2.1

For the replacements of the trifluoromethyl group, primary amine (**122**), alcohol (**121**) and trifluoromethoxy (**120**) substituents were all inactive against FXIIa. Considering the electron donating effect, they all belong to the electron-donating functional group. These results indicated that the EDG groups indeed have negative influence on the inhibitory potency of compounds, which lead to inactive compounds against FXIIa.

When the extension of the pyrrolidine ring length was attempted, the synthesis of compound **123** was not successful via coupling reaction. A new approach for compound **123** synthesis is required. The first binding mode requires further investigation.

3.4.2. Molecules chosen to validate second binding mode

After analysis of the second binding mode, it was determined that the trifluoromethyl functional group pointed toward the S1' pocket. The ERH functional group is extended to the FXIIa S1 pocket and showed a strong binding interaction between ERH and the S1 pocket.

The S1 pocket is commonly found in all trypsin-like serine proteases. Asp189, as the key amino acid, was located in the specificity pocket S1. It was discovered that the Arginine amino acid can form a salt bridge with Asp189 in the FXIIa S1 pocket.¹ A series of urea compounds (**124-127**) which the mimic arginine functional group (benzamidine) was designed and synthesised (**Figure 3.14**). The ERH functional group is the combination of aliphatic side chain and benzamidine. The length of the aliphatic side chain was therefore considered. Finally, in order to distinguish the difference between within and without the benzamidine functional group, a series of urea compounds associated with

compound (**124-127**) but without benzamidine ((**128-131**) was synthesised. The IC_{50} of FXIIa inhibitors (**4** and **124-131**) were listed in **Table 3.10**.

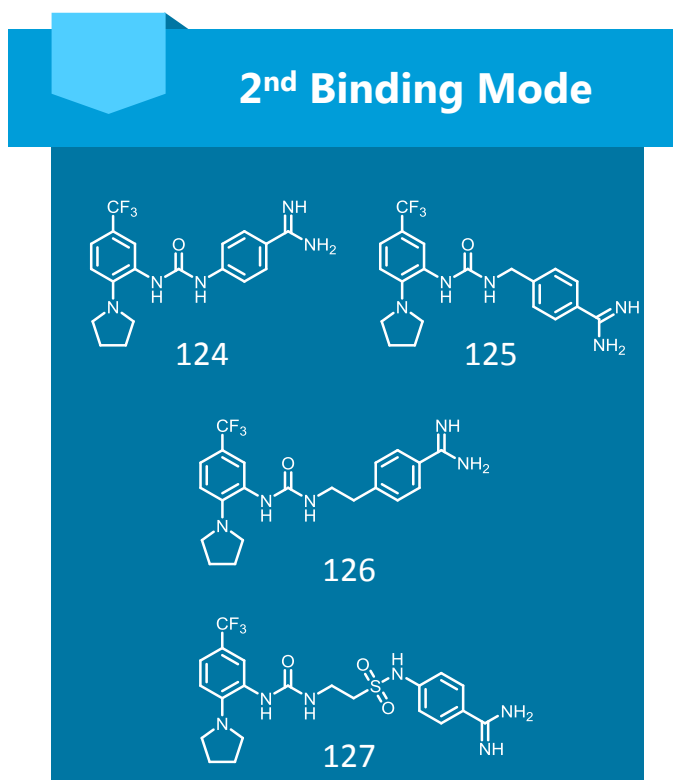


Figure 3.14 New molecules chosen for second binding mode. They were designed based on the results of molecular docking in section 3.3.2.

Table 3.10 Potency of new designed molecules for second binding mode

Compd	Physical properties	ERH groups	IC_{50} (μM) ^{a, b}	
			α -FXIIa	β -FXIIa
124	benzamidine	4-aminobenzamidine	12.1 $\pm$ 2.6	13.9 $\pm$ 3.1
125		4-(aminomethyl)benzamidine	7.5 $\pm$ 1.8	8.3 $\pm$ 2.9
126		4-(2-aminoethyl)benzamidine	3.7 $\pm$ 3.9	3.7 $\pm$ 4.1
127		4-((2-aminoethyl)sulfonamido)benzamidine	5.7 $\pm$ 0.1	5.9 $\pm$ 0.2
128	Without benzamidine	4-aminobenzonitrile	NA ^c	NA
129		4-(aminomethyl)benzonitrile	NA	NA
130		4-(2-aminoethyl)benzonitrile	NA	NA
131		2-amino-N-(4-cyanophenyl)ethane-1-sulfonamide	NA	NA
4		2-amino-N-phenylethane-1-sulfonamide	10.3 $\pm$ 0.8	11.4 $\pm$ 1.3

^a The data given are mean values derived from three replicates.

^b Results are expressed in mean $\pm$ SD

^c NA = not active: inhibition <10% at 100 μM

Compared to compounds without benzamidine (**128-131**), the inhibitory potency of compounds **124-127** had great difference from inactive compounds to active compounds (**Table 3.10**). These results indicated the benzamidine functional group was critical for the compounds activity. In comparison with compound **4**, the inhibition of compound **127** is slightly increased to 5.7 ± 0.1 μM (α -FXIIa) and 5.9 ± 0.2 μM (β -FXIIa), which became the most potent urea compound against FXIIa to date.

In order to get a better understanding of the relationship between structure and activity of compounds **124-127**, a series of molecular docking was undertaken and used the same protocol described in section 3.3. By analysis of all interactions of compounds **124-127**, they all presented almost the same results. Therefore, urea compound **126** was chosen for the explanation of the interaction between compounds and FXIIa in details (**Figure 3.15**).

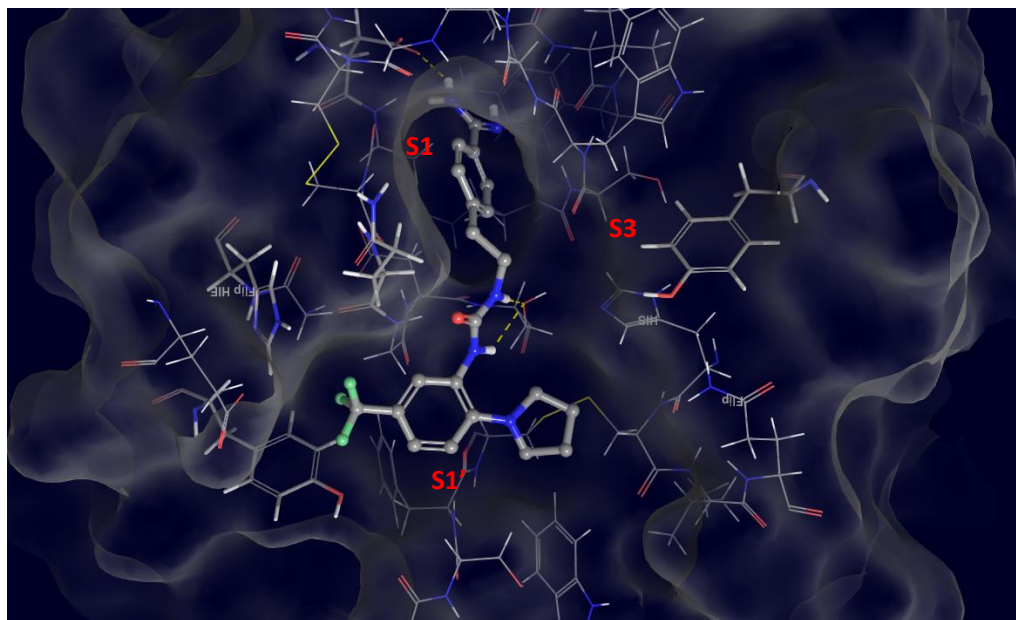
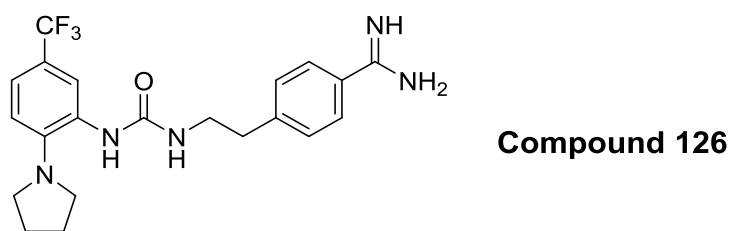


Figure 3.15: The best binding pose of compound **126** in the active site of the FXIIa hybrid model. The FXIIa model is shown with the key surface pockets labeled. Compound **126** is presented in brown CPK and amino acid residues around ligands are depicted in grey CPK. (Glide score = -8.142)

Similar to compound **3** (section 3.2.2.2), as depicted in **Figure 3.15**, the trifluoromethyl functional group in compound **126** was pointing towards specificity S1' pocket and the pyrrolidine ring was also located in the S1' pocket. Based on the binding pose, the ERH functional group (4-(2-aminoethyl)benzamidine) and urea moiety of compound **126** was in the direction of the FXIIa S1 pocket. There is clearly a strong interaction between benzamidine and the S1 pocket of enzyme FXIIa. The details of molecular interactions between the ERH group and the S1 pocket are illustrated in **Figure 3.16**.

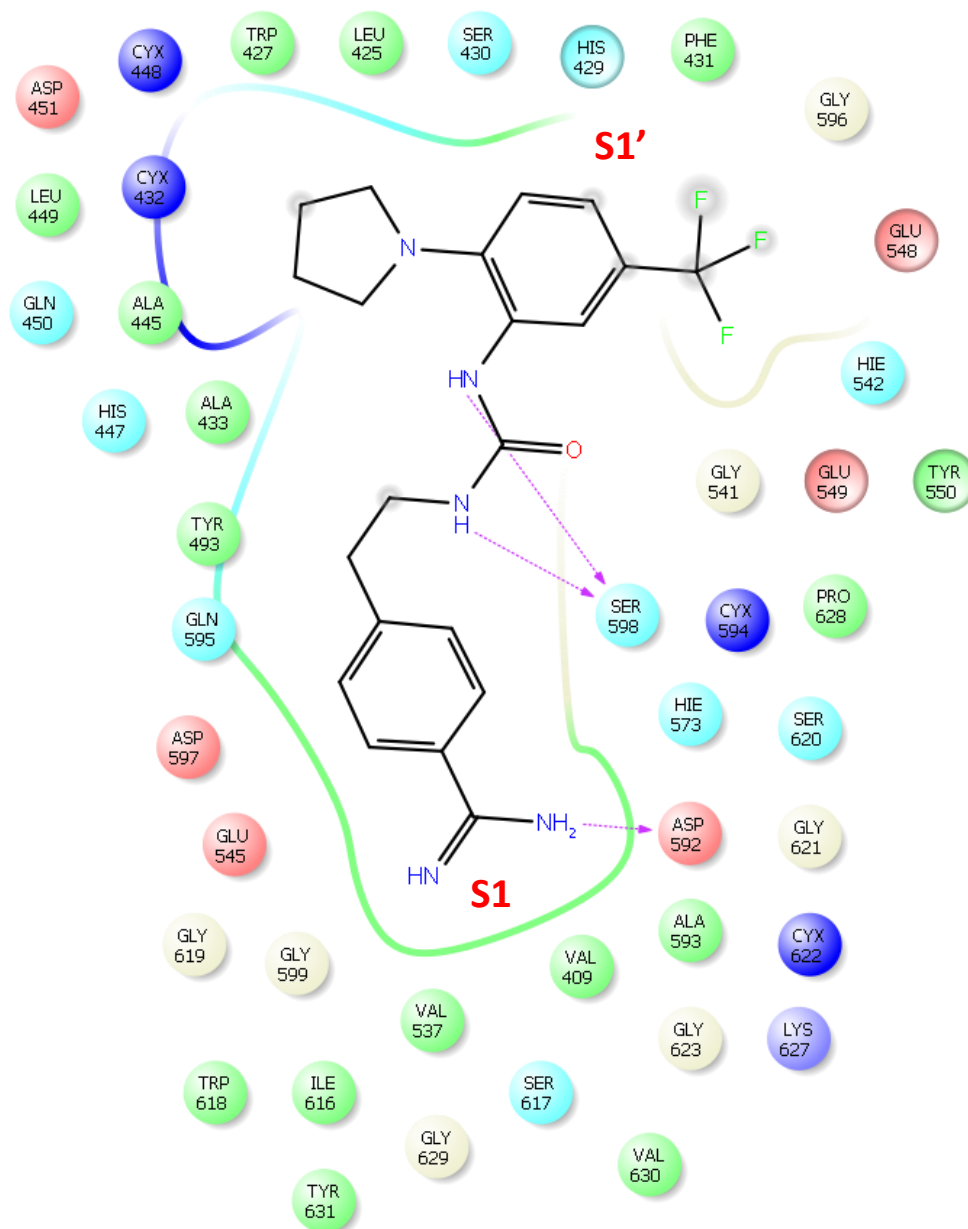


Figure 3.16: Interaction between FXIIa active site and compound **126**. The Figure is generated by schrödinger (Glide) Ligand interaction diagram. The hydrogen bonding interactions (NH---O_{Ser598}, distance = 3.0 Å; NH---O_{Ser598}, distance = 2.1 Å) between the two NH groups of compound **126** and SER598 is displayed by dotted lines (purple). Also, benzamidine has a hydrogen binding interaction which is represented by dotted lines (purple) (NH---O_{Asp592}, distance = 1.8 Å).

For the ERH functional group, according to compound **126**-FXIIa interaction (**Figure 3.16**), 4-(2-aminoethyl) benzamidine formed a strong salt bridge with ASP592 (NH---O_{Asp592}, distance = 1.8 Å). Two hydrogen atoms of the NH

groups have strong hydrogen bonds formed with SER598, (NH---O_{Ser598}, distance = 3.0 Å; NH---O_{Ser598}, distance = 2.1 Å). It was known that mimic arginine group (benzamidine) can have strong electrostatic effect with aspartic acid (ASP592), which leads to strong interaction between ligand and receptor. Via the increased inhibitory potency of compound **126** became the most active lead compound, it indicated that second binding mode is most likely to be the right binding way for ligands interacted with enzyme. Therefore, second binding mode will be used for further research in future.

3.5. Selectivity for other plasma proteins (Factor II, VII, X, XI)

3.5.1. Overview

In summary, a total of 134 urea compounds were designed and synthesised. Via the relationship of structure and activity of urea compounds, the top 25 most active compounds were selected and used for further selectivity research. They were all screened against other plasma proteins. For those plasma proteins, they included thrombin (THR, FIIa), FVIIa, FXa and FXIa.

3.5.2. Selectivity study of top 25 urea compounds against FXa and FXIIa

As depicted in **Table 3.11**, 4 out of the top 25 compounds were active against FXa. Via analysis of SARs, it was found that three of compounds (**124**, **126** and **127**) commonly contained benzamidine functional group except for compound **15** (**Figure 3.17**). As mentioned in section 3.3.3, the benzamidine group imitated arginine and interacted with aspartic acid (ASP189) in the FXIIa S1 pocket.¹ It has been confirmed that the S1 pocket commonly existed in all plasma serine protease. It is very likely there are strong electrostatic interaction between

compounds (**124**, **126** and **127**) and activated FXa. Compared to inhibitory potency of compounds against FXIIa, both compounds (**124** and **126**) showed decreased potency against FXa. This may be because binding sites in FXa and FXIIa have big differences, even though S1 pockets in both activated enzymes are very similar. The active compounds for FXIIa inhibition therefore may not preferably bind to FXa. For the compound **127**, it unexpectedly showed that compound against factor X (inhibition for $IC_{50} = 1.9 \pm 0.6 \mu M$) has better inhibitory potency (2-fold) compared to compound against FXIIa (inhibition for $IC_{50} = 5.7 \pm 0.1 \mu M$). Since the crystal structure of FXa has been crystallised and published, molecular modelling of compound **127** with FXa would be necessary for further understanding the interaction between ligand and enzyme in section 3.5.3.1.

For compound **15**, it was shown that its inhibitory potency of FXIIa and FXa are very similar. In section 3.3.2 docking analysis, it was believed that a series of tetrahydrofuran urea compounds has binding interaction with activated FXIIa by following the first binding mode. The first binding mode required more evidence to support the assumption of first binding mode. Since compound **15** showed its activity against FXa, the docking study could be helpful to understand the interaction between compound **15** and enzyme FXa. Furthermore, this may give us some ideas for its interaction with FXIIa as well.

Table 3.11 Potency of top 25 compounds against α -FXIIa and FXa

Compd	IC ₅₀ (μ M) ^a	
	α -FXIIa	FXa
65	55.7 $\pm$ 9.6	NA
51	35.4 $\pm$ 4.4	NA
54	34.1 $\pm$ 9.6	NA
70	29.7 $\pm$ 7.8	NA
33	29.1 $\pm$ 6.9	NA
15	20.5 $\pm$ 2.2	19.5 $\pm$ 4.9
18	20.2 $\pm$ 2.2	NA
46	19.0 $\pm$ 3.4	NA
69	15.0 $\pm$ 10.6	NA
59	15.6 $\pm$ 6.5	NA
64	14.1 $\pm$ 3.8	NA
131	13.8 $\pm$ 1.7	NA
132	13.7 $\pm$ 0.3	NA
1	13.2 $\pm$ 2.4	NA ^b
4	10.3 $\pm$ 0.8	NA
124	12.1 $\pm$ 2.6	141.7 $\pm$ 16.5
61	8.4 $\pm$ 2.9	NA
2	8.0 $\pm$ 1.4	NA
41	7.8 $\pm$ 1.6	NA
46	7.5 $\pm$ 1.8	NA
3	6.9 $\pm$ 1.0	NA
126	6.7 $\pm$ 3.9	58.7 $\pm$ 2.8
5	6.7 $\pm$ 1.9	NA
127	5.7 $\pm$ 0.1	1.9 $\pm$ 0.6

^aThe data given are mean values derived from three replicates (FXIIa). For FXa biological assays, IC₅₀ values derived from three replicates; whereas two replicates are for inactive compounds.

^bNA = not active: inhibition <10% at 100 μ M

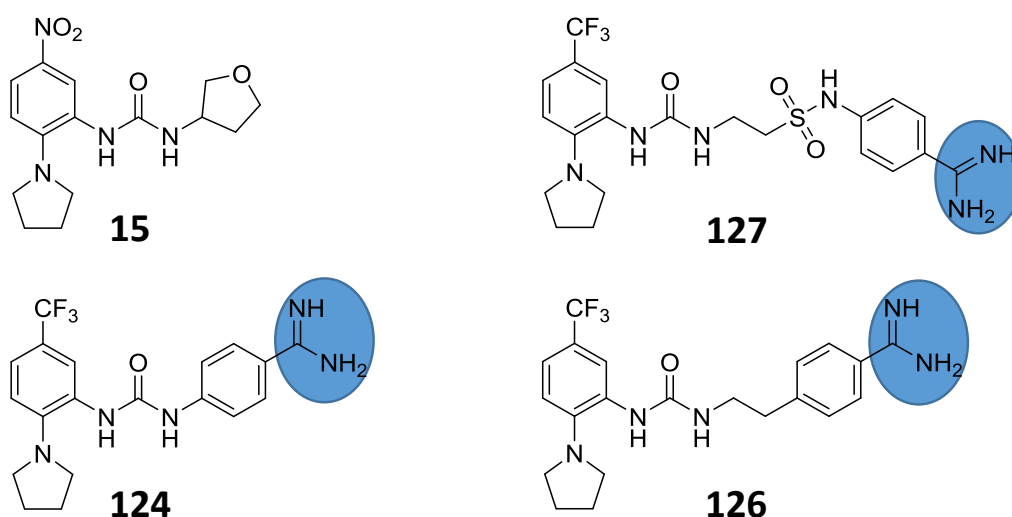


Figure 3.17 2D structures of compounds **15**, **124**, **126** and **127**

3.5.3. Docking analysis

3.5.3.1. Docking study of compound **86** with FXa

As depicted in **Figure 3.18**, the trifluoromethyl functional group and the pyrrolidine ring of compound **127** were located in hydrophobic pocket S4. Fluorine atoms of trifluoromethyl could have strong interaction with key amino acids backbones ($F \cdots H-C_{PHE448} = 2.1 \text{ \AA}$, $F \cdots H-C_{HIS447} = 2.4 \text{ \AA}$, $F \cdots H-C_{GLN450} = 2.1 \text{ \AA}$) (**Figure 3.19**). The aromatic ring which is next to the trifluoromethyl group, showed strong π -interactions with amino acid PHE448 and HIS447. The pyrrolidine ring has a reasonably strong carbon-hydrogen backbone, resulted in a strong van der Waals interactions with hydrophobic S4 pocket.

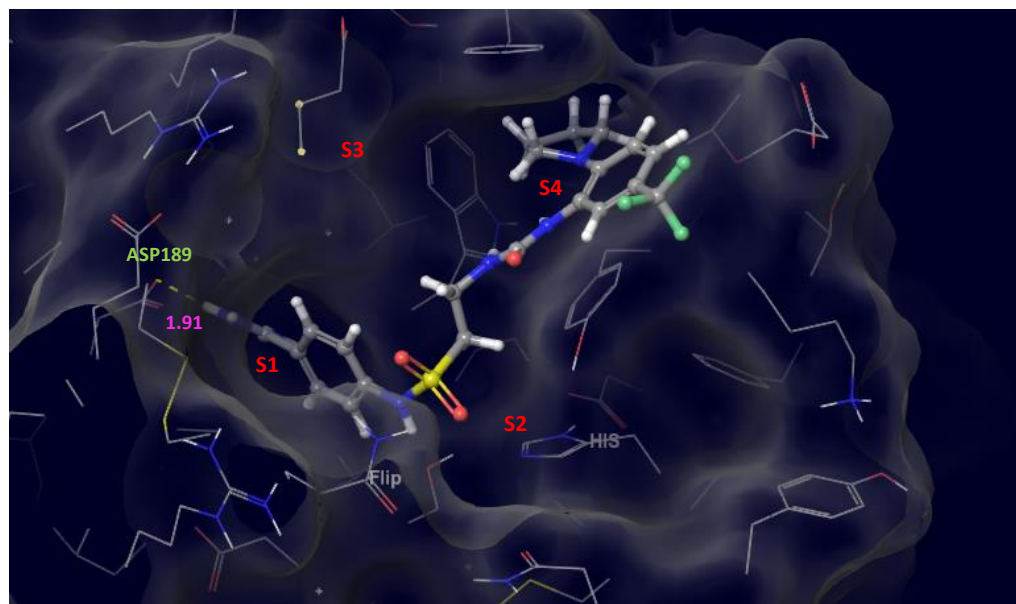


Figure 3.18: The best binding pose of compound **127** in the active site of the FXIIa crystal structure. This figure was generated by using the same approach of FXIIa docking study (schrödinger (Glide) with default parameters in docking simulation). All of ligands are minimized with lowest binding energy in the active site. FXa model is shown with the key surface pockets labeled. Compound **127** is presented in grey CPK. (Glide score = -7.125)

The NH group that was located between sulfonyl and the aromatic ring, had hydrogen binding with TYR631 ($\text{NH} \cdots \text{O}=\text{C}_{\text{TYR631}} = 2.8 \text{ \AA}$). At the bottom of the S1 specificity pocket of FXa enzyme ASP189 was located, which participates in ionic and hydrogen-bond interactions with the positively charged benzimidamide. NH_2 group of benzimidamide formed a salt bridge with ASP189 and showed a strong electrostatic interaction ($\text{NH} \cdots \text{O}-\text{O}=\text{C}_{\text{ASP189}} = 1.9 \text{ \AA}$). It also interacted with GLY219 and formed a hydrogen bond ($\text{NH} \cdots \text{O}=\text{C}_{\text{GLY219}} = 2.4 \text{ \AA}$). The NH group of benzimidamide had hydrogen bond with ASP633 ($\text{NH} \cdots \text{O}-\text{O}=\text{C}_{\text{ASP189}} = 1.6 \text{ \AA}$). Finally, the aromatic ring in the ERH functional group also showed strong π - interactions with amino acid TYR636. All the interactions between compound **127** and FXa were presented in details in **Figure 3.19**.

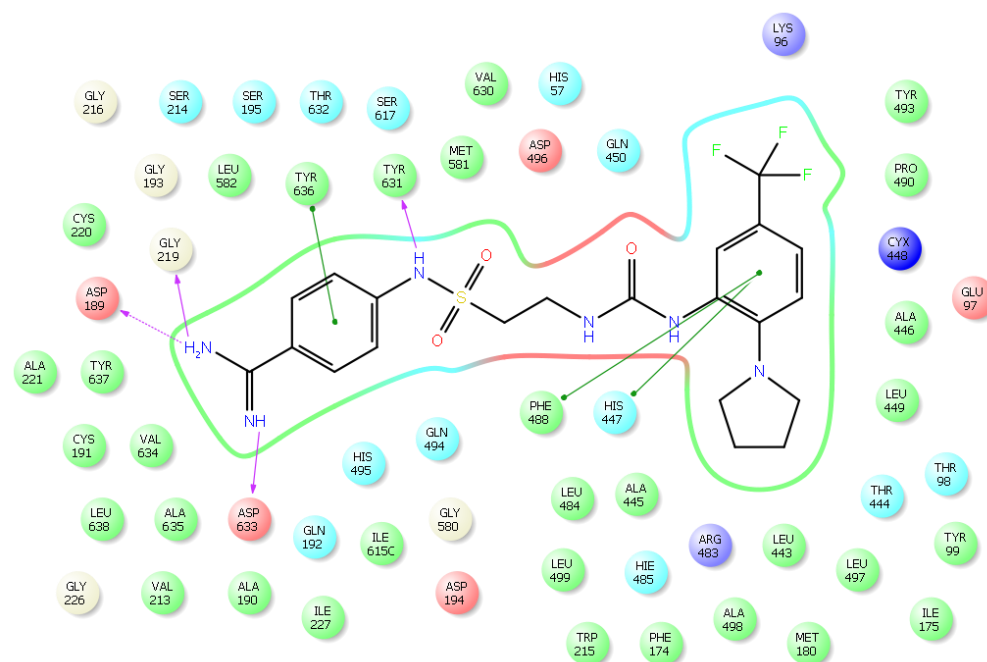


Figure 3.19: Interaction between FXa active site and compound **127**. The Figure is generated by schrödinger (Glide) Ligand interaction diagram. The hydrogen binding interaction between the NH group (adjacent to benzamidine) and TYR636 is displayed by dotted lines (purple). Also, benzamidine has hydrogen binding interactions with amino acids (GLY219, ASP189 and ASP633) which is represented by dotted lines (purple). The aromatic ring of the ERH group has π - interaction with TYR636 and is depicted with solid line (green). The aromatic ring (adjacent to the trifluoromethyl group), PHE488 and HIS447 also have strong π - interaction which is reprented by solid line. (green)

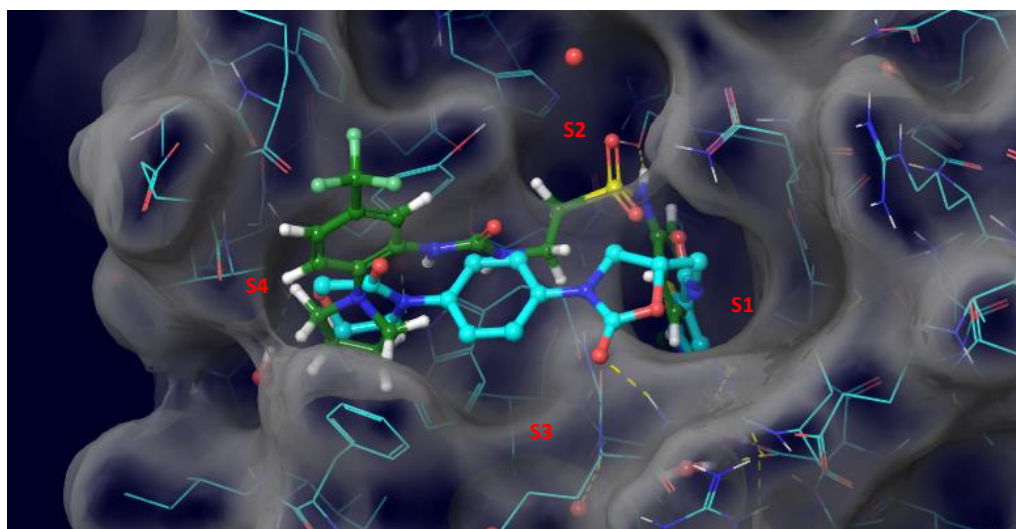


Figure 3.20 Overlay of compounds **127** and Rivaroxaban. This figure was generated by using Schrödinger (Glide) with default parameters in docking simulation. Compound **127** was presented in green in cartoon representation. Rivaroxaban was presented in blue in cartoon representation.

The chlorine atom of Rivaroxaban participated in a hydrophobic edge-to-face Cl- π interaction with the arene of TYR228. Also, it displaced a water molecule positioned at this site, providing a further entropic binding contribution. The carbonyl group of morpholinone led to the morpholinone ring vertical twisting that was relative to the arene and polarized the ring CH₂ group above TRP215, lead to reinforce the CH₂--- π interaction.

Overlay of Rivaroxaban and compound **127**, it was observed that both of them have interaction in specificity pockets S1 and S4. Compound **127** showed a clash at the binding site of S2 pocket, indicating that ligand did not have binding position to interact with FXa. This could be an explanation for the activity of compound **127** towards FXa in a micromolar range.

4. Conclusion and future work

4.1. Conclusion

The deficiency of FXII in the intrinsic pathway shows valuable interests of preventing thrombosis without having bleeding risk. The finding and development of FXIIa inhibitors can be useful for clinical applications involved with the exposure of patients to artificial surfaces. To date, small molecule inhibitors of FXIIa are not clinically available. This project aims to design and synthesise small molecule FXIIa inhibitors (**Figure 4.1**).

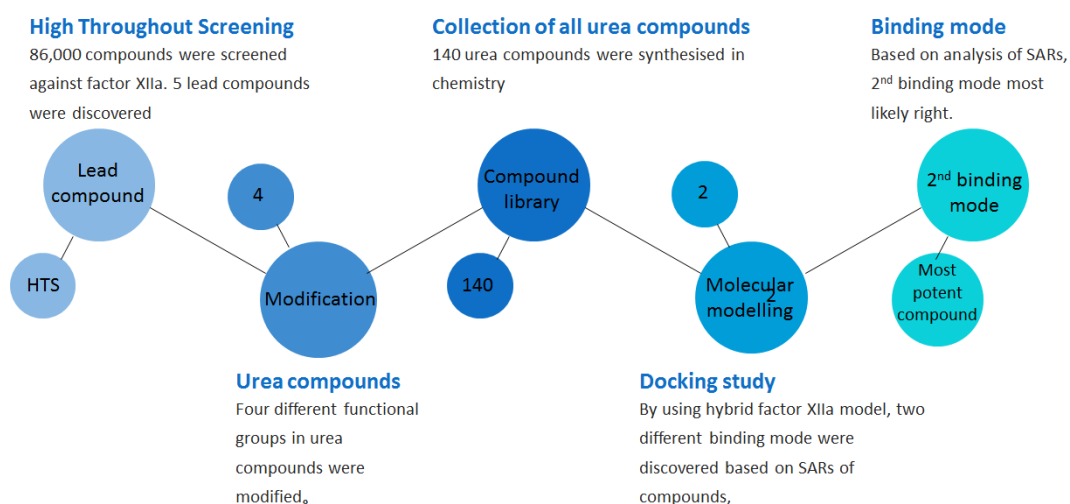


Figure 4.1: schematic procedure of small molecule inhibitor of FXIIa discovery

In high throughput screening studies, a series of urea compounds was discovered and used as lead compounds. The inhibitory potency of compounds against FXIIa was in the micromolar range. In order to improve this potency, it was necessary to modify the lead compounds. Several hypotheses were proposed and extensive modifications of the lead compounds were carried out (**Figure 4.2**). Firstly, diverse synthetic procedures (Lossen rearrangement) were developed

and used for the preparation of the designed molecules. Secondly, via a series of lead compound modifications, the optimal group in each position was identified.

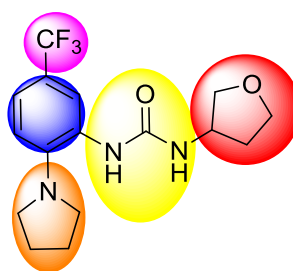


Figure 4.2 An example of lead compound structure. Trifluoromethyl group (purple), pyrrolidine ring (orange), tetrahydrofuran group (Extended right hand; red), urea moiety (yellow) and positions between trifluoromethyl group and pyrrolidine ring (blue).

Firstly, substitution of the trifluoromethyl functional group and the pyrrolidine ring have limitations. For the modification of the trifluoromethyl group, only a strong electron-withdrawing group (nitro moiety) can replace the trifluoromethyl group. The replacement of the trifluoromethyl group by nitro functional group in urea compounds has decreased activity in comparison with compounds containing the trifluoromethyl group. The inhibitory potency of urea compounds will be completely lost either when the trifluoromethyl group is absent or it is replaced by EDG or other weak EWG. For the replacement of the pyrrolidine ring, it was successful only if the pyrrolidine ring was substituted by either a smaller size of ring (azetidine) or a slightly modified pyrrolidine derivative (2-methylpyrrolidine). Similar to the trifluoromethyl group, the replacement of the pyrrolidine ring reduced or completely abolished the inhibitory potency of the urea compounds.

Secondly, various substitutions of the ERH functional group are available. All active urea compounds have limited inhibition against FXIIa in a micromolar range. For the modification of the urea moiety, the replacement of either NH groups in the urea compounds would cause the compound's inhibition to either decrease or be abolished. The derivatives of urea compounds (thiourea and cyclised urea) also suggested that neither of them are a good choice for the improvement of urea compounds potency.

Thirdly, there is a strictness in the position between the trifluoromethyl group, the pyrrolidine ring and the ERH functional group. Only when the trifluoromethyl functional group and the pyrrolidine were at a *para*-position, urea compounds may be active against FXIIa. It indicated that the binding area for urea compounds is limited at the active site of FXIIa.

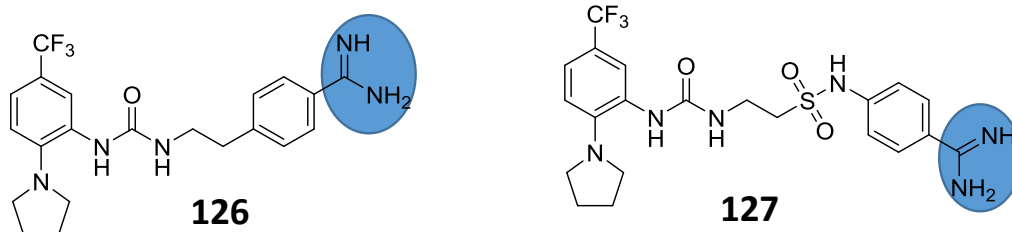
Finally, for the replacement of the trifluoromethyl group, the pyrrolidine ring and the ERH functional group, the results demonstrated that the trifluoromethyl group and the pyrrolidine ring are both important for the inhibitory potency of compounds. Urea compounds have potency against FXIIa only if the trifluoromethyl and pyrrolidine group were retained in the compound simultaneously. If either of them are missing, it will lead to a complete loss of activity of the urea compounds.

The combination of all synthetic urea compounds leads to a development of a compound library with their bio-chemical profiles. In order to get a better understanding of the relationship between urea compounds structures and activities, a molecular modelling study was undertaken. To this end, a hybrid model of FXIIa was generated by our crystallography group (Emsley group, The University of Nottingham). This hybrid FXIIa model was developed from the

published crystal structure of zymogen formed FXIIa, unpublished data (crystal structure of β -FXII) and a homology model based on the HGFA X-ray structure. Via bio-chemical validation, a canonical model of the FXII-recombinant CTI variants was used in this circumstance. These CTI inhibitors had inhibition of FXIIa enzymatic activity in a substrate cleavage assay. Therefore, the accuracy of the homology model was validated. This hybrid model can assist with the discrimination of active compounds from inactive compounds within the series detailed in this project.

Two binding modes were discovered during the molecular modelling. Considering the trifluoromethyl group and pyrrolidine are constrained, the possible modification for the improvement of urea compounds inhibition can only be in the ERH functional group. In this case, a series of molecules were designed to demonstrate two binding modes. It turned out the second binding mode is more likely to be the correct binding mode. Further research into the two binding modes is required.

In the compound library, the top 25 most active urea compounds were selected and used for selectivity experiments against FXIIa and FXa. These results showed that 4 out of 25 urea compounds are inhibitors of both enzymes.



Based on the second binding mode, newly designed compounds **126** and **127** are the most potent FXIIa inhibitors. Both compounds also have inhibitory potency against FXa, with compound **127** having the most potent inhibition against FXa.

Ligand (compound **127**) showed the similar binding with FXa inhibitor (rivaroxaban) at the active site of FXa. The mimic arginine group (benzamidine) particularly interacted at the bottom of specificity pocket S1. The trifluoromethyl group and the pyrrolidine ring was located in the hydrophobic S4 pocket and also showed strong π - π interaction. This indicated that the benzamidine group in compound **127** could be possible to bind to the S1 pocket in FXIIa, which means the second binding more is very likely to be the correct ligand/receptor binding pose.

4.2. Future work

A set of urea-derived compounds were synthesised to serve as inhibitors for FXIIa. These ligands were modified at the trifluoromethyl group, the pyrrolidine ring, the urea moiety and the ERH functional group. A series of biological plasma assays were developed and optimised in order to have the full set of SARs. Screening the top 25 most potent FXII inhibitors identified four inhibitors that are also potent against FXa. X-ray crystallography techniques validated homology model ‘hybrid FXIIa’ based on the unpublished crystal structure of the β -FXIIa and a canonical model of the FXII-recombinant CTI variants. It indicated that FXIIa has distinctive S1’ pocket site in comparison with other plasma proteases. These results explained the inhibition of FXIIa and could help design new selective FXIIa inhibitors.

There are still many areas to explore in this project but to enumerate a few. For the selectivity of the top 25 active urea-derived FXIIa inhibitors, multiple plasma assays will be undertaken, including FVIIa, thrombin and FIIa. Currently, the protocols of the biological assays have been established.

Molecular modelling is necessary since 4 out of 25 urea compounds were discovered to be active to FXa. It would be helpful to give a better understanding of the interaction between ligands and FXIIa/FXa.

Considering the distinctive S1’ pocket in FXIIa, increasing the number of binding sites between the urea compounds and FXIIa would be helpful for the increase of FXIIa inhibition.

It was known that the S3 pocket in FXIIa has similarities with FXa. Based on the published FXa inhibitors, urea-derived FXIIa inhibitors could have

possibilities to interact with S3 pocket. New designed molecules are requested based on computational drug design.

Compound **127** includes a benzamidine functional group. It was demonstrated that it is more water soluble than the original urea lead compounds by Dekker group and has strong interaction at the binding site of the S1 pocket in plasma enzyme assay. Therefore, X-ray crystallography experiments for ligand-enzyme complex would be of high importance to determine the binding mode for the existing and new candidates.

5. Experimental

5.1. Chemistry

Instrumentation. NMR spectra were collected on a Bruker Advance 400 spectrometer. ^1H is at 400.13 MHz, ^{13}C at 100.61 MHz and ^{19}F at 376.50 MHz. 1D carbon and all fluorine spectra were proton decoupled unless stated otherwise. Data were analysed using Bruker TOPSPIN 3 software. Chemical shifts are reported in ppm using the residual solvent proton signal as an internal reference. Coupling constants (J values) are expressed in Hz and are quoted to the nearest 0.1 Hz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Analytical LC-MS was performed using a Shimadzu UFLCXR system coupled to an Applied Biosystems API2000 mass spectrometer. The column by default used was Phenomenex Gemini-NX 3 μm -110 A C18, 50x2 mm at a flow rate of 0.5 mL/min. UV detection was recorded at 220 (channel2) and 254 nm (channel1). Short gradient: Pre-equilibration run for 1 min at 5% B; then: 5 to 98% solvent B in 2 min, 98% B for 2 min, 98 to 10% B in 0.5 min then 10% for 1 min. Solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in acetonitrile.

The purity of compounds were greater than 90% and was measured using Analytical HPLC-MS, which was performed using a Shimadzu UFLCXR system coupled to an Applied Biosystems API2000 mass spectrometer. The column by default used was Phenomenex Gemini-NX 3 μm -110 A C18, 50 $\times$ 2 mm at a flow rate of 0.5 mL/min. UV detection was recorded at 220 nm (channel 2) and 254 nm (channel 1). Short gradient: Pre-equilibration run for 1 min at 5%

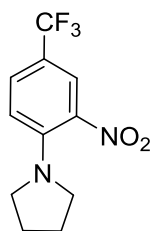
B; then: 5 to 98% solvent B in 2 min, 98% B for 2 min, 98 to 10% B in 0.5 min then 10% for 1 min. Solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in acetonitrile. Flash column chromatography was performed using prepacked silica gel cartridges (FlashMaster systems) and was supplied by Biotage (Cardiff, Wales). Melting points were determined with an Electrothermal melting point apparatus. Sonication was performed using a Camlab Transonic T460/4.

General Chemical Conditions. All commercial materials were purchased from Sigma Aldrich (UK), Alfa Aesar, VWR International, Apollo Scientific, Acros, Fluorochem and Fischer Scientific. They were used in reactions without further purifications. Solvents were purchased from Fisher (UK), VWR (UK), Sigma Aldrich (UK) and Cambridge Isotope Laboratories Inc. (UK) and they were either analytical or HPLC grade. Anhydrous solvents such as DMF, THF, DCM and pyridine were used when necessary. ISOLUTE scaffold (International Sorbent Technology, UK) were used for column and flash chromatography. Reactions were monitored by thin layer chromatography (TLC) plates (Merck) Kieselgel 60 F254. Results of TLC were visualized using a UV (λ_{254} nm or λ_{366} nm) lamp. For other non-visible compound under UV lamp, several dyes were used for staining, including: ninhydrin (solution in n-butanol), KMnO_4 and phosphomolybdic acid (PMA).

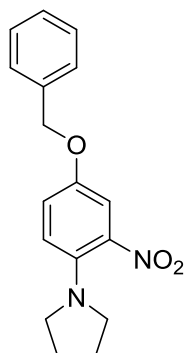
All volatiles removed *in vacuo* refer to the use of a Büchi rotary evaporator, including a dry-ice pump and a water bath at less than 40°C. The phrase ‘dried’ refers to a sample left overnight on a high vacuum pump or freeze dryer.

5.1.1. General procedures for the preparation of nitro benzene

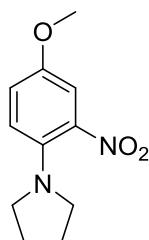
A solution of heterocyclic amine (12 mmol) in THF (30 mL) was treated with nitrobenzene (10 mmol) and sodium bicarbonate (1.680 g, 20 mmol). The reaction was stirred at room temperature and monitored by TLC for disappearance of starting material and formation of product. The mixture was concentrated, diluted with DCM (2×50 mL), washed with saturated aqueous sodium chloride solution (2×50 mL). Drying with anhydrous Na_2SO_4 , was followed by filtration and concentration to afford the product. The mixture was then purified by flash chromatography using either methanol and DCM or petroleum ether and ether acetate.



1-(2-Nitro-4-(trifluoromethyl) phenyl) pyrrolidine (2a). From 1-fluoro-2-nitro-4-(trifluoromethyl)benzene (10 mmol, 2.091 g) and pyrrolidine (12 mmol, 0.853 g). Yellow Oil (98.1%). HPLC-MS: $t_R = 2.85$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 1.93 (m, 4H, NCH_2CH_2), 3.20 (t, 4H, $J = 9$ Hz, NCH_2CH_2), 7.18 (d, 1H, $J = 6.5$ Hz, ArH), 7.71 (d, 1H, $J = 9.6$ Hz, ArH), 8.04 (s, 1H, ArH); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ 25.60, 50.83, 115.04, 114.38, 11.84, 123.36, 129.63, 135.50, 144.43; ^{19}F NMR (377 MHz, $\text{DMSO}-d_6$): δ -59.63; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$ 261.1, found 261.1

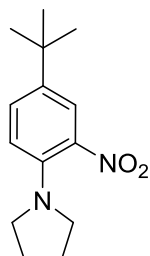


1-(4-(Benzyloxy)-2-nitrophenyl)pyrrolidine (7a). From 4-(benzyloxy)-1-fluoro-2-nitrobenzene (10 mmol, 2.471 g) and pyrrolidine (12 mmol, 0.853 g). Yellow oil (87%). HPLC-MS: t_R = 3.15 min, purity 96%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.40 (dt, J = 10.3, 7.4 Hz, 5H), 7.31 (t, J = 7.1 Hz, 1H), 7.22 (dd, J = 9.3, 3.0 Hz, 1H), 7.00 (d, J = 9.3 Hz, 1H), 5.07 (s, 2H), 3.05 (t, J = 6.1 Hz, 4H), 1.86 (t, J = 6.1 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 148.36, 137.81, 136.85, 135.76, 128.39, 127.83, 127.62, 122.74, 117.61, 110.40, 70.03, 50.12, 25.20. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_3$ 299.1, found 299.1

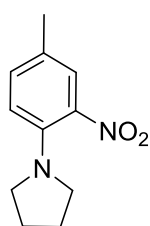


1-(4-Methoxy-2-nitrophenyl)pyrrolidine (8a). A solution of 4-bromo-3-nitroanisole (1 eq, 1 mmol, 0.232 g) and pyrrolidine (4 eq, 4 mmol, 0.284 g, 0.33 mL) in ethanol (2 mL) was heated at reflux for 30 h. After completion of the reaction, the resulting mixture was extracted with chloroform (3×20 mL). All of organic layers were combined and dried with anhydrous Na_2SO_4 . The solvent was concentrated to an oily residue and purified by flash chromatography using methanol and dichloromethane. Yellow oil (85%). HPLC-MS: t_R = 2.90 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.28 (d, J = 2.9 Hz, 1H), 7.16

(dd, $J = 9.3, 3.0$ Hz, 1H), 7.03 (d, $J = 9.3$ Hz, 1H), 3.73 (s, 3H), 3.07 (t, $J = 6.2$ Hz, 4H), 1.89 (t, $J = 6.2$ Hz, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 149.95, 138.16, 136.34, 122.55, 118.22, 109.32, 56.24, 50.62, 25.69. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}_3$ 223.1, found 222.8



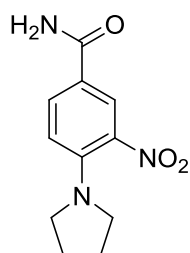
1-(4-(tert-Butyl)-2-nitrophenyl)pyrrolidine (9a). From 4-chloro-3-nitro-tert-butylbenzene (1 eq, 10 mmol, 2.131 g) and pyrrolidine (1.2 eq, 0.853 g, 12 mmol). Yellow oil (98%). HPLC-MS: $t_R = 3.22$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.62 (d, $J = 2.4$ Hz, 1H), 7.51 (dd, $J = 8.9, 2.5$ Hz, 1H), 6.98 (d, $J = 9.0$ Hz, 1H), 3.09 (t, $J = 6.5$ Hz, 4H), 1.91 – 1.84 (m, 4H), 1.24 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 140.97, 138.54, 136.34, 131.36, 122.75, 122.30, 116.75, 50.47, 31.32, 25.69; MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_2$ 249.2, found 248.9



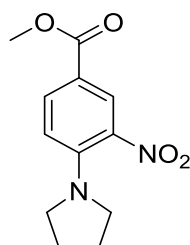
1-(4-Methyl-2-nitrophenyl)pyrrolidine (10a). From 1-fluoro-4-methyl-2-nitrobenzene (1 eq, 10 mmol, 1.551 g) and pyrrolidine (1.5 eq, 12 mmol, 0.853 g). Yellow oil (87%). HPLC-MS: $t_R = 3.12$ min, purity 100%. ^1H NMR (400 MHz, CDCl_3): δ 7.56 (s, 1H), 7.19 (d, $J = 6.5$ Hz, 1H), 6.88 (d, $J = 8.6$ Hz, 1H), 3.20 (t, $J = 6.6$ Hz, 4H), 2.28 (s, 3H), 1.98 (m, 4H); ^{13}C NMR (101 MHz,

CDCl₃): δ 140.63, 134.21, 127.13, 126.45, 125.91, 119.86, 116.39, 50.65, 25.73;

MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₅N₂O₂ 207.1, found 207.1

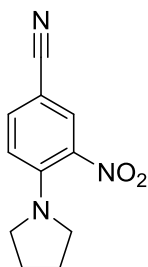


3-Nitro-4-(pyrrolidin-1-yl)benzamide (13a). A solution of pyrrolidine (3 eq, 9 mmol, 0.640 g) in THF (5 mL) was treated with 4-chloro-3-nitrobenzamide (1 eq, 3 mmol, 0.600 g) and sodium bicarbonate (3 eq, 9 mmol, 0.756 g). The reaction was stirred in microwave at 130 °C for 1 h. The mixture was concentrated, diluted with dichloromethane (30 mL), washed with saturated aqueous sodium chloride solution. Drying with anhydrous Na₂SO₄, was followed by filtration and concentration to afford the product. Yellow oil (64%). HPLC-MS: t_R = 2.46 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.28 (d, J = 1.8 Hz, 1H), 7.99 – 7.86 (m, 2H), 7.24 (s, 1H), 7.05 (d, J = 9.0 Hz, 1H), 3.18 (s, 4H), 1.92 (s, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 166.06, 143.59, 135.38, 131.98, 126.07, 120.67, 115.95, 50.28, 25.18. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₄N₃O₃ 236.2, found 236.0

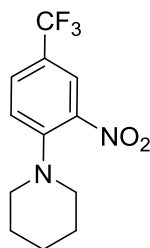


Methyl 3-nitro-4-(pyrrolidin-1-yl)benzoate (14a). From methyl-4-fluoro-3-nitrobenzoate (1.991 g, 10 mmol) and pyrrolidine (0.852 g, 12 mmol). The mixture was purified by flash chromatography using petroleum ether and ethyl

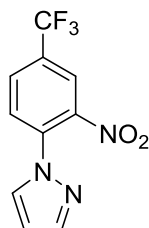
acetate (4:1). Yellow oil (75%). HPLC-MS: t_R = 2.88 min, purity 100%. ^1H NMR (400 MHz, CDCl_3) δ 8.38 (d, J = 2.1 Hz, 1H), 7.94 (dd, J = 9.0, 2.1 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 5.29 (s, 1H), 3.87 (s, 3H), 3.27 (t, J = 6.5 Hz, 4H), 2.08 – 1.92 (m, 4H). ^{13}C NMR (101 MHz, CDCl_3) δ 165.85, 144.98, 136.27, 133.50, 129.07, 116.90, 115.51, 52.08, 50.68, 25.75. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{15}\text{N}_2\text{O}_4$ 251.2, found 251.1



3-Nitro-4-(pyrrolidin-1-yl)benzonitrile (16a). A solution of pyrrolidine (2.5 eq, 10 mmol, 0.712 g, 0.84 mL) in THF (10 mL) was treated with 4-fluoro-3-nitrobenzonitrile (1 eq, 4 mmol, 0.664 g) and sodium bicarbonate (3 eq). The reaction was stirred in microwave at 100 °C for 10 min. The mixture was filtered and concentrated, diluted with dichloromethane (20 mL), washed with saturated aqueous sodium chloride solution. Drying with Na_2SO_4 , was followed by filtration and concentration to afford the product. Yellow oil (89%). HPLC-MS: t_R = 2.81 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.24 (d, J = 1.9 Hz, 1H), 7.75 (dd, J = 9.0, 1.9 Hz, 1H), 7.12 (d, J = 9.1 Hz, 1H), 3.20 (d, J = 6.0 Hz, 4H), 1.93 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 143.86, 135.51, 135.22, 131.15, 118.39, 117.38, 95.82, 50.41, 25.08. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_2$ 218.1, found 218.1

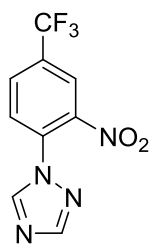


1-(2-Nitro-4-(trifluoromethyl)phenyl)piperidine (19a). From 1-fluoro-2-nitro-4-(trifluoromethyl)benzene (10 mmol, 2.091 g) and piperidine (12 mmol, 1.022 g). Yellow oil (98.9%). HPLC-MS: t_R = 3.09 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 6.93 (s, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 4.97 (s, 2H), 3.05 (t, J = 6.0 Hz, 4H), 1.85 (m, 4H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 142.09, 140.63, 126.85, 124.16, 117.14, 113.58, 110.99, 49.87, 24.23; ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$): δ -60.09; MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_2$ 275.1, found 275.1

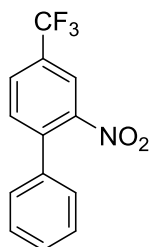


1-(2-Nitro-4-(trifluoromethyl)phenyl)-1H-pyrazole (23a). A solution of 1H-pyrazole (1.2 eq, 3.6 mmol, 0.246 g) in THF (10 mL) was added with sodium hydride (1.2 eq, 3.6 mmol, 0.144 g) and trimethylamine (2 eq, 2.4 mmol, 0.180 g) at 0 °C. The mixture was stirred for 30 min and a solution of 1-fluoro-2-nitro-4-(trifluoromethyl)benzene (1 eq, 1.2 mmol, 0.251 g) in acetonitrile (3 mL) was added into the mixture and monitored by TLC. The reaction was stirred at room temperature. After completion of the reaction, the mixture was quenched with water and extracted with dichloromethane. All of organic layers were combined. The solvent was concentrated and purified by flash chromatography using petroleum ether and ethyl acetate. Yellow oil (90%). HPLC-MS: t_R = 2.82 min,

purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.47 (d, J = 2.2 Hz, 2H), 8.23 – 8.17 (m, 1H), 8.09 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 1.7 Hz, 1H), 6.64 (dd, J = 2.5, 1.8 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 143.57, 142.65, 135.09, 131.30, 128.33, 126.20, 124.63, 123.15, 121.92, 109.43. ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$) δ -61.14. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{10}\text{H}_7\text{F}_3\text{N}_3\text{O}_2$ 258.2, found 258.0

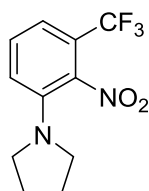


1-(2-Nitro-4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (24a). From 1,2,4-triazole (1.2 eq, 3.6 mmol, 0.249 g) and 1-fluoro-2-nitro-4-(trifluoromethyl)benzene (1 eq, 1.2 mmol, 0.251 g) (the same procedure with compound **23a**). Yellow oil (89%). HPLC-MS: t_R = 2.62 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.27 (s, 1H), 8.58 (d, J = 1.5 Hz, 1H), 8.36 – 8.29 (m, 2H), 8.21 (d, J = 8.4 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 153.83, 145.68, 143.43, 132.42, 131.19, 128.05, 124.42, 123.40, 119.00. ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$) δ -61.49. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_9\text{H}_6\text{F}_3\text{N}_4\text{O}_2$ 259.2, found 259.0



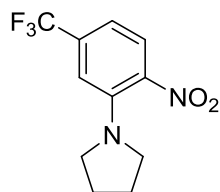
2-Nitro-4-(trifluoromethyl)-1,1'-biphenyl (25a). 1-bromo-2-nitro-4-(trifluoromethyl)benzene (1 eq, 1 mmol, 0.270 g, 0.15 mL) was dissolved in dioxane/ H_2O (6 mL, V:V = 2:1) and potassium carbonate (2 eq, 2 mmol, 2 mL)

was added in the reaction, followed by the addition of phenylboronic acid (1.2 eq, 1.2 mmol, 0.146 g). The reaction mixture was stirred for 2 min. Tetrakis(triphenylphosphine)palladium(0) ($\text{Pd(PPh}_3)_4$, 0.05 eq, 0.05 mmol, 0.058 g) was then added and the flask was flushed with Nitrogen gas, sealed and allowed to stir at 90 °C for 12 h. The reaction mixture was evaporated and extracted with ethyl ether. All organic layers (ethyl ether) were combined and washed with saturated aqueous sodium chloride. The organic layers were dried with Na_2SO_4 , followed by filtration and concentration. The crude product was purified by flash chromatography using petroleum ether and ethyl acetate to give the desired product. Yellow oil (43%). HPLC-MS: t_R = 3.07 min, purity 97%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.41 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.53 – 7.44 (m, 3H), 7.39 (dd, J = 7.1, 2.1 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 149.47, 139.25, 136.01, 133.64, 129.61, 129.40, 129.35, 128.16, 124.83, 122.12, 121.76. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{13}\text{H}_9\text{F}_3\text{NO}_2$ 268.2, found 268.0

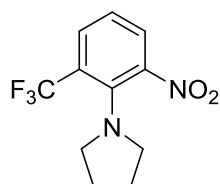


1-(2-Nitro-3-(trifluoromethyl)phenyl)pyrrolidine (76a). From 1-fluoro-2-nitro-3-(trifluoromethyl)benzene (2.091 g, 10 mmol) and pyrrolidine (0.853 g, 12 mmol). Yellow oil (99%) HPLC-MS: t_R = 3.08 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.57 – 7.47 (m, 1H), 7.30 (d, J = 8.7 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 3.21 (t, J = 6.6 Hz, 4H), 2.00 – 1.83 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 141.18, 135.76, 133.20, 131.22, 122.14, 121.63, 113.36,

48.66, 25.05. ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$) δ -59.35; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$ 261.2, found 261.2

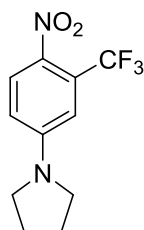


1-(2-Nitro-5-(trifluoromethyl)phenyl)pyrrolidine (77a). From 2-fluoro-1-nitro-4-(trifluoromethyl)benzene (2.091 g, 10 mmol) and pyrrolidine (0.853 g, 12 mmol). The mixture was purified by flash chromatography using petroleum ether and ethyl acetate (4:1). Yellow oil (79%). HPLC-MS: t_R = 3.10 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.91 (dd, J = 8.5, 0.7 Hz, 1H), 7.25 (d, J = 0.8 Hz, 1H), 7.01 (dd, J = 8.5, 1.6 Hz, 1H), 3.18 (t, J = 6.5 Hz, 4H), 2.68 – 2.41 (m, 2H), 2.10 – 1.70 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 141.71, 137.94, 127.49, 124.77, 122.06, 113.16, 110.97, 50.14, 25.17. ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$) δ -61.92. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$ 261.2, found 261.2

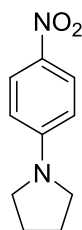


1-(2-Nitro-6-(trifluoromethyl)phenyl)pyrrolidine (78a). From 2-fluoro-1-nitro-3-(trifluoromethyl)benzene (2.091 g, 10 mmol) and pyrrolidine (0.853 g, 12 mmol). Yellow oil (99%). HPLC-MS: t_R = 3.18 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.12 (dd, J = 8.1, 1.3 Hz, 1H), 8.01 (dd, J = 8.0, 1.5 Hz, 1H), 7.57 (td, J = 8.1, 0.6 Hz, 1H), 3.10 (t, J = 6.5 Hz, 4H), 1.91 – 1.83 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 151.55, 140.90, 131.72, 129.69, 127.04,

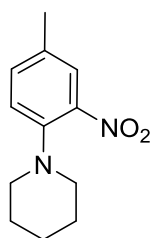
125.02, 122.31, 52.40, 26.30. ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$) δ -59.94; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$ 261.2, found 261.1



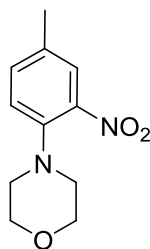
1-(4-Nitro-3-(trifluoromethyl)phenyl)pyrrolidine (79a). From 4-fluoro-1-nitro-2-(trifluoromethyl)benzene (2.091 g, 10 mmol) and pyrrolidine (0.853 g, 12 mmol). Yellow oil (79%). HPLC-MS: t_R = 3.02 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.08 (d, J = 9.2 Hz, 1H), 6.85 (d, J = 2.2 Hz, 1H), 6.78 (dd, J = 9.3, 2.6 Hz, 1H), 3.41 (t, J = 6.5 Hz, 4H), 1.99 (t, J = 6.6 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 150.31, 133.21, 129.24, 124.72, 121.22, 118.51, 109.84, 47.88, 24.91. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$ 261.1, found 261.0



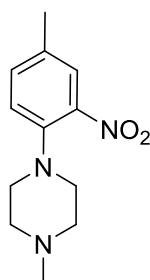
1-(4-Nitrophenyl)pyrrolidine (80a). From 1-fluoro-4-nitrobenzene (1.410 g, 10 mmol) and pyrrolidine (0.853 g, 12 mmol). Yellow oil (95%). HPLC-MS: t_R = 2.31 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.05 (d, J = 9.3 Hz, 1H), 6.61 (d, J = 9.3 Hz, 1H), 3.38 (t, J = 6.6 Hz, 2H), 1.98 (t, J = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 151.82, 135.24, 126.00, 110.89, 47.71, 24.91. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{10}\text{H}_{13}\text{N}_2\text{O}_2$ 193.1, found 193.1



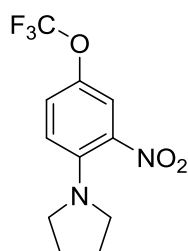
1-(4-Methyl-2-nitrophenyl)piperidine (86a). From 1-fluoro-4-methyl-2-nitrobenzene (10 mmol, 1.551 g) and piperidine (12 mmol, 1.022 g). Yellow Oil (93.2%). HPLC-MS: t_R = 3.12 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 7.38 (s, 1H), 7.37 (d, J = 2.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 2.88 (t, J = 5.5 Hz, 4H), 2.28 (s, 3H), 1.58 (m, 4H), 1.52 (m, 2H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 144.46, 143.79, 134.60, 132.06, 125.36, 122.09, 53.30, 26.15, 23.95, 20.16; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{17}\text{N}_2\text{O}_2$ 221.1, found 221.1



4-(4-Methyl-2-nitrophenyl)morpholine (87a). From 1-fluoro-4-methyl-2-nitrobenzene (10 mmol, 1.551 g) and morpholine (12 mmol, 1.042 g). Yellow oil (87.3%). HPLC-MS: t_R = 2.32 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 7.44 (s, 1H), 7.42 (d, J = 2.6 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 3.67 (t, J = 4.6 Hz, 4H), 2.92 (t, J = 3 Hz, 4H), 2.30 (s, 3H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 144.27, 143.28, 134.74, 133.17, 125.42, 122.22, 66.69, 52.42, 20.21; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}_3$ 223.1, found 223.1



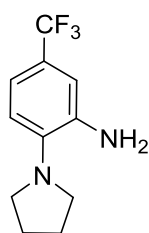
1-Methyl-4-(4-methyl-2-nitrophenyl)piperazine (88a). From 1-fluoro-4-methyl-2-nitrobenzene (10 mmol, 1.551 g) and 1-methylpiperazine (12 mmol, 1.202 g). Yellow oil (94.5%). HPLC-MS: t_R = 3.01 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.59 (s, 1H), 7.39 (d, J = 11 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 2.92 (t, J = 9.6 Hz, 2H), 2.40 (m, 2H), 2.29 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ 144.01, 143.45, 134.62, 132.59, 125.38, 122.09, 55.10, 51.86, 46.16, 20.18; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{18}\text{N}_3\text{O}_2$ 236.1, found 236.1



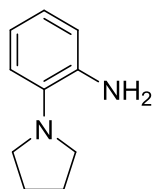
1-(2-Nitro-4-(trifluoromethoxy)phenyl)pyrrolidine (120a). From 2-fluoro-5-(trifluoromethoxy)nitrobenzene (1 eq, 3 mmol, 0.675 g) and pyrrolidine (1.5 eq, 4.5 mmol, 0.38 mL). Yellow oil (93%). HPLC-MS: t_R = 3.08 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.76 (d, J = 2.3 Hz, 1H), 7.48 (dd, J = 9.3, 2.1 Hz, 1H), 7.11 (d, J = 9.4 Hz, 1H), 3.13 (t, J = 6.2 Hz, 4H), 1.91 (t, J = 6.2 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 141.46, 136.37, 134.81, 126.92, 121.54, 119.23, 117.72, 50.35, 25.25. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_3$ 277.1, found 277.0

5.1.2. General procedures for the preparation of anilines

To a solution of amine (1eq, 5 mmol) in methanol (20 mL) was added Pd/C (5-10 wt. %). H₂ (g) was bubbled through the solution at R.T. overnight with vigorous stirring and monitored by TLC. After completion of the reaction, the mixture was filtered and the solvent was removed to give the desired product. The mixture was then purified by flash chromatography using methanol and DCM.

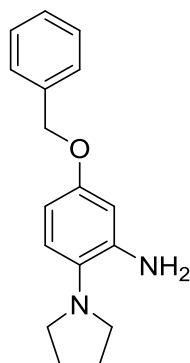


2-(Pyrrolidin-1-yl)-5-(trifluoromethyl) aniline (2b). From 1-(2-nitro-4-(trifluoromethyl) phenyl) pyrrolidine (**2a**) (5 mmol, 1.300 g). Brown oil (89%). HPLC-MS: t_R = 2.71 min, purity 95%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 6.93 (s, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 4.97 (s, 2H), 3.05 (t, J = 6.0 Hz, 4H), 1.85 (m, 4H); ¹³C NMR (101 MHz, DMSO-*d*₆): δ 142.09, 140.63, 126.85, 124.16, 117.14, 113.58, 110.99, 49.87, 24.23; ¹⁹F NMR (377 MHz, DMSO-*d*₆): δ -60.09; MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₄F₃N₂ 231.11, found 231.08

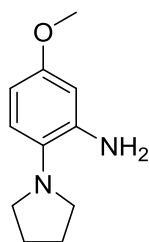


2-(Pyrrolidin-1-yl)aniline (6b). From 1-(2-nitrophenyl)pyrrolidine (5 mmol, 0.965 g). Brown oil (91%). HPLC-MS: t_R = 2.62 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.85 (dd, J = 7.8, 0.8 Hz, 1H), 6.77 – 6.70 (m, 1H), 6.65

(dd, $J = 7.7, 1.3$ Hz, 1H), 6.51 (td, $J = 7.8, 1.4$ Hz, 1H), 4.58 (s, 2H), 2.94 (t, $J = 5.3$ Hz, 4H), 1.89 – 1.78 (m, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 142.04, 136.45, 122.66, 117.64, 116.54, 114.57, 49.92, 23.52. MS (ESI $^+$): m/z [M+H] $^+$, calcd for C₁₀H₁₅N₂ 163.1, found 163.0

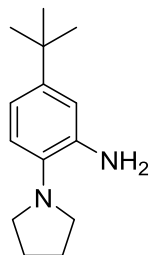


5-(Benzyloxy)-2-(pyrrolidin-1-yl)aniline (7b). From 1-(4-(benzyloxy)-2-nitrophenyl)pyrrolidine (**7a**) (5 mmol, 1.492 g). Brown oil (81%). HPLC-MS: $t_R = 2.20$ min, purity 96%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.36 – 7.23 (m, 5H), 6.80 (d, $J = 8.4$ Hz, 1H), 6.34 (d, $J = 2.5$ Hz, 1H), 6.15 (dd, $J = 8.6, 2.5$ Hz, 1H), 4.94 (s, 2H), 2.87 (s, 4H), 1.81 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.27, 143.75, 137.70, 128.42, 127.67, 127.50, 119.18, 115.02, 102.17, 101.51, 69.07, 51.12, 23.53. MS (ESI $^+$): m/z [M+H] $^+$, calcd for C₁₇H₂₁N₂O 269.1, found 268.9

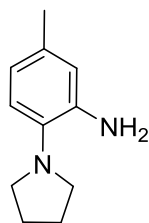


5-Methoxy-2-(pyrrolidin-1-yl)aniline (8b). From 1-(4-methoxy-2-nitrophenyl)pyrrolidine **8a** (5 mmol, 1.111 g). Brown oil (89%). HPLC-MS: $t_R = 0.53$ min, purity 94%. ^1H NMR (400 MHz, DMSO- d_6) δ 6.85 (d, $J = 8.6$ Hz, 1H), 6.27 (d, $J = 2.2$ Hz, 1H), 6.12 – 6.07 (m, 1H), 3.63 (d, $J = 6.1$ Hz, 3H), 3.44

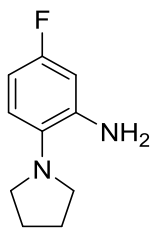
(s, 2H), 2.91 (s, 4H), 1.84 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.24, 143.55, 129.51, 119.23, 111.30, 100.55, 54.80, 51.24, 23.45. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{17}\text{N}_2\text{O}$ 193.1, found 193.1



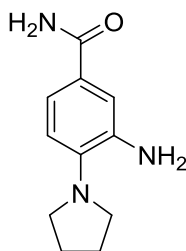
5-(tert-Butyl)-2-(pyrrolidin-1-yl)aniline (9b). From 1-(4-(tert-butyl)-2-nitrophenyl)pyrrolidine (**9a**) (5 mmol, 1.242 g). Brown oil (95%). HPLC-MS: t_R = 2.15 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 6.86 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 2.1 Hz, 1H), 6.59 (dd, J = 10.5, 4.3 Hz, 1H), 5.11 (s, 3H), 3.01 (s, 4H), 1.87 (s, 4H), 1.23 (s, 12H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 151.62, 146.27, 141.49, 133.44, 128.85, 118.19, 114.25, 51.23, 31.78, 23.97. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{14}\text{H}_{23}\text{N}_2$ 219.0, found 218.9



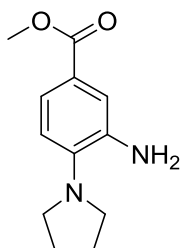
5-Methyl-2-(pyrrolidin-1-yl)aniline (10b). From 1-(4-methyl-2-nitrophenyl)pyrrolidine (**10a**) (5 mmol, 1.031 g). Brown oil (91%). HPLC-MS: t_R = 2.75 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 6.74 (d, J = 8 Hz, 1H), 6.46 (s, 1H), 6.30 (d, J = 6.5 Hz, 1H), 4.53 (s, 2H), 2.89 (t, J = 6.6 Hz, 4H), 2.12 (s, 3H), 1.82 (m, 4H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 142.49, 134.52, 131.98, 119.18, 117.52, 115.69, 50.68, 23.96, 21.14; MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{17}\text{N}_2$ 177.1, found 177.2



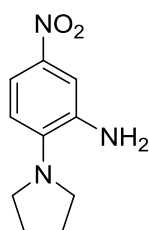
5-Fluoro-2-(pyrrolidin-1-yl)aniline (12b). From 1-(4-fluoro-2-nitrophenyl)pyrrolidine (5 mmol, 1.051 g). Brown oil (67%). HPLC-MS: t_R = 0.68 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 6.84 (dd, J = 8.5, 6.1 Hz, 1H), 6.42 (dd, J = 11.1, 2.9 Hz, 1H), 6.24 (td, J = 8.6, 2.9 Hz, 1H), 4.97 (s, 2H), 2.94 – 2.79 (m, 4H), 1.89 – 1.74 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 160.16, 144.27, 132.54, 118.84, 101.27, 100.34, 50.42, 23.47. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{10}\text{H}_{14}\text{FN}_2$ 181.2, found 181.4



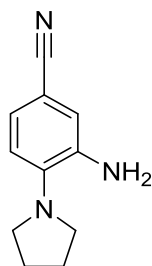
3-Amino-4-(pyrrolidin-1-yl)benzamide (13b). From 3-nitro-4-(pyrrolidin-1-yl)benzamide (**13a**) (2 mmol, 0.470 g). Brown oil (31%). HPLC-MS: t_R = 0.58 min, purity 80%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.29 (d, J = 2.0 Hz, 1H), 7.94 (dd, J = 8.9, 2.0 Hz, 2H), 7.26 (s, 1H), 7.05 (d, J = 9.0 Hz, 1H), 3.36 (s, 2H), 3.19 (t, J = 6.1 Hz, 4H), 1.92 (t, J = 6.1 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 166.11, 143.61, 135.39, 132.00, 126.09, 120.67, 115.96, 50.30, 25.20. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{16}\text{N}_3\text{O}$ 206.2, found 206.1



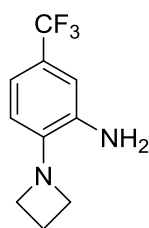
Methyl 3-amino-4-(pyrrolidin-1-yl)benzoate (14b). From methyl 3-nitro-4-(pyrrolidin-1-yl)benzoate (**14a**) (3 mmol, 0.751 g). Brown oil (95%). HPLC-MS: t_R = 2.32 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.28 (d, J = 1.8 Hz, 1H), 7.18 (dd, J = 8.2, 1.8 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 4.75 (s, 2H), 3.75 (s, 3H), 3.11 (s, 4H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 166.68, 141.71, 140.32, 121.87, 118.85, 115.79, 115.52, 51.42, 49.35, 23.99. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{17}\text{N}_2\text{O}_2$ 221.1, found 220.9



5-Nitro-2-(pyrrolidin-1-yl)aniline (15b). A solution of 2-fluoro-5-nitroaniline (1 eq, 10 mmol, 1.560 g) in DMF (4 mL) was added with pyrrolidine (2.5 eq, 25 mmol, 1.775 g) in a microwave tube. The mixture was stirred at 120 °C for 30 min. After completion of the reaction, the mixture was washed with water and extracted with dichloromethane. The solvent was removed by vacuum and purified by flash chromatography using petroleum ether and ethyl. Brown oil (79%). HPLC-MS: t_R = 2.70 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.51 (d, J = 2.8 Hz, 1H), 7.46 (dd, J = 8.8, 2.8 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 5.04 (s, 2H), 3.32 – 3.24 (m, 4H), 1.90 – 1.83 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 162.75, 161.05, 144.68, 140.02, 114.51, 109.78, 50.03, 24.95. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{10}\text{H}_{14}\text{N}_3\text{O}_2$ 208.2, found 208.0

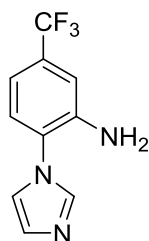


3-Amino-4-(pyrrolidin-1-yl)benzonitrile (16b). A solution of 3-nitro-4-(pyrrolidin-1-yl)benzonitrile (**16a**) (1 eq, 5 mmol, 1.086 g) in methanol (15 mL) was added with zinc (1 eq, 5 mmol, 0.325 g) and ammonium chloride (2 eq, 10 mmol, 0.525 g). The mixture was stirred and reflux at 60 °C overnight and monitored by TLC. After completion of the reaction, the mixture was filtered and the solvent was removed to give the desired product. Brown oil (89%). HPLC-MS: t_R = 2.55 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 6.97 – 6.87 (m, 2H), 6.81 (d, J = 8.6 Hz, 1H), 4.93 (s, 2H), 3.12 (s, 4H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 141.59, 141.07, 121.33, 120.29, 116.74, 116.51, 102.07, 49.26, 24.05. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{14}\text{N}_3$ 188.1, found 188.1

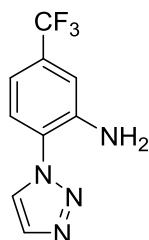


2-(Azetidin-1-yl)-5-(trifluoromethyl)aniline (18b). From 1-(2-nitro-4-(trifluoromethyl)phenyl)azetidine (5 mmol, 1.231 g). Brown oil (67%). HPLC-MS: t_R = 2.75 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 6.87 (d, J = 1.2 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.46 (d, J = 8.1 Hz, 1H), 4.73 (s, 2H), 3.82 (t, J = 7.2 Hz, 4H), 2.16 (p, J = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ

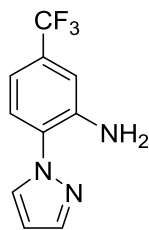
142.28, 138.40, 126.55, 123.87, 120.14, 112.81, 110.61, 52.73, 16.75. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₀H₁₂F₃N₂ 217.1, found 217.1



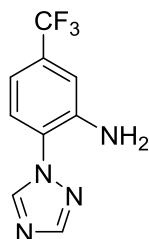
2-(1H-Imidazol-1-yl)-5-(trifluoromethyl)aniline (21b). From 1-(2-nitro-4-(trifluoromethyl)phenyl)-1H-imidazole (5 mmol, 1.286 g). Brown oil (69%). HPLC-MS: t_R = 1.07 min, purity 90%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.83 (s, 1H), 7.37 (s, 1H), 7.25 (d, *J* = 8.1 Hz, 1H), 7.20 (s, 1H), 7.13 (s, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 5.50 (s, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 144.51, 137.78, 130.17, 129.86, 129.71, 128.28, 125.60, 123.20, 120.52, 112.68. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₀H₉F₃N₃ 228.2, found 228.0



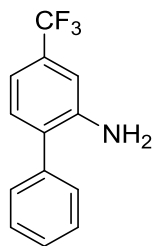
2-(1H-1,2,3-Triazol-1-yl)-5-(trifluoromethyl)aniline (22b). From 1-(2-nitro-4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole (5 mmol, 1.291 g). Brown oil (87%). HPLC-MS: t_R = 2.75 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.54 (s, 1H), 8.00 (s, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.29 (s, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 5.94 (s, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 143.48, 134.18, 130.89, 130.57, 126.90, 124.68, 123.05, 113.49, 112.42. ¹⁹F NMR (377 MHz, DMSO-*d*₆) δ -61.71. MS (ESI⁺): m/z [M+H]⁺, calcd for C₉H₈F₃N₄ 229.1, found 228.9



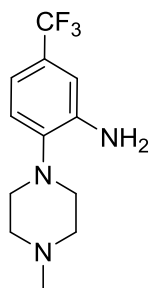
2-(1H-Pyrazol-1-yl)-5-(trifluoromethyl)aniline (23b). From 1-(2-nitro-4-(trifluoromethyl)phenyl)-1H-pyrazole (**23a**) (5 mmol, 1.286 g). Brown oil (93%). HPLC-MS: t_R = 2.78 min, purity 93%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.22 (d, J = 2.0 Hz, 1H), 7.81 (d, J = 1.0 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 7.24 (s, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.55 (s, 1H), 6.13 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 142.55, 141.02, 131.08, 128.94, 127.85, 125.98, 124.69, 123.28, 112.57, 107.35. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{10}\text{H}_8\text{F}_3\text{N}_3$ 228.1, found 228.1



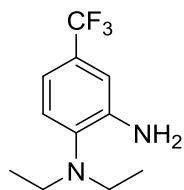
2-(1H-1,2,4-Triazol-1-yl)-5-(trifluoromethyl)aniline (24b). From 1-(2-nitro-4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**24a**) (5 mmol, 1.291 g). Brown oil (83%). HPLC-MS: t_R = 2.52 min, purity 98%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.96 (s, 1H), 8.29 (s, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.24 (s, 1H), 6.96 (d, J = 8.8 Hz, 1H), 5.93 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 152.73, 145.23, 143.41, 130.24, 126.52, 125.80, 124.65, 123.10, 112.39. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_9\text{H}_8\text{F}_3\text{N}_4$ 229.1, found 229.1



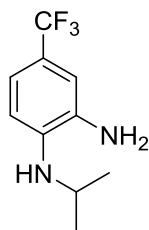
4-(Trifluoromethyl)-[1,1'-biphenyl]-2-amine (25b). From 2-nitro-4-(trifluoromethyl)-1,1'-biphenyl (**25a**) (2 mmol, 0.534 g). Brown oil (63%). HPLC-MS: t_R = 3.03 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.49 (d, J = 7.4 Hz, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.42 (s, 1H), 7.39 (t, J = 7.1 Hz, 1H), 7.16 (d, J = 7.8 Hz, 1H), 7.09 (s, 1H), 6.90 (d, J = 7.8 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 145.87, 138.22, 130.88, 129.07, 128.91, 128.50, 127.49, 125.84, 123.14, 112.33, 111.01. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{13}\text{H}_{11}\text{F}_3\text{N}$ 238.1, found 237.9



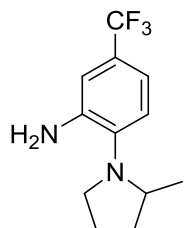
2-(4-Methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (27b). From 1-methyl-4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (5 mmol, 1.446 g). Brown solid (80.0%), mp: 178.5 °C. HPLC-MS: t_R = 2.78 min, purity 100%. ^1H NMR (400 MHz, CDCl_3): δ 7.02 (s, 1H), 6.97 (d, J = 6.8 Hz, 1H), 6.93 (d, J = 1.9 Hz, 1H), 4.06 (s, 2H), 2.98 (m, 2H), 2.57 (m, 2H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3): δ 141.51, 123.06, 119.63, 115.43, 115.39, 111.49, 111.45, 55.64, 50.44, 46.08; ^{19}F NMR (377 MHz, CDCl_3): δ -62.17; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{17}\text{F}_3\text{N}_3$ 260.1, found 260.1



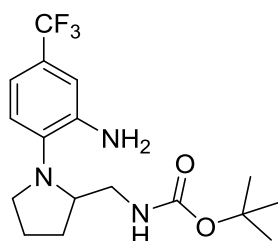
N¹, N¹-Diethyl-4-(trifluoromethyl) benzene-1, 2-diamine (29b). From N¹, N¹-diethyl-2-nitro-4-(trifluoromethyl)aniline (5 mmol, 1.311 g). Brown oil (71%). HPLC-MS: t_R = 2.71 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.03 (d, J = 8.1 Hz, 1H), 6.99 (s, 1H), 6.81 (dd, J = 8.1, 1.3 Hz, 1H), 5.14 (s, 2H), 2.92 (q, J = 7.1 Hz, 4H), 0.91 (t, J = 7.1 Hz, 6H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 144.90, 138.98, 138.97, 126.15, 124.68, 122.22, 112.26, 110.13, 45.43, 12.00. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₇F₃N₂ 233.1, found 233.2



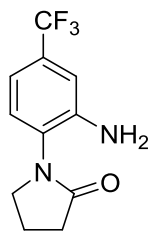
N¹-Isopropyl-4-(trifluoromethyl)benzene-1, 2-diamine (30b). From N-isopropyl-2-nitro-4-(trifluoromethyl)aniline (5 mmol, 1.240 g). Brown oil (98%). HPLC-MS: t_R = 2.75 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.92 (dd, J = 4.1, 2.3 Hz, 2H), 6.84 – 6.78 (m, 1H), 4.93 (s, 2H), 3.12 (t, J = 6.0 Hz, 4H), 1.85 (t, J = 6.2 Hz, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 141.58, 141.07, 121.32, 120.28, 116.74, 116.50, 102.09, 49.26, 24.05. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₀H₁₄F₃N₂ 219.2, found 219.0



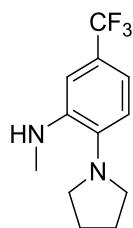
2-(2-Methylpyrrolidin-1-yl)-5-(trifluoromethyl)aniline (32b). From 2-methyl-1-(2-nitro-4-(trifluoromethyl)phenyl)pyrrolidine (5 mmol, 1.370 g). Brown oil (64%). HPLC-MS: t_R = 2.81 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 6.95 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.1 Hz, 1H), 5.02 (s, 2H), 3.65 – 3.50 (m, 2H), 2.55 – 2.50 (m, 1H), 2.10 (ddd, J = 11.9, 9.5, 3.7 Hz, 1H), 1.94 – 1.81 (m, 1H), 1.80 – 1.62 (m, 1H), 1.54 – 1.39 (m, 1H), 0.90 (d, J = 6.0 Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 143.51, 138.60, 126.30, 123.18, 119.10, 112.77, 110.23, 54.08, 50.85, 32.65, 22.54, 18.54. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{16}\text{F}_3\text{N}_2$ 245.2, found 245.1



tert-Butyl ((1-(2-amino-4-(trifluoromethyl)phenyl)pyrrolidin-2-yl)methyl)carbamate (33b). From tert-butyl ((1-(2-nitro-4-(trifluoromethyl)phenyl)pyrrolidin-2-yl)methyl)carbamate (3 mmol, 1.077 g). Brown oil (32%). HPLC-MS: t_R = 2.75 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.07 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 1.6 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.71 (t, J = 5.5 Hz, 1H), 5.12 (s, 2H), 3.69 – 3.51 (m, 2H), 3.04 – 2.93 (m, 1H), 2.68 (dt, J = 12.7, 6.5 Hz, 1H), 1.82 (ddd, J = 39.1, 26.1, 12.3, 7.4 Hz, 5H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.81, 143.90, 139.15, 126.25, 119.53, 112.74, 110.34, 77.53, 58.71, 54.91, 51.69, 42.85, 28.44, 28.23, 23.28. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{25}\text{F}_3\text{N}_3\text{O}_2$ 360.2, found 360.1

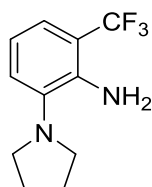


1-(2-Amino-4-(trifluoromethyl)phenyl)pyrrolidin-2-one (34b). From 1-(2-nitro-4-(trifluoromethyl)phenyl)pyrrolidin-2-one (3 mmol, 0.823 g). Brown oil (71%). HPLC-MS: t_R = 4.71 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.17 (d, J = 8.1 Hz, 1H), 7.06 (s, 1H), 6.84 (d, J = 8.1 Hz, 1H), 5.46 (s, 2H), 3.61 (t, J = 7.0 Hz, 2H), 2.41 (t, J = 8.0 Hz, 2H), 2.12 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 174.27, 145.31, 128.37, 127.17, 125.70, 122.99, 120.29, 111.95, 48.53, 30.76, 18.44. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_1$ 245.2, found 245.1

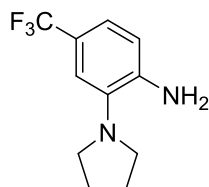


N-Methyl-2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (68b). A solution of methyl (2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)carbamate (**68a**) (1 eq, 1 mmol, 0.288 mmol) in diethyl ether (10 mL) was dried and filled with nitrogen gas. Lithium aluminium hydride (LiAlH_4 , 1 eq, 0.038 g) was added in the mixture with caution. The mixture was refluxed for 2 h. After completion of the reaction, the mixture was filtered and the solvent was removed to give the desired product. Brown oil (33%). HPLC-MS: t_R = 3.15 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 6.97 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.63 (s, 1H), 5.13 (d, J = 4.9 Hz, 1H), 3.02 (s, 4H), 2.74 (d, J = 5.1 Hz, 3H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 143.56, 140.55, 126.49, 123.10,

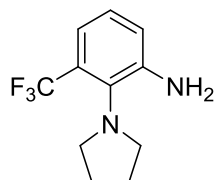
116.51, 112.59, 104.60, 49.80, 30.27, 23.73. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₂H₁₆F₃N₂ 245.1, found 245.0



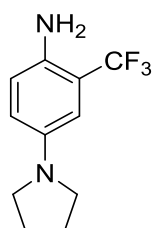
2-(Pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (76b). From 1-(2-nitro-3-(trifluoromethyl)phenyl)pyrrolidine (**76a**) (5 mmol, 1.300 g). Brown oil (95%). HPLC-MS: t_R = 3.00, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.46 (t, *J* = 10.3 Hz, 2H), 7.31 (t, *J* = 7.7 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 5H), 7.10 (d, *J* = 7.8 Hz, 5H), 6.66 (t, *J* = 7.8 Hz, 5H), 5.07 (s, 10H), 2.97 (s, 20H), 1.88 (s, 21H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 140.63, 133.61, 127.05, 123.05, 121.12, 116.33, 115.20, 51.24, 24.04. ¹⁹F NMR (377 MHz, DMSO-*d*₆) δ -61.30. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₄F₃N₂ 231.1, found 230.9



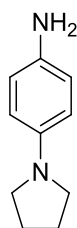
2-(Pyrrolidin-1-yl)-4-(trifluoromethyl)aniline (77b). From 1-(2-nitro-5-(trifluoromethyl)phenyl)pyrrolidine (**77a**) (5 mmol, 1.300 g). Brown oil (73%). HPLC-MS: t_R = 2.83 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.08 (s, 1H), 7.06 (s, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 5.28 (s, 2H), 2.98 (t, *J* = 5.6 Hz, 3H), 1.95 – 1.69 (m, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 145.78, 136.21, 126.73, 124.05, 119.82, 116.51, 113.68, 49.77, 23.60. ¹⁹F NMR (377 MHz, DMSO-*d*₆) δ -59.14. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₄F₃N₂ 231.0, found 230.7



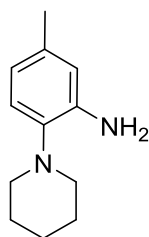
2-(Pyrrolidin-1-yl)-3-(trifluoromethyl)aniline (78b). From 1-(2-nitro-6-(trifluoromethyl)phenyl)pyrrolidine (**78a**) (5 mmol, 1.300 g). Brown oil (90%). HPLC-MS: t_R = 3.03 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.02 (dd, J = 12.1, 4.6 Hz, 1H), 6.99 (t, J = 4.1 Hz, 1H), 6.79 (dd, J = 7.1, 1.9 Hz, 1H), 5.02 (s, 1H), 3.06 (s, 2H), 1.92 (t, J = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 148.79, 131.33, 131.06, 130.42, 123.72, 119.87, 113.87, 49.04, 26.24. ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$) δ -59.93. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{14}\text{F}_3\text{N}_2$ 231.1, found 230.8



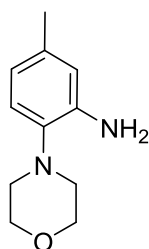
4-(Pyrrolidin-1-yl)-2-(trifluoromethyl)aniline (79b). From 1-(4-nitro-3-(trifluoromethyl)phenyl)pyrrolidine (**79a**) (5 mmol, 1.300 g). Brown oil (91%). HPLC-MS: t_R = 2.04 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 6.80 (d, J = 8.7 Hz, 1H), 6.66 (d, J = 8.5 Hz, 1H), 6.49 (d, J = 1.7 Hz, 1H), 4.68 (s, 2H), 3.11 (s, 4H), 1.90 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 139.85, 136.13, 126.73, 119.00, 117.86, 112.13, 107.75, 47.84, 24.73. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{14}\text{F}_3\text{N}_2$ 231.1, found 230.9



4-(Pyrrolidin-1-yl)aniline (80b). From 1-(4-nitrophenyl)pyrrolidine (5 mmol, 0.965 g). Brown oil (95%). HPLC-MS: t_R = 0.52 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 6.51 (d, J = 8.7 Hz, 1H), 6.36 (d, J = 8.7 Hz, 1H), 4.26 (d, J = 7.4 Hz, 1H), 3.09 (t, J = 6.3 Hz, 2H), 1.90 (t, J = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 140.53, 138.61, 115.56, 113.00, 48.05, 24.66. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{10}\text{H}_{15}\text{N}_2$ 163.2, found 163.1

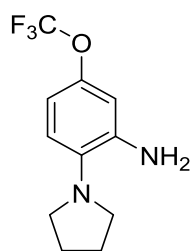


5-Methyl-2-(piperidin-1-yl)aniline (86b). From 1-(4-methyl-2-nitrophenyl)piperidine (**86a**) (5 mmol, 1.100 g). Brown oil (79%). HPLC-MS: t_R = 2.78 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 6.74 (d, J = 7.8 Hz, 1H), 6.46 (s, 1H), 6.34 (d, J = 2.6 Hz, 1H), 4.60 (s, 2H), 2.68 (m, 4H), 2.12 (s, 3H), 1.63 (m, 4H), 1.49 (m, 2H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 142.20, 137.28, 132.56, 119.07, 117.26, 114.99, 52.32, 26.43, 24.11, 20.90; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{19}\text{N}_2$ 191.2, found 191.2



5-Methyl-2-morpholinoaniline (87b). From 4-(4-methyl-2-nitrophenyl)morpholine (**87a**) (5 mmol, 1.110 g). Brown solid (81%), mp: 130.3 °C. HPLC-MS: t_R = 2.62 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 6.76 (d, J = 7.9 Hz, 1H), 6.48 (s, 1H), 6.35 (d, 1H, J = 9 Hz), 4.69 (s, 2H), 3.72 (t, J = 9 Hz, 4H), 2.73 (t, J = 9 Hz, 4H), 2.13 (s, 3H); ^{13}C NMR (101 MHz,

DMSO-*d*₆): δ 142.60, 136.16, 133.38, 119.38, 117.63, 115.52, 67.18, 51.63, 21.22; MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₇N₂O 193.1, found 193.2



2-(Pyrrolidin-1-yl)-5-(trifluoromethoxy)aniline (120b). From 1-(2-nitro-4-(trifluoromethoxy)phenyl)pyrrolidine (**120a**) (2.5 mmol, 0.690 g). Brown oil (95%). HPLC-MS: t_R = 2.61 min, purity 94%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.87 (d, J = 8.6 Hz, 1H), 6.60 (s, 1H), 6.41 (d, J = 8.5 Hz, 1H), 5.03 (s, 2H), 2.98 – 2.88 (m, 4H), 1.85 (d, J = 13.2 Hz, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 144.13, 143.64, 135.43, 121.51, 118.21, 107.65, 106.29, 49.93, 23.52. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₁H₁₄F₃N₂O 247.1, found 246.8

5.1.3. General procedures for the preparation of urea

5.1.3.1. Method A

A solution of amine (1 eq, 1 mmol) in dichloromethane (8 mL) was added with isocyanate (2 eq, 2 mmol) under an atmosphere of N₂ (g). The reaction was stirred at room temperature overnight and was monitored by TLC for the disappearance of starting material and formation of product. The mixture was purified by chromatography using petroleum ether and ethyl acetate.

5.1.3.2. Method B

4-NBsOXY: A stirred solution of Oxyrna (1 eq, 5 mmol) in dichloromethane (10 mL) was added with 4-nitrobenzenesulfonyl chloride (1 eq, 5 mmol) and DIPEA (1 eq, 5 mmol) under an atmosphere of N₂ (g). Then the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was monitored by

TLC for the disappearance of starting material and formation of product. After completion of the reaction, the reaction mixture was extracted with dichloromethane (2×10 mL) and 5% citric acid (2×5 mL), washed with saturated sodium chloride solution (2×5 mL), and dried over anhydrous Na_2SO_4 . It was then evaporated *in vacuo* and was recrystallised by using hexane. The crude product were pure enough for next step without further chromatography purification.

Synthesis of urea: A stirred solution of carboxylic acid (1 eq, 1 mmol) in THF (5 mL) was added with 4-NBsOXY (1 eq, 1 mmol), DIPEA (1 eq, 1 mmol) and DMAP (0.1 eq, 0.1 mmol) at room temperature. The reaction mixture was stirred for 30 min, followed by the addition of hydroxylamine hydrochloride (3 eq, 3 mmol) in acetonitrile (6 mL) and DIPEA (3 eq, 3 mmol). The reaction was monitored by TLC for the disappearance of starting material and formation of product. Then 4-NBsOXY (1 eq, 1 mmol) and DIPEA (1.5 eq, 1.5 mmol) and was added to the reaction mixture. The reaction was stirred at 0 °C for 90 min, followed by the addition of amine (1.5 eq, 1.5 mmol) and stirring at room temperature for more 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using a rotary evaporator and purified with flash chromatography using petroleum and ethyl acetate.

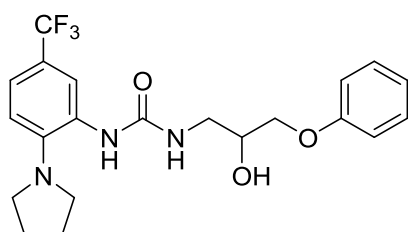
5.1.3.3. Method C

A solution of di-*tert*-butyl dicarbonate (1.4 eq, 0.7 mmol, 0.152 g) in acetonitrile (2 mL) was treated successively with a solution of 4-dimethylaminopyridine (DMAP, 1 eq, 0.5 mmol, 0.061 g) in acetonitrile (2 mL) and a solution of aniline (1 eq, 0.5 mmol) in acetonitrile (2 mL). The reaction mixture was stirred for 10

min vigorously at 0 °C. The amine (1.5 eq, 0.75 mmol) in acetonitrile (2 mL) was added into the reaction mixture. The mixture was stirred for overnight from 0 °C to room temperature. After completion of the reaction, the mixture was purified by chromatography using petroleum and ethyl acetate and concentrated to afford the product.

5.1.3.4. Method D

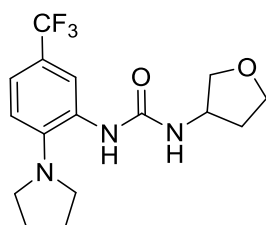
A stirred solution of carboxylic acid (1 eq, 1 mmol) in acetonitrile (10 mL) was added with NMM (4-methylmorpholine, 2 eq, 2 mmol, 0.22 mL) at 0 °C. T3P (50% in EtOAc, 1.2 eq, 1.2 mmol, 0.71 mL) was then added in the reaction mixture. The mixture was stirred at the same temperature for 15 min, followed by the addition of hydroxylamine (1.2 eq, 1.2 mmol, 0.083 g, obtained by neutralization of the corresponding hydroxylamine hydrochloride salt (1.2 eq, 1.2 mmol) with DIPEA (1.2 eq, 1.2 mmol, 0.2 mL) in acetonitrile (2 mL). After completion of the reaction, T3P (1.5 eq, 1.5 mmol, 0.89 mL) and NMM (1.5 eq, 1.5 mmol, 0.16 mL) were added to the reaction mixture and the reaction was stirred for another 30 min. Then the amine (1.5 eq, 1.5 mmol) was added and the mixture was refluxed for 3 h. The solvent was removed under reduced pressure and the mixture was purified by flash chromatography using petroleum and ethyl acetate.



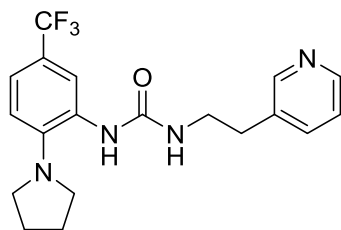
1-(2-Hydroxy-3-phenoxypropyl)-3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)urea (1). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (2b) (0.5 mmol, 0.115 g) and 1-amino-3-

phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (63%). HPLC-MS: t_R = 2.89 min, purity 88%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.93 (s, 1H), 7.85 (s, 1H), 7.28 (t, J = 7.9 Hz, 2H), 7.21 (d, J = 8.1 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.96 – 6.89 (m, 3H), 6.86 (t, J = 5.4 Hz, 1H), 5.27 (d, J = 3.7 Hz, 1H), 3.95 – 3.83 (m, 3H), 3.39 (d, J = 5.4 Hz, 1H), 3.17 (dd, J = 18.8, 4.1 Hz, 5H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 158.59, 155.78, 145.19, 130.09, 129.47, 126.18, 123.49, 120.58, 120.12, 119.92, 116.64, 114.46, 70.00, 68.23, 50.15, 42.60, 24.48. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{25}\text{F}_3\text{N}_3\text{O}_3$ 424.4, found 424.1

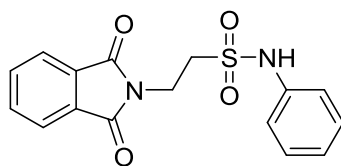


1-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (2). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (1.5 mmol, 0.345 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method B). White solid (89%). HPLC-MS: t_R = 2.75 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.04 (s, 1H), 7.67 (s, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.13 – 7.00 (m, 2H), 4.25 – 4.14 (m, 1H), 3.84 – 3.65 (m, 3H), 3.49 (dd, J = 8.9, 3.3 Hz, 1H), 3.13 (t, J = 6.0 Hz, 4H), 2.13 (td, J = 15.2, 7.5 Hz, 1H), 1.90 (t, J = 6.1 Hz, 4H), 1.77 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.12, 144.68, 130.79, 126.15, 123.46, 119.54, 118.73, 117.08, 73.03, 66.16, 50.34, 50.20, 32.70, 24.44. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_2$ 344.2, found 344.1



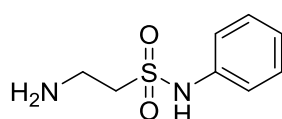
1-(2-(Pyridin-3-yl)ethyl)-3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)urea (3). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). White solid (71%). HPLC-MS: t_R = 2.27 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.48 – 8.38 (m, 2H), 7.85 (s, 1H), 7.66 (d, J = 10.9 Hz, 2H), 7.32 (dd, J = 7.6, 4.9 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 8.5 Hz, 1H), 6.69 (t, J = 5.6 Hz, 1H), 3.40 – 3.35 (m, 2H), 3.12 (t, J = 6.1 Hz, 4H), 2.77 (t, J = 6.8 Hz, 2H), 1.87 (t, J = 6.2 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.42, 150.00, 147.38, 145.32, 136.43, 136.29, 135.02, 129.76, 127.04, 123.39, 120.17, 119.99, 116.50, 50.12, 40.20, 32.90, 24.50. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{22}\text{F}_3\text{N}_4\text{O}$ 379.2, found 379.2

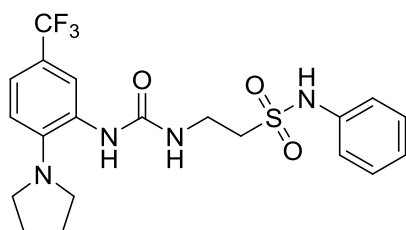


2-(1, 3-Dioxoisindolin-2-yl)-N-phenylethane-1-sulfonamide (4a). A solution of aniline (5 eq, 5 mmol, 0.45 mL) in chloroform at 0 °C. 2-(1, 3-dioxoisindolin-2-yl)propanoyl chloride (1 eq, 1 mmol, 0.274 g) was added in the solution of aniline. The reaction was allowed to warm up to room temperature and stirred for 48 h. After the completion, the resulting mixture was extracted with saturated ammonium chloride (2 × 15 mL) and dried with anhydrous Na_2SO_4 . The organic layers were combined and concentrated. The

residue was recrystallized with ethanol (30 mL) to give the crude product. White solid (73%). HPLC-MS: t_R = 2.69 min, purity 100%. ^1H NMR (400 MHz, CDCl_3) δ 7.88 – 7.82 (m, 1H), 7.78 – 7.69 (m, 1H), 7.37 – 7.29 (m, 2H), 7.21 – 7.09 (m, 1H), 4.12 – 4.05 (m, 1H), 3.50 – 3.43 (m, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 168.49, 136.73, 134.53, 131.89, 129.92, 125.34, 123.83, 120.27, 47.99, 32.77. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{15}\text{N}_2\text{O}_4\text{S}$ 331.2, found 331.2

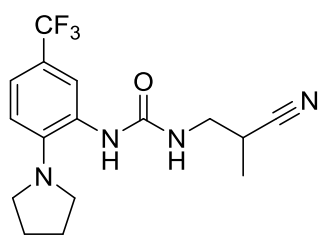


2-Amino-N-phenylethane-1-sulfonamide (4b). A suspension of 2-(1, 3-Dioxoisindolin-2-yl)-N-phenylethane-1-sulfonamide (**4a**) (1 mmol, 0.331 g) was heated at reflux in ethanol (5 mL). Hydrazine (1.1 mmol, 0.055 g, 0.053 mL) was added. After 3h, the crude product was obtained by filtration. White solid (61%). HPLC-MS: t_R = 0.51 min, purity 81%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.32 (dd, J = 14.0, 7.4 Hz, 2H), 7.22 (dd, J = 13.7, 7.7 Hz, 2H), 7.09 (dd, J = 16.6, 8.0 Hz, 1H), 4.84 (s, 1H), 3.54 (dd, J = 13.3, 6.4 Hz, 1H), 3.35 – 3.22 (m, 1H), 3.15 (t, J = 6.9 Hz, 1H), 2.90 (t, J = 6.9 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 135.74, 129.31, 125.19, 119.65, 53.68, 36.46. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_8\text{H}_{13}\text{N}_2\text{O}_2\text{S}$ 201.1, found 201.1



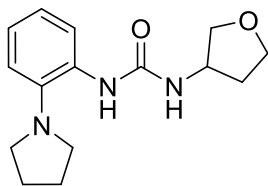
N-Phenyl-2-(3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)ethane-1-sulfonamide (4). From 2-

(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-Amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.151 g) (Method C). White solid (83%). HPLC-MS: t_R = 2.90 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.82 (s, 1H), 7.89 (s, 1H), 7.78 (s, 1H), 7.32 (t, J = 7.8 Hz, 2H), 7.22 (d, J = 7.4 Hz, 3H), 7.10 (t, J = 7.3 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 6.79 (t, J = 5.6 Hz, 1H), 3.48 (dd, J = 12.6, 6.3 Hz, 2H), 3.24 (t, J = 6.6 Hz, 2H), 3.16 (s, 4H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.52, 145.70, 138.02, 129.30, 128.85, 126.16, 124.01, 123.47, 120.58, 119.99, 119.25, 116.39, 50.83, 50.06, 34.31, 24.58. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_3\text{S}$ 457.2, found 457.2

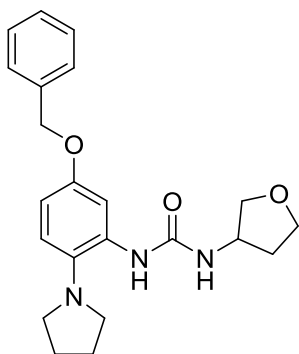


1-(2-Cyanopropyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea

(5). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 3-amino-2-methylpropanenitrile (0.6 mmol, 0.051 g) (Method C). White solid (79%). HPLC-MS: t_R = 2.81 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.87 (s, 2H), 7.25 (d, J = 7.7 Hz, 1H), 7.07 (t, J = 5.8 Hz, 1H), 7.02 (d, J = 8.5 Hz, 1H), 3.32 – 3.25 (m, 2H), 3.18 (s, 4H), 3.09 – 2.95 (m, 1H), 1.89 (s, 4H), 1.21 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.02, 146.05, 129.73, 126.61, 123.92, 122.94, 120.94, 120.01, 117.04, 50.62, 42.44, 26.84, 25.02, 15.38. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{20}\text{F}_3\text{N}_4\text{O}$ 341.1, found 340.9

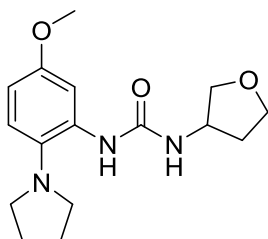


1-(2-(Pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (6). From 2-(pyrrolidin-1-yl)aniline (**6b**) (1.5 mmol, 0.243 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method B). White solid (81%). HPLC-MS: t_R = 2.91 min, purity 98%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.19 (s, 1H), 8.11 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.91 (d, J = 5.6 Hz, 2H), 4.20 (s, 1H), 3.75 (dd, J = 14.9, 6.2 Hz, 3H), 3.48 (d, J = 8.5 Hz, 1H), 2.16 – 2.07 (m, 1H), 1.69 (d, J = 4.9 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.51, 150.37, 128.14, 124.38, 121.55, 120.03, 114.69, 73.01, 66.14, 50.07, 44.35, 32.70, 29.99. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{22}\text{N}_3\text{O}_2$ 276.2, found 276.1



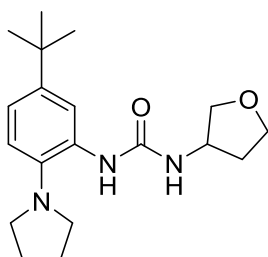
1-(5-(Benzyloxy)-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (7). From 5-(benzyloxy)-2-(pyrrolidin-1-yl)aniline (**7b**) (1.5 mmol, 0.403 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (71%). HPLC-MS: t_R = 2.30 min, purity 97%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.88 – 7.82 (m, 2H), 7.40 (dt, J = 14.7, 7.3 Hz, 5H), 7.31 (t, J = 7.1 Hz, 1H), 7.03 (t, J = 6.6 Hz, 1H), 6.50 (dd, J = 8.7, 2.9 Hz, 1H), 5.00 (s, 2H), 4.25 – 4.14 (m, 1H), 3.84 – 3.67 (m, 3H), 3.49 (dd, J = 8.9, 3.3 Hz, 1H), 2.87 (s, 4H), 2.12

(td, $J = 15.3, 7.5$ Hz, 1H), 1.89 (s, 4H), 1.75 – 1.67 (m, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.85, 154.70, 137.42, 136.12, 132.24, 128.36, 127.65, 127.49, 120.10, 106.71, 105.35, 72.99, 69.16, 66.17, 52.18, 50.08, 32.62, 23.95. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{22}\text{H}_{28}\text{N}_3\text{O}_3$ 382.1, found 381.9



1-(5-Methoxy-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (8).

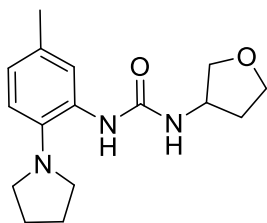
From 5-methoxy-2-(pyrrolidin-1-yl)aniline (**8b**) (1.5 mmol, 0.288 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (76%). HPLC-MS: $t_R = 0.90$ min, purity 95%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.88 (s, 1H), 7.77 (d, $J = 2.6$ Hz, 1H), 7.39 (d, $J = 6.5$ Hz, 1H), 7.03 (d, $J = 8.7$ Hz, 1H), 6.42 (dd, $J = 8.7, 2.7$ Hz, 1H), 4.20 (s, 1H), 3.85 – 3.78 (m, 1H), 3.78 – 3.67 (m, 3H), 3.66 (s, 3H), 3.49 (dd, $J = 8.8, 2.9$ Hz, 1H), 2.86 (s, 4H), 2.12 (dq, $J = 15.2, 7.5$ Hz, 1H), 1.88 (s, 4H), 1.76 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 156.16, 155.38, 136.65, 132.43, 120.66, 106.35, 104.76, 73.48, 66.65, 55.44, 52.71, 50.56, 33.08, 24.41. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_3$ 306.2, found 306.2



1-(5-(tert-Butyl)-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea

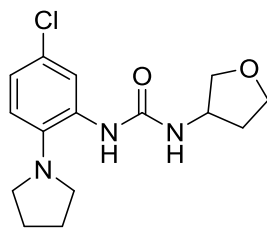
(9). From 5-(tert-butyl)-2-(pyrrolidin-1-yl)aniline (**9b**) (1 eq, 0.5 mmol, 0.109

g) and 3-aminotetrahydrofuran (1.5 eq, 1.5 mmol, 0.093 g, 0.75 mmol) (Method C). White solid (88%). HPLC-MS: t_R = 2.25 min, purity 98%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.00 (d, J = 2.1 Hz, 1H), 7.62 (s, 1H), 7.16 (d, J = 6.5 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.88 (dd, J = 8.4, 2.2 Hz, 1H), 4.24 – 4.14 (m, 1H), 3.84 – 3.64 (m, 4H), 3.47 (dt, J = 8.3, 4.1 Hz, 1H), 2.93 (d, J = 5.7 Hz, 3H), 2.12 (td, J = 15.2, 7.5 Hz, 1H), 1.88 (s, 3H), 1.74 – 1.64 (m, 1H), 1.22 (s, 7H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.20, 144.74, 137.33, 133.04, 128.28, 117.86, 109.32, 73.12, 66.20, 51.29, 48.62, 34.01, 31.32, 31.15, 24.03. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{30}\text{N}_3\text{O}_2$ 332.2, found 332.3

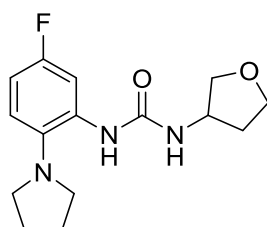


1-(5-Methyl-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (10).

From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (1.5 mmol, 0.266 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method B). White solid (85%). HPLC-MS: t_R = 1.78, purity 96%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.77 (s, 1H), 7.64 (s, 1H), 7.19 (d, J = 6.5 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 7.9 Hz, 1H), 4.18 (dd, J = 9.4, 6.6 Hz, 1H), 3.84 – 3.65 (m, 3H), 3.48 (dd, J = 8.8, 3.3 Hz, 1H), 2.92 (s, 4H), 2.19 (s, 3H), 2.11 (dt, J = 15.2, 7.6 Hz, 1H), 1.88 (s, 4H), 1.70 (dt, J = 12.4, 5.3 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.04, 137.25, 133.58, 131.42, 122.14, 120.25, 118.31, 73.06, 66.17, 51.39, 50.14, 32.66, 24.01, 20.86. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_2$ 290.2, found 290.2

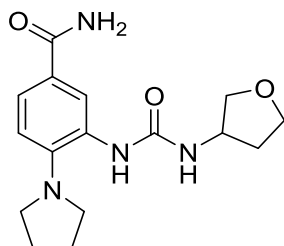
**1-(5-Chloro-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (11).**

From 5-chloro-2-(pyrrolidin-1-yl)aniline (1 eq, 0.5 mmol, 0.099g) and 3-aminotetrahydrofuran (1.5 eq, 0.75 mmol, 0.093 g) (Method C). White solid (43%). HPLC-MS: t_R = 2.45 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.03 (d, J = 2.5 Hz, 1H), 7.77 (s, 1H), 7.32 (d, J = 6.6 Hz, 1H), 7.04 (d, J = 8.6 Hz, 1H), 6.88 (dd, J = 8.5, 2.5 Hz, 1H), 4.20 (qt, J = 7.0, 3.7 Hz, 1H), 3.84 – 3.65 (m, 3H), 3.49 (dd, J = 8.9, 3.2 Hz, 1H), 2.95 (t, J = 5.6 Hz, 4H), 2.19 – 2.06 (m, 1H), 1.91 (d, J = 13.2 Hz, 4H), 1.70 (ddd, J = 12.5, 8.3, 5.3 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.75, 138.66, 134.84, 126.33, 121.03, 119.84, 118.81, 72.98, 66.16, 51.17, 50.14, 32.64, 24.05. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{ClN}_3\text{O}_2$ 310.2, found 310.2

**1-(5-Fluoro-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (12).**

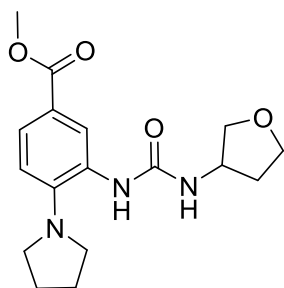
From 5-fluoro-2-(pyrrolidin-1-yl)aniline (**12b**) (1.5 mmol, 0.270 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (71%). HPLC-MS: t_R = 1.95 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.92 (dd, J = 12.6, 3.3 Hz, 2H), 7.46 (d, J = 6.6 Hz, 1H), 7.12 (dd, J = 8.6, 6.2 Hz, 1H), 6.64 (td, J = 8.5, 2.8 Hz, 1H), 4.26 – 4.13 (m, 1H), 3.87 – 3.63 (m, 3H), 3.50 (dd, J = 8.9, 3.1 Hz, 1H), 2.90 (s, 4H), 2.13 (dq, J = 15.3, 7.6 Hz, 1H), 1.90

(s, 4H), 1.77 – 1.57 (m, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 157.25, 154.69, 136.27, 135.09, 120.35, 106.68, 104.83, 72.95, 66.15, 51.89, 50.09, 32.61, 23.96. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{FN}_3\text{O}_2$ 294.2, found 294.2



4-(Pyrrolidin-1-yl)-3-(3-(tetrahydrofuran-3-yl)ureido)benzamide (13).

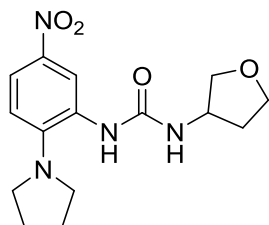
From 3-amino-4-(pyrrolidin-1-yl)benzamide (**13b**) (1.5 mmol, 0.308 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (37%). HPLC-MS: t_R = 2.78 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.96 (d, J = 1.8 Hz, 1H), 7.66 (s, 1H), 7.53 (s, 1H), 7.48 (dd, J = 8.5, 2.0 Hz, 1H), 6.99 (s, 1H), 6.83 (d, J = 8.5 Hz, 2H), 4.19 (s, 1H), 3.78 – 3.65 (m, 3H), 3.49 – 3.45 (m, 1H), 3.16 (dd, J = 13.5, 7.3 Hz, 4H), 2.11 (dt, J = 20.3, 7.5 Hz, 1H), 1.88 (t, J = 6.3 Hz, 4H), 1.70 (td, J = 12.4, 5.0 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 167.84, 155.67, 145.43, 128.02, 125.20, 125.09, 123.57, 115.24, 73.10, 66.17, 53.30, 50.07, 30.71, 24.62. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{23}\text{N}_4\text{O}_3$ 319.1, found 319.1



Methyl 4-(pyrrolidin-1-yl)-3-(3-(tetrahydrofuran-3-yl)ureido)benzoate (14).

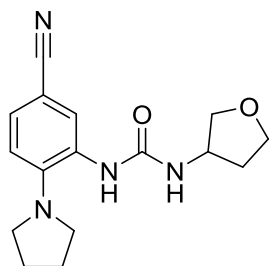
From methyl 3-amino-4-(pyrrolidin-1-yl)benzoate (**14b**) (0.5 mmol, 0.093 g) and 3-aminotetrahydrofuran (1.5 eq, 1.5 mmol, 0.093 g, 0.75 mmol) (Method

C). White solid (79%). HPLC-MS: t_R = 2.47 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.02 (d, J = 1.7 Hz, 1H), 7.55 (dd, J = 8.6, 1.9 Hz, 1H), 7.50 (s, 1H), 6.86 (d, J = 8.6 Hz, 1H), 6.75 (d, J = 6.8 Hz, 1H), 4.24 – 4.12 (m, 1H), 3.71 (ddd, J = 16.4, 11.4, 5.8 Hz, 3H), 3.47 (dd, J = 8.8, 3.4 Hz, 1H), 3.23 (s, 4H), 2.12F (td, J = 15.2, 7.5 Hz, 1H), 1.88 (s, 4H), 1.70 (ddd, J = 12.4, 7.4, 5.4 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 166.22, 155.62, 147.21, 127.47, 126.77, 125.74, 119.41, 115.41, 73.08, 66.17, 51.52, 49.98, 32.76, 24.75. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{24}\text{N}_3\text{O}_4$ 334.1, found 334.1



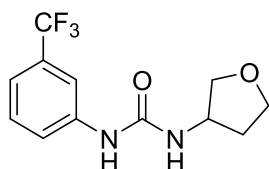
1-(5-Nitro-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (15).

From 5-nitro-2-(pyrrolidin-1-yl)aniline (**15b**) (1.5 mmol, 0.311 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (84%). HPLC-MS: t_R = 2.45 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.14 (d, J = 2.5 Hz, 1H), 7.86 (dd, J = 9.2, 2.7 Hz, 1H), 7.65 (s, 1H), 6.84 (d, J = 9.3 Hz, 1H), 6.75 (d, J = 6.8 Hz, 1H), 4.19 (s, 1H), 3.81 – 3.66 (m, 3H), 3.48 (dd, J = 8.9, 3.4 Hz, 1H), 3.42 – 3.35 (m, 4H), 2.12 (dt, J = 20.1, 7.6 Hz, 1H), 1.90 (s, 4H), 1.78 – 1.65 (m, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.69, 149.57, 137.11, 125.36, 122.47, 121.15, 114.59, 101.26, 73.00, 66.17, 50.15, 32.69, 24.95. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{N}_4\text{O}_4$ 321.1, found 321.1



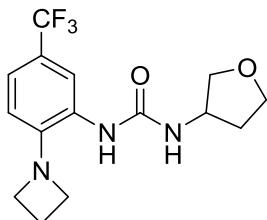
1-(5-Cyano-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (16).

From 3-amino-4-(pyrrolidin-1-yl)benzonitrile (**16b**) (1.5 mmol, 0.281 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (87%). HPLC-MS: t_R = 2.44 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.75 (d, J = 1.7 Hz, 1H), 7.58 (s, 1H), 7.34 (dd, J = 8.5, 1.9 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 6.8 Hz, 1H), 4.23 – 4.14 (m, 1H), 3.80 – 3.67 (m, 3H), 3.47 (dd, J = 8.9, 3.4 Hz, 1H), 3.23 (t, J = 6.3 Hz, 4H), 2.11 (dq, J = 15.3, 7.5 Hz, 1H), 1.88 (t, J = 6.4 Hz, 4H), 1.75 – 1.63 (m, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.46, 146.98, 128.51, 128.28, 128.13, 119.86, 116.41, 99.59, 73.04, 66.18, 50.28, 49.96, 32.71, 24.78. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{N}_4\text{O}_2$ 301.1, found 301.1

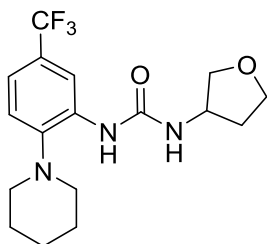


1-(Tetrahydrofuran-3-yl)-3-(3-(trifluoromethyl)phenyl)urea (17). From 1-isocyanato-3-(trifluoromethyl)benzene (1 eq, 0.5 mmol, 0.094 g) and 3-aminotetrahydrofuran (1.5 eq, 1.5 mmol, 0.093 g, 0.75 mmol) (Method A). White solid (89%). HPLC-MS: t_R = 2.63 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.70 (s, 1H), 7.97 (s, 1H), 7.49 – 7.38 (m, 2H), 7.21 (d, J = 7.1 Hz, 1H), 6.55 (d, J = 6.7 Hz, 1H), 4.26 – 4.13 (m, 1H), 3.84 – 3.64 (m, 3H), 3.50 (dd, J = 8.9, 3.5 Hz, 1H), 2.12 (dq, J = 15.3, 7.5 Hz, 1H), 1.79 – 1.64 (m, 1H).

^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.81, 141.18, 129.73, 125.64, 122.94, 121.21, 117.38, 113.63, 72.86, 66.18, 50.18, 32.57. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_2$ 275.2, found 275.1

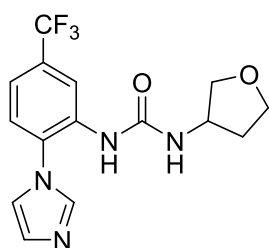


1-(2-(Azetidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (18). From 2-(azetidin-1-yl)-5-(trifluoromethyl)aniline (**18b**) (1.5 mmol, 0.324 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (79%). HPLC-MS: t_R = 2.63 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.76 (s, 1H), 7.37 (s, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.83 (d, J = 6.7 Hz, 1H), 6.65 (d, J = 8.4 Hz, 1H), 4.25 – 4.12 (m, 1H), 3.89 (t, J = 7.2 Hz, 4H), 3.81 – 3.68 (m, 3H), 3.47 (dd, J = 8.9, 3.2 Hz, 1H), 2.23 (p, J = 7.2 Hz, 2H), 2.12 (td, J = 15.3, 7.5 Hz, 1H), 1.69 (dt, J = 12.4, 5.3 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.63, 147.60, 127.41, 126.68, 123.99, 120.91, 119.39, 113.92, 73.49, 66.60, 53.59, 50.65, 33.19, 17.11. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_2$ 330.2, found 330.2

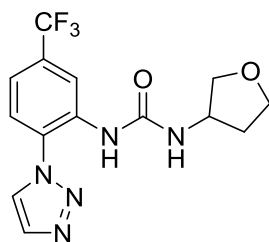


1-(2-(Piperidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (19). From 2-(piperidin-1-yl)-5-(trifluoromethyl)aniline (1.5 mmol, 0.366 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method B). White solid (79%). HPLC-MS: t_R = 2.95 min, purity 95%. ^1H NMR (400 MHz,

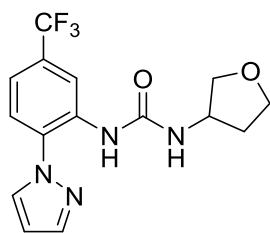
DMSO- d_6) δ 8.47 (s, 1H), 7.84 (s, 1H), 7.64 (d, J = 6.5 Hz, 1H), 7.23 (q, J = 8.4 Hz, 2H), 4.27 – 4.17 (m, 1H), 3.88 – 3.67 (m, 3H), 3.53 (dd, J = 9.0, 3.2 Hz, 1H), 2.76 – 2.71 (m, 4H), 2.15 (dq, J = 15.3, 7.5 Hz, 1H), 1.74 (dd, J = 14.3, 9.1 Hz, 5H), 1.55 (d, J = 4.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.72, 145.18, 135.15, 125.89, 124.38, 120.53, 117.90, 114.43, 72.88, 66.19, 52.62, 50.24, 32.59, 25.48, 23.58. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{17}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2$ 358.2, found 358.2



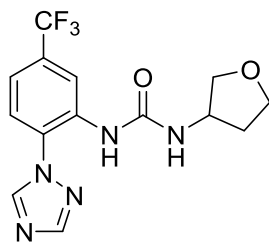
1-(2-(1H-Imidazol-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (21). From 2-(1H-imidazol-1-yl)-5-(trifluoromethyl)aniline (**21b**) (1.5 mmol, 0.341 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (73%). HPLC-MS: t_R = 1.91 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.64 (s, 4H), 7.89 (s, 4H), 7.74 (s, 4H), 7.41 (s, 12H), 7.25 (s, 4H), 7.18 (s, 5H), 4.13 (d, J = 25.5 Hz, 5H), 3.69 (d, J = 4.9 Hz, 17H), 2.09 (s, 6H), 1.65 (s, 6H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.36, 137.85, 136.15, 129.66, 129.15, 128.52, 125.23, 123.17, 120.81, 118.27, 116.96, 72.80, 66.06, 50.16, 32.50. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{15}\text{H}_{16}\text{F}_3\text{N}_4\text{O}_2$ 341.2, found 341.2



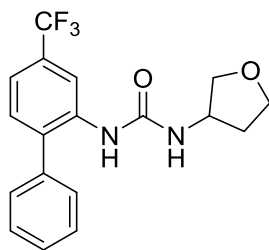
1-(2-(1H-1, 2, 3-Triazol-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (22). From 2-(1H-1, 2, 3-triazol-1-yl)-5-(trifluoromethyl)aniline (**22b**) (1.5 mmol, 0.342 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (73%). HPLC-MS: t_R = 2.54 min, purity 93%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.71 (s, 1H), 8.61 (d, J = 0.8 Hz, 1H), 8.08 (s, 2H), 7.62 (d, J = 8.3 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 6.6 Hz, 1H), 4.23 – 4.09 (m, 1H), 3.76 – 3.65 (m, 3H), 3.46 (dd, J = 9.0, 3.1 Hz, 1H), 2.10 (dq, J = 15.4, 7.6 Hz, 1H), 1.67 (dt, J = 12.5, 8.1 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.16, 135.34, 134.19, 130.25, 128.24, 126.91, 122.37, 118.24, 117.64, 72.72, 66.07, 50.21, 32.39. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{14}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$ 342.1, found 342.0



1-(2-(1H-Pyrazol-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (23). From 2-(1H-pyrazol-1-yl)-5-(trifluoromethyl)aniline (**23b**) (1.5 mmol, 0.341 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (77%). HPLC-MS: t_R = 2.71 min, purity 99%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.05 (s, 1H), 8.69 (s, 1H), 8.31 (d, J = 1.8 Hz, 1H), 7.91 (d, J = 1.1 Hz, 1H), 7.63 (d, J = 7.7 Hz, 2H), 7.40 (d, J = 8.3 Hz, 1H), 6.64 (s, 1H), 4.18 (s, 1H), 3.83 – 3.71 (m, 2H), 3.68 (dd, J = 13.8, 8.2 Hz, 1H), 3.49 (dd, J = 8.9, 3.3 Hz, 1H), 2.10 (dq, J = 15.1, 7.6 Hz, 1H), 1.79 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.38, 141.70, 133.96, 131.58, 130.66, 128.08, 127.76, 124.92, 122.62, 117.70, 107.71, 72.73, 66.19, 50.36, 32.28. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{16}\text{F}_3\text{N}_4\text{O}_2$ 341.1, found 340.9

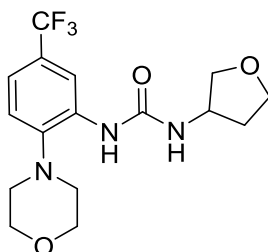


1-(2-(1H-1, 2, 4-Triazol-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (24). From 2-(1H-1, 2, 4-triazol-1-yl)-5-(trifluoromethyl)aniline (**24b**) (1.5 mmol, 0.342 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (79%). HPLC-MS: t_R = 2.50 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.02 (s, 1H), 8.71 (s, 1H), 8.38 (s, 1H), 8.16 (s, 1H), 7.65 (d, J = 8.2 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 6.5 Hz, 1H), 4.25 – 4.09 (m, 1H), 3.80 – 3.60 (m, 3H), 3.48 (dd, J = 9.0, 2.9 Hz, 1H), 2.16 – 2.01 (m, 1H), 1.75 – 1.62 (m, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.17, 153.01, 145.63, 135.32, 133.36, 128.02, 127.18, 125.14, 122.43, 117.20, 72.74, 66.11, 50.26, 32.41. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{14}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$ 342.1, found 342.0



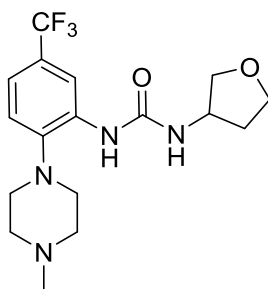
1-(Tetrahydrofuran-3-yl)-3-(4-(trifluoromethyl)-[1, 1'-biphenyl]-2-yl)urea (25). From 4-(trifluoromethyl)-[1, 1'-biphenyl]-2-amine (**25b**) (1.5 mmol, 0.356 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (73%). HPLC-MS: t_R = 2.87 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.59 (s, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.53 (t, J = 7.3 Hz, 2H), 7.47 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 7.0 Hz, 2H), 7.34 – 7.29 (m, 2H), 7.22 (d, J = 6.5 Hz, 1H), 4.19 (d, J = 2.3 Hz, 1H), 3.78 – 3.59 (m, 3H), 3.47 (dd, J = 8.9, 2.7

Hz, 1H), 2.09 (dq, $J = 15.3, 7.5$ Hz, 1H), 1.66 (dt, $J = 12.3, 8.6$ Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.85, 137.72, 137.20, 134.44, 131.23, 129.08, 128.64, 128.32, 128.19, 125.70, 123.00, 116.56, 72.94, 66.12, 50.26, 32.60. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_2$ 351.1, found 351.0



1-(2-Morpholino-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea

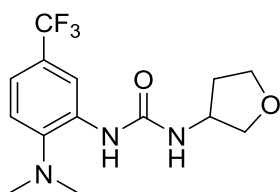
(26). From 2-morpholino-5-(trifluoromethyl)aniline (1.5 mmol, 0.369 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method B). White solid (81%). HPLC-MS: $t_R = 2.73$ min, purity 97%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.50 (s, 1H), 7.92 (s, 1H), 7.60 (d, $J = 6.6$ Hz, 1H), 7.30 (d, $J = 8.3$ Hz, 1H), 7.23 (d, $J = 8.3$ Hz, 1H), 4.27 – 4.17 (m, 1H), 3.83 (t, $J = 4.2$ Hz, 4H), 3.82 – 3.65 (m, 3H), 3.53 (dd, $J = 9.0, 3.1$ Hz, 1H), 2.83 – 2.77 (m, 4H), 2.21 – 2.06 (m, 1H), 1.74 (ddd, $J = 12.6, 9.1, 5.5$ Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.67, 143.75, 135.24, 128.53, 125.83, 120.72, 118.00, 114.63, 72.90, 66.09, 51.52, 50.25, 32.63, 24.44. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_3$ 360.1, found 360.2



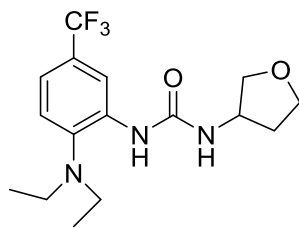
1-(2-(4-Methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)-3-

(tetrahydrofuran-3-yl)urea (27). From 2-(4-methylpiperazin-1-yl)-5-

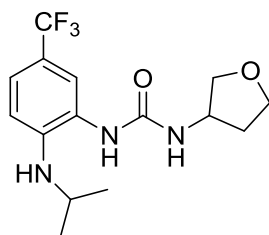
(trifluoromethyl)aniline (**27b**) (1.5 mmol, 0.389 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method B). White solid (57%). HPLC-MS: t_R = 2.11 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.13 (s, 2H), 7.98 (s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.69 (d, J = 8.0 Hz, 1H), 4.21 – 4.11 (m, 1H), 3.86 – 3.64 (m, 4H), 3.50 (dd, J = 8.8, 3.9 Hz, 4H), 2.92 (s, 4H), 2.68 (s, 3H), 2.20 (s, 3H), 2.08 (dq, J = 15.1, 7.6 Hz, 1H), 1.80 (dq, J = 7.2, 5.2 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.08, 136.85, 135.21, 134.09, 121.47, 120.20, 118.68, 106.94, 72.94, 66.33, 52.88, 50.21, 42.88, 32.28, 21.16. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_2$ 373.2, found 373.2



1-(2-(Dimethylamino)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (28). From N^1,N^1 -dimethyl-4-(trifluoromethyl)benzene-1,2-diamine (1 eq, 0.5 mmol, 0.102 g) and 3-aminotetrahydrofuran (1.5 eq, 0.75 mmol, 0.093 g) (Method C). White solid (69%). HPLC-MS: t_R = 2.74 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.49 (d, J = 1.3 Hz, 1H), 8.14 (s, 1H), 7.46 (d, J = 6.6 Hz, 1H), 7.27 (d, J = 8.3 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 4.20 (qt, J = 7.0, 3.7 Hz, 1H), 3.85 – 3.66 (m, 3H), 3.49 (dd, J = 8.9, 3.3 Hz, 1H), 2.62 (d, J = 5.9 Hz, 6H), 2.13 (dq, J = 15.4, 7.5 Hz, 1H), 1.71 (ddd, J = 12.5, 8.1, 5.0 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.24, 145.64, 135.45, 126.37, 124.54, 120.37, 118.16, 114.60, 114.56, 73.46, 66.60, 50.54, 44.00, 33.04. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{14}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_2$ 318.2, found 318.1

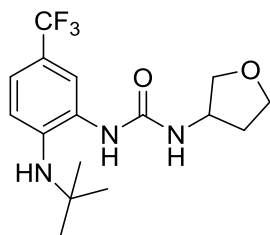


1-(2-(Diethylamino)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (29). From N^1 , N^1 -diethyl-4-(trifluoromethyl)benzene-1, 2-diamine (**29b**) (1.5 mmol, 0.348 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (81%). HPLC-MS: t_R = 2.85 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.54 (d, J = 1.4 Hz, 1H), 8.30 (s, 1H), 7.58 (d, J = 6.5 Hz, 1H), 7.32 (d, J = 8.2 Hz, 1H), 7.22 – 7.16 (m, 1H), 4.19 (qt, J = 7.0, 3.7 Hz, 1H), 3.84 – 3.65 (m, 3H), 3.50 (dd, J = 8.9, 3.3 Hz, 1H), 2.94 (q, J = 7.1 Hz, 4H), 2.13 (dq, J = 15.4, 7.5 Hz, 1H), 1.72 (ddd, J = 12.5, 8.7, 5.2 Hz, 1H), 0.88 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.78, 141.13, 138.14, 125.87, 125.09, 123.72, 117.16, 113.77, 72.96, 66.18, 50.14, 47.45, 32.55, 11.82. MS (ESI $^+$): m/z $[M+H]^+$, calcd for $C_{16}H_{23}F_3N_3O_2$ 346.2, found 346.0

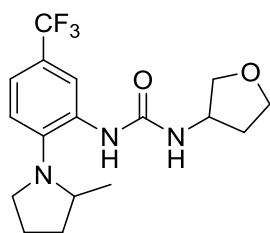


1-(2-(Isopropylamino)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (30). From N^1 -isopropyl-4-(trifluoromethyl)benzene-1, 2-diamine (**30b**) (1.5 mmol, 0.327 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (77%). HPLC-MS: t_R = 2.73 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.73 (s, 1H), 7.56 (s, 1H), 7.19 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 6.55 (d, J = 6.6 Hz, 1H), 4.94 (d, J = 7.2 Hz, 1H),

4.25 – 4.14 (m, 1H), 3.86 – 3.60 (m, 4H), 3.52 (dd, $J = 8.9, 3.3$ Hz, 1H), 2.13 (dq, $J = 15.2, 7.5$ Hz, 1H), 1.80 – 1.64 (m, 1H), 1.19 (d, $J = 6.2$ Hz, 6H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.36, 142.38, 125.28, 120.82, 119.06, 115.36, 110.53, 72.91, 66.15, 50.29, 43.26, 32.59, 22.27. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_2$ 332.1, found 332.1

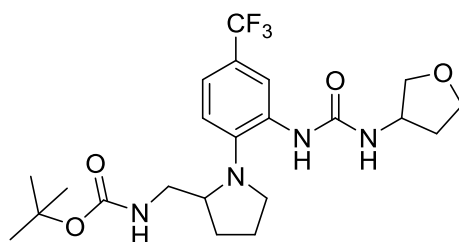


1-(2-(tert-Butylamino)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (31). From N^1 -(tert-butyl)-4-(trifluoromethyl)benzene-1, 2-diamine (1.5 mmol, 0.348 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (69%). HPLC-MS: $t_R = 2.58$ min, purity 93%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.73 (d, $J = 9.7$ Hz, 2H), 7.20 (d, $J = 8.1$ Hz, 1H), 7.02 (d, $J = 8.5$ Hz, 1H), 6.77 (d, $J = 7.1$ Hz, 1H), 4.19 (s, 1H), 3.86 – 3.63 (m, 3H), 3.51 (dd, $J = 8.9, 3.3$ Hz, 1H), 2.13 (dq, $J = 15.2, 7.5$ Hz, 1H), 1.74 (td, $J = 12.4, 5.1$ Hz, 1H), 1.36 (s, 1H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.43, 141.95, 128.12, 123.67, 119.52, 117.27, 115.56, 112.48, 72.92, 66.19, 51.17, 50.32, 32.60, 29.13. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2$ 346.2, found 346.2



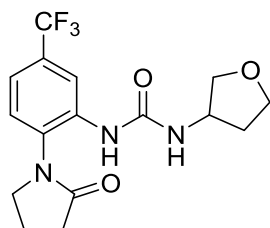
1-(2-(2-Methylpyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (32). From 2-(2-methylpyrrolidin-1-yl)-5-

(trifluoromethyl)aniline (**32b**) (1.5 mmol, 0.366 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (61%). HPLC-MS: t_R = 2.90 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.36 (s, 1H), 7.85 (d, J = 2.0 Hz, 1H), 7.39 (d, J = 6.6 Hz, 1H), 7.20 (s, 2H), 4.25 – 4.16 (m, 1H), 3.85 – 3.64 (m, 3H), 3.62 – 3.42 (m, 3H), 2.62 (td, J = 8.7, 5.4 Hz, 1H), 2.14 (dq, J = 15.4, 7.7 Hz, 2H), 2.01 – 1.88 (m, 1H), 1.86 – 1.64 (m, 2H), 1.54 (dq, J = 17.8, 8.9 Hz, 1H), 0.92 (d, J = 5.9 Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.81, 141.95, 135.07, 135.05, 126.03, 122.95, 120.07, 118.19, 115.65, 72.92, 66.17, 55.50, 52.55, 50.20, 32.97, 22.87, 18.54. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2$ 358.2, found 358.2



tert-Butyl ((1-(2-(3-(trifluoromethyl)phenyl)pyrrolidin-2-yl)methyl)carbamate (**33**). From tert-butyl ((1-(2-amino-4-(trifluoromethyl)phenyl)pyrrolidin-2-yl)methyl)carbamate (**33b**) (1.5 mmol, 0.539 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (63%). HPLC-MS: t_R = 2.94 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.33 (d, J = 5.8 Hz, 1H), 7.93 (s, 1H), 7.32 (d, J = 7.0 Hz, 1H), 7.29 (s, 1H), 7.20 (d, J = 8.2 Hz, 1H), 6.74 (d, J = 5.3 Hz, 1H), 4.23 (s, 1H), 3.87 – 3.69 (m, 3H), 3.66 (d, J = 13.9 Hz, 1H), 3.53 (d, J = 8.9 Hz, 1H), 3.47 (d, J = 6.9 Hz, 1H), 3.04 – 2.90 (m, 1H), 2.86 – 2.72 (m, 1H), 2.63 (dd, J = 14.8, 7.6 Hz, 1H), 2.12 (ddd, J = 15.9, 12.6, 5.7 Hz, 2H), 1.93 (d, J = 5.8 Hz, 1H), 1.84 – 1.64 (m, 3H), 1.34 (s, 10H). ^{13}C NMR

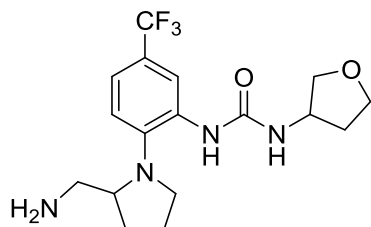
(101 MHz, DMSO- d_6) δ 155.78, 154.93, 142.82, 134.96, 128.71, 126.01, 123.32, 118.48, 116.22, 77.63, 72.91, 66.18, 59.99, 53.62, 50.22, 43.01, 32.63, 28.94, 28.18, 23.53. MS (ESI⁺): m/z [M+H]⁺, calcd for C₂₂H₃₁F₃N₄O₄ 473.4, found 473.3



1-(2-(2-Oxopyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-

(tetrahydrofuran-3-yl)urea (34).

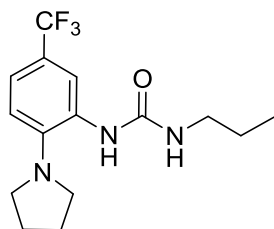
From 1-(2-amino-4-(trifluoromethyl)phenyl)pyrrolidin-2-one (**34b**) (1.5 mmol, 0.366 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (77%). HPLC-MS: t_R = 2.50 min, purity 100%. ¹H NMR (400 MHz, DMSO- d_6) δ 8.31 (s, 1H), 7.75 (s, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 6.0 Hz, 1H), 3.85 – 3.79 (m, 1H), 3.69 (dd, J = 15.1, 7.8 Hz, 5H), 3.53 (d, J = 8.4 Hz, 1H), 2.24 – 2.12 (m, 5H), 1.74 (s, 1H). ¹³C NMR (101 MHz, DMSO- d_6) δ 175.18, 154.44, 137.34, 133.09, 131.09, 128.06, 125.44, 118.03, 116.22, 72.86, 66.11, 50.20, 49.46, 32.58, 30.75, 18.55. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₁₉F₃N₃O₃ 358.1, found 358.1



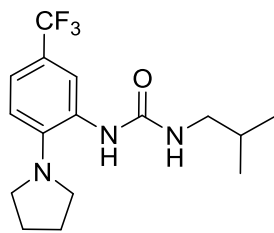
1-(2-(2-(Aminomethyl)pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-

(tetrahydrofuran-3-yl)urea (35). From tert-butyl ((1-(2-(3-(tetrahydrofuran-3-yl)ureido)-4-(trifluoromethyl)phenyl)pyrrolidin-2-yl)methyl)carbamate (**33**)

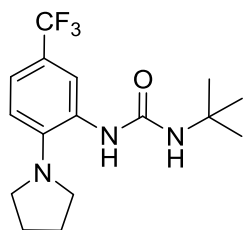
(0.5 mmol, 0.273 g). White solid (79%). HPLC-MS: t_R = 2.12 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.77 (s, 1H), 8.43 (d, J = 8.8 Hz, 1H), 8.22 (s, 1H), 8.11 (s, 2H), 7.29 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 4.18 (s, 1H), 3.81 – 3.78 (m, 1H), 3.68 (dt, J = 14.4, 8.3 Hz, 3H), 3.51 – 3.43 (m, 2H), 2.76 (d, J = 28.3 Hz, 2H), 2.18 – 1.95 (m, 3H), 1.93 – 1.60 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.25, 148.31, 142.17, 135.99, 125.95, 120.19, 117.93, 115.34, 72.19, 66.31, 62.83, 53.59, 50.18, 43.66, 32.47, 27.26, 23.56. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_2$ 373.1, found 373.1



1-Propyl-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (36). From 1-isocyanatopropane (1 eq, 0.5 mmol, 0.043 g) and 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (1.5 mmol, 0.345 g) (Method A). Brown solid (64%). mp: 161.8 °C. HPLC-MS: t_R = 2.78 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 0.88 (t, 3H, J = 7.4 Hz, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.44 (m, 2H), 1.90 (m, 4H), 3.05 (q, J = 6.8 Hz, 2H), 3.15 (t, J = 6.4 Hz, 4H), 6.73 (t, J = 5.5 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 7.22 (dd, J = 1.7 Hz, 1.6 Hz, 1H), 7.67 (s, 1H), 7.98 (d, J = 1.9 Hz, 1H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 11.80, 23.42, 24.90, 41.40, 50.41, 117.25, 120.56, 120.88, 123.95, 126.64, 131.6, 145.37, 155.94; ^{19}F NMR (377 MHz, $\text{DMSO-}d_6$): δ -59.90; MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{F}_3\text{N}_3\text{O}$ 316.2, found 316.2

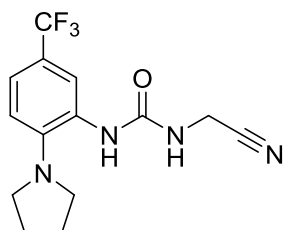


1-Isobutyl-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (37). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl) aniline (**2b**) (1 mmol, 0.230 g) and isopropyl isocyanate (1.2 mmol, 1.2 mL) (Method A). White solid (73%), mp: 204.8 °C. HPLC-MS: t_R = 2.82 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6): δ 1.09 (d, J = 6.5 Hz, 6H), 1.90 (m, 4H), 3.12 (m, 4H), 3.75 (m, 1H), 6.67 (d, J = 7.3 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 7.6 (s, 1H), 8.05 (s, 1H, ArH); ^{13}C NMR (DMSO- d_6): δ 20.47, 24.86, 41.54, 50.75, 117.46, 119.10, 119.77, 120.86, 121.17, 123.93, 126.63, 131.53, 145.06, 155.22; MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{23}\text{F}_3\text{N}_3\text{O}$ 320.2, found 320.1



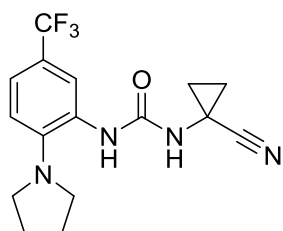
1-(tert-Butyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (38). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl) aniline (**2b**) (1 mmol, 0.230 g) and 2-isocyanato-2-methylpropane (1.2 mmol, 0.119 g) (Method A). White solid (79%). HPLC-MS: t_R = 2.72 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.04 (s, 1H), 7.57 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 8.5 Hz, 1H), 6.65 (s, 1H), 3.12 (t, J = 6.2 Hz, 4H), 1.90 (t, J = 6.3 Hz, 4H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.32, 144.62, 136.02, 131.28, 128.03, 124.18,

116.97, 102.71, 50.27, 49.51, 29.07, 24.40. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₂₃F₃N₃O 330.2, found 330.1



1-(Cyanomethyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea

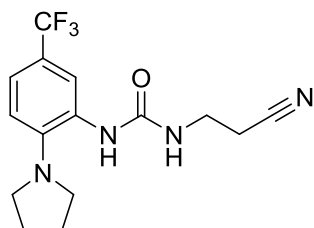
(39). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and aminoacetonitrile hydrochloride (0.75 mmol, 0.069 g) (Method C). White solid (77%). HPLC-MS: *t_R* = 2.74 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.12 (s, 1H), 7.74 (s, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.12 (t, *J* = 5.6 Hz, 1H), 6.99 (d, *J* = 8.6 Hz, 1H), 4.14 (d, *J* = 5.8 Hz, 2H), 3.20 (d, *J* = 6.0 Hz, 4H), 1.89 (d, *J* = 6.1 Hz, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.42, 146.18, 127.69, 126.13, 123.44, 121.93, 121.42, 118.55, 116.36, 50.03, 28.36, 24.67. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₄H₁₆F₃N₄O 313.2 found 313.2



1-(1-Cyanocyclopropyl)-3-(2-(pyrrolidin-1-yl)-5-

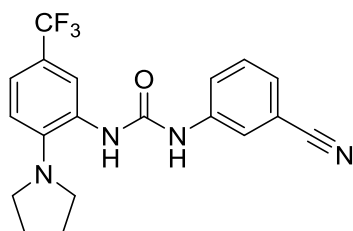
(trifluoromethyl)phenyl)urea (40). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 1-aminocyclopropane-1-carbonitrile (0.75 mmol, 0.062 g) (Method C). White solid (69%). HPLC-MS: *t_R* = 2.80 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.94 (s, 3H), 7.80 (s, 3H), 7.64 (s, 3H), 7.29 (d, *J* = 8.5 Hz, 3H), 7.01 (d, *J* = 8.5 Hz, 3H), 3.18 (s, 11H), 1.89 (s, 12H), 1.41 (d, *J* = 2.4 Hz, 5H), 1.10 (d, *J* = 2.4 Hz, 5H). ¹³C NMR

(101 MHz, DMSO-*d*₆) δ 156.74, 155.29, 145.89, 128.13, 126.12, 121.53, 119.23, 116.64, 50.16, 24.64, 20.64, 20.53, 16.21, 16.17. MS (ESI⁺): *m/z* [M+H]⁺, calcd for C₁₆H₁₈F₃N₄O 339.2, found 339.2



1-(2-Cyanoethyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea

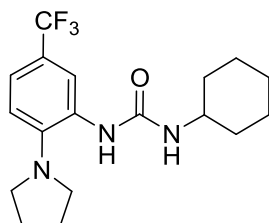
(41). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (74%). HPLC-MS: *t_R* = 2.75 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.87 (d, *J* = 7.7 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 1H), 7.01 (t, *J* = 7.7 Hz, 2H), 3.32 (s, 2H), 3.19 (s, 4H), 2.69 (t, *J* = 6.3 Hz, 2H), 1.90 (s, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.56, 145.64, 129.10, 126.16, 123.47, 120.58, 119.77, 119.54, 116.55, 50.15, 35.58, 24.57, 18.46. MS (ESI⁺): *m/z* [M+H]⁺, calcd for C₁₅H₁₈F₃N₄O 327.1, found 327.1



1-(3-Cyanophenyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea

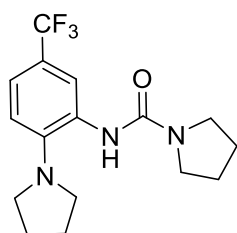
(42). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 3-aminobenzonitrile (0.75 mmol, 0.089 g) (Method C). White solid (89%). HPLC-MS: *t_R* = 3.04 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.46 (s, 1H), 8.12 (s, 1H), 8.01 (s, 1H), 7.88 (s, 1H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.41 (d, *J* = 7.2 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.05

(d, $J = 8.4$ Hz, 1H), 3.24 (s, 4H), 1.91 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 152.90, 148.26, 146.15, 140.72, 134.90, 130.20, 127.92, 125.31, 122.74, 121.35, 120.64, 119.65, 118.85, 116.62, 111.62, 50.25, 24.66. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{18}\text{F}_3\text{N}_4\text{O}$ 375.1, found 374.9



1-Cyclohexyl-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (43).

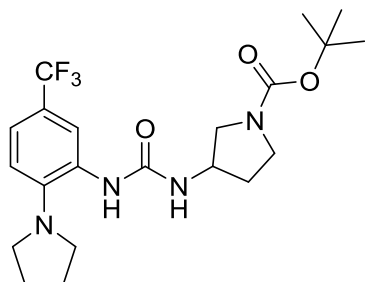
From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and cyclohexyl isocyanate (2eq, 1 mmol, 0.15 mL) (Method C). White solid (70%), mp: 190.6 °C. HPLC-MS: $t_R = 2.74$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6): δ 1.90 (m, 4H), 3.13 (t, 4H, $J = 6.4$ Hz, 4H), 3.45 (m, 1H), 6.70 (d, $J = 7.7$ Hz, 1H), 7.05 (d, $J = 8.4$ Hz, 1H), 7.19 (dd, $J = 1.72$ Hz, 1H), 7.6 (s, 1H), 8.03 (d, $J = 1.96$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 24.83, 24.89, 25.73, 33.46, 48.34, 50.73, 117.40, 119.24, 119.81, 123.94, 126.64, 131.48, 145.14, 155.15; MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{25}\text{F}_3\text{N}_3\text{O}$ 356.19, found 356.1905



N-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)pyrrolidine-1-

carboxamide (44). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method B). White solid (85%). HPLC-MS: $t_R = 2.92$ min, purity 96%. ^1H NMR (400 MHz, DMSO-

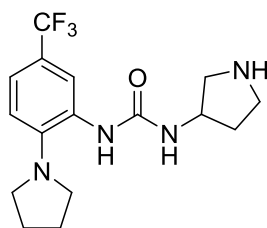
d_6) δ 7.71 (s, 1H), 7.46 (s, 1H), 7.30 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 3.31 (dt, J = 16.4, 6.3 Hz, 8H), 1.86 (dd, J = 8.1, 5.0 Hz, 8H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.91, 147.54, 126.48, 125.47, 123.60, 122.27, 117.53, 115.18, 49.77, 45.55, 25.09, 24.97. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}$ 328.1, found 328.3



***tert*-Butyl**

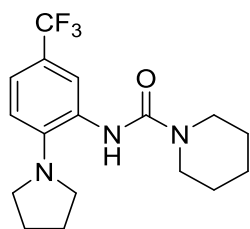
3-(3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)pyrrolidine-1-carboxylate (45). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and *tert*-butyl 3-aminopyrrolidine-1-carboxylate (0.75 mmol, 0.140 g) (Method C). White solid (67%). HPLC-MS: t_R = 3.02 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.01 (s, 1H), 7.67 (s, 1H), 7.22 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 4.14 (s, 1H), 3.52 – 3.40 (m, 1H), 3.30 (d, J = 6.7 Hz, 2H), 3.10 (dd, J = 16.6, 8.5 Hz, 5H), 2.11 – 1.97 (m, 1H), 1.90 (s, 4H), 1.82 – 1.66 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.13, 153.63, 144.89, 130.48, 126.15, 123.46, 119.79, 119.07, 117.00, 78.40, 51.93, 50.32, 49.52, 43.68, 30.48, 28.19, 24.48. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{30}\text{F}_3\text{N}_4\text{O}_3$ 443.2, found 443.2

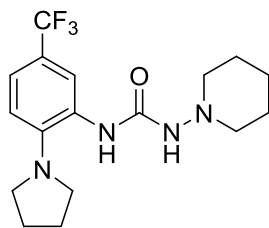


1-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(pyrrolidin-3-yl)urea

(46). From tert-butyl 3-(3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)pyrrolidine-1-carboxylate **(45)** (0.5 mmol, 0.222 g). White solid (79%). HPLC-MS: t_R = 2.17 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.45 (s, 2H), 8.10 (s, 1H), 7.91 (s, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 8.5 Hz, 1H), 4.29 – 4.16 (m, 1H), 3.41 – 3.10 (m, 7H), 3.01 (dd, J = 11.4, 5.6 Hz, 1H), 2.14 (dq, J = 14.1, 7.2 Hz, 1H), 1.96 – 1.75 (m, 5H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.53, 144.50, 129.95, 126.08, 123.39, 120.92, 120.49, 117.42, 51.03, 49.56, 49.06, 43.49, 30.30, 24.52. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{22}\text{F}_3\text{N}_4\text{O}$ 343.4, found 343.3

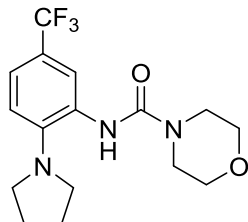
**N-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)piperidine-1-**

carboxamide (47). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline **(2b)** (0.5 mmol, 0.115 g) and piperidine (0.75 mmol, 0.064 g) (Method C). White solid (74%). HPLC-MS: t_R = 3.03 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.00 (s, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.24 (s, 1H), 6.79 (d, J = 8.7 Hz, 1H), 3.39 (d, J = 5.4 Hz, 4H), 3.31 (t, J = 6.1 Hz, 4H), 1.86 (t, J = 6.3 Hz, 4H), 1.57 (d, J = 4.7 Hz, 2H), 1.46 (d, J = 3.8 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.98, 148.32, 127.05, 125.53, 122.93, 116.65, 116.33, 114.58, 49.51, 44.55, 25.68, 25.11, 24.17. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{23}\text{F}_3\text{N}_3\text{O}$ 342.1, found 342.0



1-(Piperidin-1-yl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea

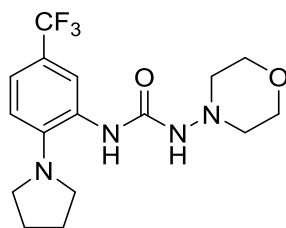
(48). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and piperidin-1-amine (0.75 mmol, 0.075 g) (Method C). White solid (73%). HPLC-MS: $t_R = 3.15$ min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.13 (s, 1H), 7.32 (d, $J = 8.6$ Hz, 1H), 7.25 (s, 1H), 6.80 (d, $J = 8.7$ Hz, 1H), 3.63 – 3.50 (m, 4H), 3.42 – 3.37 (m, 4H), 3.31 (t, $J = 6.2$ Hz, 4H), 1.94 – 1.76 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.36, 148.45, 127.32, 126.32, 124.86, 123.22, 116.24, 114.57, 66.12, 49.53, 44.07, 25.15. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{24}\text{F}_3\text{N}_4\text{O}$ 357.1, found 357.0



N-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)morpholine-4-

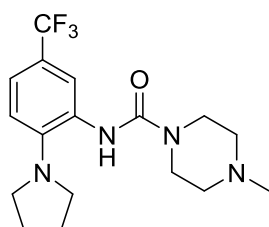
carboxamide (49). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and morpholine (0.75 mmol, 0.075 g) (Method C). White solid (71%). HPLC-MS: $t_R = 2.77$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.75 (s, 1H), 8.32 (s, 1H), 7.98 (s, 1H), 7.24 (d, $J = 6.9$ Hz, 1H), 7.17 (d, $J = 8.4$ Hz, 1H), 3.31 (t, $J = 6.3$ Hz, 4H), 3.12 (t, $J = 5.8$ Hz, 4H), 2.96 (s, 2H), 1.93 (s, 4H), 1.60 (dd, $J = 29.3, 22.7$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.57, 143.15, 131.93, 126.08, 123.39, 121.99, 118.14, 115.83,

56.70, 50.52, 25.85, 24.34, 22.77. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₂₁F₃N₃O₂ 344.2, found 344.2



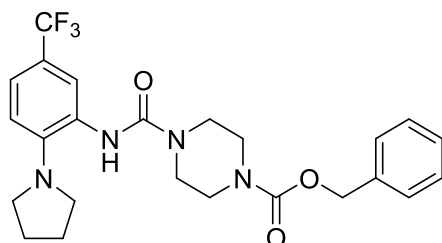
1-Morpholino-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (50).

From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and morpholin-4-amine (0.75 mmol, 0.077 g) (Method C). White solid (79%). HPLC-MS: *t_R* = 2.87 min, purity 95%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.72 (s, 1H), 8.23 (s, 1H), 8.07 (s, 1H), 7.27 (d, *J* = 8.3 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 3.92 – 3.46 (m, 4H), 3.16 (t, *J* = 5.7 Hz, 4H), 2.79 (d, *J* = 54.3 Hz, 4H), 1.94 (s, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 154.66, 143.76, 131.01, 126.10, 123.41, 119.64, 117.82, 117.10, 66.34, 55.84, 50.52, 24.43. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₂₂F₃N₄O₂ 359.1, found 359.0

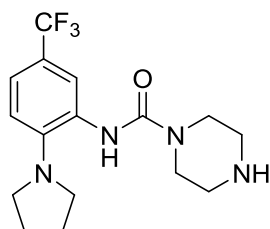


4-Methyl-N-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)piperazine-1-carboxamide (51). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 1-methylpiperazine (0.75 mmol, 0.075 g) (Method C). White solid (76%). HPLC-MS: *t_R* = 2.11 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.09 (s, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 7.23 (s, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 3.49 – 3.39 (m, 4H), 3.31 (t, *J* = 5.9 Hz, 4H), 2.32 – 2.24 (m, 4H), 2.19 (s, 3H), 1.86 (d, *J* = 6.0 Hz, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 156.11,

148.42, 127.29, 126.33, 125.06, 123.13, 116.23, 114.54, 54.65, 49.51, 45.80, 43.52, 25.13. MS (ESI⁺): *m/z* [M+H]⁺, calcd for C₁₇H₂₄F₃N₄O 357.4, found 357.3

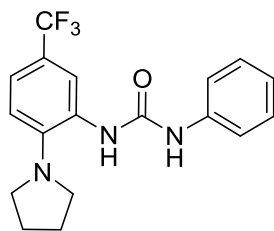


Benzyl 4-((2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)carbamoyl)piperazine-1-carboxylate (52). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and benzyl piperazine-1-carboxylate (0.75 mmol, 0.165 g) (Method C). White solid (69%). HPLC-MS: *t_R* = 3.04 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.18 (s, 1H), 7.38 (d, *J* = 4.3 Hz, 4H), 7.32 (dd, *J* = 11.6, 5.2 Hz, 2H), 7.26 (s, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 5.11 (s, 2H), 3.43 (s, 8H), 3.31 (d, *J* = 6.3 Hz, 4H), 1.91 (s, 1H), 1.86 (d, *J* = 6.1 Hz, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 156.09, 154.51, 148.43, 136.83, 128.46, 127.91, 127.64, 127.28, 126.32, 124.84, 123.63, 116.53, 114.54, 66.37, 49.52, 43.38, 25.14. MS (ESI⁺): *m/z* [M+H]⁺, calcd for C₂₄H₂₈F₃N₄O₃ 477.1, found 477.1

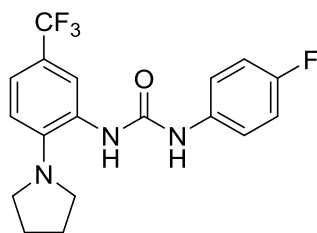


N-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)piperazine-1-carboxamide (53). From Benzyl 4-((2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)carbamoyl)piperazine-1-carboxylate (**52**) (0.5 mmol, 0.239 g). White solid (83%). HPLC-MS: *t_R* = 2.11 min, purity 97%. ¹H NMR

(400 MHz, DMSO- d_6) δ 8.12 (d, J = 70.8 Hz, 1H), 7.38 – 7.19 (m, 2H), 6.84 – 6.75 (m, 1H), 3.47 – 3.28 (m, 12H), 2.59 (s, 1H), 1.86 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 156.24, 148.38, 127.17, 126.34, 125.32, 123.66, 116.63, 114.56, 49.52, 44.85, 43.64, 25.13. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{22}\text{F}_3\text{N}_4\text{O}$ 343.4, found 343.3

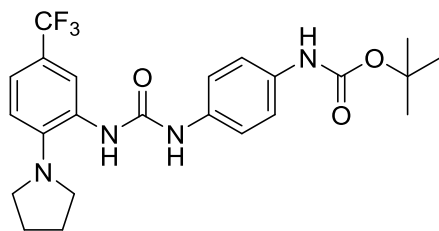


1-Phenyl-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (54). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and aniline (0.75 mmol, 0.070 g) (Method C). White solid (70%). mp: 208.4 °C. HPLC-MS: t_R = 3.06 min, purity 90%. ^1H NMR (400 MHz, DMSO- d_6): δ 1.91 (m, 4H), 3.21 (t, J = 6.2 Hz, 4H), 6.96 (t, J = 7.3 Hz, 1H), 7.06 (d, J = 8.5 Hz, 1H), 7.27 (t, J = 7.4 Hz, 3H), 7.47 (d, J = 7.6 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 9.17 (s, 1H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 25.02, 50.76, 118.57, 120.60, 120.79, 121.08, 122.30, 123.90, 126.59, 129.25, 12.59, 140.25, 146.13, 153.37; MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{19}\text{F}_3\text{N}_3\text{O}$ 350.2, found 350.1

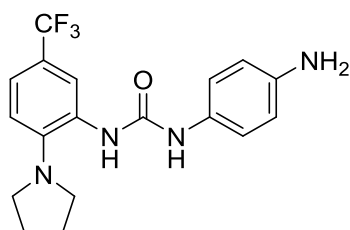


1-(4-Fluorophenyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (55). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 4-fluoroaniline (0.75 mmol, 0.083 g) (Method C). White solid (83%). HPLC-MS: t_R = 2.68 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.19

(s, 1H), 7.96 (d, $J = 12.6$ Hz, 2H), 7.47 (dd, $J = 9.0, 4.9$ Hz, 2H), 7.29 (d, $J = 8.4$ Hz, 1H), 7.15 – 7.02 (m, 3H), 3.22 (t, $J = 6.3$ Hz, 4H), 1.92 (t, $J = 6.3$ Hz, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 152.96, 145.72, 144.27, 136.09, 128.93, 128.74, 120.74, 119.83, 119.75, 116.76, 115.38, 115.16, 108.26, 50.28, 24.58. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{18}\text{H}_{18}\text{F}_4\text{N}_3\text{O}$ 368.2, found 368.1

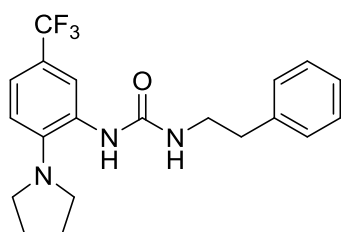


tert-Butyl (4-(3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)phenyl)carbamate (56). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and tert-butyl (4-aminophenyl)carbamate (0.75 mmol, 0.156 g) (Method C). White solid (69%). HPLC-MS: $t_R = 3.11$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.18 (s, 1H), 9.04 (s, 1H), 8.00 (s, 1H), 7.92 (s, 1H), 7.40 – 7.30 (m, 4H), 7.26 (d, $J = 8.0$ Hz, 1H), 7.07 (d, $J = 8.5$ Hz, 1H), 3.18 (d, $J = 5.9$ Hz, 4H), 1.91 (s, 4H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 152.90, 145.40, 134.22, 133.96, 129.62, 128.83, 126.14, 123.45, 120.35, 120.04, 119.80, 118.71, 116.92, 78.76, 50.35, 28.18, 24.53. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{23}\text{H}_{28}\text{F}_3\text{N}_4\text{O}_3$ 465.4, found 465.3



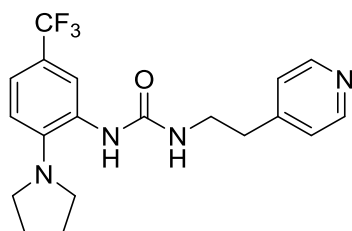
1-(4-Aminophenyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (57). From tert-butyl (4-(3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)phenyl)carbamate (**56**) (0.5 mmol, 0.233 g). White solid (79%). HPLC-MS: t_R = 2.40 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.00 (d, J = 117.8 Hz, 3H), 8.30 (s, 1H), 7.88 (s, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.30 (d, J = 8.6 Hz, 3H), 7.04 (d, J = 8.5 Hz, 1H), 3.57 (s, 11H), 3.24 (s, 4H), 1.90 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 153.13, 145.96, 140.01, 128.23, 126.18, 124.76, 123.78, 123.49, 121.21, 118.76, 116.62, 50.32, 24.68. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{20}\text{F}_3\text{N}_4\text{O}$ 365.2, found 365.2



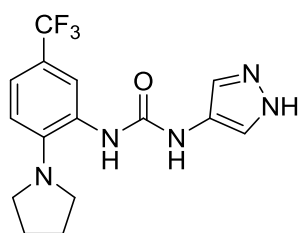
1-Phenethyl-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (58).

From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-phenylethan-1-amine (0.75 mmol, 0.091 g) (Method C). White solid (79%). HPLC-MS: t_R = 3.06 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.89 (s, 1H), 7.69 (s, 1H), 7.30 (t, J = 7.4 Hz, 2H), 7.21 (dd, J = 13.4, 6.8 Hz, 4H), 7.01 (d, J = 8.5 Hz, 1H), 6.68 (t, J = 5.5 Hz, 1H), 3.32 (d, J = 6.8 Hz, 2H), 3.12 (d, J = 5.9 Hz, 4H), 2.74 (t, J = 7.0 Hz, 2H), 1.89 (d, J = 12.5 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.45, 145.23, 139.60, 129.99, 128.78, 126.07, 123.51, 119.94, 116.59, 50.16, 40.73, 35.89, 24.50. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{23}\text{F}_3\text{N}_3\text{O}$ 378.1, found 378.0

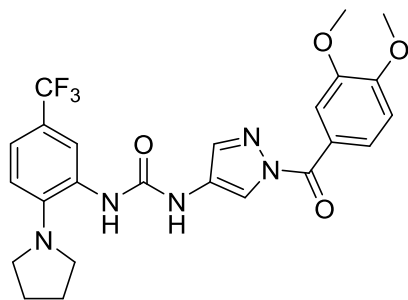


1-(2-(Pyridin-4-yl)ethyl)-3-(2-(pyrrolidin-1-yl)-5-

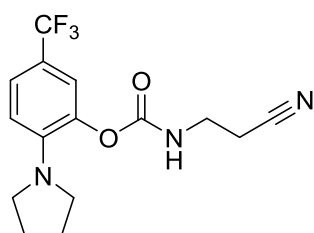
(trifluoromethyl)phenyl)urea (59). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-(pyridin-4-yl)ethan-1-amine (0.75 mmol, 0.092 g) (Method C). White solid (81%). HPLC-MS: t_R = 2.20 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.45 (d, J = 5.5 Hz, 2H), 7.88 (s, 1H), 7.69 (s, 1H), 7.27 – 7.21 (m, 3H), 6.98 (d, J = 8.5 Hz, 1H), 6.71 (t, J = 5.7 Hz, 1H), 3.40 – 3.35 (m, 2H), 3.10 (t, J = 5.9 Hz, 4H), 2.77 (t, J = 6.8 Hz, 2H), 1.86 (d, J = 6.0 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.54, 149.42, 148.64, 145.40, 135.00, 129.75, 126.23, 124.41, 123.54, 116.53, 111.03, 50.15, 42.85, 35.10, 31.26. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{22}\text{F}_3\text{N}_4\text{O}$ 379.2, found 379.2

**1-(1H-Pyrazol-4-yl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea**

(60). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (1.5 mmol, 0.345 g) and 1H-pyrazole-4-carboxylic acid (1 mmol, 0.112 g) (Method D). White solid (79%). HPLC-MS: t_R = 2.68 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.41 (s, 1H), 8.17 (s, 1H), 7.95 (s, 1H), 7.57 (s, 2H), 7.23 (d, J = 8.2 Hz, 1H), 7.02 (d, J = 8.3 Hz, 1H), 3.19 (s, 4H), 1.88 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 153.47, 145.79, 136.56, 129.94, 126.64, 124.28, 123.95, 122.12, 120.47, 120.02, 117.08, 53.68, 24.98. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{17}\text{F}_3\text{N}_5\text{O}$ 340.1, found 340.1

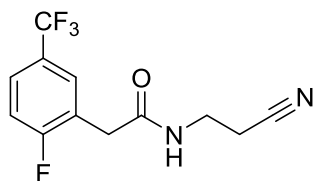


1-(1-(3, 4-Dimethoxybenzoyl)-1H-pyrazol-4-yl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (61). From 1-(1H-pyrazol-4-yl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (**60**) (1 eq, 0.5 mmol, 0.170 g) and 3, 4-dimethoxybenzoyl chloride (1.1 eq, 0.55 mmol, 0.110 g). White solid (31%). HPLC-MS: t_R = 3.02 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.43 (s, 2H), 8.46 (s, 2H), 8.27 (s, 2H), 8.00 (s, 2H), 7.88 (s, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.65 (s, 2H), 7.56 (s, 3H), 7.30 (d, J = 8.3 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H), 3.87 (s, 12H), 3.23 (s, 8H), 1.90 (s, 8H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.14, 152.62, 148.32, 148.02, 146.11, 137.61, 128.06, 126.18, 125.83, 123.19, 123.00, 121.42, 116.70, 116.54, 114.24, 111.90, 110.98, 110.71, 55.79, 55.45, 50.22, 24.69. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{24}\text{H}_{25}\text{F}_3\text{N}_5\text{O}_4$ 504.1, found 504.1



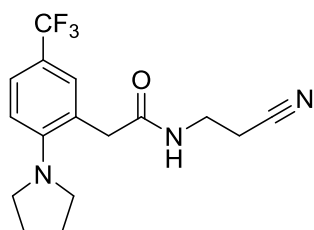
2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl (2-cyanoethyl)carbamate (62). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenol (**66-c**) (1.5 mmol, 0.347 g) and 3-cyanopropanoic acid (1 mmol, 0.099 g) (Method D). White solid (67%). HPLC-MS: t_R = 2.89 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.19 (t, J = 5.8 Hz, 1H), 7.34 (d, J = 8.9 Hz, 1H), 7.18 (s, 1H), 6.78 (d, J = 8.8

Hz, 1H), 3.37 (d, $J = 6.1$ Hz, 4H), 3.32 (s, 2H), 2.71 (t, $J = 6.3$ Hz, 2H), 1.87 (t, $J = 6.4$ Hz, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.80, 143.92, 140.87, 136.49, 130.69, 122.87, 118.79, 114.84, 112.05, 49.44, 36.67, 24.99, 17.90. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{17}\text{F}_3\text{N}_3\text{O}_2$ 328.3, found 328.0



N-(2-Cyanoethyl)-2-(2-fluoro-5-(trifluoromethyl)phenyl)acetamide (63-a).

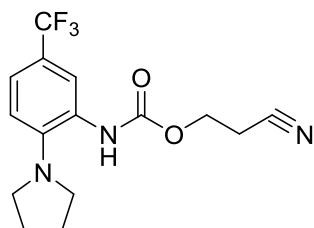
From 3-aminopropanenitrile (1.5 mmol, 0.105 g) and 2-(2-fluoro-5-(trifluoromethyl)phenyl)acetic acid (1 mmol, 0.222 g) (Method D). White solid (77%). HPLC-MS: $t_R = 2.58$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.53 (s, 1H), 7.77 (d, $J = 6.4$ Hz, 1H), 7.74 – 7.64 (m, 1H), 7.40 (t, $J = 9.1$ Hz, 1H), 3.63 (s, 2H), 3.33 – 3.27 (m, 2H), 2.65 (t, $J = 6.4$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 168.95, 161.45, 129.35, 126.26, 124.72, 124.55, 122.58, 119.19, 116.32, 35.04, 34.84, 17.53. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{12}\text{H}_{11}\text{F}_4\text{N}_2\text{O}$ 275.1, found 275.1



N-(2-Cyanoethyl)-2-(2-(pyrrolidin-1-yl)-5-

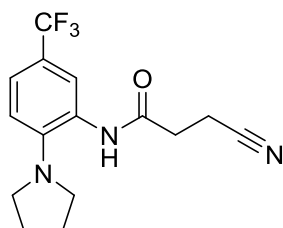
(trifluoromethyl)phenyl)acetamide (63). From 3-aminopropanenitrile (1.5 mmol, 0.105 g) and 2-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)acetic acid (**69-a**) (1 mmol, 0.273 g) (Method D). White solid (87%). HPLC-MS: $t_R = 2.71$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.33 (t, $J = 5.4$ Hz, 1H), 7.39 (d, $J = 7.9$ Hz, 2H), 6.94 – 6.88 (m, 1H), 3.61 (s, 2H), 3.32 – 3.21 (m, 7H),

2.64 (t, $J = 6.3$ Hz, 2H), 1.87 (t, $J = 5.9$ Hz, 5H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 170.94, 151.95, 129.37, 126.43, 124.24, 124.01, 119.26, 118.48, 115.34, 50.76, 46.07, 35.06, 25.07, 17.53. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_3\text{O}$ 326.2, found 326.1



2-Cyanoethyl (2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)carbamate

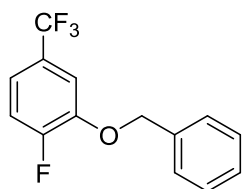
(64). From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 3-hydroxypropanenitrile (0.75 mmol, 0.053 g, 0.05 mL) (Method C). White solid (89%). HPLC-MS: $t_R = 2.89$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.07 (s, 1H), 7.37 (d, $J = 8.7$ Hz, 1H), 7.30 (s, 1H), 6.81 (d, $J = 8.7$ Hz, 1H), 4.23 (t, $J = 5.7$ Hz, 2H), 3.36 (s, 4H), 2.89 (s, 2H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.51, 148.15, 126.87, 126.14, 124.05, 122.71, 118.61, 116.19, 114.80, 59.30, 49.56, 25.10, 17.93. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{17}\text{F}_3\text{N}_3\text{O}_2$ 328.2, found 328.1



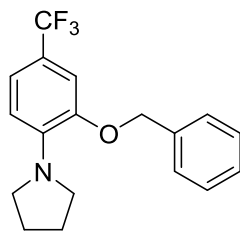
3-Cyano-N-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)propanamide

(65). A solution of 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.55 mmol, 0.127 g) was added with 3-cyanopropanoic acid (0.5 mmol, 0.049 g). Pyridine (1.64 mmol, 0.14 mL), ethyl acetate (0.28 mL) and T3P (50 wt. % EtOAc, 1 mmol, 0.59 mL) were added in the mixture. The reaction was

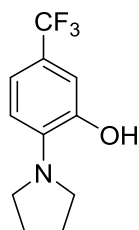
monitored by TLC. After completion of the reaction, the resulting mixture was concentrated and purified by flash chromatography using petroleum ether and ethyl acetate. White solid (85%). HPLC-MS: t_R = 2.78 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.62 (s, 1H), 7.41 (s, 1H), 7.36 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 3.31 (s, 4H), 2.72 (s, 4H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 168.99, 147.82, 128.88, 125.96, 123.42, 120.83, 120.34, 116.74, 114.95, 49.74, 30.94, 25.11, 12.48. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{17}\text{F}_3\text{N}_3\text{O}$ 312.1, found 312.1



2-(Benzyloxy)-1-fluoro-4-(trifluoromethyl)benzene (66-a). A solution of 2-fluoro-5-(trifluoromethyl)phenol (1 eq, 1.11 mmol, 0.14 mL) in acetonitrile (5 mL) was added with benzyl bromide (1 eq, 1.11 mmol, 0.15 mL) and DIPEA (1 eq, 1.11 mmol, 0.74 mL). The reaction was monitored by TLC and stirred at R.T overnight. After completion of the reaction, the resulting mixture was concentrated and purified by flash chromatography using petroleum ether. White solid (73%). HPLC-MS: t_R = 3.17 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.61 (d, J = 7.2 Hz, 1H), 7.42 (ddd, J = 23.3, 15.4, 7.1 Hz, 7H), 5.28 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 152.71, 146.79, 135.95, 128.50, 128.22, 127.97, 125.08, 122.38, 118.56, 116.96, 112.43, 70.65. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{14}\text{H}_{11}\text{F}_4\text{N}$ 271.2, found 271.1

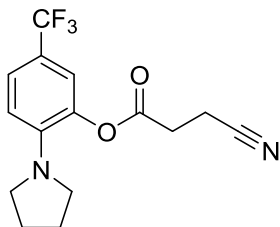


1-(2-(Benzyloxy)-4-(trifluoromethyl)phenyl)pyrrolidine (66-b). A solution of pyrrolidine (2.5 eq, 2.5 mmol, 0.178 g, 0.21 mL) in THF (2 mL) was treated with 2-(benzyloxy)-1-fluoro-4-(trifluoromethyl)benzene (**66-a**) (1 eq, 1 mmol, 0.271 g) and sodium bicarbonate (3 eq, 3 mmol, 0.252 g). The reaction was stirred in microwave at 130 °C for 1.5 h. The mixture was filtered and concentrated, diluted with DCM (20 mL), washed with saturated aqueous sodium chloride solution. Drying with Na₂SO₄, followed by filtration and concentration to afford the product without further purification. White solid (91%). HPLC-MS: t_R = 3.36 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.47 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.2 Hz, 2H), 7.37 – 7.31 (m, 1H), 7.19 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 5.10 (s, 2H), 3.35 (s, 4H), 1.81 (s, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 147.20, 142.65, 136.76, 128.42, 127.87, 126.33, 123.64, 118.82, 117.18, 113.62, 110.01, 70.75, 50.04, 24.85. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₈H₁₉F₃NO 322.1, found 322.1



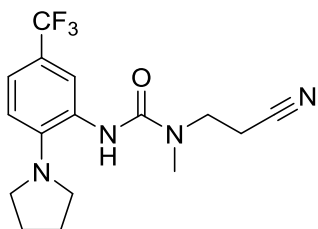
2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenol (66-c). From 1-(2-(benzyloxy)-4-(trifluoromethyl)phenyl)pyrrolidine (**66-b**) (1 mmol, 0.322 g). White solid (78%). HPLC-MS: t_R = 2.42 min, purity 98%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.99 – 6.91 (m, 2H), 6.63 (d, J = 8.3 Hz, 1H), 3.36 (d, J = 6.4 Hz,

4H), 1.84 (t, $J = 6.5$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 146.30, 141.37, 126.39, 123.70, 116.79, 113.93, 111.38, 49.66, 24.67. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{13}\text{F}_3\text{NO}$ 232.1, found 231.9



2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl 3-cyanopropanoate (66).

From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenol (**66-c**) (0.75 mmol, 0.174 g) and 3-cyanopropanoic acid (0.5 mmol, 0.055 g) (Method D). White solid (93%). HPLC-MS: $t_R = 2.98$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.38 (d, $J = 8.7$ Hz, 1H), 7.30 (d, $J = 1.6$ Hz, 1H), 6.83 (d, $J = 8.7$ Hz, 1H), 3.35 (d, $J = 6.3$ Hz, 4H), 3.03 (t, $J = 6.9$ Hz, 2H), 2.81 (t, $J = 6.9$ Hz, 2H), 1.87 (t, $J = 6.4$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 170.05, 143.78, 135.89, 125.94, 123.75, 121.30, 119.76, 115.59, 115.13, 49.54, 29.52, 25.05, 12.27. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_2$ 313.1, found 312.9

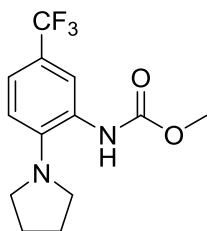


1-(2-Cyanoethyl)-1-methyl-3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)urea (67).

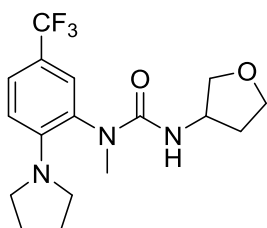
From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 3-(methylamino)propanenitrile (0.75 mmol, 0.063 g) (Method C). White solid (53%). HPLC-MS: $t_R = 2.82$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.07 (s, 1H), 7.32 (d, $J = 8.7$ Hz, 1H), 7.28 (s, 1H), 6.80 (d, $J = 8.7$ Hz, 1H),

3.56 (t, $J = 6.6$ Hz, 2H), 3.32 (d, $J = 5.9$ Hz, 4H), 2.99 (s, 3H), 2.74 (t, $J = 6.6$ Hz, 2H), 1.85 (t, $J = 6.2$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.46, 148.42, 127.32, 126.32, 124.95, 123.24, 119.29, 116.56, 114.61, 49.52, 44.15, 34.48, 25.13, 16.14. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{20}\text{F}_3\text{N}_4\text{O}$ 341.1, found 340.9



Methyl (2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)carbamate (68a).

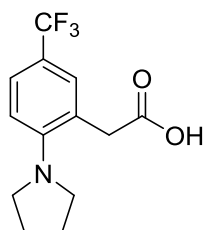
From 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and methanol (0.75 mmol, 0.024 g, 0.03 mL) (Method C). White solid (61%). HPLC-MS: $t_R = 2.96$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.84 (s, 1H), 7.37 – 7.31 (m, 2H), 6.82 (d, $J = 8.5$ Hz, 1H), 3.62 (s, 3H), 3.32 (t, $J = 6.1$ Hz, 4H), 1.84 (d, $J = 9.0$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.54, 147.94, 141.63, 128.35, 126.17, 123.49, 114.89, 110.52, 51.87, 49.60, 25.06. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{13}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_2$ 289.1, found 288.9



1-Methyl-1-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-

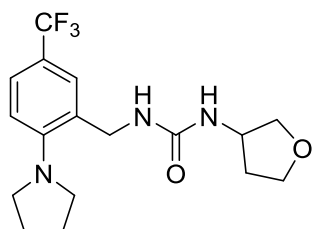
(tetrahydrofuran-3-yl)urea (68). From N-methyl-2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**68b**) (0.75 mmol, 0.183 g) and tetrahydrofuran-3-carboxylic acid (0.5 mmol, 0.058 g) (Method D). White solid (51%). HPLC-MS: $t_R = 3.16$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.51 (s, 1H),

8.18 (d, $J = 5.6$ Hz, 1H), 8.12 (d, $J = 8.5$ Hz, 1H), 7.99 (d, $J = 8.4$ Hz, 1H), 4.22 (s, 1H), 3.76 – 3.68 (m, 3H), 3.48 – 3.43 (m, 1H), 3.00 (dt, $J = 16.7, 8.5$ Hz, 4H), 2.18 (t, $J = 7.3$ Hz, 1H), 1.96 (dd, $J = 15.2, 7.8$ Hz, 4H), 1.24 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 175.16, 169.75, 162.98, 148.50, 136.12, 122.62, 114.65, 111.80, 69.71, 67.45, 43.43, 41.06, 29.85, 29.24, 25.15. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2$ 358.2, found 358.2

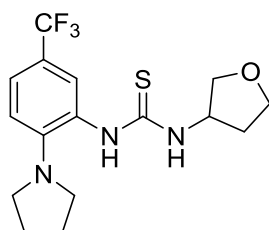


2-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)acetic acid (69-a).

Pyrrolidine (excess, 1 mL) was treated with 2-fluoro-5-(trifluoromethyl)phenyl acetic acid (1 eq, 1 mmol, 0.222 g). The reaction was stirred in microwave at 130 °C for 1.5 h. The mixture was filtered and concentrated, diluted with DCM (30 mL), washed with saturated aqueous sodium chloride solution. Drying with Na_2SO_4 , was followed by filtration and concentration to afford the product. White solid (78%). HPLC-MS: $t_R = 2.76$ min, purity 96%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.70 (s, 3H), 7.38 (d, $J = 5.9$ Hz, 8H), 6.89 (d, $J = 9.4$ Hz, 4H), 3.25 (dd, $J = 11.7, 5.2$ Hz, 17H), 1.82 (ddd, $J = 20.2, 11.1, 6.6$ Hz, 24H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 169.17, 151.81, 129.59, 126.44, 125.10, 124.23, 118.09, 115.30, 50.59, 45.59, 25.05. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{13}\text{H}_{15}\text{F}_3\text{NO}_2$ 274.1, found 274.1

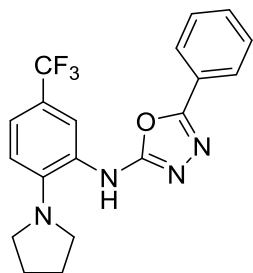


1-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)benzyl)-3-(tetrahydrofuran-3-yl)urea (69). A solution of 2-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)acetic acid (**69-a**) (1 eq, 0.5 mmol, 0.136 g) in anhydrous acetonitrile (5 mL) was added with diphenylphosphoryl azide (1 eq, 0.5 mmol, 0.137 g, 0.11 mL) and trimethylamine (1 eq, 0.5 mmol, 0.051 g, 0.07 mL). The reaction mixture was stirred at R.T. for 30 min and then was reflux for another 30 min. After the addition of tetrahydrofuran-3-amine hydrochloride (1.5 eq, 0.75 mmol, 0.083 g) and DIPEA (0.26 mL), the reaction was monitored by TLC. After completion of the reaction, the mixture was quenched with water and extracted with DCM. All of organic layers are combined. The solvent was concentrated and purified by flash chromatography using petroleum ether and ethyl acetate. White solid (68%). HPLC-MS: t_R = 2.81 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.39 (d, J = 9.2 Hz, 2H), 6.90 (d, J = 8.3 Hz, 1H), 6.31 (d, J = 6.9 Hz, 1H), 6.21 (t, J = 5.7 Hz, 1H), 4.26 (t, J = 8.4 Hz, 2H), 4.17 – 4.03 (m, 1H), 3.80 – 3.54 (m, 3H), 3.26 (t, J = 6.0 Hz, 4H), 2.06 (td, J = 15.2, 7.5 Hz, 1H), 1.90 (t, J = 6.1 Hz, 4H), 1.64 (ddd, J = 17.1, 7.4, 5.3 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 157.77, 150.97, 128.69, 126.52, 125.43, 124.15, 123.84, 118.54, 118.23, 114.93, 73.12, 66.17, 50.88, 50.31, 40.98, 32.72, 25.09. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_2$ 358.4, found 358.1



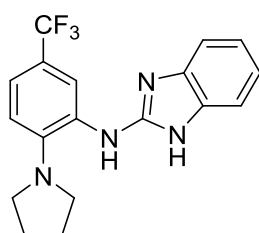
1-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)thiourea (70). A solution of 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) in anhydrous acetonitrile (3 mL) was added with 1, 1'-

thiocarbonyldiimidazole (0.75 mmol, 0.134 g) at 0 °C. The reaction was allowed to warm up to R.T. and stirred for 1 h, followed by the addition of tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g). After completion of the reaction, the resulting mixture was concentrated and purified by flash chromatography using petroleum ether and ether acetate. White solid (71%). HPLC-MS: t_R = 2.87 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.96 (s, 1H), 7.40 – 7.23 (m, 3H), 6.84 (d, J = 8.7 Hz, 1H), 4.07 (dd, J = 15.9, 9.1 Hz, 1H), 3.79 (ddd, J = 16.7, 9.2, 4.1 Hz, 3H), 3.59 (d, J = 3.3 Hz, 1H), 3.46 (s, 4H), 2.18 (dd, J = 13.0, 7.3 Hz, 1H), 1.87 (s, 4H), 1.65 (dd, J = 14.2, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 181.96, 148.70, 137.31, 126.64, 124.98, 124.41, 115.51, 108.48, 72.92, 66.83, 53.94, 49.77, 32.65, 25.64. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{OS}$ 360.1, found 360.1



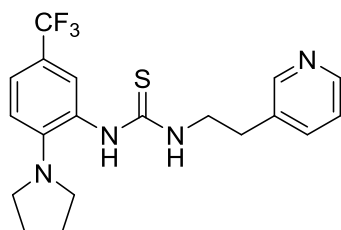
5-Phenyl-N-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-1, 3, 4-oxadiazol-2-amine (72). A solution of 2-(pyrrolidin-1-yl)-5-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) in anhydrous dichloromethane (3 mL) was added with 1, 1'-thiocarbonyldipyridin-2(1H)-one (0.5 mmol, 0.116 g) at 0 °C for 1 h. The reaction was allowed to warm up to R.T. and stirred for another 1 h. After completion of the reaction, the resulting mixture was concentrated. The residue was dissolved in THF (5 mL) and benzohydrazide (0.5 mmol, 0.068 g) was added in the mixture. The mixture was stirred for 16 h at R.T and was monitored by TLC. The resulting mixture was concentrated and

re-dissolved in anhydrous acetonitrile (5 mL), followed by the addition of 4-toluenesulfonyl chloride (TosCl, 0.6 mmol, 0.114 g), pyridine (1.05 mmol, 0.083 g, 0.08 mL). The mixture was stirred at 70 °C for 6 h purified by flash chromatography using petroleum ether and ether acetate. White solid (43%). HPLC-MS: t_R = 3.11 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.86 – 7.80 (m, 2H), 7.77 (s, 1H), 7.57 – 7.50 (m, 3H), 7.43 (dd, J = 34.9, 8.0 Hz, 2H), 6.96 (d, J = 8.7 Hz, 1H), 3.31 (t, J = 6.0 Hz, 4H), 1.85 (t, J = 6.2 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 161.95, 158.22, 147.06, 141.21, 130.86, 130.12, 129.35, 128.20, 126.10, 125.38, 125.12, 124.03, 118.11, 115.82, 49.95, 24.98. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{18}\text{F}_3\text{N}_4\text{O}$ 375.1, found 375.0



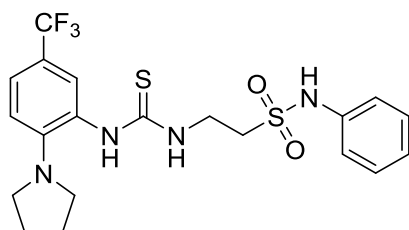
N-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (73). A solution of 1-(2-aminophenyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)thiourea (**132**) (1 eq, 0.20 mmol, 0.075 g) in acetonitrile (2 mL) was added with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.5 eq, 0.30 mmol, 0.056 g). The reaction mixture was refluxed for 3 h at 80 °C. After completion of the reaction, the reaction was concentrated *in vacuo* and purified by flash chromatography using petroleum ether and ethyl acetate. White solid (49%). HPLC-MS: t_R = 2.31 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.79 (s, 1H), 8.32 (s, 1H), 7.36 (d, J = 7.0 Hz, 1H), 7.30 – 7.26 (m, 2H), 7.11 (dd, J = 15.4, 5.8 Hz, 3H), 6.82 (d, J = 7.8 Hz, 1H), 3.24 (d, J = 6.1 Hz, 4H), 1.90 (t, J = 6.2 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 151.43, 148.00, 142.60, 134.91, 132.97, 132.23, 127.58, 126.40, 122.30, 120.14, 117.96,

116.90, 116.40, 109.46, 50.27, 24.55. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₈H₁₈F₃N₄ 347.1, found 347.1



1-(2-(Pyridin-3-yl)ethyl)-3-(2-(pyrrolidin-1-yl)-5-

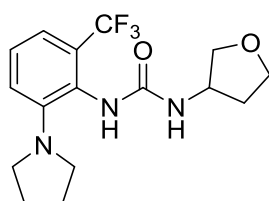
(trifluoromethyl)phenyl)thiourea (74). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (The same procedure with compound **70**). White solid (71%). HPLC-MS: *t_R* = 2.35 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.10 (s, 1H), 8.41 (d, *J* = 3.1 Hz, 2H), 7.62 (d, *J* = 7.4 Hz, 1H), 7.43 – 7.25 (m, 3H), 7.18 (s, 1H), 6.80 (d, *J* = 8.7 Hz, 1H), 3.71 (s, 2H), 3.30 (s, 4H), 2.86 (t, *J* = 7.0 Hz, 2H), 1.83 (s, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 181.32, 149.88, 148.40, 147.41, 136.20, 134.77, 128.02, 126.16, 124.48, 123.37, 116.41, 115.11, 49.29, 44.96, 31.81, 25.18. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₉H₂₂F₃N₄S 395.2, found 395.2



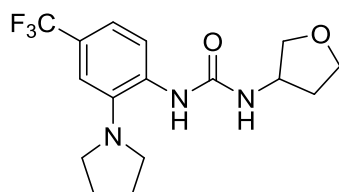
N-Phenyl-2-(3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)thioureido)ethane-1-sulfonamide (75). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (The same procedure with compound **70**). White solid (63%). HPLC-MS: *t_R* = 2.96 min,

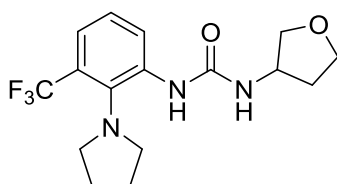
purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.95 (s, 1H), 7.84 (s, 1H), 7.31 (d, J = 6.8 Hz, 4H), 7.21 (d, J = 6.3 Hz, 4H), 7.10 (s, 1H), 6.81 (d, J = 8.8 Hz, 1H), 3.95 (d, J = 7.6 Hz, 2H), 3.44 (s, 2H), 3.29 (d, J = 4.7 Hz, 4H), 1.81 (d, J = 6.1 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 167.33, 148.28, 137.92, 134.42, 131.67, 129.32, 124.29, 124.08, 123.08, 120.29, 119.96, 115.31, 59.78, 49.26, 32.09, 25.18. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_2\text{S}_2$ 473.1, found 473.1



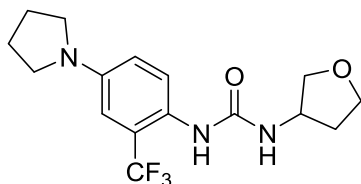
1-(2-(Pyrrolidin-1-yl)-6-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (76). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**76b**) (0.5 mmol, 0.115 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (79%). HPLC-MS: t_R = 2.38 min, purity 97%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.32 (s, 1H), 7.23 (t, J = 7.9 Hz, 1H), 6.95 (d, J = 7.5 Hz, 2H), 6.32 (s, 1H), 4.13 (s, 1H), 3.77 (dd, J = 15.1, 7.4 Hz, 1H), 3.73 – 3.58 (m, 2H), 3.45 – 3.37 (m, 1H), 3.32 (s, 3H), 2.08 (dt, J = 19.8, 7.4 Hz, 1H), 1.84 (s, 4H), 1.66 (d, J = 4.8 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.85, 149.11, 130.53, 127.94, 125.94, 122.14, 119.31, 114.08, 73.56, 66.59, 50.77, 50.03, 33.18, 25.63. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_2$ 344.2, found 344.1



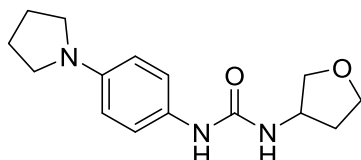
1-(2-(Pyrrolidin-1-yl)-4-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (77). From 2-(pyrrolidin-1-yl)-4-(trifluoromethyl)aniline (**77b**) (0.5 mmol, 0.115 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (79%). HPLC-MS: t_R = 2.82 min, purity 97%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.15 (d, J = 8.4 Hz, 1H), 7.87 (s, 1H), 7.37 (d, J = 6.5 Hz, 1H), 7.26 (s, 1H), 7.24 (d, J = 9.3 Hz, 1H), 4.22 (s, 1H), 3.85 – 3.60 (m, 3H), 3.50 (dd, J = 8.9, 2.7 Hz, 1H), 3.03 (s, 4H), 2.14 (dq, J = 15.1, 7.6 Hz, 1H), 1.92 (s, 4H), 1.72 (d, J = 4.4 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.06, 140.57, 137.41, 126.42, 123.72, 122.08, 119.85, 115.10, 73.43, 66.61, 51.38, 50.62, 33.11, 24.59. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_2$ 344.1, found 344.0



1-(2-(Pyrrolidin-1-yl)-3-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (78). From 2-(pyrrolidin-1-yl)-3-(trifluoromethyl)aniline (**78b**) (0.5 mmol, 0.115 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (86%). HPLC-MS: t_R = 2.92 min, purity 99%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.27 (d, J = 7.6 Hz, 1H), 7.61 (s, 1H), 7.49 (d, J = 6.7 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.26 – 7.20 (m, 1H), 4.28 – 4.15 (m, 1H), 3.82 (q, J = 7.5 Hz, 1H), 3.72 (ddd, J = 16.5, 11.4, 7.0 Hz, 2H), 3.52 (dd, J = 8.9, 3.0 Hz, 1H), 3.06 (s, 4H), 2.20 – 2.08 (m, 1H), 1.98 (s, 4H), 1.73 (ddd, J = 16.7, 10.8, 5.6 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.22, 141.71, 135.45, 129.74, 126.60, 124.78, 120.11, 73.34, 66.63, 51.00, 50.69, 33.13, 26.25. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_2$ 344.1, found 344.1

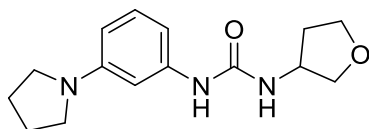


1-(4-(Pyrrolidin-1-yl)-2-(trifluoromethyl)phenyl)-3-(tetrahydrofuran-3-yl)urea (79). From 4-(pyrrolidin-1-yl)-2-(trifluoromethyl)aniline (**79b**) (0.5 mmol, 0.115 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (91%). HPLC-MS: t_R = 2.72 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.41 (d, J = 8.9 Hz, 1H), 7.35 (s, 1H), 6.74 (d, J = 6.9 Hz, 2H), 6.63 (d, J = 2.2 Hz, 1H), 4.21 – 4.10 (m, 1H), 3.83 – 3.64 (m, 3H), 3.45 (dd, J = 8.8, 3.3 Hz, 1H), 3.23 (t, J = 6.1 Hz, 4H), 2.10 (dq, J = 15.3, 7.5 Hz, 1H), 1.95 (t, J = 6.2 Hz, 4H), 1.68 (dt, J = 12.4, 5.2 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.74, 144.41, 129.20, 125.52, 124.16, 122.90, 115.23, 107.17, 73.05, 66.13, 50.19, 47.45, 32.67, 24.96. MS (ESI⁺): m/z [M+H]⁺, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_2$ 344.1, found 343.9

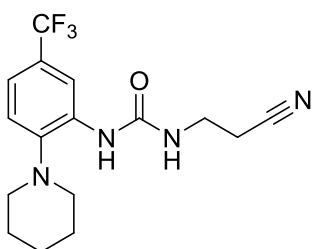


1-(4-(Pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (80). From 4-(pyrrolidin-1-yl)aniline (**80b**) (0.5 mmol, 0.081 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (91%). HPLC-MS: t_R = 1.35 min, purity 96%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.30 (s, 2H), 8.07 (s, 1H), 7.15 (d, J = 8.6 Hz, 2H), 6.43 (d, J = 8.7 Hz, 2H), 4.16 (s, 1H), 3.59 (s, 3H), 3.49 (s, 1H), 3.14 – 2.98 (m, 4H), 2.08 (dt, J = 19.8, 7.4 Hz, 1H), 1.72 – 1.61 (m, 1H), 1.51 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.41, 143.46,

129.22, 120.01, 111.76, 73.05, 66.16, 53.29, 47.61, 30.68, 24.87. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₅H₂₁N₃O₂ 276.2, found 276.1

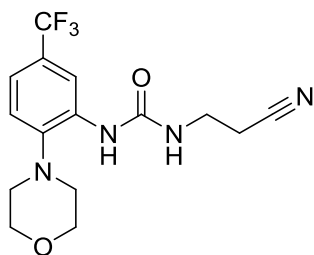


1-(3-(Pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (81). From 3-(pyrrolidin-1-yl)aniline (0.5 mmol, 0.081 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (91%). HPLC-MS: *t_R* = 2.85 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.13 (s, 1H), 6.97 (t, *J* = 8.0 Hz, 1H), 6.68 (s, 1H), 6.55 (d, *J* = 7.9 Hz, 1H), 6.30 (d, *J* = 6.7 Hz, 1H), 6.10 (d, *J* = 8.1 Hz, 1H), 4.23 – 4.11 (m, 1H), 3.80 – 3.67 (m, 3H), 3.46 (dd, *J* = 8.8, 3.3 Hz, 1H), 3.17 (d, *J* = 5.9 Hz, 4H), 2.09 (ddt, *J* = 22.0, 14.5, 7.3 Hz, 1H), 1.93 (s, 4H), 1.69 (td, *J* = 12.3, 5.3 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 154.89, 148.16, 140.98, 129.01, 105.24, 105.19, 101.02, 73.00, 66.13, 49.97, 47.25, 32.71, 24.92. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₅H₂₂N₃O₂ 276.2, found 276.1



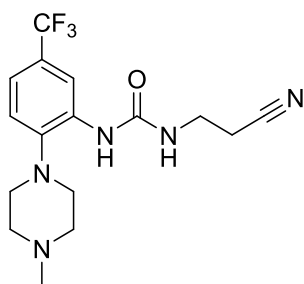
1-(2-Cyanoethyl)-3-(2-(piperidin-1-yl)-5-(trifluoromethyl)phenyl)urea (82). From 2-(piperidin-1-yl)-5-(trifluoromethyl)aniline (0.5 mmol, 0.122 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (69%). HPLC-MS: *t_R* = 2.93 min, purity 90%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.42 (s, 1H), 7.95 (s, 1H), 7.77 (t, *J* = 5.6 Hz, 1H), 7.28 – 7.20 (m, 2H), 3.40 – 3.35 (m, 2H), 2.78 – 2.65 (m, 6H), 1.75 (s, 4H), 1.54 (s, 2H). ¹³C NMR

(101 MHz, DMSO- d_6) δ 154.98, 145.45, 134.84, 125.86, 124.31, 120.53, 119.60, 118.23, 114.75, 52.57, 35.50, 25.47, 23.59, 18.38. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₂₀F₃N₄O 341.0, found 340.8



1-(2-Cyanoethyl)-3-(2-morpholino-5-(trifluoromethyl)phenyl)urea (83).

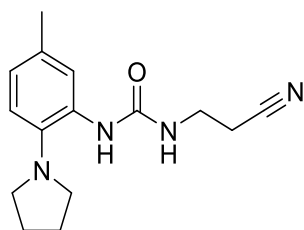
From 2-morpholino-5-(trifluoromethyl)aniline (0.5 mmol, 0.123 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (63%). HPLC-MS: t_R = 2.70 min, purity 90%. ¹H NMR (400 MHz, DMSO- d_6) δ 8.44 (s, 1H), 8.04 (s, 1H), 7.71 (t, J = 5.7 Hz, 1H), 7.29 (dd, J = 19.1, 8.3 Hz, 2H), 3.83 (s, 4H), 3.41 – 3.35 (m, 2H), 2.82 (d, J = 4.0 Hz, 4H), 2.71 (t, J = 6.3 Hz, 2H). ¹³C NMR (101 MHz, DMSO- d_6) δ 154.94, 144.09, 134.86, 128.47, 124.73, 120.71, 119.63, 118.41, 115.12, 66.05, 51.43, 35.46, 18.37. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₅H₁₈F₃N₄O₂ 343.1, found 342.8



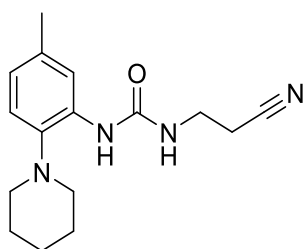
1-(2-Cyanoethyl)-3-(2-(4-methylpiperazin-1-yl)-5-

(trifluoromethyl)phenyl)urea (84). From 2-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (**27b**) (0.5 mmol, 0.130 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (57%). HPLC-MS: t_R = 2.07 min, purity 90%. ¹H NMR (400 MHz, DMSO- d_6) δ 8.40 (s, 1H), 7.92 (s,

1H), 7.75 (t, $J = 5.6$ Hz, 1H), 7.27 (dd, $J = 18.4, 8.4$ Hz, 2H), 3.34 (d, $J = 6.0$ Hz, 2H), 2.82 (d, $J = 4.1$ Hz, 4H), 2.71 (t, $J = 6.3$ Hz, 2H), 2.59 (s, 4H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.96, 144.44, 134.72, 125.82, 124.16, 120.53, 119.61, 118.39, 115.14, 54.34, 50.92, 45.72, 35.47, 18.36. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_5\text{O}$ 356.1, found 356.1

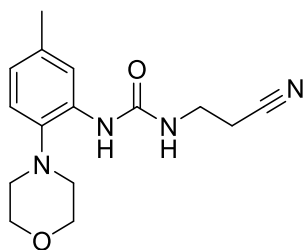


1-(2-Cyanoethyl)-3-(5-methyl-2-(pyrrolidin-1-yl)phenyl)urea (85). From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (0.5 mmol, 0.088 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (66%). HPLC-MS: $t_R = 0.92$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.77 (s, 1H), 7.68 (s, 1H), 7.23 (t, $J = 5.7$ Hz, 1H), 6.92 (d, $J = 8.1$ Hz, 1H), 6.70 (d, $J = 8.0$ Hz, 1H), 3.34 – 3.28 (m, 2H), 2.94 (s, 4H), 2.67 (t, $J = 6.3$ Hz, 2H), 2.19 (s, 3H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.35, 137.97, 132.67, 131.01, 122.77, 121.31, 119.64, 118.03, 51.22, 35.54, 24.06, 20.78, 18.50. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{N}_4\text{O}$ 273.1, found 273.1

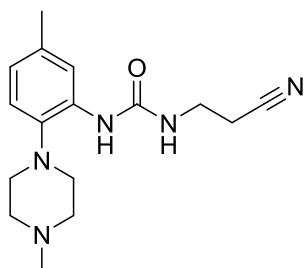


1-(2-Cyanoethyl)-3-(5-methyl-2-(piperidin-1-yl)phenyl)urea (86). From 5-methyl-2-(piperidin-1-yl)aniline (**86b**) (0.5 mmol, 0.095 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (69%). HPLC-MS: $t_R = 2.00$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6)

δ 7.89 (s, 1H), 7.83 (s, 1H), 7.63 (t, J = 5.6 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.69 (d, J = 7.7 Hz, 1H), 3.36 – 3.28 (m, 2H), 2.67 (dd, J = 15.1, 5.5 Hz, 6H), 2.21 (s, 3H), 1.72 (s, 4H), 1.51 (s, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.09, 139.56, 134.30, 132.96, 121.88, 119.81, 119.63, 118.93, 53.29, 35.56, 25.83, 23.74, 21.05, 18.46. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{23}\text{N}_4\text{O}$ 287.1, found 287.1



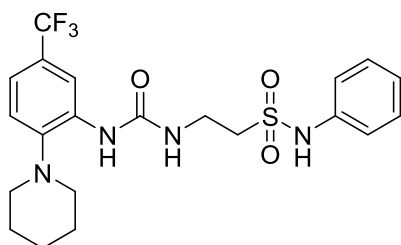
1-(2-Cyanoethyl)-3-(5-methyl-2-morpholinophenyl)urea (87). From 5-methyl-2-morpholinoaniline (**87b**) (0.5 mmol, 0.096 g) and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (68%). HPLC-MS: t_R = 2.46 min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.88 (s, 2H), 7.57 (t, J = 5.6 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 7.9 Hz, 1H), 3.84 – 3.74 (m, 4H), 3.34 (s, 2H), 2.75 – 2.63 (m, 6H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.04, 138.25, 134.33, 133.47, 122.07, 119.98, 119.67, 119.26, 66.34, 52.17, 35.48, 21.04, 18.46. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{15}\text{H}_{21}\text{N}_4\text{O}_2$ 289.2, found 289.0



1-(2-Cyanoethyl)-3-(5-methyl-2-(4-methylpiperazin-1-yl)phenyl)urea (88).

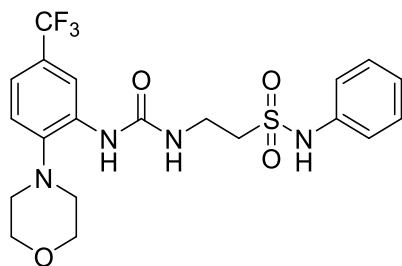
From 5-methyl-2-(4-methylpiperazin-1-yl)aniline (**88b**) (0.5 mmol, 0.103 g)

and 3-aminopropionitrile (0.75 mmol, 0.052 g, 0.05 mL) (Method C). White solid (43%). HPLC-MS: t_R = 1.46 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.86 (s, 1H), 7.77 (s, 1H), 7.61 (t, J = 5.6 Hz, 1H), 7.00 (d, J = 8.0 Hz, 1H), 6.71 (d, J = 7.5 Hz, 1H), 3.33 (dd, J = 12.4, 6.3 Hz, 4H), 2.69 (dd, J = 12.0, 5.5 Hz, 6H), 2.54 (s, 2H), 2.25 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.06, 138.51, 134.21, 133.16, 122.03, 119.79, 119.65, 119.28, 54.75, 51.67, 45.85, 35.51, 21.02, 18.44. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{24}\text{N}_5\text{O}$ 302.2, found 302.2

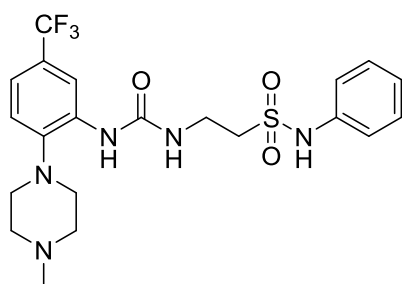


N-Phenyl-2-(3-(2-(piperidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)ethane-1-sulfonamide (89). From 2-(piperidin-1-yl)-5-(trifluoromethyl)aniline (0.5 mmol, 0.122 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (74%). HPLC-MS: t_R = 3.07 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.82 (s, 1H), 8.37 (s, 1H), 7.90 (s, 1H), 7.87 – 7.78 (m, 1H), 7.58 (t, J = 5.6 Hz, 1H), 7.30 (d, J = 7.9 Hz, 2H), 7.22 (dd, J = 12.8, 7.1 Hz, 4H), 7.09 (t, J = 7.2 Hz, 1H), 3.54 – 3.41 (m, 2H), 3.27 (t, J = 6.7 Hz, 2H), 2.73 (d, J = 4.7 Hz, 4H), 1.73 (s, 4H), 1.53 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 167.31, 154.86, 145.34, 138.00, 134.84, 131.66, 129.30, 124.02, 123.07, 119.95, 118.15, 114.62, 52.52, 50.52, 34.20, 25.49, 23.59. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{26}\text{F}_3\text{N}_4\text{O}_3\text{S}$ 471.2, found 471.2

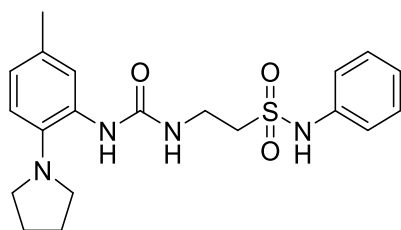


2-(3-(2-Morpholino-5-(trifluoromethyl)phenyl)ureido)-N-phenylethane-1-sulfonamide (90). From 2-morpholino-5-(trifluoromethyl)aniline (0.5 mmol, 0.123 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (72%). HPLC-MS: t_R = 2.86 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.83 (s, 1H), 8.38 (s, 1H), 7.99 (s, 1H), 7.52 (t, J = 5.6 Hz, 1H), 7.45 (dd, J = 11.0, 4.4 Hz, 1H), 7.32 – 7.23 (m, 5H), 7.09 (t, J = 7.3 Hz, 1H), 3.81 (s, 4H), 3.50 (dd, J = 12.7, 6.4 Hz, 2H), 3.28 (t, J = 6.7 Hz, 2H), 2.80 (s, 4H), 1.41 (s, 1H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 154.85, 144.04, 137.99, 134.83, 129.32, 125.80, 124.04, 123.10, 120.61, 119.99, 118.35, 115.07, 66.07, 51.37, 34.20, 27.41. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_4\text{S}$ 473.2, found 473.2

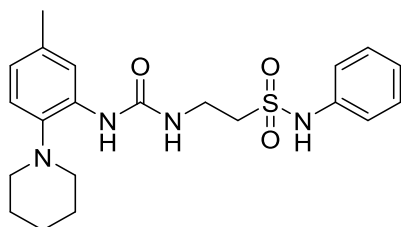


2-(3-(2-(4-Methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)ureido)-N-phenylethane-1-sulfonamide (91). From 2-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (**27b**) (0.5 mmol, 0.130 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (63%). HPLC-MS: t_R = 2.26 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.35 (s, 1H), 7.89 (s, 1H), 7.59 (t, J = 5.6 Hz, 1H), 7.27 (dt, J = 15.1, 7.9 Hz,

6H), 7.09 (t, $J = 7.2$ Hz, 1H), 3.48 (d, $J = 6.4$ Hz, 2H), 3.40 (s, 1H), 3.28 (d, $J = 6.8$ Hz, 2H), 2.82 (s, 4H), 2.63 (s, 4H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.88, 144.25, 138.02, 134.74, 129.32, 125.84, 124.04, 123.14, 120.50, 119.98, 118.29, 115.05, 54.26, 50.68, 50.52, 45.51, 34.23. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{27}\text{F}_3\text{N}_5\text{O}_3\text{S}$ 486.1, found 485.9

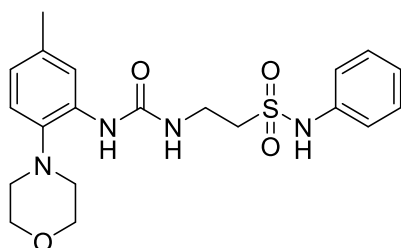


2-(3-(5-Methyl-2-(pyrrolidin-1-yl)phenyl)ureido)-N-phenylethane-1-sulfonamide (92). From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (0.5 mmol, 0.088 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (71%). HPLC-MS: $t_R = 2.21$ min, purity 100%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.82 (s, 1H), 7.71 (d, $J = 26.0$ Hz, 1H), 7.60 (s, 1H), 7.36 – 7.26 (m, 2H), 7.23 (d, $J = 7.6$ Hz, 2H), 7.10 (t, $J = 7.3$ Hz, 1H), 7.03 (t, $J = 5.6$ Hz, 1H), 6.87 (t, $J = 14.7$ Hz, 1H), 6.69 (d, $J = 7.7$ Hz, 1H), 3.46 (dd, $J = 12.8, 6.3$ Hz, 2H), 3.22 (dd, $J = 17.5, 10.9$ Hz, 2H), 2.93 (s, 4H), 2.18 (s, 3H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.31, 138.04, 132.44, 130.85, 129.32, 124.04, 122.83, 121.53, 120.01, 119.82, 117.91, 51.15, 50.85. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{27}\text{N}_4\text{O}_3\text{S}$ 403.2, found 403.2



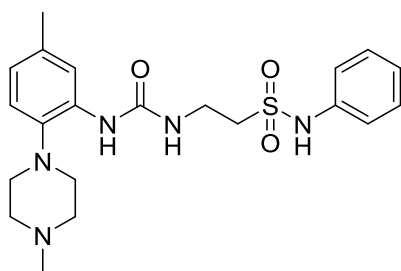
2-(3-(5-Methyl-2-(piperidin-1-yl)phenyl)ureido)-N-phenylethane-1-sulfonamide (93). From 5-methyl-2-(piperidin-1-yl)aniline (**86b**) (0.5 mmol,

0.095 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (77%). HPLC-MS: t_R = 2.33 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.82 (s, 1H), 7.83 (s, 1H), 7.77 (s, 1H), 7.42 (t, J = 5.6 Hz, 1H), 7.32 (t, J = 7.8 Hz, 2H), 7.25 – 7.21 (m, 2H), 7.10 (t, J = 7.3 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 7.9 Hz, 1H), 3.52 – 3.42 (m, 2H), 3.25 (t, J = 6.9 Hz, 2H), 2.68 – 2.58 (m, 4H), 2.19 (s, 3H), 1.69 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 167.33, 154.97, 139.46, 138.03, 134.29, 132.98, 129.34, 124.06, 121.83, 119.97, 118.77, 53.26, 50.66, 34.21, 25.84, 23.72, 21.06. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{29}\text{N}_4\text{O}_3\text{S}$ 417.2, found 417.1

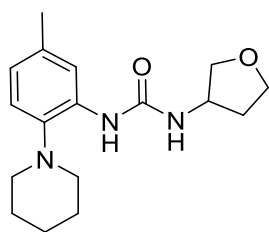


2-(3-(5-Methyl-2-morpholinophenyl)ureido)-N-phenylethane-1-

sulfonamide (94). From 5-methyl-2-morpholinoaniline (**87b**) (0.5 mmol, 0.096 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (61%). HPLC-MS: t_R = 2.72 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.76 (s, 2H), 7.84 (s, 1H), 7.32 (t, J = 7.1 Hz, 3H), 7.24 (dd, J = 7.2, 1.3 Hz, 3H), 7.10 (dd, J = 10.3, 4.2 Hz, 2H), 7.00 (d, J = 8.0 Hz, 1H), 6.71 (d, J = 7.2 Hz, 1H), 3.77 (s, 4H), 3.49 – 3.45 (m, 2H), 3.26 (t, J = 6.9 Hz, 2H), 2.69 (s, 4H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 168.12, 154.98, 138.20, 135.75, 134.33, 129.36, 127.52, 124.08, 122.05, 120.01, 119.20, 66.38, 52.15, 50.71, 34.21, 21.06. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{27}\text{N}_4\text{O}_4\text{S}$ 419.2, found 419.2

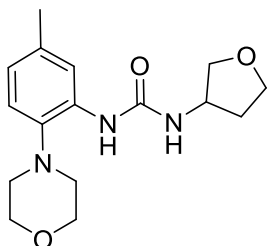


2-(3-(5-Methyl-2-(4-methylpiperazin-1-yl)phenyl)ureido)-N-phenylethane-1-sulfonamide (95). From 5-methyl-2-(4-methylpiperazin-1-yl)aniline (**88b**) (0.5 mmol, 0.103 g) and 2-amino-N-phenylethane-1-sulfonamide (**4b**) (0.75 mmol, 0.150 g) (Method C). White solid (51%). HPLC-MS: t_R = 2.18 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.84 (s, 1H), 7.80 (d, J = 13.3 Hz, 2H), 7.46 (d, J = 5.7 Hz, 1H), 7.31 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.5 Hz, 2H), 7.10 (t, J = 7.3 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 6.9 Hz, 1H), 3.47 (s, 2H), 3.23 (d, J = 6.6 Hz, 2H), 2.79 (s, 8H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.96, 138.03, 137.85, 134.31, 133.54, 131.92, 129.33, 124.05, 121.99, 119.98, 119.91, 119.19, 54.19, 50.64, 44.53, 34.20, 21.04, 17.45. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{30}\text{N}_5\text{O}_3\text{S}$ 432.2, found 432.2

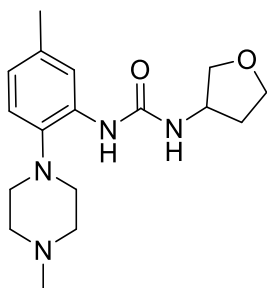


1-(5-Methyl-2-(piperidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (96). From 5-methyl-2-(piperidin-1-yl)aniline (**86b**) (0.5 mmol, 0.095 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (71%). HPLC-MS: t_R = 2.07 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.91 (s, 1H), 7.72 (s, 1H), 7.45 (d, J = 6.4 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 6.67 (d, J = 7.8 Hz, 1H), 4.20 (s, 1H), 3.84 – 3.65 (m, 3H), 3.50 (dd, J

= 8.8, 3.1 Hz, 1H), 2.64 (s, 4H), 2.18 (d, J = 11.5 Hz, 3H), 2.16 – 2.05 (m, 1H), 1.71 (s, 5H), 1.51 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.88, 139.36, 134.56, 132.95, 121.60, 119.80, 118.71, 72.94, 66.22, 53.31, 50.21, 32.60, 25.81, 23.72, 21.07. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{26}\text{N}_3\text{O}_2$ 304.1, found 304.0

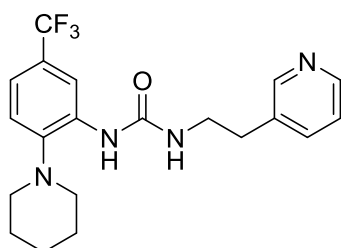


1-(5-Methyl-2-morpholinophenyl)-3-(tetrahydrofuran-3-yl)urea (97). From 5-methyl-2-morpholinoaniline (**87b**) (0.5 mmol, 0.096 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (63%). HPLC-MS: t_R = 2.47 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.94 (s, 1H), 7.78 (s, 1H), 7.42 (d, J = 6.5 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 4.24 – 4.16 (m, 1H), 3.86 – 3.65 (m, 7H), 3.51 (dd, J = 8.9, 3.4 Hz, 1H), 2.74 – 2.64 (m, 4H), 2.20 (s, 3H), 2.17 – 2.07 (m, 1H), 1.72 (ddd, J = 12.5, 7.5, 5.5 Hz, 1H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.82, 137.96, 134.66, 133.52, 121.75, 120.01, 118.89, 72.95, 66.36, 66.22, 52.23, 50.21, 32.63, 21.08. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_3$ 306.4, found 306.3

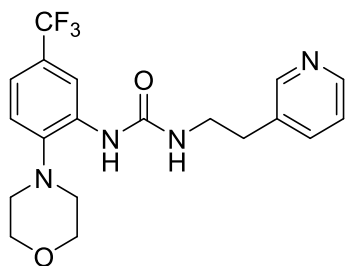


1-(5-Methyl-2-(4-methylpiperazin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (98). From 5-methyl-2-(4-methylpiperazin-1-yl)aniline (**88b**) (0.5

mmol, 0.103 g) and tetrahydrofuran-3-amine hydrochloride (0.75 mmol, 0.093 g) (Method C). White solid (54%). HPLC-MS: t_R = 1.93 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.51 (d, J = 1.1 Hz, 3H), 8.39 – 8.27 (m, 6H), 7.27 (d, J = 8.3 Hz, 3H), 7.21 (d, J = 8.2 Hz, 3H), 4.24 – 4.12 (m, 4H), 3.79 (ddd, J = 16.0, 14.3, 7.4 Hz, 10H), 3.51 (d, J = 3.7 Hz, 4H), 3.29 (s, 12H), 2.99 (d, J = 4.2 Hz, 13H), 2.60 (s, 9H), 2.09 (dq, J = 15.2, 7.6 Hz, 3H), 1.80 (dq, J = 7.3, 5.2 Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.01, 143.04, 135.80, 123.13, 121.00, 117.74, 114.44, 72.88, 70.35, 66.33, 52.86, 50.26, 49.15, 43.44, 32.28. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{26}\text{N}_4\text{O}_2$ 319.2, found 319.2

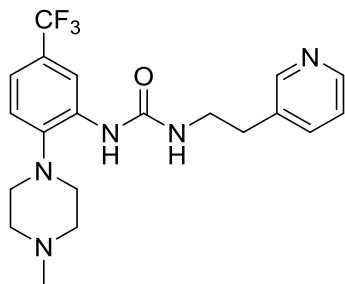


1-(2-(Piperidin-1-yl)-5-(trifluoromethyl)phenyl)-3-(2-(pyridin-3-yl)ethyl)urea (99). From 2-(piperidin-1-yl)-5-(trifluoromethyl)aniline (0.5 mmol, 0.122 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). White solid (81%). HPLC-MS: t_R = 2.51 min, purity 98%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.46 (s, 1H), 8.42 (d, J = 4.4 Hz, 1H), 8.36 (s, 1H), 7.78 (s, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.41 (t, J = 5.5 Hz, 1H), 7.31 (dd, J = 7.5, 4.9 Hz, 1H), 7.20 (s, 2H), 3.39 (d, J = 6.7 Hz, 2H), 2.79 (t, J = 6.9 Hz, 2H), 2.76 – 2.66 (m, 4H), 1.72 (s, 4H), 1.52 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 154.91, 149.97, 147.40, 145.40, 136.29, 134.93, 128.60, 124.19, 123.39, 120.23, 118.00, 114.93, 77.54, 52.45, 32.83, 28.20, 25.44, 23.60. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{24}\text{F}_3\text{N}_4\text{O}$ 393.2, found 393.2



1-(2-Morpholino-5-(trifluoromethyl)phenyl)-3-(2-(pyridin-3-yl)ethyl)urea

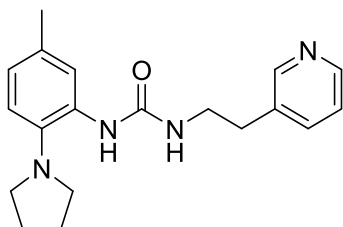
(100). From 2-morpholino-5-(trifluoromethyl)aniline (0.5 mmol, 0.123 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). White solid (73%). HPLC-MS: t_R = 2.26 min, purity 96%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.42 (dd, J = 13.2, 8.9 Hz, 2H), 8.37 (s, 1H), 7.86 (s, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.36 – 7.22 (m, 4H), 3.85 – 3.77 (m, 4H), 3.41 – 3.35 (m, 2H), 2.79 (s, 4H), 1.34 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.85, 149.99, 147.43, 144.06, 136.34, 134.92, 125.83, 124.61, 123.45, 120.42, 118.17, 115.28, 66.03, 51.33, 32.80, 28.23. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{22}\text{F}_3\text{N}_4\text{O}_2$ 395.2, found 395.2



1-(2-(4-Methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)-3-(2-(pyridin-3-

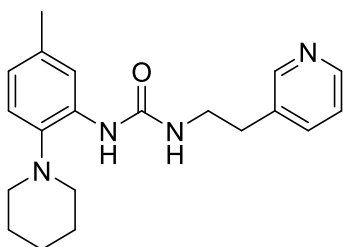
yl)ethyl)urea (101). From 2-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (**27b**) (0.5 mmol, 0.130 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). White solid (79%). HPLC-MS: t_R = 1.38 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.49 – 8.38 (m, 2H), 8.33 (s, 1H), 7.75 (s, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 5.6 Hz, 1H), 7.32 (dd, J = 7.6, 4.9 Hz, 1H), 7.24 (q, J = 8.4 Hz, 2H), 3.38 (s, 2H),

2.80 (dd, $J = 13.9, 5.9$ Hz, 6H), 2.60 (s, 4H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.87, 149.98, 147.41, 144.33, 136.33, 135.04, 134.80, 125.85, 124.37, 123.43, 120.28, 118.14, 115.24, 54.26, 50.70, 45.61, 32.81. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{25}\text{F}_3\text{N}_5\text{O}$ 408.4, found 408.3



1-(5-Methyl-2-(pyrrolidin-1-yl)phenyl)-3-(2-(pyridin-3-yl)ethyl)urea (102).

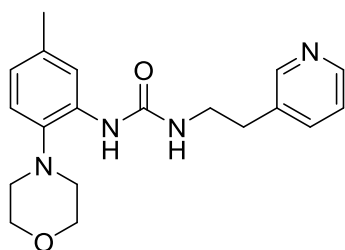
White solid (79%). From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (0.5 mmol, 0.088 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). HPLC-MS: $t_R = 0.47$ min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.47 – 8.38 (m, 2H), 7.65 (d, $J = 5.3$ Hz, 2H), 7.58 (s, 1H), 7.31 (dd, $J = 7.6, 4.9$ Hz, 1H), 6.89 (d, $J = 7.8$ Hz, 2H), 6.67 (d, $J = 8.0$ Hz, 1H), 3.33 (d, $J = 6.7$ Hz, 2H), 2.90 (d, $J = 5.4$ Hz, 4H), 2.76 (t, $J = 6.9$ Hz, 2H), 2.18 (s, 3H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.33, 149.97, 147.36, 137.86, 136.28, 135.14, 132.88, 130.88, 123.41, 122.42, 121.33, 117.78, 51.09, 40.23, 32.96, 23.99, 20.77. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{25}\text{N}_4\text{O}$ 325.2, found 325.2



1-(5-Methyl-2-(piperidin-1-yl)phenyl)-3-(2-(pyridin-3-yl)ethyl)urea (103).

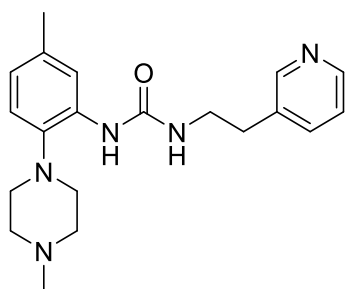
From 5-methyl-2-(piperidin-1-yl)aniline (**86b**) (0.5 mmol, 0.095 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C).

White solid (71%). HPLC-MS: t_R = 1.13 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.48 – 8.38 (m, 2H), 7.82 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.35 – 7.21 (m, 2H), 6.94 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 7.8 Hz, 1H), 3.36 – 3.30 (m, 2H), 2.78 (t, J = 6.9 Hz, 2H), 2.67 – 2.57 (m, 4H), 2.19 (s, 3H), 1.69 (s, 4H), 1.50 (s, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.07, 149.95, 147.36, 139.56, 136.27, 135.17, 134.38, 132.75, 123.39, 121.65, 119.55, 119.16, 53.17, 40.31, 32.93, 25.79, 23.75, 21.03. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{27}\text{N}_4\text{O}$ 339.2, found 339.2

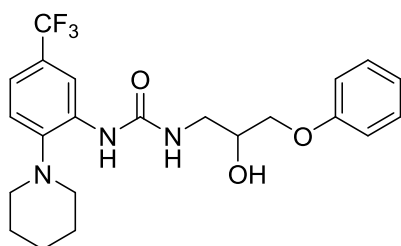


1-(5-Methyl-2-morpholinophenyl)-3-(2-(pyridin-3-yl)ethyl)urea (104).

From 5-methyl-2-morpholinoaniline (**87b**) (0.5 mmol, 0.096 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). White solid (69%). HPLC-MS: t_R = 2.02 min, purity 92%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.48 – 8.37 (m, 2H), 7.83 (s, 1H), 7.71 (s, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.32 (dd, J = 7.6, 4.9 Hz, 1H), 7.21 (t, J = 5.5 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 3.82 – 3.69 (m, 4H), 3.33 (d, J = 6.7 Hz, 2H), 2.78 (t, J = 6.9 Hz, 2H), 2.72 – 2.62 (m, 4H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.03, 149.97, 147.38, 138.23, 136.30, 135.14, 134.42, 133.27, 123.42, 121.83, 119.68, 119.47, 66.31, 52.07, 40.24, 32.91, 21.03. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{25}\text{N}_4\text{O}_2$ 341.2, found 341.2

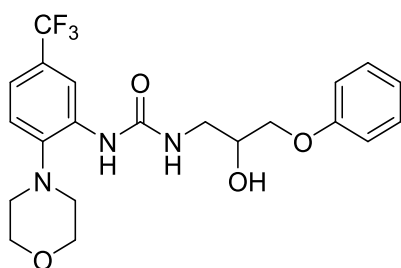


1-(5-Methyl-2-(4-methylpiperazin-1-yl)phenyl)-3-(2-(pyridin-3-yl)ethyl)urea (105). From 5-methyl-2-(4-methylpiperazin-1-yl)aniline (**88b**) (0.5 mmol, 0.103 g) and 2-(pyridin-3-yl)ethan-1-amine hydrochloride (0.75 mmol, 0.092 g) (Method C). White solid (54%). HPLC-MS: t_R = 0.53 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.47 – 8.37 (m, 2H), 7.79 (s, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.59 (s, 1H), 7.32 (dd, J = 7.6, 4.9 Hz, 1H), 7.25 (t, J = 5.5 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 7.9 Hz, 1H), 3.32 (d, J = 6.7 Hz, 2H), 2.77 (t, J = 6.9 Hz, 2H), 2.70 (t, J = 4.5 Hz, 4H), 2.53 (s, 4H), 2.25 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.05, 149.95, 147.37, 138.48, 136.30, 135.16, 134.30, 132.98, 123.41, 121.80, 119.54, 119.50, 54.70, 51.52, 45.80, 40.29, 32.92, 21.02. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{28}\text{N}_5\text{O}$ 354.4, found 354.3

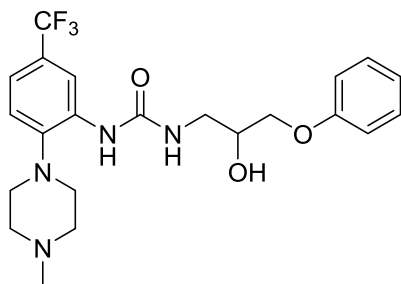


1-(2-Hydroxy-3-phenoxypropyl)-3-(2-(piperidin-1-yl)-5-(trifluoromethyl)phenyl)urea (106). From 2-(piperidin-1-yl)-5-(trifluoromethyl)aniline (0.5 mmol, 0.122 g) and 1-amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (74%). HPLC-MS: t_R = 3.07 min, purity 96%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.42 (s, 1H),

7.97 (s, 1H), 7.54 (s, 1H), 7.31 – 7.18 (m, 4H), 6.93 (t, $J = 8.9$ Hz, 3H), 5.28 (d, $J = 2.8$ Hz, 1H), 3.95 – 3.87 (m, 3H), 3.40 (dd, $J = 11.6, 7.2$ Hz, 1H), 3.17 (dd, $J = 12.2, 6.3$ Hz, 1H), 2.73 (d, $J = 4.7$ Hz, 4H), 1.74 (s, 4H), 1.54 (d, $J = 5.2$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.60, 155.30, 145.41, 135.18, 129.48, 125.92, 124.20, 123.22, 120.59, 117.90, 114.50, 70.02, 68.17, 52.53, 42.59, 25.41, 23.64. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{22}\text{H}_{27}\text{F}_3\text{N}_3\text{O}_3$ 438.1, found 438.0

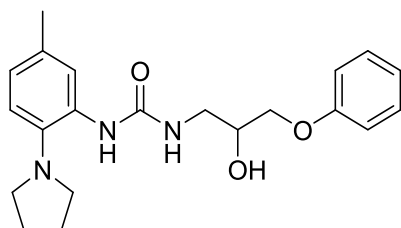


1-(2-Hydroxy-3-phenoxypropyl)-3-(2-morpholino-5-(trifluoromethyl)phenyl)urea (107). From 2-morpholino-5-(trifluoromethyl)aniline (0.5 mmol, 0.123 g) and 1-amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (69%). HPLC-MS: $t_R = 2.88$ min, purity 90%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.46 (s, 1H), 8.07 (s, 1H), 7.50 (s, 1H), 7.32 – 7.26 (m, 3H), 7.23 (d, $J = 8.4$ Hz, 1H), 6.93 (t, $J = 8.9$ Hz, 3H), 5.31 (s, 1H), 3.91 (s, 3H), 3.83 (d, $J = 1.7$ Hz, 4H), 3.45 – 3.37 (m, 1H), 3.21 – 3.10 (m, 1H), 2.86 – 2.74 (m, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.59, 155.25, 143.99, 135.24, 129.49, 125.86, 124.37, 123.16, 120.61, 118.00, 114.51, 70.01, 68.16, 66.01, 51.42, 42.58. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{25}\text{F}_3\text{N}_3\text{O}_4$ 440.2, found 440.2



1-(2-Hydroxy-3-phenoxypropyl)-3-(2-(4-methylpiperazin-1-yl)-5-

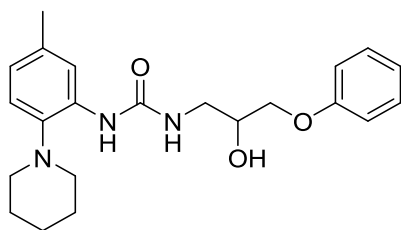
(trifluoromethyl)phenyl)urea (108). From 2-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (**27b**) (0.5 mmol, 0.130 g) and 1-amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (75%). HPLC-MS: t_R = 2.28 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.40 (s, 1H), 7.93 (s, 1H), 7.52 (s, 1H), 7.29 – 7.19 (m, 4H), 6.94 (d, J = 8.7 Hz, 3H), 5.29 (s, 1H), 3.90 (d, J = 7.6 Hz, 3H), 3.15 (dd, J = 15.5, 8.2 Hz, 2H), 2.81 (s, 4H), 2.57 (s, 4H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 158.59, 155.26, 154.00, 149.09, 144.44, 135.02, 129.48, 120.59, 115.22, 114.50, 106.70, 70.01, 68.13, 54.31, 50.91, 45.78, 44.27, 42.59. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{22}\text{H}_{28}\text{F}_3\text{N}_4\text{O}_3$ 453.1, found 453.1



1-(2-Hydroxy-3-phenoxypropyl)-3-(5-methyl-2-(pyrrolidin-1-

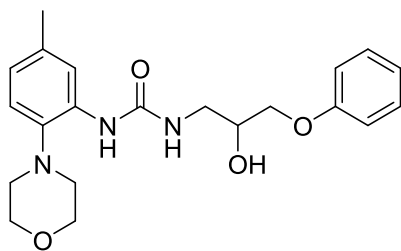
yl)phenyl)urea (109). From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (0.5 mmol, 0.088 g) and 1-amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (74%). HPLC-MS: t_R = 2.22 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.76 (s, 1H), 7.68 (d, J = 1.1 Hz, 1H), 7.31 – 7.24 (m, 2H), 7.02 (t, J = 5.5 Hz, 1H), 6.92 (dd, J = 15.1, 8.0 Hz, 4H),

6.68 (dd, $J = 8.0, 1.2$ Hz, 1H), 5.25 (d, $J = 4.2$ Hz, 1H), 3.94 – 3.83 (m, 3H), 3.20 – 3.10 (m, 1H), 2.93 (d, $J = 2.5$ Hz, 4H), 2.18 (s, 3H), 1.86 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.62, 155.73, 137.86, 133.05, 130.90, 129.48, 122.41, 121.27, 120.56, 117.91, 114.48, 70.04, 68.39, 51.15, 42.58, 24.02, 20.79. MS (ESI $^+$): m/z [M+H] $^+$, calcd for C₂₁H₂₈N₃O₃ 370.3, found 370.3



1-(2-Hydroxy-3-phenoxypropyl)-3-(5-methyl-2-(piperidin-1-

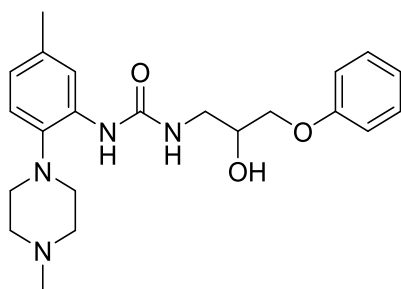
yl)phenyl)urea (110). From 5-methyl-2-(piperidin-1-yl)aniline (**86b**) (0.5 mmol, 0.095 g) and 1-amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (69%). HPLC-MS: $t_R = 2.32$ min, purity 94%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.85 (s, 1H), 7.82 (s, 1H), 7.37 (s, 1H), 7.28 (t, $J = 7.9$ Hz, 2H), 6.94 (q, $J = 7.4$ Hz, 4H), 6.67 (d, $J = 7.9$ Hz, 1H), 5.25 (d, $J = 3.6$ Hz, 1H), 3.95 – 3.85 (m, 3H), 3.19 – 3.10 (m, 1H), 2.69 – 2.59 (m, 4H), 2.19 (s, 3H), 1.71 (s, 4H), 1.49 (s, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.63, 155.51, 139.61, 134.59, 132.80, 129.49, 121.64, 120.57, 119.71, 119.13, 114.50, 70.08, 68.39, 53.26, 42.64, 25.75, 23.77, 21.07. MS (ESI $^+$): m/z [M+H] $^+$, calcd for C₂₂H₃₀N₃O₃ 384.1, found 384.1



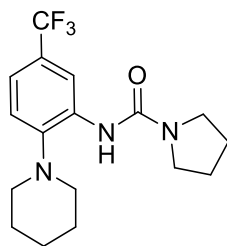
1-(2-Hydroxy-3-phenoxypropyl)-3-(5-methyl-2-morpholinophenyl)urea

(111). From 5-methyl-2-morpholinoaniline (**87b**) (0.5 mmol, 0.096 g) and 1-

amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (61%). HPLC-MS: t_R = 2.72 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.90 (s, 2H), 7.30 (dd, J = 19.9, 12.2 Hz, 3H), 6.96 (dt, J = 15.8, 7.9 Hz, 4H), 6.70 (d, J = 7.8 Hz, 1H), 5.27 (d, J = 2.7 Hz, 1H), 3.90 (d, J = 8.1 Hz, 3H), 3.79 (s, 4H), 3.39 (s, 1H), 3.15 (dd, J = 12.1, 6.2 Hz, 1H), 2.70 (s, 4H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 158.61, 155.45, 138.21, 134.68, 133.32, 129.49, 121.76, 120.58, 119.84, 119.34, 114.49, 70.06, 68.37, 66.28, 52.15, 42.61, 21.06. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_4$ 386.1, found 386.1

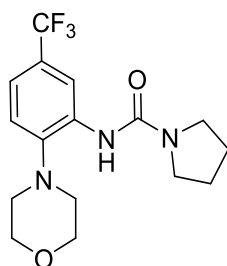


1-(2-Hydroxy-3-phenoxypropyl)-3-(5-methyl-2-(4-methylpiperazin-1-yl)phenyl)urea (112). From 5-methyl-2-(4-methylpiperazin-1-yl)aniline (**88b**) (0.5 mmol, 0.103 g) and 1-amino-3-phenoxypropan-2-ol hydrochloride (0.75 mmol, 0.153 g) (Method C). White solid (53%). HPLC-MS: t_R = 2.16 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.84 (s, 1H), 7.77 (s, 1H), 7.35 (d, J = 5.4 Hz, 1H), 7.28 (t, J = 7.9 Hz, 2H), 6.95 (dt, J = 15.8, 7.9 Hz, 4H), 6.68 (d, J = 7.9 Hz, 1H), 5.27 (s, 1H), 3.91 (dd, J = 13.8, 6.9 Hz, 3H), 3.34 (s, 1H), 3.15 (dd, J = 12.3, 6.5 Hz, 1H), 2.70 (d, J = 4.3 Hz, 4H), 2.54 (s, 4H), 2.22 (d, J = 17.9 Hz, 6H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 158.63, 155.50, 148.56, 138.55, 134.49, 133.01, 129.48, 121.80, 120.57, 119.52, 114.50, 70.08, 68.36, 54.66, 51.58, 45.79, 42.64, 21.04. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{31}\text{N}_4\text{O}_3$ 399.2, found 399.2



N-(2-(Piperidin-1-yl)-5-(trifluoromethyl)phenyl)pyrrolidine-1-

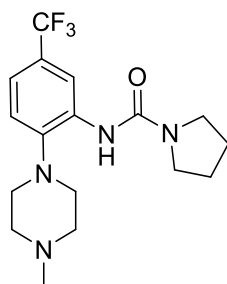
carboxamide (113). From 2-(piperidin-1-yl)-5-(trifluoromethyl)aniline (0.5 mmol, 0.122 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (73%). HPLC-MS: t_R = 3.19 min, purity 98%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.53 (s, 1H), 7.81 (s, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H), 3.39 (d, J = 5.7 Hz, 4H), 2.83 – 2.71 (m, 4H), 1.91 (s, 4H), 1.67 (d, J = 4.5 Hz, 4H), 1.55 (d, J = 4.7 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 152.74, 144.87, 135.32, 124.94, 123.11, 121.07, 118.03, 113.42, 52.53, 45.36, 30.41, 26.46, 23.47. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{23}\text{F}_3\text{N}_3\text{O}$ 342.2, found 342.1



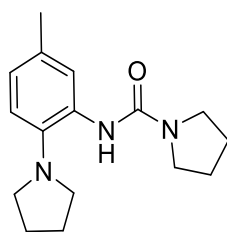
N-(2-Morpholino-5-(trifluoromethyl)phenyl)pyrrolidine-1-carboxamide

(114). From 2-morpholino-5-(trifluoromethyl)aniline (0.5 mmol, 0.123 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (81%). HPLC-MS: t_R = 2.90 min, purity 97%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.53 (d, J = 1.4 Hz, 1H), 7.85 (s, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 3.80 – 3.72 (m, 4H), 3.41 (s, 4H), 2.89 – 2.77 (m, 4H), 1.91 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 152.74, 143.59, 135.47, 125.40, 123.04, 121.39, 118.16, 113.85,

113.81, 66.83, 51.44, 45.36, 25.12. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₂₁F₃N₃O₂ 344.2, found 344.0

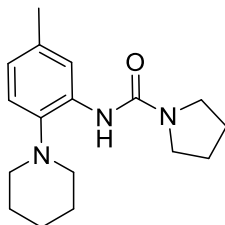


N-(2-(4-Methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)pyrrolidine-1-carboxamide (115). From 2-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (**27b**) (0.5 mmol, 0.130 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (69%). HPLC-MS: *t_R* = 2.12 min, purity 100%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.54 (d, *J* = 1.4 Hz, 1H), 7.75 (s, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 3.39 (dd, *J* = 14.1, 7.7 Hz, 8H), 2.85 (t, *J* = 4.6 Hz, 4H), 2.25 (s, 3H), 1.91 (s, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 152.72, 143.86, 135.29, 125.14, 123.07, 121.14, 118.17, 113.70, 55.39, 50.98, 45.73, 45.39, 25.08. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₇H₂₄F₃N₄O 357.2, found 357.2



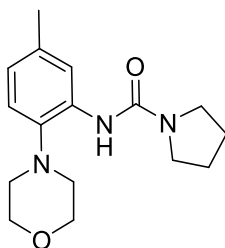
N-(5-Methyl-2-(pyrrolidin-1-yl)phenyl)pyrrolidine-1-carboxamide (116). From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (0.5 mmol, 0.088 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (91%). HPLC-MS: *t_R* = 1.90 min, purity 95%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.61 (s, 2H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H), 3.34 (s, 4H), 2.98 (t, *J* = 5.7 Hz,

4H), 2.20 (s, 3H), 1.86 (d, $J = 2.7$ Hz, 8H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 153.68, 138.16, 132.58, 131.02, 123.15, 121.88, 118.19, 51.33, 45.27, 25.12, 24.18, 20.75. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}$ 274.2, found 274.1



N-(5-Methyl-2-(piperidin-1-yl)phenyl)pyrrolidine-1-carboxamide (117).

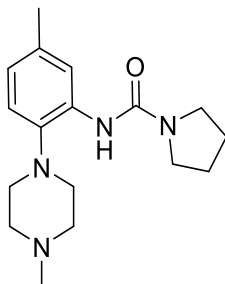
From 5-methyl-2-(piperidin-1-yl)aniline (**86b**) (0.5 mmol, 0.095 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (73%). HPLC-MS: $t_R = 2.30$ min, purity 96%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.98 (s, 1H), 7.88 (s, 1H), 7.04 (d, $J = 8.0$ Hz, 1H), 6.70 (d, $J = 7.9$ Hz, 1H), 3.37 (t, $J = 6.4$ Hz, 4H), 2.73 – 2.63 (m, 4H), 2.22 (s, 3H), 1.90 (s, 4H), 1.71 – 1.60 (m, 4H), 1.52 (d, $J = 3.9$ Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 152.91, 138.93, 134.75, 133.64, 121.61, 120.31, 117.47, 53.20, 45.30, 26.79, 25.13, 23.56, 21.18. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{26}\text{N}_3\text{O}$ 288.2, found 288.1



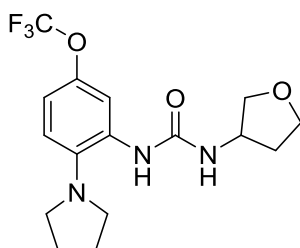
N-(5-Methyl-2-morpholinophenyl)pyrrolidine-1-carboxamide (118).

From 5-methyl-2-morpholinoaniline (**87b**) (0.5 mmol, 0.096 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (82%). HPLC-MS: $t_R = 2.68$ min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.99 (s, 1H), 7.88 (s, 1H), 7.09 (d, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 7.9$ Hz, 1H), 3.79 – 3.65 (m, 4H), 3.39 (t, $J = 6.3$ Hz, 4H), 2.79 – 2.65 (m, 4H), 2.23 (s, 3H), 1.90 (s, 4H). ^{13}C NMR (101 MHz,

DMSO- d_6) δ 152.91, 137.64, 134.90, 134.19, 121.78, 120.62, 117.77, 67.13, 52.13, 45.31, 25.16, 21.19. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₆H₂₄N₃O₂ 290.2, found 290.0

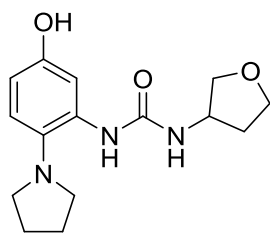


N-(5-Methyl-2-(4-methylpiperazin-1-yl)phenyl)pyrrolidine-1-carboxamide (119). From 5-methyl-2-(4-methylpiperazin-1-yl)aniline (**88b**) (0.5 mmol, 0.103 g) and pyrrolidine (0.75 mmol, 0.053 g) (Method C). White solid (87%). HPLC-MS: t_R = 1.94 min, purity 88%. ¹H NMR (400 MHz, DMSO- d_6) δ 7.98 (s, 1H), 7.81 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 3.37 (d, J = 9.3 Hz, 8H), 2.74 (t, J = 4.5 Hz, 4H), 2.22 (s, 6H), 1.90 (s, 4H). ¹³C NMR (101 MHz, DMSO- d_6) δ 152.89, 137.78, 134.78, 133.91, 121.71, 120.37, 117.69, 55.78, 51.64, 45.82, 45.34, 25.14, 21.18. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₇H₂₇N₄O 303.2, found 303.2



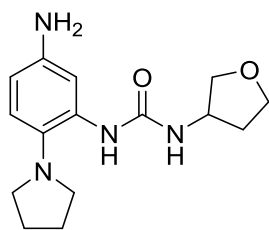
1-(2-(Pyrrolidin-1-yl)-5-(trifluoromethoxy)phenyl)-3-(tetrahydrofuran-3-yl)urea (120). From 2-(pyrrolidin-1-yl)-5-(trifluoromethoxy)aniline (**120b**) (1.5 mmol, 0.369 g) and tetrahydrofuran-3-carboxylic acid (1 mmol, 0.116 g) (Method D). White solid (89%). HPLC-MS: t_R = 2.77 min, purity 98%. ¹H NMR (400 MHz, DMSO- d_6) δ 8.05 (s, 1H), 7.87 (s, 1H), 7.40 (d, J = 6.7 Hz, 1H), 7.13

(d, $J = 8.8$ Hz, 1H), 6.81 (d, $J = 8.7$ Hz, 1H), 4.25 – 4.14 (m, 1H), 3.84 – 3.62 (m, 3H), 3.50 (dd, $J = 8.9, 3.2$ Hz, 1H), 2.96 (s, 4H), 2.13 (dt, $J = 15.4, 7.5$ Hz, 1H), 1.88 (d, $J = 13.4$ Hz, 4H), 1.77 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 154.77, 143.61, 143.59, 138.52, 135.12, 121.53, 119.54, 113.43, 111.50, 73.00, 66.18, 51.32, 50.16, 32.67, 24.05. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_3$ 360.1, found 360.1



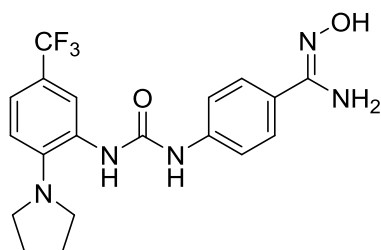
1-(5-Hydroxy-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (121).

A solution of 1-(5-(benzyloxy)-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (**7**) (1eq, 1 mmol, 0.381 g) in methanol (20 mL) was added with Pd/C (5-10 wt. %). H_2 (g) was bubbled through the solution at R.T. overnight with vigorous stirring and monitored by TLC. After completion of the reaction, the mixture was filtered and the solvent was removed to give the desired product without further purification. White solid (75%). HPLC-MS: $t_R = 0.54$ min, purity 95%. ^1H NMR (400 MHz, DMSO- d_6) δ 7.38 (s, 1H), 7.18 (d, $J = 8.6$ Hz, 1H), 6.86 (s, 1H), 6.63 (dd, $J = 8.5, 2.0$ Hz, 1H), 4.94 (s, 1H), 4.01 (t, $J = 6.9$ Hz, 4H), 3.68 – 3.62 (m, 3H), 3.52 (s, 1H), 2.91 – 2.84 (m, 4H), 2.04 (d, $J = 7.5$ Hz, 1H), 1.78 (d, $J = 8.4$ Hz, 2H), 1.71 (s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 161.23, 152.54, 148.97, 110.47, 109.92, 108.52, 103.87, 72.26, 66.29, 42.52, 32.67, 25.68, 23.07. MS (ESI $^+$): m/z [M+H] $^+$, calcd for $\text{C}_{15}\text{H}_{22}\text{N}_3\text{O}_3$ 292.2, found 292.2



1-(5-Amino-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (122).

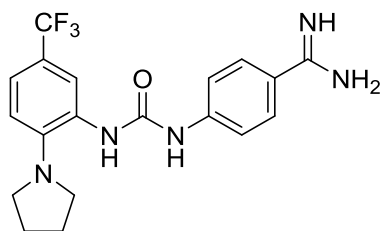
A solution of 1-(5-nitro-2-(pyrrolidin-1-yl)phenyl)-3-(tetrahydrofuran-3-yl)urea (**15**) (1 eq, 1 mmol, 0.321 g) in methanol (20 mL) was added with Pd/C (5-10 wt. %). H₂ (g) was bubbled through the solution at R.T. overnight with vigorous stirring and monitored by TLC. After completion of the reaction, the mixture was filtered and the solvent was removed to give the desired product without further purification. White solid (71%). HPLC-MS: t_R = 0.33 min, purity 90%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.79 (d, J = 13.0 Hz, 1H), 7.41 (dd, J = 14.5, 2.3 Hz, 1H), 7.34 – 7.23 (m, 1H), 6.85 (dd, J = 22.1, 8.7 Hz, 1H), 6.09 (dd, J = 8.4, 2.5 Hz, 1H), 5.17 (s, 1H), 4.67 (s, 1H), 4.17 (s, 1H), 3.74 (ddt, J = 16.4, 13.9, 7.9 Hz, 3H), 3.47 (dd, J = 8.8, 3.3 Hz, 1H), 3.00 – 2.69 (m, 4H), 2.11 (dt, J = 20.3, 7.5 Hz, 1H), 1.83 (d, J = 22.6 Hz, 4H), 1.70 (dd, J = 17.2, 12.4 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 154.96, 145.13, 136.02, 128.04, 120.34, 106.98, 73.05, 66.23, 52.67, 32.59, 30.46, 23.99. MS (ESI⁺): m/z [M+H]⁺, calcd for C₁₅H₂₃N₄O₂ 291.1, found 291.1



(Z)-N'-Hydroxy-4-(3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)benzamidine (124a). A solution of 1-(4-cyanophenyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (**128**) (1

eq, 1mmol, 0.374 g) in the mixture of ethanol and water (ratio: 3 mL:0.3 mL) was added with hydroxylamine hydrochloride (3.7 eq, 3.7 mmol, 0.257 g) and sodium bicarbonate (1.7 eq, 1.7 mmol, 0.180 g). The reaction was gently stirred for 5 min and then heated under reflux for 3 h. After completion of the reaction, the resulting mixture was filtered and concentrated *in vacuo*. White solid (78%). HPLC-MS: $t_R = 2.35$ min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.49 (s, 1H), 9.28 (s, 1H), 8.05 (s, 1H), 7.96 (s, 1H), 7.59 (t, $J = 6.2$ Hz, 2H), 7.47 (d, $J = 8.7$ Hz, 2H), 7.31 (d, $J = 8.2$ Hz, 1H), 7.09 (d, $J = 8.5$ Hz, 1H), 5.72 (s, 2H), 3.23 (s, 4H), 1.92 (d, $J = 12.5$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 152.84, 150.61, 144.07, 140.41, 135.75, 128.85, 127.83, 125.96, 121.75, 117.44, 117.25, 116.80, 104.21, 50.32, 24.62. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{21}\text{F}_3\text{N}_5\text{O}_2$ 408.2, found 408.2



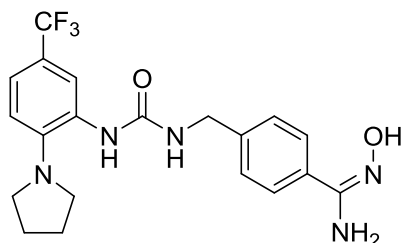
4-(3-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)benzamidinium

(124).

(Z)-N'-hydroxy-4-(3-(2-(pyrrolidin-1-yl)-5-

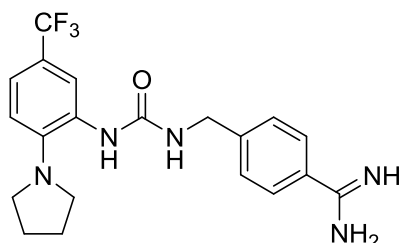
(trifluoromethyl)phenyl)ureido)benzamidinium **(124a)** (1 eq, 0.1 mmol, 0.041 g) was dissolved in acetic acid (1 mL). Acetic anhydride (1.1 eq, 0.11 mmol, 0.011 g) was added into the reaction at R.T. After 5 min, Pd/C (5-10 wt. %) was added into the mixture. H_2 (g) was bubbled through the solution at R.T. overnight with vigorous stirring and monitored by TLC. After completion of the reaction, the mixture was filtered and the solvent was removed to give the desired product. White solid (79%). HPLC-MS: $t_R = 2.31$ min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.88 (s, 1H), 7.81 – 7.58 (m, 7H), 7.52 (dd, $J = 23.2, 8.4$ Hz, 1H),

7.31 (d, $J = 8.3$ Hz, 1H), 6.94 (d, $J = 8.7$ Hz, 1H), 3.28 (s, 4H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 165.09, 153.63, 146.93, 145.68, 128.76, 126.67, 123.37, 121.94, 120.44, 118.42, 117.26, 115.77, 111.07, 49.93, 24.85. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{21}\text{F}_3\text{N}_5\text{O}$ 392.1, found 392.1



(E)-N'-Hydroxy-4-((3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)methyl)benzamidinium (125a). From 1-(4-cyanobenzyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (**129**) (1 mmol, 0.388 g) (The same procedure with compound **124a**). White solid (57%). HPLC-MS: $t_R = 2.28$ min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.58 (s, 1H), 7.93 (s, 1H), 7.83 (s, 1H), 7.63 (d, $J = 8.2$ Hz, 2H), 7.29 (d, $J = 8.1$ Hz, 2H), 7.26 – 7.15 (m, 2H), 7.03 (d, $J = 8.5$ Hz, 1H), 5.77 (s, 2H), 4.32 (t, $J = 7.8$ Hz, 2H), 3.16 (s, 4H), 1.88 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.66, 150.68, 141.05, 135.54, 133.52, 131.81, 129.91, 127.50, 126.84, 125.69, 125.38, 120.13, 116.78, 50.24, 42.65, 24.52. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{23}\text{F}_3\text{N}_5\text{O}_2$ 422.4, found 422.3



4-((3-(2-(Pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)methyl)benzamidinium (125). From (E)-N'-

hydroxy-4-((3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)methyl)benzamidinium (**125a**) (0.1 mmol, 0.042 g)

(The same procedure with compound **124**). White solid (71%). HPLC-MS: t_R =

2.25 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.04 (s, 1H), 7.97 –

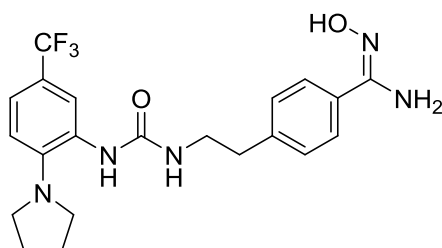
7.62 (m, 4H), 7.52 – 7.28 (m, 3H), 7.24 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.4 Hz,

1H), 4.36 (dd, J = 13.1, 5.9 Hz, 2H), 3.18 (s, 4H), 1.89 (s, 4H), 1.71 (s, 2H). ^{13}C

NMR (101 MHz, $\text{DMSO-}d_6$) δ 167.81, 155.60, 145.67, 143.85, 136.86, 130.51,

127.52, 127.31, 127.20, 124.97, 122.59, 120.23, 116.59, 108.83, 50.16, 42.53,

24.55. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{20}\text{H}_{23}\text{F}_3\text{N}_5\text{O}$ 406.2, found 406.2



N'-Hydroxy-4-((3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)ethyl)benzamidinium (126-a**).** From 1-(4-

cyanophenethyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea (**130**) (1

mmol, 0.402 g) (The same procedure with compound **124a**). White solid (69%).

HPLC-MS: t_R = 2.28 min, purity 91%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.55

(s, 1H), 7.89 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 5.9 Hz, 1H), 7.59 (t, J = 11.0 Hz,

2H), 7.22 (dd, J = 8.2, 3.6 Hz, 3H), 7.00 (d, J = 8.5 Hz, 1H), 6.70 (t, J = 5.6 Hz,

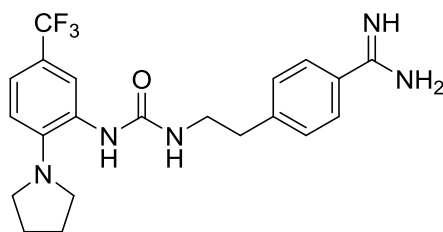
1H), 5.75 (s, 2H), 3.36 (s, 2H), 3.13 (s, 4H), 2.76 (t, J = 6.9 Hz, 2H), 1.88 (s,

4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.43, 150.70, 145.24, 140.40, 131.23,

129.95, 128.49, 127.53, 126.19, 125.31, 123.50, 120.06, 116.56, 50.14, 35.65,

35.59, 24.49. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{25}\text{F}_3\text{N}_5\text{O}_2$ 436.2, found

436.2



4-(2-(3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)ethyl)benzamidinium (126). N'-hydroxy-4-(2-

(3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)ethyl)benzamidinium

(126-a) (0.1 mmol, 0.044 g) (The same procedure as compound **124**). White

solid (43%). HPLC-MS: t_R = 2.21 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.19 – 7.53 (m, 7H), 7.47 (dd, J = 13.3, 5.2 Hz, 2H), 7.31 (d, J = 7.9 Hz,

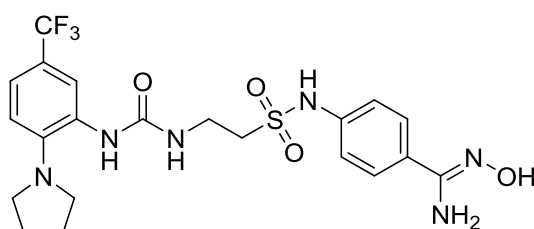
1H), 7.25 – 7.16 (m, 1H), 6.99 (d, J = 8.7 Hz, 1H), 3.39 (s, 2H), 3.27 – 3.07 (m,

4H), 2.80 (dd, J = 17.6, 11.2 Hz, 2H), 1.87 (t, J = 27.0 Hz, 4H). ^{13}C NMR (101

MHz, $\text{DMSO-}d_6$) δ 164.53, 156.06, 148.28, 146.15, 135.04, 129.76, 129.05,

128.10, 127.28, 124.30, 118.44, 116.27, 111.49, 50.54, 43.29, 26.18, 24.96. MS

(ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{25}\text{F}_3\text{N}_5\text{O}$ 420.2, found 420.2



(Z)-N'-Hydroxy-4-((2-(3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)ureido)ethyl)sulfonamido)benzamidinium (127a).

From N-(4-cyanophenyl)-2-(3-(2-(pyrrolidin-1-yl)-5-

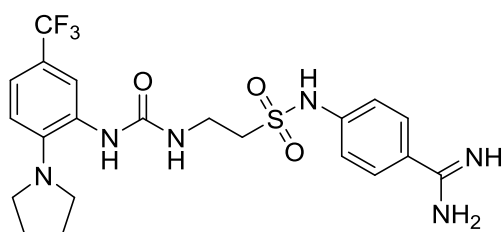
(trifluoromethyl)phenyl)ureido)ethane-1-sulfonamide (**131**). (1 mmol, 0.481 g)

(The same procedure as compound **124a**). White solid (63%). HPLC-MS: t_R =

2.28 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.02 (s, 1H), 9.57 (s,

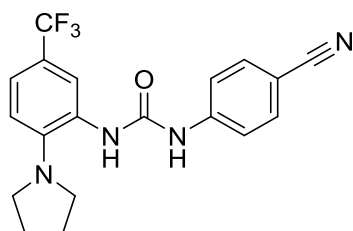
1H), 7.90 (s, 1H), 7.78 (s, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.22 (dd, J = 13.8, 8.6

Hz, 3H), 6.98 (d, $J = 8.5$ Hz, 1H), 6.80 (t, $J = 5.6$ Hz, 1H), 5.74 (s, 2H), 3.48 (dd, $J = 12.6, 6.3$ Hz, 2H), 3.27 (t, $J = 6.6$ Hz, 2H), 3.15 (s, 4H), 1.86 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.58, 150.38, 145.79, 138.64, 128.95, 128.80, 126.44, 123.50, 121.13, 119.57, 119.16, 118.15, 116.42, 51.12, 50.10, 34.35, 24.62. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{26}\text{F}_3\text{N}_6\text{O}_4\text{S}$ 515.4, found 515.3



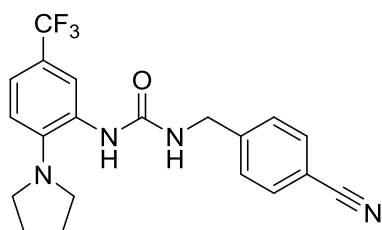
4-((2-(3-(2-(Pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)ethyl)sulfonamido)benzamidinium (127).

From (Z)-N'-hydroxy-4-((2-(3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)ethyl)sulfonamido)benzamidinium (127a) (0.1 mmol, 0.052 g) (The same procedure as compound 124). White solid (43%). HPLC-MS: $t_R = 2.26$ min, purity 95%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.51 (s, 2H), 8.12 – 7.41 (m, 6H), 7.22 (d, $J = 7.5$ Hz, 1H), 6.92 (ddd, $J = 100.7, 44.1, 7.8$ Hz, 4H), 4.18 (d, $J = 6.6$ Hz, 2H), 3.20 – 2.95 (m, 6H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 164.66, 161.21, 155.65, 148.64, 145.78, 128.99, 126.85, 118.89, 118.76, 118.36, 118.04, 116.31, 111.09, 50.06, 42.88, 33.17, 25.76. MS (ESI $^+$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{26}\text{F}_3\text{N}_6\text{O}_3\text{S}$ 499.4, found 499.3

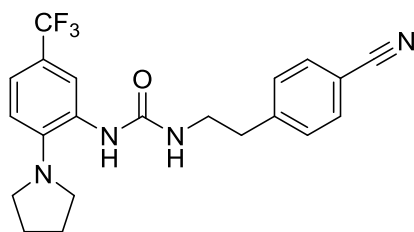


1-(4-Cyanophenyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea

(128). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 4-aminobenzonitrile (0.75 mmol, 0.089 g) (Method C). White solid (79%). HPLC-MS: t_R = 3.31 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.61 (s, 1H), 8.16 (s, 1H), 7.86 (s, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 7.05 (d, J = 8.6 Hz, 1H), 3.24 (s, 4H), 1.91 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 152.67, 146.31, 144.31, 133.47, 133.33, 127.64, 121.54, 119.34, 119.25, 118.01, 116.62, 113.48, 103.26, 50.26, 24.70. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{19}\text{H}_{18}\text{F}_3\text{N}_4\text{O}$ 375.1, found 374.9

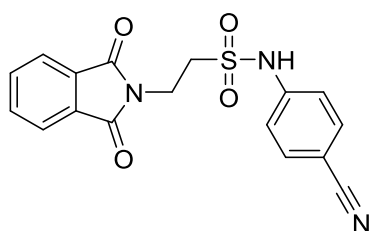
**1-(4-Cyanobenzyl)-3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)urea**

(129). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 4-(aminomethyl)benzonitrile (0.75 mmol, 0.099 g) (Method C). White solid (71%). HPLC-MS: t_R = 2.94 min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.91 (d, J = 7.6 Hz, 1H), 7.81 (s, 2H), 7.67 (d, J = 8.4 Hz, 1H), 7.45 (s, 1H), 7.28 (dd, J = 13.0, 7.1 Hz, 2H), 7.04 (d, J = 8.2 Hz, 1H), 6.75 (t, J = 6.6 Hz, 1H), 3.18 (s, 4H), 3.02 (t, J = 7.6 Hz, 2H), 1.90 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 164.54, 157.82, 156.19, 146.98, 132.74, 128.32, 120.93, 119.40, 118.44, 117.12, 116.23, 111.50, 109.89, 50.64, 43.30, 25.00. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{22}\text{H}_{20}\text{F}_3\text{N}_4\text{O}$ 389.2, found 389.2



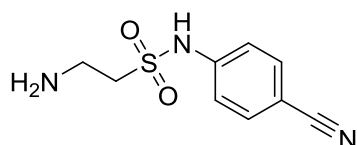
1-(4-Cyanophenethyl)-3-(2-(pyrrolidin-1-yl)-5-

(trifluoromethyl)phenyl)urea (130). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 4-(2-aminoethyl)benzonitrile (0.75 mmol, 0.110 g) (Method C). White solid (91%). HPLC-MS: t_R = 2.95 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.84 (s, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.66 (s, 1H), 7.44 (d, J = 7.9 Hz, 2H), 7.21 (d, J = 8.2 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.66 (s, 1H), 3.38 (d, J = 6.5 Hz, 2H), 3.11 (s, 4H), 2.85 (t, J = 6.6 Hz, 2H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.43, 145.80, 145.35, 132.13, 129.97, 129.68, 126.17, 123.48, 120.08, 119.66, 119.00, 116.49, 108.95, 59.77, 50.10, 35.89, 24.49. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{22}\text{F}_3\text{N}_4\text{O}$ 403.1, found 403.1

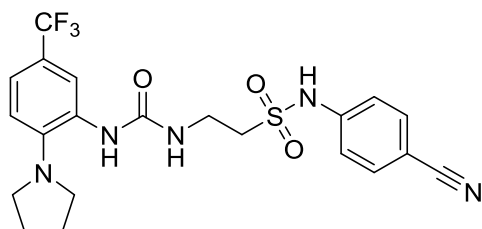


N-(4-Cyanophenyl)-2-(1, 3-dioxoisindolin-2-yl)ethane-1-sulfonamide (131a). A solution of 4-aminobenzonitrile (5 eq, 2.5 mmol, 0.295 g) in THF (5 mL) was treated with sodium hydride (60% in mineral oil, 5 eq, 2.5 mmol, 0.100 g) at 0 °C. After 10 min, 2-(1, 3-dioxoisindolin-2-yl)ethane-1-sulfonyl chloride (1 eq, 0.5 mmol, 0.137 g) was added in the reaction. The reaction was allowed to warm up to R.T and stirred for 48 h. After completion of the reaction, the resulting mixture was concentrated and purified by flash chromatography using

petroleum ether and ethyl acetate. White solid (32%). HPLC-MS: t_R = 2.63 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.70 (s, 1H), 7.84 (s, 4H), 7.74 (d, J = 8.7 Hz, 2H), 7.27 (d, J = 8.7 Hz, 2H), 3.97 (t, J = 6.9 Hz, 2H), 3.65 (t, J = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 167.33, 142.39, 134.50, 133.78, 131.58, 123.12, 118.83, 118.39, 105.29, 48.56, 32.18. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{17}\text{H}_{14}\text{N}_3\text{O}_4\text{S}$ 356.1, found 356.1

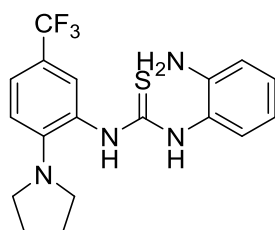


2-Amino-N-(4-cyanophenyl)ethane-1-sulfonamide (131b). A suspension of N-(4-cyanophenyl)-2-(1, 3-dioxoisindolin-2-yl)ethane-1-sulfonamide (**131a**) (1 eq, 2 mmol, 0.710 g) was heated under reflux in ethanol (5 mL), followed by the addition of hydrazine hydrate (64% in water, 1.1 eq, 2.2 mmol, 0.1 mL). After 3 h, the crude product was obtained by filtration. Brown solid (65%). HPLC-MS: t_R = 0.56 min, purity 88%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.07 (dd, J = 5.9, 3.3 Hz, 1H), 7.86 (dd, J = 5.9, 3.3 Hz, 1H), 5.87 (s, 2H), 3.23 – 2.87 (m, 2H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 154.95, 132.46, 127.42, 125.20, 119.79, 49.84, 35.87. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_9\text{H}_{12}\text{N}_3\text{O}_2\text{S}$ 226.1, found 226.1



N-(4-Cyanophenyl)-2-(3-(2-(pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ureido)ethane-1-sulfonamide (131). From 2-(pyrrolidin-1-yl)-6-(trifluoromethyl)aniline (**2b**) (0.5 mmol, 0.115 g) and 2-

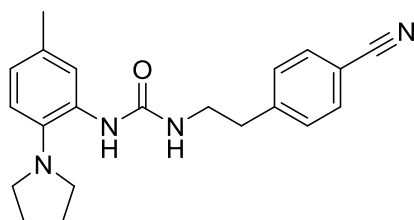
amino-N-(4-cyanophenyl)ethane-1-sulfonamide (**131b**) (0.75 mmol, 0.169 g) (Method C). White solid (75%). HPLC-MS: t_R = 2.87 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.56 (s, 1H), 7.88 (s, 1H), 7.82 – 7.66 (m, 3H), 7.32 (d, J = 8.7 Hz, 2H), 7.23 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.77 (t, J = 5.6 Hz, 1H), 3.53 – 3.45 (m, 2H), 3.40 (t, J = 6.7 Hz, 2H), 3.15 (s, 4H), 1.87 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.51, 145.37, 133.72, 132.25, 129.72, 128.81, 127.12, 123.52, 120.65, 119.58, 118.35, 116.40, 109.61, 51.55, 50.07, 34.41, 24.59. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{23}\text{F}_3\text{N}_5\text{O}_3\text{S}$ 482.1, found 482.1



1-(2-Aminophenyl)-3-(2-(pyrrolidin-1-yl)-5-

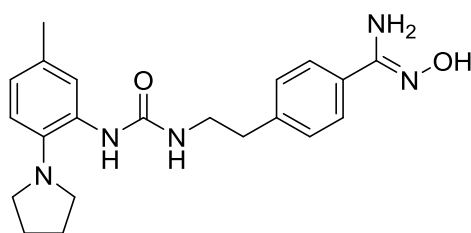
(trifluoromethyl)phenyl)thiourea (132). A stirred solution of 1,1'-thiocarbonyldiimidazole (1.5 eq, 0.75 mmol, 0.134 g) in acetonitrile (2 mL) at 0 °C was added with a solution of 2-(pyrrolidin-1-yl)-5-(trifluoromethyl) aniline (1.0 eq, 0.5 mmol, 0.115 g) in acetonitrile (1 mL) dropwise over 10 min. The reaction was stirred for 15 mins and warmed up to room temperature. After 3 h, 1, 2- phenylenediamine (1.5 eq, 0.75 mmol, 0.081 g) was added and the reaction was heated to 50 °C for 3 h. The reaction was stirred at ambient temperature for 16 h. After completion of reaction, the mixture was purified by chromatography (petroleum ether and ethyl acetate) and concentrated to afford the product. White solid (69%). HPLC-MS: t_R = 2.90 min, purity 100%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.97 (d, J = 40.6 Hz, 2H), 7.44 (s, 1H), 7.36 (d, J = 8.8 Hz, 1H), 6.99 (dd, J = 11.6, 7.1 Hz, 3H), 6.82 (d, J = 8.7 Hz, 1H), 6.74 (d, J = 7.9 Hz,

1H), 6.56 (t, $J = 7.5$ Hz, 1H), 4.87 (s, 2H), 3.38 (s, 4H), 1.86 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 181.32, 144.49, 132.27, 128.82, 127.60, 126.29, 124.24, 123.61, 122.36, 120.58, 116.36, 115.76, 115.15, 109.52, 49.46, 25.19. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{20}\text{F}_3\text{N}_4\text{S}$ 381.1, found 381.1



1-(4-Cyanophenethyl)-3-(5-methyl-2-(pyrrolidin-1-yl)phenyl)urea (133).

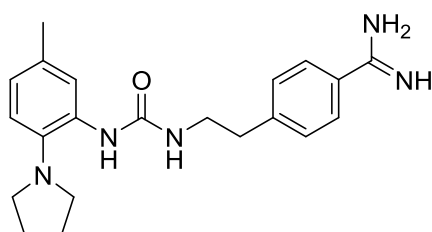
From 5-methyl-2-(pyrrolidin-1-yl)aniline (**10b**) (0.5 mmol, 0.088 g) and 4-(2-aminoethyl)benzonitrile (0.75 mmol, 0.110 g) (Method C). White solid (73%). HPLC-MS: $t_R = 2.26$ min, purity 90%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.76 (d, $J = 8.1$ Hz, 2H), 7.61 (s, 1H), 7.54 (d, $J = 6.2$ Hz, 1H), 7.44 (d, $J = 8.1$ Hz, 2H), 6.91 – 6.81 (m, 2H), 6.68 (d, $J = 8.0$ Hz, 1H), 3.34 (d, $J = 6.6$ Hz, 2H), 2.91 (d, $J = 14.6$ Hz, 4H), 2.84 (t, $J = 6.8$ Hz, 2H), 2.18 (s, 3H), 1.84 (d, $J = 6.2$ Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 155.36, 145.93, 137.95, 132.76, 132.18, 130.86, 129.98, 122.53, 121.48, 119.05, 117.78, 108.93, 51.08, 35.95, 24.00, 20.78. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{25}\text{N}_4\text{O}$ 349.2, found 349.2



(Z)-N'-Hydroxy-4-(2-(3-(5-methyl-2-(pyrrolidin-1-

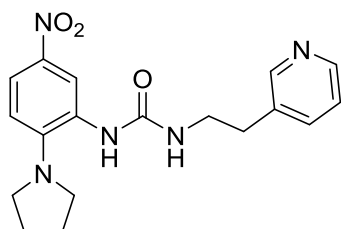
yl)phenyl)ureido)ethyl)benzamidinium (134). From 1-(4-cyanophenethyl)-3-(5-methyl-2-(pyrrolidin-1-yl)phenyl)urea (**133**) (1 mmol, 0.349 g) (the same procedure as compound **124**). White solid (64%). HPLC-MS: $t_R = 0.76$ min,

purity 90%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.56 (s, 1H), 7.66 (s, 1H), 7.60 (d, J = 8.4 Hz, 3H), 7.22 (d, J = 8.1 Hz, 2H), 6.88 (t, J = 7.9 Hz, 2H), 6.67 (d, J = 8.0 Hz, 1H), 5.75 (s, 2H), 3.34 – 3.27 (m, 2H), 2.91 (s, 4H), 2.75 (t, J = 7.0 Hz, 2H), 2.19 (s, 3H), 1.86 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 155.37, 150.74, 140.54, 137.84, 132.97, 131.23, 130.93, 128.50, 125.34, 122.40, 121.31, 117.82, 51.11, 40.58, 35.68, 23.99, 20.79. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{28}\text{N}_5\text{O}_2$ 382.2, found 382.2



4-(2-(3-(5-Methyl-2-(pyrrolidin-1-yl)phenyl)ureido)ethyl)benzamidinium

(135). From (Z)-N'-hydroxy-4-(2-(3-(5-methyl-2-(pyrrolidin-1-yl)phenyl)ureido)ethyl)benzamidinium (**134**) (0.5 mmol, 0.191 g) (the same procedure as compound **124**). White solid (64%). HPLC-MS: t_R = 2.97 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.74 (t, J = 7.8 Hz, 3H), 7.65 (s, 1H), 7.61 (s, 1H), 7.42 (dd, J = 18.7, 7.9 Hz, 3H), 6.95 (d, J = 5.5 Hz, 1H), 6.89 (d, J = 8.1 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 3.35 (s, 2H), 2.91 (s, 4H), 2.85 (t, J = 6.9 Hz, 2H), 2.18 (s, 3H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 165.78, 155.39, 145.69, 137.85, 132.92, 130.93, 129.31, 127.56, 127.03, 122.45, 121.29, 117.86, 51.12, 46.62, 35.78, 24.01, 20.79. MS (ESI^+): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}$ 366.4, found 366.3



1-(5-Nitro-2-(pyrrolidin-1-yl)phenyl)-3-(2-(pyridin-3-yl)ethyl)urea (136).

From 5-nitro-2-(pyrrolidin-1-yl)aniline (**15b**) (1.5 mmol, 0.311 g) and 3-(pyridin-3-yl)propanoic acid (1 mmol, 0.151 g) (Method D). White solid (79%). HPLC-MS: t_R = 1.97 min, purity 95%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.54 (s, 1H), 8.51 (d, J = 3.8 Hz, 1H), 7.99 (d, J = 2.3 Hz, 1H), 7.87 (s, 2H), 7.85 (s, 1H), 7.48 (dd, J = 7.6, 5.1 Hz, 1H), 6.78 (d, J = 9.3 Hz, 1H), 6.60 (d, J = 4.8 Hz, 1H), 3.40 (d, J = 6.5 Hz, 2H), 2.81 (t, J = 6.7 Hz, 2H), 1.86 (s, 4H), 1.28 (dd, J = 12.5, 6.7 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 156.52, 150.41, 148.48, 145.95, 139.20, 137.19, 136.62, 125.25, 124.60, 122.17, 114.71, 63.73, 50.50, 42.09, 33.36, 25.45. MS (ESI⁺): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{22}\text{N}_5\text{O}_3$ 356.2, found 356.2

5.2. Biology

Equipment: Polypropylene centrifuge tubes (15 mL and 50 mL), 96 well polypropylene plates and white solid polystyrene flat bottomed 384 well plates were purchased from Greiner brand. Plate reader was multilabel reader from Perkin Elmer including the software EnVision 2014. Single channel Gilson pipette and 8 channel Matrix pipettes were used in biological experiment.

Material: PBS Tablets (phosphate buffered saline) was purchased from Oxoid. Tween 20 and anhydrous DMSO- d_6 were obtained from Sigma Aldrich. Human α -FXIIa and β -FXIIa were purchased from enzyme research laboratories. FXIIa substrate (Pro-Phe-Arg-7AMC) was purchased from Sigma Aldrich. PPACK dihydrochloride (D-Phe-Pro-Arg-CMK) was obtained from BioScience.

5.2.1. Procedure of FXIIa biological assay

5.2.1.1. Preparation of stock solution

Preparation of buffer: 10 tablets of PBS were dissolved with 100 mL distilled water. Once PBS tablets were dissolved, all 100 mL of 10 times PBS solution was made to 1 L with distilled water. Buffer was stored at room temperature and used within a week.

Preparation of 1% (weight/volume, w/v) Tween 20 PBS buffer: Tween 20 (0.100 g) was added to PBS (9.9 mL) in a centrifuge tube (15 mL). The tube was then rolled for approximately 20 min. 1% (w/v) Tween 20 PBS buffer was stored at 4°C and used within 2 weeks.

5.2.1.2. Preparation of enzymes

Enzymes buffer (0.03% (v/v) Tween 20 PBS): Tween 20 PBS (1% (w/v), 1.5 mL) and the solution of PBS (48.5 mL) were added into a centrifuge tube (50 mL). This enzyme buffer was prepared daily.

α -FXIIa: Lyophilised α -FXIIa (0.5 mg/vial) was dissolved in enzyme buffer (0.03% (v/v) Tween 20 PBS, 625 μ L) by rolling approximately 20 min to give a solution of α -FXIIa (0.8 mg/mL). α -FXIIa enzymes were then aliquoted in 24 μ L volumes and stored at -80°C . When α -FXIIa is ready to use, 24 μ L of α -FXIIa (0.8 mg/mL) was thawed and then pulsed in a microfuge. After addition of 0.03% (v/v) Tween 20 PBS Buffer (16 mL), α -FXIIa was prepared at a concentration of 1.2 ng/ μ L.

β -FXIIa: Lyophilised β -FXIIa (0.1 mg/vial) was dissolved in enzyme buffer (0.03% (v/v) Tween 20 PBS, 250 μ L) by rolling approximately 20 min to give a solution of β -FXIIa (0.4 mg/mL). β -FXIIa enzymes were then aliquoted in 18 μ L volumes and stored at -80°C . When β -FXIIa was ready to use, 18 μ L of β -FXIIa (0.4 mg/mL) was thawed and pulsed in a microfuge. After addition of 0.03% (v/w) Tween 20 PBS Buffer (16 mL), β -FXIIa was prepared at a concentration of 0.45 ng/ μ L.

5.2.1.3. Preparation of substrate

Substrate (25 mg) was dissolved in DMSO- d_6 (2.17 mL), which lead to substrate at a concentration of 20 mM. Substrate was then aliquoted in 50 μ L volumes and stored at -20°C . When substrate was ready to use, 50 μ L of substrate (20 mM) was thawed and pulsed in a microfuge. After addition of PBS buffer (33.274 mL, no Tween), substrate was prepared at a concentration of 30 μ M.

5.2.1.4. Standard control of PPACK protease inhibitor

PPACK (5 mg) was dissolved in DMSO- d_6 (954 μ L), which lead to PPACK at a concentration of 10 mM. PPACK was then aliquoted in 20 μ L volumes and stored at -20°C . When PPACK was ready to use, 10 μ L of PPACK was diluted with 90 μ L of DMSO- d_6 and lead to PPACK at a concentration of 1mM.

5.2.1.5. Preparation of test compounds

Step 1: Dissolution of test compounds: Test compounds were dissolved in DMSO- d_6 and was made up to a concentration of 10 mM.

Step 2: Serial dilution in DMSO- d_6 : For a serial dilution in DMSO- d_6 , 80 μ L of DMSO- d_6 was multiple-dispensed into a 96 well polypropylene plate (wells A7 to G12). 50 μ L of test compounds (10 mM) was added to plate (wells H8 to H12) and then 50 μ L of PPACK (1 mM) was added to well H7 in plate. 37 μ L of stock was transferred from wells H7/H12 to the 80 μ L DMSO- d_6 in G7/G12 and the solution was mixed by pipetting. The process was repeated from G7/G12 to F7/F12, F7/F12 to E7/E12. This gave curves with a top working concentration of 300 μ M. Then process continued and repeated from E7/E12 to D7/D12, D7/D12 to C7/C12, C7/C12 to B7/B12 and B7/B12 to A7/A12.

Step 3: Aqueous PBS dilution: After serial dilution in DMSO- d_6 , 6 μ L of each curves was transferred into an empty polypropylene plate followed by addition of PBS (194 μ L, no Tween). This generated an 8 point half log serial dilution and mixed on plate shaker.

5.2.1.6. Combination of substrate, test compounds and enzyme

9 times substrate (10 μ L, 30 μ M) was added into a White solid white polystyrene flat bottomed 384 well plate. Inhibitor curves (10 μ L) from 5.2.5.1 were added in triplicate to substrate. Enzyme (10 μ L) from 5.2.1.2 was then added in triplicate last.

5.2.1.7. IC₅₀ screen on plate reader

The 384 well plate was placed on a plate shaker for 30 seconds before reading for the initial time point (T1) at 380/460 nm with a triplicate read and incubated for 2.5 h. In the end, read of plate was finished at T-end with another triplicate.

5.2.2. Procedure of FXa biological assay

The procedure of FXa bio-assay was similar to FXIIa enzyme assay. The only difference is the preparation of substrate, enzyme and incubation time.

5.2.2.1. Preparation of FXa

FXa (0.8 mg/vial) was dissolved in enzyme buffer (0.03% (v/v) Tween 20 PBS, 800 μ L) by rolling approximately 20 min, which lead to FXa at a concentration of 1 mg/mL. FXa enzymes were then aliquoted in 20 μ L volumes and stored at -80°C. When FXa was ready to use, 10 μ L of FXa (1 mg/mL) was thawed and pulsed in a microfuge. After addition of Tween 20 PBS Buffer (0.03% (v/w), 14.48 mL), the concentration of FXa was made to 0.69 ng/ μ L.

5.2.2.2. Preparation of substrate

Substrate (5 mg) was dissolved in DMSO- d_6 (342 μ L) to give a concentration at 20 mM. Then the substrate was aliquoted to volumes (20 μ L) and stored at -20°C. When the substrate was ready to use, 20 μ L of substrate (20 mM) was thawed. After addition of PBS buffer (13.31 mL, no Tween), the concentration of stock was 30 μ M.

5.2.2.3. IC₅₀ screen on plate reader

The 384 well plate was placed on a plate shaker for 30 seconds before reading for the initial time point (T1) at 380/460 nm with a triplicate read and incubated for 1 h. In the end, read of plate was finished at T-end with another triplicate.

6. Appendix

6.1. Procedure of FXIa biological assay

The procedure of FXIa bio-assay was similar to FXIIa enzyme assay. The only difference is the preparation of substrate, enzyme and incubation time.

6.1.1. Preparation of FXIa

FXIa (10 μ L, 0.6 mg/mL) was dissolved in enzyme buffer (0.03% (v/v) Tween 20 PBS, 625 μ L) by rolling approximately 20 min to give a solution of FXIa (0.12 mg/mL). FXIa enzymes were then aliquoted in 10 μ L volumes and stored at -80°C. When FXIa was ready to use, 10 μ L of FXIa (0.12 mg/mL) was thawed and then pulsed in a microfuge. After addition of 0.03% (v/v) Tween 20 PBS Buffer (11.99 mL), FXIa was prepared at a concentration of 0.10 ng/ μ L.

6.1.2. Preparation of substrate

Substrate (25 mg) was dissolved in PBS (2.32 mL), which lead to substrate at a concentration of 20 mM. Substrate was then aliquoted in 100 μ L volumes and stored at -20°C. When substrate was ready to use, 100 μ L of substrate (20 mM) was thawed and pulsed in a microfuge. After addition of PBS buffer (11.01 mL, no Tween), substrate was prepared at a concentration of 180 μ M.

6.1.3. IC₅₀ screen on plate reader

The 384 well plate was placed on a plate shaker for 30 seconds before reading for the initial time point (T1) at 405 nm with a triplicate read and incubated for 1 h. In the end, read of plate was finished at T-end with another triplicate.

6.2. Procedure of FIIa biological assay

The procedure of FIIa bio-assay was similar to FXIIa enzyme assay. The only difference is the preparation of substrate, enzyme and incubation time.

6.2.1. Preparation of FIIa

FIIa (10 μ L, 1.0 mg/mL I) was dissolved in enzyme buffer (0.03% (v/v) Tween 20 PBS, 990 μ L) by rolling approximately 20 min to give a solution of FIIa (0.01 mg/mL). FIIa enzymes were then aliquoted in 10 μ L volumes and stored at -80°C. When FIIa was ready to use, 10 μ L of factor Ila (0.01 mg/mL) was thawed and then pulsed in a microfuge. After addition of 0.03% (v/v) Tween 20 PBS Buffer (16.63 mL), FIIa was prepared at a concentration of 0.00601 ng/ μ L.

6.2.2. Preparation of substrate

Substrate (5 mg) was dissolved in DMSO- d_6 (0.367 mL), which lead to substrate at a concentration of 20 mM. Substrate was then aliquoted in 15 μ L volumes and stored at -20°C. When substrate was ready to use, 15 μ L of substrate (20 mM) was thawed and pulsed in a microfuge. After addition of PBS buffer (9.99 mL, no Tween), substrate was prepared at a concentration of 30 μ M.

6.2.3. IC₅₀ screen on plate reader

The 384 well plate was placed on a plate shaker for 30 seconds before reading for the initial time point (T1) at 400/405 nm with a triplicate read and incubated for 1 h. In the end, read of plate was finished at T-end with another triplicate.

6.3. Procedure of FVIIa biological assay

The procedure of FVIIa bio-assay was similar to FXIIa enzyme assay. The only difference is the preparation of enzyme, substrate and incubation time.

6.3.1. Preparation of FVIIa

FVIIa (0.1 mg/mL l) was dissolved in enzyme buffer (0.03% (v/v) Tween 20 PBS, 250 μ L) by rolling approximately 20 min to give a solution of FVIIa (0.4 mg/mL). FVIIa enzymes were then aliquoted in 20 μ L volumes and stored at -80°C. When FVIIa was ready to use, 20 μ L of FVIIa (0.04 mg/mL) was thawed and then pulsed in a microfuge. After addition of 0.03% (v/v) Tween 20 PBS buffer (5.313 mL), FVIIa was prepared at a concentration of 1.5 ng/ μ L.

6.3.2. Preparation of substrate

6.3.2.1. Preparation of 1.5 mM (v/v) calcium buffer

Calcium chloride (0.111g) was added to distilled water (10 mL) in a centrifuge tube to make up calcium chloride solution at a concentration of 100 mM. 150 μ L of calcium solution (100 mM) was added to PBS (9.85 mL) in a centrifuge tube. The tube was then rolled for approximately 20 min and used it immediately with enzyme stock.

6.3.2.2. Preparation of substrate

Substrate (5 mg) was dissolved in DMSO- d_6 (356.5 μ L), which lead to substrate at a concentration of 21.7 mM. Substrate was then aliquoted in 37.65 μ L volumes and stored at -20°C. When substrate was ready to use, 37.65 μ L of substrate (21.7 mM) was thawed and pulsed in a microfuge. After addition of PBS calcium buffer (4.5 mL, 1.5 mM Ca), substrate was prepared at a concentration of 180.1 μ M.

6.3.3. IC₅₀ screen on plate reader

The 384 well plate was placed on a plate shaker for 30 seconds before reading for the initial time point (T1) at 400/405 nm with a triplicate read and incubated for 2.5 h. In the end, read of plate was finished at T-end with another triplicate.

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