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Original article

Synthesis and structure–activity relationship of potent, selective and orally active anthranilamide-based factor Xa inhibitors: Application of weakly basic sulfoximine group as novel S4 binding element☆

Vrajesh Pandya [a,b,*], Mukul Jain [a], Ganes Chakrabarti [a], Hitesh Soni [a], Bhavesh Parmar [a], Balaji Chaugule [a], Jigar Patel [a], Tushar Jarag [a], Jignesh Joshi [a], Nirav Joshi [a], Akshyaya Rath [a], Vishal Unadkat [a], Bhavesh Sharma [a], Haresh Ajani [a], Jeevan Kumar [a], Kalapatapu V.V.M. Sairam [a], Harilal Patel [a], Pankaj Patel [a]

[a] Zydus Research Centre, Sarkhej-Bavla N.H. 8A, Moraiya, Ahmedabad 382210, India
[b] Department of Chemistry, Faculty of Science, M.S. University of Baroda, Vadodara 390002, India

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ABSTRACT

A novel series of potent and efficacious factor Xa inhibitors which possesses sulfoximine moiety as novel S4 binding element in anthranilamide chemotype has been identified. Lead optimization at this novel P4 group led to many potent factor Xa inhibitors with excellent anticoagulant activity in human plasma. Selected compounds were dosed orally in rats and checked for their *ex vivo* prothrombin time prolonging activity, which resulted in identification of compound 5-chloro-*N*-(5-chloropyridin-2-yl)-2-(4-(*N*-(2-(diethylamino)acetyl)-*S*-methylsulfonimidoyl)benzamido)benzamide (**18f**). The detailed pharmacokinetic evaluation and subsequent metabolism study of **18f** suggested the presence of an active metabolite. The compound **18f** and its active metabolite **18b** demonstrated excellent *in vivo* efficacy in both arterial and venous thrombosis model in rats and were found to be highly selective against related serine proteases. Based on this promising profile, compound **18f** was selected for further evaluation.

1. Introduction

Thrombosis is a pathological process and it occurs when the functioning of hemostatic pathway exceeds the normal regulatory counterbalance by fibrinolytic pathway or other anticoagulant factors, which are supposed to restrict and localize thrombus formation to the area of injury. Occlusions of coronary and cerebral arteries are associated with platelet activation and coagulation triggered by the rupture of atherosclerotic plaque, resulting in myocardial infarction and ischemic stroke [1]. There is a significant association between myocardial ischemia commonly known as ACS (Acute Coronary Syndrome) and AF (Atrial Fibrillation), one form of cardiac arrhythmia and patients with AF are at higher risk of cerebral stroke [2].

Venous thrombotic occlusions are associated with DVT (Deep Vein Thrombosis) and PE (Pulmonary Embolism), which comprises VTE (Venous Thromboembolism). It affects more than one million individuals in the European union each year and is responsible for at least 500,000 deaths [3]. Platelets play a major role in arterial thrombosis which is a leading cause of MI (Myocardial Infarction) and stroke, and antiplatelet therapy improves survival of patients with this arterial thrombotic disorders [4]. Anticoagulants hold promise in both arterial and venous thrombotic disorders as they prevent fibrin formation required for stable clot formation. The drawbacks of warfarin, an oral anticoagulant has provoked extensive research for identification of safe and orally bioavailable anticoagulant which can fulfill the high unmet need of oral anticoagulants.

The discovery process for oral anticoagulant has been dominated by directly targeting two specific protease enzymes, coagulation factor Xa (FXa) and thrombin (FIIa). Due to its unique position in coagulation cascade and limited role outside, FXa has always been considered to have an edge over thrombin in terms of safety in bleeding [5,6]. However, race to be the first selective oral anticoagulant in the market won by Dabigatran etexilate [7], a thrombin inhibitor. Enormous research activity in developing novel orally bioavailable Factor Xa inhibitor resulted in several clinical candidates, which are listed in Fig. 1.

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* Corresponding author. Zydus Research Centre, Sarkhej-Bavla N.H. 8A, Moraiya, Ahmedabad 382210, India. Tel.: +91 2717 250801; fax: +91 2717 250606.
E-mail address: vrajeshpandya@zyduscadila.com (V. Pandya).

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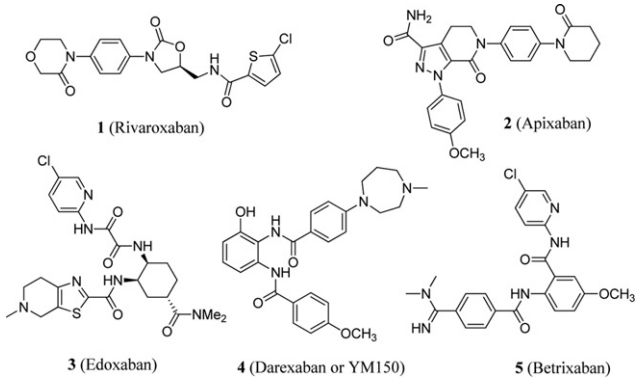


Fig. 1. Oral factor Xa inhibitors.

Rivaroxaban (**1**) is the first oral FXa inhibitor to be approved in Europe and US for the prevention of VTE in patients who have had elective total hip or knee replacement surgery [8]. Recently, it has also been approved by US for stroke prophylaxis in patients with non-valvular AF. Clinical trial for secondary prevention of ACS is in progress. Apixaban (**2**) is approved in Europe for the prevention of VTE in patients who have had elective total hip or knee replacement surgery [9]. Edoxaban (**3**) is approved in Japan for the prevention of VTE after major orthopedic surgery [10]. Recently, Darexaban (**4**), which was in Phase III for the prevention of VTE after major orthopedic surgery is discontinued by Astellas due to commercial reason [11]. Betrixaban (**5**) is in Phase II for the treatment of DVT and PE, and for the prevention of stroke related to chronic AF [12]. Letaxaban which was in Phase II for VTE prevention in patients underwent knee replacement surgery is discontinued by Takeda [13].

These newly discovered FXa inhibitors are devoid of a highly basic amidine group (except Betrixaban), a known culprit for poor oral bioavailibility and was earlier used successfully as both S1 and S4 ligand [14]. Vicinal diamide based FXa inhibitors (Anthranilamide and cis-diamine based inhibitors) have been explored by many research groups as they provide U or V shape of the molecule, which is ideal for FXa binding [15–20]. Three molecules based on vicinal diamide scaffold are already in clinical trials. Edoxaban (**3**), which is recently approved in Japan has the 5-chloro-2-aminopyridine group as S1 ligand and the methyltetrahydrothiazolo[5,4-*c*]pyridine group as S4 ligand [21]. Betrixaban (**5**) has the 5-chloro-2-aminopyridine group as S1 ligand, but possesses classical amidine group as S4 ligand [22]. Darexaban (**4**) contains the 4-methoxyphenyl group as S1 ligand and the 4-methylhomopiperazine group as S4 ligand [11]. Several vicinal diamide-based FXa inhibitors with diversified S4 ligands are reported by many research groups [23–25].

As a part of our research endeavor to develop viable therapies for the treatment of thrombotic diseases through inhibition of coagulation FXa, we envisioned less basic sulfoximine group in anthranilamide chemotype as S4 binding element, and that works as novel replacement of highly basic amidine group of Betrixaban. The optimized 5-chloro-2-aminopyridine group as S1 ligand has been retained in our designed compounds. In this paper, we describe the synthesis and structure–activity relationships for **I** (Fig. 2), and identified compound **18f** and its active metabolite **18b** as potent and selective FXa inhibitor with excellent *in vivo* efficacy and favorable oral bioavailability, thus making it a promising drug candidate for further evaluation.

2. Chemistry

The synthesis of first set of compounds **15a–g** is outlined in Scheme 1. Ester derivatives **6a–d** were chosen as starting materials to prepare sulfoximine fragment. Controlled oxidation of compounds **6a–d** using H_2O_2 gave sulfoxides **7a–d**, which upon treatment with sodium azide and sulfuric acid [26] were converted into sulfoximine derivatives **8a–d**. Sulfoximine group was then protected by reacting it with benzyloxycarbonyl chloride using pyridine as base to get intermediates **9a–d**. Alkaline hydrolysis of **9a–d** gave key acid intermediates **10a–d**. Commercially available 2-nitrobenzoic acid derivatives **11a–c** were condensed with 5-chloro-2-aminopyridine (**12**) to get intermediates **13a–c**, which upon reduction using $SnCl_2$ furnished anilines **14a–c**. Synthesis of **14d** was accomplished by chlorination of **14c** using *N*-chloro succinimide (NCS). The compounds **15a–g** were obtained by converting **10a–d** into acid chlorides using oxalyl chloride followed by condensation with appropriate anilines (**14a–d**) and subsequent deprotection of Cbz group using sulfuric acid or BBr_3.

The compounds **16a–e** were synthesized as shown in Scheme 2. Compound **15a** was reacted with methanesulfonyl chloride, acetyl chloride and methoxyacetyl chloride to obtained **16a**, **16b** and **16c** respectively. Reaction of **15a** with cyanogen bromide (BrCN) gave **16d**, which after treatment with sulfuric acid produced amide derivative **16e**.

The aminoacyl derivatives (**18a–s**) and aminoalkyl derivatives (**22a–c**) were synthesized as shown in Scheme 3. Anthranilamide derivatives **15a**, **15e**, and **15f** were reacted with the chloroacetyl chloride to get key intermediates **17a**, **17e**, and **17f** respectively. The displacement of chloro group from **17a**, **17e**, and **17f** with appropriately substituted amines gave desired compounds **18a–s**. Alternatively, **15a** was reacted with methyl bromoacetate using sodium hydride to get **19**. Ester group of **19** was reduced using $NaBH_4$ to get alcohol derivative **20**. The hydroxyl group of **20** was then converted into leaving group using triphenylphosphine (TPP) and CBr_4 as brominating agent to get the bromo derivative **21**. The compounds **22a–c** were obtained by displacement of bromo group from **21** with appropriate amines.

3. Results and discussion

The compounds synthesized were evaluated for their *in vitro* inhibitory activity of human FXa, expressed as IC_{50} or K_i values or % inhibition at 0.1 μM and their anticoagulant activity in human and rat plasma was measured as prolongation of prothrombin time (PT), expressed as the concentration of the compound required to double the clotting time ($PTCT_2$) in the PT assay. Prediction of oral bioavailability and simultaneous efficacy of the compounds has been achieved by measuring *ex vivo* PT prolonging activity in rats. PT prolongation was measured at 2 h after oral administration to rat at a dose of 30 mg/kg and expressed as fold prolongation with respect to control group.

3.1. In vitro and ex vivo structure–activity relationships

Table 1 shows the SAR of initial compounds **15a–g**, where the effect of introduction of the sulfoximine group, effect of alkyl substituent at S atom and effect of hydrophilicity in the central phenyl ring was studied.

The simplest sulfoximine compound **15a** with methyl substituent at S atom and chloro substituent at central phenyl ring displayed 76% inhibition of FXa activity at 0.1 μM. Insertion of methylene group between phenyl ring and sulfoximine group in **15a** was not tolerated and led to the inactive compound **15b**. Increasing alkyl chain at S atom was also not tolerated as reflected from the data of **15c** (*S*-ethyl) and **15d** (*S*-hydroxyethyl). We then evaluated SAR at central phenyl ring of compound **15a** (Table 1). Replacement of neutral chloro substituent in **15a** with hydrophilic methoxy substituent produced compound **15e** with diminished potency (42% inhibition at 0.1 μM).

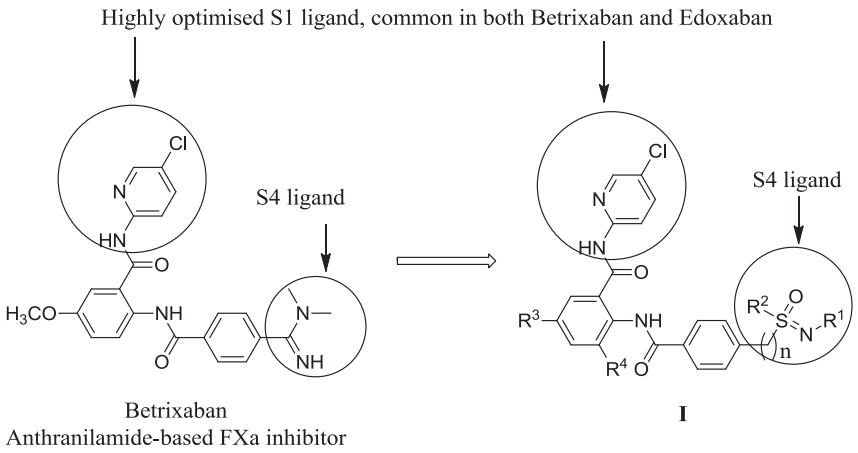
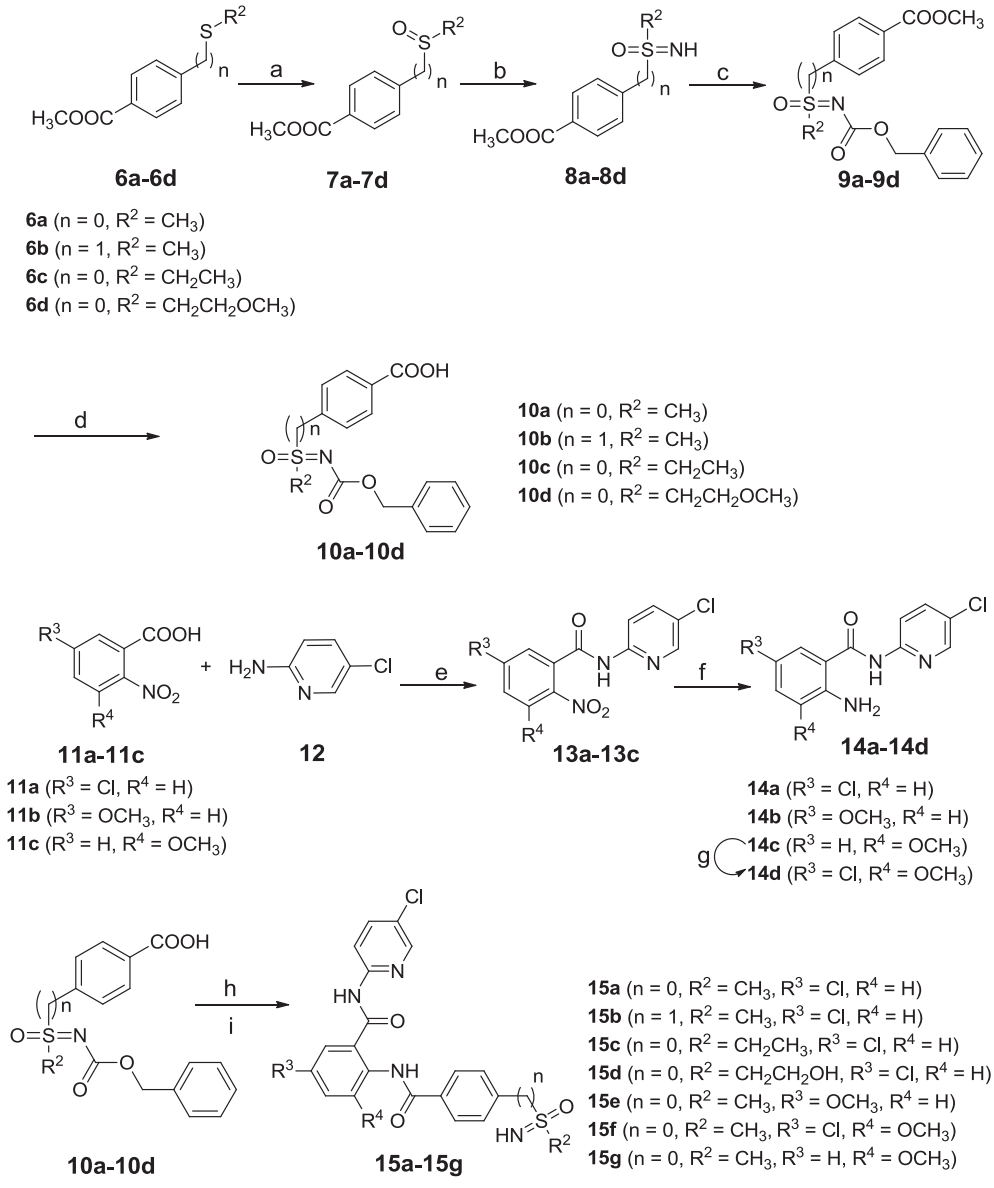


Fig. 2. Anthranilamide derivatives possessing sulfoximine group as novel S4 binding element.



6a (n = 0, $R^2 = CH_3$)
6b (n = 1, $R^2 = CH_3$)
6c (n = 0, $R^2 = CH_2CH_3$)
6d (n = 0, $R^2 = CH_2CH_2OCH_3$)

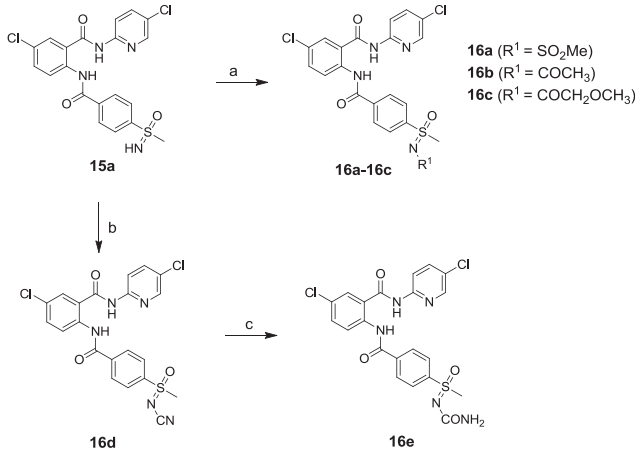
10a (n = 0, $R^2 = CH_3$)
10b (n = 1, $R^2 = CH_3$)
10c (n = 0, $R^2 = CH_2CH_3$)
10d (n = 0, $R^2 = CH_2CH_2OCH_3$)

11a (R^3 = Cl, R^4 = H)
11b ($R^3 = OCH_3$, R^4 = H)
11c (R^3 = H, $R^4 = OCH_3$)

14a (R^3 = Cl, R^4 = H)
14b ($R^3 = OCH_3$, R^4 = H)
14c (R^3 = H, $R^4 = OCH_3$)
14d (R^3 = Cl, $R^4 = OCH_3$)

15a (n = 0, $R^2 = CH_3$, R^3 = Cl, R^4 = H)
15b (n = 1, $R^2 = CH_3$, R^3 = Cl, R^4 = H)
15c (n = 0, $R^2 = CH_2CH_3$, R^3 = Cl, R^4 = H)
15d (n = 0, $R^2 = CH_2CH_2OH$, R^3 = Cl, R^4 = H)
15e (n = 0, $R^2 = CH_3$, $R^3 = OCH_3$, R^4 = H)
15f (n = 0, $R^2 = CH_3$, R^3 = Cl, $R^4 = OCH_3$)
15g (n = 0, $R^2 = CH_3$, R^3 = H, $R^4 = OCH_3$)

Scheme 1. Reagents and conditions: (a) H_2O_2, cat. V_2O_5, acetonitrile, 0–25 °C; (b) NaN_3, H_2SO_4, $CHCl_3$, −20 °C to 45 °C; (c) ClCOOBn, CH_2Cl_2, pyridine, 0–25 °C; (d) NaOH, THF, H_2O, 25 °C; (e) $POCl_3$, pyridine, acetonitrile, 25 °C; (f) $SnCl_2$, ethyl acetate, 25 °C; (g) NCS, benzene, 60–65 °C; (h) Oxalyl chloride, DCM, 25–30 °C, then **14a**–**d**, THF, 25–30 °C, 2 h; (i) sulfuric acid, 0 °C–25 °C, or BBr_3, DCM, −30 °C to 25 °C, (for **15d**).

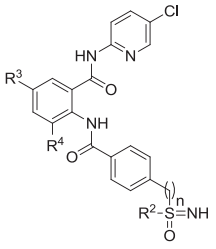


Scheme 2. Reagents and conditions: (a) $MeSO_2Cl$, pyridine, DCM, 0–25 °C (for **16a**); CH_3COCl, pyridine, DCM, 0–25 °C (for **16b**); CH_3OCH_2COCl, pyridine, DCM, 0 °C–25 °C (for **16c**); (b) BrCN, DCM, 25–30 °C; (c) Sulfuric acid, 0–30 °C.

Further addition of methoxy substituent in central ring of **15a** had no advantage in improving FXa inhibitory activity, which can be seen from the data of compound **15f** (69% inhibition at 0.1 μM). Removal of chloro substituent from **15f** produced inactive compound (**15g**) suggesting the importance of chloro substituent and its position on the central phenyl ring.

Next, SAR at N atom of sulfoximine group was evaluated. Initially, simple substituents were introduced at N atom to get compounds **16a–e** (Table 2). Compound **16a** with methanesulfonyl substituent inhibited FXa with slightly lower potency compare to unsubstituted sulfoximine derivative **15a**. However, anticoagulant activity of **16a** in human plasma was found to be much better than **15a** ($PTCT_2$ = 7.2 μM for **15a** vs 2.6 μM for **16a**), which was in line with the fact that anticoagulant activity of FXa inhibitors has been observed to be a function not only of potency, but also of

Table 1
In vitro FXa inhibitory activity data of compounds **15a–g**.

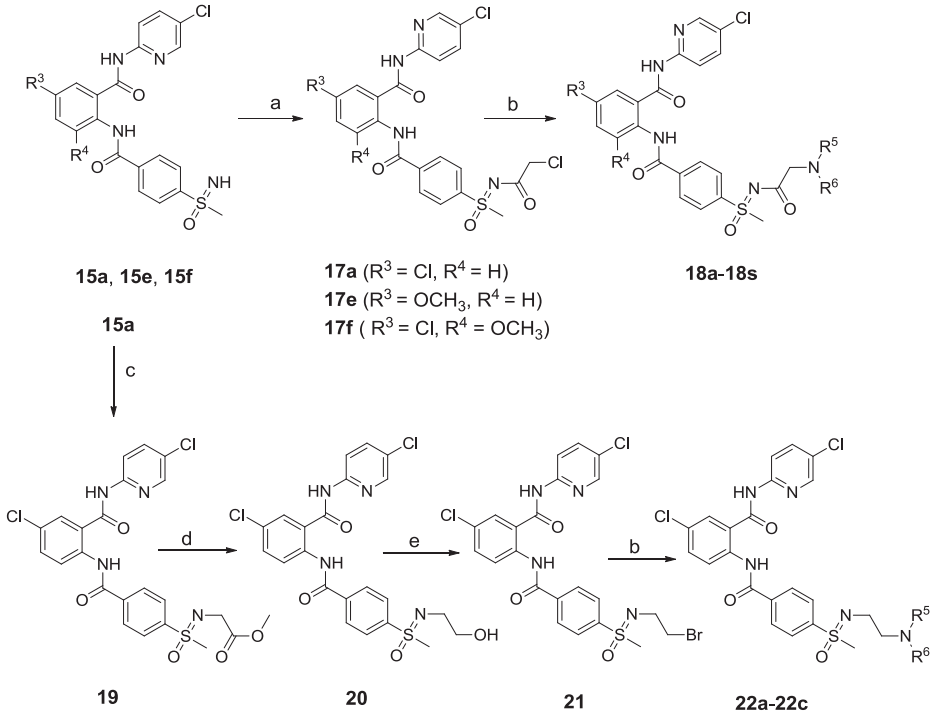


Compound	*n*	R^2	R^3	R^4	% Inhibition[a] at 0.1 μM
15a	0	CH_3	Cl	H	76
15b	1	CH_3	Cl	H	No inhibition
15c	0	C_2H_5	Cl	H	No inhibition
15d	0	CH_2CH_2OH	Cl	H	No inhibition
15e	0	CH_3	OCH_3	H	42
15f	0	CH_3	Cl	OCH_3	69
15g	0	CH_3	H	OCH_3	No inhibition

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

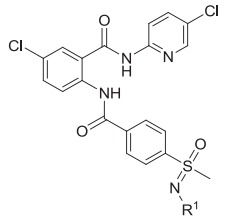
lipophilicity and plasma protein binding. Compound **16b** with acetyl substituent showed 61% inhibition of FXa activity at 0.1 μM, but displayed poor anticoagulant activity ($PTCT_2$ = 7 μM). Methoxyacetyl derivative **16c** with increased polarity showed improvement in anticoagulant activity ($PTCT_2$ = 3 μM). Compound **16d** with cyano group showed improved inhibition of FXa (76% inhibition) and appreciable anticoagulant activity ($PTCT_2$ = 2.3 μM). Conversion of cyano group of **16d** to amide group (**16e**) was found to be detrimental for both FXa inhibitory activity (41% inhibition) and anticoagulant activity ($PTCT_2$ = 4.1 μM).

Taking clue from the difference observed in anticoagulant activity for compound **16b** and **16c**, we decided to replace methoxy group of **16c** with several alkylamino substituents to see its effect



Scheme 3. Reagents and conditions: (a) chloroacetyl chloride, TEA, THF, 25–30 °C; (b) NHR^5R^6, DMF, 25–30 °C; (c) NaH, Methyl bromoacetate, DMF, 40 °C; (d) $NaBH_4$, DMSO, 60 °C; (e) TPP, CBr_4, DCM, 25–30 °C.

Table 2
In vitro FXa inhibitory and anticoagulant activity data of compounds **15a**, **16a**–**e**.



Compound	R^1	% Inhibition[a] at 0.1 μM	$PTCT_2$[b] using human plasma (μM)
15a	H	76	7.2
16a	SO_2Me	55	2.6
16b	$COCH_3$	61	7
16c	$COCH_2OMe$	48	3
16d	CN	76	2.3
16e	$CONH_2$	41	4.1

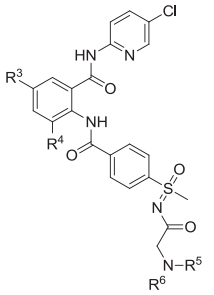
[a] Inhibitory activity against human FXa. Values shown are the mean of duplicate measurements.
[b] Concentration of the compound required to double the clotting time in the PT assay using human plasma. $PTCT_2$ values shown are the mean of duplicated measurements.

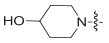
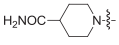
on anticoagulant potential and subsequently several aminoacyl derivatives **18a**–**s** were synthesized. *In vitro* FXa inhibitory activity and anticoagulant activity in human plasma of **18a**–**s** are listed in Table 3. Compound **18a** with methylamino substituent showed 93% inhibition at 0.1 μM with improved anticoagulant activity in human plasma ($PTCT_2 = 0.77$ μM). Increasing alkyl chain from methyl (**18a**, $PTCT_2 = 0.77$ μM) to ethyl (**18b**, $PTCT_2 = 0.87$ μM) and to isopropyl (**18c**, $PTCT_2 = 0.95$ μM) was well tolerated for both FXa inhibitory and anticoagulant activity as shown in Table 3. Further increase in bulk with cyclopentylamino substituent (**18d**) was found to be detrimental for anticoagulant activity even though FXa inhibitory potency was retained ($PTCT_2 = 2.26$ μM). Dialkyl amino groups were also well tolerated with comparable anticoagulant activity as evident from data of **18e** (dimethylamino, $PTCT_2 = 1.2$ μM) and **18f** (diethylamino, $PTCT_2 = 0.68$ μM). However, compound **18g** with diisopropylamino substituent showed decreased anticoagulant activity ($PTCT_2 = 1.45$ μM). Compound **18h** with pyrrolidinyl substituent showed potent FXa inhibitory activity and excellent anticoagulant activity ($PTCT_2 = 0.46$ μM). Ring expansion in **18h** (5 membered pyrrolidine) to **18i** (6 membered piperidine) retained FXa inhibitory activity, but showed significant reduction in anticoagulant activity ($PTCT_2 = 0.46$ μM of **18h** vs 1.73 μM of **18i**). Substitution with morpholine group (**18j**) resulted in a diminished activity with only 45% FXa inhibition at 0.1 μM. Compound **18k** with 4-methylpiperazine substituent showed 88% inhibition of FXa activity, but failed to show improvement in anticoagulant activity ($PTCT_2 = 2.03$ μM). Ring expansion in **18k** produced compound **18l** (4-methylhomopiperazine derivative) with similar anticoagulant activity ($PTCT_2 = 1.82$ μM). Introduction of additional hydrophilic substituent in **18i** afforded compounds **18m** (4-hydroxypiperidine) and **18n** (piperidine-4-carboxamide), with inferior FXa inhibitory and anticoagulant activity (**18m**: $PTCT_2 = 2.16$ μM, **18n**: $PTCT_2 = 2.54$ μM).

Further refining of FXa inhibition and anticoagulant effect have been achieved by studying effect of chloro and methoxy substituents on central phenyl ring, which represent neutral and hydrophilic substituents respectively and are also frequently used in anthranilamide compounds.

Replacement of chloro substituent in **18f** with methoxy substituent produced compound **18o** with reduced potency

Table 3
In vitro FXa inhibitory and anticoagulant activity data of compounds **18a**–**s**.



Compound	NR^5R^6	R^3	R^4	% Inhibition[a] at 0.1 μM	$PTCT_2$[b] using human plasma (μM)
18a		Cl	H	93	0.77
18b		Cl	H	100	0.87
18c		Cl	H	97	0.95
18d		Cl	H	100	2.26[c]
18e		Cl	H	85	1.2
18f		Cl	H	100	0.68
18g		Cl	H	79	1.45
18h		Cl	H	95	0.46
18i		Cl	H	97	1.73[c]
18j		Cl	H	45	ND[d]
18k		Cl	H	88	2.03[c]
18l		Cl	H	75	1.82[c]
18m		Cl	H	69	2.16[c]
18n		Cl	H	49	2.54[c]
18o		OCH_3	H	56	1.44
18p		Cl	OCH_3	92	0.58
18q		Cl	OCH_3	100	0.74
18r		Cl	OCH_3	97	0.92
18s		Cl	OCH_3	90	0.53

[a] Inhibitory activity against human FXa. Values shown are the mean of duplicate measurements.
[b] Concentration of the compound required to double the clotting time in the PT assay using human plasma. $PTCT_2$ values shown are the mean of duplicated measurements unless otherwise indicated.
[c] Single determination.
[d] Not determined.

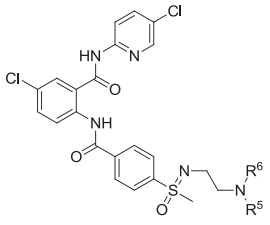
(56% inhibition at 0.1 μM, $PTCT_2$ = 1.44 μM), which matches with our earlier observation for compound **15e**. Incorporating additional methoxy substituent along with chloro substituent has produced compounds with almost similar FXa inhibitory and anticoagulant activity, when compared with only chloro substituted compounds as evident from compounds **18p–s** (**18p** vs **18a**, **18q** vs **18b**, **18r** vs **18f**, and **18s** vs **18h**).

We then synthesized compounds **22a**, **22b** and **22c** by replacing aminoacyl group with aminoalkyl group in compounds **18e**, **18f** and **18h** respectively and their biological data is summarized in Table 4. Compound **22a** with dimethyl amino substituent showed 82% inhibition of FXa activity at 0.1 μM and moderate anticoagulant activity ($PTCT_2$ = 1.14 μM). Compounds with diethylamino substituent (**22b**) and pyrrolidinyl substituent (**22c**) showed similar FXa inhibition but their anticoagulant potency decreased significantly ($PTCT_2$ = 1.78 μM for **22b** and 2.16 μM for **22c**).

The compounds selected on the basis of FXa inhibition and anticoagulant activity from Tables 3 and 4 were evaluated for their *ex vivo* PT prolonging activity in rats, *in vitro* anticoagulant activity in rat plasma, and IC_{50} value determination. Results are summarized in Table 5. All tested compounds have their IC_{50} value for human FXa inhibition less than 12 nM. They all displayed relatively inferior anticoagulant activity in rat plasma compare to human plasma as reflected in their $PTCT_2$ values. However, compound **18f** (IC_{50} = 2.1 nM) showed balanced anticoagulant activity in both rat and human plasma ($PTCT_2$ = 0.8 μM for rat vs 0.68 μM for human). Compound **18e** (IC_{50} = 9 nM) is another compound with similar anticoagulant activity in both species ($PTCT_2$ = 1.4 μM for rat vs 1.2 μM for human).

The *ex vivo* PT prolonging activity in rats was determined at 2 h after oral administration to rat at a dose of 30 mg/kg and the data are summarized in Table 5. Compounds **18f**, **18e**, and **18c** have shown PT prolongation 2.2, 1.6 and 1.5 fold respectively, while remaining compounds failed to show significant PT prolongation which could be due to their poor oral bioavailability.

Table 4
In vitro FXa inhibitory and anticoagulant activity data of compounds **22a–22c**.



Compound	NR^5R^6	% Inhibition[a] at 0.1 μM	$PTCT_2$[b] using human plasma (μM)
22a		82	1.14
22b		77	1.78
22c		74	2.16

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

Table 5
In vitro and *ex vivo* anticoagulant activities of potent factor Xa inhibitors.

Compound	IC_{50} (nM)[a]	Human $PTCT_2$[b]	Rat $PTCT_2$[b]	*Ex vivo* rat PT ratio[c]
18a	5.4	0.77	1.7	1.1
18b	2.7	0.81	2.1	1.3
18c	4	0.95	1.9	1.5
18e	9	1.2	1.4	1.6
18f	2.1	0.68	0.8	2.2
18h	3.2	0.46	1.57	1.4
18p	5.6	0.58	0.9	1.1
18q	2.4	0.74	1.15	1.1
18r	3.4	0.92	1.3	1.1
18s	6.4	0.53	1.1	1.1
22a	11.6	1.14	2.01	1.0
1	1.6	0.39	1.5	ND[d]

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

3.2. Profiling studies including in vivo antithrombotic efficacy

Based on *ex vivo* anticoagulant activity of **18f**, it was selected for further evaluation which includes pharmacokinetics evaluation in rats, selectivity against related proteases and CYP3A4 inhibition.

However, to the contrary, hydrochloride salt of **18f** showed low plasma levels (less than 100 ng/mL) when dosed orally at 30 mg/kg in Wistar rats. The lack of correlation between plasma concentration and *ex vivo* anticoagulant activity prompted us to search the possibility for an active metabolite. From thorough metabolism study, it was found that de-ethylated compound is generated from **18f**, which is same to compound **18b** (Table 3), further confirmed by matching UPLC retention time. In a separate study, plasma levels of compounds **18f** and its metabolite **18b** were estimated by dosing hydrochloride salt of **18f** orally in rats at 30 mg/kg (Table 6). The plasma levels of **18b** were found to be excellent (C_{max} = 3.28 μg/mL) in comparison with parent compound **18f**. The absorption and elimination pattern of **18f** with simultaneous formation of metabolite **18b** are shown in Fig. 3. The half life of **18b** (4.79 h) justifies once a day dosage regime and the low peak vs trough ratio in PK profile may mitigate the bleeding liability associated with this therapeutic class.

Selectivity against related serine proteases is a decisive criteria for developing FXa inhibitors and then we examined the effect of compounds **18f** and its metabolite **18b** against related serine proteases. Both the compounds **18f** and **18b** were found to be more than 10^4 fold selective against thrombin, plasmin, trypsin, t-PA and aPC (Table 7). Additionally, compounds **18f** and **18b** were checked for their effect on human cytochrome P450 enzyme (CYP3A4), which is known to be a primary factor responsible of metabolism of most drugs. Both the compounds showed low inhibition of CYP3A4 at 10 μM (18% for **18f** and 27% for **18b**).

Table 6
Pharmacokinetic parameters[a] for compound **18f**[b] and its metabolite **18b**.

Compound	C_{max} (μg/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC (0–24) (h μg/mL)
18f (**18b**)[c]	3.28 ± 0.26	2.75 ± 0.47	4.79 ± 0.2	43.51 ± 2.81

[a] Data are expressed as the mean ± SEM.
[b] Monohydrochloride salt was used.
[c] Compound **18f** was dosed in fasted male Wistar rats at 30 mg/kg po formulated with a Tween-80:PEG:CMC (5:5:90% v/v) and plasma levels of metabolite **18b** was determined (n = 4).

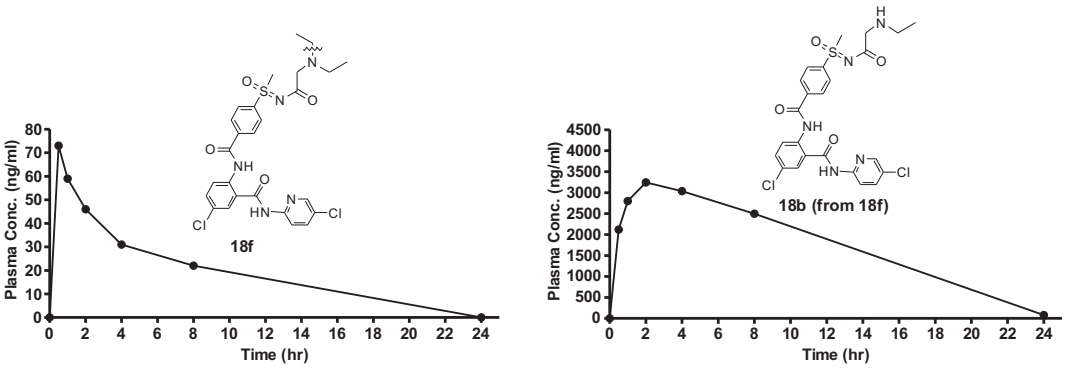


Fig. 3. Absorption and elimination pattern of **18f** and its metabolite **18b** in fasted male Wistar rats at 30 mg/kg po.

Further, the *in vivo* antithrombotic efficacy of compound **18f** was evaluated in rats using $FeCl_3$-induced arterial and venous thrombosis model. Dose dependent thrombus weight reduction was found in both arterial and venous thrombosis model, when **18f** was dosed orally at 10 mg/kg and 30 mg/kg in rats (Fig. 4 and Fig. 5). In an arterial thrombosis model, **18f** reduced thrombus weight by 32% and 71% at 10 mg/kg and 30 mg/kg respectively (Fig. 4). In venous thrombosis model, reduction in thrombus weight was 45% and 81% at 10 mg/kg and 30 mg/kg respectively (Fig. 5). Rivaroxaban (**1**) was used as positive standard at single dose of 10 mg/kg. In an arterial thrombosis model, **1** reduced thrombus weight by 50% and in venous thrombosis model, reduction in thrombus weight was 63%.

3.3. Molecular modeling study

To predict the binding mode of compound **18f** and its active metabolite **18b**, a docking study was carried out. Fig. 6 shows inhibitors **18f** (A) and **18b** (B) docked in the active site of FXa. The proposed binding model indicated that the 5-chloro-2-aminopyridyl moiety deeply occupied the S1 pocket with chlorine atom pointing toward the center of the Tyr228 aromatic ring, while the substituted sulfoximine group fits in the S4 pocket formed by the residues Phe174, Trp215, and Tyr99.

We have also observed that there are several π–π and CH–π interactions in **18f** and **18b** with Trp215 and Tyr99. These two molecules form a CH–π interaction with Tyr99 with a distance of 3.6 Å between aromatic CH and centroid of Tyr99, at the same time an inclined π–π interaction between Trp215 and phenyl ring attached to sulfoximine group with a distance of 5.4 Å. The –NH– of ethylamino group attached to sulfoximine group of **18b** showed H-bond interaction with Glu97, which is at the periphery of the S4 site. The model further suggested that the NH group of the amide bond linked to the 5-chloro-2-aminopyridyl moiety forms H-bond with Gly218 and likewise, the carbonyl group of the amide bond connected to the sulfoximine group substituted phenyl ring forms H-bond with Gly216. Additionally, carbonyl group of amide bond linked to the sulfoximine group substituted phenyl ring forms a H-bond with Gln192.

4. Conclusion

Application of sulfoximine group as novel S4 ligand in anthranilamide chemotype resulted in identification of lead molecule **15a**. Further optimization was achieved by synthesizing several derivatives with substituents at both S and N atom of sulfoximine group. Biological evaluation of synthesized compounds led to identification of compound **18f**, which has shown strong human factor Xa inhibitory activity and anticoagulant activities in both rat and human plasma. Oral dosing of this compound in rats produced active metabolite **18b**, which was responsible for potent *ex vivo* anticoagulant activity of compound **18f**. *In vivo* antithrombotic efficacy was determined using $FeCl_3$-induced arterial and venous thrombosis model in rats. Dose dependent reduction in thrombus weight was observed with the compound **18f**. Both the compounds (**18f** and its metabolite **18b**) were found to be more than 10^4 fold selective against related serine proteases and have low effect on CYP3A4. The PK profile of **18f** along with its metabolite showed fairly long half life and low peak vs trough ratio. Based on these encouraging data compound **18f** was selected for further evaluation.

5. Experimental section

5.1. Chemistry

5.1.1. General methods

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using commercial silica gel (100–200 or 230–400 mesh). Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (V_{max} in cm^{-1}, using KBr pellets, CCl_4 or $CHCl_3$). The ^1H NMR spectra were recorded on a Brucker Avance-300 spectrometer (300 MHz) and Brucker Avance-400 spectrometer (400 MHz). The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, in either $CDCl_3$ or DMSO-d_6 solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), bd (broad

Table 7
Selectivity profile for compounds **18f** and **18b** against related serine proteases[a] and effect on CYP3A4.

Compound K_i (nM)	FXa	Thrombin	Plasmin	Trypsin	t-PA	aPC	CYP3A4
18f	1.1	>20000[b]	>20000[b]	>20000[b]	>20000[b]	>20000[b]	18% Inhibition at 10 μM
18b	1.5	>20000[b]	>20000[b]	>20000[b]	>20000[b]	>20000[b]	27% Inhibition at 10 μM

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

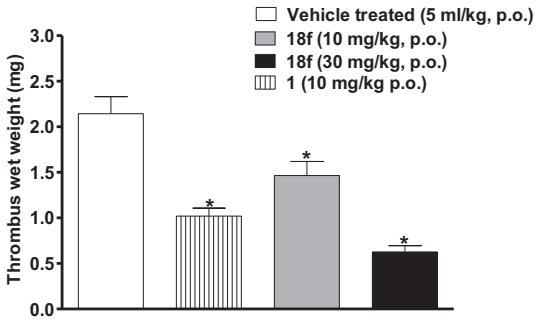


Fig. 4. Effect of **18f** (monohydrochloride) and **1** on $FeCl_3$-induced carotid artery thrombus weight after 2 h of oral administration in male Wistar rats ($n = 10$). $^*p < 0.01$ vs vehicle control, ANOVA followed by Dunnett's test.

doublet), and m (multiplet). ^{13}C NMR spectra were recorded on Brucker Avance-400 at 100 MHz in DMSO-d_6 solution. Mass spectra (ESI-MS) were obtained on Shimadzu LC-MS 2010-A spectrometer. Purity of compounds were determined by Ultra Performance Liquid Chromatography (UPLC) (Column, BEH C-18, 2.1 × 100 mm; UV detection, 220 nm; eluent, 0.05% TFA buffer:ACN (gradient); flow rate, 0.4 mL/min) or by HPLC analysis (column ODS C-18, 150 nm × 4.6 nm × 4 μ on AGILENT 1100 series; UV detection, 220 nm; eluent, 0.05% TFA buffer:ACN (gradient); flow rate, 1 mL/min) purity of all tested compounds was found to be >95%.

*5.1.1.1. Methyl 4-(methylsulfinyl)benzoate (**7a**).* To a stirring solution of methyl 4-(methylthio)benzoate (**6a**) (10 g, 0.0548 mol) and V_2O_5 (100 mg, 0.0004 mol) in acetonitrile (10 v/w) at −20 to −25 °C under nitrogen atmosphere, 50% hydrogen peroxide (2.05 g, 0.0604 mol) was added drop wise to the above solution in 20–30 min. The reaction mixture was stirred at 25–30 °C for 5 h. Reaction mixture was diluted with water (30 v/w) and extracted with ethyl acetate (30 v/w). The organic phase was dried over sodium sulfate and evaporated to afford the title compound **7a** (9.5 g, 88%) as white solid: mp 120–122 °C; purity by HPLC: 94.19%; IR (KBr) 2991, 2952, 1718, 1595, 1429, 1400, 1373, 1274, 1045, 860, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.78 (s, 3H), 3.87 (s, 3H), 7.83 (d, $J = 8.4$ Hz, 2H), 8.12 (d, $J = 8.4$ Hz, 2H); ESI/MS m/z 198.9 $(M + H)^+$.

*5.1.1.2. Methyl 4-((methylsulfinyl)methyl)benzoate (**7b**).* This compound was prepared from **6b** by means of the procedure similar to that reported for **7a** as off white solid; yield: 52%; mp 80–83 °C; purity by HPLC: 95.35%; IR (KBr) 2999, 2949, 1718, 1610, 1575, 1508, 1434, 1280, 1182, 1031, 862, 709 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.48 (s, 3H), 3.84 (s, 3H), 4.03 (d, $J = 12.8$ Hz, 1H), 4.24 (d, $J = 12.4$ Hz, 1H), 7.45 (d, $J = 8.4$ Hz, 2H), 7.94–7.96 (dd, $J = 1.6$ and 6.4 Hz, 2H); ESI/MS m/z 212.8 $(M + H)^+$.

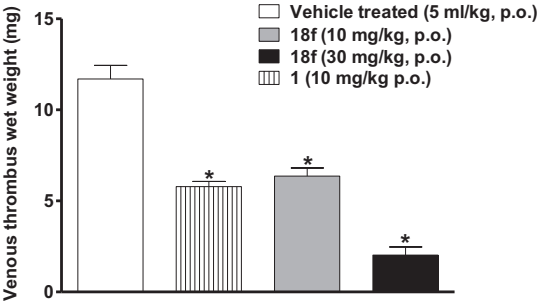


Fig. 5. Effect of **18f** (monohydrochloride) and **1** on partial stasis combined with $FeCl_3$-induced venous thrombus weight after 2 h of oral administration in male Wistar rats ($n = 10$). $^*p < 0.01$ vs vehicle control, ANOVA followed by Dunnett's test.

*5.1.1.3. Methyl 4-(ethylsulfinyl)benzoate (**7c**).* This compound was prepared from **6c** by means of the procedure similar to that reported for **7a** as off white solid; yield: 80%; mp 72–74 °C; purity by HPLC: 91.35%; IR (KBr) 2952, 2933, 1718, 1595, 1488, 1436, 1398, 1278, 1045, 860, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.00 (t, 3H), 2.74–2.83 (m, 1H), 3.04–3.13 (m, 1H), 3.87 (s, 3H), 7.78 (d, $J = 8.4$ Hz, 2H), 8.12 (d, $J = 8.4$ Hz, 2H); ESI/MS m/z 212.9 $(M + H)^+$.

*5.1.1.4. Methyl 4-((2-methoxyethyl)sulfinyl)benzoate (**7d**).* This compound was prepared from **6d** by means of the procedure similar to that reported for **7a** as off white solid; yield: 72%; mp 60–63 °C; purity by UPLC: 94.50%; IR (KBr) 2842, 1724, 1595, 1571, 1442, 1396, 1280, 1195, 1172, 1047, 860, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.94–3.0 (m, 1H), 3.19–3.22 (m, 1H), 3.24 (s, 3H), 3.53–3.58 (m, 1H), 3.68–3.74 (m, 1H), 3.87 (s, 3H), 7.82 (d, $J = 8.4$ Hz, 2H), 8.12 (d, $J = 8.4$ Hz, 2H); ESI/MS m/z 242.9 $(M + H)^+$.

*5.1.1.5. Methyl 4-(S-methylsulfonimidoyl)benzoate (**8a**).* To a stirring solution of **7a** (6.8 g, 0.034 mol) in chloroform (10 v/w) at 25 °C was added sodium azide (6.63 g, 0.102 mol). To this was added sulfuric acid (20 g, 0.204 mol) at −20 to −25 °C under nitrogen atmosphere in 20–30 min. The reaction mixture was stirred at 25–30 °C for 12 h and then at 45–50 °C for 3 h. Chloroform was removed from reaction mixture and remaining residue was made alkaline by using aqueous potassium carbonate solution. Ethyl acetate was added to it and organic layer was separated out. The organic phase was dried over sodium sulfate and evaporated to afford the title compound **8a** (6.75 g, 92%) as white solid: mp 113–115 °C; purity by HPLC: 97.48%; IR (KBr) 3280, 2999, 2923, 1703, 1595, 1573, 1438, 1398, 1298, 1220, 1097, 1008, 856, 756 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.10 (s, 3H), 3.89 (s, 3H), 4.42 (s, 1H), 8.06 (d, $J = 8.4$ Hz, 2H), 8.14 (d, $J = 8.4$ Hz, 2H); ESI/MS m/z 213.9 $(M + H)^+$.

*5.1.1.6. Methyl 4-((S-methylsulfonimidoyl)methyl)benzoate (**8b**).* This compound was prepared from **7b** by means of the procedure similar to that reported for **8a** as off white solid; yield: 65%; mp 131–133 °C; purity by HPLC: 91.68%; IR (KBr) 3255, 2956, 2914, 1718, 1612, 1573, 1510, 1436, 1417, 1280, 864, 705 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.77 (s, 3H), 3.72 (s, 1H), 3.84 (s, 3H), 4.42–4.50 (q, $J = 12.8$ Hz, 2H), 7.57 (d, $J = 8$ Hz, 2H), 7.94–7.96 (dd, $J = 2.0$ and 6.8 Hz, 2H); ESI/MS m/z 227.7 $(M + H)^+$.

*5.1.1.7. Methyl 4-(ethylsulfonimidoyl)benzoate (**8c**).* This compound was prepared from **7c** by means of the procedure similar to that reported for **8a** as off white solid; yield: 80%; mp 77–80 °C; purity by HPLC: 93.33%; IR (KBr) 3267, 2956, 2916, 1720, 1593, 1571, 1434, 1396, 1296, 1217, 972, 866, 731 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.04 (t, $J = 7.2$ Hz, 3H), 3.13–3.18 (q, $J = 7.2$ Hz, 2H), 3.88 (s, 3H), 4.40 (s, 1H), 8.01 (d, $J = 8.4$ Hz, 2H), 8.14 (d, $J = 8.4$ Hz, 2H); ESI/MS m/z 228 $(M + H)^+$.

*5.1.1.8. Methyl 4-(2-methoxyethylsulfonimidoyl)benzoate (HCl salt) (**8d**).* This compound was prepared from **7d** by means of the procedure similar to that reported for **8a**. Crude product was stirred with HCl:diethyl ether to obtained hydrochloride salt of title compound as off white solid; yield: 67%; mp 143–145 °C; purity by UPLC: 95.16%; IR (KBr) 3441, 1732, 1573, 1550, 1438, 1282, 1114, 864, 734 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.99 (s, 3H), 3.65–3.69 (m, 2H), 3.90 (s, 3H), 4.06–4.13 (m, 2H), 8.13–8.26 (m, 4H); ESI/MS m/z 257.9 $(M + H)^+$.

*5.1.1.9. Methyl 4-(N-((benzyloxy)carbonyl)-S-methylsulfonimidoyl) benzoate (**9a**).* To a stirring solution of **8a** (2 g, 0.0093 mol) in dichloromethane (5 v/w) was added pyridine (1.1 g, 0.0139 mol).

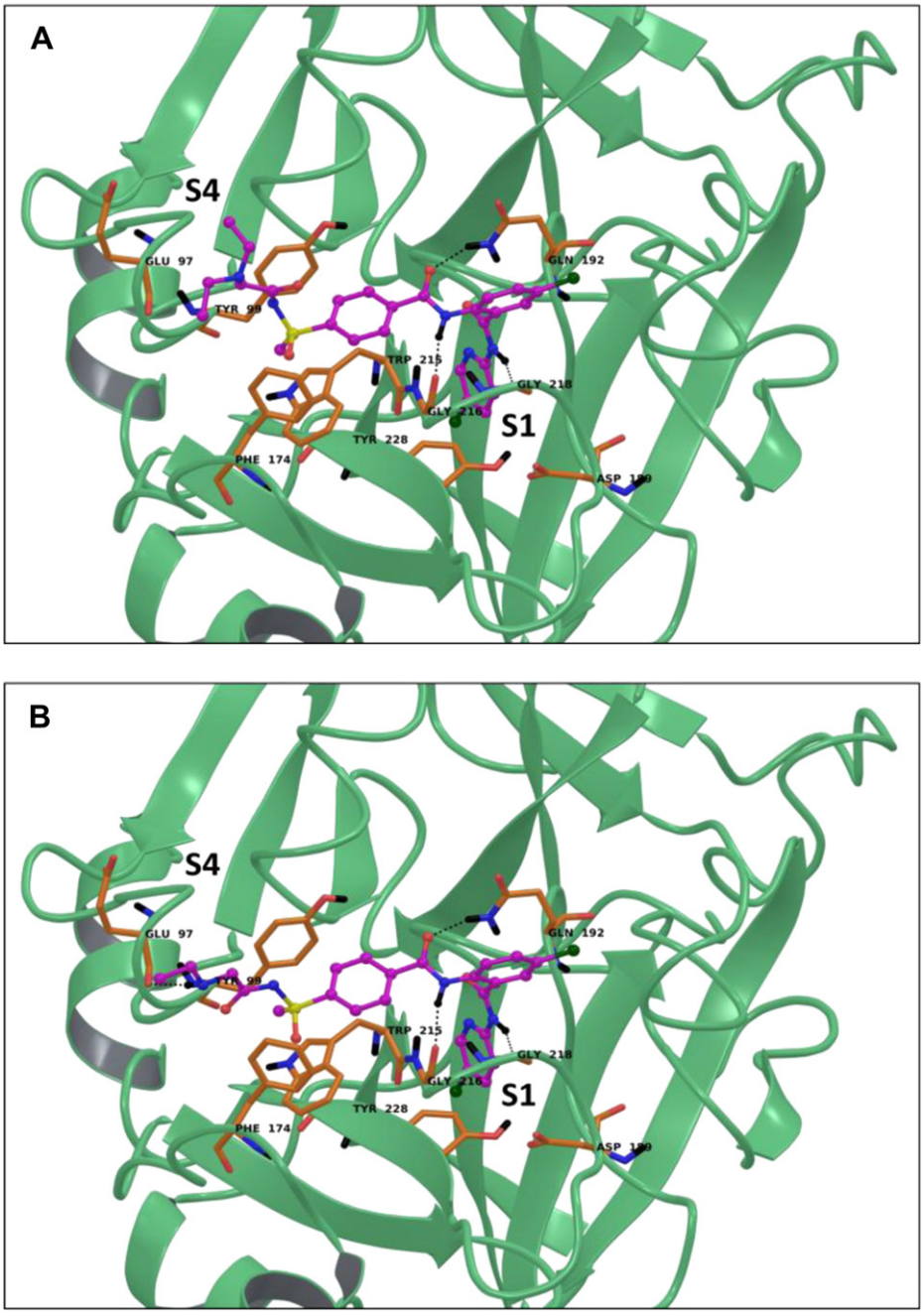


Fig. 6. Docking study of **18f** (A) and **18b** (B) in the active site of FXa depicting the surface of FXa.

To this was added benzyloxycarbonyl chloride (50% in toluene) (1.9 g, 0.0116 mol) at 15–20 °C under nitrogen atmosphere. The reaction mixture was stirred at 25–30 °C for 3 h and then diluted with dichloromethane (10 v/w). The organic phase was separated, dried over sodium sulfate and evaporated to afford the title compound **9a** (3.1 g, 95%) as off white solid: mp 141–143 °C; purity by UPLC: 98.13%; IR (KBr) 1718, 1668, 1573, 1496, 1286, 1255, 1219, 1085, 896, 752 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.52 (s, 3H), 3.91 (s, 3H), 4.88–4.97 (q, J = 12 Hz, 2H), 7.19–7.21 (m, 2H), 7.27–7.33 (m, 3H), 8.06–8.09 (dd, J = 2 and 6.8 Hz, 2H), 8.16–8.18 (dd, J = 2 and 6.8 Hz, 2H); ESI/MS m/z 369.9 $(M + Na)^+$.

*5.1.1.10. Methyl 4-((N-((benzyloxy)carbonyl)-S-methylsulfonimidoyl) methyl)benzoate (**9b**).* This compound was prepared from **8b** by means of the procedure similar to that reported for **9a**; yield: 85%; mp 116–118 °C; purity by HPLC: 96.13%; IR (KBr) 1720, 1666, 1610, 1573, 1500, 1288, 1209, 1114, 869, 785 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.19 (s, 3H), 3.85 (s, 3H), 4.97–5.06 (m, 4H), 7.30–7.38 (m, 5H), 7.54 (d, J = 8 Hz, 2H), 7.95 (d, J = 8.4 Hz, 2H); ESI/MS m/z 384 $(M + Na)^+$.

*5.1.1.11. Methyl 4-(N-((benzyloxy)carbonyl)ethylsulfonimidoyl)benzoate (**9c**).* This compound was prepared from **8c** by means of the procedure similar to that reported for **9a**; yield: 82%; purity by HPLC: 94.98%; IR (KBr) 2976, 2943, 1720, 1658, 1575, 1498, 1382, 1271, 1087, 860, 752 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.09 (t, J = 7.2 Hz, 3H), 3.58–3.69 (m, 2H), 3.91 (s, 3H), 4.87–4.97 (q, J = 12.4 Hz, 2H), 7.18–7.20 (m, 2H), 7.28–7.37 (m, 3H), 8.03 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H); ESI/MS m/z 362 $(M + H)^+$.

*5.1.1.12. Methyl 4-(N-((benzyloxy)carbonyl)-2-methoxyethylsulfonimidoyl)benzoate (**9d**).* This compound was prepared from **8d** by means of the procedure similar to that reported for **9a**; yield: 88%; mp 95–97 °C; purity by UPLC: 92.66%; IR (KBr) 3020, 1728, 1678, 1517, 1400, 1215, 1116, 759, 669 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.98 (s, 3H), 3.60–3.67 (m, 2H), 3.91 (s, 3H), 3.94–3.99 (m, 2H), 4.89–4.98 (q, J = 12.4 Hz, 2H), 7.19–7.21 (m, 2H), 7.27–7.30 (m, 3H), 8.02–8.04 (dd, J = 2.0 and 6.8 Hz, 2H), 8.13–8.15 (dd, J = 2.0 and 6.8 Hz, 2H); ESI/MS m/z 391.8 $(M + H)^+$.

*5.1.1.13. 4-(N-((Benzyloxy)carbonyl)-S-methylsulfonimidoyl)benzoic acid (**10a**).* To a stirring solution of sodium hydroxide (0.518 g, 0.01296 mol) in the solvent mixture of 15 mL water and 15 mL THF was added **9a** (3 g, 0.00864 mol). Reaction mixture was stirred at 25–30 °C for 3 h and then diluted with water (10 v/w). Aqueous layer was washed with methyl *tert*-butyl ether (10 v/w). Aqueous layer was then cooled to 0 °C and acidified with diluted HCl. Aqueous layer was extracted with ethyl acetate, which on drying over sodium sulfate and evaporation afforded title compound (2.6 g, 90%) as off white solid: mp 172–174 °C; purity by HPLC: 95.20%; IR (KBr) 3435, 1693, 1664, 1602, 1575, 1375, 1272, 1228, 1087, 746, 698 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.51 (s, 3H), 4.89–4.97 (q, J = 12.4 Hz, 2H), 7.19–7.21 (m, 2H), 7.25–7.33 (m, 3H), 8.06 (d, J = 8.8 Hz, 2H), 8.16 (d, J = 8.4 Hz, 2H), 13.56 (bs, 1H); ESI/MS m/z 333.8 $(M + H)^+$.

*5.1.1.14. 4-((N-((Benzyloxy)carbonyl)-S-methylsulfonimidoyl)methyl) benzoic acid (**10b**).* This compound was prepared from **9b** by means of the procedure similar to that reported for **10a**; yield: 82%; mp 180–183 °C; purity by HPLC: 95.55%; IR (KBr) 3444, 1676, 1641, 1427, 1382, 1259, 1103, 790, 696 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.19 (s, 3H), 4.96 (s, 2H), 4.98–5.06 (q, J = 12.4 Hz, 2H), 7.30–7.38 (m, 5H), 7.50 (d, J = 8 Hz, 2H), 7.93 (d, J = 8 Hz, 2H); ESI/MS m/z 370 $(M + Na)^+$.

*5.1.1.15. 4-(N-((Benzyloxy)carbonyl)ethylsulfonimidoyl)benzoic acid (**10c**).* This compound was prepared from **9c** by means of the procedure similar to that reported for **10a**; yield: 84%; purity by HPLC: 97.91%; IR (KBr) 3435, 1720, 1633, 1500, 1456, 1392, 1294, 1228, 1085, 902, 734 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.07 (t, J = 7.6 Hz, 3H), 3.58–3.67 (m, 2H), 4.88–4.98 (q, J = 12.4 Hz, 2H), 7.18–7.20 (dd, J = 2.0 and 7.6 Hz, 2H), 7.25–7.32 (m, 3H), 7.98 (d, J = 8.4 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H); ESI/MS m/z 347.9 $(M + H)^+$.

*5.1.1.16. 4-(N-((Benzyloxy)carbonyl)-2-methoxyethylsulfonimidoyl) benzoic acid (**10d**).* This compound was prepared from **9d** by means of the procedure similar to that reported for **10a** as oily compound; yield: 73%; purity by UPLC: 97.37%; IR (KBr) 3433, 1718, 1637, 1500, 1388, 1286, 1230, 1105, 1082, 898, 796, 731 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.00 (s, 3H), 3.57–3.68 (m, 2H), 3.90–4.01 (m, 2H), 4.90–4.98 (q, J = 12.4 Hz, 2H), 7.20–7.32 (m, 5H), 8.02 (d, J = 8.4 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H); ESI/MS m/z 377.8 $(M + H)^+$.

*5.1.1.17. 5-Chloro-N-(5-chloropyridin-2-yl)-2-nitrobenzamide (**13a**).* To a stirring solution of **11a** (5 g, 0.02487 mol) and 2-amino-5-chloro pyridine (**12**) (3.2 g, 0.02487 mol) in 20 mL acetonitrile at 25–30 °C was added pyridine (5.9 g, 0.0746 mol). The reaction mixture was then cooled to 0–10 °C under nitrogen atmosphere. To this was added $POCl_3$ (4.57 g, 0.0298 mol) drop wise by maintaining exothermicity. After stirring at 25–30 °C for 1 h, reaction mixture was poured in cooled water and filtered. Solid obtained was stirred in saturated solution of sodium bicarbonate for 10 min. Filtration and drying afforded title compound **13a** (7 g, 90%) as pale yellow solid: mp 182–183 °C; purity by HPLC: 96.28%; IR (KBr) 1691, 1575, 1525, 1461, 1375, 1299, 1114, 1016, 914, 842, 761,678 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.80–7.83 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (s, 1H), 7.96–7.99 (dd, J = 2.4 and 8.8 Hz, 1H), 8.16–8.19 (dd, J = 4.0 and 8.8 Hz, 2H), 8.41 (s, 1H), 11.43 (s, 1H); ESI/MS m/z 309.8 $(M - H)$.

*5.1.1.18. N-(5-Chloropyridin-2-yl)-5-methoxy-2-nitrobenzamide (**13b**).* This compound was prepared from **11b** by means of the procedure similar to that reported for **13a** as off white solid; yield: 94%; mp 155–158 °C; purity by UPLC: 97.65%; IR (KBr) 1691, 1579, 1517, 1461, 1377, 1234, 1110, 1070, 835, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.91 (s, 3H), 7.19–7.24 (m, 2H), 7.96–7.99 (dd, J = 2.4 and 8.8 Hz, 1H), 8.16–8.22 (m, 2H), 8.40 (d, J = 1.6 Hz, 1H), 11.30 (s, 1H); ESI/MS m/z 329.8 $(M + Na)^+$.

*5.1.1.19. N-(5-Chloropyridin-2-yl)-3-methoxy-2-nitrobenzamide (**13c**).* This compound was prepared from **11c** by means of the procedure similar to that reported for **13a** as off white solid; yield: 97%; mp 198–200 °C; purity by UPLC: 99.22%; IR (KBr) 1691, 1577, 1537, 1473, 1375, 1307, 1276, 1058, 852, 792 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.92 (s, 3H), 7.44 (d, J = 7.6 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.66 (t, J = 8 Hz, 1H), 7.94–7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.07 (d, J = 8.8 Hz, 1H), 8.44 (d, J = 2.4 Hz, 1H), 11.47 (s, 1H); ESI/MS m/z 306.7 $(M - H)$.

*5.1.1.20. 2-Amino-5-chloro-N-(5-chloropyridin-2-yl)benzamide (**14a**).* To a stirring solution of **13a** (5 g, 0.016 mol) in 50 mL ethyl acetate at 25–30 °C was added stannous chloride dihydrate (18 g, 0.08 mol). After stirring at same temperature for 2 h, reaction mixture was diluted with ethyl acetate (10 v/w) and made alkaline with aqueous ammonia solution. Reaction mixture was then filtered through hyflow bed and organic layer was dried over sodium sulfate. Evaporation of solvent afforded **14a** (3.2 g, 71%) as pale yellow solid; mp 182–184 °C; purity by UPLC: 99.22%; IR (KBr) 3489, 3377, 1658, 1614, 1571, 1373, 1294, 1087, 850, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 6.54 (s, 2H), 6.78 (d, J = 8.8 Hz, 1H), 7.20–7.23 (dd, J = 2.4 and 8.8 Hz, 1H), 7.76 (d, J = 2.4 Hz, 1H), 7.90–7.93 (dd, J = 2.4 and 8.8 Hz, 1H), 8.08–8.10 (dd, J = 0.4 and 8.8 Hz, 1H), 8.412–8.419 (dd, J = 0.4 and 2.8 Hz, 1H), 10.78 (s, 1H); ESI/MS m/z 303.7 $(M + Na)^+$.

*5.1.1.21. 2-Amino-N-(5-chloropyridin-2-yl)-5-methoxybenzamide (**14b**).* This compound was prepared from **13b** by means of the procedure similar to that reported for **14a** as off white solid; yield: 75%; mp 69–71 °C; purity by UPLC: 99.16%; IR (KBr) 3462, 3367, 1658, 1593, 1571, 1514, 1375, 1298, 1159, 1091, 856, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.72 (s, 3H), 6.01 (s, 2H), 6.73 (d, J = 8.8 Hz, 1H), 6.87–6.90 (dd, J = 2.8 and 8.8 Hz, 1H), 7.20 (d, J = 2.8 Hz, 1H), 7.90–7.93 (dd, J = 2.4 and 8.8 Hz, 1H), 8.11–8.14 (dd, J = 0.4 and 8.8 Hz, 1H), 8.40–8.41 (dd, J = 0.8 and 2.8 Hz, 1H), 10.73 (s, 1H); ESI/MS m/z 277.8 $(M + H)^+$.

*5.1.1.22. 2-Amino-N-(5-chloropyridin-2-yl)-3-methoxybenzamide (**14c**).* This compound was prepared from **13c** by means of the procedure similar to that reported for **14a** as off white solid; yield: 60%; mp 137–139 °C; purity by UPLC: 98.34%; IR (KBr) 3493, 3367, 1658, 1612, 1587, 1552, 1500, 1373, 1226, 1080, 846, 740 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.80 (s, 3H), 6.14 (s, 2H), 6.55 (t, J = 8 Hz, 1H), 6.96 (t, J = 6.8 Hz, 1H), 7.37–7.40 (dd, J = 1.2 and 8.4 Hz, 1H), 7.90–7.93 (dd, J = 2.8 and 8.8 Hz, 1H), 8.10–8.12 (dd, J = 0.4 and 9.2 Hz, 1H), 8.40–8.41 (dd, J = 0.8 and 2.8 Hz, 1H), 10.54 (s, 1H); ESI/MS m/z 277.9 $(M + H)^+$.

*5.1.1.23. 2-Amino-5-chloro-N-(5-chloropyridin-2-yl)-3-methoxybenzamide (**14d**).* To a stirring solution of **14c** (15.5 g, 0.0558 mol) in 155 mL benzene at 25–30 °C was added NCS (8.17 g, 0.0614 mol). Reaction mixture was then heated at 60–65 °C for 24 h. Excess solvent

was removed under vacuum. Water was added to it and product was extracted with ethyl acetate. Ethyl acetate was dried over sodium sulfate and evaporated to afford the title compound **14d** (12.3 g, 71%) as off white solid: mp 116–118 °C; purity by UPLC: 98.15%; IR (KBr) 3485, 3367, 1712, 1666, 1593, 1573, 1519, 1375, 1303, 1238, 1051, 833, 740 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.83 (s, 3H), 6.24 (s, 2H), 6.99 (d, J = 2 Hz, 1H), 7.47 (d, J = 2 Hz, 1H), 7.91–7.94 (dd, J = 2.4 and 8.8 Hz, 1H), 8.07–8.09 (dd, J = 0.8 and 9.2 Hz, 1H), 8.41–8.42 (dd, J = 0.8 and 2.8 Hz, 1H), 10.73 (s, 1H); ESI/MS m/z 312 $(M + H)^+$.

*5.1.1.24. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methylsulfonimidoyl)benzamido)benzamide (**15a**).* To a stirring solution of **10a** (2 g, 0.0060 mol) and DMF (one drop) in DCM (10 v/w) cooled at 10–15 °C was added oxalyl chloride (0.91 g, 0.0072 mol) under nitrogen atmosphere. Reaction mixture was stirred at 25–30 °C for 3 h and then evaporated to dryness. Acid chloride obtained was dissolved in dry THF (8 mL) and then added to the solution containing **14a** (1.52 g, 0.0054 mol) in 6 mL THF cooled at 10–15 °C. After stirring at 25–30 °C for 2 h, reaction mixture was quenched with water (10 v/w). Product was extracted with chloroform which on drying over sodium sulfate, evaporation gave solid product.

The crude product obtained above was added to sulfuric acid (5 v/w) cooled at 0–5 °C. The reaction mixture was stirred at same temperature for 15–20 min. Above reaction mixture was slowly poured in chilled water and basified using aqueous potassium carbonate solution. Precipitated product was filtered and washed with water. Drying afforded **15a** (1 g, 64%) as off white solid: mp 239–242 °C; purity by UPLC: 98.19%; IR (KBr) 3315, 1685, 1651, 1602, 1573, 1519, 1460, 1371, 1292, 1217, 1030, 1006, 833, 746, 677 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.11 (s, 3H), 4.40 (s, 1H), 7.66–7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.94–7.97 (dd, J = 2.4 and 8.8 Hz, 1H), 8.07 (bs, 4H), 8.12 (d, J = 4.4 Hz, 1H), 8.14 (d, J = 4.4 Hz, 1H), 8.44 (d, J = 2.4 Hz, 1H), 11.19 (s, 1H), 11.27 (s, 1H); ESI/MS m/z: 462.9 $(M + H)^+$.

*5.1.1.25. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-((S-methylsulfonimidoyl)methyl)benzamido)benzamide (**15b**).* This compound was prepared from **10b** and **14a** by means of the procedure similar to that reported for **15a** as off white solid; yield: 63%; mp 217–219 °C; purity by HPLC: 96.07%; IR (KBr) 3178, 1674, 1652, 1602, 1575, 1521, 1460, 1375, 1296, 1209, 1035, 831, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.78 (s, 3H), 3.69 (s, 1H), 4.41–4.50 (q, J = 13.6 Hz, 2H), 7.59 (d, J = 8.8 Hz, 2H), 7.64–7.67 (dd, J = 2.4 and 8.8 Hz, 1H), 7.89 (d, J = 8 Hz, 2H), 7.91–7.95 (m, 2H), 8.11 (d, J = 8 Hz, 1H), 8.19 (d, J = 8.8 Hz, 1H), 8.43 (d, J = 2.4 Hz, 1H), 11.18 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 499 $(M + Na)^+$.

*5.1.1.26. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(ethylsulfonimidoyl)benzamido)benzamide (**15c**).* This compound was prepared from **10c** and **14a** by means of the procedure similar to that reported for **15a** as off white solid; yield: 67%; mp 213–215 °C; purity by HPLC: 97.47%; IR (KBr) 3259, 1735, 1666, 1602, 1583, 1571, 1517, 1438, 1373, 1218, 1002, 829, 756 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.05 (t, J = 7.2 Hz, 3H), 3.14–3.19 (q, J = 7.2 Hz, 2H), 4.37 (s, 1H), 7.65–7.68 (dd, J = 2.4 and 8.8 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.92–7.95 (dd, J = 2.8 and 8.8 Hz, 1H), 8.01 (d, J = 8.8 Hz, 2H), 8.07 (d, J = 8.4 Hz, 2H), 8.09–8.12 (dd, J = 2.4 and 8.8 Hz, 2H), 8.43 (d, J = 2.8 Hz, 1H), 11.17 (s, 1H), 11.25 (s, 1H); ESI/MS m/z 477 $(M + H)^+$.

*5.1.1.27. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(2-hydroxyethylsulfonimidoyl)benzamido)benzamide (**15d**).* This compound was prepared from **10d** and **14a** by means of the procedure similar to that reported for **15a**. Deprotection of CBZ group and methoxy group was achieved as follows: To a stirring solution of 2-(4-(*N*-benzyloxycarbonyl-2-methoxyethylsulfonimidoyl)benzamido)-5-chloro-*N*-(5-chloropyridin-2-yl)benzamide (300 mg, 0.00046 mol) in 3 mL DCM cooled at −30 °C was added BBr_3 (0.577 g. 0.0023 mol) under nitrogen atmosphere. Reaction mixture was stirred at 25–30 °C for 3 h. Reaction mixture was diluted with water and basified with aq. sodium carbonate solution. Organic layer was separated, dried and distilled out to get crude product, which was column purified using 100–200 silica gel and 2% methanol in chloroform as mobile phase producing title compound as off white solid; yield: 28%; mp 207–210 °C; purity by UPLC: 96.94%; IR (KBr) 3019, 1731, 1672, 1601, 1584, 1513, 1403, 1375, 1215, 1024, 757, 699 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.33 (t, J = 6.4 Hz, 2H), 3.62–3.67 (m, 2H), 4.47 (s, 1H), 4.79 (t, 1H), 7.65–7.68 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93–7.95 (dd, J = 2.4 and 8.8 Hz, 1H), 8.01–8.04 (m, 4H), 8.10–8.12 (dd, J = 2.8 and 8.8 Hz, 2H), 8.43 (d, J = 2.4 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 492.6 $(M + H)^+$.

*5.1.1.28. N-(5-Chloropyridin-2-yl)-5-methoxy-2-(4-(S-methylsulfonimidoyl)benzamido)benzamide (**15e**).* This compound was prepared from **10a** and **14b** by means of the procedure similar to that reported for **15a** as off white solid; yield: 61%; mp 247–250 °C; purity by UPLC: 95.78%; IR (KBr) 3435, 3288, 1676, 1651, 1608, 1523, 1458, 1373, 1298, 1218, 1072, 835, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.10 (s, 3H), 3.84 (s, 3H), 7.16–7.19 (dd, J = 2.8 and 9.2 Hz, 1H), 7.40 (d, J = 2.8 Hz, 1H), 7.92–7.97 (m, 2H), 8.04 (s, 4H), 8.14 (d, J = 8.8 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 10.97 (s, 1H), 11.11 (s, 1H); ESI/MS m/z 458.9 $(M + H)^+$.

*5.1.1.29. 5-Chloro-N-(5-chloropyridin-2-yl)-3-methoxy-2-(4-(S-methylsulfonimidoyl)benzamido)-benzamide (**15f**).* This compound was prepared from **10a** and **14d** by means of the procedure similar to that reported for **15a** as off white solid; yield: 75%; mp 188–190 °C; purity by UPLC: 97.66%; IR (KBr) 3269, 1664, 1577, 1458, 1375, 1307, 1228, 1064, 839, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.08 (s, 3H), 3.84 (s, 3H), 4.36 (s, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.85–7.88 (dd, J = 2.4 and 8.8 Hz, 1H), 7.98–8.06 (m, 5H), 8.36 (d, J = 2.4 Hz, 1H), 9.98 (s, 1H), 10.87 (s, 1H); ESI/MS m/z 492.6 $(M + H)^+$.

*5.1.1.30. N-(5-Chloropyridin-2-yl)-3-methoxy-2-(4-(S-methylsulfonimidoyl)benzamido) benzamide (**15g**).* This compound was prepared from **10a** and **14c** by means of the procedure similar to that reported for **15a** as off white solid; yield: 65%; mp 185–187 °C; purity by UPLC: 98.82%; IR (KBr) 3277, 1662, 1583, 1518, 1469, 1375, 1266, 1062, 1004, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.08 (s, 3H), 3.82 (s, 3H), 4.36 (s, 1H), 7.26 (d, J = 8 Hz, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 8 Hz, 1H), 7.85–7.88 (dd, J = 2.8 and 9.2 Hz, 1H), 7.99–8.10 (m, 5H), 8.35 (d, J = 2.8 Hz, 1H), 9.92 (s, 1H), 10.62 (s, 1H); ESI/MS m/z 458.8 $(M + H)^+$.

*5.1.1.31. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(methylsulfonyl)sulfonimidoyl)benzamido)benzamide (**16a**).* To a stirring solution of **15a** (0.5 g, 0.0010 mol) and pyridine (0.12 g, 0.0015 mol) in 3 mL DCM cooled at 10–15 °C was added methanesulfonyl chloride (0.137 g, 0.0012 mol) under nitrogen atmosphere. Reaction mixture was stirred at 25–30 °C for 3 h and then quenched with water (10 v/w). Product was extracted with DCM which on drying over sodium sulfate and evaporation gave solid product. Product obtained was column purified using ethyl acetate:hexane mobile phase (0–60%) and 100–200 silica gel to get title compound as off white solid; yield: 51%; mp: 220–222 °C; purity by UPLC: 98.96; IR (KBr) 3342, 1676, 1653, 1599, 1502, 1460, 1371, 1307, 1072, 848, 752 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.04 (s, 3H), 3.66 (s, 3H), 7.67–7.70 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.8 and 8.8 Hz, 1H), 8.08–8.19 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.21 (s, 1H), 11.27 (s, 1H); ESI/MS m/z: 540.9 $(M + H)^+$.

*5.1.1.32. 2-(4-(N-Acetyl-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl) benzamide (**16b**).* This compound was prepared from **15a** and acetyl chloride by means of the procedure similar to that reported for **16a** as off white solid; yield: 61%; mp 215–217 °C; purity by UPLC: 96.00%; IR (KBr) 3431, 1637, 1602, 1575, 1512, 1460, 1373, 1296, 1217, 1033, 831, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.97 (s, 3H), 3.46 (s, 3H), 7.67–7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.4 and 8 Hz, 1H), 8.09–8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.19 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 503.1 (M − H).

*5.1.1.33. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-methoxyacetyl)-S-methylsulfonimidoyl) benzamido)benzamide (**16c**).* This compound was prepared from **15a** and methoxyacetyl chloride by means of the procedure similar to that reported for **16a** as off white solid; yield: 69%; mp: 206–208 °C; purity by UPLC: 96.98%; IR (KBr) 2926, 2823, 1666, 1604, 1573, 1516, 1462, 1373, 1296, 1217, 1116, 922, 837, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.26 (s, 3H), 3.52 (s, 3H), 3.94 (d, J = 2.8 Hz, 2H), 7.66–7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.0 Hz, 1H), 7.93–7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.09–8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.20 (s, 1H), 11.25 (s, 1H); ESI/MS m/z 532.9 (M − H).

*5.1.1.34. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-cyano-S-methylsulfonimidoyl)benzamido)benzamide (**16d**).* To a stirring solution of **15a** (200 mg, 0.000432 mol) in 3 mL DCM was added catalytic amount of DMAP followed by cyanogen bromide (58 mg, 0.000476 mol) under nitrogen atmosphere. Reaction mixture was stirred at 25–30 °C for 6 h and then quenched with water (10 v/w). Product was extracted with DCM which on drying over sodium sulfate and evaporation gave solid product. Product obtained was column purified using ethyl acetate:hexane mobile phase (0–40%) and 100–200 silica gel to get off white solid; yield: 57%; mp: 166–168 °C; purity by UPLC: 96.44%; IR (KBr) 2195, 1710, 1676, 1654, 1602, 1516, 1460, 1375, 1246, 1114, 827, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.80 (s, 3H), 7.67–7.70 (dd, J = 2.4 and 8.4 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.8 and 8.8 Hz, 1H), 8.08 (d, J = 8.8 Hz, 1H), 8.14 (d, J = 8.8 Hz, 1H), 8.19–8.24 (m, 4H), 8.44 (d, J = 2.4 Hz 1H), 11.21 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 487.8 $(M + H)^+$.

*5.1.1.35. 2-(4-(N-Carbamoyl-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl)benzamide (**16e**).* **16d** (100 mg, 0.000205 mol) was added to 2 mL sulfuric acid. Reaction mixture was stirred at 25–30 °C for 6 h and then quenched with water (10 v/w). Solid obtained was filtered, washed with water and dried to get off white solid; yield: 45%; mp 205–208 °C; purity by HPLC: 96.78%; IR (KBr) 3423, 1654, 1600, 1577, 1510, 1460, 1375, 1298, 1226, 1118, 835, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.37 (s, 3H), 6.05 (bs, 1H), 6.45 (bs, 1H), 7.65–7.68 (dd, J = 2.4 and 8.4 Hz, 1H), 7.91 (d, J = 2.8 Hz, 1H), 7.92–7.95 (dd, J = 2.4 and 8.8 Hz, 1H), 8.03–8.11 (m, 5H), 8.13 (d, J = 4.4 Hz, 1H), 8.43 (d, J = 0.4 Hz, 1H), 11.19 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 527.8 $(M + Na)^+$.

*5.1.1.36. 5-Chloro-2-(4-(N-(2-chloroacetyl)-S-methylsulfonimidoyl) benzamido)-N-(5-chloropyridin-2-yl)benzamide (**17a**).* To a stirring solution of **15a** (2 g, 0.0042 mol) and TEA (1.06 g, 0.0105 mol) in 20 mL THF cooled at 0 °C was added chloroacetyl chloride (0.71 g, 0.0063 mol). Reaction mixture was stirred at 25–30 °C for 2 h. Reaction mixture was diluted with water (10 v/w). Precipitated product was filtered and purified by refluxing in solvent mixture of 6 v/w hexane and 3 v/w ethyl acetate. Filtration at 25–30 °C afforded **17a** (1.86 g, 80%) as off white solid; mp 190–193 °C; purity by UPLC: 95.83%; IR (KBr) 1687, 1660, 1602, 1573, 1516, 1460, 1404, 1375, 1298, 1209, 1008, 839, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.57 (s, 3H), 4.27 (s, 2H), 7.67–7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.94–7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.08–8.13 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.19 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 538.9 $(M + H)^+$.

*5.1.1.37. 2-(4-(N-(2-Chloroacetyl)-S-methylsulfonimidoyl)benzamido)-N-(5-chloropyridin-2-yl)-5-methoxybenzamide (**17e**).* This compound was prepared from **15e** by means of the procedure similar to that reported for **17a** as off white solid; yield: 80%; mp 178–180 °C; purity by UPLC: 98.74%; IR (KBr) 1681, 1652, 1610, 1573, 1515, 1461, 1373, 1207, 1028, 839, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.56 (s, 3H), 3.84 (s, 3H), 4.26 (s, 2H), 7.16–7.19 (dd, J = 2.8 and 9.2 Hz, 1H), 7.39 (d, J = 2.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.93–7.95 (dd, J = 2.8 and 5.6 Hz, 1H), 8.10–8.14 (m, 5H), 8.42 (d, J = 2.4 Hz, 1H), 10.97 (s, 1H), 11.11 (s, 1H); ESI/MS m/z 557.1 $(M + Na)^+$.

*5.1.1.38. 5-Chloro-2-(4-(N-(2-chloroacetyl)-S-methylsulfonimidoyl) benzamido)-N-(5-chloropyridin-2-yl)-3-methoxybenzamide (**17f**).* This compound was prepared from **15f** by means of the procedure similar to that reported for **17a** as off white solid; yield: 82%; mp 143–145 °C; purity by UPLC: 90%; IR (KBr) 1656, 1573, 1521, 14,601, 1375, 1315, 1226, 1064, 842, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.54 (s, 3H), 3.85 (s, 3H), 4.26 (s, 2H), 7.29 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 7.85–7.88 (dd, J = 2.8 and 8.8 Hz, 1H), 8.05–8.10 (m, 5H), 8.36 (d, J = 2.8 Hz, 1H), 10.09 (s, 1H), 10.89 (s, 1H); ESI/MS m/z 592.6 $(M + Na)^+$.

*5.1.1.39. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(methylamino)acetyl)sulfonimidoyl)benzamido)benzamide (**18a**).* To a stirring solution of **17a** (1 g, 0.0018 mol) in 5 mL DMF was added 40% aq. CH_3NH_2 solution (0.576 g, 0.0185 mol) followed by catalytic amount of KI. The reaction mixture was stirred at 25–30 °C for 12 h and then diluted with water. Filtration and drying afforded title compound, which was column purified using 230–400 silica gel and methanol:DCM mobile phase (0–3%); yield: 42%; mp: 174–176 °C; purity by UPLC: 98.72%; IR (KBr) 3392, 1683, 1649, 1602, 1573, 1525, 1460, 1373, 1296, 1217, 1114, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.38 (s, 3H), 3.47 (bs, 2H), 3.57 (s, 3H), 7.65 (d, J = 6.8 Hz, 1H), 7.93–7.96 (m, 2H), 8.12–8.20 (m, 6H), 8.45 (d, J = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 33.64, 42.87, 53.56, 116.17, 124.65, 125.68, 126.41, 126.87, 127.58, 128.51, 129.15, 131.52, 137.72, 140.42, 140.75, 146.41, 151.01, 164.01, 166.01, 175.67; ESI/MS m/z 533.9 $(M + H)^+$.

*5.1.1.40. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(ethylamino)acetyl)-S-methylsulfonimidoyl) benzamido)benzamide (**18b**).* 10 mol eq. 70% ethylamine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 48%; mp: 158–160 °C; purity by UPLC: 99.38%; IR (KBr) 3433, 1681, 1639, 1602, 1523, 1458, 1373, 1296, 1224, 1116, 922, 835, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.0 (t, J = 7.2 Hz, 3H), 2.57–2.66 (m, 2H), 3.42 (d, J = 9.6 Hz, 2H), 3.54 (s, 3H), 7.60 (bd, J = 9.2 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93–7.95 (dd, J = 2.8 and 8.0 Hz, 1H), 8.10 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 1H), 8.25 (bs, 3H), 8.45 (d, J = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.92, 42.67, 42.95, 53.30, 116.17, 124.60, 125.35, 126.40, 126.78, 127.37, 128.65, 129.11, 131.39, 137.65, 140.40, 140.77, 146.44, 151.25, 164.37, 165.97, 178.05; ESI/MS m/z 547.8 $(M + H)^+$.

*5.1.1.41. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(isopropylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (**18c**).* 2 mol eq. isopropylamine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 57%; mp: 176–178 °C; purity by UPLC: 99.24%; IR (KBr) 3313, 1681, 1637, 1600, 1523, 1458, 1373,

1296, 1226, 1093, 833, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.97–1.00 (q, 6H), 2.78–2.84 (m, 1H), 3.42 (s, 2H), 3.54 (s, 3H), 7.62 (bd, 1H), 7.91–7.95 (m, 2H), 8.11 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.8 Hz, 1H), 8.31 (bs, 3H), 8.45 (d, J = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 21.71, 42.91, 48.09, 51.35, 116.16, 124.66, 125.43, 126.32, 126.61, 127.40, 128.62, 129.13, 131.42, 137.69, 140.40, 140.72, 146.44, 151.10, 164.33, 165.96, 178.23; ESI/MS m/z 561.9 $(M + H)^+$.

*5.1.1.42. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(cyclopentylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (**18d**).* 2 mol eq. cyclopentylamine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 40%; mp: 141–143 °C; purity by UPLC: 97.52%; IR (KBr) 3416, 1681, 1651, 1602, 1510, 1458, 1373, 1296, 1219, 1114, 920, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.31–1.35 (m, 2H), 1.43–1.46 (m, 2H), 1.54–1.59 (m, 2H), 1.67–1.71 (m, 2H), 3.05–3.08 (m, 1H), 3.41 (s, 2H), 3.54 (s, 3H), 7.64 (bd, 1H), 7.92–7.94 (m, 2H), 8.09–8.19 (m, 6H), 8.45 (d, J = 2.8 Hz, 1H); ESI/MS m/z 587.8 and 589.9 $(M + H)^+$.

*5.1.1.43. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(dimethylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (**18e**).* 10 mol eq. 50%. dimethylamine solution was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 66%; mp: 134–136 °C; purity by UPLC: 99.19%; IR (KBr) 1683, 1647, 1602, 1518, 1458, 1373, 1294, 1217, 1116, 922, 839, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.20 (s, 6H), 3.09 (s, 2H), 3.48 (s, 3H), 7.64–7.66 (dd, J = 2 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.92–7.94 (dd, J = 2.8 and 8.8 Hz, 1H), 8.06–8.13 (m, 6H), 8.43 (d, J = 2.8 Hz, 1H), 11.23 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 42.94, 44.70, 63.86, 116.18, 124.47, 125.80, 126.70, 127.47, 127.87, 128.39, 129.17, 131.63, 137.76, 139.01, 141.58, 146.35, 150.58, 163.97, 166.06, 177.91; ESI/MS m/z 548 $(M + H)^+$.

*5.1.1.44. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (**18f**).* To a stirring solution of **17a** (1 g, 0.0018 mol) in 5 mL DMF was added diethylamine (0.263 g, 0.0036 mol) followed by catalytic amount of KI. The reaction mixture was stirred at 25–30 °C for 16 h and then diluted with water. Filtration and drying afforded title compound, which was column purified using 230–400 silica gel and 0–3% methanol in chloroform as mobile phase to get **18f** (0.710 g, 66%) as off white solid; mp: 170–172 °C; purity by UPLC: 98.91%; IR (KBr) 1687, 1666, 1600, 1573, 1510, 1464, 1398, 1375, 1294, 1217, 1114, 920, 831, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.94 (t, J = 7.2 Hz, 6H), 2.54–2.59 (q, J = 7.2 Hz, 4H), 3.26 (s, 2H), 3.49 (s, 3H), 7.65–7.68 (dd, J = 2.0 and 8.4 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.92–7.95 (dd, J = 2.8 and 8.8 Hz, 1H), 8.07–8.14 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.24 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 12.16, 42.95, 46.87, 57.70, 116.19, 124.52, 125.80, 126.79, 127.48, 127.85, 128.40, 129.19, 131.63, 137.77, 139.07, 141.66, 146.37, 150.64, 164.02, 166.07, 178.89; ESI/MS m/z 576 and 577.6 $(M + H)^+$.

To a stirring solution of **18f** (0.5 g, 0.000869 mol) in 5 mL DCM cooled at 10–15 °C was added HCl:diethyl ether (12% w/w, 0.2 mL). The reaction mixture was stirred at 25–30 °C for 1 h and then diluted with diethyl ether. Precipitated solid was filtered and dried to get monohydrochloride salt of **18f** (0.490 g, 92%) as off white solid; purity by UPLC: 98.14%; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.15–1.23 (m, 6H), 3.06–3.17 (m, 4H), 3.66 (s, 3H), 4.08 (d, J = 5.2 Hz, 2H), 7.67–7.70 (dd, J = 2.4 and 8.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.94–7.97 (dd, J = 2.8 and 8.8 Hz, 1H), 8.09 (d, J = 4.8 Hz, 1H), 8.11 (d, J = 4.8 Hz, 1H), 8.15–8.21 (m, 4H), 8.45 (d, J = 2.4 Hz, 1H), 9.43 (bs, 1H), 11.28 (s, 2H); ESI/MS m/z 575.9 $(M + H)^+$.

*5.1.1.45. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diisopropylamino)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (**18g**).* 2 mol eq. diisopropylamine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 86%; mp: 151–153 °C; purity by UPLC: 97.94%; IR (KBr) 1680, 1656, 1602, 1518, 1460, 1375, 1296, 1213, 1116, 920, 831, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.92 (d, J = 4.8 Hz, 12H), 2.95–2.98 (m, 2H), 3.17 (s, 2H), 3.46 (s, 3H), 7.67 (d, J = 8 Hz, 1H), 7.92–7.95 (dd, J = 2.4 and 8.8 Hz, 2H), 8.06–8.14 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.25 (bs, 2H); ESI/MS m/z 604 $(M + H)^+$.

*5.1.1.46. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(pyrrolidin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (**18h**).* 2 mol eq. pyrrolidine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 69%; mp: 133–135 °C; purity by UPLC: 99.47%; IR (KBr) 1685, 1656, 1602, 1573, 1518, 1458, 1373, 1296, 1203, 1116, 922, 839, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.67 (bs, 4H), 2.60–2.61 (bd, 4H), 3.51 (s, 3H), 7.65–7.68 (dd, J = 2.0 and 8.4 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.93–7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.08–8.14 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.25 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 23.26, 42.94, 53.25, 60.20, 116.20, 124.60, 125.80, 126.95, 127.51, 127.93, 128.43, 129.20, 131.63, 137.79, 139.06, 141.49, 146.39, 150.61, 164.01, 166.06, 177.39; ESI/MS m/z 574 and 575.7 $(M + H)^+$.

*5.1.1.47. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(piperidin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (**18i**).* 2 mol eq. piperidine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 91%; mp: 125–127 °C; purity by UPLC: 98.90%; IR (KBr) 1681, 1654, 1600, 1510, 1458, 1374, 1296, 1217, 1112, 920, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.29 (bs, 2H), 1.34–1.46 (bs, 4H), 2.42–2.50 (bs, 4H), 3.15 (bs, 2H), 3.50 (s, 3H), 7.68 (d, J = 7.2 Hz, 1H), 7.91–7.96 (m, 2H), 8.07–8.13 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.27 (bd, 2H); ESI/MS m/z 587.9 and 589.9 $(M + H)^+$.

*5.1.1.48. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-morpholinoacetyl) sulfonimidoyl)benzamido)benzamide (**18j**).* 2 mol eq. morpholine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 60%; mp: 135–137 °C; purity by UPLC: 98.71%; IR (KBr) 1681, 1656, 1600, 1575, 1510, 1458, 1373, 1296, 1215, 1112, 918, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.45 (t, J = 4.4 Hz, 4H), 3.12 (s, 2H), 3.50 (s, 3H), 3.53 (t, J = 4.8 Hz, 4H), 7.66–7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.07–8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.19 (s, 1H), 11.27 (s, 1H); ESI/MS m/z 590.1 $(M + H)^+$.

*5.1.1.49. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(4-methylpiperazin-1-yl)acetyl) sulfonimidoyl)benzamido)benzamide (**18k**).* 2 mol eq. *N*-methylpiperazine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 60%; mp: 178–180 °C; purity by UPLC: 98.83%; IR (KBr) 1689, 1653, 1602, 1516, 1460, 1375, 1296, 1224, 1010, 920, 823, 742 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.14 (s, 3H), 2.33 (bs, 4H), 2.45 (bs, 4H), 3.10 (s, 2H), 3.49 (s, 3H), 7.67 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.92–7.95 (dd, J = 2.8 and 9.2 Hz, 1H), 8.07–8.15 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.25 (bs, 2H); ESI/MS m/z 603.2 $(M + H)^+$.

*5.1.1.50. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(4-methyl-1,4-diazepan-1-yl)acetyl)sulfonimidoyl)benzamido)benzamide (**18l**).* 2 mol eq. *N*-methylhomopiperazine was used and this

compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 69%; mp: 177–180 °C; purity by UPLC: 98.57%; IR (KBr) 1654, 1600, 1575, 1510, 1460, 1373, 1298, 1215, 1112, 920, 833, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.86 (bs, 2H), 2.68 (s, 3H), 2.78 (bs, 2H), 2.91 (bs, 2H), 2.99–3.15 (m, 4H), 3.50 (s, 2H), 3.65 (s, 3H), 7.69 (d, J = 7.6 Hz, 1H), 7.92–7.96 (bd, 2H), 8.10–8.12 (bd, 6H), 8.45 (s, 1H), 11.27 (bs, 2H); ESI/MS m/z 616.8 and 618.8 $(M + H)^+$.

*5.1.1.51. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(4-hydroxypiperidin-1-yl)acetyl)-S-methylsulfonimidoyl)benzamido) benzamide (**18m**).* 2 mol eq. 4-hydroxyopiperidine was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 75%; mp: 204–206 °C; purity by UPLC: 96.75%; IR (KBr) 3417, 1681, 1653, 1604, 1573, 1521, 1460, 1373, 1294, 1215, 1114, 920, 837, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.37 (bs, 2H), 1.66 (bs, 2H), 2.20 (bs, 2H), 2.72 (bs, 2H), 3.12 (bs, 2H), 3.35–3.40 (m, 1H), 3.50 (s, 3H), 4.55 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.4 and 8.8 Hz, 1H), 8.08–8.14 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.20 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 604.1 and 605.8 $(M + H)^+$.

*5.1.1.52. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(4-aminocarbonyl-1-piperidinyl)acetyl)-S-methylsulfonimidoyl)benzamido)benzamide (**18n**).* 2 mol eq. piperidine-4-carboxamide was used and this compound was prepared from **17a** by means of the procedure similar to that reported for **18a** as off white solid; yield: 42%; mp: 246–248 °C; purity by UPLC: 98.55%; IR (KBr) 3408, 3155, 1689, 1662, 1602, 1521, 1460, 1373, 1294, 1215, 1120, 922, 839, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.49–1.55 (m, 2H), 1.59–1.62 (m, 2H), 1.96–2.08 (m, 3H), 2.82 (bs, 2H), 3.11 (bs, 2H), 3.49 (s, 3H), 6.7 (s, 1H), 7.19 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.8 and 8.8 Hz, 1H), 8.07–8.14 (m, 6H), 8.44 (d, J = 2.8 Hz, 1H), 11.19 (s, 1H), 11.25 (s, 2H); ESI/MS m/z 630.8 $(M + H)^+$.

*5.1.1.53. N-(5-Chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methylsulfonimidoyl) benzamido)-5-methoxybenzamide (**18o**).* 2 mol eq. diethylamine was used and this compound was prepared from **17e** by means of the procedure similar to that reported for **18a** as off white solid; yield: 41%; mp: 113–115 °C; purity by UPLC: 99.58%; IR (KBr) 1656, 1610, 1573, 1529, 1460, 1375, 1299, 1220, 1074, 837, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.92 (t, J = 7.2 Hz, 6H), 2.49–2.54 (m, 4H), 3.21 (s, 2H), 3.47 (s, 3H), 3.84 (s, 3H), 7.18 (d, J = 8 Hz, 1H), 7.39 (s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 8.05–8.14 (m, 5H), 8.41 (s, 1H), 10.96 (s, 1H), 11.09 (s, 1H); ESI/MS m/z 572 $(M + H)^+$.

*5.1.1.54. 5-Chloro-N-(5-chloropyridin-2-yl)-3-methoxy-2-(4-(S-methyl-N-(2-(methylamino)acetyl)sulfonimidoyl)benzamido) benzamide (**18p**).* 10 mol eq. 40% aq. CH_3NH_2 solution was used and this compound was prepared from **17f** by means of the procedure similar to that reported for **18a** as off white solid; yield: 64%; mp: 157–160 °C; purity by UPLC: 97.68%; IR (KBr) 3263, 1662, 1573, 1529, 1458, 1375, 1307, 1222, 1064, 840, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.22 (s, 3H), 3.20 (d, 2H), 3.47 (s, 3H), 3.84 (s, 3H), 7.30 (d, J = 2.4 Hz, 1H), 7.36 (s, 1H), 7.85–7.88 (dd, J = 2.4 and 8.8 Hz, 1H), 8.02–8.09 (m, 5H), 8.36 (d, J = 2.4 Hz, 1H), 10.10 (bs, 1H), 10.90 (bs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 34.63, 42.92, 54.96, 56.63, 114.31, 115.24, 120.14, 123.03, 125.47, 127.32, 128.65, 131.40, 135.17, 137.83, 138.61, 141.03, 146.34, 150.60, 155.35, 164.77, 177.61; ESI/MS m/z 563.7 $(M + H)^+$.

*5.1.1.55. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(ethylamino) acetyl)-S-methylsulfonimidoyl) benzamido)-3-methoxybenzamide (**18q**).* 10 mol eq. 70% ethylamine was used and this compound was prepared from **17f** by means of the procedure similar to that reported for **18a** as off white solid; yield: 53%; mp: 178–180 °C; purity by UPLC: 96.77%; IR (KBr) 3433, 1664, 1573, 1529, 1460, 1375, 1307, 1224, 1114, 1064, 840, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.10 (t, J = 7.2 Hz, 3H), 2.79 (t, J = 7.2 Hz, 2H), 3.58 (s, 3H), 3.86 (s, 3H), 7.31 (s, 1H), 7.39 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H), 8.12 (s, 4H), 8.38 (s, 1H), 10.10 (bs, 1H), 10.81 (bs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 11.83, 42.05, 42.85, 51.04, 56.66, 114.35, 115.28, 120.19, 123.07, 125.51, 127.44, 128.70, 131.47, 135.22, 137.83, 138.74, 140.59, 146.38, 150.62, 155.37, 164.82, 177.63; ESI/MS m/z 578.0 $(M + H)^+$.

*5.1.1.56. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)acetyl)-S-methylsulfonimidoyl)benzamido)-3-methoxybenzamide (**18r**).* 2 mol eq. diethylamine was used and this compound was prepared from **17f** by means of the procedure similar to that reported for **18a** as off white solid; yield: 64%; mp: 168–170 °C; purity by UPLC: 98.31%; IR (KBr) 1666, 1573, 1521, 1458, 1375, 1307, 1220, 1114, 1066, 839, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.95 (t, J = 3.2 Hz, 6H), 2.49–2.52 (m, 4H), 3.47 (s, 2H), 3.86 (s, 3H), 7.30 (d, J = 2.0 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 7.86–7.89 (dd, J = 2.4 and 8.8 Hz, 1H), 8.01–8.10 (m, 5H), 8.37 (d, J = 2.4 Hz, 1H), 10.07 (s, 1H), 10.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 12.14, 42.98, 46.88, 56.63, 57.64, 114.29, 115.33, 120.45, 122.95, 125.46, 127.20, 128.62, 131.44, 135.24, 137.85, 138.43, 141.46, 146.31, 150.59, 155.36, 164.75, 177.83; ESI/MS m/z 605.9 $(M + H)^+$.

*5.1.1.57. 5-Chloro-N-(5-chloropyridin-2-yl)-3-methoxy-2-(4-(S-methyl-N-(2-(pyrrolidin-1-yl)acetyl)sulfonimidoyl)benzamido)benzamide (**18s**).* 2 mol eq. pyrrolidine was used and this compound was prepared from **17f** by means of the procedure similar to that reported for **18a** as off white solid; yield: 74%; mp: 154–156 °C; purity by UPLC: 97.66%; IR (KBr) 1687, 1656, 1575, 1521, 1460, 1377, 1309, 1215, 1066, 842, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.67 (bs, 4H), 2.58 (bs, 4H), 3.48 (s, 3H), 3.86 (s, 3H), 7.30 (s, 1H), 7.38 (s, 1H), 7.86–7.89 (dd, J = 2.4 and 8.8 Hz, 1H), 8.02–8.10 (m, 5H), 8.37 (d, J = 1.6 Hz, 1H), 10.07 (s, 1H), 10.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 23.28, 42.97, 53.19, 56.62, 60.29, 114.29, 115.23, 120.12, 123.10, 125.45, 127.20, 128.62, 131.41, 135.20, 137.84, 138.45, 140.97, 146.31, 150.58, 155.35, 164.74, 177.43; ESI/MS m/z 604.0 $(M + H)^+$.

*5.1.1.58. 2-(4-(N-Methoxycarbonylmethyl-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl)benzamide (**19**).* To a stirring solution of **15a** (5 g, 0.0108 mol) in 15 mL DMF cooled at 10–15 °C was added sodium hydride (1.76 g, 0.0367 mol) under nitrogen atmosphere. To this was added methyl bromoacetate (2.48 g, 0.0162 mol) in one lot. Reaction mixture was stirred at 40 °C for 3 h and cooled to 20 °C. Product was diluted with water and extracted with DCM. Crude product was column purified using 100–200 silica gel and 0–50% ethyl acetate in hexane as mobile phase to get **19** (1.1 g, 20%) as off white solid; mp: 211–213 °C; purity by UPLC: 94.47%; IR (KBr) 1747, 1678, 1656, 1600, 1572, 1512, 1460, 1375, 1296, 1153, 837, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.26 (s, 3H), 3.49–3.66 (q, J = 16.8 Hz, 2H), 3.55 (s, 3H), 7.66–7.69 (dd, J = 2.8 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.94–7.97 (dd, J = 2.8 and 8.8 Hz, 1H), 8.01–8.13 (m, 6H), 8.44 (d, J = 2.4 Hz, 1H), 11.17 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 532.9 $(M - H)$.

*5.1.1.59. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-hydroxyethyl)-S-methylsulfonimidoyl)benzamido)benzamide (**20**).* To a stirring solution of **19** (2 g, 0.00374 mol) in 10 mL DMSO at 20–25 °C was added sodium borohydride (0.71 g, 0.0149 mol). Reaction mixture was stirred at 60 °C for 3 h and cooled to 25 °C. Product was diluted with water and extracted with DCM.

The organic phase was dried over sodium sulfate and evaporated to afford crude product. Product obtained was column purified using 100–200 silica gel and 0–2% methanol in DCM to get **20** (1.02 g, 54%) as off white solid; mp: 210–212 °C; purity by UPLC: 96.52%; IR (KBr) 3416, 1680, 1656, 1602, 1514, 1460, 1373, 1294, 1220, 1139, 1070, 920, 831, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.70–2.75 (m, 1H), 2.83–2.89 (m, 1H), 3.18 (s, 3H), 3.37–3.42 (m, 2H), 4.42 (t, J = 6 Hz, 1H), 7.66–7.69 (dd, J = 2.8 and 8.8 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.94–7.97 (dd, J = 2.4 and 8.8 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 8.13 (d, J = 8.8 Hz, 2H), 8.44 (d, J = 2.0 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 506.9 and 508.6 $(M + H)^+$.

*5.1.1.60. 2-(4-(N-(2-Bromoethyl)-S-methylsulfonimidoyl)benzamido)-5-chloro-N-(5-chloropyridin-2-yl) benzamide (**21**).* To a stirring solution of **20** (1.2 g, 0.0023 mol) in 15 mL DCM at 25–30 °C was added triphenylphosphine (0.92 g, 0.0035 mol) and carbon tetrabromide (1.1 g, 0.0035 mol). Reaction mixture was stirred at 30 °C for 3 h and cooled to 20 °C. Product was diluted with water and extracted with DCM. The organic phase was dried over sodium sulfate and evaporated to afford crude product. Product obtained was column purified using 100–200 silica gel and 0–40% ethyl acetate in hexane to get **21** (0.270 g, 20%) as off white solid; mp: 198–200 °C; purity by HPLC: 96.15%; IR (KBr) 1676, 1600, 1573, 1514, 1458, 1373, 1294, 1130, 920, 833, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.02–3.09 (m, 1H), 3.16–3.22 (m, 1H), 3.24 (s, 3H), 3.48 (t, J = 6.8 Hz, 2H), 7.66–7.69 (dd, J = 2.4 and 8.8 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.93–7.96 (dd, J = 2.8 and 9.2 Hz, 1H), 8.02–8.04 (bd, 2H), 8.09–8.13 (m, 4H), 8.44 (d, J = 2.4 Hz, 1H), 11.18 (s, 1H), 11.26 (s, 1H); ESI/MS m/z 570.6 $(M + H)^+$.

*5.1.1.61. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(dimethylamino)ethyl)-S-methylsulfonimidoyl)benzamido)benzamide (**22a**).* 10 mol eq. 50% dimethylamine was used and this compound was prepared from **21** by means of the procedure similar to that reported for **18a** as off white solid; yield: 26%; mp: 131–133 °C; purity by UPLC: 99.06%; IR (KBr) 1684, 1635, 1575, 1507, 1459, 1374, 1296, 1229, 835, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.11 (s, 6H), 2.31–2.38 (bd, 2H), 2.72–2.78 (m, 1H), 2.87–2.93 (m, 1H), 3.17 (s, 3H), 7.64 (d, J = 8.8 Hz, 1H), 7.91 (bs, 2H), 7.99 (d, J = 8.4 Hz, 2H), 8.12–8.14 (bd, 4H), 8.42 (d, J = 2.0 Hz, 1H), 11.93 (bs, 1H); ESI/MS m/z 533.9 $(M + H)^+$.

*5.1.1.62. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(N-(2-(diethylamino)ethyl)-S-methylsulfonimidoyl)benzamido)benzamide (**22b**).* 2 mol eq. diethylamine was used and this compound was prepared from **21** by means of the procedure similar to that reported for **18a** as off white solid; yield: 60%; mp: 63–65 °C; purity by UPLC: 98.34%; IR (KBr) 1654, 1577, 1506, 1458, 1373, 1294, 1072, 831, 744 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.98 (t, J = 7.2 Hz, 6H), 2.46–2.53 (m, 4H), 2.64 (t, J = 4.0 Hz, 2H), 2.87–2.95 (m, 1H), 3.04–3.09 (m, 1H), 3.12 (s, 3H), 7.58–7.61 (dd, J = 2.4 and 8.8 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.76–7.79 (dd, J = 2.8 and 8.8 Hz, 1H), 8.08 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H), 8.28–8.38 (m, 2H), 8.87 (d, J = 8.8 Hz, 1H), 11.93 (bs, 2H); ESI/MS m/z 561.6 $(M + H)^+$.

*5.1.1.63. 5-Chloro-N-(5-chloropyridin-2-yl)-2-(4-(S-methyl-N-(2-(pyrrolidin-1-yl)ethyl)sulfonimidoyl)benzamido)benzamide (**22c**).* 2 mol eq. pyrrolidine was used and this compound was prepared from **21** by means of the procedure similar to that reported for **18a** as off white solid; yield: 64%; mp: 88–90 °C; purity by UPLC: 99.03%; IR (KBr) 1656, 1573, 1521, 1460, 1375, 1315, 1226, 1064, 842, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.72 (bs, 4H), 2.47 (bs, 4H), 2.59–2.74 (m, 2H), 2.93–3.00 (m, 1H), 3.06–3.18 (m, 1H), 3.13 (s, 3H), 7.58–7.61 (dd, J = 2.4 and 9.2 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.77–7.80 (dd, J = 2.8 and 9.2 Hz, 1H), 8.08 (d, J = 8.4 Hz, 2H), 8.17 (d, J = 8.4 Hz, 2H), 8.19–8.32 (m, 2H), 8.87 (d, J = 8.8 Hz, 1H), 11.95 (bs, 1H); ESI/MS m/z 559.6 and 561.4 $(M + H)^+$.

5.2. Biology

5.2.1. In vitro factor Xa inhibition assay

The inhibitory activity of different compounds against purified serine proteases was measured using chromogenic substrates in 96-well microtiter plates at RT. The enzymes were incubated with test compound or its solvent, dimethyl sulfoxide (DMSO). The plate was incubated at 37 °C for 45 min. At the end of incubation the plate was read at 405 nm using a Spectra Max. The following buffer (final concentrations) was used in the assay: human FXa (10 ng), 50 mM Tris–HCl buffer pH 7.5, 150 μM NaCl, and 1 mM calcium chloride and 500 μM substrate (S-2765) (Hyphen Biomed), 2.5% DMSO with varying concentrations of test compound. The % inhibition was calculated using wells without any inhibitor [only substrate, enzyme and buffer (Test Well)], wells without any enzyme [only substrate and buffer (served as substrate blank wells)]. The IC_{50} was the amount of inhibitor required to inhibit 50% enzymatic activity compared to control.

5.2.2. In vitro prothrombin time (PT) prolongation in human and rat plasma

Blood was obtained from healthy volunteers or rat and anticoagulated with 3.8% sodium citrate. Plasma was obtained after centrifugation at 2000*g* for 10 min. An initial stock solution of the inhibitor was prepared in DMSO. Subsequent dilutions were done in plasma. Clotting time was determined on control plasma and plasma containing five to seven different concentrations of inhibitor. Prothrombin time (PT) measurement was performed in a temperature-controlled automated coagulation device (Sysmex CA50, Dade-Behring) using Thromborel-S (Dade Behring) kit according to the reagent instructions. Determinations at each plasma concentration were done in duplicate. Anticoagulant activity was defined as the concentration required to double the prothrombin time [PTCT2].

5.2.3. Ex vivo PT prolongation in rats

Male rats weighing 230–280 g were used in these studies. In rats, the test drug was formulated in polyethyleneglycol (PEG)-400:0.5% sodium carboxymethylcellulose (1:9) and administered to animals orally at 30 mg/kg and 5 mL/kg dosing volume using a gastric tube. Citrated blood was collected from retro orbital plexus 2 h after oral administration. Platelet-poor plasma was prepared by centrifugation for measurement of PT. All data were expressed as relative fold values, compared with the baseline value of the vehicle group in rat.

5.2.4. Pharmacokinetic study in rats

The hydrochloride salt of **18f** was formulated with Tween-80:PEG:CMC (5:5:90% v/v), A graduated dose volume (5 mL/kg) of suspension was administered to fasted male Wistar rats at 30 mg/kg po. The animals were anesthetized for blood sample collection from retro-orbital plexus. Serial blood samples were collected into heparinised containers at various time points and blood centrifuged to yield plasma. Plasma concentration was determined by using LC–MS/MS method.

5.2.5. Enzyme selectivity of *18f* and *18b*

Reaction mixtures were prepared in 96-well plates containing the chromogenic substrate and test compound. The reaction was initiated by the addition of enzyme, and the color was continuously

monitored at 405 nm using a microplate reader Spectra Max 340PC (Molecular Devices, CA, U.S.) at 37 °C. Each enzyme was used at final concentration as follows: 0.024 U/mL FXa, 0.080 U/mL thrombin, 0.040 μg/mL trypsin, 0.040 U/mL plasmin, 4000 U/mL t-PA and 0.12 μg/mL aPC. The enzymatic activities were assessed by the amidolysis of the following chromogenic substrates for the corresponding protease: S-2765 (FXa), S-2238 (thrombin), S-2222 (trypsin), S-2302 (plasmin), S-2288 (t-PA) and S-2366 (aPC). The rate of substrate hydrolysis (mOD min^{-1}) was measured at 37 °C. The mode of inhibition was estimated from a Lineweaver–Burk plot. The K_i was determined from a Dixon plot by plotting the reciprocal of the initial reaction velocities at different substrate concentrations against different inhibitor concentrations.

5.2.6. $FeCl_3$-induced arterial thrombosis in rats

Male rats ($n = 10$) were treated orally with the hydrochloride salt of **18f** at 10 and 30 mg/kg and 5 mL/kg dosing volume using a gastric tube and then subjected to $FeCl_3$-induced arterial thrombosis after 2 h of administration. Rats were anaesthetized with urethane (1.25 g/kg, intraperitoneally). A midline cervical incision was made on the ventral side of the neck, and left carotid artery was isolated. A 2 × 3 mm strip of Whatman filter paper no. #1 saturated with 35% (w/v) $FeCl_3$ was kept on the carotid artery for 5 min. One hour after removal of the filter paper, the arterial thrombus was excised, blotted of excess blood and immediately weighed.

5.2.7. Partial stasis combined with $FeCl_3$-induced venous thrombosis in rats

Male rats ($n = 10$) were treated orally with the hydrochloride salt of **18f** at 10 and 30 mg/kg and 5 mL/kg dosing volume using a gastric tube and then subjected to $FeCl_3$-induced arterial thrombosis after 2 h of administration. Male Wistar rats (180–250 g) were anesthetized with urethane (1.25 g/kg, intraperitoneally) after 2 h of compound administration. The abdomen was opened by making an incision along the linea alba toward the sternum, followed by exposition of the posterior vena cava. Partial stasis was induced in the posterior vena cava by tying a cotton thread together with a blunt needle (21 G, BD) just caudally of the junction of the posterior vena cava and left renal vein. The needle was then removed. A round piece of Whatmann #1 filter paper saturated with 7 μl of 6% w/v ferric chloride solution was then applied to the external surface of the posterior vena cava for 5 min and then removed. Warm saline was sprayed over tissues, and muscle layer and skin were provisionally closed. One hour after removal of the filter paper, ligatures were applied near the bifurcation of the posterior vena cava and around all side branches of the ligated posterior vena cava segment. The ligated venous segment was excised, the thrombus removed, blotted of excess blood and immediately weighed.

5.3. Docking study

Docking studies of the molecules **18f** and **18b** were carried out to understand the binding mode and intermolecular hydrogen bonding interactions of the molecules with FXa. Glide version 5.7 (GLIDE 5.7, Schrodinger, LLC, New York, NY, 2011) with default parameters and OPLS2005 force-field was used. Molecules were minimized with LigPrep version 2.5 (LigPrep 2.5, Schrodinger, LLC, New York, NY, 2011) and coordinates for the docking study was obtained using 1MQ6 [27] crystal structure from the RCSB protein data bank.

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