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Design, synthesis, and biological activity of non-amidine factor Xa inhibitors containing pyridine *N*-oxide and 2-carbamoylthiazole units

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Abstract—Based on the both of results for X-ray studies of tetrahydrothiazolopyridine derivative **1c** and **FXV673**, we synthesized a series of thiazol-5-ylpyridine derivatives containing pyridine *N*-oxide and 2-carbamoylthiazole units to optimize the S4 binding element. *N*-Oxidation of thiazol-5-ylpyridine increased the anti-fXa activity more than 10-fold independent on the position of *N*-oxide. The 4-pyridine *N*-oxide derivatives **3a** and **3d** excelled over the tetrahydrothiazolopyridine **1b** in potency. 2-Methylpyridine *N*-oxide **3d** exhibited 49-fold selectivity over thrombin. Our modeling study proposed a binding mode that the pyridine *N*-oxide ring of **3a** stuck into the 'cation hole', and the oxide anion of **3a** occupied in the almost same space to that of **FXV673**. From observations of the SAR and modeling studies, we suggested the possibilities that the formation of hydrogen bond with the oxide anion in the 'cation hole' and the affinity of cationic pyridine ring to S4 subsite were responsible for increase in anti-fXa activity.

1. Introduction

Factor Xa (fXa) is a serine protease that plays an important role in converting prothrombin to thrombin to lead blood clot formation within the coagulation cascade. This enzyme is situated at a central position of the intrinsic and extrinsic pathways. Thus, fXa has emerged as an attractive target for development of new antithrombotic agents.[1] We have explored an orally active fXa inhibitor without the amidine group based on the concept that this group was a possible cause for low bioavailability and poor absorption owing to its strong basicity.[2]

We have previously reported[3] our discovery of an orally bioavailable fXa inhibitor **1c** starting from the lead compounds **1a** and **1b** containing the 6-chloronaphthalen as the S1 binding element,[4] which takes the place of amidinobenzene or amidinonaphthalen. In our exploratory studies, X-ray analysis for the crystal complex of Gla-less fXa and **1c** revealed the binding mode in which tetrahydrothiazolopyridine and 6-chloronaphthalene, respectively, functioning as S1 and S4 binding elements.[3b] Furthermore, we had confirmed the existence of an intramolecular S–O close contact on the 2-carbamoylthiazole moiety of **1c** in this crystal complex as shown in Figure 1. It is known that an intramolecular S–O close contact plays an important role on the mechanism of several biological effects.[5] We had suggested that the restriction of conformation by this contact contributed to the affinity of tetrahydrothiazolopyridine for the S4 subsite.

Elevating the potency of these (6-chloronaphthalen-2-yl)sulfonylpiperazine series, we focused our synthetic effort on converting tetrahydrothiazolopyridine leaving the 2-carbamoylthiazole unit to optimize the S4 binding element. Guertin and co-workers reported to the identification of **FXV673**, a potent fXa inhibitor containing

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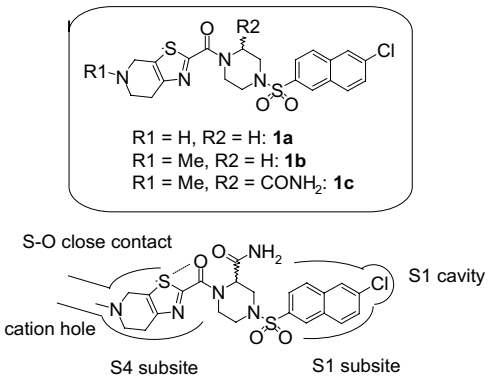


Figure 1. Structures of tetrahydrothiazolopyridine derivatives **1a**–**c** and the outline for X-ray crystal complex of Gla-less fXa and **1c**.

pyridine *N*-oxide, which was the most preferred S4 binding element.[6] Their X-ray study had found that the pyridine *N*-oxide ring was located in the 'cation hole' in the terminus of S4 subsite.[6b] Pyridine *N*-oxide has a dipole-charge, so that cationic pyridine is expected to interact with the aromatic S4 by 'cation-π interaction'.[7] This functional group is a neutral group, it does not contribute to the poor absorption responsible for the ionic form in intestine. In fact, various antagonists, and inhibitors containing this group showed good bioavailabilities.[8] We prompt to utilize the pyridine *N*-oxide unit for design of a fXa inhibitor incorporating or fusing to the 2-carbamoylthiazole. Because since the 2-carbamoylthiazole unit with conformational restriction by S–O close contact is thought to fix the direction of substituents on the thiazole ring, the pyridine *N*-oxide unit is expected to effectively contribute to increase potency occupying the appropriate position in S4. Based on the results of the X-ray study for **1c**, we designed thiazolopyridine *N*-oxides **2** that were expected to have a similar binding mode as **1c** in terms of the fused-cyclic ring adopted the parallel lying to Trp 215 in S4. On the other hand, our strategy directed the design of biphenyl type such as thiazol-5-ylpyridine *N*-oxides **3** in which the pyridine *N*-oxide ring as the 5-positional substituent on the thiazole ring was expected to direct the 'cation hole' like that of **FXV673** (Fig. 2).

In this report, we will discuss the design, synthesis and structure activity relationships (SAR) of non-amidine fXa inhibitors containing thiazolopyridine *N*-oxide and 5-thiazolylpyridine *N*-oxide.

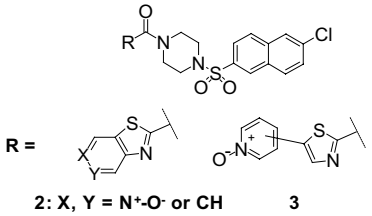
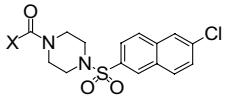


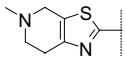
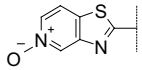
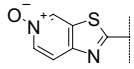
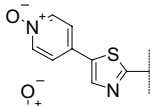
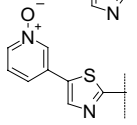
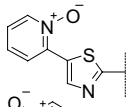
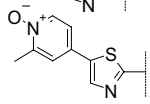
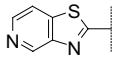
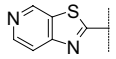
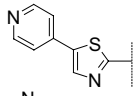
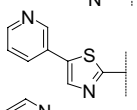
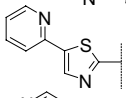
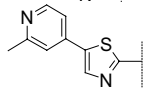
Figure 2. Our inhibitor design utilizing the prydine *N*-oxide and 2-carbamoylthiazole units.

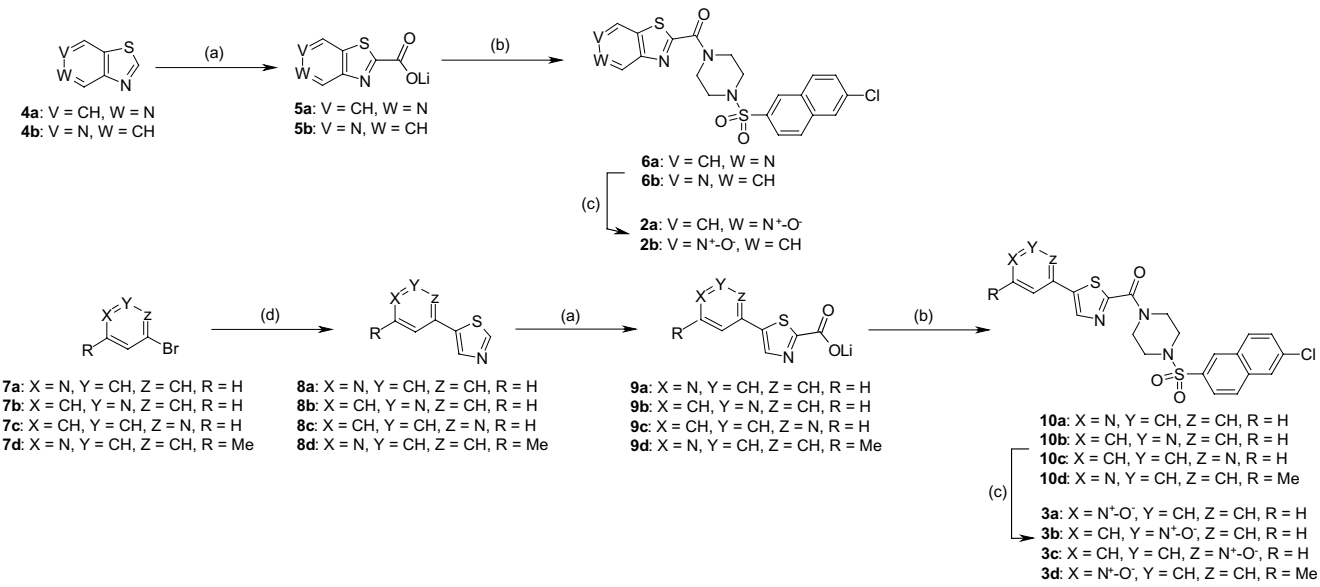
2. Chemistry

Synthetic routes to compounds in Table 1 are shown in Scheme 1. Thiazolopyridines **4a** and **4b**[9] were treated with *n*-BuLi and CO_2 to give lithium thiazolopyridin-2-carboxylates **5a** and **5b**. Bromopyridines **7a**–**d**[10] were coupled with 5-trimethylstanylthiazole[11] by Stille coupling. The resulting 5-pyridylthiazoles **8a**–**d** were treated with *n*-BuLi and CO_2 to give lithium 5-pyridylthiazole-2-carboxylates **9a**–**d**. Condensations of lithium carboxylates with 1-(6-chloronaphthalen-2-yl-sulfonyl)piperazine afforded compounds **6a**,**b**, and **10a**–**d**. The resulting pyridine derivatives were oxidized with *m*-CPBA to give *N*-oxides **2a**,**b**, and **3a**–**d**.

Table 1. In vitro anti-fXa activities for synthetic compounds



Entry	X =	Anti-fXa IC_{50} (nM)
1b		22
2a		>10,000
2b		>10,000
3a		8.6
3b		37
3c		160
3d		13
6a		>10,000
6b		>10,000
10a		96
10b		400
10c		>10,000
10d		160



Scheme 1. Syntheses of **2**, **3**, **6**, and **10**. Reagents and conditions: (a) *n*-BuLi/Et_2O, then CO_2 gas, quant.; (b) 1-(6-chloronaphthalen-2-yl)sulfonylpiperazine hydrochloride, WSCI, HOBt, Et_3N/DMF, 37–82%; (c) *m*-CPBA/CH_2Cl_2, 55–89%; (d) 5-trimethylstanylthiazole, $Pd(PPh_3)_4$/benzene refluxed, 68–92%.

3. Results and discussion

In Table 1, in vitro anti-fXa activities are summarized. Thiazolopyridines **6a**,**b**, and their *N*-oxides **2a**,**b** showed less than 50% of inhibition rates at 10 μM. Maignan and co-workers reported that thienopyridine and pyrrolopyridine functioning as S4 binding elements, which have structural similarity to thiazolopyridine in terms of the fused bi-cyclic pyridine, adopted the vertical standing to Trp 215 in the crystal complex.[12] We had predicted that binding modes of thiazolopyridine **6a**,**b** were similar to that of the tetrahydrothiazolopyridine derivative **1c**, which is the parallel lying to Trp 215.[3b] It is possible that the fused aromatic system, which is parallel lying does not allow for the affinity to S4 subsite. On the other hand, the thiazolylpyridine derivatives showed a different tendency from the thiazolopyridines. Although pyridine compounds **10a**–**d** exhibited weak inhibitory effects, corresponding *N*-oxidations increased potencies more than 10-fold independent on the position of *N*-oxide. It is possible that the cationic pyridine ring interacts with S4 subsite composed of aromatic amino acids. Especially, 4-pyridine *N*-oxide **3a** had the most potent activity and excelled over tetrahydrothiazolopyridine **1b**. 2-Methylpyridine *N*-oxide **3d**, which was expected to interact hydrophobically with S4 had 2-fold less activity relative to **3a**. To aid in our understanding the SAR of the thiazolylpyridines, a modeling study of **3a** with fXa was executed by using the *Cerius2* program. The X-ray coordinate of tetrahydrothiazolopyridine **1c** and Gla-less fXa (PDB code: **1V3X**) was used as the template.[3b] As expected, the 4-pyridine *N*-oxide moiety was located in S4 sticking into the 'cation hole' (Fig. 3). In the X-ray study of **FXV673**, pyridine *N*-oxide was making a hydrogen bond via H_2O with the carbonyl oxygen of main chain of Ile175 in the 'cation hole' as shown in Figure 4.[6b] The superposition of compound

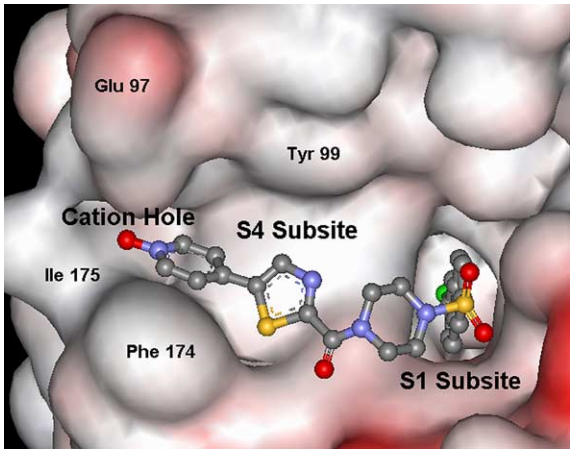


Figure 3. Proposed binding mode of **3a** with fXa. The surface view is the active site of fXa. The ball & stick drawn (gray: carbon, blue: nitrogen, red: oxygen, yellow: sulfur) indicate compound **3a** omitted protons.

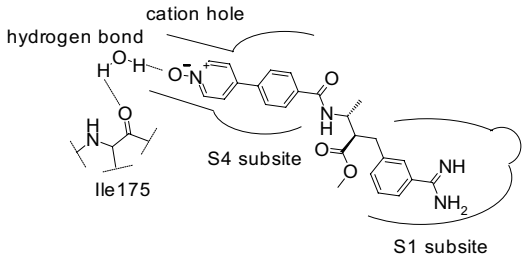


Figure 4. The outline for X-ray crystal complex of Gla-less fXa and **FXV673**.

3a and **FXV673** indicated that oxide anion of **3a** occupied in the almost same space to that of **FXV673**, where

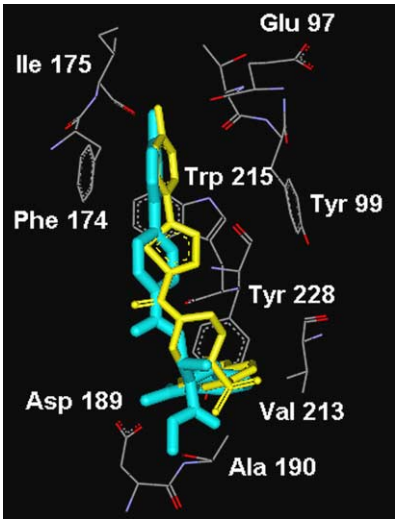


Figure 5. The superposition of compound **3a** and **FXV673**. The structure of **FXV673** picked up from X-ray coordinates (PDB code: **1ksn**), was superposed on the coordinates for the modeling of **3a**. The pale blue line was **FXV673**. The yellow one was compound **3a**.

this hydrogen bond was possible to form (Fig. 5). On the other hand, the carboxylate of Glu 97 veered away from the oxide anion of **3a**, it was possible to cause the charge repulsion. The order of activities for pyridine *N*-oxide derivatives (4-Pyr > 3-Pyr > 2-Pyr) may relate to the distance from the *N*-oxide to the carboxylate of Glu 97. From the results of SAR and modeling studies, we thought possibilities that the formation of hydrogen bond with the oxide anion in the 'cation hole' and the affinity of cationic pyridine ring to S4 subsite were responsible for increase in anti-fXa activity.

Although 4-pyridine *N*-oxide **3a** exhibited the most potent anticoagulant activity in human plasma as shown in Table 2, it did only the weak in rat plasma. Selectivity over thrombin changed depending on the position of the *N*-oxide and the substituent on the pyridine ring. The structural difference of S4 subsite between fXa and thrombin may relate with this change of selectivity.

Table 2. In vitro anti-fXa, antithrombin, and anticoagulant activities of the synthesized compounds

Compd	Anti-fXa IC_{50} (nM)	Anti-thr IC_{50} (nM)	$PTCT_2$ in human plasma (μM)[a]	$PTCT_2$ in rat plasma (μM)[a]
3a	8.6	150	4.0	11.1
3b	37	>5000	7.4	>10
3d	13	640	7.7	>10

[a] Anticoagulant activities in human and rat plasma were evaluated with the plasma clotting time doubling concentration ($PTCT_2$).

The oral activity of compound **3a** was examined by the administration to rats, at a dose of 10 mg/kg. As shown in Table 3, compound **3a** displayed durable and potent anti-fXa activity, and an evident prolongation effect of prothrombin time regardless of its weak anticoagulant activity in vitro. This result may relate with the oral absorption of compound **3a**.

4. Conclusion

Based on the both of results for X-ray studies of tetrahydrothiazolopyridine derivative **1c** and **FXV673**, we synthesized a series of thiazolopyridine and thiazol-5-ylpyridine derivatives containing pyridine *N*-oxide and 2-carbamoylthiazole units to optimize the S4 binding element. *N*-Oxidation of thiazol-5-ylpyridine considerably increased the anti-fXa activity independent on the position of *N*-oxide. The 4-pyridine *N*-oxide derivatives **3a** and **3b** excelled over the tetrahydrothiazolopyridine in potency. Our modeling study proposed a binding mode that the pyridine *N*-oxide ring of **3a** stuck into the 'cation hole', and the oxide anion of **3a** occupied in the almost same space to that of **FXV673**. From observations of the SAR and modeling studies, we suggested the possibility that the formation of hydrogen bond with the oxide anion in the 'cation hole' and the affinity of cationic pyridine ring in S4 subsite were responsible for increase in anti-fXa activity.

5. Experimental section

5.1. Anti-fXa activity in vitro

Anti-fXa activity in vitro was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Cosmo Bio-ERL). Five percent aqueous DMSO (10 L) or inhibitors in aqueous DMSO (10 μL) and 0.05 U/mL human fXa (10 μL) were mixed with 0.1 M Tris–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL). The reaction was started by the addition of 0.75 M S-2222 (40 L). After the mixture was stirred for 10 s at rt, the increase of optical densities (OD/min) were measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows: anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained by plotting the inhibitor concentration against the anti-fXa activity.

Table 3. Ex vivo anti-fXa and anticoagulant activity for compound **3a**

At 10 mg/kg (p.o.) to rats					
Anti-fXa activity (%)			Prolongation effect of PT (% of control)		
1 h	2 h	4 h	1 h	2 h	4 h
81.2 ± 1.4	78.6 ± 0.9	70.9 ± 2.2	109.05 ± 0.25	106.99 ± 0.44	107.05 ± 0.41

Values expressed as mean ± SE from four rats.

5.2. Antithrombin activity in vitro

Antithrombin activity in vitro was measured by using chromogenic substrate S-2266 (Chromogenix, Inc.) and human thrombin (Sigma Chemical, Inc.). Five percent aqueous DMSO (10 μL) or inhibitors in aqueous DMSO (10 μL) and 4 U/mL human thrombin (10 μL) were mixed with 0.1 M Tris–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL). The reaction was started by the addition of 0.50 M S-2266 (40 μL). After the mixture was stirred for 10 s at rt, the increase of optical densities (OD/min) were measured at 405 nm. Antithrombin activity (inhibition %) was calculated as follows: antithrombin activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained from the inhibition % on the statistical probability paper.

5.3. Anticoagulant activity in vitro

Anticoagulant activity in vitro was evaluated with the plasma clotting time doubling concentration for prothrombin time (PTCT2). Plasma (20 μL) was mixed with inhibitors in saline (20 μL) in the process tube. The coagulation was started by the addition of SIMPLASTIN (Organon Teknica, Inc.) (40 μL).

5.4. Anti-fXa activity and anticoagulant activity ex vivo

Male wister rats were fasted overnight. Synthetic compounds were dissolved in polyethylene glycol and administered orally to rats with a stomach tube. For control rats, polyethylene glycol was administered orally. Rats were anesthetized with ravonal at several time points when blood samples were collected in the presence of trisodium citrate. After blood samples were centrifuged, the platelet poor plasma samples were used for measuring their anti-fXa activities or anticoagulant activities. Anti-Xa activity: Plasma (5 μL) was mixed with 0.1 M Tris–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL) H_2O (5 μL) and 0.1 U/mL human fXa (10 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at rt, the increase of optical densities (OD/min) were measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows; anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. Anticoagulant activity: Plasma (20 μL) was mixed with inhibitors in saline (20 μL) in the process tube. The coagulation was started by the addition of SIMPLASTIN (40 μL). Anticoagulant activity was evaluated with the prolongation rate of prothrombin time versus control (% of control).

5.5. Modeling study

Modeling study of compound **3a** and fXa was performed with *Cerius2* program (ver. 4.6, Accelrys Inc.) using the CFF95 force field. Starting conformation of **3a** was constructed with the template picked up from the X-ray coordinate of tetrahydrothiazolopyridine derivative **1c** and Gla-less fXa (PDB code: **1V3X**, Ref. 3b). Some water molecules around the active site of fXa were removed from the complex model, because they seem to have unsuitable contacts with compound **3a**. During the energy minimization of the complex model, the pyridine *N*-oxide ring of **3a**, side chain atoms of Glu97, Thr98, Tyr99, Phe174, Ile175, Ile176, Thr177, Met180, and Trp215 of Gla-less fXa, were allowed to move.

5.6. Chemistry

All solvents and reagents were used as acquired from commercial sources without purification. Melting points were determined on a Yanagimoto apparatus and are uncorrected. Column chromatography was performed on Merck silica gel 60 (0.063–0.200 mm). Thin layer chromatography (TLC) was performed on Merck TLC plates pre-coated with silica gel 60 F_{254}. ^{1}H NMR spectra were recorded on a JEOL JNM-EX400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane, which was used as the internal standard. Mass spectra were performed using a JEOL JMS-AX505W (EI) or a JEOL JMS-HX110 (FD, FAB) spectrometer.

5.6.1. Lithium thiazolo[4,5-*c*]pyridin-2-carboxylate (5a). To a stirred solution of **4a** (600 mg, 4.41 mmol) in dry THF (50 mL) was added *n*-BuLi (1.54 M in hexanes; 3.00 mL, 4.63 mmol) at −78 °C under an argon atmosphere. The reaction mixture was stirred for 20 min at −78 °C. After the bubbling of CO_2 gas for 15 min, the reaction was warmed up to rt. Evaporation of the solvent gave **5a** (1.06 g, quant.) as a pale brown amorphous solid: ^{1}H NMR (DMSO-d_6) δ 8.07 (1H, d, J = 5.4 Hz), 8.48 (1H, d, J = 5.4 Hz), 9.22 (1H, s).

5.6.2. Lithium thiazolo[5,4-*c*]pyridin-2-carboxylate (5b). Starting with **4b** and following the procedure for the preparation of **5a** gave **5b** (quant.) as a pale brown amorphous solid: ^{1}H NMR (DMSO-d_6) δ 7.91 (1H, d, J = 5.9 Hz), 8.56 (1H, d, J = 5.9 Hz), 9.25 (1H, s).

5.6.3. 1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(thiazolo[4,5-*c*]pyridin-2-yl)carbonyl]piperazine hydrochloride (6a). To a mixture of **5a** (1.00 g, 4.41 mmol), 1-(6-chloronaphthalen-2-yl)sulfonylpiperazine hydrochloride (1.13 g, 2.94 mmol) and 1-hydroxybenzotriazole hydrate (450 mg, 2.94 mmol) in DMF (50 mL) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (732 mg, 3.82 mmol). The reaction mixture was stirred for overnight at rt and concentrated in vacuo. To the residue was added CH_2Cl_2 and H_2O. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue using column chromatography (CH_2Cl_2/MeOH, 10/1) gave **6a** (485 mg, 38%) as a colorless solid. Analytical sample of **6a** was obtained according to the following procedure: To the solution of **6a** (80 mg, 0.17 mmol as a free) in CH_2Cl_2 (10 mL) was added 1 N HCl/EtOH (2 mL) and evaporation of the solvent gave a HCl salt of **6a** (65 mg, 77%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 3.27 (4H, br s), 3.90–4.03 (2H, m), 4.61–4.73 (2H, m), 7.58 (1H, dd, J = 8.8, 2.0 Hz), 7.79 (1H, dd, J = 8.8, 2.0 Hz), 7.85–8.01 (4H, m), 8.34 (1H, s), 8.59 (1H, d, J = 5.4 Hz), 9.35 (1H, d, J = 1.0 Hz). MS (FAB) m/z 473 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}ClN_4O_3S_2$·

0.5HCl·0.4H_2O: C, 50.61; H, 3.70; Cl, 10.67; N, 11.24; S, 12.87. Found: C, 50.82; H, 3.85; Cl, 10.40; N, 11.08; S, 12.65.

5.6.4. 1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(thiazolo-[5,4-*c*]pyridin-2-yl)carbonyl]piperazine hydrochloride (6b). Starting with **5b** and following the procedure for the preparation of **6a** gave **6b** (48%) as a colorless solid. Analytical sample was obtained according to the same procedure as **6a**: ^{1}H NMR (DMSO-d_6) δ 3.10–3.30 (4H, m), 3.84 (2H, m), 4.32 (2H, m), 7.69 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8, 2.0 Hz), 8.10–8.30 (4H, m), 8.51 (1H, s), 8.79 (1H, d, J = 5.9 Hz), 9.62 (1H, s). MS (FAB) m/z 473 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}ClN_4O_3S_2$·HCl: C, 49.51; H, 3.56; N, 11.00; Cl, 13.92; S, 12.59. Found: C, 49.45; H, 3.71; N, 11.20; Cl, 13.67; S, 12.55.

5.6.5. 2-[[4-[(6-Chloronaphthalen-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thiazolo[4,5-*c*]pyridine 5-oxide (2a). To a solution of **6a** (355 mg, 0.75 mmol as a free) in CH_2Cl_2 (50 mL) was added *m*-chloroperbenzoic acid (388 mg, 2.25 mmol) at 0 °C. After stirring of the reaction mixture for 2 h, satd Na_2SO_3 (20 mL) was added. The separated mixture was stirred for 1 h. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4. After evaporation of the solvent, to the residue was added H_2O. Collecting the percipitate gave **2a** (330 mg, 88%) as a colorless solid: mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 3.15 (4H, br s), 3.80 (2H, br s), 4.32 (2H, br s), 7.70 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8, 2.0 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.18 (1H, d, J = 8.8 Hz), 8.22 (1H, s), 8.25 (1H, d, J = 8.8 Hz), 8.30 (2H, d, J = 8.8 Hz), 8.51 (1H, s), 9.03 (1H, d, J = 1.5 Hz). MS (FAB) m/z 489 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}ClN_4O_4S_2$·0.5H_2O: C, 50.65; H, 3.64; Cl, 7.12; N, 11.25; S, 12.88. Found: C, 50.94; H, 3.33; Cl, 7.21; N, 10.92; S, 13.10.

5.6.6. 2-[[4-[(6-Chloronaphthalen-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thiazolo[5,4-*c*]pyridine 5-oxide (2b). Starting with **6b** as a free and following the procedure for the preparation of **6a** gave **2b** (76%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 3.15 (4H, br s), 3.80 (2H, br s), 4.39 (2H, br s), 7.69 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8, 1.5 Hz), 8.03 (1H, d, J = 8.3 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.21 (1H, d, J = 1.5 Hz), 8.25 (1H, d, J = 8.8 Hz), 8.29 (1H, d, J = 8.3 Hz), 8.51 (1H, s), 9.09 (1H, d, J = 1.5 Hz). MS (FAB) m/z 489 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}N_4O_4S_2$·0.1CH_2Cl_2: C, 51.06; H, 3.48; Cl, 8.53; N, 11.24; S, 12.86. Found: C, 50.82; H, 3.42; Cl, 8.44; N, 11.14; S, 12.96.

5.6.7. 5-(Pyridin-4-yl)thiazole (8a). The mixture of **7a** (316 mg, 2.00 mmol), 5-trimethylstannylthiazole (496 mg, 2.00 mmol) and $Pd(PPh_3)_4$ (116 mg, 0.100 mmol) in dry benzene (20 mL) was refluxed for 48 h under an argon atmosphere. After evaporation of the solvent, purification of the residue using column chromatography (hexane/AcOEt, 3/1) gave **8a** (293 mg, 90%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.47 (2H, dd, J = 4.9, 2.0 Hz), 8.27 (1H, s), 8.65 (2H, dd, J = 4.9, 2.0 Hz), 8.89 (1H, s). MS (FAB) m/z 163 $(M+H)^+$.

5.6.8. 5-(Pyridin-3-yl)thiazole (8b). Starting with **7b** and following the procedure for the preparation of **8a** gave **8b** (68%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.37 (1H, dd, J = 8.1, 4.9 Hz), 7.88 (1H, d, J = 8.1 Hz), 8.14 (1H, s), 8.60 (1H, d, J = 4.9 Hz), 8.84 (1H, s), 8.86 (1H, s). MS (FAB) m/z 163 $(M+H)^+$.

5.6.9. 5-(Pyridin-2-yl)thiazole (8c). Starting with **7c** and following the procedure for the preparation of **8a** gave **8c** (76%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.22 (1H, t, J = 5.9 Hz), 7.67–7.78 (2H, m), 8.34 (1H, s), 8.60 (1H, d, J = 4.9 Hz), 8.84 (1H, s). MS (FAB) m/z 163 $(M+H)^+$.

5.6.10. 5-(2-Methylpyridin-4-yl)thiazole (8d). Starting with **7d** and following the procedure for the preparation of **8a** gave **8d** (56%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.62 (3H, s), 7.28 (1H, d, J = 5.1 Hz), 7.34 (1H, s), 8.25 (1H, s), 8.53 (1H, d, J = 5.1 Hz), 8.86 (1H, s). MS (FAB) m/z 177 $(M+H)^+$.

5.6.11. Lithium 5-(pyridin-4-yl)thiazole-2-carboxylate (9a). Starting with **8a** and following the procedure for the preparation of **5a** gave **9a** (quant.) as a pale yellow amorphous solid: ^{1}H NMR (DMSO-d_6) δ 7.66 (2H, d, J = 5.4 Hz), 8.37 (1H, s), 8.59 (2H, d, J = 5.4 Hz). MS (FD) m/z 213 $(M+Li+H)^+$.

5.6.12. Lithium 5-(pyridin-3-yl)thiazole-2-carboxylate (9b). Starting with **8b** and following the procedure for the preparation of **5a** gave **9b** (quant.) as a brown amorphous solid: ^{1}H NMR (DMSO-d_6) δ 7.46 (1H, dd, J = 8.1, 7.8 Hz), 8.07 (1H, d, J = 8.1 Hz), 8.21 (1H, s), 8.50–8.55 (1H, m), 8.90 (1H, s).

5.6.13. Lithium 5-(pyridin-2-yl)thiazole-2-carboxylate (9c). Starting with **8c** and following the procedure for the preparation of **5a** gave **9c** (quant.) as a pale yellow amorphous solid: ^{1}H NMR (DMSO-d_6) δ 7.31 (1H, m), 7.85 (1H, t, J = 7.8 Hz), 7.94 (1H, d, J = 7.8 Hz), 8.36 (1H, s), 8.56 (1H, d, J = 4.4 Hz).

5.6.14. Lithium 5-(2-methylpyridin-4-yl)thiazole-2-carboxylate (9d). Starting with **8d** and following the procedure for the preparation of **5a** gave **9d** (quant.) as a brown amorphous solid: ^{1}H NMR (DMSO-d_6) δ 3.34 (3H, s), 7.44 (1H, d, J = 4.6 Hz), 7.53 (1H, s), 8.32 (1H, s), 8.44 (1H, d, J = 4.6 Hz). MS (FAB) m/z 221 $(M+H)^+$.

5.6.15. 1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[[5-(pyridin-4-yl)thiazol-2-yl]carbonyl]piperazine hydrochloride (10a). Starting with **9a** and following the procedure for the preparation of **6a** gave **10a** (82%) as a pale yellow solid. Analytical sample was obtained as a HCl salt according to the same procedure as **6a**: mp 194–197 °C. ^{1}H NMR (DMSO-d_6) δ 3.14 (4H, br s), 3.79 (2H, br s), 4.41 (2H, br s), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8, 2.0 Hz), 8.11 (2H, d, J = 5.9 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.22 (1H, d,

J = 2.0 Hz), 8.25 (1H, d, J = 8.8 Hz), 8.51 (1H, s), 8.77 (1H, s), 8.79–8.85 (2H, m). MS (FAB) *m/z* 499 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}ClN_4O_3S_2 \cdot HCl \cdot 0.1H_2O$: C, 51.42; H, 3.79; Cl, 13.20; N, 10.43; S, 11.94. Found: C, 51.24; H, 3.71; Cl, 13.25; N, 10.31; S, 11.91.

5.6.16. 1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[[5-(pyridin-3-yl)thiazol-2-yl]carbonyl]piperazine hydrochloride (10b). Starting with **9b** and following the procedure for the preparation of **6a** gave **10b** (37%) as a pale yellow solid. Analytical sample was obtained as a HCl salt according to the same procedure as **6a**: mp 226–229 °C. ^{1}H NMR (DMSO-d_6) δ 3.14 (4H, br s), 3.78 (2H, br s), 4.43 (2H, br s), 7.65–7.72 (2H, m), 7.83 (1H, dd, J = 8.8, 2.0 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.23 (1H, s), 8.27 (1H, d, J = 8.8 Hz), 8.35 (1H, d, J = 7.8 Hz), 8.50 (1H, s), 8.51 (1H, s), 8.68 (1H, d, J = 5.1 Hz), 9.07 (1H, d, J = 2.0 Hz). MS (FAB) *m/z* 499 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}ClN_4O_3S_2 \cdot HCl$: C, 51.59; H, 3.76; Cl, 13.24; N, 10.46; S, 11.98. Found: C, 51.38; H, 3.68; Cl, 13.34; N, 10.38; S, 11.96.

5.6.17. 1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[[5-(pyridin-2-yl)thiazol-2-yl]carbonyl]piperazine hydrochloride (10c). Starting with **9c** and following the procedure for the preparation of **6a** gave **10c** (52%) as a pale yellow solid. Analytical sample was obtained as a HCl salt according to the same procedure as **6a**: mp 238–241 °C. ^{1}H NMR (DMSO-d_6) δ 3.13 (4H, br s), 3.77 (2H, br s), 4.42 (2H, br s), 7.37 (1H, m), 7.69 (1H, dd, J = 8.8, 2.0 Hz), 7.81 (1H, d, J = 8.8 Hz), 7.89 (1H, m), 8.03 (1H, d, J = 7.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.21 (1H, d, J = 2.0 Hz), 8.25 (1H, d, J = 8.8 Hz), 8.50 (1H, s), 8.56 (1H, s), 8.57 (1H, d, J = 4.4 Hz). MS (FAB) *m/z* 499 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}ClN_4O_3S_2 \cdot 0.5HCl \cdot 0.6H_2O$: C, 52.13; H, 3.92; Cl, 10.70; N, 10.57; S, 12.10. Found: C, 52.36; H, 3.80; Cl, 10.50; N, 10.56; S, 12.18.

5.6.18. 1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[[5-(2-methylpyridin-4-yl)thiazol-2-yl]carbonyl]piperazine hydrochloride (10d). Starting with **9d** and following the procedure for the preparation of **6a** gave **10d** (44%) as a pale yellow amorphous solid. Analytical sample was obtained as a HCl salt according to the same procedure as **6a**: ^{1}H NMR (DMSO-d_6) δ 2.70 (3H, s), 3.26 (4H, s), 3.78 (2H, br s), 4.40 (2H, br s), 7.51 (1H, dd, J = 8.8, 2.0 Hz), 7.63 (1H, dd, J = 8.8, 2.0 Hz), 7.91 (1H, d, J = 6.2 Hz), 7.96 (1H, d, J = 8.8 Hz), 8.00 (1H, s), 8.03 (1H, d, J = 2.0 Hz), 8.06 (1H, d, J = 8.8 Hz), 8.30 (1H, s), 8.51 (1H, d, J = 6.2 Hz), 9.62 (1H, s). MS (FAB) *m/z* 513 $(M+H)^+$. Anal. Calcd for $C_{24}H_{21}ClN_4O_3S_2 \cdot HCl \cdot H_2O$: C, 50.79; H, 4.26; Cl, 12.49; N, 9.87; S, 11.30. Found: C, 50.49; H, 4.17; Cl, 12.19; N, 9.66; S, 11.09.

5.6.19. 4-[2-[[4-[(6-Chloronaphthalen-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thiazol-5-yl]pyridine 1-oxide (3a). Starting with **10a** as a free and following the procedure for the preparation of **2a** gave **3a** (55%) as a pale yellow solid: mp 157–161 °C. ^{1}H NMR (DMSO-d_6) δ 3.13 (4H, br s), 3.77 (2H, br s), 4.43 (2H, br s), 7.69 (1H, d, J = 8.8 Hz), 7.76 (2H, d, J = 6.4 Hz), 7.82 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.20–8.28 (4H, m), 8.46 (1H, s), 8.50 (1H, s). MS (FAB) *m/z* 515 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}ClN_4O_4S_2 \cdot 1.5H_2O$: C, 50.97; H, 4.09; Cl, 6.54; N, 10.34; S, 11.83. Found: C, 50.75; H, 3.92; Cl, 6.93; N, 9.96; S, 11.64.

5.6.20. 3-[2-[[4-[(6-Chloronaphthalen-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thiazol-5-yl]pyridine 1-oxide (3b). Starting with **10b** as a free and following the procedure for the preparation of **2a** gave **3b** (89%) as a pale yellow solid: mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 3.13 (4H, br s), 3.77 (2H, br s), 4.41 (2H, br s), 7.48 (1H, dd, J = 7.8, 8.1 Hz), 7.64 (1H, d, J = 8.1 Hz), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8, 2.0 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.20–8.23 (2H, m), 8.26 (1H, d, J = 8.8 Hz), 8.48–8.51 (2H, m), 8.76 (1H, s). MS (FAB) *m/z* 515 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}ClN_4O_4S_2$: C, 53.64; H, 3.72; Cl, 6.88; N, 10.88; S, 12.45. Found: C, 53.27; H, 3.69; Cl, 7.16; N, 10.73; S, 12.28.

5.6.21. 2-[2-[[4-[(6-Chloronaphthalen-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thiazol-5-yl]pyridine 1-oxide (3c). Starting with **10c** as a free and following the procedure for the preparation of **2a** gave **3c** (79%) as a pale yellow solid: mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 3.14 (4H, br s), 3.78 (2H, br s), 4.41 (2H, br s), 7.47 (1H, t, J = 7.8 Hz), 7.54 (1H, t, J = 7.8 Hz), 7.68 (1H, dd, J = 8.8, 2.0 Hz), 7.84 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.20 (1H, s), 8.25 (1H, d, J = 8.8 Hz), 8.42–8.51 (3H, m), 8.95 (1H, s). MS (FAB) *m/z* 515 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}ClN_4O_4S_2 \cdot 0.5H_2O$: C, 52.72; H, 3.85; Cl, 6.77; N, 10.69; S, 12.24. Found: C, 52.84; H, 3.64; Cl, 7.09; N, 10.57; S, 12.40.

5.6.22. 4-[2-[[4-[(6-Chloronaphthalen-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thiazol-5-yl]-2-methylpyridine 1-oxide (3d). Starting with **10d** as a free and following the procedure for the preparation of **2a** gave **3d** (92%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 2.55 (3H, s), 3.23 (4H, br s), 3.91 (2H, br s), 4.59 (2H, br s), 7.31 (1H, dd, J = 6.8, 2.5 Hz), 7.41 (1H, d, J = 2.5 Hz), 7.58 (1H, dd, J = 9.0, 1.7 Hz), 7.78 (1H, J = 8.3, 1.7 Hz), 7.91 (1H, d, J = 8.3 Hz), 7.92 (1H, s), 7.93 (1H, J = 9.0 Hz), 7.98 (1H, br s), 8.25 (1H, d, J = 6.8 Hz), 8.32 (1H, br s). MS (FAB) *m/z* 529 $(M+H)^+$. Anal. Calcd for $C_{24}H_{21}ClN_4O_4S_2$: C, 54.49; H, 4.00; Cl, 6.70; N, 10.59; S, 12.12. Found: C, 54.19; H, 4.04; Cl, 6.73; N, 10.48; S, 12.23.

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