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Synthesis and biochemical evaluation of triazole/tetrazole-containing sulfonamides against thrombin and related serine proteases

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Abstract

A small library of 25 triazole/tetrazole-based sulfonamides have been synthesized and further evaluated for their inhibitory activity against thrombin, trypsin, tryptase and chymase. In general, the triazole-based sulfonamides inhibited thrombin more efficiently than the tetrazole counterparts. Particularly, compound **26** showed strong thrombin inhibition ($K_i = 880$ nM) and significant selectivity against other human related serine proteases like trypsin ($K_i = 729$ μ M). Thrombin binding affinity of the same compound was determined by ITC and demonstrated that the binding of this new triazole-based scaffold is enthalpically driven, making it a good candidate for further development.

Keywords

Thrombosis or Thrombin inhibition; Sulfonamide synthesis; Isothermal titration calorimetry; Trypsin selectivity; Triazole and tetrazole scaffolds

Even though much work has been done to prevent and treat vascular diseases, they are still the leading cause of death worldwide.¹ The national vital statistical reports published recently² showed that in 2009, cardiovascular diseases were the major reason of death in the U.S (almost 32% of the total number of deaths reported in that year) and cerebrovascular ailments along with strokes occupied the fourth place in the list of mortality. For those reasons, the development of new, safe and effective antithrombotic drugs continues to be a major priority in the pharmaceutical industry.

Traditional therapy for the treatment of thrombosis (cerebral, coronary and deep venous) and thromboembolic disorders (pulmonary embolism) has included the use of parenteral anticoagulants like unfractionated heparins (UFH), low-molecular-weight heparins (LMWH), factor Xa (FXa) inhibitors (fondaparinux), vitamin K antagonists (warfarin,

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Supplementary data

Supplementary information (detailed experimental syntheses, and biochemical assay protocols) associated with this article can be found in the online version at doi:10.1016/...

acenocoumarol, phenprocoumon) and thrombin inhibitors (hirudin, desirudin, argatroban and bivalirudin).^{3–7} Small molecular weight thrombin inhibitors with high selectivity and good bioavailability have been difficult to obtain.^{7, 8}

Thrombin, a trypsin-like serine protease, is an established target for the design of antithrombotic drugs since it plays an important role in the formation of blood clots by converting fibrinogen to fibrin, activating other coagulation factors (V, VIII, XI and XIII) present in the coagulation cascade and stimulating platelet aggregation.^{7–13}

Structurally, thrombin has three domains⁹ (the active site and exosites 1 and 2). Thrombin's active site is primarily constituted by a catalytic triad (Ser195, His57 and Asp102), a basic-recognition S1 pocket (Asp189 is the most important residue), a hydrophobic proximal S2 pocket (Tyr60A and Trp60D are the main residues) and a bigger lipophilic distal S3 pocket (Leu 99, Ile174 and Trp215).^{7, 9–12} Additionally, subsites S1', S2' and S3' and the oxyanion hole have also been considered in the design of stronger and more selective inhibitors.^{10, 14}

The development of highly potent and selective direct thrombin inhibitors (DTIs) is an active area of research.^{15, 16} We have identified a series of heterocyclic sulfonamides that inhibits the activity of thrombin with high potency and good selectivity to related serine proteases. These inhibitors do not contain the traditional highly basic functionalities (guanidine, amidine, amino, imidazole, hydroxyguanidine)^{8, 17, 18} used to interact with the Asp189 present in the specific S1 pocket of serine proteases. Herein, we report the chemical synthesis of twenty five triazole/tetrazole-based sulfonamides and their biochemical evaluation against thrombin, trypsin, trypase and chymase.

The synthesis of the triazole-containing inhibitors which is depicted in Scheme 2 involved seven steps while the preparation of the tetrazole-based analogs was achieved in just five steps. The initial step in the synthesis of the triazole sulfonamides comprised the formation of aromatic azides that were prepared by any of the three methods shown in Scheme 1.

Azides **1–3** were prepared by the reaction of their bromide precursors with sodium azide in ethanol.¹⁹ On the other hand, the synthesis of azides **4–7** was accomplished by the reaction of equimolar amounts of triphenylphosphine and their alcohol precursors with sodium azide using carbon tetrachloride and dimethylformamide as solvents. We could observe that under these reaction conditions, once the azide was formed it was gradually transformed into the respective amine as reported previously²⁰, accounting for the moderate yields obtained as shown in Scheme 1. Finally, an alternative procedure used to convert an alcohol to the corresponding azide²¹ consisted in using tosylimidazole (TsIm), with catalytic amount of TBAI for the preparation of 3-(azidomethyl)pyridine **8**. Alkylation of 3-aminophenol was performed first by protecting the amino group with (Boc)₂O and then alkylating the resulting Boc-phenol with propargyl bromide in the presence of potassium carbonate^{22, 23}, finally obtaining the Boc-protected alkyne **9** with 79% yield. Formation of triazole-containing anilines **10–17** was successfully achieved by using in sequence first a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition²⁴ between alkyne **9** and azides **1–8** and then deprotecting the resulting Boc-triazoles with TFA.²²

Synthesis of tetrazole **25** involved a 3-step route in which, 3-nitrobenzonitrile initially reacted with sodium azide and ZnBr₂ to form its corresponding nitrotetrazole²⁵ which was subsequently alkylated²⁶ by 3-chlorobenzylbromide and afterwards reduced with Zn/AcOH²⁷ to form the desired 3-(2-(3-chlorobenzyl)-2H-tetrazol-5-yl)aniline. Preparation of sulfonyl chlorides **18–24** was performed by heating the corresponding 3-substituted styrenes with sulfuryl chloride in DMF solution.²⁸ The final step in the synthesis of triazoles **26–42** and tetrazoles **43–50** consisted of the reaction of anilines **10–17** and **25** with sulfonyl chlorides **18–24** using water as the solvent and Na₂CO₃ as the base.²⁹ We observed that

when THF was added to this reaction as a co-solvent in a 1:1 ratio, the yields were considerably increased. The percentage yields of these reactions are shown in Tables 1 and 2 for triazoles and tetrazoles respectively.

The inhibitory potency and selectivity of the compounds were first evaluated by measuring the enzymatic activity (v_i/v_0) of thrombin, trypsin and chymase in the absence (v_0) and in the presence (v_i) of 10 μM inhibitor. The residual enzymatic activity is expressed as the ratio (v_i/v_0). The inhibition constants (K_i) for thrombin and trypsin were determined for some selected top compounds (compounds **26–33**, **36**, **38**, **40** and **42**) and are reported in Tables 1 and 2. According to the enzyme inhibition studies in the triazole-based series (Table 1), compounds containing a benzene-fused heterocyclic systems (see substituent R_1 of compounds **26–34**) are the most active against thrombin. We noticed that if the benzene ring of substituent R_1 of compound **26** ($v_i/v_0 = 0.06$) is removed to be transformed into analogs **35** ($v_i/v_0 = 0.27$) and **37** ($v_i/v_0 = 0.41$), the thrombin inhibitory potency drops approximately five and seven times respectively. Similarly, when the thiophene ring of substituent R_1 of compound **26** ($K_i = 0.88 \mu\text{M}$) is removed to become analog **36** ($K_i = 8.91 \mu\text{M}$), the thrombin inhibitory potency activity drops by a factor of ten. These data suggests that both rings, the benzene and thiophene, are important structural features for thrombin inhibition. We also noticed that the presence of a nitrogen atom in substituent R_1 reduces the inhibitory capacity against thrombin. For example, compounds **39–42**, which all contain a pyridine ring in R_1 , exhibited residual activities higher than 0.58. In particular, when compound **36** ($K_i = 8.91 \mu\text{M}$) is transformed into its pyridine analog **42** ($K_i = 39.2 \mu\text{M}$), the inhibitory activity drops almost four times. The adverse presence of nitrogen atom in substituent R_1 can also be illustrated by comparing compound **26** with compounds **33** and **34**. In the former case, the replacement of the benzothiophene ring of compound **26** ($K_i = 0.88 \mu\text{M}$) by a benzothiazole in compound **33** ($K_i = 5.43 \mu\text{M}$), reduces the inhibitory activity almost six times while in the second case, substitution of the same ring by an indole moiety present in compound **34**, diminishes the inhibition four times. Similar results are found by comparing compound **27** with compounds **31** and **32**.

The syntheses of compounds **26–30** were carried out in order to identify the nature of substituent R_2 needed to improve thrombin inhibition. The thrombin K_i values of compounds **26–30** reported in Table 1 indicate that R_2 decreases the enzyme activity in the following order: $\text{Cl} > \text{NO}_2 > \text{NH}_2 > \text{CF}_3 > \text{F}$. As we can see from these values, compound **26** is the best compound in this series not only because it exhibits the best K_i value against thrombin ($K_i = 0.88 \mu\text{M}$) but also because it shows remarkable selectivity against trypsin ($K_i = 729 \mu\text{M}$, K_i ratio = 828) and the other human related proteases like trypsin ($v_i/v_0 = 0.95$) and chymase ($v_i/v_0 = 0.98$) which sets it apart from its analogs **27–30**. In fact, it is worth mentioning that all compounds reported in this manuscript showed excellent selectivity against trypsin and chymase.

In an effort to evaluate the importance of the triazole ring for the inhibitory potency against thrombin, we initially synthesized eight tetrazole-based sulfonamides **43–50**. Based on these preliminary results, we noticed that the triazole-based scaffold inhibits thrombin much better than the tetrazole-based series. This observation is supported by comparing the inhibitory activity of compound **38** ($v_i/v_0 = 0.50$) with compound **50** ($v_i/v_0 = 0.95$). As noticed, replacement of the triazole ring in **38** by the tetrazole moiety in **50** increases the thrombin inhibitory activity almost twofold. To further corroborate the superiority of triazoles over tetrazoles, an overlay of the energy-minimized conformations of compounds **26**, **38** and **50** (Figure 1) was performed.³⁰ By comparing the structures of compounds **38** (blue) and **50** (red), we noticed that the main difference is found in the dissimilar orientation that the 3-chlorobenzyl moiety has in both structures. We think that the orientation observed in compound **38** allows this group to approach closer and more effectively to the S3 pocket of

thrombin giving account for the higher inhibitory activity. Comparison of the conformations of compounds **26** (green) and **38** (blue) provides additional proof that the inhibitory activity increases with the incorporation of more hydrophobic groups into the S3 pocket. For instance, the presence of an extra thiophene group in compound **26** ($K_i = 0.88 \mu\text{M}$) increases the potency almost 15 times over that of compound **38** ($K_i = 13.7 \mu\text{M}$). In addition, the triazoles show better selectivity than the tetrazoles.

In order to evaluate the thermodynamic profile of the top compound (compound **26**), its binding to thrombin was measured by isothermal titration calorimetry (ITC) (Figure 2). As shown in the figure, the binding of compound **26** to thrombin is enthalpically driven suggesting that the compound establishes strong interactions with thrombin.^{31, 32} The binding is characterized by a Gibbs energy (ΔG) of -8.3 kcal/mol , a binding enthalpy (ΔH) of -9.1 kcal/mol and an entropic contribution ($-T\Delta S$) of 0.8 kcal/mol . The thermodynamic profile of compound **26** provides a good optimization scenario since it already exhibits strong favorable enthalpy. It is well known that the binding entropy is easier to optimize³³

In summary, we discovered and synthesized a small library of 25 triazole/tetrazole-based sulfonamides which were evaluated for their inhibitory activity against thrombin, trypsin, trypsin and chymase. In particular, 5 compounds (**26–30**) showed outstanding K_i values for thrombin inhibition and most importantly, they had significant selectivity against other human related serine proteases which make them good candidates for further development. Despite the fact that tetrazoles are not as good as triazoles for inhibiting thrombin, they can still be considered for further optimization since they show remarkable selectivity against related serine proteases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
An overlay of compounds **26** (green), **38** (blue) and **50** (red). This image was generated using MOE.³⁰

Figure 2.
The thermodynamic signature for the binding of compound **26** to thrombin.

Scheme 1.

Synthesis of azides. Reagents and conditions: (a) NaN_3 , EtOH, rt, 24 h; (b) NaN_3 , PPh_3 (1 eq), CCl_4 -DMF, 80–90 °C, 4 h; (c) NaN_3 , TsIm, TBAI, TEA, DMF, 100 °C, 21 h

Scheme 2.

Synthesis of triazole and tetrazole-based sulfonamides. Reagents and conditions: (a) (Boc)₂O, THF, 61 °C, 24 h, 83% yield; (b) K₂CO₃, NaI, DMF, propargyl bromide, 68 °C, 45 h, 98% yield; (c) Na(ascb), CuSO₄·5H₂O, H₂O-EtOH (1:1), rt, 24 h, 65–99% yield; (d) TFA, DCM, rt ~2 h, >93% yield; (e) SO₂Cl₂, DMF, 60–80 °C, 3–7 h; (f) NaN₃, ZnBr₂, H₂O, reflux, 31 h, 94% yield; (g) TEA, 3-chlorobenzylbromide, AN, rt, 23 h, 34% yield; (h) Zn, AcOH, rt, 2.5 h, 97% yield; i) 1M Na₂CO₃, THF-H₂O, rt, 1.5–5 h, % yields are shown in Tables 1 and 2.

Table 1

Inhibition of thrombin and related serine proteases by triazole-based sulfonamides.

Compound	% Yield ^d	R ²	R ¹	K _i (μM)		Ratio ^b		v _i /v ₀ (10 μM) ^a		
				thrombin	trypsin	thrombin	trypsin	thrombin	trypsin	chymase
26	70	Cl		0.88	729	828	0.06	0.95	0.98	
27	84	NO ₂		1.71	122	71	0.06	1.0	0.89	
28	72	CF ₃		1.97	127	64	0.09	0.97	0.99	
29	49	NH ₂		1.75	71	41	0.11	0.99	0.88	
30	58	F		2.27	119	48	0.12	0.95	0.95	
31	56	NO ₂		6.89	nd	---	0.18	0.98	1.00	
32	52	NO ₂		4.60	187	41	0.19	1.0	0.95	
33	63	Cl		5.43	nd	---	0.19	0.98	1.00	
34	56	Cl		nd ^c	nd	---	0.24	0.99	1.00	
35	76	Cl		nd	nd	---	0.27	0.98	0.96	
36	53	Cl		8.91	nd	---	0.37	0.97	1.00

Compound	% Yield ^d	R ²	R ¹	K _i (μM)		Ratio ^b	v _i /v ₀ (10 μM) ^a		
				thrombin	trypsin		thrombin	trypsin	chymase
37	78	Cl		nd	nd	---	0.41	0.95	0.99
38	32	Cl		13.7	nd	---	0.50	0.95	0.97
39	41	CF ₃		nd	nd	---	0.58	0.96	1.00
40	47	NO ₂		62.5	nd	---	0.70	1.0	1.00
41	60	F		nd	nd	---	0.71	1.0	0.99
42	64	Cl		39.2	nd	---	0.76	0.99	0.98

^aResidual enzyme activity determined in the presence of 10μM inhibitor.

^bRatio of K_i trypsin/ K_i thrombin

^cNot determined

^d% yield of the last step shown in Scheme 2

Table 2

Inhibition of thrombin and related serine proteases by tetrazole-based sulfonamides

Compound	% Yield ^c	R ⁴	v _i /v ₀ (10 μM) ^a		
			thrombin	tryptase	chymase
43	29		0.23 ^b	1.0	0.99
44	56		0.34	0.97	0.91
45	34		0.54	1.0	0.94
46	66	CH ₃	0.55	0.97	0.95
47	44		0.68	1.0	0.97
48	81		0.81	1.0	0.95
49	63		0.93	1.0	1.00
50	61		0.95	1.0	0.97

^a Residual enzyme activity determined in the presence of 10 μM inhibitor.^b K_i value for this compound is 5.20 μM

c
% yield of the last step shown in Scheme 2

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