Bioorganic & Medicinal Chemistry Letters 24 (2014) 1111–1115

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Heterocyclic core analogs of a direct thrombin inhibitor

Timothy A. Blizzard [a,*], Sanjay Singh [b], Basanagoud Patil [b], Naresh Chidurala [b], Venukrishnan Komanduri [b], Samarpita Debnath [b], Sergei Belyakov [b], Alejandro Crespo [a], Alice Struck [a], Marc Kurtz [a], Judyann Wiltsie [a], Xun Shen [a], Lisa Sonatore [a], Marta Arocho [a], Dale Lewis [a], Martin Ogletree [a], Tesfaye Biftu [a]

[a] Merck Research Laboratories, Rahway, NJ, USA
[b] Albany Molecular Research, Singapore Research Center, Singapore

ARTICLE INFO

Article history:
Received 18 November 2013
Revised 1 January 2014
Accepted 3 January 2014
Available online 10 January 2014

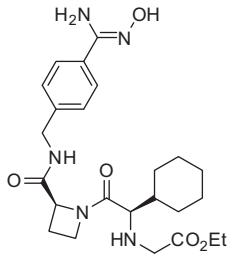
Keywords:
Thrombin inhibitor
DTI
Thrombosis
Anti-thrombotic

ABSTRACT

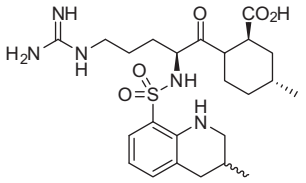
Thrombin is a serine protease that plays a key role in blood clotting. Pyrrolidine **1** is a potent thrombin inhibitor discovered at Merck several years ago. Seven analogs (**2**–**8**) of **1** in which the pyrrolidine core was replaced with various heterocycles were prepared and evaluated for activity against thrombin, clotting factors VIIa, IXa, Xa, and XIIa, and trypsin. The thiomorpholine analog **6** was the most active, essentially matching the thrombin inhibitory activity of **1** with slightly improved selectivity over trypsin.

Thrombotic events (stroke, heart attack, etc.) are an important cause of morbidity and mortality, especially in older patients.[1] A safe and effective drug that could prevent thrombotic events would clearly have a significant positive impact on life expectancy and quality of life. Fortunately, thrombogenesis is a complicated biological process involving a number of enzymes that provide attractive targets for potential new therapies.[2] Several approved drugs, including Warfarin (a vitamin K epoxide reductase inhibitor), Dabigatran (a thrombin inhibitor), Rivaroxaban and Apixaban (factor Xa inhibitors) are currently used prophylactically to reduce the risk of thrombotic events.[3] Unfortunately, current therapy suffers from significant side effects. Bleeding, in particular, remains a significant concern. Thus, there remains an unmet medical need for improved antithrombotic agents that would not have the bleeding liability of current drugs. This is a very active field of research and, in addition to those drugs already on the market, numerous compounds employing a variety of mechanisms are in development.[4–6]

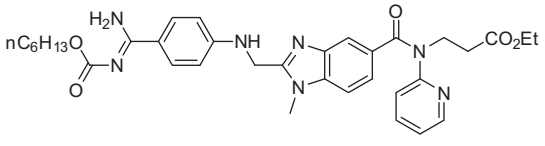
Thrombin (Factor IIa) is a serine protease that plays a key role in blood clotting. Selective thrombin inhibition is an established mechanism for thromboembolism prevention.[7,8] Three small molecule thrombin inhibitors have reached the market. The first, Ximelagatran, was subsequently withdrawn due to unacceptable liver toxicity.[7] Two newer direct thrombin inhibitors, Dabigatran and Argatroban, are currently on the market. Numerous other thrombin and Factor Xa inhibitors are in various stages of development.[9]



Ximelagatran



Argatroban

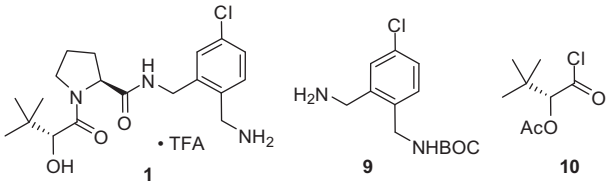


Dabigatran

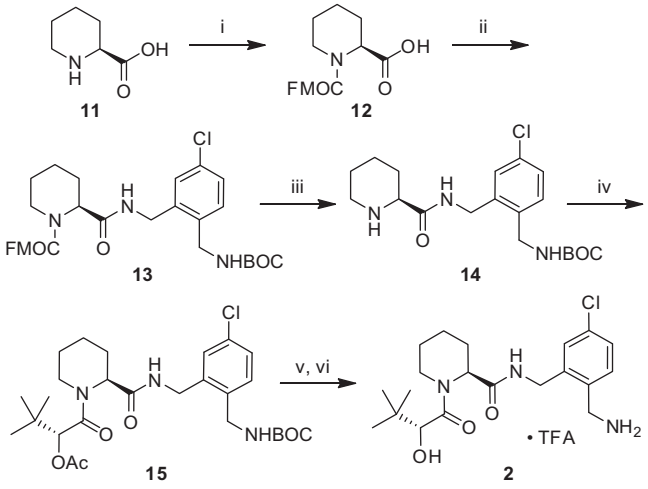
Pyrrolidine **1** is a potent thrombin inhibitor discovered[10] at Merck several years ago as part of a program directed at finding clinically useful thrombin inhibitors.[10–13]

* Corresponding author.
E-mail address: timblizzard@comcast.net (T.A. Blizzard).

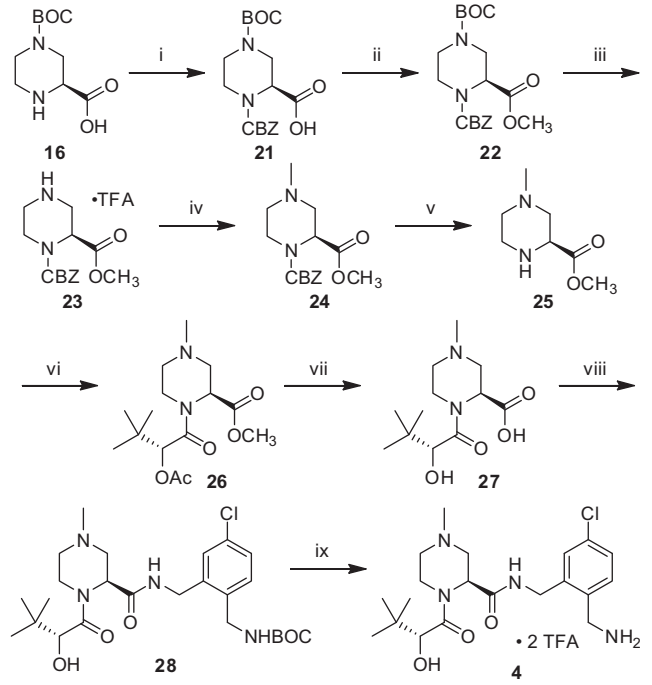
0960-894X/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2014.01.002



In their original report, Morissette et al. noted that reducing the core ring size (i.e. replacement of pyrrolidine with azetidine) resulted in reduced thrombin inhibitory activity.[10] However, the effect of ring expansion was unknown. In an attempt to discover an improved thrombin inhibitor, and to more fully elucidate the SAR of the heterocyclic core of **1**, we prepared and report herein a series of six-membered heterocyclic core analogs of **1**.

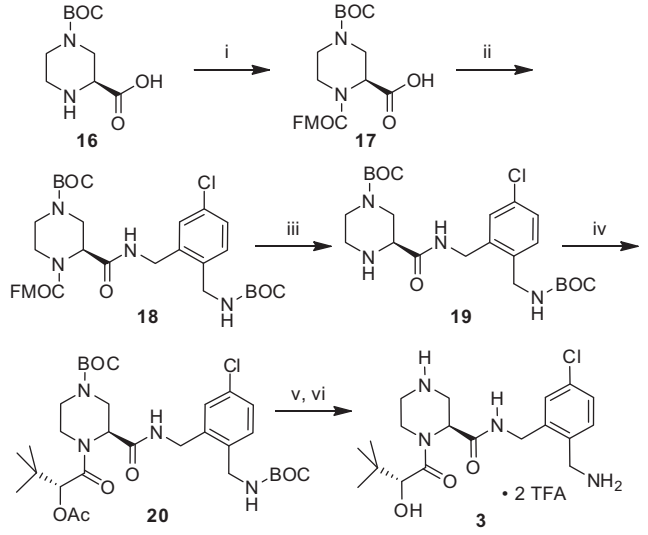


Scheme 1. Reagents and conditions: (i) FMOC-Cl, Na_2CO_3, 1,4-dioxane, water, 16 h, room temp, 52%; (ii) **9**, EDC, HOBT, DMF, 16 h, room temp, 70%; (iii) piperidine, CH_2Cl_2, 1 h, room temp, 80%; (iv) **10**, Et_3N, THF, −10 °C to room temp, 1 h, 65%; (v) K_2CO_3, CH_3OH, room temp, 30 m; (vi) TFA, CH_2Cl_2, room temp, 2 h, 30% for two steps.

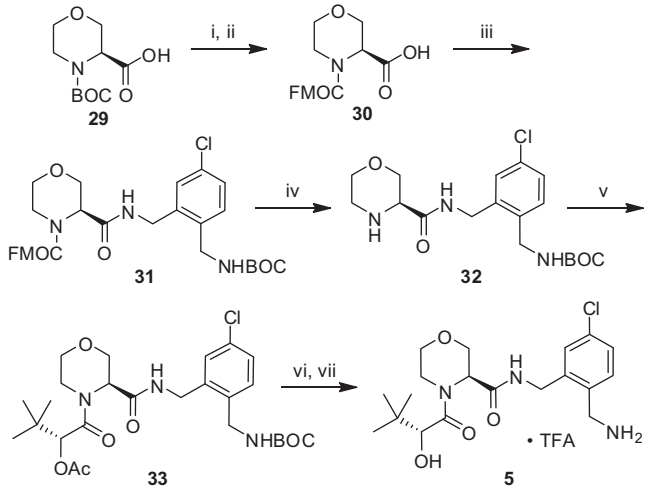


Scheme 3. Reagents and conditions: (i) CBZ-Cl, $NaHCO_3$, 1,4-dioxane, H_2O, room temp, 16 h, 82%; (ii) K_2CO_3, CH_3I, DMF, room temp, 2 h, 74%; (iii) TFA, CH_2Cl_2, room temp, 30 m, 97%; (iv) paraformaldehyde, Et_3N, $NaCNBH_3$, CH_3OH, AcOH, 0 °C to room temp, 2 h, 70%; (v) H_2 (1 atm), 10% Pd/C, CH_3OH, room temp, 3 h, 71%; (vi) **10**, Et_3N, CH_2Cl_2, room temp, 30 m, 40%; (vii) LiOH, THF, H_2O, CH_3OH, room temp, 2 h, 59%; (viii) **9**, EDC, HOBT, Et_3N, DMF, room temp, 16 h, 32%; (ix) TFA, CH_2Cl_2, room temp, 1 h, 73%.

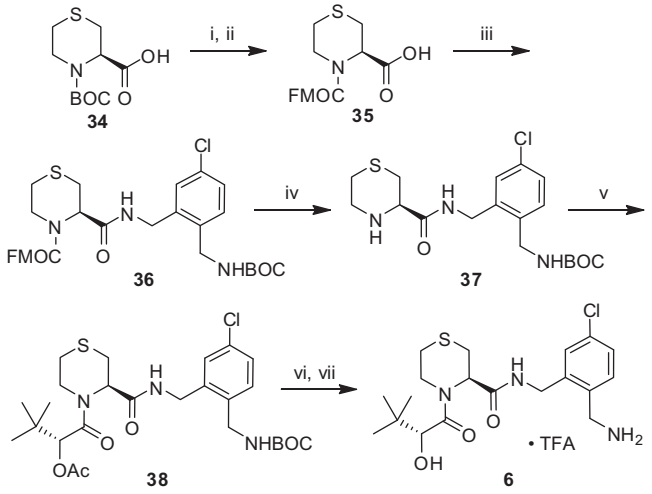
We initially targeted the piperidine analog **2**. Starting with acid **11**,[14] **2** was readily synthesized as outlined in Scheme 1. Treatment of **11** with FMOC-Cl afforded the protected aminoacid **12** in good yield. The right-hand side chain was then introduced by EDC-mediated coupling of **12** with amine **9**,[10] to afford intermediate amide



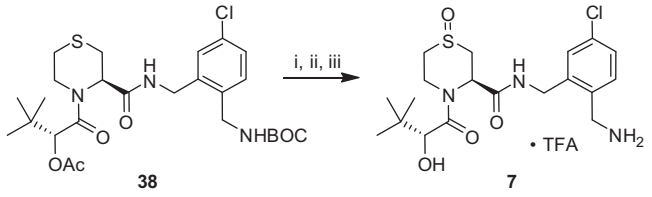
Scheme 2. Reagents and conditions: (i) FMOC-Cl, Na_2CO_3, 1,4-dioxane, water, 0 °C to room temp, 16 h, 44%; (ii) **9**, EDC, HOBT, DMF, room temp, 16 h, 78%; (iii) piperidine, CH_2Cl_2, room temp, 2 h, 73%; (iv) **10**, Et_3N, THF, −5 °C to room temp, 1 h, 51%; (v) K_2CO_3, CH_3OH, room temp, 30 m; (vi) TFA, CH_2Cl_2, room temp, 2 h, 40% for two steps.



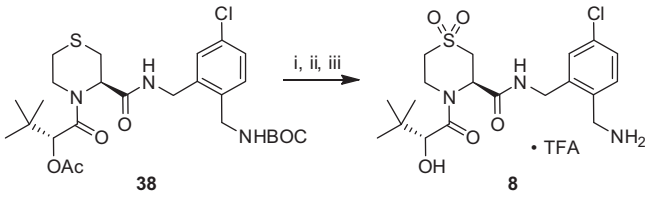
Scheme 4. Reagents and conditions: (i) TFA, CH_2Cl_2, room temp, 5 h; (ii) FMOC-Cl, K_2CO_3, 1,4-dioxane, H_2O, room temp, 3 h, 84% for two steps; (iii) **9**, EDC, HOBT, DMF, 0 °C to room temp, 5 h, 80%; (iv) piperidine, CH_2Cl_2, room temp, 1 h, 62%; (v) **10**, Et_3N, CH_2Cl_2, 0 °C to room temp, 16 h, 71%; (vi) K_2CO_3, CH_3OH, room temp, 30 m; (vii) TFA, CH_2Cl_2, 0 °C, 30 m, 48% for two steps.



Scheme 5. Reagents and conditions: (i) TFA, CH_2Cl_2 room temp, 2 h; (ii) FMOC-Cl, $NaHCO_3$, 1,4-dioxane, H_2O, room temp, 16 h, 67% for two steps; (iii) **9**, EDC, HOBT, Hunig's base, DMF, room temp, 16 h, 71%; (iv) piperidine, CH_2Cl_2, room temp, 2 h, 83%; (v) **10**, Et_3N, CH_2Cl_2, −10 °C to room temp, 1 h, 63%; (vi) K_2CO_3, CH_3OH, room temp, 30 m; (vii) TFA, CH_2Cl_2, room temp, 2 h, 27% for two steps.



Scheme 6. Reagents and conditions: (i) MCPBA (0.88 equiv), CH_2Cl_2, room temp, 30 m, 98%; (ii) K_2CO_3, CH_3OH, room temp, 30 m; (vii) TFA, CH_2Cl_2, room temp, 2 h, 35% for two steps.



Scheme 7. Reagents and conditions: (i) MCPBA (2.5 equiv), CH_2Cl_2, room temp, 4 h, 66%; (ii) K_2CO_3, CH_3OH, room temp, 30 m; (vii) TFA, CH_2Cl_2, room temp, 2 h, 29% for two steps.

13. Removal of the FMOC group, followed by introduction of the left-hand side chain by reaction of amine **14** with acid chloride **10**[15] afforded the penultimate intermediate **15**. Finally, protecting group removal completed the synthesis of piperidine analog **2**.

The corresponding piperazine analog **3** was similarly prepared from the protected piperazine acid **16**[14] and reagents **9** and **10** as outlined in Scheme 2.

The synthesis of the *N*-methyl piperazine analog **4** from **16** (Scheme 3) was more complicated due to the protecting group manipulations required to install the *N*-methyl substituent. Treatment of **16** with CBZ-Cl readily afforded the bis-protected piperazine **21**. Protection of the acid as the methyl ester followed by removal of the BOC protecting group and reductive methylation of amine **23** provided the *N*-methyl intermediate **24** in good overall yield. Removal of the CBZ group afforded amine **25**. Analog **4** was readily prepared from **25** by the synthetic route employed for **2**.

The morpholine analog **5** was prepared in a similar manner from the protected amino acid **29**,[14] as outlined in Scheme 4.

The thiomorpholine analog **6** was prepared from the protected amino acid **34** by an analogous sequence, as outlined in Scheme 5.

The corresponding sulfoxide **7** was readily prepared by MCPBA oxidation of intermediate **38** followed by deprotection to afford **7** as outlined in Scheme 6.

Finally, sulfone **8** was prepared by MCPBA oxidation of sulfide intermediate **38** under stronger conditions followed by deprotection (Scheme 7).

The new analogs were generally less potent inhibitors of thrombin than the lead pyrrolidine **1** (Table 1). The piperidine analog **2**, although differing from **1** only in the addition of a single methylene unit, was about five-fold less active than **1**. The piperazines **3** and **4**, with an additional basic nitrogen in the ring, were even weaker inhibitors. The morpholine analog **5** was comparable in activity to the piperazines. Interestingly, the thiomorpholine analog **6** was the most active of the seven analogs, exhibiting thrombin inhibition comparable to that of the pyrrolidine **1**. In addition, **6** demonstrated slightly improved selectivity for thrombin over trypsin (>13,000X for **6** vs ~3700X for **1**), although both compounds were highly selective. Finally, the sulfoxide analog **7** and the sulfone **8** were significantly less active than the thiomorpholine **6**.

Pyrrolidine **1** (Fig. 1) and the most active analog, thiomorpholine **6** (Fig. 2), were docked into a published crystal structure of thrombin with a close analog of **1** bound in the active site (2zgb.pdb).[16] As expected, **1** and **6** bind by placing the Cl-phenyl group into the P1 pocket, the core heterocyclic group into the P2 pocket, with the hydroxyl group interacting with G216 in P3 and the *tert*-butyl group in the P4 pocket. Thrombin activity differences for compounds **1**–**8** can thus be explained by the differential interaction of the heterocyclic core with the hydrophobic residues (shown as sticks in Figs. 1 and 2) framing the P2 pocket (H57, L99, Y60A and particularly W60D, which caps the pocket). The hydrophobic pyrrolidine **1**, piperidine **2**, and thiomorpholine **6** are the more potent analogs. The hydrophilic piperazine **3**, morpholine **5**, sulfoxide **7** and sulfone **8** are less active. Although *N*-methyl piperazine analog **4** is relatively hydrophobic due to N-methylation, the additional methyl group introduces a steric clash with the W60D side chain, accounting for its reduced activity.

Table 1
Enzyme inhibition data (K_i, nM) for heterocyclic core analogs **1**–**8**

Compound	Thrombin[a]	Factor VIIa[a]	Factor IXa[a]	Factor Xa[a]	Factor XIIa[a]	Trypsin[a]
1	1.5	>15,000	>3000	1400	>15,000	5600
2	7.3	15,000	nt	1800	15,000	10,600
3	63	15,000	15,000	4400	15,000	15,000
4	34	15,000	nt	3700	15,000	15,000
5	32	15,000	15,000	3900	15,000	15,000
6	1.1	15,000	nt	900	15,000	9400
7	78	nt	nt	nt	nt	nt
8	52	15,000	nt	3100	15,000	15,000

[a] Values are means of two experiments (nt = not tested).

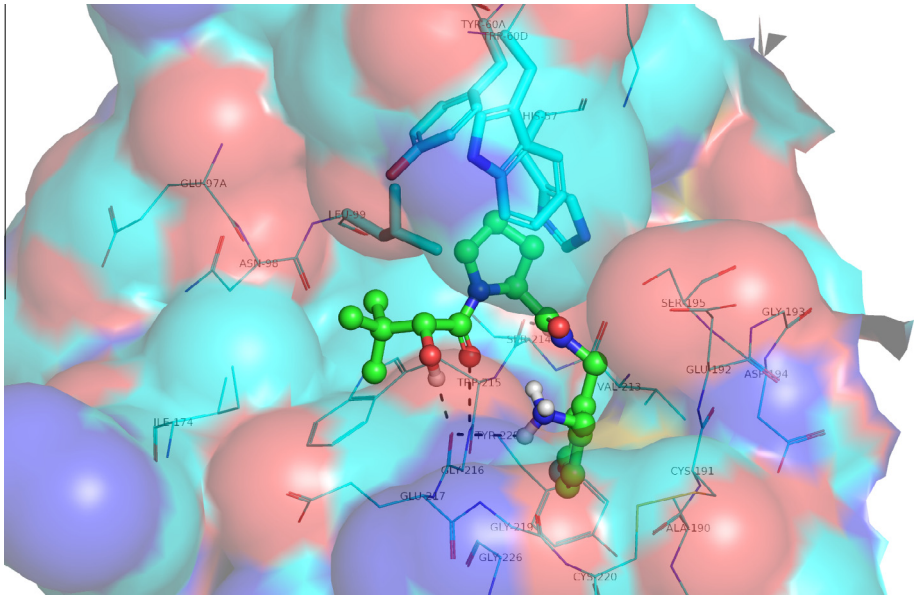


Figure 1. Suggested binding mode of pyrrolidine **1** against thrombin (2zgb.pdb).

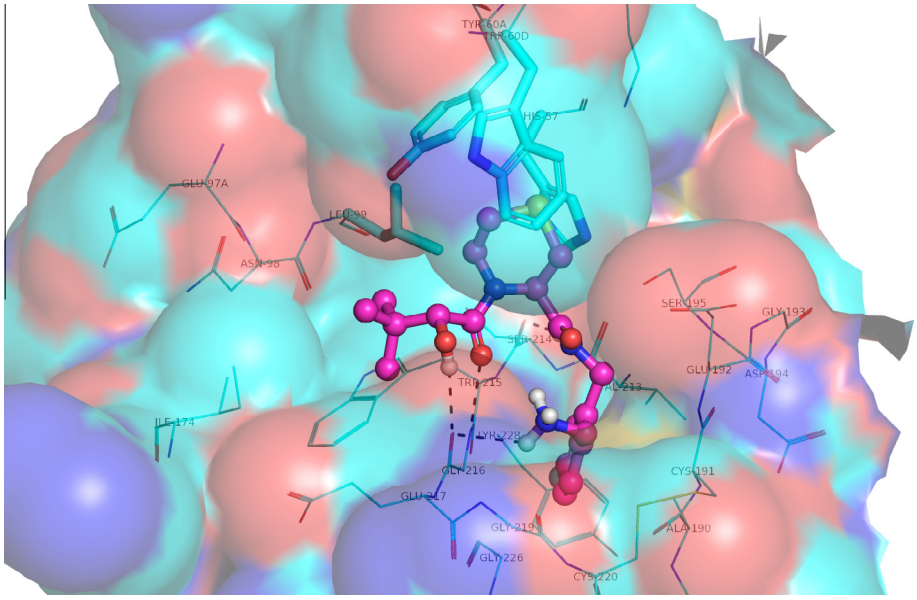


Figure 2. Suggested binding mode of thiomorpholine **6** against thrombin (2zgb.pdb).

Consistent with this binding hypothesis, the observed inhibition data correlates reasonably well with the hydrophobicity of the heterocyclic core. Figure 3 shows the correlation of thrombin activity with *c*log*D* (compounds are colored by PSA).[17] It is clear that the most potent compounds **1**, **2** and **6** are more hydrophobic, whereas the less active analogs **3**, **5**, **7** and **8** are more polar. Interestingly, although compound **4** could be assigned to the less polar group based on *c*log*D*, its activity is only around 30 nM. As noted above, modeling suggests that this reduced activity is due to an unfavorable steric interaction.

The most interesting of the novel analogs, thiomorpholine **6** was further evaluated in a dog pharmacokinetics study (IV dosing). Disappointingly, **6** exhibited poorer PK in dogs than the lead pyrrolidine **1** (Table 2). This is consistent with the reduced dog hepatocyte stability of **6** (76% remaining at $t = 90$ m) versus **1** (93% remaining). None of the analogs inhibited CYP 3A4, CYP 2D6, or CYP 2C9 at concentrations up to 50 μM. Due to its poor pharmacokinetics relative to pyrrolidine **1**, thiomorpholine **6** was not evaluated further.

In conclusion, the novel heterocyclic analogs **2**–**8** reported herein had no significant advantage over **1** as direct thrombin inhibitors. The best analog, thiomorpholine **6**, was comparable in activity to **1** and was slightly more selective but, unfortunately, had poorer pharmacokinetic properties. Thus, ring expansion of the lead pyrrolidine did not lead to the improved oral thrombin inhibitor that were the goal of this research program. However,

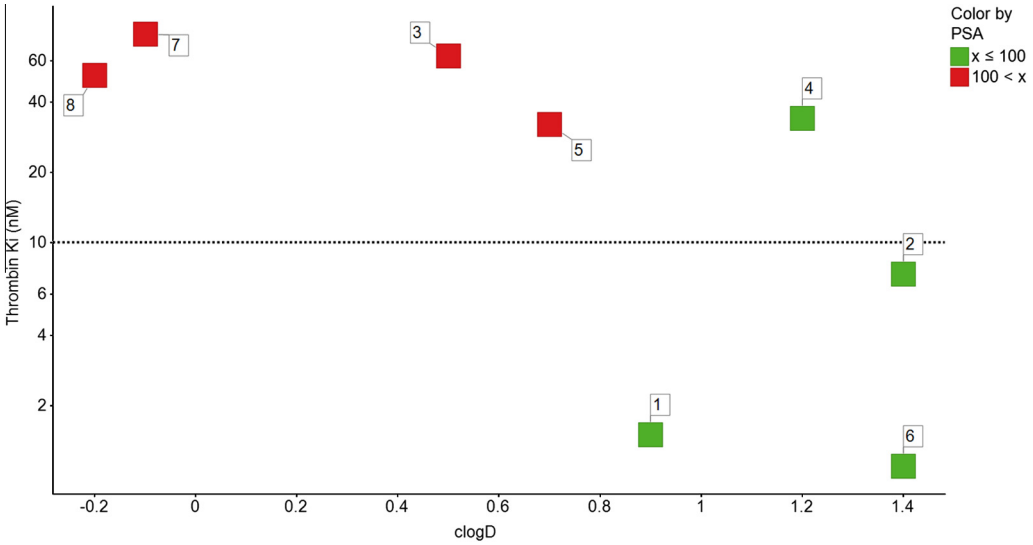


Figure 3. Correlation plot between thrombin activity (K_i, nM) and $c\log D$ (color by PSA).

Table 2
Dog PK data for pyrrolidine **1** and thiomorpholine **6**[a]

#	$t_{½}$ (h)	AUC (μM h)	Cl (mL/min/kg)	V_d (L/kg)
1	6.8	10.0	3.5	1.9
6	2.7	1.0	12.8	2.3

[a] IV dosing (0.3 mpk).

the medical need for improved prophylactic antithrombotic agents remains. Additional results in this area will be reported in future publications from this laboratory.

References and notes

1. Di Minno, M. N. D.; Momi, S.; Di Minno, A.; Russolillo, Anna *Curr. Drug Targ.* **2013**, *14*, 3.
2. Versteeg, H. H.; Heemskerk, J. W. M.; Levi, M.; Reitsma, P. H. *Physiol. Rev.* **2013**, *93*, 327.
3. Arsenault, K. A.; Hirsh, J.; Whitlock, R. P.; Eikelboom, J. W. *Nat. Rev. Cardiol.* **2012**, *9*, 402.
4. Weitz, J. I.; Eikelboom, J. W.; Samama, M. M. *Chest* **2012**, *141*, e120S.
5. Weitz, J. I. *Am. J. Hematol.* **2012**, *87*, S133.
6. Young, R. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6228.
7. Eriksson, B. I.; Quinlan, D. J.; Eikelboom, J. W. *Ann. Rev. Med.* **2011**, *62*, 41.
8. O'Brien, P. J.; Mureebe, L. J. *Cardiovasc. Pharmacol. Ther.* **2012**, *17*, 5.
9. Winstanley, L.; Chen, R. *CNS Neurol. Disorders–Drug Targets* **2013**, *12*, 242.
10. Morrissette, M. M.; Stauffer, K. J.; Williams, P. D.; Lyle, T. A.; Vacca, J. P.; Krueger, J. A.; Lewis, S. D.; Lucas, B. J.; Wong, B. K.; White, R. B.; Miller-Stein, C.; Lyle, E. A.; Wallace, A. A.; Leonard, Y. M.; Welsh, D. C.; Lynch, J. J.; McMasters, D. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4161.
11. Brady, S. F.; Stauffer, K. J.; Lumma, W. C.; Smith, G. M.; Ramjit, H. G.; Lewis, S. D.; Lucas, B. J.; Gardell, S. J.; Lyle, E. A.; Appleby, S. D.; Cook, J. J.; Holahan, M. A.; Stranieri, M. T.; Lynch, J. J.; Lin, J. H.; Chen, I. W.; Vastag, K.; Naylor-Olsen, A. M.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 401.
12. Rittle, K. E.; Barrow, J. C.; Cutrona, K. J.; Glass, K. L.; Krueger, J. A.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; McMasters, D. R.; Morrissette, M. M.; Nantermet, P. G.; Newton, C. L.; Sanders, W. M.; Yan, Y.; Vacca, J. P.; Selnick, H. G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3477.
13. Nelson, T. D.; LeBlond, C. R.; Frantz, D. E.; Matty, L.; Mitten, J. V.; Weaver, D. G.; Moore, J. C.; Kim, J.; Boyd, R.; Kim, P.-Y.; Gbewonyo, K.; Brower, M.; Sturr, M.; McLaughlin, K.; McMasters, D. R.; Kress, M. H.; McNamara, J. M.; Dolling, U. H. *J. Org. Chem.* **2004**, *69*, 3620.
14. Purchased from Aldrich Chemical Company.
15. (*R*)-1-Chloro-3,3-dimethyl-1-oxobutan-2-yl acetate (**10**) was freshly prepared using the following sample procedure: To the neat hydroxy acid[12] (86.2 mg, 0.653 mmol) was added acetyl chloride (0.15 mL, 2.11 mmol) and mixture was warmed with hot gun. Vigorous evolution of bubbles was observed. After cessation of bubbles, the mixture was heated at 60 °C for 20 min. Completion of the reaction was monitored by TLC (1:1 ethyl acetate/hexane), excess acetyl chloride was distilled off and oil was dried under high vacuum for 10 min. Thionyl chloride (0.15 mL, 2.06 mmol) was added to the residual oil and the reaction mixture was refluxed for 1.5 h. After completion of the reaction (TLC; 1:1 ethyl acetate/hexane), excess thionyl chloride was distilled off and the resulting oil was dried under high vacuum. The crude product was used without purification.
16. Docking calculations for **1** and **6** were performed against thrombin structure in complex with a close analog of pyrrolidine **1** (2zgb.pdb) using Glide (Schrödinger 2013R1) docking software with standard parameters and XP precision.
17. Physical properties were computed with ACD/Labs release 11. The correlation plot was generated using Spotfire.