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From natural products to achiral drug prototypes: Potent thrombin inhibitors based on P_2/P_3 dihydropyrid-2-one core motifs

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ABSTRACT

A series of dihydropyrid-2-ones was synthesized and tested for inhibitory activity against serine protease enzymes. Moderate to low nanomolar inhibitory activities were obtained against thrombin and excellent selectivity against trypsin was observed.

Blood coagulation is a complex multifaceted process that involves cellular and proteinaceous components.[1] Through a series of ordered events, primary hemostasis caused by platelet aggregation is followed by activation of plasma coagulation factors causing clot formation in blood vessels (secondary hemostasis). The cascade of biochemical events leading to blood coagulation involves a stepwise activation of trypsin-like proteases. The multifunctional serine protease thrombin (Factor IIa)[2] is responsible for the final cleavage of fibrinogen to fibrin, which undergoes cross-linking to form a strong fibrin clot. Under normal physiological conditions, the blood coagulation system is balanced by anticoagulation on the one hand, and fibrinolysis on the other.[3] A pathogenic imbalance that favors the activation of the coagulation system may lead to thrombosis in humans.[4] Inhibition of thrombin is widely viewed as an effective therapeutic means to prevent the formation of blood clots and related thromboembolic disorders.[5]

In previous studies we have shown that chlorodysinosin A, a natural product belonging to the aeruginosin family[6] exhibits low nanomolar in vitro inhibitory activity against thrombin, trypsin, and tissue factors VIIa and Xa (Fig. 1).[7]

An X-ray co-crystal structure of chlorodysinosin[7] (and related aeruginosins such as dysinosin A[8] and oscillarin,[9] with thrombin, revealed the importance of a hydrophobic interaction in the S_3 site. Based on these observations, hybrid molecules were designed and synthesized, showing superb in vitro inhibition of thrombin.[10] We also reported on the design and synthesis of P_2/P_3 phenolic core motifs[11] as well as P_2/P_3 pyrid-2-one core motifs (Fig. 1).[12]

Further refinement of the core motif led to the synthesis of molecules containing a dihydropyrid-2-one with impressive inhibitory activities against thrombin (Scheme 1).[13]

Protection of 4-methyl piperidine **1** as the *N*-Boc derivative, followed by oxidation with $NaIO_4$ and RuO_2 led to the lactam **2**. Formation of the Li enolate and alkylation with ethyl 2-bromoacetate gave **3** in 69% yield. Introduction of an endocyclic double bond was achieved by formation of an enolate, addition of PhSeBr and oxidative elimination. The desired product **4** was accompanied by the α,β-unsaturated ester **5**. Increasing the size of the ester (e.g., CO_2t-Bu) favors the *endo*:*exo* ratio in a 2:1 fashion albeit in lower total yield (64%).

Removal of the *N*-Boc group of **4** and treatment with *t*-BuONO and pyridine in Et_2O gave the *N*-nitroso product **6**. Reduction with Zn/AcOH followed by treatment with a diverse set of arylsulfonyl chlorides gave the corresponding *N*-sulfonamides **7a**–**i**. Hydrolysis of the ethyl ester, coupling with the *N*-Cbz-4-amino-methylbenzamidine, followed by hydrogenolysis, afforded a set of compounds (**8a**–**i**) that were evaluated for their enzymatic in vitro activity against thrombin and trypsin.

Compounds **8a**–**i** exhibited remarkably different levels of inhibitory activity against thrombin (Table 1). The weakest inhibitor

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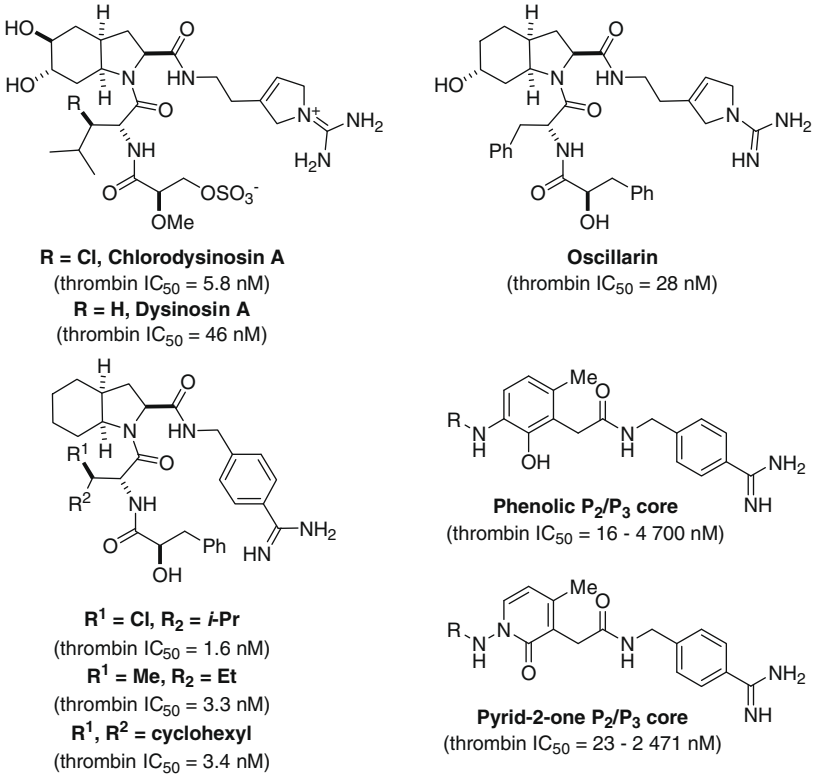
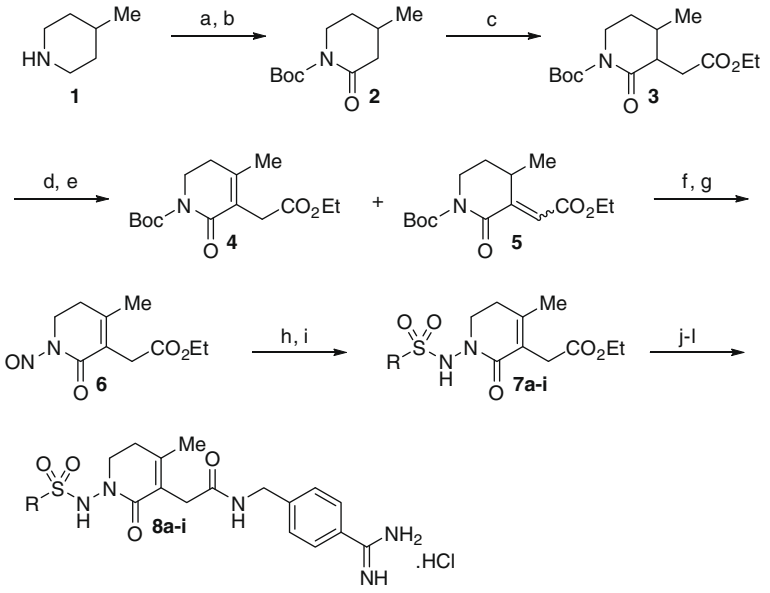


Figure 1. Structures of natural and unnatural products and their in vitro inhibitory activity.

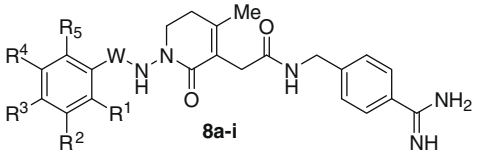


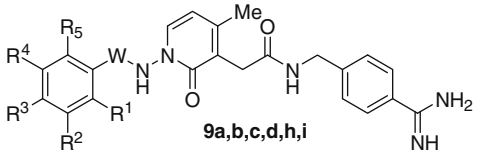
Scheme 1. Reagents and conditions: (a) Boc_2O, Et_3N, DCM, 35 °C, 73%; (b) $RuO_2{\cdot}xH_2O$, $NaIO_4$, EtOAc/H_2O, 83%; (c) LiHMDS, $BrCH_2CO_2Et$, THF, −78 °C, 69%; (d) LiHMDS, PhSeBr, −78 °C; (e) H_2O_2, pyridine, DCM, 0 °C, **4**, 35%, **5**, 41%; (f) TFA, DCM; (g) *t*-BuONO, pyridine, Et_2O, reflux, 91%; (h) Zn, AcOH, 61%; (i) RSO_2Cl, pyridine, DCM; (j) LiOH, THF/H_2O; (k) EDC, HOBt, *i*-Pr_2NEt, *N*-Cbz-4-amino-methylbenzamidine, DMF; (l) H_2, Pd/C, MeOH/HCl.

was the benzylsulfonamide **8a**. In contrast, the corresponding phenylsulfonamide **8b** was five times more active (Table 1, entries 1 and 2). Single or double substitution at the *ortho, meta,* and *para* positions were well tolerated regardless of the electronic nature of the substituents (Table 1, entries 3, 4, 6, 8). An electron withdrawing *ortho*-F substituent (**8e**) was less favorable (Table 1, entry 5). By far, the best combination of substituents were found to be at the two *ortho*-positions, exemplified by the chloro analogue **8g**, with an IC_{50} of 3 nM against thrombin, and excellent selectivity against trypsin (Table 1, entry 7). A naphthylsulfonyl group was also well tolerated (Table 1, entry 9).

There is a remarkably improved inhibition for the dihydropyrid-2-one series compared to the pyrid-2-one series.[12] Included in Table 1 are the IC_{50} values of identical compounds in both series except for the nature of the P_2/P_3 heterocyclic core. Between 25- and 100-fold enhancement of inhibitory activity was observed in each

Table 1
Inhibitory activities against thrombin and trypsin and measured pK_a of sulfonamide N for dihydropyrid-2-ones **8a–i** and corresponding pyrid-2-ones (**9a–d**, **9h**, **9i**)





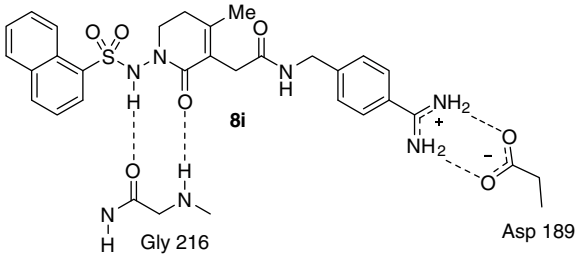
Entry	Compd	W	R^1	R^2	R^3	R^4	R^5	Thrombin[a]	Trypsin[b]	pK_a
								(IC_{50} nM)		
1	**8a** (**9a**)	CH_2SO_2	H	H	H	H	H	241 (3960)	– (14,900)	– (–)
2	**8b** (**9b**)	SO_2	H	H	H	H	H	42 (2470)	766 (5,770)	– (5.9)
3	**8c** (**9c**)	SO_2	H	OMe	H	H	H	11 (1050)	860 (15,600)	8.8 (–)
4	**8d** (**9d**)	SO_2	Me	H	H	Me	H	6 (578)	374 (7130)	8.7 (–)
5	**8e**	SO_2	F	H	Cl	H	H	106	683	–
6	**8f**	SO_2	Cl	H	H	Cl	H	10	1250	–
7	**8g**	SO_2	Cl	H	H	H	Me	3	286	–
8	**8h** (**9h**)	SO_2	OMe	H	Me	H	H	44 (1180)	117 (2020)	– (6.7)
9	**8i** (**9i**)	SO_2	CH=CH–CH=CH	H	H	H	26 (1240)	915 (17,100)	8.2 (5.8)	

[a] Human thrombin.
[b] Bovine trypsin.

case with improved selectivity against trypsin compared to the pyrid-2-one series.

In view of the otherwise virtual identity of these pairs of compounds, we attribute the greatly enhanced activity in the dihydropyrid-2-one series mainly to the higher pK_a of the sulfonamide NH group (see **8i** vs **9i** in Tables). It is possible that this results in a better antiparallel H-bonded interaction with Gly 216 (Fig. 2).[6–9,14] At physiological pH (pH ~7.4), the corresponding pyridones are deprotonated with a delocalised negative charge at the sulfonamide N and may be so also in the complex with thrombin. Thus, a direct H-bond with the Gly 216 carbonyl group is not possible. It should be noted that the thrombin assay is run at pH 7.4.

The X-ray structure of compound **9i** in complex with thrombin has been solved at 1.61 Å resolution. As seen in Figure 3, there are no direct H-bonds between the ligand and Gly 216 in thrombin. The distances between the sulfonamide nitrogen and the amide oxygen of Gly 216 and between the pyrid-2-one oxygen and amide nitrogen of Gly 216 are 4.36 and 3.87 Å, respectively. The corresponding distances in the X-ray of a dihydropyrid-2-one naphthyl



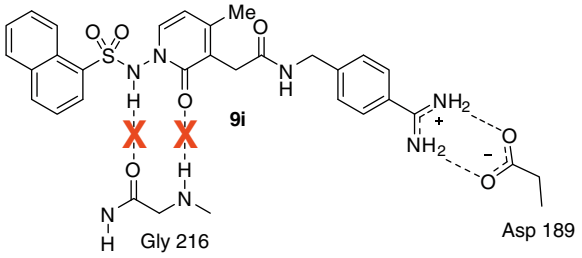


Figure 2. Proposed interactions of sulfonamide inhibitor **8i** and **9i** with Gly 216 and Asp 189 in thrombin.

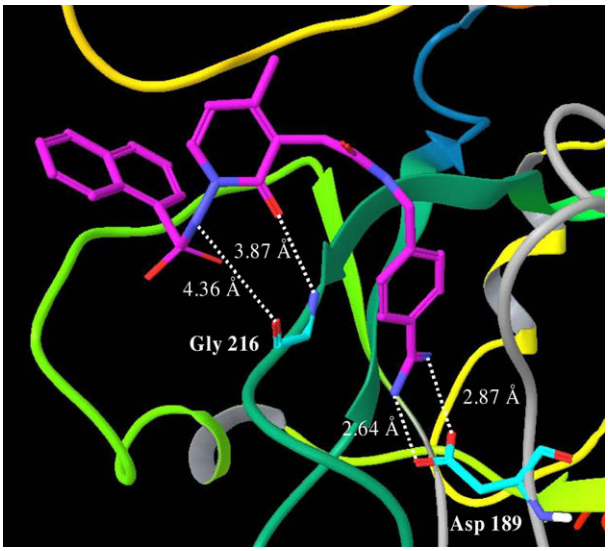


Figure 3. Co-crystal structure of **9i** with thrombin. The long non hydrogen bond distances to Gly 216 are highlighted as well as the favorable interactions between the benzamidine and Asp 189.

analogue with a different P1 moiety are 3.08 and 3.01 Å, indicative of direct H-bonding (data not shown). The pK_a of **9i** sulfonamide group is 5.8 and in the crystallization conditions the pH is 7.2–7.4, which is similar to pH 7.4 used in the thrombin activity measurements. It is therefore likely that the sulfonamide group in the pyrid-2-one **9i** is negatively charged, whereas it is neutral for the dihydropyrid-2-one analogues. These data support the hypothesis that pK_a of the sulfonamide nitrogen strongly influences the affinity to thrombin.

In conclusion, we have shown that structural information derived from co-crystal complexes of natural product inhibitors of thrombin can be used for the design and synthesis of totally achiral congeners. Dihydropyrid-2-ones with IC_{50} values below 10 nM against thrombin have been achieved which is a dramatic improvement compared to the closely related pyrid-2-one analogues. We attribute this to the pK_a differences of the sulfonamide nitrogen resulting in a productive H-bonded interaction with Gly 216 for the dihydropyrid-2-ones. Subtle differences in the nature of sub-

stituents on the aromatic ring of the sulfonamides also play a crucial role in binding and relative potency.

Further refinements that build upon these studies will be reported in due course.

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References and notes

1. (a) Coburn, C. A. *Exp. Opin. Ther. Patents* **2001**, *11*, 721; (b) Colman, R. W.; Hirsh, J.; Marder, J. V.; Clowes, A. W. Blood coagulation factors: Their complementary DNA's, genes and expressions in *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, 4th ed., Lippincott, Williams and Wilkins, 2001, p 17.; (c) Martin, T. J. *Highlights Bioorg. Chem.: Methods Appl.* **2004**, 239; (d) Dahlbäck, B. *Lancet* **2000**, *355*, 1627.
2. Davie, E. W.; Kulman, J. D. *Sem. Thromb. Hemostasis* **2006**, *32*, 3.
3. (a) Dahlbäck, B. *J. Int. Med.* **2005**, *257*, 209; (b) Sidelmann, J. J.; Gram, J.; Jespersen, J.; Kluft, C. *Sem. Thromb. Hemostasis* **2000**, *26*, 605.
4. (a) Prezelj, A.; Anderluh, P. S.; Peternel, L.; Urleb, U. *Curr. Pharm. Des.* **2007**, *13*, 287; (b) The World Health Organization (WHO) Cardiovascular Disease (CVD): Global Strategy on Diet, Physical Activity and Health, WHO, 2003.
5. See for example, (a) Schwienhorst, A. *Cell. Mol. Life Sci.* **2006**, *63*, 2773; (b) Srivastava, S.; Goswami, L. N.; Dikshit, D. K. *Med. Res. Rev.* **2005**, *25*, 66; (c) Gustafsson, D.; Bylund, R.; Antonsson, T.; Nilsson, I.; Nyström, J. E.; Eriksson, U.; Bredberg, U.; Teger-Nilsson, A. C. *Nat. Rev. Drug Disc.* **2004**, *3*, 649; (d) Steinmetzer, T.; Sturzebecher, J. *Curr. Med. Chem.* **2004**, *11*, 2297; (e) Pfau, R. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 437; (f) Kontogiorgis, C. A.; Hadjipavlou-Litina, D. *Curr. Med. Chem.* **2003**, *10*, 525; (g) Steinmetzer, T.; Hauptmann, J.; Sturzebecher, J. *Expert Opin. Invest. Drugs* **2001**, *10*, 845; (h) Sanderson, P. E. J.; Naylor-Olsen, A. M. *Curr. Med. Chem.* **1998**, *5*, 289.
6. For a recent review, see: Ersmark, K.; Del Valle, J. R.; Hanessian, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 1202.
7. Hanessian, S.; Del Valle, J. R.; Xue, Y. F.; Blomberg, N. *J. Am. Chem. Soc.* **2006**, *128*, 10491.
8. Hanessian, S.; Margarita, R.; Hall, A.; Johnstone, S.; Tremblay, M.; Parlanti, L. *J. Am. Chem. Soc.* **2002**, *124*, 13342.
9. Hanessian, S.; Tremblay, M.; Petersen, J. F. W. *J. Am. Chem. Soc.* **2004**, *126*, 6064.
10. (a) Hanessian, S.; Ersmark, K.; Wang, X.; Del Valle, J. R.; Blomberg, N.; Xue, Y.; Fjellström, O. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3480; (b) Hanessian, S.; Guillemette, S.; Ersmark, K. *Chimia* **2007**, *61*, 361.
11. Hanessian, S.; Therrien, E.; Van Otterlo, W. A. L.; Bayrakdarian, M.; Nilsson, I.; Fjellström, O.; Xue, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1032.
12. (a) Hanessian, S.; Simard, D.; Bayrakdarian, M.; Therrien, E.; Nilsson, I.; Fjellström, O. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1972; (b) Bayrakdarian, M.; Berggren, K.; Davidsson, Ö.; Fjellström, O.; Gustafsson, D.; Hanessian, S.; Inghardt, T.; Någård, M.; Nilsson, I.; Simard, D.; Therrien, E. WO 2005/075424 A1 2005-08-18.
13. Berggren, K.; Davidsson, Ö.; Fjellström, O.; Gustafsson, D.; Inghardt, T.; Någård, M.; Nilsson, I.; Therrien, E.; Hanessian, S.; Van Otterlo, W. WO 2005/058826 A1 2005-06-30; For selected synthesis of non-peptidic inhibitors containing aromatic and heteroaromatic core motifs, see: (a) Sanderson, P. E. J.; Cutrona, K. J.; Dyer, D. L.; Krueger, J. A.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; Yan, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 161; (b) Sanderson, P. E. J.; Cutrona, K. J.; Savage, K. L.; Naylor-Olsen, A. M.; Bickel, D. J.; Bohn, D. L.; Clayton, F. C.; Krueger, J. A.; Lewis, S. D.; Lucas, B. J.; Lyle, E. A.; Wallace, A. A.; Welsh, D. C.; Yan, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1441; (c) Soll, R. M.; Lu, T.; Tomczuk, B.; Illig, C. R.; Fedde, C.; Eisennagel, S.; Bone, R.; Murphy, L.; Spurlino, J.; Salemme, F. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1; (d) Sanderson, P. E. J.; Cutrona, K. J.; Dorsey, B. D.; Dyer, D. L.; McDonough, C. M.; Naylor-Olsen, A. M.; Chen, I. W.; Chen, Z.; Cook, J. J.; Gardell, S. J.; Krueger, J. A.; Lewis, S. D.; Lin, J. H.; Lucas, B. J., Jr.; Lyle, E. A.; Lynch, J. J., Jr.; Stranieri, M. T.; Vastag, K.; Shafer, J. A.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 817; (e) Tamura, S. Y.; Semple, J. E.; Reiner, J. E.; Goldman, E. A.; Brunck, T. K.; Lim-Wilby, M. S.; Carpenter, S. H.; Rote, W. E.; Oldeshulte, G. L.; Richard, B. M.; Nutt, R. F.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1543; (f) Levy, O. E.; Semple, J. E.; Lim, M. L.; Reiner, J.; Rote, W. E.; Dempsey, E.; Richard, B. M.; Zhang, E.; Tulinsky, A.; Ripka, W. C.; Nutt, R. F. *J. Med. Chem.* **1996**, *39*, 4527.
14. For X-ray crystal structures, see: (a) Sandler, B.; Murakami, M.; Clardy, J. *J. Am. Chem. Soc.* **1998**, *120*, 595; (b) Rios Steiner, J. L.; Murakami, M.; Tulinsky, A. *J. Am. Chem. Soc.* **1998**, *120*, 597; (c) Carroll, A. R.; Pierens, G. K.; Fechner, G.; De Almeida Leone, P.; Ngo, A.; Simpson, M.; Hyde, E.; Hooper, J. N. A.; Boström, S. L.; Musil, D.; Quinn, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 13340. See also Hanessian, S.; Balaux, E.; Musil, D.; Olsson, L. L.; Nilsson, I. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 243.