Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 789–794

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Thrombin Inhibitors Incorporating Non-basic Partially Saturated Heterobicyclic P_1-Arginine Mimetics

Lucija Peterlin-Mašič,[a] Gregor Mlinšek,[b] Tomaž Šolmajer,[b,c] Alenka Trampuš-Bakija,[d] Mojca Stegnar[d] and Danijel Kikelj[a,*]

[a]*Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia*
[b]*National Institute of Chemistry, Hajdrihova 19, 1115 Ljubljana, Slovenia*
[c]*Lek Pharmaceutical d.d., Drug Discovery, Verovškova 57, 1526 Ljubljana, Slovenia*
[d]*University Medical Centre, Department of Angiology, Riharjeva 24, 1000 Ljubljana, Slovenia*

Received 10 October 2002; revised 5 December 2002; accepted 8 January 2003

Abstract—The design, synthesis and biological activity of non-covalent thrombin inhibitors incorporating 4,5,6,7-tetrahydroindazole, 2-methyl-4,5,6,7-tetrahydroindazole, 4,5,6,7-tetrahydroisoindole, 5,6,7,8-tetrahydroquinazoline and 5,6,7,8-tetrahydroquinazolin-2-amine as novel, partially saturated, heterobicyclic P_1-arginine side-chain mimetics is described. The binding mode of the most potent candidate in the series co-crystallized with human α-thrombin, which exhibited an in vitro K_i of 140 nM and more that 478-fold selectivity against trypsin, is discussed.
© 2003 Elsevier Science Ltd. All rights reserved.

Thrombin is a multifunctional serine protease with trypsin-like specificity, and plays a central role in thrombosis and hemostasis by regulating the blood coagulation cascade and platelet activation processes. Serving as the terminal enzyme of the cascade, thrombin cleaves fibrinogen to fibrin, which ultimately combines with platelets and other components to form a blood clot. The limited efficacy and the side effects of established antithrombotics, including warfarin, heparin and acetylsalicylic acid, have provided the impetus for developing alternative anticoagulants.[1] Inhibition of thrombin is a prime target for therapeutic intervention of thrombosis. During the last decade, the search for orally bioavailable, potent and selective low molecular weight thrombin inhibitors has become one of the most intensively studied areas in drug discovery.[2]

The enzyme S_1 site of thrombin is a deep pocket with Asp 189 at its bottom, capable of forming ionic and hydrogen-bond interactions with positively charged residues such as arginine (**I**) and lysine.[3] Thus, thrombin prefers an arginine as P_1 moiety in its S_1 specify pocket and consequently most thrombin inhibitors contain a P_1 guanidine or amidine moiety.[1,2,4] It was often observed that thrombin inhibitors with strongly basic P_1 moieties have low selectivity and poor oral bioavailability, resulting in metabolic instability and poor absorbtion after peroral application.[1,2,4] In order to overcome the high basicity of the P_1 guanidine, amidine and aliphatic amine moieties, various amino heterocycles have been investigated as P_1 arginine mimetics. Among them, some bicyclic aromatic (amino)heterocycles, for example 1-isoquinolinamine,[5] 3-benzisoxazolamine,[6] indole,[7] benzimidazole,[7] imidazo[1,2-*a*]pyridine,[8] indazole[7,9] and pyrrolo[3,2-*b*]pyridine[10] were recently employed as P_1 moieties in tripeptidomimetic thrombin inhibitors.

In a previous paper we reported thrombin inhibitors featuring P_1 4,5,6,7-tetrahydrobenzothiazol-2-amine moiety.[11a] In this article, we report the design, synthesis, in vitro biological activities and a binding mode of novel selective thrombin inhibitors incorporating different weakly basic, partially saturated P_1-heterobicyclic arginine side-chain mimetics.[11b]

Our approach to designing thrombin inhibitors based on the D-Phe-Pro-Arg motif was to explore the S_1 pocket of thrombin with weakly basic, partially satu-

*Corresponding author. Tel.: +386-1-476-9561; fax: +386-1-476-9500; e-mail: danijel.kikelj@ffa.uni-lj.si

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00030-1

rated heterobicyclic arginine mimetics, which would increase the selectivity of inhibitors for thrombin against trypsin. L-Proline (a preferred ligand for interaction at the YPPW loop) was used as the P_2 moiety and diverse bicyclic arginine side chain mimetics as P_1 moieties (Fig. 1). Ideally, our partially saturated heterobicyclic P_1-arginine mimetics would exhibit a range of pK_a values, participate in hydrogen bonding interactions with Asp 189, and benefit from hydrophobic interactions at the S_1 binding pocket. As it has been shown previously, potent inhibitors with neutral P_1 residues would require the use of lipophilic residues at P_3, in order to add more hydrophobic binding energy to compensate losses at P_1.[12]

Arginine mimetics prepared as a part of this study are listed in Figure 1. Their calculated pK_a values[13] range from weakly basic 2-aminotetrahydroquinazoline **4** (pK_a = 4.7) and tetrahydroindazole **2** (pK_a = 4.0) to the non-basic tetrahydroquinazoline **5** (pK_a = 2.4) and H-bond donor tetrahydroisoindole **7** (pK_a = 2.9). A convenient synthetic approach to these novel partially saturated, heterobicyclic arginine side-chain mimetics **1–7**, containing a five- or six-membered *N*-heterocyclic ring optionally substituted by amino or methyl group, has been reported by us previously.[14–16] We expected that a bulky and lipophilic cyclohexane ring of **III** would introduce conformational rigidity into the P_1-moiety of the inhibitor and confer selectivity for thrombin against trypsin, since the selectivity pocket S_1 of thrombin is slightly larger and more lipophilic than that of trypsin, in which it is narrowed by Ser 190.

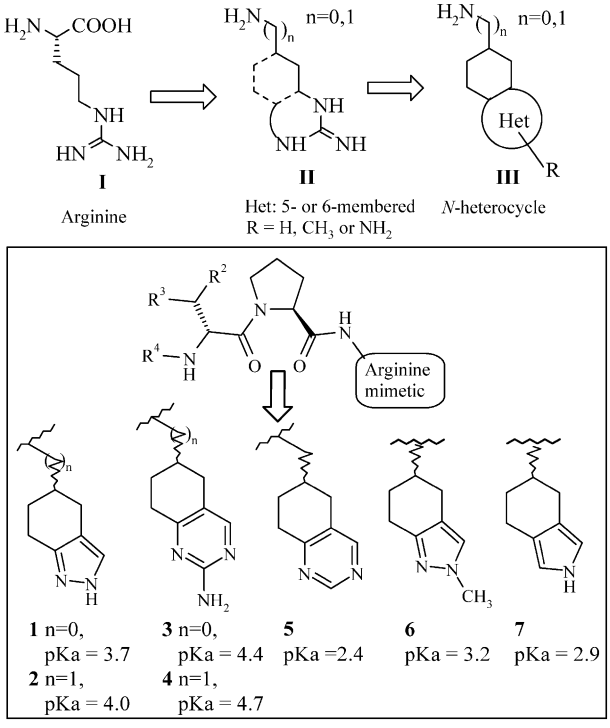
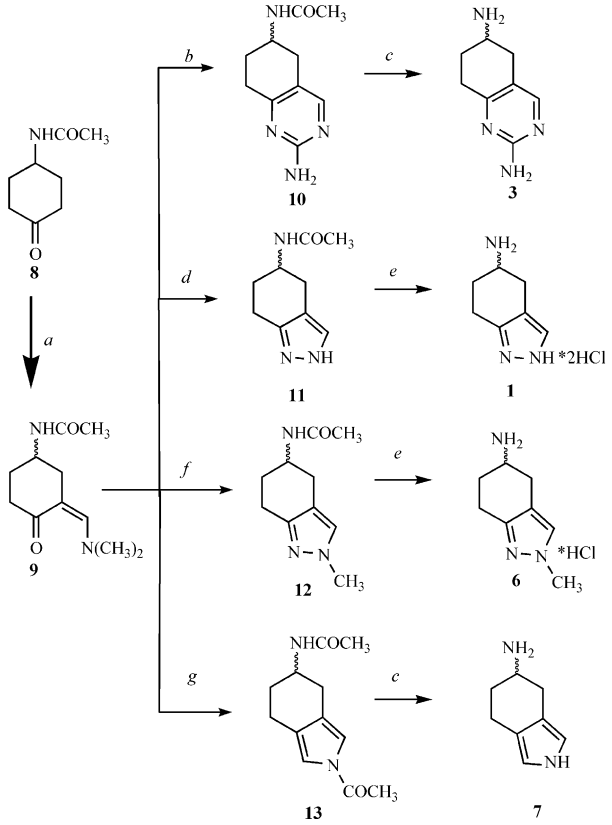


Figure 1. Evolution of thrombin inhibitors incorporating weakly basic, partially saturated heterobicyclic arginine side-chain mimetics, with their calculated pK_a values.

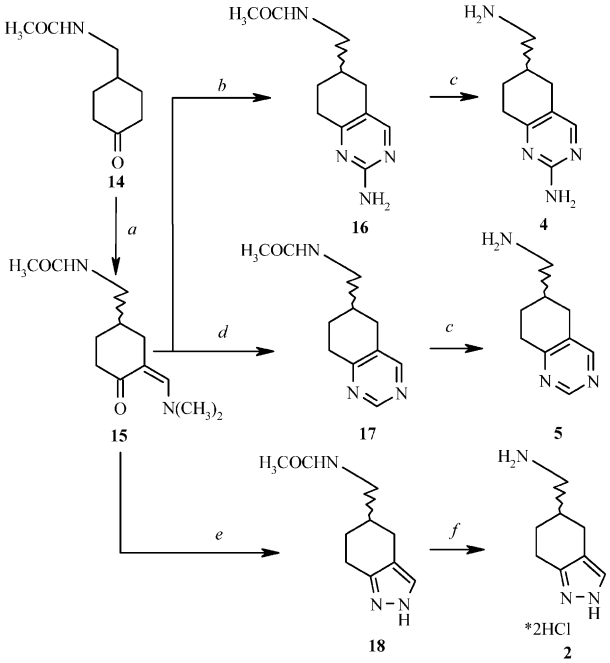
Chemistry

The preparation of arginine mimetics **1, 3, 6** and **7** is outlined in Scheme 1. Condensation of *N*-(4-oxocyclohexyl)acetamide (**8**)[16] with dimethyl-formamide dimethyl acetal (DMFDMA) provided enamino ketone **9**[16,17] which, with guanidine hydrochloride, hydrazine hydrate and *N*-methyl-hydrazine, afforded acetylated heterocycles **10**, **11** and **12**. Final basic hydrolysis of **10** gave 5,6,7,8-tetrahydro-2,6-quinazolinediamine (**3**)[16] whereas acid hydrolysis of **11** and **12** afforded 5-amino-4,5,6,7-tetrahydroindazole (**1**)[17] and 5-amino-2-methyl-4,5,6,7-tetrahydroindazole (**6**) as hydrochloride salts. Base-catalyzed reaction of enamino ketone **9** with glycine and acetic anhydride gave diamide **13** which, after basic hydrolysis, afforded 5-amino-4,5,6,7-tetrahydro-isoindole (**7**).[17]

Scheme 2 outlines the synthesis of arginine mimetics **2**, **4** and **5** with an aminomethyl group bound to the cyclohexane ring.[14,15] Condensation of *N*-[(4-oxo-cyclohexyl)methyl]acetamide (**14**)[14] with DMFDMA and reaction of the resulting enamino ketone **15** with guanidine hydrochloride, formamidine hydrochloride and hydrazine hydrate afforded protected heterocycles **16**, **17** and **18**, which, on hydrolysis, gave 6-(amino-methyl)-5,6,7,8-tetrahydro-2-quinazolinamine (**4**), 6-(amino-methyl)-5,6,7,8-tetrahydroquinazolinamine (**5**) and 4,5,6,7-tetrahydroindazol-5-ylmethan-amine (**2**).[14]



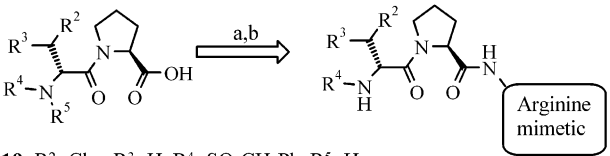
Scheme 1. Reagents and conditions: (a) DMFDMA, Et_3N, toluene, reflux, 7 h; (b) guanidine hydrochloride/NaOEt, abs EtOH, reflux, 3 h; (c) aq NaOH, MeOH, reflux 16 h; (d) hydrazine hydrate, EtOH, rt, 16 h; (e) 6 M HCl, reflux, 6 h; (f) methylhydrazine, EtOH, rt, 16 h; (g) glycine, KOH, abs EtOH, reflux, 2 h; then Ac_2O, reflux, 1 h.



Scheme 2. Reagents and conditions: (a) DMFDMA, Et_3N, toluene, reflux, 7 h; (b) guanidine hydrochloride/NaOEt, abs EtOH, reflux, 3 h; (c) aq NaOH, MeOH, reflux 16 h; (d) formamidine hydrochloride/NaOEt, abs EtOH, reflux, 4 h; (e) hydrazine hydrate, EtOH, rt, 16 h; (f) 6 M HCl, reflux, 6 h.

The final coupling and elaboration reactions between **19–22** and the various arginine mimetics **1–7** are outlined in Scheme 3. Coupling reactions were performed in DMF at room temperature using *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt) as amide bond forming reagents and *N*-methylmorpholine as base. Optional final deprotection of *N*-*tert*-butyloxycarbonyl (Boc) group was effected by hydrogen chloride in glacial acetic acid solution.

The in vitro biological activity of the inhibitors **23–38** is summarized in Table 1. The ability of new thrombin inhibitors to inhibit the enzymatic action of thrombin, trypsin and factor Xa was measured with the amidolytic enzyme assay using S-2238 as a chromogenic substrate.[18a] Values of K_i were calculated according to Cheng and Prusoff,[18b] based on IC_{50} values, or from a relation between reaction velocity equations in the absence and presence of inhibitor using the relevant



19: R^2=Chx, R^3=H, R^4=SO_2CH_2Ph, R^5=H
20: R^2=Ph, R^3=Ph, R^4=Boc, R^5=H
21: R^2=3,4-Cl_2Ph, R^3=H, R^4=Boc, R^5=H
22: R^2=Chx, R^3=H, R^4=CH_2-Boc, R^5=Boc

23-38: See Table 1

Scheme 3. Coupling and elaboration to targets **23–38**. Reagents and conditions: (a) **1–7**, HOBt, *N*-methylmorpholine, EDC, DMF; rt, 12–15 h; (b) optional clevage of Boc protecting group: for **26**, **28**, **31** and **33**: HCl_g, HOAc, rt, 0.5 h.

K_m.[18c] The selectivity for thrombin against trypsin was compared on the basis of the ratios $K_{i(trypsin)}/K_{i(thrombin)}$. The inhibitors which showed the most promising results in amidolytic test were tested in standard clotting assays including the thrombin time (TT), activated partial thromboplastin time (APTT) and prothrombin time (PT) determinations, which were used as a qualitative in vitro indicator of potential antithrombotic activity.

Results and Discussion

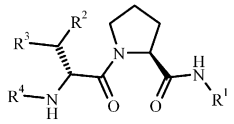
Moderate to good levels of thrombin inhibition, with K_i's between 140 and 690 nM were observed in vitro, for the best inhibitors. All new targets, which were tested against FXa, were selective against this enzyme. Compounds **23–38** also demonstrated good levels of selectivity against trypsin.

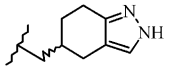
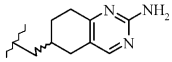
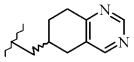
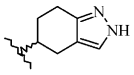
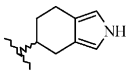
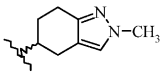
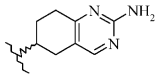
Regarding structure–activity relationship of inhibitors with different amino-linked P_1 heterobicyclic residues and fixed P_2 and P_3 moieties, in vitro potency decreased as a function of the P_1 group in the following order: tetrahydroindazole (**35**, $K_i = 5.4\,\mu M$, $pK_a = 3.7$) > *N*-methyltetrahydroindazole (**37**, $K_i = 12.8\,\mu M$, $pK_a = 3.2$) > tetrahydrisoindole (**36**, $K_i = 27.3\,\mu M$, $pK_a = 2.9$) > 2-aminotetrahydroquinazoline (**38**, $K_i > 35.1\,\mu M$, $pK_a = 4.4$). A methylene linker between the cyclohexane ring and the amino group, which allows substantial rotational freedom of the P_1 part, was beneficial for inhibitory activity against thrombin. Thus, compound **23** exhibited a 38-fold greater inhibitory potency than the inhibitor **35**. It is evident that, among tested P_1 arginine side-chain mimetics the 4,5,6,7-tetrahydroindazole moiety is preferred for binding in the S_1 selectivity pocket of thrombin.

In the 4,5,6,7-tetrahydroindazole series, the most potent compound **23**, possessing 4,5,6,7-tetrahydroindazol-5-ylmethanamine to fill the selectivity pocket, proline in the central part and a benzylsulfonyl group attached to D-cyclohexylalanine as the P_3 part of the inhibitor, was found to be the most potent, with K_i value of 140 nM and more than 478-fold selectivity for thrombin over trypsin. It doubled the TT, APTT and PT at concentrations of 11.0, 42.3 and 78.3 μM, respectively. The replacement of the benzylsulfonyl group in compound **23** by an *N*-carboxymethyl group in **24** brought a 2-fold decrease in inhibitory potency ($K_i = 280$ nM), a decrease in selectivity for thrombin over trypsin and higher activity in all plasma-based clotting assays. The compound **24** doubled the TT, APTT and PT at concentrations of 4.9, 28.0 and 55.3 μM, respectively. Inhibitor **25**, featuring Boc-D-3,3-$(Ph)_2$-Ala at P_3, displayed a K_i for thrombin of 530 nM and the corresponding free amino analogue **26** inhibited thrombin with a K_i of 360 nM. Analogue **27**, with Boc-D-3,4-Cl_2-Phe in the P_3 position, had an inhibition constant of 3.3 μM and the K_i of the corresponding free amino analogue **28** was 5-fold lower ($K_i = 0.69\,\mu M$).

In the 2-aminoquinazoline series, the most potent analogue **30**, which featured Boc-D-3,3-$(Ph)_2$-Ala at P_3,

Table 1. Inhibitory potencies of compounds **23–38**



Compd	R^4	R^3	R^2	R^1	K_i (μM)			Selectivity thrombin/trypsin	APTT	PT	TT
					Thrombin	Trypsin	FXa		(μM)		
23	SO_2CH_2Ph	H	Chx		0.14	>68.3	>75.4	>478	42.3	78.3	11
24	CH_2COOH	H	Chx		0.28	19.9	84.8	71	28	55.3	4.9
25	Boc	Ph	Ph		0.53	[a]	85.2	ND	81	105	29.2
26	H	Ph	Ph		0.36	216.6	82.1	602	43	59.8	7.7
27	Boc	H	3,4-Cl_2–Ph		3.33	>68.3	>75.4	>21	ND	ND	ND
28	H	H	3,4-Cl_2–Ph		0.69	>68.3	>75.4	>99	ND	ND	ND
29	SO_2CH_2Ph	H	Chx		>35.1	>68.3	>75.4	ND	ND	ND	ND
30	Boc	Ph	Ph		4.5	[a]	[a]	ND	ND	ND	ND
31	H	Ph	Ph		12.9	409	57.6	32	ND	ND	ND
32	Boc	H	3,4-Cl_2–Ph		>35.1	>68.3	>75.4	ND	ND	ND	ND
33	H	H	3,4-Cl_2–Ph		>35.1	>68.3	>75.4	ND	ND	ND	ND
34	SO_2CH_2Ph	H	Chx		289	342	111	1.2	ND	ND	ND
35	SO_2CH_2Ph	H	Chx		5.38	>68.3	>75.4	>13	ND	ND	ND
36	SO_2CH_2Ph	H	Chx		27.3	415.8	ND	15	ND	ND	ND
37	SO_2CH_2Ph	H	Chx		12.8	730.3	ND	57	ND	ND	ND
38	SO_2CH_2Ph	H	Chx		>35.1	>68.3	>75.4	ND	ND	ND	ND

APTT, concentration of inhibitor required to double the activated partial thromboplastin time in human plasma; PT, concentration of inhibitor required to double the prothrombin time in human plasma; TT, concentration of inhibitor required to double the thrombin time in human plasma; ND, not determined; Chx, cyclohexyl.

[a]Due to precipitation measurements, were not possible.

displayed a K_i for thrombin of 4.5 μM and the corresponding free amino analogue **31** inhibited thrombin with a K_i of 12.9 μM. Analogues **29**, **32** and **33** demonstrated no inhibitory activity against thrombin (K_i >35.1 μM) and trypsin (K_i >68.3 μM). Similarly, compound **34** with tetrahydroquinazoline at P_1 was also devoid of inhibitory activity against thrombin (K_i = 289 μM).

Crystals of thrombin with the inhibitor **23** were prepared using **23** as a diastereomeric mixture with respect to the stereogenic center at C5 of the 4,5,6,7-tetrahydroindazole ring (Fig. 2)[19]. Based on the electron density we concluded that both *R* and *S* configurations can bind into the S1 pocket. Although our QM/MM calculations indicate that *R* configuration binds energetically more favorably, the electron density is slightly more compatible with the *S* configuration.

The inhibitor **23** forms seven hydrogen bonds to the surrounding residues and two bonds to water molecules. As illustrated in Figure 3, both 4,5,6,7-tetrahydroindazole nitrogens (N1 and N2) form hydrogen bonds with Oδ1 Asp189 while contacts to Oδ2 Asp189 are not present. The bond length between N2 and Oδ1 Asp189 is 2.72 Å and between N1 and Oδ1 Asp189 is 2.67 Å. At the S_1 specify pocket other interactions of the P_1 residue include hydrogen bonds between tetrahydroindazole N1 and Ala190 (3.09 Å) and hydrogen bond between N2 of tetrahydroindazole and a water molecule 34 (3.40 Å).

The oxygen atom of the cyclohexylalanine carbonyl group forms a weak hydrogen bond to Gly216 (3.20 Å) of the thrombin β sheet. The aminomethyl group on the tetrahydroindazole moiety participates in a weak hydrogen bond interaction to the Oγ Ser 215 (3.51 Å) of

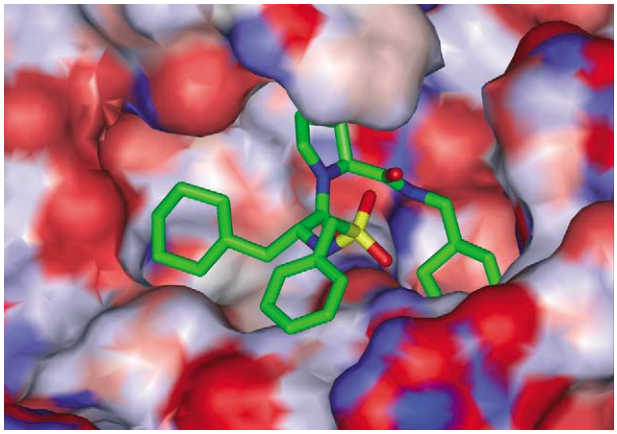


Figure 2. Connolly surface map of the X-ray structure of the α-thrombin-inhibitor **23** complex at 2.4 Å resolution. The inhibitor is shown as sticks. Colors green, blue, red and yellow identify carbon, nitrogen, oxygen and sulphur, respectively.

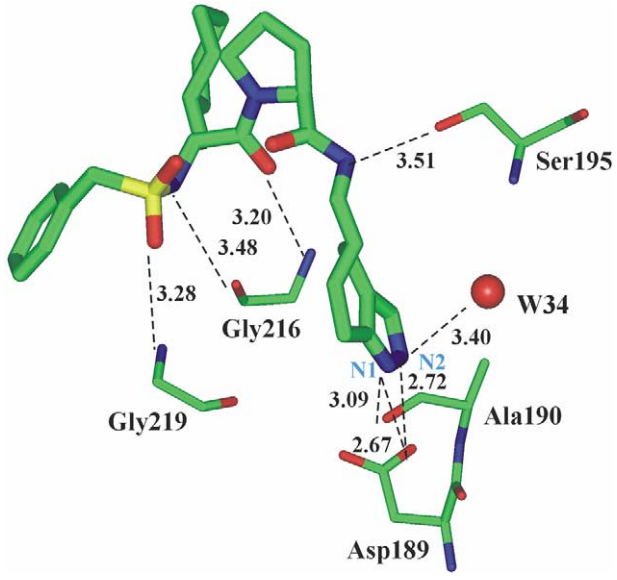


Figure 3. Schematic representation of inhibitor **23** bound in the active site of thrombin. Dashed lines indicate hydrogen bonds. Distances are given in Ångstroms.

the catalytic triad. One of the N-terminal sulfonamide oxygens forms a hydrogen bond with the NH of Gly219 (3.28 Å), while another sulfonamide oxygen participates in a hydrogen-bonding interaction with water 63 (3.34 Å). The N-terminal sulfonamide nitrogen forms a hydrogen bond interaction with Gly216 (3.48 Å) on the thrombin β sheet.

Although the inhibitor **23** forms two short hydrogen bonds between the 4,5,6,7-tetrahydroindazole nitrogens and Asp189 (1 N — Oδ1 Asp189 measures 2.67 Å and 2 N — Oδ1 Asp189 measures 2.72 Å), the lower potency of **23** and its congeners **24–28** compared to inhibitors containing highly basic P_1 guanidine and amidine functionalities[20] could be explained by the low pK_a values of the 4,5,6,7-tetrahydroindazole nitrogen atoms [calcd $pK_a = 4.0$ (N1)],[13] which are not favorable for a stronger ionic interactions with Asp189. However, thrombin inhibitors with non-basic P_1 moieties should posses better pharmacokynetic profiles than inhibitors with highly basic P_1 moities.[21] The results of our studies towards orally bioavailable thrombin inhibitors based on **23** are in progress.

In conclusion, we have designed and evaluated a novel class of non-covalent thrombin inhibitors incorporating novel, weakly basic, partially saturated heterobicyclic P_1-arginine side-chain mimetics, while maintaining the intrinsically potent P_2-proline and P_3-lipophilic pharmacophores in the S_2 and S_3 pockets. Potent and selective thrombin inhibitors were identified, with K_i for thrombin of 140 nM and a more that 478-fold selectivity against trypsin for the most potent candidate **23**, which serves, supported by the results of the crystal structure of **23** complexed in the thrombin active site, as an attractive lead for further SAR development.

Acknowledgements

The authors thank Dr. T. B. Tschopp and Dr. B. Steiner (Pharma Division, Preclinical Research, F. Hoffmann La Roche Ltd, Basel, Switzerland) for biological testing of some compounds, Dr. W. Bode (Max Planck Institut fûr Biochemie, Martinsried, Germany) for X-ray structures and Dr. Roger Pain (Jožef Stefan Institute, Ljubljana, Slovenia) for critical reading of the manuscript. Financial support of this work by the Ministry of Education, Science and Sport of the Republic of Slovenia (Grant No P0787-502 and L3-2034) and by Lek Pharmaceuticals d.d., Ljubljana is gratefully acknowledged.

References and Notes

1. (a) For reviews, see: Tapparelli, C.; Metternich, R.; Ehrhardt, C.; Cook, N. S. *Trends Pharmacol. Sci.* **1993**, *14*, 366. (b) Rewinkel, J. B. M.; Adang, A. E. P. *Curr. Pharm. Des.* **1999**, *5*, 1043. (c) Steinmetzer, T.; Hauptmann, J.; Stürzebecher, J. *Exp. Opin.* **2001**, *10*, 845. (d) Tucker, T. J.; Isaacs, R. C. A. In *Advances in Amino Acid Mimetics and Peptidomimetics*; JAI: 1999; p 53. (e) Coburn, C. A. *Exp. Opin. Ther. Pat.* **2001**, *11*, 721.
2. (a) Hauel, N. H.; Nar, H.; Priepke, H.; Ries, U.; Stassen, J. M.; Wienen, W. *J. Med. Chem.* **2002**, *45*, 1757. (b) Sanderson, P. E. J.; Naylor-Olsen, A. M. *Curr. Med. Chem.* **1998**, *5*, 289. (c) Menear, K. *Curr. Med. Chem.* **1998**, *5*, 457. (d) Wiley, M. R.; Fisher, M. *J. Exp. Opin. Ther. Pat.* **1997**, *7*, 1265. (e) Coburn, C. A.; Rush, D. M.; Williams, P. D.; Homnick, C.; Lyle, E. A.; Lewis, C. D.; Lucas, B. J.; Di Muzio-Mower, J. M.; Juliano, M.; Kreuger, J. A.; Vastag, K.; Chen, I. W.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1069.
3. Bode, W.; Mayr, I.; Baumann, U.; Huber, R.; Stone, S. R.; Hofsteenge, J. *EMBO J.* **1989**, *8*, 3467.
4. (a) Rigid P1-arginine surrogates: Peterlin-Mašič, L.; Kikelj, D. *Tetrahedron* **2001**, *57*, 7073. (b) Reiner, J. E.; Siev, D. V.; Araldi, G. L.; Cui, J. J.; Ho, J. Z.; Reddy, K. M.; Mamedeva, L.; Vu, P. H.; Lee, K. S. H.; Minami, N. K.; Gibson, T. S.; Anderson, S. M.; Bradbury, A. E.; Nolan, T. G.; Semple, J. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1203. (c) Adang, A. E. P.; Lucas, H.; de Man, A. P. A.; Engh, R. A.; Grootenhuis, P. D. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3603. (d) Wiley, M. R.; Weir, L. C.; Briggs, S. L.; Chirgadze, N. Y.; Clawson, D.; Gifford-Moore, D. S.; Schacht, A. L.; Smith, G. F.;

Vasudevan, V.; Zornes, L. L.; Klimkowski, V. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2767. (e) Rewinkel, J. B. M.; Lucas, H.; van Galen, P. J. M.; Noach, A. B. J.; van Dinther, T. G.; Rood, A. M. M.; Jenneboer, A. J. S. M.; van Boeckel, C. A. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 685.
5. Timmers, C. M.; Rewinkel, J. B. M. WO Patent Appl. 0024718, 2000; *Chem. Abstr.* **2000**, *132*, 322143.
6. Sanderson, P. E.; Lyle, T. A.; Dorsey, B. D.; Stanton, M. G.; Naylor-Olsen, A. D. WO Patent Appl. 0026210, 2000; *Chem. Abstr.* **2000**, *132,* 334472.
7. Blagg, J.; Brown, A. D.; Gautier, E. C. L.; Smith, J. D.; McElroy, A. B. European Patent Appl. 0997474, 2000; *Chem. Abstr.* **2000**, *134*, 131552.
8. Sanderson, P. E.; Naylor-olsen, A. M. WO Patent Application 9961442, 1999; *Chem. Abstr.* **2000**, *132*, 12328.
9. Ho, J. Z.; Gibson, T. S.; Semple, E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 743.
10. Sanderson, P. E.; Lyle, T. A.; Dorsey, B. D., Stanton, M. G.; Staas, D.; Coburn, C.; Naylor-Olsen, A. D.; Morrissette, M. M.; Selnick, H. G.; Nanterment, P. G.; Williams, P. D.; Stauffer, K. J.; Burgey, C.; Isaacs, R. WO Patent Appl. 0026211, 2000; *Chem. Abstr.* **2000**, *132*, 334473.
11. (a) Marinko, P.; Krbavčič, A.; Mlinšek, G.; Šolmajer, T.; Trampuš Bakija, A.; Stegnar, M.; Stojan, J.; Kikelj, D. *Bioorg. Med. Chem. Lett.* Submitted for publication. (b) Peterlin-Mašič, L.; Marinko, P.; Breznik, M.; Stegnar, M.; Trampuš-Bakija, A.; Fortuna, M. WO Patent Appl. 01/85760, 2001; *Chem. Abstr.* **2001**, *135*, 358167.
12. (a) Feng, D. M.; Gardell, S. J.; Lewis, S. D.; Bock, M. G.; Chen, Z.; Freidinger, R. M.; Naylor-Olsen, A. M.; Ramjit, H. G.; Woltmann, R.; Baskin, E. P.; Lynch, J. J.; Lucas, R.; Shafer, J. A.; Dancheck, K. B.; Chen, I.-W.; Maso, S. S.; Krueger, J. A.; Hare, T. A.; Mulichak, A. M.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 3726. (b) Tucker, T. J.; Brady, S. F.; Lumma, W. C.; Lewis, D. S.; Gardell, S. J.; Olsen, A. M. N.; Yan, Y.; Sisko, J. T.; Stauffer, K. J.; Lucas, B. J.; Lynch, J. J.; Cook, J. J.; Stranieri, M. T.; Holahan, M. A.; Lyle, E. A.; Baskin, E. P.; Chen, I. W.; Dancheck, K. B.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 3210.
13. pK_a values were calculated on model compounds of general formula Ac-NH–$(CH_2)_{0\text{-}1}$-heterobicycle using ACD/pK_a/logP software, (Version 6.0). Information about the program is accessible via the internet address http://www.acdlabs.com.
14. Peterlin-Mašič, L.; Jurca, A.; Marinko, P.; Jančar, A.; Kikelj, D. *Tetrahedron* **2002**, *58*, 1557.
15. Peterlin-Mašič, L.; Kikelj, D. *Tetrahedron Lett.* **2000**, *41*, 5589.
16. Marinko, P.; Obreza, A.; Peterlin, L.; Krbavèiè, A.; Kikelj, D. *J. Heterocycl. Chem.* **2000**, *37*, 405.
17. Bach, N. J.; Kornfeld, E. C.; Jones, N. D.; Chaney, M. O.; Dorman, D. E.; Paschal, J. W.; Clemens, J. A.; Smalstig, E. B. *J. Med. Chem.* **1980**, *23*, 481.
18. (a) Hilpert, K.; Ackermann, J.; Banner, D. W.; Gast, A.; Gubernator, K.; Hadvary, P.; Labler, L.; Muller, K.; Schmid, G.; Tschopp, T. B.; van de Waterbeemd, H. *J. Med. Chem.* **1994**, *37*, 3889. (b) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099. (c) Brandt, R. B.; Laux, J. E.; Yates, S. W. *Biochem. Med. Metab. Biol.* **1987**, *37*, 344.
19. Structure will be deposited in the Protein Data Bank (PDB).
20. Steinmetzer, T.; Batdorsdhjin, M.; Kleinwächter, P. *J. Enzyme Inhib.* **1999**, *14*, 203.
21. Cui, J. J.; Araldi, G. L.; Reiner, J. E.; Reddy, K. M.; kemp, S. J.; Ho, J. Z.; Siev, D. V.; Mamedova, L.; Gibson, T. S.; Gaudette, J. A.; Minami, N. K.; Anderson, S. M.; Bradbury, A. E.; Nolan, T. G.; Semple, J. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2925.