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Noncovalent Tripeptidic Thrombin Inhibitors Incorporating Amidrazone, Amine and Amidine Functions at P1

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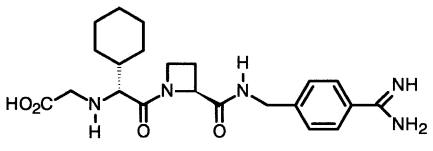
Abstract—A series of noncovalent tripeptidic thrombin inhibitors incorporating amidrazone, amine and amidine functions at P1 was investigated. While the amidrazone and the amine series displayed limited oral absorption, the amidine series demonstrated generally good oral absorption and strong antithrombotic activity; the single-digit picomolar K_i achieved from this series is among the best yet reported. The present work highlights the benzamidine compound **11f** (LB30812) that exhibits excellent overall profiles of potency, oral absorption and antithrombotic efficacy. © 2002 Elsevier Science Ltd. All rights reserved.

Thrombin, a crucial enzyme in the blood coagulation, has been a target for antithrombotic therapy. Orally active thrombin inhibitors would provide effective and safe prophylaxis for venous and arterial thrombosis. While several classes of small molecules have been developed in this research field, recent attention has been devoted to D-Phe-Pro-Agmatine-type tripeptides that lack an electrophilic serine trap.[1] It is well understood that the noncovalent inhibitors, owing to their fast-binding nature, are more efficacious and thus have a better therapeutic index than covalent inhibitors.[1b,2] Since thrombin inhibitors with strongly basic P1 tend to have poor oral absorption albeit melagatran (**1**)[3] is not the case, P1 elements with appropriate basicity (p*K*a 6.5–9) have been of research interest. In this context, several publications have appeared where aminopyridine,[4] acylguanidine[5] and imidazole[6,7] were explored at the P1 position of the noncovalent tripeptide scaffold.

Our previous research efforts for thrombin inhibitors had been focused on a class of arylsulfonyl-phenylalanine amides. LB30057 (**2**) is a prototype compound of a benzamidrazone class (pK_a ~8.9), which demonstrated good oral availability in animals and humans.[8] The benzylamine series derived from this template (e.g., **4**) is also orally bioavailable,[9] but the amidine analogues (e.g., **3**) were not orally absorbed despite good antithrombotic activity.[10] In our previous study, a rigid and mildly basic imidazolylethynyl P1 was incorporated into the tripeptide scaffold in combination with diphenylalanine at P3, leading to potent, selective and orally available thrombin inhibitors (i.e., **5**).[7] As an extension of this work, we describe SAR of the amidrazone, amine and amidine functions at the P1 position of the D-diphenyl-Ala-Pro template and identification of a number of orally available thrombin inhibitors with excellent potency.



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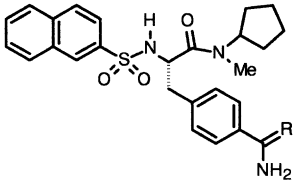
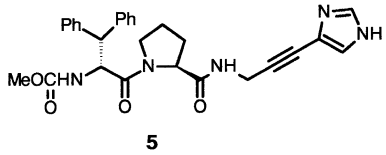


2, R = NNH_2 (Ki = 0.38 nM)
3, R = NH (Ki = 6.8 nM)
4, R = H_2 (Ki = 18.3 nM)



5

Ki = 14 nM (thrombin), 24 μM (trypsin)

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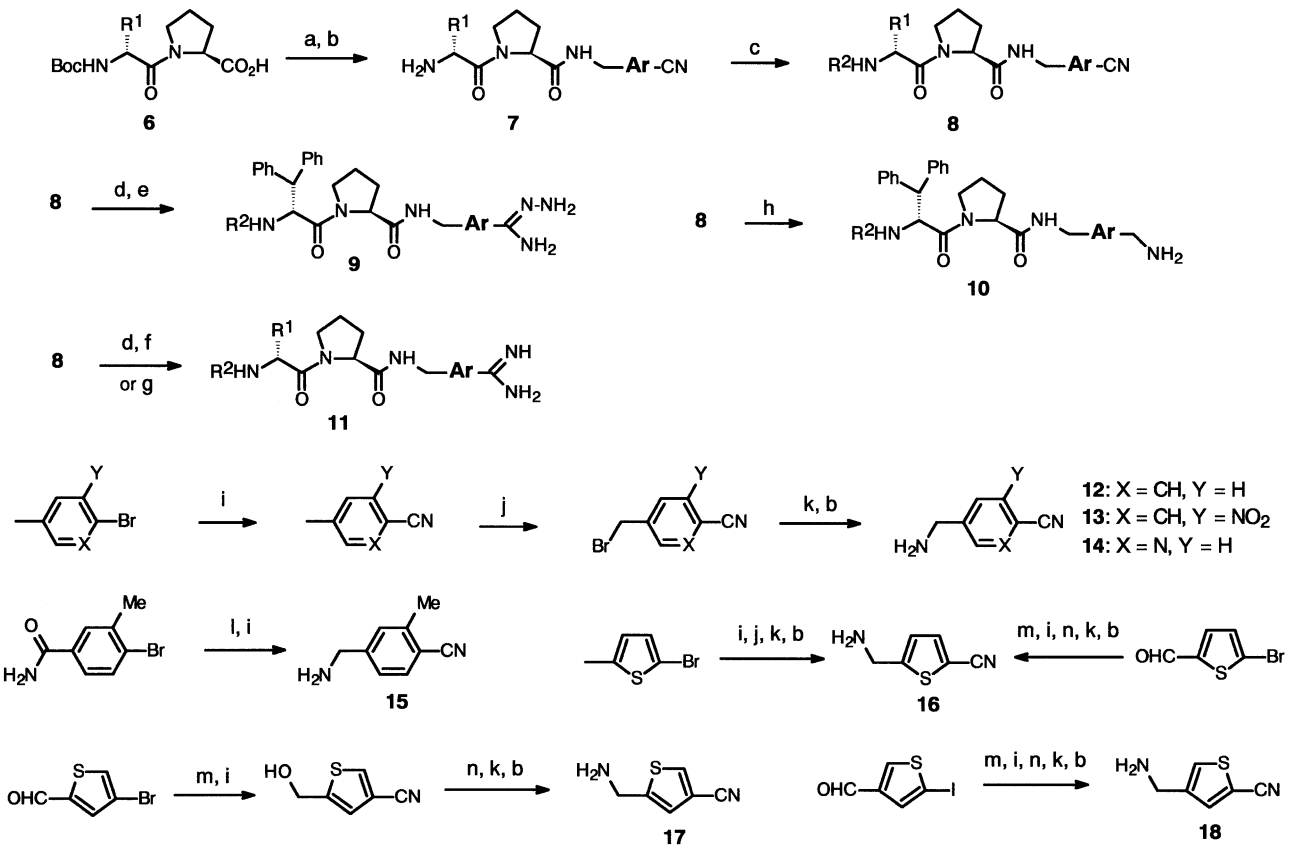


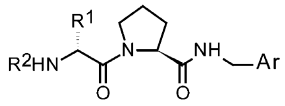
Scheme 1. (a) CN–Ar–CH_2NH_2 (**12**–**18**), NMM, EDC, HOBT, quant; (b) TFA, CH_2Cl_2 or AcCl, MeOH; (c) R^2-Cl (sulfonylchlorides, sulfamoylchlorides, chlorosulfonic acid, chloroformate, chlorophosphate) Et_3N, CH_2Cl_2; for **9g** and **11n** *t*-BuO_2CCH_2Br, DIPEA, CH_3CN, rt, 36 h; for **11k** $Pd(Ph_3P)_4$, CuI, Et_3N, K_2CO_3, $PhCH_2NEt_3Cl$, iodobenzene, DMF, 100 °C, 4 h; (d) (i) H_2S, py, Et_3N; (ii) MeI, CH_3CN, reflux; (e) $NH_2NH_2{\cdot}H_2O$, MeOH, rt; (f) NH_4OAc, MeOH, reflux; (g) (i) $NH_2OH{\cdot}HCl$, Na_2CO_3, $EtOH/H_2O$; (ii) H_2, 10% Pd/C, Ac_2O, MeOH; (h) H_2 (60 psi), concd HCl, MeOH; (i) CuCN, DMF, reflux; (j) NBS, benzoylperoxide, CCl_4, reflux; (k) $(Boc)_2NH$, NaH, THF; (l) BH_3/THF, 0 °C; (m) $NaBH_4$, MeOH, rt; (n) CBr_4, PPh_3, CH_2Cl_2.

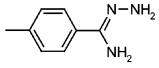
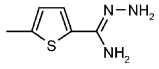
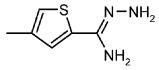
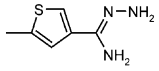
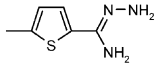
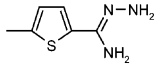
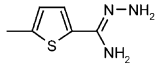
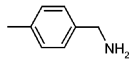
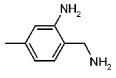
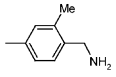
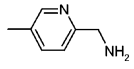
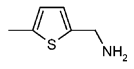
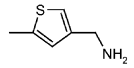
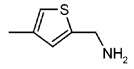
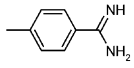
The target compounds were prepared as outlined in Scheme 1. The *N*-protected dipeptides **6** were coupled to the P1 precursors **12**–**18** and the *N*-Boc group was removed. The free amines **7** were converted to **8** by reacting with R^2-Cl in the presence of base. *N*-Phenylation of **7** was accomplished by a Pd-mediated cross coupling reaction. The intermediates **8** were then subjected to a Pinner-type reaction which proceeds via a thioimidate intermediate to produce the amidrazones **9** and the amidines **11**.[8,10] The nitrile group could also be converted to the amidine moiety by catalytic hydrogenation of an amidoxime intermediate under acetylation.[11] The amines **10** were prepared from the same intermediate by hydrogenation in the presence of HCl.[9] The requisite CN–Ar–CH_2NH_2, **12**–**14** and **16**, were prepared starting from the corresponding CH_3–Ar–Br in several steps involving CuCN-mediated cyanation, radical bromination and a Gabriel-type amination.[12] Synthesis of compounds **17** and **18** began with aldehyde reduction of 4-bromothiophene-2-carboxaldehyde and 4-iodothiophene-3-carboxaldehyde,[13] respectively: following cyanation afforded better yields of the hydroxycyano intermediates than inverse reactions. The hydroxyl group was subsequently brominated prior to amination.

The benzamidrazone compound **9a** exhibited a K_i of 47 nM for thrombin (Table 1). This moderate potency may be related with the vertical N–NH_2 of the amidrazono group which weakly interacts with Asp189 of thrombin via a lateral interaction (Fig. 1a). It was thus anticipated that five-membered heterocycles would flip up the NH_2 pendant allowing the amidrazone to favorably interact with Asp189 via a bidentate bridge (Fig. 1b). Indeed, replacement with thiophene nuclei (**9b**–**d**) resulted in a great enhancement in potency. The expected bidentate interaction was confirmed by X-ray crystallographic study on the **9b**–thrombin complex where the amidrazone group forms H-bonds to the Asp189 carboxylic oxygens (3.24 and 2.99 Å).[14] Furthermore, the thiophenes **9c** and **9d** displayed better selectivity for thrombin over trypsin. It was, however, disappointing that none of these amidrazone inhibitors revealed good absorption behavior when orally administered in rats ($C_{max} < 0.2$ μg/mL, 30 mg/kg). It appears that the amidrazone function in this series is not advantageous for gut membrane permeability. Boc-, acetyl and carboxymethyl replacement at the N-terminus (**9e**–**g**) led to improved oral absorption over **9a**–**d** but at the expense of thrombin potency.

The benzylamine **10a** showed potent thrombin inhibition ($K_i = 4$ nM). This inhibitor was also orally bioavailable to some extent when administered to rats ($C_{max} = 0.5$ μg/mL, 30 mg/kg, $n = 3$). Substitution at the

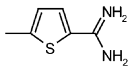
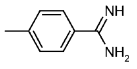
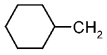
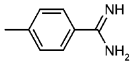
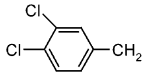
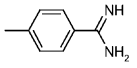
Table 1. Thrombin and trypsin inhibitory activities for compounds **9**–**11**



Compd	R^1	R^2	Ar	Thrombin[a] K_i (nM)	Trypsin[b] K_i (nM)	Factor Xa[c] K_i (nM)
9a	Ph(Ph)CH	$MeSO_2$		47	150	Inactive
9b	Ph(Ph)CH	$MeSO_2$		0.53	12	193
9c	Ph(Ph)CH	$MeSO_2$		1.8	540	Inactive
9d	Ph(Ph)CH	$MeSO_2$		2.2	250	59,000
9e	Ph(Ph)CH	Boc		84	NT	Inactive
9f	Ph(Ph)CH	Ac		105	NT	Inactive
9g	Ph(Ph)CH	HO_2CCH_2		31	1,100	Inactive
10a	Ph(Ph)CH	$MeSO_2$		4	9.9	Inactive
10b	Ph(Ph)CH	$MeSO_2$		12	11	Inactive
10c	Ph(Ph)CH	$MeSO_2$		32	110	Inactive
10d	Ph(Ph)CH	$MeSO_2$		6	220	Inactive
10e	Ph(Ph)CH	$MeSO_2$		5.8	85	Inactive
10f	Ph(Ph)CH	$MeSO_2$		2.4	68	Inactive
10g	Ph(Ph)CH	$MeSO_2$		5.3	606	100,000
11a	Ph(Ph)CH	$MeSO_2$		0.003	0.7	300

(*continued on next page*)

Table 1 (*continued*)

Compd	R^1	R^2	Ar	Thrombin[a] K_i (nM)	Trypsin[b] K_i (nM)	Factor Xa[c] K_i (nM)
11b	Ph(Ph)CH	$MeSO_2$		0.005	0.6	360
11c		$MeSO_2$		0.5	9.9	5000
11d		$MeSO_2$		0.02	1.3	200
11e		$MeSO_2$		0.05	0.8	120
Melagatran (**1**)				1.2	—	—

[a]Human thrombin.
[b]Bovine trypsin.
[c]Human factor Xa.

ortho position of the phenyl ring (**10b**,**c**) was detrimental to the potency. The pyridine heterocycle of this series (**10d**) was beneficial to the aqueous solubility, but did not improve oral absorption. The thiophene analogues **10e**–**g**, while retaining a comparable level of potency for thrombin, displayed slightly better trypsin-selectivity and oral absorption (C_{max} = 0.7–1.8 μg/mL, same conditions). When tested for their ability to inhibit thrombus formation in a rat model of venous thrombosis, compounds **10a** and **10d**–**g** exhibited only moderate antithrombotic activity (30–67% inhibition, iv bolus, 5 mg/kg, $n = 3$).[15] The overall in vivo profile made this series of compounds unsuitable for further evaluation.

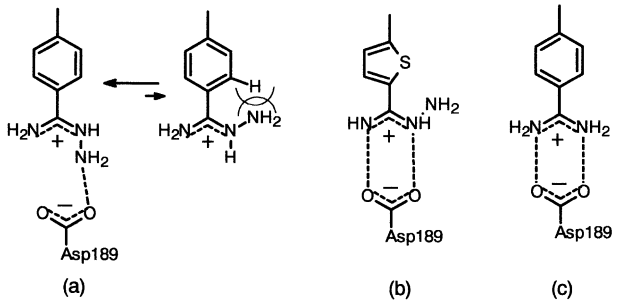
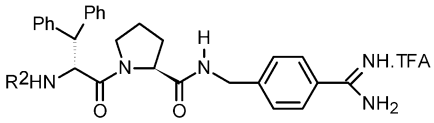


Figure 1.

Remarkable activity for thrombin inhibition was achieved from the series of the amidine analogues. The benzamidine **11a** is extremely potent (K_i = 3 pM), which favorably compares with the most potent thrombin inhibitors reported to date. The thienyl amidine **11b** is substantially equipotent. Analogues possessing cyclohexylGly, cyclohexylAla, and dichlorophenylAla (**11c**–**e**) were significantly less potent as expected, but still superior to the amidrazone and the amine analogues. This indicates high contribution of the amidine moiety to enzyme affinity via a strong salt–bridge interaction with Asp189 (Fig. 1c). Most notable is that the diphenylAla **11a** showed a good absorption profile upon oral dosing in rats (C_{max} = 2.8 μg/mL, AUC = 263 μg h/mL, 30 mg/kg) whereas the non-diphenylAla derivatives were significantly less absorbed (C_{max} = 0.1–0.9 μg/mL). In addition, the picomolar inhibitor **11a** demonstrated excellent antithrombotic activity when evaluated in the rat thrombosis model, reflecting its strong in vitro activity (70% inhibition at 1 mg/kg, iv bolus, $n = 3$).

Further SAR studies in the benzamidine series was focused on the variation of the N-terminal sulfonyl substituent as shown in Table 2. Compared to the primary lead **11a**, the sulfamide derivative **11f** displayed a similar level of enzyme inhibition and oral absorption, but superior antithrombotic activity (100 and 80% inhibition at 1 and 0.5 mg/kg, respectively, iv bolus, rat, $n = 3$). However, the more hydrophobic sulfonyl and sulfamoyl substituents (**11g**,**h**) were detrimental to oral absorption while retaining comparable thrombin inhibitory activity. Compounds possessing sulfamic acid and aniline moieties (**11i**,**k**) were also poorly absorbed, which is probably due to their poor aqueous solubility. The phosphonate **11j**, the acetamide **11l** and the carbamate **11m** showed slightly improved oral absorption but at the expense of thrombin potency. Carboxymethyl substitution (**11n**) resulted in a decrease in both potency and oral absorption as compared to those of **11a**. Also notable was the unsubstituted amine compound **11o** that was still absorbed despite its highly basic property. Overall, the sulfamide **11f** showed the best in vivo profile in this series of compounds. This inhibitor was substantially orally bioavailable when evaluated in dogs and monkeys (Fig. 2) and metabolically stable in S9 fractions from rat, dog, monkey and human species.

Table 2. Enzyme inhibitory activities and pharmacokinetic parameters for compounds **11a–o**



Compd	R^2	Thrombin[a] K_i (nM)	Trypsin[b] K_i (nM)	Plasma concentration[c]	
				C_{max} (μg/mL)	AUC (μg h/mL)
11a	$MeSO_2$	0.003	0.7	2.7	263
11f	$NH_2S(O)_2$	0.003	0.3	2.8	272
11g	$PhCH_2S(O)_2$	0.005	0.58	0.9	68
11h	Cyclohexyl-$NHS(O)_2$	0.003	0.25	<0.3	—
11i	$HOS(O)_2$	0.10	4.4	0.55	35
11j	$(MeO)_2P(O)_2$	0.044	2.9	4.0	271
11k	Ph	0.045	1.5	0.3	53
11l	Ac	0.16	NT	3.0	244
11m	MeOC(O)	0.036	0.31	3.9	594
11n	HO_2CCH_2	0.015	3.0	1.3	120
11o	H	0.036	2.5	1.4	107

[a]Human thrombin.
[b]Bovine trypsin.
[c]Concentration after oral dosing at 30 mg/kg as a water solution or as a 20–30% co-solvent (PEG/EtOH/Twin20 = 85/10/5) solution in water.

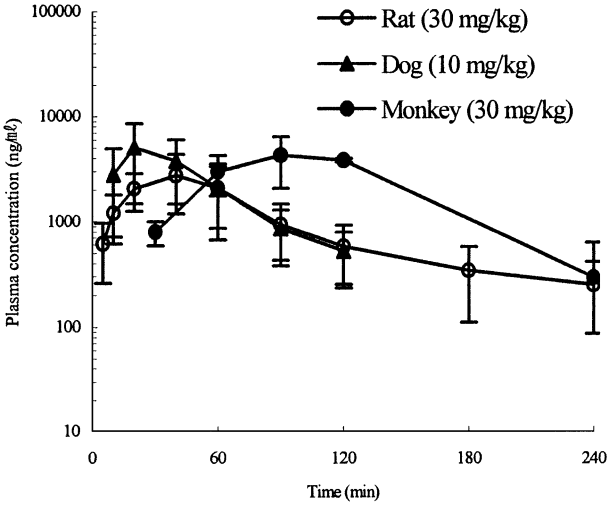


Figure 2. Plasma concentrations of compound **11f** after oral administration in rats, dogs and monkeys.

In conclusion, we have investigated a series of non-covalent tripeptidic thrombin inhibitors that incorporate amidrazone, amine and amidine functions at P1. The amidrazone and the amine series displayed limited oral absorption despite their mildly basic nature. In contrast, the amidine series demonstrated generally good oral absorption and strong antithrombotic activity; the single-digit picomolar potency achieved from this series is among the highest yet reported. The present work highlights the benzamidine compound **11f** (LB30812) that exhibits excellent overall profiles of potency, oral absorption and antithrombotic efficacy. Further SAR studies of this amidine series are in active progress and the results will be published in due course.

References and Notes

1. For recent reviews, see: (a) Hauptmann, L.; Sturzebecher, *J. Thromb. Res.* **1999**, *93*, 203. (b) Sanderson, P. E. J. *Med. Res. Rev*. **1999**, *19*, 179.
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