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Amidinohydrazones as Guanidine Bioisosteres: Application to a New Class of Potent, Selective and Orally Bioavailable, Non-amide-Based Small-Molecule Thrombin Inhibitors

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Abstract—We describe a new class of potent, non-amide-based small molecule thrombin inhibitors in which an amidinohydrazone is used as a guanidine bioisostere on a non-peptide scaffold. Compound **4** exhibits nM inhibition of thrombin, is selective for thrombin, and shows 60 and 23% bioavailability in rabbits and dogs, respectively. Crystallographic analysis of **4** bound to thrombin confirmed the amindinohydrazone binding mode.

A key strategy in new antithrombotic therapy has been directed towards the discovery of small molecule inhibitors of the coagulation cascade, in particular the serine protease thrombin, an enzyme which plays a pivotal role in both fibrin generation and platelet activation.[1] Recent years have witnessed a number of important developments with respect to the discovery of potent and selective inhibitors possessing improved pharmacokinetic characteristics in comparison to preceding generations of such agents.[2,3] However, for long-term anticoagulation, the search for direct antithrombins with good oral bioavailability and long half-life still remains a significant challenge.

In previous papers we evaluated a novel series of achiral thrombin inhibitors exemplified by **1** and **2**.[4–6] These compounds exhibited potent and selective inhibition of thrombin and are distinguished by the structural simplicity of the series. However, proper in vivo evaluation of the most interesting compounds, such as **2** (K_i 4.6 nM), was limited by poor solubility. Further, it was desirable to diversify the properties of this series in two critical ways through (a) modulation of the pK_a of the highly basic guanidino grouping given the poor in vivo profiles of known guanidino-containing thrombin inhibitors and (b) variance of the overall lipophilicity of the series since it has been appreciated that the antithrombotic performance of thrombin inhibitors is dependent inter alia upon a proper balancing between oral absorption, volume of distribution, and protein binding, all of which may be linked to the lipophilicity of the molecule.[7]

We report here further structure-based elaboration of this series through replacement of the simple guanidine grouping with an amidinohydrazone motif. This interchange was inspired by the oral activity of the guanidino-containing α_2-agonist guanabenz (**3**),[8] the depressed pK_a guanabenz (8.1),[9] and the potential for maintaining key hydrogen-bonding interactions with Asp-189 of thrombin's S1 binding pocket. This novel replacement has led to a potent, selective, and orally bioavailable, non-amide-based thrombin inhibitor series.

Compounds **4**–**9** were prepared as follows. Orcinol was first derivatized to monosulfonate **21** using the appropriate sulfonyl chloride (ca. 1 equiv) for **4**–**5**, **7**–**11**, **13**–**15**, **18** and **19** in a biphasic mixture of saturated bicarbonate and Et_2O (26–76% yield). For **6**, 5-chloro-2-methoxybenzenesulfonyl chloride was used (satd $NaHCO_3$, THF, *n*-Bu_2O (50:38:12)) followed by catalytic hydrogenation (H_2 (1 atm), 10% Pd/C, MeOH) to provide **21** in 63% yield. Monosulfonates **21** were converted in the presence of excess diol to **23** (3–4 equiv) by (a) standard Mitsunobu[10] chemistry (PPh_3 (1.5 equiv)/DEAD (1.5 equiv)) for **14** and **19** or (b) Mitsunobu

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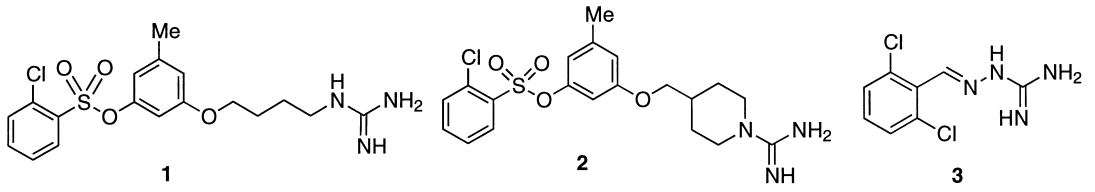


1 2 3

modification[11] for compounds **5**–**13**, **15** and **18** (PBu_3 (1.5 equiv)/ADDP (1.5 equiv) (39–100% yield)). In the case of **4**, compound **22** was first prepared using monobenzylated propanediol in the Mitsunobu reaction and then deprotected to **23** (H_2 (1 atm), 10% Pd/C, MeOH (71% yield)). Intermediate **23** was oxidized (SO_3 pyridine complex/DMSO/DIEA/CH_2Cl_2)[12] typically at 0 °C to ambient temperature (47–100% isolated yields); the aldehydes for quinoline and pyridine analogues **11** and **14** were advanced into the next reaction without purification. Conversion of **24** into the corresponding amidinohydrazones was accomplished with aminoguanidine nitrate. Compounds **5**, **7**, **8**, **12**, **13**, **15**, **16**, **18** and **19** were isolated as nitrate salts (2 equiv in ethanol; 58–98% yields). Compounds **4** and **10** were isolated as HCl salts after HCl treatment of the free base. Compounds **9**, **11**, **13** and **20** were isolated as HOAc salts after HOAc treatment of the free base and/or chromatography through a silica gel SPE column using CH_2Cl_2/MeOH/HOAc mixtures as eluants.

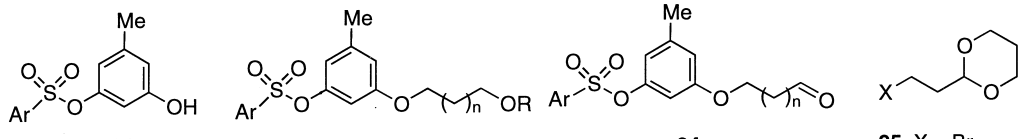
Ether analogue **16** was prepared in 4 steps: (a) benzylation of orcinol (NaH, DMF then 2-trifluorobenzyl chloride (41% yield)), (b) coupling with 1,3-propanediol under Mitsunobu conditions[10] (85% yield), (c) oxidation[11] (85% yield), and (d) amidinohydrazone formation (77% yield; nitrate salt). Analogue **17** was prepared in six steps from 3,5-dihydroxybenzaldehyde: (a) MeOH, PPh_3, DEAD, THF, 0 °C (25%), (b) olefination with **26** (LDA in cyclohexane, THF, −78°C (28% yield)), which in turn was prepared from bromide **25** (PPh_3, CH_3CN, reflux, 2 d), (c) catalytic reduction (H_2, 10% Pd/C, THF) (d) sulfonylation (2-Cl-$PhSO_2Cl$, DIEA, cat. DMAP, CH_2Cl_2 (77% yield for 2 steps), (e) oxidation[12] (15% yield) and (f) amidinohydrazone formation (obtained as HCl salt after HCl treatment of free base; 75% yield).

The compounds of the present study were evaluated for thrombin inhibition and cross-screened for selectivity against a panel of the serine proteases such as chymotrypsin, elastase, plasmin, and trypsin.[4] The thrombin inhibition data is presented in Table 1. Counter-screening concentrations varied between 1 and 27 μM and for those compounds exhibiting activity at screening concentrations, K_i's were determined. Only trypsin and/or chymotrypsin activity were observed at high concentrations (K_i's chymotrypsin: **7** (88 μM), and **17** (13.7 μM); K_i's trypsin: **7** (59 μM), **8** (17 μM), **15** (23 μM), **13** (45 μM), **16** (14.8 μM), **19** (67 μM) and **20** (17 μM). Exchanging the guanidino group of **1** (K_i 13 nM) with an amidinohydrazone (**4**; K_i 8.3 nM) produced a potent thrombin inhibitor. Critical for optimal potency is the amidinohydrazone side chain length of 3 carbon atoms. Reduction of the side chain from 3 carbons to 2 carbons or elongation to 4 carbons significantly decreased potency (**6** versus **18** and **19**). An attempt to fill the hydrophobic S1 pocket with a cyclopropyl ring resulted in 4-fold less activity (**10** versus **20**), presumably due to steric clashes with the protein.

The sulfonate group, which provides a rigid, directional link from the central orcinol scaffold to thrombin's distal aromatic binding pocket contributes to the potency of this series (**11** versus **16**). Replacing the phenolic ether linkage with a carbon atom resulted in a pronounced decrease in potency, possibly due to an increase in conformational flexibility and side chain conformational change (**4** versus **17**).

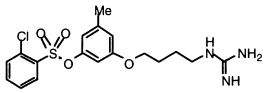
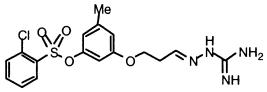
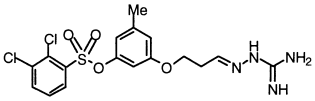
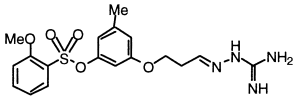
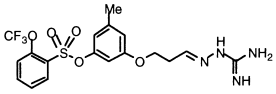
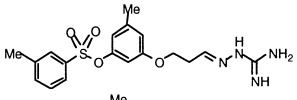
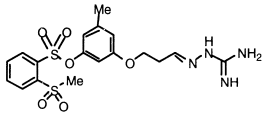
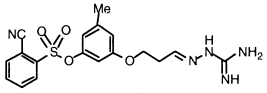
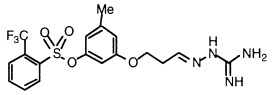
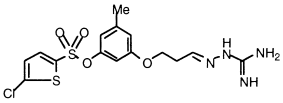
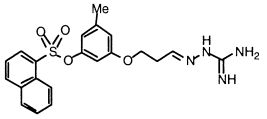
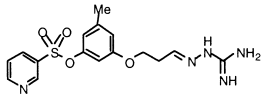
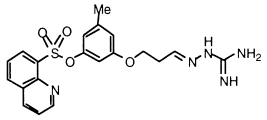
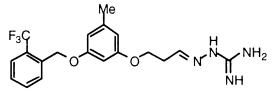
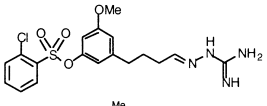
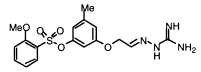
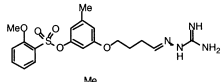
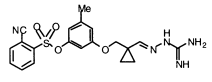
Analysis of compounds **1** and **2**, crystallographically bound to thrombin, had shown that the projection of the substituent ortho to the sulfonyl group in the aryl binding pocket was directed towards solvent[4] thereby presenting additional opportunities for property diversification. In the current series, a wide range of pendant group functionality spanning electron donating/withdrawing and hydrophilic/hydrophobic moieties is allowed and produced a host of compounds with K_i's substantially less than 50 nM. In addition, the 5-chlorothienyl (**12**; K_i 9.2 nM) and 8-quinolinyl analogue (**15**; K_i 4.7 nM) groups are well accommodated in the aryl binding pocket, but less potent are the 1-naphthyl analogue (**13**; K_i 44 nM) and the hydrophilic 3-pyridyl analogue (**14**; K_i 36 nM).

Further insights were provided by comparison of **4** and **1** crystallographically bound to thrombin as shown in the overlays in Figures 1 and 2.[13] The interactions of the S1 pocket are maintained by the amidinohydrazone group, however the hydrogen bond is now more evenly split between the terminal nitrogens than was the case in the guanidino substituent. The increased planarity of the amidinohydrazone group leads to a slight overall displacement of the remainder of the molecule such that



21 22 R = CH_2Ph 23 R = OH 24 25 X = Br 26 X = $P^+(Ph)_3I^-$

Table 1. Inhibition of thrombin by amidinohydrazones **4**–**20**

	Structure	Thrombin K_i (nM)
1		13 ± 1.6
4		8.3 ± 2.5
5		9.4 ± 0.67
6		11 ± 9.9
7		11 ± 1.5
8		6.0 ± 3.0
9		20 ± 7.6
10		9.1 ± 4.8
11		4.4 ± 2.5
12		9.2 ± 5.0
13		44 ± 3.8
14		36 ± 10
15		4.7 ± 1.8
16		420 ± 220
17		990 ± 240
18		> 1000
19		1300 ± 60
20		40 ± 3.7

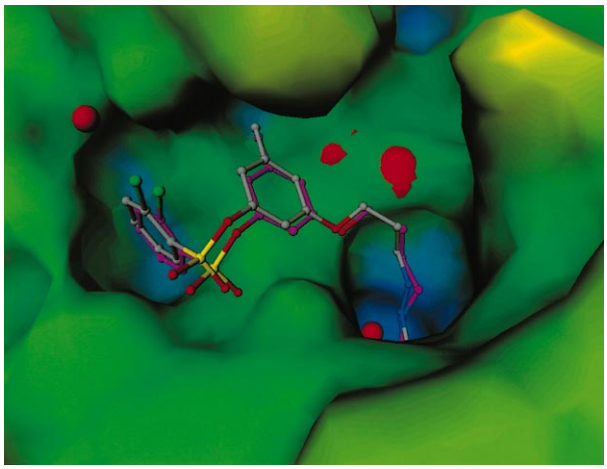


Figure 1. Overlap of crystal structures of compounds **4** (gray) and **1** (magenta) bound to thrombin; surface view.

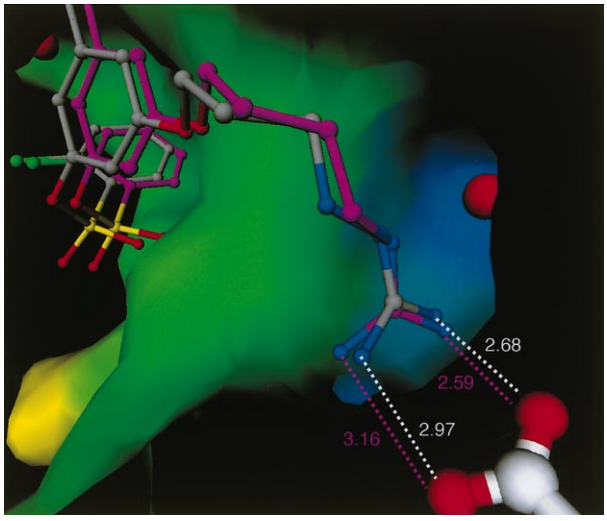


Figure 2. Overlap of crystal structures of compounds **4** (gray) and **1** (magenta) bound to thrombin; focus on S1 pocket.

edge-to-face interaction of **4** to tryptophan 215 of the distal aromatic binding pocket has increased (3.5 Å) in comparison to **1** (3.2 Å). Thus, the nearly equivalent K_i's for the two compounds suggest that the loss in positive van der Waal's packing energy due to the displacement of the P2 and aryl binding groups is more than compensated for by the loss of rotational freedom due to the amidinohydrazone group.

Pertinent physical properties of **4** and **10** along with pharmacokinetic and pharmacodynamic characteristics (evaluated in dogs) are summarized in Table 2. The amidinohydrazone motif substantially reduced the pK_a of a guanidino group.[14] Compounds **4** and **10** also possess more "drug-like" lipophilicities,[15] which may be reflected in the oral bioavailability of these compounds (23 and 21% for **4** and **10**, respectively, as determined using doses of 6 mg/kg i.v. and 30 mg/kg p.o. in 20% 2-hydroxypropyl-β-cyclodextrin formulations). The

Table 2. Physical and pharmacokinetic characteristics in dogs of **4** and **10**

Compound	pK_a	log P	$t_{1/2}$ (h) dog plasma	F (%)	$C_{max\ (oral)}$ (μM)	TCT[a] (s)	Cl (mL/min/kg)	Vss (K/kg)
4	8.71	2.34	21	23	1.8	34 ± 3[a]	39	3.5
10	8.84	2.11	19	21	0.9	101 ± 23[b]	79	23

[a]Baseline TCTs: [a]21 ± 2; [b]24 ± 1.

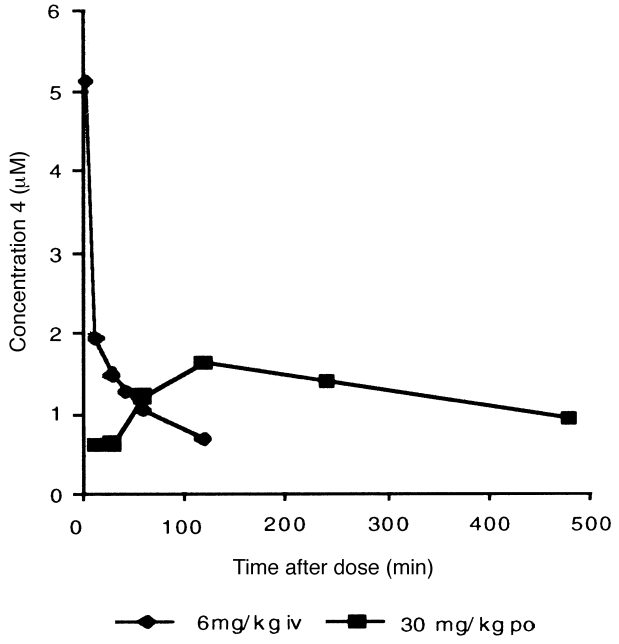


Figure 3. Bioavailability of compound **4** in rabbits.

stability of the compounds in dog plasma were comparable ($t_{1/2}$ 21 h and $t_{1/2}$ 19 h, respectively). Pharmacodynamically, **4** effected a maximal 1.6-fold increase in TCT at 2 h (30 mg/kg, p.o.). Compound **10** maximally prolonged clotting time 4-fold at 1 h (30 mg/kg, p.o.) and produced measurable anticoagulation at 8 and 24 h (1.6- and 1.2-fold, respectively). Given the high clearance and wide distribution of these compounds, both compounds produced only marginal anticoagulation effects as measured by TCT prolongation.

Compound **4** was further evaluated for bioavailability in rabbits by measuring plasma concentration of drug substance using doses of 6 mg/kg i.v. and 30 mg/kg p.o. in rabbits (Fig. 3). Compound **4** was highly orally bioavailable in rabbits (F 60%). Micromolar level of compound was observed at 8 h (termination of experiment). Pharmacodynamically, thrombin clotting times (TCTs) were prolonged to 1.9× over controls (50 ± 6.4 s at 60 min versus 26 ± 4.8 s baseline level) at 30 mg/kg p.o.

In summary, the present work demonstrates that the amidinohydrazone motif is an effective replacement of the highly basic guanidino functionality. Key hydrogen-bonding interactions of the guanidine are preserved while offering opportunities for improving overall in vivo anticoagulant properties and performance through reduction of pK_a. Additional evaluation, diversification and optimization of this novel class of achiral, non-amide-based thrombin inhibitors are in progress and will be reported in due course. The results and observations of the present work are broadly applicable to other potential targets involving such basic group interactions and are under active investigation.

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