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Oxyguanidines. Part 2: Discovery of a novel orally active thrombin inhibitor through structure-based drug design and parallel synthesis

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Abstract—Through structure-based drug design and parallel synthesis, we have discovered a novel series of nonpeptidic phenyl-based thrombin inhibitors using oxyguanidines as guanidine bioisosteres. These compounds have been found to be highly potent, highly selective, and orally bioavailable.

The serine protease, thrombin, a key mediator in the blood coagulation system, is involved in the cleavage of fibrinogen to form fibrin, the cross-linking of fibrin to form clots, the stimulation of platelet aggregation, and the autoamplification of the coagulation cascade to produce additional active thrombin.[1] The orally bioavailable antithrombotic agent, warfarin, is the current treatment but possesses a number of limitations, such as (a) the indirect mechanism of action, (b) the need for constant monitoring to assure effective drug plasma levels or avoidance of bleeding complications, and (c) potential drug–drug interactions. Other antithrombotics such as heparin and low-molecular-weight heparin (LMWH), lack intrinsic oral bioavailability.[2] There have been very few reports of orally active thrombin inhibitors, which are efficacious in animal models of thrombosis.[3–6] We have reported a series of orally active thrombin inhibitors using amidinohydrazones as guanidine bioisosteres.[7] It is now widely recognized that structure-based drug design coupled with parallel synthesis is a powerful tool and valuable asset in the drug discovery process for lead optimization. We report here the discovery and development of novel potent orally active thrombin inhibitors utilizing this combined approach.

In a previous paper,[8] we reported a novel series of potent orally active thrombin inhibitors using oxyguanidines as guanidine bioisosteres exemplified by **1** (Fig. 1). Compound **2**[9] has been disclosed by Glaxo as a potent thrombin inhibitor. By combining the left side of **2** with the right side of **1**, we have prepared compound **8a**, which has a $K_i = 21$ nM for thrombin. X-ray crystallographic studies revealed that the right side oxyguanidine of **8a** binds to the thrombin S_1 pocket in the same

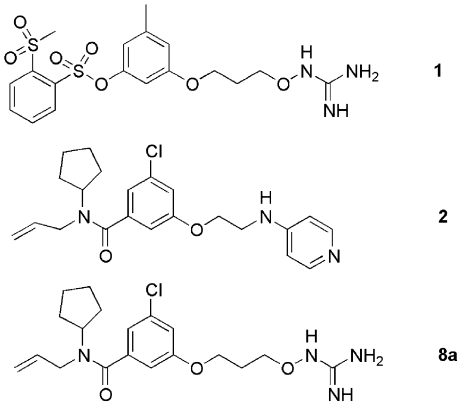

Figure 1. Chemical structures of compounds **1**, **2**, and **8a**.

Keywords: Oxyguanidine; Thrombin inhibitor; Structure-based drug design; Parallel synthesis.
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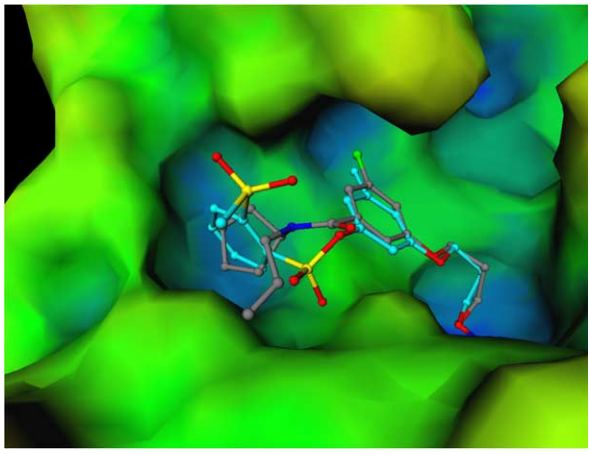


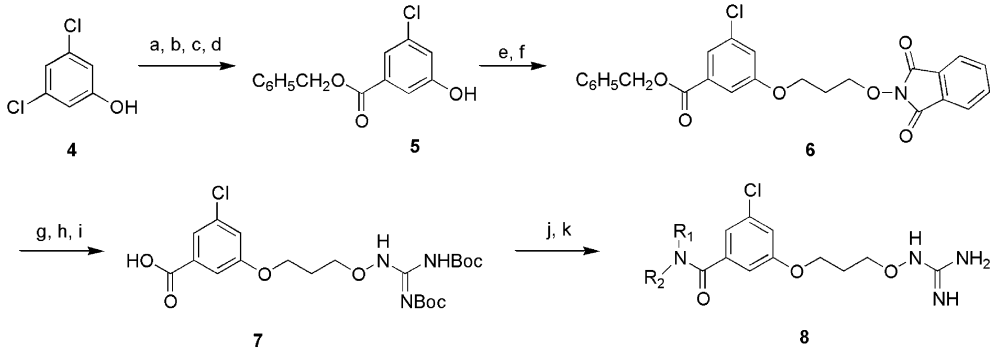
Figure 2. Overlap of crystal structures of compounds **1** (blue) and **8a** (gray) bound to thrombin.

manner as the right side of **1**, the cyclopentyl ring binds to the aryl binding pocket, and the allyl group is exposed to the solvent (Fig. 2).[10] Based on this structure and previous structure–activity relationship (SAR) information, we devised a solution-based parallel synthesis approach to explore the SAR of the aryl binding pocket.

A solution-phase library of analogs of **8** was prepared in multi-milligram quantities according to Scheme 1. The key intermediate **7** was prepared from 3,5-dichlorophenol in nine steps in overall 23% yield. 3,5-Dichlorophenol **4** was first protected with *tert*-butyldimethylsilyl chloride, treated with Rieke Mg and carbon dioxide at −78 °C, followed by oxalyl chloride and benzyl alcohol, and deprotected with tetrabutylammonium fluoride to give compound **5**. The ester **5** was reacted with 3-bromopropanol in the presence of cesium carbonate followed by Mitsunobu reaction with *N*-hydroxyphthalimide. The phthalimide **6** was deprotected with aqueous methylamine in tetrahydrofuran, treated with bis-Boc amidinopyrazole in DMF, and hydrolyzed with base (NaOH) in ethanol–water to give the intermediate **7**. Compound **7** was treated with R^1–HN–R^2, benzotriazole-1-yl-oxy-tri-(dimethylamino)phosphonium hexafluorophosphate (BOP), triethylamine, and dimethylaminomethyl-polystyrene resin in CH_2Cl_2 followed by deprotection with trifluoroacetic acid/methylene chloride. The crude products were purified by silica gel chromatography using IST VacMaster manifold and SPE cartridges to give **8** >90% pure by ^{1}H NMR, LCMS, and HPLC.

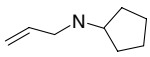
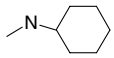
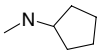
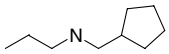
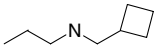
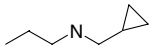
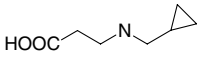
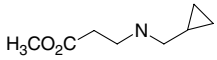
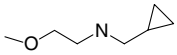
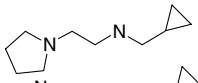
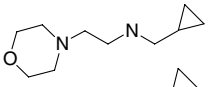
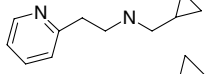
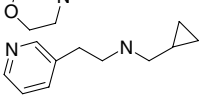
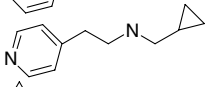
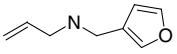
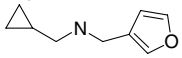
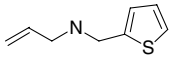
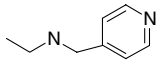
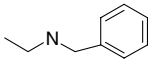
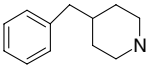
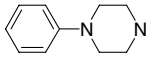
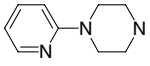
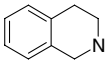
Table 1 summarizes the thrombin inhibition for selected analogs **8**. We have determined the X-ray crystal structure of several of these analogs and invariably the aryl, heteroaryl, or cycloalkyl groups with $\leqslant 1$ carbon atom attachment are determined to occupy the aryl binding pocket (**8a**,**f**, **13d**,**o**, **14**). In the case of two preferred groups as in **8p**, the aryl or heteroaryl group is the preferential group binding in the aryl binding pocket. Based on this analysis, the following SAR will be discussed. While none of these analogs were demonstrably better than **8a**, several analogs had a similar potency to **8a** (**8e**,**f**,**o**,**p**). Two of these (**8e** and **8f**) contain a small cycloalkylmethylene substituent and a small alkyl chain (propyl). The other two (**8o** and **8p**) share a 3-furylmethylene substituent with an allyl or cycloalkylmethylene substituent. Compounds that contain heteroatoms (**8h**), polar groups (**8g**,**h**), heterocyclic rings (**8j**,**n**), or heteroaryl rings (**8l**,**m**,**n**) reduce activity. The 3-furylmethylene group does appear to be significant since other aryl or heteroaryl methylene groups are less potent (**8q**–**s**). Cyclized secondary amines (**8t**–**z**) are not accommodated in the aryl binding pocket.

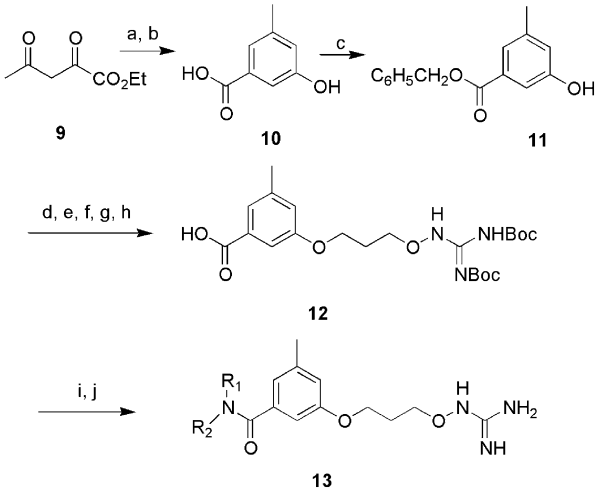
In order to assess the preference of methyl in place of chloro of **8**, compounds **13** (Scheme 2) were prepared by a similar route as compounds **8**. The requisite hydroxy benzoic acid **10** was prepared via the literature method.[11] The noncommercially available secondary amines were made through reductive amination of aldehydes and primary amines. Table 2 summarizes the thrombin inhibition for the selected analogs **13**. In general, compounds **13** are more potent than compounds **8**, and have a narrower SAR. Still, similar conclusions can be drawn from this series. The most potent compounds with K_i less than 10 nM contain either a cyclohexyl, cyclohexylmethylene, or furylmethylene substituent as R^2 and



Scheme 1. Reagents and conditions: (a) TBDMSCl, DMAP, DIEA, CH_2Cl_2, 100%; (b) Rieke Mg, CO_2, THF, −78 °C, 64%; (c) oxalyl chloride, $C_6H_5CH_2OH$, cat. DMF, CH_2Cl_2, 96%; (d) TBAF, THF, 91%; (e) 3-bromopropanol, Cs_2CO_3, CH_3CN, 50 °C, 83%; (f) *N*-hydroxyphthalimide, triphenylphosphine, DEAD, THF, 95%; (g) 40% aq CH_3NH_2, THF, 64%; (h) *N*,*N*′-bis(*tert*-butoxycarbonyl)amidinopyrazole, DMF, 85%; (i) NaOH, EtOH–H_2O, 70%; (j) R^1–NH–R^2, benzotriazole-1-yl-oxy-tri(dimethylamino)phosphonium hexafluorophosphate (BOP), dimethylaminomethyl-polystyrene resin, triethylamine, CH_2Cl_2; (k) TFA, CH_2Cl_2, 55–85% over last two steps.

Table 1. Thrombin inhibition for selected analogs **8**

Compds	R^1–N–R^2	K_i, μM	Compds	R^1–N–R^2	K_i, μM
8a		0.021	**8b**		0.033
8c		0.373	**8d**		0.038
8e		0.016	**8f**		0.023
8g		0.213	**8h**		0.038
8i		0.156	**8j**		0.204
8k		0.159	**8l**		0.059
8m		0.047	**8n**		0.052
8o		0.020	**8p**		0.017
8q		0.147	**8r**		1.60
8s		0.091	**8t**		0.880
8u		7.62	**8v**		0.470
8w		1.500	**8x**		2.900
8y		0.640	**8z**		0.171



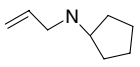
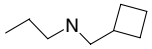
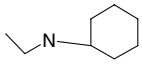
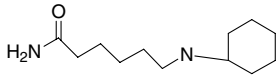
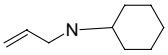
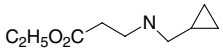
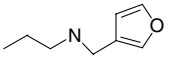
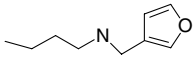
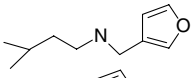
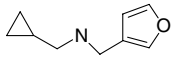
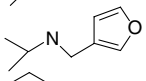
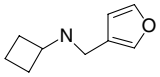
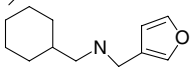
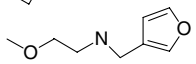
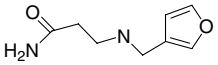
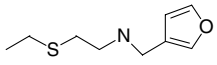
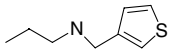
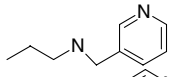
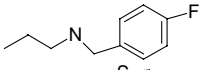
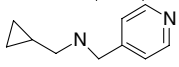
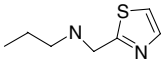
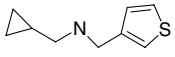
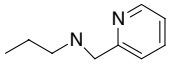
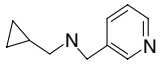
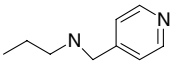
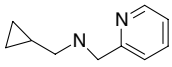
Scheme 2. Reagents and conditions: (a) AcOH, H_2O, NaOH, 100%; (b) MgO, H_2O, 100 °C, 74%; (c) $C_6H_5CH_2Br$, $NaHCO_3$, DMF, 86%; (d) 3-bromopropanol, Cs_2CO_3, CH_3CN, 50 °C, 75%; (e) *N*-hydroxyphthalimide, triphenylphosphine, DEAD, THF, 95%; (f) 40% aq CH_3NH_2, THF, 82%; (g) *N*,*N*′-bis(*tert*-butoxycarbonyl)amidinopyrazole, DMF, 90%; (h) NaOH, EtOH–H_2O, 85%; (i) R^1–NH–R^2, benzotriazole-1-yl-oxy-tri(dimethylamino)-phosphonium hexafluorophosphate (BOP), dimethylaminomethyl-polystyrene resin, triethylamine, CH_2Cl_2; (j) TFA, CH_2Cl_2, 54–85% over last two steps.

an alkyl or *S*-alkyl chain in the R^1 position (**13c**,**e**,**g**,**h**,**i**,**p**). Pyridines in the R^2 position tend to have lower activity; replacing furylmethylene with 2-pyridylmethylene gives a 8-fold less active compound (**13z** vs **13j**). In general, nonpolar alkyl chains in R^1, and smaller aromatic, heterocyclic, or cycloalkyl rings in R^2 lead to the most potent compounds. In addition, the introduction of a cyclopropyl group in the S1 binding pocket increases potency by 2–3-fold (**14** vs **13g** and **15** vs **13j**, Fig. 3 and Table 3).

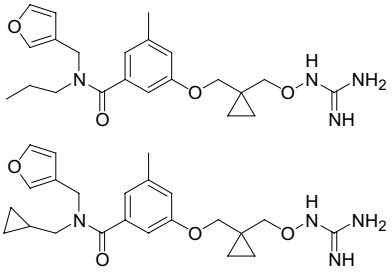
These thrombin inhibitors retain the excellent selectivity against other serine proteases (>10^3-fold) that was observed in the previous set of phenyl-based thrombin inhibitors.[7,8,12] The oxyguanidine unit of compound **13g** has a measured pK_a of 7.23. The apparent permeability coefficient (P_{app}) from the apical to basolateral side in the human Caco-2 monolayer was determined to be 4.05×10^{-6} cm/s that would be considered highly permeable. When administered as a 2 mg/kg iv infusion over 5 min to beagle dogs, the terminal half-life was 3.8 h. The C_{max} reached 4.5 μM at 1 h after 10 mg/kg by oral gavage, and the dose-corrected oral bioavailability (F) was 62% (Fig. 4).

The pharmacokinetics of several analogs in dogs are compared in Table 3. The parameters of all of them are quite similar and would be considered moderate for the dog. Firstly, this series is not plagued by low absorption potential as judged by the high permeability data in the human Caco-2 cell line. In the dog, the clearance values

Table 2. Thrombin inhibition for selected analogs **13**

Compds	R^1–N–R^2	K_i, μM	Compds	R^1–N–R^2	K_i, μM
13a		0.010	**13b**		0.017
13c		0.006	**13d**		0.008
13e		0.009	**13f**		0.065
13g		0.009	**13h**		0.008
13i		0.007	**13j**		0.011
13k		0.068	**13l**		0.022
13m		0.021	**13n**		0.028
13o		0.110	**13p**		0.006
13q		0.048	**13r**		0.052
13s		0.021	**13t**		0.024
13u		0.195	**13v**		0.031
13w		0.094	**13x**		0.034
13y		0.073	**13z**		0.086

(CL) range from 1/10 to 1/3 of hepatic blood flow ($HBF_{dog} = 38$ mL/min/kg), which would be considered in the medium range. The volume of distribution (V_{ss}) is also considered in the medium range for the dog (2–4-fold total body water of approximately 7 L). The terminal half-lives are in the medium range for the dog. As a result, the series is characterized as having very good, dose-corrected oral bioavailability (F). Based on the comparable metabolic stability in dog and human microsomes (>98%), we would expect that this series has a high potential for oral bioavailability in human.



14

15

Figure 3. Chemical structures of compounds **14** and **15**.

In regard to a biomarker in human, the apTT (activated partial thromboplastin time) anticoagulant assay using human plasma is a reasonable measure of efficacy. The potencies of these analogs (Table 3) in the apTT assay differ by three orders of magnitude from their thrombin inhibition potencies. The most likely explanation for this difference is the high plasma protein binding for this series (>98%). The plasma protein binding may limit the antithrombotic efficacy of this series in vivo.

Table 3. In vitro anticoagulant potency (2×apTT: concentration of compound required to double the human activated partial thromboplastin time) and pharmacokinetic parameters in dogs after 2 mg/kg infusion over 5 min and 10 mg/kg oral administration

Compds	K_i (μM)	Caco-2 (A to B, 10^{-6} cm/s)	2×apTT (μM)	PK (dog)				
				C_{max} (po, μM)	$T_{1/2}$ (iv, h)	CL (iv, mL/min/kg)	V_{ss} (iv, L/kg)	F (%)
13a	0.010	4.86	11.3	3.7	3.7	11.2	2.8	89
13g	0.009	4.05	11.5	4.5	3.8	10.1	2.4	62
13j	0.011	3.00	8.4	5.1	2.5	7.8	1.6	68
14	0.004	4.11	7.9	5.8	4.4	4.2	1.7	73
15	0.004	2.17	8.7	4.1	5.9	5.7	2.5	77

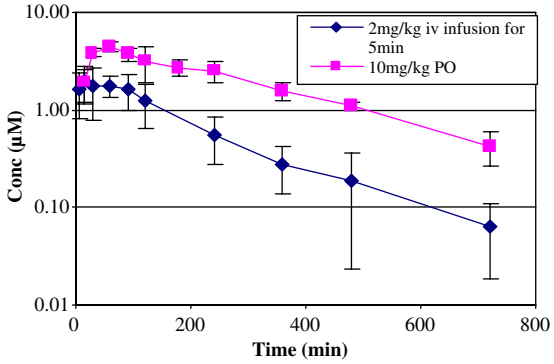


Figure 4. In vivo pharmacokinetics in dog ($n = 3$) after 2 mg/kg iv over 5 min and 10 mg/kg po of compound **13g**.

In summary, we have optimized a novel series of non-peptidic phenyl-based, highly potent, and highly selective thrombin inhibitors using oxyguanidines as guanidine bioisosteres through structure-based design and parallel synthesis. The compounds of this series are Caco-2 monolayer permeable and orally bioavailable in dog. Additional SAR and in vivo efficacy results will be reported in due course.

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