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Oxyguanidines: Application to Non-peptidic Phenyl-Based Thrombin Inhibitors

Bruce Tomczuk,* Tianbao Lu, Richard M. Soll,† Cynthia Fedde, Aihua Wang, Larry Murphy, Carl Crysler, Malini Dasgupta, Stephen Eisennagel, John Spurlino and Roger Bone

3-Dimensional Pharmaceuticals, Inc., 665 Stockton Drive, Exton, PA 19341 USA

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Abstract—Although thrombin has been extensively researched with many examples of potent and selective inhibitors, the key characteristics of oral bioavailability and long half-life have been elusive. We report here a novel series non-peptidic phenyl-based, highly potent, highly selective and orally bioavailable thrombin inhibitors using oxyguanidines as guanidine-mimetics.

A key strategy in the development of novel antithrombotic therapy has been directed towards the discovery of small molecule inhibitors of the coagulation cascade. One molecular target is thrombin (Factor IIa), which is a serine protease which plays a pivotal role in both fibrin generation penultimate to clot formation and platelet activation.[1] Although this target has been extensively researched with many examples of potent and selective inhibitors, the key characteristics of oral bioavailability and long half-life have been elusive. A first generation (intravenous only) active site thrombin inhibitor, argatroban, has recently been approved in the U.S. for HITT and HITTS.[2] It is likely that a second generation compound, such as the double-prodrug ximelagatran (**1**) by AstraZeneca or a compound from the pyridinone (**2a**) or pyrazinone series (**2b**) by Merck, will have improved oral and pharmacokinetic properties.[3–5] We have developed a series of non-peptidic phenyl-based thrombin inhibitors which have addressed these elusive properties. These inhibitors incorporate a novel oxyguanidine moiety, which is a guanidine-mimetic of the RGD-type thrombin inhibitors.[6] The oxyguanidine possesses a greatly reduced p*K*a of approximately 7.0–7.5 compared to a guanidine of 13–14.[7] In the GI tract, a basic compound of reduced p*K*a would result in a larger percentage of uncharged molecules with a greater propensity to cross cell membranes and enter the systemic circulation. This positive distinguishing characteristic will be exemplified by in vitro permeability and pharmacokinetic studies.

These compounds were synthesized starting with the monosulfonated orcinol **3**,[8] which undergoes two sequential Mitsunobu reactions, first with a diol and the second with *N*-hydroxyphthalimide.

The phthalimide **4** is deprotected with aqueous methylamine to reveal the alkoxyamine **5** (Scheme 1). Subsequent reaction with bis-Boc guanidinopyrazole gives a protected alkoxy guanidine **6**. Deprotection with TFA provides the final products **7**–**29** as TFA salts. These phenyl arylsulfonates have excellent hydrolytic stability in buffers from pH 2 to 10 at 37 °C for at least 24 h.

Tables 1 and 2 indicate the structure–activity relationships in the aryl- and the heteroaryl-sulfonate functionalities, respectively. The *ortho*-substituted derivatives (**7**–**12**) were generally potent with the exception being the anilino derivative **10**. Focus was placed on the sulfones (**11**,**12**) due to their increased aqueous solubility characteristic. The 3-methylsulfone **13** was an order of magnitude less potent than **11**. A combination of methylsulfone groups at the *ortho*- and *para*-positions (**14**) caused a loss of two orders of magnitude in

*Corresponding author. Tel.: +1-610-458-6058; fax: +1-610-458-8258; e-mail: bruce.tomczuk@3dp.com
†Present address: Ontogen Corporation, 6451 El Camino Real, Carlsbad, CA 92009, USA.

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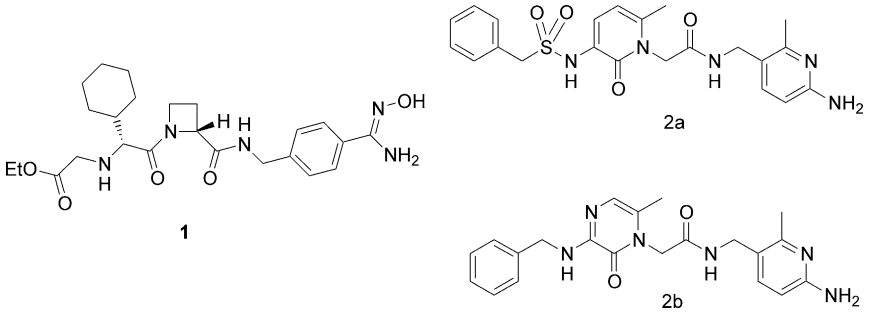
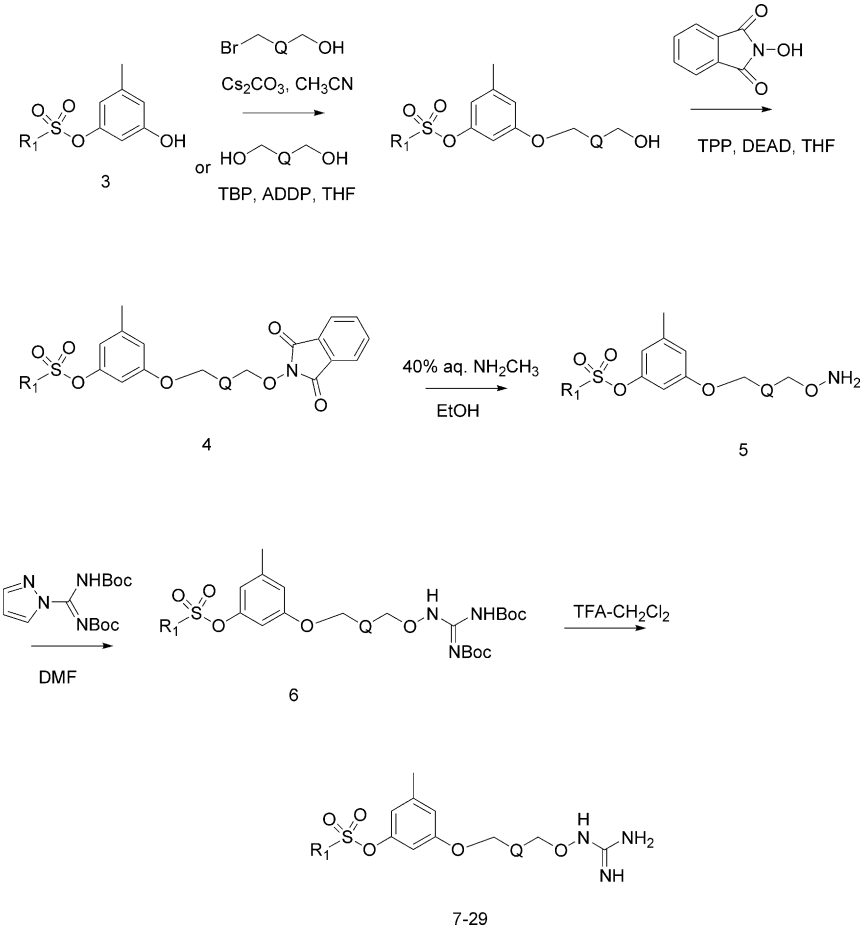


potency. In the heteroarylsulfonates shown in Table 2, the 8-substituted quinoline **15** appears to be consistently more potent than the 4-substituted isoquinoline **16**. Reduction of **15** to the tetrahydroquinoline, which is similar to that found in argatroban, actually reduced potency. Interestingly, the tetrahydrobenzothiophene-1,1-dioxide **18**, which is a constrained *ortho*-sulfone derivative, is less potent than **11** or **12**.

Focusing on the *ortho*-methylsulfones, we varied the oxyguanidine side-chain as shown in Table 3. These variations centered on the 2-position of the 1,3-propanedioxy side-chain. The only variation that did not reduce potency was the cyclopropyl **19**. The incorporation of fluorine was detrimental with a single fluorination (**21**) losing one order of magnitude and gem-difluorination (**22**) another 3-fold loss. The rigidification of the side chain with an sp^2 center as in olefin **23** or the addition of a hydrophilic hydrogen-bond donor group as in **20** were not beneficial. However, the length of the side-chain is critical as the one-carbon abrogation (**24**) lost over 800-fold in potency.

The central phenyl scaffold variations at the 3-position, shown in Table 4, indicate that a small substituent is preferential. Thus, even chloro **27** that is slightly larger is less potent than **11**. The larger groups such as methoxy **25** and ethyl **26** were also less potent. This struc-



Scheme 1. Synthesis of Alkoxyguanidines.

Table 1. Inhibition of α-human thrombin by substituted arylsulfonates

Compd	R	Thrombin inhibition K_i, μM[a]
7	2-Cl	0.0021 (±0.0001)
8	2-CN	0.0099 (±0.0005)
9	2-OCH_3	0.0067 (±0.0001)
10	2-NH_2	0.0866 (±0.0027)
11	2-SO_2CH_3	0.0119 (±0.0003)
12	2-$SO_2C_6H_5$	0.0048 (±0.0004)
13	3-SO_2CH_3	0.204 (±0.009)
14	2,4-$(SO_2CH_3)_2$	0.749 (±0.040)

[a]Values are means of three experiments, standard deviation is given in parentheses.

Table 2. Inhibition of α-human thrombin by heteroarylsulfonates

Compd	Thrombin inhibition K_i, μM[a]
15	0.0067 (±0.0005)
16	0.0132(±0.0008)
17	0.031(±0.0003)
18	0.102(±0.002)

[a]Values are means of three experiments, standard deviation is given in parentheses.

Table 3. Inhibition of α-human thrombin by 2-methylsulfonyl-arylsulfonates with side-chain variations

Compd	Q	Thrombin inhibition K_i, μM[a]
19		0.0077 (±0.0002)
20	CHOH	0.137 (±0.005)
21	CHF	0.114 (±0.005)
22	CF_2	0.364 (±0.031)
23	C=CH2	0.0324 (±0.0028)
24	bond	2.62 (±0.17)

[a]Values are means of three experiments, standard deviation is given in parentheses.

Table 4. Inhibition of α-human thrombin by 3-substituted central phenyl scaffold

Compd	X	Q	Thrombin inhibition K_i, μM[a]
25	–OCH_3	–CH_2–	0.0346 (±0.0011)
26	–CH_2CH_3	–CH_2–	0.0297 (±0.0008)
27	–Cl	–CH_2–	0.0394 (±0.0024)
28	–CH_2OH		0.403 (±0.010)
29	–F		0.0493 (±0.0001)

[a]Values are means of three experiments, standard deviation is given in parentheses.

ture–activity is consistent with that found in our previous studies with phenyl templated thrombin inhibitors.[9] The incorporation of a hydrophilic hydrogen-bonding functionality as found in **28** resulted in a 800-fold loss in potency.

These oxyguanidine phenyl-based thrombin inhibitors retain the excellent selectivity against other serine proteases that were observed in other previous work with other phenyl-based thrombin inhibitors.[10] A representative compilation of activities against other serine proteases is shown in Table 5 for compound **11**. The oxyguanidine unit is a guanidine mimetic that possesses a p*K*a of 7.05. This is the lowest p*K*a reported for any guanidine mimetic that would include various amino, amidino, and aminopyridine moieties. This is an important characteristic because it would result in a higher proportion of neutral species of the compound at the pH of the lower intestine where absorption is likely to occur. This higher absorption potential was realized when the permeability of compound **11** was quantitated in the human Caco-2 monolayer assay.[11] The apparent permeability coefficient (Papp) from the apical to basolateral side was determined to be 2.4×10^{-6} cm/s. This result would be considered in the medium range from our laboratory.

The in vitro metabolic potential of **11** in liver microsomes from several species was studied as shown in Table 6.[12] The disappearance of parent compound after incubation with liver microsomes and NADPH was determined and the rate was calculated. It appears that compound **11** has a low metabolic liability in dog microsomes. These were distinct from the results with

Table 5. In vitro inhibition of serine proteases by compound **11** (K_i (nM))

Thrombin	Fxa	Plasmin	Urokinase	Trypsin	Chymotrypsin	Elastase
11.9	>22,000	>22,000	67,000	>22,000	>22,000	>22,000

Table 6. Characterization of compound **11**

p*K*a	Caco-2	Microsomal stability (rate in pg/min/mg protein)			
	Papp ($\times 10^{-6}$ cm/s)	Dog	Rat	Mouse	Human
7.05	2.4	21.1	69.5	30.8	50.4
		Propranolol 36.2	92.0	81.8	18.9

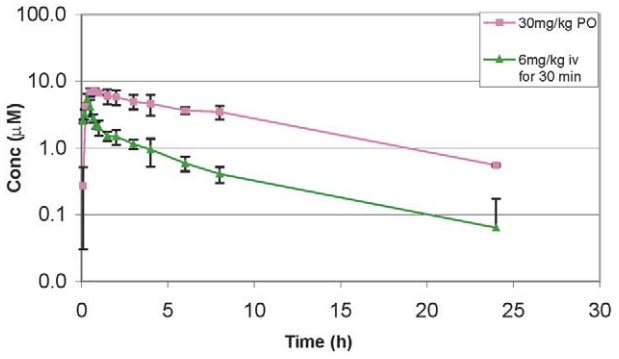


Figure 1. In vivo pharmacokinetics in dog ($n=3$) after 6 mg/kg iv over 15 min and 30 mg/kg po of compound **11**.

rat and human microsomes that have higher rates of metabolism.

Confident that the series was permeable in a human cell line and stable in microsomal metabolism in the dog, compound **11** was administered as a solution at a dose of 6 mg/kg intravenously and 30 mg/kg by oral gavage to beagle dogs.[13] The pharmacokinetic analysis indicated that the terminal half-life after iv administration was 7.3 h (Fig. 1). The calculated clearance rate was 6.9 mL/min/kg that was 1/5 the hepatic blood flow in dog. This rate would be considered medium. The calculated volumn of distribution was 4.4 L/kg that would be considered high. After oral administration, the Cmax reached 7.0 μM at 0.75 h. The dose-corrected oral bioavailability (*F*) was 95%. These pharmacokinetic parameters are among the best reported orally bioavailable thrombin inhibitors.[14]

We have discovered a novel series of phenyl-based thrombin inhibitors using oxyguanidines as guanidine mimetics. We believe that the key to the superior oral pharmacokinetics is the use of the hydrophilic, but non-basic oxyguanidine moiety.

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