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# ARGATROBAN ANALOGS: SYNTHESIS, THROMBIN INHIBITORY ACTIVITY AND CELL PERMEABILITY OF AMINOHETEROCYCLIC GUANIDINE SURROGATES

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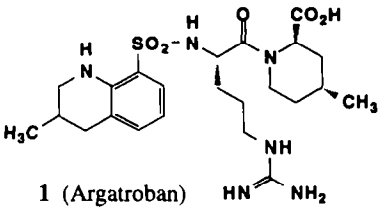
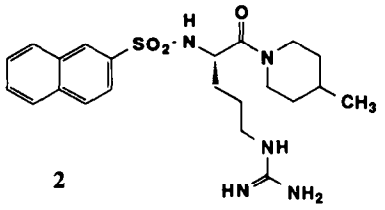
**Abstract:** A series of Argatroban analogs, **3-6**, in which the guanidino group was replaced by amino-substituted heterocycles of decreasing basicity were prepared and evaluated for their ability to inhibit human α-thrombin. Basicity was found to be important in determining inhibitory potency. Aminopyridine analog **3b** ($pK_a$~7) afforded the most potent inhibition ($I_{50}$=0.47 μM) and exhibited enhanced Caco-2 cell permeability.

Argatroban (MD-805, Novastan), **1**, is a potent, selective, efficacious reversibly-binding thrombin inhibitor[1] with a short duration of action that has been approved as a parenteral antithrombotic in Japan.[2] It has been speculated that the short duration and poor oral bioavailability of Argatroban and other thrombin inhibitors

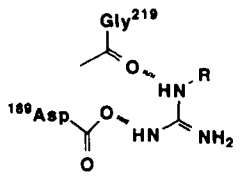



1 (Argatroban)




2

may, in part, be associated with their highly basic guanidine functionality ($pK_a$~14) and that analogs containing guanidine surrogates with attenuated basicity may possess improved oral bioavailability and pharmacokinetics.[3] Crystallographic studies have suggested that the guanidino group of Argatroban binds to Asp-189 and Gly-219



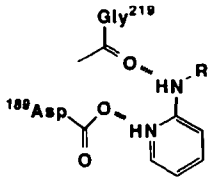
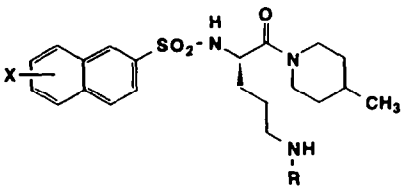
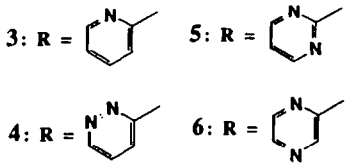




**Figure 1.** Left: binding of Argatroban guanidine to thrombin; Right: proposed binding of aminopyridine surrogate to thrombin.

of thrombin as shown in Figure 1.[4] We anticipated that it may be possible for a substituted aminoheterocycle of suitable basicity in its protonated form (e.g. 2-aminopyridine) to mimic the binding interactions of the guanidino group (Figure 1).

In order to facilitate synthesis and subsequent evaluation of this concept, the structurally simplified Argatroban analog **2**, in which the pipecolic acid was replaced by a 4-methylpiperidine and the tetrahydroquinoline ring was replaced by a 2-naphthalene ring, was employed as a surrogate standard for **1**.[5] A series of related analogs, **3-6**, incorporating aminoheterocyclic guanidine surrogates with a range of basicities were then prepared and evaluated for their thrombin inhibitory activity and ability to cross Caco-2 cell monolayers[6] and compared to **2**. This Letter describes the synthesis and *in vitro* biological evaluation of analogs of **2** containing 2-aminopyridinyl (**3**), 3-aminopyridazinyl (**4**), 2-aminopyrimidinyl (**5**) and 2-aminopyrazinyl (**6**) groups as guanidine surrogates.

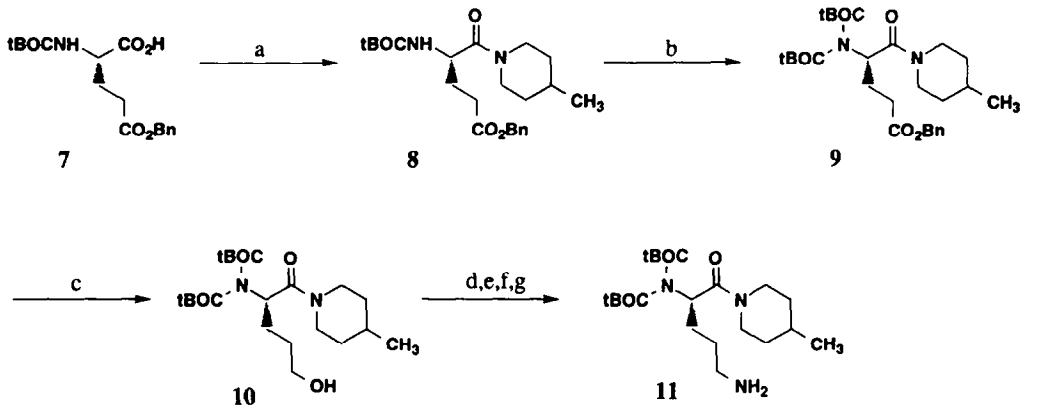




## Synthesis

Aminoheterocyclic analogs **3-6** were prepared from key synthetic intermediate amine **11**. Amine **11** was available from N-BOC-L glutamic acid-γ-benzyl ester **7** by EDAC mediated coupling with 4-methylpiperidine to

**Scheme I**

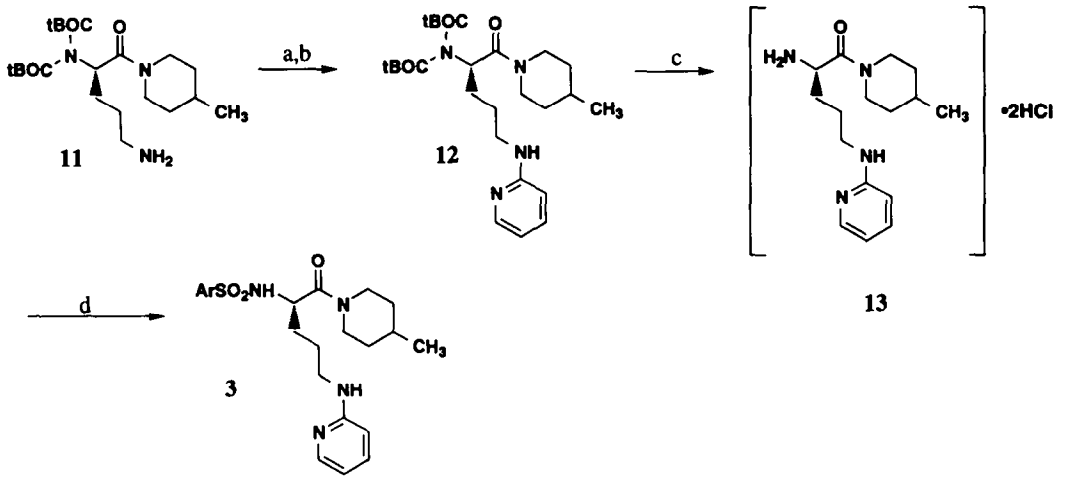


a. 4-Methylpiperidine/EDAC/HOBT/NMM/DMF, 0 to 25°, 100%; b. $(tBOC)_2O$ (10 eq)/4-pyrrolidinopyridine/$CH_3CN$, 85°, 72%; c. LiCl/$NaBH_4$/EtOH, 25°, 71%; d. MsCl/$Et_3N$/$CH_2Cl_2$, -20°, 97%; e. NaI(5 eq)/acetone, 25°, 93%; f. $NaN_3$/DMF, 25°, 100%; g. 10%Pd-C/$H_2$(1 atm)/$CH_3OH$, 100%.

afford **8**.[7] In order to prevent subsequent intramolecular cyclization with the sidechain the t-butoxycarbonyl nitrogen of **8** was fully protected at this stage by treatment with excess BOC anhydride (10-15 eq) in the presence

of 4-pyrrolidinopyridine (0.6 eq) or 4-DMAP in acetonitrile.[8] Experimentally BOC anhydride was added in portions to **8** until starting material was fully consumed as monitored by TLC. Bis-BOC amine **9** was obtained in 72% yield as a stable clear, colorless oil. The benzyl ester of **9** was reduced slowly with excess $NaBH_4$/LiCl in ethanol to afford **10** in 71% yield. Alcohol **10** was converted to key amine **11** in a standard 4-step sequence (90% yield) through reduction of an intermediate azide. Amine **11** was then treated with the appropriate chloroheterocycle in the presence of base to afford the corresponding arylated amine. Scheme II illustrates the sequence for the preparation of the 2-aminopyridine analogs **3**. Heating a solution of **11** with 2-chloropyridine-N-oxide hydrochloride (2 eq) in the presence of sodium bicarbonate (4 eq) in 1-butanol at 100° for 21 h gave the 2-aminopyridine-N-oxide in 47% yield. Reduction of the N-oxide under transfer hydrogenation conditions[9] afforded 2-aminopyridine **12** in 60% yield. Deprotection of the amine by treatment with hydrogen chloride in

**Scheme II**




a. 2-chloropyridine-N-oxide•HCl/$NaHCO_3$/1-butanol, 100°, 47%; b. 10%Pd-C/$HCO_2NH_4$/EtOH, reflux, 60%; c. HCl/dioxane, 25°, 100%; d. $ArSO_2Cl/Et_3N$ (4 eq)/$CH_2Cl_2$, 0°, 75-95%.

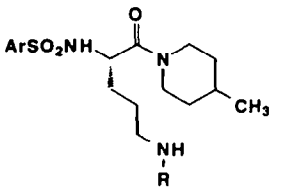
dioxane and then coupling with the desired arylsufonylchloride gave analogs **3**. By a similar sequence the pyridazine analog **4** was obtained from **11** and 2,6-dichloropyridazine (2 eq/$NaHCO_3$, 4 eq/1-butanol, 100°, 39%) followed by reduction of the resulting 3-amino-6-chloropyridizine (20%$Pd(OH)_2$-C/$H_2$(1 atm)/methanol, 25°, 54%), the pyrimidine analog **5** was obtained from **11** and 2-chloropyrimidine (3 eq/$NaHCO_3$, 2 eq/ethanol, 65°, 52%) and the pyrazine analog **6** was obtained from **11** and 2-chloropyrazine (5 eq/$NaHCO_3$, 5 eq/1-butanol, 120°, 35%).

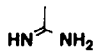
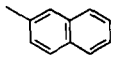
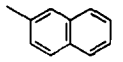
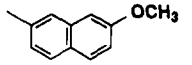
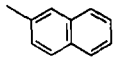
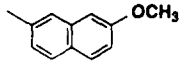
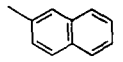
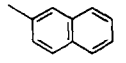
## Biological Evaluation and Discussion

Human α-thrombin[10] was incubated with inhibitors **2-6** (3 min) at room temperature and then thrombin inhibitory activity was measured by its ability to cleave the synthetic substrate S-2238 (D-Phe-Pip-Arg-pNA) as

described previously.[11] The data are expressed as an average $I_{50}$ value ($n \geq 5$) and are shown in Table 1. Examination of the results shows that it is possible to substitute the guanidino group of **2** with an appropriate aminoheterocycle and retain thrombin inhibitory activity, with potency following the order **2>3a>4a>5~6** (i.e. guanidine>2-aminopyridine> 3-aminopyridazine>2-aminopyrimidine≈2-aminopyrazine). Significantly, the rank order of thrombin inhibitory potency followed the rank order of basicity of the simple amino-substituted

**Table 1. In Vitro Thrombin Inhibition of Arylsulfonamides 2-6**




| Compound | R | Ar | ~pK$_a$[a] | Thrombin $I_{50}$ (μM)[b] |
|---|---|---|---|---|
| 2 |  |  | 14 | 2.5 |
| 3a |  |  | 6.9 | 5.5 |
| 3b |  |  | 6.9 | 0.47 |
| 4a |  |  | 5.2 | 24 |
| 4b |  |  | 5.2 | 2.3 |
| 5 |  |  | 3.5 | >115 |
| 6 |  |  | 3.1 | >40 |

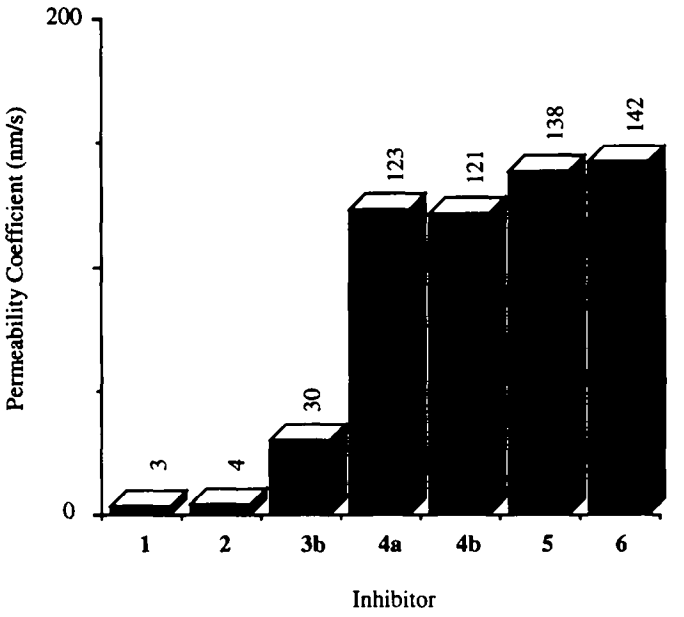
a. $pK_a$ values of the aminoheterocycles were obtained from Albert, A. "Ionization Constants" in Physical Methods in Heterocyclic Chemistry, Vol. 1, Katritzky, A. R., Ed., Academic Press, NY, 1963, pp 2-103.
b. the concentration which half-maximally inhibits thrombin cleavage of synthetic substrate, see ref. 11.

heterocycles. In particular, **3a** in which the guanidino group was replaced with a 2-aminopyridine ($pK_a$~7), was found to be only 2-fold less potent than **2** indicating that amino-substituted heterocycles of significantly reduced basicity can serve as guanidine surrogates in this series of thrombin inhibitors. The 7-methoxy-2-naphthyl analogs which are ~10-fold more potent than the 2-naphthyl analogs in the guanidine series (data not shown) were also prepared in the 2-aminopyridine (**3b**) and 3-aminopyridazine (**4b**) cases, and found also to be 10-12 fold more potent than **3a** and **4a**, respectively.

### Caco-2 Cell Permeability Studies

Inhibitors **1-6** were evaluated for their ability to cross a Caco-2 cell monolayer (n=2) as an in vitro model of intestinal absorption with the results expressed as a permeability coefficient.[6,12] Aminopyridine **3a** was not evaluated due to its poor solubility under the assay conditions. We anticipated that highly basic species would traverse cell membranes less efficiently than neutral molecules and thus the less basic aminoheterocyclic inhibitors

**Figure 2.** Permeability Across Caco-2 Cell Monolayer at pH=7.4




would show enhanced cell permeability. Shown in Figure 2 are the results from a Caco-2 cell permeability study which confirm that aminoheterocyclic analogs **3-6** do indeed exhibit significantly increased cell permeability relative to either **1** or **2**. Interestingly, the permeability increases from $pK_a$ 14 to $pK_a$ 5.2 but then plateaus at $pK_a \leq 5.2$. Presumably under the assay conditions, *i.e.* pH=7.4, the charged population for inhibitors **4a**, **4b**, **5** and **6** is no longer significant.

## Conclusion

A series of Argatroban analogs, **3-6**, in which the guanidino group ($pK_a$~14) was replaced by amino-substituted heterocycles of decreasing basicity were prepared and evaluated for their ability to inhibit human α-thrombin. The degree of basicity of the heterocycle was found to be important in determining inhibitory potency *in vitro* with the more basic heterocycles affording the most potent inhibition. The 2-aminopyridine analogs **3a** and **3b** ($pK_a$~7) gave the most potent inhibition with aminopyridine **3b** exhibiting a thrombin $I_{50}$=0.47 μM. Aminoheterocyclic analogs **3-6** also showed significantly increased Caco-2 cell permeability characteristics relative to guanidino analogs **1** or **2**.

**Acknowledgements.** We thank Drs. Steven E. Hall, S. David Kimball, Wan F. Lau and Richard A. Morrison for helpful discussions and the BMSPRI Analytical Research Department for elemental combustion analyses, mass and infrared spectra.

## Notes and References

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(c) Brandstetter, H.; Turk, D.; Hoeffken, H. W.; Grosse, D.; Stürzebecher, J.; Martin, P. D.; Edwards, B. F. P.; Bode, W. *J. Mol. Biol.* **1992**, *226*, 1085-1099.

5. Compounds **1** and **2** showed similar lipophilicities, the calculated intrinsic log P values were 1.90 and 2.09, respectively (Daylight program).

6. (a) Hildago, I. J.; Raub, T. J.; Borchardt, R. T. *Gastroenterology* **1989**,*96*, 736-749.
(b) Hilgers, A. R.; Conradi, R. A.; Burton, P. S. *Pharm. Res.* **1990**,*7* , 902-910.
(c) Vachon, P. H.; Beaulieu, J.-F. *Gastroenterology* **1992**, *103*, 414-423.

7. All compounds showed $^1H$ and $^{13}C$ NMR, mass and IR spectra consistent with their proposed structures. In addition **2-6** showed acceptable (±0.4%) elemental combustion analyses (C,H,N,S) .

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9. Balicki, R. *Synthesis* **1989**, 645-646.

10. Obtained from Sigma Chemical Company, St. Louis, MO, USA.

11. Balasubramanian, N.; St. Laurent, D. R.; Federici, M. E.; Meanwell, N. A.; Wright, J. J.; Schumacher, W. A.; Seiler, S. M. *J. Med. Chem.* **1993**, *36(2)*, 300-303. Argatroban, **1**, showed an $I_{50}$=0.038 μM under these assay conditions.

12. Aminopyridine **3b** was not sufficiently soluble at pH=7.4 and was examined at pH=5.5. For comparision purposes **2** showed a permeability coefficent of 6 nm/s at pH=5.5.

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