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Azaindoles: Moderately Basic P1 Groups for Enhancing the Selectivity of Thrombin Inhibitors

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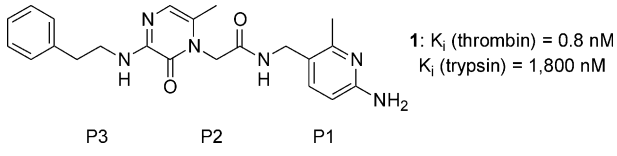
Abstract—Starting from a 2-amino-6-methylpyridine P1 group and following a strategy of enlarging it whilst reducing its polarity, we have developed a series of potent, moderately basic azaindoles which are intrinsically much more selective for thrombin versus trypsin. Certain pyrazinone acetamide azaindole derivatives have pharmacokinetic parameters after oral administration to dogs, or efficacy in vitro, comparable to an optimized pyrazinone acetamide 2-amino-6-methylpyridine derivative.

The development of an orally active thrombin inhibitor as an anticoagulant is a well established medicinal chemistry goal.[1] Selectivity versus other serine protease inhibitors, particularly those which share thrombin's preference for P1 groups containing strong bases such as guanidines and amidines, is a critical hurdle to overcome in the design of such a compound.[1a] Trypsin is arguably the most demanding serine protease in this regard owing to the similarity of their active sites and its location in the gut.

A focus in the design of selective thrombin inhibitors has been on the 'specificity pocket' (S1). Thrombin's 'specificity pocket' is identical to that of trypsin except at residue 190 which is alanine and serine, respectively. Consequently the pocket is slightly larger and more lipophilic in thrombin than it is in trypsin.[2] This fact has guided the design of inhibitors selective for thrombin and a general design strategy which involves addition of hydrophobic surface area to, and subtraction of hydrophilic groups from, a P1 group is likely, but not guaranteed,[3] to have the effect of increasing the relative affinity of an inhibitor for thrombin until the size limit of thrombin's 'specificity pocket' is reached.

Strongly basic P1 groups will tend to retard absorption across the gut wall and may be associated with unwanted side effects.[4] A reduction in the basicity of the P1 group has been used in concert with the selectivity increasing strategy mentioned above to overcome these two liabilities. For example, the 2-amino-6-methylpyridine P1 group (conjugate acid pKa = 6.9) was developed with a pyrazinone acetamide P2/P3 template to give **1**, a selective, orally active thrombin inhibitor.[5,6]



For a second generation compound we recognized the advantages inherent in a structure like **1**, with the exception that it should have an intrinsically more selective P1 group. To this end we chose to investigate bicyclic 6/5 heteroaromatic P1 groups which, in accordance with the design strategy outlined above, are larger and have fewer hydrogen bond donors and acceptors than the 2-amino-6-methylpyridine. First we looked at

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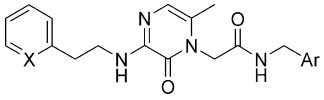
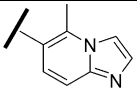
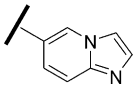
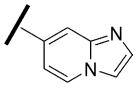
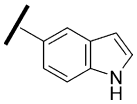
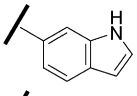
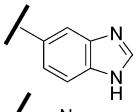
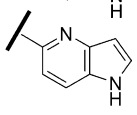
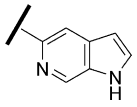
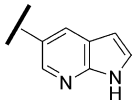
the imidazopyridine derivative **2** of aminopyridine **1** (Table 1).[7] The affinity for thrombin and trypsin both dropped and the selectivity actually diminished slightly. Deletion of the methyl group (compound **3**) resulted in a further 12- to 15-fold drop in affinities for thrombin and trypsin. The isomeric imidazopyridine **4** was no better. On the other hand, the affinity for thrombin of simple 5-linked indole **5a** was a significant improvement over imidazopyridine **3**. Moreover, it was completely inactive (>100 μM) against trypsin.[8,9] The orientation of the indole appears to be important since the isomeric 6-linked indole **6** is distinctly less active than **5a**.[10] Interestingly, benzimidazole **7** is slightly more active against thrombin than **5a** but its selectivity is eroded.[11]

Improvement in the affinity of indole **5a** for thrombin was possible by introducing a 2-pyridin-2-ylethyl P3 group to give compound **5b** ($K_i = 1.6$ nM).[13] Notably, **5b** is over five orders of magnitude more potent for thrombin than trypsin. The crystal structure of **5b** bound in thrombin's active site was determined and it is shown in Figure 1.[14] The indole binds in a conformation

Table 1. Inhibition constants for compounds **2**–**10**[12]



Compd	X	Ar	Thrombin K_i (nM)	Trypsin K_i (nM)
2	CH		11	6800
3	CH		140	100,000
4	CH		270	120,000
5a	CH		18	>100,000
5b	N		1.6	290,000
6	CH		830	>100,000
7	CH		11	2000
8a	CH		11	>100,000
8b	N		3.2	>1,000,000
9a	CH		3.7	95,000
9b	N		1.2	200,000
10a	CH		18	>100,000
10b	N		6.6	140,000

such that the 2-position is essentially equidistant (2.8–2.9 Å) to the two oxygens of the carboxyl side chain of Asp-189. A water molecule forms a hydrogen bond bridge from the indole NH to Phe-227. The indole 7-position is 3.2 Å from the side chain of Val-213. It should be noted that this binding conformation is quite different from that of the 3-chlorophenyl P1 groups in which the chlorine atom makes contact with the phenol side chain of Tyr-228.[15] The rest of the molecule binds in the expected fashion in the P2/P3 region and is similar to the reported structure of compound **1**.[5a]

The indole groups of compounds **5a** and **b** are chemically labile. To stabilize the indole and to introduce a basic group we then prepared the three possible azaindole isomers **8**, **9**, and **10**.[8] The synthesis of **8** and **9** followed our usual practice of coupling the preformed P1 amine to the preformed P2/P3 carboxylic acid under standard, EDC conditions.[5a,13] The synthesis of the 4-azaindole P1 amine starts from commercially available 6-methyl-5-nitropyridin-2-ol **11** (Scheme 1). Conversion to the nitrile **12** in two steps followed by a Batcho-Leimgruber procedure with concomitant reduction of the nitrile gave the 5-aminomethyl-4-azaindole **13**. 5-Aminomethyl-6-azaindole was prepared from commercially available 4-methyl-5-nitropyridin-2-ol using the same reaction sequence. The synthesis of the 7-azaindoles starts from 7-aza-5-bromoindoline **14**.[16] Conversion of **14** to 5-aminomethyl-7-azaindoline **15** followed by coupling with the P2/P3 acid and oxidation gave **10**.

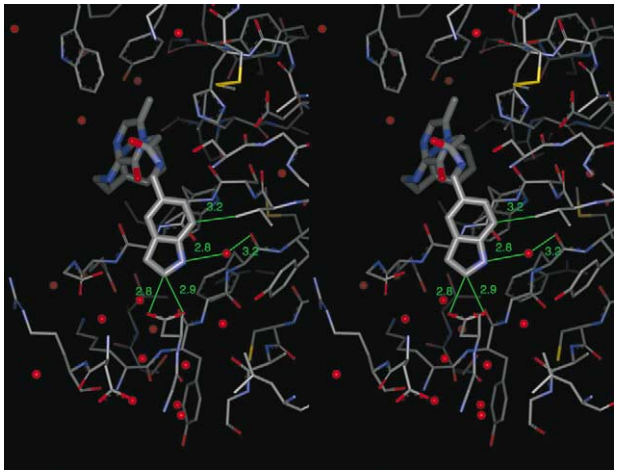
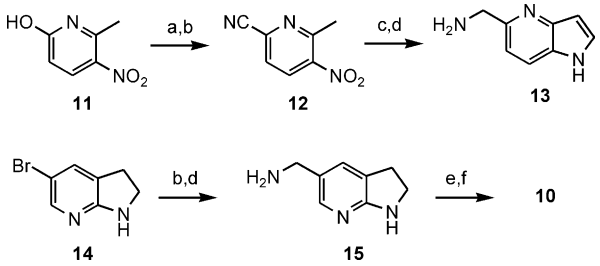


Figure 1. Crystal structure of compound **5b** bound in thrombin's active site.



Scheme 1. Synthesis of **10** and amine **13**:[8] (a) $POBr_3$, $(CHCl_2)_2$, reflux, 4 h; (b) $Zn(CN)_2$, $(Ph_3P)_4Pd$, DMF, 80 °C, 5 h; (c) DMF dimethyl acetal, DMF, 90 °C, 2 h; (d) 10% Pd/C, MeOH/6M HCl, 16 h; (e) carboxylic acid,[5a,13] EDC, HOBT, DMF, 4 h; (f) MnO_2, DMF, 2 h.

Table 2. Final assay concentration of compound required to double the human activated partial thromboplastin time (2×APTT)[12] and pharmacokinetic parameters in dogs after oral administration at 1mg/kg

Compd	2×APTT μM	Dog C_{max} (μM)	$t_{1/2}$ (h)	AUC (μM h)
1	0.41	5.37	3.18	23.1
8b	0.80	blq[a]	—	—
9a	2.63	2.69	3.60	15.2
9b	0.40	0.18	nd[b]	0.17
10b	0.95	2.10	2.42	9.1

[a]Below the level of quantitation.
[b]Not determined due to insufficient data.

The affinity of the azaindole derivatives for thrombin correlates with the acidity of the conjugate acids of the parent azaindoles.[17] Thus, while 7-azaindole derivative **10a** is equipotent to indole **5a**, 4-azaindole **8a** is slightly more potent and 6-azaindole **9a** is the most potent ($K_i = 3.7$ nM). Satisfyingly, despite the addition of a polar functional group, all three azaindoles remained highly selective. To improve the activity of the compounds, the corresponding 3-[(2-pyridin-2-ylethyl]amino)pyrazinones **8b**, **9b**, and **10b** were made and in each case a roughly three-fold improvement in the K_i was seen along with better selectivity versus trypsin.[8]

The potent azaindoles were examined in more depth for their efficacy in vitro (2×APTT) and for their pharmacokinetic parameters after oral administration in dogs (Table 2). Compound **9a** was well absorbed in dogs and was comparable to compound **1**.[5a] However, it was poorly efficacious in vitro. On the other hand pyridine **9b** was as efficacious as **1** in vitro but was absorbed very poorly. 7-Azaindole **10b** had intermediate properties with moderate efficacy and oral absorption, while 4-azaindole **8b** was moderately efficacious but was not absorbed in dogs.

In conclusion, we have developed a series of moderately basic azaindole P1 groups which are intrinsically much more selective than the aminopyridine P1 group of compound **1**. Certain of these azaindole derivatives have pharmacokinetic parameters after oral administration to dogs, or efficacy in vitro, comparable to compound **1**. The challenge remains to find a compound which combines these latter properties in the same molecule.

Acknowledgements

We thank Orn Almarsson for performing the p*K*a and solubility determinations for compound **1** and Dan McMasters for his help with the figures.

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