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Unexpected Enhancement of Thrombin Inhibitor Potency with *o*-Aminoalkylbenzylamides in the P1 Position

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Abstract—Thrombin inhibitors incorporating *o*-aminoalkylbenzylamides in the P1 position were designed, synthesized and found to have enhanced potency and selectivity in several different structural classes. X-ray crystallographic analysis of compound **24** bound in the α-thrombin–hirugen complex provides an explanation for these unanticipated results.

Thrombin, a serine protease, plays a critical, multifunctional role in the mechanism of clot formation establishing this trypsin-like enzyme as a popular drug target. Such an entity would have potential for the treatment and prevention of cardiovascular disorders such as deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke.[1,2] Existing therapies, such as orally administered warfarin and subcutaneous injection with low molecular weight heparin (LMWH), are burdened by drug and dietary restrictions, bleeding risk and required monitoring of blood parameters.[3,4] Because of these limitations, there is a clear need to develop orally administered thrombin inhibitors which are safe, effective anti-coagulants to obtain maximum patient compliance. In this communication, a series of *o*-aminoalkylbenzylamides with unexpected potency enhancing properties will be discussed as P1 groups in the search for a clinically useful antithrombotic drug.

It is recognized that formation of a salt bridge to Asp189 in the bottom of the thrombin S1 pocket with a basic P1 group such as guanidine or amidine is important for potency[5] (for example, **1a**,[6] Fig. 1). However, these moieties have been associated with poor oral bioavailability.[7–9] Alternatively, *trans*-cylclohexylamine

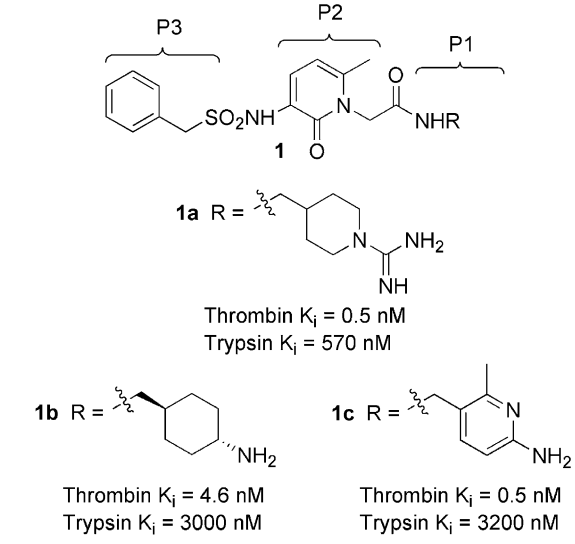

Figure 1. Potent and selective basic P1 groups.

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1b[6] and the moderately basic 2-amino-6-methyl-pyridine **1c**[10] have been used successfully as potent and selective replacements. In a search to improve on the pharmacokinetic and pharmacodynamic properties associated with these P1 groups, it was noted that *p*-aminomethylaryl groups (Fig. 2, **2a–c**[11,12]) had been incorporated into several P3P2 scaffolds with good potency. The interaction of the *meta* and *ortho* isomers within the S1 pocket, however, were unreported and merited further examination.

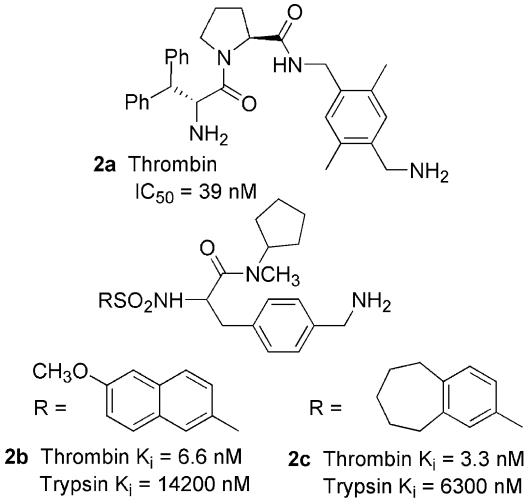
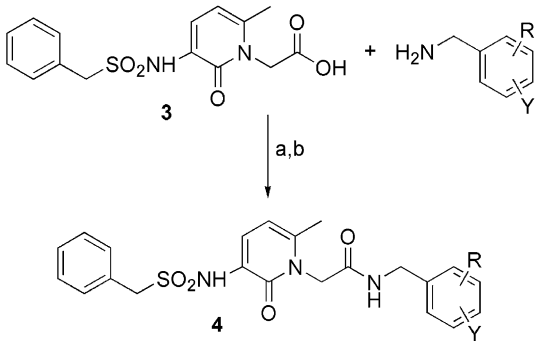
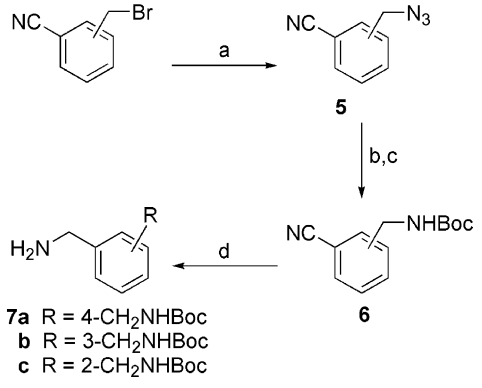


Figure 2. P3P2 scaffolds with *p*-aminomethylbenzyl in P1.



Scheme 1. (a) EDC, HOAT, Et_3N, DMF, rt, 2–18 h; (b) HCl, EtOAc, 0 °C.



Scheme 2. (a) NaN_3, THF, H_2O, rt, 18 h; (b) H_2, 5% Pd/C, 45 psi, THF, rt, 2 h; (c) Boc_2O, THF, rt, 2 h; (d) $CoCl_2$, $NaBH_4$, THF, H_2O, 35 °C, 24 h.

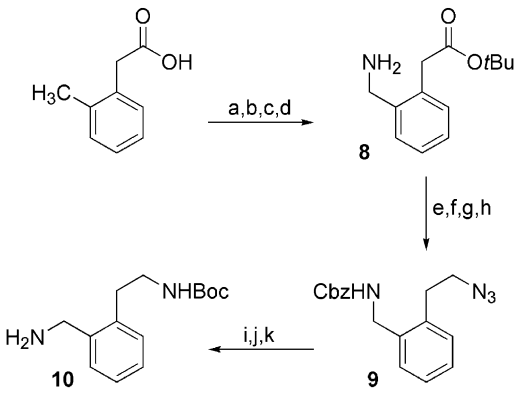
Chemistry

Compounds **1a–c** were chosen for comparison because of their reported biological profiles and the availability of acid **3**[13] which could be coupled and deprotected (Scheme 1) to give a series of aminoalkylbenzylamides **4**. The benzylamine targets for this study were prepared as in Schemes 2–5. Azide displacement of 4-cyanobenzylbromide (Scheme 2) gave **5**. Hydrogenolysis of the azide proceeded smoothly, and treatment with di-*tert*-butyl dicarbonate gave the protected amine **6**. The nitrile was reduced and the benzylamine **7a** was isolated as its hemisulfate salt with sodium hydrogen sulfate from ethyl acetate. All three positional benzylamine isomers **7a–c**[14] were prepared in this manner.

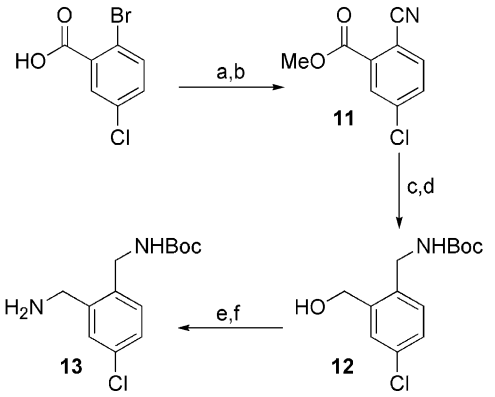
An eleven step sequence (Scheme 3) was used to prepare the 2-Boc-aminoethyl analogue of **7c**. Commercially available *o*-tolylacetic acid was reacted with NBS followed by esterification, displacement with azide and reduction to give benzylamine **8**. Treatment of **8** with benzylchloroformate followed by reduction of the ester to the alcohol, mesylation and displacement with sodium azide gave **9**. Reduction of azide **9** with triphenylphosphine, protection of the amine as its *t*-butylcarbamate and removal of the Cbz group gave the homologous benzylamine **10**.

Synthesis of the 5-chloro substituted derivative of **7c** (Scheme 4) was accomplished by esterification of 2-bromo-5-chlorobenzoic acid followed by palladium(0) mediated cyanation to give nitrile **11**. Reduction of both the nitrile and ester was achieved using $LiAlH_4$ and treatment of the amine with di-*tert*-butyl dicarbonate gave carbamate **12**. Conversion of the alcohol to azide and reduction using triphenylphosphine provided 5-chlorobenzylamine **13**.

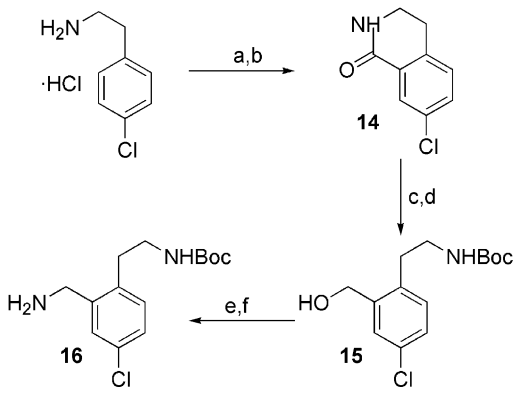
The homologue of **13** was prepared by reacting commercially available 4-chlorophenethylamine with phosgene



Scheme 3. (a) NBS, AIBN, CCl_4, 77 °C, 4 h; (b) Concd H_2SO_4, isobutylene, dioxane, rt, 4 h; (c) NaN_3, DMF, 65 °C, 3 h; (d) 5% Pd/C, H_2, 45 psi, THF, rt, 2 h; (e) $PhCH_2OCOCl$, DMAP, CH_2Cl_2, 0 °C; (f) 2.0 M $LiBH_4$, THF, 0 °C to rt, 18 h; (g) CH_3SO_2Cl, TEA, CH_2Cl_2, 0 °C to rt, 18 h; (h) NaN_3, DMF, 40 °C, 4 h; (i) Ph_3P, THF, H_2O, rt, 18 h; (j) Boc_2O, CH_2Cl_2, 0 °C to rt, 2 h; (k) H_2, 10% Pd/C, 1 atm, EtOH, rt, 2 h.



Scheme 4. (a) HCl, MeOH, rt, 18 h; (b) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 90 °C, 8 h; (c) 1.0 M $LiAlH_4$, THF, 0 °C, 0.5 h; (d) Boc_2O, CH_2Cl_2, 0 °C to rt, 2 h; (e) DPPA, DBU, THF, rt, 18 h; (f) PPh_3, THF, H_2O, 65 °C, 18 h.



Scheme 5. (a) Phosgene, ClPh, reflux; (b) $AlCl_3$, ClPh, 70 °C, 1 h; (c) Boc_2O, DIEA, DMAP, DMF, rt, 2 h; (d) 2.0 M $LiBH_4$, THF, 0 °C, 1.5 h; (e) DPPA, DBU, THF, 0 °C to rt, 3 h; (f) PPh_3, THF, H_2O, rt, 18 h.

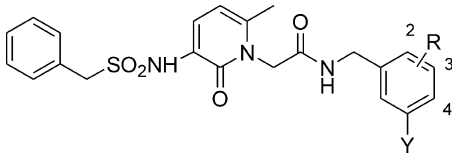
(Scheme 5) followed by treatment in situ of the resulting isocyanate with aluminum chloride to give a mixture of regioisomers from which the desired dihydroisoquinolinone **14** was obtained by fractional crystallization with toluene/heptane. Protection with Boc anhydride and reductive cleavage resulted in alcohol **15**. Azide displacement with DPPA followed by reduction gave the homologous 5-chlorobenzylamine **16**.

Results and Discussion

The results of a study to examine the effect of the aminomethyl position on the phenyl ring are shown in Table 1. Thrombin (Factor IIa) and trypsin (Tryp) inhibition constants (K_i) were determined for each compound in the new series. The concentration needed to double the activated partial thromboplastin time (2× APTT) in human plasma[15] was also determined for the more potent compounds.

Although substitution of aminomethyl in the 4-postion of benzylamide (**17**) showed a 10- to 100-fold loss in thrombin potency when compared to the basic P1 groups of **1a–c**, the result (49 nM) proved worthy of further evaluation. Moving the aminomethyl group to the 3-position (**18**) produced a 10-fold further loss in binding affinity (490 vs 49 nM) most likely due to a reduced interaction between the basic amine and Asp189. However, introduction of aminomethyl at the 2-position gave an unanticipated result in that compound **19** was nearly an order of magnitude more potent than its *para*-isomer **17** (5.2 vs 49 nM) and significantly more selective (12,885 vs 26.5-fold). In addition, it had good activity in the 2× APTT assay (0.44 μM) and had twice the thrombin selectivity (12,885 vs 6400-fold) of **1c**. This discovery was especially remarkable given that the amino group in the 2-postion was predicted to have little or no interaction with Asp189. This exciting result led to the modification of the alkyl sidechain to determine the optimal length for activity. Excision of the methylene spacer as in aniline **20** was 90-fold less potent and had decreased selectivity compared to **19.** However, the phenethylamine homologue **21** was equipotent with **19** (5.2 vs 4.1 nM) and its selectivity was improved 8-fold.

Table 1. Benzylsulfonamidopyridinone P1 analogues



Compd	R	Y	IIa K_i (nM)	Tryp K_i (nM)	Selectivity ratio $\frac{\text{Trp } K_i}{\text{IIa } K_i}$	2× APTT (μM)
1c	—	—	0.5	3200	6400	0.22
17	4-CH_2NH_2	H	49	1300	26.5	—
18	3-CH_2NH_2	H	490	23,000	46.9	—
19	2-CH_2NH_2	H	5.2	67,000	12,885	0.44
20	2-NH_2	H	470	>1,000,000	>2128	—
21	2-$CH_2CH_2NH_2$	H	4.1	43,7000	106,585	0.53
22	H	H	130	46,6000	3585	—
23	H	Cl	7.1	20,000	2786	2.8
24	2-CH_2NH_2	Cl	0.046	1850	40,217	0.16
25	2-$CH_2CH_2NH_2$	Cl	0.05	37,000	740,000	0.18

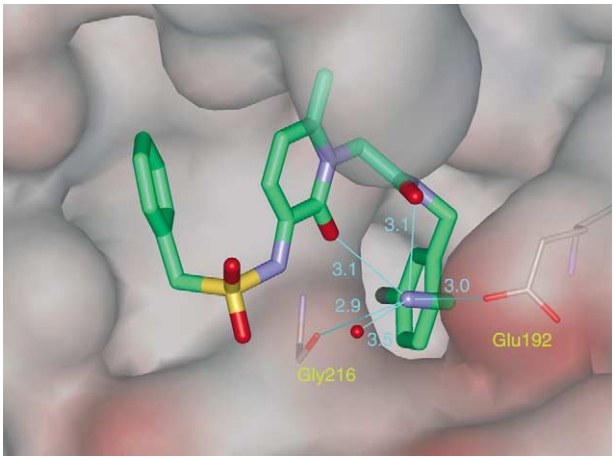
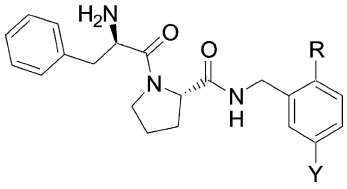


Figure 3. X-ray crystal structure of **24**.

Substitution at the 3-position of aryl P1 groups with lipophilic moieties such as chlorine can lead to large improvements in thrombin inhibitory potency because this substituent can access a lipophilic recess in the S1 pocket of the enzyme.[11] An 18-fold improvement in potency of the 3-chloro analogue **23**[16] over the unsubstituted benzylamine **22** encouraged us to incorporate this feature into the 5-position of **19** and **21.** This resulted in the synthesis of the most potent compounds (**24** and **25**, respectively) in this series. Binding affinities were improved 82 to 113-fold over the des-chloro variants and more interestingly, they were 1 to 2 orders of magnitude more potent then any of the basic P1 groups present in the lead structure **1**. Of particular note was the large improvement of **24** and **25** over **1c** in trypsin selectivity and their comparable efficacy in the 2× APTT assay (0.16/0.18 vs 0.22 μM).

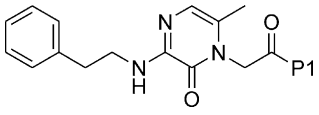
Solving the crystal structure of the thrombin-**24** complex[17] (Fig. 3) led to a better understanding of these results. As was observed for **1c**, the benzylsulfonamide group of **24** fills the distal hydrophobic pocket, while the 6-methyl substituent on the P2 pyridinone ring occupies the insertion loop of the proximal hydrophobic pocket. However, in contrast to the interactions of the aminopyridine of **1c** with Asp189, the P1 methylamine of **24** is observed to form a direct salt bridge with Glu192 (3.0 Å O–N distance), and is within hydrogen-bonding

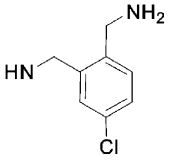
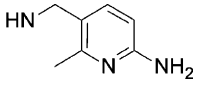
Table 2. P1 analogues with D-PhePro as P3P2 scaffold



Compd	R	Y	IIa K_i (nM)	Tryp K_i (nM)	Selectivity ratio $\frac{\text{Trp } K_i}{\text{IIa } K_i}$	2× APTT (μM)
26	H	H	4600	>1,000,000	>217	—
27	H	Cl	250	89,000	356	—
28	CH_2NH_2	H	313	10,6000	339	—
29	CH_2NH_2	Cl	3.3	6200	1908	0.34
30	$CH_2CH_2NH_2$	Cl	62	252,000	4065	—

Table 3. P1 analogues with phenethylaminopyrazinone as P3P2 scaffold



Compd	P1	IIa K_i (nM)	Tryp K_i (nM)	Selectivity ratio $\frac{\text{Trp } K_i}{\text{IIa } K_i}$	2× APTT (μM)
31		0.13	1600	12,307	0.41
32		0.8	1800	1908	0.41

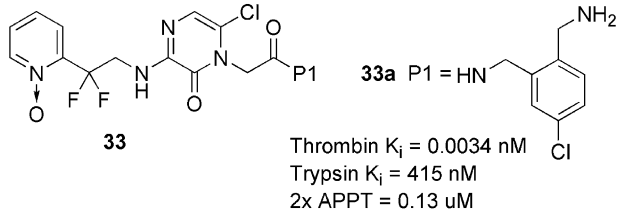


Figure 4. Pyridine *N*-oxide 6-chloropyrazinone P3P2 scaffold.

distance of four additional H-bond acceptors: the Gly216 backbone carbonyl oxygen (2.9 Å O–N), the carbonyl oxygen of the inhibitor P1P2 amide linker (3.1 Å O–N), the P2 pyridinone carbonyl oxygen (3.1 Å O–N) and a crystallographically resolved water molecule (3.5 Å O–N). The ability of the protonated amino group to engage in so many hydrogen–bonding interactions explains the extraordinary potency of **24**, and the salt bridge formed with Glu192 accounts for its dramatic selectivity over trypsin, which has a neutral glutamine residue at this position.

Investigation into the generality of this potency enhancing effect using other P3P2 scaffolds was initiated. As was found for **23** over **22**, the introduction of chlorine into the 3-position of the phenyl ring led to an 18-fold improvement in potency over its unsubstituted parent (**26** vs **27**) in the peptide series D-PhePro[18–20] (Table 2). Although the simple *o*-aminomethyl derivative **28** had modest potency (313 nM), addition of a 5-chloro group (**29**) improved potency by nearly two orders of magnitude to 3.3 nM, selectivity from 339-fold to 1908-fold and gave a very acceptable 2× APTT (0.34 μM). However, homologation to **30**, while improving selectivity by 2-fold, decreased potency by 19-fold.

The phenethylaminopyrazinone derivative **31** (Table 3) was prepared for comparison to the corresponding aminopyridine **32**.[13] Potency (0.13 vs 0.8 nM) and selectivity (12,307-fold vs 1908-fold) were also improved in this series, while efficacy in the 2× APTT assay was identical (0.41 μM).

This potency enhancing property was further exemplified by the introduction of aminomethyl-5-chlorobenzylamine in the P1 position of the recently published P3P2 scaffold **33**[21] resulting in benzylamide (**33a**), one of the most potent thrombin inhibitors to be prepared in our laboratories (Fig. 4).

Conclusion

The discovery of *o*-aminoalkylbenzylamides as a new class of thrombin inhibitor P1 groups is described. The interaction of **24** with the thrombin active site provides insights into their unanticipated potency and selectivity enhancing features, the generality of which extended over several different P3P2 scaffolds. The pharmacodynamic and pharmacokinetic properties of this new P1 class will be discussed in subsequent publications.

Acknowledgements

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