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DESIGN AND SYNTHESIS OF POTENT AND SELECTIVE 5,6-FUSED HETEROCYCLIC THROMBIN INHIBITORS

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Abstract: Thrombin, a serine protease, plays a central role in the initiation of thrombotic events. We report the design, synthesis, and antithrombotic efficacy of XU817 (**7**), a nonpeptide 5-(amidino) indole thrombin inhibitor. Utilizing the co-crystal structure of XU817 bound in the active site of thrombin we were able to synthesize analogs with enhanced thrombin affinity.

Introduction

Thromboembolic diseases remain a leading cause of mortality and morbidity in developed societies. Thrombin (fIIa) is a trypsin-like serine protease and a key enzyme in the blood coagulation cascade.[1] Thrombin has a major role in the initiation and propagation of thrombotic disease by catalyzing the conversion of fibrinogen to fibrin as well as stimulating platelet aggregation.[2] It also activates a number of other coagulation factors such as factor V, VIII, XI, and XIII. Therefore inhibition of thrombin is an attractive therapeutic target for the intervention of thrombosis.

It is clear from the current literature that the discovery of a highly selective, potent, and orally active thrombin inhibitor is a major priority of many pharmaceutical companies around the world.[3] The ideal thrombin inhibitor would be extremely selective over other relevant serine proteases such as trypsin, and would provide predictable levels of anticoagulation when administered parenterally or orally, and possess minimal danger of bleeding.

Our objective was to design and synthesize a potent and selective nonpeptide thrombin inhibitor. In designing a potent thrombin inhibitor lacking an electrophilic interaction with Ser195 the following design considerations were employed: (1) an ionic interaction with Asp189 (P_1 pocket), (2) lipophilic interaction with Trp60A (P_2 pocket), and (3) edge to face or Van der Waals interaction with Phe215 and Ile174 (P_3 pocket).[4] We envisioned a 5,6-fused heterocycle in the P_1 pocket with the appropriate P_2P_3 groups linked by an acetate group branching from the 1-position of the heterocycle. Using the Insight II® program we modeled various heterocycles in the P_1 pocket of thrombin and selected the 5-(amidino) indole as our first arginine surrogate. The indole was selected not only because it was anticipated to bind well in the P_1 pocket of thrombin, but also for ease of analog synthesis. The model suggests that an acetyl group at the 1-position of the indole would readily direct the appropriate groups into the P_2P_3 pockets. Additionally, modeling revealed that a 4-benzylpiperidine would overlap

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well with the D-Phe-Pro of DuP 714.[5] Previous to our work, the use of amidinoindoles as a arginine surrogate was published by various research groups.[6] Prior to this work the most potent example showed modest efficacy (thrombin K_i = 260 nM).[6b] We investigated amidinoindazole, amidinobenzimidazoles and 3-(amidino) indole. We envisioned utilizing the differences between the active sites of thrombin and FXa to obtain selective inhibitors for the each serine protease by manipulating the substitution pattern of the indole .

Figure 1. Electron Density of XU817 (**7**) in the Active Site of Thrombin.

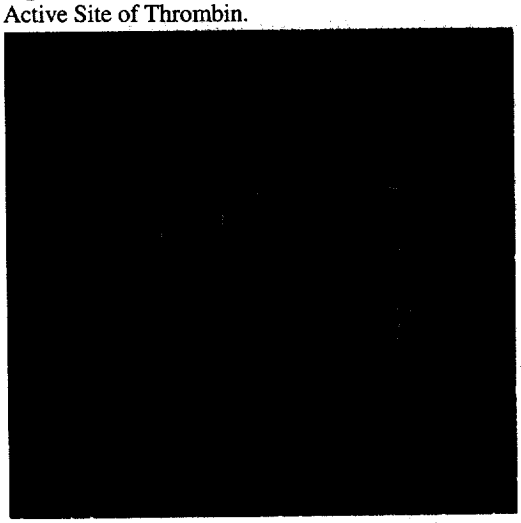
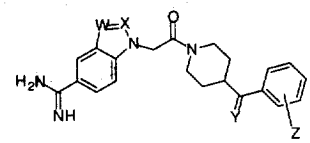


Table 1. 1-Substituted-5-(Amidino)indole/Indazole Benzimidazole Thrombin Inhibitors.



No.	W	X	Y	Z	IIa K_i nM	Trypsin K_i nM	FXa K_i nM
7	CH	CH	H,H	H	18	>15000	10300
8	CH	CH	H,H	o-F	24	ND	23000
9	CH	CH	H, H	m-F	24	ND	11000
10	CH	CH	O	H	>21000	>15000	9500
11	CH	CH	H,OH	H	>21000	>15000	16000
22	CH	N	H, H	H	140	>6000	2900
26	N	CH	H, H	H	300	ND	25000

ND = not determined

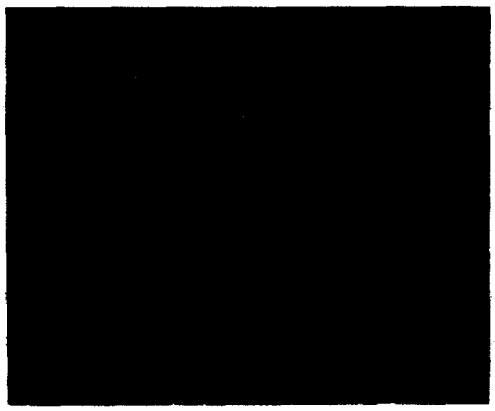
Results and Discussion

We synthesized and evaluated compound **7** (**XU817**) and related analogs. These compounds resulted in thrombin affinity values ranging from 7 nM to 200 nM with 200- to 1000- fold selectivity over trypsin, and FXa (Table 1). Compound **7** has a thrombin affinity of 18 nM with μM affinity for trypsin and FXa. Co-crystallization of compound **7** bound in thrombin revealed a very tightly fitting inhibitor explaining why this small molecule has such high affinity for thrombin (Figure 1). This inhibitor wraps very closely against the wall of P_2P_3 which explains its specificity for thrombin over FXa; FXa has a smaller P_2 pocket due to Tyr 99. Ortho- or meta-fluoro substitution on the benzyl ring was introduced to further enhance the interaction in the P_3 pocket, but an increase in affinity was not observed. Changing the hybridization of the benzylic carbon from sp^3 to an sp^2 carbon as in compound **10** or replacing the sp^3 hydrogens with a hydroxyl group as in compound **11** proved to be deleterious, affording micromolar thrombin inhibitors. The corresponding indazole (**22**) and the benzimidazole analog (**26**) of XU817 were prepared for comparison with XU817 and a 5- to 10-fold decrease in affinity for thrombin was observed in both cases.

Utilizing the co-crystal structure of XU817 (**7**) bound in thrombin we were able to enhance the affinity for thrombin by 3-fold (Table 2). It was apparent from the co-crystal structure that substitution at the 3-position of the

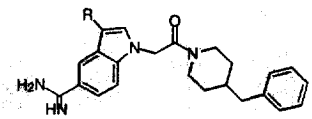
indole could provide a handle to increase the FXa affinity without generating a chiral center. Our strategy was to extend an appropriate functional group from the 3-position which could interact via a hydrogen bond with the NH of Glu192 in thrombin. Indeed, compound **16** improves the affinity for thrombin, but it also improves the affinity for FXa and trypsin. The increase in affinity for FXa and trypsin may be the result of a hydrogen bond between

Figure 2. Co-Crystal Structure of Compound **29** bound in the Active Site of Thrombin.



Electron density for compound **29**. The molecule is well defined, with the terminal phenyl ring having the least clear density.

Table 2. 1,3-Di-Substituted-5-(Amidino)indole Thrombin Inhibitors.



No.	R	IIa K_i nM	Trypsin K_i nM	FXa K_i nM
16	CH_2CO_2Me	7.4	140	400
17	CH_2CO_2H	19	ND	3000
18	CH_2CH_2OH	7.0	310	2400
19	$CH{=}CHCO_2Me$	28	280	1200

ND = not determined

the acetate carbonyl and the NH_2 of Gln192 in FXa and trypsin.[7] Interestingly, the corresponding carboxylic acid analog **17** showed a decrease in potency. However, by placing an alcohol, a hydrogen bond donor, in this position we regained the initial selectivity over FXa and trypsin while retaining the affinity for thrombin. Extending the length of the 3-acetyl group by one carbon and incorporating unsaturation as seen in compound **19** resulted in a decrease in affinity.

The 3-(amidino)indole **29** (fIIa K_i = 210 nM; fXa K_i = 3300 nM; Trypsin K_i = 1200 nM) was prepared with the rationale that the NH of the indole could potentially hydrogen bond with the hydroxyl of Ser195. Figure 2 depicts a co-crystal structure of **29** bound in thrombin showing that indeed the NH does hydrogen bond with the hydroxyl of Ser195. Unfortunately, the sulfonamide linkage does not appear to interact with the enzyme. The piperidine moiety directs the benzyl group into an edge to face interaction with Trp215. The piperidine again serves to provide the selectivity over FXa, due to steric interactions. Inhibitor **29** does not interact with the P_2P_3 pockets as well as XU817 (**7**) as illustrated by a thrombin K_i = 210 nM.

XU817 (**7**) was evaluated in the rat vena cava thrombosis model[8] to compare its efficacy relative to DuP 714, (Figure 3). Amidine indole **7** proved to be not only a potent and selective thrombin inhibitor but also efficacious in venous thrombosis with an ID_{50} = 0.8 mg/kg/h (iv administration). Relative to DuP 714, this small non-peptidic thrombin inhibitor is only 20-fold less efficacious than DuP 714 despite its 400-fold difference in K_i (Figure 4).

Figure 3. Effect of XU817 on thrombus weight in the rat vena cava thrombosis model.

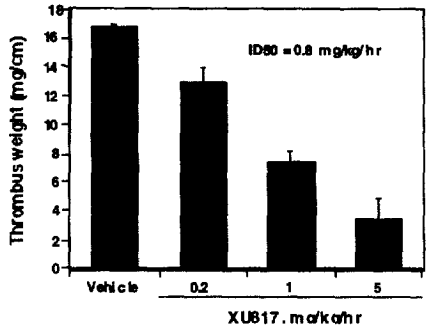
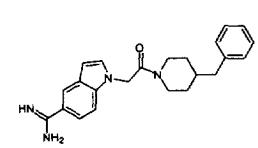
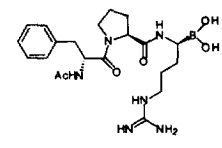


Figure 4. Antithrombic Comparison of DuP 714 and XU817.



Compound 7 (XU817)
Thrombin K_i = 18 nM
FXa K_i = 10300 nM
Trypsin K_i = >15000 nM
rat vena cava ID_{50}= 0.8 mg/kg/hr

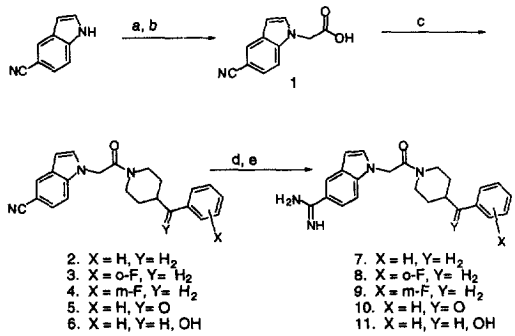


DuP 714
Thrombin K_i = 0.042 nM
FXa K_i = 9.0 nM
Trypsin K_i = 0.045 nM
rat vena cava ID_{50}= 0.04 mg/kg/hr

Chemistry

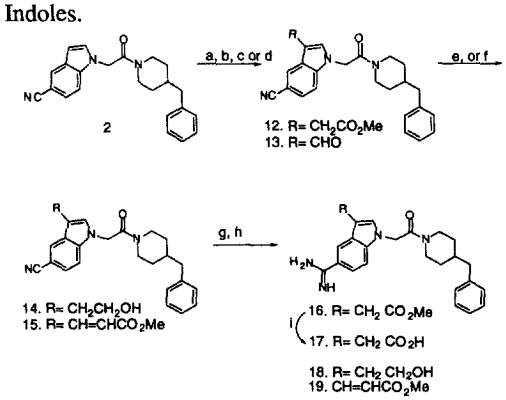
The compounds in Table 1 were prepared from commercially available 5-cyanoindole, 5-nitroindazole and 3-chloro-5-nitrobenzonitrile (Schemes 1 and 3). Alkylation of 5-cyanoindole with methyl-2-bromoacetate in the presence of NaH in DMF afforded compound **1** (Scheme 1). Saponification in MeOH/KOH followed by peptide coupling with the corresponding 4-benzylpiperidine employing DEC in methylene chloride afforded compounds **2–6**. Subjecting these compounds to the Pinner[9] conditions followed by treatment with ammonium carbonate yielded amidines **7–11**. Purification of the amidines was accomplished using preparative HPLC.

Scheme 1. Synthesis of the 5-Amidino Indoles.



(a) NaH (1.2 equiv), DMF, methyl-2-bromoacetate; (b) KOH, MeOH; (c) KOH, MeOH; (d) DEC, CH_2Cl_2, 4-benzylpiperidine and analogs; (e) MeOH, HCl(g), 0 °C to rt; (f) ammonium carbonate, MeOH.

Scheme 2. Synthesis 3-Substituted-5-Amidino Indoles.

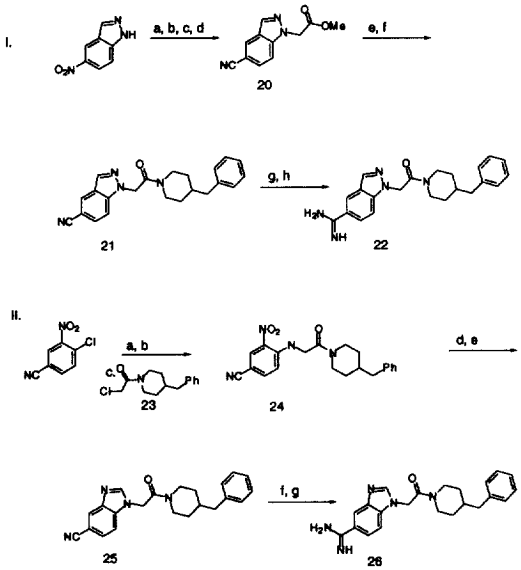


(a) oxalyl chloride (3.0 equiv), CH_2Cl_2, rt; (b) MeOH; (c) TFA, Et_3SiH, 0 °C; or (d) $POCl_3$, DMF; (e) $NaBH_4$, MeOH; (f) $Ph_3P{=}CHCO_2Me$, THF, reflux; (g) HCl_g, MeOH, (h) ammonium carbonate, MeOH; (i) TFA, water.

The 3-substituted indoles were obtained via acylation of compound **2** with oxalyl chloride in dry methylene chloride followed by treatment with methanol to afford intermediate methylketoester (Scheme 2). Selective reduction of the ketoester with triethyl silane in the presence of TFA at 0 °C afforded ester **12**.

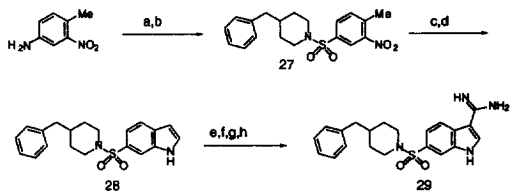
Intermediate **12** was subjected to the Pinner conditions followed by ammonium carbonate in dry MeOH to afford amidine **16**. Amidine **16** was then treated with TFA water to afford acid **17**. Reduction of ester **12** with $NaBH_4$ gave alcohol **14** which was then converted to the amidine **18** under the usual Pinner and ammonium carbonate conditions. Compound **19** was obtained by subjecting intermediate, **2**, to the Vilsmeier conditions to afford the desired aldehyde **13**. Subjecting aldehyde **13** under Wittig olefination conditions with methyl-(triphenylphosphoranylidene)acetate yielded ester **15**. Amidine formation was accomplished under the standard Pinner conditions followed by ammonium carbonate. Again all of the amidines were purified via preparative HPLC to afford the corresponding TFA salts in >95% purity.

Scheme 3. Synthesis of the 5-Amidino Indazole Series.



I. (a) $Pd(OH)_2$, MeOH, HCl; (b) HCl, $NaNO_2$; (c) CuCN, NaCN; (d) NaH, methyl-2-bromoacetate; (e) KOH, MeOH; (f) DEC, 4-benzylpiperidine; (g) HCl(g), MeOH; (h) ammonium carbonate, MeOH. II. (a) NaN_3, acetone; (b) H_2, 10% Pd/C, MeOH; (c) DMF, $NaHCO_3$, 100 °C; (d) H_2, 10% Pd/C; (e) HCO_2H; (f) HCl_g, MeOH; (g) ammonium carbonate.

Scheme 4. Synthesis 3-amidino Indole.



(a) i. $NaNO_2$ AcOH/HCl, ii. $CuCl_2$, H_2O, SO_2; (b) 4-benzylpiperidine, Et_3N, THF, 0 °C to rt, 24 h; (c) pyrrolidine, dimethylforamide dimethyl acetal, DMF, reflux, 3 h; (d) Ra Ni, THF, H_2 5 h; (e) chlorosulfonyl isocyanate, CH_3CN, 0 °C, 0.5 h; (f) dichloroethane, $POCl_3$, reflux 10 min; (g) MeOH (anh.),$HCl_{(g)}$, 0 °C, 72 h; (h) MeOH (anh.), ammonium carbonate, rt, 18 h.

The 5-amidinoindazole was prepared from the corresponding nitro compound via catalytic reduction, diazotization with $NaNO_2$/HCl, followed by CuCN displacement to afford the cyano compound (Scheme 3, part I). Alkylation with methyl α-bromoacetate yielded compound **20**.

Compound **22** was obtained following the same synthetic sequence discussed above in Scheme 1. 5-Cyanobenzimidazole was prepared from reaction of commercially available 4-chloro-3-nitro-benzonitrile in DMF in the presence of $NaHCO_3$ with α-aminobenzylpiperidine to afford intermediate **24** (Scheme 3, part II).

Cyclization under hydrogenation conditions with 10% Pd/C in MeOH in the presence of formic acid to afforded compound **25** which was converted to amidine **26** via Pinner conditions.

Compound **29** was prepared from commercially available 4-methyl-2-nitroaniline; coupling with 4-benzyl piperidine was achieved via a Sandmeyer type reaction (Scheme 4). This gave the sulfonamide **27** in good yield. The 3-position of the indole was functionalized with chlorosulfonyl isocyanate. The 3-amide results after basic work-up with KOH solution. The indole amide was then dehydrated with phosphorous oxychloride to give the 3-cyanoindole which is then converted the amidine via the Pinner conditions followed by ammonium carbonate to afford compound **29**.[10]

Conclusion

We have designed and synthesized potent and selective nonpeptide thrombin inhibitors with K_i values ranging from 7 to 210 nM. The amidino indole arginine surrogate proved to be more potent and selective than the corresponding amidinobenzimidazole, amidinoindazole and the 3-amidinoindole. XU817 would be efficacious in venous thrombosis based on the rat vena cava thombosis model. Future papers will further explore manipulation of the indole ring to obtain affinity and selectivity over different blood coagulation serine proteases.

References and Notes

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