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BIARYL SUBSTITUTED ALKYLBORONATE ESTERS AS THROMBIN INHIBITORS

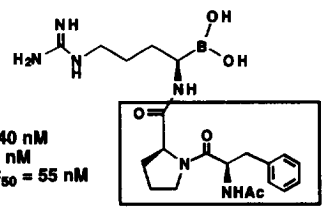
M. L. Quan,* J. Wityak, C. Dominguez, J. V. Duncia, C. A. Kettner, C. D. Ellis, A. Y. Liauw, J. M. Park, J. B. Santella, R. M. Knabb, M. J. Thoolen, P. C. Weber,‡ and R. R. Wexler.

The DuPont Merck Pharmaceutical Company, P.O. Box 80500 Wilmington, DE 19880-0500

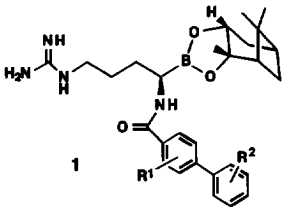
Abstract: Thrombin is a serine protease that plays an important role in the blood coagulation cascade, and is a target enzyme for new therapeutic agents. Ac-(D)-Phe-Pro-boroArg-OH (DuP 714) was found to be a highly effective thrombin inhibitor. In order to reduce the peptidic nature of DuP 714, we have designed a series of novel biaryl substituted alkylboronate esters as potent thrombin inhibitors. The most potent compounds have subnanomolar affinity for thrombin. © 1997 The DuPont Merck Pharmaceutical Company. Published by Elsevier Science Ltd.

Thrombin is a serine protease that plays an important role in the blood coagulation cascade.[1] As the last protease in the coagulation cascade, thrombin catalyzes the conversion of fibrinogen to fibrin. Additionally, it is a potent activator of platelets and a number of coagulation factors. Consequently, it is a target enzyme for new therapeutic agents with potential for the treatment of pulmonary embolism, deep vein thrombosis, unstable angina, thrombolysis, and coronary by-pass surgery.

In 1990, a series of boropeptide thrombin inhibitors based upon the D-Phe-Pro-Arg sequence was described.[2] These modified peptides have a C-terminal boronic acid moiety in place of the carboxy terminus and are among the most potent thrombin inhibitors known. Ac-(D)-Phe-Pro-boroArg-OH (DuP 714) was a highly potent compound in the series.[2] In order to reduce the peptidic nature of these inhibitors, we designed and synthesized some simple biaryl amide substituted aminoalkylboronates exemplified by **1**. The most potent compounds have subnanomolar affinity for thrombin.



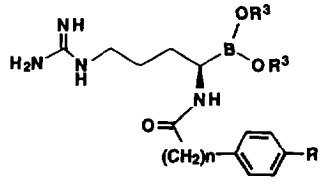
DuP 714
Thrombin K_i = 0.040 nM
Trypsin K_i = 0.045 nM
Thrombin Time IC_{50} = 55 nM



1

Replacement of the Ac-(D)-Phe-Pro portion of DuP 714 with a benzoyl group decreased thrombin inhibition from 0.04 nM to 510 nM (**2**, Table 1). However, replacement of the Ac-(D)-Phe-Pro portion with 1,1-biphenyl carbonyl afforded **3**, which had a K_i of 0.94 nM against thrombin. A greater than two order of magnitude increase in binding affinity was observed by *para*-substitution of the phenyl moiety with a second phenyl group. From molecular modeling studies it appeared that the terminal phenyl ring of the biphenyl moiety was well-positioned for a favorable lipophilic edge to face interaction[3] with Trp^{215} in the active site. The thrombin inhibition of **3** and **4** suggested that the contribution to the binding energy of the boronate esters was negligible. This was validated by X-ray crystallographic studies of boropeptide pinanediol ester-thrombin

complexes in which the pinanediol from the inhibitors bound within the active site was not detected.[4] Placing a methylene group between the carboxamide carbon and the biaryl caused a seven fold decrease in binding affinity (**3** and **5**).



(I)

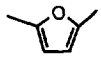
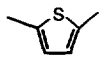
Table 1. K_i Data of Thrombin Inhibitors[a]

Ex.	R	n	R^3	Thr K_i (nM)	Tryp K_i (nM)
DuP 714			H	0.040	0.045
2	H	0	pinacol	512	70
3	Ph	0	(+) pinanediol	0.94	33
4	Ph	0	H	1.7	not tested
5	Ph	1	(+) pinanediol	6.9	not tested

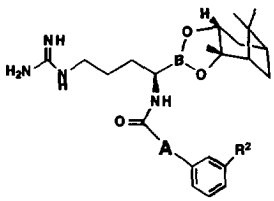
[a]see reference 2 for assay conditions.

Meta-substitution of the biphenyl moiety was also explored. As compared with **3**, a 117-fold decrease in thrombin affinity was observed when substitution was at the *meta*-position (**6**). However, when the terminal phenyl substituent was *meta*-substituted with either a methylsulfonamide or a *tert*-butyloxycarbonylamine, the thrombin activity returned to the nanomolar range (**7** and **8**). Replacement of the inner phenyl ring of **3** with a 2,5-substituted 5-membered heterocycle such as furan caused the thrombin affinity to decrease by approximately 150-fold. *Meta*-substitution of the terminal ring with a *tert*-butyloxycarbonylamine group afforded a moderate increase in activity (**10** and **11**).

Table 2. K_i Data of Thrombin Inhibitors[a]

Ex.	A	R^2	Thrombin K_i (nM)	Trypsin K_i (nM)
6		H	110	7.4
7		$NHSO_2CH_3$	3.5	3.8
8		NHBOC	8.3	35
9, free acid		H	137	not tested
10		NHBOC	30	25
11		NHBOC	42	13

[a]see reference 2 for assay conditions.



(II)

Molecular modeling studies of **3** superimposed with DuP 714 bound to thrombin indicated that an *ortho*-substituent on the benzoyl moiety of **3** might participate in a possible lipophilic edge to face interaction of with Trp^{60D} of thrombin. The addition of a methyl group at the *ortho*-position to the carbonyl caused a two fold increase in affinity (**12**). Replacement of the *ortho*-methyl with a fluoro or amino group caused the thrombin affinity to decrease by three fold, whereas a nitro- substituent at the *ortho*-- or *meta*-position caused the affinity to decrease by ten fold. A comparison of *ortho*- and *meta*- amino groups indicated a four fold decrease in

thrombin activity when the amino substituent was placed at the *meta*-position as compared to the *ortho*-position. As shown in Table 3, all compounds with an *ortho*-substituent had similar affinity for trypsin.

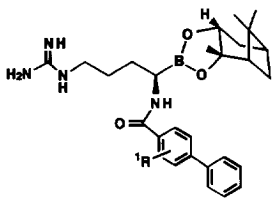
The X-ray crystal structure[5] of **12** bound to thrombin superimposed on DuP 714 (Figure 1) showed that compound **12** overlaps very well with DuP 714. The *ortho*-methylbiphenyl group occupies the same lipophilic S_2S_3 pocket as the Ac-(D)-Phe-Pro portion of DuP 714. The terminal phenyl ring is at a similar location relative to the phenylalanine of DuP 714, and it forms an edge to face interaction with Trp^{215}. The *ortho*-methyl group has a weak interaction with Trp^{60D}. Similar to DuP 714, the P_1 arginine has an ionic interaction with Asp^{189}; the boronic acid forms a covalent bond with Ser^{195} through a electrophilic interaction; and the NH group of the carboxamide is also involved in a hydrogen bonding interaction with Ser^{215}.

The crystal structure also indicated that there might be some space available for substitution at the *ortho*-position of the terminal ring, and a substituent with a hydrogen bond accepting group might be able to form a hydrogen bond with the phenolic hydroxyl group of Tyr^{60A}. To test this hypothesis, a few compounds were prepared, but they did not show an increase in affinity for thrombin (Table 4). It is possible that the substitution may have changed the conformation of the terminal ring, resulting in a weaker edge to face interaction with Trp^{215}. The new inhibitors may also have induced a conformational change in the enzyme, which might not allow such an interaction. The substituent may have also changed the angle between the two phenyl rings. Such rotation of the terminal phenyl ring might be unfavorable for the interaction with Trp^{215}. Although this substitution did not improve affinity for thrombin, the *in vitro* thrombin times were better than **12**. Compounds **18** and **21** are only two to three fold less potent than DuP 714, as indicated in Table 4.

Table 3. Substitution Effects on the Inner Phenyl Ring[a]

Ex.	R^1	Thrombin K_i (nM)	Trypsin K_i (nM)
3	H	0.94	33
12	o-CH_3	0.42	4.2
13	o-F	1.2	not tested
14	o-NH_2	1.3	5.3
15	o-NO_2	4.7	not tested
16	m-NO_2	4.9	4.8
17	m-NH_2	5.5	4.7

[a]see reference 2 for assay conditions.

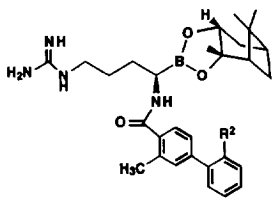


(III)

Table 4. Substitution Effects on the Terminal Phenyl Ring[a]

Ex.	R^2	Thrombin K_i (nM)	Trypsin K_i (nM)	Thr Time IC_{50} (nM)
12	H	0.42	4.2	600
18	SO_2NH-*t*-Bu	0.21	0.70	140
19	SO_2NEt_2	0.36	0.62	not tested
20	CF_3	0.46	3.1	not tested
21	SO_2NH_2	0.92	0.11	110
22	$SO_2NHCO_2CH_3$	1.1	0.64	225

[a]see reference 2 for assay conditions.

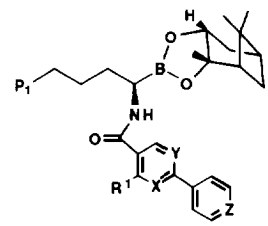


(IV)

Figure 1. X-ray Crystal Structure of Compound 12 and DuP 714 Bound to Thrombin

Table 5. Effects of Nitrogen Atom on the Phenyl Ring

Ex.	X	Y	Z	R^1	P_1	Thrombin K_i (nM)
3	CH	CH	CH	H	$NHC(NH)NH_2$	0.94
23	CH	CH	N	H	$NHC(NH)NH_2$	19
12	CH	CH	CH	CH_3	$NHC(NH)NH_2$	0.42
24	N	N	CH	CH_3	$NHC(NH)NH_2$	10
25	CH	CH	CH	CH_3	CH_2NH_2	26
26	N	CH	CH	CH_3	CH_2NH_2	580
27	N	N	CH	CH_3	CH_2NH_2	410



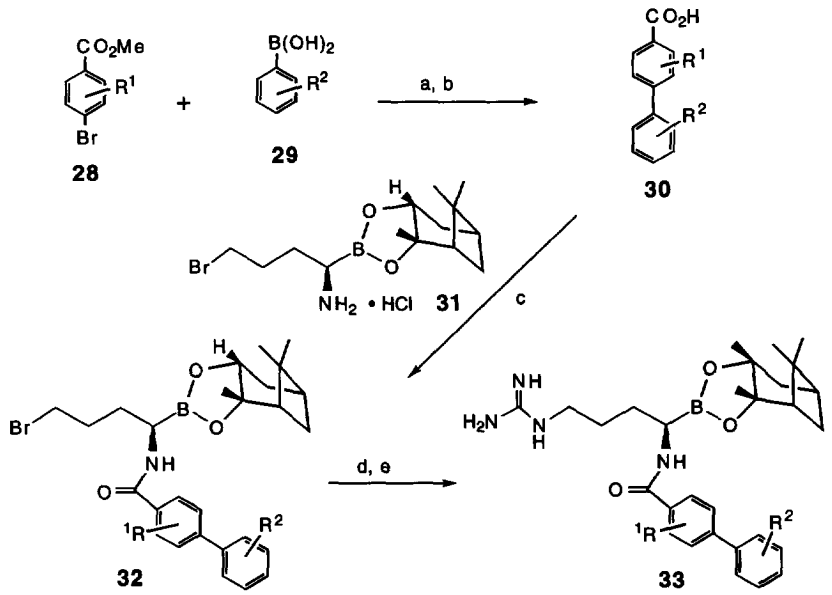
(V)

As shown in Table 5, the incorporation of nitrogen atoms into the biphenyl resulted in a 15- to 20-fold decrease in thrombin affinity. A similar effect was observed when nitrogen was either in the terminal ring or in the inner ring. The same result was also seen for the P_1 lysine analogs (**25, 26,** and **27**).

Syntheses of boroamino acid amides have been described.[2b, 6] Compounds listed in Tables 1 to Table 4 were prepared from the α-aminoboronic acid **31**, which was prepared using the Matteson route.[2b, 6, 7] The starting biaryl carboxylic acids were synthesized via palladium-catalyzed coupling of appropriately substituted 4-bromobenzoic acid with the requisite arylboronic acid (Scheme 1).[8] The phenylpyrimidine analogs in Table 5 were prepared using the methods[9] described in Scheme 2. The phenylpyridine analogs in Table 5 were prepared as described in Scheme 3. Compounds **38** and **43** were then converted to the final targets following the methods described in Scheme 1.

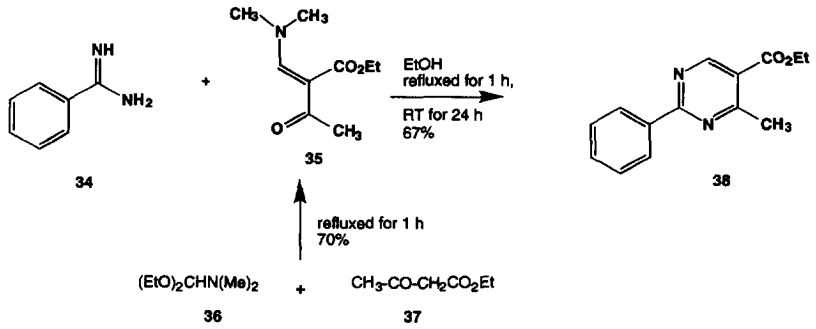
In conclusion, we have replaced the P_2P_3 peptide portion of DuP 714 with substituted biaryl groups. These compounds are potent thrombin inhibitors with the best compounds having subnanomolar affinity for thrombin. The selectivity and *in vitro* potency of our best compounds are very similar to DuP 714.

Scheme 1

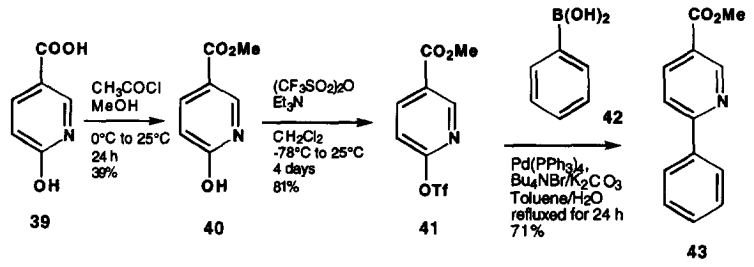


a. $Pd(PPh_3)_4$, Aq. Na_2CO_3, toluene, refluxed for 6h, ~70%;
b. NaOH/ MeOH; 90-95%;
c. HBTU, NMM, DMF, RT for 24 h, 30-50% yield; d. (1) NaN_3/DMF, 100°C for 5 h.
(2) $Pd(OH)_2$ /H_2/ MeOH/HCl, RT for 12 h. ~70%;
e. $NH_2C(NH)SO_3H$(FSA)/DMAP/EtOH, refluxed for 5 h, 90%.

Scheme 2



Scheme 3



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References and Notes

‡ Current address: Schering Plough Research, Kenilworth, NJ 07033.

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