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DESIGN, SYNTHESIS AND *IN VITRO* ACTIVITIES OF A SERIES OF BENZIMIDAZOLE/BENZOXAZOLE GLYCOPROTEIN IIb/IIIa INHIBITORS

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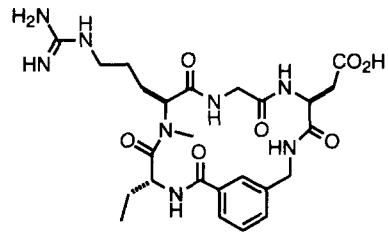
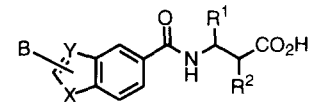
Abstract: A potent centrally constrained series of benzimidazole and benzoxazole glycoprotein IIb/IIIa inhibitors has been discovered based on the solution conformation of a cyclic RGD-containing peptide, DMP 728. The high potency of this series of compounds in the inhibition of platelet aggregation requires a benzamidine as the basic moiety and an α-carbamate or sulfonamide substituted β-alanine as the acidic moiety.

Glycoprotein IIb/IIIa (GP IIb/IIIa) is a platelet membrane protein which plays an important role in hemostasis.[1] The binding of fibrinogen to GP IIb/IIIa on the surface of activated platelets results in crosslinking of the platelets and ultimately platelet aggregation. The pathophysiological consequences of this process include ischemic heart failure, myocardial infarction, unstable angina, and stroke.[2] The GP IIb/IIIa receptor recognizes the Arg-Gly-Asp (RGD) sequence present in fibrinogen.[3] Thus, there have been considerable efforts aimed at the design of RGD mimetics to inhibit the binding of fibrinogen to the GP IIb/IIIa receptor, and a number of potent cyclic RGD containing peptides and non-peptide RGD mimetics have been reported as potential antithrombotics.[4] Among these, DMP 728 (structure **I**) binds extremely tightly to activated GP IIb/IIIa receptor (K_{diss}~0.1 nM) and is orally active in animal models of thrombosis.[5-7]



I



B=basic moiety
X=N, Y=NH
X=N, Y=O
X=O, Y=N

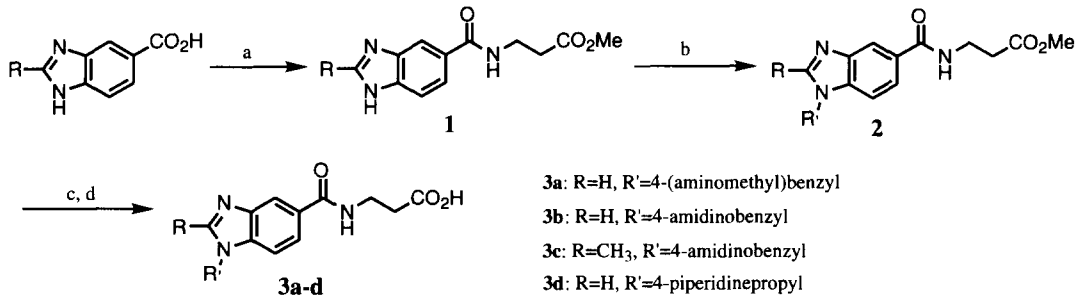
II

In our efforts to discover a RGD non-peptide mimetic with reduced peptide character and improved bioavailability, we have focused on a centrally constrained core as a template of non-peptide mimetic. Modeling studies based on the conformation[8] of DMP 728 revealed that a benzimidazole or a benzoxazole ring would be an appropriate template for a non-peptide series of inhibitors. In particular, addition of a basic moiety to the 1 or 2 position of the ring and an aspartic acid surrogate to the 5 or 6 position yielded structures of general formula **II** which overlap well with the RGD seqeunce in the cyclic ring system of **I**. This design allowed rapid assessment of structure-activity relationships through the incorporation of different basic moieties (B in **II**) and different substitutions (R^1 and R^2 in **II**) at position α or β to the carboxylic acid.

Chemistry:

Coupling of 5-benzimidazolecarboxylic acid or 2-methyl-5-benzimidazolecarboxylic acid with β-alanine methyl ester using TBTU gave the intermediates **1** (Scheme 1). Alkylation of **1** with 4-cyanobenzyl bromide or N-Cbz-4-piperidinepropyl bromide using NaH produced a mixture (~1:1) of 1,2,5-trisubstituted regioisomer (shown) as well as the corresponding 1,2,6-trisubstituted regioisomer (not shown) **2**. Hydrogenation of **2** followed by saponification afforded **3a** and **3d**. A Pinner reaction using HCl/MeOH followed by NH_3/MeOH and saponification of the resultant amidino compounds afforded **3b** and **3c**.

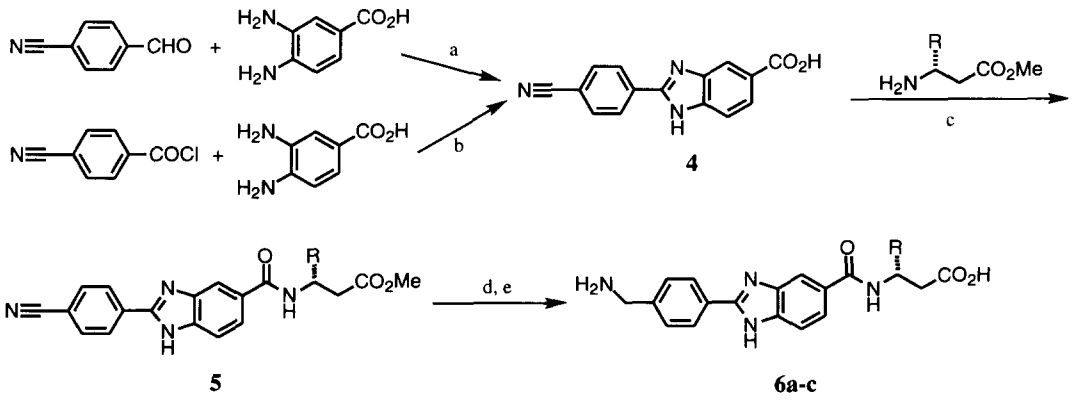
SCHEME 1



(a) β-AlaOMe, TBTU, DIEA, DMF, 80-90%; (b) 4-cyanobenzyl bromide or N-Cbz-4-piperidinepropyl bromide, NaH, DMF, 40-60%; (c) H_2, Pd/C, DMF 80-90% or (1) HCl, MeOH, (2) NH_3, MeOH, 40-60%; (d) NaOH, MeOH, 80-90%.

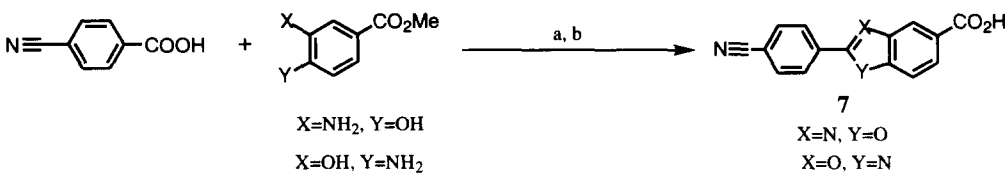
The 2-substituted benzimidazole intermediate **4** was synthesized by treating 4-cyanobenzaldehyde and 3,4-diaminobenzoic acid with molecular sieves in DMF and then refluxing the Schiff base in acetic acid in the presence of atmospheric oxygen or by refluxing 4-cyanobenzoyl chloride and 3,4-diaminobenzoic acid in acetic acid (Scheme 2). Coupling of **4** with a β-alanine derivative afforded the intermediate **5**. Hydrogenation of **5** followed by saponification yielded the products **6a-c**.

SCHEME 2



(a) (1) DMF, molecular sieves (2) CH_3CO_2H, reflux, 25%; (b) CH_3CO_2H, reflux, 30%; (c) TBTU, DIEA, DMF, 70-90%; (d) H_2, Pd/C, DMF, HCl, 80-90%; (e) NaOH, MeOH, 80-90%.

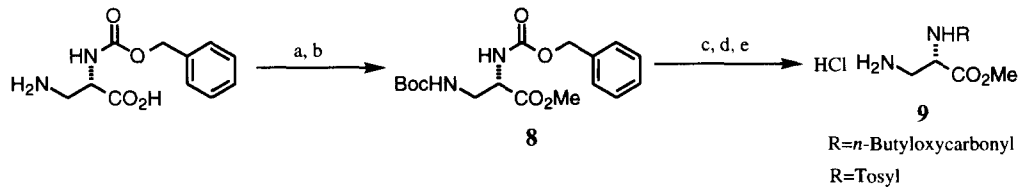
SCHEME 3



(a) Boric acid, xylene, reflux, 30-40%; (b) NaOH, MeOH, 80-90%.

We attempted to synthesize the benzoxazole derivatives **7** using the same methods used for the synthesis of benzimidazole derivatives **4** without success. However, in the presence of boric acid,[9] compounds **7** were obtained in 30-40% yield by refluxing 4-cyanobenzoic acid and methyl 3-amino-4-hydroxybenzoate or methyl 4-amino-3-hydroxybenzoate in xylene (Scheme 3).

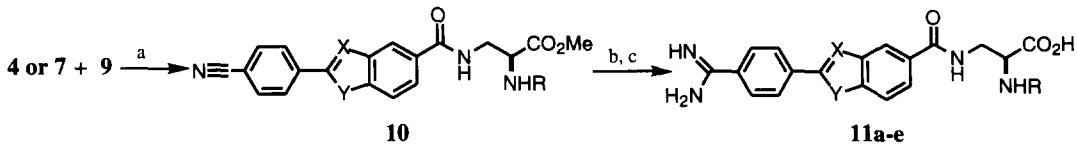
SCHEME 4



(a) MeOH, 4 N HCl/dioxane, 90%; (b) $(Boc)_2O$, DIEA, $CHCl_3$, 85%; (c) H_2, Pd/C, MeOH, 100%; (d) *n*-butyl chloroformate or *p*-toluenesulfonyl chloride, DIEA, $CHCl_3$, 60-80%; (e) 4 N HCl/dioxane, 100%.

The synthesis of L-2,3-diaminopropionic acid derivatives **9** is outlined in Scheme 4. Fisher esterification of commercially available N^2-benzyloxycarbonyl-L-2,3-diaminopropinoic acid followed by conversion to its N^3-Boc analog yielded compound **8**. The Cbz group was removed by hydrogenation, and acylation of the resultant amine with *n*-butyl chloroformate or *p*-toluenesulfonyl chloride followed by deprotection of the Boc group using 4 N HCl in dioxane afforded N^2-*n*-butyloxycarbonyl or N^2-tosyl derivatives **9**.

SCHEME 5



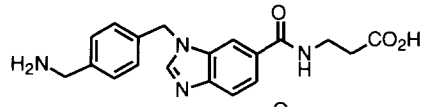
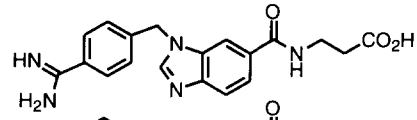
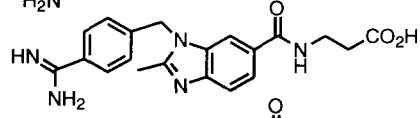
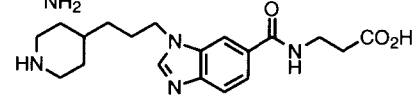
(a) TBTU, DIEA, DMF, 80-85%; (b) (1) HCl, MeOH (2) NH_3, MeOH, 40-60%; (c) NaOH, MeOH, 80-90%.

Coupling of **4** or **7** with **9** using TBTU/DIEA/DMF afforded the intermediates **10** (Scheme 5) in 80-85% yield. The cyano group in **10** was converted to an amidine by a Pinner reaction using HCl/MeOH followed by NH_3/MeOH. Saponification of the resulting amidino intermediates afforded the final products **11a-e**.

Structure Activity Relationships:

We started with compound **3a** in which the basic moiety (B=4-aminomethylbenzyl group in **II**) is at N^1 position, the carboxamide is at 5 or 6 position on the benzimidazole ring (~1:1 mixture of two regioisomers) and the acidic moiety is a β-alanine (R1=R2=H in **II**). Compound **3a** has an IC_{50} of 83 μM for inhibiting the aggregation of platelets in human platelet-rich plasma (Table 1).[10] It has been shown that a benzamidine group has a better interaction with the receptor than a benzylamine group.[11] Thus, replacement of the aminomethyl group in **3a** with an amidino group afforded compound **3b** which is ~12 fold more potent than **3a**. The 2-methylbenzimidazole analog **3c** does not improve the activity of **3b**. Replacement of the 4-aminomethylbenzyl group in **3a** with a 4-piperidinepropyl group (**3d**) also results in 5 fold enhancement in activity relative to **3a**.

Table 1. *In Vitro* Activity of Benzimidazole Analogs in the Inhibition of Platelet Aggregation Using Human Platelet-Rich Plasma.[a]

Compound[b]	Structure	IC_{50} (μM)
3a		83
3b		6.8
3c		11
3d		16

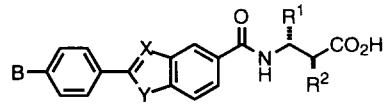
a. ~ 1:1 mixture of 1,5-disubstituted regioisomer (not shown) and 1,6-disubstituted regioisomer (shown). b. tested as TFA salts

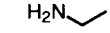
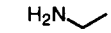
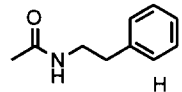
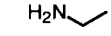
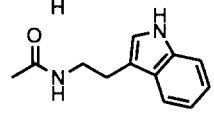
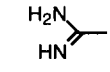
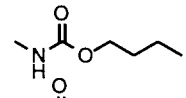
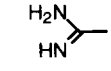
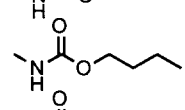
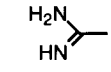
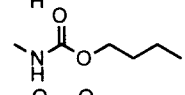
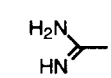
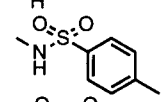
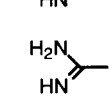
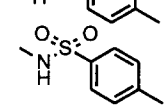
Shifting the basic moiety from the 1 position in **3a** to the 2 position of the benzimidazole ring (B=aminomethylphenyl in **II**) afforded compound **6a** (R^1=R^2=H in **II**) which is comparable to **3a** in inhibitory activity (Table 2). It has been shown[12] that introduction of a hydrophobic residue at the C-terminus of the RGD peptides increases their inhibitory activity. Surprisingly, replacement of the β-alanine with hydrophobic derivatives of aspartic acid gave rise to analogs with weak activity. A phenethyl derivative (compound **6b**) gave 16% inhibition at 100 μM. However, a 3-indoleethyl group (compound **6c**) gave a better activity, indicating that an indole ring at this position has better interaction with the receptor than a phenyl ring.[13]

It has been reported[14] that substitution at position α to the carboxylic acid significantly enhances the potency of the inhibitor due to the interaction of the α-substituent with an exosite in the receptor. We decided to pursue studies with different R^2 groups in structure **II** (R^1=H). Replacement of the aminomethyl group in **6a** with an amidino group and substitution of the α-proton in **6a** with a *n*-butyloxycarbonylamino group afforded compound **11a** which is ~2000 fold more potent in activity as compared with compound **6a** (Table 2). Replacement of the 2,5-disubstituted benzimidazole ring in **11a** with a 2,5-disubstituted benzoxazole ring (**11b**) or 2,6-disubstituted

benzoxazole ring (**11c**) resulted in some improvement in activity. When the α-substituent is a *p*-toluenesulfonamide, compounds **11d** and **11e** showed similar inhibitory activities as compared with the α-carbamate analogs **11b** and **11c** (Table 2).

Table 2. *In Vitro* Activity of Benzimidazole/Benzoxazole Analogs in the Inhibition of Platelet Aggregation Using Human Platelet-Rich Plasma.



Compound[a]	B	X	Y	R^1	R^2	IC_{50} (μM)[b]
6a		N	NH	H	H	100
6b		N	NH		H	16% at 100 μM
6c		N	NH		H	61
11a		N	NH	H		0.05
11b		N	O	H		0.02
11c		O	N	H		0.02
11d		N	O	H		0.01
11e		O	N	H		0.01

a. tested as TFA salts. b. N=2

In summary, we have discovered a series of potent glycoprotein IIb/IIIa inhibitors.[15] In this series of compounds, a benzamidine moiety at 2-position on the benzimidazole/benzoxazole ring and an α-carbamate or a sulfonamide substitution were found to be critical for high potency in the *in vitro* inhibition of platelet aggregation. Preliminary results indicated that the benzoxazole series of compounds were orally active antiplatelet agents in dogs. In addition, compound **11c** is highly fluorescent (λ_{ex}=310 nm, λ_{em}(max)=408 nm) and binds to both activated and unactivated platelets. Thus, it might serve as a convenient reagent for fluorescent-detected competitive binding assays.[16]

Acknowledgments: We thank Jeff Bozarth and Mark Forsythe for running platelet aggregation assays.

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