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CONSTRAINED β-ALANINE BASED GpIIb/IIIa ANTAGONISTS

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Abstract: The concepts of centrally constrained and peptide based fibrinogen receptor antagonists have been successfully combined into a single series of analogs which have been demonstrated to be potent inhibitors of platelet aggregation. © 1997 Elsevier Science Ltd.

Introduction:

When uncontrolled, the process of platelet aggregation, required for maintaining normal hemostasis, can be a major contributor to unstable angina, reocclusion following angioplasty, and other thrombotic disorders.[1] Platelet aggregation is dependent upon the binding of the adhesive glycoprotein fibrinogen to its platelet membrane receptor glycoprotein IIb/IIIa (GpIIb/IIIa), a member of the integrin family of cell surface receptors.[2] The binding of fibrinogen to GpIIb/IIIa has been shown to be mediated largely by the γ-chain C-terminal dodecapeptide.[3] However, small synthetic peptides containing the tripeptide integrin receptor recognition sequence Arg-Gly-Asp (RGD)[4], present in the α-chain of fibrinogen, are able to block the fibrinogen-GpIIb/IIIa interaction and are, as a consequence, capable of blocking platelet aggregation.[5] Since exposure of GpIIb/IIIa is the final common pathway of platelet aggregation, irrespective of the agonist, inhibition of the binding of fibrinogen to this receptor by mimicking the RGD sequence has been well investigated as a potential intervention point in the treatment of thrombotic disorders.[6] A number of agents of this class are currently undergoing clinical evaluation.[7]

Several key structural features are generally required for maximum affinity to GPIIb/IIIa.[8] These are, in relation to tetrapeptides like RGDV, a basic functional group that mimics the side chain of the arginine residue, a carboxylic acid to represent the aspartyl side chain and some sort of lipophilic group proximal to the carboxylic acid, which can approximate the position of the side chain of the valine, or other C-terminal amino acid. The remainder of the molecule serves largely to hold these three functional groups in the proper spatial arrangement. A number of fibrinogen receptor antagonists have been recently reported in which this has been accomplished with rigid central constraints.[9] We have successfully applied this approach to the design of a series of novel fibrinogen receptor antagonists that includes the natural Asp-Val C-terminal dipeptide. This demonstrates that design principles developed for nonpeptide GpIIb/IIIa antagonists may be successfully applied to small RGD peptides to obtain improved in vitro potency and potentially, enhanced in vivo performance.

Results and Discussion:

Compounds **1–15** were assayed for their ability to inhibit the aggregation of fixed, activated human platelets[10] and to block the binding of radiolabelled fibrinogen to activated human platelets.[11]

For this study, 4-guanidino cinnamic acid was chosen for use as a bioisostere for arginine. This readily available material is an effective arginine replacement[12] that allows for the facile preparation of RGD mimics which are equipotent with the natural peptides. A comparison of the IC_{50} values generated for the inhibition of both platelet aggregation and fibrinogen binding for RGDV and **1** illustrates this point.

The importance of the distance separating the basic functional group that mimics the arginine sidechain in RGD from the acidic functional group that mimics the aspartic acid sidechain is one of the distinguishing features of fibrinogen receptor antagonists.[13] Accordingly, the first aspect of this series that was examined was the effect of lengthening the glycine spacer. It was discovered that replacing glycine with β-alanine (**2**) led to a modest threefold enhancement in potency.

Previously[12] it had been demonstrated that alkylation of the glycine nitrogen in certain RGD mimics led to increases in in vitro potency. As a consequence, both N-methyl and N-ethyl β-alanine were prepared and substituted for β-alanine. The N-ethyl derivative (**5**) is fivefold more potent than the unsubstituted β-alanine.

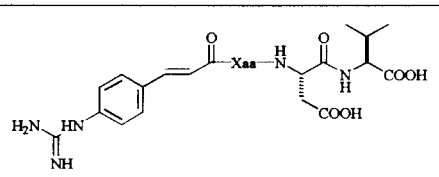
The usefulness of central constraints put in place between the acidic and basic termini of RGD based fibrinogen receptor antagonists has been well demonstrated.[9] We therefore chose to examine the effect of imposing this type of constraint upon these compounds by cyclization of the N-ethyl group of the β-alanine residue back onto either the α- or the β-carbon atom of the β-alanine framework. It should be noted that simple substitution of proline for glycine in tetrapeptides such as RGDV leads to analogs that are unable to block platelet aggregation (data not shown). A series of 2- and 3-substituted azetidinyl, pyrrolidinyl, and piperidinyl derivatives of **5** was prepared. For the five- and six-membered rings, both the *R* and the *S* enantiomers were synthesized.

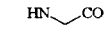
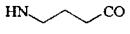
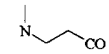
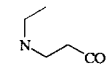
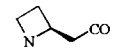
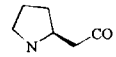
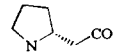
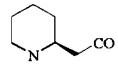
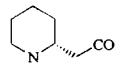
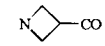
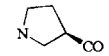
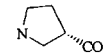
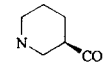
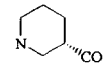
Azetidine containing analogs (**6** and **11**) are less effective platelet aggregation inhibitors than their uncyclized counterpart.

In terms of potency, for the pyrrolidinyl analogs there is a dramatic preference for the *R*-pyrrolidine, regardless of whether the N-alkyl group has been cyclized onto the α- (**12**) or β- (**8**) carbon atom of the β-alanine residue. In the case of **8** there is a fourfold improvement in potency relative to the acyclic analog **5**, and in the case of **12**, a sixfold increase. Compound **12**, which is the most effective platelet aggregation inhibitor in this series, is more than two orders of magnitude more potent than the parent tetrapeptide.

Identical trends are observed for the piperidinyl derivatives (**9–10**) and (**14–15**). However the magnitude of the increase in potency is not equal to that observed for the five membered ring congeners.

Table 1. **Centrally constrained β-alanine based RGD mimics.**

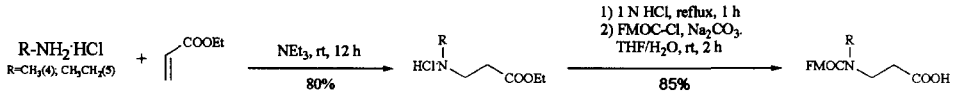


	Xaa	IC_{50} (μM) inhibition of platelet aggregation	IC_{50} (μM) inhibition of Fg binding
	RGDV	10.0	6.0
1		6.60	5.0
2		2.00	0.24
3		>25	70.0
4		0.50	0.20
5		0.41	0.22
6		3.00	0.11
7		25.0	26.0
8		.096	1.50
9		>25	>20
10		1.60	0.20
11		>25	>20
12		.065	0.003
13		3.30	3.00
14		0.18	0.07
15		0.62	0.12

Chemistry:

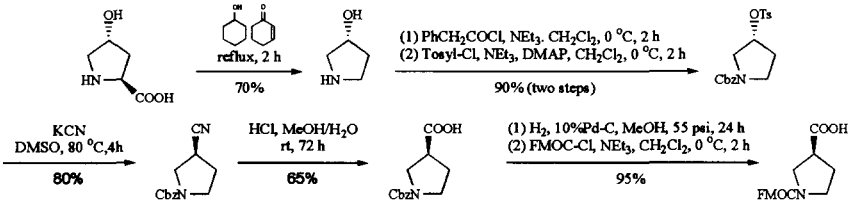
4-Guanidinocinnamic acid was prepared via guanylation of 4-aminocinnamic acid[14] and was used as its hydrochloride salt, without any further protection.

N-alkyl β-alanines were synthesized through the Michael addition of the appropriate amine hydrochloride to ethyl acrylate in the presence of triethylamine. Acid hydrolysis of the ester followed by N-protection with 9-fluorenylmethoxy chloroformate (FMOC-Cl) provided the material that was used in the subsequent coupling reactions.

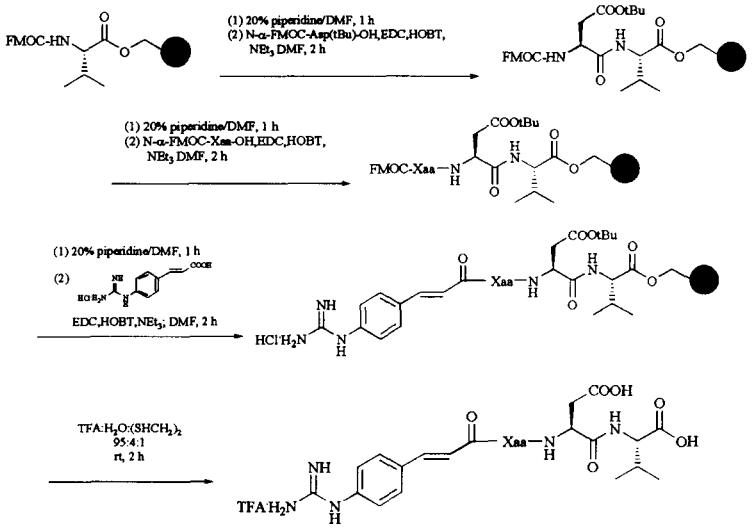


Azetidine, pyrrollidine, and piperidine 2-acetic acids (**6–10**) were prepared from the corresponding α-amino acids via a one carbon homologation.[15]

R and *S* pyrrolidine-3-carboxylic acids (**12** and **13**) were prepared from *trans*-D- or L-4-hydroxyproline using a modified version of the procedure described by Bridges.[16] Following decarboxylation, the nitrogen was protected as its benzyl carbamate. The 4-hydroxyl group was then converted to its tosylate and displaced by cyanide with inversion of configuration. Acid hydrolysis of the resulting nitrile and substitution of FMOC for Cbz as the nitrogen protecting group gave the pyrrollidine-3-carboxylic acids that were needed for the synthesis of **12** and **13**. The Scheme below illustrates the synthesis of (*S*)-pyrrollidine-3-carboxylic acid starting from trans-L-4-hydroxyproline.



With all of the necessary building blocks in hand, the compounds illustrated in Table 1 were assembled using standard FMOC based solid phase synthesis, as illustrated. The starting point was commercially prepared N-α-FMOC-L-Valine linked to a *p*-alkoxybenzylalcohol (Wang) resin.[17]



All of the syntheses were run in manual shakers. Couplings were accomplished with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and deprotections with piperidine. Reactions were followed by means of a modified ninhydrin test.[18] In general, all of the couplings were complete in two hours time and required only one pass.

Following cleavage from the resin and deprotection of the aspartyl side chain with trifluoroacetic acid (TFA), the solution containing the crude peptide was purified by reverse phase HPLC using an acetontirile/water gradient buffered with 0.1% TFA. Final yields of purified peptide (>95% area percent by analytical HPLC) based on the starting resin were typically between 70% and 80%.

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