Design of a New Class of Orally Active Fibrinogen Receptor Antagonists

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The integrin receptor recognition sequence Arg-Gly-Asp was successfully used as a template from which to develop a series of potent, selective, orally active, peptide-based fibrinogen receptor antagonists with a long duration of action. Simple modifications centered on the Arg and Gly residues quickly led to a modified peptide (**1**) with significantly enhanced ability to inhibit in vitro platelet aggregation. Substitution of the guanidino group in **1** by piperidine provided **3**, which showed not only a further increase in potency but also a modest degree of oral efficacy. Finally, exploration of the nature of the C-terminal amino acid, with respect to its side-chain functionality and the carboxy terminus, yielded a group of molecules that showed excellent in vitro potency for inhibiting platelet aggregation, excellent integrin selectivity, a high level of oral efficacy, and an extended duration of action.

Introduction

Coronary, cerebral, and peripheral vascular thrombotic disorders are together the leading cause of death in the western world and as such represent a target that remains ripe for pharmacologic intervention.[1] Platelet aggregation is the central event associated with arterial thrombus formation and is mediated by the binding of fibrinogen to its platelet membrane receptor, glycoprotein IIb/IIIa (GP IIb/IIIa).[2] As exposure of the GP IIb/IIIa binding sites is the final common event that takes place during platelet activation regardless of the agonist involved, blockade of GP IIb/IIIa represents an intervention point that is potentially superior to the inhibition of individual platelet agonists or the blockade of their individual platelet receptors.[3]

The fibrinogen−GP IIb/IIIa interaction is, in part, mediated by the tripeptide integrin recognition sequence Arg-Gly-Asp (RGD).[4] In recent years a number of cyclic peptides, peptide mimetics, and nonpeptides containing or modeled after the RGD sequence have been synthesized, some of which are in development as parenteral agents.[5] Most recently, a number of nonpeptide, orally active agents have been reported, and several of these are also undergoing clinical trials.[6]

There are well-known limitations associated with the use of peptides as drugs, particularly low oral bioavailability, poor metabolic stability, poor potency, and poor selectivity. Despite these potential difficulties, the idea of using the peptide RGD as a template from which to create an orally active fibrinogen receptor antagonist through rational modification was explored. This line of work has led to the discovery of a group of peptide-based GP IIb/IIIa antagonists that are potent, selective, and orally active with a duration of action sufficient for once or twice a day dosing.

Beginning with the tetrapeptide Arg-Gly-Asp-Val (RGDV), a number of modifications to the peptide backbone and arginine side chain led quickly to a modified peptide with substantially enhanced in vitro potency and antithrombotic efficacy relative to the parent peptide. These first analogues demonstrated little or no ex vivo inhibition of platelet aggregation upon intragastric administration to dogs. Systematic changes designed to first reduce the polar nature of these compounds and to then enhance their metabolic stability led eventually to a new class of fibrinogen receptor antagonists which still retain a large part of their peptide character while displaying the same desirable qualities as many of the recently reported nonpeptide GP IIb/IIIa antagonists.

Chemistry

The C-terminal amino acids used in the preparation of **12**, **13**, and **17**, β-(1-decalinyl)alanine, β-(2-decalinyl)-alanine, and homocyclohexylalanine, respectively, were prepared by catalytic hydrogenation of the corresponding β-(1-naphthyl)alanine, β-(2-naphthyl)alanine, and homophenylalanine over 5% rhodium on alumina in methanol/acetic acid.[7] The two β-decalinylalanines were prepared as mixtures of all possible diastereomers.

(*S*)-2-Amino-5-cyclohexylpentanoic acid, β-cyclopentylalanine, β-cycloheptylalanine, and β-cyclooctylalanine, required for the synthesis of **18**−**21**, were prepared according to the method of Evans.[8] L-Cyclohexylalaninol, used in the synthesis of **24**, was prepared by the reduction of the mixed anhydride formed from L-cyclohexylalanine and ethyl chloroformate with sodium borohydride.[9] 6-Guanidinohexanoic acid, used in the preparation of **1**, was prepared from the corresponding amine using aminoiminomethanesulfonic acid.[10]

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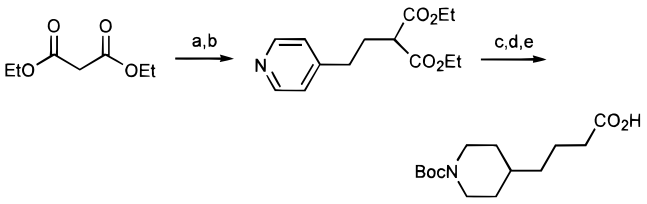
Scheme 1. Synthesis of *N*-Boc-piperidine-4-carboxylic Acid[a]



[a] (a) NaH, EtOH, 80 °C, 0.5 h; (b) 4-vinylpyridine, 80 °C, 3 h; (c) 20% HCl/H_2O, 100 °C, 16 h; (d) H_2, 5 wt % PtO_2, 95:5 MeOH/AcOH, 55 psi, 16 h; (e) 1:1 1 N NaOH/THF, Boc_2O, 0 °C to rt, 16 h.

N-Boc-4-(4-piperidinyl)butyric acid as used in **3** and **5**−**25** was prepared as shown in Scheme 1. Reaction of diethyl malonate with 4-vinylpyridine under basic conditions followed by hydrolysis and decarboxylation under acidic conditions yielded 4-(4-pyridyl)butyric acid. Reduction of the aromatic ring over platinum oxide and subsequent protection with di-*tert*-butyl dicarbonate then gave *N*-Boc-4-(4-piperidinyl)butyric acid.

N-Boc-3-(4-piperidinyl)propionic acid (for **2**) and *N*-Boc-5-(4-piperidinyl)pentanoic acid (for **4**) were prepared by sequential homologation of *N*-Boc-piperidine-4-carboxaldehyde with methyl triphenylphosphoranylideneacetate followed by catalytic hydrogenation and saponification with base.

Peptides **1**−**25** were synthesized using solution-phase chemistry as illustrated in Scheme 2. All α-amino acids used were of the L-configuration except for D-valine in **5** and D-cyclohexylalanine in **16**. The C-terminal amino acid was purchased or prepared as its benzyl ester for the synthesis of **1**−**21**. For **22**−**25**, the corresponding cyclohexylalanine ethyl ester, cyclohexylalaninamide, cyclohexylalaninol, or 2-aminoethylcyclohexane was used throughout the synthesis.

The C-terminal amino acid was coupled to *N*-α-Boc-aspartic acid-*β*-benzyl ester via the activated ester derived from isopropyl chloroformate to give the appropriately protected dipeptide. The N-terminus was deprotected with trifluoroacetic acid (TFA), and the dipeptide was coupled to *N*-α-Boc-*N*-α-ethylglycine using similar conditions to give the protected tripeptide. Prior to coupling, the amino terminus of the dipeptide was desalted by treating with saturated sodium bicarbonate solution. All of the couplings were performed on the free amine rather than the amine salt.

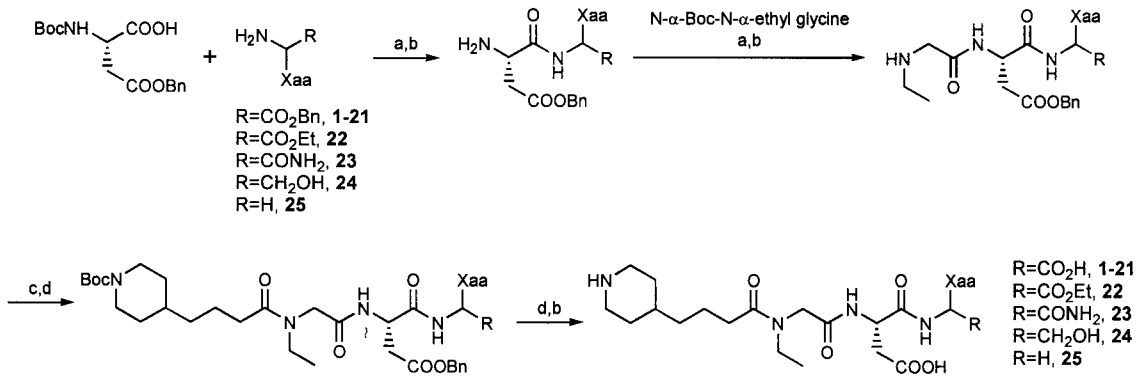
A second cycle of deprotection and coupling, this time using bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl) as the coupling agent in order to accommodate the more hindered *N*-α-ethylglycine amine, led to the fully protected tetrapeptide. In the case of **1**, 6-guanidinohexanoic acid was used in the last coupling reaction as its hydrochloride salt. In all other cases *N*-Boc-4-(4-piperidinyl)butyric acid was used. Debenzylation of the aspartic acid side chain and, where required, the C-terminal carboxylate was accomplished by treatment with hydrogen over palladium on carbon. Removal of the last Boc group with TFA then provided the deprotected tetrapeptide. All compounds were purified by reverse-phase HPLC with a gradient system of acetonitrile in water, buffered with 0.1% TFA. Lyophilization then provided each compound as a fluffy white solid, in the form of its TFA salt.

Results and Discussion

Compounds **1**−**25** were assayed for their ability to inhibit the aggregation of fixed, activated human platelets and to block the binding of radiolabeled fibrinogen to activated human platelets. To correlate the in vitro data obtained with human platelets with the ex vivo data obtained from dogs, a number of compounds were also assayed for their ability to block the ADP-induced aggregation of canine platelets. For the series of compounds described in this paper, there is excellent agreement in terms of both magnitude and rank order potency between in vitro results obtained with human and canine platelets.[11]

Early work with the peptide RGDV involved systematic changes to each of the amide bonds and conservative side-chain substitutions. This rapidly revealed a number of modifications which resulted in either retention or enhancement of in vitro potency with regard to the inhibition of platelet aggregation. These modifications included the elimination of the terminal amino group, replacement of arginine with homoarginine, and alkylation of the glycine nitrogen.[12] Combination and optimization of these features led to compound **1** (Figure 1). This analogue inhibited platelet aggregation with an IC_{50} of 470 nM and abolished cyclic flow reductions

Scheme 2. Synthesis of Compounds **1**−**25**[a]



[a] (a) Isopropyl chloroformate, *N*-methylpiperidine, CH_2Cl_2, rt, 16 h, 80−90%; (b) 1:3 TFA/CH_2Cl_2, 0 °C, 2 h, then saturated $NaHCO_3$, 100%; (c) 6-guanidinohexanoic acid (for **1**), *N*-α-Boc-3-(4-piperidinyl)propionic acid (for **2**). *N*-α-Boc-4-(4-piperidinyl)propionic acid (for **3** and **5**−**25**), *N*-α-Boc-5-(4-piperidinyl)propionic acid (for **4**); (d) BOP-Cl, NEt_3, CH_2Cl_2, 0 °C, 14 h, 70−80%; (e) H_2, 25 wt % 10% Pd−C, 9:1 MeOH/AcOH, 55 psi, 24 h, 95%.

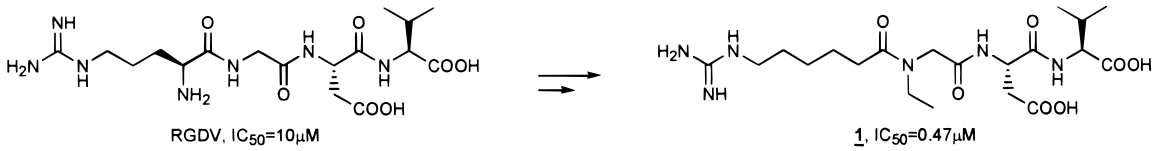


Figure 1. Early modifications to the peptide RGDV.

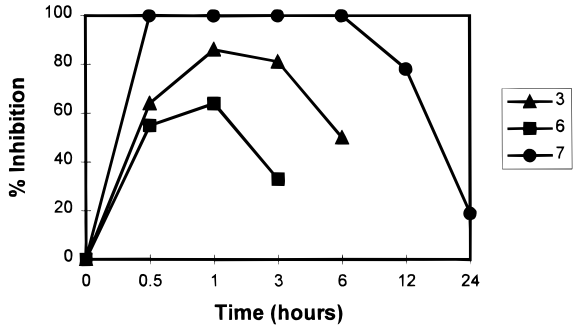
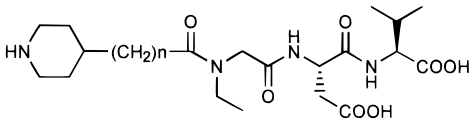


Figure 2. Effect of different C-terminal amino acids on oral efficacy (10 mg/kg ig).

Table 1. Optimization of Chain Length



	n	IC_{50}(nM)
2	2	770
3	3	27
4	4	210

in a canine model[13] of coronary artery occlusion upon parenteral administration of a bolus dose of 30 μg/kg followed by an infusion of 3 μg/kg/min for 60 min. However, following intragastric administration at 10 mg/kg no significant inhibition of ex vivo platelet aggregation was observed.

An assumption was made that the major barrier limiting the oral efficacy of **1** was likely to be poor gastrointestinal absorption owing to the very polar and highly charged nature of the molecule (log $D = -4$, pH 7.4). In the small intestine, where most of the absorption of the drug would be likely to take place, at a pH of between 6 and 8, the guanidino group of **1** (pK_a = 12.5) as well as both carboxylic acids (pK_a = 4.3, Asp side chain, and 3.8, C-terminus) should be fully ionized. To decrease the polar nature of **1**, a prodrug strategy was first attempted, involving various esters of one or both of the carboxylic acids. This approach was uniformly unsuccessful. Attention was then focused on the basic guanidino group. Previously[14] it was demonstrated that replacement of the guanidine of the arginine side chain in peptide analogues of RGDV with basic heterocycles, such as piperidine and imidazole, improves in vitro antiaggregatory activity. Piperidine-containing analogues had proven to be the most potent of these and have successfully been applied to nonpeptide GP IIb/IIIa antagonists.[15] Accordingly, the guanidino group of **1** was replaced with a piperidine ring, and the chain length used to connect this ring to the *N*-α-ethylglycine residue was optimized (Table 1).

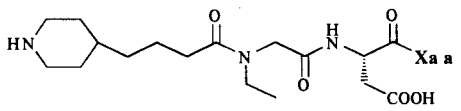
Compound **3** was almost 20-fold more potent than **1**. More importantly, upon intragastric administration at 10 mg/kg, **3** inhibited both ADP- and collagen-induced ex vivo platelet aggregation by more than 50% for at least 6 h (Figure 2). Replacing the guanidino group with piperidine made a significant difference, in terms of both in vitro potency and oral efficacy. The explanation for the improved oral efficacy displayed by **3** may simply lie in its enhanced potency relative to **1**. However, analogues of some of the compounds presented in this paper were prepared that contain a benzamidine in place of guanidine, and these also showed a dramatic improvement in potency but were not as effective orally when compared with those that contain piperidine.[16] Differences in the physical properties of piperidine relative to guanidine with regard to basicity (pK_a piperidine = 11.3, pK_a guanidine = 12.5), lipophilicity (log D **3** = −2.3, log D **1** = −4.0 at pH 7.4), and localization of the positive charge may also play a role in enhancing oral efficacy.

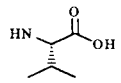
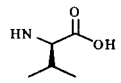
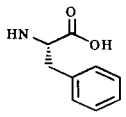
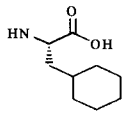
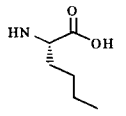
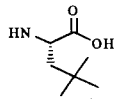
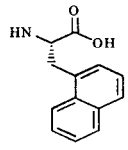
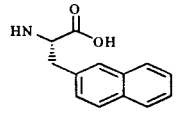
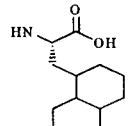
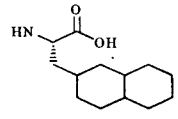
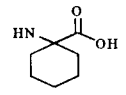
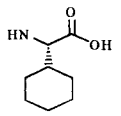
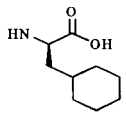
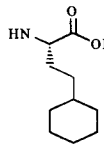
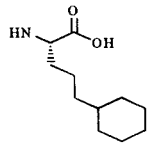
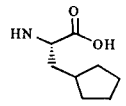
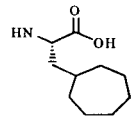
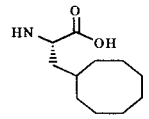
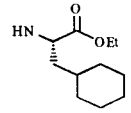
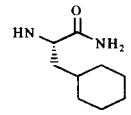
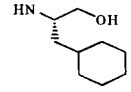
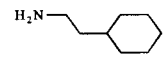
Attention was next focused on some of the other factors that may hinder the oral efficacy of this group of compounds, notably the enzymatic degradation of the natural Asp−Val peptide bond that still made up the right-hand half of these RGDV analogues. It was reasoned that the replacement of the C-terminal valine residue with another, nonproteogenic amino acid might allow for increased metabolic stability and possibly enhanced absorption across the gastrointestinal tract. To test this hypothesis, analogues **5** and **7** (Table 2) were prepared. The C-terminal phenylalanine derivative **6** was prepared for comparison with the cyclohexylalanine analogue **7**.

The D-valine analogue of **3**, compound **5**, shows a dramatic drop in in vitro potency. The L-phenylalanine analogue **6** shows about the same level of in vitro potency as **3** but has a much smaller effect on ex vivo platelet aggregation following intragastric administration. This was presumed to be due to enhanced degradation by digestive enzymes in the gut (vide infra). Conversely, **7**, with the unnatural cyclohexylalanine residue at the C-terminus, not only provides the same level of in vitro potency as **3** but also shows an impressive increase in oral efficacy. Figure 2 shows a comparison of the inhibition of ex vivo platelet aggregation following intragastric dosing of **3**, **6**, and **7** at 10 mg/kg. This result supported the hypothesis that an unnatural peptide bond (Asp−Cha) at the carboxy terminus might show enhanced oral efficacy. Attention was therefore focused on the preparation of a number of analogues with a variety of unnatural C-terminal amino acids, resulting first in the synthesis of compounds **8**−**13**.

Unnatural amino acids with simple alkyl side chains, such as norleucine and *tert*-butylalanine, provided compounds **8** and **9**, respectively. These analogues, in addition to a 3−5-fold decrease in in vitro potency relative to **7**, displayed significantly worse oral efficacy than the cyclohexylalanine analogue. These two com-

Table 2. In Vitro Activity



	Xaa	Inhibition of fixed platelet aggregation (IC_{50} in nM)	Inhibition of fibrinogen binding to activated platelets (IC_{50} in nM)
3		27	8
5		720	260
6		64	
7		26	17
8		130	11
9		82	72
10		110	22
11		68	22
12		19	11
13		96	
14		3410	
15		32	
16		1370	
17		31	11
18		53	
19		47	
20		32	
21		78	
22		64	16
23		97	28
24		129	
25		72	10

pounds performed similarly to the valine-containing analogue **3** when administered intragastrically at a dose of 10 mg/kg (data not shown). Unnatural amino acids with aryl side chains, for example, β-(1-naphthyl)-alanine (**10**) and β-(2-naphthyl)alanine (**11**), also provided analogues with in vitro potencies several times worse than that of **7**, as well as poorer oral efficacy. These two compounds exhibited oral efficacies similar to that of the phenylalanine analogue **6** when given at the same doses (data not shown).

To examine the susceptibility of some of these compounds to proteolytic degradation, **7**, **8**, and **10** were incubated with carboxypeptidase A and the rates of degradation measured (Table 3). Cyclohexylalanine analogue **7** was significantly more resistant to degradation than the other compounds. The fact that this

Table 3. Degradation of Selected Compounds by Carboxypeptidase A

compd	rate of degradation (nmol/min/unit of enzyme)
7	0.6
8	7.4
10	3.1

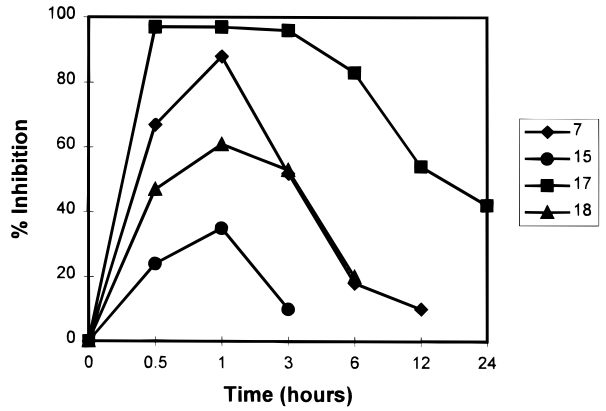


Figure 3. Effect of distance on oral efficacy (1 mg/kg ig).

compound also displays enhanced oral efficacy is consistent with the hypothesis that digestive enzymes in the gut are at least partially responsible for the lesser degree of oral efficacy shown by compounds such as **3** and **6** relative to **7**.

The saturated versions of **10** and **11**, which incorporate β-(1-decalinyl)alanine (**12**) and β-(2-decalinyl)-alanine (**13**) at the carboxy terminus, were prepared next and showed oral efficacy similar to **7**. A comparison of the oral efficacy observed for the analogues containing naphthylalanines relative to those containing decalinylalanines (**10** vs **12** and **11** vs **13**) mirrors what was observed for the two derivatives containing phenylalanine and cyclohexylalanine (**6** vs **7**). Thus it appeared as though placing unnatural amino acids that contain cyclic, nonplanar side chains at the C-terminus might offer the quickest route to compounds that display good levels of oral efficacy. Despite the good levels of activity shown by **12** and **13**, it was decided that in order to avoid the complication of introducing new chiral centers into the molecule at the C-terminal amino acid side chain, work would be confined to amino acids with simple carbocyclic side chains.

The spacing between the carbocycle at the C-terminus and the peptide backbone was the first issue to be examined, via the synthesis of compounds **14**−**18**. The analogues which incorporated either 1-amino-1-cyclohexanecarboxylic acid (**14**) or D-cyclohexylalanine (**16**) were 130 and 53 times less potent than **7** in vitro and were not examined further. Cyclohexylglycine (**15**), cyclohexylalanine (**7**), homocyclohexylalanine (**17**), and (*S*)-2-amino-5-cyclohexylpentanoic acid (**18**) all provided analogues with similar levels of in vitro potency, but very different levels of oral efficacy. Thus while the ability to block in vitro platelet aggregation is generally not affected when the distance between the cyclohexyl ring of the C-terminal side chain and the peptide backbone is varied between zero and four carbons, the ability to block ex vivo platelet aggregation can be dramatically affected (Figure 3). Oral efficacy significantly increases as the distance is increased from zero

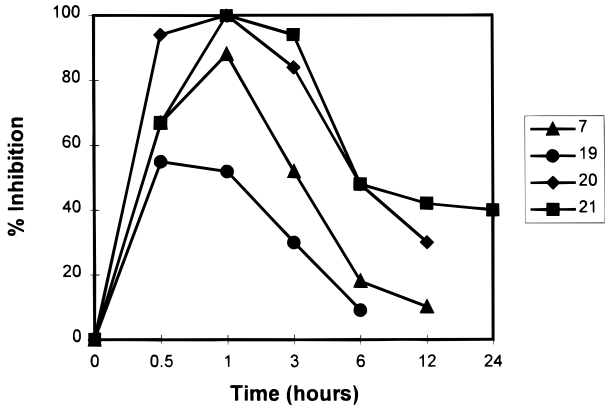


Figure 4. Effect of ring size on oral efficacy (1 mg/kg ig).

to two carbon atoms and falls off with the addition of another methylene group. Compound **17** can block ex vivo platelet aggregation by 50% or greater for 24 h following a single dose of 1 mg/kg ig.

The issue of ring size was examined next. This was accomplished by the synthesis of the five-, seven-, and eight-membered ring homologues of cyclohexylalanine and the preparation of compounds **19**−**21**. The five-, six-, seven-, and eight-membered ring-containing analogues all differ in their in vitro potencies by less than a factor of 3. Relative to cyclohexylalanine analogue **7**, the level of oral efficacy demonstrated by the compound which contains a smaller ring (**19**) is somewhat diminished, while the efficacy of those compounds containing a larger ring is enhanced (Figure 4). As with the spacing of the carbocycle in the C-terminal amino acid side chain, ring size has a minimal effect on receptor binding and consequently on in vitro activity. Both of these factors have a much more significant effect on determining the degree of oral efficacy and the level of ex vivo activity shown by this group of fibrinogen receptor antagonists. This may be explained by differences in the absorption, metabolism, and clearance of members of this class of compounds, but as of yet no definitive answers are available.

One last detail that was examined was the nature of the C-terminal carboxylic acid. It was known from previous work that the presence of this functional group was not mandatory for maintaining in vitro potency, and therefore it seemed reasonable to explore variations at this position and observe the effect these modifications might have on oral efficacy. As shown by compounds **22**−**25**, all of which are direct analogues of **7**, replacing the terminal carboxylic acid with an ester, amide, alcohol, or proton decreases in vitro potency between 2- and 5-fold. However, only the acid **7**, ester **22**, and amide **23**, all of which include a carbonyl-containing functional group at the C-terminus, show any degree of oral efficacy (Figure 5). The carboxamide was the most efficacious of these. Neither **24** nor **25** showed any significant ex vivo activity when given at a dose of 5 mg/kg ig.

Selectivity. Glycoprotein IIb/IIIa is a member of the integrin superfamily of cell surface receptors and as such shares common elements with a number of other physiologically important members of this group.[17] Selectivity for binding to the platelet fibrinogen receptor relative to other, sometimes closely related integrin receptors may therefore be of some concern during the development of fibrinogen receptor antagonists.

Table 4. Selectivity for Platelet Fibrinogen Receptor vs HUVEC Vitronectin Receptor

	IC_{50} for inhibition of fixed platelet aggregation (μM)	IC_{50} for HUVEC adhesion (μM) to plates coated with		
		fibrinogen	vitronectin	fibronectin
7	0.026	1012	>1650	>1750
17	0.031	1930	>2500	>2400
23	0.097	>2350	>2400	>2400
RGDS	82.0	3.5	22.7	>250
echistatin	0.020	0.0033	0.0125	0.042

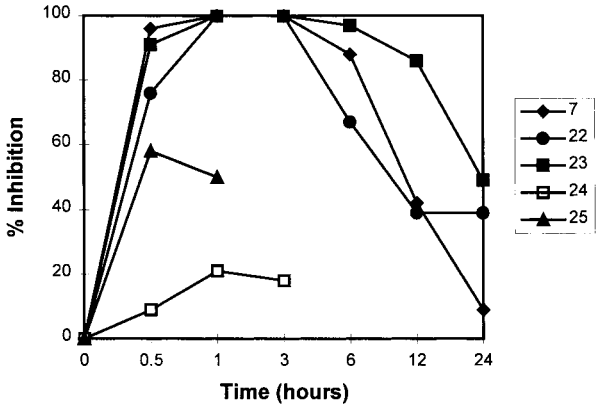


Figure 5. Effect of varying the C-terminal carboxylic acid on oral efficacy (5 mg/kg ig).

Compounds **7**, **17**, and **23** were evaluated for their ability to block the adhesion of human umbilical vein endothelial cells (HUVEC) to plates coated with the adhesive glycoproteins fibrinogen, vitronectin, and fibronectin, relative to their ability to block the interaction of fibrinogen and GP IIb/IIIa. HUVEC express the vitronectin receptor, which shares an identical β-subunit with GP IIb/IIIa. This then offers an opportunity to gauge the selectivity that has been achieved for GP IIb/IIIa over another closely related integrin receptor.[18] The snake venom protein echistatin, which is nonselective for these two receptors, was used as a control. RGDS was also evaluated as a comparison to demonstrate how the selectivity of this new class of fibrinogen receptor antagonists compares to a classic RGD-containing peptide. As shown in Table 4, this new class of compounds is between 30 000 times and 90 000 times more selective for GP IIb/IIIa than for the vitronectin receptor and offers a significant enhancement over both the potent RGD-containing protein echistatin and the small peptide RGDS.

Conclusion

We have demonstrated that the rational and systematic modification of small RGD-containing peptides can yield a group of derivatives that retain much of their peptide character but avoid some of the problems inherent in the use of small peptides as drugs. These include poor oral efficacy, poor in vitro potency, and poor selectivity.

A number of features present in this new class of fibrinogen receptor antagonists appear to play a major role in enhancing both potency and oral efficacy. This has allowed for the development of a structure−oral activity relationship for this set of compounds (see Figure 6). Two of the most notable of these characteristics are the alkylation of the glycine nitrogen in RGDV and the replacement of the guanidino group of the arginine side chain with a piperidine ring.

With respect to the alkylation of the glycine nitrogen, direct analogues of **7** containing either glycine or sarcosine in place of *N*-ethylglycine were prepared. The IC_{50} values for these compounds for the inhibition of in vitro platelet aggregation were 3.9 and 0.1 μM, respectively, compared to 0.026 μM for **7**. In addition, the glycine-containing analogue of **7**, when administered to dogs intragastrically at a dose of 5 mg/kg, resulted in an average maximal inhibition of ex vivo aggregation of only 17%, after 1 h. The increase in potency, along with the changes in the conformation of the peptide that are likely to be brought about by alkylation of the amide bond, may both play some role in the enhanced oral efficacy shown by the N-alkylated derivatives. Likewise, replacement of guanidine with piperidine also dramatically enhances both potency and oral efficacy (compare **1** and **3**). This effect on oral efficacy may again be due to the dramatic enhancement in potency but also may be due in part to the differences in the physical characteristics of piperidine and guanidine.

In any case, replacement of guanidine by piperidine or alkylation of the glycine nitrogen alone is not enough to appreciably improve upon the oral efficacy of the parent peptide. It is only when these two modifications are combined that a significant enhancement is observed, as illustrated by the four compounds in Table 5.

Further, our results suggest that it is the nature of the C-terminal amino acid that provides the most useful handle for fine-tuning oral efficacy. We demonstrate that the C-terminal amino acid is best chosen from among a group of select nonproteogenic amino acids of

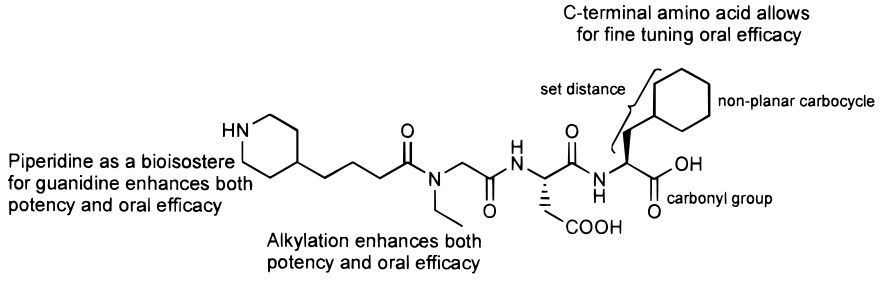
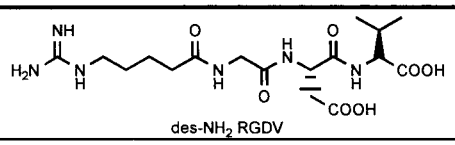
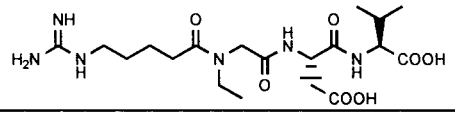
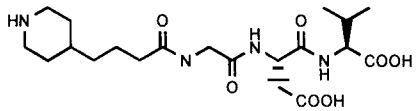
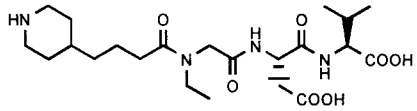


Figure 6. Structure−oral activity relationships for peptide-based fibrinogen receptor antagonists.

Table 5. Effect of Single vs Combined Structural Modifications

	IC_{50} (μM)	% Inhibition of Ex Vivo Platelet Aggregation		
		1 hour	3 hours	6 hours
 des-NH_2 RGDV	2.30	nd	nd	nd
	0.630	23	0	0
	1.06	47	0	nd
 **3**	0.027	80	66	20

the L-configuration. This modification yields RGD-based analogues which are less prone to proteolytic degradation than ones containing either natural L-amino acids or structurally related unnatural L-amino acids at the C-terminus.

The specific features of the C-terminal amino acid which play a role in the generation of orally active compounds include (1) the side-chain functionality, where a nonplanar carbocycle is preferred, (2) the spacing between the carbocycle and the peptide backbone, (3) the ring size, and (4) the presence of a carbonyl-containing functional group at the C-terminus. All of the features that contribute to the potency and oral efficacy of this new peptide-based class of fibrinogen receptor antagonists are illustrated in Figure 6. This series represents an excellent starting point for the selection of a clinically useful, orally active fibrinogen receptor antagonist.

Experimental Section

In Vitro Platelet Aggregation Studies. Blood was obtained from human volunteers, all of whom had been free of any medications for at least 14 days prior to blood donation. In all cases the first 1−2 mL of blood obtained were discarded in order to avoid the traces of thrombin that have been shown to be generated during venipuncture. The remainder of each blood sample was mixed with 10% of its volume of a 3.8% sodium citrate solution. Gel-filtered platelets were isolated following the procedures of Marguerie[19a] and Ruggeri.[19b] For the preparation of fixed, activated platelets, washed platelets were activated with human α-thrombin (Enzyme Research Laboratory, South Bend, IN) at a final concentration of 1 U/mL for 2 min at room temperature, followed by the addition of the thrombin inhibitor I-2581 (Kabi, Pharmacia Harper, Franklin, OH) at a final concentration of 20 μM. To the activated platelets was added paraformaldehyde (Sigma) to a final concentration of 0.5%, and the whole was incubated for 30 min at room temperature. The fixed, activated platelets were then collected by centrifugation at 650*g* for 15 min. Platelet pellets were washed four times with Tyrode's-HSA buffer and resuspended to 2×10^8 cells/mL in the same buffer.

Platelet aggregation was performed using fixed, activated platelets according to the turbidometric method of Born.[20] Various doses of a given compound were incubated with 0.4 mL of platelet suspension for 1 min, and aggregation was initiated by the addition of fibrinogen (Calbiochem) to a final concentration of 250 μg/mL (0.72 μM). A platelet aggregation profiler model PAP-4 (Bio Data, Hatsboro, PA) was used to record platelet aggregation. Inhibition of aggregation was expressed as the percent of the rate of aggregation in the presence of antagonists compared to that observed in the absence of antagonists. The IC_{50}'s were then calculated for each compound.[21]

Competitive [^{125}I]Fibrinogen Binding Studies. Fibrinogen (Kabi, Stockholm, Sweden) was purified according to Hawiger and Timmons[22a] and radioiodinated using a modification of the procedure of Fraker and Speck.[22b] Competitive binding assays were performed according to Hawiger and Timmons[22a] with minor modifications. Reactions were carried out in duplicate in Tyrode's buffer with 1×10^8 platelets/mL, 100 nM [^{125}I]fibrinogen, and either 100 μM TRAP (SFLLRN-NH_2) (Peninsula Laboratories, Belmont, CA) or 10 μM ADP. When inhibitors of [^{125}I]fibrinogen binding were tested, both inhibitor and [^{125}I]fibrinogen were added prior to agonist addition. Following a 30-min incubation at room temperature, the reaction mixtures were layered onto a 20% sucrose cushion and centrifuged at 10000*g* for 3 min. The reaction tubes were frozen with liquid N_2 and the tips of each tube clipped off and counted in a γ-counter.

Ex Vivo Platelet Aggregation Studies. Compounds were dissolved in deionized water and were administered to mongrel dogs of either sex by intragastric gavage via an intragastric feeding tube. Venous blood samples (4.5 mL) for measuring ex vivo platelet aggregation were obtained 10 min before administration of compound and at 1, 3, 6, 12, and 24 h after administration of compound. The 6-, 12-, and 24-h blood samples were obtained only if substantial (>50%) inhibition of ex vivo platelet aggregation was measured from samples obtained at the earlier time points. Each blood sample was collected directly into a plastic syringe containing 0.5 mL of 3.8% trisodium citrate.

Blood samples were centrifuged at 150*g* for 10 min to obtain platelet-rich plasma (PRP). After removal of the PRP the sample was centrifuged for an additional 10 min at 1500*g* to obtain platelet-poor plasma (PPP). Platelet count in the PRP was determined by using a Coulter counter (Coulter Electronics, Hialeah, FL). If the concentration of platelets in the PRP was greater than 300 000 platelets/μL, then the PRP was diluted with PPP to adjust the platelet count to 300 000 platelets/μL. Aliquots of PRP (250 μL) were then placed in siliconized glass cuvettes (Bio/Data Corp., Horsham, PA) and

incubated at 37 °C for 3 min while being stirred at 1200 rpm. Epinephrine (final concentration of 1 μM) was then added to the PRP, which was incubated for 1 min at 37 °C. The platelet agonist ADP (final concentration of 10 μM) was then added to the PRP. Platelet aggregation was monitored spectrophotometrically using a light transmission aggregometer (Bio/Data platelet aggregation profiler model PAP-4, Bio/Data Corp.). The rate of change (slope) of light transmittance was recorded in duplicate. Platelet aggregation data are reported as the percent decrease in the rate of aggregation as compared to data obtained from control PRP (obtained prior to administration of test compound). Results reported are mean values from at least two dogs for each dose of a given compound.

Selectivity Studies. Human umbilical vein endothelial cells (Clonetics, CA) between passages 4 and 7 were used for this assay.[23] Harvested monolayer cells were transferred to the wells of microtiter plates that were preincubated for 16 h at room temperature with 0.1 mL/well of either 1.5 μg/mL human fibrinogen (Calbiochem), 3 μg/mL human fibronectin (Calbiochem), or 0.9 μg/mL human vitronectin (Calbiochem). Each well received 20 000 cells, which were allowed to adhere to the ligand-coated plates in the presence of RGDS (Bachem Bioscience, PA), echistatin (Peninsula Laboratories, CA), or one of the newly synthesized compounds for 75 min in a tissue culture incubator.

The adherent cells were quantitated by measuring the endogenous hexosaminidase activity spectrophotometrically at 405 nm using *p*-nitrophenyl-*N*-acetyl-β-D-100 (Sigma) as the substrate. The percent inhibition of cell attachment was determined and an IC_{50} calculated.

Degradation of Compounds by Carboxypeptidase A. A suspension of carboxypeptidase A (CPA; bovine pancreas, Sigma) was diluted with enough 5% lithium chloride solution to provide a solution containing 2.0 U/mL CPA. The activity of the solution was measured using hippuryl-L-phenylalanine (Sigma; 1 unit of activity of CPA is defined as the amount that will hydrolyze 1 μM of hippuryl-L-phenylalanine in 1 min at 25 °C). A 1.0 mM solution of each substrate to be tested was prepared in 0.05 M Tris buffer (Sigma) containing 1.0 M sodium chloride (pH = 7.5).

Incubations with CPA were conducted as follows. Each substrate solution (2.0 mL) was combined with CPA solution (1.0 mL) in glass vials to give a total of 2.0 μM substrate with 2.0 units of CPA. Incubations were carried out at room temperature for 60 min. Hydrolysis of the substrate was monitored by HPLC at 9-min intervals. The rate of hydrolysis of each substrate was calculated and normalized to 1 unit of CPA.

Chemistry. All amino acids, solvents and other reagents were used as received from commercial sources without additional purification. Proton NMRs were recorded on a Bruker ARX 300-MHz spectrometer. Mass spectra were obtained from a Varian VG-70SE spectrometer. Preparative reverse-phase HPLC was performed with a Rainin SD-1 Dynamax system and a 2-in. C-18 reverse-phase Dynamax 60A column using a gradient of 20% acetonitrile/0.1% TFA in water to 100% acetonitrile and a flow rate of 50 mL/min. Analytical reverse-phase HPLC was performed with a Rainin HPX system and an analytical C-18 reverse-phase Dynamax 60A column using the same gradient system used for preparative work and a flow rate of 1 mL/min. Work up means drying over magnesium sulfate, filtering, and concentrating in vacuo.

Compounds **1**−**25** were obtained as hygroscopic solids following lyophilization after final reverse-phase HPLC purification. Consequently, elemental analysis was not obtained for these compounds, and purity was gauged by analytical reverse-phase HPLC of the lyophilized samples using the gradient system described above. Retention time (t_R) in this system and area percent (*A*%) are given for each compound.

***N*-Boc-piperidine-4-carboxaldehyde.** To a solution of piperidine-4-carboxylic acid (10 g, 77.5 mmol) and potassium carbonate (21.4 g, 155 mmol) in 150 mL of water was added di-*tert*-butyl dicarbonate (16.9 g, 77.5 mmol) as a solution in tetrahydrofuran (THF), dropwise via addition funnel at 0 °C. The reaction mixture was allowed to come to room temperature and stirred for 4 h. THF was removed in vacuo, and the aqueous phase was washed with ether, brought to pH = 2 with 1 N hydrochloric acid (HCl), and extracted with ethyl acetate. The combined organic extracts were worked up to provide *N*-Boc-piperidine-4-carboxylic acid (17.4 g, 98%) as a white solid.

To a solution of *N*-Boc-piperidine-4-carboxylic acid (8 g, 35 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (3.4 g, 35 mmol), and triethylamine (9.8 mL, 70 mmol) in 50 mL of dimethylformamide (DMF) was added bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) (8.9 g, 35 mmol) in a single portion at 0 °C. The reaction mixture was allowed to come to room temperature and stirred for 16 h. The reaction mixture was diluted with ethyl acetate and washed with water, 1 N HCl, saturated sodium bicarbonate, and brine. The organic layer was worked up to give the *N*,*O*-dimethylhydroxamide of *N*-Boc-piperidine-4-carboxylic acid as a white solid (6.2 g, 65%).

All of the amide from above was dissolved in 100 mL of THF, and lithium aluminum hydride (LAH; 114 mL of a 1 M solution in THF) was added slowly via syringe at room temperature. Stirring was continued for 5 h at room temperature. Excess LAH was quenched by the careful addition of 1 N HCl. Solvent was removed in vacuo and the residue taken up in ethyl acetate and washed with water and brine. The organic layer was worked up to give *N*-Boc-piperidine-4-carboxaldehyde (19.8 g, 87%) as a light-yellow oil that was used without purification. NMR ($CDCl_3$): δ 9.8 (s, 1H), 4.0 (m, 2H), 2.8 (m, 2H), 1.9 (br s, 1H), 1.7 (m, 2H), 1.4 (s, 9H), 1.2 (m, 2H).

***N*-Boc-3-(4-Piperidinyl)propionic Acid.** A solution of *N*-Boc-piperidine-4-carboxaldehyde (3.5 g, 16.4 mmol) and methyl triphenylphosphoranylideneacetate (5.5 g, 16.4 mmol) in 50 mL of chloroform was heated at reflux for 16 h. After cooling, solvent was removed in vacuo and the residue taken up in ether and filtered. The filtrate was concentrated in vacuo and subjected to flash chromatography (10% ethyl acetate in hexanes) to give methyl *N*-Boc-3-(4-piperidinyl)acrylate (3.6 g, 81.5%).

A solution of this acrylate (1.7 g, 6.4 mmol) in 25 mL of methanol containing 10% palladium on carbon (170 mg, 10 wt %) was shaken under a positive pressure of hydrogen (Parr shaker, 55 psi) for 24 h. Catalyst was removed by filtration through Celite and the filtrate concentrated in vacuo to give methyl *N*-Boc-3-(4-piperidinyl)propionate (1.7 g, 100%).

All of the methyl propionate was dissolved in 25 mL of a solution consisting of 2 parts methanol to 1 part 1 N sodium hydroxide and allowed to stir for 30 min at room temperature. The reaction mixture was brought to pH 2 with 1 N HCl and extracted with ethyl acetate. The combined organic extracts were worked up to provide *N*-Boc-3-(4-piperidinyl)propionic acid (1.5 g, 91%) as a white solid that was used without any further purification. NMR ($CDCl_3$): δ 4.1 (m, 2H), 2.7 (m, 2H), 2.3 (t, J = 7.0 Hz, 2H), 1.7 (m, 3H), 1.4 (s, 9H), 1.3 (m, 2H), 1.0 (m, 2H).

***N*-Boc-5-(4-piperidinyl)pentanoic Acid.** *N*-Boc-3-(4-piperidinyl)propionic acid was subjected to the same sequence of reactions described above to provide this two-carbon homologue. NMR ($CDCl_3$): δ 4.1 (m, 2H), 2.6 (m, 2H), 2.2 (t, J = 7.5 Hz, 2H), 1.7 (m, 5H), 1.4 (s, 9H), 1.2 (m, 2H), 0.8 (m, 2H).

4-(4-Pyridyl)butyric Acid. Sodium hydride (8.4 g, 0.21 mol) was added portionwise to 200 mL of absolute ethanol. Diethyl malonate (72 g, 0.44 mol) was added, and the solution was heated to 80 °C for 30 min and cooled to room temperature. 4-Vinylpyridine (20 g, 0.19 mol) was added and the resulting solution heated at 80 °C for 3 h. The solution was evaporated in vacuo and the residue dissolved in 200 mL of 20% HCl and heated at reflux for 16 h. Solvent was removed in vacuo and the residue taken up in hot methanol and filtered. The filtrate was evaporated to give 4-(4-pyridyl)butyric acid as a white solid (20.2 g, 64.5%). NMR (CD_3OD): δ 8.85 (d, J = 6.0 Hz, 2H), 8.05 (d, J = 6.0 Hz, 2H), 3.05 (t, J = 8.6 Hz, 2H), 2.45 (t, J = 8.6 Hz, 2H), 2.05 (m, 2H).

***N*-Boc-4-(4-piperidinyl)butyric Acid.** 4-(4-Pyridyl)butyric acid (19 g, 0.11 mol) was dissolved in 100 mL of methanol

and 5 mL of acetic acid. Platinum oxide (1 g, 0.004 mol) was added and the suspension placed on a Parr shaker under a positive pressure of hydrogen (50 psi) for 16 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give a white solid. This solid was dissolved in 150 mL of 1 N sodium hydroxide and cooled to 0 °C. To this was added di-*tert*-butyl dicarbonate (27.6 g, 0.125 mol) in 100 mL of THF, dropwise via addition funnel. The reaction mixture was allowed to come to room temperature and stirred for 16 h. THF was removed in vacuo, and the remaining aqueous phase was washed with ether. The aqueous phase was carefully acidified with 1 N HCl and extracted with ethyl acetate. The combined organic extracts were washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo to give *N*-Boc-4-(4-piperidinyl)butyric acid (29.2 g, 98%) as a white solid. This material was used without any additional purification. NMR ($CDCl_3$): δ 2.73 (t, J = 8.6 Hz, 2H), 2.35 (t, J = 8.6 Hz, 2H), 1.75 (m, 4H), 1.44 (s, 9H), 1.42 (m, 4H), 1.03 (m, 2H).

Representative Peptide Synthesis. Compounds **1**−**25** were all prepared using the same general procedure, given here for **3**, substituting the appropriate amino acids. All compounds were isolated as their trifluoroacetate salts after HPLC purification and were tested as such. Purity of final products was determined by analytical reverse-phase HPLC (20% acetonitrile/0.1% TFA in water to 100% acetonitrile gradient, run over 30 min at 1 mL/min).

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-valine (3). To a solution of *N*-Boc-L-aspartic acid β-benzyl ester (1.79 g, 5.53 mmol) in 20 mL of methylene chloride (CH_2Cl_2) was added *N*-methylpiperidine (0.64 mL, 5.27 mmol) in a single portion at 0 °C. Isopropyl chloroformate (0.6 g, 5.27 mmol) was added as a 1 M solution in toluene, dropwise via syringe at 0 °C. Stirring was continued for 2 min at 0 °C, and a solution containing L-valine benzyl ester (1.1 g, 5.27 mmol) and *N*-methylpiperidine (0.64 mL, 5.27 mmol) in 5 mL of CH_2Cl_2 was added in a single portion. The reaction mixture was allowed to come to room temperature and stirred for 16 h. Solvent was removed in vacuo and the residue partitioned between ethyl acetate (300 mL) and 1 N HCl (75 mL). The aqueous layer was discarded and the organic phase further washed sequentially with 1 N HCl (2 × 75 mL), water (1 × 75 mL), saturated sodium bicarbonate (3 × 75 mL), and brine (1 × 75 mL). Work up provided *N*-Boc-β-benzyl-aspartyl-valine benzyl ester as a colorless oil that was used without purification (2.57 g, 95.5%). NMR ($CDCl_3$): δ 7.32 (s, 10H), 7.05 (m, 1H), 5.72 (m, 1H), 5.15 (q, J = 12.8, 24.4 Hz, 2H), 5.05 (s, 2H), 4.50 (m, 2H), 3.01 (dd, J = 17.1, 4.3 Hz, 1H), 2.68 (dd, J = 17.1, 6.8 Hz, 1H), 2.18 (septet, 1H), 1.42 (s, 9H), 0.88 (d, J = 7.3 Hz, 3H), 0.83 (d, J = 7.3 Hz, 3H).

To a solution of *N*-Boc-β-benzyl-aspartyl-valine benzyl ester (2.57 g, 5.02 mmol) in 36 mL of CH_2Cl_2 was added TFA (12 mL) over 2 min at 0 °C. Stirring was continued at 0 °C for 2 h. Solvents were removed in vacuo, and the residue was partitioned between ethyl acetate (200 mL) and saturated sodium bicarbonate (75 mL). The aqueous phase was discarded, and the organic layer was washed twice more with saturated sodium bicarbonate (75 mL) and worked up to give β-benzyl-aspartyl-valine benzyl ester (1.69 g, 81.6%). This material was coupled directly to Boc-*N*-ethylglycine using a procedure identical to the one given above to provide, after flash chromatography (30% ethyl acetate/hexanes), *N*-Boc-*N*-ethylglycyl-β-benzyl-aspartyl-valine benzyl ester as a colorless oil (1.88 g, 62.6%). NMR ($CDCl_3$): δ 7.31 (m, 1H), 7.29 (s, 10H), 7.07 (m, 1H), 5.12 (q, J = 27.4, 12.8 Hz, 2H), 5.10 (s, 1H), 4.85 (m, 1H), 4.48 (dd, J = 7.7, 4.3 Hz, 1H), 3.83 (s, 2H), 3.30 (m, 2H), 3.00 (m, 1H), 2.63 (dd, J = 17.1, 6.85 Hz, 1H), 2.17 (septet, 1H), 1.45 (s, 9H), 1.09 (t, J = 6.4 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H).

The terminal amine of *N*-Boc-*N*-ethylglycyl-β-benzyl-aspartyl-valine benzyl ester was deprotected with TFA as described above to give *N*-ethylglycyl-β-benzyl-aspartyl-valine benzyl ester as a colorless oil (1.49 g, 95%) which was used without any purification.

To a solution of *N*-Boc-4-(4-piperidinyl)butyric acid (0.89 g, 3.26 mmol) in CH_2Cl_2 (15 mL) was added triethylamine (0.46 mL, 3.26 mmol) in a single portion at 0 °C. BOP-Cl (0.84 g, 3.26 mmol) was added in a single portion at 0 °C, and the reaction mixture was allowed to stir for 30 min at 0 °C. A solution of *N*-ethylglycyl-β-benzyl-aspartyl-valine benzyl ester (1.49 g, 3.00 mmol) in 5 mL of CH_2Cl_2 was added, followed by triethylamine (0.46 mL, 3.26 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 16 h. Solvent was removed in vacuo and the residue partitioned between ethyl acetate (300 mL) and 1 N HCl (75 mL). The aqueous layer was discarded and the organic phase further washed sequentially with 1 N HCl (2 × 75 mL), water (1 × 75 mL), saturated sodium bicarbonate (3 × 75 mL), and brine (1 × 75 mL). The organic phase was then subjected to workup to provide, after flash chromatography (75% ethyl acetate/hexanes), the fully protected tetrapeptide *N*-Boc-4-(4-piperidinyl)butanoyl-*N*-ethylglycyl-β-benzyl-aspartyl-valine benzyl ester as a colorless oil (1.76 g, 74.4%). NMR ($CDCl_3$): δ 7.41 (m, 1H), 7.32 (s, 10H), 7.15 (m, 1H), 5.15 (m, 4H), 4.80 (m, 1H), 4.45 (m, 1H), 4.00 (m, 4H), 3.35 (m, 2H), 3.10 (m, 2H), 2.60 (m, 3H), 2.30 (m, 2H), 2.15 (m, 1H), 1.60 (m, 6H), 1.41 (s, 9H), 1.10 (m, 6H), 0.80 (m, 6H).

The tetrapeptide was deprotected as follows. *N*-Boc-4-(4-piperidinyl)butanoyl-*N*-ethylglycyl-β-benzyl-aspartyl-valine benzyl ester (1.76 g, 2.34 mmol) was dissolved in 100 mL of a 1:1 mixture of methanol and acetic acid; 10% palladium on carbon (0.5 g) was added, and the reaction mixture was shaken under a positive pressure of hydrogen (Parr shaker, 45 psi) for 16 h. Catalyst was removed by filtration of the reaction mixture through Celite, and the filtrate was concentrated in vacuo to give *N*-Boc-4-(4-piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-valine as a colorless oil. The N-terminus of this material was deblocked using TFA, as described above, to give 4-(4-piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-valine. Final purification was achieved via reverse-phase HPLC (acetonitrile/water gradient). Final product was obtained by lyophilization to a fluffy white amorphous solid (1.14 g, 83.2%). NMR (D_2O): δ 4.10 (m, 3H), 3.90 (s, 1H), 3.30 (q, J = 8.6 Hz, 2H), 3.20 (m, 2H), 2.75 (m, 4H), 2.33 (t, J = 8.3 Hz, 2H), 2.12 (m, 1H), 2.06 (q, J = 8.1 Hz, 1H), 1.80 (m, 2H), 1.45 (m, 2H), 1.20 (m, 4H), 1.02 (t, 3H), 0.80 (d, J = 7.5 Hz, 6H). MS: m/z (FAB) 471 $(M + H)^+$. HPLC: t_R 12.78 min, 99.7 *A*%.

6-Guanidinohexanoyl-*N*-ethylglycyl-aspartyl-valine (1). NMR (D_2O): δ 4.08 (m, 3H), 3.9 (s, 1H), 3.24 (m, 2H), 3.00 (m, 2H), 2.75 (m, 2H), 2.35 (t, J = 7.7 Hz, 2H), 2.13 (t, J = 7.7 Hz, 1H), 2.04 (q, J = 7.3 Hz, 1H), 1.46 (m, 3H), 1.20 (m, 2H), 1.00 (m, 3H), 0.76 (d, J = 7.3 Hz, 6H). MS: m/z (FAB) 473 $(M + H)^+$. HPLC: t_R 14.93 min, 99.6 *A*%.

3-(4-Piperidinyl)propionyl-*N*-ethylglycyl-aspartyl-valine (2). NMR (D_2O): δ 4.62 (m, 2H), 3.90 (s, 2H), 3.33 (m, 4H), 2.66 (m, 4H), 2.37 (t, J = 7.9 Hz, 2H), 2.16 (m, 1H), 2.03 (m, 1H), 1.78 (m, 2H), 1.44 (m, 2H), 1.20 (m, 2H), 1.00 (m, 3H), 0.78 (d, J = 8.0 Hz, 6H). MS: m/z (FAB) 457 $(M + H)^+$. HPLC: t_R 11.02 min, 99.6 *A*%.

5-(4-Piperidinyl)pentanoyl-*N*-ethylglycyl-aspartyl-valine (4). NMR (D_2O): δ 4.10 (m, 2H), 4.07 (s, 1H), 3.92 (s, 1H), 3.33 (q, J = 7.2 Hz, 2H), 3.20 (m, 2H), 2.75 (m, 4H), 2.34 (t, J = 7.2 Hz, 2H), 2.06 (m, 2H), 1.80 (m, 2H), 1.45 (m, 2H), 1.20 (m, 6H), 1.06 (m, 3H), 0.81 (d, J = 6.0 Hz, 6H). MS: m/z (FAB) 485 $(M + H)^+$. HPLC: t_R 12.20 min, 98.7 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-D-valine (5). NMR (D_2O): δ 4.05 (m, 3H), 3.85 (s, 1H), 3.28 (q, J = 7.9 Hz, 2H), 3.15 (m, 2H), 2.70 (m, 4H), 2.28 (t, J = 6.9 Hz, 2H), 2.02 (m, 1H), 1.95 (m, 1H), 1.73 (m, 2H), 1.40 (m, 3H), 1.15 (m, 3H), 0.95 (m, 3H), 0.70 (m, 6H). MS: m/z (FAB) 471 $(M + H)^+$. HPLC: t_R 12.29 min, 99.1 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-phenylalanine (6). NMR (D_2O): δ 7.10 (m, 5H), 4.45 (m, 2H), 3.90 (s, 1H), 3.73 (s, 1H), 3.10 (m, 5H), 2.85 (m, 1H), 2.60 (m, 3H), 2.25 (t, J = 7.9 Hz, 2H), 1.97 (t, J = 8.0 Hz, 1H), 1.68 (m, 2H), 1.35 (m, 3H), 1.10 (m, 4H), 0.88 (m, 3H). MS: m/z (FAB) 519 $(M + H)^+$. HPLC: t_R 17.08 min, 99.1 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-cyclohexylalanine (7). NMR (D_2O): δ 4.52 (m, 1H), 4.30 (m, 1H), 4.05 (s, 1H), 3.88 (s, 1H), 3.32 (q, J = 8.6 Hz, 2H), 3.20 (m, 2H), 2.72 (m, 5H), 2.33 (t, J = 8.6 Hz, 2H), 2.10 (m, 1H), 1.75 (m, 2H), 1.50 (m, 10H), 1.18 (m, 4H), 1.00 (m, 3H), 0.70 (m, 6H). MS: *m/z* (FAB) 525 $(M + H)^+$. HPLC: t_R 18.63 min, 99.9 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-norleucine (8). NMR (D_2O): δ 4.54 (m, 1H), 4.15 (m, 1H), 4.04 (s, 1H), 3.85 (s, 1H), 3.32 (q, J = 7.9 Hz, 2H), 3.17 (m, 2H), 2.70 (m, 4H), 2.30 (t, J = 7.7 Hz, 2H), 2.09 (t, J = 8.2 Hz, 1H), 1.65 (m, 4H), 1.40 (m, 2H), 1.15 (m, 8H), 0.98 (m, 3H), 0.68 (m, 3H). MS: *m/z* (FAB) 485 $(M + H)^+$. HPLC: t_R 13.19 min, 99.9*A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-*tert*-butylglycine (9). NMR (D_2O): δ 4.60 (m, 1H), 4.25 (m, 1H), 3.88 (s, 2H), 3.33 (q, J = 7.8 Hz, 2H), 3.20 (m, 2H), 2.74 (m, 4H), 2.35 (t, J = 7.6 Hz, 2H), 2.12 (t, J = 8.1 Hz, 1H), 1.80 (m, 2H), 1.50 (m, 4H), 1.15 (m, 4H), 1.03 (m, 3H), 0.78 (s, 9H). MS: *m/z* (FAB) 499 $(M + H)^+$. HPLC: t_R 13.94 min, 99.2 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-(1-naphthyl)alanine (10). NMR (D_2O): δ 7.95 (d, J = 9.6 Hz, 1H), 7.80 (m, 1H), 7.65 (m, 1H), 7.42 (m, 2H), 7.26 (m, 1H), 7.22 (t, J = 9.6 Hz, 1H), 4.52 (m, 2H), 3.78 (s, 1H), 3.71 (s, 1H), 3.57 (m, 2H), 3.20 (m, 4H), 2.55 (m, 4H), 2.21 (t, J = 8.4 Hz, 2H), 1.78 (t, J = 7.2 Hz, 1H), 1.58 (m, 2H), 1.20 (m, 6H), 0.92 (m, 3H). MS: *m/z* (FAB) 569 $(M + H)^+$. HPLC: t_R 18.25 min, 99.3 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-(2-naphthyl)alanine (11). NMR (D_2O): δ 7.55 (m, 3H), 7.42 (s, 1H), 7.20 (m, 2H), 7.10 (d, J = 9.6 Hz, 1H), 4.52 (m, 2H), 3.57 (d, J = 6.0 Hz, 1H), 3.41 (s, 1H), 3.10 (m, 3H), 2.85 (m, 3H), 2.53 (m, 4H), 1.98 (t, J = 8.4 Hz, 2H), 1.54 (m, 3H), 1.10 (m, 6H), 0.71 (t, J = 6.0 Hz, 3H). MS: *m/z* (FAB) 569 $(M + H)^+$. HPLC: t_R 18.47 min, 95.5 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-(1-decalinyl)alanine (12). NMR (D_2O): δ 4.22 (m, 1H), 4.10 (q, J = 12.4, 8.6 Hz, 2H), 3.40 (m, 4H), 2.77 (m, 4H), 2.35 (t, J = 8.6 Hz, 2H), 2.15 (t, J = 8.6 Hz, 1H), 1.87 (m, 3H), 1.50 (m, 12H), 1.30 (m, 12H), 0.98 (m, 9H). MS: *m/z* (FAB) 579 $(M + H)^+$. HPLC: t_R 25.00 min, 99.4 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-(2-decalinyl)alanine (13). NMR (D_2O): δ 4.75 (m, 1H), 4.38 (m, 1H), 4.10 (q, J = 12.4, 8.5 Hz, 2H), 3.55 (m, 5H), 2.77 (m, 5H), 2.55 (t, J = 8.6 Hz, 2H), 2.30 (t, J = 8.5 Hz, 1H), 1.95 (d, J = 12.4 Hz, 2H), 1.55 (m, 14H), 1.15 (m, 14H), 0.72 (m, 3H). MS: *m/z* (FAB) 579 $(M + H)^+$. HPLC: t_R 18.00 min, 99.6 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-1-aminocyclohexane-1-carboxylic Acid (14). NMR (D_2O): δ 4.58 (m, 1H), 4.05 (s, 1H), 3.90 (s, 1H), 3.30 (q, J = 9.0 Hz, 2H), 3.20 (m, 2H), 2.70 (m, 4H), 2.35 (t, J = 7.2 Hz, 1H), 2.11 (t, J = 7.2 Hz, 1H), 1.80 (m, 4H), 1.60 (m, 2H), 1.40 (m, 6H), 1.13 (m, 7H), 1.40 (m, 3H). MS: *m/z* (FAB) 497 $(M + H)^+$. HPLC: t_R 12.53 min, 99.9 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-cyclohexylglycine (15). NMR (D_2O): δ 4.60 (m, 1H), 4.06 (m, 1H), 4.01 (s, 1H), 3.85 (s, 1H), 3.30 (q, J = 6.0 Hz, 2H), 3.16 (m, 2H), 2.70 (m, 4H), 2.30 (t, J = 7.2 Hz, 2H), 2.10 (t, J = 7.2 Hz, 1H), 1.70 (m, 2H), 1.45 (m, 8H), 1.00 (m, 12H). MS: *m/z* (FAB) 511 $(M + H)^+$. HPLC: t_R 14.81 min, 99.9 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-D-cyclohexylalanine (16). NMR (D_2O): δ 4.58 (m, 1H), 4.26 (m, 1H), 4.05 (s, 1H), 3.85 (s, 1H), 3.32 (q, J = 8.4 Hz, 2H), 3.18 (m, 2H), 2.72 (m, 4H), 2.32 (t, J = 7.2 Hz, 2H), 2.12 (t, J = 7.2 Hz, 1H), 1.77 (m, 2H), 1.45 (m, 10H), 0.90 (m, 12H). MS: *m/z* (FAB) 525 $(M + H)^+$. HPLC: t_R 16.94 min, 99.8 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-homocyclohexylalanine (17). NMR (D_2O): δ 4.57 (m, 1H), 4.06 (s, 1H), 4.03 (m, 1H), 3.85 (s, 1H), 3.29 (q, J = 6.0 Hz, 2H), 3.20 (m, 2H), 2.82 (t, J = 15.6 Hz, 2H), 2.70 (m, 2H), 2.29 (t, J = 7.2 Hz, 2H), 2.13 (t, J = 7.2 Hz, 1H), 1.72 (m, 3H), 1.50 (m, 10H), 1.10 (m, 6H), 1.00 (m, 3H), 0.60 (m, 4H). MS: *m/z* (FAB) 539 $(M + H)^+$. HPLC: t_R 19.50 min, 99.9 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-(*S*)-2-amino-5-cyclohexylpentanoic Acid (18). NMR (D_2O): δ 4.65 (m, 1H), 4.23 (m, 1H), 4.10 (s, 1H), 3.95 (s, 1H), 3.35 (q, J = 6.0 Hz, 2H), 3.27 (m, 2H), 2.75 (m, 4H), 2.40 (t, J = 7.2 Hz, 1H), 2.15 (t, J = 7.2 Hz, 1H), 1.82 (m, 3H), 1.60 (m, 12H), 1.25 (m, 6H), 1.00 (m, 3H), 0.72 (m, 4H). MS: *m/z* (FAB) 553 $(M + H)^+$. HPLC: t_R 19.62 min, 99.9 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-cyclopentylalanine (19). NMR (D_2O): δ 4.75 (m, 1H), 4.30 (m, 1H), 4.15 (q, J = 12.7, 8.6 Hz, 2H), 3.50 (m, 5H), 2.85 (t, J = 17.1 Hz, 2H), 2.75 (m, 3H), 2.52 (t, J = 8.5 Hz, 2H), 2.35 (t, J = 8.5 Hz, 1H), 1.95 (d, J = 8.5 Hz, 2H), 1.55 (m, 13H), 1.12 (m, 3H). MS: *m/z* (FAB) 511 $(M + H)^+$. HPLC: t_R 17.82 min, 99.8 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-cycloheptylalanine (20). NMR (D_2O): δ 4.57 (m, 1H), 4.30 (m, 1H), 4.08 (s, 1H), 3.92 (s, 1H), 3.35 (q, J = 7.2 Hz, 2H), 3.26 (m, 2H), 2.75 (m, 4H), 2.35 (t, J = 7.2 Hz, 1H), 2.18 (m, 1H), 1.82 (m, 2H), 1.40 (m, 21H), 1.00 (m, 3H). MS: *m/z* (FAB) 539 $(M + H)^+$. HPLC: t_R 17.21 min, 98.9 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-β-cyclooctylalanine (21). NMR (D_2O): δ 4.40 (m, 1H), 4.20 (q, J = 8.6, 8.6 Hz, 2H), 3.95 (m, 2H), 3.30 (m, 4H), 2.82 (m, 4H), 2.35 (t, J = 9.1 Hz, 2H), 2.12 (t, J = 8.6 Hz, 1H), 1.85 (d, J = 8.6 Hz, 2H), 1.57 (m, 14H), 1.35 (m, 5H), 1.10 (m, 12H). MS: *m/z* (FAB) 553 $(M + H)^+$. HPLC: t_R 22.30 min, 99.5 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-cyclohexylalanine Ethyl Ester (22). NMR (D_2O): δ 4.45 (m, 1H), 4.18 (q, J = 12.4, 8.6 Hz, 2H), 3.95 (m, 2H), 3.30 (m, 5H), 2.87 (m, 5H), 2.35 (t, J = 8.6 Hz, 2H), 2.25 (t, J = 8.6 Hz, 1H), 1.89 (m, 2H), 1.55 (m, 7H), 1.00 (m, 18H). MS: *m/z* (FAB) 553 $(M + H)^+$. HPLC: t_R 17.04 min, 98.3 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-cyclohexylalaninamide (23). NMR (D_2O): δ 4.43 (m, 1H), 4.20 (m, 1H), 4.08 (s, 1H), 3.92 (d, J = 2.2 Hz, 1H), 3.35 (q, J = 6.0 Hz, 2H), 3.25 (m, 2H), 2.83 (dt, J = 8.6, 2.4 Hz, 2H), 2.52 (m, 2H), 2.36 (t, J = 6.8 Hz, 2H), 2.14 (m, 1H), 1.80 (m, 2H), 1.50 (m, 10H), 1.21 (m, 4H), 1.00 (m, 3H), 0.75 (m, 5H). MS: *m/z* (FAB) 524 $(M + H)^+$. HPLC: t_R 16.15 min, 99.4 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-cyclohexylalaninol (24). NMR (D_2O): δ 4.55 (m, 1H), 4.04 (s, 1H), 3.90 (s, 1H), 3.80 (m, 1H), 3.30 (m, 6H), 2.75 (m, 4H), 2.35 (t, J = 7.2 Hz, 1H), 2.04 (m, 1H), 1.81 (m, 2H), 1.50 (m, 8H), 1.20 (m, 6H), 0.85 (m, 8H). MS: *m/z* (FAB) 511 $(M + H)^+$. HPLC: t_R 16.51 min, 98.2 *A*%.

4-(4-Piperidinyl)butanoyl-*N*-ethylglycyl-aspartyl-(2-cyclohexyl)ethylamine (25). NMR (D_2O): δ 4.10 (s, 1H), 3.90 (q, J = 12.4, 12.4 Hz, 2H), 3.35 (d, J = 8.5 Hz, 2H), 3.25 (d, J = 12.8 Hz, 2H), 2.73 (m, 8H), 2.35 (t, J = 12.8 Hz, 2H), 2.15 (t, J = 17.1 Hz, 2H), 1.83 (m, 4H), 1.57 (m, 7H), 1.10 (m, 12H), 0.75 (t, J = 21.4 Hz, 2H). MS: *m/z* (FAB) 481 $(M + H)^+$. HPLC: t_R 17.71 min, 99.3 *A*%.

References

(1) *SCRIP* **1995**, *2024/25*, 23.

(2) Phillips, D. R.; Charo, I. F.; Parise, L. V.; Fitzgerald, L. A. The Platelet Membrane Glycoprotein IIb-IIIa Complex. *Blood* **1988**, *71*, 831−843.

(3) Coller, B. S. Platelets and Thrombolytic Therapy. *N. Engl. J. Med.* **1990**, *322*, 33−42.

(4) (a) Hynes, R. O. Integrins: A Family of Cell Surface Receptors. *Cell* **1987**, *48*, 549−554. (b) Ruoslahti, E.; Pierschbacher, M. D. New Perspectives in Cell Adhesion: RGD and Integrins. *Science* **1987**, *238*, 491−497.

(5) (a) Shebuski, R. J.; Interruption of Thrombosis and Hemostasis by Antiplatelet Agents. *Toxicol. Pathol.* **1993**, *21*, 180−189. (b) Barrett, J. S.; Murphy, G.; Peerlinck, K.; DeLepeleire, I.; Gould, R. J.; Panebianco, D.; Hand, E.; Deckmyn, H.; Vermylen, J.; Arnout, J. Pharmacokinetics and Pharmacodynamics of MK-383, A Selective Non-peptide Platelet Glycoprotein-IIb/IIIa Receptor Antagonist, In Healthy Men. *Clin. Pharmacol. Ther.* **1994**, *56*, 377−388. (c) Tcheng, J. E.; Ellis, S. G.; Kleiman, N. S.; Harrington, R. A.; Mick, M. J.; Navetta, F. I.; Worley, S.; Smith, J. E.; Kereiakes, D. J.; Kitt, M. M.; Miller, J. A.; Sigmon, K. N.; Califf, R. M.; Topol, E. J. Outcome of Patients Treated with the GP IIb/IIIa Inhibitor Integrelin During Coronary Angioplasty: Results of the IMPACT Study. *Circulation* **1993**, *88*, I-595 (Abstract 3200). (d) Theroux, P.; Kouz, S.; Knudtson, M. L.; Kells,

C.; Nasmith, J.; Roy, L.; Dalle, S.; Steiner, B.; Xiao, Z.; Rapold, H. J. A Randomized Double-blind Controlled Trial with the Non-Peptidic Platelet GP IIb/IIIa antagonist Ro 44-9883 in Unstable Angina. *Circulation* **1994**, *90*, I-232 (Abstract 1243).

(6) (a) Eldred, C. D.; Evans, B.; Hindley, S.; Judkins, B. D.; Kelly, H. A.; Kitchin, J.; Lumley, P.; Porter, B.; Ross, B. C.; Smith, K. J.; Taylor, N. R.; Wheatcroft, J. R. Orally Active Non-Peptide Fibrinogen Receptor (GpIIb/IIIa) Antagonists: Identification of 4-[4-[4-(Aminoiminomethyl)phenyl]-1-piperazinyl]-1-piperidineacetic Acid as a Long-Acting, Broad Spectrum Antithrombotic Agent. *J. Med. Chem.* **1994**, *37*, 3882−3885. (b) Nicholson, N. S.; Panzer-Knodle, S. G.; Salyers, A. K.; Taite, B. B.; Szalony, J. A.; Haas, N. F.; King, L. W.; Zablocki, J. A.; Keller, B. T.; Broschat, K.; Engleman, W.; Herin, M.; Jacqmin, P.; Feigen, L. P. SC-54684A: An Orally Active Inhibitor of Platelet Aggregation. *Circulation* **1995**, *91*, 403−410. (c) Barrett, J. S.; Gould, R. J.; Ellis, J. D.; Holahan, M. M.; Stranieri, M. T.; Lynch, J. J.; Hartman, G. D.; Ihle, N.; Duggan, M.; Moreno, O. A.; Theoharides, A. D. Pharmacokinetics and Pharmacodynamics of L-703,014, a Potent Fibrinogen Receptor Antagonist, After Intravenous and Oral Administration in the Dog. *Pharm. Res.* **1994**, *11*, 426−431. (d) Duggan, M. E.; Naylor-Olsen, A. M.; Perkins, J. J.; Anderson, P. S.; Chang, C. T.-C.; Cook, J. J.; Gould, R. J.; Ihle, N. C.; Hartman, G. D.; Lynch, J. J.; Lynch, R. J.; Manno, P. D.; Schaffer, L. W.; Smith, R. L. Non-peptide Fibrinogen Receptor Antagonists. XX. Design and Synthesis of the Potent, Orally Active Fibrinogen Receptor Antagonist L-734,-217. *J. Med. Chem.* **1995**, *38*, 3332−3341. (e) Bondinell, W. E.; Keenan, R. M.; Miller, W. H.; Ali, F. E.; Allen, A. C.; DeBrosse, C. W.; Eggleston, D. S.; Erhard, K. F.; Haltiwanger, R. C.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Koster, P. F.; Ku. T. W.; Lee, C. P.; Nichols, A. J.; Ross, S. T.; Samanen, J. M.; Valocik, R. E.; Vasko-Moser, J. A.; Venslavsky, J. W.; Wong, A. S.; Yuan, C.-K. Design of a Potent and Orally Active Nonpeptide Platelet Fibrinogen Receptor (GP IIb/IIIa) Antagonist. *Bioorg. Med. Chem.* **1994**, *2*, 897−908. (f) Zablocki, J. A.; Rico, J. G.; Garland, R. B.; Rogers, T. E.; Williams, K.; Schretzman, L. A.; Rao, S. A.; Bovy, P. R.; Tjoeng, F. S.; Lindmark, R. J.; Toth, M. V.; Zupec, M. E.; McMackins, D. E.; Adams, S. P.; Miyano, M.; Markos, C. S.; Milton, M. N.; Paulson, S.; Herin, M.; Jacqmin, P.; Nicholson, N. S.; Panzer-Knodle, S. G.; Haas, N. F.; Page, J. D.; Szalony, J. A.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. Potent in Vitro and in Vivo Inhibitors of Platelet Aggregation Based Upon the Arg-Gly-Asp Sequence of Fibrinogen. (Aminobenzamidino)succinyl (ABAS) Series of Orally Active Fibrinogen Receptor Antagonists. *J. Med. Chem.* **1995**, *38*, 2378−2394. (g) Weller, T.; Alig, L.; Beresini, M.; Blackburn, B.; Bunting, S.; Hadvary, P.; Hurzeler Muller, M.; Knopp, D.; Levet-Trafit, B.; Lipari, M. T.; Modi, N. B.; Muller, M.; Refino, C. J.; Schmitt, M.; Schonholzer, P.; Weiss, S.; Steiner, B. Orally Active Fibrinogen Receptor Antagonists. 2. Amidoximes as Prodrugs of Amidines. *J. Med. Chem.* **1996**, *39*, 3139−3147. (h) Samanen, J. M.; Ali, F. E.; Barton, L. S.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, W.; Chen, L.; Erhard, K.; Feuerstein, G.; Heys, R.; Hwang, S.-M.; Jakas, D. R.; Keenan, R. M.; Ku, T. W.; Kwon, C.; Lee, C.-P.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M.; Peishoff, C. E.; Rhodes, G.; Ross, S.; Shu, A.; Simpson, R.; Takata, D.; Yellin, T. O.; Uzsinskas, I.; Venslavsky, J. W.; Yuan, C.-K.; Huffman, W. F. Potent, Selective, Orally Active 3-Oxo-1,4-benzodiazepine GPIIb/IIIa Integrin Antagonists. *J. Med. Chem.* **1996**, *39*, 4867−4870. (i) Simpfendorfer, C.; Kottke-Marchant, K.; Topol, E. J. First Experience With Chronic Platelet GPIIb/IIIa Receptor Blockade: A Pilot Study of Xemlofiban an Orally Active Antagonist in Unstable Angina Patients Eligible for PTCA. *J. Am. Coll. Cardiol.* **1995**, *27*, 242A (Abstract 969-110). (j) Xue, C.-B.; Rafalski, M.; Roderick, J.; Eyermann, C. J.; Mousa, S.; Olson, R. E.; DeGrado, W. F. Design, Synthesis and In Vitro Activities of a Series of Benzimidazole/Benzoxazole Glycoprotein IIb/IIIa Inhibitors. *Bioog. Med. Chem. Lett.* **1996**, *6*, 339−344.

(7) Lazer, E. S.; Miao, C. K.; Wong, H.-C.; Sorcek, R.; Spero, D. M.; Gilman, A.; Pal, K.; Behnke, M.; Graham, A. G.; Watrous, J. M.; Homon, C. A.; Nagel, J.; Shah, A.; Guindon, Y.; Farina, P. R.; Adams, J. Benzoxazolamines and Benzothiazolamines: Potent, Enantioselective Inhibitors of Leukotriene Biosynthesis with a Novel Mechanism of Action. *J. Med. Chem.* **1994**, *37*, 913−923.

(8) Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. The Asymmetric Synthesis of α-Amino Acids. Electrophilic Azidation of Chiral Imide Enolates, a Practical Approach to the Synthesis of (R)- and (S)-α-Azido Carboxylic Acids. *J. Am. Chem. Soc.* **1990**, *112*, 4011−4030.

(9) Ishizumi, K.; Koga, K.; Yamada, S.-I. Chemistry of Sodium Borohydride and Diborane IV. Reduction of Carboxylic Acids to Alcohols with Sodium Borohydride Through Mixed Carbonic-Carboxylic Acid Anhydrides. *Chem. Pharm. Bull.* **1968**, *16*, 492−497.

(10) Miller, A. E.; Bischoff, J. J. A Facile Conversion of Amino Acids to Guanidino Acids. *Synthesis* **1986**, *9*, 777−779.

(11) For example, compare these IC_{50} values for the inhibition of ADP-induced aggregation of canine platelets with those given in Table 2. Compound **17**, 52 nM.; compound **21**, 60 nM.; compound **23**, 102 nM.

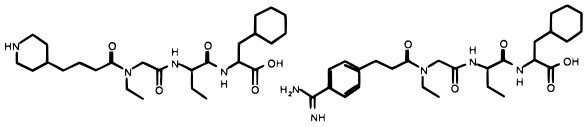
(12) Klein, S. I.; Molino, B. F.; Chu, V.; Ruggeri, Z.; Czekaj, M.; Gardner, C. J.; Newman, J.; Barrett, J. A. Novel RGD Analogues as Antithrombotic Agents. In *Peptides 1990, Proceedings of the Twenty-first European Peptide Symposium*; Giralt, E., Andreu, D., Eds.; ESCOM Science Publishers: Leiden, 1991; pp 374−375.

(13) Folts, J. D. An In-Vivo Model of Experimental Arterial Stenosis, Intimal Damage, and Periodic Thrombosis. *Circulation* **1991**, *83*, IV-3−IV-14.

(14) Klein, S. I.; Molino, B. F.; Czekaj, M.; Gardner, C. J. Antithrombotic Peptides and Pseudopeptides. U.S. Patent 5,051,405 1991.

(15) Hartman, G. D.; Egbertson, M. S.; Halczenko, W.; Laswell, W. L.; Duggan, M. E.; Smith, R. L.; Naylor, A. M.; Manno, P. D.; Lynch, R, J.; Zhang, G.; Chang, C.; Gould, R. J. Non-Peptide Fibrinogen Receptor Antagonists. 1. Discovery and Design of Exosite inhibitors. *J. Med. Chem.* **1992**, *35*, 4640−4642.

(16) For example, compare the following two compounds, which are equipotent in vitro:



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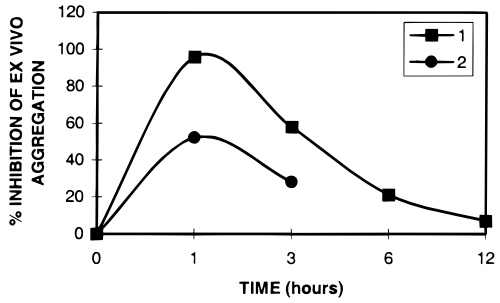

(17) Ruoslahti, R. Integrins. *J. Clin. Invest.* **1991**, *87*, 1−5.

(18) Van Mourik, J. A.; Von Dem Borne, A. E. G.; Giltay, J. G. Pathophysiological Significance of Integrin Expression by Vascular Endothelial Cells. *Biochem. Pharm.* **1990**, *39*, 233−239.

(19) (a) Marguerie, G. A.; Plow, E. F.; Edington, T. S. Human Platelets Possess an Inducible and Saturable Receptor Specific for Fibrinogen. *J. Biol. Chem.* **1979**, *254*, 5357−5363. (b) Ruggeri, Z. M.; DeMarco, L.; Gatti, L.; Bades, R.; Montgomery, R. R. Platelets Have More Than One Binding Site for Von Willebrand Factor. *J. Clin. Invest.* **1983**, *72*, 1−12.

(20) Born, G. V. R. Aggregation of Blood Platelets by Adenosine Diphosphate and its Reversal. *Nature* **1962**, *194*, 927−929.

(21) Plow, E. F.; Pierschbacher, M. D.; Ruoslahti, E.; Marguerie, G. A.; Ginsberg, M. H. The Effect of Arg-Gly-Asp Containing Peptides on Fibrinogen and Von-Willebrand Binding to Platelets. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8057−8061.

(22) (a) Hawiger, J.; Timmons, D. In *Methods In Enzymology*; Hawiger, J., Ed.; Academic Press: New York, 1992; pp 228−243. (b) Fraker, P. J.; Speck, J. C. Protein and Cell Membrane Iodinations with a Sparingly Soluble Chloroamide, 1,3,4,6-Tetrachloro-3a,6a-diphenylglycoluril. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 849−857.

(23) Ruoslahti, E.; Engvall, E. Two Active Sites with Different Characters in Fibronectin. *FEBS Lett.* **1979**, *97*, 221−224.

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