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

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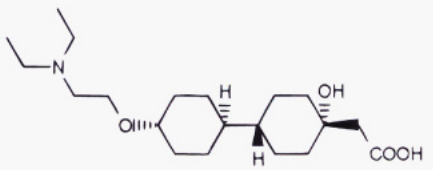
The binding of fibrinogen to its platelet receptor GpIIb/IIIa ($\alpha IIb/\beta_3$), is an essential step in the process of platelet aggregation, regardless of which agonist is involved in initiating platelet activation.[1] An agent which prevented fibrinogen binding to GpIIb/IIIa would therefore be expected to be a "broad-spectrum" antithrombotic agent, with potential advantages over agents such as aspirin, which only interfere with the actions of a single activating agonist, for example thromboxane A_2.

Platelet GpIIb/IIIa antagonists described to date fall into three classes: monoclonal antibodies which bind to GpIIb/IIIa;[2-4] peptides containing the recognition sequence arginine-glycine-aspartic acid[5-8] (RGD); and more recently, several series of non-peptide antagonists which mimic the RGD sequence.[9-11] Representatives of all three classes have been shown to inhibit thrombus formation in animal models.[2-4,12,13] In this communication we report preliminary results from our own quest for a potent, orally active non-peptide GpIIb/IIIa antagonist.

Searching of our in-house compound database for compounds containing an acetic acid moiety and a remote basic center, which might mimic the acidic and basic side chains of aspartic acid and arginine, respectively, led to the identification of the weakly active bicyclohexyl derivative **1**. We now report the development of this lead into a series of highly potent, selective, and long-acting non-peptide GpIIb/IIIa antagonists.



1

Discussion. In earlier studies with conformationally constrained cyclic RGD containing peptides,[14a] we concluded that the most active GpIIb/IIIa antagonists were those containing the RGD tripeptide in an extended conformation. Overlay of **1** with the extended RGD sequence of the cyclic peptide GR83895[14b] (Figure 1) with the carboxyl group of **1** overlaying the carboxyl of the aspartic acid side chain, indicated that the nitrogen atom of **1** could only be superimposed on the ϵ-nitrogen of the arginine guanidinyl group (not on the terminal nitrogens), suggesting that the amine to acid distance in **1** was too short. This analysis was supported by the 20-fold potency enhancement resulting from replacement of the diethylamino group of **1** by a guanidinyl group (entries **1** and **2**, Table 1). Subsequent replacements for the guanidinyl group were designed to retain the acid–base distance derived from our peptide model and the bidentate nature of the basic group, both features which we felt were more important for potency. The isothiourea moiety, for example, gave enhanced potency as predicted from our earlier peptide work.[15] In addition, compound **3** possessed a longer duration of action, as determined by inhibition of *ex vivo* platelet aggregation in our marmoset model (87 min at 5 mg/kg iv, see note *b*, Table 1), than any of our linear peptides.[15] It was, however, orally inactive. The importance of both the geometry of the hydroxycyclohexane ring and the presence of the hydroxyl group of **3** was demonstrated by the weak activity of the isomer **4** and the deshydroxy compound **5**. Replacement of the other cyclohexane ring of **3** with phenyl gave **6**, which retained a similar biological profile and provided a synthetically more accessible starting point for further analogues.

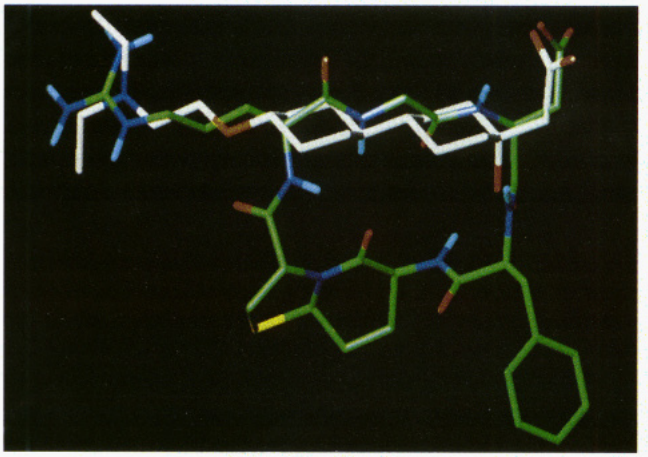
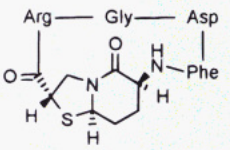


Figure 1. Overlay of the bicyclohexyl lead **1** with the RGD sequence of GR83895.



GR83895

Since we felt that a more conformationally restricted framework might provide greater potency, we replaced the flexible isothiourea chain of **6** with the more rigid benzamidine moiety, previously disclosed as an alternative mimic of the arginine side chain.[17] The resulting 33-fold increase in potency in the biphenyl amidine **7** (Table 2) was accompanied by a marked improvement in duration of action in the marmoset (5.2 h at 0.5 mg/kg iv). A similar result was obtained with the cyclohexene **8** (duration of action of 6.7 h at 1 mg/kg iv in

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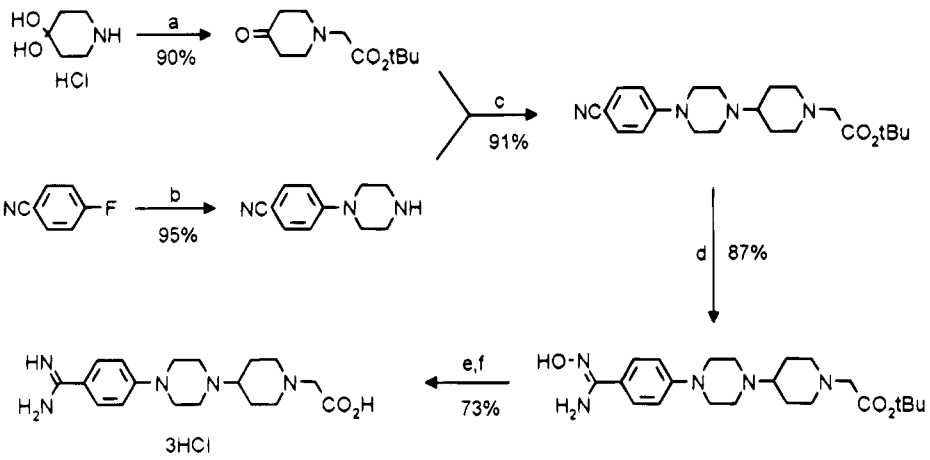
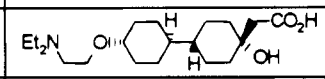
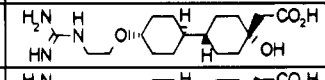
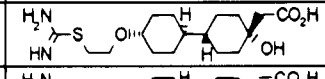
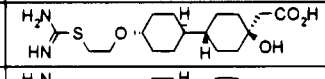
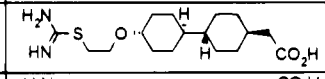
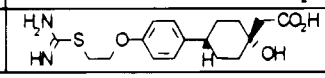
Scheme 1[a]



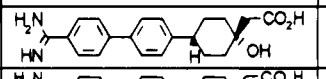
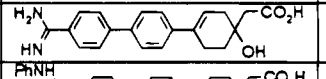
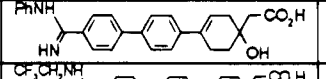
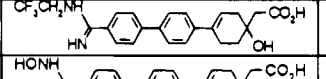
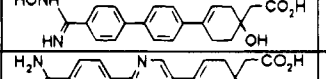
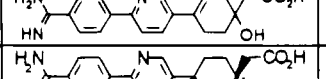
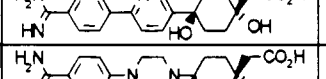
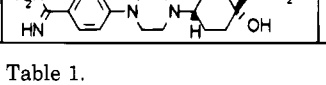
[a] (a) $ClCH_2CO_2tBu$, K_2CO_3, MeCN, reflux; (b) piperazine (5 equiv), K_2CO_3, toluene, reflux; (c) $NaBH(OAc)_3$, AcOH (1 equiv), CH_2Cl_2, room temperature; (d) $NH_2OH{\cdot}HCl$, KOtBu, MeOH, 45–50 °C; (e) H_2/Pd–C, AcOH, Ac_2O (1.5 equiv); (f) 5 N HCl.

Table 1. Optimization of the Amino Terminus

Compound	Structure	IC_{50}(nM)[a]
1		54,000
2		2,600
3		360[b]
4		25,000
5		>100,000
6		430

[a] Inhibition of ADP (10 μM)-induced aggregation in human gel-filtered platelets (GFP) containing fibrinogen (500 μg/mL) and aspirin (100 μM). IC_{50} = compound concentration producing 50% inhibition of aggregation.[16] IC_{50} values are expressed as the mean of two determinations (maximum 3-fold variation in each experiment). [b] Duration of action at 5 mg/kg iv in the marmoset was 87 min. Inhibition of ADP (10 μM)-induced aggregation measured in samples of whole blood taken before (control) and at intervals after iv or po dosing in the marmoset.[15] Duration of action is the time taken for the 10 μM ADP response to return to 50% of its predose (control) value.

Table 2. Amidine Modifications and Ring System Variations

Compound	Structure	IC_{50} (nM)[a]
7		13
8		55
9		680
10		210
11		7400
12		20
13		140
14		72

[a] See note a, Table 1.

the marmoset); however, both **7** and **8** were orally inactive. Having achieved this level of potency and duration of action, we next addressed the issue of oral activity.

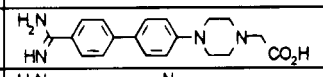
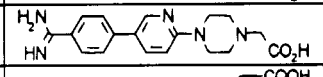
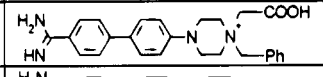
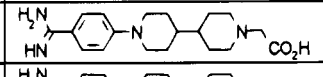
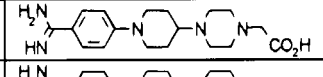
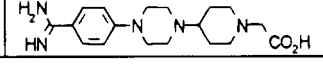
As a consequence of the long duration of action of **8**, we assumed that its lack of oral activity was due to poor absorption. Features of this molecule which we considered to contribute to its lack of oral absorption included the high basicity (pK_a 11.6)[18] of the benzamidine moiety and its poor aqueous solubility (0.004 mg/mL). Less basic amidine replacements such as phenylamidine **9** (pK_a 8.9), trifluoroethylamidine **10** (pK_a 8.0), and hydroxyamidine **11**[19] (pK_a 5.1) (Table 2) resulted in substantial reductions in *in vitro* potency.

Although introduction of the pyridine ring in **12** failed to improve its solubility over **8**, addition of an extra hydroxy group to the cyclohexyl ring gave the highly water soluble **13**. This compound showed some oral activity (duration 1 h at 10 mg/kg in the marmoset), supporting the importance of good aqueous solubility for oral absorption in this series. In an alternative approach to improving solubility, replacement of the central phenyl ring of **7** with piperazine gave **14**, which possessed sustained oral activity (duration >8 h at 5 mg/kg).

Viewing the hydroxyl group of **7** as a hydrogen bond donor, we hypothesized that a similar hydrogen bonding interaction might be provided by a protonated nitrogen atom of a piperazine or a piperidine. We therefore prepared the biphenylylpiperazine **15** and the pyridyl analogue **16** (Table 3), which in addition to maintaining excellent potency avoided the problem of geometrical isomerism. However, the potency of the quaternary salt **17**, which lacked a similar hydrogen bonding capability β to the carboxylic acid, suggested either that the original hydrogen-bonding hypothesis was incorrect or that an alternative mode of receptor binding may be available for such quaternary compounds.

Introduction of a further piperazine or piperidine ring to replace the central phenyl ring of **15** gave compounds **18**–**20**. Although the bipiperidine **18** retained good *in vitro* potency, the activity of the piperidyl piperazine **19**

Table 3. Piperazine and Piperidineacetic Acids

Compound	Structure	IC_{50}(nM)[a]
15		23
16		14
17		31
18		32
19		500
20		37

[a] See note a, Table 1.

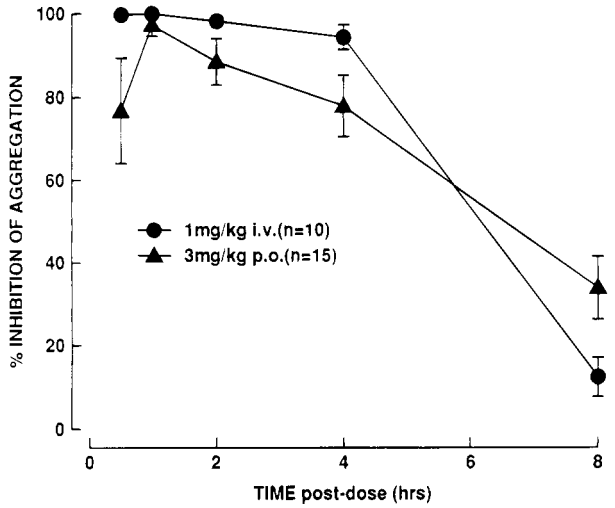


Figure 2. A comparison of inhibition of ADP (10 μM)-induced platelet aggregation by **20** *ex vivo* in the conscious marmoset following either intravenous or oral administration. (See note b), Table 1. The data are expressed as the arithmetic mean (± SEM) of n determinations.)

was poor. Potency was regained in the highly water soluble (>30 mg/mL) piperazine–piperidine **20** (GR144053). In addition to its *in vitro* potency, this compound possessed an excellent profile in the marmoset, with a duration of action of 6.6 h after iv administration at 1 mg/kg and 5.7 h after oral administration at 3 mg/kg (Figure 2). An even longer duration of action was observed in studies in the cynomolgus monkey (12 h at 1 mg/kg iv and 8 h at 3 mg/kg *po*). The synthesis of **20** is shown in Scheme 1.

From the compounds described herein, **20** was chosen for more detailed evaluation[20–22] owing to its excellent potency and duration of action after iv or oral administration and ease of synthesis. As described elsewhere,[22] **20** is highly effective in inhibiting thrombus formation in a canine model of coronary thrombosis.

In conclusion, initial modifications of the weak bicyclohexyl lead **1**, including replacement of the basic side chain with the benzamidine moiety, gave the highly potent, but orally inactive, GpIIb/IIIa antagonist **7**. Oral activity was achieved by introduction of a central piperazine ring to give compound **14**, possibly as a result of improved solubility. Both potency and oral activity were maintained when the hydroxycyclohexane ring of **14** was replaced by piperidine to give the synthetically more accessible compound **20**. **20** has been shown to be a highly effective antithrombotic agent, and its pharmacological profile will be reported in detail elsewhere.

Supplementary Material Available: Proton NMR data for **2**, **20**, and Scheme 1 intermediates, experimental details for Scheme 1, and a Figure illustrating activity of GR144053 in a canine model of coronary thrombosis. (5 pages). Ordering information is given on any current masthead page.

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