Potent Non-peptide Fibrinogen Receptor Antagonists Which Present an Alternative Pharmacophore[1]

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The cross-linking of the dimeric plasma protein fibrinogen between glycoprotein IIb/IIIa (GPIIb/IIIa) receptor complexes[2,3] on adjacent activated platelets is the final step in platelet aggregation leading to platelet-mediated thrombus formation.[4,5] By inhibiting platelet aggregation and thrombus formation, GPIIb/IIIa receptor antagonists may be useful for the treatment and prevention of acute myocardial infarction, unstable angina, or thrombotic stroke.[6,7]

Small proteins and peptides containing the sequence Arg-Gly-Asp (RGD) have been shown to be antagonists of the fibrinogen receptor and are particularly effective when the RGD sequence is conformationally constrained.[8–11] On the basis of the conformational and compositional features of the constrained RGD-containing cyclic semipeptide **2**,[8,12–14] we previously reported the design and synthesis of the highly potent non-peptide fibrinogen receptor antagonist **1**,[1] which resulted from an 'atom-for-atom' overlay with the conformational model derived for **2**.[12]

In compound **1**, the arginine mimetic[15] side chain is attached to the benzodiazepine nucleus at the 8 position. As part of our research in this area, we also prepared an isomer of **1** in which the arginine mimetic is attached to the 7 position of the benzodiazepine. This compound proved to be a surprisingly potent antagonist of the platelet fibrinogen receptor. In this communication, we describe the synthesis and biological activity of compound **3** (Figure 1) and discuss its activity in light of the receptor-bound conformational model we proposed for RGD antagonists based on the cyclic peptide antagonist **2** and the non-peptide antagonist **1**.[1]

The synthesis of the 7-carboxy-3-oxo-1,4-benzodiazepine analog **3** is illustrated in Scheme 1. Esterification of 3-methyl-4-nitrobenzoic acid (**4**) gave *tert*-butyl 3-methyl-4-nitrobenzoate,[16] which was subjected to benzylic bromination with *N*-bromosuccinimide in refluxing CCl_4 to give the corresponding benzyl bromide. Without purification, the intermediate benzyl bromide was reacted with phenethylamine, and the resulting amine was protected as its *tert*-butyl carbamate. Catalytic reduction of the nitro group gave the 2,4-disubstituted aniline **5** in good overall yield. Michael-type addition of **5** to dimethyl acetylenedicarboxylate[17] followed by catalytic hydrogenation provided the corresponding dimethyl *N*-arylaspartate intermediate. The *N*-*tert*-butyloxycarbonyl and *tert*-butyl ester groups were concurrently removed with trifluoroacetic acid to give the amino diester **6**, which was cyclized in methanol in the presence of sodium methoxide to yield methyl 7-carboxy-3-oxo-1,4-benzodiazepine-2-acetate **7**. The acid chloride formed by reaction of **7** with refluxing thionyl chloride was treated with the Cbz-protected *p*-amidinoaniline **8**[1,18] to give amide **9**. Removal of the Cbz-protecting group was effected by catalytic hydrogenation to afford **10**, which was saponified with 1.0 N sodium hydroxide in methanol to afford racemic 3-oxo-1,4-benzodiazepine-2-acetic acid **3**.

Biological evaluation revealed that **3** has good affinity for the human fibrinogen receptor and good potency in a human platelet aggregation assay (Table 1). When compared to compound **1**, compound **3** exhibits 10-fold lower binding affinity but only 2.5-fold lower antiaggregatory potency. Given the structural rigidity of these compounds, it is not possible to simultaneously superimpose the acid, amidine, and benzodiazepine nucleus of **3** onto those of **1**. These observations suggest it is unlikely that compound **3** binds in the same mode as compound **1**, and thus, the 7-substituted-3-oxo-1,4-benzodiazepine-2-acetic acid nucleus provides a new series of potent fibrinogen receptor antagonists.

In our previous report,[1] we concluded that the potent activity observed with compound **1** supports the receptor-bound conformation of peptide **2** as that predicted in our model. As such, this model, which features the "turn-extended-turn" conformation about RGD, should be predictive for potent antagonists of the fibrinogen receptor. The remarkable biological activity of compound **3**, which does not maintain an atom-for-atom mimicry with peptide **2**, challenges this conclusion and requires that our model be re-examined.

A straightforward hypothesis, which is consistent with the wide structural diversity found in non-peptide fibrinogen receptor antagonists,[19] is that the sole prerequisite for antagonist activity is a molecule which contains basic and acidic residues spaced appropriately. For non-peptides, increased potency can be obtained by adding binding elements which either mimic those found in peptide antagonists or access receptor regions not explored by the peptides.[20] The simplicity of this explanation is appealing, but it is not in total agreement with the cyclic peptide data from which our pharmacophore hypothesis was generated.[1] In cyclic peptide fibrinogen receptor antagonists, the central glycine residue in the RGD sequence cannot be replaced with other amino acids.[21] The data suggest a close contact between the receptor and the glycine Cα carbon which establishes within the receptor a region of excluded volume between the arginine and aspartic acid binding points. An overlay of compounds **1** and **3** onto cyclic peptide **2**, where the basic and acidic moieties are superimposed, places a substantial portion of the benzodiazepine nucleus of **3**, but not of **1**, within this region of excluded volume. Thus, if the binding sites for peptide and non-peptide fibrinogen receptor antagonists significantly overlap, an explanation for activity which only includes appropriately spaced basic and acidic groups does not explain the collective data.

Alternatively, by postulating an enlarged (or second distinct) cationic binding site on the receptor, the activity observed with **3** (as well as most of the other non-peptide fibrinogen receptor antagonists[19]) can be accommodated with a minor modification of our original pharmacophore hypothesis. Simple visual comparison

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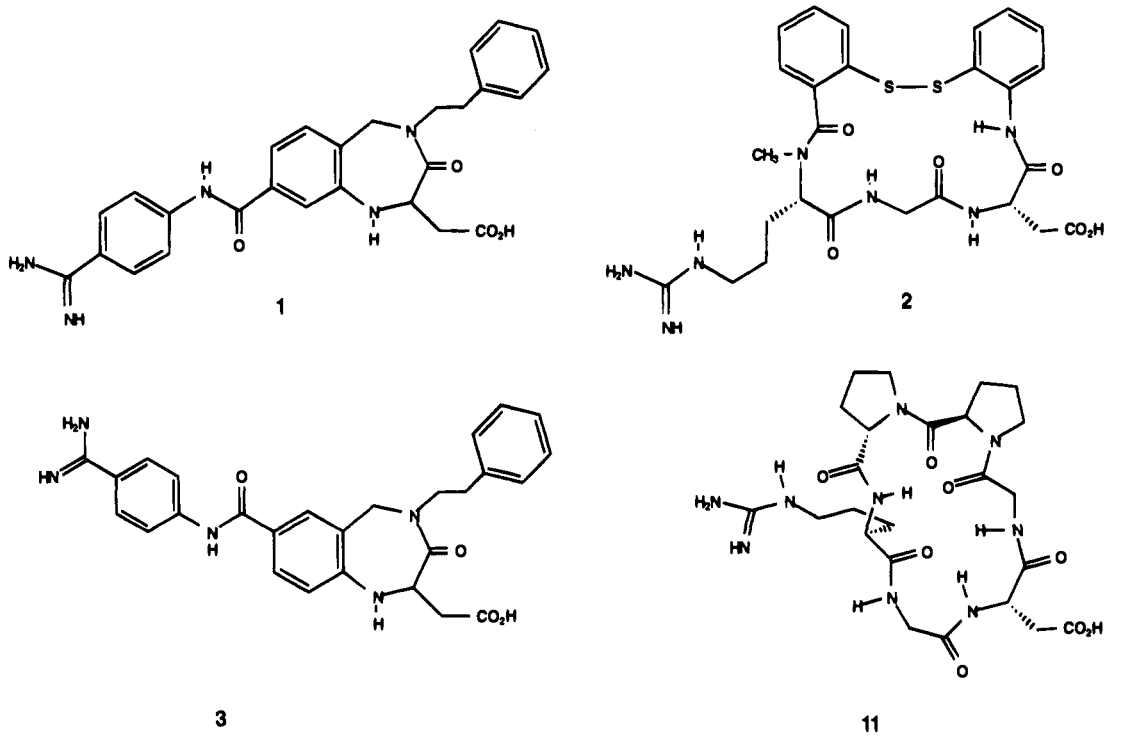
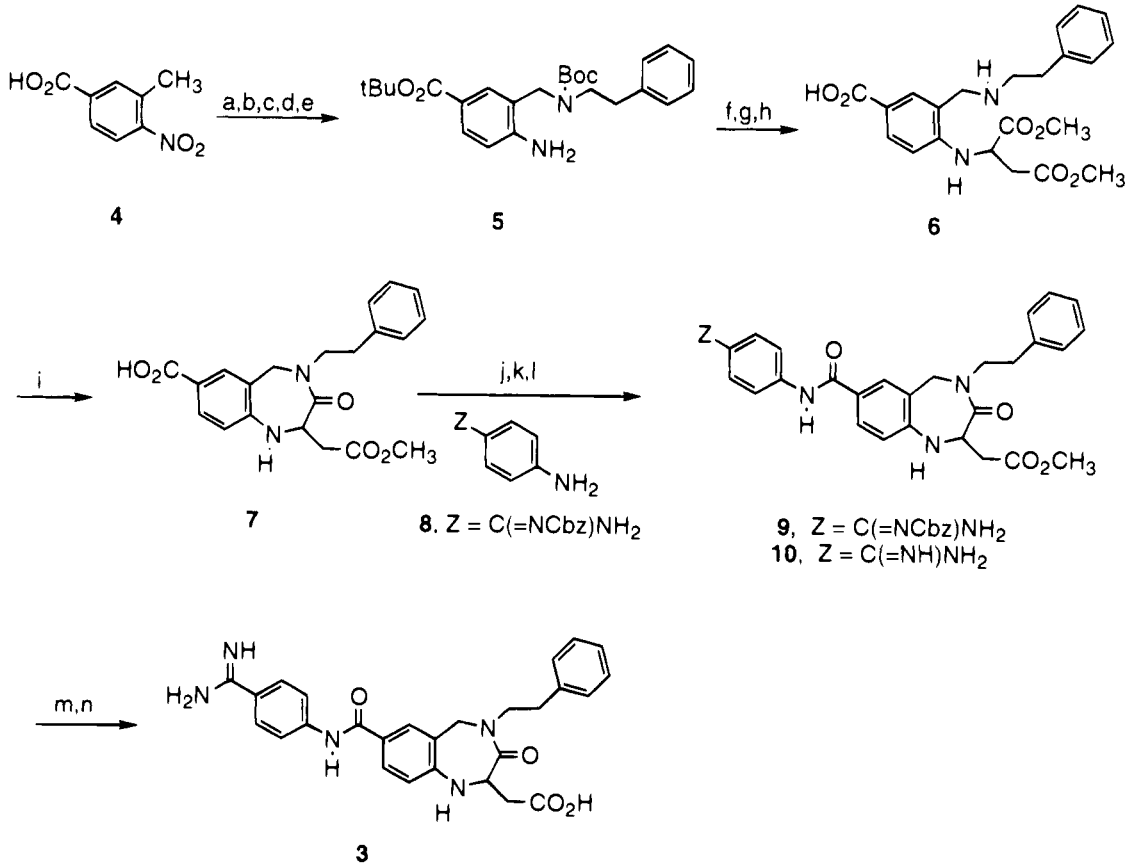


Figure 1.

Scheme 1. Synthesis of 7-(Aminoacyl)-3-oxo-1,4-benzodiazepine 3[a]



[a] (a) $PhSO_2Cl$/tBuOH/py (82%); (b) NBS/$(PhCO_2)_2$/CCl_4; (c) $Ph(CH_2)_2NH_2$/$NaHCO_3$/THF (40%); (d) $(Boc)_2O$/$CHCl_3$ (100%); (e) H_2/Pd/C/EtOAc (91%); (f) $(CCO_2CH_3)_2$/MeOH; (g) H_2/10% Pd/C (94%); (h) TFA/CH_2Cl_2; (i) $NaOCH_3$/MeOH (87%); (j) $SOCl_2$/reflux; (k) py/CH_2Cl_2 (70%); (l) H_2/Pd/C/TFA/EtOAc/MeOH; (m) 1 N NaOH/CH_3OH; (n) HPLC/TFA (50%).

of **3** with **1** (with the benzodiazepine nuclei overlaid) reveals that **3** does not position the cationic phenylamidine group in the same space as is accomplished in **1**. While our original studies with peptide **2** did not define the orientation of the arginine side chain, the greater constraint inherent in the (*p*-amidophenyl)amidine group in **1** compared to the propylguanidine group in **2** suggested that the basic residue in **1** more

Table 1. IC_{50} and K_i Values of Cyclic Peptide Ligands and Their Mimetics[a]

no.	compound	antiaggregatory activity IC_{50} (μM)[b]	binding inhibition, GPIIb/IIIa K_i (μM)[c]
1		0.15 ± 0.04[d]	0.0023 ± 0.0011[d]
2	cyclo(Mba-(N$^{\alpha}$-Me)Arg-Gly-Asp-Man)	0.09 ± 0.02[e]	0.002 ± 0.0001[e]
3		0.38 ± 0.02	0.026 ± 0.0020
11	cyclo(Pro-Arg-Gly-Asp-Gly-D-Pro)	5.26 ± 1.63[f]	1.9 ± 0.4[f]

[a] All compounds gave satisfactory ^{1}H NMR, mass spectra, and C,H,N microanalyses. [b] Inhibition of platelet aggregation in human platelet-rich plasma induced by ADP; see ref 14. [c] Inhibition of [^{3}H]SK&F 107260 binding to purified GPIIb/IIIa isolated from human platelets and reconstituted in liposomes; see ref 26. [d] Data from ref 1. [e] Data from ref 13. [f] Data from ref 24.

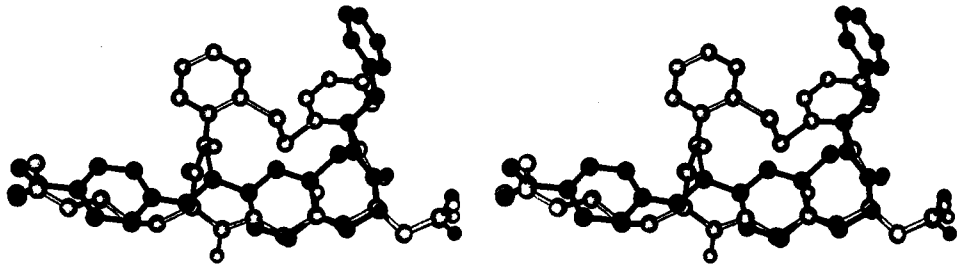


Figure 2. Stereoplot of non-peptide **3** overlaid on peptide **2** to maximize the correspondence of the benzodiazepine and Gly-Asp regions, respectively. The arginine side chain in **2** is fit to the position of the amidine group in **3**.

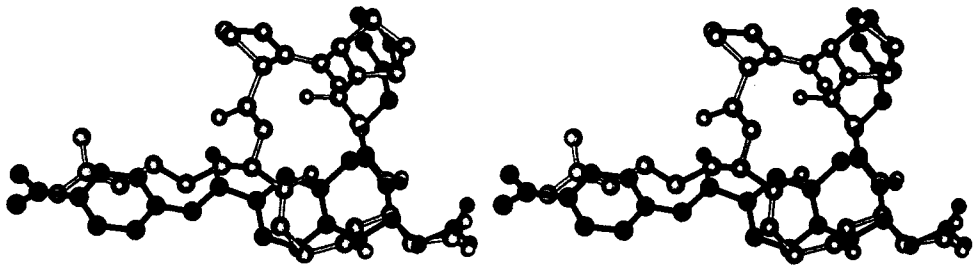


Figure 3. Stereoplot of non-peptide **3** overlaid on peptide **11**. The backbone contour of the RGD region of **11** resembles the corresponding region of **3**, yielding a similar spatial arrangement of the basic and acidic moieties in these molecules.

closely defined the optimal orientation of the cationic group in our pharmacophore model.[1] The relatively high affinity of **3** for the GPIIb/IIIa receptor suggests that there are additional regions in space where the cationic group may be positioned which give rise to an acceptable receptor interaction. This is visualized in Figure 2 where peptide **2** and non-peptide **3** are overlaid in the original Gly-Asp/benzodiazepine orientation[1] and the arginine side chain in **2** is rotated to best fit the amidino moiety in **3**.[22]

Additional support for an expanded or second cation binding site comes from studies of the cyclic peptide cyclo(Pro-Arg-Gly-Asp-Gly-D-Pro), **11**, a conformationally constrained but less active antagonist relative to cyclic peptide **2**.[23] Previously, we had observed that cyclic peptides which present the RGD sequence in either a turn-extended-turn conformation as found in **2** or in a Gly-Asp β turn as found in **11** display good activity, with examples of the former being better than those of the latter conformational type. Overlays of low-energy, ^{1}H NMR-derived conformations of molecules representing each conformational type reveal a remarkable similarity in the conformations of the Gly-Asp region but a distinct difference in the placement of the arginine residue.[24] It is intriguing that the disposition of the benzamidine and acetic acid side chains in compound **3** closely resembles respectively the arginine and aspartic acid side chains in **11** (Figure 3).[22,25]

In summary, 7-substituted-3-oxo-1,4-benzodiazepine-2-acetic acid analog **3** represents a novel non-peptide fibrinogen receptor antagonist. While this molecule appears to be chemically similar to the 8-substituted-3-oxo-1,4-benzodiazepine-2-acetic acid **1**, three-dimensional comparisons of these molecules with the highly active cyclic peptide **2** suggest compound **3** may be accessing a different portion of the cationic binding site of the receptor than **1** and **2**. Further studies are underway to better define the pharmacophore differences between these two novel families of fibrinogen receptor antagonists and to provide additional evidence for the existence of a second cation binding site.

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