Direct Design of a Potent Non-Peptide Fibrinogen Receptor Antagonist Based on the Structure and Conformation of a Highly Constrained Cyclic RGD Peptide

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In the absence of structural data or good models for the receptor, success in mimicking natural peptide ligands with potent non-peptides has been elusive, in part because of uncertain identification of the ligands' bioactive conformations. Recently, a glucose-based agonist of the somatostatin receptor[1] and a steroid-based antagonist of the fibrinogen receptor[2] were designed from presumed bioactive conformations of the peptide ligands, somatostatin and Arg-Gly-Asp (RGD), respectively. Both of these peptidomimetic molecules have activity, but at levels 3 orders of magnitude weaker than those of the peptide ligands mimicked. Because of the inherent conformational mobility of peptides, the accessibility of multiple low-energy conformations, and the potential for induced fit (e.g., FK506[3,4] and cyclosporin A[5,6]), an understanding of receptor-bound conformations and thus a clearer starting point for mimetic design may be acquired through examination of peptide ligands which are highly constrained conformationally. We report here the design, synthesis, and biological activity of benzodiazepine **1**[7] (Figure 1). This compound was designed from the constrained RGD-containing cyclic peptide **2**[8] with consideration of both 2- and 3-dimensional molecular features. Compound **1** maintains the low nanomolar affinity and activity of cyclic peptide **2**, a potent antagonist of the platelet GPIIb/IIIa receptor. We believe this to be the first example of a highly potent peptidomimetic designed directly from a peptide conformational model without the need for a traditional medicinal chemical effort to optimize potency.[9]

Our interest in GPIIb/IIIa antagonists derives from their potential use as antithrombotic agents. The plasma protein fibrinogen plays a critical role in the cross-linking of activated platelets which contributes to thrombus formation. The platelet

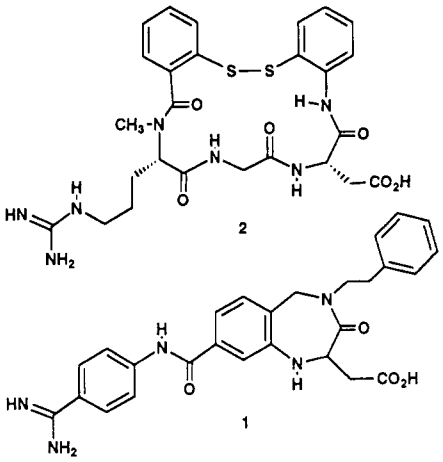

Figure 1.

receptor for fibrinogen is the glycoprotein complex GPIIb/IIIa,[10,11] a member of the integrin superfamily of adhesive protein receptors, containing a recognition site for the tripeptide sequence RGD in the fibrinogen α-chain.[12] 2-Mercaptobenzoyl-(N^{α}-methyl)-Arg-Gly-Asp-2-mercaptoanilide cyclic disulfide (**2**) is a cyclic peptide that displays high affinity for the fibrinogen receptor and considerable potency in inhibiting platelet aggregation induced by ADP in human platelet-rich plasma.[8,13]

The relative conformational rigidity of small cyclic peptides such as **2** allowed us to use ^{1}H NMR experiments to study its conformational preferences. Preliminary results indicated the presence of a C_7 turn around Asp. To test this feature, a γ-turn mimetic, which fixed the C_7 turn at Asp, was constructed and incorporated into two semipeptides. These molecules maintained both significant affinity and antiaggregatory activity, supporting the turn hypothesis.[14] Complete analysis of the ^{1}H NMR data for **2** revealed the RGD sequence to be characterized by a turn at Arg, extended ϕ, ψ angles at Gly, and a turn at Asp,[15] shown schematically in Figure 1. Two components in unequal distribution are visible in the ^{1}H NMR spectra of solutions in methanol at 203 K. Conformations calculated from nuclear Overhauser data for these components differ only in the amide plane rotation of the Asp-2-mercaptoanilide amide bond. In the major conformation, the amide plane rotation affords a C_7 turn about Asp. An X-ray crystal structure was also determined for **2**.[15] This structure affirms the "turn-extended-turn" conformation about RGD, as described for both the major and minor ^{1}H NMR-derived conformers; however, the crystal conformation matches that of the minor component in solution. Although either conformer is

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(9) Genentech scientists have disclosed, in oral presentations, the development of potent non-peptide GPIIb/IIIa antagonists using a process with similarities to that described in this manuscript. Using constrained peptides, they defined, with NMR data, a model of the peptide antagonist pharmacophore. From this model, they designed non-peptide mimetics; the first group of nonpeptides designed were virtually inactive. However, an interactive chemistry/modeling process on these initial compounds ultimately resulted in optimized structures, benzodiazepinediones, which have similar biological activity to **1**. Cf: Blackburn, B. K.; McDowell, R. S. *J. Cell Biochem.* **1993**, *Suppl. 17C*, 205. Blackburn, B.; Barker, P.; Gadek, T.; McDowell, R.; McGee, L.; Somers, T.; Webb, R.; Robarge, K. *International Patent Application*, WO 93/08174, April 29, 1993.

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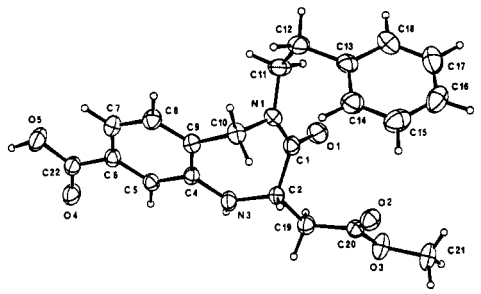
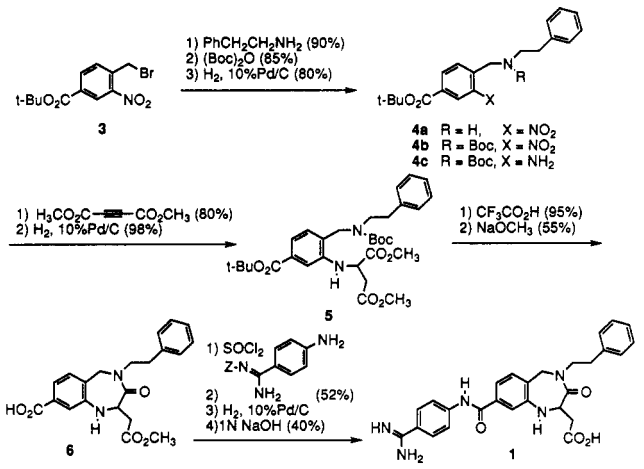


Figure 2.

Scheme I



potentially a bioactive one, we chose the major conformer calculated from the ^{1}H NMR spectra as the basis on which to design a non-peptide ligand.

The design process leading to the non-peptide antagonist **1** focused on mimicking both compositional and conformational features of the cyclic peptide. The Arg and Asp side chains or their equivalents were the critical compositional elements as determined from peptide structure/activity relationships.[8,13] In addition, a lipophilic group following Asp appeared to increase activity.[8,16] We felt that two particular conformational features of the peptide backbone, the C_7 turn at Asp and the extended Gly residue, were critical to the 3-dimensional placement of the Arg and Asp side chains, and therefore these features were maintained in the non-peptide. The 1,4-benzodiazepine nucleus offered the potential to mimic both the C_7 turn and the extended Gly in a manner that restricted conformational flexibility. Modeling indicated a substituent at the 2 position of the 1,4-benzodiazepine, which is equivalent to Asp Cα, was likely to adopt the equatorial orientation observed in the peptide. To establish both the distance and directionality necessary to maintain the Arg-to-Asp spatial relationship in the cyclic peptide, an amide group was added at position 8 of the 1,4-benzodiazepine. This group potentially provides a mimic of the corresponding Arg carbonyl. Taken together, the diazepine, the aromatic, and amide pieces of **1** represent a small molecule template that mimics the Arg–Gly–Asp backbone region of **2**. Appropriate placement of the amidinophenyl group (as an Arg side-chain equivalent[17,18]), the carboxylic acid, and the lipophilic phenethyl group completed the design.

Compound **1** was prepared in racemic form[19] from *tert*-butyl 4-(bromomethyl)-3-nitrobenzoate **3**[20] (Scheme I). Compound **3** was alkylated with phenethylamine to give benzylamine **4a**, which after protection of the amine and reduction of the nitro group gave **4c**. Michael addition of **4c** to dimethyl acetylenedicarboxylate,[21] followed by catalytic hydrogenation, yielded the dimethyl *N*-arylaspartate derivative **5**. The (*tert*-butyloxy)carbonyl group was removed with trifluoroacetic acid to give the amino ester, which was cyclized in methanol in the presence of sodium methoxide to afford benzodiazepine **6**. An X-ray structure was determined for compound **6**[22] (Figure 2) to verify that the benzodiazepine structure could mimic conformationally the Gly-Asp region of **2**.[23] The acid **6** was converted to the corresponding acid chloride in refluxing thionyl chloride, which was coupled with the Cbz-protected *p*-amidinoaniline[7] to yield the methyl ester precursor. Removal of the Cbz protecting group by catalytic hydrogenation, followed by ester hydrolysis with 1 N sodium hydroxide in methanol, produced peptidomimetic **1**.

Both high affinity for the GPIIb/IIIa receptor and potent antiaggregatory activity are exhibited by compound **1**. In competitive binding assays employing either tritiated **2**[24] or biotinylated fibrinogen[25] as the displaced ligand, **1** displays a K_i of 2.32 ± 0.11 or 1.25 ± 0.06 nM, respectively. The affinity of this molecule is comparable to that of peptide **2**, which exhibits K_is of 2.08 ± 0.10 (tritiated **2** as the ligand) and 0.62 ± 0.04 nM (biotinylated fibrinogen as the ligand).[8,16] Compound **1** also inhibits platelet aggregation induced by ADP in human platelet-rich plasma with an IC_{50} of 150 ± 40 nM. Compounds **1** and **2** (IC_{50} = 57 ± 11 nM[8,16]) are both potent inhibitors in this assay, which assesses functional inhibition of the platelet cross-linking process.

In summary, compound **1** is a high-affinity, potent, non-peptide GPIIb/IIIa antagonist designed directly from the conformational and compositional features of the constrained cyclic peptide **2**. To our knowledge, this peptidomimetic process represents a unique approach to the generation of a *high-affinity*, low-molecular-weight antagonist in that the design employs both the 2- and 3-dimensional features of a high-affinity peptide ligand. The extent to which the activity profile of the mimetic matches that of the cyclic peptide suggests that the dominant solution conformation of the latter reflects the receptor-bound conformation. We thank C. Kwon, D. Takata, T. O. Yellin, and S. Ross for chemical support; P. Koster, D. Powers, J. Stadel, J. Vasko, and A. Wong for assistance with biological assays; and M. Huddleston and E. Reich for mass spectral and elemental analyses of **1**.

Supplementary Material Available: Tables of crystal data and methods of data collection, fractional atomic coordinates; bond distances and angles, and anisotropic displacement parameters for **6**; listing of orthogonal coordinates for the ^{1}H NMR-derived conformation of **2** (13 pages). Ordering information is given on any current masthead page.

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(22) The intermediate **6** crystallized from aqueous ethanol in the monoclinic space group $P2_1/c$ with four formula units in a cell of dimensions a = 13.015(2), b = 11.461(1), and c = 12.907(1) Å, β = 105.26(4)°, V = 1857.7(3) Å^3. The structure was discovered by direct methods from 2765 unique, absorption-corrected X-ray diffraction data collected with copper radiation at 223 K on a crystal 0.3 × 0.2 × 0.15 mm on edge. Refinement (on F) of 320 variables by standard least-squares methods using 1827 observations ($I \geq 3\ \sigma(I)$) led to standard crystallographic residuals R = 0.034, R_w = 0.039, and S = 1.322. Additional details of the crystallographic experiment and metrical results, including fractional atomic coordinates, have been deposited with the Cambridge Crystallographic Data Centre.
(23) Values for the following torsion angles for the *S* enantiomer of **6** when compared to the corresponding ones in **2** (listed in parentheses) illustrate the conformational similarity of the two molecules: C6–C5, 178 (175); C5–C4, −179 (−160); C4–N3, 173 (177); N3–C2, −50 (−86); C2–C1, 73 (79); C1–N1, −6 (−14).
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