Communications to the Editor

Design and Synthesis of a C_7 Mimetic for the Predicted γ-Turn Conformation Found in Several Constrained RGD Antagonists[1]

James F. Callahan,*,† John W. Bean,‡
Joelle L. Burgess,† Drake S. Eggleston,‡
Shing Mei Hwang,§ Kenneth D. Kopple,‡
Paul F. Koster,∥ Andrew Nichols,∥
Catherine E. Peishoff,‡ James M. Samanen,†
Janice A. Vasko,∥ Angela Wong,§ and
William F. Huffman†

Departments of Peptidomimetic Research, Physical and Structural Chemistry, Pharmacology, and Cellular Biochemistry
SmithKline Beecham Pharmaceuticals
P.O. Box 1539
King of Prussia, Pennsylvania 19406

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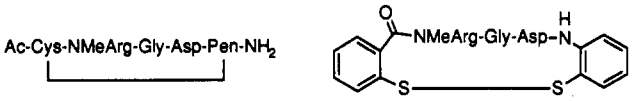
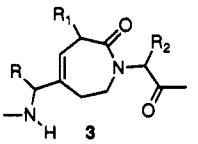
Synthetic peptides containing the Arg-Gly-Asp (RGD) sequence inhibit binding of fibrinogen to its receptor (GP-IIb/IIIa) thereby inhibiting platelet aggregation and thrombus formation.[2] Previous reports from these laboratories have outlined the development of peptide fibrinogen receptor antagonists culminating in the synthesis of two cyclic disulfide analogues, 1[3] and 2.[4] In order to more thoroughly define an antagonist pharmacophore for the GPIIb/IIIa receptor, these constrained cyclic peptides have been studied extensively by ^{1}H NMR and molecular modeling.[5] The ^{1}H NMR spectra of 2 at low temperature show two slowly exchanging conformations. The Arg-Gly-Asp region of the major form is characterized by an extended glycine residue and a C_7 conformation[6] at aspartic acid, while the minor component exhibits a similar conformation at glycine and a conformation at aspartic acid that is near α_R, similar to that found in a type I β-turn.[5] The most probable conformation of the minor form of 2 closely agrees with a structure determined by X-ray crystallography.[5] The ^{1}H NMR data also suggest that the dominant conformation of the Arg-Gly-Asp region in 1 is similar to that found in the major component of 2.[5] In order to differentiate the two conformations about the aspartic acid residue and thereby gain insight into the biologically active conformation of 2, we decided to constrain the γ-turn conformation about the aspartic acid residue with a secondary structure mimetic.[7] Our previous work has shown that the C_7 mimetic 3 locks three residues of a peptide chain into a γ-turn conformation.[8,9] The syntheses of the RGD analogues 4 and 5, incorporating the appropriate C_7 mimetic, were initiated to test the contribution of the γ-turn conformation to the antagonist pharmacophore. Analogues 4 and 5 were chosen as initial targets to evaluate the contribution of the predicted γ-turn conformation on the biologically active conformation without the additional constraint of the cyclic disulfide.



1 SK&F 106760 2 SK&F 107260



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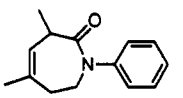

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Entry into the template for the C_7 mimetic ring system 3 is based on the key trisubstituted olefin 7 obtainable in three steps from the commercially available substituted

† Department of Peptidomimetic Research.
‡ Department of Physical and Structural Chemistry.
§ Department of Pharmacology.
∥ Department of Cellular Biochemistry.

(1) This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday.

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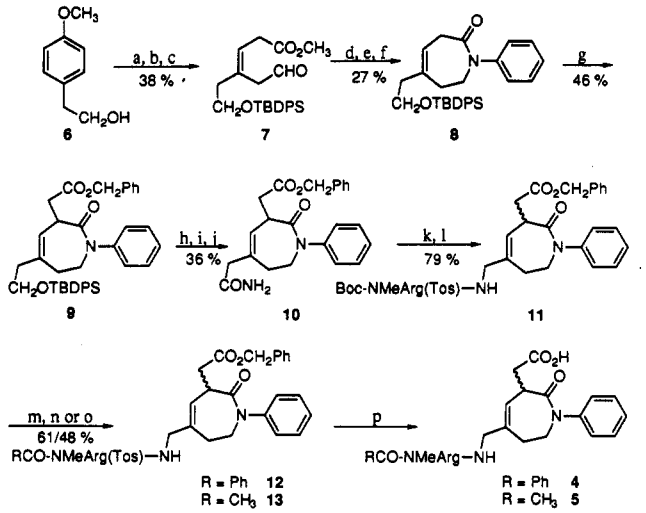
Scheme I. Synthesis of C_7 Mimetic Containing Analogue



(a) Li, NH_3, tBu-OH, −78 °C; EtOH, −78 °C to RT. (b) TBDMS, imidazole, DMF. (c) O_3, $MeOH/CH_2Cl_2$, −78 °C; Me_2S, −78 °C to RT. (d) Aniline, MeOH, $NaBH_3CN$, pH = 6, RT. (e) 1 N NaOH (aqueous), dioxane, RT; 3 N HCl (aqueous). (f) BOP, HOBt, *N*-methylmorpholine, DMF, RT. (g) $LiN(TMS)_2$, THF, −78 °C; $BrCH_2CO_2CH_2Ph$, −78 °C to RT. (h) nBu_4NF, THF, RT. (i) Jones oxidation, acetone, 0 °C. (j) EtO_2CCl, Et_3N, THF, −20 °C, NH_4OH, THF, −20° to RT. (k) $PhI(OCOCF_3)_2$, CH_3CN/H_2O, RT. (l) Boc-NMeArg(Tos)-OH, BOP, HOBt, *N*-methylmorpholine, DMF, RT. (m) 4 N HCl, dioxane, RT. (n) PhCOCl, Et_3N, DMF, RT. (o) AcCl, Et_3N, DMF, RT. (p) HF, 0 °C.

anisole **6** as shown in Scheme I.[8,10] Reductive amination of **7** with aniline followed by hydrolysis and cyclization gave **8** which incorporated the backbone portion of the C_7 mimetic. The central (i + 1) "aspartic acid" side chain was introduced via the alkylation of the anion of **8** with benzyl bromoacetate to give **9**.[11] Selective deprotection of the alcohol in **9** followed by oxidation and aminolysis gave the primary amide **10**. Modified Hofmann rearrangement[12] of **10** followed by BOP coupling[13] to BocN-MeArg(Tos)-OH[3b] gave the fully protected peptide **11**. Selective removal of the N-terminal Boc protection and treatment with benzoyl chloride or acetyl chloride gave **12** and **13**, respectively. Final deprotection of **12** and **13** with anhydrous HF yielded **4** and **5**, respectively, each as an inseparable set of diastereoisomers.[14]

The biological results for the two mimetic containing analogues **4** and **5** are summarized in Table I. The data indicate that both **4** and **5** are potent inhibitors of platelet aggregation in vitro[3b] and retain nanomolar affinity for isolated human GPIIb/IIIa receptor,[3b] showing an antagonist profile similar to that of the cyclic peptide **1**. These results suggest that the γ-turn conformation locked by the mimetic found in **4** is consistent with an antagonist pharmacophore for the GPIIb/IIIa receptor.

Table I. Affinity and Antiaggregatory Activity

compound	antiaggregatory activity canine PRP/ADP:[3b] IC_{50} (μM)[a]	binding inhibition human GPIIb/IIIa:[3b] K_i (μM)[b]
1 (SK&F 106760)	0.36 ● 0.04	0.175 ± 0.025
2 (SK&F 107260)	0.09 ± 0.02	0.004
4	1.11 ± 0.16	0.106 ± 0.007
5	0.72 ± 0.28	0.271 ± 0.003
Ac-Arg-Gly-Asp-Ser-NH_2	91.3 ● 0.10	37.01 ± 0.28

[a] Inhibition of platelet aggregation in canine platelet-rich plasma induced by ADP. [b] Inhibition of [^{3}H]-107260 binding to purified GP-IIb/IIIa isolated from human platelets that were reconstituted in liposomes.

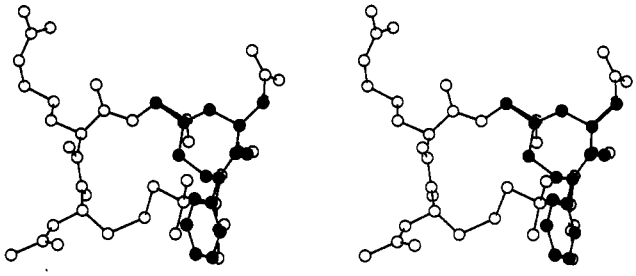


Figure 1. Overlay of mimetic fragment **14** onto the solution conformation of **1**.

In order to examine more closely the overlap of this C_7 mimetic onto the proposed γ-turn region of the cyclic peptides **1** and **2**, the lowest energy conformation of **14**,[15,16] a simplified version of the C_7 mimetic contained in **4** and **5**, was calculated.[17] This conformation of **14** can be readily superimposed on the γ-turn containing solution conformation of the cyclic antagonist **1** (Figure 1). The excellent fit of the mimetic is consistent with the comparable affinity of **4** and **1** for the GPIIb/IIIa receptor. Although mimetic in **4** fits the γ-turn conformation found in **2** equally well, it exhibits a lower affinity for the GPIIb/IIIa receptor. This partial loss of affinity can be rationalized by a variety of other factors. For example, the mimetic containing analogue **4** may lack a conformational constraint or receptor interaction that is present in the antagonist **2**. It is also possible that even though the C_7 mimetic in **4** successfully constrains the backbone of the molecule in a γ-turn conformation, the variation of functional groups inherent in the C_7 mimic design either eliminates an important binding element of the ligand or introduces a new, unfavorable interaction with the receptor. Further work to examine these and other possible explanations are currently being pursued.

In conclusion, we have successfully designed and synthesized a conformational mimetic that replaces the

(10) Compound **7** was isolated as a 80:20 mixture of the desired unsaturated aldehyde and the saturated aldehyde which resulted from overreduction during the initial Birch reaction. The saturated material was separated by chromatography after cyclization to **8**.

(11) Attempts to alkylate the hydroxymethyl-substituted cyclic lactam, which were successful in similar cases, gave only a diene side product formed via the initial acylation of the dianion at oxygen followed by elimination.

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(14) The diastereoisomers of **4** and **5** were identical under several sets of chromatographic conditions. Alkylation with an optically active benzyl ester (iodo (+)-methylbenzylacetate) followed by the same reaction sequence allowed for separation of **11** into two diastereomers. Unfortunately, acylation and final deprotection caused partial racemization at i + 1 in the two diastereoisomers of **5** as determined by ^{1}H NMR analysis.

(15) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel-An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

(16) The lowest energy conformation was calculated using the multiconformer and Batchmin utility found in MacroModel Version 3.1x, Still, W. C. Department of Chemistry, Columbia University, New York, NY 10027. The ϕ_{i+1} and ψ_{i+1} angles of the mimetic **14** were varied at 30° resolution with a closure distance (bond between bridge methylenes) of 1–5 Å. The resulting multiconformer run generated 633 conformations which were then minimized utilizing a MM2 force field in Batchmin.

(17) The calculated low energy conformation of **14** was found to be consistent with the conformation of other similarly substituted C_7 mimetics which have been determined by X-ray crystallography (see refs 8 and 9).

C-terminal region of a RGD antagonist leading to both a high affinity ligand of the GPIIb/IIIa receptor and an potent inhibitor of in vitro platelet aggregation.

Registry No. 1, 126053-71-2; 2, 136620-00-3; 4 (diastereomer 1), 142841-16-5; 4 (diastereomer 2), 142865-90-5; 5 (diastereomer 1), 142841-17-6; 5 (diastereomer 2), 142865-91-6; 6, 702-23-8; 7, 142841-18-7; 8, 142841-19-8; 9, 142841-20-1; 10, 142841-21-2; 11 (diastereomer 1), 142841-22-3; 11 (diastereomer 2), 142841-26-7; 12 (diastereomer 1), 142841-23-4; 12 (diastereomer 2), 142841-27-8; 13 (diastereomer 1), 142841-24-5; 13 (diastereomer 2), 142841-28-9; 14, 142841-25-6; TBDPS-Cl, 58479-61-1; $PhNH_2$, 62-53-3; $BrCH_2CO_2CH_2Ph$, 5437-45-6; Boc-*N*MeArg(Tos)-OH, 108695-16-5; PhCOCl, 98-88-4; AcCl, 75-36-5; Ac-Arg-Gly-Asp-Ser-NH_2, 122207-62-9; H-Arg-Gly-Asp-OH, 99896-85-2.