Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 15, pp. 1835-1840, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00243-6

NON-PEPTIDE FIBRINOGEN RECEPTOR ANTAGONISTS. 3. DESIGN AND DISCOVERY OF A CENTRALLY CONSTRAINED INHIBITOR

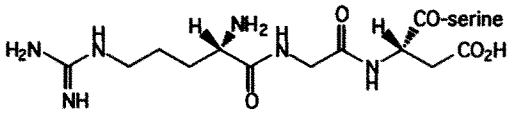
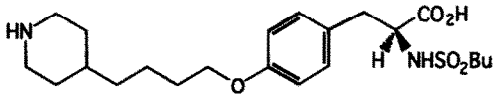
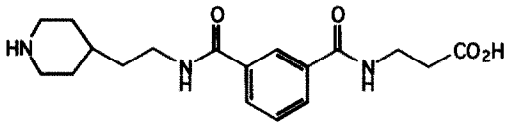
M. S. Egbertson,* A. M. Naylor,* G. D. Hartman,* J. J. Cook, R. J. Gould, M. A. Holahan, J. J. Lynch Jr., R. J. Lynch, M. T. Stranieri, L. M. Vassallo

Merck Research Laboratories, Departments of Biological Chemistry, Medicinal Chemistry, Molecular Systems, and Pharmacology, West Point, PA 19486

Abstract: Analysis of platelet aggregation inhibition results and rotational isomer preferences has provided an understanding of inhibitory potency for m-phthalic acid analogs **3-7.** Constraint of the N-terminal amide led to compound **9**, which is potent, selective, and orally active.

Small molecule inhibitors of platelet aggregation offer a potentially attractive means to prevent thrombogenic disorders such as unstable angina and myocardial infarction.[1-3] The tripeptide sequence Arg-Gly-Asp (RGD) is believed to mediate fibrinogen binding to the platelet GpIIb/IIIa receptor (Table 1)[2], and peptidomimetics[4,5] of this sequence have become the focus of research. We hypothesized that potent inhibitors could be prepared by incorporating an element of

Table 1. Small Molecule Fibrinogen Receptor Antagonists.

		IC_{50} (μM)[a]
	Arg-Gly-Asp-Ser (RGDS) 1	26
	(L-700,462) 2	0.01[6]
	3	0.53

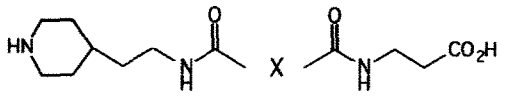
a) Inhibition of ADP-mediated platelet aggregation in human gel-filtered platelets (μM)[12]

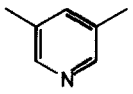
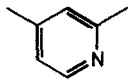
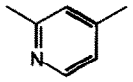
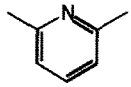
geometric (structural) constraint at the center of the molecule to direct the vectors of the N and C terminal chains. The central phenyl ring of **2** (L-700,462)[6] as well as compounds reported by Alig[4,7], Austel[8], Blackburn[4], Callahan[9], Ku[10] and others[11] have built upon this idea. We now report

the design and preparation of the low-molecular weight, centrally constrained, nanomolar fibrinogen receptor antagonist **9** (L-709,780), and describe the pharmacology of this compound.

Consideration of synthetic ease, the potential for geometric control, and the previously reported steric requirements of the central glycine of RGD[13] suggested the use of a bis-functionalized aromatic ring as the central constraining moiety. We have found that the meta-phthalic acid derivative **3** (Table 1) has an IC_{50}=0.53 μM for inhibition of platelet aggregation, and thus serves as a key lead compound.

Table 2. Pyridine Analogs of Compound **3**.

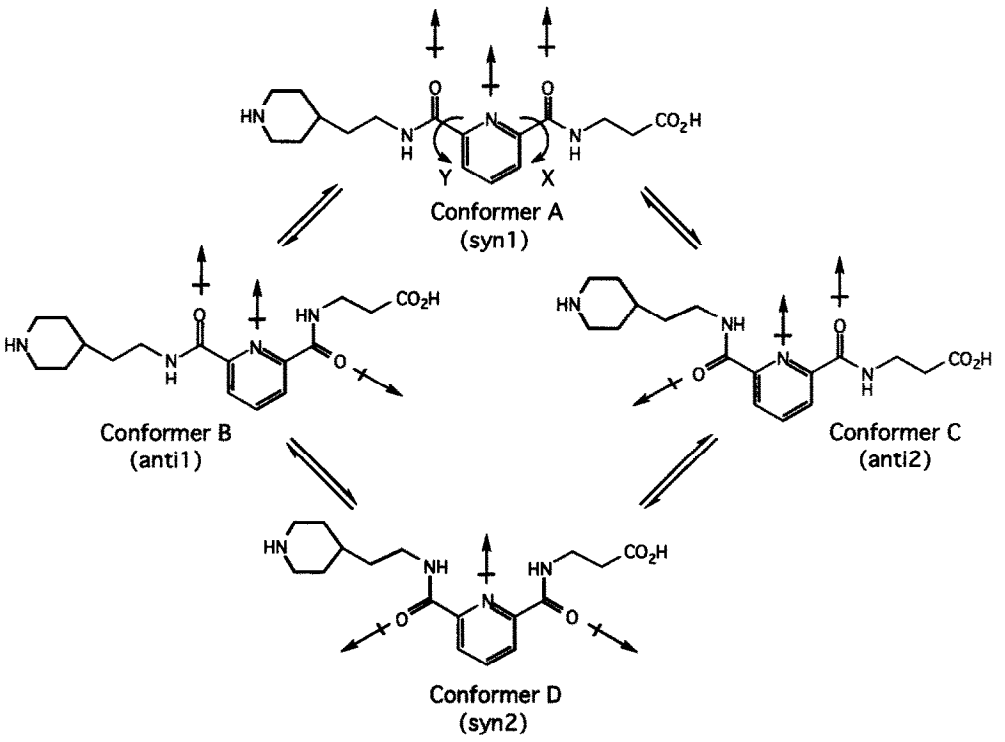


compound	X	IC_{50}[a] (μM)	compound	X	IC_{50}[a] (μM)
4		2.0	**6**		1.1
5		1.6	**7**		>300

a) Inhibition of ADP-mediated platelet aggregation in human gel-filtered platelets (μM)

As part of a program directed at systematic modification of **3**, pyridine analogs (Table 2) were prepared. While the 3,5- (**4**) and both possible 2,4-disubstituted pyridines (**5** and **6**) were of comparable potency to each other, the 2,6-isomer (**7**) was >100-fold less potent. We hypothesized that the position of the pyridine nitrogen in **7** favored an energetic preference for less potent rotational isomers. A comparison of some of the possible rotational isomers of **7** suggests that conformation D, in which dipole moments are opposed, would be of lower energy compared to conformation A, in which dipole moments are aligned (Figure 1).

Figure 1. Rotational Isomers of Compound **7**.



To investigate this hypothesis further, AM1[14] calculations were carried out on the central portion of **3-7**.[15,16] Table 3 contains the calculated energies and dipole moments for four of the possible conformations of **3'-7'**. In general, those conformations with high dipole moments display higher relative energies than those with low dipole moments, as can be seen for the anti conformations of the parent **3'**. For this structure, the barriers to rotation about the bonds X and Y are ~1.2-1.4 kcal.

Our calculations indicate that the dipole moment of **7'** is lowest for syn2, and that this form of **7'** is ~4-10kcal more stable than are the anti and syn1 forms. The barriers to rotation about the bonds X and Y of **7'** (4-6kcal) are significantly higher than are those for **3'-6'**. Therefore, it seems reasonable to conclude that conformer D is a preferred orientation for **7**, and that the poor potency of **7** is a reflection of this preference.

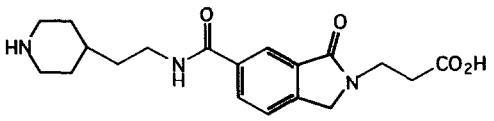
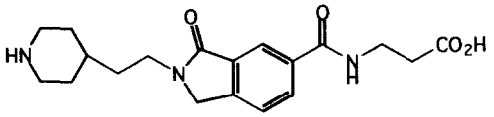
Table 3. AM1 calculated heats of formation (ΔHf) and dipole moments.[15]

Analog	Conformer	ΔHf (kcal/mol)	ΔΔHf (kcal/mol)	Dipole Moment (Debye)
3'	syn1	-45.66	1.12	6.6
	anti1,anti2	-46.78	0.00	4.3
	syn2	-46.20	0.58	4.4
4'	syn1	-35.57	0.92	5.1
	anti1,anti2	-36.49	0.00	4.2
	syn2	-35.68	0.81	6.1
5'	syn1	-34.77	1.36	5.5
	anti1	-36.13	0.00	2.2
	anti2	-30.77	5.36	5.5
	syn2	-30.23	5.90	5.5
6'	syn1	-34.77	1.36	5.5
	anti1	-30.77	5.36	5.5
	anti2	-36.13	0.00	2.2
	syn2	-30.23	5.90	5.5
7'	syn1	-25.94	10.11	7.9
	anti1,anti2	-31.87	4.18	4.8
	syn2	-36.05	0.00	2.4
8',9'	syn	-26.17	0.87	6.5
	anti	-27.04	0.00	3.0

With these results in mind we proposed that the potency of **3** could be improved by restricting the rotation of bonds X and/or Y, thus favoring either the syn1, anti1 or anti2 conformations, and suppressing the syn2 conformation. To test these ideas, the isoindolinone compounds **8** and **9** were prepared (Scheme 1).[17] Thus, although **8** had an IC_{50}=10uM, a loss of potency of 20-fold relative to **3**, **9** showed an 20-fold increase in potency, with an IC_{50}=25 nM.

Energetic calculations of compound **9'** indicate that conformations are preferred in which the carbonyl groups lie in the same plane as the phenyl ring. The anti orientation is energetically favored over the syn by ~1kcal with a low rotational barrier of 1.2kcal.

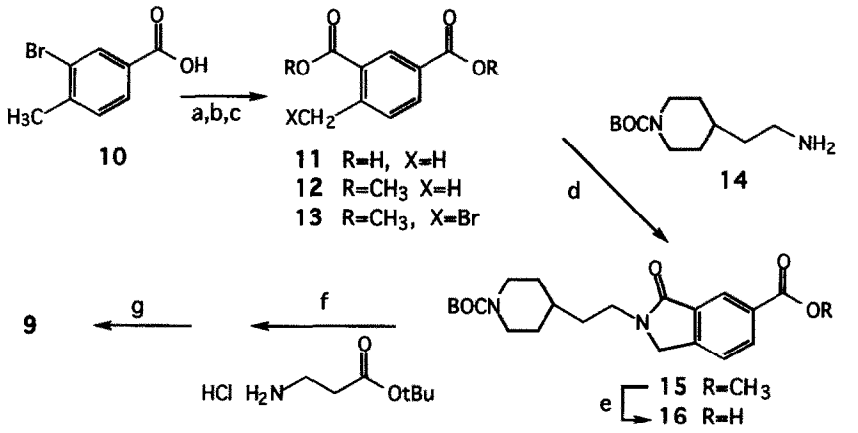
Figure 2. Constrained Amide Fibringon Receptor Antagonists.

		IC_{50} (μM)[a]
8		10 .0
9		0.025

a) Inhibition of ADP-mediated platelet aggregation in human gel-filtered platelets (μM)

The selectivity of **9** was determined by comparison of the compound's ability to inhibit platelet aggregation with its ability to inhibit the binding of human umbilical vein endothelial cells (HUVEC) to fibrinogen, vitronectin and fibronectin.[18] At concentrations of 300 uM, **9** showed no inhibition of binding of HUVEC, indicating >12,000-fold selectivity for GpIIb/IIIa.

Scheme 1. Preparation of Compound **9**.



a) 1.1eq CH_3MgBr/0°C,then 2eq nBuLi/-65°C, solid CO_2, 85%; b) CH_3OH/HCl, 95%; c) NBS, CCl_4, 80%; d) **14**, C_6H_6,reflux, 80%; e) LiOH/MeOH,THF,H_2O, quant.; f)N-methylmorpholine, BOP reagent, CH_3CN, 80%; g) HCl(g)/EtOAc, 95%.

The in vivo activity of **9** was assessed by determining ex vivo platelet aggregation responses to ADP and collagen before and after oral administration to conscious dogs, using methods described previously.[19] The oral administration of 2 mg/kg and 4 mg/kg **9** resulted in a maximal 65% and 80-90% inhibition of ex vivo platelet aggregation, with platelet aggregation returning to pretreatment levels at approximately 140 minutes and 480 minutes after oral administration, respectively.

In summary, analysis of platelet aggregation inhibition results and modeling considerations for analogs **3-7** revealed the importance of rotational isomer preference. Additional constraint of the N-terminal amide led to compound **9**, which proved to be a potent, selective, and orally active fibrinogen receptor antagonist. Further development of this lead compound is underway.

Acknowledgment: The authors acknowledge the support and encouragement of Drs. P.S. Anderson, J. Shafer and P.A. Friedman throughout the course of the project. We also thank Ms. J. Kaysen for preparation of the manuscript.

References and Notes:

1. Falk, E. *Circulation*, **1985**, *71*, 699-708. Coller, B.S. *New Eng. J. Med.* **1990**, *322*, 33-42 (in Seminars in Medicine of the Beth Isreal Hospital, Boston). Cadroy, Y.; Harker, L.A. Cardiovascular Pharmacology, 3rd ed.; M. Antonaccio, Ed.; Raven Press: New York, 1990: pp 515-539.

2. Cook, N.S.; Kottirsch, G.; Zerwes, H.G. *Drugs of the Future*, **1994**, *19(2)*, 135-159.

3. Sutton, J.; Topol, E.; Rossen, J.D.; Runge, M.; Ghannam, A.; Fitspatrick, V.; Hand, E.; Barrett, J.S.; Sax, F.L. *Clinical Research*, **1993**,*41*, 118a. Roux, S.P.; Tschopp, T.B.; Kuhn, H.; Steiner, B.; Hadváry, P. *J. Pharmacology and*

Experimental Therapeutics, **1993**,*264*, 501-508. Nicholson, N.; Taite, B.; Panzer-Knodle, S.; Salyers, A.; Haas, N.; Szalony, J.; Zablocki, J.; Feigen, L.; Glenn, K.; Keller, B.; Broschat, K.; Herin, M.; Jacqmin, P.; Lesne, M. *Thrombosis and Haemostasis THHADQ Abstracts* **1993**,*69* , 538-1455, #1558.

4. Alig, L.; Edenhoger, A.; Hadváry, P.; Hürzeler, M.; Knopp, D.; Müller, M.; Steiner, B.; Trzeciak, A.; Weller, T. *J. Med. Chem.* **1992**,*35*, 4393-4407. Blackburn, B.K.; Gadek, T.R. *Annual Reports in Medicinal Chemistry* **1993**, *28*, 79-88.

5. Himmelsbach, F.; Pieper, H.; Austel, V.; Linz, G.; Müller, T.; Eisert, W.; Weisenberger, J. European Patent Application 0496378 A1, (1991). Himmelsbach, F.; Austel, V.; Pieper, H.; Eisert, W.; Müller, T.; Weisenberger, J.; Linz, G.; Krüger, G. European Patent Application 0483667 A2, (1992). Zablocki, J.A.; Miyano, M.; Garland, R.B.; Pireh, D.; Schretzman, L.; Rao, S.N.; Lindmark, R.J.; Panzer-Knodle, S.G.; Nicholson, N.S.; Taite, B.B.; Salyers, A.K.; King, L.W.; Campion, J.B.; Feigen, L.P. *J. Med. Chem.* **1993**, *36*, 1811-1819.

6. 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.T.-C.; Gould, R.J. *J. Med. Chem.* **1992**, *35*, 4640.

7. Alig, L.; Edenhofer, A.; Müller, M.; Trzeciak, A.; Weller, T. U.S. Patent No. 5,039,805 (1991).

8. Austel, V.; Pieper, H.; Linz, G.; Müller, T.; Weisenberger, J.; Seewaldt-Becker, E. European Patent Application 0531883 A (1991).

9. Callahan, J.F.; Bean, J.W.; Burgess, J.L.; Eggleston, D.S.; Hwang, S.M.; Kopple, K.D.; Koster, P.F.; Nichols, A.; Peishoff, C.E.; Samanen, J.M.; Vasko, J.A.; Wong, A.; Huffman, W.F. *J. Med. Chem.* **1992**, *35*, 3970-3972.

10. Ku, T.W.; Ali, F.E.; Barton, L.S.; Bean, J.W.; Bondinell, W.E.; Burgess, J.L.; Callahan, J.F.; Calvo, R.R.; Chen, L.; Egglestron, D.S.; Gleason, J.G.; Huffman, W.F.; Hwang, S.M.; Jakas, D.R.; Karash, C.B.; Keenan, R.M.; Kopple, K.D.; Miller, W.H.; Newlander, K.A.; Nichols, A.; Parker, M.F.; Peishoff, C.E.; Samanen, J.M.; Uzinskas, I.; Venslavsky, J.W. *J. Am. Chem. Soc.* **1993**, *115*, 8861-8862.

11. Hirschmann, R.; Sprengeler, P.A.; Kawasaki, T.; Leahy, J.W.; Shakespeare, W.C.; Smith, A.B.III *J. Am. Chem. Soc.* **1992**, *114*, 9699-9701. Porter, B.; Eldred, C.D.; Kelly, H.A.; European Patent Application 0537980 A1 (1992). König, W.; Just, M.; Jablonka, B. European Patent Application 0449079 A (1991).

12. Platelet aggregation was measured in a functional assay which monitors the increase in light transmittance which occurs when platelets aggregate. Human gel-filtered platelets were adjusted to a concentration of 2 x 10^8/mL and mixed with 0.1 mg/mL human fibrinogen, 1 mM $CaCl_2$ and the compound of interest. Aggregation was then initiated by addition of the agonist (10 uM adenosine diphosphate (ADP)). Inhibition of platelet aggregation was determined by comparison of light transmittance values for the control and subject samples. The IC_{50} was determined as the concentration necessary to inhibit the change in light transmittance by 50%. At least two determinations were made for each compound and the IC_{50} calculated by fitting to a four parameter equation. The average standard error of the IC_{50} determinations was +/- 20%.

13. Gartner, T.K.; Bennett, J.S.; Ogilvie, M.L. *Blood*, **1987**, Suppl. 1, 351a.

14. Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J.Am.Chem.Soc.* **1985**, *107*,3902.

15. AMPAC QCPE Program version 2.1 supplied by Cray Research. AMPAC optimizations for various conformations used the keywords AM1, VECTORS, PULAY, and DENSITY. Calculations were performed by defining the torsion of the sidechain-aromatic bond as a reaction coordinate that was sampled in 30° increments from 0° to 180°.

16. The ligands studied computationally, **3'-9'**, are simplified models of **3-9** where methyl substituents are attached to the amide nitrogen atoms in place of ethypiperidine and propionic acid.

17. Compound **8** was prepared similarly to **9**, except that t-butyl-6–alanine was used in the displacement/lactam formation and **14** was used in the amide formation. All compounds were characterized by NMR, TLC, HPLC, high-resolution mass spectrometry, and H,C,N analysis.

18 Cheresh, D.A. Human Endothelial Cells Synthesize and Express an Arg-Gly-Asp-Directed Adhesion Receptor Involved in Attachment to Fibrinogen and von Wellebrand Factor. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 6471-6457.

19. Ramjit, D.R.; Lynch, J.J. Jr.: Sitko, G.R.; Mellott, M.J.; Holahan, M.A.: Stabilito, I.I.; Stranieri, M.T.; Zhang, G.; Lynch, R.J.; Manno, P.D.; Chang, C.T.-C.: Nutt, R.F.; Brady, S.F.; Veber, D.F.; Anderson, P.S.; Shebuski, R.J.; Friedman, P.A.; Gould, R.J. *J. Pharm. Exp. Thera.* **1993**, *266(3)*, 1501.

(*Received in USA* 16 *May* 1994; *accepted* 21 *June* 1994)