Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 21, pp. 2793-2798, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10085-3

NONPEPTIDE GLYCOPROTEIN IIB/IIIA INHIBITORS: 18. INDOLE ALPHA-SULFONAMIDE ACIDS ARE POTENT INHIBITORS OF PLATELET AGGREGATION

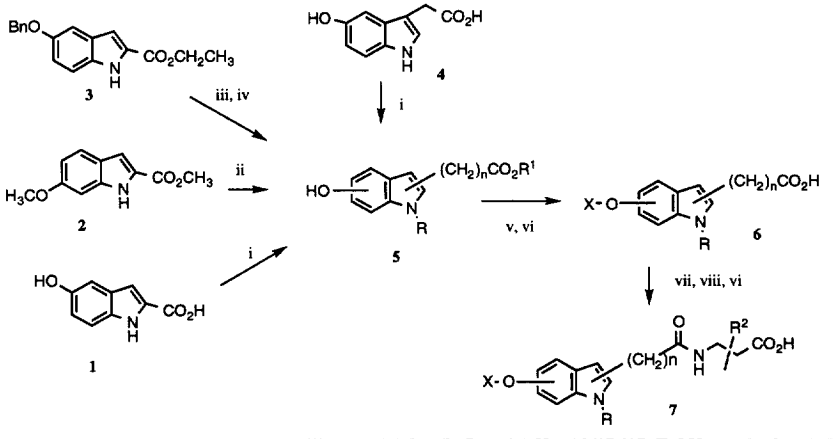
Karen M. Brashear,[a,*] Jacquelynn J. Cook,[b] Bohumil Bednar,[b] Rodney A. Bednar,[b] Robert J. Gould,[b] Wasyl Halczenko,[a] Marie A. Holahan,[b] Robert J. Lynch,[b] George D. Hartman,[a] and John. H. Hutchinson[a]

[a]Department of Medicinal Chemistry and [b]Department of Pharmacology
Merck and Co., Inc., P.O. Box 4, WP44-J100, West Point, PA 19486

Abstract: The structure–activity relationship (SAR) of a series of orally active glycoprotein IIb/IIIa antagonists containing an alkyl or aryl sulfonamide grafted onto an indole core is described. © 1997 Elsevier Science Ltd.

Platelet rich thrombus formation is involved in various vasoocclusive disorders such as unstable angina, acute myocardial infarction, and reocclusion following angioplasty.[1] The final, common event leading to thrombus formation, independent of the mechanism of platelet activation, is the binding of fibrinogen to its platelet receptor glycoprotein IIb/IIIa (GPIIb/IIIa). Nonpeptide antagonists[2] that mimic the RGD sequence on the alpha chain of fibrinogen have been shown to inhibit platelet aggregation.[3] These laboratories have previously reported the design and synthesis of potent, centrally constrained GPIIb/IIIa antagonists,[3b,4] as well as the identification of an α-sulfonamide moiety as a unique potency enhancing feature.[3a,5] This paper describes our work on a series of GPIIb/IIIa antagonists that incorporate an indole as the bicyclic central core. This has led to the identification of L-756,568, **7a**, which is a potent antagonist that shows prolonged duration of activity when dosed orally in rhesus monkeys.

Scheme 1[a]

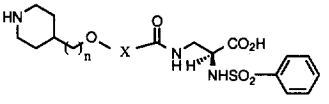


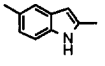
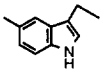
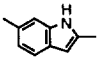
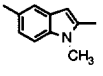
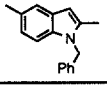
[a] Reagents: (i) CH_2N_2, EtOAc; (ii) BBr_3, THF; (iii) NaH, MeI or BnBr; (iv) H_2, 10%Pd/C, EtOH; (v) X-OH, DEAD PPh_3 in THF or X-Cl, Cs_2CO_3, DMF; (vi) 1 N NaOH, THF/MeOH; (vii) β-Alanine ester, BOP, NMM, CH_3CN; (viii) HCl (g), EtOAc.

The indole analogs **7a–q** were prepared as illustrated in Scheme 1. Standard methodologies were used in the preparation of substituted β-alanines[6] as well as the various *N*-termini.[7] The indole intermediate **5** was obtained through de-methylation or esterification of the commercially available indoles **1**, **2**, and **4**. In the case of the *N*-substituted indoles, the *O*-benzyl protected indole **3** was alkylated with the appropriate halide, followed by debenzylation to give the desired intermediate **6** (R = Me or Bn). The *N*-terminus was then installed by alkylation with the appropriate halide using cesium carbonate in DMF, or with the appropriate alcohol employing Mitsunobu chemistry. (For compounds **7a**, **7b**, **7h**, and **7j**, a Boc group was used in protection of the *N*-terminus). Saponification of the resulting ester afforded the acid intermediate **6**, which was coupled to the appropriate β-alanine derivative and deprotected to give the target compounds **7a–7q** (Tables 1, 2, and 3).

Two assays were utilized to assess the potency of the prepared antagonists. The platelet aggregation assay[8] measures the ability of compounds to inhibit ADP-induced platelet aggregation of human gel-filtered platelets. However, since the receptor concentration in the assay is approximately 20–30 nM, the limit of sensitivity of this assay is between 10–15 nM. In addition, owing to the presence of added 2% bovine serum albumin in this assay, it fails to accurately assess the intrinsic potency of highly protein bound[9] compounds. Thus, to measure the differences in affinity for the receptor between compounds, a second assay was developed based on purified receptor.[10] ED_{50} values are calculated from the competitive binding between compounds of interest and the fibrinogen receptor antagonist [^{125}I]L-692,884 to purified GPIIb/IIIa activated by coating onto yttrium silicate Scintillation Proximity Assay Fluomicrospheres (SPA-A assay).

Table 1



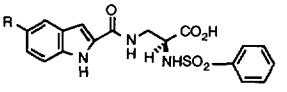
Compound	n	X	Platelet Aggregation IC_{50} (nM)	SPA-A ED_{50} (nM)	Protein Binding
7a	1		13	0.099	66%
7b	2		8	0.056	76%
7c	1		5700	-	not soluble
7d	1		15	0.49	57%
7e	1		15	-	94%
7f	2		21	-	94%
7g	1		150	-	not soluble

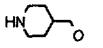
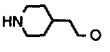
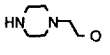
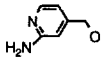
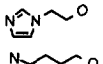
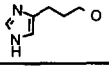
The first group of analogs (Table 1) investigated the preferred arrangement of the substituents on the central indole constraint (responsible for directing the vectors of the amino and acid termini). Substitution, as seen in compounds **7a** and **7b**, where the amino terminus is attached at the 5-position on the indole ring, and the acid

terminus extends from the 2-position, afforded the most potent compounds in both the platelet aggregation assay and the SPA-A assay. Interestingly, both the 2-carbon linker present in **7b** (ED_{50} = 0.056 nM) and the one-carbon linker of **7a** (ED_{50} = 0.099 nM) result in compounds of similar potency in the SPA-A assay. Substitution of the acid chain at the 3-position on the indole to give **7c** led to a dramatic decrease in potency, over 500-fold, compared to **7a** (5700 nM vs. 13 nM for the platelet aggregation assay). The 2,6-substituted indole **7d** could not be distinguished from the isosteric analog **7a** based on the platelet aggregation assay. However, using the SPA-A assay, it was shown to be about fivefold less potent (ED_{50} **7d** = 0.49 nM vs. ED_{50} **7a** = 0.099 nM).

Modification through *N*-substitution on the indole ring was also examined (**7e–7g**). These variations afforded a significant decrease in potency in the platelet aggregation assay with the more lipophilic benzyl group (**7g**, IC_{50} = 150 nM), while the smaller *N*-methyl substituent in **7e** and **7f** was tolerated, albeit with increased protein binding. A possible explanation for the low potency of **7g** is that it is highly bound to the bovine serum albumin present in the assay mixture. Unfortunately, **7g** was not soluble enough to accurately measure the extent of protein binding.

Table 2



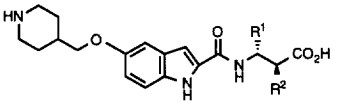
Compound	R	Platelet Aggregation IC_{50} (nM)	SPA-A ED_{50} (nM)	Protein Binding
7a		13	0.099	66%
7b		8	0.056	76%
7h		12	0.13	57%
7i		280	0.25	99%
7j		45	0.07	99%
7k		45	0.28	98%
7l		100	2	98%

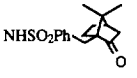
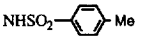
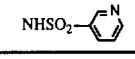
Compounds with amino termini of varying basicity and chain length were prepared and are described in Table 2. The more basic compounds **7a**, **7b**, and **7h** were shown to be the most potent in the platelet aggregation assay, whereas the weakly basic heterocycles were highly protein bound and were less potent GPIIb/IIIa antagonists. For these latter compounds (**7i**, **7j**, **7k**, and **7l**), the SPA-A assay was utilized to assess their intrinsic potency. The ED_{50} values obtained from the SPA-A assay demonstrated that the pyridine **7i** and imidazole congeners (**7k** and **7l**) were 5- to 20-fold less potent than **7b**. In general, it appears that the most basic amino heterocycles were also the most potent in both in vitro assays. It is interesting that the 2-amino pyridine analog **7j**

was found to be among the most potent compounds tested, despite the fact that 2-aminopyridine has a pKa of ~6.7 compared to that of piperidine at ~12. Perhaps this is due to the fact that the 2-aminopyridine is capable of forming a bidentate interaction with the receptor.[11]

The effect of removing the α-sulfonamide group is shown with compound **7m**, which has an IC_{50} of 1μM in the platelet aggregation assay (Table 3). Installation of a 3-pyrid-3-yl substituent has provided a significant boost in potency in other series of RGD mimic fibrinogen receptor antagonists.[12,13] However, incorporation of this functionality into the indole series afforded compounds that were only moderately more potent than the unsubstituted compound **7m**, and much less potent than the sulfonamide compounds **7a** and **7b**. We then decided to examine various alternative α-sulfonamides as shown with the analogs **7o**, **7p**, and **7q**. All of the sulfonamide analogs displayed similar potencies in the SPA-A assay, and have modest to low protein binding. Therefore, it was decided to evaluate all 4 sulfonamide analogs, as well as the two-carbon linker **7b**, for their in vivo efficacy in rhesus monkeys.

Table 3



Compound	R^1	R^2	Platelet Aggregation IC_{50} (nM)	SPA-A ED_{50} (nM)	Protein Binding
7m	H	H	1000	170	52%
7n	(*R*,*S*)-pyrid-3-yl	H	810	-	47%
7a	H	$NHSO_2Ph$	13	0.099	66%
7o	H		17	0.04	63%
7p	H		6	0.06	85%
7q	H		12	0.07	-

Our goal was to obtain compounds suitable for once-a-day dosing. Therefore, each compound was examined for its effect on ex vivo platelet aggregation when dosed orally at 0.25–0.3 mg/kg in rhesus monkeys daily for 4 days.[14] Plasma samples and platelet aggregation measurements were obtained 2 h after dosing and 0.5 h prior to the next dosing (23.5 h after previous dose). The 30 min pre-dose sample is an indicator of inhibition of platelet aggregation at trough plasma concentration of drug, while the 2 h post dose measurement is a reflection of the ex vivo aggregation at or near the peak plasma concentration of drug. Inhibition of platelet aggregation at trough plasma levels of drug are shown in Figure 1 along with exact oral doses (mg/kg) of each compound.

The inhibition of the extent of ADP-induced aggregation at 2 h post dose (data not shown) seen for **7b** and **7o** was disappointing, as these two compounds failed to maintain consistent, high levels (80–100%) of inhibitory activity. In contrast, **7a**, **7p**, and **7q** uniformly displayed total inhibition (100%) of platelet aggregation at or near peak plasma concentration of drug. However, as can be seen in Figure 1, **7p** and **7q** were unable to maintain

inhibitory activity equal to that of **7a** at trough plasma concentration of drug. Indole **7a** was very effective at a dose of 0.25 mg/kg/day in maintaining consistent, effective levels of inhibitory activity throughout a 24 h period. Inhibition of platelet aggregation at trough plasma concentration of drug ranged between 82–95%, befitting a compound suitable for once-a-day dosing.

In summary, incorporation of a potency-enhancing α-sulfonamide onto an rigid indole core has given a series of potent, structurally simple, nonpeptide RGD mimics as inhibitors of platelet GPIIb/IIIa-fibrinogen binding. Optimization of this series of compounds has culminated in the synthesis of L-756,568, a potent, orally active fibrinogen receptor antagonist with long duration of activity suitable for once daily oral administration.

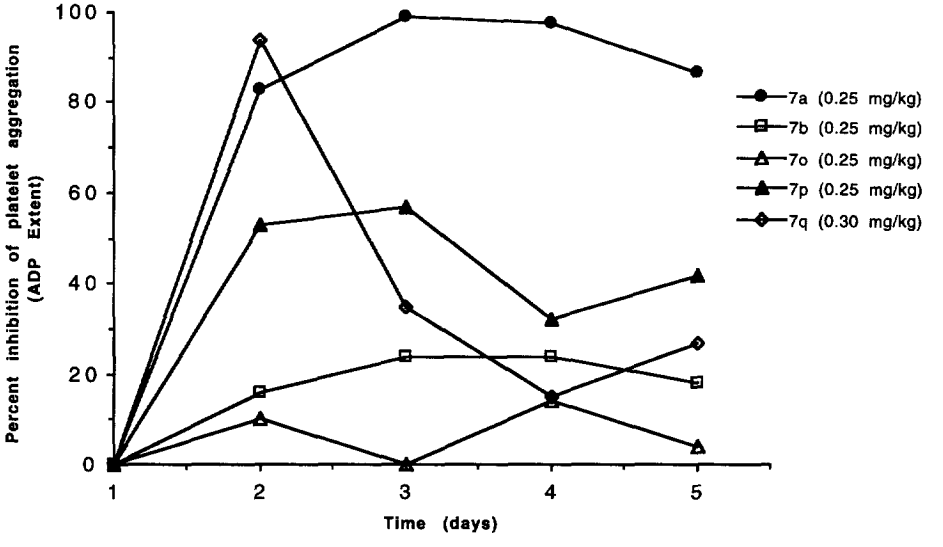


Figure 1. Inhibition of ADP-induced platelet aggregation at baseline and 23.5 h after dosing rhesus (n = 2/compound)

Acknowledgment

The authors would like to thank Joan D. Glass and Halea A. Hartman for their help in pharmacological studies, and Joy Hartzell for assistance in preparing the manuscript.

References and Notes

1. Fitzgerald, D. J.; Roy, L.; Cattalo, F.; Fitzgerald, G. A. *N. Engl. J. Med.* **1986**, *315*, 983.
2. For recent reviews, see: (a) Schror, K. *Drugs* **1995**, *50*, 7. (b) The EPIC Investigators, *New Engl. J. Med.* **1994**, *330*, 956. (c) Raddatz, P.; Gante, J. *Exp. Opin. Ther. Patents.* **1995**, *5*, 1163. (d) Ojima, I.; Chakravarty, S.; Dong, Q. *Bioorg. Med. Chem.* **1995**, *3*, 337. (e) Wityak, J.; Sielecki, T. *Exp. Opin. Ther. Patents.* **1996**, *6*, 1175.
3. (a) 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. (b) Duggan, M. E.; Naylor–Olsen, A. M.; Perkins, J. J.; Anderson, P. S.; Chang, C. T-C.; Cook, J. J.; Gould, R. J.; Ihle, N. C.; Hartman, G. D.; Lynch, J. J.; Lynch, R. J.; Manno, P. D.; Schaffer, L. W.; Smith, R. L. *J. Med. Chem.* **1995**, *38*, 3332. (c) Zablocki, J. A.; Rico, J. G.; Garland, R. B.; Rogers, T. E.; Williams, K.; Schretzman, L. A.; Shashidhar, A. R.; Bovy, P. R.; Tjoeng, F. S.; Lindmark, R. J.; Toth, M. V.; Zupec, M. E.; McMackins, D. E.; Adams, S. P.; Miyano, M.; Markos, C. S.; Milton, M. N.; Paulson, S.; Herin, M.; Jacqmin, P.; Nicholson, N. S.; Panzer–Knodle, S. G.; Haas, N. F.; Page, J. D.; Szalony, J. A.;

Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *J. Med. Chem.* **1995**, *38*, 2378. (d) Scarborough, R. M.; Naughton, M. A.; Teng, W.; Rose, J. W.; Phillips, D. R.; Nannizzi, L.; Arfsten, A.; Campbell, A. M.; Charo, I. F. *J. Biol. Chem.* **1993**, *268*, 1066. (e) Wityak, J.; Sielecki, T. M.; Pinto, D. J.; Emmett, G.; Sze, J. Y.; Liu, J.; Tobin, E.; Wang, S.; Jiang, B.; Ma, P.; Mousa, S. A.; Wexler, R. R.; Olson, R. E. *J. Med. Chem.* **1997**, *40*, 50. (f) Weller, T.; Alig, L.; Beresini, M.; Blackburn, B.; Bunting, S.; Hadvary, P.; Muller, M. H.; Knopp, D.; Levet–Trafit, B.; Lipari, M. T.; Modi, N. B.; Muller, M.; Refino, C. J.; Schmitt, M.; Schonholzer, P.; Weiss, S.; Steiner, B. *J. Med. Chem.* **1996**, *39*, 3139. (g) Himmelsbach, F; Austel, V.; Guth, B.; Linz, G.; Muller, T. H.; Pieper, H.; Seewaldt–Becker, E.; Weisenberger, H. *Eur. J. Med. Chem.* **1995**, *30*, 243s. (h) Miller, W. H.; Ku, T. W.; Ali, F. E.; Bondinell, W. E.; Calvo, R. R.; Davis, L. D.; Erhard, K. F.; Hall, L. B.; Huffman, W. F.; Keenan, R. M.; Kwon, C.; Newlander, K. A.; Ross, S. T.; Samanen, J. M.; Takata, D. T.; Yuan, C-K. *Tetrahedron Lett.* **1995**, *36*, 9433. (i) Kottirsch, G.; Zerwes, H. G.; Cook, N. S.; Tapparelli, C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 727. (j) Hoekstra, W. J.; Maryanoff, B. E.; Andrade–Gordon, P.; Cohen, J. H.; Costanzo, M. J.; Damiano, B. P.; Haertlein, B. J.; Harris, B. D.; Kauffman, J. A.; Keane, P. M.; McComsey, D. F.; Villani, F. J.; Yabut, S. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2371.

4. Askew, B. C.; McIntyre, C. A.; Hunt, C. A.; Claremon, D. A.; Gould, R. J.; Lynch, R. J.; Armstrong, D. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 339.
5. (a) Egbertson, M. S.; Chang, C. T-C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, J. J.; Lynch, R. J.; Manno, P. D.; Naylor, A. M.; Prugh, J. D.; Ramjit, D. R.; Sitko, G. R.; Smith, R. S.; Turchi, L. M.; Zhang, G. *J. Med. Chem.* **1994**, *37*, 2537. (b) Egbertson, M. S.; Hartman, G. D.; Gould, R. J.; Bednar, R. A.; Cook, J. J.; Gaul, S. L.; Holahan, M. A.; Libby, L. A.; Lynch, J. J.; Lynch, R. J.; Sitko, G. R.; Stranieri, M. T.; Vassallo, L. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2519. (c) Askew, B. C.; Bednar, R. A.; Bednar, B.; Claremon, D. A.; Cook, J. J.; McIntyre, C. J.; Hunt, C. A.; Gould, R. J.; Lynch, R. J.; Lynch, J. J.; Gaul, S. L.; Stranieri, M. T.; Sitko, G. R.; Holahan, M. A.; Glass, J. D.; Hamill, T.; Gorham, L. M.; Prueksaritanont, T.; Baldwin, J. J.; Hartman, G. D. *J. Med. Chem.* **1997**, *40*, 1779.
6. (a) For phenyl-and 4-tolyl-sulfonamides, see: ref 5c. (b) For 10-(–)camphor- and 3-pyridyl-sulfonamides, adapted from chemistry as described in Egbertson, M. S.; Bednar, B.; Bednar, R. A.; Hartman, G. D.; Gould, R. J.; Lynch, R. J.; Vassallo, L. M.; Young, S. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1415. (c) 3-Pyrid-3-yl see: Rico, J. G.; Lindmark, R. J.; Rogers, T. E.; Bovy, P. R. *J. Org. Chem.* **1993**, *58*, 7948.
7. (a) Compound **7l**, see: Bost, R. W.; Andrews, E. R. *J. Am. Chem. Soc.* **1943**, *65*, 900. (b) Compound **7k**, see: Yoshino, T.; Inaba, S.; Komura, H.; Ishido, Y. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 105. (c) For compounds **7a**, **7b**, and **7h**, the amino termini were prepared in 1-2 steps from commercially available starting materials using standard chemistry. (d) For compound **7j**, the amino terminus was prepared from 2-amino-4-picoline in 3 steps. The synthesis of this amino terminus will be described in a future publication.
8. For a description of the methods used to determine potencies see: Gan, Z.-R.; Gould, R. J.; Jacobs, J. W.; Friedman, P. A.; Polokoff, M. A. *J. Biol. Chem.* **1988**, *263*, 19827.
9. Protein Binding Assay measures the ability of a compound to bind to human albumin in pH 7.4 buffer.
10. Bednar, B.; Cunningham, M. E.; McQueney, P. A.; Egbertson, M. S.; Askew, B. C.; Bednar, R. A.; Hartman, G. D.; Gould, R. J. *Cytometry* **1997**, *28*, 58.
11. 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. G.; Feigen, L. P. *J. Med. Chem.* **1993**, *36*, 1811.
12. Hutchinson, J. H.; Cook, J. J.; Brashear, K. M.; Breslin, M. J.; Glass, J. D.; Gould, R. J.; Halczenko, W.; Holahan, M. A.; Lynch, R. J.; Sitko, G. R.; Stranieri, M. T.; Hartman, G. D. *J. Med. Chem.* **1996**, *39*, 4583.
13. Zablocki, J. A.; Tjoeng, F. S.; Bovy, P. R.; Miyano, M.; Garland, R. B.; Williams, K.; Schretzman, L.; Zupec, M. E.; Rico, J. G.; Lindmark, R. J.; Toth, M. V.; McMackins, D. E.; Adams, S. P.; Panzer–Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *Bioorg. Med. Chem.* **1995**, *3*, 539.
14. Cook, J. J.; Sitko, G. S.; Holahan, M. A.; Stranieri, M. T.; Glass, J. D.; Askew, B. C.; McIntyre, C. J.; Claremon, D. A.; Baldwin, J. J.; Hartman, G. D.; Gould, R. J.; Lynch, J. J. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 677.

(*Received in USA* 15 *August* 1997; *accepted* 22 *September* 1997)