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NONPEPTIDE GPIIB/IIIA INHIBITORS. 10. CENTRALLY CONSTRAINED ALPHA-SULFONAMIDES ARE POTENT INHIBITORS OF PLATELET AGGREGATION

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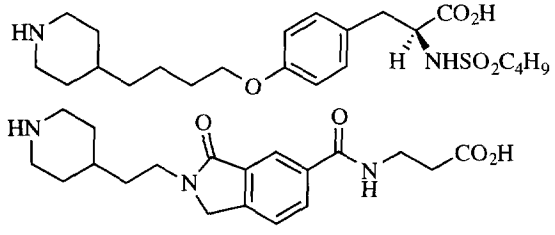
Abstract: Potency enhancing features of two series of fibrinogen receptor antagonists were combined to give analogs with improved potency and oral activity. Antagonists containing either alkyl or aryl sulfonamides and a central isoindolinone structural constraint demonstrate high affinity for both activated and unactivated platelet receptors.

The final step in the occlusive thrombotic process involves the aggregation of platelets mediated by fibrinogen binding to the platelet glycoprotein IIb/IIIa.[1] Proteins or peptides containing an RGD tripeptide and small nonpeptide mimics of RGD can inhibit this process by acting as fibrinogen receptor antagonists.[2]

Two potency-enhancing structural features emerged as we pursued nonpeptide fibrinogen receptor antagonists. First, α-carbon substitution of a tyrosine-like lead led to the discovery of the potency-enhancing effect of the sulfonamide moiety.[3] The potent n-butylsulfonamide iv clinical candidate AGGRASTAT™ (1) (Figure 1) demonstrates the utility of this approach. Modeling studies suggest that the sulfonamide functionality interacts with a binding site region not exploited by cyclic peptide inhibitors. Therefore, sulfonamide-containing inhibitors have been termed "exo-site inhibitors".[4] However, while **1** exhibits excellent *in vivo* efficacy when administered iv, it is not suitable for use as an oral agent due to its very short duration of action post oral administration (Figure 2). Our search for an orally active series focussed on optimization of a non-tyrosine backbone, and led to the discovery of a second potency enhancing structural feature. We hypothesized that potent inhibitors could be prepared by incorporating an element of geometric (structural) constraint at the center of the molecule to direct the vectors of the *N*-and *C*-terminal chains. In practice, the "centrally constrained" fibrinogen receptor antagonist L-709,780 (2) proved to be of nanomolar potency and gave good levels of platelet inhibition in dogs at oral doses of 2mg/kg (figure 2).[5] We sought further optimization of **2** to obtain an oral agent that could provide good levels of platelet inhibition in dogs at doses of less than 1 mg/kg. As described recently, combination of a centrally constrained backbone with either of two fluorescent, α-sulfonamide substituents led to potent fibrinogen receptor antagonists that were useful tools for the exploration of receptor/ligand interactions.[6] *This paper describes how a fibrinogen receptor antagonist with improved oral activity can be obtained by adding an α -substitutent to the centrally constrained compound 2.*

Figure 1. Small Molecule Fibrinogen Receptor Antagonists.

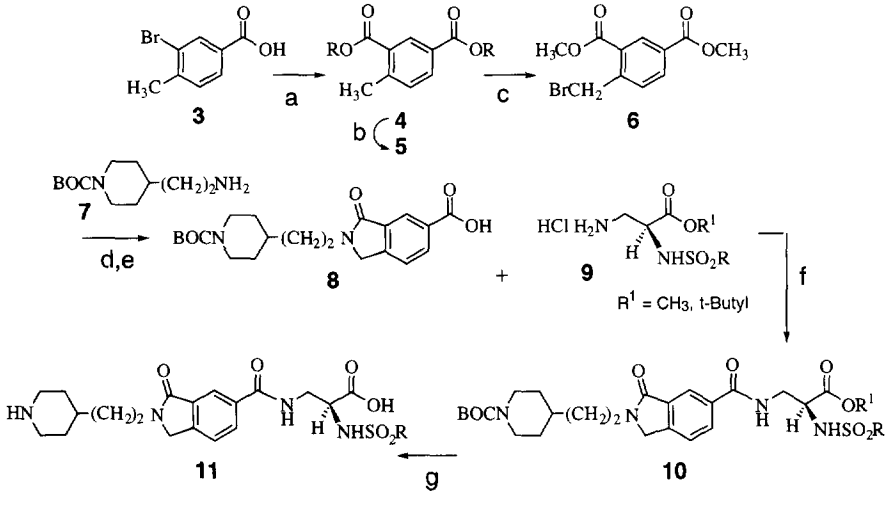


1
(L-700,462
MK-383, AGGRASTAT™)

2
(L-709,780)

α-sulfonamide analogs were prepared as outlined in Scheme 1. Initially, simultaneous deprotonation and metal halogen exchange of commercially available acid **3** was attempted with two equivalents of *n*-Butyl lithium at -65 °C, however, significant amounts of debrominated side products were encountered. Stepwise deprotonation of **3** with Grignard reagent at 0 °C, followed by metal-halogen exchange at -65 °C (two equivalents of *n*-Butyl lithium were used to minimize side reactions of the di-anion with butyl bromide), and subsequent quenching with solid CO_2 in THF gave good yields of diacid **4**. Conversion of **4** to the diester **5** and radical bromination gave crude **6** which was reacted with the amine **7**.[4] Displacement and ring closure occurred in one step, and was followed by hydrolysis of the remaining ester group to give isoindolinone **8**. BOP coupling with the previously described 2,3-diaminopropionic acid derivatives **9**[6,7] followed by one-step deprotection of the *N*- and *C*-terminal protecting groups gave the α-sulfonamide analogs **11**. We found that for methyl ester analogs, acidic rather than basic hydrolysis conditions in the final step led to optimal enantiomeric purity of the products.[8]

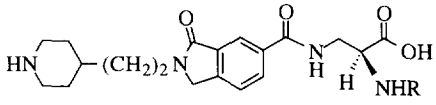
Scheme 1. Preparation of α-sulfonylamido isoindolinones.



(a) 1.1 equiv CH_3MgBr/0 °C, then 2 equiv *n*BuLi/-65 °C, solid CO_2, (85%); (b) CH_3OH/HCl, (95%); (c) 1 equiv NBS, 5 mol % dibenzoyl peroxide, CCl_4, reflux (80%); (d) **7**, C_6H_6, 1 equiv TEA, reflux, (80%); (e) 5 equiv LiOH/1:1:1 MeOH/THF/H_2O, quant; (f) BOP/DMF/N-methyl morpholine (50-80%); (g) 6 N HCl/dioxane or HCL/EtOAc (90%)

Compound **2** inhibits ADP-induced platelet aggregation with an IC_{50} of 27 nM (Table 1).[9] Gratifyingly, combining the isoindolinone central constraint with α-substituents improved potency in this series. Compounds **12-27** (Table 1) illustrate alkyl and aryl sulfonamides, amide, urea and sulfamamide derivatives **11**, all of which inhibited platelet aggregation at low nanomolar concentrations and showed a two to three-fold improvement in potency over **2**.

Table 1. Sulfonamide derivatives of Lead Compound **2**



no.	R	Inhibition Platelet Aggregation IC_{50} (nM)	SPA-A ED_{50} (nM)	Form B K_D (nM)
1	see figure 1	11	0.97	13
2	see figure 1	27	12	100
12	SO_2CH_3	14	3.4	7.4
13	$SO_2(CH_2)_3CH_3$	9	1.3	4.4
14	$SO_2(CH_2)_3CH_3$ d-isomer	14	5.5	8.8
15	$SO_2CH_2CH(CH_3)_2$	9	1.2	7.5
16	$SO_2(CH_2)_4CH_3$	9	0.88	7.6
17	$SO_2(CH_2)_2OCH_2CH_3$	8	1.1	5.1
18	$CO(CH_2)_4CH_3$	13	4.2	6.4
19	$CONH(CH_2)_3CH_3$	16	1.4	6.9
20	$CONHCH_2C_6H_5$	10	1.2	6.0
21	$SO_2NH(CH_2)_3CH_3$	19	2.8	21
22	$SO_2C_6H_5$	8	0.33	3.7
23	$SO_2$2-thienyl	7	0.53	7.7
24	$SO_2$3-pyridyl	15	0.38	3.6
25	$SO_2CH_2C_6H_5$	4	1.6	12
26	$SO_2$4-$(CO_2H)C_6H_4$	7	2	15
27	$SO_2$2-$(CO_2H)C_6H_4$	15	0.83	6.4

The *in vivo* activity of **2** and L-746,223 (24) was assessed by determining *ex vivo* platelet aggregation responses to ADP (10 μM ADP and 1 μM epinephrine) and collagen (10 μg/mL collagen and 1 μM epinephrine) before and after intravenous administration to conscious dogs.[10] Complete inhibition of *ex vivo*, ADP-induced

platelet aggregation in dogs was achieved with an intravenous bolus dose of 0.1 mg/kg **2**. For compound **24**, complete inhibition was obtained at 0.01 mg/kg. The discrepancy between the *ex vivo* inhibition following intravenous administration and the observed *in vitro* IC_{50} for **2** and **24** suggested that the potency of these α-substituted compounds was not being accurately assessed by the *in vitro* platelet aggregation assay.[11]

To measure the intrinsic differences in potency between compounds, we have isolated the unactivated form of GPIIb/IIIa from platelets and developed two assays based on purified receptor. In the first assay, 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).[12] Binding measurements with fibrinogen indicate that the SPA-A assay provides a close estimate of binding affinity for an *activated* form of GPIIb/IIIa.[13] Displacement of the fluorescent fibrinogen receptor antagonist L-736,622[6] by compounds of interest from the unactivated form of GPIIb/IIIa solubilized in Triton X100 micelles (Form B assay)[14] gave K_D values that provide a close estimate of binding affinity for an *unactivated* form of GPIIb/IIIa.

The α-unsubstituted compound **2** showed an ED_{50} of 12 nM in the SPA-A assay (Table I). α-Substituted analogs **13-27** showed ten to thirty-fold improvements in potency over **2**. They exhibited the same level of potency observed for **1**, thus demonstrating the potency enhancing ability of the α-sulfonamide moiety. Upon examination, the structural influence of the sulfonamide side chain on potency became apparent. For example, longer alkyl chain length was associated with improved potency, with the methyl sulfonamide **12** three to four-fold less potent than the *n*-pentyl sulfonamide **16** (ED_{50} = 3.4 nM versus 0.88 nM). The α-butyl sulfonamide **13** proved to be ten-fold more potent than **2**, (ED_{50} = 1.3 nM versus 12 nM). In contrast to the results observed for **1**, where the (S)-*n*-butylsulfonamide was approximately 100-fold more potent than the (R)-enantiomer analog,[4] enantiomers **13** and **14** showed only a four-fold difference in ED_{50} (1.3nM for S versus 5.5 nM for R, respectively). Incorporation of an oxygen in the chain did not negatively affect potency (**17**, ED_{50} = 1.1 nM). The alkyl derivatives in the amide, urea and sulfamamide series (**18-21**) were compared and shown to be similar in potency to the sulfonamides, with the exception of the hexanoyl derivative **18**, which appeared approximately two to four-fold less potent than the others. Aryl sulfonamides **22-27** were, in general, more potent than alkyl sulfonamides, with the phenyl sulfonamide **22** demonstrating an ED_{50} of 0.33 nM. The α-3-pyridyl sulfonamide **24** proved to be 32-fold more potent than **2**, (ED_{50} = 0.38 nM versus 12 nM), consistent with the in vivo result described above. Heterocycles **23** and **24** were equally potent, however, incorporation of a methylene between the sulfonamide and the aryl group decreased potency approximately three-fold (**25**, ED_{50} = 1.6 nM). The position of an additional carboxy group on the phenyl ring appeared to play a role in determining potency, as the 2-carboxy derivative **27** was about two-fold more potent than the 4-substituted isomer (ED_{50} = 0.83 nM versus 2 nM, respectively). The effect of alpha substituents on potency is therefore profound and complex, possibly reflecting both an electrostatic influence on the acidity of the alpha NH (which may be increased by aryl side chains), and a lipophilic component that is sensitive to requirements of size, shape, and distance.

In general, compounds demonstrated a five to ten-fold decrease in affinity for the unactivated receptor (Form B assay) relative to the activated receptor (SPA-A), however, the affinity for unactivated receptor remained in the low nanomolar range.[14] Thus α-substituted compounds tightly bind both activated and unactivated forms of the receptor.

The *in vivo* oral activity of **1**, **2** and of **24** were compared by determining *ex vivo* platelet aggregation responses to ADP and collagen before and after oral administration to conscious dogs.[10] Compound **1** exhibited mimimal oral activity when dosed at 0.2 mkg/kg. The oral administration of 2 mg/kg **2** resulted in a 60% inhibition of *ex vivo* platelet aggregation for up to 60 min after oral dosing, with platelet aggregation returning to pretreatment levels at approximately 180 min after oral administration. For compound **24** it was possible to achieve 60% inhibition of *ex vivo* platelet aggregation for up to 200 min at a dose 20-fold lower than that used for **2**, 0.1 mg/kg (figure 2). *Thus a compound with potency comparable to **1**, but with greatly improved oral activity, has been identified.*

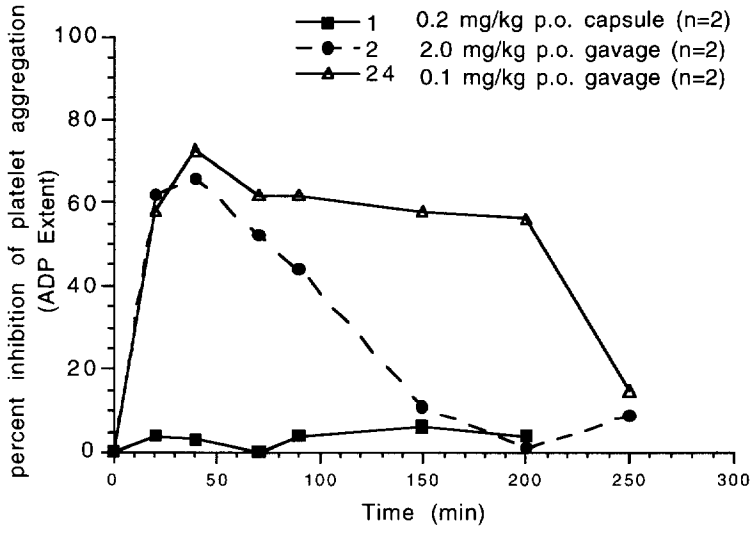


Figure 2. Effect of **1** (0.2 mg/kg), **2** (2.0 mg/kg) and **24** (0.1 mg/kg) po, on the extent of *ex vivo* platelet aggregation in response to ADP (10 μM ADP + 1 μM epinephrine) in conscious dogs.

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8. All compounds were characterized by NMR, TLC, HPLC, High Resolution Mass Spectroscopy and H, C, N analysis.
9. Platelet aggregation was measured in a functional assay that monitors the increase in light transmittance that 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 μM 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%.
10. Lynch, J. J. Jr.; Cook, J. J.; Sitko, G. R.; Holahan, M. A.; Ramjit, D. R.; Mellott, M. J.; Strainieri, M. T.; Stabilito, I. I.; Zhang, G.; Lynch, R. J.; Manno, P. D.; Chang, C. T.-C.; Egbertson, M. S.; Halczenko, W.; Duggan, M. E.; Laswell, W. L.; Vassallo, L. M.; Shafer, J. A.; Anderson, P. S.; Friedman, P. A.; Hartman, G. D.; and Gould, R. J. *J. Pharm. Exp. Ther.* **1995**, *272*, 20.
11. The potency of α-substituted compounds may approach the lower limit of accuracy of the assay for inhibition of aggregation. The lowest observed IC_{50} in a receptor binding assay can never be less than 50% of the concentration of receptors used in the assay, in this case, 4-16 nM (calculated from 2 x 10^8 platelets/mL, 20,000 to 100,000 receptors per platelet).
12. Purified GP IIb/IIIa was coated onto yttrium silicate Scintillation Proximity Assay Fluomicrospheres (Amersham RPN 143) and is abbreviated IIb/IIIa/SPA. The binding of the RGD-containing heptapeptide [^{125}I]-L-692,884 (New England Nuclear, NEX-330) to the yttrium silicate containing IIb/IIIa/SPA is detectable, without the necessity of separation of bound from free, in a Top Count Scintillation Counter. The ED_{50} for a nonradiolabeled compound was determined by competition with the binding of [^{125}I]-L-692,884 to IIb/IIIa/SPA at pH 7.5 (20 mM HEPES, 0.15 M NaCl, room temperature) with ~0.3 nM of IIb/IIIa/SPA, ~0.3 nM of [^{125}I]-L-692,884 and a wide range of concentrations of the competing non-radiolabeled compounds. After equilibration, the bound CPM are measured and the ED_{50} value determined by non-linear least square fit to CPM = $(B_{max}-B_{min})/(1+(I/ED_{50})^B) + Bmin$, where **I** is the concentration of the test compound, **B** is the Hill slope, **Bmax** is the maximum binding observed without the test compound, and **Bmin** is the non-specific binding signal. The average standard error of mean for ED_{50} determinations was ± 20%.
13. Bohumil Bednar, Rodney A. Bednar, unpublished results. The two assays described here will be discussed in greater detail in subsequent publications.
14. GPIIb/IIIa was purified from resting human outdated platelets as described in Kouns, W. C.; Hadvary, P.; Haering, P.; Steiner, B. *J. Biol. Chem.* **1992**, *267* , 18844. Competitive fluorescent displacement binding measurements were done with an inactive form of GPIIb/IIIa (typically 0.6 uM) solubilized in Triton X-1000 buffer (0.1% Triton X-100, 20 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) and 1 uM of L-736,622[6] at 20 °C. Changes in the fluorescence of the solution upon addition of nonfluorescent fibrinogen receptor antagonists were recorded and, after correction for the fluorescence of GPIIb/IIIa and subtraction of the fluorescence of L-736,622 in the buffer, were plotted as % of fluorescence of the initial solution (no nonfluorescent ligand present). The values of K_D were calculated from displacement measurements using the K_D value for L-736,622 of 3.7 nM obtained in stopped-flow measurements. Due to the high concentration of GPIIb/IIIa required, this fluorescence assay can determine K_D values in the low nanomolar, but not the sub-nanomolar range.

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