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NONPEPTIDE GLYCOPROTEIN IIB/IIIA INHIBITORS. 19. A NEW DESIGN PARADIGM EMPLOYING LINEARLY MINIMIZED, CENTRALLY CONSTRAINED, EXOSITE INHIBITORS.

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Abstract: A new series of potent, linearly-minimized, orally active, selective GPIIb/IIIa inhibitors is identified. Thus **15** (L-750,034) achieves interaction via a constrained, non-turned conformation that maintains the proper distance between its charged termini and full sulfonamide exosite interaction. The diminutive stature and the proposed linear conformation of L-750,034 define a new paradigm for the conceptualization of RGD mimics. © 1999 Published by Elsevier Science Ltd. All rights reserved.

The binding of fibrinogen to platelets via the activated, surface glycoprotein IIb/IIIa (GPIIb/IIIa) is the common, final step for platelet aggregation leading to the formation of arterial thrombi, the causative factor in ischemic pathologies such as myocardial infarction and unstable angina.[1] Although controversy exists about the role of the α- and γ-chains of fibrinogen in binding to GPIIb/IIIa, peptides that contain RGD (arginyl-glycyl-aspartyl), and nonpeptides that mimic RGD, can efficiently inhibit fibrinogen binding and, consequently modulate platelet aggregation and thrombus formation.[2,3] Several of these agents are currently in use as antithrombotic agents in the treatment of unstable angina and as adjunctive therapy during percutaneous transluminar coronary angioplasty (PTCA).[4,5]

Extensive studies of both linear[6,7] and cyclic[3,8–10] peptides using nmr, computational, and empirical SAR methodologies have sought to delineate the receptor bound conformation(s) of the RGD segment of these optimized inhibitors. The initial expectation was that within the RGD unit of peptides a specific, unique, conformation existed that would both characterize potent ligands for GPIIb/IIIa and determine selectivity for closely related integrin receptors. Although it has been claimed that RGD peptides assume a type II β-turn conformation in solution, the bulk of the data suggests that, for the inhibitors containing the RGD triad, multiple conformations exist that allow for tight binding to GPIIb/IIIa.[3,11,12] Similarly, the structurally diverse group of potent, nonpeptide inhibitors that have been reported exhibit a range of cupped,[13–15] turn-extended-turn,[10,16,17] and Gly-Asp β-turn[18] orientations in support of the notion of a flexible RGD binding motif.

Seeking to simplify matters, we sought to define the minimal linear array of atoms that would provide a potent GPIIb/IIIa ligand. Previously, we identified **1** (L-709,780)[19] as a structurally simple, yet potent and selective GPIIb/IIIa ligand, whose backbone spans 17 atoms. In this report, we identify a potent, centrally

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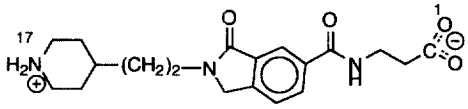
constrained, orally active, selective GPIIb/IIIa antagonist **15** (L-750,034) that possesses a linearly minimized 14-atom molecular array connecting the charged N- and C-termini.

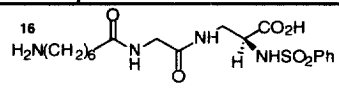
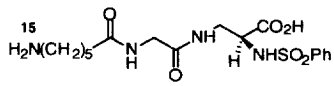
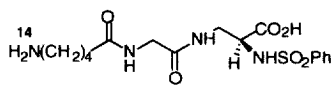
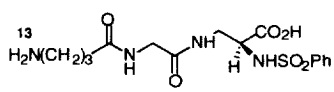


1 (L-709,780)

To date, potent inhibitors of GPIIb/IIIa have invariably displayed a linear or cyclic ensemble of 16–18 atoms encompasing the charged centers in mimicry of the thru-bond extension of RGD.[20] Consideration of alternative frameworks suggested to us that, with the proper molecular array, one might be able to bridge across the turn described above. Such a molecule could still partake of the favorable energetics at the charged termini, but would possess fewer atoms and a minimized thru-bond length. From the start, it was clear that the atomic and topograpical arrangement employed would be of key importance since numerous studies of termini distance requirements had provided the similar finding of 16–18 atoms as optimal.[9,14,18–27]

Our initial work indicated that minimizing the length of simple linear, unconstrained molecules, while maintaining potency, would be problematic. Thus, the chain shortened compounds **4**, **5**, and **6** employing, respectively, 15, 14, and 13 atom arrays proved to be, respectively 7, 76, and >79-fold less potent than the 16-atom inhibitor **3** (Table 1). Conceptually, these results were discouraging since we had anticipated that these flexible probes would offer perhaps our best opportunity to match GPIIb/IIIa with a shortened ligand.

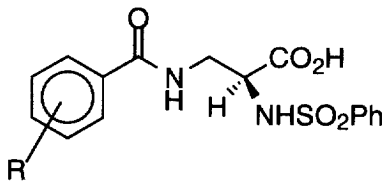
Table 1. Length vs Potency Comparison For Unconstrained Inhibitors

	Compound	Inhibition of Platelet Aggregation IC_{50} (μM)[19]
3		0.38
4		2.8
5		29
6		>30

Our attention was next directed to minimizing length in structures containing central constraint,[19] a key feature of potent inhibitors from several laboratories, in conjunction with a flexible N-terminus. Relying on the regiodifferentiated 4-hydroxybenzoyl fragment, the compounds in Table 2 were prepared. Typically, **7** (IC_{50} = 0.013 μM) and **8** (IC_{50} = 0.023 μM), utilizing 17 and 16 atoms, respectively, were the most potent compounds in the series. The shorter molecules **9** (15 atoms) and **10** (14 atoms) were four- and eightfold, respectively, less potent than the optimized inhibitor **7**. Interestingly, the meta-substituted analog **11** (13 atoms) displayed an IC_{50} = 9.2 μM, signaling a dramatic 83-fold loss in potency compared to **10**. Taken together, the data show that the

difference in potency between the centrally constrained inhibitors **8** (16 atoms) and **10** (14 atoms) was only five-fold, compared to the 76-fold difference between the unconstrained analogs **3** (16 atoms) and **5** (14 atoms). This striking comparison suggests the availability of a pseudo-optimal binding conformation at the distance provided by the 14 atom array of **10**.

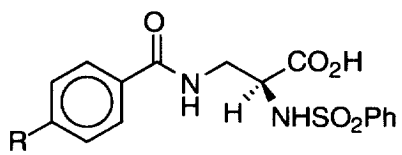
Table 2. Length vs Potency Comparison For 4-Hydroxybenzamide Analogs

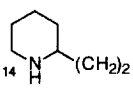
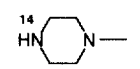
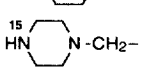
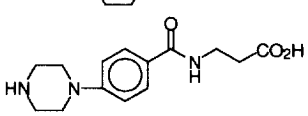


Compound	R	Inhibition of Platelet Aggregation IC_{50} (μM)[19]
7	4- $H_2N(CH_2)_5O$— (17)	0.013
8	4- $H_2N(CH_2)_4O$— (16)	0.023
9	4- $H_2N(CH_2)_3O$— (15)	0.055
10	4- $H_2N(CH_2)_2O$— (14)	0.11
11	3- $H_2N(CH_2)_2O$— (13)	9.2

To further probe molecular frameworks containing 14 atoms, we prepared carbon chain analogs of **10** (Table 3). Compound **12** (14 atoms) was 18-fold more potent than **13** (13 atoms) and each was approximately 2-fold less potent than the corresponding oxygen isosteres **10** and **11**, indicating the importance of the spatial orientation of the benzylic C-O/C-C bonds. Maintaining the 14 atom disposition of **12**, and attempting to affect subtle alteration of the C-N terminal distance, we conformationally constrained the N-terminus in analogy to previous work[12,19,21,28,29] that demonstrated potency and selectivity enhancements from appropriate N-terminal heterocycles.

Table 3. Length vs Potency Comparison For Centrally Constrained Inhibitors



Compound	R	Inhibition of Platelet Aggregation IC_{50} (μM)[19]
12	$H_2N(CH_2)_3$ — (14)	0.27
13	$H_2N(CH_2)_2$ — (13)	4.9
14		0.13
15 (L-750,034)		0.017
16		0.034
17		230

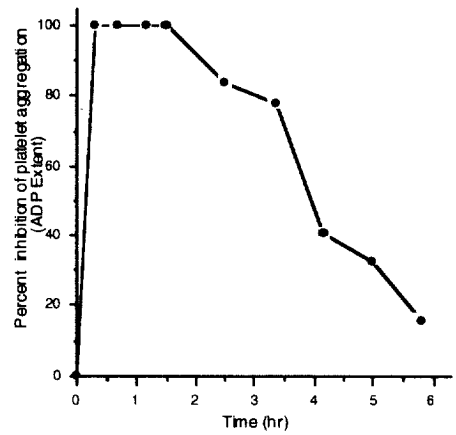
Cyclic constraint at the N-terminus of **12** afforded piperidine **14** which displayed a modest twofold increase in potency. Gratifyingly, however, the internally constrained piperazine[28] **15** (L-750,034) had IC_{50} = 0.017 μM, and was thus 16-fold more potent than the uncyclized amine **12** and >1700-fold more potent than the non-centrally/non-terminally constrained inhibitor **5**, which also spanned 14 atoms. That **15** represents an optimized molecular array is supported by the observation that the analog **16**, extended by an additional methylene unit, was 2-fold less potent than **15** in head-to-head comparisons. Similarly, analog **17**, lacking the α-sulfonamido substituent, was >13,500-fold less potent than **15** attesting to the importance of the exosite sulfonamido interaction.[21]

Compound **15** showed good oral activity in the dog with a single dose of 2.0 mg/kg inhibiting *ex vivo* platelet aggregation >40% for 4h (Figure 1). In addition, **15** displayed >100-fold selectivity for binding to GPIIb/IIIa by comparing the inhibition of attachment of HUVEC cells to fibrinogen, vitronectin and fibronectin coated surfaces.[30]

Molecular modeling studies of **15**[31] indicate that this highly constrained ligand can adopt low energy conformations that position the piperazine terminal nitrogen atom and the carboxylic acid carbon 10.1–13.0 Å apart. This separation distance range contains a subset of that distance range proposed[15] for active conformations of **1**. Superposition of conformations of **15**[32] onto those proposed, active conformations of **1**[15] reveals that **15** may act as a molecular ruler, as illustrated in Figure 2, spanning the charged centers in a more direct fashion than the cupped or ladle-shaped conformations deployed by longer GPIIb/IIIa ligands. To our knowledge, such a linear array is without precedent in the literature that describes the bound conformation of highly potent GPIIb/IIIa ligands.

Figure 1:

Effect of **15** (2 mg/kg po gavage) on *ex vivo* platelet aggregation responses to ADP (10 μM and 1 μM epinephrine) expressed as percent inhibition over time in conscious dogs. Data are mean for n = 2.



In summary, compound **15** (L-750,034) is a potent, linearly-minimized, orally active, selective GPIIb/IIIa inhibitor that achieves interaction at GPIIb/IIIa via a constrained, non-turned conformation that maintains the proper distance between the charged termini and allows full opportunity for sulfonamido exosite interaction. The diminuitive stature and the preferred linear conformation of L-750,034 at the receptor define a new paradigm for the conceptualization of RGD mimetics.

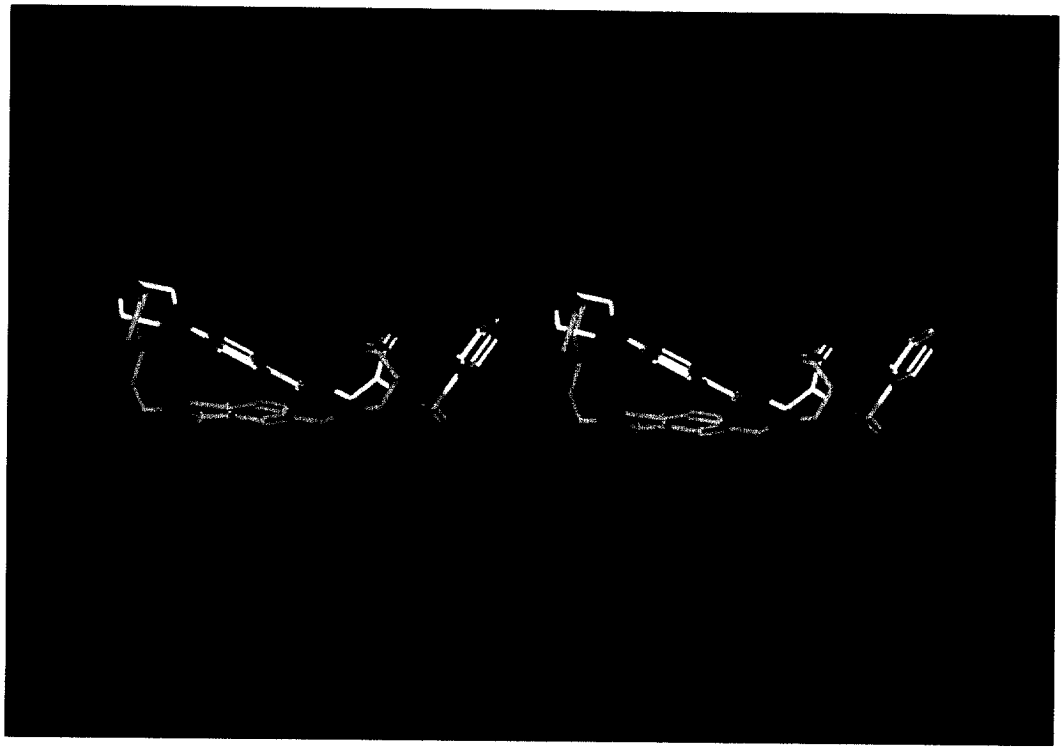


Figure 2: Superposition comparison of preferred receptor-associated conformations for **15** and **1**. L-709,780 (**1**) is in yellow and L-750,034 (**15**) is in white.

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