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Nonpeptide GPIIB/IIIA Receptor Antagonists. Part 21: C-6 Flexibility and Amide Bond Orientation are Important Factors in Determining the Affinity of Compounds for Activated or Resting Platelet Receptors

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Abstract—Compound affinity for activated and resting GPIIb/IIIa receptors may differ, and comparison of those differences determines selectivity. Structural features that influence selectivity are discussed. © 2000 Published by Elsevier Science Ltd.

Introduction

Platelets have a unique ability to transform almost instantaneously from a resting to an activated form under the influence of agonists such as thrombin or collagen.[1] The integrin receptor, glycoprotein IIb/IIIa (GPIIb/IIIa), is expressed on the surface in both forms. Platelet activation results in a conformational change of this receptor, and in a great enhancement of the receptor's ability to bind to the serum protein, fibrinogen. Fibrinogen binding results in the aggregation of platelets.[2–4] The GPIIb/IIIa antibody abciximab and inhibitors that were designed to mimic the tripeptide sequence Arg-Gly-Asp (RGD) found in fibrinogen, such as the cyclic peptide eptifibatide and the small nonpeptide tirofiban, are able to competitively inhibit the binding of fibrinogen and thus inhibit platelet aggregation.[5,6] They are now used clinically to block platelet aggregation in the treatment of unstable angina.[4,7,8]

It has been reported that the GPIIb/IIIa conformational change can be detected with antibodies,[9,10] and that the precise structure of the activated conformation depends upon the method of activation used.[11] Occupied GPIIb/IIIa, but not unoccupied resting or activated receptor, displays ligand induced antibody binding sites (LIBS).[12] There are multiple binding sites on GPIIb/IIIa, and some sites recognize small peptides or RGD-mimics, while others recognize fibrinogen and abciximab or analogues based on a 10-amino acid sequence found in fibrinogen.[13–15] The binding of RGD-type inhibitors has been observed to induce a conformational change in the receptor resulting in a high-affinity form.[16,17] Despite what has been learned thus far, the molecular basis for the selective binding of fibrinogen to activated platelets is unknown. Therefore the discovery of small molecules that show selectivity in binding to resting and activated forms of the receptor is of interest.

In the past, the potency of GPIIb/IIIa antagonists has been assessed using inhibition of platelet aggregation[18] or a solid-phase assay employing microplates coated with GPIIb/IIIa or fibrinogen.[19] However, neither assay provides information on the affinity of potent GPIIb/IIIa antagonists for resting platelets. Further, the measurement of inhibition of platelet aggregation under typical conditions (2×10^8 platelets/mL, 20,000–100,000 receptors/platelet and therefore ~16–32 nM of receptors) does not provide a true measure of affinity for highly potent compounds (K_d less then 1 nM) since the IC_{50} can never be less than ~8–16 nM (50% of the concentration of receptors). In earlier publications, we have

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reported the development of assays to characterize the tight binding[18,20,21] of the first reported high affinity inhibitors[18,22,23] and the biological effects that result from tight binding (i.e. the long duration of platelet inhibition in vivo).[18,23–25] To measure the affinity of antagonists for resting and activated form of GP IIb/IIIa, resting and activated forms of the receptor from human platelets were isolated and assays based on purified receptor were developed. It was further demonstrated that affinities obtained in assays with a purified receptor agree well with affinities determined on resting and activated platelets.[18,20,26] Using these assays we were thus able to identify two families of platelet glycoprotein IIb/IIIa inhibitors from the compounds we prepared. One family binds with significantly different affinities to the activated and resting forms of the receptor, the other shows less selectivity in binding. Below are summarized our findings regarding selectivity and structure for these series of compounds.

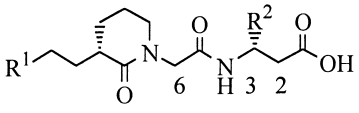
Results

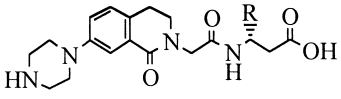
Compounds that demonstrate selectivity between resting and activated GPIIb/IIIa are shown in Tables 1 and 2. Common structural features among these compounds include substitution at the C-terminal β-alanine C3 carbon and incorporation of a flexible, unsubstituted, glycine linkage at the central portion of the molecule, encompassing the C6 carbon. Selectivity was determined by comparing the activity of the compounds in two assays. The first, the flow cytometry assay,[27] measured the affinity of compounds (K_D) for *resting* human platelets using competitive displacement of fluorescein-containing isoindolinone GPIIb/IIIa antagonist L-762,745.[18,20] In the second assay, K_D values were calculated from the competitive binding between the compounds of interest and the GPIIb/IIIa antagonist {125I}L-692,884 to purified GPIIb/IIIa activated by coating onto yttrium silicate Scintillation Proximity Assay Fluomicrospheres (SPA-Assay).[18,20,26] The ratio of these assays is used as the selectivity for binding to resting versus activated GPIIb/IIIa and is shown in the Tables as R/A.[28,29] Close examination of SAR in these series offer some interesting insights into the requirements for selectivity for resting platelet receptors.

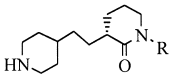
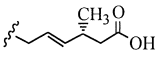
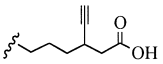
The incorporation of a lactam of the type found in **1**[33] predisposes many compounds toward selectivity (for example, see **2**, **3**, **5–7**, **9**). The β-carbon substituent may also have an effect. Comparison of **8** vs **9**[34] shows a 5-fold decrease in affinity for resting receptors upon replacement of a β-3-pyridyl group with an acetylene. A similar trend is observed in other series (Table 1, **4** vs **5**[34]) but not all (Table 2, **10** vs **11**). The C-terminal amide bond appears to have little effect on selectivity, as the selectivity of **1** can be maintained when the C-terminal amide bond of **1** is replaced by a *trans* double bond (**6**),[33a] or when the amide of **3** is replaced by ethylene (**7**).

The orientation of the N-terminal amide bond may have an effect on selectivity. Table 2 shows a comparison of compounds **2** and **3** that contain a 'forward' lactam with compounds **10** and **11** that have a 'reverse' lactam. Selectivity is reduced for the 'reverse' lactam-containing

Table 1. Patelet aggregation, binding constants and selectivity ratios for GPIIb/IIIa antagonists

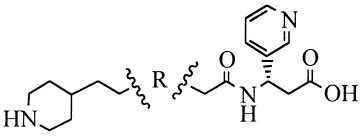


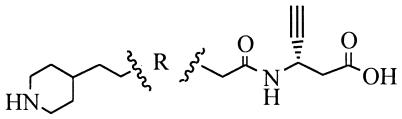


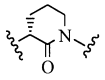
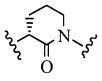
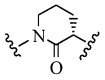
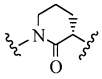
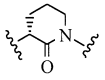
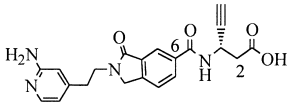
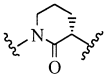
No.	R^1	R^2	Plaggin[a] IC_{50} nM	K_D nM[b] Resting	K_D nM[b] Activated	~R/A[c]	No.	Plaggin[a] IC_{50} nM	K_D nM[b] Resting	K_D nM[b] Activated	~R/A[c]
1		CH_3	32	640	4.5	140					
2			10	84	0.38	221	**4**	13	3.4	0.25	14
3			13	210	0.86	244	**5**	15	50	0.56	89
											
6			57	2300	12	192	**8**	12	23	0.77	30
7			51	3100	9	344	**9**	15	120	0.85	141

[a]See ref 30.
[b]See ref 31.
[c]See ref 32.

Table 2. Comparison of 'forward' and 'reverse' amide compounds





No.	R	Plaggin	K_D nM		~R/A	No.	R	Plaggin	K_D nM		~R/A
		IC_{50} nM	Resting	Activated				IC_{50} nM	Resting	Activated	
2		10	84	0.38	221	**3**		13	210	0.86	244
10		9	11	0.3	37	**11**		15	6.1	0.34	18
12		28	340	0.78	435	**14**		27	84	2.2	38
13		19	60	0.26	230						

compounds relative to their 'forward' lactam counterparts. Comparison of **11**, **12** and **13** offers an interesting example of how combination of the proper N-terminus and β-carbon substituent can improve selectivity, even in a series with a nonselective bias.

However, the selectivity enhancing effects of the N-terminal group and β-substituent may be modulated by replacement of the C6-flexible backbone (**13**, 230-fold selective) with a scaffold in which the C6 carbon is constrained in an aromatic ring (**14**, 38-fold selective). Further examples can be found in Table 3. The β-unsubstituted analogue **15**[22] is only ~9-fold selective, and the β-unsubstituted pyrazololactam **16**[23] is only 14-fold selective. Although the results are somewhat confounded by its racemic nature, compound **17** is unselective relative to **1**. A similar difference in selectivity between C6-flexible and constrained compounds can be observed by comparing **18** and **19**,[34] which have the same β-hydroxymethyl β-alanine C-terminus but differ in their central and N-terminal regions.

Table 4 shows a series of C6 constrained, α-substituted analogues that exhibit high affinity for both resting and activated GPIIb/IIIa and thus low selectivity. A variety of structures, such as simple phenyl rings,[22]

Table 3. C6-Constrained compounds

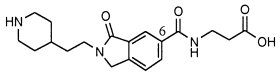
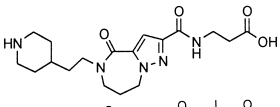
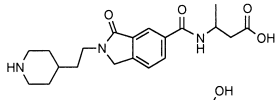
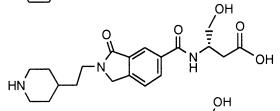
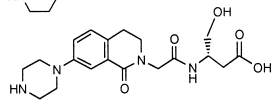
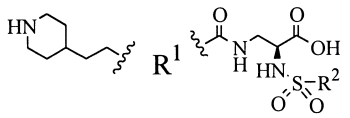
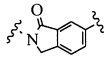
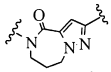
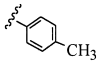
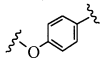
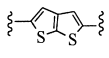
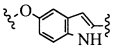
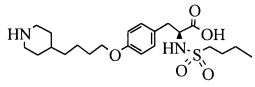
No.	Structure	Plaggin	K_D nM		R/A
		IC_{50} nM	Resting	Activated	
15		27	120	13	9
16		470	680	46	14
17		65	49	64	0.8
18		8	47	21	2
19		81	>1000	15	>67

Table 4. C6-Constrained compounds



No.	R^1	R^2	Plaggin IC_{50}nM	K_D nM Resting	K_D nM Activated	R/A
20			8	3.1	0.25	12
21			8	1.1[35]	0.08	18
22			13	2.4	0.16	15
23			8	2.1	0.09	23
24			10	1.3	0.056	24
25			15	13	1.9	7

isoindolinones, thienothiophenes,[18] pyrazololactones[23] and indoles[34a] may be employed as central constraints to afford selectivities of 7- to 25-fold.

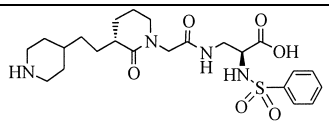
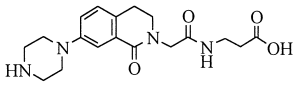
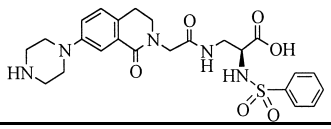
Selectivity may also be dependent on the site and structural type of C-terminal substitution. For example, C3-substituted analogue **1** displays greater selectivity for the resting receptor than does the C2-analogue **26** (Table 5). Similarly, **5** is more selective than **28**. For most series, constrained or flexible, alpha sulfonamide substitution tends to increase potency against both resting and activated platelets.

The effects of the alpha and beta substituents on potency may differ depending on whether the substituted compound is C6 constrained or is centrally flexible. α Substitution, which usually results in a profound increase in potency for the activated receptor (activated K_D) in the centrally constrained series (see **15** vs **20**, (40-fold), or **16** vs **21**, (600-fold)), has less of an effect on the potency of some non-centrally constrained compounds (see **27** vs **28** (1.4-fold) and **1** vs **26** (6-fold)). It is difficult to explain exactly why this is so in the absence of X-ray data, however, empirically it has been shown that the glycine portion of the RGD binding motif is very sensitive to substitution of any kind, with even a simple methyl group causing large losses in activity. The different steric demands that arise from more or less flexibility in the center of the molecule may determine the accessibility of binding pocket(s) to the substituents at the C terminus, and therefore have an affect on the affinity of compounds for activated receptors.

Conclusions

Selectivity is influenced by several factors. Many compounds containing a C6 flexible region and an amide bond of the correct orientation were found to be selective. Compounds possessing a C6 constrained region were

Table 5. α-Substituted C6 flexible compounds

No.	Structure	Plaggin IC_{50}nM	K_D nM Resting	K_D nM Activated	R/A
26		11	15	0.78	19
27		140	nd	9.7	—
28		35[36]	13	6.7	1.9

less selective. Selectivity in some centrally flexible compounds could be increased by the use of certain N-terminal groups and β-carbon substituents. α Sulfonamide substitution affects C6 flexible and C6 constrained series to lesser and greater degrees but in general contributes to a decrease in selectivity.

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36. Note that the plaggin value for compound **28** is different from that reported previously (ref 34). The earlier report was in error.