Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 19, pp. 2537-2542, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10014-2

DIHYDROISOQUINOLONE RGD MIMICS. EXPLORATION OF THE ASPARTATE ISOSTERE

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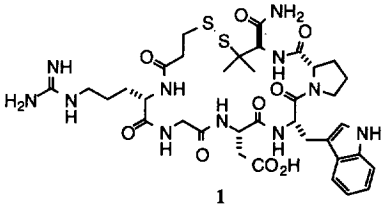
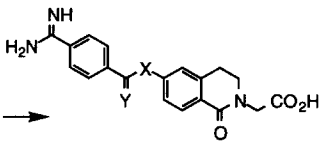
Abstract: Disubstituted 3,4-dihydroisoquinolones that contain an ether-linked benzamidine at C_6 and a β-substituted aspartate mimic at C_2 offer enhanced affinity for GPIIb-IIIa relative to the non-substituted isoquinolone propionate. Alkyl substituents afforded a 10-fold increase in intrinsic activity while aryl substituents yielded a 40-fold improvement. © 1997 Elsevier Science Ltd.

Platelets contribute to the maintenance of hemostasis by adhering to damaged vessel walls and subsequently aggregating via the plasma proteins fibrinogen and Von Willebrand factor to form a plug that prevents blood loss.[1] While this function of platelets is desirable, uncontrolled or misplaced platelet aggregation can lead to life threatening vessel occlusion. The development of agents that can modulate platelet function may therefore have significant therapeutic potential in arterial thrombosis.[2] Platelet aggregation is known to be mediated by the membrane bound integrin GPIIb-IIIa, and it is the activated form of this receptor that binds with the multivalent plasma protein fibrinogen. GPIIb-IIIa is known to bind one of two arginine-glycine-aspartic acid (RGD) sequences located on the fibrinogen α chain between residues 95-97 and 572-574.[3] The isolated tripeptide sequence RGD has been found to have affinity for GPIIb-IIIa, and as a result, this motif has become the starting point for the development of novel inhibitors of GPIIb-IIIa and platelet aggregation.[4] Many examples of modified linear RGD analogues and conformationally constrained cyclic variants have been described. The latter compounds offer a unique opportunity to evaluate the conformations of the critical RGD region with the prospect of using the information obtained in the design of potent non-peptide GPIIb-IIIa antagonists.[5]

We have recently reported on the NMR analysis of cyclic heptapeptide **1**, which exhibits potent activity towards GPIIb-IIIa.[6,7] Our investigation revealed that the RGD portion of this molecule likely exists in a Gly-Asp type II' β-turn, thus fixing the relative position of the side chains for the arginine and aspartic acid residues (Figure 1). Non-peptide mimics designed from this β-turn employed an isoquinolone nucleus substituted at C_2 and C_6 with an acetic acid residue and a tethered arginine isostere, respectively. Initial SAR studies demonstrated that substantial gains in activity could be achieved with modification of the arginine isostere and these efforts culminated in the development of a family of disubstituted isoquinolones (**2**,**3**) with high affinity for GPIIb-IIIa. Since the activity gains were realized solely by modification of the arginine isostere, the possibility that further increases in potency may result from modification of the aspartate isostere existed. In this report we describe efforts towards the optimization of the aspartate isostere found in this series of isoquinolone based RGD mimics.



1



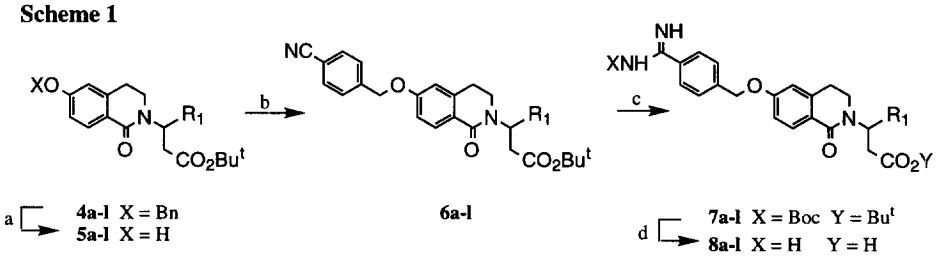
2

		ELISA IC_{50} (μM)	PRP IC_{50} (μM)
1		0.10	0.10
2	X = O, Y = H,H	0.60	30.0
3	X = NH, Y = O	0.003	0.09

Figure 1. Cyclic peptide **1** and active isoquinolone based Gly-Asp RGD mimics.

The 2,6-disubstituted isoquinolone propionates required for this study were readily synthesized from compounds **4a–l**, which were derived from 6-benzyloxy-3,4-dihydroisoquinolone using acyliminium ion chemistry as previously described.[8,9] These intermediates were subjected to the action of palladium and hydrogen affording the free phenols **5a–l** (Scheme 1). The free phenol in these derivatives was then alkylated with *p*-cyanobenzyl bromide, in the presence of K_2CO_3, which afforded the substituted analogues **6a–l**. The nitrile moiety in these compounds was converted to a readily purified BOC-protected amidine (**7a–l**) using a thio-Pinner[10] sequence. Reaction with TFA provided the desired analogs **8a–l** as their corresponding trifluoroacetate salts.

Scheme 1

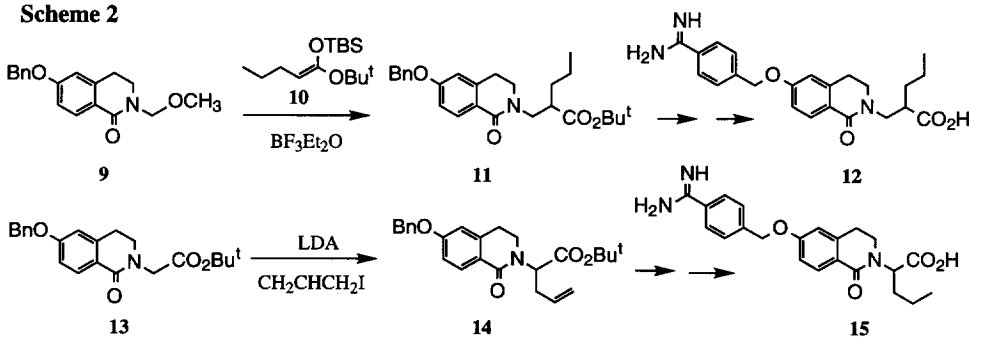


(a) H_2 Pd/C; (b) *p*-cyanobenzyl bromide-K_2CO_3; (c) H_2S-MeI-NH_4OAc-Boc_2O; (d) TFA

One example of an α-substituted isoquinolone propionate was prepared using similar chemistry as for compounds **4**. In this case, α-methoxy amide **9** was allowed to react with ketene acetal **10**, in the presence of BF_3•Et_2O, which provided **11** (Scheme 2). This intermediate was transformed into the desired amidino substituted analog **12** using the same procedure outlined for compounds **8**. One example of an α-substituted isoquinolone acetate was prepared from the known isoquinolone derivative **13** by deprotonation with LHMDS and subsequent quenching of the resulting lithium enolate with allyl iodide. This intermediate (**14**) was also transformed into the desired Gly-Asp mimic **15** using the chemistry previously outlined.

The biological activity of these constructs was evaluated in vitro by measuring the ability of the compound to inhibit the binding of fibrinogen to purified human GPIIb-IIIa in an ELISA.[11] Compounds displaying sufficient activity (IC_{50} < 0.5 μM) were further evaluated in a functional assay which determined the agents' ability to inhibit ADP induced platelet aggregation in human platelet-rich plasma (PRP).[12] Compound **2**, and the peptide **1**, from which it was derived, served as comparators for these studies (Figure 1).

Scheme 2



The initial goal was to determine the effect of a one carbon extension of the acidic side chain on receptor affinity. Activity data from compound **8a** indicates that additional length in the aspartate isostere was well tolerated affording a 2-fold increase in intrinsic activity (ELISA) and a 70-fold increase in functional (PRP) activity over the lead compound **2**. Addition of a 2-carbon aliphatic substituent β to the carboxylate yielded analog **8b** which was 7-fold more potent (by ELISA) than **8a.** Sequential elongation of this substituent from 2 to 6 atoms (**8c–f**) provided no additional gains in the observed ELISA activity and a loss of activity in PRP for the hexyl substituent.

Table 1. Activity of β-substituted isoquinolone propionates **8**.

Compound	R	ELISA IC_{50} (μM) [a,b]	PRP IC_{50} (μM) [b,c]
8a	H	0.32	0.42
8b	CH_2CH_3	0.046	0.30
8c	$(CH_2)_2CH_3$	0.047	0.23
8d	$(CH_2)_3CH_3$	0.040	0.60
8e	$(CH_2)_4CH_3$	0.024	0.57
8f	$(CH_2)_5CH_3$	0.041	3.00
8g	$(CH_2)_3OCH_2CH_3$	0.061	0.47
8h	$(CH_2)_3OCH_3$	0.056	0.60
8i	$CH_2O(CH_2)_2OCH_3$	0.090	0.68
8j	Ph	0.007	0.20
8k	p-$C_6H_4CO_2CH_3$	0.006	0.22
8l	p-$C_6H_4OCH_3$	0.008	0.26

a) Concentration required to inhibit the binding of fibrinogen to purified GPIIb-IIIa by 50%.
b) The IC_{50} values are expressed as the average of at least two determinations. The average error for the determinations was ±15%.
c) Concentration required to reduce ADP induced human platelet aggregation response by 50%.

The difference between intrinsic activity (ELISA) and functional PRP activity (plasma based assay) of these β-alkyl substituted analogs ranged from 1- to 5-fold for analogs **8a–c** to >15-fold for analogs **8d–f**. We hypothesized that the hydrophobicity of the β-substituent influenced functional activity by facilitating protein binding, thus reducing activity in plasma. In order to test this concept, the ELISA was modified by incorporating additional bovine serum albumin (BSA) in concentrations up to 4.5% (w/v). Compound **8e** and **8f** were then reevaluated with this modified assay and the data revealed that both compounds had a concentration dependent decrease in the inhibition of fibrinogen binding with increasing concentrations of BSA (see Table 2). Over the BSA concentration range studied, compound **8e** appeared to be less influenced by

additional protein (4-fold loss) than the one carbon extended congener **8f** (7.5-fold loss) suggesting that the hydrophobicity of the β-substituent can mitigate functional activity. These experiments demonstrate that exogenous plasma protein can decrease the ability of these Gly-Asp mimics to inhibit the binding of fibrinogen to GPIIb-IIIa and provides a possible explanation for the observed disparity between the ELISA and PRP activities for this series.

Table 2. Activity of **8e**, **8f**, and **8g** as a function of BSA concentration in the ELISA.

	ELISA IC_{50} (μM)[a]		
BSA Concentration	**8e**	**8f**	**8g**
0.1 %	0.024	0.041	0.061
1.0 %	0.040	0.080	0.073
4.5 %	0.10	0.31	0.10

a) See Table 1.

In an effort to minimize the difference between ELISA and PRP activity in this series, the hydrophobicity of the β-substituent was modified by the incorporation of heteroatoms. Derivative **8g**, which contains an ether linkage in the carbon chain β to the carboxylate, displayed similar intrinsic (ELISA) activity as its all carbon congener **8f**. However, functional activity was increased by 6-fold, suggesting that this compound was less affected by protein binding. Evaluation of the intrinsic activity of **8g** in the presence of BSA confirmed that the inhibition of fibrinogen binding was not effected to the same extent as compound **8f** (Table 2). Unfortunately, attempts at further increasing functional activity by removing the terminal carbon from the β-substituent (**8h**) or incorporating an additional oxygen atom (**8i**) were unsuccessful.

Replacement of the extended alkyl chain with a phenyl group (**8j**) provided a 10-fold increase in intrinsic activity relative to **8b–i** but functional activity for this compound was comparable to the most active analogs containing alkyl substitution (**8b** and **8c**). Substitution at the *para* position of the phenyl ring with a methoxy (**8l**) or carbomethoxy (**8k**) provided no additional gains in either activity parameter.

The aspartate isostere of this series was further examined by altering the length and substitution of the chain between the isoquinolone nucleus and the carboxylate (Table 3). Moving the β-alkyl chain of **8c** to the α-position afforded compound **12** (Scheme 2), which was 40-fold less active in the ELISA. Removing one carbon from the propionate chain of **8c** provided the α-substituted acetic acid derivative **15**, which was also roughly 40-fold less active than the corresponding β-substituted propionate and 2.8-fold less active than the non-substituted derivative **2**.

Table 3. α-Substituted isoquinolone analogs.

Compound	ELISA IC_{50} (μM) [a]
12	1.8
15	1.7

a) See Table 1.

Comparison of lead isoquinolone **2** and the one carbon extended congener **8a** indicates that a longer aspartate mimic is beneficial for this series of compounds. Additionally, the inclusion of a substituent β to the carboxylate significantly enhances activity. The size of the substituent seems to be of little importance for

intrinsic activity in that a small 2 carbon residue (**8b**) imparts as much benefit as a large 6 carbon residue (**8f**). Interestingly, phenyl substitution (**8j**) provides an additional 10-fold increase in intrinsic activity over alkyl substitution. A possible explanation for the observed increase in activity is that the β-substituent contained in these compounds interacts favorably with a hydrophobic binding site in the GPIIb-IIIa receptor. Hydrophobic binding sites in the receptor have long been postulated, because of the observation that the incorporation of a residue with a hydrophobic side chain, at the C-terminus of the RGD sequence, provides an increase in potency.[13] Other reports in the literature demonstrate that substitution of the aspartate isostere at the β position leads to enhanced potency.[14] If in fact the β-substituent is interacting with a hydrophobic binding site, data for **8b–l** suggest that it is tolerant of a wide variety of substituents.

An equally plausible explanation for the increase in activity in this series is that incorporation of the β-substituent changes the conformation of the aspartate isostere such that it is preferred for binding to GPIIb-IIIa. Insight into the conformation of the aspartate isostere contained in compounds **8** can be obtained from ^{1}H NMR spectral data. The β-methine proton α to the amide in these compounds is observed at 5 ppm, which indicates that it resides in the deshielding cone of the lactam carbonyl. A conformation in which the methine proton is co-planar with the lactam carbonyl requires a non-planar arrangement of the carboxylate moiety and the central constraint, which imparts a cup shape to an otherwise flat molecule. A cupped shape has been thought to be a critical feature for potent RGD mimics.[5c] The degree of conformational bias should be independent of the length of the *n*-alkyl substituent, hence the similar activities for analogs **8b–i**. Increasing the size of the β-substituent from alkyl (A-value for ethyl, 1.75)[15] to phenyl (A-value, 3.0)[15] may further enhance the conformational preference, which possibly explains the increase in activity observed for compounds with the latter substitution (**8j–l**).

Substitution at the α-carbon in the shorter acetic acid substituted series (**15**) also presumably influences the conformation of the aspartate isostere in a manner similar to the propionate series (methine proton observed at 5.4 ppm), but in this case, a loss of activity is seen. A likely explanation for this result is that the conformations accessible to **15** maintain a distance between the carboxylate and the amidine that is too short for optimal binding. In the one case studied, substitution α to the carboxylate in the propionate series (**16**) caused an erosion of activity relative to the other propionates **8**. This is in contrast to observations on α-substitution in other series of GPIIb-IIIa inhibitors. It has been shown that activity is greatly enhanced with α-sulfonamide substitution and this has been ascribed to this substituent interacting favorably with an exosite in the receptor.[16] It is recognized that an alkyl group does not posses the same electronic and hydrogen bond accepting/donating capabilities that a sulfonamide moiety does, therefore it is not surprising that activity differences between these two groups are observed. That activity in this series wanes with α-substitution indicates that either the conformation of the α-alkyl substituted aspartate is vastly different from the related α-sulfonamide congeners or that the proposed exosite cannot interact favorably with an α-alkyl group.

In conclusion, we have demonstrated that modifications of the aspartate isostere of our isoquinolone series of GPIIb-IIIa antagonists afforded substantial increases in intrinsic activity. Specifically, propionate derivatives with phenyl substitution β to the carboxylate provided an approximately 60-fold improvement over the lead compound **2**. However, we were unable to translate all of the gains in intrinsic activity to activity in plasma-based systems. We have demonstrated that this disparity in activity is likely, in part, a result of the

increased hydrophobic character of these substituted analogs allowing for increased interaction with plasma proteins.

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(*Received in USA* 29 *July* 1997; *accepted* 4 *September* 1997)