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Fused Bicyclic Gly-Asp β-Turn Mimics with Potent Affinity for GPIIb-IIIa. Exploration of the Arginine Isostere

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Abstract—6-[4-Amidinobenzoyl]amino]-tetralone-2-acetic acid is a potent antagonist of GPIIb-IIIa. Substitution in the *meta* position of the benzamidine, or replacement with a heteroaryl amidine was tolerated in this series. Use of an acyl-linked 4-alkyl piperidine as an arginine isostere also provided active compounds. Compounds from this series provided substantial systemic exposure in the rat following oral administration.

The search for a means to control thrombus formation has consumed a significant amount of resources during the last decade. Two major themes have emerged from this effort: modulation of the coagulation cascade,[1] and inhibition of platelet aggregation.[2] The latter approach has sought to control thrombosis by minimizing the platelet's ability to copolymerize with the soluble plasma protein fibrinogen.[3] The interaction between platelets and fibrinogen is known to be mediated by the membrane bound integrin GPIIb-IIIa, and it is the activated form of this receptor that binds to one of several RGD sequences located on fibrinogen and other proteins.[4] A wide variety of peptides that contain an RGD sequence can effectively inhibit the binding of fibrinogen to GPIIb-IIIa, and as a result, this tripeptide motif has become the lead structural element used in the design of novel non-peptide inhibitors of platelet aggregation.[5]

We have recently described our initial efforts towards the design and optimization of non-peptide antagonists of GPIIb-IIIa which began with the structural evaluation of a potent cyclic RGD containing peptide. The data indicated that the RGD sequence for the peptide studied was constrained in a well defined Gly-Asp type II′ β-turn.[6,7] We have shown that this structural element can be effectively mimicked with an appropriately substituted 6,6-benzofused ring system (Fig. 1). Structure–activity relationships defined for our early leads indicated that effective antagonists could be obtained when an ether-linked benzamidine was incorporated at the 6-position and an acetic acid residue was attached at the 2-position of a variety of ring-systems.[8] Linkage of the benzamidine to the bicycle with an amide provided the most potent derivatives as exemplified by compounds **1** and **2** (Fig. 1). A common feature found in the structure activity evaluation of our Gly-Asp mimics was the use of a benzamidine as an arginine isostere.[9] While this functional group provided the desired affinity for GPIIb-IIIa, we did not want to be limited to only one effective arginine isostere. Therefore, we sought to develop a series of chemically varied arginine isosteres that would also provide equivalent or enhanced activity. Towards this end, we investigated the effects that altering the phenyl portion of the benzamidine had on biological activity. We initially targeted compounds that contained substitution *meta* to the amidine moiety since the required intermediates were synthetically more

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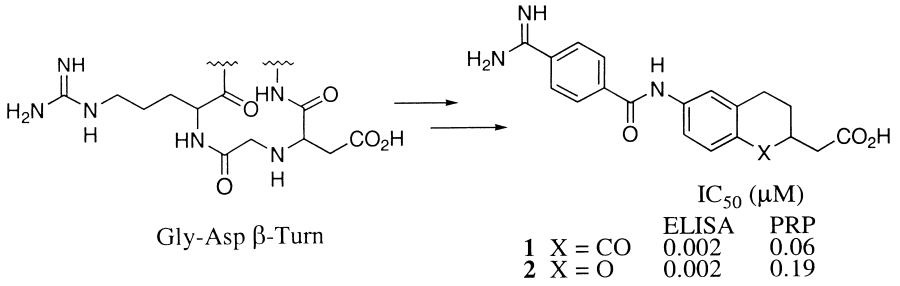
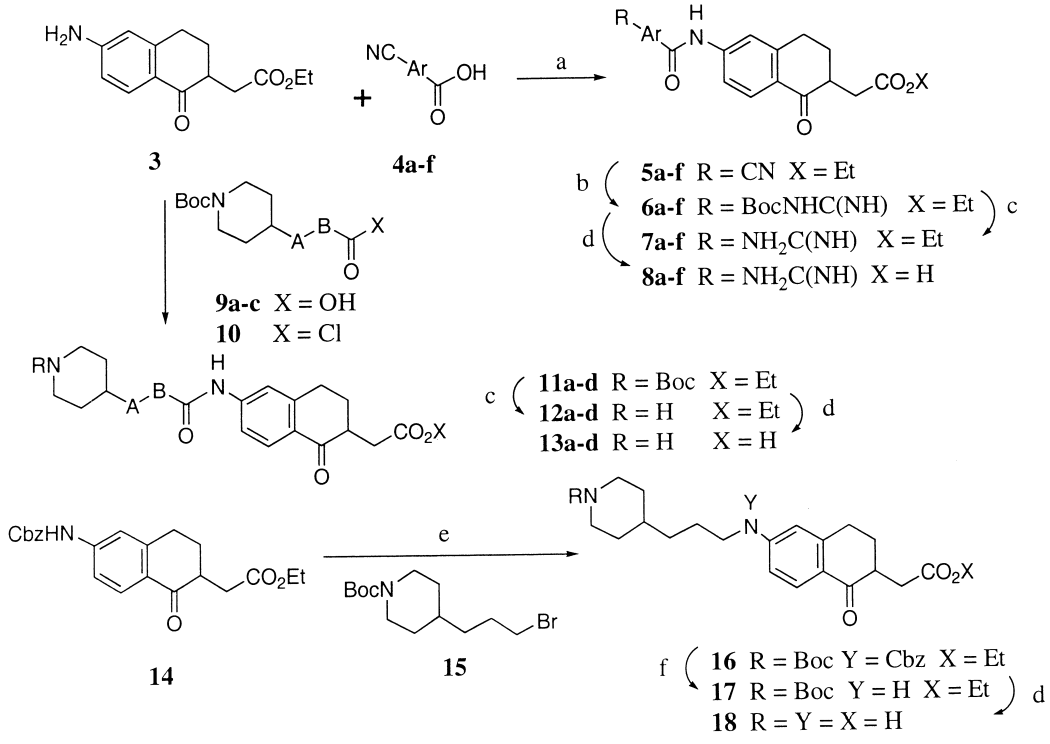


Figure 1. Gly-Asp β-turn and 6,6-based mimics.

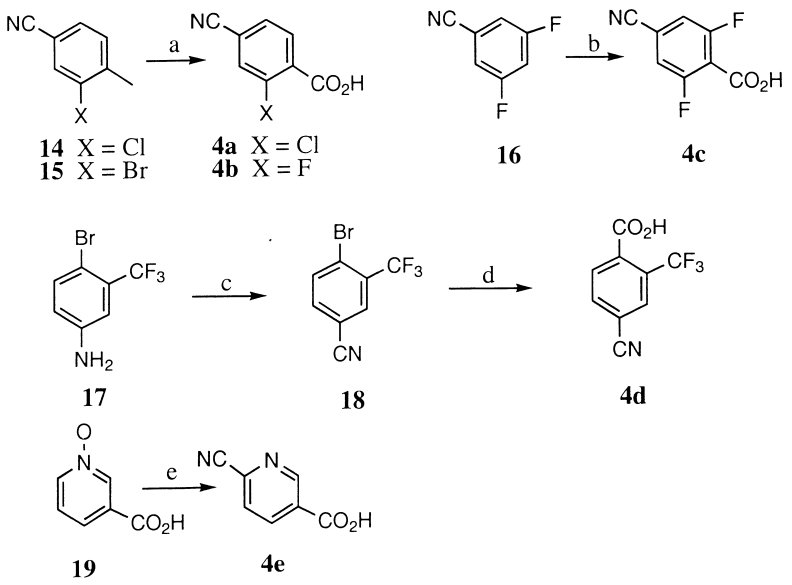
accessable, and *ortho* substituents (particulary halo[10] groups) were not generally compatible with our synthetic approach to amidine formation. Replacement of the benzamidine with a heteroaryl amidine was also of interest.

Arginine isosteres other than benzamidine have also found use in the preparation of RGD mimics. It has been shown that 4-alkyl piperidines can be employed as arginine isosteres with excellent results.[11] While we have previously shown that 6-oxo-linked 4-alkyl piperidines could be employed in this series,[6] the observed potency afforded by this structural motif was less than the corresponding benzamidine. Since the piperidine moiety offers the possibility of providing an arginine isostere with chemical features distinct from benzamidine, we chose to reinvestigate the use of this structural motif in the more potent amide linked series.

The general synthesis for the compounds disclosed in this report is outlined in Scheme 1.[12] Compounds that contain an aryl amidine were prepared by first coupling the known aniline **3**[8] with cyano-arylcarboxylic acids **4a–f** yielding amides **5a–f**. The nitrile moiety in these molecules was then transformed into a Boc-protected amidine (**6a–f**) using a modified thio-Pinner sequence that we have recently described.[6] These compounds could either be *N*-deprotected with neat TFA, providing the amidino-esters **7**, or completely deprotected by first hydrolyzing the ethyl ester with ethanolic NaOH and then *N*-deprotecting with neat TFA affording the desired parent amidino-acids **8a–f** as the TFA salts. The required aryl carboxylic acids **4a–f** were either commercially available or prepared using conventional methods as shown in Scheme 2. Compounds that contain an acyl-linked, 4-substituted piperidine (**13a–d**) were prepared by coupling the desired acid (**9a–c**) or chloroformate (**10**) with **3** in the presence of EDCI or pyridine, respectively (Scheme 1). The formed adducts **11a–d** were deprotected with methods analogous to those described for the preparation of compounds **7** and **8**. The 4-substituted piperidines required for the preparation of these compounds were either known in the literature[11] or prepared as outlined in Scheme 3. One example of an



Scheme 1. (a) EDCI; (b) H_2S; MeI; NH_4OAc; Boc_2O; (c) TFA; (d) NaOH; TFA; (e) NaH; (f) H_2/PdC.



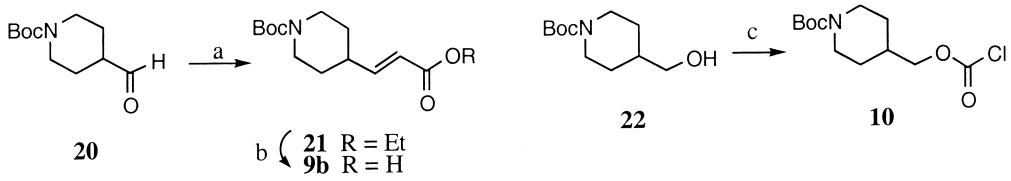
Scheme 2. (a) RuO_4, NaOCl; (b) BuLi, CO_2; (c) HONO, CuCN; (d) *t*BuLi, CO_2; (e) TMSCl, NaCN.

amine-linked, 4-alkyl piperidine was prepared as shown in Scheme 1. Alkylation of the Cbz protected analogue of **3** (**14**) with the known bromide **15** provided intermediate **16** in good yield. Removal of the Cbz group with hydrogen and palladium provided secondary amine **17**, which was transformed into the target molecule **18** using chemistry similar for the preparation of compounds **8**.

Biological activity was assessed in vitro by measuring the agents' ability to inhibit the binding of fibrinogen to purified human GPIIb-IIIa in an ELISA type assay.[13] Functional activity was subsequently determined by measuring the inhibition of ADP (5 μM) induced platelet aggregation in human platelet-rich plasma (PRP).[14] Analogues with sufficient functional activity (PRP IC_{50} <0.5 μM) were candidates for oral evaluation in the rat. For this assay, we were primarily interested in determining the peak blood level (C_{max}) and area under the curve (AUC) afforded by these compounds for the 5 hour time course following a single oral dose of 10 mg/kg. Since we had previously demonstrated that systemic exposure levels for related compounds in this series were significantly enhanced by conversion of the parent amidino-acids to ethyl ester prodrugs, we chose not to evaluate the corresponding carboxylates.[8]

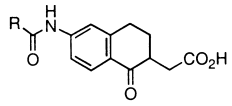
Analogue **8a**, which contains a single chlorine atom *meta* to the amidine residue, was 3-fold less active in the ELISA and 6-fold less potent in PRP than the lead tetralone **1** (Table 1). Replacement of the chlorine with fluorine afforded compound **8b**, which was equipotent with **1** in the ELISA (0.003 μM) and slightly less active (0.1 μM) in PRP. Incorporation of a second fluorine atom (**8c**) negatively impacted activity in PRP (0.24 μM) while binding affinity (ELISA) was unaffected. Incorporation of a trifluoromethyl group (**8d**) caused substantial erosion on both ELISA and PRP activity. Exchange of the phenyl moiety for the similary sized thiophene (**8f**) was detrimental for potency in general. The pyridyl amidine (**8e**) afforded good potency in the ELISA (0.009 μM) and acceptable activity in PRP (0.22 μM).

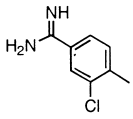
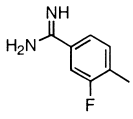
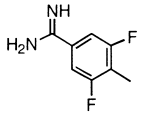
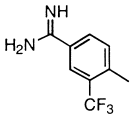
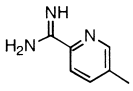
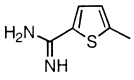
Comparison of the data contained in Table 1 indicates that substitution *meta* to the benzamidine (**8a–d**) generally results in a diminution of activity. Of the substituents examined, fluorine had the smallest effect and slightly decreased activity in PRP relative to the non-substituted tetralone **1**. Introduction of a second fluoro group had a greater negative impact on PRP activity than the ELISA, suggesting that this modification hindered the translation of receptor affinity into plasma activity, possibly by increasing non-specific interactions with other plasma constituents.[7] Substitution of larger groups such as chloro and trifluoromethyl, resulted in more substantial losses in overall activity which may suggest that steric bulk in this area of the antagonist may not be tolerated by the receptor. The trend towards lower activity is also observed for the heteroaryl



Scheme 3. (a) Triethyl phosphonoacetate; (b) NaOH; (c) phosgene.

Table 1. Amidine analogues **8**



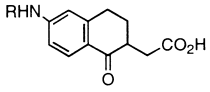
Compound	R	IC_{50} μM[a] (ELISA)	IC_{50} μM[b] (PRP)
8a		0.011	0.4
8b		0.003	0.1
8c		0.005	0.24
8d		0.072	2.0
8e		0.009	0.22
8f		0.21	9.0

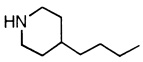
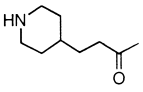
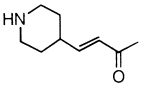
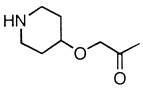
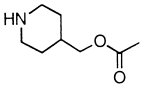
[a]Concentration required to reduce binding of fibrinogen to purified human GPIIb-IIIa by 50%. The IC_{50} values are expressed as the average of at least two determinations. The average error for the IC_{50} determinations was 15%.
[b]Concentration required to reduce ADP-induced human platelet aggregation response by 50%. The IC_{50} values are expressed as the average of at least two determinations. The average error for the IC_{50} determinations was 16%.

amidines **8e–f**. Of the two examined, the 6-membered pyridyl analogue was preferred to the 5-membered thiophene.

Consistent with earlier findings, replacement of the benzamidine moiety with a more simple, amine-linked 4-alkyl piperidine (**18**) caused a substantial loss of activity (Table 2). The amide-linked congener **13a** was 8- and 6-fold more potent in the ELISA and PRP assays, respectively. Introduction of unsaturation into the tether connecting the piperidine and tetralone moieties further increased activity in the ELISA (0.026 μM) and PRP (0.36 μM), which gives this analogue similar activity to some of the amidine containing compounds listed in Table 1. The increased potency afforded by amide **13a** and unsaturated amide **13b** relative to amine **18** demonstrates that incorporation of conformational constraint in the tether can be used to enhance activity

Table 2. Piperidine analogues **18** and **13**



Compound	R	IC_{50} μM[a] (ELISA)	IC_{50} μM[b] (PRP)
18		0.61	5.5
13a		0.071	0.91
13b		0.026	0.36
13c		1.2	18
13d		0.33	1.8

[a,b]As in Table 1.

in these piperidine analogues. Introduction of an oxygen atom into the tether connecting the piperidine and tetralone moieties as in carbamate **13c** and ether **13d** resulted in a decrease in activity relative to **13a.**

Compounds **8a–c**, **8e** and **13b** displayed sufficent activity in vitro to warrant further examination in vivo. As discussed, we chose to evaluate the ethyl ester derivatives of these compounds (**7a–c, 7e** and **12b,** respectively) orally in the rat. As shown in Table 3, all of the compounds afforded good blood levels of parent amidinoacid following an oral dose of 10 mg/kg of the corresponding ester prodrug. The data demonstrate that incorporation of a single halogen *meta* to the amidine moiety was well tolerated and compounds with this modification provided exposure levels similar to the non-substituted parent. Difluoro compound **8c** provided approximately half of the exposure of the monohalo and unsubstituted analogues. Heteroamidine **8e** provided the least exposure of the amidine series with C_{max} and AUC values approximately 3-fold less than tetralone **1**. Piperidine analogue **12b** provided moderate blood levels but overall exposure was less than the best benzamidine containing analogues. While the exposure levels varied with substitution, it is of interest to note that each analogue afforded peak concentration levels significantly greater than the corresponding IC_{50} values recorded in human plasma. This suggests that if these compounds provided similar exposure in humans, pharmacologicaly effective concentrations of active metabolite (acid) in plasma could be achieved following an oral dose of less than 10 mg/kg.

Table 3. Blood levels obtained after a single oral dose of compounds **1**, **8a–c**, **8e** and **13b**

Compound	C_{max} (ng/mL)	AUC (ng*h/mL)
1	3630	11243
8a	3108	10391
8b	4020	11868
8c	2025	6414
8e	1174	3565
13b	2700	8137

In summary, we have investigated modifications of the arginine isostere contained in lead tetralone **1.** Substitution of a single fluorine atom *meta* to the benzamidine afforded an analogue with similar activity to the parent while substitution of additional fluorines, or larger functional groups resulted in compounds with diminished activity. The trend towards lower activity was also seen with the two heteroaryl amidines studied. Conformationally restricted 4-alkyl piperidines were found to provide good activity while the more flexible analogues were less potent. Benzamidine and piperidine containing compounds from this study provided substantial systemic exposure in rats after an oral dose of 10 mg/kg. The maximum concentrations found in plasma for all of the compounds evaluated were well above what would be required to inhibit human platelet aggregation.

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