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Nonpeptide $\alpha_v\beta_3$ Antagonists. Part 2: Constrained Glycyl Amides Derived from the RGD Tripeptide

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Abstract—Mimetics of the RGD tripeptide are described that are potent, selective antagonists of the integrin receptor, $\alpha_v\beta_3$. The use of the 5,6,7,8-tetrahydro[1,8]naphthyridine group as a potency-enhancing N-terminus is demonstrated. Two 3-substituted-3-amino-propionic acids previously contained in $\alpha_{IIb}\beta_3$ antagonists were utilized to enhance binding affinity and functional activity for the targeted receptor. Further affinity increases were then achieved through the use of cyclic glycyl amide bond constraints.

The vitronectin receptor $\alpha_v\beta_3$ is a member of the integrin superfamily of receptors that is highly expressed in osteoclast cells.[1,2] These cells are responsible for resorption of bone in the remodeling cycle. Patients with osteoporosis have decreased bone mass and increased bone fragility due to an imbalance between osteoclast-mediated bone resorption and bone formation.[3] Proteins and peptides that contain the arginine-glycine-aspartic acid (RGD) tripeptide, including vitronectin, osteopontin, bone sialoprotein, and echistatin bind to $\alpha_v\beta_3$ with high affinity.[4,5] Antibodies to $\alpha_v\beta_3$, echistatin, and small-molecule $\alpha_v\beta_3$ antagonists have been shown to inhibit bone resorption in vitro and prevent bone loss in vivo.[6–14] A recent report from these laboratories described the design of a series of high-affinity $\alpha_v\beta_3$ antagonists from members of the 'centrally constrained' sulfonamide class of $\alpha_{IIb}\beta_3$ fibrinogen antagonists.[15] Central to this communication was the finding that a 5,6,7,8-tetrahydro[1,8]naphthyridine (THN) moiety could be utilized as a novel N-terminus, imparting potency, integrin selectivity, and increased lipophilicity to this structural class.

Herein, we describe the identification of a class of $\alpha_v\beta_3$ antagonists derived directly from the tripeptide RGD. We have explored the use of the THN group as an N-terminus in this series and have examined the effect of substitution at the 3-position of the propionic acid on in vitro potency. In addition, structure–activity relationship (SAR) investigations of constraints about the glycyl amide bond of these tripeptide mimetics are described.

The synthesis of three peptidomimetics is described in Scheme 1. The ketone **2** was subjected to a Friedlander condensation with 2-amino-3-formylpyridine[16] (**1**) to produce the naphthyridine **3**. Regioselective hydrogenation of the distal pyridine ring followed by ester hydrolysis gave the tetrahydronaphthyridine acid **4**. Diimide coupling of acid **4** with glycine ethyl ester, and subsequent hydrolysis gave the acid **5**. Finally, coupling of **5** to three 3-amino-propionate esters bearing different 3-substituents, followed by hydrolysis provided the target compounds **6**, **7**, and **8**.

Preparation of the pyrrolidinone-constrained antagonist **15** (Scheme 2) began with the conversion of 2-oxo-hexanoic (**9**) acid to its (*R*)-benzyl-oxazolidinone, and subsequent ketalization to give **10**. Stereoselective allylation[17] followed by ozonolysis produced the aldehyde

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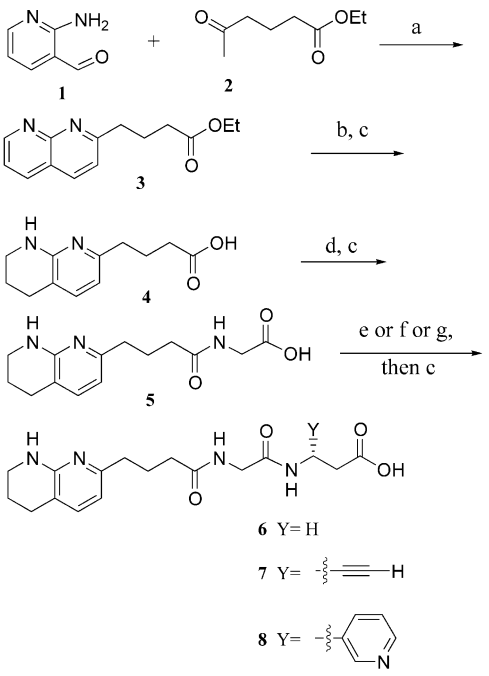
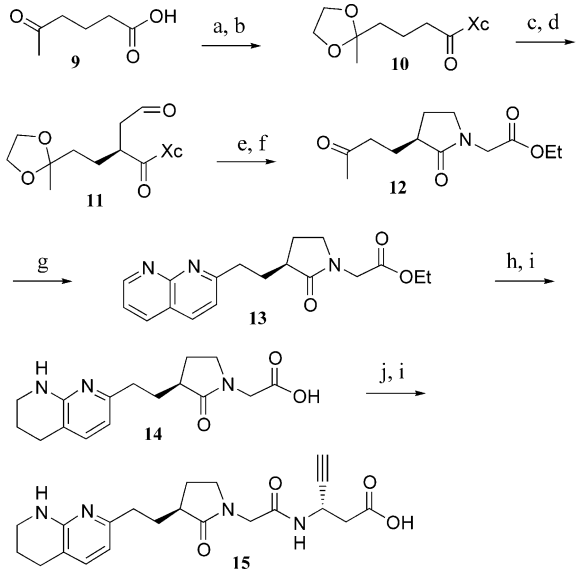
11. A facile one-pot reductive amination/cyclization with glycine ethyl ester, followed by ketal unmasking provided the pyrrolidinone-ketone **12**. A five-step sequence utilizing transformations previously described in Scheme 1 then produced the desired antagonist **15**. The corresponding 3-pyridyl analogue **30** was prepared from **14** through the same sequence, utilizing the corresponding 3(*S*)-pyridin-3-yl β-alanine ester. The diastereoisomer **31** was produced with the same protocol, using the (*S*)-antipode of the benzyl-oxazolidinone chiral auxiliary. The synthesis of a piperidinone variant **21** (Scheme 3) started with the alkylation of the enolate of *N*-(trimethysilyl)piperidin-2-one (**17**) with the iodide **16**.[18] Ketal removal then revealed ketone **18**. Friedlander condensation of **18** with 2-amino-3-formylpyridine was followed by *N*-alkylation of the piperidinone with ethyl iodoacetate, and then hydrogenation produced the THN-ester **20**. The standard three-step sequence then yielded the piperidinone analogue **21** as a mixture of diastereomers.

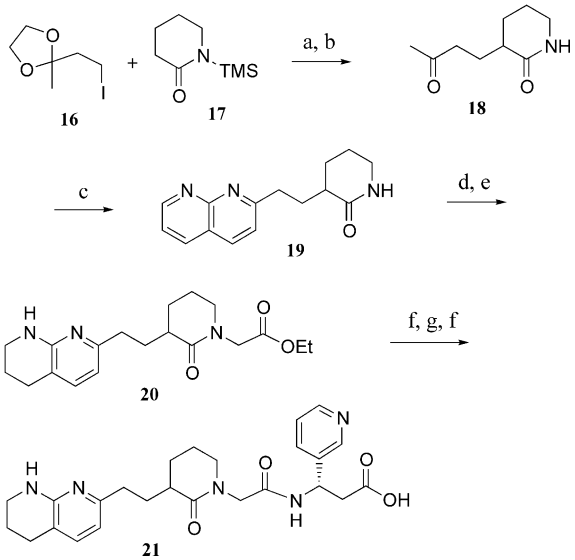


Scheme 1. (a) Proline, ethanol, reflux; (b) H_2, Pd/C; (c) 6 N HCl; (d) glycine ethyl ester hydrochloride, EDC, HOBT; (e) ethyl 3-aminopropionate, EDC, HOBT; (f) ethyl-3(*S*)-3-aminopent-4-ynoate; (g) ethyl-3 (*S*)-3-amino-3-pyridin-3-ylpropanoate, EDC, HOBT.



Scheme 2. Xc = *N*-(*R*)-(+)-4-benzyl-2-oxazolidinone (a) ethylene glycol, cat. TsOH, toluene, reflux; (b) pivaloyl chloride, triethylamine, THF, then lithium (*R*)-(+)-4-benzyl-2-oxazolidinone; (c) NaHMDS, allyl bromide; (d) ozone, then PPh_3; (e) glycine ethyl ester, sodium triacetoxyborohydride, triethylamine; (f) acetone, cat. TsOH, reflux; (g) 2-amino-3-formylpyridine, proline, ethanol, reflux; (h) H_2, Pd/C, ethanol; (i) 6 N HCl; (j) ethyl-3(*S*)-3-aminopent-4-ynoate, EDC, HOBT.

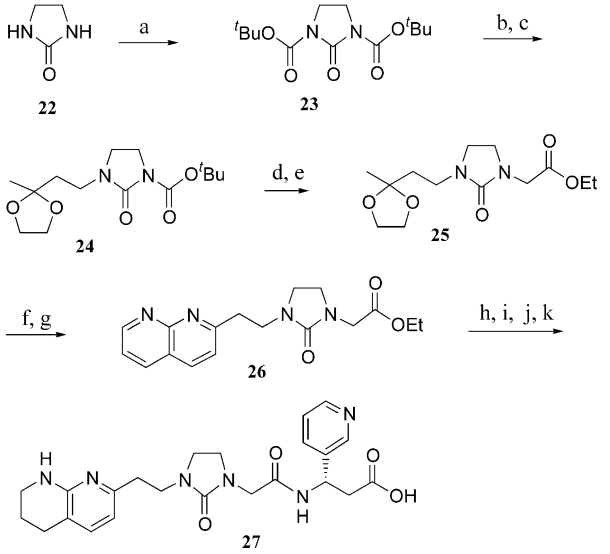
The compounds displayed in Table 1 were evaluated for their ability to displace a non-peptide radioligand from human recombinant $\alpha_v\beta_3$ (SPAV3[20]), and to inhibit the rate of ADP-stimulated aggregation of gel-filtered human platelets mediated through the integrin $\alpha_{IIb}\beta_3$ (PLAGGIN[21]). The RGD tripeptide **28**[22] and the analogue **29**,[23] which lacks the aspartic acid α-carboxylate and the arginine α-amino group, have IC_{50} values >10 μM in both in vitro assays. Measurable binding affinity to $\alpha_v\beta_3$ was attained when the guanidinomethylene N-terminal portion of **29** was replaced with 5,6,7,8-tetrahydro[1,8]naphthyridine (THN), producing compound **6**. This analogue had an IC_{50} of 111 nM in the SPAV3 assay while remaining inactive in the PLAGGIN assay at concentrations up to 10 μM.



Scheme 3. (a) LDA; (b) acetone, cat. TsOH, reflux; (c) 2-amino-3-formylpyridine, proline, ethanol, reflux; (d) NaHMDS, ethyl iodoacetate; (e) H_2, Pd/C, ethanol; (f) 6 N HCl; (g) ethyl-3(*S*)-3-amino-3-pyridin-3-ylpropanoate, BOP, NMM.

Binding affinities of this class for $\alpha_v\beta_3$ could be further improved by incorporating substituents at the 3-position of the propionic acid that have been utilized in potent fibrinogen receptor antagonists.[24] The 3(*S*)-acetylene analogue **7** and 3(*S*)-3-pyridyl analogue **8** had IC_{50} values in the SPAV3 assay of 8.8 nM and 1.3 nM, respectively. These two compounds also inhibited the formation of mouse osteoclasts in vitro (OCFORM assay[25]) with IC_{50} values of ~100–200 nM. Although both compounds demonstrated functional activity mediated through $\alpha_v\beta_3$, they were poor inhibitors of platelet aggregation. The $\alpha_{IIb}\beta_3$ antagonists from which these substituted propionic acids were drawn bore N-termini significantly different from the THN, such as guanidines, amidines, and piperidines. The finding that **7** and **8** are potent antagonists of $\alpha_v\beta_3$, yet lack activity in the PLAGGIN assay, suggests that the N-terminal moiety can play a critical role in modulating integrin selectivity among compounds that bear identical C-termini.

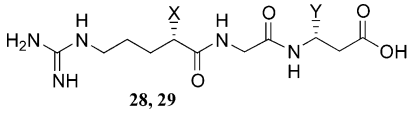
Having identified affinity-enhancing substituents at the N- and C-termini, our efforts focused on exploring the effects of constraints at the central region of the molecule. Constraining the glycyl amide of **7** through cyclization into the (*S*)-pyrrolidinone **15** yielded a 3-fold affinity enhancement in the SPAV3 assay. A similar increase was gained with the pyridine-containing (*S*)-pyrrolidinone analogue **30**, resulting in a highly potent $\alpha_v\beta_3$ antagonist with a SPAV3 IC_{50} of 0.35 nM, and an OCFORM IC_{50} of 62 nM. Selectivity over $\alpha_{IIb}\beta_3$ was maintained, as the PLAGGIN IC_{50} remained greater than 10 μM. Interestingly, the (*R*)-pyrrolidinone antipode **31** was only about 3-fold less potent in the SPAV3 assay than **30**, making it essentially equipotent with the amide parent **8**. Ring expansion to produce the piperidinone **21** offered no advantage over the amide **8**. Finally, an attempt to eliminate the pyrrolidinone stereogenic center of **30** by conversion to the imidazolidone **27** resulted in a substantial loss of binding affinity and cell-based potency (Table 2).

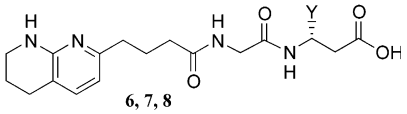


Scheme 4. (a) BOC_2O, DMAP; (b) $Mg(ClO_4)_2$, reflux; (c) LiHMDS, **16**; (d) TFA, toluene; (e) LiHMDS, ethyl iodoacetate; (f) acetone, cat. TsOH, reflux; (g) 2-amino-3-formylpyridine, proline, ethanol, reflux; (h) H_2, Pd/C, ethanol; (i) 6 N HCl; (j) ethyl-3(*S*)-3-amino-3-pyridin-3-ylpropanoate, EDC, HOBT; (k) NaOH, ethanol.

Modeling of the 3-pyridyl propionic acid analogues was carried out to compare the tertiary structural differences to the $\alpha_v\beta_3$ affinities.[26] The amide **8** and (*S*)-pyrrolidinone **30** have low energy minima that share a high degree of atom overlap throughout the chain. The increased restriction of **30** to these conformations may explain the increased affinity over **8**, assuming that these minima are similar to the integrin-bound state. For the (*R*)-pyrrolidinone **31** and piperidinone **21**, low energy

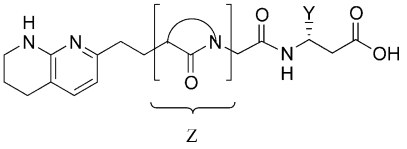
Table 1. Glycine-containing antagonists

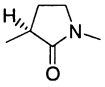
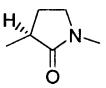
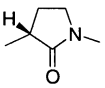
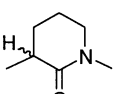
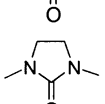




Compd	X	Y	IC_{50} (nM) SPAV3	PLAGGIN
28	NH_2	COOH	> 10,000	> 10,000
29	H	H	> 10,000	> 10,000
6	—	H	111	> 10,000
7	—	CCH	8.8	> 10,000
8	—	3-Pyridyl	1.3	> 10,000

Table 2. Glycyl-constrained antagonists



Compd	Z	Y	IC_{50} (nM) SPAV3	OCFORM	PLAGGIN
15		CCH	2.9	150	> 10,000
30		3-Pyridyl	0.35	62	> 10,000
31		3-Pyridyl	0.94	79	> 10,000
21		3-Pyridyl	1.2	480	> 10,000
27		3-Pyridyl	12	> 1000	7100

minima exist which place the THN, 3-pyridyl, and carboxylate groups in positions similar to those for the minima of **8** and **30**. Conversely, this premise does not hold for compound **27**. Models of this imidazolidone showed preferred confirmations that present the charged termini similarly to those for the above set, yet the affinity of **27** for $\alpha_v\beta_3$ was significantly reduced. Of interest is the observation that the imidazolidone **27** proved the most potent of the series in the PLAGGIN assay, indicating an erosion of integrin selectivity. The coplanar presentation of the two alkyl groups about the imidazolidone ring is unique to this compound and may be the source of its functional divergence.

With this series of compounds, we have demonstrated the design of RGD tripeptide mimetics that are highly potent antagonists of the $\alpha_v\beta_3$ receptor but poor inhibitors of $\alpha_{IIb}\beta_3$-mediated platelet aggregation in vitro. We have extended the use of the 5,6,7,8-tetrahydro[1,8]-naphthyridine group to an alternative structural class and confirmed its ability to provide potency enhancement and integrin selectivity for $\alpha_v\beta_3$. Similarly, C-terminal 3-substituents of known $\alpha_{IIb}\beta_3$ antagonists were utilized to enhance binding affinity and functional activity for the targeted receptor. Substantial potency increases were then added through the identification of optimal constraining elements of the glycyl amide bond. Additional structural classes that build on these findings will be the subject of future reports.

Acknowledgements

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26. For each molecule, 500 conformations were generated using a distance geometry method (S. Kearsley, Merck & Co., unpublished). The conformations were energy minimized using the MMFF force field as implemented in Batchmin (Mohamadi, F.; Richard, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440) with a 4r distance-dependent dielectric model. Alignments of the molecules were generated using the following procedure. Three pharmacophore points, the proximal THN nitrogen, the 3-aryl group, and the carboxylate, were assigned for each conformation of each molecule. The resulting inter-point distances were used as a molecular descriptor and clustering based on that descriptor generated 18 clusters (unique presentations of the pharmacophore points).