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N-Aryl-γ-lactams as integrin $\alpha_v\beta_3$ antagonists

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Abstract—Novel $\alpha_v\beta_3$ antagonists based on the *N*-aryl-γ-lactam scaffold were prepared. SAR studies led to the identification of potent antagonists for $\alpha_v\beta_3$ receptor with excellent selectivity against the structurally related $\alpha_{IIb}\beta_3$ receptor. Additional interactions of *N*-aryl-γ-lactam derivatives with $\alpha_v\beta_3$ were found when compared to c(-RGDf[NMe]V-) peptide antagonist. The effects of the conformation and configuration of the γ-lactam core on the binding were also assessed.
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Integrins are a family of cell surface receptors that function in cell–substrate recognition and cell–cell communication. Integrin $\alpha_v\beta_3$ recognizes a wide range of extracellular matrix ligands, including vitronectin, fibrinogen, von Willebrand Factor, and osteopontin, and is highly expressed on proliferative endothelial cells, smooth muscle cells, metastatic tumor cells, and osteoclasts.[1] In principle, small molecule $\alpha_v\beta_3$ antagonists could provide novel therapeutic strategies for the treatment of pathological conditions involving abnormal cell adhesion and neovascularization, such as cancer, restenosis, angiogenic ocular disorders, and osteoporosis.[2] Studies have shown that nonpeptide $\alpha_v\beta_3$ antagonists inhibit bone resorption in vivo, indicating that these antagonists could be useful for the treatment of osteoporosis.[3]

Like platelet-specific integrin $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$ binds to extracellular matrix proteins that contain the Arg-Gly-Asp (RGD) sequence.[4] Xiong et al. recently solved the crystal structure of the extracellular domains of $\alpha_v\beta_3$ integrin complexed with cyclic peptide antagonist c(-RGDf[NMe]V-).[5] This pioneering work depicted the main interactions in the complex to be between the positively charged guanidinium group in the ligand and the negatively charged side chains of Asp^{218} and Asp^{150} in the α subunit, and between the aspartic acid residue in the RGD ligand and the metal ion in the MIDAS region (MIDAS: metal-ion-dependent adhesion site) of the β subunit. Docking studies revealed that various peptidomimetic antagonists bind to $\alpha_v\beta_3$ in a very similar fashion as in the peptide–integrin complex.[6,7] We were interested in developing scaffolds that mimic the Arg-Gly dipeptide. Scheme 1 is a schematic representation of our design strategy. We found that a conformationally constrained, *N*-aryl-γ-lactam scaffold, when elaborated with various β-amino acids, provided potent and selective $\alpha_v\beta_3$ antagonists. Herein we detail our investigations on the binding modes of these γ-lactam derivatives.

The γ-lactam **3** was obtained from the condensation of 3-nitroaniline with itaconic acid (Scheme 2).[8] Compound **3** was then coupled with β-amino ester **9**[9] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) to afford ester **4**. A facile reduction of the nitro group in **4** under acidic condition led to aniline **5**. Guanidine analogue **1c** was prepared by the treatment of aniline **5** with thiourea **10** to give

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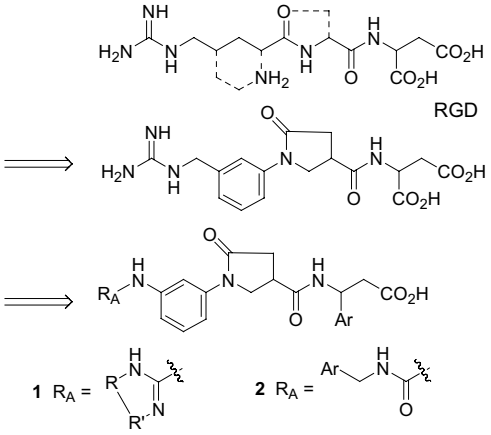
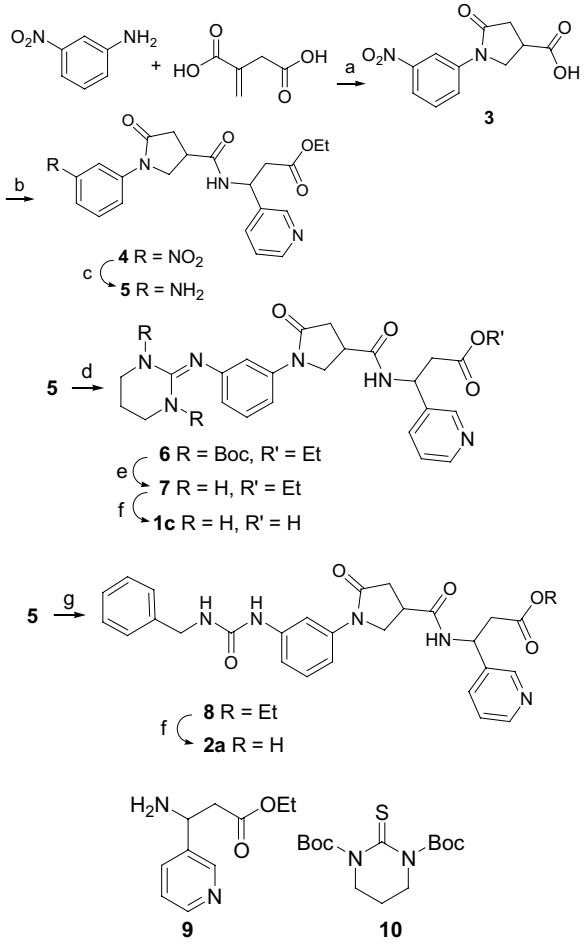
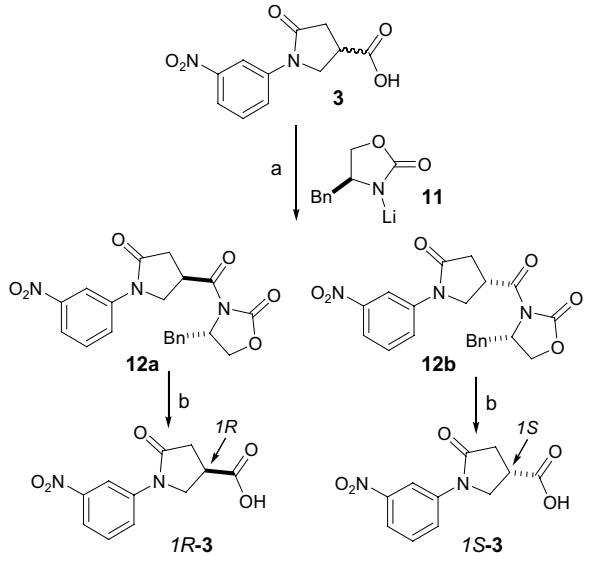


Scheme 1. From RGD to γ-lactam scaffold.



Scheme 2. Synthesis of γ-lactam derived $\alpha_v\beta_3$ antagonists: (a) neat, 110 °C, 8 h, 70%; (b) **9**, EDCI, HOBt, Et_3N, DMF, rt, 8 h, 90%; (c) Zn, AcOH, THF/H_2O, 80%; (d) **10**, cat. $HgCl_2$, DMF, 16 h, 80%; (e) 1:1 TFA in CH_2Cl_2, rt, 30 min, 100%; (f) aq NaOH, THF/MeOH; H^+, 95%; (g) benzyl isocyanate, 75%.

protected guanidine **6**.[10] Removal of the Boc groups led to compound **7**, which was converted to acid **1c** by basic hydrolysis. Urea derivative **2a** was prepared by the condensation of aniline **5** with benzyl isocyanate followed by a final hydrolysis of ester **8**.



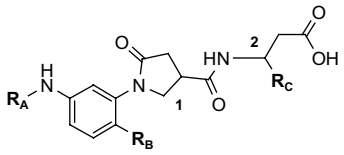
Scheme 3. Preparation of enantiopure compounds 1*R*-**3** and 1*S*-**3**. (a) $(COCl)_2$, cat. DMF; then **11**, THF, −78 °C, 70%; (b) LiOH, 95:5 H_2O:H_2O_2, 95%.

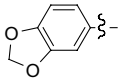
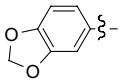
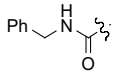
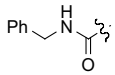
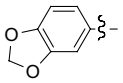
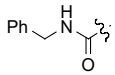
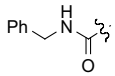
The pure enantiomers of **3** were obtained with the aid of Evans' chiral auxiliary (Scheme 3).[11] Racemic **3** was coupled with oxazolidinone **11** to afford two diastereomers **12a** and **12b**, which were readily separated by flash chromatography. The absolute configurations of **12a** and **12b** were unambiguously determined by X-ray crystallographic studies.[12] Removal of the oxazolidinone under a mild basic hydrolysis condition led to enantiomers 1*R*-**3** and *S*-**3** in excellent yields. The chiral β-amino acids such as 3-amino-3-(3-fluorophenyl)-propionic acid were prepared using enzymatic resolution method.[13] All compounds were evaluated in vitro by competitive electrochemiluminescent binding assay using vitronectin as the natural ligand for $\alpha_v\beta_3$ receptor, and fibrinogen for $\alpha_{IIb}\beta_3$ receptor.[14] These results are summarized in Tables 1 and 2.

As illustrated in Table 1, additional interactions of γ-lactam with $\alpha_v\beta_3$ were found when comparing to c(-RGDf[NMe]V-) peptide complexed with $\alpha_v\beta_3$ in the crystal structure.[5] Five-membered cyclic guanidine analogue **1b** showed superior binding affinity to acyclic guanidine **1a**. Six-membered homologue **1c** displayed further activity enhancement. The results indicate that a hydrophobic pocket near the guanidine-binding site exists, and it accommodates the methylene groups on the cyclic guanidines. The pocket extends deep into the receptor, as evidenced in urea analogue **2a**, which exhibits potent activity ($K_i = 10$ nM) despite lacking the ligand guanidinyl ionic interaction with the α subunit. Evidently, the hydrophobic contacts of the benzyl group compensate the lost guanidinyl interaction.[15]

Extra contacts were also found in the β-amino acid binding region. Docking studies suggested a well-defined pocket to accommodate the β-aromatic group on the β-amino acid.[7] A π–π stacking interaction of the same moiety with the side chain of Tyr^{178} in the α unit was also proposed.[6] We,[16] and others[17] found that a variety

Table 1. $\alpha_v\beta_3$ Binding assay results from γ-lactam derivatives*



Entry	R_A	R_B	R_C	$\alpha_v\beta_3$ $K_i \pm$ SD (nM)[a]
1a		H		16.6[b]
1b		H		5.1 ± 2.2
1c		H		1.4 ± 0.9
1d		H		0.7 ± 0.3
3a		F		2.9[b]
3b		Cl		52.7 ± 59.5
3c		F		0.5 ± 0.6
2a		H		10.4 ± 0.6
2b		H		2.4 ± 0.7
2c		H		11.1 ± 1.1
2d		H		70.9 ± 14.3

*For clarity, the two chiral centers on the γ-lactam and β-amino acid were arbitrarily assigned to number 1 and 2, respectively.

[a] Determined by competitive electrochemiluminescent binding assay using vitronectin as the natural ligand (see Ref. 17); SD of at least two K_i's determined.

[b] One determination.

Table 2. Binding activities of the optical pure stereoisomers for integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$

Entry	$\alpha_v\beta_3$ $K_i \pm$ SD (nM)[a]	$\alpha_{IIb}\beta_3$ $K_i \pm$ SD (nM)[a]
1*R*,2*S*-**2c**	10[b]	>25,000
1*R*,2*R*-**2c**	154[b]	>25,000
1*S*,2*S*-**2c**	>1000	>25,000
1*S*,2*R*-**2c**	>1000	>25,000
1*R*,2*S*-**1d**	0.1 ± 0.2	2100 ± 400
1*R*,2*R*-**1d**	7.3 ± 0.3	>25,000

[a] Determined by competitive electrochemiluminescent binding assay using vitronectin as the natural ligand (see Ref. 17); SD of at least two K_i's determined.

[b] One determination.

of β-arylamino acids can be used in $\alpha_v\beta_3$ antagonists. Moreover, our SAR results suggest that a hydrogen bond acceptor at the 3-position of the aromatic ring is beneficial to the activity, which is in agreement with the findings from other groups.[17] For example, potent antagonists **1a**–**d** contain 3-pyridyl and 1,3-benzodioxolyl groups that can form hydrogen bonds with the receptor through the N or O atom on the aromatic ring. This structural requirement is clearly demonstrated in urea analogues **2a**–**d**. Like their guanidine counterparts, 3-pyridyl and 1,3-benzodioxolyl derived urea **2a** and **2b** display excellent binding affinities for $\alpha_v\beta_3$. Fluorine at the 3-position is also beneficial to the binding, as seen with compound **2c** ($K_i = 11$ nM). In contrast, phenyl

analogue **2d**, being unable to form a hydrogen bond around the aromatic ring, shows significantly loss of binding potency ($K_i = 71$ nM).

The *N*-aryl-γ-lactam scaffold has a well-defined conformation because of the hindered rotation between the central phenyl and lactam ring.[18] The dihedral angle (ϕ) between the two rings is dictated by the *ortho*-substituent (relative to the lactam) on the phenyl ring. Different angles (ϕ) guide the guanidine and carboxylic acid groups to different relative geometries, hence influence the binding to $\alpha_v\beta_3$. This is manifested by the pyridyl analogues **1a**, **3a**, and **3b**, where a fluorine at the *ortho*-position in **3a** provides the best binding, while a smaller H in **1a** or a bigger Cl in **3b** leads to less potency.[16] Here, the guanidine and carboxylate groups act like an electrostatic clamp, holding the entire molecule in the binding pocket.[19] Only the correct conformation in the central link allows the ligand to interact with the receptor effectively. Interestingly, the fluorine adds no benefit to binding when comparing **3c** ($K_i = 0.5$ nM) with **1d** ($K_i = 0.7$ nM), suggesting the important contribution of the hydrophobic groups (i.e., the methylenes in the cyclic guanidine and 1,3-benzodioxolyl moiety) to the binding. As a result, compounds **1d** and **3c** are among the most potent $\alpha_v\beta_3$ antagonists in the γ-lactam series.

Examination of the four individual stereoisomers of compound **2c** in the $\alpha_v\beta_3$ assay revealed that the *R*-configuration on the γ-lactam is required for effective binding. The corresponding *S*-configuration is detrimental, as seen with 1*S*,2*S*-**2c** and 1*S*,2*R*-**2c** ($K_i > 1$ μM for both compounds). This result agrees with the notion that the relative geometry of the guanidine and carboxylic acid groups is an important structural parameter in the antagonistic binding (vide supra). Apparently, optimal orientations of the benzylurea (as a guanidine mimetic) and carboxylate groups are vital for the antagonists to bind with the receptor.[19] Localized structural modifications such as the chirality change on the β-amino acid do not alter the relative orientation of the two ionic groups or their mimetic groups, therefore exert less dramatic effects on the binding. This is illustrated in the compounds with the *R*-configured γ-lactam core. For example, compound 1*R*,2*S*-**2c** is about 15 times more active than its diastereomer 1*R*,2*R*-**2c**, favoring the *S*-configuration on the β-amino acid. This outcome is substantiated in more potent guanidine analogues. Thus, the favored isomer 1*R*,2*S*-**1d** binds to the receptor in picomolar range ($K_i = 0.1$ nM) while its diastereomer 1*R*,2*R*-**1d** is a less potent $\alpha_v\beta_3$ antagonist ($K_i = 7.3$ nM).

Generally, the γ-lactam derivatives are highly selective for $\alpha_v\beta_3$ versus $\alpha_{IIb}\beta_3$. Among all the compounds listed in Table 2, only compound 1*R*,2*S*-**1d** shows marginal binding ($K_i = 2100$ nm) toward $\alpha_{IIb}\beta_3$, affording more than 21,000-fold selectivity favoring $\alpha_v\beta_3$. None of the other compounds bind to $\alpha_{IIb}\beta_3$ ($K_i > 25{,}000$ nM).

In conclusion, we found that the γ-lactam derivatives were potent and selective $\alpha_v\beta_3$ antagonists. Our SAR results indicated the presence of a hydrophobic pocket near the guanidine binding site and hydrogen bonding around the aromatic moiety of the β-amino acid, which are in agreement with the findings from other groups. The effects of the conformation and configuration of the γ-lactam on the binding were also assessed. Conformational changes in the central scaffold affect the binding significantly to polar ligands, such as compounds **1a**, **3a**, and **3b**, but are less influential in more hydrophobic ligands. The favored stereochemistry on the γ-lactam is the *R*-configuration, while on the β-amino acid is the *S*-configuration. These SAR results provide a ligand $\alpha_v\beta_3$ binding model and are useful for designing new $\alpha_v\beta_3$ antagonists.

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1988, *13*, 281) and fibrinogen (Calbiochem) was labeled with ruthenium(II) tris bipyridine *N*-hydroxysuccinimide ester (Origen TAG® Ester, Igen Inc. Gaithersburg, MD) according to the manufacturers instructions. Incorporation of $\alpha_v\beta_3$, $\alpha_v\beta_5$, or $\alpha_{IIb}\beta_3$ on paramagnetic beads: 4.5 μ uncoated Dynabeads® (Dynal®, Lake Success, NY) were washed three times in phosphate buffered saline pH 7.4 (PBS) and resuspended in 50 mM Tris–HCl, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ pH 7.5 (Buffer A). Purified receptor $\alpha_v\beta_3$ and $\alpha_v\beta_5$, (Chemicon Inc.), or $\alpha_{IIb}\beta_3$ (Samanen, J., et al. *J. Med. Chem.* **1991**, *34*, 3114) were diluted in buffer A and added to the uncoated Dynabeads® at a ratio of 50 μg protein to 10^7 beads. The bead suspension was incubated with agitation overnight at 4 °C. The beads were washed three times in buffer A, 0.1% bovine serum albumin (BSA) and resuspended buffer A+3% BSA. After 3 h at 4 °C the beads were washed three times in Buffer A, 1% BSA, 0.05% azide and stored at −70 °C. Solid phase binding assay: all compounds were dissolved and serially diluted in DMSO prior to a final dilution in assay buffer (50 mM Tris–HCl pH 7.5, 100 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1% BSA, 0.05% Tween-20) containing Vitronectin-Ru or Fibrinogen-Ru and appropriate integrin coated paramagnetic beads. The assay mixture was incubated at 25 °C for 2 h with agitation and subsequently read on an Origen Analyzer® (Igen Inc. Gaithersburg, MD). Non-specific binding was determined using 1 μM Vitronectin, 1 μM Fibrinogen, or 5 mM EDTA. The data was prepared using a four-parameter fit by the Levenburg Marquardt algorithm (XLfit® ID Business Solutions). K_i values were calculated using the equation of Cheng and Prusoff (Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099).

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