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Non-Peptide $\alpha_v\beta_3$ Antagonists. Part 3: Identification of Potent RGD Mimetics Incorporating Novel β-Amino Acids as Aspartic Acid Replacements

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Abstract—Potent non-peptidic $\alpha_v\beta_3$ antagonists have been prepared incorporating various β-amino acids as aspartic acid mimetics. Modification of the β-alanine 3-substituents alters the potency and physicochemical properties of these receptor antagonists and in some cases provides orally bioavailable $\alpha_v\beta_3$ inhibitors.

Osteoporosis is a chronic skeletal disease characterized by both decreasing bone mass and increased bone fragility.[1] In healthy adults, bone mass is maintained by a homeostatic balance between the processes of bone resorption and new bone formation. In post-menopausal women, increases in osteoclast-mediated bone resorption lead to net reductions in bone mass and an increased incidence of fracture. The mechanism of bone resorption involves the attachment and migration of osteoclast cells to the bone surface in a process that is mediated by the highly expressed transmembrane integrin $\alpha_v\beta_3$.[2] This adhesion event presumably involves integrin binding to an RGD (arg-gly-asp) tripeptide sequence found in extracellular matrix proteins on the bone surface. Once osteoclasts attach to the bone matrix, a cascade of intracellular signalling events leads to the formation of a sealed acidified environment and secretion of matrix degrading proteinases at the osteoclast-bone interface.[3] Antagonism of the RGD binding domain of $\alpha_v\beta_3$ inhibits bone resorption and offers the potential of a selective, non-hormonal therapy for osteoporosis.[4]

In a previous communication, we disclosed a novel series of constrained RGD mimetics as $\alpha_v\beta_3$ antagonists.[5] These integrin antagonists exemplified by **1** incorporated three key structural features: (1) a tetrahydro-[1,8]napthyridine as a conformationally-locked guanidine replacement with reduced basicity, (2) a stereodifferentiated pyrrolidinone central constraint, and (3) a 3-substituted 3(*S*)-pyridinyl β-amino acid as an aspartic acid replacement. In spite of sub-nanomolar binding affinity of **1** to human $\alpha_v\beta_3$, poor oral pharmacokinetics emerged as a limiting feature for this compound. We envisioned replacing the 3-pyridinyl substituent in **1** with more lipophilic aryl moieties to further enhance potency in this series and to confer improved bioavailability. In this communication, we disclose novel, potent C-terminal aspartic acid replacements[6] as part of our continuing effort to discover non-peptidic $\alpha_v\beta_3$ antagonists that may be administered orally in man for the treatment and prevention of osteoporosis.

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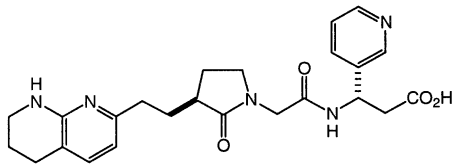

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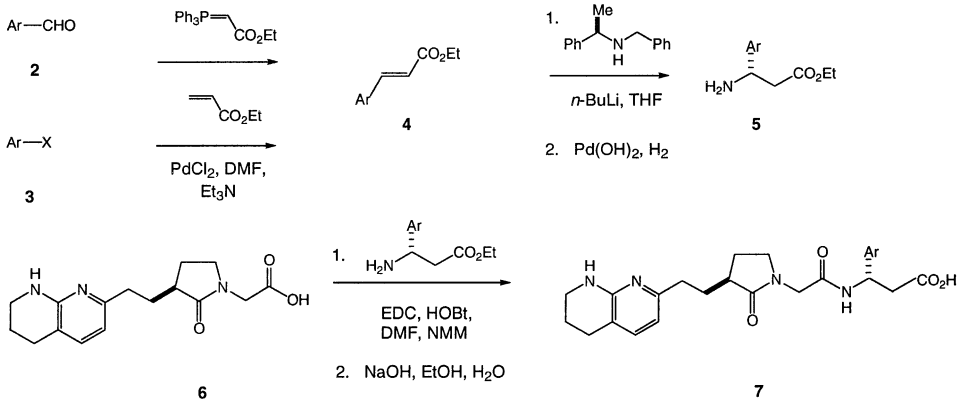
The synthesis of pyrrolidinone $\alpha_v\beta_3$ receptor antagonists described in this paper involved the union of 3-aryl β-aminoesters with carboxylic acid **6** (Scheme 1). The resultant esters were saponified to yield the final analogues (**8**–**22**) described in Table 1.[7] Enantioselective synthesis of the 3-aryl β-aminoesters was generally achieved by utilizing the Davies method[8] for heteroconjugate addition of (*R*)-(+)-*N*-benzyl-α-methylbenzylamine to the desired 3-aryl acrylates (Scheme 1). Depending on the commercial availability of starting materials, the 3-aryl acrylates were prepared by either Wittig olefination with the desired aryl aldehyde (**2**→**4**) or via a Heck cross-coupling with the aryl halide (**3**→**4**). Syntheses of the 3-pyridinyl, 5-benzodioxole, 3-quinoline, and dihydrobenzofuranyl β-amino esters are described in the literature.[9] Carboxylic acid **6** was transformed to receptor antagonists **7** by standard carbodiimide coupling and ester hydrolysis.

The compounds synthesized in this study were evaluated for their ability to inhibit the in vitro binding of a high affinity radioligand to human $\alpha_v\beta_3$ immobilized on scintillation proximity beads (SPAV3).[10] Selectivity for $\alpha_v\beta_3$ versus the fibrinogen receptor ($\alpha_{IIb}\beta_3$) was determined by measuring inhibition of the rate of ADP-stimulated platelet aggregation in human plasma.[11] This series of $\alpha_v\beta_3$ antagonists has uniformly low affinity for the fibrinogen receptor ($IC_{50} > 1$ μM).

The 3-aryl substituent is an important feature for enhancing receptor potency and modulating physical properties in this series. For example, the 3-phenyl analogue **9** binds with nearly 60-fold greater affinity relative to the unsubstituted derivative **8** (Table 1). Although the 3-pyridinyl substituent in **1** provides an additional 10-fold gain in potency versus the 3-phenyl analogue **9**, the poor pharmacokinetic profile of **1** following oral administration to dogs precluded further evaluation of this analogue in in vivo models of bone resorption. We suspected that the hydrophilic character of **1** (measured logP = −0.61) adversely affected oral absorption. Therefore, we focused our efforts on incorporation of more lipophilic, non-basic 3-aryl groups in this series with the goals of preserving sub-nanomolar potency while obtaining an acceptable pharmacokinetic profile.

In evaluating closely-related fibrinogen receptor antagonists, we and others[12] had previously identified the 3-quinolinyl β-amino acid as a potent aspartic acid mimetic. The 3-quinolinyl analogue **10** exhibited a 10-fold gain in SPAV3 potency versus the 3-pyridinyl analogue **1**. This highly potent antagonist demonstrated additional lipophilicity (measured logP 0.73) over the 3-pyridyl derivative.

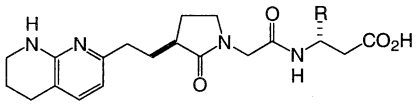
We next investigated the utility of a 3-benzodioxole β-amino acid as an aspartic acid replacement[5b] in these integrin antagonists. Incorporation of a benzodioxolyl 3-substituent in this lactam-constrained integrin antagonist series resulted in potent $\alpha_v\beta_3$ antagonist **11** (Table 1). The benzodioxole moiety has been reported to induce and inhibit human P-450 isozymes via formation of a metallocarbene complex between the dioxymethylene and heme functions.[13] Indeed, the ethyl ester prodrug of **11** was a potent time-dependent inhibitor or human CYP3A although the acid **11** was not. Nevertheless, because of these metabolic concerns, an effort to identify potent, structurally related bicyclic systems was undertaken. Receptor antagonists (**12**–**18**) that contain benzodioxole-like 3-substituents are shown in Table 1. Blocking of the metabolic activation site of the benzodioxole by difluoro-substitution to give **12** resulted in a loss of potency. Both the 5- and 6-[2,3]-dihydrobenzofurans were prepared and evaluated in our primary binding assays. We were gratified to discover that the 6-dihydrobenzofuran **14** had superior potency relative to the benzodioxole and lacked P-450 inhibition.[9d] The 5-dihydrobenzofuran isomer **13** had much lower receptor affinity. Benzofuran **14** suggests the need for heteroatom substitution in the *meta* position of the 3-aryl ring. The critical importance of the furanyl oxygen atom in analogue **14** was illustrated by evaluation of the carbocyclic pseudo-isostere **15**. Replacement of the

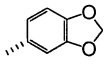
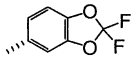
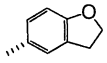
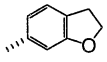
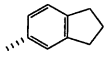
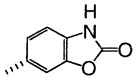
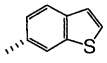
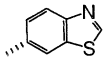
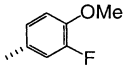
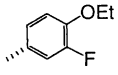


Scheme 1. Preparation of $\alpha_v\beta_3$ receptor antagonists.

furanyl oxygen atom in **14** with a methylene unit in **15** affords a 200-fold reduction in inhibitory potency. The benzoxazolidone **16** had comparable potency to the benzodioxole, while the benzothiophene **17** and benzothiazole **18** were significantly less active $\alpha_v\beta_3$ antagonists. For this series of receptor antagonists, bicyclic 3-substituents that contained a *meta*-oxygen atom were more potent than the corresponding sulfur-containing bicyclic systems.

Table 1. $\alpha_v\beta_3$ receptor antagonists



R	Entry	SPAV3 (IC_{50}, nM)	Plaggin	LogP
	**8**	230	ND	ND
	**9**	4.0	8% at 10 μM	0.07
	**1**	0.35	50% at 11 μM	−0.61
	**10**	0.04	50% at 776 μM	0.73
	**11**	0.32	50% at 5.7 μM	0.26
	**12**	5.8	3% at 10 μM	1.44
	**13**	1.1	20% at 10 μM	ND
	**14**	0.11	60% at 10 μM	0.35
	**15**	29	ND	ND
	**16**	0.29	19% at 10 μM	−0.49
	**17**	2.2	29% at 10 μM	1.15
	**18**	3.7	19% at 10 μM	0.06
	**19**	1.8	6% at 10 μM	0.43
	**20**	0.57	3% at 10 μM	>3.7
	**21**	0.49	30% at 10 μM	0.45
	**22**	0.31	28% at 10 μM	0.91

Replacement of the 3-pyridinyl substituent with 3(3-fluorophenyl) group provided analogue **19** (Table 1) which had diminished potency and increased lipophilicity. Efforts to improve the potency of **19** centered on exploring the potency enhancement effects of additional substituents on the fluorophenyl ring (Table 1). Our results with the dihydrobenzofuran **14** and 3-quinoline suggested that we could tolerate additional substitution at the 4-position of the aryl ring. Indeed, we discovered that incorporation of an aryl or alkoxy substituent in the 4-position of the 3-fluorophenyl moiety significantly enhances potency (2- to 6-fold). In particular, analogues that contained a 4-alkoxy-3-fluorophenyl β-amino acid (**21** and **22**) were more potent than **19** while maintaining comparable physical properties. The potency of analogue **22** is comparable to our initial lead receptor antagonists **1** and **11**.

From these studies, a clearer picture has emerged of some of the structural features required in the C-terminus for good receptor potency (Fig. 1). A heteroatom in the *meta*-position is significantly potency enhancing (compare **14** to **13**). The observation that the 6-[2,3]-dihydrobenzofuran **14** is 200 times more active than the corresponding carbocyclic isostere **15** suggests that either a specific hydrogen bond contact or other polar interaction may be important for high affinity binding. Lipophilic substituents in the 4-position are also potency enhancing possibly interacting with a hydrophobic pocket in the receptor binding site. This is clearly evident in both the fluorobiphenyl analogue **21** which accommodates a phenyl group in the 4-position and in the quinoline analogue **10**.

Pharmacokinetic parameters for key $\alpha_v\beta_3$ antagonists are shown in Table 2. Selected compounds were dosed orally and by intravenous injection to conscious dogs. The benzodioxole **11** was well absorbed (F = 30%) and exhibited moderate clearance and modest half-life. In contrast, the corresponding dihydrobenzofuran analogue **14** underwent more rapid plasma clearance with lower oral bioavailability. Oral bioavailability, plasma clearance, and half-life were all substantially improved

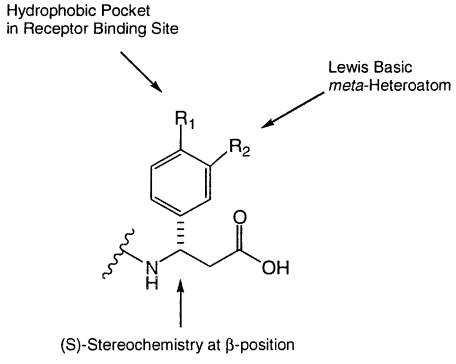


Figure 1. β-Alanine 3-aryl substituents.

Table 2. Pharmacokinetic parameters for selected $\alpha_v\beta_3$ antagonists in dogs[a]

Entry	F (%)	Cl (mL/min/kg)	$T_{1/2}$ h
1	>10	42	1.0
10	1	34	0.5
11	30	25	1.2
14	6	33	0.7
19	44	15	1.5
20	12	20	0.8

[a]Compounds dosed orally as aqueous solutions at 1 mpk and by iv at 0.2 mpk.

for compound **19**. The more potent substituted 3-fluorophenyl analogues such as **20** had attenuated pharmacokinetic profiles.[14]

In conclusion, we have identified a new class of highly potent, selective $\alpha_v\beta_3$ antagonists based on a non-peptidic RGD motif. We have identified several novel 3-substituted β-amino acids that function in these integrin antagonists as potent aspartic acid mimetics. Importantly, polar substituents in the *meta*-position of these 3-aryl substituents greatly enhance receptor binding. β-Amino acids that possess 3-heterobicyclics (dihydrobenzofuran, benzoxazolidone, and quinoline) and 3-fluorophenyl β-substituents are noteworthy. In particular, the 3-fluorophenyl derivative **19** displayed the best pharmacokinetic profile of the compounds studied. Further improvements in potency have been realized in this series by inclusion of additional 4-aryl substituents. Relating these structural modifications to effects on physicochemical properties and pharmacokinetics is currently under study.

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14. In the cases of compounds **10** and **19**, pharmacokinetic studies in bile-duct cannulated dogs indicated rapid biliary clearance of unchanged drug (>70%) as the main route of clearance.