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Synthesis and biological evaluation of nonpeptide integrin antagonists containing spirocyclic scaffolds

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Abstract—Analogues of isoxazoline $\alpha_v\beta_3$ antagonist **1** designed to further restrict the four carbon alkyl tether were prepared by incorporating two spirocyclic scaffolds, 1-oxa-2-azaspiro[4,5]dec-2-ene and 1-oxa-2,7-diazaspiro[4,4]non-2-ene. Additional optimization provided potent antagonists of both $\alpha_v\beta_3$ and $\alpha_5\beta_1$ which are selective over GPIIb/IIIa.

Integrins are heterodimeric transmembrane glycoprotein receptors which mediate various cell–cell and cell–extracellular matrix interactions.[1] As such, integrins are attractive targets for therapeutic intervention in diseases involving cell-adhesion processes[2] including thromboembolic disorders,[3] metastasis[4] and osteoporosis.[5] The glycoprotein IIb/IIIa receptor, which is ultimately responsible for platelet aggregation during thrombosis,[6] was the focus of early integrin antagonist research, and numerous small molecule inhibitors have now been studied in clinical trials.[7] More recently, another integrin, $\alpha_v\beta_3$, has moved into the forefront of integrin antagonist research. The $\alpha_v\beta_3$ integrin or vitronectin receptor binds to a variety of intrinsic ligands including vitronectin, fibronectin, von Willebrand factor, thrombospondin and osteopontin,[8] and has been implicated in a variety of pathological conditions involving abnormal cell adhesion including osteoporosis, restenosis and diseases involving neovascularization, for example cancer metastasis, diabetic retinopathy, and macular degeneration.[7] The $\alpha_5\beta_1$ integrin receptor may serve as an attachment point for cell penetration of certain viruses[9] and bacteria,[10] and is therefore of interest as a target for antiinfective therapy. The $\alpha_5\beta_1$ integrin has also been shown to affect $\alpha_v\beta_3$-mediated endothelial cell migration and angiogenesis via regulation of $\alpha_v\beta_3$ integrin function.[11] A recent study with $\alpha_v\beta_3$ and $\alpha_5\beta_1$ specific antibodies further demonstrated that the simultaneous blockade of both these integrins inhibits smooth muscle cell spreading and invasive migration (processes important in restenosis), whereas blocking $\alpha_v\beta_3$ alone was ineffective.[12] Similarly, it was reported that combined antagonism of both $\alpha_v\beta_3$ and $\alpha_5\beta_1$, as opposed to $\alpha_v\beta_3$ alone, induced apoptosis of angiogenic endothelial cells on type I collagen.[13]

All of these integrins recognize a putative RGD binding site on their respective ligands. Extremely potent inhibitors of GPIIb/IIIa and $\alpha_v\beta_3$ have been identified through the use of conformationally restricted cyclic peptides.[14] While generally unsuitable as drugs due to their poor bioavailability, these cyclic peptides provided structural insight for the design of small molecule peptidomimetics that incorporate mimics of the RGD sequence and are much more attractive as potential therapeutics. Many heterocyclic scaffolds have been successfully employed to provide the desired conformational constraint to maintain the acidic and basic ends of these linear molecules at the appropriate distance and in a conformation suitable for binding.[15] An ongoing interest in $\alpha_v\beta_3$ as a therapeutic target is evident in the number of $\alpha_v\beta_3$ antagonists that continue to be described in the literature.[16] On the other hand, selective small molecule antagonists of $\alpha_5\beta_1$ or dual $\alpha_5\beta_1$–$\alpha_v\beta_3$ antagonists have yet to be described in detail.

We have recently reported on a series of potent $\alpha_v\beta_3$ integrin antagonists which incorporate an isoxazoline ring as the conformational constraint.[17] An early example in this series was compound **1**.

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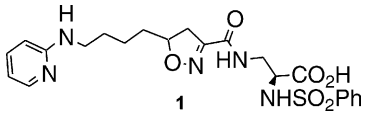

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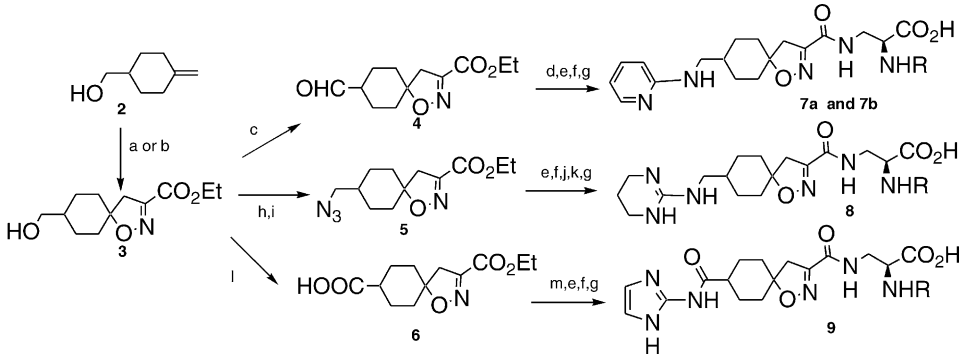
Both diastereomers of **1** had comparable potency in an $\alpha_v\beta_3$ binding assay, which suggested that the binding of the basic group occurs within the common conformational space shared by both isomers. Based on this observation, we felt that the introduction of an additional steric constraint in the alkylene linker to restrict the guanidine mimic to this common space would provide more potent analogues. The steric constraint we envisioned was a spiro-fused ring system, wherein the open chain tether between the isoxazoline and the guanidine mimic would be replaced by a spiro-fused carbocyclic or heterocyclic ring.

Spirocyclic scaffolds which incorporate an isoxazoline ring are readily available from 1,3-dipolar cycloaddition of a nitrile oxide with exomethylene carbocycles or heterocycles. An appropriately functionalized 6,5-spiro-isoxazoline scaffold was prepared from commercially available 4-methylene cyclohexanol **2** as shown in Scheme 1.[18,19] The resulting alcohol **3** was obtained as a 3:2 mixture of *cis*/*trans* diastereomers around the cyclohexyl ring. Swern oxidation gave the corresponding mixture of aldehydes **4** which were separated into single diastereomers by careful flash chromatography. Reductive amination with 2-aminopyridine in the presence of sodium triacetoxyborohydride[20] followed by hydrolysis of the ethyl ester, coupling with *t*-butyl 3-amino-2-phenylsulfonylaminopropionate[18] and TFA deprotection gave the initial targets **7**. Synthesis of analogues with alternate basic groups was accomplished by substitution of other aromatic 2-aminoheterocycles for the 2-aminopyridine with appropriate modification of the reductive amination conditions. The reductive amination could be carried out with unprotected 2-aminobenzimidazole using $Ti(OiPr)_4$ as a catalyst for imine formation followed by reduction in situ with $NaBH_4$. In the case of 2-aminoimidazole, initial formation of the intermediate imine using 1-trityl-2-aminoimidazole in the presence of anhydrous $MgSO_4$ in refluxing benzene, followed by reduction of the isolated imine was found to be superior to the one-pot procedure.[18] Unfortunately, racemization at the carbon adjacent to the aldehyde could not be avoided and the resulting products were obtained as mixtures of diastereomers. Non-aromatic heterocyclic guanidine mimics were prepared from alcohol **3** via conversion the tosylate and displacement with sodium azide to give **5**. Hydrolysis of the ester and coupling to an aminopropionate was carried out as described above. Reduction of the azide[21] provided an amine which was heated with methyl-(tetrahydropyrimidin-2-ylidene)-sulfonium iodide in DMF followed by deprotection to provide the 2-aminotetrahydropyrimidine analogue **8**. Compounds with a carbonyl in place of the methylene linker were also prepared from alcohol **3** by Jones oxidation, followed by coupling with 2-aminoimidazole sulfate in the presence of Castro's reagent. Elaboration of the acidic end as previously described provided **9**.

In the initial comparison of the spirocyclic compounds with the diastereomers of corresponding open chain compound **1**, the affinity for the $\alpha_v\beta_3$ integrin was determined using a purified $\alpha_v\beta_3$ receptor binding assay ($\alpha_v\beta_3$ ELISA).[22] Isomer **7a**, in which the carbon substituents on the cyclohexyl ring are *trans*, was comparable in potency (2 nM vs 1.3 and 0.9 nM, respectively, for the isomers of **1**) to both diastereomers of **1** and about 25X more potent that the *cis* isomer **7b**. Having established some confidence in the design of the spirocyclic linker, we proceeded to elaborate the SAR of this series with respect to the basicity of the guanidine mimic and the aryl sulfonamide alpha to the carboxy terminus. These subsequent compounds were evaluated as mixtures of diastereomers using a functional $\alpha_v\beta_3$ antagonism assay, involving adhesion of β_3-transfected 293 cells to fibrinogen ($\alpha_v\beta_3$ 293β_3).[23] Inhibition of platelet aggregation in human platelet-rich plasma (GPIIb/IIIa hPRP) was used to assess the selectivity of compounds for the $\alpha_v\beta_3$ over the GPIIbIIIa integrin receptor.[24] Compounds were also screened for $\alpha_5\beta_1$ antagonism using an ELISA assay.[25] The data are summarized in Table 1.

Varying the pK_a of the basic moiety did not significantly alter the potency. Holding the basic group constant at imidazole and varying the substituents on the aryl sulfonamide at N2 provided compounds **10**–**14**. While improved potency in the $\alpha_v\beta_3$ cell adhesion assay was



Scheme 1. Reagents and conditions: (a) ethyl oximidoacetate, $NaHCO_3$, THF, 0 °C; (b) diethyl nitromalonate, mesitylene, 175 °C; (c) $(COCl)_2$, DMSO; (d) 2-aminopyridine, $Na(OAc)_3BH$, DCE; (e) 1 M LiOH, THF; (f) *t*-butyl 3-amino-2-phenylsulfonylaminopropionate, Castro's reagent, NMM, DMF; (g) TFA, CH_2Cl_2; (h) TsCl, pyr; (i) NaN_3, DMF; (j) Ph_3P, H_2O; (k) methyl-(tetrahydropyrimidin-2-ylidene)-sulfonium iodide DMF, 95 °C; (l) Jones reagent; (m) 2-aminoimidazole sulfate, Castro's reagent, DMF, NMM, 70 °C.

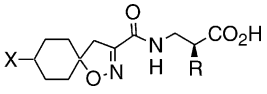
obtained with des-methyl compound **11** and the analogous 2,6-dichloro analogue **12**, the selectivity over IIb/IIIa for these compounds was only 5–15-fold. Improved selectivity over IIb/IIIa was obtained with compounds **13** and **14** which have biphenylsulfonamide groups, however, the addition of the second phenyl ring significantly reduced the aqueous solubility of these compounds. Also, the examples in Table 1 were 2–10× less potent than the corresponding open-chain compounds previously reported.[18]

Based on the above results, a spirocyclic scaffold based on 3-hydroxyproline was designed to further rigidize the molecule and set the absolute stereochemistry of the products. The substituent on the second nitrogen in the spirocycle was also viewed as an opportunity to fine tune the properties of the molecules. Synthesis of a spiro-fused 5,5-system was accomplished as shown in Scheme 2 starting from *N*-Cbz-4-hydroxy-*cis* L-proline **15**. Reduction of the acid with borane–methyl sulfide complex[26] provided the diol which was selectively protected on the primary alcohol followed by Swern oxidation of the remaining secondary hydroxyl to provide ketone **16**. A Wittig reaction generated the *exo*-methylene compound which underwent 1,3-dipolar cycloaddition with ethyl oximidoacetate to give a 7:1 mixture of diastereomers that were readily separated by flash chromatography to give the single isomers. NOE experiments on the deprotected alcohol **17b** established that the carbon substituents on the spiro system were *cis* in the major diastereomer. The major diastereomer therefore has (*S*,*S*) absolute stereochemisty, and the minor product is the (*R*,*S*) isomer. The remaining steps were carried out as described above on the individual isomers to provide the compounds shown in Table 2.

The (*S*,*S*,*S*) isomer **18a** was >20× more potent than the (*S*,*R*,*S*) isomer **18b**. As previously observed, varying the basic group in compounds **19**–**21** did not significantly affect the $\alpha_v\beta_3$ potency, however improved selectivity over IIb/IIIa was obtained by lowering the pK_a of the basic moiety. The proline-derived compounds with R^1 = Cbz exhibited 3–6-fold better potency versus $\alpha_v\beta_3$ as compared to the analogous compounds in Table 1 and had similar potency to the open chain isoxazolines with increased selectivity over IIb/IIIa. Removal of the Cbz group to provide **22** resulted in a drop in $\alpha_v\beta_3$ potency as well as a 100-fold loss of selectivity over IIb/IIIa. The $\alpha_v\beta_3$ potency was retained in the methylcarbamate **23**, but activity dropped off 3-fold as the size of the alkyl group was increased to *n*-butyl in **24**. The inverse was true for selectivity over IIb/IIIa. Pyridylcarbamate **25** was equipotent to **19** and shows good aqueous solubility, although there was some loss in selectivity in going from phenyl to pyridyl.

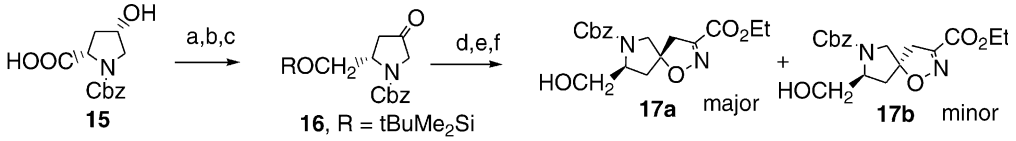
The introduction of a second lipophilic substituent at the basic end of the molecule also resulted in a significant increase in activity against $\alpha_5\beta_1$. In contrast to the IIb/IIIa selectivity, the larger, more lipophilic carbamates (Cbz > CO_2Bu > CO_2Me > H) and less basic guanidine mimics (benzimidazole≈pyridine > imidazole > imidazoline) resulted in more potent $\alpha_5\beta_1$ antagonists. Both **20** and **27** were subnanomolar antagonists in the $\alpha_5\beta_1$ ELISA assay. When compared to the corresponding open-chain analogue (example 3e in ref 18), compound **20** is equipotent in the 293β3 $\alpha_v\beta_3$ assay but >1000-fold more potent in the $\alpha_5\beta_1$ ELISA assay.[27] Consistent with its increased potency versus $\alpha_5\beta_1$, compound **27** was shown to inhibit the in vitro streptococcal

Table 1.



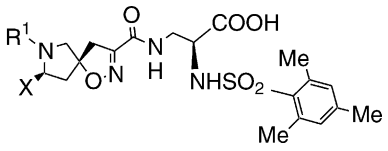
Compd[a]	X	R	αvβ3 293β3 IC_{50}±SEM (nM)[b]	GPIIb/IIIa hPRP IC_{50} (nM)	$\alpha_5\beta_1$ ELISA IC_{50} (nM)
7c	2-Imidazolyl $NHCH_2$	SO_2mesityl	170±120 (4)	1000	1500
7d	2-Benzimidazolyl $NHCH_2$	SO_2mesityl	440±160 (3)	7100	170
8	3,4,5,6-Tetrahydro-2-pyrimidinyl $NHCH_2$	SO_2mesityl	160±28 (3)	800	NT
9	2-ImidazolylNHCO	SO_2mesityl	106±31	33,000	2700
10	2-Imidazolyl $NHCH_2$	SO_2Ph	130±48 (5)	830	4700
11	2-Imidazolyl $NHCH_2$	SO_2(2,6-dimethylphenyl)	56±24 (4)	310	1800
12	2-Imidazolyl $NHCH_2$	SO_2(2,6-dichlorophenyl)	34±8.6 (4)	490	3000
13	2-Imidazolyl $NHCH_2$	SO_2(2,6-dimethyl-4-biphenyl)	93±45 (5)	8300	670
14	2-Imidazolyl $NHCH_2$	SO_2(2,6-dichloro-4-biphenyl)	17±7.4 (4)	8300	310

[a] All compounds were purified by reverse-phase HPLC and isolated as TFA salts. Satisfactory spectral and analytical data were obtained for all compounds.
[b] Values are means of number of experiment in parentheses.



Scheme 2. Reagents and conditions: (a) BH_3·Me_2S, THF; (b) *t*$BuMe_2SiCl$, TEA, CH_2Cl_2; (c) $(COCl)_2$, DMSO; (d) $Ph_3PCH_3^+Br^-$, KO*t*Bu, THF; (e) ethyl oximidoacetate, TEA, CH_2Cl_2; (f) HOAc/THF/H_2O.

Table 2.



Compd[a]	X	R^1	αvβ3 293β3 IC_{50}±SEM (nM)[b]	GPIIb/IIIa hPRP IC_{50} (nM)	$\alpha_5\beta_1$ ELISA IC_{50} (nM)
18a	2-Dihydroimidazolyl $NHCH_2$	Cbz	57±35	7300	13
18b	2-Dihydroimidazolyl $NHCH_2$[f]	Cbz	>1000	9200	1200
19	2-Imidazolyl $NHCH_2$[e]	Cbz	41±26	34,000	18
20	2-Pyridyl$NHCH_2$	Cbz	49±24	>100,000	0.18
21	2-Benzimidazolyl $NHCH_2$	Cbz	74±28	>100,000	NT
22	2-Imidazolyl $NHCH_2$[e]	H	180±74	1400	230
23	2-Imidazolyl $NHCH_2$[e]	CO_2Me	57±35[c]	7300	21
24	2-Imidazolyl $NHCH_2$[e]	CO_2nBu	130±70	11,000	14
25	2-Imidazolyl $NHCH_2$[e]	CO_2CH_2(3-pyr)	32±29[d]	5600	39
26	2-Benzimidazolyl $NHCH_2$	CO_2Me	34±15	>100,000	1.4
27	2-Benzimidazolyl $NHCH_2$	CO_2nBu	87±38	>100,000	0.39

[a] All compounds were purified by reverse-phase HPLC and isolated as TFA salts.Satisfactory spectral and analytical data were obtained for all compounds.
[b] *n* of 3.
[c] *n* of 4.
[d] *n* of 6.
[e] Mix of diastereomers at C7.
[f] (*S*,*R*,*S*) isomer.

invasion of mammalian epithelial cells, a process mediated by the $\alpha_5\beta_1$ integrin.[28]

In conclusion, we have identified two spirocycles which can serve as novel conformational constraints in RGD peptidomimetics. With appropriate substitutions, the 1-oxa-2,7-diazaspiro[4,4]non-2-ene scaffold provided potent and selective antagonists of both the $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin receptors.

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