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RAPID SYNTHESIS OF RGD MIMETICS WITH ISOXAZOLINE SCAFFOLDS ON SOLID PHASE: IDENTIFICATION OF αvβ3 ANTAGONISTS LEAD COMPOUNDS

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Abstract: Isoxazoline containing RGD mimetics were rapidly synthesized on a solid phase to optimize linkers, regioisomers of isoxazoline scaffolds, and exosite binding groups to yield lead αvβ3 antagonists.

Integrins are a widely expressed family of cell surface adhesion proteins that participate in cell–cell adhesion or cell to extracellular matrix adhesion events. These adhesion events play important physiological roles during development and pathogenesis.[1] Integrins are expressed on a wide variety of cell types such as monocytes, osteoclasts, activated lymphocytes, endothelial cells, platelets, smooth muscle cells, melanomas, and breast carcinomas. The ability to selectively interfere with the functions of the cell adhesion receptors by small molecules offers many opportunities for therapeutic intervention of diseases as diverse as thrombosis, inflammation, osteoporosis, diabetic retinopathy, restenosis and cancer.[2]

The fibrinogen receptor (GPIIb/IIIa), which belongs to the family of integrin receptor proteins, has been implicated in platelet aggregation. GPIIb/IIIa has been extensively investigated over the past decade in efforts to find antithrombotic agents.[3] These efforts have resulted in clinical investigation of several nonpeptide GPIIb/IIIa antagonists for the treament of thromboembolic disorders.[4] The vitronectin receptor (αvβ3) is closely related to GPIIB/IIIa where the β3 subunit is shared by both integrins. Integrins αvβ3 and GPIIb/IIIa recognize RGD (Arg-Gly-Asp) motifs of extracellular ligands such as fibronectin, fibrinogen, vitronectin, and osteopontin. The αvβ3 which is expressed on osteoclasts, endothelial cells, smooth muscle cells and tumor cells has been shown to mediate several biologically relevant processes including angiogenesis, adhesion of osteoclasts to the bone matrix, and migration of smooth muscle cells. Consequently, αvβ3 antagonists may have potential use in the treatment of restenosis, ocular neovascularization related to diabetic retinopathy, age-related macular degeneration, neovascularization associated with tumor growth, and metastasis.[5]

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During the lead identification phase of the αvβ3 antagonists program, we not only screened the collection of compounds from the IIb/IIIa program[7b, 7c], but also became interested in using parallel synthesis technology on solid phase. The basic and acidic groups make major contributions to the binding of RGD mimetics to the integrin receptor via charge–charge interactions. The distance between the positively charged ammonium group and the negatively charged carboxylate group is important for binding. The maximum possible distance will be dictated by the most extended conformation of the tripeptide RGD itself. Synthesis of a library of RGD mimetics was undertaken (Scheme 1) where the 3- and 5-positions of isoxazoline heterocycle were utilized for displaying positively charged ammonium and negatively charged carboxylate groups. There were four variables selected for library construction viz. linker L_1 , linker L_2, regioisomers of isoxazoline A and B, and the substituent alpha to the carboxylic acid group (exosite binding group) R^1 (Figure 1).

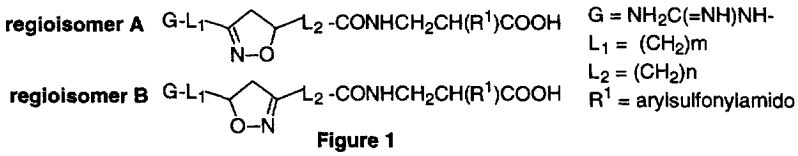
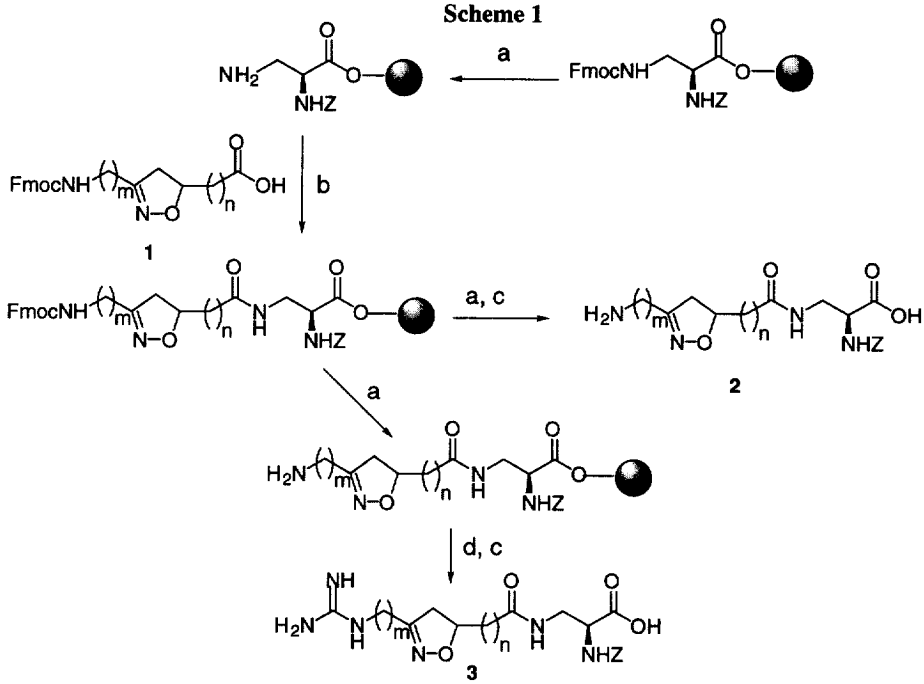


Figure 1

We were interested in maintaining the iterative process involved in the classical medicinal chemistry approaches where the choice of the next set of synthetic targets is determined by the biological data on the previous set of compounds. Initially, we chose to optimize the distance between the basic and acidic groups by changing linkers L_1 and L_2. Isoxazoline building blocks with appropriate linkers were individually synthesized (as racemic mixtures) using literature procedures since the specialized building blocks were not commercially available.[6] The subsequent set of compounds was synthesized on solid phase using the optimized regioisomer of isoxazoline with optimized linkers while varying the substituent alpha to the carboxylic acid group. Diaminopropionic acid was selected as an aspartic acid mimic due to its success in the IIb/IIIa antagonists.[7]

α-N-CBZ-β-N-Fmoc-L-diaminopropionic acid (DAP) was attached to the bromomethyl Wang resin as described previously[8] and the Fmoc group was removed by treatment with 20% piperidine in DMF. The appropriate Fmoc protected isoxazoline derivative was then coupled to the DAP derivative on solid phase using HBTU as a coupling reagent. The Fmoc group was removed using 20% piperidine in DMF and the amine product was obtained by cleavage from the solid phase using trifluoroacetic acid in dichloromethane. The deprotected amines on the solid phase were guanylated using N,N-*bis*-Boc-S-ethylthiourea and the final products were obtained in 70-80% yields and 70-95% purities (HPLC method) by cleavage from the solid phase using trifluoroacetic acid in dichloromethane (Scheme 1). The crude products were characterized by mass spectrometry and HPLC and initial binding to αvβ3 was determined by an ELISA assay.[9] The primary amines bound to αvβ3 integrin with low affinity giving <50% inhibition at 10 μM level. Guanidines were much more active, binding in the submicromolar range. The compounds listed in Table 1 were purified[10] to homogeneity by reverse phase HPLC and final binding constants were measured.

Scheme 1



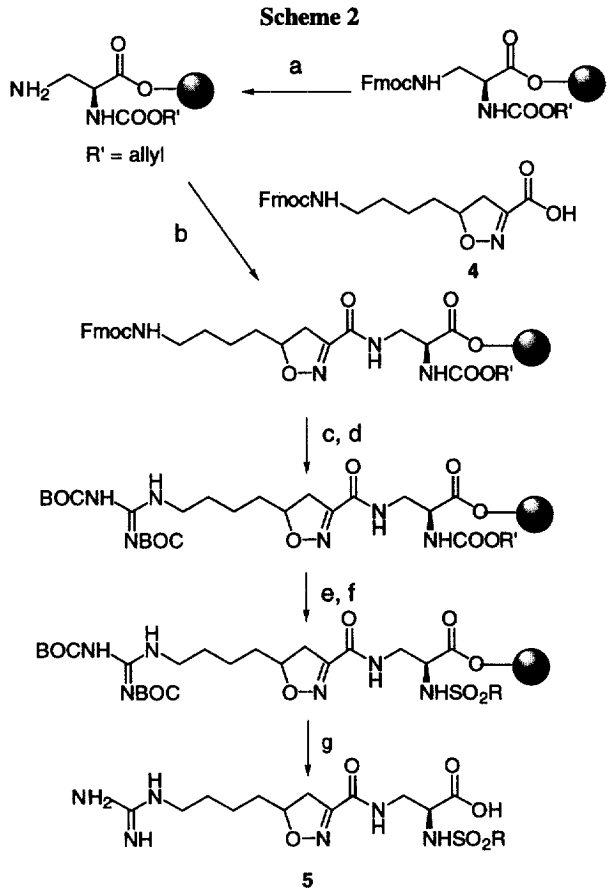
(a) 20% piperidine in DMF/ 25 °C/ 10 min; (b) HBTU/ DIEA in DMF/ 25 °C/ 18 h; (c) 50% TFA in CH_2Cl_2/ 25 °C/ 2 h; (d) N,N-*bis*-Boc-S-ethylthiourea in DMF/ DIEA/ 25 °C/ 18 h.

Table 1. Optimization of linkers and regiosomers of isoxazoline

Compound	m	n	regioisomer	αvβ3 (ELISA) IC50 nM	GPIIb/IIIa (PRP) IC_{50} μM
3a	4	0	A	81	55
3b	3	1	A	72	12
3c	2	2	A	480	23
3d	3	0	A	110	>100
3e	2	1	A	900	12
3f	5	0	A	710	13
3g	3	2	A	100	ND
3h	4	1	A	180	0.8
3i	5	1	A	470	1.5
3j	4	2	A	300	3.6
3k	2	0	A	2600	>100
3l	4	0	B	23	ND
3m	2	2	B	87	17
3n	3	0	B	140	53
3o	1	2	B	310	>100
3p	2	0	B	66	ND

Compounds **3a**, **3b**, **3l** and **3m**, in which the combined chain length (m + n) for the linkers was four atoms exhibited the best activity. Interestingly, compound **3p** that has chain length of two atoms was also as active as **3a**. One possible explanation is that the guanidine moiety of compound **3p** may be binding to the receptor through a water molecule. Nevertheless, we concluded that the combined optimum chain length for the linkers is four atoms. The αvβ3 integrin activity was dependent on the regiochemistry of isoxazoline. The isoxazoline with the carboxamido group attached to the sp2 carbon (3 position) is more potent than the isoxazoline with the carboxamido group substituted at sp3 carbon (5 position).

Scheme 2



(a) 20% piperidine in DMF/ 25 °C/ 20 min; (b) HBTU/ DIEA in DMF/ 25 °C/ 18 h; (c) 20% piperidine in DMF/ 25 °C/ 20 min; (d) N,N-*bis*-Boc-S-ethylthiourea in DMF/ DIEA/ 25 °C/ 18 h; (e) $(Ph_3P)_4Pd$, HOAc, 4-N-methylmorpholine in DMF/ CH_2Cl_2/ 25 °C/ 3 h; (f) $ArSO_2Cl$, DIEA in DMF/ 25 °C/ 18 h; (g) 50% TFA in CH_2Cl_2/ 25 °C/ 2 h.

The optimized features of the first set were incorporated into a second set of compounds where the substituent alpha to the carboxylate was varied through parallel synthesis. The synthesis of this set of compounds on solid phase is shown in Scheme 2. α-N-Alloc-β-N-Fmoc-L-diaminopropionic acid was attached to the Wang resin through displacement of the bromide as described earlier.[8] The Fmoc group was deprotected by treatment with 20% piperidine in DMF. The Fmoc-protected isoxazoline derivative **4** (regioisomer A) was coupled to the resin using HBTU as a coupling reagent. The removal of the Fmoc group followed by guanylation furnished intermediates in which the guanidino group was protected as bis-BOC. The allyloxycarbonyl was then removed using the standard conditions known for solution phase synthesis.[12] Preliminary results indicated that sulfonamides are about 10-fold more potent than carbamates. Several selected sulfonyl chlorides were then coupled in DMF using diisopropylethylamine as a Lewis base. The compounds were obtained in 75-90% yields and >80% purities after the cleavage from the resin by treatment with TFA in dichloromethane. The ELISA data on the unpurified compounds is summarized in Table 2.

Table 2. Optimization of alpha substituent to the carboxylic acid group on regiosomer A.

Compound	R	αvβ3 ELISA IC_{50} nM	GPIIb/IIIa PRP IC_{50} μM
5a	phenyl	11	0.78
5b	4-fluorophenyl	15	1.3
5c	4-chlorophenyl	12	2.6
5d	4-methoxyphenyl	12	0.46
5e	4-nitrophenyl	140	43
5f	4-tert-butylphenyl	36	47
5g	2-bromophenyl	4	0.17
5h	2,4,6-trimethylphenyl	0.7	0.34
5i	1-naphthyl	16	3.5
5j	1-(5-N,N-dimethylamino)quinolyl	7	7.9
5k	2-thienyl	23	0.36
5l	3-(2,5-dichloro)thienyl	24	1.9
5m	5-(2-pyridyl)thienyl	8	0.69
5n	3-(1-methyl)pyrazolyl	96	3.6
5o	3-(2-chloro-1,4-dimethyl)pyrazolyl	2	0.22
5p	4-(3,5-dimethyl)isoxazolyl	14	0.85

Arylsulfonamides (**5a-p**; Table 2) were found to be more potent than benzylcarbamate **31** and among the group of arylsulfonamides, 2,4,6-trimethylphenylsulfonamide **5h** was found be the most potent αvβ3 antagonist. The lead compound **5h** was then further optimized by conventional medicinal chemistry approaches.[12] In conclusion, parallel synthesis was found to be useful in rapid identification of lead compounds as antagonists of the αvβ3 integrin receptor.

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