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Synthesis of pyrazoles and isoxazoles as potent $\alpha_v\beta_3$ receptor antagonists

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Abstract—We describe a series of pyrazole and isoxazole analogs as antagonists of the $\alpha_v\beta_3$ receptor. Compounds showed low to sub-nanomolar potency against $\alpha_v\beta_3$, as well as good selectivity against $\alpha_{IIb}\beta_3$. In HT29 cells, most analogs also demonstrated significant selectivity against $\alpha_v\beta_6$. Several compounds showed good pharmacokinetic properties in rats, in addition to anti-angiogenic activity in a mouse corneal micropocket model. Compounds were synthesized in a straightforward manner from readily available glutarate precursors.

The integrin $\alpha_v\beta_3$ is a heterodimeric transmembrane receptor found on the surface of activated endothelial cells, smooth muscle cells, and many tumor cells. $\alpha_v\beta_3$ recognizes the RGD tripeptide sequence on numerous extracellular matrix proteins[1] allowing endothelial cells and tumor cells to interact with these proteins. A number of antagonists of $\alpha_v\beta_3$ have been reported to demonstrate anti-angiogenic activity, and thus have potential utility in inhibiting tumor growth.[2] $\alpha_v\beta_3$ is also the predominant integrin found on the surface of osteoclasts, which are responsible for cellular attachment, and subsequent bone resorption.[3] Antagonists of $\alpha_v\beta_3$ have been shown to block the degradation of bone in animal models of osteoporosis[4] and thus may also have utility in the treatment of this disease.

We previously described an RGD-based peptidomimetic $\alpha_v\beta_3$ antagonist, SC-68448, which demonstrated antitumor efficacy in a mouse Leydig cell tumor model.[5] We later reported a series of conformationally restricted cinnamic acid analogs such as **1** in which the glycine-3-aminopropionic acid functionality was replaced by the bioisosteric 4-aminocinnamic acid moiety.[6] In two recent papers, this work was extended to a series of oxadiazole- and thiazole-containing analogs (**2** and **3**).[7,8] These compounds were shown to be potent and selective antagonists of $\alpha_v\beta_3$ with a reasonable pharmacokinetic profile in rats. To further expand upon this work, two additional heterocyclic series, pyrazoles and isoxazoles, were investigated using intermediates available from the previously described heterocyclic series.[7,8]

The glutarate portion of the molecule was synthesized from readily available aryl or heteroaryl aldehydes (Scheme 1). Condensation with ethylacetoacetate provided the cyclic compound **26**, which could be converted to either glutaric diacid **27** or diester **28**. Treatment of **27**

Keywords: Integrin antagonist; $\alpha_v\beta_3$; RGD peptidomimetic.
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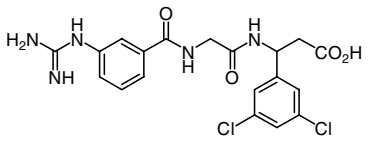
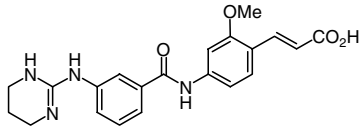


SC-68448



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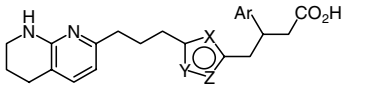
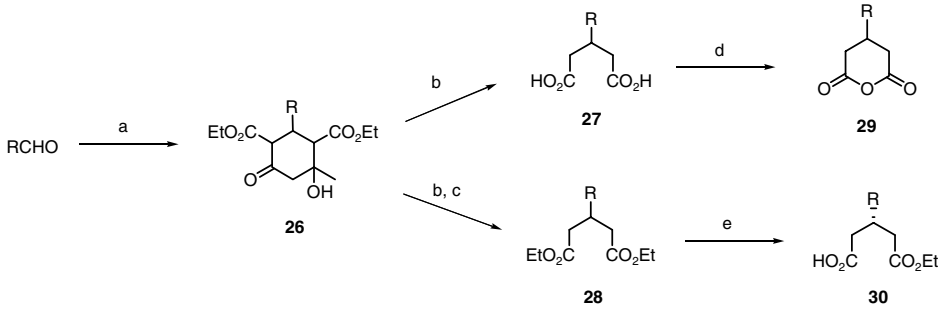


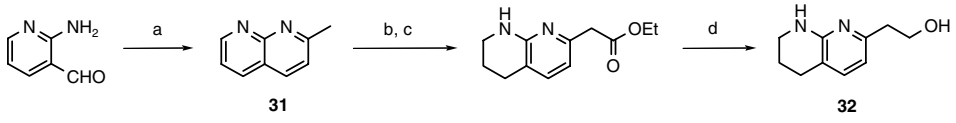
2 X=N, Y=N, Z=O
3 X=N,Y=S, Z=CH

with acetic anhydride gave glutaric anhydride **29**, while enzymatic resolution followed by chiral chromatography provide chiral mono-ester **30**. The 1,8-tetrahydronaphthyridine guanidine mimetic **32** was synthesized using conditions somewhat modified from what was previously described[10] (Scheme 2). 2-Amino-3-pyridine carboxaldehyde was treated with acetone under Friedlander conditions to provide **31**, which was deprotonated and reacted with diethyl carbonate. Catalytic hydrogenation followed by LAH reduction provided alcohol **32**. The synthesis of other guanidine isosteres detailed in the tables has been described previously.[7–9] Scheme 3 describes the general synthesis of racemic pyrazoles and isoxazoles. The lithium enolate of ethyl acetate was added to anhydride **29** to give β-ketoester **33**. Condensation with hydroxylamine, followed by esterification, gave hydroxyisoxazole **34**. Mitsunobu reaction with a guanidine-containing alcohol **35**, followed by saponification, gave the desired isoxazole acids (**4** and **6**–**10**). Alternately, condensation of **33** with hydrazine or a mono-substituted hydrazine gave, after esterification, hydroxypyrazole **36**. Mitsunobu reaction with **35** or alkylation with bromide **37** provided ester **38**. Saponification gave the desired pyrazole acids (**5**, **11**–**19**, and **22**–**24**). Hydroxypyrazole **36** could also be converted to thiol **39** using Lawesson's reagent. Alkylation with **37** gave ester **40**, which was saponified to acid **20** or oxidized with Oxone® and saponified to give acid **21**. The (*S*)-isomer of pyrazole **11** was prepared under somewhat modified conditions (Scheme 4). Chiral acid **30** was condensed with Meldrum's acid to give, after refluxing in ethanol, diester **41**. Condensation with methylhydrazine, Mitsunobu reaction with **32**, and saponification gave (*S*)-**11**.

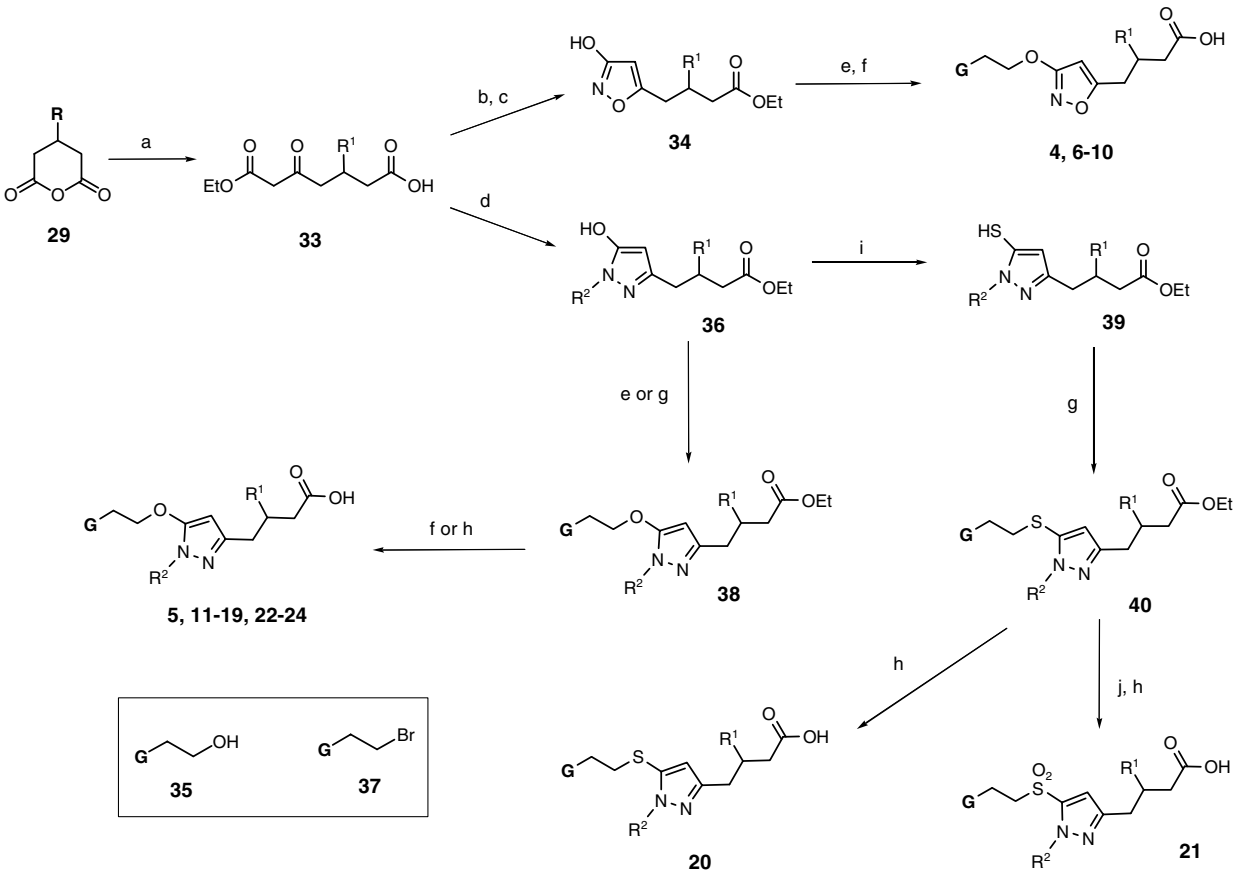
To expand upon the previously described oxadiazole and thiazole series of inhibitors,[7,8] related pyrazole and isoxazole series were investigated. The initial synthesis focused on analogs that maintained a 3,4-methylenedioxyphenyl β-substituent and the 1,8-tetrahydronaphthyridine guanidine isostere (**4** and **5**). Isoxazole **4** and unsubstituted pyrazole **5** (Tables 1 and 2) both showed very similar low-nanomolar potency in the $\alpha_v\beta_3$ solid-phase receptor assay (SPRA). Compounds **4** and **5** also demonstrated reasonable selectivity against the other β_3 integrin, $\alpha_{IIb}\beta_3$. In addition, these compounds showed excellent cellular activity against $\alpha_v\beta_3$ in 293 cells[11] and were selective versus $\alpha_v\beta_6$ in HT29 cells. Based on the promising potency and selectivity profiles of **4** and **5**, as well as ease of synthesis, more detailed SAR studies were carried out on these two heterocyclic classes. Table 1 describes the SAR of a series of isoxazole analogs. β-substituents that had previously demonstrated good potency against $\alpha_v\beta_3$ in other heterocyclic series were chosen for further study. In addition to the relatively optimized tetrahydronaphthyridine group, several other guanidine isosteres were also investigated. These included additional groups with reduced basicity relative to a simple guani-



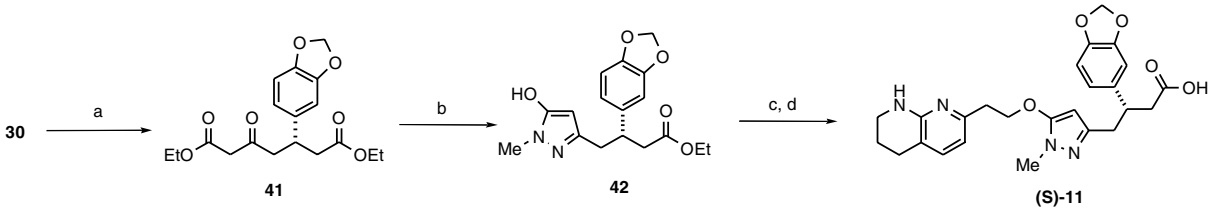
Scheme 1. Reagents: (a) ethyl acetoacetate, piperidine; (b) i—10% NaOH, EtOH, Δ; ii—HCl; (c) EtOH, HCl(g); (d) Ac_2O, Δ; (e) i—Chirazyme L-2, ii—chiral chromatography.



Scheme 2. Reagents and conditions: (a) acetone, L-proline, EtOH, Δ; (b) LiHMDS, THF, diethyl carbonate, −40 to 0 °C; (c) 10% Pd/C, H_2, EtOH; (d) LAH, THF.



Scheme 3. Reagents and conditions: (a) LDA, EtOAc, −78 °C; (b) NH_2OH–HCl, NaOH, H_2O; (c) 4 N HCl, dioxane, EtOH; (d) R^2NHNH_2, EtOH, Δ; (e) diethyl azodicarboxylate, Ph_3P, **35**, THF; (f) 1 N NaOH, THF; (g) Na_2CO_3, **37**, DMF; (h) HCl, H_2O, acetone, Δ; (i) Lawesson's reagent; (j) Oxone®.

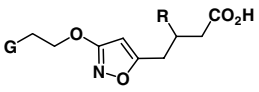


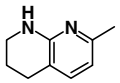
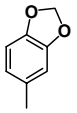
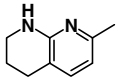
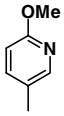
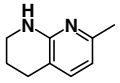
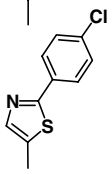
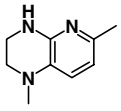
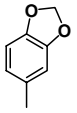
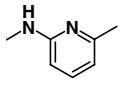
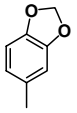
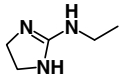
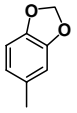
Scheme 4. Reagents: (a) i—diethyl cyanophosphonate, Meldrum's acid, Et_3N, DMF, ii—EtOH, Δ; (b) $MeNHNH_2$, EtOH, Δ; (c) **32**, diisopropyl azodicarboxylate, Ph_3P, THF; (d) HCl, H_2O, acetone, Δ.

dine group, as well as a more basic cyclic guanidine (i.e., **10**). These analogs demonstrated excellent potency, both in SPRA and cellular assays. In addition, good selectivity versus $\alpha_v\beta_6$ in cells was observed, particularly with aminopyridine **9** and cyclic guanidine **10**. In general, significant selectivity versus $\alpha_v\beta_5$ was not obtained.

N-substituted analogs of pyrazole **5** were also investigated (Table 2). Several diverse substituents on the pyrazole ring nitrogen were well tolerated, providing analogs with low to sub-nanomolar potency in both $\alpha_v\beta_3$ SPRA and cellular assays. Both sterics and electronics seemed to have minimal effect on potency. However, for the substituted analogs, selectivity versus $\alpha_{IIb}\beta_3$ was somewhat diminished, while selectivity versus $\alpha_v\beta_6$ was generally maintained. Since **11** was identified as one of the more potent analogs, particularly in 293 cells, the (*S*)-enantiomer (in previous series, consistently the more potent isomer) was synthesized, and as expected, showed a modest increase in potency. Based on the excellent potency of **11**, further modifications of this *N*-methyl analog were investigated, focusing on alternate β-substituents and guanidine isosteres (Table 3). In addition, a thioether (**20**) and sulfonyl (**21**) linker were explored in place of the ether linkage. All were very potent inhibitors of $\alpha_v\beta_3$, in both SPRA and 293 whole cell assays. As before, aminopyridine and cyclic

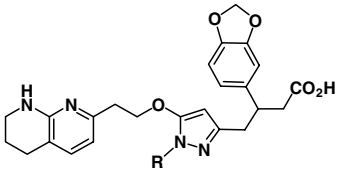
Table 1. In vitro data for isoxazole analogs



Compound	G	R	IC_{50}[a] (nM)			
			$\alpha_v\beta_3$ SPRA	$\alpha_v\beta_3$ 293	$\alpha_v\beta_5$ 293	$\alpha_v\beta_6$ HT29
4			1.0	0.34	1.2	350
6			0.9	—	—	66.4
7			1.1	—	—	131
8			1.2	0.69	1.47	323
9			2.4	2.49	17.5	8225 (2)
10			0.9	0.58	9.4	6830

[a] Average of at least three determinations except where noted.

Table 2. In vitro data for N-substituted pyrazole analogs



Compound	R	IC_{50}[a] (nM)				
		$\alpha_v\beta_3$ SPRA	$\alpha_{IIb}\beta_3$ SPRA	$\alpha_v\beta_3$ 293	$\alpha_v\beta_5$ 293	$\alpha_v\beta_6$ HT29
5	–H	1.2	81.9	0.71	2.1	674
11	–Me	1.1	23.8	0.21	0.77	754
(*S*)-**11**	–Me	0.44	—	—	—	347
12	–*n*-Bu	2.7	39.4	1.25	3.0	1633 (2)
13	$-CH_2CF_3$	1.5	32.7	1.09	1.03	465
14	$-CH_2CH_2OH$	1.0	—	—	—	25.9
15	$-CH_2CO_2H$	0.6	—	—	—	491
16	$-CH_2Ph$	2.6	13.5	0.9	1.84	2108
17	–Ph	1.4	24.4	1.18	3.27	352

[a] Average of at least three determinations except where noted.

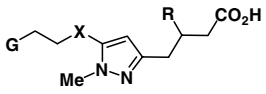
guanidine analogs (**24** and **25**) also demonstrated excellent selectivity against $\alpha_v\beta_6$ in HT29 cells, along with thioether analogs **20** and **21**.

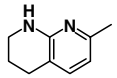
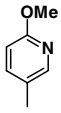
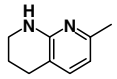
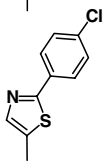
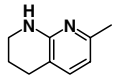
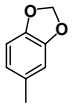
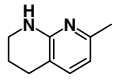
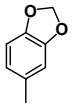
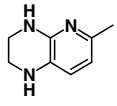
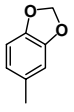
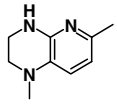
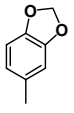
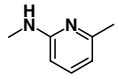
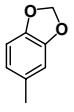
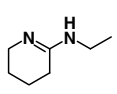
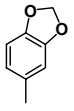
Rat pharmacokinetic data, along with anti-angiogenic efficacy data in a mouse corneal micropocket model[12] are shown for select analogs in Table 4. In general, most compounds in these series demonstrated good pharmacokinetic properties in rats, with excellent oral bioavailability and a reasonable half-life. The exception was for those analogs that contained a more basic guanidine moiety, such as **10**, which was poorly absorbed orally and had a relatively short half-life.

Compounds **4**, **10**, and **11** all showed good efficacy in the micropocket model when dosed by osmotic mini-pump (OMP) for 5 days at doses from 50 to 100 mg/kg/day. Although **4** and **11** showed good oral bioavailability in the rat, oral dosing was not investigated in this model. However, **23** was dosed orally at 30 mg/kg/day and demonstrated reasonable efficacy.

In conclusion, we have described a new series of $\alpha_v\beta_3$ receptor antagonists with excellent potency in a solid-phase receptor assay as well as in a cellular assay. These analogs also showed reasonable selectivity versus $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_6$, and a very good rat pharmacokinetic profile. In addition, several analogs also demonstrated efficacy in an in vivo model of angiogenesis, further supporting the concept of using $\alpha_v\beta_3$ receptor antagonists for select disease states.

Table 3. In vitro data for pyrazole analogs



Compound	G	X	R	IC_{50}[a] (nM)				
				$\alpha_v\beta_3$ SPRA	$\alpha_{IIb}\beta_3$ SPRA	$\alpha_v\beta_3$ 293	$\alpha_v\beta_5$ 293	$\alpha_v\beta_6$ HT29
18		–O–		0.8	—	—	—	164
19		–O–		1.4	57.6	0.9	18	189
20		–S–		1.5	37.6 (1)	0.8	5.43	4623 (2)
21		$–SO_2–$		2.6	—	1.69	1.86	1394 (2)
22		–O–		0.83	—	—	—	216
23		–O–		1.2	36.2	0.75	2.58	1433
24		–O–		2.6	80.3 (1)	1.77	11	4762 (2)
25		–O–		21	16.2 (1)	—	—	>10,000 (2)

[a] Average of at least three determinations except where noted.

Table 4. Pharmacokinetic and in vivo efficacy data

Compound	Rat PK		Mouse CMP
	F (%)	$t_{1/2}$ (h)	(% inhib of angiogenesis)
4	86	1.3	25% at 100 mpk/d (OMP)
10	8.5	0.5	50% at 50 mpk/d (OMP)
11	95	4.9	41% at 100 mpk/d (OMP)
23	100	5.0	33% at 30 mpk/d (po, bid)

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