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Synthesis of cinnamic acids and related isosteres as potent and selective αvβ3 receptor antagonists

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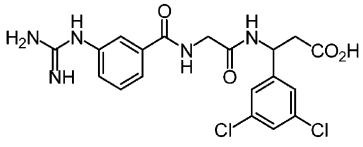
Abstract—We describe a series of conformationally-restricted cinnamic acid peptidomimetics as well as several cinnamic acid isosteres, including 3-phenylpropionic acids, 2-amino-3-phenylpropionic acids, phenoxyacetic acids and 2-phenylcyclopropylcarboxylic acids. Several analogues demonstrated low to sub-nanomolar potencies against $\alpha_v\beta_3$ and greater than 200-fold selectivity against the other β_3 integrin $\alpha_{IIb}\beta_3$. In whole 293 cells, many of these analogues also showed modest selectivity against other α_v integrins such as $\alpha_v\beta_1$ and $\alpha_v\beta_5$. These compounds were synthesized from readily available starting materials using either Heck or Mitsunobu coupling conditions.

The integrin $\alpha_v\beta_3$ is a non-covalently linked, heterodimeric transmembrane receptor found on the surface of activated endothelial cells, smooth muscle cells and many tumor cells. $\alpha_v\beta_3$ recognizes the arginine-glycine-aspartic acid (RGD) tripeptide sequence on numerous extracellular matrix proteins. This process allows endothelial cells and tumor cells to interact with a wide variety of extracellular matrix components such as vitronectin, fibronectin, fibrinogen, thrombospondin, osteopontin, bone sialoprotein, and denatured collagen.[1] Angiogenesis, a process extensively studied over the last two decades, is the mechanism by which new blood vessels are formed from pre-existing blood vessels. Antagonists of $\alpha_v\beta_3$ have demonstrated potent anti-angiogenetic activity, and thereby 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] $\alpha_v\beta_3$ also plays a significant role in other pathophysiological conditions, including restenosis after angioplasty,[5] ocular neovascularization[4,6] and rheumatoid arthritis.[7] Antagonists of this integrin may prove to be beneficial in the treatment of these diverse disease states.



SC-68448

We have previously described an RGD-based peptidomimetic $\alpha_v\beta_3$ antagonist, SC-68448, which demonstrated anti-tumor efficacy in a mouse Leydig cell tumor model.[8] We now report a series of conformationally-restricted cinnamic acid analogues in which the glycine-3-aminopropionic acid functionality was replaced by the bioisosteric 4-aminocinnamic acid moiety. This work was also extended to additional isosteres, including

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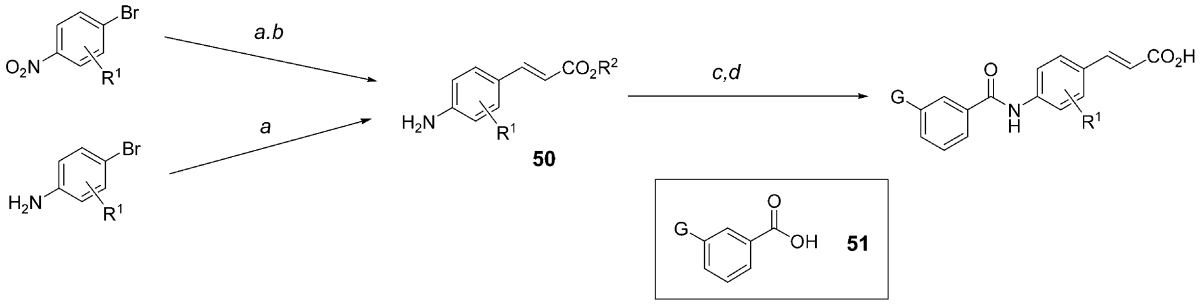
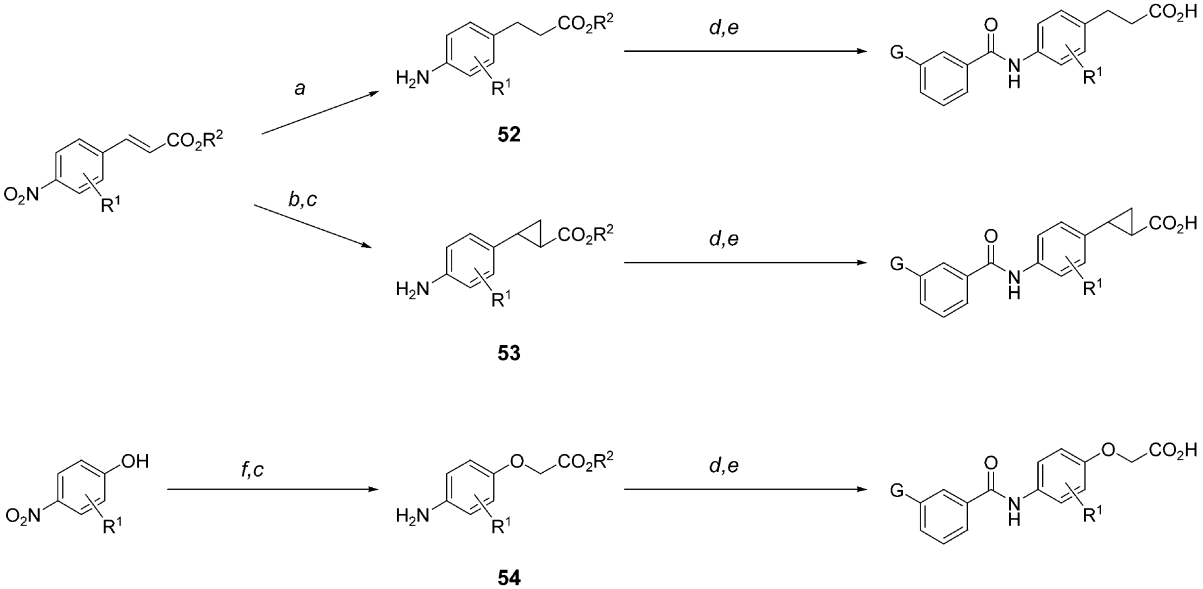
3-phenylpropionic acids, phenoxyacetic acids and 2-phenylcyclopropylcarboxylic acids.

The cinnamic acid analogues described were synthesized as shown in Scheme 1. An appropriately substituted 4-bromoaniline or 4-bromo-1-nitrobenzene was coupled with either methyl or ethyl acrylate under standard palladium-catalyzed Heck conditions to give the corresponding 4-amino or 4-nitrocinnamic esters. In the case of the nitro analogues, reduction using $SnCl_2$ provided the 4-amino ester **50**. Coupling of **50** with a 3-guanidinobenzoic acid **51** using isobutyl chloroformate, followed by saponification, provided the desired cinnamic acid analogues. 3-Phenylpropionic acid, phenoxyacetic acid and 2-phenylcyclopropylcarboxylic acid analogues were synthesized as described in Scheme 2. The 4-nitrocinnamic acid esters were hydrogenated to provide phenylpropionic ester **52**, while cyclopropanation using diazomethane and reduction with $SnCl_2$ gave the corresponding cyclopropyl analogue **53**. Coupling of **52** or **53** with a 3-guanidinobenzoic acid **51**, using isobutyl chloroformate, followed by saponification, gave the desired peptidomimetics. The phenoxyacetic acid analogues were synthesized from the appropriate 4-nitrophenol. Mitsunobu coupling with an α-hydroxyester gave, after reduction, the 4-aminophenoxyacetic ester **54**. Coupling and saponification as before, provided the desired acids. The α-aminopropionic acid analogues were synthesized as shown in Scheme 3. Palladium-catalyzed coupling of **55** with a 4-nitro-1-bromobenzene gave amino ester **56**. Alternately, **56** could be prepared from a 4-nitro benzaldehyde and *N*-benzoyl glycine. Reduction and derivatization of the α-amino group gave **57**. Coupling and saponification provided the desired α-amino acid analogues.

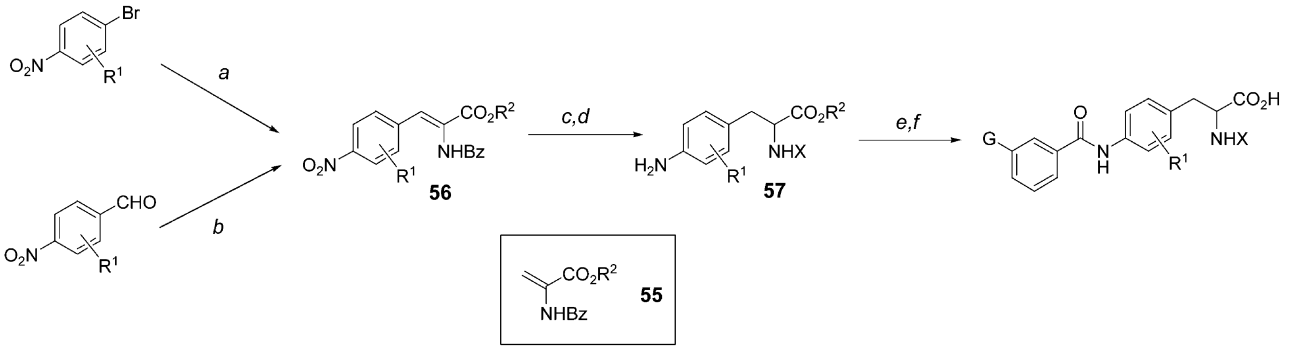
A series of substituted cinnamic acids containing a simple phenylguanidine was synthesized and tested in both the $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ solid-phase receptor binding assays (SPRA).[4] Our goal was to demonstrate at least 100-fold selectivity over the related platelet integrin, $\alpha_{IIb}\beta_3$. These analogues, which explored phenyl ring substituent effects, are detailed in Table 1. The simple cinnamic acid analogue **1** showed only moderate $\alpha_v\beta_3$ potency and no selectivity over $\alpha_{IIb}\beta_3$. Several substituents, including 2-CO_2H (**6**), 3-methyl (**8**), 3-ethyl



Scheme 1. (a) Methyl or ethyl acrylate, cat. $Pd(OAc)_2$, $P(o\text{-}tol)_3$, *i*-Pr_2NH, Δ; (b) $SnCl_2$, EtOH, Δ; (c) **51**, isobutyl chloroformate, *N*-methylpiperidine, CH_2Cl_2; (d) LiOH or NaOH.

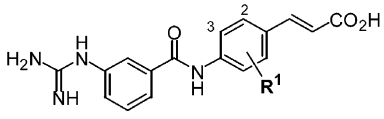


Scheme 2. (a) H_2, Pd/C, EtOH; (b) CH_2N_2, $Pd(OAc)_2$, Et_2O; (c) $SnCl_2$, EtOH, Δ; (d) **51**, isobutyl chloroformate, *N*-methylpiperidine, CH_2Cl_2; (e) LiOH or NaOH; (f) $HOCH_2CO_2R^2$, DEAD, Ph_3P, THF.



Scheme 3. (a) **55**, cat. $Pd(OAc)_2$, P(o-tol)$_3$, DMF, Δ; (b) (1) Ac_2O, *N*-benzoylglycine, Δ; (2) MeOH, K_2CO_3, Δ; (c) H_2, Pd/C, MeOH; (d) **X**Cl; (e) **51**, isobutyl chloroformate, *N*-methylpiperidine, CH_2Cl_2; (f) LiOH or NaOH.

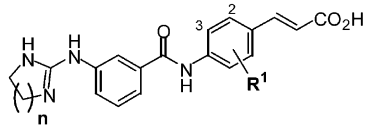
Table 1. SPRA data for simple guanidine cinnamic acid analogues



Compd	R^1	IC$_{50}$ (nM)[a] $\alpha_v\beta_3$ SPRA	IC$_{50}$ (nM)[a] $\alpha_{IIb}\beta_3$ SPRA
1	H	10.6	7.0
2	2-Me	73	121
3	2-Cl	23	45
4	2-CF_3	122 (1)	152 (1)
5	2-OMe	9.7	69
6	2-CO_2H	1.2	9.1
7	2-CO_2Me	32 (1)	80 (1)
8	3-Me	4.0	9.6
9	3-Et	3.0	15.1
10	3-F	7.1	14.1
11	3-Cl	3.5	11
12	3-CF_3	155 (2)	670 (2)
13	3-OMe	10.3	38
14	2,6-diCl	1830 (1)	25,000 (1)
15	3,5-diMe	23	2170

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

Table 2. SPRA data for cyclic guanidine cinnamic acids



Compd	*n*	R^1	IC$_{50}$ (nM)[a] $\alpha_v\beta_3$ SPRA	IC$_{50}$ (nM)[a] $\alpha_{IIb}\beta_3$ SPRA
16	1	3-Cl	0.31	80
17	1	3-Et	0.49	125
18	1	3,5-diMe	2.8	1830
19	2	H	2.0	528
20	2	2-Cl	3.9	641
21	2	2-OMe	2.2	661
22	2	2-CO_2H	0.45	97
23	2	2-CO_2Me	5.7	817
24	2	3-Me	0.46	181
25	2	3-Et	0.53	439
26	2	3-Cl	0.48	233
27	2	3,5-diMe	4.9	8270

[a] Average of at least three determinations.

Table 3. 293 Cell selectivity data for cinnamic acid analogues

Compd	IC$_{50}$ (nM)[a] $\alpha_v\beta_1$ 293 cell	IC$_{50}$ (nM)[a] $\alpha_v\beta_3$ 293 cell	IC$_{50}$ (nM)[a] $\alpha_v\beta_5$ 293 cell
1	109	13.1	39
2	505	19.4	49
10	426	9.6	45
11	426	5.9	60
15	2070	21	116
18	264	3.0	29
19	219	1.8	7.6
20	507	3.8	26
24	60	1.7	9.6
26	85	1.7	13.0

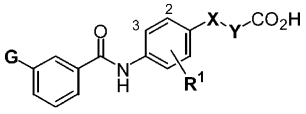
[a] Average of at least three determinations.

(**9**), and 3-chloro (**11**) showed increased $\alpha_v\beta_3$ potency in the 1–4 nM range. However, these analogues were still relatively non-selective versus $\alpha_{IIb}\beta_3$. Although 3,5-disubstituted analogue **15** was less potent for $\alpha_v\beta_3$, it did demonstrate nearly 100-fold selectivity. Incorporation of a cyclic guanidine (both tetrahydropyrimidine and dihydroimidazole) was found to provide a significant increase (5- to 10-fold, on average) in potency, as well as an increase in selectivity (Table 2). Several analogues demonstrated sub-nanomolar potency as well as $\alpha_{IIb}\beta_3$ selectivity of >200-fold. As before, the 2-CO_2H (**22**), 3-methyl (**24**), 3-ethyl (**17**,**25**), and 3-chloro (**16**,**26**) analogues showed superior potency, all in the 0.3 to 0.5 nM range. As before, 3,5-disubstitution gave the best selectivity, but with diminished potency. Selected analogues were also assayed for binding to $\alpha_v\beta_1$-, $\alpha_v\beta_3$-, and $\alpha_v\beta_5$-expressing 293 cells[9] to probe selectivity against other α_v integrins (Table 3). In general, whole cell $\alpha_v\beta_3$ potencies were similar to those obtained in the SPRA assay and $\alpha_v\beta_5$ potencies were all within an order of magnitude of $\alpha_v\beta_3$. However, $\alpha_v\beta_1$ potencies were significantly lower, generally 50- to 100-fold. Table 4 highlights both the SPRA data for $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, as well as 293 whole cell binding data for $\alpha_v\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ for a series of cinnamic acid isosteres. In general, similar trends were observed between the cinnamic acid series described in the previous Tables and the phenylpropionic acid, phenoxyacetic acid and phenylcyclopropylcarboxylic acid

series described in Table 4. Phenyl ring substitution tended to increase $\alpha_v\beta_3$ potency and incorporation of a cyclic guanidine improved both $\alpha_v\beta_3$ potency and selectivity. While the phenylpropionic acid analogues **28**–**32** were significantly less potent than the corresponding cinnamic acid analogues, the phenoxyacetic acid class demonstrated improved potency, particularly when containing a 2-methoxy substituent and a cyclic guanidine (**35**). Cyclopropyl analogues (**36**,**37**), on the other hand, showed diminished potency. In general, in 293 cells, $\alpha_v\beta_3$ potency was similar to SPRA potency, while significant selectivity was observed versus $\alpha_v\beta_1$ and in many cases $\alpha_v\beta_5$, as well. Based on extensive literature precedence, an α-amino moiety was introduced to the phenylpropionic acid class (Table 5). While a simple α-amino group showed poor potency (**38**), *N*-derivatized analogues showed excellent potency. Carbamates (**40**,**41**) and sulfonamides (**43**,**44**) demonstrated the optimal $\alpha_v\beta_3$ potency, while the potency of amides (**39**) and ureas (**42**) was somewhat diminished. However, most analogues in Table 5 showed relatively poor $\alpha_{IIb}\beta_3$ selectivity. Introduction of a cyclic guanidine dramatically increased $\alpha_v\beta_3$ potency to the sub-nanomolar level (**46**–**48**). In addition, 2-methoxy-substituted analogue **46**, was not only a 0.16 nM $\alpha_v\beta_3$ antagonist, but also demonstrated >6000-fold selectivity over $\alpha_{IIb}\beta_3$. The pK_a-modulated imidazolone **49** also demonstrated good potency and selectivity. Unlike the compounds described in Table 4, in 293 cells, all α-amino analogues showed relatively poor selectivity versus the other α_v integrins.

In summary, we have developed a series of simple, readily accessible cinnamic acid analogues, and by appropriate phenyl substitution and guanidine manipulation have found several analogues with low to sub-nanomolar $\alpha_v\beta_3$ potency and >200-fold selectivity over $\alpha_{IIb}\beta_3$. In addition, we have developed several new classes

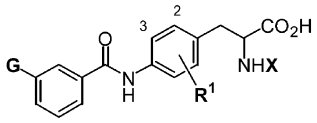
Table 4. SPRA and whole cell data for cinnamic acid isosteres

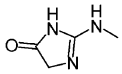


Compd	G	R^1	X–Y	IC_{50} (nM)[a]				
				$\alpha_v\beta_3$ SPRA	$\alpha_{IIb}\beta_3$ SPRA	$\alpha_v\beta_1$ 293 cell	$\alpha_v\beta_3$ 293 cell	$\alpha_v\beta_5$ 293 cell
28		H	$-CH_2CH_2-$	21	93	758 (2)	10.9	135 (2)
29		2-OMe	$-CH_2CH_2-$	24	172	ND	ND	ND
30		2-CO_2H	$-CH_2CH_2-$	9.0	57	ND	ND	ND
31		2-OMe	$-CH_2CH_2-$	5.8	3190	162	5.6	16.6
32		2-CO_2H	$-CH_2CH_2-$	6.1	4710	ND	ND	ND
33		H	$-OCH_2-$	19.1	128	351 (2)	11.9	144 (2)
34		2-OMe	$-OCH_2-$	3.2	54	222	4.3	102
35		2-OMe	$-OCH_2-$	0.43	772	82	1.7	29
36		H		31	533	339 (2)	65	149 (2)
37		H		19	4810	320	40	69

ND, Not determined.
[a] Average of at least three determinations except where noted in parentheses.

Table 5. SPRA and whole cell data for α-aminophenylpropionic acid analogues



Compd	G	R^1	X	IC_{50} (nM)[a]				
				$\alpha_v\beta_3$ SPRA	$\alpha_{IIb}\beta_3$ SPRA	$\alpha_v\beta_1$ 293 cell	$\alpha_v\beta_3$ 293 cell	$\alpha_v\beta_5$ 293 cell
38		–H	–H	4760 (1)	21900 (1)	ND	ND	ND
39		–H	–C(O)Me	2.7	148	16 (2)	2.6	40 (2)
40		–H	$-CO_2i$-Pr	0.80	22	0.47 (2)	2.6	0.76 (2)
41		–H	$-CO_2t$-Bu	0.51	14.3	6.7	2.5	1.0
42		–H	–C(O)NH*t*-Bu	3.2	285	4.8	2.9	3.0
43		–H	$-SO_2$Me	0.63	15.6	29	0.98	3.1 (2)
44		–H	$-SO_2$Ph	0.63	0.71	18.3 (2)	0.20	8.1
45		2-OMe	$-CO_2i$-Pr	1.1 (2)	253 (2)	1.1 (2)	0.32	0.38 (2)
46		2-OMe	$-CO_2i$-Pr	0.16	1000	0.68	0.35	0.41
47		–H	$-SO_2$Ph	0.27	12.5	ND	ND	ND
48		–H	$-SO_2$Ph	0.11	6.0	10.4 (2)	0.12	0.96 (2)
49		–H	$-CO_2i$-Pr	1.6	1270	17.7 (2)	0.94	0.89 (2)

ND, Not determined.
[a] Average of at least three determinations except where noted in parentheses.

of $\alpha_v\beta_3$ antagonists that contain bioisosteres of the cinnamic acid moiety. Particularly promising was the α-aminophenylpropionic acid class which demonstrated excellent potency against $\alpha_v\beta_3$ as well as very good selectivity versus $\alpha_{IIb}\beta_3$.

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