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N-(Arylacetyl)-biphenylalanines as Potent VLA-4 Antagonists

Bing Li,[a,*] Stephen E. de Laszlo,[a] Theodore M. Kamenecka,[a] Ihor E. Kopka,[a] Philippe L. Durette,[a] Thomas Lanza, Jr.,[a] Malcolm MacCoss,[a] Sharon Tong,[a] Richard A. Mumford,[b] Ermengilda D. McCauley,[b] Gail Van Riper,[b] John A. Schmidt[b] and William K. Hagmann[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Inflammation and Rheumatology Research, Merck Research Laboratories, Rahway, NJ 07065, USA*

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Abstract—A series of potent *N*-(aralkyl-, arylcycloalkyl-, and heteroaryl-acyl)-4-biphenylalanine VLA-4 antagonists was prepared by rapid analogue methods using solid-phase chemistry. Further optimization led to several highly potent compounds (IC_{50} <1 nM). Evaluation of rat pharmacokinetic revealed generally high clearance.

VLA-4 ($\alpha_4\beta_1$; CD49d/CD29) is a heterodimeric integrin expressed on the surface of lymphocytes.[1] Its ligands include vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated endothelial cells at sites of inflammation and produced in response to inflammatory cytokines, and an alternatively spliced (CS-1) domain of fibronectin (FN). The adhesion of lymphocytes to VCAM-1 and subsequent migration to extravascular spaces appear to be critical steps in the inflammatory response.[2] Several reports have demonstrated that blocking this interaction in animals with α_4 antibodies or VLA-4 antagonists can have therapeutic utility in a variety of disease models, including models of airways hyperresponsiveness, inflammatory bowel disease, multiple sclerosis, and arthritis.[3–6]

VLA-4 has been shown to bind to the sequences -Ile-Asp-Ser- (-IDS-) in the C-D loop of VCAM-1 and -Leu-Asp-Val- (-LDV-) in the CS-1 domain of FN.[7,8] It is believed that the center aspartic acid makes an essential binding contact with VLA-4. In our search for unique antagonists of VLA-4, we and others have reported series of substituted acylated and sulfonylated phenylalanine derivatives as potent VLA-4 antagonists.[3,9–14] Substituted biphenylalanines emerged as one potency enhancing pharmacophore for both VLA-4 and the related integrin, $\alpha_4\beta_7$.[12,14] Acyl components could be relatively small and include *N*-benzylpyroglutamate, substituted benzoyls, and tetrahydrofuroyl.

In our search for unique leads that retained potency and had improved pharmacokinetic characteristics, a solid-phase chemistry strategy for the synthesis of single compounds was pursued. (L)-2′-Methoxy-4-biphenylalanine had been identified as a potency enhancing acid component and was initially chosen to load on the resin. For the acyl component, commercially available carboxylic acids were chosen from among substituted aryl acetyls. The corresponding benzoyl derivatives have previously been disclosed.[15]

The acids were incorporated via amide bond coupling to the derivatized resin to afford the desired products after cleavage from the resin (Scheme 1). (L)-4-Iodophenylalanine *t*-butyl ester was reacted with FmocCl in the presence of diisopropylethylamine to give *N*-Fmoc-(L)-4-iodophenylalanine *t*-butyl ester which was reacted with 2-methoxyphenylboronic acid under Suzuki aryl coupling conditions to yield (L)-4-(2′-methoxy)-biphenylalanine *t*-butyl ester.[16] The ester was removed by treatment with trifluoroacetic acid to afford the free acid which was subsequently loaded onto a Wang resin using standard conditions.[12] Approximately 3 g of the free acid gave about 3 g of loaded resin which was enough to prepare ~100 compounds. The *N*-Fmoc protecting group on the resin-loaded amino acid was

*Corresponding author. Fax: +1-732-594-6090; e-mail: bing_li@merck.com

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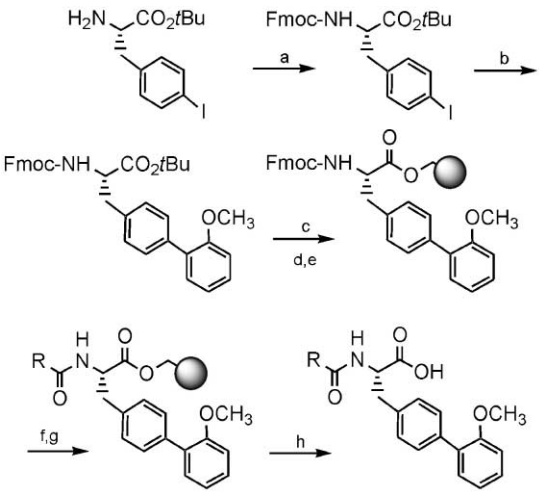


Scheme 1. (a) FmocCl, DIEA, CH_2Cl_2; (b) 2-OCH_3Ph–$B(OH)_2$, $Pd(PPh_3)_4$, KBr, Na_2CO_3, toluene; (c) TFA, CH_2Cl_2; (d) Wang resin, 2.0 equiv EDC, 1.0 equiv DMAP; (e) 4.0 equiv AcOH, 4.0 equiv EDC, 2.0 equiv DMAP; (f) 20% piperdine, DMF; (g) 4.0 equiv acid, 4.0 equiv HBTU, 4.0 equiv HOBt, 5.0 equiv DIEA; (h) 95% TFA/5% water.

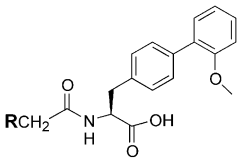
removed by treatment with 20% piperidine in DMF followed by coupling with the acid component. After washing, the desired product was removed from the resin with trifluoroacetic acid/water (95:5). The solvents were removed in vacuo and the products characterized by HPLC and mass spectrometry. The inhibition of VLA-4 binding to a soluble radiolabeled VCAM-1-Ig fusion protein[12] by these compounds was listed in Table 1.

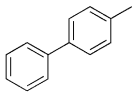
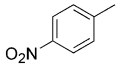
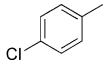
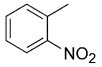
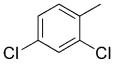
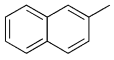
The addition of a phenyl ring **2** onto the acetamide **1** had little effect on potency. Only when the conformation about the α-carbon was changed by *ortho* substitution (**5**, **6**, **7**, and **14**) did potency improve. Potency was also greatly enhanced by replacement of phenyl with a heteroaryl. Interestingly, all three positional isomers of pyridine (**10**, **19**, and **20**) were equipotent. Tetrazolyl- (**11**) and dichloro-imidazolyl- (**21**) acetyl derivatives were also more potent than the phenyl derivative (**2**).

Conformational constraints offered by α-substitution on the phenylacetyl group also significantly improved VLA-4 potency (Table 2). The binding appeared to be sensitive to the bond angle between the phenyl carbon and carbonyl carbon at α-position noted in Table 2. The methoxy compound (**22**) may have another specific binding interaction between VLA-4 and the methoxyl.

The obvious hybrid molecules incorporating the potency enhancing effects of phenyl to 3-pyridyl as well as methylene to cyclopentyl were prepared. The desired 3-pyridyl cycloalkane and heterocycle derivatives were prepared as illustrated in Scheme 2. Ethyl 3-pyridyl acetate was treated with excess potassium hydride followed by alkylation with a dihaloalkane to afford the cyclized acetate. Saponification of the ester followed by standard coupling conditions with (L)-4-(2′-methoxy) biphenylalanine, *t*-butyl ester afforded the desired

Table 1. Inhibition of VLA-4 by substituted arylacetyl biphenylalanine derivatives (IC_{50}, nM)

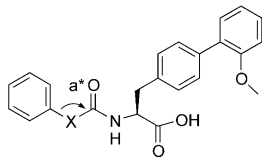


No.	R	HPLC Purity%	VLA-4	No.	R	HPLC Purity%	VLA-4
1	H	92	262	**12**		95	607
2		85	264	**13**		95	44.9
3		92	315	**14**		84	4.8
4		84	328	**15**		81	64.9
5		86	36.8	**16**		84	246
6		92	8.0	**17**		87	129
7		89	16.6	**18**		89	801
8		84	145	**19**		93	15.8
9		87	30.9	**20**		92	12.2
10		87	12.9	**21**		85	6.15
11		81	14.4				

products. The nature of substitution on the pyridyl ring as well as other heteroaryls was explored and results were listed in Table 3. Activity of this series against the related $\alpha_4\beta_7$ integrin was also included.

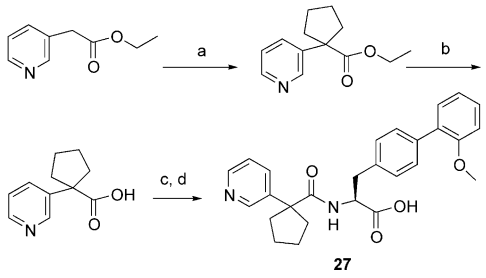
It appeared from the results in Table 3 that the unsubstituted 3-pyridyl **27** or equivalent 4-pyridazinyl **35** derivatives were the most potent VLA-4 antagonists of this series of heteroaryls. Interestingly, the nature of the specific binding interaction of the 3-pyridyl was not entirely clear. Whereas the pyridyl nitrogen might be expected to be a hydrogen bond acceptor, the pyridones **29** and **30** were also very potent. Likewise, with the possible exception of isoquinolinyl **37**, most substitutions and positional isomers had comparable potencies. If there was a specific polar interaction of VLA-4 with

Table 2. Inhibition of VLA-4 by substituted phenylacetyl biphenylalanine derivatives (IC_{50}, nM)



No.	X	VLA-4	a*	No.	X	VLA-4	a*
2		264	111.4	**24**		90.7	115.5
22		7.1	110.3	**25**		3.2	106.6
23		14.1	108.9	**26**		6.5	106.6

*a represents degree of phentl carbon–carbon–carbonyl carbon angle.



Scheme 2. (a) 3 equiv KH; $Br(CH_2)_4Br$, THF; (b) NaOH, CH_3OH; (c) L-4-(2′-methoxy)biphenylalanine, *t*-butyl ester, HBTU, HOBt, DIEA, CH_2Cl_2; (d) TFA, CH_2Cl_2.

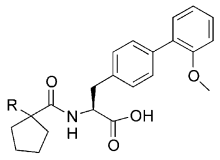
this portion of the molecule, it appeared to be fairly nonspecific or was spread over an area that was not unidirectional.

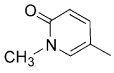
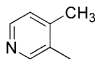
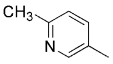
Most of the analogues in Table 3 were not very specific for VLA-4 over $\alpha_4\beta_7$. The most specific compound was the 5-bromo analogue **34** ($\sim$100×). In an attempt to improve specificity while retaining potency, the nature of the spirocyclic ring was explored with substituents and heterocyclic replacements. These derivatives were prepared in an analogous fashion to **27** (Scheme 2). Inhibition data was reported in Table 4.

As can be seen in Table 4, modification of the spirocyclopentyl portion of **27** had little effect on VLA-4 potency or specificity with respect to $\alpha_4\beta_7$. There was $\sim$10-fold increase in specificity with the pyranyl analogue **44**. The major contribution of the spirocyclic portion of the molecule might be largely conformational.

The pharmacokinetic (PK) properties of several VLA-4 antagonists from this study were measured in rats (Table 5). Whereas the unsubstituted phenyl (**23** and **25**) and pyridyl (**27**) derivatives had very promising PK characteristics, any change to the heterocycle or the

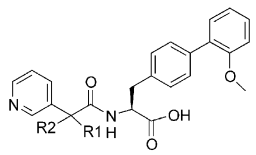
Table 3. Inhibition of VLA-4 and $\alpha_4\beta_7$ by heteroaryl cyclopentyl acetyl biphenylalanine derivatives (IC_{50}, nM)



No.	R	VLA-4	$\alpha_4\beta_7$
25		3.2	110
27		0.5	9.9
28		3.2	180
29		1.4	26
30		2.1	18
31		1.5	44
32		1.2	22
33		1.3	36.3
34		1.6	165
35		0.5	33
36		3.0	215
37		9.8	120
38		3.9	98
39		0.7	49
40		0.8	15

spirocycle resulted in high plasma clearance rates and generally poor oral bioavailability. It was hoped that if the pyridyl nitrogen in **27** was a target for metabolic oxidation that the ortho methyl substitution in **31** and **33** might reduce this metabolism. The data clearly showed that this was not the case.

Table 4. Inhibition of VLA-4 and $\alpha_4\beta_7$ by substituted 3-pyridylacetyl biphenylalanine derivatives (IC_{50}, nM)



No.	R1/R2	VLA-4	$\alpha_4\beta_7$
27		0.5	9.9
41		0.9	16.7
42[a]		0.7	94
43		1.4	158
44		0.5	94
45		5.1	83
46		1.0	51
47		0.7	30

[a]Mixture of four isomers.

Table 5. Pharmacokinetic parameters of selected compounds[a]

No.	F[b] (%)	Cl_p (mL/kg/min)	$t_{1/2}$[c] (h)
23	94	7.5	2.0
25	55	21.5	1.0
27	35	24	1.1
29	1	>100	ND[d]
31	11	>100	0.6
32	1	>100	0.2
33	12	>100	0.3
36	<1	>100	ND
39	17	>100	2.5
43	6	82	0.4
44	<1	>100	ND

[a]Sprague–Dawley rats.
[b]Dose: 1 mg/kg iv; 2 mg/kg po.
[c]$t_{1/2}$ = plasma half-life$_{(0-8\ h)}$.
[d]ND, not determined.

In summary, a series of potent *N*-(aralkyl-, arylcycloalkyl-, and heteroaryl-acyl)-4-biphenylalanine VLA-4 antagonists was prepared by rapid analogue methods using solid phase chemistry. Further optimization led to several highly potent compounds (IC_{50} <1 nM). Evaluation of rat pharmacokinetics revealed generally high clearance.

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