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Substituted Tetrahydrofuroyl-1-phenylalanine Derivatives as Potent and Specific VLA-4 Antagonists

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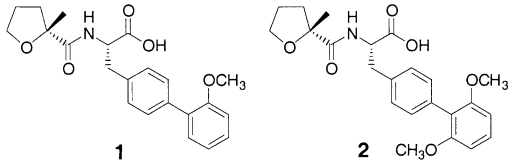
Abstract—A series of substituted tetrahydrofuroyl-1-phenylalanine derivatives was prepared and evaluated as VLA-4 antagonists. Substitution of the α carbon of the tetrahydrofuran with aryl groups increased the specificity for VLA-4 versus $\alpha_4\beta_7$ while amide substitution increased the potency of the series without increasing the specificity. Substitution of the β carbon of the tetrahydrofuran with keto or amino groups slightly improved the specificity for VLA-4 versus $\alpha_4\beta_7$ but with a significant loss in binding affinity for VLA-4.

VLA-4 ($\alpha_4\beta_1$; CD49d/CD29; 'very late antigen-4') is a key cell surface integrin present on leukocytes except neutrophils and platelets that binds vascular cell adhesion molecule-1 (VCAM-1) on endothelial cell surfaces and leads to leukocyte infiltration to extravascular tissue. Antibodies against VLA-4 have been shown to block leukocyte infiltration and prevent tissue damage in inflammatory disease models of asthma,[1] multiple sclerosis,[2] rheumatoid arthritis (RA),[3] and inflammatory bowl disease (IBD).[4] Orally active small molecule inhibitors of VLA-4 might therefore serve as useful agents in the treatment of these diseases.

In a previous communication,[5] we disclosed the identification of **1** and **2** as potent VLA-4 antagonists. In the rhesus monkey, compound **2** exhibited excellent oral bioavailability and low plasma clearance ($F = 51\%$; $Cl_p = 2.9$ mL/kg/min). When the binding assay was run in the presence of 5% plasma, a 10-fold shift in the IC_{50} was observed (0.35 nM vs 3.61 nM) indicating a high level of protein binding, a factor which may be responsible for the lower plasma clearance number. Compound **2** also showed good affinity for the closely related integrin $\alpha_4\beta_7$ (7.1 nM). Although this integrin may itself have therapeutic value, a more selective compound was desired.[6] In an effort to further increase the potency of this series, reduce plasma protein binding and improve the specificity for VLA-4, a systematic study of substitution at the α- and β-carbons of the tetrahydrofuran ring was undertaken.



The alkyl substituted tetrahydrofuroic acids (**3**–**5**) were prepared via simple alkylation of tetrahydrofuroic acid methyl ester. The alkanol and alkyl amine substituted derivatives were prepared as shown in Scheme 1. The lithium enolate of tetrahydrofuroic benzyl ester was alkylated with the *tert*-butyl dimethyl silyl protected iodoalkanols which provided the alkylated products in modest yield. After removal of the benzyl ester, PyBOP coupling with the dimethoxybiphenylalanine *tert*-butyl ester[7] produced the coupled products as mixtures of diastereomers. Tetrabutylammonium fluoride deprotection of the silyl ether gave the alcohol, which was

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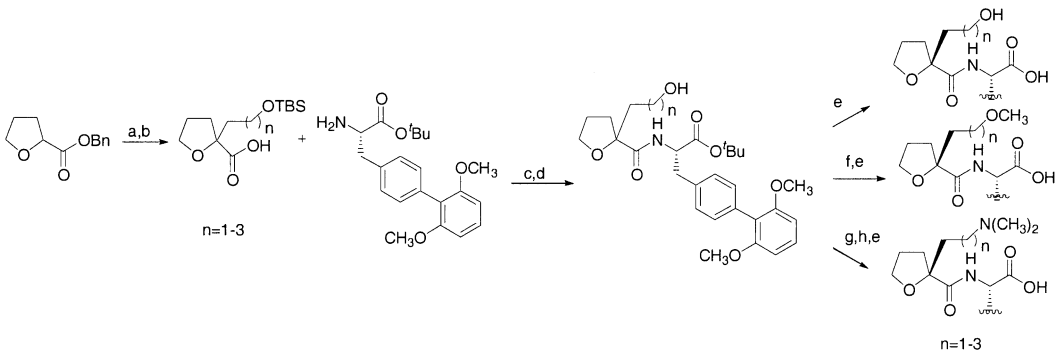


Scheme 1. (a) LDA, HMPA, $I(CH)_nOTBS$; (b) Pd/C, H_2, NEt_3, MeOH; (c) PyBOP, DIPEA, CH_2Cl_2; (d) TBAF, THF; (e) TFA, CH_2Cl_2; (f) MeOTf; (g) oxalyl chloride, DMSO, NEt_3; (h) $HNMe_2$, $NaBH(OAc)_3$, CH_2Cl_2.

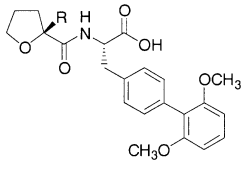
converted to the methyl ether and also to the dimethylamino derivatives. The *tert*-butyl ester was cleaved and the diastereomeric products separated via prep TLC. The more polar diastereomer proved to be the more active isomer. The stereochemistry of the more active isomer was assigned as (*R*) in analogy to **1**, which was rigorously proven by X-ray crystallography.[8]

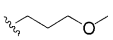
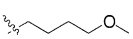
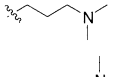
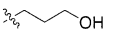
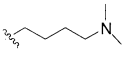
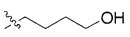
Alkyl substitution (**3**–**5**) did little to increase the potency or specificity of the series (Table 1). Dimethyl amine (**12**–**14**), methyloxy (**9**–**11**), and hydroxy (**6**–**9**) were appended to the α-position of the tetrahydrofuran with a two, three and four carbon spacer. The three carbon spacer was optimal with activity greatly tailing off as the chain length was increased. The ethers were more potent than the alcohols and amines, but were only equipotent to methyl, with no improvement in specificity. The introduction of the ether functionality did reduce plasma protein binding, with **10** showing only a 2-fold decrease in activity in the presence of 5% plasma protein (0.35 nM vs 0.61 nM). This reduction in protein binding also resulted in a significant loss in rat pharmacokinetics ($F = 1.9\%$; $Cl_p = 81.1$ mL/kg/min).

The aryl substituted tetrahydrofurans **15**–**23** were prepared from the reaction of potassium cyanide with 4-chlorobutyrophenones followed by hydrolysis the resulting cyanides (Scheme 2). Coupling of the acids with biphenylalanine ester produced the coupled products as mixtures of diastereomers which were separated by prep TLC after hydrolysis of the ester.

The phenyl substituted derivative **15** (Table 2) showed equal potency to the methyl derivative **1**, but was starting to show an increased specificity for $\alpha_4\beta_1$ (26× vs 12×). Substitution of the *meta* or *para* position increased the specificity even more. The trifluoromethyl group proved to be the most effective substituent for

Table 1. Inhibition of VLA-4[a] and $\alpha_4\beta_7$[b] by alkyl substituted tetrahydrofuroyl-1-phenylalanine derivatives



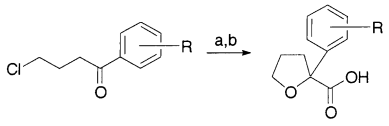
Compd	R	IC_{50} (nM)			Ratio	Compd	R	IC_{50} (nM)			Ratio
		$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]	β_7/β_1			$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]	β_7/β_1
2	CH_3	0.34	3.61	7.09	21	**9**		0.7	1.99	16.1	40
3		0.48	1.46	13.8	29	**10**		0.35	0.61	7.8	22
4		0.7	7.46	6.1	9	**11**		0.62	1.27	22.0	35
5		1.15	4.02	22.3	19	**12**		15.5	19.5	80.0	5
6		1.56	7.59	16.3	10	**13**		9.18	—	67.0	7
7		2.12	3.36	35.3	17	**14**		16.3	22.9	226.5	14
8		255	870	245	1						

[a]VCAM-Ig.
[b]MAdCAM-Ig.
[c]Plus 5% human plasma.

increasing specificity, but due to its large molecular weight and high hydrophobicity, chlorine was chosen as the optimal substituent.

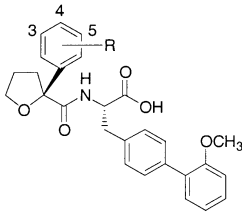
The dimethoxybiphenylalanine derivative **24** (Table 3) showed the predicted 10-fold increase in potency ($\alpha_4\beta_1$ $IC_{50} = 4.09$ nM vs 0.55 nM) compared with **20** and maintained good specificity ($\alpha_4\beta_7/\alpha_4\beta_1 = 95\times$). In the rat this compound exhibited moderate clearance and excellent bioavailability ($Cl_p = 19.3$ mL/min/kg, $F = 100\%$). Unfortunately, the good pharmacokinetics were accompanied by a highly level of protein binding. As seen with **2**, a 9-fold shift in activity was seen when the binding assay was run with 5% plasma (0.55 nM vs 4.75 nM). To address the issue of protein binding, polar groups were appended onto the phenyl ring. Nitro substituents were well tolerated, but interestingly, its reduction to the corresponding amine (**27**) led to a significant loss in potency without any improvement in protein binding. The dimethoxy analogue **28** was also synthesized with the anticipation that these substituents would reduce protein binding, but unfortunately this analogue showed a significant reduction in binding affinity.

The acyl substituted derivatives **30**–**35** were prepared next using a similar protocol to the alkyl series (Scheme 3). Tetrahydro-2-furoic acid methyl ester was acylated with benzyl chloroformate followed by hydrogenolysis to the acid **29**. After coupling to the amino acid, the methyl ester was hydrolyzed and converted to the amides.



Scheme 2. (a) KCN, MeOH; (b) KOH, ethylene glycol, 200 °C.

Table 2. Inhibition of VLA-4[a] and $\alpha_4\beta_7$[b] by aryl substituted tetrahydrofuroyl-1-phenylalanine derivatives



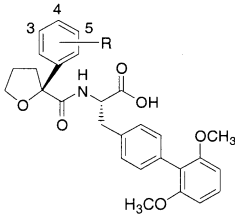
Compd	R	IC_{50} (nM)			Ratio β_7/β_1
		$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]	
15	H	2.2	—	56.4	26
16	4-*t*-Bu	1.8	29	99.5	55
17	4-F	4.3	48	45.7	11
18	4-CF_3	5.31	120	504	95
19	4-Br	3.62	53	232	64
20	4-Cl	4.09	46	275	67
21	3-CF_3	4.65	290	327	70
22	3,4-Me	6.18	34	374	60
23	3,5-CF_3	4.62	1105	499	108

[a]VCAM-Ig.
[b]MAdCAM-Ig.
[c]Plus 5% human plasma.

Substitution of the α-carbon of the tetrahydrofuran ring with acyl groups provided a significant increase in potency and a significant decrease in protein binding, but without a significant gain in specificity (Table 4). Amide substitution **31**–**33** proved to be better than ester **30** or ketone **35**, with the best amide being the morpholine amide **31**. The homologue compound **34** was significantly less potent. Unfortunately, the rat pK for **31** ($Cl_p = 87$ mL/min/kg, $F = 8.6\%$) was quite poor and an increase in specificity was not seen ($\alpha_4\beta_7/\alpha_4\beta_1 = 16\times$). In an attempt to merge the potency increase and protein binding decrease of the amide series with the good pK profile and specificity of the aryl substituted series, thiazole and benzoxazole substitution were examined. These were synthesized from acid **29** via standard heterocyclic chemistry.[9] Unfortunately, neither the benzoxazole **36** nor the thiazole **37** showed the potency enhancements seen in the amide series, although the benzoxazole exhibited remarkable specificity ($\alpha_4\beta_7/\alpha_4\beta_1 = 897\times$).

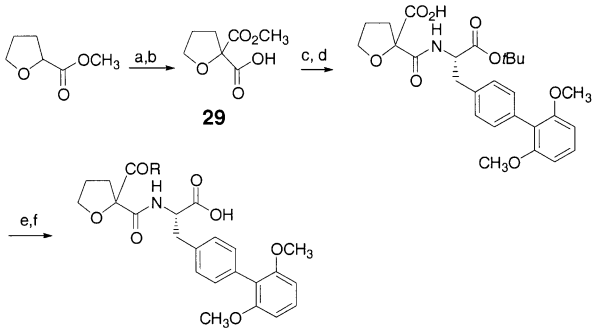
Substitution of the β carbon of the tetrahydrofuran ring was briefly explored. As outlined in Scheme 4, the first step toward 3-keto, 5-alkyl-substitued tetrahydrofuranoyls was the rhodium acetate insertion of the acyclic diazo compound. It was found that in order to

Table 3. Inhibition of VLA-4[a] and $\alpha_4\beta_7$[b] by aryl substituted tetrahydrofuroyl-1-phenylalanine derivatives



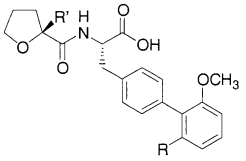
Compd	R	IC_{50} (nM)			Ratio β_7/β_1
		$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]	
24	4-Cl	0.55	4.75	52.2	95
25	3-NO_2,4-Cl	0.47	2.41	45.6	97
26	3-NO_2	0.23	0.95	19.6	85
27	3-NH_2	0.92	5.63	26.6	29
28	3,5-Dimethoxy	1.48	5.77	—	—

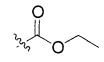
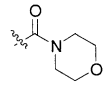
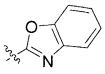
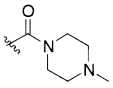
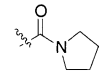
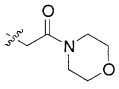
[a]VCAM-Ig.
[b]MAdCAM-Ig.
[c]Plus 5% human plasma.



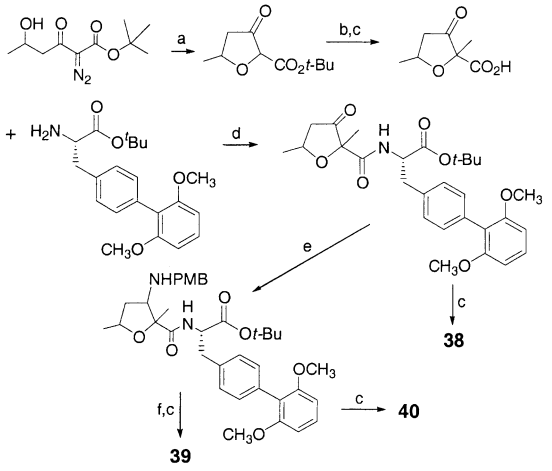
Scheme 3. (a) LDA, HMPA, $ClCO_2Bn$; (b) Pd/C, H_2, MeOH; (c) PyBOP, DIPEA, amino acid, CH_2Cl_2; (d) NaOH, MeOH; (e) PyBOP, ROH or R_2NH; (f) TFA, CH_2Cl_2.

Table 4. Inhibition of VLA-4[a] and $\alpha_4\beta_7$[b] by acyl and heteroaryl substituted tetrahydrofuroyl-1-phenylalanine derivatives



Compd	R	R′	IC_{50} (nM)			Ratio β_7/β_1	Compd	R	R′	IC_{50} (nM)			Ratio β_7/β_1
			$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]					$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]	
1	H	Me	3.9	60.6	45.3	12	**2**	OCH_3	Me	0.34	3.6	7.09	21
30	H		4.06	19	51.1	13	**35**	OCH_3		0.67	—	11.24	22
31	H		0.54	1.0	8.49	16	**36**	OCH_3		0.42	2.4	376.9	897
32	H		0.59	1.0	23.2	39	**37**	OCH_3		0.31	—	14.5	47
33	H		1.33	4.42	22.95	17							
34	H		23.15	—	11.0	11							

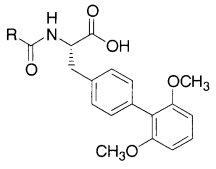
[a]VCAM-Ig.
[b]MAdCAM-Ig.
[c]Plus 5% human plasma.

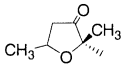
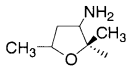
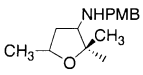


Scheme 4. (a) $\{Rh(OAc)_2\}_2$, benzene, 1,2-DCE, 80–100 °C; (b) K_2CO_3, MeI, MeCN, reflux; (c) TFA, DCM, rt; (d) PyBOP, DIEA, DCM, rt; (e) *p*-OMe-benzyl amine, $NaBH(OAc)_3$, acetic acid, DCM, rt; (f) $Pd(OH)_2$, $HCO_2^-(NH_4)^+$, MeOH, reflux.

further derivatize the keto compound, one must methylate the 2-position of the tetrahydrofuran. This was accomplished by treating the insertion reaction product with anhydrous potassium carbonate and iodomethane in acetonitrile. After hydrolysis of the *tert*-butyl ester and subsequent amide coupling, the desired 3-keto compound **38** was obtained. The 3-amino derivatives **39**

Table 5. Inhibition of VLA-4[a] and $\alpha_4\beta_7$[b] binding by 3-substituted tetrahydrofuroyl-1-phenylalanine derivatives



Compd	R	IC_{50} (nM)			Ratio β_7/β_1
		$\alpha_4\beta_1$[a]	$\alpha_4\beta_1$[a,c]	$\alpha_4\beta_7$[b]	
2		0.3	3.6	7.1	24
38		0.7	1.32	24.5	35
39		2.6	—	149	57
40		3.4	14.9	733	225

PMB, *p*-methoxybenzyl.
[a]VCAM-Ig.
[b]MAdCAM-Ig.
[c]Plus 5% human plasma.

and **40** were also explored. These were prepared by reductive amination of the **38** followed by hydrogenolysis to remove the *p*-methoxybenzyl group. Binding data for VLA-4 and $\alpha_4\beta_7$ are shown in Table 5. Substitution at the β-carbon resulted in some improvement in specificity. However, the improved specificity was achieved at the expense of decreased potency in VLA-4 binding affinity.

In summary, α-substituted tetrahydrofuroyl-1-phenylalanine derivatives were examined as VLA-4 antagonists. The introduction of alkyl groups on the tetrahydrofuran ring did not effect either the potency or the specificity. Amide substitution produced significantly more potent inhibitors of VLA-4 but with a deterioration of clearance and oral bioavailability. The most promising compounds were the aryl and heteroaryl substituted derivatives. These derivatives maintained the potency seen in the lead compound, but with improved specificity and good pharmacokinetics. Substitution at the β-carbon resulted in some improvement in specificity, but with an unacceptable decrease in binding affinity for VLA-4.

References and Notes

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