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The Discovery of Sulfonylated Dipeptides as Potent VLA-4 Antagonists

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Abstract—Directed screening of a carboxylic acid-containing combinatorial library led to the discovery of potent inhibitors of the integrin VLA-4. Subsequent optimization by solid-phase synthesis afforded a series of sulfonylated dipeptide inhibitors with structural components that when combined in a single hybrid molecule gave a sub-nanomolar inhibitor as a lead for medicinal chemistry. Preliminary metabolic studies led to the discovery of substituted biphenyl derivatives with low picomolar activities. SAR and pharmacokinetic characterization of this series are presented.

VLA-4 ($\alpha_4\beta_1$; CD49d/CD29; 'very late antigen-4') is a member of the integrin family and is expressed on all leukocytes, except platelets.[1] Its ligands include vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated endothelial cells at sites of inflammation and is produced in response to inflammatory cytokines, and the CS-1 domain of fibronectin (FN), which arises by alternative splicing within a region of FN. These cell adhesion interactions may be required for the activation, migration, proliferation, and differentiation of leukocytes during normal and/or pathophysiological processes. Thus, inhibition of VLA-4 may produce a reduction in the migration and/or activation of cell types important to sustaining a prolonged inflammatory response.[2]

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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.[3,4] Potent acyclic and cyclic VLA-4 antagonists based upon these binding sequences have been reported.[5,6] The common feature among these and all reported VLA-4 antagonists is the presence of a free carboxylic acid moiety, which presumably mimics the aspartic acid in the VCAM-1 and CS-1 binding sequences. In our search for unique antagonists of VLA-4, we initially focused our screening efforts on carboxylate-containing compounds. From a combinatorial library that had been prepared for a different molecular target, sulfonylated dipeptides **1**–**3** were identified after deconvolution (Fig. 1).[7]

Given the limited structural diversity in this library and the objective to optimize these lead compounds, we chose to prepare single compounds by solid-phase methods employing commercially available α- and β-amino acids

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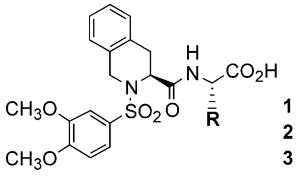
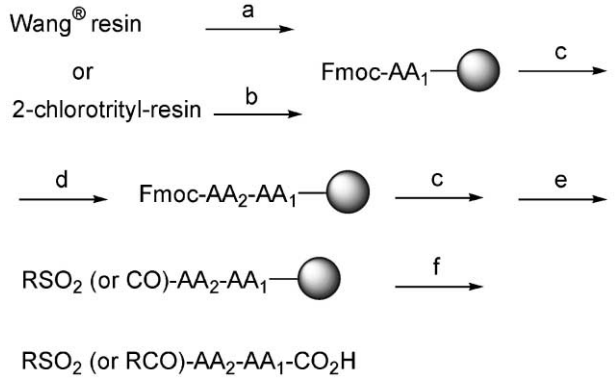


	R	IC_{50} (nM)[11]
1	$(CH_2)_3CH_3$	58
2	$(CH_2)_2CO_2H$	257
3	$(CH_2)_3NHC(=NH)NH_2$	360

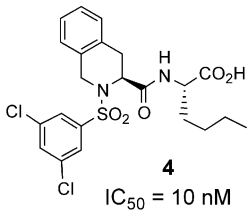
Figure 1. Lead structures obtained from combinatorial library.

and acyl chlorides and sulfonyl chlorides. As outlined in Scheme 1, an appropriate resin was loaded with an *N*-Fmoc-amino acid. The Fmoc protecting group was removed with 20% piperidine followed by coupling with another *N*-Fmoc-amino acid. Subsequent removal of the Fmoc protecting group again with 20% piperidine followed by reaction with a sulfonyl chloride or acyl chloride gave the capped dipeptide on resin. The desired product was removed from the resin with trifluoroacetic acid/thioanisole/ethanedithiol. The solvents were removed in vacuo, the residue purified by reverse-phase HPLC, and the product characterized by mass spectrometry.

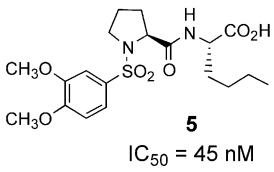
Approximately 300 compounds were prepared in this effort and led to the following SAR: (i) sulfonamides were much more potent than their acyl analogues; (ii) 3-substituted arylsulfonamides (RSO_2 in Scheme 1) were preferred as the capping group; (iii) the second amino acid AA_2 preferred cyclic (*S*)-α-amino acids; and (iv) the first AA_1 preferred the (*S*) configuration with hydrophobic side chains. Three compounds, **4**–**6**, illustrate the most potent substituents at AA_1, AA_2, and the capping sulfonamide (RSO_2). The 3,4-dichlorobenzene-dimethoxybenzene-sulfonamide **1** was replaced by the 3,5-sulfonamide in **4** with a ~6-fold improvement in potency. The AA_2 tetrahydroisoquinoline group was replaced with the smaller (*S*)-prolyl group in **5** with little change in potency. 2(*S*)-β-Naphthylalanine in **6** provided an ~8-fold improvement in potency.



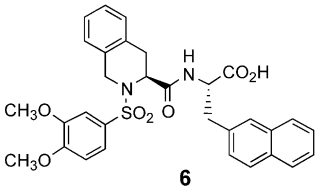
Scheme 1. (a) *N*-Fmoc-AA1, DCC, HOBt, DMF; (b) *N*-Fmoc-AA1, $EtN(iPr)_2$, DMF; (c) 20% piperidine, DMF; (d) *N*-Fmoc-AA_2, HOBt, HATu, $EtN(iPr)_2$, DMF; (e) RSO_2Cl (or RCOCl), $EtN(iPr)_2$, CH_2Cl_2; (f) TFA/thioanisole/ethanedithiol (38:1:1).



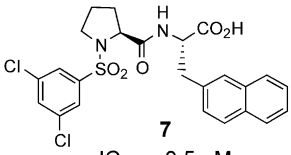
4
IC_{50} = 10 nM



5
IC_{50} = 45 nM



6
IC_{50} = 7 nM



7
IC_{50} = 0.5 nM

The individual contributions to binding from the new substituents found in **4**–**6** proved to be nearly additive. The combined hybrid structure **7** was nearly 100-fold more potent that the original lead **1**. Compound **7** was determined to be reasonably specific with respect to other integrins ($\alpha_4\beta_7$ IC_{50} = 789 nM; $\alpha_5\beta_1$ IC_{50} > 50 μM; $\alpha_v\beta_3$ IC_{50} > 50 μM). In pharmacokinetic studies in rats, **7** was determined to have low oral bioavailability (F = 15%), high plasma clearance (Cl_p = 49 mL/min/kg), and a short plasma half-life ($t_{1/2}$ = 0.6 h). It was found to be highly protein bound in human plasma (99.6%).

Initially, the relatively poor pharmacokinetic parameters of **7** were considered from a drug metabolism perspective. Preincubation of **7** in rat hepatocytes or rat liver microsomes led to a rapid loss of parent molecule and the appearance of several oxidized species. Most of these metabolites could be attributed to oxidation of the naphthyl group in the AA_1 position. Rather than attempt to block oxidation with substituted naphthylalanine analogues, 4-biphenyl replacements for the labile naphthylene were prepared.

Substituted 4-biphenylalanines were prepared from arylboronates and *N*-Boc-(L)-*O*-trifluoromethanesulfonyl-tyrosine or *N*-Boc-(L)-4-iodophenylalanine ester as reported.[8] Alternatively, arylhalides were reacted with 4-trimethylstannyl-(L)-phenylalanine to form the desired biaryl-amino acid derivative.[9] The *N*-Boc group was removed in the presence of concentrated sulfuric acid in *tert*-butanol to afford the free amine,[10] which was subsequently coupled to the appropriate *N*-sulfonylated proline. Removal of the ester gave the free acids listed in Tables 1–4. Binding data for two compounds reported to be a potent, selective VLA-4 antagonist (BIO-1211)[12] and a dual $\alpha_4\beta_7$/VLA-4 antagonist (TR-14035)[13] are included in Table 2 for comparison to the data reported herein.

A survey of Table 1 suggests that substitution on the distal ring of the biphenylalanine with polar groups (**11a**, **13a**, **16a**, **17a**, **18a**, **20a**, and **21a**) may have a slight potency enhancing advantage over the unsubstituted parent **8**. This potency enhancing effect is not as dramatic as that reported for a series of substituted *N*-benzoyl-biphenylalanine derivatives that are dual $\alpha_4\beta_7$/VLA-4

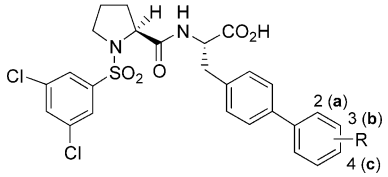
antagonists.[13] The addition of a second methoxy group to the 6′-position (**21a**, **23**, and **31**) gave a significant improvement in potency. Interestingly, this same compound had been prepared via a different optimization strategy.[14]

Analogues with a 2(*S*)-methyl group on the central (L)-proline were prepared in order to prevent the possible cleavage of the central amide bond in vivo. As seen in Table 2, the effect of this methyl group was variable compared to the des-methyl proline derivatives in Table 1 (e.g., **10a** vs **22**; **14a** vs **29**; **20a** vs **35**). The 2,6-dimethoxy substitution in **23** again gave the most potent analogue of the series. Little difference was noted in the potency between **21a** and **23**. In order to assess specificity, binding data for the related $\alpha_4\beta_7$ integrin were added in Table 2. As can be seen, the compounds in Table 2 are $\geq$100-fold specific for VLA-4 with respect to $\alpha4\beta7$. This class of compounds also does not inhibit VLA-5 ($\alpha5\beta1$, data not shown).

The azetidine ring analogues **36** and **37** were prepared to examine the effect of contracting the central pyrrolidine ring (Table 3). These compounds have potency and specificity similar to their proline analogues (**21a** and **23**).

In an attempt to mimic the polar substitutions on the distal ring of the biphenylalanine and seek alternatives to metabolically labile methoxy groups, heteroaryl analogues were prepared (Table 4). With respect to the biphenyl parent **8** or 2′-methoxy **22**, the 2- and 3-pyridyls **38** and **41** and thiazole **42** demonstrated some

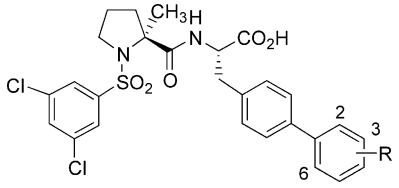
Table 1. Inhibition of VLA-4[a] by substituted sulfonylated proline biphenylalanine derivatives (IC_{50}, nM)



No.	R	2 (**a**)	3 (**b**)	4 (**c**)
8	H	0.63		
9a,c	CH_3	1.43		1.53
10a	OCH_3	0.64		
11a,b,c	CHO	0.22	0.23	0.38
12a,b	CO_2H	0.95	0.54	
13a,b	CO_2CH_3	0.15	0.40	
14a,b	CN	0.46	0.39	
15a,c	F	1.02		0.90
16a	$CONH_2$	0.09		
17a	$CONHCH_3$	0.10		
18a	$CON(CH_3)_2$	0.13		
19a	SCH_3	0.36		
20a	SO_2CH_3	0.10		
21a	$2,6(OCH_3)_2$	0.09		

[a]^{125}I-VCAM-Ig was used as the ligand in a binding assay; see ref 11.

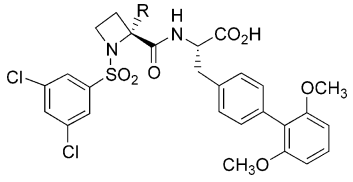
Table 2. Inhibition of VLA-4 and $\alpha_4\beta_7$ by substituted sulfonylated 2(*S*)-methyl-proline biphenylalanine derivatives (IC_{50}, nM)



No.	R	VLA-4[a]	$\alpha_4\beta_7$
22	$2-OCH_3$	0.52	242[a]
23	$2,6-(OCH_3)_2$	0.08	2.98,[a] 11.5[b]
24	$2,6-(OH)_2$	0.50	669.9[b]
25	$2,6-(OCO-i-C_3H_7)_2$	4.50	3551.1[b]
26	2-Cl	1.92	2260[a]
27	2-F	2.4	>4000[a]
28	2-F, 5-CN	1.01	1235[a]
29	2-CN	0.92	344.5[a]
30	3-CN	1.12	1140.8[a]
31	2-CN, $6-OCH_3$	0.28	77[b]
32	$2-CONH_2$	0.14	24.32[a]
33	$2-CON(CH_3)_2$	0.22	82.7[a]
34	$2-SCH_3$	0.65	78.25[a]
35	$2-SO_2CH_3$	0.16	13.2[a]
BIO-1211		0.13	433,[a] 862[b]
TR-14035		0.11	0.75[b]

[a]^{125}I-VCAM-Ig was used as the ligand in a binding assay; see refs 11 and 15.
[b]^{125}I-MAdCAM-Ig was used as the ligand in a binding assay; see ref 15.

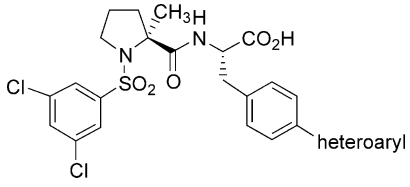
Table 3. Inhibition of VLA-4 and $\alpha_4\beta_7$ by sulfonylated 2(*S*)-azetidinyl-4-biphenylalanine derivatives (IC_{50}, nM)



No.	R	VLA-4[a]	$\alpha_4\beta_7$[b]
36	H	0.08	10.6
37	CH_3	0.07	14.2

[a]^{125}I-VCAM-Ig was used as the ligand in a binding assay; see refs 11 and 15.
[b]^{125}I-MAdCAM-Ig was used as the ligand in a binding assay; see ref 15.

Table 4. Inhibition of VLA-4 and $\alpha_4\beta_7$ by sulfonylated 2(*S*)-methyl-proline 4-heteroaryl-phenylalanine derivatives (IC_{50}, nM)



No.	Heteroaryl	VLA-4[a]	$\alpha_4\beta_7$
38	2-Pyridyl	0.15	97.1[a]
39	3-CN-2-pyridyl	0.14	ND[c]
40	$3-CH_3O-2$-pyridyl	0.11	52.5[b]
41	3-Pyridyl	0.35	311[a]
42	2-Thiazolyl	0.24	413[a]
43	3-(1,2,4)-Triazolyl	0.52	948.8[b]
44	7-Indolyl	1.33	5720[a]

[a]^{125}I-VCAM-Ig was used as the ligand in a binding assay; see refs 11 and 15.
[b]^{125}I-MAdCAM-Ig was used as the ligand in a binding assay; see ref 15.
[c]ND, not determined.

Table 5. Pharmacokinetic parameters[a] of selected compounds

No.	F[b] (%)	Cl_p (mL/kg/min)	$t_{1/2}$[c] (h)
10a	3	39	ND[d]
14a	4	21	ND
22	20	20	ND
23	23	>100	2.2
29	33	5	1.5
32	13	26	1.1
36	11	>100	2.4
37	9	>100	1.5
38	31	17	1.0
40	2	71	0.5

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

increase in potency. The addition of a cyano (**39**) or a methoxy (**40**) at the 3′-position of the 2-pyridyl had little effect. Specificity with respect to $\alpha_4\beta_7$ was >100-fold.

The pharmacokinetic (PK) properties of representative VLA-4 antagonists were measured in rats (Table 5). Generally, the compounds had low to moderate oral bioavailability and moderate to fast plasma clearance rates. Compounds **23**, **36**, and **37** had exceptionally high clearance rates (equal to or exceeding rat hepatic bloodflow!), yet appeared to have comparatively long plasma half-lives. The plasma concentration vs. time curves (AUC) for these compounds exhibited a very rapid drop in plasma concentration followed by very low but sustained circulating concentrations (~1 nM).

Given the apparent attractive PK profile of compound **29** in rats, it was examined in beagle dogs and rhesus monkeys. In both species, the oral bioavailability was low, plasma clearance was moderate to high, and half-lives moderate to long (dog: F = 4%; Cl_p = 25 mL/kg/min; $t_{1/2}$ = 3.4 h; monkey: F = 17%; Cl_p = 12 mL/kg/min; $t_{1/2}$ = 5.3 h). Compound **29** was found to be highly protein bound (>99.5% in human and rat plasma) and much less potent in a plasma shift assay where plasma was added to the binding assay, suggesting that the attractive pharmacokinetic profile of **29** may be a result of high plasma protein binding.

In summary, substituted sulfonylated dipeptides as VLA-4 antagonists were discovered from directed screening of a combinatorial library. Single compound synthesis by solid phase chemistry and subsequent optimization led to very potent compounds (IC_{50}'s < 100 pM) that could not be distinguished by the current assay. Initial pharmacokinetic evaluations suggest that these compounds are very rapidly cleared from plasma but may have some use in evaluating the role of VLA-4 in animal models of disease.

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11. Details of a competitive binding assay between suspended human Jurkat cells and a soluble radiolabeled ^{125}I-VCAM-immunoglobulin fusion protein (^{125}I-VCAM-Ig) have been disclosed (Durette, P. L; Hagmann, W. K.; MacCoss, M.; Mills, S.; Mumford, R. PCT Application WO98/53817A1, 1998; *Chem. Abstr.* **1998**, *130*, 52736s). Briefly, human Jurkat cells were suspended in binding buffer (25 mM HEPES, 150 mM NaCl, 3 mM KCl, 2 mM glucose, 0.1% bovine serum albumin, pH 7.4) supplemented with $MnCl_2$ (1 mM), placed in Millipore MHVB multiscreen plates + compounds in DMSO, incubated at room temperature for 30 min, filtered on a vacuum box, and washed with 100 μL of binding buffer containing 1 mM $MnCl_2$. After insertion of the plates into adapter plates, 100 μL of Microscint-20 (Packard cat #6013621) was added to each well. The plates were then sealed, placed on a shaker for 30 s, and counted on a Topcount microplate scintillation counter (Packard). Control wells containing DMSO alone were used to determine the level of VCAM-Ig binding corresponding to 0% inhibition. Control wells in which cells were omitted were used to determine the level of binding corresponding to 100% inhibition. Percent inhibition was then calculated for each test well and the IC_{50} was determined from a ten point titration using a validated four parameter fit algorithm. All titrations were run in duplicate.
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15. Details of a competitive binding assay between human RPMI-8866 cells (a human B-cell line $\alpha_4^+ \beta_1^- \beta_7^+$ was a gift from Prof. John Wilkins, University of Manitoba, Canada) and radiolabeled ^{125}I-VCAM-immunoglobulin fusion protein (^{125}I-VCAM-Ig) have been disclosed (ref 8) and are similar to the VLA-4 binding assay. Likewise, a competitive binding assay between RPMI-8866 cells and a radiolabeled MAdCAM-immunoglobulin fusion protein (^{125}I-MAdCAM-Ig) similar to the above assay was also employed.