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Substituted *N*-(3,5-Dichlorobenzenesulfonyl)-L-prolyl-phenylalanine Analogues as Potent VLA-4 Antagonists

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Abstract—A series of substituted *N*-(3,5-dichlorobenzenesulfonyl)-L-prolyl- and α-methyl-L-prolyl-phenylalanine derivatives was prepared as VLA-4/VCAM antagonists. The compounds showed excellent potency with a wide variety of neutral, polar, electron withdrawing or donating groups on the phenylalanine ring ($IC_{50} \sim 1$ nM). Heteroaryl ring substitution for phenylalanine was also well tolerated. Pharmacokinetic studies in rat were performed on a representative set of compounds in both series.

The integrin VLA-4 ('very late antigen-4', $\alpha_4\beta_1$) is expressed on all leukocytes except platelets.[1] Its ligands include vascular cell adhesion molecule-1 (VCAM-1), which is a member of the immunoglobulin (Ig) super family, as well as peptides derived from the type III connecting segment (CS-1) of fibronectin. Antibodies to α_4 are effective inhibitors of leukocyte infiltration and prevent tissue damage in several animal models of inflammation.[2] Inhibition of VLA-4 may reduce the migration and/or activation of cell types critical in sustaining a prolonged inflammatory response. VLA-4 inhibitors may be considered in the treatment of asthma,[3] multiple sclerosis[4] and rheumatoid arthritis.[5]

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.[6,7] We previously reported[8] that sulfonylated dipeptides which incorporate 2-(*S*)-α-naphthylalanine (**1**) and 4-biphenyl replacement (**2**) provided substantial improvement over initial screening leads in blocking binding of VLA-4 to VCAM-1 (Fig. 1).

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The relatively poor pharmacokinetic characteristics of this series of compounds, coupled with the appearance of oxidized species attributed to the naphthyl moeity, pointed to the need to replace this group with a metabolically more stable entity. In this paper, we replace the 2-naphthylalanine in **1** with a variety of substituted phenyl and heterocyclic alanine derivatives.[8] Our goal was to retain potency in the inhibition of VLA-4, reduce serum protein binding that was observed with the biphenyl series, and improve the pharmacokinetic characteristics of these VLA-4 antagonists.

In Scheme 1, we outline the synthesis of substituted phenylalanine VLA-4 inhibitors. In general, most of our analogues were assembled from commercially available amino acids. Treatment of the free amino acids with thionyl chloride in cold methanol affords the corresponding

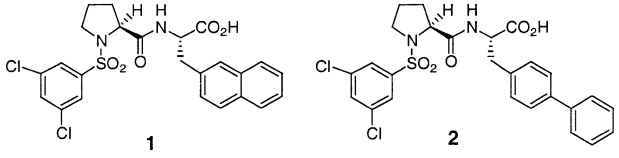

Figure 1.

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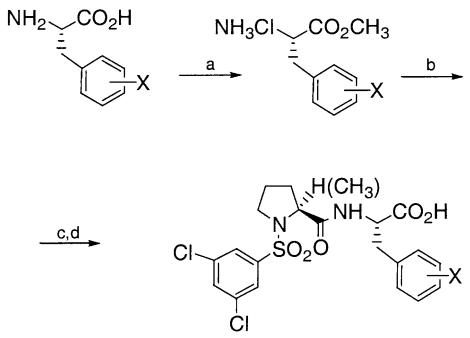


Scheme 1. (a) SO_2Cl, MeOH, 0°C; (b) *N*-(3,5-Cl_2PhSO_2)-L-Pro, PyBOP, DCM; (c) LiOH, EtOH/H_2O; (d) H^+.

methyl esters. The amino acid ester hydrochlorides are then coupled to *N*-(3,5-dichlorobenzenesulfonyl)-L-proline. Hydrolysis of the ester with lithium hydroxide in water/ethanol and subsequent acidification gave the free acids which are listed in Table 1.

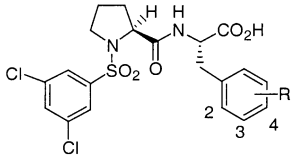
Analogues with a 2-(*S*)-methyl-L-proline were prepared to block potential cleavage of the central amide bond in vivo. Table 2 consists of a cognate set of substituted phenylalanine derivatives coupled to *N*-(3,5-dichlorobenzenesulfonyl)-2-(*S*)-methyl-proline. Binding data for two literature compounds described as a potent, selective VLA-4 antagonist (BIO-1211)[9] and a dual $\alpha_4\beta_7$/VLA-4 antagonist (TR-14035)[10] are included in Table 2 for comparison to the data reported herein.

An examination of the binding data in Table 1 suggests that 4-substituted phenylalanine analogues of **2** are relatively insensitive to both the steric and electronic character of the substituent pattern, consistent with the 4-biphenyl derivative **2**. Sterically hindered derivative **8e** is essentially equipotent with the methyl ether **6e** or the tyrosine derivative **5e.** Receptor binding for electron withdrawing VLA-4 antagonists **12e**, **13e**, and **15e** is similar with either neutral or electron-rich derivatives like **4e**, **5e**, and **6e**. The 3,4- and 3,5-difluoro derivatives **12b** and **12c**, respectively, are nearly equipotent with the other analogues.

Analogues of **3**–**14** were prepared using 2-(*S*)-methyl-proline in place of (*S*)-proline in order to compare potency, selectivity in vitro and resistance to central amide bond hydrolysis in vivo. As seen in Table 2, the impact of this 2-(*S*)-methyl-proline substitution ranged from a negligible to modest loss of potency within each paired set. The most potent analogues across both paired groups were the 4-oxo derivatives **5e**, **6e**, **8e** and **18**, **19**, **21**, respectively. Binding data for the related $\alpha_4\beta_7$ integrin are included in Table 2 in order to assess specificity. The compounds in Table 2 are several orders of magnitude more specific for VLA-4 with respect to $\alpha_4\beta_7$.

A series of heteroaryl analogues was also prepared to assess the effect heteroaryl substitution for phenylalanine has on the inhibition of VLA-4 (Table 3). The results obtained for VLA-4 binding and $\alpha_4\beta_1/\alpha_4\beta_7$ selectivity from this limited set closely parallel those seen in Tables 1 and 2. All are approximately equipotent against VLA-4 (~1 nM) with the same modest loss in VLA-4 binding potency among 2-(*S*)-proline and 2-(*S*)-methyl-proline pairs **27a**, **27b** and **31a**, **31b**, respectively. Only analogue **29** showed a marked drop in potency.

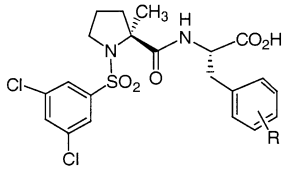
Table 1. Inhibition of VLA-4[a] by substituted *N*-(3,5-dichlorobenzene-sulfonylated prolyl-phenylalanine derivatives (IC_{50}, nM, $n=3$)



Compd	R	2 (**a**)	3,4 (**b**) 3,5 (**c**)	3 (**d**)	4 (**e**)
1			−0.5		
2			−0.65		
3a	H	1.4	—	—	—
4e	C_2H_5	—	—	—	0.44
5e	OH	—	—	—	0.34
6e	OCH_3	—	—	—	0.37
7e	OCF_3	—	—	—	0.86
8e	O-tC_4H_9	—	—	—	0.22
9e	I	—	—	—	0.33
10e	Br	—	—	—	0.92
11a,**d**,**e**	Cl	0.99	—	3.6	0.85
12a,**b**,**d**,**e**	F	1.3	0.48	0.67	0.48
12c	F_2	—	1.1	—	—
13e	CN	—	—	—	0.79
14d	CF_3	—	—	3.2	
15e	NO_2	—	—	—	0.3

[a]VCAM-Ig.

Table 2. Inhibition of VLA-4[a] and $\alpha_4\beta_7$[b] by substituted *N*-sulfonylated 2-(*S*)-methyl-prolyl-phenylalanine derivatives (IC_{50}, nM, $n=3$)



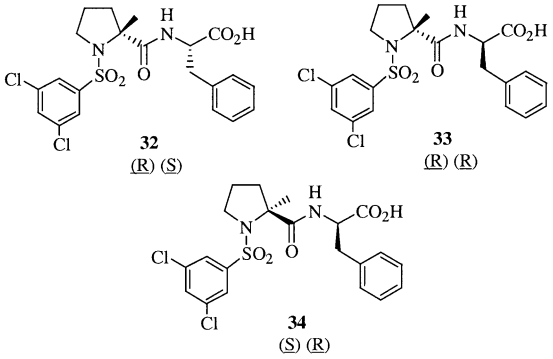
Compd	R	VLA-4	$\alpha_4\beta_7$
16a	H	1.0	3546
17	C_2H_5	1.88	2%[c]
18	OH	0.86	—
19	OCH_3	0.59	16%[c]
20	OCF_3	3.8	—
21	O-tC_4H_9	0.27	646
22	I	4.77	58%[d]
23a	*o*-Cl	1.8	<16%[c]
23b	*m*-Cl	1.7	<16%[c]
23c	*p*-Cl	2.3	<16%[c]
24a	*o*-F	1.7	<5%[c]
24b	*m*-F	0.9	<5%[c]
24c	*p*-F	1.2	<5%[c]
25	CN	1.6	29%[c]
26	CF_3	3.9	25%[c]
BIO-1211		0.13	862[b]
TR-14035		0.11	0.75[b]

[a]VCAM-Ig.
[b]MadCAM-Ig.
[c]% inhibition @ 0.1 μM.
[d]% inhibition @ 20 μM.

Since there was little difference observed in the binding of most of our antagonists against the VLA-4 receptor, we sought to differentiate these derivatives based on their susceptibility to metabolism in vitro as well as their in vivo pharmacokinetic properties. A preliminary examination of the metabolism of compounds in this class suggests that there are oxidation products associated with the central proline ring as well as hydroxylation of the aromatic phenyl ring as determined by preincubation of **16a** in rat hepatocytes or rat liver microsomes (10 μm/30 min).[8] The pharmacokinetic properties of a representative subset of VLA-4 derivatives from the previous tables were measured in rats and are reported in Table 4.

The compounds described generally had low to moderate oral bioavailability and moderate to fast plasma clearance rates. Several compounds (**26** and **29**) had clearance rates approaching rat hepatic blood flow (~60 mL/min). The plasma concentration versus time curves (AUC) for these compounds (**3a**, **16a**, and **24c**) exhibited a very rapid drop in plasma concentration (data not shown) followed by low sustained circulating concentrations (~1 nM).

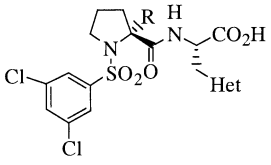
The attractive pharmacokinetic profile of **16a** encouraged us to explore the structure–function relationship of all its stereoisomers.[11] By introducing the unnatural (*R*) configuration for either phenylalanine or α-methyl-proline, we hoped to increase the circulating plasma levels of drug without unduly affecting VLA-4 binding affinity. The stereoisomers of **16a** were synthesized by the route described in Scheme 1. Binding data and pharmacokinetic parameters of all four compounds are described in Table 5.



The data from Table 5 show that only **16a** with the (*S*,*S*) configuration has reasonable binding potency against VLA-4. The other three isomers show moderate to good oral bioavailability, but suffer from exceptionally high clearance rates with the previously observed rapid drop in plasma concentration versus time (AUC) followed by very low but sustained circulating concentrations. The low but sustained serum levels may be due to high serum protein binding (~99.5% in human and rat plasma). Compound **16a** was less potent in a plasma shift assay (9.2 nM @ 10% rat plasma). Most of the compounds are rapidly cleared by the liver and excreted intact in the bile. The pharmacokinetic properties of **16a** were studied in beagle dogs, rhesus monkeys and sheep. Table 6 summarizes these results.

We observed modest to good oral bioavailability in all four species. The clearance rates across all species were moderate, with acceptably long half-lives. The major

Table 3. Inhibition of VLA-4 and $\alpha_4\beta_7$[b] by substituted sulfonylated proline heterocyclic amino acid derivatives (IC_{50}, nM, $n=3$)



Compd	R	Heterocycle	VLA-4[a]	$\alpha_4\beta_7$[a]
27a	H		0.67	45% @ 20 μM
27b	CH_3		1.31	13% @ 0.1 μM
28	H		0.86	2100
29	H		26.5	ND
30	H		0.73	2350
31a	H		0.92	2850
31b	CH_3		1.61	45% @ 20 μM

[a]VCAM-Ig.

Table 4. Pharmacokinetic parameters[a] of selected compounds

Compd	F[b] (%)	Cl_p (mL/kg/min)	Vds (L/kg)	$t_{1/2}$[c] (h)
3a	46	32	0.56	1.1
16a	59	30	1.25	1.0
19	9	47	1.4	0.6
20	34	36	1.4	0.9
23b	32	22	0.36	0.5
24c	53	44	1.2	1.2
26	55	53	ND[d]	0.9
29	5	58	0.48	0.3

[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.

Table 5. Pharmacokinetic parameters[a] of (*R*)- and (*S*)-α-methyl-proline and phenylalanine VLA-4 inhibitors

Compd	VCAM-Ig (IC_{50}, nM)	F[b] (%)	Cl_p (mL/kg/min)	Vdss (L/kg)	$t_{1/2}$[c] (h)
16a	1.0	59	30	1.25	1.0
32	2055	26	60	3.5	0.7
33	28450	91	62	5.6	2.6
34	152	22	84	2.0	0.8

[a]Sprague–Dawley rats.
[b]Dose: 1 mg/kg iv; 2 mg/kg po.
[c]$t_{1/2}$ plasma half-life (0–8 h).

Table 6. Pharmacokinetic parameters[a] of **16a** in different animal species

Species	F[b] (%)	Cl_p (mL/kg/min)	Vdss (L/kg)	$t_{1/2}$[c] (h)
Rat[a]	59	30	1.25	1.0
Dog[d]	39	15.4	2.6	6.7
Monkey[e]	23	16.3	2.7	7.9
Sheep[f]	42	20	4.2	6.9

[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]Beagle (male) dose: 1 mg/kg iv; 2 mg/kg po.
[e]Rhesus (male) dose: 1 mg/kg iv; 2 mg/kg po.
[f]Abraham Sheep (female) dose: 1 mg/kg iv; 2 mg/kg po.

metabolism product of **16a** in all the species was the acylglucuronide of the carboxylic acid.

In summary, we described a series of potent and selective VLA-4 inhibitors that have good oral bioavailability in several species and low but sustained circulating plasma drug levels in vivo. Further work on related sulfonylated dipeptide derivatives will be reported in due course.

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