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N-Tetrahydrofuroyl-(L)-Phenylalanine Derivatives as Potent VLA-4 Antagonists

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Abstract—Given the proposed involvement of VLA-4 in inflammatory processes, a program to identify orally active VLA-4 antagonists was initiated. Herein, we report the discovery of a *N*-tetrahydrofuroyl-(L)-phenylalanine derivative (**17**) and related analogues as potent VLA-4 antagonists with good oral bioavailability. © 2002 Elsevier Science Ltd. All rights reserved.

VLA-4 ($\alpha_4\beta_1$; 'very late antigen-4') is a heterodimeric cell-surface protein expressed at high levels on lymphocytes. VLA-4 binds to an alternatively spliced segment of fibronectin (CS-1) on extracellular matrix and to vascular cell adhesion molecule-1 (VCAM-1) on endothelium. Both of these ligand molecules are expressed in inflamed tissues and actively participate in physiologic and pathologic responses in inflammatory and autoimmune diseases.

The binding of VLA-4 to VCAM-1 leads to lymphocyte infiltration to extravascular tissues. Antibodies against VLA-4 have been demonstrated to block lymphocyte infiltration and prevent tissue damage in animal models of inflammatory diseases such as asthma,[1] multiple sclerosis,[2] rheumatoid arthritis,[3] and inflammatory bowel disease.[4] Therefore, an orally active VLA-4 antagonist might be useful in the treatment of these diseases.

In a previous communication,[5] we described sulfonylated prolyl biphenylalanine derivatives **1** as potent VLA-4 antagonists. In this paper, we wish to report our discovery of a series of low molecular weight dipeptides that emerged from the development of **1**. Apart from being potent VLA-4 antogonists, such dipeptides show good bioavailability as well. Structure **1** consists of three distinctive regions of pharmacophore depicted as P1, P2, and P3 (Fig. 1). Presently, we wish to report our efforts in the optimization of P1, P2, and P3.

That sulfonamides in the P1 region were not essential for potency came as a surprise during a Suzuki-coupling reaction (Fig. 2) of a sulfonylated pyrazolidine. Analysis of the major product of this coupling showed that the sulfonyl group had been eliminated giving rise to the corresponding dehydropyrazolidine **3**, which showed interesting VLA-4 activity.[5] The minor product was the desired coupling product **4**.

To ensure that the observed activity was not due to contamination from the potent sulfonylated pyrazolidine **4**, the dehydropyrazolidine **3** was synthesized via an alternative route (Scheme 1). Starting from the

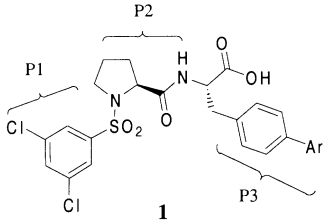

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Figure 1. Binding domains of sulfonylated dipeptides.

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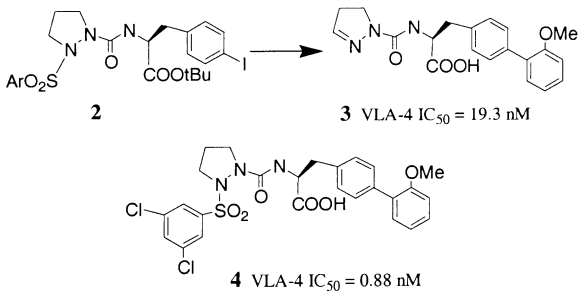
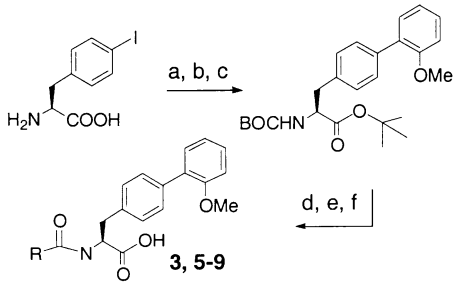


Figure 2. Discovery of potent VLA-4 antagonists lacking a P1 substituent.

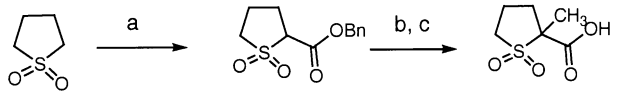
commercially available 4-iodo-phenylalanine, the carboxyl and amino groups were initially protected. A subsequent Suzuki coupling with an arylboronic acid gave the biaryl-alanine derivative. The BOC group was then removed and the resulting amine salt was converted to *p*-nitrophenylcarbamate and reacted with pyrazoline. Finally, deprotection of the *t*-butyl ester furnished pure **3**. The potency of this material was approximately the same as that of the first batch ($IC_{50} = 26.8$ nM) and it cannot be contaminated by **4**. Thus, we demonstrated that potent VLA-4 ligands may be prepared lacking the P1 binding interaction.

Small cyclic P2 analogues devoid of the 3,5-dichlorophenylfulfonyl[5] group were prepared by methods similar to those in Scheme 1. Compound **6** was prepared via the *p*-nitrophenylcarbamate intermediate. Compound **7** was synthesized from enantiomerically pure and commercially available BOC-L-proline as a single diastereomer demonstrating no racemization occurred during the Suzuki coupling reaction. The requisite sulfones were prepared as shown in Scheme 2. Their binding affinities for VLA-4 are shown in Table 1.[6]

Comparison of compounds **6** and **7** to **5** suggests that the presence of the nitrogen atom improved potency. Potency increased even further when other heterocycles such as tetrahydrofuran **8**, pyroglutamate **9**, and sulfone



Scheme 1. (a) Isobutylene, diglyme, H_2SO_4 (60%); (b) BOC_2O, DIEA (100%); (c) (2-methoxyphenyl) boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH; (d) HCl/EtOAc; (e) *p*-nitrophenylchloroformate, DIEA, pyrazoline or PyBOP, RCOOH, DIEA; (f) TFA/CH_2Cl_2.



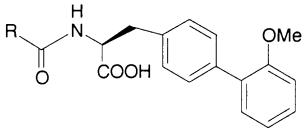
Scheme 2. (a) $ClCO_2Bn$, 2 equiv LiHMDS, (64%); (b) LiHMDS, MeI, (78%); (c) H_2, Pd/C, EtOAc, (75%).

10 were incorporated. *N*-Benzyl pyroglutamate derivatives as potent VLA-4 antagonists have been reported.[7] Increasing the ring size resulted in a loss of potency (**8** versus **11**). Shifting the oxygen in **8** to an adjacent position in the ring (**12**) also decreased potency. These results suggest a specific binding interaction between the ring oxygen and VLA-4.

The corresponding α-methyl analogues (**13** and **14**) of the two most potent compounds (**8** and **10**) were also found to be quite potent. Compound **13** had an excellent PK profile in rats ($F = 72\%$; $CL_p = 5.3$ mL/min/kg; $t_{1/2} = 1.4$ h). The PK profile of the sulfone **14** was by far less impressive in rats ($F = 6\%$; $CL_p = 82$ mL/min/kg; $t_{1/2} = 2.3$ h).

Keeping the α-methyl-tetrahydrofuranoyl constant, further optimization of substituted phenylalanine analogues was explored. The disasteromers of **13** were separated via preparative TLC and the more active isomer **15** was assigned as R as proven by X-ray crystallographic analysis of the corresponding resolved *R*-tetrahydrofuroic acid.[8] The less active *S* isomer has an IC_{50} of 13.7 nM in VCAM binding assay. The preference of *R* configuration was a surprise because when the P1 region is occupied by a large group (3,5-dichlorophenylsulfonyl) like **1** it was the *S* proline that gave the more active diastereomer. This reversal of stereo preference may suggest that the oxygen atom in the tetrahydrofuran ring is interacting with the P2 binding site instead of the P1 binding region as the oxygens of the sulfonyl group. The existence of the P2 binding site was also discussed in a previous report.[9] The enantiomerically pure 2-(*R*)-tetrahydrofuryl acid was employed for the preparation of compounds in Table 2 and was obtained through resolution as has been described.[8] The

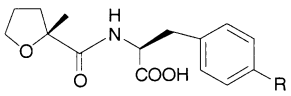
Table 1. Inhibition of VLA-4 by compounds

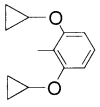
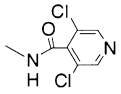
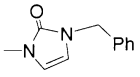


Compd	R	IC_{50}[a] (nM)	Compd	R	IC_{50}[a] (nM)
5		63	**10**		2.1
6		17	**11**		4.9
7		11	**12**		36
8		1.7	**13**		3.9
9		2.8	**14**		1.3

[a]^{125}I-VCAM-Ig was used as the ligand in a binding assay; see ref 5.[6]

Table 2. Inhibition of VLA-4 binding by compounds **15**–**22** and pharmacokinetic parameters[b]

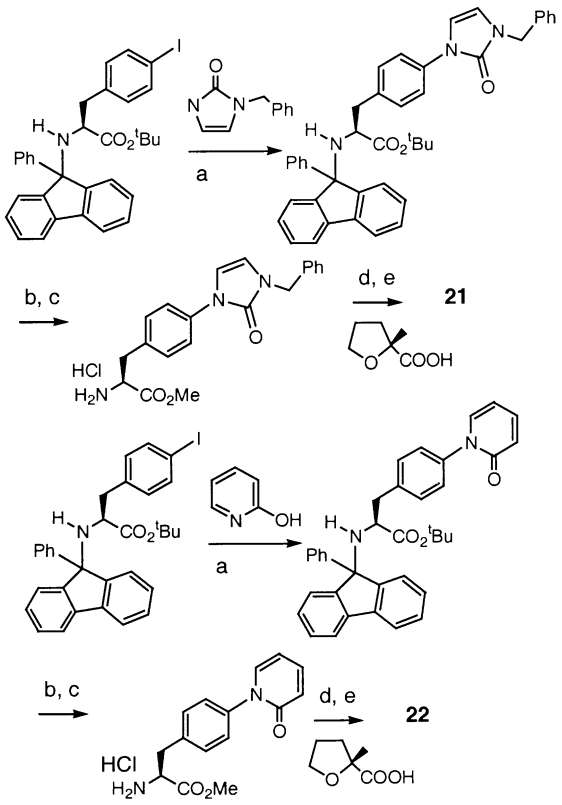


Compd	R	VLA-4[a] IC_{50} (nM)	F[b] (%)	CL_p[c]	$t_{1/2}$ (h)
15		2.7	57	17	0.6
16		11	ND[d]		
17		0.3	42	58	3.4
18		3.9	76	14	0.6
19		2.4	ND[d]		
20		0.2	3	11	0.4
21		4.1	ND[d]		
22		12.9	27	66	0.5

[a]^{125}I-VCAM-Ig was used as the ligand in a binding assay; see ref 5.
[b]Spraguen–Dawley rats, dose: 1 mg/kg iv; 2 mg/kg po.
[c]CL_p, mL/kg/min.
[d]ND, not determined.

biaryl phenylalanine derivatives (**15**–**22**) were prepared according to the methodology outlined in Scheme 1. The dichloro-isonicotinoyl group in **20** was prepared according to literature procedures.[10] The syntheses of the imidazolone **21** and the pyridone **22** are illustrated in Scheme 3. The successful execution of the Goldberg variant[11] of the Ullmann reaction[12] en route to **21** and **22**, which proceeded without concomitant epimerization at the phenylalanine stereogenic center is noteworthy.[13] The results of this P3 substituted optimization are shown in Table 2 along with selected pharmacokinetic (PK) parameters from rats.

Comparison of **15** and **16** showed that replacing the methoxy group with a cyano group decreased potency. An 8-fold increase in potency was observed when a second methoxy group was introduced (**15**–**17**) as it was reported previously.[5,14] Di-methoxy-biphenyl **17** displayed an increased plasma clearance rate compared to that of **15**. In order to address the metabolic stability of the methoxy groups, more stable equivalents were sought. Cyclopropyloxy **18**[15] indeed showed a better PK



Scheme 3. (a) CuI, Cs_2CO_3, DMF, 150 °C, 66%; (b) TFA, anisole DCM, 25 °C, 70%; (c) $SOCl_2$, MeOH, 75 °C, 80%; (d) PyBOP, DIEA, DCM, 75%; (e) NaOH, MeOH, rt, 95%.

profile in rats but its corresponding di-substituted analogue **19**[15] did not exhibit the expected increase in potency. Given the attractive PK of compound **17** in rats, it was examined in rhesus monkey and it showed a low plasma clearance (3.3 mL/min/kg) and high oral bioavailability ($F = 51\%$).

To further improve potency and pharmacokinetic parameters, 4-amino phenylalanine derivatives such as compounds **20**–**22** were prepared. The most potent of this series of compounds, **20** was equipotent in VLA-4 binding to compound **17**. However, it had a less favorable PK profile than that of the di-methoxy analogue **17**.

In summary, the synthesis, biological properties, and pharmacokinetic profile of several interesting VLA-4 antagonists containing a tetrahydrofuranoyl have been described.

Acknowledgements

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preparation. We thank Linus Lin and Thomas Lanza, Jr. for supplying the boronic acid intermediate used for the preparation of compound **18**.

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