Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 729–731

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-Aryl 2,6-Dimethoxybiphenylalanine Analogues as VLA-4 Antagonists

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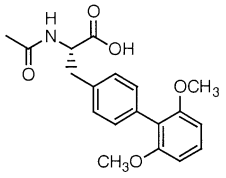
Received 8 October 2001; accepted 12 December 2001

Abstract—A series of *N*-arylated phenylalanine derivatives has been synthesized and has been shown to be potent inhibitors of the integrin VLA-4. *N*-phenyl and *N*-heteroaryl derivatives with hydrogen bond acceptors in the *meta* position demonstrated low nanomolar activity against VLA-4.

VLA-4 ($\alpha_4\beta_1$; CD49d/CD29; 'very late antigen-4') is a key cell surface integrin present on leukocytes and platelets, which binds vascular cell adhesion molecule-1 (VCAM-1) on endothelial cell surfaces and leads to leukocyte infiltration into extravascular tissue. Antibodies against VLA-4 have been shown to block leukocyte infiltration and prevent tissue damage in inflammatory disease models of asthma,[1,2] multiple sclerosis,[3,4] rheumatoid arthritis (RA),[5] and inflammatory bowl disease (IBD).[6] Orally active small molecule inhibitors of VLA-4 might therefore serve as useful agents in the treatment of these diseases.

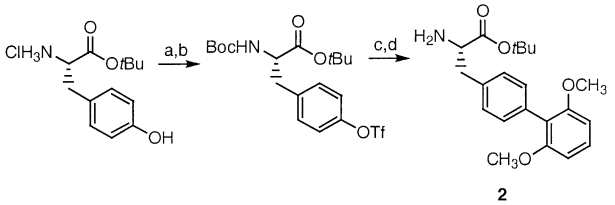
Initial efforts in our laboratories led to the discovery of the acylated phenylalanine derivative **1** as a low nanomolar inhibitor of VLA-4.[7] The amide bond is very important, but it was unclear if the amide carbonyl was serving as a hydrogen bond acceptor, if the amide nitrogen N–H was serving as a hydrogen bond donor, or both. In an effort to test the hypothesis that the amide bond hydrogen was providing a key binding interaction with VLA-4 and also to move away from peptide-like inhibitors of VLA-4, we investigated a series of *N*-arylated derivatives of amino acid **2**. These arylated derivatives provide a N–H bond whose hydrogen bonding potential could be modified by substitution on the aryl group or by different heteroaryl groups.



1, IC_{50} = 12 nM

The phenylalanine derivative **2** was readily prepared in multigram quantities. The nitrogen of (L)-tyrosine-*tert*-butyl ester was initially protected as a *tert*-butyl carbamate then the phenol hydroxyl converted to the corresponding triflate. The triflate underwent standard Suzuki coupling with 2,6-dimethoxyphenylboronic acid, followed by deprotection of the a *tert*-butyl carbamate group in the presence of the *tert*-butyl ester[8] to give rise to the aminoester **2** (Scheme 1).

Using this amino acid as the building block, a series of *N*-arylated derivatives was synthesized. Coupling between the amino ester and a series of aryl halides



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Scheme 1. (a) Boc_2O; (b) Triflic anhydride, pyridine, CH_2Cl_2, 0 °C; (c) 2,6-dimethoxyphenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, EtOH, $PhCH_3$; (d) H_2SO_4, *t*BuOAc.

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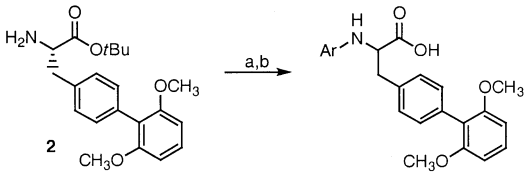
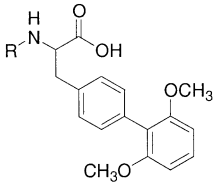
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00009-4

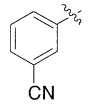
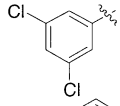
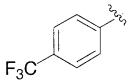
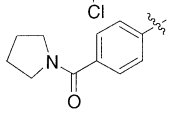
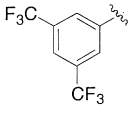
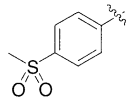
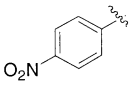
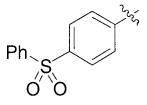
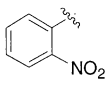
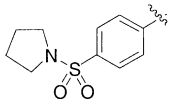
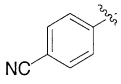
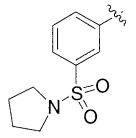
utilizing conditions described by Buchwald[9] and Hartwig,[10] followed by deprotection of the *tert*-butyl ester, provided swift access to the desired arylated derivatives (Scheme 2). Under these conditions, the amino acid was racemized as determined by the synthesis of the (*S*)-(−)-1-phenylethyl ester of **15**. Inhibition of ^{125}I-VCAM-Ig binding to VLA-4 on Jurkat cells by these compounds is shown in Table 1.[7]



Scheme 2. (a) ArX, NaO*t*Bu, (*rac*)-BINAP, Pd_2dba_3, $PhCH_3$, 75 °C; (b) CF_3CO_2H, CH_2Cl_2.

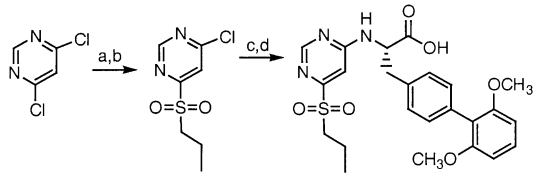
Table 1. Inhibition of VLA-4/VCAM binding[a] by *N*-aryl phenylalanine derivatives



Compd	R	IC_{50} (nM)	Compd	R	IC_{50} (nM)
1[b]	CH_3CO	12	**10**		4360
3[b]	PhCO	1.2			
4		8870	**11**		6490
5		45% at 4 μM	**12**		320
6		46% at 4 μM	**13**		97
7		5250	**14**		240
8		3550	**15**		91
9		4470	**16**		12

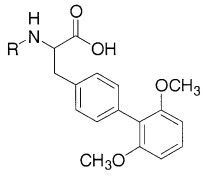
[a]VCAM-Ig[7].
[b]L-phenylalanine derivative.

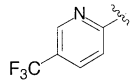
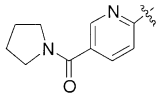
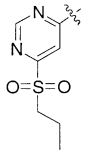
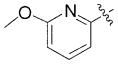
The simplest *N*-phenyl substituted analogue **4** showed a significant loss of potency compared with the acylated derivative **1** or the benzoylated derivative **3**. Since aniline is a much poorer hydrogen bond donor than acetamide,[11] it was felt that the basicity of the aryl amine needed to be reduced to make it a better hydrogen bond donor relative to the amide. With this in mind, a number of aryl amines substituted with electron withdrawing groups were synthesized. Trifluoromethyl, nitro, cyano and chloro substitution did little to increase the potency of this series (**5**–**11**). Interestingly, a significant increase in potency was seen with sulfonyl and carboxamide substitutions (**12**–**15**). This increase in potency was attributed to a possible binding interaction between these hydrogen bonding substituents and VLA-4 and not from an increased contribution from the aniline N–H interaction. A sulfonamide in the *para* position (**15**) showed a 3-fold increase in potency as compared with the corresponding carboxamide (**12**). Moving the sulfonamide from the *para* position (**15**) to the *meta* position (**16**) provided a further increase in potency. Thus, the introduction of a *meta* sulfonamide



Scheme 3. (a) Propanethiol, NaH, THF; (b) MCPBA, CH_2Cl_2; (c) **2**, NEt_3; (d) CF_3CO_2H, CH_2Cl_2.

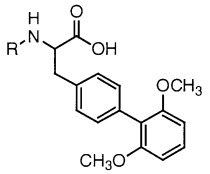
Table 2. Inihibition of VLA-4/VCAM binding[a] by *N*-heteroaryl phenylalanine derivatives

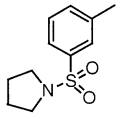
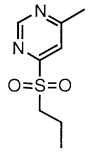


Compd	R	IC_{50} (nM)	Compd	R	IC_{50} (nM)
17		610	**21**		3190
18		820	**22**		23
19		170	**23**		0.39
20		4180			

[a]VCAM-Ig[7].

Table 3. Sprague–Dawley rat pharmacokinetic parameters



Compd	R	F%	Clp (mL/min/kg)	$t_{1/2}$ (h)	Vdss (L/kg)
16		24	73	0.9	0.55
23		6	17	0.8	0.28

substituent provided an increase in potency of ~800-fold from the parent aniline derivative (**4**).

A series of heteroaryl amines was examined next since arylamines were not functioning as very effective amide bond mimetics. The pyridyl substituted derivatives (**17**–**22**, Table 2) were made using a procedure similar to that used to make the phenyl series[12] and a slight modification of this procedure was used to make the substituted pyridine derivatives (**21**–**22**).[13] A *meta* substituted pyrimidine **23** was synthesized for comparison as illustrated in Scheme 3.[14]

The pyridyl derivatives (**17**–**22**) proved to be significantly more potent than the corresponding phenyl derivative (**4**). This increase in potency was seen in all three positional isomers, which suggested it was due to the aminopyridine hydrogen atom more effectively mimicking the amide N–H. The 4-pyridyl isomer (**19**) was more potent than the 2- or 3-pyridyl isomers (**17**–**18**) indicating an interaction between the pyridyl nitrogen and VLA-4 could be occurring. Substitution with a carboxamide (**22**) again led to a further increase in potency. The *meta* substituted pyrimidine derivative **23** proved to be the first subnanomolar VLA-4 inhibitor in this series.

The pharmacokinetic profiles of the *meta*-sulfonamide substituted aniline **16** and the pyrimidine derivative **23** were measured in rats (Table 3). Both compounds showed low bioavailability and high clearance (Table 3). These pharmacokinetic profiles are very similar to our sulfonylated proline series,[7] which contain an amide bond. Thus, replacement of the amide bond of our VLA-4 inhibitors with an aryl or heteroaryl amine did not lead to a significant improvement in pharmacokinetics.

To date, most small molecule inhibitors of VLA-4 have been acylated amino acids, and many retain significant peptide character.[15] We have demonstrated that N-aryl phenylalanine derivatives with significantly less peptide character can serve as potent inhibitors of VLA-4.

Acknowledgements

The authors are grateful to Zhen Wang, Junying Wang, and Song Zheng for formulation and mass spectral analysis of pharmacokinetic samples and to Marcie Donnelly for dosing of animals for pharmacokinetic evaluation.

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14. Similar *N*-arylated compounds have been reported: Head, J. C.; Porter, J. R.; Warrellow, G. J.; Archibald, S. C.; Hutchinson, B. W. WO 0018759, 2000.
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