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The Discovery of Acylated β-Amino Acids as Potent and Orally Bioavailable VLA-4 Antagonists

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Abstract—Acylated β-amino acids are described as potent, specific and orally bioavailable antagonists of VLA-4. The initial lead was identified from a combinatorial library. Subsequent optimization using a traditional medicinal chemistry approach led to significant improvement in potency (up to 8-fold) while maintaining good pharmacokinetic properties. © 2002 Elsevier Science Ltd. All rights reserved.

VLA-4 ($\alpha_4\beta_1$; CD49d/CD29; 'very late antigen-4') is a member of the integrin family that 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). The binding interactions between VLA-4 and its ligands (VCAM-1 and FN) are proposed to be key mediators of cell–cell and cell–matrix adhesion. As such, these cell adhesion interactions may be required for the activation, migration, proliferation, and differentiation of leukocytes during normal pathophysiological processes. Thus, inhibition of VLA-4 may produce a reduction in the migration activation of cell types important to sustaining a prolonged inflammatory response. Anti-α_4 antibodies and blocking peptides have been reported to be efficacious in a number of animal models of inflammatory diseases, including antigen-induced bronchial hyperresponsiveness, experimental allergic encephalomyelitis, adjuvant arthritis, and spontaneous chronic colitis.[2] Therapeutic targets for such an agent might include asthma, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease.

Guided by a VLA-4 direct binding assay,[3] compound **1** was discovered as our initial lead by screening a combinatorial library (Fig. 1).[4] In order to further optimize the potency of this lead, a follow-up single compound library was designed employing commercially available α- and β-amino acids, acyl chlorides, and sulfonyl chlorides.[4] The results of this effort suggest: (a) 3-substituted arenesulfonamides are essential for potency and (b) (L)-proline can be used to replace the tetrahydroisoquinolin-2-carboxyl (Tic) group. Two structures of particular interest were identified (**2** and **3a**) from the library, which showed marked improvement in potency.

While follow-up studies of **2** are reported elsewhere,[4–6] further examination of the above library revealed useful structure–activity relationships (SAR) for the β-amino

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acid lead **3a**. As summarized in Figure 1, the phenyl substituent is clearly preferred over other substituents (**3b**, **3c**, and **3d**) or no substituent (**4c**). The *R*-configuration of the β-amino acid moiety is also essential for binding to VLA-4 (**3a** vs **4a** and **3b** vs **4b**). This finding is particularly intriguing considering that the opposite stereochemistry is preferred for the β-amino acid unit in a series of phenylureidophenacetyl-capped peptides (e.g., **5**).[7] It is likely that the arylsulfonylproline moiety of **3a** binds to a different region of VLA-4 than the phenylureidophenacetyl-Leu unit of **5**.[8]

Encouraged by these results, we set out to further optimize the potencies and pharmacokinetic properties of this new lead, focusing on modifications of the phenyl group of the β-amino acid portion of the molecule. Two synthetic routes were utilized for the synthesis of the requisite β-amino acids (Scheme 1). The sulfinimide route, reported by Ellman[9] and Davies,[10] works well with aldehydes as well as methyl ketones. As reported in the literature, the enolate addition step proceeded with high diastereoselectivity (>95%, based on NMR analysis). The minor diastereomer, if present, can be easily removed by silica gel chromatography after the amide bond coupling step. The Michael addition route, reported by Davies,[11] also worked uneventfully in our hands.

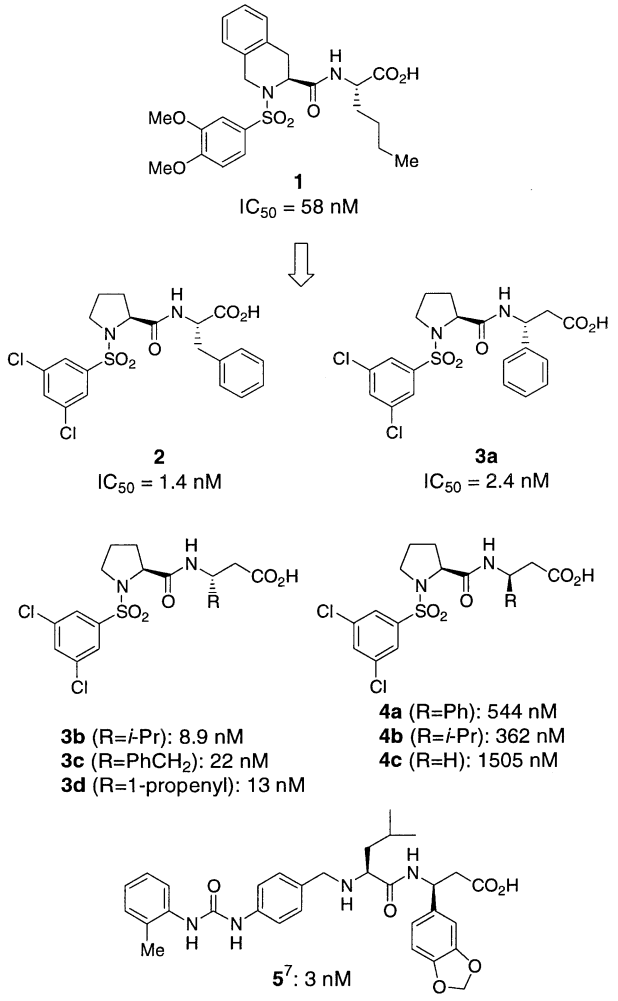
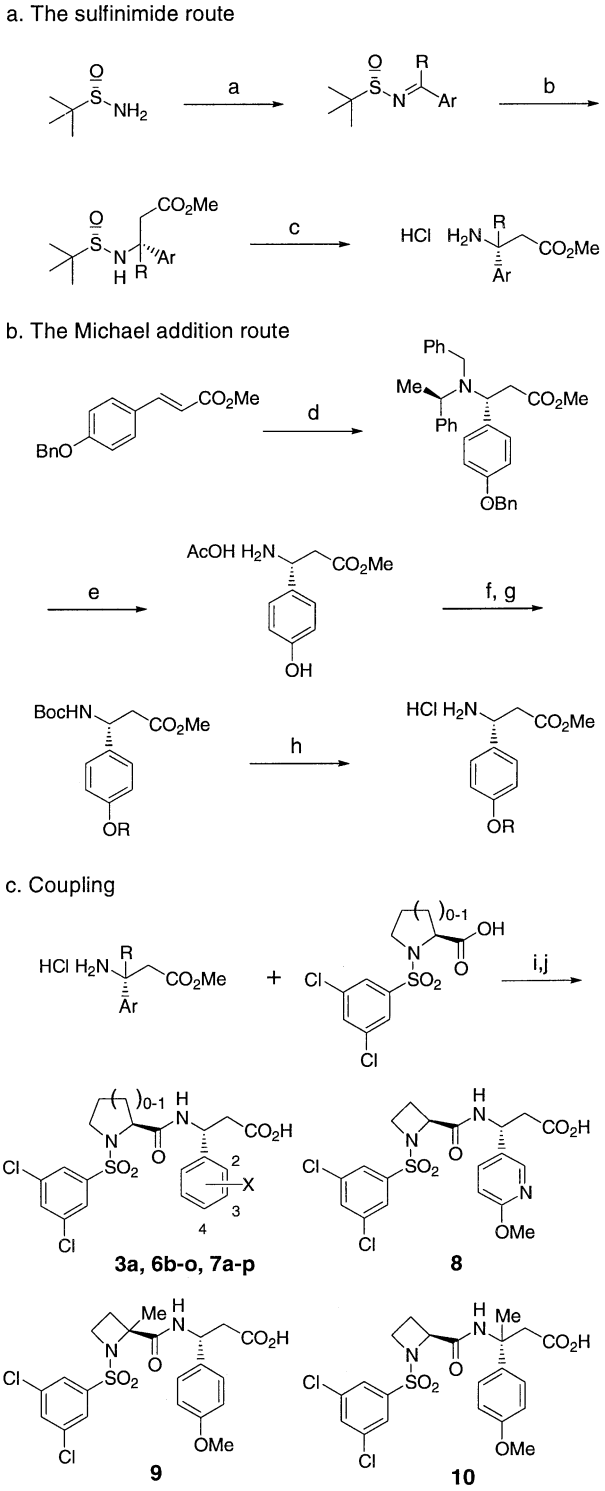


Figure 1.

With the phenol in hand, *O*-alkylation was accomplished under either base promoted conditions or Mitsunobu conditions. Subsequent coupling afforded the final product after hydrolysis.



Scheme 1. (a) ArCHO or ArCOMe, $Ti(OEt)_4$, THF, rt; (b) MeOAc, LDA/$TiCl(OiPr)_3$ or NaHMDS, THF, −78 °C; (c) HCl/MeOH/EtOAc, rt; (d) amine, BuLi, THF, −78 °C; (e) H_2 (40 psi), $Pd(OH)_2$, MeOH/AcOH, rt; (f) $(Boc)_2O$, iPr_2NEt, DMF, rt; (g) MeI, EtI, CF_3CH_2OMs or cyclopentyl bromide, Cs_2CO_3, DMF or $MeSCH_2Cl$, Cs_2CO_3, DMF, rt then XeF_2, $ClCH_2CH_2Cl$, rt; (h) HCl/EtOAc, rt; (i) PyBop, iPr_2NEt, CH_2Cl_2, 0 °C to rt; (j) LiOH, MeOH/THF/H_2O, rt.

Table 1. Binding data and pharmacokinetic properties in the rat[a]

Compd	R	Ring size	$\alpha_4\beta_1$ IC_{50} (nM)	Clearance (mL/kg/min)	F%
2		5	1.4	32	47
3a	H	4	2.4	27	49
7a	H	5	2.6	—	—
6b	4-F	4	3.2	—	—
7c	3-F,4-Cl	4	11	—	—
7d	4-Br	4	2.1	—	—
7e	4-$MeSO_2$	5	0.55	92	1.3
6f	4-HO	5	0.74	22	1.2
6g	4-MeO	4	0.39	39	36
7g	4-MeO	4	0.49	17	43
7h	4-EtO	5	0.27	40	44
6i	4-*t*BuO	5	0.53	—	—
6j	4-*cyclo*-PrO	4	0.31	70	30
7j	4-*cyclo*-PrO	5	0.85	26	52
6k	4-*cyclo*-pentyl-O	4	0.50	165	5
7l	4-PhO	4	3.1	—	—
7m	4-FCH_2O	4	0.72	—	—
6n	4-F_2CHO	4	1.3	—	—
7o	4-F_3CO	5	2.6	22	42
7p	4-F_3CCH_2O	4	1.4	43	38
8			3.0	55	22
9			1.5	7	42
10			26	46	19

[a]Sprague–Dawley rats; dose: 1 mg/kg iv; 2 mg/kg po; volume distribution: 1–2 L/kg.

The binding results to VLA-4 are summarized in Table 1. Among the three pairs of azetidine and proline analogues (**3a**/**7a**, **6g**/**7g**, **6j**/**7j**), the azetidine analogues ($n=0$) showed comparable or slightly lower potency than the proline analogues. An α-methyl analogue (**9**) was also prepared, and found to be 3-fold less potent than the parent compound **7g**, which is consistent with findings of the corresponding α-amino acid series.[4,5] In comparison, methylation of the β-amino acid resulted in 50-fold loss of potency (**10** vs **7g**).

The phenyl group of the β-amino acid was studied in more detail, and several trends are noteworthy. A 4-substituent capable of hydrogen bonding seems to be preferred for potency (HO/RO and SO_2 vs H, Cl, Br). Limited modifications at the 3-position (**7c** and **8**) resulted in significant loss of potency. In the 4-hydroxy/alkoxy series, the binding affinity seems to improve as the hydrogen bonding affinity of the oxygen increases. For example, an increase in potency was observed as the hydroxy hydrogen was substituted with Me and Et group [**6f** (R = OH), 0.74 nM; **6g/7g** (R = MeO), 0.39/0.49 nM; **7 h** (R = EtO), 0.27 nM]. However, further substitution led to no further increase in potency [**6i** (R = *t*-BuO) and **6k** (R = cyclopentoxy) vs **6h** (R = EtO)] as the steric hindrance around the oxygen increases. The electronic effect was also evident in a number of cases. For example, significant loss of potency was observed for **7m** (R = FCH_2O), **7n** (R = F_2CHO) and **7o** (R = F_3CO) in comparison with **7g** (R = MeO), as the methyl hydrogen was sequentially replaced with an election withdrawing fluorine atom. Similarly, the trifluoroethoxy analogue **7p** is significantly less potent than the ethoxy analogue **7h**. More subtly, loss of potency from **7h** (R = EtO) to **7j** (R = cycloPrO) to **7l** (R = PhO) was observed, presumably due to increase in *s*-character of the carbon substituent in the series.

Selected compounds were also tested against the $\alpha_4\beta_7$ integrin,[12] and were found to be > 500-fold specific.

The pharmacokinetic properties of selected compounds were evaluated in the rat (Table 1). Studies of the corresponding α-amino acid analogues suggest that the amide bond may be susceptible to cleavage by a carboxypeptidase in vivo.[4,13,14] It is presumed that the unnatural β-amino acid derivatives may be less favorable substrates for such a peptidase. Indeed, no hydrolysis products were observed in vivo based on HPLC analysis of rat plasma, bile, and urine samples.[14] Nevertheless, the α-methyl analogue **9** showed much reduced clearance in comparison with the parent compound **7g**, which may be attributed to increase in rat plasma protein binding. Related findings were made in the α-amino acid series and were confirmed by protein binding experiments.[4,5]

In vitro rat microsomal studies have shown that the methoxy group of **6g** was cleaved, which is consistent with the observed increase in clearance for **7 h** (R = EtO) and **6k** (R = cyclopentoxy). It is known that cyclopropyloxy and fluoromethoxy groups are more metabolically stable than a methoxy group.[15] Indeed, both **7j** (R = *cyclo*-PrO) and **7o** (R = F_3CO) showed reduced clearance at the expense of potency.[16]

In summary, an acylated β-amino acid lead **3a** was discovered from a combinatorial library. Optimization using traditional medicinal chemistry approaches led to significant improvement in potency while maintaining good pharmacokinetic properties. Notable examples include **7g**, **7h**, and **7j** as potent, specific and orally bioavailable antagonists of VLA-4.

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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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