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N-Aryl-prolyl-dipeptides as Potent Antagonists of VLA-4

Theodore M. Kamenecka,[a,*] Thomas Lanza, Jr.,[a] Stephen E. de Laszlo,[a] Bing Li,[a] Ermengilda D. McCauley,[b] Gail Van Riper,[b] Linda A. Egger,[c] Usha Kidambi,[c] Richard A. Mumford,[b] Sharon Tong,[a] Malcolm MacCoss,[a] John A. Schmidt[b] and William K. Hagmann[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Inflammation and Rheumatology Research, Merck Research Laboratories, Rahway, NJ 07065, USA*
[c]*Department of Pharmacology, Merck Research Laboratories, Rahway, NJ 07065, USA*

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Abstract—The design, synthesis, and biological evaluation of *N*-arylprolyl-dipeptide derivatives as small molecule VLA-4 antagonists is described. Potency against VLA-4 and $\alpha_4\beta_7$ and rat pharmacokinetic evaluation revealed some advantages over the related *N*-(arylsulfonyl)-prolyl-dipeptide analogues.

The cell adhesion molecule VLA-4 (very late antigen, $\alpha_4\beta_1$, CD49d/CD29) is an integrin constitutively expressed on circulating lymphocytes.[1] VLA-4 interacts with the alternatively spliced CS-1 domain of fibronectin in the extracellular matrix and with VCAM-1 (vascular cell adhesion molecule-1) expressed on endothelial cells. These ligands are upregulated in response to inflammatory cytokines and are expressed at sites of inflammation. The binding of these ligands with VLA-4 is thought to be required for cell trafficking, activation, and development during normal and/or pathophysiological processes. As such, VLA-4 antagonists may be useful in the treatment of diseases which are characterized by a prolonged inflammatory responses where cell trafficking and activation are important such as asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.[2–4]

Previously, *N*-arylsulfonylated dipeptide derivatives have been reported to be potent VLA-4 antagonists.[5] Typical of this class of compounds is derivative **1** (Fig. 1). Compounds of this structure class generally have poor pharmacokinetic (PK) properties, characterized by low oral bioavailability ($F < 5\%$) and high plasma clearance rates ($Cl_p > 100$ mL/min/kg. They also show roughly 50- to 100-fold specificity for VLA-4 over the related $\alpha_4\beta_7$ integrin.

In the search for new leads, we found that migration of the sulfonyl residue from the proline nitrogen in **1** to the α-carbon (as in **A** to **B**, Fig. 2) led to potent compounds with better pharmacokinetic profiles.[6] Furthermore, the sulfonyl group was deemed not necessary, as direct attachment of the aryl group to the α-carbon (as in **B** to **C**) led to compounds that retained their potency against VLA-4 and also led to further improvements in PK.[7] In searching for the structural elements that contributed to the poor PK characteristics of compounds like **1**, the *N*-sulfonyl group was proposed to be unnecessary for potency (as in **C** to **D**) since α-aryl-cycloalkanoyl derivatives (**C**) could be made which were potent. This sulfonyl elimination would lead to a new class of *N*-aryl-prolyl derivatives that might have better PK characteristics and is the subject of this report.

The desired *N*-arylated prolines were constructed by two different strategies outlined in Schemes 1 and 2. (L)-proline could be *N*-arylated in the presence of K_2CO_3 and CuI without loss of enantiomeric excess as described in the literature.[10] This procedure worked well for iodobenzene as well as for 2-, 3- and 4-substituted phenyl halides and provided the *N*-arylated carboxylic acids upon workup. Standard peptide coupling with the biphenylalanine aminoester using the HATU/HOAt

*Corresponding author. Fax: +1-858-202-5753; e-mail: theodore_kamenecka@merck.com

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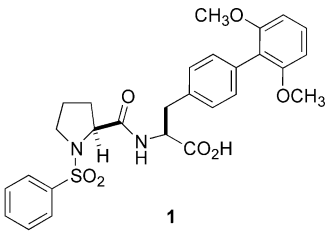

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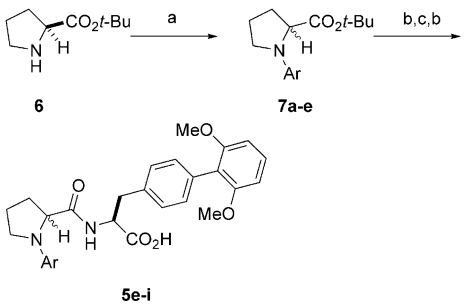
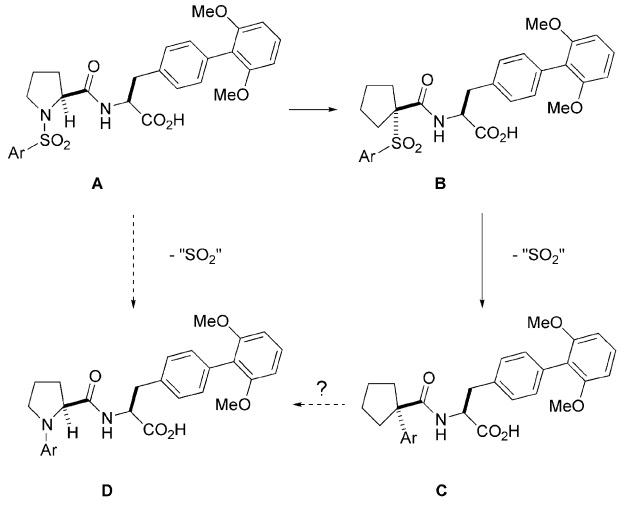
VLA-4: IC_{50} = 0.05 nM
$\alpha_4\beta_7$: IC_{50} = 2.5 nM

Figure 1. Initial *N*-arylsulfonylated dipeptide lead.



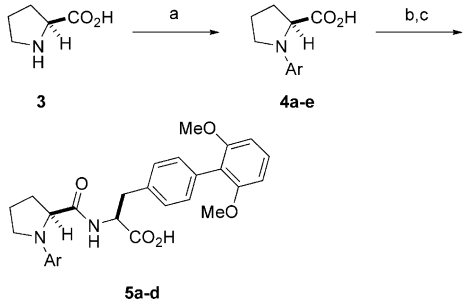
Scheme 2. (a) Aryl-halide, Pd_2dba_3, NaOtBu, BINAP, toluene, 80 °C; (b) TFA, CH_2Cl_2; (c) 2,6-dimethoxybiphenyl alanine *tert*-butyl ester, HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2.



Ar = Ph	VLA-4[a]	$\alpha_4\beta_7$[b]	rat PK[c] Cl_p[d]	F%
A	0.05	2.5	127	2
B	0.11	5.0	122	22
C	3.20	110	22	55

[a]VCAM-Ig IC_{50} nM. See ref 8.
[b]MAdCAM-Ig IC_{50} nM. See ref 9.
[c]Sprague–Dawley rats.
[d]mL/min/kg

Figure 2. New leads based on parent molecule A.



Scheme 1. (a) Aryl-halide, CuI, K_2CO_3, DMAC, 90 °C; (b) HATU, HOAt, 2,6–dimethoxybiphenyl alanine *tert*-butyl ester, *i*-Pr_2NEt, CH_2Cl_2; (c) TFA, CH_2Cl_2.

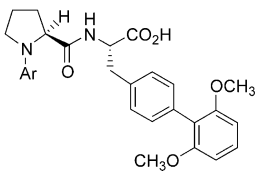
method gave the dipeptides.[11] Deprotection of the *tert*-butyl ester with TFA provided the final products.

This method, however, failed to work for heteroaromatic halides such as 2-bromopyridine and 5-iodopyrimidine. In the CuI method, a catalytic cycle is proposed wherein the amino acid salt forms a chelate with the CuI. A π-complex is formed with the aromatic halide which facilitates intramolecular displacement of the halide by the amino acid nitrogen atom. The avidity of pyridine and other aromatic amine containing heterocycles for copper salts may disrupt this mechanism and might explain why these systems failed.

Fortunately, other methods for *N*-arylation are feasible as described in Scheme 2. (L)-Proline *tert*-butyl ester was coupled with pyridyl-like halides in the presence of sodium *tert*-butoxide and a suitable palladium source to afford the desired *N*-arylated prolines in good yield.[12] Not surprisingly, under the basic reaction conditions, complete loss of stereochemical integrity was observed giving racemic products. Interestingly, attempted coupling with proline methyl or benzyl ester gave much lower yields of product if at all. Deprotection of the *tert*-butyl ester with TFA, standard HATU coupling, and ester hydrolysis afforded the desired products in good yield. Since the proline *tert*-butyl ester was racemized in the *N*-arylation step, the compounds made using this method were 1:1 mixtures of diastereomers at the proline α-center. The diastereomers were not easily separated and so they were tested as mixtures. Compounds prepared by the methods outlined in Schemes 1 and 2 were evaluated as inhibitors of VLA-4 and the related integrin, $\alpha_4\beta_7$ and are outlined in Table 1.

The unsubstituted *N*-phenyl proline derivative (**5a**) exhibited a 10-fold loss in potency relative to sulfonamide **1**. This loss in potency is somewhat less than what was observed (30-fold loss) in going from **B** to **C** as described in Figure 2. Clearly, the interaction of the sulfonyl group with some portion of VLA-4 is important for binding, but it is not critical for activity. Attempts to find this interaction via substitution of the phenyl ring at the 2- and 3-positions (**5b**,**c**) with hydrogen bond acceptors led to small improvements in potency, while

Table 1. Inhibition of VLA-4 and $\alpha_4\beta_7$ by *N*-aryl-prolyl biphenylalanine derivatives (IC_{50}, nM)



Compd	Ar	VLA-4[b]	$\alpha_4\beta_7$[c]
5a	Phenyl	0.61	21
5b	2-CN-phenyl	0.37	3.5
5c	3-CH_3O-phenyl	0.41	5.8
5d	4-NO_2-phenyl	1.1	17.1
5e	2-Pyridyl[a]	1.9	18
5f	3-Pyridyl[a]	0.34	5.5
5g	2-Pyrazinyl[a]	0.17	8.9
5h	5-Pyrimidinyl[a]	0.73	18.8
5i	4-Isoquinolyl[a]	1.1	45
5j	4,6-$(NO_2)_2$–2-pyridyl[a]	0.38	17

[a](*R*,*S*)-Proline mixture of diastereomers.
[b]VCAM-Ig ligand.
[c]MAdCAM-Ig ligand.

Table 2. Pharmacokinetic parameters[a] of selected *N*-aryl-prolyl derivatives

Compd	F (%)[b]	CL_p (mL/min/kg)	$t_{1/2}$ (h)[c]
1	2	127	0.6
5a	14	78	0.6
5b	<1	69	0.3
5c	3	64	0.7
5d	<1	>100	1.4
5e	2	29	0.3
5f	<1	43	0.2

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

4-substitution was deleterious. Efforts to find the sulfonyl binding site by using the lone pair on nitrogen via the 2-pyridyl derivative (**5e**) was unsuccessful giving a compound 3-fold less active. It is not clear what is responsible for this loss in activity, as the 2-pyridyl nitrogen atom is the closest proximation in space to one of the sulfonyl oxygen atoms. However, the basicity of aminopyridine **5e** might be what is responsible for its loss in activity. The potential for an intramolecular hydrogen bonding interaction between the pyridyl nitrogen atom in **5e** and the amide NH bond may alter the conformation of the molecule. This would be less likely to occur with the less basic pyrizine derivative (**5g**) or with the non-basic sulfonamide **1**. The other analogues do not have a heteroatom capable of such an interaction.[13] One of the most potent derivatives was 3-pyridyl proline (**5f**) which showed a 2-fold improvement in potency over **5a**. The heteroatom is apparently better situated to pick up the hydrogen bonding interaction within VLA-4 and improves activity. However, there are spatial limitations within the receptor as 4-isoquinolyl derivative (**5i**) is apparently too large and potency is lost (Table 1).

Although one pyridyl nitrogen is good as in **5f**, two is not advantageous as pyrimidinyl analogue **5h** was less active. The 2-pyrazinyl proline (**5g**), which is essentially the combination of 2- and 3-pyridine, was the most potent analogue.[14] Introduction of another heteroatom in the ring will reduce the basicity of the system and this may be enough to lessen intramolecular hydrogen bonding if that is an issue. Also of note is that analogues **5e** through **5j** are a mixture of diastereomers and one enantiomer is likely to be more potent than the other. In summary, these results confirm our earlier findings that the sulfonyl residue is not necessary for potency as evidenced by the conversion of **B** to **C**, but now also in the conversion of **C** to **D** (or for that matter, **A** to **D**). The potency lost by removal of the sulfonyl moiety from **1** is almost completely recovered in *N*-aryl proline **5g**.

Despite improvements in potency on VLA-4 from lead **5a**, these compounds showed little specificity with regard to $\alpha_4\beta_7$. Most compounds were in the range of 10- to 30-fold specific for VLA-4 with the most potent derivative **5g** exhibiting $\sim$50-fold specificity. These results are not surprising given the lack of specificity ($\sim$10- to 100-fold) also observed for compounds in class **C** (Fig. 1) and suggests that perhaps the interactions which confer specificity are located in a different part of the molecule.

The pharmacokinetic properties of selected derivatives were determined in rats (Table 2). As hoped, removal of the sulfonyl group (**1** to **5a**) leads to a compound with improved oral bioavailability (14%) and a lower plasma clearance rate. With the exception of **5d**, all compounds examined had plasma clearance rates lower than that of the corresponding sulfonylated proline **1**, however they were still high.[15] Despite the apparent lower clearance rates, recovery of potency comes at the expense of oral bioavailability. As the potency of pyridyl analogues **5e** and **5f** approaches that of **1**, so does their lack of oral bioavailability. This common trend was not only observed here, but also in other efforts aimed at targeting unique leads.[4,5]

In summary, a new lead was discovered based on sulfonamide **1**, lacking the sulfonyl group. Although removal of the sulfonyl residue led to a 10-fold drop in potency, it was not required for binding to the receptor. Proper selection of the aryl group on nitrogen allowed for recovery of potency in the binding assays with modest improvements in plasma clearance rates. *N*-aryl proline derivatives **5f** and **5g** represent two such compounds which may be further optimized as potent VLA-4 antagonists.

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9. Details of a competitive binding assay between human RPMI-8866 cells (a human B-cell line $\alpha_4\beta_7$ was a gift from Professor John Wilkins, University of Manitoba, Canada) and radiolabeled ^{125}I-MAdCAM-immunoglobulin fusion protein (^{125}I-MAdCAM-Ig) have been disclosed[5] and are similar to the VLA-4 binding assay.
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14. The other combination of 2- and 3-pyridines leading to a pyridazinyl derivative was not made.
15. Although not fully investigated, these compounds most likely suffer from similar metabolism issues as already described. For a discussion, see ref 5c and: Kopka, I. E.; Young, D. N.; Lin, L. S.; Mumford, R. A.; Magriotis, P. A.; MacCross, M.; Mills, S. G.; Van Ripper, G.; McCauley, E.; Egger, L. E.; Kidambi, U.; Schmidt, J. A.; Lyons, K.; Stearns, R.; Vincent, S.; Colletti, A.; Wang, Z.; Tong, S.; Wang, J.; Owens, K.; Levorse, D.; Hagmann, W. K. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 637.