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Amidines as amide bond replacements in VLA-4 antagonists

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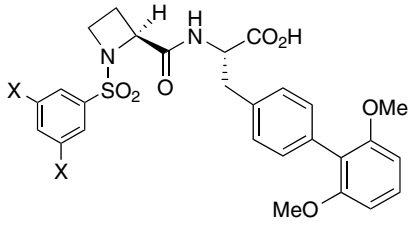
Abstract—VLA-4 ($\alpha_4\beta_1$, very late activating antigen-4), a key cell surface integrin plays an important role in inflammation by promoting leukocyte attachment and extravasation from the vasculature into the peripheral tissues. As such, VLA-4 antagonists may be useful in the treatment, prevention, and suppression of diseases where cell adhesion and migration are important such as asthma, rheumatoid arthritis, and multiple sclerosis. Herein, we report on the discovery, synthesis, and biological evaluation of amidines as small molecule antagonists of VLA-4.

The adhesion molecule VLA-4 (very late activating antigen-4, $\alpha_4\beta_1$, CD49d/CD29) is a member of the integrin family that is expressed on all circulating leukocytes except platelets.[1] VLA-4 is a receptor for VCAM-1 (vascular cell adhesion molecule-1) on the endothelium and the alternatively spliced CS-1 domain of fibronectin in the extracellular matrix. VCAM-1 expression is upregulated on endothelial cells by inflammatory cytokines and is highly expressed at sites of inflammation. The interaction between adhesion molecules on the leukocyte surface and specific counter receptors on the vascular endothelium may be important for the activation, migration, proliferation, and differentiation of leukocytes during normal and pathophysiological processes.[2] Monoclonal antibodies directed against the α_4-integrin have been shown to be effective in animal models of autoimmune and allergic diseases such as experimental autoimmune encephalomyelitis, collagen-induced arthritis, and eosinophil recruitment.[3] As such, VLA-4 antagonists may be useful in the treatment, prevention, and suppression of diseases where cell adhesion and extravasation are important such as multiple sclerosis, rheumatoid arthritis, and allergic asthma.

Previously, we reported *N*-phenylsulfonylproline biphenylalanine derivatives to be potent, specific VLA-4 antagonists.[4] Characteristic of this class of compounds are derivatives **1a**,**b** (Fig. 1). Despite their desirable potencies and specificities, compounds of this type (sulfonylated dipeptides) suffer from generally poor pharmacokinetic properties.

Typically, they have low oral bioavailability (F <10%) and very high plasma clearance in the rat (Cl_p >90 mL/



X = Cl (**1a**)	X = H (**1b**)
VLA-4: 0.08 nM	VLA-4: 0.10 nM
$\alpha_4\beta_7$: 11 nM	$\alpha_4\beta_7$: 78 nM
Cl_p: 102 mL/min/kg	Cl_p: 107 mL/min/kg
F%: 11	F%: 1.4
log D: 4	log D: 2.6

Figure 1. Potent amide VLA-4 antagonists.

Keywords: VLA-4; Integrin; Asthma; Inflammation.
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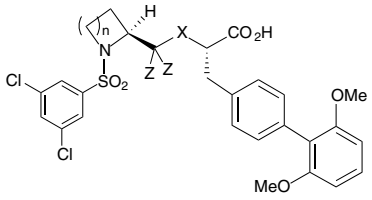
Table 1. Inhibition of VLA-4[a] by amide bond replacements (IC_{50}, nM)

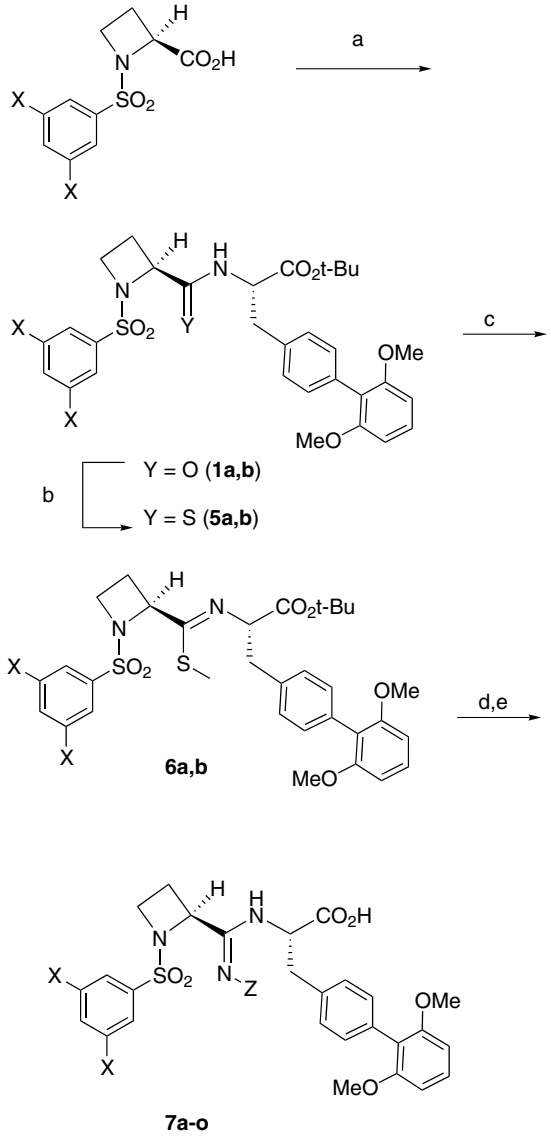


Number	*n*	X	Z	VLA-4[a]	$\alpha_4\beta_7$[b]
1a	1	NH	O	0.08	11
2	1	O	O	15	3000
3	1	NMe	O	350	ND[c]
4	2	NH	H,H	140	8400

[a] VCAM-Ig IC_{50} nM.
[b] MAdCAM-Ig IC_{50} nM.
[c] Not determined.

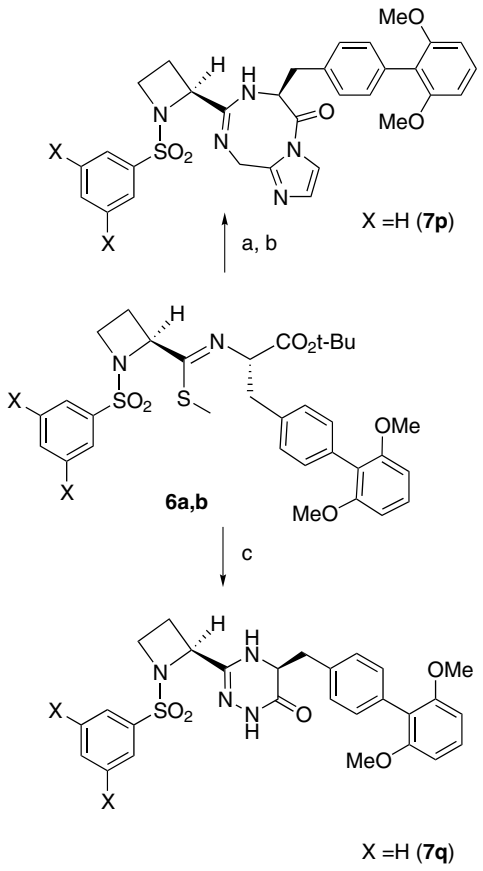
min/kg). We wondered if the poor pharmacokinetic properties of this class of compounds was the result of the amide bond and made efforts to remove or modify this group (Table 1). Replacing the amide bond with an ester group (**2**) led to a 200-fold drop in potency, while N-methylation (**3**) led to a 4000-fold drop. This clearly indicates the necessity of the NH group for potency. Removal of the amide carbonyl group as in **4** also leads to a 2000-fold drop in potency. Thus, the NH bond must also have the appropriate pK_a. The secondary amine may be too basic to engage in the necessary hydrogen bonding interaction or perhaps a conformational change in the molecule is responsible for the loss in potency. Nonetheless, it was still not known if the amide bond oxygen atom was needed, only that the amide NH was required as a hydrogen bond donor. We envisioned amidines as possible amide bond replacements with the hope that attenuation of the basicity of the group on nitrogen would have positive effects on potency and pharmacokinetics.

The synthesis of amidine derivatives is outlined in Schemes 1 and 2. Synthesis of **1a**,**b** has already been described.[4] Conversion of the amides into thioamides **5a**,**b** was accomplished with Lawessons reagent and S-methylation was best carried out by stirring with an excess of dimethyl sulfate in the presence of an inorganic base. Gram quantities of thioimidates **6a**,**b** were prepared in this manner. Exposure of the thioimidate to an amine nucleophile in the presence of base afforded the amidine substitution product and ester cleavage provided final products (Table 2). If the amine nucleophile contained another reactive site, side reactions were sometimes observed. For instance, when hydrazine was used, the cyclic product (**7q**) was obtained (Scheme 2). This cyclization not only occurred in the substitution reaction, but sometimes in the ester cleavage step, as evidenced by the formation of **7p**. The cyclized products are much less active against VLA-4 as the acid moiety is required for potency, however, this could potentially lend itself to a prodrug approach if the *N*-acyl bond were hydrolyzed in vivo.[4] The binding results are summarized in Table 2.



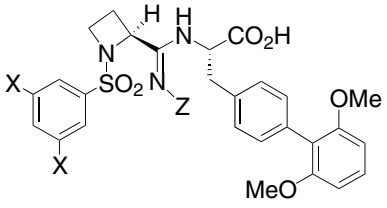
Scheme 1. (a) HATU, HOAt, 2,6-dimethoxybiphenyl alanine *tert*-butyl ester, *i*-Pr_2NEt, CH_2Cl_2; (b) Lawesson's reagent, $C_6H_5CH_3$, 70 °C; (c) K_2CO_3, Me_2SO_4, acetone, rt; (d) ZNH_2, Et_3N, MeOH, 50 °C; (e) TFA, CH_2Cl_2 [NB: for Z = SO_2Me (**7n**), amidine (**7b**) was treated with MsCl,TEA].

Interestingly, changing the oxygen atom in **1a** to an NH gives a compound (**7a**) with equal to better potency and specificity. This substitution confirms that the amide bond oxygen atom is not critical for potency, however, it does not negate the fact that the amide oxygen or amidine nitrogen may still act as a hydrogen bond acceptor. Despite the slight loss in potency (**7a**,**c** vs **7b**,**d**), the chlorine atoms were removed from the aryl sulfonyl group to reduce both molecular weight and lipophilicity, which might hopefully improve pharmacokinetics. A methyl group (**7c**,**d**) on nitrogen gives compounds equipotent to **1a** or **7a**, but larger alkyl groups lead to a slight loss in potency (**7g**–**m**). Regardless, it is remarkable the range of substituents that is tolerated while still maintaining sub-nanomolar potency. As the amide NH-bond is critical for potency, it was postulated that a



Scheme 2. (a) 2-Aminomethyl-1,3-imidazole, MeOH, Et_3N; (b) TFA, CH_2Cl_2; (c) hydrazine hydrochloride, MeOH, Et_3N.

Table 2. Inhibition of VLA-4[a] by N-sulfonylated-proline biphenyl-alanine derivatives: amidines (IC_{50}, nM)



Number	X	Z	VLA-4[a]	$\alpha_4\beta_7$[b]
7a	Cl	H	0.05	16
7b	H	H	0.10	19
7c	Cl	Me	0.05	21
7d	H	Me	0.12	40
7e	H	OH	0.14	98
7f	H	OMe	0.17	35
7g	H	CH_2CF_3	0.13	27
7h	H	$CH_2CH(CH_3)_3$	0.21	120
7i	H	CH_2CH_2OMe	0.28	65
7j	H	CH_2CO_2H	0.17	78
7k	H	$CH_2CH(CH_2CH_2O)_2$	0.28	43
7l	H	$CH_2$2-pyridyl	0.15	35
7m	H	$CH_2CH_2$1-triazole	0.16	69
7n	H	SO_2Me	0.07	2.2
7o	H	CN	0.09	4.1
7p	H	CH_2-2-imidazole–[c]	85% inh[d]	4090
7q	H	NH–[c]	58	ND[e]

[a] VCAM-Ig ligand.
[b] MAdCAM-Ig ligand.
[c] Cyclized product.
[d] At 10 μM.
[e] ND = not determined.

better hydrogen bond donor might provide for tighter binding. Hence, an electron withdrawing group on nitrogen should increase the acidity of the amidine NH and thus, **7n**,**o** were prepared. As anticipated, these were the two most potent amidines prepared bearing the simple phenyl sulfonyl residue. The amidines also maintain their roughly 100-fold specificity for VLA-4 versus $\alpha_4\beta_7$. The cyclized amidines (**7p**,**q**) were much less potent as it was already established that the acid residue is critical for potency.

The physical properties of the amidines as a class are interesting (Table 3). Despite the wide range of substitution patterns, log *D* proved difficult to modify.[5] Although relevant in the amide case (**1a** to **1b**), removing the two chlorine atoms from the phenyl sulfonyl ring in the amidines (**7c** to **7d**) did not grossly affect log *D*. Even highly basic amidines such as **7c**,**d** had similar log *D*'s to **1b**. This may be due to the fact that this small polar residue (amidine group) is buried in hydrophobic residues on both sides. Only when large hydrophobic groups are incorporated does the log *D* increase as observed for **7g**,**h**. Additionally, compounds with the same log *D* values (**7d** vs **7n**) could have very different basicities as indicated by their respective pK_a values. The two most potent amidines (**7n**,**o**) are, in fact, not basic at all based on their pK_a's and are physically more like the hydrophobic acid leads **1a**,**b**.

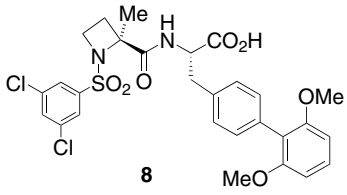
Table 3. Physical properties and rat pharmacokinetics of amidines

Number	log *D* (pH 7.3)	pK_a	$F^{a,b}$ (%)	Cl_p (mL/min/kg)
7a	4.1	—	0.3	90
7b	ND[c]	—	0.2	61
7c	2.7	10.2	0.8	85
7d	2.4	10.5	0.7	81
7e	2.3	—	ND	ND
7f	2.8	<4	9.3	79
7g	3.3	—	1.8	45
7h	3.7	—	0	64
7i	ND	—	0	81
7j	1.9	—	ND[d]	ND[d]
7k	2.6		0.6	67
7l	2.8	—	ND[d]	ND[d]
7m	2.4	—	0	111
7n	2.4	−2	1.6	103
7o	2.5	−2	3.0	93
7p	ND	—	0.6	382
7q	3.2	—	3.3	86

[a] Sprague–Dawley rats.
[b] Dose: 1 mg/kg i.v.; 2 mg/kg p.o.
[c] ND = not determined.
[d] Compound not stable.
[e] Cyclized product.

As a class, the amidines have poor pharmacokinetics in the rat characterized by high plasma clearances (60–100

mL/min/kg) and low oral bioavailability (0–10%), similar to the corresponding amides. Amidines **7g**,**h** may have reduced clearances due to their lipophilicity (higher log *D*'s) resulting from increased protein binding. Cyclic amidines **7p**,**q** also display poor pharmacokinetics. Whether this is an inherent property of the cyclic substrate or whether they are hydrolyzed to the acyclic acids in vivo and consequently suffer from the same problems as all the other amidines is uncertain.[5] Studies were undertaken to determine the reasons for the poor pharmacokinetic profile of compounds of this type (i.e., **8**, **7a**).[6] In a study with Wistar rats dosed with **8** and 1-aminobenzotriazole (1-ABT, a non-specific mechanism based inhibitor of P-450's), no change in AUC (plasma concentration vs time curves) or plasma clearance was observed.[7]



8

VLA-4: 0.07 nM
$\alpha_4\beta_7$: 14 nM
Cl_p: 110 mL/min/kg

However, dosing **8** along with cyclosporin (a known inhibitor of CYP3A, P-glycoprotein, and mrp-2)[8] led to a 5-fold drop in clearance rate as well as a 5-fold increase in AUC.[9] These results suggested that an active transport mechanism may be responsible for the high clearance rate of **8** and compounds in this class. In a parallel study using a mutant strain of Wistar transporter deficient rats (TR^- rats are deficient in the organic ion transporter mrp-2) with **8**, a similar 5-fold drop in clearance and 5-fold increase in AUC was observed supporting the cyclosporin results.[10] Such dramatic changes in pharmacokinetics were not only observed for **8**, but for other amides as well. Interestingly, the pharmacokinetics of amidine **7a** in Wistar rats ($Cl_p = 49$ mL/min/kg) were no different from that in TR^- rats ($Cl_p = 58$ mL/min/kg) implying that the organic ion transporter mrp-2 may not be responsible for the clearance of these amidines in rats.[11] These results may prove useful in the development of a compound with improved and acceptable pharmacokinetics.

In summary, we have successfully identified potent and specific non-amide containing VLA-4 antagonists. While ester and amine substitutions failed, amidines were equipotent if not more so than their corresponding amides. A variety of substitution patterns are acceptable allowing one to fine tune physical properties such as log *D* and pK_a. Compounds such as **7b** and **7g** are potent examples, which have slightly better pharmacokinetic profiles than **1a** and **8**. Additionally, the amidines as a class appear not to suffer from the same clearance mechanism (mrp-2) experienced by the amides. This may have advantages in developing an oral VLA-4 antagonist when active transporters such as mrp-2 are involved.

References and notes

1. Hemler, M. E.; Elices, M. J.; Parker, C.; Takada, Y. *Immunol. Rev.* **1990**, *114*, 45.
2. Elices, M. J. *Curr. Opin. Anti-inflamm. Immunol.* **1999**, *1*, 15.
3. (a) Kent, S. J.; Karlik, S. J.; Cannon, C.; Hines, D. K.; Yednock, T. A.; Fritz, L. C.; Horner, H. C. *J. Neuroimmunol.* **1995**, *58*, 1; (b) Zeidler, A.; Brauer, R.; Thoss, K.; Bahnsen, J.; Heinrichs, V.; Jablonski-Westrich, D.; Wroblewski, M.; Rebstock, S.; Hamann, A. *Autoimmunity* **1995**, *21*, 245; (c) Pretolani, M.; Ruffie, C.; Lapa E Silva, J.-R.; Joseph, D.; Lobb, R. R.; Vargaftig, B. B. *J. Exp. Med.* **1994**, *180*, 795.
4. Hagmann, W. K.; Mumford, R. A.; Durette, P. L.; Lanza, T.; de Laszlo, S.; Kopka, I.; Lin, L.; Chang, L.; Truong, Q.; Wilson, J.; Li, B.; Kamenecka, T.; Yang, G.; MacCoss, M.; Mills, S.; Kevin, N. J.; Van Riper, G.; Egger, L.; Schmidt, J. A.; Lyons, K.; Vincent, S.; Tong, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2709.
5. Plasma levels of the corresponding acyclic acids were not determined. Although acyl glucuronidation of some acids was observed in vivo, this was not the major contributor to the high plasma clearance rates.
6. The α-methyl group in **8** was initially installed to prevent amide hydrolysis, but compounds lacking this steric protection suffered relatively little hydrolysis anyways.
7. (a) Ortiz de Montellano, P. R.; Mico, B. A.; Mathews, J. M.; Kunze, K. L.; Miwa, G. T.; Lu, A. Y. H. *Arch. Biochem. Biophys.* **1981**, *210*(2), 717; (b) Mico, B. A.; Federowitz, D. A.; Ripple, M. G.; Kerns, W. *Biochem. Pharmacol.* **1988**, *37*(13), 2515.
8. For a review of P-glycoprotein and mrp-2 (multidrug resistance associated protein-2), see: Van Zuylen, L.; Nooter, K.; Sparreboom, A.; Verweij, J. *Invest. New Drugs* **2000**, *18*(3), 205–220.
9. (a) Gutmann, H.; Fricker, G.; Torok, M.; Michael, S.; Beglinger, C.; Drewe, J. *Pharm. Res.* **1999**, *16*(3), 402; (b) Gutmann, H.; Fricker, G.; Drewe, J.; Toeroek, M.; Miller, D. S. *Mol. Pharmacol.* **1999**, *56*(2), 383.
10. Paulusma, C. C.; Elferink, R. P. O. *J. Mol. Med.* **1997**, *75*(6), 420.
11. Elferink, R. P. O.; Jansen, P. L. *Pharmacol. Ther.* **1994**, *64*(1), 77.