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Sulphonamide-Based Small Molecule VLA-4 Antagonists

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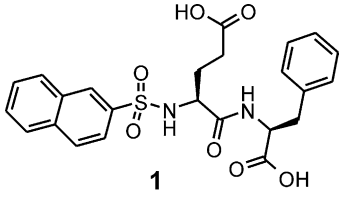
Abstract—The discovery of a sulphonamide by-product with VLA-4 antagonistic activity led to a series of potent, small molecule VLA-4 antagonists. Synthesis, SAR and in vivo evaluation of the selected compound will be presented.

Integrins comprise a large family of cell-surface receptors that bind to the extracellular matrix proteins, such as fibronectin (FN), vitronectin, fibrinogen, collagen, laminin and invasin, and to the cell-surface ligands such as vascular cell adhesion molecule-1 (VCAM-1) or intercellular adhesion molecules (ICAMs) to mediate cell-matrix and cell-cell interactions. These interactions are important for the regulation of cell migration. Integrins are large membrane glycoproteins consisting of two subunits, α and β. VLA-4 (CD49d/CD29), which consists of α_4 and β_1 subunits, is a member of β_1 or VLA subfamily. It is expressed on monocytes, T- and B-lymphocytes, basophils and eosinophils.[1] VLA-4 mediates both the initial tethering as well as firm adhesion of cells to the walls of the inflamed vessels through the interaction with cytokine-inducible vascular cell adhesion molecule VCAM-1.

VLA-4 antagonists inhibit leukocyte migration into lung tissues during inflammatory responses and lymphocyte trafficking. Hence, these agents may reduce tissue damage caused by inflammation and could potentially be effective therapies for asthma. Animal studies also indicate that antagonists of α_4-containing integrins may have utility in the treatment of other inflammatory diseases, including multiple sclerosis, rheumatoid arthritis, heart failure and inflammatory bowel disease.[2–6]

VLA-4 has received considerable attention as a target for the design of small molecule anti-inflammatory therapeutics.[7] Our initial effort to identify a potent VLA-4 antagonist was based on the belief that we could mimic the LDV motif in the CS-1 segment of FN[8] or the IDS motif in the C-D loop of VCAM-1[9] using our peptide secondary structure mimetic scaffolds.[10]



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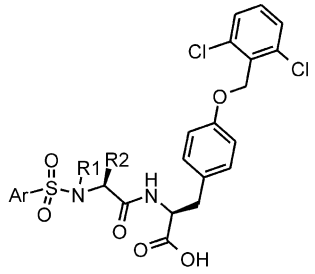
However, during the course of our work we discovered that the 2-naphthylsulphonyl amide **1**, a minor byproduct in a tested crude material, was a potent VLA-4 antagonist



2, Ar=2-naphthyl, R1=H, R2=$CH_2CH_2CO_2H$
3, Ar=2-naphthyl, R1=Me, R2=$CH_2CH_2CO_2H$
4, Ar=Ph, R1=Me, R2=$CH_2CH_2CO_2H$
5, Ar=Ph, R1=Me, R2=CH_2CO_2H
6, Ar=Ph, R1=CH_2CO_2H, R2=H
7, Ar=4-F-Ph, R1=CH_2CO_2H, R2=H
8, Ar=2-pyridinyl, R1=CH_2CO_2H, R2=H
9, Ar=2-thienyl, R1=CH_2CO_2H, R2=H

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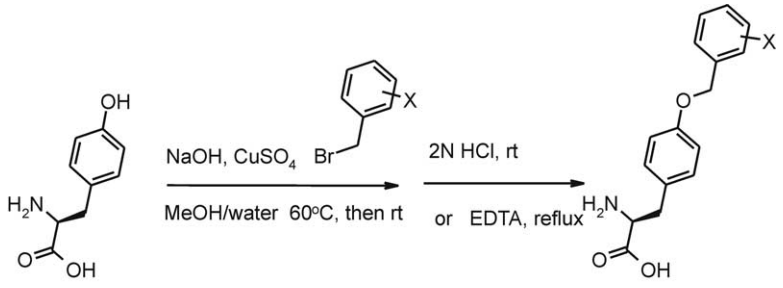
with an IC_{50} of 180 nM in the RAMOS/CS-1 cell-adhesion assay.[11] This discovery prompted us to further explore a series of sulphonylated dipeptides. Herein, we disclose the SAR and the identification of the potent VLA-4 antagonist **6** that is orally available in cynomologous monkey and efficacious in the murine model of asthma.

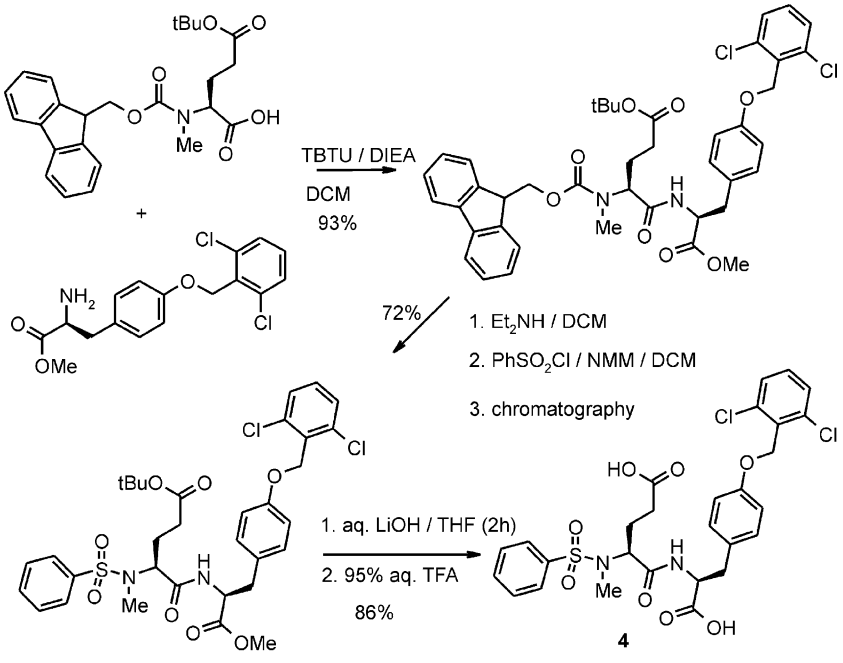
One of the early modifications involved the introduction of a *para*-substituent at the phenylalanine aromatic ring. Several groups have reported[12] a dramatic potency enhancement achieved by linking an aryl group to that position. The obvious choice was to utilize tyrosine to introduce diversity at that site. This selection was motivated by the ease of synthesis of analogues, for example, by alkylation (Scheme 1) or Mitsunobu reactions. We favored using an ether linkage at that position (vs amide or ester linkage) due to the benign nature (i.e., resistance to esterases or amidases) and that could be advantageous in terms of 'drug-likeness'. To our satisfaction, the introduction of the 2,6-dichlorobenzyloxy moiety led to compound **2** which was 90-fold more potent than **1**.

In the attempt to reduce the number of hydrogen-bond donors and to restrict some of the flexibility of the molecule, we synthesized compound **3** which is the *N*-methyl sulfonamide analogue of compound **2**. This compound displayed a 2-fold increase in activity over compound **2**. Importantly, the replacement of the naphthyl moiety in **3** by a smaller, phenyl substituent produced compound **4** that was 10-fold more potent (IC_{50} 0.1nM) than **3**. To ascertain the stereoselectivity of the interaction the compound with D-isomer at the tyrosine moiety was also synthesized and was found to be 50-fold less active than the L-isomer (**4**) at that position. The synthesis of compound **4** (Scheme 2) exemplifies the methodology we had used in the synthesis of analogues (Table 1).

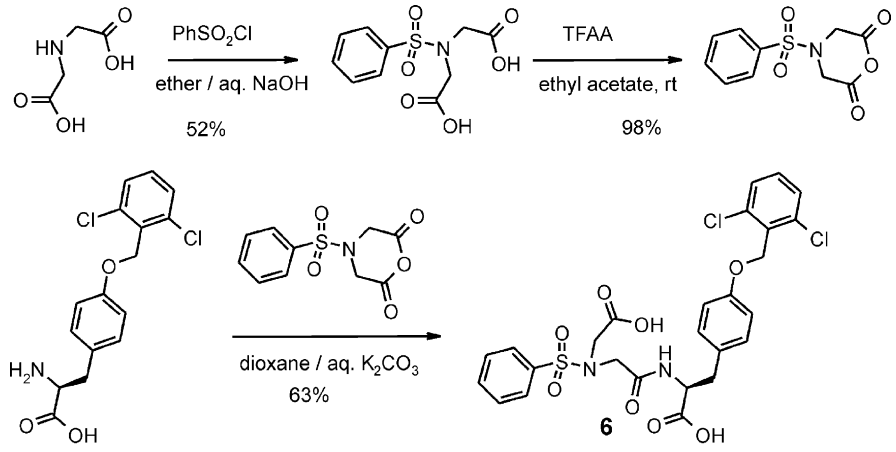
In further optimization we investigated the influence of the proximity of the N-terminal carboxyl group to the phenyl moiety on the activity. The replacement of the glutamic acid with the aspartyl moiety led to compound **5** and a significant decrease in potency.



Scheme 1.



Scheme 2.



Scheme 3.

Table 1. IC_{50} data for the selected compounds in the cell adhesion assay[11] utilizing VLA-4-expressing Ramos cells and immobilized, biotinylated CS-1 fragment

Compd	Ramos/CS-1 IC_{50} (nM)	Ramos/CS-1 with 10%FBS IC_{50} (nM)
1	180	ND
2	2	ND
3	1	ND
4	0.1	2
5	2	ND
6	0.6	8
7	0.7	10
8	1.5	10
9	0.8	10

FBS, fetal bovine serum; ND, not determined.

Table 2. Compound **6** inhibition of eosinophil infiltration in the murine asthma model[13]

	Saline	OVA	in-5	in-10	po-10	po-25	DEX-ip-1
EOS count (1.0×E5)	0.01	8.73	6.26	4.36	2.67	2.16	3.79
EOS inhibition (%)	—	—	28	50	69	75	57

Table 3. PK data of **6** in cynomologous monkey

	Cmax (ng/mL)	$t_{1/2}$ (min)	CL (mL/min/kg)	Vdss (L/kg)	*F* (%)
2	528 (iv) 101 (po)	6.6	58.8	0.303	26.9

Interestingly, transposition of the aspartic acid side chain to the sulphonamide nitrogen produced compound **6** with the potency almost in the same range as for compound **4**. Additional advantages of compound **6** are the elimination of one center of chirality and simplification of the synthesis (see Scheme 3). Several other analogues (i.e., **7**, **8**, and **9**) in which the sulphonamide aryl group was varied, were also prepared and were found to be almost equipotent to compound **6**. This was a pleasant discovery since one can potentially utilize this part of the molecule to improve the PK profile without compromising the potency of the compound.

Compound **6** displayed sufficiently good in vitro ADME properties (pH, plasma and microsomal stability, as well as no inhibition of several CYP450 isoforms up to 50 μM) to warrant in vivo efficacy study in murine asthma model and pharmacokinetic study in primate. The murine asthma model (with sensitization by ovalbumin and dexamethasone as a control) data is shown in Table 2. Compound **6** reduced eosinophil influx by 50% at 10 mpk (in) and by 69% at 10 mpk (po). It also reduced leukocyte infiltration into BALF by 39 and 59%, respectively (data not shown). The pharmacokinetic study in cynomologous monkey (Table 3) revealed that although **6** had acceptable oral availability, it suffered from high clearance and a very short elimination-phase half life, both of which were detrimental to further in vivo study in monkey.

In summary, we have developed SAR around the sulphonamide chemotype of VLA-4 antagonists and devised an expedient synthesis of the most promising compound (**6**) in the series. This compound has shown good in vivo efficacy as indicated by inhibition of eosinophil and leukocyte lung infiltration in the murine asthma model.

References and Notes

1. Hemler, M. *Annu. Rev. Immunol.* **1990**, *8*, 365.
2. Lobb, R. R.; Abraham, W. M.; Burkly, L. C.; Ma, W.; Knight, J. A.; Leone, D. R.; Antognetti, G.; Pepinsky, R. B. *Ann. N.Y. Acad. Sci.* **1996**, *796*, 113.
3. Yusf-Makagianasar, H.; Andersen, M. E.; Song, X.; Murray, J. S.; Siahaan, T. J. *Curr. Top. Biochem. Res.* **2000**, *2*, 33.
4. Gonzalez-Amaro, R.; Portales-Perez, D.; Baranda, L.; Redondo, J. M.; Martinez-Martinez, S.; Yanez-Mo, M.; Garcia-Vicuna, R.; Cabanas, C.; Sanchez-Mardrid, F. J. *Immunol.* **1998**, *161*, 65.
5. Furuzawa-Carballeda, J.; Alcocer-Varela, J. *Scand. J. Immunol.* **1999**, *50*, 215.
6. Allison, M. C.; Poulter, L. W. *Clin. Exp. Immunol.* **1991**, *85*, 504.
7. For a review, see: (a) Adams, S. P.; Lobb, R. R. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed., Academic: New York, 1999; Vol. 34, p 179. (b) Lobb, R. R.; Adams, S. P. *Exp. Opin. Invest. Drugs* **1999**, *8*, 935. (c) Holland, G. W.;

Biediger, R. J.; Vanderslice, P. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic: New York, 2002; Vol. 37, p 65.
8. Komoriya, A.; Green, L. J.; Mervic, M.; Yamada, S. S.; Yamada, K. M.; Humphries, M. J. *J. Biol. Chem.* **1991**, *266*, 15075.
9. Wang, J. H.; Pepinsky, R. B.; Stehle, T.; Liu, J.-H.; Karpusas, M.; Browning, B.; Osborn, L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5714.
10. (a) For a review, see: Kim, H.-O.; Kahn, M. *Res. Adv. In Organic Chem.* **2000**, *1*, 43. (b) Kim, H.-O.; Kahn, M. *Comb. Chem. High Throughput Screening* **2000**, *3*, 167.
11. Assay conditions were developed based on the publication by Vanderslice, P.; Ren, K.; Revelle, J. K.; Kim, D. C.; Scott, D.; Bjercke, R. J.; Yeh, E. T. H.; Beck, P. J.; Kogan, T. P. *J. Immunol.* **1997**, *158*, 1710. BiotinGPEILDVPST-amide was allowed to bind to black NeutrAvidin-coated, 96-well plates (Pierce #15117) for 1 h at rt at the concentration of 10 μg/mL, 100 μL/well, in the following assay buffer: 50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$. The plate was washed three times with water and blocked overnight with 200 μL of the same buffer plus 3% BSA. The plate was washed the same way just prior to use. RAMOS cells were incubated with Calcein-AM (Molecular Probes) in the dark in DPBS for 1 h prior to use at 10 million cells/mL, then spun down and resuspended in assay buffer at 500,000 cells/mL with 5 μM ethidium homodimer. Cells were incubated at 37 °C in the presence or absence of drugs for 30 min at 37 °C and cell lysis was assessed by fluorescence at 535/617 nM. Unbound cells were rinsed free with three gentle additions of assay buffer followed by quick inversion. Cells were then lysed with 50 μL/well of lysis solution: 1% Nonidet P-40, 50 mM Tris (pH 7.5), 5 mM EDTA, and remaining cells were quantified by fluorescence at 485/535 nM. Cyclic hexapeptide (with disulfide bond) CWLDVC was used as a control, with a reproducible IC_{50} of 100 nM.
12. (a) Chen, L.; Tilley, J. W.; Guthrie, R. W.; Mennona, F.; Huang, T.-N.; Kaplan, G.; Trilles, R.; Miklowski, D.; Huby, N.; Schwinge, V.; Wolitzky, B.; Rowan, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 729. (b) Hagmann, W. K.; Durette, P. L.; Lanza, T.; Kevin, N. J.; de Laszlo, S. E.; Kopka, I. E.; Young, D.; Magriotis, P. A.; Li, B.; Yang, G.; Kamenecka, T.; Chang, L. L.; Wilson, J.; MacCoss, M.; Mills, S. G.; Van Riper, G.; McCauley, E.; Egger, L. A.; Kidambi, U.; Lyons, K.; Vincent, S.; Steans, R.; Colletti, A.; Teffera, J.; Tong, S.; Fenyk-Melody, J.; Owens, K.; Levorse, D.; Kim, P.; Schmidt, J. A.; Mumford, R. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2709. (c) Porter, J. R.; Archibald, S. C.; Childs, K.; Critchley, D.; Head, J. C.; Linsley, J. M.; Parton, T. A. H.; Robinson, M. K.; Shock, A.; Taylor, R. J.; Warrellow, G. J.; Alexander, R. P.; Langham, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1051. (d) Kopka, I. E.; Lin, S. L.; Mumford, R. A.; Lanza, T.; Magriotis, P. A.; Young, D.; de Laszlo, S. E.; MacCoss, M.; Mills, S. G.; Van Riper, G.; McCauley, E.; Lyons, K.; Vincent, S.; Egger, L. A.; Kidambi, U.; Steans, R.; Colletti, A.; Teffera, J.; Tong, S.; Owens, K.; Levorse, D.; Schmidt, J. A.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2415.
13. The measurement of eosinophil influx into broncoalveolar lavage (BAL) was done as follows: BALB/C female mice received compound **6** on day 15, 18 and 21 at 15 min before OVA challenge. Twenty-four hours after final OVA challenge, the mice underwent exsanguinations by posterior vena cava puncture and then BAL (0.4 mL of saline three times) was performed on the right lung after tying of the left lung at the mainstem bronchus. Total BAL fluid cells were counted from a 0.05 mL aliquot and the remaining fluid was centrifuged at 1200 rpm (Microcentrifuge, 5415 C, Eppendorf) for 5 min. The cell pellets were resuspended in saline containing 10% BSA (Calbiochem, San Diego, CA, USA) and smears were made on glass slides. Eosinophiles were stained for 5–8 min with Discombe's diluting fluid containing (0.05% aqueous eosin and 5% (v/v) acetone in distilled water, and then counterstained with 0.07% methylene blue.