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Heterocycle-substituted proline dipeptides as potent VLA-4 antagonists

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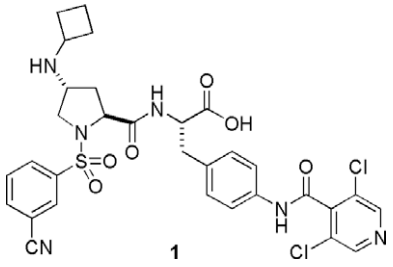
ABSTRACT

A variety of N-linked tertiary amines and heteroarylamines were examined at the 4-position of sulfonylated proline dipeptides in order to improve VLA-4 receptor off-rates and overcome the issue of CYP3A4 time-dependent inhibition of ester prodrugs. A tight-binding inhibitor **5j** with a long off-rate provided sustained receptor occupancy despite poor oral pharmacokinetics.

The adhesion molecule VLA-4 ($\alpha_4\beta_1$; CD49d/CD29; 'very late antigen-4') is a member of the integrin family and is expressed on all circulating leukocytes except platelets.[1] VLA-4 binds to vascular cell adhesion molecule-1 (VCAM-1) which is expressed on activated endothelial cells and is upregulated in response to inflammatory cytokines. The specific interaction between VLA-4 on leukocytes and VCAM-1 on the vascular endothelium may be required for the activation, migration, proliferation, and differentiation of leukocytes during normal and pathophysiological processes.[2] Inhibition of VLA-4 may therefore be effective in preventing recruitment and infiltration of cell types required for a prolonged inflammatory response. Indeed, monoclonal antibodies and blocking peptides against the α_4-integrin have been shown to be effective in animal models of asthma,[3] rheumatoid arthritis,[4] and multiple sclerosis.[5] The humanized α_4 monoclonal antibody natalizumab (Tysabri, Biogen Idec/Elan) was recently approved for the treatment of multiple sclerosis and inflammatory bowel disease.[6]

Previous communications from our laboratories have detailed the development of small molecule inhibitors of VLA-4.[7] Compound **1** is representative of a class of sulfonylated dipeptides that exhibit strong potency against both the activated and resting states of VLA-4.[8] Extensive SAR led to identification of the 3-cyanobenzenesulfonyl proline group coupled to a *N*-isonicotinoyl-(L)-4-aminophenylalanine unit as an optimal scaffold for potency against VLA-4.[8] Additionally, the presence of a basic amine at the 4-position of the proline ring is crucial for minimizing plasma protein binding.



1

In general, this class of inhibitors suffers from poor oral bioavailability and high plasma clearance in preclinical species. Despite the poor pharmacokinetics of **1**, it was anticipated that its slow off-rate (90% bound at 1 h) from the VLA-4 receptor might lead to a sustained receptor occupancy (RO) in vivo and the potential for a prolonged pharmacological effect. Indeed, after a 5 mpk oral dose of **1** to rats, 67% RO was observed at 12 h. Furthermore, administration of the ethyl ester prodrug of **1** under the same parameters gave higher RO (77%) of the acid at 12 h.[8] The ester, however, was found to be a time-dependent inhibitor of CYP3A4 which may pose a risk for clinical drug–drug interactions.[9]

Oxidative dealkylation of the secondary amine on proline was thought to be a key step in a pathway leading to the species responsible for CYP3A4 time-dependent inhibition (TDI).[10] Our strategy to minimize this event was to replace the secondary alkyl amine of **1** with either a tertiary amine or an N-linked aromatic

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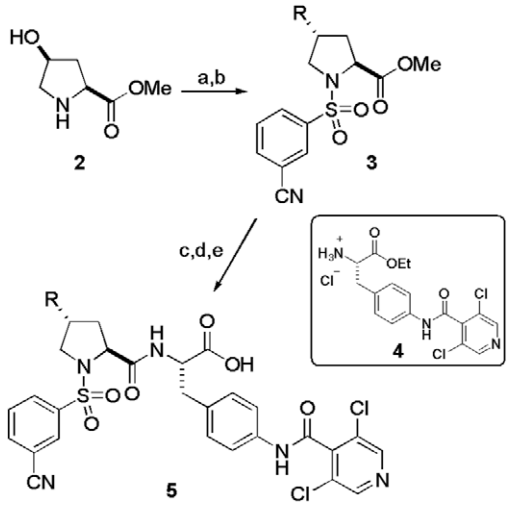


Scheme 1. Reagents and conditions: (a) 3-cyanobenzenesulfonyl chloride, NEt_3, CH_2Cl_2; (b) (i) Tf_2O, iPr_2NEt, CH_2Cl_2, −78 °C, (ii) amine (R), −30 °C to rt; (c) (i) LiOH, $MeCN/H_2O$, (ii) HCl; (d) **4**, HATU, NMM, DMF; (e) LiOH, $MeCN/H_2O$.

heterocycle, thus slowing dealkylation and reducing TDI. Herein, we describe our efforts to identify potent VLA-4 antagonists with slow receptor off-rates and high receptor occupancy, and whose ethyl ester prodrugs have low risk of TDI.[11]

Our general synthetic approach to compounds **5a**-**s** is outlined in Scheme 1. After N-sulfonylation of *cis* (4*S*)-hydroxyproline methyl ester, the alcohol was converted to the triflate and reacted with heteroarylamines or secondary amines to provide products **3**.[12] Hydrolysis of the methyl ester was carried out with LiOH and the resulting acid was coupled to *N*-isonicotinoyl-(L)-4-aminophenylalanine ethyl ester **4** to give the sulfonylated dipeptide ethyl esters. Conversion to the carboxylic acids was readily achieved through hydrolysis with lithium hydroxide.

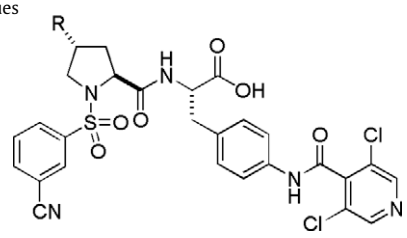
Acids **5a**–**s** were tested for potency in an in vitro binding assay against the resting state of human VLA-4.[13,14] The effect of plasma protein binding was evaluated by repeating the assay in the presence of 90% human plasma. Finally, the corresponding ethyl esters were counter-screened for TDI of CYP3A4.

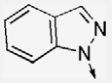
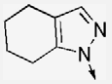
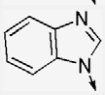
Table 1 summarizes the results for heteroaryl-substituted analogues. Tetrazole **5d** and benzimidazole **5g** displayed the strongest binding affinity to VLA-4 with IC_{50} values of 80 pM. With the exception of indazole **5e**, all compounds in this series had reduced TDI relative to secondary amine-substituted proline derivatives.

We next examined cycloalkylamine substitution on proline and the effect of ring size on inhibition of VLA-4. As illustrated in Table 2, compounds **5h–l** exhibited subnanomolar inhibition of VLA-4 and, with the exception of eight-membered ring analogue **5l**, had minimal potency shift in the presence of 90% plasma. The greater hydrophobicity of the cyclooctylamine group in **5l** likely results in higher plasma protein binding and the observed potency shift. Among this group, piperidine **5j** had the best overall profile with no plasma shift in the binding assay and the lowest TDI value for a corresponding ethyl ester.

Building on this result, a number of additional N-linked six-membered ring heterocycles were attached to the proline core. Morpholine **5m** and thiomorpholine **5n** along with methylpiperidine compounds **5o–s** were all very potent in the VLA-4 binding

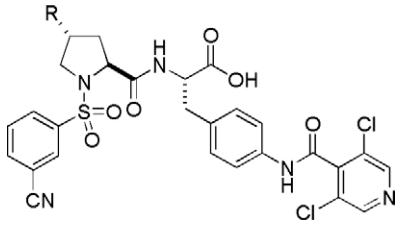
Table 1
Inhibition of VLA-4 by heteroaryl-substituted proline analogues

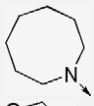
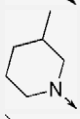
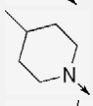
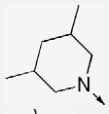
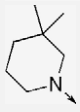


Compd	R	VLA-4[a] (IC_{50}, nM)	90% Plasma[b] (IC_{50}, nM)	TDI[c] (min^{-1})
5a		0.22	0.48	0.009
5b		0.81	100% 100 nM	0.012
5c		0.55	93% 62.5 nM	0.007
5d		0.08	100% 100 nM	0.008
5e		0.25	2.01	0.038
5f		0.35	4.75	0.016
5g		0.08	0.50	0.019

[a] Competitive binding assay against the resting state of VLA-4.
[b] See Ref. 8 for a description of this assay.
[c] Values for CYP3A4 time-dependent inhibition (k_{obs}, 10 μM) were determined from the ethyl ester.

Table 2
Effect of ring size and substitution on inhibition of VLA-4



Compd	R	VLA-4[a,b] (IC_{50}, nM)	90% Plasma[b] (IC_{50}, nM)	TDI[c] (min^{-1})
5h		0.34	0.42	0.022
5i		0.16	0.22	0.024
5j		0.11	0.12	0.016
5k		0.42	0.45	0.021
51		0.25	0.81	0.023
5m		0.13	0.15	0.020
5n		0.13	0.23	0.002
5o[d]		0.11	0.20	0.002
5p[d]		0.13	0.17	0.019
5q		0.16	0.17	0.009
5r[d]		0.14	0.31	0.019
5s		0.16	0.41	0.011

[a] Competitive binding assay against the resting state of VLA-4.
[b] See Ref. 8 for a description of this assay.
[c] Values for CYP3A4 time-dependent inhibition (k_{obs}, 10 μM) were determined from the ethyl ester.
[d] Mixture of diastereomers

assay with minimal shifts in the presence of 90% plasma. Additionally, the ethyl esters of all the compounds in this group had favorable TDI rates of 0.020 min^{-1} or lower. Thiomorpholine **5n** and 2-methylpiperidine **5o** had particularly low TDI values, suggesting that oxidative dealkylation to a primary amine may be mitigated with these substructures.

A receptor off-rate assay was utilized to further differentiate between these numerous potent, non-plasma shifted compounds.[14] These results are presented as the percentage of compound bound to receptor after 3 h and are shown in Table 3. Compounds **5j**, **5p**, and **5s** emerged as the tightest binding antagonists as they remained 85% bound to receptor after 3 h.

The subnanomolar potency, absence of plasma shift, and slow off-rate of **5j**, in addition to reduced TDI risk for its ethyl ester (**5j–Et**), highlights the desirable profile of this acid/ester pair. We utilized a rat receptor occupancy model to evaluate the potential for target engagement in vivo.[14] As shown in Figure 1, a 5 mpk oral dose of acid **5j** resulted in receptor occupancy of nearly 80% at 12 h post-dose, decreasing to 20% at 24 h. The same dose of prodrug **5j–Et** significantly improved receptor occupancy to >90% at 12 h and remained at 60% after 24 h.

Plasma levels of **5j** were determined in systemic circulation and in the portal vein after oral dosing of **5j** or **5j–Et**. Table 4 shows the systemic and portal vein C_{max} of acid **5j** after a 5 mpk oral dose of **5j**

Table 3
Off-rate determination for selected compounds

Compd	Off-rate[a] (%)
5d	10
5g	35
5i	65
5j	85
5k	60
5m	15
5n	34
5o	38
5p	85
5q	67
5r	72
5s	85

[a] Bound at 3 h; see Ref. 8 for a description of this assay

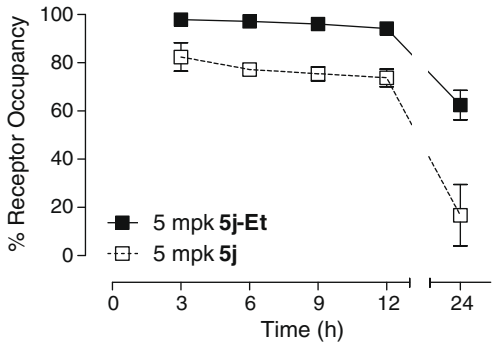


Figure 1. Rat receptor occupancy of **5j** from dosing of acid **5j** or ethyl ester prodrug **5j–Et**

Table 4
Rat pharmacokinetics of **5j**

Compound dosed (5 mpk po)	Systemic C_{max} (nM)	Portal vein C_{max} (nM)
Acid **5j**	3	245
Ester **5j–Et**	12	397

or prodrug **5j–Et** in rats. Levels of **5j** are substantially higher in the portal vein than in systemic circulation. This is critical as we believe that the slow off-rate and initial exposure of **5j** to lymphocytes bearing VLA-4 in the portal vein combine to provide the observed high levels of receptor occupancy in rats.

In summary, tertiary heterocycle-substituted proline dipeptides were shown to be potent antagonists of VLA-4. Acid **5j** had the optimal profile, displaying excellent potency and a slow off-rate from human VLA-4. The ester prodrug **5j–Et** had reduced TDI of CYP3A4 and gave sustained (12–24 h) receptor occupancy when dosed to rats. We have recently characterized the intrinsic and pharmacologic properties of this compound against equine VLA-4.[15] Results described therein suggest that **5j** may have utility for the treatment of recurring airway obstruction in horses.

Acknowledgment

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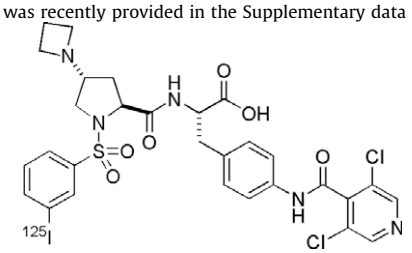
11. We targeted k_{obs} (10 μM) values of less than 0.020 min^{-1} for TDI by ethyl ester prodrugs. By comparison, the TDI rate for the ethyl ester of **1** was 0.040 min^{-1}.
12. For discussions on the regioselectivity of 1,2,4-triazole and tetrazole alkylation, see: (a) Bulger, P. G.; Cottrell, I. F.; Cowden, C. J.; Davies, A. J.; Dolling, U.-H. *Tetrahedron Lett.* **2000**, *41*, 1297; (b) Ulhaq, S.; Chinje, E. C.; Naylor, M. A.; Jaffar, M.; Stratford, I. J.; Threadgill, M. D. *Bioorg. Med. Chem. Lett.* **1998**, *6*, 2139.
13. We have previously demonstrated that this class of compounds generally has a higher affinity for the activated state of VLA-4. Thus, potency was examined only against the resting state of VLA-4 to best differentiate compounds.
14. The [^{125}I]-tracer shown below was utilized to assess binding potency, receptor off-rate, and rat receptor occupancy. A detailed description of each of these experiments was recently provided in the Supplementary data of Ref. 8.



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