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Discovery and Evaluation of Potent, Tyrosine-based α4β1 Integrin Antagonists

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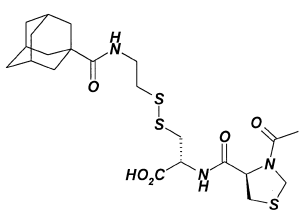
Abstract—Using disulphide cysteine-based inhibitors as lead structures, this communication describes our strategy for identifying more stable, potent antagonists of the α4β1 integrin. These studies ultimately discovered potent, low molecular weight inhibitors based on D-thioproline-L-tyrosine.

Introduction

The integrin α4β1 is currently a major target for the design of novel therapeutic treatments for chronic inflammatory diseases such as asthma, IBD, MS and arthritis.[1,2] In the previous communication,[3] we described our discovery of low and sub-micromolar antagonists of the α4β1 integrin against one of its ligands, the endothelial surface protein vascular cell adhesion molecule (VCAM). These inhibitors are disulphide derivatives of cysteine and the most potent analogue was the L-thioproline-L-cysteine derivative **1**. We were concerned that the chemical and metabolic instability of the disulphide group would preclude their development as therapeutic drugs. We therefore sought to identify analogues that were more stable. This communication describes the discovery of the tyrosine-derivative **2** a stable inhibitor of the α4β1 integrin possessing low nanomolar potency and good efficacy in an animal model of inflammation.



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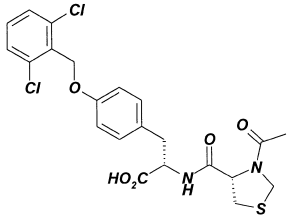

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Results and Discussion

Having previously established the importance of the *N*-acetyl-L-thioproline residue in conveying activity against the α4β1 integrin, we decided to derivatize this moiety with a number of α-amino acids. The desired products were readily prepared using the corresponding amino-ester under standard peptide coupling conditions (EDC, NMM, DMF) followed by ester hydrolysis (aqueous LiOH). Table 1 displays some of the prepared compounds and their activity in protein-based, ligand binding[4] and cell-based, adhesion[5] assays.

One of the early derivatives, the thioether derivative **3**, showed reduced activity (IC_{50} 8 μM in the cell-based assay) compared to the disulphide **1**, but was investigated further since it represented a more stable lead. However, subsequent studies in which the *S*-thioether substituent was varied did not afford any analogues with improved activity. The phenylalanine analogue **4**, showed a modest level of potency, but the unsubstituted tyrosine analogue **5** was much less active. Interestingly, when the phenolic oxygen of **5** was methylated **6** much of the activity was regained. Recognising that none of these analogues were accessing the same region of space as the adamantylcarbonyl group in the lead compound **1** we synthesised the *O*-benzylated tyrosine analogue **7** which afforded improved potency in both the protein and cell based assays.

An important breakthrough was the discovery that an 'L to D' switch in the stereochemistry of the thioproline residue afforded a magnitude improvement in activity (**8**, Table 2).

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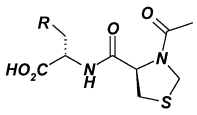
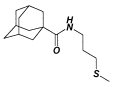
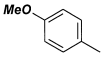
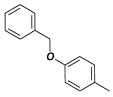
Table 1.



Compound	*R*	α4β1/VCAM protein assay IC_{50} (nM)[4]	α4β1/VCAM cell assay IC_{50} (nM)[5]
3		90	8040
4		360	35250
5		2740	263000
6		310	76570
7		220	19520

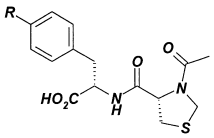
When the analogous stereochemical switch was performed on the tyrosine residue in a related analogue a decrease in activity of two orders of magnitude was observed.

We proceeded by preparing analogues based on *N*-acetyl-D-thioproline-L-tyrosine (Table 2). The adamantyl-carbonyl **9** and benzoyl **10** analogues were synthesised since the adamantyl group and ester linkage were present in the lead compound **1**. Both of these ester derivatives showed significant improvement in activity relative to **8**. However, rat plasma stability studies[6] revealed they were readily hydrolysed, with half-lives of 2.3 and 1.6 h respectively whereas the ether derivative **8** was stable. In an attempt to stabilise the benzoyl analogue **10** from presumed esterase attack, the 2,6-dichlorobenzoyl derivative **11** was prepared. Not only did this afford plasma stability ($t_{1/2}$ >12 h) but also potency was improved particularly in the cell-based assay. Completing a logical sequence of modifications, the stable 2,6-dichlorobenzyl derivative **2** was prepared which exhibited low nanomolar activity in both protein and cell-based assays.

Further SAR work around the benzyl ether, Table 3, showed that the type of substituent was not crucial, the 2,6-dimethoxy **13** and the 2,6-difluoro **14** analogues being essentially equipotent with the 2,6-dichlorobenzyl ether **2**. A substituent in the 4-position could also be accommodated as shown by the 2,4,6-trichloro derivative **16**. However, a loss of potency, particularly in the cell based assay, was observed with monosubstitution (the 2-trifluoromethyl analogue **15**) and 3,5-disubstitution (the 3,5-dichloro analogue **17**).

Cross-screening against a panel of other integrins showed that compound **2** was very selective for α4β1, with the exception of the related integrin α4β7 where it displayed a 5-fold selectivity, Table 4.

Table 2.



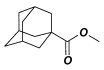
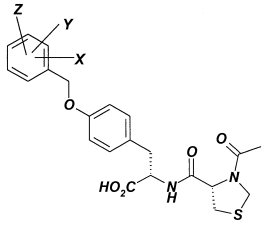
Compound	*R*	α4β1/VCAM protein assay IC_{50} (nM)[4]	α4β1/VCAM cell assay IC_{50} (nM)[5]	Stability in plasma half-life (h)[6]
8		25	1480	>12
9		12	140	2.3
10		4	170	1.6
11		6	24	>12
2		5	34	>12

Table 3.



Compound	X	Y	Z	α4β1/VCAM protein assay IC_{50} (nM)[4]	α4β1/VCAM cell assay IC_{50} (nM)[5]
2	2-Cl	6-Cl	H	5	35
13	2-MeO	6-MeO	H	2	40
14	2-F	6-F	H	3	60
15	2-CF_3	H	H	15	170
16	2-Cl	4-Cl	6-Cl	9	35
17	3-Cl	5-Cl	H	17	150

Sheep that are naturally sensitised to *Ascaris suum* exhibit an early and late phase bronchoconstriction followed by an airway hyperresponsiveness to inhaled methacholine. It has been shown that this antigen induced bronchoconstriction and airway hyperresponsiveness can be inhibited by either α4 antibodies or α4β1 antagonists.[7] Intravenous administration of compound **2** (0.03, 0.3, 3.0 mg/kg in basic saline), 1 h prior to

Table 4.

Assay (IC_{50} μM)	α4β1 cell	αIIbβ3 cell	α5β1 cell	αLβ2 cell	αMβ2 cell	αVβ3 cell	α4β7 cell
Compound **2**	0.035	>400	>800	>200	>400	>800	0.19

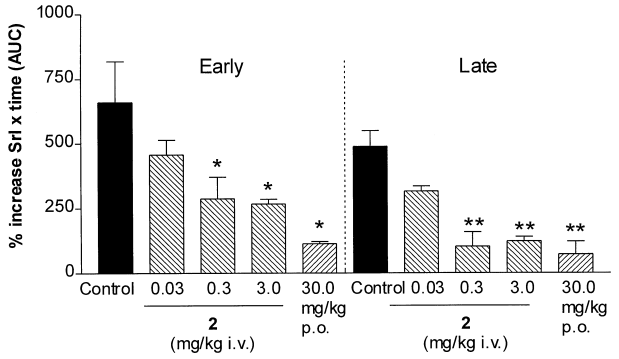


Figure 1. Area under curves (AUC): antigen induced early and late phase responses in allergic sheep: inhibition by compound **2**. $n=3$ each group. Compound **2** given intravenously (iv) 1 h prior to antigen challenge. Compound **2** given orally (po) 2 h prior to antigen challenge. * $P<0.05$, ** $P<0.01$ Students' *t*-test.

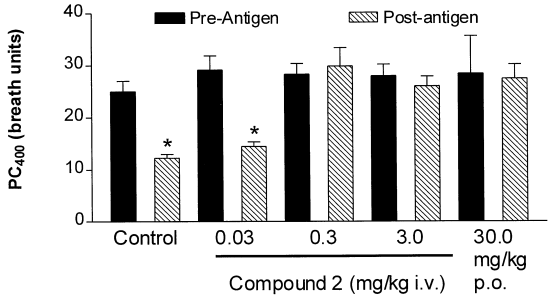


Figure 2. Antigen induced airway hyperresponsiveness to methacholine in allergic sheep: inhibition by compound **2**.$n=3$ each group. Compound **2** given intravenously (iv) 1 h prior to antigen challenge. Compound **2** given orally (po) 2 h prior to antigen challenge. * $P<0.05$, ** $P<0.01$ Students' *t*-test.

antigen challenge, in this model caused a dose related inhibition of both early (0–4 h) and late (4–8 h) phase bronchoconstriction (Fig. 1) as measured by the area under the curves (AUC) of the percentage increase in specific lung resistance (Srl) multiplied by time. Airway hyperresponsiveness was measured by the amount of methacholine required to induce a 400% increase in Srl pre- and post-antigen challenge. The expression of airway hyperresponsiveness was completely prevented at doses of compound **2** of 0.3 and 3.0 mg/kg (Fig. 2). When administered orally, 2 h prior to antigen challenge, compound **2** (30 mg/kg) caused a marked inhibition of both early and late phase bronchoconstriction (Fig. 1) and blocked the expression of airway hyperresponsiveness (Fig. 2).

In conclusion we have identified a potent and selective antagonist of the integrin α4β1 which shows efficacy in an animal model of airway inflammation. Further modifications and structural diversification of the VLA4 antagonist **2** have since been performed and will form the basis of future publications.

References and Notes

1. For recent reviews see (a) M. Lobb, R. R.; Adams, S. P. *Exp. Opin. Invest. Drugs* **1999**, *8*, 935. (b) Zimmerman, C. N. *Exp. Opin. Ther. Patents* **1999**, *9*, 129
2. (a) Bochner, B. S. In *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*: Mousa, A. S., Ed.; Springer-Verlag and R. G. Landes Co., 1998; pp 113–131. (b) Elices, M. J. In *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*: Mousa A. S., Ed.; Springer-Verlag and R. G. Landes Co., 1998; pp 133–147.
3. Archibald, S. C.; Head J. C.; Linsley, J. M.; Porter J. R.; Robinson, M. K.; Shock, A.; Warrellow, G. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 993.
4. α4β1 (from HL60 lysate) was immobilised on a plate with a non-blocking anti-β1 antibody (TS2/16). The test compounds were titrated into a solution of 2-domain VCAM-Fc-Ig in a separate plate and added to the wells. The assay was carried out in TBS, 1% BSA, 1 mM $MnCl_2$, 0.1% Tween. After incubation for 2 h at room temperature the plates were washed and residual VCAM visualised with peroxidase coupled anti-human Fc.
5. A Jurkat cell line expressing α4β1 was incubated at 37 °C for 30 min with human 2-domain VCAM-1-FC immobilised on a plate with anti-human FC in the presence of the test compounds. The plates were washed and residual cells were stained with Rose Bengal.
6. Test substances were incubated in rat plasma at room temperature at initial concentrations of 10, 1 and 0.1 μM. After initial mixing and at several timepoints, 50 μL aliquots were taken and added to 200 μL acetonitrile. An internal standard was added and the extracts were analysed by LC-MS/MS. Half-lives were calculated from exponential regressions through the timepoints.
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