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

Sarah C. Archibald, John C. Head, Janeen M. Linsley, John R. Porter,* Martyn K. Robinson, Anthony Shock and Graham J. Warrellow

Celltech Chiroscience, 216 Bath Road, Slough SL1 4EN, UK

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Abstract—Acyclic, disulphide derivatives of cysteine have been identified as moderately potent antagonists of α4β1-mediated leukocyte cell adhesion to VCAM. This communication describes how they were discovered from a simple L-cystine derivative and using the structure–activity data of C*DThioPC* related cyclic peptides.

Introduction

α4β1 (VLA-4, very late antigen-4, CD49d/CD29) is a member of the integrin family, a group of cell surface receptors mediating cell-cell and cell-matrix interactions.[1] The integrin, α4β1 is expressed on most leukocytes including lymphocytes, monocytes, eosinophils, and basophils although absent, or in low levels, on neutrophils.[2] The former group of leukocytes play an essential role in chronic diseases of autoimmune origin and α4β1 is believed to be involved in the cell adhesion, migration and activation of these cell-types at sites of inflammation.[1,2] Importantly, anti-α4 antibodies have shown efficacy in a number of animal models of chronic inflammatory diseases including asthma, rheumatoid arthritis, and multiple sclerosis.[3] Hence, small molecule α4β1 antagonists represent an attractive target for the therapeutic treatment of chronic inflammatory diseases, particularly asthma.

The primary ligands for α4β1 are the endothelial surface protein vascular cell adhesion molecule (VCAM)[4] and the extracellular matrix protein fibronectin (FN).[5] α4β1 appears to recognise key sequences within these ligands, namely QIDS in VCAM[7a] and LDV in the alternatively spliced connecting segment (CS-1) of FN.[6] X-ray crystal structures of domains 1 and 2 of VCAM have been reported,[7] showing a surface exposed loop between the C and D β-strands containing the QIDS sequence. The aspartic acid (D) residue present within these sequences is postulated to bind to a magnesium cation held within the ligand-binding region of α4β1,[8] the structure of this region is unclear but Springer has proposed a 7 bladed beta-propeller model for the α chain subunit.[9]

A number of cyclic peptide antagonists of α4β1 have been reported in the literature,[10] either based on the LDV or RGD motifs and cyclised through cysteine disulphide linkages. For example, the cyclic peptide R*CDThioPC*[11] has activity against α4β1 but it also binds to the related integrin α5β1 (VLA-5), presumably because of its origin with the RGD sequence. Replacing the arginine residue with lipophilic groups led to more potent and selective analogues, for example the cyclic tetrapeptide (adamantylcarbonyl)-C*DThioPC*-OH **1**. This paper describes our initial SAR studies based on this compound and the subsequent identification of simplified, potent α4β1 antagonists.

Results and Discussion

Activity data in a cell-based α4β1/VCAM[12] assay for the cyclic tetrapeptide (adamantylcarbonyl)-C*DThio PC*-OH **1** and some key analogues are shown in Table 1. Substituting the thioproline residue of **1** with proline **2** was found to give approximately a 4-fold drop in activity in the cell-based assay with VCAM, though the homoproline analogue **3** is significantly more active than **1**. Interestingly, replacing the aspartic acid residue of **2** with alanine, to give **4** does not appreciably affect activity, while modification of the carboxylic acid group of the cysteine residue in **4** to the primary amide **5** gave a drop in potency of two orders of magnitude. This

*Corresponding author: Tel.: +44-1753-534655; fax: +44-1753-536632; e-mail: jporter@celltech.co.uk

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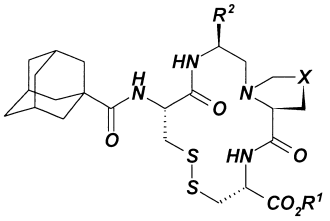
Table 1.



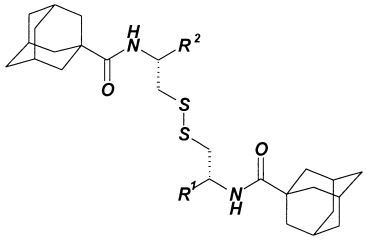
Compound	R^1	R^2	X	α4β1/VCAM IC_{50} (nM)[12]
1	H	CH_2CO_2H	S	47
2	H	CH_2CO_2H	CH_2	170
3	H	CH_2CO_2H	CH_2CH_2	12
4	H	CH_3	CH_2	330
5	NH_2	CH_3	CH_2	41000

clearly established which of the carboxylic acid groups in **1** was important for binding and this residue presumably mimics the key aspartic acid residue in the recognition sequence of α4β1′s ligands.

Although cyclic peptides have been developed as therapeutic drugs they generally suffer from poor pharmacokinetics and rapid metabolism. Indeed, plasma stability studies on **1** revealed rapid degradation ($t_{1/2}=3$ min). Although depeptidisation and stabilising modifications were considered, we took the view that this approach to identifying a drug candidate would be both demanding and without sufficient guarantee of success. Instead we attempted to determine which were the key pharmacophoric groups in a cyclic peptide such as **4** and use this information to design simpler, acyclic structures more amenable to structure–activity studies.

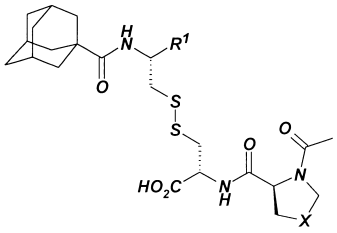
During the initial experiments intended to address this matter we isolated *N*,*N*-diadamantylcarbonyl-L-cystine (**6**), Table 2, as a by-product. This compound displayed significant activity (α4β1/VCAM cell IC_{50} 7.3 μM) prompting further investigation. As expected, the analogue **7**, Table 2, in which both acid groups are esterified is devoid of activity. The mono-acid mono-ester **8** however, has a slightly improved potency relative to the

Table 2.



Compound	R^1	R^2	α4β1/VCAM IC_{50} (nM)[12]
6	CO_2H	CO_2H	7300
7	CO_2Me	CO_2Me	>800000
8	CO_2H	CO_2Me	5100
9	CO_2H	H	4100

Table 3.



Compound	R^1	X	α4β1/VCAM IC_{50} (nM)[12]
10	CO_2Me	CH_2	25200
11	H	CH_2	2340
12	H	S	280
13	H	CH_2CH_2	425

disulphide **6**. More interestingly, the analogue **9** in which the one of the carboxylic acid groups is removed completely is also slightly more active and represents a significantly simplified structure.

The inhibitors **6**–**9** bear a clear relationship to the potent cyclic peptides **1**–**5** with the essential cysteine-derived carboxylic acid common to both structures and the proximal adamantylcarbonyl group of the acyclic analogues mimicking the (thio)proline residues of the cyclic peptides. Support for this idea was delivered by making the appropriate proline acyclic analogues, Table 3.

The first analogue prepared, **10**, was 6-fold less active than its adamantyl counterpart **8**. It was postulated that **10** could not adopt the same configuration as **8** due to a steric clash between the *N*-acetyl proline group and the methyl ester and the analogue **11** lacking the ester group proved to be more active (α4β1/VCAM cell assay IC_{50} 2.3 μM). Incorporation of thioproline to give **12**, a modification that gave enhanced potency in the cyclic peptide series, was also significantly more active in this series as well (α4β1/VCAM cell assay IC_{50} 280 nM). Interestingly, replacement of the thioproline with homoproline to give **13** brought a reduction in activity, suggesting that the orientation of the two amide carbonyl groups adjacent to the proline moiety is optimum in **12**.

We subsequently prepared a number of analogues in which the adamantyl carbonyl and thioproline groups were varied but none of these were more active than **12**. Cross screening against the integrins α5β1 and αIIb/βIIIa showed that these compounds were selective (IC_{50} >800 and 400 μM respectively) for α4β1.

Conclusions

Starting from a cyclic peptide and by a process of defining the key pharmacophoric groups we have identified a series of sub-micromolar antagonists of α4β1 with a much simplified structure. As the disulphide moiety in these compounds was expected to be insufficiently stable (chemically and metabolically) to allow further development of this class of inhibitors we next

set out to identify more stable and potent antagonists of α4β1 integrin. This work is described in the following communication.

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