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Discovery and Evaluation of *N*-(triazin-1,3,5-yl) Phenylalanine Derivatives as VLA-4 Integrin Antagonists

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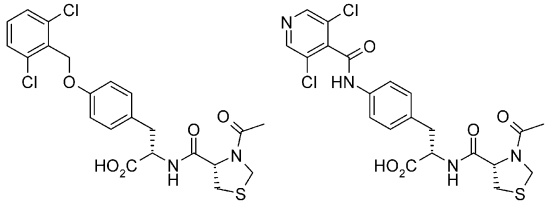
Abstract—SAR studies aimed at improving the rate of clearance of a series of VLA-4 integrin antagonists by the introduction of a 1,3,5-triazine as an amide isostere are described.

The integrin very late antigen-4 (VLA-4) is a heterodimeric ($\alpha4\beta1$) adhesion molecule, expressed on the surface of many leukocytes, including T-lymphocytes and eosinophils, but not neutrophils. The binding of VLA-4 to ligands such as vascular cell adhesion molecule-1 (VCAM-1) expressed on endothelial cells is recognised as a key step in the processes of adhesion, migration and activation of inflammatory leukocytes at sites of inflammation.[1] Blocking such an interaction would be expected to be of therapeutic benefit in a variety of inflammatory and autoimmune diseases. Anti-$\alpha4$ antibodies have shown efficacy in animal models of a number of inflammatory diseases and the results from early stage clinical trials are promising.[2] Thus, small molecule VLA-4 receptor antagonists represent an attractive target for the treatment of various chronic inflammatory diseases such as asthma, multiple sclerosis and rheumatoid arthritis.[3]

We have recently described the discovery of the thioproline CT5219,[4] **1**, a small molecule VLA-4 antagonist that showed excellent efficacy in inhibiting antigen-induced airway responses in the allergic sheep model. The potency of **1** could be further enhanced by the replacement of the 2,6-dichlorobenzyl ether with a 3,5-dichloropyridylamide group, **2**. Depeptidisation by replacement of the thioproline group with a benzamide or pyridyl amide as in, for example, compound **3** also gave a series of potent compounds. Disappointingly, these compounds displayed rapid clearance in a number of species thereby hindering further development. That this rapid clearance was a result of an active transport mechanism was shown by the presence in the bile of only trace amounts of metabolites compared to the parent compounds.[5]



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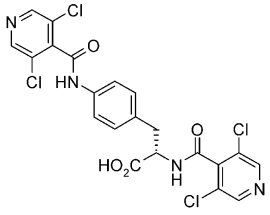

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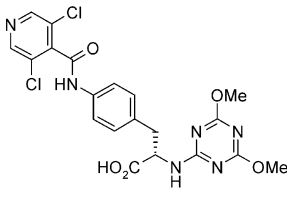

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We postulated that the α-amido group, although apparently essential for potency, was responsible for the rapid clearance in this series. In an attempt to move away from the lead acyl derivatives but retain the features believed necessary for inhibitory activity, we envisaged incorporating the carbonyl of the α-aminocarbonyl

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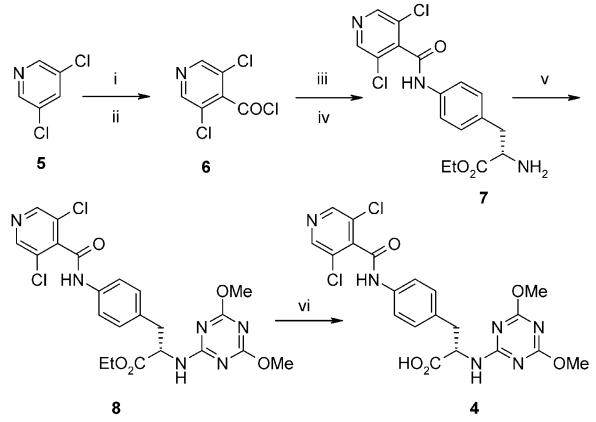
group into a heterocycle, to obtain *N*-(triazin-1,3,5-yl)-phenylalanine derivatives such as **4**. In this way, we retained a degree of structural and electrostatic similarity but also ensured that the α-amino group remained non-basic and also possessed a hydrogen atom. These features were felt to be necessary for potent VLA-4 antagonists based on a number of earlier structure–activity studies which included attempts to replace the α-aminocarbonyl group with isosteres. These included CH_2NH, $–CH_2O–$, –CH=CH– and *N*-methylation of the α-aminocarbonyl. All such substitutions lead to a significant reduction in activity.

In order to gain some understanding of the SAR for clearance it was considered essential to have access to a relatively high throughput screen that could provide this data. The method chosen was the isolated perfused rat liver[6] (IPRL), whereby five compounds (including a reference compound) could be dosed as a cassette. The elimination of each compound from the perfusate is expressed in terms of a rate constant, *k*, and normalised to the reference compound. The higher the value of *k* the more rapidly the compound was cleared. Compounds were assayed for their ability to inhibit the binding of VLA-4 to VCAM-1 in a protein-based, ligand binding[7] and a cell-based, adhesion[8] assay.

We opted, in the first instance, to retain the dichloropyridyl amide derivatised analogues of phenylalanine for the core of our structures. Incorporation of this group had given consistently the most potent VLA-4 antagonists in other series. The compounds were readily prepared as outlined in Scheme 1. The lithiated 3,5-dichloropyridine **5** was quenched with CO_2 to generate the carboxylic acid that was converted to the acid chloride **6** by treatment with thionyl chloride. Coupling of **6** to the α-*N*-Boc protected ethyl ester of 4-aminophenylalanine in the presence of base gave, after removal of the Boc group, the amine **7**. The amino group of this intermediate **7** could then be reacted with a range of reactive, commercially available heterocycles as shown, for example with 1,3-dimethoxy-5-chloro triazine to give the ester **8**. Aqueous LiOH hydrolysis of this ester afforded the target compound **4**.



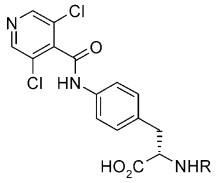
Scheme 1. (i) LDA, THF, −78 °C, 30 min then CO_2; (ii) $SOCl_2$, DCM; (iii) α-*N*-Boc 4-aminophenylalanine ethyl ester, *N*-methyl morpholine, DCM; (iv) 3 M HCl in EtOAc; (v) 1,3-dimethoxy-5-chloro triazine, DIPEA (1 equiv), DCM; (vi) LiOH, THF, H_2O.

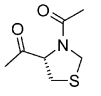
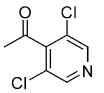
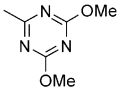
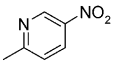
Table 1 shows some of these compounds and compares their activities against VLA-4 and their rate of clearance with the amides **2** and **3**. Although the potencies of the *N*-heteroaryl derivatives **4**, **9** and **10** are much reduced compared to the *N*-acyl derivatives **2** or **3**, there is a marked improvement (reduction) in their rate of bilary clearance. We initially focused on optimising the *N*-triazin-1,3,5-yl derivative **4**, because of the combination of reasonable potency, low rate of clearance, promising DMPK and ease of synthesis of analogues of this type of compound. The triazine **4** displayed a clearance of 3 mL/min/kg, a $t_{1/2}$ of 1.6 h and 9% bioavailability in the rat (10 mg/kg po and iv).

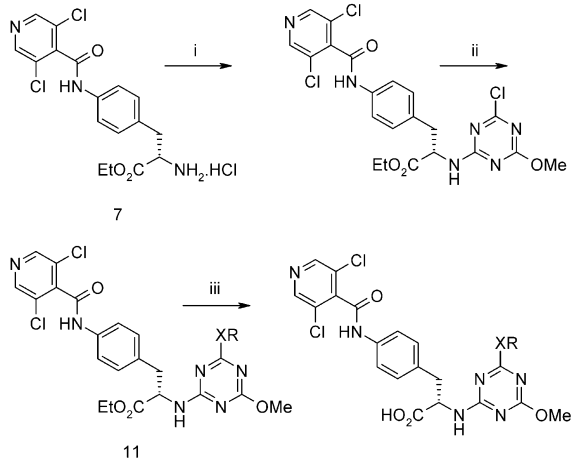
Further derivatisation of the triazines was achieved by condensation of the appropriate 1,3-dialkoxy-5-chloro triazine with the amino ester **7** in a similar fashion to that shown in Scheme 1. Alternatively, condensation of 1,3-dichloro-5-methoxy triazine with the amino ester **7** followed by displacement of the remaining chloro atom with either amines or alcohols gave the differentially substituted triazines **11** as shown in Scheme 2. In both routes the esters were hydrolysed under aqueous conditions to generate the target acids.

The resulting data for some of the prepared analogues is given in Table 2. It is evident that a wide range of alkoxy and amino groups are tolerated with regard to potency and it was possible to improve potency 3–4 fold

Table 1. Potency and rate of clearance for lead compounds

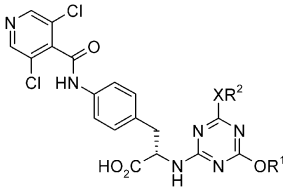


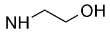
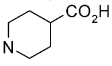
Compd	R	VLA-4 protein[7] IC_{50} (nM)	VLA-4 cell[8] IC_{50} (nM)	IPRL k (h^{-1})
2		0.6	0.5	3.7
3		0.9	0.5	3.9
4		8	900	1.1
9		80	1700	2.1
10		260	2500	1.0



Scheme 2. (i) 1,3-Dichloro-5-methoxy triazine, DIPEA (2 equiv), DCM; (ii) RXH, NaH, THF or RNH_2, DIPEA, DCM; (iii) LiOH, H_2O, dioxan, H^+.

Table 2. Potency and rate of clearance for triazine compounds

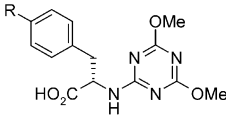


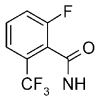
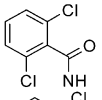
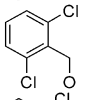
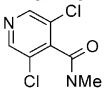
Compd	R^1	XR^2	VLA-4 protein[7] IC_{50} (nM)	VLA-4 cell[8] IC_{50} (nM)	IPRL k (h^{-1})
13	*n*Pr	OnPr	50	740	0.2
14	Me	OnPr	45	300	2.3
15	Me	OPh	30	320	0.6
16	Me	NMe_2	16	590	1.1
17	Me	NEt_2	70	1620	nd
18	Me	NHnPr	25	740	2.4
19	Me		7	320	1.8
20	Me		14	510	nd
21	Me		25	1000	2.1
22	Me		6	240	2.1

(with respect to activity in the cell-based assay) in the best examples (**14**, **15**, **19** and **22**). Notably all analogues exhibited the sought-after, low rates of clearance, with some compounds displaying exceptionally low values (for example, **13** and **15**). In general the more lipophilic the substituents the better the potency and the lower the rate of clearance though this may be a result of the enhanced plasma protein binding of these compounds. Interestingly the incorporation of polar functionality, for example the carboxylic acid **22**, also has a profound effect on potency whilst retaining a reasonable rate of clearance suggesting that the SAR for both properties in this region of the molecule is quite complex.

The advantage of the dichloropyridyl amide derivatised analogues of phenylalanine was demonstrated by the

Table 3. Potency and rate of clearance for phenylalanine substituents



Compd	R	VLA-4 protein[7] IC_{50} (nM)	VLA-4 cell[8] IC_{50} (nM)	IPRL k (h^{-1})
23		50	4600	0.6
24		30	4000	0.7
25		1500	nd	2.2
26		5500	nd	nd

preparation of analogues where this group was replaced with structurally similar moieties (Table 3). In all cases potency was lost when compared to the parent **4**, although the rates of clearance remained similar, suggesting that this feature is not key for recognition by the active transporter. The amide in this series appears to be crucial as replacing it with an ether, for example compound **25**, effectively abolishes potency. It is also interesting to note the effect of methylating the amide, compound **26**, also gives significantly less potency. Possibly the combination of conformational twist induced by the two *ortho* substituents and the presence of the relatively non-basic pyridyl nitrogen is optimal for these compounds.

This work, and additional studies employing parallel synthesis, failed to identify any analogues with potencies less than 100 nM in the cell-based assay. Potencies of this order were considered appropriate to show efficacy in animal models and consequently this series was not developed further. However, we had demonstrated that it was possible to identify compounds that had reasonable potencies in blocking the adhesion of VLA-4 to VCAM-1, yet were not subject to rapid clearance in the rat. Our efforts in optimising the pyrimidinyl and pyridine series are described in the following communication.

Acknowledgements

This paper is dedicated to the memory of Alex Zomaya.

References and Notes

1. (a) Elices, M. J. In *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*; Mousa, S A., Ed.;

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5. Unpublished data.
6. Frink, E. J., Jr.; Kramer, T. H.; Banchy, S. M.; Brown, B. R. *Anesth. Analg.* **1990**, *71*, 484.
7. VLA4 (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 two-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.
8. A Jurkat cell line expressing VLA4 was incubated at 37 °C for 30 min with human two-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.