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N-(Pyrimidin-4-yl) and *N*-(Pyridin-2-yl) Phenylalanine Derivatives as VLA-4 Integrin Antagonists

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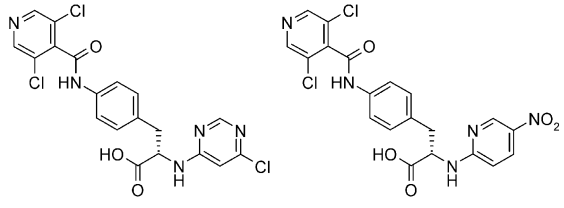
Abstract—The SAR studies to optimise both potency and rate of clearance in the rat for a series of pyrimidine and pyridine based VLA-4 antagonists are described.

The integrin very late antigen-4 (VLA-4) is a heterodimeric ($\alpha 4\beta 1$) adhesion molecule, expressed on the surface of many leukocytes. 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. Consequently the design of VLA-4 antagonists is currently a major target for the pharmaceutical industry.[2]

We have recently reported on our initial discovery of *N*-acyl derivatives of phenylalanine that afforded highly potent antagonists of VLA-4 binding to VCAM-1.[3] However, virtually all such analogues suffered from rapid bilary clearance from the body by an as yet undetermined active transport mechanism. Postulating that the central amide bond was playing a key role in the recognition of these structures by the transporter, we sought to replace this functionality with a suitable isostere. The preceding communication[4] describes our efforts to incorporate the amide carbonyl group into a heterocycle system to give *N*-(triazin-1,3,5-yl)-phenylalanine derivatives. These compounds displayed much reduced clearance, but initial analogues possessed only modest potency as VLA-4 antagonists. We also described the *N*-(pyrimidin-4-yl) and *N*-(pyridin-2-yl) phenylalanine lead structures, **1** and **2**. This communication outlines the SAR studies aimed at optimising the potency and rate of clearance on this series of compounds.



1 **2**

Our initial structure–activity study focused on optimising the *N*-pyrimidin-4-yl compound **1**. Once again we opted to retain the dichloropyridyl amide derivatised analogues of phenylalanine for the core of our structures and to explore the effect of introducing substituents in the 2- and 6-positions of the pyrimidine. Compounds in this series were prepared by condensation of the appropriately substituted pyrimidine with the 3,5-dichloropyrid-4-ylcarboxamido substituted phenylalanine ester **3**, followed by aqueous LiOH hydrolysis of the ester to give the target acid **5** as shown in Scheme 1. 4-Chloro or sulphonylalkyl substitutents on the pyrimidine were found to be acceptable leaving groups for this transformation, and where not commercially available were prepared as shown in Scheme 2. Additionally, where a 2- or a 6-sulphonyl functionality was required this group could be introduced by mCPBA oxidation of the ester **4** (where R = thioalkyl).

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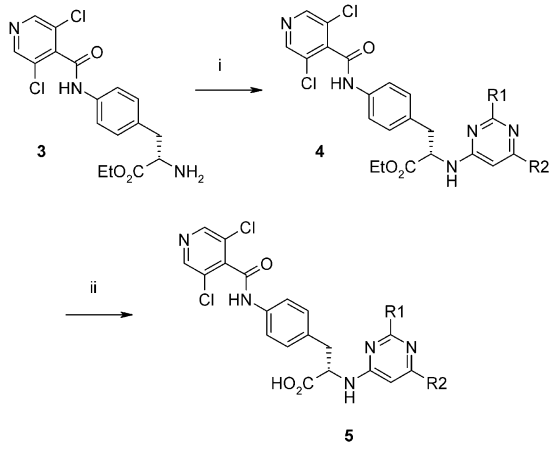
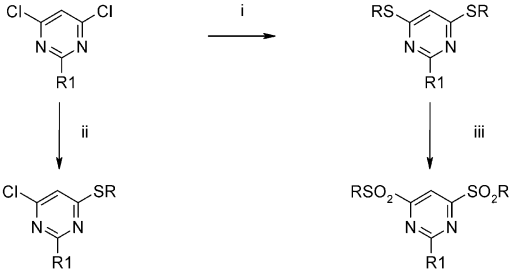


Scheme 1. (i) Pyrimidine, DIPEA, DCM; (ii) LiOH, water, dioxan.

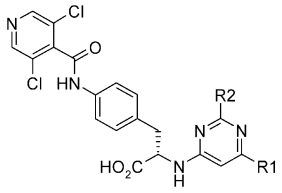


Scheme 2. (i) NaSR (2 equiv), EtOH; (ii) NaSR (1 equiv), EtOH; (iii) mCPBA, DCM.

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[5] (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[6] and a cell-based, adhesion[7] assay.

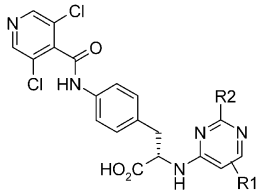
Initially we looked at a range of 6-substituted pyrimidines as shown in Table 1. Disappointingly, although these compounds were more potent in the cell based assay than the lead compound, **1**, they all displayed relatively rapid rates of clearance. However, the greatly improved potency of the sulphonyl derivative **7** was striking and prompted the synthesis of a number of sulphonyl analogues. In general an alkylsulphonyl substituent afforded high potency in either the 2- or 6-pyrimidine position, and the larger alkylsulphonyl group was more active than the methylsulphonyl analogue (compare **7** with **11** and **15** with **16**). Interestingly, the sulphinyl analogue **8** is equipotent to the sulphonyl equivalent **7**. However, despite the variation in the physicochemical properties (logP, pK_a) of these *N*-pyrimidinyl analogues, all such compounds displayed relatively rapid clearance; for example compound **7** had a clearance of 27 mL/min/kg, a $t_{1/2}$ of 0.5 h and an oral bioavailability of 4% after dosing in the rat (10 mg/kg po and iv).

Table 1. Potency and rate of clearance for 2- and/or 6-substituted pyrimidines



Compd	R1	R2	VLA-4 protein[6] IC_{50} (nM)	VLA-4 cell[7] IC_{50} (nM)	IPRL k (h^{-1})
1	Cl	H	80	1700	2.1
6	*n*PrS	H	60	460	nd
7	*n*PrSO$_2$	H	1	40	4.4
8	*n*PrSO	H	6	50	4.3
9	PhCH$_2$	H	40	1100	6.4
10	PhO	H	20	520	3.1
11	MeSO$_2$	H	6	670	4.0
12	PhCH$_2$SO$_2$	H	10	50	5.2
13	H	MeS	60	3400	nd
14	H	*n*PrSO$_2$	40	430	4.4
15	MeO	MeSO$_2$	7	1000	4.9
16	MeO	*n*PrSO$_2$	4	140	5.6
17	Me	*n*PrSO$_2$	2	140	6.5

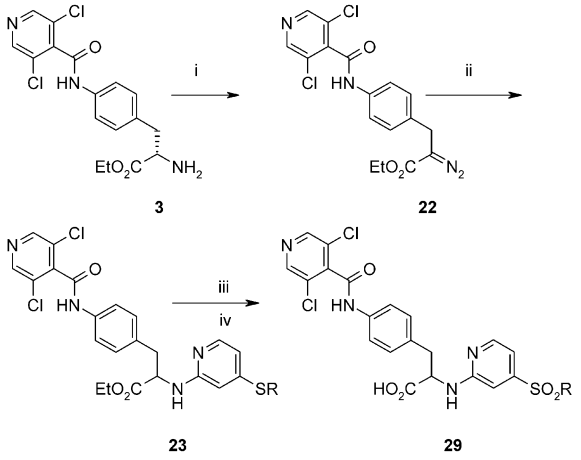
Table 2. Potency and rate of clearance for carboxylic acid substituted pyrimidines



Compd	R1	R2	VLA-4 protein[6] IC_{50} (nM)	VLA-4 cell[7] IC_{50} (nM)	IPRL k (h^{-1})
18	5-CO$_2$H	MeS	660	32,000	1.3
19	6-CO$_2$H	*n*PrSO$_2$	4	180	0.9
20	6-CO$_2$H	*n*HexSO$_2$	14	1500	3.1
21	6-CO$_2$H	*i*BuSO$_2$	30	520	5.9

It had previously been observed in related and unrelated series[8] that incorporation of carboxylate functionality in this region of the molecule could have a profound effect upon the rate of clearance with only moderate loss in potency. When we incorporated this modification into the pyrimidine series, we observed a similar effect (Table 2). Compound **18** had a clearance of 3 mL/min/kg, and a $t_{1/2}$ of 0.8 h after dosing in the rat (1 mg/kg iv).

The corresponding *N*-pyridin-2-yl analogues were also investigated. Their synthesis by nucleophilic substitution was limited to analogues in which the pyridine precursor bore a sufficiently powerful electron-withdrawing group. Hence an alternative route based on Rh-catalysed carbene insertion[9] was developed; the



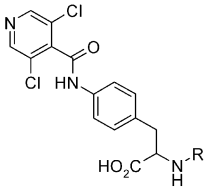
Scheme 3. (i) Isoamyl nitrite, $CHCl_3$, AcOH, reflux; (ii) 2-amino-4-thiopropylpyridine, $Rh_2(OAc)_2$, toluene, 80 °C; (iii) mCPBA, DCM; (iv) LiOH, water, dioxan.

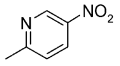
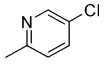
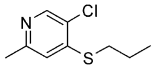
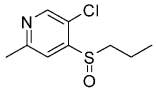
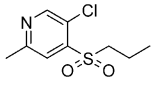
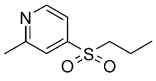
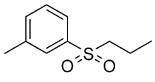
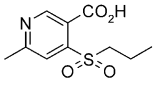
synthesis of the 4-sulphonyl substituted analogue **29** is illustrative (Scheme 3). Treatment of the 3,5-dichloropyrid-4-ylcarboxamido substituted phenyl alanine ester **3** with isoamyl nitrite in chloroform at reflux for 1 h gave the diazo compound **22** in good yield. Heating with the appropriately substituted pyridine in the presence of catalytic rhodium (II) acetate in toluene afforded the pyridyl compound **23**. Oxidation (where the pyridyl substituent was a thioalkyl group) was performed with mCPBA, and aqueous hydrolysis with LiOH gave the target acid **29**. Although this method affords relatively easy access to the desired pyridine analogues it does destroy the chiral centre, all reported data for these compounds (Table 3), is for the racemic mixture.

The activity of **28** and **29** (Table 3) demonstrated again the potency-enhancing effect of the alkylsulphonyl group relative to the alkylthio or chloro substituents. The alkylsulphinyl group of **27** is equipotent and cleared at the same rate as the sulphonyl analogue **28**. The greatly reduced (though not abolished) potency of the phenyl analogue **30** [compared to the corresponding pyridinyl or pyrimidinyl analogues (**29** and **7**)] illustrates the importance of having at least one electronegative nitrogen atom in the *ortho* ring position. This atom presumably mimics the oxygen of the carbonyl in the original *N*-acyl lead structures. Although the potent antagonist **28** displays a relatively good clearance value, this study generally showed that alkylsulphonyl substituted pyridine analogues, like their pyrimidine counterparts, suffer from high clearance rates. Again, it is interesting to note that the combination of sulphonyl and carboxylate functionality, **31**, gives a compound that has a respectable potency and a much slower rate of clearance.

In conclusion, we have identified a novel series of VLA-4 antagonists and have gained useful insights into their SAR for potency and rate of clearance. In particular the incorporation of sulphonyl or sulphinyl functionality appears to enhance potency, possibly as these hydrogen bond acceptor groups can access a similar region of space to the acetyl or sulphonyl group in a potent series of proline based antagonists.[3] We have also observed that the presence of carboxylate functionality can improve the rate of clearance in the rat. The reason for this is not clear, though it is thought unlikely to be a result of plasma protein binding. Possibly the transporter cannot recognise the more polar molecule. However, the increased polarity of these molecules suggests that they will be poorly absorbed following oral administration.

Table 3. Potency and rate of clearance for pyridines



Compd	R	VLA-4 protein[6] IC_{50} (nM)	VLA-4 cell[7] IC_{50} (nM)	IPRL k (h^{-1})
2		260	2400	1.0
24		50	1300	nd
25		410	53,000	0.2
26		1400	nd	nd
27		7	80	4.6
28		4	70	2.1
29		2	50	5.2
30		50	1600	nd
31		10	90	0.2

Acknowledgements

This paper is dedicated to the memory of Alex Zomaya.

References and Notes

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6. VLA4 (from HL60 lysate) was immobilised on a plate with a non-blocking anti-β1 antibody (TS2/16). The test comounds 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.

7. A Jurkat cell line expressing VLA4 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.

8. Unpublished data.

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