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Bioorganic & Medicinal Chemistry Letters 13 (2003) 805–808

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Dehydrophenylalanine Derivatives as VLA-4 Integrin Antagonists

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Received 10 October 2002; revised 3 January 2003; accepted 7 January 2003

Abstract—We describe a series of dehydrophenylalanine derivatives where the *Z* isomers are potent VLA-4 antagonists but are subject to rapid biliary clearance and the *E* isomers have poor activity but have a slower rate of clearance. These configurationally constrained molecules have led to the design of a novel class of benzodiazepine VLA-4 antagonists.

VLA-4 (α4β1 or CD49d/CD29) is a member of the integrin family[1] of glycoprotein cell surface receptors whose principal function is to mediate cell-cell and cell-matrix interactions. VLA-4 recognises a number of ligands[2] including vascular cell adhesion molecule (VCAM),[3] expressed on endothelial cells in response to pro-inflammatory cytokines (such as IL-1, IL-4 and TNF-α). VLA-4 is present on most leukocytes that play an essential role in chronic diseases of autoimmune origin, where it is believed to be involved in the adhesion, migration and activation of these cell-types at sites of inflammation.[4] Consequently, blocking the action of VLA-4 would be expected to be of therapeutic benefit in the treatment of a variety of inflammatory diseases. Anti-α4 antibodies have shown efficacy in a number of animal models of chronic inflammatory diseases including asthma, rheumatoid arthritis, and multiple sclerosis.[5] A humanised monoclonal antibody has shown activity in Phase II clinical trials for multiple sclerosis and ulcerative colitis,[6] and a number of small molecule antagonists are believed to be in pre-clinical development.[7]

We have recently described the discovery of *N*-acetyl D-thiaproline-(*O*-2,6-dichlorobenzyl)-*L*-tyrosine, CT5219, a potent VLA-4 integrin antagonist that showed efficacy in an animal model of asthma.[8] However, we felt unable to develop this compound as it displayed a poor pharmacokinetic profile in the rat. Further work on similar phenylalanine based antagonists revealed that the observed rapid biliary clearance appeared to be a characteristic of these structures, a feature that has also been reported by a number of other workers in this field.[9]

In an attempt to gain an understanding of the SAR for the clearance we set about screening a library of phenylalanine analogues to identify compounds that displayed acceptable rates of clearance. In order to get a relatively high throughput screen we chose the isolated perfused rat liver (IPRL) assay,[10] 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. We calculated that an IPRL $k = 1\ h^{-1}$ would equate to an observed clearance rate of around 10 mL/min/kg in the rat, a figure that we felt would be acceptable.

An initial library of over 50 compounds was screened in this assay, only a few of which displayed an acceptably slow rate of clearance. Of these, the dehydrophenylalanine analogues, **1** and **2**, attracted our attention. We were intrigued to notice that although both compounds displayed relatively poor potency, the *E*-isomer, **2**, had a significantly slower rate of clearance when compared to the *Z*-isomer, **1**. Encouraged by these results we prepared a series of dehydrophenylalanine analogues to see if we could improve their potency as determined by their ability to inhibit

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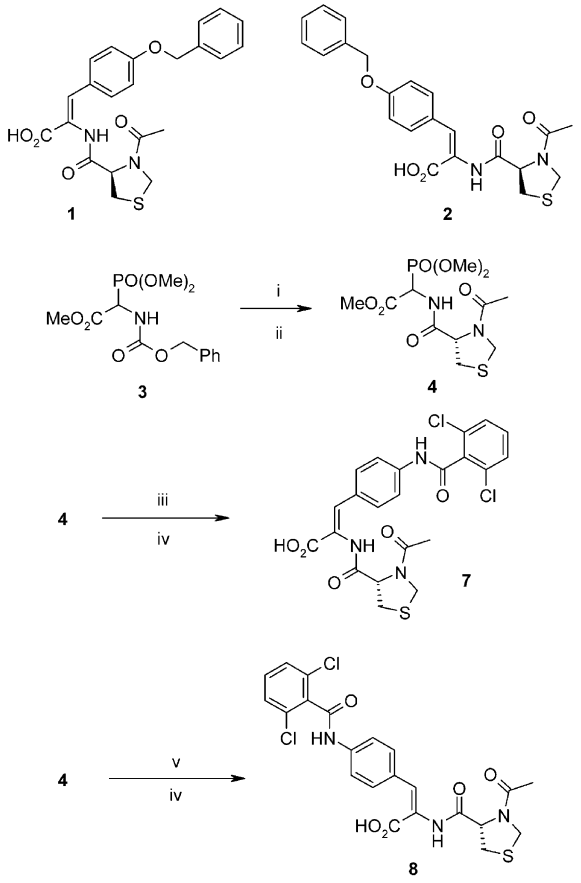
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00019-2

the binding of VLA-4 to VCAM-1 in a protein-based, ligand binding[11] and a cell-based, adhesion assay.[12] We now wish to report our observations on this series of compounds that also lead to some insights on the active conformation of these antagonists in the ligand-binding site.

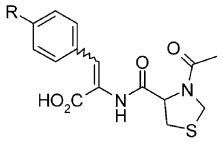
The compounds were prepared in a similar fashion to that depicted in Scheme 1 for the benzamides **7** and **8**. The commercially available phosphonate **3** was deprotected by hydrogenation and coupled to the *N*-acetyl-D-thiaproline using standard amide coupling conditions to give the phosphonate **4**. Treatment of a solution of the phosphonate **4** and the requisite benzaldehyde with DBU gave the dehydroamino ester. NMR analysis of the crude reaction mixture showed the presence of only a single double bond isomer whilst nOe experiments[13] on the purified products determined that this was the *Z* isomer. (At temperatures below 300 K both isomers exist as a 60:40 mixture of rotamers as a result of restricted rotation about the thiaproline *N*-acetyl bond.) Hydrolysis of the ester under basic conditions gave the target acid **7**. In order to obtain quantities of the *E* isomer the phosphonate **4** was treated with sodium hydride in THF, followed by the requisite benzaldehyde to furnish approximately 1:1 mixtures of the *E* and *Z* dehydro aminoesters as determined by NMR analysis of the crude reaction mixture. The pure *E* isomer was isolated by column chromatography then hydrolysed to the target acid **8** as before.

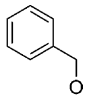
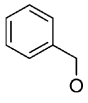
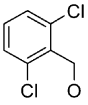
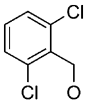
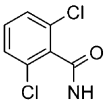
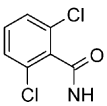
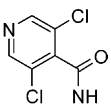
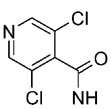


Scheme 1. (i) H_2, 10% Pd/C, MeOH; (ii) *N*-Acetyl-D-thiaproline, HOBT, EDC, *N*-methyl morpholine DCM; (iii) DBU, 2,6-dichloro-*N*-(4-formylphenyl)benzamide, DCM; (iv) LiOH, THF, H_2O; (v) LDA, THF then 2,6-dichloro-*N*-(4-formylphenyl)benzamide, THF, DMF.

We have shown in earlier series[8] that the potency of the antagonists could be significantly enhanced by the replacement of the benzyl ether with 2,6-dichlorobenzyl ether or, more preferably, 2,6-dichlorobenzyl amide analogues. Additionally we had shown that reversal of the stereochemistry at the thiaproline α-centre gave a further enhancement to the potency. Gratifyingly, incorporation of these modifications into the dehydrophenylalanine series had a similar effect, Table 1. For example, the 2,6- dichlorobenzyl amide, *Z* isomer, **7**, is 500-fold more potent in the cell-based assay than the benzyl ether, *Z* isomer, **1**, whilst the 3,5-dichloropyridyl amide, *Z* isomer, **9**, is more potent still. Interestingly, this enhancement in potency is not observed for the *E* isomer analogues, where both the ether and amide analogues display poor activity in the cell based assays. (The apparent improvement in potency for the

Table 1. Potency and rate of clearance for thiaprolines

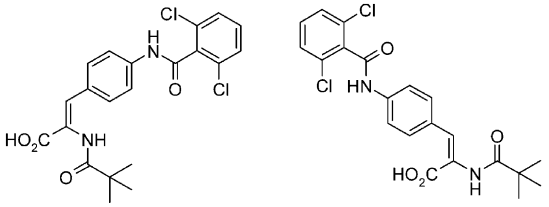


Compd	R	*E*/*Z*	Thioproline	VLA-4 protein IC_{50} nM	VLA-4 cell IC_{50} nM	IPRL k h^{-1}
1		*Z*	L	2200	71,000	3.8
2		*E*	L	1500	20,000	0.6
5		*Z*	D	2800	5000	4.1
6		*E*	D	7400	83,000	0.4
7		*Z*	D	5	140	3.9
8		*E*	D	400	29,000	0.7
9		*Z*	D	2	25	4.4
10		*E*	D	60	200	1.2

3,5-dichloropyridyl amide, *E* isomer, **10**, is a result of the presence of a small amount of the potent *Z* isomer as an impurity.) What is perhaps more significant is that the *E* isomers all display a markedly slower rate of clearance than their *Z* isomer counterparts.

In order to demonstrate that this effect was independent of the α-amide substituent a series of nicotinamides were prepared by a similar route to that described in Scheme 1. Although the data set is more limited a similar trend was observed, Table 2, with the more potent 3,5-dichloropyridyl amide *Z* isomer, **13**, having a much faster rate of clearance than the less potent *E* isomer **14**.

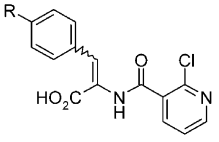
A similar effect was observed with the *t*-butyl amides **15** and **16**. Whereas the *E* isomer, **16**, showed poor activity (VLA-4/VCAM protein IC_{50} 2.7 μM; VLA-4/VCAM cell IC_{50} 80 μM) the *Z* isomer, **15**, was significantly more potent (VLA-4/VCAM protein IC_{50} 0.05 μM; VLA-4/VCAM cell IC_{50} 3.7 μM, IPRL *k* 4.8 h^{-1}).

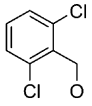
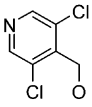
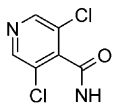
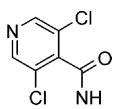


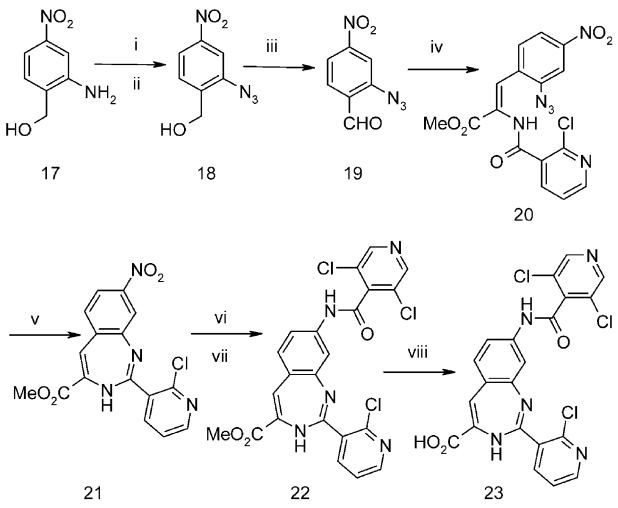
15 16

Obviously the dehydrophenylalanines are conformationally far more rigid than their saturated analogues. Whilst the extra flexibility of the saturated systems probably accounts for their easier accommodation in the integrin ligand binding site and hence their greater potencies,[8] the data from the unsaturated systems suggests that the shape of the antagonist can have a profound effect upon both potency and clearance. The observed difference in clearance is presumably a result of the difference in binding to plasma protein, for example the slowly cleared *E* isomers **6** and **14** are both greater than 99% bound to plasma whereas the rapidly cleared *Z* isomers **9** and **15** are 70 and 87% bound respectively. We have observed a similar effect in a series of squaric acid derivatives.[14]

Table 2. Potency and rate of clearance for nicotinamides



Compd	R	*E*/*Z*	VLA-4 protein IC_{50} nM	VLA-4 cell IC_{50} nM	IPRL *k* h^{-1}
11		*Z*	220	7100	3.7
12		*Z*	140	14,000	nd
13		*Z*	1.4	70	3.9
14		*E*	66	8500	1.5



17 18 19 20 21 22 23

Scheme 2. (i) HCl, $NaNO_2$, H_2O; (ii) NaOAc, NaN_3, H_2O; (iii) MnO_2, DCM; (iv) *N*-(2-chloronicotinoyl)-α-phosphonoglycine trimethyl ester, DBU, DCM; (v) PPh_3, toluene; (vi) $SnCl_2{\cdot}2H_2O$, MeOH; (vi) 3,5-dichloroisonicotinoyl chloride, Et_3N, DCM; (viii) LiOH, THF, H_2O.

We were interested to see whether it would be possible to effect further conformational restraint in these compounds and retain potency. Consequently, we prepared the 1,3-benzodiazepine, **23**, as shown in Scheme 2. The azidobenzaldehyde **19**, prepared in two steps from the alcohol **17**, was treated with the phosphonoglycine in the presence of DBU to give the dehydroalanine **20**. Cyclisation to the benzodiazepine **21** by means of an intramolecular aza-Wittig reaction[15] was achieved in 80% yield and was followed by reduction of the nitro group and coupling with the acid chloride to give the amide **22**. Hydrolysis of the ester gave the target acid **23**.

The benzodiazepine, **23**, did show pleasing levels of potency (VLA-4/VCAM protein IC_{50} 55 nM; VLA-4/VCAM cell IC_{50} 410 nM) but, as may have been predicted from the conformation, it was subject to rapid clearance having an IPRL clearance rate constant of 5.3 h^{-1}, corresponding to a clearance of 140 mL/min/kg in the rat (dosing at 10 mg/kg). This compound is, however, the first of a novel structural class of VLA-4 antagonists.

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11. 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 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.
12. 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.
13. The identity of the geometric isomers was confirmed by recording NOESY data in phase sensitive mode with a mixing time of 350 ms. For example with compound **6** the olefinic ^1H resonances in the two conformers are isochronous at 6.60 ppm. Medium strength, positively phased NOESY cross peaks are observed between this signal and the two NH signals at 9.77 ppm (major) and 10.05 ppm (minor). This identifies **6** as the *E* isomer. With compound **5**, the olefinic ^1H resonances are resolved at 7.31 ppm (major) and 7.40 ppm (minor). In the NOESY data no crosspeaks are observed corresponding to these resonances. Weak, positively phased nOe crosspeaks are observed between the two NH resonances in each conformer at 9.39 ppm (major) and 9.66 ppm (minor) and the higher frequency phenylalanine AA′BB′ multiplet resonance centred at 7.67 ppm. This identifies **5** as the *Z* isomer. Parallel data was recorded for the other isomeric pairs of molecules.
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