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Bioorganic & Medicinal Chemistry Letters 17 (2007) 597–601
Bioorganic & Medicinal Chemistry Letters

Highly constrained bicyclic VLA-4 antagonists

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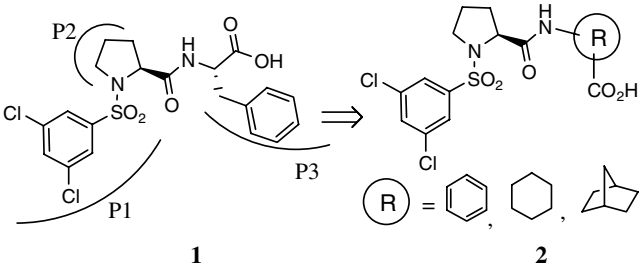
Received 3 October 2006; revised 2 November 2006; accepted 3 November 2006
Available online 7 November 2006

Abstract—VLA-4 is implicated in several inflammatory and autoimmune disease states. A series of cyclic β-amino acids (β-aa) was studied as VLA-4 antagonists. Binding affinity was highly dependent on the dihedral angle (ϕ) between the amino and the carboxyl termini of the β-aa. Compound **5m** where the β-aa is embedded in a bicycle possesses the most preferred ϕ (120°). It is a potent and bioavailable VLA-4 antagonist (VCAM-Ig α4β1 IC_{50} = 54 nM, rat po F = 49%).

The integrin VLA-4 (very late antigen-4, $\alpha_4\beta_1$, CD49d/CD29) is a heterodimeric cell surface glycoprotein transmembrane receptor.[1] It is expressed on all leukocytes except platelets and neutrophils, and is a key mediator in cell–cell and cell–matrix interactions. It mediates cell adhesion to vascular cell adhesion molecule-1 (VCAM-1), an immunoglobulin (IgG) expressed on endothelial cells in response to proinflammatory cytokines at sites of inflammation, and it binds extracellularly to CS-1, an alternatively spliced form of fibronectin (Fn).[2] These cell adhesion interactions may be required for the activation, migration, proliferation, and differentiation of leukocytes during normal and/or pathophysiological processes. Thus, inhibition of VLA-4 may produce a reduction in the migration and/or activation of cell types important to sustaining a prolonged inflammatory response.[2]

Therapeutic efficacy in several animal models of inflammation and autoimmune diseases, such as asthma, multiple sclerosis, and Crohn's disease, via the inhibition of the interaction between VLA-4 and its ligands has been validated by both anti-VLA-4 monoclonal antibodies and low molecular weight antagonists.[3] Thus, there is a substantial interest in developing small-molecule VLA-4 antagonists.[4]

Derivatization of VLA-4 antagonists, which emerged from screening of a combinatorial library,[5] led to more potent compounds such as **1** (IC_{50} = 1.4 nM).[6] Starting from such capped dipeptides, our objective was to conceive structures which would enhance the pharmacokinetic profile compared to reference compounds such as **1**.[6a] One of the ideas followed was to replace the P3 phenylalanine in **1** with a non-natural-amino acid; for example, aminobenzoic acid derivatives or their saturated counterparts, as depicted in **2**. In this basic design, the benzyl group found in P3 of **1** (or its equivalent), which is also present in many VLA-4 antagonists,[4b–f] is absent (Fig. 1).



1 **2**

Figure 1.

Keywords: VLA-4 antagonists; Integrin; α4β1; Anti-inflammatory; Autoimmune; β-Amino acid; Conformational restriction; Bioavailable; Bicyclic.
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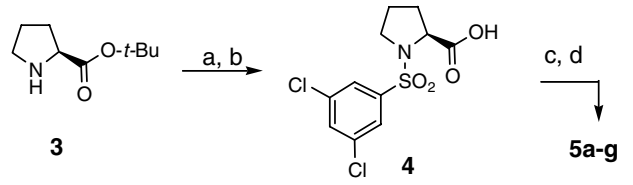
doi:10.1016/j.bmcl.2006.11.011



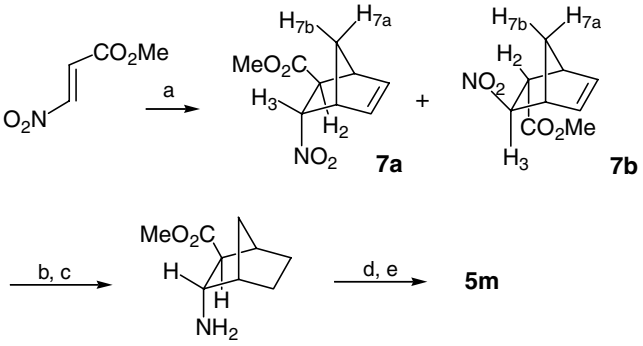
Scheme 1. Reagents and conditions: (a) $(3,5\text{-}Cl_2)C_6H_3SO_2Cl$, $NEt(i\text{-}Pr)_2$, DMAP, DCM, 94%; (b) 1:1 TFA/DCM, rt, 40–98%; (c) $R(NH_2)(CO_2Et)$, EDC, HOBt, NMM, DCM, 80–93%; (d) NaOH/MeOH, rt, 4 h, 70–90%.

Herein, we show the evolution of this approach, from inactive entities to structurally novel, orally bioavailable compounds with good potency for VLA-4.

Most of the compounds studied were prepared by sulfonylation of a protected P-2 amino acid such as **3**, followed by coupling of the resulting key intermediate **4**[7] with an appropriate P3 amino acid. Subsequent base hydrolysis furnished **5a**–**g**. Compounds **6a**, **b** were prepared analogously, utilizing suitably protected P2-amino acids.

For **5h**–**k**, **n** and **o**, the diastereomeric esters (Scheme 1, step c) were hydrolyzed separately with excess base at 27 °C overnight. This resulted in partial racemization of the α-carbon in each case. For **5j**, the more active isomer of **5j**–**k**, these isomers were separated by preparative HPLC. The structural assignments of the resulting isomers **5l**, **m** were made by ^{1}H NMR spectral correlation with spectra of isomerically pure intermediates and via decoupling experiments. The more active isomer **5m**, derived from the epimerization, showed a W-coupling between the aforementioned α-proton (*endo*) and one of the protons (*anti*) on the apical carbon of the bicyclo[2.2.1] system.

A stereochemically unambiguous synthesis of **5m** was undertaken to ascertain its stereochemical assignment. As shown in Scheme 2, methyl (*E*)-3-nitroacrylate[8] was reacted with cyclopentadiene to provide the Diels–Alder adducts **7a** (*endo*-nitro) and **7b** (*exo*-nitro) in a 6:1 ratio.[9] The structure of the major component was deduced to be **7a** as described below. Based on literature precedents[9,10] the H_3's in **7a** and **b** were assigned as the lower field methyne protons (δ 5.43 and 4.73), while the H_2's were assigned as the higher field ones (δ 3.08 and 3.72). Initial attempts to assign the stereochemistry at C2 and C3 were based on long-range W-coupling in the ^{1}H NMR (500 MHz) spectra. In **7a**, a definitive W coupling is observed between H_2 (δ 3.08) and H_{7a} (δ 1.65), analogous to that observed in **5m** earlier. For this compound, no W coupling is observed between H_3 (δ 5.43) and H_{7a}. The minor component **7b** exhibits a W coupling between H_3 (δ 4.73) and H_{7a} (δ 1.74) but not between H_2 (δ 3.72) and H_{7a}. Concurrent NOE studies demonstrated that in **7a**, an NOE is observed between H_3 and H_{7b}, but not between H_2 and H_{7b}, while in **7b**, there is an NOE between H_2 and H_{7b}, but not between H_3 and H_{7b}. In this manner, **7a** was unambiguously assigned as the major product from the Diels–Alder reaction.



^{1}HNMR chemical shifts (δ) for H_2, H_3, H_{7a}, and H_{7b} in **7a** and **7b**.

cpd	H_2	H_3	H_{7a}	H_{7b}
7a	3.08	5.43	1.65	1.72
7b	3.72	4.73	1.74	1.92

Scheme 2. Reagents and conditions: (a) cyclopentadiene, Et_2O, 0 °C, 40 min, 80%; (b) H_2, PtO_2, EtOAc, quant.; (c) HCO_2NH_4, 10% Pd/C, MeOH, 30%; (d) **4**, EDC, HOBt, NMM, DCM, 82%; (e) 1 equiv NaOH/MeOH, rt, 2 h, 80%.

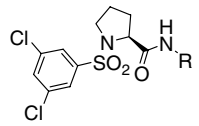
Reduction of the double bond in **7a** was followed by transfer hydrogenation of the nitro group to provide the aminoester which was coupled with **4**. Hydrolysis of the product provided the desired compound **5m**, spectrally identical to **5m** obtained previously, verifying the initial structural assignment of **5m** from HPLC separation. Compounds **5p**, the bicyclo[2.2.2] analog of **5m**, and **q**, the oxygen isostere of **5m**, were also synthesized according to Scheme 2. For **5p**, the Diels–Alder reaction required prolonged heating with excess 1,3-cyclohexadiene at 130 °C. Under these conditions, the *endo*-nitro:*exo*-nitro stereoisomeric products (analogous to **7a** and **b**) were obtained in a 4:1 ratio.

The VLA-4 binding affinity of the compounds discussed was assessed by measuring the reduction in binding of ^{125}I-VCAM-Ig to a suspension of Jurkat cells (a human $\alpha_4\beta_1$ T cell line) in the presence of the test compound, as previously described.[6b] All assays were run at least in duplicate.

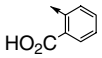
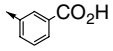
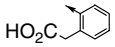
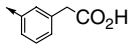
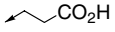
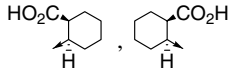
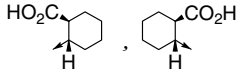
To begin, a series of aminobenzoic acids and (aminophenyl)acetic acids was examined (Table 1, **5a**–**d**). These were inactive as VLA-4 antagonists. This lack of activity compared to the parent **5e** was thought to be due to the rigidity of the aromatic ring. To increase the flexibility of P3 and to diverge from a planar configuration between the amino and the carboxy moieties of P3, cyclohexyl systems with the amino and the carboxyl groups in a β-*cis* (**5f**) and β-*trans* (**5g**) relationship were evaluated.

Table 1 shows **5f**, **g** have significantly improved binding affinities over **5a**. These results suggest that the dihedral angle (ϕ) between the amino and the carboxylic acid groups in P3 could play an important role in optimizing the interaction of the ligand with the receptor. In the case of **5a**, **b**, $\phi = 0°$ and the compounds were inactive. For **5c**, **d**, the methylene spacer provided some flexibility in ϕ. For **5e**, **f**, the outcome was much improved.

Table 1. VLA-4 binding affinities of (*N*-arylsulfonyl)prolyl amino acids **5a–g**



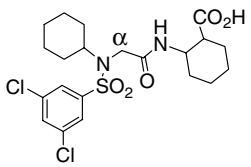
5

Compound	R	VLA-4 IC_{50} (nM)
5a		0% inhibition at 100 μM
5b		2% inhibition at 100 μM
5c		11% inhibition at 100 μM
5d		43% inhibition at 100 μM
5e		1500
5f		9460
5g		525

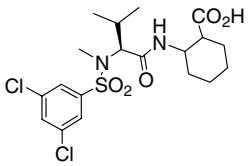
In these compounds, $\phi = 60°$ assuming the chair conformation predominates, implying that $\phi = 60°$ is preferred to $\phi = 0°$.

In an effort to optimize P2 in the presence of a cyclic P3, we opened the pyrrolidyl ring in **5g**, providing more freedom in this putative β-turn region. The data suggest that substitution at the α-position is critical for maintaining binding (**6a** vs **6b**, Fig. 2). A comparison of data from **5g** versus **6b** shows that there is no significant advantage in opening up the proline ring.

Meanwhile, we felt a closer examination of the data from cyclohexane amino acids **5f** and **g** was in order. The 18-fold difference in binding affinity between these compounds was intriguing considering that in the chair conformation, both sets of compounds have ϕ of ~60°. We hypothesized that the difference in receptor binding was due to either the availability of a *trans*-diaxial relationship ($\phi \approx 180°$) between the amino and the carboxy groups of **5g**, or differences in the dihedral angles in the boat and twist boat conformations between **5f** and **5g**, to the extent that they are available. Considering the sterics involved, the *trans*-compounds **5g** would be expected to achieve the boat/twist boat conformations (ϕ = 120–150°) more easily than the *cis*-compounds **5f** ($\phi \sim 0$–30°). This line of reasoning in combination with the data for **5f**, **g** suggests that the optimal dihedral angle lies between 60° and 180°.



6a: inactive @ 100 μM



6b: IC_{50} = 462 nM

Figure 2.

A set of conformationally constrained bicyclic systems was examined to further study the issue. The rigid bicyclo[2.2.1] system served as the starting point. As shown in Table 2, the *exo*-norbornane system gave rise to two epimeric pairs, **5h**, **i**; likewise, the *endo*-norbornane system gave rise to **5j**, **k**. Pairwise, these bicyclo[2.2.1] compounds were more active than their cyclohexyl counterparts (**5f**, **g**). This could be due to conformational constraint and/or the added hydrophobicity of the bicyclo system. Compounds **5h**, **i**, where the amino group is *exo* on the bicyclic moiety, are less active than **5j**, **k**, where the amino group is *endo*.

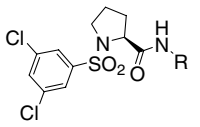
The most active pair of the 4 epimeric pairs, **5j**, was separated into **5l** (*endo*-acid) and **5m** (*exo*-acid). The data suggest that the *endo*-amino-*exo*-carboxylic acid combination (**5m**) represents the most preferred spatial arrangement for receptor–ligand interaction among the cyclic systems studied. The dihedral angle in this case is close to 120°, within the range of our prediction.

The data from compounds **5n–q** show that the integrin binding affinity is affected substantially by steric changes in this region and/or by small changes in the dihedral angle (**5n–p**). The bicyclo[2.2.2] system (**5p**) is much more flexible than the bicyclo[2.2.1] (norbornane) system. Therefore, the operative dihedral angle is less well defined in **5p** compared to **5m**, contributing to the decrease in binding affinity. An exquisite sensitivity to increases in polarity is demonstrated by **5q**. Overall, data from **5m** and the unsubstituted system **5e** show that by judiciously building conformational constraints into a linear system via cyclic entities, a 30-fold increase in receptor–ligand binding was achieved.

Compound **5m** is a selective VLA-4 antagonist versus $\alpha_4\beta_7$ (VCAM-Ig $\alpha_4\beta_7$ IC_{50} = 13 μM) and exhibits an excellent pharmacokinetic profile in Sprague–Dawley rats with 49% oral bioavailability (using a cassette-dosing regimen, Table 3).[11]

This study demonstrates the feasibility of designing non-natural cyclic amino acids to fit into receptor–ligand binding sites normally requiring L-amino acids for a series of VLA-4 antagonists. Conformational analysis of these cyclic β-amino acids was instrumental in arriving at a preferred dihedral angle of ~120° between the amino and the carboxylic acid functionalities for optimal

Table 2. VLA-4 binding affinities of (*N*-arylsulfonyl)prolyl amino acids **5g–q**



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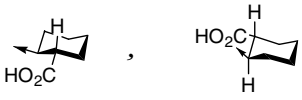
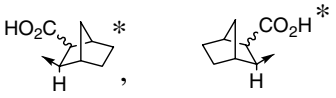
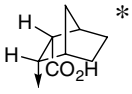
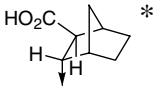
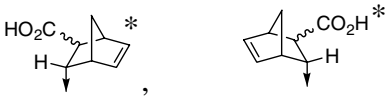
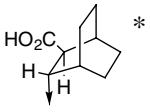
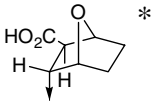
Compound	R	VLA-4 IC_{50} (nM)
5g		525
5h, **i**		299, 1490
5j, **k**		95, 322
5l		1597
5m		54
5n, **o**		657, 2160
5p		2740
5q		25,600

*The absolute stereochemical assignment is arbitrary.

Table 3. Pharmacokinetic data parameters of **5m**

Route[a]	Dose[b]	CL_p (ml/min/kg)	$t_{1/2}$ (h)[c]	AUC (μM h kg/mg)	*F* (%)
iv	1 mpk	3.3	1.9	9.1	—
po	2 mpk	—	1.7	5.5	49

[a] Sprague–Dawley rats.
[b] Cassette-dosing.
[c] Plasma half-life (0–8 h).

receptor–ligand interactions in this class of compounds. In this manner, the β-alanine moiety of a micromolar compound **5e** was transformed to a norbornane system, leading to a series of compounds lacking the substituted phenyl or benzyl group typically found in this class of VLA-4 antagonists.[4b] The best of these was **5m**, a 54 nM orally bioavailable and selective VLA-4 receptor antagonist.

Acknowledgments

We thank Dr. Linus Lin for the preparation of compound **5q**. We thank Ms. Amy Bernick for mass spectral analysis.

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7. This and all other compounds prepared were characterized by mass spectrum (FAB or LC/MS), and 300 MHz, 400 MHz, or 500 MHz ^{1}H NMR.
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11. The cassette dosing regimen that we used was a mixture of five compounds in equal amounts; each at 2 mpk po and 1 mpk iv. Whereas there is the possibility for drug–drug interactions in mixture dosing in related structures, we did not observe significant differences in PK parameters between single or mixture dosing.