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Constraining the amide bond in N-Sulfonylated dipeptide VLA-4 antagonists

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Abstract—The integrin VLA-4 is implicated in several inflammatory disease states. In search of non-peptidic antagonists of VLA-4, rotational constraints were imposed on the amide bond of prototypical N-sulfonylated dipeptide VLA-4 antagonists. By judicious structural modification of the side chains, trisubstituted imidazoles with moderate binding potencies were obtained, for example, **19**, VLA-4 IC_{50} = 237 nM.

The integrin VLA-4 (very late antigen-4; $\alpha_4\beta_1$, CD49d/CD29) is a heterodimeric cell surface glycoprotein transmembrane receptor.[1] Expressed on all leukocytes except platelets and neutrophils, it is a key mediator in cell–cell and cell–matrix interactions. It is involved in leukocyte recruitment, activation, proliferation, and differentiation during normal and/or pathophysiological processes. Blockade of the interaction between VLA-4 and its ligands, vascular cell adhesion molecule-1 (VCAM-1) and CS-1, an alternatively spliced form of fibronectin, may reduce the vascular extravasation of inflammatory cells into tissues during prolonged inflammation.[2]

Recent Phase III clinical data of natalizumab, the humanized anti-α4 antibody, in multiple sclerosis validate the potential for α4 integrin antagonists in human disease.[3] In addition, peptidyl VLA-4 antagonists have proven effective in several animal models of inflammation and autoimmune diseases.[4] These results led to much effort in the development of small molecule VLA-4 antagonists.[5]

Initial efforts at Merck led to derivatives such as **1a** (IC_{50} = 1.4 nM),[6b] **1b** (IC_{50} = 0.64 nM),[6a] and **1c** (IC_{50} = 0.56 nM),[6a] all containing a central amide bond. Indeed, the equivalent of this amide bond is featured in many of the VLA-4 antagonists that have been identified, for example, **2**, **3**.[5a,c] We were interested in transforming compound **1** into active compounds where the amide bond is incorporated into various cyclic structures as shown in Figure 1. The first route (I) connected the amide nitrogen to the C-3 position of the proline

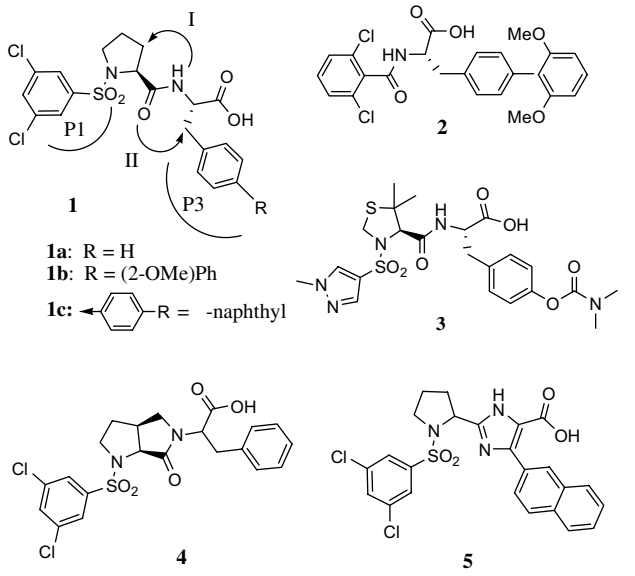

Figure 1.

Keywords: VLA-4; Peptide bond mimetic; Bicyclic VLA-4 antagonists; Imidazole VLA-4 antagonists.
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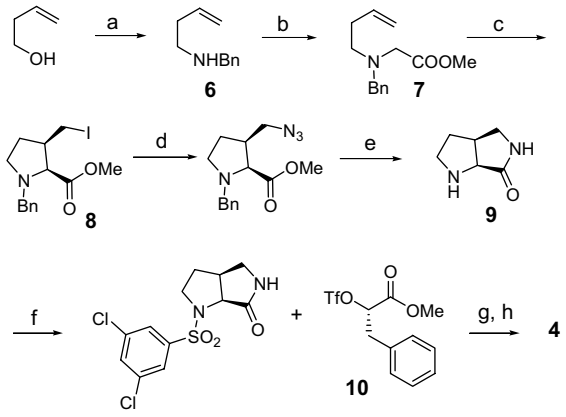
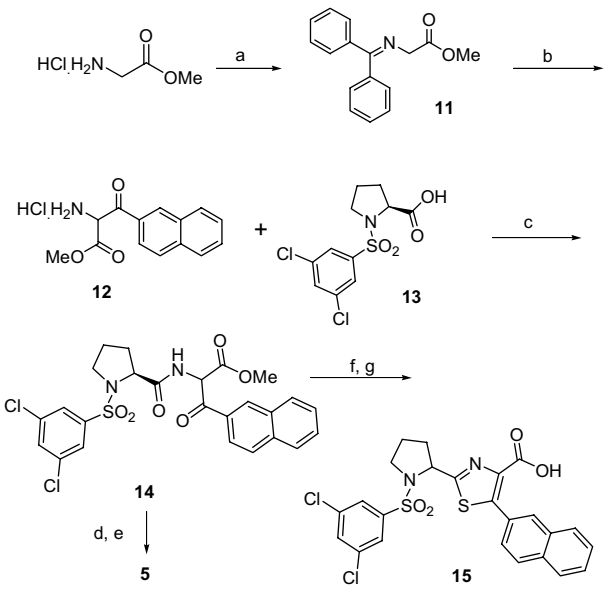


Scheme 1. Reagents and conditions: (a) i—MsCl, NEt_3, DCM; ii—$BnNH_2$, 80%; (b) $BrCH_2CO_2Me$, NEt_3, DMSO, rt, 12 h, 69%; (c) i—LDA, −78 °C; ii—$ZnBr_2$, −90 °C; iii—I_2, 80%; (d) NaN_3, DMF, rt, 16 h, 71%; (e) H_2, Pd/C, EtOAc, 98%. H_2, Pd/C, formic acid, MeOH, 99%; (f) $(3,5\text{-}Cl_2)C_6H_3SO_2Cl$, $NEt(iPr)_2$, DMAP, DCM/THF (1:1), 41%; (g) NaH, THF/DMF, 51%; (h) NaOH/MeOH, 94%.



Scheme 2. Reagents and conditions: (a) $(C_6H_5)_2C{=}NH$, DCM, rt, 77%; (b) KO-*t*-Bu, THF, −70 °C, β-naphthyl-COCl; 2 N HCl, 92%; (c) NMM, THF; *t*-Bu-OCOCl, NMM, −20 °C, 74%; (d) NH_4OAc, HOAc, 3A sieves, xylene, reflux, 16 h, 55%; (e) excess TMSI, 100 °C, 1–3 h, 40%; (f) Lawesson's Reagent, THF, 3A sieves, reflux, 14 h. 69%; (g) 2 equiv NaOH/MeOH, rt, 50%.

(P2) via a carbon bridge, as in **4**.[7] The second approach (II) incorporated the amide bond and the β-carbon of the P3 side chain into a 5-membered heteroaryl ring, as in **5**.[8] Both approaches introduced a considerable amount of conformational restriction to the resulting non-peptidic entities. Herein, we describe the effects of such constraints on receptor-ligand binding in this class of VLA-4 antagonists.

Compound **4**, the target from route I, contains a novel diaza-5,5-fused ring system which provided considerable synthetic challenges. The successful route began with the conversion of homoallylic alcohol to the corresponding homoallylic benzylamine **6**,[9] which was reacted with methyl bromoacetate to yield the key intermediate **7** (Scheme 1). Deprotonation of **7** was followed by the putative complexation of the resulting enolate oxygen, amine nitrogen, and the homoallyl double bond with zinc bromide.[10] Subsequent treatment with iodine provided the substituted proline methyl ester **8**, possessing an iodomethyl group. The iodo functionality was converted to an azide[11] which was hydrogenated to compound **9**, containing the desired restricted amide bond. Thus, the transformation from **8** to the strained, unsubstituted 5,5-fused ring system **9** was achieved in one pot via reductive cyclization followed by N-debenzylation.[12]

Subsequently, the 3,5-dichlorobenzenesulfonyl moiety (P1) was attached to the pyrrolidine nitrogen. Alkylation of the lactam nitrogen with the triflate of methyl (L)-3-phenyllactate **10** (obtained from methylation of (L)-3-phenyllactic acid and sulfonylation of the hydroxyl group) followed by base hydrolysis of the methyl ester provided the racemate **4**.

The imidazole **5** was prepared as shown in Scheme 2. Glycine methyl ester was converted to its benzophenone Schiff base **11**.[13] Addition of the corresponding potassium enolate to a solution of β-naphthoyl chloride at −70 °C provided the α-imino-β-keto compound which was hydrolyzed to intermediate **12**. Mixed anhydride coupling of **13** (prepared from proline *t*-butyl ester by N-sulfonylation and ester hydrolysis) with **12** provided the key intermediate **14**.[14] The imidazole ring formation was achieved by treatment of **14** with acidic ammonium acetate in refluxing xylene. Removal of the methyl ester was best accomplished by treatment with iodotrimethylsilane, providing the imidazole **5**.

The corresponding oxazole methyl ester was prepared by treating a solution of **14** and DBU in a mixture of carbon tetrachloride, pyridine, and acetonitrile with triphenylphosphine.[15] Unfortunately this compound could not be hydrolyzed to the corresponding acid cleanly under a variety of conditions. In contrast, treatment of **14** with Lawesson's reagent followed by base hydrolysis readily provided the thiazole **15**.

Compounds **16**–**20** were prepared from iminoglycine **11** using sequences analogous to Scheme 2 by the appropriate choice of acylating agent (in step b) and *N*-sulfonylated amino acid (in step c) for amide coupling.

The $\alpha_4\beta_1$ integrin binding affinity of the reported compounds was assessed by measuring the reduction in binding of ^{125}I-VCAM-Ig to a suspension of Jurkat cells in the presence of the test compound as described previously.[6c] All test compounds were assayed at least in duplicate.

The data in Table 1 show that a significant amount of binding was lost by the introduction of conformation constraints via route I or route II (Fig. 1) relative to the parent dipeptides (**1a** vs **4**, and **1c** vs **5**, **15**). Also, N-methylation of the amide bond N—H in **1b** resulted in a compound with IC_{50} of 2400 nM, equivalent to a

Table 1. VLA-4 binding affinities of prototype conformationally restrained compounds, **4**, **5**, **15** and reference compounds **1a**, **b**

Compound	**1a**	**1b**	**1c**	**4**	**5**	**15**
VLA-4 IC_{50} (nM)	1.4	0.64	0.56	2100	8% inhibition at 100 μM	19% inhibition at 100 μM

3750-fold loss in binding affinity versus **1b**. By comparison, the data for **4** and **1a** suggest that constraining the P2 amide bond in a 5,5-fused system provides limited advantage in binding. These data suggest that free N—H is required for adequate binding with VLA-4.[17]

In compound **4**, the methylene unit in P3 is freely rotating. In contrast, in compounds **5** and **15**, the equivalent P3 methylene group is incorporated into the heterocyclic ring (cf. **1c**) and the resulting structure is much more rigid at that point. In fact, in these compounds, the P3 side chain extends out linearly from the heterocyclic moiety. This rigidity contributed to the drastic loss of potency in **5**. Thus, the data from **4**, **5**, and **15** suggest that in order to achieve reasonable binding with VLA-4, the equivalent of the P2 amide bond in **1** should not be quasi-coplanar with the P3 side chain.

We were intrigued by the possibility of transforming inactive but non-peptide entities derived from peptides such as **1** into moderately potent compounds. The trisubstituted imidazole **5**, which contains only one chiral center and retains the N—H of the amide bond was chosen as the platform for this study. We monitored the effects of relaxing the rigidity of the substituents around the imidazole ring on binding affinity.

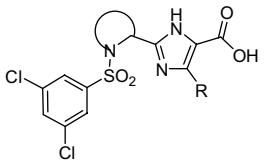
To begin, a spacer was introduced between the naphthyl group and the imidazole ring, as in **16** (Table 2). This resulted in no improvement in binding affinity (**16** vs **5**). The rigidity conferred by the pyrolidinyl group of the prolyl moiety (P2) in **16** was then relaxed. Thus, the spatially diverse (*N*-methyl)leucyl and (*N*-cyclohexyl)glycyl groups[16] were chosen as prolyl replacements while the β-naphthylmethyl group was retained in P3 (**17**, **18**). As shown in Table 2, by opening up the proline ring, a compound that was inactive at 100 μM (**16**) could be transformed to a modest antagonist (**18**).

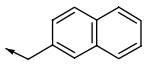
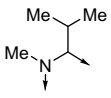
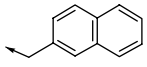
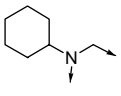
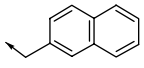
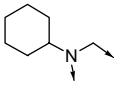
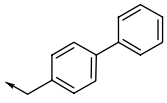
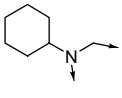
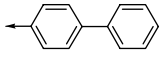
Retaining the (*N*-cyclohexyl)glycyl moiety in **18**, we examined 4′-biphenyl groups with and without a methylene linker to the imidazole as a surrogate of the β-naphthyl group (**19**, **20**). The biphenylmethyl compound **19** was at least as active as the β-naphthyl compound (**18**), suggesting a rather broad spatial tolerance for hydrophobic groups beyond the methylene spacer. Analogous to **5**, compound **20** which has no spacer between the imidazole ring and the aryl substituent lost all activity. This underlines the importance of the methylene spacer to provide the necessary flexibility to P3 and to confer activity thereby.

Taken together, these data show that reasonable flexibility built into both the P2 and the P3 moieties of **5** is required in order to have a reasonable affinity for the VLA-4 receptor. This flexibility compensates for the constraint inherent in the imidazole ring compared to an amide bond. In this manner, these dipeptide-derived imidazoles can be transformed from totally inactive entities to submicromolar VLA-4 antagonists. Our data show that this can be achieved while retaining the structural characteristics of the side chains present in the original capped dipeptide lead.

Many medicinal chemistry leads contain at least one amide bond. As lead optimization progresses, non-peptide entities are often sought. This investigation demonstrates that, in a series of N-sulfonylated dipeptide VLA-4 antagonists, an imidazole has the potential to act as an amide bond surrogate when care is taken to introduce adequate flexibility to its substituents and to array them with appropriate binding motifs.

Table 2. VLA-4 binding affinities of conformationally restrained imidazoles **16**–**20**



Compound		R	VLA-4 IC_{50} (nM)
16			30% inhibition at 100 μM
17			2535
18			285
19			237
20			45% inhibition at 100 μM

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