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Bioisosteric replacement of anilide with benzoxazole: potent and orally bioavailable antagonists of VLA-4

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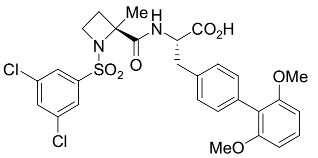
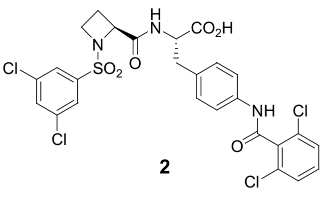
Abstract—We have designed and synthesized a series of heterocyclic bioisosteres for an anilide based on molecular modeling. Excellent potency was retained in the benzoxazole and the benzimidazole derivatives, where a hydrogen bond acceptor is appropriately positioned to mimic the amide bond oxygen. The deletion of the hydrogen bond donor (N–H) led to improved lipophilicity and bioavailability. In the process, **9a** was identified as a potent, specific, and bioavailable VLA-4 antagonist, while **9c** was found to be a potent and bioavailable dual antagonist of VLA-4 and $\alpha_4\beta_7$.

Integrins are a super family of structurally related heterodimeric glycoproteins, which are involved in cell adhesion and cell trafficking. The $\alpha_4\beta_1$ integrin (very late antigen-4, VLA-4) is expressed on many leukocytes including eosinophils, basophils, and monocytes, but not on mature neutrophils. Therefore, VLA-4 antagonists have potentials for the treatment of allergic diseases such as asthma, and other chronic inflammatory diseases.[1] The $\alpha_4\beta_7$ integrin is found primarily on mucosal lymphocytes, and blockade of $\alpha_4\beta_7$ may be beneficial in the treatment of inflammatory bowl disease or Crohn's disease.[2] In general, a specific inhibitor of either $\alpha_4\beta_1$ or $\alpha_4\beta_7$ may be more desirable for safety concerns, though a dual antagonist may be advantageous to achieve maximum efficacy.[3]

Previously, we reported a series of *N*-substituted dipeptides and found that the *N*-arylsulfonyl group is essential for high specificity against $\alpha_4\beta_1$ versus $\alpha_4\beta_7$.[4] Further optimization led to the discovery of **1** and **2** as potent VLA-4 antagonists against both the Mn^{2+} activated states (0.07 and 0.27 nM, respectively), and the unactivated states (Ca^{2+}/Mg^{2+} states: 0.27 and 0.31 nM, respectively).[5] Both compounds are ca. 100× or more selective for $\alpha_4\beta_1$ versus $\alpha_4\beta_7$. However, both compounds suffer from poor pharmacokinetic properties. Specifically, **1** seems to be absorbed but rapidly cleared in the rat (Clp 97 mL/min/kg, F% 9). On the other hand, **2** is more slowly cleared in vivo but poorly absorbed (Clp 33 mL/min/kg, F% 0.7). We reasoned that the hydrophilic nature of the anilide bond may be responsible for the poor bioavailability of **2**, and started an effort to replace the anilide functionality with an appropriate heterocycle.[6]



1



2

We considered all four possible modes of cyclization for the heterocycle formation. Each mode would restrict the two dihedral angles (α and β) to a different degree. In particular, mode *a* is likely to disrupt the *trans*-amide bond configuration. Our goal is to maintain the

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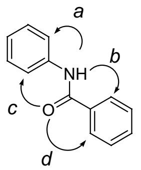
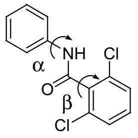
preferred orientation of the amide bond and the two aryl rings. Mechanical calculations were then performed to help to differentiate modes *b*, *c*, and *d*.



A



B

The results of the molecular modeling (MacroModel v5.5) are illustrated in Figure 1. It appears that the amide bond prefers to be coplanar with the aniline ring ($\alpha = 0°$ or 180°), while the dichlorophenyl group prefers to be perpendicular to the amide bond ($\beta = 90°$ or 270°), which constitutes the lowest energy conformation. In contrast, the torsional strain reaches a maximum of 99 kcal/mol when the amide bond is perpendicular to the aniline ring ($\alpha = 90°$ or 270°), and is coplanar with the dichlorophenyl ring ($\beta = 0°$ or 180°). These results can be understood by maximizing the resonance effect between the amide bond and the aromatic ring while avoiding steric repulsions between the chlorine atoms and the amide bond. Clearly, neither mode *b* nor *d* cyclization is desirable in maintaining the preferred dihedral angle β at 90° or 270°, and only mode *c* is capable of preserving the preferred conformation of the aryl anilide. Therefore, mode *c* cyclization was pursued in our synthesis.

Synthesis of the benzoxazole derivatives followed the procedures illustrated in Scheme 1. The key steps are benzoxazole formation[7] and cinchona alkaloid-catalyzed alkylation of glycinate developed by Corey et al.[8] Compound **7** was studied in the binding assays and was found to be significantly less active than **2** (1.7 nM vs 0.27 nM) against VLA-4. Since little conformational change from anilide to benzoxazole was expected, it was postulated that the loss of potency was due to less optimal binding to the oxazole versus the original amide. Assuming that the amide bond oxygen is interacting with the VLA-4 receptor through hydrogen-bonding interactions, a better hydrogen bond acceptor than the oxygen of benzoxazole **7** may be more desirable. To test this hypothesis, the regioisomeric benzoxazole and the benzimidazole derivative were synthesized (Scheme 2). In both cases, moderate to significant improvement in potency against activated and unactivated states of VLA-4 were observed (Table 1: **7** vs **9a** and **14a**: 1.7 nM, vs 0.74 nM and 0.54 nM (Mn^{2+} states); 33 nM vs 3.6 nM, and 1.8 nM (Ca^{2+}/Mg^{2+} states)), which is consistent with the improved hydrogen bonding capability of the oxygen of benzoxazole **9** and the nitrogen of benzimidazole **14**. Three other compounds (**9b**,**c**, and **14b**) were also prepared based on literature precedents,[9] and they were also found to be potent VLA-4 antagonists. Significant activities against $\alpha_4\beta_7$ were also observed for these three compounds, which is consistent with previous findings that arylsulfonyl group is essential for $\alpha_4\beta_1$ selectivity.[4]

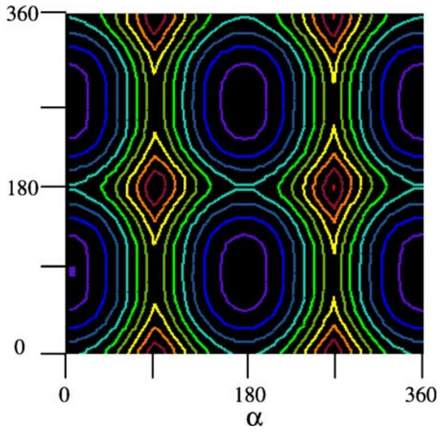
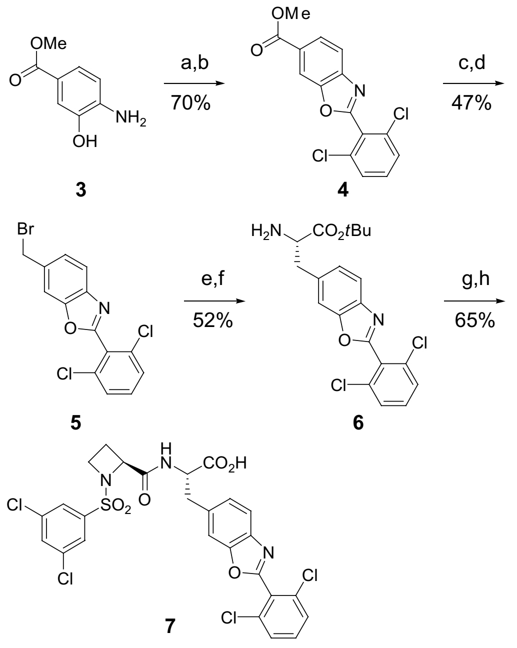
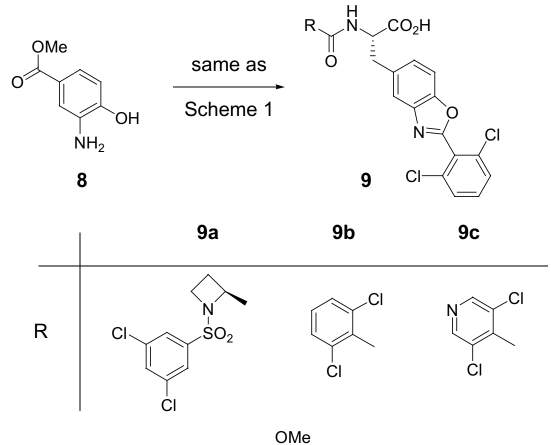


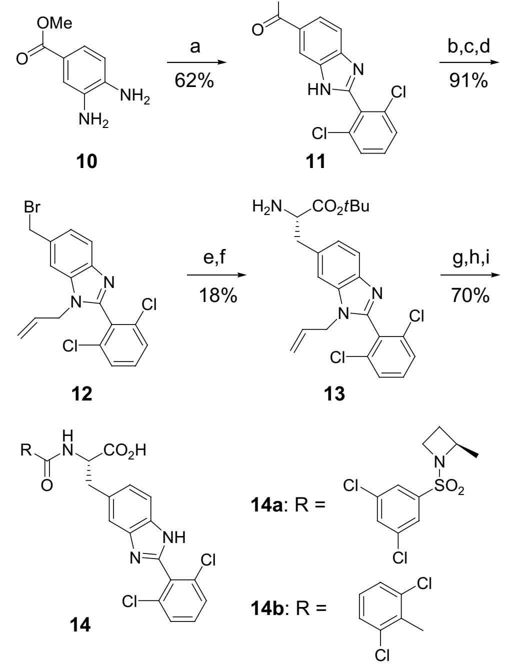
Figure 1. Torsional strain of fragment B as a function of dihedral angle α and β (MacroModel v5.5). The energy level is color coded with purple being the lowest at 219 kcal/mol and red being the highest at 318 kcal/mol.



Scheme 1. Reagents and conditions: (a) 2,6-Cl_2–PhCHO, toluene, reflux; (b) $Pb(OAc)_4$, AcOH 0 °C to rt, 4 h; (c) $LiAlH_4$, THF, −30 to 0 °C; (d) NBS, Me_2S, CH_2Cl_2, −20 °C to rt; (e) $Ph_2C{=}NCH_2CO_2t$Bu, CsOH monohydrate, catalyst, CH_2Cl_2 (Ref. 8); (f) AcOH/THF/H_2O, 1:1:1, rt, 30 min; (g) RCO_2H, PyBop, *i*Pr_2NEt, 0 °C to rt; (f) TFA, CH_2Cl_2, rt.

The pharmacokinetic properties of these new compounds were evaluated in the rat (Table 1).[10] The bioavailability of benzoxazole **9a** improved significantly in comparison with **2** (F%: 15 vs 0.7), suggesting that the polar anilide bond may be responsible for the poor bioavailability of **2**. This is also consistent with the observed decrease in bioavailability of **14a**, which is less lipophilic than both **2** and **9a** (log *D* for **2**, **9a**, and **14a**:





Scheme 2. Reagents and conditions: (a) 2,6-Cl_2–PhCHO, $PhNO_2$, toluene, reflux; (b) allyl bromide, Cs_2CO_3, CH_3CN, rt, 2 h, 1:1 mixture of regioisomers (only one is shown); (c)–(g) same as in Scheme 1; (h) $PhSiH_3$, $Pd(PPh_3)_4$, AcOH, CH_2Cl_2, rt, 4 h; (i) TFA, CH_2Cl_2.

Table 1. Summary of binding and pharmacokinetic properties[10]

Compound	$\alpha_4\beta_1$		$\alpha_4\beta_7$	Clearance (mL/kg/min)	F%
	Mn^{2+} IC_{50} (nM)	Ca^{2+}/Mg^{2+} IC_{50} (nM)	Mn^{2+} IC_{50} (nM)		
1	0.07	0.27	14	97	9
2	0.27	0.31	22	33	0.7
7	1.7	33	1695	39	8
9a	0.74	3.6	179	24	15
9b	0.79	52	5.5	9	20
9c	0.34	21	4.3	25	25
14a	0.54	1.8	105	21	0.3
14b	0.69	22	3.2	24	1.2

3.0, 4.3, and 2.9, respectively).[11] Two aryl derivatives **9b** and **9c** were also studied in the rat. In particular, **9c** emerged as a potent dual $\alpha_4\beta_1$ and $\alpha_4\beta_7$ antagonist with good pharmacokinetic properties in the rat. These compounds have much-reduced molecular weight and are very attractive candidates for further evaluations.

In summary, we have designed and synthesized a number of heterocyclic mimetics for the anilide bond based on molecular modeling. Excellent potency was retained by positioning a suitable hydrogen bond acceptor to mimic the amide bond oxygen. The removal of the hydrogen bond donor (N–H) led to improved lipophilicity and bioavailability. In the process, **9a** was identified as a potent, specific, and bioavailable VLA-4 antagonist, while **9c** was found to be a potent and bioavailable dual antagonist of VLA-4 and $\alpha_4\beta_7$.

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