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Specific and Dual Antagonists of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ Integrins

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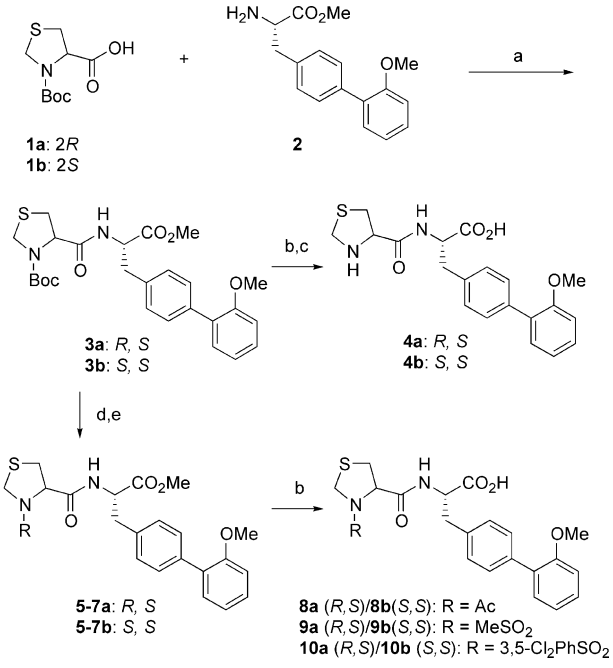
Abstract—*N*-(3,5-Dichlorophenylsulfonyl)-(*R*)-thioprolyl biarylalanine **10a** has been identified as a potent and specific antagonist of the $\alpha_4\beta_1$ integrin. Altering the configuration of thioproline from *R* to *S* led to a series of dual antagonists of $\alpha_4\beta_1$ and $\alpha_4\beta_7$, and the *N*-acetyl analogue **8b** was found to be the most potent dual antagonist. A binding site model for $\alpha_4\beta_1$ and $\alpha_4\beta_7$ is proposed to explain the structure–activity relationship.

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 or VLA-4) is expressed on many leukocytes including eosinophils, basophils, and monocytes, but not on platelets. VLA-4 antagonists may have potential 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 bowel disease.[2] Although a specific inhibitor of either $\alpha_4\beta_1$ or $\alpha_4\beta_7$ may be desirable, a dual antagonist may be advantageous to achieve maximum efficacy.[3] In this paper, we wish to report a series of *N*-substituted thioprolyl biarylalanine derivatives as specific or dual antagonists of VLA-4 and $\alpha_4\beta_7$, and the specificity can be modulated by varying the *N*-substituent and the stereochemistry of thioproline. A binding site model was developed, and was used to design more potent VLA-4 antagonists.

The synthesis of these molecules is illustrated in Scheme 1. Either 2(*R*)- or 2(*S*)-thioproline (**1a** or **1b**) was reacted with **2**[4] to provide the respective diastereomers **3a**/**3b**. Deprotection and hydrolysis provided **4a**/**4b**. Acylation or sulfonylation afforded derivatives **8**–**10** after hydrolysis.

The inhibition of VLA-4[5] or $\alpha_4\beta_7$[6] was determined for each compound (Table 1).

In the 2(*R*)-thioproline series (**4a** and **8a**–**10a**), the potency against VLA-4 increased as the *N*-substituent



Scheme 1. (a) EDC, HOBt, *N*-methylmorpholine, CH_2Cl_2; (b) LiOH, MeOH/THF/H_2O; (c) TFA/CH_2Cl_2; (d) HCl/EtOAc; (e) Ac_2O, iPr_2-NEt, CH_2Cl_2/THF, 0 °C to rt.

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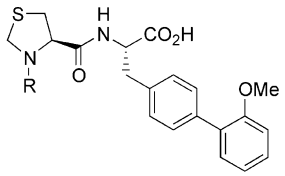
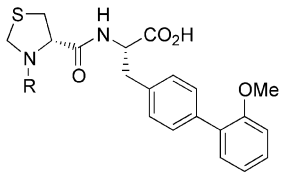
Table 1. Binding results of **4**, **8**, **9**, and **10**





Compd	R	$\alpha_4\beta_1$ IC_{50} (nM)	$\alpha_4\beta_7$ IC_{50} (nM)	$\frac{\alpha_4\beta_7}{\alpha_4\beta_1}$	Compd	R	$\alpha_4\beta_1$ IC_{50} (nM)	$\alpha_4\beta_7$ IC_{50} (nM)	$\frac{\alpha_4\beta_7}{\alpha_4\beta_1}$
4a	H	13	1232	95	**4b**	H	8.2	197	24
8a	Ac	0.90	50	56	**8b**	Ac	0.20	2.1	10
9a	$MeSO_2$	0.42	13	31	**9b**	$MeSO_2$	0.39	8.9	23
10a	3,5-Cl_2PhSO_2	0.30	306	1020	**10b**	3,5-Cl_2PhSO_2	6.0	160	27

changed from a hydrogen to the larger 3,5-dichlorophenylsulfonyl group, suggesting specific interactions between the arenensulfonyl group and VLA-4. The potency against the $\alpha_4\beta_7$ integrin varied with **9a** being the most potent. In the 2(*S*)-thioproline series (**4b** and **8b**–**10b**), potencies against both VLA-4 and $\alpha_4\beta_7$ varied as the size of the *N*-substituent increased.

To explain these results, a working hypothesis was formulated as illustrated in Figure 1. It is proposed that there are three major binding sites (S_1, S_2, and S_3) for both $\alpha_4\beta_1$ and $\alpha_4\beta_7$. The common biarylalanine segment anchors the compounds into the S_3 site as shown. The two receptors may also share an S_2 site, which are comparable in size. The major difference between the two integrins may be the size discrepancy of their respective S_1 site. Thus, in the 2(*R*) series, binding interactions with $\alpha_4\beta_1$[7] increased as the size of the *N*-substituent changes from hydrogen to 3,5-dichlorophenylsulfonyl group due to extra contacts with the S_1 site of $\alpha_4\beta_1$, and **10a** is the most potent in the series. The S_1 site of $\alpha_4\beta_7$ may be smaller, and it cannot accommodate the larger 3,5-dichlorophenylsulfonyl group. As a result, **10a** is also the more specific antagonist of VLA-4. In the 2(*S*)-thioproline series, it is assumed that the configuration alteration causes a conformation change, and the S_1 site is no longer accessible by the *N*-substituent. Instead, the *N*-substituent is better oriented to fit into the S_2 site. Since the S_2 site may be comparable in size for both $\alpha_4\beta_1$ and $\alpha_4\beta_7$, similar potency was observed against both receptors for the series. Due to moderate size of the S_2 site, the *N*-acetyl analogue **8b** is likely to fit the best, and it is the most potent dual antagonist. A related structure was reported by Archibald and co-workers as a dual $\alpha_4\beta_1$ and $\alpha_4\beta_7$ antagonist,[3e] which is also consistent with this binding site model.

To support this binding site model, two pairs of compounds were prepared to probe the S_2 site of $\alpha_4\beta_1$ by incorporating a hydroxy group at the 3-position of proline (Scheme 2). It is reasonable to assume that the conformation of (L)-proline is similar to that of (*R*)-thioproline.[8]

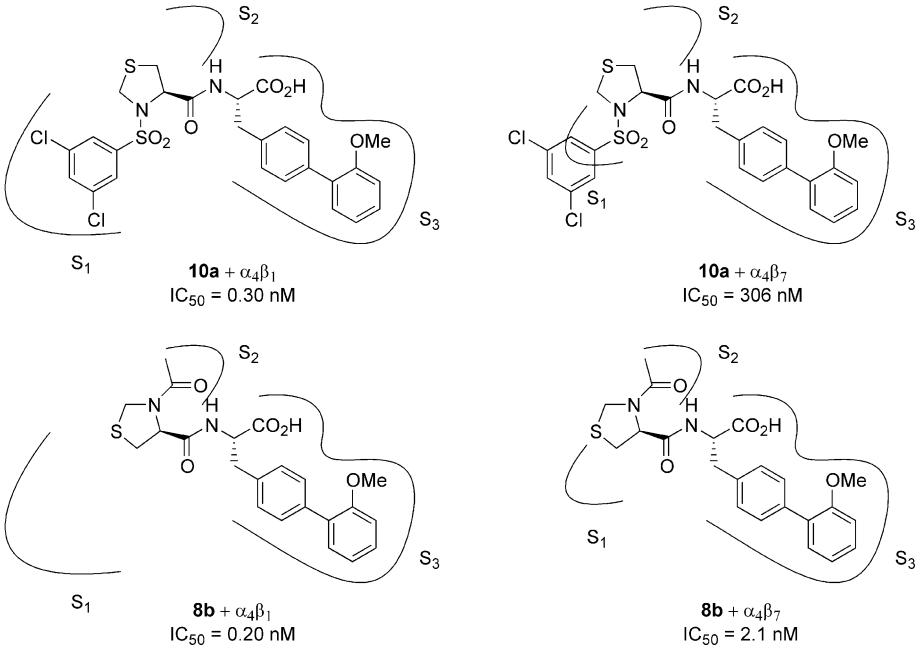
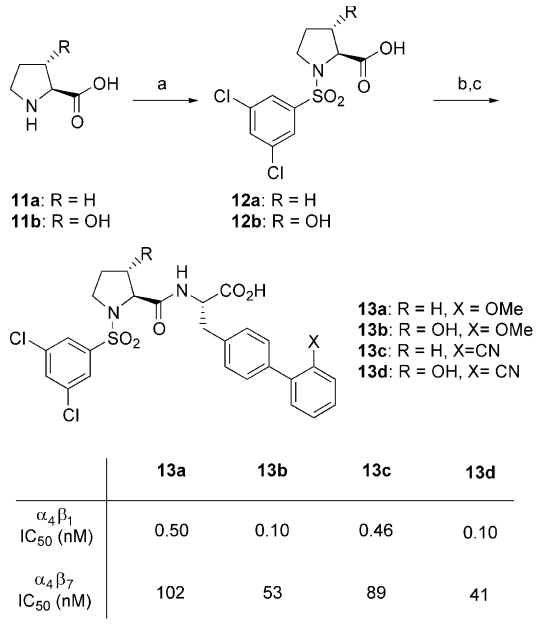


Figure 1. Proposed binding interactions of $\alpha_4\beta_1$ and $\alpha_4\beta_7$.



	13a	13b	13c	13d
$\alpha_4\beta_1$ IC_{50} (nM)	0.50	0.10	0.46	0.10
$\alpha_4\beta_7$ IC_{50} (nM)	102	53	89	41

Scheme 2. (a) 3,5-Cl_2–$PhSO_2Cl$, Na_2CO_3, H_2O, rt; (b) PyBop, *i*Pr_2NEt, CH_2Cl_2; (c) LiOH, MeOH/THF/H_2O.

When studied in the binding assays, the 3-hydroxyproline derivatives (**13b**/**13d**) are significantly more potent (5-fold) against $\alpha_4\beta_1$ than the proline derivative (**13a**/**13c**), which is in agreement with the hypothesis that an S_2 binding site exists for the $\alpha_4\beta_1$ receptor. A 2-fold increase in potency was also observed against $\alpha_4\beta_7$, which is consistent with additional contacts with the S_2 site of the $\alpha_4\beta_7$ integrin. The absolute binding affinities for $\alpha_4\beta_7$ are still relatively poor, presumably due to unfavorable binding to the S_1 site as described earlier. Dappen and co-workers have reported $\alpha_4\beta_1$ potency enhancement of a 3,3-dimethyl but not the 3-(*S*)-phenyl substituted proline analogue over the unsubstituted proline derivative.[9] The former may be explained by additional interactions between the methyl groups and the S_2 site of $\alpha_4\beta_1$. In the latter case, the phenyl group may be too bulky to fit into the S_2 site as also seen with **9b**/**10b**.[10] This binding site model was further explored for the design of a series of extremely potent VLA-4 antagonists, which will be the subject of another report.

In summary, we have identified the *N*-3,5-dichlorophenylsulfonyl (*R*)-thioprolyl biarylalanine derivative **10a** as a potent and specific antagonist of VLA-4. Altering the configuration of thioproline from *R* to *S* led to a series of dual antagonist of VLA-4 and $\alpha_4\beta_7$, and **8b** was found to be the most potent dual antagonist. Structure–activity relationship analysis led to a binding site model, which served as a guide in the design of more potent antagonists of VLA-4 and $\alpha_4\beta_7$.

Acknowledgements

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5. Details of a competitive binding assay between human Jurkat cells and a radiolabeled ^{125}I-VCAM-immunoglobulin fusion protein (^{125}I-VCAM-Ig) have been disclosed (Durette, P. L; Hagmann, W. K.; MacCoss, M.; Mills, S.; Mumford, R. PCT Application WO98/53817A1, 1998; *Chem. Abstr.* **1998**, *130*, 52736s). Briefly, human Jurkat cells were suspended in binding buffer (25 mM HEPES, 150 mM NaCl, 3 mM KCl, 2 mM glucose, 0.1% bovine serum albumin, pH 7.4) supplemented with $MnCl_2$ (1 mM), placed in Millipore MHVB multiscreen plates±compounds in DMSO, incubated at room temperature for 30 min, filtered on a vacuum box, and washed with 100 μL of binding buffer containing 1 mM $MnCl_2$. After insertion of the plates into adapter plates, 100 μL of Microscint-20 (Packard cat# 6013621) was added to each well. The plates were then sealed, placed on a shaker for 30 s, and counted on a Topcount microplate scintillation counter (Packard). Control wells containing DMSO alone were used to determine the level of VCAM-Ig binding corresponding to 0% inhibition. Control wells in which cells were omitted were used to determine the level of binding corresponding to 100% inhibition. Percent inhibition was then calculated for each test well and the IC_{50} was determined from a 10-point titration using a validated four parameter fit algorithm. All titrations were run in duplicate.
6. Details of a competitive binding assay between human RPMI-8866 cells (a human B-cell line $\alpha_4^+\beta_1^-\beta_7$ was a gift from Professor John Wilkins, University of Manitoba, Canada) and radiolabeled ^{125}I-MAdCAM-immunoglobulin fusion protein (^{125}I-MAdCAM-Ig) have been disclosed[5] and are similar to the VLA-4 binding assay.
7. The *N*-substituents (H, MeCO and RSO_2) also differ in hydrogen bonding capability, hybridization of the nitrogen and therefore the orientation the substituent, etc. Various alkyl and aryl including heteroaryl sulfonyl groups were studied,[8] and the SAR of these compounds are best correlated with the size of the alkyl or aryl group.
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10. The substituents that can favorably interact with the S_2 site include small groups such as Ac, $MeSO_2$, Me and OH, but not groups that can ionize under physiological conditions such as COOH and NH_2. More detailed studies will be the subject of another report.