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Aza-bicyclic amino acid sulfonamides as $\alpha_4\beta_1/\alpha_4\beta_7$ integrin antagonists

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Abstract—The design, synthesis, and biological activity of novel $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin antagonists, containing a bridged azabicyclic nucleus, are reported. Conformational analysis of targets containing an azabicyclo[2.2.2]octane carboxylic acid and known integrin antagonists indicated that this azabicycle would be a suitable molecular scaffold. Variation of substituents on the pendant arylsulfonamide and phenylalanine groups resulted in potent $\alpha_4\beta_1$-selective and dual $\alpha_4\beta_1/\alpha_4\beta_7$ antagonists. Potent compounds **11i**, **11h**, and **14** were effective in the antigen-sensitized sheep model of asthma. ©2003 Elsevier Science Ltd. All rights reserved.

Integrins are heterodimeric receptors, with α and β components, that are involved in cell adhesion. Two representatives of the α_4 integrin family, $\alpha_4\beta_1$ and $\alpha_4\beta_7$, are important in the adhesion of lymphocytes to the extracellular matrix.[1] Integrin $\alpha_4\beta_1$ (VLA-4) binds to vascular cell adhesion molecule-1 (VCAM-1), which is expressed on endothelial cell surfaces. The successful blockade of leukocyte infiltration by antibodies to VLA-4 indicates the potential therapeutic utility of VLA-4 antagonists in treating inflammatory disorders, such as asthma, multiple sclerosis, and rheumatoid arthritis. Integrin $\alpha_4\beta_7$ plays a key role in lymphocyte homing in the intestinal mucosa via interaction with mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which is expressed on epithelial cell surfaces. The blockade of this interaction was found to be beneficial in the treatment of inflammatory bowel and Crohn's diseases.[1] In addition to binding to MAdCAM-1, $\alpha_4\beta_7$ integrins interact with VCAM-1 and fibronectin, albeit to a lesser extent.

There are many reports in the scientific and patent literature of small-molecule α_4 integrin antagonists.[2] A major structural class is the *N*-acylphenylalanines, with the *N*-acyl group frequently resembling proline. Representative compounds **1**,[3] **2**,[4] **3**,[5] **4**,[6] and **5**[7] (Fig. 1) have exhibited good in vitro activity in cell adhesion and ELISA assays. We sought to investigate a novel molecular scaffold for the *N*-acyl core in the form of 2-azabicyclo[2.2.2]octane (**6**, Fig. 2). Initially, we were concerned that the 2-azabicyclic unit might adversely affect the bioactive conformation of the $\alpha_4\beta_1$ target molecule. Thus, it was important to study the preferred conformations of known $\alpha_4\beta_1$ integrin antagonists, such as **2**–**4**, to determine whether the bicyclic targets would be suitable. Extensive conformational analysis was performed on condensed[8] versions of compounds in Figure 1 by using the Monte Carlo Multiple Minimum (MCMM)[9] search algorithm, followed by energy minimization. The OPLS-AA force field[10] was used in all calculations, with 25,000 steps for the searches and 2000 iterations for minimizations. With regard to protonation of the ionizable groups, we assumed full ionization at physiological pH. To assure that the Monte Carlo simulations converged to a global minimum for each compound, we carried out additional independent Monte Carlo simulations or increased the number of steps in those cases where one of the five low-energy conformers had not converged. Because the low-lying

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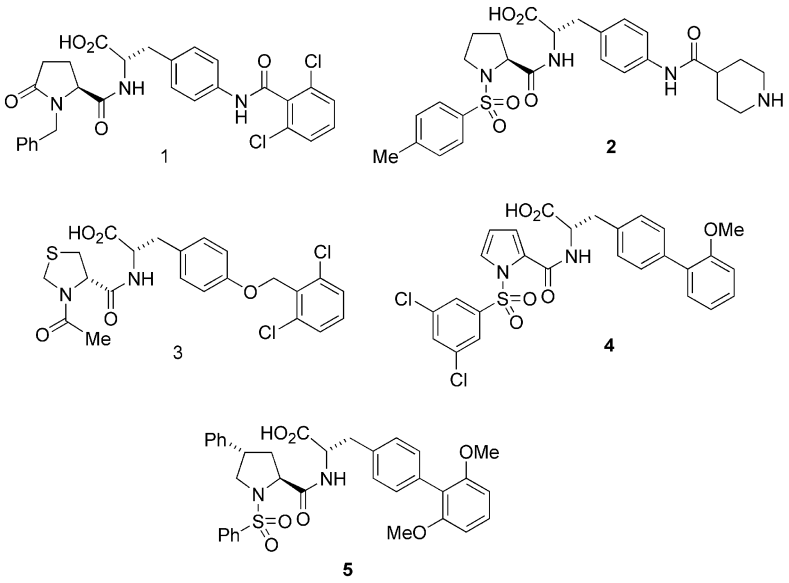


Figure 1. Nonpeptide $\alpha_4\beta_1/\alpha_4\beta_7$ integrin antagonists.

minima were identified several hundred times, it is likely that the sampling was exhaustive.

The global minima of the modeled compounds had the key functional groups, carboxylate, amide nitrogen, and phenyl ring (phenylalanine, Phe), oriented in a similar fashion. This was particularly gratifying, given that **3** (Fig. 1) has a different stereochemistry in the acyl group. This can be appreciated from Figure 3, where a root-means-square fitting approach was used for the overlay.

Next, the conformation of target molecule **6** was compared to pyrrolidine **2**. Figure 4 shows a representative overlap of **6** and **2**, which suggests that the bicyclic moiety does not prevent **6** from achieving the preferred conformation. It should also be noted that the ring nitrogen atoms are positioned on top of each other, which implies that the electron distribution has not been changed.

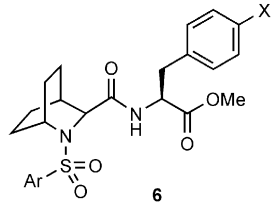


Figure 2. Prototype azabicyclic target **6**.

We have described the successful replacement of proline by azabicyclic amino acids in novel vasopressin receptor antagonists.[11] The synthetic methodology for preparing the requisite bridged bicyclic amino acid **7** in enantiomerically enriched form is well developed.[12] Arylsulfonylation, followed by hydrolysis,[13] then provided key intermediate **8**, and the coupling of **8** with substituted Phe esters provided **9** (Scheme 1), which was used to obtain the targets.

Examples of subsequent transformation of **9** are presented in Schemes 2–4. In Scheme 2, reduction of the nitro group in **9a**, $X = NO_2$, with Zn powder, followed by acylation of the aniline and hydrolysis of the ester group afforded target **11** in 75–90% yield. The preparation of phthalimide **14** is presented in Scheme 3. Because the phthalimide was opened to **13** on basic hydrolysis, treatment with acetic acid was required to regenerate

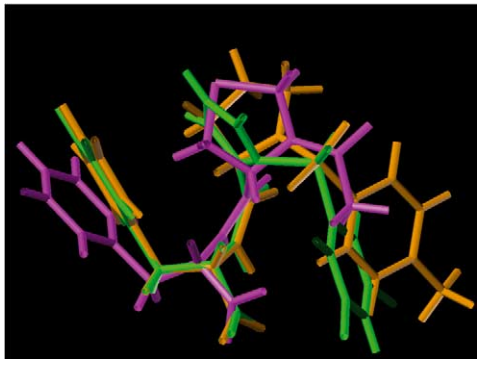


Figure 3. Superposition of the low-energy conformations of **2** (orange), **3** (magenta), and **4** (green).

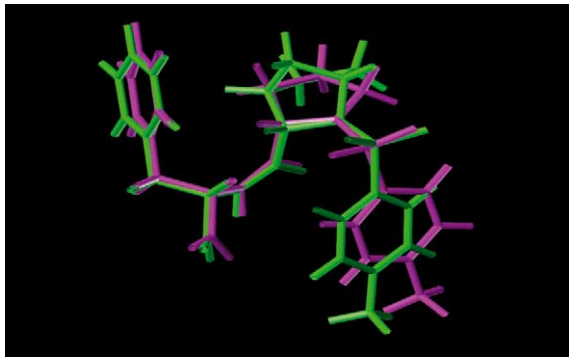
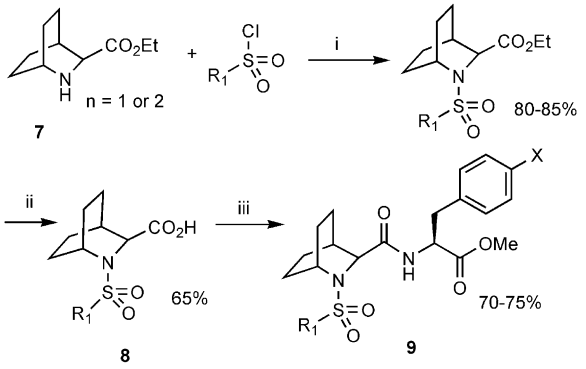


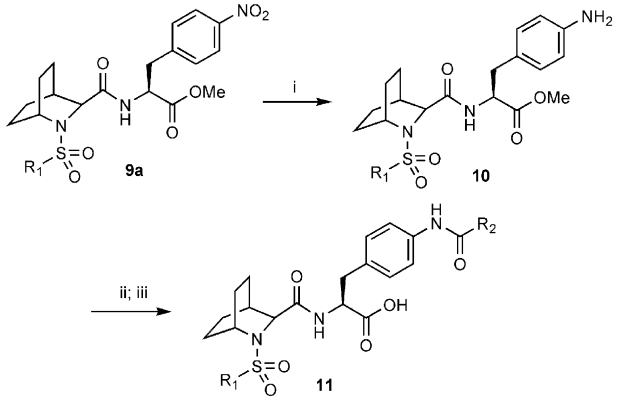
Figure 4. Overlay of **2** (magenta) and **6** (green).

the phthalimide. The Suzuki transformation of **9b** (X = Br) to the aryl-substituted Phe derivative **15** is presented in Scheme 4.

The azabicyclic target compounds were tested for antagonism of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins. The results of the cellular adhesion assays are presented in Tables 1 and 2. The SAR in this series are similar to other N-acyl derivatives of 4-substituted phenylalanine.[14] High potency of the benzamide analogues against the $\alpha_4\beta_1$ integrin was dependent on the presence of 2,6-disubstitution (e.g., **11b**) in the phenyl ring. Monosubstituted analogues demonstrated moderate potency, whereas the unsubstituted compound **11a** has low activity. Substitution of phenyl group of Phe in the 2-position or 3-position, leaving the 4-position unsubstituted, or inversion of the chirality of Phe resulted in a larger than 100-fold decrease in potency.

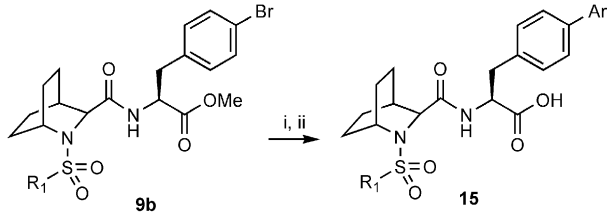


Scheme 1. (i) Et_3N, CH_2Cl_2, 23 °C; (ii) LiOH or NaOH, MeOH, water, 60 °C; (iii) substituted Phe methyl ester, EDAC/HOBt, *i*-Pr_2NEt/CH_2Cl_2, 23 °C.

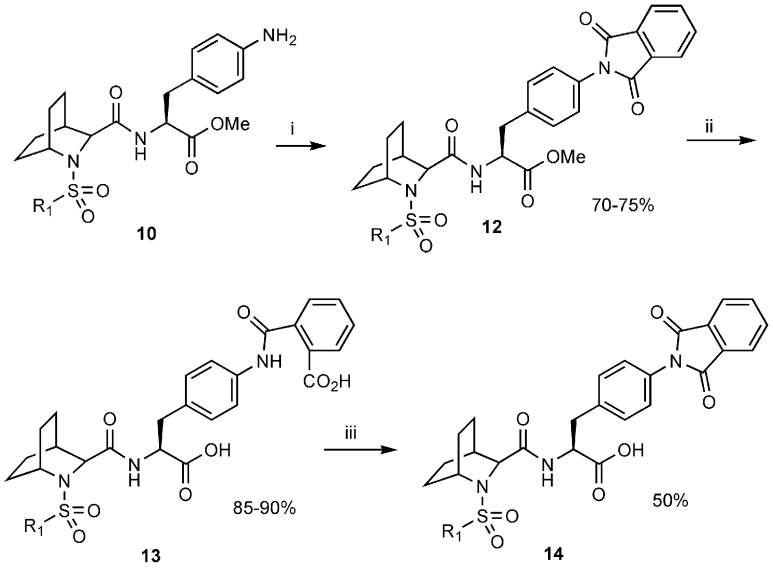


Scheme 2. (i) Zn, NH_4Cl, MeOH, reflux, 3 h; (ii) RCOCl, Et_3N, CH_2Cl_2; (iii) LiOH, MeOH, water, 23 °C.

The configuration of the chiral center of the bicyclic amino acid strongly affects in vitro activity as well. The (*S*)-enantiomers are significantly more active than the *R*-enantiomers (**11f** vs **11g**). Additional substitution of the 4-position of the benzamide (**11c**) does not affect the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ activities, while introduction of the basic nitrogen atom improves potency, compensating for the lack of 2,6-disubstitution (**11h**). Linkers between the aromatic ring carbonyl and R_2, such as CH_2 (**11d**) or NH (**11e**), dramatically decreased in vitro potency. Various arylsulfonamide groups could be introduced to provide compounds with better dual activity, with phenyl (e.g., **11i**) and 2-thienyl (e.g., **11k**) derivatives being the best. Phthalimide[15] **14** was a strong $\alpha_4\beta_1$ antagonist, with the carbonyl groups probably playing a similar role to the 2,6-disubstitution of other analogues. The disubstituted biphenyl **15** has good potency,

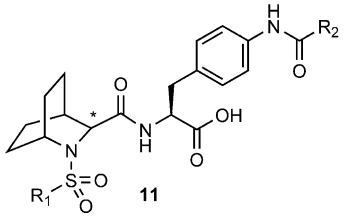


Scheme 4. (i) $ArB(OH)_2$, $Pd(PPh_3)_4$, 50–65%; dimethoxyethane; (ii) LiOH, MeOH, water, 75–90%.



Scheme 3. (i) phthalic anhydride, AcOH, 120 °C; (ii) NaOH, MeOH/water, rt; (iii) AcOH, 120 °C.

Table 1. Inhibition of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins by *N*-arylsulfonyl[2.2.2] azabicyclooctane amide derivatives (IC_{50})



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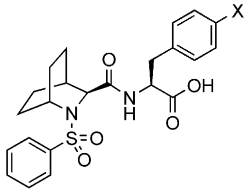
Compd	R_1	R_2	Chirality*	$\alpha_4\beta_1$/VCAM-1 (μM)	$\alpha_4\beta_7$/MAdCAM-1 (μM)
11a	4-MeC_6H_4	Ph	*RS*	0.90	30% @ 5
11b	4-MeC_6H_4	2,6-$Cl_2C_6H_3$	*RS*	0.045±0.006	0.32
11c	4-MeC_6H_4	2,4,6-$Cl_3C_6H_2$	*RS*	0.051±0.019	0.43±0.23
11d	4-MeC_6H_4	CH_2-2,6-$Cl_2C_6H_3$	*RS*	1.1	28% @ 5
11e	4-MeC_6H_4	NH-2,6-$Cl_2C_6H_3$	*RS*	1.6	NT
11f	Ph	2,6-$Cl_2C_6H_3$	*S*	0.019±0.003	0.094±0.065
11g	Ph	2,6-$Cl_2C_6H_3$	*R*	1.6	NT
11h	Ph	4-pyridyl	*S*	0.036±0.009	0.175±0.107
11i	Ph	2,6-Dichloro-4-pyridyl	*S*	0.012±0.004	0.013±0.008
11j	2-Thienyl	2,6-$Cl_2C_6H_3$	*S*	0.030±0.011	0.062±0.029
11k	2-Thienyl	2,6-Dichloro-4-pyridyl	*S*	0.005±0.001	0.032±0.019
11l	5-Cl-2-thienyl	2,6-Dichloro-4-pyridyl	*RS*	0.011±0.002	0.064±0.001
11m	4,5-Cl_2-2-thienyl	2,6-Dichloro-4-pyridyl	*RS*	0.069±0.011	0.014±0.002
11n	2,6-$Cl_2C_6H_3$	2,6-Dichloro-4-pyridyl	*RS*	0.105	0.011±0.003
11o	2-$CF_3C_6H_4$	2,6-Dichloro-4-pyridyl	*RS*	0.081±0.015	0.102±0.045
11p	8-Isoquinolinyl	2,6-Dichloro-4-pyridyl	*S*	0.012±0.002	0.022±0.011
11r	8-Quinolinyl	2,6-Dichloro-4-pyridyl	*S*	0.15	0.151±0.009
3				0.41±0.14	0.090±0.031

Data represent inhibition of binding of $\alpha_4\beta_1+$ cells to immobilized VCAM-1 or $\alpha_4\beta_7+$ cells to immobilized MAdCAM-1. Confidence intervals were usually calculated with $N=3$. All compounds were characterized by using NMR and MS data; they also gave satisfactory elemental analyses. For **3**, the reported literature value for $\alpha_4\beta_1$: $IC_{50}=0.034$ μM.[5]

Table 2. IC_{50} data for other azabicyclic $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin antagonists



Compd	X	$\alpha_4\beta_1$/VCAM-1 (μM)	$\alpha_4\beta_7$/MAdCAM-1 (μM)
14	Phthalimido	0.026±0.003	0.312±0.056
15	2,6-$(MeO)_2C_6H_3$	0.081	0.090
5		0.030±0.004	0.119±0.013

For **5**, the reported literature values for $\alpha_4\beta_1$ and $\alpha_4\beta_7$ are, respectively: $IC_{50}=0.002$ and 0.26 μM.[7]

whereas the unsubstituted analogue of **15** lost activity; similar correlations were observed for Pro analogues.[16]

On the basis of in vitro potency and chemical diversity, we selected **11h**, **11i** and **14** for in vivo studies, and evaluated them in the antigen-sensitized sheep model of asthma.[17] In untreated sheep, aerosol challenge with *Ascaris suum* results in an immediate increase in specific lung resistance (SR_L), which persists for 2–4 h (early-phase bronchoconstrictive response). Between 6 and 8 h post challenge, SR_L increases a second time in the late-phase bronchoconstrictive response, similarly to that observed in humans. Airway hyperreactivity is then examined 24 h post antigen challenge by the amount of carbachol required to provoke a 400% increase in SR_L (PC 400). The test compounds were administered in a single 10-mg aerosol dose 0.5 h prior to antigen challenge and our results are presented in Figure 5. In the control animals, antigen challenge caused an increase in airway resistance in the early phase (EP), and again during the late phase (LP). Airway hyperreactivity (AHHR) was increased at 24 h post challenge. Compound **11h** ($N=3$) and **11i** ($N=3$) exhibited similar degrees of efficacy and potency. Both compounds completely blocked the LP and the AHHR responses. Neither compound affected the EP response. In contrast, **14** completely blocked both the LP and AHHR response and also demonstrated inhibition of the EP response. Results are similar to those reported by Archibald et al. for **3**,[5] which demonstrated efficacy of an $\alpha_4\beta_1$ antagonist in the sheep model at 0.3 mg/kg administered iv 1 h prior to antigen challenge.

In summary, we have discovered novel, potent $\alpha_4\beta_1$-selective and $\alpha_4\beta_1/\alpha_4\beta_7$ dual integrin antagonists by incorporating azabicyclic amino acid group as a proline-mimetic scaffold. Selected compounds exhibited good in vivo activity in the sheep model of asthma, which could reflect their potential in the treatment of asthma and other inflammatory diseases in humans.

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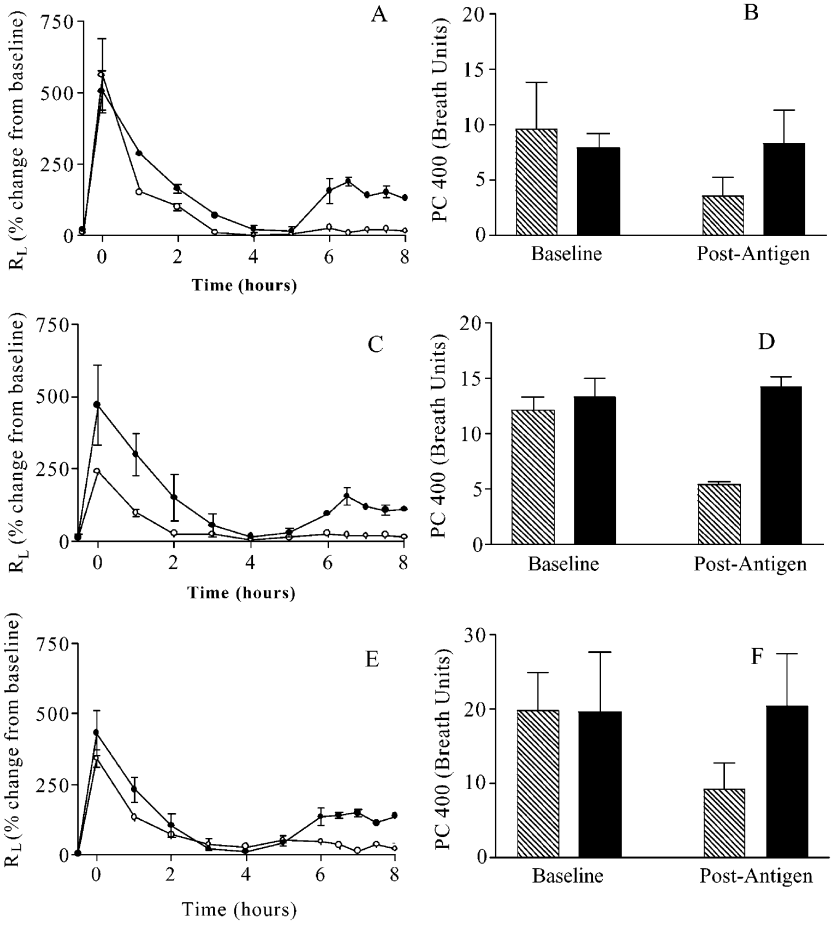

Figure 5. Antigen-induced early-phase and late-phase responses (A, C, E), and airway hyperresponsiveness to carbachol (B, D, F) in the allergic sheep: Solid circles represent control trial and open circles represent treatment trial in the same animals (A, C, E). Hatched bars represent control trial and solid bars represent treatment trial in the same sheep (B, D, F). Results for **11i** are shown in A and B; **14** in C and D; **11h** in E and F. Antigen-induced LAR was completely inhibited by all three compounds (A, C, E) while **14** also inhibited the EAR (C). Each compound completely reversed the airway hyperresponsiveness (indicated by the decrease in post-antigen PC400) to carbachol (B, D, F). A 10-mg dose in the sheep model is equivalent to 0.25 mg/kg based on sheep weights.[5]

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