

## Design of LFA-1 antagonists based on a 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one scaffold

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**Abstract**—A new class of lymphocyte function-associated antigen-1 (LFA-1) antagonists is described. Elaboration of the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one scaffold resulted in the synthesis of potent inhibitors of the LFA-1/ICAM-1 interaction. Along with the *in vitro* activity, we present the X-ray crystal structure of the complex of compound **9b**, in a novel binding mode to the I-domain of LFA-1.

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The cell adhesion molecule LFA-1 (lymphocyte function-associated antigen-1), also known as CD11a/CD18, is known to play a crucial role in many immunological functions including antigen presentation, leukocyte trafficking, B- and T-cell activation. LFA-1 belongs to the  $\beta_2$  integrin family and is found on all leukocytes.<sup>1</sup> The ligands for LFA-1, the ICAMs 1–3 (intercellular adhesion molecules), are expressed on both leukocytes and the endothelium. In particular, the interaction of LFA-1 with ICAM-1 results in leukocyte adhesion to the endothelium and facilitates their extravasation to tissue sites.<sup>2,3</sup> The LFA-1/ICAM interaction has been proposed to be an attractive therapeutic target in the treatment of several inflammatory disorders such as psoriasis and transplant rejection.<sup>4,5</sup> The recent approval of efalizumab (Raptiva®), a fully humanized anti-LFA-1 antibody for the treatment of moderate to severe psoriasis, provides excellent validation of the target.<sup>6</sup>

In the search for small molecules that would interfere with the LFA-1/ICAM-1 interaction, we and others

have disclosed several diverse chemical series of compounds.<sup>7,8</sup> Some representative structures are shown in Figure 1. NMR and X-ray co-crystallographic studies have established that many of these inhibitors bind to the IDAS site (I-domain allosteric site) of the I-domain located on the CD11a unit.<sup>7–9</sup> The X-ray structure of our clinical candidate BMS-587101<sup>7a</sup> in complex with the I-domain of LFA-1 shows the following: (i) the

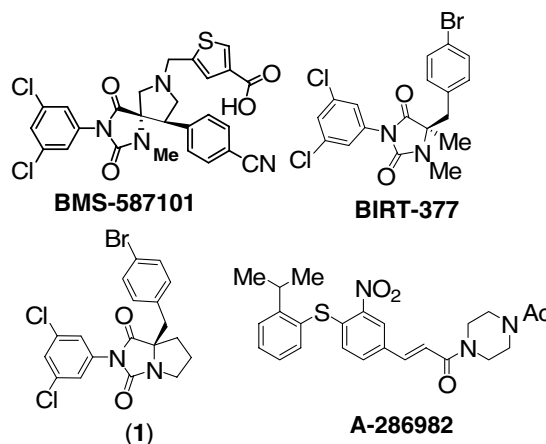


Figure 1. Representative LFA-1 antagonists reported in the literature.

**Keywords:** LFA-1 antagonists; 2,3-Dihydro-1*H*-pyrrolizin-5(7*aH*)-one; LFA-1/ICAM interaction.

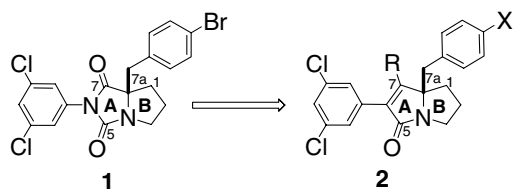
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*p*-cyanophenyl aromatic ring forms a favorable edge-to-face  $\pi$ - $\pi$  interaction with the 3,5-dichlorophenyl ring; (ii) the urea carbonyl is hydrogen bonded to the amino acid residues Lys-NH<sub>2</sub> 305, Lys-NH<sub>2</sub> 287, and Glu-COOH 284 via a water molecule. Unlike the urea carbonyl, the amide carbonyl does not appear to make any discernible contacts with the protein.

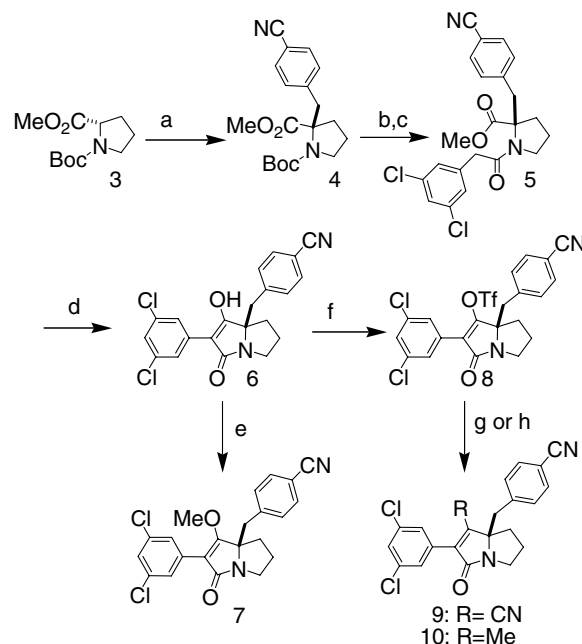
As a continuation of our work<sup>7b</sup> on the modification of the reported bicyclic[5.5]hydantoin core **1**,<sup>8c</sup> we herein describe exploration of the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one core **2** as a suitable surrogate in the design of LFA-1 inhibitors. Replacement of the N-6 nitrogen of the urea with a carbon atom and introduction of unsaturation in ring A provide structural rigidity similar to the bicyclic[5.5]hydantoin class of compounds, while retaining the all important carbonyl (C-5) as well as the 3,5-dichlorophenyl and benzyl groups necessary for high affinity. In addition, the pyrrolizinone core allows for elaboration at the C-7 position, thus providing an understanding of the SAR at this position (Fig. 2).

The synthesis of the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-ones is outlined in Schemes 1 and 2. In our initial approach (Scheme 1), homochiral *N*-*boc*-L-proline methyl ester (**3**) was elaborated to give 7-hydroxy pyrrolizin-5-one **6** in reasonable overall yield. Compound **6** served as the key intermediate for the synthesis of compounds **7**, **9**, and **10**. Unfortunately, C–C coupling of the *O*-triflate **8** was not general and failed with copper mediated alkyl/aryl Grignards or lithium reagents. This necessitated the development of a more general approach utilizing **3** as the starting material (Scheme 2). The new sequence (Scheme 2) was used to prepare compounds **15**–**18**.

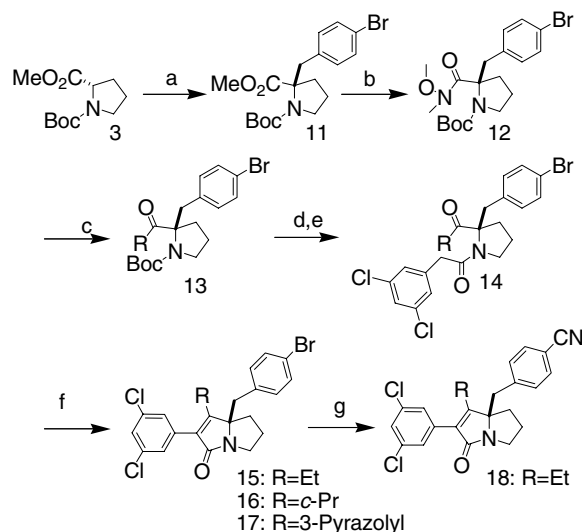
Inhibition of the LFA-1/ICAM binding interaction using the HeLa/HSB assay<sup>7b</sup> for a limited number of racemic 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-ones is shown in Table 1. The data clearly suggest that the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one core is an excellent surrogate for the bicyclic[5.5]hydantoin system. The enol **6** was essentially inactive, as was the bulky 3-pyrazolyl derivative **17**. Compound **7** with a H-bond accepting methoxy group at C-7 displayed sub-micromolar potency in the HeLa/HSB assay. The potency increase was significantly more pronounced for compounds **9** (R = CN) and **18** (R = Et), which displayed IC<sub>50</sub>s of 40 and 52 nM, respectively, in the HeLa/HSB binding assay. The C-7 SAR for the cyano analog (**9**) and the ethyl analog (**18**) was intriguing considering the hydrophilic and hydrophobic nature of these two substituents. These data suggested that either (i) the C-7 position of



**Figure 2.** Bicyclic[5.5]hydantoin core (**1**) and 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one core (**2**).



**Scheme 1.** Reagents and conditions: (a) i—LiHMDS, THF,  $-78^{\circ}\text{C}$ , ii—4-cyanobenzyl chloride, THF, 30%; (b) 4 N HCl-dioxane; (c) (3,5-dichlorophenyl)acetic acid, EDCI, DIPEA, DMF, 95%; (d) LiHMDS, THF, 90%; (e) (MeO)<sub>3</sub>PO, K<sub>2</sub>CO<sub>3</sub>, reflux, 10 h, 30%; (f) Tf<sub>2</sub>O, DIPEA, DCM,  $-10^{\circ}\text{C}$ , 60%; (g) Pd(PPh<sub>3</sub>)<sub>4</sub>, Zn(CN)<sub>2</sub>, DIPEA, DMF,  $195^{\circ}\text{C}$ , 10 min microwave, 75%; (h) (CH<sub>3</sub>)<sub>2</sub>(CN)CuLi, THF,  $-78^{\circ}\text{C}$ , 15%.



**Scheme 2.** Reagents and conditions: (a) i—LiHMDS, THF,  $-78^{\circ}\text{C}$ , ii—4-bromobenzyl bromide, THF, 85%; (b) LiN(OMe)Me, THF,  $-10^{\circ}\text{C}$ ; (c) RMgBr or lithium pyrazol-1-ylid-3-yl lithium, THF rt; (d) 4 N HCl-dioxane; (e) (3,5-dichlorophenyl)acetic acid, EDCI, DIPEA, DMF, 90–95%; (f) 5% KOH in MeOH, reflux (60–80%); (g) Pd(PPh<sub>3</sub>)<sub>4</sub>, Zn(CN)<sub>2</sub>, DMF,  $200^{\circ}\text{C}$ , 20 min microwave (80%).

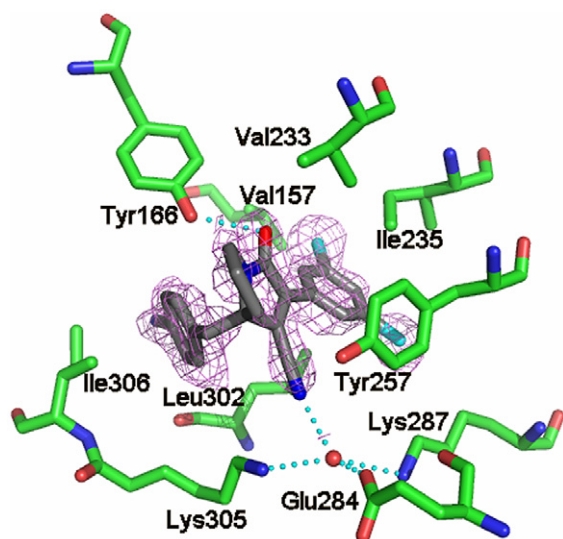
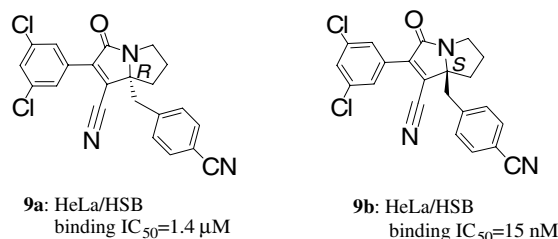
the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one core tolerates a variety of lipophilic and hydrophobic substituents or (ii) the binding mode for these compounds with the I-domain of LFA-1 may be different. To further understand this discrepancy in the SAR, the cyano analog (**9**) was co-crystallized with the I-domain of LFA-1.

**Table 1.** In vitro activity of racemic 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-ones

| Compound        | R                | X  | HeLa/HSB binding <sup>a</sup><br>(IC <sub>50</sub> or % inhibition) |
|-----------------|------------------|----|---------------------------------------------------------------------|
| <b>BIRT-377</b> |                  |    | 26 nM <sup>b</sup>                                                  |
| <b>6</b>        | OH               | CN | 27% at 1 μM                                                         |
| <b>7</b>        | OCH <sub>3</sub> | CN | 430 nM                                                              |
| <b>9</b>        | CN               | CN | 40 nM                                                               |
| <b>10</b>       | CH <sub>3</sub>  | CN | 660 nM                                                              |
| <b>15</b>       | Et               | Br | 106 nM                                                              |
| <b>16</b>       | Cyclopropyl      | Br | 200 nM                                                              |
| <b>17</b>       | 3-Pyrazolyl      | Br | 30% at 10 μM                                                        |
| <b>18</b>       | Et               | CN | 52 nM                                                               |

<sup>a</sup> Values are averages of at least two determinations.<sup>b</sup> K<sub>d</sub> value in an LFA-1/ICAM-1 binding assay as reported in the literature.<sup>9</sup>

When racemic **9** was co-crystallized with the I-domain of LFA-1, a single enantiomer (*S*) was found bound to the I-domain. Figure 3 shows the X-ray co-crystal structure of this enantiomer (designated **9b**) determined at 1.75 Å resolution.<sup>10</sup> The X-ray co-crystal structure revealed a novel binding mode for compound **9b** when compared to the hydantoin class of LFA-1 antagonists such as BMS-587101 and BIRT-377, where the absolute stereochemistry at the equivalent of the C-7*a* center is (*R*)<sup>9</sup>. This ‘flipped’ binding mode for compound **9b** is a direct result of the vinyl cyano group at C-7*a* forming a hydrogen bonding network through a water molecule to Lys-NH<sub>2</sub> 287 and Lys-NH<sub>2</sub> 305 and Glu-COOH 284, unlike BMS-587101 where the urea carbonyl, which corresponds to the C-5 carbonyl of **9b**, forms the same hydrogen bonding network. In addition, the Tyr-OH 166, which is too far from the C-2 carbonyl of BMS-587101 to form a H-bond, has now moved in the direction of the carbonyl of **9b**, and is capable of forming a direct H-bond. Interestingly, the 3,5-dichlorophenyl

**Figure 3.** X-ray structure of **9b** complexed with the I-domain of LFA-1. Also shown is the initial electron density (magenta: 2Fo-Fc 1) prior to fitting compound **9b**. The water molecule is shown as a red sphere and hydrogen bonds are shown as small cyan spheres. Figure created using PyMol.<sup>11</sup>**Figure 4.** Absolute stereochemistry of **9a** and **9b**.

group at C-6 is not positioned as deeply in the hydrophobic pocket between α-helices 1 and 7 and β-strands 1, 3, and 4 as was found for BMS-587101.

Compound **9** was separated into its individual enantiomers **9a** and **9b** employing a Chiralpak AD column and the compounds tested for LFA-1/ICAM binding inhibition in the HeLa/HSB assay. As expected, there was a marked difference in the inhibition potential of these enantiomers. The faster eluting enantiomer, **9a**, was much less active and displayed an IC<sub>50</sub> of 1.4 μM, while the slower eluting enantiomer, **9b**, had an IC<sub>50</sub> of 15 nM (Fig. 4). The absolute stereochemistry for **9b** was established as *S* at the 7*a* position employing vibrational circular dichroism (VCD) spectroscopic analysis.<sup>12</sup> This result is consistent with the absolute stereochemistry of the compound in the X-ray co-crystal structure (vide supra).

The accommodation of **9b** in a binding mode opposite to that of the hydantoin class of LFA-1 antagonists is evidence of the flexibility of the I-domain of the LFA-1 protein. Since only **9** was subjected to X-ray crystallographic analysis, we cannot be certain that this opposite ‘flipped’ binding mode is an inherent property of the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one core or just unique to the C-7 cyano substituted compounds in this series. Racemic **18**, containing an ethyl group at C-7, was equipotent to **9** (IC<sub>50</sub> = 52 nM) in the HeLa/HSB binding assay. The lack of hydrogen-bonding ability of the ethyl group does not preclude the possibility that this compound may be binding in the ‘traditional’ mode similar to the hydantoin class of LFA-1 antagonists.

In conclusion, we have designed a novel class of LFA-1/ICAM antagonists based on the 2,3-dihydro-1*H*-pyrrolizin-5(7*aH*)-one scaffold. X-ray crystal structural analysis of our most active compound **9b** revealed a ‘flipped’ binding mode to the I-domain of the LFA-1 protein. The stereochemistry of **9b** was found to be 7*a*(*S*), which is opposite to that found in the hydantoin class of LFA-1 antagonists.

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