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Design of LFA-1 antagonists based on a 2,3-dihydro-1*H*-pyrrolizin-5(7aH)-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(7aH)-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 β_2 integrin family and is found on all leukocytes.[1] 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.[2,3] 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.[4,5] 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.[6]

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.[7,8] 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.[7–9] The X-ray structure of our clinical candidate BMS-587101[7a] in complex with the I-domain of LFA-1 shows the following: (i) the

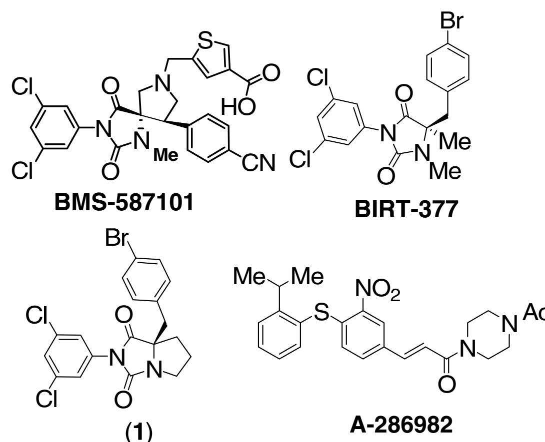

Figure 1. Representative LFA-1 antagonists reported in the literature.

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

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p-cyanophenyl aromatic ring forms a favorable edge-to-face π–π interaction with the 3,5-dichlorophenyl ring; (ii) the urea carbonyl is hydrogen bonded to the amino acid residues Lys-NH_2 305, Lys-NH_2 287, 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[7b] on the modification of the reported bicyclic[5.5]hydantoin core **1**,[8c] we herein describe exploration of the 2,3-dihydro-1*H*-pyrrolizin-5(7aH)-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(7aH)-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[7b] for a limited number of racemic 2,3-dihydro-1*H*-pyrrolizin-5(7aH)-ones is shown in Table 1. The data clearly suggest that the 2,3-dihydro-1*H*-pyrrolizin-5(7aH)-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_{50}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 the 2,3-dihydro-1*H*-pyrrolizin-5(7aH)-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.

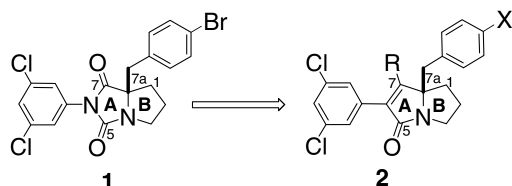
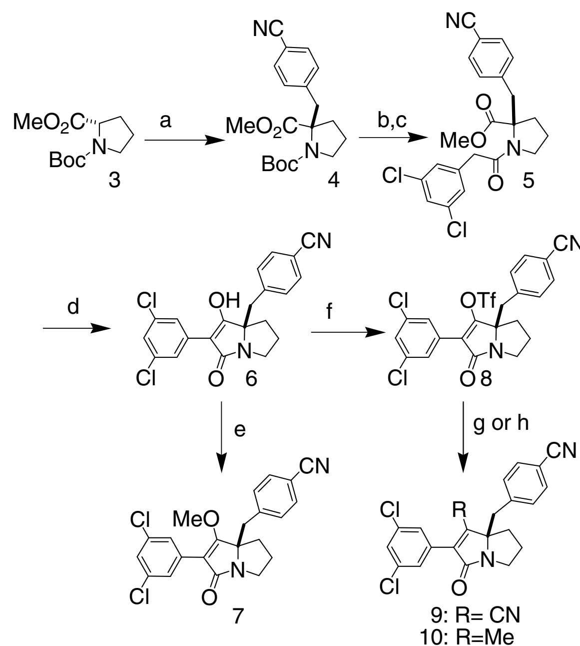
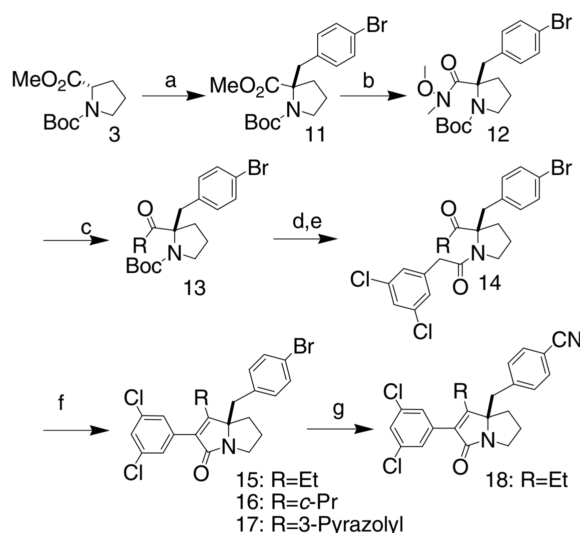


Figure 2. Bicyclic[5.5]hydantoin core (**1**) and 2,3-dihydro-1*H*-pyrrolizin-5(7aH)-one core (**2**).



Scheme 1. Reagents and conditions: (a) i—LiHMDS, THF, −78 °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) $(MeO)_3PO$, K_2CO_3, reflux, 10 h, 30%; (f) Tf_2O, DIPEA, DCM, −10 °C, 60%; (g) $Pd(PPh_3)_4$, $Zn(CN)_2$, DIPEA, DMF, 195 °C, 10 min microwave, 75%; (h) $(CH_3)_2(CN)CuLi$, THF, −78 °C, 15%.



Scheme 2. Reagents and conditions: (a) i—LiHMDS, THF, −78 °C, ii—4-bromobenzyl bromide, THF, 85%; (b) LiN(OMe)Me, THF, −10 °C; (c) RMgBr or lithium pyrazol-1-id-3-yllithium, 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) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 200 °C, 20 min microwave (80%).

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

Compound	R	X	HeLa/HSB binding[a] (IC_{50} or % inhibition)
BIRT-377			26 nM[b]
6	OH	CN	27% at 1 μM
7	OCH_3	CN	430 nM
9	CN	CN	40 nM
10	CH_3	CN	660 nM
15	Et	Br	106 nM
16	Cyclopropyl	Br	200 nM
17	3-Pyrazolyl	Br	30% at 10 μM
18	Et	CN	52 nM

[a] Values are averages of at least two determinations.
[b] K_d value in an LFA-1/ICAM-1 binding assay as reported in the literature.[9]

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.[10] 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-7a center is (*R*)[9]. This 'flipped' binding mode for compound **9b** is a direct result of the vinyl cyano group at C-7a forming a hydrogen bonding network through a water molecule to Lys-NH_2 287 and Lys-NH_2 305 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 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.

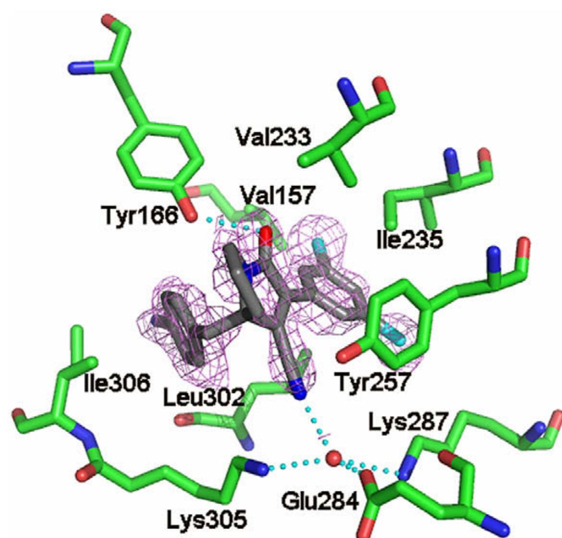


Figure 3. X-ray structure of **9b** complexed with the I-domain of LFA-1. Also shown is the initial electron density (magenta: 2Fo-Fc 1) prior to fitting compound **9b**. The water molecule is shown as a red sphere and hydrogen bonds are shown as small cyan spheres. Figure created using PyMol.[11]

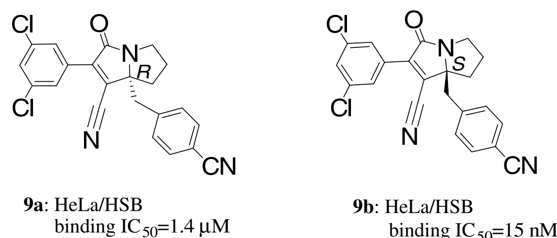


Figure 4. Absolute stereochemistry of **9a** and **9b**.

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_{50} of 1.4 μM, while the slower eluting enantiomer, **9b**, had an IC_{50} of 15 nM (Fig. 4). The absolute stereochemistry for **9b** was established as *S* at the 7a position employing vibrational circular dichroism (VCD) spectroscopic analysis.[12] 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(7aH)-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_{50} = 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(7aH)-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 7a(*S*), which is opposite to that found in the hydantoin class of LFA-1 antagonists.

Acknowledgments

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