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De novo design, synthesis, and in vitro activity of LFA-1 antagonists based on a bicyclic[5.5]hydantoin scaffold

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Abstract—LFA-1 (leukocyte function-associated antigen-1), is a member of the β_2-integrin family and is expressed on all leukocytes. The LFA-1/ICAM interaction promotes tight adhesion between activated leukocytes and the endothelium, as well as between T cells and antigen-presenting cells. Evidence from both animal models and clinical trials provides support for LFA-1 as a target in several different inflammatory diseases. This paper describes the de novo design, synthesis and in vitro activity of LFA-1 antagonists based on a bicyclic[5.5]hydantoin scaffold.

LFA-1 (leukocyte function-associated antigen-1), also known as CD11a/CD18 and $\alpha_L\beta_2$ is a member of the β_2-integrin family and is expressed on all leukocytes.[1] It is a heterodimeric transmembrane glycoprotein consisting of α and β subunits. The ligands, ICAM (intercellular adhesion molecule)-1, -2, and -3, are differentially expressed and regulated on both leukocytes and the endothelium. The LFA-1/ICAM interaction promotes tight adhesion between activated leukocytes and the endothelium, as well as between T cells and antigen-presenting cells. Leukocyte activation results in both a clustering of LFA-1 on the cell surface and a conformational shift in the LFA-1 molecule that correlates with an increase in affinity for binding to ICAM-1.[2,3]

In vivo studies using anti-LFA-1 antibodies or in LFA-1-deficient mice demonstrate that LFA-1 plays a vital role in extravasation of lymphocytes, eosinophils, and neutrophils.[4,5] Similarly, in vitro studies demonstrate that LFA-1 plays a critical role during T-cell activation and proliferation, and is required for efficient cytolysis by cytotoxic T cells.[6] Clinical trials using a humanized anti-LFA-1 antibody Efalizumab (Raptiva®) provide excellent proof-of-principle for this target.[7] In Phase III clinical trials, 29% of subjects demonstrated 75% or greater psoriasis area and severity index (PASI 75) score improvement and 56% achieved PASI 50 when efalizumab was given sc weekly at 1 mg/kg.[8] US approval was granted for Raptiva® in October 2003 for moderate-to-severe psoriasis. In addition to psoriasis, the humanized anti-LFA-1 antibody is in development for prevention of solid organ transplant rejection.

Because of the strong evidence from both animal models and clinical trials for LFA-1/ICAM as a target in several different inflammatory diseases, there has been an intense effort to identify orally available, small molecule inhibitors of this interaction.[9] Figure 1 lists selected analogs from several recently disclosed, structurally diverse chemical series. Literature reports demonstrate that some of these chemical series are in fact allosteric inhibitors of the LFA-1/ICAM interaction. Specifically, both

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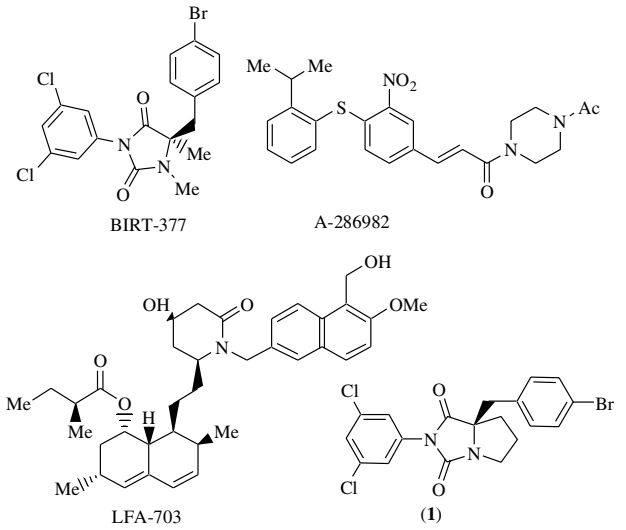


Figure 1. Representative LFA-1/ICAM inhibitors known from the literature.

NMR and X-ray crystallography studies show binding to the IDAS site (I-domain allosteric site) of the I-domain on the CD11a unit, locking the conformation of LFA-1 in a low affinity state thereby preventing its binding to ICAM-1.[10–12] Elegant studies by Takagi and Springer revealed the importance of the conformational switch of the I-domain in binding to LFA-1.[13]

Of the compounds outlined in Figure 1, the structure of the bicyclic compound (**1**)[14] was intriguing with respect to its symmetry and relationship to BIRT377. Moving the *p*-bromophenyl residue away from the quaternary center and placing it on the cyclopentyl ring system of compound **1** would reveal a pseudo *C*-2 symmetric, bicyclic[5.5]hydantoin system that would adapt a somewhat rigid 'half-open book' conformation (Fig. 2) Therefore, the question was whether we could access the *p*-bromophenyl pocket of BIRT-377 by optimizing the linker in the bicyclic[5.5]hydantoin system. This report describes the synthesis and in vitro SAR of novel LFA-1 antagonists based on the aforementioned de novo design.

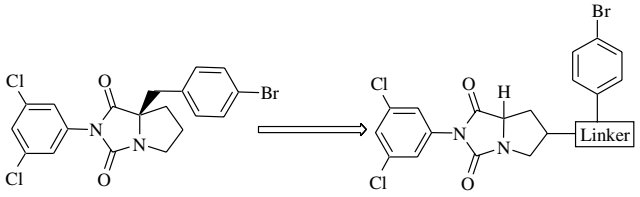
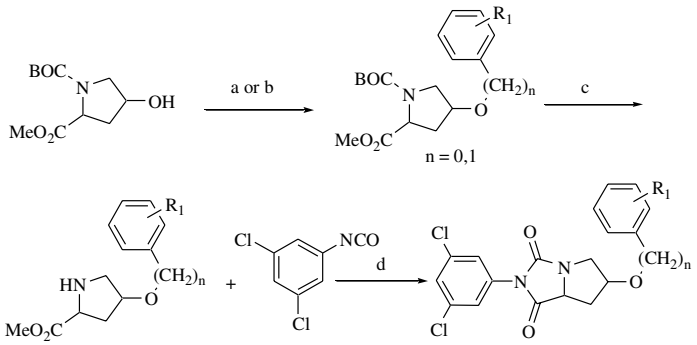


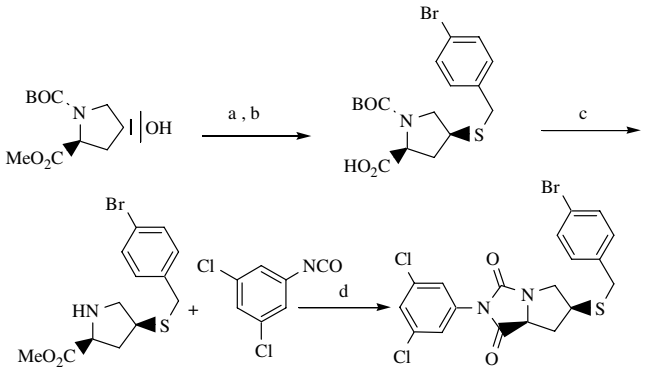
Figure 2. De novo design of LFA-1 antagonists based on the bicyclic-[5.5]hydantoin scaffold.

The synthetic pathways utilized in the preparation of the bicyclic[5.5]hydantoins are outlined in Schemes 1–3. Commercially available homochiral hydroxyproline served as the starting material for the synthesis of the various compounds outlined in Table 1. The amino substituted bicyclic hydantoins (Table 1, **12**–**17**) were synthesized by reductive amination of the ketone followed by separation of diastereomers by silica gel column chromatography (Scheme 3).

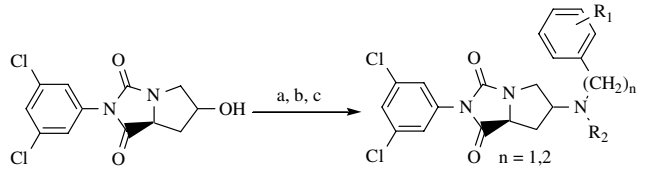
Table 1 displays the in vitro inhibitor potencies against the LFA-1/ICAM interaction for a series of bicyclic[5.5]hydantoins.[15] As the data indicates, the length and nature of the linker, stereochemistry at the 5- and 7a-positions of the hydantoin scaffold, and the position and substitution on the phenyl ring (R in Table 1) all contribute significantly to the overall activity of this series of compounds. For example, of the oxygen, sulfur, and nitrogen atoms, the oxygen-containing linker appears to be optimal (compare compound **5** with **11** and **16**). Altering the linker length leads to a significant loss of activity. This can be seen in the comparison of **5** with **3** and **14**, respectively. Table 1 also indicates that the 4-Br substitution on the phenyl ring is optimal (compare compound **5** with **7**). Substitution of the 4-bromo residue with a cyano moiety leads to a threefold drop in potency (compare **5** and **8**). Significant reduction in potency is observed when the 4-bromo substituent on the phenyl ring is replaced by bulky lipophillic groups such as in compound **2**. We next examined the stereochemical preferences for the residues at positions 7a and 5 of the bicyclic[5.5]hydantoin scaffold. It is clear from Table 1 that compounds with *S* absolute configuration at the 7a- and 5-positions are significantly more potent than the corresponding 7a*S*/5*R* or 7a*R*/5*R* analogs (compare compound **5** with **4**, **6**, and **9** and compound **12** with **13**). From the series of compounds



Scheme 1. Reagents and conditions: (a) dry KOH, DMSO, subst. BnBr, rt, 3 h, 60–70%; (b) DIAD, Ph_3P, THF, subst. phenol, rt, 18 h, 60–70%; (c) TFA, CH_2Cl_2, rt, 2 h, 70–90%; (d) K_2CO_3, DMF, rt, 18 h, 30–40%, or K_2CO_3,CH_2Cl_2, triethylamine, rt, 24 h, 70–90%.



Scheme 2. Reagents and conditions: (a) MsCl, CH_2Cl_2, Et_3N, rt, 12 h, 90%; (b) MeONa, MeOH, (4-bromobenzyl)mercaptan, reflux, 18 h, 50–70%; (c) $SOCl_2$, MeOH, rt, 24 h, 70%; (d) K_2CO_3, DMF, rt, 18 h, 20–30%.



Scheme 3. Reagents and conditions: (a) PDC, CH_2Cl_2, rt, 36 h, 60–70%; (b) $Na(OAc)_3BH$, mol. sieves, CH_2Cl_2, benzylamine, rt, 18 h, 40–50%; (c) separation of diasteromers by SiO_2 column chromatography.

outlined in Table 1, compound **5** has been identified as the most potent LFA-1 antagonist.

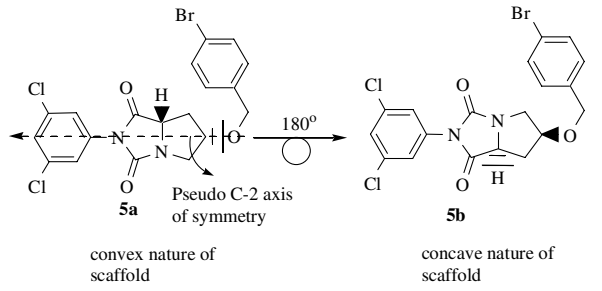


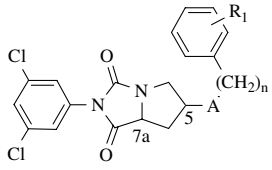
Figure 3. Two different orientations of the bicyclic[5.5]hydantoin, **5**.

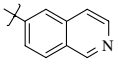
The pseudo *C*-2 symmetric bicyclic[5.5]hydantoin system in compound **5** that would adapt a 'half-open book' conformation, can have two different orientations as shown in Figure 3.

In order to determine the preferred bound conformation, we examined a binding model of BIRT-377 bound to LFA-1.[12] In this model, the inhibitor adopts a conformation orienting the 3,5-dichlorophenyl and the *p*-bromophenyl aromatic rings in a favorable edge-to-face π–π interaction. Molecular modeling suggests that of the two symmetry-related orientations of the bicyclic hydantoin moiety (Fig. 4, **5a** and **5b**), only **5b** is consistent with the BIRT-377 model, and thus may represent the bound conformation of compound **5** to LFA-1 (Fig. 4).

As further evidence of the importance of the 'half-open book' conformation of the bicyclic[5.5]hydantoin, the urazole analog (**18**), in which the bicyclic ring system

Table 1. In vitro activity of bicyclic[5.5]hydantoins



Compd	A	*n*	R_1	Stereo	HeLa/HSB (IC_{50} or % inhib. @ 1 μM)
BIRT-377					26 nM[a]
2	O	1		7a*S*,5*S*	28% (2)
3	O	0	4-Br–Phe	7a*S*,5*S*	480 nM (3)
4	O	1	4-Br–Phe	7a*S*,5*R*	935 nM (1)
5	O	1	4-Br–Phe	7a*S*,5*S*	85 nM (3)
6	O	1	4-Br–Phe	7a*R*,5*R*	275 nM (3)
7	O	1	3-Br–Phe	7a*S*,5*S*	755 nM (3)
8	O	1	4-CN–Phe	7a*S*,5*S*	270 nM (3)
9	O	1	4-Cl–Phe	7a*R*,5*R*	620 nM (1)
10	O	1	4-(2-CNPh)	7a*S*,5*S*	14% (2)
11	S	1	4-Br–Phe	7a*S*,5*S*	290 nM (2)
12	NH	1	4-CN–Phe	7a*S*,5*S*	175 nM (2)
13	NH	1	4-CN–Phe	7a*S*,5*R*	9% (2)
14	NH	2	4-Br–Phe	7a*S*,5*S*	10% (2)
15	NH	2	4-Br–Phe	7a*S*,5*R*	3% (2)
16	NMe	1	4-Br–Phe	7a*S*,5*S*	175 nM (2)
17	NEt	1	4-CN–Phe	7a*S*,5*S*	730 nM (1)

Values in parenthesis indicate the number of determinations.
[a] K_d value in an LFA-1/ICAM-1 binding assay as reported in the literature.[12]

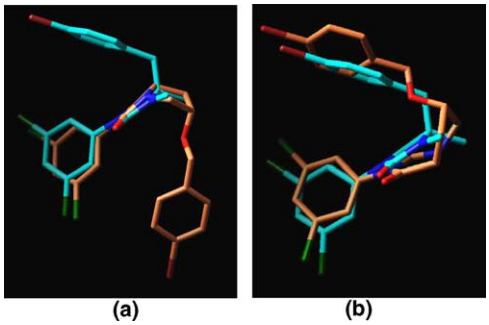


Figure 4. Overlay of two symmetry-related orientations with the putative bioactive conformation of BIRT-377 proposed by Last-Barney et al.[12]

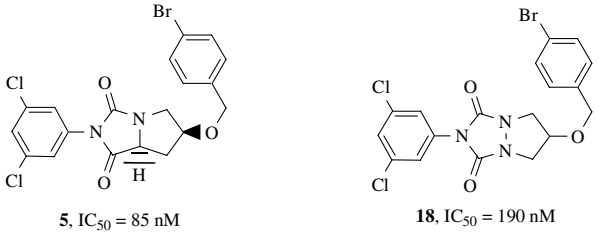


Figure 5. Relative potencies of hydantoin and urazole analogs.

is more planar, is about twofold less potent than compound **5** (Fig. 5).[16]

In conclusion, we have identified a novel series of LFA-1 antagonists based on de novo design employing the bicyclic[5.5]hydantoin scaffold. Optimization of the length and nature of the linker, stereochemistry at the 5- and 7a-positions of the hydantoin scaffold, and the position and substitution on the phenyl ring led to the identification of compound **5** as a potent inhibitor of the LFA-1/ICAM interaction.

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15. Adhesion of the HSB-2 human lymphoblast cell line to H1-Hela cells was used to characterize LFA-1 antagonists. H1-HeLa cells have high constitutive expression of ICAM-1 and are competent to support LFA-1-dependent adhesion (Suchard et al., unpublished observation). Prior to adhesion assays, HSB-2 cells were labeled with calcein AM and treated with PMA for 30 min. The labeled, PMA-activated cells were added to a monolayer of H1-HeLa cells in the presence or absence of compound or anti-human LFA-1 antibody. Adherent HSB-2 cells were quantified as previously described (Akeson, A. L.; Woods, C. W. J. *Immunol. Methods* **1993**, *163*, 181). Adhesion in the presence of vehicle alone was considered equivalent to 0% inhibition, and adhesion in the presence of an excess of anti-human LFA-1 antibody was considered equivalent to 100% inhibition. IC_{50} values were determined using Excel fit (Microsoft Corp., Bellevue, WA).
16. Compound **18** was synthesized using the synthetic protocol outlined below. (a) NH_2–$NHCO_2Me$, toluene, 25 °C, 28 h (82%), 4 M KOH, reflux, 1 h (100%); (b) epichlorohydrin, $HgCl_2$ (0.05 equiv), 155 °C, 11 h (56%); (c) A, NaOMe, MeOH then B, DMF reflux, 2 h (30%).