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1,4-Diazepane-2,5-diones as novel inhibitors of LFA-1

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Abstract—1,4-Diazepane-2,5-diones (**2**) are found to be a new class of potent LFA-1 inhibitors. The synthesis, structure, and biological evaluation of these 1,4-diazepine-2,5-diones and related derivatives are described.
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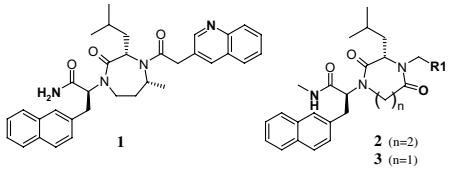
Lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$) is a heterodimeric adhesion receptor belonging to the β_2 integrin family. LFA-1 is expressed on all leukocytes. Interactions between LFA-1 and intercellular adhesion molecules, such as ICAM-1, play central roles in mediating immune and inflammatory responses and are essential in the pathogenesis of many diseases.[1] Together with other adhesion receptors, LFA-1 mediates leukocyte migration to sites of inflammation and antigen exposure.[2–4] LFA-1 is involved in both firm adhesion and locomotion of leukocytes.[5] Moreover, during an immune response, LFA-1 binding to ICAM-1 can enhance T-cell-receptor-dependent activation and proliferation of T-cells.[6] Blockade of LFA-1 interactions with their ligands is an attractive therapeutic target. The intervention of integrin LFA-1 by monoclonal antibodies (mAbs) has shown efficacy in animal models of inflammation and autoimmune disease, for example, arthritis,[7] ischemia/reperfusion injury,[8] and transplant rejection.[9] Clinical studies suggest that anti-LFA-1 therapy is beneficial in bone marrow and solid organ transplantation.[10,11] Recent data show that a humanized monoclonal antibody of the CD11a subunit of LFA-1 inhibits the activation of T cells and is efficacious in patients suffering from moderate to severe plaque psoriasis[12a] and in allergic asthma.[12b] Because of the importance of this integrin and its implication in various disease processes, efforts toward the design and synthesis of orally bioavailable small molecule inhibitors have intensified in recent years.[1,13] In addition, understanding the mechanism of LFA-1 binding to ligands has become fundamental in developing therapeutic agents against this integrin.[1d]

In a previous communication,[14] we disclosed the identification of **1**, as a potent LFA-1 antagonist, from a structure-based combinatorial library. This compound exhibited nanomolar binding activity in an LFA-1/ICAM-1 ELISA-type binding assay.[15] As part of an effort to optimize **1** and identify novel LFA-1 antagonists, we sought to explore the structure–activity relationship (SAR) of related templates (**2** and **3**). The efforts led to the discovery of a new class of potent LFA-1 antagonists whose central core is comprised of 1,4-diazepane-2,5-diones (**2**). Although we introduced a second sp^2 carbon atom in **2** and **3** and shortened the ring in **3**, a molecular modeling analysis suggested that the side chains in **2** and **3** can adopt an arrangement similar to that in **1**. Herein, we describe the synthesis and initial SAR studies of this novel series.



1 **2** (n=2) **3** (n=1)

Keywords: Integrin; LFA-1; Diazepanediones; I-domain; L-site binding.
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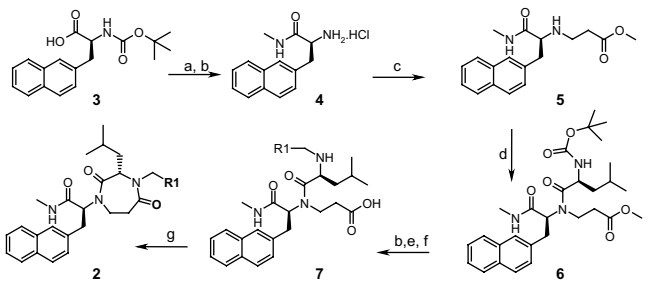
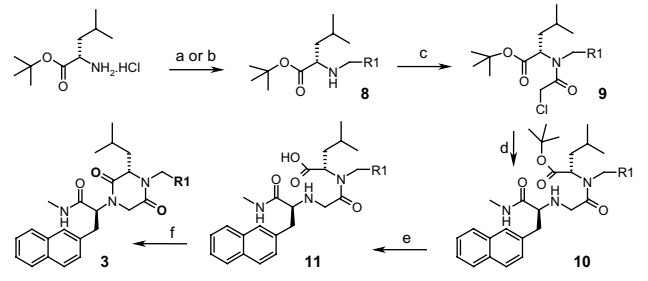


Scheme 1. Regents and conditions: (a) DCCI (2 equiv), HOBt (2 equiv), methylamine (2 equiv), NMM (1 equiv), DMF, rt, 12 h; (b) HCL (gas), CH_2Cl_2, rt, 2 h; (c) methyl acrylate (excess), DIPEA (2 equiv), MeOH, rt, 14 h; (d) *N*-*t*-Boc-leucine (1 equiv), HATU (5 equiv), DIPEA (5 equiv), DMF, 0 °C–rt, 48 h; (e) R1–CHO, $Na(OAc)_3BH$ (1.5 equiv), HOAc (0.5 equiv), THF, rt, 1 h; (f) LiOH (2.5 equiv), THF, water, rt, 1 h; (g) HATU (1.2 equiv), DIPEA (1.2 equiv) DMF, 0 °C–rt, 3 h.

1,4-Diazepane-2,5-diones were synthesized as outlined in Scheme 1. The synthesis starts with the conversion of Boc-amino acid **3** into the amino intermediate **4**, after removal of the Boc protective group. 1,4-Addition of **4** to methyl acrylate leads to **5**. Coupling of **5** with *N*-*t*-Boc protected leucine and coupling conditions, such as PyBroP/NMM/DMAP/DMF/rt,[16a] DIPC/DMF/rt, CIP/HOBt/DIEA/THF/rt,[16b] and DCC/HOBt/NMM/rt,[16c] gave only trace amounts or low yields of the desired coupling product **6**. Good yields (75–90%) of the coupling reaction, however, could be obtained if excess amounts of HATU were used. Deprotection of **6**, reductive amination, and ester hydrolysis afforded the amino acid intermediate **7**. Cyclization of **7** led to the desired 1,4-diazepane-2,5-diones (**2**).[17] The overall yield of this unoptimized sequence is in the range of 10–19%.

The synthesis of piperazine-2,4-diones (**3**) is shown in Scheme 2. Alkylation or reductive amination of L-leucine *t*-butyl ester hydrochloride gave amino acid intermediate **8** (30–40%). Coupling of **8** with chloroacetic acid (90%) and reaction of the resulting chloro compound **9** with **4** gave compound **10** (35–40%). Ester deprotection of **10** followed by cyclization afforded (15–20%) the piperazine-2,5-diones (**3**).[18]



Scheme 2. Regents and conditions: (a) R1–CHO, $Na(OAc)_3BH$ (1.5 equiv), HOAc (0.5 equiv), THF, rt, 1 h; (b) R1–CH_2Br, DIEA, DMF, rt, 16 h; (c) chloroacetic acid, DIPCl (2 equiv), DMF; (d) **4**, DIEA, DMF, 40 °C, 16 h; (e) TFA, CH_2Cl_2, rt, 3 h; (f) HATU (1.2 equiv), DIPEA (1.2 equiv) DMF, 0 °C–rt, 4 h.

Compounds **2**, **3**, and related derivatives were evaluated for their activity in the LFA-1/ICAM-1 binding assay.[15b] The results are outlined in Table 1. Initially, we investigated the SAR of **2** around the R1 moiety, because this moiety has been shown in **1** to be vital for binding potency.[14] The phenyl derivative (**2a**) is a weak LFA-1 inhibitor. Substitution of the benzene ring in the 4-bromophenyl (**2b**) derivative provides no improvement in potency. The bulky 3-benzyloxyphenyl derivative (**2c**) is less active. Polar substitution in the 3-hydroxyphenyl (**2d**), 4-hydroxy-3-methoxyphenyl (**2e**), and 4-dimethylaminophenyl (**2f**) derivatives, however, shows a two or threefold binding improvement. The 4-pyridyl derivative (**2g**) is totally inactive. As observed earlier in **1** for the effect of a 3-quinolyl group, the 3-quinolyl derivative (**2h**) shows a remarkable enhancing ability in inhibiting ICAM-1 and LFA-1 interactions (IC_{50} = 69 nM). It is as active as the previous lead structure (**1**). The 6-quinolyl derivative (**2i**) is twofold less active. Compound **2j**, which is a longer chain analogue of **2h**, is about 30-fold less active. The 4-pyridylethylene analogue (**2k**), however, is also an excellent inhibitor of LFA-1 (IC_{50} = 24 nM). The corresponding amide analogue (**2l**) is more than 100-fold less active. Data of the piperazine-2,4-dione series (**3a**–**c**) indicated that they are only weak inhibitors of LFA-1. Interestingly, while the corresponding 4-bromophenyl derivatives (**2b** and **3a**) are comparable in potency, a 187-fold loss in potency was observed with the 6-quinolyl derivative (**3b**) in the six-membered ring series.

Three other stereoisomers of **2h** also have been synthesized according to Scheme 1 and were found to be much less active (Table 2), suggesting that the *S*,*S* configuration of the chiral centers in this series of compounds is optimal for binding potency.

Compound **2h** is a potent and selective inhibitor of LFA-1 with no inhibition of binding to the closely related integrin Mac-1 (CD11b/CD18), with an IC_{50} value greater than 100 μM (cell-free ICAM-1/Mac-1 binding assay).[19] It is also inactive (IC_{50} > 100 μM) in a VLA-4

Table 1. In vitro cell-free LFA-1/ICAM-1 binding assay[15b]

Compd	R1	IC_{50} (nM)[a]
1	—	70
2a	Phenyl	2850
2b	4-Bromophenyl	2950
2c	3-Benzyloxyphenyl	6600
2d	3-Hydroxyphenyl	1300
2e	4-Hydroxy-3-methoxyphenyl	950
2f	4-Dimethylaminophenyl	800
2g	4-Pyridyl	33,000
2h	3-Quinolinyl	69
2i	6-Quinolinyl	130
2j	3-Quinolinylmethyl	890
2k	4-Pyridyl–CH=CH–	24
2l	4-Pyridyl–NHCO–	3700
3a	4-Bromophenyl	2950
3b	6-Quinolinyl	24,400
3c	4-(3-Pyridyl)phenyl	13,300

[a] Each value is a mean of two experiments.

Table 2. In vitro cell-free LFA-1/ICAM-1 binding assay of stereoisomers of **2h**

Compound	Chiral configurations[a]	IC_{50} (nM)[b]
2h	*S*,*S*	69
2m	*S*,*R*	510
2n	*R*,*S*	5700
2o	*R*,*R*	4200

[a] Configurations of the naphthylalanine side chain and the leucine side chain are listed, respectively, for each isomer.
[b] Each value is a mean of two experiments.

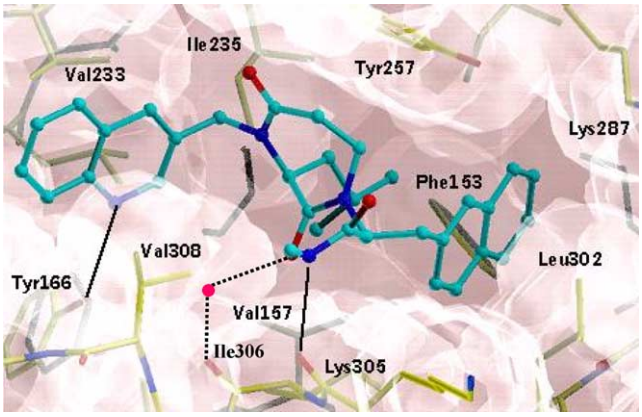


Figure 1. X-ray crystallographic representation of the complex of compound **2h** with the I-domain of LFA-1. The continuous and dotted black lines indicate, respectively, direct and water-mediated intermolecular hydrogen-bond interactions.

dependent adhesion assay, which quantifies the binding of fluorescently labeled Ramos cells to immobilized recombinant human VCAM-1.[19b] Compound **2h** exhibits a high degree of protein binding (>95%) in both rat and human plasma. Moreover, it has low oral bioavailability in mice (7.0%) and in rats (8.6%).[20]

To understand where and how this class of compounds is bound to LFA-1 and to verify the absolute stereochemistry, a high-resolution X-ray crystallographic analysis was performed. Compound **2h** was co-crystallized with the I-domain of LFA-1.[21] The crystal structure of the complex, solved to 1.8 Å resolution, shows that **2h** binds to the so-called L-site (lovastatin site)[15] of the I-domain (Fig. 1).[22] Binding to this site has been previously shown[23] to block a high-affinity conformational change important for LFA-1 function, suggesting an allosteric mode of inhibition for this class of inhibitors.[1a]

The X-ray structure shows that **2h** makes many favorable interactions with the LFA-1 I-domain. The seven-membered 1,4-diazepane-2,5-dione ring of **2h** has van der Waals interactions with the aromatic side chain of Tyr257, and as expected, the ring conformation (difficult to predict by modeling) crucially determines the positions of its substituents. The naphthyl moiety of **2h** occupies a hydrophobic pocket formed mainly by the side chains of Phe153, Ile259, Leu289, Leu295, Leu298, Leu302, Ile306, and the aliphatic moiety of Lys287. The Leu side chain of the ligand binds in a hydrophobic subsite formed mainly by the side chains of Phe153, Val157, Ile235, Tyr257, Ile259, and Ile306. The quinolyl moiety binds to a pocket that is formed mainly by the side chains of Val130, Leu132, Tyr166, Thr231, Val233, and Ile255. There are two direct hydrogen bonds between **2h** and the I-domain, specifically from the amide-nitrogen atom to the Lys305 carbonyl group and from the quinolyl nitrogen atom to Tyr166-OH. In addition, there is a system of water-mediated weak hydrogen bonds between the amide oxygen atom and both Glu284 and Tyr257. There is also a weak water-mediated hydrogen bond between the oxygen atom at position 2 of the diazepane ring and the carbonyl group of Ile306. An analysis of these interactions provides a better understanding of the SAR and offers a basis for further optimization of this class of inhibitors.

In summary, we have discovered 1,4-diazepane-2,5-diones as a new class of inhibitors of LFA-1. Compounds **2h** and **2k** are potent inhibitors of LFA-1 and ICAM-1 interactions. The X-ray crystal structure reveals the L-site as the binding site of **2h** with the I-domain of LFA-1. Further optimization of this novel class of compounds is in progress and will be reported in due course.

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