Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 499–502

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

1,4-Diazepane-2-ones as Novel Inhibitors of LFA-1

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Received 9 September 2002; accepted 8 October 2002

Abstract—The design, synthesis, and biological evaluation of 1,4-diazepane-2-ones as novel LFA-1 antagonists from a scaffold-based combinatorial library are described. Initial optimization of the library lead has resulted in high-affinity antagonists of the LFA-1/ICAM-1 interaction, such as compounds **18d** and **18e** with IC_{50} values of 110 and 70 nM, respectively.

Antagonism of cell surface receptors belonging to the integrin superfamily is a new paradigm for drug discovery in transplantation and cancer, as well as in other diseases.[1] 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. Similar to other integrins, LFA-1 must undergo affinity and/or avidity changes to bind to its ligands of the immunoglobulin superfamily ICAM-1 (CD54), -2 (CD102), -3 (CD50), -4, and -5. 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] Recent studies employing LFA-1 deficient mice or cell-based assay systems show a requirement for LFA-1 in CD8+ T-cell activation and effector function.[7–9] LFA-1 is an attractive therapeutic target: blockade of integrin LFA-1 by monoclonal antibodies (mAbs) has shown efficacy in animal models of inflammation and autoimmune disease, for example, arthritis,[10] ischemia/reperfusion injury[11] and transplant rejection.[12] Clinical studies suggest that anti-LFA-1 therapy is beneficial in bone marrow and solid organ transplantation.[13,14] Moreover, recent data show that a humanized anti-LFA-1 mAb is efficacious in patients suffering from moderate to severe plaque psoriasis.[15b] Because of the importance of this integrin and its implication in various disease processes, efforts toward the design and synthesis of orally bioavailable low molecular weight (LMW) inhibitors have intensified in recent years.[15]

We recently reported that lovastatin (mevinolin), a known HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitor, also blocks LFA-1 function,[16a] and we have shown by NMR spectroscopy and X-ray crystallography that lovastatin binds to a unique site on the I-domain (inserted domain) of the LFA-1 α chain (Fig. 1). This lovastatin-binding site (L-site)[16b] is distant from the metal ion binding domain called metal ion dependent adhesion site (MIDAS) and is situated at the proposed I-domain allosteric site (IDAS).[16a,17] Discovery of this small molecule-binding pocket within the I-domain of LFA-1 thus provides an opportunity for structure-based design of LFA-1 antagonists, which prompted us to employ a combinatorial library approach in identifying novel antagonists using small molecule scaffolds. 1,4-Diazepane-2-one **1** (Fig. 2) is an interesting, nonplanar library scaffold that has many potential sites of diversity with which we selected to explore interactions within the L-site pocket of the LFA-1 I-domain. Herein, we report the discovery of potent and selective LFA-1 antagonists from this diazepane-based library.

The 1,4-diazepane-2-one library was generated on Rink-amide MBHA resin (Scheme 1). Coupling of the Rink

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PII: S0960-894X(02)00991-5

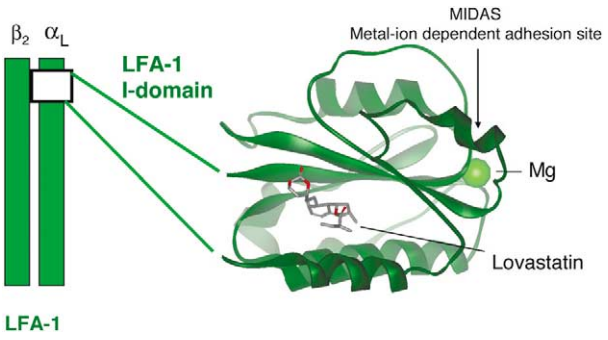


Figure 1. X-ray structure of the I-domain of LFA-1 with lovastatin.

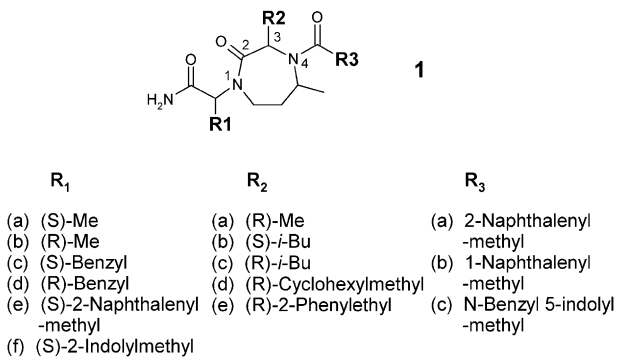
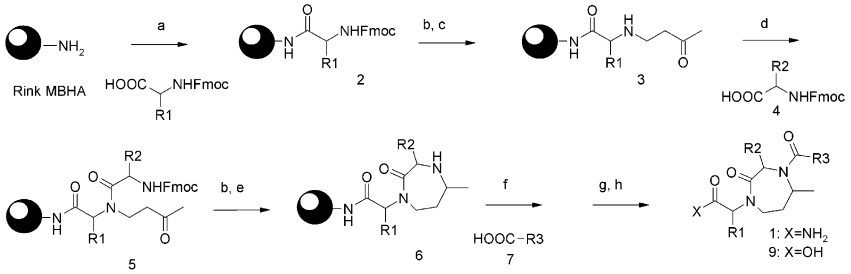


Figure 2. 1,4-Diazepane-2-one scaffold, showing three sites of diversity, for creating the combinatorial library of 90 compounds.

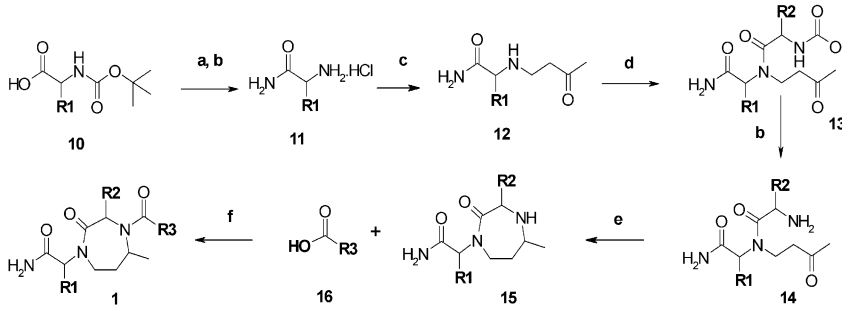
resin with amino acids using standard HOBt/DICD conditions gave the polymer-supported amino acids **2**. The completion of the reaction can be monitored by using the bromophenol blue test or by titration of the FMOC residue. The aminoacids **2** were then deprotected with 20% piperidine in DMF and treated with a 0.5 M methyl vinylketone solution in DMF or CH_2Cl_2 to give **3**. The conditions are applicable to a variety of substituents including BOC-protected tryptophan. It is interesting to note that even though a large excess of methyl vinylketone was used (12–20 equiv), no Michael di-adduct was detected after TFA cleavage from the resin. Acylation of **3** with activated amino acids **4** using the above HOBt/DICD/DMF conditions gave unsatisfactory yields of **5**. However, better yields could be obtained by using a 0.5 M mixture of resin/aminoacid/DICD (1:10:5) in DMF. After FMOC deprotection of **5**, the diazepine ring cyclization was achieved by the reductive amination ($NaBH_3CN$ in DMF buffered with 1% acetic acid). The resulting cyclic amines **6** were then acylated with acids **7** using DICD in DMF. TFA cleavage in CH_2Cl_2 gave diazepane-2-ones **1**.[18] Only a single diastereomer of **1**, in which the methyl group on the 5-position of the diazepane ring is in a *syn* relationship with the substituent on the 3-position (i.e., the R_2 group), was detected.[19] It is conceivable that the reductive amination is selectively controlled by the chiral center of the R_2 group present in the ring.[20] Minor amounts of the corresponding acids **9** were also observed.

The solution phase synthesis was also developed for selected 1,4-diazepane-2-ones (Scheme 2). The synthesis starts with the conversion of BOC-amino acid **10** into the corresponding amide **11**. After removal of the BOC protecting group, a 1,4-addition of the amino intermediate to methyl vinylketone leads to **12**. Coupling of **12** with an *N*-BOC protected amino acid gives **13**, after silica gel purification. Deprotection and intramolecular reductive amination of **14** affords the seven-membered ring intermediate **15**. Acylation with carboxylic acid **16** leads to the desired diazepane **1**. The overall yield of this unoptimized sequence is in the range of 5–10%.

The design of the library was based on an idea to use the diazepane ring as a scaffold to which various interacting moieties could be attached. One of the R groups should correspond to the decalin system of lovastatin and fill most of the large hydrophobic pocket within the L-site.[21] For an exploration of possible substituents for the R_1, R_2, and R_3 groups on the diazepane template, docking studies based on the X-ray crystal structure of the LFA-1 I-domain in complex with lovastatin were performed.[22] It became obvious that the diazepane scaffold would serve very well as a template, making only a few contacts with the protein, while the binding affinity would have to arise from interactions involving appropriate hydrophobic groups in R_1, R_2, and R_3. Information from superposition analyses utilizing other weak hits from HTS indicated that large hydrophobic substituents on the R_3 position might be necessary.[23] Molecules with large hydrophobic groups in R_3 were then docked manually into the binding site and the corresponding complexes were geometry optimized.[24] Figure 3a shows such a structure in which the naphthalenyl-methyl moiety in R_3 points deeply into the binding site, occupying an area close to that of the decalin system in lovastatin. This binding mode would suggest



Scheme 1. (a) FMOC-amino acid (6 equiv), HOBt (6 equiv), DICD (6 equiv), DIPEA (1 equiv), DMF, rt, 12 h; (b) 20% piperidine, DMF, rt, 20 min; (c) 0.5 M methyl vinylketone, DMF, rt, 12 h; (d) **4** (8 equiv), DICD (4 equiv), DMF, rt, 48 h; (e) 0.5 M $NaBH_3CN$, DMF, rt, 12 h; (f) **7** (10 equiv), DICD (10 equiv), DMF, rt, 12 h; (g) TFA/CH_2Cl_2 1/1, rt, 60 min; (h) freeze dried in CH_3CN/H_2O.



Scheme 2. Solution phase synthesis of 1,4-diazepane-2-ones: (a) DCCI (1.2 equiv), HOBt (1.2 equiv), 25% NH_4OH (1.2 equiv), DMF, rt, 16 h; (b) TFA (neat), 0 °C, 15 min, then 3 N HCl/diethylether; (c) methyl vinylketone (1.5 equiv), dioxane, DIPEA (1.2 equiv), rt, 16 h; (d) BOC-amino acid (1 equiv), DIPC (4 equiv), NMM (4 equiv), DMF, rt, 16 h; (e) sodium cyanoborohydride (2 equiv), dioxane–water (4:1), pH 5.4, 30 min; (f) DIPEA (1 equiv), EADC (2 equiv), CH_2Cl_2, rt, 16 h.

relatively small hydrophobic groups in the R_2 position, but no conclusion could be drawn about the R_1 group, because it points out of the L-site. An alternative binding hypothesis achieved by additional docking (Fig. 3b) revealed the possibility that the R_1 naphthyl group of this diazepane template and the decalin moiety of lovastatin could occupy the same binding pocket in the L-site and provided information about other substituents on this scaffold. Because we intended to cover a broad range of substituents in our combinatorial library to gain insights into binding requirements of this scaffold, no attempts were made to determine the optimal combination of substituent groups or to evaluate other binding conformations in greater detail. The above considerations led to the substituent selection on the R_1, R_2, and R_3 positions of the diazepane template as shown in Figure 2 for the first library consisting of 90 compounds.

Initial screening of the crude products in the 90-compound diazepane library (Fig. 4) using an LFA-1/ICAM-1 ELISA-type binding assay[16b] identified **17a** (**1**; R_1, R_3 = e, a; R_2 = b) as the most active compound. Compound **17a** and its carboxylic acid **17b** could be purified directly from the crude library sample. **17a** was also independently synthesized by solution-phase chemistry (Scheme 2) and was shown to have an IC_{50} value (2 μM) comparable to that of lovastatin in the cell-free LFA-1/ICAM-1 adhesion assay. Its corresponding acid **17b** is much less active (IC_{50} = 44 μM). Despite its relatively weak activity, the structural novelty and available high throughput chemistry prompted us to use **17a** as a lead for further optimization. NMR investigations indicated that **17a** binds to the L-site of the LFA-1 I-domain[25] suggesting that either the R_1 or R_2 naphthyl group of **17a** might occupy the binding site used by the decalin portion of lovastatin. However, NMR analysis of **17a** was limited due to the presence of two almost identical residues (i.e., two 2-naphthyl groups) on its R_1 and R_3 positions and suggested the need to differentiate these two groups. The SAR trend from the above library (Fig. 4) suggested the requirement of a large hydrophobic group on the R_1 position and a medium-sized group on the R_2 position in this series of compounds. The preference for the (*S*)-isobutyl over the (*R*)-isobutyl group at the R_2 position was also indicated. No information, however, was available for smaller R_3 groups. Accordingly, our initial optimization effort was focused primarily on reducing the size and lipophilicity of the R_3 group in **17a** (Table 1). The total loss of potency of compound **15a** (Scheme 2) suggests the importance of an acyl group in this R_3 position.

Comparable activities were observed for **17a** and the 4-bromophenyl (**18b**) replacement. A slight improvement in potency was seen for the phenyl (**18c**) and 4-pyridyl (**18a**) derivatives. A significant improvement in potency, however, was observed for the quinoline derivatives. Both 6-quinolyl (**18d**) and 3-quinolyl (**18e**)

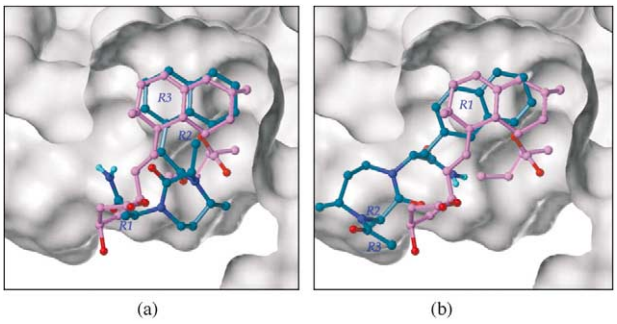


Figure 3. Superposition of representative diazepane-2-ones [structure **1**; $R_1 = R_2$ = Me in panel (a); $R_2 = R_3$ = Me in panel (b)] and lovastatin in the L-site of LFA-1. Compound **1** is drawn in green. Lovastatin is drawn in magenta.

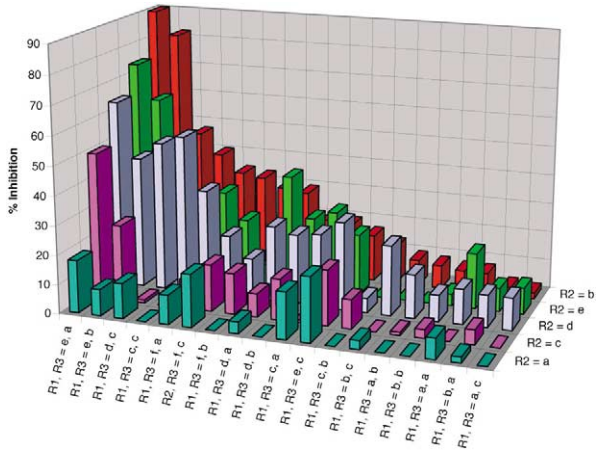
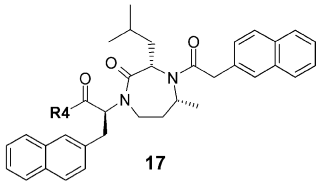
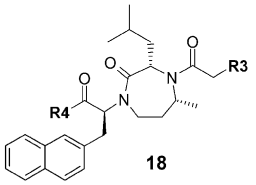


Figure 4. The 90-compound diazepane library screened against the LFA-1/ICAM-1 assay. Each compound was screened at 12 μM.

Table 1. In vitro cell-free LFA-1 assay (LFA-1/ICAM-1 binding assay)[16b]





Compd	Substituent[a]	IC_{50} (μM)[b]
15a	R_1 = (*S*)-2-naphthyl; R_2 = (*S*)-*i*-Bu	>20
17a	$R_4 = NH_2$	2
17b	R_4 = OH	>20
18a	R_3 = 4-pyridyl; $R_4 = NH_2$	0.97
18b	R_3 = 4-bromophenyl; $R_4 = NH_2$	1.30
18c	R_3 = phenyl; $R_4 = NH_2$	0.75
18d	R_3 = 6-quinolyl; $R_4 = NH_2$	0.11
18e	R_3 = 3-quinolyl; $R_4 = NH_2$	0.07
18f	R_3 = 6-quinolyl; R_4 = OMe	2.60

[a]All compounds have been synthesized according to Scheme 2.
[b]Values are means of three experiments.

derivatives showed more than an 18- and 28-fold increase, respectively, in potency in comparison to that of the library lead **17a**. These binding improvements suggested that the nitrogen atom in the quinoline ring of **18d** and **18e** might be involved in hydrogen-bond interactions within the L-site pocket of the LFA-1 I-domain. NMR studies confirm that **18d** binds to the L-site pocket. However, specific residues that could be involved in hydrogen-bond formation were not revealed and await further I-domain-ligand crystallographic evidence. The methyl ester (**18f**) of **18d** is less active.

In summary, we have discovered diazepanes as novel antagonists of LFA-1 by a combinatorial library approach. Preliminary optimization of the initial library lead has resulted in high-affinity antagonists of the LFA-1/ICAM-1 interaction, such as compounds **18d** and **18e** with IC_{50} values of 110 and 70 nM, respectively. Further exploration of this novel series is in progress and will be reported in due course.

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18. The crude products **1** were shown to be in the range of 14–72% purity by HPLC.
19. NOESY study clearly showed the presence of an NOE effect between H3 and H5, suggesting a *cis* relationship between these two protons.
20. Compounds without the R_2 substituents show a 1:1 mixture of two diastereomers. These stereochemical results support the selectivity induction by the chiral center in the diazepane ring: Wattanasin, S.; Roche, D. Novartis; unpublished results.
21. Hydrophobic interactions between lovastatin's decalin ring system and the I-domain seem to be the major binding determinants. For specific interactions of various moieties of lovastatin with the protein residues in the I-domain/lovastatin complex, please see ref 16a.
22. Lovastatin was removed from the structure and the diazepane system was docked manually into the L-site using the in-house program WitnotP written by: Widmer, A. Novartis; unpublished results.
23. The HTS hits are Novartis proprietary structures.
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25. Binding of the compounds to the LFA-1 L-site was confirmed by the chemical shift changes shown by the ^{15}N-HSQC NMR spectra of the isolated LFA-1 I-domain described, please see ref 16a.