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Review Article

Inhibitors of LFA-1/ICAM-1 interaction: from monoclonal antibodies to small molecules

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CONTENTS

Summary

The interaction between leukocyte function-associated antigen-1 (LFA-1), a member of β_2-integrin family, and intercellular adhesion molecule-1 (ICAM-1) plays a critical role in mediating cell adhesion, leukocyte transmigration and augmentation of T-cell receptor signaling. Inhibitors of LFA-1/ICAM-1 interaction represent a new therapeutic area for treatment of diseases such as rheumatoid arthritis, psoriasis and ischemic reperfusion injury, and have evolved from monoclonal antibodies (MAbs) to small molecules as potential therapeutic agents. This review will summarize the MAbs that have been or are being evaluated in clinical trials and will examine the discovery of the small-molecule antagonists. The SAR of several of the leading series and the mechanism of action for some of these molecules will also be discussed.

Introduction

The family of leukocytes includes neutrophils, lymphocytes (mostly B- and T-cell subtypes), monocytes, eosinophils and basophils. During an inflammatory response, peripheral blood leukocytes are recruited to the site of inflammation or injury by a series of specific cellular interactions. The initiation and maintenance of immune functions are regulated by intercellular adhesive interactions as well as signal transduction resulting from interactions between leukocytes and other cells (1-3). Leukocyte adhesion to vascular endothelium and transmigration from the circulation to sites of inflammation is a critical step in the inflammatory response (4-6). For this purpose, leukocytes utilize 3 major groups of adhesion molecules, namely, carbohydrate-binding selectins, integrins and members of the immunoglobulin (Ig) supergene families.

Integrins are a large superfamily of heterodimeric transmembrane glycoproteins that attach cells to extracellular matrix proteins of the basement membrane or to ligands on other cells. Integrins contain large (α; 120-170 kDa) and small (β; 90-100 kDa) subunits (7, 8). Like other members of the β_2-integrin family, leukocyte function-associated antigen-1 (LFA-1) is expressed exclusively on leukocytes and shares a common β chain. The 4 members that differ in their α chains are LFA-1 (CD11a/CD18 and $\alpha_L\beta_2$), Mac-1 (CD11b/CD18, $\alpha_M\beta_2$), p150,95 (CD11c/CD18, $\alpha_X\beta_2$) and CD11d/CD18 ($\alpha_D\beta_2$) (9). As a cell-surface adhesion receptor, LFA-1 has been identified as one of the major integrins that supports inflammatory and specific T-cell immune responses through mediating cell adhesion and leukocyte transmigration, activation of helper T cells and cytotoxic T cells (10). These functions are achieved through interactions with its counterreceptors: intercellular adhesion molecule (ICAM)-1, -2 and -3 which are members of the Ig supergene family located on endothelial, leukocytes and other cell types (11, 12). Even with the more recent identification of ICAM-4 and -5, ICAM-1 is still perhaps the most important among the ICAMs since it is the most widely distributed and its expression appears to be strictly regulated.

The interaction of LFA-1 to ICAM-1 is a vital step in the normal functioning of the immune system. A genetic condition termed leukocyte adhesion deficiency (LAD) has been characterized as the lack of appropriate expression of functional adhesion molecules of the CD18 family. Thus, patients with LAD have recurrent bacterial infections due to the inability to effectively recruit granulocytes in response to infections (13). Consistent with this background, ICAM-1 knockout homozygous mice have numerous abnormalities in their inflammatory responses (14). Interestingly, the LFA-1 α-chain knockout homozygous mouse has a selective defect in induction of peripheral immune responses whereas responses to systemic infection are normal (15). The interaction of LFA-1 with

ICAM-1 has been directly implicated in numerous pathological states, such as dermatitis, psoriasis, asthma, rheumatoid arthritis, graft rejection, tumor metastasis, reperfusion injury, myocardial infarction, *etc*. Therapeutic prevention of LFA-1 binding to ICAM-1 has potential in the prophylaxis or treatment of these disease states (16-20).

Structural biology of integrin activation and LFA-1/ICAM-1 interaction

The α subunit of LFA-1 belongs to a subgroup of integrin α subunits (α_1, α_2, α_L, α_M, α_X, α_D, α_E) that contain an N-terminal stretch of 200 amino acids which is called the inserted I or A domain and is homologous to the A-domains present in von Willebrand factor, several collagen and complement proteins, and cartilage matrix protein. Mutagenesis and antibody epitope mapping studies indicated that the I domain of LFA-1 is critical for specific interaction with ICAMs (21-23). The most extracellular portion of the LFA-1 I domain contains a magnesium cation-binding domain called the metal ion dependent adhesion site (MIDAS), which is involved in the direct interaction with ICAMs. Residues Asp-137, Ser-139, Ser-141, Thr-206 and Asp-239, together with a chloride ion, provides the hexa-coordination to the divalent magnesium cation and constitute part of the ligand binding surface. After expression at the cell surface of leukocytes in an inactive form, LFA-1 must be converted to an active state to interact with ICAMs. Two mechanisms have been put forward for the activation of LFA-1 (24). The first one suggests an incremental increase in the intrinsic affinity of the integrin for its ligands concomitant with a conformational change in the extracellular domain, and the second one suggests that the avidity of LFA-1 is increased as a result of increased integrin receptor clustering at the cell surface.

The crystal structures of the CD11a and CD11b I domains have been revealed, allowing for a greater insight into their functions. The CD11b I domain exhibited 2 different conformations depending on the crystal contacts (25, 26). This led to the proposal that one of the observed conformations is a mimetic of the activated ligand bound form while the other represents the unligated state of the protein. In crystallography studies, differences in the position of the α-helix of CD11a I domain were also observed in the presence or absence of divalent cation (27, 28). The proposal that these conformations reflect a regulatory site was supported by a recent structural study of an I domain of $\alpha_2\beta_1$/collagen peptide complex in which the ligated I domain is in the proposed activated form (29). In the activated ligand bound state, the C-terminal helix of the I domain drops downwards by 10 Å, whereas in the unligated state, this helix moves away from the β-sheet core of the protein to expose a hydrophobic cleft. On the basis of a series of site-directed mutagenesis studies of amino acid residues in this cleft, Abbott and ICOS scientists have proposed that this site (termed the I domain allosteric site or IDAS), defined by amino acid residues Ile-259, Leu-298, Ile-235, Val-157, Leu-161 and Lle-306 in the I domain (30), could act as a regulatory site for the LFA-1 activation process (31).

Although ICAM-1 has 5 extracellular Ig-like domains, only the first Ig domain of ICAM-1 is involved in direct interaction with LFA-1 I domain according to point mutation (32), antibody binding studies (33) and x-ray crystal structure analysis of domains 1 and 2 of ICAM-1 (34). The second Ig domain of ICAM-1 has a role in maintaining the structure of the LFA-1 ligand-binding site in the first domain of ICAM-1, but does not appear to have a direct role in ligand binding (35). Two working models for the relatively flat interacting epitope of ICAM-1 with LFA-1 have been generated. The residues of first Ig domain of ICAM-1 involved in the direct association with LFA-1 include 7 amino acids: Lys-39, Glu-34, Met-64, Tyr-66, Gln-73, Leu-30 and Asn-68 (36). In particular, Glu-34 plays a critical role in the cation-dependent binding through displacement of the chloride ion and coordination to the Mg^{2+} of LFA-1 I domain.

Monoclonal antibodies against LFA-1 or ICAM-1

It has been demonstrated that antagonism of the LFA-1/ICAM-1 interaction can be realized by antibodies directed against either protein or either subunit of LFA-1 (37). These molecules effectively inhibit inflammatory responses both *in vitro* and *in vivo*. More significantly, 5 monoclonal antibodies have been examined in human clinical trials for a variety of indications. Some of the clinical efficacy results are summarized below.

Odulimomab, a murine IgG_1 monoclonal antibody directed against the CD11a of LFA-1, was developed by Immunotech. Odulimomab has been launched in France for the prevention of graft-*versus*-host disease (38) and kidney transplant rejection (39) by Pasteur-Meireux Connaught as Antilfa™. One European phase III clinical trial in renal transplant patients administered odulimomab has been completed by IMTIX, the transplant division of Pasteur-Meireux Connaught (40). A multicenter phase III study involving 760 kidney recipients is being conducted throughout the U.S., Canada and Europe. It is focusing on patients at a risk of rejection due to suboptimal grafts which is the population of patients who responded best to odulimomab treatment in a previous trial.

Enlimomab (R6.5), a murine IgG_{2a} monoclonal antibody which binds to domain 2 of ICAM-1, was discovered by Isis Pharmaceuticals and was developed by Boehringer Ingelheim in Europe and the U.S. Phase III trials of the drug for stroke were disappointing as trial results reported an adverse effect on neurological outcomes in stroke patients which may have been associated with immunogenicity problems (41). Enlimomab has been in phase III clinical trials for use in renal transplantation (42) and phase II trials for use against graft-*versus*-host disorders and liver transplant rejection (43). It was in phase III trials in stroke and was investigated in the U.S.

as a therapy for rheumatoid arthritis in phase II clinical trials (44).

Erlizumab (rhuMAb CD18) (45) is a recombinant humanized anti-CD18 monoclonal antibody developed by Genentech. The Limitation of Myocardial Injury following Thrombolysis in Acute Myocardial Infarction (LIMIT AMI) phase II study of erlizumab for the treatment of acute myocardial infarction failed to reach its primary objectives, thus prompting Genentech to discontinue development of the agent (46). Roche was also carrying out phase II trials for the treatment of hemorrhagic shock. However, its development was discontinued in January 1998 (47).

Genentech and Xoma are collaborating on the development of Xanelim™ (efalizumab, Hu1124), a humanized anti-CD11a monoclonal antibody. The drug showed significant therapeutic benefit in comparison with placebo in a completed phase II study in Canada that enrolled 145 patients with moderate-to-severe psoriasis (48, 49). Subsequently, 2 phase III clinical trials in patients with moderate to severe psoriasis are under way at about 80 sites with more than 1000 patients being evaluated in the U.S. and Canada. Initial results from these 2 trials are very encouraging, with a BLA submission to the FDA expected by year-end or in the first quarter of 2002 (50). Efalizumab is also being developed for organ transplant rejection in a phase I/II multiple-dose trial.

LeukArrest™ (Hu23F2G) is a humanized leukointegrin monoclonal antibody developed by Icos Corporation that binds to CD11/CD18 adhesion molecules. Hu23F2G was in phase II trials in the U.S. for the treatment of trauma-induced hemorrhagic shock. Results from a 150-patient trial failed to meet its primary endpoints, however, the product was well tolerated (51). The highest dose inhibited pulmonary and cardiac failure and reduced the incidence of adult acute respiratory distress syndrome (ARDS). A single-dose phase II study of Hu23F2G in patients with multiple sclerosis undergoing acute exacerbations has been completed (52). No significant clinical benefit was seen in this trial. An additional phase II trial has been completed using a multiple dosing regimen. Again, recovery of neurological function was not enhanced, but formation of new brain lesions was reduced by Hu23F2G. Preliminary results from a phase II trial in 420 patients with myocardial infarction have shown that although Hu23F2G was well tolerated, it had little effect on infarct size (53). A phase III trial in North America involving patients with acute ischemic stroke has been terminated prematurely since the interim results did not show any clinical benefit and failed to meet the trial's primary endpoints; the agent, however, was well tolerated.

Monoclonal antibodies against the LFA-1/ICAM-1 association have been indispensable in establishing the pathological roles that this interaction plays in human. However, these antibodies have apparent deficiencies as therapeutic agents which would limit their chronic use. These deficiencies include the inability to deliver the agents orally and the potential for immunoreactivity (54) although the liability of immunogenicity could be alleviated by producing humanized antibodies. Furthermore, protein-based therapeutics are generally expensive to produce. Therefore, the last 5 years have witnessed substantial research activities in the pharmaceutical industry aiming at discovery of small molecule antagonists of the LFA-1/ICAM-1 interaction to provide therapeutic agents with a broader application.

Small-molecule antagonists of LFA-1/ICAM-1 interaction

In the early 1990s, several series of small molecules have been reported to prevent cell adhesion by either inhibiting transcription of ICAM-1 and other adhesion proteins or by acting intracellularly to inhibit the activation of integrins via an unknown mechanism (although they do not directly antagonize the association of ICAM-1 with LFA-1) (55, 56). The pyrazole-based RWJ-50271 was reported as the first true antagonist of the LFA-1/ICAM-1 association, although it lacks the potency to be useful therapeutically (57). More recently, a diverse set of small molecules began to emerge as potent antagonists of the LFA-1/ICAM-1 interaction with defined mechanism of action and potential therapeutic utilities.

Antagonists mimicking the ICAM-1 ligand binding site

Based on the homology modeling of the interaction epitope of the first Ig domain of ICAM-1 with LFA-1 (36), scientists from Genentech and Hoffmann-La Roche have designed a series of diaminopropionic acid derivatives as ICAM-1 1st Ig domain mimetics capable of interrupting LFA-1/ICAM-1 interaction with nanomolar IC_{50} values in a cell-free binding assay (Table I) (58). Among the large number of examples claimed in the patents (59, 60), the critical structural features include α-amino group acylated with halogenated or methylated terephthalic acids, which in turn are coupled to 3-aminomethyl phenol (**1**), aminomethyl heterocycles (**2**) or 1-naphth-1-ylethyl amine (**3**). The β-amino group can be capped with a variety of alkyl, acyl and sulfonyl groups which constitutes a region for modulating the selectivity for LFA-1 over Mac-1 (**4-6**). The compounds exhibit 10- (**4**) to over 100-fold (**5**) selectivity for inhibiting the LFA-1/ICAM-1 interaction over the Mac-1/ICAM-1 interaction. The 3-atom linker appears to be rather tolerant of different variations. The cellular activity of compound **4** decreased somewhat in a neutrophil (expressing both LFA-1 and Mac-1)/ICAM-1 adhesion assay with an IC_{50} value of 16 nM, while compound **6** showed an IC_{50} of 0.1 nM in the same cellular adhesion assay.

The *in vitro* and *in vivo* immunomodulatory activity of these compounds compares favorably to both antibodies directed against LFA-1 and ciclosporin (58). For example, compound **6** inhibits a mouse mixed lymphocyte reaction

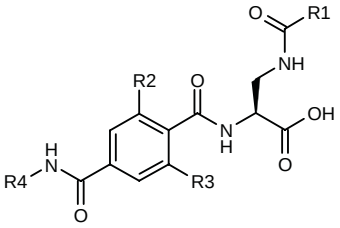
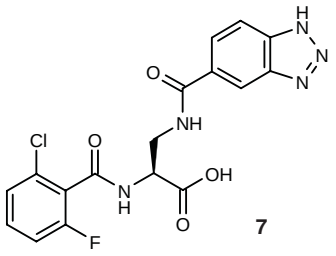


Table I: Diaminopropionic acid derivatives.

	R_1	R_2	R_3	R_4	IC_{50} (nM) LFA-1/ICAM-1	IC_{50} (nM) Mac-1/ICAM-1	IC_{50} (nM) MLR
1	2-thienyl	Br	H	3-OH-PhCH_2	1.3	ND	160
2	2-thienyl	Br	H	(indole-4-yl)methyl	2.7	25	420
3	2-thienyl	Br	H	1-naphth-CH(Me)	4.8	68	1535
4	2-thienyl	Cl	H	3-OH-PhCH_2	0.5	6.4	70
5	9*H*-fluoren-9-yl-methyl	Cl	H	3-OH-PhCH_2	1.8	273	12500
6	3,5-$(OH)_2$-Ph	Cl	Cl	3-OH-PhCH_2	0.2	ND	2



7

IC_{50} = 47 nM (LFA-1/ICAM-1)
IC_{50} = 273 nM (Mac-1/ICAM-1)

Fig. 1.

(MLR) with an IC_{50} of 2 nM as compared to IC_{50} values of 0.138 and 3.35 nM for anti-LFA-1 (M17) and anti-CD18 (HB226) antibodies, respectively. The only available *in vivo* efficacy data on this series of ICAM-1 mimetics come from compound **7**, a truncated, 100-fold less potent analogue of **4** (Fig. 1). Compound **7** exhibited efficacy comparable to the anti-LFA-1 antibody in inhibiting the paw swelling response in a mouse model of delayed type hypersensitivity (DTH) when the compound was dosed s.c. at 9 μM via a minipump for one day. In a croton oil-induced dermatitis murine model, **7** also inhibited the ear swelling response in a dose-dependent manner when administered s.c. at 4 μM for 3 days via a minipump; efficacy was comparable to the anti-CD18 antibody (60).

Detailed molecular analysis suggests that the binding sites within LFA-1 for ICAM-1, -2 and -3 are overlapping but not identical. Moreover, the binding faces for LFA-1 on top of ICAM-1, -2 and -3 appear to be distinct (61). ICAM-1 first Ig domain mimetics are potentially advantageous for offering selective antagonists against other ICAMs, although such selectivity information is not available for compounds **1-7**.

Antagonists targeting the LFA-1 I domain

HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors possessing cholesterol-lowering activity, such as mevinolin (lovastatin) **8**, have been found to be inhibitors of LFA-1/ICAM-1 interaction with low micromolar IC_{50} values in a cell-free binding assay and have displayed reduced potency in a cellular adhesion assay by Novartis (Fig. 2) (62). The inhibitory activities of these compounds are mainly derived from the lactone form because the ring-opening hydroxyacid is 10-fold less potent. Some hydroxyamide derivatives (**9**,**10**) have potencies similar to **8** in a Jurkat cell (LFA-1 expressing)/ICAM-1 cellular binding assay. In a murine thioglycollate induced peritonitis model, **9** fully inhibited the neutrophil migration when administered s.c. at a dose of 0.1 mg/kg (63).

A further improvement was made on the mevinolin derivatives wherein the lactone ring was modified to lactam or cyclic carbamate to provide compounds with improved affinity in the adhesion assay and oral efficacy in some inflammatory disease models (64). Compound **11** had IC_{50} values of 50 and 200 nM in a cell free adhesion assay and a human MLR assay, respectively. In the murine thioglycollate-induced peritonitis model, **11** has an ED_{50} value of 0.1 mg/kg p.o. It also provided 41% inhibition when given at 3 mg/kg b.i.d. in an allergic contact dermatitis (ACD) model. Without any information on the pharmacokinetic profiles of these compounds, it is interesting to note that compound **12**, showing an IC_{50} value of 190 nM in the cell-free assay, provided the same degree of inhibition (41%) when given at 1 mg/kg b.i.d. in the ACD model.

The binding site of **8** on LFA-1 has been determined using both NMR spectroscopy and x-ray crystallography (62). The region of the interaction between the protein and ligand **8** is a crevice formed by helix 1 and 7, and strands 4 and 5, consisting of hydrophobic residues Leu-

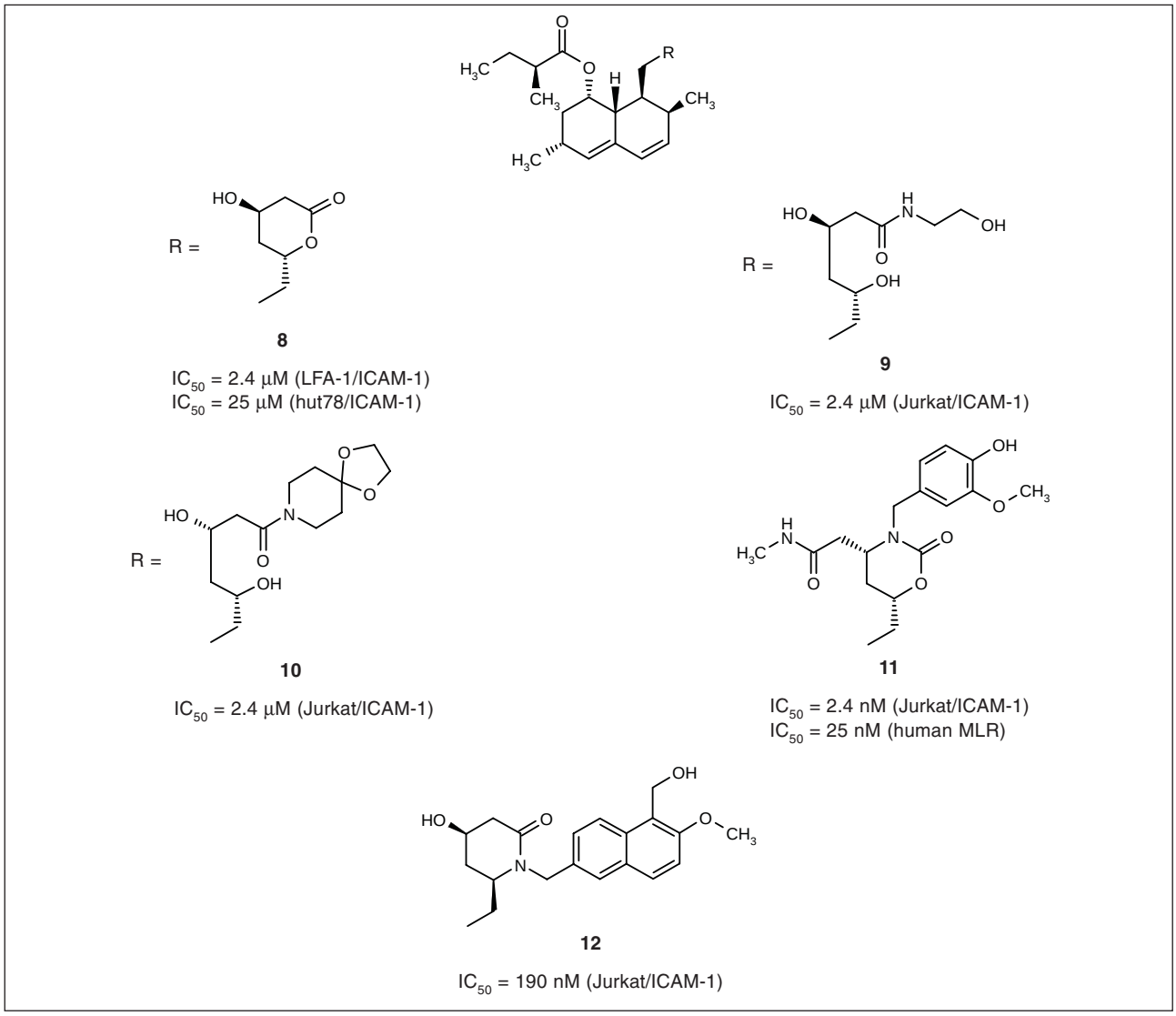


Fig. 2. Mevinolin analogs.

132, Phe-153, Ile-235, Tyr-257, Lys-287, Leu-298, Glu-301, Leu-302 and Lys-305. This crevice is located at a face opposite of the MIDAS motif which coincides with the proposed allosteric site, the IDAS of LFA-1. It has been suggested by the original authors that **8** may inhibit LFA-1/ICAM interactions by an indirect mechanism. It is plausible that the binding of **8** may inhibit structural changes that occur due to either ICAM-1 binding or the poorly understood activation process.

5-Substituted-3-phenylhydantoin derivatives have been reported as LFA-1 inhibitors by Boehringer Ingelheim (65). This series of compounds were discovered through optimization of a HTS hit with low micromolar potency in a cell-free adhesion assay. The most potent compounds in the series (**13-17**) inhibit the binding of LFA-1 to ICAM-1 with affinities in the range of 20-60 nM in the binding assay (Table II). They were also tested in 2 cellular assays. The inhibitory activities against LFA-1-induced aggregation of JY cells correlate well with the potency derived from the cell-free binding assay. However, the compounds are substantially weaker as inhibitors of both LFA-1- and VLA-4-mediated adhesion of SKW3 cells to ICAM-1, a possible reflection of the selective antagonism of LFA-1 mediated adhesion.

Compound **15**, BIRT-377, was characterized in detail both *in vitro* and *in vivo*. It is highly selective for LFA-1-mediated events (*i.e.*, no Mac-1/ICAM-1 inhibitory activity was observed) and it did not exhibit significant cytotoxicity in a cellular toxicity MTT assay (LD_{50} > 250 μM). **15** has an IC_{50} value of 0.85 μM in inhibiting the production of IL-2 by stimulated human peripheral blood lymphocytes. **15** also inhibited the SEB-induced production of IL-2 in a dose-dependent manner at 25 and 50 mg/kg doses p.o. in a mouse model, with the higher dose reducing the level

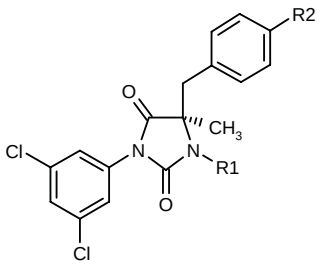


Table II: 3-Phenylhydantoin derivatives.

	R_1	R_2	K_d (nM)	IC_{50} (nM) JY cells	IC_{50} (nM) SWK-3 cells
13	$(CH_2)_4SO_3H$	Br	100	< 1	
14	$(CH_2)_4N(CH_3)_2$	Br	50	200	2
15	CH_3	Br	20	100	3
16	H	Br	60	100	8
17	Ac	Br	60	1300	35
18	CH_3	3-Pyr	40	30	0.5

of serum IL-2 by approximately 64% which is the same degree observed for an anti-LFA-1 Ab (M17.2; 200 μg i.p.). The N-1 position of the hydantoin seems to be the position for addressing the poor aqueous solubility of the overall molecule and metabolic liability of **15** which may be achieved by attaching charged groups as in compounds **13** and **14** (66). The binding site of **15** was only established as the CD11a chain in the original communication (65), although our modeling work suggests that compound **15** also binds to the IDAS and inhibits LFA-1 activation in a similar fashion as **8**.

Biaryl systems were explored using the Suzuki reaction of the aryl bromide **15** with various aryl boronic acid (67). Among the analogs examined, 3-pyridyl-based compound **18** appears to have the best *in vitro* profile with insignificant cellular toxicity (Table II). It is selective for ICAM-1 binding in the SKW3 cell adhesion assay and does not inhibit fibronectin. It also shows good activity in the JY cell aggregation assay with an IC_{50} of 30 nM obtained.

Structural modification of the hydantoin core of **15** led to the discovery of 2 new series of LFA-1 antagonists (Fig. 3). 1,3-Dihydropyrrolo[2,3-*b*]pyridin-2-one derivatives, such as **19**, and 1,3-dihydroindol-2-one derivatives, such as **20**, antagonize the binding of ICAM-1 to LFA-1 with IC_{50} values of 0.375 and 0.705 μM, respectively, in a cell-free binding assay (68). The discovery that tetrahydropyrrolo[1,2-*c*]imidazole-1,3-dione and tetrohydroimidazo[1,5-*b*]pyridine-1,3-dione derivatives were LFA-1 antagonists, such as **21** and **22**, was also disclosed (69). Although no specific potency data were reported for these compounds, the 10 examples claimed were reported to have K_d values below 10 μM.

Diaryl sulfide-based LFA-1 inhibitors represent the most extensively explored and reported small molecule antagonists to date. IC-52593 (**23**) was discovered from a high throughput screening by Icos Corp. (Fig. 4) (70). Initial SAR indicates that both the sulfide and the anilino group are required for the affinity. Compound **23** has an IC_{50} value of 2.1 μM in a cell-free binding assay, and exhibited an IC_{50} value of 1.5 μM for inhibiting JY cell (LFA-1 expressing)/ICAM-1 binding. Compound **23** also blocks the interaction of LFA-1 with other ICAMs, such as ICAM-3, in the JY cell-based assay with an IC_{50} value of 0.5 μM.

Compound **23** was developed into several series of potent, selective and orally bioavailable LFA-1

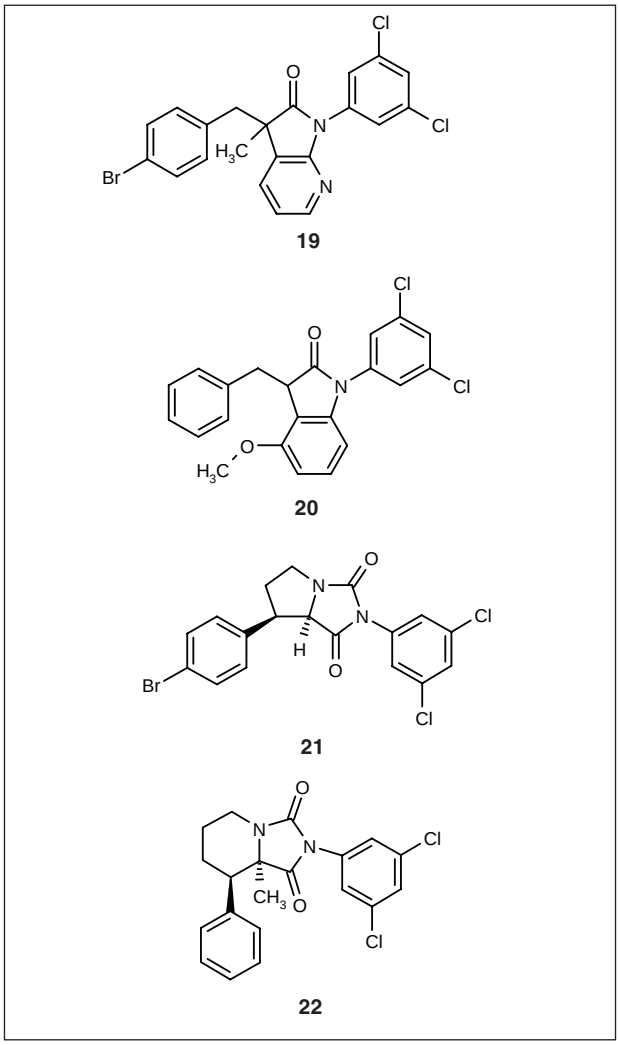


Fig. 3. New Boehringer Ingelheim LFA-1 inhibitors.

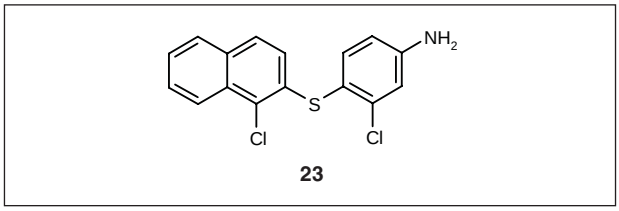


Fig. 4

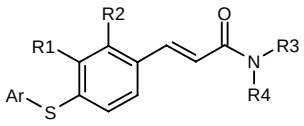


Table III: p-*Arylthio cinnamide derivatives*.

	Ar	R_1	R_2	IC_{50} (nM) NR_3R_4	IC_{50} (nM) LFA-1/ICAM-1	JY cells
24	2-i-Pr-Ph	NO_2	H	4-Ac-1-Piz	44	35
25	2,3-dihydrobenzo-[1,4]dioxin-6-yl	Cl	H	4-Ac-1-Piz	40	80
26	2,3-dihydrobenzo[1,4]dioxin-6-yl	CF_3	H	3-CO_2H-1-Pip	25	140
27	2,3-dihydrobenzo[1,4]dioxin-6-yl	CF_3	CF_3	4-CO_2H-1-Pip	3.0	0.5
28	2-MeO-Ph	CF_3	CF_3	4-CO_2H-1-Pip	5.0	0.1
29	1-Me-5-indolyl	Cl	Cl	4-CO_2H-1-Pip	6.0	4.0

antagonists by Abbott Laboratories (71, 72). The anilino diaryl sulfide template was transformed into the *p*-arylthio cinnamide series through identification of an additional binding pocket (Table III). Parallel optimization of the 3 segments of the molecule led to the discovery of A-286982 (**24**), which has IC_{50} values of 44 and 35 nM in a cell-free binding assay and the JY cell adhesion assay, respectively (73). The poor solubility associated with **24** was addressed through 2 structure-based approaches. One was the identification of more hydrophilic isopropyl phenyl ring replacements using a NMR fragment screening technique. Incorporation of these new fragments, mainly heterocycles, into the parent cinnamide template led to analogues with marginally increased aqueous solubility and improved pharmacokinetic profiles in rats, as exemplified by A-292949 (**25**) (74). Additional SAR efforts on the heterocycles failed to further advance the potency of the resulting analogs. The second approach was attaching a negatively charged carboxy group to the solvent-exposing piperidine/piperazine-based cyclic amides. A-304470 (**26**) has an aqueous solubility of greater than 3 mg/ml (pH 7.4) while maintaining the affinity of **24**. In contrast, attachment of positively charged groups to the cyclic amides did not improve the solubility of the resulting analogs. Compound **26** also exhibited a very good pharmacokinetic profile with bioavailabilities of 28 and 55% observed in rats and dogs, respectively (75). In a rat model of stroke, **26** administered at 50 mg/kg i.v. prior to reperfusion provided a statistically significant reduction of infarct size (Infarct Size/Area at Risk = 44%) compared to the control (IS/AAR = 63%).

Improvements in potency of over 10-fold were achieved with the introduction of a second chlorine or trifluoromethyl group to the 3-position of the central phenyl ring to fill up the cavity remaining in the binding pocket (Table III). The most potent compounds (**27** and **28**) contain 2 CF_3 groups and have IC_{50} values of 0.5 and 0.1 nM, respectively, in the JY cell adhesion assay. In a murine model of allergen-induced asthma, compound **29** (F = 23% in rats) had a significant and dose-dependent inhibitory effect on eosinophilia at concentrations as low as 1 mg/kg p.o. When **29** was given at 10 mg/kg p.o. at

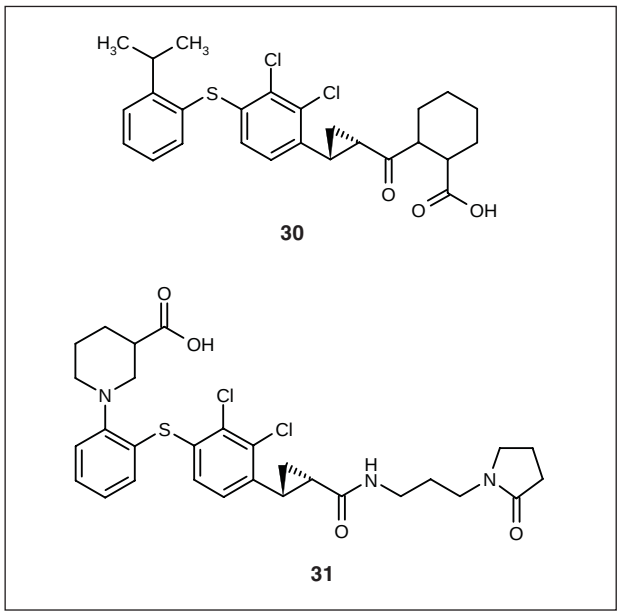


Fig. 5. Diaryl sulfide cyclopropylamides.

–1 and +7 h after allergen inhalation, eosinophil trafficking was inhibited by more than 60% (76). In a staphylococcus enterotoxin A-induced neutrophil trafficking model in the rat, compound **29** inhibited neutrophil migration in an apparent dose-responsive manner, with a significant inhibitory effect seen at a concentration of 100 mg/kg.

A series of diarylsulfide cyclopropylamides was discovered through modification of the double bond of **24** to a *trans*-cyclopropyl group to circumvent the potential metabolism liability of the cinnamide (Fig. 5) (77). Initially, the IC_{50} value of compound **30** dropped more than 2 orders of magnitude (from 13 nM to 1.48 μM) when the medium of the assay was changed from serum-free to 50% FBS. Compound **30** was virtually inactive at a concentration of 4 μM in the JY adhesion assay. Alteration of the polarity of the 2 termini of the molecule yielded compound **31** (A-324920) with maximized potency and minimized protein-binding properties. In the cell-free assay,

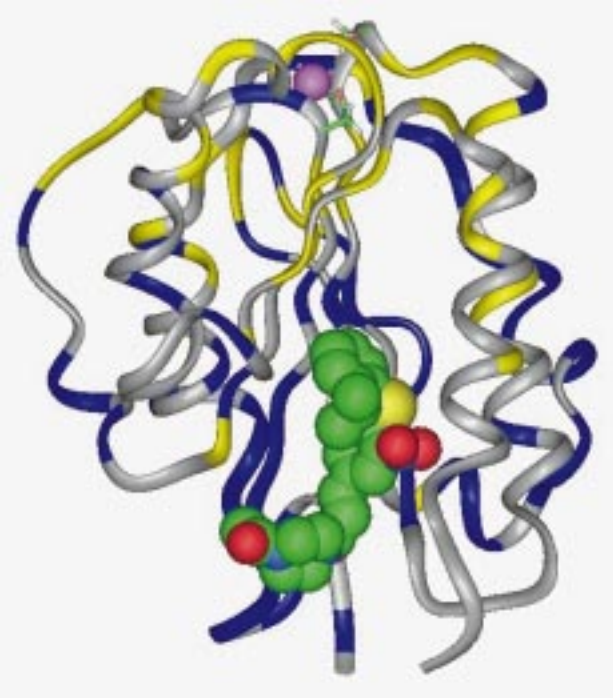


Fig. 6. NMR-derived structural model for A-286982 with LFA-1 I domain.

the IC_{50} value of **31** decreased only 5-fold, from 5 nM in the absence of serum to 27 nM in the presence of 50% FBS. In the JY cell adhesion assay, the degree of the decreased in potency was comparable, from an IC_{50} of 9 nM (–serum) to 50 nM (+50% serum). **31** also exhibited a bioavailability of 27% and an oral half-life of 1.2 h in rat, which justifies further refinement of this series of compounds.

The *p*-arylthio cinnamide **24** was found to bind to the IDAS of LFA-1 distal from the MIDAS, on the basis of multidimensional NOE studies (Fig. 6) (74). Thus, this series of compounds is predicted to inhibit adhesion of LFA-1 to ICAM-1 by lowering the affinity of LFA-1 for ICAM-1 through an increase in the K_d of LFA-1 or through stabilizing the low affinity state of LFA-1. Recently, disulfide bonds were introduced to lock the LFA-1 I domain in the predicted open, ligand binding or closed, nonbinding conformations, respectively (78). It was found that **8** does not inhibit the adhesion of the locked open I domain to ICAM-1, which again suggests that the mechanism of action of **8**, **15** and **24**, is to stabilize the I domain in the closed conformation and block the downward shift of the C-terminal α-helix that occurs in the transition to the open conformation. The presence of the I domain in the α-subunits of other integrins suggests that it is possible to target individually the regulatory sites within those I domains and identify small molecules which are capable of interrupting biological processes involving the activation of those integrins.

Antagonists with unknown site of action

Diazepanes derived from conformationally restricted dipeptides have been shown by Novartis to inhibit the LFA-1/ICAM-1 interaction (Fig. 7) (79). On the basis of the examples claimed in the patent, the large hydrophobic groups on the two opposite ends of the molecules seem to be critical for the intrinsic activities of this series of compounds. For example, compounds **32** and **33** have IC_{50} values of 440 and 70 nM, respectively, in a cell-free binding assay. In the ACD mouse model, **33** had an inhibitory effect of 40% when administered p.o. at a dose of 2 x 3 mg/kg or 50% when applied topically at a dose of 10 mM.

Novel thiadiazole amides (80) and thiadiazolyl urea (81) have been reported to be LFA-1 and Mac-1 inhibitors by Pharmacia & Upjohn (Fig. 8). The amide-based compounds appear to have very inconsistent inhibitory selectivity for LFA-1 and Mac-1. For example, compound **34** has IC_{50} values of 0.2 and 2.4 μM against LFA-1 and Mac-1, respectively, in the cell-free binding assays. However, in the cellular assay, the selectivity of **34** was reversed, with IC_{50} values of 2.0 and > 20 μM against PMN (Mac-1 expressing human neutrophil) and JY cells, respectively. The urea-based inhibitors seems to be more Mac-1 selective, such as compound **35**, which had IC_{50}s of 0.3 and 0.1 μM against LFA-1 and Mac-1, respectively, in the cell-free binding assay. The selectivity for Mac-1 inhibition exhibited by **35** was magnified to IC_{50} values of 0.8 and 5.0 μM for Mac-1 and LFA-1, respectively, in the cellular assays.

A novel benzopyran compound **36** obtained from *Streptomyces* sp. Mer-88 was claimed by Daiichi Seiyaku (Fig. 9). Compound **36** inhibited the association of LFA-1 and ICAM-1 in a dose-dependent manner *in vitro* with 92.3% of the binding inhibited at a final concentration of 12.8 μM (82).

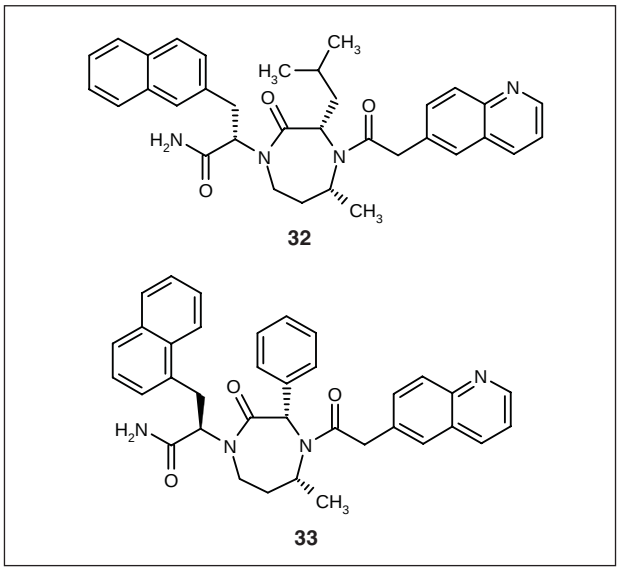


Fig. 7. Diazepane derivatives.

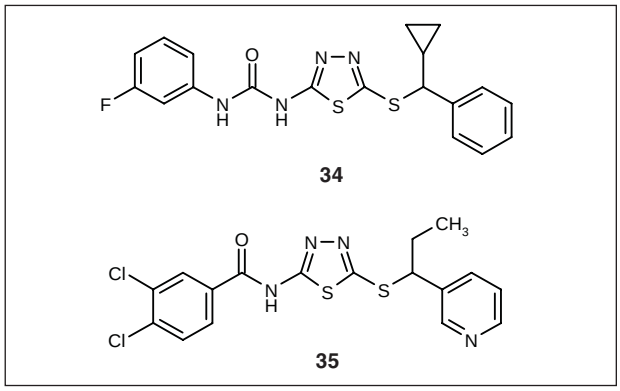


Fig. 8. Thiadiazole amide and urea derivatives.

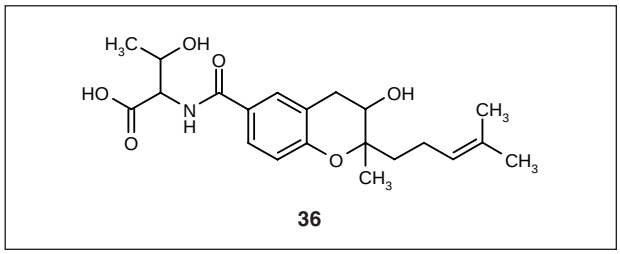


Fig. 9. Benzopyran derivative.

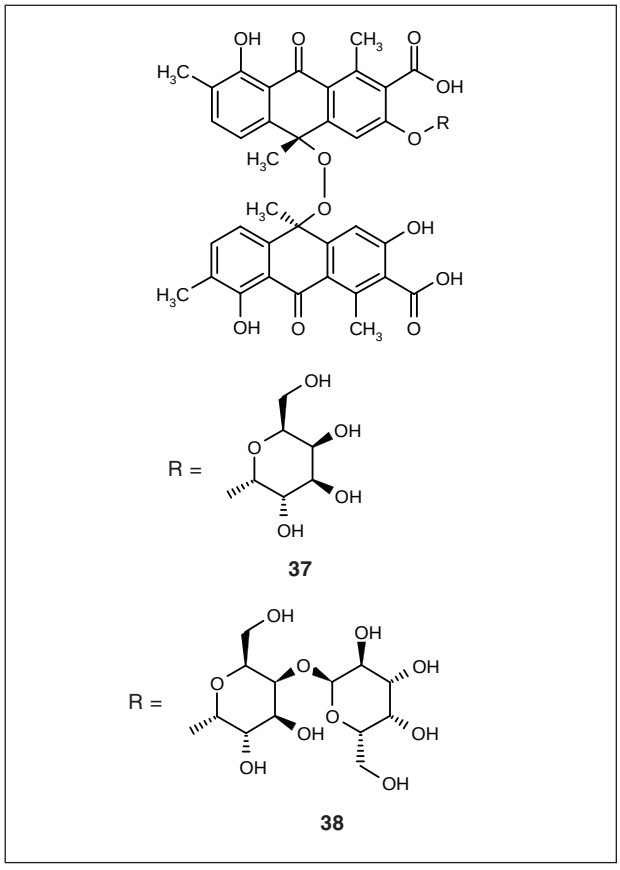


Fig. 10. Adxanthromycin A and B.

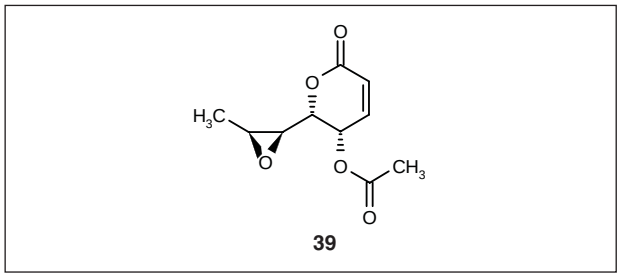


Fig. 11. Asperlin.

Scientists from Teikyo University have reported the isolation of Adxanthromycin A (**37**) and B (**38**) from the cultured broth of strain *Streptomyces* sp. NA-148 as novel inhibitors of the ICAM-1/LFA-1 interaction (Fig. 10) (83). Compounds **37** and **38** inhibited SKW-3 adhesion to soluble ICAM-1 with IC_{50}s of 18.8 and 25.0 μg/ml, respectively. They also completely and dose-dependently inhibited homotypic aggregation of JY cells at a concentration of 6.25 μg/ml. These compounds exhibited much weaker inhibition in the cell-free assay, which raises the possibility of involvement of additional molecular targets.

Asperlin (**39**) was found to be a β_2-integrin cell adhesion molecule inhibitor by American Home Products (Fig. 11) (84). Compound **39** inhibited the adhesion of activated β_2-integrin-expressing HL60 cells to recombinant soluble ICAM-1 with an IC_{50} value of approximately 12.5 mcg/ml, and blocked the homotypic binding of LFA-1 and ICAM-1 expressing 8866 cells in a dose-dependent manner. **39** also exhibited selectivity over other integrins and selectins. For example, **39** did not inhibit the adhesion of activated β_1-integrin-expressing U937 cells to human fibronectin, nor did it inhibit the adhesion of HL60 cells to E-selectin. **39** was not cytotoxic to HL60 cells in a MTT cytotoxicity assay, despite the potential reactive structural features of the compound.

Aminoalkylphosphonic ester derivatives and malonic diester derivatives have been claimed as ICAM-1-mediated cell adhesion inhibitors by Kyorin Pharmaceutical Co. (Fig. 12) (85, 86). For example, compounds **40** and **41** at 10 μM inhibited the binding (88 and 100%, respectively) of U937 cells to human umbilical vein endothelial cells (HUVEC) which were treated with human IL-1b to induce ICAM-1 and VCAM-1, respectively. In a mouse DTH model, **28** and **29** reduced swelling by 66 and 78%, respectively, over controls when dosed b.i.d. at 30 mg/kg for 5 days. An uncertainty pertaining to these 2 compounds is the possible involvement of other adhesion molecules, such as VCAM-1 and selectins, as the molecular targets for these inhibitors.

Conclusions

The LFA-1/ICAM-1 interaction has been implicated in many pathological processes, most notably inflammatory diseases and ischemic reperfusion injury. Small molecule

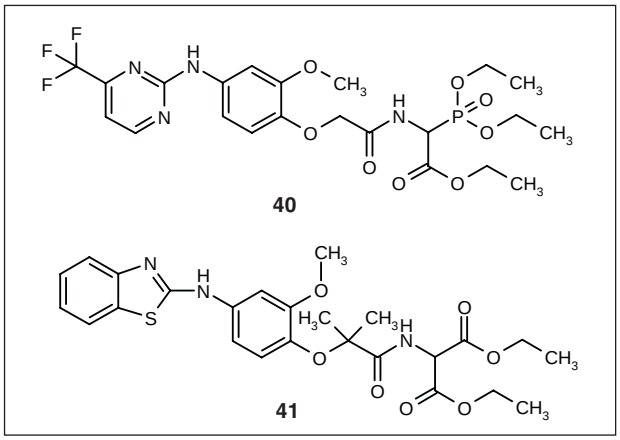


Fig. 12. Phosphonic ester and malonic ester derivatives.

antagonists may be useful in the treatment of psoriasis, rheumatoid arthritis and stroke. Extensive research is being conducted in this exciting new area as evidenced by the increasing numbers of related patents and by the different classes of antagonists reported to date. At present, IC-747 appears to be the most advanced compound since it is currently undergoing clinical development for the treatment of psoriasis in a phase I trial initiated in July 2001 (87). These early benchmark compounds will help to better establish the therapeutic utility of LFA-1 antagonists.

The activation and downregulation of leukocyte adhesion are still relatively poorly understood, although much is already known about the binding sites in integrins for ICAMs and other adhesion molecules, and reciprocally, about the integrin recognition sites in the ICAMs. The advances of basic research in leukocyte adhesion combined with the increasing amounts of structural data, should result in second generation antagonists with improved pharmaceutical properties and better specificities for greater clinical applications.

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