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Review Article

VLA-4 antagonists

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CONTENTS

Introduction

The integrins constitute a large class of heterodimeric cell-surface molecules consisting of α- and β-chains, each of which has a large extracellular domain and a short cytoplasmic tail. There are at least 14 different α-chains and 8 β-chains known which combine in a restricted manner depending on cell type to give approximately 23 members of the integrin family, each of which binds specific peptide ligands. Integrins mediate a variety of cell functions, including adhesion, migration, activation and survival.

Lymphocytes and leukocytes, with the exception of neutrophils, constitutively express the integrin VLA-4 ($\alpha_4\beta_1$, very late activating antigen-4, CD-49d/CD-29) and are capable of expressing the closely related integrin $\alpha_4\beta_7$ (LPAM, lymphocyte Peyer's patch cellular adhesion molecule) (1). VLA-4 interacts with both VCAM-1 (vascular cell adhesion molecule-1, CD106) or an alternatively spliced portion of the type III connecting segment of fibronectin known as the CS-1 region. The predominant form of VCAM-1 contains 7 extracellular immunoglobulin domains, a transmembrane domain and a short cytoplasmic tail. There is homology between domains 1-3 and 4-6, suggesting that they arose from a gene duplication event. The critical epitope of VCAM-1 for recognition of VLA-4 or $\alpha_4\beta_7$ is the sequence "QID(40)SPL" in domain 1, of which Asp 40 is essential (2-4). A crystal structure of a fragment containing the two *N*-terminal domains shows that this sequence lies on an exposed area of the C-D loop (5, 6). A related sequence on the CS-1 segment of fibronectin "LDV" is responsible for its interaction with VLA-4 (7-9). Compounds based on these sequences have fueled much of the effort in the development of small-molecule VLA-4 antagonists.

A recent report that VLA-4 also binds to the *N*-terminal fragment of osteopontin resulting from thrombin cleavage, but not full-length osteopontin, may also be of significance, as both osteopontin and thrombin are present at sites of inflammation. The osteopontin cleavage fragment (OPN 17-168) or either of the two peptides ELVTDFPTDLPAT (OPN 131-143) or SVVYGLR (OPN 162-168), identified by fragment screening, is independently able to inhibit adhesion of VLA-4-expressing cells (10).

The integrin $\alpha_4\beta_7$ also interacts with the same epitopes as VLA-4 in both VCAM and the CS-1 region of fibronectin, as well as with the mucosal addressin cell adhesion molecule-1 (MadCAM). The opposite is not true; VLA-4 does not interact with MadCAM. The expression of MadCAM is limited to high endothelial venules of gut-associated lymphoid organs and lamina propria venules and is involved in the trafficking of $\alpha_4\beta_7$-bearing lymphocytes to these mucosal tissues (11).

Integrins are involved in two-way signaling. The cytoplasmic tail of VLA-4 is linked to intracellular cytoskeletal proteins as well as kinases (12). While the details of this signaling process are largely unknown, the affinity of VLA-4 for its ligands can be modulated by intracellular pathways linked to chemokine interactions with G-coupled protein receptors on leukocytes in a process referred to as outside-in signaling (13, 14). In addition, binding of leukocytes to VCAM or fibronectin causes marked changes in cell morphology and is crucial for regulating cell motility (outside-in signaling) (15). Both α- and β-subunits are required for ligand binding, with affinity determined by their juxtaposition under the control of their contacts with proteins associated with the cytoskeleton (16).

Little is known about the structure of integrins, although a β-propeller motif in which the 7 *N*-terminal repeats are arranged in a torus has been proposed for the α-subunit (17). Sequence-swapping experiments support this model and indicate that elements of the β-propeller are involved in ligand binding (18). While many integrins, including the α-subunit of leukocyte function antigen-1 (LFA-1), display a well-defined metal ion-dependent adhesion site (MIDAS) which clearly mediates ligand affinity, this structural feature is absent from the α-subunit of α_4 integrins. It is apparent that the subunits are conformationally mobile, moving between high- and low-affinity states in response to physiological stimuli (19, 20).

Potential disease targets

In response to inflammatory stimuli, locally released chemokines and cytokines activate VLA-4 and the related integrin LFA-1 on the surface of circulating leukocytes and upregulate the expression of VCAM-1 on endothelial cells, respectively. While VLA-4 is constitutively expressed on leukocytes, CD4+ T-cells (21) and eosinophils (22), it may be upregulated in response to inflammation. Contact between E-, L- and P-selectins and their carbohydrate ligands on leukocyte and endothelial surfaces in concert with VCAM causes leukocytes to stick and begin to roll when in contact with blood vessel walls (23-25). This process provides an opportunity for firm adhesion to occur between these leukocytes and VCAM-1 expressed on the endothelial cell surface, and leads to flattening of the cells followed by their transmigration from the blood vessels to the extracellular matrix. Here they bind fibronectin and possibly other elements of the extracellular matrix, and migrate along a chemokine gradient to the site of inflammation. Since many inflammatory diseases are characterized by an influx of lymphocytes and leukocytes, there is keen interest on the part of the pharmaceutical industry in finding and testing compounds which have the potential to attenuate this process.

Although the ligand for LFA-1, intracellular adhesion molecule-1 (ICAM-1), is also expressed on endothelial cells and is involved in mediating leukocyte, including neutrophil, adherence, the LFA-1/ICAM interaction is less specific. Compounds which inhibit this interaction are being targeted to neutrophil-mediated diseases, particularly psoriasis. Adhesion receptors and their role regulating cell trafficking were recently reviewed (26).

Asthma is an inflammatory disease characterized by accumulation of activated CD-4+ T-cells and eosinophils in lung airways, resulting in the restriction of air flow and hyperreactivity of airways smooth muscle. VCAM expression and levels of soluble VCAM are increased in asthma patients (27, 28). Furthermore, antibodies directed against VLA-4 block eosinophil accumulation, hyperreactivity and inflammation in mouse (29, 30), rat (31) and guinea pig (32) models of allergic asthma. More recently, the peptide VLA-4 antagonists CY-9607 (33) and Bio-1211 (34) were shown to block the late-phase airways response and to attenuate carbachol-induced airways hyperresponsivity in a sheep model of allergic asthma.

Recent data support an important role for upregulated endothelial VCAM-1 in monocyte migration into the subintimal space during the early stages of atherosclerosis (35, 36). Mice in which the fourth domain of VCAM-1 was knocked out (VCAM-$1^{D4D/D4D}$) are viable, but produce only 2-8% of the VCAM-1 or VCAM-1 mRNA levels compared with wild-type mice. When crossed with LDL receptor-null mice, the resulting $LDLR^{-/-}$ VCAM-$1^{D4D/D4D}$ animals were paired with $LDLR^{-/-}$ $VCAM^{+/+}$ littermates and were fed a high-cholesterol diet for 8 weeks. While lipoprotein and cholesterol levels were comparable in the two groups, the extent of the atherosclerotic lesions in the aorta was significantly reduced in the $LDLR^{-/-}$ VCAM-$1^{D4D/D4D}$ mice (37). Treatment of normal or $LDLR^{-/-}$ mice on an atherogenic diet with a peptide mimetic derived from the LDV sequence over the course of 3 weeks markedly reduced the amount of lipid accumulation in their aortic sinuses (38). An antioxidant compound, AGI-1067, whose structure has not yet been published, is claimed to inhibit both chemokine MCP-1 and VCAM-1 gene expression and is being developed jointly by AtheroGenics and Schering-Plough. It is currently in phase II clinical trials for atherosclerosis (39).

Multiple sclerosis (MS) and Guillain-Barré syndrome, autoimmune diseases of the central and peripheral nervous systems, respectively, are characterized by an infiltration of activated T-cells and demyelination leading to neuronal degeneration. Anti-VLA-4 and anti-β_1 antibodies have been shown to be effective in a number of animal models of experimental autoimmune encephalo-myelitis (EAE) (40), although the timing of administration may be critical (41-43). Recently, preliminary results from the first human trials of the anti-α_4 antibody natalizumab were reported and will be summarized in the next section.

Treatments directed to VLA-4 and $\alpha_4\beta_7$ integrins may have a role in rheumatoid arthritis, inflammatory bowel disease, and possibly reperfusion injury. Fibroblast-like synovial cells express VCAM-1 and are involved in the chemotaxis and survival of B- and T-cells which mediate rheumatoid arthritis (44-46). Antibodies to VLA-4 are effective in rat models of adjuvant arthritis (47). As noted in the following sections, a few small-molecule VLA-4 antagonists have shown efficacy in animal models as well.

Inflammatory bowel disease is thought to be mediated at least in part by VCAM and MadCAM expressed locally in gut mucosal tissues (48, 49). Antibodies against $\alpha_4\beta_7$ have been shown to reduce the spontaneous development of ulcerative colitis in the cotton top tamarin (50) and inflammation in mouse models of colitis (51, 52). Anti-VCAM is also effective in the mouse dextran sulfate sodium (DSS)-induced colitis model (53). Recent publications indicate a possible role for VLA-4 in cardiac (54, 55) and cerebral (56) reperfusion damage. In the former case, murine neutrophils were shown to express VLA-4 after penetrating the extracellular matrix. Although these studies suggest VLA-4 is not important for adhesion, it may be involved in the coupling of superoxide release with cell-cell contact.

In addition to the above, VCAM/VLA-4 interactions play a role in regulating the trafficking of hematopoietic cells (58, 59) and may help render myeloma and lymphoma cells resistant to apoptosis (59-61). VCAM expression is required for embryogenesis; VCAM knockout mice display placental and heart defects and, with the exception of a very small percentage which develop to adulthood, die by embryonic day 11.5-12.5 (62, 63). However, animals in which the VCAM-1 gene was deleted after birth are viable and show defects in the homing of hematopoietic cells to bone marrow (64, 65). Mice in which VCAM expression is markedly attenuated are viable (37), and while there is little published information

on the possible reproductive toxicity of compounds targeting VLA-4 or $\alpha_4\beta_7$, several companies have drugs undergoing chronic studies in phase II, suggesting that these compounds are free of major reproductive liabilities.

Antibodies as drugs – natalizumab

Natalizumab (*Antegren*®) is a humanized monoclonal antibody developed by Protein Design Labs from a murine anti-alpha4 antibody which is currently under joint development by Biogen and Elan (66). Pharmacokinetic studies have been carried out in patients with MS at doses of 0.1-6 mg/kg i.v. The compound exhibits a long half-life (70-177 hours at doses of 1 and 6 mg/kg) and a dose-dependent AUC. Only mild adverse effects were observed and these consisted of transient headache and fatigue (67, 68).

A group of 72 patients with relapsing-remitting or secondary progressive MS were treated with i.v. infusions of 3 mg/kg natalizumab or placebo at weeks 0 and 4 and were followed for 24 weeks. Patients were evaluated for general well-being and MS symptoms every 2 weeks for the first 8 weeks and then every 4 weeks. MRI revealed a significant reduction in new active (1.8 *vs.* 3.6 per patient) and new enhancing lesions (1.6 *vs.* 3.3 per patient) during the first 12 weeks of the study, with no difference in the second 12 weeks. The number of treated patients with acute exacerbations was not different from placebo during the first 12 weeks, but was significantly higher during the 12-week follow-up (14 *vs.* 3) (69).

A phase II study was carried out in a group of 244 patients with moderately severe Crohn's disease (CDAI, or Crohn's Disease Activity Index, scores of 220-450). Treatment groups received placebo, a single 3 mg/kg infusion or two infusions of 3 or 6 mg/kg of natalizumab 4 weeks apart and were followed for 12 weeks. Patients were assessed by means of the CDAI and changes in quality of life as reflected in an inflammatory bowel disease questionnaire (IBDQ). The treatment group which received 3 mg/kg showed an improvement in the CDAI score of > 70 points by week 2 that was maintained throughout the study. Remission as defined by a CDAI score of < 150 was achieved by 46% of the patients who received 3 mg/kg compared with 27% in the placebo-treated group. There were also significant responses as determined by IBDQ scores at weeks 6 and 12 in the patients who received two infusions of natalizumab (70). Five of 10 ulcerative colitis patients achieved a positive clinical response in a small safety trial of a single 3 mg/kg i.v. dose (71). It is not clear whether further work in ulcerative colitis is ongoing with this drug.

It is encouraging that patients exposed to natalizumab for 6-10 weeks do not exhibit any significant side effects and that there is a suggestion of efficacy in MS and Crohn's disease. Biogen and Elan intend to start phase III clinical trials for both indications later this year.

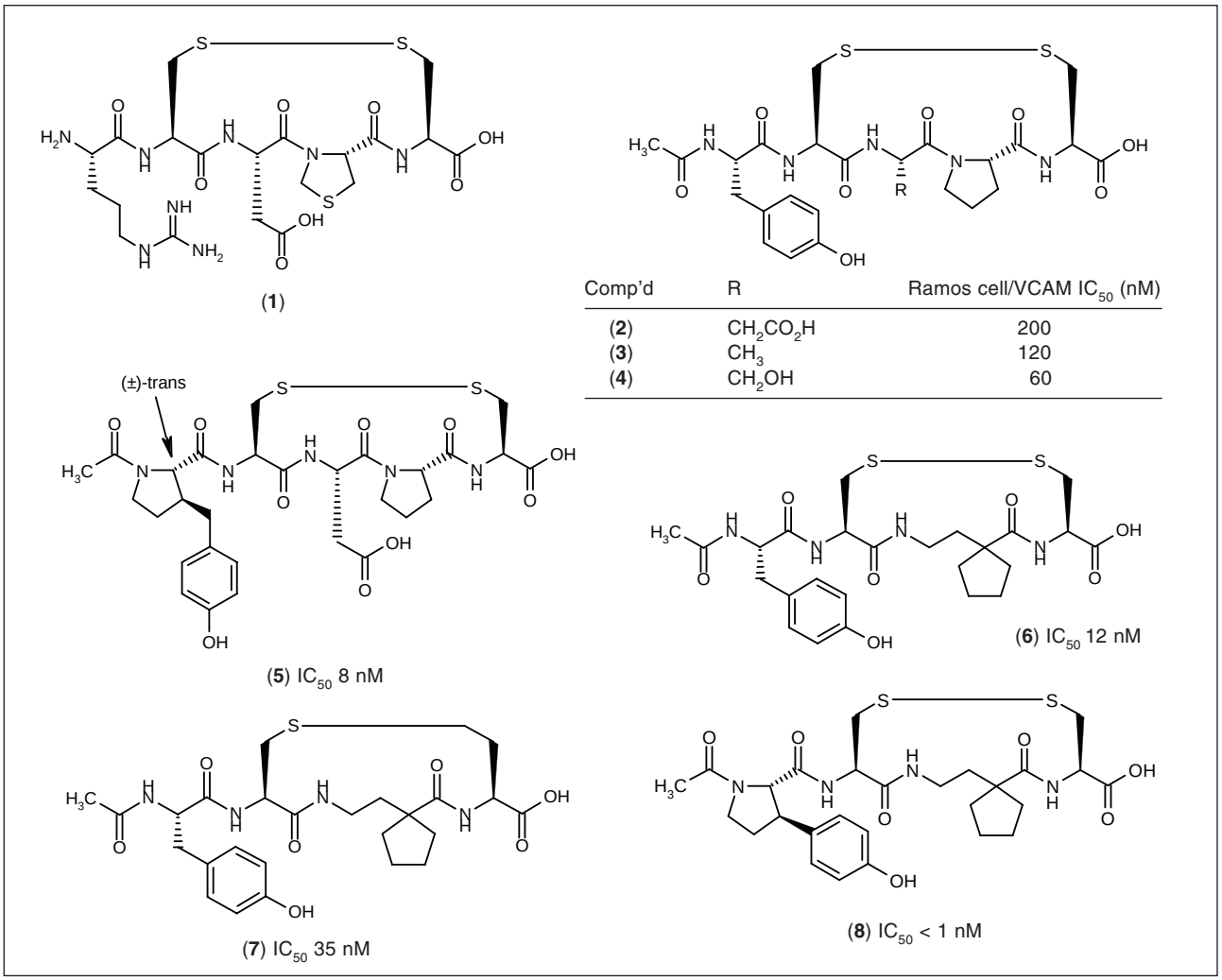
Small molecules as drugs

There has been intense interest among pharmaceutical companies over the past decade in discovering and evaluating new candidate VCAM/VLA-4 inhibitors. The early work on peptide derivatives, mostly based on variants of the LDV sequence, has been published and has led to a concerted effort directed particularly to phenylureido-LDV mimetics, as summarized below. Acyl-phenylalanines constitute the second major class of active compounds, many of which are capable of inhibiting both VLA-4- and $\alpha_4\beta_7$-mediated interactions with varying degrees of selectivity. In addition, there are individual reports of compounds of other chemical classes, one of which is summarized in the section on miscellaneous compounds. In general, insufficient data are available to evaluate the relevance of these compounds. While reports are starting to appear in peer-reviewed journals, most of the novel structures are only available through the patent literature and poster presentations at scientific meetings. In the sections that follow, the binding data that are cited have been gathered under widely different conditions and protocols and are not useful for directly comparing compounds with one another except where they are members of a series from one organization.

Cyclic peptide derivatives

An early publication (72) from Tanabe reported the cyclic peptide **1**, which was presumably originally designed as a constrained RGD mimic and is a potent inhibitor of both the VLA-4 and $\alpha_5\beta_1$ integrins. Structure-activity studies carried out at Genentech established that neither the arginine nor the aspartic acid moieties were necessary for VLA-4-antagonist activity. For example, compound **2** in which the *N*-terminal arginine was replaced with tyrosine had an IC_{50} of 200 nM in a Ramos cell/VCAM-1 binding assay. Further gains in potency could be obtained by substituting the aspartic acid with various amino acids, including alanine (**3**; IC_{50} = 120 nM) and serine (**4**; IC_{50} = 60 nM), while removal of the arginine and aspartic acid resulted in loss of affinity for the $\alpha_5\beta_1$ integrin (73). A further enhancement in potency was gained by incorporation of constraint into the tyrosine to give **5** as a mixture of diastereomers (IC_{50} = 8 nM). NMR analysis indicates that the *N*-terminal amino acid is disordered, but that the ring system exists in a well-defined, relatively planar conformation. Administra-tion of 0.5 mg i.v. of **4** to mice inhibited the homing of ^{51}Cr-labeled lymph node cells to Peyer's patches (Fig. 1).

Using the SAR and NMR models, Roche workers further simplified the structure of these cyclic peptides by substituting 1-(aminoethyl)cyclopentylcarboxylic acid for the Asp-Pro dipeptide of **2** to give the tetrapeptide analog **6** (IC_{50} = 12 nM in the same Ramos cell/VCAM binding assay employed at Genentech) (74). Compound **7**, in which one of the sulfur atoms was replaced by $-CH_2-$, suffered only a modest loss in potency (IC_{50} = 20 nM)



Comp'd	R	Ramos cell/VCAM IC_{50} (nM)
(**2**)	CH_2CO_2H	200
(**3**)	CH_3	120
(**4**)	CH_2OH	60

Fig. 1.

(75). The Roche team also prepared the constrained tyrosine analog in a stereochemically defined 2-*S*,3-*R*-configuration and incorporated it to give the extremely potent cyclic peptide **8** (IC_{50} < 1 nM) (76) (Fig. 1).

From an extensive series of cyclic peptides built around the LDV motif, AstraZeneca chemists prepared the cyclic hexapeptide **9** (ZD-7349), which potently inhibited the binding of MOLT-4 cells to VCAM (IC_{50} = 260 nM) and to fibronectin (IC_{50} = 330 nM) (77) (Fig. 2). Further profiling of **9** in animal models indicates that it inhibits the development of ovalbumin-induced delayed-type hypersensitivity (DTH) in mice when given via osmotic minipumps at a rate of 0.01 mg/kg/day s.c., as a 3-10 mg/kg s.c. injection 4 hours prior to challenge or as an i.v. bolus 20 hours after challenge. ZD-7349 given chronically to mice in a 14-day minipump at 10 mg/kg/day also inhibited the development of collagen-induced arthritis at 3 mg/kg/day and delayed the onset of symptoms in an experimental autoimmune encephalomyelitis (EAE) model.

ZD-7349 was chosen for development in a prototype depot formulation through incorporation into a 50:50 poly(*d*,*l*-lactide-co-glycolide) depot form suitable for s.c. implantation. Unlike some potent compounds in the series, for example **10** (78) (Fig. 2), which was unstable at pH 3, ZD-7349 has adequate stability and is released from the polymer matrix at a steady state over the course of 17 days (77).

Linear peptides – LDV mimics

Starting with the 10-amino-acid CS-1-derived peptide GPEILDVPST (IC_{50} = 150 uM), which inhibits the binding of ^{51}Cr-labeled Jurkat cells to the CS-1 25-mer, Arrhenius and coworkers examined a series of truncated analogs and showed that the minimal fragment retaining activity was the sequence ILDVP (IC_{50} = 250 μM) (79). Replacement of isoleucine with phenylalanine and inversion of the stereochemistry of the *C*-terminal proline led to

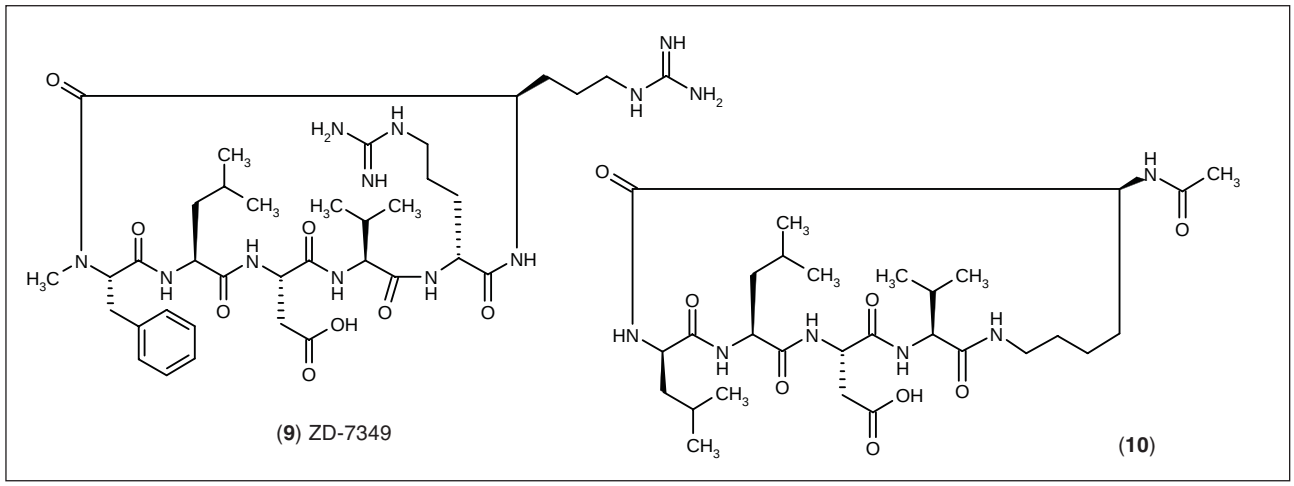


Fig. 2.

the sequence FLDVp (IC_{50} = 25 μM), prompting a more extensive investigation of analogs based on LDV and culminating in the identification of the *N*-terminal phenyl acetyl analog **11** (IC_{50} = 0.5 μM) (Fig. 3). Compound **11** at 6 mg/kg over 24 hours was active in inhibiting the oxazolone-induced DTH in mice (80). It also inhibits both the early- and late-phase increase in bronchial resistance in sheep sensitive to *Ascaris suum,* as well as the development of hyperreactivity in these animals, when given at a dose of 30 mg/kg by aerosol b.i.d. for 3 days and 30 minutes prior to challenge and 4 hours postchallenge on day 4 (33).

CY-9701 (**12**) (Fig. 3) is an analog in which an *N*-methyl group and a *C*-terminal piperazine were added to improve proteolytic stability. CY-9701 (100 mg/kg intranasally 5 minutes before ovalbumin challenge) decreased eosinophil influx to the lung, bronchial hyperresponsiveness and the concentrations of IL-4, IL-5 and eotaxin in the bronchoalveolar lavage fluid in a murine allergy model 72 hours after challenge (81).

Starting from the *N*-(4-hydroxyphenylacetyl)-LDV peptide **13** (IC_{50} = 5000 nM in the Jurkat cell/CS-1 binding assay), Biogen medicinal chemists systematically varied the *N*-terminal residue and discovered that addition of a urea terminating in 2-methylphenyl, as in **15** (IC_{50} = 6 nM), led to a marked increase in potency. The profound role of the phenylureidophenylacetyl moiety is evident from its adoption into LDV mimics by many pharmaceutical companies. Addition of a *C*-terminal proline led to Bio-1211 (**16**), which is a highly selective inhibitor of VLA-4-mediated binding (IC_{50} = 4 nM). It is approximately 500-fold selective for VLA-4 over alpha4beta7 integrins and is inactive in binding assays for inhibition of $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_{IIb}\beta_{IIIa}$ and $\alpha_L\beta_2$. After a single nebulized dose of 0.1 mg/kg to sheep sensitized to *Ascaris suum*, **16** inhibited the early-phase and virtually eliminated the late-phase increase in bronchial resistance 24 hours after challenge (34) (Fig. 3).

Bio-1211 was licensed to Merck & Co. and was taken into the clinic for the treatment of asthma with disappointing efficacy results in early phase II trials. Extensive work to find less peptidic analogs has been carried out at Biogen, one example of which is the more potent derivative Bio-1272 (**17**) (82) (Fig. 3).

A very interesting observation made by Biogen scientists working with [^{3}H]-Bio-1211 is that its dissociation constant and off-rate for binding to Jurkat cells are highly dependent on the affinity state of the integrin. *In vitro*, this is modulated by cation concentrations. In the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+}, the K_d of Bio-1211 was 20-40 nM and improved to 100 pM in the presence of 2 mM Mn^{2+}. A further enhancement of K_d to 18 pM was observed when the binding experiments were run in the presence of 2 mM Mn^{2+} and 10 mg/ml of the activating antibody TS2/16. The effect is due exclusively to modulation of the off-rates; on-rates did not vary widely among experiments. Similar results were observed for Jurkat cells binding to the CS-1 peptide or to an Ig-VCAM conjugate, suggesting that regulation of off-rates by ion concentrations, and likely by the activation state of cells expressing VLA-4, may be general (83).

Several companies have pursued related phenylureidophenacyl-LDV mimics; the most advanced of these being IVL-745 (**18**) (Fig. 4), which Aventis has taken into the clinic as an inhaled antiasthmatic agent. Compound **18** is a potent inhibitor of Ramos cell/fibronectin and Ramos cell/VCAM-1 binding, with little effect on $\alpha_5\beta_1$- or $\alpha_4\beta_7$-mediated adhesion (84). VCAM-1 binding to human lymphocytes and eosinophils (85, 86) and the proliferation of human T-cells induced by culture in the presence of immobilized anti-CD3 and rh-VCAM-1 are inhibited in a concentration-dependent manner (87). When administered 4-6 hours prechallenge or up to 4 hours postchallenge, inhaled doses of 3-10 mg/kg prevented typical lung histopathological changes and eosinophil accumulation in allergic Brown-Norway rats challenged with ovalbumin (88).

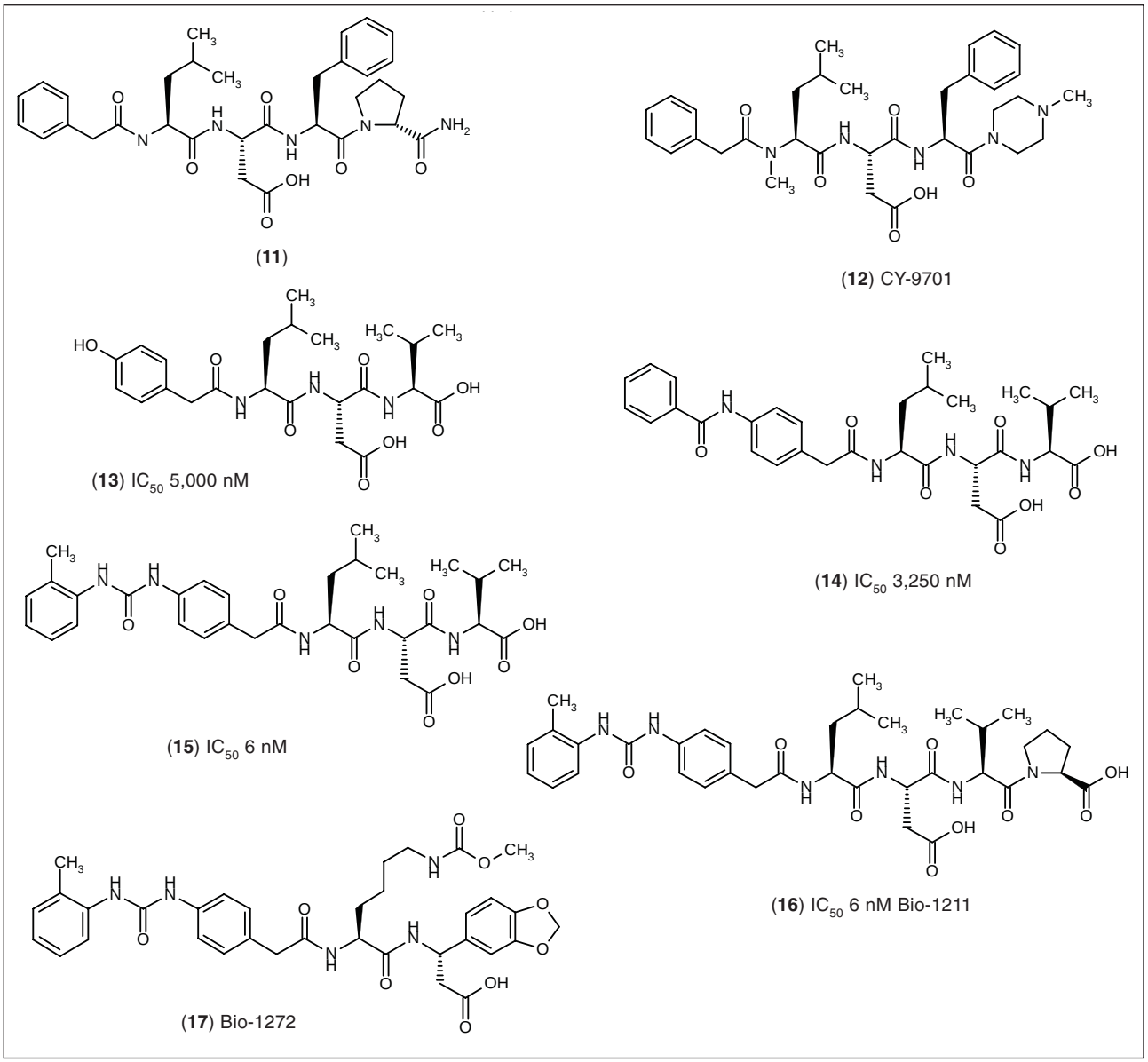


Fig. 3

A second member of the class, the 3-fluoroprolinol **19**, was active in VCAM/VLA-4 binding assays and demonstrated a bioavailability of 16% in the mouse and 59% in the rat after doses of 10 mg/kg p.o. At a dose of 50 mg/kg p.o. t.i.d. for 2 days **19** inhibited eosinophil accumulation in bronchoalveolar lavage fluid of mice sensitive to *Ascaris suum* by 46%. At a dose of 50 mg/kg b.i.d. it also limited pulmonary eosinophilia in rats induced over 24 hours by treatment with compound 48/80 (89). A number of other related compounds, including the phenylurea **20** (90) and the benzoxazoles **21** (91) and **22** (92), have been reported in the patent literature, but with insufficient biological data to assess their relevance. One of a number of hydantoins patented by Aventis, **23** was reported to inhibit the interaction between U-937 cells and human VCAM with an IC_{50} of 2.5 nM (93) (Fig. 4).

Texas Biotechnology has reported a series of ureas, including **24-26,** which are very potent antagonists (IC_{50} = 0.4-1.5 nM) of VLA-4 binding to the CS-1 peptide and over 1,000-fold selective for VLA-4 over $\alpha_4\beta_7$ (94-96). TBC-3486 appears to be the lead compound; 3 mg/kg intranasally and 3 and 10 mg/kg i.p. were effective in a mouse lung inflammation model and TBC-3486 was also active in a mouse adjuvant arthritis model. The related analog **27** has an IC_{50} of 7 nM in the VLA-4/CS-1 binding assay (97). Texas Biotechnology has licensed its VLA-4 program to Schering-Plough and is collaborating with the

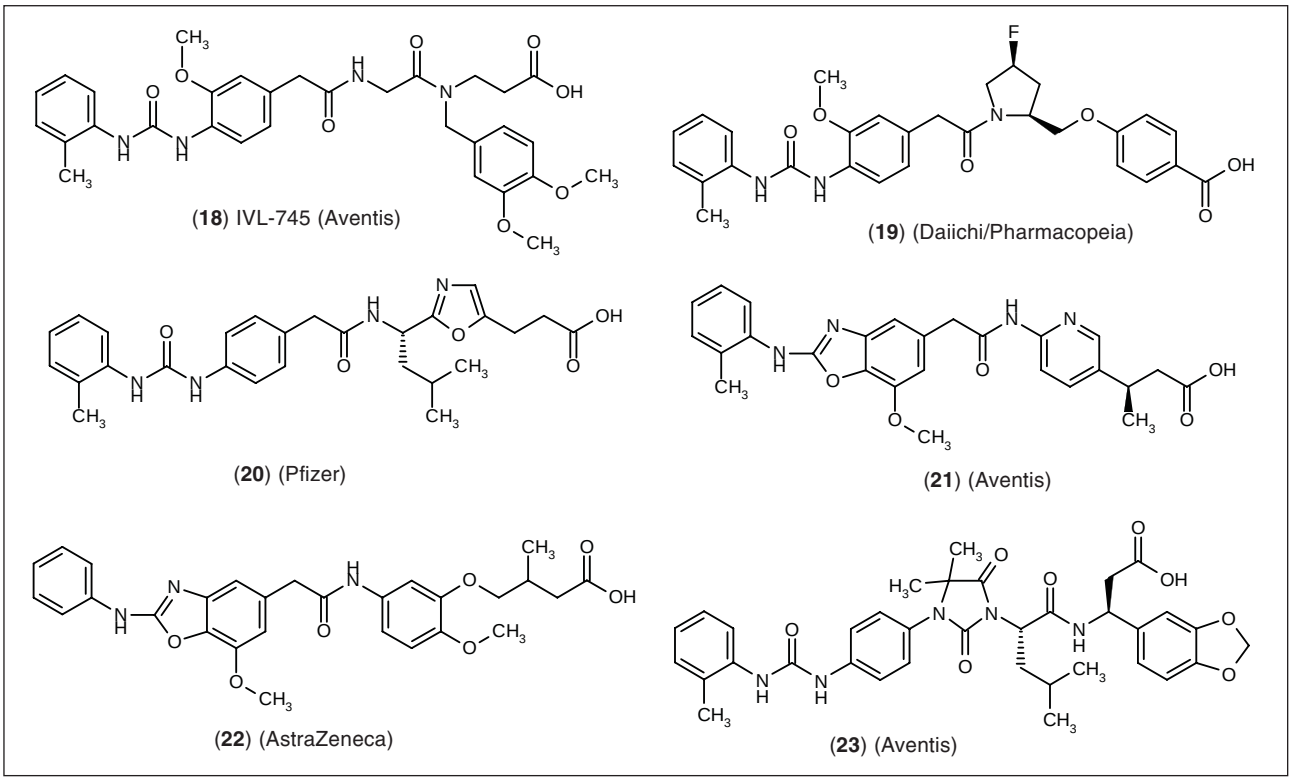


Fig. 4.

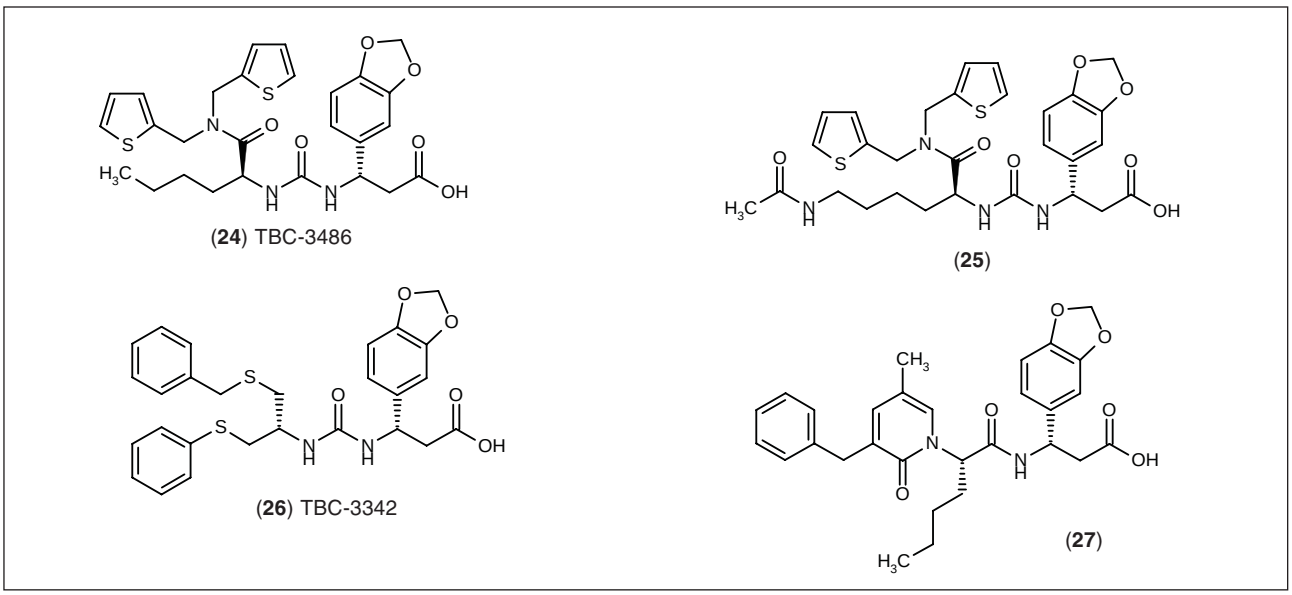
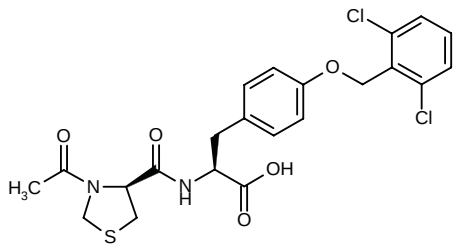


Fig. 5.

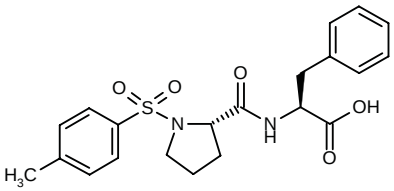
latter company on selecting candidates for development for the treatment of asthma (Fig. 5).

Acylphenylalanines

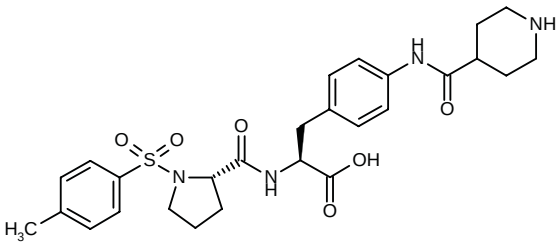
Acylphenylalanines constitute a second major class of $\alpha_1\beta_4$/VCAM antagonists (Fig. 6). The acylphenylalanine core seems to be critical for activity, but tolerates a remarkably broad array of substituents in the 4-position and on the phenylalanine nitrogen atom. Also, unlike the LDV mimics which are generally highly selective for VLA-4, many members of the acylphenylalanine class are dual inhibitors of VLA-4/VCAM and $\alpha_4\beta_7$/MadCAM, depending on the particular substitution pattern. Since relative activities are determined using different cell lines which express VLA-4 (typically Ramos or Jurkat cells) or



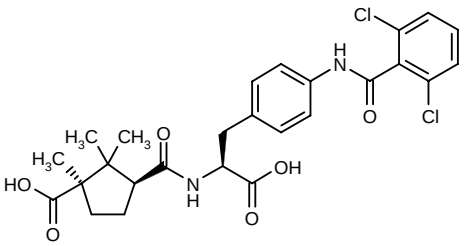
(**28**) (Celltech Chiroscience)
CT-5219



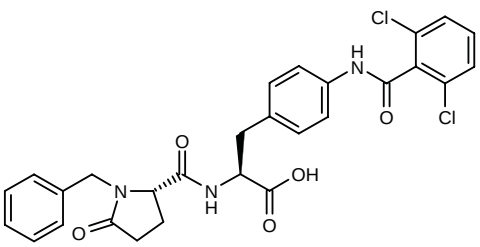
(**29**) (American Home Prod./Elan)
CT-757



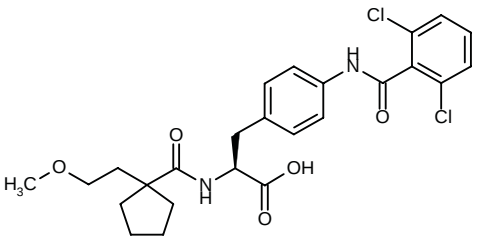
(**30**) (American Home Prod./Elan)
CT-767



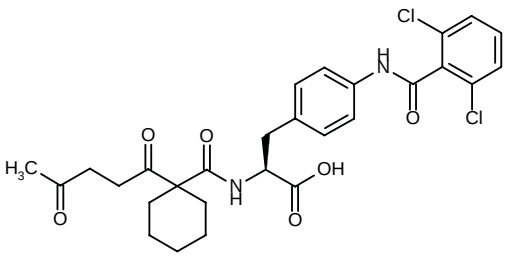
(**31**) (Tanabe Seiyaku)
TR-9109



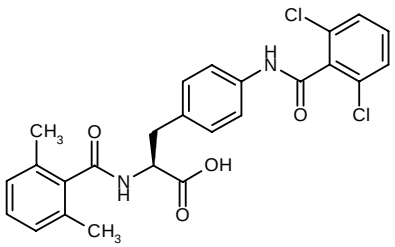
(**32**) (Hoffmann-La Roche)



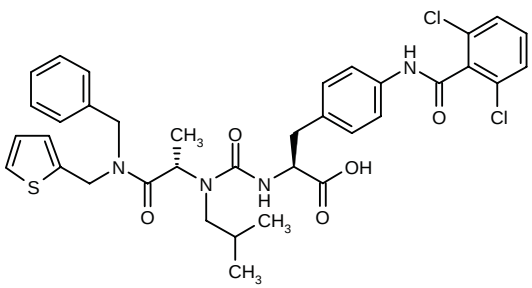
(**33**) (Hoffmann-La Roche)



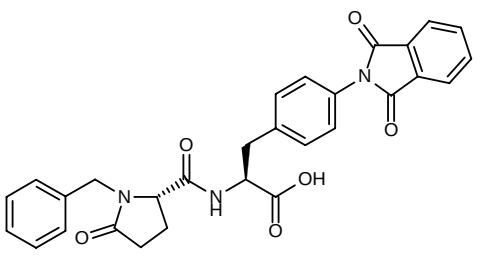
(**34**) (Tanabe Seiyaku/Pharmacia)



(**35**) (Hoffmann-La Roche)



(**36**) (Kaken Pharmaceuticals)



(**37**) (Hoffmann-La Roche)

Continued

Fig. 6.

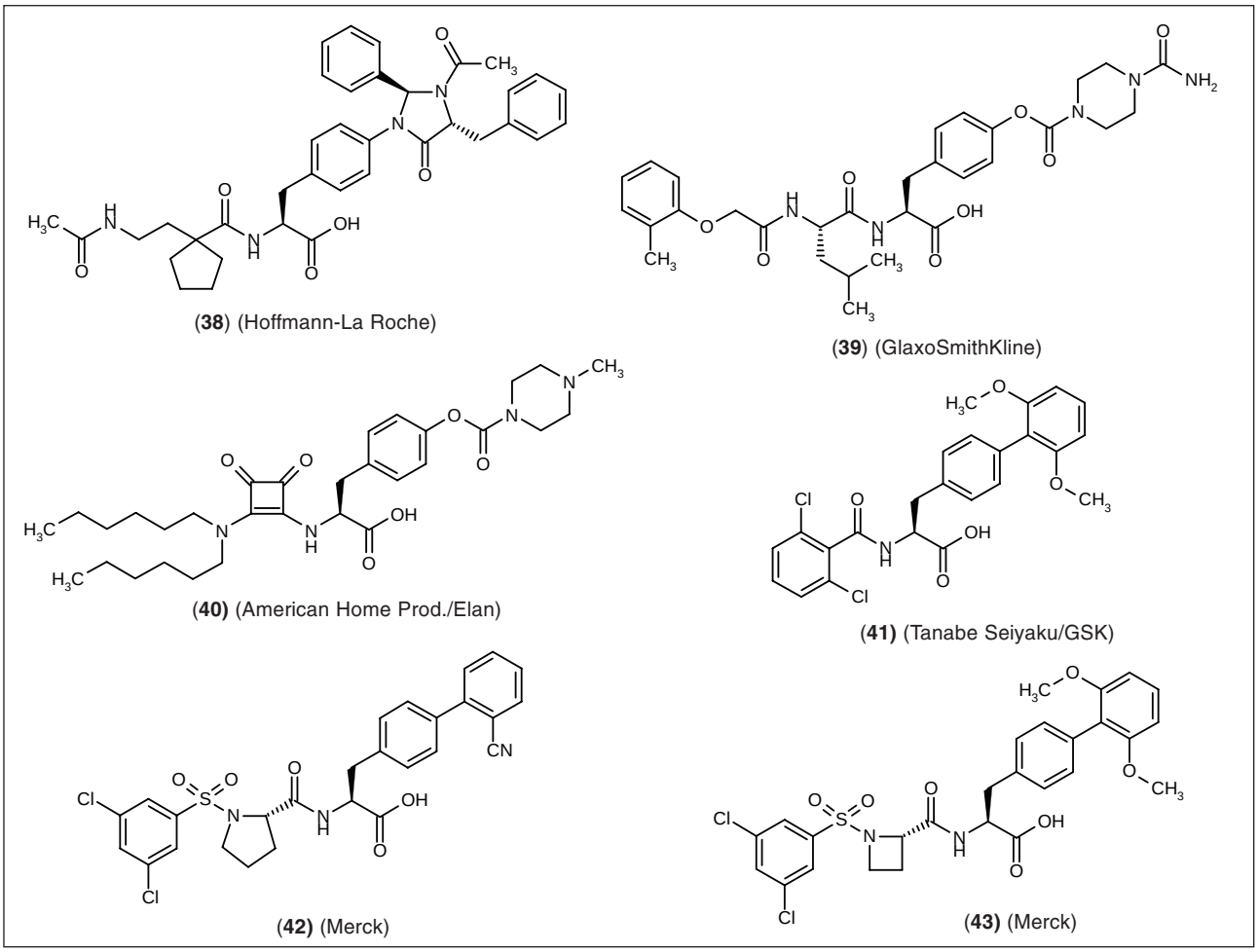


Fig. 6. Continuation.

$\alpha_4\beta_7$ (RPMI 8866 cells), it is not possible to quantitate them, except by comparison of different compounds in the same assays.

Of a series of thiaproline-tyrosine esters and ethers, the 2,6-dichlorobenzyl ether **28** was among the most potent in a Jurkat cell/VCAM-1 binding assay (IC_{50} = 35 nM). It was inactive against other integrins except $\alpha_4\beta_7$ (IC_{50} = 190 nM) and was highly stable to plasma esterases. Given to sheep at doses of 0.03-3 mg/kg i.v., **28** inhibited both the early- and late-phase increase in airways resistance caused by administration of antigen in a dose-dependent manner and also inhibited the increase in airways hyperreactivity at the higher doses (98). It has poor oral bioavailability in rats and sheep and is subject to rapid elimination, particularly in rats (99).

Elan and American Home Products have been collaborating on VLA-4/VCAM antagonists for some time. Tos-Pro-Phe (**29**, CT-757) was an early hit from screening which was extensively investigated. Oral bioavailability was 10-15% in rats, with most of the drug being rapidly eliminated in the bile. Both rats and cynomolgus monkeys hydrolyzed approximately 25% of the administered drug at the Pro-Phe bond (100). Further SAR work led to the identification of the piperidine **30** (CT-767) which was more potent and, importantly, less protein-bound, as demonstrated in the Jurkat cell/HUVEC assay in the presence and absence of serum (101).

Many investigators have identified the 2,6-dichlorobenzoylamino moiety as a highly favorable substituent for the 4-position of various acylphenylalanines. One of the first to be described was the camphoric acid derivative **31** (TR-9109) from Tanabe Seiyaku (102). A number of such compounds with *N*-benzylpyroglutamyl- (**32**) (103), *N*-(1-substituted cycloalkylcarbonyl)- (**33** and **34**) (104, 105), *N*-(2,6-disubsituted benzoyl)-, (**35**) (106) and substituted ureido **36** (107) acyl groups, all gave low-nanomolar inhibition in a cell-based assay of the Ramos cell/VCAM interaction. Other 4-substituents which are associated with high potency in the same assay include imides (**37**) (108) and the imidazolidinone **38** (104).

The phenoxyacyl-Leu-Phe derivative **39** (109) and the squaric acid analog **40** (110) represent still further examples of structure types associated with potent activity in cell-based assays. The former compound was also

reported to reduce both eosinophil infiltration and airways hyperreactivity in a guinea pig asthma model when administered intratracheally at 0.2 mg/kg 0.5 hours before and 6 hours after ovalbumin challenge. GlaxoSmithKline has an inhaled VLA-4 antagonist in clinical trials which could be **39** or a close analog.

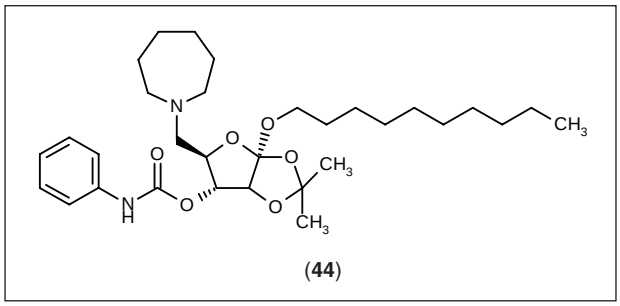
TR-14035 (**41**) represents the first example of a new variation on the acylphenylalanines in which the 2,6-dichlorobenzamide typical of many of the more potent analogs is replaced by a phenyl ring with a hydrogen bond acceptor in the *ortho* position. The increased lipophilicity thus gained translates into improved oral bioavailability: in rats, F = 60% and $t_{1/2}$ = 5 hours, and in dogs, F = 25% and $t_{1/2}$ = 2.5 hours (111). Like other acylphenylalanines, TR-14035 is cleared rapidly, largely through biliary excretion. IC_{50}s in Jurkat cell/CS-1 and RPMI cell/CS-1 assays for VLA-4 and alpha4beta7-inhibitory activities, respectively, were 56 nM and 6 nM, suggesting that TR-14035 is somewhat selective for $\alpha_4\beta_7$ over VLA-4. Consistent with this finding, it also potently inhibits RPMI cell/VCAM and MadCAM interactions.

TR-14035 inhibited DNFB-induced delayed-type hypersensitivity in mice at doses of 10-100 mg/kg p.o. and MLN cell migration to the DSS-induced inflamed colon of mice at doses of 10-25 mg/kg p.o. A dose of 30 mg/kg p.o. b.i.d. over 29 days caused a significant decrease in paw swelling relative to controls in a rat peptidoglycan/polysaccharide (PGPS) model of polyarthritis. TR-14035 has been licensed to GlaxoSmithKline worldwide except for parts of Asia and has been renamed SB-683698. It has completed phase I clinical trials, with phase II expected to start at the beginning of 2002 for asthma, inflammatory bowel disease, rheumatoid arthritis and multiple sclerosis (112).

Several patents from competing companies have also claimed biphenylalanines, but with insufficient biological data to assess their potential. One publication from Merck describes phenylsulfonylprolyl- and azacyclobutyl-biphenylalanines such as **42** and **43** (113). These compounds have IC_{50}s very close to those of Bio-1211 and TR-14035, used as standards in a Jurkat cell/VCAM-Ig conjugate binding assay, and are at least 100-fold selective over $\alpha_4\beta_7$/RPMI 8866 cell binding. They have moderate to poor oral bioavailability and generally rapid clearance approaching hepatic blood flow in rats. Compound **42** stood out in this series as having an oral bioavailability of 33% in the rat and low clearance (5 ml/kg/min). Clearance values in dogs and monkeys were comparable and likely a consequence of its high protein binding (> 99.5% in both rat and human plasma). In support of this conclusion, the potency of **42** in the binding assay was decreased markedly when the assay was carried out in the presence of plasma.

Miscellaneous

There are a number of compounds of other chemical classes reported to inhibit VLA-4/VCAM-1 binding, but they are either very weak inhibitors relative to the LDV and acylphenylalanine derivatives described in the previous sections, or they are described in the patent literature with no associated biological data. One exception is a patent from Ranbaxy that describes sugars such as **44** (Fig. 7). This compound is active in a Jurkat cell/human VCAM binding assay (IC_{50} = 7.5 nM) and is also highly effective in the mouse DNFB-induced delayed hypersensitivity assay when administered i.v., s.c. or orally (114). It remains to be determined whether these compounds work largely or solely through effects on VLA-4 or whether other mechanisms are involved.



(**44**)

Fig. 7.

Conclusions

There is a great deal of anticipation concerning the role of VLA-4/VCAM-1 inhibitors with and without concomitant inhibition of $\alpha_4\beta_7$-mediated interactions in the treatment of various human inflammatory diseases. As patents are issued and programs begin to mature, we can expect a considerable volume of data from animal models employing compounds now in early development in the near future, with clinical data following over the next 2-3 years. It will be interesting to note whether new structural classes emerge and, given the rapid clearance of many of the compounds identified to date, what the relationship between their pharmacokinetics and pharmacodynamics will be.

References

1. Erle, D.J., Briskin, M.J., Butcher, E.C., Pardo, A., Lazarovits, A.I., Tidwell, M. *Expression and function of the MadCAM- 1 receptor, integrin $\alpha_4\beta_7$, on human leukocytes.* J Immunol 1994, 153: 517-28.

2. Renz, M.E., Chiu, H.H., Jones, S., Fox J., Kim, K.J., Presta, L.G., Fong, S. *Structural requirements for adhesion of soluble recombinant murine vascular cell adhesion molecule-1 to $\alpha_4\beta_1$.* J Cell Biol 1994, 125: 1395-406.

3. Clements, J.M., Newham, P., Shepherd, M. et al. *Identification of a key integrin-binding sequence in VCAM-1 homologous to the LDV active site in fibronectin.* J Cell Sci 107: 2127-35.

4. Chiu, H.H., Crowe, D.T., Renz, M.E., Presta, L.G., Jones, S., Weissman, I.L., Fong, S. *Similar but nonidentical amino acid residues on vascular cell adhesion molecule-1 are involved in the interaction with $\alpha_4\beta_1$ and $\alpha_4\beta_7$ under different activity states.* J Immunol 1995, 155: 5257-67.

5. Jones, E.Y., Harlos, K., Bottomley, M.J. et al. *Crystal structure of an integrin-binding fragment of vascular cell adhesion molecule-1 at 1.8 Å resolution.* Nature 1995, 373: 539-44.

6. Wang, J.H., Pepinsky, R.B., Stehle, T. et al. *The crystal structure of an N-terminal two domain fragment of vascular cell adhesion molecule 1 (VCAM-1): A cyclic peptide based on the domain 1 C-D loop can inhibit VCAM-1 α-4 integrin interaction.* Proc Natl Acad Sci USA 1995, 92: 5714-8.

7. Makarem, R., Newham, P., Askari, J.A. et al. *Competitive binding of vascular cell adhesion molecule-1 and the HepII/IIIC S domain of fibronectin to the integrin α-4 β-1.* J Biol Chem 1994, 269: 4005-11.

8. Komoriya, A., Green, I.J., Mervic, M. et al. *The minimal essential sequence for a major cell type-specific adhesion site (CS-1) within the alternatively spliced type III connecting segment domain of fibronectin is leucine-aspartic acid-valine.* J Biol Chem 1991, 266: 15075-9.

9. Jongewaard, I.N., Tsai, P.M., Smith, J.W. *The type III connecting segment of fibronectin contains an aspartic acid residue that regulates the rate of binding to integrin $\alpha_4\beta_1$.* Cell Adhes Commun 1996, 3: 487-95.

10. Bayless, K.J., Davis, G.E. *Identification of dual α_4 β_1 integrin binding sites within a 38 amino acid domain in the N-terminal thrombin fragment of human osteopontin.* J Biol Chem 2001, 276: 13483-9.

11. Briskin, M., Winson-Hines, D., Shyjan, A. et al. *Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue.* Am J Pathol 1997, 151: 97-110.

12. Liu, S., Thomas, S.M., Woodside, D.G., Rose, D.M., Kiosses, W.B., Pfaff, M., Ginsberg, M.H. *Binding of paxillin to α_4 integrins modifies integrin-dependent biological responses.* Nature 1999, 402: 676-81.

13. Hughes, P.E., Pfaff, M. *Integrin affinity modulation.* Trends Cell Biol 1998, 8: 359-64.

14. Bochner, B.S. *Road signs guiding leukocytes along the inflammation superhighway.* J Allergy Clin Immunol 2000, 106: 817-28.

15. Schwartz, M.A., Schaller, M.D., Ginsberg, M.H. *Integrins: Emerging paradigms of signal transduction.* Ann Rev Cell Dev Biol 1995, 11: 549-99.

16. Lu, C., Takagi, J., Springer, T.A. *Association of the membrane proximal regions of the α and β subunit cytoplasmic domains constrains an integrin in the inactive state.* J Biol Chem 2001, 276: 14642-8.

17. Springer, T.A. *Folding of the N-terminal, ligand-binding region of integrin α-subunits into a β-propeller domain.* Proc Natl Acad Sci USA 1997, 94: 65-72.

18. Irie, A., Kamata, T., Takada, Y. *Multiple loop structures critical for ligand binding of the integrin α-4 subunit in the upper face of the β-propeller mode 1.* Proc Natl Acad Sci USA 1997, 94: 7198-203.

19. Leitinger, B., Hogg, N. *From crystal clear ligand binding to designer I domains.* Nat Struct Biol 2000, 7: 614-6.

20. Humphries, M.J. *Integrin cell adhesion receptors and the concept of agonism.* Trends Pharmacol Sci 2000, 21: 29-32.

21. Pacheco, K.A., Tarkowski, M., Klemm, J., Rosenwasser, L.J. *CD49d expression and function on allergen-stimulated T cells from blood and airway.* Am J Respir Cell Mol Biol 1998, 18: 286-93.

22. Bocchino, V., Bertorelli, G., D'Ippolito, R. et al. *The increased number of very late activation-4 positive cells correlates with eosinophils and severity of disease in the induced sputum of asthmatic patients.* J Allergy Clin Immunol 2000, 105: 65-70.

23. MacKay, I.R., Rosen, F.S. *T-cell function and migration.* N Engl J Med 2000, 343: 1020-34.

24. Berlin, C., Bargatze, R.F., Campbell, J.J. et al. *α_4 Integrins mediate lymphocyte attachment and rolling under physiologic flow.* Cell 1995, 80: 413-22.

25. Johnston, B., Issekutz, T.B., Kubes, P. *The α_4-integrin supports leukocyte rolling and adhesion in chronically inflamed postcapillary venules in vivo.* J Exp Med 1996, 183: 1995-2006.

26. Wardlaw, A.J. *Adhesion receptors.* Drugs Fut 1999, 24: 279-86.

27. Ohkawara, Y., Yamauchi, K., Maruyama, N. et al. *In situ expression of the cell adhesion molecules in bronchial tissues from asthmatics with airflow limitation: In vivo evidence of VCAM-VLA-4 interaction in selective eosinophil infiltration.* Am J Respir Cell Mol Biol 1995, 12: 4-12.

28. Zangrilli, J.G., Shaver, J.R., Cirelli, R.A. et al. *VCAM-1 levels after segmental challenge correlate with eosinophil influx and IL-4 and IL-5 production, and the late phase response.* Am J Respir Crit Care Med 1995, 151: 1346-53.

29. Borchers, M.T., Crosby, J., Farmer, S., Sypek, J., Ansay, T., Lee, N.A., Lee, J.J. *Blockade of CD49d inhibits allergic airway pathologies independent of effects on leukocyte recruitment.* Am J Physiol 2001, 280(4, Pt. 1): L813-21.

30. Henderson, W.R., Chi, E.Y., Albert, R.K. et al. *Blockade of CD-49d (α_4 integrin) on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma.* J Clin Invest 1997, 100: 3083-92.

31. Richards, I.M., Kolbasa, K.P., Hatfield, C.A. et al. *Role of very late activation antigen-4 in the lungs and airway lumen of sensitized Brown Norway rats.* Am J Respir Cell Mol Biol 1996, 15: 172-83.

32. Sagara, H., Matsuda, H., Wada, N. et al. *A monoclonal antibody against very late activation antigen-4 inhibits eosinophil accumulation and late asthmatic response in a guinea pig model of asthma.* Int Arch Allergy Immunol 1997, 112: 287-94.

33. Abraharn, W.M., Ashfaq, A., Sielczak, M.W., Narita, M., Arrhenius, T., Elices, M.J. *Blockade of late-phase airway response and airway hyperresponsiveness in allergic sheep with a small-molecule peptide inhibitor of VLA-4.* Am J Respir Crit Care Med 1997, 156: 696-703.

34. Lin, K.-C., Ateeq, H.S., Hsiung, S.H. et al. *Selective tight-binding inhibitors of integrin α_4 β_1 that inhibit allergic airway responses.* J Med Chem 1999, 42: 920-34.

35. Krause, Brian R., Auerbach, Bruce J., Bocan, T.M.A. *Direct vascular targets for atherosclerosis prevention.* Curr Med Chem: Immunol, Endocr Metab Agents 2001, 1: 39-46.

36. Saxena, U., Medford, R.M. *Vascular adhesion molecule-1 (VCAM-1), an inflammatory gene target for new therapeutics.* Curr Opin Cardiovascular Pulmonary Renal Invest Drugs 2000, 2: 258-62.

37. Cybulsky, M.I., Iiyama, K., Li, H., Zhu, S., Chen, M., Iiyama, M., Davis, V., Gutierriz-Ramos, J.-C., Connelly, P.W., Milstone, D.S. *A major role for VCAM-1, but not ICAM-1, in early atherosclerosis.* J Clin Invest 2001, 107: 1255-62.

38. Shih, P.T., Brennan, M.-L., Vora, D.K., Territo, M.C., Strahl, D., Elices, M.J., Lusis, A.J., Berliner, J.A. *Blocking of very late antigen-4 intergrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet.* Circ Res 1999, 84: 345-51.

39. AtheroGenics Press Release available at www.atherogenics.com.

40. Archelos, J.J., Previtali, S.C., Hartung, H.-P. *The role of integrins in immune-mediated diseases of the nervous system.* Trends Neurosci 1999, 22: 30-8.

41. Theien, B.E., Vanderlugt, C.L., Eagar, T.N., Nickerson-Nutter, C., Nazareno, R., Kuchroo, V.K., Miller, S.D. *Discordant effects of anti-VLA-4 treatment before and after onset of relapsing experimental autoimmune encephalomyelitis.* J Clin Invest 2001, 107: 995-1006.

42. Kanwar, J.R., Harrison, J.E.B., Wang, D., Leung, E., Mueller, W., Wagner, N., Krissansen, G.W. *β_7 Integrins contribute to demyelinating disease of the central nervous system.* J Neuroimmunol 2000, 103: 146-52.

43. Brocke, S., Piercy, C., Steinman, L., Weissman, I.L., Veromaa, T. *Antibodies to CD44 and integrin α_4, but not L-selectin, prevent central nervous system inflammation and experimental encephalomyelitis by blocking secondary leukocyte recruitment.* Proc Natl Acad Sci USA 1999, 96: 6896-901.

44. Reparon-Schuijt, C.C., Van Esch, W.J.E., Van Kooten, C., Rozier, B.C.D., Levarht, E.W.N., Breedveld, F.C., Verweij, C.L. *Regulation of synovial B cell survival in rheumatoid arthritis by vascular cell adhesion molecule 1 (CD 106) expressed on fibroblast-like synoviocytes.* Arthritis Rheum 2000, 43: 1115-21.

45. Kitani, A., Nakashima, N., Izumihara, T. et al. *Soluble VCAM-1 induces chemotaxis of Jurkat and synovial fluid T cells bearing high afflnity very late antigen-4.* J Immunol 1998, 161: 4931-8.

46. Tokuhlra, M., Hosaka, S., Volin, M.V., Haines, G.K. III, Katschke, K.J. Jr., Kim, S., Koch, A.E. *Soluble vascular cell adhesion molecule 1 mediation of monocyte chemotaxis in rheumatoid arthritis.* Arthritis Rheum 2000, 43: 1122-33.

47. Seiffge, D. *Protective effects of monoclonal antibody to VLA-4 on leukocyte adhesion and course of disease in adjuvant arthritis in rats.* J Rheumatol 1996, 23: 2086-91.

48. Brandtzaeg, P., Haraldsen, G., Helgeland, L., Nilsen, E.M., Rugtveit, J. *New insights into the immunopathology of human inflammatory bowel disease.* Drugs Today 1999, 35(Suppl. A, Crossing Borders in IBD): 33-70.

49. Jones, S.C., Banks, R.E., Haidar, A., Gearing, A.J.H., Hemingway, I.K., lbbotson, S.H., Dixon, M.F., Axon, A.T.B. *Adhesion molecules in inflammatory bowel disease.* Gut 1995, 36: 724-30.

50. Hesterberg, P.E., Winsor-Hines, D., Briskin, M.J. et al. *Rapid resolution of chronic colitis in the cotton-top tamarin with an antibody to a gut homing integrin α_4 β_7.* Gastroenterology 1996, 111: 1373-80.

51. Kato, S., Hokari, R., Matsuzaki, K. et al. *Amelioration of murine experimental colitis by inhibition of mucosal addressin cell adhesion molecule-1.* J Pharmacol Exp Ther 2000, 295: 183-9.

52. Picarella, D., Hurlbut, P., Rottman, J., Shi, X., Butcher, E., Ringler, D.J. *Monoclonal antibodies specific for P7 integrin and mucosal addressin cell adhesion molecule-1 (MADCAM- 1) reduce inflammation in the colon of scid mice reconstituted with CD45RB high CD4+ T cells.* J Immunol 1997, 158: 2099-106.

53. Soriano, A., Salas, A., Salas, A. *VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade arneliorates DSS-induced colitis in mice.* Lab Invest 2000, 80: 1541-51.

54. Poon, B.Y., Ward, C.A., Giles, W.R., Kubes, P. *Emigrated neutrophils regulate vertricular contractillity via α-4 integrin.* Circ Res 1999, 84: 1245-51.

55. Poon, B.Y., Ward, C.A., Cooper, C.B., Giles, W. R., Bums, A. R. *α-4 Integrin mediates neutrophil-induced free radical injury to cardiac myocytes.* J Cell Biol 2001, 152: 857-66.

56. Reiton, J.K., Sloan, K.E., Frew, E.M., Whalley, E.T., Adams, S.P., Lobb, R.R. *Inhibition of α-4 integrin protects against transient focal cerebral ischemia in normotensive and hypertensive rats.* Stroke 2001, 32: 199-205.

57. Papayannopoulou, T., Craddock, C., Nakamoto, B., Priestley, G.V., Wolf, N.S. *The VLA4/VCAM-1 adhesion pathway defines contrasting mechanisms of lodgement of transplanted murine hemopoietic progenitors between bone marrow and spleen.* Proc Natl Acad Sci USA 1995, 92: 9647-51.

58. Chan, J.Y.-H., Watt, S.M. *Adhesion receptors on haematopoietic progenitor cells.* Br J Haematol 2001, 112: 541-57.

59. Weekes, C.D., Kuszynski, C.A., Sharp, J.G. *VLA-4 mediated adhesion to bone marrow stromal cells confers chemoresistance to adherent lymphoma cells.* Leuk Lymphoma 2001, 40: 631-45.

60. Michigami, T., Shimizu, N., Williams, P.J., Niewolna, M., Dallas, S.L., Mundy, G.R., Yoneda, T. *Cell-cell contact between marrow stromal cells and myeloma cells via VCAM-1 and α-4 β-1 integrin enhances production of osteoclast-stimulating activity.* Blood 2000, 96: 1953-60.

61. Damiano, J.S., Cress, A.E., Hazlehurst, L.A., Shtil, A.A., Dalton, W.S. *Cell adhesion mediated drug resistance (CAM-DR): Role of integrins and resistance to apoptosis in human myeloma cell lines.* Blood 1999, 93: 1658-67.

62. Kwee, L., Baldwin, H.S., Shen, H.M., Stewart, C.L., Buck, C.A., Labow, M.A. *Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM-1) deficient mice.* Development 1995, 121: 489-503.

63. Gurtner, G.C., Davis, V., Li, H., McCoy, M.J., Sharpe, A., Cybulsky, M.I. *Targeted disruption of the murine VCAM 1 gene: Essential role of VCAM-1 in chorioallantoic fusion and placentation.* Genes Dev 1995, 9: 1-14.

64. Koni, P.A., Joshi, S.K., Temann, U.-A., Olson, D., Burkly, L., Flavell, R.A. *Conditional vasucular cell adhesion molecule 1 deletion in mice: Impaired lymphocyte migration to bone marrow.* J Exp Med 2001, 193: 741-53.

65. Leuker, C.E., Labow, M., Muller, W., Wagner, N. *Neonatally induced inactivation of the vascular cell adhesion molecule-1 gene impairs B cell localization and T cell-dependent humoral immune response.* J Exp Med 2001, 193: 755-67.

66. Elices, M. *Natalizumab Elan*. Curr Opin Anti-inflammatory Immunomodulatory Invest Drugs 2000, 2: 228-35.

67. Carter, J.L., Agius, M., Mass, M., Romine, J.S., Taylor, J., Libonati, M., Shah, J. *A placebo-controlled, pharmacodynamic,*

pharmacokinetic, tolerability and safety study of three doses of intravenous natalizumab in multiple sclerosis. Neurology 2000, 54(Suppl. 3): A259.

68. Sheremata, W.A., Vollmer, T.L., Stone, L.A., Willmer-Hulme, A.J., Koller, M. *A safety and pharmacokinetic study of intravenous natalizumab in patients with MS.* Neurology 1999, 52: 1072-4.

69. Tubridy, N., Behan, P.O., Capildeo, R. et al. *The effect of anti-α-4 integrin antibody on brain lesion activity in MS.* Neurology 1999, 53: 466-72.

70. Ghosh, S., Goldin, E., Malchow, H.A. et al. *A randomized double-blind, placebo-controlled, pan-European study of a recombinant humanized antibody to α-4 integrin (Antegren®) in moderately to severe active Crohn's disease.* Gastroenterology 2001, 120(Suppl. 1): A127-28. (A company press release with more details can be found at: www.biogen.com.)

71. Gordon, F.H., Hamilton, M.I., Free, R. et al. *Treatment of active ulcerative colitis with a recombinant humanized antibody to α-4 integrin (Antegren®).* Gastroenterology 1999, 116: A726.

72. Nowlin, D.M., Gorcsan, F., Moscinski, M., Chiang, S., Lobl, T.J., Cardarelli, P.M. *A novel cyclic pentapeptide inhibits α-4/β-1 and α-5/β-1 integrin-mediated cell adhesion.* J Biol Chem 1993, 268: 20352-9.

73. Jackson, D.Y., Quan, C., Artis, D.R. et al. *Potent α-4 β-1 peptide antagonists as potential anti-inflammatory agents.* J Med Chem 1997, 40: 3359-68.

74. Fotouhi, N., Joshi, P., Fry, D. et al. *The design and synthesis of potent cyclic peptide VCAM/VLA-4 antagonists incorporating an achiral Asp-Pro mimetic.* Bioorg Med Chem Lett 2000, 10: 1171-3.

75. Fotouhi, N., Joshi, P., Tilley, J.W. et al. *Cyclic thioether peptide mimetics as VCAM-VLA-4 antagonists.* Bioorg Med Chem Lett 2000, 10: 1167-9.

76. Chen, L., Fotouhi, N., Jackson, D.J. et al. (F. Hoffmann-La Roche AG). *Pyrrolidincarbonyl-amino cyclic disulfides.* WO 0063234.

77. Dutta, A.S., Gormley, J.J., Coath, M. et al. *Potent cyclic peptide inhibitors of VLA-4 ($\alpha_4\beta_1$ integrin)-mediated cell adhesion. Discovery of compounds like cyclo(MePhe-LeuAsp-Val-dArg-dArg) (ZD7349) compatible with depot formulation.* J Pept Sci 2000, 6: 398-412.

78. Dutta, A.S., Mandy, C., Gormley, J.J. et al. *Potent cyclic monomeric and dimeric peptide inhibitors of VLA-4 (α-4 β-1 integrin)-mediated cell adhesion based on the Ile-Leu-Asp-Val tetrapeptide.* J Pept Sci 2000, 6: 321-41.

79. Arrhenius, T., Chiem, A., Elices, M., He, Y.-B., Jia, L., Maewal, A., Greta, F. *Small molecule inhibitors of the leukocyte integrin VLA-4.* Pept: Chem, Struct Biol, Proc Am Pept Symp, 14 th 1996: 337-9.

80. Arrhenius, T.S. Elices, M.J., Gaeta, F.C.A. (Cytel Corp). *CS-1 peptidomimetics, compositions and methods of using same.* US 5688913.

81. Richard, B., Way, W., Huyghe, B. et al. *CY-9701, a small molecule VLA-4 inhibitor modulates proinflammatory cytokines in an allergic mouse asthma model.* Am J Respir Crit Care Med 1999, 159(Suppl., Pt. 2): 327.

82. Zimmerman, C.N., Lin, K.-C. *SAR studies of the leucine portion of Bio-1050, a potent and selective nanomolar antagonist of the integrin VLA-4 (α-4 β-1).* 213th ACS Natl Meet (April 13-17, San Francisco) 1997, Abst MEDI 076.

83. Chen, L.L., Whitty, A., Lobb, R.R., Adains, S.P., Pepinsky, R.B. *Multiple activation states of integrin α-4 β-1 detected through their different affinities for a small molecule ligand.* J Biol Chem 1999, 274: 13167-75.

84. Lockey, P., Stoppard, A., White, E., White, M., Wong, W. *IVL-745, a potent, selective, low molecular weight antagonist of VLA-4 mediated cell adhesion.* Am J Respir Crit Care Med 2000, 161: A201.

85. Bahra, P., Ebsworth, K., Lawrence, C.E., Webber, S.E., Williams, R.J. *The VLA-4 agtagonist IVL-745 is a novel and potent inhibitor of human lymphocyte adhesion to VCAM1.* Am J Respir Crit Care Med 2000, 161: A201.

86. Cairns, J.A., Kaik, K., Lloyd, J., Webber, S.E., Williams, R.J. *The VLA-4 antagonist, IVL-745 is a novel and potent inhibitor of eosinophil adhesion to VCAM.* Am J Respir Crit Care Med 2000, 161: A202.

87. Ebsworth, K., Lawrence, C.E., Webber, S.E., Williams, R.J. *The VLA-4 antagonist IVL-745 is a novel and potent inhibitor of VCAM-1 co-stimulated human T-cell activation.* Am J Respir Crit Care Med 2000, 161: A201.

88. Underwood, S.L., Prince, L., Page, K., Webber, S.E., Foster, M.L. *In vivo profile of the novel VLA-4 antagonist IVL-745 in antigen induced airway inflammation in the rat.* Am J Respir Crit Care Med 2000, 161: A199.

89. Baldwin, J.J., McDonald, E., Moriarty, K.J., Sarko, C.R., Machinaga, N. (Daiichi Pharmaceutical Co., Ltd.; Pharmacopeia, Inc.). *VLA-4 inhibitor compounds.* WO 0100206.

90. Duplantier, A.J., Milici, A.J., Chupak, L.S. (Pfizer Products Inc.). *Non-peptidyl inhibitors of VLA-4 dependent cell binding useful in treating inflammatory autoimmune and respiratory diseases.* WO 0000477.

91. Clark, D.E., Eastwood, P.R., Harris, N.V. et al. (Aventis Pharma Ltd.). *Substituted bicyclic heteroaryl compounds as integrin antagonists.* WO 0061580.

92. Brittain, D.R., Johnstone, C., Davies, G.M., Large, M.S. (Aventis Pharma Deutschland GmbH). *Chemical compounds.* WO 0005224.

93. Wehner, V., Stilz, H.U., Schmedt, W., Seiffge, D. (Texas Biotechnology Corp.). *Preparation of substituted imidazolidine compounds for use as anti-inflammatory drugs in pharrnaceutical preparations.* DE 19751251.

94. Holland, G.W., Kassir, J.M., Scott, I.L. et al. *TBC3486: Novel highly potent, and selective VLA-4 antagonist.* 219th ACS Natl Meet (March 26-30, San Francisco) 2000, Abst MEDI 10.

95. Kassir, J.M., Scott, I.L, Biediger, R.J. et al. *Novel N,N-disubstituted amides that are highly potent VLA-4 antagonists.* 219th ACS Natl Meet (March 26-30, San Francisco) 2000, Abst MEDI 284.

96. Scott, I.L., Raju, B.G., Ren, K. et al. *Novel urea derivatives that are potent VLA-4 antagonists.* 219th ACS Natl Meet (March 26-30, San Francisco) 2000, Abst MEDI 283.

97. Biediger, R.J., Holland, G.W., Kassir, J.M. et al. (Zeneca Ltd.). *Preparation of propanoic acid peptide derivatives that inhibit the binding of integrins to their receptors.* WO 0068188.

98. Archibald, S.C., Head, J.C., Gozzard, N. et al. *Discovery and evaluation of potent, tyrosine-based α_4 β_1 integrin antagonists.* Bioorg Med Chem Lett 2000, 10: 997-9.

99. Porter, J. *From cyclic peptide to small molecule antagonists of VLA-4.* 11th Symp Med Chem Eastern England (April) 2000.

100. Semko, C.M., Dressen, D.B., Grant, F.S. et al. *Development of CT757: A potent and highly selective small molecule inhibitor of VLA-4.* 220th ACS Natl Meet (Aug 20-24, Washington DC) 2000, Abst MEDI 133.

101. Sarantakis, D., Baudy, R.B., Bicksler, J.J. et al. *Acylated p-amino-L-phenylalanines with VLA-4/VCAM-1 inhibitory activity.* 220th ACS Natl Meet (Aug 20-24, Washington DC) 2000, Abst MEDI 136; Sarantakis, D., Bicksler, J.J., Cannon, C. et al. *VLA-4/VCAM inhibitors - Dipeptide p-amino-L-phenylalanine amides.* 220th ACS Natl Meet (Aug 20-24, Washington DC) 2000, Abst MEDI 137.

102. Teegarden, B.R., Rishton, G.M., Yamagishi, M. et al. *Discovery of TR-9109: A novel small molecule inhibitor of $\alpha_4\beta_1$ integrin mediated cell adhesion.* 218th ACS Natl Meet (Aug 22-26, New Orleans) 1999, Abst MEDI 58.

103. Chen, L., Tilley, J.W., Guthrie, R.W. et al. *N-Benzylpyroglutamyl-L-phenylalanine derivatives as VCAM/VLA-4 antagonists.* Bioorg Med Chem Lett 2000, 10: 729-33.

104. Chen, L., Guthrie, R.W., Huang, T.-N., Hull, K.G., Sidduri, A., Tilley, J.W. (F. Hoffmann-La Roche AG). *Acylphenylalanine derivatives.* WO 9910312.

105. Teegarden, B.R., Jayakumar, H., Matuski, K., Chrusciel R.R., Fisher, J.F., Tanis, S.P., Thomas, E.W., Blinn, J.R. (Tanabe Seiyaku Co., Ltd.; Pharmacia & Upjohn Co.). *Inhibitors of $\alpha_4\beta_1$ mediated cell adhesion.* WO 0037429.

106. Chen, L., Guthrie, R.W., Huang, T.-N., Hull, K.G., Sidduri, A., Tilley, J.W. (F. Hoffmann-La Roche AG). *Acylphenylalanine derivatives.* WO 9910313.

107. Okuyama, A., Ikegami, S., Harada, T., Maruyama, Y. et al. (Kaken Pharmaceutical Co., Ltd.). *Preparation of urea derivatives as VLA-4 antagonists.* WO 0132610.

108. Tilley, J.W., Kaplan, G., Rowan, K., Schwinge, V., Wolitzky, B. *Imide and lactam derivatives of N-benzylpyroglutamyl-L-phenylalanine as VCAM/VLA-4 antagonists.* Bioorg Med Chem Lett 2001, 11: 1-4.

109. Annour, D.R., Brown, D., Congreave, M.S. et al. (Glaxo Group Ltd.). *Compounds useful in the treatment of inflammatory diseases.* WO 0037444.

110. Lombardo, L.J., Sabalski, H. (American Home Products Corp.). *3,4-Diamino-3-cyclo-butene-1,2-dione derivatives which inhibit leukocyte adhesion mediated by VLA-4.* US 6166050.

111. Sircar, I., Gudmundsson, K., Martin, R. et al. *Discovery of TR-14035; an orally active dual α-4-β-1 integrin antagonist.* 218th ACS Natl Meet (Aug 22-26, New Orleans) 1999, Abst MEDI 59 and MEDI 60.

112. GlaxoSmithKline Corporate Web Site: www.gsk.com.

113. Hagmann, W.K., Durette, P.L., Lanza, T. et al. *The discovery of sulfonated dipeptides as potent VLA-4 antagonists.* Bioorg Med Chem Lett 2001, 11(20): 2709.

114. Arora, S.K., Tanwar, M.P., Gupta, J.B., Sharma, G. (Ranbaxy Laboratories Ltd.). *Derivatives of monosaccharides as cell adhesion inhibitors.* WO 0042053.