Discovery of small molecule leads in a biotechnology datastream

Thomas R. Gadek and Robert S. McDowell

A case study of the discovery of small molecule antagonists to the integrins GPIIbIIIa ($\alpha II_B\beta 3$), $\alpha v\beta 3$, LFA-1 ($\alpha L\beta 2$), $\alpha 4\beta 1$ and $\alpha 4\beta 7$ is presented from the perspective of a biotechnology research organization. A strategy incorporating protein mutagenesis and structural studies to develop a structure–activity relationship (SAR) that described the 'epitope' of the integrin ligand was crucial to the identification of peptide analogs of these proteins, and subsequently, through parallel trends in SAR, to the identification of small molecule mimetics of these peptides, which are active analogs of the protein ligands themselves.

Thomas R. Gadek
Genentech
One DNA Way
South San Francisco
CA 94080, USA
e-mail: trg@gene.com
Robert S. McDowell
Sunesis Pharmaceuticals
341 Oyster Point Boulevard
South San Francisco
CA 94080, USA

▼ Drug discovery over the past 50 years has seen the refinement of medicinal chemistry, traditionally practiced in larger pharmaceutical research organizations, and the emergence of biotechnology, typically the preserve of smaller 'biotech' organizations [1]. More recently, large and small pharmaceutical research organizations have attempted to combine the strengths of these two disciplines to better identify and validate protein therapeutic targets during the discovery and development of small-molecule leads. In this review, some examples of the successful identification of small-molecule leads at the interface of medicinal chemistry and biotechnology will be provided, focusing on small-molecule antagonists to the integrins. Furthermore, the background, strategy and datastream that a biotechnology company can contribute to this area will be discussed.

The integrins are a family of proteins involved in cell adhesion whose tissue distribution and expression on cells suggests a role in moderating disease processes [2]. Their function, generally at the end of a chain of cellular processes that contribute to the manifestation of a disease state, makes them attractive therapeutic targets. For example, the activation of platelets to a pro-aggregatory state by any of several thrombotic agents [e.g. ADP, Paf (platelet activating factor), arachidonic acid and prostaglandins] involves several independent signaling cascades that lead to the interaction of the integrin glycoprotein IIbIIIa on the platelet surface with fibrinogen in serum and the subsequent formation of a thrombus [3]. Conceptually, it is easier to control thrombosis via a single antagonist of the integrin rather than via a panel of antagonists tailored to each of the thrombotic stimuli. The marketing approval granted to the GPIIbIIIa antagonists Reopro® (Lilly; http://www.lilly.com), Integrilin® (Millenium; http://www.mlnm.com) and Aggrestat® (Merck; http://www.merck.com) validates this integrin as a therapeutic target and exemplifies the therapeutic and commercial utility of antibodies, peptides and small molecules at the research interface between biotechnology and medicinal chemistry [3,4]. Reports linking integrins to other disease states have since emerged and thus other members of this protein family have become attractive therapeutic targets. Additional research and development efforts have led to the current clinical evaluation of both antibody and small-molecule antagonists of $\alpha v\beta 3$ in osteoporosis and oncology, $\alpha 4\beta 1$ in multiple sclerosis (MS) and asthma, $\alpha 4\beta 7$ in gastrointestinal disorders and lymphocyte functional antigen 1 (LFA-1; $\alpha L\beta 2$) antagonists in psoriasis and rheumatoid arthritis. In this article, a general approach to the identification of small-molecule antagonists of these integrins will be described, followed by a detailed analysis of the most recent example, LFA-1, which illustrates all aspects of this general approach.

Identifying orally available antagonists of integrin–ligand binding

Efforts to identify lead, orally active antagonists of the integrins began with the design of a panel of assays to study the binding and

1359-6446/03/$ – see front matter PII: S1359-6446(03)02735-1

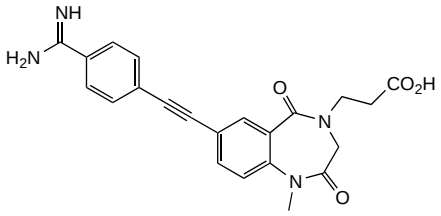
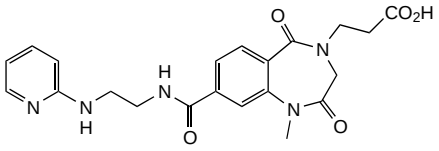
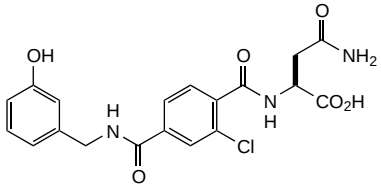
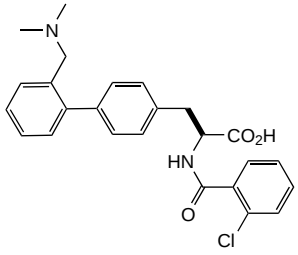
inhibition of the integrin–ligand interaction. At Genentech (http://www.gene.com), full-length integrin heterodimers based on the human sequence were expressed in 293 cells, and clonal cell lines were established that were stable in their expression of >1,000,000 copies of the integrin per cell [5,6]. These cells were banked and used in large fermentor runs during the course of the programs and, in the case of GPIIbIIIa, provided >4g of purified protein for assay and screening purposes. An advantage of establishing stable cell lines that expressed high levels of integrin was the ease of separation and purification of the isolated protein products. The low level of background-related integrin sequences in the 293 cells (e.g. <10,000 copies per cell) avoided contamination issues and increased the quality of the data obtained from the binding assays. The purified proteins were used in ELISA-based assays that measured the ability of compound(s) to inhibit the direct binding of the integrin to its native ligand [e.g. GPIIbIIIa and fibrinogen, LFA-1 and intercellular adhesion molecule (ICAM.)] [7]. The sensitivity and dynamic range of these assays typically spanned nearly six orders of magnitude in IC_{50} values (between 1 nM and 500 μM). This enabled the identification of relatively weak inhibitors that were subsequently optimized through the establishment of a structure–activity relationship (SAR) within the identified compounds using an assay that could reliably discriminate twofold differences in IC_{50} values in molecules differing by a single atom [8]. The 293 cell lines also enabled the construction of secondary cell attachment assays, which test the ability of compounds to inhibit the attachment of the integrin to its ligand under the high avidity conditions provided by the high surface expression of the integrin. These binding and attachment assays, in combination with functional assays, enabled the study of compound binding to the integrin in increasingly more complex biological situations. It was believed that this panel of assays would link the binding to and functional antagonism of the integrin, the *in vitro* affinity and selectivity of the inhibitor for the target integrin and integrin function on cells *in vitro* and in the *in vivo* disease situation. In the case of GPIIbIIIa, the correlation of binding affinity of the test compound in the ELISA assays to the effect of the test compound on cell attachment and platelet function enabled the identification of classes of compounds with correlating high affinity for GPIIbIIIa and antithrombotic activity [6]. This is in contrast to competitor efforts that identified GPIIbIIIa antagonists with a degree of pro-aggregatory activity [9].

The search for leads began with an analysis of the integrin–ligand system. In general, the ligand is present in great excess relative to the integrin in the disease state, and the integrin is capable of recognizing several ligands. Consequently, it is easy to envisage a significant biological response arising from an antagonist targeting the integrin rather than the ligand. It is also reasonable to envisage mimics or analogs of the cognate ligand(s) as a source of antagonist leads to each of the integrins (i.e. analogs of a known binder as a source of antagonists). Antibodies can be used as pharmacological tools to validate targets, assays and animal models of human disease. In the case of the integrins, several attempts to use antibodies to the ligands (e.g. fibrinogen and ICAM-1) have met with limited success [10], whereas function-blocking antibodies targeting LFA-1, αvβ3, α4β1, α4β7 have entered human trials and, in the case of the anti-GPIIbIIIa antibody Reopro®, have reached the market [4].

Determining integrin–ligand interaction

In a first-order analysis, the interaction between the integrin and its ligand can be considered a ground state that is dominated by interactions between amino acid side-chains rather than the backbone of the proteins involved [11]. A clustering of the amino acid side-chains of the ligand on the surface of the integrin define the binding 'epitope' of the integrin ligand. This epitope can be identified by alanine-scanning mutagenesis and can be recognized as a cluster of polar and non-polar residues that, when sequentially mutated to alanine, diminish the binding of the ligand by more than tenfold [12]. These alanine mutagenesis data, when combined with structure information of the ligand determined by crystallography, NMR or computational models, can define a SAR of the protein ligand. In this context, peptides that display the appropriate side-chain functionality of the epitope show the same or similar SAR. Such peptides are in fact analogs of the native protein epitope and, for this purpose, can be considered a 'privileged scaffold' [13] in the search for analogs of proteins with agonist or antagonist activities.

Examples of these analogs are the RGD peptides, which are antagonists of GPIIbIIIa and αvβ3, and other peptides that have been derived from the epitopes of the ligands vascular cellular adhesion molecule (VCAM), mucosal addressin cell adhesion molecule 1 (MAdCAM) and ICAM [4,14]. The RGD peptides were isolated and identified by classical protein chemistry methods whereas the other peptides were synthesized from analysis of the structure of the protein ligand and alanine mutagenesis data. The identification of peptide analogs of protein ligands that exhibit the same SAR as the native ligand is most common for linear protein epitopes such as the RGD sequence. More recently, the successful identification of ICAM analogs provided an example of a non-linear but contiguous protein epitope that could be displayed in a smaller cyclic peptide [15]. These efforts can be viewed as an extension of the studies on peptide hormone analogs and the search for agonists and antagonists of hormone receptors [e.g. Losartan (Merck; http://www.merck.com) as an antagonist of

Integrin–ligand	Epitope	Peptide lead	Small molecule lead
GPIIbIIIa–fibrinogen	RGD	cyclo-AcyRGDC-CO_2H	
αvβ3–vitronectin	RGD	GRGDSP	
LFA-1–ICAM-1	E34, K39, M64, Y66, N68, Q73	cyclo-CGY$^{(m)}$DMPC	
α4β1–VCAM	RTQIDSPL	cyclo-TQIDSPNG cyclo-YCDPC	

Drug Discovery Today

Figure 1. List of integrins, their ligands, peptides displaying aspects of the ligand's epitope and small-molecule antagonist leads against GPIIbIIIa–fibrinogen [8], αvβ3–vitronectin (39), LFA-1–ICAM-1[35] and α4β1–VCAM [14, 21] interactions. Abbreviation: ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte functional antigen 1; VCAM, vascular cellular adhesion molecule.

the angiotensin II receptor] [16–18]. Similarly, peptide analogs of the native protein ligands have been used as lead structures in the identification of non-peptidic agents capable of antagonizing the integrin–ligand interaction. Figure 1 shows the structures of antagonists of GPIIbIIIa, αvβ3, α4β1 and LFA-1, which have been identified through peptide analogs of the native protein ligand [7,15,19–22].

Crucial to this transformation from peptide to non-peptide ligand was a detailed understanding of the structure of the peptide, including all main-chain and side-chain rotamers, and, in particular, a dataset to develop a testable hypothesis about the likely bound conformation(s) of the peptide in a complex with the integrin. X-ray crystallography, NMR and computational studies have been applied in this regard [15,23,24]. In the case of the integrins, crystallography has recently provided some very interesting insights into the bound state of an RGD peptide complexed with αvβ3 [25]. It is interesting to note that this conformation of the RGD is well represented in low-energy conformations of molecules believed to have advanced into clinical trials [26]. In general, the integrins are too large (~250 kD) for NMR structural studies, although in the case of LFA-1, a substructural domain of the α subunit, the I-domain, which is involved in ICAM binding, has been stably expressed and studied by NMR [27]. In the absence of crystallographic or NMR-derived structural data, we have successfully used homology modeling to define the SAR of the integrin ligands for identifying interesting peptide leads [22,28,29].

A SAR describing these peptide leads was developed for potency and selectivity. The establishment of some structural bias in the conformational population represented in these peptides was crucial. In each case (GPIIbIIIa, αvβ3, α4β1, α4β7 and LFA-1) there was a trend to increased conformational definition in the backbone of the more potent peptides [14,15,23]. However, even those peptides with a defined backbone conformation frequently exhibited dynamic

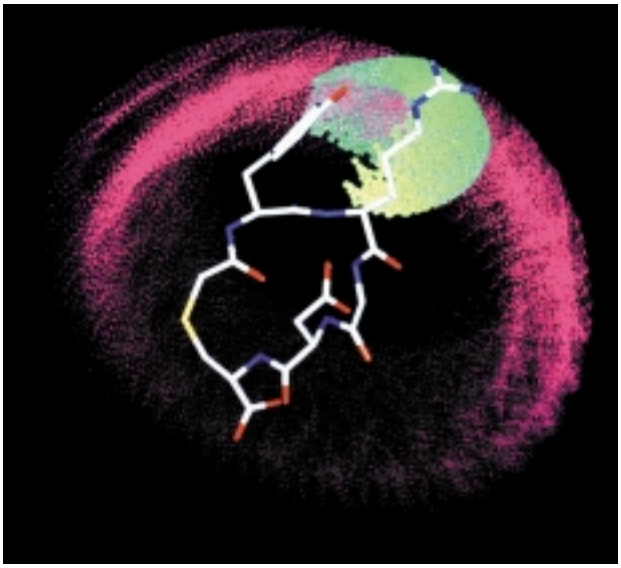


Figure 2. Location of the zeta-carbon of the arginine side-chain relative to the aspartate side-chain carboxylic acid carbon determined by sampling during molecular dynamics trajectories of a linear RGD peptide (purple) and a more constrained cyclic peptide, cyclo-AcyRGDC-CO_2H (green). The correlation of the binding of the integrin GPIIbIIIa to the relative locations of the guanidine of the arginine and the carboxylic acid of the aspartate by ensemble dynamics identified the conformation shown and enabled the design of small molecule leads (see refs [23,24]).

side-chain rotamer populations (Fig. 2). In the absence of a cocrystal structure, we turned to molecular dynamics simulations of an ensemble of flexible peptide and non-peptide ligands that were all known to bind to the target integrin [15,24]. This collection of compounds was selected from the total collection of molecules shown to bind to the integrin and that showed a level of diversity in the connectivity between functional groups shown, from the SAR of the compounds, to be points of contact with the receptor. The members of the ensemble did not necessarily need to be the most potent molecules made, but it was important that they represented structurally diverse connectivities that are capable of presenting aspects of a binding epitope in a common three-dimensional (3D) array. The resulting ensemble of low-energy binding conformations share a common 3D array of binding contacts and defines the bound conformation of each compound at a level of precision that can enable the design of active analogs. In our experience, this has been particularly useful in the transition from peptide to non-peptide analogs.

In vitro and *in vivo* lead validation

When lead compounds were identified, they were tested in animal models of human disease where the target integrin had been implicated in the disease process. Access to antibodies, both monoclonal and polyclonal, anti-human and anti-animal, proved invaluable in defining these models, and throughout the programs discussed in this review [30,31]. Non-function blocking antibodies directed against the human integrin acted as reagents for detection in the ELISA assays. In cell-based assays, anti-human antibodies targeting the integrin were used to 'calibrate' the assay, and, in all instances, humanized versions of these anti-human antibodies were taken into human clinical studies by several companies. Data from these trials could aid target validation, and in some instances, help define dosage levels of the emerging small molecule antagonists.

The preceding discussion has outlined the issues, strategy and organization common to the GPIIbIIIa, αvβ3, α4β1, α4β7 and LFA-1 programs run at Genentech. The following section discusses the details of the LFA-1 program, which took advantage of all the nuances and strategies used and developed throughout the other integrin programs.

LFA-1 antagonists – a case study

Our interest in LFA-1 as a target was piqued by the efforts of colleagues who had been developing antibodies against LFA-1 [32]. Preclinical experiments with these antibodies suggested that a selective inhibitor of LFA-1 could be useful in psoriasis, rheumatoid arthritis and other autoimmune diseases. Orally active agents based on this hypothesis would provide large market opportunities and represent a significant clinical advance. We began to think of strategies for assay development and lead identification.

LFA-1 and the related receptor Mac1 ($\alpha_M\beta2$) were cloned in 293 cells, and stable cell lines were established that showed high expression levels of these proteins, as noted earlier. These cells provided a source of purified protein for ELISA assays. Taking advantage of immunoadhesin technology developed at Genentech [33], ICAM-1 was expressed as a fusion protein composed of the first five immunoglobulin domains of ICAM-1 with the Fc fragment of an IgG. In addition, mutagenesis studies of both LFA-1 and ICAM-1 were used to define their binding epitopes [28,34]. Early data from an alanine-scanning study of the first domain of ICAM-1 indicated that two residues, Glu34 and Lys39, were crucial to the interaction of ICAM-1 and LFA-1. In parallel with the mutagenesis studies, a series of staggered decapeptides was produced, moving through the sequence in steps of two amino acids at a time. A linear peptide epitope could not be identified using this approach and no significant binding of the decapeptides was observed, even in the sequences containing Glu34 and Lys39. Further efforts to constrain the sequence encompassing residues 34 through 39 failed to show any promise. Unfortunately, at the time, none of the crystal structures of ICAM-1 was available. However, our colleagues working on

the mutagenesis of ICAM-1 included an expert in the folding of immunoglobulin domains. After some conversations outlining our plan to find leads from ICAM-1 via peptide analogs and our lack of success in identifying a linear sequence of interest, a structural model of the first domain of ICAM-1 was constructed based on its homology to known immunoglobulin structure (L. Presta, pers. commun., [28]). This model served as a template upon which testable hypotheses were defined. One supposition was that the modeled protein could be traversed, from α carbon to α carbon, by a linear tripeptide that bore no direct sequence homology to ICAM-1. When this hypothesis was tested and confirmed, it provided the basis of a lead discovery process [35]. Strategically, we focused on acquiring an understanding of peptide–integrin binding, particularly on the definition of their bound conformation, rather than enhancing the affinity of these peptides for LFA-1. The resulting peptide SAR defined at least two binding modes that involved a carboxylic acid contact with LFA-1. Using this carboxylate contact as a constant, the peptides were recast in three different backbone scaffolds to enable NMR and ensemble dynamics studies to identify a common low energy 3D array of the functional groups that were shown to be points of contact with LFA-1 in the peptide SAR (i.e. the meta-phenol, the proline side-chain and a carboxylic acid) [35].

Issues related to lead discovery from a biotechnology datastream

When comparing the lead discovery process with lead discovery from a biotechnology datastream, several features of the process are worthy of note. One is the use of recombinant full-length LFA-1 in the primary assay. This enabled the definition of a novel set of antagonists that were competitive antagonists of ICAM-1 binding [15, Keating *et al*, unpublished]. This was in contrast to several other programs that had used the I domain of LFA-1 in assays to find a common set of allosteric antagonists [36]. The second feature was that in the efforts to convert the peptide lead to a non-peptide lead, we attempted to interpret assay data from compound libraries in the context of the SAR of the peptides we derived from the SAR of ICAM-1. This again used structural considerations as a template upon which were formed hypotheses and this allowed the elaboration of 2-bromo-benzoyltryptophan into the meta-phenol series of analogs (Fig. 1). These acted as small molecule leads and were ultimately optimized for affinity, selectivity and pharmacokinetic properties. The perspective gained from an overlay of 2-bromo-benzoyltryptophan onto the peptide, and our knowledge of the SAR of the peptides, enabled us to rapidly graft that SAR onto the non-peptide leads. The final aspect of the process worth noting regards the use of analogs in the optimization process. From the SAR of ICAM-1, it was initially hypothesized that a carboxylate and an amine would be necessary functionalities. Peptides were found that contained both a positive and a negative charge and an SAR that was consistent with that of ICAM-1. Through a series of analogs, all of which retained the carboxylic acid, a preference for a meta-phenol as a contact with LFA-1 was identified. These analogs used a common carboxylate as a link to the protein surface and probed the surface to first find an appropriate backbone conformational contour via a glycine residue and then an appropriate side-chain functionality with the phenol of a tyrosine. This well defined SAR led to >100 fold increase in potency between cyclo-H_2N-CRGDMPC-CO_2H and cyclo-H_2N-CGY$^{(m)}$DMPC-CO_2H [35]. There was no direct correlation for the unnatural amino acid meta-tyrosine (Y$^{(m)}$) to an amino acid in the ICAM-1 SAR. These peptides were recast in a non-peptide scaffold that retained the meta-phenol and carboxylic acid. Optimization of these compounds proceeded with an awareness of, but without overriding attempts to fit, the bound conformation of the peptides, as determined by ensemble dynamics, and the structures of ICAM-1 that began to appear in the literature. However, when the optimized non-peptides emerged with functionalities that could be seen as similar in function (i.e. H-bonding, lipophilic, negatively charged and polarizable) to the functional groups of the amino acid side-chains of the residues defining the ICAM-1 epitope, we were intrigued. If the proposed overlay of the lead compounds on the structure of ICAM-1 is correct, then it is interesting to consider that the analog-driven SAR processes that medicinal chemists have for the optimization of the binding of compounds to the surface of proteins is actually a well conceived search strategy. Certainly, the circumstantial evidence offered by their success over the years has argued for this.

Questions have arisen as to whether the antagonists derived from the SAR of ICAM-1 are in fact binding to LFA-1 in a manner consistent with the proposed overlay used in the program [35]. Two additional studies lend credence to our work. One is work describing the cocrystal structure of the first domain of ICAM-1 with an enhanced affinity form of the I-domain of LFA-1 [37]. In this structure, the backbone of the first domain of ICAM-1 is similar to previously published structures of ICAM-1 alone. Consequently, the bound structure of ICAM-1 seems close to a ground-state structure whose side-chains at the contact interface have been rotated slightly. These side-chain changes include a rotation of Glu34 from that found in the published structures [38] to that proposed in the overlay of our lead compound on ICAM-1 [15]. In addition to this structural study, an investigation of the binding of ICAM-1 and the small molecule and peptide leads has determined that they do compete for a common binding site on LFA-1 [Keating *et al*, unpublished].

Conclusion

The preceding discussion has attempted to describe aspects of small molecule lead discovery efforts that incorporated the datastream of a biotechnology company. This has captured technological strengths in the study of protein structure and function and has attempted to extend the use of active analogs of a native ligand to include analogs of proteins. Although we have discussed several successful programs targeting integrins, we do not believe the successes are unique to this family of proteins. In fact, the integrins represent a mechanistically related family of high-value targets that afford an economy of scale in our research programs. However, it is unlikely that all protein targets are amenable to this approach and we have not discussed any limitations or failures of this approach. The work described here augments and even extends the more traditional approaches, but will not replace them. Given the number of novel targets emerging from the genome and the absence of lead molecules, it does seem that the discovery of leads from a protein datastream could be important over the next ten years.

References

1 Hirschmann, R.F. (2003) *Foreward to: The Practice of Medicinal Chemistry* (2nd edn) (Wermuth, C., ed.), Elsevier
2 Hillis, G.S. and MacLeod, A.M. (1996) Integrins and disease. *Clin. Sci.* 91, 639–650
3 Vorchheimer, D.A. *et al.* (1999) Platelet glycoprotein IIb/IIIa receptor antagonists in cardiovascular disease. *J. Am. Med. Assoc.* 281, 1407–1414
4 Ockey, D.A. and Gadek, T.R. (2002) Inhibitors of protein–protein interactions. *Expert Opin. Ther. Pat.* 12, 393–400
5 Bodary, S.C. *et al.* (1989) Expression of recombinant platelet glycoprotein IIbIIIa results in a functional fibrinogen-binding complex. *J. Biol. Chem.* 264, 18859–18862
6 Fei, D.T.W. *et al.* (1993) RGD-containing peptides inhibit adhesion of 293 cells transfected with GpIIb/IIIa to fibrinogen: comparison to inhibition of platelet aggregation. *Blood Coagul. Fibrinolysis* 4, 255–262
7 Barker, P.L. *et al.* (1992) Cyclic RGD peptide analogs as antiplatelet antithrombotics. *J. Med. Chem.* 35, 2040–2048
8 McDowell, R.S. *et al.* (1994) From peptide to non-peptide. 2. The *de novo* design of potent, non-peptidal inhibitors of platelet aggregation based on a benzodiazepinedione scaffold. *J. Am. Chem. Soc.* 116, 5077–5083
9 Dickfeld, T. *et al.* (2001) Differential antiplatelet effects of various glycoprotein IIb-IIIa antagonists. *Thromb. Res.* 101, 53–64
10 Furuya, K. *et al.* (2001) Examination of several potential mechanisms for the negative outcome in a clinical stroke trial of enlimomab, a murine anti-human intercellular adhesion molecule-1 antibody: a bedside-to-bench study. *Stroke* 32, 2665–2674
11 Kimura, S.R. *et al.* (2001) Dynamical view of the positions of key side chains in protein–protein recognition. *Biophys. J.* 80, 635–642
12 Pearce, K.H., Jr *et al.* (1996) Structural and mutational analysis of affinity-inert contact residues at the growth hormone-receptor interface. *Biochemistry* 35, 10300–10307
13 Smith, A.B. 3rd *et al.* (1999) Molecular modeling, synthesis, and structures of N-methylated 3,5-linked pyrrolin-4-ones toward the creation of a privileged nonpeptide scaffold. *Bioorg. Med. Chem.* 7, 9–22
14 Jackson, D.Y. (2002) Alpha 4 integrin antagonists. *Curr. Pharm. Des.* 8, 1229–1253
15 Gadek, T.R. *et al.* (2002) Generation of an LFA-1 antagonist by the transfer of the ICAM-1 immunoregulatory epitope to a small molecule. *Science* 295, 1086–1089
16 Freidinger, R.M. (1999) Nonpeptidic ligands for peptide and protein receptors. *Curr. Opin. Chem. Biol.* 3, 395–406
17 Ripka, A.S. and Rich, D.H. (1998) Peptidomimetic design. *Curr. Opin. Chem. Biol.* 2, 441–452
18 Gadek, T.R. and Nicholas, J.B. (2003) Small molecule antagonists of proteins. *Biochem. Pharmacol.* 65, 1–8
19 Gadek, T.R. and McDowell, R.S. (1996) Controlling the three dimensional display of the RGD adhesive epitope in peptides and nonpeptides. Book of Abstracts, 211th ACS National Meeting, New Orleans, LA, March 24-28, MEDI-235.
20 Quan, C. *et al.* (1998) Transfer of a protein binding epitope to a minimal designed peptide. *Biopolymers* 47, 265–275
21 Jackson, D.Y. *et al.* (1997) Potent α4β1 peptide antagonists as potential anti-inflammatory agents. *J. Med. Chem.* 40, 3359–3368
22 Fong, S. *et al.* (1997) Mucosal addressin cell adhesion molecule-1 (MAdCAM-1). Its binding motif for α4β7 and role in experimental colitis. *Immunol. Res.* 16, 299–311
23 McDowell, R.S. and Gadek, T.R. (1992) Structural studies of potent constrained RGD peptides. *J. Am. Chem. Soc.* 114, 9245–9253
24 McDowell, R.S. *et al.* (1994) From peptide to non-peptide. 1. The elucidation of a bioactive conformation of the arginine-glycine-aspartic acid recognition sequence. *J. Am. Chem. Soc.* 116, 5069–5076
25 Xiong, J-P. *et al.* (2002) Crystal structure of the extracellular segment of integrin αvβ3 in complex with an Arg-Gly-Asp ligand. *Science* 296, 151–155
26 Duggan, M.E. and Hutchinson, J.H. (2000) Ligands to the integrin receptor αvβ3. *Expert Opin. Ther. Pat.* 10, 1367–1383
27 Legge, G.B. *et al.* (2000) NMR solution structure of the inserted domain of human leukocyte function associated antigen-1. *J. Mol. Biol.* 295, 1251–1264
28 Fisher, K.L. *et al.* (1997) Identification of the binding site in intercellular adhesion molecule 1 for its receptor, leukocyte function-associated antigen 1. *Mol. Biol. Cell* 8, 501–515
29 Renz, M.E. (1994) Structural requirements for adhesion of soluble recombinant murine vascular cell adhesion molecule-1 to alpha 4 beta 1. *J. Cell Biol.* 125, 1395–1406
30 Nakakura, E.K. *et al.* (1993) Potent and effective prolongation by anti-LFA-1 monoclonal antibody monotherapy of non-primarily vascularized heart allograft survival in mice without T cell depletion. *Transplantation* J, 412–417
31 Gottlieb, A. *et al.* (2000) Effects of administration of a single dose of a humanized monoclonal antibody to CD11a on the immunobiology and clinical activity of psoriasis. *J. Am. Acad. Dermatol.* 42, 428–435
32 Werther, W.A. *et al.* (1996) Humanization of an anti-lymphocyte function-associated antigen (LFA)-1 monoclonal antibody and reengineering of the humanized antibody for binding to rhesus LFA-1. *J. Immunol.* 157, 4986–4995
33 Ashkenazi, A. and Chamow, S.W. (1997) Immunoadhesins as research tools and therapeutic agents. *Curr. Opin. Immunol.* 9, 195–200
34 Edwards, C.P. *et al.* (1998) Mapping the intercellular adhesion molecule-1 and -2 binding site on the inserted domain of leukocyte function-associated antigen-1. *J. Biol. Chem.* 273, 28937–28944
35 Gadek, T.R. *et al.* (2002) Generation of an LFA-1 antagonist by the transfer of the ICAM-1 immunoregulatory epitope to a small molecule. *Science* 295, 1086–1089
36 Liu, G. (2001) Small molecule antagonists of the LFA-1/ICAM-1 interaction as potential therapeutic agents. *Expert Opin. Ther. Pat.* 11, 1383–1393
37 Shimaoka, M. *et al.* (2003) Structures of the αL I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell* 112, 99–111
38 Bella, J. *et al.* (1998) The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. *Proc. Natl. Acad. Sci. U. S. A.* 95, 4140–4145
39 Ali, F.E. *et al* (1997) Pyridine derivatives and analogs useful as vitronectin receptor antagonists. *PCT Int. Appl.* WO 9724124, p 133