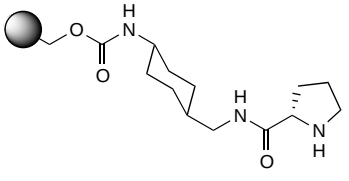
Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

Orally active thrombin inhibitor

Thrombin inhibitors, which block the blood coagulation cascade by inhibiting the conversion of fibrinogen to fibrin, offer the potential for developing novel agents for the treatment of thrombogenic disorders. Scientists at Merck Research Laboratories (West Point, PA, USA) have previously shown that various peptidomimetic structures containing the basic P_1 moiety are highly potent and selective thrombin inhibitors (K_i <5 nM). However, these molecules were found to have poor oral bioavailability in rats and dogs, which limits their potential therapeutic use.

In an attempt to overcome this prob-



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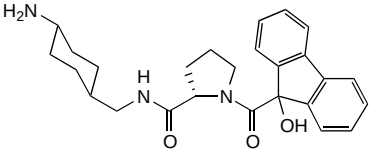
lem, the group prepared a range of analogues of the initial structures in which the P_3 residue was replaced by lipophilic carboxylic amides using a resin-based synthetic methodology [Brady, S.F. *et al. J. Med. Chem.* (1998) 41, 401–406]. The compounds were prepared using a common P_1–P_2 template linked to a polystyrene 'Wang' resin support by an acid-labile carbamate (**1**). From the total pool of 200 compounds prepared using this method, *N*-(9-hydroxy-9-fluorenecarboxy)prolyl *trans*-4-aminocyclohexylmethyl amide (L372460, **2**) was found to be the most potent (K_i = 1.5 nM). This molecule exhibited efficacy in a rat model of arterial thrombosis and good oral bioavailability in both dogs (74%) and monkeys (39%) with a serum half-life of ~4 h.



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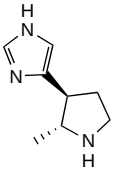
H_3 receptor agonist

The H_3 receptor is located presynaptically on histaminergic neurones, providing a negative feedback pathway that modulates the synthesis and release of histamine and other neurotransmitters. Although initial studies *in vitro* suggested that (*R*)-α-methylhistamine is a potent selective H_3 agonist (H_3/H_1 ratio ~ 10,000), subsequent *in vivo* studies have shown substantial H_1 activity (ED_{50} = 0.1 mg kg^{-1}; H_3/H_1 ratio = 17). Workers from Schering-Plough Research Institute (Kenilworth, NJ, USA) have previously used conformational analysis to identify a novel pyrrolidine (**3**) with improved specificity for the H_3 receptor (H_3/H_1 ratio >>550) [Shih, N-Y. *et al. J. Med. Chem.* (1995) 38, 1593–1599]. The group have recently reported an SAR study that led to the discovery of *trans*-4-methyl-3-imidazole pyrrolidine (**4**) as an alternative potent and highly selective H_3 agonist (ED_{50} = 0.3 mg kg^{-1}; H_3/H_1 ratio >>330) [Shih, N-Y. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 243–248].



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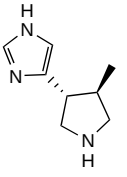

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Proteasome inhibitors

The proteasome is a multicatalytic protease responsible for intracellular protein turnover in eukaryotic cells. It is involved with degradation of damaged proteins and processing of regulatory proteins for various cell functions. Unregulated proteasome activity leads to pathological conditions, such as cancer and inflammatory diseases, as a consequence of disregulation of cellular processes. For example, in cancer the unregulated degradation of cyclins, cyclin-dependent kinase inhibitors and

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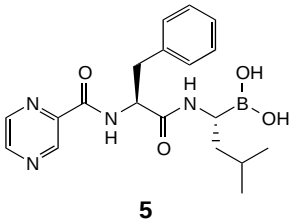
  1359-6446/98/$19.00. PII: S1359-6446(98)01192-1

tumour suppressor genes involved in cell cycle regulation causes accelerated and uncontrolled cell division.

Workers from ProScript (Cambridge, MA, USA) have reported the discovery of a series of potent and selective dipeptidyl boronic acid proteasome inhibitors, exemplified by **5** [Adams, J. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 333–338]. This compound has been shown to have potent inhibitory activity of proteasome enzymatic function *in vitro* (K_i = 0.62 nM), to modulated proteasome-dependent physiological processes both in cell culture and *in vivo*, and to have antitumour and anti-inflammatory efficacy in several different animal models. It would therefore appear to offer potential for the future development of new therapeutic agents for the treatment of cancer and inflammatory diseases.



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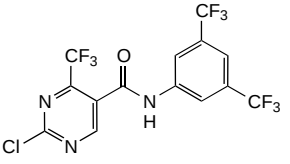
Potent inhibitor of NF-κB- and AP-1-mediated gene expression

In certain immunoinflammatory diseases the continuous activation of T cells leads to the self-perpetuating destruction of normal tissues and organs. The transcription factors nuclear factor κB (NF-κB) and activator protein 1 (AP-1) control the production of cytokines and other cellular regulators that are upregulated upon activation of T cells. Both NF-κB and AP-1 are therefore attractive therapeutic targets for the possible regulation of immunoinflammatory disorders. Workers from Signal Pharmaceuticals (San Diego, CA, USA) have described the identification of a novel series of compounds that inhibit the activation of NF-κB and AP-1 in T cells and the production of IL-2 and IL-8 [Sullivan, R.W. *et al. J. Med. Chem.* (1998) 41, 413–419].

2-Chloro-4-(trifluoromethyl)pyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]-carboxamide (**6**) was the most potent inhibitor identified (IC_{50} = 50–100 nM). Studies have demonstrated that the inhibitory activity of this compound is specific to T cells. *In vivo* studies in several animal models of inflammation and immunosuppression have demonstrated that the compound is active in a dose-dependent manner when administered intraperitoneally [Goldman, M.E. *et al. Transplant. Proc.* (1996) 28, 3106–3109]. These results suggest that these compounds may be useful as novel immunoinflammatory agents.



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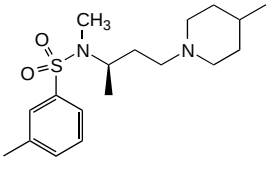
5-HT$_7$ receptor antagonist

The 5-HT$_7$ receptor is the most recently identified 5-HT receptor. The receptor has been cloned from a variety of mammalian cDNA and although it shows a high degree of interspecies homology it is not particularly homologous with other 5-HT receptors. Although various pharmacological roles for this receptor subtype have been postulated, the biological function of it is still poorly understood. Workers from SmithKline Beecham Pharmaceuticals (Harlow, UK) have recently reported the first selective 5-HT$_7$ receptor antagonist (**7**) following the high-throughput screening of the SmithKline Beecham Compound Bank against the cloned human 5-HT$_7$ receptor and subsequent SAR studies [Forbes, I.T. *et al. J. Med. Chem.* (1998) 41, 655–657]. This compound will be a useful tool for the further characterization of the biological role of this receptor subtype.



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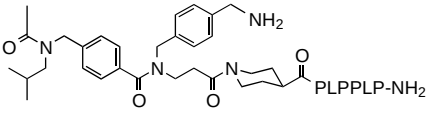
Combinatorial chemistry

Ligands for the SH3 domain

The *src*-homology 3 (SH3) domains are noncatalytic structural features that act as receptors in a number of inter- and intracellular protein interactions. These selective recognition processes are necessary for many significant biological functions including cellular signalling, mitogenesis and oncogenesis. The peptide ligands of SH3 domains so far characterized bind by occupying two hydrophobic Leu-Pro pockets and a specificity pocket. Continuing a long-standing study by Schreiber at Harvard University, a recent publication from his laboratory [Morken, J.P. *et al. J. Am. Chem. Soc.* (1998) 102, 23–29] describes the use of combinatorial chemistry in generating nonpeptide ligands (NLs) that explore the specificity pocket.



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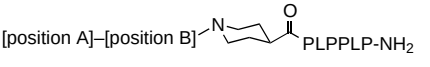

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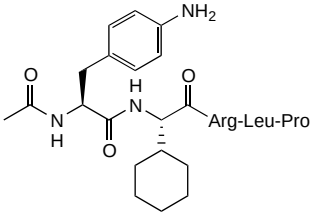
Based on a previously reported ligand, NL-1 (**1**) of the Src SH3 domain, an encoded library of 2,499 compounds (**2**), was constructed on TentaGel resin using split-pool synthesis. The library was synthesized from 50 monomers in position A and 50 monomers in position B, including deletions in each position. Incubating the library whilst still attached to the resin beads with a biotinylated Src SH3 domain and using a colorimetric assay to identify beads that tightly bound compounds revealed several new ligands, some showing improvements in affinity over NL-1. The same library was used to show that it is readily possible to find different structures with selectivity for the Hck SH3 domain, despite this protein possessing a 55% sequence identity.

1359-6446/98/$19.00. PII: S1359-6446(98)01195-7

Factor Xa inhibitors

Blood loss following injury is limited by a complex interaction of two interdependent processes leading to the generation of a blood clot. Both pathways converge at the formation of factor Xa, a proteolytic enzyme that converts prothrombin to thrombin. Thus inhibition of factor Xa is seen as an alternative to the inhibition of thrombin as a method of controlling inappropriate thrombus formation. The Selectide group have described the use of a peptide combinatorial library for the discovery of potent and selective factor Xa inhibitors [Ostrem, J.A. *et al. Biochem.* (1998) 37, 1053–1059; Al-Obeidi and Ostrem *Drug Discovery Today* (1998) 3, 223–231].

A library of octapeptides synthesized from naturally occurring L-amino acids was prepared using split-pool synthesis on TentaGel resin and screened by incubation with biotinylated human factor Xa conjugated with streptavidin alkaline phosphatase. Beads containing peptide sequences that bound to factor Xa were detected by a colorimetric assay, and were subjected to Edman degradation to sequence the attached ligand. The sequences identified showed a remarkable conservation of the tripeptide sequences, YIR or FIR, with K_i values ranging from 4 to 15 μM.



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Further modification of the active octapeptide YIRLAAFT demonstrated that removing three or four residues from the C-terminal had little effect on affinity and that inverting the tyrosine stereochemistry enhanced the potency. Furthermore, this and related sequences had no affinity for trypsin – a selectivity essential in the design of thrombin inhibitors. Using a combination of secondary combinatorial libraries based on the YIR motif and rational design eventually led to the discovery of the pentapeptide, SEL2489 (**3**), with a K_i = 25 nM against factor Xa.

Molecularly imprinted polymers

Molecular imprinting is a recent technique that allows the creation of polymers containing synthetic receptors that can mimic natural binding sites. By polymerizing functionalized monomers in the presence of cross-linking agents and a template molecule, a molecular imprinted polymer (MIP) can be formed. Extensive washing to remove the template generates artificial receptors that can bind the original molecule with high specificity. This technique has been recently used to identify specific members of a small library of steroid molecules [Ramström, O. *et al. Anal. Commun.* (1998) 35, 9–11].

MIPs were generated using either 11α-hydroxyprogesterone or corticosterone, and then packed into a stainless steel hplc column. A mixed library of 12 related steroid structures was generated and eluted through each of the columns. Using a column containing a control polymer generated without imprinting, the 12 steroids were not substantially retained during elution. However, the affinity of 11α-hydroxyprogesterone for its respective MIP resulted in the column retaining the compound longer than the other steroids, including the structurally closely related 11β-hydroxyprogesterone. The same effect was observed for the corticosterone and its respective templated MIP.

These synthetic receptors offer the opportunity for the initial screening of combinatorial libraries, especially when the natural receptor is either poorly characterized or has proven difficult to purify.

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Genomics

Gene chip collaboration

Affymetrix (Santa Clara, CA, USA) has entered into an agreement with the Genetics Institute (Cambridge, MA, USA) to make its GeneChip® expression monitoring arrays available as part of the Genetics Institute's DiscoverEase® package. The new agreement, termed EasyAccess, will allow the Genetics Institute, as well as pharmaceutical and biotechnology companies who license DiscoverEase®, to utilize the GeneChip® for the identification of human secreted proteins as drug targets and the discovery of new compounds that perturb gene expression of secreted proteins. The Genetics Institute has negotiated an initial three-year licence from Affymetrix to use and market the GeneChip® technology on a subscription basis.

The details of the technology and its application for drug discovery have been described previously in this column [Wallace, R.W. *Drug Discovery Today* (1997) 2, 557–558]. Its utility is for quickly resequencing known genes to search for mutations, or to follow the expression of many different genes simultaneously. For the latter application, it works much the same way as a Southern blot, except that with the GeneChip® it is possible to monitor tens or even hundreds of thousands of genes simultaneously by the hybridization status of thousands of distinct DNA probes encoded at a tiny region – an address – on a silicon chip about the size of a postage stamp. Affymetrix expects that the system will allow drug researchers to obtain tens of millions of data points routinely.

Human protein library

The initial GeneChip® product offered through EasyAccess will be a panel of human secreted proteins. The Genetics Institute has a major molecular biology project under way to construct a comprehensive library containing information on novel human secreted proteins. The goal is to identify and determine the function of large numbers

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of secreted proteins and the genes that encode them. Secreted proteins were targeted by the Genetics Institute because they represent nine of the ten top-selling biotechnology products and they are targets for eight of the ten top-selling, small-molecule drugs. The company anticipates that useful drug targets remain to be discovered amongst the panel of secreted human proteins. 'Now that we and our collaborators have broad access to Affymetrix' GeneChip probe arrays, we can utilize information to characterize secreted proteins and make better decisions about their relevance for pharmaceutical development,' notes Adelene Perkins, Director of the DiscoverEase program at the Genetics Institute.

Database access

Subscribers to EasyAccess will receive entry to the library that is being developed by the Genetics Institute and to a relational database of information for the various secreted proteins. To date, the Genetics Institute has licensed access to the DiscoverEase program to seven commercial companies including Chiron (Emeryville, CA, USA), Genetech (South San Francisco, CA, USA), Kirin Brewery Co. (Tokyo, Japan), Chugai Pharmaceutical Co. (Tokyo, Japan), Ontogeny (Cambridge, MA, USA), Bayer (West Haven, CT, USA) and Scios (Mountain View, CA, USA).

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Emerging molecular targets

Interleukin 13

The T helper 2 (Th2) lymphocytes have an important role in the immune response to allergic reactions and parasitic worm infections. Th2 cells secrete various cytokines [interleukin 4 (IL-4), IL-5 and IL-13] that induce the production of IgE antibodies and eosinophils, which in turn protect the body against parasitic infections.

The need to understand better the relative importance of the different cytokines in Th2-cell-mediated responses has prompted McKenzie, A.N. and coworkers to investigate the effects of the absence of IL-13 on parasitic worm infections in mice [*Curr. Biol.* (1998) 8, 339–342]. Mice lacking the ability to produce IL-13 were found to be unable to resist the challenge of a parasitic worm infection effectively, despite secreting normal levels of the other Th2 cytokines in response to the infection. However, normal resistance to the parasitic worm infection was restored on treating of the mice with IL-13.

The group observed a decrease in the size and number of mucus-secreting goblet cells in the intestines of the mice unable to produce IL-13 and suggested that IL-13 may impart resistance by increasing mucus production, which is known to assist the expulsion of parasitic infections. On the basis of this work it will be interesting to explore the role of this cytokine in other Th2-cell-mediated responses such as allergies and asthma. This may lead to the identification of novel therapeutic targets for a variety of immunological conditions.

HIV immune evasion

A second recent paper in *Current Biology* reports important findings relating to the general mechanisms of HIV infection [Phillips, R. *et al. Curr. Biol.* (1998) 8, 355–358]. The binding of HIV to $CD4^+$ T helper lymphocytes primarily involves the binding of the HIV outer envelope glycoprotein gp120 to the CD4 receptor on the surface of the lymphocytes. The entry of the virus into the cells also requires the involvement of several co-receptors. β-Chemokines, which are released from cytotoxic T lymphocytes ($CD8^+$ T cells), exert an antiviral effect by binding to these co-receptors thereby preventing their participation in the gp120–CD4 interaction.

A research group from the John Radcliffe Hospital (Oxford, UK) have now reported the mechanism of chemokine release from $CD8^+$ cells. The group have identified a receptor expressed by the $CD8^+$ that binds to a peptide derived from HIV-1. Upon treatment of the $CD8^+$ cells with this peptide, the group observed both the release of β-chemokine and the stimulation of $CD8^+$-mediated lysis of infected $CD4^+$ T cells. The group also assessed the effects of natural variants of the HIV-1 $CD8^+$-stimulatory peptide and found that peptide variants that did not stimulate cytolysis also failed to induce β-chemokine release. This suggests that some HIV variants that express non-stimulating peptides promote the spread of the virus by avoiding $CD8^+$-cell-mediated lysis and facilitating viral entry by reducing the release of β-chemokine. This work has important implications for our basic understanding of the mechanisms of HIV infections and for the future development of novel therapeutic antiviral agents.

Andrew Lloyd

Contributions to *Profiles*

We welcome contributions for the *Profiles* series, which gives a commentary on promising lines of research, new technologies and progress in therapeutic areas. Articles should provide an accurate summary of the essential facts together with an expert commentary to provide a perspective. Brief outlines of proposed articles should be directed to the *Monitor* Editor (see below). Articles for publication in *Monitor* are subject to peer review and occasionally may be rejected or, as is more often the case, authors may be asked to revise their contribution. The *Monitor* Editor also reserves the right to edit articles after acceptance.

All suggestions or queries relating to Monitor should be addressed to Dr Andrew Lloyd, *Monitor* Editor, Department of Pharmacy, University of Brighton, Moulsecoombe,Brighton, UK BN2 4GL. tel: +44 1273 642049, fax: +44 1273 679333, e-mail: a.w.lloyd@brighton.ac.uk

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