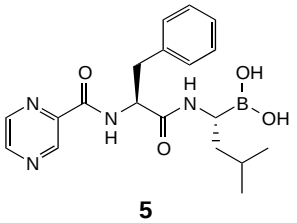
tumour suppressor genes involved in cell cycle regulation causes accelerated and uncontrolled cell division.

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.

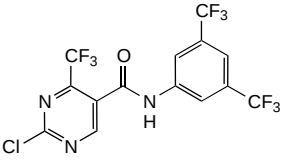


5

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].

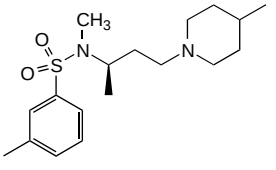
2-Chloro-4-(trifluoromethyl)pyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]-carboxamide (**6**) was the most potent inhibitor identified (IC_{50} = 50–100 nM). Studies have demonstrated that the inhibitory activity of this compound is specific to T cells. *In vivo* studies in several animal models 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.



6

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.

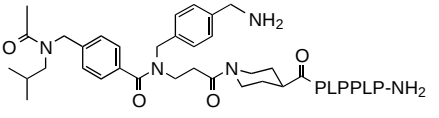


7

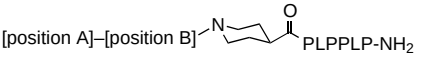
Combinatorial chemistry

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.



1



2

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.

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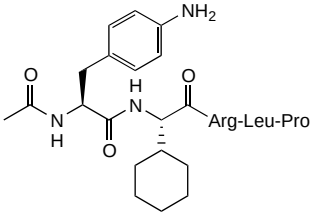
Factor Xa inhibitors

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.



3

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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