STRUCTURE-BASED DRUG DESIGN

X-rated

Biophysical methods, such as NMR and X-ray crystallography, are becoming increasingly popular early in lead generation, owing to their effectiveness in identifying low-affinity, low-molecular-mass 'fragments' and aiding their development into potent compounds. Lesuisse *et al.* now highlight the potential of such approaches by using X-ray-based screening of molecular fragments to develop nanomolar inhibitors of the SH2 domain of SRC, a kinase involved in bone resorption that represents a potential target for the treatment of osteoporosis.

The SH2 domain of SRC, which is involved in protein–protein interactions, seems to be crucial to its bone-resorbing activity, and this has therefore been the target of previous drug discovery efforts. However, although potent inhibitors that mimic the natural phosphate-containing peptide sequence that is recognized by the SRC SH2 domain — pYEEI — have been reported, the presence of the phosphotyrosine group that is necessary for strong binding in these cases is highly undesirable for drug candidates, as this group is readily hydrolysed, and has low membrane permeability.

The authors' search for compounds without these undesirable properties was prompted by two observations: first, that the binding of phenylphosphate (a fragment of phosphotyrosine) could be detected using conventional binding-assay techniques; and second, that crystals of SRC could be obtained that had the appropriate nature and quality to allow fragments to be soaked into them, such that the resultant structure could be determined by X-ray crystallography.

Two types of fragment — more than 150 in total — were screened both for binding affinity and by soaking them into SRC crystals to establish their binding mode: phenylphosphate-related fragments to elucidate optimal modification of the phenyl ring, and fragments that contained carboxylic acids, which have been reported as phosphate mimics. In the case of the acids, although no binding could be detected in the conventional assays, analysis of the crystal structures revealed that some of the fragments were forming interactions analogous to those involved in the binding of the natural ligand.

Incorporating the best phosphotyrosine mimetics indicated by these studies into the previously discovered potent inhibitors that mimicked the remainder of the pYEEI sequence resulted in several compounds with low nanomolar affinity for the SH2 domain, which could prove valuable in the cellular validation of the concept of SH2-domain inhibition.

Peter Kirkpatrick

References and links

ORIGINAL RESEARCH PAPER
Lesuisse, D. *et al.* SAR and X-ray. A new approach combining fragment-based screening and rational drug design: application to the discovery of nanomolar inhibitors of Src SH2. *J. Med. Chem.* 2002 May 2 (doi: 10.1021/jm010927p)

FURTHER READING Blundell, T. *et al.* High-throughput crystalllography for lead discovery in drug design. *Nature Rev. Drug Discov.* **1**, 45–54 (2002)

IN BRIEF

LIGAND-GATED ION CHANNELS

Ivermectin and nodulosporic acid receptors in *Drosophila melanogaster* contain both γ-aminobutyric acid-gated Rdl and glutamate-gated GluClα chloride channel subunits

Ludmerer, S. W. *et al. Biochemistry* **41**, 6548–6560 (2002)

Ligand-gated chloride channels are the targets for several insecticides, such as ivermectin and nodulisporic acid (see the Review by Raymond and Satelle on page 427 of the June issue), but the subunit composition of these channels is poorly characterized. Ludmerer *et al.* provide the first biochemical and immunological evidence of co-assembly of subunits from two different subclasses of ligand-gated-ion-channel subunit.

HIGH-THROUGHPUT SCREENING

High-throughput NMR-based screening with competition binding experiments

Dalvit, C. *et al. J. Am. Chem. Soc.* 2002 June 6 (doi: 10.1021/ja020174b)

In recent years, NMR has emerged as a powerful method for the detection of small molecules that interact with macromolecular targets of therapeutic interest (see the Review by Wüthrich and colleagues on page 211 of the March issue). However, ligand-based NMR screening methods are limited in their ability to detect high-affinity molecules. Dalvit *et al.* describe an approach that can overcome this limitation, and which also permits detection of high-affinity molecules that are only marginally soluble, thus enlarging the diversity of compounds that are amenable to NMR screening.

ANTIPYRETICS

Determinants of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H2 synthases

Boutard, O. *et al. Proc. Natl Acad. Sci. USA* **99**, 7130–7135 (2002)

Acetaminophen has anti-fever properties, but unlike other inhibitors of the biosynthesis of prostaglandins, it has little effect on inflammation or platelets. Boutard *et al.* provide evidence that high concentrations of hydroperoxide byproducts counteract the inhibitory effect of acetaminophen on prostaglandin H synthase in platelets and inflammatory cells, indicating that the properties of acetaminophen might be accounted for by differing concentrations of hydroperoxides in cells associated with the inflammatory response and cells associated with fever.

RATIONAL DRUG DESIGN

Disabling receptor ensembles with rationally designed interface peptidomimetics

Berezov, A. *et al. J. Biol. Chem.* 2002 May 14 (doi: 10.1074/jbc.M202880200)

Homo- and heterodimerization of erbB-family receptor tyrosine kinases — which are overexpressed in various cancers — is known to be important in receptor signalling. Berezov *et al.* describe the design of peptides derived from potential dimerization surfaces in an extracellular subdomain of the erbB receptors, which can suppress growth in cells overexpressing erbB receptors.