LEAD DISCOVERY

Get a grip

Dynamic combinatorial chemistry (DCC) is an emerging strategy for lead discovery, in which the binding site of a target macromolecule is used as a template for the self-assembly of its own inhibitor. Writing in *Nature Biotechnology*, Erlanson *et al.* now describe the use of a novel extension of this strategy to generate nonpeptidic inhibitors of the cysteine protease caspase-3, a key mediator of apoptosis, and therefore a therapeutic target in a wide range of conditions, such as stroke and neurodegenerative diseases.

A related approach to DCC called 'tethering', in which a cysteine residue in the binding site of the target protein is covalently modified with small-molecule fragments under reversible 'thiol exchange' conditions, has also recently been shown to be useful in lead discovery. Here, the formation of a covalent bond allows fragments that bind too weakly to be identified by traditional means (such as high-throughput screening) to be detected by mass spectrometry. But could combining the benefits of the two approaches by using a covalent tether in the binding site as the substrate for DCC — effectively giving small-molecule fragments greater 'grip' in the binding pocket — further facilitate lead generation?

To answer this question, the authors first covalently modified a cysteine residue in the active site of caspase-3 with small-molecule 'extenders' designed to possess elements necessary for binding to caspase-3. Each extender also contained a protected thiol group. After deprotection to give the free thiol, the protein–extender complexes were then screened against a ~7,000-member library of disulphide-containing fragments under thiol-exchange conditions, and those that formed the most stable disulphide bonds were identified by mass spectrometry. Several novel hits based on a range of extenders were found.

Next, on the basis of X-ray crystallography studies of the protein–extender–fragment structures, selected fragments were combined with the binding elements of their respective extenders to create reversible inhibitors. This was achieved by replacing the disulphide bonds with more pharmaceutically appropriate linkages and replacing the covalent linkage between the extender and the protein with a reversible linkage. The resulting compounds inhibited caspase-3 with K_is in the high nanomolar to low micromolar range. The K_i of one such compound was then optimized further by a factor of tenfold (2.8 μM to 0.2 μM) simply by rigidifying the linker portion. Although such potency is known to be too low for effective inhibition of apoptosis in cells, putting back a group that binds irreversibly to caspase-3 gave a compound that had better caspase-3-inhibitory activity than that of the widely used irreversible peptidic caspase inhibitor Z-VAD-FMK, and that also inhibited apoptosis in cells.

So, this combination of tethering and DCC allows the rapid identification of potent caspase inhibitors using relatively small libaries. Notably, the compounds discovered were novel and essentially nonpeptidic, whereas most other reported caspase inhibitors have been constructed by converting the known peptide substrate into a peptidomimetic. Moreover, the approach should be applicable to other important target families — for example, kinases and phosphatases — that have known covalent-binding fragments that could be converted to appropriate extenders.

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References and links

ORIGINAL RESEARCH PAPER Erlanson, D. A. *et al. In situ* assembly of enzyme inhibitors using extending tethering. *Nature Biotech.* **21**, 308–314 (2003)

FURTHER READING Ramström, O. & Lehn, J.-M. Drug discovery by dynamic combinatorial libraries. *Nature Rev. Drug Discov.* **1**, 26–32 (2002)

IN BRIEF

ANTICANCER DRUGS

Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab.

Cho, H.-S. *et al. Nature* **421**, 756–760 (2003)

Overexpression of the receptor tyrosine kinase HER2 is found in 20–30% of breast cancer cases, and trastuzumab (Herceptin; Genentech) — a monoclonal antibody against HER2 — has been approved for the treatment of such patients. The authors report the crystal structure of human HER2 complexed with the Herceptin antigen-binding fragment, which reveals a region of the receptor that could be a promising target for the development of drugs with improved therapeutic properties.

COMPUTATIONAL CHEMISTRY

Absorption classification of oral drugs based on molecular surface properties.

Bergström, C. A. S. *et al. J. Med. Chem.* **46**, 558–570 (2003)

The recognition that poor pharmacokinetic properties have been an important cause of costly late-stage failures in drug development has led to increasing interest in computational approaches for the prediction of such properties as early as possible in the drug discovery process. The authors describe computational models that can rapidly predict the solubility and permeability of structurally diverse molecules with high accuracy from calculated molecular surface properties alone.

STROKE

Activated protein C blocks p53-mediated apoptosis in ischemic human brain and is neuroprotective.

Cheng, T. *et al. Nature Med.* 3 Feb 2003 (doi: 10.1038/nm826)

Activated protein C (APC) is a systemic anticoagulant and anti-inflammatory factor, a recombinant form of which has recently been approved for the treatment of severe sepsis. Cheng *et al.* show that APC can protect the brain from ischaemic injury by directly inhibiting apoptosis in brain cells, indicating that APC could be valuable in the treatment of stroke and other neurodegenerative diseases.

ANTICANCER DRUGS

Selective killing of cancer cells by β-lapachone: direct checkpoint activation.

Li, Y. *et al. Proc. Natl Acad. Sci. USA* **100**, 2674–2678 (2003)

The cell-proliferation cycle has inbuilt checkpoints at which apoptosis is activated if DNA damage is irreparable, thereby safeguarding genomic integrity. Many traditional anticancer drugs kill cancer cells by causing nonselective DNA damage, leading to activation of checkpoint-mediated apoptosis, but are therefore also toxic to normal cells. Li *et al.* report that β-lapachone can selectively induce apoptosis in cancer cells through a regulatory pathway that links checkpoint activation with apoptosis, indicating that direct checkpoint activation could be a novel anticancer strategy.