Monitor

Monitor provides an insight into the latest developments in the pharmaceutical and biotechnology industries. ***Chemistry*** examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while ***Profiles*** provides commentaries on promising lines of research, new molecular targets and technologies. ***Biology*** reports on new significant breakthroughs in the field of biology and their relevance to drug discovery. ***Business*** reports on the latest patents and collaborations, and ***People*** provides information on the most recent personnel changes within the drug discovery industry.

Chemistry

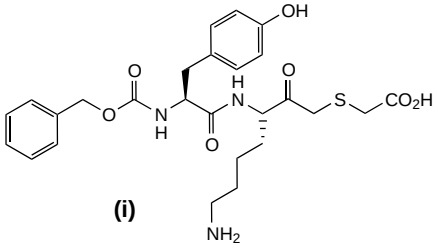
Combinatorial chemistry

Cysteine protease inhibitors

Cysteine proteases are important therapeutic targets because of their role in several diseases, such as cathepsin B in tumour growth and cathepsin K in osteoporosis. Cysteine proteases catalyze the hydrolysis of amide bonds in peptides and proteins through nucleophilic attack by the active-site cysteine thiol on the amide carbonyl. Most inhibitors of cysteine proteases which exploit this mechanism contain electrophilic functionality, such as carbonyl groups, or Michael acceptors that react with the active-site cysteine residue. In an effort to discover inhibitors of the cysteine protease, cathepsin B, which is implicated in cancer, a library of mercaptomethyl ketones was synthesized [1].

A library of 2016 compounds was synthesized on solid phase. Screening was undertaken against cathepsin B at a concentration of 1 μM. Of the compounds screened, 110 library members inhibited >50% of the enzyme activity upon a five minute incubation with cathepsin B (as determined by rates of cleavage of the fluorescent substrate Cbz-Phe-Arg-AMC). Enzyme activity was monitored by the release of the fluorescent aminomethylcoumarin (AMC) group. Of those 110 library members re-screened at a concentration of 333 nM, 18 were found to cause >50% inhibition of the enzyme.

One of the most potent compounds was compound **i**, which possessed a K_i of 2 nM. This work has delivered potent inhibitors against cathepsin B, and the 2016-membered library should be a rich source of inhibitors against other cysteine proteases because of the diverse functionality incorporated within the library.



1 Ellman, J. A. *et. al.* (2003) Synthesis of a diverse library of mechanism-based cysteine protease inhibitors. *J. Combi. Chem.* 5, 869–880

Homocysteine *S*-methyltransferase

The challenge of functional genomics and proteomics is to translate sequencing data into a precise understanding of how proteins function in cells, tissues or whole organisms. Small ligands that are able to interact in a specific manner with proteins can be effective tools in the search for proteome function. Given the high number of proteins in mammalian organisms, HTS procedures have been developed to handle this complicated task.

The potential drawback of such methods is that the full range of proteins that the chosen ligands might interact with are not discovered if screening is performed with only one or a few proteins. A lack of information regarding how many proteins a given ligand can interact with precludes our ability to completely understand the full spectrum of effects that ligands could have in a complex medium such as a living cell. Thus, approaches that study the effects of ligands in whole cells are becoming increasingly important. To discover novel protein–ligand interactions, Collinsova *et al*. have designed a method based on affinity capture principles coupled to combinatorial chemistry [2].

Phosphinic pseudopeptides have been shown to function as transition state analogues of zinc metalloproteases and these compounds are highly potent inhibitors of this protein family. Given the ability of the phosphoryl group to interact with zinc atoms, phosphinic peptides might also interact with other zinc metalloenzymes not belonging to this family of zinc proteases.

Thus, Collinsova *et al*. tested this idea by preparing affinity columns harbouring 361 different phosphinic peptides and using them to isolate all interacting proteins from crude rat-liver homogenates. By applying a deconvolution process, the authors were able to identify the most specific ligand within the phosphinate peptide library that had the highest affinity toward one newly discovered protein target, betaine: homocysteine *S*-methyltransferase (BHMT).

The deconvolution process resulted in the synthesis of 19 different phosphinic

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pseudopeptides of general formula Ac-Val-DL-Ala-ψ[PO_2^--CH_2]-DL-Leu-X_{aa}'-NH_2. The ability of these pseudopeptides to inhibit human recombinant BHMT (at 100 μM) showed compound **ii** to be one of the most potent compounds, capable of an 80% inhibition of human recombinant BHMT. The phosphinic pseudopeptide inhibitors of BHMT developed in this study could be promising tools for studying the physiological function of BHMT, and further work in this area is warranted.

Val-Phe-ψ[PO_2^--CH_2]-Leu-His-NH_2

(ii)

2 Collinsova, M. *et al.* (2003) Combining combinatorial chemistry and affinity chromatography: highly selective inhibitors of human betaine: homocysteine *S*-methyltransferase. *Chem. Biol.* 10, 113–122

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Biology

Cancer biology

Survivin: antiapoptotic function by inhibiting AIF-mediated events

Inhibitors of apoptosis (IAPs) function by inhibiting caspases through their characteristic baculovirus IAP repeat (BIR). Survivin contains a single BIR domain and its expression is associated with tumour aggression. However, it remains uncertain if the anti-apoptotic properties of Survivin can be attributed to caspase inhibition.

To gain insight into the mechanism of Survivin-mediated protection in melanoma cells, Liu *et al.* [1] determined the temporal sequence of apoptotic events by employing an inducible, dominant-negative Survivin BIR mutant (T34A-Sur). The authors showed that inhibition of Survivin by induction of T34A-Sur promotes activation of caspases-3, -8 and -9. In addition, the pan-caspase inhibitor z-VAD-fmk only partially rescued T34A-Sur-induced cells from apoptosis, as determined by propidium iodide staining and flow cytometry, pointing to the existence of an apoptotic component that is caspase-independent.

It was also demonstrated that T34A-Sur is able to trigger nuclear translocation of apoptosis-inducing factor (AIF). AIF can induce caspase-independent DNA fragmentation as well as apoptotic mitochondrial events that lead to subsequent caspase activation. The authors suggest that the primary anti-apoptotic function of Survivin is likely to be the suppression of AIF function, which is a departure from the conventional view that the anti-apoptotic function of IAPs is due to caspase inhibition.

It would now be of interest to determine if the inhibition of AIF by Survivin was direct or via an intermediate. Identifying the subcellular localization of Survivin might also lend further credence to its role as a physiological regulator of mitochondrial and AIF-dependent apoptotic pathways. In addition, given the high sequence homology shared by the IAPs, one ponders the possibility that the inhibition of AIF is a general function of the IAPs or a unique property of Survivin.

1 Liu, T. *et al.* (2004) Rapid induction of mitochondrial events and caspase-independent apoptosis in Survivin-targeted melanoma cells. *Oncogene* 23, 39–48

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Reductions in apoptosis do not always enhance tumour formation *in vivo*

The importance of cell death by apoptosis as tumour-suppressive mechanism *in vivo* has been demonstrated in several ways. Selective loss of proapoptotic lesions as well as gain of antiapoptotic lesions is observed in many neoplasms. In animal models, the loss of p53, ARF, BAX or gain of Bcl-xL have been shown to cooperate with deregulated c-*myc* expression to form tumours *in vivo*, by virtue of suppressed apoptosis. This put forth the hypothesis that any significant suppression of apoptosis can cooperate with oncogenes in tumour formation.

Caspase-9 and Apaf-1 are known to have an important role in the mitochondrial pathway of apoptosis. Scott *et al.* [2] investigated their *in vivo* role in lymphomagenesis, using an IgH enhancer-driven c-myc transgene in Apaf-1–/– and caspase-9–/– mice. Due to perinatal lethality, Emicro-*myc* transgenic Apaf-1–/– or caspase-9–/– foetal liver cells were used to reconstitute lethally irradiated recipient mice. No differences were seen in rate, incidence or severity of lymphoma with loss of Apaf-1 or caspase-9, and Apaf-1 was not a crucial determinant of anticancer drug sensitivity of c-*myc*-induced lymphomas. Loss of Apaf-1 only mildly reduced the sensitivity of these cells to apoptotic stimuli for 48 hours.

This study leads us to important questions. What are the crucial differences between antiapoptotic lesions that cooperate with oncogenes *in vivo* and lesions that do not allow tumour formation *in vivo*?

Is it only the extent to which apoptosis is suppressed, complete versus partial? This is a possible explanation based on the data in this study. Or is it crucial where the lesion affects apoptosis, upstream or downstream of the mitochondria? Or do the cooperative lesions exert effects in addition to their antiapoptotic effect, for example stimulation of cell cycle progression? This study has opened the way to start addressing these questions *in vivo*.

2 Scott, C.L. *et al.* (2004) Apaf-1 and caspase-9 do not act as tumour suppressors in *myc*-induced lymphomagenesis or mouse embryo fibroblast transformation. *J. Cell Biol.* 164, 89–96

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Rad51C: a component of the Holliday junction resolvasome in mammalian cells

Despite the importance of homologous recombination for the repair of double-strand breaks in DNA, little is known about the processing of Holliday junctions (HJs) in mammalian systems. Liu *et al.* [3] have shown that Rad51C is required for HJ resolution and