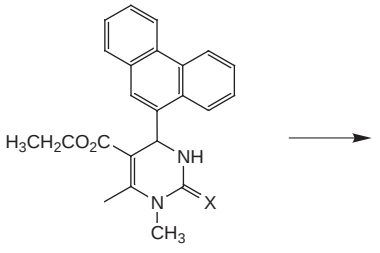
Combinatorial chemistry

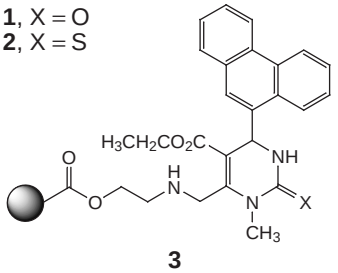
Novel chiral stationary phases

The principle of reciprocity introduced by Pirkle proposes that if a single molecule of a chiral substance has different affinities for the enantiomers of another chiral molecule, then single enantiomers of the latter can be used to select enantiomers of the former. Bringing this principle together with combinatorial chemistry has led to the development of novel chiral stationary phases suitable for the resolution of drug molecules [Lewandowski, K. *et al. J. Chem. Soc. Chem. Commun.* (1998), 2237–2238].

Initially a combinatorial library of 4-aryl-1,4-dihydropyrimidines (DHPM) was prepared in solution using the Biginelli multicomponent reaction. Applying the large range of available precursors allowed the production of 140 racemic products that were subsequently screened against a chiral molecule, (*S*)-(3,5-dinitrobenzoyl)-leucine, attached to polymeric resin beads. By applying retention times of the enantiomers to calculate the separation factor, α, it was evident that the racemic mixtures were separated to different degrees by the chiral stationary phase. In particular, the highest values of α were achieved for two DHPMs (**1**, **2**).



1, X = O
2, X = S



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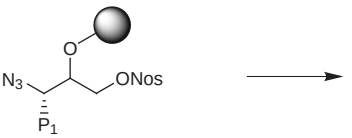
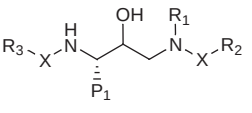
Single enantiomers of these particular DHPMs were then employed in the production of chiral stationary phases by attachment to a solid support. Columns packed with these materials (**3**) have proven to be highly effective at separating several different chiral substances including amino acids, non-steroidal anti-inflammatory drugs and dihydropyrimidines.

Cathepsin D inhibitors from libraries

Aspartyl proteases are a ubiquitous class of enzyme. The presence of many of these enzymes has been implicated in disease, and potent and selective inhibitors may offer opportunities for therapy. A recent paper describes the use of combinatorial chemistry in the search for inhibitors of cathepsin D, an enzyme that plays a role in breast tumour metastasis [Lee, C.E. *et al. J. Am. Chem. Soc.* (1998) 120, 9735–9747].

Known inhibitors of aspartyl proteases usually employ a secondary alcohol as a mimic of the tetrahedral intermediate in peptide hydrolysis and, consequently, this functional group was maintained as an invariant part of the library structures. Using a solid-phase synthetic approach, key intermediates were linked to the support through the secondary alcohol, and the synthesis designed such that four side-chains in the products (**4**) could be varied. In particular, a method for variation of the P1 side chain has been developed.





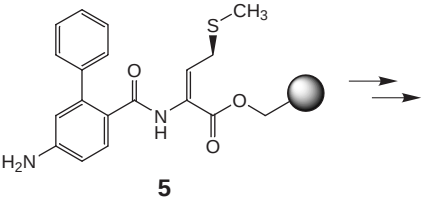
4, X = CO, $CONH_2$, SO_2

A library of 204 compounds was synthesized and the compounds screened for cathepsin D inhibition. Many of the most potent analogues were resynthesized on a larger scale and several of these had K_i values of below 3 nM. This synthetic approach is now being used for a study of the inhibitors that bind to the malarial protease plasmepsins I and II.

Inhibitors of protein farnesyltransferase

Ras proteins are found in a large proportion of human tumours. The Ras protein needs to undergo several post-translational modifications to be able to function, one of which is farnesyltransferase-mediated farnesylation. Thus, inhibition of this enzyme is a possible method for blocking the uncontrolled mitogenic signalling pathway. Many inhibitors have been previously reported, but these have limited utility because of the presence of a vulnerable thiol residue. Combinatorial chemistry has been applied in the discovery of protein farnesyltransferase inhibitors that lack this thiol group [Augeri, D.J. *et al. J. Med. Chem.* (1998) 41, 4288–4300].

Several derivatives of the Wang resin-linked derivative (**5**) were synthesized leading ultimately to a lead (**6**) with K_i = 64 nM. This compound had limited activity in a cell-based assay,



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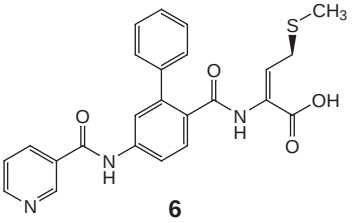

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and routine medicinal chemistry was used in the discovery of more potent farnesyltransferase inhibitors that inhibited Ras processing in whole cells.

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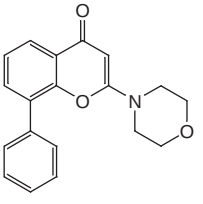
Molecular cancer target: PI-3-kinase

Many endogenous growth factors and oncogenes use the ubiquitous phosphoinositide (PI) signalling pathway for inducing proliferation of their target cells. This primary cellular communication pathway has thus become a favorite therapeutic target for the treatment of cancer. The simplest way to interfere with PI signalling involves inhibition of phosphatidylinositol-specific phospholipase C (PI-PLC), which hydrolyses membrane phosphatidylinositol-4,5-diphosphate [$PI(4,5)P_2$], generating two intracellular signalling molecules: inositol-1,4,5-triphosphate (IP_3) and diacylglycerol. The first regulates intracellular calcium ion levels and the second activates protein kinase C, which regulaties several cellular events. Consequently, PI-PLC inhibitors are also toxic to non-proliferating cells, as many other cellular functions, besides mitogenesis, utilize this signalling path.

Targeting phosphatidylinositol-3-kinase

An attractive cancer drug target is phosphatidylinositol-3-kinase (PI-3-kinase) that phosphorylates PI to generate phosphatidylinositol-3-phosphate [PI(3)P], which is further phosphorylated to $PI(3,4)P_2$ and $PI(3,4,5)P_3$. These membrane phospholipids participate in acute cellular responses activated by growth factors and oncogenes. Hence, reducing their cell membrane levels by inhibition of PI-3-kinase should in theory affect cancer cells more than nonmalignant cells. Indeed, both the fungal metabolite wortmannin and the natural bioflavinoid quercetin (known inhibitors of PI-3-kinase) exhibit potent antitumour activities *in vivo*. Unfortunately, these nonselective drugs also inhibit other kinases, resulting in toxicity towards nonmalignant cells. Lilly Research Laboratories have introduced the quercetin-like chromone, **1** [LY294002; 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one], as a selective and potent (IC_{50} = 1.4 μM) PI-3-kinase inhibitor [Vlahos, C.J. *et al. J. Biol. Chem.* (1994) 269, 5241–5248]. Compound **1** induced apoptosis in Ramos-Burkitt lymphoma B cells, indicating a potential application in the treatment of B cell lymphomas [Curnock, A.P. and Knox, K.A. *Cell. Immunol.* (1998) 187, 77–87]. Yet, at higher concentrations, **1** inhibits adrenal cortex type II PI-4-kinase, required for normal adrenal function [Downing, G.J. *et al. Biochemistry* (1996) 35, 3587–3594]. This could indicate that the therapeutic window of **1** might be relatively small.



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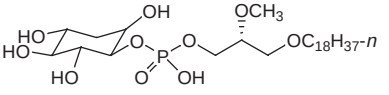
3-substituted myo-inositol derivatives

More recently Lixin Qiao and his colleagues of the Drug Discovery Program at Georgetown University Medical Center (Washington, DC, USA) and the Arizona Cancer Center (Tucson, AZ, USA) have described several 3-substituted myo-inositol derivatives that selectively inhibit PI-3-kinase. These inhibitors leave other aspects of the myo-inositol-signalling pathway intact, thereby limiting damage to non-malignant cells [Qiao, L. *et al. J. Med. Chem.* (1998) 41, 3303–3306]. However, such myo-inositol derivatives are sensitive to endogenous phospholipases, which minimizes their bioavailability. To overcome this problem, ether lipid analogues of 3-deoxy-PI were synthesized using 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycerol. The resulting ether lipid 3-deoxy-PI analogue (**2**) potently inhibited bovine



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brain p110/p85 PI-3-kinase (IC_{50} = 2.5 μM), while concentrations tenfold higher inhibited bovine PI-PLC. This compound exhibited a similarly low IC_{50} value (2.1 μM) in a soft agarose colony formation assay using HT-29 human colon adenocarcinoma cells. This antitumour activity was also evident *in vivo*: HT-29 cells injected subcutaneously into immunodeficient *scid* mice formed solid tumours; but daily treatment with the ether lipid 3-deoxy-PI analogue **1** at 150 mg kg^{-1} i.p. reduced tumour volume by 67% ten days after inoculation. Thus, lipid analogues of 3-deoxy-PI are useful antitumour drug candidates with minimal toxicity towards normal cells. Moreover, their high lipophilicity allows good bioavailability and tumour penetration. Future designs of similar ether lipid analogues of 3-deoxy-PI should improve selectivity, further reducing inhibition of PI-PLC.

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