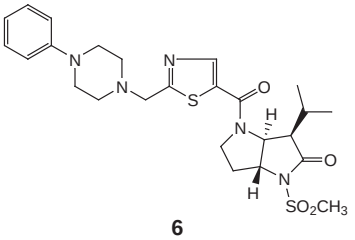
Mycobacterium tuberculosis and other atypical mycobacteria.

Leukocyte elastase and macrophage metalloelastase inhibitors

The destruction of the alveolar walls by leukocytes as part of the inflammatory process in the lung has been long associated with the development of pulmonary emphysema particularly in smokers. The destruction of the alveolar cell walls has been traditionally associated with the degradation of the fibrous protein elastin by human leukocyte elastase. However, recent evidence has suggested that tissue macrophages might play a role in this process as these cells have a greater lifetime in the tissue, and macrophage metalloelastases have been shown to be more effective at degrading elastin than leukocyte elastase. Inhibitors of both these enzymes could therefore have a role in the treatment of pulmonary emphysema.

The therapeutic potential of leukocyte elastase and macrophage metalloelastase inhibitors in this field has been reviewed in an extensive article by Skiles, J.W. and Jeng, A.Y. [*Expert Opin. Ther. Pat.* (1999) 9, 869–895]. This review describes the array of compounds described in the conventional and patent literature over recent years that have been shown to be effective inhibitors of both these enzymes. Some of the most interesting compounds highlighted by this review are those leukocyte elastase inhibitors, such as (**6**), recently patented by Glaxo Group Ltd. Some of these agents have been shown to have an effective oral dose of less than 10 mg kg^{-1} and provide a duration of action of at least six hours in an *in vivo* hamster assay.



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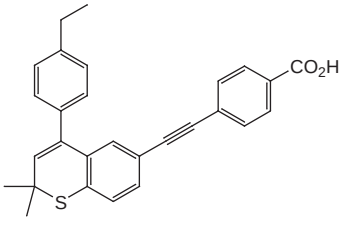
High-affinity retinoic acid receptor-antagonists

Recent retinoid research has demonstrated the importance of these molecules in the control of normal cellular processes. Retinoids function as modulators of gene transcription both in embryogenesis and in the subsequent maintenance of cellular functions such as proliferation and differentiation. There are two known retinoid receptors, the retinoic acid receptors and the retinoid X-receptors. The natural ligands for these receptors differ in their geometric isomeric forms with all-*trans* retinoic acid being the endogenous ligand for the retinoic acid receptors and the 9-*cis*-retinoic acid the proposed endogenous ligand for the retinoid X-receptors.

Retinoic acid receptor-antagonists have potential uses in the prevention of retinoid-induced toxicity caused by systemic retinoid treatment such as Accutane™. As part of a programme of research into the development of novel retinoid receptor ligands, workers at Allergan Inc. (Irvine, CA, USA) have synthesized and evaluated a range of novel retinoic acid receptor antagonists [*Bioorg. Med. Chem.* (1999) 7, 1321–1338]. Binding, transcriptional and *in vivo* assays have identified the 2,2-dimethylthiochromene analogue (**7**) as a potential antidote for the treatment of retinoid-induced activity. This compound is presently in preclinical development as a topical agent for the treatment and prevention of mucocutaneous toxicity caused by systemic retinoids.



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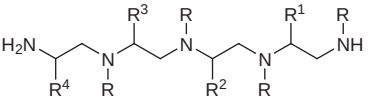
Combinatorial chemistry

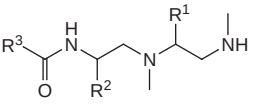
Novel antitumour agents

A new strategy has been described that combines the combinatorial synthesis of several libraries and the testing of these compounds against 60 different cell-based antitumour screens at the National Cancer Institute (Bethesda, MD, USA) [Appel, J.R. *et al.* (1999) *Mol. Divers.* 4, 91–102]. Five different combinatorial libraries consisting of peptides, peptidomimetics, polyamines or small molecules were initially prepared and tested against three representative cell lines to identify the most active library types. Following this investigation, the search was narrowed down to two libraries based on *N*-perbenzylated pentamine structures (**1**) and *N*-acylated permethylated triamines (**2**).

The libraries were prepared on solid support using the 'tea-bag' method for simultaneous multiple synthesis. The



1 R = benzyl



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mixtures were generated by using a range of building blocks in proportions previously shown to yield equimolar ratios of products. The *N*-perbenzylated pentamines were constructed from a total of 52 building blocks, giving a final library size of 7,311,616 compounds. The *N*-acylated permethylated triamines also used 52 monomers to give a library size of 454,272 compounds. In each case, the synthetic route employed a borane reduction to reduce peptide intermediates to polyamines.

Active compounds were tested in mice to determine the maximum tolerated dose, followed by screening against

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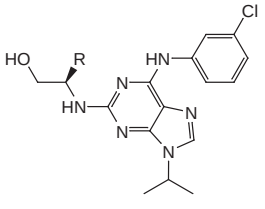
human tumour cells cultivated in hollow fibres. Three compounds have been identified that are currently being evaluated in human tumour xenografts.

Purine CDK inhibitors

Purines occur at relatively high concentrations in all living organisms where they play critical roles as cofactors and signalling molecules in modulating protein function. Furthermore, purines have been a fruitful source of cyclin-dependent kinase (CDK) inhibitors, targets that are especially attractive because of their key role in regulating the cell cycle. A recent publication describes the use of both solution- and solid-phase methods for the synthesis of purine-based libraries and the results of screening these compounds against CDK [Chang, Y-T. *et al.* (1999) *Chem. Biol.* 6, 361–375].

Supporting purine precursors on solid-phase allowed the combinatorial variation of two substituent positions. The effects of substituents in the 2-, 6- and 9-positions are additive and the results of screening the binary libraries against starfish oocyte CDK1–cyclin B enabled the identification of potent trisubstituted purine products. The most active compounds were subsequently tested for their ability to inhibit growth of U937 human leukaemia cells. In general, the cell-based IC_{50} values were higher than the *in vitro* values, presumably as a result of competition with high concentrations of intracellular ATP.



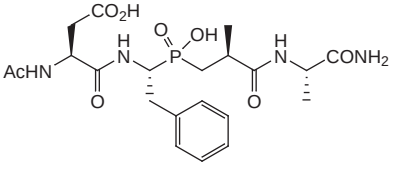
3 R = isopropyl
4 R = H

From the several hundred compounds prepared, four highly specific and potent CDK inhibitors were identified. These various purines act selectively on different biochemical pathways affecting cell-cycle progression. For example, compound (**3**) arrests the cell cycle specifically in the G2-phase whereas compound (**4**) induces M-phase arrest.

Selective ACE inhibitors

Inhibitors of angiotensin converting enzyme (ACE) have been employed for many years as effective treatments for hypertension, cardiac failure and diabetic nephropathy. These drugs block the renin-angiotensin cascade and prevent the formation of the hypertensive peptide, angiotensin II. Recently, it has been shown that the two catalytic domains of ACE might have slightly different functions. Evidence suggests that the *N*-domain might be responsible for the breakdown of peptides such as acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), a negative regulator of haematopoietic stem cell differentiation and proliferation.

To help define the distinct roles of the two active sites, combinatorial chemistry has been used to identify compounds that selectively inhibit the *N*-domain site [Dive, V. *et al.* (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 4330–4335]. A phosphinic peptide library has been used to identify a compound (**5**) that can differentiate the two ACE active sites



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(*N*-domain site K_i = 12 nM, *C*-domain site K_i = 25 μM). Further studies with this compound might reveal the contribution of the ACE *N*-domain active site to the breakdown of AcSDKP.

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Bioinformatics

Rapid searching of sequence databases

Searching sequence databases is one of the most common tasks for any scientist with a newly discovered protein or nucleic acid sequence and is used to determine or infer:

- If the sequence has been found and already exits in a database
- The structure (secondary and tertiary)
- Its function or chemical mechanism
- The presence of an active site, ligand-binding site or reaction site
- Evolutionary relationships (homology).

Sequence database searching is different from database interrogation searching. Generally, sequence searching involves searching for a sequence in a database of sequences. By contrast, interrogation searching involves searching for keywords or other text in the text information (labelled the 'header') associated with each sequence in a database (SRS at EMBL http://www.emblheidelberg.de/srs/srsc is an example of a database interrogation program).

When comparing just two sequences, rigorous pairwise alignment algorithms can be used such as the Needleman and Wunsch algorithm [Needleman, S.B. and Wunsch, C. (1970) *J. Mol. Biol.* 48, 443–453] for optimal global alignments, and the Smith and Waterman algorithm [Smith, T.F. and Waterman, M.S. (1981) *J. Mol. Biol.* 147, 195–197] for optimal local alignments.

However, these two algorithms are computationally intensive and usually much too slow (with single central processing unit computer architecture) for searching a large database of sequences with a query (or unknown) sequence. Computers using parallel multi-processors can run these programs faster (see Box 1), but these computer architectures are expensive. Other algorithms, however, which employ heuristics (which essentially means they use some sort of assump-

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