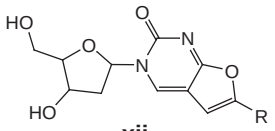
subtype-selective ligands with modifications at the N^5-, N^7- and N^8- positions, enabling modulation of both affinity and selectivity for these different subtypes.

7 Baraldi, P.G. *et al.* (1999) Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine derivatives as highly potent and selective human A_3 adenosine receptor antagonists. *J. Med. Chem.* 42, 4473–4478

Varicella-zoster virus inhibitor

Workers from the Welsh School of Pharmacy, University of Wales College of Cardiff (Cardiff, UK) have recently reported a new category of potent antiviral agents based on novel deoxynucleoside analogues with unusual bicyclic base moieties exemplified by (**xii**)[8].



xii

These compounds are potent and selective inhibitors of varicella-zoster virus (VZV) *in vitro*. Optimal activity requires a long alkyl side chain of C8–C10 giving EC_{50} values against VZV of 3–9 nM. These compounds are 300-fold more potent against VZV than acyclovir and show little or no *in vitro* cytotoxicity. The ease of synthesis of these novel compounds, coupled with their excellent antiviral activity, makes them particularly interesting lead structures for the future development of antiviral agents.

8 McGuigan, C. *et al.* (1999) Potent and selective inhibition of varicella-zoster (VZV) by nucleoside analogues with an unusual bicyclic base. *J. Med. Chem.* 42, 4479–4484

Antitumour therapy: Patent focus

Ecker G. has provided an interesting patent focus on agents for antitumour therapy[9]. The paper highlights the most interesting patent disclosures in the field of antitumour therapy for the period of May to October 1999. The review highlights the focus on inhibitors of signal induction pathways, particularly inhibitors of tyrosine, serine/threonine and cyclic-dependent kinases and RAS-farnesyltransferases, inhibitors of adhesion and angiogenesis, and novel peptides for breast cancer therapy and diagnostics. The most interesting approach to cancer chemotherapy is modified antibody direct enzyme prodrug therapy (ADEPT) using cyclodextrins for detoxification to reduce side effects. Although multi-drug resistance is recognized as an increasing barrier to effective anticancer therapy, only two patent applications in the past six months address this issue. This review provides a useful patent-focused review for anyone undertaking an initial analysis of this field.

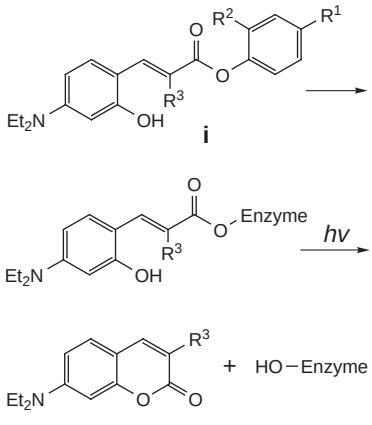
9 Ecker, G. (1999) Patent focus on agents for tumour therapy: May–October 1999. *Exp. Opin. Ther. Patents* 9, 1627–1639

Andrew Lloyd

Combinatorial chemistry

Separation of serine proteases

A strategy that enables the modification of the catalytic residue of serine proteases and their subsequent light-catalyzed reactivation has been described (**Scheme I**)[1]. The *o*-hydroxycinnamate structures (**i**) were prepared by either solution- or solid-phase techniques and react specifically with the key serine hydroxy-group of two of the coagulation cascade enzymes, thrombin and factor Xa.



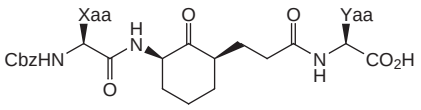
Scheme I

In particular, the side-chain R^2 can be modified to enhance selectivity for serine proteases of interest. A library of 112 compounds has been prepared using different dipeptide residues in this side-chain, and selective inhibitors for thrombin and factor Xa isolated. With a biotin derivative in the R^3 position, an avidin column can be used to selectively capture either of the enzymes. A judicious use of this inhibition strategy has enabled the isolation of each enzyme from a mixture of the two.

1 Porter, N.A. *et al.* (1999) Selective inhibition, separation, and purification of serine proteases: A strategy based on a photoremovable inhibitor. *J. Am. Chem. Soc.* 121, 7716–7717

Library of serine and cysteine protease inhibitors

A combinatorial library of 400 compounds based on the cyclohexanone pharmacophore (**ii**) has been prepared



ii

and used to find selective inhibitors of several serine and cysteine protease inhibitors[2]. This pharmacophore allows derivatization in two directions, enabling the introduction of side chains that mimic the S2 (Xaa) and S2′ (Yaa) residues of the enzyme's natural peptide substrates.

The library was prepared using the 'split synthesis' approach, with the 20 naturally occurring amino acids but replacing cysteine and methionine with hydroxyproline and ornithine. The library was subsequently screened against cathepsin B, plasmin, urokinase,

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kallikrein and papain, all but papain being implicated in the progression of cancer. Among the many active compounds detected following iterative deconvolution of the library, one component (Xaa = Trp, Yaa = Trp; 5μM) was found to be an inhibitor of the serine protease plasmin.

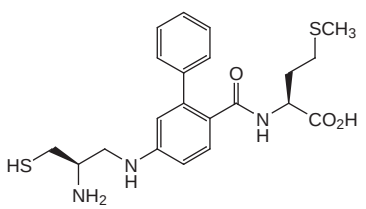
The data demonstrated that for plasmin, the S2′ subsite preferentially binds hydrophobic and, especially, aromatic amino acids. By contrast, the S2′ subsites of cathepsin B and papain do not appear to have strong preferences for any particular amino acid.

2 Abato, P. *et al.* (1999) Combinatorial library of serine and cysteine protease inhibitors that interact with both the S and S′ binding sites. *J. Med. Chem.* 42, 4001–4009

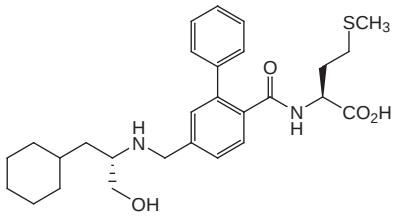
Protein farnesyltransferase inhibitors

The GTPase, RAS p21, is a molecular switch that fulfils a key function in cellular message transduction. Many human tumours, including lung, colon and pancreatic cancers, appear to contain a RAS mutant that is permanently in the 'on' state. As prenylation, catalyzed by protein farnesyltransferase (FTase), is an essential RAS post-translational modification, there has been much interest in the discovery of inhibitors of this enzyme as anticancer agents.

Using known active FTase inhibitor structures, combinatorial library chemistry has been employed in the discovery of novel and more potent inhibitors[3]. Using the known inhibitor, FTI276 (**iii**) as a starting point, a library of benzylic amines has been prepared via the reductive amination of a resin-bound aldehydic intermediate, followed by trifluoroacetic acid (TFA)-catalyzed cleavage from the resin. The preferred compound from this series (**iv**) is an inhibitor of FTase at nanomolar concentrations. Orthodox solution-phase medicinal chemistry has been employed to generate potent analogues that attenuate tumour growth in a nude mouse xenograft model of human pancreatic cancer.



iii



iv

3 Henry, K.J. *et al.* (1999) Discovery of a series of cyclohexylethylamine-containing protein farnesyltransferase inhibitors exhibiting potent cellular activity. *J. Med. Chem.* 42, 4844–4852

Nick Terrett
Discovery Chemistry
Pfizer Central Research
Sandwich, Kent, UK
fax: +44 1304 655419
e-mail: nick_terrett@sandwich.pfizer.com

A genome analysis production line

Rational approaches to speed up the drug discovery process using bioinformatic analyses of genomic DNA are intriguing, as they inherently deliver all the targets with all their associated information. Hence, an assay developer is able to select bioinformatically proposed targets that might not have become obvious from classical experiments.

Automated strategy of Directed Minimal Sequencing

As the time and cost pressures for genomic sequence analysis rapidly increase, Lion Biosciences AG (Heidelberg, Germany) has automated crucial process steps in their Directed Minimal Sequencing strategy. This approach sequences small genomic DNA fragments, which are pre-ordered by high-throughput hybridization. A 'minimal tiling path' is selected from a clone map to generate a sequence scan of the genome or a genomic region at homogeneous minimum coverage. On demand, a patent-quality sequence can be obtained, with bioinformatic analyses predicting potentially interesting loci, and the overall sequencing being reduced by >80%. Sequencing can be further reduced if, for example, a cDNA of interest is already known and can be hybridized to the clone map. Hence, the clones representing the exons and the regions in-between can be selected for directed genomic gene sequencing.

Advantages

By comparison with the classical shotgun sequencing, described by Fleischmann R.D. and coworkers[1], the Directed Minimal Sequencing strategy is suggested to have three major technical advantages.

Firstly, accurate (99.5%, mostly double-stranded) contiguous genomic sequences are produced online, independent of the genome size. Hence, the genomic DNA structure can be analyzed and gene-finding programs activated immediately after the first sequences are obtained.

Secondly, integration with automated genome assembly and functional whole-genome annotation (bioSCOUT™) enables the maximum utilization of the minimal sequencing results with little human intervention: assembly is reduced to a 'click' of the mouse, as clones have already been assembled in the mapping process. Optional finishing is performed on clones selected from the present maps instead of using PCR or additional backbone libraries. Open reading frames are automatically predicted and annotated.

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