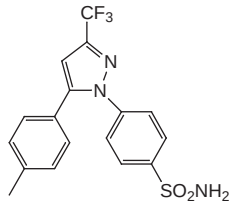
Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

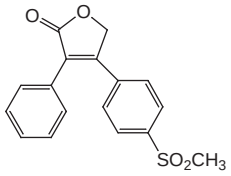
Molecules

Cyclooxygenase inhibitors

As highlighted in *Monitor* last month, there is considerable interest in the therapeutic potential of cyclooxygenase-2 (COX-2) inhibitors as non-steroidal anti-inflammatory drugs with reduced side effects, such as gastrointestinal toxicity[1]. Two compounds in this class, celecoxib (**i**) and rofecoxib (**ii**), have recently been approved by

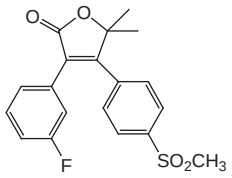


i



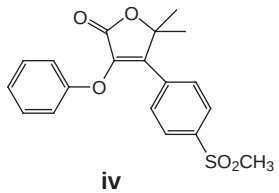
ii

the Food and Drug Administration (FDA). Workers at the Merck Frost Centre for Therapeutic Research (Quebec, Canada) have previously reported an analogue of rofecoxib, 5,5-dimethyl-3-(3-fluorophenyl)-4 -(4-methanesulfonylphenyl)-2(5*H*)-furanone (**iii**), as a highly potent and selective COX-2 inhibitor[2].



iii

A recent paper from the group has evaluated a range of spacer units inserted between the 3-fluorophenyl and lactone ring of (**iii**)[3], including -O-, -S-, -NH-, -CH_2- and -CO-. Their evaluation in whole cell assays using either transfected Chinese hamster ovary (CHO) cells expressing human COX-1 and COX-2 or human whole blood expressing COX-1 and COX-2, identified the oxygen linker as having the greatest COX-2-inhibiting potency (**iv**). Synthesis of a range of analogues of (**iv**) led to the identification of (**v**), which has potent *in vivo* efficacy in the rat in a paw oedema assay (ED_{50} = 0.1 mg kg^{-1}), a pyresis assay (ED_{50} = 0.5 mg kg^{-1}), a hyperalgesia assay (ED_{50} = 1.3 mg kg^{-1}) and an adjuvant arthritis assay (ED_{50} = 0.2 mg kg^{-1}). Furthermore,



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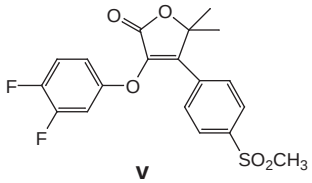
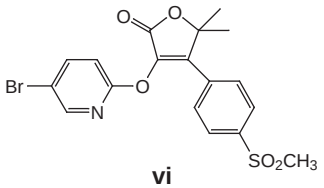


v

gastrointestinal examination using the rat ^{51}Cr assay showed no loss of gastrointestinal integrity with 100 mg kg^{-1} twice-a-day dosing for five days.

In a second paper, this group describe a further diversification of the furanone template through the synthesis and evaluation of a series of O-linked heterocycles[4]. Using the same assays, they identified (**vi**) as a potent, orally active and selective COX-2 inhibitor that has no ulcerogenic effect at 100-times



vi

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the dose required for anti-inflammatory, analgesic and antipyretic activity.

1 Ho, L. and Pasinetti, G.M. (2000) The potential of specific COX-2 inhibition. *Drug Discovery Today* 5, 88–89

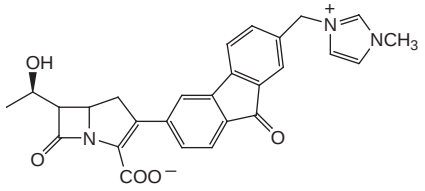
2 Riendeau, D. *et al.* (1997) Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. *Br. J. Pharmacol.* 121, 105–117

3 Li, C-S. *et al.* (1999) A new structural variation on the methane sulfonylphenyl class of selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* 9, 3181–3186

4 Lau, C.K. *et al.* (1999) Synthesis and biological evaluation of 3-heteroaryloxy-4-phenyl-2-(5*H*)-furanones as selective COX-2 inhibitors. *Bioorg. Med. Chem. Lett.* 9, 3187–3192

Potent anti-MRS agents

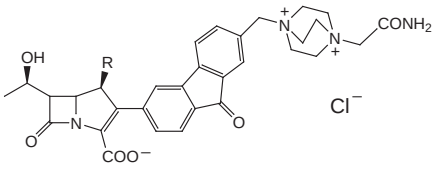
Methicillin-resistant staphylococci (MRS) infections continue to be of concern worldwide. These organisms display multiple resistance to the most common antibiotics including β-lactams. Of particular concern are the recent reports of vancomycin-resistant MRS. Merck Research Laboratories (Rathway, NJ, USA) have previously reported the discovery of 2-metabiphenylcarbopenems with potent activity against MRS both *in vitro* and *in vivo*[5]. Although these agents [such as L695256 (**vii**)] have



vii

potent activity, their development has been hindered by low aqueous solubility. Hence, the group has recently investigated the replacement of the methylimidazolium group with a dicationic substituent[6].

Several cationic groups were examined and it was found that a dicationic moiety derived from 1,4-diazabicyclo [2,2,2]octane produced compounds with the most favourable properties: showing acceptable aqueous solubility whilst maintaining activity against MRS. A structure–activity study led to the identification of L741462 (**viii**) as a water-soluble agent with a similar activity against MRS as vancomycin. Improved pharmacokinetics were subsequently attained by introducing a 1-β-methyl substituent to produce L742728 (**ix**). Unfortunately, the



viii R = H
ix R = CH_3

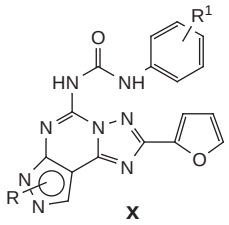
company has been unable to pursue the development of L42728 as a consequence of immuno-based toxicity. However, these studies will provide valuable information for the future development of similar antibiotics with appropriate aqueous solubility.

5 Chambers, H.F. (1995) *In vitro* and *in vivo* antistaphylococcal activities of L-695,256, a carbapenem with high affinity for the penicillin-binding protein PBP 2A. *Antimicrob. Agents Chemother.* 39, 462–466

6 Greenlee, M.L. *et al.* (1999) Dicationic 2-fluorenonylcarpenems: Potent anti-MRS agents with improved solubility and pharmacokinetic properties. *Bioorg. Med. Chem. Lett.* 9, 3225–3230

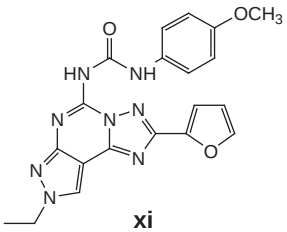
Adenosine A_3-receptor antagonists

Activation of the adenosine A_3 receptor modulates secondary messenger responses within cells and triggers the release of inflammatory agents such as histamine from mast cells. Animal model studies also suggest that A_3 receptors might play a role in brain ischaemia, immunosuppression and bronchospasm. These results suggest that A_3-receptor antagonists could be useful in the treatment of asthma and inflammation. Previous work in this field has primarily focused on four types of non-xanthine structures: dihydropyridine and pyridine analogues, flavanoids, isoquinolines and triazoloquinaxoline derivatives. In a recent paper, Baraldi, P.G. and coworkers described a novel class of highly potent and selective human A_3-receptor antagonists based on pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives (**x**)[7].



x

Structure–activity studies confirmed that a substituted phenylcarbamoyl moiety confers affinity and selectivity for the A_3 receptor, as the unsubstituted N^5-derivative had no selectivity for the human A_3 receptor but displayed high affinity for the A_1- and/or A_{2A}-receptor subtypes. Alkyl substitution at the N^δ-position enhanced both the affinity and selectivity for the A_3-receptor subtype and led to the identification of a potent and selective A_3-receptor antagonist (**xi**) (K_i = 0.28 nM; $rA_1/hA_3 > 35000$; $rA_{2A}/hA_3 > 35000$; $hA_1/hA_3 > 3600$; $hA_{2A}/hA_3 > 3600$).



xi

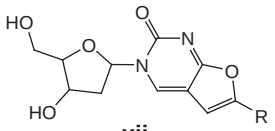
These results also indicate that the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine nucleus might be a useful template for generating other adenosine receptor

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.

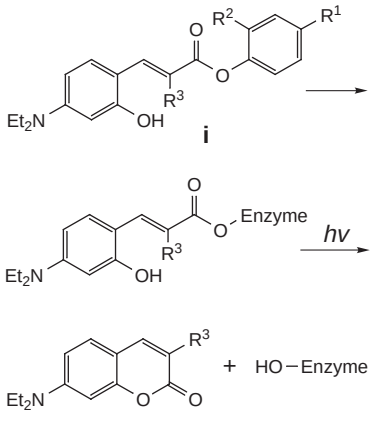
9 Ecker, G. (1999) Patent focus on agents for tumour therapy: May–October 1999. *Exp. Opin. Ther. Patents* 9, 1627–1639

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.



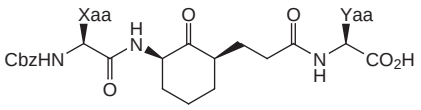
Scheme I

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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