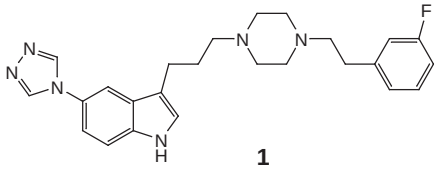
Monitor: molecules and profiles

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.

3-(Piperazinylpropyl)indoles: h5-HT$_{1D}$ receptor agonists

The antimigraine drug, sumatriptan, which has revolutionized the treatment of migrane, exhibits binding affinity for both the h5-HT$_{1D}$ and the h5-HT$_{1B}$ receptors. It is believed that the clinical efficacy of this and related drugs is mediated through either one or both of these receptors. In the search for more effective antimigraine agents, workers from Merck, Sharp & Dohme Research Laboratories (Harlow, UK) have synthesized and evaluated a novel series of 3-(piperazinylpropyl)indoles as potential h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor ligands [Chambers, M.S. *et al.* (1999) *J. Med. Chem.* 42, 691–705].

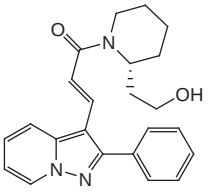


1

This class of compounds has been shown to have subnanomolar affinity as an agonist for the h5-HT$_{1D}$ receptor, with a 200-fold selectivity for this receptor subtype over the h5-HT$_{1B}$ receptor. The optimal compound was shown to be 1-(3-[5-(1,2,4-triazol-4-yl)-1*H*-indol-3-yl]propyl)-4-(2-(3-fluorophenyl) ethyl)piperazine (**1**), with a high selectivity for the h5-HT$_{1D}$ receptor over the h5-HT$_{1B}$ receptor and good oral bioavailability in the rat [27%, (t_{max} 2 h)], dog (25%) and rhesus monkey (24%). This compound is presently undergoing further evaluation as a potential development candidate.

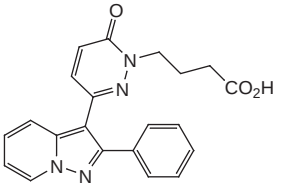
Novel non-xanthine adenosine A$_1$ receptor antagonist

The purine nucleoside, adenosine, exerts a wide range of physiological functions through interaction with the four distinct adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3). Although many adenosine receptor agonists and antagonists have been studied, the only clinically approved compound is adenosine itself, and is used for the treatment and diagnosis of cardiac arrhythmias and the diagnosis of ischemic heart disease. Other compounds have generally failed as a consequence of their poor bioavailability, which is due to hydrophobicity and limited water solubility. A recent review by Baraldi, P.G. and coworkers provides a useful overview of the known A_1 receptor agonists [*Expert Opin. Ther. Pat.* (1999) 9, 515–527].



2

There is also considerable interest in the identification of selective, potent and bioavailable non-xanthine adenosine receptor agonists and antagonists. FK453 (**2**) had previously been shown to be an A_1 receptor antagonist with potent diuretic and renal vasodilatory activity but poor oral bioavailability as a consequence of rapid first-pass metabolism.



3

Akahane, A. and coworkers have recently reported the design and synthesis of a novel series of pyrazolo[1,5-*a*]pyridines using the X-ray crystal

Monitor Editor: **Andrew W. Lloyd**, School of Pharmacy and Biomolecular Sciences, University of Brighton, Cockcroft Building, Moulsecoomb, Brighton, UK BN2 4GJ. tel: +44 1273 642049, fax: +44 1273 679333, e-mail: a.w.lloyd@brighton.ac.uk

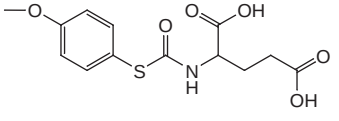
 1359-6446/99/$ – see front matter PII: S1359-6446(99)01370-7

structure of FK453 as a template [*J. Med. Chem.* (1999) 42, 779–783]. The most potent of these compounds (**3**) was shown to have a high affinity for the A_1 receptor, with good oral bioavailability and water solubility. This compound is presently undergoing phase II clinical trials as a diuretic antihypertensive agent.

Novel inhibitors of carboxypeptidase G_2: applications in ADEPT therapy

The recently developed technique of antibody-direct enzyme prodrug therapy (ADEPT) offers a powerful means of cancer treatment through the specific delivery of cytotoxic agents to targeted tumours. This technique involves the initial administration of a tumour-specific antibody–enzyme complex. Following plasma clearance of the excess conjugate, a prodrug specific for the targeted enzyme is administered, which leads to the generation of the cytotoxic agent solely at the tumour site. However, this therapy is often compromised by nonspecific enzyme activity leading to myelosuppression.

One strategy that has been successfully employed to overcome this problem involves the use of a galactosylated antibody directed towards the enzyme component of the antibody–enzyme conjugate. This enhances both the clearance of the free antibody–enzyme conjugate and the deactivation of any circulating enzyme.



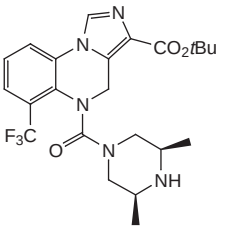
4

An alternative approach has recently been described by Khan, T.H. and coworkers. This group has investigated the potential use of small-molecule enzyme-inactivating compounds for the carboxypeptidase G_2 (CPG_2) [*J. Med. Chem.* (1999) 42, 951–956]. The group initially synthesized a range of thiocarbamates as potent inhibitors of CPG_2. The most potent inhibitor, *N*-(*p*-methoxybenzenethiocarbonyl)amino-L-glutamic acid (**4**), was used in a preliminary investigation of the ability to abrogate the cytotoxicity of a combination of the A5B7–CPG_2 conjugate and the prodrug *N*-*p*-[*N*,*N*-bis(2-chloroethyl)amino]phenoxycarbonyl-L-glutamate (PGP) against LS174T cells. The results suggest that this novel noncompetitive inhibitor of CPG_2 is an ideal candidate for assessing whether small-molecule inhibitors can be used as an enzyme-clearing strategy in ADEPT.

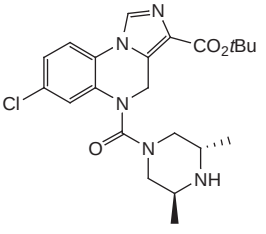
High-affinity $GABA_A$ receptor ligands

γ-Aminobutyric acid (GABA) acts on the $GABA_A$ chloride ion channel controlling excitation of many CNS pathways. Various classes of compounds, such as the benzodiazepines, neurosteroids and barbiturates, interact with this macromolecular ionophore complex which comprises several different receptor subunits (α,β,γ,δ). There is considerable interest in the development of specific ligands that will interact selectively with the different $GABA_A$ receptor subtypes. Such agents might allow discrimination between the useful anxiolytic and hypnotic effects and the undesirable side-effects that are often reported for existing $GABA_A$ agonists, partial agonists, inverse agonists and antagonists.

A recent paper from Upjohn Laboratories (MI, USA) describes the development of a series of imidazo [1,5-*a*]quinoxaline piperizine ureas [Jacobsen, E.J. *et al.* (1999) *J. Med. Chem.* 42, 1123–1144]. Specific compounds from this group were found to have high affinity for the $GABA_A$/benzodiazepine receptor complex with efficacies ranging from full agonists to inverse agonists. Of particular interest are the compounds found to be partial agonists which have been shown to have an unusual bell-shaped dose-response profile.



5



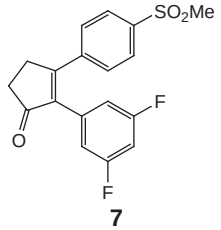
6

Many of these compounds were found to have pharmacological profiles consistent with anticonvulsant and anxiolytic-like activity. The group identified two orally active partial agonists (**5**,**6**) with good activity in anxiolytic models, acceptable pharmacokinetics and minimal benzodiazepine-type side-effects.

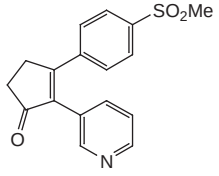
Selective cyclooxygenase-2 inhibitors

As has been reported previously in this column, the identification of two isoforms of the cyclooxygenase enzyme (COX-1 and COX-2) has stimulated considerable interest in the development of ligands that are selective for either isoform. COX-1 is usually constitutively produced in a range of tissues and is important in the normal homeostatic control of, for example, gastric cytoprotection. COX-2 is usually induced by inflammatory stimuli, and is therefore thought to be responsible for prostanoid production associated with inflammation. COX-2-specific inhibitors might therefore have useful

anti-inflammatory activity without the ulcerogenic side-effects often associated with current non-steroidal anti-inflammatory agents.



7



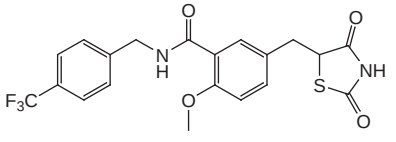
8

A recent paper from Merck Research Laboratories (NJ, USA) describes the synthesis and evaluation of cyclopentenones containing a 4-(methylsulfonyl)phenyl group in the 3-position and a phenyl ring in the 2-position as selective inhibitors of COX-2 [Black, W.C. *et al.* (1999) *J. Med. Chem.* 42, 1274–1281]. Through iterative structural optimization, the group have identified two compounds (**7**,**8**) as potential anti-inflammatory agents. These agents have been shown to have good oral bioavailability and efficacy in rat models of pain, fever and inflammation with reduced gastrointestinal side-effects.

(3-substituted benzyl)thiazolidine-2,4-diones: new antihyperglycaemic agents

Non-insulin-dependent diabetes mellitus (type 2 diabetes) is often associated with obesity, hypertension and hyperlipidaemia in middle age. Evidence suggests that this metabolic disorder, characterized by hyperglycaemia and/or insulin resistance, requires tight control of blood glucose levels in the early stages of the disease. However, current therapies often fail as a consequence of poor compliance, efficacy and hypoglycaemic responses. Therefore, there is still a need for an effective, orally active agent that can be used to control both insulin and glucose levels.

A recent publication from Kyorin Pharmaceutical Co. Ltd (Tochigi, Japan) has described the synthesis of a series of 3-[2,4-(dioxothiazolidin-5-yl)methyl] benzamide analogues as part of a programme to identify novel antidiabetic agents [Nomura, M. *et al.* (1999) *Bioorg. Med. Chem. Lett.* 9, 533–538]. 5-[(2,4-(dioxothiazolidin-5-yl)methyl]-2-methoxy-*N*-[[4-trifluoromethyl)phenyl]methyl]-benzamide (**9**) was identified as a suitable lead compound for further evaluation for the treatment of diabetes mellitus.

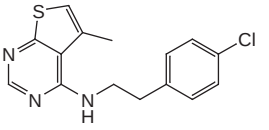


9

This compound showed potent antihyperglycaemic, antihyperinsulinaemic and antihyperlipidaemic activity on oral administration to mice at doses of less than 1 mg kg^{-1} day^{-1}. Further studies have demonstrated that this agent activates both the peroxisome proliferator-activated receptor (PPAR) α and γ isoforms (K_d = 228 nM and 326 nM, respectively). As classical thiazolidine-2,4-diones specifically bind to and activate the PPARγ isoform, which is known to be responsible for the regulation of adipocyte differentiation, the enhanced pharmacological activity of (**9**) might be due to its ability to bind to both isoforms.

Novel anti-osteoporosis agent

Osteoclasts play a major role in bone resorption and are therefore a primary target in the treatment of osteoporosis. A recent study by Katada, J. and coworkers describes the synthesis and screening of a new series of thienopyrimidines for cytotoxic activity against haematopoietic-derived leukaemia cell lines [*Bioorg. Med. Chem. Lett.* (1999) 9, 797–802]. These agents were found to be active against P388 cells and J774 cells but not cytotoxic to non-haematopoietic adhesive cells (ECV-304 and A437 cell lines).



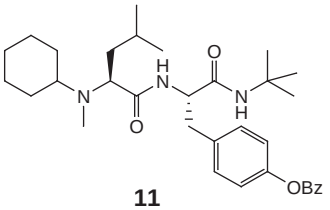
10

Osteocytes are also derived from haematopoietic precursor cells and investigation of the activity of the thienopyrimidines against this cell type identified compound (**10**) as having submicromolar cytotoxic activity. Further studies have demonstrated that this compound is also able to reduce bone resorption *in vitro* and might therefore be a useful lead for the future development of anti-osteoporosis agents.

N-type calcium channel blockers

Intracellular calcium concentrations, regulated by voltage-sensitive calcium channels, affect various important neuronal functions including cellular excitability, neurotransmitter release, intracellular metabolism and gene expression. The neuronal voltage-sensitive calcium channels (N-type) are classified into six subtypes that differ in protein structure, function, conductance, activation/inactivation and sensitivity to drugs and toxins. The N-type calcium channels, associated with the presynaptic terminals of central and peripheral neurones, regulate calcium flux associated with depolarization-evoked release of transmitters from synaptic endings. Existing N-type calcium channel blockers have been shown to be efficacious in the treatment of traumatic brain injury, focal cerebral ischaemia and pain in animal models.

As part of a programme to identify small-molecule N-type calcium channel blockers for therapeutic use in the



11

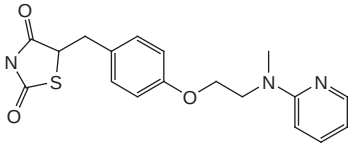
treatment of stroke and pain, Hu, L-Y. and coworkers have identified a series of *N,N*-dialkyl-dipeptidylamines, exemplified by (**11**), with potent functional activity at N-type voltage-sensitive calcium channels. These compounds have also been shown to possess efficacy in the audiogenic-seizure mouse model. Structure–activity studies have demonstrated that the N-type calcium channel blocking activity correlates with lipophilicity and the molecular size of the *N*-substituents.

Combinatorial chemistry

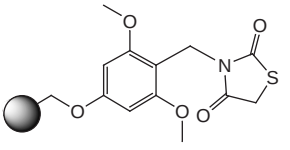
Solid-phase synthesis of BRL49653

Obesity is an increasingly common disease in the western world and incidence has been associated with a range of other diseases including hypertension, atherosclerosis and type 2 (non-insulin dependent) diabetes. However, little is known about the proteins that mediate gene control of lipid metabolism. One possible clue is the class of peroxisome proliferator activated receptors (PPARs), a family of nuclear receptors that respond to long-chain fatty acids and prostaglandins and that have been a subject of this column in previous months. Further study of this family of receptors might yield vital clues in the understanding of the signalling pathways that regulate energy balance.

One agent known to bind to the mPPARg is the antidiabetic thiazolidinedione, BRL49653 (**1**), and it was judged that a common route to the synthesis of analogues might help clarify an understanding of the role of PPARs. A recent paper describes the solid-phase synthesis of BRL49653 and indicates how this route might be used for the preparation of other analogues [Brummond, K.M. and Lu, J. (1999) *J. Org. Chem.* 64, 1723–1726].



1



2

The key to the synthesis of BRL49653 was the discovery that the 2,4-thiazolidinedione moiety could be attached to a polystyrene support through the 3,5-dimethoxyphenol linker (**2**), thus allowing final cleavage under mildly acidic conditions. The molecule was built up by Knoevenagel condensation, Mitsunobu chemistry and nucleophilic aromatic substitution. The authors are currently using this synthetic route in the preparation of structurally diversified analogues of BRL49653.

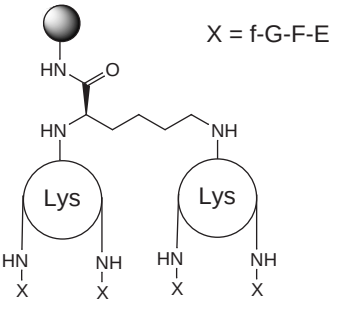
Novel protein kinase inhibitors

Calcium is key to the regulation of a diverse range of cellular functions, often through a calcium receptor protein such as the well-characterized calmodulin (CaM). However, the ubiquity with which CaM functions as a calcium receptor makes it an unattractive molecular target for drug design. A recent paper describes the use of combinatorial chemistry to optimize peptide inhibitors of a protein kinase that clearly do not function through the calcium receptor [Lukas T.J. *et al.* (1999) *J. Med. Chem.* 42, 910–919].

Although myosin light-chain kinase (MLCK) possesses a CaM–calcium-binding region, functional genomic studies were used to identify a lead peptide inhibitor based on a core autoinhibitory sequence. The lead peptide, Arg-Lys-Lys-Tyr-Met-Ala-Arg-Arg-Lys-NH_2, with an IC_{50} value of 1.2 μM, was modified using the mix-and-split library method. As the basic amino acid residues were important for selective inhibition of MLCK, these were held constant and the library focused on variation of the central three residues. Using 18 amino acid residues, the first round of synthesis generated 18 pools, each of 5832 possible peptide sequences. Recursive deconvolution eventually revealed a peptide sequence (Arg-Lys-Lys-Tyr-Lys-Tyr-Arg-Arg-Lys-NH_2) with an IC_{50} value of 50 nM, and 4000-fold selectivity over CaM kinase II, thus demonstrating that this inhibitor has little affinity for the calcium-binding protein. Such inhibitors add to the tools available for the deconvolution of complex signal-transduction pathways.

Inhibition of RAS farnesylation

Much is known about the structure and function of RAS proteins. Although they have a central function in normal cell processes, point mutations activate the oncogenic potential of RAS genes. The proteins undergo a series of post-translational modifications at their carboxy terminals, including farnesylation and subsequent terminal tripeptide cleavage. Oncogenic RAS proteins have been considered as reasonable targets for the discovery of cancer therapeutics, often using the inhibition of farnesyl transferase as a pharmacological



3

1359-6446/99/$ – see front matter PII: S1359-6446(99)01369-0