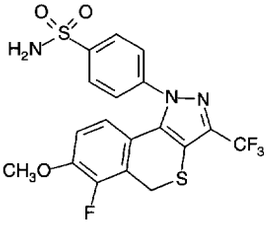
Monitor: molecules and profiles

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, 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, therapeutic advances, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

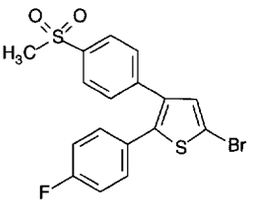
Selective COX-2 inhibitors

The widely used nonsteroidal anti-inflammatory drugs (NSIADs) inhibit the enzyme cyclooxygenase, which converts arachidonic acid into prostaglandins. However, the prostaglandins produced by the constitutive form of cyclooxygenase (COX-1) have important roles in normal platelet, gastric and renal function. As a consequence, inhibition of COX-1 results in undesirable side effects. Recent evidence has suggested that selective inhibitors of the second isoform of cyclooxygenase (COX-2) may offer an advantage over the existing non-selective NSAIDs by reducing the associated renal and gastric toxicity. Bertenshaw, S.R. and coworkers [*Bioorg. Med. Chem. Lett.* (1996) 6, 2827–2830] have described the synthesis and evaluation of series of 1,5-diarylpyrazones containing either a sulphone or sulphonamide moiety as COX-1 and COX-2 inhibitors. The series of benzothiopyranopyrazoles, exemplified by **1**, were found to be both selective COX-2 inhibitors *in vitro* and antiinflammatory agents *in vivo* in the air-pouch model of inflammation.



1

In a recent paper, a group from DuPont Merck (Wilmington, DE, USA) has described another new class of COX-2 inhibitors, the terphenyls, which they discovered while seeking to improve the *in vitro* selectivity of their COX-2 selective inhibitor, diarylthiophen **2** (DuP697) [Pinto, D.J.P. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 2907–2912]. The terphenyl compound **3** was identified as a potential lead compound having good COX-2 selectivity and a better pharmacokinetic profile than DuP697.



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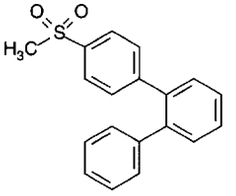
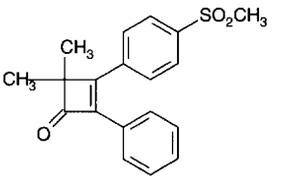


3

Another group [Friesen, R.W. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 2677–2682] has evaluated a series of novel 1,2-diarylcyclobutenes as potential selective COX-2 inhibitors. 4,4-Dimethyl-2-phenyl-3-[4-(methylsulphonyl)phenyl]cyclobutenone (**4**) was shown to be particularly selective for COX-2 and orally active in the rat paw oedema model (ED_{50} = 2.4 mg/kg).



4

AMPA receptor antagonists

AMPA receptor antagonists have potential use in the treatment of pathophysiological disorders including stroke and epilepsy in which the ionotropic glutamate receptors, such as the NMDA and AMPA receptors, are excessively activated. Although some quinoxaline-like AMPA antagonists have been previously identified, the therapeutic use of these agents is often limited by their poor water solubility and poor penetration of the blood–brain barrier, which lead to undesirable side effects. Lubisch, W., Behl, B. and Hofmann, H.P. [*Bioorg. Med. Chem. Lett.* (1996) 6, 2887–2892] have reported the discovery of a series of pyrrolylquinoxalinediones, including **5**. Some of these compounds were shown to have receptor affinities that are comparable with the existing antagonist NBQX *in vitro* and to be equipotent with NBQX in inhibiting AMPA-induced lethal convulsions in mice.

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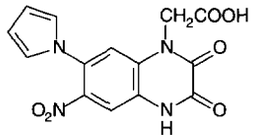
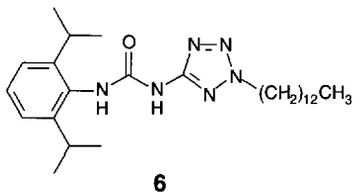


5

Heterocyclic ureas as hypocholesterolaemic agents

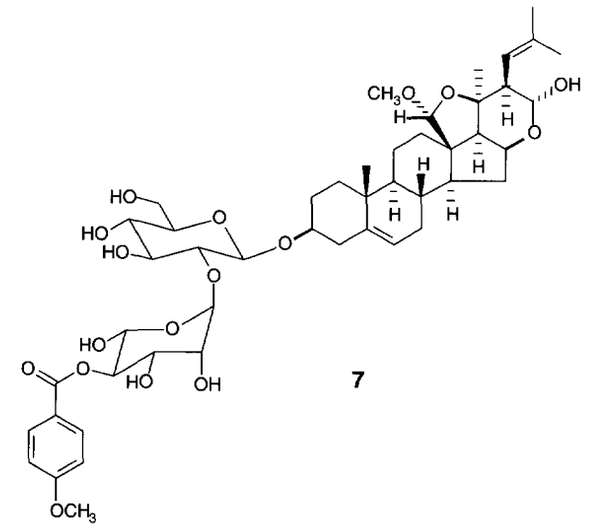
In the search for hypocholesterolaemic agents for the prevention of coronary heart disease in the western world, attention has recently been directed towards inhibitors of the enzyme acyl-CoA:cholesterol *O*-acyltransferase (ACAT). These inhibitors block the intracellular formation of cholesteryl esters and therefore adsorption of cholesterol from the gastrointestinal tract. White, A.D. and coworkers [*J. Med. Chem.* (1996) 39, 4382–4395] have investigated the ability of a series of aryl-substituted heterocyclic ureas to inhibit ACAT *in vitro* and to lower total plasma cholesterol in cholesterol-fed rats and dogs *in vivo*. The most effective compound (**6**) was found to reduce total plasma cholesterol by 67% in the acute cholesterol-fed rat model and by 47% in cholesterol-fed dogs on administration at 3 mg/kg.



6

Novel cytostatic rearranged cholestane glycoside

Mimaki, Y. and coworkers [*Bioorg. Med. Chem. Lett.* (1996) 6, 2635–2638] have described the isolation and characterization of a novel rearranged cholestane glycoside (**7**) from *Ornithogalum saundersiae* (Liliaceae) bulbs, which exhibits potent cytostatic activity against human promyelocytic leukemia HL-60 (IC_{50} = 9.2 nM) and human T-lymphocytic leukemia MOLT-4 cells (IC_{50} = 3.2 nM). Evidence from morphological and DNA fragmentation studies indicate that the cytostatic activity of this compound may be mediated through the induction of apoptosis and suggests that the induction of apoptosis may be an efficient strategy for cancer chemotherapy.



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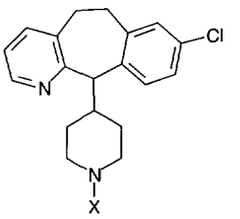
Ras farnesyl protein transferase inhibitors

The p21 Ras proteins play a major role in the control of cell growth and differentiation through intracellular signal transduction. Inactivation of signal transduction is normally mediated by an intrinsic Ras GTPase. However, this enzymatic site is often impaired in Ras mutants leading to unregulated cell proliferation and malignant transformation. Because mutated Ras genes of this nature have been found in a wide variety of cancers, inhibition of Ras-protein-mediated signal transduction represents a possible approach to the development of potential anticancer agents.

For activity the Ras p21 proteins must be bound to the plasma membrane. This membrane binding occurs through a sequence of post-translational modifications including the farnesylation of a cysteine residue on the Ras p21 proteins by farnesyl protein transferase. Farnesyl protein transferase inhibitors may therefore have use as chemotherapeutic agents by preventing the association of the Ras p21 proteins with the cell membrane, thereby blocking the signal transduction pathway.

A group from the Schering-Plough Research Institute (Kenilworth, NJ, USA) have described a new class of tricyclic aminoacetyl and sulphonamide farnesyl protein transferase inhibitors, illustrated by **8** and **9** [Njoroge, F.G. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 2977–2982]. These compounds have submicromolar farnesyl protein transferase activity but do not inhibit the closely related geranylgeranyl protein transferase to any great extent.



8 X= -COR
9 X= $-SO_2R$

HTS in Monitor

From the April issue, a new regular column on high-throughput screening contributed by Dr Mark Rogers (Group Leader, Cellular Bioassay Design, Glaxo Wellcome, Stevenage, UK) will complement our established features on combinatorial chemistry (Dr Nick Terrett, Head of New Leads Chemistry, Pfizer, Sandwich, UK) and emerging molecular targets (Dr Bob Wallace, Wallace & Associates, New Milford, CT, USA).

Combinatorial chemistry

Anticancer agents from OBOC libraries

The one bead–one compound (OBOC) approach to combinatorial library synthesis is a highly effective method for the rapid production of large numbers of compounds. This procedure has been used in the synthesis of a library used in the high-volume cellular screening for cytotoxic anticancer agents [Salmon, S.E. *et al. Molecular Diversity* (1996) 2, 57–63]. The split-synthesis procedure was employed to prepare less than 100,000 compounds in three different libraries: tetrameric and trimeric peptide libraries (using both natural and unnatural amino acids) and a trimeric nonpeptidic library. These compounds were linked to the solid phase through two orthogonally cleavable linkers that could selectively release compounds at different pH.

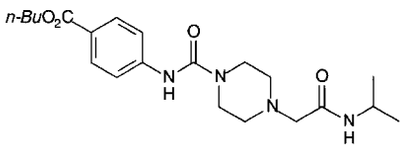
To perform the biological assay, the resin beads were mixed with tumour cells and these were plated in soft-agarose-containing tissue culture medium. At the neutral pH of the tissue culture medium, a 'reverse diketopiperazine' linker permitted release of one aliquot of compound from the beads. Within 48 hours, cytotoxic compounds could be detected by the clear ring of tumour cell lysis surrounding the active beads. The bead thus identified was isolated, the second aliquot of compound cleaved by base-catalysed cleavage of an ester linkage, and the structure determined by either Edman degradation for the peptides or LC-MS for the other structures.

Following screening of the three libraries, a compound with activity against P-388 leukaemia was identified from the tetrameric peptide library. A particular unnatural amino acid in the trimeric peptide library appeared to confer activity against MCF-7 breast cancer and several other solid tumour lines. The nonpeptide library yielded compounds active against 8226 human myeloma cells. No structures are revealed in the paper.

Human rhinovirus inhibitors

Combinatorial chemistry techniques have been used in a search for inhibitors of human rhinovirus, a primary cause of the common cold. Workers from Lilly (Indianapolis, IN, USA) have prepared a library of 4,000 ureas using the solution reaction of amines with an excess of isocyanates [Kaldor, S.W. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 3041–3044]. In particular, they have used functionalized resin beads as 'covalent scavengers' to purify the library products.

The 4,000 ureas were prepared as 400 ten-compound mixtures by reacting 1.25 equivalents of an isocyanate with a limiting amount of an equimolar mixture of ten amines. After completion of the reaction, the excess isocyanate was removed by the addition of aminomethylpolystyrene resin. Filtration to remove the resin and evaporation gave the product mixtures free of isocyanate. The library products were tested for activity against human rhinovirus-14 (HRV-14) in a whole-cell assay, and resynthesis of the constituent components of the most active mixture revealed novel low to submicromolar inhibitors (**1**, **2**) of human rhinovirus.



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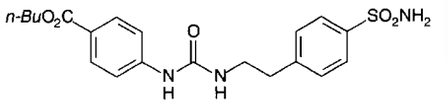

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Emerging molecular targets

Cell-permeable peptide import for use in target validation

A 'magic bullet' compound, capable of specifically blocking a cellular reaction, often serves as an enormously valuable reagent to prove the efficacy of an enzyme or protein as a new drug target. But, reagents with the specificity and potency needed to qualify as 'magic bullets' can be difficult to obtain, sometimes requiring as much chemistry and biology effort as the discovery of a drug candidate itself.

To get around this difficulty, drug targets for which an 'experiment of nature' exists are often selected. This 'experiment of nature' may be a particular enzyme or protein that exists, preferably in a population of human subjects, in a mutated form that mimics the action of the proposed drug. In such a case, the 'magic bullet' reagent is not needed to prove the efficacy of the target. The experimental phenotype exists naturally, and the effects of inactivating the particular protein or enzyme are apparent by observing the population with the mutation. In other cases, it is possible to use an animal model in which a particular gene has been 'knocked out' to determine if inactivating a potential drug target will have the effect predicted from theory. But there still remain many potential targets for which a 'magic bullet' reagent would be an invaluable tool.

Peptides as 'magic bullets'. Despite their well deserved reputation as unlikely drugs and challenging lead compounds for drug development, peptides sometimes serve as excellent 'magic bullet' reagents for proof-of-principle studies. Especially when designed on the basis of a naturally occurring sequence from a protein with multifunctional domains, peptides have proven to be of immense value for dissecting functional activity. Some protein kinases and phosphatases, for example, contain autoinhibitory domains as part of their primary structure. Such domains mimic the natural substrate of the enzyme and interact with the catalytic site to keep the kinase or phosphatase activity suppressed. But when an appropriate regulatory molecule – calcium ion, calmodulin or cAMP, for example – binds to the enzyme, its conformation is altered, so that the auto-inhibitory domain no longer blocks the active site, liberating the signaling activity of the kinase or phosphatase.

Peptides that mimic the autoinhibitory domains of such enzymes are often highly specific and potent kinase or phosphatase inhibitors. Because the peptides are not covalently connected to the other domains of the enzyme, their inhibitory activity is not released by the binding of the regulatory molecule. Such peptides can be extremely useful for determining the role a particular kinase or phosphatase plays in a complex metabolic mixture