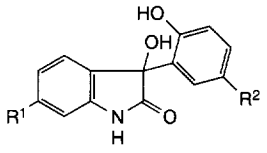
Monitor: molecules and profiles

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.

Novel BK channel openers

The large conductance calcium-activated potassium (BK) channels are found on many excitable cells including neurones and various smooth muscle cells. The important role these channels play in the regulation of cell excitability and function has led to increasing interest in modulators of these channels that may be used to treat various CNS and smooth muscle related disease states. Hewawasam, P. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 1255–1260] have synthesized both racemic and chiral 3-aryl-3-hydroxyindoles, exemplified by **1**, and examined their electrophysiological properties using the cloned BK channel *mSlo* expressed in *Xenopus laevis* oocytes and shown the 3-aryl-3-hydroxyindol-2-ones to be a novel class of BK channel openers.



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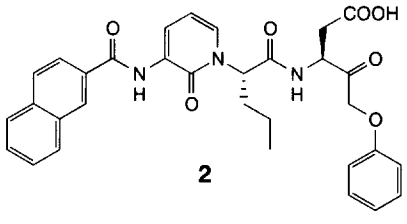
Pyridone-based peptidomimetic ICE inhibitors

The intracellular cysteine protease interleukin-1β-converting enzyme (ICE) is involved in the activation of the pro-inflammatory cytokine IL-1β and has also been shown to be important in triggering apoptosis, or programmed cell death. This enzyme is therefore a potential target for the development of both anti-inflammatory and anti-apoptosis agents. Semple, G. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 1337–1342] have described the synthesis and evaluation of new potent inhibitors of ICE with a significantly reduced peptide character. The P_2-P_3 amino acids of the native substrate were replaced with pyridone and pyrimidone heterocycles and the activity optimized by manipulation of the alkyl and aryl substituents to yield potent compounds such as **2** (K_i = 1.4 nM). The ability of these compounds to release mature IL-1β from lipopolysaccharide-stimulated cell lines is presently being investigated.



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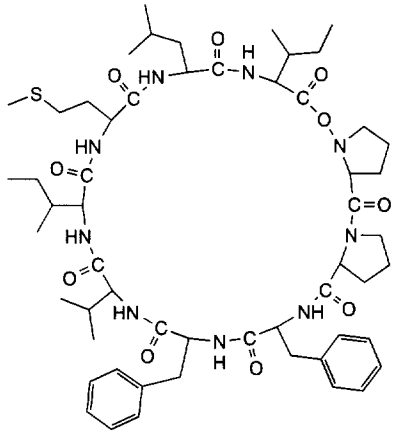
Immunosuppressive cyclic nonapeptide

Morita, H. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 1269–1272] have reported the isolation and structure elucidation of a new cyclic nonapeptide, cyclolinopeptide B, *cyclo* (Pro-Pro-Phe-Phe-Val-Ile-Met-Leu-Ile) (**3**), from the seeds of *Linum usitatissimum*. This compound has been shown to possess potent immunosuppressive activity, comparable with that of cyclosporin A, against human peripheral blood lymphocytes (IC_{50} = 44 ng/ml).



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Novel antiparasitic binaphthalene

The ever-increasing problem of resistance development to antiparasitic agents continues to drive the search for novel compounds to combat such infections. The naturally occuring binaphthalenes are known to be the active components of a number of antiparasitic

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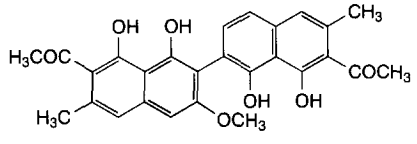
traditional medicines, but the use of these compounds has been limited by their mammalian toxicity. A group from Merck Research Laboratories (Rahway, NJ, USA) has reported the isolation and characterization of sporandol (7,7′-diacetyl-1,1′,8,8′-tetrahydroxy-3,3′-dimethoxy-6,6′-dimethyl-2,2′-binaphthalene, **4**), a novel parasiticide from *Chrysosporium meridarium*. [Tsipouras, A. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1279–1282]. Sporandol was shown to be active against a range of parasites, including the endoparasite (fluke) *Fasciola hepatica* and the ectoparasite *Dipetalogaster maximus*, in the mouse at 190 mg/kg. Furthermore, there were no signs of gross toxicity following administration of sporandol to mice at 620 mg/kg for 6 days, suggesting that this agent has less mammalian toxicity than other binaphthalenes.



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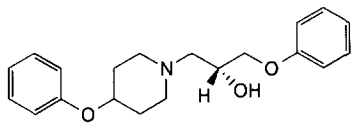
Selective D_4 receptor antagonists

The potential use of selective dopamine D_4 receptor antagonists as atypical antipsychotic agents for the treatment of psychotic disorders such as schizophrenia continues to generate interest in this field. Workers from Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA) have identified 3-(4-benzylpiperidinyl)-1-naphthoxy-2-propanol as a selective D_4 receptor ligand as part of a high-volume screening programme [Wright, J.L. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1377–1380]. The replacement of the benzyl group with phenoxy and the naphthalene with phenyl was found to improve the activity of this compound tenfold. The *R*-enantiomer of this optimized compound (**5**; K_i = 2 nM) was shown to be more potent than the *S*-enantiomer and was shown to be 100 times more selective for the D_4 receptor over the D_2 and D_3 receptors. This compound was shown to antagonize the quinpirole (a dopamine agonist) stimulation of mitogenesis in D_4-transfected Chinese hamster ovary (CHO) p-5 cells and to have no agonistic activity in the same *in vitro* assay. Unlike nonselective dopamine antagonists this compound did not affect dopamine synthesis in the hippocampal or striatal regions of the brain on administration to rats at 10 mg/kg intraperitoneally. In addition to being a potential antipsychotic agent in humans, this compound should aid our further understanding of the role of dopamine D_4 receptors.



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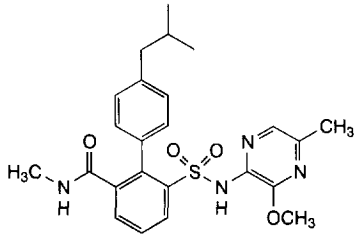
ET_A-selective endothelin antagonist



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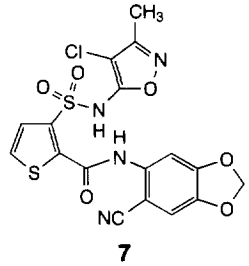
The endothelin family of closely related isopeptides have been implicated in a number of disease states including renal failure, cerebral vasospasm and pulmonary hypertension. Two endothelin receptor subtypes have been identified: the ET_A receptor, which mediates vasoconstriction and smooth muscle proliferation in vascular smooth muscle, and the ET_B receptor, which primarily mediates vasodilatation in vascular beds. A group from Zeneca Pharmaceuticals (Macclesfield, UK) have reported the discovery of a novel class of orally active benzenesulphonamide-based ET_A-selective endothelin antagonists exemplified by *N*-methyl-2-[4-(2-methylpropyl)phenyl]-3-(3-methoxy-5-methylpyrazin-2-ylsulphamoyl)benzamide (**6**) [Mortlock, A.A. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1399–1402]. This compound was shown to have good *in vivo* oral potency and, when dosed at 2.5 mg/kg in the conscious rat, to have a duration of action in excess of 4 h.

Other ET_A-selective endothelin antagonists have been described in two back-to-back papers from Immuno-Pharmaceutics (San Diego, CA, USA) and Texas Biotechnology Corporation (Houston, TX, USA). The first paper describes the structure–activity relationships of *N*-2-aryl-3-(isooxazolylsulphamoyl)-2-thiophenecarboxamides. The combination of *ortho* substitution on a benzo[*d*][1,3]dioxole yielded a series of compounds, exemplified by **7**, with *in vitro* potency, ET_A selectivity, *in vivo* activity and moderate half-life [Wu, C. *et al. J. Med. Chem.* (1997) 40, 1682–1689].

The second paper describes the replacement of the amide group of amidothiophenesulphonamides with an acetyl group to yield a series of compounds that maintain their *in vitro* binding affinity and *in vivo* activity but provide oral bioavailability and longer duration of action. The most effective compound was **8**, which was shown to competitively bind to ET_A receptors (K_i = 430 ± 30 pM; IC_{50} = 1.4 nM) with 9,000-fold selectivity for the ET_A receptor over the ET_B receptor. The compound was shown to have 60–100% oral bioavailability and a serum half-life in the dog of 6–7 hours [Wu, C. *et al. J. Med. Chem.* (1997) 40, 1690–1697]. These and other



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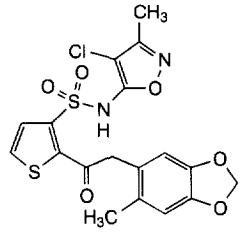

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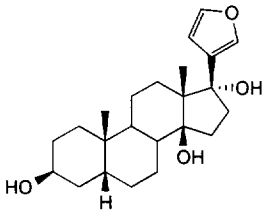
potent, selective ET_A receptor antagonists will provide tools for the more detailed evaluation of the pharmacological and clinical roles of such receptors in diseases.

Novel antihypertensive agent

Recent evidence has suggested the existence of an endogenous ouabain-like inhibitor of the Na^+, K^+-ATPase that may be involved in the pathogenesis of essential hypertension. Compounds that selectively counteract the pressor effect caused by this endogenous factor may have potential as hypertensives with reduced side effects resulting from interactions with other mediators of blood pressure regulation. Quadri, L. and coworkers have identified 17β-(3-furyl)-5β-androstane-3β,14β,17α-triol (PST2238; **9**) as a potent antihypertensive agent [*J. Med. Chem.* (1997) 40, 1561–1564]. This compound was shown to antagonize the *in vitro* binding of ouabain to isolated Na^+, K^+-ATPase and reduce systolic blood pressure *in vivo* when orally administered chronically in animal models of hypertension. The hypotensive effect of **9** on genetically hypertensive adult rats was found to have a delayed onset of several days. Also, the effect was long-lasting, suggesting that this compound does not act on mechanisms that acutely modulate blood pressure, but acts via antagonism of the long-term pressor effect of an ouabain-like agent.



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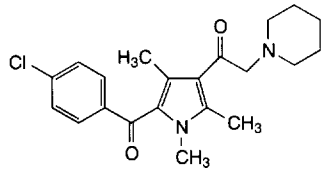
New class of anticonvulsant agents

Although a large number of anti-epilepsy drugs are available on the market, it is still not possible to adequately control seizures in all patients and there is therefore scope for the development of new classes of anticonvulsants. Carson, J.R. and coworkers [*J. Med. Chem.* (1997) 40, 1578–1584] have described a new class of such drugs based on aroyl(aminoacyl)pyrroles. These compounds were shown to be active in the mouse and rat maximal electroshock tests, but not in the mouse metrazol test. The lead compound **10** was shown to be as potent as phenytoin and carbamazepine with a similar therapeutic index.



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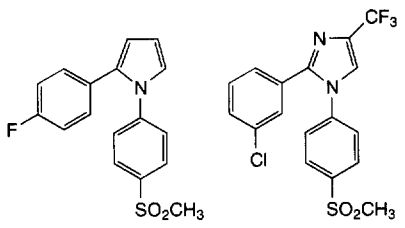
COX-2 inhibitors

The recent identification of two isoforms of cyclooxygenase with different physiological and pathological functions has driven the search for compounds that specifically inhibit the individual isoenzymes. Pharmaceutical companies are particularly interested in specific inhibitors of COX-2, which is deemed to be responsible for inflammatory conditions in response to inflammatory and mitogenic stimuli. A group from Searle Research and Development (Skokie, IL, USA) have described the identification of 1,2-diarylpyrroles as potent, selective inhibitors of COX-2 (IC_{50} = 15–100 nM) [Khanne, I.K. *et al. J. Med. Chem.* (1997) 40, 1619–1633]. *In vivo* testing of these compounds in the rat carrageenan-induced paw oedema model established that the compounds were orally active anti-inflammatory compounds with the most potent inhibitor of oedema (**11**) having an ED_{50} of 4.7 mg/kg and a 200-fold selectivity for COX-2 over COX-1.

In a second paper Khanne, I.K. and coworkers have described the synthesis and evaluation of 1,2-diarylimidazoles as COX-2 inhibitors [*J. Med. Chem.* (1997) 40, 1634–1647]. These compounds were also found to be potent and highly selective inhibitors of the human COX-2 enzyme. Several of these compounds, exemplified by **12**, were found to exhibit excellent inhibition in the adjuvant-induced arthritis model (ED_{50} = 0.02 mg/kg). The 1,2-diarylimidazoles were also shown to inhibit carrageenan-induced rat paw oedema and hyperalgesia, with several orally active compounds showing no gastro-intestinal toxicity in either the rat or mouse at up to 200 mg/kg, suggesting that these compounds offer potential as anti-inflammatory agents with reduced side effects.



11 **12**

HTS NEWS

In mid-July **FluorRx** (Carmel, IN, USA) announced it has agreed on a technology licensing deal with **LJL BioSystems** (Sunnyvale, CA, USA). The licensing agreement will allow LJL, a developer of automated assay systems for high-throughput applications, to incorporate FluorRx's fluorescence lifetime (FLT) sensing technology into LJL's HTS systems. FluorRx's FLT technology measures the excited state lifetime, rather than the intensity, of proprietary fluorophores by a method termed phase modulation. The reagents and instrumentation are covered by nine US patents, to which LJL now has the exclusive worldwide rights.

Microcide Pharmaceuticals (Mountain View, CA, USA), a biopharmaceutical company specializing in the development of novel antibiotics, will to provide Tularik (South San Francisco, CA, USA) with at least 40,000 extracts from its natural product collection for use in Tularik's screening programs. Tularik is precluded from using the samples in antibacterial or antifungal assays.

Combinatorial chemistry

Combinatorial polymers

A recent paper describes the use of combinatorial chemistry for the synthesis of structurally related polymers [Brocchini, S. *et al. J. Am. Chem. Soc.* (1997) 119, 4553–4554]. A library of 112 different A-B copolymers were individually synthesized from 14 tyrosine-derived diphenols and eight aliphatic diacids. Several hundred milligrams of each polymer was prepared using up to 32 simultaneous reactions in separate vessels set up in a water shaker bath. In the set of diphenols, the monomers differed by the nature of a pendant R group and by the spacing between the terminal phenols. In the other set of monomers, the diacids were varied by the nature and size of the spacer group between the carboxylic acids.

Overall, the variation in the monomers provided polymers with a range of polymer free volume, bulkiness, flexibility and lipophilicity. In particular, polymers were examined for their surface wettability (as measured by the air–water contact angle) and cellular response in *in vitro* cell proliferation studies. In the latter experiments, the proliferation of rat lung fibroblasts was studied in a group of 42 polymers. It was found that there was a linear correlation between the cell proliferation and the surface contact angle, with cell proliferation decreasing with increasing surface hydrophobicity.

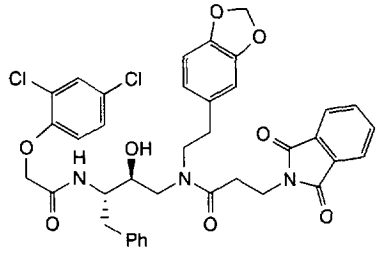
This is one of the first combinatorial studies of polymer properties and offers the opportunity to prepare and evaluate new speciality materials. The results presented suggest that such studies could have a profound effect on the future design of biomedical polymers.

Cathepsin D inhibitor library

A comprehensive example of the discovery and optimization of cathepsin D inhibitors using combinatorial chemistry has recently been published [Kick, E.K. *et al. Chem. Biol.* (1997) 4, 297–307]. Cathepsin D is an aspartyl protease that has been implicated in tumour metastasis in breast cancer, melanoma metastasis and Alzheimer's disease. An X-ray crystallographic structure of the enzyme with the natural inhibitor pepstatin complexed to the active site was the starting point for the selection of building blocks to attach to a hydroxyethyl scaffold.

Three sets of ten monomers were chosen using a molecular modelling program entitled CombiBuild. These monomers were used to prepare a *directed* combinatorial library of 1,000 analogues in parallel on polystyrene bead solid support. For comparative purposes a *diverse* set of 1,000 compounds were also selected without using structure-based design methods. Both sets were tested for inhibitory activity against cathepsin D leading to seven compounds from the *directed* library with an IC_{50} below 100 nM compared with just one compound of similar activity from the *diverse* library.

From the active compounds, a second generation library of 39 compounds was designed, picking monomers from the same clusters that earlier had yielded active monomers. This optimization process gave rise to compounds with a five- to sixfold improvement in potency, including the inhibitor **1** (IC_{50} = 14 nM).



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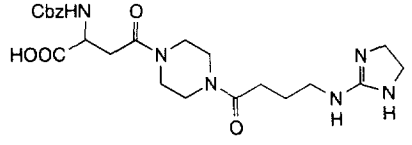
This study demonstrates the rapidity with which novel enzyme inhibitors may be discovered and optimized through a synergistic combination of molecular modelling and combinatorial synthesis.

A selective $\alpha_v\beta_3$ integrin antagonist library

RGD peptide mimics have been a favourite subject for combinatorial libraries. A recent paper describes the synthesis of a library of such compounds with antagonistic activity against the $\alpha_v\beta_3$ integrin [Corbett, J.W. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1371–1376]. The $\alpha_v\beta_3$ integrin has been implicated in melanoma development and is a significant receptor for mediating the attachment of the osteoclasts to bone during resorption. Selective integrin antagonists may have utility for the therapy of diseases including osteoporosis and diabetic retinopathy.

The library compounds contained three components: a β-alanine derivative that mimicked the carboxylic acid of aspartic acid, previously optimized to contain a 2-carbobenzyloxyamine group, a linking diamine chain, and thirdly an amine or guanidine to represent the basic side chain of arginine. The compounds were synthesized in parallel on Wang resin and assessed for their ability to inhibit the $\alpha_v\beta_3$ integrin receptor. Many active compounds were discovered, including **2** (IC_{50} = 1.1 nM)



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Next month in Monitor

Molecules:
Novel thrombin inhibitors
GPIIb–IIIa antagonists
Farnesyltransferase inhibitors
Selective dopamine D_4 agonists
A_1-adenosine antagonists

Combinatorial chemistry:
Water-soluble synthetic polymers
Linker for amidines
Nonpeptide stromelysin inhibitors