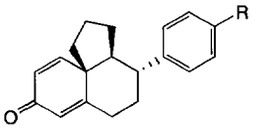
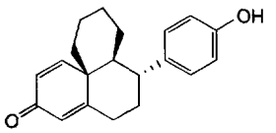
Monitor: molecules and profiles

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.

Aromatase inhibitors



1 R = F
2 R = H
3 R = OH



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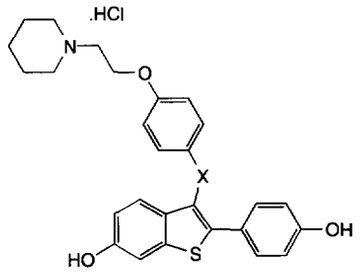
Oestrogens have been shown to have an important role in the development and growth of certain types of carcinomas such as breast cancers. The enzyme aromatase is involved in the biogenesis of such mammalian sex hormones through the conversion of androgens to oestrogens. Aromatase inhibitors are therefore recognized as potential therapeutic targets for reducing oestrogen levels and thereby modulating the growth of particular carcinomas. Hobbs-Mallyon, D., Li, W. and Whiting, D.A. [*J. Chem. Soc., Perkin Trans. 1* (1997) 1511–1516] have described the synthesis and evaluation of aryltricyclospirodienones (**1–4**) as novel steroid mimetic inhibitors of aromatase. These compounds may offer the clinical advantage over other aromatase inhibitors of being less rapidly metabolized. The activities of compounds **2** and **3** were found to be comparable with that of aminoglutethimide, which is presently used in the clinic.

Oestrogen receptor modulator



5 X = CO
6 X = O

Over recent years a number of compounds such as raloxifene (**5**) have been shown to have oestrogen-like actions on bone tissue and serum lipids while acting as potent oestrogen antagonists in breast and uterine tissue. These selective oestrogen receptor modulators (SERMs) may therefore have potential uses in the treatment of diseases such as osteoporosis, breast cancer and gynaecological disorders. As part of an ongoing strucure–activity study of raloxifene, workers from Lilly Research Laboratories (Indianapolis, IN, USA) have identified novel, highly potent SERM **6**, which was found to be 10 times more potent than raloxifen as an antagonist in an *in vitro* oestrogen-dependent human breast MCF-7 cancer cell proliferation assay (IC_{50} = 50 pM). This compound was also shown to inhibit the uterine proliferative response to exogenous oestrogen in immature rats, to produce a significant reduction in total cholesterol and to have a protective effect on bone *in vivo* using ovariectomized aged rats [*J. Med. Chem.* (1997) 40, 1407–1416].

Heterocyclic fused pyridazinones as PDE 4 inhibitors

Phosphodiesterases have important roles in the regulation of cell functions such as growth and metabolism through their responsibility for the hydrolysis of the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) secondary messengers. Recently, attention in this field has focused on inhibitors of the phosphodiesterase 4 (PDE 4) isoenzyme that is particularly abundant in the brain and immunocompetent cells, as such inhibitors may have uses in the treatment of asthma, inflammation and CNS disorders. Piaz, V.D. and coworkers [*J. Med. Chem.* (1997) 40, 1417–1421] have reported the synthesis and evaluation of a series of novel heterocyclic, fused pyridazinones **7** as

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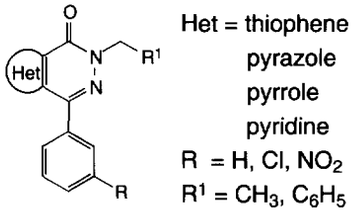
selective PDE 4 inhibitors. The biological evaluation of these compounds showed good selectivity for the PDE 4 isoenzyme with reduced binding to the rolipram binding site, which suggests that these compounds may offer fewer side effects than other agents.



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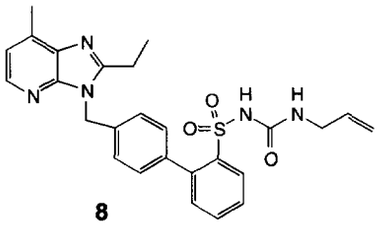
Angiotensin II (AT_1) antagonists

The angiotensin II (AT_1) receptor has recently been identified as a potential target for the treatment of hypertension [*Drug Discovery Today* (1997) 1, 39–40]. This has led to the identification of a range of selective AT_1 antagonists by various groups, including the highly potent biphenyl-2′-tetrazoles and the biphenyl-2′-acylsulphonamide-substituted imidazole[4,5-*b*]pyridines developed by Merck. A group from Hoechst AG (Frankfurt, Germany) have described the synthesis and evaluation of novel methylbiphenylimidazole[4,5-*b*]-pyridines with a sulphonylurea or sulphonylcarbamate group as bioisosteres for the biphenyl moiety present in losartan [Heitsch, H. *et al. Bioorg. Med. Chem.* (1997) 5, 673–678]. These compounds have been shown to be potent AT_1-selective angiotensin II receptor antagonists. They exhibit nano- and subnanomolar binding affinities to the AT_1-receptor subtype *in vitro* and potent activity *in vivo* when evaluated using normotensive pithed rats. Compounds **8–10** were shown to be more potent than losartan and their previously described 4-thiomethylimidazole homologues [Deprez, P. *et al. J. Med. Chem.* (1995) 38, 2357–]. In particular, the cyclopropylmethyl-substituted biphenylsulphonylurea **9** is one of the most effective angiotensin II (AT_1) antagonists reported to date with ED_{50}s of 14 μg/kg and 12 μg/kg when administered intravenously and intradermally, respectively.



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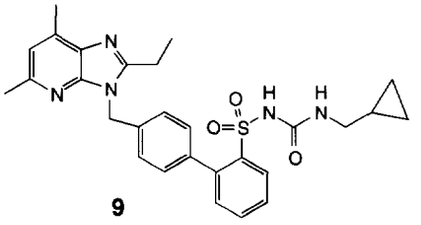

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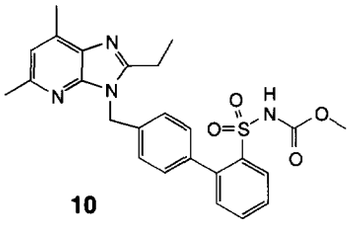

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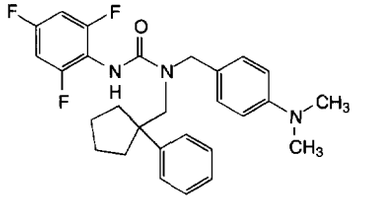
Trisubstituted ureas as ACAT inhibitors

Evidence indicating that inhibition of arterial macrophage acyl-CoA:cholesterol acyltransferase (ACAT) reduces the rate of atherosclerotic lesion progression has driven the development of specific inhibitors for this enzyme for the treatment of atherogenesis. A group from Parke-Davis (Ann Arbor, MI, USA) have described the discovery of several novel trisubstituted ureas, exemplified by **11**, which inhibit ACAT in the range of 20–100 nM and lower total plasma cholesterol on oral administration in an acute model of hypercholesterolaemia in rats [Purchase, T.S. *et al. Bioorg. Med. Chem.* (1997) 5, 739–747].



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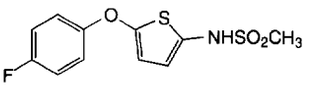
Thiophene-2-alkylsulphonamides as 5-LO inhibitors

5-Lipoxygenase (5-LO) is the first enzyme in the leukotriene biosynthetic pathway and has therefore received much attention as a potential target for the treatment of numerous inflammatory disorders involving overproduction of leukotrienes. A group from the R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ, USA) has described the identification of *N*-alkyl-5-aryloxythiophene-2-sulphonamides, such as **12**, as a new class of potent 5-LO inhibitors [Beers, S.A. *et al. Bioorg. Med. Chem.* (1997) 5, 779–786]. These compounds were shown to possess *in vitro* activity equal to or greater than that of Zileuton, giving nanomolar IC_{50}s in the rat basophilic leukaemia (RBL-1) cell homogenate assay and submicromolar IC_{50}s in the RBL-1 and human peripheral blood leukocyte cell assays. Compound **12** was also evaluated in the adjuvant arthritic rat and found to have significant anti-inflammatory activity on oral administration at 3–30 mg/kg.



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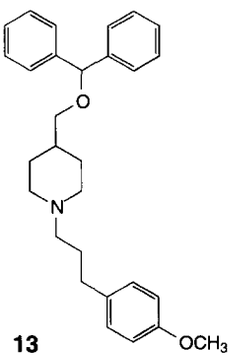
Human T cell Kv1.3 potassium channel blocker

The voltage-gated potassium channel Kv1.3, expressed on human T cells, is involved in the control of membrane potential, IL-2 production and T cell proliferation, and is therefore regarded as a possible therapeutic target for



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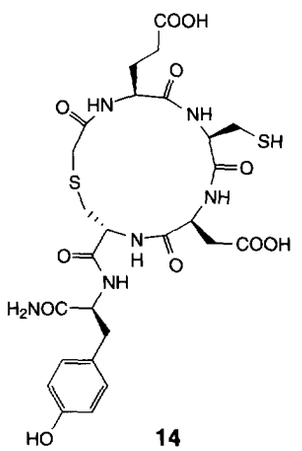
immunosuppressive therapy. A human T cell ^{86}Rb efflux high-throughput screen of compound libraries at Pfizer (Groton, CT, USA) identified UK78282 (**13**) as a potent, selective blocker of the human T cell voltage-gated potassium channels and inhibitor of T cell activation [Burgess, L.E. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1047–1052]. Structure–activity relationship studies have revealed that there are three critical elements required for activity, a benzhydryl head group, a lipophilic tail and a central basic nitrogen.

Fertilization inhibitor

The extracellular, membrane-bound, heterodimeric protein fertilin is found on the surface of sperm. It has been shown to have both disintegrin and metalloprotease domains, the disintegrin domain being located on the β-subunit. Unlike other disintegrins, such as the P-II disintegrin domain of the snake venom metalloproteases (SVMP), the fertilin disintegrin does not contain the usual RGD peptide sequence. Pyluck, A. and coworkers examined the ability of several reduced cyclic and linear peptides, such as **14**, containing the ECD peptide sequence – a possible alternative binding sequence on the disintegrin domain of fertilin – to inhibit sperm-egg fusion in an *in vitro* mouse fertilization assay at 500 μM concentrations [*Bioorg. Med. Chem. Lett.* (1997) 7, 1053–1058]. These studies suggest that the ECD peptide sequence forms part of the fertilin disintegrin binding sequence. However, evidence suggests that the binding site of fertilin is not as restricted as that of SVMP P-II RGD disintegrins in that more than three amino acids of the fertilin disintegrin domain are probably recognized by its receptor.



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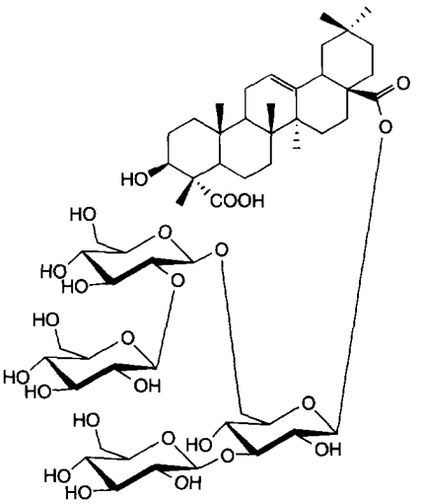
New natural products

Uterine stimulating saponin

A vast range of new natural products are being continually identified as possible lead structures. The seeds of *Vaccaria segetalis* have been used historically to increase blood flow, promote milk secretion and to treat amenorrhoea and breast infections in China. Morita, H. and coworkers have isolated and characterized a new triterpenoid saponin, vaccaroid A (**15**), from these seeds and shown that this compound elicits rat uterine contraction activity at 60 μg/ml [*Bioorg. Med. Chem. Lett.* (1997) 7, 1095–1096].



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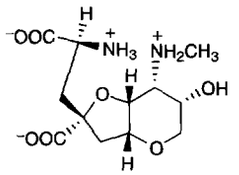
New neurotoxic amino acid

Other workers from Japan [Sakai, R. *et al. J. Am. Chem. Soc.* (1997) 119, 4112–4116] have described the isolation and characterization of a new neurotoxic amino acid, dysiherbaine (**16**) from the Micronesian sponge *Dysidea herbacea*. Systemic administration of dysiherbaine to mice caused neurotoxic symptoms typical of neuroexcitory amino acids such as domoic acid. *In vitro* studies showed that this compound blocked the binding of [^{3}H]-kainic acid and [^{3}H]-1-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) but not a *N*-methyl-D-asparatic acid (NMDA) receptor antagonist to rat brain synaptic membranes. This suggests that dysiherbaine may be a selective non-NMDA type glutamate receptor agonist in the CNS.



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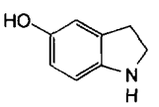
Dopamine D_4 receptor-selective compounds

In another field, a group from Schering Plough Research Institute (Kenilworth, NJ, USA) [Hegde, V.R. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1207–1212] have described the isolation and characterization of four dopamine D_4 receptor-selective compounds (**17–20**) from the fruit of the Chinese plant *Phoebe chekiangensis*. Compounds **17** and **20** were found to have better selectivity for the D_4 receptor over the D_2 receptor than standard dopaminergic antagonists such as clozapine. Evidence suggests that compound **17** is an agonist at this recpetor subtype while compound **20** is an antagonist.



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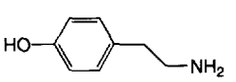

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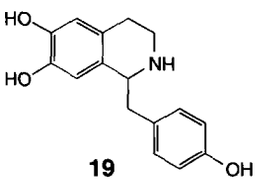

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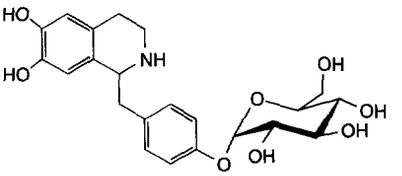

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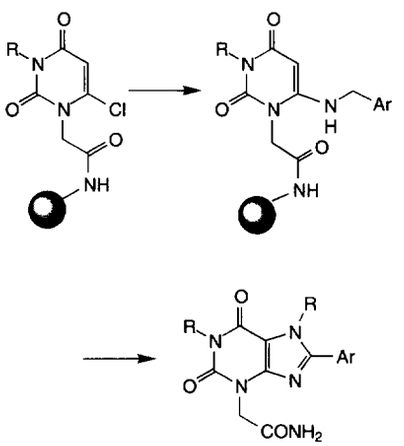
Combinatorial chemistry

Xanthines as a library scaffold

Xanthines, such as caffeine and theophylline, are a class of naturally occurring heterocycles with a range of pharmacological activities. A new synthetic approach on solid phase has recently been published that allows the generation of combinatorial libraries of these compounds [Heizmann, G. and Eberle, A.N. *Molecular Diversity* (1996) 2, 171–174].

The synthetic route involves the initial coupling of bromoacetic acid to Rink amide resin and then derivatizing with a 4-chlorouracil (Scheme 1). A key step in the synthesis is the fusion of the second ring, which was achieved by a nucleophilic displacement of the chlorine with an amine followed by a concomitant nitrosylation and cyclization. An important feature in the synthesis of a combinatorial library of these compounds is the ability to vary three positions within the molecule. The products can be cleaved from the resin support under acid catalysis, and chromatographic purification finally gives the fully substituted xanthines. Ten examples were synthesized in moderate yields but good purities.

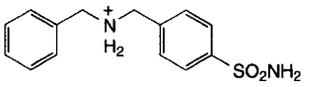


Scheme 1

Virtual combinatorial libraries

If a combinatorial library can be defined as the compounds actually synthesized from a given number of monomeric precursors, a virtual combinatorial library (VCL) can be visualized as the *potential* library that could theoretically be formed from a set of monomers. If the most active compounds in a library can be selectively produced by the presence of the target protein, it may not be necessary to make every compound in the library. This concept has been explored through the search for inhibitors of the enzyme carbonic anhydrase [Huc, I. and Lehn, J-M. *Proc. Natl. Acad. Sci. U. S. A.* (1997) 94, 2106–2110]. A number of synthetic precursors were combined in the presence of carbonic anhydrase in the expectation that the enzyme would catalyse the formation of compounds with the highest enzyme affinity. Mixing a set of three aldehydes and four amines led to the *in situ* formation of imines that could be irreversibly reduced with sodium cyanoborohydride. The reaction was carried out both with and without carbonic anhydrase and the product mixtures examined by HPLC.

The HPLC traces varied slightly but sufficiently to demonstrate that one particular aldehyde generated a different proportion of reduced products in the presence of the enzyme. Of the twelve possible products, the benzylamine derivative (**1**) was identified as having the greatest affinity for carbonic anhydrase. This novel combinatorial approach holds promise for the discovery of other enzyme inhibitors from VCLs.



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Laser optical encoding

Just about every type of encoding strategy has been explored for tagging combinatorial library components. Among the many techniques that are non-invasive, and therefore cannot affect the library chemistry, is the use of laser optical synthesis chips (LOSC) [Xiao, X-Y. *et al. Angew. Chem., Int. Ed. Engl.* (1997) 36, 780–782]. A readable two-dimensional bar code has been generated by laser-etching with a carbon dioxide laser onto an inert 3 × 3 mm alumina ceramic plate. Around this plate is a polypropylene square of 10 × 10 mm radiolytically grafted to provide a support for synthesizing library compounds.

A 'directed sorting' approach has been used for the synthesis of a library of 27 oligonucleotides on these chips. In the directed sorting strategy, there is zero redundancy among the library members leading to just one chip for each compound in the library. The library was prepared by scanning and sorting the chips before each synthetic step, and at the end of the synthesis the structure of each compound was determined by reference to the etched code. Integration of LOSC technology with automation will enhance the utility of this library synthesis approach.

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News on HTS deals

An alliance has been established between **Chiroscience** (Cambridge, UK) and the specialist ophthalmics company **Alcon Laboratories** (USA) to develop novel therapeutics. In the collaboration Chiroscience will provide focused compound libraries to Alcon, who will build assays and carry out high-throughput screening for the discovery of new leads.

IRORI Quantum Microchemistry (La Jolla, CA, USA) has entered into a collaboration with **Dupont Merck Pharmaceuticals** (Wilmington, DE, USA) to develop new drug leads. Under the agreement IRORI will produce small-molecule libraries based on pharmacophores selected by Dupont Merck's scientists, who will screen the libraries for activity. Libraries will be produced using IRORI's Micromemory-based Directed Sorting™ technology, which employs a memory microreactor consisting of a remote electromagnetic semiconductor fused to a solid support polymer as a tagging device.

Mark V. Rogers/Henriëtte Willems