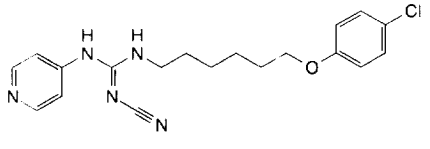
Monitor: molecules and profiles

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.

Oral antitumour agents

A group from Leo Pharmaceutical Products (Ballerup, Denmark) have recently reported an investigation into the potential use of cyanoguanidines as antitumour agents [Schou, C. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 3095–3100]. Initial studies demonstrated that *N*-(5-phenoxypentyl)-*N'*-cyano-*N''*-4-pyridylguanidine, an analogue of the potassium channel opener Pinacidil, was able to increase longevity in a rat model with Yoshida ascites sarcoma cell tumours.

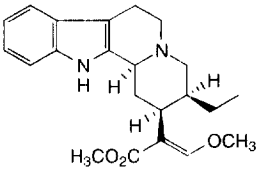
Chemical synthesis of a range of derivatives led to the identification of a novel series of cyanoguanidines with hydrophobic aromatic side-chains that showed potent antitumour activity *in vitro* using the human breast and lung cancer cell lines MCF-7, NYH and H460. The most potent compounds identified in the structure activity study were those with a 6–8-carbon side-chain and a terminal, substituted phenoxy function. Compound **1** caused complete remission of tumours following oral administration once daily for two weeks in a therapeutic treatment model in which nude mice were inoculated with the NYH human lung cancer cells.



1

Influenza A antiviral

Takayama, H. and coworkers from various groups have identified a plant indole alkaloid with potent anti-influenza virus activity against a particular strain of the influenza A virus *in vitro* [*Bioorg. Med. Chem. Lett.* (1997) 7, 3149–3152]. The alkaloid hirsutine (**2**) is a major constituent of *Uncaria rhynchophylla* MIQ, the original plant of the Chinese 'Kampo' medicine used for the treatment of hypertension. The paper also reports on the group's investigations into the SARs of natural and synthetic analogues of hirsutine that indicated that the β-alkoxyacrylic acid ester function is essential for anti-influenza A activity.



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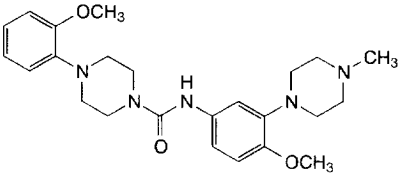
5-HT$_{1B}$ receptor antagonists

Substantial evidence suggests that most antidepressant drugs act by enhancing serotonin (5-HT) neurotransmission. Since 5-HT$_{1B}$ receptors are involved in suppressing the synthesis and release of 5-HT from 5-HT terminals, selective inhibition of this receptor subtype should elevate the release of 5-HT. Antagonists to the 5-HT$_{1B}$ receptor have therefore been suggested as potential fast-acting antidepressant drugs. Although several 5-HT$_{1B}$ receptor antagonists have previously been identified, these compounds have been found to have limited effect on 5-HT release *in vivo*. A group from the Neurobiology Centre de Recherche Pierre Fabre (Castres, France) have recently reported the synthesis and evaluation of a novel series of arylpiperazide derivatives of phenylpiperazines as potential 5-HT$_{1B}$ receptor antagonists [Jorand-Lebun, C. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 3183–3188].

In vitro studies using cloned human 5-HT$_{1B}$ receptors were used to demonstrate the potent and selective activity of these compounds. The *O*-methoxyphenylpiperazide (**3**) was found to be a particularly promising drug candidate as studies *in vivo* demonstrated that this compound was able to reverse the hypothermia induced by a 5-HT$_{1B}$ agonist in the guinea pig following oral administration and, more importantly, was shown to increase the extracellular brain 5-HT on systemic administration.



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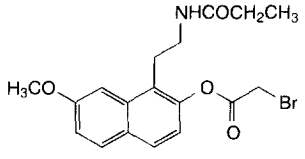
Mel_{1b} melatonin receptor ligand

The hormone melatonin has an important role in synchronizing circadian and seasonal rhythms. The hormone is secreted by the pineal gland under the control of the suprachiasmatic nucleus of the hypothalamus, which regulates release in relation to environmental light. To date, two melatonin receptor subtypes have been identified (ML-1 and ML-2) and two G-protein coupled receptors of the ML-1 subtype have been cloned and classified as Mel_{1a} and Mel_{1b}. Until recently, investigations into the physiological role played by the Mel_{1b} receptor have been hindered by the lack of a specific marker for the receptor subtype.

Witt-Enderby, P.A. and coworkers from Duquesne University (Pittsburg, PA, USA) have described the development of a high-affinity ligand that binds irreversibly to the Mel_{1b} receptor [*J. Med. Chem.* (1997) 40, 4195–4198]. The group found that *N*-[2-[2-(bromoacetoxy)-7-methoxynaphthyl]ethyl]propionamide (**4**) selectively alkylated human Mel_{1b} melatonin receptors expressed in Chinese hamster ovary cells. This agent will therefore be a useful affinity ligand for both molecular characterization and determining the tissue distribution of the Mel_{1b} melatonin receptor.



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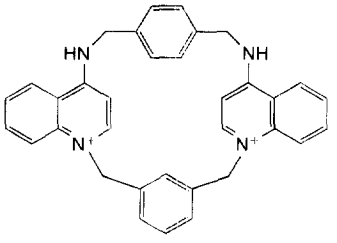
Bis-quinolinium cyclophanes

Ganellin's group at University College London, UK, have reported the synthesis and evaluation of a series of bis-quinolinium cyclophanes as potential small-conductance calcium-activated potassium-channel (SK_{Ca}) blockers [Rosa, J.C. *et al. J. Med. Chem.* (1998) 41, 2–5]. Recent evidence suggests that such blockers may have therapeutic applications in a wide range of disorders including memory disorders, muscular dystrophy and alcohol abuse.

The group have extended their previous work by using aromatic rings to restrict the quinolinium groups conformationally. The SK_{Ca} blockade was evaluated by assessing the ability of the compounds to inhibit the post-hyperpolarization in cultured rat sympathetic neurones. Compound **5** was found to be the most potent non-peptide SK_{Ca} blocker discovered to date (IC_{50} = 3 nM). It is 100 times more potent than dequalinium and as potent as the natural peptidic toxin apamin.



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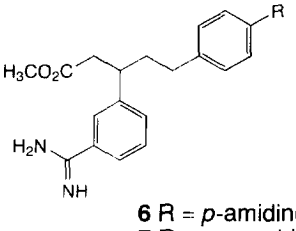
Factor Xa inhibitors

Factor Xa has a central role in the fibrinolytic coagulation cascade – the generation of thrombin from prothrombin by proteolysis. As it is estimated that a molecule of Factor Xa can activate 138 molecules of thrombin, inhibitors of Factor Xa have been suggested as potential potent antithrombic agents. Maduskuie, T.P. and coworkers have reported the molecular modeling, rational design and synthesis of a novel series of bisphenylamidine carboxylate compounds as inhibitors of Factor Xa [*J. Med. Chem.* (1998) 41, 53–62].

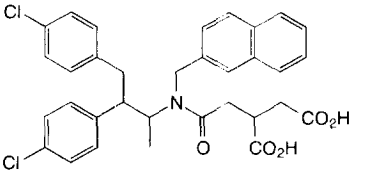


6 R = *p*-amidine
7 R = *p*-guanidine

The group used the Factor Xa dimer crystal structure to model the interaction of candidate molecular fragments with the S1 and S4 aryl-binding subsites. The optimized molecular fragments were linked together so as to retain the orientation of the fragments in their respective pockets. This led to the identification of an initial compound **6** with significant inhibitory activity (K_i = 34 nM). The optimization of this lead compound using rational drug design led to the identification of **7**, a very active Factor Xa inhibitor (K_i = 9 nM) with 400-fold selectivity for Factor Xa over thrombin (K_i = 3.1 μM) and tenfold selectivity over trypsin (K_i = 96 nM).

Farnesyltransferase inhibitors

The development of novel farnesyltransferase inhibitors continues to feature heavily in the literature. This enzyme catalyses the post-translational prenylation of the Ras protein before its localization in the plasma membrane. As *ras* mutations have been shown to be involved in many human cancers, inhibitors of farnesyltransferase that block the activation of the mutated Ras protein have potential as anticancer agents. Although several compounds have been shown to suppress tumour growth both in cell culture and in animal models, a compound with clinical therapeutic benefit in humans has yet to reported. Aoyama, T. and coworkers from the Tsukuba Research Institute, Banyu Pharmaceutical Co. (Tsukuba, Japan) have reported the development of a new class of farnesyl diphosphate-based competitive farnesyltransferase inhibitors following chemical optimization of **8** [*J. Med. Chem.* (1998) 41, 143–147]. The most potent compound identified



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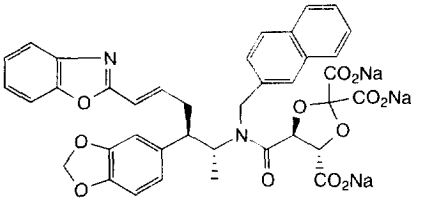

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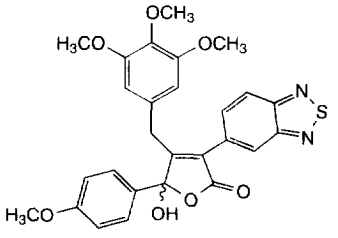
was **9**, which was found to have potent activity against Ras-activated tumour cells in culture and to inhibit tumour growth in a nude mouse xenograft model. This compound was also shown to have high selectivity for the Ras farnesyltransferase, only weakly inhibiting other enzymes that utilize prenyldiphosphate as a substrate, and it showed no toxicity against 3T3 mouse fibroblast cells at 100 μM.

Endothelin antagonists

The endothelins are a family of potent endogenous peptidic vasoconstrictor and pressor agents that appear to play important roles in several disease states including hypertension and heart failure. Over recent years a wide range of non-peptide endothelin antagonists have been reported by various groups. In general, the most potent of these compounds contain a methylendioxyphenyl group. This group is common in natural and synthetic medicinal compounds providing an electronegative function that is non-polar and relatively unreactive. However, this functional group undergoes cytochrome P450-mediated metabolism resulting in the irreversible binding of the substrate to haem iron of cytochrome P450. This metabolism may cause drug–drug interactions or nonlinear pharmacokinetics.

In an attempt to overcome this problem a group from Merck (Darmstadt, Germany) have used self-organizing neural networks to analyse the molecular electrostatic potentials of existing endothelin receptor ligands in order to identify a suitable bioisosteric group for methylendioxyphenyl [Anzali, A. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 11–16]. Using the Kohonen neural network to generate Kohonen maps of bioisosteric candidates, the group identified benzothiadiazole as a potential bioisosteric substitute.

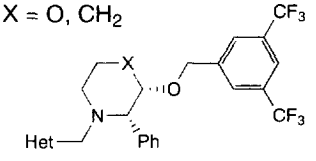
In a subsequent paper the group describes the synthesis and biological properties of a series of different methylendioxyphenyl, benzothiadiazole and benzofurazan derivatives [Mederski, W.W.K.R. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 117–122]. These studies confirmed the hypothesis of the use of a benzothiadiazole as a bioisoster of methylendioxyphenyl in the development of endothelin receptor antagonists and led to the discovery of EMD122801, the sodium salt of **10**, as a potent selective ET_A receptor antagonist (IC_{50} = 0.3 nM).



10

NK_1 receptor antagonists

Recent studies have shown that the introduction of heterocyclic moieties into piperidine- and morpholine-derived human NK_1 receptor antagonists, to give compounds of type **11**, leads to improved potency *in vivo* and *in vitro*. Owens, A.P. and coworkers have extended this approach by investigating the effects of heterocyclic replacement of the carboxamido group in phenylglycinol-derived human NK_1 receptor antagonists (**12**) [*Bioorg. Med. Chem. Lett.* (1998) 8, 51–56].



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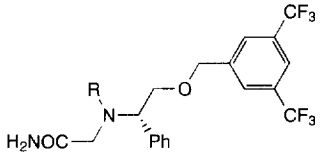

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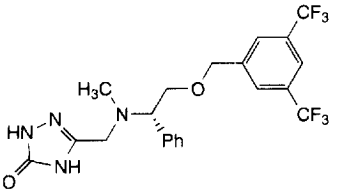

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The group substituted the carboxamido group with triazole, triazolinone and tetrazole heterocycles to give a series of compounds. Compound **13** was found to be the most potent (IC_{50} = 430 pM) and was shown to have excellent selectivity for the NK_1 receptor over the other neurokinin receptors (NK_2, NK_3; IC_{50} > 1 mM) and low-affinity binding to the calcium channel (IC_{50} > 1 mM). Studies in rats demonstrated that the compound has modest oral bioavailability on dosing at 3 mg kg^{-1} and has a plasma elimination half-life of 0.8 h following *in vivo* administration of the same dose. Studies *in vitro* have demonstrated that the major metabolic pathway involves *N*-demethylation, suggesting that oral bioavailability may be improved by replacing the *N*-methyl group with less metabolically labile substituents.

Combinatorial chemistry

Library analysis by mass spectrometry

Electrospray mass spectrometry (ESMS) has been successfully employed in the rapid analysis of combinatorial libraries of enzyme inhibitors [Wu, J. *et al. Chem. Biol.* (1997) 4, 653–657]. The enzyme in question was β-1,4-galactosyltransferase, responsible for the production of *N*-acetyllactosamine (LacNAc), a major component of glycoconjugates that function as cell-recognition molecules. All 20 compounds of an initial library were individually incubated with the enzyme, reactants and a standard. The ability of each compound to inhibit the enzyme was assessed by using MS to measure the ratio of the intensities of the product and reference ions.

It was found that the presence of a diphosphate was essential for inhibitory activity, with uridine derivatives being especially active. From the initial set of compounds, uridine-5′-diphospho-(2-deoxy-2-fluoro)galactose (UDP-2F-Gal) (**1**) was identified as the most potent.

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