Monitor: molecules and profiles

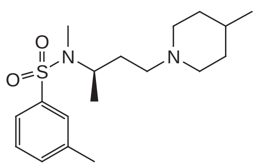
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and 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.

Molecules

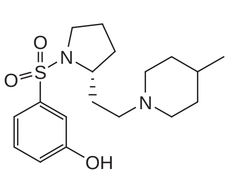
Novel, selective and potent 5-HT$_7$-receptor antagonists

The serotonin 5-HT$_7$ receptor is a member of the seven-transmembrane G protein-coupled receptor superfamily. Various studies have indicated that the 5-HT$_7$ receptor is primarily found in the brain where it is localized to the thalamus, hypothalamus and various limbic and cortical regions. Studies have further indicated that this receptor is involved in mediating 5-HT-induced phase shifts of neuronal activity in the suprachiasmatic nucleus of the hypothalamus, suggesting a potential role in the control of circadian rhythms. However, further characterization of the pharmacological role of this receptor subtype has been hindered by a lack of selective ligands.

Workers from SmithKline Beecham Pharmaceuticals (Harlow, Essex, UK) have previously reported the identification of the sulfonamide, SB258719 (**i**), as the first potent 5-HT$_7$-receptor antagonist with 100-fold selectivity over a wide range of receptors, including most 5-HT receptor subtypes, α_{1b}, D2 and D3 receptors[1]. As an extension of this work, the group has recently undertaken a conformational study of the flexible side-chain of (**i**)[2]. This led to the identification of a particularly low energy conformation in which the two methyl groups are gauche to each other. The synthesis of conformational mimics resulted in the identification (*R*)-3-(2-(2-(4-methylpiperidin-1-yl) ethyl)pyrrolidine-1-sulfonyl)phenol (SB269970) (**ii**), which has a pK$_i$ of 8.9 for the 5-HT$_{7(a)}$ receptor and >250-fold selectivity for this receptor subtype over 13 other receptors apart from the 5-HT$_{5a}$ receptor where 50-fold selectivity was observed.



(i)



(ii)

A functional model of 5HT$_7$ receptor activation, which examined adenylate cyclase activity in HEK 293 cells stably expressing the human 5-HT$_{7(a)}$ was used to confirm that this compound was an antagonist. This novel, potent and selective 5-HT$_7$-receptor antagonist will be a useful tool to further evaluate the therapeutic potential of 5-HT$_7$-receptor ligands.

1 Forbes, I.T. *et al.* (1998) (*R*)-3,*N*-Dimethyl-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzenesulfonamide: The first selective 5-HT$_7$ receptor antagonist. *J. Med. Chem.* 41, 655–657

2 Lovell, P.J. *et al.* (2000) A novel, potent, selective 5-HT$_7$ antagonist: (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol (SB269970). *J. Med. Chem.* 43, 342–345

Cocaine antagonist

Cocaine abuse is becoming of considerable concern to governments and medical associations in the developed world as the number of addicts with impaired ability to function at work or socially increases. Cocaine acts by inhibiting the reuptake of dopamine into dopaminergic neurons through a specific interaction with the dopamine transporter protein.

Wang, S. and coworkers recently used 3-D-database pharmacophore searching to identify a novel dopamine transport inhibitor, 4-hydroxy-1-methyl-

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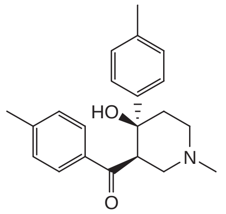
4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone (**iii**) with K_i values of 492 and 360 nM for dopamine transporter binding and dopamine reuptake inhibition, respectively[3]. This compound has significant functional antagonistic effects against cocaine and a different *in vitro* pharmacological profile from cocaine at dopamine, serotonin and norepinephrine transport sites. An examination of the structure–activity relationships and the use of molecular modelling led to the identification of a high-affinity analogue (**iv**).



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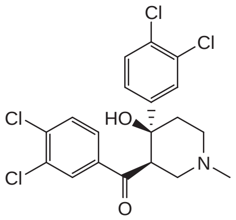
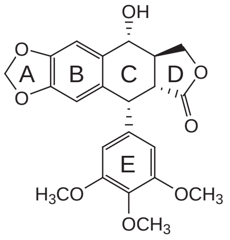


(iv)

This compound partially mimics the effect of cocaine in increasing locomotor activity in mice but lacked the cocaine-like discriminator stimulus in rats. This interesting observation warrants the further investigation of compounds of similar nature as a potential treatment of cocaine abuse.

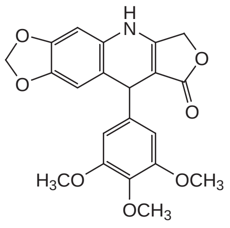
Aza-podophyllotoxin as potent cytotoxic agents

The inhibition of microtubule assembly offers a potential approach for the development of novel anticancer drugs. Podophyllotoxin (**v**) is an antitumour ligand found mainly in the plants *Podophyllum peltatum* and *Podophyllum emodi* and prevents microtubule assembly through an interaction with tubulin.

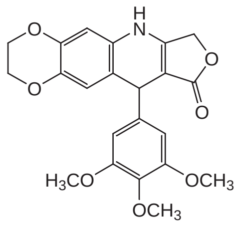


(v)

Hitotsuyanagi, Y. and coworkers have described the synthesis and cytotoxic activity of a series of 4-aza-2,3-dehydro-4-deoxypodophyllotoxin analogues against P-388 leukaemia cells[4].



(vi)

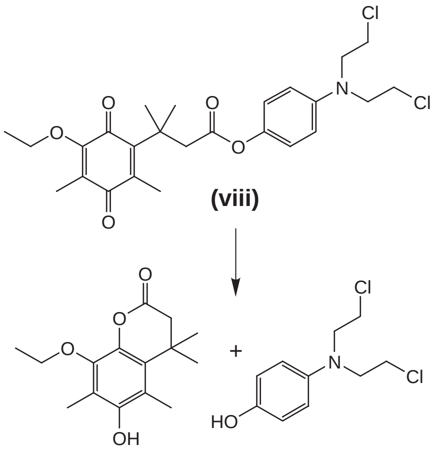


(vii)

These studies demonstrated that the steric effects of substituents on the B ring on activity are greater than their electronic effects. Modification to the E ring also indicated that the methoxy-group on this ring is not essential for potent cytotoxic activity. Analogues (**vi**) and (**vii**) were found to be more than twice as potent (IC_{50} = 1.8 and 1.7 ng ml^{-1}, respectively) than the natural podophyllotoxin (**v**).

Hypoxia-sensitive antitumour compounds

The poor vascularization of solid tumours provides an opportunity to use bioreductive prodrugs to target the hypoxic environment of the tumour. These drugs usually combine a redox sensitive moiety such as a quinone, *N*-oxide or nitro group with a cytotoxic species, which is then released on one- or two-electron reduction of the promoiety. An extension of this approach using a novel dual prodrug approach is reported in a recent patent evaluation[5]. The approach involves the enzymatic reduction of a (1,4-benzoquinonyl) alkanoic acid (**viii**) followed by an internal substitution reaction to give a coumarin (**ix**) and the activated nitrogen mustard (**x**).



(viii)

(ix) **(x)**

This offers the advantage over the use of reduction-only prodrugs of enabling the release of the same nitrogen

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[3] Wang, S. *et al.* (2000) Discovery of a novel dopamine transporter inhibitor, 4-hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone, as a potential cocaine antagonist through 3D-database pharmacophore searching, molecular modeling, structure–activity relationships, and behavioural pharmacological studies. *J. Med. Chem.* 43, 351–360

[4] Hitotsuyanagi, Y. *et al.* (2000) 4-Aza-2,3-dehydro-4-deoxypodophyllotoxins: simple aza-podophyllotoxin analogues possessing potent cytotoxicity. *Bioorg. Med. Chem. Lett.* 10, 315–317

mustard from a structurally diverse range of parent molecules. This enables the adjustment of the parent compounds redox potential to match that of the hypoxic cancer cell without interfering with the DNA crosslinking ability of the DNA mustard.

5 Anon (2000) Nitrogen mustard derivatives of (1,4-benzoquinonyl)alkanoic acids as hypoxia-sensitive antitumour agents. *Exp. Opin. Ther. Patents* 10, 507–511

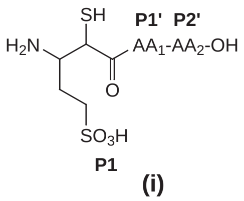
Andrew Lloyd

Combinatorial chemistry

Subsite preferences in aminopeptidase A

Aminopeptidase A (APA) or EC 3.4.11.7, is a membrane-bound zinc metallopeptidase that specifically cleaves acidic N-terminal amino acids from peptide substrates. The enzyme has significant homology with aminopeptidase N (APN), another peptidase that cleaves hydrophobic and basic N-terminal residues from peptides. To gain a better understanding of the physiological function of APA in brain and peripheral tissues it is necessary to identify efficient and selective inhibitors. A combinatorial approach has been used to investigate the subsite preferences of APA (Ref. 1).

Amastatin is generally used as an APA inhibitor but this compound is not selective and, in particular, has affinity for APN. This study investigated the combinatorial synthesis of thiol-containing compounds of the structure (**i**). It was found that the introduction of a sulphonate into the P1 position, a hydrophobic group into P1′, and a (3*R*)-carboxyproline in P2′ gave rise to highly selective and efficient inhibitors of APA.



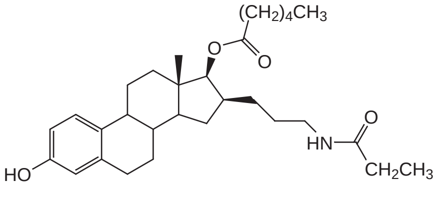
(i)

1 David, C. *et al.* (1999) Investigation of subsite preferences in aminopeptidase A (EC 3.4.11.7) led to the design of the first highly potent and selective inhibitors of this enzyme. *J. Med. Chem.* 42, 5197–5211

Solid-phase synthesis of phenolic steroids

Both 17β-hydroxysteroid dehydrogenase and the oestrogen receptor can bind oestradiol as a natural ligand/substrate and appear to play key roles in oestrogen-sensitive diseases such as breast and endometrium cancers. These two targets can be blocked by two different drugs or the same drug, offering a novel therapy for these cancers. A recent paper describes a method for the solid-phase combinatorial synthesis of phenolic steroids with relevance for these protein targets[2].

A survey of possible solid-phase linker methods for the steroid phenol group revealed that a photolabile *o*-nitrobenzyl ether linker was most effective in generating products (e.g. **ii**) in excellent yields and purities. This study of library methods has set the scene for the generation of libraries of oestradiol-related compounds that could be tested for affinity for inhibition of the oestradiol binding proteins.



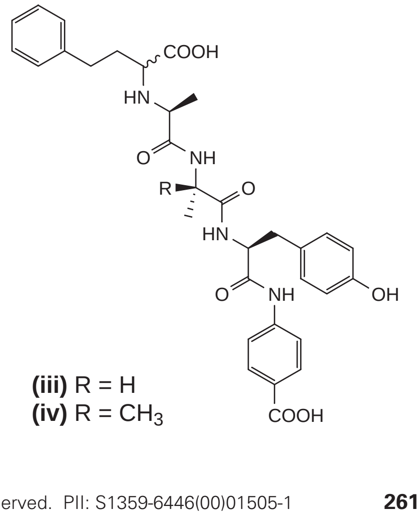
(ii)

2 Tremblay, M.R. and Poirier, D. (2000) Solid-phase synthesis of phenolic steroids: from optimization studies to a convenient procedure for combinatorial synthesis of biologically relevant estradiol derivatives. *J. Comb. Chem.* 2, 48–65

Inhibitors of EC 3.4.24.15

In vitro, the neutral metalloendopeptidase EC 3.4.24.15 hydrolyses several biologically active peptides including bradykinin and neurotensin. Although it might have a physiological role in brain and endocrine function, further investigations of its function have been restricted by the lack of a stable potent inhibitor. To date, the use of the most frequently used inhibitor, *N*-[1-(*R,S*) carboxy-3-phenylpropyl]-Ala-Ala-Tyr-*p*-aminobenzoate (cFP, **iii**), has been limited by rapid hydrolysis of the Ala-Tyr bond. Therefore, a recent study has investigated the design and solid-phase synthesis of novel stable inhibitors of EC 3.4.24.15 (Ref. 3).

cFP was used as a template for the solid-phase preparation of compounds in which the scissile bond has been replaced by groups that are more stable to hydrolysis. The compound in which the Ala-Tyr amide bond had been reduced to an aminomethyl group had a reduced affinity for the enzyme by some thousand-fold. However, replacement of the Ala residue with aminoisobutyric acid produced compound (**iv**) with a K$_i$ of 23 nM. Furthermore, this compound is stable to hydrolysis and does not inhibit angiotensin converting enzyme, or other related thermolysin-like or neutral endopeptidases. The compounds therefore provide a valuable tool for the further investigation of the physiological function of EC 3.4.24.15.



(iii) R = H
(iv) R = CH$_3$