Monitor: molecules and profiles

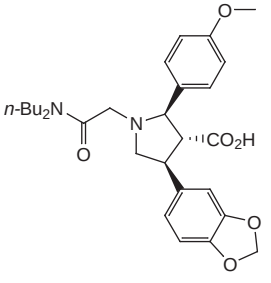
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and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

ET_A-receptor antagonists

The endothelins ET-1, ET-2 and ET-3 are potent vasoconstricting and mitogenic peptides that might be involved in a variety of disease states including hypertension, congestive heart failure, restinosis and renal failure. There are two endothelin receptor subtypes ET_A and ET_B. ET_A receptors mediate vasoconstriction and smooth muscle cell proliferation whilst ET_B receptors mediate various effects depending on the tissue type, including stimulating nitric oxide release from endothelial cells, which in turn causes a vasodilatory effect. There is clearly an advantage in developing selective ET_A-receptor antagonists as therapeutic agents to avoid the inhibition of the potentially useful action of the endothelins on ET_B receptors. Such agents would also be useful for determining the role of endothelin in different tissues.

A recent paper from workers at Abbott Laboratories (Abbott Park, IL, USA) describes the discovery of a series of pyrrolidine-based endothelin-receptor antagonists with enhanced ET_A-receptor selectivity [Boyd, S.A. *et al.* (1999) *Bioorg. Med. Chem.* 7, 991–1002].

The pyrrolidine carboxylic acid A127722 (**1**) has previously been disclosed as a potent ET_A-selective receptor antagonist that is presently undergoing clinical trials. Studies examining the 2-substituents on the pyrrolidine showed that the 2-alkyl substituents had improved selectivity for the ET_A receptor compared to that attained with A127722 (1400-fold selectivity). The most effective of these compounds showed >10,000-fold selectivity for the ET_A receptor over the ET_B receptor. One such compound (**2**) was shown to retain good oral bioavailability (39%) in rats despite the introduction of lipophilic substituents.



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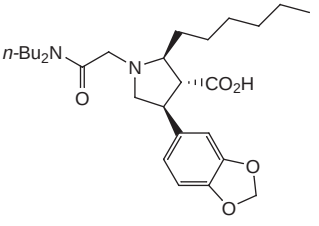

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Dual acting α_1-adrenoreceptor antagonist and steroid 5α-reductase inhibitor

Benign prostatic hyperplasia commonly occurs in aged males. The disease gives rise to a range of urological symptoms including increased frequency of urination, poor urine stream and delayed initial urine flow. The symptoms may be alleviated through treatment with α_1-adrenoreceptor antagonists that relax the smooth muscle of the prostate and urethra. The hyperplastic growth of the prostate is thought to be because of the action of dihydrotestosterone, which is produced from testosterone by 5α-reductase. As a consequence, a number of 5α-reductase inhibitors are under development and in clinical trials in the US and Europe. The development of a dual-acting agent that acts as an antagonist at the α_1-adrenoreceptor and inhibits 5α-reductase would offer some advantages in the treatment of urinary tract disorders caused by benign prostatic hyperplasia.

Workers at Zeria Pharmaceuticals Co. Ltd (Saitama, Japan) have recently

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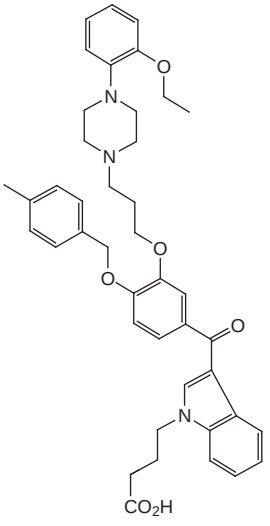
reported a study of a series of arylpiperazines as potential dual-acting agents [Sato, H. *et al.* (1999) *Bioorg. Med. Chem. Lett.* 9, 1553–1558]. Structure activity studies have led to the identification of (**3**) as a potential lead compound with a pA_2 value of 7.5 for α_1-adrenoreceptor antagonism and an IC_{50} value of 1.5 nM for 5α-reductase inhibition. The group also studied the effect of this agent on phenylephrine-induced increases in uretheral pressure in anaesthetized rabbits with (**3**) showing a dose-dependent inhibition at a dose of 0.03–0.3 mg kg^{-1}. This compound was also shown to cause inhibition of prostatic 5α-reductase in rats in a dose-dependent manner with an ED_{50} of 7.9 mg kg^{-1}.



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Emerging molecular targets

Mast cell tryptase inhibitors

Asthma affects approximately 15 million people in the US alone. Although this disease is generally not fatal, the economic burden on healthcare providers for the provision of treatments for sufferers is substantial, as is the effect of the disease on quality of life. This disease results in airway inflammation and associated airflow obstruction in response to normally tolerated stimuli. Existing treatments usually combine the use of bronchodilators for sympathomatic relief of bronchochonstricion to temporarily improve pulmonary function and inhaled corticosteroids that reduce airway inflammation by inducing eosinophil and mast cell apoptosis. Although such treatments are clinically effective, the use of steroids is limited by their associated side effects. These problems have led to the development of novel therapeutic approaches based on the selective inhibition of inflammatory mediators.

Mast cell activation and degranulation plays an important role in the pathogenesis of asthma. Activated mast cells release potent proinflammatory mediators that result in bronchoconstriction and granulocyte influx. Tryptase is a serine protease with trypsin-like activity found almost exclusively, in relatively high concentrations, in the secretory granules of mast cells.

A recent review of the proteolytic and cellular activities of tryptase clearly indicates that this enzyme is responsible for a wide range of pro-inflammatory processes [Elrod, K.C. and Numerof, R.P. (1999) *Emerg. Ther. Targets* 3, 203–212]. The review also highlights evidence from studies on tryptase inhibitors in animal models and Phase II clinical trials, which support the further development of such agents as novel treatments for the control of airway inflammation in asthma.

DNA-ligase IV

A recent paper from a group at the University of Sussex (Brighton, UK) describes the identification of a defect in DNA-ligase IV in cells from a leukaemic patient [Riballo, E. *et al.* (1999) *Curr. Biol.* 9, 699–702]. The defect in this protein impairs the ability of the enzyme to repair double-stranded breaks in damaged DNA and might therefore cause predisposition to leukaemia. The patient from whom the cells were derived had died following radiotherapy for the leukaemia. The impaired DNA-ligase IV undoubtedly contributed to the patient's acute radiosensitivity, preventing the repair of DNA damage to normal cells caused by the radiotherapy. Patient variation in clinical radiosensitivity is a major barrier to optimizing cancer radiotherapy. These results suggest that this might, in part, be attributed to variations in the ability to repair damaged DNA and might offer a means of screening for potential radiosensitivity through the quantification of DNA-ligase IV activity in patients undergoing radiotherapy.

Furthermore it might ultimately be possible to utilize gene therapy to correct the gene deficiency resulting in the DNA-ligase IV defect to reduce the onset of leukaemia. Furthermore, it might be possible to increase radioresistance of normal cells through the upregulation of DNA-ligase IV.

Cathepsin K – a target for the treatment of osteoporosis

Osteoporosis is characterized by low bone density, high porosity and brittleness leading to increased bone fragility and the likelihood of fractures. The disease affects all individuals regardless of sex or race after 35 years of age. However, accelerated bone loss is most predominant in postmenopausal women. The disease is caused by an imbalance in the bone remodelling process.

Bone remodelling is an ongoing process involving the deposition and resorption of bone matrix. Bone resorption is mediated by osteoclasts, which are multinucleated giant cells of the monocyte–macrophage lineage. The cells solubilize the bone matrix through the secretion of proteolytic enzymes into a low-pH sealed cleft formed between the cell and the underlying bone matrix. Cathepsin K is the most abundant protease secreted by osteoclasts and, therefore, appears to play a pivotal role in the degradation of bone-associated proteins.

A recent review has discussed the potential use of cathepsin K inhibitors as an alternative to oestrogen replacement therapy for the treatment of postmenopausal osteoporosis [Smith, W.W. and Abdel-Meguid, S.S. (1999) *Exp. Opin. Ther. Patents* 9, 683–694]. The review highlights the successful application of modern drug discovery processes through reference to recent papers and patents, including genomics and structure-based drug design, the identification of this novel molecular target, and rapid identification of novel, potent and selective inhibitors of cathepsin K.

Inhibitors of this cysteine protease might also have uses in adjuvant therapy following the implantation of orthopaedic implants where inhibition of bone resorption might offer a means of enhancing the rate of osteointegration of implanted devices.

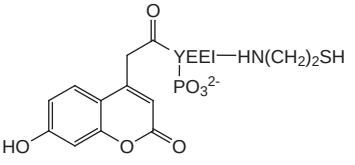
Andrew Lloyd

Combinatorial chemistry

High-affinity SH2-targeted ligands

The Src-homology-2 (SH2) domain plays a key role in the recognition of specific protein sequences that contain a phosphotyrosine residue. Attempts to design agents that bind to the SH2 domain has been driven by a desire to interrupt signalling pathways that are known to be responsible for various diseases. Although the affinity of peptides for the SH2 domain lies in the 200–800 nM range, non-peptidic ligands are generally one to three orders of magnitude less active.

A recent publication describes the parallel solid-phase synthesis of peptide and non-peptide conjugates that have a high affinity for the SH2 domains of LCK and FYN [Lee, T.R. and Lawrence, D.S. (1999) *J. Med. Chem.* 42, 784–787]. Using the Fmoc-protection protocol, a phosphotyrosine-containing peptide attached to the solid phase through a disulfide linker was derivatized by a range of carboxylic acids to generate 900 analogues. Cleavage of the products allowed screening of their ability to bind to SH2 domains using an enzyme-linked immunosorbent assay (ELISA). Although the majority of compounds failed to display any affinity in the ELISA assay, several compounds, including the coumarin derivative (**1**) and the sulfonated compound (**2**), are among the most potent SH2-targeted agents yet identified.



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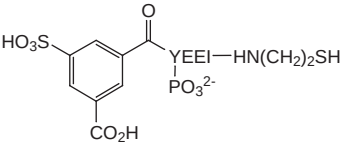

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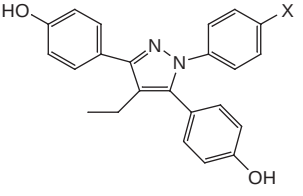
These two key compounds also demonstrated an unprecedented ability to discriminate between the SH2 domains of two members of the Src family of protein kinases.

Oestrogen-receptor ligands

Natural and synthetic compounds affecting oestrogen levels have an important role as agents for the control of fertility, hormone-responsive breast cancer and for menopausal hormone replacement therapy. Most synthetic oestrogens retain a phenolic function, but there are many other synthetic oestrogens including both cyclic and acyclic systems. A key goal of medicinal chemistry has been to obtain agents that have high levels of tissue selectivity. The latest chapter in this process is the design of novel oestrogen-receptor ligand-templates that can be synthesized by simple condensation reactions typical of those used in combinatorial chemistry [Fink, B.E. *et al.* (1999) *Chem. Biol.* 6, 205–219].

Many oestrogen-receptor ligands display a central core structure containing peripheral structural elements and in this paper, a number of 1,2- and 1,3-azole heterocycles (imidazoles, thiazoles, oxazoles and pyrazoles) were prepared and screened as receptor ligands. The binding values, obtained from a competitive radiometric-binding assay using tritiated oestradiol, demonstrated that of all these systems, pyrazoles had the highest binding affinity. In particular, compounds (**3**) and (**4**) had high levels of binding and showed close conformational relationships to the non-steroidal ligand, raloxifene. The solid-phase synthesis of related pyrazole libraries is under way in the search for more potent and selective oestrogen-receptor ligands.



3 X = H
4 X = OH

Thymidylate synthase inhibitors

Thymidylate synthase (TS) catalyzes the final step in the biochemical pathway leading to thymidylate and has been nominated as a target for the discovery of new anticancer and antimicrobial drugs. The X-ray structure of TS obtained from *Lactobacillus casei* (LcTS) has been derived, and was the starting point for the design of novel library-derived inhibitors [Tondi, D. *et al.* (1999) *Chem. Biol.* 6, 319–331]. 153,516 compounds from the available chemicals directory were screened against the X-ray structure using the molecular-docking program, DOCK, and five high-ranking compounds were selected for screening. Of these, dansyltyrosine was selected for solid-phase synthesis of 33 analogues.

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