Monitor: molecules and profiles

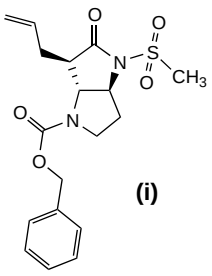
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and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

Inhibition of intracellular neutrophil elastase

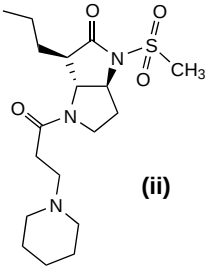
Human neutrophil elastase (HNE) is a serine protease that is stored in granules within neutrophils until recruitment and activation of the neutrophil, when it is released. Excessive sustained elastase activity causes damage to tissue, particularly lung elastin; there is therefore a great deal of interest in the inhibition of HNE to treat respiratory diseases such as emphysema and chronic bronchitis.

A group at GlaxoWellcome (Stevenage, UK) had previously disclosed that pyrrolidine *trans*-lactams are inhibitors of serine proteases such as elastin[1]. Molecule **(i)** was co-crystallized with porcine pancreatic elastase and the structure shows ring-opening of the lactam and acylation of the catalytic serine (Ser195)[2]. The catalytic histidine residue (His57) is displaced out of position, which contributes to the observed prolonged stability of the acylated enzyme.



(i)

The lactam *N*-substituent serves as an activating group for nucleophilic opening of the lactam ring. An electron-withdrawing group, which has a tetrahedral geometry adjacent to the nitrogen, was found to be essential for activity. The methylsulfonamide and 2-naphthylsulfonamide were found to be the most potent, with the methyl analogue proving to be the most stable to liver microsomal enzymes and, therefore, preferred. To improve aqueous solubility, the benzyloxycarbonyl group of **(i)** was replaced by the basic piperidine derivative to give compound **(ii)**.



(ii)

Compound **(ii)** was found to be orally active, exhibiting 76% and 41% elastase inhibition in hamster broncheolar lavage at 20 and 10 mg kg^{-1}, respectively. Clearance of **(ii)** was still found to be high (140 mg min^{-1} kg^{-1}) upon intravenous administration in the hamster, yet with a high oral bioavailability of 49%. This suggests that the clearance is not predominantly hepatic, and was later determined to be a result of the opening of the lactam ring, giving a short half-life ($t_{1/2}$) of 0.2 h. Despite the short half-life, the inhibitory effect on elastase is prolonged to >20 h. This is because the inhibitor penetrates the circulating neutrophils and irreversibly inhibits the stored elastase. When the neutrophils are recruited they release inactive elastase. There is thus no need for a sustained therapeutic concentration of the inhibitor.

A suitable development candidate with lowered clearance and improved *in vivo* potency will be described in due course.

1 Macdonald, S.J. *et al.* (1998) Syntheses of *trans*-5-oxo-hexahydro-pyrrolo[3,2-b]pyrroles and *trans*-5-oxo-hexahydro-furo[3,2-b]pyrroles (pyrrolidine *trans*-lactams and *trans*-lactones): new pharmacophores for elastin inhibition. *J. Med. Chem.* 41, 3919–3922

2 Macdonald, S.J. *et al.* (2001) Intracellular inhibition of human neutrophil elastase by orally active pyrrolidine-*trans*-lactams. *Bioorg. Med. Chem. Lett.* 11, 243–246

Microsomal triglyceride transfer-protein inhibitors

The main cause of death in the Western world, despite recent medical advances, is coronary heart disease (CHD). An important risk factor for CHD is elevated low-density lipoprotein (LDL) cholesterol. The treatment of hypercholesterolemia with statin therapy has been shown to reduce the incidence of CHD significantly. Other risk factors include low levels of high-density lipoprotein (HDL) and high

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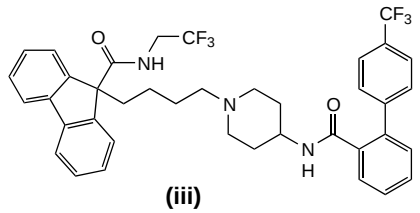
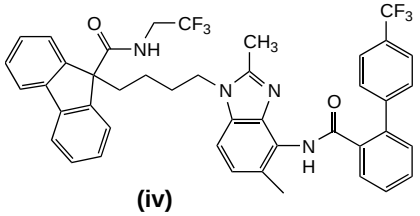
levels of LDL triglycerides. There is increasing focus on trying to further reduce CHD by augmenting statin therapy with the treatment of associated factors such as hyper-triglyceridemia.

Microsomal triglyceride-transfer protein (MTP) is involved in the synthesis of very-low-density lipoprotein (VLDL), the direct precursor to LDL. An inhibitor of MTP, BMS201038 **(iii)**, has entered clinical trials. The Bristol-Myers Squibb (BMS) Pharmaceutical Research Institute (Princeton, NJ, USA) sought to identify a suitable back-up candidate[3]. A concern was the piperidine ring, a nearly ubiquitous pharmacophore for G-protein-coupled receptors. A whole range of alternative functionalities was tried, with a particular focus on improving the aqueous solubility of such lipophilic molecules. Heteroaryl scaffolds facilitate the incorporation of additional substituents and compound **(iv)** was eventually identified.



(iii)



(iv)

Compound **(iv)** was found to be a more potent inhibitor of human MTP both *in vitro* (IC_{50} = 1 nM for **(iv)** and = 8 nM for **(iii)**, MTP triglyceride-transfer assay) and in a cell-based assay (IC_{50} = 0.03 nM for **(iv)** and = 0.8 nM for **(iii)**, apoB secretion from HepG2 cells). *In vivo*, **(iv)** was found to be a potent lipid-lowering agent reducing total cholesterol, VLDL and LDL cholesterol, HDL cholesterol and triglyceride by >50% in golden syrian hamsters and cynomolgus monkeys. A pharmacokinetic profile indicated good oral bioavailability in rats (81%), and reasonable oral bioavailability in the cynomolgus monkey (21%). The ED_{50} value for total cholesterol-lowering in the monkey was found to be 0.38 mg kg^{-1} compared with 2 mg kg^{-1} for the original candidate **(iii)**. Based on the superior potency of **(iv)** for lipid-lowering *in vivo* and an acceptable pharmacokinetic and safety profile compound, **(iv)** has been chosen as a back-up clinical candidate.

3 Robl, J.A. *et al.* (2001) A novel series of highly potent benzimidazole-based microsomal triglyseride transfer protein inhibitors. *J. Med. Chem.* 44, 851–856

D-Morphine: possible clinical implications for the management of neuropathic pain

There has been much interest in opioids that act as non-competitive antagonists at the *N*-methyl-D-aspartate (NMDA) receptor, in view of their possible use in the treatment of pain, either alone or in combination with opioid μ-receptor agonists. Clinical use of the most potent NMDA competitive antagonists (K_is in the nanomolar range) is frustrated by their neurotoxic effects. Electro physiological and receptor-binding studies have demonstrated that some opioids (e.g. methadone, ketobemidone and dextromethorphan) have non-competitive NMDA receptor activity similar to ketamine[4]. Other opioid-receptor agonists and antagonists (e.g. L-morphine, codeine, etorphine, fentanyl and naloxone) have no such activity[5]. However, relatively few of the opioids in current use have been examined in the NMDA tests.

Recently, Stringer *et al.*[6] have examined a wide range of opioids and their enantiomers, with the aim of providing further guidance in the choice of opioids for the treatment of neuropathic pain. Inhibition of [^{3}H] MK801 binding to rat forebrain synaptic membranes was used in the assay. Among others, they studied natural L-morphine, D-morphine, L-morphine-3-glucoronide (M3G) and L-morphine-6-glucoronide (M6G). Moreover, they introduced DL-ketamine and imipramine for comparison. Surprisingly, D-morphine showed a significant affinity for the NMDA receptor (K_i = 4.7 μM), comparable to that of ketamine (K_i = 0.8 μM). By contrast, the displacement by natural L-morphine was 47-times less (K_i = 160 μM). No significant displacement of the binding of the competitive NMDA-receptor antagonist [^{3}H] CGS19755 was observed with D-morphine.

These data suggest that DL-morphine could have application in the treatment of neuropathic pain states in place of natural L-morphine. This would possibly result in a reduction in analgesic tolerance and physical dependence. However, this approach, although attractive, must await the development of efficient means to prepare the racemate. Indeed, the racemization of natural L-morphine presents many problems.

An alternative approach that is being explored is to combine dextromethorphan, which has little μ-opioid receptor agonist activity but appreciable NMDA receptor antagonist activity, with a potent μ-opioid receptor agonist, lacking the NMDA-receptor antagonist activity (e.g. natural L-morphine). Open and double-blind studies in patients with chronic pain have already shown that one such combination (Morphidex®, Algos Pharmaceuticals Corporation, Neptune, NJ, USA) provides satisfactory pain relief at a morphine dose lower than that required using morphine alone.

4 Ebert, B. *et al.* (1995) Ketobemidone, methadone and pethidine are non-competitive NMDA antagonists in the rat cortex and spinal cord. *Neurosci. Lett.* 187, 165–168

5 Yamakura, T. *et al.* (1999) Direct inhibition of the *N*-methyl-D-aspartate receptor channel by high concentrations of opioid. *Anesthesiology* 91, 1053–1063

6 Stringer, M. *et al.* (2000) D-Morphine, but not L-morphine, has low micromolar affinity for the non-competitive *N*-methyl-D-aspartate site in rat forebrain. Possible clinical implications for the management of neuropathic pain. *Neurosci. Lett.* 295, 21–24

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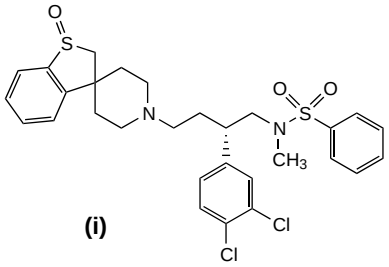
Novel antiviral molecules

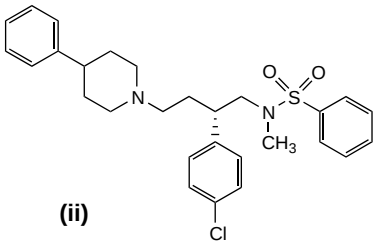
CCR5 antagonists as anti-HIV agents

Antagonists of the CCR5 chemokine receptor have attracted a lot of attention as potential anti-HIV agents. This is because HIV-binding to this receptor is required for viral entry and infection. Although the CXCR4 chemokine receptor also has a role in HIV-infection, it is CCR5 that has garnered most of the attention because HIV-1 variants that recognize this receptor predominate during early stages of infection. In fact, viruses that exclusively rely on the CXCR4 receptor for entry are rarely isolated from patients.

Two papers from researchers at Merck (Rahway, NJ, USA) describe the identification and SARs of a series of compounds that bind CCR5 and prevent infection by HIV[1,2]. In the first report, compounds similar to **(i)** were identified through screening of the corporate compound collection. This compound inhibits the binding of macrophage inflammatory protein-1α (MIP-1α) to the CCR5 receptor with an IC_{50} value of 35 nM, thereby inhibiting viral replication in tissue culture with an IC_{95} value of 6–12 μM. Initial SAR studies of this chemotype revealed that the spirocyclic sulfoxide could be simplified, as in **(ii),** but the phenyl ring and the sulfonamide were sensitive to modification. The *N*-methyl-phenylsulfonamide appears to be optimal for this portion of the molecule because all attempts at isosteric replacement resulted in a loss in potency. Substitution of the phenyl ring had a dramatic effect on activity with the 3-monosubstituted phenyl ring and unsubstituted phenyl ring being preferred.



(i)



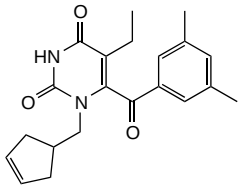
(ii)

1 Dorn, C.P. *et al.* (2001) Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 1. Discovery and initial structure–activity relationships for 1-amino-2-phenyl-4-(piperidinyl-1-yl)butanes. *Bioorg. Med. Chem. Lett.* 11, 259–264

2 Finke, P.E. *et al.* (2001) Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 2. Structure–activity relationships for substituted 2-aryl-1-(*N*-(methyl)-*N*-(phenylsulfonyl)amino]-4-(piperidin-1-yl)butanes. *Bioorg. Med. Chem. Lett.* 11, 265–270

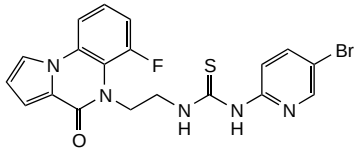
Non-nucleoside reverse transcriptase (NNRTI) inhibitors

A unique non-nucleoside reverse transcriptase inhibitor (NNRTI), SJ3366 (**iii**), has been developed by Buckheit and colleagues[3]. Although the compound is structurally related to the 1-[(2-hydroxyethoxy)methyl]-6-(phenyl-thio)thymine (HEPT)-based inhibitors, its uniqueness comes from the fact that it is active against both HIV-1 and HIV-2 and has a dual mode of action: inhibition of reverse transcriptase (RT) and inhibition of viral entry. The compound inhibits isolated RT derived from HIV-1, with a K_i value of 3.2 nM, but is inactive against RT from HIV-2. Its main mode of action against HIV-2, therefore, appears to be as an entry inhibitor. SJ3366 was evaluated against several laboratory and clinically isolated strains in established or fresh human cell preparations, yielding inhibition activities of 0.6–10 nM (HIV-1) and 3–480 nM (HIV-2) with little toxicity. Reduced activity was observed against HIV-1 strains possessing the Y181C, K103N and Y188C mutations in the RT enzyme, however, the compound selected for a virus having the Y181C mutation after five cell passages.

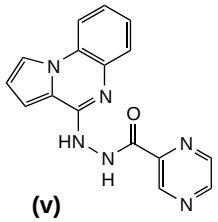


(iii)

In a separate study, quinoxalinylethylthioureas (QXPTs) such as compound **(iv)**, have been identified as potent, non-nucleoside inhibitors of HIV-1. Unfortunately, **(iv)** and related analogues display poor pharmacokinetic properties, having low oral bioavailability. In recent work by Campiani and coworkers[4], this chemotype is further explored to



(iv)



(v)

increase potency and enhance oral bioavailability. SAR studies generated several potent analogues based on the QXPT chemotype; however, like the parent compound, these displayed poor bioavailability. Presumably this is because of first-pass metabolism associated

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