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

Respiratory syncytial virus inhibitor

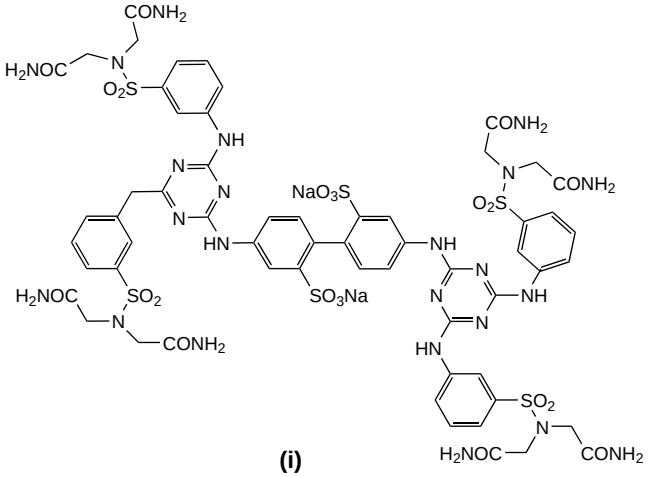
Although respiratory syncytial virus (RSV) is known to be a significant cause of respiratory infection, only one small molecule drug, ribavirin, is licensed for treatment. Potential toxicity and high cost restrict the use of this agent. Therefore, to further expand the range of therapeutic agents available against RSV, several groups are engaged in the search for new inhibitors directed at various viral targets. In a recent publication, researchers at Wyeth-Ayerst (Pearl River, NY, USA) describe the discovery and pre-clinical activity of a fusion inhibitor, RFI-641 **(i)** (Ref. 1). This compound was found to inhibit six laboratory and 18 clinically isolated strains of RSV at concentrations of between 0.008 and 0.11 μM. Furthermore, the compound was found to reduce viral titers in several animals, such as mice, cotton rat and African green monkeys, resulting in a 1.7 log drop in titer in the African green monkeys. Based on its preclinical profile, this compound has been advanced to Phase I clinical trials.

1 Nikitenko, A.A. *et al.* (2001) The discovery of RFI-641 as a potent and selective inhibitor of the respiratory syncytial virus. *Bioorg. Med. Chem. Lett.* 11, 1041–1044

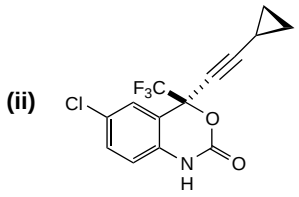
Novel NNRTI analogues as anti-HIV-1 agents

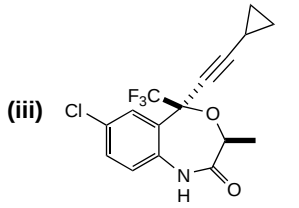
Although non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine, delavirdine and efavirenz have proven to be effective in the context of highly active anti-retroviral therapy (HAART), several patients develop resistance to these drugs. Interestingly, resistance to this class of drug develops rapidly and is most often associated with a single mutation, most prominently Y181C or K103N, in HIV reverse transcriptase[2]. As a result, several groups have taken up the cause to develop second-generation NNRTIs, which are active against the K103N mutant strain and other resistant strains of HIV.

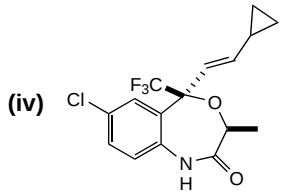
With this in mind, a group at DuPont (Wilmington, DE, USA) has synthesized and examined derivatives of efavirenz **(ii)**, where the oxazinone-ring system has been modified[3]. Thus, compounds **(iii)** and **(iv)**, having a benzoxazepinone in place of the oxazinone ring, were synthesized using chemistry similar to that



(i)







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used for the synthesis of **(ii)**. Both compounds were highly potent against a wild-type strain of the HIV virus in cell culture (IC_{90} = 2.2 and 2.5 nM, respectively). Both compounds were 10-fold less active against virus carrying the K103N mutation (IC_{90} = 29 and 35 nM, respectively), but were deemed potent enough to warrant evaluation *in vivo*. Here they demonstrated sufficient plasma levels at 24 h after dosing [C_{24h} = 1.14 and 1.4 μM (dose = 10 mg kg^{-1}), respectively].

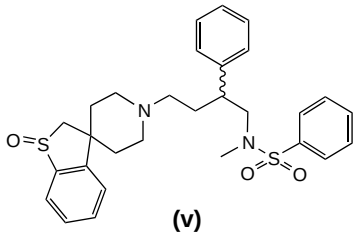
2 Deeks, S.G. (2001) Nonnucleoside reverse transcriptase inhibitor resistance. *J. Acquired Immune Defic. Syndr.* (Suppl.) 26, 25–33

3 Cocuzza, A.J. *et al.* (2001) 4,1-Benzoxazepinone analogues of efavirenz (Sustiva™) as HIV-1 reverse transcriptase inhibitors. *Bioorg. Med. Chem. Lett.* 11, 1389–1392

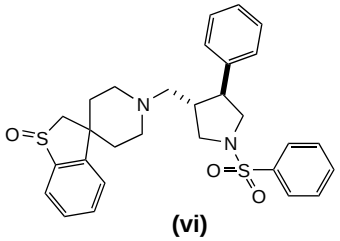
Pyrrolidine-based CCR5 receptor antagonists

Current therapy against HIV infection involves combination therapy with up to three antiviral compounds. These agents target either viral reverse transcriptase or HIV-protease, but the development of resistance to these drugs continues to be a significant problem. In order to overcome this, several groups have sought other viral targets to attack so that the success achieved with combination therapy can be extended to those for whom current treatment fails. Not only that, in recent years AIDS has become more appreciated as a worldwide public health problem, necessitating new drugs that can be developed easily and at less expense.

One of the more promising areas of research to emerge in the last few years has been the development of compounds that inhibit the entry of HIV, thus preventing infection. Macrophage-tropic strains of the virus, those responsible for early infection, bind to the host-cell receptor, CC chemokine receptor 5 (CCR5), which is required for viral entry. An important observation was the discovery that the endogenous ligands to this receptor, macrophage inflammatory protein-1α (MIP-1α), MIP-1β and RANTES (regulation upon-activation, normal T-cell expressed and secreted), were shown to inhibit infection of CD4+ T-cells. Since then, several companies have found small organic molecules that bind to the CCR5 receptor and thus act as antiviral agents[4]. A promising class of compounds, 2-aryl-4-(piperidin-1-yl)butanamine analogues, represented by compound **(v)**, has been disclosed by Merck (Rahway, NJ, USA)[5]. This compound binds to CCR5, inhibiting the binding of labelled [^{125}I]-MIP-1α (IC_{50} = 68 nM). As such, it is suggested that this binding will also inhibit viral entry, just like the endogenous ligand it displaces.



(v)



(vi)

A recent report from Merck[6] further explores this series of compounds. More specifically, it explores cyclic analogues, which possess the same pharmacophoric elements as **(v)** but employ a pyrrolidine-based scaffold. Of all the possible stereoisomers of the pyrrolidine, compound **(vi)** (IC_{50} = 26 nM) was found to be the most active at displacing [^{125}I]-MIP-1α from CCR5. The corresponding enantiomer of **(vi)** and the analogue with a cis-configuration around the pyrrolidine were found to be inactive or significantly less active, respectively. These results might provide some insight into the spatial relationship between the pharmacophoric elements attached to **(v)** and **(vi)**.

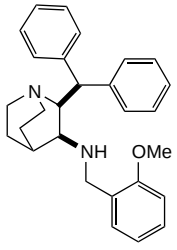
4 Blair, W.S. *et al.* (2000) HIV-1 entry – an expanding portal for drug discovery. *Drug Discov. Today* 5, 183–194

5 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

6 Hale, J.J. *et al.* (2001) 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 1: Discovery of the pyyrolidine scaffold and determination of its stereochemical requirements. *Bioorg. Med. Chem. Lett.* 11, 1437–1440

Substance P antagonist inhibits HIV replication

Substance P (SP) has been suggested to have a role in HIV infection of monocyte-derived macrophages. This is supported by the presence of SP receptors on these cells and their production of SP. As such, SP is believed to have an autocrine role in the regulation of macrophage function. In a recent paper by Lai *et al.*[7], it is suggested that SP maintains CCR5 expression and thus supports HIV infection.



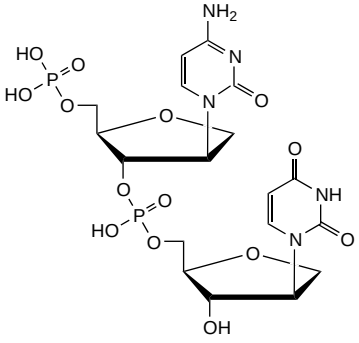
(vii)

Evidence for this comes from the fact that SP enhanced the replication of HIV in human blood-isolated phagocytes. At the same time, CP96345 **(vii)**, an SP antagonist, was found to inhibit HIV infection of these cells. Furthermore, only R5 (CCR5-dependent) strains were significantly inhibited by CP96345, suggesting that this drug inhibits an early event in the viral lifecycle. This, coupled with the observation that SP96345 also down-regulates CCR5 expression, leads to the conclusion that reduced receptor-expression, induced by the drug, is able to inhibit HIV infection.

7 Lai, J.P. *et al.* (2001) Substance P antagonist (CP-96,345) inhibits HIV-1 replication in human mononuclear phagocytes. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3970–3975

Dinucleotide inhibitors of HIV-integrase

HIV-integrase appears to be an optimal target for the development of antiviral agents effective against HIV. It is required for viral replication and there is no functional equivalent of HIV integrase in human cells, making it a rather unique enzyme. Integrase is multi-functional, catalyzing both the processing of the HIV cDNA genome by cleaving two nucleotides from the 3′-end of each strand and the insertion of the resulting double-stranded DNA into the host genome.



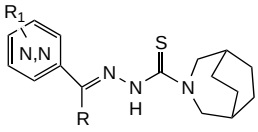
(viii)

A recent report by Taktakishvili and coworkers[8] describes the synthesis and initial evaluation of a non-natural dinucleotide capable of inhibiting both functions of HIV-integrase. The dinucleotide **(viii)** contains the non-natural nucleotide deoxyisouridine, wherein the base is attached to the 2′-position of the ribose template. *In vitro*, **(viii)** inhibits the cDNA-processing reaction, with IC_{50} = 7.5 μM, and the insertion reaction, with IC_{50} = 5.9 μM. Moreover, this compound is completely stable to hydrolysis by mammalian 3′- and 5′-exonucleases.

8 Taktakishvili, M. *et al.* (2001) Discovery of a nuclease-resistant, non-natural dinucleotide that inhibits HIV-1 integrase. *Bioorg. Med. Chem. Lett.* 11, 1433–1435

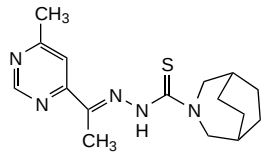
Cytotoxicity and antitumor activity of copper(II) and iron(II) complexes of thiosemicarbazones

The antitumor agent hydroxyurea (HU) is a clinically useful drug for the treatment of a wide range of solid tumors, as well as acute and chronic leukemia. HU is a specific inhibitor of DNA synthesis, its primary site of action being the enzyme ribonucleotide reductase (RR)[9]. Because HU is a relatively poor inhibitor of RR and has a short serum half-life, the search for more effective inhibitors was undertaken by several groups. Among others, a large series of thiosemicarbazone derivatives (TSCs) were reported and, in particular, the 5-hydroxypyridine TSC was chosen for clinical evaluation. However, as well as showing weak activity, this compound had a short half-life in man. In addition, it was toxic and poorly soluble in water[10]. Subsequently, it was found that complexation of the TSCs with iron and copper resulted in more potent compounds[11], which led to a new interest for this class. As a continuation of their studies in this field, Easmon and coworkers[12] recently reported a series of acyldiazinyl TSCs **(ix–xvi)**, which have an ^4N-azabicyclo[3.2.2]nonane group, and were investigated for their antiproliferative and antitumor activities.



(ix-xvi)

The *in vitro* cytotoxicity studies were performed on human acute lymphoblastic leukemia (CCRF-CEM) and colon adenocarcinoma (HT29) cell lines. The free ligands **(ix–xvi)** exhibited potent cytotoxic activity against CCRF-CEM cells (with IC_{50} values in the range of 0.005–0.77 μM) and HT29 cells (with IC_{50} values in the range of 0.011–2.22 μM). In all cases, the pyrimidine and the pyrazine



(xiii)

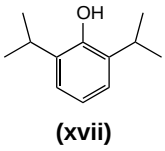
nucleus proved to be better than the pyridazine ring. Complexation of the ligands with copper(II) resulted in a significant improvement in their cytotoxicity. Selected compounds were further tested for their antiproliferative activity in a clonogenic assay, using human-tumor xenografts, and were found to be significantly active. Finally, compound **(xiii)** was tested *in vivo* using the large-cell lung carcinoma, LXFL529. Results from a combined dose-finding–antitumor study suggest that 30 mg kg^{-1} d^{-1} represents the maximal tolerable dose, at which a T/C (test/control) value of 58% was found. By contrast, at 10 mg kg^{-1} d^{-1} no effect on tumor growth was observed. Some conclusions were also drawn on the possible mechanism of action of this class of compounds. Although indications are given that the primary target for these compounds is not RR, it is suggested that induction of apoptosis could be involved in their mechanism of action.

9 Krakoff, I.H. *et al.* (1968) Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. *Cancer Res.* 28, 1559–1565

10 Agraval, K.C. *et al.* (1968) Potential antitumor agents III. Sodium salt of alpha-(*N*)heteroaromatic carboxyaldehyde thiosemicarbazones. *J. Pharm. Sci.* 57, 1948–1951

11 Saryan, L.A. *et al.* (1979) Comparative cytotoxicity and biochemical effects of ligands and metal complexes of alpha-(*N*)heteroaromatic carboxyaldehyde thiosemicarbazones. *J. Med. Chem.* 22, 1218–1221

12 Easmon, J. *et al.* (2001) Synthesis, cytotoxicity and antitumor activity of copper(II) and iron(II) complexes of ^4N-azabicyclo[3.2.2]nonane thiosemicarbazones derived from acyl diazines. *J. Med. Chem.* 44, 2164–2171



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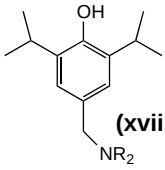
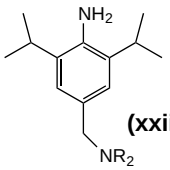


(xviii–xxii)



(xxiii–xxv)

NR_2 **(xviii)** = Morpholine
(xix) = Thiomorpholine
(xx) = Ethyl isonipecotate
(xxi) = N-acetylpiperazine
(xxii) = N-methylbenzylamine

NR_2 **(xxiii)** = Morpholine
(xxiv) = Thiomorpholine
(xxv) = Ethyl isonipecotate

Water-soluble analogues of the anaesthetic propofol

Propofol **(xvii)** is a widely used intravenous anaesthetic, whose mechanism of action involves the positive allosteric modulation of the neurotransmitter γ-aminobutyric acid (GABA) at $GABA_A$ receptors. The main advantages of propofol are favourable operating conditions (induction of anaesthesia is rapid and maintenance can be achieved by continuous infusion) and a rapid recovery. However, the compound shows cardiovascular side-effects and its injection is painful[13]. Recently, it has been suggested that propofol analogues containing a *para* substituent still retain good activity at the $GABA_A$ receptor[14].

On this basis, Cooke and coworkers[15] have synthesized several *para*-alkylamino-substituted analogues of propofol **(xviii–xxii)**, with the aim of obtaining water-soluble compounds with good anaesthetic activity. All the compounds were tested *in vivo* and *in vitro* as their hydrochloride salts. The anaesthetic potency of the compounds was determined upon their intravenous administration to mice when their hypnotic $dose_{50}$ (HD_{50}) was determined: propofol (HD_{50} = 68 μM kg^{-1}) was used as a positive control. All the *para* compounds compared favourably with propofol, the most potent being **(xx)**, which has an HD_{50} value of 19 μM kg^{-1}. To explore the SARs of this series further, several compounds **(xxiii–xxv)**, that have an amino group instead of the phenolic group at position 1, were synthesized and tested. They all showed good hypnotic potency, the most interesting being **(xxv)**, which had an HD_{50} value of 14.4 μM kg^{-1}.

However, further modifications of the amino group (e.g. removal, conversion to an amide or mono- or dimethylation) were not tolerated. The *in vitro* effect of the compounds at $GABA_A$ receptors was assessed by determining their ability to inhibit [^{35}S]-tert-butyl-bicyclophosphorothionate (TBPS) binding to rat whole-brain membranes. However, most of the compounds were found to be weakly active or inactive, which suggests that their *in vivo* anaesthetic activity is mediated by a non-GABAergic mechanism.

13 Trapani, G. *et al.* (2000) Propofol in anaesthesia. Mechanism of action, structure–activity relationships, and drug delivery. *Curr. Med. Chem.* 7, 249–271

14 Trapani, G. *et al.* (1998) Propofol analogues. Synthesis, relationships between structure and affinity at $GABA_A$ receptor in rat brain, and differential electrophysiological profile at recombinant human $GABA_A$ receptors. *J. Med. Chem.* 41, 1846–1854

15 Cooke, A. *et al.* (2001) Water-soluble propofol analogues with intravenous anaesthetic activity. *Bioorg. Med. Chem. Lett.* 11, 927–930

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Drug delivery

System for time and/or site-specific oral drug delivery

Time and/or site-specific drug delivery can be advantageous in the treatment of certain diseases. The symptoms of bronchial asthma and rheumatoid arthritis, for example, follow circadian rhythms, recurring primarily at night or in the early morning. A delayed-release formulation that can be taken before bedtime is advantageous in these cases. Delayed release is typically achieved through osmotic mechanisms, with tablets that contain a drug-loaded core which is surrounded by outer layers that slowly erode and then release the core. Alternatively, site-specific release is often used as a method to achieve drug delivery into specific regions of the gastrointestinal (GI) tract. In this regard, colon-specific release has some potential advantages as a strategy for improvement of the oral bioavailability of peptide drugs. The local concentration of peptidases is lower in the colon than in the small intestine. Although physiologically it is not the ideal site for absorption when compared to the small intestine, the colon is the site of significant absorption, and some of the absorptive disadvantages are offset by the long residence time. In addition, colon-specific delivery of drugs represents an advantageous approach for the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease. To date, several different strategies have been used to achieve colon-specific delivery. Most of these strategies rely on prodrugs or polymers, designed to selectively degrade under the microbiological or pH conditions unique to the colonic environment versus the other portions of the GI tract and/or the relative reproducibility of small intestinal transit time (SITT).

Sangalli and colleagues have recently reported the application of a novel oral drug delivery system designed for delayed, colon-specific release[1]. The system

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