Monitor: molecules and profiles

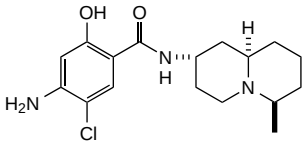
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and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

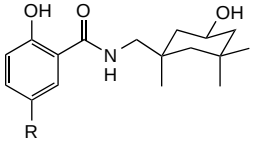
Inhibitors of influenza virus fusion

Despite the availability of a vaccine, the search is still ongoing for effective antiviral agents to combat the annual winter outbreaks of influenza. Infection is associated with significant morbidity and mortality. The outbreaks are driven in part by the ever-changing nature of the virus as a result of antigenic drift and antigenic shift. Therefore, the success of prophylactic vaccination depends on the ability to accurately predict the anticipated circulating virus strain. Effective drugs participate by offering an alternative means for the prevention and treatment of infection.

Recently, a group from Bristol-Myers Squibb (Wallingford, CT, USA) identified BMY27709 [compound **(i)**], as a potent inhibitor of the H1 and H2 subtypes of influenza A [1]. Mechanistically, the compound was found to inhibit viral fusion by preventing the low pH-induced conformational rearrangement of the viral haemagglutinin into its fusogenic state. Initial SAR studies highlighted the importance of the salicylic-acid moiety and, in particular, the 2-hydroxyl group. Researchers maintained this group and conducted a search for alternative amine components using parallel synthetic techniques. In this manner, compounds **(ii)** and **(iii)** were identified as potent inhibitors of influenza virus infectivity in cell culture (EC_{50} values of 0.04 and 0.03 μM, respectively).



(i)



(ii) R = Me
(iii) R = Cl

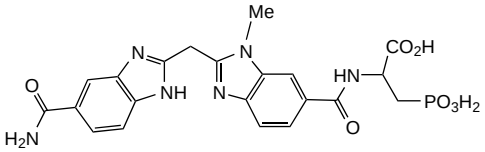
1 Desphande, M.S. *et al.* (2001) An approach to the identification of potent inhibitors of influenza virus fusion using parallel synthesis methodology. *Bioorg. Med. Chem. Lett.* 11, 2392–2396

Bisbenzimidizole based inhibitors of HCV protease

The hepatitis C virus (HCV) was identified in 1989 as the etiological agent responsible for non-A and non-B hepatitis. HCV infection is often chronic and has been associated with the development of cirrhosis and hepatocellular carcinoma. Current treatment involves interferon in combination with ribavirin. Although this therapy provides some relief, it is less than optimal because of toxicity and low long-term response rates.

The search for new agents that are effective against HCV has centered on targeting the enzymes expressed and required by the virus. One of the more prominent targets is the HCV NS3 protease. This enzyme is required for processing the viral polypeptide synthesized during infection. Several interesting leads have been identified. These compounds mainly take advantage of the protease's susceptibility to strong product inhibition. Not surprisingly, the majority of these leads are peptidomimetics that resemble the protease cleavage product.

By contrast, a recent disclosure by Yeung and coworkers from Bristol-Myers Squibb (Wallingford, CT, USA) reveals a non-peptide based inhibitor of the HCV NS3 protease [2]. This compound is APC6336 [compound **(iv)**; $EC_{50} = 0.2$ μM], which was identified in a screen of bisbenzimidazole-like analogs. Interestingly, inhibition is dependent upon the presence of Zn^{2+}. The Zn^{2+} ion is believed to direct coordination of the bisbenzimidazole



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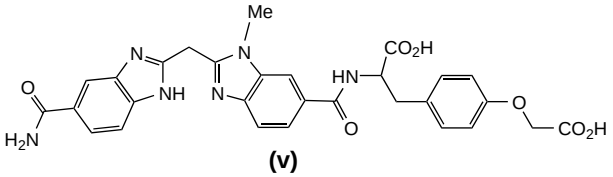


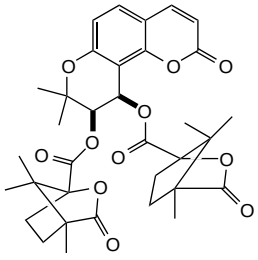
(v)

core with the active-site serine and histidine residues. An SAR investigation of the phosphonoalanine side-chain identified compound **(v)**, which is only slightly less active (IC_{50} = 1 μM).

2 Yeung, K-S. *et al.* (2001) Structure–activity relationship studies of a bisbenzimidazole-based, Zn^{2+}-dependent inhibitor of HCV NS3 serine protease *Bioorg. Med. Chem. Lett.* 11, 2355–2359

DCK anti-HIV agents

3′,4′-Di-*O*-(*S*)-camphanoyl-(3′*R*,4′*R*)-(+)-*cis*-khellactone [DCK; **(vi)**] was identified as a potent anti-HIV agent with an EC_{50} value of 2.6×10^{-4} μM. Unfortunately, this compound is poorly water-soluble, which limited further development. To overcome this, Lee and coworkers set out to introduce water-solubilizing groups at the 3-position. Earlier work had shown that substitution at this site enhanced anti-HIV activity [3]. Not surprisingly, in the current study the introduction of an hydroxymethyl moiety at the 3-position, to yield compound **(vii)**, was well tolerated ($EC_{50} = 1.9 \times 10^{-4}$ μM) [4].



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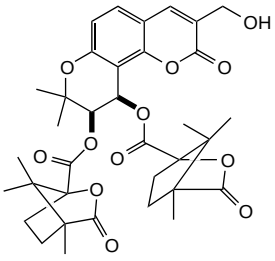


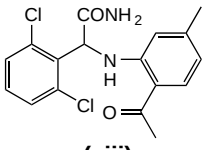
(vii)

3 Xie, L. *et al.* (1998) Anti-AIDS agents. Part 33: Synthesis and anti-HIV activity of mono-methyl substituted 3′4′-di-*O*-(–)-camphanoyl-(+)-cis-khellactone (DCK) analogues *Bioorg. Med. Chem. Lett.* 8, 2151–2156

4 Xie, L. *et al.* (2001) Anti-AIDS agents. Part 47: Synthesis and anti-HIV activity of 3-substituted 3′,4′-di-*O*-(*S*)-camphanoyl-(3′*R*,4′*R*)-(+)-*cis*-khellactone derivatives *Bioorg. Med. Chem. Lett.* 11, 2291–2293

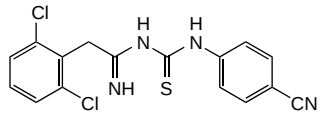
Discovery and evolution of a new class of NNRTIs

The discovery and chemotype evolution of a new series of non-nucleoside reverse transcriptase inhibitors (NNRTIs) has recently been disclosed from the laboratories of Janssen Pharmaceutica (Spring House, PA, USA) [5–7]. This study was initiated by the discovery of the α-anilinophenylacetamide (α-APA) series of inhibitors, represented by compound **(viii)**. Although potent against HIV, this compound showed no advantage over approved NNRTI-based drugs in Phase II clinical trials. Therefore, a new series of compounds was sought.



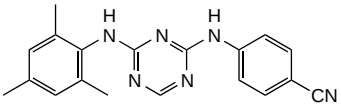
(viii)

Using compound **(viii)** as a starting point, the imidoylthiourea (ITU) chemotype was synthesized. Compound **(ix)** was found to be the most active compound in this series. The SAR around this template is rather specific in that there seems to be little tolerance for structural changes. Nonetheless, **(ix)** showed superior activity against NNRTI-resistant mutant strains of HIV (EC_{50} values of 0.002–0.589 μM) when compared with the clinically approved agents, delavirdine and nevirapine. Unfortunately, this compound proved to be hydrolytically and oxidatively unstable during formulation studies.



(ix)

Stability was achieved by replacing the central amidine and thiourea groups with a heterocycle, as in compound **(x)**. This compound maintained high potency in initial assays [EC_{50} (HIV_{LAI}) = 0.0003 μM] but, unfortunately, was not active against double-mutant strains of HIV. Interestingly, this problem was solved by replacing the central triazine ring with pyrimidine to yield the so-called DAPY (diaminopyrimidine) chemotype. Compound **(xi)** proved to be one of the more active analogs in this series [EC_{50} (HIV_{LAI}) = 0.0014 μM] and was equally active against mutant and double-mutant strains (EC_{50} values = 0.0012–0.019 μM).



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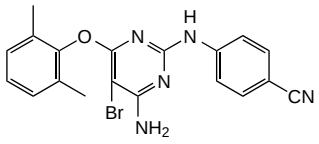


(xi)

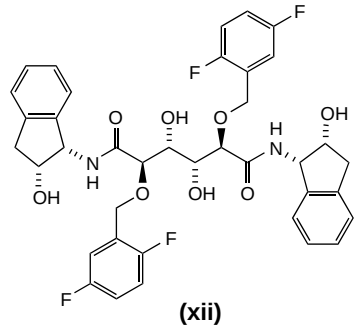
5 Ludovici, D.W. *et al.* (2001) Evolution of anti-HIV drug candidates. Part 1: From α-anilinophenylacetamide (α-APA) to imidoyl thiourea (ITU). *Bioorg. Med. Chem. Lett.* 11, 2225–2228

6 Ludovici, D.W. *et al.* (2001) Evolution of anti-HIV drug candidates. Part 2: Diaryltriazine (DATA) analogues. *Bioorg. Med. Chem. Lett.* 11, 2229–2234

7 Ludovici, D.W. *et al.* (2001) Evolution of anti-HIV drug candidates. Part 3: Diarylpyrimidine (DAPY) analogues. *Bioorg. Med. Chem. Lett.* 11, 2235–2239

C_2-Symmetric HIV-protease inhibitors

A series of C_2-symmetric inhibitors of HIV-protease derived from the carbohydrate L-mannaric acid has been disclosed, which highlights the effects of fluoro substitution [8]. In particular, compound **(xii)** is described, which is a moderate inhibitor of the enzyme (K_i = 3.29 nM) and weaker than the corresponding des-fluoro analogue (K_i = 1.22 nM). Nonetheless, in tissue culture the fluorinated compound exhibits a substantially improved antiviral potency over the des-fluoro analogue (ED_{50} = 0.02 and 0.1 μM, respectively). These results suggest that the fluoro groups increase cellular uptake and could prove to be an effective tool for increasing antiviral activity.



(xii)

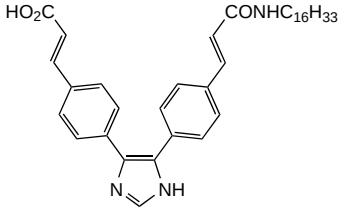
8 Pyring, D. *et al.* (2001) Design and synthesis of potent C_2-symmetric diol-based HIV-1 protease inhibitors: Effects of fluoro substitution. *J. Med. Chem.* 44, 3083–3091

Non-carbohydrate imidazole-based selectin inhibitors

During an inflammatory response immune cells are attracted to the site of tissue injury or infection. The first step is the rolling of leukocytes and lymphocytes along the endothelium and this is mediated by the selectins. The natural ligand of the selectins is the sialylated and fucosylated tetrasaccharide sialyl lewis^x.

A selectin inhibitor is expected to attenuate the inflammatory response and many sugar or peptide inhibitors are known. In general, these have poor pharmacokinetic properties and there is a need for small molecules that are able to selectively interfere with the selectin–ligand interaction.

High-throughput screening by a group from the Ontogen Corporation (Carlsbad, CA, USA), using an ELISA assay, identified the imidazole derivative **(xiii)** (IC_{50} = 17 μM ± 26 μM) against P-selectin binding [9]. Evaluation of SARs established the requirement for a Ca^{2+} binding group, served by the carboxylic acid, a rigid core, and a hydrophobic moiety, the hexadecyl side chain. A number of core heterocycles were tolerated but the imidazole was found to give the best aqueous solubility. Substitution with phenyl derivatives at the 2-position of the imidazole improved potency, giving compound **(xiv)** (IC_{50} = 0.29 μM ± 0.33 μM), and activity in a cell-based assay for P-selectin binding (IC_{50} = 9 μM ± 16 μM). The compound is water soluble as the *bis*-sodium salt (15 mg ml^{-1}), and was tested *in vivo* in a mouse peritonitis model and found to give a 30–50% reduction in cell infiltration in a dose-dependent manner. This is the first reported non-carbohydrate small-molecule selectin inhibitor with *in vivo* efficacy.



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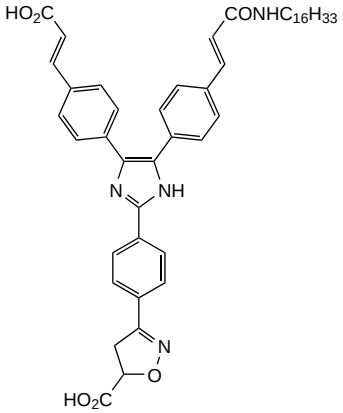


(xiv)

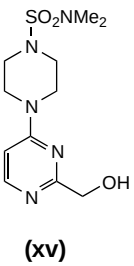
9 Slee, D.H. (2001) Development of potent non-carbohydrate imidazole-based small molecule selectin inhibitors with anti-inflammatory activity. *J. Med. Chem.* 44, 2094–2107

Sorbitol dehydrogenase inhibitors to treat diabetes

In patients with diabetes, prolonged elevated levels of blood glucose leads to tissue degeneration manifested as neuropathy, retinopathy, and so on. One approach to protecting vulnerable tissue is to block excessive glucose metabolism through the polyol pathway. This pathway involves the reduction of glucose to sorbitol, by aldose reductase, and the subsequent oxidation of sorbitol to fructose catalyzed by sorbitol dehydrogenase. This reaction involves the conversion of NAD^+ to NADH and it has been postulated that the increase in NADH:NAD^+ ratio from chronic excessive flux through this pathway leads to reductive stress (pseudohypoxia), which triggers biochemical changes that ultimately contribute to the observed pathology in affected tissue.

Compound **(xv)** is a known inhibitor of sorbitol dehydrogenase (IC_{50} = 246 nM), and has been shown to cause a dose-dependent increase in sorbitol concentration in the sciatic nerve of normal and streptozoticin-diabetic rats (ED_{50} = 14 mg kg^{-1}).

A group from the cardiovascular and metabolic disease laboratories (Pfizer, Groton, CT, USA) sought to improve the properties of compound **(xv)** [10]. Sorbitol dehydrogenase is a zinc-requiring enzyme and they proposed that the N1 and oxygen atoms were important in coordinating to the zinc. The conformation of the hydroxymethyl side-chain would thus be expected to have a significant impact on binding; therefore, the group sought to examine the effect of chirality on the hydroxymethyl carbon.



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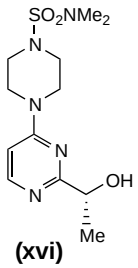

(xvi)

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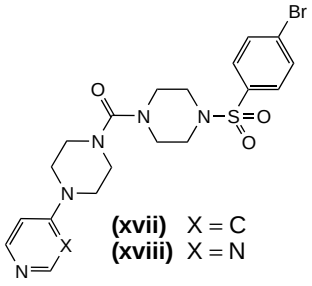
Compound **(xvi)** was prepared, and the *R*-isomer, shown, was found to be 10-fold more potent than its isomer and compound **(xv)**. The absolute configuration was determined by total synthesis from *R*(+) lactic acid. Compound **(xvi)** also exhibited a dose-dependent inhibition of fructose accumulation in diabetic rat sciatic nerve [ED_{50} = 1.6 mg kg^{-1}, compared to 14 mg kg^{-1} for compound **(xv)**]. However, the half-life of these compounds is short because of the *N*-demethylation of the sulfonamide. The reported large-scale synthesis of compound **(xvi)** will facilitate the discovery of sorbitol dehydrogenase inhibitors with a longer serum half-life.

10 Myalari, B.L. (2001) Sorbitol dehydrogenase inhibitors (SDIs): a new potent, enantiomeric SDI, 4-[2-1*R*-hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic acid dimethylamide. *J. Med. Chem.* 44, 2695–2700

Consideration of pK_a in the identification of new agents to lower cholesterol

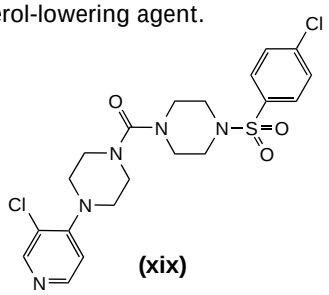
Considerable success in the treatment of cardiovascular disease has been achieved through the lowering of plasma cholesterol levels using HMG-CoA reductase inhibitors, also known as statins.

This success has focused attention on identifying inhibitors of later steps in the biosynthetic pathway to cholesterol, including 2,3-oxidosqualene lanosterol synthase (OSC). A group at AstraZenca (Macclesfield, UK) have previously described *N*-pyridyl and *N*-pyrimidinyl piperidines, **(xvii)** and **(xviii)**, respectively, as potent inhibitors of microsomal OSC from both human and rat [11]. The compounds demonstrated a similar reduction in cholesterol biosynthesis to the statin drug, simvastatin, in the rat. However, at higher oral doses the pyridyl derivative was found to cause a reduction of food intake of 64% (50 mg kg^{-1}), whereas the pyrimidinyl derivative only gave a 17% (100 mg kg^{-1}) reduction in food intake. Thus, the pyridine would not be permitted to enter clinical development because safety testing at higher doses would be precluded.



(xvii) X = C
(xviii) X = N

The mechanism for the effect on feeding behaviour is unknown, and the group hypothesized that the pK_a values of the terminal pyridine and pyrimidine might be important [12]. The pK_a for the pyridine is 9.2 and for the pyrimidine is 6.1. The group thus set out to lower the pK_a of the pyridine ring. One approach was to replace the electron-donating nitrogen of the piperidine with a carbon. A second approach was halogenation of the 3-position of the pyridine to give, for example, the chloro-derivative **(xix)**, which has a reduced pK_a of 5.8, is a potent inhibitor of microsomal OSC (87% at 100 nM) and exhibited good reduction of cholesterol biosynthesis via oral administration in the rat (ED_{80} = 1.4 mg kg^{-1}). The compound induced an improved feeding profile (14% reduction in feeding at 100 mg kg^{-1}) and is a development candidate as a novel cholesterol-lowering agent.



(xix)

11 Brown, G.R. (2000) A novel series of 4-piperidinopyridine and 4-piperidinopyrimidine inhibitors of 2,3-oxidosqualene cyclase-lanosterol synthase. *J. Med. Chem.* 43, 4964–4972

12 Brown, G.R. (2001) Novel 4-piperidinopyridine inhibitors of oxidosqualene cyclase-lanosterol synthase derived by consideration of inhibitor pK_a. *Bioorg. Med. Chem.* 11, 2213–2216

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Drug Delivery

Preparation of an experimental animal model by use of a drug delivery system

The pharmaceutical industry relies heavily on experimental animal models of disease states. These animal models are used to assay the potential effectiveness of new drug candidates. However, some diseases prove extremely difficult for which to develop appropriate models. One such disease is chronic hyperendotoxemia, a complication of Gram-negative bacterial infection, which can develop after trauma or surgery. Research aimed at the development of a treatment for chronic hyperendotoxemia has been hampered by the lack of a good animal model. Considerable research has been conducted to develop an appropriate model but, to date, a convenient, reproducible preparation of such a model has not been accomplished.

Lipopolysaccharide (LPS) is involved in the immune cascade and can induce a state of chronic hyperendotoxemia in animal models, but controlling the amount of LPS has proven to be difficult. Small doses of LPS cannot prevent Gram-negative sepsis, whereas overdose results in septic shock and multi-organ failure. To date, the application of an appropriate dosage form of LPS to induce chronic hyperendotoxemia has not been achieved. The controlled release of LPS could provide a suitable animal model.

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