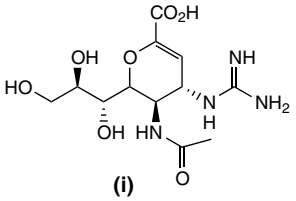
Monitor: molecules and profiles

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.**

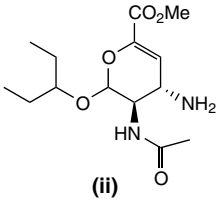
Molecules

Influenza neuraminidase inhibitor

During the normal course of infection, the influenza virus must balance its attraction for its cellular target with the necessity to migrate freely. Influenza 'sticks' to its target by using haemaglutinin, a sialic-acid recognizing protein. Although this enables infection of the cell, it is problematic for the progeny virus escaping the cell as it becomes tangled in the sialic-acid coated surface of the cell. To overcome this problem, influenza carries the enzyme neuraminidase, which cleaves sialic acid from the surface glycoproteins. Inhibition of this process, namely by using a neuraminidase inhibitor, has proven to be effective as a method for combating influenza. Two anti-influenza agents that target this enzyme have recently reached the market, zanamivir [Relenza; **(i)**] and oseltamivir [Tamiflu; **(ii)**].

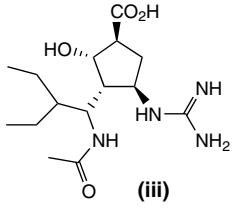


(i)



(ii)

Babu and coworkers (BioCryst Pharmaceuticals, Birmingham, AL, USA) have described a new neuraminidase inhibitor, BCX1812 (RWJ270201), which is currently in human clinical trials[1]. Similar to its predecessors, this compound was rationally designed with the aid of X-ray crystallographic structures of the enzyme complexed with inhibitors, but differs in that it possesses a five-membered ring rather than a six-membered ring. The stereochemistry of BCX1812 **(iii)** was deduced by co-crystallization of the racemic mixture with the enzyme. A different relative orientation for the substituents attached to the ring was predicted by the crystal structure of an earlier prototype bound to neuraminidase.



(iii)

The new compound combines the highly charged guanidine group found on zanamivir with the hydrophobic pentyl side chain of oseltamivir, to give a potent and orally bioavailable inhibitor. Inhibitory activity (IC_{50}) was 0.1–1.4 nM and 0.6–11.0 nM against neuramidase from flu-A and flu-B, respectively. The compound also performed well when dosed orally (0.1–1.0 mg^{-1} kg^{-1} day^{-1}) in the mouse influenza model.

1 Babu, Y.S. *et al.* (2000) BCX-1812 (RWJ-27021): discovery of a novel, highly potent, orally active, and selective influenza neuraminidase inhibitor through structure-based drug design. *J. Med. Chem.* 43, 3482–3486

HIV protease inhibitor

The benefits of combination therapy [commonly referred to as highly active anti-retroviral therapy (HAART)] in the treatment of HIV infection has made a significant impact in the reduction of AIDS-related deaths. However, viral resistance continues to be a problem.

Similar to other components of combination therapies, the HIV-protease inhibitors elicit viral mutations leading to resistance. Tyndall and coworkers (Centre for Drug Design, University of Queensland, Brisbane, Australia) have described a method for potentially overcoming resistance to HIV-protease inhibitors using modifications to existing inhibitors[2]. Three protease inhibitors **[(iv)–(vi)]** were constructed by grafting macrocyclic-tripeptide units onto active site-spanning elements taken from the acyclic inhibitors, VX478 (amprenavir), L735524 (indinavir) and Ro318959 (saquinavir). The newly designed compounds display activity comparable with their respective parent-inhibitors, both *in vitro* [K_i = 1.7 nM **(iv)**, 0.6 nM **(v)**, 0.3 nM **(vi)**] and *in vivo* against HIV-1 infected PBMCs [IC_{50} = 45 nM **(iv)**, 56 nM **(v)**, 95 nM **(vi)**].

These compounds might overcome viral resistance; the inhibitors bind

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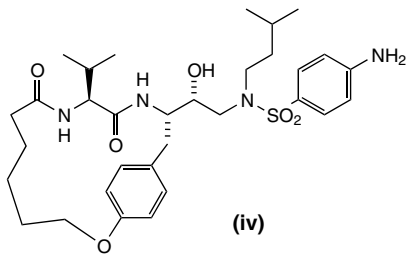
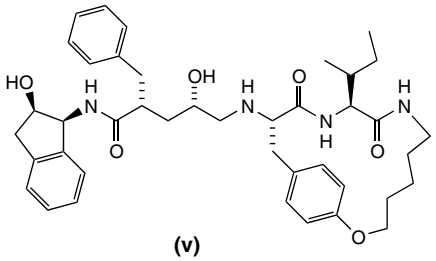
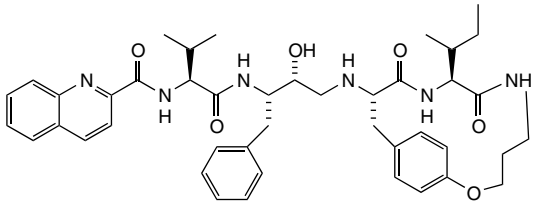


(iv)



(v)



(vi)

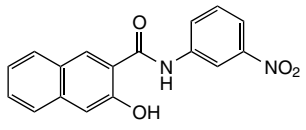
HIV-protease and the macrocycle increases stability. X-ray crystal structures of **(iv)** and **(v)**, bound to protease, show that the macrocyclic-tripeptide portion of the inhibitors mimics the corresponding portion of the substrate. By minimizingthe inhibitor structure to occupy only the substrate domain, it is hoped that mutations that reduce inhibitor binding would also be detrimental to substrate processing. Moreover, macrocyclization not only reduces the entropic cost of binding, by freezing the peptide portion into its bound conformation, but also imparts chemical and proteolytic stability to the inhibitor. The macrocyclic tripeptides showed no evidence of degradation when they were incubated with a variety of proteases, for up to 24 h (37°C); these conditions led to rapid hydrolysis of the corresponding acyclic peptides. This might lead to an increase in bioavailability and serum-half life.

2 Tyndall, J.D. *et al.* (2000) Synthesis, stability, antiviral activity, and protease-bound structures of substrate-mimicking constrained macrocyclic inhibitors of HIV-1 protease. *J. Med. Chem.* 43, 3405–3504

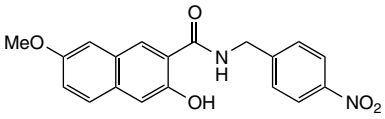
Inhibitors of HCMV polymerase

It is estimated that >40% of the population is infected with the human cytomegalovirus (HCMV), a member of the herpes virus family. After primary infection, the virus remains with the individual in a benign latent stage, which presents no harm unless it is reactivated. Individuals who are immunocompromised, such as those receiving organ or bone marrow transplants and those undergoing chemotherapy treatment, are most at risk from viral reactivation. When the latent virus is reactivated, infection can lead to pneumonia, gastorenteritis, retinitis and encephalitis. Despite the significant morbidity and mortality associated with the disease, there are currently no orally administered therapies approved for HCMV.

Researchers at Pharmacia (Kalamazoo, MI, USA) have been investigating inhibitors of HCMV-polymerase. They have identified naphthalene carboxamides, such as [**(vii)**; IC_{50} = 12.3 μM], by screening of their compound collection. A preliminary study of the SAR of this chemotype has recently been published[3]. Some simple modifications to the naphthalene and the amide substituent led to a dramatic improvement in the inhibitory activity against the enzyme. For example, **(viii)**, a representative analogue in this series, has an IC_{50} value of 0.82 μM against HCMV-pol and is completely inactive against a human DNA polymerase, α-pol. More importantly, **(viii)** has moderate antiviral activity in tissue culture [IC_{50} (plaque reduction) = 3.7 μM].



(vii)

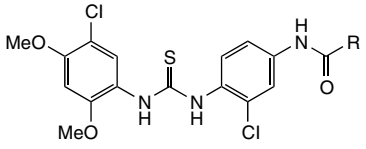


(viii)

3 Vaillancourt, V.A. *et al.* (2000) Naphthalene carboxamides as inhibitors of human cytomegalovirus DNA polymerase. *Bioorg. Med. Chem. Lett.* 10, 2079–2081

Novel herpes-virus inhibitors

Drug-resistant HSV strains have been isolated in increasing numbers from immunocompromised patients treated with viral polymerase inhibitors such as acyclovir and pencyclovir. Because infection by the herpes virus (HSV-1 and HSV-2) is widespread, there is increasing impetus to discover new anti-viral agents, preferably targeting alternative events in the virus life cycle. Such agents could potentially be used in combination with current therapies to suppress the emergence of resistant strains. Researchers at Wyeth-Ayerst (Pearl River, NY, USA) have discovered a novel class of compounds, thioureas CL253824 **(ix)** and WAY1500138 **(x)**, which inhibit HSV-1 (IC_{50} = 0.43–7.9 μM, against various strains)[4].



(ix, x)

Viral DNA replication is believed to progress through a 'rolling-circle mechanism', yielding concatameric DNA. This must be cleaved before it can be packaged into progeny viral capsids. One of the seven HSV-1 gene products believed to be involved in this process is UL6. Time-of-addition studies, electron microscopic evaluation of capsid morphology

and mutational analysis of resistant virus are consistent with the notion that this protein is targeted by CL253824 and WAY1500138. The exact mode of action on the protein has not yet been determined.

4 Van Zeijl, M. (2000) Novel class of thiourea compounds that inhibit herpes simplex virus type 1 DNA cleavage and encapsidation: resistance maps to the UL6 gene. *J. Virol.* 74, 9054–9061

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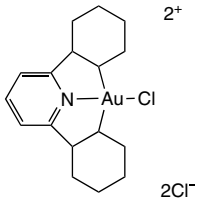
Novel antitumour molecules

Novel antitumour metal complexes related to cisplatin

Cisplatin and carboplatin, which are platinum-based drugs, play a leading role as cancer chemotherapeutic agents. Recently there has been a growing interest in the development of structurally related complexes of other metals (e.g. Ti, Au, Cu, Sn and Rh) as potential antitumour drugs. Two recent reports highlight such developments. Orioli and coworkers (University of Florence, Florence, Italy) have reported results on the use of gold (III) complexes with multidentate ligands as potential antitumour agents[1]. The use of multidentate ligands was found to impart a high degree of stability under physiological conditions, a problem that has hampered the development of gold (III) complexes previously. The *in vitro* cytotoxic properties were tested against the human ovarian tumour cell line A2780, either wild-type or cisplatin resistant. Typically, the investigated compounds had IC_{50} values in the 0.2–10 μM range and were able to overcome cisplatin resistance when tested on the relevant cell line. However, in the most potent complexes tested, the free terpyridine **(i)** and phenanthroline ligands were found to be more cytotoxic than the gold (III) complexes, rendering the interpretation of the cytotoxicity data complicated.

A related report by Caruso and coworkers (Consiglio Nazionale delle Ricerche, Rome, Italy and University of Camerino, Camerino, Italy) describes the characterization and biological testing of the coordination complex *cyclo*-tetrakis[bis(1-phenyl-3-methyl-4-benzoylpyrazolon-5-ato)μ-oxotitanium (IV)] as a potential antitumour drug[2]. The authors conclude, on the basis of both *in vitro* and *in vivo* results in CF-1 and AJ mice, that a Ti complex–liposome system might be a promising drug candidate.

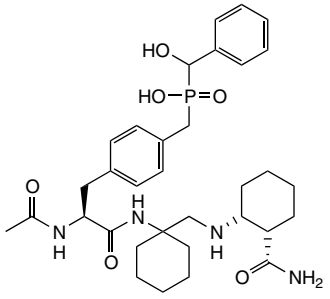


(i)

1 Messori, L. *et al.* (2000) Gold (III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold (III) compounds. *J. Med. Chem.* 43, 3541–3548
2 Caruso, F. *et al.* (2000) Synthesis, structure and antitumor activity of a novel tetranuclear titanium complex. *J. Med. Chem.* 43, 3665–3670

Phosphinate isosteres of phosphotyrosine for incorporation in Grb2–SH2 inhibitors

The signal transduction processes mediated by Grb2 (growth factor receptor-bound protein 2) are essential for the activity of growth factors involved in cell proliferation and differentiation. Deregulation of the pathways mediated by Grb2 can lead to uncontrolled cellular proliferation and ultimately tumour formation. Inhibiting the binding interactions between tyrosine kinase growth factor receptors and the Src homology 2 (SH2) domain of Grb2 therefore constitutes an attractive strategy in the search for new anticancer drugs. Recent research by Furet and colleagues[3] (Novartis, Basel, Switzerland) and Walker and coworkers[4] (Novartis, Horsham, UK) describe the structure-based design and synthesis of a series of phosphinate isosteres of phosphotyrosine and their incorporation into a short inhibitory peptide sequence of the Grb2–SH2 domain. The phosphinate isosteres were designed as less charged and phosphatase-resistant pTyr replacements without the loss of binding affinity for the Grb2–SH2 domain. The resulting compounds, most notably **(ii)**, were found to inhibit binding to Grb2–SH2 as potently as the corresponding doubly charged (phosphonomethyl)phenylalanine analogue.



(ii)

3 Furet, P. *et al.* (2000) Structure-based design and synthesis of phosphinate isosteres of phosphotyrosine for incorporation in Grb2–SH2 domain inhibitors. Part 1. *Bioorg. Med. Chem. Lett.* 10, 2337–2341
4 Walker, C.V. *et al.* (2000) Structure-based design and synthesis of phosphinate isosteres of phosphotyrosine for incorporation in Grb2–SH2 domain inhibitors. Part 2. *Bioorg. Med. Chem. Lett.* 10, 2343–2346

Inhibitors of the Src SH2 domain and Src tyrosine kinase as potential anticancer drugs

The Src homology 2 (SH2) domain of proteins recognizing phosphotyrosine (pTyr) sequences of tyrosine kinases play a crucial role in many intracellular signalling cascades. The non-receptor tyrosine kinase, Src, is involved in signalling pathways controlling cell proliferation, migration and angiogenesis. Elevated levels of kinase activity have been associated with several different cancers including breast and colon. Using

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