Monitor: molecules and profiles

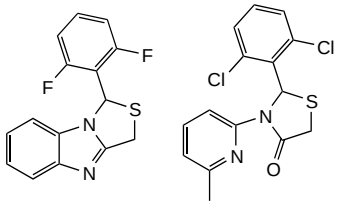
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and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

Novel antiviral molecules

Thiazolidinone based non-nucleoside inhibitors of HIV-reverse transcriptase

Non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs) have proven effective in the treatment of HIV infection. Unfortunately, resistance is emerging to the therapeutic drugs currently used in the fight against HIV, providing the impetus to develop novel agents. Towards this goal, a new chemotype based on a thiazolidinone template, which is active as an NNRTI, has recently been investigated by Barreca and coworkers[1].

The origin of the thiazolidinone template is actually derived from an earlier study of a fused heterocyclic analog **(i)** [EC_{50} (HIV-1 III_B) = 0.35 μM]. The minimal pharmacophoric elements presented by this chemotype were determined to be the two aromatic rings aligned in a 'butterfly-like' conformation and the thiazolidine nitrogen atom[2]. From this information, a new thiazolidinone chemotype was designed. Studies of SARs at the 2- and 3-positions of the ring yielded compound **(ii)** [EC_{50} (HIV-1 III_B) = 0.04 μM] as the most active derivative. A *bis*-chlorinated phenyl ring is preferred at the C1 position and a methylpyridyl group is optimal at the nitrogen position. The thiazolidinone template appears to be intrinsically more active than the fused tricyclic template represented by **(i)**, which suggests that the fused ring system is too rigid to allow the most optimal fit. As expected, compound **(ii)** is active against HIV reverse transcriptase *in vitro* and is selectively active against HIV-1 but not HIV-2 in cell culture, which is consistent with its proposed mechanism of action.



(i) **(ii)**

1 Barreca, M.L. *et al.* (2001) Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents. *Bioorg. Med. Chem. Lett.* 11, 1793–1796

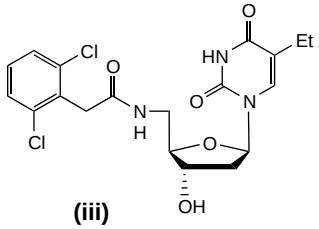
2 Barreca, M.L. *et al.* (1999) Comparative Molecular Field Analysis (CoMFA) and Docking Studies of Non-nucleoside HIV-1 RT Inhibitors (NNI). *Bioorg. Med. Chem. Lett.* 7, 2283–2292

HSV-thymidine-kinase inhibitors to prevent virus reactivation

Reactivation of latent virus, after the initial resolution of infection, is an insidious problem associated with herpes simplex virus (HSV). Infection by HSV-1 causes orofacial lesions, whereas HSV-2 generally causes genital infections. Usually, the initial infection resolves within 10 days but, unfortunately, by that time the virus has migrated to neuronal tissues where it will remain in a latent stage until reactivated.

Current drugs against HSV, such as acyclovir, work well in attacking actively replicating virus but lack activity against the latent form of the virus. Clearly, it would be desirable to develop a therapy that would be active against latent virus, or at least prevent reactivation.

Virally encoded thymidine kinase (TK), although not essential for viral replication in rapidly dividing cells has been implicated as being involved in HSV reactivation in neuronal cells. In dividing host-cell populations, cellular TK provides thymidine triphosphate for viral replication; however, in non-dividing nerve cells the virus must rely on its own TK to replicate. This makes HSV-TK an attractive target for the development of drugs that prevent the reactivation of the virus from latency. Three related series of inhibitors, **(iii)**, **(iv)** and **(v)**, have recently been reported by a group at Roche (Welwyn Garden City, UK)[3].



(iii)

All three inhibitors are designed around a 2′-deoxy-5-ethyluridine template. This nucleoside was chosen for its selective binding to HSV-TK over the analogous cellular enzyme. Beginning with **(iii)** as a lead structure that inhibits HSV-1 TK and HSV-2 TK with IC_{50} values of 1.0 μM and 3.0 μM, respectively, SAR

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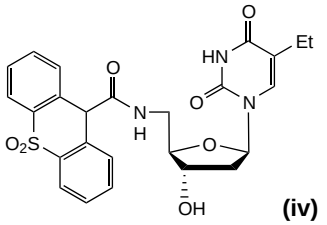
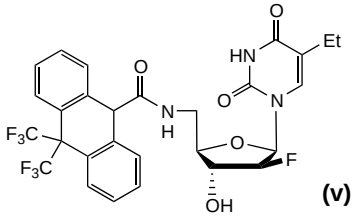
studies centered on the aromatic substituent attached to the acetamide sidechain. A tricyclic ring system, such as that in **(iv)** proved to be most effective at inhibiting HSV-1 TK and HSV-2 TK (IC_{50} = 0.95 and 0.13 μM, respectively). Further manipulations involved replacing the SO_2 group by $C(CF_3)_2$ to yield **(v)**, with IC_{50} values for HSV-1 TK, HSV-2 TK of 0.19 and 0.11 μM, respectively. No inhibition of host-cellular TK was observed at concentrations up to 10 μM.



(iv)



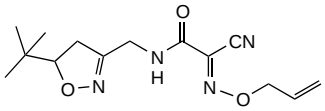
(v)

3 Martin, J.A. *et al.* (2001) Nucleoside analogues as highly potent and selective inhibitors of herpes simplex virus thymidine kinase. *Bioorg. Med. Chem. Lett.* 11, 1655–1658

Novel antiviral chemotype active against influenza virus A

Influenza is still a major health concern despite the availability of a vaccine and two classes of drugs. Vaccination only provides protection against the predominate strain of the virus during influenza season, which usually changes from year to year. Efforts are under way to develop novel agents effective against influenza A and influenza B, which are responsible for annual epidemics and pandemics.

A novel series of compounds with activity against influenza A has recently been disclosed by Kai and coworkers[4]. The new chemotype is represented by compound **(vi)** and the paper[4] details the SAR investigation of the isoxazoline ring and the oxime moiety. Compound **(vi)** contains the optimal structural features for activity observed to date, namely, the tert-butyl group attached to the isoxazoline ring and the allyl group present on the oxime oxygen atom. Modifications to the isoxazoline heterocycle were not tolerated. Inhibition of the influenza virus (A/WSN/33 strain) in a cell-protection assay (involving the treatment of Madin-Darby bovine kidney cells with influenza and measurement of viral-toxicity prevention for the compound of interest), was observed with an EC_{50} value of 3 μg ml^{-1}. The mechanism of action is currently unknown.



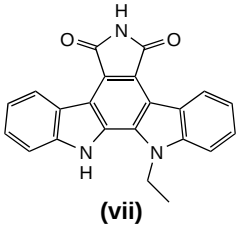
(vi)

4 Kai, H. *et al.* (2001) Anti-influenza virus activities of 2-alkoxyimino-N-(2-isoxazolin-3-ylmethyl)acetamides. *Bioorg. Chem. Med. Lett.* 11, 1997–2000

Indolocarbazole based inhibitors of CMV

Human cytomegalovirus (HCMV) presents a significant health threat to immunocompromised individuals. These include recipients of bone marrow and renal transplants, as well as those infected with HIV. As with AIDS patients, recent advances in therapy, namely the introduction of highly active retroviral therapy (HAART), have led to immune reconstitution, which is able to keep HCMV in check. Nonetheless, the emergence of HIV resistance leading to HCMV relapse remains an ever present threat to these patients. Current treatment options available for HCMV include ganciclovir (GCV), foscarnet (PFA), cidofovir (HPMPC) and formivirsen. These drugs are beneficial but possess side effects and have poor oral bioavailability indicating the need for new and improved anti-HCMV agents.

Recently, a group from GlaxoSmithKline (Stevenage, UK) reported that indolocarbazoles are highly active inhibitors of HCMV in cell culture, which could eventually lead to a new class of drugs[5]. The most active analog to emerge from this study is compound **(vii)**, which had an IC_{50} value of 19 nM. Because the template is similar to a known class of protein kinase C (PKC) inhibitors, the activity against this enzyme was also evaluated. Compound **(vii)** inhibited PKC at a much higher concentration (IC_{50} = 10 μM) and was not cytotoxic in cell culture, indicating a high therapeutic index (>6500). Further studies showed that **(vii)** was active against GCV-resistant strains of HCMV suggesting that it inhibited the virus at a different site of action than GCV. Unfortunately, compound **(vii)** was found to be poorly soluble precluding further development of the compound. However, it is hoped that this compound can prove useful as a lead structure and that related analogs showing improved solubility will eventually be derived from this series.

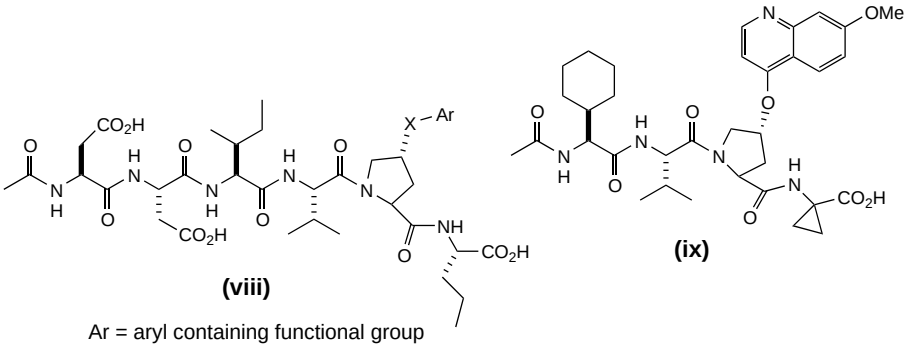


(vii)

5 Slater, M.J. *et al.* (2001) Synthesis of *N*-alkyl substituted indolocarbazoles as potent inhibitors of human cytomegalovirus replication. *Bioorg. Med. Chem. Lett.* 11, 1993–1995

Solid-phase synthesis of HCV-protease inhibitors

The NS3-serine protease expressed by the hepatitis C virus (HCV) has been proven to be absolutely essential for viral replication, leading several groups to develop inhibitors of this enzyme. Most of the inhibitors disclosed have been designed using the N-terminal cleavage product of the protease as a template. A group from Boehringer Ingelheim (Quebec, Canada) have investigated a series of tetrapeptide analogs, such as **(viii)**, and in doing so have uncovered a hydrophobic binding pocket located at the P2 position (Schecter and Berger nomenclature[6]).



(viii)

(ix)

Ar = aryl containing functional group
X = CH_2 or O

In a recent publication, the same group focussed on optimizing this position using combinatorial chemistry[7]. A tetrapeptide was attached to a solid support and used as a scaffold for the synthesis of peptidomimetic inhibitors. The researchers then took advantage of the Mitsunobu reaction to introduce hydroxy-aryl groups at P2. Further modifications to the aryl group were achieved using the Suzuki cross-coupling reaction. SARs derived from this library suggest that, in addition to a hydrophobic interaction, a dipole–quadrupole interaction between the enzyme and the P2 substituent contributes to binding. Compound **(ix)**, having a quinoline-based P2 group (IC_{50} = 0.8 μM) was the most active analog disclosed.

6 Schecter, I. and Berger, A. (1967) On the size of the active site in proteases. I. Papain. *Biophys. Res. Commun.* 27, 157–162

7 Poupart, M-A. *et al.* (2001) Solid-phase synthesis of peptidomimetic inhibitors for the hepatitis C virus NS3 protease *J. Org. Chem.* 66, 4743–4751

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Drug delivery

A new drug delivery system for protein drugs using silicone

In general, macromolecular drugs such as proteins have an extremely short half-life in the body. Formulations that are designed to protect the drug from physiological effects until its release can be advantageous in these situations. Sustained release formulations that are designed to maintain an appropriate concentration in the body over a long period of time can also be advantageous. In this regard, a constant, zero-order release rate is usually ideal. Zero-order release formulations of lipophilic drugs can usually be achieved using formulations that contain hydrophobic polymers, but there has been limited success developing zero-order release formulations of protein drugs.

Recently, Kajihara and coworkers[1] reported the use of a new drug delivery system for protein drugs using silicone. Although this matrix formulation possessed some advantages over typical hydrophobic polymers, it still exhibited first-order release; that is, the concentration of drug released decreases over time. More recently, this group reported on the use of an improved formulation that is referred to as a covered-rod type formulation, which exhibits zero-order release characteristics for a model protein drug[2]. This latest report compares the release characteristics of the matrix- and covered-rod-type formulations. For a model drug, interferon (IFN) was chosen. Most protein drugs do not diffuse easily through silicone, especially when present in low concentrations. However, it was found that high loadings of simple, inexpensive, water-soluble proteins, such as human serum albumin (HSA) facilitate the release of protein drugs, such as IFN, from silicone. IFN–HSA powders containing a small amount of IFN were loaded into the silicone formulations and the resulting release characteristics of IFN were studied. For some *in vitro* studies, Texas-Red (sulforhodamine 101 acid chloride)-labeled HSA was used to facilitate visualization by confocal laser microscope (CLSM) analysis.

Matrix formulations were prepared by packing a mixture of silicone–IFN–HSA and additives into a polytetrafluoroethylene tube and removed after curing (hardening). Covered-rod-type formulations were made by preparing two silicone mixtures, one containing IFN–HSA and additives and another containing none of the drug mixture. The two silicone mixtures were loaded into separate syringes and extruded through a concentrically arranged die so that the silicone containing the drug mixture formed the inner, rod-shaped part, and the drug-free mixture formed the outer part. The resulting extrusion was cured at room temperature for three days. The configuration is called a covered-rod-type formulation because the inner, rod-shaped silicone that contains the drug is covered by drug-free silicone, except on the ends. Studies were then conducted to compare the matrix and covered-rod-type formulations.

The covered-rod-type formulation is prepared under mild conditions that require no heat or organic solvents. Protein drugs, including IFN, are denatured by heat and/or organic solvents. A covered-rod-type formulation was crushed in a grinder and, when the pieces were immersed in buffer, almost 100% of the IFN was recovered. This indicates that IFN is stable to the preparation conditions of the covered-rod-type formulation.

Parameters in the control of release rates

In vitro release rates of IFN from the covered-rod-type formulations were studied and compared with the release rate from matrix-type formulations. Matrix-type formulations released IFN at a rate that was initially high and gradually