Monitor: molecules and profiles

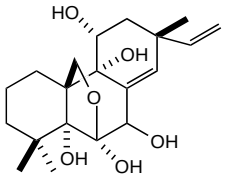
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and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

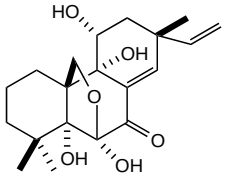
Novel antimycobacterial agents from the fungus *Diaporthe* Sp.

The incidence of tuberculosis infection has increased rapidly and multidrug resistant strains of *Mycobacterium tuberculosis* have emerged [1]. Therefore, the search for new drugs is of great importance. Dettrakul and collaborators [2] have reported the characterization of two new antitubercular pimarane diterpenes, diaporthein A (**i**) and diaporthein B (**ii**), from the culture broth of the fungus *Diaporthe* sp. BCC 6140. The structures of these compounds were determined from spectroscopic data. However, the stereochemistry at the C-7 of **i** could not be established from the available data and was tentatively defined as β by comparison with structurally related sphaeropsidins [3].

Compounds **i** and **ii** were tested for their antimycobacterial activity against *M.tuberculosis* H37Ra [4]. The antimycobacterial drugs Isoniazid [minimum inhibitory concentration (MIC) = 0.04–0.09 μg ml^{-1}] and kanamycin sulfate (MIC = 2.0–5.0 μg ml^{-1}) were used as reference drugs. In addition, the cytotoxicity of compounds **i** and **ii** was determined according to the colorimetric assay described by Skehan and co-workers [5]. The results indicated that diaporthein A has only weak antimycobacterial activity (MIC = 200 μg ml^{-1}). By contrast,



(i)



(ii)

diaporthein B is a potent inhibitor of *M. tuberculosis* growth (MIC = 3.1 μg ml^{-1}). Because the only difference between compound **i** and **ii** is the C-7 substituent, it can be assumed that the carbonyl moiety of diaporthein B is essential for the antimycobacterial activity of these compounds.

When compounds **i** and **ii** were tested for cytotoxicity in Vero cell lines, a trend similar to their antitubercular activity was seen (IC_{50} values >50 μg ml^{-1} and 1.5 μg ml^{-1}, respectively).

These compounds, therefore, have potential as novel antitubercular compounds that might be active against the numerous multidrug resistant strains of *M.tuberculosis*.

1 Bradford, W.Z. *et al.* (1996) The changing epidemiology of acquired drug-resistant tuberculosis in San Francisco. *Lancet*, 348, 928–931
2 Dettrakul, S. *et al.* (2003) Antimycobacterial pimarane diterpenes from the fungus *Diaporthe* Sp. *Biorg. Med. Chem. Lett.* 13, 1253–1255
3 Evidente, A. *et al.* (2002) Sphaeropsidins D and E, two other pimarane diterpenes, produced *in vitro* by the plant pathogenic fungus *Sphaeropsis sapinea* f. sp. *cupressi*. *Phytochemistry*, 59, 817–823
4 Collins, L. *et al.* (1997) Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* 41, 1004–1009
5 Skehan, P. *et al.* (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* 82, 1107–1112

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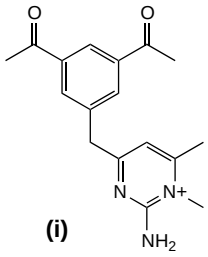
Novel antiviral molecules

Inhibitors of HIV-1 nuclear import

Translocation of transcribed viral DNA from the cytosol into the nucleus of an infected cell is required for HIV replication in non-dividing cells. HIV viral DNA is contained in a large complex of proteins referred to as the pre-integration complex (PIC), which includes integrase, reverse transcriptase, viral protein R and the matrix antigen (MA). There are two stretches of amino acids within the MA that are



(i)

similar in sequence to known nuclear localization signal (NLS) sites located on other proteins, suggesting that this protein is responsible for translocation of the PIC to the nucleus via interaction with the transporter karyopherin α.

According to a recent report, arylene bis(methylketone) compounds such as **i** (ITI-H0294) function as inhibitors of HIV-1 nuclear import [1]. In a cell culture assay that measured the inhibition of viral replication in macrophages, this compound had an IC_{50} of 100 nM. These compounds block translocation by interacting with the NLS located within the MA and also show some binding activity to HIV reverse transcriptase. The bis(methylketone) portion of the template is hypothesized to react with lysine residues that are located within the basic amino acid stretches of the NLS, forming two Schiff bases. Analogues of **i** where the ketone groups were replaced by alcohol or carboxylic acid-based functional groups showed little or no activity. Additionally, treating a reaction mixture consisting of MA and **i** with the reducing agent $NaBT_4$ yielded a labeled MA adduct, presumably through reduction of the Schiff bases.

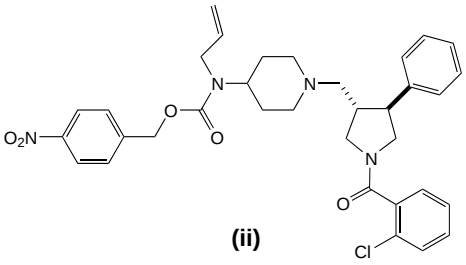
The SAR studies and labeling experiments support the hypothesis that ITI-H0294 reacts with MA to form a Schiff base. Reactions of ketones with the surface lysine residues of proteins is generally considered non-selective. However, selectivity of **i** for MA over other proteins is believed to arise from an interaction of the pyrimidine side chain with HIV reverse transcriptase, a neighboring protein in the PIC. Given the unique target and mechanism exploited by these agents to inhibit HIV, they represent interesting leads towards the development of new agents to combat AIDS.

1 Al-Abed, Y. *et al.* (2002) Inhibition of HIV-1 nuclear import via Schiff base formation with arylene bis(methylketone) compounds. *Bioorg. Med. Chem. Lett.* 12, 3117–3119

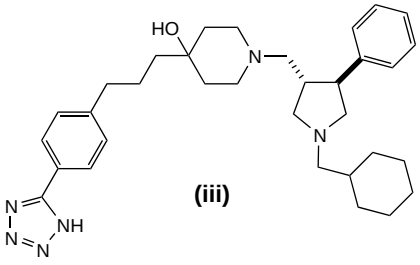
Anti-HIV CCR5 receptor antagonists

Blocking the binding of HIV to the CCR5 chemokine receptor has been identified as a means of inhibiting the entry of the virus into the host cell and thus represents a potential antiviral target. Tri-substituted pyrrolidines have been disclosed by Merck (http://www.merck.com) as CCR5 antagonists capable of binding to the CCR5 receptor and inhibiting the entry of HIV-1 into peripheral blood mononuclear cells (PBMCs). A lead compound in this series was compound **ii**, which had a high affinity for CCR5 and was capable of inhibiting the binding of the endogenous ligand MIP-1α with an IC_{50} of 0.8 nM [2]. In addition, compound **ii** also inhibited HIV infection of HeLa cells and PBMCs with a CIC_{90} (the concentration of compound that inhibits virus antigen production by 90% relative to untreated controls) of 1 and 31 nM, respectively. However, this compound suffered from a high rate of metabolic clearance in the rat (Cl = 41 ml/min/kg). Efforts to overcome this problem by replacing potentially labile functionalities have recently been reported [3,4].

A similar trisubstituted pyrrolidine scaffold was discovered through a targeted combinatorial chemistry campaign, ultimately leading to compound **iii,** which had an IC_{50} of 0.2 nM in the MIP-1α binding assay and inhibited HIV infection in HeLa cells with a CIC_{90} of 4 nM [3]. Unlike compound **ii**, this new lead contains an acidic tetrazole group. The presence of the acidic tetrazole group and the basic piperidine results in the formation of a zwitterion in solution. This chemical feature appears to be important for antiviral activity in cell culture. In a second series of inhibitors, it was



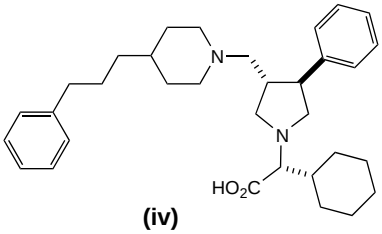
(ii)



(iii)

found that the acidic group could be translocated to the N1-pyrrolidinyl position, such as in compound **iv**, to yield compounds with comparable activity [IC_{50} (MIP-1α) = 0.1 nM, CIC_{90} (HeLa) = 11 nM] [4]. In the rat, compound **iii** suffered from negligible oral bioavailability (F ≈ 0%) and from high clearance (Cl = 61 ml/min/kg), whereas compound **iv** had more desirable pharmacokinetic characteristics (F = 26% and Cl = 17.6 ml/min/kg).

Although compound **iv** is not fully optimized for use in anti-HIV therapy, it does represent an improvement in structure over the original lead and the exploration of this series proved useful in identifying chemical features that yield improved cell culture activity.



(iv)

2 Hale, J.J. *et al.* (2001) 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 2: lead optimization affording selective, orally bioavailable compounds with potent ant-HIV activity. *Bioorg. Med. Chem. Lett.* 11, 2741–2745

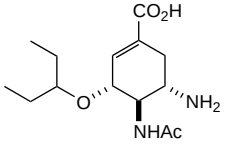
  PII: S1359-6446(03)02720-X

3 Hale, J.J. *et al.* (2002) 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 3: polar functionality and its effect on anti-HIV-1 activity. *Bioorg. Med. Chem. Lett.* 12, 2997–3000

4 Lynch, C.L. *et al.* (2002) 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 4: synthesis of N-1 acidic functionality affording analogues with enhanced antiviral activity against HIV. *Bioorg. Med. Chem. Lett.* 12, 3001–3004

Novel influenza neuraminidase inhibitors

Inhibition of the influenza neuraminidase enzyme by compounds such as GS-4071 (**v**, oseltamivir) has proven to be effective for the treatment of infection. A basic group was assumed to be required for binding to the enzyme. Indeed, X-ray crystallographic analysis of GS-4071 bound to neuraminidase showed that



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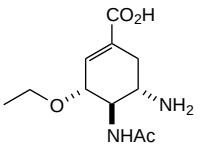
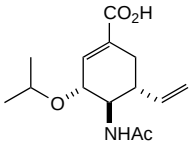


(vi)



(vii)

the NH_2 group interacts with the conserved acidic residues Glu119, Glu227 and Asp151. However, a recent publication challenges this assumption and provides evidence that uncharged hydrophobic groups bind to this site with equal facility [5]. A comparison of the inhibitory activity of compound **vi** with compound **vii** illustrates this point. Compound **vi** is identical to GS-4071 except for the smaller alkyl group attached to C-3, which results in a reduced binding affinity to neuramindase (K_i = 0.18 μM). By contrast, compound **vii** has a larger C-3-ether group but, more importantly, the C-5-amino group is replaced by ethylene. Despite the uncharged nature of this group, the resulting compound shows a greater inhibition of neuraminidase activity (K_i = 0.045 μM). An X-ray crystal structure of compound **vii** bound to neuraminidase shows the ethylene group in close proximity to Asp151 and Glu119, confirming its role as a replacement for the NH_2 group.

The successful replacement of an amino group by ethylene appears to be a rather unique modification that could potentially be applied to other enzyme inhibitors that bind to acidic protein residues.

5 Hanessian, S. *et al.* (2002) Design, synthesis, and neuraminidase inhibitory activity of GS-4071 analogues that utilize a novel hydrophobic paradigm. *Bioorg. Med. Chem. Lett.* 12, 3425–3429

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