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.**

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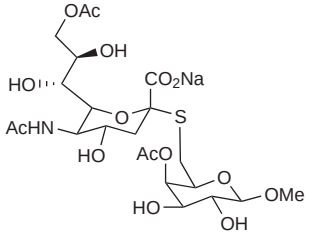
Molecules

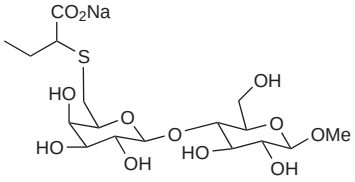
Rotavirus inhibitors

Rotaviruses are responsible for causing the severe gastroenteritis in infants that results in 600,000 deaths annually worldwide. The virus is highly host-cell specific and this is believed to occur through its selective recognition of sialic acid (*N*-acetylneuraminic acid) residues expressed on the host-cell surface. Inhibition of this adhesion process is, therefore, an attractive target for drug development.

One approach is to design inhibitors that mimic the cellular sialic-acid-containing recognition sites located on the cell. Towards this goal, a group led by Mark von Itzstein has reported thioglycoside **(i)** as an inhibitor of rotavirus infection in cell culture, although this activity was strain selective [1]. Unfortunately, although this inhibitor proved to be active against a bovine (NCDV) and a simian (SA11) strain of the virus, it was inactive against a human strain (Wa). However, a recent paper by the same group describes the synthesis and antiviral activity of compound **(ii)** [2]. This compound is derived from a simplified sialic-acid mimic, ethylacetic acid, attached via a thioether bond to a disaccharide. Unlike the first compound, **(ii)** is active against both bovine (NCDV) and human (Wa) rotavirus strains. More importantly, although **(ii)** is only a modest inhibitor of rotavirus infection in cell culture, this appears to be the first report of a simple carbohydrate derivative with activity against a human strain of the virus.



(i)



(ii)

1 Kiefel, M.J. *et al.* (1996) Synthesis and biological evaluation of *N*-acetylneuraminic acid-based rotavirus inhibitors. *J. Med. Chem.* 39, 1314–1320

2 Fazil, A. *et al.* (2001) Synthesis and biological evaluation of sialylmimetics as rotavirus inhibitors. *J. Med. Chem.* 44, 3292–3301

New HIV-1 protease inhibitors

HIV-1 encodes an aspartic protease that is essential for viral maturation. This has proved to be a useful target for the development of antiviral agents, with five HIV-1 protease inhibitors currently approved for therapy. However, because of the emergence of resistance and side effects associated with such therapies, efforts remain focused on developing new and improved protease inhibitors.

HIV protease is a C_2-symmetrical homodimeric protein, with the dimer interface forming the active site. Two novel approaches that take advantage of this dimeric nature in the design of new protease inhibitors have recently been reported.

In the first report, the C_2-symmetrical structure of the substrate binding site is used in the design of C_2-symmetrical inhibitors [3]. This is not a new concept [4]; however, what might set this report apart from others is the carbohydrate-based scaffold used. This enables the rapid exploration of the putative P1/P1′ sites of the inhibitor. The current report features P1/P1′ substituents linked to the scaffold via thioether bonds. As such, compound **(iii)** was found to exhibit potent activity against both the HIV protease (K_i = 0.5 nM) and HIV itself (ED_{50} = 0.027 μM) in HIV-1-infected MT-4 cells.

The second report describes the synthesis of peptide **(iv)** and the peptidomimetic **(v)**, which act by inhibiting protease dimerization [5]. These compounds were specifically designed to disrupt dimerization by binding to the interfacial domains of the protease monomer in a β-strand fashion. Kinetic analysis revealed that **(iv)** and **(v)** inhibited dimerization with a K_{id} value of 5.4 μM and 9.1 μM, respectively. Compound **(v)**

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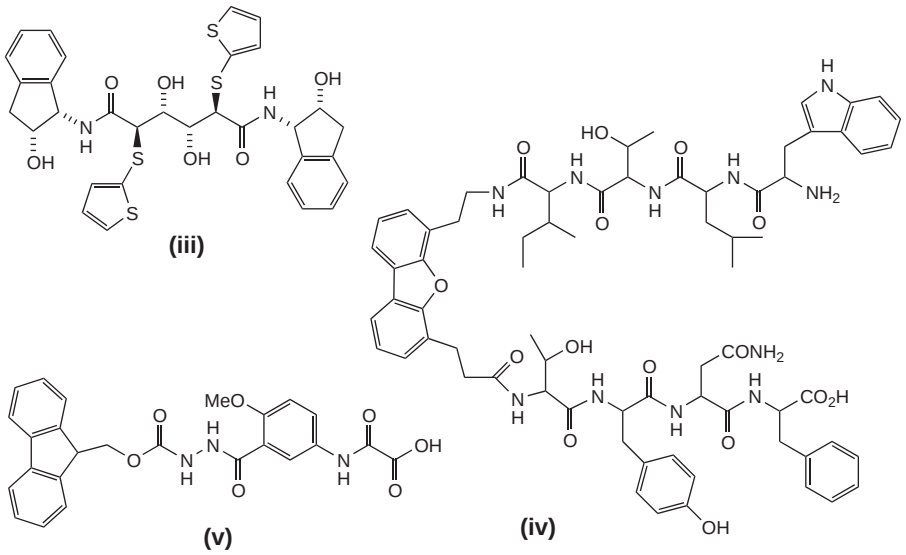

(iii)

(v)

(iv)

is the first reported non-peptide-based HIV-protease dimerization inhibitor.

3 Mühlman, A. *et al.* (2001) Synthesis of potent C2-symmetric, diol-based HIV-1 protease inhibitors. Investigation of thioalkyl and thioaryl P1/P1′ substituents. *J. Med. Chem.* 44, 3402–3406

4 Kempf, D.J. (1994) Design of symmetry-based, peptidomimetic inhbitors of human immunodeficiency virus protease. *Methods Enzymol.* 241, 334–354

5 Song, M. *et al.* (2001) Design and synthesis of new inhibitors of HIV-1 protease dimerization with conformationally constrained templates. *Bioorg. Med. Chem. Lett.* 11, 2465–2468

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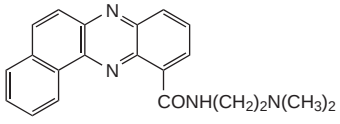
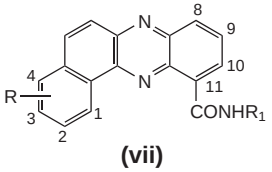
Novel angular benzophenazines as potential anti-cancer agents

The topoisomerases are essential enzymes in the regulation of DNA topology, and are important cellular targets for several chemotherapeutic agents [6]. Drugs that target topoisomerase II (e.g. doxorubicin and etoposide) have been widely used in cancer therapy and, specifically, those that target topoisomerase I (e.g. camptothecin analogues) have made an important impact on the treatment of colon cancer [7]. However, a common shortfall of these compounds is their inability to overcome multidrug resistance (MDR) [8,9].

Several dual inhibitors of topoisomerase I and II have been identified. Among others, the acridine derivative XR5000 (DACA) [10] and TAS103 [11] are currently in clinical trials. In addition, the benzo[*a*]phenazinecarboxamide **(vi)** showed cytotoxic potency *in vitro* when evaluated against L1210 and P388 leukaemia cell lines [12]. Vicker and collaborators [13] extended their studies to a series of novel, substituted, angular tetracyclic phenazines **(vii)**, and tested their cytotoxicity in four cell lines: (1) H69 parental (H69/P) human small-cell lung carcinoma; (2) H69/LX4 drug-resistant human small-cell lung carcinoma [which overexpresses P-glycoprotein (P-gp)]; (3) COR-L23 parental (COR-L23/P) human non-small-cell lung carcinoma cell line; and (4) COR-L23/R drug-resistant human non-small-cell lung carcinoma cell line [which overexpresses multidrug-resistance-associated protein (MRP)].



(vi)

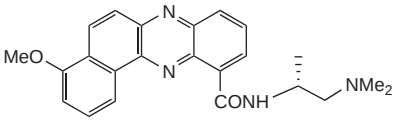


(vii)

R = hydroxy, alkoxy, amido, ester, halogen, amino, nitro, cyano groups.

An extensive structure–activity relationship (SAR) study was carried out, which initially considered the effects of substitution at the 2–4- and 8–10-positions. Because previous reports indicated that substitution at position 1 was poorly tolerated, this position was not further investigated [12]. By contrast, preliminary data had indicated that substitution at position 4 is generally most favoured and, therefore, tolerance at this position was examined in detail. Eventually, the SAR of the amide side-chain variation, including the steric tolerance in the α position to the amide group, was explored.

Selected compounds were then studied for their ability to stabilize cleavable DNA complexes in the presence of either topoisomerase I or II [10]. The enantiomerically pure **(viii)**, [(*R*); XR11576], which inhibited both topoisomerase I and II in a dose-dependent manner at a concentration range of 0.03–1 μM, was found to be a potent cytotoxic agent; IC_{50} values for H69/P and H69/LX4 were 23 nM and 29 nM, respectively. In the same experiment, the IC_{50} values for TAS-103 with H69/P and H69/LX4 were 21 nM and 22 nM, respectively. In addition, compound **(viii)** had good oral bioavailability (~72%) in female mice and demonstrated linear pharmacokinetics after intravenous dosing. Finally, compound **(viii)** given intravenously daily for seven days of three cycles at



[(*R*); XR11576]

(viii)

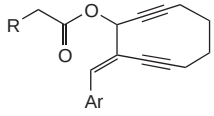
30 and 45 mg kg^{-1} evoked a dose-related tumour growth delay in female mice.

6 Wang, J.C. (1996) DNA topoisomerases. *Annu. Rev. Biochem.* 65, 635–692
7 Dancey, J. *et al.* (1996) Current perspectives on camptothecins in cancer treatment. *Br. J. Cancer* 74, 327–338
8 Seeber, S. *et al.* (1982) *In vivo* resistance towards anthracyclines, etoposide, and *cis*-diamine-dichloroplatinum (II). *Cancer Res.* 42, 4719–4725
9 Hendricks, C.B. *et al.* (1992) Effect of P-glycoprotein expression on the accumulation and cytotoxicity of Topotecan (SK & F104864), a new camptothecin analogue. *Cancer Res.* 52, 2268–2278
10 Finlay, G.J. *et al.* (2001) From ansacrine to DACA (*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide): selectivity for topoisomerases I and II among acridine derivatives. *Eur. J. Cancer* 32A, 708–714
11 Utsugi, T. *et al.* (1997) Antitumor activity of a novel quinoline derivative, Tas-103, with inhibitory effects on topoisomerases I and II. *Jpn. J. Cancer Res.* 88, 992–1002
12 Rewcastle, G. *et al.* (1987) Synthesis and antitumor activity of substituted phenazine-1-carboxamides. *J. Med. Chem.* 30, 843–851
13 Vicker, N. *et al.* (2002) Novel angular benzophenazines: dual topoisomerase I and topoisomerase II inhibitors as potential anticancer agents. *J. Med. Chem.* 45, 721–739

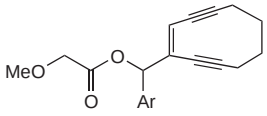
A novel class of enediyne prodrugs

The enediyne antitumour antibiotics represent a family of natural products that consist of a (*Z*)-hex-1,5-diyn-3-ene moiety embedded in either a 9- or a 10-membered ring [14,15]. An apoprotein is normally associated with the labile nine-membered ring enediyne through non-covalent interaction. It prevents the enediyne moiety from decomposition and facilitates its delivery to its target DNA in the nucleus for cleavage chemistry to occur [16]. The naturally occurring, 10-membered-ring enediynes are stabilized by various 'locking devices' with strategically engineered molecular architectures. Bioactivation is generally required for the activity of these enediyne antitumour antibiotics, and thus they are referred to as prodrugs [17].

Further to their previous results [18], Dai and collaborators have recently reported the DNA-cleavage potency and cytotoxicity of a series of (*E*)-3-acyloxy-4-(arylmethylidene)cyclodeca-1,5-diynes [**ix (a–d), x (a–d)**] [19]. The DNA cleavage activity of the compounds was tested using ΦX174 RF1 supercoiled DNA (form I). Previous experiments had revealed a time-dependent DNA scission and an increase in potency of the compounds with time over a period of 48 h; therefore, in this study the samples were allowed to react with DNA for 72 h at 37°C. Compound **x(a)** was found to have almost the same level of activity as **xi(a)** over the concentration range of 5–100 μM. These results suggest that **x(a)** and its analogues might undergo an allylic rearrangement to give a 10-membered-ring enediyne.



(ix) R = H
(x) R = OMe



(xi) **(a)** Ar = Ph
(b) Ar = 1-Naph
(c) Ar = 2-Naph
(d) Ar = *p*-MeOC$_6$H$_4$

The DNA cleavage results showed that, when tested at 20 μM, the phenyl derivatives are much weaker cleavers than the naphthyl derivatives. However, because the DNA binding constants of the two drug types are similar, the reason for this result is unclear. The cytotoxicity of the compounds was assayed using the P388 mouse T-cell-leukaemia cell line. In this test, the phenyl derivatives, correlating with their poor DNA cleaving activity, were less potent than the corresponding naphthyl analogues. The most interesting compounds were **ix(b)** and **ix(c)**, which had low micromolar IC_{50} values (IC_{50} = 2.4 and 2.8 μM, respectively). IC_{50} values for **xi(b)** and **xi(c)** were 3.8 and 9.5 μM, respectively.

14 Lhermitte, H. *et al.* (1996) The enediyne and dienediyne-based antitumor antibiotics. Methodology and strategies for total synthesis and construction of bioactive analogues. Part 1 and Part 2. *Contemp. Org. Synth.* 3, 41–46
15 Lhermitte, H. *et al.* (1996) The enediyne and dienediyne-based antitumor antibiotics. Methodology and strategies for total synthesis and construction of bioactive analogues. Part 1 and Part 2. *Contemp. Org. Synth.* 3, 93–124
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18 Dai, W-M. *et al.* (1999) Synthesis and DNA cleavage study of a 10-membered ring enediyne formed via allylic rearrangement. *J. Org. Chem.* 64, 682–683
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