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Monitor: molecules and profiles

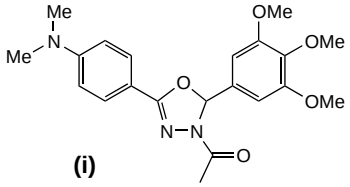
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and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

New tubulin-binding antimitotic compounds

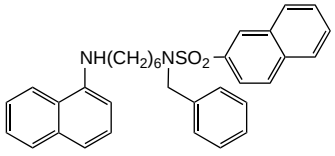
The major protein component of microtubules, tubulin, has proven to be an important target for several antitumour drugs. Examples of such agents are provided by the *vinca* alkaloids, the taxanes and the colchicines. Although these classes of agent differ in the way in which they affect normal tubulin polymerization, the consequence of tubulin binding is the same for all three classes, namely metaphase arrest in dividing cells followed by the induction of apoptosis.

An important drawback in the clinical use of these agents is the development of drug resistance, which often occurs through the expression of the membrane exporter protein, P-glycoprotein (P-gp). The development of microtubule inhibitors that are effective in drug-resistant tumours has, therefore, become an important area of research. Tahir and coworkers [1] reported the discovery of A204197 **(i)**, a novel antiproliferative tubulin-binding oxadiazoline derivative, which has the same activity (IC_{50} = 36–48 nM) *in vitro* in cell lines resistant to paclitaxel, vinblastine or colchinine [1]. A204197 was found to induce cell-cycle arrest in G_2–M phase, increase phosphorylation of certain G_2–M checkpoint proteins and induce apoptosis. In addition, A204197 competed with [^{3}H]-labelled colchinine (but not [^{3}H]-labelled paclitaxel) for binding to tubulin, and prevented tubulin polymerization in a dose-dependent manner.



(i)

In related work, Chibale and coworkers [2] synthesized sulfonamide derivatives of the multidrug resistance reversal (MDRR) antimalarial drugs chloroquine and primaquine, and evaluated their potential to chemosensitize cancer cells to paclitaxel. Most notably, the chloroquine derivative **(ii)** exhibited 99% MDRR activity in human breast cancer cells *in vitro* when co-administered with paclitaxel at 1 μM. Molecular modelling studies show that these new analogues share a common pharmacophore with other taxane MDRR agents.



(ii)

1 Tahir, S.K. *et al.* (2001) A-204197, a new tubulin-binding agent with antimitotic activity in tumor cell lines resistant to known microtubule inhibitors. *Cancer Res.* 61, 5480–5485

2 Chibale, K. *et al.* (2001) Modulation of human mammary cell sensitivity to paclitaxel by new quinoline sulfonamides. *Biorg. Med. Chem. Lett.* 11, 2457–2460

Novel bioreductive–hypoxia-selective agents

NAD(P)H:quinone oxidoreductase (NQO1), also known as DT-diaphorase, catalyzes the two-electron reduction of quinones using either NADH or NADPH as cofactor. NQO1 can protect cells against the toxic effects of quinones but, importantly, is also involved in the bioreductive activation of several cytotoxic quinone anticancer agents [e.g. mitomycin C (MMC) and cyclopropamitosenes]. A clear correlation between NQO1 activity and MMC sensitivity in human lung- and breast-cancer cell-lines has been observed, providing validation of NQO1 as a useful antitumour target.

Swann and coworkers [3] described the synthesis of a range of indolequinones bearing various functional groups (selected on the basis of the X-ray crystal structure of human NQO1) and the effect of substituents on the metabolism of quinones by recombinant human NQO1. In general, compounds with electron-withdrawing groups at the indole 3-position were among the best substrates for the enzyme, and compounds with groups larger than methyl at indole N-1 were tolerated. Substrates with a leaving group at the 3-indolyl methyl position generally inactivated the

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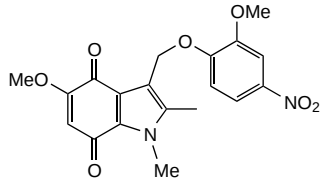
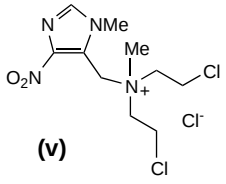
enzyme and were the most cytotoxic and selective compounds towards human colon carcinoma cells with high NQO1 activity (BE-NQ) compared with colon cells with no detectable NQO1 activity (BE-WT). For example, for compound **(iii)** the IC_{50} value for BE-NQ is 0.51 μM, whereas the IC_{50} value for BE-WT is 1.5 μM.



(iii)

In a related report, Na *et al.* [4] reported the synthesis and antitumour evaluation of a series of dimeric mitomycins in which the mitomycin units are tethered at either the C-7 amino [e.g. **(iv)**] or aziridine nitrogen positions. Comparison of the efficiency of DNA-crosslinking for the dimeric mitomycins (using a denaturing-gel-electrophoresis-based assay) with their *in vitro* cytotoxicity in various human-tumour cell-lines revealed a poor correlation. Indeed, the mitomycins with the highest levels of DNA crosslinked adducts displayed the weakest cytotoxicities, indicating that DNA adduction is probably just one of a number of parameters governing mitomycin efficacy.

The development of hypoxia-selective cytotoxins as selective antitumour agents is described by Tercel and coworkers [5]. The group had previously described the potential of nitrobenzyl quaternary salts of nitrogen mustards as hypoxia-selective cytotoxins, and have now reported the synthesis and evaluation of a series of heterocyclic analogues, including pyrrole, imidazole, thiophene and pyrazole examples. Candidate compounds were chosen to cover a range of one-electron reduction potentials (from –277 to –511 mV) and substitution patterns. All compounds tested were less toxic *in vitro* than mechlorethamine and were more cytotoxic under hypoxic than aerobic conditions, although differentials were highly variable within the series. Most notably, compound **(v)** produced DNA-crosslinks in hypoxic RIF-1 cells, and showed *in vivo* activity in combination with radiation or cisplatin. However, further development of this agent has been restricted by its unpredictable *in vivo* toxicity.



(v)

3 Swann, E. *et al.* (2001) Indolequinone antitumour agents: correlation between quinone structure and rate of metabolism by recombinant human NAD(P)H:quinone oxidoreductase. Part 2. *J. Med. Chem.* 44, 3311–3319

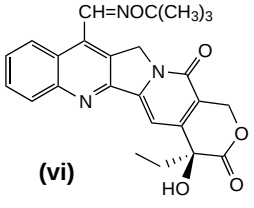
4 Na, Y. *et al.* (2001) Synthesis, DNA cross-linking activity and cytotoxicity of dimeric mitomycins. *J. Med. Chem.* 44, 3453–3462

5 Tercel, M. *et al.* (2001) Hypoxia-selective antitumor agents. 16. Nitroarylmethyl quaternary salts as bioreductive prodrugs of the alkylating agent mechlorethamine. *J. Med. Chem.* 44, 3511–3522

Novel camptothecin analogues

New derivatives of the camptothecin (CPT) class of topoisomerase I (topo I) inhibitors continue to attract interest as antitumour agents. Topo I is an essential enzyme for topological DNA modifications during crucial cellular processes, such as replication, transcription and repair. Camptothecin is able to stabilize topo I-DNA 'cleavable' complexes, resulting in topo I-mediated DNA strand breaks. Two CPTs, topotecan and CPT-11, have been approved for clinical use and several other CPTs are in clinical development at present.

Dallavalle and coworkers reported the synthesis and antitumour activity of topo 1 inhibitors of a series of oxyiminomethyl derivatives in position 7 of camptothecin [6]. The compounds were tested *in vitro* against the human non-small-cell lung carcinoma cell line H460, and in 24 out of 37 cases the activity was in the 0.01–0.3 μM range. Quantitative-SAR (QSAR) analysis indicated that lipophilicity was the most important parameter contributing to *in vitro* cytotoxicity. Analysis of the DNA-topo I-drug cleavable complex indicated that for the most potent compounds [e.g. compound **(vi)**] the cytotoxicity was partially related to the stabilization and persistence of the cleavable complex. *In vivo* activity of **(vi)** was compared with topotecan in human lung-tumour xenograft mouse-models. Compound **(vi)** exhibited superior activity in both H460 and LX1 xenografts in terms of tumour growth inhibition and log-cell kill. At the maximum tolerated dose for **(vi)**, 3 mg kg^{-1}, all treated mice bearing the lung carcinoma LX1 achieved complete regression.



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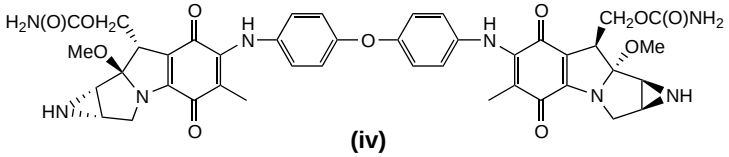

(iv)

Fan *et al.* [7] reported their studies on antitumour-QSARs in 167 camptothecin analogues tested in the National Cancer Institute (Bethesda, MD, USA) *in vitro* 60 human cancer cell-line panel. The

analogues were selected from >70,000 screened compounds, yielding rich information on mechanisms of drug action and drug resistance. The average pairwise Pearson correlation coefficient within the CPT set was found to be 0.70 (indicating similar patterns of antitumour activity across the 60 cell lines) and coherence between structures and their activity patterns was observed. QSAR studies on 58 compounds using GI_{50} values for 60 cell lines as the dependent variables were performed. The application of various statistical methods to construct QSAR models indicated that the fully cross-validated genetic function approximation (GFA) method performed best in terms of correlation coefficients and cross-validation analysis. Several molecular descriptors, including partial atomic charges and three interatomic distances that refine the relative spatial dispositions of three significant atoms were correlated with antitumour activity. The cross-validated r^2 for the final GFA model was 0.783, indicating a predictive QSAR model.

6 Dallavalle, S. *et al.* (2001) Novel 7-oxyiminomethyl derivatives of camptothecin with potent *in vitro* and *in vivo* antitumor activity. *J. Med. Chem.* 44, 3264–3274

7 Fan, Y. *et al.* (2001) Quantitative structure-antitumor activity relationships of camptothecin analogues: cluster analysis and genetic algorithm-based studies. *J. Med. Chem.* 44, 3254–3263

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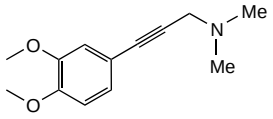
Combinatorial chemistry

Semicarbazide-sensitive amino oxidase inhibitors

The semicarbazide-sensitive amine oxidases (SSAOs) are a widespread group of enzymes found in plants and animals, which possess a copper atom and an oxidized tyrosine residue (topaquinone) in their active site. Although they appear to be involved in the oxidative deamination of endogenous amines, their precise function is unknown. In humans, SSAOs have been implicated in the physiopathology of diabetes.

Elevated levels of SSAOs have been reported in diabetics and it is believed that vascular damage could result from the toxicity of formaldehyde and hydrogen peroxide formed by the action of SSAOs on endogenous methylamine. Drugs that inhibit SSAOs could thus prove useful as prophylactics that prevent the long-term retinal damage associated with diabetes mellitus [1].

A small library of 20 single aryl propargyl amines was synthesized in solution. Testing the compounds in this library for their ability to inhibit bovine plasma SSAO identified several potent compounds. One of the most potent isolated was **(i)**, which possessed a K_i value of 2.9 μM.



(i)

The structure–activity relationship (SAR) obtained from studying this library of compounds suggests that binding to the active site occurs by coordination of the amine to the proximal copper (II) and formation of a π-complex between topaquinone and the electron-rich aryl group of the inhibitor. Further putative inhibitors are currently being synthesized, their design based on the SAR obtained from the small library discussed here, to test this hypothesis.

1 Conn, C. *et al.* (2001) Combinatorial synthesis of SSAO inhibitors using Sonogashira coupling: SAR of aryl propargylic amines. *Bioorg. Med. Chem. Lett.* 11, 2565–2568

Tyrosine and dual-specificity protein phosphatase inhibitors

Tyrosine phosphorylation of proteins is a fundamental mechanism of intracellular signal transduction involved in important cellular events such as cell growth and differentiation. The phosphorylation states of proteins are strictly controlled by various protein tyrosine-kinases (PTKs) and protein tyrosine-phosphatases (PTPs). PTPs represent a diverse family of enzymes that exist as integral membrane and non-receptor forms. Disorders of PTPs are likely to be related to several diseases such as cancer, autoimmune diseases and diabetes, and the characterization of PTPs are, therefore, expected to be not only useful tools for clarifying the biological functions of the PTPs themselves, but also candidates for novel therapeutics.

Recently, a subgroup of PTP enzymes that dephosphorylate both phosphotyrosine and phosphoserine/threonine have attracted attention. In particular, CDC25 phosphatases (CDC25-A, -B, and -C) are considered important members of a family of dual-specificity phosphatases (DSPs) that are known to be key enzymes in cell-cycle progression. CDC25 dephosphorylates and activates cyclin-dependent kinases. To find a selective inhibitor of CDC25, as well as other biologically important PTPs or DSPs, a focussed library was synthesized that used the structural features of PTPs and DSPs for its design [2].

A small library of 32 single compounds was synthesized in solution. Screening assays were performed against a recombinant vaccinia virus (VH-1)-related phosphatase (VHR) protein, using *p*-nitrophenyl phosphate as a substrate to determine the compounds' ability to act as VHR inhibitors. VHR is itself is a DSP that has been found to dephosphorylate and inactivate members of the mitogen-activated protein-kinase (MAPK) family. Additionally, compounds were tested for their ability to inhibit a recombinant CDC25B protein, using

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