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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.

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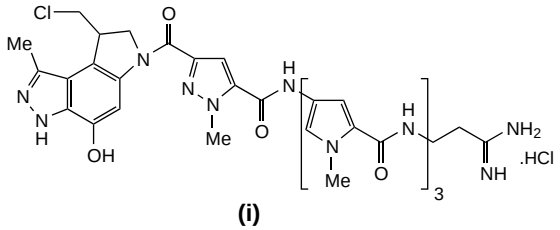
New antitumour molecules

Antitumour cyclopropylindole (CPI)-lexitropsin hybrid molecules

The search for small molecule sequence-selective DNA alkylators is an area of intense current research activity. The antibiotics Distamycin A and (+)-CC1065 are examples of such sequence-selective agents (for AT-rich regions of DNA), and CC1065 in particular has attracted interest as an antitumour agent. CC1065 exerts its antitumour effect through binding to double-stranded B-DNA within the minor groove at AT-rich sequences, and selectively alkylating adenine through its cyclopropylpyrroloindole (CPI) subunit.

Baraldi and coworkers at the Università di Ferrara (Ferrara, Italy) have reported the synthesis and biological evaluation of a series of water-soluble conjugate molecules, comprising either two CPI pyrazole analogues and three mixed pyrazole–pyrrole lexitropsins structurally related to distamycin A (Ref. 1), to increase DNA affinity and extend sequence selectivity. It was found that tethering the pyrazole CPI analogues to the DNA-binding lexitropsins afforded, with few exceptions, conjugate molecules that showed enhanced cytotoxic activity against five different cancer cell lines *in vitro*. In particular, conjugate molecule **(i)** was particularly active against L1210 murine leukaemic cells (IC_5 = 7.4 nM) and T- and B-human lymphoblast cells (IC_{50} = 71 nM and 8.8 nM, respectively). In addition, high-resolution denaturating gel electrophoresis indicated that **(i)** selectively alkylates the third adenine of the 5'-ACAAAAATCG-3' motif within a 400 bp DNA fragment, the strongest and most highly sequence-specific DNA alkylation activity observed. This high sequence selectivity suggests that this type of hydrid molecule might in time, be able to target a single gene.

1 Baraldi, P.G. *et al.* (2001) Design, synthesis, DNA binding, and biological evaluation of water-soluble hybrid molecules containing two pyrazole analogues of the alkylating cyclopropylpyrroloindole (CPI) subunit of the antitumor agent CC-1065 and polypyrrole minor-groove binders. *J. Med. Chem.* 44, 2536–2543



(i)

Purine-based estrogen sulfotransferase inhibitors

Substantial evidence now exists to suggest that sulfated biomolecules contribute to several disease states, including chronic inflammation, HIV-1 infection and hormone-dependent breast tumour growth. Estrogen sulfotransferase (EST) catalyzes the transfer of a sulfuryl group from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to estrogen and related compounds in the cytosol. A delicate balance of sulfated and non-sulfated estrogens is crucial to maintain hormone homeostasis; unusually high levels of estrogen sulfate, however, are found in breast tumour cells, implicating EST as a potential therapeutic target in breast cancer.

The X-ray crystal structure of EST bound with estrogen and 3',5'-diphosphoadenosine (PAP, a product of the sulfation reaction) has been solved and several EST inhibitors have previously been reported; however, in many of these cases secondary biological activities, such as disruption of hormone action, are prevalent. Bertozzi and coworkers at the University of California (USA) have reported the screening of a 275-membered purine library (originally targeted against the cyclin-dependent protein kinase, CDK2) for EST inhibitors using a dual assay procedure. This involves MS analysis of pre- and post-incubation mixtures of enzyme immobilized on an agarose

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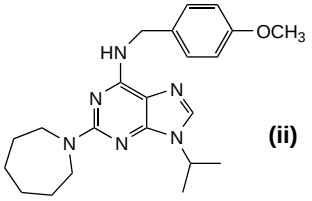
column and purine (higher throughput), and a radiolabel transfer assay[2]. The most potent compound in this series, **(ii)**, had an IC_{50} value of 500 nM against EST, but no inhibitory activity against three representative members of the related carbohydrate sulfotransferase family at a concentration of 200 μM.



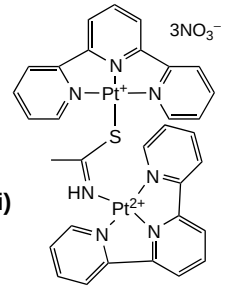
(ii)

2 Verdugo, D.E. *et al.* (2001) Discovery of estrogen sulfotransferase inhibitors from a purine library screen. *J. Med. Chem.* 44, 2683–2686

Inhibition of human thioredoxin reductase by (2,2':6',2''-terpyridine) platinum (II) complexes

Human thioredoxin reductase (hTrxR) is an FAD-dependent homodimeric oxidoreductase involved principally in the reduction of the 12 kDa protein thioredoxin, but also a range of other low molecular weight compounds such as lipoamide, hydrogen peroxide and *S*-nitrosoglutathione. Reduced thioredoxin (Trx) provides reducing equivalents for several processes, such as the formation of deoxyribonucleotides, a key step in DNA synthesis and, therefore, also in cellular proliferation. In addition, the thioredoxin–thioredoxin-reductase system maintains cellular redox homeostasis, regulates the activity of transcription factors and certain enzymes, and modulates protein biosynthesis. Many tumour cells are known to have several-fold increased Trx and TrxR levels, and in some cases tumour cells can secrete reduced Trx, which then acts as an autocrine growth stimulator, implicating the thioredoxin–thioredoxin-reductase system as a potential target for novel anticancer drugs. Previous work has shown the known antitumour agents carmustin (BCNU) and auranofin to be efficient hTrxR inhibitors.

(2,2':6',2''-Terpyridine)platinum (II) complexes are known to possess DNA-intercalating activity and are potential chemotherapeutic agents. Becker and coworkers (Giessen University, Giessen, Germany) have now identified TrxR as a major target of (2,2':6',2''-terpyridine) platinum (II) complexes[3], and have synthesized new complexes to optimize this inhibition. The NADPH-reduced enzyme is inhibited almost stoichiometrically by the complexes, forming both a reversible competitive and an irreversible tight-binding component; the closely related enzyme glutathione reductase however, was >1000-times less inhibited. The most potent inhibitor in the series was found to be *N*,S-*bis*(2,2':6',2''-terpyridine)platinum (II)-thioacetamide trinitrate **(iii)**, for which the K_i for the competitive component of the inhibition was 4 nM and the K_i for the tight-binding component was 2 nM after a five-minute incubation time. Growth inhibition (IC_{50}) *in vitro* in the low micromolar range was observed on three glioma cell lines (NCH37, NCH82 and NCH89) and on two head and neck squamous cell lines (HNO97 and HNO199); in the case of NCH37 a corresponding decrease in TrxR activity was observed. The potential of (2,2':6',2''-terpyridine)platinum (II) complexes acting simultaneously at two different intracellular targets – hTrxR and DNA – as antitumour agents is thus suggested.

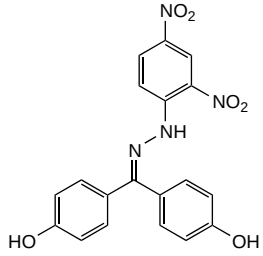


(iii)

3 Becker, K. *et al.* (2001) Human thioredoxin reductase is efficiently inhibited by (2,2':6',2''-terpyridine)platinum (II) complexes. Possible implications for a novel antitumor strategy. *J. Med. Chem.* 44, 2784–2792

4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007) double salts as antitumour agents

4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007) **(iv)** is known to possess anticancer properties *in vitro* and *in vivo* when applied topically to metastatic cancer spread to the skin. Morgan and coworkers (DEKK-TEC and University of New Orleans, New Orleans, LA, USA) have synthesized a series of A-007 double salts with phenothiazin-5-ium salts, such as **(v)**, which have improved *in vitro* activity against primary human cancer cell cultures (breast, melanoma, ovarian and non-small-cell lung)[4]. In addition, the stable salt between **(iv)** and **(v)** allowed diffusion into the dermis layers of the skin in rats, and it is anticipated that these salts could allow A-007 to penetrate into the deep lymphatic or vascular channels of the dermis, thereby improving *in vivo* anticancer properties.



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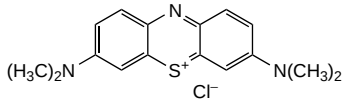

(v)

4 Morgan, L.R. *et al.* (2001) Anticancer properties for 4,4´-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007)/3,7-diaminophenothiazin-5-ium double salts. *Bioorg. Med. Chem. Lett.* 11, 2193–2195

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