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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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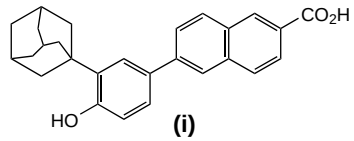
## Molecules

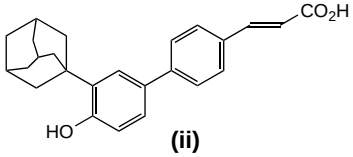
### An 'atypical' retinoid with pro-apoptotic and antitumour activity

The development of drug resistance through the reduced ability of tumour cells to undergo drug-induced apoptosis is a major limiting factor in chemotherapy efficacy. As a result, there is growing interest in the identification of novel strategies to facilitate drug-induced apoptosis. The 'atypical' retinoids represent one apoptosis-based therapeutic approach because some retinoid-related compounds possess unique mechanism(s) of action, such as the ability to activate p53-independent apoptosis (p53 mutations are frequently observed in tumour cells).

Atypical retinoids are known to activate certain retinoic acid receptors (RARs) and exert growth inhibitory or apoptogenic activities that are not receptor mediated; hence, they do not fit into the classical concept of ligand–receptor interaction. Compound **i** is an atypical retinoid that is known to be RARγ-selective; however, the role of RARs in mediating biological activity is uncertain.




**(i)**




**(ii)**

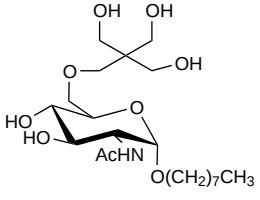
Cincinelli and co-workers [1] have reported the discovery of an atypical retinoid (compound **ii**), which has potent anti-proliferative and pro-apoptotic properties that are more favourable than those of compound **i**. Compound **ii** was tested *in vitro* in a panel of tumour cell lines and was found to be at least twice as potent ($IC_{50}$) as compound **i**. Values for 50% Growth inhibition ($IC_{50}$) ranged from 0.1–0.3 μM, and tumour cell activities were found to be independent of p53 status. Prostate carcinoma cells (such as DU145) in particular exhibited exquisite sensitivity to compound **ii**. Following treatment of sensitive cells with compound **ii** at a concentration causing antiproliferative effects ($IC_{80}$), apoptosis induction was observed after 24 hours. Cell accumulation was present in the S-phase of the cell cycle, suggesting drug interference with DNA replication. Compound **ii** also caused a significant tumour growth inhibition in a human ovarian carcinoma, A2780/DX, and in a human melanoma, MeWo, growing in nude mice. Further development of the antitumour properties of this lead compound are anticipated.

1 Cincinelli, R. *et al.* (2003) A novel atypical retinoid endowed with proapoptotic and antitumour activity. *J. Med. Chem.* 46, 909–912

### Synthetic glycosides for the treatment of CNS tumours

Tumours of the CNS are notoriously difficult to treat, and drug delivery issues often further complicate this field of study. Inhibitors of astroblast and astrocytoma division, with glycidic epitopes that are immunologically related to those of the epidermal growth-factor receptor and of blood groups, have been found in brain extracts. Based on these findings, a tetrasaccharide and, later, a series of α–L-Fuc(1,3)-D-GlcNAc disaccharide derivatives have been synthesized and tested as inhibitors of human glioma growth [2]. Fernández-Mayoralas and co-workers have now reported that octyl 2-acetamido-2-deoxy-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucopyranoside (compound **iii**) inhibits the growth of cultured human astroblastoma cells (from a biopsy sample) and causes a progressive decrease in volume of a primitive neuroectodermal tumour implanted in rats [3].

Bonding of glycoside drugs with synthetic polymers has emerged as a promising strategy for controlled, targeted




**(iii)**



 PII: S1359-6446(03)02722-3

delivery. The effect of polymer disks [a graft copolymer of poly(ε-caprolactone) on poly(methyl methacrylate)] loaded with compound **iii** on cell cultures, obtained from the biopsy of malignant astroblastoma that were unresponsive to surgery was measured. After a four day incubation, observation of the cultures revealed that the glycoside **iii** was released from the polymer disk and the astroblastoma cells were destroyed efficiently. In addition, intracerebral implantation of drug-loaded disks in pigs demonstrated a notable lack of acute clinical toxicity.

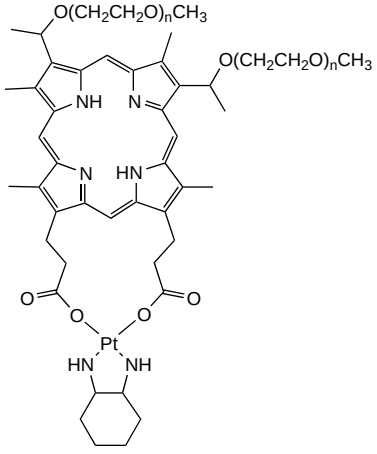
2 Aguilera, B. *et al.* (1998) Novel disaccharide inhibitors of human glioma cell division. *J. Med. Chem.* 41, 4599–4606
3 Fernández-Mayoralas, A. *et al.* (2003) Central neural tumor destruction by controlled release of a synthetic glycoside dispersed in a biodegradable polymeric matrix. *J. Med. Chem.* 46, 1286–1288

### Antitumour hematoporphyrin-platinum(II) conjugates

Cisplatin is an extremely effective anticancer drug used mainly for the treatment of ovarian, testicular, and head and neck cancers. Severe toxic side effects, largely due to lack of drug selectivity for tumour tissue, remain a problematic feature of this class of agent, despite several attempts to increase tumour selectivity.

Certain porphyrins are known to selectively accumulate in tumour tissue via an unknown mechanism, probably involving receptor-mediated endocytosis of low-density lipoproteins (LDL) (cancer cells express elevated levels of LDL receptors). The tumour-targeting properties of porphyrins are often compromised by their insolubility in aqueous solution, nevertheless, some amphiphilic porphyrins are known to selectively accumulate in tumour tissues. Previous studies into selective delivery of platinum(II) complexes to tumour cells have involved the use of sulfanato-porphyrin-platinum(II) conjugates that exhibited a significant tumour-targeting effect (tumour:muscle ratio = 7) [4]. Kim and co-workers have described the synthesis, biodistribution and antitumour activity of hematoporphyrin-platinum(II) conjugates [5]. Poly(ethylene glycol) (PEG) side chains with differing lengths were attached to hematoporphyrin to improve water solubility and for modulation of the hydrophilicity–hydrophobicity balance [5]. Chelation of an antitumour (diamine)Pt(II) moiety via the porphyrin carboxylate groups produced the desired hematoporphyrin-platinum (II) conjugates.

The porphyrin-platinum(II) conjugates were tested *in vitro* and *in vivo* against a variety of human cancer cell lines, and were found to exhibit high antitumour activity, improved water solubility (compared to cisplatin) and considerable lipophilicity. For example, compound **iv** exhibited more potent *in vivo* activity than cisplatin against a leukaemia L1210 cell line and had excellent water solubility.



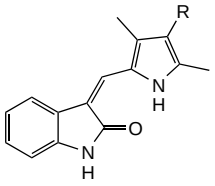

**(iv)** $(MPEG350)_2Hp(COO)_2Pt(dach)$

4 Song, R. *et al.* (2002) Synthesis and selective tumor targeting properties of water-soluble porphyrin-Pt(II) conjugates. *J. Inorg. Biochem.* 89, 83–88
5 Kim, Y-S. *et al.* (2003) Synthesis, biodistribution and antitumour activity of hematoporphyrin-platinum(II) conjugates. *Bioorg. Med. Chem.* 11, 1753–1760

### Indolin-2-ones as novel receptor tyrosine kinase inhibitors

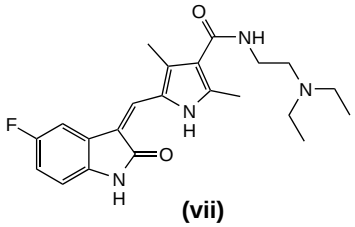
Several growth factor receptor tyrosine kinases are known to have crucial roles in tumour growth and survival, and as such are validated targets for the development of novel cancer therapeutics. For example, the vascular endothelial growth-factor (VEGF) and platelet-derived growth-factor (PDGF) receptors are both known to be expressed on tumour cells, to directly affect tumour cell proliferation and to have prominent roles in angiogenesis.

In recent years, Sun and co-workers have developed two indolin-2-one derivatives for clinical development as a VEGF-R inhibitor compound **v** and a PDGF-Rβ inhibitor (compound **vi**) [6]. However, further development of these compounds was compromised by their low aqueous solubility and/or protein binding properties.




**(v)** R = H
**(vi)** $R = (CH_2)_2COOH$

The same group have since reported the development of indolin-2-one analogues that show potent and broad inhibitory activity against both VEGF-R2 and PDGF-Rβ (simultaneous inhibition of both targets was expected to result in more efficacious antitumour activity), and desirable pharmacological properties with regard to solubility and protein binding [7]. Of the range of analogues synthesized, compound **vii** gave the best overall profile in terms of potency for the VEGF-R2 and PDGF-Rβ tyrosine kinase at biochemical and cellular levels (nanomolar $IC_{50}$ values), inhibitory activity against ligand-induced cellular proliferation (as measured by BrdU incorporation in 3T3 cells), cellular cytotoxicity ($LD_{50}$ = 49 μM), and solubility at pH 2 and pH 6.




**(vii)**

Compound **vii** also possessed good oral bioavailability, was highly efficacious in several preclinical tumour models, and was well tolerated at efficacious doses [8]. This compound is currently in Phase I clinical trials as a new therapeutic agent in the treatment of cancer.


6 Sun, L. *et al.* (1999) Design, synthesis, and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-ylmethylidenyl]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF receptor tyrosine kinases. *J. Med. Chem.* 42, 5120–5130

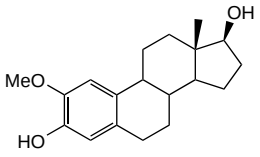
7 Sun, L. *et al.* (2003) Discovery of 5 [5-fluoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. *J. Med. Chem.* 46, 1116–1119

8 Mendel, D.B. *et al.* (2003) *In vivo* anitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting VEGF and PDGF receptors: determination of a pharmacokinetic / pharmacodynamic relationship. *Clin. Cancer Res.* 9, 327–337


### Novel antitumour 2-methoxyestradiol analogues

Tumour angiogenesis has become a popular target for the development of more specific, less toxic anticancer drugs than those currently in clinical use. Targeting of the genetically stable endothelial cells of the developing tumour is an approach that holds great promise. It could produce effective therapies that circumvent the commonly encountered multidrug resistance that is observed when genetically unstable tumour tissue is treated using current cytotoxic agents. Recent data show that some of the more effective anticancer drugs in use presently, have anti-angiogenic properties [9] and several anti-angiogenic agents are currently being evaluated in the clinic.

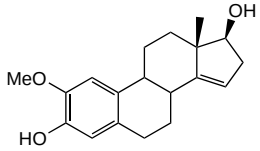
2-Methoxyestradiol (2-ME2) (compound **viii**), a natural metabolite of estrogen devoid of estrogenic effects, is known to exert both anti-angiogenic and antitumour activity in *in vitro* and *in vivo* models. 2-ME2 is currently being evaluated in Phase I and II clinical trials




**(viii)**

against a variety of human tumours; however, its mechanisms of action are unclear and are likely to be multifactoral. Analogues of 2-ME2 have been synthesized and found to be more potent inhibitors of tumour proliferation and tubulin assembly than 2-ME2. Tinley and co-workers [10] have reported the synthesis and evaluation of new 2-ME2 analogues for activities that precede anti-angiogenic and antitumour effects [11].

One analogue in particular, 2-methoxy-14-dehydroestradiol (compound **ix**), was found to be 6–15-fold more potent than compound **viii** when tested for inhibitory activity against endothelial cell proliferation ($IC_{50}$ = 0.05 μM) and invasion ($IC_{50}$ = 0.29 μM) in human umbilical vein endothelial cells (HUVEC). Compound **ix** was




**(ix)**

also found to be ~15-fold more potent than compound **viii** when tested for inhibition of proliferation and cytotoxicity in a variety of human tumour cell lines. When tested *in vivo* in the murine xenograft MDA-MB-435 model (administered intraperitoneally daily for 30 days), **compound ix** provided a 29% inhibition of tumour burden. Mechanistic studies indicate that the active analogues caused mitotic spindle disruption, mitotic arrest, microtubule depolymerization and inhibition of purified tubulin assembly. The novel analogues were also found to cause Bcl-2 phosphorylation and activation of mitogen-activated protein kinase signaling pathways.


9 Miller, K.D. *et al.* (2001) Redefining the target: chemotherapeutics as antiangiogenics. *J. Clin. Oncol.* 19, 1195–1206

10 Rao, P.N. *et al.* (2002) Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs. *Steroids* 67, 1079–1089

11 Tinley, T.L. *et al.* (2003) Novel 2-methoxyestradiol analogues with antitumor activity. *Cancer Res.* 63, 1538–1549



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