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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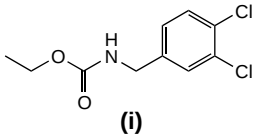
Novel antitumour molecules

Direct activation of the apoptosis machinery

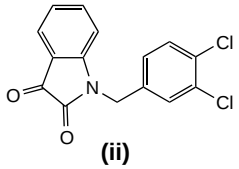
Apoptosis, or programmed cell death, is a key regulator of development and homeostasis, and defects in apoptotic pathways are implicated in both tumorigenesis and multidrug resistance. Two distinct pathways for apoptosis have been characterized. The intrinsic (drug-induced) pathway involves the release of cytochrome c from mitochondria, followed by assembly of the large oligomeric protein complex, known as the apoptosome and consisting of units of the multidomain protein Apaf-1. The apoptosome recruits and activates procaspase-9, which, in turn, activates downstream effector caspases (cysteine aspartyl proteases), such as caspase-3, resulting in engagement of the full apoptotic programme leading to selective cell death.

Many cancer chemotherapeutic agents exert their cytotoxic activity by indirectly engaging apoptosis via the p53 pathway. The *p53* gene is the most frequently mutated gene in cancer (30–70% of clinical tumour samples), therefore, efforts have recently been directed towards finding inhibitors of the anti-apoptotic members of the Bcl-2 family, Bcl-2 and Bcl-X_L, known to be overexpressed in several tumour types. Nguyen and Wells have reported the use of a cell-free assay to simultaneously target multiple components of the apoptosis pathway, screening against a library of ~3500 small molecules [1]. Of the 42 compounds found to activate procaspase-3 processing (monitored by cleavage of a fluorogenic substrate, DEVD-AFC, or DEVDase activity), compound **i** was found to be the most active and was used as the starting point for structure–activity relationship studies. Further biochemical studies have indicated that this class of agent promoted the oligomerization of Apaf-1 into the mature apoptosome, leading to the activation of caspases 9 and 3.



(i)

Compound **ii**, in particular, was found to exert cytostatic and cytotoxic effects on several transformed cell lines (e.g. leukaemic, breast, lung, ovarian and epidermal origin) and several normal cell lines (e.g. peripheral blood lymphocytes, human mammary epithelial cells, non-transformed mammary fibroblasts). IC_{50} values for the leukaemic cell panel, for example, were in the range 4–9 μM, whereas values against normal cell lines were generally >50 μM. Compounds such as **i** and **ii,** that act downstream in the apoptotic pathway and do not require transcription for activity, provide an alternative strategy for targeting cancer cells. This is because they are not dependent on the induction of apoptosis as a by-product of either DNA damage or tubulin blockade and, thus, might provide a basis for overcoming the drug resistance mechanisms that are often associated with traditional chemotherapeutic agents.



(ii)

1 Nguyen, J.T. and Wells, J.A. (2003) Direct activation of the apoptosis machinery as a mechanism to target cancer cells. *Proc. Natl. Acad. Sci. USA* 100, 7533–7538

Novel antimicrotubule agents

Microtubules are hollow tubes, predominantly consisting of tubulin (a heterodimer of α- and β-tubulin), that have a key role in cellular metabolism, intracellular transport, and mitosis. Novel agents that interfere with microtubule assembly, for example, by inhibiting tubulin polymerisation or causing depolymerisation on binding to tubulin, have proven to be efficacious antitumour agents in the clinic [e.g. Taxol® (paclitaxel)]. P-glycoprotein-mediated drug resistance and limited solubility in aqueous systems for natural-product-derived taxanes have initiated further research against this attractive

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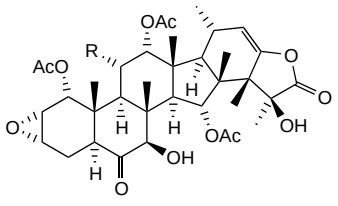
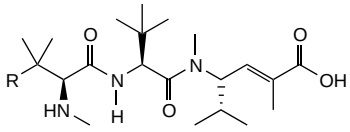
anticancer target. The search for antimicrotubule drugs with superior properties to paclitaxel has yielded several structurally unrelated classes of agent from natural sources, most notably from the marine environment, for example, discodermolide, eleutherobin and the sarcodictyins.

Recent reports detail the discovery and development of further novel classes of antimicrotubule agents. Tinley and co-workers have reported the isolation and purification of the highly oxygenated steroids, taccalonolides E (**iii**) and A (**iv**) from *Tacca chantrieri* during the course of a mechanism-based screening programme designed to identify new microtubule-disrupting agents from natural products [2]. The taccalonolides, like paclitaxel, increased the density of cellular microtubules in interphase cells. Evaluation of *in vitro* antiproliferative effects in drug-sensitive and multidrug-resistant cells has revealed that taccalonolide E (**iii**) was slightly more potent than A (**iv**), and that both compounds were poorer substrates for transport by P-glycoprotein than paclitaxel. In addition, the ability of **iii** and **iv** to circumvent mutations in the Taxol-binding region of β-tubulin (using the PTX 10, PTX 22, and 1A9/A8 cell lines) suggest little cross-resistance and overlap of binding sites, particularly for taccalonolide A. The taccalonolides, in common with other antimitotics, caused G_2-M accumulation, Bcl-2 phosphorylation and initiation of apoptosis. They represent the first plant-derived microtubule-stabilising agents to be identified since paclitaxel and the first natural steroids to show this activity.



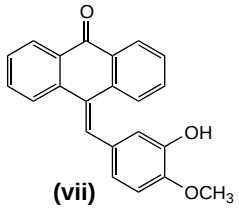
(iii) R=H
(iv) R=OAc

The *Vinca* alkaloids (e.g. vinorelbine, vinblastine and vincristine) are a widely-studied class of agents that bind to the tubulin dimer, block the formation of new microtubules, and lead to the depolymerisation of existing microtubules. The hemiasterlins are a family of highly modified tripeptides, originally isolated from marine sponges, and hemiasterlin (**v**) has been reported to be a potent inhibitor of cell growth through binding to the *Vinca*-peptide site in tubulin, resulting in depolymerisation of microtubules and G_2-M cell cycle arrest. Loganzo and co-workers have reported their studies on the antitumour profile of a synthetic analogue of hemiasterlin, HTI-286 (**vi**) [3]. HTI-286 was found to be a potent antimicrotubule agent, inducing mitotic arrest and subsequent apoptosis. Studies against 18 human cancer cell lines have established HTI-286 as a potent inhibitor of proliferation (mean IC_{50} value = 2.5 nM) and a poorer substrate for P-glycoprotein than currently used antimicrotubule agents, such as paclitaxel and vinorelbine. Pronounced *in vivo* activities were also reported; HTI-286 inhibited the growth of human tumour xenografts (e.g. HCT-15, DLD-1, MX-1W and KB-8-5) in athymic mice (whereas paclitaxel and vincristine were ineffective because of P-glycoprotein-mediated drug resistance). These preclinical properties make this class of agents promising.



(v) R = 1-methylindol-3-yl
(vi) R = phenyl

A further report detailing the antitumour properties of novel antimicrotubule agents, in this case, of synthetic rather than natural-product origin, has been presented by Prinz and co-workers [4]. They note that despite a wealth of reports detailing the discovery and development of antimicrotubule antitumour agents of both natural product (e.g. paclitaxel, colchicine, combretastatin A-4, epothilones A and B) and synthetic origin (e.g. indanocine, phenstatin, acridinyl-9-carboxamide D-82318), no new tubulin polymerisation inhibitors of low molecular weight and synthetic accessibility have yet achieved market approval. Prinz *et al.* report the single-step synthesis and antitumour evaluation of a series of 10-benzylidene-9(10*H*)-anthracenones and 10-(phenylmethyl)-9(10*H*)-anthracenones that were initially screened for antiproliferative activity against K562 leukaemic cells. The most active compound in this assay, compound **vii** (IC_{50} = 20 nM), was found to induce cell cycle arrest in G2/M and induce apoptosis (through monitoring for dose-dependent caspase-3-like protease activity in K562 and MCF-7/Casp-3 cells). Seven compounds of the new series, including compound **vii**, were found to strongly inhibit tubulin polymerisation with increased or comparable activity to those of the reference compounds colchicine, podophyllotoxin and nocodazole, through displacement of [^{3}H]colchicine from its tubulin binding site. In general, antiproliferative activity was found to correlate with inhibition of tubulin polymerisation, and therefore, this novel series of synthetic small-molecule antimicrotubule agents demand further study.



(vii)

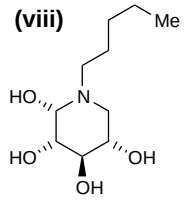
2 Tinley, T.L. *et al.* (2003) Taccalonolides E and A: plant-derived steroids with microtubule-stabilising activity. *Cancer Res.* 63, 3211–3220
3 Loganzo, F. *et al.* (2003) HTI-286, a synthetic analogue of the tripeptide hemiasterlin, is a potent antimicrotubule agent that circumvents P-glycoprotein-mediated resistance in vitro and in vivo. *Cancer Res.* 63, 1838–1845
4 Prinz, H. *et al.* (2003) Novel benzylidene-9(10*H*)-anthracenones as highly active antimicrotubule agents. Synthesis, antiproliferative activity, and inhibition of tubulin polymerisation. *J. Med. Chem.* 46, 3382–3394

A novel inhibitor of glucosylceramide synthase

The tumour-derived gangliosides are a class of sialic acid-containing glycosphingolipids that are a component of the outer leaflet of the cell membrane. Active shedding of gangliosides, and their take-up by host cells in the tumour microenvironment, has been implicated in the enhancement of tumour formation and progression. Biological properties and actions of tumour gangliosides include potent immunosuppressive activity, proangiogenic properties, and enhancement of growth factor-mediated fibroblast and vascular endothelial cell proliferation. In addition, a correlation has been established between the expression and/or shedding of gangliosides and enhanced tumour progression. Further evidence of the *in vivo* link between tumour ganglioside synthesis and shedding, and tumour formation and progression, has been established experimentally; transfection of an antisense sequence to glucosylceramide synthase (a key enzyme involved in the synthesis of glycosphingolipids) was found to inhibit tumour formation in a mouse melanoma model [5].

Weiss and co-workers have now reported the antitumour properties of a novel inhibitor of glucosylceramide synthase, the imino sugar OGT2378 (**viii**). Treatment of the host orally with OGT2378 was found to inhibit MEB4 melanoma tumour growth in a syngeneic, orthotopic murine model. Exposure to 20 μM OGT2378 *in vitro* reduced the glucosylceramide and ganglioside content of MEB4 cells by 93 and >95% respectively. When adminsistered in the diet of C57BL/6 mice (2500 mg kg day^{-1}) three days before intradermal MEB4 tumour innoculation, OGT2378 was well tolerated and depleted host tissue (hepatic) gangliosides by 82% and tumour gangliosides by >98%. This treatment resulted in a tenfold lower mean tumour volume at the end of treatment (four weeks). Marked reduction in tumour volume was also observed when treatment

(viii)



with compound **viii** was initiated seven days after tumour innoculation, suggesting that inhibition of glucosylceramide synthase using OGT2378 is a promising and feasible therapeutic approach to the inhibition of tumour progression.

5 Deng, W. *et al.* (2002) Transfection of glucosylceramide synthase antisense inhibits mouse melanoma formation. *Glycobiology* 12, 145–152

6 Weiss, M. *et al.* (2003) Inhibition of melanoma tumor growth by a novel inhibitor of glucosylceramide synthase. *Cancer Res.* 63, 3654–3658

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