Monitor

Monitor **provides an insight into the latest developments in the pharmaceutical and biotechnology industries.** ***Chemistry*** **examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while** ***Profiles*** **provides commentaries on promising lines of research, new molecular targets and technologies.** ***Biology*** **reports on new significant breakthroughs in the field of biology and their relevance to drug discovery.** ***Business*** **reports on the latest patents and collaborations, and** ***People*** **provides information on the most recent personnel changes within the drug discovery industry.**

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Chemistry

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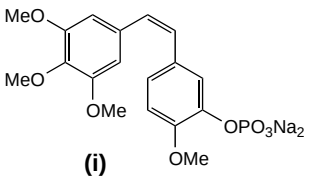
Antitumour molecules

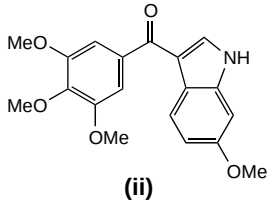
Novel antimitotic agents

Microtubules are dynamic hollow tubular structures that are comprised of α- and β-tubulin heterodimers and have an important role in a number of fundamental cellular functions, including regulation of motility and cell division [1]. Because microtubules have crucial roles in the regulation of mitotic spindle formation, the disruption of microtubule function can have quite drastic effects on cell viability, leading to cell cycle arrest in M phase and induction of apoptosis. The importance of microtubules as an anticancer drug target is underscored by the clinical use of several natural product-derived inhibitors of microtubule dynamics in cancer treatment, including taxanes (e.g. paclitaxel) and Vinca alkaloids (e.g. vincristine). Although they are effective in several malignancies, the clinical potential of the taxane and Vinca alkaloid classes of agent is limited by the development of multidrug resistance (MDR) through, for example, overexpression of the P-glycoprotein transmembrane efflux pump, which can reduce the intracellular drug concentration to a non-toxic level. The development of new antitmitotic tubulin inhibitors that overcome resistance mechanisms has become a topic of great interest in this area in recent years.

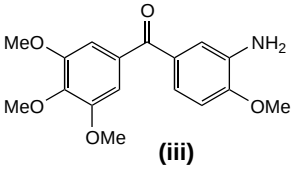
One of the most important tubulin-binding sites of those identified for the various antimitotic agents in clinical development is the colchicine-binding site. Combretastatin A-4 (CA-4) is a naturally occurring antitumour stilbene that inhibits tubulin polymerisation through binding at the colchicine-binding site: the water-soluble phosphate prodrug of CA-4 (CA-4P; **i**) is currently in clinical development [2]. As part of a discovery programme designed to synthesise and test new heterocyclic CA-4 analogues, Kuo and co-workers [3] (National Health Research Institutes, Taiwan; http://www.nhri.org.tw/index/eindex.php3) have reported the discovery and subsequent development work associated with their lead compound in this class (BPR0L075; **ii**). Screening of **ii** in a variety of human cancer cell lines has revealed IC_{50} values in the low nanomolar range. In human cervical carcinoma KB cells, cell cycle arrest in the G_2-M phase and apoptosis were observed. Importantly, several KB-derived cell lines were found to be resistant to vincristine, paclitaxel and colchicine, but not BPR0L075. BPR0L075 displayed potent activity against several human xenograft tumours at *intra venous* doses of 50 mg kg^{-1} in nude mice.



(i)



(ii)



(iii)

In related work, Liou *et al.* [4] (National Health Research Institutes, Taiwan) have examined the synthesis and structure–activity relationships of a series of 3-aminobenzophenones as potential antimitotic agents. This new series was designed to mimic the aminocombretastatin molecular skeleton, which are agents known to act as potent tubulin inhibitors through binding to the colchicine-binding site. Liou and colleagues concluded that an amino group at the B-ring C-3 position and a methoxy group at the C-4 position were required for optimal inhibition of tubulin polymerisation. For example, compound **iii** inhibited the growth of human cancer cell lines KB (oral epidermoid), HT29 (colorectal), TSGH (stomach) and KB-VIN10 (P-glycoprotein 170, overexpressing MDR) with IC_{50} values in the range 31–33 nM, with a tubulin polymerisation inhibition IC_{50} of 0.3 μM.

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1 Amos, L.A. (2000) Focusing-in on microtubules. *Curr. Opin. Struct. Biol.* 10, 236–241
2 Griggs, J. *et al.* (2001) Targeting tumour vasculature: the development of combretastatin A4. *Lancet Oncol.* 2, 82–87
3 Kuo, C-C. *et al.* (2004) BPR0L075, a novel synthetic indole compound with antimitotic activity in human cancer cell lines, exerts effective antitumoral activity *in vivo*. *Cancer Res.* 64, 4621–4628
4 Liou, J-P. *et al.* (2004) Synthesis and structure–activity relationships of 3-aminobenzophenones as antimitotic agents. *J. Med. Chem.* 47, 2897–2905

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Biology

Microbiology

Stimulation of the SOS response by β-lactam antibiotics can provide temporary protection for bacteria

β-lactam antibiotics inhibit penicillin binding proteins (PBPs) and prevent the synthesis of cell wall peptidoglycan, but they are only active against dividing bacteria. Resistance arises through production of β-lactamases, low-affinity PBPs or decreased uptake and/or increased efflux. Miller *et al.* describe a previously unrecognized pathway that enables *Escherichia coli* to partially escape the bactericidal activity of β-lactams by activating the SOS response and inhibiting cell division [1].

This study began with the unexpected observation that exposure of *E. coli* to ampicillin, cephalexin or piperacillin caused induction of the *dpiB–dpiA* two-component signal-transduction system, whereas several other classes of antibiotics had no effect. Growth of a temperature-sensitive PBP3 mutant at 42°C also induced the *dpiBA* operon. Although the primary function of these genes in *E. coli* is unknown, DpiA can activate the *recA*-dependent SOS response regulon, which includes SfiA, a protein that inhibits formation of the septation ring and blocks cell division.

dpiA, *recA* and *sfiA* mutants were tested to determine whether DpiA-mediated growth inhibition could protect bacteria from β-lactams. All three mutants exhibited a tenfold decrease in viability compared with wild type when cells were incubated in ampicillin for 1–2 hours. These observations suggest that β-lactam inactivation of PBP3 can inhibit cell division through a *dpiA–recA–sfiA* pathway and that this response can provide partial protection from the bactericidal activity of β-lactam antibiotics. Although induction of the *dpiA* pathway provides only temporary protection, it might permit bacteria to buy time while they activate more-potent resistance mechanisms.

1 Miller, C. *et al.* (2004) SOS response induction by β-lactams and bacterial defense against antibiotic lethality. *Science* 305, 1629–1631

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Co-expressed attack and defense by group B streptococci

Group B *Streptococcus* (GBS) is a major cause of serious infections in human newborns. The surface-associated β-hemolysin/cytolysin (βH/C) is thought to contribute to pathogenesis by its ability to form pores in host cells membranes. The gene responsible for βH/C production, *cylE*, is also necessary for a distinguishing feature of GBS, production of a carotenoid pigment. GBS has the ability to persist within phagocytic cells, but the underlying mechanisms are not known.

Liu *et al.* [2] generated isogenic *cylE* mutants with the dual phenotype lack of βH/C and carotenoid. These mutants were tested for virulence and in intracellular-persistence assays. Mice infected with *cylE* mutants had a much higher survival rate. Mutants are cleared more rapidly, both in mice and in whole human blood inoculated with bacteria. *cylE* mutants are more sensitive to exposure to neutrophils or macrophages than wild types, and this is dependent on the cytolytic activity of βH/C. *cylE* mutants have decreased survival within macrophages and neutrophils.

At higher bacterial inoculums, the wild type, but not the *cylE* mutant causes apoptosis of macrophages. *cylE* mutants are more sensitive than wild types to oxidative killing within phagocytes and to oxidants *in vitro*. The mutants could be rescued *in vitro* by addition of carotenoid from wild type, indicating that it contributes to oxidative resistance.

This study clearly demonstrates that the *cylE* gene is important for evasion of phagocytic killing in two ways: expression of the β-hemolysin/cytolysin that attacks the cell by cytolysis and apoptosis induction; and the carotenoid pigment that defends the bacteria from potent reactive oxygen species.

2 Liu, G.Y. *et al.* (2004) Sword and shield: Linked group B streptococcal ß-hemolysin/cytolysin and carotenoid pigment function to subvert host phagocyte defense. *Proc. Natl. Acad. Sci .U. S. A.* 101 14491–14496

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The hexameric structure of a retroviral capsid has implications for HIV assembly

Retroviruses cause a number of human diseases including AIDS. In the final step of the life cycle, the viral capsids are formed from proteolytic cleavage of the Gag polyprotein, but it is still not understood how the mature virions assemble. Mortuza *et al.* have solved the structure of a hexamer of the N-terminal domain of the capsid protein from the murine leukaemia virus, showing how the monomers form hexamers in the mature virus [3].

Electron microscopy has shown that retroviruses are comprised from a network of hexameric rings, and the contacts were thought to be mediated by the N-terminal domains. This structure now shows that the N-terminal domains alone are capable of forming this hexameric structure.

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