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

Bcl-2 inhibitors

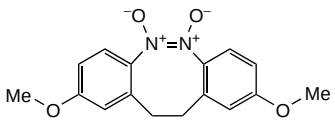
The Bcl-2 family of proteins has a crucial role in regulating apoptosis, which is of fundamental importance for normal cellular development, host defence and suppression of oncogenesis. Members of the Bcl-2 family identified, to date, include both anti-apoptotic molecules such as Bcl-2 and Bcl-X_L and pro-apoptotic molecules such as Bax, Bak, Bid and Bad. The ability of Bcl-2 and Bcl-X_L to form heterodimers with these pro-apoptotic proteins is thought to be essential for the anti-apoptotic function of Bcl-2 and Bcl-X_L.

Bcl-2 has been implicated as an anti-cancer drug target because of its observed over-expression in several tumour types, including breast cancer (70% of cases), prostate cancer (30–60% of cases), B-cell lymphomas (80% of cases) and colorectal adenocarcinomas (90% of cases). In addition, the expression levels of Bcl-2 proteins correlate with resistance to a wide variety of chemotherapeutic agents and γ-radiation therapy, and enhanced tumour cell chemosensitivity has been demonstrated using antisense oligonucleotides or single-chain antibodies targeted at Bcl-2.

Although it is often thought that blocking protein–protein interactions (such as those between Bcl-2 and pro-apoptotic proteins) using small molecules is not feasible, several reports of small-molecule inhibitors of Bcl-2–Bcl-X_L have been published in recent years. The three-dimensional (3D) structure of Bcl-2 has been modelled based on the high-resolution NMR solution structure of the closely related Bcl-X_L by Wang and co-workers at the Georgetown University Medical Center (Washington, DC, USA) and National Cancer Institute (Frederick, Maryland, USA).

The group then employed a structure-based computer screening approach to search the National Cancer Institute 3D database of >200,000 compounds to identify potential small-molecule Bcl-2 inhibitors that bind to the BH3 binding site of Bcl-2 [1]. Seven compounds (from 35 tested) were found to be fairly potent Bcl-2 inhibitors *in vitro*, based on their ability to inhibit binding of a Bak BH3 peptide to Bcl-2.

The seven active compounds were tested for anti-proliferative activity using a human myeloid leukaemia cell line, HL60 (which expresses high levels of Bcl-2 protein) from which compound **(i)** emerged as the most potent (IC_{50} = 4 μM). Furthermore, compound **(i)** was found to induce apoptosis in cancer cells with high levels of Bcl-2 expression and, using NMR methods, was found to bind to the BH3 binding site in Bcl-X_L. Compound **(i)**, therefore, represents both a valuable tool to elucidate Bcl-2 function and a lead compound for further design and optimization.



(i)

1 Enyedy, I.J. *et al.* (2001) Discovery of small-molecule inhibitors of Bcl-2 through structure-based computer screening. *J. Med. Chem.* 44, 4313–4324

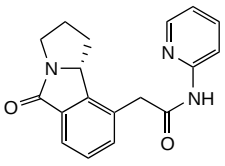
Cyclin-dependent kinase inhibitors

The cyclin-dependent kinases (Cdks), a group of proteins involved in cell cycle regulation, are themselves regulated by subunit proteins called cyclins, which bind to and activate their Cdk partners. For example, D-type cyclins, which have been shown to be amplified or over-expressed in several tumours, associate with Cdk4 and Cdk6; the resulting complexes phosphorylate the retinoblastoma protein (pRB) and regulate the cell cycle during G_1–S transition. The importance of Cdks to the process of cell division has stimulated interest in them as targets for cancer, psoriasis and restenosis, and for the prevention of chemotherapy-associated side effects such as alopecia.

Several Cdk inhibitors have been reported, from which two non-specific Cdk inhibitors, UCN-01 and flavopiridol, have entered clinical trials. Research interest is now largely focussed on selective Cdk inhibitors (from the hundreds of

homologous protein kinases known) that might be expected to cause cell cycle arrest more specifically.

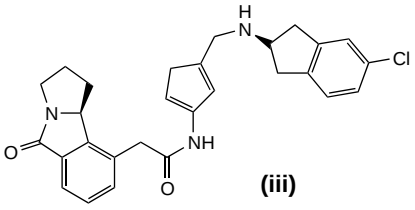
Two recent papers from Honma and co-workers at the Banyu Tsukuba Research Institute (Ibaraki, Japan) in collaboration with Merck Research Laboratories (Tokyo, Japan) illustrate this approach to selective Cdk4 inhibition [2,3]. The first part of this study describes structure-based lead generation using a Cdk4 homology model constructed according to an X-ray structure of an activated form of Cdk2. The authors then used the *de novo* design program LEGEND (University of Tokyo, Japan) with their in-house structure selection supporting system SEEDS (designed to select candidate compounds based on commercial availability and/or synthetic accessibility) to generate new scaffold candidates. Four classes of scaffold candidates were identified, from which a library of candidates of one of these scaffolds, the diarylureas, was synthesized according to the structural requirements of the ATP binding pocket of the Cdk4 model. The most potent compound to emerge from this diarylurea library was **(ii)**, which had an IC_{50} value of 0.042 μM against Cdk4. X-ray analysis of a Cdk2–**(ii)** complex confirmed the expected binding mode.



(ii)

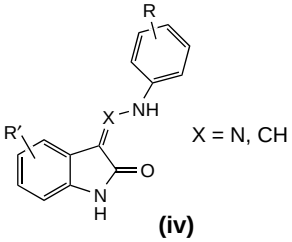
A structure-based approach for designing highly selective Cdk4 inhibitors was then applied. Identification of specifically altered amino acid residues around the ATP binding pocket in Cdk4 (compared to 390 other protein kinases) was followed by the prediction of appropriate positions to introduce substituents in the type of lead diarylurea already identified.

Library design supported by *de novo* design programs and synthesis and testing of candidate compounds lead to the identification of **(iii)** as a highly selective and potent Cdk4 inhibitor (IC_{50} = 0.0023 μM). Compound **(iii)** showed high selectivity for Cdk4 over Cdk1 or 2 (780-fold and 190-fold, respectively) and also over many other kinases tested. Compound **(iii)** was found to cause cell cycle arrest of a cancer cell line expressing pRB in the G_1 phase, and is a useful antitumour lead structure.



(iii)

In related work, Kuyper and co-workers at GlaxoSmithKline (Research Triangle Park, North Carolina, USA) have reported the design and synthesis of two closely related classes of oxindole-based compounds, represented by **(iv)**, as Cdk2 inhibitors [4].



(iv)

Crystallographic analysis of the initial lead compound bound to Cdk2 and a semi-automated method of ligand docking was used to select compounds for synthesis. Several compounds with low nanomolar IC_{50} values against Cdk2 were identified; in addition, potency against Cdk2 was approximately tenfold greater than against Cdk1 for leading structures. Members of this series cause cell cycle arrest, exhibit a selective cytotoxic effect on several tumour cell lines *in vitro* and show potential use in the prevention of chemotherapy-induced alopecia.

2 Honma, T. *et al.* (2001) Structure-based generation of a new class of potent Cdk4 inhibitors: new *de novo* design strategy and library design. *J. Med. Chem.* 44, 4615–4627

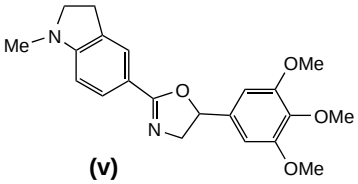
3 Honma, T. *et al.* (2001) A novel approach for the development of selective Cdk4 inhibitors: library design based on locations of Cdk4-specific amino acid residues. *J. Med. Chem.* 44, 4628–4640

4 Bramson, H.N. *et al.* (2001) Oxindole-based inhibitors of cyclin-dependent kinase 2 (Cdk2): design, synthesis, enzymatic activities, and X-ray crystallographic analysis. *J. Med. Chem.* 44, 4339–4358

Novel anti-mitotic agents

Tubulin is a heterodimer consisting of two closely related and tightly linked globular polypeptides (α- and β-tubulin) that polymerize parallel to a cylindrical axis to form microtubules during cellular processes such as mitosis. The formation of microtubules and the mitotic spindle is a crucial event in cellular replication, and in recent years microtubules have become an important subcellular target for the development of novel anti-cancer agents. Well-known anti-cancer agents of this class, often called anti-mitotic agents or spindle poisons, include both microtubule-stabilizing agents (taxanes, epothilones and eleutherobin) and drugs that inhibit tubulin polymerization (vinca alkaloids and colchicines).

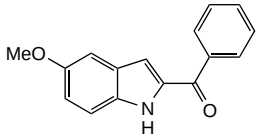
Szczepankiewicz and co-workers at the Abbott Laboratories (Abbott Park, IL, USA) have reported a series of new tubulin-binding compounds based on a lead oxadiazoline that was previously reported by this group to possess good cytotoxic activity against non-multidrug-resistant and multidrug-resistant cancer cell lines, but had a short *in vivo* half-life. Structure–activity relationship (SAR) studies led to the discovery of compound **(v)**, which maintained the *in vitro* activity of the original lead compound, but also



(v)

demonstrated a better pharmacokinetic profile, dose-dependent *in vivo* activity and a threefold increase in the lifespan of tumour-implanted mice after oral dosing (28-day study) [5].

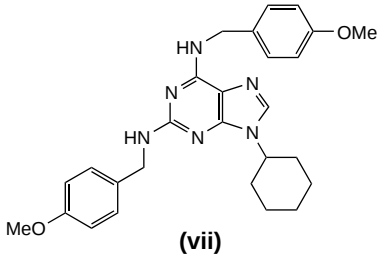
Mahboobi and co-workers at the University of Regensburg (Regensburg, Germany) have reported a new class of anti-mitotic compounds based on 2-aroylindoles [6]. Compound **(vi)** (as well as several others in the series) had a nanomolar IC_{50} value against the growth of human HeLa/KB cervical, SK-OV-3 ovarian and U373 astrocytoma carcinoma cell lines *in vitro*. Inhibition of proliferation correlated with arrest during the G2 and M phases of the cell cycle and inhibition of tubulin polymerization. Notably, compound **(vi)** did not significantly affect the GTPase activity of β-tubulin, as is the case for colchinine, vincristine, nocodazole and taxol. Of particular interest was the observation that selected compounds in the series also inhibited angiogenesis in the chorioallantoic membrane (CAM) assay, which suggests another potential anticancer target for these compounds.



(vi)

In xenograft studies, compound **(vi)** was highly active against human amelanocytic melanoma MEXF989 cells (oral administration, athymic nude mice). 2-Arolylindoles, therefore, constitute an interesting class of antitumour agent for further preclinical development.

Chang and co-workers (New York University and Scripps Research Institute, La Jolla, CA, USA) have reported the development of a series of trisubstituted purines, based on the lead structure myoseverin, as anti-mitotic agents [7]. Notably compound **(vii)** (myoseverin B) was found to be a significantly improved



(vii)

inhibitor of microtubule assembly (IC_{50} for tubulin polymerization inhibition = 2 μM) and exhibited low cytotoxicity in most cell types *in vitro* (using the National Cancer Institute 60-cell panel), suggesting that this molecule might be useful as a cytostatic antitumour agent.

5 Szczepankiewicz, B.G. *et al.* (2001) New anti-mitotic agents with activity in multidrug-resistant cell lines and *in vivo* efficacy in murine tumor models. *J. Med. Chem.* 44, 4416–4430

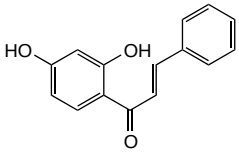
6 Mahboobi, S. *et al.* (2001) Synthetic 2-aroylindole derivatives as a new class of potent tubulin-inhibitory, anti-mitotic agents. *J. Med. Chem.* 44, 4535–4553

7 Chang, Y-T. *et al.* (2001) Synthesis and biological evaluation of myoseverin derivatives: microtubule assembly inhibitors. *J. Med. Chem.* 44, 4497–4500

Flavonoids: anti-proliferative activity against breast cancer cells

Naturally occurring flavonoids occur in many foods including fruits, vegetables, spices, tea and soy-based products, and have attracted widespread interest as anti-proliferative, anti-aromatase, anti-estrogenic and cancer chemopreventative agents.

Chulia and co-workers at the Faculté de Pharmacie (Limoges, France) have synthesized a range of 2′-hydroxychalcones, flavanones, flavones and flavan-4-ols with wide variation in A-ring substitution patterns (H, OH, OMe) and evaluated their antitumour activity *in vitro* against human MCF-7 breast cancer cells [8],



(viii)

In general, 2′-hydroxychalcones (e.g. compound **(viii)**; IC_{50} = 16 μM) and methoxylated flavonones were found to exhibit the most potent anti-proliferative activity in MCF-7 cells.

8 Chulia, C. *et al.* (2001) Flavonoids: structural requirements for anti-proliferative activity on breast cancer cell lines. *Bioorg. Med. Chem. Lett.* 11, 3095–3097

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Combinatorial chemistry

Telomerase inhibitors

Telomerase is the enzyme responsible for maintaining telomere length and its activity is not observed in normal somatic cells. By contrast, high expression of telomerase is observed in approximately 85–90% of human tumour cells; therefore, telomerase is regarded as a specific target for the development of cancer chemotherapeutic agents. There are several types of inhibitor known, for example, antisense oligodeoxynucleotides and compounds that exhibit potent inhibition of telomerase in the picomolar range. Despite this research, there have been no clinical trials of inhibitors, to date.

Recent developments have highlighted new telomerase inhibitors based on the bisindole unit **(i)** [1]. These new inhibitors are a simple assembly of a phosphate with a hydrophobic group and the bisindole unit, with a long alkyl spacer between them. The simple structural feature of these inhibitors has led to the search for more potent inhibitors [2].

A small library of 42 single compounds was synthesized on Merrifield solid-phase resin. Upon cleavage, evaluation of the library compounds' ability to inhibit telomerase, by testing in a quantitative stretch PCR assay using telomerase