# 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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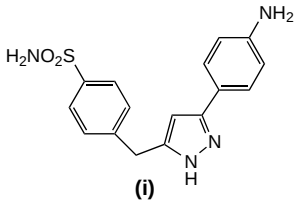
## Novel antitumour molecules

### New protein kinase inhibitors

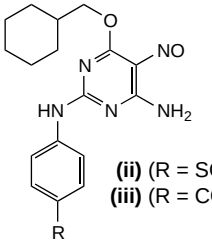
Protein kinases and protein kinase-mediated phosphorylation have a fundamental role in a multitude of cellular signal transduction pathways, controlling numerous biological events, such as cell growth, differentiation and apoptosis. Aberrant kinase activity is thought to contribute to the initiation and progression of several diseases, including cancer, diabetes, and neurodegenerative and inflammatory disorders. Protein kinases catalyse the transfer of a phosphate group from ATP to a specific substrate amino acid residue (tyrosine, serine, threonine or histidine), and the majority of drug discovery research in this area has involved the search for small molecules that mimic ATP and bind competitively at its binding site. Historically, there have been doubts as to whether selectivity within a target class can be achieved; however, the discovery and ultimate development of potent and selective inhibitors, such as the anticancer drugs Gleevec® and Iressa® have helped to validate kinase inhibition as a therapeutic strategy. A themed issue of *Bioorganic and Medicinal Chemistry Letters* provides a collection of original articles from major authors in the kinase field [1], including work in the area of cyclin-dependent kinase (CDK) inhibition (highlighted below), tyrosine kinase receptor (KDR) inhibition as an anti-angiogenic strategy, platelet-derived growth factor (PDGR) receptor inhibition, and inhibition of Ras-MAPK signalling and DNA-dependent protein kinase.

The cyclin-dependent kinases (CDKs) are a family of serine or threonine kinases, whose activity is governed by binding to specific cyclin partner proteins, controlling entry to and progression through the cell cycle. A review on the biology that underpins CDK inhibition strategies in cancer has recently been published [2]. To date, studies using small-molecule CDK inhibitors that target the enzyme ATP-binding domain have demonstrated single-agent activity and synergistic activity (with cytotoxics) in preclinical tumour models. However, kinase specificity, both with the CDK class and more generally, remains an important challenge, as does potency against tumour cells both *in vitro* and *in vivo*. CDK inhibitors feature prominently in this issue, and include the following contributions:

- development of substituted 4,6-bis anilino pyrimidines and substituted 2,4-bis anilino pyrimidines (through chemical modification and X-ray crystallography) as potent and selective CDK4 inhibitors by Beattie and co-workers [3] and Breault and co-workers (AstraZeneca) [4].
- identification of imidazo[1,2-*a*]pyridines as a potent and selective class of CDK4 and, in particular, CDK2 inhibitors by Anderson and co-workers [5];
- anilinopyrazoles as selective CDK2 inhibitors (over CDK1, where enzymes share 90% identity in ATP-binding pocket); for example, compound **i**, where the $IC_{50}$ (CDK2) value is 0.34 nM and the $IC_{50}$ (CDK1) value is 501 nM (Tang and co-workers [6]);




**(i)**




**(ii)** (R = $SO_2NH_2$)
**(iii)** (R = $CONH_2$)

- Structure-based design, synthesis and evaluation of 2-arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidines as potent inhibitors of CDK1 and CDK2 by Sayle and co-workers [7]. For example, compounds **ii** and **iii,** bearing sulfonamide and carboxamide groups at the 4'-position gave $IC_{50}$ values against CDK2 of 1.1 and 34 nM, respectively;
- 2,6,8,9-Tetrasubstituted purines related to the antitumour agent roscovitine (currently in clinical trials) as CDK1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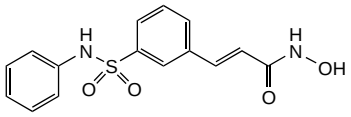
inhibitors by Moravec and co-workers [8].

1 Metz, W.A. (2003) A perspective on protein kinase inhibitors. *Bioorg. Med. Chem. Lett.* 13, 2953
2 Mclaughlina, F. *et al.* (2003) The cell cycle, chromatin and cancer: mechanism-based therapeutics come of age. *Drug Discov. Today* 8, 793–802
3 Beattie, J.F. *et al.* (2003) Cyclin-dependent kinase 4 inhibitors as a treatment for cancer. Part 1: identification and optimisation of substituted 4,6-bis anilino pyrimidines. *Bioorg. Med. Chem. Lett.* 13, 2955–2960
4 Breault, G.A. *et al.* (2003) Cyclin-dependent kinase 4 inhibitors as a treatment for cancer. Part 2: identification and optimisation of substituted 2,4-bis anilino pyrimidines. *Bioorg. Med. Chem. Lett.* 13, 2961–2966
5 Anderson, M. *et al.* (2003) Imidazo[1,2-*a*]pyridines: a potent and selective class of cyclin-dependent kinase inhibitors identified through structure-based hybridisation. *Bioorg. & Med. Chem. Lett.* 13, 3021–3026
6 Tang, J. *et al.* (2003) Anilinopyrazole as selective CDK2 inhibitors: design, synthesis, biological evaluation, and X-ray crystallographic analysis. *Bioorg. Med. Chem. Lett.* 13, 2985–2988
7 Sayle, K.L. *et al.* (2003) Structure-based design of 2-arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidine inhibitors of cyclin-dependent kinases 1 and 2. *Bioorg. Med. Chem. Lett.* 13, 3079–3082
8 Moravec, J. *et al.* (2003) 2,6,8,9-Tetrasubstituted purines as new CDK1 inhibitors. *Bioorg. Med. Chem. Lett.* 13, 2993–2996



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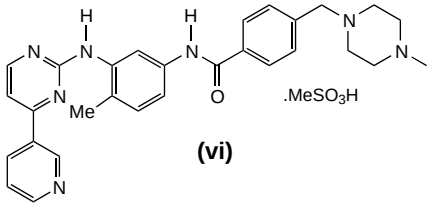
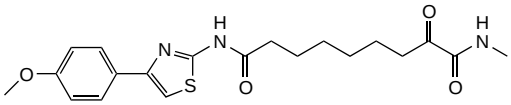


(vi)



(v)

## Histone deacetylase inhibitors

Histone deacetylases (HDACs) catalyse deacetylation of lysine residues in the $-NH_2$ terminal tails of the core nucleosomal histones, leading to upregulation of target gene transcription in genes that are associated with cell-cycle progression and differentiation. HDAC inhibition can lead to reversal of the malignant phenotype in transformed cells, through activation of differentiation programs, cell-cycle inhibition, induction of apoptosis and angiogenesis blockade [2]. For these reasons, HDAC inhibition is regarded as a promising anticancer drug target, and several experimental agents are in early clinical trials.

In general, HDAC inhibitors described to date consist of three parts: a zinc-chelating group, a (hydrophobic) spacer group and an 'enzyme binding' group (generally aromatic in character). Plumb and co-workers have made use of these three characteristics in the design and synthesis of novel, achiral and synthetically accessible HDAC inhibitors [9]. Most notably, compound PXD101 (**iv**) was found to inhibit histone deacetylase activity in HeLa cell extracts with an $IC_{50}$ value of 27 nM and induced a concentration-dependent increase in acetylation of histone H4 in tumour cell lines. Compound **iv** was found to be cytotoxic *in vitro* in several tumour cell lines (e.g. ovarian A2780, $IC_{50}$ = 200 nM). Significant dose-dependent growth delay with no obvious signs of toxicity was observed following daily treatment over seven days of nude mice bearing ovarian and colon tumour xenografts [10–40 mg kg $day^{-1}$ administered intraperitoneally (i.p.)]. Xenografts of cisplatin-resistant ovarian tumour cells were also found to be responsive using this agent, and a marked increase in acetylation of histone H4 was detected in blood and tumour of mice following treatment with PXD101. Measurement of histone acetylation in blood cells could provide a possible future clinical trial pharmacodynamic endpoint, and the promising activity associated with PXD101 make this agent worthy of further study.

In related studies, Wada and co-workers have synthesized and tested a series of alpha-keto esters and amides as potent inhibitors of HDAC [10]. Alterations to the linker and aryl group produced HDAC inhibitors in the nanomolar $IC_{50}$ range with low micromolar $IC_{50}$ values against cellular proliferation. Compound **v** displayed significant antitumour activity in an HT1080 mouse tumour model (i.p. administration at 30 and 100 mg $kg^{-1}$ every other day), despite the short half-life of this compound.

LAQ824 is a previously reported cinnamyl hydroxamic acid analogue HDAC inhibitor (Novartis Pharmaceuticals) [11]. Nimmanapalli and co-workers have now reported that LAQ824 can deplete the mRNA and protein expression of Bcr-Abl in human chronic myeloid leukaemia blast crisis (CML-BC) cells [12]. The *bcr-abl* fusion gene, generated *via* the reciprocal chromosomal translocation t (9;22)(q34;q11), is the molecular hallmark in 95% of CML cases. Moreover, the dysregulated Bcr-Abl tyrosine kinase activity contributes towards the malignant phenotype in the chronic and advanced blastic phases of CML. However, impressive and well-documented activity associated with STI 571 (Gleevec®, **vi**) in the chronic phase of CML (95% of patients achieving durable complete clinical remissions) is not so apparent in the accelerated and blast crisis of the disease – a phenomenon that is explained by resistance to Gleevec®, due to mutations in the kinase domain of Bcr-Abl and amplification of the *bcr-abl* gene. Acetylation of heat shock protein 90 (hsp90) by LAQ824 inhibited the chaperone association of Bcr-Abl with hsp90, thereby promoting the proteasomal degradation of Bcr-Abl and, importantly,

co-treatment of LAQ824 increased Gleevec®-induced apoptosis of CML-BC cells. LAQ824 also down-regulated levels of mutant Bcr-Abl, relevant to blast crisis, and induced apoptosis of Gleevec®-resistant primary CML-BC cells, suggesting that LAQ824 might be a promising agent in the treatment of Gleevec®-sensitive or -refractory CML.


9 Plumb, J.A. *et al.* (2003) Pharmacodynamic response and inhibition of growth of human tumour xenografts by the novel histone deacetylase inhibitor PXD101. *Mol. Cancer Therap.* 2, 721–728

10 Wada, C.K. *et al.* (2003) α-Keto amides as inhibitors of histone deacetylase. *Bioorg. Med. Chem. Lett.* 13, 3331–3335

11 Catley, L. *et al.* (2002) LAQ824 is a novel histone deacetylase inhibitor with significant activity against multiple myeloma: results of a prelinical evaluation. *Blood* 100, 391

12 Nimmanapalli, R. *et al.* (2003) Histone deacetylase inhibitor LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib mesylate-sensitive or -refractory chronic myelogenous leukaemia-blast crisis cells. *Cancer Res.* 63, 5126–5135



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## Molecules

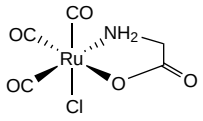
### Carbon monoxide-releasing metal carbonyls: a new class of pharmaceuticals?

Carbon monoxide (CO) is produced naturally in humans at a rate of between 3 and 6 $cm^3$ per day, and this rate is increased in certain inflammatory states and pathological conditions that are associated with red blood cell haemolysis and oxidant-mediated stress. This endogenous CO is derived from the degradation of intracellular haem by a family of constitutive (HO-2) and inducible (HO-1) haem oxygenase enzymes.

Over the past ten years, interest in the biological effects of CO has greatly increased and CO is now regarded as a versatile signaling molecule, having essential regulatory roles in a variety of physiological and pathophysiological processes that take place within the cardiovascular, nervous and immune systems.

Research into the biological effects of CO and its potential therapeutic exploitation has been hampered by the practical inconvenience and danger involved in administering low doses of the toxic gas. However, a group of scientists from the Northwick Park Institute for Medical Research and the University of Sheffield (http://www.shef.ac.uk) have developed a family of compounds that overcomes these problems.

Initially, it was shown that certain transition metal carbonyls reported in the literature were able to liberate CO and mimic the effects of CO gas in biological systems [1]. Subsequently, novel forms of 'CO-RMs' (carbon monoxide-releasing molecules) have been developed. As a prototype of this class of compounds, $[Ru(CO)_3Cl(glycinate)]$ or CORM-3 (**i**) is a stable, solid, water-soluble Ru(II) complex, which liberates CO by ligand exchange [2]. This compound is able to induce a range of functions that are described for CO gas, and has been evaluated in a model of ischaemic reperfusion damage, where its presence during




**(i)**

reperfusion enhances heart function and reduces muscle infarction [3].

Ruthenium-centered organometallic compounds are also undergoing intense development as anti-cancer agents [4]. The successful development of marketed drugs from this class of compound should open up a floodgate of interest in the whole field of bioorganometallic chemistry, turning it into a mature subject to be taken seriously by the pharmaceutical industry.


1 Motterlini, R. *et al.* (2002) Carbon monoxide–releasing molecules: characterization of biochemical and vascular activities. *Circ. Res.* 90, e17–e24

2 Johnson, T.R. *et al.* (2003) Metal carbonyls: a new class of pharmaceuticals? *Angew. Chem. Int. Ed.* 42, 3722–3729

3 Clark, J.E. *et al* (2003) Cardioprotective actions by a water-soluble carbon monoxide–releasing molecule. *Circ. Res.* 93, e2–e8

4 Cocchietto, M. *et al* (2003) Primary tumor, lung and kidney retention and antimetastasis effect of NAMI-A following different routes of administration. *Invest. New Drugs* 21, 55–62



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