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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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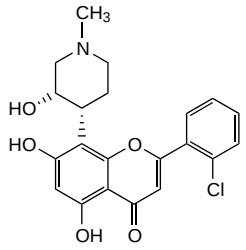
Novel antitumour molecules

Cyclin-dependent kinase (CDK) inhibitors

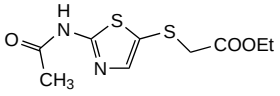
Progression through the cell cycle is governed by the well-orchestrated activation of cyclin-dependent kinases (serine–threonine protein kinases) in association with appropriate cyclin subunits. Different phases of the cell cycle are characterized by the presence and activity of distinct CDK-cyclin complexes; for example, CDK2 activity, together with the co-activator cyclin E, is required for progression through G1 to S phase of the cell cycle. This is an important part of the G1 checkpoint, which serves to maintain the proper sequence of cell cycle events and enable the cell to respond to either inappropriate damage or to proliferative signals. Because loss of checkpoint control has been shown to contribute to tumourigenesis, and because certain CDKs (such as CDK2) are frequently misregulated in a wide variety of human cancers, small molecule ATP-competitive inhibitors of CDK2 and its regulatory pathways have become the focus of many cancer drug-discovery programmes in recent years. For example, the non-selective CDK inhibitor flavopiridol (compound **i**) is currently undergoing clinical evaluation.

Kim and co-workers (Bristol-Myers Squibb Pharmaceutical Research Institute; http://www.bms.com) have reported the discovery and development of



(i)

aminothiazole inhibitors of CDK2 [1]. HTS for CDK inhibitors identified the 2-amino-5-thio-substituted thiazole (**ii**) as a selective CDK2 inhibitor (IC_{50} versus CDK2-cyclin E = 0.17 μM; 10- and 100-fold selective compared to CDK1 and CDK4, respectively). Compound **ii**, however, was found to be inactive in cells and unstable in plasma and was stabilized to metabolic hydrolysis by replacing the ester function with a 5-ethyl substituted oxazole, as in compound **iii**. Combinatorial and parallel synthesis



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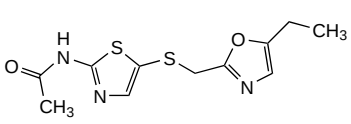

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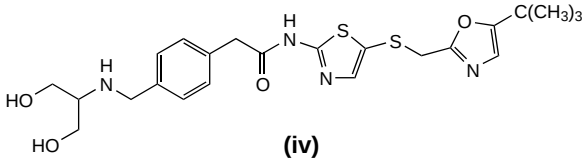

(iv)

provided access to a large number of analogues possessing potent and selective CDK2-inhibitory activity, and X-ray crystallography of lead compounds bound to CDK2 protein, combined with molecular modelling, provided insight into the binding mode of these inhibitors.

Compound **iii** exhibited fairly potent cytotoxicity *in vitro* against a variety of human cancer cell lines and reduced the phosphorylation of CDK2 substrates, such as retinoblastoma protein (RB) and histone H1 in the A2780 ovarian cell line. Optimization of pharmacokinetic properties led to the identification of the water-soluble compound **iv**, which demonstrated good efficacy *in vivo* in several human cancer cell line xenograft models, as well as a murine tumour cell line driven by cyclin E overexpression.

The functions of CDK-cyclin complexes are negatively regulated by a host of regulatory proteins, including members of the CIP–KIP family, such as $p21^{WAF1}$ and $p27^{KIP1}$. A binding groove in the cyclin subunit is, in part, responsible for recognition of CDK-cyclin complexes by $p21^{WAF1}$, and this groove is involved in the recruitment of CDK substrates, such as pRB and E2F. Blockade of this cyclin

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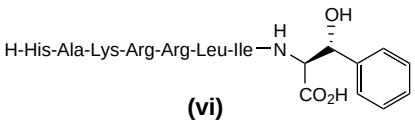
recruitment site thus offers a strategy for the recognition and phosphorylation of CDK substrates, and a therapeutic approach towards restoration of $p21^{WAF1}$-like tumour suppression.

Previous reports have described an octapeptide, ^{152}HSKRRLIF^{159}, located at the C-terminus of $p21^{WAF1}$, which displayed potent CDK-cyclin inhibitory activity through binding to the cyclin recruitment site. SAR studies then led to the identification of the lead compound **v**, where the native Ser was replaced by an Ala residue [2]. Atkinson and co-workers (University of Nottingham; www.nottingham.ac.uk/pharmacy) have now reported the synthesis and CDK2-cyclin A inhibitory activity of a series of octapeptides, based on compound **v**, that target the cyclin recruitment site [3]. Replacement of the C-terminal Phe residue by α- and / or β-modified variants was achieved using solid-phase chemistry. Both the L-*threo*-β-hydroxyphenylalanine (β-phenylserine, Pse) (**vi**) and (2S)-phenylalanol (**vii**) derivatives were found to be competitive inhibitors at the cyclin recruitment site and displayed superior inhibitory activity towards the CDK2-cyclin A complex, and serve as starting points for further structural modification.

H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-OH

(v)



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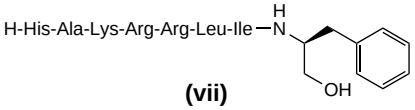
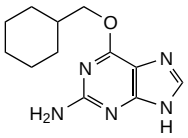


(vii)

Further recent work in the CDK inhibitor area has been reported by Gibson and co-workers [4] [University of Newcastle, (http://www.newcastle.ac.uk); AstraZeneca (http://www.astrazeneca.com); University of Oxford (http://www.newcastle.ac.uk/cancer.research)]. *O*^{6}-Substituted guanines,



(viii)

such as *O*^{6}-cyclohexylmethylmethylguanine (**viii**), are known to be ATP-competitive inhibitors of CDK1–cyclin B1 and CDK2–cyclin A, the *O*^{6}-substituent occupying the kinase ribose-binding site. *O*^{6}-substituted guanines were synthesized to probe the ribose pocket and the structures of four representative compounds bound to monomeric CDK2 were determined by X-ray crystallography. However, none of the newly synthesized compounds improved inhibitory potency in CDK-inhibitory assays.

1 Kim, K.S. *et al.* (2002) Discovery of aminothiazole inhibitors of cyclin-dependent kinase 2: synthesis, X-ray crystallographic analysis and biological activities. *J. Med. Chem.* 45, 3905–3927

2 Fischer, P.M. *et al.* (2001) Peptide inhibitors of cyclin-dependent kinases derived from p21 (WAF1): delineation and structural insight into their interactions with cyclin A. *Clin. Cancer Res.* 7 (Suppl.), P824

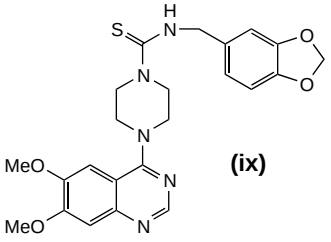
3 Atkinson, G.E. *et al.* (2002) Peptide inhibitors of CDK2-cyclin A that target the cyclin recruitment-site: structural variants of the C-terminal Phe. *Bioorg. Med. Chem. Lett.* 12, 2501–2505

4 Gibson, A.E. *et al.* (2002) Probing the ATP ribose-binding domain of cyclin-dependent kinases 1 and 2 with *O*^{6}-substituted guanine derivatives. *J. Med. Chem.* 45, 3381–3393

Antagonists of the PDGFR tyrosine kinase family

The receptor tyrosine kinase family is characterized by having an extracellular ligand-binding region, a single transmembrane spanning region and intracellular tyrosine kinase domains. The association of aberrant tyosine kinase activity with cellular proliferation, and tumour formation and maintenance is well documented and several tyrosine kinase inhibitors are in advanced clinical trials or have recently received regulatory approval; for example, the Novartis (http://www.novartis.com) compound Gleevec® (Bcr-Abl and c-Kit tyrosine kinase inhibition) and the epidermal growth factor receptor inhibitors Iressa® (AstraZeneca; http://www.astrazeneca.com) and Tarceva® (OSI Pharmaceuticals; http:///www.osip.com). The platelet-derived growth factor receptor (PDGRF) family, part of the larger receptor tyrosine kinase family, includes members such as αPDGFR and βPDGFR, colony-stimulating factor 1 receptor, Flt-3 and c-Kit. Abnormal PDGF-induced cell proliferation has been implicated in several proliferative diseases, such as atherosclerosis, glomerulosclerosis, liver cirrhosis and certain cancers.

Several adenosine 5′-triphosphate (ATP) competitive inhibitors of PDGFR phosphorylation have been previously reported, including the 4-[4-(*N*-substituted thiocarbamoyl)-1-piperazinyl]-6,7-dimethoxyquinazolines, such as CT52923 (**ix**). Previous SAR studies in this series have identified structural features of these inhibitors that contribute to their specific βPDGFR inhibitory activity. Pandey and co-workers at Millenium Pharmaceuticals (http://www.mlmn.com) have now reported their studies directed towards enhancement of the intrinsic potency of this series in human plasma and retention of kinase specificity, while achieving desirable pharmacokinetic properties, such as oral bioavailability and long plasma half-life [5]. Most notably, compound **x** was found to possess potent *in vitro* PDGFR inhibitory activity (IC_{50} = 0.03 μM) and long plasma half-life. Oral administration of **x** at 60 mg kg^{-1} showed significant delay in murine death in the nude mouse model of chronic myelomonocytic leukaemia (CMML). On the basis of its *in vitro* activity, microsomal stability, oral bioavailability and half-life



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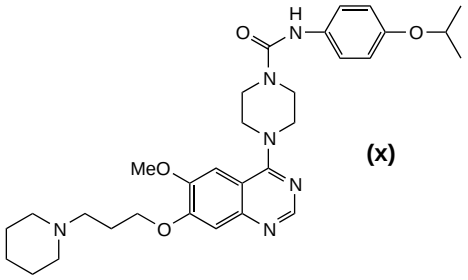


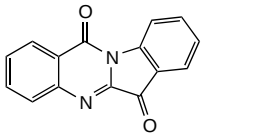
(x)

in vivo, compound **x** has been chosen as a developmental candidate for clinical evaluation. Initial studies will focus on the use of this compound for the treatment of acute myelogenous leukaemia (AML) as a result of its pronounced activity in the Flt–ITD (internal tandem duplication mutation)-mediated leukaemia mouse model.

5 Pandey, A. (2002) Identification of orally active, potent, and selective 4-piperazinylquinazolines as antagonists of the platelet-derived growth factor receptor tyrosine kinase family. *J. Med. Chem.* 45, 3772–3793

Novel antitumour indolo[2,1-*b*]quinazoline analogues

In a search to identify novel anticancer compounds from Indian medicinal plants, compound **xi** from various plant sources (including *Wrightia tinctoria*) has been reported to possess antibacterial, antifungal and weak cytotoxic activity against B-16 melanoma cancer cell lines. Although this compound does not possess potent cytotoxic activity, it bears some structural resemblance to the known potent cytotoxic agent batracyclin, and was thus considered as a useful lead compound for further biological studies by Sharma and co-workers (Dr. Reddy's Research Foundation; http://www.drreddys.com). The authors report the synthesis and antitumour evaluation of a range of indolo[2,1-*b*]quinazoline derivatives [6]. *In vitro* testing of this series, in a panel of eight human cancer cell lines of distinct tissue origin, revealed that many compounds showed GI_{50} activity in the 1–5 μM range. One of the most active compounds *in vitro*, compound **xii**, was also found to perform well in a preliminary modified hollow fibre assay [12 human cancer cell lines implanted in the subcutaneous (sc) and intraperitoneal (ip) compartments in mice], and was also found to be active *in vivo* in the HT-29 colon cancer xenograft model.



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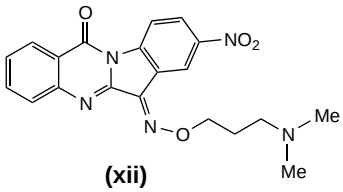

(xii)

6 Sharma, V.M. *et al.* (2002) Novel indolo[2,1-*b*]quinazoline analogues as cytostatic agents: synthesis, biological evaluation and structure-activity relationship. *Bioorg. Med. Chem. Lett.* 12, 2303–2307

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