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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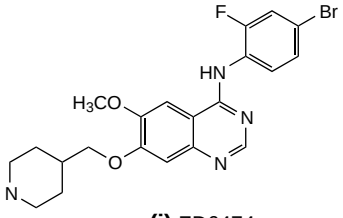
Novel antitumour molecules

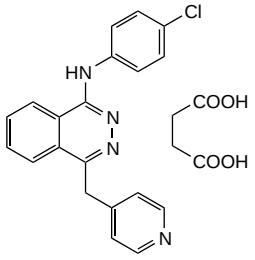
New angiogenesis inhibitors and vascular targeting agents

The growth of new capillaries from existing blood vessels, a process known as angiogenesis, is a crucial step in the survival mechanism of the growing tumour, providing a vasculature for the supply of nutrients and removal of waste products. In addition, angiogenesis has an important role in the metastatic spread of tumours beyond their site of origin. Vascular endothelial growth factor (VEGF) is a pro-angiogenic cytokine, which when released binds to VEGF receptors (members of the receptor tyrosine kinase family) and initiates a signal transduction cascade that leads to induction of angiogenic activity.

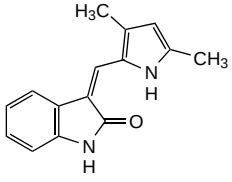
The receptor tyrosine kinase family is characterized by an extracellular ligand binding region, a single transmembrane spanning region and intracellular tyrosine kinase domains. Upon VEGF binding, dimerization of VEGF receptor (VEGFR) leads to activation of the tyrosine kinase domains, binding of ATP, catalysis of γ-phosphate transfer to the hydroxyl group of tyrosine and initiation of the signal transduction cascade. The most promising strategy for VEGF receptor inhibition to date (as with other tyrosine kinase receptors) has been to use selective small-molecule ATP-competitive inhibitors to block the VEGF-mediated signalling process. Several VEGF receptor kinase inhibitors of this type are currently undergoing clinical evaluation (**i–iii**).



(i) ZD6474

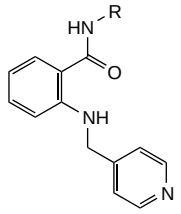


(ii) PTK 787 / ZK222584; vatalanib



(iii) SU 5416; semaxanib

Manley and co-workers (Novartis Pharma AG, http://www.novartisoncology.com; and Schering AG, http://www.schering.de) have described the synthesis and biological evaluation of two novel anthranilic acid amides (**iv** and **v**) as novel anti-angiogenic VEGF receptor kinase inhibitors [1]. These new agents potently and selectively inhibit recombinant VEGFR-2 and VEGFR-3 kinases. For example, for compound **iv**, IC_{50} for VEGFR-2 (human KDR) = 23 nM; IC_{50} for VEGFR-3 (Flt-4) = 18 nM; activity against VEGFR-1 and a panel of other kinases gave IC_{50} >100 nM. In addition, these anthranilamides readily penetrate cells and are absorbed following once-daily oral administration to mice. Both compounds potently inhibit VEGF-induced angiogenesis in immunocompetent mice with ED_{50} values of 7 mg kg^{-1}, and potently inhibit both the growth of primary tumour and the formation of spontaneous peripheral metastases in a mouse orthotopic model of melanoma. Anthranilamides **iv** and **v** are, therefore, novel VEGFR kinase inhibitors worthy of further study.



(iv) R = 3-$C_6H_4CF_3$
(v) R = 3-isoquinolinyl

In related work, Fraley and co-workers (Merck Research Laboratories, http://www.merck.com) have described the optimization of a pyrazolo[1,5-*a*]pyrimidine class of VEGFR-2 (KDR) kinase inhibitors

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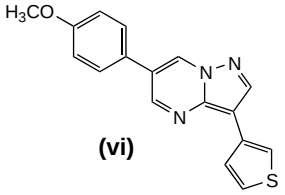
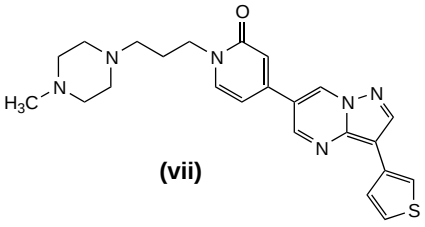
based on the initial lead compound **vi** [2]. The introduction of solubilizing functionality to the 3,6-disubstituted pyrazolo[1,5-*a*]pyrimidine core significantly improved the physical properties of these compounds, producing marked increases in cellular activity and more favourable pharmacokinetics in rats. These improvements in activity are illustrated by compound **vii** (IC_{50} for KDR = 7 nM; IC_{50} in the VEGF stimulated endothelial cell mitogenesis assay = 20 nM; logP = 1.6).



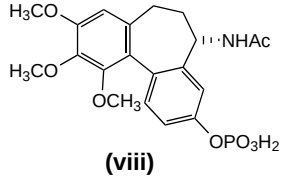
(vi)



(vii)

An alternative antitumour approach to the anti-angiogenic strategies described above is to target existing tumour vasculature. Opportunities for selective disruption of tumour blood flow leading to tumour death exist because of differences in the physiology of immature tumour versus mature normal vasculature. A potential advantage of this approach lies in the targeting of central regions of tumour that are normally resistant to conventional therapy.

Previous studies have identified tubulin-binding agents that induce vascular damage in mouse tumour models, the best known example of which is combretastatin A-4 phosphate where vascular damaging activity was demonstrated at one-tenth of the maximum tolerated dose (MTD) [3]. A new study by Davis and co-workers (Angiogene Pharmaceuticals, http://www.angiogene.co.uk; AstraZeneca, http://astrazeneca.com; and Gray Cancer Institute, http://www.gci.ac.uk) report the synthesis and biological evaluation of a water-soluble phosphate prodrug (compound **viii**; ZD6126) of the tubulin-binding agent *N*-acetylcolchinol as a novel vascular targeting agent that causes selective destruction of tumour vasculature [4]. *In vitro* studies show the induction of pronounced, reversible changes in endothelial cell morphology at sub-cytotoxic doses (0.1 μM), but no effect on the growth of human umbilical vein endothelial cells (HUVEC) at concentrations below 100 μM. Murine *in vivo* studies (single administration well below the maximum tolerated dose) indicated a large reduction in vascular volume, induction of extensive necrosis in tumours, and a reduced tumour cell yield in a clonal excision assay. After single dose administration, a viable rim of tumour remained and there was minimal tumour growth delay observed; however, well tolerated, multiple administration regimens lead to substantial tumour growth delay. Synergistic growth delay effects were also observed for compound **viii** in combination with paclitaxel. ZD6126 is thus a promising antivascular agent worthy of further study.



(viii)

1 Manley, P.W. *et al.* (2002) Anthranilic acid amides: a novel class of antiangiogenic VEGF receptor kinase inhibitors. *J. Med. Chem.* 45, 5687–5693

2 Fraley, M.E. *et al.* (2002) Optimization of a pyrazolo[1,5-*a*]pyrimidine class of KDR kinase inhibitors: improvements in physical properties enhance cellular activity and pharmacokinetics. *Bioorg. Med. Chem. Lett.* 12, 3537–3541

3 Dark, G.G. *et al.* (1997) Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature. *Cancer Res.* 57, 1829–1834

4 Davis, P.D. *et al.* (2002) ZD6126: a novel vascular-targeting agent that causes selective destruction of tumor vasculature. *Cancer Res.* 62, 7247–7253

Cell permeable 2′-*O*-methoxyethyl oligonucleotides as telomerase inhibitors

The ribonucleoprotein telomerase catalyses the addition of repeat units of telomeric DNA (TTAGGG in humans) to the ends of chromosomes to maintain genomic integrity during rounds of cell division. Telomerase has been put forward as a promising anticancer drug target because most tumour cells are able to switch on telomerase to prevent induction of senescence, whereas telomerase activity is absent from most normal somatic cells. The development of potent telomerase inhibitors has been the subject of intense research activity in recent years; inhibitors successfully tested to date include agents that interact with G-quadruplex structures and oligonucleotides. Oliognucleotides seem well suited as telomerase inhibitors because the template sequence of the RNA component of telomerase must bind the telomere to function, making the template sequence accessible to hydridization and functionally critical.

An obstacle to the development of antisense oligonucleotides has been the prevailing view that oligonucleotides are unable to efficiently enter cultured cells in the absence of cationic lipid carrier molecules. Chen and co-workers (University of Texas Southwestern Medical Center, http://www.swmed.edu; and ISIS Pharmaceuticals, http://www.isip.com) now report that 2′-methoxyethyl (MOE) RNA oligonucleotides (sequence 5′-CAGUUAGGGUUAG containing phosphorothioate linkages) can enter cultured cancer cells, cause telomeres to shorten and reduce cell proliferation [5].

In four different human cancer cell lines (DU145 prostate, LNCaP prostate, A549 small cell lung and MCF-7 breast) oligonucleotide concentrations in the nanomolar to low micromolar range are sufficient for telomerase inhibition. One explanation for the lack of need for an optimized delivery system and response to low concentrations of oligonucleotide

could be that telomerase is not a typical antisense target in that it does not bind and negate mRNA transcripts, but rather has a direct effect on inhibiting the binding of the enzyme to its substrate via the essential RNA component of human telomerase. *In vivo* validation of this strategy and preclinical studies are eagerly anticipated.

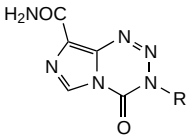
5 Chen, Z. *et al.* Telomerase inhibition, telomere shortening and decreased cell proliferation by cell permeable 2'-*O*-methoxyethyl oligonucleotides. *J. Med. Chem.* 45, 5423–5425

Antitumor imidazotetrazines

Malignant brain tumours, such as anaplastic astrocytoma and glioblastoma multiforme, are among the most aggressive of tumours and are considered largely incurable by surgical or radiotherapeutic intervention. The role of cytotoxic chemotherapeutic drugs continues to be investigated and one of the most effective anticancer agents in this class to emerge in recent years is the prodrug temozolomide (compound **ix**), developed by Stevens and co-workers, initially at the University of Aston and latterly at the University of Nottingham (http://www.nottingham.ac.uk).

In 1998, temozolomide was licensed for clinical use in Europe in the treatment of glioblastoma multiforme; approval for use in the treatment of anaplastic astrocytoma followed in 1999 in Europe and the USA.

The mechanism of action of prodrug **ix**, has been the subject of extensive investigation [6]. Detailed studies indicate that temozolomide, given orally, is rapidly absorbed systemically and undergoes nucleophilic attack by water at the 4-carbonyl position of the tetrazinone ring to give an intermediate triazene that further reacts with water to liberate 5-aminoimidazole-4-carboxamide plus the highly reactive methyldiazonium cation. The methyldiazonium cation preferentially methylates DNA at the O^6 position of guanine in guanine-rich regions.



(ix) R = CH_3; temozolomide
(x) R = $(CH_2)_2Cl$; mitozolomide

The development of temozolomide (and the related antitumour drug mitozolomide **x**) has been extensively published. In the final part of the *Antitumor Imidazotetrazines* series (Part 41), Arrowsmith *et al.* (Universities of Nottingham and Aston; http://www.nottingham.ac.uk/pharmacy) have described the conjugation of temozolomide and mitozolomide to a series of peptides and lexitropsins bearing DNA major- and minor-groove-binding structural motifs [7]. However, *in vitro* evaluation of these novel conjugates failed to demonstrate any enhanced growth-inhibitory activity compared to the unconjugated drug; sites of alkylation at tracts of multiple guanines were also unaffected. In the preceding paper in the journal, Brown *et al.* (Imperial College of Science, Technology and Medicine, London, http://www.ic.ac.uk; and University of Nottingham) describe the radiosyntheses of [4-^{11}C-*carbonyl*]- and [3-*N*-^{11}C-*methyl*]-8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Temozolomide) for use in positron emission tomography (PET) imaging studies [8].

6 Wheelhouse, R.T. *et al.* (1995) NMR and molecular modelling studies on the mechanism of action of the antitumour drug temozolomide. *Contrib. Oncol.* 49, 40–49
7 Arrowsmith, J. *et al.* (2002) Antitumor imidazotetrazines. 41. Conjugation of the antitumor agents mitozolomide and temozolomide to peptides and lexitropsins bearing DNA major and minor-groove binding structural motifs. *J. Med. Chem.* 45, 5458–5470
8 Brown, G.D. *et al.* (2002) Antitumor imidazotetrazines. 40. Radiosyntheses of [4-11C-carbonyl]- and [3-*N*-11C-*methyl*]-8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Temozolomide) for use in positron emission tomography (PET) studies. *J. Med. Chem.* 45, 5448–5457

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Contributions to *Monitor*

We welcome recommendations of papers for review within *Monitor*, in the fields of combinatorial chemistry, pharmacogenomics, pharmacoproteomics, bioinformatics, new therapeutic targets, high throughput screening, new drug delivery technologies and other promising lines of research.

Details of recent papers or those *in press* should be directed to Dr Steve Carney, Editor, *Drug Discovery Today*, Elsevier Science London, 84 Theobald's Road, London, UK WC1X 8RR. tel: +44 207 611 4132, fax: +44 207 611 4485, e-mail: DDT@elsevier.com