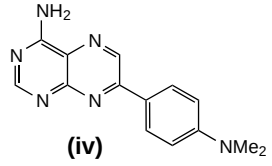
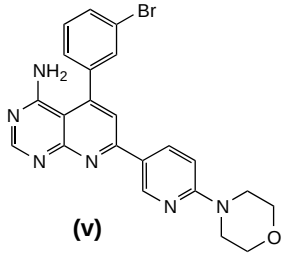
extra-cellular adenosine. It is an intracellular enzyme that catalyzes the phosphorylation of adenosine to adenosine monophosphate (AMP), presumably trapping it within the cell. Inhibition of AK augments the concentration and effect of adenosine locally at sites of injury. This indirect approach might avoid the actions of adenosine in other tissues and thus give a greater therapeutic margin.

A group at Abbott Laboratories (Abbott Park, IL, USA) identified the screening hit **(iv)** as an inhibitor of AK with an IC_{50} value of 400 nM (Ref. 3). Molecule **(iv)** was chosen as a starting point for optimization rather than the natural ligand adenosine because it lacked the polar sugar unit and is thus more likely to give membrane penetration and improve metabolic stability.



(iv)

Altering the 1,4-pyrazine ring to a pyridine allowed groups to be incorporated at position 5, which greatly improved potency. Changing the phenyl ring to a pyridine results in improved water solubility and leads to a series of molecules exemplified by compound **(v)**. Molecule **(v)** inhibited cytosolic AK with an IC_{50} value of 2 nM and, in an intact cell based assay, inhibited AK with an IC_{50} value of 50 nM. Upon oral administration it is active in a thermal hyperalgesia model and a formalin test.



(v)

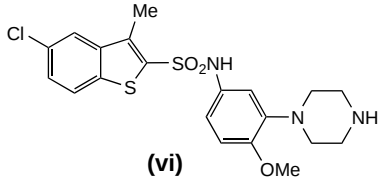
The series is being developed to identify potent oral analgesics and to gain selectivity over side effects shown by some analogues by reducing spontaneous locomotor activity.

3 Cowart, M. *et al.* (2001) Structure–activity studies of 5-substituted pyridopyrimidines as adenosine kinase inhibitors. *Bioorg. Med. Chem. Lett.* 11, 83–86

Selective 5-HT_6 antagonists for cognitive disorders

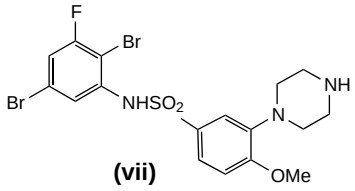
The 5-hydroxytryptamine (5-HT, serotonin) superfamily consists of seven classes of receptors and embraces 14 human subclasses. The latest to be cloned is the 5-HT_6 receptor, the biological function of which is poorly understood. However, its distribution in the brain, in addition to its high affinity for a variety of psychiatric drugs, has generated a great deal of interest with a potential role in the treatment of schizophrenia, depression and learning and memory disorders.

A group at SmithKline Beecham (Harlow, UK) previously reported the sulfonamide **(vi)** to be a potent 5-HT_6 antagonist in Phase I clinical trials for the treatment of cognitive disorders[4]. The group continued to study the SAR around the bisaryl-sulfonamides including the reverse sulfonamide exemplified by molecule **(vii)**[5].



(vi)

Substituted phenyl groups on the left-hand side were favoured in this series with 2- and 3-substitution giving improved potency over 4-substitution. Substitution at both positions 3 and 5 gave low clearance and high oral bioavailability but a low brain:blood ratio. CNS penetration was improved by substitution at position 2, with the 2,5-dibromo-3-fluoro analogue shown proving optimal. Molecule **(vii)** is a highly selective, competitive antagonist ($pK_i = 8.54$), with 19% CNS penetration, low clearance (14 ml min^{-1} kg^{-1}) and good oral bioavailability (65%) in the rat. Attempts to improve the brain penetration, by cyclizing onto the polar sulfonamide NH, led to rapid clearance. On the basis of its biological profile, molecule **(vii)** (SB357134) has been selected for further clinical evaluation.



(vii)

4 Bromidge, S. *et al.* (1999) 5-Chloro–*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulphonamide (SB-271046): A potent,selective and orally bioavailable 5-HT_6 receptor antagonist. *J. Med. Chem.* 42, 202–205

5 Bromidge, S. *et al.* (2001) Phenyl benzenesulphonamides are novel selective 5-HT_6 antagonists: identification of *N*-(2,5-dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-ylbenzensulphonamide (SB-357134). *Bioorg. Med. Chem. Lett.* 11, 55–58

Steven Langston
Millennium Pharmaceuticals
Merrifield Centre
Rosemary Lane
Cambridge, UK CB1 3LQ
tel: +44 (0)1223 722400
e-mail: steve.langston@mpi.com

Novel antitumour molecules

Polyamine analogues as chemotherapeutic agents

The polyamines spermidine, spermine and putrescine are polycationic compounds that are found in significant amounts in nearly every prokaryotic and eukaryotic cell type. Their biosynthetic pathways provide important targets for therapeutic intervention because depletion of polyamines results in the disruption of several cellular functions and, in specific cases, cytotoxicity. The role of terminally alkylated polyamines as antitumour and/or antiparasitic agents has been reviewed by researchers at Wayne

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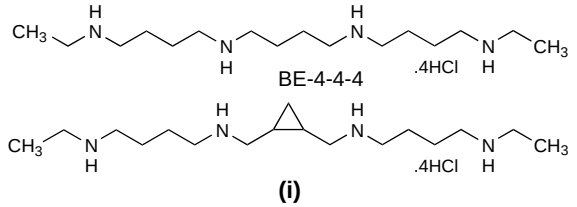
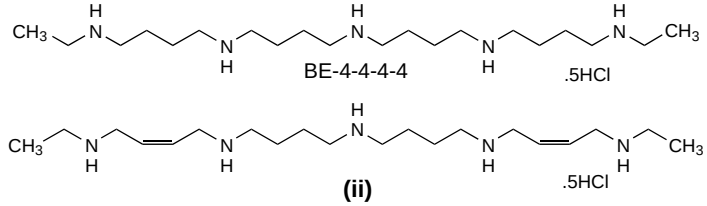
State University (Detroit, MI, USA)[1]. The authors propose that in terms of future drug discovery and development, research efforts will probably be concentrated in the specific areas of polyamine transport (elevated in rapidly dividing cells), new target validation (particularly for antitumour agents) and refinement of SARs (development of agents that bind specifically to non-enzymatic polyamine binding sites).

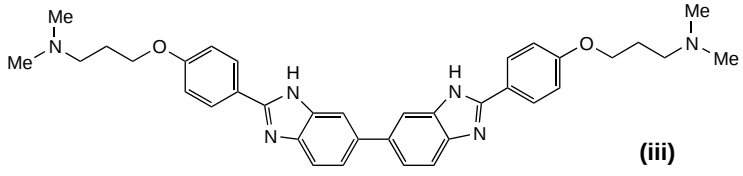
Two recent reports by Basu and colleagues[2,3] illustrate the potential of alkylated polyamine analogues as potential antitumour agents. In the first report[2], analogues of $^{1}N,^{14}N$-bisethylhomospermine (BE-4-4-4) with restricted conformations were synthesized in the search for chemotherapeutic agents with higher cytotoxic activities and lower systemic toxicities than BE-4-4-4. Four new tetramines, for example the *cis*-cyclopropyl analogue **(i)** were more cytotoxic (ID_{50} = 0.08 μM) than BE-4-4-4 in the human prostate cell line DuPro *in vitro* (the prostate gland is one of the major sites of polyamine biosynthesis), leading the authors to propose several new tetramines with wider therapeutic windows than BE-4-4-4.

In their second report, the group describe the synthesis and evaluation of pentamine analogues of the antineoplastic agent, 3,8,13,18,23-penta-azapentacosane (BE-4-4-4-4), in which *cis*-double bonds were introduced into all possible sites in the saturated penta-azapentacosane structure[3]. Previous molecular dynamic studies on spermine–DNA interactions suggested that in a minimum energy conformation, spermine binds to DNA in a cisoidal conformation that wraps around the major groove of the double helix. The cellular uptake, effects on cellular polyamine levels and cytotoxicity of these pentamines were examined in the DuPro prostate cancer cell line *in vitro* using a colony-forming efficiency (CFE) assay. Two pentamines, one a monounsaturated and one a diunsaturated **(ii)**, in which the double bonds were placed at the terminal butane segments of the penta-azapentacosane chain, were shown to be nearly as potent growth inhibitors as the fully saturated analogue, BE-4-4-4-4 (ID_{50} = 0.2 μM).

1 Casero, R.A. Jr. and Woster, P.M. (2001) Terminally alkylated analogues as chemotherapeutic agents. *J. Med. Chem.* 44, 1–26







2 Basu, H.S. *et al.* (2001) Conformationally restricted analogues of $^{1}N,^{14}N$-Bisethylhomospermine (BE-4-4-4): synthesis and growth inhibitory effects on human prostate cancer cells. *J. Med. Chem.* 44, 390–403

3 Basu, H.S. *et al.* (2001) *cis*-Unsaturated analogues of 3,8,13,18,23-pentaazapentacosane (BE-4-4-4-4): synthesis and growth inhibitory effects on human prostate cancer cell lines. *J. Med. Chem.* 44, 404–417

Antitumour bisbenzimidazole-based DNA minor groove-binding agents

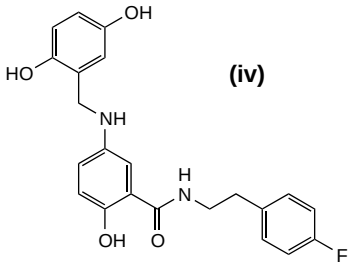
The discovery of potential new chemotherapeutic agents by studies on the non-covalent interactions of small molecules with the minor groove of DNA continues to attract research interest. A variety of minor-groove-binding bisbenzimidazole compounds have been reported, most notably Hoechst 33258, which has been evaluated in clinical trials but failed to show any objective responses in Phase II. Neidle and coworkers have now reported the synthesis and evaluation of a novel head-to-head bisbenzimidazole compound **(iii)**, which was found using X-ray crystallographic analyses to bind in the A/T minor groove region of a B-DNA duplex[4]. The compound showed potent growth inhibition in human ovarian carcinoma cell lines (mean IC_{50} = 0.31 μM) with no significant cross-resistance in two acquired cisplatin-resistant cell lines and a low level of cross-resistance in the P-glycoprotein-overexpressing acquired-doxorubicin-resistant cell line. In addition **(iii)** was found to have significant *in vivo* activity in the hollow fibre assay and tumour xenografts (CH1 cells).

4 Neidle, S. *et al.* (2001) A new class of symmetric bisbenzimidazole-based DNA minor groove-binding agents showing antitumor activity. *J. Med. Chem.* 44, 138–144

Lavendustin A analogues as inhibitors of tubulin polymerization, EGFR and Syk tyrosine kinases

Cushman and coworkers have reported the synthesis and antitumour evaluation

of a series of novel *N*-alkylamide analogues of the novel protein-tyrosine-kinase (PTK) inhibitor, Lavendustin A (Ref. 5). As anticipated, several compounds in this series, for example **(iv)**, were shown to be effective inhibitors of the epidermal growth factor receptor (EGFR) PTK and the non-receptor-PTK, Syk. However, the authors questioned whether their inhibitory effects on these kinases could account for the cytotoxic properties observed in a variety of human cancer cell cultures. Analysis of the cytotoxicity profile using the NCI COMPARE analysis algorithm uncovered a substantial correlation with known tubulin polymerization inhibitors in the database, a correlation supported by experimental studies in which the compounds were shown to be moderate inhibitors of tubulin polymerization. The studies provide an intriguing insight into an agent previously known only as PTK inhibitor and the relationship between cytotoxicity and PTK inhibition.

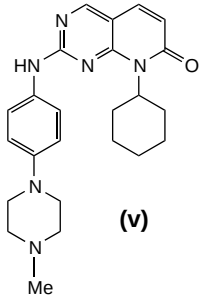


(iv)

5 Cushman, M. *et al.* (2001) Design, synthesis, and biological evaluation of a series of lavendustin A analogues that inhibit EGFR and Syk tyrosine kinases, as well as tubulin polymerization. *J. Med. Chem.* 44, 441–452

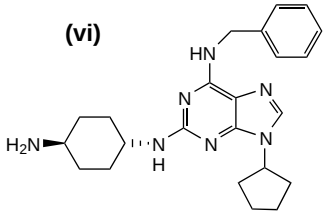
Cyclin-dependent kinase inhibitors

Cyclin-dependent kinases (CDKs) are regulatory proteins of the eukaryotic cell cycle that associate with different cyclins in differing concentrations to regulate cell-cycle progression. Uncontrolled cell proliferation, one of the hallmarks of cancer, can occur through the malfunction of the cell cycle control mechanism; indeed, several cancer types have been associated with mutations in genes encoding CDK inhibitory proteins (CKIs). Therefore, CDKs have become a popular target for the development of selective antitumour agents. Two recent reports illustrate this approach[6,7]. First, the report by Toogood and colleagues puts forward the hypothesis that a selective inhibitor of cyclin D/CDK4 will inhibit cell proliferation at the G_1 checkpoint. They tested this hypothesis through the synthesis and evaluation of CDK4 inhibitors on the basis of the pyrido[2,3-*d*]pyrimidine template[6]. Analysis of >60 analogues of this class revealed some clear SAR trends that might be exploited in the design of more potent CDK inhibitors. The most potent compound in this series, **(v)**, inhibits CDK4 with an IC_{50} value of 0.004 μM, with modest selectivity being exhibited between different CDKs. X-ray crystallographic analyses of representative compounds bound to the related kinase, CDK2, revealed that the compounds occupy the ATP-binding site. Second, a report from Dreyer and Kim and coworkers[7] describes the crystal structure of human CDK2 in complex with the selective adenine-derived inhibitor H717 **(vi)** (IC_{50} = 48nM; Ref. 7) providing further basis for the design of more potent inhibitory drugs.



(v)

(vi)



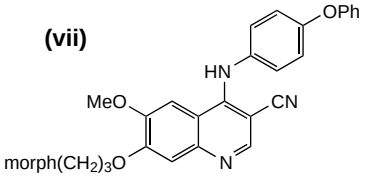
6 Toogood, P. *et al.* (2000) Pyrido[2,3-*d*]pyrimidin-7-one inhibitors of cyclin-dependent kinases. *J. Med. Chem.* 43, 4606–4616

7 Dreyer, M.K. *et al.* (2001) Crystal structure of human cyclin-dependent kinase 2 in complex with the adenine-derived inhibitor H717. *J. Med. Chem.* 44, 524–530

MEK (MAPKK) inhibitors

Mitogen-activated protein kinase kinase (MAPKK) or MEK is a key member of the kinase signalling cascade from growth factors to nuclear transcription. MEK is activated on phosphorylation of key serine residues by upstream kinases such as Raf. Once activated, it catalyzes phosphorylation of MAP kinase or ERK which then activates transcription factors in the nucleus. Because constitutively active MEK mutants are known to produce tumours in nude mice and because overexpression of MEK or ERK protein is associated with various human cancers including kidney, breast and colon, MEK has become an interesting target for therapeutic intervention. A report by Zhang and coworkers[8] details the synthesis of a series of 3-cyano-4-(phenoxyanilino)quinolines and their evaluation as MEK inhibitors. The most potent compound in the series **(vii)** had a low nanomolar IC_{50} value against MEK and potent cell growth inhibitory activity in three human colon tumour cell lines *in vitro* (Colo205, Lovo and SW620).

(vii)



8 Zhang, N. *et al.* (2000) Synthesis and structure–activity relationships of 3-cyano-4-(phenoxyanilino)quinolines as MEK (MAPKK) inhibitors. *Bioorg. Med. Chem. Lett.* 10, 2825–2828

Andrew Westwell
Cancer Research Laboratories
University of Nottingham
Nottingham
UK NG7 2RD
tel: +44 (0)115 9513419
fax: +44 (0)115 9513412
e-mail: andrew.westwell@nottingham.ac.uk