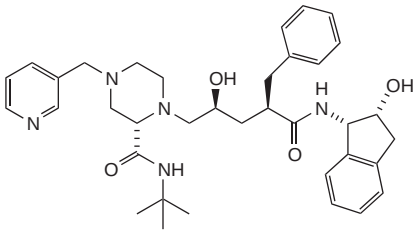
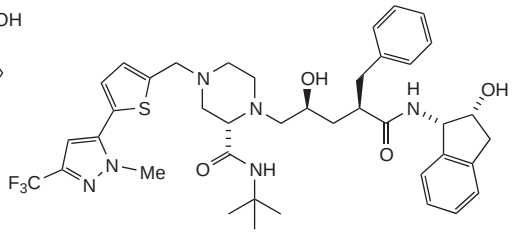
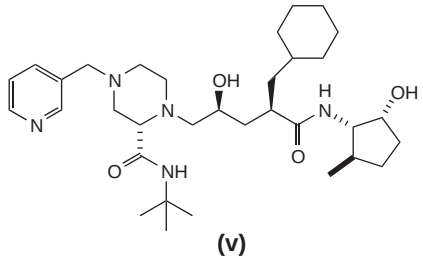


(iii)



(iv)



(v)

inhibitors, compounds need to be developed that have improved pharmacokinetics and potency against drug-resistant strains.

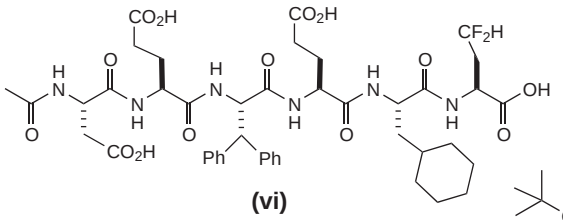
A group from Merck (Rahway, NJ and West Point, PA, USA) has accomplished this by using combinatorial chemistry as a means of rapidly finding molecules with the desired properties [4]. Three regions of indinavir **(iii)** were examined using solid-phase chemistry to synthesize the library. Compounds were assayed for activity and pharmacokinetic properties as mixtures of 24 compounds. The best mixtures were then evaluated and re-tested to yield the optimized compounds. For example, **iv** was found to be more potent than **iii** against wildtype and drug-resistant HIV-protease, with IC_{50} values of <0.1 and <0.6 nM, respectively. Compound **v** ($t_{1/2}$ = 49 min) has an improved half-life in dogs (dose = 10 mg kg^{-1}) compared with indinavir ($t_{1/2}$ = 39 min).

4 Cheng, Y. *et al.* (2002) A combinatorial library of Indinavir analogues and its *in vitro* and *in vivo* studies. *Bioorg. Med. Chem. Lett.* 12, 529–532

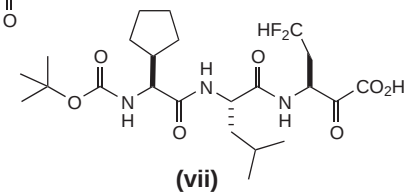
Design of tripeptide HCV-protease inhibitors

The serine protease (NS3-protease) expressed by hepatitis C virus (HCV) has emerged as a key target in the development of agents to be used in the treatment HCV infection. The enzyme is required for processing of the non-structural elements of the viral proteome and is crucial in the HCV lifecycle. For this reason, several groups are currently involved in the discovery of potent inhibitors.

It is known that the NS3-protease is susceptible to product-based inhibition. In fact, the peptidomimetic compound **vi**, which was designed based on this knowledge, is a highly potent inhibitor of the protease, with a K_i value of 40 nM. Unfortunately, **vi** suffers from several structural features that make it unattractive as a potential drug; namely, the large size of the molecule and the potentially reactive cysteine residue at the C-terminus. Two recent publications offer solutions to both problems [5,6].



(vi)



(vii)

It was found that a CF_2H group could replace the cysteine thiol (SH) group of the peptide because it has similar steric and electrostatic properties. Furthermore, changing the C-terminal carboxylic acid into an α-ketoacid converts it into a reversible covalent inhibitor. This enables the N-terminus to be trimmed, yielding tripeptide **vii** with an IC_{50} value of 0.4 μM after additional optimization of the other amino acid side chains.

5 Narjes, F. *et al.* (2002) A designed P_1 cysteine mimetic for covalent and non-covalent inhibitors of HCV protease. *Bioorg. Med. Chem. Lett.* 12, 701–704

6 Colarusso, S. *et al.* (2002) Evolution, synthesis and SAR of tripeptide α-ketoacid inhibitors of the hepatitis C virus NS3/NS4A serine protease. *Bioorg. Med. Chem. Lett.* 12, 705–708

Michael A. Walker
Bristol-Myers Squibb
Pharmaceutical Research Institute
Wallingford
CT 06492, USA
tel: +1 203 677 6686
fax: +1 203 677 7702
e-mail: walkerma@bms.com

Novel antitumour molecules

Non-genotoxic enediynes as potential anticancer therapeutics

Adduct formation between anticancer agents and non-genotoxic species such as proteins, glutathione (GSH) and water often does not have profound genetic and cytotoxic consequences compared with the formation of direct DNA adducts, and is often regarded as a detoxification process. GSH is the most prevalent intracellular thiol known to function in many biological phenomena, such as the suppression of apoptosis. In addition, GSH and glutaredoxin reductase combine

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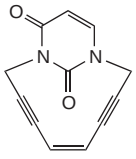
to maintain the small redox protein glutaredoxin in its reduced form, which contributes to the synthesis of deoxyribonucleotide precursors of DNA (along with the related thioredoxin system). GSH inhibitors might therefore possess anticancer activity through the suppression of ribonucleotide synthesis and induction of apoptosis.

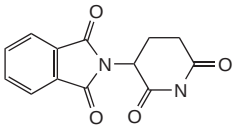
Hakimelahi and co-workers (Academia Sinica, Taipei, Taiwan and University of Alberta, Edmonton, Canada) have synthesized a novel enediyne **(i)** that is reactive with methyl thioglycolate (a representative thiol), is stable to cycloaromatization at 80°C and has interesting antitumour properties [1]. By comparison, cycloaromatization of the well-known enediyne antitumour antibiotics such as neocarzinostatin to a biradical genotoxic intermediate is generally accepted as the mechanism by which the natural product enediyne class exert their potent antitumour activity. In addition, compound **i** was found to deplete the GSH content of human leukaemia Molt-4 cells, and the apoptotic morphology of these cells suggested that modification of GSH content might play a role in their apoptosis. Compound **i** also exhibited low-to-submicromolar *in vitro* activity against a range of murine L1210 and P388 leukaemias, sarcoma 180 and human CCRF-CEM lymphoblastic leukaemia, but was non-toxic up to a level of 50 μM on non-cancerous human erythroleukemia (HEL) cells. Synergistic antitumour effects were observed when **i** was combined with the clinically used anticancer agents adriamycin and ara-C.



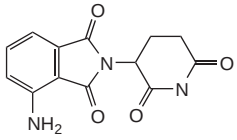
(i)

1 Hakimelahi, G.H. *et al.* (2002) A novel approach towards studying non-genotoxic enediynes as potential anticancer therapeutics. *Bioorg. Med. Chem.* 10, 1321–1328

Angiogenesis inhibitors related to thalidomide



(ii)



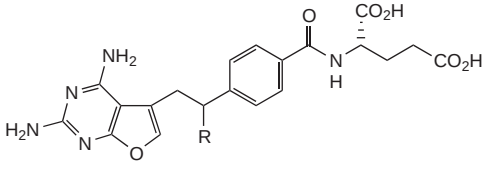
(iii)

Thalidomide **(ii)** was originally developed as a sedative, but achieved notoriety when withdrawn from the market because of its association with teratogenicity. The recent discovery of both immunomodulatory and antiangiogenic activity associated with thalidomide has revived interest in this compound as an anticancer agent. Notably thalidomide has recently been shown to be useful in the treatment of multiple myeloma and might prove useful in the treatment of other hemotological malignancies. D'Amato and co-workers (Harvard Medical School, Boston, MA, USA and EntreMed, Rockville, MD, USA) have now reported the discovery of a thalidomide derivative **(iii)** with dual activity against B-cell neoplasias [2]. Compound **iii** was able to directly inhibit the proliferation of myeloma and Burkitt's lymphoma cell lines *in vitro* without showing toxicity to normal bone marrow stromal cells or hematopoietic progenitor cells, and treatment of drug-resistant myeloma cell tumours in mice was able to produce complete and sustained regressions without any observed toxicity. In Burkitt's lymphoma complete regressions were observed with **iii**. Furthermore, in the murine corneal micropocket model, **iii** inhibited angiogenesis more potently than thalidomide. Compound **iii** is thus a powerful anti-myeloma and anti-B-cell-lymphoma agent possessing both antiproliferative and antiangiogenic effects.

2 Lentzsch, S. *et al.* (2002) *S*-3-Aminophthalimido-glutarimide inhibits angiogenesis and growth of B-cell neoplasias in mice. *Cancer Res.* 62, 2300–2305

Novel antifolates

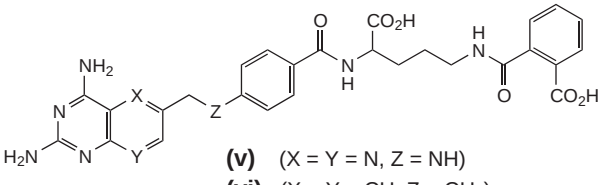
The crucial role of folate metabolism in the biosynthesis of nucleic acid precursors has made inhibition of folate-dependent enzymes (e.g. dihydrofolate reductase, DHFR) an attractive anticancer strategy that has resulted in several clinically used agents. Gangjee and co-workers (Duquesne University, Pittsburgh, PA, USA; Roswell Park Cancer Institute, Buffalo, NY, USA and Tufts University School of Medicine, Boston, MA, USA) have previously reported the synthesis of *N*-[4-[1-methyl-2-(2,4-diaminofuro[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid **(iva)** and its C9-H analogue **(ivb)**, and found the C9-methyl analogue to be twice as inhibitory against recombinant human (rh) DHFR and approximately tenfold greater in growth inhibitory potency against various tumour cells *in vitro* (e.g. CCRF-CEM human leukaemic lymphoblast cells). The increased potency of **iva** was attributed to increased hydrophobic interaction of the C-9 methyl group with DHFR and to increased lipophilicity. Molecular modelling studies suggested that extending the C9-methyl to an ethyl moiety would further enhance DHFR inhibitory activity and lead to more potent cell growth inhibitory activity. Synthesis and antitumour evaluation of the C9-ethyl derivative **(ivc)** verified these predictions; compound **ivc** doubled



(iva) (R = Me)
(ivb) (R = H)
(ivc) (R = Et)

the inhibitory potency against rh DHFR when compared with the C9-methyl analogue and demonstrated increased growth inhibitory potency *in vitro* against several human tumour cell lines (GI_{50} values = $<1.0 \times 10^{-8}$ M [3]. Compounds **ivc** and **iva** were also efficient substrates for human folylpolyglutamate synthetase (FPGS), an important mechanism for trapping classical folates and antifolates within the cell, thus maintaining high intracellular concentrations.

N$^{\alpha}$-(4-amino-4-deoxypteroyl)-*N*$^{\gamma}$-hemiphthaloyl-L-ornithine [PT523, **(v)**] is an unusually potent antifolate currently in advanced preclinical development. Notable pharmacological features of **v** include tight binding to DHFR and efficient use of the reduced folate carrier (RFC) membrane transport protein responsible for cellular uptake of both folates and classical antifolates. Interestingly, compound **v** was found to overcome a clinically relevant ten- to 30-fold level of resistance to the classical antifolate methotrexate (MTX) in cultured cells. In contrast to antifolates such as MTX and compounds **(iva–c)**, compound **v** is not a substrate for FPGS and in this respect resembles non-classical antifolates such as trimetrexate. In addition, tumour cells resistant to several of the newer antifolates, such as the thymidylate synthase inhibitor ZD1694 (raltitrexed), were found to be minimally cross-resistant to **v**. Rosowsky and coworkers (Harvard Medical School, Boston, MA, USA) have now extended their studies on compounds related to **v** to further optimize DHFR and cell-growth inhibitory properties [4]. Compounds **vi** and **vii** were synthesized and found to possess IC_{50} values of 0.69 nM and 1.3 nM, respectively, compared with previously reported values of 4.4 nM for aminopterin and 1.5 nM for compound **v** against CCRF-CEM human leukaemic lymphoblast cells. DHFR inhibitory activity for compounds **vi** and **vii** were found to be comparable with **v**.



(v) (X = Y = N, Z = NH)
(vi) (X = Y = CH, Z = CH_2)
(vii) (X = Y = N, Z = $CHCH_2CCH$)

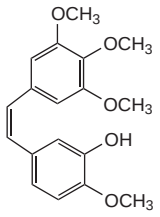
3 Gangjee, A. *et al.* (2002) Synthesis of *N*-[4-[1-ethyl-2-(2,4-diaminofuro[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid as an antifolate. *J. Med. Chem.* 45, 1942–1948

4 Vaidya, C.M. *et al.* (2002) Synthesis and *in vitro* antitumor activity of new deaza analogues of the nonpolyglutamatable antifolate *N*$^{\alpha}$-(4-amino-4-deoxypteroyl)-*N*$^{\gamma}$-hemiphthaloyl-L-ornithine (PT523). *J. Med. Chem.* 45, 1690–1696

Potent, orally active heterocycle-based combretastatin A-4 analogues

Antimitotic agents interfere with the normal microtubule polymerization or depolymerization process, causing mitotic arrest of eukaryotic cells, and agents such as vincristine and taxol have wide clinical usage and have established antimitotics as one of the major classes of cancer chemotherapeutic drugs. However, vincristine and taxol (and other related agents) suffer from undesirable side effects and a lack of efficacy in multidrug resistant (MDR) cancer cell lines, and many research groups are involved in the search for new agents in this class with fewer side effects, improved pharmacokinetic properties and better efficacy against MDR cell lines.

Combretastatin A-4 [CA-4, **(viii)**] exhibits strong antitubulin activity, potent activity against a broad spectrum of human cancer cell lines (including those that are MDR) and is not a substrate for the cellular MDR pump. However, poor pharmacokinetics resulting from its high



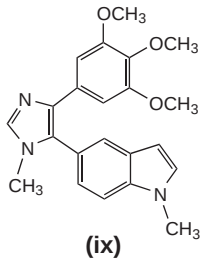
(viii)

lipophilicity and low water solubility limit *in vivo* efficacy (e.g. against murine colon 26 adenocarcinoma), and the susceptibility of the *cis* double bond to isomerize to the inactive *trans* isomer limit potential clinical applications. Considerable effort has, therefore, gone into modifying CA-4 to improve its *in vivo* efficacy, for example through the preparation of water soluble prodrugs and the replacement of the *cis* double bond by more stable entities. Wang and co-workers (Abbott Laboratories, Abbott Park, IL, USA) have now reported the synthesis and structure–activity relationship studies on a series of compounds with heterocycles in place of the CA-4 *cis* double bond [5]. Evaluation of cytotoxicities of the new series against NCI-H460 and HCT-15 human cancer cell lines led to the conclusion that 3-amino-4-methoxyphenyl and *N*-methyl-indol-5-yl were the best replacements for the 3-hydroxy-4-methoxyphenyl group in CA-4, and that 4,5-disubstituted imidazole was the best replacement for the *cis* double bond. In particular, compounds **ix** and **x** were found to be 32% and 82% bioavailable, respectively, in the rat, and were orally efficacious with an increase in lifespan of 38.5% and 40.5%, respectively, against murine M5076 reticulum sarcoma in mice.

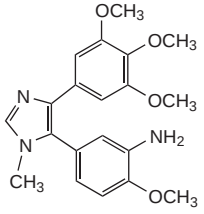
5 Wang, L. *et al.* (2002) Potent, orally active heterocycle-based combretastatin A-4 analogues: synthesis, structure–activity relationship, pharmacokinetics, and *in vivo* antitumor activity evaluation. *J. Med. Chem.* 45, 1697–1711

Novel dual topo I and II inhibitors as potential anticancer agents

Topoisomerases I and II (topo I and II) are essential enzymes in the regulation



(ix)

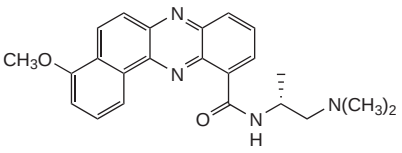


(x)

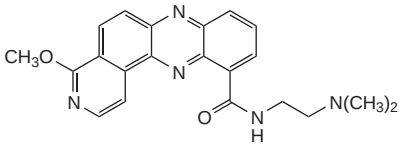
of DNA topology, and thereby have a crucial role in cellular proliferation. Several small molecule inhibitors of topo I and II have found clinical or advanced preclinical application; for example, doxorubicin and etoposide (topo-II inhibitors) and the camptothecin class (targeting topo I). However, one major drawback of these specific topo I or II inhibitors is their inability to overcome multidrug resistance (MDR) mechanisms. For this reason, several dual topo I and II inhibitors (that act at different points in the cell cycle) have been developed that have also found clinical application; for example, intoplicine, XR5000 (DACA) and TAS103.

Charlton and co-workers at Xenova (Slough, Berkshire, UK) and the Auckland Cancer Society Research Centre (Auckland, New Zealand) have described the synthesis and antitumour evaluation of a series of angular benzophenazines based on the previously reported XR5000**,** and a series of phenazine-1-carboxamides [6]. The new analogues were evaluated *in vitro* against the H69 parental human small-cell-lung carcinoma cell line and the H69–LX4 resistant cell line, which overexpresses P-glycoprotein (Pgp). Selected analogues were also evaluated against the CORL23 parenteral human non-small-cell-lung carcinoma cell line and the CORL23–R resistant cell line, which overexpresses Pgp. Many of these new angular benzophenazines were found to be potent cytotoxic agents in these cell lines, showing dual inhibition of both topo I and II, and could circumvent multidrug resistance mechanisms. Most notably, compound **xi** (XR11576) displayed potent activity in the H69 and H69–LX4 cell lines (with IC_{50} values of 23 nM and 29 nM, respectively), was shown to be orally bioavailable and demonstrated tumour growth delay in female mice by both the oral and intravenous routes. XR11576 has been selected as a development candidate for further evaluation.

In related work, Denny and co-workers at the Auckland Cancer Society Research Centre and Xenova have examined a series of heterocyclic phenazinecarboxamides structurally related to XR5000 [7]. The new compounds showed similar (potent) inhibition for paired cell lines



(xi)



(xii)

that underexpressed topo II or overexpressed Pgp, indicating a non-topo II mechanism of cytotoxicity and circumvention of Pgp induced multidrug resistance. Among the compounds tested, the pyrido[4,3-*a*]phenazines were the most potent, and compound **xii** also showed modest growth delays in H69–P xenografts with oral dosing.

6 Vicker, N. *et al.* (2002) Novel angular benzophenazines: dual topoisomerase I and topoisomerase II inhibitors as potential anticancer agents. *J. Med. Chem.* 45, 721–739

7 Gamage, S.A. *et al.* (2002) Structure-activity relationships for pyrido-, imidazo-, pyrazolo-, pyrazino- and pyrrolophenazinecarboxamides as topoisomerase-targeted anticancer agents. *J. Med. Chem.* 45, 740–743

Andrew D. Westwell
School of Pharmaceutical Sciences
University of Nottingham
Nottingham, UK NG7 2RD
tel: +44 115 951 3419
fax: +44 115 951 3412
e-mail: andrew.westwell@nottingham.ac.uk