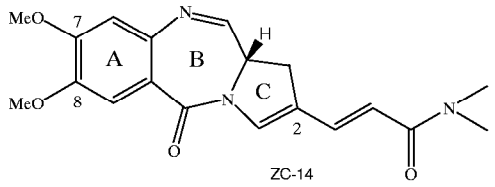


through NMR coupling constants.ZC-14 is remarkably cytotoxic in a number of cell lines with,for example,GI50 values of between 9 and 52 nanomolar in MALME3M, HCT116,SKOV3,SKMEL28,H460 and MCF7 cell lines. The complete synthesis and full *in vitro* evaluation of ZC-14 will be described and compared to a series of closely related analogues.DNA footprinting studies are currently underway to elucidate the effect of the extended C2-acrylamido side chain on sequence-selectivity,and these results will be described.

414

Design and optimization of topoisomerase poison conjugates of triple helix-forming oligonucleotides for sequence-specific DNA cleavage

P.B. Arimondo[1], D. Guianvarc'h[1], C. Bailly[2], A. Boutorine[1], J.S. Sun[1], M. Kuwahara[3], S. Hecht[3], C. Giovannangeli[1], T. Garestier[1], C. Hélène[1]. *[1]Muséum National d'Histoire Naturelle, Laboratoire de Biophysique-CNRS UMR8646, Paris, France; [2]COL, IRCL, INSERM UR524; [3]University of Virginia, Department of Chemistry, Charlottesville, USA*

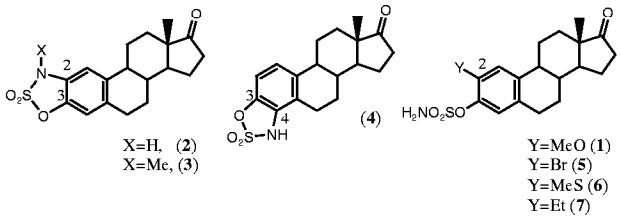
To achieve a sequence-specific DNA cleavage by topoisomerase I, derivatives of the antitumor drug camptothecin have been covalently linked to sequence-specific DNA ligands, such as triplex-forming oligonucleotides (TFO) and hairpin polyamides (MGB), that bind in a sequence-specific manner to the major and minor groove of double-helical DNA, respectively. The binding of the DNA ligand moiety of the conjugate at the target sequence positions the drug selectively at the target site, thereby stimulating topoisomerase I-mediated DNA cleavage at this site. In a continuous effort to optimize this strategy, a broad set of conjugates consisting of (i) 16-20 bases long oligonucleotides, (ii) hairpin polyamides of different length, (iii) alkyl linkers of variable length, and (iv) camptothecin derivatives substituted on the A or B quinoline ring, were designed and synthesized. Analysis of the cleavage sites at nucleotide-resolution reveals that the specificity and efficacy of cleavage depends markedly on the length of both the triple-helical structure and the linker between the oligonucleotide and the poison. Even though the CPT moiety is brought from the major groove side of DNA by the TFO and from the minor groove side by the MGB, both approaches provide a suitable route to guide a cytotoxic agent to a selected sequence in DNA. The optimized hybrid molecules induced strong and highly specific cleavage. Such rationally designed camptothecin conjugates could provide useful antitumor drugs directed selectively against genes bearing the targeted binding site. In addition, they represent a powerful tool to probe the molecular interactions in the DNA/topoisomerase I complex.

415

A-ring Analogues of Oestrone 3-O-Sulphamate as Potent Steroid Sulphatase Inhibitors and Potential Anti-Cancer Agents

L.W.L. Woo[1], M.P. Leese[1], B. Leblond[1], A. Purohit[2], L. Wood[3], G. Packham[3], J. Robinson[1], N. Vicker[1], M.J. Reed[2], B.V.L. Potter[1]. *[1]University of Bath, Pharmacy and Pharmacology, Bath; [2]Endocrinology and Metabolic Medicine, Faculty of Medicine, Imperial College; [3]CRC Wessex Medical Oncology Unit, Centre Block, Level F, Southampton General Hospital, Southampton, UK*

There is strong evidence to suggest that a concomitant inhibition of steroid sulphatase (STS), which converts oestrone (E1) sulphate to E1 and also dehydroepiandrosterone (DHEA) sulphate to DHEA, will further attenuate oestrogenic stimulation to hormone-dependent cancers. E1-3-O-sulphamate (EMATE) was the first potent, orally active, irreversible STS inhibitor developed by us but, unexpectedly, it was found to be also highly oestrogenic. Hence, the challenge has been to design non-oestrogenic STS inhibitors with comparable, or even superior, potency. Whilst there has been success in achieving this goal through the development of the non-steroidal coumarin sulphamates (notably 667COUMATE),1 we have also shown in a series of A-ring modified analogues of EMATE that 2-MeO-EMATE (1) is a highly potent STS inhibitor like EMATE, but in contrast, is devoid of oestrogenicity.2 Here, we further investigate the effects of A-ring modification of EMATE by preparing the 5-ring analogues of EMATE (2 - 4) and a series of 2-substituted EMATE (F, Cl, Br, I, CN, MeS and Et). The most potent STS inhibitor here is 2-Br-EMATE (5) whose IC_{50} of 1.7 nM from a placental microsomes preparation is some 11-fold lower than that of EMATE. Preliminary results from a luciferase reporter gene-expression assay have shown that (5) did not induce oestrogenic activity up to a concentration of 100 nM (c.f. 10 nM for EMATE) indicating that (5) is less oestrogenic than EMATE. A homology model of the human STS has been built from the crystal structure of ASA and the human STS sequence in order to understand the relationship between the binding of these compounds to the active site and their STS inhibitory activities. Although 2-MeS- (6) (IC_{50} = 120 nM) and 2-Et-EMATE (7) (IC_{50} = 820 nM) were found to be less potent than EMATE as STS inhibitors *in vitro*, like (1), these compounds have been shown to induce apoptosis (presumably via tubulin disruptions) in a MCF-7 cells proliferation assay (42%, 53% and 51% inhibition at 1 mM for 1, 6 and 7 respectively). Hence, 1, 6 and 7 are antiproliferative agents with potential applications for treating both hormone dependent- and hormone independent cancers.



This work is funded by Sterix Ltd. LWLW, BVLP, AP and MR are stockholders of Sterix.

References

[1] Woo et.al. (2000) Chem. & Bio. 7: 773.
[2] Purohit et.al. (1998) J. Steroid Biochem. Mol. Bio., 64: 269.

416

Novel Antineoplastic Complexes of Bismuth(III), Cerium(III) and Lanthanum(III)

M.A. Jakupec[1], V. Arion[1], W. Berger[2], S. Wild[3], H. Zorbas[4], B.K. Keppler[1]. *[1]Inst. of Inorganic Chemistry, Vienna University, Wien, Austria; [2]Institute for Cancer Research, Vienna University, Wien, Austria; [3]Max Planck Institute of Biochemistry, Martinsried, Germany; [4]Institute of Biochemistry, Ludwig-Maximilian University Munich, München, Germany*

In a search for new lead structures for development of non-platinum antineoplastic metallopharmaceuticals we have identified acetatobis[1-(azepanyl)-4-(2-pyridyl)-2,3-diazapenta-1,3-dien-1-thiolato-N',N3,S]bismuth(III) (KP1255), trans-[aquachlorobis(1,10-phenantroline)cerium(III)] dichloride (KP776) and [tris(1,10-phenantroline)lanthanum(III)] trithiocyanate (KP772) as potent agents among series of related complexes, which have been synthesized in our laboratory.

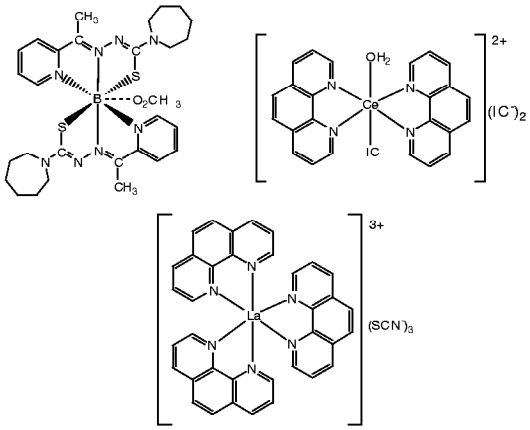


Figure 1. Structures of the bismuth complex KP1255 (top left), the cerium complex KP776 (top right) and the lanthanum complex KP772 (bottom).

Unlike platinum drugs the bismuth complex KP1255 displays rather flat dose-response curves with mean IC_{50} values in the low nanomolar range

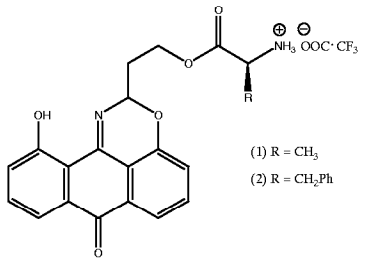
in panels of human tumor cell lines. Activities of the cerium complex KP776 and the lanthanum complex KP772 are similar to each other with IC_{50} values mainly in the low micromolar range. In pairs of chemosensitive parental tumor cells and P-glycoprotein- or MRP1-overexpressing cell clones derived from them as *in vitro* models of multidrug resistance collateral sensitivity to the latter two compounds has been observed. All three compounds produce DNA interstrand cross-links, but with much lower efficiency than platinum drugs like cisplatin. The lanthanum complex KP772 induces DNA strand breaks without altering the secondary structure of DNA. For the complexes KP1255 and KP776 neither induction of DNA strand breaks nor alterations of the secondary structure of DNA could be detected. In conclusion, it remains doubtful whether DNA is the critical target site of these novel agents and further possible mechanisms of action are being explored.

417

Oxa-aza-benzo[de]anthracenes: design, synthesis and evaluation of a structurally new class of dual topoisomerase inhibitors

D.J. Mincher[1], A. Di Salvo[1], G. Barsacq[1], G. Kay[1], L. Young[1], M.C. Bibby[2]. [1]*Napier University, School of Life Sciences, Edinburgh, United Kingdom;* [2]*University of Bradford, Cancer Research Unit, Bradford, United Kingdom*

Human DNA topoisomerase I and II are valid targets in cancer therapy and inhibitors of these enzymes include the clinically active doxorubicin (topo II) and the camptothecins topotecan and irinotecan (topo I). The clinical utility of the camptothecin class of compounds is limited by the rapid *in vivo* conversion to inactive metabolites as a result of inherently labile structural features of the active drug molecules and the ease of reversibility of cleavable complex formation. Towards the design of non-camptothecin inhibitors of topo I with increased structural stability, and which also target topo II thereby potentially circumventing acquired drug resistance associated with altered expression of a single topoisomerase, we report the rational design and synthesis of a series of oxa-aza-benzo[de]anthracenes with angular ring systems that do not bind strongly to DNA.



The 2H-3-oxa-1-aza-benzo[de]anthracen-7-ones (1) and (2) are representatives of a new class of dual topo I and II inhibitors with cytotoxic activity against human and animal cell lines *in vitro*; for example the L-alanine conjugate (1) is active against the human leukaemic HL60 cell line (IC_{50} 7μM) and completely inhibited the topo I and topo II-mediated relaxation of supercoiled pBR322 DNA at 50 and 25μM respectively, as shown by changes in the electrophoretic mobility of the plasmid *in vitro*. Furthermore (1) stimulated topo I-mediated DNA cleavable complex formation at 25μM, comparable to camptothecin at 10μM. The chemosensitivity and enzyme inhibitory properties are modulated by the nature of the amino acid sidechain (R-group). Correlations are drawn between chemical structure, cytotoxic potency, DNA binding and topoisomerase I inhibition for this novel class of inhibitor that lacks the structural lability of the camptothecins.

418

Synthesis and evaluation of renal dipeptidase inhibitors as biomarkers for colon cancer

H. Hallur Gurulingappa, P. Buckhaults, K.W. Kinzler, B. Vogelstein, S.R. Khan. *The Sidney Kimmel Comprehensive Cancer Center at Jo, Johns Hopkins Medical Institutions, Baltimore, USA*

Colon cancer is the second most common cancer in the U.S. and kills more than 50,000 people each year, but it is also one of the most preventable and curable cancers. The best prevention is getting screened on a regular basis. With regular screening, precancerous polyps can be detected early and removed, thus preventing the development of colon cancer in the first place. Current screening tests such as sigmoidoscopy, colonoscopy and detection of fecal occult blood have significant problems which have stimulated the search for more specific non-invasive tests for the early detection of colorectal cancers. In recent serial analysis of gene expression (SAGE) studies carried on normal, adenomatous and cancerous colonic epithelium, the gene renal dipeptidase (RDP) was found to be overexpressed in both benign and malignant tumors compared with normal colonic epithelium. RDP has been extensively analyzed with respect to its catalytic mechanism and inhibition kinetics by variety of synthetic inhibitors. RDP is unique among the dipeptidase in that it can cleave amide bonds in which the COOH terminal partner is a D-amino acid, providing an excellent opportunity for the development of specific probes for its detection *in vivo*. Based on these findings we designed and synthesized alkylaminophosphonic acid derivatives with iodinated aromatic ring as one of the side chains as inhibitors of RDP, in order to use them as biomarkers to detect colon cancer at earlier stage. The Ki values of the substrates were determined using colon cancer lysate *in vitro* and were in the range of 0.6-10 nM. The synthesis of 125iodine alkylaminophosphonic acid is under way. A full update of the enzyme assay results of radiolabelled molecules will be provided at the meeting.

419

WP744, a novel anthracycline highly active against STI-571-resistant tumors

W. Priebe, G. Evrony, S. Lee, I. Fokt, M. Talpaz, N. Donato. *The University of Texas M.D. Anderson Cancer Center, Department of Bioimmunotherapy, Houston, USA*

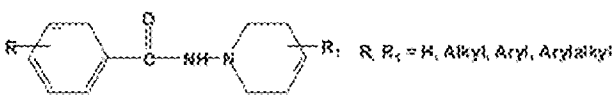
STI-571, a BCR-ABL inhibitor, is effective in chronic myelogenous leukemia, but advanced disease often progresses despite therapy, suggesting that combinations of STI-571 with another agent may be required to control disease progression. We tested one such agent-WP744, a novel anthracycline with greater proapoptotic and cytotoxic properties than its parent compound, doxorubicin (DOX). WP744 has been tested against a panel of DOX-sensitive and MDR-type leukemia and solid tumor cell lines, and has also shown to inhibit colony formation of blasts isolated from fresh bone marrow samples of patients with acute myelogenous leukemia. In this study, we found that WP744 was active not only against the STI-571-resistant cell line K562-R, but also against 4 cell lines (WDT-1, WDT-2, WDT-3, and WDT-4) isolated from peripheral blood of patients with advanced chronic myelogenous leukemia that had progressed after STI-571 therapy. In all of these cell lines, WP744 inhibited cell growth and induced apoptosis to a greater extent than did DOX. These results suggest that WP744 may be effective in treating advanced leukemia that has progressed after STI-571 therapy.

420

The synthesis and biological evaluations of n-aminotetrahydropyridines as anticancer agents

K. Redda[1], K. Yoon[1], T. Wilson[1]. [1]*Florida A&M University, College of Pharmacy, Tallahassee, USA*

The objective of our research is to develop effective chemotherapeutic agents that can be utilized for the treatment of lung and colon cancers. The role of non steroidal anti-inflammatory agents (NSAIDs) such as aspirin, piroxicam, and sulindac in colon cancer has been well-documented in epidemiological and animal studies. Accumulating evidence indicates that the inhibition of colon tumor development by NSAIDs is mediated through the modulation of arachidonic acid metabolism via the cyclooxygenase enzymes, which in turn inhibit immune responsiveness.



The increased expression of cyclooxygenase-2 (COX-2) enzyme has been reported to correlate with the malignant changes observed in a variety of human cancers, including colorectal, gastric, esophageal, brain, and lung tumors. Our earlier published work established that the N-aminocarbonyl-1,2,3,6-tetrahydropyridine analogs we synthesized were effective non steroidal anti-inflammatory agents with strong cyclooxygenase-1 (COX-1) and (COX-2) inhibitory activities. It was of interest to us to investigate if these analogs showed any anticancer activities. Dry substituted pyridines were reacted with 1-chloro-2,4-dinitro benzene under reflux using acetone and gave invariably crystalline N-(2,4-dinitrophenyl)pyridinium chloride salts. The salts were further reacted with benzoyl hydrazides or benzene sulfonyl hydrazides to furnish an anilino derivative which hydrolyzed in wa-