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.

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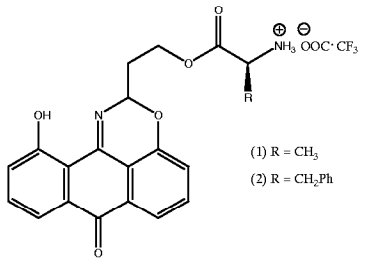
Oxa-aza-benzo[de]anthracenes: design, synthesis and evaluation of a structurally new class of dual topoisomerase inhibitors

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.

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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.

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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.

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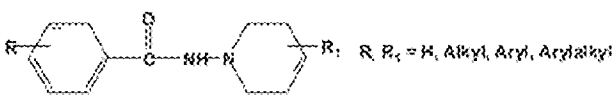
The synthesis and biological evaluations of n-aminotetrahydropyridines as anticancer agents

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-

ter and dioxane mixture to the corresponding pyridinium ylides The ylides provided the substituted N-(pyridyl/phenyl) carbonyl/sulfonylamino-1,2,3,6-tetrahyropyridines when reduced using sodium borohydride in ethanol at ice-bath temperature. The anti-inflammatory activities of these tetrahydropyridines were determined using the rat paw edema assay with Indomethacin as the reference compound. Inhibition of the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes were also conducted. Several analogs were found to possess significant anti-inflammatory activities with varying degrees of COX-2/COX-1 ratios. The investigation was supported by a grant from the US National Institutes of Health (NIH), GM 08111 and RR 03020.

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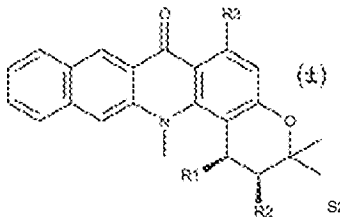
Relationships between DNA alkylation, perturbation of the cell cycle and cytotoxicity in a series of benzoacronycine derivatives

A. Pierre[1], S. Leonce[1], C. Bailly[2], M. Koch[3], F. Tillequin[3], B. Pfeiffer[1], J.A. Hickman[1]. [1]*Institut de Recherches Servier, Cancer research, Suresnes, France;* [2]*INSERM U524 and Centre Oscar Lambret, Lille, France;* [3]*Universite Rene Descartes, Paris, France*

Due to outstanding antitumor properties in orthotopic models of human solid tumors, the synthetic benzoacronycine derivative S23906-1 has been selected for advanced preclinical development. This compound was recently shown to bind covalently to DNA in the minor groove and alkylate the guanine residues at the N2 position. This unusual property, coupled with uncommon cell cycle perturbations characterized by a cell cycle arrest in the S or G2+M phases, raises the question regarding the precise molecular mechanism underlying the antitumor properties of S23906-1. To address this question, a resistant cell line was established by stepwise exposure of KB-3-1 epidermoid carcinoma cells to S23906-1. The resistant KB/S23-500 line, which does not display the classical MDR phenotype, was used to screen the derivatives. A set of selected compounds was studied for their i) cytotoxic properties in L1210, KB-3-1 and KB/S23-500 cells in culture measured by the MMT assay, ii) perturbation of cell cycle measured by flow cytometry and iii) *in vitro* DNA alkylation as determined by reduction of the electrophoretic mobility of reacted DNA.



S23906-1 R_1 = R_2 = OAc R_3 = OMe

The most cytotoxic compounds possessed a methoxy group at the C3 position and at least one leaving group at the C1 and C2 positions. These cytotoxic derivatives formed covalent adducts with DNA *in vitro*. The resistant KB/S23-500 cells were cross resistant to the compounds that induced an arrest in S and G2+M phases, in contrast to compounds arresting cells only in G2+M phases. A tight correlation between cytotoxicity, arrest in S phase, cross resistance of the KB/S23-500 cell line and DNA alkylation was thus observed for compounds with a good leaving group. These observations strongly suggest that DNA is actually an important target for this class of compounds.

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2-Benzimidazolylhydrazones derived from alpha-(N)-acyl heteroaromatics: RNA synthesis inhibitors with camptothecin-like activity

J. Easmon[1], G. Peurstinger[1], A. Schmidt[1], J. Hofmann[2], G. Heinisch[1], Z.L. Liao[3], Y. Pommier[3]. [1]*University of Innsbruck, Pharmaceutical Chemistry Deparment, Innsbruck, Austria;* [2]*University of Innsbruck, Institute of Medical Chemistry and Biochemistry, Innsbruck, Austria;* [3]*Center for Cancer Research, NCI, NIH, USA*

Recently we have shown that replacement of the thiocarbamoyl moiety in 2-acetylpyridine thiosemicarbazone (TSC) by a 2-benzothiazole (BZT) ring results in compounds with high cytotoxic activity (factor ~ 10) [J. Easmon et al. Eur. J. Med. Chem. 1997, 32, 397-408]. In a classical bioisosteric follow up studies, hydrazones bearing a 2-benzimidazolyl moiety had been synthesised and their antiproliferative activity evaluated *in vitro* against a panel of human tumor cell lines using the MMT assay. For this class of agents, hydrazone derivatives of 2-acylpyridines inhibited cell proliferation at lower concentrations (IC_{50} = 0.006-1.36 μM) compared to the acetyl diazine and quinoline derived compounds (IC_{50} = 0.21-3.37 μM). Moreover, the novel hydrazones are not substrate for the MDR eflux system and do not show cross resistance to hydroxyurea resistant KB cells. However, in the 2 day NCI *in vitro* assay both types of compounds were found to be equipotent with a mean GI50 of -7.27 to -6.44 for the acylpyridine derived compounds and a mean GI50 of -;7.31 to -5.71 for the acetyl diazine derived hydrazones. Based on the *in vitro* assay results, several of the compounds were then evaluated in the *in vivo* Hollow Fiber Assay. Compounds fulfilling one or more of the several criteria used to identify positive result are EPH 97-NSC 703101 (IP: 6, SC: 8), EPH 241-NSC 703106 (IP: 6, SC: 8), EPH 307-NSC 720194 (IP: 12, SC: 0), and EPH 316-NSC 720195 (IP: 12, SC: 8) with no net cell kill observered. In a study of the effects of the compounds on macromolecular synthesis in L1210 lymphoid leukemia cells, RNA synthesis was preferentially inhibited (e g. EHP 61, IC_{50} <5 μM) whilst DNA and protein synthesis are not affected (IC_{50} >100 μM). Using EPH 103 (NSC 703104) and other analogues as a seed in the COMPARE analysis, a positive correlation to camptothecin and analogues was obtained with a PCC of 0.75 - 0.60. The hydrazone derivatives have been tested in topoisomerase I-deficient cells. Preliminary results suggest that resistance to topoisomerase I-deficient cells and this would be consistent with topoisomerase-I targeting. The synthesis, structure activity-relationships, and biochemical studies relating to this class of compounds will be presented. Financial support was provided in part by the Austrian Science Foundation (FWF), project No. P12384-MOB.

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Novel pteridine-based inhibitors of cAMP phosphodiesterases: promising antineoplastic agents

K. Merz[1], N.Graf v. Keyserlingk[2], S. Vatter[1], M. Habermeyer[1], G. Eisenbrand[1]. [1]*University of Kaiserslautern, Department of Chemistry, Division of Food Chemistry and Environmental Toxico;* [2]*Faustus Forschungs Compagnie Translational Cancer R, Leipzig, Germany*

The second messenger cAMP is involved in a multitude of cellular processes, including for example cell growth and cell differentiation. The intracellular level of cAMP is regulated by adenylate cyclases and cAMP phosphodiesterases (PDEs). Many human and non human tumor cells exhibit markedly enhanced cAMP phosphodiesterase activity, concomitant with low intracellular cAMP levels. This suggests that inhibition of cAMP PDE might be exploitable for antitumor treatment. Specific amine substituted pteridines have been identified as highly potent PDE inhibitors, with preference for the PDE4 isoenzyme family, the predominant isoenzyme family in many tumors. This had been demonstrated previously for pteridine derivatives bearing different substituents in the 4- and 7-position of the bicyclic core, showing efficient growth inhibition in various tumor cell lines. Effective intracellular inhibition of PDE activity was achieved together with enhanced cAMP levels and subsequent induction of apoptosis. In the course of our structure-activity-studies we identified pteridine derivatives with potent PDE4 inhibitory properties, bearing identical amino substituents in 4- and 7- position of the pteridine ring system. These compounds were found to be more easily accessible by chemical synthesis. Novel compounds were tested in cell lines expressing different levels of total PDE-activity, with PDE4 representing the highest cAMP hydrolyzing activity. Efficient growth inhibitory activity in different tumor cell lines (IC_{50} down to 1 mM) was observed. The project was supported by EFRE of the EU and by funds from the Freistaat Sachsen (P-Nr: 6306).

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Synthesis and biological evaluation of phosphoramide mustard (PM) prodrugs

D. Farquhar[1], C. Ballatore[1], J. Nelson[2], B. Andersson[3]. [1]*University of Texas M.D. Anderson Cancer Center, Experimental Therapeutics, Houston, USA;* [2]*University of Texas M.D. Anderson Cancer Center, Education, Houston, USA;* [3]*University of Texas M.D. Anderson Cancer Center, Bone Marrow Transplantation, Houston, USA*

Cyclophosphamide and ifosfamide are bifunctional alkylating agents used for the chemotherapeutic management of many common human malignancies. These agents are not active in their own right but are oxidatively biotransformed, mainly in the liver, by cytochome P-450 dependent mixed-function oxidases to unstable intermediates that are believed to transport the ultimate active metabolites, phosphoramide mustards (PM's), into cells.