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

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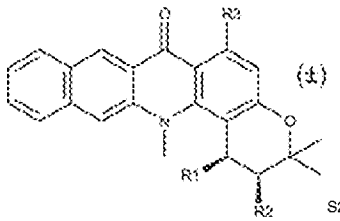
Due to outstanding antitumor properties in orthotopic models of human solid tumors, the synthetic benzoacronycine derivative S23906-1 has been selected for advanced preclinical development. This compound was recently shown to bind covalently to DNA in the minor groove and alkylate the guanine residues 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

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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

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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

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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.

Despite their broad spectrum activity, the clinical usefulness of cyclophosphamide and ifosfamide is limited by the formation of toxic byproducts. Both agents generate acrolein, a metabolite that has been implicated in kidney and bladder toxicity. In addition, ifosfamide gives rise to chloroacetaldehyde, a metabolite believed to cause CNS toxicity. To avoid toxicologic problems associated with the formation of such byproducts, we have investigated alternative strategies to deliver PM's into cells. Various prodrug formulations of PM's were prepared including compounds that are activated by carboxylate esterase, β-glucuronidase and β-galactosidase. GRAPH. In the absence of activating enzymes, most of the prodrugs were fairly stable and showed a low order of biological activity. In the presence of activating enzymes, however, the prodrugs were rapidly converted to PM's. The increased toxicity of the prodrugs to human tumor cells in the presence of activating enzymes varied from as little as 10-fold to as much as 500-fold. In addition to providing an alternative cell-delivery strategy for PM's, some of these prodrugs offer potential for use in conjunction with gene therapy approaches to tumor-selective drug activation. (Supported by grant CA RO1 89386).

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Design of a DNA damaging molecule "programmed" to release multiple high affinity inhibitors of EGFR tyrosine kinase under hydrolytic conditions: A novel antitumour drug combination strategy

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The altered protein expression and activity of receptor tyrosine kinases (TK) are implicated in the progression of various types of cancers. One such dysfunction is the overexpression of the epidermal growth factor receptor (EGFR) that correlates with aggressive tumor progression and poor prognosis. Recently, we developed a novel strategy that seeks to combine DNA damaging properties and EGFR TK inhibitory activities into single molecules termed "combi-molecules" designed to kill EGFR-expressing tumour cells (Matheson et. al., J. Pharm. Exp. Ther, 296, 832-840, 2001 Brahimi et al., ibid, 2002, in press). In order to enhance the EGFR inhibitory potency and stability of these compounds, we designed a novel strategy termed "cascade release" (CR) that seeks to mask the combi-molecule into a stable carrier "programmed" to release the antitumour species by hydrolytic cleavage. Since these molecules henceforth referred to as "cascade release molecules" (CRM) are also designed to retain EGFR affinity on their own, this principle leads to molecular systems whereby three generations of inhibitors can arise from the hydrolysis of the parent CRM. To study this model, we recently designed and synthesized RB24 (IC_{50} competitive binding=130 nM), which was a masked form of RB14 (IC_{50}=100 nM), a hydrolabile triazene capable of generating the combi-molecule ZR08 (IC_{50}=44 nM). The latter was found to further degrade into RB10, another potent inhibitor of EGFR (IC_{50}=40 nM). Kinetic studies using UV spectrophotometry demonstrated that the parent CRM, RB24, was hydrolyzed with a t1/2=42 min. Western blot analysis demonstrated potent inhibition of EGFR autophosphorylation by the CRM in the carcinoma of the vulva cell line, A431 (IC_{50}=2 uM). Studies on serum stimulated growth using a pair of isogenic cells [NIH3T3 and HER14 (engineered to overexpress EGFR)] showed that RB24 selectively induced approximately 5-fold stronger growth inhibitory activity in the EGFR-transfectant when compared with its parent NIH3T3, indicating significant EGFR selectivity. The results in toto suggest that RB24 is the first ever molecule capable of being an EGFR TK inhibitor (I), while being the parent of two other EGFR inhibitors (I2) and (I3), the latter being the precursor of another stable inhibitor (I4) + a DNA damaging fragment. Further studies are ongoing in our laboratory to determine the effects of the CR system on the sustainability and reversibility of EGFR TK inhibition.

Drug delivery

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Taxane-monoclonal antibody covalent conjugates for targeted chemotherapy of cancer

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In efforts to develop taxane derivatives capable of tumor-specific drug delivery (Safavy, US Patent 6,191,290 B1), we previously reported the synthesis and cytotoxicity results of a paclitaxel (PTX) monoclonal antibody (MAb) C225 (ErbituxTM, ImClone Systems, Somerville, NJ) conjugate (Figure 1) (Safavy et al., Eighth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer, Princeton, NJ, 2000) with enhanced cytotoxicity of PTX against A431, UM-SCC-1, and UM-SCC-6 human cell lines.

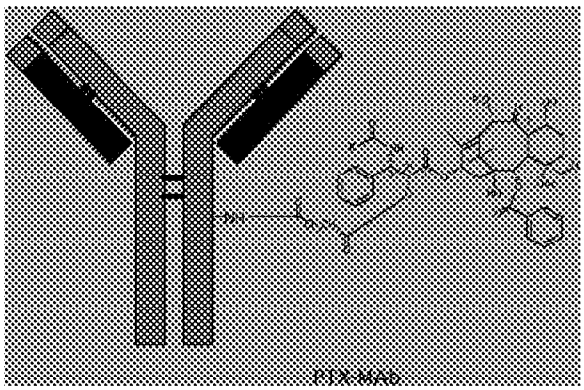
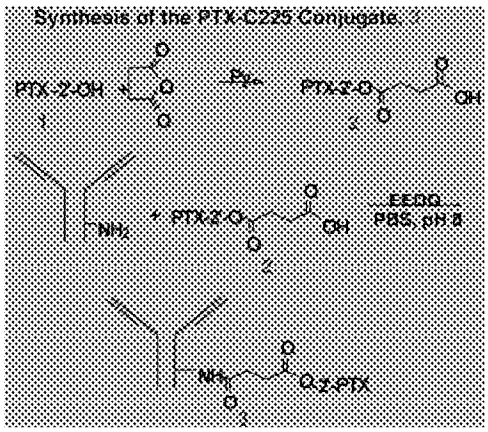


Figure 1

Here, conjugates of PTX with the anti-epidermal growth factor receptor antibody, C225 and anti HER2/neu antibody, Herceptin (Her, Trastuzumab, Genentech, South San Francisco, CA) were synthesized by the procedure shown in Scheme 1. The purity and number of drugs per antibody (PTX: MAb) were evaluated by HPLC and MALDI-TOF mass spectrometry, respectively.



Scheme 1

Purities of *98% and PTX: MAb of 2.5 were detected for both conjugates. These conjugates were then tested in cell binding and cytotoxicity experiments using MDA-MB-468 (human breast carcinoma) and LNCAP and DU145 (human prostate carcinoma) cell lines to determine the effect of receptor-targeted delivery in enhancing the drug efficacy. To demonstrate the retention of antigen-binding ability, the parent MAb and conjugates were radiolabeled with 125I and screened in binding inhibition assays. The percent cell binding (%B) of the PTX-C225 conjugate, as compared to the unconjugated MAb (parenthesized values) were 49 (88), 41 (87), and 40 (87) in MDA-MB 468, LNCAP, and DU145 cells, respectively. Herceptin and the PTX-Her conjugate showed a %B of 21 (36) to LNCAP cells with no appre-