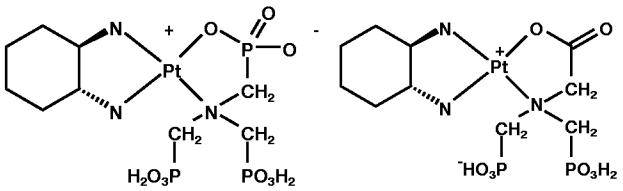
fects within this class of compounds involve modifications of both the leaving phosphonate-containing group, which is responsible for the osteotropic properties, and the non-leaving amine group, which is decisive for the cellular processing of DNA adducts. Structure-activity relationships have been investigated *in vitro* within a series of complexes of the general formula cis-[PtA2X2], where A2 is either two ammine ligands or one bidentate ethanediamine or isomerically pure cis-, trans-R,R- or trans-S,S-1,2-diaminocyclohexane (DACH) and X2 is one bidentate aminopolymethylenephosphonate, either aminotris(methylenephosphonate) (ATMP) or bis(phosphonomethyl)aminoacetate (BPMAA). In the cisplatin-sensitive human ovarian tumor cell line CH1 the complexes of ATMP display a 2-20fold higher potency than their BPMAA-containing counterparts. Within the series of ATMP complexes potency decreases depending on the amine ligand in the following order: trans-R,R-DACH > trans-S,S-DACH > cis-DACH ≈ diammine > ethanediamine. Within the BPMAA-containing series the order of decreasing potency is somewhat different: trans-R,R-DACH > trans-S,S-DACH ≈ ethanediamine > cis-DACH.



Thus, in both series the complexes of trans-R,R-DACH (Fig. 1) prove to be superior to those of other isomers of DACH and those of ethanediamine, which is consistent with published findings for oxaliplatin and other DACH-containing platinum compounds. As complexes of this latter type usually exhibit low levels of cross-resistance with diammine platinum drugs like cisplatin and carboplatin, we expect that the activity of the phosphonate-containing derivatives is retained in cells resistant to cisplatin.

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Covalent binding of the acronycine derivative S23906-1 to glutathione prevents DNA alkylation and reduces cytotoxicity

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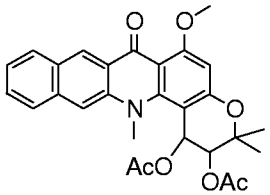
The benzoacronycine derivative S23906-1 has been recently identified as a potent anticancer drug active against a variety of human tumor xenograft models in mice and has been selected for advanced preclinical evaluation (1). This promising new anticancer agent derives from the alkaloid acronycine isolated from a plant distributed in Australia. The parent tetracyclic alkaloid is weakly cytotoxic to a wide range of tumor cells *in vitro* and displays moderate antitumor activities *in vivo*. Clinical testing of acronycine itself showed insufficient antitumor responses and the development of this compound was discontinued. Nevertheless, the antitumor potential of this compound has stimulated the synthesis of more potent and more active analogues, such as S23906-1 which is the lead synthetic compound in these new series.



From the mechanistic point of view, S23906-1 was recently characterized as a DNA alkylating agent reacting irreversibly with guanine residues in double stranded DNA (2). The covalent binding to DNA is thought to be responsible for the cytotoxic action and the capacity of the drug to trigger apoptosis in tumor cells (3). However, covalent binding to other intracellular reactive nucleophilic species may also occur. In the course of our ongoing studies aimed at characterizing the interaction of S23906-1 with biologically significant molecules, the binding and bonding to glutathione (GSH) was examined. Direct measurements by mass spectrometry as well as competition experiments with DNA demonstrated that S23906-1 forms covalent adducts with GSH, but not with its glutathione disulfide (GSSG). However, the drug binds non covalently to GSSG. Circular dichroism measurements revealed that S23906-1 form very stable complexes with both GSH and GSSG. A range of GSH derivatives was use to delineate the portion of the GSH molecule responsible for the binding and bonding interaction with S23906-1. The cytotoxicity of the GSH-S23906-1 covalent adducts was evaluated using human KB epidermoid carcinoma cells sensitive and resistant to S23906-1 (KB-3-1 and KB/S23-500, respectively). The formation of covalent complexes between GSH and S23906-1 decreases the formation of potentially lethal DNA cross-links, thereby modulating the cytotoxic action of the drug.

References

[1] Guilbaud et al., Clin. Cancer Res. 7, 2573-2580, 2001.
[2] David-Cordonnier et al., Biochemistry, in press, 2002.
[3] Leonce et al., Mol. Pharmacol. 60, 1383-1391, 2001.

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Gamma-Gutamyltransferase-dependent extracellular detoxification of cisplatin by human kidney proximal tubule cells

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Elevated nephrotoxicity is the main limiting factor for utilization of the anticancer agent cisplatin. *In vivo*, the administration of the cysteine-containing tripeptide GSH has been found to reduce nephrotoxicity, but the precise biochemical mechanism of this protective action is not fully understood. The aim of the present study was to gain insights into the mechanism by which GSH prevents cisplatin nephrotoxicity, and in particular whether the protective action of GSH is mediated by products of the extracellular breakdown of GSH operated by gamma-glutamyl transpeptidase (GGT), an enzyme activity highly expressed in kidney tubular cells. HK-2 cells, derived from immortalization of human kidney proximal tubule cells, were challenged with cisplatin in the presence of extracellular GSH, in conditions capable of enhancing or inhibiting GGT anzyme activity. Cisplatin cytotoxicity was judged by its antiproliferative action as assessed by WST-1 reduction test. HK-2 cells exhibited a high GGT activity, corresponding to that normally found in the proximal convolute tubule. The antiproliferative effect of cisplatin was only little affected by addition of GSH. However, when the antiproliferative assay was performed in the presence of glycyl-glycine, to serve as transpeptidation acceptor and thus to stimulate GGT-mediated GSH catabolism, cisplatin-induced growth inhibition was prevented to a large extent. This effect was not mediated through an increase of intracellular GSH levels, which were not affected by glycyl-glycine supplementation. The thiol dipeptide cysteinyl-glycine, i.e. the GSH catabolite generated by GGT activity, showed a higher reactivity against cisplatin *in vitro* than GSH, as shown by the quicker oxidation of its ?SH groups. Neither the cisplatin/GSH nor the cisplatin/cysteinyl-glycine adducts displayed an antiproliferative effect. However, 2h pre-complexing with GSH in the presence of GGT, or directly with the GSH catabolite cysteinyl-glycine decreased the antiproliferative effect of cisplatin and drug-induced DNA platination to a greater extent than pre-complexing with GSH alone. The results support that extracellular metabolism of GSH by GGT plays a role in modulating cisplatin nephrotoxicity. A better understanding of these reactions might help to devise strategies o reduce cisplatin nephrotoxicity without impairing its terapeutic efficacy.

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Enhanced antitumor activity of irofulven in combination with gemcitabine against the MV522 human lung carcinoma xenograft

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Effective therapy for cancer often requires a multi-modal approach combining therapies with differing mechanisms of action to enhance antitumor activity. The novel antitumor agent, irofulven (HMAF, MGI 114), has demonstrated both preclinical and clinical antitumor activity as monotherapy. Its activity has been shown to be independent of resistance mechanisms such as p53 and p21 mutations, MDR or MRP expression, and bcl-2

over-expression. Irofulven has demonstrated efficacy including partial and complete responses against the MV522 lung carcinoma xenograft model, an aggressive metastatic tumor that is refractory to treatment with standard chemotherapeutic agents. In this study, irofulven and gemcitabine (a pyrimidine antimetabolite) were combined to elucidate the efficacy of these two agents against MV522 lung carcinoma *in vitro* and *in vivo*. As determined by median-effect principle analyses, additive and synergistic antitumor activity was observed *in vitro* when MV522 cells were treated for 48 hours concurrently with combinations of irofulven (ranging from 25 to 250 ng/ml) and gemcitabine (ranging from 0.5 to 8 ng/ml). Female athymic nude mice implanted subcutaneously with MV522 tumors were treated with irofulven and/or gemcitabine on an intermittent dosing schedule (q3dx4). Mice administered 20 mg/kg/d of gemcitabine demonstrated 2 partial responses (PR) (mean tumor shrinkage of 69%), whereas 40 mg/kg/d gemcitabine produced 2 PR (mean tumor shrinkage of 42%). Irofulven given at 3 mg/kg/d demonstrated no PR or CR (mean tumor growth inhibition (TGI) of 43 %) and at 4.5 mg/kg/d doses displayed a mean TGI of 48% with 1 CR. In contrast to the limited activity produced by this sub-MTD dosing as monotherapy, marked antitumor activity was observed when the two agents were combined. Irofulven administered at doses of 3 mg/kg/d combined with 40 mg/kg/d gemcitabine produced 6 PR (mean tumor shrinkage 57%) and 1 CR. Furthermore, administration of 4.5 mg/kg doses of irofulven plus either 20 mg/kg or 40 mg/kg gemcitabine demonstrated 3 PR (mean tumor shrinkage 67%) and 6 CR, or 2 PR (mean tumor shrinkage 79%) and 8 CR, respectively. Minimal body weight loss (maximum -6.3%) demonstrated the limited toxicity of this combination. In summary, the combination of irofulven and gemcitabine produces greater than additive antitumor activity. This combination is currently being evaluated in a Phase I clinical trial.

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Characterisation of the roles of Topoisomerase I and II in the mechanism of action of novel anti-tumour agents XR11576 (MLN576) and XR5944 (MLN944)

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Topoisomerase I and II (Topo I and Topo II) are the targets of many antitumour agents currently used in the clinic. Their main mechanism of cytotoxicity involves stabilising an otherwise reversible DNA-topoisomerase covalent complex (cleavable complex). Collisions of DNA tracking proteins convert these complexes into permanent single or double strand breaks thus triggering cell death. XR11576 (MLN576) and XR5944 (MLN944) are two novel DNA targeting agents with potent activity against a panel of human tumour cell lines and human tumour xenografts in mice. Both compounds retain good activity in cell lines expressing either P-gp or MRP multidrug resistance *In vitro* cleavage data shows XR11576 and XR5944 stabilise cleavable complexes for both Topo I and Topo II in a dose dependent fashion. The agents are also able to overcome 'atypical' resistance due to down-regulation of Topo II alpha. These data therefore suggest that XR11576 and XR5944 can act as 'dual' inhibitors of both Topo I and Topo II. Cleavable complex formation by XR11576 and XR5944 has been analysed in human leukaemic K562 cells using the TARDIS assay. Data obtained showed that both drugs induced cleavable complex formation for both Topo I and Topo II (alpha and beta) in a dose and time dependent manner. The levels of XR11576 and XR5944 induced cleavable complexes were significantly higher than untreated controls. Interestingly, the cleavable complex formation was detectable only after a minimum of 24 hours of drug exposure and, at present, the reason for the marked time dependency remains unclear. XTT growth inhibition assays were performed at comparable exposure times to the TARDIS assay to correlate cytotoxicity with cleavable complex formation. Future studies will further investigate the role of Topo I and Topo II in XR11576 and XR5944 induced cell death.

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Release of GST-pi promoter hypermethylation and activity of brostallicin in human prostate cancer cells

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The a-bromoacrylic DNA minor groove binder brostallicin (PNU-166196), is a new promising anticancer agent which has shown an outstanding preclinical activity profile and is currently in Phase II clinical evaluation. Differently from several antineoplastic drugs, the activity of brostallicin is increased, either *in vitro* or *in vivo*, in cells expressing high levels of glutathione (GSH) and glutathione S-transferases (GST). Among the isoenzymes, GST-pi is the stronger activator of brostallicin efficacy. It has been reported that in a high percentage of human prostate cancers, the levels of GST-pi are negligible because of hypermethylation of the promoter region of GST-pi gene. Evidences have been provided that the treatment of prostate cancer cells with DNA methyltransferase inhibitors resulted in a demethylation and activation of the GST-pi gene and, consequently, the intracellular level and activity of the GST-pi protein increases. The cytotoxic activity of brostallicin has been tested against the non-GST-pi-expressing human prostate cancer cell line (LNCaP) where the GST-pi promoter is completely methylated. Brostallicin is five times less cytotoxic on LNCaP cells compared with the GST-pi-expressing (with methylated promoter) Du145 human prostate cancer cells (IC_{50} 200 and 38 ng/ml, respectively). Aim of this work was to verify *in vitro* whether treatment with agents releasing the expression of GST-pi such as 5'aza-2'deoxy cytidine, procainamide or HDAC inhibitors could activate the expression of GST-pi in LNCaP cells and, consequently, increase the antitumor activity of brostallicin. Our results indicate that pre-treatment with procanaimide (7 days pretreatment, a scheme previously reported to induce hypomethylation of GST-pi promoter) results in an increased cytotoxicity of brostallicin compared to untreated LNCaP cells. These data indicate that the association of brostallicin with hypomethylating agents could be synergistic in prostate cancer cells

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Analysis of *in vitro* and *in vivo* activity of a newly synthesized psorospermin analog

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Psorospermin (Ps) is a natural product isolated from the African plant Psorospermum febrifugum. This compound was previously shown to be active against drug-resistant leukemia lines and AIDS-related lymphoma (Cassady, J.M., et al. JNCI, Vol #53, 23-41). Previous studies have also shown that Ps alkylates the N7 position of guanine weakly, but in the presence of Topo II, alkylation is greatly enhanced at specific sequences determined by Topo II (Kwok, Y., et al., PNAS, Vol #95, 13531-13536). Because of this distinctive mechanism of action and activity profile in the NCI Compare program, we synthesized the diastereomeric pairs of O5-methyl-(±)-(2'R,3'R)-Ps (PsMeO). The diastereomeric pair enriched in the active compound showed *in vitro* activity in AML (IC_{50} = 0.3 μM), CML (0.4 μM), multiple myeloma (0.3 μM), pancreatic (0.6μM), breast (2 μM), and ovarian cancers (0.7 μM). Cytotoxicity studies using matched pairs of multidrug resistant and wild-type tumor cell lines showed the resistant cells were considerably more sensitive to PsMeO than the selecting agent (doxorubicin, mitoxantrone). Cell cycle studies show a reduced number of cells in G1 and G2 and a concurrent increase of cells in S phase, suggesting that PsMeO accelerates G1/S entry and/or delays cell exit from S to G2/M stages. Apoptosis was also increased 6-12 hours post treatment. To determine whether the drug effects we and others have observed *in vitro* can be achieved following the systemic administration *in vivo*, we examined the antitumor activity of PsMeO in severe combined immune deficient (SCID) mice bearing established human pancreatic cancer MiaPaCa-2 xenografts. There was a considerable decrease in tumor volume in mice treated with PsMeO compared to untreated controls. These results, together with the Topo II alkylation specificity and activity in several tumor types, demonstrate PsMeO to have a unique mechanism of action that distinguishes it from current chemotherapeutic agents.

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Design and biological evaluation of new fluoroquinolones with a dual mechanism of action against topoisomerase II and G-quadruplex

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We have designed and synthesized four new fluoroquinoanthroxazines (FQAs) in order to investigate the combined effect of single compounds having dual mechanisms of action; i.e., G-quadruplex-interactive compounds with topoisomerase II poisons. We have extended the aromatic system of A62176 by introduced a naphthyl group at the cis(C) or trans (T) depending on the orientation of the extension. Also the chirality at the 3-C aminopyrrolidine carbon in these compounds yields two enantiomers, S- and R-, and therefore four novel stereoisomeric FQAs were prepared: FQA-CS, FQA-