an acetonitrile/ ammonium formate gradient with a reversed-phase phenyl column and fluorescence detection, a limit of detection for SJG-136 of 1 nM in serum has been achieved. Extraction efficiencies from serum were >60% across a range of concentrations (1-100 nM). All *in vivo* studies have been approved by the UK Home Office. In pilot pharmacokinetic studies where SJG-136 was administered i.p. to NMRI mice at the MTD of 0.2 mg/kg, the drug could be observed at detectible levels with a Cmax of 336 nM after 30 min in mouse plasma. A calculated terminal t1/2 of 0.98 h and an AUC of 0.34 uM h resulted in a clearance of 17.72 ml / min kg. Preliminary plasma protein-binding studies demonstrate that the agent is poorly bound to proteins (≤20 %), suggesting that SJG-136 is readily bioavailable in the blood with peak plasma concentrations substantially higher than those needed for *in vitro* cytotoxicity. Studies are currently in progress to establish the levels of SJG-136 that can be achieved in tumours.

**79**

## Brostallicin potentates the antitumor activity of other cytotoxic antineoplastic agents in experimental tumor models

C. Geroni[1], T. Colombo[2], S. Marchini[2], M.A. Sabatino[2], C. Fowst[1], J. Tursi[1], M. Broggini[2]. [1]*Pharmacia Corporation Oncology, External Research, Nerviano (Milan), Italy;* [2]*Istituto "Mario Negri", Molecular Pharmacology, Milan, Italy*

Brostallicin (PNU-166196) is a synthetic a-bromoacrylic, second generation DNA minor groove binder, currently in Phase II clinical evaluation. Unlike other cytotoxics, its antitumor activity is increased both in the presence of high levels of glutathione (GSH) and glutathione S-transferases (GST) and the GSH/GST system is involved in its mechanism of DNA interaction (Geroni C. Cancer Res.; 62:2332, 2002). Moreover, brostallicin is fully active against DNA-mismatch repair deficient tumor cells and circumvents resistance to akylating agents and camptothecins. Multiple combinations of brostallicin with compounds belonging to major classes of antitumor agents have been studied on the basis of brostallicin's newly determined mode of action and ability to overcome drug-resistance. In nude mice bearing the human colon carcinoma HCT-116 model, the sequential combination of cisplatin (day1) and brostallicin (day3) yields a delay in tumor growth (14 days) that is significantly superior (p=0.02) to the best delays caused by either drug alone (2 and 3 days, respectively). In terms of toxicity, the maximum tolerated dose of each agent could be administered without additional toxicity. Synergism with doxorubicin (DX) is observed on a murine leukemia model (L1210) when DX treatment is given 24h before brostallicin. Both brostallicin and DX administered as a single agent shows 33% increase in life span (ILS); conversely, in combination the antitumor activity is significantly higher (100%ILS). An increase in toxicity is observed when DX and brostallicin are administered simultaneously. Supraadditive antitumor effect is shown when brostallicin is tested in combination (simultaneous, single i.v. treatment) with gemcitabine on L1210 leukemia (58,50,117 %ILS for brostallicin and gemcitabine alone and in combination, respectively). The antitumor activity of simultaneous administration of brostallicin and taxotere has been tested on human NSCLC xenograft model (A549). Clear additivity is shown, both in terms of % of tumor regression and tumor growth delay at all tested doses, without any additive toxicity. Further combination studies are ongoing. Although the precise mechanism of interaction has not yet been identified, a clear therapeutic gain is observed in preclinical models when brostallicin is combined with other anticancer agents. These results indicate the value of brostallicin in cancer combination treatment protocols.

**80**

## Structure-activity relationships of oxalatoplatinum(II) complexes: identification of oxaliplatin derivatives with improved activity

M. Galanski[1], S. Slaby[1], A. Yasemi[1], M.A. Jakupec[1], B.K. Keppler[1]. [1]*Inst. of Inorganic Chemistry, Vienna University, Wien, Austria;* [2]*Faustus Forschungs Compagnie Translational Cancer R, Leipzig, Germany*

Oxaliplatin, (trans-R,R-1,2-diaminocyclohexane)oxalatoplatinum(II), has been the first representative of the extensively studied class of diaminocyclohexane(DACH)-containing platinum complexes to become established in clinical practice. This class of compounds is known to display activity profiles different from those of cis/carboplatin, which has been confirmed by the clinical activity of oxaliplatin in colorectal cancer. Structure-activity relationships are well-explored with respect to the leaving group and the stereoisomers of DACH. However, despite the key role of the stable amine ligand for the altered activity profile no attempts have been made to improve the pharmacological properties by structural modifications of DACH so far. Ligands derived from DACH by stepwise substitution of cyclohexane have been used to prepare new oxalatoplatinum(II) derivatives in order to define the structural requirements essential for the oxaliplatin-like activity and to explore possibilities of improving this activity. Results obtained from cytotoxicity assays in human colon (SW480) and ovarian (CH1) cancer cell lines demonstrate that increasing the steric demand by introduction of substituents to cyclohexane is a promising strategy to this end. Racemic mixtures of (trans-1,2-diamino-4-alkylcyclohexane)oxalatoplatinum(II) (alkyl = methyl, ethyl) show equivalent to slightly higher potency compared to the enantiomerically pure oxaliplatin. Since trans-R,R-1,2-DACH-containing platinum complexes generally display a superior activity compared to their trans-S,S-1,2-DACH-containing congeners, we expect activity to be further improved by use of the more active enantiomer. Evaluation of the pure enantiomers and of further derivatives *in vitro* and *in vivo* will be presented.

**81**

## Growth arrest, apoptosis and potentiation of 5-fluorouracil and Raltitrexed cytotoxic effect induced by histone deacetylase inhibitor SAHA in colorectal cancer cells

A. Budillon[1], E. Di Gennaro[1], S. Pepe[2], F. Bruzzese[1], G. Romano[1], I. Pelagianis[2], P. Mascagni[3], F. Leoni[3]. [1]*Istituto Nazionale Tumori Fondazione G. Pascale, Experimental Oncology, Napoli, Italy;* [2]*Università Federico II, Endocrinology and Molecular and Clinical Oncology, Napoli, Italy;* [3]*Italfarmaco, SpA, Research Centre, Cinisello Balsamo, Italy*

Histone deacetylase (HDAC) inhibitors have been recently shown to induce growth arrest and apoptosis, in a variety of human cancer cells by mechanism that cannot be solely attributed to the level of histone acetylation. Suberoylanilide hydroxamic acid (SAHA), an orally active HDAC inhibitor, has shown promising preclinical effect in human cancer cells and phase I clinical studies have been recently completed. To determine if SAHA has potential clinical applications as antitumor agent for patients with colorectal cancer we analyzed the effect of SAHA on growth, apoptosis and cell cycle regulation in four human colorectal cancer cells. SAHA induced growth inhibition in a time and dose-dependent manner in all cells with $IC_{50}$ values ranging from 0.5uM to 10 uM independently of p53 status. Cell cycle analysis revealed an increased percentage of cells in G1 after 24 h and up to 72 h. Moreover SAHA induced time and dose-dependent apoptotic cell death beginning after 24 h of incubation. To investigate the mechanism of SAHA induced growth arrest and cell cycle perturbation we examined the expression of p27 and p21 cyclin-dependent kinase inhibitors in untreated and treated mut-p53 HT29 and wt-p53 LoVo colon cancer cells. In both cell lines SAHA induced time dependent upregulation of both of p27 and p21, beginning after 12 h of treatment with a peak between 24 and 48 h. Interestingly, SAHA treatment led to reduced expression of mut-p53 in HT29 and to upregulation of wt-p53 in LOVO cells. In addition protein expression of Thymidilate Synthase, a critical target for chemotherapeutic agents active in colorectal cancer such as 5-fluorouracil (5FU) and Raltitrexed, was downmodulated by SAHA treatment. On the basis of this observations, we have investigated if the combination of SAHA and Raltitrexed or 5FU enhanced cell growth inhibition compared to single drug schedule in HT29 and LoVo cell lines.Preliminary results show that simultaneous exposure to SAHA and either Raltitrexed or 5FU produced a supra-additive to additive antiproliferative effect, as demonstrated by median drug effect analysis calculating a combination index. Overall these results demonstrated that SAHA has antiproliferative and proapototic activity in human colorectal cancer derived cells. Moreover, SAHA can be combined with cytotoxic drugs currently used for colorectal cancer treatment and it should be further investigated for therapeutic use in patients with this malignancy.

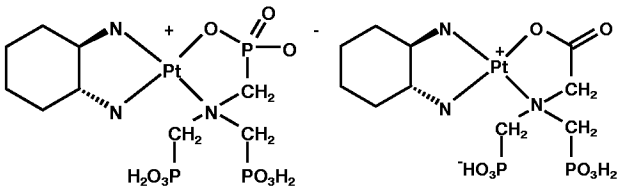
**82**

## Structure-activity relationships of platinum(II) phosphonate compounds for the treatment of bone malignancies

M.A. Jakupec, M. Galanski, S. Slaby, B.K. Keppler. *Inst. of Inorganic Chemistry, Vienna University, Wien, Austria*

In order to produce platinum complexes with selective activity in primary and secondary bone tumors aminobis/trismethylenephosphonates with high affinity for the mineral bone matrix have been used as ligands for platinum(II). Previously, accumulation in bone tissue has been confirmed by autoradiography and therapeutic activity superior to cisplatin has been found in an orthotopically transplanted rat osteosarcoma model which disseminates to the lung producing lethal osteoid-forming metastases. Current attempts to optimize the pharmacological ef-

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.



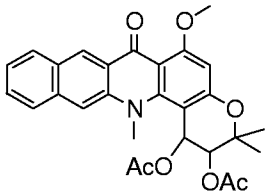
Thus, in both series the complexes of trans-R,R-DACH (Fig. 1) prove to be superior to those of other isomers of DACH and those of ethanediamine, which is consistent with published findings for oxaliplatin and other DACH-containing platinum compounds. As complexes of this latter type usually exhibit low 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.

## 83

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

M.-H. David-Cordonnier[1], A. Joubert[1], W. Laine[1], S. Léonce[2], A. Pierré[2], J.A. Hickman[2], C. Bailly[1]. *[1]Institute for Cancer Research, INSERM U-524, Lille, France; [2]Institut de Recherches Servier, Cancer Division, Suresnes, France*

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.

## 84

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

A. Pompella[1], A. Paolicchi[1], M. Franzini[1], E. Lorenzini[1], M. Sotiropoulou[2], N. Romiti[1], E. Chieli[2], F. Zunino[3]. *[1]University Of Pisa, Experimantal Pathology, Pisa, Italy; [2]University Of Ioannina, Dept. Of Biochemistry, Ioannina, Greece; [3]Istituto Nazionale Tumori, Div. Di Oncologia Sperimentale, Milan, Italy*

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.

## 85

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

E.S. Van Laar[1], M.J. Kelner[2], T. Woldetsadik[3], B. Hollister[3], J.R. MacDonald[1], S.J. Waters[1]. *[1]MGI Pharma, Inc, R & D; [2]University of California, Department of Pathology, San Diego, USA; [3]Piedmont Research Center, Inc, Morrisville, USA*

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