Potential New Antitumor Agents from an Innovative Combination of Demethylcantharidin, a Modified Traditional Chinese Medicine, with a Platinum Moiety

Yee-Ping Ho,*,† Kenneth K. W. To,†
Steve C. F. Au-Yeung,*,‡ Xinning Wang,†,‡
Ge Lin,§ and Xiuwen Han||

School of Pharmacy and Department of Pharmacology, Faculty of Medicine, and Department of Chemistry, Faculty of Science, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region, and The State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, The People's Republic of China, 116023

Received November 7, 2000

Abstract: A combination of demethylcantharidin, a modified component of a traditional Chinese medicine (TCM), with a platinum moiety has produced a series of TCM-based platinum compounds $[Pt(C_8H_8O_5)(NH_2R)_2]$ **1**−**5**, which demonstrate selective cytotoxicity toward SK-Hep-1 (human liver) cell line, and circumvention of cross-resistance. The inclusion of demethylcantharidin rendered the compounds highly active as protein phosphatase (PP2A) inhibitors. The new TCM-Pt compounds may possess a novel dual mechanism of antitumor action: inhibition of PP2A and platination of DNA.

Traditional Chinese medicine (TCM) has been practiced by Chinese communities worldwide for many generations, and there is a wealth of literature information available related to the therapeutic use of TCM. In recent years, there has been a global surge in the popularity of herbal/traditional medicine, and there is enormous interest in developing new pharmaceutical products from such resources.

In this study, our objective was to exploit the benefits of TCM in designing a new chemical entity (NCE) to create a therapeutic agent with specific biological activity. A potential candidate identified was cantharidin, which is the active principle of *Epicanta gorhami* or *Mylabris* ("blister beetles"), which has long been used as a TCM for the treatment of liver, lung, intestinal, and digestive tract tumors. Unfortunately, cantharidin has severe side effects such as dysphagia, hematemesis, and dysuria.[1] Earlier studies reported that cantharidin and its derivatives have strong affinity and specificity for a "cantharidin-binding" protein, which has been isolated and identified as protein phosphatase 2A (PP2A).[2] It is also known that the liver cytosol is one site that is rich in PP2A,[3] and from in vitro experiments, the level of PP2A inhibition parallels cytotoxicity.[4]

Demethylcantharidin (norcantharidin) is a synthetic analogue of cantharidin and has potent antitumor activity but without the latter's adverse effects.[1] Demethylcantharidin was therefore chosen as *the* principal component in our design of a NCE which, based on literature findings, should demonstrate potential antitumor properties and, more significantly, selectivity toward liver cancer. A difficulty remained in identifying an appropriate vehicle to ensure the delivery of the TCM to cellular material. A possible solution would be to take advantage of the established antitumor drug, cisplatin, and its chemistry: that is, to integrate the structures of demethylcantharidin in the form of a *ligand* together with cisplatin into a single NCE. Because of the known mechanism of cytotoxic action of cisplatin, this new TCM-platinum (Pt) complex is hypothesized to have a dual mechanism of cytotoxic action, i.e., inhibition of PP2A by demethylcantharidin (and/or its hydrolyzed diacid form) and DNA binding by Pt.

Since its discovery as an antitumor agent, cisplatin *cis*-$[PtCl_2(NH_3)_2]$ has revolutionized cancer chemotherapy[5] and is now widely used in the treatment of testicular, ovarian, bladder, and head/neck tumors.[6] However, there are several major disadvantages which include low aqueous solubility, severe nephrotoxicity,[7] and drug resistance.[8] These limitations lead to the development of new Pt analogues which displayed improved therapeutic properties.[9] One such analogue is the less toxic carboplatin $[Pt(C_6H_6O_4)(NH_3)_2]$, which is routinely used as an antitumor drug, but the problem of cross-resistance remains.[10] Another significant analogue is oxaliplatin, which contains a 1,2-diaminocyclohexane (DACH) carrier ligand and demonstrates antitumor activity in cell lines with acquired cisplatin resistance.[11]

With our drug design philosophy, we strived to develop new TCM-based Pt compounds that are distinct from the classical cisplatin and its existing analogues, which operate under a different mechanism. The new compounds should have (i) acceptable selectivity, (ii) no cross-resistance, (iii) high potency, (iv) minimal side effects, and (v) good aqueous solubility.

This paper describes the results of an innovative approach to the design of a new series of NCEs based on an active chemical component from a traditional Chinese medicine (TCM) with known therapeutic value but purposely integrates into it a Pt moiety as a vehicle. We report on their in vitro biological activity that includes high potency toward human breast and liver cancers, which are normally unresponsive to cisplatin, and, more significantly, an inference of a novel mechanism of antitumor activity which may also explain why there is no demonstration of cross-resistance.

Demethylcantharidin was readily prepared by a first-step Diels−Alder reaction between furan and maleic anhydride, followed by Pd−C catalyzed hydrogenation. Novel compounds $[Pt(C_8H_8O_5)(NH_2R)_2]$ (**1**−**5**) were synthesized[12] by reacting demethylcantharidin with a series of $(NH_2R)_2Pt(NO_3)_2$, which was prepared from treatment of K_2PtCl_4 with potassium iodide and appropriate primary amines, followed by reaction with silver nitrate (Scheme 1). The complexes were characterized by infrared, ^1H and ^{13}C NMR, and mass spectroscopy and elemental analysis. Growth-inhibitory activity (IC_{50}) of

* To whom correspondence should be addressed. Tel: (852)-2609-6831. Fax: (852)-2603-5295. E-mail: yeepingho@cuhk.edu.hk.
† School of Pharmacy, The Chinese University of Hong Kong.
‡ Department of Chemistry, The Chinese University of Hong Kong.
§ Department of Pharmacology, The Chinese University of Hong Kong.
|| Dalian Institute of Chemical Physics, Chinese Academy of Sciences.

10.1021/jm000476t CCC: $20.00
Published on Web 05/18/2001

Table 1. Growth-Inhibitory Activity ($IC_{50}/\mu M \pm$ sd; $n = 12-16$ from Three to Four Independent Experiments) of Complexes **1**−**5** in Selected Cancer Cell Lines

cell line[a]	cisplatin	carboplatin	**1**	**2**	**3**	**4**	**5**
L1210	0.51 ± 0.11	10.14 ± 3.71	2.71 ± 0.40	12.08 ± 2.35	6.19 ± 2.67	5.92 ± 1.94	0.12 ± 0.05
COLO-320-DM	11.90 ± 1.50	188.03 ± 35.75	37.25 ± 7.14	84.68 ± 9.68	48.92 ± 10.51	48.46 ± 9.95	10.10 ± 4.38
SK-Hep1	54.15 ± 9.81	500.56 ± 86.57	20.59 ± 5.89	17.18 ± 8.07	15.44 ± 6.04	19.99 ± 5.52	2.97 ± 0.45
MDA-MB-231	65.74 ± 4.39	1082.66 ± 156.81	63.80 ± 8.28	94.45 ± 9.61	49.43 ± 9.42	62.53 ± 13.32	36.53 ± 13.81
NCI:H460	11.32 ± 4.93	60.26 ± 16.37	21.14 ± 10.65	26.46 ± 8.09	3.44 ± 1.98	3.91 ± 2.65	3.14 ± 0.25
SK-OV-3	15.16 ± 11.75	148.73 ± 34.13	40.75 ± 14.27	77.65 ± 19.81	37.19 ± 13.39	62.61 ± 33.51	41.93 ± 14.96
NTERA-S-cl-D1	0.19 ± 0.10	2.75 ± 1.13	0.36 ± 0.25	1.44 ± 0.41	0.84 ± 0.20	0.60 ± 0.24	0.15 ± 0.09

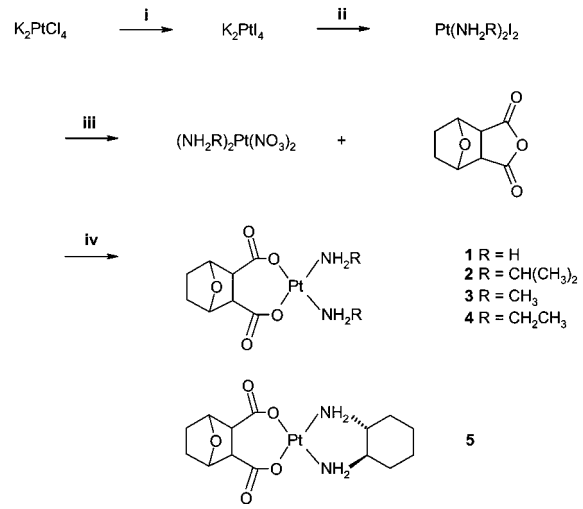
[a] L1210 mouse leukemia; COLO-320-DM human colon; SK-Hep-1 human liver; MDA-MB-231 human breast; NCI:H460 human lung; SK-OV-3 human ovarian; NTERA-Scl-D1 human testicular cancer.

Table 2. Cytotoxicity[a] of Complexes **1**−**5** toward Cisplatin-Resistant L1210 Mouse Leukemia and NCI:H460 Human Nonsmall Cell Lung Cancer ($IC_{50}/\mu M \pm$ sd; $n = 12-16$)

	cisplatin	carboplatin	**1**	**2**	**3**	**4**	**5**
L1210-sensitive	1.01 ± 0.04	17.78 ± 2.05	5.97 ± 0.44	11.54 ± 2.71	12.66 ± 2.79	12.24 ± 1.85	0.12 ± 0.05
L1210-resistant[b]	14.49 ± 0.72 (14)	138.0 ± 13.73 (7.8)	6.01 ± 1.05 (NA)	12.96 ± 2.76 (NA)	12.57 ± 0.54 (NA)	13.05 ± 1.22 (NA)	0.12 ± 0.04 (NA)
NCI:H460-sensitive	0.59 ± 0.02	17.78 ± 2.56	2.88 ± 0.43	11.76 ± 1.23	12.59 ± 0.76	9.43 ± 0.85	1.23 ± 0.04
NCI:H460-resistant[b]	4.96 ± 0.52 (8.30)	54.60 ± 4.96 (3.07)	10.52 ± 0.58 (3.65)	20.41 ± 0.96 (1.74)	23.29 ± 1.56 (1.85)	16.69 ± 1.20 (1.77)	1.40 ± 0.05 (1.14)

[a] Cytotoxicity assessed by the MTT assay after 72 h of drug exposure for L1210 and 96 h of drug exposure for NCI:H460 cell lines. [b] Resistance level (*n*-fold) in parentheses. The fold resistance equals the IC_{50} of the resistant cells divided by the IC_{50} of parental cells for various drugs.

Scheme 1[a]



[a] Reagents: (i) KI; (ii) RNH_2 (**1**−**4**), *trans*-$C_6H_{10}(NH_2)_2$ (**5**); (iii) $AgNO_3$; (iv) NaOH.

these compounds against murine L1210 leukemia and a range of human cancer cell lines was determined using the standard tetrazolium (MTT) assay[13] where drug treatment was started 24 h after cells were seeded and the duration of drug exposure was 72 h, after which the MTT test was performed (Table 1). Cross-resistance of the compounds toward cisplatin was examined using L1210 and nonsmall cell lung cancer NCI:H460 cell lines (Table 2). In vivo toxicity assessments (LD_{50}) were performed with ICR mice, and all five compounds were considerably less toxic than cisplatin. A preliminary assessment of the in vivo antitumor activity of the novel complexes, using a human liver tumor xenograft, was performed in male nude mice according to standard procedures[14] whereby the drugs were administered once the inoculated tumor size reached 5 mm × 5 mm (Table 3). In the in vivo study, the choice of vehicle for drug administration was made in order to maximize the drug's stability; that is, the vehicle for cisplatin was normal saline, and for all other Pt complexes, dextrose (5%) was used. Control xenografts were treated with either normal saline or 5% dextrose, and no differences were observed. Cisplatin and carboplatin were used as standards for all in vitro and in vivo evaluations.

The integration of demethylcantharidin into these TCM-Pt complexes appears to play an important and significant role in overcoming Pt resistance. Using a model of the cisplatin-resistant L1210 cell line, we found that complexes **1**−**5** were devoid of cross-resistance, whereas the subline demonstrated a 14-fold resistance to cisplatin (Table 2). Resistance in tumor cells to platinum anticancer drugs tends to arise from one or more of the following mechanisms: (i) reduced total cellular platinum accumulation;[15] (ii) increased cytoplasmic detoxification by glutathione GSH and/or metallothioneins;[16] and (iii) increased DNA repair and/or tolerance of platinum-DNA adducts.[17] Current evidence points to a multifactorial mode of resistance, because each of the above mechanisms has been demonstrated in one or more cell lines. This phenomenon of multiple mechanisms of resistance may be due to the fact that the resistant cell lines are established by continuous stepwise exposure to cisplatin over prolonged periods of time. In our cisplatin-resistant L1210 model, all of the above-mentioned three mechanisms have been found to contribute to the acquired resistance.

Since it is a relatively common observation with several other platinum analogues that they are able to retain cytotoxic activity against an acquired cisplatin-resistant subline of mouse L1210 leukemia, the TCM-Pt compounds were also tested against a human nonsmall cell (NSC) lung cancer cell line NCI:H460 where cisplatin resistance has been acquired through intermittent drug exposure.[18] The use of this NCI:H460 cisplatin-resistant subline may clinically represent a more relevant mechanism of cisplatin resistance. Complexes **1**−**5** were found, on the whole, to be devoid of cross-resistance toward the NSC lung cancer cell line, whereas the subline demonstrated an 8-fold resistance to cisplatin (Table 2). In this study, we have established that

Table 3. Preliminary Results for the in Vivo Antitumor Activity of Complexes **1**−**5** in SK-Hep-1 sc-Inoculated Xenograft in Male Nude Mice

compd	treatment schedule	dose (mg/kg per dose)	% wt change at nadir	*T/C* (%)[a]	*N* (no. of deaths)
cisplatin	ip D 1, 5, 9	2	NA	87.5	1 (0)
		4	−25	81.1	4 (0)
		8	−35	47.1	4 (1)
		10	−40	39.6	4 (2)
carboplatin	ip D 1, 5, 9	25	NA	87.5	1 (0)
		50	NA	108.7	2 (0)
		75	NA	44.9	2 (0)
		100	−21	67.8	5 (0)
		120	−40	62.8	4 (2)
1	ip D 1, 5, 9	25	NA	46.3	1 (0)
		50	NA	44.9	3 (0)
		75	−14	44.4	4 (0)
		90	−20	17.4	3 (1)
		100	−30	7.5	2 (1)
2	ip D 1, 5, 9	25	NA	42.7	1 (0)
		50	NA	50.0	2 (0)
		75	NA	15.9	1 (0)
		100	NA	15.9	2 (0)
		120	NA	14	3 (0)
		140	NA	8	2 (1)
3	ip D 1, 5, 9	25	NA	66.8	1 (0)
		50	NA	51.4	2 (0)
		75	NA	97.7	1 (0)
		100	NA	32.6	2 (0)
		120	NA	31.2	3 (0)
		140	NA	28.9	2 (0)
		160	NA	22.6	2 (0)
4	ip D 1, 5, 9	25	NA	52.6	1 (0)
		50	NA	60.2	2 (0)
		75	NA	30.9	1 (0)
		100	NA	61.6	2 (0)
		120	NA	69.9	1 (0)
		140	NA	32.6	2 (0)
		160	NA	12.3	2 (0)
5	ip D 1, 5, 9	12.5	NA	45.9	1 (0)
		25	NA	31.2	3 (0)
		50	NA	14.6	4 (0)
demethyl-cantharidin	ip D 1, 5, 9	2	NA	63.2	1 (0)
		4	NA	66.1	4 (0)
		8	−20	34.8	5 (1)
		10	acutely toxic	ND	3 (2)

[a] The percentage *T/C* is the ratio of the relative tumor volume in the Tx group over the control group at *day 60* after drug Tx (deaths are excluded from this calculation).

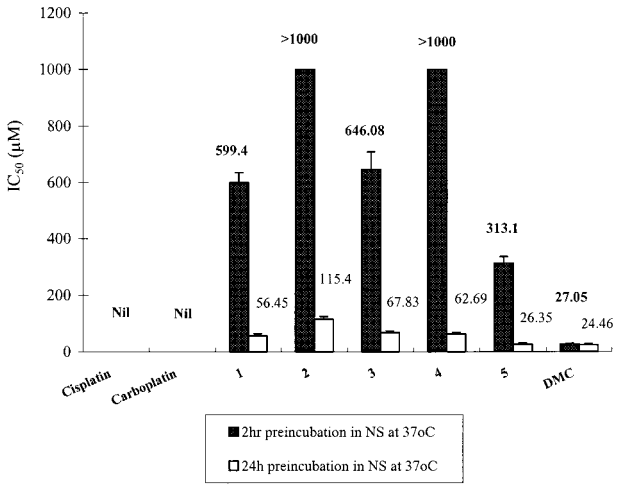


Figure 1. Protein phosphatase (PP2A) inhibitory activity of complexes **1**−**5** and demethylcantharidin ($IC_{50}/\mu M$). The inhibition of purified PP2A (Upstate Biotechnology) is reported at a concentration of 0.1 units per reaction. Demethylcantharidin (DMC) was used as control. The PP2A inhibitory effect remained unchanged with incubation time. Results represent the mean of three independent experiments.

at least two mechanisms appear to contribute to the acquired cisplatin resistance phenotype in this lung cancer cell line, namely a decreased total cellular platinum accumulation[15] and 2-fold increases for glutathione (GSH)[19] and glutathione S-transferease (GST)[20] levels. Pretreatment of our resistant cells with buthionine sulfoximine (BSO), an inhibitor of GST, showed partial reversal of cisplatin resistance as demonstrated by a dose-modifying factor (DMF) of 2.78 in the resistant NCI:H460 cell line, which further confirmed the role of an increased GSH content in cisplatin resistance. Therefore, the lack of cross-resistance of compounds **1**−**5** implies that their mechanism of action is most likely to be different from cisplatin.

Recent literature findings suggest that protein phosphatase inhibitors (PPI) can usurp the nucleotide excision repair (NER) mechanism that is responsible for DNA repair in Pt resistance, where the removal of Pt-adduct from damaged DNA is inhibited by PPIs.[21] Cantharidin and its derivatives have been reported as potent PPIs[22] and, indeed, our in vitro experiments have demonstrated compellingly that complexes **1**−**5**, after incubation in normal saline (NS) for 2 and 24 h, *do* possess substantial protein phosphatase (PP2A) inhibitory activity, which is in sharp contrast to cisplatin and carboplatin showing none (Figure 1). In this experiment, demethylcantharidin was used as the control and its PP2A inhibitory activity was unchanged with incubation time. This implies that as the demethylcantharidin ligand is released (as a diacid) from the TCM-Pt compounds over 24 h, the IC_{50} of **1**−**5** for inhibition of PP2A approached that of demethylcantharidin. The release of the diacid from compounds **1**−**5** over 24 h has been confirmed using a newly developed gas chromatographic (GC) method with flame ionization detection (FID). Demethylcantharidin in aqueous sodium hydroxide immediately hydrolyzes to the corresponding diacid, which is readily detected at time zero using our GC-FID method. Compounds **1**−**5** released demethylcantharidin progressively over a 24 h period, attaining a concentration of the ligand almost equivalent to the control.

Protein phosphatases are involved in the dephosphorylation of serine and threonine residues of cellular phosphoproteins, which control a multitude of diverse cellular events, including DNA replication. By inhibiting PP2A activity, demethylcantharidin may regulate the cell cycle at two restriction points (G1/S and G2/M phase transitions) and arrest DNA replication.[23] Hence, in addition to the observed apparent circumvention of Pt resistance, the experimental results also strongly suggest that these TCM-Pt complexes might exert a dual mechanism of antitumor action by (i) the release of the ligand demethylcantharidin, in the diacid form, which acts as a PPI and having its own cytotoxic effect; and (ii) platination of DNA, similar to the well-established alkylating action of cisplatin; which is further evidenced

by the higher potencies of the novel complexes in some of the cell lines tested (Table 1).

The TCM active principle employed is mainly used for treatment of liver and digestive tract tumors. Complexes **1**−**5** (IC_{50} range: 3.0−20.6 μM) are significantly more potent, by 18−2.5 times, than cisplatin (IC_{50}: 54 μM) and 140−25 times more potent than carboplatin (500 μM) against the SK-Hep-1 human liver cancer cell line, one which usually responds poorly to cisplatin. The design approach adopted in this study does appear to have introduced an important element of selectivity into the TCM-Pt compounds. The experimental data support emphatically our hypothesis for using demethylcantharidin, in the design of the NCEs, in attaining selectivity, a dual mechanism of antitumor action, lack of cross-resistance, higher potency, and lower levels of toxicity (LD_{50}).

Interestingly, these compounds freshly prepared in normal saline, do not exhibit any PP2A inhibition activity; thus suggesting that, in vivo, an activation step is most likely required in order to release the TCM component (demethylcantharidin), which would then contribute an additional cytotoxic effect. Preliminary assessment of the in vivo antitumor activity using a human liver tumor xenograft in nude mice shows that these compounds demonstrate tumor retardation and regression at nontoxic doses and with negligible weight loss. This is in stark contrast to cisplatin and carboplatin, where tumor regression is accomplished only at a dose close to its LD_{50} (cisplatin) and resulting in animal distress, significant weight loss (more than 20%), and drug-related premature deaths.

In conclusion, we have achieved a series of new TCM-Pt complexes, which structurally integrates a chemical component of TCM, that have undeniable potential to be further developed as effective antitumor agents. This study is the first to report a potential new dual mechanism of antitumor activity for TCM-Pt supported complexes, encompassing inhibition of PP2A and Pt platination of DNA; the former might also explain the observed circumvention of resistance. We are currently evaluating the in vivo properties and pharmacokinetics of these compounds and the TCM and conducting an in-depth investigation into the apparent dual mechanism of cytotoxic action.

Acknowledgment. We thank the Chinese University of Hong Kong (CUHK) for the provision of studentships (to K.T. and X.W.) and the Patent Committee of CUHK for financial support. The technical assistance of Dr. Bruce Whitney of the Hong Kong Cancer Institute, Prince of Wales Hospital, Shatin, Hong Kong, is also gratefully acknowledged.

Supporting Information Available: Experimental details, characterization, Pt accumulation, GSH and GST contents, cytotoxicity in the presence and absence of BSO, and GC-FID data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wang, G. S. Medical Uses of Mylabris in Ancient China and Recent Studies. *J. Ethnopharm*. **1989**, *26*, 147−162.
(2) Li, Y. M.; Casida, J. E. Cantharidin-binding protein: Identification as protein phosphatase 2A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11867−11870.
(3) Graziano, M. J.; Waterhouse, A. L.; Casida, J. E. Cantharidin poisoning associated with specific binding site in liver. *Biochem. Biophys. Res. Commun*. **1987**, *149*, 79−85.
(4) Graziano, M. J.; Pessah, I. N.; Matsuzawa, M.; Casida, J. E. Partial characterization of specific cantharidin binding sites in mouse tissues. *Mol. Pharmacol*. **1988**, *33*, 706−712.
(5) Rosenberg, B.; van Camp, L.; Thomas, K. Inhibition of Cell Division in *Escherichia coli* by Electrolysis Products from a Platinum Electrode. *Nature* **1965**, *205*, 698−699.
(6) Loehrer, P. J.; Einhorn, L. H. Drugs Five Years Later, Cisplatin. *Ann. Intern. Med*. **1984**, *100*, 704−713.
(7) Daley-Yates, P. T.; McBrien, D. C. H. Cisplatin Metabolites in Plasma, a Study of their Pharmacokinetics and Importance in the Nephrotoxicity and Antitumor Activity of Cisplatin. *Biochem. Pharmacol*. **1984**, *33*, 3063−70.
(8) Scanlon K. J.; Kashani-Sabet, M.; Tone T.; Funato, T. Cisplatin Resistance in Human Cancers. *Pharmacol. Ther*. **1991**, *52*, 385−406.
(9) Wong, E.; Giandomenico, C. M. Current Status of Platinum-Based Antitumor Drugs. *Chem. Rev*. **1999**, *99*, 2451−2466.
(10) Harrap, K. R. Preclinical Studies Identifying Carboplatin as a Viable Cisplatin Alternative. *Cancer Treat. Rev*. **1985**, *12*, 21−33.
(11) Tashiro, T.; Kawada, Y.; Sakurai, Y.; Kidani, Y. Antitumour Activity of a New Platinum Complex, Oxalato (*trans*-1-1,2-diaminocyclohexane) platinum(II): New Experimental Data. *Biomed. Pharmacother*. **1989**, *43*, 251−260.
(12) Hall, I. H.; Holshouser, M. H.; Loeffler, L. J. Effects of cis-Malonato-diammino Platinum (II) on P-388 Lymphocytic Leukemia Cell Metabolism. *J. Pharm. Sci*. **1980**, *69* (10), 1160−1163.
(13) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines using a Microculture Tetrazolium Assay. *Cancer Res*. **1988**, *48*, 589−601.
(14) Ovejera, A. A.; Houchens, D. P. Human Tumor Xenografts in Athymic Nude Mice as a Preclinical Screen for Anticancer Agents. *Semin. Oncol*. **1981**, *8*, 386−393.
(15) Bungo, M.; Fujiwara, Y.; Kasahara, K.; Nakagawa, K.; Ohe, Y.; Sasaki, Y.; Irino, S.; Saijo, N. Decreased Accumulation as a Mechanism of Resistance to Cis-diamminedichloroplatinum(II) in Human Nonsmall Cell Lung Cancer Cell Lines: Relation to DNA Damage and Repair. *Cancer Res*. **1990**, *50*, 2549−53.
(16) Meijer, C.; Mulder, N. H.; Hospers, G. A. P.; Uges, D. R. A.; de Vries, E. G. E. The Role of Glutathione in Resistance to Cisplatin in a Human Small Cell Lung Cancer Cell Line. *Br. J. Cancer* **1990**, *62*, 72−7.
(17) Eastman, A.; Schulte, N. Enhanced DNA Repair as a Mechanism of Resistance to Cis-diamminedichloroplatinum(II). *Biochemistry* **1988**, *27*, 4730−4.
(18) Lai, S. L.; Hwang, J.; Perng, R.-P.; Whang-Peng, J. Modulation of Cisplatin Resistance in Acquired-Resistance Nonsmall Cell Lung Cancer Cells. *Oncol. Res*. **1995**, *7*, 31−8.
(19) O'Dwyer, P. J.; Hamilton, T. C.; Young, R. C.; LaCreta, F. P.; Carp, N.; Tew, K. D.; Padavic, K.; Comis, R. L.; Ozols, R. F. Depletion of Glutathione in Normal and Malignant Human Cells in vivo by Buthionine Sulfoximine: Clinical and Biochemical Results. *J. Natl. Cancer Inst*. **1992**, *84*, 264−7.
(20) Habig, W. H.; Pabst M. J.; Jakoby, W. B. Glutathione-S-transferase. *J. Biol. Chem*. **1974**, *249*, 7130−9.
(21) Ariza, R. R.; Keyse, S. M.; Moggs, J. G.; Wood, R. D. Reversible Protein Phosphorylation Modulates Nucleotide Excision Repair of Damaged DNA by Human Cell Extracts. *Nucleic Acids Res*. **1996**, *24* (3), 433−40.
(22) Honkanen, R. E. Cantharidin, another natural toxin that inhibits the activity of serine/threonine protein phosphatases types 1 and 2A. *FEBS Lett*. **1993**, *330*, 283−286.
(23) Lin, X.-H.; Scheidtmann, W. J.; Ohst, K.; Newport, J.; Walter, G. Protein Phosphatase 2A is Required for the Initiation of Chromosomal DNA Replication. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14693−8.

JM000476T