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## Rational Design of Platinum(IV) Compounds to Overcome Glutathione-S-Transferase Mediated Drug Resistance

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Despite the success of *cisplatin* and its analogues as anticancer drugs used in 70% of all cancer treatments, drug resistance remains one of the most serious and challenging problems to overcome. Much progress had been made to understand the mechanisms involved in drug resistance, which are multifactorial processes.<sup>1</sup> One important enzyme responsible for drug resistance in some cancers, is glutathione-S-transferase (GST).<sup>2</sup> Cytosolic GST enzymes constitute the main cellular defense against xenobiotics, and they are known to catalyze the conjugation of glutathione (GS-H) with cisplatin in vitro,<sup>3</sup> the first step in the mercapturic acid pathway that leads to elimination of toxic compounds. Studies also found GST enzymes, specifically GST- $\pi$  isozymes, to be overexpressed in cisplatin-resistant cell lines,<sup>4</sup> and the inhibition of these enzymes has led to the reversal of drug resistance.<sup>5</sup>

A broad range of GST inhibitors, with varying degrees of isozyme specificity, are known, and some of them have been systemically tested in combination with a range of alkylating agents against multiple drug resistant (MDR) cancers as an adjuvant.<sup>6</sup> In particular, *ethacrynic acid* (EA), a diuretic in clinical use, has been extensively studied and found to effectively inhibit all GST isozymes, but to different extents.<sup>7</sup> Clinical trials involving EA in combination with *chlorambucil* and *thiotepa* against a range of cancers were of some success.<sup>8</sup>

Satraplatin is a promising Pt(IV) anticancer drug,<sup>9</sup> currently undergoing Phase III clinical trials for combination treatment in patients with hormone-refractory prostate cancer. It functions as a prodrug, releasing its cytotoxic Pt(II) payload, after losing its axial acetato-ligands by reduction in vivo. Satraplatin was even found to be suitable for oral administration as it is stable enough to survive the harsh conditions in the gastrointestinal environment. Based on the studies summarized above, we decided to tether EA to platinum to give a satraplatin-like compound, **1**, capable of targeting GST enzymes in human cancer cells. On uptake, the compound should be reduced in vivo releasing the EA moiety (inhibiting the GST enzyme) as well as a cytotoxic Pt(II) center, thus reversing cisplatinassociated drug resistance.

The synthesis of **1** was achieved via the acylation of *cis,cis,trans*-Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(OH)<sub>2</sub> with an excess of the EA chloride *without the addition of an amine base.* This method represents a deviation from a reported procedure in which pyridine is needed to quench the free HCl in the acylation process,<sup>10</sup> which failed to give **1**. It has been reported that in the absence of an adequate HCl-acceptor the formation of Pt<sup>IV</sup>(amine)<sub>2</sub>Cl<sub>4-x</sub>(O<sub>2</sub>CR)<sub>x</sub> (x = 0-2) mixtures takes place.<sup>11</sup> However, by controlling and optimizing the reaction conditions, the target compound can be readily isolated in moderate yield (see Supporting Information). Acylation of *cis,cis,trans*-





Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(OH)<sub>2</sub> using EA anhydride was also unsuccessful. The ability of 1 to inhibit GST activity was studied in vitro using the established 1-chloro-2,4-dinitrobenzene (CDNB)-glutathione (GSH) spectrophotometric assay (see Supporting Information). Briefly, A549 lung carcinoma cells were exposed to cisplatin, EA, or 1 for a short duration, sufficient for drug uptake, but insufficient for postexposure modification or cell death, then disrupted to extract their cytosolic contents. The GST activity of the extracts were determined and compared to untreated cells. Interestingly, it was found that the GST activity of the A549 cells exposed to 1 decreased to 22.6% of the control levels, while those exposed to EA and cisplatin decreased to 78.5% and 63.6%, respectively, providing the first indication that 1 could be a potent GST inhibitor as well as an anticancer drug. This could be due in part to higher drug uptake in cells treated with 1, which showed 10-fold higher Pt levels compared to those treated with cisplatin (see Supporting Information), presumably as a result of higher lipophilicity conferred by the large organic ligands.<sup>12</sup> Based on drug uptake at low temperature, 1 appears to enter the cells via passive diffusion.

To eliminate peripheral effects such as cellular drug uptake or interference from other cytosolic entities, the compounds were tested directly against specific GST isozymes. The inhibition of GSTP1-1 and GSTA1-1 (GST- $\alpha$  isozymes are also associated with drug resistance)<sup>13</sup> by the test compounds were studied using the CDNB-GSH assay (see Supporting Information). The results showed that **1** is a potent inhibitor, more than EA itself, and capable of reducing the activity of both isozymes to less than 10% of the original activity, even at low compound concentration. To our knowledge, this represents the first example of a Pt(IV) compound tailor-made to inhibit the activity of relevant drug resistant enzymes. The results

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**Figure 1.** Comparison of deconvoluted spectra (ESI-QT) of GSTA1-1 (bottom), GSTA1-1 treated with 10-fold excess of **1** (top), and GSTA1-1 treated with 10-fold excess of EA (middle).

also suggest that cisplatin is not capable of inhibiting GSTP1-1, via active site occupancy or otherwise, which, in vivo, could allow it to be rapidly conjugated to GSH and deactivated.

The interactions between 1 and the GST isozymes were probed using mass spectrometry. The ESI mass spectrum (deconvoluted to reveal the parent mass) of GSTA1-1 is shown in Figure 1 (bottom) revealing a parent mass of ca. m/z 25 504. Incubation of GSTA1-1 with a 10-fold excess of EA leads to a mass spectrum containing the intact enzyme and an additional peak at m/z 25 807 corresponding to the formation of the enzyme-EA adduct (center). No adducts were observed when GSTA1-1 was incubated with cisplatin, but with a 10-fold excess of 1, both the enzyme-EA and enzyme-1 adducts are observed. The spectrum suggest a GSTinduced cleavage of the Pt-EA carboxylate bond, although these results give no indication of the mechanism of carboxylate bond cleavage which may not involve the same catalytic functional groups as GSH conjugation. This is plausible since GSTP1-1 and GSTA1-1 have been reported to catalyze the hydrolysis of thiol esters of EA, albeit at a much slower rate compared to the conjugation to GSH.<sup>14</sup> Furthermore, the crystal structure of GST-EA complexes show EA bound nonproductively in the active site, with the carboxylate group directed outward to the solvent region.15 A similar mode of binding may occur with 1, possibly weakening the carboxylate bond between the Pt(IV) center and EA due to electronic and steric effects of EA binding. A similar observation was made when GSTP1-1 was incubated with EA or 1, but with the formation of multiple adducts. Similarly, no significant interactions with cisplatin were observed.

The growth inhibition of **1** against a range of established cancer cell lines, including the cisplatin-resistant breast MCF7 and T47D, lung A549, and colon HT29 human carcinoma cells, was studied

**Table 1.** Comparison of Cytotoxicities of *Cisplatin* and *Ethacraplatin* (1) on Selected Cancer Cell Lines

	IC <sub>50</sub> ° (μM)							
	MCF7		T47D		HT29		A549	
test compound	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
cisplatin ethacraplatin (1)	>80 31.85	36.02 32.15	>80 31.56	58.82 34.88	>80 32.63	16.82 12.96	>80 78.59	31.43 32.09

 $^{a}$  IC<sub>50</sub>, drug concentration that inhibits cell growth by 50%. Only the results for 24 h and 72 h exposure are displayed (see Supporting Information for other details). Each value is the mean of three independent experiments.

using MTT assays over periods of 24, 48, and 72 h. The growth of T47D cells decreased at lower concentrations of **1** compared to cisplatin, and growth inhibition in the other cells was accelerated on exposure to **1** (Table 1). After 24 h of exposure, growth inhibition was observed in all cells exposed to **1**, but not in cells exposed to cisplatin, suggesting a role for GST in this faster effect of **1**. As GST has been involved in several cellular pathways regulating growth, besides its known involvement in resistance to chemotherapeutics agents,<sup>16</sup> further experiments will be necessary to understand the cell mechanisms involved in the accelerated effect of **1**.

In conclusion, a novel fast-acting cytotoxic Pt(IV) compound with the capacity to inhibit GST activity has been prepared, representing a strategy of utilizing the Pt(IV) carboxylate framework to build customized compounds capable of delivering multiple modes of pharmacological effects.

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**Supporting Information Available:** Experimental procedures and spectroscopic data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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