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ORIGINAL PAPER

# In silico approach to cisplatin toxicity. Quantum chemical studies on platinum(II)–cysteine systems

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**Abstract** The behaviour of cisplatin in serum, and the drastic differences between the properties of this drug and its trans-isomer were the main motivations for this work. In a search for model "thiol–platin(II)" interactions, the first steps of the following reaction systems were evaluated: (1) cisplatin–thiomethanol; (2) transplatin–thiomethanol; (3) cisplatin–cysteine; and (4) transplatin–cysteine. In each case, calculations for the associative mode of reactions were performed. The electronic structure of these molecular systems was studied at the non-empirical all-electron level using density functional theory (DFT) within the Huzinaga and WTBS basis sets including polarisation Gaussian functions and full geometry optimisation. B3LYP or EPBO density functionals were applied throughout. The calculated molecular electrostatic potentials are presented graphically. Assuming that electrostatic effects are dominant, cisplatin should interact more strongly with the sulfur atom of $CH_3S^-$ and deprotonated CYS-$S^-$ than transplatin. This fact has been documented in the supermolecule model of the relevant interaction energies in both gas phase as well as within the solvent polarisable continuum model. The opposite relationship was observed when we compared values of energy differences between products and substrates for both isomers. The data obtained here could be applied to search for correlation between the biological activity of platinum complexes and their properties as estimated by various physico-chemical and in silico methodologies.

**Keywords** Cisplatin · Transplatin · Interactions with deprotonated thiomethanol and cysteine

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## Introduction

Cisplatin - a simple inorganic compound that became serendipitously the first metal-containing anticancer drug - remains the leading platinum-based medicine [1–3]. Cisplatin could be even more successful if its harmful side effects were reduced; its therapeutic effects are limited by cumulative nephrotoxicity and neurotoxicity. Improving the potency of cisplatin and continuing the search for new platinum-based drugs devoid of undesirable side effects have been the main thrust of investigations conducted over the last three decades on the family of cytotoxic platinum complexes. Unfortunately, despite real achievements, success has been limited: thousands new platinum compounds were evaluated but only a few have been introduced into higher clinical procedures.

One of the main reasons for the low efficiency of selection of new candidates with clinical potential has been the difficulty in obtaining platinum complexes with reduced toxicity, resistance and other harmful side effects [3–7]. This is partly because the search for potential new platinum drugs has concentrated on selection of the most cytotoxic agents without simultaneously screening them for low toxicity; this situation is exacerbated by the lack of suitable models on which to carry out tests. Also, in experimentally based mechanistic studies, more attention was paid to

explaining the anti-cancer activity than to side effects [7–10]. The same tendency can be noted in theoretical approaches to the evaluation of cisplatin biological activity. For example, in silico quantum chemical studies have so far focussed mainly on the interaction of cisplatin with DNA and its constituents. Such lines of research have been inspired by experimentally based fundamental statements that nuclear DNA is the critical target that binds cisplatin covalently, giving rise to adducts with a high level of selectivity in killing cancer cells [11]. Gradually, interest has turned to other processes of drug biotransformation. It has been shown that cisplatin participates in a series of chemical reactions during its transportation, and less than 5% of the dosed cisplatin is bound with DNA [8, 12–14]. Most platinum species interact with sulfur-biomolecules, which are present in abundance outside as well as inside cells.

Outside the cell, sulfur donors can interact with cisplatin occurring in dichloro-form [6, 9]. The unchanged [Pt$(NH_3)_2Cl_2$] form prevails in blood plasma conditions where the concentration of chloride ions is sufficiently large (100–150 mM) to inhibit cisplatin hydrolysis. However, in its original formulation, the drug is ready to exchange Cl-ligands with electron donors stronger than $H_2O$ [15, 16]. Potential extracellular ligands include thioether- or thiol-containing amino acids (methionine, cysteine), peptides (glutathione) and proteins (albumins, metallothioneins, enzymes). It has been shown that reaction of cisplatin with sulfur-containing molecules does not require prior hydrolysis [6, 8, 12].

Although the reactivity of Pt(II) with thioethers (e.g. methionine) is kinetically more favourable than with thiols, the thermodynamic stability of the resulting products is diametrically opposed [17]. Thus, the affinity of Pt(II) for thiols results in replacement of one of the chloride ions by thiolate and formation of irreversible Pt–S binding, which may lead to conformational changes and thus permanent inactivation of some enzymes and other proteins [6, 9, 12, 17]. The nephrotoxicity of cisplatin is attributed to the destructive action on SH-bearing surface enzymes in renal proximal tubular cells of cisplatin circulating in the blood following drug administration [13, 18–21].

The above processes taking place outside the cell can significantly reduce the amount of Pt-species able to penetrate the cytoplasmic membrane and enter the cell. Inside the cell, cisplatin undergoes hydrolysis, which can occur spontaneously due to the low concentration of chloride ion (4–80 mM). This results in the formation of hydrated-products that are more reactive than cisplatin in its original dichloro-form [22]. Of these, the most reactive towards DNA and other cellular nucleophiles has been shown to be $[Pt(NH_3)_2Cl(H_2O)]^+$. Kinetic data reveal that hydrolysis of the first chloride ion is the rate-limiting step for initial binding to DNA at the N7 position of adjacent guanines [22–24]. This reaction is the first step towards the successful killing of tumour cells by the platinum complex. However, the cytotoxic effect is seriously limited by reaction of the hydrated complex with non-DNA targets. Cellular thiols may cause the inactivation of the drug, initiating other, mainly harmful, processes. Thus, the cellular processes leading to both therapeutic and harmful effects have the same initial step, and are differentiated only by the sequence of further reactions due to kinetic and thermodynamic competition between the responsive drug–target systems. Moreover, the harmful side effects may be enhanced inside the cell because the non-hydrolysed drug is able to interact with sulfur donors, whereas DNA reacts only with activated cisplatin. The reaction of the "pro-drug" with thiols may occur in bodily fluids as well as in the cells of tissues [25–27].

Progress in understanding the mechanism of action of cisplatin and its congeners has been continuously supported

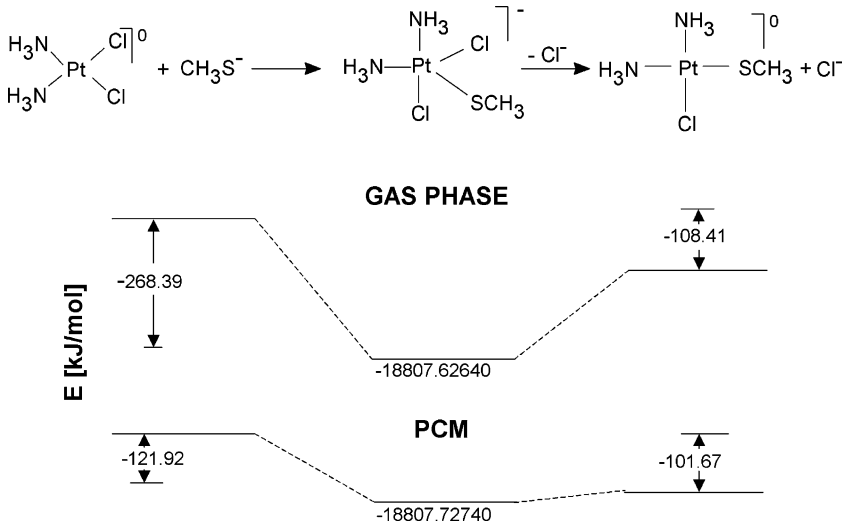


**Fig. 1** *Upper panel* Reaction of cisplatin with deprotonated thiomethanol. *Lower panel* Total energies (in atomic units) for substrates, transition complex, and products. *Top* Gas phase, *bottom* in solution (water)

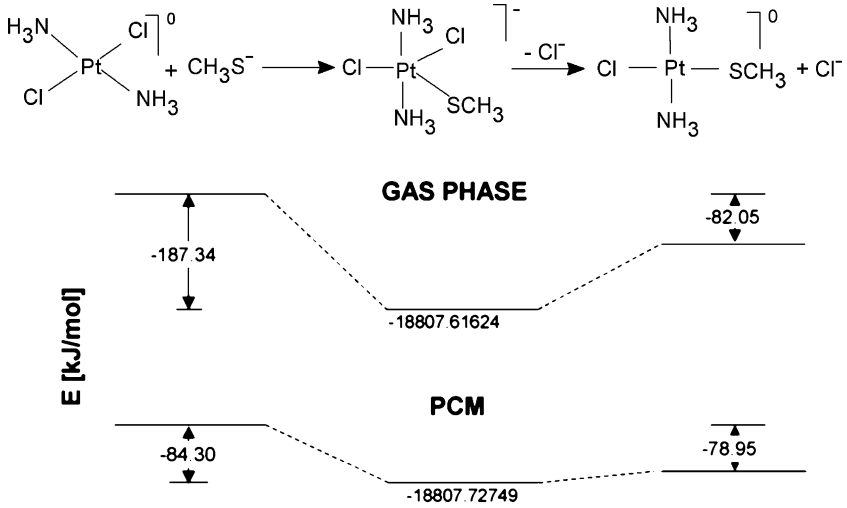


**Fig. 2** *Upper panel* Reaction diagram of transplatin with deprotonated thiomethanol. *Lower panel* Total energies (in atomic units) for substrates, transition complex, and products. *Top* Gas phase, *bottom* in solution (water)

by theoretical studies. Recently, such studies have expanded to include not only individual platinum compounds and their adducts with DNA components but also the processes of pro-drug hydrolysis and the interaction of hydrated forms with DNA and its fragments.

Other aspects of the biological action of platinum-based complexes have been researched rather more sporadically. However, some authors have been successful in developing in silico investigations directed towards the molecular modelling of drug interactions with non-DNA targets including small sulfur-bearing bio-molecules, cysteine and methionine, or simple inorganic nucleophiles such as $NH_3$ and $H_2S$ [28–31]. All these reacting systems might be treated as models of the entire metabolic processes of platinum drugs, including reactions prior to cell uptake, and deactivation prior to DNA binding.

In the present work, we attempted an in silico evaluation of the initial reactions responsible for the harmful side-effects of cisplatin. It is thought that the various undesirable side effects are initiated during transportation of the drug through the blood and lymph, where cisplatin occurs predominantly in the non-hydrolysed $[Pt(NH_3)_2Cl_2]$ form [8, 12–14].

As starting reagents, we used both cis- and trans-isomers and two model thiols: cysteine and thiomethanol. Calculations were performed for the following systems: cisplatin–thiomethanol, transplatin–thiomethanol, cisplatin–cysteine and transplatin–cysteine. The transplatin–cysteine system

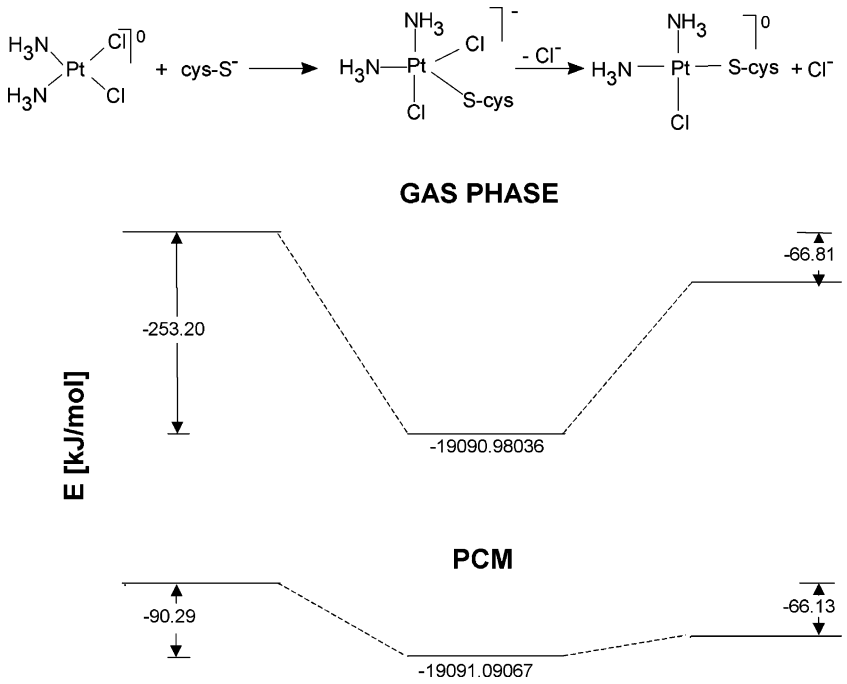


**Fig. 3** *Upper panel* Reaction diagram of cisplatin with deprotonated cysteine. *Lower panel* Total energies (in atomic units) for substrates, transition complex, and products. *Top* Gas phase, *bottom* in solution (water)

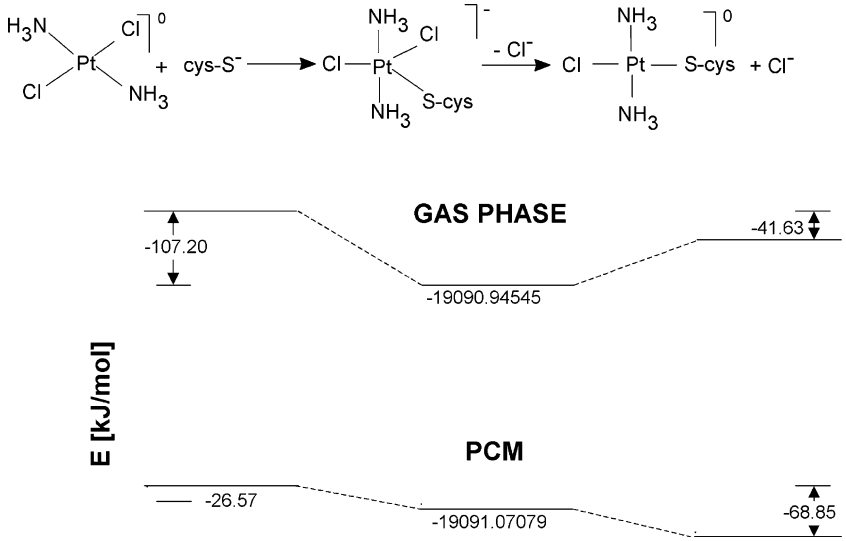


**Fig. 4** *Upper panel* Reaction diagram of transplatin with deprotonated cysteine. *Lower panel* Total energies (in atomic units) for substrates, transition complex, and products. *Top* Gas phase, *bottom* in solution (water)

was included in order to allow comparison of the parameters calculated for reactions of both isomers regarding their various biological and kinetic properties. It is known that these isomers exhibit intriguing differences: cisplatin has high antitumour activity and strong toxicity, especially towards kidneys, whereas the trans isomer is therapeutically inefficient and non nephrotoxic [32]. On the other hand, biochemical studies have shown dramatic differences in reactivity with thiols: transplatin was captured rapidly by glutathione, approximately 300-fold faster than was cisplatin [33]. As a result, the more rapid reactions of transplatin compared to cisplatin cause transplatin to be consumed before reaching its targets (DNA or renal enzymes), hence the retardation of biological activity (both cytotoxic and nephrotoxic) [22]. The other two systems investigated, consisting of cis- or trans-platin and $CH_3SH$, were chosen to simulate drug–thiol interactions on the simpler applied models.

**Table 1** Interaction energies (kcal $mol^{-1}$) for complexes of cisplatin and transplatin with deprotonated CH3-$S^-$ and CYS-$S^-$

| | Cisplatin | | Transplatin | |
|---|---|---|---|---|
| | Ab initio | PM6 | Ab initio | PM6 |
| $CH_3$-$S^-$ | −64.18 (−22.88) | −43.11 (−126.35) | −44.79 (−20.16) | −25.81 (−147.28) |
| | −85.91[a] (−62.22)[a] | | −113.85[a] (−109.80)[a] | |
| CYS-$S^-$ | −60.54 (−21.59) | −49.61 (−1.08) | −25.63 (−6.35) | −33.22 (−7.49) |

Polarisable continuum model (PCM) calculations—the respective results of calculations obtained within the PCM model where the solvent (water) effect is taken into account—are given in parentheses

[a] Møller-Plesset (MP2) results

## Computational procedures

The electronic structure of the model systems presumed responsible for the intermediate intermolecular interactions in the process of anticancer activity, was studied for complexes of cis and transplatin(II) with deprotonated thiomethanol and cysteine. The molecular structures, along with diagrams showing the total energies of substrates, intermediate complexes and products, are depicted in Figs. 1, 2, 3, and 4.

The present study is based on the supermolecular approach where reactants and products are considered as one molecular system in the singlet electronic ground state. The electronic structure of these molecular systems was studied at the non-empirical all-electron level using density functional theory (DFT) or Møller-Plesset (MP2) methods within the Huzinaga basis set with polarisation functions or the correlation consistent cc-pVTZ basis set [34]. Furthermore, in the case of the platinum atom, the WTBS basis set, which gives energy values several atomic units lower than other available Gaussians, was used [35].

In order to avoid a long optimisation process, in the first stage optimisation was performed with the all-valence MOPAC-PM6 method [36] following B3LYP DFT or MP2 formalism in the next step without symmetry

**Table 2** Energy differences between products and substrates [kcal $mol^{-1}$] for complexes of cisplatin and transplatin with deprotonated CH3-$S^-$ and CYS-$S^-$

| | Cisplatin | Transplatin |
|---|---|---|
| $CH_3$-$S^-$ | −25.92 (−18.04) | −19.62 (−18.88) |
| CYS-$S^-$ | −15.97 (−15.81) | −9.95 (−16.46) |

Results of calculations obtained within the PCM model where the solvent (water) effect is included are given in parentheses

constraints. The B3LYP and OPBE density functionals embedded in the GAUSSIAN package [37] were applied, leading to comparable results. Solvent effects were studied using the polarisable continuum model (PCM) method, and molecular electrostatic potential (MEP) maps were obtained by using the MOLEKEL-5.3 computer program [38].

## Results and conclusions

The behaviour of cisplatin in serum, and the drastic differences between the properties of this drug and its trans-isomer triggered our interest in this work. The aim of this work was to conduct a comparative study of cis- and transplatin using in silico methodology. We evaluated the first step substitution reaction in the following systems: (1) cisplatin–thiomethanol; (2) transplatin–thiomethanol; (3) cisplatin–cysteine; (4) transplatin–cysteine. The reactions presented (Figs. 1, 2) describe the substitution of the labile ligand $Cl^-$ by a simple deprotonated thiol ($CH_3S^-$), applied here as a model to represent SH-bearing biological molecules; the reactions (Figs. 3, 4) describe substitution of $Cl^-$ by nucleophiles existing in the physiological milieu (deprotonated cysteine, CYS-$S^-$). In all cases, the calculations were performed for the associative mode of reaction.

The importance of sulfur-containing bio-ligands as potential targets for cisplatin and its congeners suggested substitution at the Pt centre as being crucial for the fate of the drug in vivo. Therefore the reactions of ligand exchange occurring immediately after introduction of the drug into the bloodstream were chosen as the object of quantum-chemical calculations. We believe that reactions of the unchanged drug with deprotonated thiols represent adequate models of the initial processes responsible for the inactivation and toxicity of cisplatin.

The results of our calculations obtained for Huzinaga, cc-pVTZ and 6–31G(d,p) Gaussians are basis-set-dependent but qualitatively similar (H.C., unpublished results). Furthermore, similar to the results of Burda et al. [39], our preliminary calculations indicate no essential differences in the conclusions drawn if Gibbs free energies are considered instead of total electronic energies. In some cases, our semiempirical MOPAC-PM6 results (Table 1) are in rather qualitative agreement with the relevant ab initio calculations. We found some differences between the results of B3LYP density functional and the relevant MP2 calculations (Table 1); however, the general tendency is about the same.

Table 1 lists the interaction energies in the complexes under consideration as the energy difference between transition complexes and substrates, whereas the energy differences between products and substrates are listed in Table 2. In accordance with the results of Burda et al. [39], in all cases, the reactions under consideration are exothermic. Furthermore, in all cases the interaction energies are clearly lower in the solvent (water) than in the gas phase. A

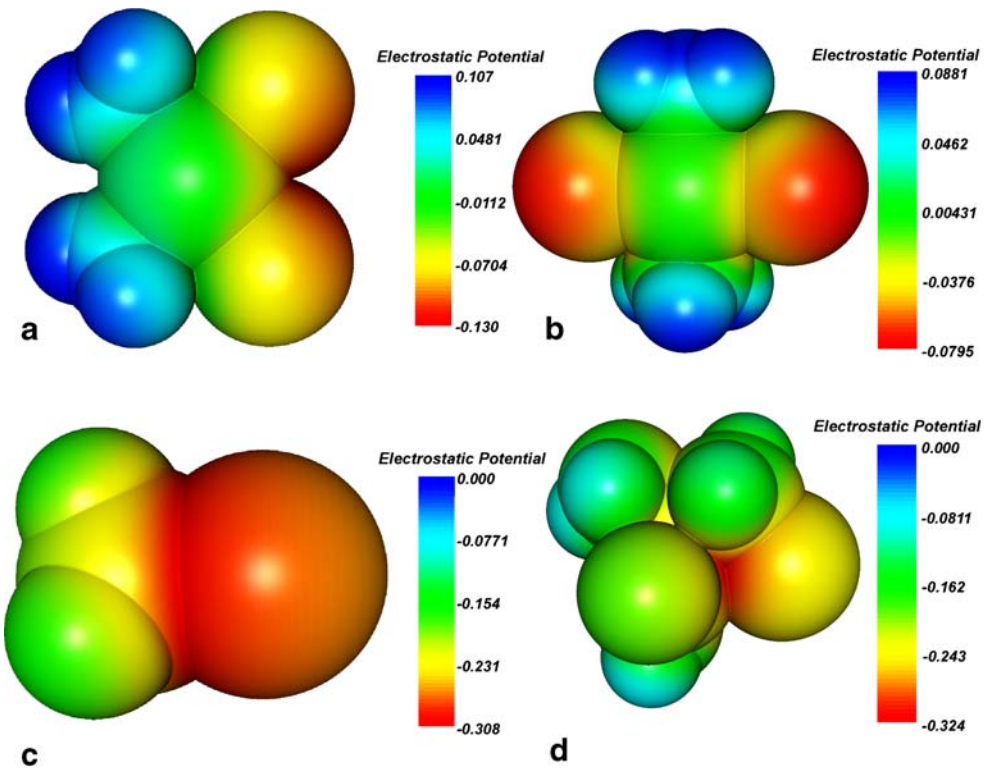


**Fig. 5** Electrostatic molecular potential maps of **a** cisplatin, **b** transplatin, **c** deprotonated thiomethanol, **d** deprotonated cysteine. *Dark blue regions* correspond to the most positive potential values whereas *red areas* correspond to surfaces with negative potential

similar situation seems to exist in the case of the energy differences between products and substrates. In the transplatin-cys-$S^-$ complex (Fig. 4), the total energy for products was found to be even lower than that of the transition complex.

Molecular electrostatic maps of cisplatin, transplatin, deprotonated thiomethanol ($CH_3$-$S^-$) and cysteine (CYS-$S^-$) are depicted in Fig. 5, in which blue denotes the most positive and red the most negative regions. According to the chemical features, this figure shows that cisplatin is more polar than transplatin. Therefore, assuming that electrostatic effects are dominant, cisplatin should interact more strongly with the sulfur atom of deprotonated $CH_3$-$S^-$ and deprotonated CYS-$S^-$ than transplatin. This fact has been documented in the supermolecule model of the relevant interaction energies in both the gas phase as well as within the PCM model (Table 1) when the solvent effect is taken into account.

The results obtained in this work confirm the significant differences between cis- and transplatin reactivity towards thiols. This indicates the likely existence of a "cause and effect" relationship between the chemical reactivity of both isomers and their biological response. However, we could not expect that the calculated values of descriptors presented here would allow formulation of any "structure–activity relationship" type correlation. The biological effects, and thus the therapeutic responses, must result from many overlapping partial cellular and sub-cellular processes, each of which offers a challenge for exploration using theoretical methodologies.

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