The Second-Generation Anticancer Drug Nedaplatin: A Theoretical Investigation on the Hydrolysis Mechanism

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The hydrolysis reaction processes of the second-generation platinum derivative Nedaplatin have been studied using density functional theory (DFT) combined with the conductor-like dielectric continuum model (CPCM) approach, in order to obtain detailed data on its mechanism of action. The first and the second hydrolysis of Nedaplatin, corresponding to the ring opening followed by the loss of the ligand, respectively, have been explored in neutral and acid conditions. The influence of an extra water molecule which could assist the degradation processes has also been considered including in our models an explicit water molecule other than the reactive one. The computed potential energy surfaces show that the rate limiting step in neutral conditions is the first hydrolysis process and, consequently, the double hydrated complex is suggested to be the species reacting with the DNA purine bases, while in acid conditions the trend is different, with the second hydrolysis process being the rate limiting step. The results obtained in this work allow us to make a comparison with the trends previously found for the other platinum anticancer drugs currently used in the medical protocols.

Introduction

The discovery of an inhibitory effect of a soluble platinum complex on the division of living cells led to the development of platinum-based drugs to treat a wide range of cancers.[1,2]

Interaction with DNA and formation of cross-links with adjacent purine bases are considered to be the crucial steps in the antitumor activity of this class of complexes.[3]

Cisplatin, the first platinum and the world's best selling anticancer drug, began to be used in treatment in 1977. Testicular cancer was found to be susceptible to treatment with Cisplatin, and there were other successes with ovarian, head, bladder, cervical, and neck cancers.[4]

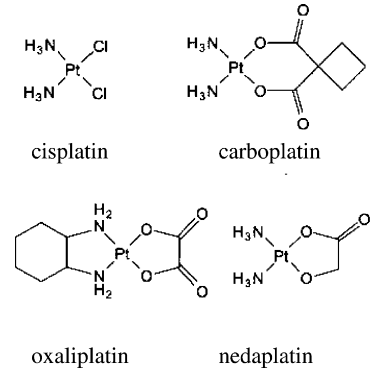
Unfortunately, its therapeutic efficacy is somewhat compromised by the occurrence of serious side effects such as nausea/vomiting and nephro-, oto-, and neurotoxicity.[5−7]

In order to overcome these limitations, many studies have been done to find other platinum drugs with an equivalent or improved range of activity but with less toxic side effects.[3,8−10]

Since the discovery of Cisplatin, a large number of platinum compounds (more than 3000) has been screened for antitumor activity, but only four of them are currently registered for clinical use. *cis*-Diammine(1,1-cyclobutanedicarboxylato) platinum II (Carboplatin),[9] *trans*-L-1,2-diaminocyclohexaneoxalatoplatinum II (Oxaliplatin),[9] and *cis*-diammineglycolatoplatinum (Nedaplatin)[9] are analogues of Cisplatin and show a lowered nephrotoxicity compared with Cisplatin.[8,10] The chemical structures of these platinum-containing drugs are shown in Scheme 1.

Carboplatin is effective against about the same tumor types as Cisplatin, but in contrast, it presents less side effects.[11] Unlike Cisplatin, that should bind to DNA monohydrated, Carboplatin has been proposed to reach DNA in its double hydrated form.[12] Nevertheless, both species are believed to exert their cytotoxicity by coordinating bifunctionally to DNA through the N7 atoms of two adjacent guanines on the same strand (intrastrand cross-links), arresting DNA replication.[4,13,14]

SCHEME 1



Recent research has sought to identify new platinum compounds which will treat tumors which do not respond to or which become resistant to Cisplatin and Carboplatin. The first of these drugs to reach commercialization is Oxaliplatin.[15] It shows activity against some tumors that are primarily resistant to the other two platinum compounds, being the first antineoplastic agent for the treatment of metastatic colorectal cancer.[16,17] Oxaliplatin in combination therapy has been evaluated in clinical trials against metastatic breast cancer,[18] recurrent ovarian cancer,[19] and pancreatic and esophagogastric cancer.[20,21] It has been suggested that the third-generation drug Oxaliplatin acts as an alkylating agent on DNA, forming essentially three types of cross-link adducts: intrastrand, interstand, and DNA−protein. The most commonly adduct is nevertheless the intrastand bridging two adjacent purine bases (1,2-GG or AG) at the N7 position.[3,13] As Carboplatin, in a previous work, it has been suggested that Oxaliplatin, would reach DNA in its fully hydrolyzed complex.[22] Important differences are observed

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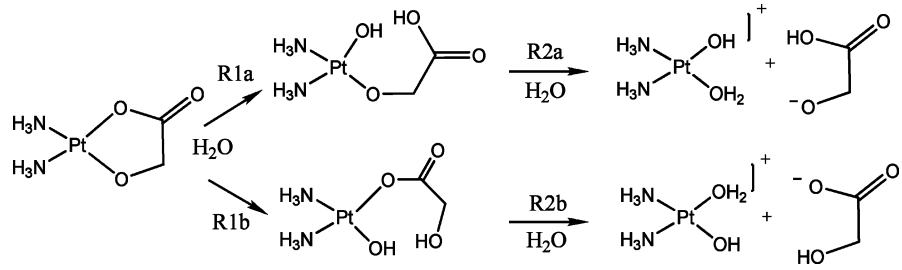
SCHEME 2: Investigated Reaction Pathways for the Hydrolysis of Nedaplatin in Neutral Conditions



between Cisplatin and Carboplatin adducts with DNA when compared with Oxaliplatin DNA ones; these adducts are more bulky and hydrophobic that the other ones and subsequently are more effective in inhibiting DNA synthesis and usually more cytotoxic than *cis*-diamine-platinum adducts.[23] Nevertheless, Oxaliplatin shows several limitations such as a reversible peripheral neuropathy characterized by paraesthesia and dysaesthesia in hands, feet, and the oral region.[3,8,10] The acute neurotoxic side effects have been suggested to involve voltage-gated ion channels.[24,25] Due to the presence of the kinetically less labile carboxylate or oxalate present in Carboplatin and Oxaliplatin, these compounds are expected to be responsible for the reduced side effects observed in patients compared with those treated with Cisplatin.[11]

Nedaplatin (*cis*-diammine-glycolate-O,O′-platinum II) is a second-generation platinum derivative, with relevant antineoplastic activity.[26−28] It contains a novel ring structure in which glycolate is bound to the platinum by a bidentate ligand and forms reactive platinum complexes that bind to nucleophilic groups in DNA, resulting in intrastrand and interstrand DNA cross-links, apoptosis, and cell death. Nedaplatin has shown superior antitumor activity and less renal or gastrointestinal toxicity when compared to Cisplatin in several Japanese clinical studies in which the drug was used to treat head and neck, lung, and cervical cancers.[26−28] This agent appears to be also less nephrotoxic and neurotoxic compared to both Cisplatin and Carboplatin. It has been available for use there since 1995 and shows pronounced efficacy against lung, head and neck, testicular, and gynecological cancers.[29,30]

In this Article, we focus our attention on the degradation pathway of Nedaplatin, in both neutral and acid conditions, in order to individuate the species that probably will react with DNA.

The kinetic studies of the hydrolysis of Cisplatin and its analogues in various solution conditions have been the object of continuous interest, becoming a substantial part of contemporary medicinal inorganic chemistry. With the development of computational transition metal chemistry, the necessity to obtain an accurate picture of the hydrolysis mechanisms of anticancer drugs is increased. The employed tool (DFT-B3LYP) can be considered the state of the art of the modern theoretical methods, providing an excellent compromise between accuracy of the results and the requested computational efforts. It has been previously used in a huge number of chemical systems including the aquation processes of metal-containing anticancer drugs.[12,31−37]

According to our results, the degradation process should take place in two consecutive steps: first, water addition with ring opening, followed by the loss of the ligand by reaction with the second water molecule.

Computational Details

All calculations were performed with the Gaussian 03[38] program at the density functional theory level, using the hybrid B3LYP functional, composed of Becke's[39] three-parameter hybrid exchange functional (B3) and the correlation functional of Lee, Yang, and Parr (LYP).[40] Geometry optimizations without symmetry constraints and in solvent ($\varepsilon = 80$) were carried out with a 6-31G(d) basis set for all atoms except the platinum atom, which was described by the quasi-relativistic Stuttgart−Dresden pseudopotentials[41] with the pseudo-orbital basis set augmented by a set of diffuse functions, $\alpha_s = 0.0075$, $\alpha_p = 0.013$, and $\alpha_d = 0.025$, and polarization functions, $\alpha_f = 0.98$.[42] In order to confirm proper convergence to equilibrium and transition state geometries, vibrational frequency analysis was done on the basis of analytical second derivatives of the Hamiltonian at this level of theory. Solvent effects were taken into account by the CPCM method.[43] Klamt radii were used for constructing the solute cavity.[44]

More accurate energies were obtained by performing single-point calculations with the larger basis set 6-31++G(2df,2pd). Similarly, the platinum valence basis set was augmented with diffuse ($\alpha_f = 0.46$) and polarization ($\alpha_g = 1.21$) functions.[42] Potential energy profiles were estimated from total electronic energies at the 6-31++G(2df,2pd) level adding zero point energy (ZPE) and enthalpy corrections at room temperature (298.15 K).

Results and Discussion

The hydrolysis reactions of the platinum(II) derivative anticancer drugs belong to the class of second-order nucleophilic substitution (S_N2) reactions. These reactions for square-planar complexes proceed via a collision between the reactant with two consecutive nucleophilic species attacking the metal center to release the ionic ligand. In such a process, a transition state in which the entering molecule, the leaving group, and the metal complex result weakly bound can be found, suggesting that the associative mechanism may be preferred and are consistent with the experimental studies on the aquation of some Pt(II) complexes with in vivo anticancer activities.[45] The equatorial plane of the five-coordinated TS structure plays an important role in determining the hydrolysis behavior. As introduced previously, the Nedaplatin degradation process should take place in two consecutive steps: first, water addition with ring opening followed by the loss of the ligand as a consequence of the second entering water molecule. We studied this kind of mechanism in both neutral and acid conditions in order to establish the most favorable energy path. A comparison between the results obtained previously[12,22,32,34,37] on similar compounds is reported in order to spotlight possible common or different behavior. In analogy with previous studies on Cisplatin-like anticancer drugs, one of the purposes is moreover to identify which is the species that could reach DNA in each environment.

1. Energetic Profiles of Hydrolysis Reactions of Nedaplatin in Neutral Conditions. The elucidation of the degradation mechanisms, in neutral conditions, of the second anticancer drug Nedaplatin, represents the first step of our investigation. The

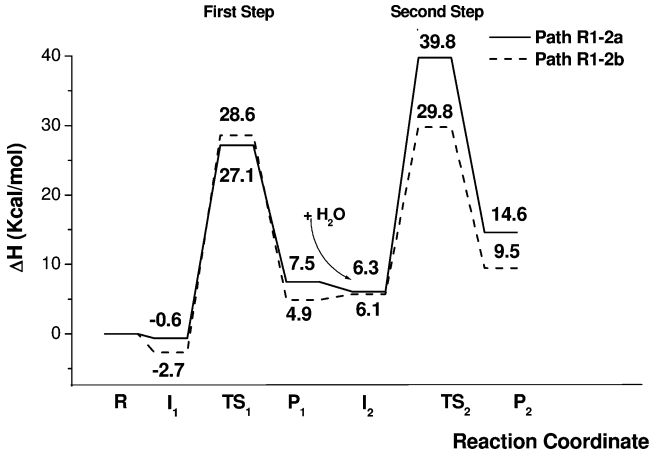


Figure 1. Activation enthalpy profiles (at 298.15 K) for the hydrolysis of Nedaplatin in neutral conditions, in the water phase.

investigated reaction paths for the hydrolysis of Nedaplatin under these conditions are depicted in Scheme 2.

The detachment of the ligand can occur in two different ways, by rupture of the bond that involves the oxygen in α to the carbonyl group (R1a) or by breaking the other Pt−O bond (R1b). The products obtained in this step show a proton transfer from the entered water molecule to the close oxygen of the ligand (O_L). Both of the products are expected to undergo a second hydrolysis process through different pathways (R2a and R2b), leading to the loss of the ligand and to the formation of *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ complexes. The hydroxo complex is accessible at physiological pH and temperature, even if in the vicinity of macromolecules the local pH could be influenced, reflecting its effect on hydrolysis rates.[46,47]

We will discuss first the water addition and the consequent ring opening.

The potential energy surfaces are depicted in Figure 1, and the optimized structures for both reactions are depicted in Figure 2.

We considered separated reactants (R) as a reference state rather than reactant adducts (I_1) (see also Figure 2), to predict the activation barriers, since it is well accepted that considering at the beginning of the reaction, the water molecule in the second coordination shell of the metal, it is acceptable only in vacuo but seems an artifact in solvent.[34,37] The potential energy profiles, for the first step, show that both of the intermediates lie under the reactant molecules; nevertheless, the most stable is that obtained in the R1b path due to the stronger interaction of the water molecule with the oxygen of the glycolate ligand (Figure 2). The energies of the transition states for the steps R1a and R1b are very similar. The first reaction requires 27.1 kcal/mol, while step R1b shows an activation barrier of 28.6 kcal/mol and conduces to the most stable product. The imaginary frequencies observed in the transition states are about 207i cm^{-1} for TS_{1a} and 340i cm^{-1} for TS_{1b}, and the analysis. of these vibrational modes clearly indicates the rupture of the bond between platinum and the ligand (Pt−O_L), and the simultaneous formation of the metal−water (Pt−O_W) bond.

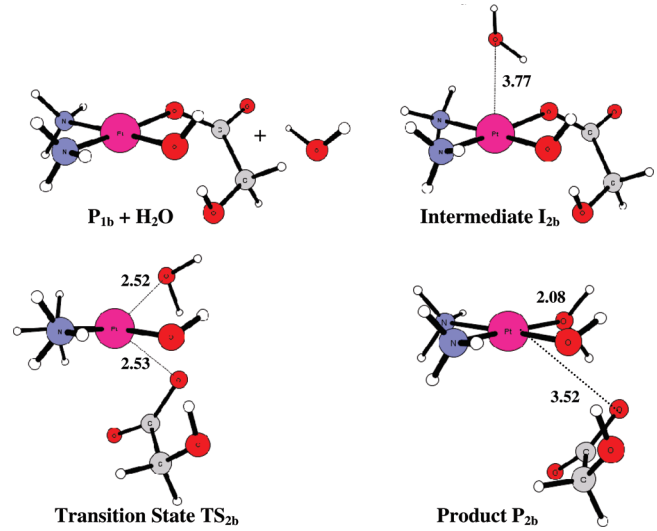


Figure 3. Optimized structures and selected structural parameters for the addition of the second water molecule to Nedaplatin (R2b) in neutral conditions, in the water phase. The distances reported are in angstroms.

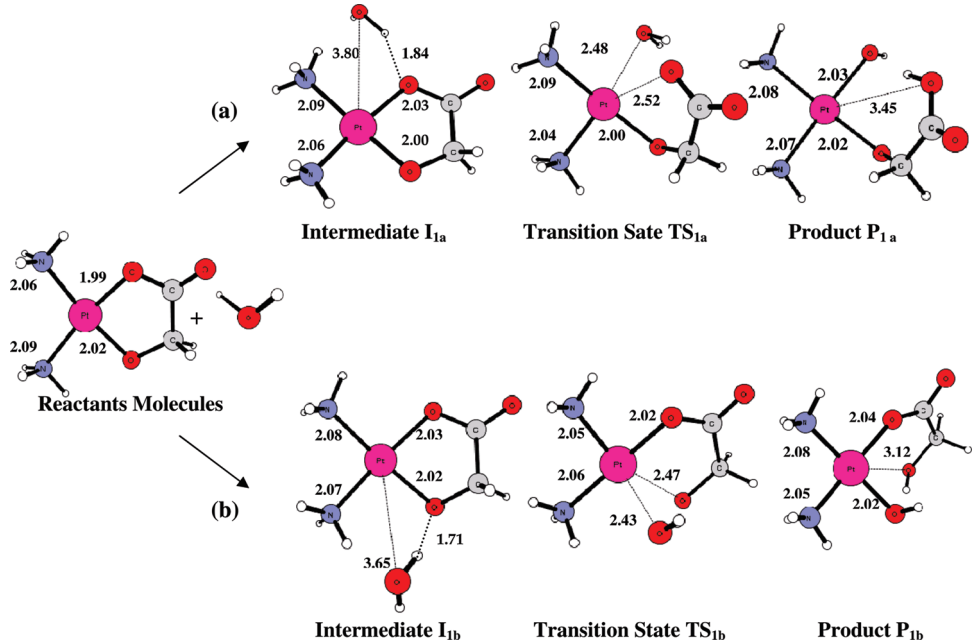


Figure 2. Optimized structures and selected structural parameters for the addition of the first water molecule to Nedaplatin in neutral conditions, in the water phase: (a) path R1a; (b) path R1b. The distances reported are in angstroms.

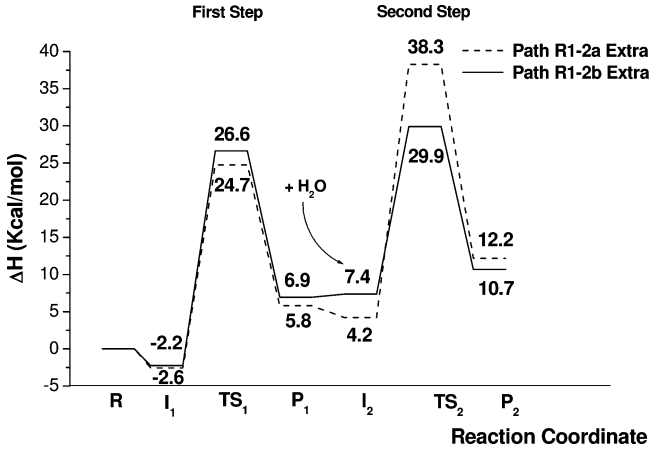


Figure 4. Activation enthalpy profiles (at 298.15 K) for the hydrolysis of Nedaplatin in neutral conditions, with an extra water molecule in the system. The energies refer to the water phase.

Both of the transition states show a penta-coordinated structure, according with an associative S_N2 reaction. The N−Pt−O_L angle becomes greater than 90° in order to allow the entering of the water molecule in the opposite side of the leaving group. The final products for the first step of the reaction are obtained by overcoming similar activation barriers, and both are expected to undergo a second hydrolysis process. Moreover, it can be observed that both display a proton transfer from the water molecule to the oxygen atom O_L and the hydroxo complex re-establishes its square-planar conformation.

In the second step of the reaction, the addition of a second water molecule to both of the final products of the first step leads to the loss of the glycolate ligand. Therefore, we considered the second water molecule entering in two different orientations and following the steps R2a and R2b, as proposed in Scheme 2. As shown in Figure 1, the most energetically favorable path is R2b, with an activation barrier of 29.8 kcal/mol with respect to the initial reactant molecules, which conduces also to the most stable product. The optimized structures for the R2b reaction are displayed in Figure 3, while those relative to the R2a path are reported in Figure S1 of the Supporting Information.

In the intermediate structure, the water molecule approaches the platinum center with a distance of 3.77 Å that becomes 2.52 Å in the TS and 2.08 Å in the product. The imaginary frequency for the transition state (TS_{2b}) is 225i cm^{-1} and corresponds to the Pt−O_L and Pt−O_W bonds being broken and formed, respectively. The activation barrier is slightly higher than the first one, being 29.8 kcal/mol, and the reaction exothermicity is 9.5 kcal/mol, considering the initial reactant molecules. Nevertheless, considering as a reference state the product obtained in the previous step and a separate water molecule, as shown in Figure 3, and according to other works on similar systems,[34,37] we obtain an activation energy of 24.9 kcal/mol and an exothermicity of 4.6 kcal/mol (see section 3). Thus, the reaction proceeds along path R1b in which the product P_{1b} obtained in the previous step reacts with another water molecule, leading to the loss of the ligand.

In order to verify the possible influence of an extra water molecule which could assist the hydrolysis processes, we conducted an additional study including in our systems another water molecule acting as an explicit solvent. This model incorporates the effects of hydrogen bonding and provides a more accurate description of the stabilization of the leaving group. The obtained paths for each step of reaction (R1a, R1b, R2a, and R2b) are reported in Figure 4. The results show that the main effect of considering explicitly an extra water molecule together with solvent bulk effects is observable on the energetics of the process. The activation barriers result all lowered compared with previous results, but the general trend of the reaction was not affected. Also, in this case, the ring-opening reaction (first step) requires an amount of energy very similar for the two paths (R1a and R1b); as a consequence, both of the products can be obtained and can undergo second hydrolysis. The most favorable path for the second step is again R2b.

The optimized structures of the stationary points for the ring-opening reaction as well as for the loss of the glycolate ligand are reported in Figure S2 of the Supporting Information.

It can be observed that the critical bond lengths in the transition states and in the intermediates, considering this model, do not change significantly.

2. Energetic Profiles of Hydrolysis Reactions of Nedaplatin in Acid Conditions. The degradation pathway of the second-generation drug Nedaplatin in acid conditions has also been investigated. In these conditions, the glycolate ligand results protonated. The investigated reaction paths are illustrated in Scheme 3.

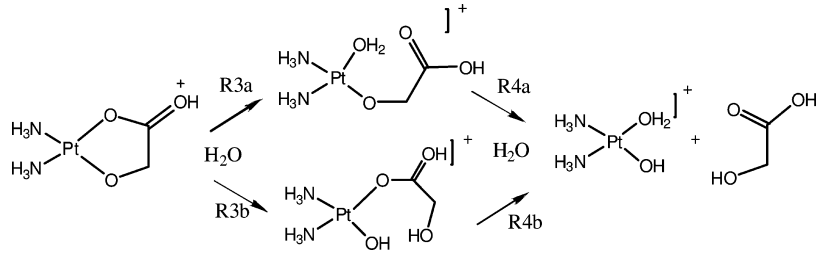
Also, in this case, the addition of the first water molecule can occur in two different ways, leading to the ring opening of the ligand. In step R3a, the detachment takes place by rupture of the bond that involves the oxygen in α to the protonated carbonyl group, while, in R3b, by breaking the other Pt−O bond. The following steps conduce to the loss of the protonated glycolate and to the formation of the products by steps R4a and R4b.

It is important to notice that two orientations of the protonated carbonyl group are possible for Nedaplatin. The most stable structure of the reagent has the proton oriented toward the oxygen in α to the carbonyl, and in our calculations, we considered this one.

The potential energy profiles for the hydrolysis processes in acid conditions are depicted in Figure 5.

The activation barriers for the first aquation processes are substantially different in acid conditions and the ring-opening caused by a water molecule entering on the same side of the protonated carbonyl group (R3a) results favored. Therefore, in acid condition, the rupture of the bond that involves the oxygen

SCHEME 3: Investigated Raction Pathways for the Hydrolysis of Nedaplatin in Acid Conditions



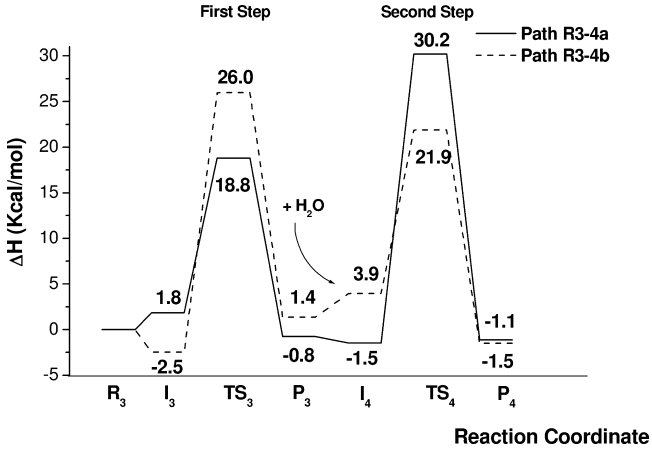


Figure 5. Activation enthalpy profiles (at 298.15 K), for the hydrolysis of Nedaplatin in acid conditions, in the water phase.

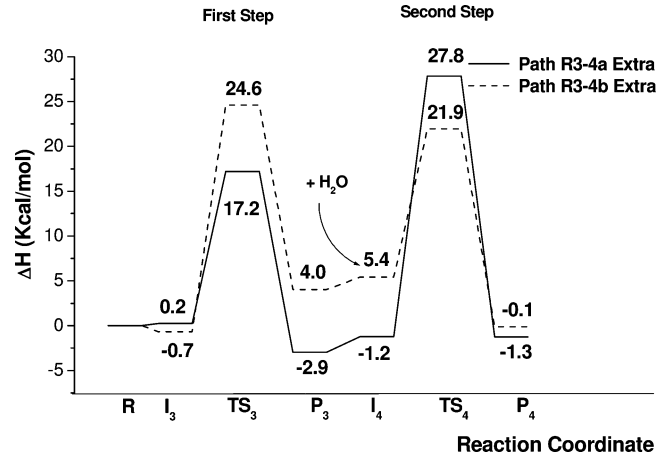


Figure 6. Activation enthalpy profiles (at 298.15 K) for the hydrolysis of Nedaplatin in acid conditions, with an extra water molecule in the system. The energies refer to the water phase.

in α to the protonated carbonyl group seems to be unequivocally favored, requiring 18.8 kcal/mol instead of 26.0 kcal/mol, which is necessary for the other path.

The optimized structures of the stationary points along R3a are reported in Figure S3a of the Supporting Information, while those relative to the R3b path are reported in Figure S3b.

It can be observed that when the reacting water molecule approaches the platinum center, the protonated carbonyl group changes its orientation to minimize the repulsion, adopting the other diastereoisomeric form. The distance of the water molecule from the metallic center in the intermediate structure is 3.85 Å and becomes 2.50 Å in the transition state. Also, in this case, the transition state results in a penta-coordinated-like structure with the entering and leaving group equidistant from platinum. The imaginary frequency for this transition state is 170i cm^{-1}. The activation barrier is lower than the neutral one, by 8.3 kcal/mol.

The next step leads to the release of the fully protonated glycolate ligand that takes place as the second water molecule approaches the metal. Our results show that, since the most stable path for the first step in acid condition is R3a, the product obtained in that step reacts with the second water molecule, leading to the loss of the ligand following path R4a. As can be seen from Figure 5, the second hydrolysis process is highly unfavorable, being the rate limiting step of the reaction. The optimized structures along path R4a are shown in Figure S4a of the Supporting Information, while stationary points along the R4b path are reported in Figure S4b.

In the intermediate structure, the water molecule approaches the platinum center with a distance of 3.62 Å interacting also with the oxygen bonded to the metal (1.77 Å). In the transition state, that distance become shorter, reaching 2.42 Å and the analysis of the obtained vibrational frequency (250 cm^{-1}) clearly indicates the rupture of the $Pt-O_L$ bond and the formation of the new $Pt-O_W$ one. The final product is obtained with the release of the ligand, and a proton transfer from the water molecule to the leaving group is observed. These results indicate that the rate limiting step under acid conditions is the loss of the ligand and suggest that probably Nedaplatin reaches DNA in its monohydrated form.

Also, in this case, we considered the influence of an explicit extra water molecule included in the considered reactions. The obtained results (Figure 6), for the first and the second hydrolysis processes, demonstrate that the main effect is observable, again, in the energetics of the process, lowering the activation barriers, while no significant changes are appreciable in the optimized geometries and, above all, in the trend of the reaction. In particular, the barriers for the preferred path are now 17.2 and 21.9 kcal/mol for the first and second hydrolysis processes, respectively.

The optimized structures of the stationary points for the ring-opening reaction as well as for the loss of the ligand are reported in Figure S5 of the Supporting Information section.

3. Comparison with Other Pt-Containing Anticancer Drugs. Previous studies on Pt(II)-containing anticancer drugs[12,22,32,34,37] reported that these kinds of complexes degrade, as Nedaplatin, according to a biphasic process and the mono- or diaquated form could reach DNA. Therefore, we can make a direct comparison between the results presented in this work and the other ones. To do this, we need to consider the activation barriers computed as the difference from the reactant molecules, for the first hydrolysis process, and, for the second one, from the product obtained in the previous step and the nucleophilic water molecule. In this way, we can report consistent values, that are collected in Figure 7. Moreover, this way to proceed is well accepted and several works concord with this way to reproduce activation energies.[34,37]

Note that, although several computational studies have been done with the aim of contributing to the common effort of deriving a better understanding of how Cisplatin reaches DNA, it is still not established whether the monoaquated or diaquated complex acts as an alkylating species on DNA. In Figure 7, we report the data obtained in a similar computational work in order to make a direct comparison, but other studies arrived at different conclusions predicting a similar activation barrier for both the hydrolysis step of Cisplatin and suggesting the diaqua complex as an active species.[34,37] Comparison of the neutral hydrolysis barriers for Carboplatin, Oxaliplatin, and Nedaplatin reveals that the rate limiting step is the ring opening, suggesting that the second- and third-generation analogues of Cisplatin should reach DNA in their fully hydrolyzed forms. Moreover, the Cisplatin-like compounds show all slower hydrolysis rates compared to Cisplatin due to the introduction of the kinetically less labile carboxylate, oxalate, and glycolate and to the presence of a large group in the NH_3 position in the case of Oxaliplatin. Therefore, a slower hydration could be the reason for the lower side effects displayed from the second- and third-generation anticancer drugs. It is important to note that this consideration can be done according to all of the activation energies proposed for the hydrolysis of Cisplatin and not only to the values reported in Figure 7.[34,37]

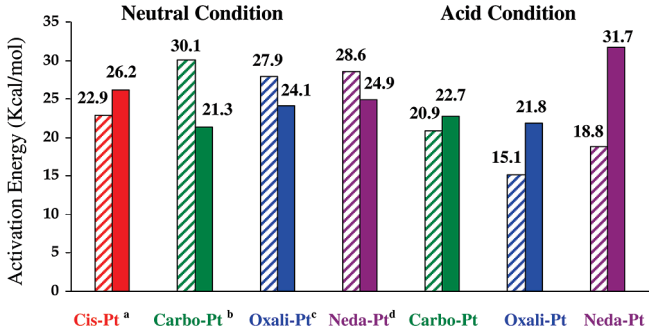


Figure 7. Comparison between calculated activation energies for Cisplatin, Carboplatin, Oxaliplatin, and Nedaplatin in neutral and acid conditions. Note that the striped column indicates the first aquation process, while the filled column indicates the second aquation process. [a]From ref 32. [b]From ref 12. [c]From ref 22. [d]From this work.

In acid conditio,n we can observe that, again, Carboplatin and Oxaliplatin follow the same trend of Nedaplatin, with the loss of the ligand becoming the rate limiting step. Nevertheless, there are some differences; in the case of Carboplatin, the activation barriers are similar, so the possibility that the diaqua species reacts with DNA cannot be excluded, while for Oxaliplatin and Nedaplatin the gap became greater, suggesting that they reach DNA monohydrated.

Conclusions

In this work, we have performed a detailed mechanistic study of the hydrolysis reactions of the second-generation anticancer drug Nedaplatin by means of density functional theory and employing the CPCM approach in order to take into account the bulk solvent effect. Both neutral and acid conditions have been studied in order to establish the most favorable energy path. The degradation mechanism of Nedaplatin proceeds via a collision between the reactant with two consecutive nucleophilic species attacking the metal center to release the ionic ligand, by a classical second-order nucleophilic substitution (S_N2) reaction. The transition states found for each step of the reaction show a pentacoordinated structure, suggesting that the associative mechanism may be preferred.

In the first step of the reaction, the detachment of the ligand can occur in two different ways, by rupture of the bond that involves the oxygen in α to the carbonyl group or by breaking the other Pt−O bond. The latest one, in neutral conditions, should be the preferable path with an activation barrier of 28.6 kcal/mol and the product obtained in this step reacting with another water molecule, leading to the formation of the *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ complex. In acid condition, we were able to ascertain that the reaction begins with the rupture of the ligand between the metal center and the oxygen in α to the carbonyl group and proceed with the loss of the ligand. In those conditions, the second hydrolysis process is the rate limiting step with an activation barrier of 31.7 kcal/mol.

In order to verify the possible influence of an extra water molecule which could assist the hydrolysis processes, we conducted an additional study including in our systems another water molecule acting as explicit solvent, obtaining for the reactions the same trends. That model incorporates the effects of hydrogen bonding and provides a more accurate description of the stabilization of the leaving group.

A comparison between Nedaplatin and the other Pt(II)-containing anticancer drugs currently used in the medical protocols reveals some common trends. In neutral condition, in analogy with the other second-generation anticancer drug Carboplatin and with the third-generation drug Oxaliplatin, the rate limiting step is the first aquation process. We can therefore hypothesize that the fully hydrolyzed complexes should be the main products reacting with DNA. In acid condition, again, Carboplatin, Oxaliplatin, and Nedaplatin reveal the same behavior, and in these conditions, they are supposed to reach DNA in their monoaquated form.

Supporting Information Available: Figures showing optimized structures of the stationary points along various reaction pathways. This material is available free of charge via the Internet at http://pubs.acs.org.

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