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Stability and proton transfer in DNA base pairs of AMD473–DNA adduct

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ABSTRACT

We investigate the energetics of four different adducts of cisplatin analogue *cis*-[$PtCl_2(NH_3)$(2-picoline)] (AMD473) with a duplex DNA using DFT/ONIOM methods to probe their stabilities. Further, we study the possibilities of proton transfer between DNA base pairs of the most stable drug–DNA adduct. The adduct **b** (2-picoline *trans* to 3′-G and 2-methyl group directs to the DNA major groove) is found to be the most stable configuration among all the possible adducts. From the proton transfer analysis we found that the single proton transfer between N1 position of guanine (G) and N3 position of cytosine (C) of each GC pair gives a structure energetically as stable as the original one.

1. Introduction

Cisplatin [1], is one of the most widely used anticancer drugs and is particularly active in treating several kinds of cancer, such as testicular and ovarian cancers. Although cisplatin is one of the most successful anticancer drugs, its toxic side effects, intrinsic and acquired cellular resistance and limited solubility in aqueous solution have motivated searches for structurally and functionally analogues alternatives. In this way, second- and later third-generation drugs (like carboplatin, oxaliplatin and dinuclear- or trinuclear species) were discovered. Unfortunately, these drugs also suffer from resistance and other side effects [2,3]. One new promising anticancer agent, *cis*-[$PtCl_2(NH_3)$(2-picoline)], known as AMD473, has now entered the worldwide phase II and III clinical trials [4]. This orally administrated drug is less toxic than cisplatin and possesses activity against cisplatin-resistant cell lines. Specifically, the N7 atom of purine bases is the main binding site, with guanine being preferred over adenine. Indeed, as water is more labile than chloride, the reactive form of these molecules are believed to be the aqua species, which results from substitutions of one or both the chloride leaving groups by water molecules. Among several possible adducts, GpG adducts being the major and ApG cross-links being the next most abundant products.

Over the last few years, considerable theoretical efforts have been focused on cisplatin–DNA interaction to provide detailed insight into the binding mechanism at molecular level. There is a large amount of modeling studies on electronic structure and spectral analyses [5,6], structure–activity studies [7–9], aquation processes [10–14] structural properties of cisplatin–DNA complexes [15–19], effect on DNA base pairing [20,21] and chemical reactions responsible in developing toxic side-effects and resistance [22,23] of cisplatin and congeners. Despite all the effort in understanding cisplatin reactions and to some extent carboplatin [24] and oxaliplatin [25], a very few studies are devoted on binding mechanism of sterically hindered drug AMD473 [26,27] and its interaction with DNA [28].

Due to the asymmetric structure of AMD473 it can form four stereoisomers with DNA. Chen et al. [29] performed NMR studies of these four stereoisomers and reported that the reactions of AMD473 with the 14-mer DNA duplex give predominantly a single stereoisomer. In this study we have investigated the stabilities of these AMD473–DNA adducts by discussing the energies and structural differences between these adducts in detail. Further we have investigated the possibilities of proton transfer between DNA bases of the most stable drug–DNA adduct.

2. Methodology

The calculations are based on the experimental NMR structure [30] (PDB: 1A84)5−d($C_1C_2T_3C_4T_5G_6$*G_7*$T_8C_9T_{10}C_{11}C_{12}$)3−d($G_{24}G_{23}A_{22}G_{21}A_{20}C_{19}C_{18}A_{17}G_{16}A_{15}G_{14}G_{13}$) in which we have manually replaced amine ligand with 2-picoline group. The NMR solution structure of AMD473 with DNA is the A.T rich 14-mer DNA duplex [29]. We have used the cisplatin–DNA duplex calculated from the NMR data [30], as it is reported that this duplex has essentially the same structural characteristics as the platinated 14-mer DNA structure [29]. Four different models of drug–DNA adducts are, **a** (2-picoline *trans* to 3′G_7 and 2-methyl group directs to the DNA backbone), **b** (2-picoline *trans* to 3′G_7 and 2-methyl group directs to the DNA major groove center), **c** (2-picoline *trans* to 5′G_6 and 2-methyl group directs to the DNA backbone) and **d** (2-picoline *trans* to 5′G_6 and 2-methyl group directs to the DNA major groove center). All the structures were solvated by 600 water molecules. Sodium counter ions were used to balance the DNA backbone charges. We adopted QM/MM based ONIOM method to optimize

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Table 1
Binding energies (BE) and optimized geometries[a] of the four adducts.

	a	b	c	d
BE (kcal/mol)	742.47	749.36	717.82	723.96
Pt–$N7_{amine}$	2.03	2.04	2.03	2.03
Pt–$N7_{pico}$	2.02	2.01	2.01	2.02
Pt–$N7G_6$	2.04	2.01	2.02	2.02
Pt–$N7G_7$	2.03	2.02	2.01	2.03
$N7G_6$–Pt– $N7G_7$	85.3	89.0	86.4	85.4
$N7_{amine}$– Pt– $N7_{pico}$	88.6	91.5	91.3	88.0

[a] The unit of distance is angstroms, the unit of angle is degrees.

the final configurations of 4 adducts where QM (high level) includes *cis*-$[Pt(NH_3)(2\text{-picoline})]^{2+}$ part, G_6 and G_7 while the remaining DNA bases, sugar-phosphate backbone and water molecules were treated with UFF (low level). On the basis of ONIOM method, the total energy of the entire system can be obtained from three separate calculations:

$$E^{\mathrm{ONIOM2}} = E^{\mathrm{low}}_{\mathrm{real\ system}} + E^{\mathrm{high}}_{\mathrm{model\ system}} - E^{\mathrm{low}}_{\mathrm{model\ system}}$$

The *real* system represents the full molecular geometry including all atoms and it is treated using a low-level of theory. The *model* system contains the part of the system that is treated at the high level. Superscripts 'high' and 'low' mean high- and low-level of calculations used in the ONIOM method.

All the structures were optimized by HF and DFT methods with BHandH [31] and mPW1PW91 [32] functionals using GAUSSIAN 03 program [33]. We have used LanL2DZ basis set as described by Hay and Wadt [34] to treat Pt whereas all other atoms of the

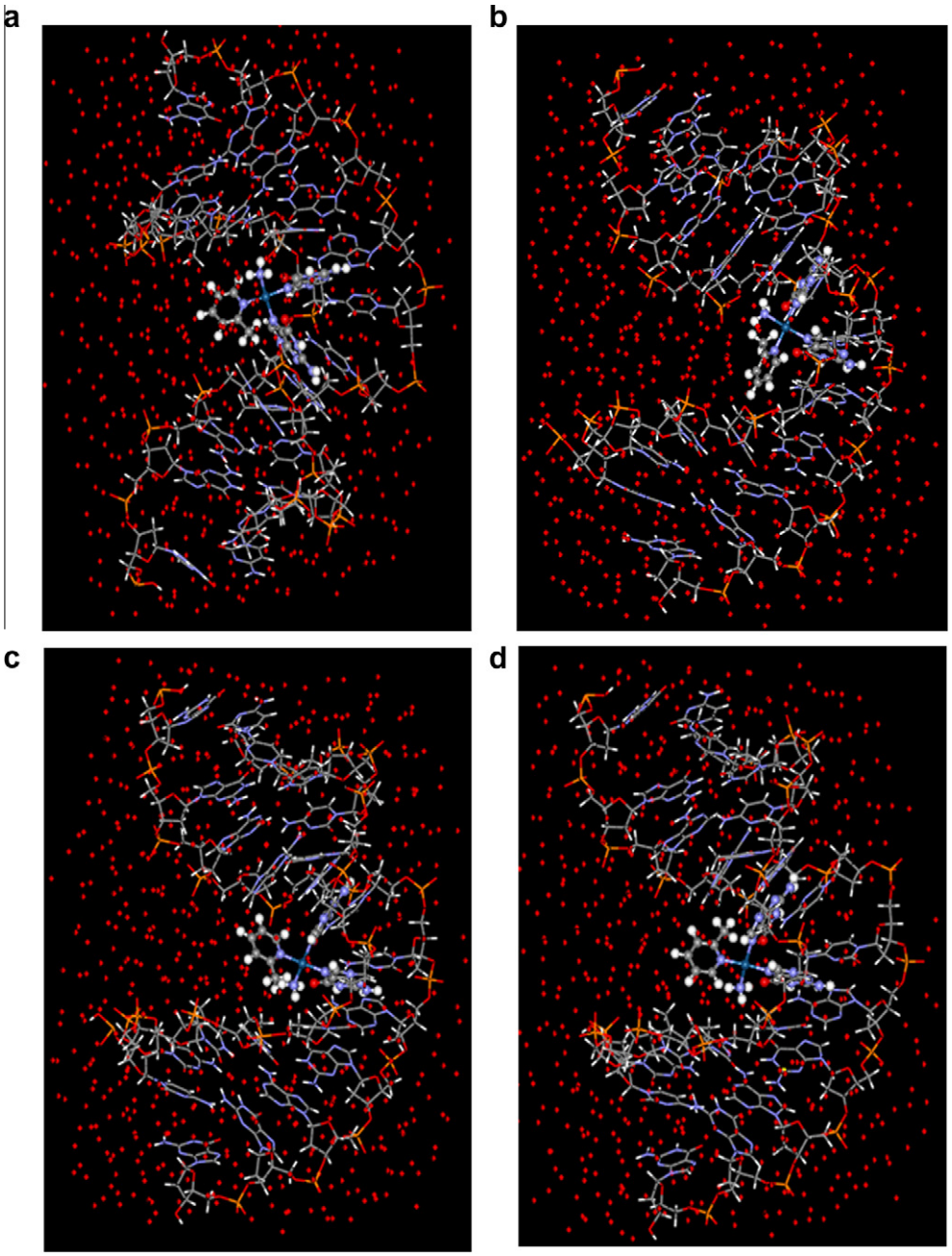

Figure 1. Structures of the AMD473 drug–DNA adducts optimized by QM/MM method. Drug, G_6, and G_7 belong to the QM region (in balls and sticks), the rest of the DNA and the solvent (only oxygen atoms are shown for clarity) (in lines) belong to the MM region.

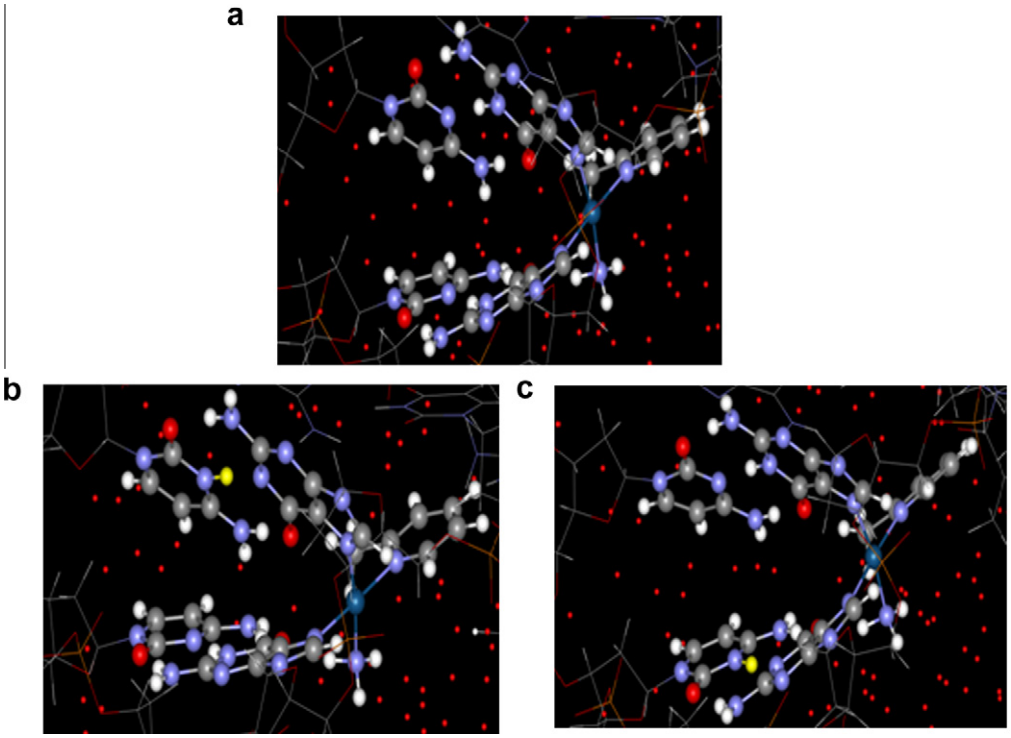

Figure 2. The optimized structures of (1) *cis*-(G_6C)–Pt–(G_7C), (2) *cis*-($G_6^{PT}C$)–Pt–(G_7C), and (3) *cis*-(G_6C)–Pt–($G_7^{PT}C$) of AMD473–DNA adduct. The transferred proton is represented by yellow colour. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

complexes were treated with 6-31G(d,p). In proton transfer calculations QM part has been extended up to two base pairs (G_6C and G_7C) and the other atoms were treated with lower level. The binding energies of real system at low level and model system at high and low levels are calculated from single point calculations of their optimized geometries. These values are then used to calculate the total binding energies of the structures **a–d**.

3. Results and discussion

According to the results obtained by QM/MM method for all configurations calculated at different levels of theory, we found model **b** as the lowest energy structure. The *cis*-[$PtCl_2(NH_3)$(2-picoline)G∗G∗] (QM part) moiety of the adduct **b** also has the lowest energy which suggests that this adduct has the most stable configuration. This observation is in agreement with the experimental studies by Chen et al. [29] where they found the favorable formation of configuration **b** from both thermodynamic and kinetic factors. This is mainly due to the steric selection of picoline ring which fits perfectly into the major groove of the DNA duplex. It is observed that there is an energy difference of 9.4 kcal/mol between **a** and **b** at BHandH level whereas the model **b** is almost 52.6 and 22.5 kcal/mol stable than **c** and **d**, respectively, obtained at the same level. Despite the energy differences, different level of theories predict similar trend of stability as **b** > **a** > **d** > **c**.

Although the energies calculated at mPW1PW91/[LANL2DZ-ECP+6-31G(d,p)] level have the lowest values, all the results along with the proton transfer analysis are discussed at BHandH/[LANL2DZ-ECP+6-31G(d,p)] level, as BHandH functional is shown to be efficient for the study of π-stacked systems like DNA. The binding energies for the bifunctionals adduct of AMD473 with DNA, along with other geometrical parameters are presented in the Table 1. From the calculated binding energies, we found **b** as the most stable structure with the highest value. It is seen that the model **a** with binding energy 7 kcal/mol lower, being the next stable structure. Comparing the values, the binding energy of the less stable structures **c** and **d** are about 31 and 25 kcal/mol lower than that of the structure **b**.

The optimized structures of four possible adducts of AMD473 drug with DNA are presented in Figure 1. The picoline ring forms (H_3C)C–N(picoline)–Pt–N(ammonia) dihedral angle equal to 111.7° in model **a**. The deviation of the picoline ligand from the perpendicular orientation with the Pt square plane shortens the distance between amine and the 2-methyl of the picoline group. The angles between Pt square plane and the planes of G_6 and G_7 are 61° and 73.2°, respectively.

In **b**, the picoline ring fits perfectly into the DNA major groove and lies along the phosphate backbone in accordance with the experimental observation [29]. The picoline ring is found to be perpendicular to the molecular plane with the dihedral angle equal to 102.3° and H_3C– Pt distance equal to 3.19 Å in agreement with previous theoretical and experimental results [35,36]. The angle between picoline ring and G_7 is 57.2° whereas it forms an angle of 77.8° with G_6. The distance between a hydrogen atom of the amine and the $06G_7$ is 1.91 Å and N–H–O is 150.3°, which indicates that a hydrogen bond exist, indeed. This gives an additional stability to the model. The Pt–$N7_G$ bond is also slightly shorter and stronger in **b** compared to that of the other structures (Table 1). The steric hindrance provided by the axial 2-picoline ligand in **b** is more prominent than that in others and thus the activity against cisplatin-resistant cell lines is more effective. The high stability of **b** may be a reason behind the lack of cross-resistance between AMD473 and cisplatin. In **c**, the angle between the Pt square plane and picoline ligand is larger (113.5°) than that of **a** and **b** and this increases the distance between amine and the 2-methyl of picoline group. Model **d** has the angle of 117.6° between the Pt square plane and picoline ring. The 2-picoline ligand forms an angle of ~90° with the planes of G_6 and G_7 bases in both models **c** and **d**. The perpendicular orientation of picoline ring to the planes of nearby

Table 2
Hydrogen bond distances (Å) of experimental and computed non-proton transferred and single proton transferred structures.

	cis (G_6C)–Pt–(G_7C)		*cis* ($G_6^{PT}C$)–Pt–(G_7C)		*cis*(G_6C)–Pt–($G_7^{PT}C$)		1A84[30]	
	G_6C	G_7C	G_6C	G_7C	G_6C	G_7C	G_6C	G_7C
O_6–N_4	2.79	2.84	2.70	2.87	2.86	2.69	2.90	2.90
N_1–N_3	2.8	2.82	2.70	2.83	2.83	2.74	2.53	2.93
N_2–O_2	2.72	2.69	2.81	2.70	2.71	2.89	2.34	2.84

Table 3
The sum of NBO charges on bases and ligands.

	cis (G_6C)–Pt–(G_7C)	*cis* ($G_6^{PT}C$)–Pt–(G_7C)	*cis*(G_6C)–Pt–($G_7^{PT}C$)
G_6 or G_6^{PT}	0.211	−0.491[a]	0.213
G_7 or G_7^{PT}	0.327	0.231	−0.466[a]
$C(G_6)$	0.054	0.782[b]	0.080
$C(G_7)$	0.008	0.103	0.807[b]
NH_3	0.273	0.244	0.248
2-picoline	0.280	0.261	0.253

[a] The sum does not contain transferred H atom.
[b] The sum contains transferred H atom.

Table 4
Energetic of the PT products (kcal/mol).

cis (G_6C)–Pt–(G_7C)	0
cis ($G_6^{PT}C$)–Pt–(G_7C)	+2.3
cis(G_6C)–Pt–($G_7^{PT}C$)	+1.5

bases, resulting in an unfavorable steric interaction which destabilizes the adducts. Thus from the observations, concerning both energetical and structural properties of the four models, we found **b** as the most stable configuration.

Further, we have investigated the energetic of intermolecular proton transfer (PT) in Watson–Crick base pairs by considering the most stable drug–DNA adduct (**b**) in water environment. This investigation may allow us to reveal a realistic picture of mispairing of base pairs which leads to the mutation of DNA. We observed that the adduct undergoes single proton transfer between N1 (G) and N3 (C) while the simultaneous single proton transfer in two stacked base pairs is not found to be stable. This is in agreement with the previous study on four base pairs model of cisplatin guanine adduct [21]. Figure 2 shows the optimized structures of (1) *cis* (G_6C)–Pt–(G_7C), (2) *cis* ($G_6^{PT}C$)–Pt–(G_7C) and (3) *cis* (G_6C)–Pt–($G_7^{PT}C$), where G_6^{PT} and G_7^{PT} denotes the proton donor guanine.

The drug binding brings some changes in base-pairing geometries of all the bases in the QM region. This is expected from previous studies on smaller model complexes, which report strong perturbation of the base-pairing by cation binding. Although the G_7C pair slightly deviates from planarity, the other G_6C pair greatly distorted. This may be due to the *cis* orientation of G_6C pair with respect to the bulky 2-picoline ring. Table 2 summarizes the hydrogen bond distances of all the optimized structures and compares them with the experimental data of base pairs (NMR structure, PDB: 1A84). The distance between O_6 (G) and N_4 (C) reduces by about 0.09 and 0.15 Å in the proton transferred GC pairs of (2) and (3), respectively, in comparison with the original GC pairs (1). The hydrogen bonding length between N_1 (G) and N_3 (C) also reduces whereas the hydrogen bond N_2–O_2 increases. Similar trends have been observed in the previous theoretical study.

To analyze the changes produced on the charge distribution of the QM part upon proton transfer, we present in Table 3 the sum of the NBO (Natural Bond Orbital) charges on the bases and the ligands. The QM regions are extracted from their corresponding overall structures, link atoms replaced with hydrogen and then single point calculations are carried out to calculate the NBO charges. The guanine bases in (1) are positive whereas the cytosine bases remained almost neutral. The positive value of G is mainly due to the charge transfer from the bases to Pt atom of the drug. After proton transfer, the sum of the charges on G in (2) and (3) becomes negative. On the other hand, the entire charge on C in PT products has a positive value which causes a charge separation between G and C leading to a stable $G^{PT}C$ pair due to Coulomb attraction between them. The simultaneous single proton transfer has been prevented since the two stacked guanine bases become negatively charged and both the cytosine bases bear positive charge which results Coulomb repulsions among the bases.

The energetic details of the proton transferred products are given in Table 4. We observed that the single proton transfer between G and C in which the guanine molecule is *trans* to 2-picoline group (3) is energetically more favorable than that of *trans* to amine (2). This difference is may be due to their difference in planarity. The *cis* (G_6C)–Pt–(G_7C) and *cis*(G_6C)–Pt–($G_7^{PT}C$) pairs are almost equal in energy. The energy differences between the products (2 and 3) and the original structure (1) are about 2–1.3 kcal/mol.

4. Conclusions

Our calculations analyze the stabilities of four stereoisomers formed by binding of AMD473 with DNA. The orientations of picoline group with respect to the planes of two platinated guanine bases destabilize the adducts **c** and **d** compared to **a** and **b**. The picoline plane is almost perpendicular to the Pt square plane in **b** and the picoline ligand perfectly fits into the DNA major groove. Also there is a favorable hydrogen bonding between NH_3 and O_6 of G_7 in model **b**. All these features enhance the stability of **b** than the other configurations. Also from the calculated energetics we found **b** as the most stable adduct. The conclusions obtained from intermolecular proton transfer reactions in DNA base pairs are that no simultaneous single proton transfer is energetically found to be stable while *cis*(G_6C)–Pt–($G_7^{PT}C$) pair is as stable as the original structure. The small difference in energy between the proton transferred products and the original one explains the influence of drug binding to induce DNA damage through base pair modification.

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