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Cisplatin-related drugs for nongenomic targets: Forcing the reactivity with nucleobases*

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Abstract: The products obtained by forcing the reaction with nucleosides (guanosine, Guo, and adenosine, Ado) of potential anticancer drugs for nongenomic targets [PtCl(*O*,*O*′-acac)(L)] (L = dimethyl sulfoxide, DMSO; dimethyl sulfide, DMS), closely related to their very powerful organometallic analogues [Pt(*O*,*O*′-acac)(γ-acac)(L)], have been studied. [PtCl(*O*,*O*′-acac)(L)] and [Pt(*O*,*O*′-acac)(γ-acac)(L)] complexes were reported unreactive toward nucleobases. Aquo species $[Pt(O,O'\text{-acac})H_2O(L)]^+$, obtained from [PtCl(*O*,*O*′-acac)(L)] by Ag^+ driven coordinated Cl^- removal, gave access to [Pt(*O*,*O*′-acac)(L)(nucleoside)]$^+$ ([Pt(*O*,*O*′-acac)(DMSO)(Guo)]$^+$, [Pt(*O*,*O*′-acac)(DMS)(Guo)]$^+$, [Pt(*O*,*O*′-acac)(DMSO)(Ado)]$^+$). The effect of the chelate oxygen donor acac (with respect to a chelate diammine), the role of the sulfur ligand (DMSO, DMS), and the influence of the purinic nucleoside itself on the coordinated Guo or Ado dynamic motions in [Pt(*O*,*O*′-acac)(L)(nucleoside)]$^+$ complexes have been investigated by NMR spectroscopy. Interestingly, a slow rotation of nucleobase around the Pt–N(7) bond with formation of two rotamers was observed already at room temperature only in the case of [Pt(*O*,*O*′-acac)(DMSO)(Guo)]$^+$. On the other hand, no hindered rotation at room temperature was detected in the analogous [Pt(*O*,*O*′-acac)(DMS)(Guo)]$^+$ and [Pt(*O*,*O*′-acac)(DMSO)(Ado)]$^+$ complexes. Data suggest that rotation of the nucleoside in [Pt(*O*,*O*′-acac)(L)(nucleoside)]$^+$ is very different with respect to the analogous [Pt(diammine)(L)(nucleoside)]$^{2+}$ systems, due to specific interactions between the acac chelate ligand, the DMSO, and the nucleobase.

Keywords: bioinorganic chemistry; cisplatin analogues; coordination chemistry; drug design; electronic interactions; NMR; nucleosides; platinum; platinum acac complexes; rotamers; steric interactions; sulfur ligands.

INTRODUCTION

Since its first clinical use, cisplatin is still one of the most widely used drugs in anticancer chemotherapy, and its mechanism of action has been explained in all essential aspects related to interaction

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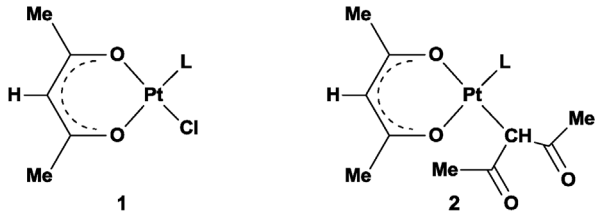
with DNA [1]. It is generally accepted that the main cellular target of cisplatin is nuclear DNA, where the drug binds mainly to the N(7) of purine bases, forming stable inter- and intra-strand cross-links. Nevertheless, some key chemical processes, taking place before cisplatin reaches DNA, are still to be clarified. Indeed, many other nongenomic biomolecules could be potential targets for platinum [2,3]. Sulfur-rich biomolecules, including free amino acids (cysteine and methionine), oligopeptides (glutathione), and proteins represent good targets for a soft metal such as Pt [4,5]. On the other hand, the need to improve the cisplatin clinical therapy drives much research into better understanding of its antitumor activity mechanism [6]. Moreover, in order to overcome acquired cellular resistance to cisplatin, many efforts are currently devoted to the discovery of new Pt anticancer drugs. In the last decades, over 3000 Pt(II) and Pt(IV) complexes have been synthesized and tested for their biological activity but, at present, only a few compounds are registered as marketed drugs (cisplatin, carboplatin, oxaliplatin, and nedaplatin) and only one compound (oxaliplatin) has been approved by the U.S. Food and Drug Administration (FDA) (for colorectal cancer) since the release of cisplatin and carboplatin [1,7,8]. Many studies [9–11], some carried out by our research group [12,13], aimed to understand not only the nuclear but also the cytoplasmic events taking place in cisplatin-treated cells and able to induce apoptosis. Our research has long been involved in both (i) the synthesis and preliminary evaluation of biological activity of new Pt complexes and (ii) the subsequent studies of intracellular signal transduction, triggered by these molecules and by cisplatin itself [3,14–16]. Recently, we have synthesized and studied new Pt(II) complexes containing acetylacetonate ligand (acac) in the metal coordination sphere: [PtCl(*O*,*O*′-acac)(DMSO)] (**1a**) (DMSO = dimethyl sulfoxide) with only one oxygen-bonded chelate acac (*O*,*O*′-acac) and [Pt(*O*,*O*′-acac)(γ-acac)(DMSO)] (**2a**) containing both an *O*,*O*′-acac and a σ-bonded acac (γ-acac) together with their dimethyl sulfide (DMS) analogues (**1b** and **2b**) having the same key structures (Scheme 1).



Scheme 1 Chemical structure of *O*,*O*′-chelated acac complexes: **1a** and **2a**, L = DMSO; **1b** and **2b**, L = DMS.

Since complexes **1a,b** and **2a,b** were all reported unreactive toward nucleobases, we studied, by NMR spectroscopy, the products obtained by forcing the reaction of **1a,b** with nucleosides (guanosine, Guo, and adenosine, Ado). Access to the target complexes $[Pt(O,O'\text{-acac})(L)(\text{nucleoside})]^+$ (L = DMSO, nucleoside = Guo, **4a**; L = DMS, nucleoside = Guo, **4b**; L = DMSO, nucleoside = Ado, **5a**) was gained by Ag^+ driven coordinated Cl^- removal from **1a,b**. The role of the chelate oxygen donor ligand (acac with respect to ethylendiammine), the effect of the sulfur ligand (DMSO, DMS), and the influence of the purinic nucleoside itself (Guo or Ado) on the coordinated nucleosides rotational conformer distribution have been investigated, and the results are here reported.

BIOLOGICAL ACTIVITY OF THE Pt(II) COMPLEXES WITH *O,O′*-ACAC AS CARRIER LIGAND

The new complexes have shown interesting biological activities (including in vitro antimetastatic) [17–21]. The [Pt(*O*,*O*′-acac)(γ-acac)(DMS)] complex (**2b**), an organometallic species with two acetylacetonate ligands, one *O*,*O*′-chelate and the other σ-linked to the metal by the methine, is the more

active among the tested complexes. This compound not only is able to induce apoptosis in endometrial cancer cells (HeLa), with activity up to about 100 times higher than that of cisplatin, but also shows high cytotoxicity in cisplatin-resistant breast cancer cell lines (MCF-7) [18,19]. It rapidly produces a sustained apoptotic response characterized by: mitochondrial depolarization, cytosol accumulation of cytochrome c, proapoptotic translocation of proteins (Bax and truncated form of Bid) from cytosol to mitochondria, activation of caspase-7 and -9, chromatin condensation, DNA fragmentation, and generation of reactive oxygen species, ROS. Moreover, **2b** is able to increase the level of free $[Ca^{2+}]_i$ in MCF-7 cells, altering the homeostasis of Ca^{2+}, an effect that is likely to be linked to its ability to trigger rapid apoptosis [22]. Differently from cisplatin, whose activity appears to be associated with cellular accumulation and DNA linking, the cytotoxicity of the new compounds is related to intracellular accumulation only [18]. By atomic absorption spectrometry, the total cellular and nuclear Pt contents for cisplatin and **2b** in HeLa and MCF-7 cells were determined. Interestingly, using doses that give the same intracellular Pt content (100 and 17 μM for cisplatin and **2b** in HeLa cells; 100 and 10 μM for cisplatin and **2b** in MCF-7 cells), even showing a similar distribution in the nucleus with respect to cisplatin, **2b** resulted in being unable to link DNA. However, at the same doses and for the same incubation times, **2b** retains its cytotoxic effect on cancer cells higher than cisplatin [18,19]. Moreover, the well-known Salmonella-*his* reversion test (Ames' test, a standard reverse mutation assay on the mutagenic capability of the complexes) on two *Salmonella typhimurium* strains, TA98 and TA100, were performed in order to assess the mutagenic capability of the new complexes, using cisplatin as positive control. Indeed, the bacteria-reversed mutation assay (Ames' test), which is normally used to evaluate the mutagenic properties of test substrates, can be also used to assess the ability of tested compounds to interact with DNA. Interestingly, whereas cisplatin exhibits the well-known mutagenic activity, the new complexes do not show the presence of significant revertants colonies even at the highest tested doses [23]. In addition to the cytotoxic studies, the in vivo effects of **2b** on the CNS (central nervous system), during rat postnatal development, were also investigated. This was done in order to compare the neurotoxicity of novel Pt compound with respect to cisplatin and to identify cellular events associated with this phenomenon. Although brain Pt content is notably higher after treatment with **2b** than after comparable cisplatin administration, the new complex induces less severe changes on the fundamental events of neuroarchitecture development. No high apoptotic events, less-altered granule cell migration, and Purkinje cell dendrite growth indicate a low neurotoxicity of **2b** for normal CNS [24–26]. On the other hand, further studies on the cytotoxic effects and intracellular transduction apoptosis mechanism of **2b** on brain cancer cells (SH-SY5Y human neuroblastoma cell line) show cytotoxicity on neuroblastoma cells with effects ca. 10-fold greater than that observed for cisplatin [27]. The broad in vitro cytotoxic activities and the mild in vivo neurotoxicity propose the organometallic species **2b** as a potential alternative to cisplatin, indicating that the development of a new Pt complex with different subcellular targets with respect to cisplatin could be a strategy to overcome resistance and to prevent severe side effects associated with the clinical therapy of Pt compounds. All these data suggest that the cytotoxicity mechanisms of the new β-diketonate complexes may not necessarily require interaction with DNA and that their biological activity is connected to the reaction with nongenomic biological targets. Besides their specific biological activity, Pt(II) complexes with *O*,*O'*-acac carrier ligand (**1a,b** and **2a,b**) show an interesting and selective chemical reactivity toward nucleophiles with different HSAB (hard–soft acid–base) character [20]. These complexes show also selective substitution of DMSO or DMS with soft biological nucleophiles, such as L-methionine, and negligible reactivity with nucleobases (Guo and 5'-GMP). Interestingly, the closely related to **1a** $[PtCl(diammine)(sulfoxide)]^+$ [28] complexes are well known to react with DNA, and $[Pt(diammine)(sulfoxide)(nucleoside)]^{2+}$ species have been also studied as model molecules [29].

REACTIONS WITH NUCLEOBASES

The reaction of **1a** complex with nucleosides was performed in an NMR tube in CD_3OD and monitored by ^1H NMR spectroscopy, using Guo or Ado as nucleobase derivative. Although the reaction with the chloro species **1a** proved to be very difficult, nucleoside coordination on the related aquo species, [Pt(*O*,*O'*-acac)(DMSO)H_2O](NO_3) (**3a**) (obtained by reaction of **1a** complex with $AgNO_3$) to give [Pt(*O*,*O'*-acac)(DMSO)(Guo)]$^+$ (**4a**) or [Pt(*O*,*O'*-acac)(DMSO)(Ado)]$^+$ (**5a**) was almost immediate and quantitative. The selective ligand-exchange reactivity of **1a,b**, **2a,b** complexes toward nucleophiles (including biological, such as nucleobases derivatives and amino acids) was previously studied [20]. In the [PtCl(*O*,*O'*-acac)(L)] (L = DMSO, **1a**; DMS, **1b**) complexes, containing two ligands with different hard/soft character on the same metal, selective substitution reaction in the presence of a further ligand is observed. The more hard ligand replaces the harder one, and the more soft replaces the softer one. When only one soft exchangeable ligand is present, as for [Pt(*O*,*O'*-acac)(γ-acac)(L)] (L = DMSO, **2a**; DMS, **2b**) complexes, the reaction takes place only in the presence of soft nucleophiles, otherwise no reaction occurs. In the case of **1a,b** complexes, the replacement of Cl^- with hard ligands such as nucleoside is kinetically and thermodynamically less favored with respect to the substitution reaction of DMS or DMSO with soft-type ligands, therefore **4a** or **5a** was accessed via the aquo species **3a**. Accordingly, even in the presence of an excess free base, the possible formation of the bis substituted complex [Pt(*O*,*O'*-acac)(nucleoside)$_2$]$^+$ from [Pt(*O*,*O'*-acac)(DMSO)(nucleoside)]$^+$ was negligible, as detectable from the low concentration of free DMSO in solution. Relevant ^1H NMR data are reported in Table 1. The ^1H NMR spectrum of **4a** species in CD_3OD, in the presence of excess Guo (Fig. 1), showed the typical H8 signal for a purine base coordinated to Pt at 8.72 ppm, deshielded with respect to free base (7.98 ppm). The deshielding of ~0.7 ppm confirmed the coordination of the nucleobase through the N(7) nitrogen atom [30]. The presence of a single, slightly broad signal for the H8 signal could suggest fast rotation of Guo around the Pt–N(7) bond on the NMR time scale and the presence of a single species in solution.

Table 1 ^1H NMR chemical shifts of acac complexes with nucleosides in CD_3OD.

	H/Cγ	Me(acac)	DMSO/DMS	H8	H2	H1'
Guo				7.98 s		5.83 d
Ado				8.64 s	8.4 s	6.10 d
3a	5.83 s	2.10 s 2.07 s	3.35[22]* s			
3b	5.70 s	1.98 s 1.94 s	2.30[45]			
4a	5.86 d	2.14 s 2.02 s, 2.03 s	3.51 s 3.50 s 3.40 s 3.39 s	8.72 ps. s		5.92 ps. t
4b	5.78 s	2.06 s 1.97 s	2.41[20] s 2.39[20] s	8.61 s		5.93 d
5a	5.96	2.17 s 2.02 s	3.48 ps. s	9.50 s	8.53 s	6.20 d

$J_{H–Pt}$ (*) are reported in square brackets [Hz], where measurement was possible.

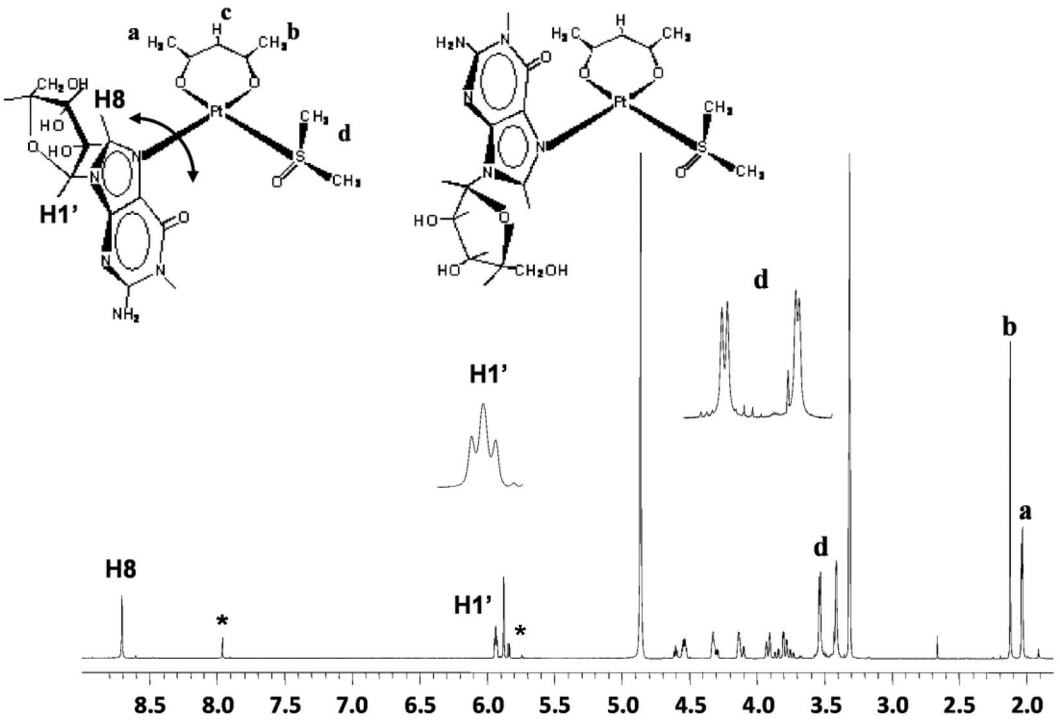


Fig. 1 ^{1}H NMR spectrum in CD_3OD of [Pt(*O*,*O'*-acac)(DMSO)(Guo)]$^{+}$ (**4a**) (* = free Guo).

A single H1' ribose proton doublet, and a single signal, or eventually a small diastereotopic splitting for the two methyl groups of DMSO, due only to the ribose chirality, could confirm the presence of a single species in solution. On the contrary, the ^{1}H NMR spectrum of **4a** showed at room temperature four different DMSO resonances (3.51, 3.50, 3.40, and 3.39 ppm) and three signals for the two methyls of acac chelate (one singlet at 2.14 ppm and two close singlets at 2.02 and 2.03 ppm, almost overlapping and integrating together as the former). Furthermore, the H1' signal of ribose (5.92 ppm) appeared as a pseudo triplet rather than a typical doublet caused by the single coupling with the H2' proton. All these data could be explained assuming that the metal-coordinated Guo has two possible orientations with respect to the Pt coordination plane. In this case, the magnetic inequivalence causing the diastereotopic splitting of the methyl groups of DMSO results by the asymmetry of the metal rather than the sugar chirality (far away from the DMSO ligand). In order to give complete assignments of the methyl groups, 2D [^{1}H,^{13}C]-HETCOR (heteronuclear correlation) and [^{1}H,^{13}C]-long-range HETCOR NMR experiments were performed. Figure 2 shows an expansion of the two NMR experiments, concerning the C–H correlations of the DMSO methyl groups. Both the methyl groups at 3.50 and 3.51 ppm on the ^{1}H axis showed a $^{1}J_{C-H}$ correlation peak with the carbon at 42.5 ppm and a $^{3}J_{C-H}$ long-range correlation peak with the carbon at 42.0 ppm on the ^{13}C axes; whereas both the methyl groups at 3.39 and 3.40 ppm on the ^{1}H axis exhibited a $^{1}J_{C-H}$ correlation peak with the carbon at 42.0 ppm and a $^{3}J_{C-H}$ long-range correlation peak with the carbon at 42.5 ppm on the ^{13}C axes. These patterns of signals suggested that two rotational conformers were present in solution and that the DMSO ligand of each conformer had one more deshielded and one more shielded methyl groups with a $\Delta\delta$ of 0.1 ppm.

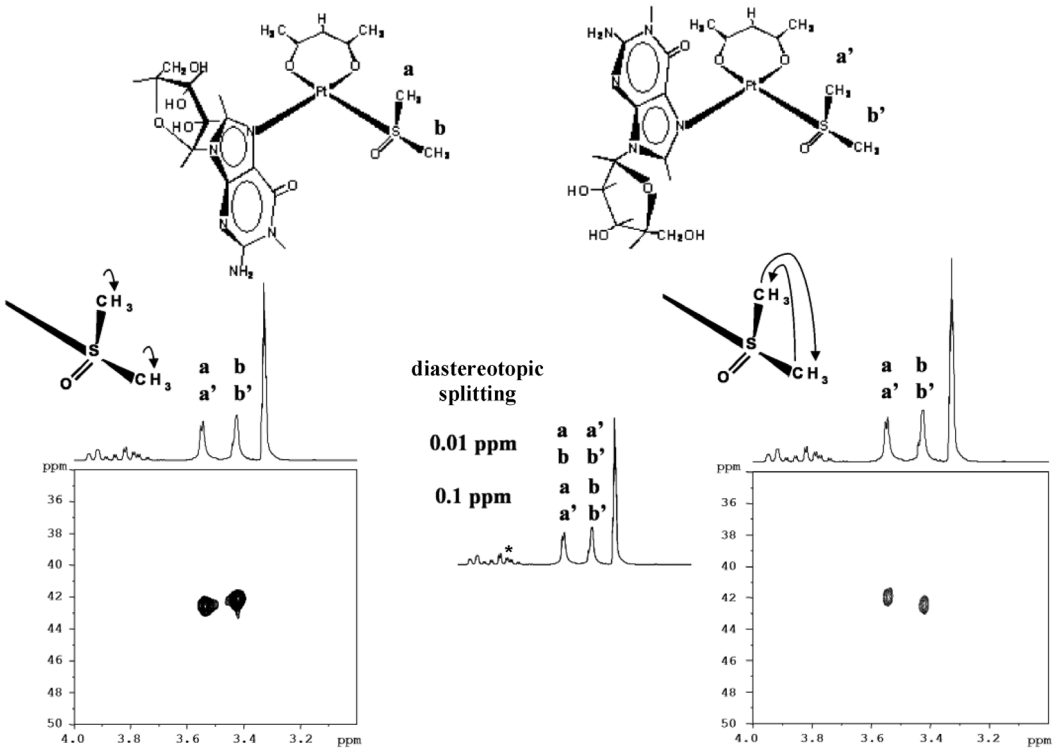


Fig. 2 Expansions of 2D [^{1}H,^{13}C]-HETCOR (left) and [^{1}H,^{13}C]-long-range HETCOR (right) NMR spectra in CD_3OD of [Pt(*O*,*O'*-acac)(DMSO)(Guo)]$^+$ (**4a**) (* = free Guo).

The considerably enhanced diastereotopic splitting exhibited by the two methyl groups of DMSO accounts for two rotamers due to the slow rotation around the Pt–N(7) bond, which results in a chiral center on the metal. Therefore, the ribose H1' signal (5.92 ppm) appeared as a pseudo triplet, due to the partial overlap of the two different sugars moieties, one for each conformer. Further evidence of the presence of rotational isomers in solution was given by variable-temperature ^{1}H NMR experiments (Fig. 3). Lowering the sample temperature (to 268 K), the dynamic motions of the molecule were slowed down to decoalescence also for the H8 signals of the two rotamers. The ΔG (14.7 Kcal mol^{-1}) for the exchange process (Eyring equation) was calculated from chemical shift difference ($\Delta\nu$) of the H8 signals and coalescence temperature (T_c 293 K). Although in a few cases the interconversion energy of nucleobase rotamers has been determined, the activation rotational barrier observed in this work is similar to data already reported [31]. Interestingly, both H8 resonances show a deshielding at lower temperatures. The same effects of splitting and deshielding was observed on the methine protons of acac chelate. These effects probably depend on the steric and electronic constraints of the frozen atropisomers. Moreover, the temperature lowering contributed to a better separation of ribose H1' doublets, though no deshielding effect was observed, probably due to the distance of the sugar moiety from the Pt coordination plane. A better separation was also observed at low temperature for the low-frequencies acac methyl signals, although the higher-frequencies methyl appeared still synchronous at the temperature reached, suggesting their position being *trans* to the coordinated Guo. A 2D ^{1}H-NOESY NMR spectrum confirmed this attribution (cross-peaks correlation between the acac methyl signals at 2.14 ppm with the DMSO methyls and the acac methyl singlets at 2.02 and 2.03 ppm with the H8 signals).

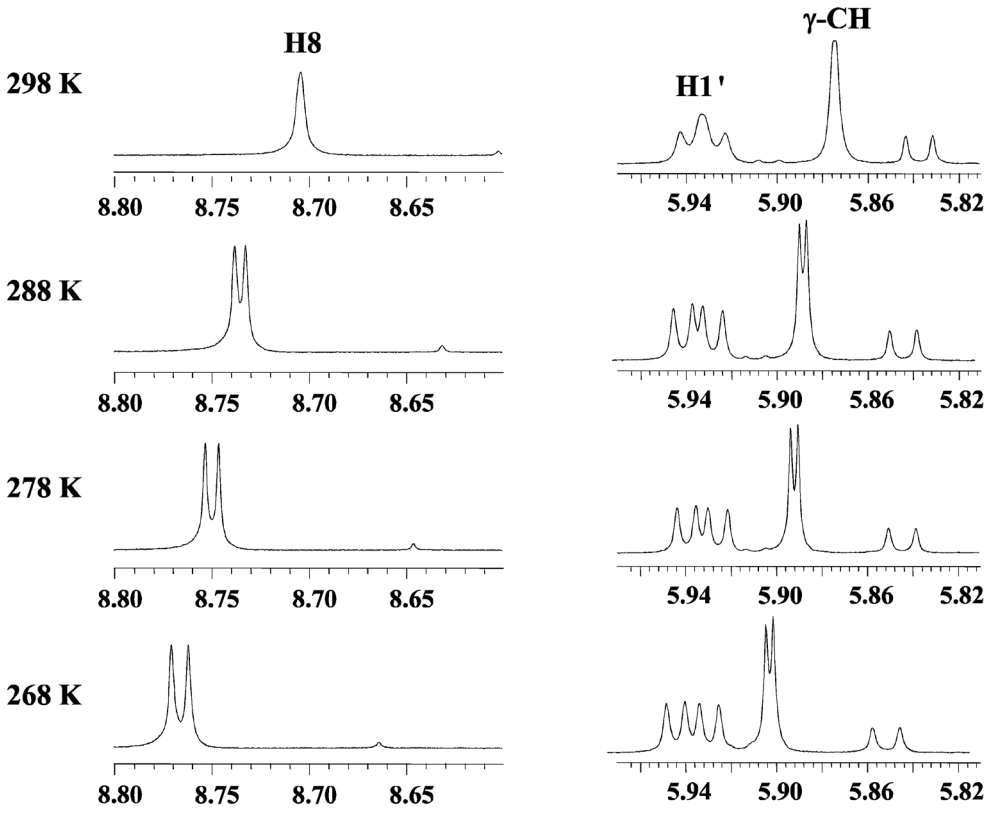


Fig. 3 Variable temperature of ^{1}H NMR spectra in CD_3OD of [Pt(*O*,*O'*-acac)(DMSO)(Guo)]$^+$ (**4a**).

It should be noted that the new β-diketonate complexes are also very interesting due to the presence of the chelate oxygen atoms characterized by acceptor rather than donor hydrogen bond ability. Two *cis* leaving groups of appropriate lability, two *cis* ammines or a chelating diammine, and the presence of hydrogen atoms on the non-leaving amine ligand have been considered, for a long time, the key features for classical Pt drug design. Residual hydrogen atoms on the non-leaving ammine ligand were required in order to form hydrogen bonds with guanine O6 and/or phosphate groups. Nevertheless, the preferred conformation of guanine derivatives in *cis*-PtA_2G_2 adducts (A_2, two ammines or diammine ligand; G guanine derivative), has also been attributed to the small size of the amino group substituents in the carrier ligand, rather than specific hydrogen bonding [32,33].

In the related Pt diammine system [Pt(en)(DMSO)(Guo)]$^{2+}$, no evidence of hindered rotation around the Pt–N(7) bond was observed down to 274 K [29]. The slow rotation of the nucleobase around the Pt–N(7) observed for [Pt(*O*,*O'*-acac)(DMSO)(Guo)]$^+$ with respect to the analogous diammine complex necessarily arise from both the steric and/or electronic effects of the *O*,*O'*-chelated acac and not simply to the presence of a *cis* DMSO ligand.

The acac ligand could have electronic effects, and at the same time it could induce steric hindrance in the coordination plane. The two sp^2 oxygen donor electron lone pair may confer to the ligand peculiar ability in stabilizing rotational conformers of nucleobases coordinated to the metal. On the other hand, **1a** does not have hydrogen atoms available for hydrogen bonds with the O6 of Guo. Nevertheless, the chelate acac electron lone pairs could cause repulsion in the coordination plane with electron-rich groups, resulting in the slow rotation of the Guo around the Pt–N(7) bond. Moreover, the six-membered ring formed by acac with Pt is more rigid and with a greater bite angle [20] with respect

to the five-membered ring formed by the chelate ethylendiammine. Crystallographic data indicate a 93.3º O–Pt–O average angle for **1a** to be compared with 82.0º for the N–Pt–N bond angle in the analogous complex [PtCl(en)(DMSO)][$PtCl_3$(DMSO)] [34], while the Pt–S distance is very close in both species (2.219 Å and 2.201 in [Pt(en)Cl(DMSO)][$PtCl_3$(DMSO)] and **1a** complex, respectively [20,34]). In order to assess the effects of DMSO for the Guo base mobility, the analogous DMS complex, [Pt(*O*,*O'*-acac)(DMS)(Guo)]$^+$ (**4b**), was synthesized and studied by NMR spectroscopy. The aquo species [Pt(*O*,*O'*-acac)(DMS)H_2O](NO_3) (**3b**) was prepared by reaction of **1b** complex with silver nitrate. The reaction in NMR tube of **3b** in CD_3OD with an excess of Guo easily affords the **4b** species. The strong deshielding of the H8 (8.61 ppm) of the purine base indicates Guo coordination by N(7) atom, but no evidence for hindered rotation of the coordinated Guo was observed in the ^{1}H NMR spectrum of **4b** at room temperature. Only one singlet for H8 of nucleoside and a single doublet for the sugar H1' suggest the presence of a single rotamer in CD_3OD solution, confirmed by the presence of only two DMS signals (2.41 and 2.39 ppm) with a small diastereotopic splitting ($\Delta\delta$ = 0.02 ppm) generated by the chiral centers on the ribose moiety. The presence of a single rotamer for **4b** at room temperature, if compared with previous results for **4a** and [Pt(en)(DMSO)(Guo)]$^+$ complexes [29], clearly indicates that beside the chelate also the sulfur ligand plays a role for Guo rotation in these systems. In order to verify the influence of base-related steric and electronic factors in **4a**, the analogous complex containing adenosine [Pt(*O*,*O'*-acac)(DMSO)(Ado)]$^+$ (**5a**) was synthesized via the aquo species **3a** and studied by NMR spectroscopy. It should be noted that the coordinated Ado is characterized by a NH_2 group in 6 position in place of the O6 of Guo. To allow the metal coordination only at the N(7) of the nucleobase, the experiment was performed at pH 2. Time monitoring the reaction, the formation of **5a** species was observed. The notable deshielding of H8 ($\Delta\delta$ = 0.86 ppm) and the slight deshielding of H2 ($\Delta\delta$ = 0.13 ppm) signals with respect to the free base account for the coordination of Ado through N(7). The presence of one sharp singlet for H8 and H2 protons, respectively, and only one doublet for H1' of ribose suggests a fast rotation of nucleobase around the Pt–N(7) bond with respect to the NMR time scale. Interestingly, in the **5a** complex the NH_2 group in 6 position of Ado could be able to form a hydrogen bond with the sp^2 σ-bonded oxygens of acac chelate. This may favor the nucleobase orientation coplanar with the coordination plane and reduce the activation energy required for the rotation around the Pt–N(7) bond [35]. All data suggest that the slow rotation of the nucleobase in [Pt(*O*,*O'*-acac)(DMSO)(Guo)]$^+$ (**4a**) is due to a combination of electronic and steric reasons, due to specific interactions between the acac chelate ligand, the DMSO and the base, and not merely to one single factor such as the sulfur bound ligand. Probably the O6 of Guo may interfere with the lone pairs of the acac chelate sp^2 σ-bonded oxygens contributing to slowing down rotation about the Pt–N(7) bond. This is not the case of the related chelate diammine system [Pt(en)(DMSO)(Guo)]$^{2+}$. Moreover, also the oxygen atom of DMSO may repulsively interact with the guanine O6 at the acac rather than diammine chelate bite angle, consequently slowing down the rotation of the nucleobase. These latter repulsive interactions are removed in the analogous sulfide complex [Pt(*O*,*O'*-acac)(DMS)(Guo)]$^+$ (**4b**). Interestingly, in **5a**, where the Ado NH_2 group could form a hydrogen bond with the sp^2 σ-bonded oxygens of acac chelate, free rotation of the nucleobase around the Pt–N(7) bond was observed.

CONCLUSIONS

Previously reported chemical and biological studies indicate that [PtCl(*O*,*O'*-acac)(L)] (**1**) and [Pt(*O*,*O'*-acac)(γ-acac)(L)] (**2**) (L = DMSO, **a**, DMS, **b**) complexes are unreactive toward nucleobase. On the other hand, [PtCl(diammine)(sulfoxide)]$^+$ reacts with DNA and [Pt(diammine)(sulfoxide)(nucleoside)]$^{2+}$ systems were used as model molecules for the occurring reaction. The present work demonstrated that [Pt(*O*,*O'*-acac)(L)(nucleoside)]$^+$ (L = DMSO, nucleoside = Guo, **4a**; L = DMS, nucleoside = Guo, **4b**; L = DMSO, nucleoside = Ado, **5a**) complexes, once formed from **1a,b** via the aquo species, exhibited rotational behaviour of the nucleoside about the Pt–N(7) bond different with respect to the analogous [Pt(diammine)(sulfoxide)(nucleoside)]$^{2+}$. The 1D, 2D, and variable-temperature NMR

data demonstrated that the steric and electronic interactions between the acac chelate ligand, the DMSO, and the specific coordinated nucleobase account for rotamers interconversion in these systems. In particular, the O6 of Guo may interfere with the lone pairs of the acac chelate sp^2 σ-bonded oxygens contributing to slowing down rotation about the Pt–N(7) bond in **4a** much more than that in the related chelate diammine system $[Pt(en)(DMSO)(Guo)]^{2+}$. On the other hand, the oxygen atom of DMSO may stronger interact with the guanine O6 at the acac rather than diammine chelate bite angle. Consistently, rotation resulted much slower at room temperature for $[Pt(O,O'\text{-acac})(DMSO)(Guo)]^{+}$ (**4a**) not only if compared with $[Pt(en)(DMSO)(Guo)]^{2+}$ but also with respect to both $[Pt(O,O'\text{-acac})(DMS)(Guo)]^{+}$ (**4b**) and $[Pt(O,O'\text{-acac})(DMSO)(Ado)]^{+}$ (**5a**). Finally, this work suggests that **1a,b** complexes, even when forced to react to DNA in a form of activated prodrug (aquo species), may exhibit different reactivity and biological activity with respect to analogous [PtCl(en)(DMSO)]Cl, due to the molecular dynamic profiles found in the $[Pt(O,O'\text{-acac})(L)(nucleoside)]^{+}$ complexes (L = DMSO, DMS).

EXPERIMENTAL SECTION

Physical measurements: Measurements of pH were performed on a Crison Research pHmeter, equipped with an Hamilton 3-mm Spintrode electrode. ^{1}H 1D NMR spectra were recorded on a Bruker Avance DPX 400MHz at 298 K. The variable time experiments and ^{1}H NOESY, [^{1}H,^{13}C]-HETCOR 2D spectra were conducted on the Bruker Avance DRX 500 WB with a variable-temperature unit BVT300 module on the temperature range from 298 to 268 K. CD_3OD were used as solvent, and chemical shift were referenced to TMS by the residual protic solvent peaks as internal references.

Starting materials: Acetylacetone, guanosine, adenosine, and deuterated solvents were purchased from Aldrich, and used without further purification. [PtCl(O,O'-acac)(DMSO)] (**1a**) [17] [PtCl(O,O'-acac)(DMS)] (**1b**) [20] were prepared according to previously reported procedures.

*Preparation of the acquospecies [Pt(O,O'-acac)(DMSO)(H_2O)](NO_3) (**3a**) [Pt(O,O'-acac)(DMS)(H_2O)](NO_3) (**3b**)*: To a CD_3OD solution (10 mL) of the complex **1a,b** (0.02 g, 0.05 mmol), stoichiometric amount of $AgNO_3$ was added. After 12 h under stirring at room temperature in dark condition, the mixture was filtered through celite. The resulting solution was used without further purification.

Reaction in NMR tube: To a CD_3OD solution of **3a** or **3b** (~5 mM in Pt) a nucleoside excess was added and time monitored recording ^{1}H NMR spectra. In the case of reaction of **3a** complex with Ado the experiments were performed at pH 2 using a nitric acid-d solution (DNO_3 65 % wt in D_2O, 99 % D).

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