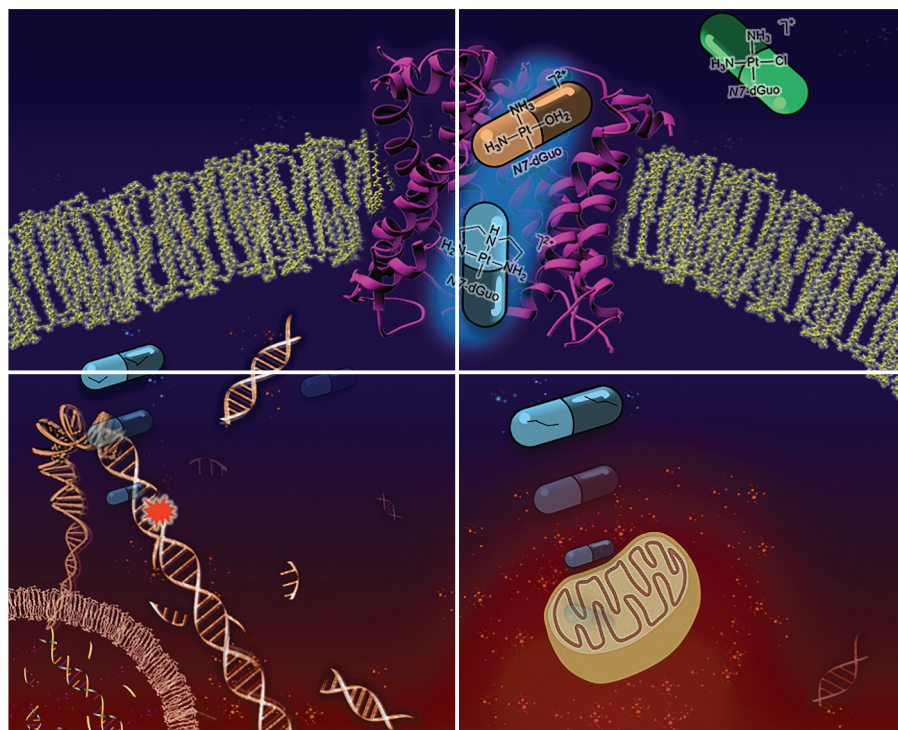




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Exploring the interactions of N7-platinated guanosines with the hCNT3 transporter: a molecular dynamics study

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The translocation of nucleosides across cellular membranes requires a specific category of integral membrane proteins, known as nucleoside transporters. These proteins are crucial for the absorption of endogenous nucleosides as well as nucleoside-derived pharmaceuticals, including antineoplastic drugs. This research examined the comparative dynamic behavior of [Pt(dien)(N7-dGuo)]²⁺ (**1**), *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (**2**), and *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (**3**) (where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine) platinated nucleosides in the human concentrative nucleoside transporter type 3 (hCNT3), utilizing molecular dynamics (MD) simulations. The membrane protein hCNT3 is involved in the cellular absorption of numerous therapeutic nucleoside derivatives used in the treatment of both viral infections and malignancies. These simulations facilitated the characterization of the ligand-binding site, underscoring the critical role of the transmembrane domain TM9 in promoting nucleoside translocation across the membrane and in determining the interaction of certain platinated nucleosides within the transporter channel. Overall, this work underscores the potential of platinum-based modifications to enhance antitumor therapies based on nucleoside analogues by synergistically targeting hCNT transporters, metabolic pathways of natural nucleosides, and DNA functionality. Notably, the latter interactions, in the case of complexes **2** and **3**, could also produce the classical 1,2-intrastrand cross-link lesions with DNA, which are considered responsible for the antitumor activity of cisplatin and analogous complexes. These considerations could lay the groundwork for new strategies aimed at developing antitumor drugs characterized by enhanced antitumor activity, selectivity for tumor cells, and reduced systemic side effects.

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Introduction

Nucleoside Transporters (NTs) are integral membrane proteins that facilitate the translocation of nucleosides, the fundamental building blocks of nucleic acids such as DNA and RNA, across cell membranes. These proteins play pivotal roles in various biological processes, including nucleotide salvage pathways,¹ signal transduction,² and the regulation of extracellular and intracellular nucleotide concentrations.³ Dysfunctions or altered expressions of nucleoside transporters can result in a range of medical conditions. Changes in their expression can impact the efficacy of nucleoside-based che-

motherapies,⁴ while their dysfunction can lead to drug resistance.⁵ Mutations in genes encoding nucleoside transporters have been implicated in a range of severe metabolic disorders, including H syndrome, pigmented hypertrichosis, and insulin-dependent diabetes mellitus,^{6,7} evidencing their critical physiological role.

Nucleoside transport across cell membranes is mediated by two major families: the human Equilibrative Nucleoside Transporters (hENTs, SLC29 family) and the human Concentrative Nucleoside Transporters (hCNTs, SLC28 family).⁸ Despite their structural differences, these two families share similar substrate specificities. ENTs operate as sodium-independent uniporters, facilitating the bidirectional transport of nucleosides across cell membranes along their concentration gradients without energy input. They lack definite prokaryotic orthologues and include four isoforms: ENT1 of the solute carrier (SLC) family 29 (SLC29A1), ENT2 (SLC29A2), ENT3 (SLC29A3), and ENT4 (SLC29A4).⁹ Notably,

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ENT1 and ENT2 are pivotal for the pharmacokinetics of anti-viral and anticancer nucleoside analogs, whereas ENT3 and ENT4 are involved in intracellular transport and lysosomal function.

In contrast, CNTs^{10,11} are evolutionarily conserved energy-dependent symporters that require an inwardly directed sodium- or proton-dependent coupling to actively transport nucleosides against their concentration gradients. This energy-dependent mechanism allows for intracellular accumulation of nucleosides crucial for DNA/RNA synthesis and cellular homeostasis. hCNT1 (SLC28A1), hCNT2 (SLC28A2), and hCNT3 (SLC28A3) represent the primary isoforms of human CNTs in the solute carrier (SLC) family 28. Among these three, hCNT3 is distinguished by its high transport efficiency. Indeed, it can intracellularly concentrate nucleosides ten times more efficiently than CNT1 or CNT2, due to its 2:1 ratio of Na⁺ to nucleoside coupling. It is located on the apical membrane of the intestine and liver epithelia and is broadly expressed in tissues including the intestine, kidney, liver, and pancreas,¹² making it of particular interest for systemic nucleoside uptake and drug absorption.

hCNT3 is a transporter for both purine and pyrimidine nucleosides, while CNT1 and CNT2 prefer pyrimidine and purine nucleosides, respectively.¹³ It plays a crucial role in drug response and the development of resistance to anti-cancer drugs. hCNT3 plays a crucial role in the chemotherapeutic strategies that utilize modified nucleosides, such as enhancing the uptake and transport of various antileukemic drugs, including cladribine and fludarabine, through the cell membrane.^{14,15} Nucleoside analog drugs, such as gemcitabine and cytarabine, are dependent on nucleoside transporters for their entry into cancer cells. Inhibition of these transporters may impede the absorption of these drugs, diminishing their effectiveness and potentially resulting in drug resistance. One potential mechanism linking these transporters to cancer is the upregulation of nucleoside transporters in cancer cells, which facilitates the uptake of nucleosides essential for accelerated DNA synthesis and cellular proliferation. Inhibiting these transporters could potentially decrease the intracellular levels of nucleosides, thereby impeding cancer cell growth.¹⁶

To enhance the effectiveness of cancer treatments, a deeper understanding of how chemotherapeutic agents exert their action is essential. Platinum-based compounds, such as cisplatin, carboplatin, and oxaliplatin, remain the cornerstone of approximately half of all cancer treatment regimens,^{17–20} underscoring their clinical relevance. Recent advances suggest that combining these potent agents with nucleic acids may lead to groundbreaking therapeutic strategies. Platinated nucleosides may offer an intriguing approach, potentially merging the cytotoxic impact of platinum drugs with the targeted functionality of nucleoside analogues (NAs) into the same molecule.^{21,22}

Our previous studies demonstrated that N7-platinated guanosine derivatives can enter HeLa cells *via* Na⁺-dependent concentrative transporters located at the plasma

membrane.^{23,24} Building on these insights, the current study explores the role of the hCNT3 nucleoside transporter,¹⁴ one of the most efficient nucleoside transporters among the CNT family, in facilitating the cellular uptake of modified nucleosides based on N7-platinated guanosine derivatives, which have potential as antitumor/antiviral drugs.

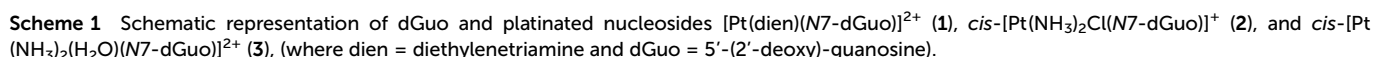
In this context, three complexes of particular interest were selected for the present study. The [Pt(dien)(N7-dGuo)]²⁺ (1) complex (where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine), has been extensively studied in previous works.^{25–32} This complex serves as an established model for N7-platinated purines. Previous studies have highlighted its significance as an experimental prototype for understanding the chemical behaviour and mechanisms of interaction of platinated purines with cellular substrates, as well as their potential therapeutic properties. For instance, it has been demonstrated that the related model complex [Pt(dien)(N7-dGTP)] (where dGTP = 5'-(2'-deoxy)-guanosine triphosphate) can be recognized by DNA polymerases and incorporated into newly synthesized DNA.^{23–27}

Alongside this species, the chloro-species *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2), originally studied for its antitumor activity by Hollis *et al.*,³³ has been recently reconsidered in our studies to better understand the mechanism of its proven antitumor activity.^{24,28,29} Indeed, *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2) could serve as a suitable precursor for other active metabolites that could be generated after administration, such as the here considered aquated *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3) hydrolytic product.

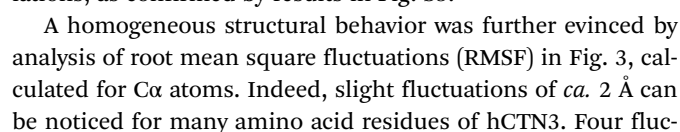
Building on previous research on the binding site interactions between Taq DNA polymerase and three different N7-platinated deoxyguanosine triphosphates,²⁸ we conducted a Molecular Dynamics (MD) investigation to examine the platinated nucleosides [Pt(dien)(N7-dGuo)]²⁺ (1), *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2), and *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3) (see Scheme 1) as potential substrates for the hCNT3 transporter. To better understand the specificity of platinated nucleoside uptake by this *trans*-membrane transporter and its potential access to specific cell types and intracellular compartments, we also studied the canonical dGuo for comparison. Our explicit MD simulations on the hCNT3 with and without nucleosides allowed us to explore the effects of platinated nucleosides on the structural and dynamic behaviour of hCNT3, and to investigate its inhibition mechanism compared to dGuo natural nucleoside.

This computational approach provides valuable insights into how platinum coordination affects substrate binding, enzyme stability, and the fidelity of DNA replication. Understanding these mechanisms is essential for designing novel therapeutic agents that leverage both the cytotoxic properties of platinum complexes and the specificity of nucleotide incorporation. The structural features of the active site and the pathways for ligand access and release can significantly affect substrate selectivity in these types of proteins.





In order to construct a model of the transporter systems within the cell membrane, we utilized CHARMM-GUI,⁴⁴ a web-based tool. The Membrane Builder option was employed to generate the necessary sequence of CHARMM inputs for constructing a protein/membrane complex and conducting molecular dynamics simulations. To mimic the composition of the plasma membrane, each model was embedded in a bilayer membrane system composed of the following components: phosphatidylcholine (23%), cholesterol (34%), sphingomyelin (17%), phosphatidylethanolamine (11%), and phosphatidylserine (8%).⁴⁵ The model was further solvated with TIP3P water molecules, which were positioned within 24 Å of the protein's matrix and cytoplasmic sides (see Fig. 1 for further details). The approximate number of atoms in each system were as follows: 58780 for the apo-form, 58812 for hCNT3-dGuo, 58833



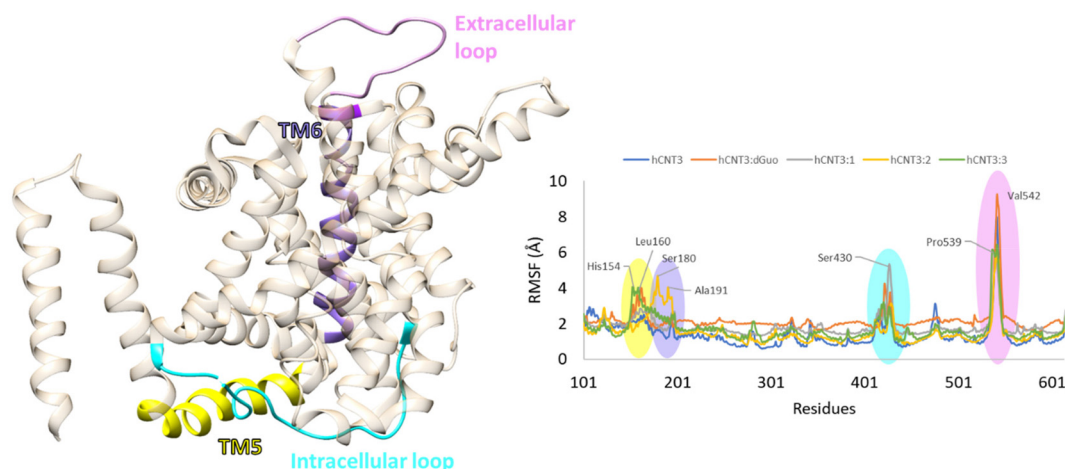
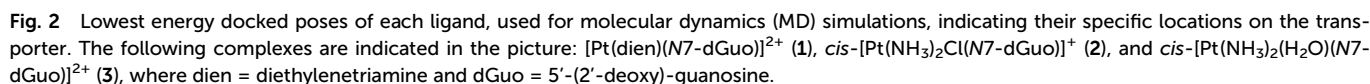


Fig. 3 Root mean square fluctuation (RMSF) calculated for the backbone atoms of the investigated systems. The peaks corresponding to the intra-cellular (green) and extracellular (purple) loops are circled. The residues corresponding to these peaks are highlighted. The following complexes are indicated in the picture: [Pt(dien)(N7-dGuo)]²⁺ (1), *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2), and *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3), where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine.

started with detecting the number of hydrogen bonds formed between nucleosides and the membrane protein, for each system during the 2×200 ns of simulation (a donor-acceptor cutoff distance within 3.0 Å coupled to a maximum donor-hydrogen-acceptor angle of 20° was applied in the calculation). The follow-up is displayed as occupancy percentages of residues involved in the H-bonds in Fig. 4.

In the hCNT3:dGuo system, the most prevalent H-bonds involve the residues E344 (30.29%), T342 (27.11%), and E520 (24.92%), acting as acceptors, while multiple H-bonds are formed between the dGuo nucleoside, acting as an acceptor,

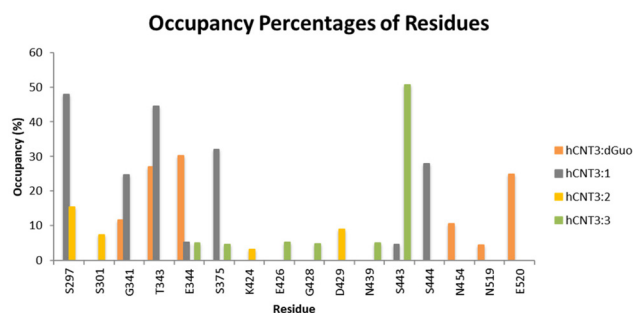


Fig. 4 Occupancy percentages of hCNT3 residues involved in H-bonds during the molecular dynamics (MD) simulation for dGuo, and [Pt(dien)(N7-dGuo)]²⁺ (1), *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2), and *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3), species (where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine).

and donor residues such as G341, suggesting a wide network of stabilizing interactions. In addition, N454 from TM9 also interacts with the dGuo moiety, albeit with a lower occupancy of 10.52% (see Fig. 4).

In the case of the Pt(dien)-containing system, more frequent hydrogen bond interactions were identified with the hydroxyl moiety of S297 and T343, with percentages of 47.95% and 44.55%, respectively. Similarly, the hCNT3:*cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ system displays the highest occupancy of 50.67% for H-bond interaction with S443 of TM9 and the highest percentage of hCNT3:*cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ is 15.18% for H-bond with the hydroxyl moiety of S297 (see Fig. 4).

Among the platinated systems, the [Pt(dien)(N7-dGuo)]²⁺ (1) complex was found to be crucial in forming frequent and stabilizing H-bond interactions with the transporter. This complex generates highly stable interactions that can significantly affect the translocation of species 1 through the transporter channel in contrast to the nucleoside dGuo characterized by weaker interactions. In particular, H-bonds between serine residues and the ligand resulted in playing a crucial role in stabilizing specific regions of the protein's structure as will be examined in detail in the section devoted to TM9 behavior.

In the case of Pt-containing compounds, a shift from the initial docked pose was further observed, most likely because of the establishment of many interactions as discussed above (see RMSD in Fig. S1). In this movement, the ligands had the opportunity to interact further with negatively charged residues, such as E344, E426, and E520 (see Fig. 4). The former, in particular, has been identified in all the trajectories with occupancies ranging from *ca.* 30–5% of occupancy. Furthermore, the radial distribution function (RDF) calculated for platinated nucleosides and negatively charged residues demonstrates that the electrostatic interaction at ~ 1.8 Å is more prevalent with the platinated guanosine derivative [Pt(dien)(N7-dGuo)]²⁺ (1), followed by *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2) and *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3). The neutral, natural nucleobase exhibits the lowest frequency (See Fig. S4).

During the simulation time, several water molecules come into interactions with the ligands, as evidenced by the analysis of the radial distribution function of O_w-nucleoside pairs (Fig. S5). The RDF for the presence of dGuo nucleoside shows a lower density of water molecules at shorter distances (~ 3.0 Å) compared to the Pt-containing species. This indicates that water is less densely distributed around the natural nucleoside, which is engaged in H-bonds with multiple protein residues as discussed above. In the case of 1, 2 and 3 instead, a peak at *ca.* 2.5 Å can be noticed, suggesting a higher probability of finding water molecules in proximity to metal complexes (Fig. S5). An explanation for this result resides in the shift of the species during the molecular dynamics, with Pt-containing compounds moving in proximity of the intracellular loop in proximity of TM5 (see Fig. 2) and facing a higher number of water molecules, with respect to the embedded dGuo.

Puckering of ribose moiety

The analysis of each system reveals significant features that help rationalize the experimental outcomes.²⁵ The structure demonstrates to be able to locate [Pt(dien)(N7-dGuo)]²⁺ (1) deep within the transporter, into the nucleoside-binding site, and close to the cytoplasmic side of the membrane, regardless the presence of a very bulky moiety on N7. As previously discussed, the interactions can be divided into two groups: those involving the ribose moiety and those with the nucleobase. Therefore, we analyzed their behavior in terms of the contact area and of the dihedral angles in the case of the sugar ring. This allows us to highlight the conformational preference of hCNT3 with respect to the sugar ring of the natural and modified pyrimidine nucleosides examined. This aspect can cause significant differences in the binding affinities of target proteins.⁵³

The conformation of the ribose moiety during the MD simulation (see Fig. 5) reveals the structural effects of the backbone in the platinated nucleosides compared with the natural one. In particular, a major rigidity of the sugar in the case of [Pt(dien)(N7-dGuo)]²⁺ is observed, caused by its bulky group, which, pointing away from TM7 with respect to the natural dGuo, affects the ribose interactions with the transporter. The other two systems exhibit greater mobility of the ribose, in terms of puckering (Fig. S6).

In the case of Pt-containing compounds, [Pt(dien)(N7-dGuo)]²⁺ is very bulky and is first accommodated by interacting with a number of serine residues, mainly from the TM9 domain within the pocket, as will be discussed in the next section in detail. [Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3) tends to establish interactions with water molecules and negatively charged residues more frequently. Because of such networks, the species considered can display different orientations of the ribose moiety, characterized by 3'-*endo* and 3'-*exo* conformations (or N- and S-conformation respectively, see Fig. 5).

Preliminary quantum mechanics calculations on the investigated species, including the ribose as a reference point, showed that the dihedral interchange can occur with a small



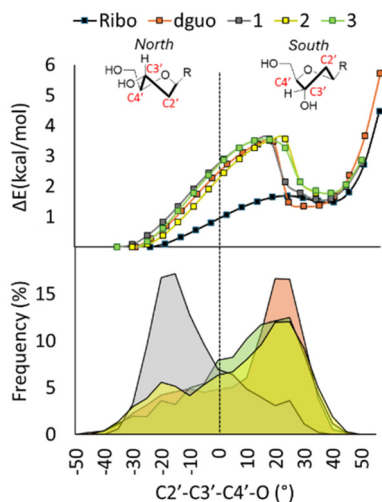


Fig. 5 Bottom: Frequency of C2'–C3'–C4'–O dihedral observed during molecular dynamics (MD) simulations. Top: Density functional theory (DFT) energy profile calculated for C2'–C3'–C4'–O dihedral for the current ligands. The following complexes are indicated in short in the picture: [Pt(dien)(N7-dGuo)]²⁺ (1), *cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺ (2), and *cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ (3), where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine.

energy penalty of 1.5 kcal mol^{−1}, from N- to S-conformation (see Fig. 5). However, for the systems with a nucleobase an energy barrier of *ca.* 3.5 kcal mol^{−1} can be noticed. Interestingly, dGuo and complex 2 have a similar behaviour, with an S-conformation observed for almost the whole trajectory, while in the case of complex 1, the N-conformation is the most populated (see Fig. 5). This set of QM calculations was performed coupling B3LYP-D3, 6-31G(d,p) basis for all atoms and the SMD implicit model ($\epsilon = 78$).^{54,55} Although the analysis of the energies at the QM level of theory revealed a small energy gap between the two conformations, it is interesting to note that the bulkier ligand on complex 1 imposes some limitations on the intramolecular reorganization of the structure, such as energy costless rearrangement of the ribose ring.

Behavior of the TM9 during MD

A more thorough structural analysis of the transporter segment TM9 (residues L433–F476) was carried out to deepen its role in ligand recognition and transport. As discussed above, many amino acids were found to come into contact with the ligands, with particular attention on the serine residues S442, S443, S445, and S446. A closer view of the structure of TM9 further highlighted that when the ligand lies in proximity to these serine residues, the α -helix is slightly unwound, as evidenced by the Ramachandran plots calculated for the populated clustered geometries from MD simulations reported in Fig. S7. The main reason for this conformational reorganization resides in the internal hydrogen bond networks that distinguish the TM9.

In detail, the measurements of the canonical hydrogen bonds (O–HN) between residues S443–L447 and S444–Val448

are fundamental for maintaining the structural organization of the helix. Each system shows a progressive increase in these distances, indicating the unwinding of the α -helix at these points (see Fig. 6).

Looking at the representative clustered structures obtained from the MD, in the case of the apo-form of hCNT3, the values of the H-bond distances S443–L447 and S444–V448 were 2.47 Å and 1.88 Å, in agreement with the available crystallographic structures.^{34,51,52} In the hCNT3:dGuo system, the distances experience a slight change (S443–L447: 4.60 Å and S444–V448: 2.49 Å), keeping the typical values observed in a stable α -helix anyway.

In the case of hCNT3:[Pt(dien)(N7-dGuo)]²⁺ system, the distance between S443 and Leu447 is measured at 6.68 Å, while the distance between S444 and V448 is 3.28 Å. These values shift from the ideal helix distances, particularly at the S444–V448 position where the distance of 3.60 Å suggests an unwound section of the helix caused by the presence of the metal complex. Similarly, in the hCNT3:*cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)]²⁺ complex, the distances S444–V448 and S443–L447 were measured at 2.36 Å and 5.62 Å, respectively (see Fig. 6).

To further quantify the impact of these variations of inherent hydrogen-bond networks of the α -helix, the RMSD of TM9 (residues L433–F476) was calculated, and comparable values were observed for all the systems (Fig. S6). In addition, by investigating the length of the α -helix by measuring the head-tail distance, significant variations were not detected with respect to the apoform, with values ranging from 62–65 Å for the ligand-bound system (*ca.* 63 Å in the apo form, see Fig. S7). As a result, it was determined that TM9 can locally adjust to ligand passing-through, specifically promoting the migration of metal-containing complexes from the matrix to the intracellular environment.

The analysis of the MD simulations thus revealed the involvement of ligand-TM9 interactions, in particular of S443–V448 segment, in the migration of metal-containing species from extra- to intracellular environment, suggesting that its consideration could be relevant for design of new drugs.

The binding free energy (ΔG_{total}) values calculated using MM-PBSA (Table S2) also provide valuable insights into the stability of the systems inside the transporter channel since the considered different contributions can be strictly linked with the analysis of hydrogen bonds.

The obtained ΔG_{total} values reveal significant differences in the stability of the ligand within the membrane-protein system. The ΔG_{total} for dGuo is −31 kcal mol^{−1}, which is less negative than that of hCNT3:[Pt(dien)(N7-dGuo)]²⁺ (−78.70 kcal mol^{−1}), likely due to the extended hydrogen bond network established with surrounding residues. The hCNT3:*cis*-[Pt(NH₃)₂(H₂O)(N7-dGuo)] system shows a slightly negative ΔG_{total} of −4.11 kcal mol^{−1} that becomes positive +11.49 kcal mol^{−1} in hCNT3:*cis*-[Pt(NH₃)₂Cl(N7-dGuo)]⁺, (Table S2) likely due to reduced H-bonds with the transporter emerged by the H-bond analysis (see Fig. 4).

Furthermore, umbrella sampling simulations able to force the exploration along a predefined reaction coordinate were



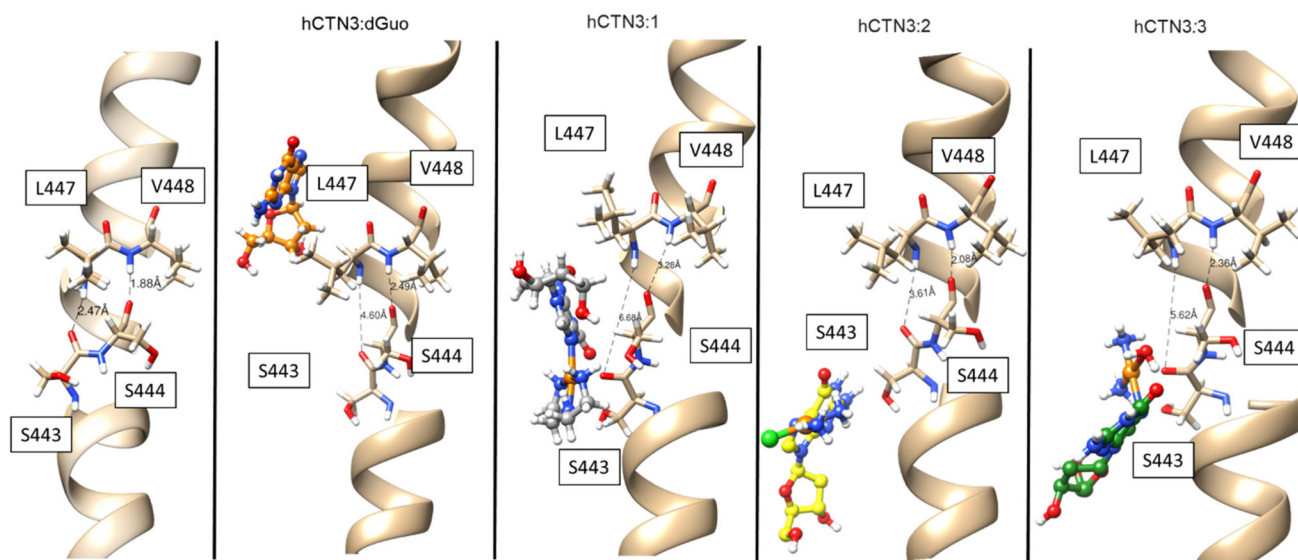


Fig. 6 Illustration of the canonical H-bonds involved in the formation of the α -helix between S443:L447 and S444:V448. The following complexes are indicated in the picture: $[\text{Pt}(\text{dien})(\text{N7-dGuo})]^{2+}$ (1), $\text{cis-}[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{N7-dGuo})]^+$ (2), and $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})(\text{N7-dGuo})]^{2+}$ (3), where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine.

finally carried out to evaluate the energy cost of the observed movements, once the hCNT3:ligand complexes are obtained already, focusing on the energetic pathway for releasing the dGuo and N7-platinated complexes in the intracellular environment. Based also on the better affinity of $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})(\text{N7-dGuo})]^{2+}$ for hCNT3 with respect to $\text{cis-}[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{N7-dGuo})]^+$, the umbrella sampling simulations were performed only on complex 1 and complex 2 in comparison to dGuo. The hydrogen bond occurring between the $-\text{NH}_2$ at the C4 position of the nucleobase and the carboxylate group of E520 (chosen as a reference point for leaving the transporter), starting from clustered geometries from MD, was the selected reaction coordinate (see Fig. 7), in order to get a reliable estimate of the potential of mean force (PMF).

The plot of PMF calculated for the dGuo-containing system clearly highlights that the existing hydrogen bond, at *ca.* 3.5 Å, stabilizes the substrate in the transporter's environment and that its cleavage requires 6 kcal mol⁻¹ for releasing the nucleotide in the cell environment (see Fig. 7). Interestingly, the plot for the platinated compounds presented a different shape, with respect to the natural nucleotide. In detail, in the case of the Pt-aquo complex, a lower-energy minimum was localized at *ca.* 7.5 Å, with an almost flat energy profile in the range 2.5–4.0 Å in which the interaction should take place. In the case of the Pt-dien complex, finally, two isoenergetic minima were identified, at 6.5 Å and 7.5 Å, linked by a low energy barrier of 4 kcal mol⁻¹. This suggests that, according to the selected reaction coordinate, the hCNT3 can drive the release of considered Pt-containing compounds through the cellular membrane, due to low-energy interactions between the protein and the metal complexes, which indeed during the unbiased MD shifted from their initial position as discussed above, in

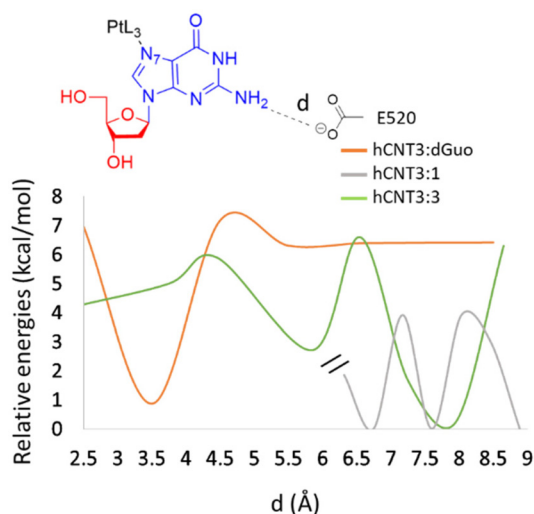


Fig. 7 Relative energies for the hydrogen bond interaction between the C4- NH_2 group of the investigated ligands and the carboxylate group of the E520 residue, obtained from umbrella sampling (US) simulations. The following complexes are indicated in the picture: $[\text{Pt}(\text{dien})(\text{N7-dGuo})]^{2+}$ (1, $\text{PtL}_3 = \text{Pt}(\text{dien})$), $\text{cis-}[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{N7-dGuo})]^+$ (2, $\text{PtL}_3 = \text{Pt}(\text{NH}_3)_2\text{Cl}$), and $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})(\text{N7-dGuo})]^{2+}$ (3, $\text{PtL}_3 = \text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})$), where dien = diethylenetriamine and dGuo = 5'-(2'-deoxy)-guanosine.

agreement with experimental results.²³ This can be justified mainly considering the bulky nature of Pt-containing arms of the complexes that branch off from N7 position of the nucleobase. Remarkably, for dien-containing complex it was not possible to investigate the PMF region at shorter distances (from 2.5 to 6 Å), due to the bulky nature of the ligand that does not allow any conformational reorganization of the molecule. This structural analysis, together with the energy behav-



ior discussed above, highlights how the protein channel crossing of complex **1** can occur at low energy cost, thus ensuring the pass through of the molecule in cellular environment. This aspect is nicely in line with the experimental outcomes and helps the rationalization of results from the cellular assays previously performed, in which the highest uptake by HeLa cells resulted for complex **1**, with respect to $[\text{Pt}(\text{dien})(\text{N7-dGTP})]^{2-}$ and $[\text{Pt}(\text{dien})(\text{N7-dGMP})]$ species.²³

Conclusions

This study provides insights into the interactions of N7-platinated guanosines with the hCNT3 transporter through molecular dynamics, highlighting their potential as therapeutic agents. Distinct behaviors were observed among the considered platinated derivatives, with $[\text{Pt}(\text{dien})(\text{N7-dGuo})]^{2+}$ (**1**) exhibiting a more hydrophobic nature compared to *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{N7-dGuo})]^+$ (**2**) and *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})(\text{N7-dGuo})]^{2+}$ (**3**). The results align well with experimental observations regarding the uptake of **1** at the plasma membrane level, suggesting hCNT3 as a potential transporter for this and related platinated species. The conformational changes observed for **1** compared to dGuo could be associated with their different steps along the transport cycle, as also observed for complexes **2** and **3**.

Several structural aspects of hCNT3 in the presence of complexes **1–3** were analyzed. Key mechanistic insights reveal that the platinated nucleosides alter the dynamics of the transporter, particularly in the intracellular loop and TM9 helix, leading to decreased flexibility and structural reorganization. This reduced flexibility may hinder nucleoside transport, highlighting the potential of these compounds to modulate transporter activity. Water molecules and hydrogen bonding were found to play significant roles in stabilizing the interactions, enhancing the inhibitory properties of **1–3** derivatives.

The findings propose a novel perspective based on the inhibitory potential of N7-platinated purine nucleoside derivatives, which could modulate the uptake of nucleosides at the cellular membrane level. These findings suggest that the previously tested complex **2** and its analogues could generate cytotoxicity in cancer cells not only by a classical mechanism involving interactions with cellular DNA, but also through a synergistic inhibitory activity affecting the activity of nucleoside transporters localized in the plasma membrane. Healthy cells are expected to be less sensitive to a decrease in the uptake of semi-essential nucleosides.¹⁶

This dual parallel mechanism could explain the observed low to moderate cytotoxicity associated with enhanced selectivity towards cancer cells, compared to healthy cells, recently observed on different cell lines for complexes in the series of *cis*- $[\text{Pt}(\text{NH}_3)_2\text{X}(\text{N7-Guo/dGuo})]^+$ (X = halido ligand) derivatives.²⁹ A role of Organic Cation Transporters (OCT) previously found to be responsible for the cell uptake of the Phenanthriplatin (*cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{phenanthridine})]^+$) antitumor drug cannot be excluded even for *cis*- $[\text{Pt}(\text{NH}_3)_2\text{X}(\text{N7-Guo/$

dGuo)]⁺ (X = halido ligand) derivatives, due to the same positive charge and high structural similarities.⁵⁶

Overall, this work underscores the potential of platinum-based modifications to enhance antitumor therapies based on nucleoside analogs by synergistically targeting hCNT transporters, metabolic pathways of natural nucleosides, and DNA functionality. Notably, the latter two interactions, in the case of complexes **2** and **3**, could also produce the classical 1,2-intrastrand cross-link lesions with DNA, which are responsible for the antitumor activity of cisplatin and analogous complexes.¹⁷ These considerations could lay the groundwork for new strategies aimed at developing antitumor drugs characterized by enhanced antitumor activity, selectivity for tumor cells, and lower systemic side effects.

The exploration of platinated nucleosides within the context of nucleoside transporters presents a novel and promising strategy for cancer chemotherapy.^{57–59} This dual-function approach utilizes the specific uptake pathways of nucleoside transporters, enabling platinated nucleosides to overcome some limitations of classical platinum agents such as cisplatin, which primarily depend on DNA binding.¹⁷ This strategy combines the targeted nature of nucleoside analogues, selectively taken up by rapidly dividing cells *via* nucleoside transporters, with the potent cytotoxic properties of platinum drugs. The outcome is a multifunctional therapeutic entity that not only enhances selective drug delivery and potentially minimizes systemic toxicity but also bypasses some common resistance mechanisms associated with traditional platinum treatments. Despite its higher selectivity for cancer cells, *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{N7-dGuo})]^+$ is less cytotoxic than cisplatin.²⁴ This is likely due to the presence of only one leaving chlorido ligand in its coordination sphere, compared to the two found in cisplatin, an aspect that could contribute to a reduction in unwanted side effects.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the SI. Docking results and additional analysis of Molecular Dynamics simulations. Supplementary information is available. See DOI: <https://doi.org/10.1039/d5qi01630d>.

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