Mechanism of Action of Anticancer Titanocene Derivatives: An Insight from Quantum Chemical Calculations

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Titanocene derivatives exhibit high potential in the treatment of cisplatin-resistant tumor types. Density functional theory calculations were performed on the hydrated form of five drug candidates differing in the pendant arms attached to the aromatic rings. A qualitative correlation has been found between the experimentally measured anticancer activity of alkylammonium-functionalized titanocene derivatives and the computed free energy change of the proton-induced dissociation reaction of these compounds. The results indicate that differences in the cytotoxic activities could be related to the solvation properties of the protolysis products, whereas no correlation was found with gas-phase properties of these molecules. Contrary to the free energy change of the protolysis reaction, other molecular properties, such as the geometrical parameters or the binding energies of the cyclopentadienyl rings in solution, do not correlate with the in vitro cytotoxic activity of these drug candidates.

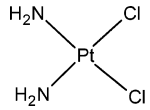
Introduction

Antitumor activity of titanocene has been discovered in the 1980s, and since then it has attracted continuous attention in the experimental literature.[1] This long-standing interest stems from the advantageous properties of these drug candidates. They are less toxic against healthy cells than cisplatin (for the structure see Chart 1) and offer a solution to overcome the platinum resistance of certain tumor types. Titanocenes also represent an important class of metallodrugs that is of interest from the basic physical and bioinorganic chemistry point of view.

Notwithstanding its close structural similarity with cisplatin, titanocene is suggested to function in an entirely different way.[2] DNA is believed to be the ultimate target of the drug, but presumably not the DNA bases due to the lowered affinity of titanium toward N-donors as compared to that toward O-donors.[3] Nevertheless, let us mention that in the early stages of the research even the cyclopentadienyls were suggested to be the active species,[4] although this view was soon rejected.[3a] The most recent results suggest that the serum transport protein transferrin mediates the uptake of Ti(IV) from titanocene via binding to the octahedral binding sites in its C- and N-lobes.[5] This event implies the loss of cyclopentadienyl rings and is believed to play a key role in the mechanism of action.

The major obstacle impeding clinical introduction of titanocene-based drugs is their low solubility. Recent studies report an effective method to increase the solubility of these compounds by attaching ionic pendant arms to the cyclopentadienyl rings.[6] In this way several new drug candidates were isolated, and some of them exhibited even higher antitumor activity than titanocene itself. The pendants used all included alkylammonium functionalities. Surprisingly, the drug activity was heavily dependent on the type of the alkylammonium group attached to the cyclopentadienyl ring. Yet, analysis of the crystal geometries of these compounds did not find any correlation between the X-ray structural parameters and the in vitro cytotoxicities of the substituted titanocenes measured on cell lines H209, A2780, H209/CP, and A2780/CP.[6a] This led to the conclusion that the active form of these drug candidates might be quite different from that observed in the crystal.

CHART 1



Titanocene dichloride [$TiCp_2Cl_2$] (Cp = η^5-C_5H_5) is well-known to hydrolyze in aqueous solution yielding [$TiCp_2(H_2O)_2]^{2+}$.[7] This species has an uncompensated positive charge of +2 that makes its chemical properties strikingly different from those of the neutral chloride form. Therefore, we will consider the hydrated form throughout this study.

Several literature examples illustrate the success of computational quantum chemistry at studying the mechanism of action of various anticancer metallodrugs such as cisplatin[8] and ruthenium complexes.[9] Metallodrugs are characterized by complex electronic structure. Thus, classical empirical force fields are of rather limited use for these systems, and their reliable description requires explicit consideration of molecular orbitals. However, quantum chemical calculations are typically done for small systems in the gas phase, which may lead to entirely incorrect conclusions since the properties of metal-cation-containing complexes are sharply modulated by the environment. In the current study we employ the density functional theory (DFT) computational technique to analyze the geometry and thermodynamic stability of titanocene dichloride

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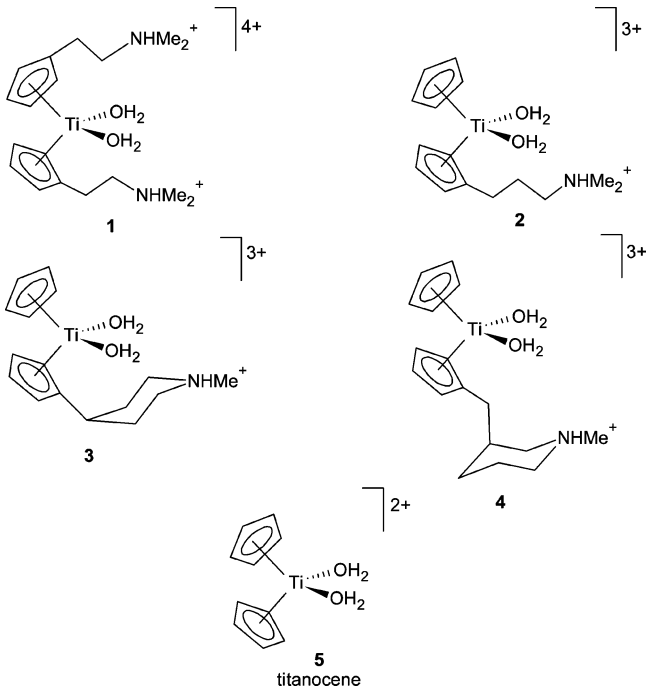
CHART 2



derivatives in their water-substituted forms with the aim to find out the possible molecular basis of the anticancer activity of these drug candidates. Our study complements the experimental work started in ref 6a by scrutinizing the geometry of the hydrated forms and the solution stability of these structures. We show that differences in the anticancer activities of titanocene derivatives can be correlated with the different solvation properties of the protolysis products, while they do not correlate with gas-phase properties of the studied molecules.

Computational Methods

All computations were performed with the Gaussian03 computer code.[10] Geometries were obtained from gradient optimization at the DFT level of theory with relaxation of all structural parameters. Initial geometries of the complexes were constructed on the basis of the X-ray structures given in ref 6a by replacing the Cl atoms with water molecules. Chart 2 gives a summary of all complexes considered in this study. In addition, we optimized the structure of the isolated water molecule, hydronium, and $[Ti(H_2O)_6]^{4+}$ cations, as well as that of the cyclopentadienyl anion along with its alkylammonium-substituted forms constituting compounds **1**−**4** (see Chart 2).

The density functional used in the current study is composed of Becke's three-parameter exchange functional[11] and Lee−Yang−Parr's correlation functional.[12] For geometry optimization we used the standard 6-31G* basis set on the C, N, O, and H atoms, while the Stuttgart relativistic pseudopotential (known as ECP 10 MDF) has been imposed on Ti.[13] This pseudopotential substitutes for the inner 10 electrons of Ti and was applied along with a polarized valence basis set of (8s,7p,6d)/[6s,5p,-3d] quality. To get more reliable energy data we have carried out single-point energy calculations on the optimized geometries by extending the basis set of Ti with an f-function of the exponent 0.5.[13]

Presence of polar solvent ($\epsilon = 78.4$, water) was mimicked with single-point calculations employing the COSMO continuum solvent model[14] in the standard parametrization supported by the Gaussian03 program: the average surface of a tesserae was 0.4 Å^2, and the minimum radius of the added spheres used to create the solvent-excluded surface was 0.2 Å. The united atom topological model[15] and a scaling factor of 1.2 were used to define the atomic radii. Among the numerous continuum dielectric methods the COSMO approach is specific in that it considers the solvent as a conductor with an infinite ϵ.[16] A direct consequence of this is that the total potential on the cavity surface is 0 in contrast to, e.g., the popular PCM (polarized continuum) treatment, which works with finite ϵ values.[17] Benchmark calculations of Chipman[18] revealed that the COSMO method in the formalism implemented in Gaussian03 gives satisfactory results for a wide range of systems, independently on the total charge of the solutes. In addition, the accuracy of the computed solvation free energies is not much dependent on the value of ϵ. On the contrary, performance of the PCM model is considerably poorer for ionic solutes. In the frame of the COSMO approach, the solvent contribution to the stability of the solvated species can be obtained as the difference of $\langle\Phi|\hat{H} + \hat{V}/2|\Phi\rangle$ and $\langle\Phi|\hat{H}|\Phi\rangle$, where Φ is the wave function optimized in solution, $\hat{H}$ represents the Hamilton operator of the isolated gas-phase system, and $\hat{V}$ is the operator representing the potential created by the solvent.

All energy data include zero-point correction as well as thermal correction to the Gibbs free energy calculated for 298 K.

Results and Discussion

Geometries in Hydrated Form. We have optimized the geometry of the hydrolyzed forms for the original and four substituted titanocene drug candidates (Chart 2) taken from the paper of Causey et al. (ref 6a) at DFT level of theory (Cartesian coordinates of the optimized structures are listed in the Supporting Information). Similar to the crystal data, the optimized geometric parameters of the hydrolyzed forms (listed in columns 1−8 of Table 1) do not reveal any systematic changes that could be correlated with the in vitro cytotoxic activity of the substituted titanocenes (see the human solid cell viability data (IC_{50}) in the last three columns of Table 1) reported in ref 6a. For example, bond distances listed in rows 2−4 of Table 1 are almost identical, whereas the activity of **4** is clearly superior to that of **2** and **3**. Therefore, we decided to examine the electronic and energetic consequences of the pendant group attachment to the cyclopentadienyl ring.

Electron Withdrawal by the Pendants. It has been suggested that alkylammonium functionalities withdraw electronic density from the Ti(IV) center and thereby make it more susceptible toward biological target molecules.[6a] To quantify the net effect of the electron withdrawal we computed NBO charges for the Ti(IV) centers in titanocene and its alkylammonium-substituted derivatives. As Table 2 illustrates, the lowest NBO charge was found for the nonsubstituted form (+1.11 au), while upon the presence of the alkylammonium pendants the charge of the Ti(IV) centers increased only slightly, by about 0.04−0.12 au. Thus, the charge of Ti(IV) centers can be considered to be nearly identical in the substituted forms, showing that the electron withdrawal affects mainly the cyclopentadienyl ring(s) and does not extend farther toward the central metal cation.

Binding Strengths of the Cyclopentadienyl Groups in Solution. It is reasonable to expect that the primary consequence of the electron withdrawal from the cyclopentadienyls is the deterioration of the Ti(IV)−cyclopentadienyl contact because of the decreased electrostatic interaction between these two parts. This, on the other hand, supports the view that dissociation of

TABLE 1: Optimized Geometrical Parameters (*d*, Distances in Å) and Human Solid Cell Viabilities (IC_{50}, μM) Measured for Cell Lines H209, A549, and A2780 from Ref 6a[a]

	d(Ti−C)[c]								IC_{50}		
	ring 1			ring 2							
compound[b]	max	min	ave	max	min	ave	*d*(Ti−O1)[d]	*d*(Ti−O2)[d]	H209	A549	A2780
1	2.58	2.32	2.44	2.58	2.32	2.44	2.12	2.12	100, 107	>200	>200,173
2	2.50	2.36	2.40	2.40	2.37	2.38	2.13	2.13	>200	>200	>200
3	2.53	2.35	2.41	2.40	2.37	2.38	2.13	2.14	129,158	>200	106,125
4	2.50	2.36	2.40	2.40	2.37	2.39	2.12	2.13	38 ± 19	91 ± 22	28, 21
5	2.40	2.37	2.38	2.40	2.37	2.38	2.13	2.13	89,151	≥200	109,131

[a] Structures of **1**−**5** were optimized at the Becke3LYP level of theory. [b] Schematic structures listed in Chart 2. [c] Longest (max), shortest (min), and average (ave) distance between the central Ti^{IV} and the C atoms of the cyclopentadienyl rings. In the monosubstituted compounds ring 1 carries the alkylammonium functionality. [d] Distances between Ti^{IV} and the O atoms of the water molecules.

TABLE 2: Computed NBO Charges (au) of the Ti Centers in Compounds 1−5[a]

compound[b]	NBO charge on Ti
1	1.23
2	1.16
3	1.18
4	1.15
5	1.11

[a] Geometries were optimized at the Becke3LYP level of theory. [b] Schematic structures are listed in Chart 2.

TABLE 3: Free Energy Change for the Dissociation Reaction in Solution Calculated According to Eq 1 (ΔG_1^{sol}), Free Energy Change for the Proton-Induced Cyclopentadiene Loss in Solution (ΔG_2^{sol}) and Gas-Phase (ΔG_2^{gas}) Calculated According to Eq 2, and Human Solid Cell Viabilities (IC_{50}, μM) Measured for Cell Lines H209, A549, and A2780[a]

				IC_{50}		
compound[b]	ΔG_1^{sol} [c]	ΔG_2^{sol} [c]	ΔG_2^{gas} [d]	H209	A549	A2780
1	85.9	−27.8	129.8	100,107	>200	>200,173
2	99.7	−13.6	282.2	>200	>200	>200
3	92.2	−22.9	271.8	129,158	>200	106,125
4	100.2	−19.8	281.4	38 ± 19	91 ± 22	28, 21
5	98.4	−15.5	372.7	89,151	≥200	109,131

[a] Total cell viabilities are taken from ref 6a. All energies are in kcal/mol. [b] Schematic structures are listed in Chart 2. [c] Computed at the Becke3LYP level using the COSMO continuum solvation model and gas-phase optimized geometries. [d] Computed at the Becke3LYP level.

the cyclopentadienyl rings might be an essential step in the anticancer action of titanocene derivatives. To assess the binding strength of the cyclopentadienyls (Cp′ and Cp″) in aqueous solution, we have calculated the thermodynamic driving force (ΔG_1^{sol}) for the dissociation of the considered drug candidates (eq 1) using the COSMO continuum solvation method.

$$[Cp'Cp'Ti(H_2O)_2]^{(4-x-y)+} + 4H_2O = [Ti(H_2O)_6]^{4+} + Cp'^{x-} + Cp''^{y-} \quad (1)$$

where *x*, *y* = 0, 1 for alkylammonium-functionalized and nonsubstituted cyclopentadienyl ligands, respectively. The computed data listed in the first column of Table 3 do not correlate with the observed trends in the anticancer activity (cytotoxicity data expressed as the total cell viabilities, IC_{50}, are listed in columns 4−6 of Table 3) of the tested drug candidates. ΔG_1^{sol} calculated for the inactive complex (**2**, 99.7 kcal/mol) is almost identical with that obtained for one of the most active substituted forms (**4**, 100.2 kcal/mol). Thus, on the basis of our computations, there is no correlation between the binding strength of the cyclopentadienyls and the activity of the studied drug candidates.

Hydrolytic Dissociation of Titanocene Is Endothermic. The high positive values of ΔG_1^{sol} indicate that hydrolysis of titanocene derivatives according to eq 1 is a strongly endothermic process. Thus, it is not likely to occur spontaneously in living organisms. Enzymatic catalysis can make the process exothermic; nevertheless, it cannot change the relative trends in the variation of ΔG along the studied series of compounds. In general, uptake of Ti(IV) by proteins may be divided into two steps: (i) dissociation of the cyclopentadienyl rings according to eq 1 followed by (ii) the binding of hydrated Ti(IV) to the active site. As the second step is independent of the type of the cyclopentadienyl ligand present in the nondissociated form of the titanocene compound, relative values of the total ΔG reflect the trends in ΔG of the dissociation step (eq 1). For the same reason, binding to the transport protein transferrin by simple dissociation of the drug candidates, suggested in ref 5b, cannot account for the observed reactivity trends.

Protons Make the Dissociation Exothermic. Above, we have shown that the binding strength of cyclopentadienyls in the hydrated form does not reflect the trends observed in the anticancer activity of the studied compounds. Although the average pH is close to 7 under physiological conditions, the local pH might be significantly lower near the surface of proteins. In the next step we therefore decided to check whether protons may play a role in the anticancer activity of titanocene derivatives. We have calculated the thermodynamic driving force (ΔG_2^{sol}) of the dissociation process under acidic conditions according to eq 2:[19]

$$[Cp'Cp''Ti(H_2O)_2]^{(4-x-y)+} + 2H_2O + 2H_3O^+ = [Ti(H_2O)_6]^{4+} + HCp'^{1-x} + HCp''^{1-y} \quad (2)$$

where *x*, *y* = 0, 1 for alkylammonium-functionalized and nonsubstituted cyclopentadienyl ligands, respectively.

The second column of Table 3 lists the computed ΔG_2^{sol} values for the proton-induced ligand exchange in aqueous solution. In contrast to the reaction described in eq 1 the protolysis of titanocene derivatives is an exothermic reaction. The absolute value of ΔG_2^{sol} is the lowest for **2**, followed by the nonsubstituted titanocene. For the other three titanocene derivatives the absolute value of ΔG_2^{sol} is at least 6 kcal/mol higher than that computed for **2**. This is quite significant considering the overall subtle free energy scale of biochemical processes.

We attempted to compare the computed ΔG_2^{sol} values with the experimentally measured cytotoxic activity (IC_{50}, see columns 4−6 of Table 3) of the studied titanocene derivatives. The lowest $|\Delta G_2^{sol}|$ was found for **2**, which is the only inactive compound in the studied series. According to the cytotoxicity data **4** is more active than **5**, and in line with this the computed

$|\Delta G_2^{sol}|$ of **4** (19.8 kcal/mol) is larger than that of **5** (15.5 kcal/mol). Obviously, no direct correlation can be expected between the thermodynamic and cytotoxicity data measured on cell lines. One of the main reasons lies in the relatively high error of the in vitro experiments. As the data listed in columns 4−6 of Table 3 show, sometimes the error might be commensurate with the actual value of IC_{50}. Similarly, it would be much too ambitious to expect that the differences of the IC_{50} values measured in different cell lines can be interpreted with thermodynamics of the dissociation process. Nevertheless, the results indisputably indicate that the only absolutely inactive compound (**2**) has a lowered $|\Delta G_2^{sol}|$ value relative to that of the other four studied complexes.

Thus, our computations suggest that protolysis of the cyclopentadienyl rings might be an essential part of the mechanism of action of antitumor titanocene derivatives, and perhaps, the true active agent is the protolysis product of the drug, i.e., $[Ti(H_2O)_6]^{4+}$. The dissociative scenario may explain why the activity spectrum of the structurally different titanium-based anticancer drug candidate budotitane (*cis*-$[(CH_3CH_2O)_2(bzac)_2$-Ti], where bzac is 1-phenylbutane-1,3-diketonate) is so similar to that of the titanocene derivatives.[20]

Let us note, however, that the correlation is not perfect. For example, the highest $|\Delta G_2^{sol}|$ is computed for **1**, while the IC_{50} values suggest only moderate activity for this complex. This suggests that other factors, e.g., the kinetics of the dissociation, might modify the in vitro cytotoxic activity of these compounds. In the studied series of titanocene derivatives, **1** is the only complex carrying a total charge of +4. It is reasonable to expect that removal of the cyclopentadienyl rings from a complex with a charge of +4 requires higher activation energy than that from a complex with lower charges, i.e., +3 or +2.

Role of Solvent. To see whether the experimentally observed reactivity trend correlates with the intrinsic properties of the single drug molecules, we have computed ΔG for the proton-induced hydrolysis reaction (see eq 2) of the five selected titanocene derivatives in the gas phase (ΔG_2^{gas}). Data in the third column of Table 3 confirm that the reactivity trend observed in solution does not stem from the intrinsic properties of the substituted titanocene molecules, as ΔG_2^{gas} is almost identical for the inactive compound **2** (282.2 kcal/mol) and the quite active compound **4** (281.4 kcal/mol).

In contrast, activity of the monofunctionalized drugs can be correlated with the solvent contribution to the stability of the cyclopentadienes formed as the protolysis products of titanocenes (definition of this quantity can be found in the Computational Methods). The substituted cyclopentadiene formed from the inactive compound **2** is apparently less stabilized by the aqueous solution than those derived from the two active forms, i.e., **4** and **3** (solvent contribution to the stability is −51.0, −55.1, and −56.6 kcal/mol, for the species formed from **2**, **4**, and **3**, respectively). This might have an important practical implication for the design of anticancer titanocene derivatives: our results suggest that one may create more potent drug candidates by improving the solubility of the protonated and substituted cyclopentadienyl rings.

Role of Protons. In fact, in solution protonation makes the whole thermodynamics exothermic, and moreover, it provides with an extra stability for the dissociated functionalized cyclopentadienes. Protonation of the cyclopentadienyl anion produces the neutral species, which is definitely worse solvated in aqueous solution than its nonprotonated form. The functionalized forms, which are nominally neutral, carry a charge of +1 after protonation, whose solvation is much more favorable than that of a neutral species. This might be the reason why most of the substituted drug candidates exhibit higher activities than the nonsubstituted form. Certainly, by increasing the number of alkylammonium functionalities to two the solvent contribution will be doubled, which may explain why the bifunctionalized drugs are usually more active than their monosubstituted counterparts.[6a]

Conclusions

This contribution represents the first quantum chemical effort dealing with the mechanism of action of anticancer titanocene derivatives. Model compounds formed by pendant arm attachment to the cyclopentadienyl rings of titanocenes were studied with DFT calculations. Their in vitro anticancer activity was then correlated with structural, electronic, and energetic properties computed in solution.

We have shown that proton-induced loss of the cyclopentadienyl rings might be a key step in the mechanism of action of these drugs. The thermodynamic driving force of this process can be correlated with the in vitro antitumor activity of the various drug candidates. In contrast, no correlation was found without including protons into the dissociation scheme.

Thus, our results support the view that titanocene might dissociate prior to binding to DNA, and most likely the active agent responsible for the anticancer activity is $[Ti(H_2O)_6]^{4+}$.

The experimentally observed anticancer activities of the monosubstituted titanocene derivatives can be associated with the solvation properties of the protolysis products rather than with the gas-phase behavior of these molecules. This puts an emphasis on the role of environment on the anticancer action of titanocene derivatives. In addition, it suggests that the search toward more effective titanocene-based drug candidates should consider finding substituents which substantially increase the solubility of the cyclopentadienes yielded by the protolytic dissociation of these compounds.

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Supporting Information Available: Cartesian coordinates of the optimized structures listed in Chart 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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