Drug Design

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Photoswitching the Cytotoxic Properties of Platinum(II) Compounds**

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Abstract: *The photoactivation of potential anticancer metal complexes is a hot topic of current research as it may lead to the development of more selective drugs. Photoactivated chemotherapy (PACT) with coordination compounds is usually based on a (photo)chemical reaction taking place at the metal center. Herein, a new strategy is exploited that consists of "photomodifying" a ligand coordinated to metal ions. Platinum(II) complexes from photoswitchable 1,2-dithienylethene-containing ligands have been prepared, which exhibit two interconvertible photoisomeric forms that present distinct DNA-interacting properties and cytotoxic behaviors.*

Transition-metal complexes represent an attractive class of compounds for medicinal or biological applications.[1] For instance, cisplatin and its derivatives have proven successful as metal-based anticancer drugs.[2] One major drawback of chemotherapy is the development of unpleasant treatment-related side effects, which are due to the poor selectivity of the therapeutic agents.[3] In that context, photoactivated chemotherapy (PACT) constitutes an elegant approach towards better control of drug-action specificity through spatial and temporal activation.[4,5] The photoactivation of metal-containing compounds is fundamentally metal-centered,[5] with various mechanisms of action.[6] To the best of our knowledge, potential PACT based on the photochemical modification of ligand(s) coordinated to a metal ion has not been reported so far.

1,2-Dithienylethene derivatives are photochromic molecules which can be converted into either their open or closed forms upon exposure to visible or UV light.[7] These thermally stable photoswitching moieties exhibit clear differences regarding specific physical properties, for example, molecule size, conformational flexibility, and electronic energy levels.[8] Consequently, this remarkable category of molecular switches has found numerous applications in several fields.[9]

Herein, we report on the preparation of two platinum(II) complexes obtained from photoswitchable diarylethene ligands, that is, $\mathbf{L}^{H}$[10] and $\mathbf{L}^{F}$[11] (Figure 1a), and the investigation of their DNA-binding abilities and cytotoxic properties. The studies reveal that the open/closed forms of the Pt/$\mathbf{L}^{H}$ and Pt/$\mathbf{L}^{F}$ complexes display distinct behaviors.

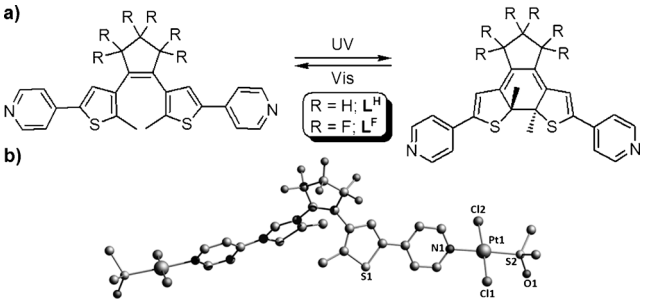

Figure 1. a) The light-driven switching process for 1,2-bis[2-methyl-5-(4-pyridyl)-3-thienyl]-cyclopentene ($\mathbf{L}^{H}$) and 1,2-bis[2-methyl-5-(4-pyridyl)-3-thienyl]-perfluorocyclopentene ($\mathbf{L}^{F}$). b) X-ray structure of the open form of **2**, with partial atom numbering. H atoms are omitted for clarity.

The ligands $\mathbf{L}^{H}$ and $\mathbf{L}^{F}$ (which are not water soluble) were synthesized by applying common procedures used to generate such molecules (see the Supporting Information).[12]

The crystal structures of one coordination compound from $\mathbf{L}^{H}$[13] and five coordination compounds from $\mathbf{L}^{F}$[10,14] have been reported so far, but none of them with platinum as metal. We have prepared platinum(II) complexes with each ligand by reaction of two equivalents of *cis*-[$PtCl_2$-$(DMSO)_2$][15] and one equivalent of either $\mathbf{L}^{H}$ or $\mathbf{L}^{F}$, in methanol at room temperature. The compounds, identified as

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trans-$[Pt_2Cl_4(DMSO)_2(\mathbf{L}^R)]$ (R = H for **1**, R = F for **2**), were fully characterized, and the structure of open **2** was obtained by single-crystal X-ray diffraction (see the Supporting Information).

A representation of the molecular structure of **2** is depicted in Figure 1 b. Crystallographic data, and selected bond lengths, and angles are summarized in Tables S1 and S2 in the Supporting Information. As expected, **2** is a dinuclear platinum(II) compound with two symmetry-related metal centers, which exhibit a slightly distorted square-planar geometry. It is notable that while the platinum precursor has a *cis* configuration, the two metal ions in **2** display a *trans* configuration. Such *cis*-to-*trans* isomerization has been observed, and is due to the *trans* effect of S-bonded dimethyl sulfoxide ligands.[16] The Pt–N, Pt–Cl, and Pt–S bond lengths (see Table S2) are in normal ranges.[17] The slight distortion of the square plane likely arises from steric hindrance induced by the DMSO methyl groups. The analytical data for **1** suggest that its structure is analogous to that of **2**.

The switching properties of $\mathbf{L}^H$ and $\mathbf{L}^F$, and the platinum complexes **1** and **2**, have next been examined by UV/Vis spectroscopy. The corresponding spectra are shown in Figures S1 ($\mathbf{L}^H$ and $\mathbf{L}^F$), S2 (**1**), and S3 (**2**). Before UV irradiation at $\lambda = 365$ nm, strong absorption bands are observed below $\lambda =$ 400 nm ($\lambda = 230$, 285 and 323 nm for $\mathbf{L}^H$; and $\lambda = 265$, 275 and 300 nm for $\mathbf{L}^F$), and are due to π–π* transitions of the open form of the ligands. Upon irradiation with UV light, two new absorption bands are observed at $\lambda = 378$ and 550 nm for $\mathbf{L}^H$, and $\lambda = 381$ and 592 nm for $\mathbf{L}^F$. These are ascribed to π–π* transitions of the closed form of the ligands.[18] Upon coordination to platinum, the π–π* transitions of both ligands are red shifted. The solution (in dichloromethane) UV/Vis spectrum of closed **1**, that is **1***, exhibits characteristic π–π* transition bands at $\lambda = 412$ and 622 nm (Figure S2), and that of **2*** at $\lambda = 404$ and 627 nm (Figure S3). Upon irradiation with visible light, the initial spectra are recovered for **1** and **2** (as for $\mathbf{L}^H$ and $\mathbf{L}^F$), therefore indicating that the photocyclization process is reversible. The stability of the closed forms of the two coordination compounds has been also evaluated by UV/Vis spectroscopy. Thus, solutions of **1** and **2** in CH_2Cl_2 have been kept both in daylight and in the dark for 24 hours. UV/Vis spectra have been subsequently recorded (Figures S2 and S3), and show that the closed complexes are stable in the absence of light. In contrast, in daylight, a clear decrease of the π–π* transition bands of the closed compounds is observed as a result of the partial opening of the coordinated ligands $\mathbf{L}^H$ and $\mathbf{L}^F$. The stability of the closed metal complexes in the dark is of paramount importance regarding the study of their potential DNA-interacting and cytotoxicity properties.

The potential interactions between DNA and the open/closed forms of **1** and **2** have been investigated by fluorescence spectroscopy. Competitive binding studies using ethidium bromide (EB) bound to calf thymus DNA (ct-DNA) have been carried out. Displacement of EB from the fluorescent EB-DNA adduct by a DNA-interacting molecule will induce fluorescence quenching.[19] Fluorescence spectra have been recorded at constant [ct-DNA] and [EB] (15 and 75 μM, respectively), and using increasing amounts of **1** and **2** (in the range 1–-25 μM). A clear decrease in emission intensity is observed, especially for the closed complexes. The data obtained thus show that the coordination compounds do interact with the biomolecule, and most importantly, that their open/closed forms exhibit drastically distinct behaviors.

The affinity of the compounds for DNA has been evaluated and compared using the Stern–Volmer quenching constant, K_{SV}, by applying Equation (1).

$$\frac{I_0}{I} = 1 + K_{SV}[\text{complex}] \quad (1)$$

Herein, I_0 and I are the emission intensities *without and with* complex, respectively. A plot of I_0/I vs. [complex] will give a straight line whose slope is equal to K_{SV}. The corresponding plots for **1** and **2** (open/closed forms) are depicted in Figure S4. The compound **1** displays a slightly better DNA affinity than **2**, with $K_{SV} = 6.6 \times 10^3 \pm 0.8\,\text{M}^{-1}$ ($\log K_{SV} = 3.82$) for **1**, while it is $4.8 \times 10^3 \pm 0.9\,\text{M}^{-1}$ ($\log K_{SV} = 3.68$) for **2**. This difference may be due to the fluorinated cyclopentenyl ring of $\mathbf{L}^F$, whose fluorine atoms may induce unfavorable electrostatic repulsion with the phosphate backbone of the double helix.[20] The most striking and important feature is the significantly different behaviors of the open and closed forms of both complexes; the K_{SV} value for closed **1*** is $42.5 \times 10^3 \pm 0.6\,\text{M}^{-1}$ ($\log K_{SV} = 4.63$), which is 6.5 times higher than that for open **1**. An analogous behavior is observed for **2*** with $K_{SV} = 26.8 \times 10^3 \pm 0.9\,\text{M}^{-1}$ ($\log K_{SV} = 4.43$), which is about 5.5 times the value obtained for **2**.

The greater interaction of the closed complexes with duplex DNA may be explained structurally. The X-ray structure of **2*** could not be obtained, however, the structures of the open/closed forms of one of the simplest members of the 1,2-dithienylethene family, namely 1,2-bis[2,5-dimethyl(3-thienyl)]-perfluorocyclopentene have been reported (see Figure S5).[21] The open form is distinctly more voluminous than the closed one (about 12 % longer and 25 % wider), and the molecule is clearly more planar upon ring closure, with a greater π conjugation. Consequently, it may be reasonable to expect that the **1*** and **2*** will have an increased proficiency to expel EB, owing to a sterically favored interaction with DNA. Furthermore, the electronic changes taking place upon ring closure will modify the coordination properties of the ligand, and therefore affect the metal center (which may modulate its DNA-binding properties).

It should be mentioned here that analogous studies with the free ligands $\mathbf{L}^H$ and $\mathbf{L}^F$ could not be performed, since they are not soluble in aqueous media or even buffer solutions containing up to 10 % DMSO. Therefore, the involvement of the metal ions is crucial and the mechanism of action (of the DNA interaction) is currently being investigated.

The interaction of the photoisomeric forms of **1** and **2** with DNA has then been studied by agarose gel electrophoresis. Electrophoretic-mobility measurements with pBR322 plasmid DNA were carried out, after a 24 hour incubation of increasing quantities of **1**/**1*** and **2**/**2*** with the biomolecule in the dark. The corresponding gels are depicted in Figure 2. The free plasmid DNA is principally composed of form I, that is, the normal supercoiled form, and some form II or relaxed circular form (Figure 2, lanes 1). As reported earlier,[22] the electrophoretic mobility of form II increases after incubation

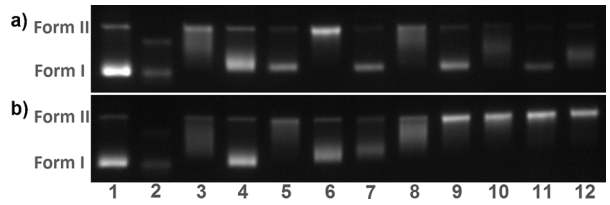

Figure 2. Agarose gel electrophoresis images of pBR322 DNA incubated for 24 h at 37 °C with increasing concentrations of a) **1** and b) **2** (open and closed* forms). Lane 1: pure plasmid DNA; lane 2: DNA + cisplatin; lanes 3,4: 0.5 equiv complex (op./cl.*); lanes 5,6: 1 equiv complex (op./cl.*); lanes 7,8: 1.5 equiv complex (op./cl.*); lanes 9,10: 2 equiv complex (op./cl.*); lanes 11,12: 2.5 equiv complex (op./cl.*). Each sample contains [DNA] = 15 μM.

with cisplatin (lanes 2) because of a shortening effect.[23] Incubation of 0.5 equivalents ([complex] = 7.5 μM) of either **1** or **2** with DNA appears to decrease the electrophoretic mobility until the complex/DNA adduct co-migrates with relaxed circular DNA (lanes 3). This phenomenon, ascribed to the unwinding of the double helix,[24] seems to be slightly more pronounced for **1**. In contrast, 0.5 equivalents of either **1*** or **2*** has almost no effect on supercoiled DNA. Only initiation of the reduction of the electrophoretic mobility is noticed (lanes 4). When 1 equivalent of the complex is used, clear disparities are observed between **1** and **2** (lanes 5). Indeed, while **1** nearly does not affect the DNA form I, **2** induces the disappearance of form I in favor of form II. Remarkably, when using 1 equivalent of **1*** and **2***, opposite results are achieved (lanes 6), that is, **1*** leads to the exclusive formation of form II, whereas **2*** initiates the alteration of the electrophoretic mobility of form I. Using 1.5 equivalents of the complexes causes comparable features (lanes 7 and 8). Open **1** and **2** mediate the reduction of the electrophoretic mobility of form I, with **2** being slightly more efficient than **1**. Both **1*** and **2*** cause an unwinding of DNA form I (smears are observed in lanes 8), thus decreasing its electrophoretic mobility. For higher complex quantities, (2 and 2.5 equivalents; lanes 9–12), the same behavior is observed for **1** and **1***, that is, form I and II are observed for **1**, while form I has disappeared for **1*.** A vanishing of the bands is also discerned, and may originate from the initial precipitation of the DNA/complex adduct. For **2** and **2***, the high concentration of complex leads, in all cases, to a total conversion of form I into form II. The open/closed forms of the complexes have drastically distinct DNA-interacting behaviors, with the closed ones modifying the DNA shape more efficiently. The closed **2*** performs better than **1***, which is reversed compared to the competitive binding studies.

Cell-viability assays were carried out subsequently. First, each compound (open/closed forms) was screened by means of single-point assays, using [complex] = 50 μM, with six cancer cell lines, that is A549 (lung adenocarcinoma), A375 (melanoma), DMS53 (small cell lung cancer, SCLC), GLC4 (SCLC), MCF7 (breast adenocarcinoma), and PC3 (prostate adenocarcinoma). The corresponding results, obtained after an incubation time of 48 hours, are shown in Figure 3 and Table S3.

The compounds **1**/**1*** exhibit very poor cytotoxicity properties. At best, a cell viability of 72 % is achieved with

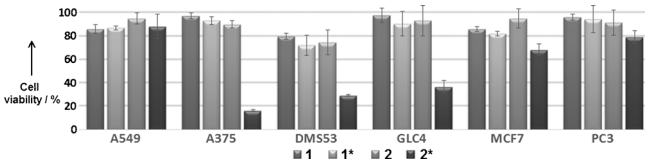

Figure 3. Cell-viability assays of the open and closed forms of the complexes with different cancer cell lines, that is, A549 (lung adenocarcinoma), A375 (melanoma), DMS53 (SCLC), GLC4 (SCLC), MCF7 (breast adenocarcinoma), and PC3 (prostate adenocarcinoma), using [complex] = 50 μM (single-point screening) and an incubation time of 48 hours (the results are given in % of cell viability; see Table S3).

DMS53 cells, using **1***. Furthermore, no important difference is noticed between **1** and **1***. For **2**, clear differences are observed between the open and closed forms. While open **2** does not show any significant cytotoxicity properties for all cancer cell lines tested, closed **2*** is active against most of them. The best result for **2** is obtained with SCLC DMS53, with a cell viability of 74 %. However, **2*** displays very good cytotoxic properties towards melanoma and SCLC DMS53, and to a lesser extent for adenocarcinoma. In particular, a cell viability of 16 % is achieved with skin-cancer A375 cells (melanoma), whereas it is 89 % for **2**. Significant disparities between the open/closed photoisomers are also observed in SCLC DMS53 (74 % vs. 29 %) and GLC4 (93 % vs. 36 %), and in MCF7 (95 % vs. 68 %).

IC_{50} values have been determined for the SCLC DMS53 cell line, which is the most common and most virulent of neuroendocrine (NE) cancers. The corresponding results, after incubation times of 48 and 72 hours, are shown in Figure S6, and listed in Table 1. The data obtained corroborate those achieved by single-point screening measurements. The behavior of **1**/**1*** is similar (identical IC_{50} values are obtained, and the same trend is observed for the IC_{25} and IC_{75} values in Table S4). As previously observed, **2**/**2*** exhibit distinct cytotoxicity properties. After an incubation time of 48 hours, **2** displays an IC_{50} value analogous to those of **1**/**1***, but **2*** is more than twice as active, with IC_{50} = 34.40 μM versus 75.87 μM (Table 1). After an incubation time of 72 hours, a marked difference between **2** and **2*** is still noted, albeit it somewhat less pronounced. Compounds **1**/**1*** are weakly cytotoxic, and this behavior is not altered by a longer incubation time. Compared to cisplatin, **2*** is about four to eight times less active. It should be taken into account,

Table 1: IC_{50} values[a] (μM) of open/closed **1**, **2** and cisplatin for the DMS53 cell line (SCLC), after 48 h and 72 h of incubation. ± SD of three independent experiments.

Compound[b]	48 h	72 h
1	77.37 ± 3.27	75.00 ± 5.39
1*	76.40 ± 5.26	73.59 ± 4.22
2	75.87 ± 5.42	55.00 ± 6.34
2*	34.40 ± 3.74	30.46 ± 4.38
cisplatin	8.53 ± 1.59	3.97 ± 0.31

[a] IC_{25} and IC_{75} have been determined as well, and are listed in Table S4. [b] Closed complexes are indicated by an asterisk (*).

however, that contrary to cisplatin, **2** is a photoactivatable molecule.

Finally, since these platinum(II) compounds show fluorescence properties, their cellular uptake in affected (A375 and DMS53) and unaffected (PC3 and A549) cell lines has been examined by confocal microscopy. The fluorescence data obtained clearly indicate that all compounds are capable of entering the cells. However, their behavior inside the cells is not the same, and is apparently associated with their cytotoxic activity. When the cell-viability assays reveal a poor cytotoxicity, vesicular structures are detected (A549, DMS53 and PC3 cells; see Figures S7, S8 and S9, respectively). In contrast, a diffuse cytoplasmic staining is observed when the compound is cytotoxic, as seen in Figure 4 for **2*** in A375.

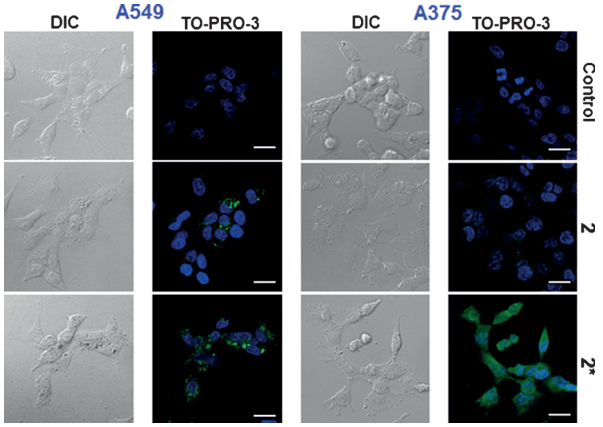

Figure 4. Uptake of **2**/**2*** by A549 and A375 cells, (24 h incubation at 37 °C; [c] = 25 μM). The merged confocal images, obtained from z-stacks, of the compounds (green) and the nuclear marker TO-PRO-3 (blue) are shown (right) together with the corresponding differential interference contrast (DIC) images (left). The white bar symbolizes a length of 20 μm.

Since platinum(II) drugs are expected to target DNA, intensity profile line scans have been performed to confirm whether the compound efficacy may be associated with its actual localization in the nucleus. Thus, pixel-intensity profiles of the compounds (green) and the nucleus (TO-PRO-3, blue) have been recorded, and the corresponding plots are depicted in Figure S10 (the intensity data have been recorded along the white arrows). Remarkably, **2*** is the sole compound which is found colocalized with the nucleus of A375 cells. In fact, **2*** is the most active molecule for this cell line (Figure 3), and suggests that its high cytotoxicity may be linked (at least in part) to its ability to reach the nucleus. Comparatively, **2**, which is much less active than **2*** against A375 cells (Figure 3), is not found in the nucleus (see Figure 4 and Figure S10).

In summary, a new family of photoactivatable cytotoxic molecules has been developed. The coordination compounds described herein present two photoisomers which interact differently with DNA duplex. Even more interesting is the fact that each isomer (particularly for **2**) exhibits a different cytotoxic behavior against various cancer cell lines, one of them (i.e. the closed form) being notably more active than the other. These first (unprecedented) results open up a new perspective in the field of PACT. We are currently preparing new members of this new category of molecules, and the study of the effect of the photoswitching process inside cells is being set up.

Keywords: cancer · cytotoxicity · drug design · photochemistry · platinum

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