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Dual-Functional Analogous *cis*-Platinum Complex with High Antitumor Activities and Two-Photon Bioimaging

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Supporting Information

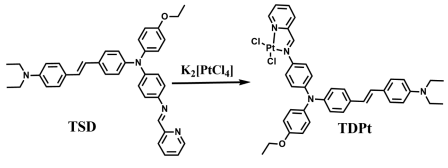
ABSTRACT: Here we have designed and synthesized a novel analogous *cis*-platinum complex (TDPt) with strong two-photon absorption properties and higher stability upon laser irradiation. Interestingly, a higher cytotoxicity against three types of cancer cells compared to that of commercial *cis*-platinum was observed. The initial confocal micrographs showed that lysosomes may be the biological targets of such TDPt, except the conventional presumed DNA. This hypothesis was further confirmed by the two-photon microscopy and transmission electron microscopy micrograph. These results form an important basis for future "on-site observation" of the anticancer mechanism of the Pt(II) complex.

Platinum-based drugs are among the most active anticancer agents and have been widely used in the treatment of a variety of human tumors, including ovarian, genitourinary, lung, head, and neck cancers.[1−3] Over the past 30 years, much progress in understanding the anticancer mechanisms involved in *cis*-platin and second-generation analogues such as carboplatin and oxaliplatin was made.[4−6] One of those is *cis*-platin, and its analogues bind to DNA preferentially at the N7 position of the guanine base,[7,8] inhibiting DNA replication[9,10] and transcription[11−13] and then resulting in cell apoptosis, which is widely accepted. Another possible mechanism is the disruption of lysosomes,[14] resulting in the release of their acidic contents into the cytoplasm, which will cause disruption of the plasma membrane and ultimately the death of the cell.[15,16] However, cellular resistance and systemic toxicity are two major defects of platinum-based anticancer drugs.[17,18] To address these issues, it is important to understand the pathway of the activities and toxicity of the drugs.

Recently, two-photon microscopy (2PM) has become a promising technique for monitoring the dynamic behavior of subcellular components and biological process in cells, because of its two conspicuous advantages over conventional confocal laser scanning microscopy.[19−21] First, the stimulating light beam has a high penetration depth because of low Rayleigh scattering and low tissue absorption of the near-infrared (NIR) light used.[22,23] Second, excitation takes place only at the focus, because of the scaling of the probability of simultaneous photon absorption with the square of the local light intensity.[24−26] This spatially confined excitation also decreases the level of photobleaching and photodamage of biological samples, affording high-contrast three-dimensional (3D) images. The more compact and economical Ti-sapphire ultrafast laser used as the excitation source led to microscopes designed for two-photon fluorescence microscopy imaging, which are available from the major microscope manufacturers.[27−30] Those features of 2PM made it useful for a wide range of bioimaging studies and held great promise for many useful applications such as chemical and structural imaging of the eye,[24] imaging and analysis of dynamic processes in living cells and tissues,[31−33] noninvasive diagnostic procedures for the detection of malignancy in organs, cancer imaging,[34] lysosomal[19,35] and vascular imaging,[36] diagnostic tools for tissue engineering and drug delivery,[37] and two-photon fluorescent probes for metal ion[38−40] and acidic vesicles.[41] 2PM has rapidly emerged as a powerful technique in the toolbox of the cell biologist over the past decade.

In addition to the interesting photophysical studies, the potential applications of the square Pt(II) complexes in nonlinear optical materials[42,43] have been recently explored. The selection of cyclometalated platinum(II) complexes for two-photon emission live-cell imaging has been very limited.[44,45] However, to the best of our knowledge, the Pt(II) complex with both anticancer activity and two-photon biological imaging functions, which is more conductive for understanding the anticancer mechanism, has been scarcely reported previously. For this purpose, an analogous *cis*-platinum complex (TDPt) was designed and prepared in this work (Scheme 1). The design concept for the title complex was as

Scheme 1. Synthetic Routes for the Ligand and Its Metal Complexes



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follows. First, triphenylamine is selected as the core group, because the electron-rich and high oxidizability of the nitrogen center of triphenylamine make it an ideal building block for constructing two-photon absorption molecular materials. Second, the diethylaminophenylethyne group is introduced to extend the π-conjugation length and act as an electron donor (D), and ethyoxyl groups work as auxiliary electron donors to improve intramolecular charge transfer (ICT). Finally, a pyridine Schiff base is used as a bidentate ligand, strongly coordinating to Pt(II) ion to form a square-planar analogous *cis*-platinum complex (TDPt).

The electronic absorption spectrum of complex TDPt shows strong absorption bands at 350−400 nm and two moderately intense bands at 450−525 and 530−625 nm, respectively, at 298 K in an ethanol solution. With reference to previous spectroscopic studies of platinum(II) complexes,[46,47] the high-energy intense absorption band at 350−400 nm is assigned to the intraligand (IL) transition of the Schiff base ligands (TSD). The band at 450−525 nm is assigned to a $d\pi^*(Pt) \rightarrow \pi^*(TSD)$ metal-to-ligand charge-transfer (MLCT) transition, while the lowest-energy absorption band at 530−625 nm is assigned to a $\pi^*(C{=}N) \rightarrow \pi^*(TSD)$ ligand-to-ligand charge-transfer (LLCT) transition, probably mixed with some MLCT contribution. The relatively low energy of the LLCT absorptions is attributed to the good electron donating ability of the triphenylamine moieties. Alternatively, the transition can be viewed as an intramolecular charge-transfer transition from the triphenylamine as a strong donating group to pyridine with a good π-accepting unit over the platinum metal center.

Moreover, TDPt was studied by transient absorption spectroscopy following excitation at 350 nm with a 150 fs laser pulse in ethanol solutions, and difference absorption spectra with different decay times are shown in Figure 1b.

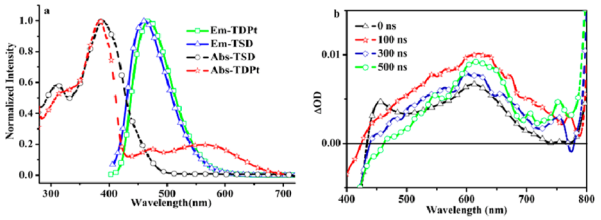

Figure 1. (a) Normalized linear absorption and one-photon excited fluorescence spectra of all compounds in a 1×10^{-5} M benzene solution. (b) Time-resolved femtosecond transient difference absorption spectra of TDPt in ethanol. λ_{ex} = 350 nm.

Single-exponential fits to the transient absorption decay gave a T_1 lifetime of 15 ns. Those results showed that the complex exhibits the ^{1}MLCT transition absorption (430−680 nm), which is consistent with the steady-state absorption spectra.[48] The emission spectra of TSD and TDPt were obtained in different solvents and are shown in Figures S4 and S5 of the Supporting Information, and the data for the emission spectra are summarized in Table S1 of the Supporting Information. The emission of TSD occurs at 469 nm with a high quantum yield (Φ_{em}) of 0.423 in benzene, and the emission energies for TDPt resemble those of the corresponding ligand because of the similar conjugated structure and the charge-transfer nature of the ligand and Pt(II) complex.. It is expected that TSD and TDPt exhibit at least moderately strong two-photon absorption (2PA).

There is no absorption in the NIR wavelength region (Figure 1). Consequently, the nonlinear absorption in the region is attributed to the two-photon-induced excited-state absorption. Figure 2 shows the typical nonlinear absorption data and the

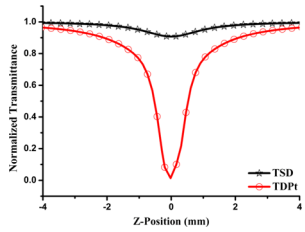

Figure 2. Open-aperture Z-scan experimental and theoretical fit curve of ligand TSD and complex TDPt at a wavelength of 820 nm.

fitting curves at 820 nm for TSD and TDPt. Under the experimental conditions described herein, the σ values are of actually effective cross sections that include contributions from both the absorption from S_1 and the absorption from S_2, obtained from a Z-scan method using the femtosecond laser.[49] The σ values for TDPt are much larger (almost 10-fold) than those of its free ligand TSD (Table S2 of the Supporting Information). To the best of our knowledge, the σ values should be the largest compared with those of the reported platinum complexes.[48] With the aid of the two-photon absorption theoretical calculation, the largest theoretical two-photon absorption cross sections obtained for the compounds are 2090 GM for TSD and 14212 GM for TDPt (1 GM = 10^{-50} cm^4 s photon^{-1}). The trend is in excellent agreement with their Z-scan experimental results. The large σ values in the NIR region as well as the good photophysical properties of the complex TDPt promoted us further to explore its potential biological applications.

Will 2PA microscopy (via the Z-scan method) supplant 2PEF as the standard microscopic method for biological imaging? To answer this question, we investigated the two-photon microscopy biological imaging application of TDPt. Syto9 costaining micrographs showed TDPt was clearly observed in live cells (Figure 3a), suggesting that the complex could be internalized with intracellualr cytoplasm without alternating the cell viability within short incubation windows. The colocalization profile shows minimal overlap among TDPt,

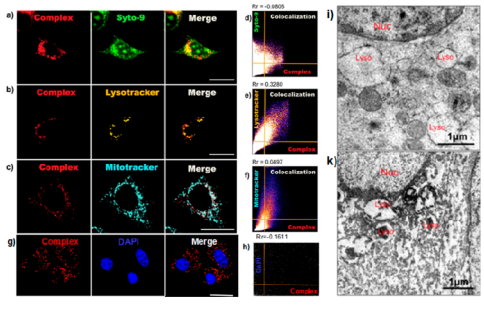

Figure 3. Confocal microscopy showing subcellular localization of TDPt (red) and colocalization with (a) Syto9 (green), (b) Lysotracker Yellow (yellow), and (c) Mitotracker Red (cyan). (d−g) Complex colocalization level with Syto9, Lysotracker, Mitotracker, and DAPI, respectively, indicated via a scatter plot Pearson's correlation (R_r). A TEM cell ultrathin section further revealed TDPt subcellular localization. (g) DAPI in MCF-7 cells. (i) Untreated cells. (k) Cells treated with TDPt.

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Syto9, and DAPI [Pearson's correlation (R_r) values of −0.0806 (Figure 3d) and −0.1611 (Figure 3h)], again suggesting TDPt has less internalization with cellular genetic material including DNA and RNA. In contrast, cells incubated with TDPt and then costained with Lysotracker showed relatively greater signal overlap [R_r = 0.3280 (Figure 3e)], which suggested that the TDPt was mostly uptake and closely associated with intracellular lysosomes. Furthermore, Mitotracker costaining experiments, shown in Figure 3f, reveal much less overlap with intracellular mitochondria (R_r = 0.0497). These results offer further support to the hypothesis that TDPt lysosome targeting is the reason for cancer cell apoptosis. It is worth noting that the Pt(II) cation factually has a geometrical environment of a planar square. This coordination configuration around Pt(II) leads to a high degree of structural symmetry. The remarkable change in the TPEF bioimage as well as emission characteristics induced by changes in the solvent may be regarded as a $\pi-\pi$ stacking effect preventing combination of TDPt and DNA.

As a third-row transition metal with an extremely high electron density, platinum complex TDPt is able to scatter the electron beam and serve as an electron microscopy (EM) agent. In this context, to further confirm whether the lysosome is the cellular destination of TDPt, MCF-7 cells were incubated with complex TDPt, and then the fixed cell pellet ultrathin sections were examined via transmission election microscopy (TEM). Via comparison with untreated cells (Figure 3i), it could be demonstrated that TDPt was distributed throughout the cell cytosol but clearly found at a much higher density within or closely associated with lysosome regions, as shown by the dark signal (Figure 3k). In further comparison with control cells, the cellular lysosomal morphology after treatment with TDPt also indicated a certain extent of membrane damage. As previously outlined, such lysosomal morphology could be an explanation for the unusually high anticancer activity of TDPt. As the pH of cellular lysosomes is known to be ∼5.0, we carefully presumed that the lysosomal binding and/or accumulation of TDPt is probably due to the N atom of the triphenylamine, Schiff base, or diethylamine providing both electron affinity and alkalinity leading to an acid-loving compound. Further lysosomal disruption is probably caused by osmatic shock, because of the significant increase in the level of local substances within the cellular lysosome, leading to the lysosomal morphology observed via TEM.

To date, most current platinum-based anticancer drugs are adducted in the nuclear DNA solely with single-photon emission characteristics. However, the DNA binding complex generally displays relatively higher cellular toxicity, hence bringing unwanted side effects for additional translational purposes. It is noteworthy that cellular lysosome stands out in a unique fashion against all other subcellular constituents. It is a membrane-bound organelle containing acid hydrolase enzymes that are capable of breaking down all types of biological polymers such as proteins, nucleic acids, carbohydrates, and lipids. It also plays a critical role in endocytosis cargo sorting, apoptosis, fertilization, and cell membrane repair. As a consequence in cancer therapy, drugs possessing the lysosome binding/breakdown property might potentially regulate and control the vigorous metabolism of the tumor and minimize the side effects incurred by healthy tissues.

In conclusion, we present the synthesis of the analogous *cis*-platinum complex (TDPt) with strong 2PA and high stability upon laser irradiation. It also showed cytotoxicity against three cancer cell lines higher than that of *cis*-platinum. The confocal micrographs showed that lysosomes are the subcellular targets of TDPt, not the conventional presumed DNA; this was further confirmed by the TEM micrographs of ultrathin sections of MCF cells. The possible anticancer mechanism is the entry and disruption of lysosomes, which has long been identified as a trigger for cell apoptosis. These results form an important basis for future "on-site observation" of the antitumor mechanism of the Pt(II) complex. Significantly, herein, the reported complex has dual functions, antitumor and high 2PA activity in the NIR region. The results provide an excellent starting point for designing and exploring the other medical probe for further in vivo applications.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, photophysical properties, and IC_{50} values (micromolar) for *cis*-platin, complex TDPt, etc. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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