All-in-One Molecular AIE Theranostics: Fluorescence Image Guided and Mitochondria Targeted Chemo- and Photodynamic Cancer Cell Ablation

Bing Guo,†a,b Min Wu,†a Qi Shi,†a Tianjiao Dai,†a Shidang Xu,†a Jianwen Jiang,†a Bin Liu* a,c

† Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore, 117585
E-mail: cheliub@nus.edu.sg
b School of Science, Harbin Institute of Technology (Shenzhen), Shenzhen, 518055 China
c Joint School of National University of Singapore and Tianjin University, International Campus of Tianjin University, Binhai New City, Fuzhou 350207, China

KEYWORDS . Aggregation-induced emission, cisplatin, mitochondria targeting, fluorescence imaging, photodynamic and chemo-therapy

‡ B. Guo and M. Wu contributed equally to this work.

ABSTRACT: Molecular theranostic platforms with precise molecular structure and multiple functions hold great promise for cancer therapy. Different from current strategy to incorporate various components into single entities with the risk of compromised efficacy and poor reproducibility, herein, molecular aggregation-induced-emission (AIE) photosensitizers with ingenious integration of AIE fluorophore and cisplatin are facilely synthesized for synergetic anticancer therapy. Through adjusting donor structures coordinated with cisplatin moiety and balancing the hydrophobic-hydrophilic property, donor-acceptor strength, and intramolecular charge transfer effect, the newly designed AIE photosensitizer TNPT exhibits good cellular uptake with predominant mitochondria location of cancerous cells, high chemotherapeutic efficacy similar to cisplatin, and strong reactive oxygen species (ROS) generation capability better than chlorin e6 (Ce6). Importantly, TNPT demonstrates synergetic photodynamic and chemo-therapy on C6 glioma cells, showing 2.4-fold more potent than cisplatin upon white light irradiation (15 J/cm²). Further cell cycle analysis and apoptosis assay indicate that the photodynamic and chemo-therapeutic functions of TNPT synergistically inhibit DNA replication and cause cell apoptosis. In addition, TNPT exhibits selective uptake on cancerous cells rather than normal cells, contributing to significantly lower cytotoxicity to normal cells as compared to free cisplatin. This study provides a facile strategy to design molecular theranostic agents.

INTRODUCTION

Among different treatment modalities for the life threatening cancers, chemotherapy is the dominant modality in clinics.1-4 To inhibit offensive tumors, repeated high dose chemotherapeutics are often conducted, but this would cause severe side effects and even trigger multiple drug resistance (MDR).5-7 As an alternative, combination therapy with simultaneous administration of different chemotherapeutic drugs and/or other modality of drugs has become the mainstay in cancer care to amplify therapeutic outcomes with minimized side effect, owing to the integrated merits from each drug with intrinsically distinct anticancer mechanisms.7-10 Nevertheless, highly efficient co-administration of different therapeutic agents is hurdled by their different pharmacokinetics profiles and the overall process is difficult to control.10-12 To circumvent these problems, dual-action drugs have been developed and proved quite effective, which often assemble different therapeutic agents into one entity with a single pharmacokinetic profile.13-15
Photodynamic therapy (PDT) has advantages in good spatiotemporal controllability, negligible MDR and minimal side-effect.\textsuperscript{8,46-49} Its efficacy is heavily dependent on both reactive oxygen species (ROS) generation capability and the distribution profile of photosensitizers (e.g., porphyrins, bodipy, etc.).\textsuperscript{10-20} However, oxygen-dependent PDT normally exhibits unsatisfactory therapeutic efficacy for hypoxic tumors, and could hardly realize efficacy on the infiltrating cancer cells distal from the primary tumor for the limited light penetration depth.\textsuperscript{19-20} To circumvent these problems, combinatorial PDT and chemotherapy is able to eliminate a wide range of cancer cells with high efficacy and introduce minimal potential in MDR.\textsuperscript{8,21-23} So far, most combinatorial “PDT + chemotherapy” agents appear in nanoparticles with physical encapsulation of different functional drugs,\textsuperscript{8,9,21-23} but these nanoparticles are far less preferable for FDA approval than small therapeutic molecules.\textsuperscript{53} The main reason is that nanoparticles generally exhibit dismal reproducibility and suboptimal combinatorial therapeutic outcomes with unpredictable drug concentration and location in different particles and varied release rate for different drugs.\textsuperscript{23-24} Recently, multifunctional molecular “PDT + chemotherapy” agents constructed by covalent linking between photosensitizers and chemotherapeutic drugs have shown promising dual-action anticancer performance, but most of them required tedious synthetic routes,\textsuperscript{25-27} and some showed significantly compromised chemotherapeutic potency as compared to free chemotherapeutic drugs.\textsuperscript{28-32}

It is known that the mode of action and efficacy of therapeutic agents is fundamentally influenced by their localization and uptake in the target cells.\textsuperscript{34-35} Rational design of organelle (e.g., nuclei and mitochondria) targeted therapeutic agents for combinatorial therapy is of great help to overcome MDR, decrease the therapeutic threshold dosage, minimize the cytotoxicity to normal organs and improve the therapeutic outcomes.\textsuperscript{34-35} As compared to nucleus targeted therapy in which efflux pumps generated on nuclei membrane and nucleotide excision repair mechanism often stimulate MDR development, mitochondria targeted therapy in the absence of both mechanisms is advantageous to execute MDR.\textsuperscript{36-39} Meanwhile, to achieve mitochondria targeting, it generally requires decoration of mitochondria-targeting peptide and/or moieties like triphenylphosphonium cation on therapeutic agents,\textsuperscript{5,6,8,25-27,41-44} adding additional synthesis workload. How to design simple molecular structures for mitochondria targeted combination therapy becomes our research interest.

Recently, we and others have demonstrated that aggregation-induced emission (AIE) photosensitizers, with intrinsic single-molecular theranostic characteristics,\textsuperscript{44-47} bright fluorescence and strong ROS generation capability in aggregate state,\textsuperscript{47} which is opposite to conventional photosensitizers or organelle trackers that often suffer from aggregation-caused quenching (ACQ) effect with quenched fluorescence and inhibited ROS generation properties in cell experiments.\textsuperscript{48} In addition, although AIE photosensitizer has been conjugated to Pt prodrug via a GSH responsive linker earlier,\textsuperscript{27} the photosensitizer and Pt worked separately at different locations without specificity. Therefore, simple molecular AIE platforms with synergetic mitochondria targeted combinatory “PDT + chemotherapy” without compromised individual therapy are promising to exert “all-in-one” theranostic anticancer performance.

In this contribution, we present a new molecular design strategy to fabricate highly efficient molecular theranostics, such as TNPT (Figure 1a) integrated with fluorescence imaging, mitochondria targeting, high chemotherapeutic efficacy and strong photodynamic performance to ablate cancer cells. The molecular design concept is illustrated thorough experimental and simulative investigation of the structure and property difference among cisplatin (PT), AIE donors (TN and TM), and complex molecules (TNPT and TMPT) (Figure 1a). Owing to the intrinsic differences in hydrophobic/hydrophilic property, donor-acceptor strength, and intramolecular charge transfer effect (ICT), the performance of the four molecules (TN, TNPT, TM and TMPT) were comparatively evaluated with focus on photophysical properties, ROS generation capability, cellular uptake, mitochondria targeting mechanisms, cancerous cell selection performance, anticancer pathways, and ultimate combined chemotherapeutic and photodynamic therapy.

RESULTS AND DISCUSSION

Molecular Design Principle. The concept is to compactly integrate PT moiety with AIE propeller through simple ionic coordination to generate new photosensitizers with potential in AIE fluorescence image guided mitochondrial targeted combinatory photodynamic and chemo-therapy (Figure 1). PT containing heavy atoms of Pt and Cl is a famous chemotherapeutic anticancer drug, which binds to DNA and interfere with its replication.\textsuperscript{49} Once PT is covalently linked to AIE fluorophore in a molecular complex, a donor-acceptor (D-A) structure will form between the AIE fluorophore as the donor and PT moiety as the acceptor, offering increased ICT effects with red-shifted absorption and emission, as compared to the AIE donor. In addition, the molecular complex would inherit chemotherapeutic function of PT moiety, and the AIE feature would inhibit non-radiative decay pathway for exciton energy showing bright emission at aggregate state.\textsuperscript{47} Furthermore, the heavy atom effect on the molecular complexes would enhance the spin-orbit perturbations and reduce the energy gap of $\Delta E_{ST}$ for the interset system crossing (ISC) process from the lowest excited single state (S$_1$) to the lowest triplet state (T$_1$), leading to improved ROS generation as compared to the pristine AIE donors.\textsuperscript{75-78} The presence of strong D-A strength would be helpful to separate the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) distribution and further reduce the $\Delta E_{ST}$, which can favour ROS generation.\textsuperscript{77-78} Moreover, the ionic coordination between PT moiety and pyridine group of AIE donor would bring in lipophilic and cationic feature with mitochondria-specific targeting function, leading to im-
proved cellular uptake for the complexes as compared to the hydrophobic AIE donors.

Since fluorophores with long wavelength absorption and emission are preferable for biomedical applications, we selected two pyridine group-containing AIE donors including 4-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)pyridine (TN) and (E)-4,4’-(2-phenyl-2-(4-(2-(pyridin-4-yl)vinyl)phenylene-1,1-diyl)bis(1,2-naphthalen-1-yl))amidine (TM), with the later having higher electron donating capability than the former (Figure 1a). After linking PT moiety with the pyridine group, the formed TMPT would have higher D-A strength and more red-shifted absorption and emission than those of TNPT. The structure-property relationship is discussed and their performance in cell imaging and ablation is evaluated.

**Synthesis and Characterization.** TNPT was synthesized via four steps as shown in Scheme 1 (supporting information). Briefly, 4,4’-(2-(4-bromophenyl)-2-phenylethene-1,1-diyi)bis(methoxybenzene) (TPE-Br) was synthesized via McMurry cross coupling reaction between bis(4-methoxyphenyl)methanone and (4-bromophenyl)(phenyl)methane in 35% yield. 4-(4-(2,2-Bis(4-methoxyphenyl)-1-phenylvinyl)styryl)pyridine (TN) was produced from TPE-Br and 4-vinylpyridine as the reactants by Heck coupling reaction in 83% yield. The PT-NO₃ compound which was yielded from -NO₃ substitution of chloride atom in PT molecule, reacted with TN to offer the product of TNPT in 87% yield. Similarly, TM was prepared via reaction between 4,4’-(2-(4-bromophenyl)-2-phenylethene-1,1-diyl)bis(1,2-naphthalen-1-yl)amine and vinylpyridine in 53% yield, which was further reacted with PT-NO₃ to give TMPT in 83% yield (Scheme S2, supporting information).

The photophysical properties of TN, TM, TNPT and TMPT in water were studied with UV-vis and photoluminescence (PL) spectrometer (Figures 1b-c and S1-S2). For the donor TN, it has two absorption peaks at 303 and 370 nm, and an emission peak around 536 nm, with a Stokes shift of 166 nm. For the other donor TM, it has two absorption maxima at 315 and 413 nm, and an emission peak around 510 nm, with a Stokes shift of 197 nm. These results indicate that TM would exhibit stronger electron donating capability than TN in the molecular complexes. For TNPT, it exhibits more red-shifted absorption maxima at 312 and 383 nm and an emission peak around 595 nm with a Stokes shift of 212 nm (Figures 1b-c). For TMPT, it has an absorption maximum near 329 nm and a shoulder at around 420 nm with an extended tail even beyond 560 nm, while its emission is around 686 nm with a Stokes shift of 266 nm. For these molecules, the absorption maximum near 310 nm is attributed to the typical π-π* transition of the substituted tetraphenyl ethylene (TPE), while the long wavelength absorption is due to ICT from AIE donor to acceptor (PT moiety). The ICT effect is evidenced by the red-shifted PL spectra in more polar solvents (Figures S1 and S2). The long wavelength absorption and emission of TMPT is about 37 and 91 nm red-shifted than those of TNPT, respectively, suggesting that the TM group in TMPT is able to assist stronger ICT than that of TN group in TNPT. The fluorescence quantum yields (QYs) of TNPT and TMPT in water were measured to be 9.2% and 0.7%, which are lower than their precursors including TN (16.6%) and TM (2.1%), respectively. These QYs were measured using (dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4-hydroxynaphthalene as the reference (Figures 1 and Supporting Information).

Figures S1a and S1d show the PL spectra of TN and TNPT at different fₚ conditions, respectively. For TN, the emission was significantly quenched at initial stage when fₚ is ≤ 40%, because the intense rotation of the AIE donor could consume exciton energy through non-radiative decay pathway. Upon further increasing fₚ to ≥ 50%, the emission intensity was sharply enhanced with a peak around 532 nm, revealing the AIE feature (Figure S3). For TNPT, upon adding water into its DMSO solution, the emission intensity was gradually increased and emission maxima was ultimately blue-shifted from around 650 to 595 nm, as a result of aggregation formation and less polar environment inside the aggregates (Figure id). As to the PL spectra of TM and TMPT in DMSO/water mixture, their emission changes at different fₚ (Figures 1e and S3) are quite similar to those of TN and TNPT, respectively.

To examine the photodynamic therapeutic performance of the AIE molecules in vitro, we evaluated the ROS generation capability under white light irradiation with 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) as the ROS indicator. The ABDA decomposition rate at different time points was continuously monitored by UV/vis
spectrometer (Figures 1f and S5). It is found that the indicator decomposition rate of TNPT is 2.0 and 1.4-fold higher than TN and Ce6, respectively, while the indicator decomposition rate of TMPT is 3.3-fold higher than TM, suggesting that the covalent attachment of PT moiety on the AIE rotors successfully improved the ROS generation rate, in good agreement with the expectation. Notably, the decomposition rate of TNPT is 16-fold higher than TMPT. The main reason is that the stronger D-A strength leads to a higher ICT effect, which competes with the fluorescence and ROS generation processes (Figures 1f, 3 and S5). These results suggest that when introducing a D-A strength into TNPT, the ICT and \( \Delta E_{\text{ ICT}} \) should be carefully balanced to achieve optimal ROS generation for PDT application. In addition, both TNPT and TMPT were found to be more stable than Ce6 (Figures 1g and S6), indicating good photostability of these AIE molecules.

![Figures 2. Optimized confirmation of structures from top-view and side-view for TN (a), TNPT (b), TM (c), and TMPT (d), respectively. The torsion angles are labelled along the molecular backbones.](image_url)

![Figure 3. (a) HOMO and LUMO wave functions at \( S_1 \) in the geometrically optimized structures (B3LYP/6-31G(d), B3LYP/6-311G(d) + SDD for Pt), scf (SMD, solvent = water). (b) \( \Delta E_{\text{HOMO-LUMO}}, \Delta E_{\text{S1-T1}}, QY \) values of the compounds.](image_url)

**Molecular Modelling.** To further understand the photophysical properties of TN, TM, TNPT, and TMPT, density functional theory (DFT) calculation was conducted (Figures 2–3). As shown in Figure 2, the optimized geometry results suggest that the electron-rich substituted TPE units of all the four compounds exhibit highly twisted conformations with large torsion angle between adjacent linkages along the molecular backbones, while the electron-deficient parts of the molecules show quite planar structure which would facilitate ICT among molecular backbones. Notably, the twisted conformation characteristics derived from substituted TPE units would play an important role to support their AIE features and/or good ROS generation capability in aggregate states, by reducing \( \pi-\pi \) intramolecular stacking in aggregate states, which consists well with the experimental results in Figure 1. For the electronic structures of the four compounds, their HOMO wave functions are mainly distributed among the electron-rich part of substituted TPE units, while the LUMO wave functions tend to delocalize on the electron-deficient parts like vinyl pyridine for TN and TM, and vinyl pyridine-PT complex for TNPT and TMPT. As compared to TN and TM, the LUMO wave functions of TNPT and TMPT are more intensively localized on the electron-deficient parts. This indicates that PT coordination effectively increased D-A strength to yield red-shifted absorption and emission, which agrees with the experimental results (Figures S1–2). Furthermore, the \( \Delta E_{\text{ ICT}} \) values of TNPT and TMPT were calculated to be around 0.658 and 0.472 eV, which were lower than that of TN (0.798 eV) and TM (0.547 eV), respectively. The fact that TMPT did not generate higher ROS production than TNPT (Figure 1f) suggests that the ROS generation capability of photosensitizers could be affected by both \( \Delta E_{\text{ ICT}} \) and ICT.

**Imaging.** Cellular uptake is important for drugs to exert their imaging and therapy functions. We used confocal laser scanning microscopy (CLSM) imaging to real-time visualize cellular uptake and location profiles of the AIE compounds (TN, TNPT, TM and TMPT) in C6 glioma cells. As shown in Figures 4 and S7, C6 cells were clearly taken up by cells than that of TN and TM molecules, respectively. To further investigate the mitochondria specificity of the AIE compounds, commercial MitoTrackers were used to co-stain cells. While hydrophobic TN and TM molecules were randomly distributed among cytoplasm, the TNPT signals overlapped well with the signals of MitoTracker in cells. This suggests that the positively charged lipophilic PT coordination could successfully drive TNPT molecules to preferentially accumulate inside mitochondria, which would facilitate TNPT to locally destroy mitochondria to yield high cytotoxicity.
stably, although TMPT and TNPT have almost the same PT coordination structure, TMPT did not show obvious mitochondria targeting, but instead showed random distribution in cytoplasm. The overlay between the presented dyes and MitoTracker dye was quantitatively evaluated with the Pearson’s correlation coefficient (R) (Figure 4). For TNPT group, the Pearson’s coefficient of TNPT and MitoTracker is 0.85, while for all other groups, the coefficient is even lower than 0.1. To understand the significant difference in cellular location between TNPT and TMPT, DFT modelling was further conducted to understand the hydrophobic-hydrophilic properties of the four molecules (TN, TNPT, TM and TMPT) in water (Figure 5). As all the molecules share the same core, the hydrophobic-hydrophilic properties could be studied through the estimation of interaction energy between the respective functional groups and water molecules. Therefore, N, N-4-trimethylaniline, 1-methoxy-4-methylbenzene, 4-methylpyridine, and PT-pyridine coordinated moiety were selected to compare their interaction energy with water. Notably, the interaction energy of PT-pyridine coordinated moiety..water (-0.368 eV) is stronger than that of 4-methylpyridine..water (-0.303 eV), indicating that TNPT and TMPT are much more hydrophilic than TN and TM, respectively. This is in good agreement with the dynamic laser scattering results that the sizes of TNPT and TMPT in water were much smaller than those of TN and TM in water, respectively (Figure S4). Meanwhile, it is found that the interaction energy of 1-methoxy-4-methylbenzene..water (-0.279 eV) is higher than that of N,N-4-trimethylaniline..water (-0.259 eV), which suggests that TNPT molecule is more hydrophilic than TMPT. The higher hydrophilicity of TNPT allows it to be better dispersed in cellular environment, which facilitates mitochondria targeting, relative to TMPT (Figure 4).

Flow cytometry measurements were carried out to monitor cellular uptake of drugs at different time points (Figures S7). It shows that C6 cells exhibit much faster uptake rate and result in much higher intracellular accumulation of TNPT than that of TN, which is consistent with the CLSM results shown in Figures 4c and 4d. These results further suggest that well balanced hydrophobic-hydrophilic and cationic structure is of high importance in achieving enhancement in cellular uptake, imaging, mitochondria targeting, and ultimately high anti-cancer efficacy.

Cancer Cell Ablation. After demonstrating that TNPT has typical AIE properties, strong ROS generation capability and effective mitochondria targeted fluorescence imaging function, we studied the cell viability by MTT assay after TNPT treatment under dark and light, respectively (Figure 6). The control groups included TN, free PT, TN+PT, TM, TM+PT, and TMPT groups. After 48 h incubation under dark conditions, TN and TM (40 μM) treated groups showed more than 81% and 95% cell survival, respectively, suggesting low dark cytotoxicity. In contrast, under the same conditions, TNPT and TMPT groups demonstrated more than 35.4% and 55.6% cell inhibition, much higher than that of TM and TN groups, respectively. The IC_{50} of TNPT group is determined to be 19.3 μM, which is quite close to the chemotherapeutic efficacy of free PT (IC_{50} = 16.8 μM). The result of high dark potency of TNPT is much better than its analogues such as TMPT, BODIPY-Pt conjugates, and platinum-porphyrin conjugates, probably owing to its balanced hydrophilic-hydrophobic property, and resultantly good cellular uptake and mitochondria targeting function for exerting potent cytotoxicity under dark.
After white light irradiation (50 mW/cm², 5 min), more than 65% and 94% survival rates were found for cells treated with 40 µM of TN and TM, respectively, mainly because of their suboptimal cellular uptake and low ROS generation rate. In contrast, the IC_{50} value of TNPT was as low as 8.3 µM, which is 2.1-fold lower than that of free PT (IC_{50} = 17.2 µM) under the same conditions. Moreover, the IC_{50} value of TNPT under light irradiation (15 J/cm²) is 2.3-fold lower than that under dark, which demonstrates that the merits of TNPT including intrinsically high dark cytotoxicity similar to PT, strong ROS generation capability and good mitochondria targeting functionality, synergistically contributes to its significant cytotoxicity under light exposure. Notably, the light cytotoxicity of TMPT is almost as low as its dark cytotoxicity, which is mainly attributed to its random location in cytoplasm and low ROS generation performance. These results demonstrate that the well customized structures and functionalities facilitate TNPT to exhibit good combinatory chemotherapy and PDT performance.

![Figure 6](image)

**Figure 6.** Inhibitory effects of TM, TMPT, PT, and TN+PT on C6 cell under dark (a) and light irradiation at 50 mW/cm² for 5 min at 6 h (b). The total time of incubation is 48 h. Inhibitory effects of TN, TNPT, PT, and TN+PT on C6 cell under dark (c) and light irradiation (15 J/cm²) at 50 mW/cm² for 5 min (d). The total time of incubation is 48 h.

To further understand the C6 cell death pathways with combinatory photodynamic therapy and chemotherapy of TNPT, Annexin V-fluorescein isothiocyanate (AV-FITC, green fluorescence) and propidium iodide (PI, red fluorescence) double-staining apoptotic analysis were conducted to identify apoptosis of cells, the fractions of different cell states, including viable, early apoptotic, early necrotic, and dead cells were characterized by flow cytometry (Figures 7a and S8). Healthy cells are impermeable and cannot be stained by neither PI nor AV-FITC, while necrotic cells can be marked by PI which intercalates with nucleus DNA. For the late apoptotic cells, they could uptake both PI and AV-FITC, while for the early apoptotic cells, they only uptake AV-FITC. The cells were separated into different groups including control, TN, PT, TN+PT, TNPT groups under dark and light irradiation, respectively. As the statistical analysis is shown in Figure S8, the lower left is for healthy cells and the upper left is for dead cells, while the lower right represents early apoptotic cells and the upper right describes the late apoptotic cells. Under dark conditions, TN did not cause apoptosis or necrosis due to its low cytotoxicity. Under the same dark conditions, both TNPT and TN+PT treatment is in the similar potent level as PT treatment alone, with around 30% apoptotic death of cells, in which most are early apoptosis. This suggests that TNPT with mitochondria targeting capability has comparable therapeutic efficacy as free PT to induce early apoptotic damage to C6 cells. Upon light irradiation, TNPT treatment induced more severe early and late apoptosis of cells with an apoptotic population of around 67%. Notably, the potency of TNPT under light irradiation is 4.5, 2.4, and 1.6-fold higher than that of the TN, PT, and TN+PT treatment. These results demonstrate that the mitochondria targeted chemotherapeutic and photodynamic functionalities of TNPT synergistically contribute to the high anticancer efficacy.

To understand the therapeutic efficacy of the synthesized photosensitizers on C6 cell progression, we studied the cell cycle arrest ability of TNPT under dark and light, respectively (Figures 7b and S9). Cell cycle distribution in different groups including control, TN, PT, TNPT and TN+PT groups was distinguished by the fluorescence intensity of PI in flow cytometry. As shown in Figure 7a, under dark conditions, TN did not obviously affect the cell cycle, which is in good agreement with its low dark cytotoxicity (Figure 6). In contrast, TNPT arrests the cell cycle at the Go-Gi phase for C6 cells to a certain degree, which is similar to that of PT group and TN+PT group. The blocking of the cell cycle at the Go-Gi phase indicates that TNPT exert its cytotoxicity via inhibiting DNA function in mitochondria, which is cellular major powerhouse and plays a critical role in apoptosis, and resultantly suppress nucleus DNA replication in S phase and further inhibit cell division in G2-M phase. Under light conditions, it is noticed that TNPT arrested the cell cycle at Go-Gi phase more effectively than TN, TN+PT and PT groups. This indicates that combinatory PDT and chemotherapy of TNPT effectively inhibited the mitochondria function, leading to significant cell death.

![Figure 7](image)

**Figure 7.** (a) Apoptosis analysis of C6 cells by flow cytometry. C6 cells were treated with TN, PT, TN+PT and TNPT for 48 h, then incubated with AV-FITC (green) and PI. (b) Cell cycle arrest of C6 cells by flow cytometry. After treatment with TN, PT, TN+PT and TNPT (10 µM) for 48 h, C6 cells were fixed and stained with PI, while the DNA content was tested with flow cytometry.

The targeting and killing efficacy of TNPT between cancerous and normal cells was further investigated (Figure 8). Notably, the fluorescence intensity for the C6 cell...
group was much higher than that of normal cell groups (HEK 293 and NIH-3T3). This indicates the intrinsic and good selectivity of TNPT on cancerous cells rather than normal cells, because of the more active metabolism, the more intense uptake behavior and the higher mitochondrial membrane potential in cancerous cells, relative to normal cells.\textsuperscript{28} Figures 8d-g illustrate the TNPT toxicity on normal cells. For NIH-3T3 cells under dark and light conditions, the TNPT (40 \textmu M) treated group showed above 88.8\% and 72.5\% survival rate, respectively, suggesting the low cytotoxicity. In contrast, the PT (40 \textmu M) treated groups for NIH-3T3 cells, the survival rate under dark and light conditions is 21.8\% and 22.1\%, respectively. For HEK 293 cells, after the TNPT (40 \textmu M) treatment, the survival rate was calculated to be 66.9\% and 53.8\% under dark and light conditions, respectively, while that of PT (40 \textmu M) groups are determined to be 15.8\% and 17.0\%, under dark and light conditions, respectively. Taken together, TNPT could selectively destructs C6 cells with efficacy higher than PT, but brings far less damage to normal cells than PT, which is highly preferable for future clinic translation.

TNPT in cells was indicated by the fluorescent signals corresponding to the compound TNPT. Inhibitory effects of TNPT or PT at different concentrations on NIH-3T3 cells under dark (d) and light irradiation (50 mW/cm\textsuperscript{2}, 5 min) (e) at 6 h, respectively. Inhibitory effects of TNPT or PT at different concentrations on HEK 293 cells under dark (f) and light irradiation (50 mW/cm\textsuperscript{2}, 5 min) (g) at 6 h, respectively.

CONCLUSIONS

A simple molecular theranostic platform was established to show AIE fluorescence image guided mitochondria targeted combinatory photodynamic and chemotherapy. The coordination of cisplatin to AIEgens not only introduces lipophilic cation, but also increases donor-acceptor strength with enhanced ICT effect, red-shifted absorption and emission, and improved ROS generation capability. The fact that TMPT has lower fluorescence quantum yield, narrower \( \Delta E_{ST} \) value, but less ROS generation capability than TNPT revealed that for photosensitizer design, both the donor-acceptor strength and ICT should be well considered to achieve optimal photosensitization performance. Importantly, good ROS generation rate for the cisplatin-coordinated AIEgen such as TNPT rather than TMPT could be achieved by balancing the donor-acceptor strength and ICT effect. In addition, through experimental and modeling results, it is concluded that TNPT has good water-dispersity, excellent cellular uptake, high mitochondria targeting, potent chemotherapy similar to free cisplatin and strong PDT efficacy on cancerous cells, while showing significantly inhibited side effects on normal cells. In addition, the cell cycle assay and apoptotic analysis revealed that the therapeutic mechanism of TNPT is to significantly inhibit DNA replication and cause cell apoptosis. This study points out new insights and directions for future development of highly effective dual-action molecular cancer drugs.

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.*

AUTHOR INFORMATION

Corresponding Author

*Bin Liu

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore, 117585

E-mail: cheliub@nus.edu.sg

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The authors are grateful to the Singapore NRF Competitive Research Program (R279-000-483-28J), National University...
REFERENCES


(38) Browning, R. J.; Reardon, P. J. T.; Parhizkar, M.; Pedley, R. B.; Edirisinghe, M.; Knowles J. C.; Stride, E. Drug Delivery Strategies for Platinum-Based Chemotherapy. ACS Nano 2017, 11, 8560-8578.


All-in-One Molecular AIE Theranostics: Fluorescence Image Guided and Mitochondria Targeted Chemo- and Photodynamic Cancer Cell Ablation

Bing Guo,‡ Min Wu,‡ Qi Shi, Tianjiao Dai, Shidang Xu, Jianwen Jiang, Bin Liu*

A molecular theranostic agent (TNPT) was synthesized and characterized for synergetic AIE fluorescence image guided mitochondria targeted combinatory photodynamic and chemotherapeutic therapy.