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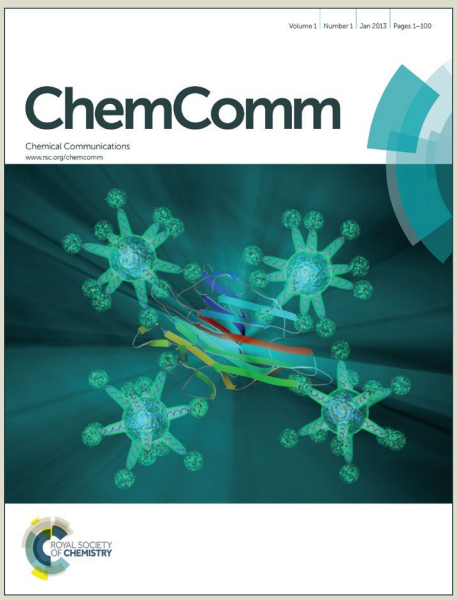

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A Novel Azopyridine-based Ru(II) Complex with GSH-responsive DNA Photobinding Ability

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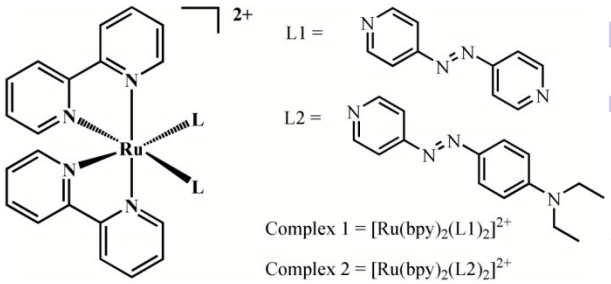
A novel azopyridine-based Ru(II) complex $[Ru(bpy)_2(L1)_2]^{2+}$ (bpy = 2,2'-bipyridine, L1 = 4,4'-azopyridine) was designed and synthesized as a potential glutathione (GSH)-responsive photoactivated chemotherapy (PACT) agent, the DNA covalent binding capability of which can be only activated after GSH reduction and visible light irradiation.

Cisplatin has been clinically used for decades for the treatment of many types of cancer, however, the efficacy is limited severely by its notorious side effects as the result of its poor selectivity toward cancer tissues.[1] Among the strategies that have been utilized to solve this problem, photoactivated chemotherapy (PACT), which uses light as the trigger of drug activity, is particularly appealing.[2] By spatial and temporal control of the irradiation, the toxicity may be confined within the diseased sites. While several novel Pt complexes have been examined as promising PACT agents,[3] more efforts have extended to non-Pt transition metal complexes to fully make use of their rich and tunable photophysical and photochemical properties.[4] Among them, Ru(II) complexes with photolabile ligand(s) are drawing increasing attention, which can covalently bind to DNA in a manner very similar to cisplatin after photoinduced ligand dissociation.[4b-l] More intriguingly, in some Ru-based PACT agents, the photolabile ligand itself is anti-cancer species, leading to the so called *dual-activity* PACT agents in which two or even more anti-cancer active species are released upon irradiation.[5]

Though PACT owns unique selectivity, undesired irradiation, especially sunlight, may lead to severe damage to skin if the PACT agents accumulate in but cannot be clear of in a proper period of time. This drawback is also universal in photodynamic therapy (PDT),[6] another type of photoactivation cancer treatment modality that has got clinical application. To address this issue in PDT,



Scheme 1. Chemical structures of the complexes **1** and **2**.

additional tumor-related factors, including lower extracellular pH value,[7] reducing intracellular environment,[8] over-expressed enzymes,[9] are often involved in drug activation in combination with light irradiation. Such *dual-activation* strategy, in which drug activity can be only switched on by two types of triggers, may improve PDT selectivity further. Though widely applied in developing new PDT agents, to the best of our knowledge, the *dual-activation* PACT agents in which the photoinduced ligand dissociation process is initiated by two types of stimuli, have not been reported so far. As a proof-of-concept, we herein report an azopyridine-based Ru(II) polypyridyl complex $[Ru(bpy)_2(L1)_2]^{2+}$ (complex **1** in Scheme 1, bpy = 2,2'-bipyridine, L1 = 4,4'-azopyridine) as a novel redox-responsive PACT candidate. It is well known that the monodentate ligand pyridine is photolabile in $[Ru(bpy)_2(py)_2]^{2+}$ (py = pyridine), and its photodissociation occurs from a low-lying ^{3}MC (metal-centered) state, which is thermally accessed from the lowest lying ^{3}MLCT (metal to ligand charge transfer) state.[10] By tethering redox-active azo groups on the pyridine ligands, **1** is endowed with the following two important properties. First, as an efficient energy acceptor,[11] the azo groups can quench the ^{3}MLCT state and thus block the monodentate ligand photodissociation effectively. Secondly, upon reduction of the azo groups by reducing agents, such as glutathione (GSH),[12] the photolabile activity of the monodentate ligand can be switched on accordingly. Given that many tumor cells are in reducing microenvironment as the result of elevated GSH concentration, which is usually several times higher than that in normal cells,[13] and that this characteristic has been widely utilized for tumor-targeted drug release[14] and PDT selectivity improvement,[8] the GSH-responsive feature of complex **1** is of

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†Electronic Supplementary Information (ESI) available: Synthesis, ^{1}H NMR and ESI-MS spectra of 1 and 2 before and after irradiation, theoretical calculation and DNA gel electrophoresis. See DOI: 10.1039/x0xx00000x

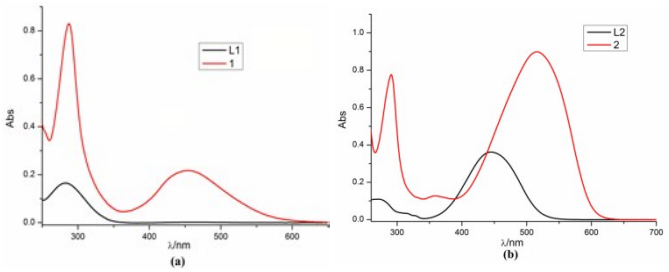

Figure 1. Absorption spectra of 1, L1 (a) and 2, L2 (b) in CH_3CN (10 μM).

importance and represents the first potential PACT agent with dual-activation property.

Complex **1** was synthesized as PF_6 salt (ESI). As a negative control, complex **2** was also prepared. Compared to L1, L2 is expected to be reduced less effectively by GSH due to the presence of strong electron-donating group of *N,N*-diethylaniline.

Both complexes show strong absorption in the region of 400-600 nm (Figure 1), with absorption maxima at 453 nm for **1** and 516 nm for **2**, respectively. Compared with L1 and L2, this visible absorption band may be attributed to both ^{1}IL (intraligand) transition and ^{1}MLCT transition for **2**, but mainly ^{1}MLCT transition for **1**.

Unlike $[Ru(bpy)_2(py)_2]^{2+}$ which undergoes py dissociation upon irradiation (Figure S1 and S2),[10] **1** and **2** are quite stable under irradiation. After 45 min irradiation (> 470 nm), the ^{1}H NMR spectra of **1** and **2** (Figure S3 and S4) in CD_3COCD_3/D_2O (4/1) showed no observable changes. Similar results were also obtained in their ESI-MS spectra (Figure S5 and S6). Lack of the monodentate ligand photodissociation in **1** and **2** may be ascribed to the quenching effect of the azo groups. Theoretical calculation on **1** supports the assumption very well. As shown in Figure S7, HOMO, HOMO-1 and HOMO-2 are mainly ruthenium centered, while HOMO-3 and HOMO-4 are azopyridine ligand in character. The electron densities of LUMO and LUMO+1 are localized on azopyridine ligand, while LUMO+2 is bpy-based. The triplet excited states were also calculated using time-dependent density functional theory (Table S1). The first and second lowest lying triplet excited states can be ascribed to L1-based transitions, with energy level at about 1.66 eV. This is different with common Ru(II) polypyridyl complexes, in which ^{3}MLCT state is usually the lowest lying one.[15] In contrast, the third and fourth lowest lying states are ^{3}MLCT in character, with an energy gap of around 0.4 eV with the L1-based triplet excited states. Thus, energy transfer from the ^{3}MLCT states to the L1-based triplet excited states are thermodynamically allowed, leading to efficient quenching of the ^{3}MLCT states. Accordingly, the thermal population

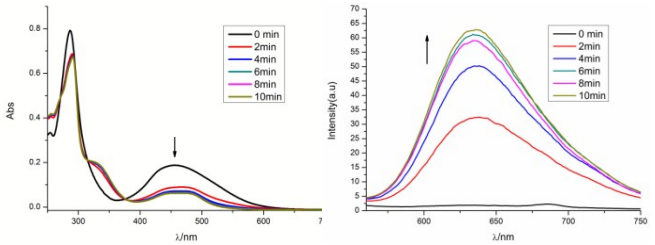

Figure 2. Absorption and emission spectra changes of **1** (10 μM) in H_2O upon addition of GSH (1 mM).

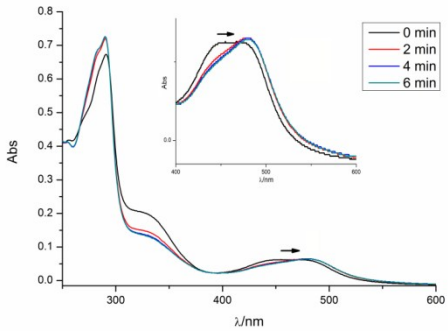

Figure 3. Absorption spectra changes of **1**-red in H_2O upo irradiation (λ > 470 nm). Inset was the amplification of 400-600 nm The **1**-red solution was obtained through addition of 1 mM GSH to (10μM in H_2O) and stirring for 20 min.

of the ^{3}MC state is shut down and the ligand photodissociation is blocked totally. Turro, Dunbar and coworkers also found that [Ru(bpy)(PAP)$(CH_3CN)_2]^{2+}$ (PAP = 2-(phenylazo)pyridine) l photochemical ligand exchange property.[16]

We then examined the responses of **1** and **2** toward GSH. As shown in Figure 2, upon addition of GSH, the absorption an luminescence spectra of **1** in aqueous solution changed quickly. The absorbance in the region of 400-600 nm decreased dramatical within 10 min. Meanwhile, the non-luminescent **1** turned to b weakly emissive with emission peak at 633 nm. After 20 min, the absorption and emission spectra of the solution did not change an more, suggesting the completion of the reduction reaction. At th moment, the ESI-MS spectrum of the solution showed a m/z peak at 393.1105 (Figure S8), assignable to $[Ru(bpy)_2(py\text{-}NH\text{-}NH\text{-}py)_2]^{+}$ (**1**-red, py-NH-NH-py = 1,2-di(pyridine-4-yl)hydrazine), meanwhile the m/z peak of **1** disappeared. The ^{1}H NMR spectrum also showe **1** was fully transformed to **1**-red (Figure S9). Interestingly, the free ligand L1 is less reactive than the coordinated one in GSH reductio 75% L1 remained unchanged under the same condition (Figure S10). Clearly, metal coordination reduces the electron density on the group, making L1 easier to be reduced.[17] Generally, the reduction o an azo group was stepwise, i.e. from R-N=N-R' to R-NH-NH-R' at first, and then to RNH_2 and $R'NH_2$.[12, 18] In our ESI-MS experiment no NH-NH bond cleavage products were observed, indicating tha the reduction stopped at the first stage. In sharp contrast, **2** canno be reduced by GSH, as evidenced by the lack of spectra changes in absorption, emission as well as ESI-MS of **2** upon addition of GS We found that the replacement of the terminal pyridine groups in by *N,N*-diethylaniline group leads to a remarkably cathodic shift in reduction potential, from -0.62 V (vs. SCE) for **1** to -0.86 V (vs. SC for **2,** demonstrating that the reduction of **2** will be far less efficien than that of **1**.

Without the quenching effect of the azo groups, **1**-red beco es photolabile again. As shown in Figure 3, light irradiation gave rise to a gradual red shift of the absorption maximum of **1**-red from abo t 450 nm to 482 nm, a common phenomenon of ligand exchan e with water for Ru(II) complexes.[4b-i] We also examined the photoinduced ligand exchange of **1**-red in CH_3CN. In this cas irradiation led to appearance of a new m/z peak at 320.5783 (Figure S12), which can be ascribed to $[Ru(bpy)_2(py\text{-}NH\text{-}NH\text{-}py)(CH_3CN)]^{2+}$. No $[Ru(bpy)_2(CH_3CN)_2]^{2+}$ based m/z peak was observed, indicatin

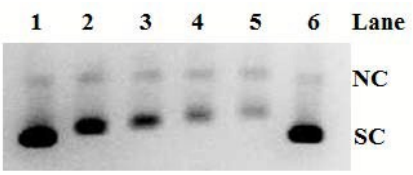

Figure 4. Agarose gel electrophoresis pattern of supercoiled pUC19 DNA (40 μg/ml) in Tris-CH_3COOH-EDTA buffer (pH = 7.4) in the presence of GSH (1 mM, Lane 2-6) and varied concentrations of **1**. All samples were kept in the dark for 20 min and then irradiated (λ > 470 nm) for 25 min. Lane 1: DNA alone; Lane 2-6: the concentrations of **1** are, respectively, 20, 40, 60, 80, and 0 μM. SC and NC denote supercoiled circular and nicked circular forms, respectively.

that only one monodentate ligand undergo photodissociation, which was in good agreement with many other photolabile Ru complexes, e.g. $[Ru(bpy)_2(py)_2]^{2+}$.[10]

The DNA photobinding abilities of **1** and **1**-red were studied using agarose gel electrophoresis. The covalent binding of Ru(II) complexes to DNA will decrease DNA mobility in electrophoresis and bleach the corresponding DNA band.[4] As shown in Figure S13, **1** had no influence on DNA mobility either in the dark or under light irradiation, consistent with its photo-stability. However, in the presence of GSH, the in-situ formed **1**-red can retard DNA mobility and bleach the SC band significantly upon irradiation (Figure 4). Control experiments indicate that **1**-red cannot covalently bind to DNA without irradiation (Figure S14), and GSH alone has no effect on DNA. Our experimental results fully demonstrate that the DNA covalent binding potential of **1** can only be turned on in the conditions where both GSH and light irradiation are present.

In summary, we, for the first time, designed and synthesized a novel azopyridine-based Ru(II) complex as a potential GSH-responsive PACT agent, the DNA covalent binding capability of which can be only activated after GSH reduction and visible light irradiation. Such dual-activation behavior is expected to be more beneficial for enhancing the selectivity of anticancer drugs.

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