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Phosphorescent ruthenium complexes with bromopyrene unit that enhance oxygen sensitivity

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ABSTRACT

Ruthenium complexes are very useful phosphorescent probes for the visualization of hypoxia. We designed and synthesized three ruthenium complexes possessing bromopyrene, naphthalene, or anthracene units to improve the oxygen response. These ruthenium complexes provided strong phosphorescence under hypoxic conditions, while an increase in oxygen concentration led to a decrease in phosphorescence intensity. Among the ruthenium complexes, that with a bromopyrene unit (Ru-BrPy) had the best properties. This showed good cellular uptake and bright emission in cells, and had the highest sensitivity for molecular oxygen. Thus, Ru-BrPy is a promising candidate as a molecular probe for detecting cellular hypoxia.

1. Introduction

Hypoxia, a condition characterized by an insufficient supply of oxygen to cells and tissues, is caused by metabolic abnormalities and by interruption of blood flow. The condition is related to various diseases, including cancers and vascular diseases, and plays an important role in pathological processes.[1–4] Therefore, the development of probes to visualize hypoxia is imperative in developing therapies.

Many imaging modalities have been developed for detecting hypoxia, such as magnetic resonance imaging (MRI), positron emission tomography (PET), Doppler optical coherence tomography (DOCT), single-photon emission computed tomography (SPECT), and immunostaining.[5–9] Among them, optical imaging has received most attention because of its advantages including high sensitivity, high resolution, ability to provide real-time monitoring, specificity, noninvasiveness, and low cost. In particular, several phosphorescent complexes, such as ruthenium complexes, iridium complexes and porphyrin derivatives, have been developed extensively as oxygen sensors.[10–18] We recently developed novel phosphorescent ruthenium complexes to detect hypoxia.[10] These show robust emissions under hypoxic conditions that are quenched promptly by the addition of molecular oxygen because of energy transfer between the triplet excited state of ruthenium complexes and the triplet ground state of oxygen. In addition, they show good cell permeability and high water solubility. In light of these favorable properties, we applied them to visualize hypoxia in tumor cells.

In the course of this research, we found that modification of the ligands of ruthenium complexes led to improved oxygen sensitivity of their phosphorescence emission.[10] For instance, ruthenium complexes bearing a pyrene unit at the ligand site (Ru-Py) showed longer-lived phosphorescence than did complexes without any modification. According to Stern–Volmer relationships, a long phosphorescence lifetime is linked to high oxygen sensitivity, so Ru-Py showed favorable properties as an oxygen sensor in cells and tissues. These findings prompted us to investigate the effect of modification of ruthenium complexes at their ligand sites in detail to further improve their oxygen sensitivity. Here, we synthesized three novel ruthenium complexes bearing naphthalene (Ru-Nap), anthracene (Ru-Ant), or bromopyrene units (Ru-BrPy) (Fig. 1), and characterized their photochemical properties and biological behavior in living cells. We found that Ru-BrPy showed good properties as an oxygen sensor for detecting hypoxic cells.

2. Results and discussion

As shown in Scheme 1, we synthesized three derivatives of ruthenium complexes. Reductive amination of the corresponding aldehyde **1a**–**c** by means of aminophenathroline gave **3a**–**c**. Subsequent complexation with Ru(bpy)$_2$ furnished the desired ruthenium complexes: Ru-BrPy, Ru-Nap, and Ru-Ant. We also prepared conventional ruthenium complexes, Ru-Py and Ru-Me$_2$, to compare their properties with these newly designed ruthenium complexes.

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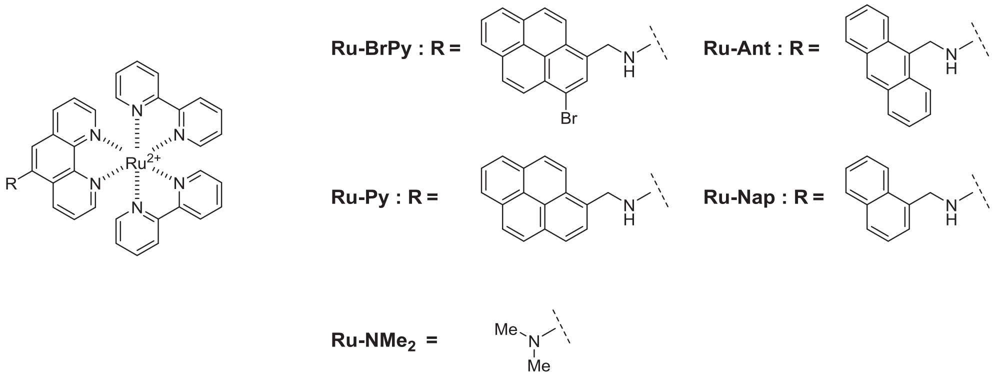
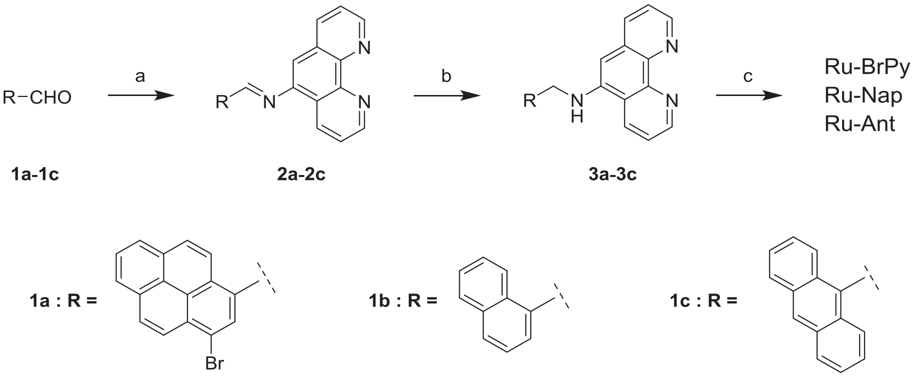


Fig. 1. Chemical structures of ruthenium complexes used in this study.



Scheme 1. Reagents and conditions: a) 1,10-phenanthrolin-5-amine, EtOH, CH_2Cl_2, AcOH, RT, 41% (for **2a**), 95% (for **2b**), 72% (for **2c**); b) LiAlH4, dry THF, 0 °C, 75% (for **3a**), 50% (for **3b**), 24% (for **3c**); c) $[Ru(bpy)_2Cl_2]$, MeOH, heated to reflux, 52% (for Ru-BrPy), 83% (for Ru-Nap), 24% (for Ru-Ant).

Initially, we evaluated the emission behaviors of the ruthenium complexes in aqueous solution using light excitation at a wavelength of 450 nm. This allowed the selective excitation of ruthenium complexes even when aromatic substituents were incorporated into the phenanthroline ligand. Fig. 2a shows representative emission spectra of Ru-BrPy. Ru-BrPy showed a broad and strong emission around 600 nm, which is attributed to the triplet metal-to-ligand charge-transfer phosphorescence of ruthenium complexes. The emission wavelength of Ru-BrPy was indistinguishable from those of other ruthenium complexes, indicating that all the complexes had similar triplet energy levels (Fig. S1a). Strong phosphorescence of Ru-BrPy was observed under hypoxic conditions, while an increase in oxygen concentration led to a decrease in phosphorescence intensity from the quenching effect of molecular oxygen. The phosphorescence emission of ruthenium complexes under aerobic conditions recovered to its original level after replacing oxygen with nitrogen gas (Fig. 2b). These results indicate that the phosphorescence emission of Ru-BrPy was regulated reversibly by changing the oxygen concentration. We also confirmed similar oxygen concentration-dependent emissions of Ru-Nap and Ru-Ant (Fig. S1a).

The responses of phosphorescence intensity to oxygen concentration obeyed Stern–Volmer relationships. As shown in Fig. 2c, the oxygen concentration was proportional to the ratio of the phosphorescence intensities of Ru-BrPy (I_0/I, where I_0 = intensity under anoxic conditions and I = intensity under the indicated oxygen concentration). The Stern–Volmer coefficient (K_{sv}) of Ru-BrPy was estimated to be $9.0 \times 10^3\ M^{-1}$. Re-estimation of the K_{sv} value for a ruthenium complex bearing a pyrene unit (Ru-Py, $6.7 \times 10^3\ M^{-1}$), which showed the highest performance in our previous study,[10] revealed that Ru-BrPy had the highest K_{sv} value among these ruthenium complexes, and thus showed the highest oxygen sensitivity. By contrast, K_{sv} values for Ru-Nap and Ru-Ant were lower than for Ru-BrPy (Ru-Nap, $2.0 \times 10^3\ M^{-1}$; Ru-Ant, $1.9 \times 10^3\ M^{-1}$), although their responses to oxygen also obeyed Stern–Volmer relationships. Given that the K_{sv} value of Ru-Me$_2$ was $2.6 \times 10^3\ M^{-1}$, the introduction of naphthalene or anthracene units into a ruthenium complex induced negligible effects on K_{sv} values. As noted, we previously reported that Ru-Py had a high K_{sv} value and showed oxygen-dependent phosphorescence with high sensitivity.[10] The mechanism for enhanced oxygen sensitivity was deduced as follows. The triplet energy level of pyrene units is similar to that of ruthenium complexes, leading to efficient energy redistribution between the excited state of the ruthenium chromophore and pyrene. Fast decay of the metal triplet state and energy transfer from pyrene to the ruthenium unit results in the formation of a long-lived triplet state of the ruthenium complexes, giving enhanced K_{sv} values. Here, we introduced a bromine atom into a pyrene unit. It is well documented that the introduction of bromine atoms into aromatic rings such as naphthalene or anthracene leads to a slight decrease (1–2 kcal/mol) in the energy levels of the triplet excited state.[19–23] Therefore, it is most likely that the energy level of a bromopyrene unit is slightly lower than that of pyrene, leading to the formation of a more stable triplet state. Eventually, the stable triplet excited state of the bromopyrene unit might form further long-lived triplets of ruthenium complexes, leading to the higher K_{sv} value of Ru-BrPy. On the other hand, the triplet energy levels of naphthalene and anthracene units are much higher and lower, respectively, than those of pyrene and ruthenium complexes.[24–26] Thus, efficient energy distribution and transfer between excited ruthenium complexes and aromatic rings on phenanthroline ligands might not occur, resulting in a