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Original article

Cyclometallated iridium phosphors with amino acid ancillary ligand for intracellular imaging

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ABSTRACT

Two new iridium complexes, $(dfppy)_2Ir$(L-alanine) (dfppy = 2-(2,4-difluorophenyl)pyridine) and $(piq)_2Ir$(L-alanine) (piq = 1-phenylisoquinoline) were prepared with L-alanine as ancillary ligand. The two complexes show bright greenish-blue and red emission respectively. Theoretic study demonstrated that the emission nature of these complexes is mainly determined by the main ligand. And their improved aqueous solubility and the retained quantum yield favor their application in cell imaging. Intracellular imaging suggested that these two complexes have fine cell membrane permeability and is mainly distributed in cytoplasm. This study displayed a new strategy to design aqueous soluble phosphorescent cyclometallated Ir(III) complex *via* introducing amino acid as ancillary ligand.

1. Introduction

Owing to the tunable emitting properties and the flexibility for chemical modifications, phosphorescent iridium complexes have been intensively studied as emitting dopants in organic light emitting diodes (OLEDs) and light emitting electrochemical cells (LECs) [1–5]. In addition, certain iridium complexes were also found to be applied in phosphorescence imaging in live cells [6–12]. Compared with the fluorescent organic dyes, phosphorescent iridium complexes display the advantages such as large Stokes shift, fine photostability, high internal quantum yield (nearly 100% in common organic solvent due to triplet harvesting effect). Moreover, their long lifetime is helpful to eliminate the interference from autofluorescence of cells and tissues [13]. Moreover, the distorted octahedral structure of Ir(III) complexes is helpful to avoid the aggregation-caused quenching (ACQ) effect that is commonly observed for the planar organic fluorophores [14]. Although some Ir(III) phosphorescent complexes have been reported as imaging agents, most of them, such as $[(ppy)_2Ir(bpy)]^+(PF_6)^-$ (ppy = 2-phenylprydine, bpy = 2,2′-bipyridyl, PF_6 = hexafluorophosphate) show relative low quantum yield due to their cationic nature [15]. On the other hand, the neutral iridium phosphors with high quantum yield, such as *fac*-$Ir(ppy)_3$, FIrpic (bis[(4,6-difluorophenyl)-pyridinate-*N*,C^2]-picolinate) and $(piq)_2Ir(acac)$ (piq = 1-phenylisoquinoline, acac = acetylacetonate), usually have low water affinity, which disfavor the cell staining. Exploring Ir(III) complexes bearing both the high quantum yield and fine aqueous solubility is challenging and helpful for their application in cell imaging.

It was reported that the homoleptic Ir(III) complexes being modified with the hydrophilic amino acid shows the improved aqueous solubility [16], yet the modification of the ligand results in the tedious preparation procedure of these Ir(III) complexes. Considering amino acids (AA) are fine chelator with carboxyl and amino group as metal coordination groups, amino acid might serve as ancillary ligand directly to form the $Ir(ppy)_2(AA)$ complex. Therefore, both the molecular volume and charge can be reduced, and the high internal quantum efficiencies can be expected. In this work, two neutral Ir(III) complexes, $(dfppy)_2Ir$(L-alanine) (dfppy = 2-(2,4-difluorophenyl)pyridine) and $(piq)_2Ir$(L-alanine) were synthesized readily with L-alanine as ancillary ligand (Scheme 1). Due to the fine biocompatibility and water solubility of the L-alanine ligand, these new Ir(III)

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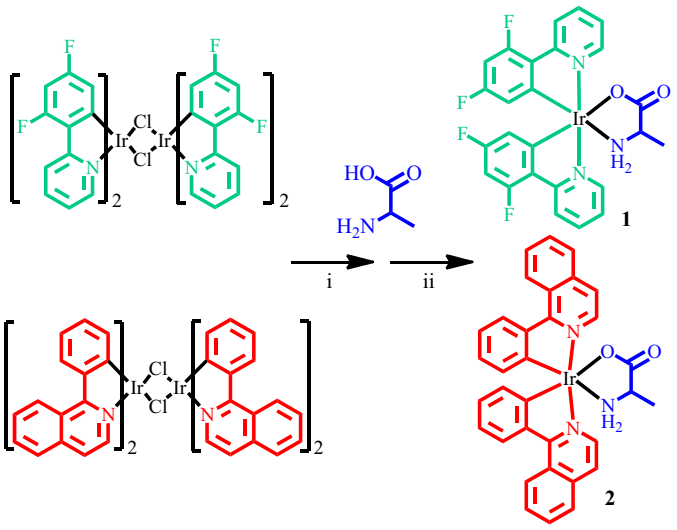

Scheme 1. Chemical structures and the general synthetic scheme of compounds **1** and **2**. (i) *t*-BuOK, MeOH, room temperature; (ii) iridium μ-Cl dimer complexes, 2-ethoxylethanol, 120 °C.

complexes were applied practically in cell imaging as the phosphorescent agent.

2. Experimental

2.1. General information

All reactions were carried out under nitrogen atmosphere. An electrospray ionization (ESI) mass spectrometer (LCQ fleet, Thermo Fisher Scientific) was used to record mass spectra and high-resolution mass spectra were measured with an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS. ^{1}H NMR spectra were measured on a Bruker AM 300 spectrometer. UV–vis absorption and photoluminescence spectra were obtained using a Shimadzu UV-3100 and a Hitachi F-4600 spectrophotometer respectively.

2.2. Syntheses

The general synthetic procedure for these complexes was shown in Scheme 1. Main ligands dfppy (2-(2,4-difluorophenyl)-pyridine) and piq were adopted for their ability to endow the Ir(III) complexes with greenish-blue and red emission [17–19]. The cyclometallated main ligands dfppy and piq were synthesized with modified Suzuki cross coupling and the intermediate iridium μ-Cl dimer complexes were obtained directly by reacting main ligands with iridium chloride according to the reported methods [2–6]. L-Alanine (2.4 mmol g) was dissolved in 10 mL methanol and 5 mL methanol solution of *t*-BuOK (2.4 mmol g) was added to the amino acid solution dropwise at room temperature for 2 h. Then the methanol solution of L-alanine potassium salt was added to a stirred solution of 1 mmol Ir(III) dimer in 20 mL 2-eyhoxyethanol at 120 °C dropwise and the reaction was kept at 120 °C for 12 h. Later, the solvent was removed in vacuum and the crude product was extracted with water and dichloromethane. The organic layer was collected and condensed. Rapid chromatography (silicon, eluent:ethyl acetate/hexane = 1/2 v/v) and recrystallization (methanol/dichloromethane) were performed to obtain pure product $(dfppy)_2Ir$(L-alanine) and $(piq)_2Ir$(L-alanine).

$(dfppy)_2Ir$(L-alanine): Yellow powder, 0.62 g, yield 47%. ^{1}H NMR (500 MHz, DMSO): δ 9.15 (dd, 1H, J = 44.7, 5.5 Hz), 8.64 (dd, 1H, J = 18.4, 5.8 Hz), 8.26 (d, 2H, J = 7.8 Hz), 8.20–8.04 (m, 2H), 7.76–7.41 (m, 2H), 6.71 (dt, 2H, J = 19.9, 9.4 Hz), 5.85–5.67 (m, 1H), 5.51–5.31 (m, 1H), 2.51 (s, 4H). ESI-MS calcd.: m/z 662.1 for $[M+H]^+$ ($C_{25}H_{19}F_4IrN_3O_2^+$), found m/z 662.1. High-resolution EI-MS calcd.: m/z 662.1037 for $[M+H]^+$ ($C_{25}H_{19}F_4IrN_3O_2^+$), found m/z 662.1036.

$(piq)_2Ir$(L-alanine): Red powder, 0.70 g, yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (t, 2H, J = 19.6 Hz), 8.78 (d, 1H, J = 12.9 Hz), 8.45 (d, 1H, J = 13.2 Hz), 8.26–8.05 (m, 2H), 8.00 (d, 1H, J = 5.4 Hz), 7.95–7.84 (m, 1H), 7.83–7.62 (m, 5H), 7.61–7.37 (m, 2H), 6.87 (t, 2H, J = 11.9 Hz), 6.71–6.42 (m, 3H), 6.15 (d, 1H, J = 8.4 Hz), 1.79 (s, 3H). ESI-MS calcd.: m/z 690.2 for $[M+H]^+$ ($C_{33}H_{27}IrN_3O_2^+$), found m/z 690.4. High-resolution EI-MS calcd.: m/z 690.1727 for $[M+H]^+$ ($C_{33}H_{27}IrN_3O_2^+$), found m/z 690.1725.

2.3. Theoretical calculations

The density functional theory calculations were carried out using Gaussian 09 software package. The basis set of LANL2DZ and 6-31G** were chosen for iridium atom and non-iridium atoms respectively. The geometries of the ground state were fully optimized with the B3LPY exchange-correlation functional both in vacuum and in DCM solution. Solvent effect was taken into consideration by using C-PCM solvent model. Vibrational frequency calculations were performed to validate that the optimized structures were minima on potential energy surface.

2.4. Cell imaging experiment

Human gastric cancer SGC-7901 cells were cultured in glass bottom dishes and seeded at a density of 1×10^6 cells/mL in RPMI 1640 supplemented with 10% FBS, $NaHCO_3$ (2 g/L) and 1% antibiotics (penicillin/streptomycin, 100 U/mL) containing 10 μmol/L iridium probes (in DMSO/culture medium, 1/99, v/v) for 60 min at 37 °C. One day before imaging, cells were passed and plated on 18-mm glass bottom dishes. Cell imaging was carried out after washing cells with PBS for three times. Confocal fluorescence imaging studies were performed with a ZEISS Laser Scanning Microscope (Zeiss LSM 710). The cell-imaging was investigated under 405 nm excitation.

3. Results and discussion

Since amino acid is not the conventional ancillary ligand for iridium phosphor complex, DFT calculations were carried out to gain more reasonable insight of the electronic structures [20]. From the calculation results (Fig. 1) we found that the HOMOs of the complexes are mainly consisted of π orbitals of phenyl rings in cyclometallated ligands and iridium atom, the LUMOs are located

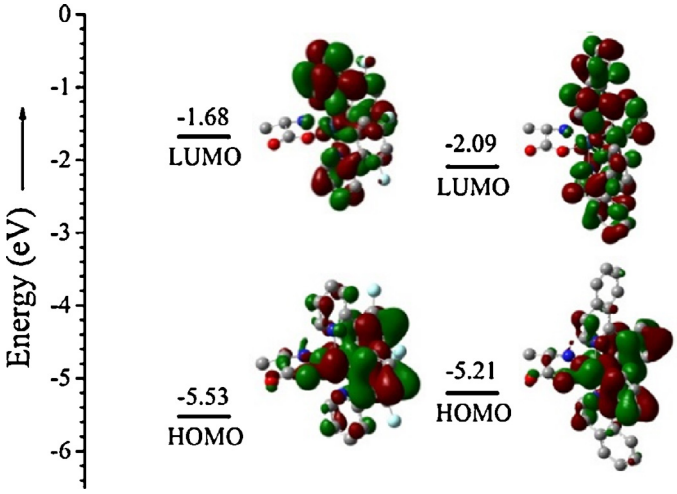

Fig. 1. Frontier molecular orbitals of complexes **1** and **2** (B3LYP/6-31G**, LANL2DZ).