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# Utilizing a series of *fac*-Re$(CO)_3$ core based quinonoid containing complexes for photophysical and cell imaging studies

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ABSTRACT

A series of N∩O bis-chelating bridging quinone ligands ($\mathbf{H_2L^1}$, $\mathbf{H_2L^2}$ and $\mathbf{H_2L^3}$) have been utilized to synthesize four dinuclear *fac*-Re$(CO)_3$ based neutral complexes (**1**–**4**) by their reaction with Re$(CO)_5$(OTf), where the N-donors 4-dimethylaminopyridine (for **1**), 3,5-dibromopyridine (for **2**) and acetonitrile (for **3** and **4**) act as ancillary ligands. The complexes were characterized by FTIR, $^1$H NMR, ESI-MS and elemental analysis. The structures of complexes **1**–**3** were analysed by X-ray diffraction methods. Photophysical investigations revealed that all the complexes display strong visible-light absorption with absorption maxima at around 500 nm ($\varepsilon = 40000–50000\ M^{-1}\ cm^{-1}$), whereas in the solid state the complexes display high intensity broad bands in the entire UV–Vis–NIR region. Complexes **1**–**4** were found to be luminescent in fluid solutions at room temperature. Favorable photophysical properties allowed their utilization as luminescent probes for live-cell imaging using HeLa cells, where complexes **2** and **4** were found to display good cellular uptake with co-localization in lysosomal compartments, which was observed as yellow fluorescence. Complexes **2** and **4** displayed very low cytotoxicity, as suggested by LDH production experiments. The photophysical properties, uptake pathway, localization properties and cytotoxicity of the complexes are discussed in detail.

## 1. Introduction

The photophysical properties of *fac*-Re$(CO)_3$ core based acyclic complexes, such as visible light absorption, large luminescence lifetimes, NIR region absorption/emission and photo-stability, have been extensively investigated and applied in a variety of areas, such as electro- and chemi-luminescence, light harvesting, photo-dynamic therapy, photo-catalysis, photonics and OLEDs etc [1]. These applications require visible region excitations and hence a strong absorption in the visible range is one of the essential criteria. Recently, a number of *fac*-[Re$(CO)_3$] complexes have been applied as luminescent live cell imaging agents owing to their favorable biological properties, such as kinetic inertness, lipophilicity, large Stokes shifts, photo-stability, membrane permeability and low cytotoxicity [2]. Particularly, Coogan and co-workers demonstrated the first application of fluorescent Re(I) complexes in cell-imaging by utilizing bipyridine and phenanthroline based lipophilic and hydrophilic mono-nuclear bisimine complexes [2a–d]. The current research in this area aims at the development of novel fluorescent, bio- and photo-active, photo-stable and non-cytotoxic variants of Re(I) complexes.

The properties of Re(I) complexes possessing bridging quinone ligands, which constitute an important class of potential photo- and redox-active bis-chelating ligands, has remained unexplored. Previously, 2,5-dihydroxy-1,4-benzoquinone derivatives have been used in various *fac*-Re$(CO)_3$ core based metallacycles where they play solely a structural role [3]. Recently, with an aim to induce novel properties into the metal complexes, our group and others have started investigating metal complexes containing ligands where the two hydroxyl groups of the 2,5-dihydroxy-1,4-benzoquinone ligand are replaced by the isoelectronic [NR] group to generate two [N∩O] chelating sites (Fig. 1) [4]. Moreover, variations in the “R” group provide an additional opportunity to influence the solubility and properties of the final complex by varying the charge density on the [N]-donor.

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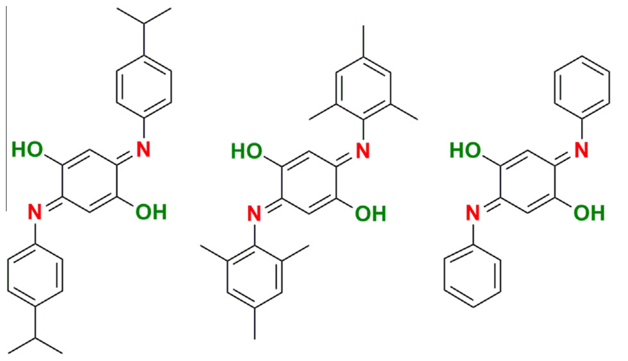

$H_2L^1$ $H_2L^2$ $H_2L^3$

**Fig. 1.** Three bis-chelating [N∩O] ligands utilized for the synthesis of complexes **1**–**4**.

To the best of our knowledge, the synthesis, photophysical properties and biological evaluations of dinuclear Re(I) complexes possessing bis-chelating imino-quinone ligands have never been investigated. Herein we report on the synthesis, characterization, photophysical properties and biological evaluations of four dinuclear *fac*-Re(CO)$_3$ based metal complexes containing the doubly deprotonated form of the 2,5-di-[4′-(isopropyl)-anilino]-1,4-benzoquinone (**$H_2L^1$**), 2,5-di-[2′,4′,6′-(tri-methyl)-anilino]-1,4-benzoquinone (**$H_2L^2$**) and anilino-1,4-benzoquinone (**$H_2L^3$**) ligands. The complexes display a strong visible light absorption ability and cellular uptake properties with the retention of luminescence in the cells.

## 2. Materials and methods

All the chemicals were used as received unless otherwise mentioned. The quinone-based bis-chelating ligands were synthesized as per the literature [4c]. All the solvents were dried and degassed by the Freeze–Thaw pump method prior to use. All the reactions were carried out under an inert atmosphere of nitrogen using Schlenk techniques. The $^1H$ NMR spectra were recorded on a Jeol ECS 400 spectrometer. IR spectra were obtained with a Nicolet 6700 FTIR instrument. Elemental analyses were performed on a Perkin–Elmer Analyzer 240 and with an Elementar Vario EL III. Mass spectrometry was performed on an Agilent 6210 ESI-TOF. SHELXS-97 was used to solve and refine the structure. Electronic absorption spectra were recorded on a SPECORD 250-222P145 spectrophotometer. The emission spectra were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer at room temperature. The photomultiplier tube voltage for fluorescence spectrophotometry was kept at 600 V using a slit width of 5 nm for both excitation and emission measurements. The lifetime measurements were done on a Horiba JobinYvon Fluorocube at room temperature.

### 2.1. Protocols for confocal imaging in live-cells

A stock solution of 100 μg/ml of both compounds in DMSO (Sigma) was prepared and incubated with a 1/50 dilution over previously seeded HeLa Cells (epithelial cells) on cover slips in a 24 well cell culture plate for 20 h at 37° C with 5% CO$_2$. The next day, the cells were fixed and mounted onto glass slides and observed using a confocal microscope with excitation and emission wavelengths of 542 and 590 nm respectively.

### 2.2. Protocols for co-localization in live-cells

HeLa cells were seeded onto cover slips in a 24 well cell culture plate and incubated with the respective compound for 20 h at 37 °C and 5% CO$_2$. The cells were fixed using 2.5% PFA (paraformaldehyde) permeablized with 0.1% Triton X-100w in PBS and incubated with lysotracker (green) for 1 h at room temperature. The cells were subsequently washed with PBS and were observed for localization using a confocal microscope under 100× oil immersion objective.

### 2.3. Synthesis of rhenium(I)-based dinuclear complexes **1** and **2** (Method I)

2,5-Bis(4′-(isopropyl)anilino)-1,4-benzoquinone (40 mg, 0.1068 mol) and potassium *tert*-butoxide (24 mg, 0.2138 mol) were placed in a schlenk flask, which was evacuated and refilled with N$_2$, followed by the addition of 10 mL of THF. This was allowed to stir at RT overnight. Following the deprotonation of the ligand, THF was removed from the reaction mixture, followed by the addition of pentacarbonylrhenium(I)triflate (100 mg, 0.2104 mol) and the pyridyl ligand (4-dimethylaminopyridine (for **1**) and 3,5-dibromopyridine (for **2**)) (0.2597 mmol). The resulting mixture was refluxed for 24 h in DCM, giving a dark brown solution and a small amount of a white precipitate (potassium triflate) at the bottom of the flask, which was filtered and discarded. The solvent was vacuum evaporated using a rotary evaporator to give a dark green, microcrystalline product which was air and moisture stable.

### 2.4. Solvothermal synthesis of complexes **1** and **2** (Method II)

A mixture of Re$_2$(CO)$_{10}$ (0.15 mmol), 2,5-bis(4′-(isopropyl) anilino)-1,4-benzoquinone (0.15 mmol) and 4-dimethylaminopyridine (for **1**) or 3,5-dibromopyridine (for **2**) (0.30 mmol) was placed in a Teflon vial which was filled with 20 mL of freshly distilled toluene/mesitylene. The vial was placed in an oven maintained at 160 °C for 48 h and cooled to RT. The product was filtered, washed with hexane and collected as green crystals.

**1** – Yield: 68.3%. FTIR (C≡O), cm$^{-1}$: 2001, 1868. $^1H$ NMR (acetone-$d_6$, 400 MHz, 25 °C, TMS, $\delta$, ppm): 7.88 (d, 4H, $^3J_{H-H}$ = 8 Hz, pyridyl-H), 7.39 (br, 8H, pyridyl-H), 6.60 (d, 4H, $^3J_{H-H}$ = 8 Hz, phenyl-H), 5.24 (s, 2H, quinone), 3.03 (s, 12H, pyridyl-CH$_3$), 1.28 (d, 20H, $^3J_{H-H}$ = 8 Hz, phenyl-CH$_3$). ESI-MS (*m/z*), Calc. for $[C_{44}H_{44}N_6O_8Re_2]^+$: 1157.2333. Found: 1157.2366. Elemental Analysis Calc. for $C_{44}H_{44}N_6O_8Re_2{\cdot}1H_2O$: C, 44.97; H, 3.95; N, 7.15. Found: C, 45.06; H, 3.93; N, 7.093%.

**2** – Yield: 71.5% FTIR (C≡O), cm$^{-1}$: 2009, 1877. $^1H$ NMR (DMSO-$d_6$, 400 MHz, 25 °C, TMS, $\delta$, ppm): 8.68 (dd, 4H, pyridyl-H), 8.43 (dd, 2H, pyridyl-H), 7.32 (d, $^3J_{H-H}$ = 8 Hz, phenyl-H), 6.95 (d, $^3J_{H-H}$ = 8 Hz, phenyl-H), 5.12 (m, 2H, quinone-H), 2.91 (m, 2H, *iso*-propyl(methylene)), 1.20 (d, 12H, $J_{H-H}$ = 8 Hz, *iso*-propyl(methyl)). ESI-MS (*m/z*), Calc. for $[C_{40}H_{30}Br_4N_4O_8Re_2^+]$: 1386.7843. Found: 1386.7898. Elemental Analysis Calc. for $C_{40}H_{30}Br_4N_4O_8Re_2{\cdot}0.4C_9H_{12}$: C, 36.50; H, 2.44; N, 3.90. Found: C, 36.28; H, 2.344; N, 3.837%.

### 2.5. Synthesis of acetonitrile based dinuclear complexes **3** and **4** (Method I)

2,5-Bis(2′,4′,6′-(trimethyl)anilino)-1,4-benzoquinone (for **3**) or 2,5-bis(4′-(isopropyl)anilino)-1,4-benzoquinone (for **4**) and potassium-*tert*-butoxide were placed in a schlenk flask, which was evacuated and refilled with N$_2$, followed by addition of DCM (20 mL). The resulting mixture was stirred overnight at RT. Following the deprotonation of the ligand, DCM was removed and 20 mL of acetonitrile was added to the same flask. Re(CO)$_5$(OTf) was added and stirred at RT overnight. The solvent was allowed to evaporate at RT, resulting in dark red crystals within a few days.