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Luminescent Re(I) Carbonyl Complexes as Trackable PhotoCORMs for CO delivery to Cellular Targets

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Abstract

A family of Re(I) carbonyl complexes of general formula $[\text{ReX}(\text{CO})_3(\text{phen})]^{0/1+}$ (where X = Cl^- , CF_3SO_3^- , MeCN, PPh_3 , and methylimidazole) derived from 1,10-phenanthroline (phen) exhibits variable emission characteristics depending on the presence of the sixth ancillary ligand/group (X). All complexes but with X = MeCN exhibit moderate CO release upon irradiation with low-power UV light and are indefinitely stable in anaerobic/aerobic environment in solution as well as in solid state when kept under dark condition. These CO donors liberate three, one, or no CO depending on the nature of sixth ligand upon illumination as studied with the aid of time-dependent IR spectroscopy. Results of excited-state density functional theory (DFT) and time-dependent DFT calculations provided insight into the origin of the emission characteristics of these complexes. The luminescent rhenium(I) photoCORMs uniformly displayed efficient cellular internalization by the human breast adenocarcinoma cells, MDA-MB-231, while the complex with PPh_3 as ancillary ligand showed moderate nuclear localization in addition to the cytosolic distribution. These species hold significant promise as theranostic photoCORMs (photoinduced CO releasing molecules), where the entry of the pro-drug can be tracked within the cellular matrices.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.6b02999. Crystal packing diagrams, IR spectra of **1–5**, UV–vis absorption spectra, ^1H NMR spectra of **1**, **2**, (in CDCl_3), and **5** (in CD_3CN), emission spectra of complexes **1–5** in CH_2Cl_2 solution, changes in absorption spectrum of **1** in PBS solution, changes in IR spectrum of **1**, groundstate optimized structures of **1–5**, NTOs for **1–5**, total spin density plots of the triplet optimized structures for **1–5**, key structural parameters, calculated key excitations, their oscillator strengths, and nature of transitions of **1–5**, and all calculated vertical excitations of **1–5** (PDF)

X-ray crystallographic data in CIF format (CIF)

INTRODUCTION

The diverse photophysical and photochemical properties of Re(I) tricarbonyl complexes make them invaluable for a range of applications such as light-emitting devices,¹ nonlinear optical materials,² radiopharmaceuticals,³ CO₂ reduction,⁴ and photopolymerization.⁵ These CO complexes are often desirable for their facile synthesis, superior stability in various organic media, and strong luminescence.^{6–8} The convenient syntheses of [Re(CO)₃X(L)] type complexes (where L = α -diimine ligand and X = monodentate ancillary ligand) and amenability of the α -diimine ligands toward various derivatization enable systematic tuning of the electronic properties of these complexes.^{9,10} Recently, the vast literature on these organometallic systems and their rich photochemistry have encouraged us to design and synthesize new rhenium carbonyl-based photoactive CO-releasing materials (photoCORMs) for site-specific delivery of CO to biological targets.^{11–13} When exposed to light (of various frequencies), these photoCORMs provide sustained fluxes of CO, which, in moderate concentrations, elicit various salutary effects in mammalian pathophysiology.¹⁴ We,^{11,12} and others,¹⁵ have shown applications of such photoCORMs toward rapid eradication of aggressive malignant cells and restoration of oxidatively damaged cell through light-induced CO delivery. Re(I) carbonyls bearing appropriate fluorogenic ligands are often found to be luminescent. This property arises from facile intersystem crossing (ISC), a step that readily enables the system to attain the metal-to-ligand charge transfer (³MLCT) state and dissipate energy in form of radiative relaxation, namely, phosphorescence.¹⁶ As a consequence, these species present a good prospect of developing theranostic (both therapeutic and diagnostic) CO delivery agents that could be tracked in biological milieu.

The recent advent of various sophisticated imaging techniques has prompted a surge in the development of suitable metallopharmaceuticals as diagnostics in addition to their therapeutic context. Among others, application of d⁶ transition metal complexes in biological imaging has emerged as a research area with significant importance.¹⁷ The large Stokes shifts, long luminescence lifetime, and resistance to photobleaching of the Re(I) complexes^{18,19} make them excellent candidates for such applications. Large Stokes shifts are crucial to eliminate autofluorescence without any loss of signal intensity, which can arise from the endogenous fluorophores such as NADPH, flavin, and chlorophyll. The relatively long luminescence lifetime is also useful to screen out autofluorescence, which usually has a shorter lifetime. Finally, photobleaching (usually common for organic molecules) upon

intracellular irradiation causes significant loss of signal intensity and thereby can compromise any time-resolved imaging studies. Rhenium complexes bearing small peptides,²⁰ biotin,²¹ and cobalamine²² have been utilized for cell imaging. A rhenium complex containing a chloroethyl group has been found to target mitochondria of the MCF-7 human breast cancer cells.²³ A family of Re(I) polypyridine glucose complex has shown efficient glucose transporters (GLUT) mediated cellular uptake in two transformed and two nontransformed human cell lines.²⁴ In a relatively recent study a fluorescent rhenium(I) naphthalimide complex has shown considerable intracellular localization within human osteoarthritic cells and a fish parasite *Spirotrunculus vortens*.²⁵ Ion-responsive Re(I)-based cellular imaging agents have also been reported. For example, a family of tyramine-derived luminescent Re(I) complexes exhibited both enhancement of luminescence intensity and prolonged emission lifetime in the presence of Zn(II) and Cd(II) ions.²⁶ It is important to note that, despite significant developments toward synthesis and design of luminescent rhenium(I) complexes for bioimaging applications, no systematic studies have been conducted to assess and compare the efficacy of cellular internalization employing structurally similar complexes bearing different ancillary coligands, variable charge, and lipophilicity.

In the present work, we synthesized a set of five Re(I) carbonyl complexes as potential theranostic photoCORMs with tunable luminescent properties. These photoCORMs readily deliver CO under the control of light, and at the same time the entry of the pro-drug into the cellular targets can be tracked by virtue of their luminescence properties. The complexes were derived from 1,10-phenanthroline (phen) with variable ancillary ligands, namely, [ReCl(CO)₃(phen)] (**1**), [Re(CF₃SO₃)(CO)₃(phen)] (**2**), [Re(CO)₃(MeCN)(phen)](CF₃SO₃) (**3**), [Re(CO)₃(PPh₃)(phen)](CF₃SO₃) (**4**), and [Re-(CO)₃(MeIm)(phen)](CF₃SO₃) (**5**) (MeIm = methyl imidazole) (Scheme 1). All these complexes except **3** release CO upon illumination with low-power UV light. Interestingly, the remaining four complexes liberate one or all three CO molecules depending on the nature of the sixth ancillary ligand. Moreover, these complexes uniformly bearing *fac*-Re(CO)₃ moiety and the same fluorogenic phen ligand exhibit variable luminescence properties depending on the presence of such ancillary ligands. Herein we report the syntheses, crystal structures, and CO release properties of all five complexes. We also report the results of more systematic study on the internalization of the Re complexes of varying charge, size, and lipophilicity within human breast adenocarcinoma MDA-MB-231 cells with the aid of fluorescence confocal microscopy. In addition, excited-state density functional theory (density functional theory (DFT) and time-dependent (TD) DFT) calculations were performed to gain insight into the origin of the emission characteristics of these complexes.

EXPERIMENTAL SECTION

Materials and Reagents

[ReCl(CO)₅], horse heart myoglobin (Mb), PPh₃, AgCF₃SO₃, and methylimidazole (MeIm) were purchased from Sigma-Aldrich and used without further purification. 1,10-Phenanthroline (phen) was purchased from Ark Pharm, Inc. Solvents were purified and/or dried by standard techniques prior to use.²⁷

[ReCl(CO)₃(phen)] (1)—A batch of 200 mg (0.55 mmol) of [Re(CO)₅Cl] and 100 mg (0.55 mmol) of phenanthroline (phen) was added to a 100 mL Schlenk flask, and to it was added a mixture of 30 mL of methanol (MeOH) and 10 mL of chloroform (CHCl₃). The mixture was then heated to reflux for 18 h. The yellow suspension thus obtained afforded a yellow precipitate upon standing for an additional 2 h. This precipitate was filtered using a small frit and washed three times with cold MeOH. Finally the orange solid was dried in vacuo to obtain 218 mg (81% yield) of the product. Anal. Calcd (%) for C₁₅H₈N₂O₃ClRe (485.90): C 37.08, H 1.66, N 5.77; Found: C 37.12, H, 1.64, N 5.82. IR (KBr disk, cm⁻¹): 2017(s), 1930(s), 1900(s), 1425(w), 853(w), 723(w), 644(w), 547(w). UV/vis (MeCN), λ_{max} (ε, M⁻¹ cm⁻¹): 265 (16 000), 370 (3000). ¹H NMR data (CDCl₃): δ 9.41 (d, 2H, 5 Hz), 8.54 (d, 2H, 10 Hz), 8.02 (s, 2H), 7.87 (m, 2H).

[Re(CF₃SO₃)(CO)₃(phen)] (2)—A batch of 172 mg (0.67 mmol) of Ag(CF₃SO₃) was added to a suspension of 163 mg (0.34 mmol) of [ReCl(CO)₃(phen)] in 60 mL of dichloromethane (CH₂Cl₂) contained in a 100 mL Schlenk flask. The suspension was then stirred at room temperature for 24 h. During this period the Schlenk flask was covered with aluminum foil to avoid exposure to any ambient light. Next the yellow suspension was filtered on a bed of diatomaceous earth. The residue (consisting of AgCl and excess Ag(CF₃SO₃)) was discarded. The filtrate was then transferred into another 100 mL Schlenk flask, and the solvent was completely removed under reduced pressure to isolate 178 mg (88.6% yield) of [Re(CF₃SO₃)(CO)₃(phen)] as a yellow solid. Anal. Calcd (%) for C₁₆H₈N₂O₆SF₃Re (599.52): C 32.06; H 1.34; N 4.67; Found: C 32.10, H 1.29, N 4.71. IR (KBr disk, cm⁻¹): 2032(s), 1923(s), 1896(s), 1428(w), 1325(m), 1232(w), 1208(m), 1180(w), 1011(m), 847(w), 723(w), 630(w). UV/vis (MeCN), λ_{max} (ε, M⁻¹ cm⁻¹): 275 (24 000), 360 (3000). ¹H NMR data (CDCl₃): δ 9.44 (d, 2H, 5 Hz), 8.64 (d, 2H, 10 Hz), 8.06 (s, 2H), 7.95 (m, 2H).

[Re(MeCN)(CO)₃(phen)](CF₃SO₃) (3)—A batch of 45 mL of freshly distilled acetonitrile (MeCN) was added to 178 mg (0.30 mmol) of [Re(CF₃SO₃)(CO)₃(phen)] in a 100 mL Schlenk flask. This reaction mixture was then heated to reflux for 20 h. During this time the color of the solution turned orange. The reaction mixture was then cooled to room temperature. Evaporation of the solvent under reduced pressure resulted in a sticky brownish-orange product. This material was finally triturated with diethyl ether (5 mL) five to six times to obtain 160 mg (84% yield) of the product as a fluffy yellow solid. Anal. Calcd (%) for C₁₈H₁₁N₃O₆SF₃Re (640.57): C 33.75; H 1.73; N 6.56, found: C 33.59, H 1.68, N 6.61. IR (KBr disk, cm⁻¹): 2038(s), 1927(s), 1919(s), 1430(w), 1280(m), 1253(m), 1162(w), 1029(m), 851(w), 724(w), 669(w), 640(m), 546(w), 518(w). UV/vis (MeCN), λ_{max} (ε, M⁻¹ cm⁻¹): 275 (32 000), 360 (5000). ¹H NMR data (CDCl₃): δ 9.44 (d, 2H, 5 Hz), 8.64 (d, 2H, 10 Hz), 8.07 (s, 2H), 7.95 (m, 2H), 1.58 (s, 3H).

[Re(PPh₃)(CO)₃(phen)](CF₃SO₃) (4)—A solution of 180 mg (0.30 mmol) of [Re(MeCN)(CO)₃(phen)](CF₃SO₃) and 788 mg (3.0 mmol) of triphenylphosphine (PPh₃) in 30 mL of CHCl₃ was heated to reflux in a 100 mL Schlenk flask for 20 h. Next the volume of the solution was reduced to ~5 mL. Addition of 25 mL of diethyl ether to this solution resulted in precipitation of a yellow solid. The supernatant was then decanted carefully, and

the solid was dried in vacuo. The excess PPh_3 from this solid was removed through recrystallization by layering hexanes over its CH_2Cl_2 solution. A total of 185 mg (72% yield) of pale yellow needles of $[\text{Re}(\text{PPh}_3)(\text{CO})_3(\text{phen})](\text{CF}_3\text{SO}_3)$ was obtained after 72 h. Anal. Calcd (%) for $\text{C}_{34}\text{H}_{23}\text{N}_2\text{O}_6\text{SF}_3\text{PRe}$ (861.80): C 47.39, H 2.69, N 3.25, found: C 47.41, H 2.73, N 3.29. IR (KBr disk, cm^{-1}): 2040(s), 1955(s), 1920(s), 1432(w), 1264(s), 1225(w), 1154(m), 1030(w), 857(w), 725(w), 697(w), 637(w), 517(w). UV/vis (MeCN), λ_{max} (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 270 (19 000), 370 (2000). ^1H NMR data (CDCl_3): δ 8.84 (d, 2H, 5 Hz), 8.79 (d, 2H, 5 Hz), 8.27 (s, 2H), 7.70 (m, 2H), 7.33 (m, 3H), 7.19 (m, 6H), 6.91 (t, 6H, 5 Hz).

$[\text{Re}(\text{Melm})(\text{CO})_3(\text{phen})](\text{CF}_3\text{SO}_3)$ (5**)**—A solution of 100 mg (0.16 mmol) of $[\text{Re}(\text{MeCN})(\text{CO})_3(\text{phen})](\text{CF}_3\text{SO}_3)$ and 64 mg (0.78 mmol) of methyl imidazole (MeIm) in 30 mL of CHCl_3 was heated to reflux in a 100 mL Schlenk flask for 18 h. Next the volume of the solution was reduced to ~5 mL and subjected to a silica gel column prepared with toluene. The column was then flushed with toluene to remove any excess MeIm. The desired complex was then recovered from the column by using a 50:50 mixture of toluene/acetonitrile. The yellow solution thus obtained afforded a sticky orange residue upon removal of the solvent. Finally this residue was triturated with ethyl ether (5 mL) five to six times to obtain 98 mg (92% yield) of the product as orange microcrystalline solid. Anal. Calcd (%) for $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_6\text{SF}_3\text{Re}$ (681.62): C 35.24, H 2.07, N 8.22, found: C 35.29, H 2.11, N 8.25. IR (KBr disk, cm^{-1}): 2030(s), 1931(s), 1885(s), 1431(w), 1265(m), 1154(w), 1031(w), 845(w), 637(w). UV/vis (MeCN), λ_{max} (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 270 (14 000), 330 (3000). ^1H NMR data (CD_3CN): δ 9.32 (d, 2H, 5 Hz), 8.64 (d, 2H, 10 Hz), 8.00 (s, 2H), 7.86 (m, 2H), 7.05 (s, 1H), 6.54 (s, 1H), 6.26 (s, 1H), 3.18 (s, 3H).

Physical Measurements

The ^1H NMR spectra were recorded at 298 K on a Varian Unity Inova 500 MHz instrument. A PerkinElmer Spectrum-One FT-IR was employed to monitor the IR spectra of the reported complexes. UV-vis spectra were obtained with a Varian Cary 50 UV-vis spectrophotometer. Luminescence spectra were recorded with a Cary Eclipse Spectrometer. Microanalyses (C, H, and N) were performed using a PerkinElmer 2400 Series II elemental analyzer. Cyclic voltammograms were recorded with a CH Instruments electrochemistry system using Pt electrode, and tetraethylammonium perchlorate as supporting electrolyte. X-band electron paramagnetic resonance (EPR) spectra were recorded with a Bruker EMX Spectrometer.

Photolysis Experiments

The apparent rates of CO release (k_{CO}) upon exposure to low-power UV light were measured with solutions of complexes **1**, **2**, **4**, and **5** in a 1 cm $\times$ 1 cm quartz cuvette. The UV light source was a UVP Benchtop 2 UV Transilluminator. The power of the source on the sample (5 mW cm^{-2}) was measured with a Field MaxII-TO laser power meter (Coherent, Portland, OR). The k_{CO} values in acetonitrile were measured by recording the electronic absorption spectra and monitoring changes in the respective spectra following exposure to UV light at regular intervals. The k_{CO} values were then calculated from $\ln(C)$ versus time (t) plots.

Fluorescence Experiments

The luminescence spectra of the solutions of complexes **1–5** in acetonitrile and dichloromethane (at concentration 4.5×10^{-4} M) were recorded at room temperature with a quartz cuvette (1 cm $\times$ 1 cm). The emission spectra were recorded with excitation either at 360 or 370 nm.

Cell Culture and Imaging

The human breast cancer cell line MDA-MB-231 was purchased from American Type Culture Collection (ATCC), cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), plated onto 35 mm imaging dish (from ibidi Labwares), and allowed to grow overnight. The cells were then treated with 25 μ M of the complexes and allowed to incubate at 37 $^{\circ}$ C with 5% CO₂(g) for 1 h. Next, the cells were washed carefully three times with phosphate-buffered saline (PBS) buffer under dark condition. Finally, the cells were imaged in 2 mL of fresh PBS with the aid of Leica SP5 Confocal Microscope through a PL Apo 40x/1.25 n.a. Oil DIC objective. Illumination (405 nm) was used at 30% power. Gain and offset were set such that the brightest pixels were just below saturation and there were only a few zero-intensity pixels. The detection gate was set to 500–600 nm. The zoom and frame size was set to give pixels that were $\sim$ 115 nm (just below Nyquist). Images were collected at 340 nm intervals for Z-stacks. Scan speed was set to 100 Hz, and 2 $\times$ line averaging was used. Differential Interference Contrast (DIC) images were collected in parallel. Application Suite Advanced Fluorescence software was used to acquire the initial images. The bright field images were acquired in DIC or phase contrast mode. The images were further processed with Fiji-ImageJ version 1.52c and ImageJ (micromanager) version 1.49h software.²⁸

Density Functional Theory Studies

Solvent-phase structure optimizations were performed with the M06L²⁹ functional as implemented in the Gaussian09 program.³⁰ The polarizable continuum model (PCM)³¹ was used for solvation treatments with acetonitrile as solvent ($\epsilon = 35.688$). The ECP60MDF pseudopotential and ECP60MDF_VDZ basis sets were used for the Re,³² and Def2-TZVP basis sets were used for other atoms.^{33,34} All geometry optimizations were performed with no restrictions. Vibrational frequency calculations were performed to confirm the optimized structures as minima (i.e., no imaginary frequencies). Excited-state calculations were performed using the TDDFT. The M06-2X³⁵ functional and the basis sets described above were employed. Nonequilibrium PCM solvation was used to calculate vertical excitations, whereas equilibrium PCM solvation was employed to optimize the first excited singlet state and the first excited triplet state of the Re complexes. In TDDFT calculations, the “ultraFine” integration grid was used, and the two-electron integral accuracy parameter was set to 12.

X-ray Crystallography

Single crystals of complexes **1–5** were grown by layering hexanes over CH₂Cl₂ solutions of the respective complexes under ambient conditions. One suitable crystal of each complex was then selected and fixed on top of MiTiGen micromount using Paratone Oil and

transferred to the diffractometer. Data were collected on a Bruker APEX II single-crystal X-ray diffractometer with graphite-monochromated Mo K α radiation ($\lambda = 0.710\,73\text{ \AA}$) using ω -scan technique in the range of $3 \leq 2\theta \leq 48$ for complexes **1**–**5**. Multiscan absorption corrections were applied to all the data set using SADABS.³⁶ The structures were generated using SHELXT (intrinsic phasing)³⁷ and subsequently refined by full-matrix least-squares procedure on F^2 with SHELXL.³⁸ All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in calculated positions on the C atoms to which they are bonded, with C–H = 0.93 Å and $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C})$. Calculations and molecular graphics were performed using SHELXTL 2014 program package.³⁸ Crystal data and structure refinement parameters are listed in Table 1. CCDC deposition numbers: 1505722 (complex **1**), 1505723 (complex **2**), 1505724 (complex **3**), 1505725 (complex **4**), and 1505726 (complex **5**).

RESULTS AND DISCUSSION

Synthesis and Molecular Structures

Complex **1** was prepared by the reaction of equimolar ratio of $[\text{ReCl}(\text{CO})_5]$ and phen in MeOH– CHCl_3 solvent mixture under refluxing condition. Chloride abstraction with silver trifluoromethanesulfonate from **1** resulted in the trifluoromethanesulfonate-coordinated complex **2** in good yield. Although **2** is stable in solvents like CHCl_3 and CH_2Cl_2 , it slowly converts to the acetonitrile-bound complex **3** in MeCN. Indeed, complex **3** was isolated in appreciable yield upon refluxing **2** in dry MeCN. The reaction of **3** with excess PPh_3 in boiling CHCl_3 afforded complex **4**. Complex **5** was synthesized by the reaction of **3** with MeIm. All these reactions are summarized in Scheme 2. Structures of all complexes were authenticated by single-crystal X-ray diffraction studies. The molecular structures of complexes **1**–**5** with atom labeling scheme are shown in Figures 1–5, and selected bond distances are listed in the respective Figure captions. In all the structures Re center resides in an octahedral coordination environment with the three CO ligands disposed *facially*. Moreover, in all five complexes, the two N atoms of the phen ligand and two CO molecules constitute the equatorial plane, while the axial positions are occupied by one CO and an ancillary X ligand (where X = Cl^- , CF_3SO_3^- , MeCN, PPh_3 , and MeIm).

In complex **1** the equatorial plane composed of N1, N1A, C2, and C2A atoms is perfectly planar, while the equatorial plane composed of N1, N2, C2, and C3 atoms in **2** and **3** are almost planar with mean deviations of 0.002 and 0.007 Å, respectively. In complexes **4** and **5**, the corresponding equatorial planes showed relatively higher degree of deviation from planarity (0.024 and 0.033 Å, respectively). In complexes **2** and **4**, the five-membered chelate rings composed of Re1, N1, N2, C14, and C15 atoms is almost planar (mean deviation 0.009 Å), while the same chelate ring in complex **5** shows slight distortion from planarity (mean deviation 0.012 Å). The corresponding chelate rings in complexes **1** and **3** deviate significantly from planarity (mean deviations 0.026 and 0.031 Å, respectively). In complexes **2**–**5** the phen rings are marginally deviated from planarity (in all cases mean deviation, 0.020 Å), while in complex **1** the phen ligand plane is slightly more distorted with mean deviation of 0.040 Å. Only in case of **4**, the Re–C1 bond distance is noticeably longer (1.949(5) Å) compared to the other two Re–C distances. This can be attributed to the *trans*-

effect of the PPh_3 group, which invokes π -competition with the CO ligands across the Re–P bond for the same metal orbitals and hence preferentially lengthens the axial Re–CO bond. The coordinated acetonitrile molecule is almost linear (N–C–C angle, $178.7(14)^\circ$) in **3**. Interestingly, the average Re–C distance in **1** ($1.915 \text{ \AA}$, Figure 1) is somewhat shorter compared to the structure of the same $[\text{ReCl}(\text{CO})_3(\text{phen})]$ complex reported previously where the average Re–C distance was $1.945 \text{ \AA}$.³⁹ The average Re–N distance in **1** (shown in Figure 1) is also slightly longer ($2.173 \text{ \AA}$) compared to the previously reported average Re–N distance ($1.916 \text{ \AA}$).³⁹ The overall refinement parameters indicate that the current structure is of slightly better quality, which might lead to the minor differences in certain metric parameters with relatively superior standard deviations. The average Re–C and Re–N distances ($1.916 \text{ \AA}$ and $2.172 \text{ \AA}$, respectively) of **3** are comparable to a structurally similar complex, $[\text{Re}(\text{CO})_3(\text{MeCN})(\text{DMDPP})](\text{PF}_6)$ (where DMDPP = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline).⁴⁰ Likewise the average Re–C and Re–N distances of complex **4** (1.929 and $2.188 \text{ \AA}$, respectively) are comparable to an analogous complex, namely, $[\text{Re}(\text{CO})_3(\text{PPh}_3)(\text{bpy})](\text{PF}_6)$ (bpy = bipyridine).⁴¹ Complex **5** crystallized in triclinic $P\bar{1}$ space group with two crystallographically independent molecules in the asymmetric unit. In this case the average Re–C and Re–N distances (1.916 and $2.182 \text{ \AA}$, respectively) are similar to those obtained for a structurally comparable complex, namely, $[\text{Re}(\text{CO})_3(\text{im})(\text{phen})]_2\text{SO}_4$ (where im = imidazole).⁴²

Spectroscopic Properties

The three facially disposed CO ligands in the present complexes (**1–5**) exhibit CO stretching bands in the expected regions (see Experimental Section and Supporting Information, Figures S6–S10). It is noteworthy to mention that both position and intensity of the IR stretching bands associated with the triflate anion (CF_3SO_3^-) differs significantly when coordinated (as in complex **2**, see Supporting Information, Figure S7) versus when present as a counterion. A detailed vibrational study on various triflate salts clearly revealed a strong stretch around 1270 cm^{-1} assigned to asymmetric SO_3 vibration in cases where CF_3SO_3^- resides as counteranion.⁴³ This is in good agreement with the IR spectra recorded for complexes **3**, **4**, and **5**. Quite in contrast, complex **2** (with CF_3SO_3^- coordinated to the Re center) reveals a complex pattern pertaining to the triflate vibrations spanning the region of $1180\text{--}1325 \text{ cm}^{-1}$.

Solutions of complexes **1**, **3**, **4**, and **5** in CH_2Cl_2 and MeCN are indefinitely stable in the absence of light. These solutions are also stable under aerobic conditions, a fact crucial for their applications in biological experiments. The electronic absorption spectra of the complexes consist of two bands in such solutions (see Supporting Information, Figures S11–S14). In acetonitrile, the lower energy bands with relatively low molar absorptivity appear at $330\text{--}400 \text{ nm}$. This absorption arises from MLCT transition(s). The higher energy bands, spanning the range of $265\text{--}300 \text{ nm}$, correspond to ligand-centered (LC) transition(s) (vide infra). Well-resolved ^1H NMR spectra (see Supporting Information, Figures S15–S17) confirm the diamagnetic ground state of Re(I) in all five complexes. Finally, complexes **1–5** display luminescence in MeCN and CH_2Cl_2 solutions upon excitation around 360 nm . The emission spectra of **1**, **3**, **4**, and **5** in MeCN are shown in Figure 6, while the spectra for **1–5** in CH_2Cl_2 are included in the Supporting Information (Figure S18). Complex **1** exhibits a

broad emission band centered at 605 nm upon excitation at 370 nm, while **3** displays its emission maxima at 532 nm upon excitation at 360 nm. The solutions of **4** and **5** exhibit emission band maxima at 518 and 578 nm, respectively, upon excitation at 370 nm. As shown in Figure 6, **3** exhibits significantly higher emission intensity compared to the other three complexes.

CO Release Rates

Exposure of MeCN solutions of the complexes to low-power (5 mW cm^{-2}) UV light (centered at 302 nm) at regular intervals results in systematic changes in their absorption spectra. Myoglobin assay confirms loss of CO from those complexes associated with such spectral changes (except for complex **3**, where no CO release was noted under the same experimental condition). Despite the presence of an absorption band around 350 nm, liberation of CO from complexes **1** and **5** is only realized when solutions of such complexes are illuminated with UVB light of $\lambda < 315 \text{ nm}$. Ishitani and co-workers have performed extensive studies on photochemical ligand substitution (PLS) reactions of rhenium-(I) carbonyl complexes of type $[\text{Re}(\text{X}_2\text{bpy})(\text{CO})_3\text{Y}]$ ($\text{X} = \text{H}, \text{CF}_3, \text{OEt}, \text{and Ph}$ and $\text{Y} = \text{Cl}, \text{py}, \text{and PR}_3$, py = pyridine, and bpy = bipyridine).⁴⁴ These authors have shown that complexes like $[\text{ReCl}(\text{CO})_3(\text{bpy})]^+$ and $[\text{Re}(\text{CO})_3(\text{bpy})\text{py}]^+$ containing either σ -donating or moderately π -accepting ancillary ligands require higher energy UVB light ($\lambda < 313 \text{ nm}$) for CO photorelease.⁴⁵ However, complexes of type $[\text{Re}(\text{CO})_3(\text{bpy})(\text{PR}_3)]^+$ with a strong π -accepting coligand release CO upon exposure to UVA light of relatively lower energy ($\lambda = 360 \text{ nm}$).^{44,46} This difference in sensitivity has been attributed to the weaker *trans*-labilizing ability of Cl^- or py (pyridine) ligand compared to a phosphorus donor (as in complex **4**). Along the same line, the present complexes containing either σ -donating or moderately π -accepting ancillary ligands (Cl^- and MeIm) exhibit stability toward lower energy UV light, while complex **4** with PPh_3 as ancillary ligand releases CO upon illumination with UVA light ($\lambda = 365 \text{ nm}$). In all cases, UV illumination is required to augment the forward internal conversion rate between the $^3\text{MLCT}$ and thermally accessible photoexcited higher energy ^3LF state.⁴⁴ The latter state is productive in terms of CO dissociation. The apparent CO release rates (k_{CO}) from **1**, **4**, and **5** were determined spectrophotometrically. In MeCN solutions, complexes **1**, **4**, and **5** afford k_{CO} values of $0.07 \pm 0.02 \text{ min}^{-1}$ (concentration $4.50 \times 10^{-4} \text{ M}$), $1.59 \pm 0.02 \text{ min}^{-1}$ (concentration $4.50 \times 10^{-4} \text{ M}$), and $0.07 \pm 0.02 \text{ min}^{-1}$ (concentration $2.25 \times 10^{-4} \text{ M}$), respectively. From the k_{CO} values it is evident that complex **4** (with PPh_3 as the sixth ligand) not only shows sensitivity toward low-energy UVA light but also exhibits much faster CO release rate. Selected physical properties of these complexes are listed in Table 2.

Time-Dependent IR Studies and Nature of the Photoproducts

The design of the luminescent photoCORMs in this study includes a stable fluorogenic bidentate ligand (phen) that can provide either a “turn off” or a shift in the fluorescence signals upon irradiation (following liberation of CO from the complexes). The inherent blue fluorescence of the phen ligand could also serve as an indicator to identify any deligation of phen upon CO release. We hypothesized that such choice would aid in elucidating the nature of the photoproduct(s) present in the corresponding photolyzed solutions. Time-resolved IR spectroscopic studies were performed to assess the number of CO molecules release from

these species upon illumination. These studies revealed that in case of complex **1** exhaustive photolysis with UVB light (5 mW cm^{-2}) results in *release of all three CO molecules* (see Supporting Information, Figure S20), whereas complexes **4** and **5** *liberate only one CO molecule under same experimental condition*. Complex **4** also releases CO upon exposure to UVA light. In such IR studies, MeCN solutions of these complexes were taken in a glass Petri dish and irradiated with low-power UV light from the top. Aliquots from such irradiated solutions were taken at regular intervals and dried under vacuum, and the IR spectra of the solids thus obtained were carefully recorded within KBr matrices. Results from one representative case are shown in Figure 7. These two IR spectra in case of **4** clearly demonstrate that 3 h of UV illumination results in abrogation of the CO stretching band around 2040 cm^{-1} with emergence of a new band at 1863 cm^{-1} . This dicarbonyl species is highly photostable, and hence no additional change could be observed upon further illumination. The exhaustively photolyzed solutions of all complexes also exhibit no EPR signal(s) and confirm retention of the Re(I) oxidation state in the respective photoproducts.

Taken together, it is now apparent that, for complexes with π -accepting ancillary ligands (complexes **4** and **5**), the PPh_3/MeIm preferentially labilizes the CO *trans* to it. After liberation of one CO molecule from such complexes, the presence of sufficiently π -accepting coordinated PPh_3/MeIm ligands (and two CO molecules) can effectively stabilize the Re(I) state in the corresponding dicarbonyl photoproducts and thereby do not show further photorelease of CO upon illumination. However, for complexes with considerable σ -donating ancillary ligands (such as Cl^- in complex **1**), once one CO molecule dissociates upon illumination, the solvent MeCN takes this position. The presence of one MeCN ligand, in addition to the σ -donating sixth ligand and two other CO molecules, most possibly does not provide enough stability to the Re(I) center via π -backbonding and subsequently results in the loss of the other two CO molecules from the corresponding photoproducts of such complexes. Further studies on the intermediate species are however required to prove this hypothesis, and such studies are currently in progress in this research group.

Computational Studies

The DFT-optimized ground-state structures of Re complexes **1–5** are shown in Figure S21, and the key structural parameters of the X-ray structure, optimized ground-state (S_0) structure, optimized lowest singlet excited-state structure (S_1), and lowest triplet excited-state (T_1) structure are summarized in Table S1. Calculated S_0 structures of the Re complexes are in good agreement with their X-ray structures. Bond distances of the optimized S_1 and optimized T_1 structures of the Re complexes are also rather similar to the corresponding distances of the optimized S_0 structures, whereas bond angles show minor deviations. Figure 8 shows the Kohn–Sham orbital energies in the frontier region and highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of ground state (S_0) optimized Re complexes **1–5**. Calculated HOMO–LUMO gaps of **1** (0.09 eV), **2** (0.09 eV), **3** (0.10 eV), **4** (0.10 eV), and **5** (0.09 eV) are rather similar. In all five systems, HOMO is a metal-based orbital, while LUMO is a ligand-based orbital. We used ground-state optimized structures (S_0) of the complexes to calculate the vertical excitations. Selected key vertical excitations are summarized in Table S2. To analyze the nature of the absorption, natural transition orbital (NTO) analysis was performed.⁴⁷ The NTOs that

correspond to the key transitions are shown in Figure S22. All calculated vertical excitations are also depicted in the Supporting Information. Our calculations suggest that the absorption band at 300–325 nm region for all five complexes have $^1\text{MLCT}$ character, while the absorption band at 220–250 nm can be characterized as either metal-centered (^1MC) charge transfer or ligand-centered (^1LC) charge transfer. The emission of the Re complexes **1–5** is expected to originate from the lowest-lying triplet excited state (i.e., phosphorescence), because spin–orbit coupling facilitates ISC from singlet to the triplet state lying below. We used DFT to optimize the triplet states of all five Re complexes. Calculated total spin densities of the triplet states, shown in Figure S23, suggest that the spin densities are partially localized on the metal in addition to delocalization over the ligands. Table 3 summarizes the calculated wavelengths at lowest singlet excited-state (S1) optimized structures, the lowest triplet excited (T1) optimized structures, and experimental emission wavelengths. The emission band of all five Re complexes originate from the lowest triplet excited state (T1), and it is associated with $^3\text{MLCT}$ character. Calculated emission wavelengths (from T1) of **2** (521 nm), **3** (520 nm), and **4** (532 nm) are in good agreement with the experimental values, while calculated emission of **1** (523 nm) and **5** (518 nm) showed reasonable agreement. The relatively weak intensity for **1** and **5** (Figure 6) suggests that these two complexes fluctuate more easily at the excited state.

Cellular Studies and Fluorescence Confocal Microscopy

Following determination of the physical parameters of the luminescent photoCORMs we focused on the possibility of their applications in cellular imaging studies. This stems from our interest toward developing theranostic photoCORMs that could be trackable within biological matrices.¹¹ In the present study, fluorescence confocal microscopy was employed to elucidate the uptake and internalization of the rhenium complexes by mammalian cancer cells. In such studies triple negative human breast cancer cells MDA-MB-231 were incubated with 25 μM of complexes **1**, **3**, **4**, and **5** for an hour in phenol red-free DMEM medium. Solutions of the complexes were prepared with MeCN–PBS (40:60 v/v mixture). After incubation, the medium was aspirated, the cells were washed thrice carefully with PBS, and finally fresh PBS was added to each dish for imaging experiments (for details see Experimental Section). Also prior to the imaging Trypan blue was added to quench any extracellular fluorescence. The results of this investigation show that the rhenium complexes both neutral and cationic, with ancillary ligands of varied lipophilicity, exhibit appreciable cellular uptake (Figure 9).

Visual inspection of the images in Figure 9 indicates considerable extent of cellular internalization of all four complexes. For cationic complexes (**3**, **4**, and **5**) the superior cellular uptake was expected due to relatively higher propensity of membrane permeability of the positively charged species.⁴⁸ In the present study even for neutral complex **1**, significant uptake was also noted (Figure 9, extreme left panel of second and third row). All the images indicated distribution of complexes primarily within the cytoplasm. However, careful inspection reveals that unlike other species, complex **4** exhibits a significant extent of nuclear uptake in addition to cytosolic distribution (Figure 10, micrograph c). Interestingly, another structurally similar complex, namely, $[\text{Re}(\text{CO})_3(\text{PPh}_3)(\text{pbt})](\text{CF}_3\text{SO}_3)$ (where pbt = 2-pyridyl(benzothiazole)), showed no such nuclear accumulation despite internalization into

MDA-MB-231 cells (Figure 11).¹² This clearly suggests that the appropriate combination of ligand/ancillary ligand has led to an optimum lipophilicity resulting in significant extent of nuclear uptake of complex **4** within just 1 h of incubation (Figures 10c and 11b). In a recent report, Ford and co-workers have reported one Re(I) biocompatible photoCORM, namely, [Re(bpy) (CO)₃(L)](CF₃SO₃) (bpy = bipyridine and L = the water-soluble phosphine P(CH₂OH)₃), which is internalized by human prostatic carcinoma cells PPC-1 (showing no apparent toxicity by itself) and exhibits strong fluorescence around 500 nm.⁴⁹ This photoCORM has been shown to release CO upon exposure to 405 nm light and allows multiplex imaging of the cells by confocal microscopy. However, no nuclear uptake of this phosphine complex has been reported.

Currently, very few luminescent transition metal complexes have been reported that show nuclear uptake capability.^{50,51} Among the limited examples there is a family of Ir(III) dipyrroquinoxaline complexes that stains the nucleoli of fixed MDCK cells upon relatively longer incubation time.⁵² However, no selectivity in accumulation of these complexes in nucleus over the cytoplasm was realized (much like for complex **4**). A triphenylphosphonium-functionalized derivative of a Pt(II) complex has also shown higher affinity for the cell nucleolus.⁵³ The imaging studies discussed above clearly demonstrate that present complexes are internalized by cancer cells quite readily irrespective of their charge and lipophilicity. In addition, complex **4** with relatively more lipophilic character exhibits its ability to stain the nuclei, albeit to a lesser extent (Figure 10c and 11b).

CONCLUSIONS

In summary, five luminescent Re(I) carbonyl complexes derived from the same rigid α -diimine ligand (phen) have been synthesized and characterized. All complexes except **3** exhibit moderate CO release upon illumination with low-power UV light. IR and EPR spectroscopy have been employed to examine the nature of the photoproducts. The results demonstrate that depending on the nature of the sixth ancillary ligands these photoCORMs release three, one, or no CO upon photoirradiation. Excited-state DFT and TDDFT calculations have been performed to describe the variable emission characteristics of these CO donors. The complexes uniformly display efficient cellular internalization within the human breast cancer cells (MDA-MB-231) as noted from confocal microscopy studies. Among them complex **4** (with PPh₃ as the sixth ligand) exhibits moderate nuclear accumulation in addition to the cytosolic distribution. Taken together, results of the present study establish that minor changes in the design of such photoCORMs can alter the capacity of CO release from these complexes significantly. Moreover, appropriate ligand/coligand combination is critical to achieve optimal lipophilic character that can lead to preferential accumulation of such species within the nuclei of cells. Further research toward development of more effective theranostic Re-based photo-CORMs with capacity for organelle-specific accumulation is in progress at present in this laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Thermal ellipsoid plot of complex **1** shown with 50% probability ellipsoids. Hydrogen atoms are omitted for the sake of clarity. Selected bond distances [Å]: Re1–C1, 1.913(8); Re1–C2, 1.917(5); Re1–C2A, 1.917(5); Re1–N1, 2.173(3); Re1–N1A, 2.173(3), Re1–Cl, 2.4738(19).

Figure 2.

Thermal ellipsoid plot of complex **2** shown with 50% probability ellipsoids. Hydrogen atoms are omitted for the sake of clarity. Selected bond distances [Å]: Re1–Cl1, 1.877(6); Re1–C2, 1.917(6); Re1–C3, 1.921(6); Re1–N1, 2.180(4); Re1–N2, 2.170(4); Re1–O4, 2.197(3).

Figure 3. Thermal ellipsoid plot of the cation of complex **3** shown with 50% probability ellipsoids. Hydrogen atoms and the counteranion are omitted for the sake of clarity. Selected bond distances [Å]: Re1–C1, 1.897(12); Re1–C2, 1.912(13), Re1–C3, 1.940(11); Re1–N1, 2.168(8), Re1–N2, 2.175(7); Re1–N3, 2.132(9).

Figure 4. Thermal ellipsoid plot of the cation of complex **4** shown with 50% probability ellipsoids. Hydrogen atoms and the counteranion are omitted for the sake of clarity. Selected bond distances [Å]: Re1–C1, 1.949(5); Re1–C2, 1.917(6); Re1–C3, 1.922(5); Re1–N1, 2.185(4); Re1–N2, 2.191(4), Re1–P, 2.5116(13).

Figure 5.

Thermal ellipsoid plot of the cation of complex **5** shown with 50% probability ellipsoids. Hydrogen atoms and the counteranion are omitted for the sake of clarity. Selected bond distances [Å]: Molecule A, Re1–C1, 1.908(6); Re1–C2, 1.924(5), Re1–C3, 1.912(5); Re1–N1, 2.177(4); Re1–N2, 2.188(4); Re1–N3, 2.184(4). Molecule B, Re1–C20, 1.933(6); Re1–C21, 1.901(5); Re1–C22, 1.916(6), Re1–N5, 2.181(4); Re1–N6, 2.181(4); Re1–N7, 2.188(4).

Figure 6.
Emission spectra of **1**, **3**, **4**, and **5** in MeCN at 298 K.

Figure 7.

Changes in IR spectra during photolysis in MeCN solution of **4** upon irradiation with low-power UV light for 0 (left) and 3 (right) h and showing conversion of [Re(CO)₃(phen)(PPh₃)]⁺ to [Re(CO)₂(phen)(PPh₃)(MeCN)]⁺.

Figure 8. Kohn-Sham orbital energies at the frontier region of ground-state optimized complexes **1–5** and their HOMO and LUMO.

Figure 9. Emission spectra (upper), fluorescence images (middle), and the overlaid fluorescence and transmitted light images (bottom) of MDA-MB-231 cells with complexes **1**, **3**, **4**, and **5** (from left to right).

Figure 10. Magnified fluorescence images of MDA-MB-231 cells with complexes (a) **1**, (b) **3**, (c) **4**, and (d) **5**.

Figure 11. Fluorescence images of MDA-MB-231 cells with (a) $[\text{Re}(\text{CO})_3(\text{PPh}_3)(\text{pbt})](\text{CF}_3\text{SO}_3)$ and (b) complex **4**.

Scheme 1.
Complexes Reported in This Work

Scheme 2.
Steps for the Synthesis of Complexes 1–5

Table 1
Crystal Data and Structure Refinement Parameters for 1, 2, 3·H₂O, 4·CH₂Cl₂, and 5

	1	2	3·H ₂ O	4·CH ₂ Cl ₂	5
empirical formula	C ₁₅ H ₈ ClN ₂ O ₃ Re	C ₁₆ H ₈ F ₃ N ₂ O ₆ ReS	C ₁₈ H ₁₃ F ₃ N ₃ O ₇ ReS	C ₃₃ H ₂₅ Cl ₂ F ₃ N ₂ O ₆ PRes	C ₂₀ H ₁₄ F ₃ N ₄ O ₆ ReS
formula weight	485.89	599.51	658.57	946.71	681.62
<i>T</i> (K)	298	298	298	298	298
λ (Å)	0.710 73	0.710 73	0.710 73	0.710 73	0.710 73
crystal system	monoclinic	monoclinic	monoclinic	monoclinic	triclinic
space group	<i>C2/m</i>	<i>P2(1)/n</i>	<i>C2/c</i>	<i>P2(1)/c</i>	<i>P1</i>
<i>a</i> (Å)	15.8984(13)	6.6738(4)	20.2923(14)	14.8378(5)	10.6208(19)
<i>b</i> (Å)	11.8800(10)	14.0156(9)	17.2933(12)	15.0784(6)	13.166(3)
<i>c</i> (Å)	7.9938(7)	19.3333(12)	14.6464(10)	16.9677(6)	16.427(2)
α (deg)	90	90	90	90	75.616(13)
β (deg)	107.1770(10)	94.8850(10)	107.580(1)	111.02(5)	86.112(13)
γ (deg)	90	90	90	90	89.034(14)
<i>V</i> (Å ³)	1442.5(2)	1801.82(19)	4899.7(6)	3543.6(2)	2219.9(8)
<i>Z</i>	8	4	8	4	2
<i>D</i> _{calc} (Mg m ⁻³)	2.237	2.210	1.780	1.775	2.039
absorption coefficient (mm ⁻¹)	8.622	6.928	5.108	3.748	5.640
No. of unique reflections	1209	2865	3041	5616	7525
goodness-of-fit ^a on <i>F</i> ²	0.933	1.044	1.072	1.030	0.660
<i>R</i> ₁ ^b	0.0216	0.0239	0.0506	0.0326	0.0240
<i>wR</i> ₂ ^c	0.0459	0.0576	0.1576	0.0846	0.0759
largest diff peak and hole	0.601, −0.444	1.348, −0.396	1.317, −0.704	1.351, −0.532	0.565, −0.633

^a GOF = $[\sum(\alpha F_0^2 - F_c^2)^2 / (\sum(N_0 - N_v))]^{1/2}$ (*N*₀ = number of observations, *N*_v = number of variables).

^b $R_1 = \sum|F_0| - |F_c| / \sum|F_0|$.

^c $wR_2 = [(\sum\alpha F_0^2 - F_c^2)^2 / \sum(F_0^2)]^{1/2}$.

Table 2

Excitation and Emission Wavelengths, Apparent CO Release Rates, and Redox Potentials for Complexes 1, 3, 4, and 5

complex	λ_{ex} (nm)	λ_{em} (nm)	k_{CO} (min ⁻¹) ^a	E_{pa} ^b (V)
1	370	605	0.07 ± 0.02 (4.50×10^{-4} M)	1.80
3	360	532	no CO photorelease	1.84
4	370	518	1.59 ± 0.02 (4.50×10^{-4} M)	1.84
5	370	578	0.07 ± 0.02 (2.25×10^{-4} M)	1.67

^aConcentrations are shown in parentheses.

^bIrreversible Re^I/Re^{II} response.

Table 3
 Calculated^a Wavelengths at the Optimized 1st Singlet Excited States (S1), Optimized 1st Triplet Excited States (T1), and the Experimental Emission

	1	2	3	4	5
S1/nm	439	418	393	442	434
T1/nm	523	521	520	532	518
λ_{em} (exp)/nm	605	532	532	518	578

^aTDDFT.