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Photoswitchable alkoxy-bridged binuclear rhenium(I) complexes – a potential probe for biomolecules and optical cell imaging†

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We describe the solvothermal synthesis, structural characterization, photophysics and potential applications as probes of two alkoxy-bridged binuclear Re(I) complexes, $[\{Re(CO)_3(1,4\text{-NVP})\}_2(\mu_2\text{-OR})_2]$ (**1**, R = C_4H_9; **2**, R = $C_{10}H_{21}$; 1,4-NVP = 4-(1-naphthylvinyl)pyridine). Irradiation of **1** and **2** at 365 nm leads to an interesting *trans-cis* photoisomerization process, which was examined by ^{1}H NMR, UV-vis, emission and time-resolved techniques. Compounds **1** and **2** exhibit photoswitchable luminescence enhancement arising from photoinduced intramolecular energy transfer from the ^{3}MLCT state of the Re(I) chromophore to the triplet excited state of the ligand 1,4-NVP. In addition, these Re(I) complexes serve as excellent probes for the ultrasensitive detection of biological molecules, including bovine serum albumin (BSA) and a platelet derived growth factor (PDGF) binding aptamer. Our results also suggest that, since these Re(I) complexes have low cytotoxicity and fluorescence properties under physiological conditions, they could be a useful probe for optical imaging of cancer cells by confocal laser scanning microscopy.

Introduction

The design and generation of photoactive materials capable of performing useful functions is a subject of considerable interest.[1] A photoactive molecule, functioning as a photoswitch or a bioimaging probe upon illumination with light, would be highly rewarding.[2] In the design of molecules that are activated by light, transition metal complexes offer several advantages.[3] For example, complexes containing d^6 metal ions such as Ru^{II} and Re^{I} display: (i) absorption and emission behaviors in the visible region due to the metal-to-ligand charge transfer (MLCT) state, (ii) various redox states and (iii) moderate excited state lifetimes. Furthermore, the optical properties of these complexes can be fine tune by the judicious choice of ligands and introduction of suitable substituents.[4]

The coordination of an isomerizable ligand to a metal center is a novel approach to sensitizing the isomerization process of an organic component to visible light and to find applications as photoswitches.[5] Rhenium(I) complexes are able to function as photoswitches, because of their luminescent characteristics and good photostability conferred on them.[6] Hence, rhenium(I) complexes have the potential to function as light-induced molecular switching devices when a photoisomerizable ligand is coordinated to the rhenium metal center. For the development of photoinduced molecular devices, rhenium(I) complexes coordinated to a stilbene-like ligand have been studied by following the *trans-cis* photoisomerization process of the ligands that are coordinated to the rhenium(I)-tricarbonyl unit.[7,8] This photoinduced *trans-cis* isomerization is triggered by either direct irradiation or sensitization *via* an inter- or intra-molecular energy transfer process. A mechanism for the reaction of rhenium(I)-tricarbonyl diimine complexes under photoirradiation, based on *ab initio* calculations[9] and the excited state dynamics of rhenium(I) complexes, has recently been proposed.[10] Although stilbene-like ligands coordinated with rhenium have been studied,[7,8,11] the 4-(1-naphthylvinyl)pyridine (1,4-NVP) incorporated rhenium complex has not. While several mononuclear,[12] binuclear[13] and tetranuclear[14] rhenium(I) complexes have been previously reported by our group, this work is focused on rhenium(I) complexes carrying a long alkyl chain in combination with a photoisomerizable ligand. It should be

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† Electronic supplementary information (ESI) available: Crystallographic data of complex **1** (CCDC 919830), UV-vis and emission spectra of 1,4-NVP ligand, UV-vis and emission spectral changes of complex **1** upon photoirradiation, lifetime decay profile of complex **2**, ^{1}H NMR spectra of **2** before and after irradiation at 365 nm, change in emission spectrum of **1** and **2** with PDGF aptamer. See DOI: 10.1039/c3ra42627k

noted that rhenium(I) complexes coordinated with alkoxy ligands having long alkyl chains provide lipophilic characteristics to the complex[15] and they serve as sensitive probes for quinones and amines.[14c,16] Hence, we anticipated that the coordination of a photoisomerizable 1,4-NVP ligand and long alkyl chains with rhenium could result in a metal complex with interesting photophysical properties that could be used as a probe for proteins and as a bioimaging agent for cells.

In recent years, much focus has been directed towards the design of aptamer-based sensor systems for use in the area of biomedical research, particularly cancer diagnosis, disease related protein detection and, targeted drug delivery.[17] Aptamers are single-stranded DNA or RNA molecules that can specifically bind to small molecules,[18] peptides, proteins and cells[19] with high affinity and specificity. PDGF, a ubiquitous mitogen found in human platelets, regulates cell growth and proliferation through the specific binding to its receptor on the cell surface.[20] It plays a crucial role in cell transformation and the growth of malignant tumors and has growth-promoting activity for fibroblasts, smooth muscle cells and glial cells.[21] Aptamers can be selected to bind the protein PDGF selectively, called PDGF binding aptamer. Bovine serum albumin (BSA) is a well-known heart shaped protein containing a single polypeptide chain that consists of 583 amino acid residues with 17 disulfide bonds and one free SH group.[22] The tertiary structure of BSA is composed of three domains I–III carrying two tryptophan residues Trp134 and Trp212[23] and each domain consists of subdomains A and B. The former, Trp134, is located in a hydrophilic environment, close to the protein surface while the latter, Trp212, is surrounded by a hydrophobic environment within a protein pocket. It has been used as a model protein in numerous biochemical studies[24] and plays a major role in various physiological functions in living organisms. BSA facilitates the metabolism, transport and distribution of exogenous and endogenous substances.[25] The binding of rhenium(I) metal complexes to proteins[26] and DNA[27] was studied by Yam[28] and Lo *et al.*[29] Our interest in this area was to attempt the utilization of these binuclear rhenium(I) complexes as selective biological probes for the detection of the PDGF binding aptamer and BSA.

The novel discovery that fluorescent staining probes with high sensitivity, noninvasive properties and low cytotoxicity can serve as diagnostic agents in the visualization of pathological conditions quickly prompted significant breakthroughs in biological imaging studies as well as in biomedical applications. Rhenium(I) complexes have shown great potential as luminophores for applications in living cells.[30] We envisage that the biocompatibility and cellular uptake property of rhenium(I) complexes might exhibit qualitative efficiency comparable to carboxyfluorescein diacetate succinimidyl ester (CFSE), a commercial dye for fluorescence labeling of cells.

In this paper we report on the synthesis of alkoxy-bridged binuclear rhenium(I) complexes containing a photoactive 1,4-NVP ligand by a solvothermal method. The photoassisted *trans-cis* isomerization behavior of the 1,4-NVP ligand coordinated to rhenium has been studied. In addition, these rhenium complexes function as a selective sensor for BSA and PDGF binding aptamer as well as a fluorescent probe for cell imaging.

Experimental section

Materials

The materials $Re_2(CO)_{10}$, 1-butanol, 1-decanol and 4-(1-naphthylvinyl)pyridine (1,4-NVP) were procured from commercial sources and used as received. BSA and PDGF aptamer were obtained from Genei and Ocimum biosciences. Infrared spectrum was recorded on a Perkin-Elmer FT-IR spectrometer PARAGON 1000 and ^{1}H NMR spectra on Bruker AMX-400 FT-NMR spectrometer. FAB-MS data were obtained using a JEOL, JMS-700 double focusing mass spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer.

Methods

Stock solution for complexes **1** and **2** (1×10^{-3} M) were prepared in dichloromethane for photolysis and in aqueous DMSO for biological studies. For binding studies with biomolecules, complexes **1** and **2** were dissolved in 2% DMSO–98% water (v/v). The sample solution containing BSA (1×10^{-3} M) and the PDGF aptamer (100 pM) was prepared using phosphate–saline (PBS) buffer (pH 7.4). The PBS buffer solution was prepared using a mixture of disodium hydrogenphosphate, sodium dihydrogenphosphate and sodium chloride at pH 7.4. MilliQ water was used for preparing PBS buffer. Freshly prepared sample solutions were used for each measurement.

Photophysical measurements

Electronic absorption spectra were recorded on Analytik Jena Specord S100 spectrophotometer using 1 cm pathlength cuvette. In photoisomerization studies, irradiation of the complexes **1** and **2** was carried out in dichloromethane at 365 nm using a 200 W Hg(Xe) lamp in Heber Microphotoreactor. Emission spectra were recorded using JASCO FP6300 spectrofluorimeter. HPLC grade dichloromethane and DMSO were employed in all photophysical and photochemical measurements. Emission quantum yields were measured in deoxygenated dichloromethane solutions by integration of the emission spectra relative to that of $[Ru(bpy)_3](PF_6)_2$ ($\Phi = 0.062$ in CH_3CN).[31]

Excited state lifetime measurement

Fluorescence decays were recorded by time correlated single photon counting (TCSPC) method using the following set up. A diode pumped millena CW laser (Spectra Physics) 532 nm was used to pump a Ti:Sapphire rod in a Tsunami picosecond mode locked laser system (Spectra Physics). The 750 nm (8 MHz) line was taken from the Ti:Sapphire laser and passed through a pulse picker (Spectra Physics, 3980 2s) to generate 80 kHz pulses. The second harmonic output (375 nm) was generated by a flexible harmonic generator (Spectra Physics, GWU 23 ps). The vertically polarised 375 nm laser was used to

excite the sample. The fluorescence emission at the magic angle (54.7°) was dispersed in a monochromator (f/3 aperture), counted by a MCP PMT (Hamamatsu R 3809) and processed through CFD, time-to-amplitude converter (TAC) and multi channel analyzer (MCA). The instrument response function for this system is ≈ 52 ps and the fluorescence decay was analyzed using the software provided by IBH (DAS-6) and PTI global analysis software.

Transient absorption spectral study

Transient absorption measurements were made with a laser flash photolysis technique using an Applied Photophysics SP-Quanta Ray GCR-2(10) Nd:YAG laser as the excitation source (pulse width ~8 ns and energy 50 mJ per pulse). The time dependence of the luminescence decay was observed using a Czerny-Turner monochromator with a stepper motor control and a Hamamatsu R-928 photomultiplier tube. The production of the excited state on exposure to 355 nm was measured by monitoring (pulsed Xenon lamp of 250 W) the change in absorbance. The change in the absorbance of the sample on laser irradiation was used to calculate the rate constant as well as to record the time-resolved absorption transient spectrum.

Cell culture

The human breast carcinoma cell line MDA-MB-231 (BCRC 60425, Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan) was maintained in Dulbecco's Modified Eagle's medium (DMEM; HyClone) containing 10% fetal bovine serum (FBS; Biological Industries), 2 mM L-glutamine and antibiotics (100 mg/L Streptomycin, 100 U/mL Penicillin G, and 0.25 mg/L Amphotericin B) at 37 °C in a humidified atmosphere with 5% CO_2 in air.

Cell viability assay

MDA-MB-231 cells were seeded at a density of 1.0×10^4 per well in 96-well culture plates for 24 h. Cells were treated with different concentrations of Re(I) complexes or DMSO (vehicle control) for 4 and 24 h, respectively, in triplicate. After incubation, the medium was removed and the cells were washed with PBS, followed by incubating with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 1 mg/mL) solution at 37 °C for 4 h. The medium was removed, and the formazan was solubilized in DMSO and determined spectrophotometrically (Molecular Devices SPECTRAmax PLUS384) at 540 nm. The percentage of viable cells was estimated by comparison with untreated control cells.

Confocal laser scanning microscopy

Briefly, cancer cells were plated at a density of 4.0×10^5 cells on 17-mm glass cover slips in culture medium. Carboxyfluorescein diacetate succinimidyl ester (CFSE) is the most commonly used reagent for the fluorescence labeling of cells. The distribution of CFSE is observed in different locations of cells including membrane, cytosol and nucleus by binding to cellular proteins and PKH26. CFSE is co-localized with compound **2** to serve as a positive control. Twenty-four hours after seeding, the MDA-MB-231 cells were incubated for 30 min with free **2** in the presence or absence of CFSE at a final concentration of compound **2** of 20 μM. Subsequently, cells were washed with phosphate buffer solution (PBS) and images were collected by confocal fluorescence microscopy (Zeiss LSM 510 META Confocal Laser Scanning Microscope equipped with a Ti:Sa multiphoton laser 690–1020 nm for two-photon excitation). The excitation wavelengths (nm) used for compound **2** and CFSE were 760 and 488 nm, respectively. Emission signals were detected at 435–485 nm for compound **2** and 525 nm for CFSE. A 40× oil-immersion objective lens was used to observe the fluorescence intensities of the samples.

Preparation of $[\{Re(CO)_3(1,4\text{-NVP})\}_2(\mu_2\text{-}OC_4H_9)_2]$ (1)

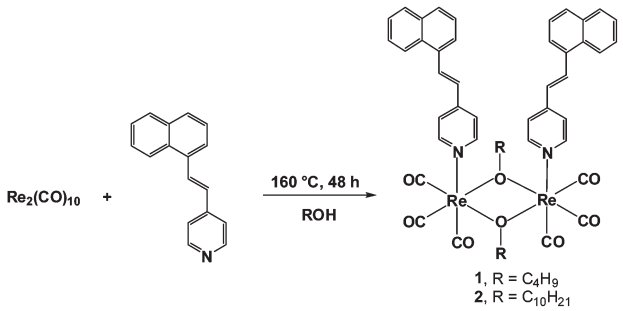
A mixture of $Re_2(CO)_{10}$ (217 mg, 0.33 mmol) and 4-(1-naphthylvinyl)pyridine (154 mg, 0.66 mmol) was suspended in 1-butanol (9 mL) in a Teflon flask and placed in a steel bomb. The steel bomb was kept in an oven and heated at 160 °C for 48 h. Upon cooling to room temperature, light-yellow colored crystals were obtained. Yield: 264 mg, 70%. IR (KBr, cm^{-1}): 2015.8, 2000.8, 1895.8, 1867.4; ^1H NMR (400 MHz, DMSO-d_6, δ/ppm): 8.32 (m, 2H, H^h), 8.29 (d, J = 15.9 Hz, 2H, H^d), 8.25 (d, J = 6.2 Hz, 4H, H^a), 7.83 (m, 2H, H^k), 7.70 (d, J = 7.7 Hz, 2H, H^g), 7.60 (d, J = 6.2 Hz, 4H, H^b), 7.45 (m, 4H, $H^{i,j}$), 7.32 (d, J = 7.7 Hz, 2H, H^e), 7.05 (d, J = 15.9 Hz, 2H, H^c), 6.86 (t, J = 7.7 Hz, 2H, H^f), 4.35 (t, J = 7.6 Hz, 4H, H^1), 2.04 (m, 4H, H^2), 1.48 (m, 4H, H^3), 1.07 (t, J = 7.2 Hz, 6H, H^4); FAB-MS: m/z = 1148.23 (M^+); Anal. Calcd. for $C_{48}H_{44}N_2O_8Re_2$: C, 50.16; H, 3.86; N, 2.44%; Found: C, 50.08; H, 3.75; N, 2.35%. CCDC 919830.

Preparation of $[\{Re(CO)_3(1,4\text{-NVP})\}_2(\mu_2\text{-}OC_{10}H_{21})_2]$ (2)

Complex **2** was self-assembled by adopting the procedure used for the synthesis of **1**, except that 1-decanol was used in the place of 1-butanol. Yellow colored crystals were obtained upon cooling to room temperature. Yield: 263 mg, 60%. IR (KBr, cm^{-1}): 2015.5, 2002.1, 1885.1, 1865.8; ^1H NMR (400 MHz, DMSO-d_6, δ): 8.31 (m, 2H, H^h), 8.29 (d, J = 15.9 Hz, 2H, H^d), 8.24 (d, J = 5.8 Hz, 4H, H^a), 7.83 (m, 2H, H^k), 7.70 (d, J = 7.7 Hz, 2H, H^g), 7.59 (d, J = 5.8 Hz, 4H, H^b), 7.45 (m, 4H, $H^{i,j}$), 7.32 (d, J = 7.7 Hz, 2H, H^e), 7.05 (d, J = 15.9 Hz, 2H, H^c), 6.86 (t, J = 7.7 Hz, 2H, H^f), 4.32 (t, J = 7.6 Hz, 4H, H^1), 2.05 (m, 4H, H^2), 1.24–1.44 (m, 28H, H^{3-9}), 0.86 (t, J = 6.1 Hz, 6H, H^{10}); FAB-MS: m/z = 1318.41 (M^+); Anal. Calcd. for $C_{60}H_{68}N_2O_8Re_2$: C, 54.69; H, 5.20; N, 2.13%; Found: C, 54.23; H, 5.01; N, 2.10%.

X-Ray crystallographic determination

Single-crystal X-ray diffraction analysis was performed using an Enraf Nonius CAD4 diffractometer equipped with graphite monochromatized Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods and refined by the full-matrix least-squares method on F^2 values using the WINGX[32] and SHELX-97[33] program packages. Experimental details for X-ray data collection and the refinements are summarized in Table S1 in ESI.†



Scheme 1 Preparation of two photoswitchable alkoxy-bridged Re(I) complexes **1** and **2**.

Results and discussion

Syntheses of complexes 1 and 2

Complexes **1** and **2** were synthesized using a one pot self-assembly method by mixing $Re_2(CO)_{10}$ and 4-(1-naphthylvinyl)pyridine in 1-butanol (for **1**) or 1-decanol (for **2**) in a Teflon flask kept in a steel bomb, which was heated at 160 °C for 48 h (Scheme 1).

Upon cooling to room temperature, light-yellow colored crystals of **1** and yellow colored crystals of **2** were obtained. The complexes were characterized by IR, ^{1}H NMR, FAB-MS, and elemental analysis. The IR spectra of complexes **1** and **2** showed bands in the region 2015–1885 cm^{-1} corresponding to the *facial* orientation of the carbonyl groups. Four bands are obtained in this region because of D_{2h} symmetry. The ^{1}H NMR spectra of **1** and **2** showed two sets of peaks corresponding to aromatic rings and aliphatic chains. The peaks in the up-field region are assigned to the aliphatic chains. The peak near 4.3 ppm is assigned to the proton attached to the alkoxy carbon. The peaks in the down-field region are due to naphthyl ring protons and vinyl protons. The FAB-MS spectra of **1** and **2** showed molecular ion (M^+) peaks at m/z 1148.23 and m/z 1318.41, respectively. The purity of the complexes was confirmed by elemental analysis and the results obtained were satisfactory. The structure of **1** was further confirmed by single-crystal X-ray diffraction analysis (Fig. 1).

The crystal structure showed that the two rhenium(I) centers adopt a distorted octahedral structure with a NC_3O_2 coordination environment. The rhenium(I) centers with a *facial* arrangement of three carbonyl groups are bridged through alkoxy ligands and the distance between the two metal centers is 3.38 Å, indicative of the presence of intramolecular Re···Re interactions. The 1,4-NVP ligand is coordinated to each rhenium atom by a pyridine nitrogen and lies vertical to the plane connecting the two rhenium atoms. The two face-to-face pyridyl ligands of 1,4-NVP in **1** exhibit weak π–π-stacking interactions (centroid···centroid distance is 3.5 Å), which confer a significant stabilizing effect on the structure of **1**. Furthermore, the *trans* conformation of the 1,4-NVP ligand in **1** is confirmed. The crystal packing diagram showed the presence of weak intra- and inter-molecular π–π stacking interactions (~3.6 Å) between the pyridyl rings of **1** and the pyridyl and naphthyl moieties of adjacent molecules, respectively (Fig. 2).

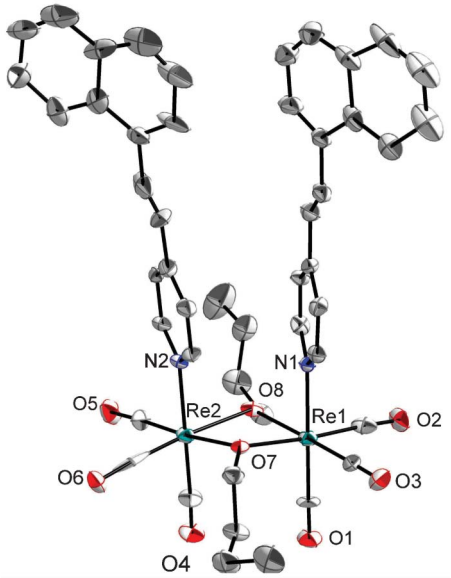


Fig. 1 Perspective drawing of **1** with the atomic numbering scheme. Thermal ellipsoids are shown at the 30% probability level.

Electronic absorption and emission spectra

The electronic absorption spectra of **1** and **2** in dichloromethane at 298 K showed intense bands in the high energy region (200–300 nm) and a low energy band at 355 nm, which

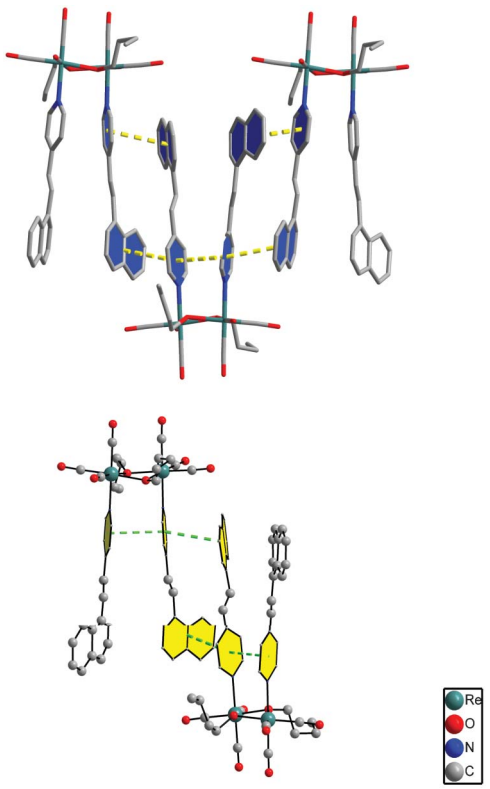


Fig. 2 Crystal packing diagram of **1**. Hydrogen atoms are omitted for clarity.

Table 1 Absorption and emission spectral data, lifetime and quantum yield for Re(I) binuclear complexes **1**, **2** and 1,4-NVP ligand at 298 K

Compounds	λ^{abs}_{max}, nm ($\varepsilon \times 10^4$ dm^3 mol^{-1} cm^{-1})	λ^{em}_{max}, nm	τ, ns	Φ_{em}
1	235 (5.8), 261 (3.7), 355 (3.5)	420	0.67	6.0×10^{-4}
2	232 (3.9), 262 (2.2), 355 (2.2)	420	0.76	7.0×10^{-4}
1,4-NVP	231 (2.5), 328 (1.4)	410	<1	7.8×10^{-1}

are ascribed to a ligand centered and a dπ(Re) $\rightarrow$ π^*(1,4-NVP) metal-to-ligand charge transfer (^{1}MLCT) transition, respectively (Table 1 and Fig. S1, ESI†). It is noteworthy that the molar absorption coefficient (ε) of the MLCT band of **1** and **2** was found to be in the order of 10^4 dm^3 mol^{-1} cm^{-1}, which is higher than the commonly observed value 10^3 dm^3 mol^{-1} cm^{-1} for the Re(I)-diimine ^{1}MLCT transition.[34]

This large ε value observed here is due to the overlap of ^{1}MLCT and the ligand centered transition of the 1,4-NVP ligand. The free 1,4-NVP ligand shows an absorption maximum at 330 nm (see Fig. S1, ESI†). Upon excitation at 355 nm, complexes **1** and **2** exhibited a weak emission at 420 nm. The emission intensity is quenched in complexes **1** and **2**, due to an efficient energy transfer quenching pathway from the ^{3}MLCT state to the ^{3}IL state of the 1,4-NVP moiety. In addition, the low quantum yields and short lifetimes of **1** and **2** are assigned to the presence of a low-lying $\pi \rightarrow \pi^*$ transition localized on the 1,4-NVP ligand.

On irradiation, complex **1** produces clean UV-vis spectral changes with two isosbestic points at 305 and 405 nm and red-shifts the ^{1}MLCT band by 30 nm, from 355 nm to 385 nm (see Fig. S3, ESI†). The irradiation of **2** in dichloromethane at 365 nm results in spectral changes with two clean and well-defined isosbestic points at 302 and 404 nm, and λ_{max} value of the ^{1}MLCT is red shifted by approximately 20 nm from 355 nm (Fig. 3). These spectral changes are attributed to the *trans-cis* isomerization of the –CH=CH-moiety.[34a] The previous results observed in our laboratory show that Re(I)-containing molecular rectangles with long alkyl chains are not soluble in water but are soluble in organic solvents and are weakly emissive.[14,35]

In the present study also we observed that complexes **1** and **2** are not soluble in water and are weakly emissive. When solutions of **1** and **2** are irradiated at 365 nm a dramatic color change from colorless to yellowish green can be observed by the naked eye (Fig. S4, ESI†). Interestingly, although the emission intensity of **1** and **2** is weak, it was greatly enhanced after a steady-state photolysis for 1 h and the emission maximum is red shifted by *ca.* 42 nm from 420 to 462 nm (see Fig. 4 and Fig. S5, S6 in the ESI†).

The increase in emission intensity with red shifted emission maximum and structureless emission features suggest excimer formation of naphthalene moiety in 1,4-NVP ligand. The excimer formation could be induced by *trans-cis* photoisomerization of 1,4-NVP ligand. It is clear from several other reports[36] that naphthalene excimer exhibits a broad and red shifted emission feature at *ca.* 400 nm. Further evidence for the formation of naphthalene excimer is obtained from transient absorption spectra (*vide infra*). Since a dilute solution is used for emission studies, it can be inferred that an intramolecular excimer is formed, as the compounds **1** and **2** contain two 1,4-NVP units each. The luminescence quantum yield (Φ) of **1** and **2** were calculated and found that Φ obtained after irradiation is considerably higher than that before irradiation. For compound **2**, Φ after irradiation (7.4×10^{-3}) was found to be *ca.* 10 times more than that before irradiation (7.0×10^{-4}).

Indeed, the luminescent ''switch on'' behavior is preferred to ''switch off'', particularly as a sensor system for biomolecules, because the former provides a high signal output. Due to the heavy atom effect in Re(I) complexes, the triplet pathway becomes more competitive. However, in these systems we

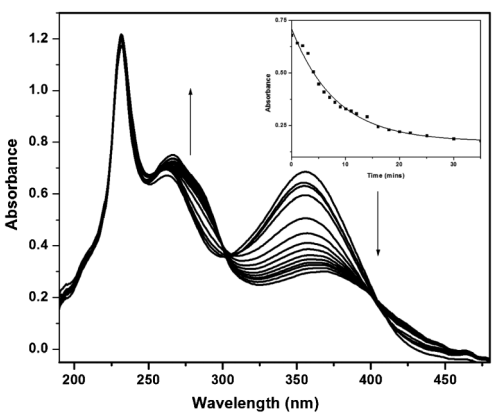


Fig. 3 UV-vis spectral changes of **2** (3×10^{-5} M) in CH_2Cl_2 upon irradiation at 365 nm. (Inset: Time *versus* absorption intensity (365 nm) plot).

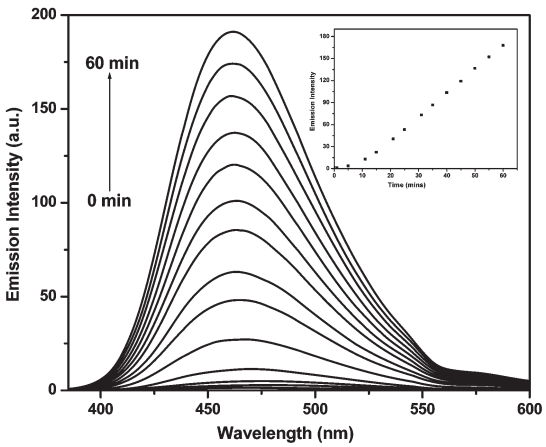


Fig. 4 Enhancement in emission intensity upon 365 nm irradiation of **2** (3×10^{-5} M) in CH_2Cl_2. (Inset: Emission intensity *versus* time plot).

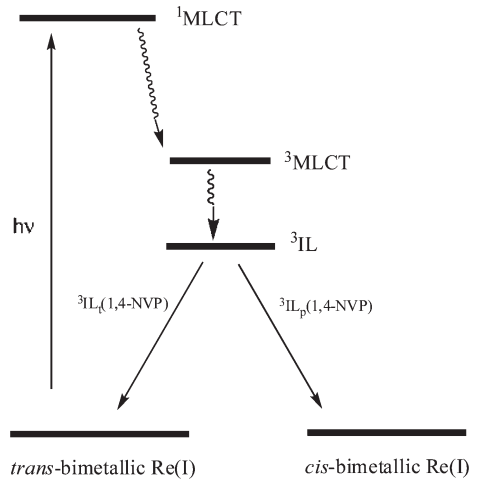


Fig. 5 Proposed scheme of the mechanism of the *trans-cis* photoisomerization of the complexes **1** and **2**.

conclude that the typical triplet mechanism involves the intramolecular energy transfer from the ^{3}MLCT state of Re(I) to the triplet excited state of the *trans* 1,4-NVP ligand, which is responsible for the photoswitchable *trans-cis* isomerization.[7b,8a,8c] When the olefins are in a *trans* conformation, the complexes are weakly emissive or nonemissive due to the presence of lowest nonemissive $^3\pi$–π^* excited states. Excitation into the ^{1}MLCT excited state sensitizes the 1,4-NVP ligand, and that could lead to the *trans-cis* isomerization of the ligand.[5b,11b,37] The presence of rhenium(I) facilitates $^1\pi$ π^*–$^3\pi$ π^* intersystem crossing, which is followed very swiftly by an intramolecular charge transfer from the Re(I) chromophore to the triplet excited state of the *trans* isomer of the 1,4-NVP ligand. The proposed scheme is depicted in Fig. 5. This mechanism is based on a previous report by Vlček and co-workers[8a] and further supported by quantum mechanical calculations reported by Bossert and Daniel.[38]

The transient absorption spectrum of complex **2** in dichloromethane at room temperature is shown in Fig. 6. The transient absorption spectrum recorded before irradiation shows an absorption maximum at 380 nm, which can be due to triplet–triplet absorption of naphthalene monomer.[39] When the transient spectrum is recorded after irradiation at 365 nm, a broad absorption is seen in the region 600–780 nm, in addition to absorption at 410–490 nm. Though the high energy band can be due to naphthalene monomer, the broad absorption in the low energy region can be attributed to triplet excimer formation of the naphthalene moiety in the 1,4-NVP ligand.[39]

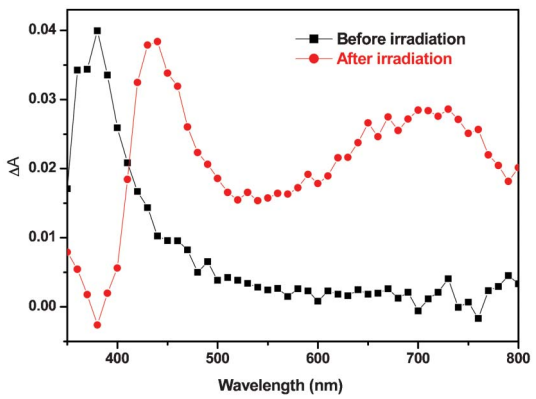


Fig. 6 Transient (difference) absorption spectra obtained by 355 nm laser flash photolysis excitation before and after irradiation of **2** in degassed CH_2Cl_2.

Here, the naphthalene excimer formation could be induced by *trans-cis* isomerization of 1,4-NVP ligand in **2**. Time resolved studies reveal that the decay monitored before the irradiation of compound **2** shows a biexponential behavior with a lifetime of less than 1 ns. After irradiation, complex **2** shows a new species with a biexponential behavior of short lifetime of 3.18 ns and a longer lifetime of 10 ns. This indicates that the lifetime of the *cis* isomer in the excited state is longer than that for the *trans* isomer (Fig. S7 in ESI†). ^{1}H NMR spectroscopic studies provide further evidence for the photoinduced *trans-cis* isomerization of the 1,4-NVP ligand in complexes **1** and **2**, in that there are obvious changes in the chemical shift values and coupling constants of the olefin protons in the *trans* and *cis* forms. The ^{1}H NMR spectral changes observed for the 365 nm irradiation of **2** are shown in Fig. S8 in the ESI.† Upon irradiating **2** at 365 nm, the intensity of signals corresponding to the *trans*-isomer decreased, while those for the *cis*-isomer increased, due to the isomerization of the coordinated 1,4-NVP ligand until a photostationary state is reached. The proton signals of olefin protons, H^c and H^d, appeared at 7.07 and 8.10 ppm, respectively with a coupling constant of 16 Hz, are assigned to the *trans* form. However, after irradiation the signals of $H^{c'}$ and $H^{d'}$ protons are shifted up-field to δ 6.80 and 7.30 ppm, respectively with a coupling constant of 12 Hz indicating a *trans-cis* conversion. These results corroborate the previous reports[10a,11a,b,40] and the ^{1}H NMR spectral study clearly showed the photoisomerization of 1,4-NVP in the alkoxy bridged rhenium complexes.

As a probe for biomolecules

The detection of biomolecules by luminescent probes is a powerful analytical technique for studying biological events.[41] The interesting photoswitching properties of **1** and **2** can be used as an advantage to develop them as bioprobes for protein and DNA (aptamer) detection. Interestingly, with the addition of BSA the luminescence intensity of **2** increased by 15 fold and the λ_{em} was blue shifted from 441 to 421 nm (Fig. 7).

In addition, we titrated complex **2** with several proteins and calculated the binding constant using the Benesi-Hildebrand eqn (1).[42]

$$I_o/(I - I_o) = b/(a - b)\,[1/K_b[M] + 1] \quad (1)$$

where I_o and I are the luminescence intensity of the Re(I) complex in the absence and in the presence of BSA/aptamer, [M] is the concentration of BSA or aptamer, K_b is the binding constant and a and b are constants. The intercept of the graph gives $b/(a - b)$ and the slope is $b/K_b\,(a - b)$. The values of slope and intercept are obtained by plotting $I_o/I - I_o$ against the inverse of the concentration term, M^{-1}. Hence, K_b can then be

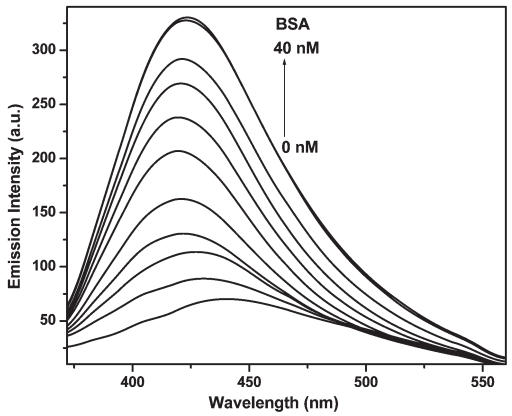


Fig. 7 Emission spectral changes of **2** (20 μM) with the addition of BSA (0 to 40 nM) in PBS buffer (pH 7.4).

calculated from the values of slope and intercept. Among the several proteins used, the change with emission intensity was a maximum with BSA compared to the others and the binding constant was determined to be 3×10^7 M^{-1} (Fig. S9 in ESI†). This value is quite high compared to other rhenium complexes reported by us. BSA is of more hydrophobic nature than other proteins used in the present study.[43] Hence, compound **2** could be a promising candidate as luminescent turn-on biological probe for BSA detection with a high selectivity and sensitivity. The detection limit of BSA is found to be 2.1 nM (Fig. S10 in ESI†). The enormous increase in emission intensity may be attributed to the strong binding of Re(I) complex with a protein through hydrophobic interactions and the rigidity of their local surroundings upon binding to the protein. This high binding constant confirms the strong interaction between Re(I) complexes with BSA. These complexes do not emit luminescence in aqueous solution upon the excitation of MLCT band, but do emit luminescence in the presence of BSA and thus serve as ''light switch'' complex for biomolecules. This kind of ''light switch'' behavior was observed with ruthenium(II)-dipyridophenazine complexes upon binding with DNA.[44]

Apart from their role as luminescent probe for BSA, both complexes **1** and **2** also serve as bioprobes for the sensitive detection of the PDGF aptamer at PBS buffer (pH 7.4). It should be noted that complexes **1** and **2** are weakly luminescent in PBS buffer, with an emission maximum at 442 nm. The emission intensity is increased gradually when the concentration of aptamer is increased from 0 to 0.3 pM (Fig. S11 and S12 in the ESI†). It is noteworthy that a remarkable emission enhancement was observed when the rhenium complexes were titrated with the PDGF aptamer. A 12-fold increase in the emission intensity was found for complexes **1** and **2** while the concentration of aptamer reached 0.3 pM. The light switching behavior of **1** and **2**, on mixing with the aptamer, is due to the intercalation of the planar aromatic ring of **1** and **2** between the base pairs in the DNA aptamer.[45] It is well known that a DNA aptamer can form a duplex structure in the presence of metal ions that facilitate strong binding. The binding constant was calculated using eqn (1) and the value for the PDGF aptamer was determined to be 6×10^9 M^{-1}. This represents a high binding constant value compared to other rhenium systems.[27,28b,46] It is proposed that the probe molecules are efficiently protected by the aptamer since the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the probes, and the mobility of the probe is restricted at the binding site, leading to a decrease in the vibrational modes of relaxation.[47] These results show that the complexes **1** and **2** can interact strongly with an aptamer DNA molecule. Thus, these binuclear complexes show promise as luminescent probes in aptamer based sensor technology and studies in this area are in progress.

In vitro cell viability effects of 1,4-NVP ligand and rhenium(I) complex 2

The utility of rhenium(I) complexes as luminophores for applications in living cells has recently been recognized.[30] Interest in these alkoxy-bridged binuclear Re(I) complexes **1** and **2** results from the fact that they display enhanced fluorescence intensity in the presence of biomolecules (Fig. 7, and Fig. S10–S11 in the ESI†) to provide possible visible wavelength excitation for imaging applications in target cancer cells through confocal fluorescence microscopy. As part of a program aimed at the development of living cell imaging, the cytotoxicity of the 1,4-NVP ligand and the Re(I) complex **2** on human breast cancer cells (MDA-MB-231) and breast epithelium cells (M10) was evaluated using MTT assays over a period of 4 and 24 h, respectively (Fig. 8 and Fig. S13 in ESI†).

No noticeable effects were observed when MDA-MB-231 cells were incubated with the 1,4-NVP ligand or the Re(I) complex **2** even at the highest concentrations (20 μM). In contrast, 1,4-NVP ligand or Re(I) complex **2** showed a slight cytotoxicity toward breast epithelium cells, M10, after only 24 h treatment at the highest concentration (20 μM). Therefore, the Re(I) complex **2** showed minimal toxicity in MDA-MB-231 cells within the tested concentration range, suggesting that Re(I) complexes are biocompatible fluorophores and could be used in conjunction with live cell imaging studies.

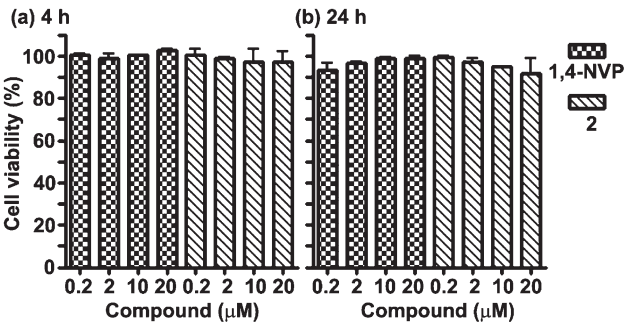


Fig. 8 *In vitro* cell viability effects of 1,4-NVP and rhenium(I) complex **2** in inhibiting the growth of MDA-MB-231 cells at 4 and 24 h.

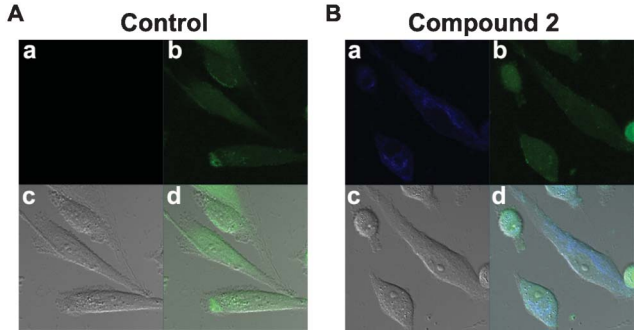


Fig. 9 Cellular localization and uptake of **2** in MDA-MB-231 cells. Images were acquired by fluorescence confocal microscopy using a 40× oil-immersion objective lens. (A) MDA-MB-231 cells were untreated (a) or incubated for 30 min with CFSE (b) at a final CFSE concentration of 2 μM. Bright-field (c) and fluorescence overlay (d) images of MDA-MB-231 cells are shown in the bottom panel. (B) MDA-MB-231 cells were treated for 30 min with complex **2** (a) or incubated for 30 min with CFSE (b) at a final concentration of 20 or 2 μM, respectively. Bright-field (c) and fluorescence overlay (d) images of MDA-MB-231 cells are shown in the bottom panel. Excitation wavelengths (nm) used for compound **2** and CFSE were 760 and 488 nm, respectively. The detection (emission) wavelengths used for imaging were 435–485 nm for complex **2** and 525 nm for CFSE.

Cellular uptake

To gain insights into the molecular mode of action involved in live cell imaging, the effect of complex **2** on cellular uptake was investigated using confocal laser scanning microscopy (Fig. 9). In control experiments, the bright green fluorescence (Fig. 9A) indicated that the MDA-MB-231 cells were stained by carboxyfluorescein diacetate succinimidyl ester (CFSE), a relatively non-toxic cytosolic marker. Notably, the analysis of breast cancer cells implied that a strong intracellular fluorescence signal (blue) from **2** was effectively observed (Fig. 9B), indicating that it was internalized by the cells. From these images, it appeared that complex **2** was homogeneously localized in the cytoplasm, which was confirmed by the overlay images of CFSE and complex **2** (Fig. 9B).

The significance of this work is found in the discovery of excitation wavelengths (nm) of 760 nm for complex **2** but only 488 nm for CFSE. The former wavelength was utilized through a two-photon excitation (TPE) by a 760-nm femtosecond laser, whereas the latter wavelength is excited through a single-photon excitation (SPE) by a 488-nm laser. TPE with an infrared laser rather than SPE is known to have the best cell/tissue penetration ability, leading to less collateral damage to normal cell/tissue. It is also noteworthy that, when highly metastatic MDA-MB-231 cells were treated with complex **2**, they displayed typical elongated and spindle shapes (mesenchymal morphology), suggesting that rhenium(I) complexes with low cytotoxicity could be a useful probe in optical imaging studies in cancer cells and clinical applications.

Conclusions

In summary, two alkoxy-bridged binuclear rhenium complexes having a photoactive 1,4-NVP unit were efficiently synthesized by a one-step self-assembly process. The light induced *trans-cis* photoisomerization behavior of the 1,4-NVP ligand coordinated to rhenium(I) is clearly demonstrated in this study. Intramolecular energy transfer takes place from Re(I) chromophore to the excited state of the 4-(1-naphthylvinyl)pyridine ligand and the Re(I) moiety acts as an intramolecular sensitizer. Furthermore, these complexes could be promising candidates for use as luminescent turn-on biological probes for proteins and PDGF aptamer (DNA) detection with high selectivity and sensitivity. In addition, these rhenium(I) complexes, with good biocompatibility and cellular uptake properties, could, perhaps, serve as an efficient optical image system in biomedical applications. This is an interesting case in which a bimetallic rhenium complex functions as a photoswitch, an ultrasensitive sensor for BSA, aptamer and a cell imaging probe.

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