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Rhenium(I) polypyridine complexes functionalized with a diaminoaromatic moiety as phosphorescent sensors for nitric oxide†

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A series of rhenium(I) polypyridine complexes functionalized with a diaminoaromatic moiety has been developed as phosphorescent sensors for nitric oxide (NO). The diamine complexes $[Re(N^N)(CO)_3(py\text{-}diamine)](CF_3SO_3)$ [py-diamine = 3,4-diaminopyridine; N^N = 1,10-phenanthroline (phen) (**1a**), 2,9-dimethyl-1,10-phenanthroline (Me_2-phen) (**2a**), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen) (**3a**), 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen) (**4a**)] were synthesized and characterized. These complexes were only weakly emissive due to the diaminoaromatic moiety that quenches the ^{3}MLCT [$d\pi(Re) \rightarrow \pi^*(N^N)$] emission by photoinduced electron transfer (PET). However, in the presence of NO, these diamine complexes were converted to the triazole derivatives $[Re(N^N)(CO)_3(py\text{-}triazole)](CF_3SO_3)$ [py-triazole = 1*H*-1,2,3-triazolo[4,5,*c*]pyridine; N^N = phen (**1b**), Me_2-phen (**2b**), Me_4-phen (**3b**), Ph_2-phen (**4b**)], which revealed intense emission upon excitation. The emission of the complexes was independent to pH under neutral and basic conditions (pH > 6). The cytotoxicity and cellular uptake properties of these complexes were studied by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay and ICP-MS, respectively. The potential application of these complexes as intracellular NO sensors was also investigated.

Introduction

Nitric oxide (NO) is an important free radical that is involved in a variety of biological processes such as neurotransmission, muscle relaxation and blood pressure and immune response regulation.[1] It is endogenously produced in different mammalian cells by nitric oxide synthase (NOS) which catalyzes the conversion of L-arginine to NO and L-citrulline.[2] Three isoforms of NOS, namely neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS), are present in biological systems. High intracellular concentrations of NO, which triggers the formation of reactive nitrogen species (RNS), have been implicated in carcinogenesis and several neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and Huntington's disease.[3] Since NO has a short physiological lifetime ($t_{1/2}$ = *ca.* 5 s) and rapidly diffuses through most cells and tissues,[1a,4] it is a challenging task to monitor the formation and migration of NO in biological systems. Among different detection methods, fluorescence detection allows imaging of intra- and intercellular NO with high spatiotemporal resolution.[5] Thus, it is an ideal method to study the production, diffusion processes and biological roles of NO.

In the past decade, two distinct groups of fluorescent NO sensors have emerged.[6] The first involves the use of a paramagnetic metal ion, such as Cu^{2+}, as a quencher to suppress the emission of a fluorophore.[7] In the presence of NO, the metal ion is reduced and displaced from the fluorophore, resulting in emission enhancement. The second group exploits an electron-rich diaminoaromatic moiety as both a recognition unit for NO and a quencher for the emission of fluorophores through photoinduced electron-transfer (PET).[5a] The diaminoaromatic moiety has been covalently linked to different organic fluorophores such as fluorescein,[8] rhodamine,[9] BODIPY[10] and cyanine[11] to yield interesting NO sensors with various optical properties. Despite the development of these reagents, the possibility of using phosphorescent transition metal diamine

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complexes as intracellular NO sensors is scarce.[12] The advantages of using phosphorescent transition metal complexes as biological probes include their intense and long-lived emission, large Stokes shifts and high photostability.[13] In the past few years, there has been a fast-growing interest in the cellular uptake properties of phosphorescent rhenium complexes with a focus on their potential as cellular imaging and therapeutic reagents.[14–19] With our ongoing interest in phosphorescent rhenium(I) polypyridine complexes as biomolecular and cellular probes,[19] we envisage that the modification of these complexes with a diaminoaromatic moiety will generate a new class of phosphorogenic sensors for NO.

Herein, we report the synthesis, characterization and electrochemical and photophysical properties of four phosphorescent rhenium(I) polypyridine diamine complexes $[Re(N^N)(CO)_3(py\text{-}diamine)](CF_3SO_3)$ [py-diamine = 3,4-diaminopyridine; N^N = 1,10-phenanthroline (phen) (**1a**), 2,9-dimethyl-1,10-phenanthroline (Me_2-phen) (**2a**), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen) (**3a**), 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen) (**4a**)] and their triazole counterparts $[Re(N^N)(CO)_3(py\text{-}triazole)](CF_3SO_3)$ [py-triazole = 1*H*-1,2,3-triazolo[4,5,*c*]pyridine; N^N = phen (**1b**), Me_2-phen (**2b**), Me_4-phen (**3b**), Ph_2-phen (**4b**)] (Chart 1). The effects of pH on the emission intensity of the Ph_2-phen complexes **4a** and **4b** were examined. The NO-sensing properties of the diamine complex **4a** were also investigated. The cytotoxicity and cellular uptake properties of the diamine complexes were studied by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay and ICP-MS, respectively. The intracellular NO-sensing properties of complex **4a** were also investigated.

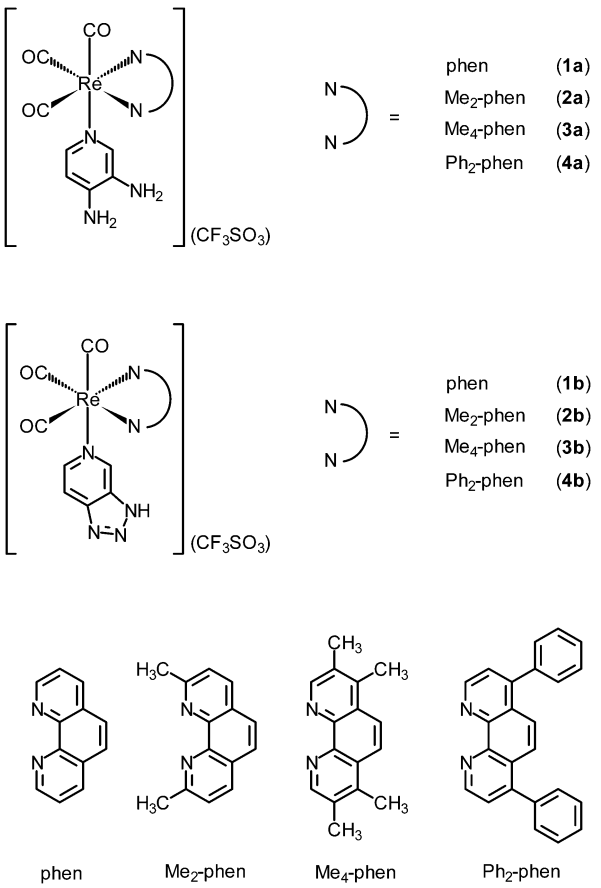

Chart 1 Structures of the rhenium(I) polypyridine diamine complexes **1a–4a** and the triazole analogues **1b–4b**.

Results and discussion

Synthesis of complexes

The diamine complexes **1a–4a** were obtained from the reaction of $[Re(N^N)(CO)_3(CH_3CN)](CF_3SO_3)$ with py-diamine in refluxing THF, followed by column chromatographic purification and recrystallization from CH_2Cl_2/diethyl ether. The triazole complexes **1b–4b** were prepared by bubbling purified NO gas into a solution of the diamine complexes **1a–4a** in CH_2Cl_2 and subsequently purified by recrystallization from CH_2Cl_2/diethyl ether. All the complexes were stable in air and soluble in most organic solvents such as CH_2Cl_2, CH_3CN and alcohol. They were characterized by ^1H NMR, positive-ion ESI-MS and IR spectroscopy and gave satisfactory microanalysis.

Electrochemical properties

The electrochemical properties of the complexes were studied by cyclic voltammetry and the electrochemical data are summarized in Table 1. The diamine complexes displayed two irreversible waves at +0.94 to +1.14 V and +1.16 to +1.51 V *vs.* SCE. Since these features were absent in the triazole complexes **1b–4b**, we assigned them to the oxidation of the diaminoaromatic ligand py-diamine. With reference to the previous electrochemical studies of related rhenium(I) polypyridine complexes,[20–22] the reversible or quasi-reversible couples occurred at +1.57 to +1.76 V were ascribed to the rhenium(II/I) oxidation. It is noteworthy that the rhenium(II/I) oxidation couple of the diamine complexes generally occurred at less positive potentials (+1.57 to +1.63 V) compared with their triazole counterparts (+1.62 to +1.76 V), which is probably due to the strong electron-donating effects of the diaminoaromatic ligand. The first reduction couples and waves at −1.17 to −1.48 V were assigned to the reduction of the diimine ligands. This argument is supported by the observation that the potential of the first reduction wave of the rhenium complexes followed the order, Ph_2-phen ≈ phen > Me_2-phen > Me_4-phen,

Table 1 Electrochemical data of the rhenium(I) polypyridine complexes[a]

Complex	Oxidation, $E_{1/2}$ or E_a/V	Reduction, $E_{1/2}$ or E_c/V
1a	+0.94,[b] +1.23,[b] +1.63[b]	−1.19, −1.60,[b] −1.88,[b] −2.10[c]
2a	+1.14,[b] +1.51,[b] +1.62[b]	−1.27,[b] −1.65,[b] −1.93[c]
3a	+1.12,[b] +1.45,[b] +1.57[b]	−1.46, −1.66,[b] −1.79,[b] −1.94[b]
4a	+1.01,[b] +1.16,[b] +1.61[b]	−1.17, −1.44, −1.65,[c] −2.03[b]
1b	+1.76[b]	−1.19,[b] −1.63,[c] −1.91,[c] −2.27[c]
2b	+1.62[b]	−1.43,[c] −1.62,[c] −1.91[c]
3b	+1.67[b]	−1.48,[c] −1.70,[b] −1.95,[b] −2.23[b]
4b	+1.74[b]	−1.17,[b] −1.43,[b] −1.69,[c] −2.02[b]

[a] In CH_3CN (0.1 mol dm^{-3} TBAP) at 298 K, glassy carbon electrode, sweep rate 100 mV s^{-1}, all potentials *versus* SCE. [b] Irreversible waves. [c] Quasi-reversible couples.

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which is in accordance with the stability of the π* orbitals of the diimine ligands.

Electronic absorption and emission properties

The electronic absorption properties of all the complexes were studied and the data are summarized in Table S1 (ESI†). In general, both the diamine and triazole complexes displayed similar electronic absorption spectra, indicating that the conversion of the diamine complexes to their triazole counterparts did not significantly affect the electronic absorption properties. Similar to other rhenium(I) polypyridine complexes,[19–27] all the complexes displayed intense absorption bands at *ca.* 251–342 nm and weaker absorption shoulders at *ca.* 370–396 nm, which were assigned to spin-allowed intraligand (^{1}IL) (π→π*) (N^N and pyridine ligands) and spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) [dπ(Re) → π*(N^N)] transitions, respectively.

Upon irradiation, all the complexes exhibited long-lived ^{3}MLCT [dπ(Re) → π*(N^N)] emission. The photophysical data are summarized in Table 2 and the emission spectra of the triazole complexes **1b–4b** in CH_3CN at 298 K are shown in Fig. 1. The structural features and long emission lifetimes of the Me_4-phen complexes **2a** and **2b** in alcohol glass at 77 K suggest the involvement of ^{3}IL (π → π*) (Me_4-phen) character in their emissive states. It is noteworthy that the diamine complexes showed significantly lower emission quantum yields and shorter emission lifetimes compared with their triazole counterparts (Table 2), which is most likely a consequence of

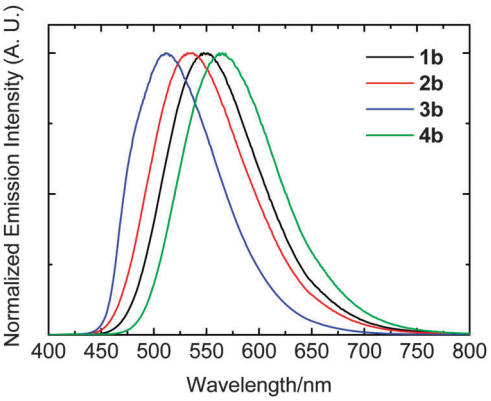

Fig. 1 Emission spectra of the rhenium(I) triazole complexes in CH_3CN at 298 K.

Table 2 Photophysical data of the rhenium(I) polypyridine complexes[a]

Complex	Medium (*T*/K)	λ_{em} (nm)	τ_o (μs)	Φ_{em}
1a[b]	CH_2Cl_2 (298)	556	0.18	0.010
	CH_3CN (298)	554	0.15	0.00081
	Glass[c] (77)	513	10.85	
2a[b]	CH_2Cl_2 (298)	527	2.32	0.036
	CH_3CN (298)	528	1.58	0.00096
	Glass[c] (77)	490	11.31	
3a[b]	CH_2Cl_2 (298)	522	0.73	0.045
	CH_3CN (298)	521	0.21	0.00038
	Glass[c] (77)	470 (max), 501, 534 sh	29.02 (76%), 90.32 (24%)	
4a[b]	CH_2Cl_2 (298)	561	2.73	0.028
	CH_3CN (298)	564	1.70	0.0023
	Glass[c] (77)	525, 556 sh	17.07	
1b	CH_2Cl_2 (298)	533	2.95	0.28
	CH_3CN (298)	549	1.34	0.097
	Buffer[d] (298)	550	0.49	0.0053
	Glass[c] (77)	497	11.03	
2b	CH_2Cl_2 (298)	518	3.83	0.37
	CH_3CN (298)	537	1.54	0.072
	Buffer[d] (298)	538	0.52	0.050
	Glass[c] (77)	493	16.55	
3b	CH_2Cl_2 (298)	515	10.01	0.62
	CH_3CN (298)	514	10.89	0.48
	Buffer[e] (298)	518	8.00	0.42
	Glass[c] (77)	466 (max), 499, 536 sh	34.19 (64%), 112.29 (36%)	
4b	CH_2Cl_2 (298)	545	8.57	0.38
	CH_3CN (298)	565	4.90	0.068
	Buffer[d] (298)	565	1.22	0.021
	Glass[c] (77)	511, 536 sh	22.85	

[a] The concentrations of the complexes were adjusted so that the absorbance at the excitation wavelength (355 nm) was equal to 0.1. [b] The emission quantum yields of the diamine complexes **1a–4a** in aqueous buffer were too low for accurate determination. [c] EtOH/MeOH (4 : 1 v/v). [d] 50 mM potassium phosphate buffer pH 7.4/MeOH (9 : 1 v/v). [e] 50 mM potassium phosphate buffer pH 7.4/MeOH (7 : 3 v/v).

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emission quenching due to the presence of the diaminoaromatic moiety. From the potentials of the diimine-based reduction of the diamine complexes **1a–4a** (−1.17 to −1.46 V, Table 1) and their low-temperature emission energy (E^{00} = 2.36 to 2.64 eV, Table 2), the excited-state reduction potentials ($E^{o}[Re^{+*/0}]$) of the rhenium complexes were calculated to be *ca.* +1.18 to +1.26 V *vs.* SCE. On the basis of these potentials and the first oxidation potential of the diamine complexes (+0.94 to +1.14 V), reductive quenching of the excited complexes is favored by *ca.* 0.06 to 0.29 eV. This indicates that the emission quenching mechanism of the diamine complexes is most likely electron transfer in nature.

pH dependent emission studies

The effects of pH on the emission intensity of the diamine complex **4a** and its triazole counterpart complex **4b** were investigated and the results are shown in Fig. 2. The emission intensity of both complexes was relatively independent to pH under neutral and basic conditions (pH > 6). Upon decreasing the pH of the solution, the diamine complex displayed emission enhancement, which was ascribed to the suppression of PET quenching due to the protonation of the diaminoaromatic moiety. Although the pK_a value could not be determined (Fig. 2), it should be much smaller than that of 1,2-diaminobenzene (pK_a = 4.52).[28] This is reasonable because of the reduced electron density on the amine nitrogen atoms due to the electron-withdrawing effects of the rhenium(I) center. Interestingly, similar to the diamine complex, the triazole analogue complex **4b** also displayed pH-dependent emission (Fig. 2). However, the effect of pH on the emission intensity is less significant compared with that of the diamine complex ($I_{pH=2.5}$: $I_{pH=12}$ = 13 and 1.4 for complexes **4a** and **4b**, respectively). The change of emission intensity in the acidic region fits an apparent pK_a value of 4.73 ± 0.05 for complex **4b**. We tentatively assigned the weaker emission in the alkaline region to the deprotonation of the triazole moiety, yielding the triazolate form, which suppresses the emission of the complex. It is noteworthy that the pK_a value is lower than that of benzotriazole (pK_a = 6.7),[9] which is not unexpected since the triazolate moiety is stabilized by the electron-withdrawing pyridine ligand and the positively charged rhenium(I) center. Interestingly, a blue shift of the emission maxima was observed as the pH was decreased (Fig. 2), which originates from the weaker electron-donating effects of the triazole ligand and hence more stabilized dπ(Re) orbitals.

NO-sensing properties

The NO-sensing ability of the diamine complexes was investigated by luminescence titrations using 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine (NOC-7) as a titrant. NOC-7 is a stable NO-amine compound that spontaneously releases two equivalents of NO under physiological conditions ($t_{1/2}$ = 10 min, PBS buffer at pH 7.4).[29] Upon addition of NOC-7 to a solution of the diamine complex, significant emission enhancement (I/I_o = 31) was observed (Fig. 3). The emission enhancement was attributed to the conversion of the diamine complex to its triazole counterpart, which was subsequently confirmed by ESI-MS. The large emission enhancement factor and low emission quantum yields of the diamine complexes render them potential phosphorogenic sensors for NO. The diamine complex **4a** showed good signal linearity with NOC-7 concentrations ranging from 0 to 50 μM (R^2 = 0.99386). The detection limit was determined to be *ca.* 300 nM, which is comparable to a ruthenium(II)-based NO sensor.[12] It is

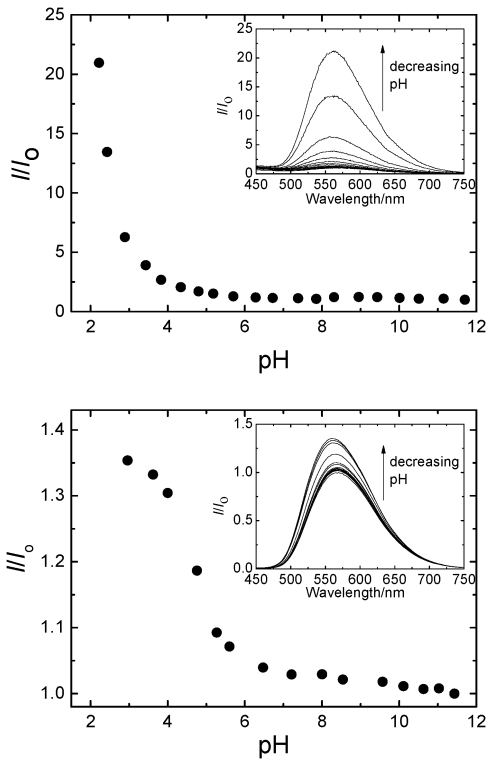

Fig. 2 pH titration curves for complexes **4a** (top) and **4b** (bottom) (10 μM) in aerated 100 mM KCl (aq)/MeOH (9 : 1 v/v) at 298 K. The emission intensity of complexes **4a** and **4b** was monitored at 568 and 560 nm, respectively. The inset shows the emission spectral traces of the complexes upon decreasing pH.

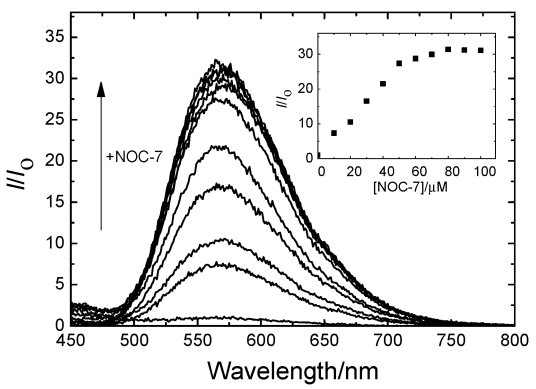

Fig. 3 Emission spectral traces of complex **4a** (5 μM) in aerated potassium phosphate buffer (50 mM, pH 7.4)/MeOH (9 : 1 v/v) at 298 K in the presence of 0 to 100 μM NOC-7. The inset shows the emission titration curve of complex **4a** with NOC-7. The emission intensity of complex **4a** was monitored at 568 nm.

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noteworthy that the end point for the titration of the diamine complex **4a** with NO was not 1 : 1 (Fig. 3, inset), which is commonly observed in other diaminoaromatic compounds.[5,6,12] This suggests that a complicated reaction mechanism may be involved in the formation of the triazole complex. In fact, diaminoaromatic compounds are known to react with the oxidized products of NO such as nitrous anhydride (N_2O_3) instead of NO itself.[5a] Nevertheless, since those oxidized products can only be generated by NO under physiological conditions, diaminoaromatic compounds are still widely employed in the design of fluorescent NO sensors.[5,6]

Biological properties and NO-sensing in living cells

We investigated the possibility of using the diamine complexes **1a–4a** as phosphorescent sensors for intracellular NO. First, the cellular uptake properties of these complexes were examined by ICP-MS. Upon incubation with the complexes ([Re] = 10 μM) at 37 °C for 1 h, an average HeLa cell (volume = 3.4 pL) contained 0.40 to 2.43 fmol of rhenium (Table 3). Due to their more lipophilic diimine ligands, the Ph_2-phen, Me_2-phen and Me_4-phen complexes displayed more effective cellular uptake than the phen complex. Similar results were also observed in related rhenium(I) complexes.[19h,j] It is important to point out that the intracellular rhenium concentrations of all four complexes (0.12 to 0.72 mM) were much higher than that in the medium before uptake (10 μM), indicative of the cellular accumulation of the complexes. The cytotoxicity of the diamine complexes **1a–4a** toward HeLa cells over an incubation period of 48 h was investigated by the MTT assay (Table 3). The Ph_2-phen, Me_2-phen and Me_4-phen complexes exhibited higher cytotoxicity, which was in accordance with their higher uptake efficiency.[19h,j]

The diamine complex **4a** was used to visualize intracellular NO that was exogenously generated by a donor (NOC-7) in HeLa cells. Upon incubation with complex **4a** for 1 h, weak intracellular emission was observed in the cytoplasmic region of the HeLa cells (Fig. 4). Owing to the highly emissive nature of the triazole complexes, the intracellular conversion of the diamine complexes to their triazole derivatives should lead to enhanced intracellular luminescence. However, after the cells were further incubated with NOC-7 (100 μM) for 30 min, the intracellular emission of the HeLa cells displayed no significant difference (Fig. 4). Similar results were also observed upon further increasing the concentration of NOC-7 (500 μM) and incubation time (1 h) (data not shown). Under the same conditions, significant emission enhancement was observed for the HeLa cells which were incubated with a commercially available NO sensor 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM diacetate). Also, we found that the HeLa cells incubated with the triazole complex **4b** displayed strong intracellular luminescence (Fig. 5), despite the less efficient uptake by HeLa cells (1.67 fmol per average cell) than its diamine counterpart (2.43 fmol per average cell). All these observations indicated that complex **4a** could not be converted to the triazole derivative under intracellular conditions. This is probably related to the short intracellular lifetime of NO ($t_{1/2}$ = *ca.* 5 s)[1a,4] and the rather low reactivity of the complex. Nevertheless, the cellular studies showed that the complex was able to penetrate the cellular membrane and accumulate in the HeLa cells. With appropriate modification of the molecular structure, related rhenium(I) diamine complexes are expected to function as phosphorescent sensors for NO in an intracellular environment.

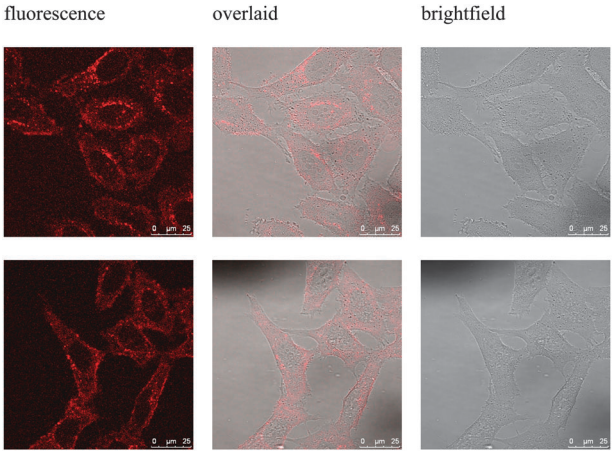

Fig. 4 Laser-scanning confocal microscopy images of HeLa cells incubated with complex **4a** (10 μM) at 37 °C for 1 h. The cells were post-incubated with (top row) and without (bottom row) NOC-7 (100 μM) in PBS at 37 °C for 30 min.

Table 3 Cellular uptake and cytotoxicity (IC_{50}, 48 h) of the rhenium(I) diamine complexes and cisplatin toward the HeLa cell line

Complex	No. of mole[a] (fmol)	Concentration (mM)	IC_{50} (μM)
1a	0.40 ± 0.02	0.12 ± 0.01	51.0 ± 4.6
2a	1.05 ± 0.01	0.31 ± 0.01	17.6 ± 0.5
3a	1.91 ± 0.07	0.56 ± 0.02	6.1 ± 0.3
4a	2.43 ± 0.04	0.72 ± 0.04	2.5 ± 0.1
Cisplatin	N.A.	N.A.	21.6 ± 1.4

[a] Number of moles of rhenium(I) associated with a typical HeLa cell upon incubation with the complexes (10 μM) at 37 °C for 1 h.

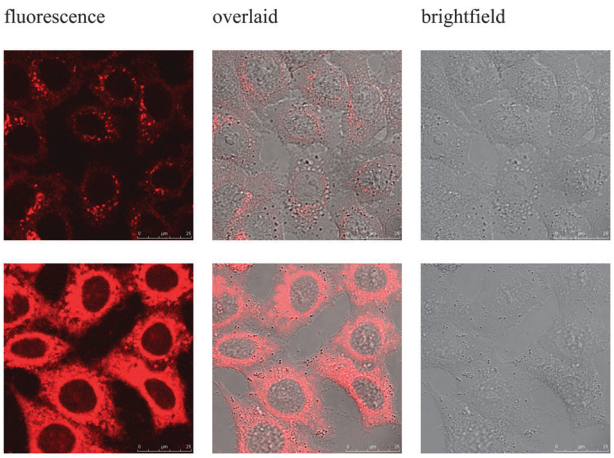

Fig. 5 Laser-scanning confocal microscopy images of HeLa cells incubated with complexes **4a** (top row) and **4b** (bottom row) (10 μM) at 37 °C for 1 h.

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Conclusion

In this work, a new class of NO sensors have been developed from phosphorescent rhenium(I) polypridine complexes. Upon reaction with NO, the weakly emissive diamine complexes were converted into the triazole derivatives, resulting in significant emission enhancement. The emission of the complexes was independent of pH under neutral and basic conditions. These complexes were found to accumulate in HeLa cells with moderate cytotoxicity. The large emission enhancement factors and low emission quantum yields of the diamine complexes cause them to function as phosphorogenic sensors for NO. Since the reactivity of the complexes toward NO is determined by the electron density of the diaminoaromatic moiety,[10] complexes with an attached electron-rich diaminoaromatic ligand are anticipated to function as highly reactive NO sensors. To achieve this, we believe that different electron-donating groups, such as a methoxy moiety can be attached to the diaminoaromatic ligands to increase their electron density. Additionally, the diaminoaromatic moiety should be separated from the electron-withdrawing rhenium(I) metal center through an insulating spacer-arm. The design of related phosphorescent transition metal diamine complexes as intracellular NO sensors is under way.

Experimental

Materials and synthesis

All solvents were of analytical reagent grade and purified according to standard procedures.[30] Diimine ligands including phen, Me_2-phen, Me_4-phen and Ph_2-phen, $AgCF_3SO_3$ and cisplatin were purchased from Acros. $Re(CO)_5Cl$ and py-diamine were obtained from Aldrich. MTT was purchased from Sigma. NOC-7 was obtained from Merck. Purified NO gas was obtained from Scientific Gas Engineering Co., Ltd. All these chemicals were used without further purification. $[Re(N^N)(CO)_3(CH_3CN)](CF_3SO_3)$ was prepared as described previously.[31] Autoclaved Milli-Q water was used for the preparation of aqueous solutions. All buffer components were of biological grade and used as received. HeLa cells were obtained from American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin-EDTA, DAF-FM diacetate and penicillin/streptomycin were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin.

$[Re(N^N)(CO)_3(py\text{-}diamine)](CF_3SO_3)$ [N^N = phen (1a), Me_2-phen (2a), Me_4-phen (3a), Ph_2-phen (4a)]

A mixture of $[Re(N^N)(CO)_3(CH_3CN)](CF_3SO_3)$ (0.30 mmol) and py-diamine (0.30 mmol) in THF (30 mL) was refluxed under an inert atmosphere of nitrogen for 12 h. The mixture was evaporated to dryness to give a yellow solid, which was purified by column chromatography on alumina. The desired product was eluted with a mixture of CH_2Cl_2 and methanol and it was subsequently recrystallized from a mixture of CH_2Cl_2 and diethyl ether. Complex **1a** was isolated as yellow crystals. Yield: 169 mg (80%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 9.81 (d, 2H, J = 5.1 Hz, H2 and H9 of phen), 9.08 (d, 2H, J = 8.4 Hz, H4 and H7 of phen), 8.36–8.30 (m, 4H, H3, H5, H6 and H8 of phen), 7.67 (s, 1H, H2 of pyridine), 7.38 (d, 1H, J = 6.0 Hz, H6 of pyridine), 6.30 (d, 1H, J = 6.0 Hz, H5 of pyridine), 5.79 (br, 2H, NH_2 at C4 of pyridine), 4.44 (br, 2H, NH_2 at C3 of pyridine). IR (KBr) ν/cm^{-1}: 3367 (br, N–H), 2026 (s, C≡O), 1912 (s, C≡O), 1165 (m, $CF_3SO_3^-$), 1030 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 559 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{21}H_{15}N_5O_6SF_3 \cdot H_2O$: C, 34.72; H, 2.36; N, 9.64. Found: C, 34.85; H, 2.69; N, 9.98. Complex **2a** was isolated as yellow crystals. Yield: 188 mg (85%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 8.82 (d, 2H, J = 8.1 Hz, H4 and H7 of Me_2-phen), 8.21 (d, 2H, J = 8.4 Hz, H3 and H8 of Me_2-phen), 8.13 (s, 2H, H5 and H6 of Me_2-phen), 7.16 (s, 1H, H2 of pyridine), 6.89 (d, 1H, J = 6.0 Hz, H6 of pyridine), 6.18 (d, 1H, J = 6.0 Hz, H5 of pyridine), 5.75 (br, 2H, NH_2 at C4 of pyridine), 4.35 (br, 2H, NH_2 at C3 of pyridine), 3.45 (s, 6H, CH_3 of Me_2-phen). IR (KBr) ν/cm^{-1}: 3368 (br, N–H), 2027 (s, C≡O), 1911 (s, C≡O), 1162 (m, $CF_3SO_3^-$), 1028 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 587 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{23}H_{19}N_5O_6SF_3$: C, 37.50; H, 2.60; N, 9.51. Found: C, 37.80; H, 2.85; N, 9.57. Complex **3a** was isolated as yellow crystals. Yield: 205 mg (89%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 9.53 (s, 2H, H2 and H9 of Me_4-phen), 8.42 (s, 2H, H5 and H6 of Me_4-phen), 7.71 (s, 1H, H2 of pyridine), 7.41 (d, 1H, J = 6.3 Hz, H6 of pyridine), 6.30 (d, 1H, J = 6.0 Hz, H5 of pyridine), 5.75 (br, 2H, NH_2 at C4 of pyridine), 4.39 (br, 2H, NH_2 at C3 of pyridine), 2.91 (s, 6H, CH_3 at C4 and C7 of Me_4-phen), 2.78 (s, 6H, CH_3 at C3 and C8 of Me_4-phen). IR (KBr) ν/cm^1: 3369 (br, N–H), 2026 (s, C≡O), 1910 (s, C≡O), 1161 (m, $CF_3SO_3^-$), 1031 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 615 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{25}H_{23}N_5O_6SF_3$: C, 39.27; H, 3.03; N, 9.16. Found: C, 39.34; H, 3.18; N, 9.42. Complex **4a** was isolated as orange crystals. Yield: 188 mg (73%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 9.87 (d, 2H, J = 5.7 Hz, H2 and H9 of Ph_2-phen), 8.27–8.23 (m, 4H, H3, H5, H6 and H8 of Ph_2-phen), 7.80–7.66 (m, 11H, H2 of pyridine and C_6H_5 of Ph_2-phen), 7.49 (d, 1H, J = 6.3 Hz, H6 of pyridine), 6.36 (d, 1H, J = 6.3 Hz, H5 of pyridine), 5.96 (br, 2H, NH_2 at C4 of pyridine), 4.66 (br, 2H, NH_2 at C3 of pyridine). IR (KBr) ν/cm^{-1}: 3372 (br, N–H), 2023 (s, C≡O), 1909 (s, C≡O), 1158 (m, $CF_3SO_3^-$), 1030 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 711 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{33}H_{23}N_5O_6SF_3$: C, 46.05; H, 2.69; N, 8.14. Found: C, 46.00; H, 3.02; N, 8.25.

$[Re(N^N)(CO)_3(py\text{-}triazole)](CF_3SO_3)$ [N^N = phen (1b), Me_2-phen (2b), Me_4-phen (3b), Ph_2-phen (4b)]

Purified NO was bubbled slowly into a solution of $[Re(N^N)(CO)_3(py\text{-}diamine)](CF_3SO_3)$ (0.070 mmol) in CH_2Cl_2 (10 mL) for 10 min. The solution was evaporated to dryness to give a yellow solid. Recrystallization of the product from CH_2Cl_2/diethyl ether afforded the complex as yellow crystals. Complex **1b**. Yield: 42 mg (83%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 10.05 (d, 2H, J = 5.4 Hz, H2 and H9 of phen), 9.51

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(s, 1H, H2 of pyridine), 9.09 (d, 2H, J = 8.1 Hz, H4 and H7 of phen), 8.59 (d, 1H, J = 6.6 Hz, H6 of pyridine), 8.40–8.32 (m, 4H, H3, H5, H6 and H8 of phen), 7.82 (d, 1H, J = 6.6 Hz, H5 of pyridine). IR (KBr) ν/cm^{-1}: 3448 (br, N–H), 2035 (s, C$\equiv$O), 1906 (s, C$\equiv$O), 1164 (m, $CF_3SO_3^-$), 1029 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 570 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{21}H_{12}N_6O_6SF_3{\cdot}0.5C_2H_5OC_2H_5$: C, 36.52; H, 2.27; N, 11.11. Found: C, 36.52; H, 2.21; N, 11.08. Complex **2b**. Yield: 42 mg (81%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 8.96 (s, 1H, H2 of pyridine), 8.81 (d, 2H, J = 8.1 Hz, H4 and H7 of Me_2-phen), 8.28 (d, 2H, J = 8.7 Hz, H3 and H8 of Me_2-phen), 8.11–8.06 (m, 3H, H6 of pyridine and H5 and H6 of Me_2-phen), 7.71 (d, 1H, J = 6.6 Hz, H5 of pyridine), 3.56 (s, 6H, CH_3 of Me_2-phen). IR (KBr) ν/cm^{-1}: 3449 (br, N–H), 2033 (s, C$\equiv$O), 1925 (s, C$\equiv$O), 1155 (m, $CF_3SO_3^-$), 1029 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 599 $\{M - PF_6^-\}^+$. Anal. Calcd for $ReC_{23}H_{16}N_6O_6SF_3{\cdot}0.5H_2O$: C, 36.52; H, 2.27; N, 11.11. Found: C, 36.54; H, 2.33; N, 11.28. Complex **3b**. Yield: 47 mg (87%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 9.79 (s, 2H, H2 and H9 of Me_4-phen), 9.53 (s, 1H, H2 of pyridine), 8.63 (d, 1H, J = 6.6 Hz, H6 of pyridine), 8.42 (s, 2H, H5 and H6 of Me_4-phen), 7.78 (d, 1H, J = 6.6 Hz, H5 of pyridine), 2.91 (s, 6H, CH_3 at C4 and C7 of Me_4-phen), 2.81 (s, 6H, CH_3 at C3 and C8 of Me_4-phen). IR (KBr) ν/cm^{-1}: 3448 (br, N–H), 2032 (s, C$\equiv$O), 1947 (s, C$\equiv$O), 1159 (m, $CF_3SO_3^-$), 1029 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 626 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{25}H_{20}N_6O_6SF_3{\cdot}1.5H_2O$: C, 37.41; H, 2.89; N, 10.47. Found: C, 37.47; H, 2.71; N, 10.72. Complex **4b**. Yield: 44 mg (72%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ 10.10 (d, 2H, J = 5.4 Hz, H2 and H9 of Ph_2-phen), 9.58 (s, 1H, H2 of pyridine), 8.70 (d, 1H, J = 6.9 Hz, H6 of pyridine), 8.32 (d, 2H, J = 5.7 Hz, H3 and H8 of Ph_2-phen), 8.20 (s, 2H, H5 and H6 of Ph_2-phen), 7.89 (d, 2H, J = 6.6 Hz, H5 of pyridine), 7.69–7.66 (m, 10H, C_6H_5 of Ph_2-phen). IR (KBr) ν/cm^{-1}: 3448 (br, N–H), 2027 (s, C$\equiv$O), 1918 (s, C$\equiv$O), 1159 (m, $CF_3SO_3^-$), 1030 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion clusters at m/z 722 $\{M - CF_3SO_3^-\}^+$. Anal. Calcd for $ReC_{33}H_{20}N_6O_6SF_3{\cdot}CH_3CN{\cdot}2H_2O$: C, 44.31; H, 2.87; N, 10.33. Found: C, 44.28; H, 2.77; N, 10.25.

Instrumentation and methods

^{1}H NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer at 298 K. Positive-ion ESI mass spectra were recorded on a Perkin-Elmer Sciex API 365 mass spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. Elemental analyses were carried out on a Vario EL III CHN elemental analyzer. Electronic absorption and steady-state emission spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer and a SPEX FluoroLog 3-TCSPC spectrophotometer equipped with a Hamamatsu R928 PMT detector, respectively. Emission lifetimes were measured in the Fast MCS or the MCS lifetime mode with a NanoLED N-375 as the excitation source. All the solutions for photophysical studies were degassed with at least four successive freeze-pump-thaw cycles and stored in a 10 cm^3 round-bottomed flask equipped with a side-arm 1 cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by the optically dilute method[32] using a degassed CH_3CN solution of $[Re(phen)(CO)_3(pyridine)](CF_3SO_3)$ (Φ_{em} = 0.18, λ_{ex} = 355 nm) as the standard solution.[33] Details of the MTT assays and ICP-MS have been reported previously.[19g]

pH dependent emission studies

A 20 mL stock solution of complexes **4a** and **4b** (10 μM), respectively, in a mixture of 10 mM KOH and 100 mM KCl(aq)/MeOH (9 : 1 v/v) was prepared. The pH of the stock solution was increased gradually by addition of appropriate amounts of 1, 0.5 0.25, 0.13, 0.07, 0.04 or 0.02 N HCl(aq). After a desired pH value was obtained, 2 mL of the stock solution was transferred to a quartz cuvette for emission measurements. The solution was then returned to the stock and the pH of the solution was further adjusted. The overall increase in the volume of the stock solution due to pH adjustment was kept below 2%.

Detection of NO in aqueous buffer solutions

A series of solutions containing different concentrations of NOC-7 (0 to 100 μM) and the diamine complex **4a** (5 μM) in potassium phosphate buffer (50 mM, pH 7.4)/MeOH (9 : 1 v/v) was prepared. The solutions were gently stirred at room temperature for 2 h and their emission spectra were then measured.

Live-cell confocal microscopy and imaging of intracellular NO

HeLa cells in growth medium were seeded on a sterilized coverslip in a 60 mm tissue culture dish and grown at 37 °C under a 5% CO_2 atmosphere for 48 h. The culture medium was then removed and replaced with medium/DMSO (99 : 1 v/v) containing complex **4a** (10 μM). After incubation for 1 h, the medium was removed and the cell layer was washed with PBS (1 mL × 3). The coverslip was mounted onto a sterilized glass slide and then imaged using a Leica TCS SPE confocal microscope. In the NO imaging experiment, after being treated with complex **4a**, the cells were incubated with NOC-7 (100 μM) in PBS for 30 min and finally washed with PBS (1 mL × 3).

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