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Luminescent rhenium(I) diimine indole conjugates – photophysical, electrochemical and protein-binding properties†

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Two novel luminescent rhenium(I) diimine indole complexes have been designed and their properties studied; these conjugates can be recognised by indole-binding proteins including bovine serum albumin, lysozyme and tryptophanase.

Indole and its derivatives such as the fluorescent amino acid tryptophan, the plant growth regulator indole-3-acetic acid and the neurotransmitter serotonin, play an important role in plant, animal and human physiology.[1] Development of new probes for monitoring the interactions of these molecules with transport- and response-mediating proteins is urgently required. In this context, detection and isolation of indole-binding proteins have been investigated with specially designed indole derivatives.[2] We believe that luminescent indole conjugates with long-lived emission in the visible region are attractive in the development of biological probes for these proteins. Here we report the synthesis, crystal structures, photophysical, electrochemical and protein-binding properties of two rhenium(I) diimine indole conjugates $[Re(Me_4\text{-phen})(CO)_3(L)](CF_3SO_3)$ (Me_4-phen = 3,4,7,8-tetramethyl-1,10-phenanthroline; L = *N*-(3-pyridoyl)-tryptamine (**1**), *N*-[*N*-(3-pyridoyl)-6-aminohexanoyl]tryptamine (**2**)). The properties of these two complexes have also been compared to those of their indole-free counterpart $[Re(Me_4\text{-phen})(CO)_3(py\text{-}CONH\text{-}Et)](CF_3SO_3)$ (py-CONH-Et = *N*-ethyl-(3-pyridyl)formamide (**3**)).

Reaction of $[Re(Me_4\text{-phen})(CO)_3(CH_3CN)](CF_3SO_3)$ with the corresponding pyridine ligands in refluxing THF afforded **1–3** in moderate yields.† The crystal structures of **1** and **2** have been investigated.‡ The rhenium(I) centers of both **1** and **2** adopt a distorted octahedral coordination geometry and the carbonyls are arranged in a *facial* orientation (Figs. 1 and 2). The bond lengths and angles are normal.[3] It is interesting to note that while the indole moiety of **1** exhibits a dihedral angle of *ca*. 6.5° with the Me_4-phen ligand, these two units in **2** are almost perpendicular to each other, with a dihedral angle of *ca*. 82.9°. Remarkably, intermolecular stacking interactions are observed between the indole ring of one molecule and the Me_4-phen ligand of a neighbouring molecule.† Interplanar dihedral angles of 6.5 and 10.1° are noticed for **1** and **2**, respectively. The shortest distances between an atom on the ring plane of one molecule and the ideal ring plane of the adjacent molecule are *ca*. 3.1 and 3.4 Å for **1** and **2**, respectively.

Complexes **1–3** display quasi-reversible Re(II/I) oxidation couples at *ca*. +1.7 V *vs*. SCE (Table 1). Additional irreversible waves/quasi-reversible couples are also observed at *ca*. +1.1 and +1.3 V for **1** and +1.1 V for **2**. These features are assigned to oxidation of the indole-containing pyridine ligands since similar waves are observed for the uncoordinated ligands. The first reduction at *ca*. −1.4 V for all three complexes is assigned to reduction of the Me_4-phen ligand. Upon visible-light excitation, complexes **1–3** in alcohol glass at 77 K show very similar intense and structured emission (Table 1). Bi-exponential decays are observed for all three complexes, and the longer- and shorter-lived components are assigned to ^{3}IL ($\pi \rightarrow \pi^*(Me_4\text{-phen})$) and ^{3}MLCT ($d\pi(Re) \rightarrow \pi^*(Me_4\text{-phen})$) excited states, respectively.[4] In CH_3CN at 298 K, complex **3** shows intense ^{3}MLCT/^{3}IL luminescence at 515 nm with an emission lifetime of *ca*. 14 μs, in marked resemblance to its structural analogue $[Re(Me_4\text{-phen})(CO)_3(py)]^+$.[4] It is noteworthy that **3** exhibits emission quenching in the presence of unsubstituted indole, and a bi-molecular quenching rate constant k_q of 5.6×10^8 dm^3 mol^{-1} s^{-1} is determined from the Stern–Volmer analysis. The transient absorption difference spectrum of **3** and indole in CH_3CN displays an absorption band at *ca*. 410 nm and a broader one of comparable intensity at *ca*. 550 nm, both attributable to the absorption of the indolyl radical.[5] This

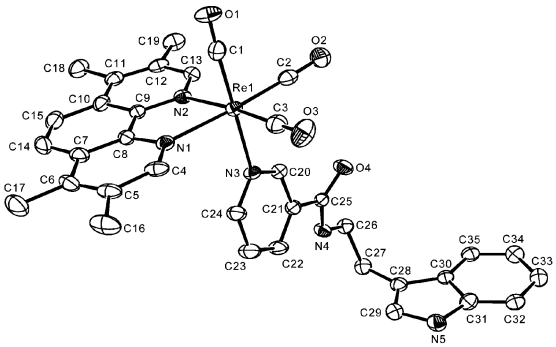

Fig. 1 Perspective drawing of the complex cation of **1**. Thermal ellipsoids are set at 20% probability. Selected bond distances (Å) and angles (°) Re(1)–C(1) 1.924(6), Re(1)–C(2) 1.888(7), Re(1)–C(3) 1.930(6), Re(1)–N(1) 2.179(5), Re(1)–N(2) 2.185(4), Re(1)–N(3) 2.253(3), C(1)–Re(1)–C(2) 86.0(2), C(1)–Re(1)–C(3) 89.0(2), C(1)–Re(1)–N(1) 93.8(2), C(1)–Re(1)–N(2) 91.73(18), C(1)–Re(1)–N(3) 178.12(18), C(2)–Re(1)–C(3) 87.6(2), C(2)–Re(1)–N(1) 173.62(18), C(2)–Re(1)–N(2) 98.61(19), C(2)–Re(1)–N(3) 93.50(18), C(3)–Re(1)–N(1) 98.8(2), C(3)–Re(1)–N(2) 173.8(2), C(3)–Re(1)–N(3) 92.78(19), N(1)–Re(1)–N(2) 75.02(15), N(1)–Re(1)–N(3) 86.49(15), N(2)–Re(1)–N(3) 86.55(14).

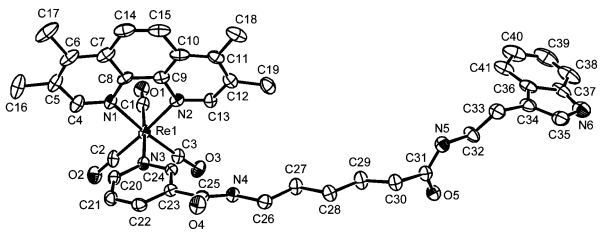

Fig. 2 Perspective drawing of the complex cation of **2**. Thermal ellipsoids are set at 20% probability. Selected bond distances (Å) and angles (°) Re(1)–C(1) 1.920(9), Re(1)–C(2) 1.902(9), Re(1)–C(3) 1.918(7), Re(1)–N(1) 2.172(5), Re(1)–N(2) 2.167(6), Re(1)–N(3) 2.229(5), C(1)–Re(1)–C(2) 88.1(3), C(1)–Re(1)–C(3) 88.7(3), C(1)–Re(1)–N(1) 93.0(2), C(1)–Re(1)–N(2) 94.5(3), C(1)–Re(1)–N(3) 175.7(2), C(2)–Re(1)–C(3) 88.3(3), C(2)–Re(1)–N(1) 99.0(3), C(2)–Re(1)–N(2) 173.8(2), C(2)–Re(1)–N(3) 92.0(3), C(3)–Re(1)–N(1) 172.6(3), C(3)–Re(1)–N(2) 97.3(3), C(3)–Re(1)–N(3) 95.6(2), N(1)–Re(1)–N(2) 75.4(2), N(1)–Re(1)–N(3) 82.78(19), N(2)–Re(1)–N(3) 85.00(19).

† Electronic supplementary information (ESI) available: synthetic procedures and characterisation data of **1–3**, crystal data of **1** and **2**, figures showing the intermolecular stacking interactions in **1** and **2**, and details of the cumulative emission titrations, energy transfer quenching experiments and inhibition assays. See http://www.rsc.org/suppdata/cc/b3/b306914a/

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Table 1 Electrochemical and photophysical data of complexes **1–3**

Complex	Medium (T/K)	Oxidation, $E_{1/2}$ or E_a/V[a]	Reduction, $E_{1/2}$ or E_c/V[a]	λ_{em}/nm[b]	τ_o/μs[b]	Φ[c]
1	CH_3CN (298)	+1.10,[d] +1.29,[e] +1.71[e]	−1.43,[d] −1.63,[e] −1.80,[e] −1.92,[e] −2.25[e]	518	8.21	0.0085
	Glass[f] (77)			464, 497, 530 sh	86.38 (52%), 19.77 (48%)	
2	CH_3CN (298)	+1.09,[d] +1.68[e]	−1.41,[e] −1.64,[e] −1.80,[e] −1.96,[e] −2.28[e]	514	6.33	0.0091
	Glass[f] (77)			465, 497, 533 sh	95.17 (49%), 21.43 (51%)	
3	CH_3CN (298)	+1.69[e]	−1.42,[e] −1.63,[e] −1.78,[e] −1.93,[e] −2.25[e]	515	14.12	0.54
	Glass[f] (77)			464, 497, 534 sh	117.65 (44%), 28.98 (56%)	

[a] In CH_3CN (0.1 M $^nBu_4NPF_6$), glassy carbon electrode, sweep rate 100 mV s^{-1}, all potentials *versus* SCE. [b] λ_{ex} = 355 nm, [Re] = 50 μM. [c] λ_{ex} = 355 nm, $A_{355\,nm}$ = 0.1, $[Ru(bpy)_3]Cl_2$ in aerated H_2O as the standard. [d] Irreversible waves. [e] Quasi-reversible couples. [f] EtOH/MeOH (4 : 1 v/v).

observation is in agreement with the facile oxidation of tryptophan residues by the ^{3}MLCT excited state of luminescent rhenium(I) diimines.[6]

In CH_3CN at 298 K, complexes **1** and **2** also display ^{3}MLCT/^{3}IL emission at an energy similar to that of **3** with lower luminescence quantum yields (Table 1). When excited in the ultra-violet region, complexes **1** and **2** ([Re] = 18 μM) exhibit additional emission due to the indole-containing ligands at *ca.* 365 nm (for example, at λ_{ex} = 250 nm, $I_{517\,nm}/I_{365\,nm}$ = *ca.* 21 and 3 for **1** and **2**, respectively). The weak indole emission can be accounted for by the fact that both complexes absorb quite strongly at *ca.* 365 nm, resulting in resonance-energy transfer from the indole to the luminophore.[7] We observe that the emission lifetimes of **1** and **2** are concentration-dependent. At [Re] = 50 μM, the emission lifetimes of **1** and **2** in CH_3CN at 298 K are 8.21 and 6.33 μs, respectively (Table 1). Self-quenching rate constants k_{sq} (obtained from a plot of τ^{-1} *vs.* [Re]) are determined to be 9.6×10^8 and 1.5×10^9 $dm^3\ mol^{-1}\ s^{-1}$ for **1** and **2**, respectively. These values are not substantially larger than the k_q for the emission quenching of **3** by indole. Intermolecular electron transfer appears to play a key role in the self-quenching of **1** and **2**. Interestingly, compared to other rhenium(I)-quencher systems,[8] intramolecular quenching of **1** and **2** is not significant, and rather long-lived emission is still observed for these two complexes.

We have studied, from different approaches, the possible interactions of **1** and **2** with indole-binding proteins including bovine serum albumin (BSA) and lysozyme.[9] Cumulative emission titration experiments show that the luminescence of both **1** and **2** is enhanced in the presence of BSA.† At [BSA] = 1 mM, **1** and **2** (240 μM) reveal a 2- and 17-fold increase in emission intensity, respectively, and the emission lifetimes are also increased by factors of 1.7 and 2.0. These observations are ascribed to the binding of the indole moiety of the complexes to the protein as no changes are observed for the indole-free complex **3**. The larger binding constant of **2** (*ca.* $1.7 \times 10^4\ M^{-1}$) than that of **1** (*ca.* $1.0 \times 10^4\ M^{-1}$) suggests that the presence of a spacer-arm in **2** could alleviate possible steric hindrance between the complex and the protein.

On the other hand, the emission lifetimes of **1** and **2** (65 μM) in degassed buffer containing lysozyme (5 μM) are 9.9 and 6.5 μs, respectively. However, when lysozyme conjugated with the energy acceptor Malachite Green (MG) is used instead, the lifetimes of **1** and **2** are reduced to 6.1 and 5.2 μs, respectively.† A similar decrease in lifetimes is not observed when only free MG is present. It is conceivable that the emission quenching is due to the binding of **1** and **2** to the modified protein, leading to distance-dependent resonance-energy transfer from the donors (**1** and **2**) to the acceptor (lysozyme–MG). On the basis of the spectral data, a Förster distance of *ca.* 34 Å is estimated for both complexes. Under the same experimental conditions, **3** does not show any noticeable decrease in its emission lifetime (*ca.* 12.0 μs), suggesting the lack of recognition of this complex by the modified protein.

The inhibition properties of **1** and **2** to another indole-binding protein, tryptophanase, have been investigated by a standard assay based on the conversion of L-serine to pyruvate by the enzyme.[10] Under our experimental conditions, free indole can inhibit 53% of the enzyme activities, while **1**, **2** and **3** can cause 61, 74 and 3% inhibition, respectively.† These results reveal that tryptophanase can interact with both **1** and **2** rather than **3**, and reflect again the importance of the spacer-arm in **2** on the binding interaction.

In summary, we have designed two novel luminescent rhenium(I) indole conjugates that can be recognised by indole-binding proteins, rendering these complexes as potential probes for this class of proteins.

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Notes and references

‡ Crystal data for **1**: $(C_{36}H_{31}F_3N_5O_7ReS)$: M = 920.92, monoclinic, $P2_1/c$, a = 11.670(2), b = 9.570(2), c = 32.349(7) Å, β = 97.33(3)°, V = 3583.3(12) Å^3, Z = 4, ρ_{calcd} = 1.707 g cm^{-3}, μ(Mo–Kα) = 3.521 mm^{-1}, F(000) = 1824, T = 253 K, λ = 0.71073 Å, 17513 measured reflections, 5870 independent reflections, 486 parameters, R_1 = 0.0331 and wR_2 = 0.0716. CCDC 204011. For **2**: $(C_{45}H_{48}F_3N_6O_9ReS)$: M = 1092.15, monoclinic, $P2_1/c$, a = 11.656(2), b = 24.084(5), c = 17.124(3) Å, β = 99.44(3)°, V = 4742.0(15) Å^3, Z = 4, ρ_{calcd} = 1.530 g cm^{-3}, μ(Mo–Kα) = 2.678 mm^{-1}, F(000) = 2200, T = 253 K, λ = 0.71073 Å, 25815 measured reflections, 7027 independent reflections, 587 parameters, R_1 = 0.0369 and wR_2 = 0.0965. CCDC 204012. See http://www.rsc.org/suppdata/cc/b3/b306914a/ for crystallographic data in .cif or other electronic format.

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