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Rhenium(I) phenanthrolines bearing electron withdrawing CF_3 substituents: synthesis, characterization and biological evaluation†

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Rhenium(I) tricarbonyl complexes bearing a 2,4,7,9-tetraphenylphenanthroline ligand, where the 2,9-phenyl groups have either *meta*- or *para*-CF_3 groups, have been screened against a selection of cell lines. The *meta* derivative shows anticancer activity against HeLa and A549 cell lines, whereas the *para* derivative causes proliferation of HL-60 cells, whilst showing toxicity towards A549 cells.

One of the main goals in the field of metal-based anti-tumor drugs is to overcome the major limitations associated with cisplatin, namely drug resistance, poor selectivity and adverse side effects.[1] Amongst the metals under consideration, rhenium has shown promising apoptosis and anti-tumor activity in a variety of oxidation states.[2] Furthermore, rhenium complexes have become the logical choice for therapeutic applications, given the close similarities with the chemistry of technetium,[3] and a number of complexes are now under clinical consideration.[4] Whilst the most extensive chemistry reported for these group VII congeners involves the $[MO]^{3+}$ core (metal in oxidation state +V), other studies have highlighted the potential of the organometallic fragment $[fac\text{-}Re(CO)_3]^+$.[5] This fragment is readily accessible as the air stable $[Re(CO)_3(H_2O)_3]^+$ species, for which the aqua ligands are substitutionally labile. Furthermore, the small size of the core allows for tagging with bioactive moieties, whilst retaining bioactivity and specificity. Radio-imaging techniques do not permit visualization at the cellular level, however by replacing the radioisotope with a fluorophore, it is possible to use fluorescent microscopy to identify cellular targets and thereby correlate results with *in vivo* radio-imaging experiments. A number of conjugated chelating ligands have recently been bound to the $[fac\text{-}Re(CO)_3]$ core, including benzoimidazole, diimine and indole.[6] To-date, results for the diimine systems have been restricted due to limited visible adsorption (generally emit only in the yellow/green region),[7] this despite reports on rhenium(I) tricarbonyl diimines of the type $[Re(CO)_3(N\text{–}N)X]$ (N–N = diimine, X = halide) dating back to the 1970s.[8] Given the relatively low sensitivity of the HOMO energy to changes to the diimine ligand, the emission energy of the rhenium(I) complexes is, broadly speaking, proportional to the reduction potential of the ligand. The colour of the emission can therefore be shifted by lowering the LUMO energy, *i.e.* by making the ligand reduction potential more positive – the so-called energy-gap law.[9] One way in which this can be accomplished is to introduce electron withdrawing substituents at the ligand, thereby shifting the emission toward the red. Transitions to a ligand localized LUMO has recently been utilized in azadipyrromethane rhenium tricarbonyl complexes to bring about red emission.[10] In the case of phenanthroline ligand systems, this can be achieved by the addition of phenyl groups, for example anionic complexes of the type $[Re(CO)_3$(bathophenanthroline sulphate)(py-3-R)] (R = H, CH_2OH; bathophenanthroline = 4,7-diphenyl-1,10-phenanthroline) exhibit red shifts of about 20 nm for excitation and 10 nm for emission.[11] It has been noted in related biquinoline chemistry that the use of electron withdrawing substituents can also lead to red shifts.[12] It was therefore expected that for bathophenanthroline-derived systems, phenyl groups possessing fluorine containing substituents such as CF_3 groups would produce a red shift. The effect is more pronounced if the aryl group is not positioned *meta* to the Re–N bond. This red emission is of particular interest in biological systems, as human tissue is more transparent to red and near infra-red light – potentially allowing such materials to be used in therapeutic

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settings,[13] as well as in OLEDs (organic light emitting diodes) which was originally our entry into such ligand sets.[14]

As a result, we report herein two such ligands, 2,9-bis(3-trifluoromethylphenyl)-4,7-diphenyl-1,10-phenanthroline and 2,9-bis(4-trifluoromethylphenyl)-4,7-diphenyl-1,10-phenanthroline, and the rhenium(I) carbonyl chloro complexes thereof (illustrated in Scheme 1). We were attracted to the use of the chloro complexes given their desirable photophysical properties as well as the tendency to form air stable complexes.[15]

The cellular uptake of the two complexes in several cell lines has been investigated by fluorescence microscopy, and their cytotoxicity towards a variety of cell lines has been examined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay.

Complexes **1** and **2** are readily synthesized by heating the ligand and rhenium pentacarbonyl chloride in equimolar quantities in toluene for several hours. Both complexes **1** and **2** can be purified by sublimation under high vacuum between 260 and 280 °C, though complex **2** exhibits decomposition and shows the presence of the free ligand in the products.

Crystals of **1** suitable for analysis by X-ray diffraction were grown at ambient temperature from a saturated solution of **1** in toluene; a thermal ellipsoid projection is shown in Fig. 1 (alternative views are given in the ESI, see Fig. S1–S4†). The structure adopts the expected *fac*-CO arrangement with a distorted octahedral rhenium centre, where the main distortion occurs as a result of the *N*,*N*-chelate bite angle (75.48(1)°). The Re–C and C–O bond lengths are typical of those observed in such *fac*-tricarbonyl rhenium(I) diimine complexes.[8]

The development of specific cancer drugs depends on a detailed understanding of how the drugs interact with different cell types. We therefore undertook a detailed analysis of the effects of these complexes bearing electron withdrawing groups on different cell types (Table 1). For toxicity screening compounds were dissolved in DMSO (1%), following which the emission shifted to the yellow/green. Given this, we denote the post-DMSO complexes as **1′** and **2′**.

For complexes **1** and **2**, the PL spectra are consistent with them acting as red emitters (solution emission for **1** and **2** were 635 nm and 596 nm, respectively, see for example ESI, Fig. S5† *cf.* about 565 nm for [Re(CO)$_3$(bathophenanthroline sulphate)(py-3-R)] (R = H, CH$_2$OH)[11]); quantum yield values were 9.7 and 7.2% (ESI, Fig. S6–S8†), respectively. For UV spectra for **1** and **2**, see ESI Fig. S9 and S10.†

Both **1′** and **2′** show photoluminescence emission peaks at 451 and 462 nm respectively (ESI, Fig. S11 and S12†). The photoluminescence emission wavelength for both samples reduces in intensity over 21 days at ambient temperature. **1′** shows a reduction in the intensity of the luminescence after 7 days and after 14 shows a change in the shape of the peak with the development of two shoulders at 373 and 415 nm (ESI, Fig. S11†). Complex **2′** shows a reduction in intensity after 3 and 7 days; the luminescence then stabilized from 7 to 21 days (ESI, Fig. S12†).

Depending on the cell types used, the IC$_{50}$ for **1′** and **2′** differs. Both are particularly toxic for human adenocarcinoma cells (A549), whereas **2′** does not show any toxicity towards CHO

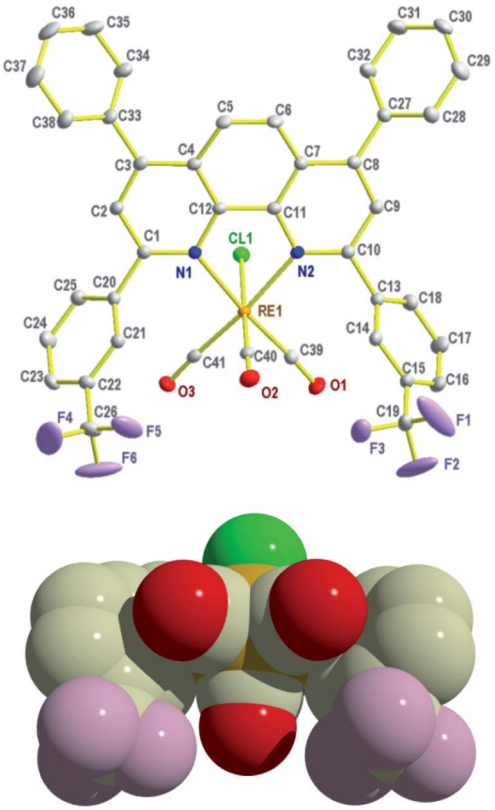
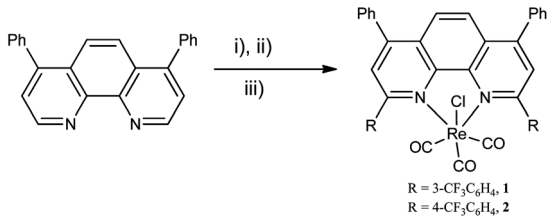


Fig. 1 Two views of molecular structure of **1**. Selected bond lengths (Å) and angles (°): Re(1)–N(1) 2.213(3), Re(1)–N(2) 2.208(3), Re(1)–Cl(1) 2.4724(9), Re(1)–C(39) 1.924(4), Re(1)–C(40) 1.929(4), Re(1)–C(41) 1.924(4), N(1)–Re(1)–N(2) 75.48(11), Cl(1)–Re(1)–N(1) 83.00(8), Cl(1)–Re(1)–N(2) 81.63(8), Cl(1)–Re(1)–C(40) 177.53(11).

Table 1 Toxicity screening for **1′** and **2′**[a]

Cell type	Compound **1′** (IC$_{50}$)	Compound **2′** (IC$_{50}$)
A549	3.62 ± 0.8 μM	4.76 ± 2.5 μM
THP-1	33 ± 16 μM	14 ± 4.1 μM
HeLa	4.56 ± 3 μM	83 ± 80 μM
CHO	12.17 ± 6.1 μM	Non-toxic
HL60	Leads to proliferation	Leads to proliferation

[a] Cells were tested for proliferation after 72 hours using an MTS kit. DMSO (1%) was used as a control in all studies, data represent mean ± SEM of at least three independent experiments done in duplicate. The IC$_{50}$ was calculated using GraphPad Prism 5 (GraphPad Software), when data was fitted to a nonlinear regression curve.



Scheme 1 (i) RLi, Et$_2$O–toluene, −78 °C to 0 °C; (ii) H$_2$O, MnO$_2$; (iii) Re(CO)$_5$Cl, toluene, reflux.[16]

cells and is less toxic towards HeLa cells than **1′**. Perhaps the most interesting observation of these experiments is that the rhenium complexes **1′** and particularly **2′** encourage the proliferation of HL-60 cells over 72 hours. Preliminary data in 16HBE cell lines also showed that **1′** and **2′** encourage cell growth (ESI, Fig. S13†). We tested both compounds on the monolayer cell line CHO and the suspension cells HL60, and monitored whether or not each can enter cells. The spontaneous fluorescence of the compounds exhibited after activation under a fluorescence microscope was not very bright, but it allowed us to predict cellular up-take of the compounds is indeed occurring in the HL60 cells (ESI, Fig. S14†) (Fig. 2).

We investigated how **1′** and **2′** can induce cell death in the CHO cells and incubated the cells for 24 hours with the compounds and stained with a nucleus and an actin fibre marker (ESI, Fig. S15†).

Neither compounds affected the actin polymerization or the shape and consistence of the nuclei in the cells, *i.e.* no fragmentation of the nuclei, which can be a sign of early apoptosis, was visible.

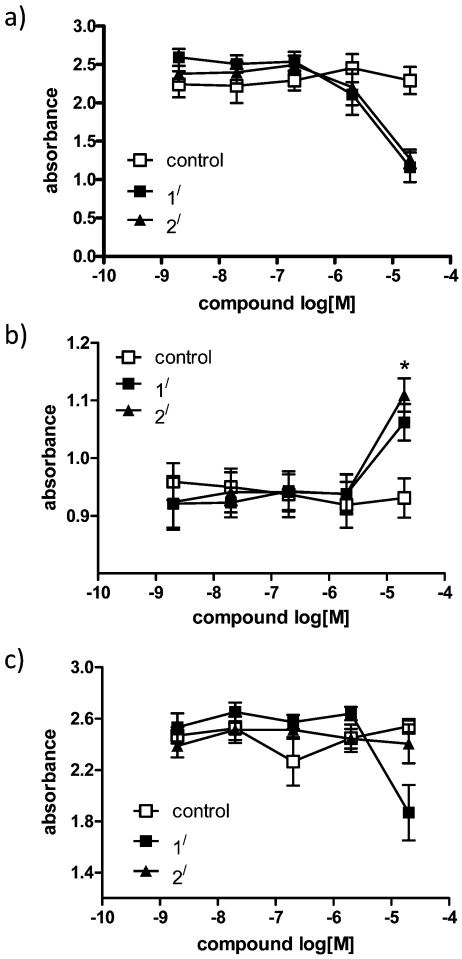


Fig. 2 Cell viability assay in (a) A549 cells, (b) HL-60 cells and (c) CHO cells, tested for proliferation after 72 hours using an MTS kit. DMSO used as a control in all studies, data represent mean ± SEM of at least three independent experiments done in duplicate. Absorbance shown is directly proportional to the number of living cells in culture.

These results therefore suggest that neither **1′** nor **2′** induce programmed cell death (apoptosis) in CHO cells, but more work needs to be done to elucidate the actual ways how these compounds kill the cells.

The cytotoxicity of a number of other complexes containing the [*fac*-Re(CO)$_3$] core has been examined, including phosphine of the form [Re(CO)$_3$(diphosphine)Br].[17] For pyridyl or phenanthroline containing complexes, screening against the HeLa cell lines afforded IC_{50} values (μM) of about 30 for [Re(CO)$_3$(2-appt)Cl] (2-appt = 2-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine)[18] and ranging from 17.5–28.5 for a series of thiourea complexes,[19a] 2.8 to >150 for a series of polypyridyl glucose complexes,[19b] 3.6 to 40 for a series of polypyridyl florous complexes,[19c] and 3.6 to >1151.7 for a series of polypyridyl poly(ethylene glycol) complexes.[19d]

We have attempted to isolate the DMSO complexes **1′** and **2′** using the method of Mayer.[20] Our initial attempts however have resulted in oily materials, though in the case of **1**, trituration with hexane afforded a yellow/brown glass. The IR spectrum contained a stretch at 950 cm^{-1} which we tentatively assign to the νS–O stretch of an O-bound DMSO in the complex **1′**.[21] We note that DMSO complexes bound to Re(CO)$_3$(I) have been previously structurally characterized.[22]

In summary, we have shown that rhenium(I) tricarbonyl complexes bearing a 2,4,7,9-tetraphenylphenanthroline ligand, where the 2,9-phenyl groups have either *meta* or *para* CF_3 groups are red emitters. Following treatment with DMSO (1%), the emission is shifted to the yellow/green. In the case of the *meta* derivative, evaluation against several cell lines revealed anti-cancer activity against HeLa and A549 cell lines; the *para* derivative caused proliferation of HL-60 cells, and toxicity towards A549 cells. Further work is in progress to determine the exact nature of **1′** and **2′**, and also the cytotoxicity profiles of the *meta*- and *para*-CF_3 ligands described herein in combination with other metals.

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