Heteronuclear d-d and d-f Ru(II)/M complexes [M = Gd(III), Yb(III), Nd(III), Zn(II) or Mn(II)] of ligands combining phenanthroline and aminocarboxylate binding sites: combined relaxivity, cell imaging and photophysical studies.


Experimental Supporting Information

Fig. S1. $^1$H NMR spectrum (400 MHz, d6-acetone) of Ru•E. $H_b$ = Proton on bipyridine ligand; $H_p$ = proton on phenanthroline ligand; $H_{py}$ = proton on pyridine ring.

Fig. S2. High-resolution ES mass spectrum of Ru•E
Fig. S3. $^1$H NMR spectrum (500 MHz, d6-DMSO) of Ru•L. $H_b$ = Proton on bipyridine ligand; $H_p$ = proton on phenanthroline ligand; $H_{py}$ = proton on pyridine ring.

Fig. S4. High-resolution ES mass spectrum of Ru•L.
**Fig. S5.** $^1$H NMR spectrum (400 MHz, d$_6$-acetone) of Ru•E$_2$ at 298 K. $H_b$ = proton on bipyridine ligand, $H_p$ = proton on phenanthroline ligand, $H_{py}$ = proton on pyridine ring.

**Fig. S6** High-resolution ES mass spectrum of Ru•E$_2$
Fig. S7. $^1$H NMR spectrum (400 MHz, D$_2$O) of Ru•L$_2$ at 298 K. $H_b$ = proton on bipyridine ligand, $H_p$ = proton on phenanthroline ligand, $H_{py}$ = proton on pyridine ring.

Fig. S8. High-resolution ES mass spectrum of Ru•L$_2$
Fig. S9. Part of the high-resolution ES mass spectrum of Ru•Gd showing (top) observed and (below) calculated signals for \([C_{49}H_{37}N_9O_8RuGd + H]^{2+}\).

Fig. S10. Part of the high-resolution ES mass spectrum of Ru•Gd\(_2\) showing (top) observed and (bottom) calculated signals for \([C_{66}H_{50}N_{12}O_{16}RuGd_2 + 2H]^{2+}\).
**Fig. S11.** Part of the high resolution ES mass spectrum of Ru•Nd showing the signal for \([\text{C}_{49}\text{H}_{37}\text{N}_9\text{O}_8\text{RuNd} + \text{H}]^{2+}\).

**Fig. S12.** Part of the high resolution ES mass spectrum of Ru•Yb showing the signal for \([\text{C}_{49}\text{H}_{37}\text{N}_9\text{O}_8\text{RuYb} + \text{H}]^{2+}\).
Fig. S13. Part of the high resolution ES mass spectrum of Ru•Nd\textsubscript{2} showing the signal for [C\textsubscript{66}H\textsubscript{50}N\textsubscript{12}O\textsubscript{16}RuNd\textsubscript{2} + 2H]\textsuperscript{2+}.

Fig. S14. Part of the high resolution ES mass spectrum of Ru•Yb\textsubscript{2} showing the signal for [C\textsubscript{66}H\textsubscript{50}N\textsubscript{12}O\textsubscript{16}RuYb\textsubscript{2} + 2H]\textsuperscript{2+}.
Fig. S15. $^1$H NMR spectrum (400 MHz, D$_2$O) of Ru•Zn at 298 K. ($H_b =$ proton on bipyridine ligand, $H_p =$ proton on phenanthroline ligand, $H_{py} =$ proton on pyridine ring).

Fig. S16. Part of the high resolution ES mass spectrum of Ru•Zn showing the signal for [C$_{49}$H$_{37}$N$_9$O$_8$RuZn+2H]$^{2+}$.
Fig. S17. Part of the high resolution ES mass spectrum of **Ru•Mn** showing the signal for [C$_{49}$H$_{37}$N$_{9}$O$_{8}$RuMn+2H]$^{2+}$.

Fig. S18. $^1$H NMR spectrum (400 MHz, D$_2$O) of **Ru•Zn$_2$** at 298 K. (H$_b$ = proton on bipyridine ligand, H$_p$ = proton on phenanthroline ligand, H$_{py}$ = proton on pyridine ring).
**Fig. S19.** Part of the high resolution ES mass spectrum of Ru•Zn$_2$ showing the signal for [C$_{66}$H$_{50}$N$_{12}$O$_{16}$RuZn$_2$]$^{2-}$.

**Fig. S20.** Part of the high resolution ES mass spectrum of Ru•Mn$_2$ showing the signal for [C$_{66}$H$_{50}$N$_{12}$O$_{16}$RuMn$_2$]$^{2-}$. 
**Fig. S21.** UV/Vis absorption spectra in MeOH, normalised at 435 nm, of Ru•E (green) and Ru•E₂ (red), emphasising the difference in the ¹MLCT absorption region (500 – 550 nm). For extinction coefficients see main text.

**Fig. S22.** Excitation spectra recorded in D₂O at 298K of (a) Ru•Nd and (b) Ru•Nd₂, monitoring excitation at 1060 nm in both cases, showing overlap of the main feature of the excitation spectra with the Ru-based ¹MLCT absorption manifold.
Fig. S23. Decay-associated spectra obtained by global analysis of the transient absorption spectral data of Ru•Zn (left) and Ru•Mn, in water at RT.

Fig. S24. Evolution-associated spectra, illustrating the appearance of the experimental TA spectra at different time delays, for Ru•Zn (left) and Ru•Mn (right), in water at RT.
**Fig. S25.** Plot of relaxation rate of water protons (s$^{-1}$) vs. concentration of Gd(III)-containing complex (mM) for **Ru•Gd** (red dots), **Ru•Gd$_2$** (blue dots) and Magnevist (green dots) (400 MHz, 298 K, D$_2$O).

**Fig. S26.** Clonogenic toxicity assay of HeLa cells incubated with **Ru•Gd** (red line) or **Ru•Gd$_2$** (blue line) at concentrations of 50 µM and 200 µM for 4h. Incubations were carried out in full DMED at 37 °C under an atmosphere of CO$_2$/air (5/95, v/v) for 7 – 10 days until visible cell colonies had formed. Error bars represent the standard deviation of six data points (three repeats of each of duplicate datasets).
Fig. S27. Plot of concentration (mM) vs. relaxation rate of water protons (s\(^{-1}\)) to determine the concentration-normalised longitudinal relaxivity values for Ru•Mn (orange) and Ru•Mn\(_2\) (purple). The data for Ru•Gd (red), and Ru•Gd\(_2\) (blue) and the commercial MRI contrast agent Magnevist\(^\text{®}\) (green) are also included for comparison (400 MHz, 298 K, D\(_2\)O).