Supporting Information

Using Nanoscopy to Probe the Biological Activity of Antimicrobial Leads That Display Potent Activity Against Pathogenic, Multi-drug Resistant, Gram-negative Bacteria.

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S1a. UV-Vis absorption data

(a) (b)

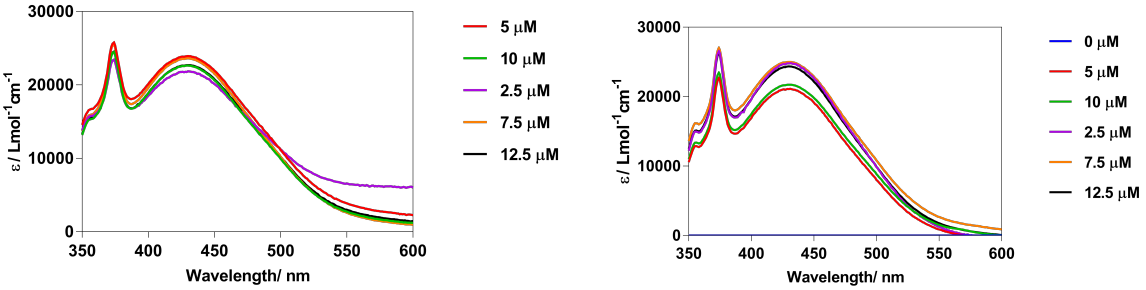


Figure 1. UV-Vis absorption spectrum showing the change in molar extinction coefficient upon increasing concentration of [{Ru(3,4,7,8-tetramethyl-1,10-pheananthroline)$_2$}$_2$(tpphz)] in MeCN (a) and water (b). Conducted on a Cary 300 UV/Vis spectrophotometer at 27.5 °C.

Table 1. UV-Vis spectroscopy data showing the molar extinction coefficient and absorption maxima for the four [{Ru(N-N)$_2$}$_2$(tpphz)] in water (a) and MeCN (b). Conducted on a Cary 300 UV/Vis spectrophotometer at 27.5 °C.

Complex	Λ_{max}/nm	ε/$M^{-1}cm^{-1}$	Transition
^{a}**1**$^{4+}$	450	34000	MLCT
^{a}**2**$^{4+}$	Not water soluble	-	-
^{a}**3**$^{4+}$	454	25300	MLCT
^{a}**4**$^{4+}$	430	22833	MLCT
^{b}**1**$^{4+}$	450	27000	MLCT
^{b}**2**$^{4+}$	450	41000	-
^{b}**3**$^{4+}$	449	22700	MLCT
^{b}**4**$^{4+}$	432	19498	MLCT

S1b. Emission data

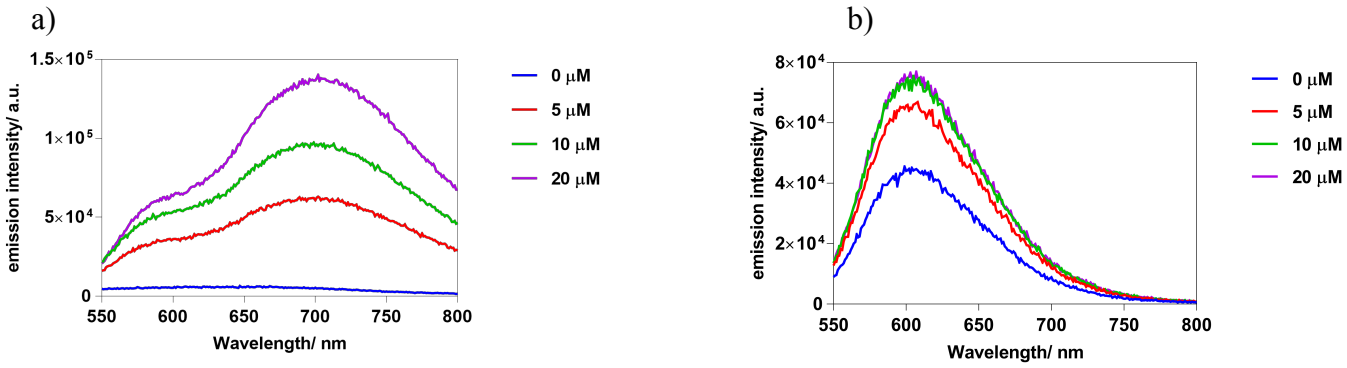


Figure 2. Emission spectra at increasing concentration of [{Ru(3,4,7,8-tetramethyl-1,10-pheananthroline)$_2$}$_2$(tpphz)] between 550-800 nm in MeCN (a) and water (b). Conducted on a Fluoromax 3 fluorimeter at 27.5 °C.

Table 2. Luminescent emission data showing the molar extinction coefficient and absorption maxima for the four [{Ru(N-N)$_2$}$_2$(tpphz)] in water) and MeCN. Conducted on a Fluoromax 3 fluorimeter at 27.5 °C.

Complex	Λ_{max}/nm (MeCN)	Λ_{max}/nm (water)
1^{4+}	710	-
2^{4+}	660	Not water soluble
3^{4+}	670	600
4^{4+}	670	650

*Data from reference 10. – signifies quenched luminescence.

S1c. Comparison of LogP

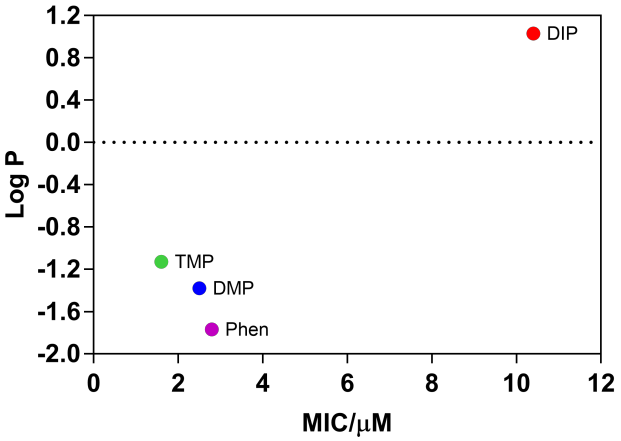


Figure 3. A comparison of the LogP values determined and the activity (MIC) of each complex against the pathogenic E. coli strain EC958, to show the increase in activity with relative increase in lipophilicity. LogP data was collected using the shake flask procedure.

S1d. CFU/mL counts for the uptake experiments

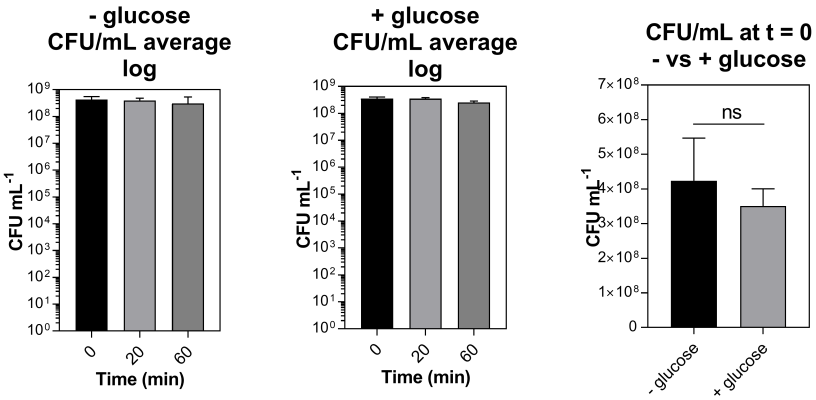


Figure 4. CFU/mL counts for the accumulation experiment of $\mathbf{4}^{4+}$ by EC958, to ensure that the number of bacteria in the solution maintained constant between each time point.

S1e. Accumulation effects ICP-AES

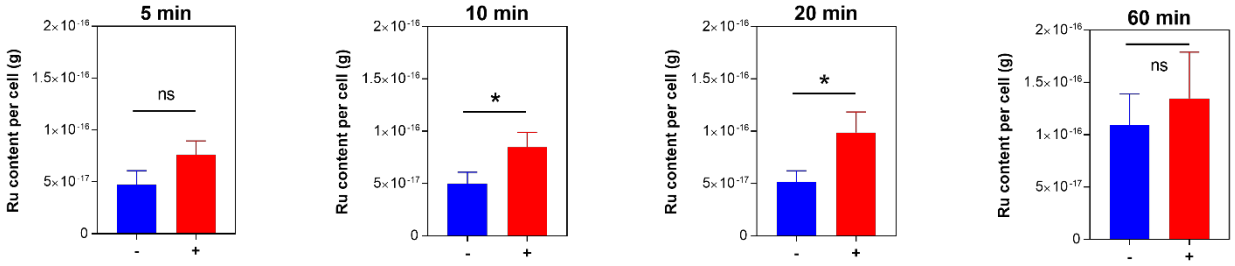


Figure 5. The difference in Ru content per cell (g) at each time point (+) with glucose and (-) without glucose. Showing significant differences in accumulation of ruthenium in the (+) with glucose sample at 10 and 20 minutes. Ruthenium content per cell determined via ICP-AES.

S1f. DNA binding studies with CT-DNA

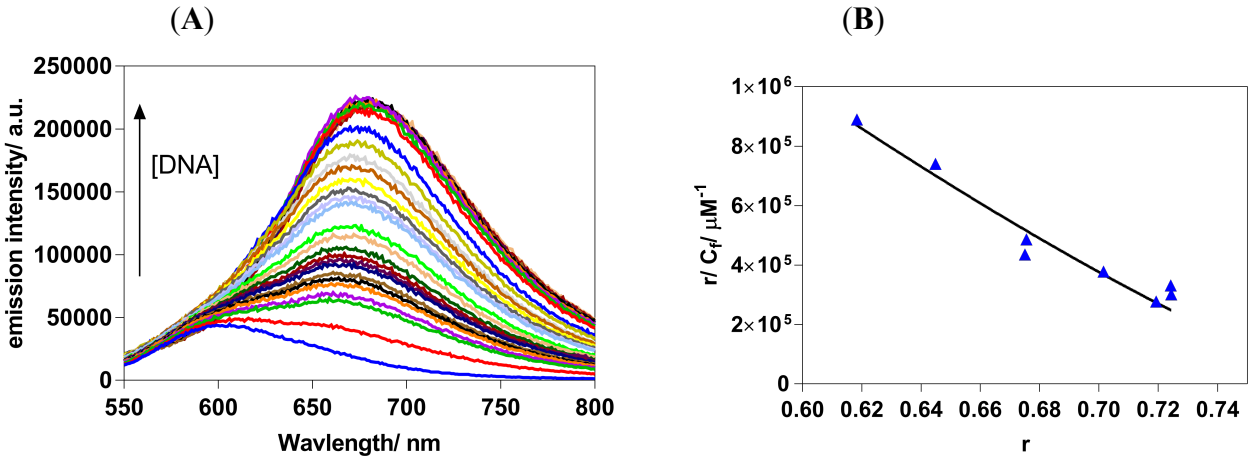


Figure 6. (A) DNA binding titration using increasing concentrations of CT-DNA and $\mathbf{4}^{4+}$ in Tris buffer at 27 ºC, conducted on a Fluoromax 3 (B) Scatchard plot and McGhee Von Hippel fit, to find the binding constant of $\mathbf{4}^{4+}$

Table 3 DNA binding constants, and site sizes of all four compounds in the study

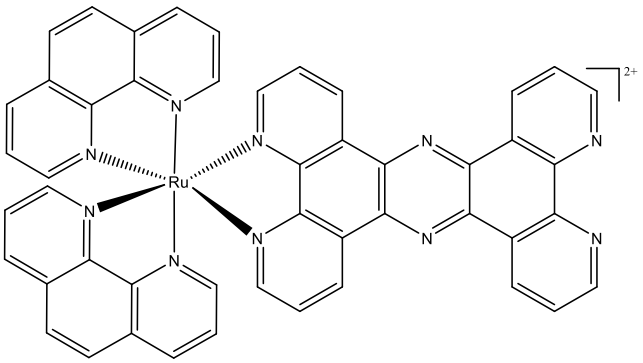
Complex	I_b/I_f	K_b	n
$\mathbf{1}^{4+}$	60	1.1×10^7	2.9
$\mathbf{2}^{4+}$	17	5.6×10^6	1.26 ± 0.02
$\mathbf{3}^{4+}$	27	6.7×10^6	1.45 ± 0.02
$\mathbf{4}^{4+}$	10	2.4×10^6	0.92 ± 0.02

S1g. *Galleria Mellonella* supplementary data

Table 4 Log-rank (Mantel-Cox) tests on the Kaplan-Meier survival curves to determine whether a significant difference is observed between the control (water) and compound injected Galleria survival percentages. Galleria were injected with 10 μL of compound (0-80 mg/kg) and stored at 37.5 degrees for 120 hours.

Concentration/ mg/kg	Chi square	P	Survival curve sig different
1	0.83	0.36	No
5	0.83	0.36	No
10	0.83	0.36	No
20	0.87	0.35	No
40	0.87	0.35	No
80	0.02	0.88	No

S1h. Chemical structure of $[Ru(phen)_2(tpphz)]^{2+}$



S1i. BacLight™ membrane polarisation assay

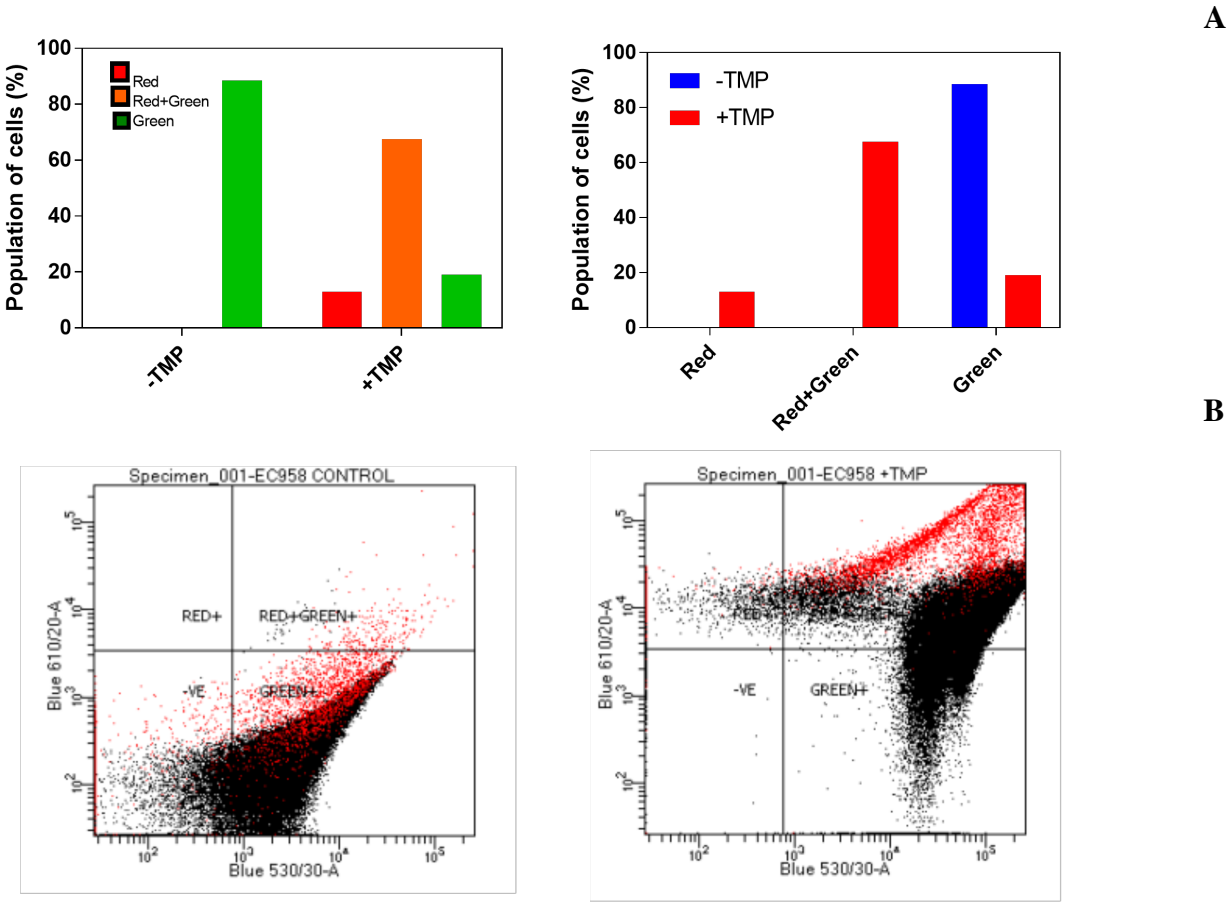


Figure 7. (A) Detection of membrane potential in *E. coli* EC958 cells. Percentage population of cells (%) containing red/red+green/green fluorescence are given. Cells were incubated with 30 µM of Di-$OC_2(3)$ for 30 minutes in the presence or absence of 1.6 µM of $\mathbf{4}^{4+}$. (B) The analysis of the flow cytometry data using red and green fluorescence parameters. The red-versus-green fluorescence dot plots were collected with log amplification.

S2. Instrumentation

S2a. Microscopy

Super resolutions microscopy images were taken on a DeltaVision/GE OMX optical microscope (version 4) for structured illumination (SIM) at the Wolfson Microscope Facility in the University of Sheffield. Images were reconstructed using DeltaVision OMX SoftWoRx 6.0 software. All images were analysed using Fiji and Image J software.

S2b. Photochemistry

Absorption spectra at 200-800 nm for the compounds were obtained with a Cary 50 Scan UV-vis-NIR Spectrophotometer, double beam mode (spectral band width = 2 nm), medium scan speed – 600 nm.

Emission spectra and DNA binding studies for the compound were conducted on the Jobin Yvon Hariba Group FluoroMax®-3 Fluorimeter. DNA binding data was fitted using Origin Software.

UV-Vis absorbance spectroscopy for LogP values were conducted via a SpectraMax M2 Microtitre Plater Reader (Molecular Devices UK).

Turbidity measurements were performed on a Jenway Cary 7350 Spectrophotometer.

S2c. Mass Spectroscopy

All Mass Spectroscopy was conducted at the Mass Spectroscopy Facility at the University of Sheffield

Mass spectra and accurate mass for the compounds were conducted using a Krato MS80 mass spectrometer working in positive ion mode.

Uptake experiment data was collected using Spectro CirosCCD (Spectro Analysis) Inductively-Coupled Plasma-Atomic Emission Spectrophotometer.

S2d. NMR Spectroscopy

^{1}H NMR spectra were taken on a Bruker AV400 machine and a Bruke AVIIIHD400 machine, both working in Fourier transform mode. All spectra were analysed using “Mestrenova” software.

S2e. Electron Microscopy

Samples were examined on a FEI Tecnai instrument operating at 80 kV equipped with a Gatan 1 k CCD Camera.