Supplementary Material (ESI) for CrystEngComm

Molecular architecture of redox-active half-sandwich Ru(II) cyclic assemblies. Interactions with biomolecules and anticancer activity.

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SUPPORTING INFORMATION

Experimental details

Characterisation and physical measurements

The ^{1}H NMR experiments carried out for characterizing compounds **2a**, **2b** and **2c** and for studying the interaction between cyclic systems, AMP and cysteine were performed in D_2O solutions with 10 mg of compound and 0.75 mL of solvent at 293 K. ^{1}H NMR spectra were recorded with a BRUKER ARX 400 (400 MHz) (Centre of Scientific Instrumentation of the University of Granada). Elemental (C, H, N) analyses were obtained at a FISONS-CARLO ERBA EA 1008 analyzer in the Centre of Scientific Instrumentation of the University of Granada.

X-ray crystallography

The single crystal X-ray diffraction data for species **2a** was acquired at 293 K from an orange prism single crystal of approximate 0.24×0.18×0.14 mm dimensions, on a Bruker APEX automated diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data collection was performed in the $1.42<\theta<24.7°$ range by applying the ω-scan mode. A total of 12282 unique and 10200 observed [$I>2\sigma(I)$] reflections were collected [R(int) = 0.056, R(sigma) = 0.036], and used for the structure solution and the structure refinement (against 1251 parameters). The data were corrected for absorption[i] and Lorenz-polarization effects. The structure was solved by direct methods[ii] and refined by full-matrix least-squares on F^2.[iii] All the non-hydrogen atoms were refined anisotropically, but those of the solvent and of the triflate anions (S excluded). Hydrogen atoms were made riding their parent atoms with an isotropic temperature factor 1.2 times that of their parent atoms. Crystallographic data (excluding structure factors) for species **2a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no.s ?. Copies of the data

can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

DNA binding studies

Calf-thymus DNA (ct-DNA) was purchased from Sigma/Aldrich. The ct-DNA was dissolved in water without any further purification and kept frozen until the day of the experiment. The ct-DNA concentration (moles of bases per litre) was determined spectroscopically by using the molar extinction coefficients at the maximum of the long-wavelength absorbance (ct-DNA ε_{258}=6600 $cm^{-1}mol^{-1}dm^3$). Concentrations of stock solutions of compounds **1a**, **1c**, **2a** and **2c** were determined from accurately weighed samples of these materials. A stock sodium cacodylate buffer (100 mM) was prepared by mixing a 50 mL solution of sodium cacodylate (0.2 M, 4.24 g of $Na(CH_2)_2AsO_2{\cdot}3H_2O$ in 100 mL) with 9.3 mL of hydrochloric acid (0.2 M), and diluting to a total of 100 mL. Stock solutions of **1a**, **1c**, **2a** and **2c** (500 μM) were prepared. All ct-DNA experiments were conducted in sodium cacodylic buffer (1 mM) and NaCl (20 mM). Spectroscopic titration series experiments keeping the ct-DNA concentration constant were undertaken by adding the salt, buffer, water and the cyclic assembly to the ct-DNA. The circular dichroism (CD) spectra were produced by using a Jasco J-715 spectropolarimeter. UV-Vis was performed and visualized by ThermoSpectronic UV300 using 2 mL of an aqueous solution of ct-DNA (150 μM) in NaCl (20mM) and sodium cacodylate buffer (1 mM). The previously described solutions were used to register the UV/Vis spectra adding increased quantities of compounds **2a** and **2b** and keeping the ct-DNA concentration constant (ct-DNA/metal-complexes mixing ratios range from 200:1 to 5:1).

Ethidium bromide (EB) displacement by the cyclic assemblies was calculated by measuring the quenching of the EB fluorescence as it leaves the protection of the ct-

DNA. A ct-DNA/salts/buffer solution with EB (ct-DNA/EB 4:5, 4 mM:5 mM) was prepared. The emission spectrum was recorded as a function of **1a**, **1c**, **2a** and **2c** concentration by using a Variant mod. Cary Eclipse Luminescence spectrometer and the ruthenium complex concentration was slowly increased for ct-DNA/metal-complex ratios from 70:1 to 1:1 keeping the ct-DNA and EB concentrations constant. After each addition the fluorescence and UV-visible spectra were recorded (parameters: emission: 600 nm; excitation: 540 nm; excitation slit: 10.0 nm; emission slit:15.0 nm).

Biological assays

Cytotoxic studies were performed at the UNIDAD DE EVALUACIÓN DE ACTIVIDADES FARMACOLÓGICAS, Instituto de Farmacia Industrial, Facultad de Farmacia, University of Santiago de Compostela 15782 Santiago de Compostela, SPAIN. The tumor cell lines A2780, A2780cisR, were cultured at 37 ºC in RPMI 1640 medium (Gibco) supplemented with 10% FBS (Fetal Bovine Serum) and L-Glutamine 2 mM in an atmosphere of 95% of air and 5% CO_2. Cell death was evaluated by using a system based on the tetrazolium compound MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*tetrazolium bromide], which is reduced by living cells to yield a soluble formazan product that can be detected colorimetrically. Cells were seeded in 96-well sterile plates at a density of 4000 cells/ well in 100 μL of medium and were incubated 24 h. Complexes dissolved in DMSO were added to final concentrations ranging from 0 to 1.1 10^{-4} M in a volume of 100 μL/well. The final concentration of DMSO in cell culture was maintained in all cases at 1%. 96 h later, 10 μL of a freshly diluted MTT solution (2.5 mg/mL) was pipetted into each well and the plate was incubated at 37 °C in a humidified 5% CO_2 atmosphere. After 5 h, the medium was removed and the obtained formazan product was dissolved in 100 μL of DMSO. The cell viability was evaluated by measurement of the absorbance at 595 nm. IC_{50} values (compound

concentration that produces 50% of cell growth inhibition) were calculated from curves constructed by plotting cell survival (%) versus drug concentration (μM). All experiments were made in triplicate.

Experimental

General remarks. The dinuclear arene ruthenium complexes $[(Cy)_2Ru_2(\mu\text{-}OO,O'O'\text{-}L)Cl_2]$ (**1a**: 1,4-benzoquinone-2,5-diolato; **1b**: 2,5-dichloro-*p*-benzoquinone-3,6-diolato; **1c**: 1,4-naftoquinone-5,8-diolato) were prepared according to published methods.[iv,v] All other reagents were commercially avaliable and used as received. General synthetic method for $[(Cy)_4Ru_4(\mu\text{-}OO,O'O'\text{-}L)_2(\mu\text{-phen})_2](CF_3SO_3)_4$. A mixture of **1** (1 mmol) and 2 equiv of $AgCF_3SO_3$ (2 mmol) in methanol (20 mL) is stirred at 40ºC for 2 h and filtered to remove AgCl. 4,7-phenanthroline (1 mmol) is added to the filtrate. The mixture is stirred at room temperature for 24 h to give a solid which is filtered off and washed with methanol and ether (Yield, 85%). Crystals of **2a** suitable for X-ray diffraction study were obtained by slow evaporation of a methanol solution of the complex at room temperature.

Cy: *p*-cymene

phen: 4,7-phenantroline

dhbq: 1,4-benzoquinone-2,5-diolato

chloranilato: 2,5-dichloro-*p*-benzoquinone-3,6-diolato

dhbq: 1,4-naftoquinone-5,8-diolato

A) Elemental analysis.

$[(Cy)_2Ru_2(dhbq)Cl_2]$ (**1a).** Elemen. Anal. Calcd (%) for $[(C_{10}H_{14})_2Ru_2(C_6H_2O_4)Cl_2](H_2O)_3$: C, 42.57; H, 4.94. Found: C, 40.11; H, 4.36.

$[(Cy)_2Ru_2(chloranilato)Cl_2]$ (**1b).** Elemen. Anal. Calcd (%) for $[(C_{10}H_{14})_2Ru_2(C_6Cl_2O_4)Cl_2](H_2O)_2(CH_3OH)_2$: C, 39.63; H, 4.75. Found: C, 39.10; H, 4.76.

$[(Cy)_2Ru_2(dhnq)Cl_2]$ (**1c).** Elemen. Anal. Calcd (%) for $[(C_{10}H_{14})_2Ru_2(C_{10}H_4O_4)Cl_2](H_2O)_2(CH_3OH)_2$: C, 46.32; H, 5.34. Found: C, 45.93; H, 5.34.

$[(Cy)_4Ru_4(dhbq)_2(phen)_2](CF_3SO_3)_4$ (**2a).** Elemen. Anal. Calcd (%) for $[(C_{10}H_{14})_4Ru_4(C_6H_2O_4)_2(C_{12}N_2H_8)_2](CF_3SO_3)_4(C_{12}N_2H_8)(H_2O)_{12}$: C, 42.98; H, 4.23; N, 3.27; S, 5.00. Found: C, 42.16; H, 4.15; N, 3.51; S, 6.27.

$[(Cy)_4Ru_4(chloranilato)_2(phen)_2](CF_3SO_3)_4$ (**2b).** Elemen. Anal. Calcd (%) for $[(C_{10}H_{14})_4Ru_4(C_6Cl_2O_4)_2(C_{12}N_2H_8)_2](CF_3SO_3)_4(C_{12}N_2H_8)(H_2O)_3(CH_3OH)_2$: C, 43.26; H, 3.63; N, 3.22; S, 4.91. Found: C, 43.13; H, 3.99; N, 3.49; S, 4.74.

$[(Cy)_4Ru_4(dhnq)_2(phen)_2](CF_3SO_3)_4$ (**2c).** Elemen. Anal. Calcd (%) for $[(C_{10}H_{14})_4Ru_4(C_{10}H_4O_4)_2(C_{12}N_2H_8)_2](CF_3SO_3)_4(C_{12}N_2H_8)_4(H_2O)_{20}(CH_3OH)_6$: C, 48.63; H, 4.66; N, 4.79.; S, 3.81. Found: C, 48.91; H, 3.91; N, 5.07; S, 3.66.

B) Crystallographic data

Table S1. Crystallographic data and refinement parameters for species **2a**.

	2a
formula	$C_{92}H_{88}F_{12}N_6O_{22}Ru_4S_4$
FW (g mol^{-1})	2390.2
T (K)	293(2)
λ (Å)	0.71073
crystal system	Monoclinic
space group	$P\,2_1$
a (Å)	12.424(3)
b (Å)	27.645(6)
c (Å)	14.359(3)
α (deg)	90
β (deg)	93.55(3)
γ (deg)	90
V(Å^3)	4922.2(2)
Z	2
ρ(calc) (Mg m^{-3})	1.613
μ (Mo-Kα, mm^{-1})	0.782
F(000)	2412
Sample Size (mm^3)	0.24×0.18×0.14
2θ range (deg)	1.42 - 24.7
hkl range	$-14 \le h \le 14$ $-16 \le k \le 32$ $-16 \le l \le 16$
unique, observed reflections	12282, 10200
R(int), R(sigma)	0.056, 0.036
data, restrains, parameters	12282, 1, 1251
$\chi(F^2)$ [a]	1.611
$R(F)$, $wR(F^2)$ for $I > 2\sigma(I)$ [a]	0.054, 0.139
$R(F)$, $wR(F^2)$ for all reflections [a]	0.071, 0.152
highest peak, deepest hole (e Å^{-3})	0.837, -0.479

[a] $\chi(F^2) = [\Sigma w(F_o^2 - F_c^2)^2/(n-p)]^{1/2}$ where n is the number of reflections, p the number of parameters and $w = 1/[\sigma^2(F_o^2) + (0.019P)^2 + 1.88P]$ with $P = (F_o^2 + 2F_c^2)/3$. $R(F) = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $wR(F^2) = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma wF_o^4]^{1/2}$.

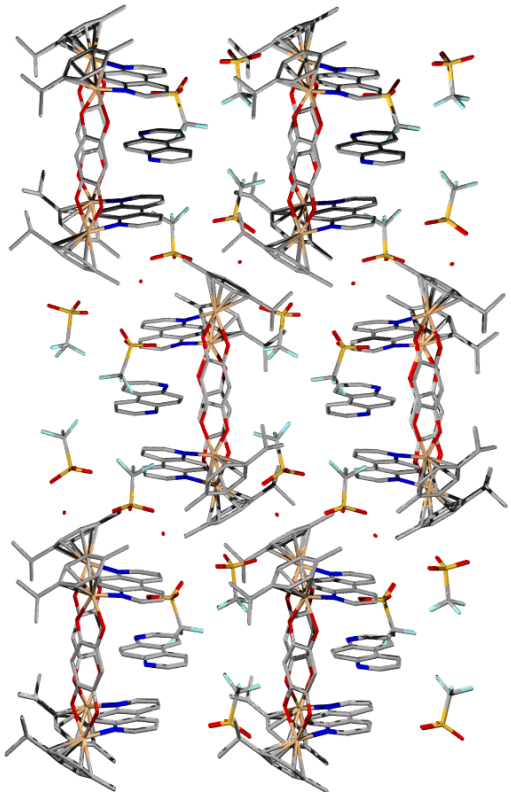
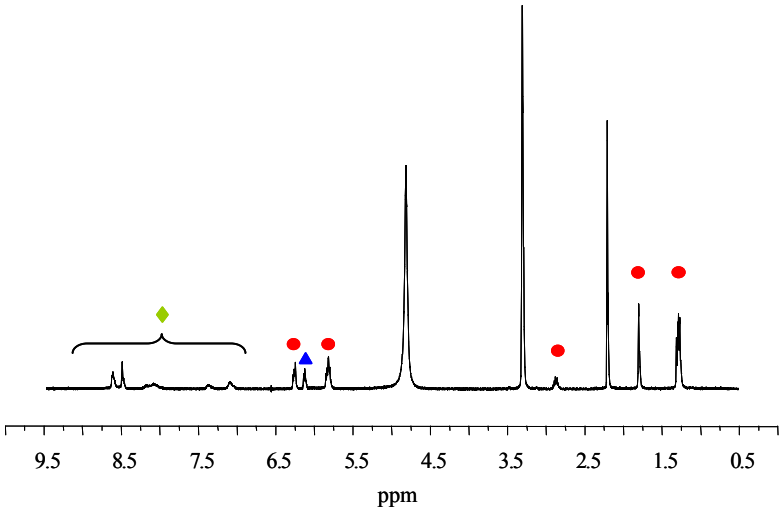


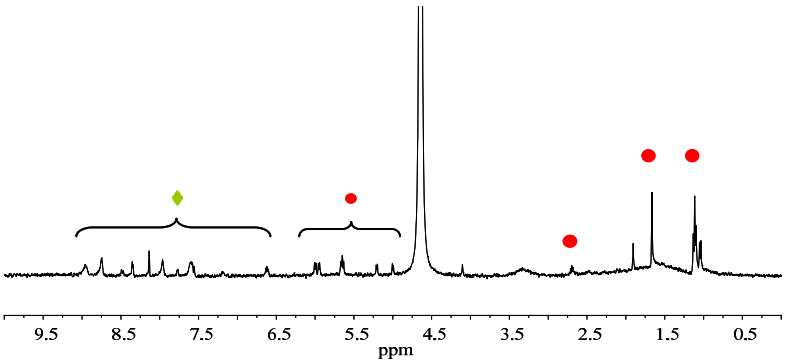
Figure S1. Perspective view of the packing of **2a** along the *b*-axis. The tetranuclear coordination rectangles build supramolecular zig-zag chains supported by π-π interactions established between the *p*-cymene moieties of adjacent assemblies.

C) ^{1}H NMR Spectra

a)



b)



c)

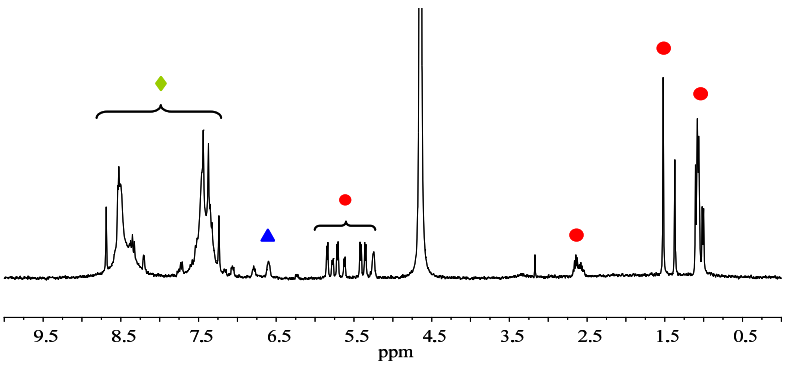
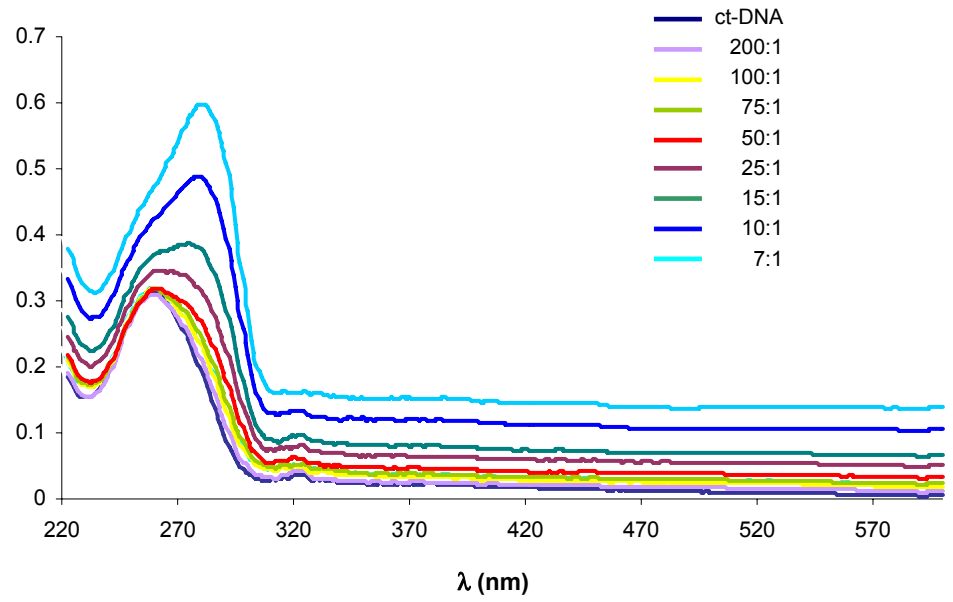


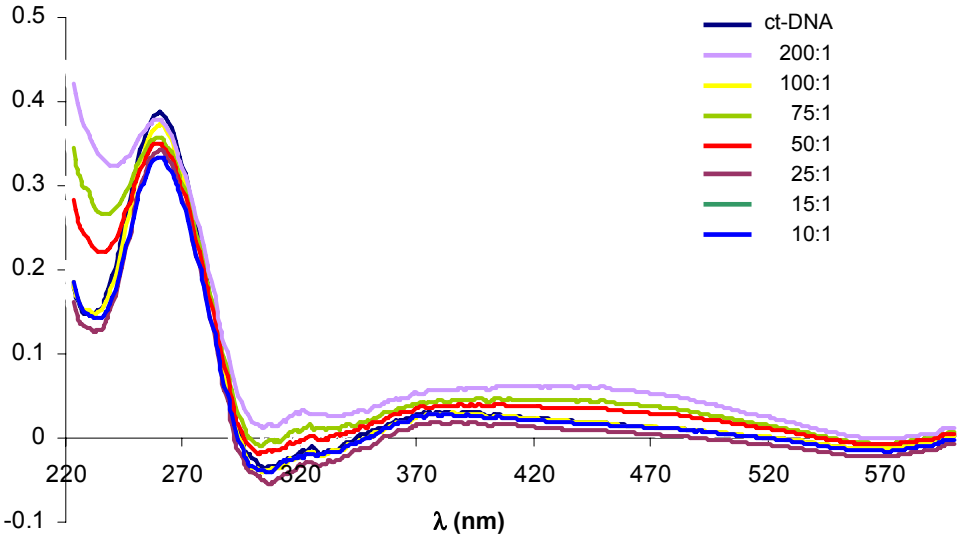
Figure S2. a) $[(Cy)_4Ru_4(dhbq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2a**), b) $[(Cy)_4Ru_4(chloranilato)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2b**) and c) $[(Cy)_4Ru_4(dhnq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2c**). (Relevant signals corresponding to Cy peaks (red circles), ligand (blue triangles); 4,7-phenantroline group (green diamonds) are marked).

D) UV-vis absorption spectra showing the interaction of *ct*-DNA with water soluble compounds 1a, 1c, 2a and 2c.

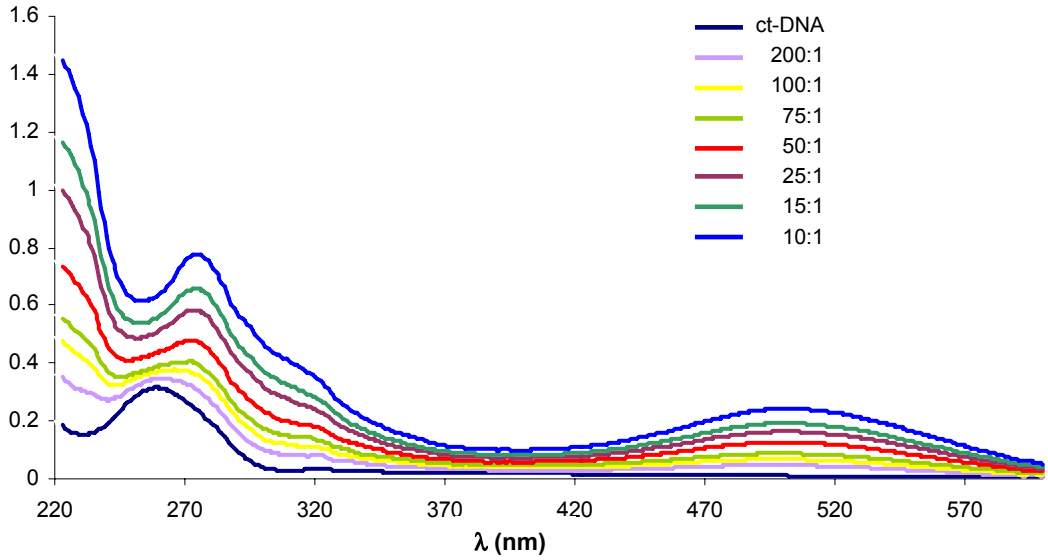
a)



b)



c)



d)

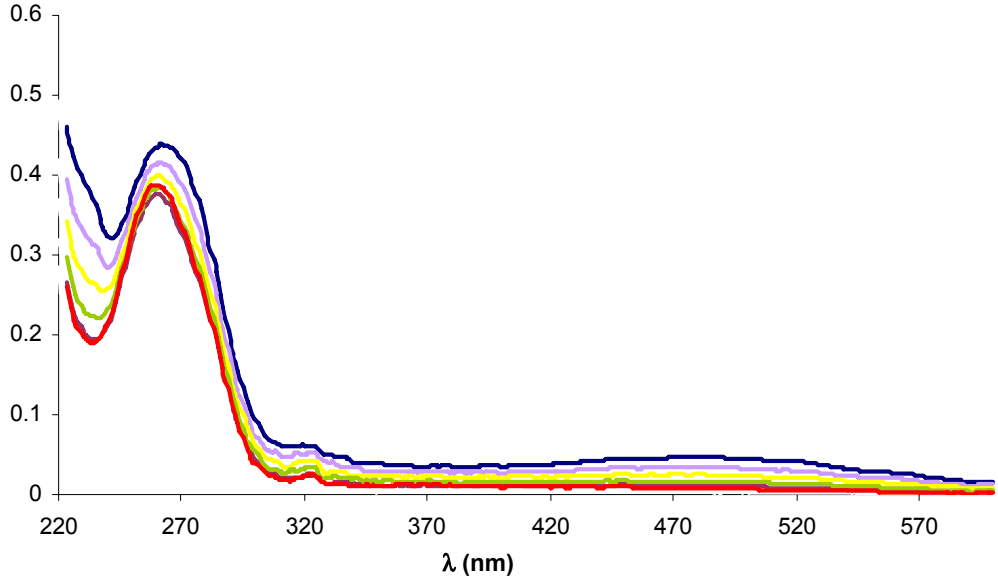
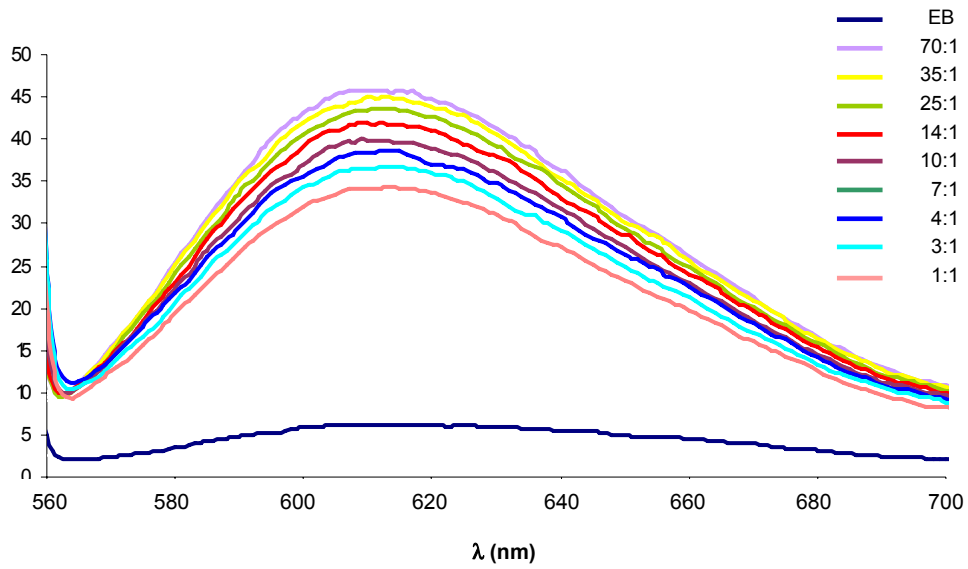


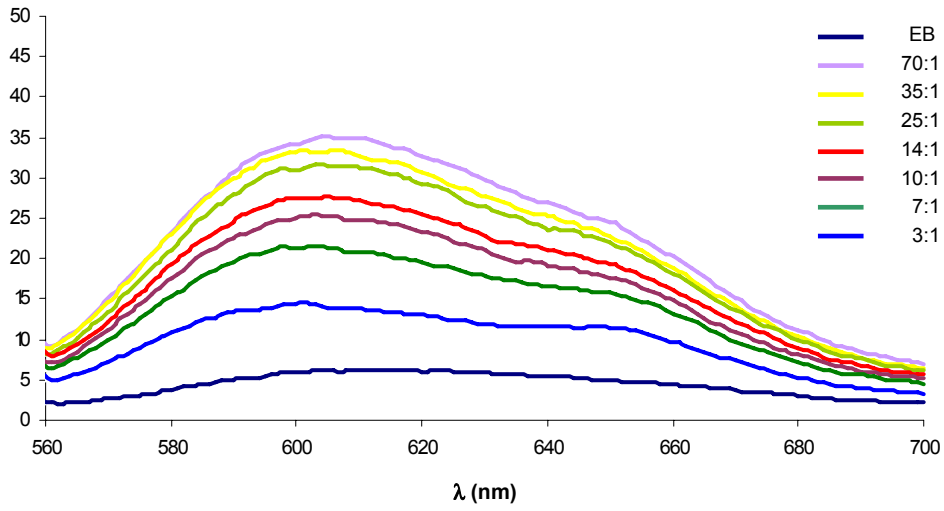
Figure S3. Effect of addition of the coordination assemblies a) $[(Cy)_2Ru_2(dhbq)Cl_2]$ (**1a**), b) $[(Cy)_2Ru_2(dhnq)_2Cl_2]$ (**1c**), c) $[(Cy)_4Ru_4(dhbq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2a**) and d) $[(Cy)_4Ru_4(dhnq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2c**), to a solution of *ct*-DNA (48 μM in 1 mM sodium cacodylate buffer and 20 mM NaCl) on the UV-vis spectra in a 200:1 to 7:1 ratio. Subtraction of the free coordination assemblies spectra has been made.

E) Fluorescence Spectra corresponding to the competitive binding assays with ethidium bromide.

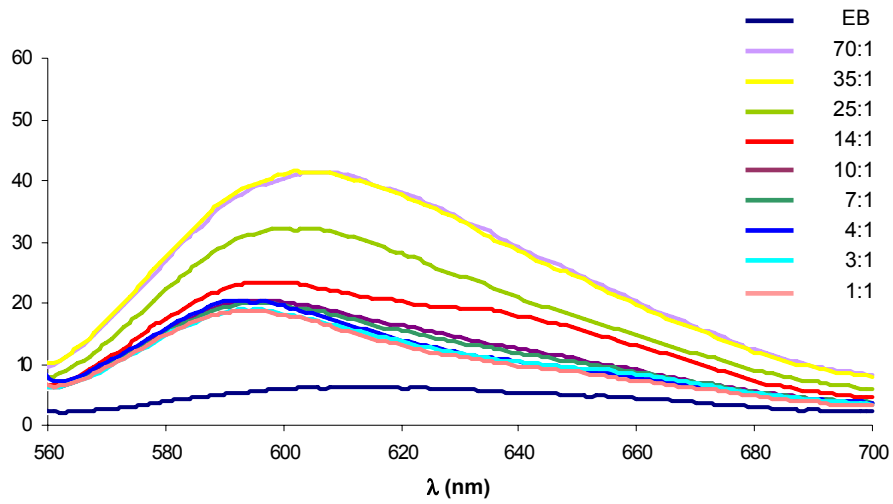
a)



b)



c)



d)

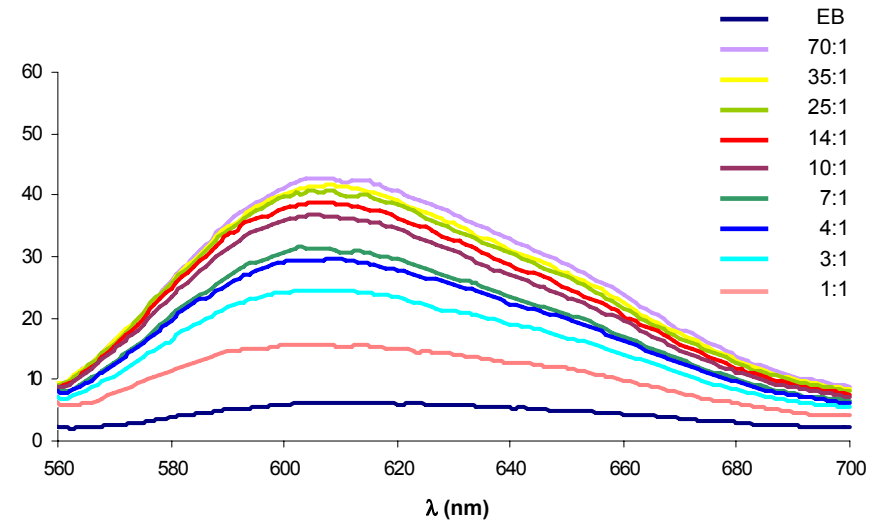
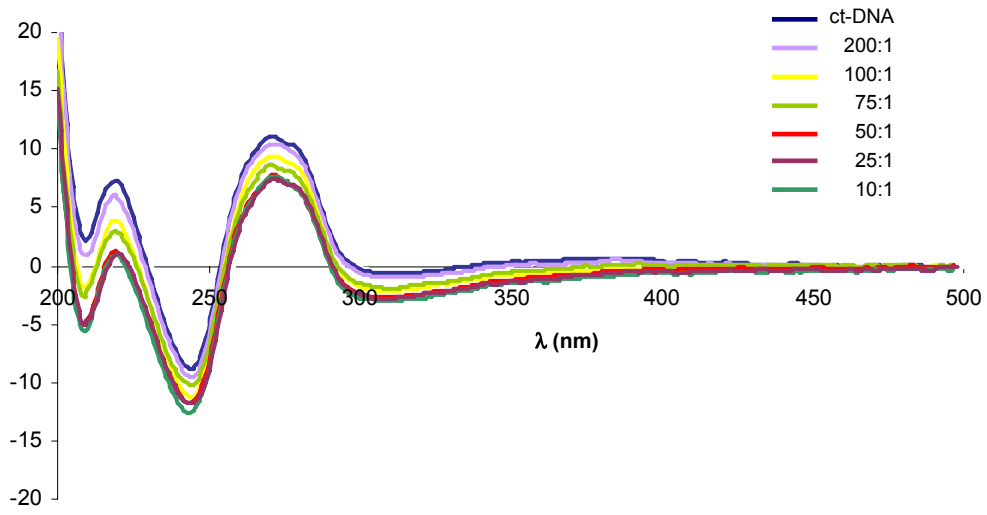


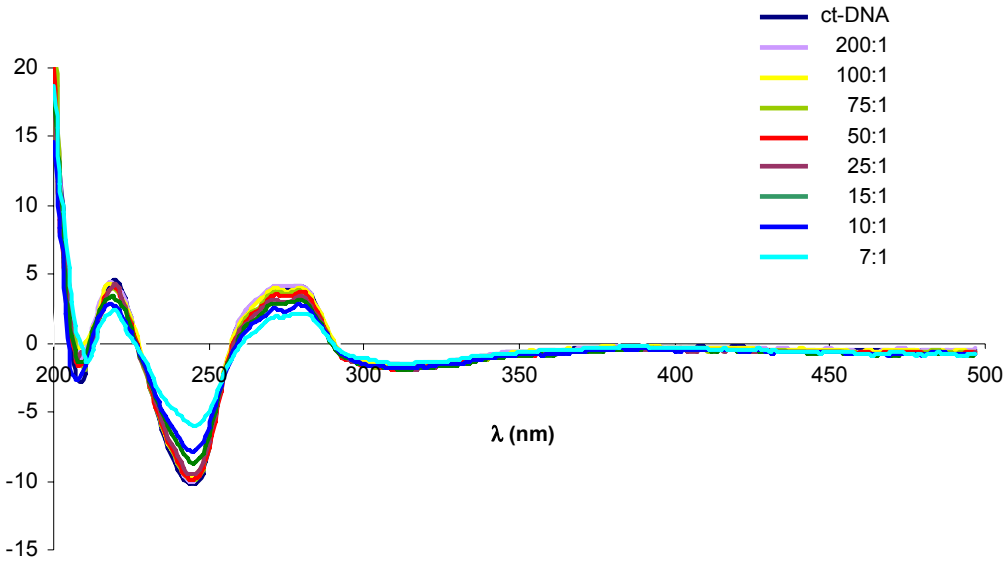
Figure S4. Competitive binding assays: fluorescence spectra of intercalated ethidium bromide (5 μM) in DNA (4 μM) upon addition of increasing amounts of a) **1a**, b) **1c**, c) **2a** and d) **2c** in 1 mM sodium cacodylate buffer and 20 mM NaCl. Emission of DNA free EB is shown as a dark blue line in all the graphs.

F) Circular Dichroism

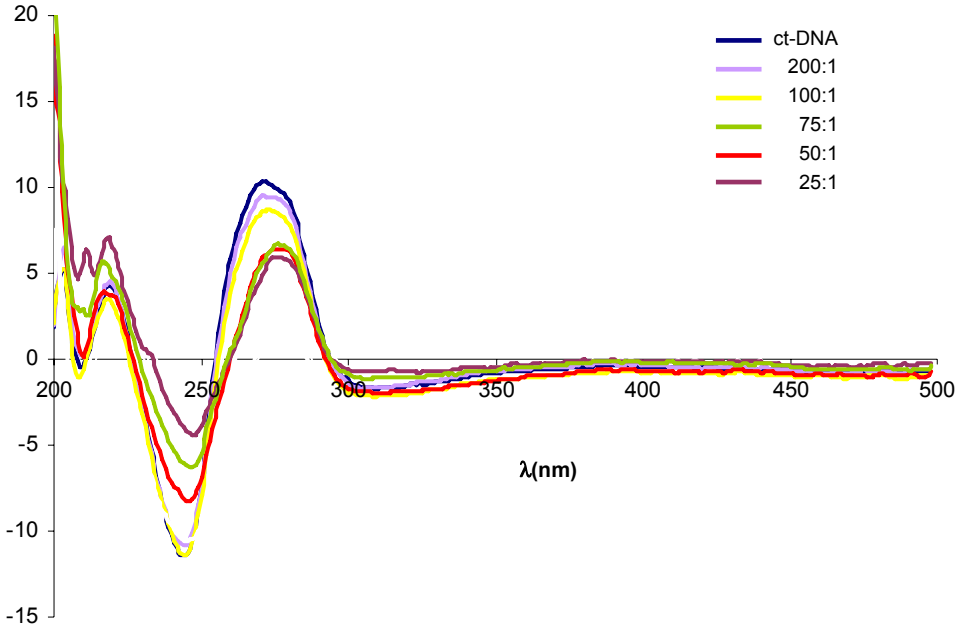
a)



b)



c)



d)

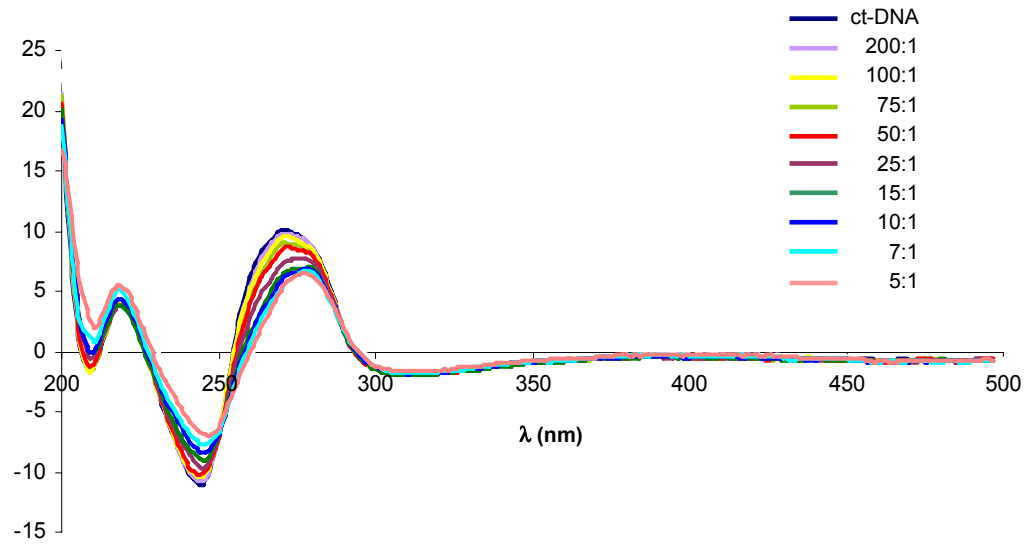


Figure S5. CD spectra of the tritation of *ct*-DNA (150 μM in a 1 mM sodium cacodylate buffer and 20 mM NaCl) with a) $[(Cy)_2Ru_2(dhbq)Cl_2]$ (**1a**), b) $[(Cy)_2Ru_2(dhnq)_2Cl_2]$ (**1c**), c) $[(Cy)_4Ru_4(dhbq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2a**) and d) $[(Cy)_4Ru_4(dhnq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2c**), in 200:1 to 5:1 ratio.

G) Reactivity with S-donor cysteine

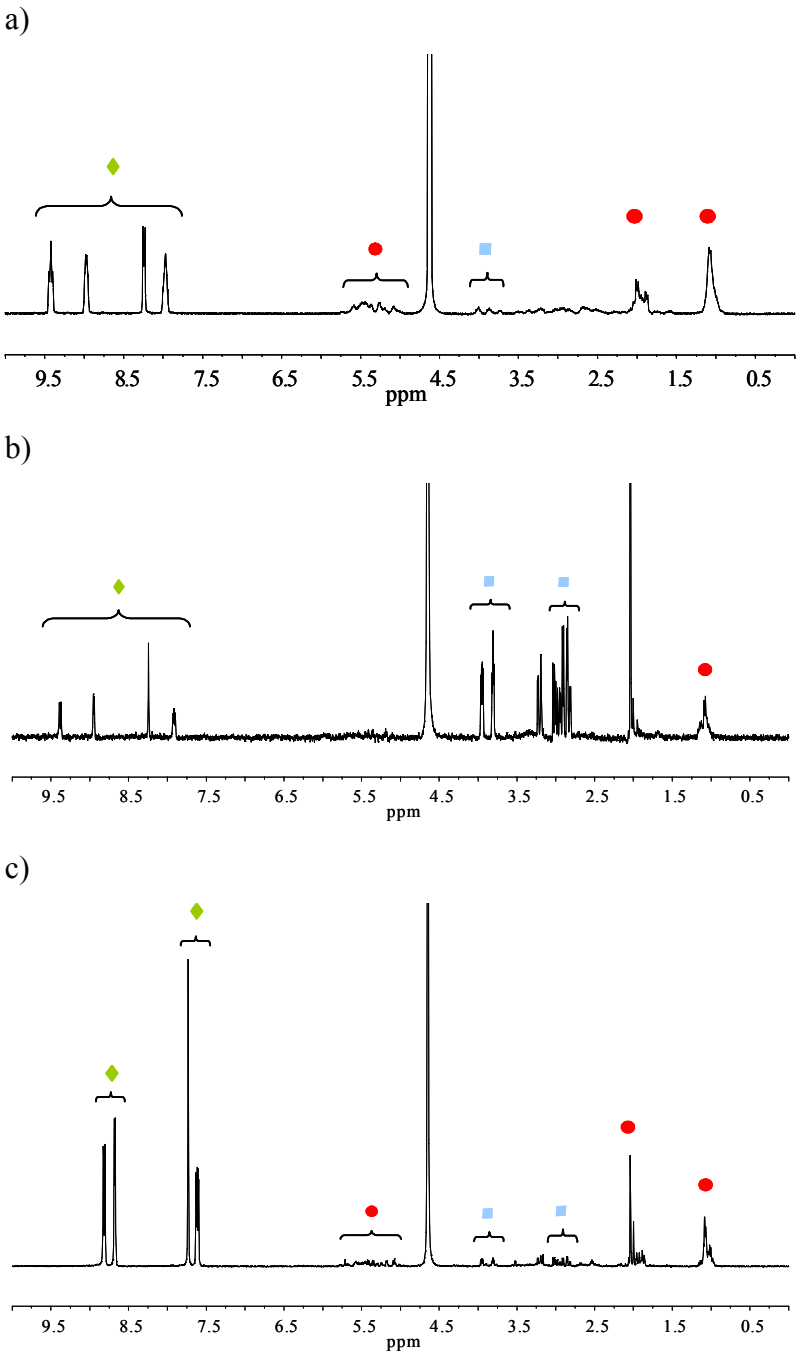


Figure S6. NMR spectra of the tetranuclear species incubated at 37 ºC (during two hours) with 4 equivalents of cysteine. ^{1}H NMR signals of free phenantroline (green diamonds) can be appreciated: a) $[(Cy)_4Ru_4(dhbq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2a**), b) $[(Cy)_4Ru_4(chloranilato)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2b**) and c) $[(Cy)_4Ru_4(dhnq)_2(4,7\text{-phen})_2](CF_3SO_3)_4$ (**2c**). (Relevant signals corresponding to Cy peaks (red circles); free 4,7-phenantroline (green diamonds) and cysteine (blue squares) are marked).

[i] Sheldrick, G. M. SADABS, software for empirical absorption correction, **1996**, University of Göttingen, Göttingen, Germany.

[ii] SIR97, Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115.

[iii] Sheldrick, G. M. SHELX-97, Program for crystal structure determination, **1997**, University of Göttingen, Göttingen, Germany.

[iv] F. Linares, M. A. Galindo, S. Galli, M. A. Romero, J. A. R. Navarro and E. Barea, *Inorg. Chem*. 2009, **48**, 7413.

[v] N. P. E. Barry and B. Therrien, *Eur. J. Inorg. Chem*. 2009, 4695.