Electronic Supplementary Material (ESI) for Dalton Transactions


# Supporting Information

# Chiral ruthenium(II) complexes with phenolic hydroxyl groups as dual poisons of topoisomerases I and IIα

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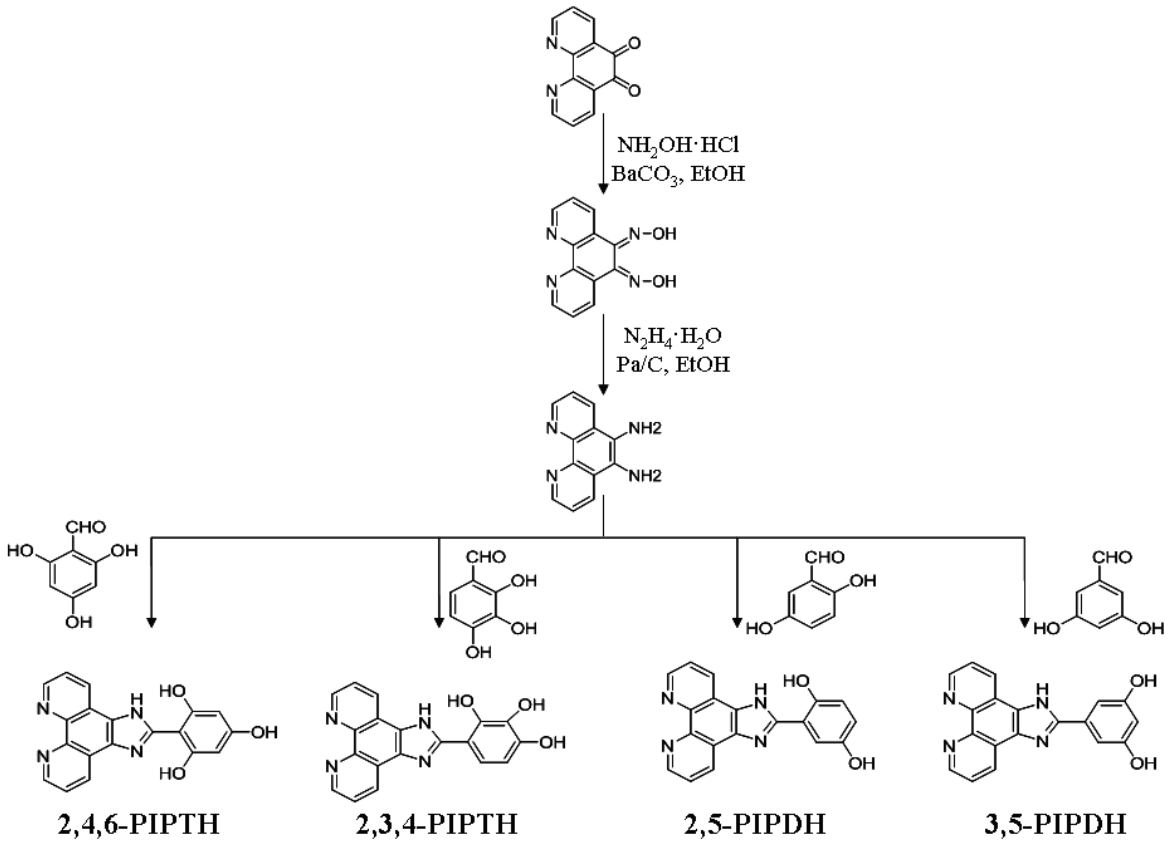



**Scheme S1**. Synthetic routes of ligands.

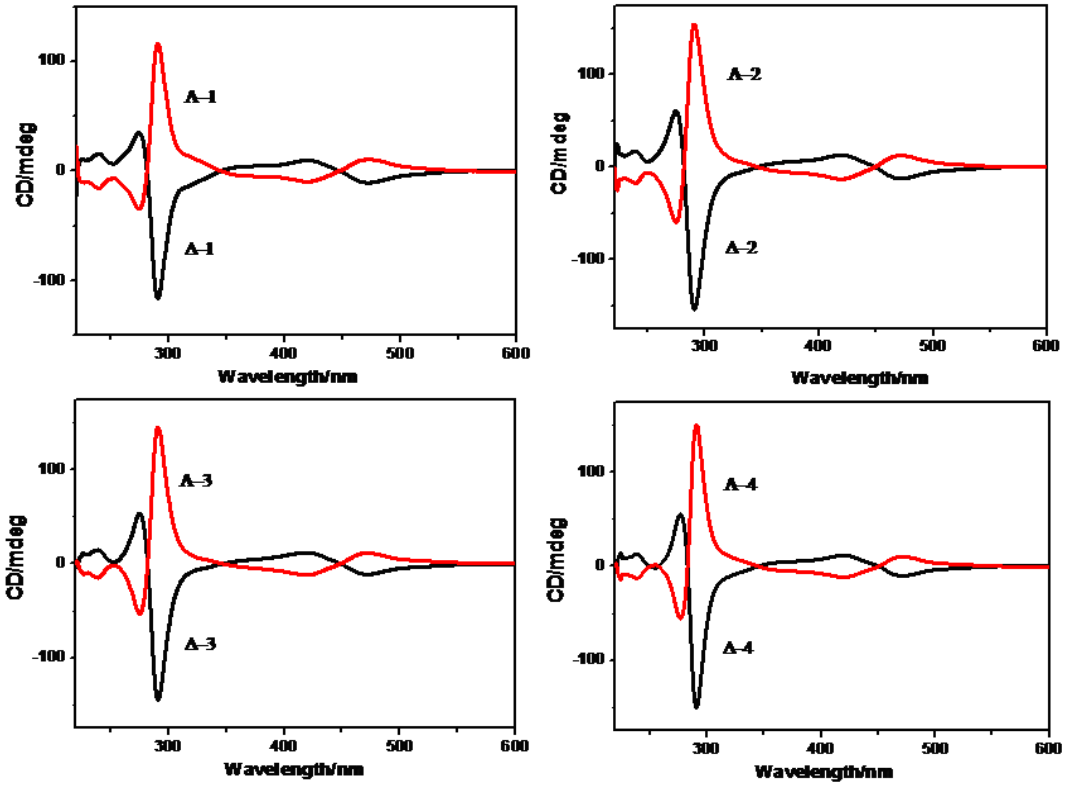
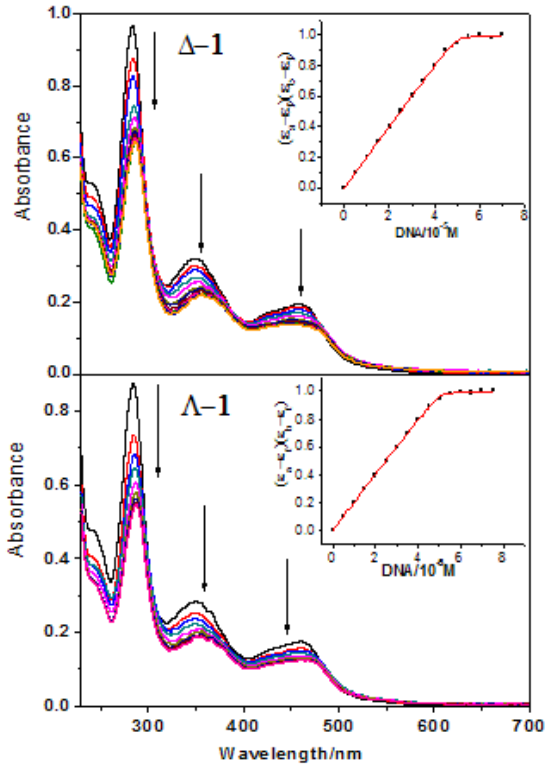
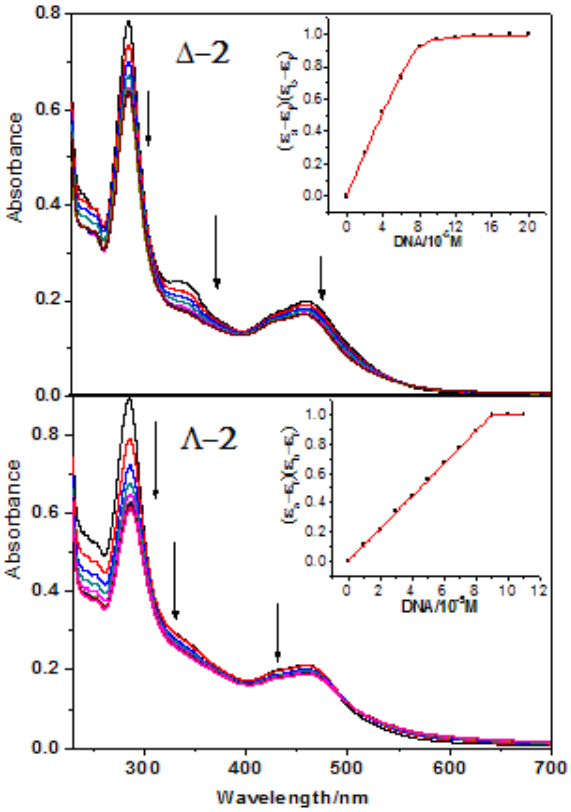
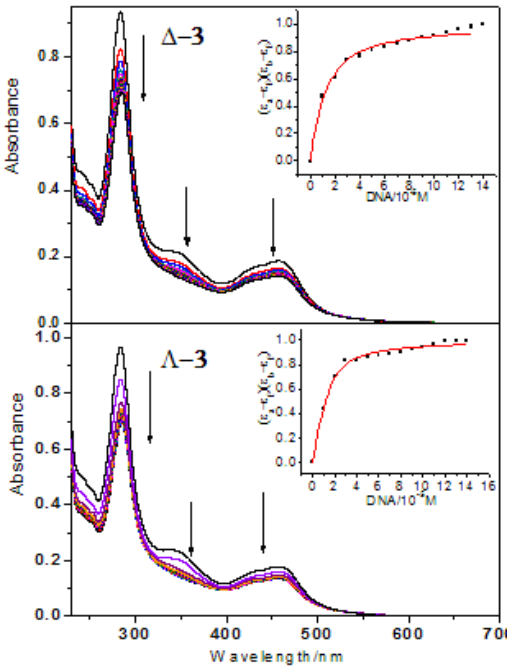



**Fig. S1**. CD spectra of ruthenium(II) complexes Δ-1 (black) and Λ-1 (red); Δ-2 (black) and Λ-2 (red); Δ-3 (black) and Λ-3 (red); Δ-4 (black) and Λ-4 (red) in $H_2O$, [Ru] = 50 μM.





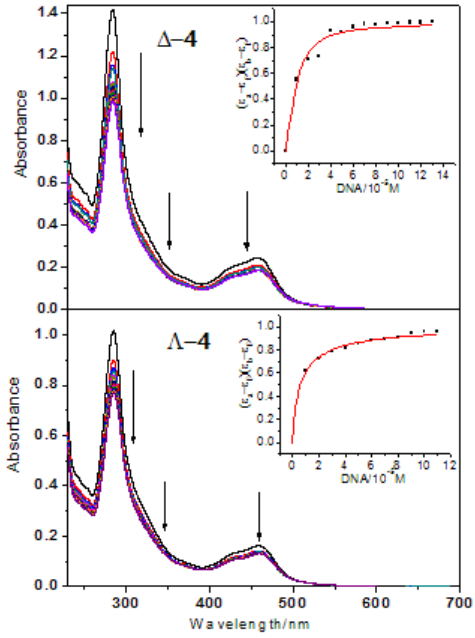




**Fig. S2**. Absorption spectra of ruthenium(II) complexes Δ-1 and Λ-1; Δ-2 and Λ-2; Δ-3 and Λ-3; Δ-4 and Λ-4. Arrows indicate the change in absorbance upon increasing the DNA concentration. ([Ru] = 10 μM, [DNA] = 0-170 μM).

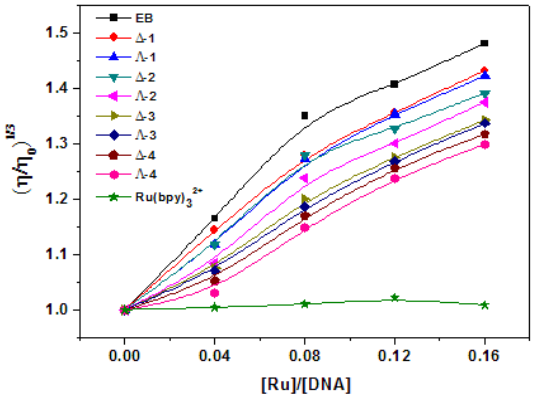



**Fig. S3**. Effect of increasing amounts of ethidium bromide (EB), [Ru(bpy)3]2+, Ru(II) complexes (Δ-1 and Λ-1; Δ-2 and Λ-2; Δ-3 and Λ-3; Δ-4 and Λ-4) on the relative viscosity of calf thymus DNA at 29±0.1 $^{o}$C. The total concentration of DNA is 0.25 mM.

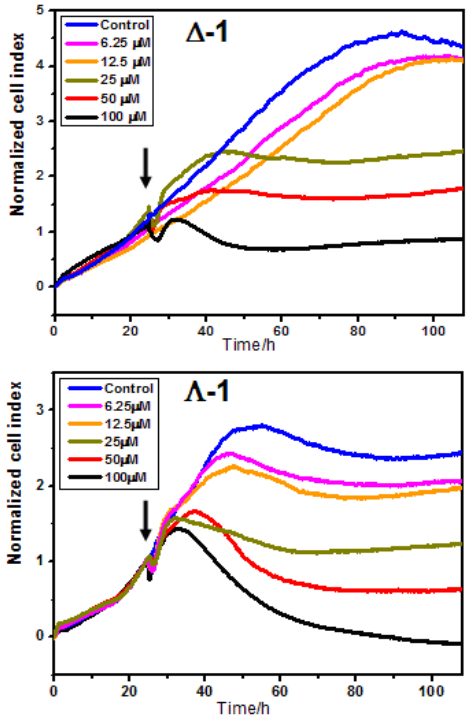



**Fig. S4.** Kinetics of cytotoxicity responsed for Ru(II) complexes in cells monitored by the xCELLigence System. Δ-**1** and Λ-**1** in Hep-G2 (5,000 cells/well) were plated in 16-well strips for the RT-CES cytotoxicity assay. Downward arrows mean the time of complexes addition.

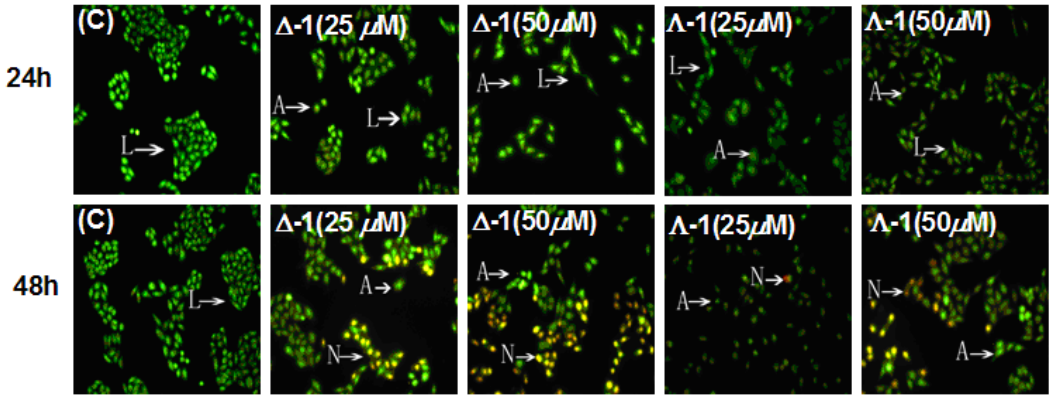



**Fig. S5.** Hep-G2 cells were stained by AO/EB and observed under fluorescence microscope: (C) Hep-G2 cells without treatment; in the presence of Ru(II) complexes Δ-**1** and Λ-**1** incubated at 37 °C and 5% $CO_2$ for 24 h and 48 h. Arrows pointed to the cells representing certain cell viable status: L—the live cells, A—the apoptotic cells, and N—the necrotic cells.

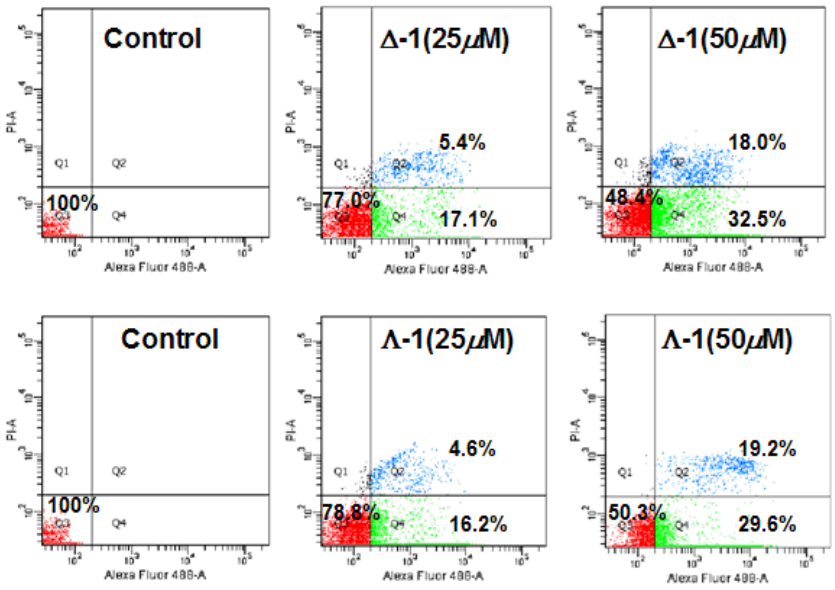



**Fig. S6**. Ru(II) complexes Δ-1 and Λ-1 induced apoptotic cell death were examined by Annexin V/PI assay. Hep-G2 cells were treated with different concentrations of Ru(II) complexes for 48 h.

**Table S1**. Electronic spectra, hypochromism (H%) and DNA-binding constants $K_b$ of Ru(II) complexes.

| Complex | λ/ nm | | Δλ / nm | H% | $K_b \times 10^5$ $M^{-1}$ |
|---|---|---|---|---|---|
| | Free | Bound | | | |
| Δ-1 | 460 | 463 | 3 | 23.7 | 9.6 |
| Λ-1 | 460 | 464 | 4 | 24.2 | 9.0 |
| Δ-2 | 458 | 459 | 1 | 10.7 | 5.1 |
| Λ-2 | 459 | 460 | 1 | 10.0 | 4.8 |
| Δ-3 | 457 | 459 | 1 | 13.7 | 5.7 |
| Λ-3 | 457 | 460 | 3 | 12.2 | 4.5 |
| Δ-4 | 457 | 460 | 3 | 14.7 | 5.5 |
| Λ-4 | 458 | 460 | 2 | 13.7 | 3.7 |

**Table S2**. Ruthenium concentrations determined in hypoxic cells after 1 h, 2 h and 4 h of exposure to 40 μM Δ-1 and Λ-1 by ICP-MS.[a]

| Time | | Ru(pg/cell) | | | | |
|---|---|---|---|---|---|---|
| | | Whole cell | Nuclues | Cytoplasm | Mitochondrion | Cytoplsma matrix |
| Δ-1 | 1 h | 5.401±0.51 | 3.708±0.26 | 1.668±0.16 | 1.207±0.09 | 0.460± 0.01 |
| | 2 h | 6.710±0.31 | 4.702±0.11 | 1.982±0.13 | 1.416±0.14 | 0.548±0.01 |
| | 4 h | 7.947±0.14 | 5.406±0.06 | 2.470±0.19 | 1.802±0.13 | 0.607±0.04 |
| Λ-1 | 1 h | 3.922±0.39 | 2.619±0.41 | 1.222±0.23 | 1.001±0.10 | 0.320±0.05 |
| | 2 h | 5.339±0.10 | 3.456±0.31 | 1.833±0.34 | 1.402±0.02 | 0.484±0.02 |
| | 4 h | 7.380±0.17 | 5.281±0.25 | 2.013±0.17 | 1.643±0.12 | 0.490±0.01 |

[a]Data are presented as mean values±standard deviations, and cell viability is assessed after 48 h of incubation.

**Table S3.** JC-1 red/green ratio signals of Hep-G2 cells after 12 h of incubation.

| Complex | $\mu$M | Red (%) | Green (%) | red/green ratio |
|---|---|---|---|---|
| Control | 0 | 89.4 | 10.6 | 8.43 |
| Δ-**1** | 25 | 79.8 | 20.2 | 3.95 |
| | 50 | 47.5 | 52.5 | 0.90 |
| Λ-**1** | 25 | 74.8 | 25.2 | 2.96 |
| | 50 | 48.7 | 51.3 | 0.94 |

# References

1 M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc.*, 1989, **111**, 8901–8911.

2 J. B. Chaires, N. Dattagupta and D. M. Crothers, *Biochem.*, 1982, **21**, 3933–3940.