Electronic Supplementary Material (ESI) for Metallomics

Supplementary Information

Contrasting cellular uptake pathways for chlorido and iodido iminopyridine ruthenium arene anticancer complexes

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Tables S1 – S11

Table S1. Extent of aquation for complexes **1** and **2** after 24 h, using 2 mM solutions of each complex in phosphate buffer (pH 7.2) Each value represents the mean ± SD for three independent NMR experiments at 310 K.

Compound		% aquation
1	[Ru(η^6-*p*-cym)(*p*-Impy-NMe_2)Cl]PF_6	66 ± 6
2	[Ru(η^6-*p*-cym)(*p*-Impy-NMe_2)I]PF_6	63 ± 3

Table S2. Time dependence. Total accumulation of Ru in A2780 cells for complexes **1, 2** and CDDP after various periods of drug exposure at 310 K with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells						
	Drug exposure time (h)						
	1	**4**	**8**	**24**	**48**	**72**	**96**
CDDP	0.0011 ± 0.0001	0.014 ± 0.004	0.11 ± 0.08	0.25 ± 0.03	0.29 ± 0.02	0.26 ± 0.01	0.25 ± 0.02
1	1.32 ± 0.09	3.5 ± 0.4	4.2 ± 0.1	7.6 ± 0.8	8.9 ± 0.6	5.8 ± 0.4	4.0 ± 0.2
2	6.5 ± 0.4	8.2 ± 0.4	9 ± 2	11 ± 1	13.0 ± 0.7	10.7 ± 0.3	7.4 ± 0.1

Table S3. Temperature dependence. Total accumulation of Ru in A2780 cells for complexes **1, 2** and CDDP after 8 h of drug exposure at various temperatures with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells		
	Temperature (K)		
	277	**293**	**310**
CDDP	N/D	0.005 ± 0.002	0.12 ± 0.03
1	0.14 ± 0.04	0.61 ± 0.06	4.6 ± 0.6
2	0.8 ± 0.1	4.1 ± 0.9	10.7 ± 0.6

Table S4. Concentration dependence. Total accumulation of Ru in A2780 cells for complexes **1, 2** and CDDP after 24 h of drug exposure at 310 K with no recovery time.

	ng Ru/Pt x10^6 cells					
	Concentration (μM)					
	0.16 x IC_{50}	**0.33 x IC_{50}**	**1.6 x IC_{50}**	**3.2 x IC_{50}**	**6.4 x IC_{50}**	**9.6 x IC_{50}**
CDDP	0.16 ± 0.02	0.28 ± 0.05	2.1 ± 0.3	4.8 ± 0.5	10 ± 1	11 ± 3
1	4.1 ± 0.8	8.0 ± 0.3	40 ± 7	95 ± 3	N/V	N/V
2	5 ± 1	11.4 ± 0.4	42 ± 5	57 ± 6	N/V	N/V

Table S5. Extent of efflux. Total accumulation of Ru in A2780 cells for complexes **1** and **2** after 24 h of drug exposure and various recovery times at 310 K. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells			
	Recovery Time (h)			
	0	**24**	**48**	**72**
1	7.8 ± 0.1	3.0 ± 0.3	1.7 ± 0.1	1.7 ± 0.4
2	11.8 ± 0.8	3.7 ± 0.2	2.8 ± 0.3	2.79 ± 0.07

Table S6. Inhibition of efflux. Total accumulation of Ru in A2780 cells for complexes **1** and **2** after 24 h of drug exposure and 24 h of recovery time in drug-free medium that contained various concentrations of verapamil. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells				
	Cellular Accumulation[A]	**Verapamil (μM)**			
		0[B]	**5[C]**	**10[D]**	**20[E]**
1	7.5 ± 0.5	3.0 ± 0.3	4.1 ± 0.5	4.9 ± 0.2	5.3 ± 0.4
2	11.9 ± 0.8	3.7 ± 0.2	4.8 ± 0.3	6.1 ± 0.4	7.2 ± 0.2

Table S7. Role of Na^+/K^+ pump in cellular metal accumulation, as a facilitated diffusion endocytosis pathway. Total accumulation of Ru in A2780 cells when co-incubated with complexes **1, 2,** CDDP and various concentrations of ouabain after 24 h of drug exposure at 310 K with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells					
	Ouabain (μM)					
	0	**5**	**10**	**20**	**100**	**200**
CDDP	0.24 ± 0.05	0.22 ± 0.02	0.21 ± 0.04	0.27 ± 0.06	0.18 ± 0.0.5	0.12 ± 0.03
1	7.5 ± 0.2	5.7 ± 0.4	5.5 ± 0.2	5.1 ± 0.6	4.9 ± 0.6	3.8 ± 0.3
2	11.9 ± 0.3	9.7 ± 0.4	9.2 ± 0.3	8.9 ± 0.5	8.5 ± 0.4	7.5 ± 0.2

Table S8. Role of CTR1 in cellular metal accumulation. Total accumulation of Ru in A2780 cells when co-incubated with complexes **1, 2,** CDDP and various concentrations of copper(II) chloride after 24 h of drug exposure at 310 K with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells					
	Copper(II) chloride (μM)					
	0	**10**	**20**	**40**	**100**	**200**
CDDP	0.24 ± 0.05	0.22 ± 0.02	0.19 ± 0.03	0.15 ± 0.04	0.10 ± 0.2	0.08 ± 0.01
1	7.5 ± 0.2	7.0 ± 0.3	6.8 ± 0.5	6.7 ± 0.3	5.2 ± 0.5	4.6 ± 0.3
2	11.9 ± 0.3	11.0 ± 0.5	10.8 ± 0.2	10.1 ± 0.6	9.5 ± 0.3	8.8 ± 0.4

Table S9. Effect of ATP depletion in cellular metal accumulation. Total accumulation of Ru in A2780 cells when co-incubated with complexes **1, 2,** CDDP and 5 μM of antimycin A_1 after 24 h of drug exposure at 310 K with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells	
	Antimycin A_1 (μM)	
	0	**5**
CDDP	0.24 ± 0.05	0.22 ± 0.02
1	7.5 ± 0.2	32 ± 2
2	11.9 ± 0.3	13.6 ± 0.4

Table S10. Membrane disruption by amphotericin B as a model for protein-mediated uptake. Total accumulation of Ru in A2780 cells when co-incubated with complexes **1, 2,** CDDP and various concentrations of amphotericin B after 24 h of drug exposure at 310 K with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells			
	Amphotericin B (μM)			
	0	**1**	**5**	**10**
CDDP	0.24 ± 0.05	0.28 ± 0.03	0.35 ± 0.05	0.49 ± 0.05
1	7.5 ± 0.2	7.7 ± 0.2	9.8 ± 0.3	10.2 ± 0.5
2	11.9 ± 0.3	13.0 ± 0.4	18.5 ± 0.7	25.4 ± 0.6

Table S11. The role of caveolae endocytotic pathway in metal accumulation. Total accumulation of Ru in A2780 cells when co-incubated with complexes **1, 2,** CDDP and various concentrations of β-methyl cyclodextrin after 24 h of drug exposure at 310 K with no recovery time. Equipotent concentrations used were CDDP = 0.4 μM, **1** = 5 μM and **2** = 1 μM.

	ng Ru/Pt x10^6 cells				
	β-methyl cyclodextrin (μM)				
	0	**10**	**20**	**500**	**1000**
CDDP	0.24 ± 0.05	0.20 ± 0.06	0.26 ± 0.03	0.23 ± 0.05	0.27 ± 0.02
1	7.5 ± 0.2	7.8 ± 0.4	7.5 ± 0.2	7.6 ± 0.4	7.3 ± 0.3
2	11.9 ± 0.3	11.2 ± 0.5	11.9 ± 0.5	12.2 ± 0.4	12.1± 0.6