Supporting Informations

Mitigating UVA Light Induced Reactivity of 6-Thioguanine through Formation of a Ru(II) Half-Sandwich Complex

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Materials and Methods:

$RuCl_3 \cdot xH_2O$, 6-mercaptopurine monohydrate (6-MP) and 6-thioguanine (6-TG) were obtained from Sigma-Aldrich (India). α-Phellandrene was brought from Merck, India. Precursors for ruthenium complexes were prepared according to the literature procedures.[1, 2] All reactions were carried out in an atmosphere of dry nitrogen using standard Schlenk and vacuum line techniques, and the solvents were dried by standard methods.[3] ^1H, and $^{13}C\{H\}$ NMR spectra were recorded using Bruker AMX 400 operating at 400 MHz for ^1H, 100 MHz for ^{13}C NMR in $[D_6]$-DMSO. HRMS of all samples were recorded in Agilent 6538 Ultra High Definition (UHD) Accurate-Mass Q-TOF. Elemental analyses were performed using Thermo Scientific Flash EA 2000 CHNS Analyzer. IR spectra were recorded on Bruker ALPHA 10 FT-IR spectrometer using neat samples using the diamond-ATR. UV-Vis spectra were recorded using a Perkin-Elmer Lamda 35 UV-Visible spectrophotometer.

Synthesis and Characterization:

[Ru(η^6-cymene)Cl(6-mercaptopurine)]Cl (RuMPCl):

6-Mercaptopurine monohydrate (83.5 mg, 0.50 mmol) was added to a solution of $[(\eta^6\text{-cymene})RuCl_2]_2$ (150 mg, 0.25 mmol) in dry DCM (10 mL). Within 1 h of stirring at room temperature, an orange colored precipitate was formed. The heterogeneous mixture was stirred for another 3 h. After that, it was filtered, washed with DCM followed by diethyl ether and dried in air to get a free flowing bright yellow colored powder. A needle shaped single crystal suitable for X-ray diffraction was obtained from diffusion of diethyl ether into DMF solution of the complex. Yield : 78%. ^1H NMR (400 MHz, $[D_6]$-DMSO, 20 °C): δ = 1.11 (dd, J = 14.8 Hz, J = 7.2 Hz, 6H), 2.12 (s, 3H), 2.72 (sept, 1H), 5.75 (d, J = 6.0 Hz, 1H), 5.88 (d, J = 6.0 Hz, 1H), 5.97 (d, J = 6.0 Hz, 1H), 6.06 (d, J = 6.0 Hz, 1H), 8.65 (s, 1H), 9.42 ppm (s, 1H); ^{13}C NMR (100 MHz, $[D_6]$-DMSO, 20 °C): δ = 19.3, 22.6, 23.1, 31.4, 81.9, 82.8, 82.9, 84.0, 100.8, 104.0, 137.7, 145.3, 148.1, 149.6, 172.2 ppm; IR: $\tilde{\upsilon}$ = 850, 1640 cm^{-1}; UV-Vis in MeOH [λ_{max}, nm (ε, $M^{-1}cm^{-1}$)]: 334 (6,200); element alanalysis calcd (%) for $C_{15}H_{18}Cl_2N_4RuS$: C 39.3, H 4.0, N 12.2, S 7.0; found: C 39.6, H 4.4, N 11.6, S 7.4; Q-TOF HRMS: *m/z*, analysis calcd for $C_{15}H_{17}N_4RuS^+$: 387.0217 $[M\text{-}2Cl^-\text{-}H^+]^+$; found: 387.0213.

[Ru(η^6-cymene)Cl(6-thioguanine)]Cl (RuTGCl):

6-Thioguanine (86 mg, 0.50 mmol) was added to a solution of $[(\eta^6\text{-cymene})RuCl_2]_2$ (150 mg, 0.25 mmol) in dry DCM (10 mL). Within 3 h of stirring at room temperature, an orange

colored precipitate was formed. The heterogeneous mixture was stirred for another 9 h. After that, it was filtered, washed with DCM followed by diethyl ether and dried in air to get a free flowing bright yellow colored powder. A needle shaped single crystal suitable for X-ray diffraction was obtained from diffusion of diethyl ether into DMF solution of the complex. Yield : 77%. ^{1}H NMR (400 MHz, [D_6]-DMSO, 20 °C): δ = 1.10 (dd, J = 11.2 Hz , J = 5.2 Hz, 6H), 2.11 (s, 3H), 2.69 (sept, 1H), 5.69 (d, J = 6.0 Hz, 1H), 5.82 (d, J = 6.0 Hz, 1H), 5.92 (d, J = 6.0 Hz, 1H), 5.99 (d, J = 6.0 Hz, 1H), 7.59 (s br 2H; NH_2), 9.00 (s, 1H), 13.99 ppm (s br 2H; 2 NH); ^{13}C NMR (100 MHz, [D_6]-DMSO, 20 °C): δ = 19.2, 22.6, 23.0, 31.4, 81.5, 82.6, 82.9, 83.8, 100.8, 103.6, 131.2, 145.4, 148.4, 157.2, 172.0 ppm; IR: $\tilde{\upsilon}$ = 815, 1598, 3366, 3317 cm^{-1}; UV-Vis in MeOH [λ_{max}, nm (ε, $M^{-1}cm^{-1}$)]: 344 (7,300); elemental analysis calcd (%) for $C_{15}H_{19}Cl_2N_5RuS.H_2O$: C 36.7, H 4.3, N 14.3, S 6.5; found: C 36.8, H 4.5, N 15.0, S 7.7; Q-TOF HRMS: *m/z*, analysis calcd for $C_{15}H_{18}N_5RuS^+$: 402.0326 [M-2Cl^--H^+]$^+$; found: 402.0326.

[Ru(η^6-cymene)Br(6-mercaptopurine)]Br (RuMPBr):

6-Mercaptopurine monohydrate (21.8 mg, 0.12 mmol) was added to a solution of [(η^6-cymene)$RuBr_2$]$_2$ (50 mg, 0.06 mmol) in dry methanol (10 mL). The mixture was refluxed for 2 h. After that, it was concentrated to 2 mL and diethyl ether was added to get an orange colored precipitate. The precipitate was washed with DCM followed by diethyl ether and dried in air. A needle shaped single crystal suitable for X-ray diffraction was obtained from diffusion of diethyl ether into DMF solution of the complex. Yield : 55%. ^{1}H NMR (400 MHz, [D_6]-DMSO, 20 °C): δ = 1.13 (dd, J = 10.8 Hz, J = 4.0 Hz, 6H), 2.18 (s, 3H), 2.78 (sept, 1H), 5.78 (d, J = 5.2 Hz, 1H), 5.88 (d, J = 5.2 Hz, 1H), 5.98 (d, J = 6.0 Hz, 1H), 6.06 (d, J = 6.0 Hz, 1H), 8.68 (s, 1H), 9.45 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]-DMSO, 20 °C): δ = 19.7, 22.7, 23.1, 31.6, 82.3, 82.8, 82.9, 83.9, 100.6, 104.7, 137.8, 145.5, 148.3, 149.9, 172.1 ppm; UV-Vis in MeOH [λ_{max}, nm (ε, $M^{-1}cm^{-1}$)]: 338 (6,500); element alanalysis calcd (%) for $C_{15}H_{18}Br_2N_4RuS$: C 33.0, H 3.3, N 10.3, S 5.9; found: C 33.6, H 3.5, N 10.0, S 5.1; Q-TOF HRMS: *m/z*, analysis calcd for $C_{15}H_{17}N_4RuS^+$: 387.0217 [M-2Br^--H^+]$^+$; found: 387.0211.

[Ru(η^6-cymene)Br(6-thioguanine)]Br (RuTGBr):

6-Thioguanine (27.9 mg, 0.16 mmol) was added to a solution of [(η^6-cymene)$RuBr_2$]$_2$ (66 mg, 0.08 mmol) in dry methanol (10 mL). The mixture was refluxed for 2 h. After that, it was concentrated to 2 mL and diethyl ether was added to get an orange colored precipitate. The

precipitate was washed with DCM followed by diethyl ether and dried in air. Yield : 73%. ^{1}H NMR (400 MHz, [D_6]-DMSO, 20 °C): δ = 1.09 (dd, J = 24.8 Hz, J = 6.8 Hz, 6H), 2.18 (s, 3H), 2.75 (sept, 1H), 5.72 (d, J = 5.6 Hz, 1H), 5.82 (d, J = 6.0 Hz, 1H), 5.93 (d, J = 5.6 Hz, 1H), 5.99 (d, J = 6.0 Hz, 1H), 7.49 (s br, 2H; NH_2), 9.01 (s, 1H), 13.88 ppm (s br, 2H; 2 NH) ; ^{13}C NMR (100 MHz, [D_6]-DMSO, 20 °C): δ = 19.7, 22.7, 23.0, 31.6, 81.9, 82.8, 82.9, 83.7, 100.5, 104.3, 131.3, 145.8, 148.5, 157.1, 170.5 ppm; UV-Vis in MeOH [λ_{max}, nm (ε, $M^{-1}cm^{-1}$)]: 378 (4,700);elemental analysis calcd (%) for $C_{15}H_{19}Br_2N_5RuS$: C 32.0, H 3.4, N 12.5, S 5.7; found: C 32.2, H 3.4, N 12.2, S 5.1; Q-TOF HRMS: *m/z*, analysis calcd for $C_{15}H_{18}N_5RuS^+$: 402.0326 $[M\text{-}2Br^-\text{-}H^+]^+$; found: 402.0331.

Single crystal X-ray crystallography:

Single crystals of complexes were separately glued to the tip of glass fibers along the largest dimension. Data were collected on a Bruker AXS single crystal diffractometer controlled by the SMART[4] software package with Kappa APEX CCD detector and a sealed Mo Kα (λ = 0.71073) source working at 2.2 KW and 50/35 (KV/mA). Intensity data were collected at room temperature. Crystallographic computations were performed using the WinGX (1.63.02) package.[5] The data were corrected for Lorentz and polarization effects. The structures were solved by direct methods (SIR-92) followed by the full-matrix least square procedure of F^2 for all reflections (SHELXL-97).[6] All non hydrogen atoms were refined by anisotropic displacement parameters and hydrogen atoms were located or, fixed at idealized positions. Structures were drawn using ORTEP-3 for Windows.[7]

Table S1: Selective bond distances (Å) and angles (°) for **RuMPCl** and **RuMPBr**

Bond distance/ angles	**RuMPCl** (X1 = Cl)	**RuMPBr** (X1 = Br)
Ru1-S1	2.4443(16)	2.4498(15)
Ru1-X1	2.3917(15)	2.5346(9)
Ru1-N1	2.108(4)	2.124(4)
N1-Ru1-X1	83.78(11)	84.02(10)
N1-Ru1-S1	83.22(12)	83.48(11)
S1-Ru1-X1	86.65(6)	86.28(4)

Table S2: Selective bond distances (Å) and angles (°) for **RuTGCl.DMF**

Bond distance/ angles	**RuTGCl.DMF**		
Ru1-S1	2.4409(11)	Ru2-S2	2.4552(13)
Ru1-Cl1	2.3952(12)	Ru2-Cl2	2.3919(12)
Ru1-N5	2.113(3)	Ru2-N10	2.114(3)
N5-Ru1-Cl1	83.31(8)	N10-Ru2-Cl2	83.51(8)
N5-Ru1-S1	83.75(7)	N10-Ru2-S2	83.36(7)
S1-Ru1-Cl1	86.20(4)	S2-Ru2-Cl2	86.70(4)

Table S3: Crystal data and structure refinement parameters for **RuMPCl**, **RuMPBr** and **RuTGCl.DMF**

Crystal identification	**RuMPCl**	**RuMPBr**	**RuTGCl.DMF**
Formula	$C_{15}H_{18}Cl_2N_4RuS$	$C_{15}H_{18}Br_2N_4RuS$	$C_{18}H_{26}Cl_2N_6ORuS$
Mol. Wt.	458.4	547.3	546.5
Crystal color	Yellow	Yellow	Yellow
Crystal dim.	0.20×0.06×0.04	0.14×0.05×0.02	0.10×0.07×0.05
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	P 1 21/c 1	P 1 21/c 1	P -1
a (Å)	11.151(3)	11.282(5)	12.222(5)
b(Å)	12.316(3)	12.335(5)	13.369(5)
c(Å)	13.284(4)	13.697(5)	17.069(5)
α (deg)	90.0	90.0	68.138(5)
β (deg)	102.498(16)	101.374(5)	73.503(5)
γ (deg)	90.0	90.0	68.533(5)
V (Å^3)	1781.1(8)	1868.7(13)	2373.4(15)
Z	4	4	4
d (g.cm^{-3})	1.709	1.945	1.512
μ (mm^{-1})	1.300	5.230	0.994
F (000)	920	1064	1088
Radiation(λ = 0.71073 Å)	Mo-Kα	Mo-Kα	Mo-Kα
Temp (K)	296 (2)	296(2)	293(2)
θ range (deg)	1.87-30.65	1.84-30.54	1.72-30.66
Scan type	φ/ω	φ/ω	φ/ω
Measured reflection	13824	16989	63855
No of unique reflection	5363	5706	14297
Max and min transmittance	0.878 and 0.739 (R_{int}=0.0828)	0.9026 and 0.5280 (R_{int}=0.0635)	0.942 and 0.932 (R_{int}=0.0488)
Refinement method	Full matrix least squares on F^2	Full matrix least squares on F^2	Full matrix least squares on F^2
Goodness of fit on F^2	0.961	0.968	1.014
Final R indices	R_1= 0.0664, wR_2 = 0.0769	R_1= 0.0463, wR_2 = 0.0870	R_1 = 0.0446, wR_2 = 0.1048
R indices (all data)	R_1= 0.2041, wR_2 = 0.1025	R_1= 0.1108, wR_2 =0.1046	R_1= 0.0887, wR_2 = 0.1259

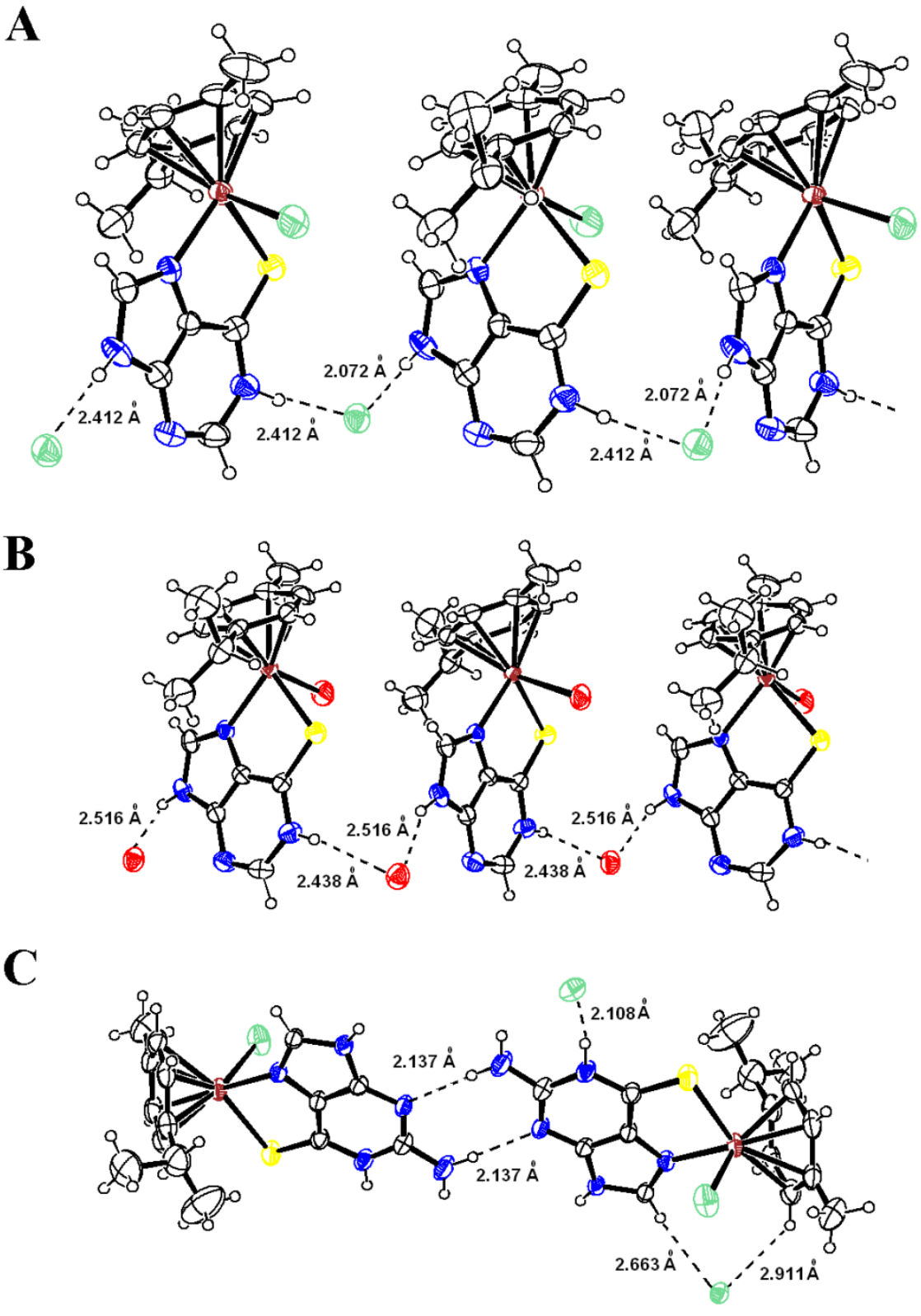

Figure S1: Intermolecular hydrogen bonding interaction (DH···A) between NH (DH) and the acceptor (A = Cl, Br or, N) in **RuMPCl** (**A**), **RuMPBr** (**B**) and **RuTGCl.DMF**(**C**).

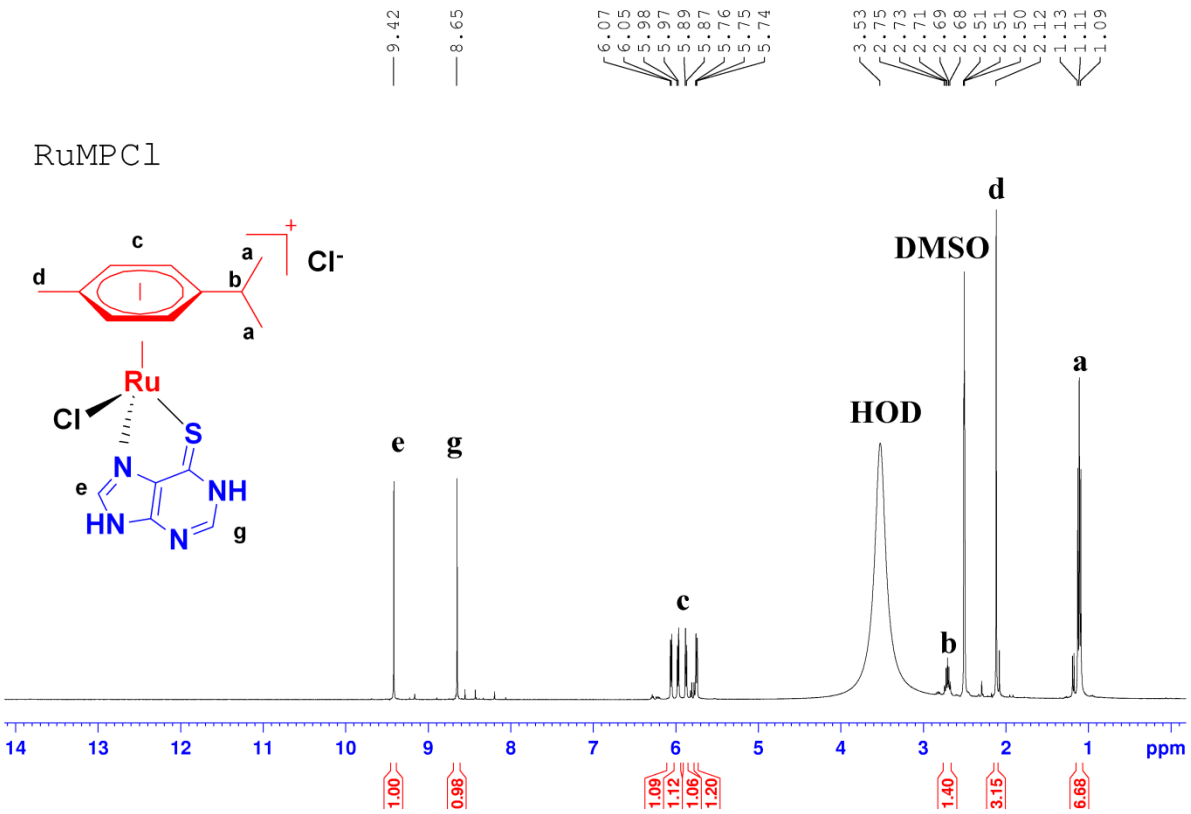

Figure S2a: ^{1}H NMR spectrum of **RuMPCl** in d_6-DMSO.

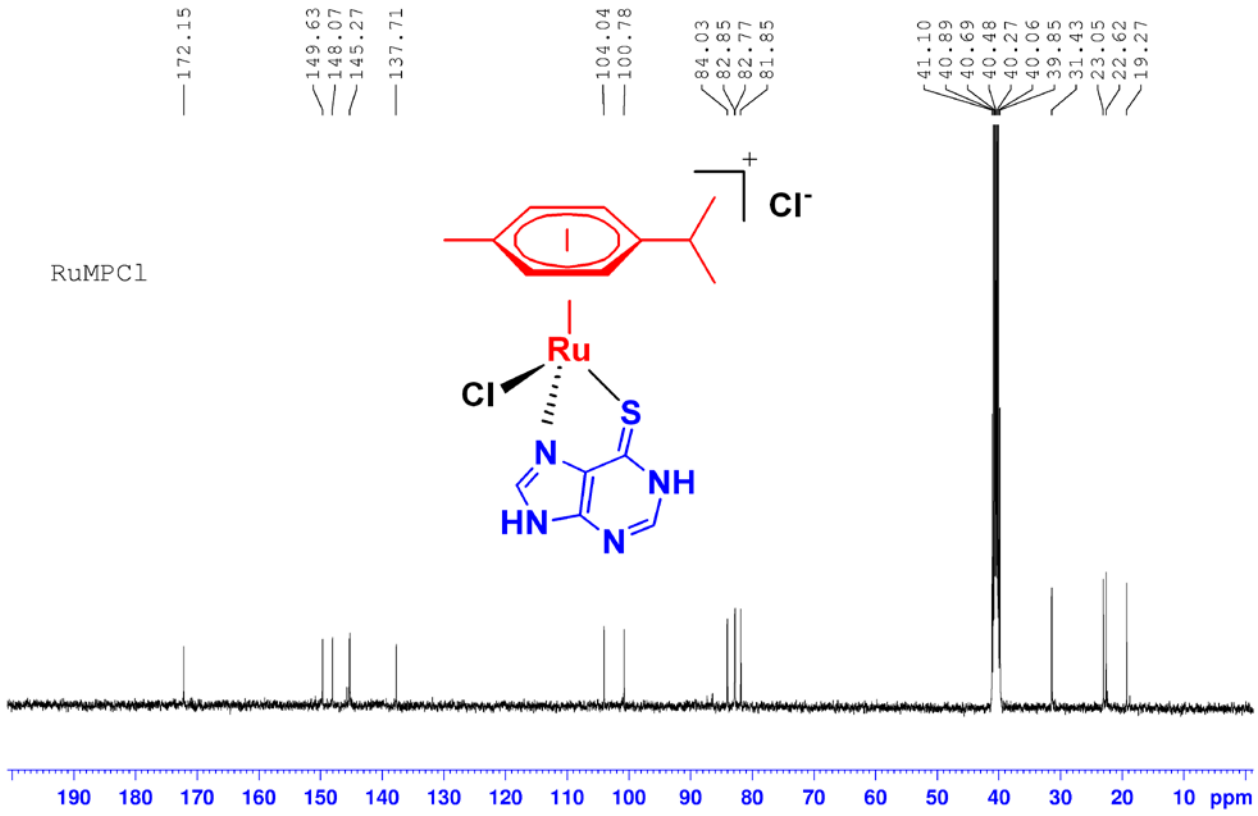

Figure S2b: ^{13}C NMR spectrum of **RuMPCl** in d_6-DMSO.

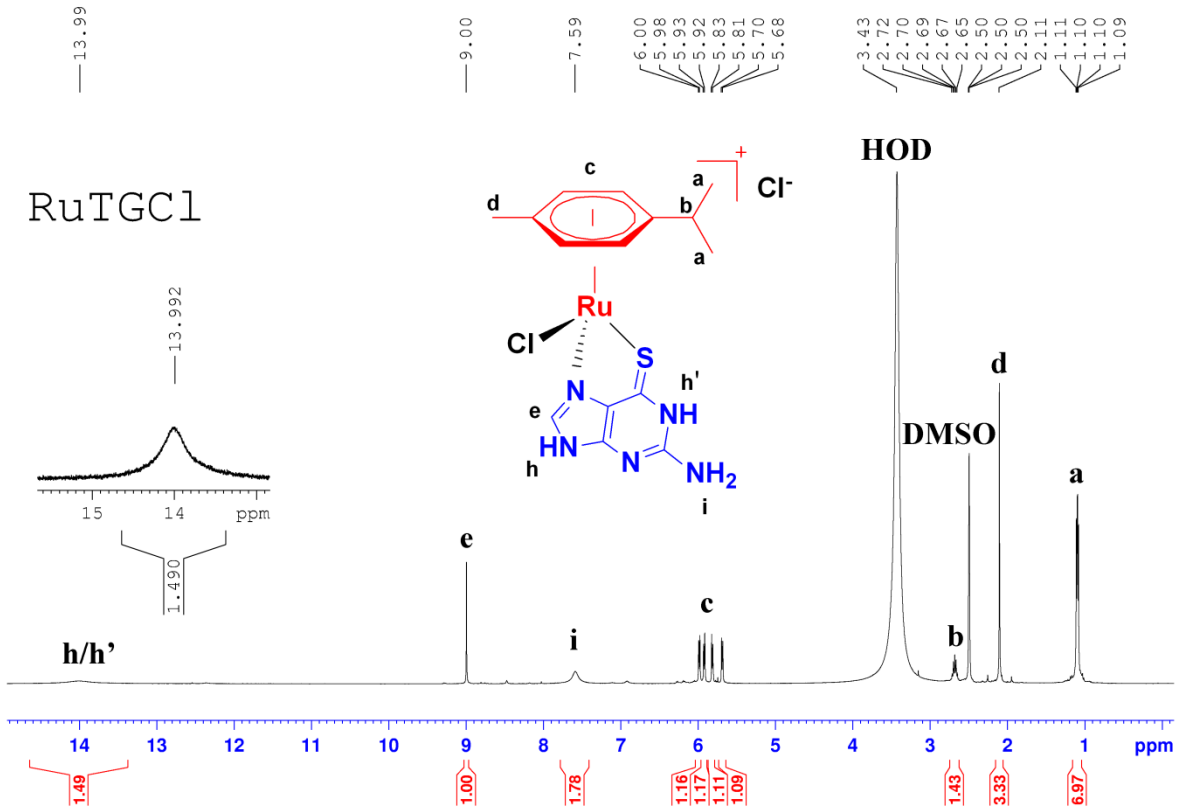

Figure S2c: ^{1}H NMR spectrum of **RuTGCl** in d_6-DMSO.

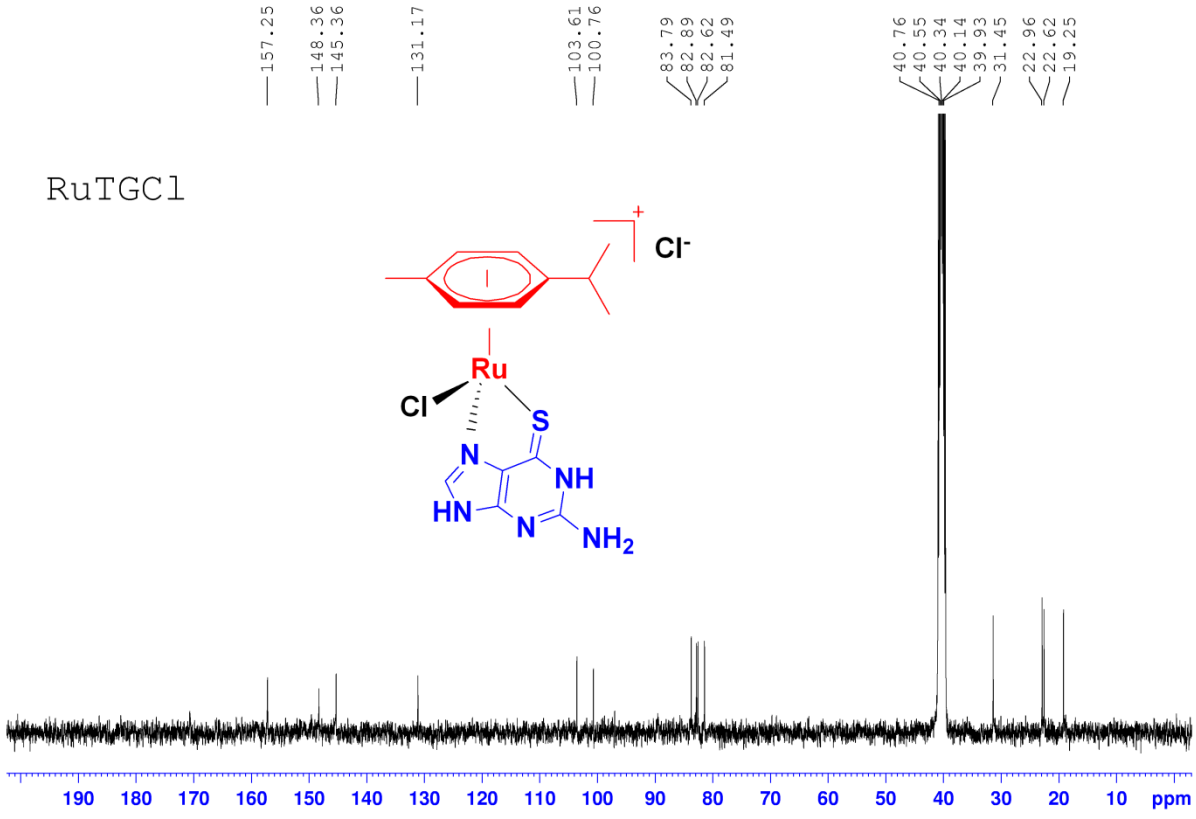

Figure S2d: ^{13}C NMR spectrum of **RuTGCl** in d_6-DMSO.

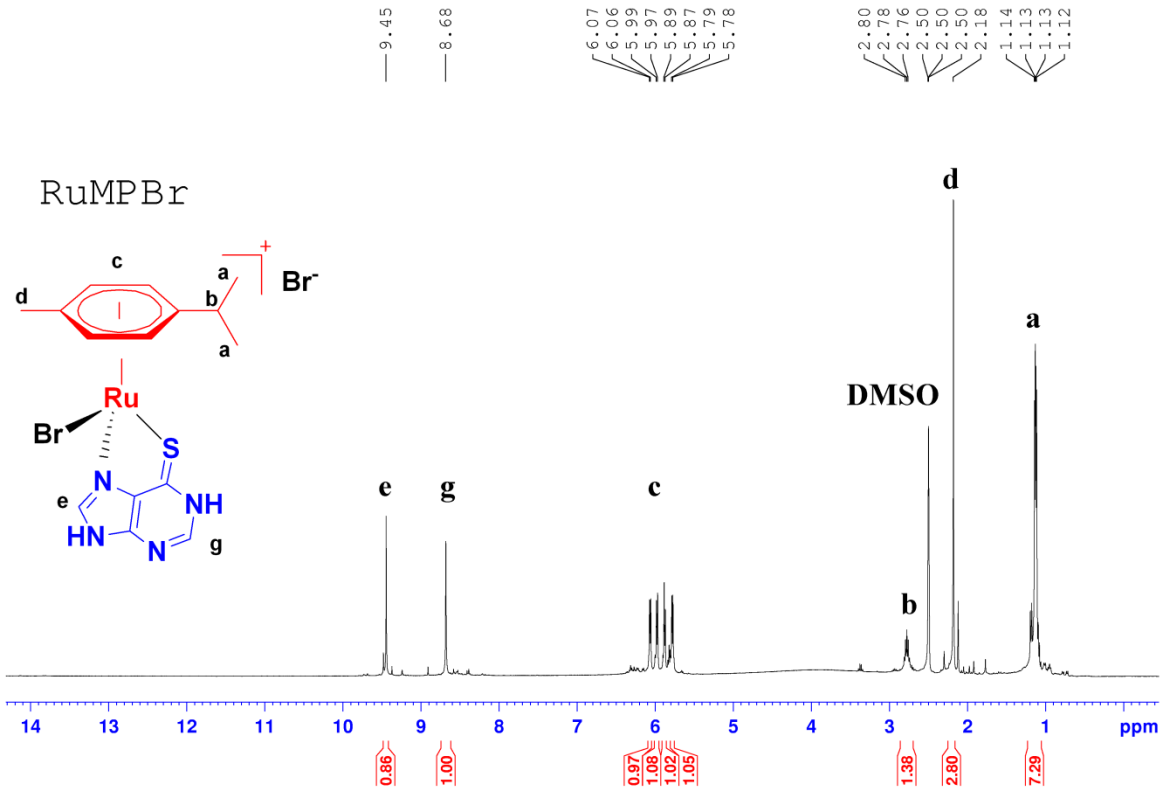

Figure S2e: ^{1}H NMR spectrum of **RuMPBr** in d_6-DMSO.

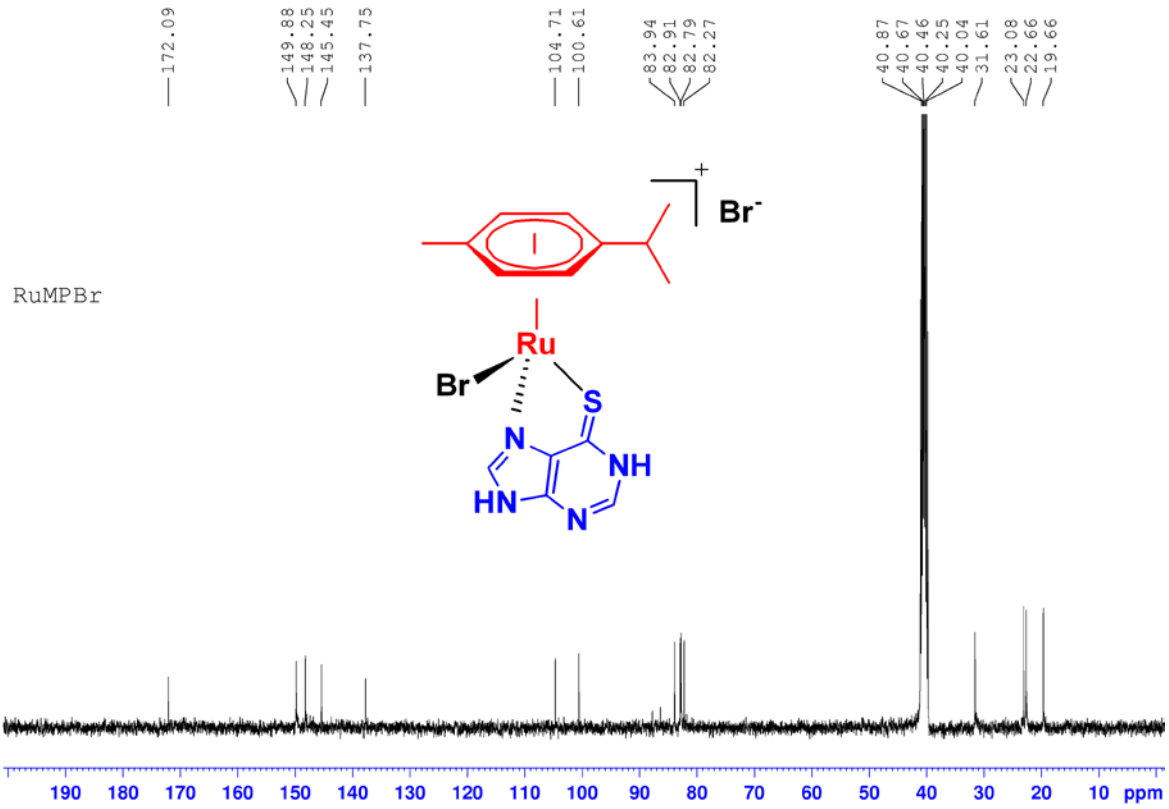

Figure S2f: ^{13}C NMR spectrum of **RuMPBr** in d_6-DMSO.

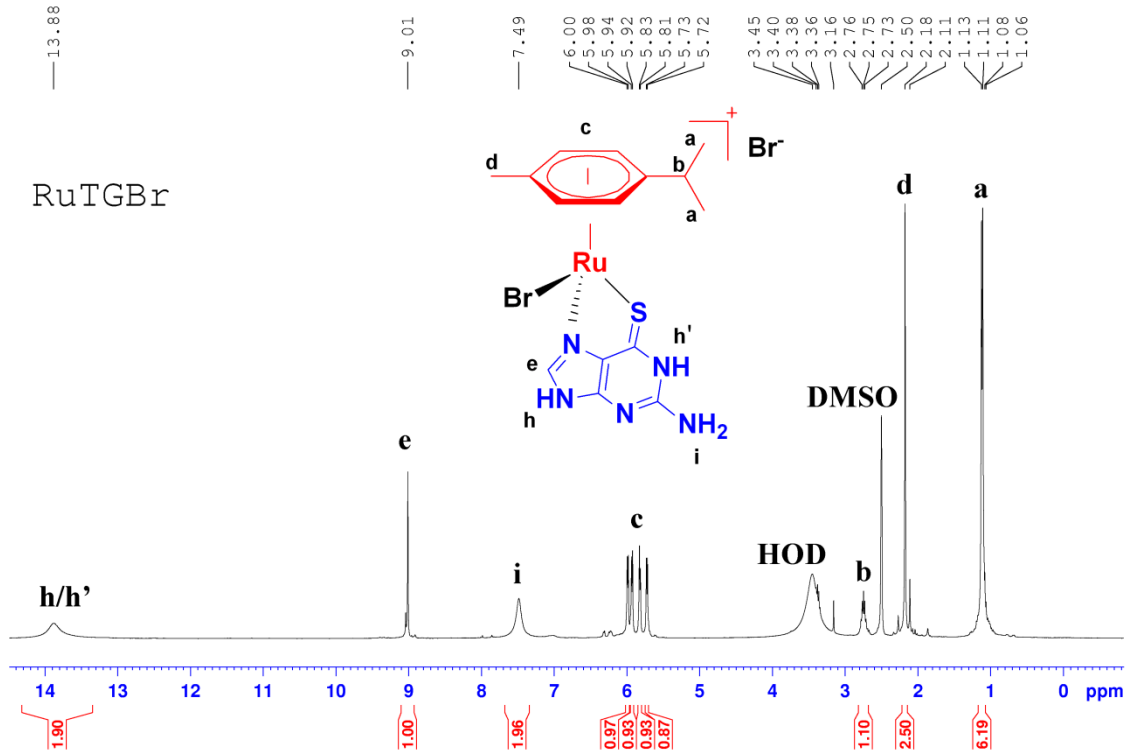

Figure S2g: ^{1}H NMR spectrum of **RuTGBr** in d_6-DMSO.

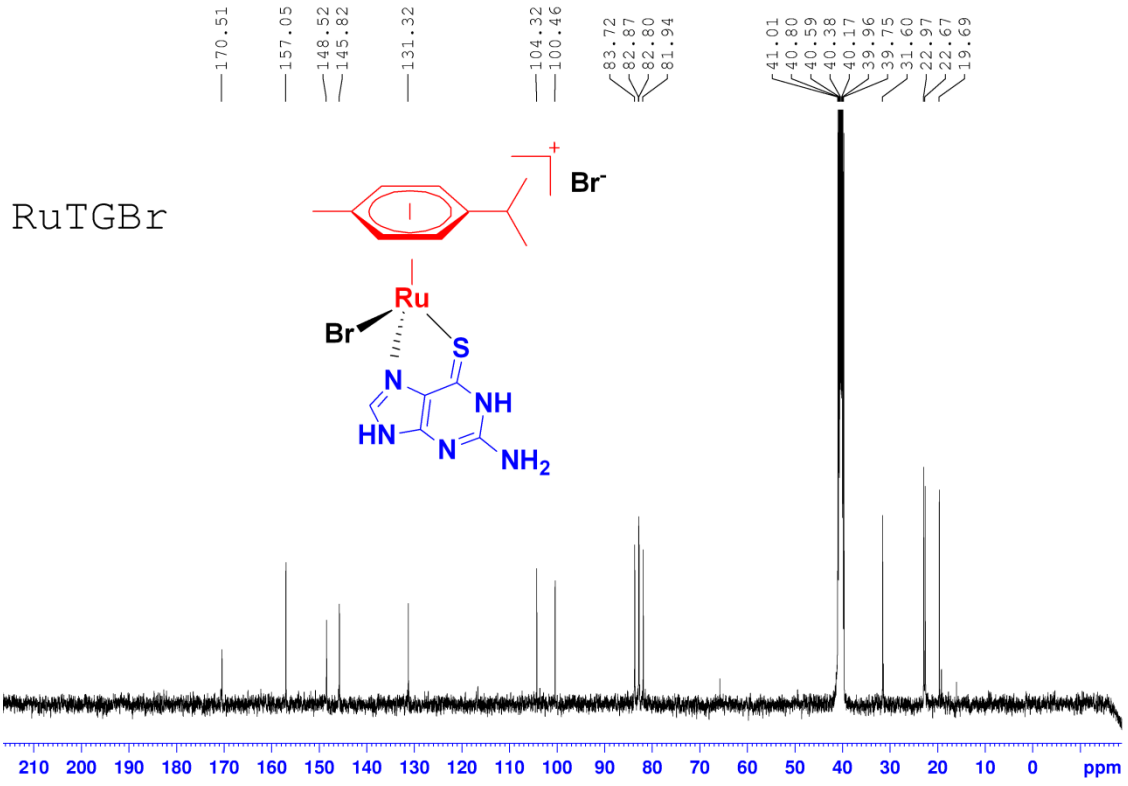

Figure S2h: ^{13}C NMR spectrum of **RuTGBr** in d_6-DMSO.

Lipophilicity (LogP) Mesurments:

The lipophilicity of the complexes was measured by the standard "shake flask technique".[8-10] Experiments were carried out at 37 ± 1 °C in triplicates. Approximately ~3 mg (1 mg/mL) of each of the complexes was dissolved in Milli Q water (3 mL). The stock solution was divided into two parts (1 mL and 2 mL) in glass vials. One part of the solution (1 mL) was taken and the absorbance recorded to get A_0 after incubating at 37 °C for 4 h. To another part (2 mL) of the solution, 2 mL of octanol was added and stirred at 37 °C for 4 h. The water layer was separated and the absorbance was recorded to obtain A_0-A ($A_{octanol}$). LogP values were calculated using the following formula:

$$\mathrm{LogP} = \mathrm{Log}[\frac{\mathrm{A_{octanol}}}{\mathrm{A_{water}}}] = \mathrm{Log}[\frac{\mathrm{A_0 - A}}{\mathrm{A_0}}] = \mathrm{Log}[\frac{[\mathrm{Ru}]_{\mathrm{oct}}}{[\mathrm{Ru}]_{\mathrm{water}}}]$$

Hydrolysis Studies:

To monitor the hydrolysis by ^{1}H NMR, the complex was taken in a NMR tube. After dissolving the complex in D_2O/H_2O (20%/80%) mixture, the ^{1}H NMR was recorded immediately. The NMR tube was kept inside the probe of the NMR spectrometer at 293 K during the entire course of the experiment.

To detect the actual hydrolysed products of ruthenium complexes, they were dissolved in LC-MS grade water to get a concentration of 2 mM. From this stock solution 10 µL was added to 990 µL of Milli Q water. This solution was infused in the ESI-MS using an auto injection module (Agilent 1290 infinity) attached to the ESI-MS (Agilent 6538 Ultra High Definition (UHD) Accurate-Mass Q-TOF). The same solution was infused after 6 h and 24 h incubation at 298 K. Mobile phase: 80% acetonitrile (LC-MS grade, Fluka)/20% water (purified using a Millipore system) containing 5 mM ammonium formate (LC-MS grade, Agilent) with a flow rate of 0.3 mL min^{-1}. The capillary voltage and the fragmentor voltage were kept at 4.0 kV and 200 V, respectively. The capillary temperature was 350 °C with a 10 L h^{-1} flow of nitrogen drying gas. ESI-MS data were processed using the MassHunter software.

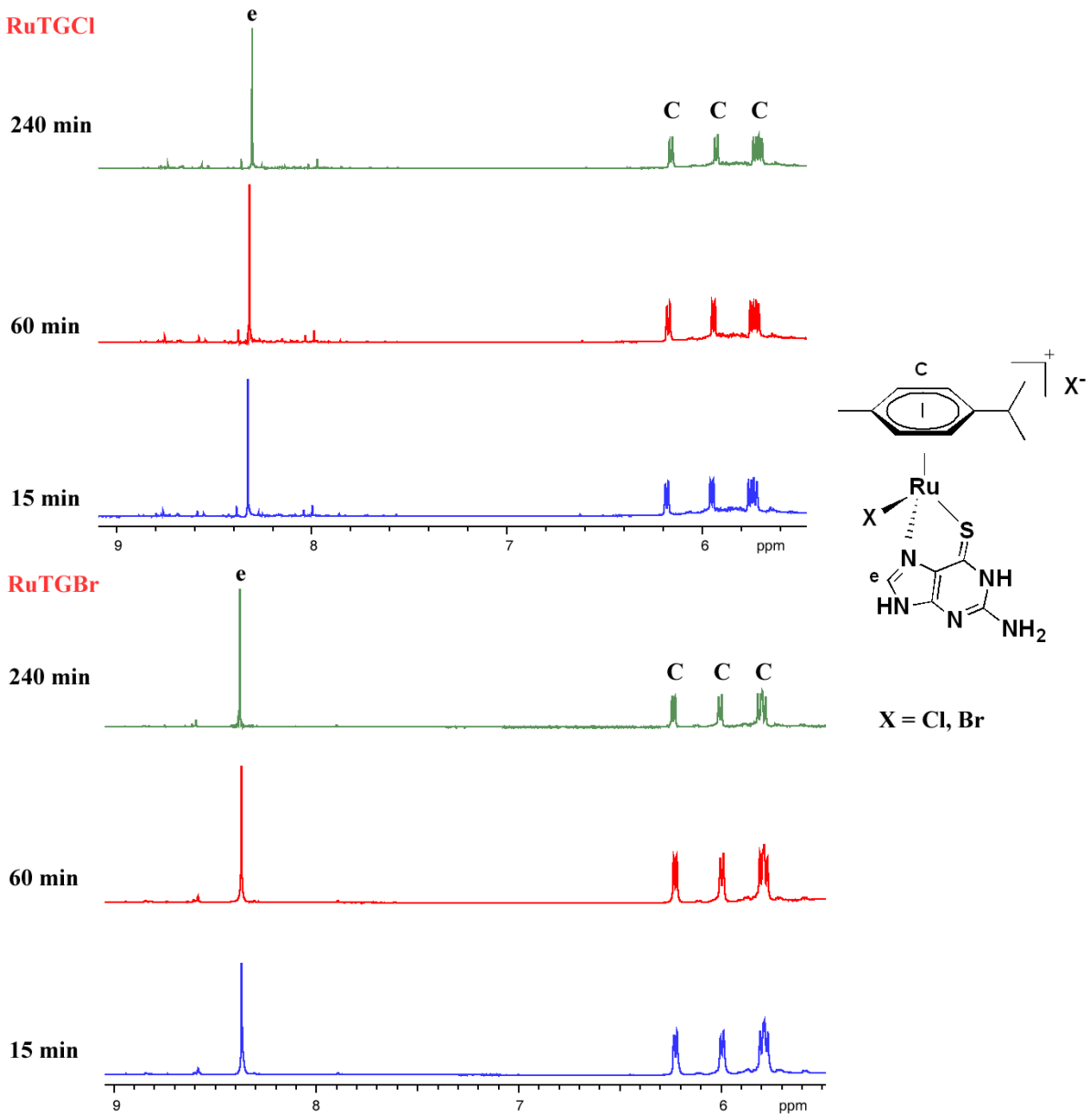

Figure S3a: ^{1}H NMR spectra of **RuTGCl** and **RuTGBr** complexes at different time intervals in 20% D_2O + 80% H_2O. Aromatic regions in the ^{1}H NMR spectra are shown.

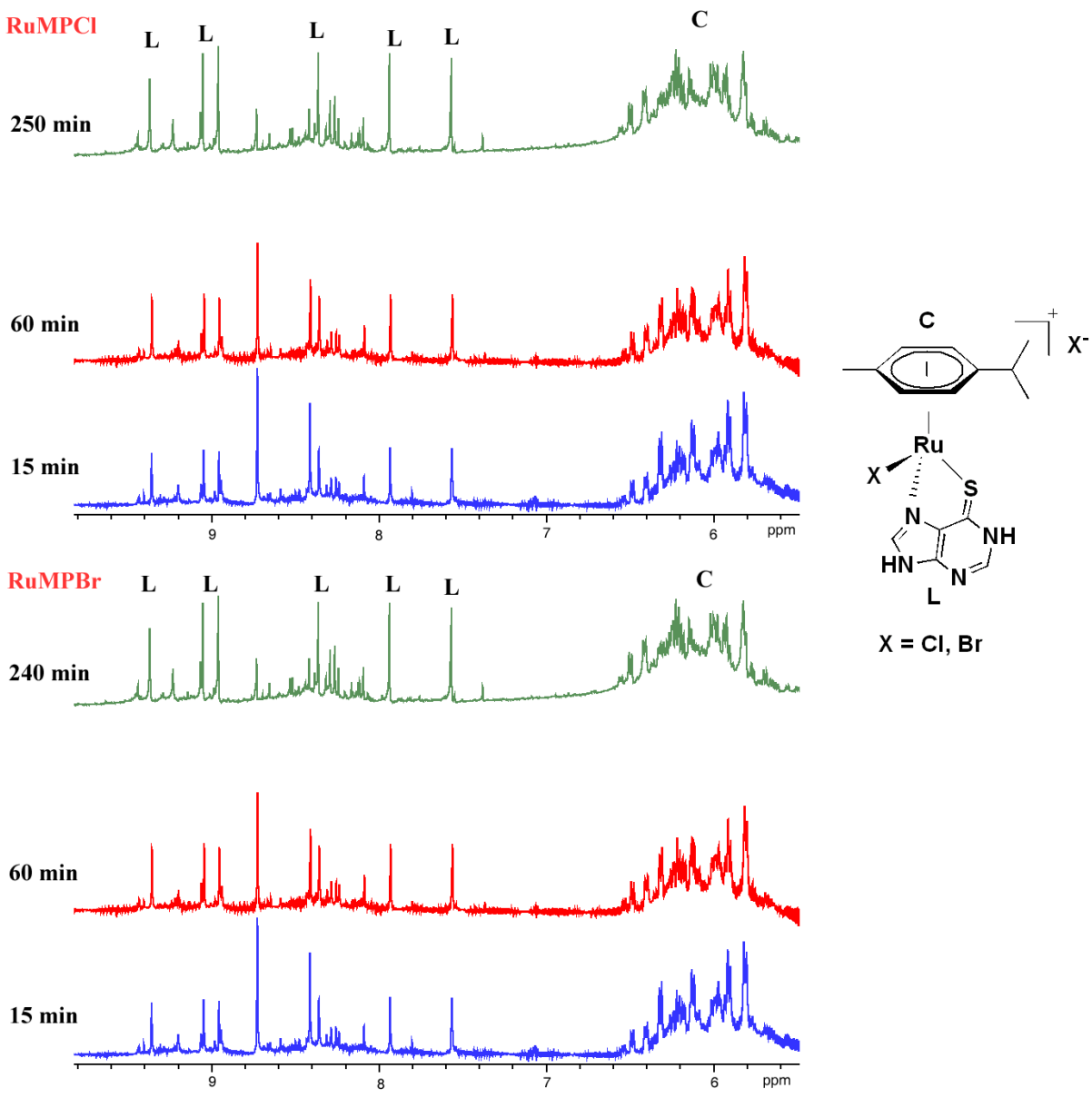

Figure S3b: ^{1}H NMR spectra of **RuMPCl** and **RuMPBr** complexes at different time intervals in 20% D_2O + 80% H_2O. Aromatic regions in the ^{1}H NMR spectra are shown.

Table S5a: *m/z* of Ru complexes in aqueous solution after 24 h incubation at 298 K

Complex	Observed peak $[M]^+$	Theoretical formula (Calculated m/z)	Found m/z (Abundance in %)[a]
RuMPCl	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}MP}\text{-H})]^+$	$[C_{15}H_{17}N_4SRu]^+$ (387.0217)	387.0215 (62)
	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}MP}\text{-H})]_2 + H^+$	$[C_{30}H_{33}N_8S_2Ru_2]^+$ (773.0365)	773.0383 (38)
RuTGCl	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}TG}\text{-H})]^+$	$[C_{15}H_{18}N_5SRu]^+$ (402.0324)	402.0311 (87)
	$[(\eta^6\text{-cym})Ru(\mathbf{TG}\text{-H})]_2 + H^+$	$[C_{30}H_{35}N_{10}S_2Ru_2]^+$ (803.0583)	803.0557 (13)
RuMPBr	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}MP}\text{ -H})]^+$	$[C_{15}H_{17}N_4SRu]^+$ (387.0217)	387.0217 (63)
	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}MP}\text{-H})]_2 + H^+$	$[C_{30}H_{33}N_8S_2Ru_2]^+$ (773.0365)	773.0392 (37)
RuTGBr	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}TG}\text{ -H})]^+$	$[C_{15}H_{18}N_5SRu]^+$ (402.0324)	402.0318 (89)
	$[(\eta^6\text{-cym})Ru(\mathbf{6\text{-}TG}\text{-H})]_2 + H^+$	$[C_{30}H_{35}N_{10}S_2Ru_2]^+$ (803.0583)	803.0565 (11)

[a]Percentages of mononuclear and dinuclear species were calculated from the relative abundances in the ESI-MS spectra

Table S5b: Amount of mononuclear and dinuclear ruthenium species in aqueous solution as observed in ESI-MS[a]

Complex	**After 10 min**		**After 24 h**	
	Monomer	Dimer	Monomer	Dimer
RuMPCl	89%	11%	63%	37%
RuTGCl	88%	12%	88%	12%
RuMPBr	75%	25%	64%	36%
RuTGBr	92%	8%	87%	13%

[a]Percentages of mononuclear and dinuclear species were calculated from the relative abundances in the ESI-MS spectra

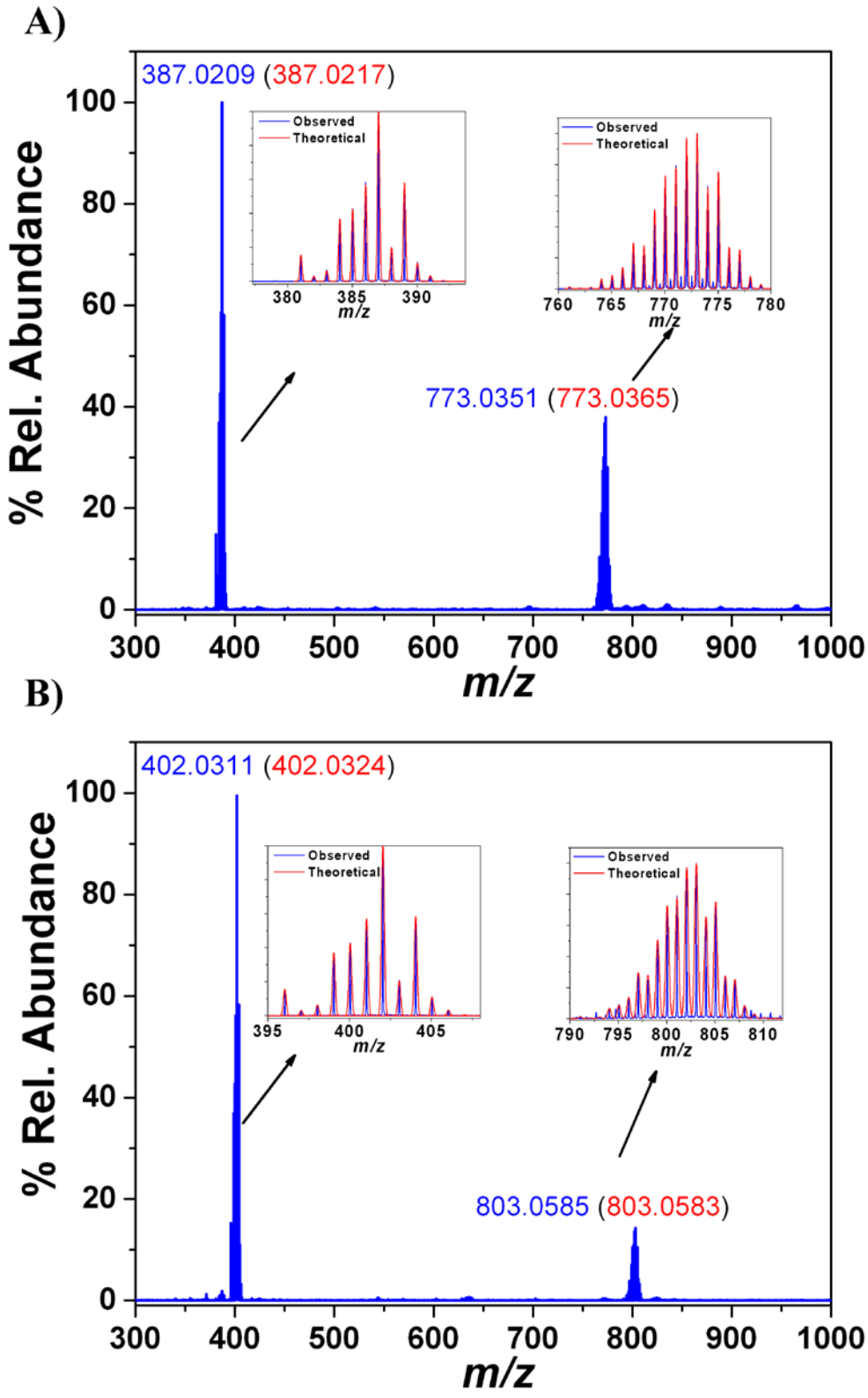

Figure S4a: HRESI-MS spectra of **RuMPCl** (**A**) and **RuTGCl** (**B**) in water incubated for 24 h at 298 K.

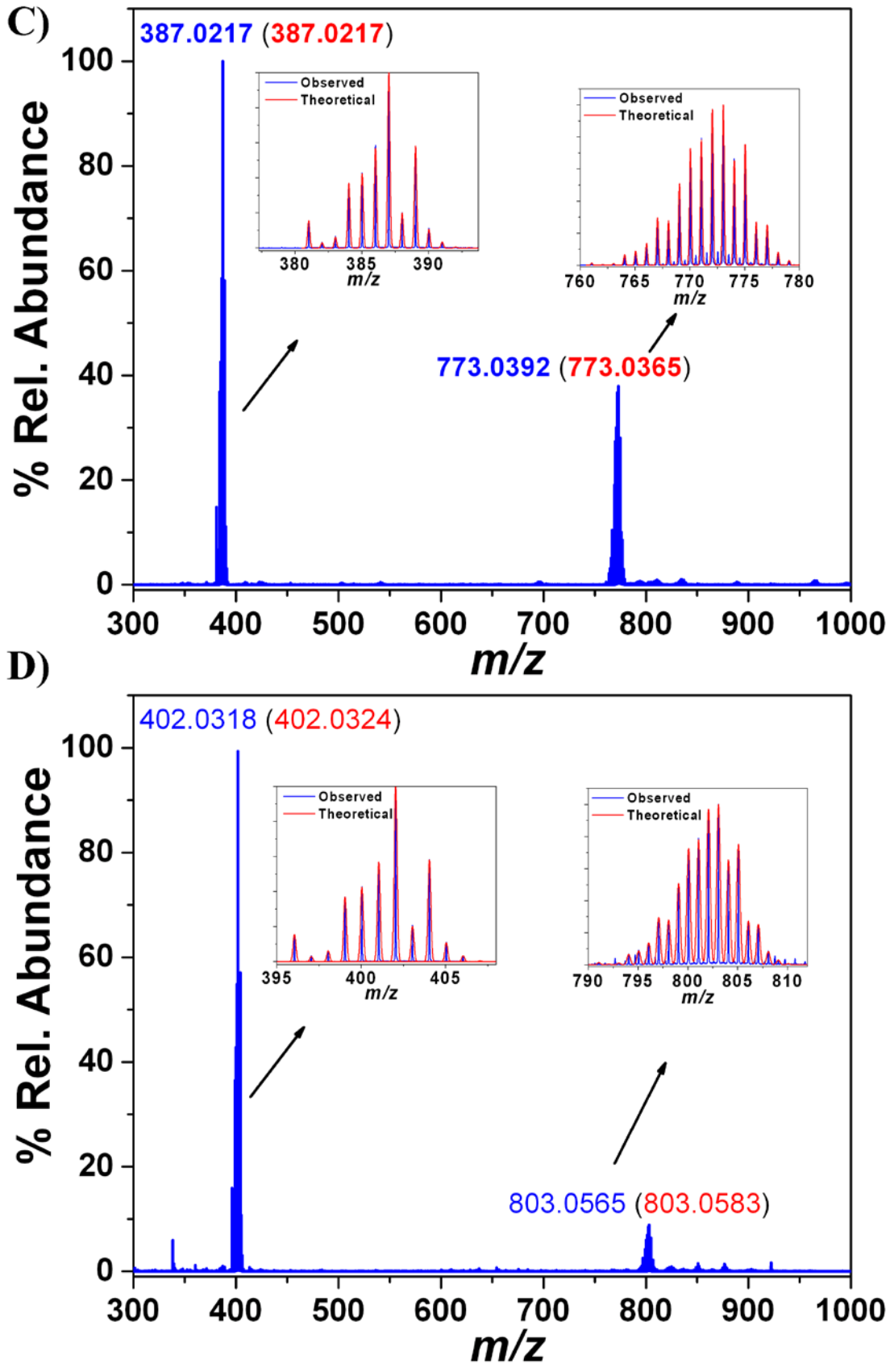

Figure S4b: HRESI-MS spectra of **RuMPBr** (**C**) and **RuTGBr** (**D**) in water incubated for 24 h at 298 K.

Photochemistry:

Standard ferrioxalate actinometry was performed to standardize the UV radiation.[11] Briefly, 1-1.5 mg of $K_3[Fe(C_2O_4)_3].3H_2O$ was directly dissolved in 2.5 mL of 0.05 M H_2SO_4 solution in a 1 cm cuvette. Absorbance of the solution was measured using UV-Vis spectrophotometer. The cuvette was exposed to the UV light source (λ_{max} = 365 nm; 400 W high-pressure mercury vapor lamp) with continuous stirring for 10 min and then absorbance was recorded. The HPMV lamp was continuously cooled with ice-cold water to avoid heating of the sample. Change in the absorbance value at 390 nm was used to calculate the photon flux (q_p) from the following equation:

$$q_p = \left(\frac{dA_{390}}{dt}\right)\frac{N_A.V}{\varphi_{365}\,.\,\varepsilon_{390}\,.\,l}$$

Where dA_{390}/dt indicates changes in the absorbance with respect to time at 390 nm, N_A stands for Avogadro's number (6.02214×10^{23} mol^{-1}), V stands for volume of the solution (0.0025 dm^3), φ_{365} indicates the quantum yield at 365 nm (1.26), ε_{390} indicates the absorption coefficient at 390 nm (312 mol^{-1} dm^3 cm^{-1}) and ℓ stands for the length of the cuvette (1 cm).

Ruthenium complexes (**RuTGCl** and **RuTGBr**) and **6-TG** of 0.1 mM concentration in 5 mL of Milli Q water were irradiated for 10 min (7±1 kJ m^{-2}) in a round bottom flask with continuous stirring. After irradiation, UV-Vis spectra and HRMS of the solution were recorded. To get ^{1}H NMR spectra of irradiated products, 8 mg of the ruthenium complex was dissolved in 5 mL water. The solution was irradiated for 0 min (no irradiation), 10 min (7±1 kJ m^{-2}) or, 30 min (20±2 kJ m^{-2}) in a round bottom flask with continuous stirring. Then the aqueous solution was lyophilized to get a free flowing yellow powder, which was dissolved in D_2O and ^{1}H NMR was recorded.

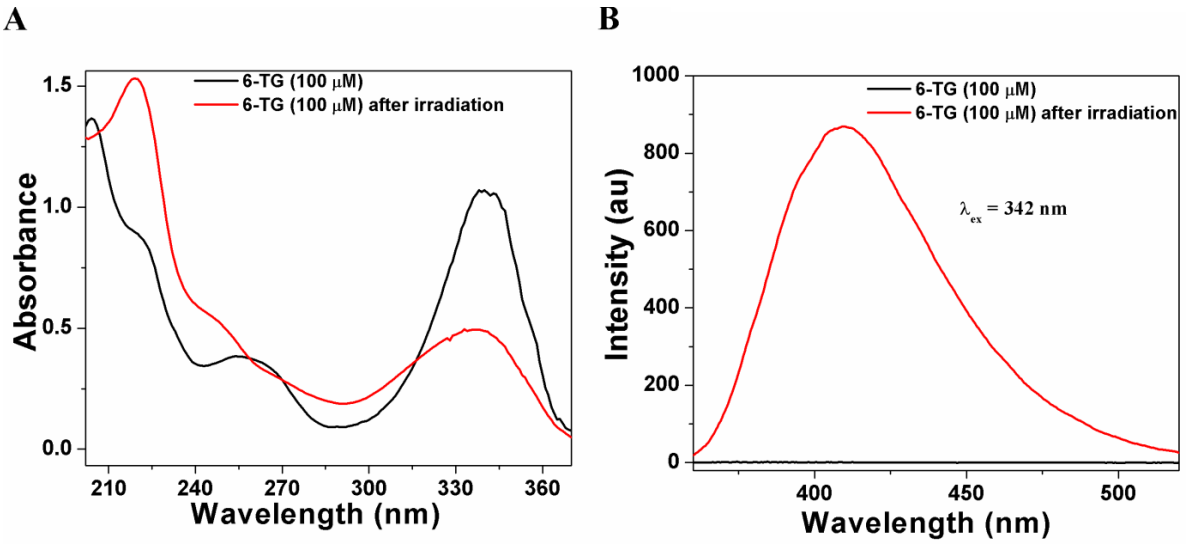

Figure S5: UV-Vis (**A**) and fluorescence (**B**) spectra of **6-TG** before and after irradiation with 7±1 kJm^{-2} of UVA light.

Figure S6: Photooxidized products of **6-TG** identified by ESI-MS after irradiation with 7±1 kJm^{-2} of UVA light. Percentages of all species were calculated from the relative abundances in the ESI-MS spectra.

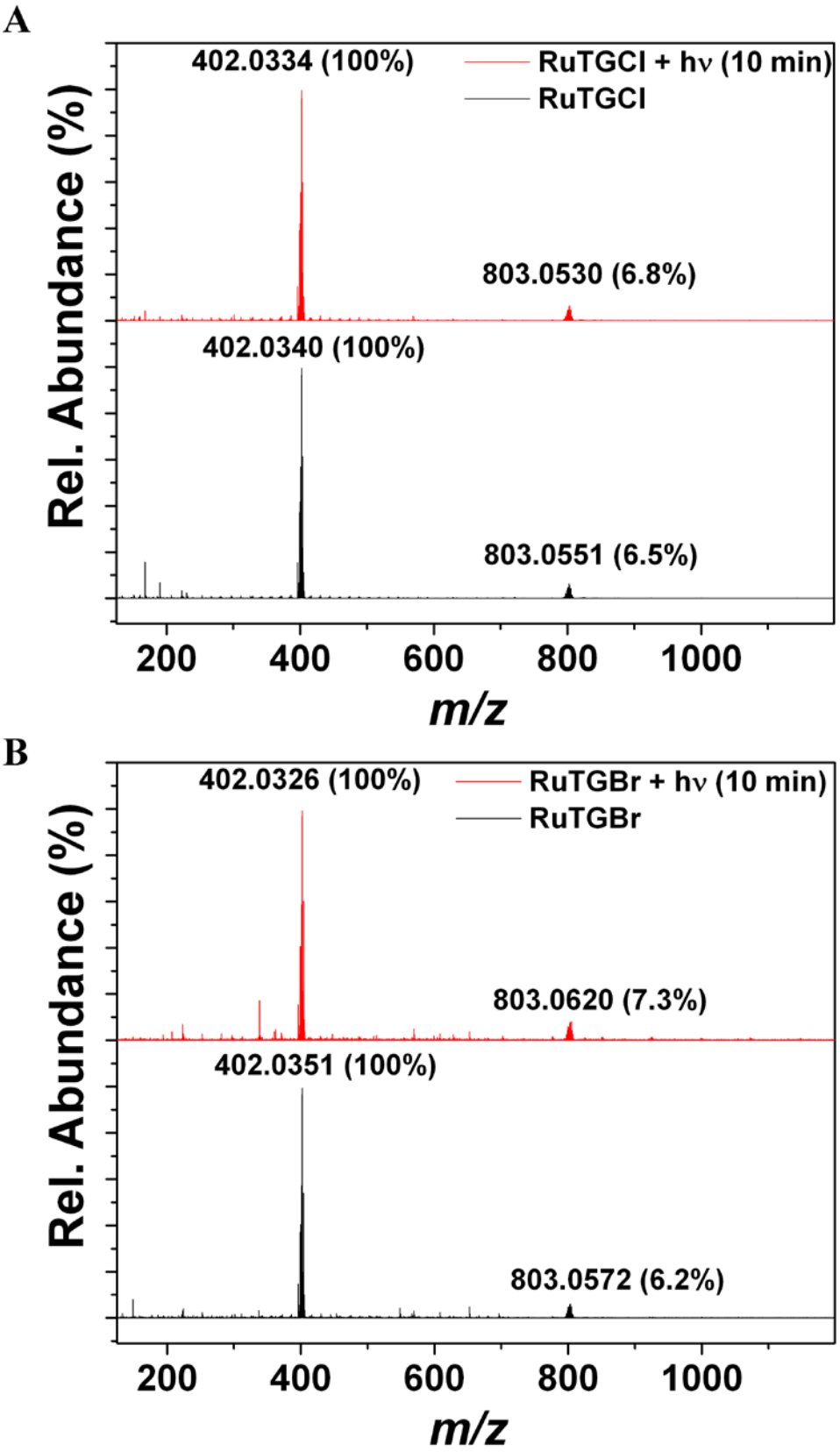

Figure S7: High resolution ESI-MS spectra of **RuTGCl** (**A**) and **RuTGBr** (**B**) in water before and after irradiation with UVA light for 10 min.

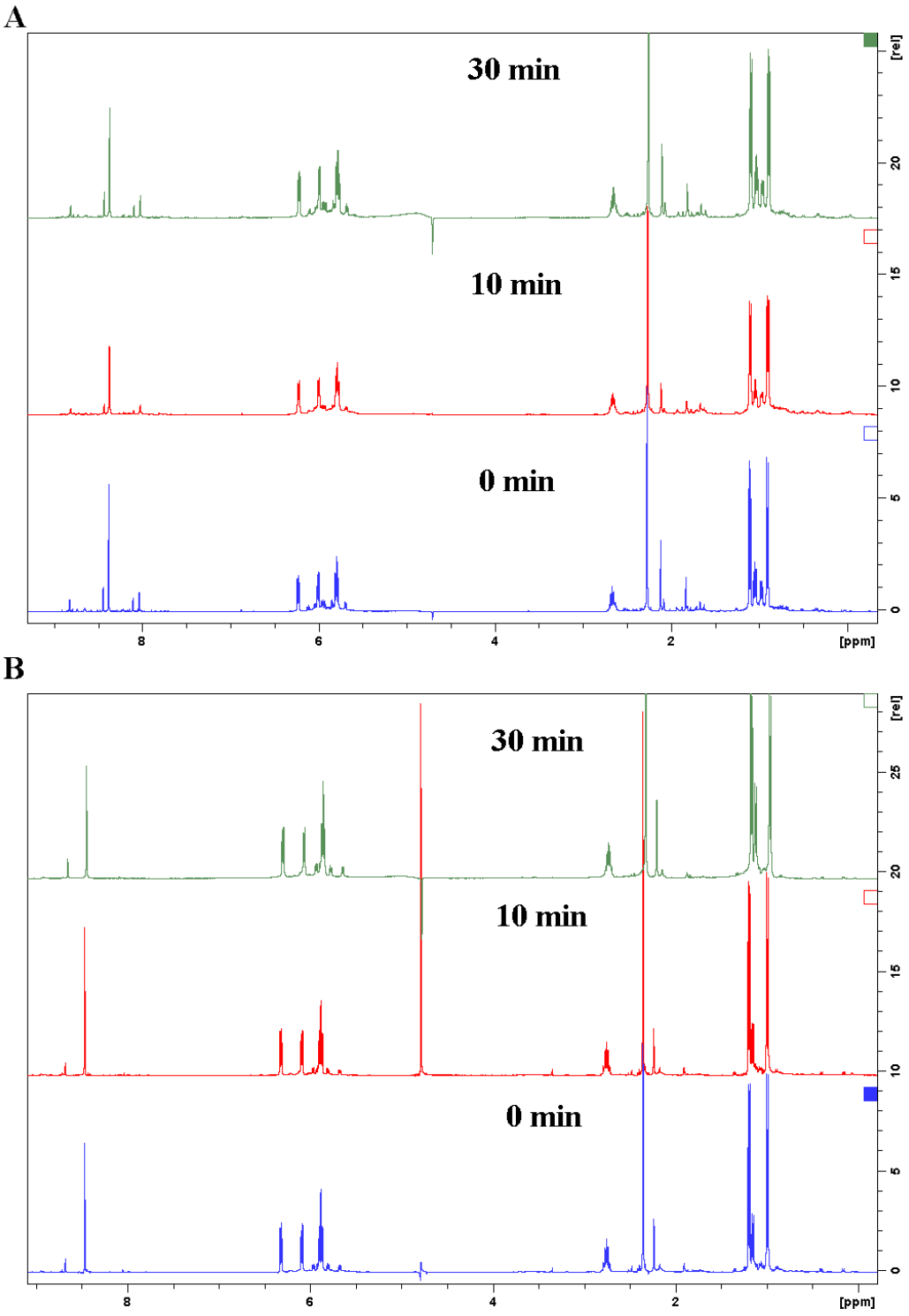

Figure S8: ^{1}H NMR spectra of **RuTGCl** (**A**) and **RuTGBr** (**B**) in D_2O after irradiation with UVA light for 0 min, 10 min and 30 min and followed by lyophilization. Peak at δ 4.79 ppm corresponds to residual solvent, HOD.

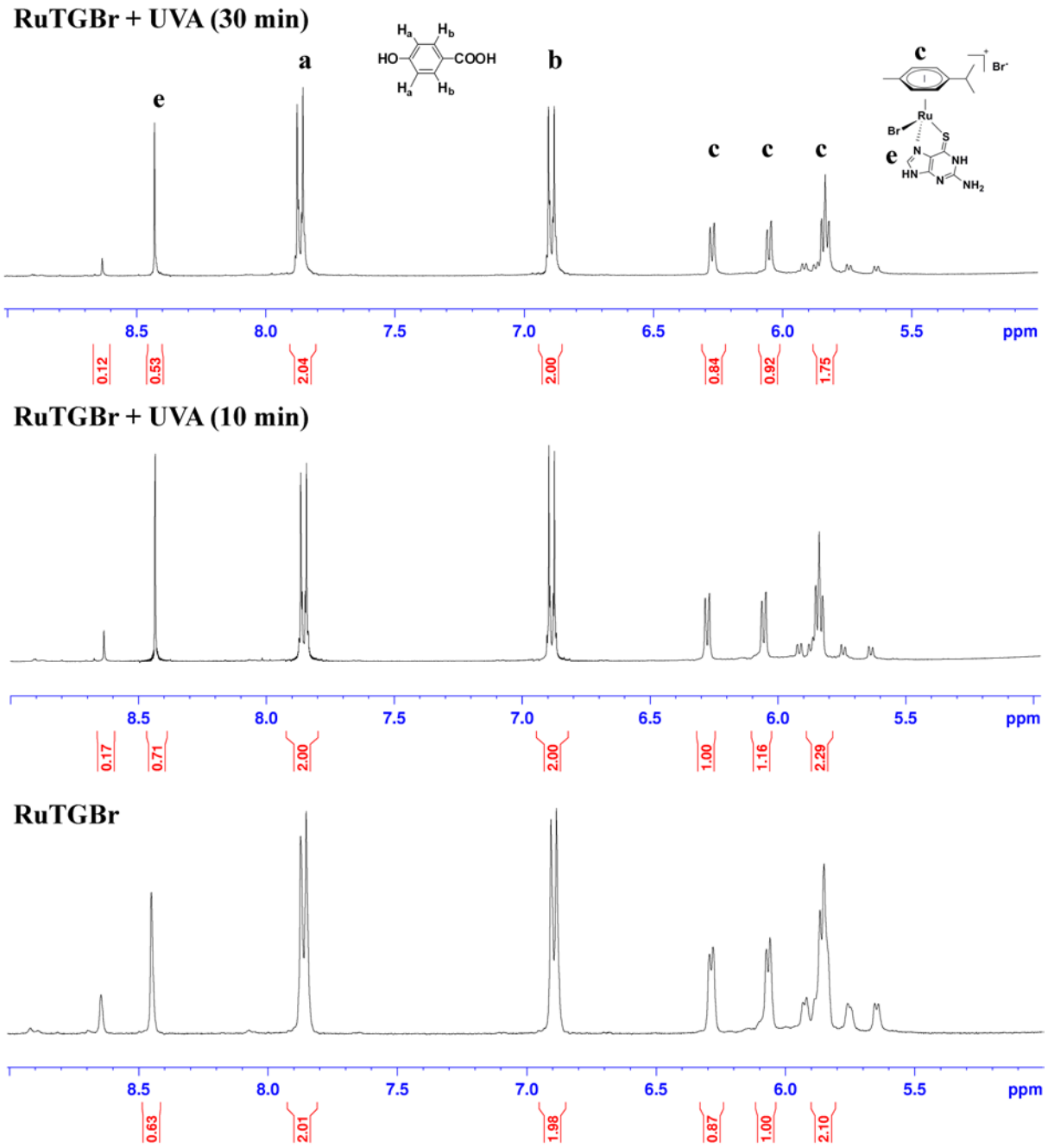

Figure S9: Quantification of photodegradation of **RuTGBr** by ^{1}H NMR spectroscopy using 4-hydroxy benzoic acid as an internal standard**.** After photoirradiation of 8.1 mg **RuTGBr** in 5 mL of water for 10 min or 30 min, water was removed by lyophilization. Then 0.5 mL of D_2O was added to the lyophilized yellow powder (28.8 mM) along with 2.0 mg (28.8 mM) of 4-hydroxy benzoic acid and ^{1}H NMR spectra was recorded. Similarly, a control sample (bottom spectrum in the Figure) was also prepared with the same amount of standard.

Growth Inhibition (GI_{50}) SRB Assays:[12]

Human tumor cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2mM L-glutamine and were maintained in a CO_2 incubator in an atmosphere of 5% CO_2 in tissue culture flasks at 37 °C. Single cell suspension of these tumor cells was made and cell count was adjusted to 1 $\times 10^5$ to 5 $\times 10^5$ cells/mL. Cell number for seeding was derived from a calibration curve set up with known number of cells, for each cell line. 96-well plate was seeded with this cell suspension, each well receiving 90 µl of it. The plate was then incubated at 37 °C temperature in CO_2 incubator for 24 h to ensure adequate cell growth prior to determination of cell growth inhibition. The drugs (10 µl) were then added at appropriate concentrations, followed by further incubation for 48 h. Experiment was terminated by gently layering the cells in the wells with 50 µL of 30% (w/v) cold TCA. The plates were kept in refrigerator (4 °C) for 1 h. The supernatant was discarded and was washed thoroughly with tap water and air dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4 % (w/v) in 1% acetic acid was added to each of the wells and plates were incubated at room temperature for 20 min. Excess SRB dye was removed by washing the plates, 3 to 4 times, with 1% acetic acid and plates were air dried. The bound SRB was eluted with Tris (10 mM, pH 10.5). Absorbance was read at 540 nm with 690 nm reference wavelength, in the ELISA-plate reader. Optical density of drug-treated cells was compared with that of control cells and growth inhibition was calculated as percent values. Each compound was tested at four different concentrations (0.1, 1.0, 10 & 100 µM), in triplicate, on the human malignant cell lines. For each of the experiments, a known anticancer drug adriamycin was used as a positive control.

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