In vitro and *in vivo* Evaluation of Water-soluble Iminophosphorane Ruthenium(II) Compounds. A Potential Chemotherapeutic Agent for Triple Negative Breast Cancer

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Supporting Information

Contents:

1. Crystallographic Data for Compound 1

Table S1. Crystal Data and Structure Refinement for compound **1**

formula	$C_{34}H_{33}ClF_6N_2OP_2Ru$
Fw	798.08
T [K]	293 (2)
λ ($Mo_{K\alpha}$)[Å]	0.71073
crystal system	Monoclinic
space group	C2/c
a [Å]	37.8820(15)
b [Å]	10.8170(5)
c [Å]	17.8540(4)
α [°]	90
β [°]	108.766(2)
γ [°]	90
V [Å]^3	6927.1(4)
Z	8
D_{calcd} (g cm^{-3})	1.531
μ (mm^{-1})	0.685
GOF	0.951
$R_1[I > 2\sigma]$	0.0551
wR_2(all data)	0.1658

2. ^{1}H NMR spectra of compounds 2-4, 6-8

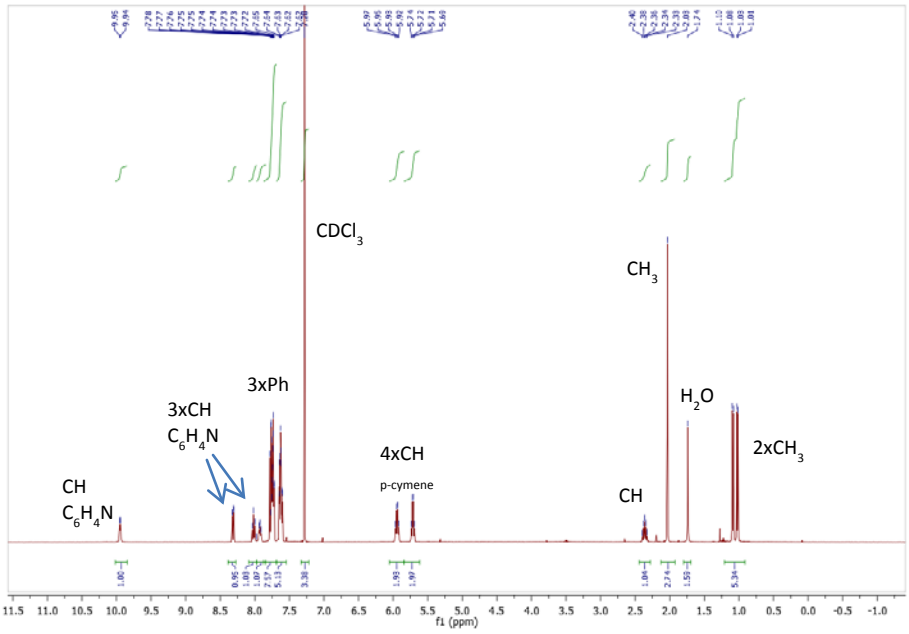


Figure S1. ^{1}H NMR spectra of compound **2** in $CDCl_3$.

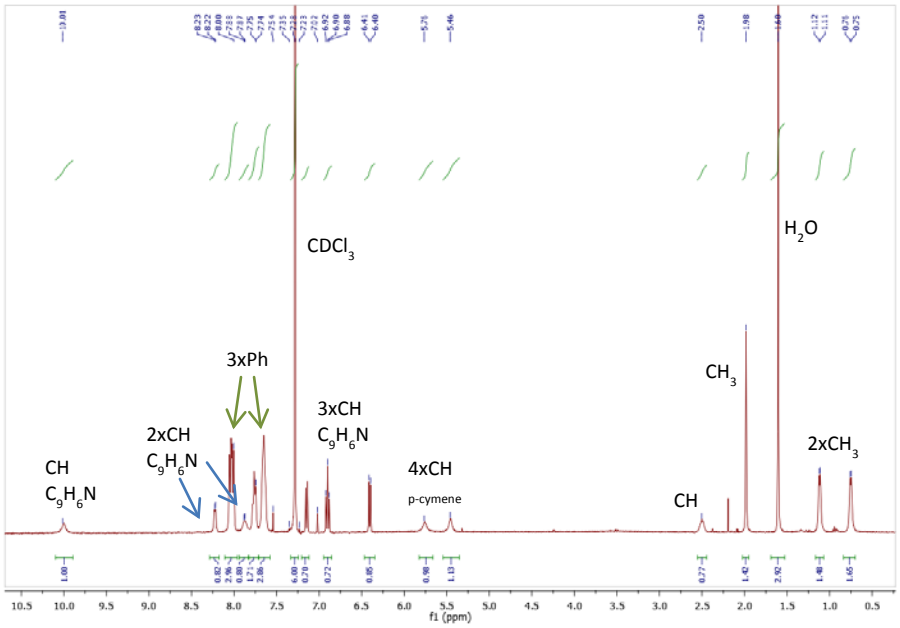


Figure S2. ^{1}H NMR spectra of compound **3** in $CDCl_3$.

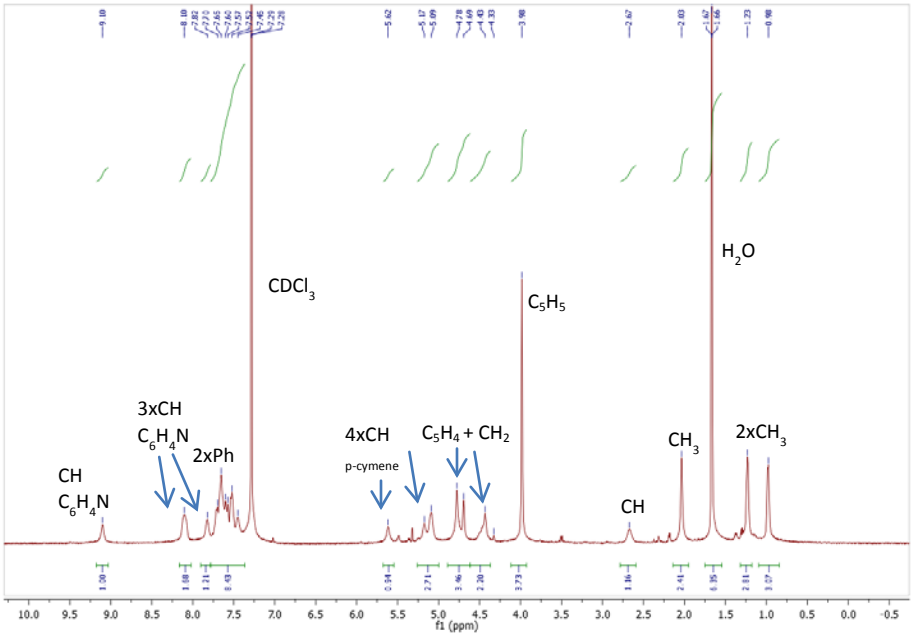


Figure S3. ^{1}H NMR spectra of compound **4** in $CDCl_3$.

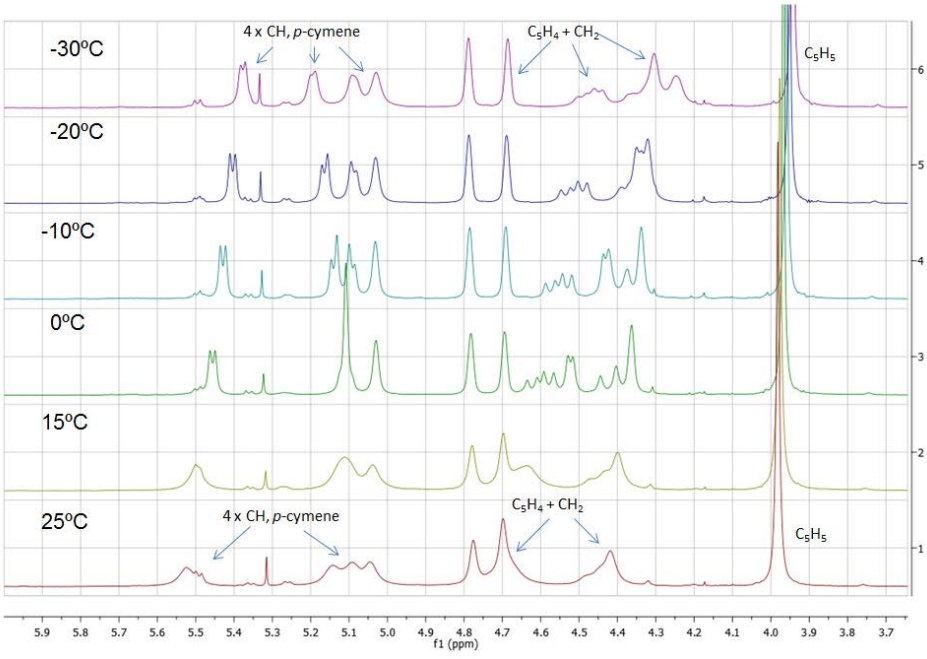


Figure S4. Variable temperature ^{1}H NMR spectra of compound **4** in $CDCl_3$ (magnification zone 6.1-3.6 ppm).

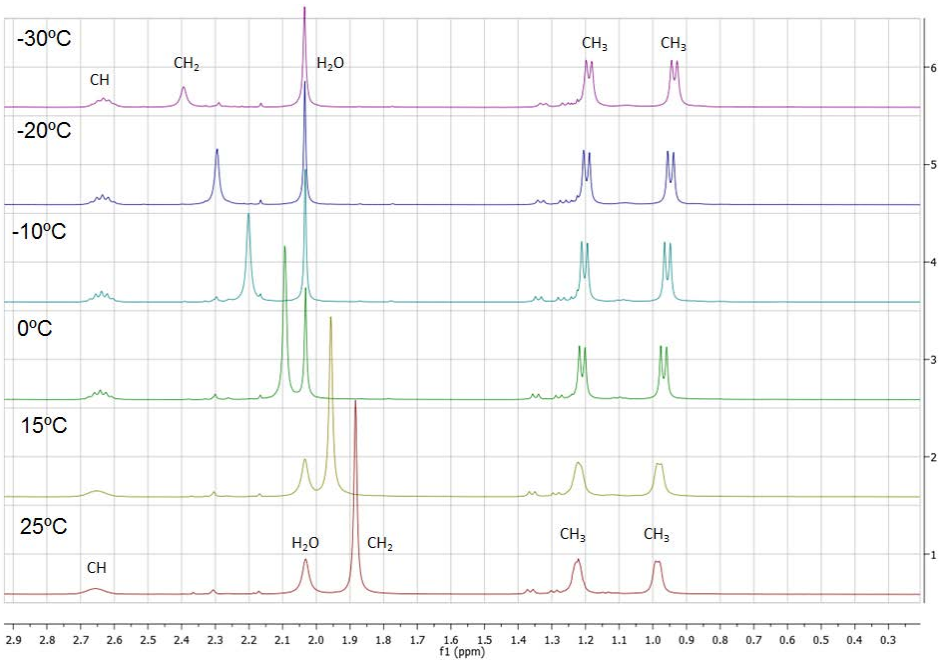


Figure S5. Variable temperature ^{1}H spectra of compound **4** in $CDCl_3$ (magnification zone 2.9-0.1 ppm).

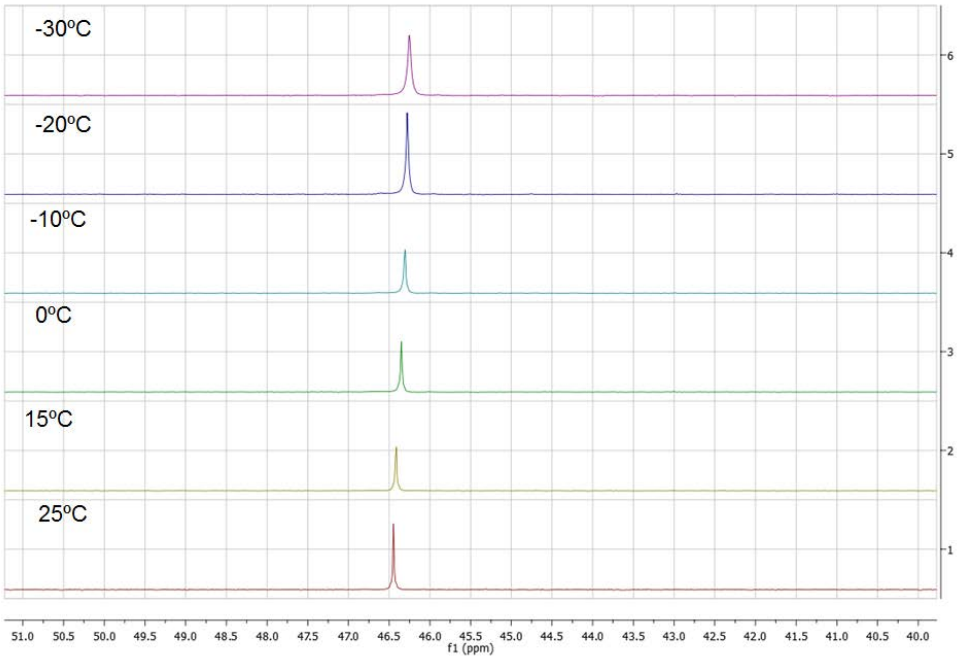


Figure S6. Variable temperature $^{31}P\{^{1}H\}$ NMR NMR spectra of compound **4** in $CDCl_3$ (δ 46.45 (s) ppm).

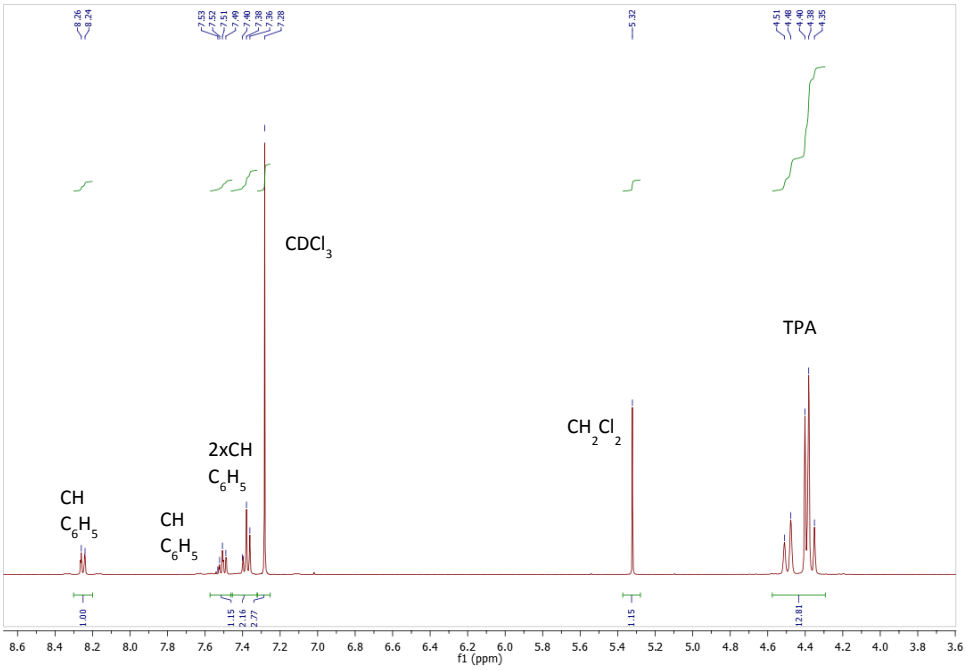


Figure S7. ^{1}H NMR spectra of compound **7** in $CDCl_3$.

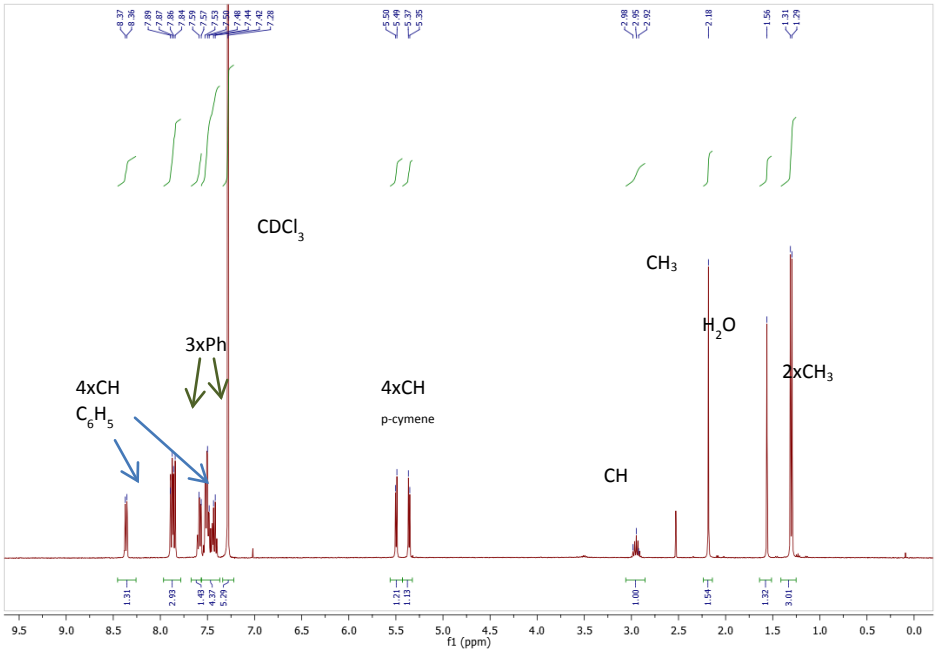


Figure S8. ^{1}H NMR spectra of compound **8** in $CDCl_3$.

3. Stability of compounds 1-4, 8 and 9 in d^6-DMSO and D_2O solution overtime assessed by $^{31}P\{^1H\}$ NMR spectroscopy[a]

	d^6-DMSO								
	2.5 hr	1 day	6 days	1 week	2 weeks	3 weeks	1 month	1.5 months	Half life (50%)
1					>99%		91%		**months**
2						>99%	87%		**months**
3								>99%	**months**
4							>99%		**months**
8			>99%	58%		25%	0%		**~9 days**
9	>99%	85%		50%	27%		6%		**~1 week**

a) % of decomposition determined by integration of all the signals present in the $^{31}P\{^1H\}$ NMR spectra, the sum being set to 100%.

	D_2O	
	3 days	Half lives (50%)
1		**insoluble**
2		**2.5 days**
3		**b**
4	>99%	**c**

a) % of decomposition determined by integration of all the signals present in the $^{31}P\{^1H\}$ NMR spectra, the sum being set to 100%.
b) Compound hydrolyzes when dissolved in water (45:55 ratio).
c) Compound precipitates after 3 days.

4. $^{31}P\{^{1}H\}$ NMR spectra showing the stability of compounds 1-4, 8, 9 in d^6-DMSO overtime

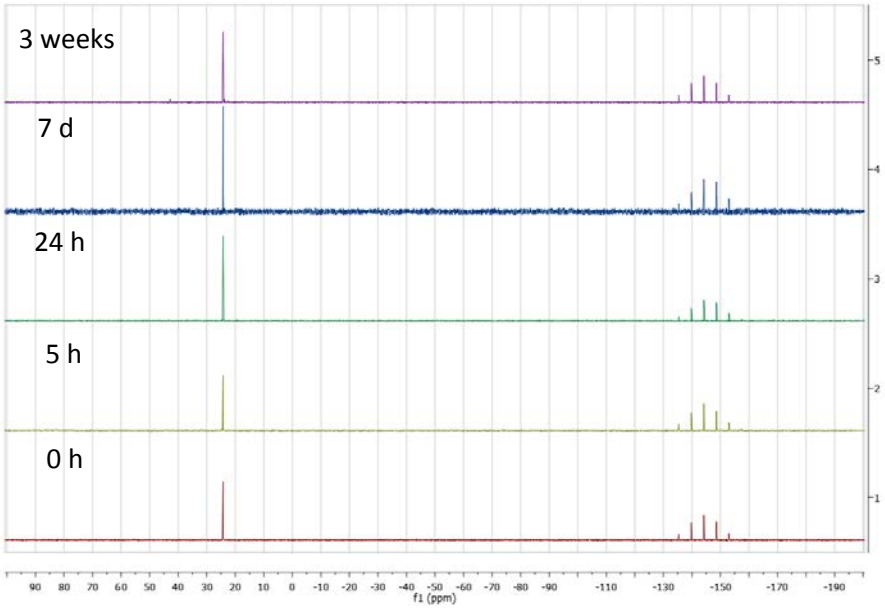


Figure S9. $^{31}P\{^{1}H\}$ NMR spectra of compound **1** in d^6-DMSO (δ 24.27 (s) and – 144.20 (h) ppm) overtime.

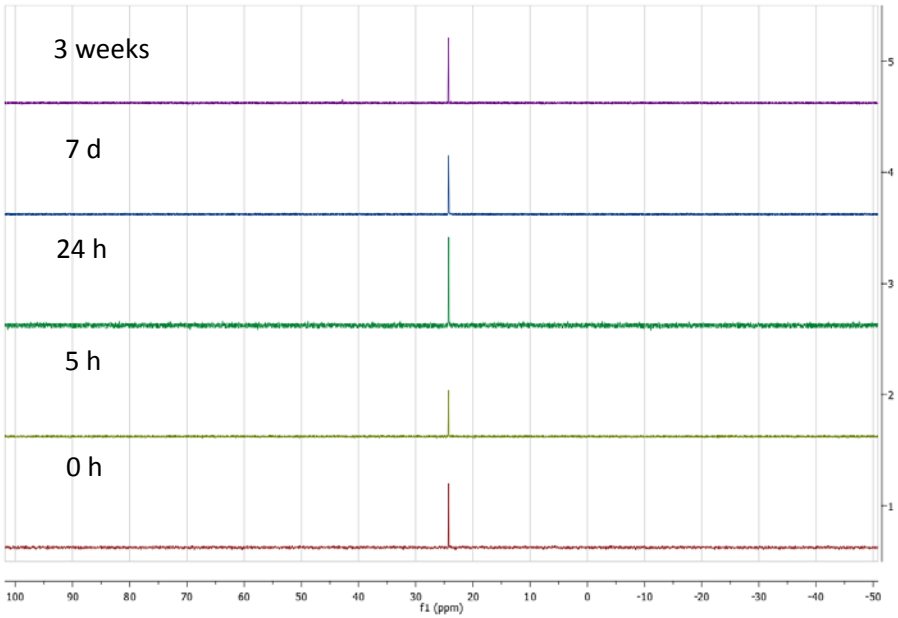


Figure. S10. $^{31}P\{^{1}H\}$ NMR spectra of compound **2** in d^6-DMSO (δ 24.26 (s) ppm) overtime.

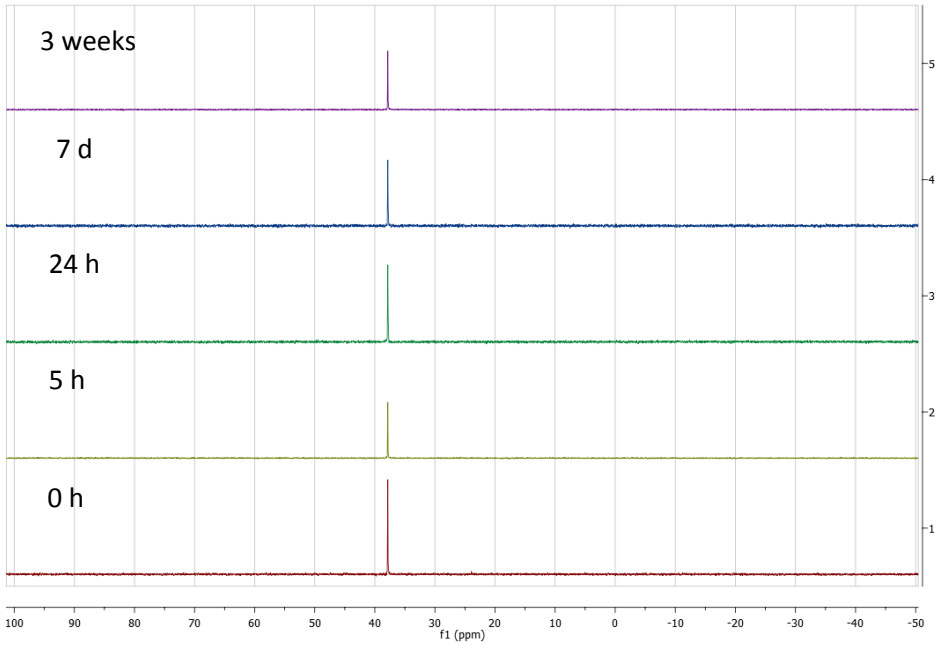


Figure S11. $^{31}P\{^{1}H\}$ NMR spectra of compound **3** in d^{6}-DMSO (δ 37.84 (s) ppm) overtime.

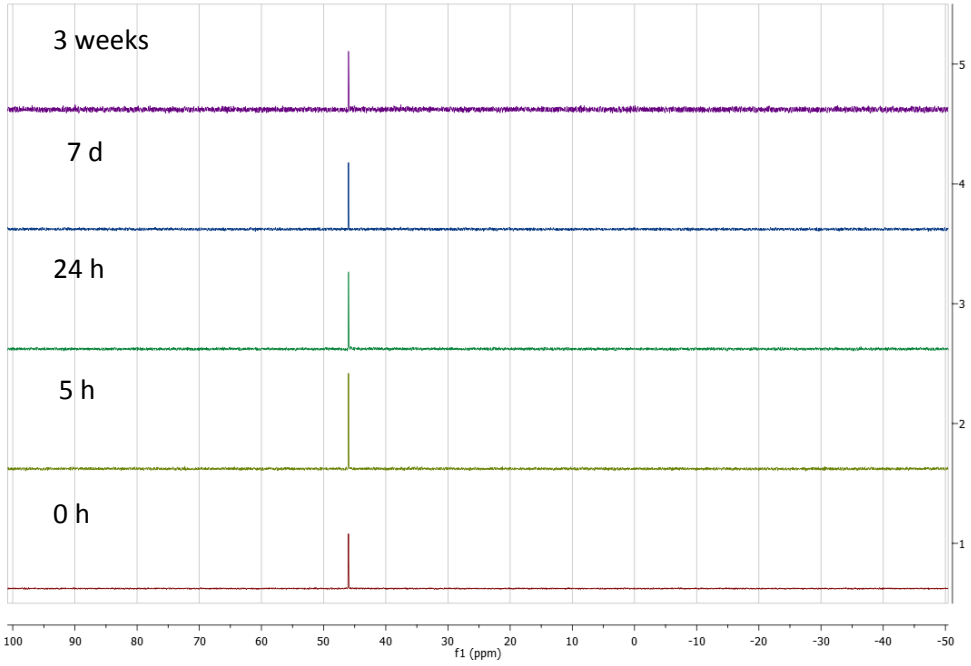


Figure S12. $^{31}P\{^{1}H\}$ NMR spectra of compound **4** in d^{6}-DMSO (δ 45.99 (s) ppm) overtime.

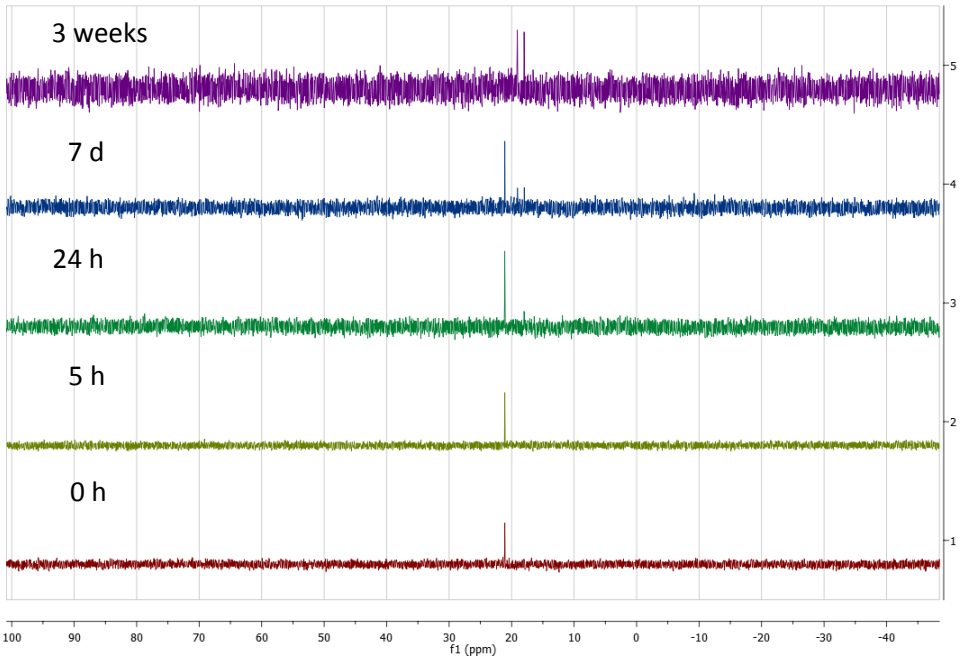


Figure S13. $^{31}P\{^{1}H\}$ NMR spectra of compound **8** in d^{6}-DMSO (δ 21.09 (s) ppm) overtime.

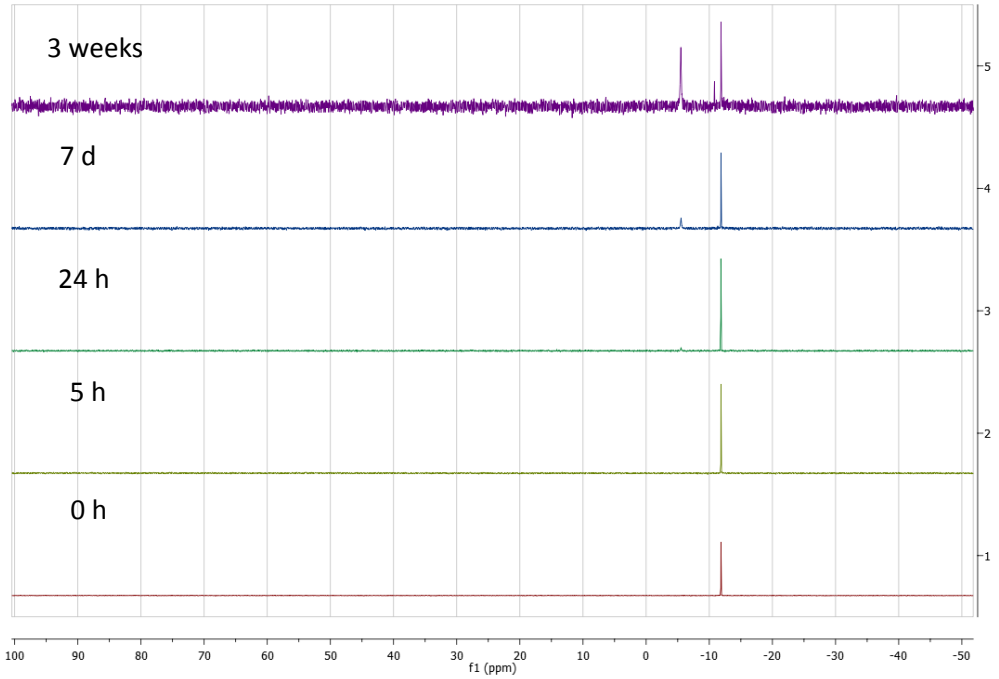


Figure S14. $^{31}P\{^{1}H\}$ NMR spectra of compound **9** in d^{6}-DMSO (δ -11.85 (s) ppm) overtime.

5. $^{31}P\{^{1}H\}$, ^{1}H NMR and ^{13}C NMR spectra of compounds 2, 3 and 4 in D_2O overtime

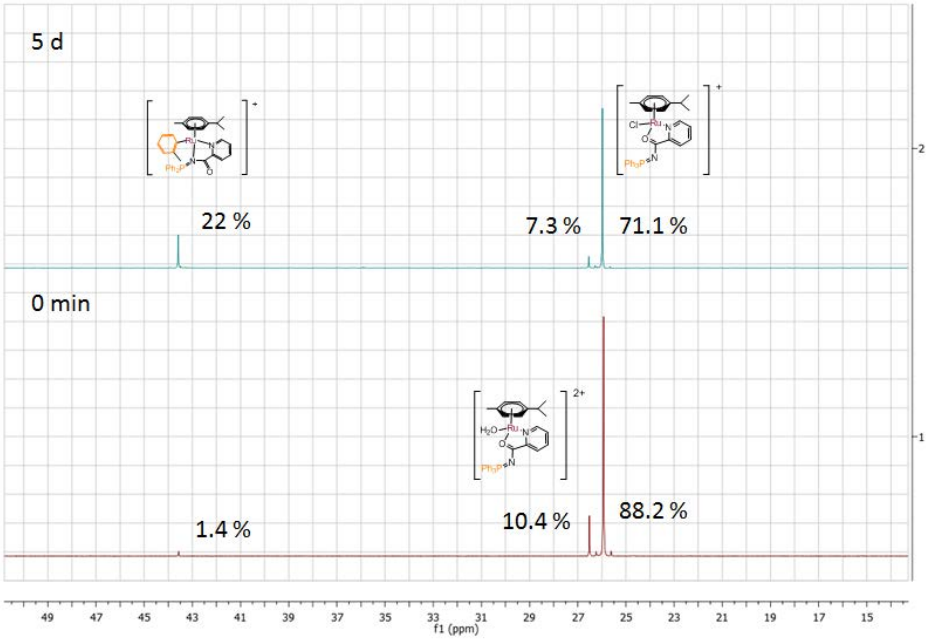


Figure S15. $^{31}P\{^{1}H\}$ NMR spectra of compound **2** in D_2O (δ 26.37 (s) ppm) overtime.

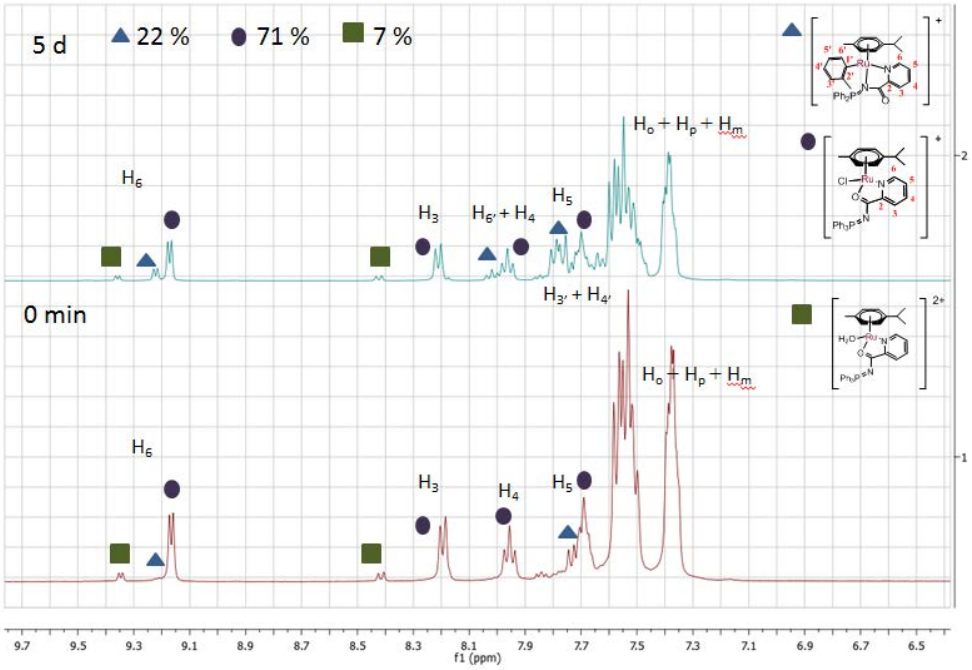


Figure S16. ^{1}H NMR spectra of compound **2** in D_2O at t = 0 and after 5 days.

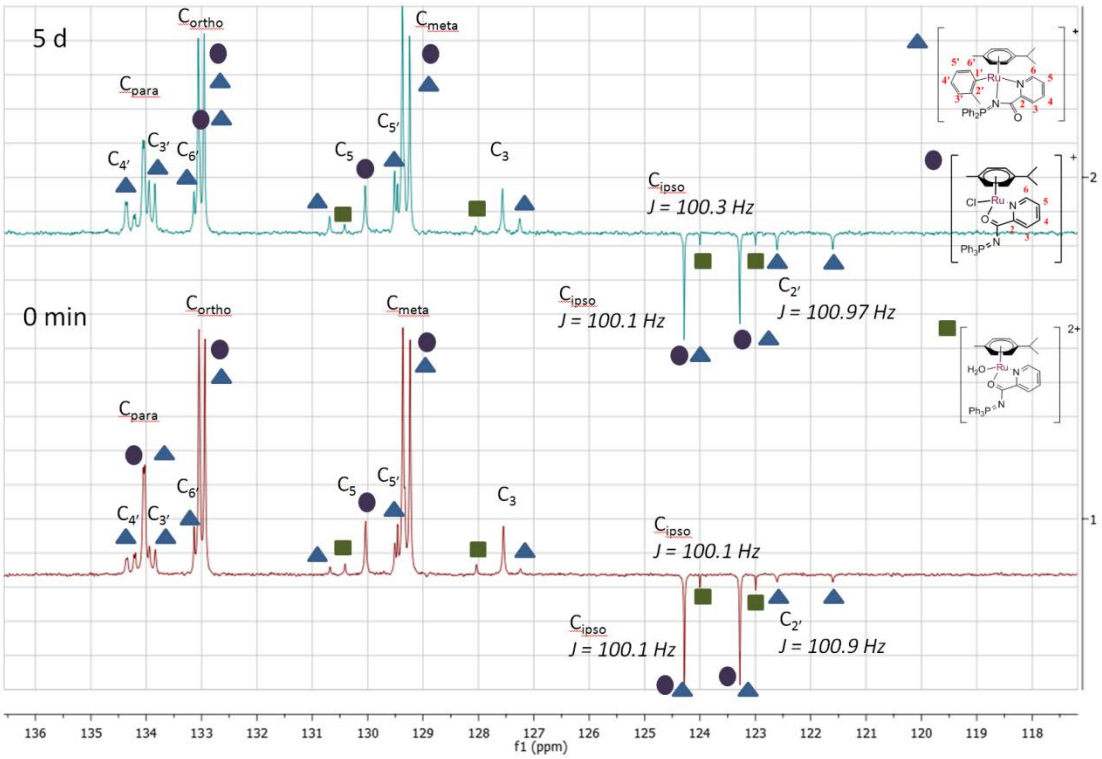


Figure S17. ^{13}C NMR spectra of compound **2** in D_2O at t = 0 and after 5 days.

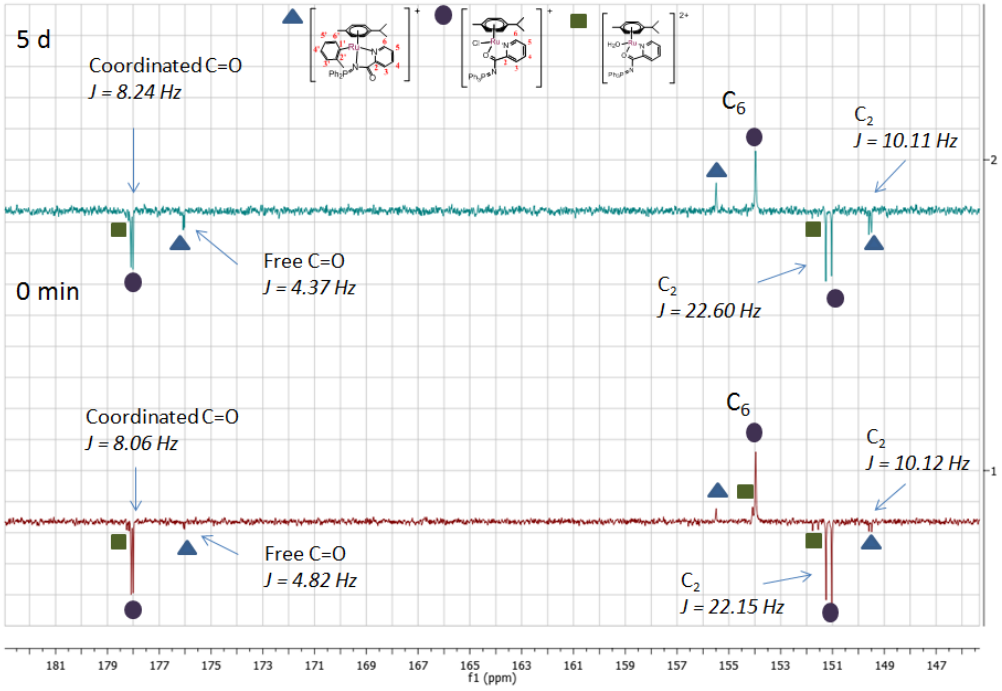


Figure S18. ^{13}C NMR spectra of compound **2** in D_2O at t = 0 and after 5 days.

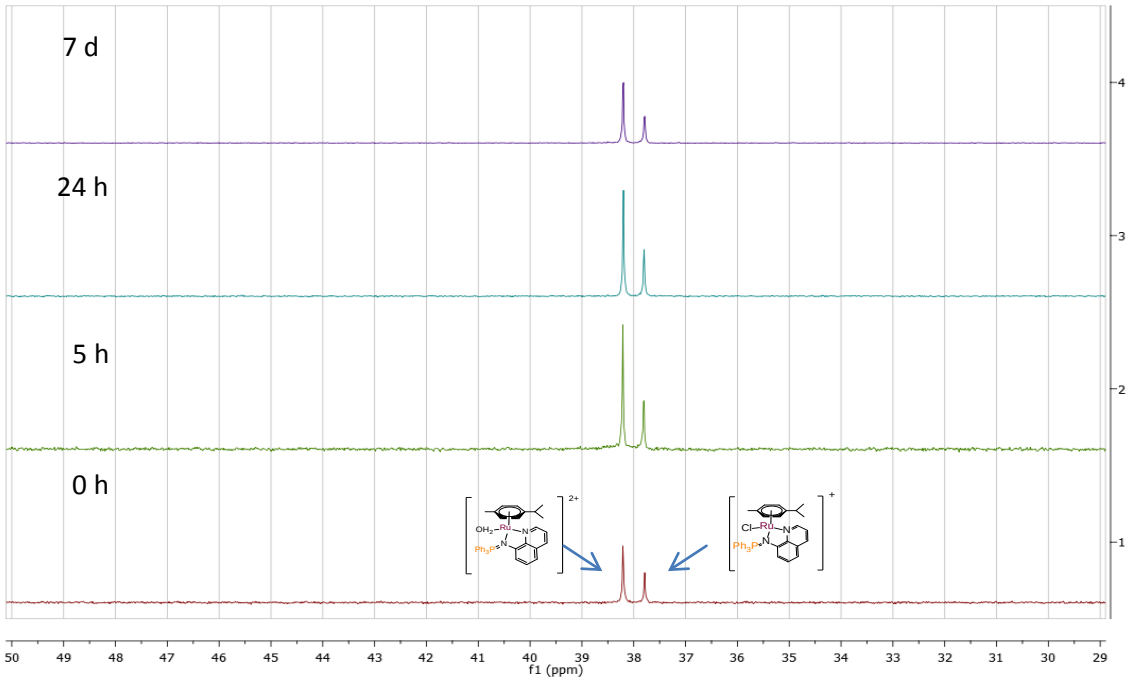


Figure S19. $^{31}P\{^{1}H\}$ NMR spectra of compound **3** in D_2O (δ 37.79 (s) ppm) overtime.

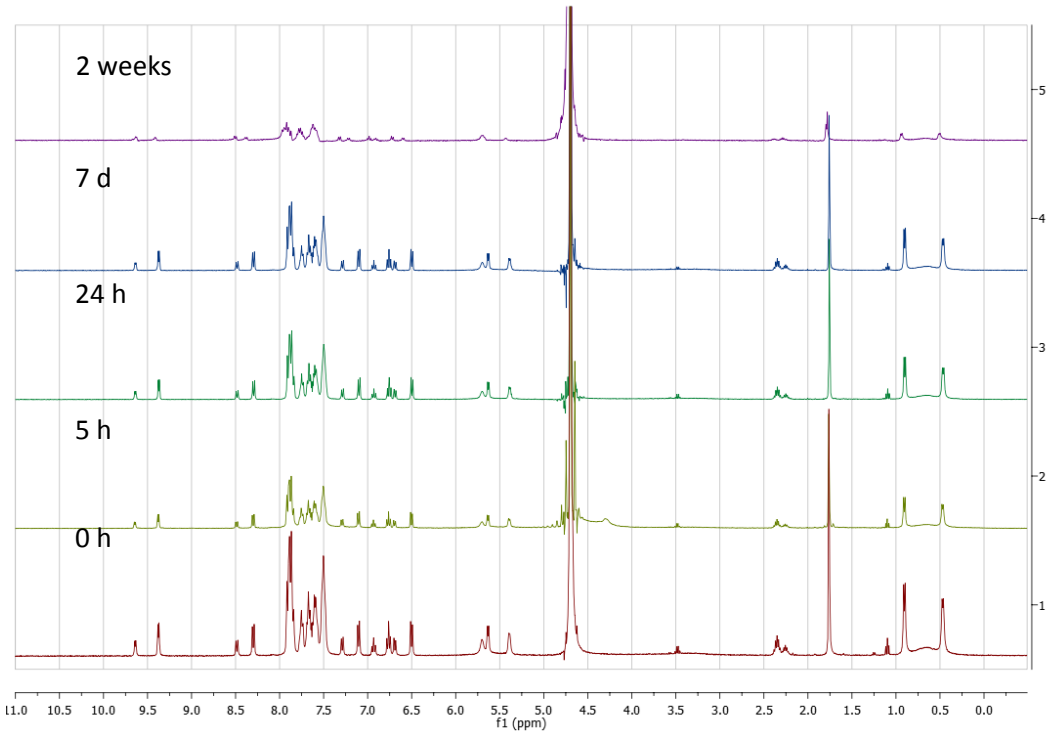


Figure S20. ^{1}H NMR spectra of compound **3** in D_2O overtime.

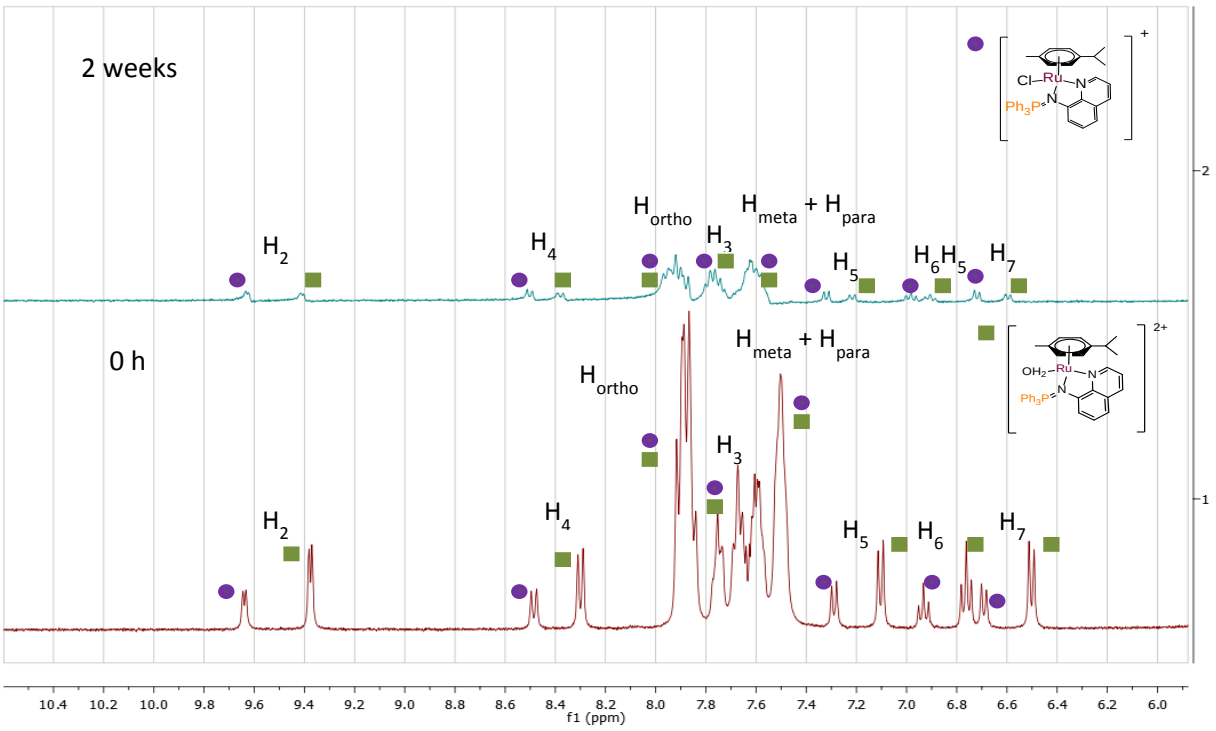


Figure S21. ^{1}H NMR spectra of compound **3** in D_2O overtime.

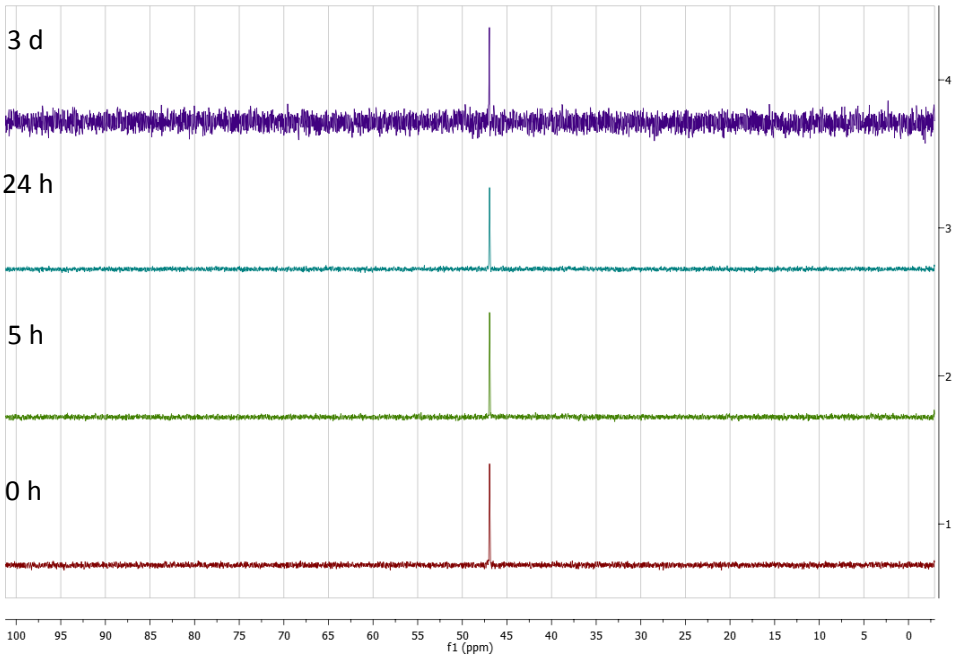


Figure S22. $^{31}P\{^{1}H\}$ NMR spectra of compound **4** in D_2O (δ 46.95 (s) ppm) overtime.

6. $^{31}P\{^{1}H\}$ NMR spectra of compounds 2 in a 100 mM NaCl/D_2O solution overtime

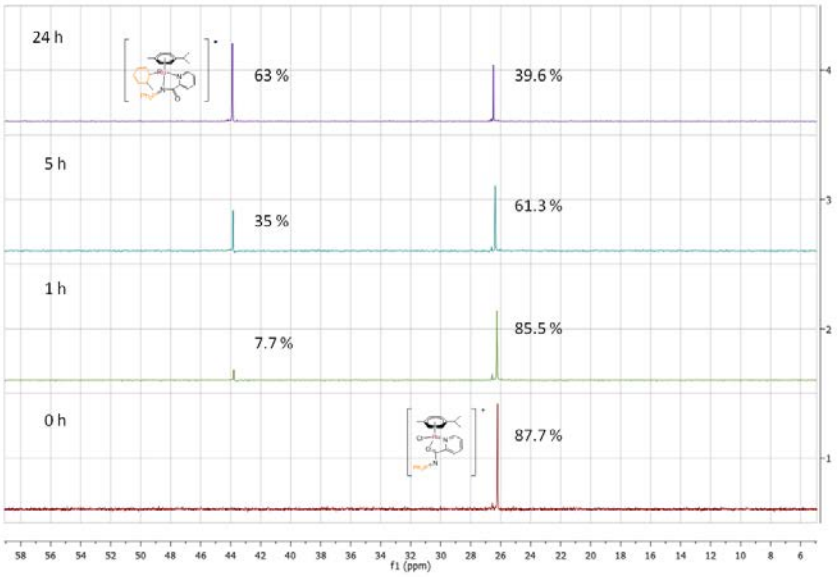


Figure S23. $^{31}P\{^{1}H\}$ NMR spectra of compound **2** in 100mM NaCl/D_2O (δ 26.21 (s) ppm) overtime.

7. $^{31}P\{^{1}H\}$ and ^{1}H NMR spectra of compounds 2 and 3 in a D_2O solution at 80°C during 1 h

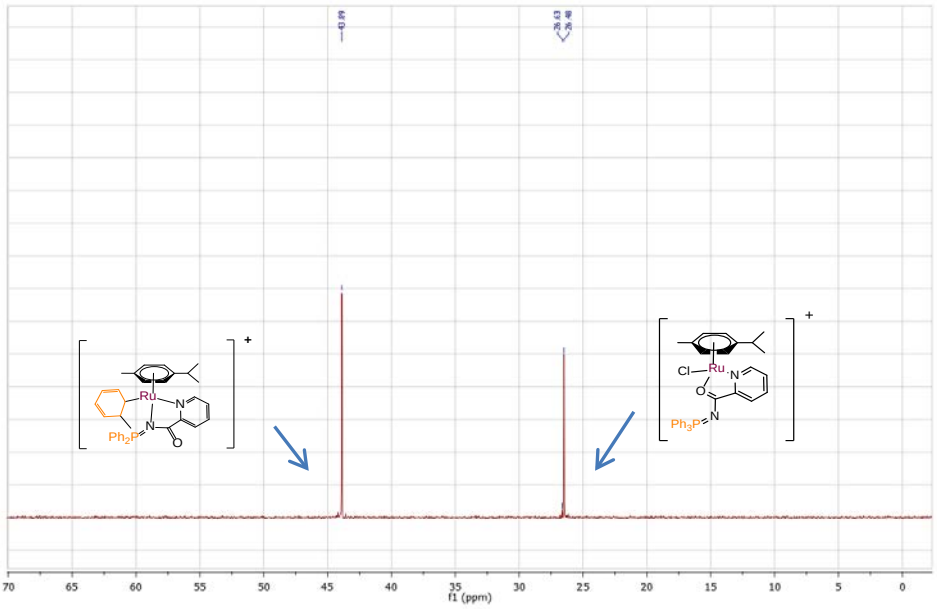


Figure S24. $^{31}P\{^{1}H\}$ NMR spectra of compound **2** in D_2O (δ 26.48 (s) ppm) after heating at 80°C for one hour.

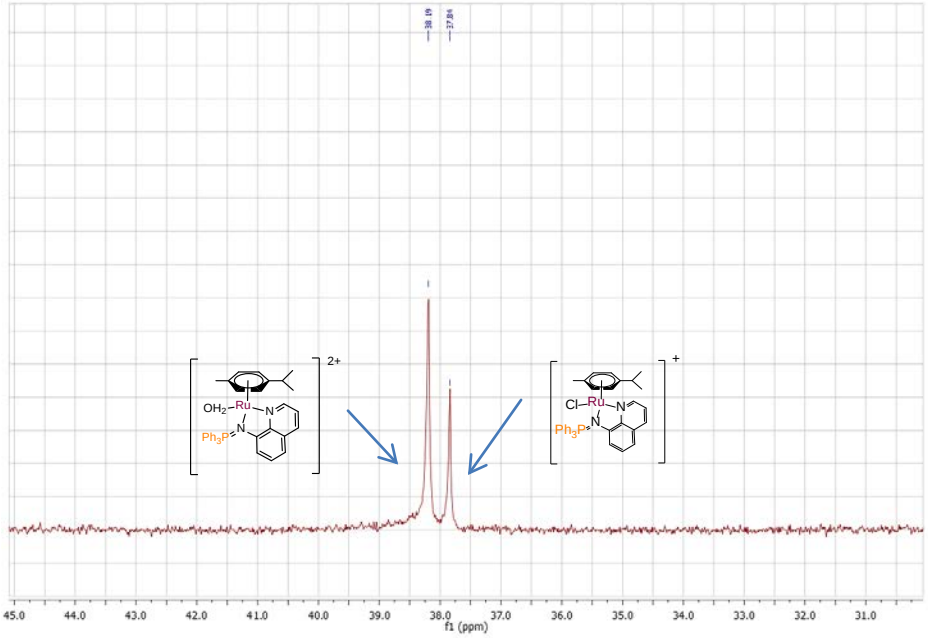


Figure S25. $^{31}P\{^{1}H\}$ NMR spectra of compound **3** in D_2O (δ 37.84 (s) ppm) after heating at 80°C for one hour.

8. Mass spectra (ESI+) of compound 2 in H_2O solution overtime (5 days)

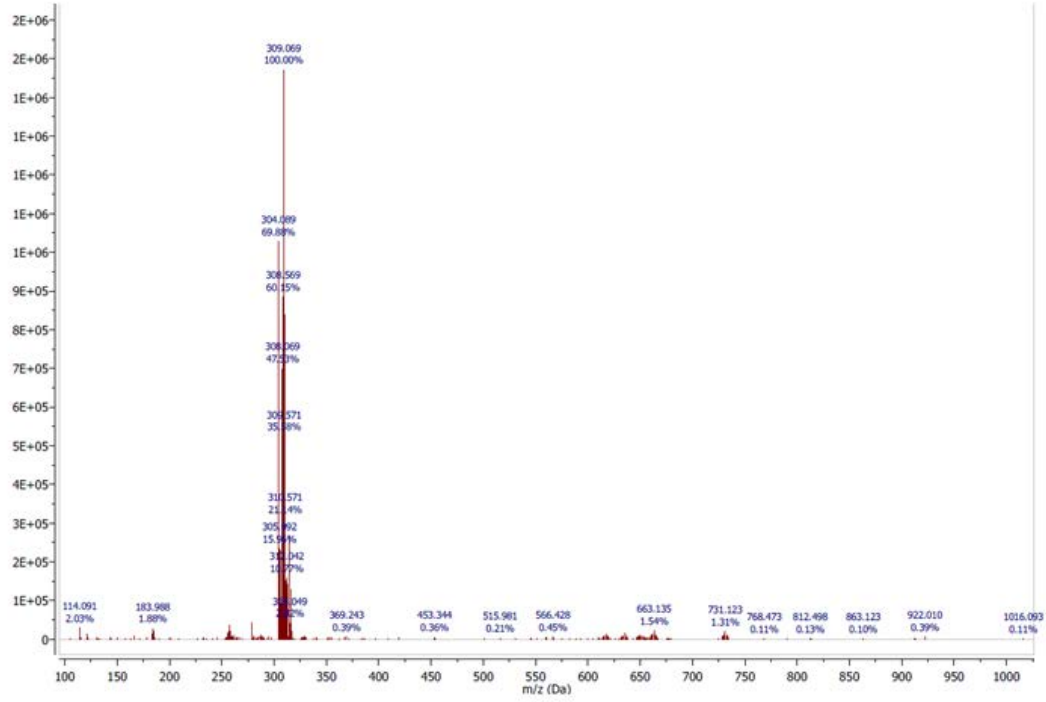


Figure S26. MS ESI+ of compound **2** in H_2O solution at t=0.

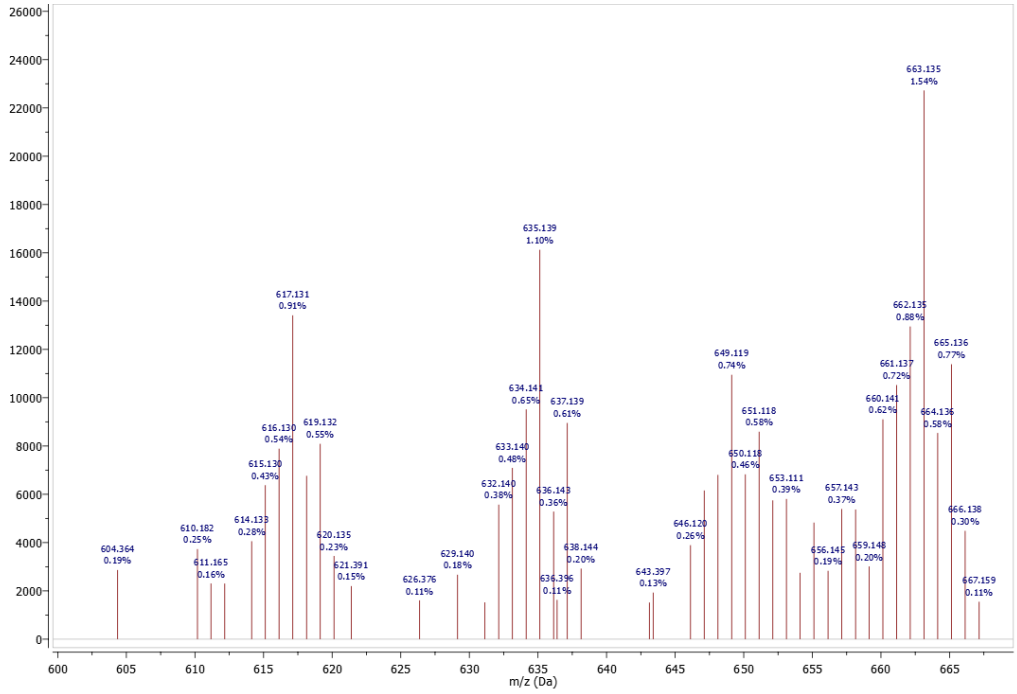


Figure S27. Magnification of [m/z]: 617.1 from 600 to 665 of compound **2** in H_2O solution at t=0.

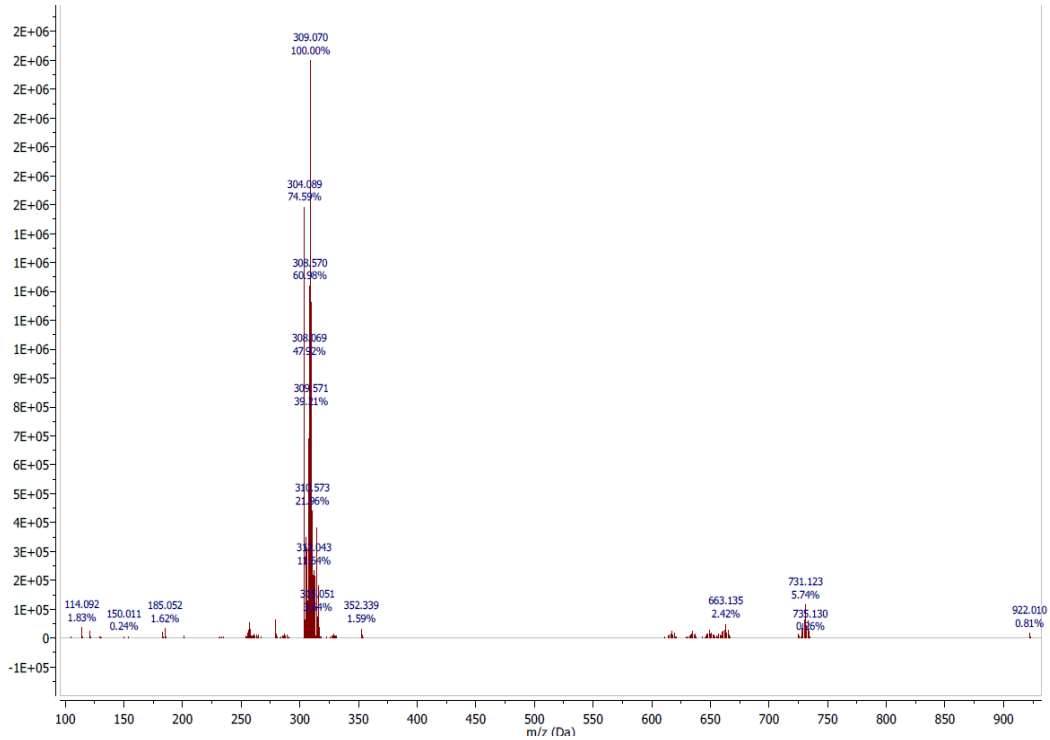


Figure S28. MS ESI+ of compound **2** in H_2O solution at t= 5 days.

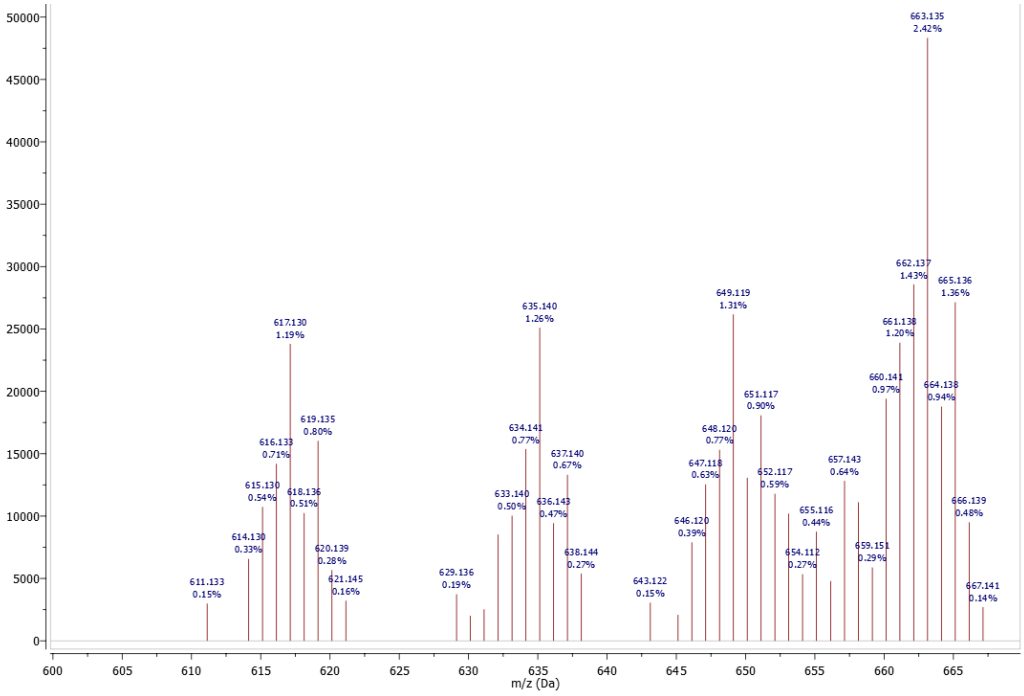


Figure S29. Magnification of [m/z]: 617.1 from 600 to 665 of compound **2** in H_2O solution at t=5 days.

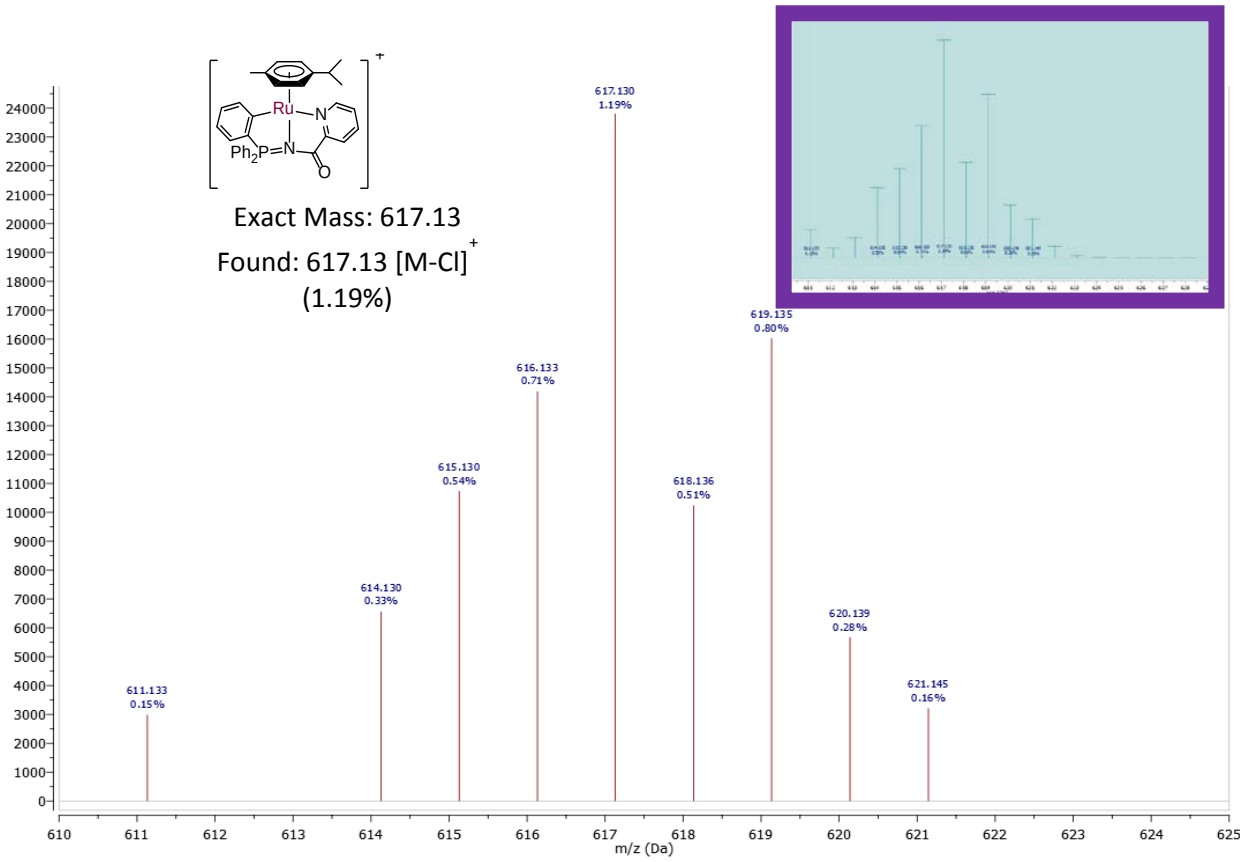
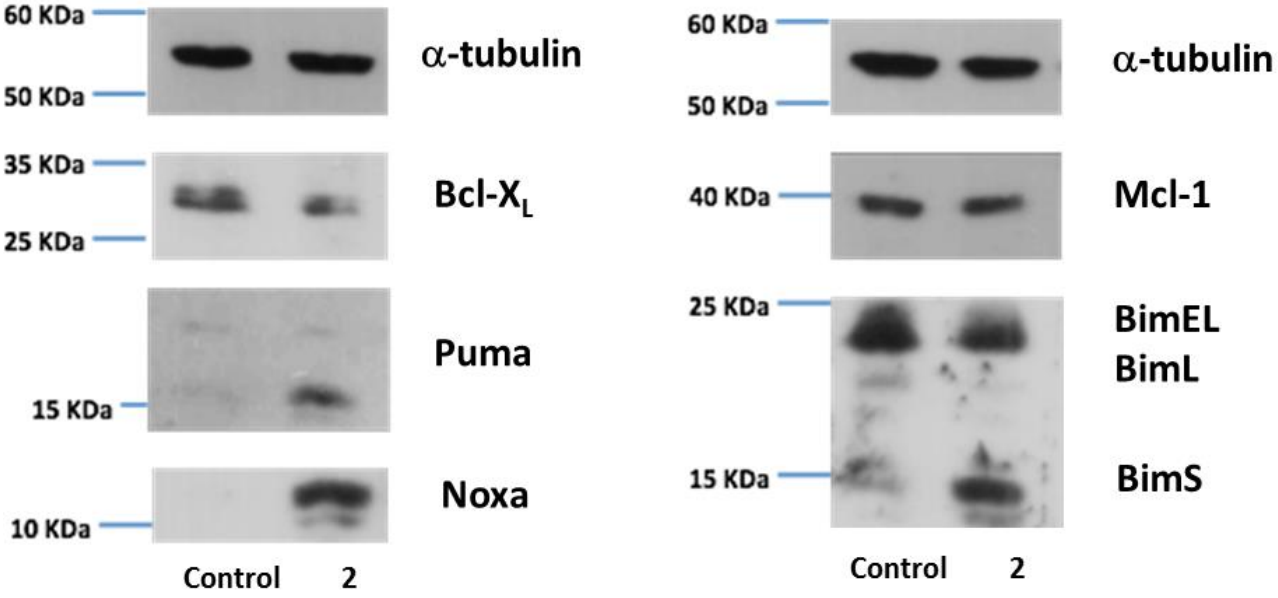


Figure S30. Magnification of peak at [m/z]: 617.1 corresponding to species [(η^6-*p*-cymene)Ru(IM-k-C,N-C_6H_4(PPh_2=N-CO-2-N-C_5H_4)]$^+$ in the MS ESI+ spectrum of compound **2** in H_2O solution at t= 5 months. Insert: theoretical isotopic distribution.

9. Study of the effect of 2 in the levels of proteins of the Bcl-2 family



S31. Effect of **2** in the levels of proteins of the Bcl-2 family. Jurkat cells were left untreated (control) or incubated for 6 h with compound **2** (1 μM). At the end of incubations total protein extracts were prepared as described in the Experimental section and analyzed by Western Blot with specific antibodies as indicated. Blots are representative of three independent experiments.

10. Experiments to assess the interaction of compounds 2-4 with CT DNA by circular dichroism

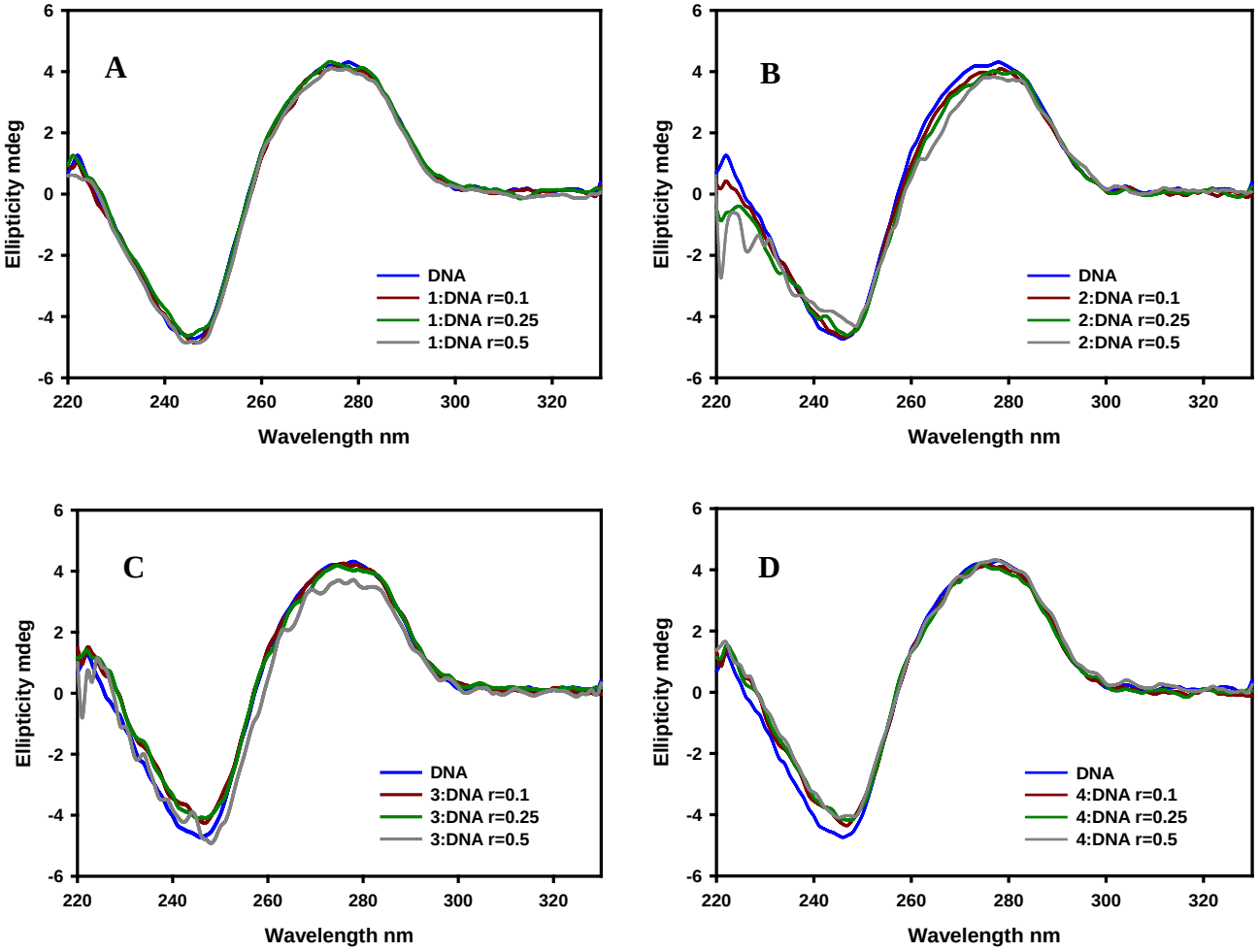


Figure S32. CD spectra of CT DNA (48 μM) and CT DNA incubated with 0.1, 0.25 and 0.5 equivalents of compounds **1** (A), **2** (B), **3** (C) and **4** (D) for 20 h at 37 °C.