Electronic Supplementary Material (ESI) for Dalton Transactions.

Anticancer activity of a *cis*-dichloridoplatinum(II) complex of a chelating nitrogen mustard: Insight to unusual guanine binding mode and low deactivation by glutathione

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Contents

Table S1 Crystal Data, data Collection and refinement parameters for complex **2**

2	
Empirical formula	$C_{10}H_{14}Cl_4N_2Pt$
Formula weight	499.12
Temperature (K)	100.01(10)
Wavelength (Å)	0.7107
Crystal system,	Monoclinic
space group	$P2_1/c$
a (Å)	8.5829(11)
b (Å)	10.4134(11)
c (Å)	15.2859(16)
α (deg.)	90.00
β (deg.)	98.85(10)
γ (deg.)	90.00
Volume (Å^3)	1350.0(3)
Z, Calculated density (mg/mm^3)	4, 2.456
F(000)	936.0
μ/mm^{-1}	11.163
Max. and min. transmission	0.518 and 1.000
Goodness-of-fit on F^2	1.008
Final R indices [$I>2\sigma(I)$]	$^aR_1 = 0.0326$, $^bwR_2 = 0.0665$
R indices (all data)	$^aR_1 = 0.0391$, $^bwR_2 = 0.0712$

$^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. $^bwR_2 = [\Sigma[w(F_o^2 - F_c^2)^2]/ \Sigma w(F_o^2)^2]^{1/2}$

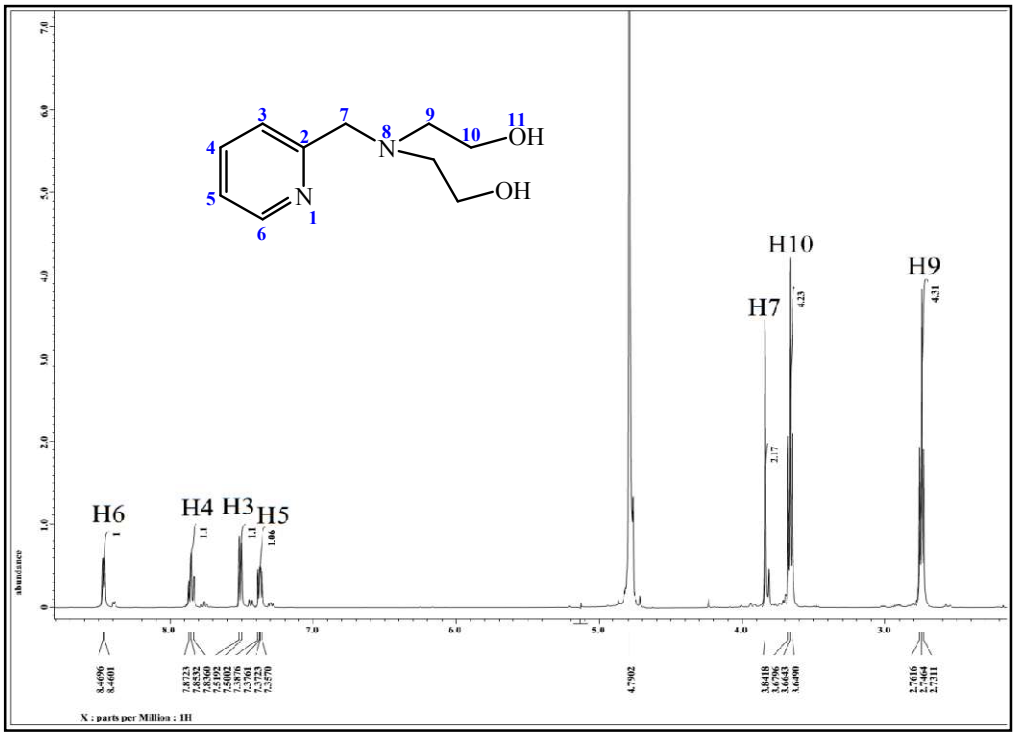


Fig. S1 ^{1}H NMR of L1 in D_2O, 400 MHz.

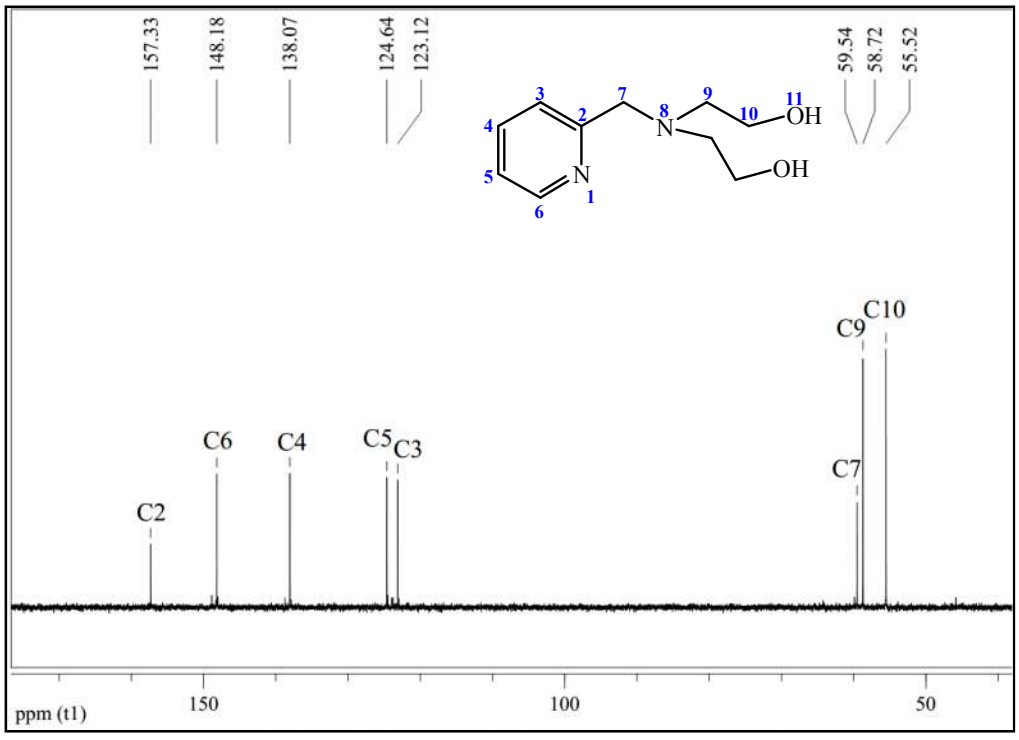


Fig. S2 ^{13}C NMR of L1 in D_2O, 125 MHz.

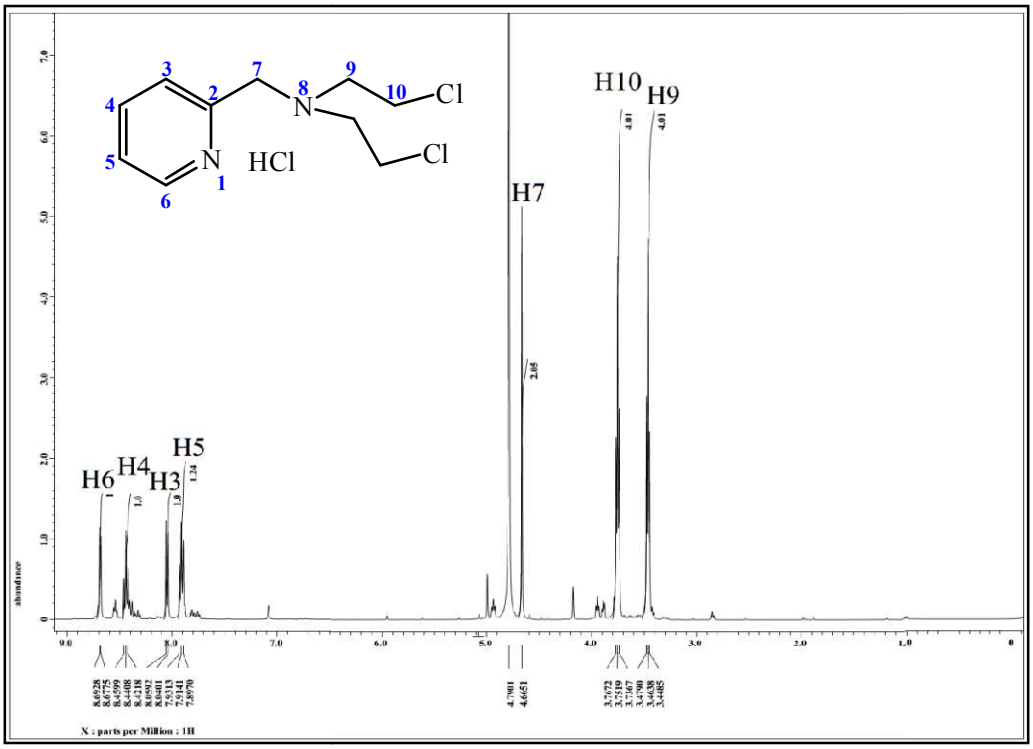


Fig. S3 ^{1}H NMR of L2.HCl in D_2O, 400 MHz.

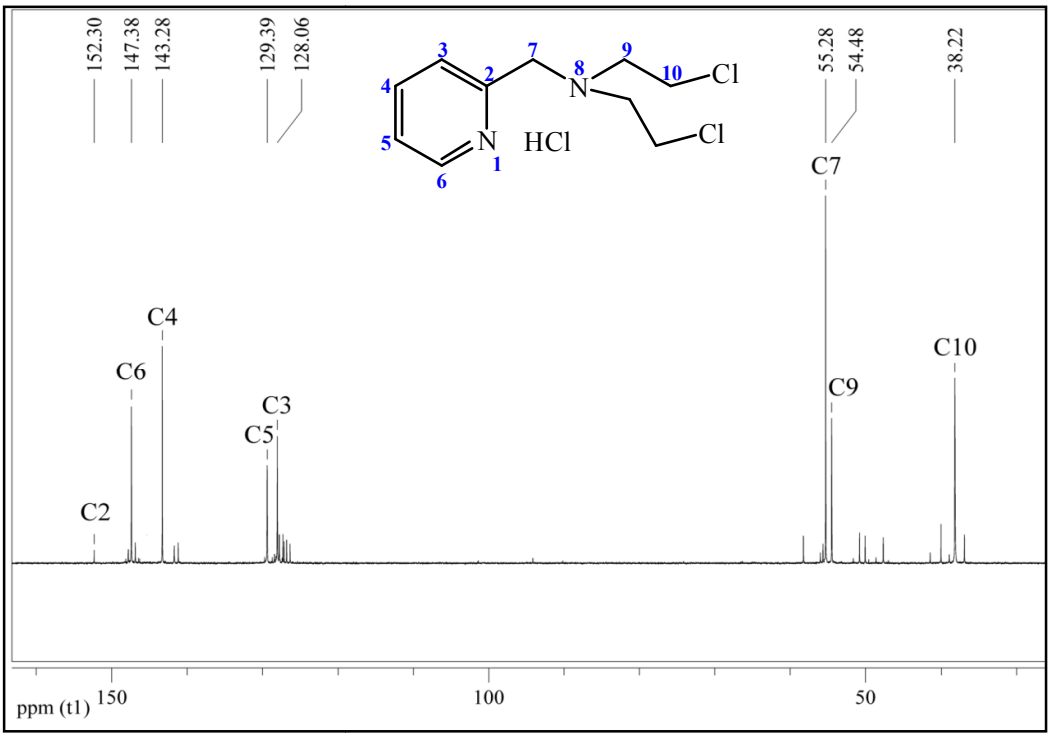


Fig. S4 ^{13}C NMR of L2.HCl in D_2O, 125 MHz.

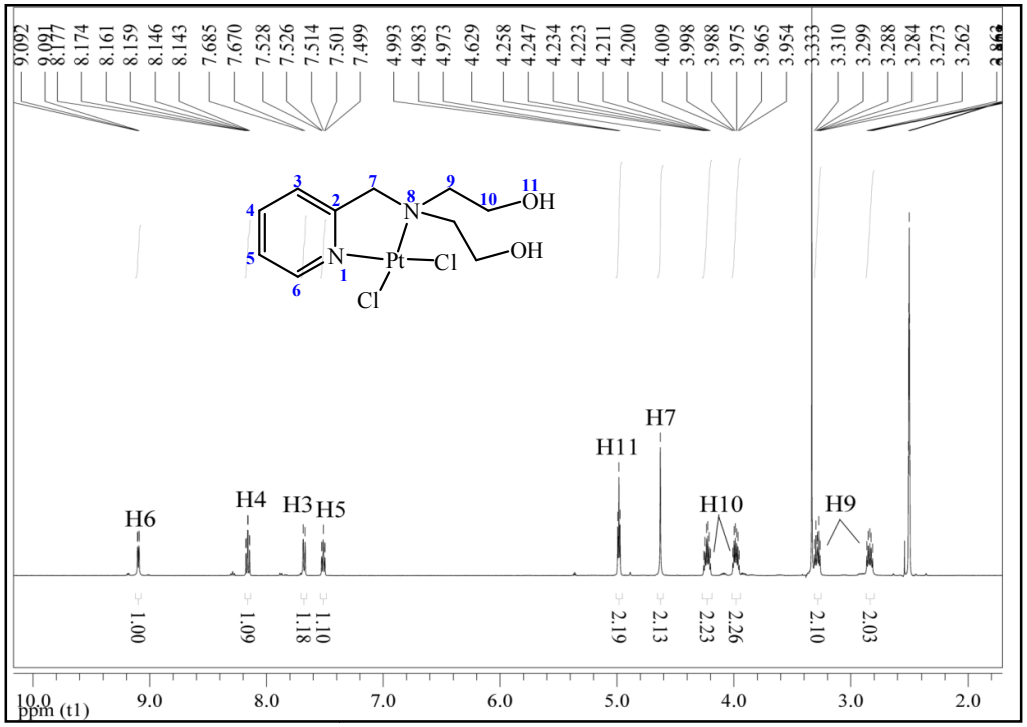


Fig. S5 ^{1}H NMR of **1** in DMSO-d_6, 500 MHz.

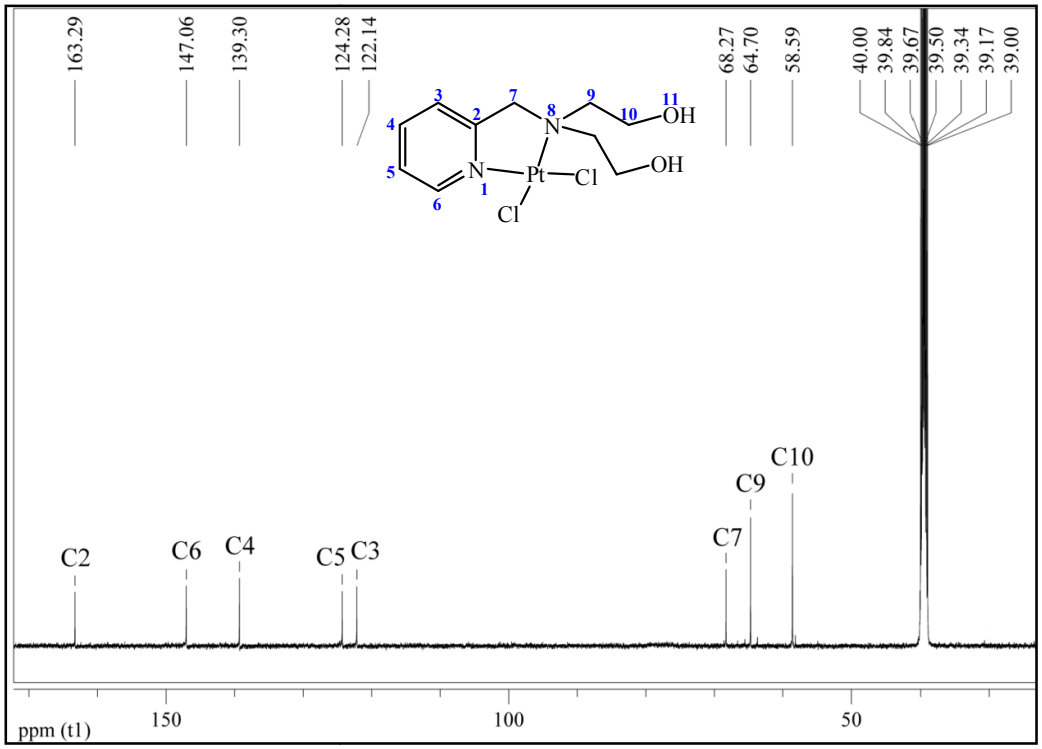


Fig. S6 ^{13}C NMR of **1** in DMSO-d_6, 125 MHz.

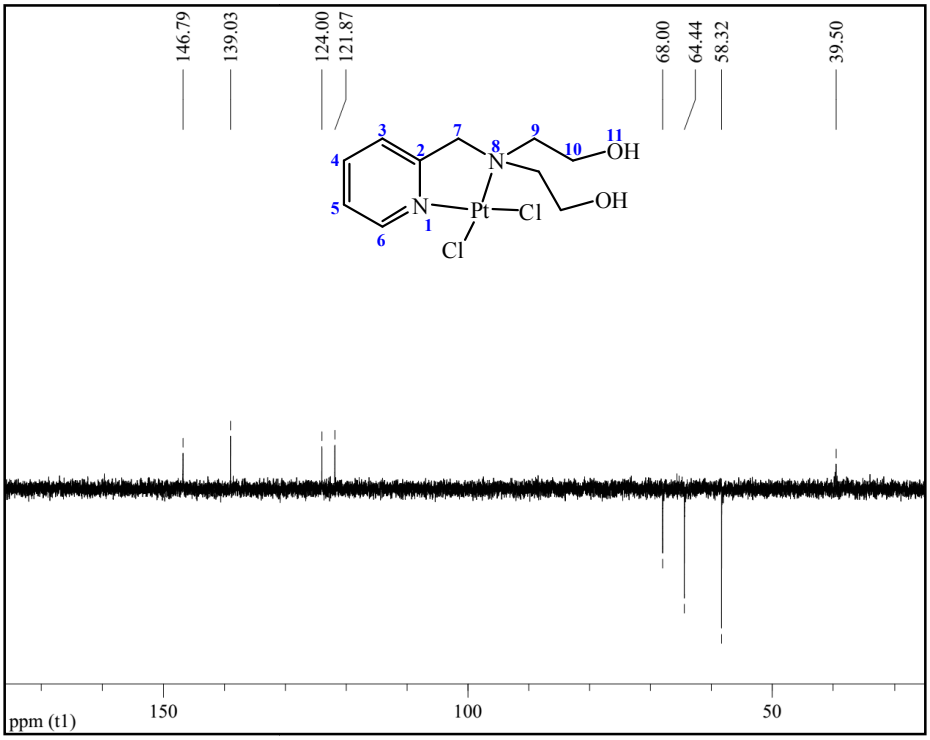


Fig. S7 ^{13}C DEPT-135 of **1** in DMSO-d_6, 125 MHz.

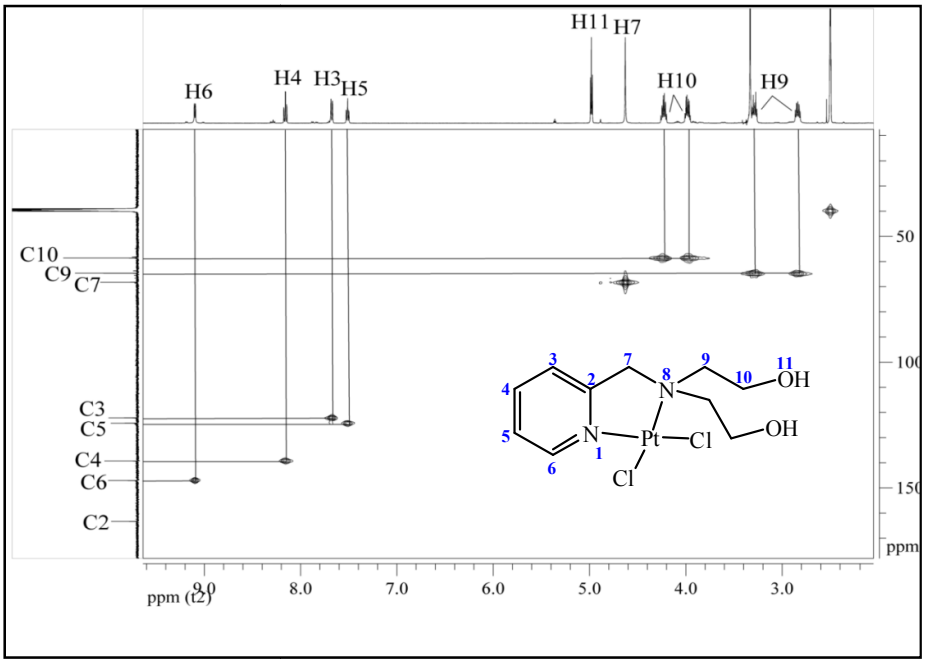


Fig. S8 HMQC of **1** in DMSO-d_6.

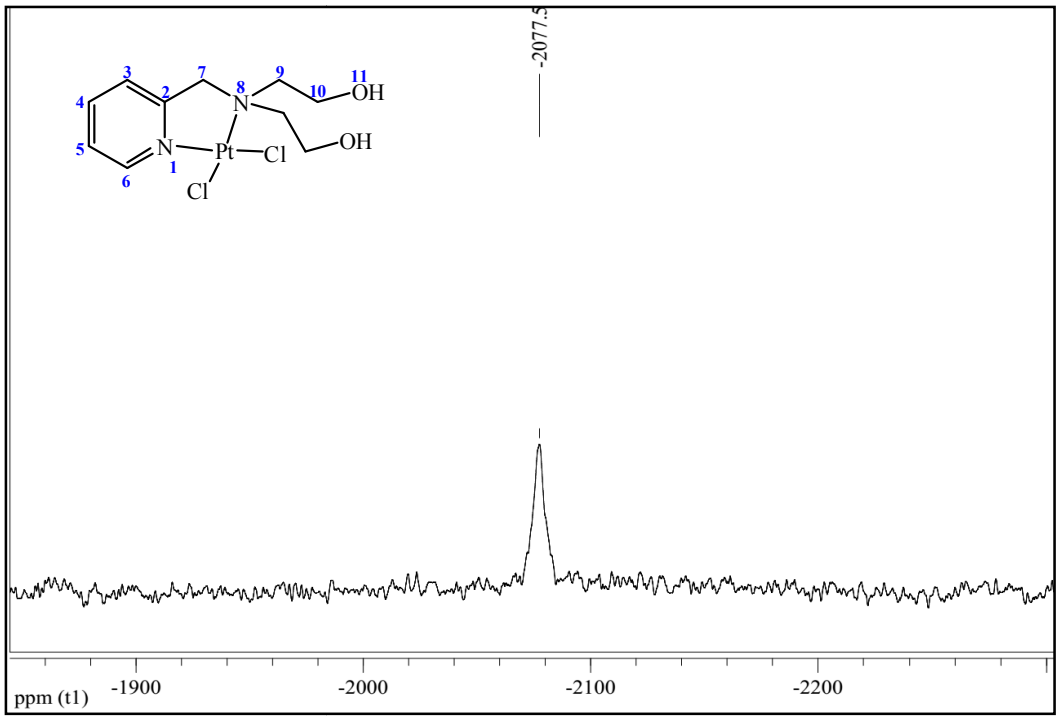


Fig. S9 ^{195}Pt NMR of **1** in DMSO-d_6, 107.5 MHz.

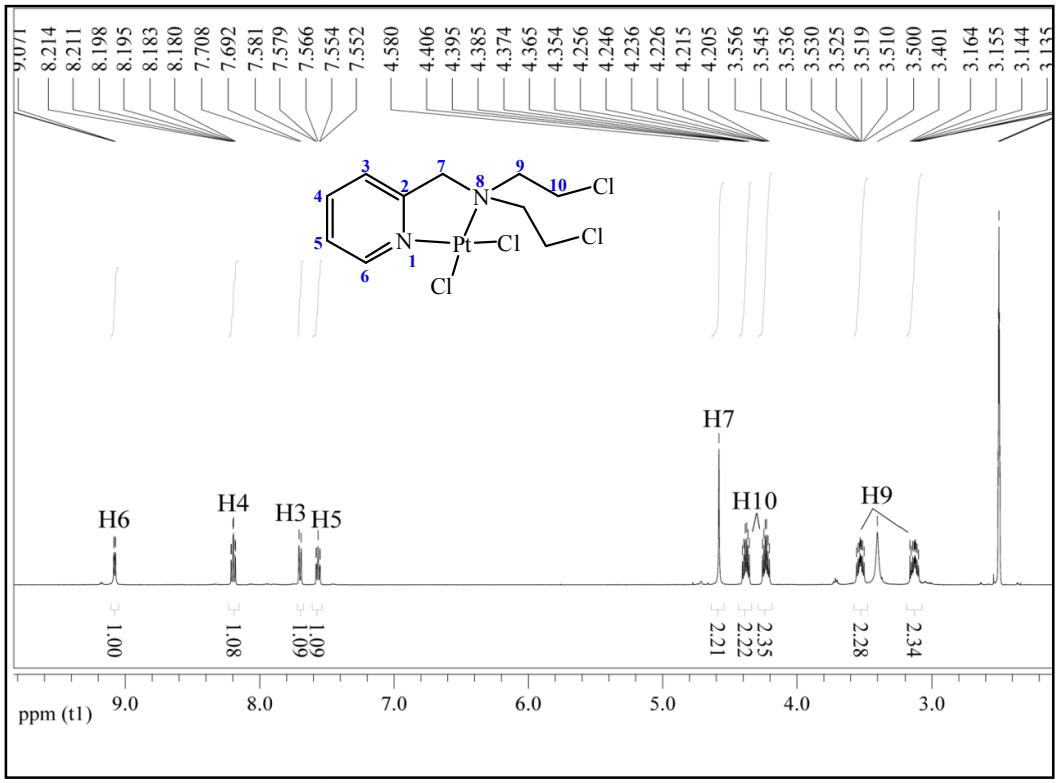


Fig. S10 ^{1}H NMR of **2** in DMSO-d_6, 500 MHz.

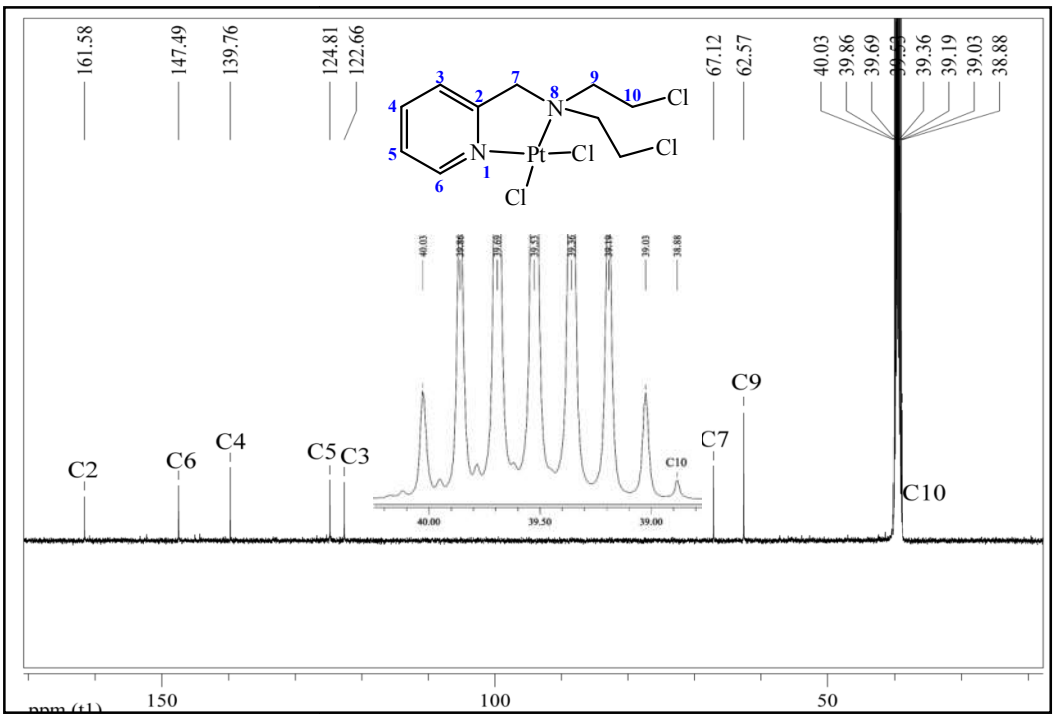


Fig. S11 ^{13}C NMR of **2** in DMSO-d_6, 125 MHz.

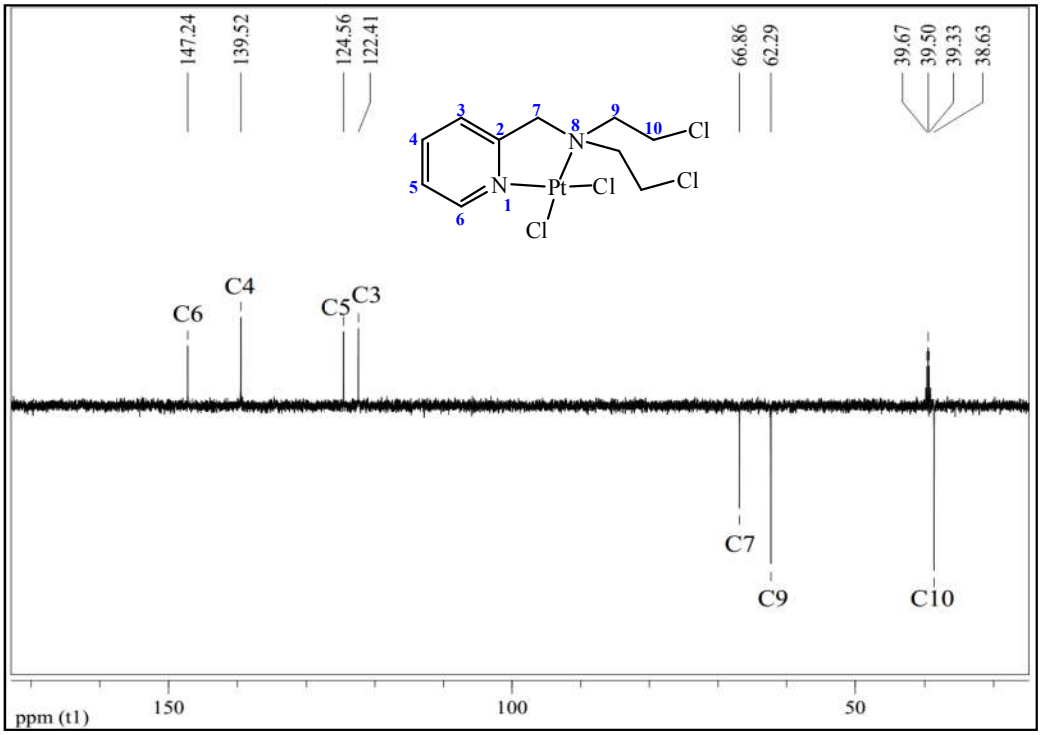


Fig. S12 ^{13}C DEPT-135 of **2** in DMSO-d_6, 125 MHz.

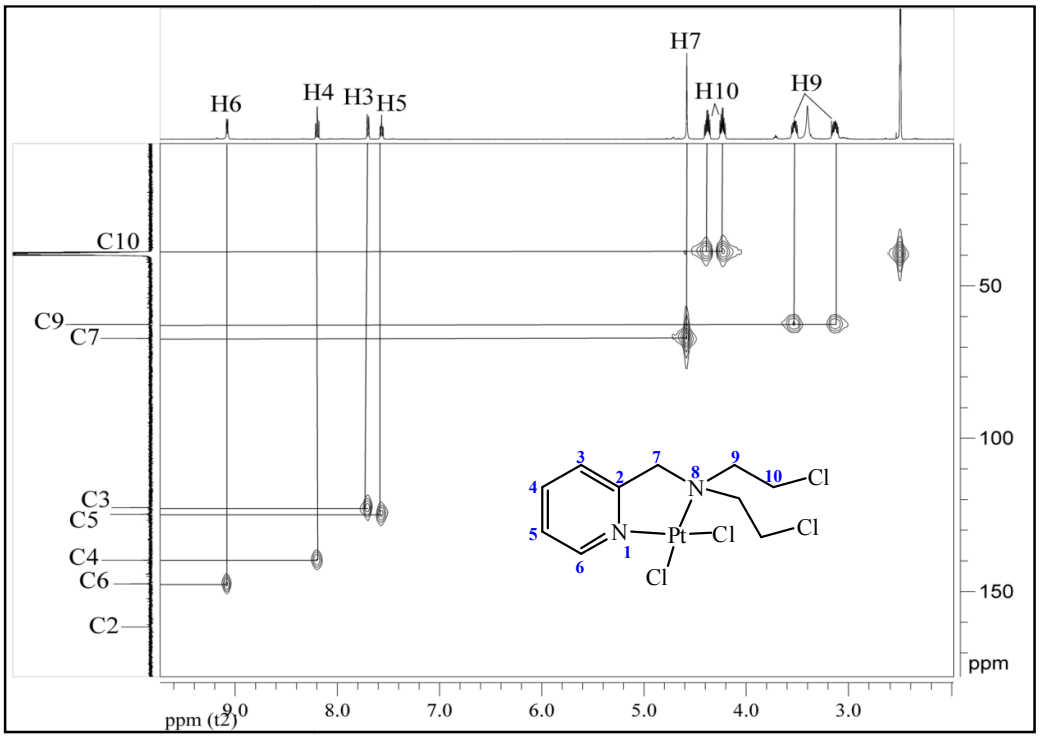


Fig. S13 HMQC of **2** in DMSO-d_6.

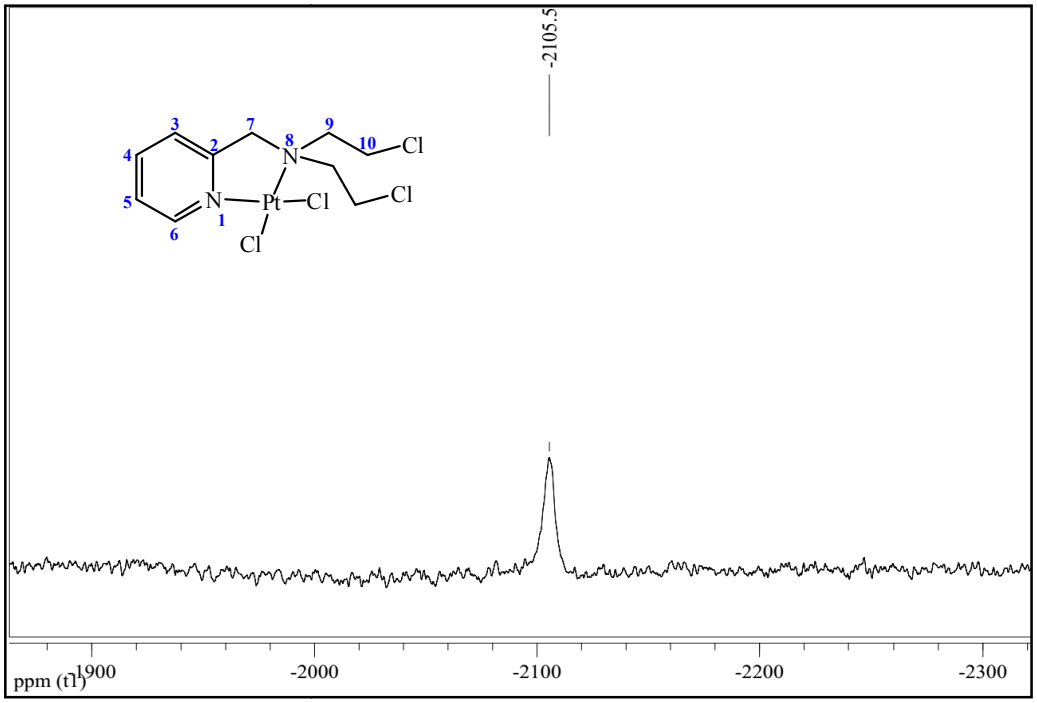


Fig. S14 ^{195}Pt NMR of **2** in DMSO-d_6, 107.5 MHz.

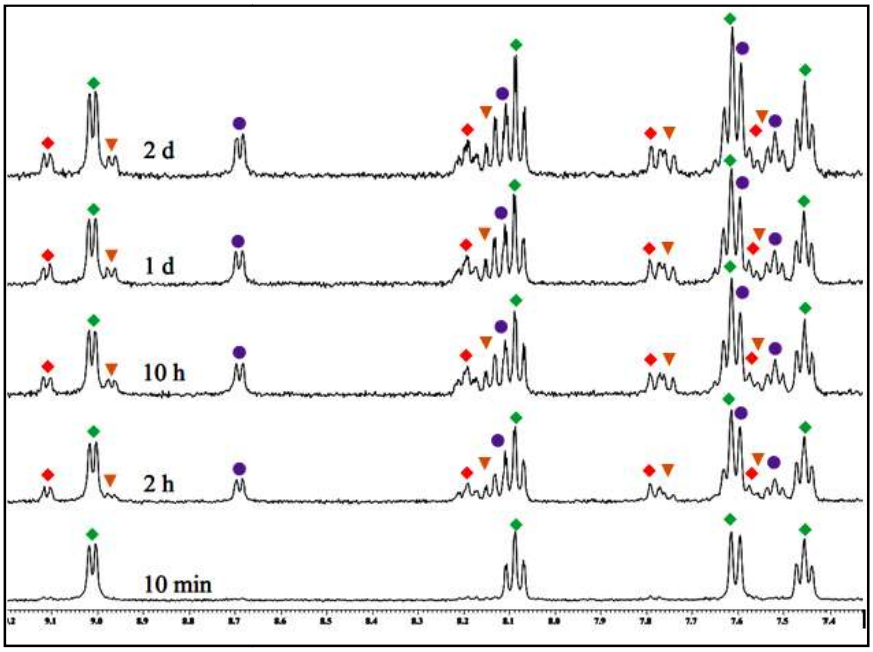


Fig. S15 Stack plot of aromatic region during the stability kinetics study of complex **1** in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture by ^1H NMR, where ♦ & ● indicate the intact complex **1** & complex **1b** and ▲ & ▼ indicate the aquated complex **1a** & **1c** respectively.

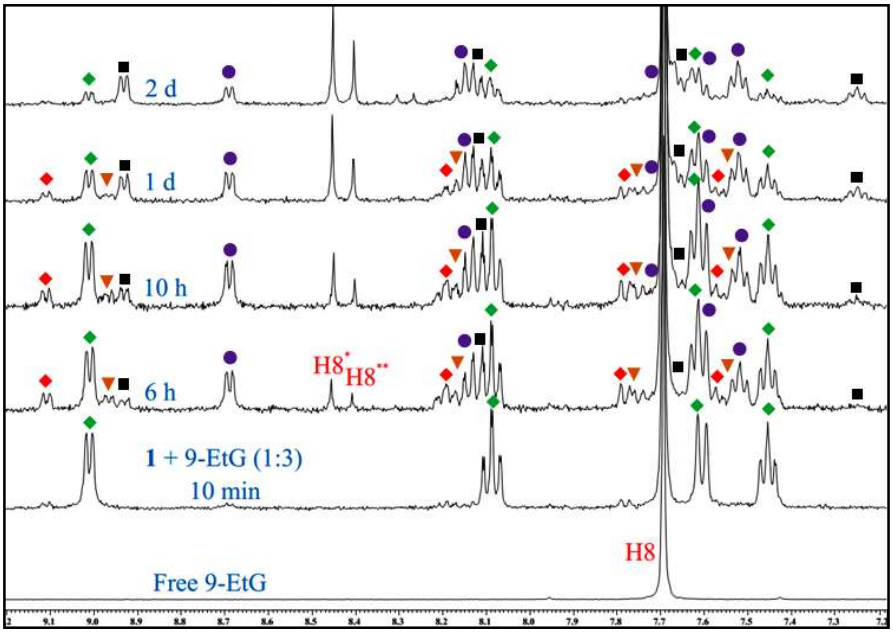


Fig. S16 Stack plot of aromatic region during the binding kinetics study of **1** with 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^1H NMR, where ♦ & ● indicate the intact complex **1** & complex **1b** and ▲ & ▼ indicate the aquated complex **1a** & **1c** respectively. ▪ indicates the signals of 9-EtG bound complexes **1d** & **1e**. H8 of free 9-EtG is shifted downfield to $H8^*$ & $H8^{**}$ of 9-EtG bound complexes **1d** & **1e** respectively.

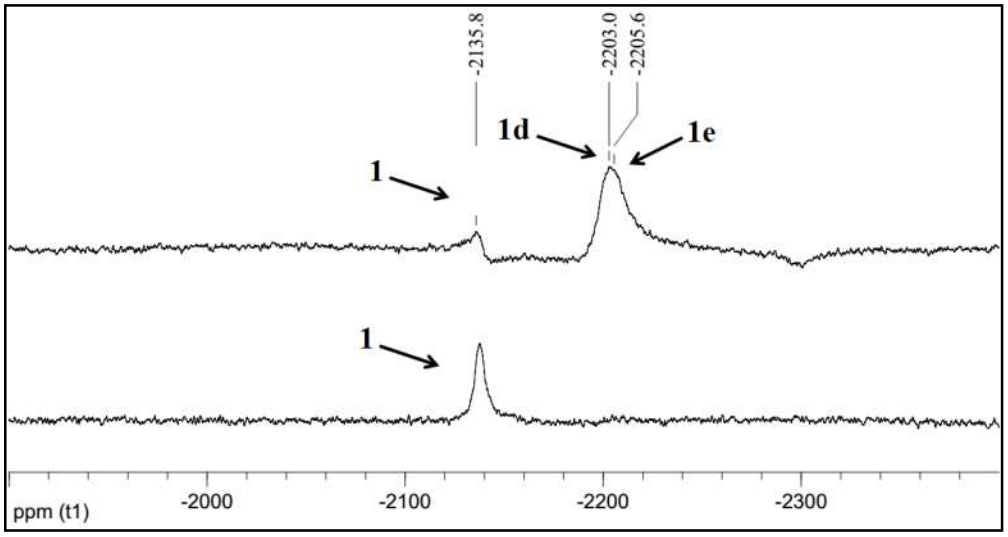


Fig. S17 Stack plot of binding kinetics study of **1** with 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{195}Pt NMR. The data taken after 2 h shows no binding of 9-EtG with **1**. After 24 h complex **1** signal at −2135.8 ppm diminished and two poorly resolvable signals of 9-EtG bound complexes **1d** & **1e** at −2203.0 & −2205.6 ppm respectively appear.

Table S2 Species found in ESI-MS during the stability/binding kinetics studies of **1** by ^{1}H NMR

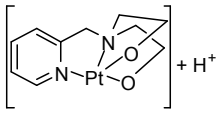
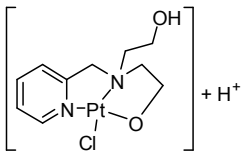
Species no.	Drawings	m/z_{calc}	Experiments	
			1 in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6	**1** + 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6
			m/z_{obs}	
I-a		390.0782	390.0811	390.0747
I-b		427.0539	427.0484	427.0508

Table S2 contd.

Table S2 Species found in ESI-MS during the stability/binding kinetics studies of **1** by ^{1}H NMR

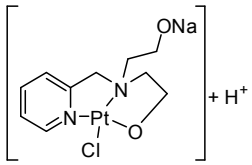
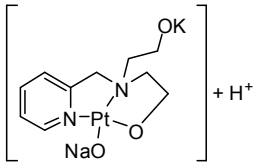
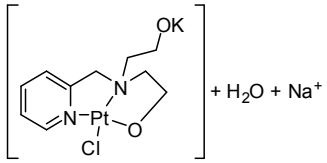
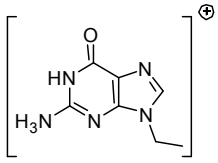
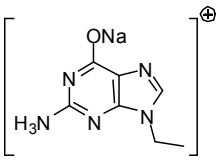
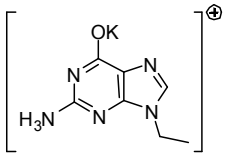
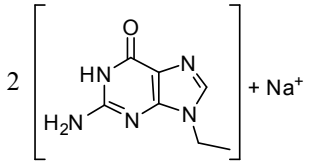
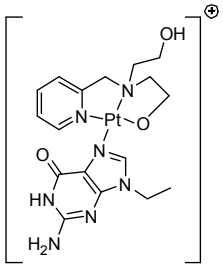
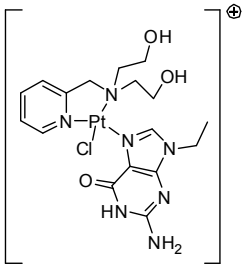
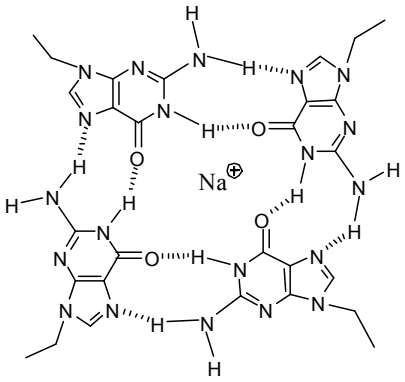
Species no.	Drawings	m/z_{calc}	Experiments	
			1 in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6	**1** + 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6
			m/z_{obs}	
I-c		449.0359	449.0396	449.0314
I-d		468.0266	468.0757	468.0773
I-e		505.0022	505.0659	505.0778
I-f		180.0885	-	180.0867
I-g		202.0705	-	202.0697
I-h		218.0444	-	218.0405
I-i		381.1512	-	381.1513

Table S2 contd.

Table S2 Species found in ESI-MS during the stability/binding kinetics studies of **1** by ^{1}H NMR

Species no.	Drawings	m/z_{calc}	Experiments	
			1 in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6	**1** + 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6
			m/z_{obs}	
I-j		569.1589	-	569.1595
I-k		606.1349	-	606.1368
I-l		739.3126	-	739.3171

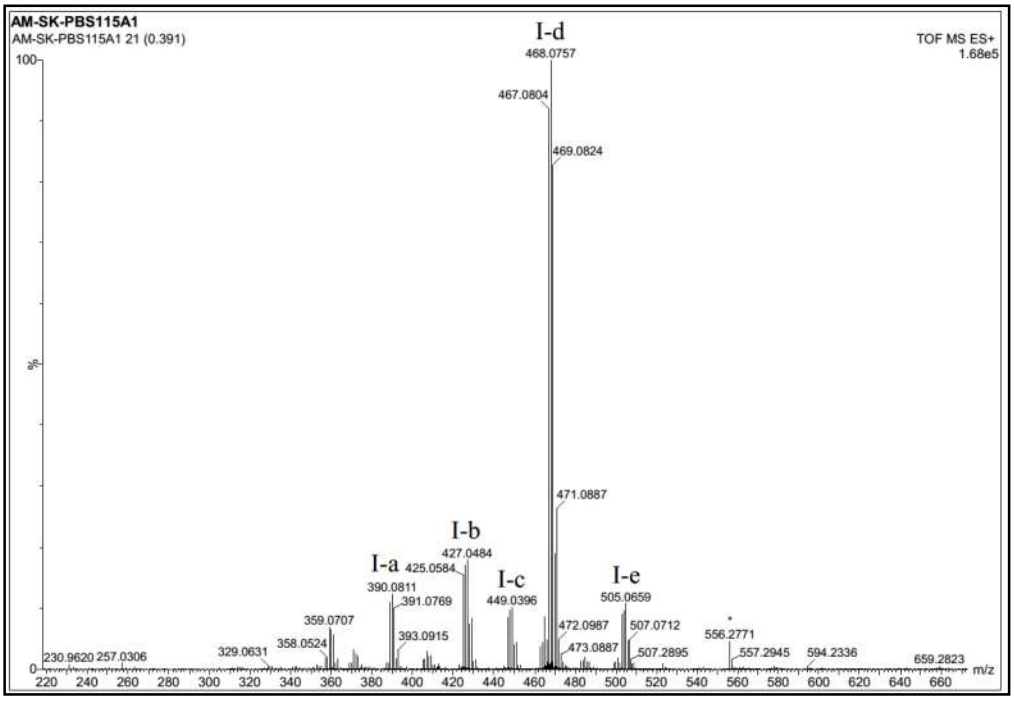


Fig. S18 ESI-MS speciation recorded during monitoring of stability kinetics of the complex **1** by ^{1}H NMR after 3 h in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

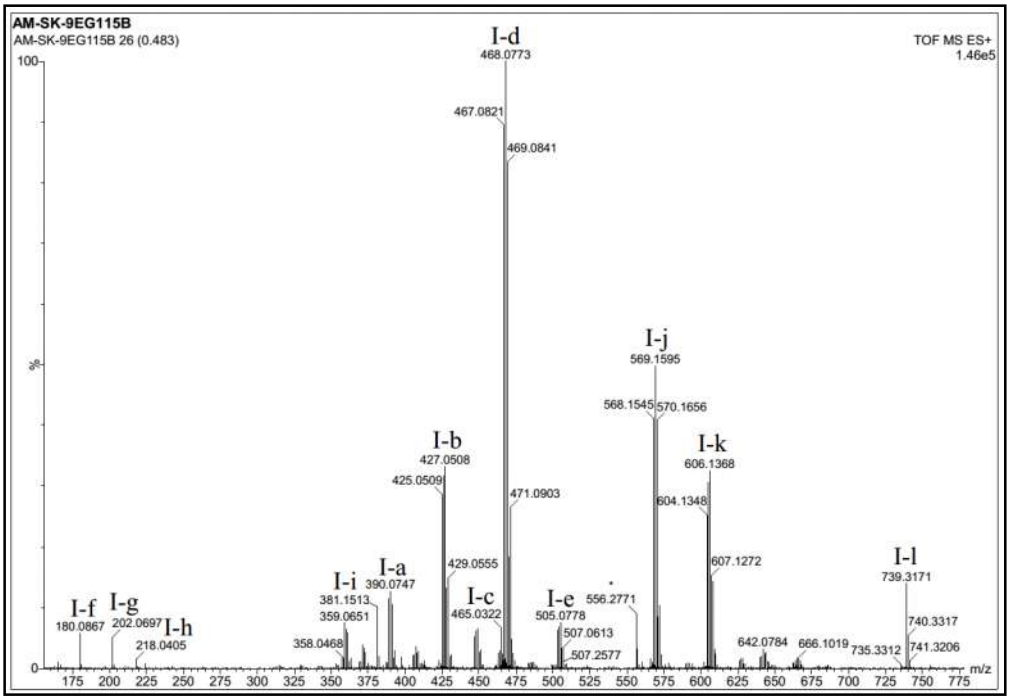


Fig. S19 ESI-MS speciation recorded during monitoring of the 9-EtG binding kinetics with complex **1** by ^{1}H NMR after 1 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

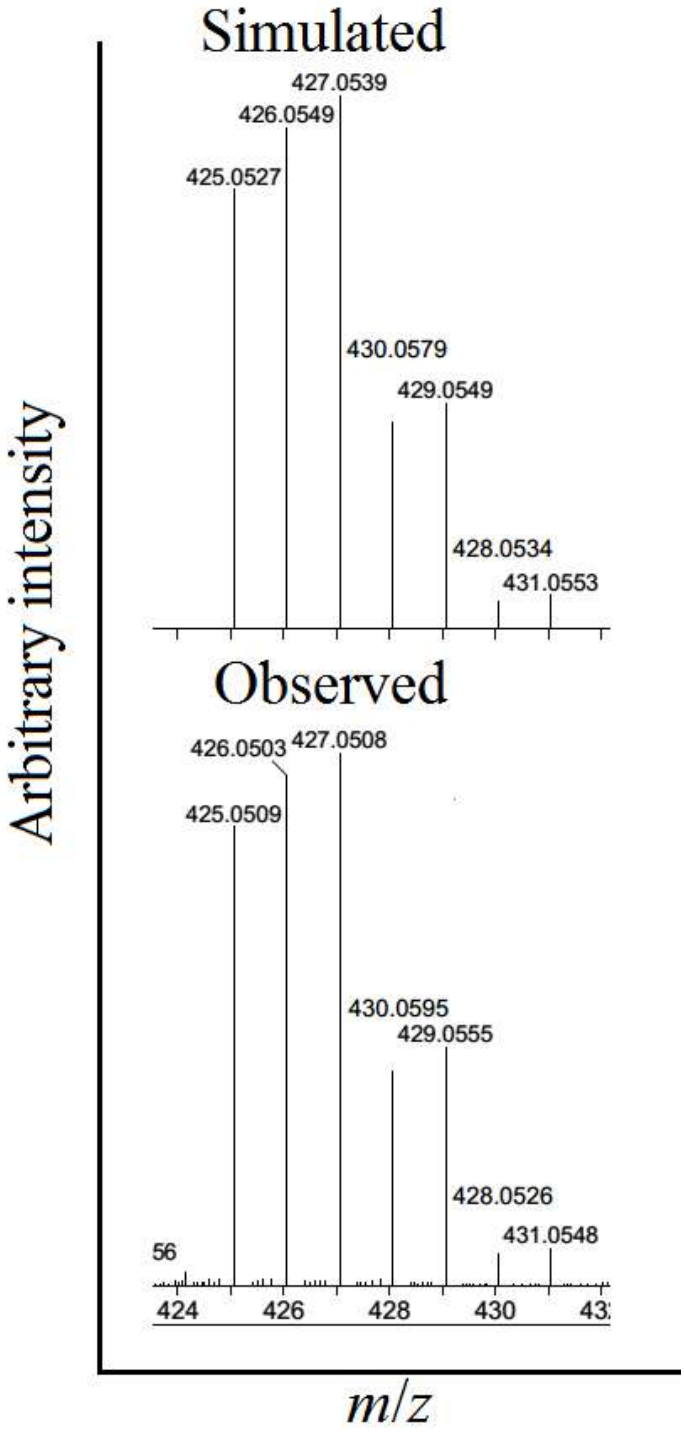


Fig. S20 Observed and simulated isotopic pattern of [**1b** (Scheme 4) + H^+] found in ESI-MS.

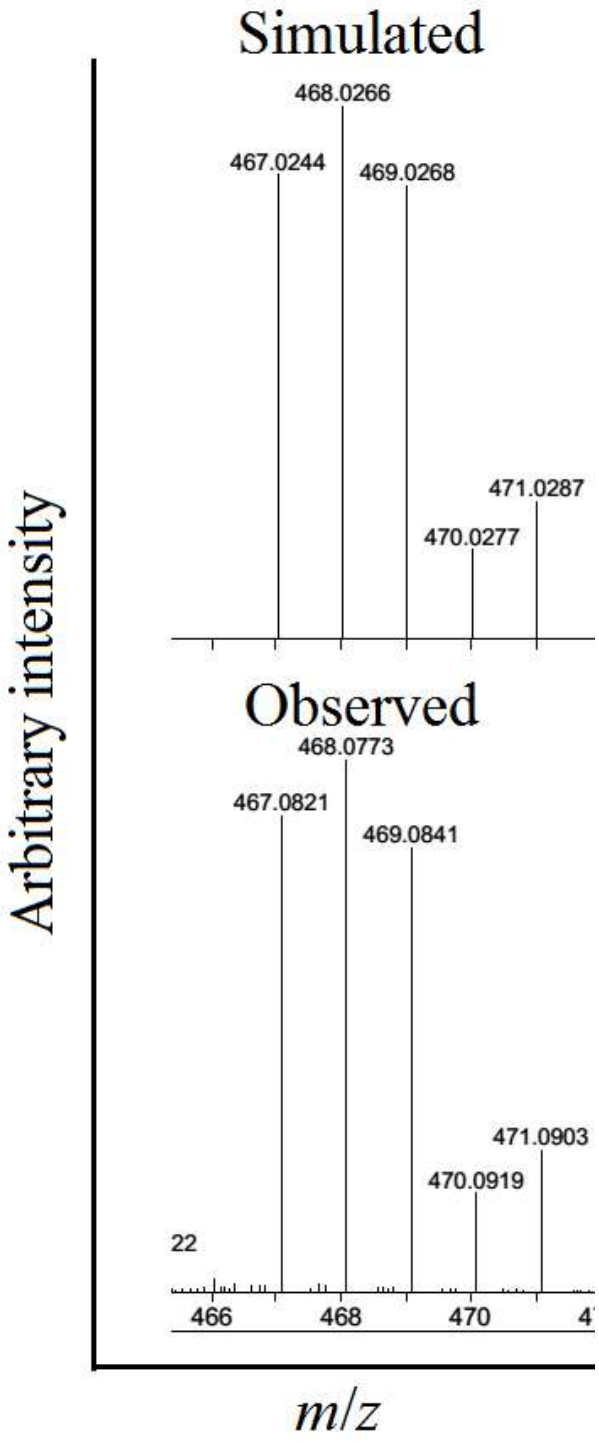


Fig. S21 Observed and simulated isotopic pattern of [**1c** (Scheme 4) − $2H^+ + Na^+ + K^+$] found in ESI-MS.

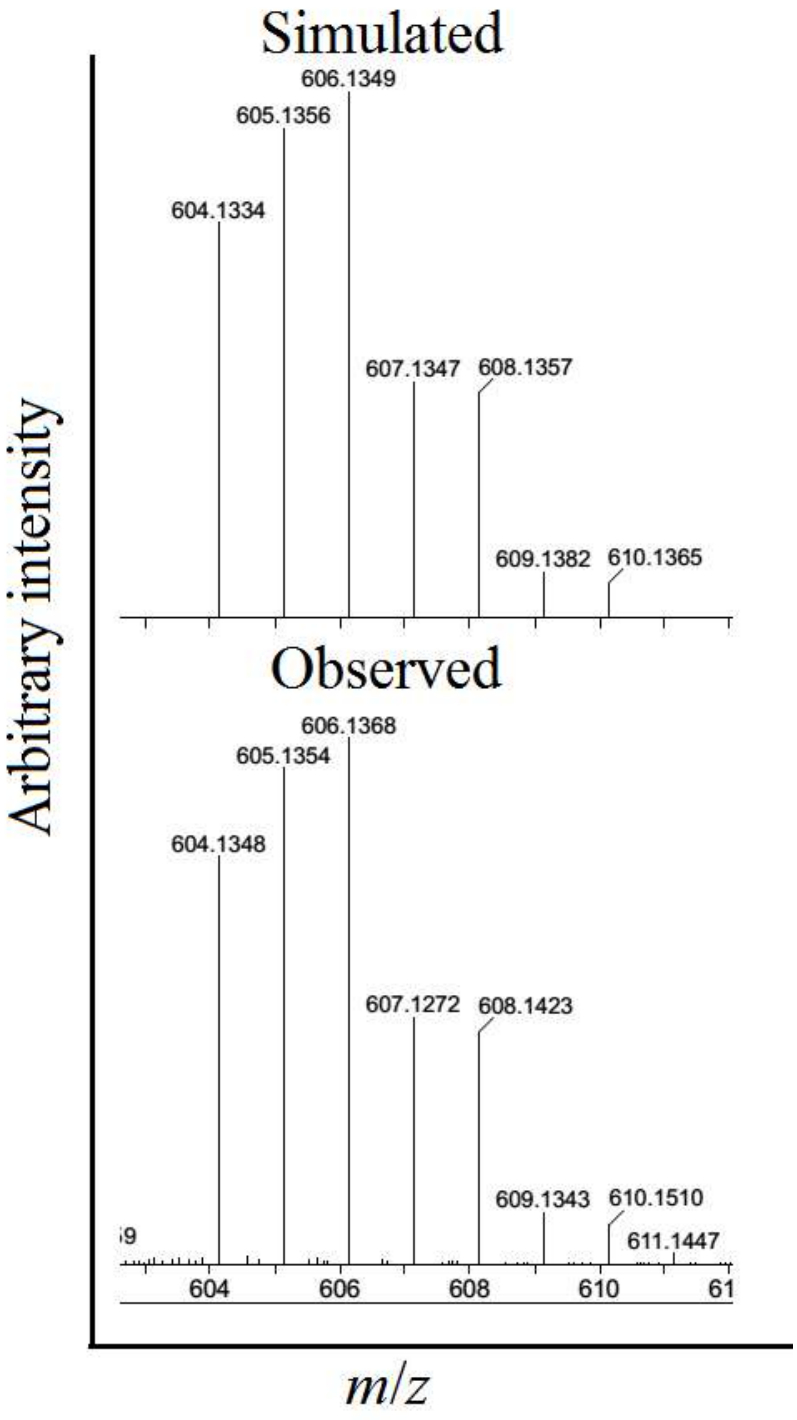


Fig. S22 Observed and simulated isotopic pattern of [**1d** (Scheme 4)] found in ESI-MS.

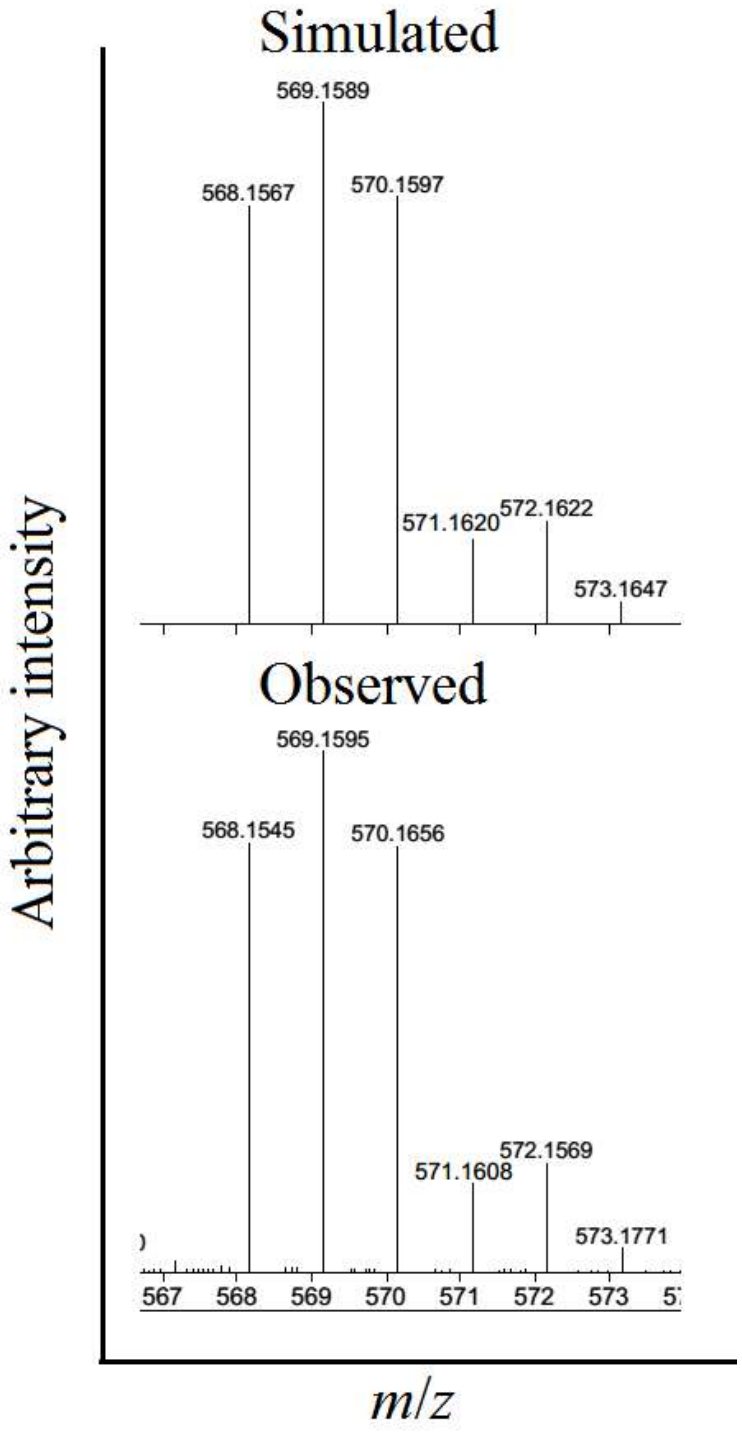


Fig. S23 Observed and simulated isotopic pattern of [**1e** (Scheme 4)] found in ESI-MS.

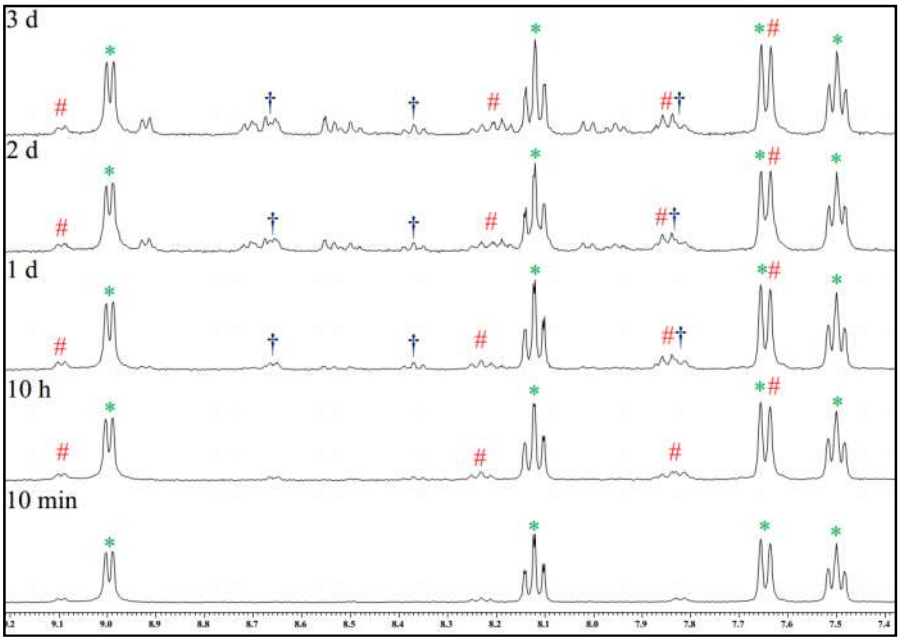


Fig. S24 Stack plot of aromatic region during the stability kinetics study of complex **2** in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture by ^{1}H NMR, where ∗, # and † indicate the signals of intact complex **2**, hydrolyzed complex **2a** and aziridinium ion **2b** respectively.

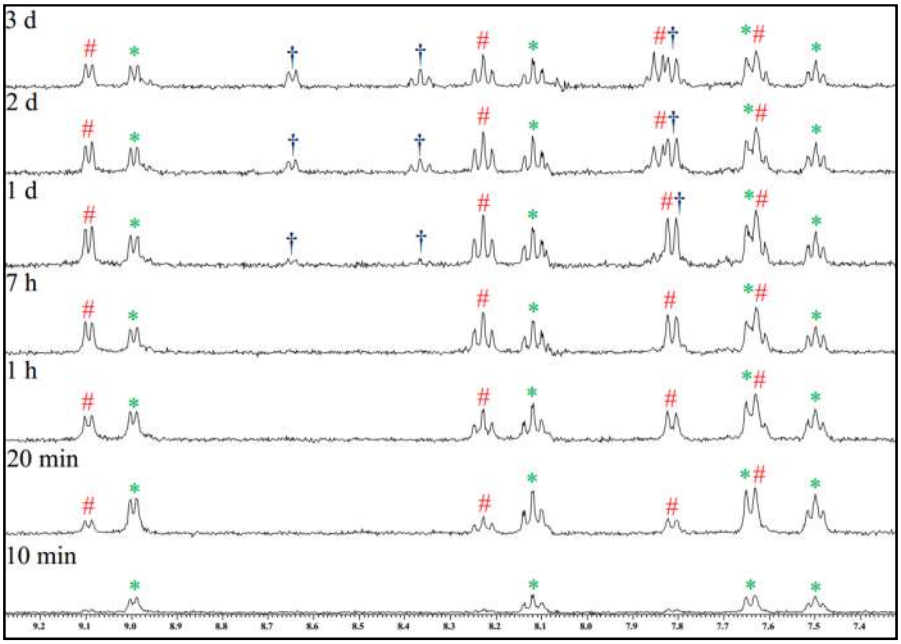


Fig. S25 Stack plot of aromatic region during the stability kinetics study of complex **2** in 20% water – DMSO-d_6 mixture by ^{1}H NMR, where where ∗, # and † indicate the signals of intact complex **2**, hydrolyzed complex **2a** and aziridinium ion **2b** respectively.

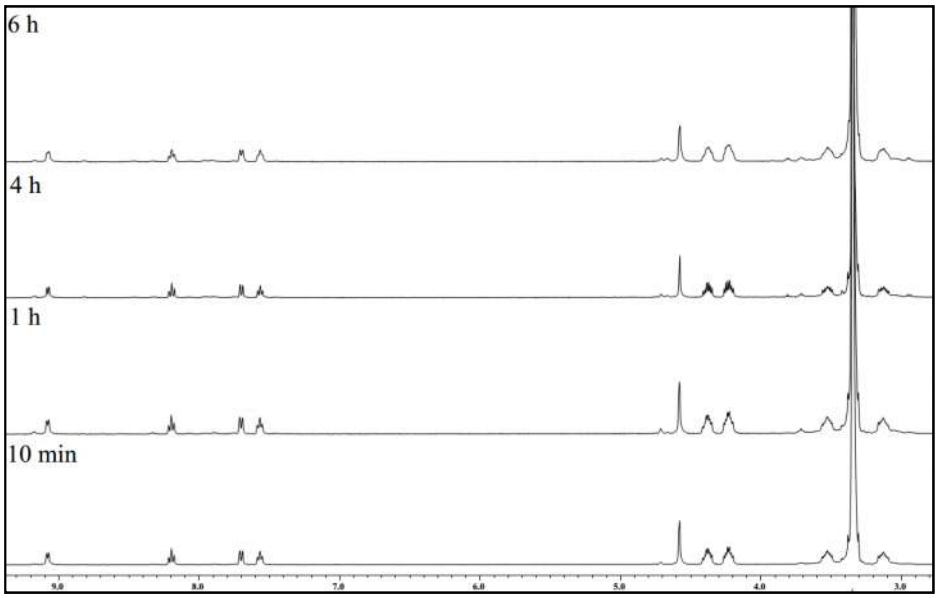


Fig. S26 Stack plot of stability kinetics study of complex **2** in DMSO-d_6 by ^{1}H NMR.

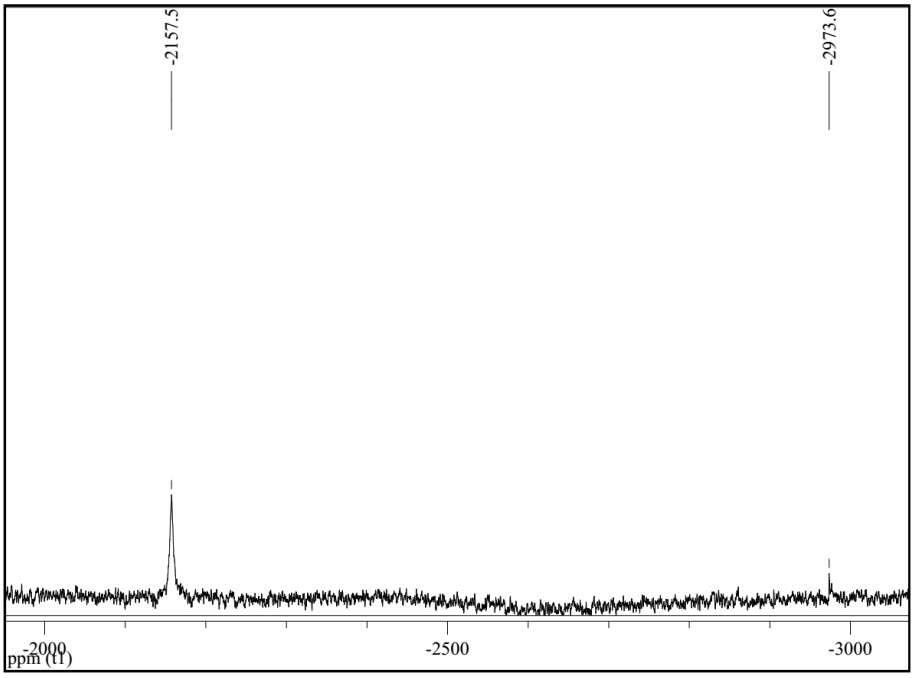


Fig. S27 ^{195}Pt NMR of complex **2** in DMSO-d_6 after 8 h where peak at −2157.5 and −2973.6 ppm represent the chemical shift of **2** and DMSO bound complex **2** respectively.

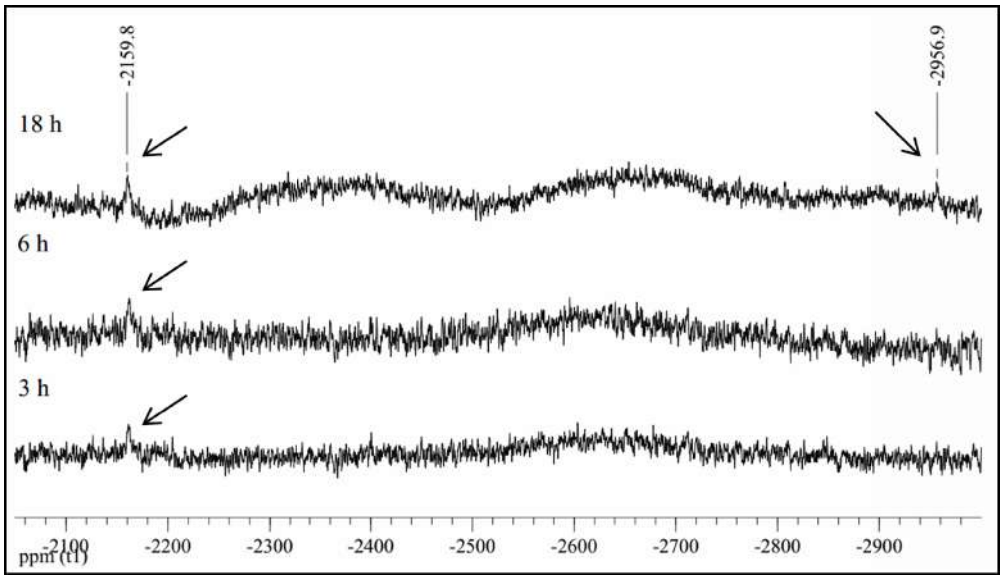


Fig. S28 Stack plot of stability kinetics study of **2** (6 mM) in 40% DMEM-DMSO-d_6 by ^{195}Pt NMR. The peak at −2159.8 ppm is for intact complex **2** and peak at −2956.9 may signify S–bonded **2**. During experimentation some amount of the native complex precipitated.

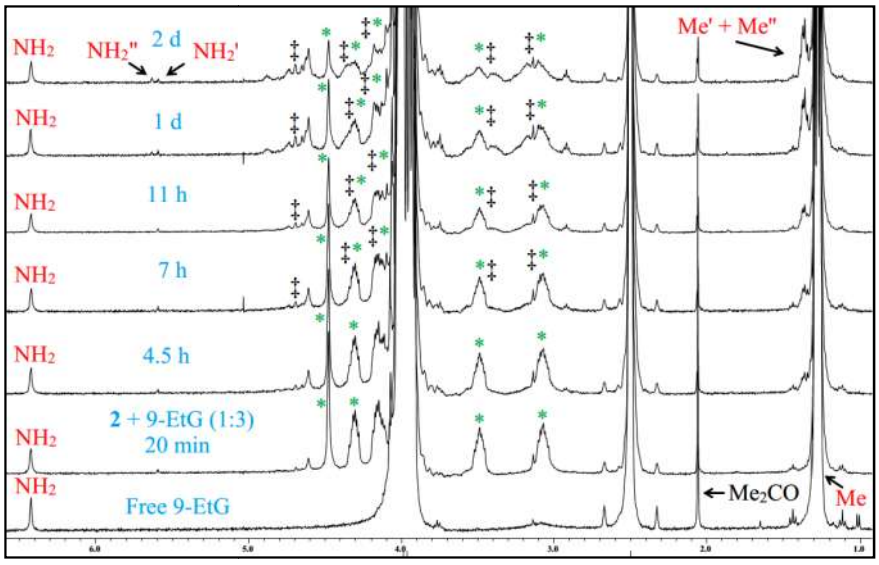


Fig. S29 Stack plot of aliphatic region during the binding kinetics study of **2** with 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR, where * and ‡ indicate the signals of intact complex **2** and 9-EtG bound complexes **2d** & **2e** respectively. NH_2 of free 9-EtG is shifted upfield to NH_2' & NH_2" of 9-EtG bound complexes **2d** & **2e** respectively. Me of free 9-EtG is shifted downfield to Me' & Me" of 9-EtG bound complexes **2d** & **2e** respectively.

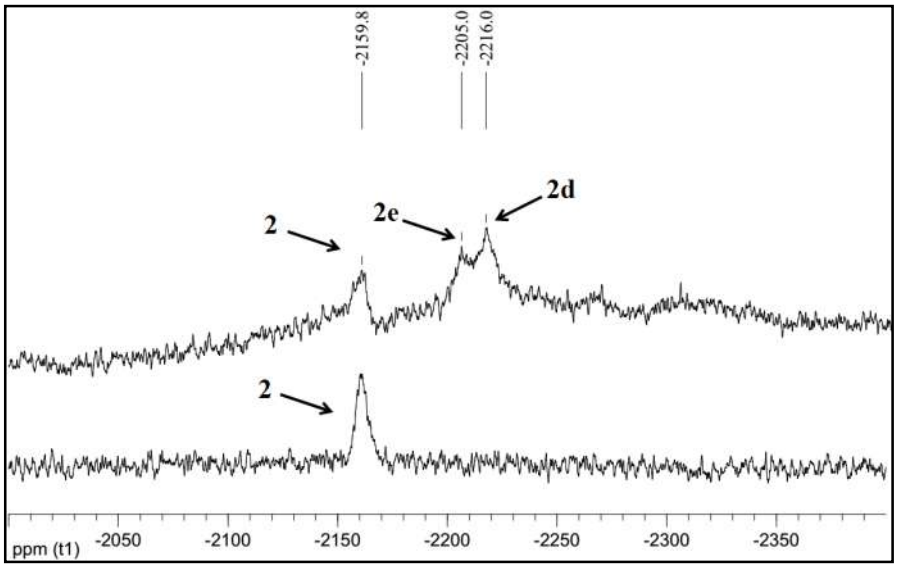


Fig. S30 Stack plot of binding kinetics study of **2** with 9-EtG (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{195}Pt NMR. The data taken after 2 h shows no binding of 9-EtG with **2**. After 24 h complex **2** signal at −2159.8 ppm diminished and two closely spaced signals of 9-EtG bound complexes **2d** & **2e** at −2216.0 & −2205.0 ppm respectively appear.

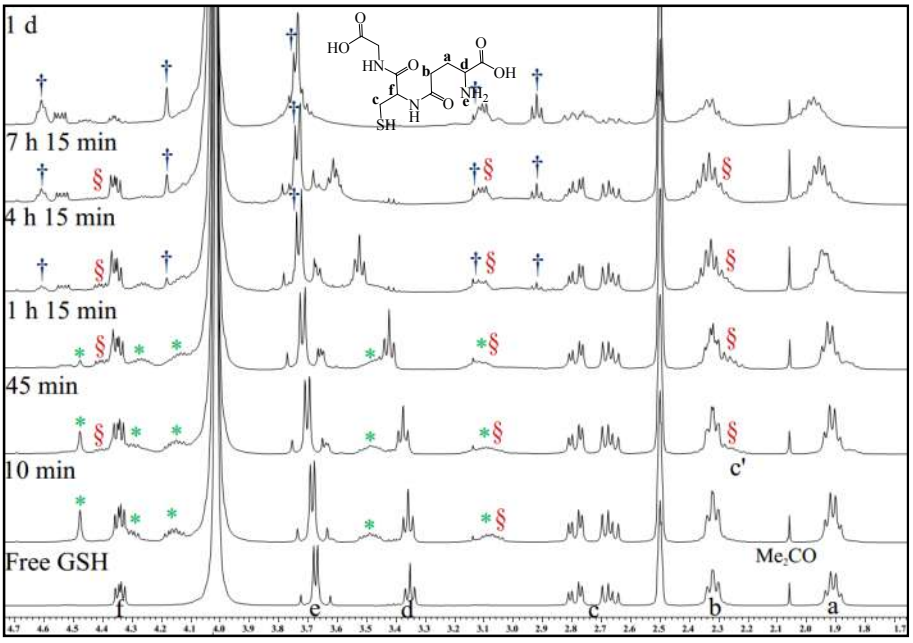


Fig. S31 Stack plot of aliphatic region during the binding kinetics study of **2** with GSH in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR, where ∗, § and † indicate the signals of intact complex **2**, GSH bound complex **2c** and aziridinium ion **2b** respectively.

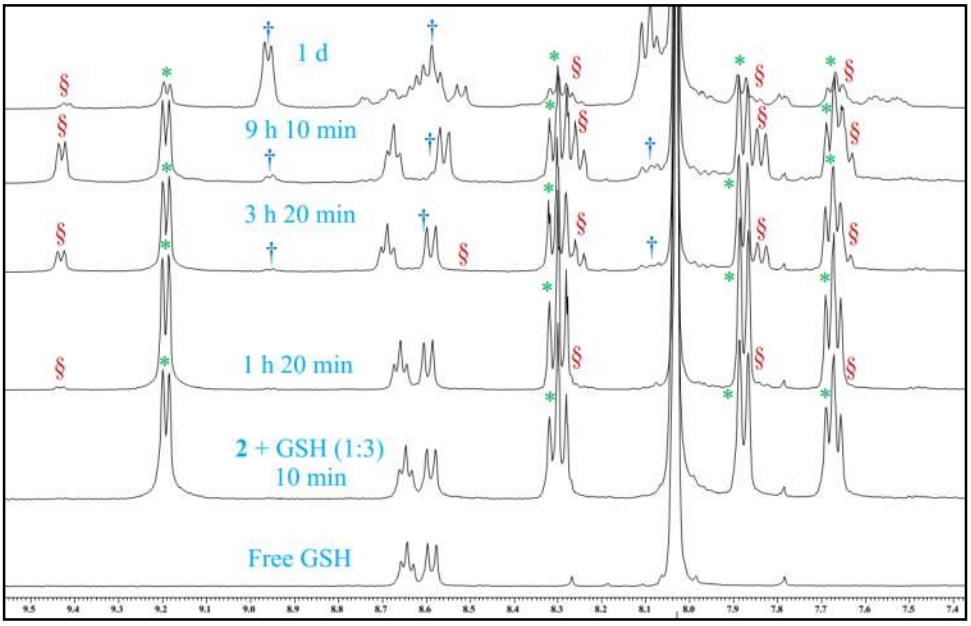


Fig. S32 Stack plot of aromatic region during the binding kinetics study of **2** with GSH in 20% PBS (pD 7.4, prepared in D_2O) – DMF-d_7 by ^{1}H NMR, where ∗, § and † indicate the signals of intact complex **2**, GSH bound complex **2c** and aziridinium ion **2b** respectively.

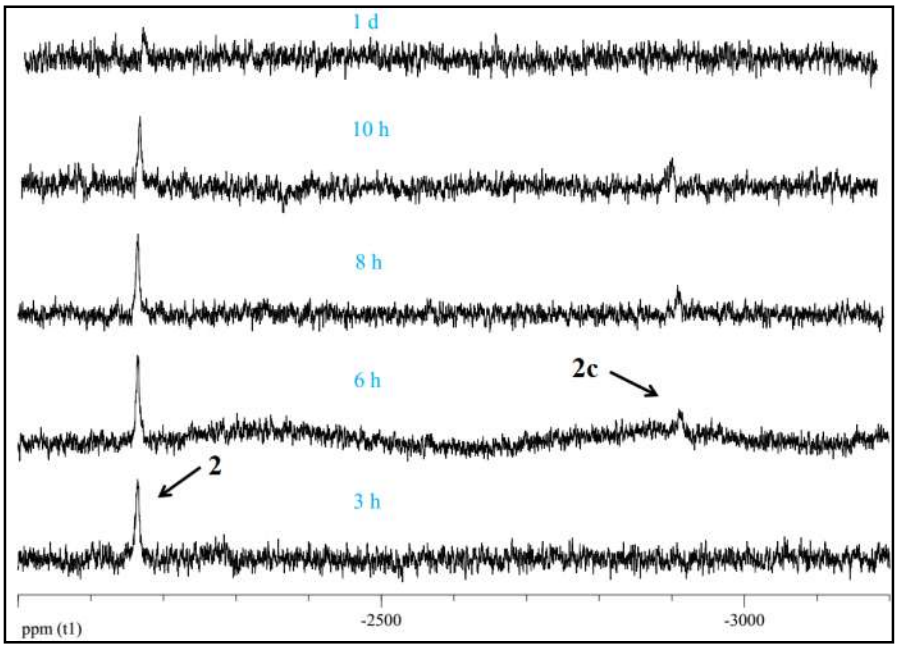
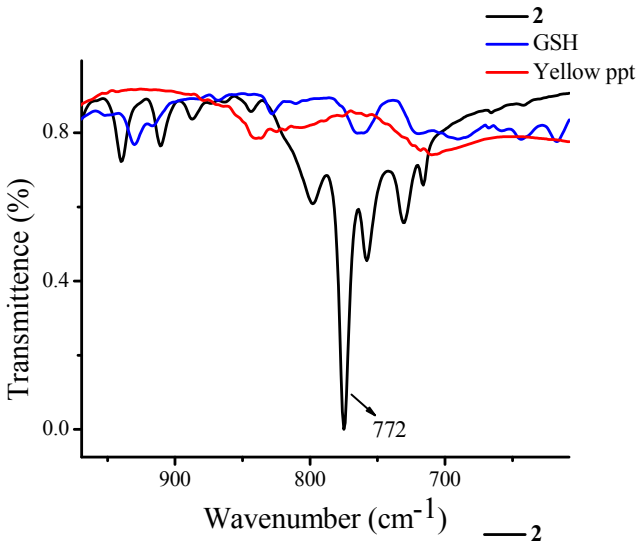
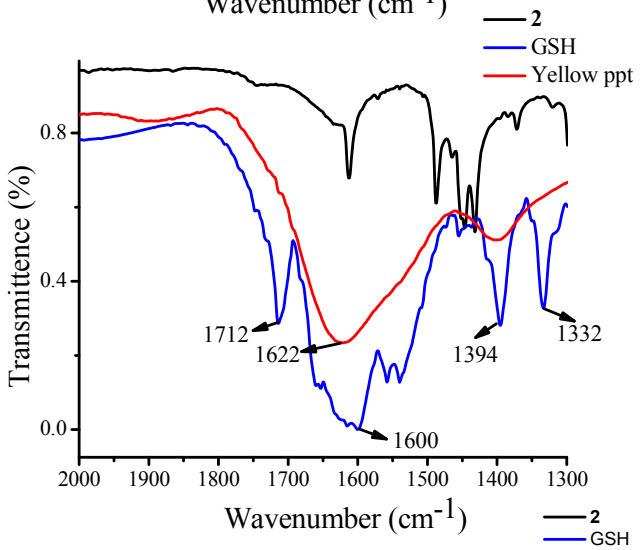


Fig. S33 Stack plot of binding kinetics study of **2** with GSH (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMF-d_7 by ^{195}Pt NMR.. After 6 h complex signal GSH bound complex **2c** at −2910.9 ppm appears along with initial signal of complex **2** at −2164.6 ppm. After a day the ^{195}Pt signal of **2c** vanishes with very small amount of unreacted complex **2** signal.

A)



B)



C)

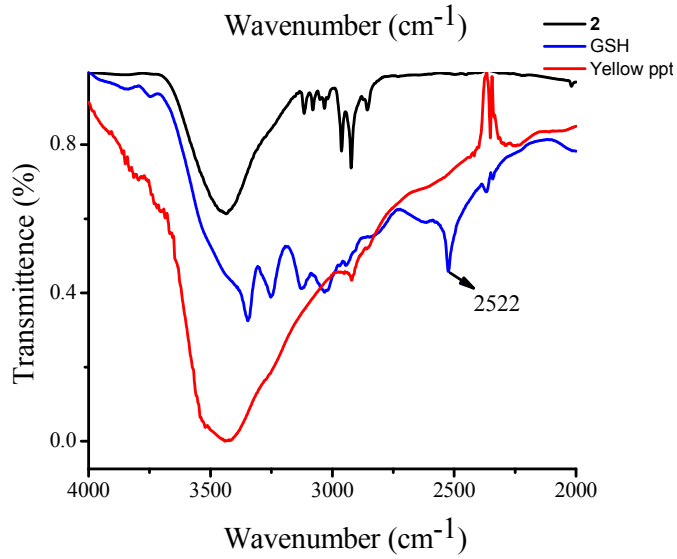
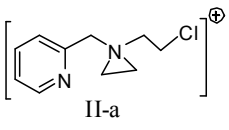


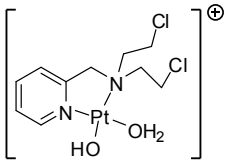
Fig. S34 Stack plot of IR spectra of **2**, GSH and yellow ppt found in the reaction of **2** with GSH (1:3) in 20% PBS (pD 7.4, prepared in D_2O) – DMF-d_7. A) Sharp band of C–Cl stretching frequency 772 cm^{-1} of **2** vanished in yellow ppt. B) Carbonyl stretching band of GSH and yellow ppt. C) S–H stretching band at 2522 cm^{-1} in GSH vanished in yellow ppt and a broad band at 3550–2700 cm^{-1} for polymeric nature of yellow ppt.

Table S3 Species found in ESI-MS during the stability/binding kinetics studies of **2** by ^{1}H NMR. The drawings of the respective species are given below the tabulated data

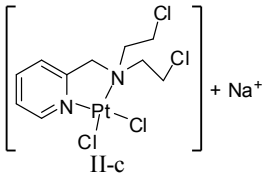
Species no.	m/z_{calc}	Experiments			
		2 in 20% PBS in DMSO-d_6	**2** in 20% H_2O in DMSO-d_6	**2** + 9-EtG (1:3) in 20% PBS in DMSO-d_6	**2** + GSH (1:3) in 20% PBS in DMSO-d_6
		m/z_{obs}			
II-a	197.0845	197.0846	197.0832	-	197.0832
II-b	463.0297	462.9756	462.9728	462.9782	462.9832
II-c	520.9429	520.9438	520.9869	520.9448	520.9427
II-d	540.9330	540.9924	540.9805	540.9927	-
II-e	606.0896	-	-	606.0881	-
II-f	642.0657	-	-	642.0645	-
II-g	179.1184	-	-	-	179.0498
II-h	233.0612	-	-	-	233.0599
II-i	308.0916	-	-	-	308.0837
II-j	330.0736	-	-	-	330.0706
II-k	352.0555	-	-	-	352.0511
II-l	374.0375	-	-	-	374.0371
II-m	734.0927	-	-	-	734.1046
II-n	770.0688	-	-	-	770.0684
II-o	792.0508				792.0432
II-p	814.0327				814.0229
II-q	836.0146				836.0093
I-b	427.0539	427.0096	427.0059	427.0033	-
I-f	180.0885	-	180.0859	180.0859	-
I-g	202.0705	-	211.0636	211.0636	-
I-h	218.0444	-	218.0458	218.0458	
I-i	381.1512	-	-	381.1545	-
I-l	739.3126	-	-	739.3130	-



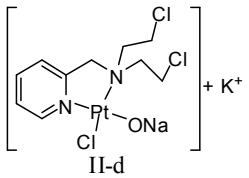
II-a



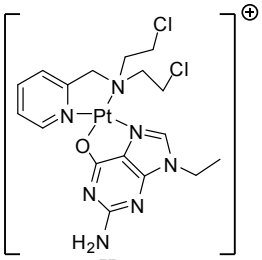
II-b



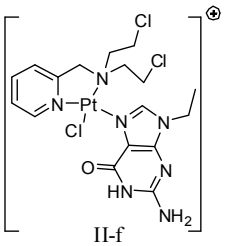
II-c



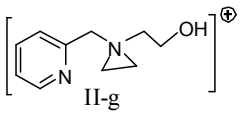
II-d



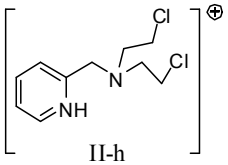
II-e



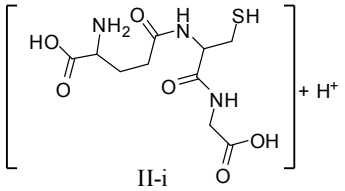
II-f



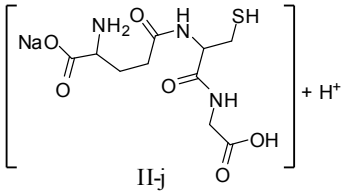
II-g



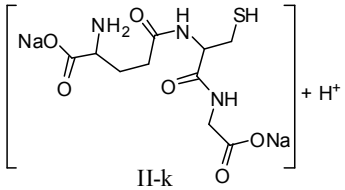
II-h



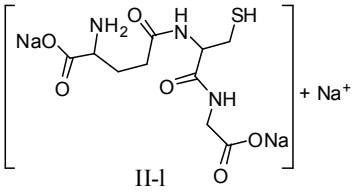
II-i



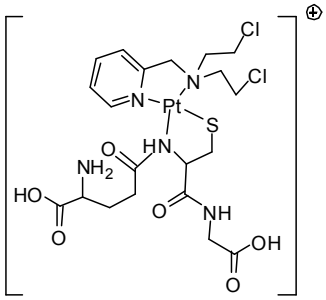
II-j



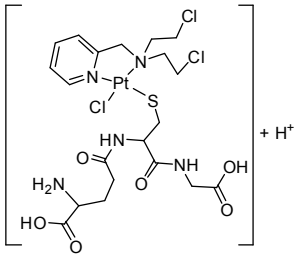
II-k



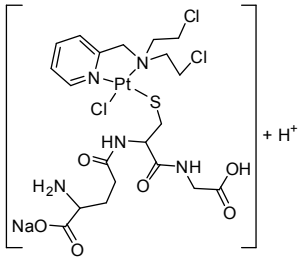
II-l



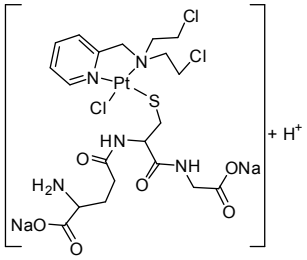
II-m



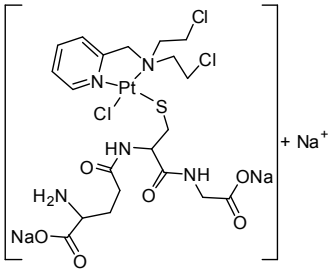
II-n



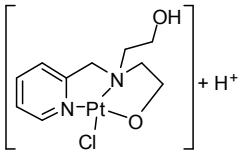
II-o



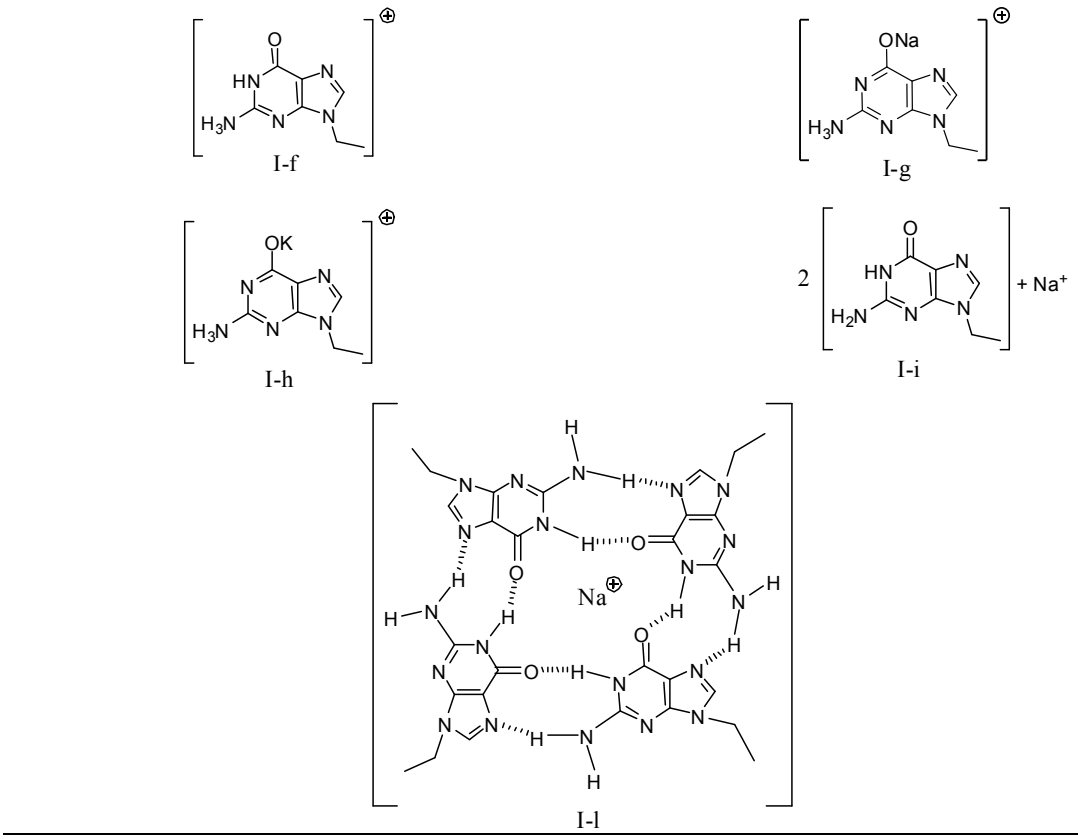
II-p



II-q



I-b



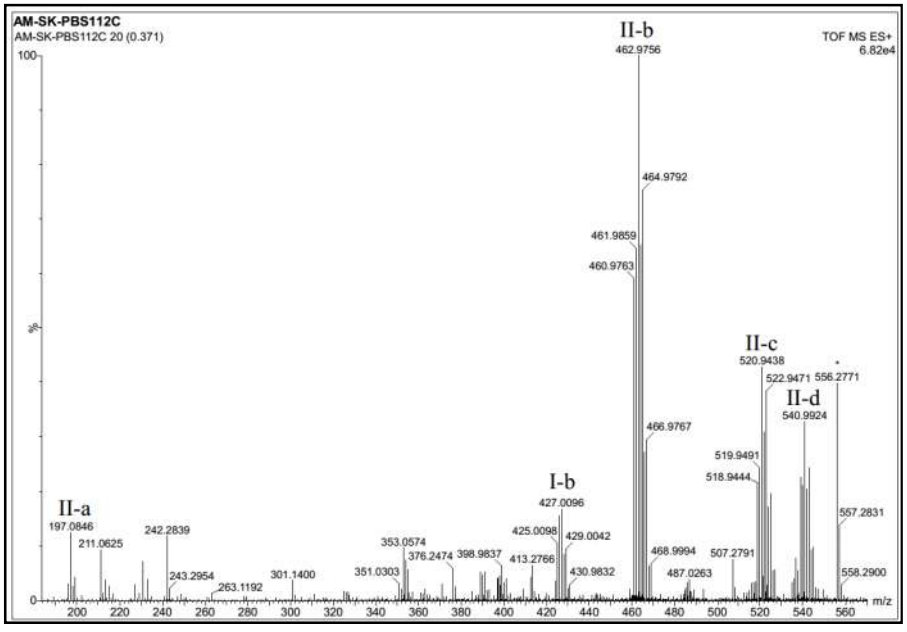


Fig. S35 ESI-MS speciation recorded during monitoring of stability kinetics of the complex **2** by ^{1}H NMR after 1 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

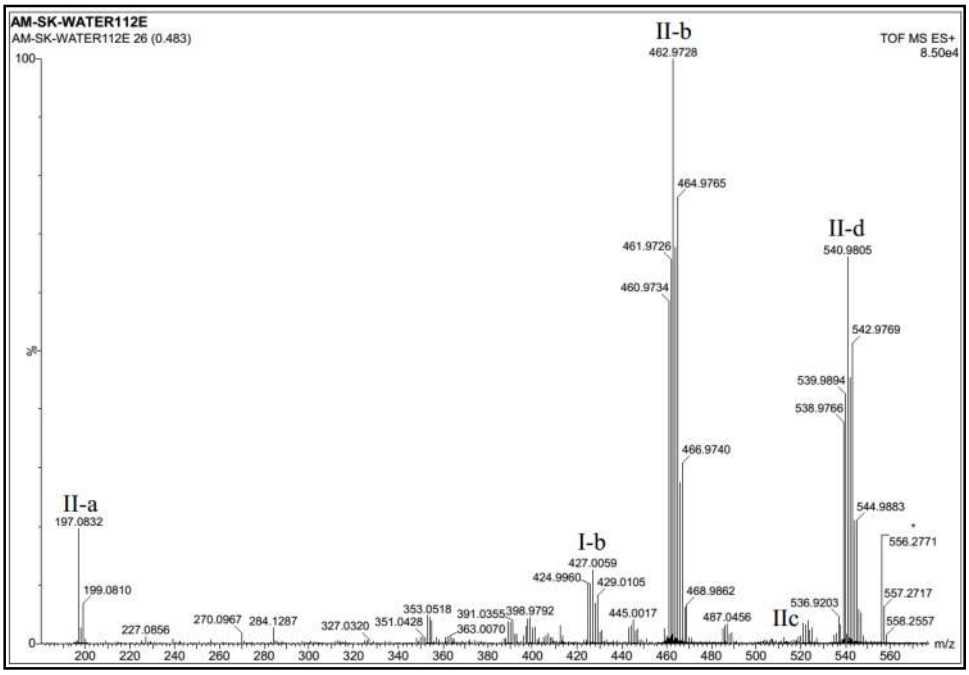


Fig. S36 ESI ESI-MS speciation recorded during monitoring of stability kinetics of the complex **2** by ^{1}H NMR after 3 d in 20% water – DMSO-d_6 mixture.

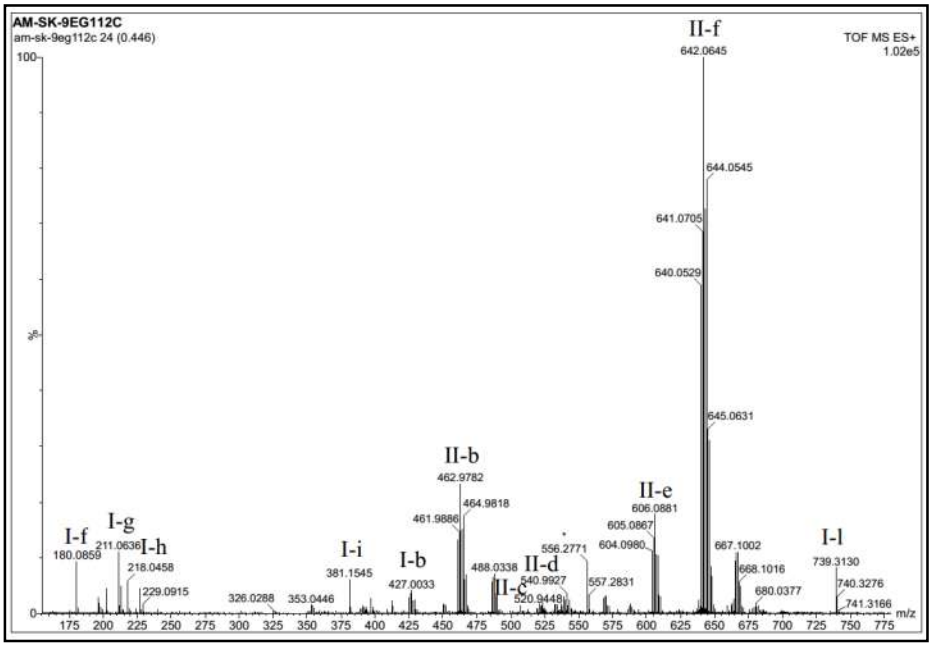


Fig. S37 ESI-MS speciation recorded during monitoring of the 9-EtG binding kinetics with complex **2** by ^{1}H NMR after 2 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

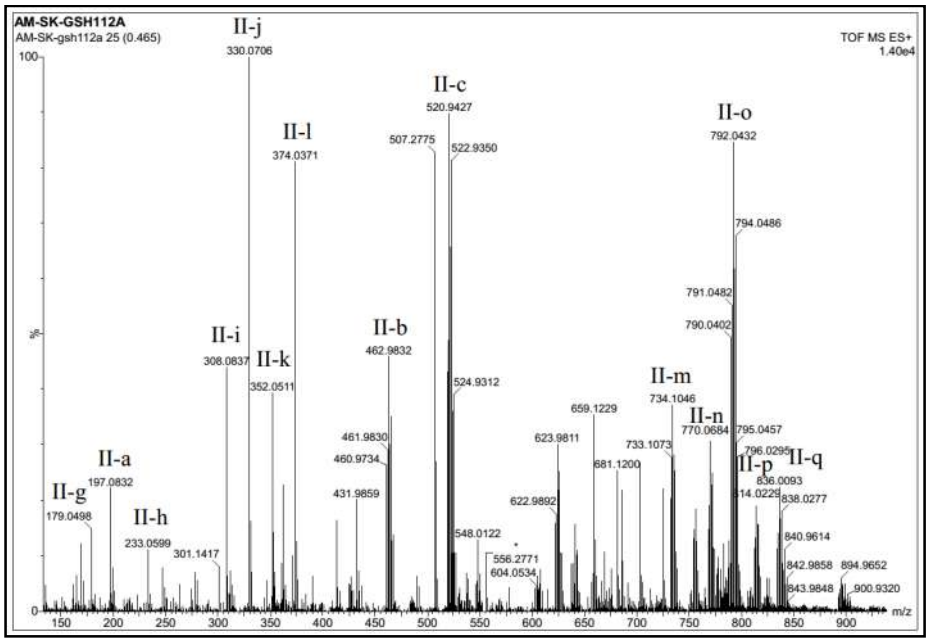


Fig. S38 ESI-MS speciation recorded during monitoring of the GSH binding kinetics with complex **2** by ^{1}H NMR after 2 h in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

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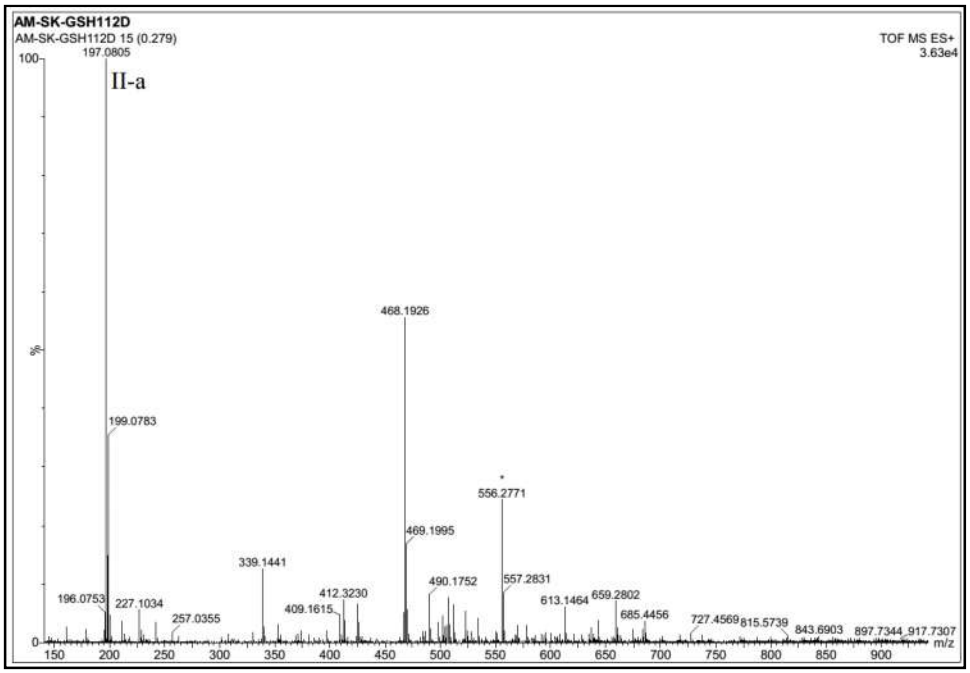

Fig. S39 ESI-MS speciation recorded during monitoring of the GSH binding kinetics with complex **2** by ^{1}H NMR after 1 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

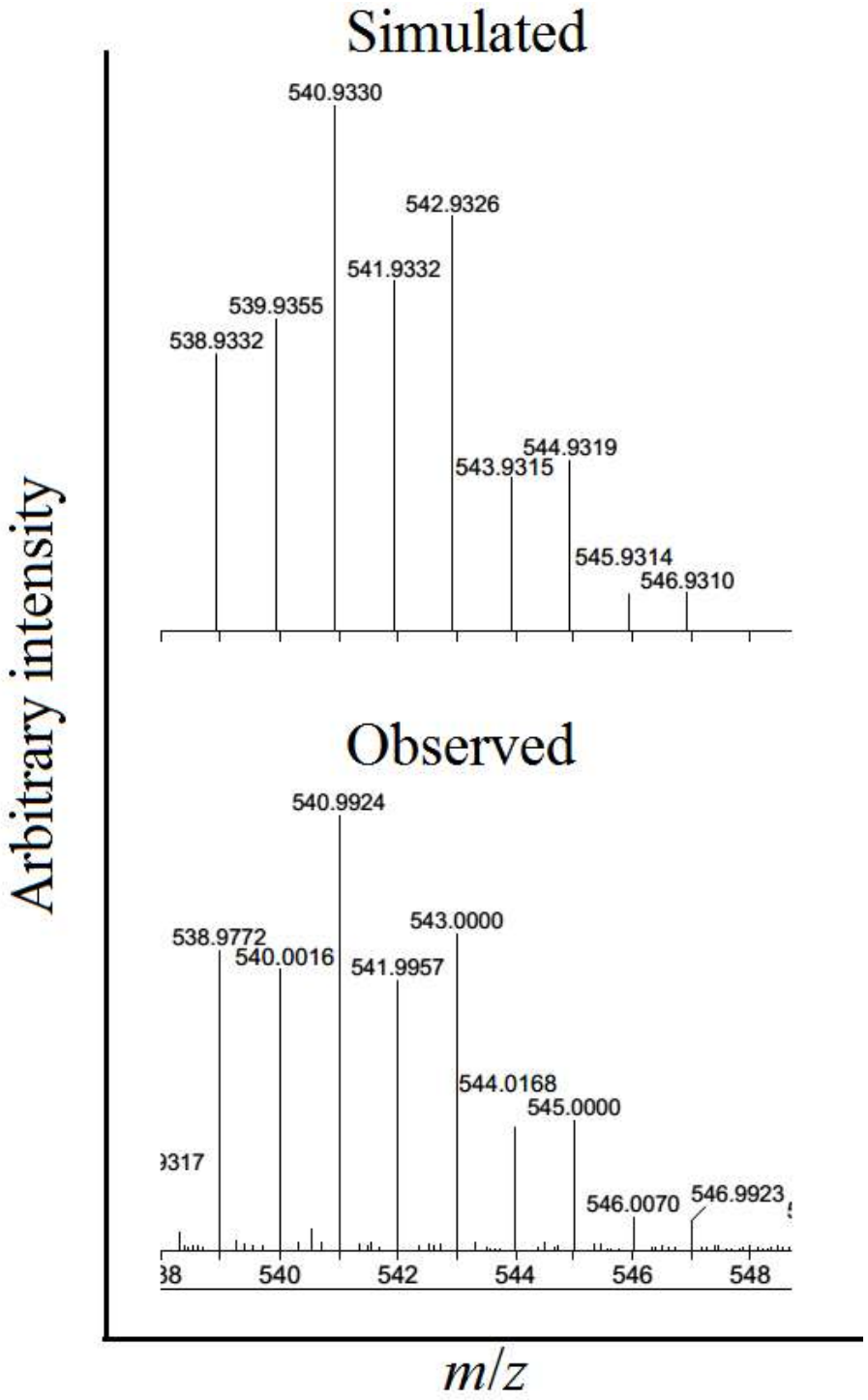

Fig. S40 Observed and simulated isotopic pattern of [**2a** (Scheme 5) − $2H^+ + Na^+ + K^+$] found in ESI-MS.

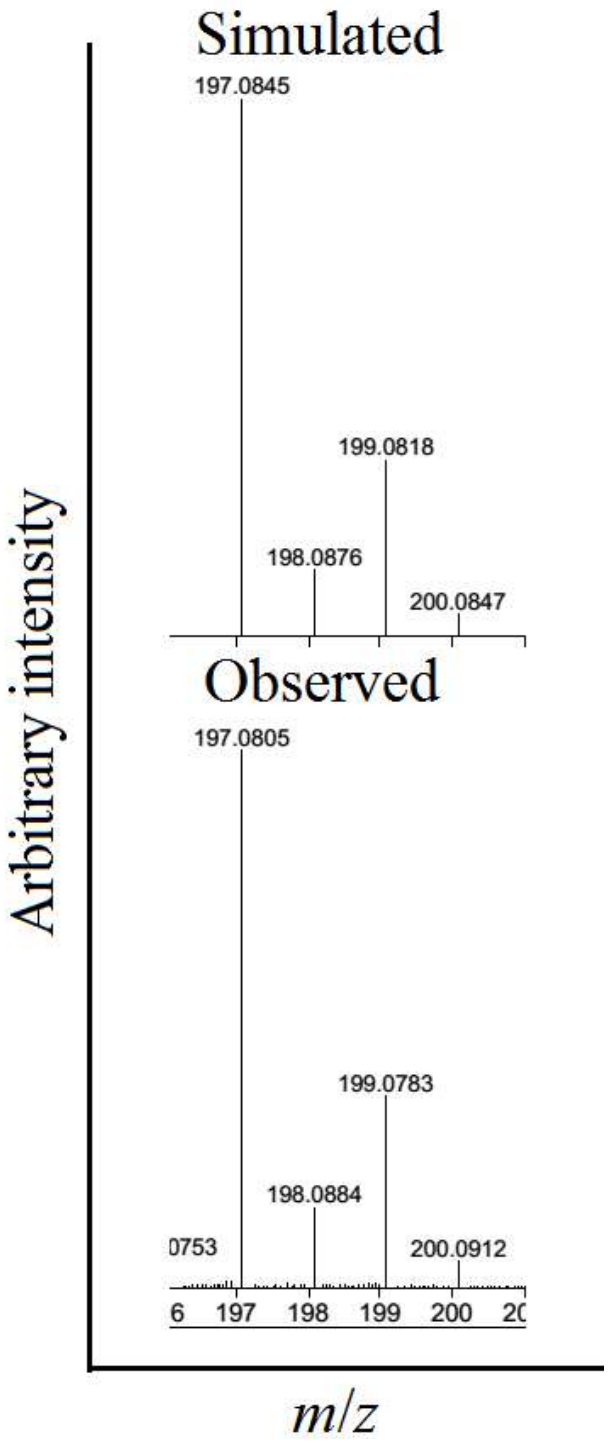

Fig. S41 Observed and simulated isotopic pattern of [**2b** (Scheme 5)] found in ESI-MS.

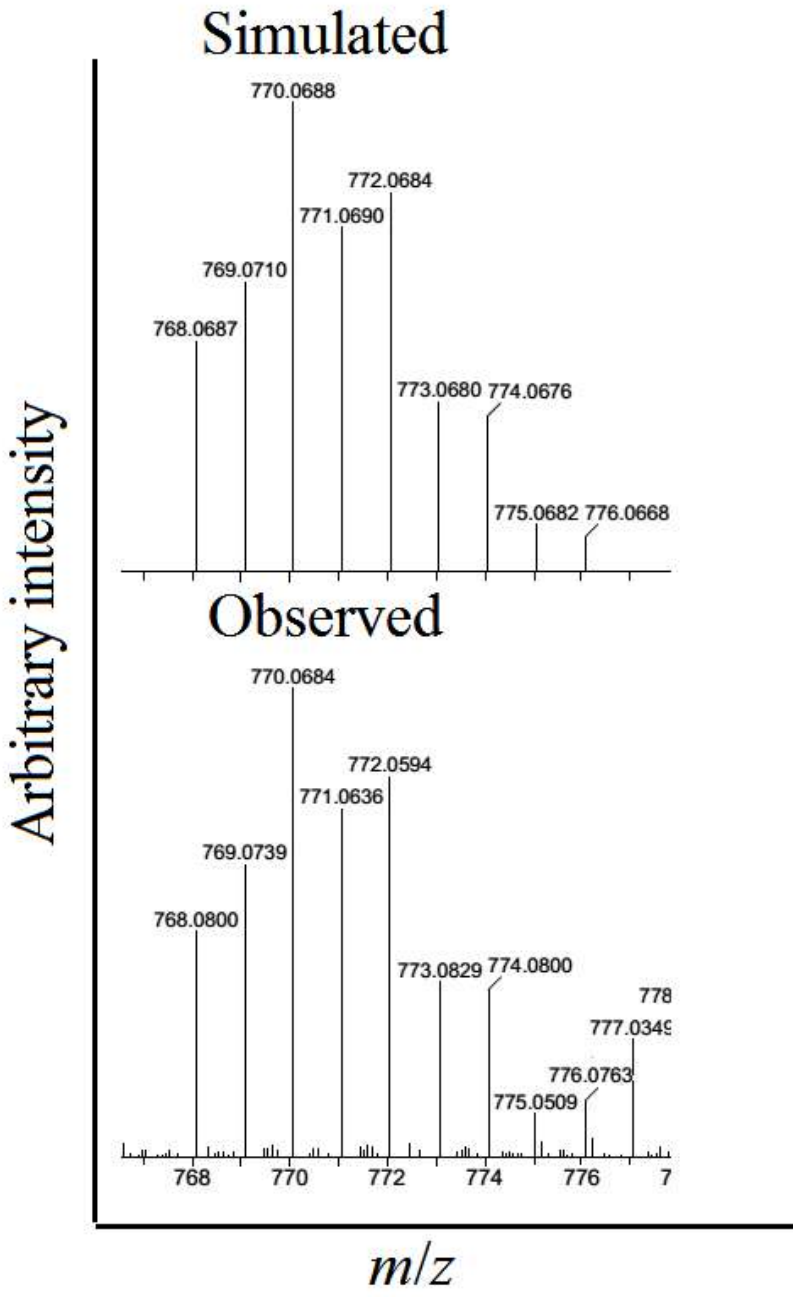

Fig. S42 Observed and simulated isotopic pattern of [**2c** (Scheme 5)] found in ESI-MS.

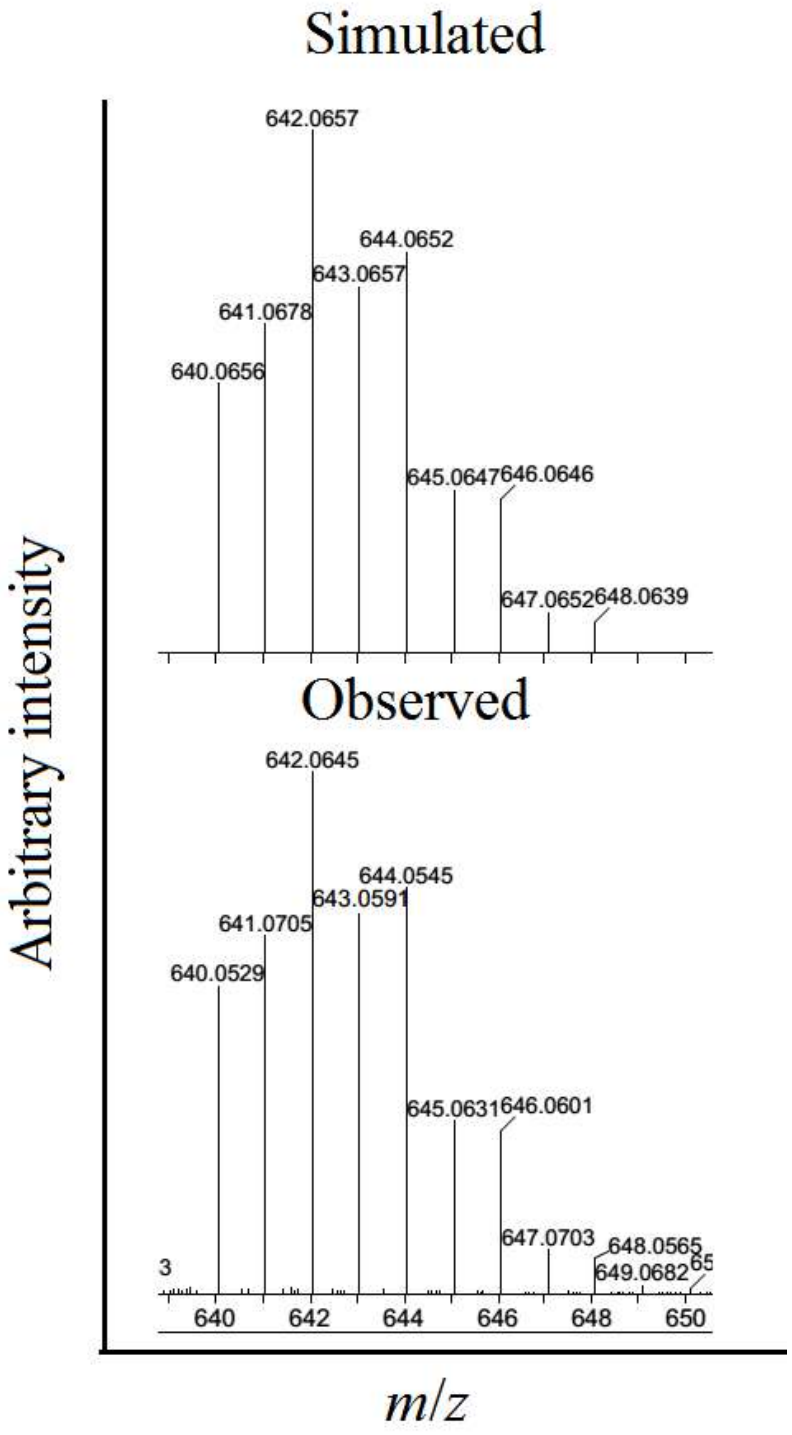

Fig. S43 Observed and simulated isotopic pattern of [**2d** (Scheme 5)] found in ESI-MS.

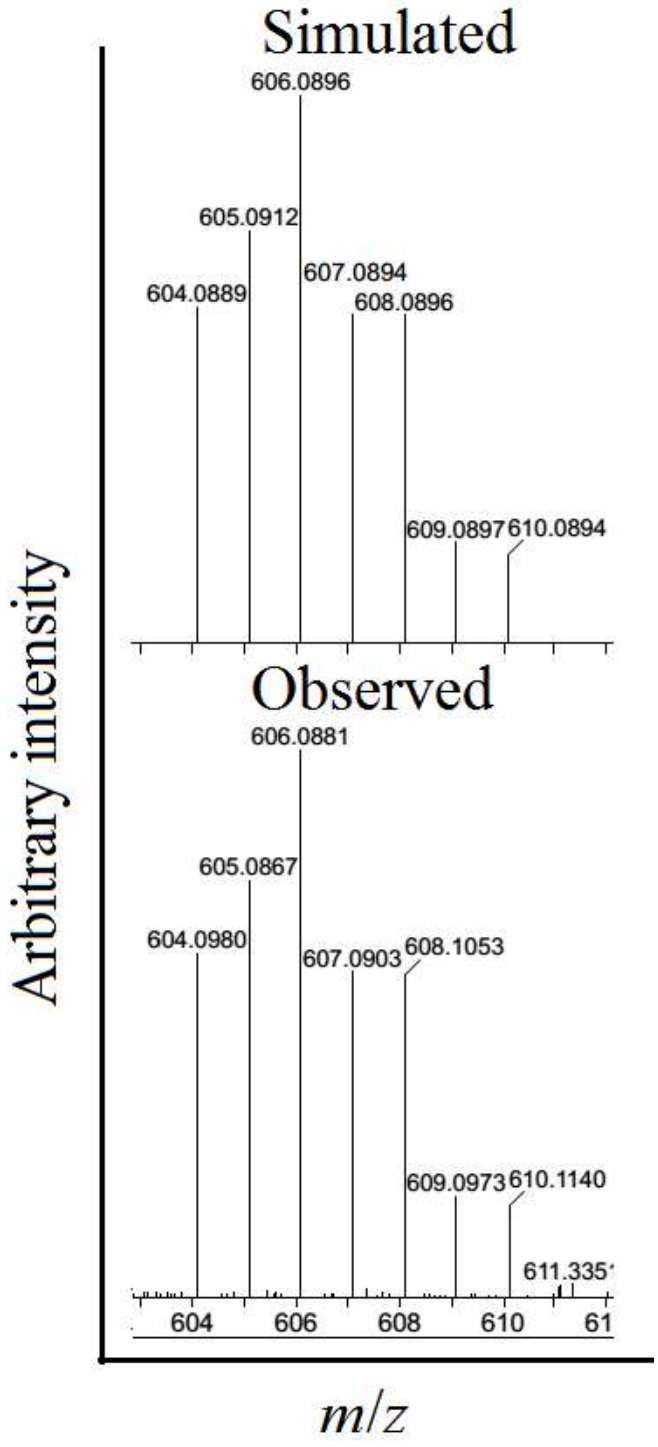

Fig. S44 Observed and simulated isotopic pattern of [**2e** (Scheme 5)] found in ESI-MS.

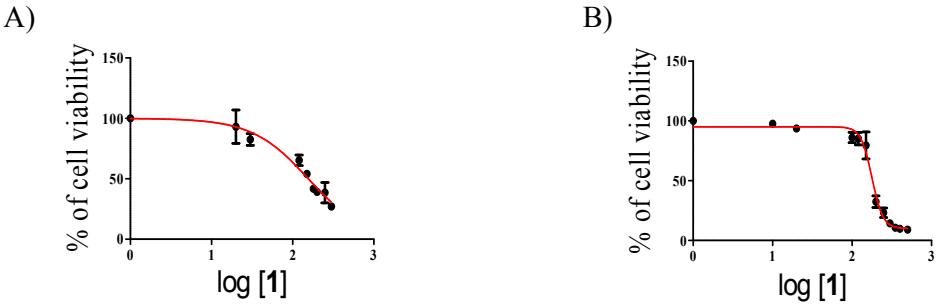

Fig. S45 Plots of cell viability (%) *vs*. log of μM concentrations of **1** against A) MCF-7 and B) HeLa WT cell lines after incubation for 48 h determined from MTT assays under normoxic condition. The plots provided are for one independent experiment out of the three independent experiments.

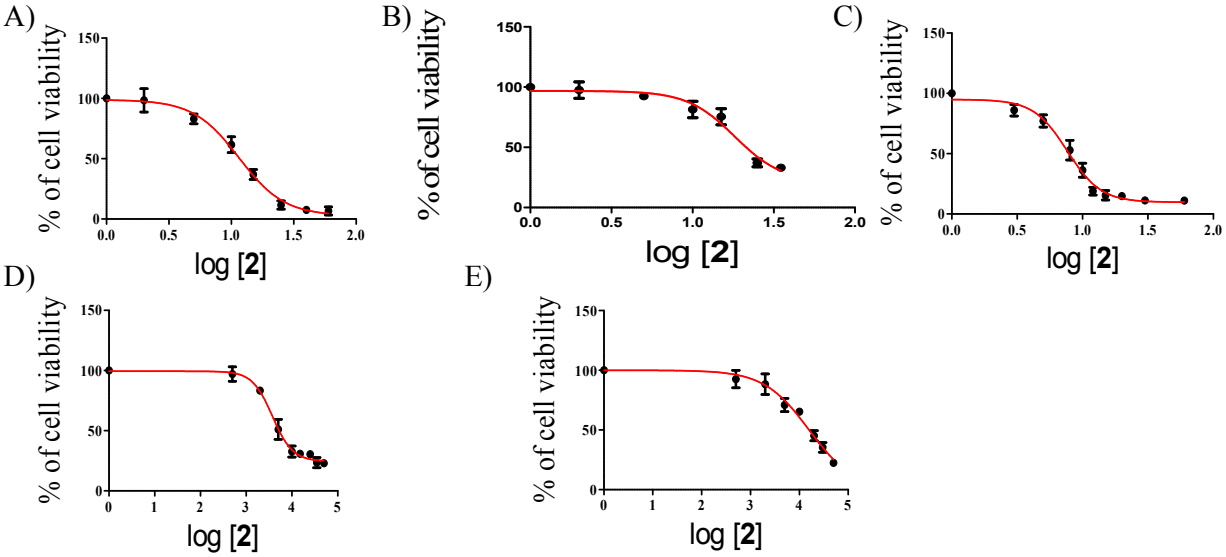

Fig. S46 Plots of cell viability (%) *vs*. log of μM concentrations of **2** against A) MCF-7, B) A549, C) HeLa WT, D) MIA PaCa2 and E) HEK293 cell lines after incubation for 48 h determined from MTT assays under normoxic condition. The plots provided are for one independent experiment out of the three independent experiments.

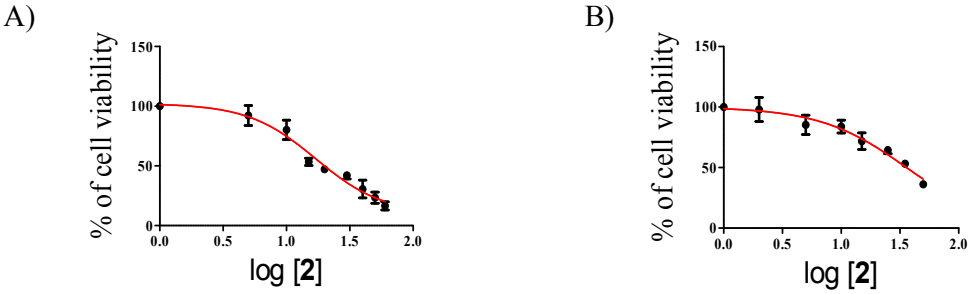

Fig. S47 Plots of cell viability (%) *vs*. log of μM concentrations of **2** against A) MCF-7 and B) A549 cell lines after incubation for 48 h determined from MTT assays under normoxic condition in presence of 250 μM and 400 μM GSH respectively. The plots provided are for one independent experiment out of the three independent experiments.

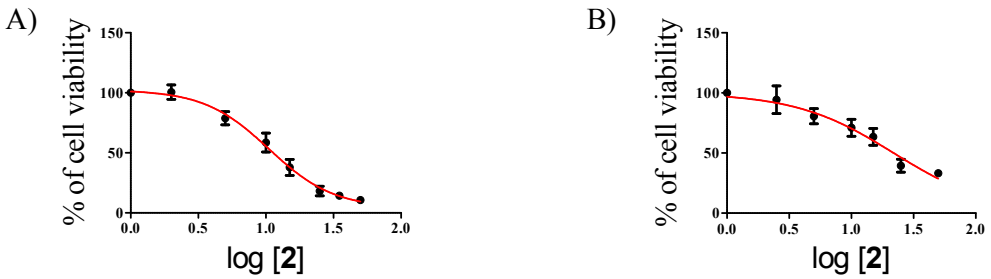

Fig. S48 Plots of cell viability (%) *vs*. log of μM concentrations of **2** against A) MCF-7 and B) A549 cell lines after incubation for 48 h determined from MTT assays under hypoxic condition. The plots provided are for one independent experiment out of the three independent experiments.

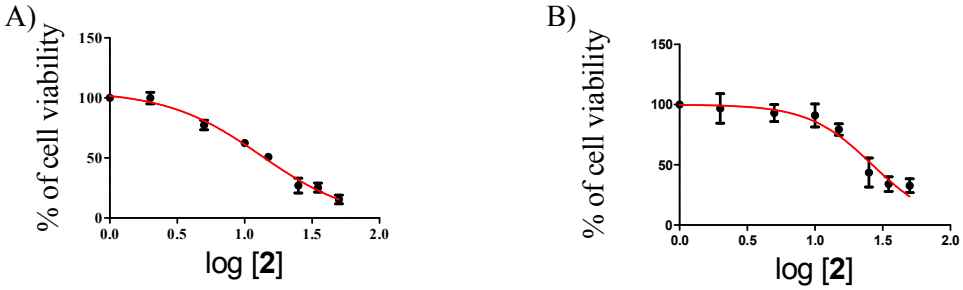

Fig. S49 Plots of cell viability (%) *vs*. log of μM concentrations of **2** against A) MCF-7 and B) A549 cell lines after incubation for 48 h determined from MTT assays under hypoxic condition in presence of 250 μM and 400 μM GSH respectively. The plots provided are for one independent experiment out of the three independent experiments.

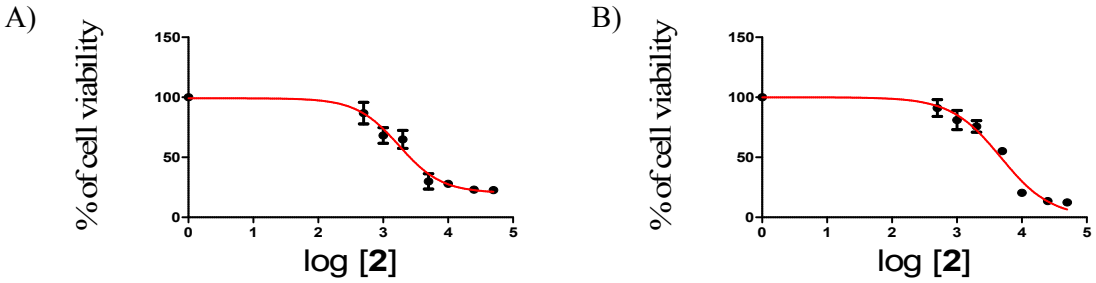

Fig. S50 Plots of cell viability (%) *vs*. log of μM concentrations of **2** against A) MIA PaCa2 cell line after incubation for 48 h determine from MTT assays under hypoxic condition and B) MIA PaCa2 cell lines after incubation for 48 h under hypoxic condition in presence of 100 μM GSH determined from MTT assays. The plots provided are for one independent experiment out of the three independent experiments.

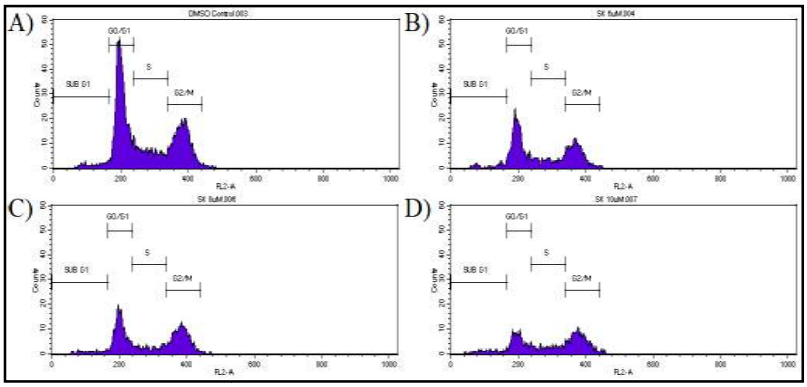

Fig. S51 Cell cycle arrest of MCF-7 treated with **2** for 24 h. (A) DMSO control, (B) 6 μM, (C) 8 μM and D) 10 μM of **2** treated cells. The figure represents one independent experiment.

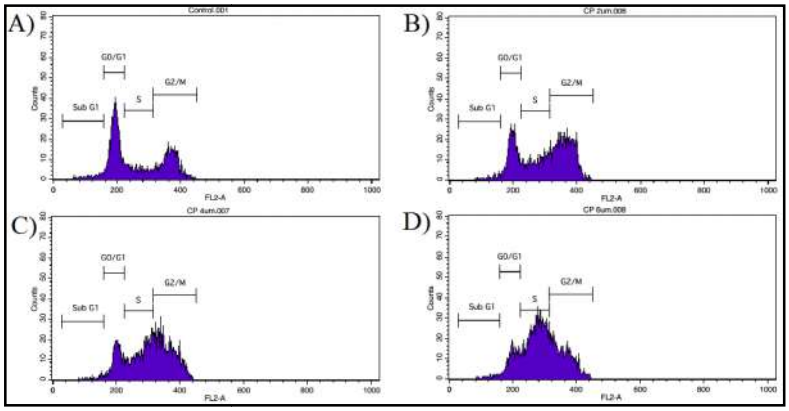

Fig. S52 Cell cycle arrest of MCF-7 treated with cisplatin for 24 h. (A) DMSO control, (B) 2 μM, (C) 4 μM and D) 6 μM of cisplatin treated cells. The figure represents one independent experiment.

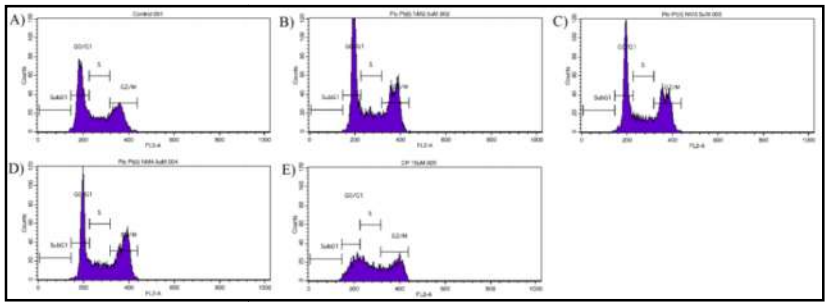

Fig. S53 Cell cycle arrest of MIA PaCa2 treated with **2** and cisplatin respectively for 24 h. (A) DMSO control, (B) 2.5 μM, (C) 3.5 μM and D) 4.5 μM of **2** and 15 μM of cisplatin treated cells. The figure represents one independent experiment.

Table S4 Cell cycle analysis of MCF-7 cells treated with **2**[a]

	Sub G1	G0/G1	S	G2/M
DMSO control[b]	3.9	43.9	22.4	29.8
2, 6 μM[b]	5.6	35.4	20.4	38.6
2, 8 μM[b]	6.5	30.4	19.3	43.8
2, 10 μM[b]	8.1	24.7	22.1	45.1

[a]Cells were treated with the complex for 24 h. Cell populations were expressed as the percentage of cells in each phase. [b]The data represents the average of two independent experiments.

Table S5 Cell cycle analysis of MCF-7 cells treated with cisplatin[a]

	Sub G1	G0/G1	S	G2/M
DMSO control[b]	1.2	50.9	17.4	30.5
Cisplatin, 2 μM[b]	1.2	24.5	26.6	47.7
Cisplatin, 4 μM[b]	1.1	18.9	44.2	35.8
Cisplatin, 6 μM[b]	1.0	19.2	58.7	21.1

[a]Cells were treated with the complex for 24 h. Cell populations were expressed as the percentage of cells in each phase. [b]The data represents the average of two independent experiments.

Table S6 Cell cycle analysis of MIA PaCa2 cells treated with **2** and cisplatin[a]

	Sub G1	G0/G1	S	G2/M
DMSO control[b]	1.0	47.6	21.8	29.6
2, 2.5 μM[b]	1.0	44.3	19.3	35.4
2, 3.5 μM[b]	1.0	41.2	18.5	39.3
2, 4.5 μM[b]	1.0	35.9	20.0	43.1
Cisplatin, 15 μM[b]	1.0	27.1	32.9	39.0

[a]Cells were treated with the complex for 24 h. Cell populations were expressed as the percentage of cells in each phase. [b]The data represents the average of two independent experiments.