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Anticancer activity of a chelating nitrogen mustard bearing tetrachloridoplatinum(IV) complex: better stability yet equipotent to the Pt(II) analogue

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Table S1 Selected crystallographic parameters for complex **1a**

1a	
Empirical formula	$C_{10}H_{14}Cl_6N_2Pt$
Formula weight	570.02
Temperature (K)	100.01(10)
Wavelength(Å)	0.7107
Crystal system,	Monoclinic
space group	*I*2/*a*
a (Å)	13.2902(3)
b (Å)	7.45130(17)
c (Å)	31.5320(7)
α (deg.)	90.00
β (deg.)	93.040(2)
γ (deg.)	90.00
Volume (Å^3)	3118.19(13)
Z, Calculated density (mg/mm^3)	8, 2.428
F(000)	2144.0
μ/mm^{-1}	10.014
Max. and min. transmission	0.821 and 1.000
Goodness-of-fit on F^2	1.066
Final *R* indices [$I \geq 2\sigma(I)$]	$^aR_1 = 0.0197$, $^bwR_2 = 0.0471$
Final *R* indices (all data)	$^aR_1 = 0.0220$, $^bwR2 = 0.0490$

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

Table S2 Selected bond lengths (Å)and angles (°) of **1a**

Bond lengths (Å)		Bond angles (°)			
Pt1–Cl1	2.3286(7)	Cl4–Pt1–Cl1	175.84(3)	N2–Pt1–N1	80.60(10)
Pt1–Cl4	2.3183(7)	Cl2–Pt1–Cl1	89.43(3)	N1–Pt1–Cl1	89.21(7)
Pt1–Cl2	2.3165(7)	Cl2–Pt1–Cl4	87.12(3)	N1–Pt1–Cl4	88.75(7)
Pt1–Cl3	2.3178(7)	Cl2–Pt1–Cl3	88.66(3)	N1–Pt1–Cl2	94.55(7)
Pt1–N1	2.027(2)	Cl3–Pt1–Cl1	91.00(3)	N1–Pt1–Cl3	176.79(7)
Pt1–N2	2.155(2)	Cl3–Pt1–Cl4	91.24(3)		
		N2–Pt1–Cl1	88.72(7)		
		N2–Pt1–Cl4	94.52(7)		
		N2–Pt1–Cl2	174.83(7)		
		N2–Pt1–Cl3	96.20(7)		

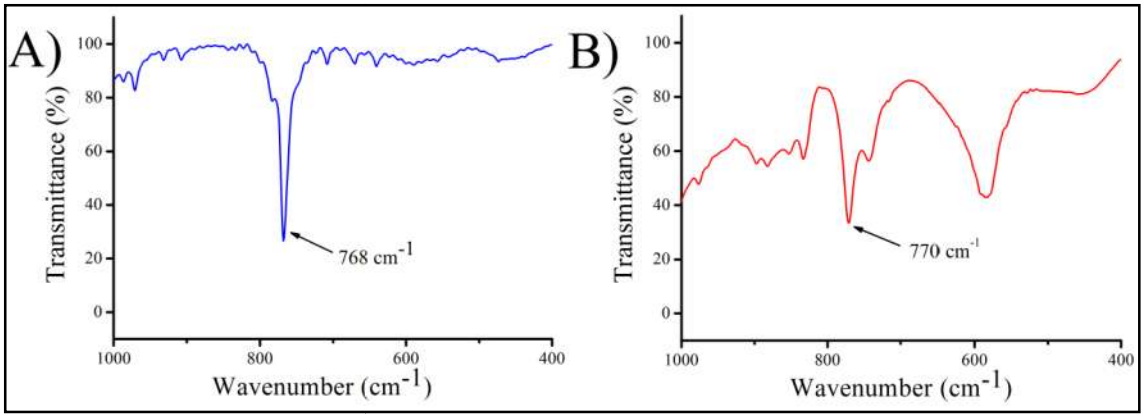


Fig. S1 Infrared C–Cl stretching frequency showing the presence of nitrogen mustard moiety in the synthesized two Pt(IV) complexes A) **1a** and B) **2a** respectively.

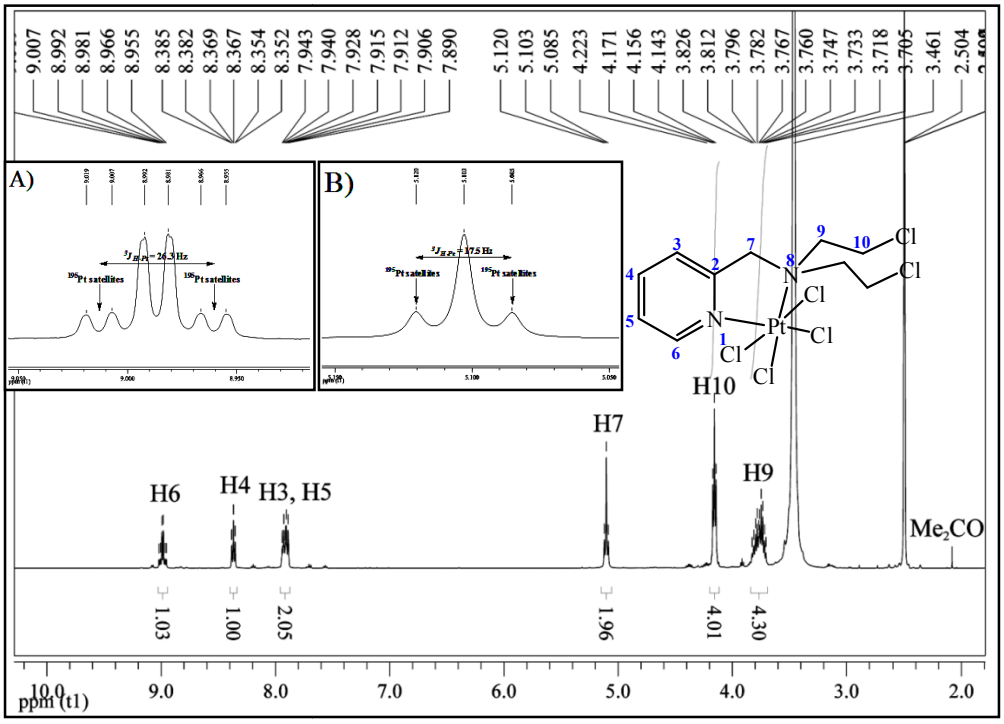


Fig. S2 ^{1}H NMR of **1a** in DMSO-d_6, 500 MHz . Insets are showing the ^{195}Pt satellites corresponding to A) H6 and B) H7 protons respectively.

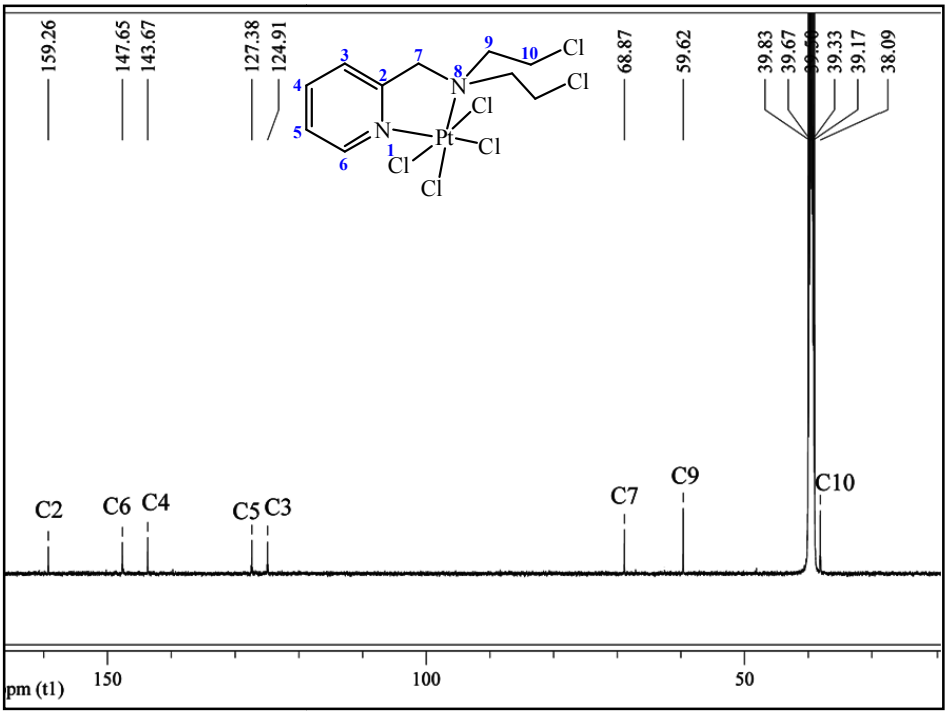


Fig. S3 ^{13}C NMR of **1a** in DMSO-d_6, 125 MHz.

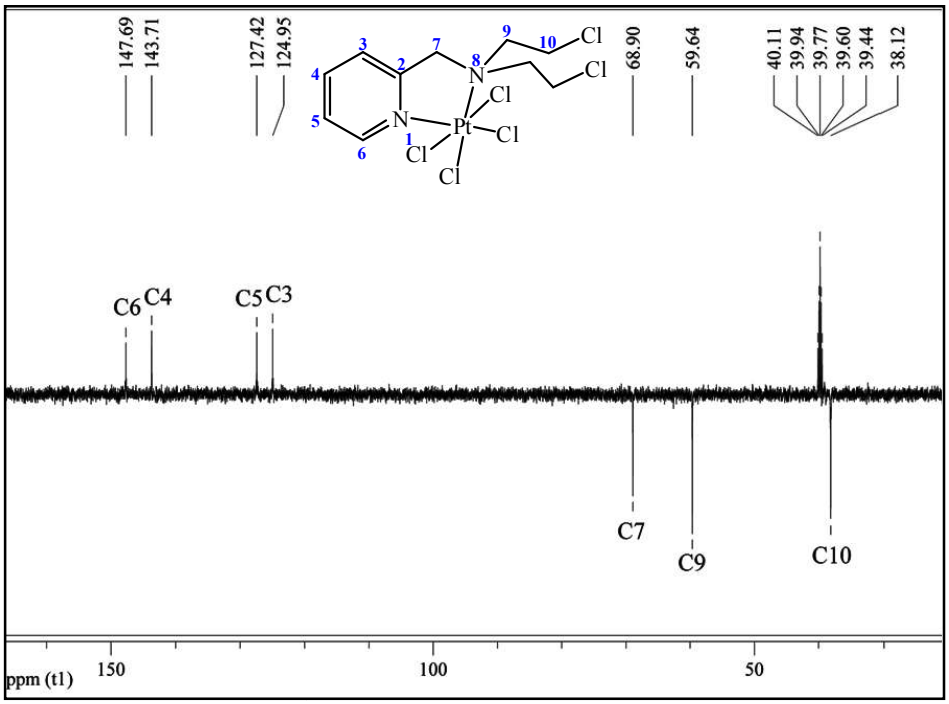


Fig. S4 ^{13}C DEPT-135 of **1a** in DMSO-d_6, 125 MHz.

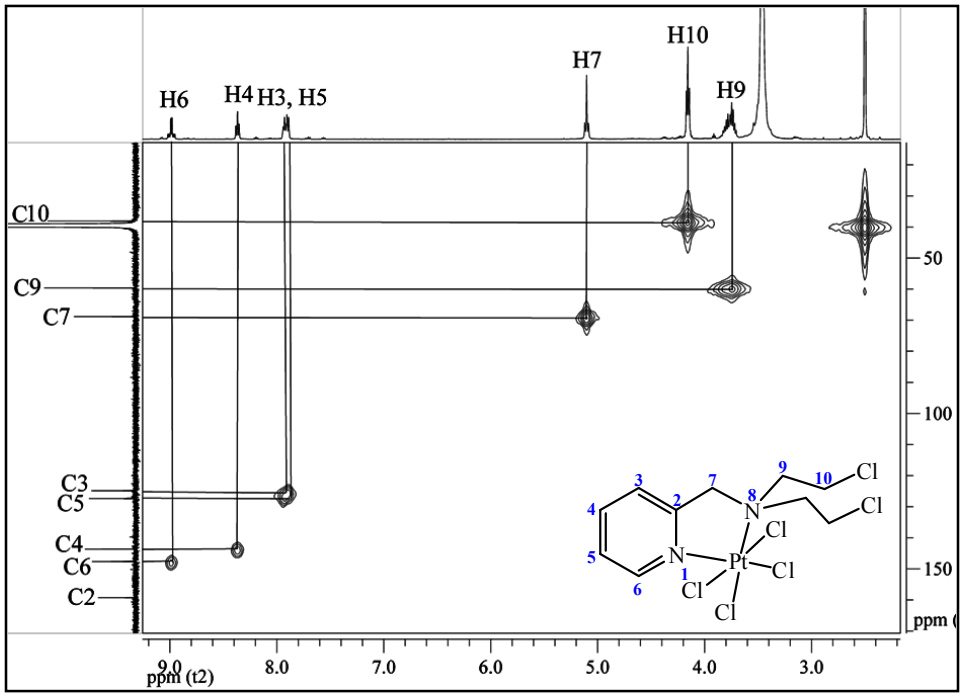


Fig. S5 HMQC of **1a** in DMSO-d_6.

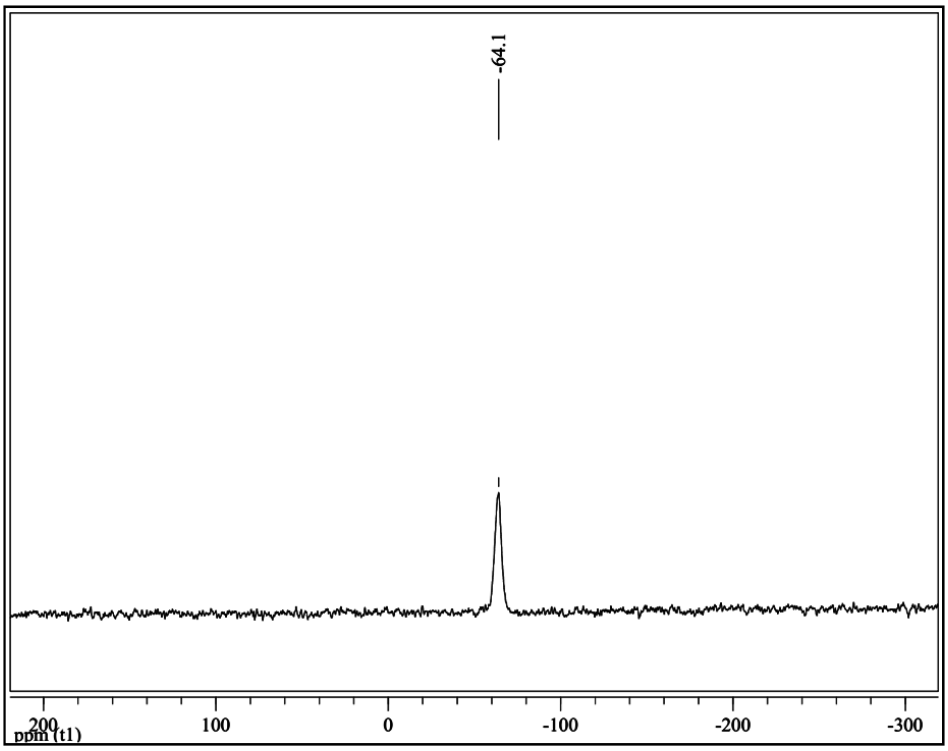


Fig. S6 ^{195}Pt NMR of **1a** in DMSO-d_6, 107.5 MHz.

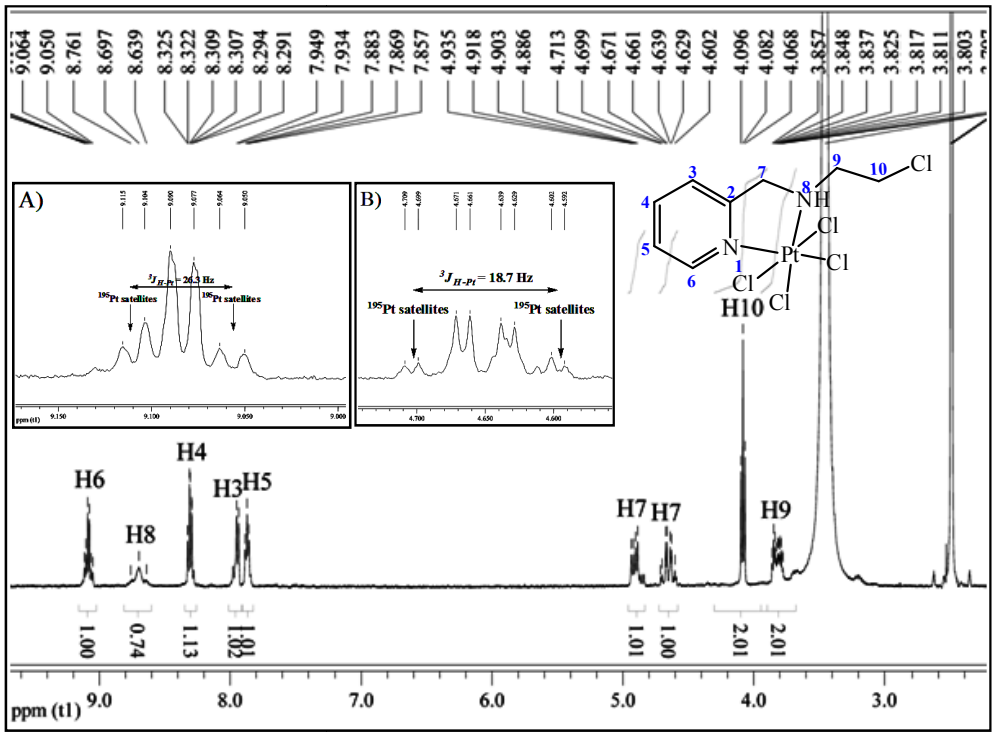


Fig. S7 ^{1}H NMR of **2a** in DMSO-d_6, 500 MHz . Insets are showing the ^{195}Pt satellites corresponding to A) H6 and B) H7 (right) protons respectively.

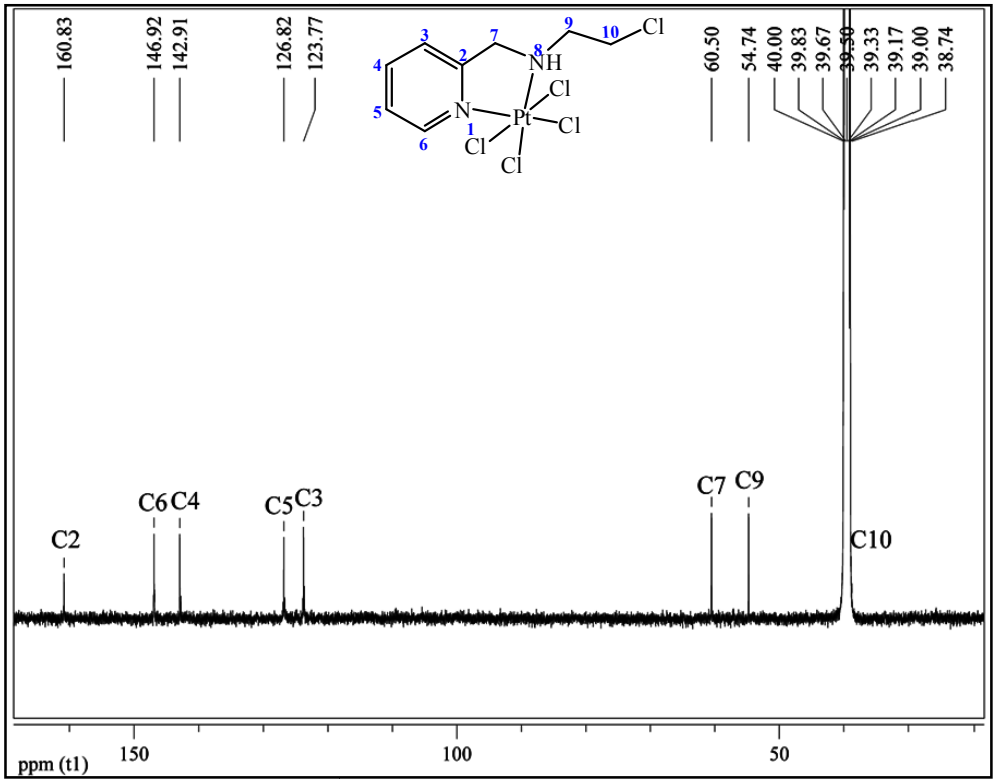


Fig. S8 ^{13}C NMR of **2a** in DMSO-d_6, 125 MHz.

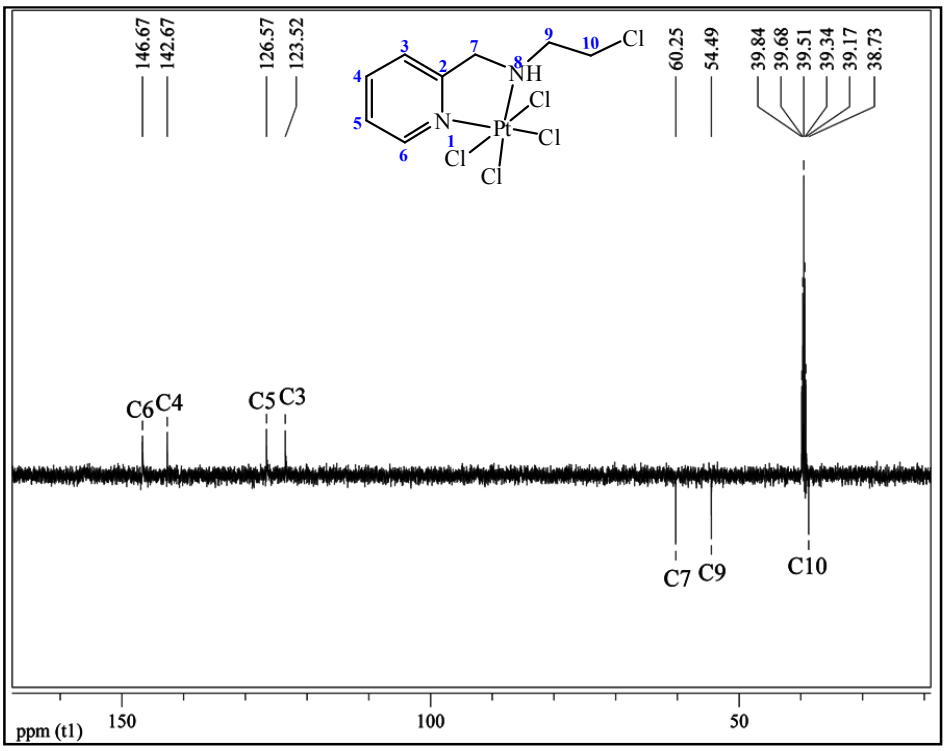


Fig. S9 ^{13}C DEPT-135 of **2a** in DMSO-d_6, 125 MHz.

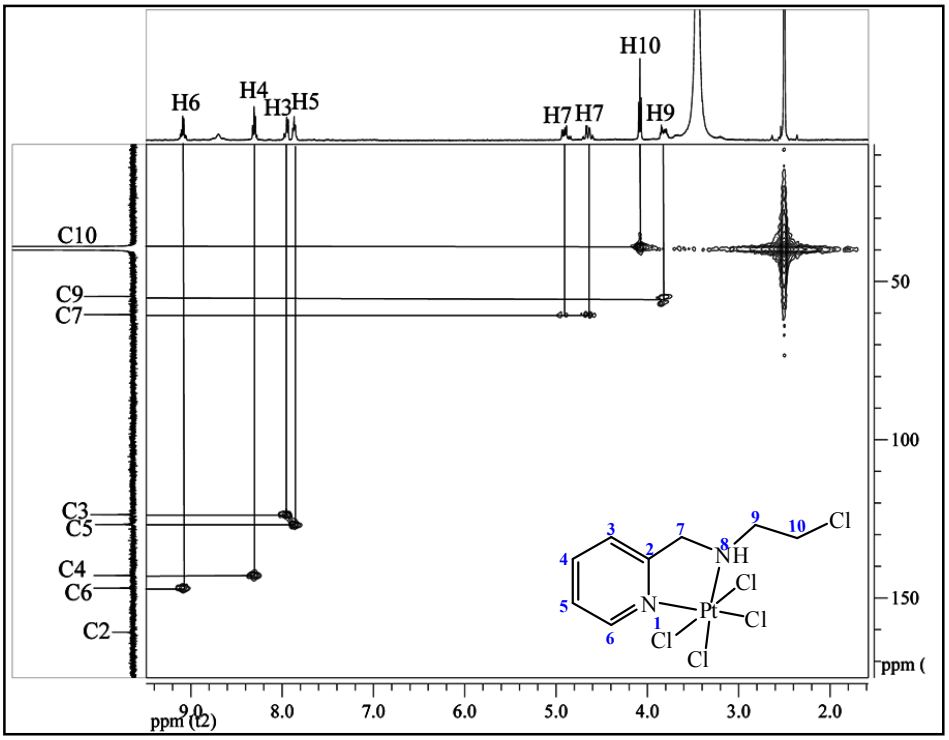


Fig. S10 HMQC of **2a** in DMSO-d_6.

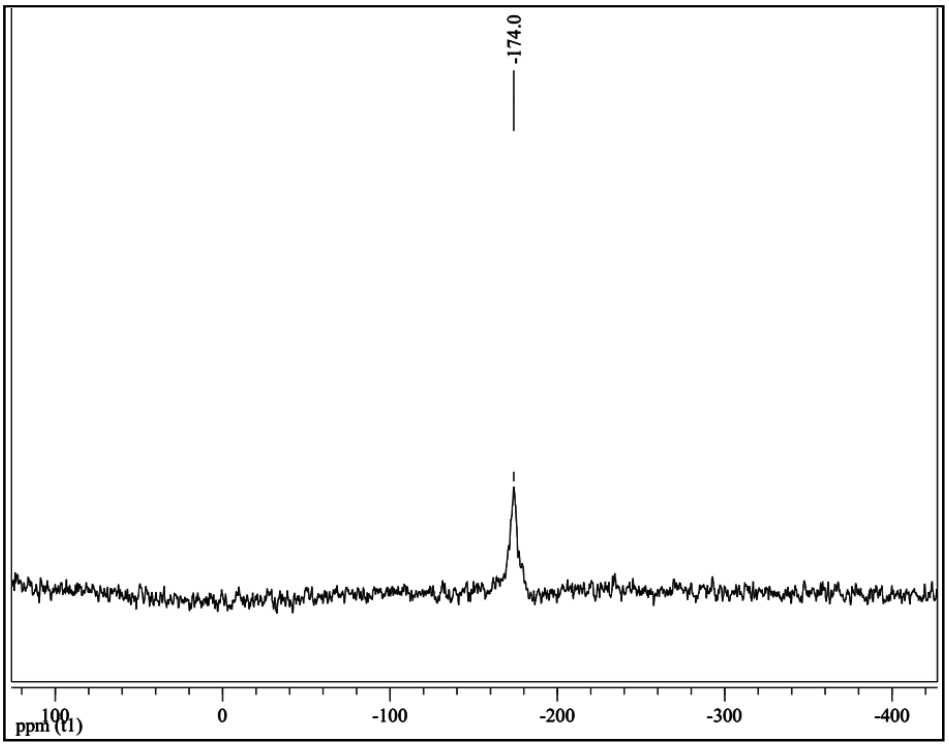


Fig. S11 ^{195}Pt NMR of **2a** in DMSO-d_6, 107.5 MHz.

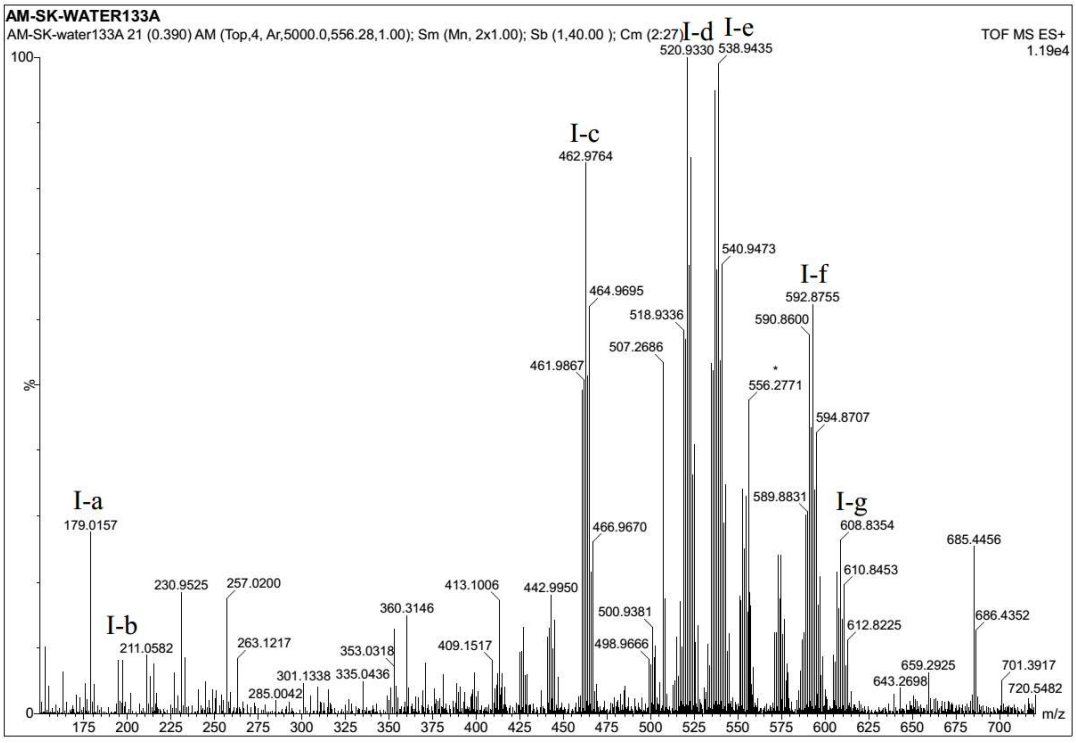


Fig. S12 ESI-MS speciation recorded during monitoring of stability kinetics of **1a** by ^{1}H NMR after 3 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

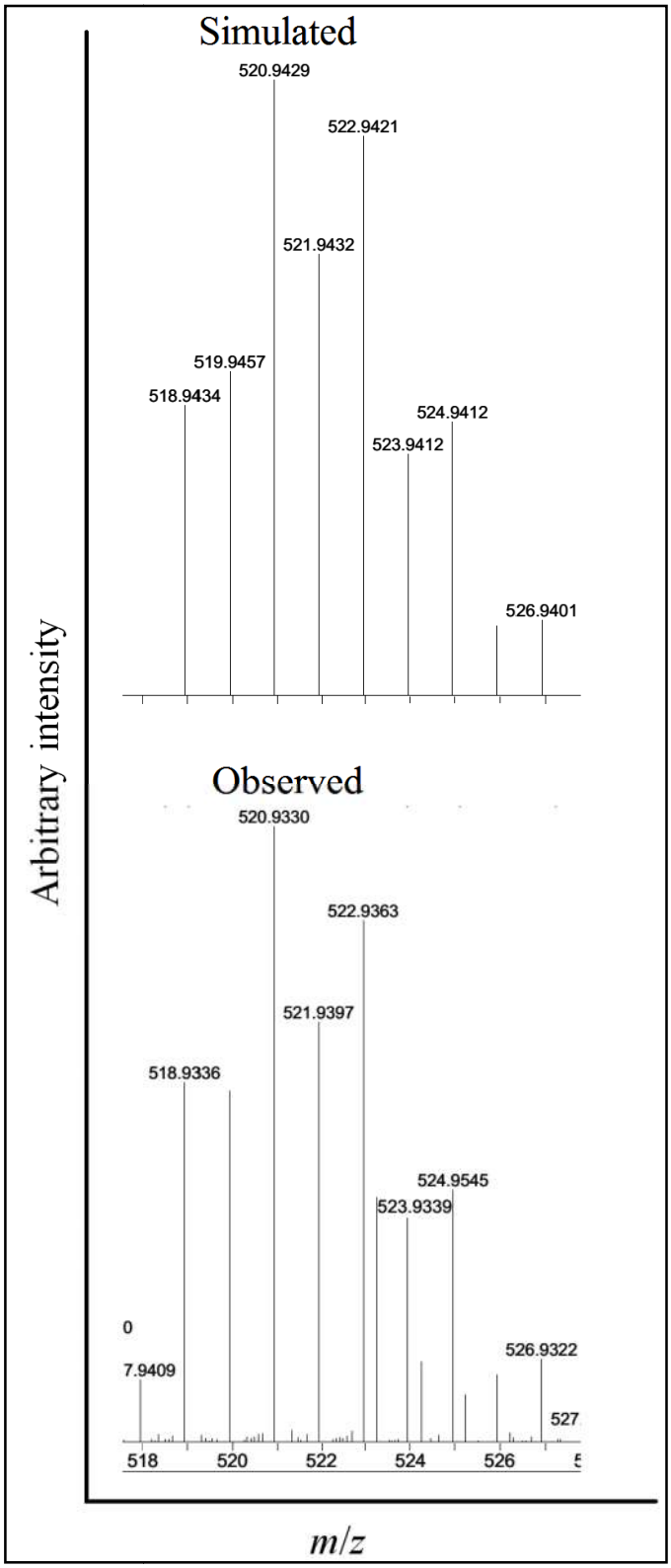


Fig. S13 Observed and simulated isotopic pattern of [**3** + Na^+]$^+$ found in ESI-MS during monitoring of stability kinetics of **1a** by ^{1}H NMR after 3 d.

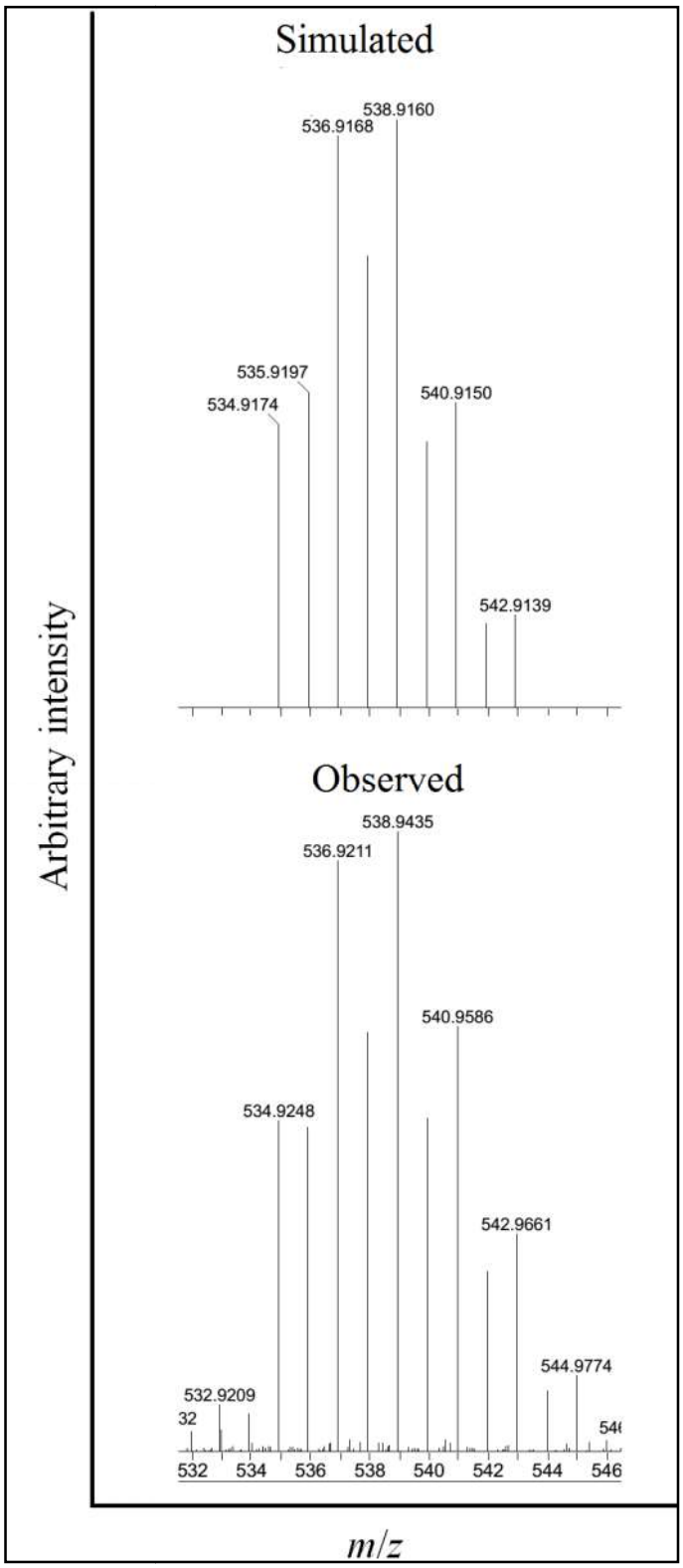


Fig. S14 Observed and simulated isotopic pattern of [**3** + K^+]$^+$ found in ESI-MS during monitoring of stability kinetics of **1a** by ^{1}H NMR after 3 d.

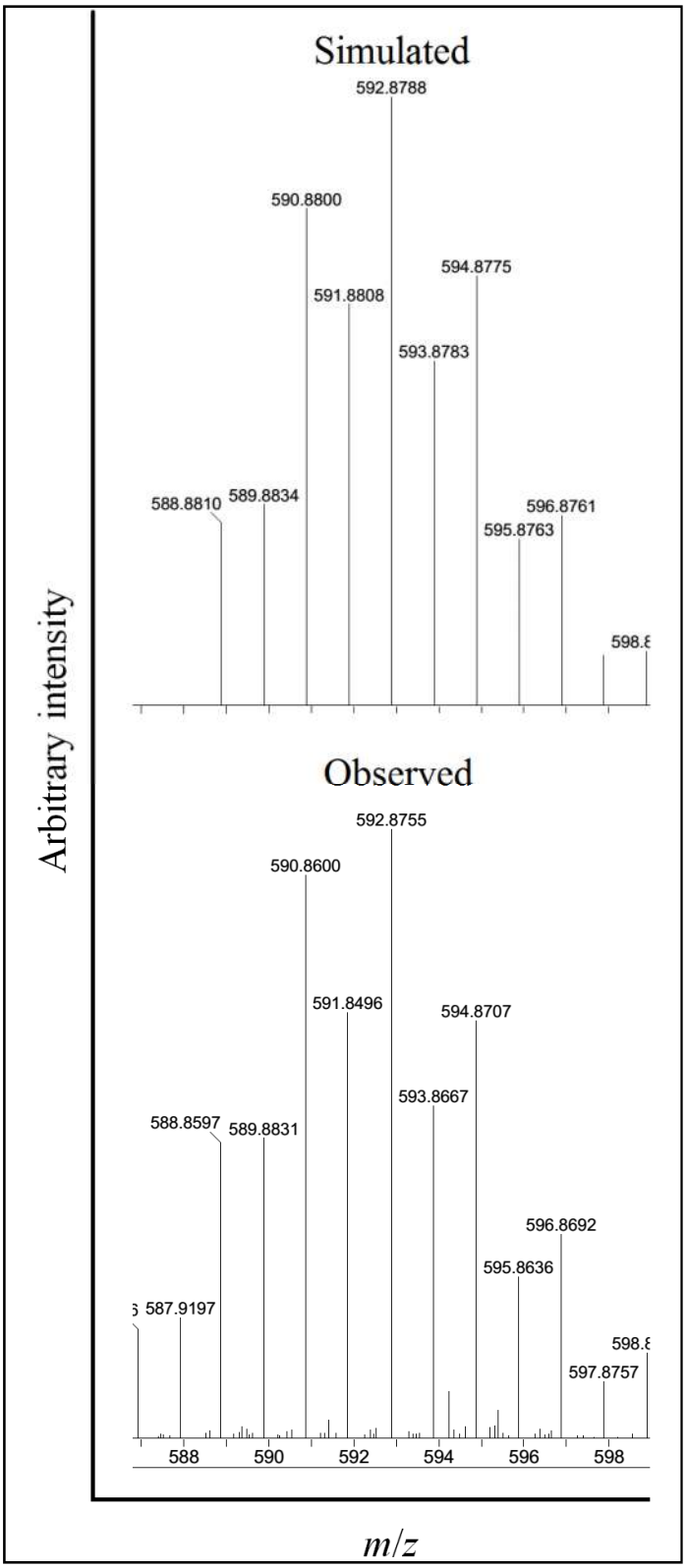


Fig. S15 Observed and simulated isotopic pattern of [**1a** + Na^+]$^+$ found in ESI-MS during monitoring of stability kinetics of **1a** by ^{1}H NMR after 3 d.

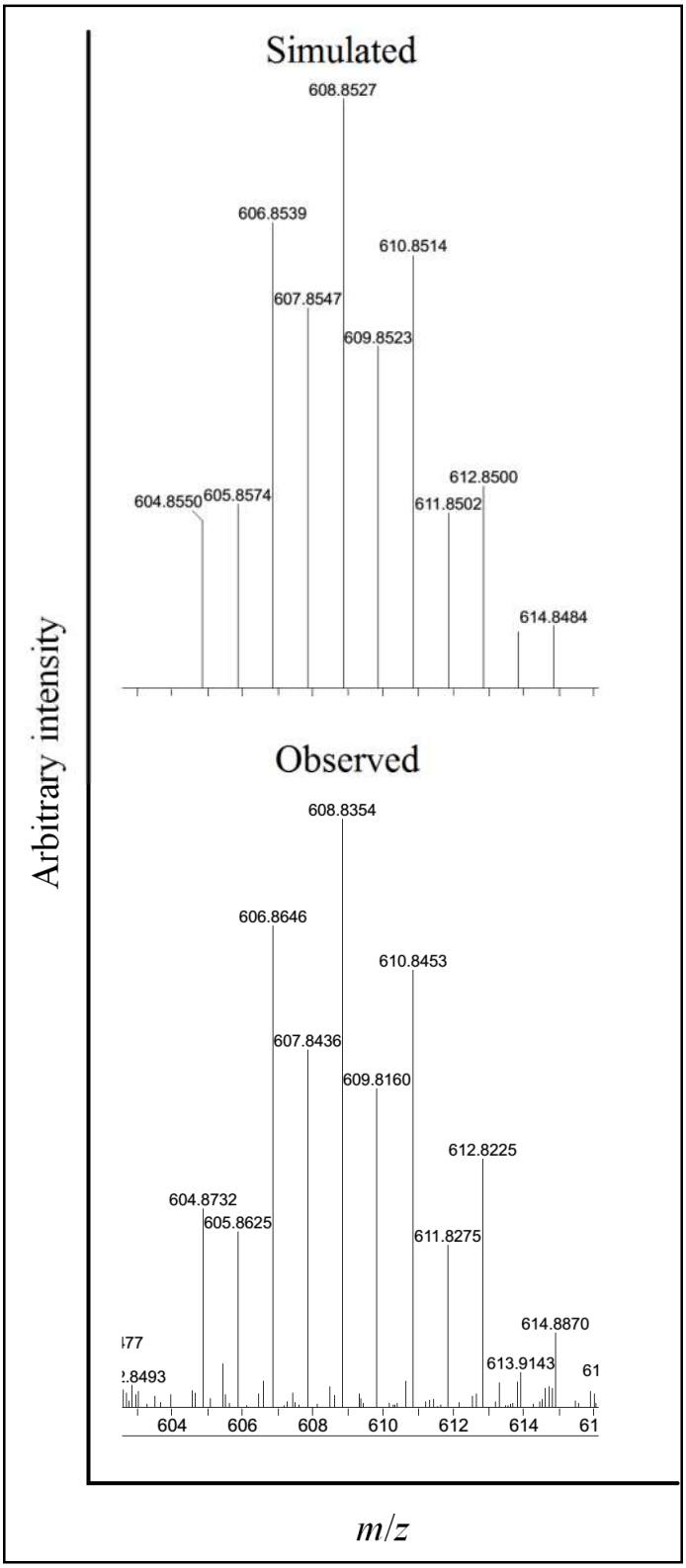


Fig. S16 Observed and simulated isotopic pattern of [**1a** + K^+]$^+$ found in ESI-MS during monitoring of stability kinetics of **1a** by ^{1}H NMR after 3 d.

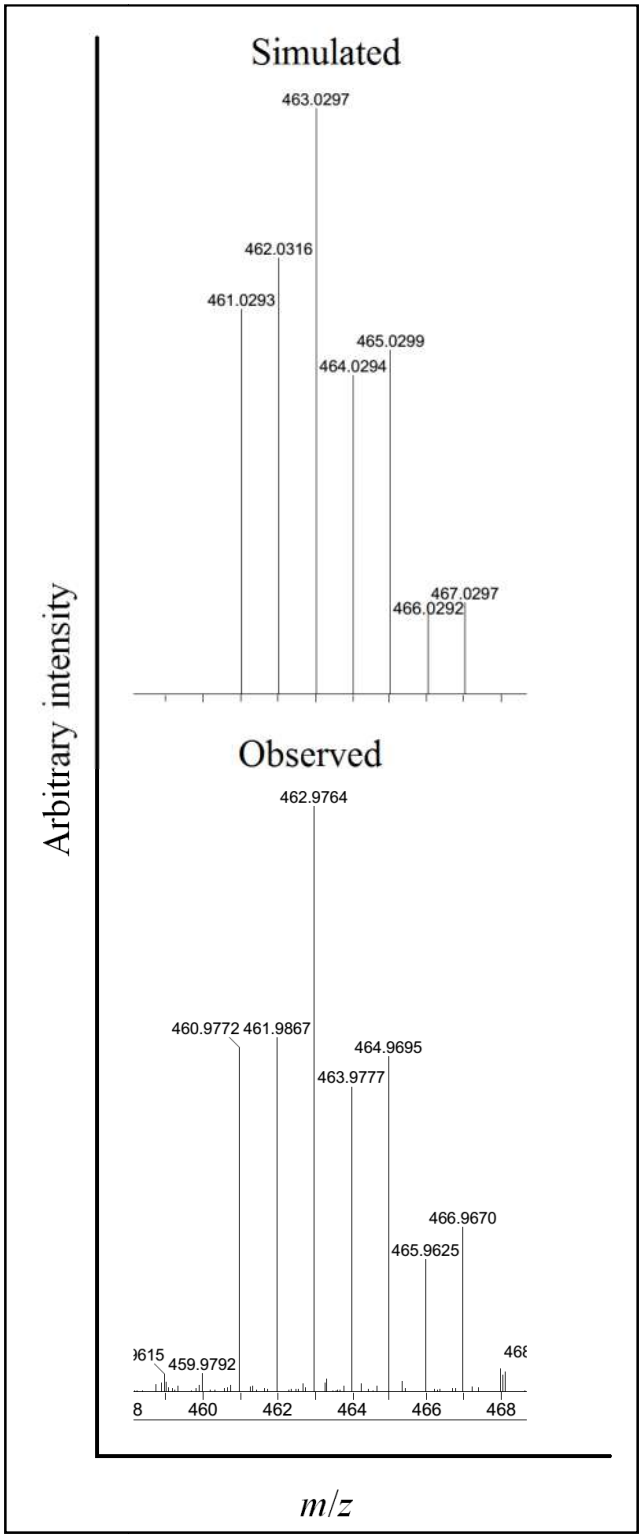


Fig. S17 Observed and simulated isotopic pattern of $[\mathbf{3} - 2Cl^- + 2OH^- + H^+]^+$ found in ESI-MS during monitoring of stability kinetics of **1a** by ^1H NMR after 3 d.

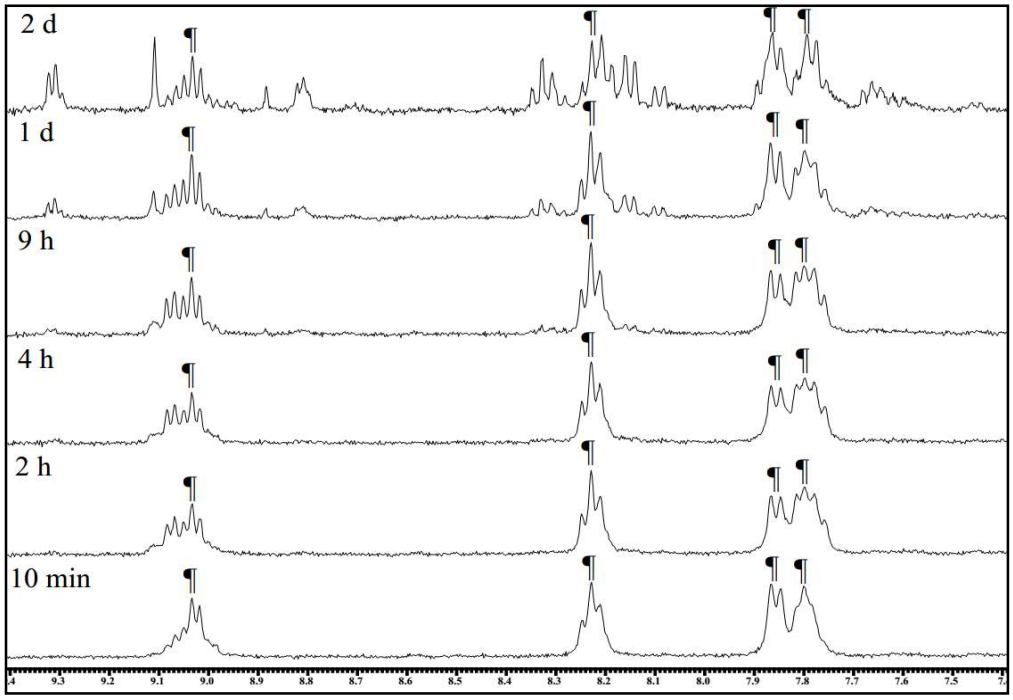


Fig. S18 Stack plot of stability kinetic study of the **2a** by ^{1}H NMR in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture. ¶ indicates unchanged **2a**.

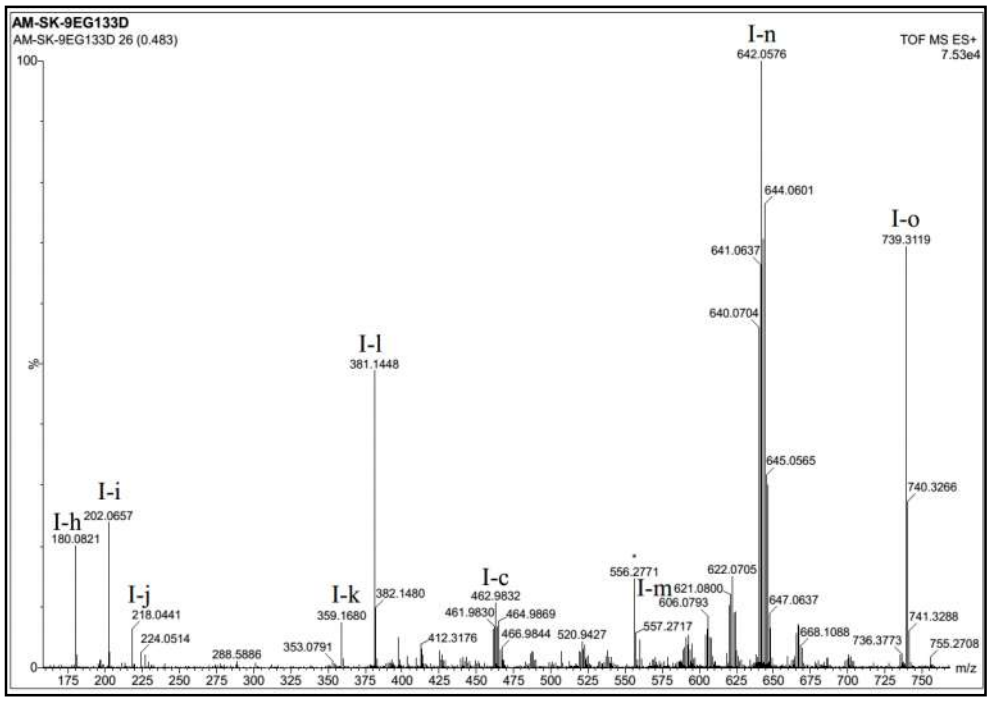


Fig. S19 ESI-MS speciation recorded during monitoring of the 9-EtG binding study with **1a** by ^{1}H NMR after 4 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

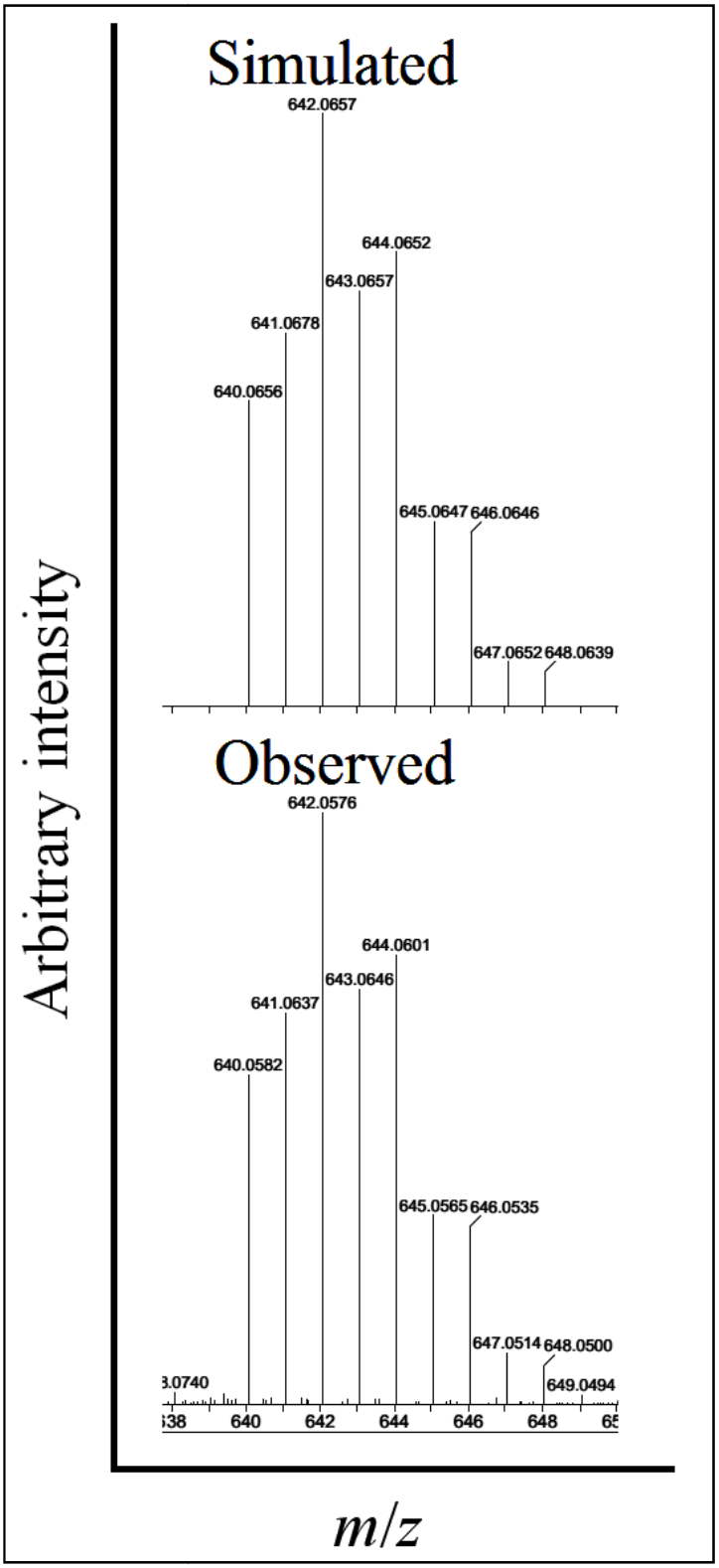


Fig. S20 Observed and simulated isotopic pattern of [**3d** (Scheme 3)] found in ESI-MS during monitoring of the 9-EtG binding study with **1a** by ^{1}H NMR after 4 d.

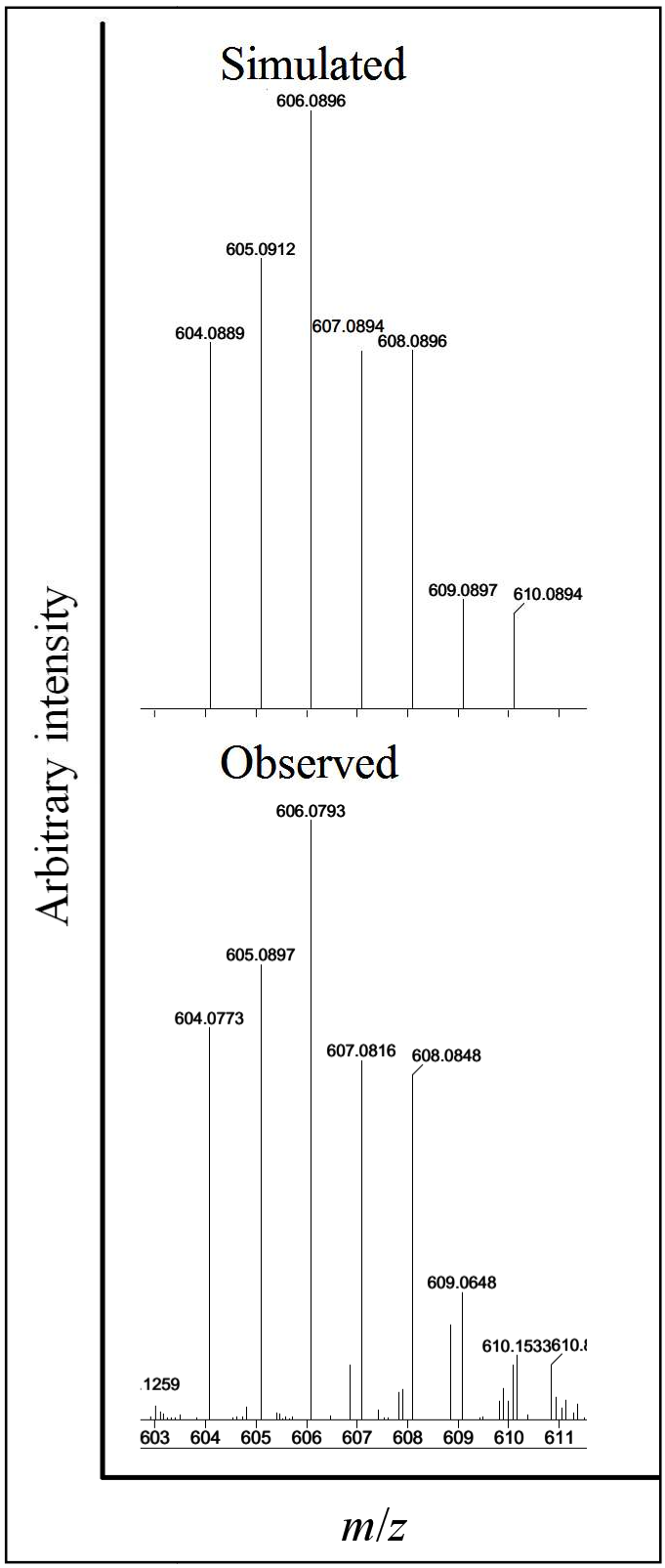


Fig. S21 Observed and simulated isotopic pattern of [**3e** (Scheme 3)] found in ESI-MS during monitoring of the 9-EtG binding study with **1a** by ^{1}H NMR after 4 d.

Table S3 Speciation of **1a** observed during the hydrolysis, 9-EtG and GSH binding experiments, as detected by ESI-MS. The speciation studies were performed using aliquots from NMR tube while studying the kinetics using NMR.

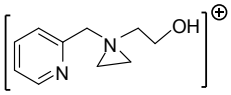
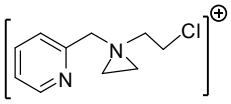
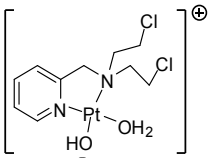
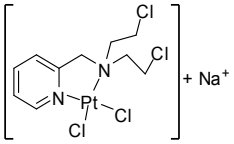
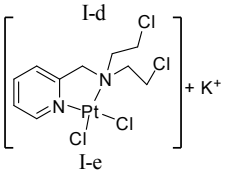
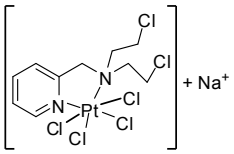
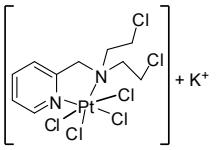
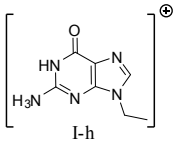
Species drawings	m/z_{calc}	Experiments		
		1a in 20% PBS in DMSO-d_6	**1a** + 9-EtG (1:6) in 20% PBS in DMSO-d_6	**1a** + GSH (1:6) in 20% PBS in DMSO-d_6
		m/z_{obs}		
 I-a	179.1184	179.0157	–	179.0481
 I-b	197.0845	197.0669	–	197.0805
 I-c	463.0297	462.9764	462.9832	462.9868
 I-d	520.9429	520.9330	–	–
 I-e	538.9160	538.9435	–	–
 I-f	592.8788	592.8755	–	–
 I-g	608.8527	608.8354	–	–
 I-h	180.0885	–	180.0821	–

Table S3 contd.

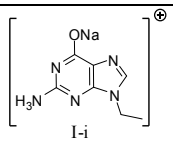
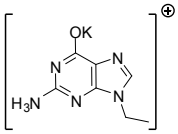
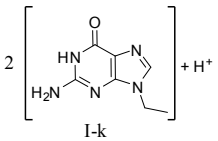
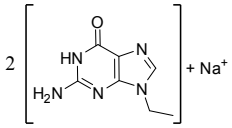
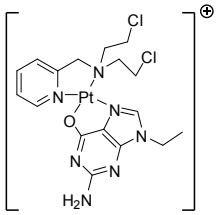
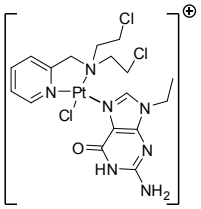
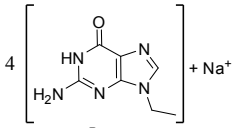
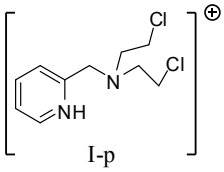
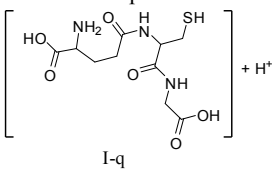
Species drawings	m/z_{calc}	Experiments		
		1a in 20% PBS in DMSO-d_6	**1a** + 9-EtG (1:6) in 20% PBS in DMSO-d_6	**1a** + GSH (1:6) in 20% PBS in DMSO-d_6
			m/z_{obs}	
 I-i	202.0705	–	202.0657	–
 I-j	218.0444	–	218.0441	–
 I-k	359.1693	–	359.1680	–
 I-l	381.1512	–	381.1448	–
 I-m	606.0896	-	606.0793	-
 I-n	642.0657	-	642.0576	-
 I-o	739.3126	-	739.3119	-
 I-p	233.0612	-	-	233.0555
 I-q	308.0916	-	-	308.0838

Table S3 contd.

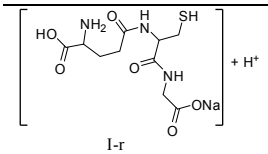
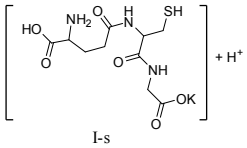
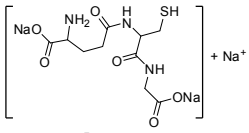
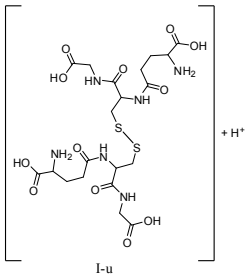
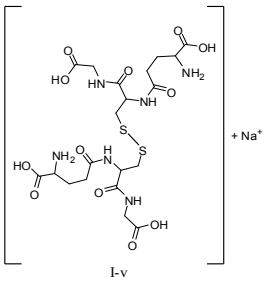
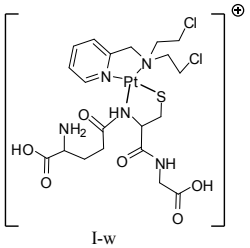
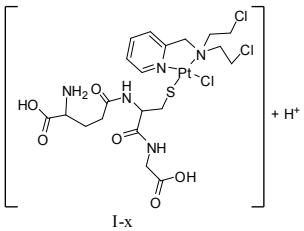
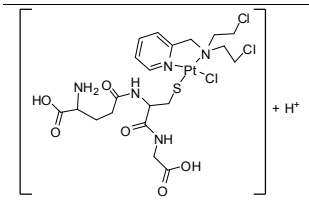
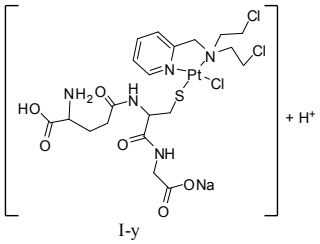
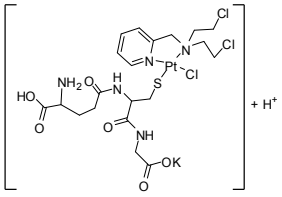
Species drawings	m/z_{calc}	Experiments		
		1a in 20% PBS in DMSO-d_6	**1a** + 9-EtG (1:6) in 20% PBS in DMSO-d_6	**1a** + GSH (1:6) in 20% PBS in DMSO-d_6
			m/z_{obs}	
 I-r	330.0736	-	-	330.0698
 I-s	346.0475	-	-	346.0396
 I-t	374.0375	-	-	374.0345
 I-u	613.1598	-	-	613.1584
 I-v	635.1417	-	-	635.1370
 I-w	734.0927	-	-	734.0967
 I-x	770.0688	-	-	770.0723

Table S3 contd.

Species drawings	m/z_{calc}	Experiments		
		1a in 20% PBS in DMSO-d_6	**1a** + 9-EtG (1:6) in 20% PBS in DMSO-d_6	**1a** + GSH (1:6) in 20% PBS in DMSO-d_6
			m/z_{obs}	
 I-x	770.0688	-	-	770.0723
 I-y	792.0508	-	-	792.0598
 I-z	808.0247	-	-	808.0373

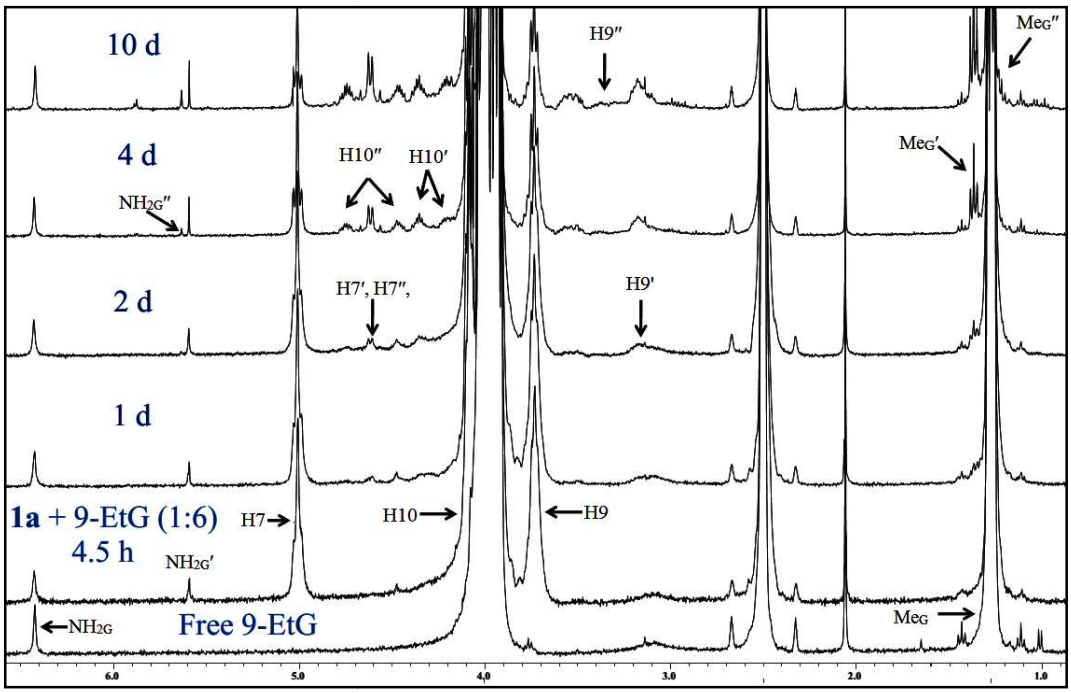


Fig. S22 Stack plot of aliphatic region during the binding kinetics study of **1a** with 9-EtG (1:6) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR. The chemical shifts are depicting the presence of excess reactants (**1a** & 9-EtG) and two types of 9-EtG bound adducts (**3d** & **3e**) after 10 d.

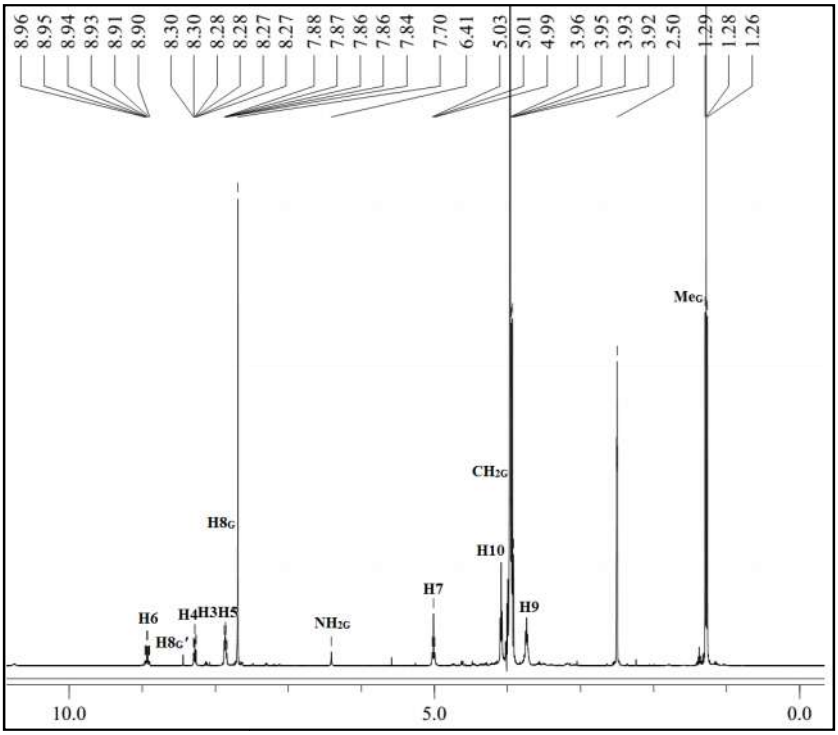


Fig. S23 ^{1}H NMR data during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR just after mixing of reactants.

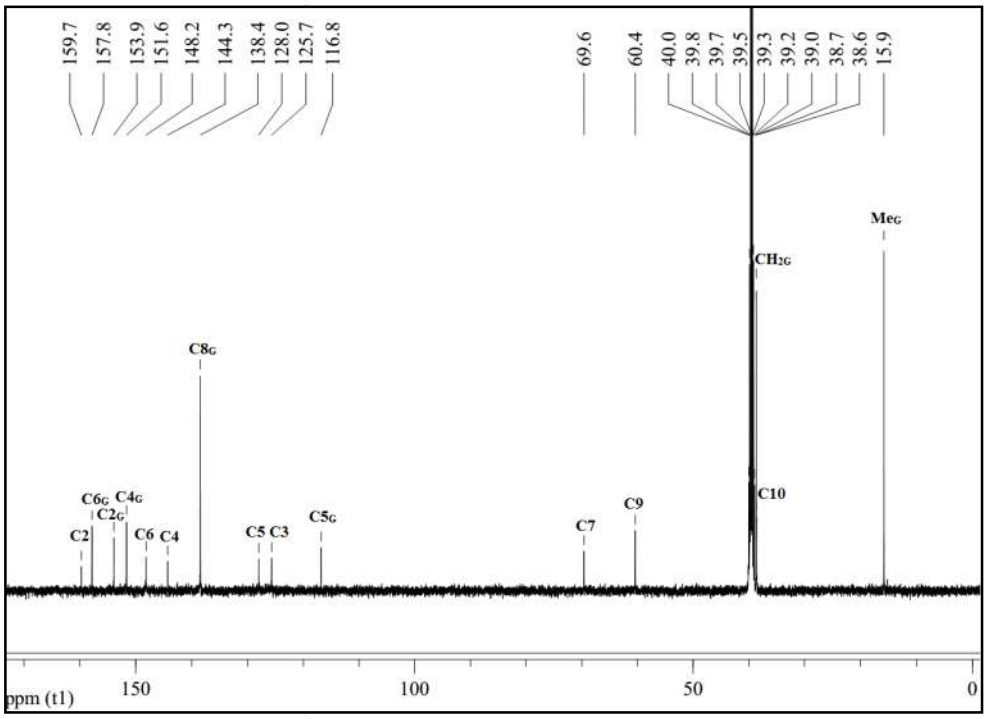


Fig. S24 ^{13}C NMR data during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR just after mixing of reactants.

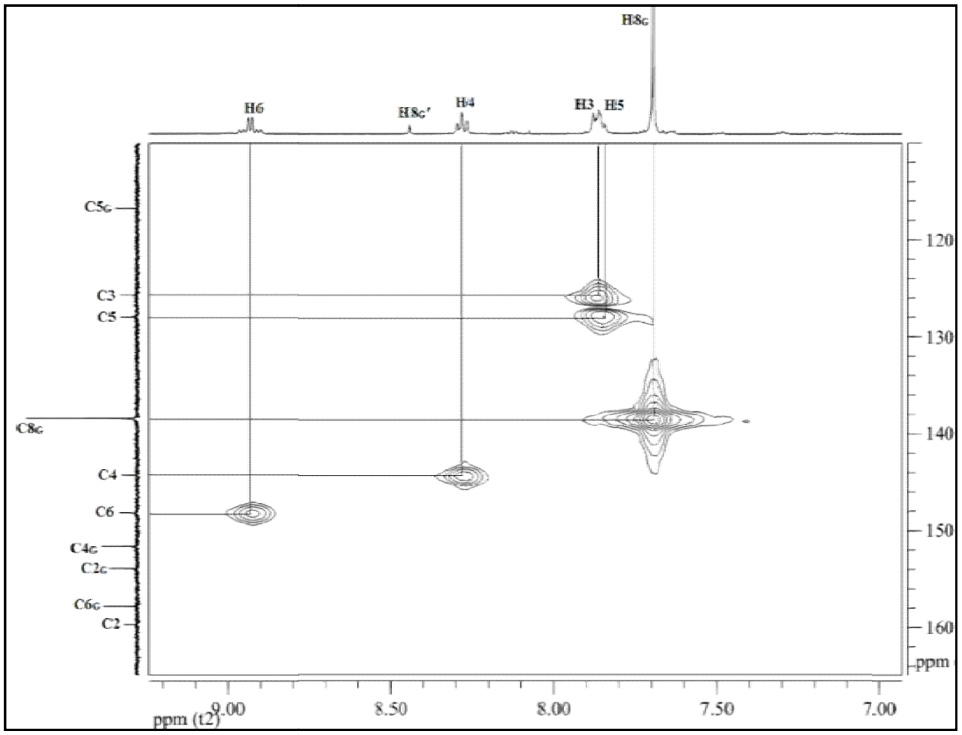


Fig. S25 HMQC data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR just after mixing of reactants.

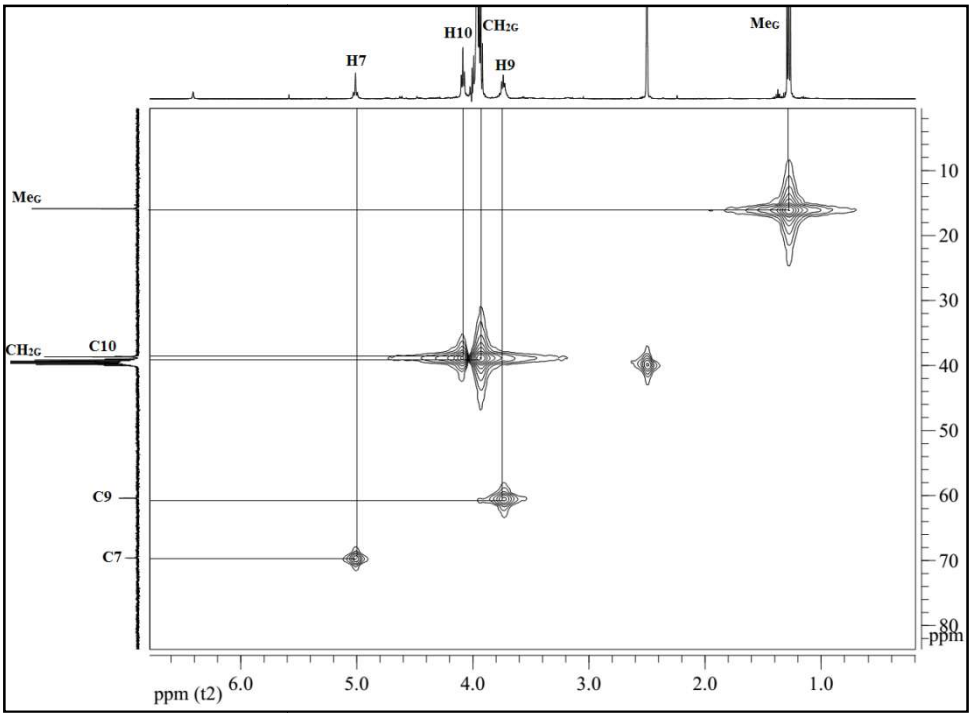


Fig. S26 HMQC data of aliphatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR just after mixing of reactants.

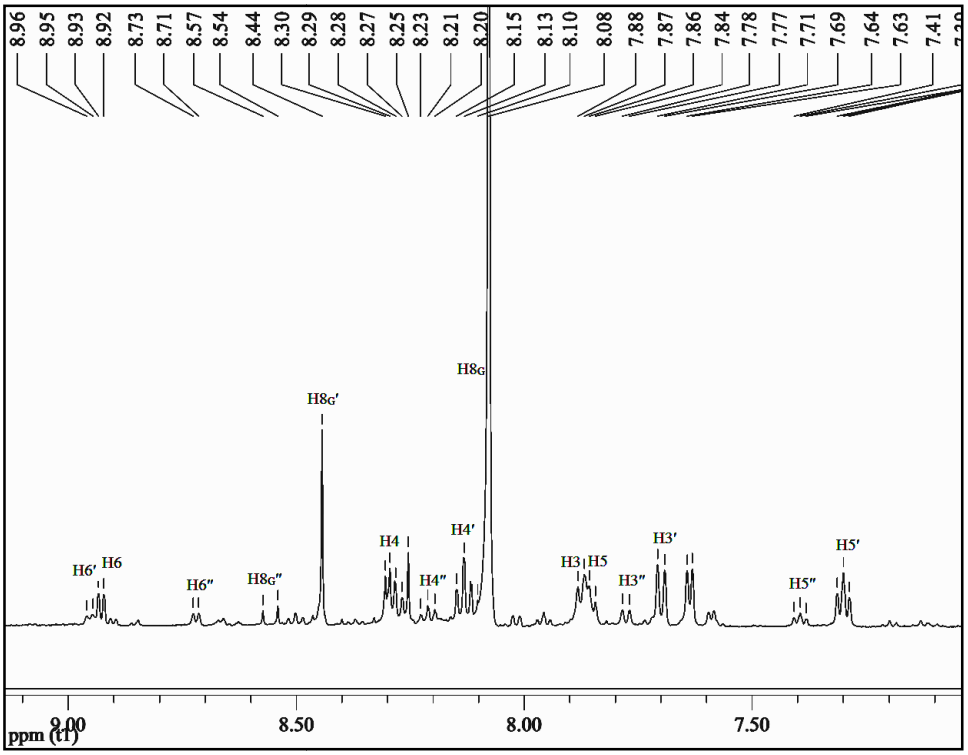


Fig. S27 ^{1}H NMR data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

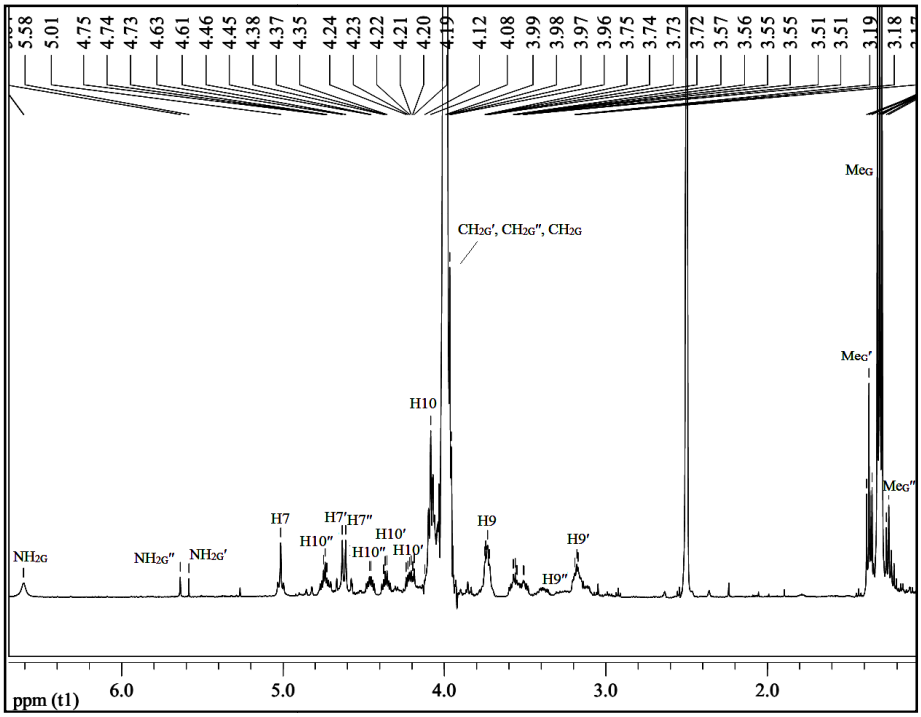


Fig. S28 ^{1}H NMR data of aliphatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

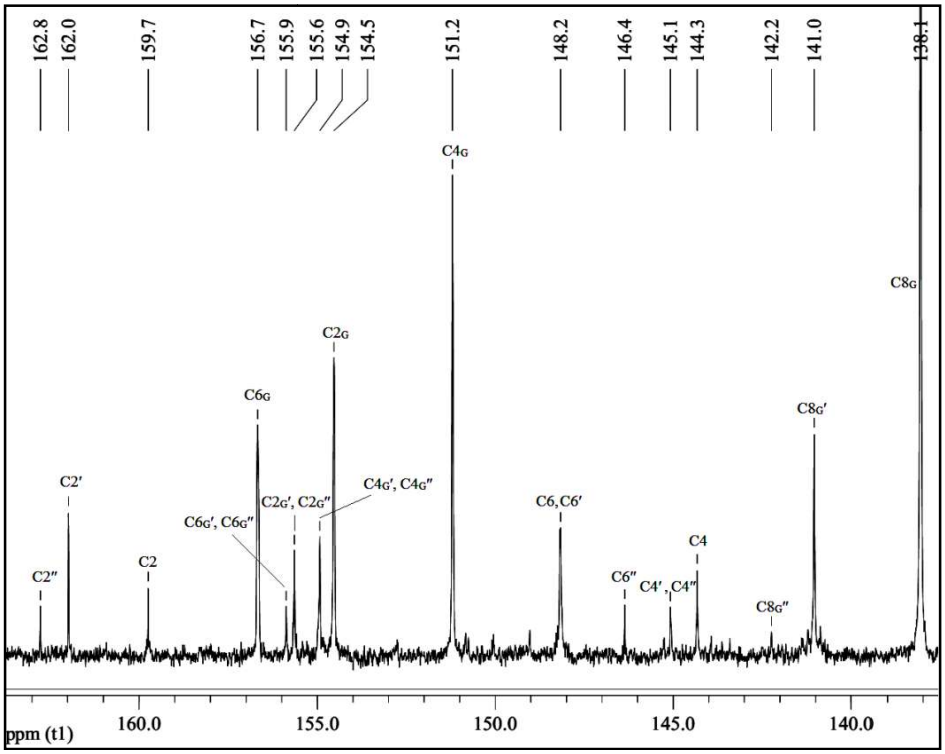


Fig. S29 ^{13}C NMR data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

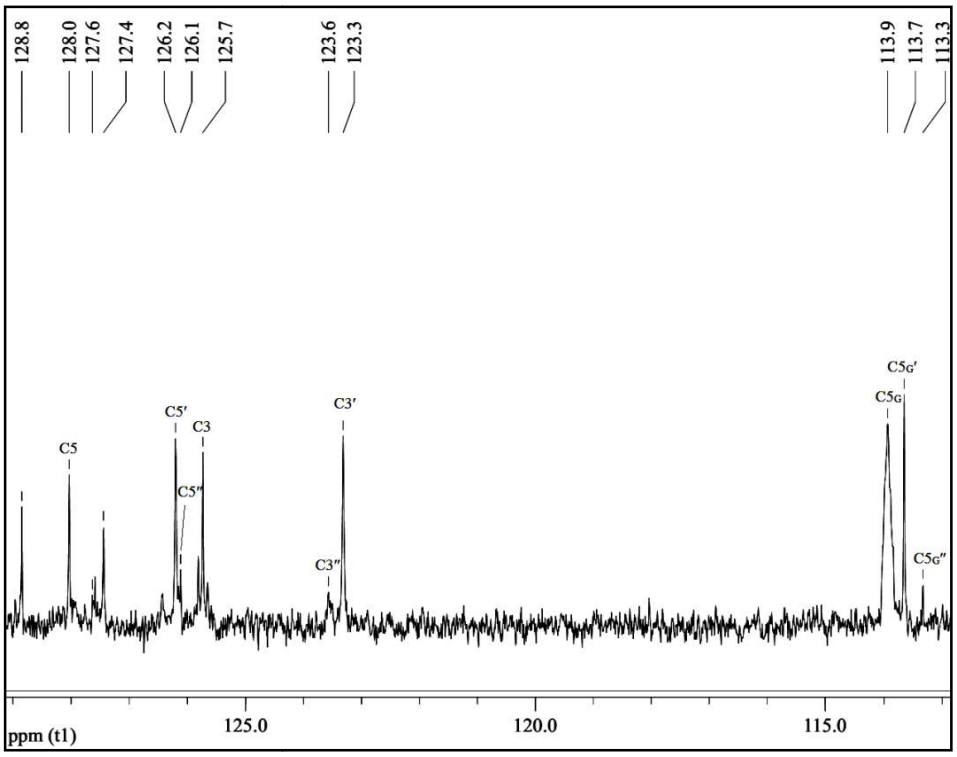


Fig. S30 ^{13}C NMR data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

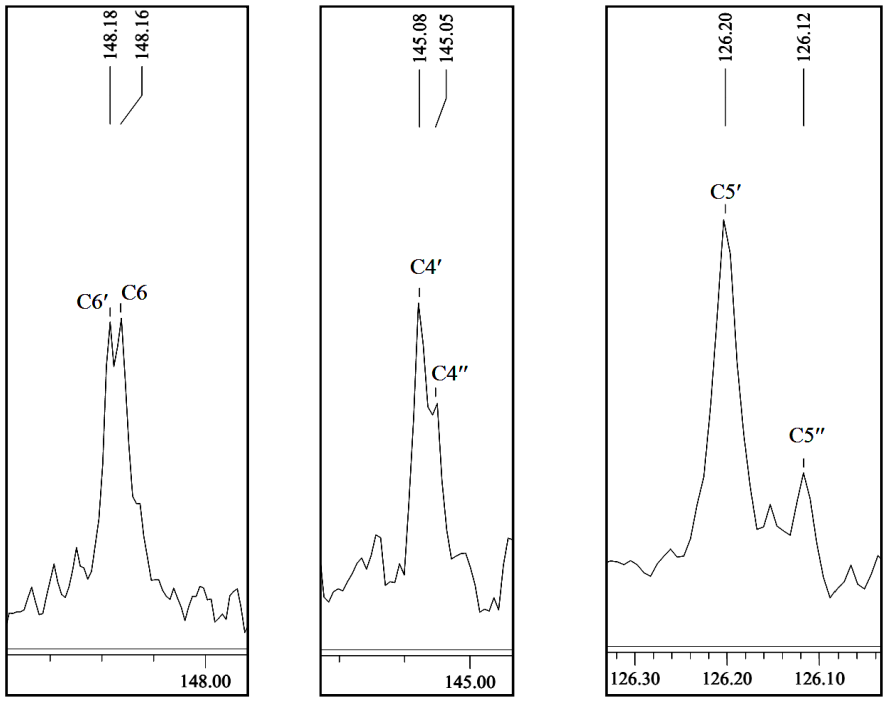


Fig. S31 ^{13}C NMR data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

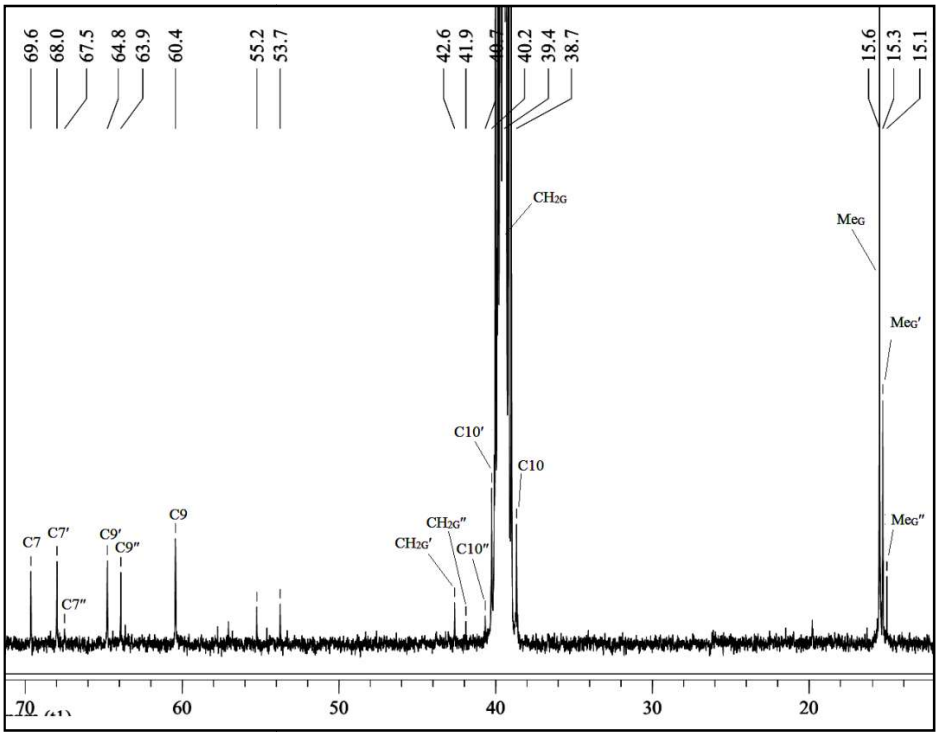


Fig. S32 ^{13}C NMR data of aliphatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

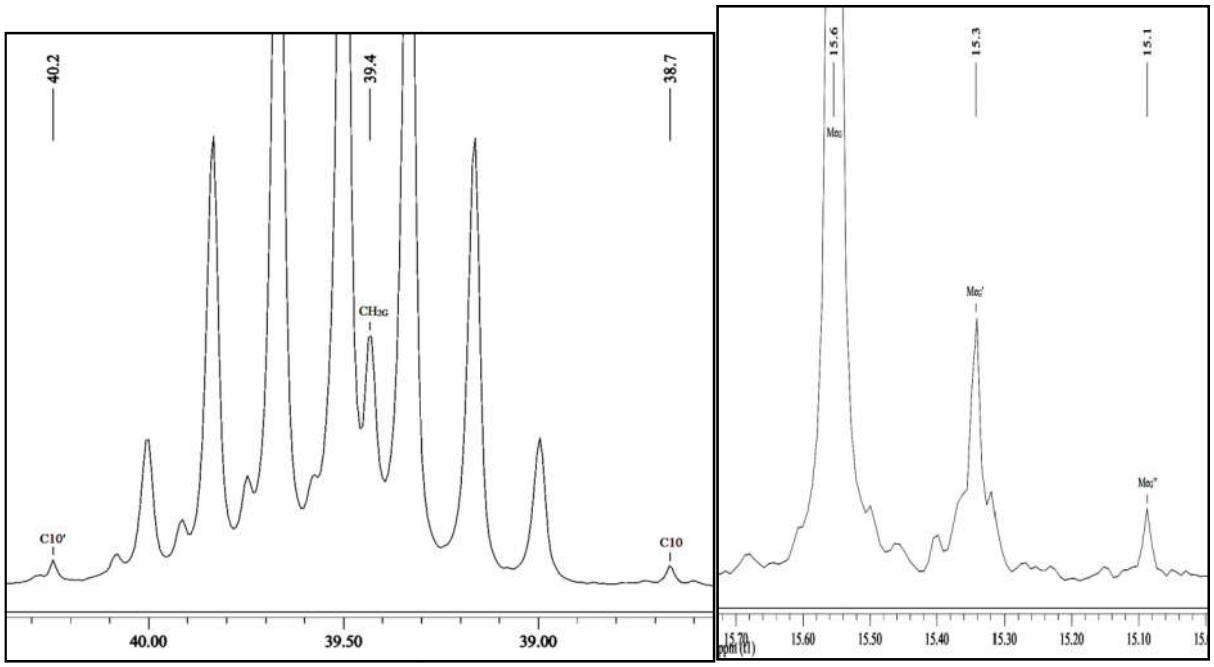


Fig. S33 ^{13}C NMR data of aliphatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

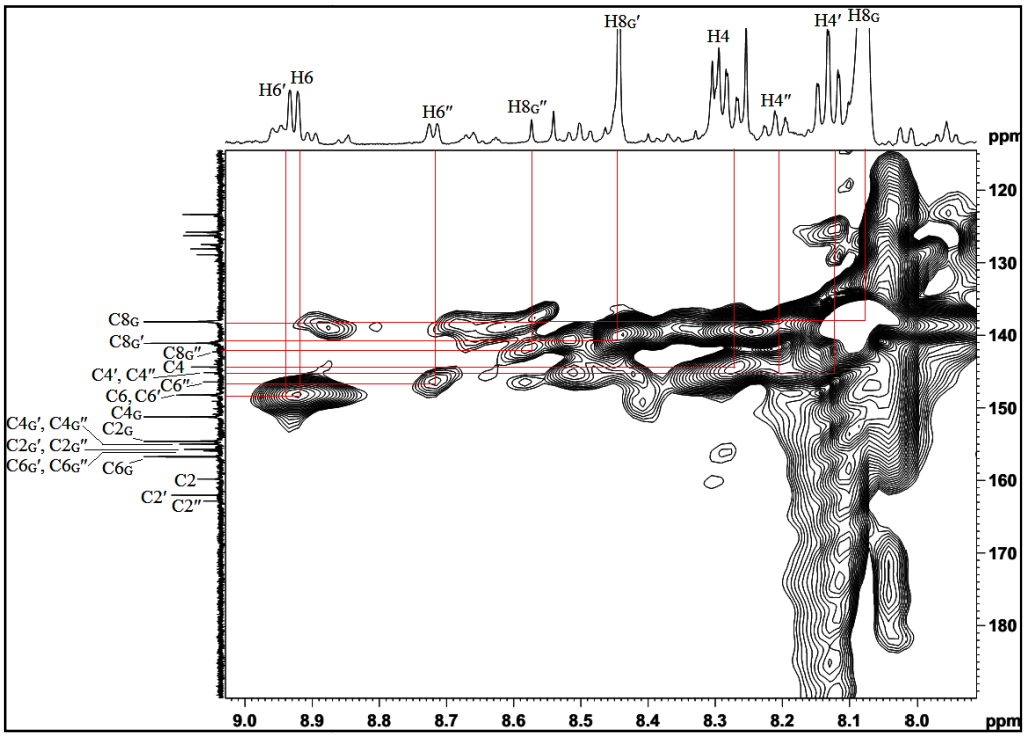


Fig. S34 HMQC data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

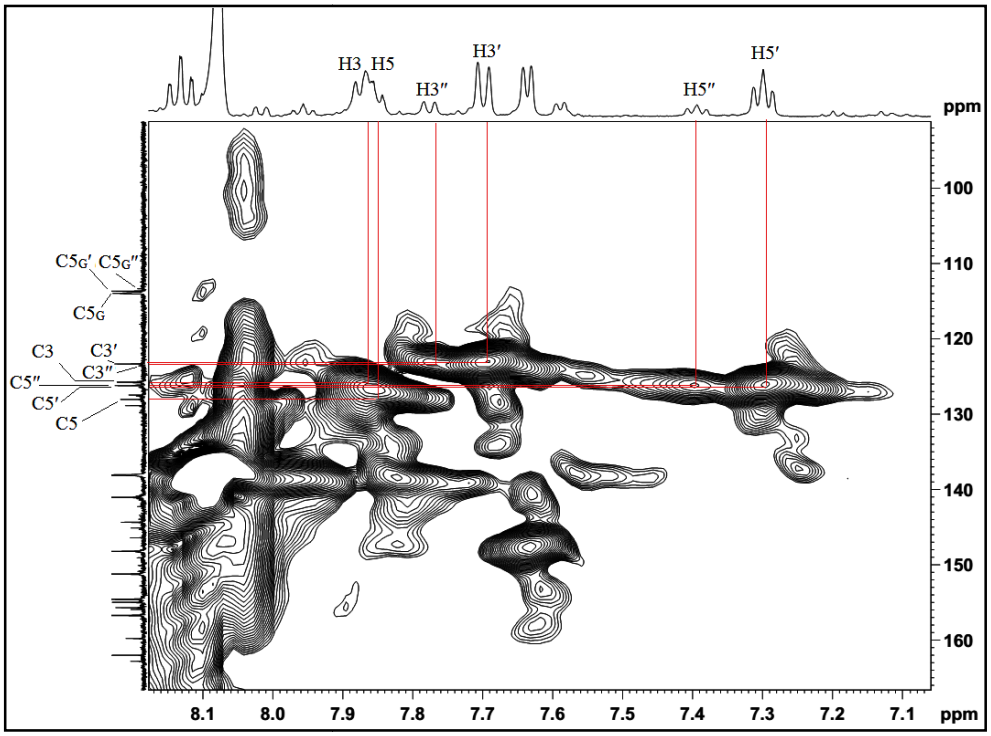


Fig. S35 HMQC data of aromatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

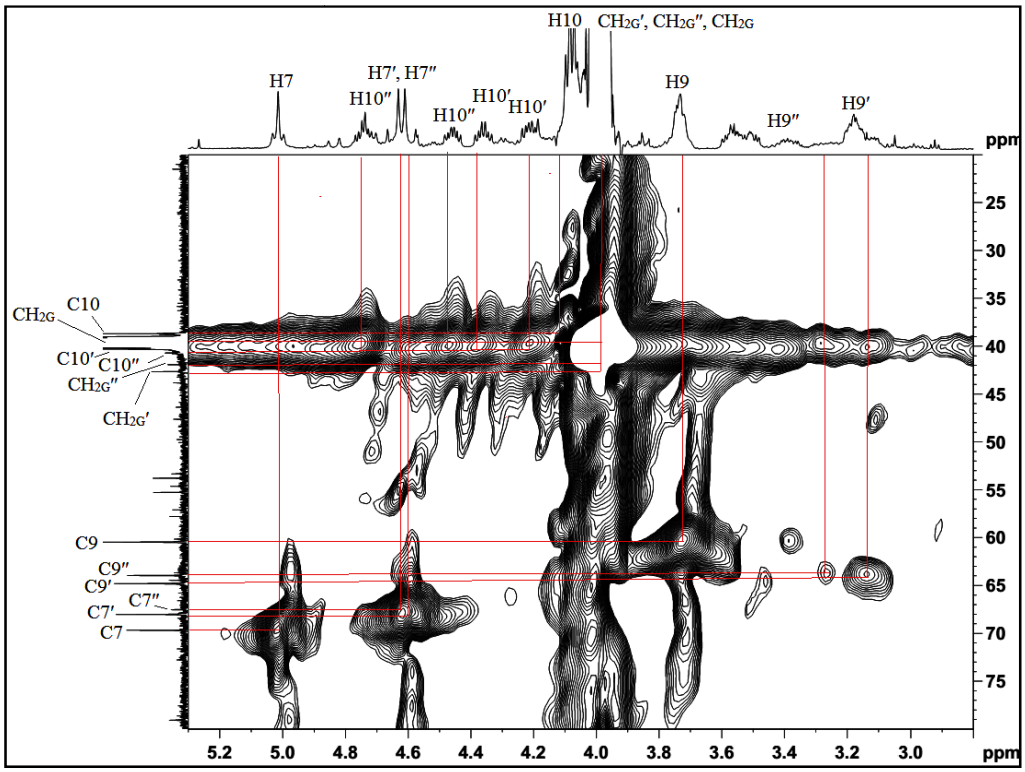


Fig. S36 HMQC data of aliphatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

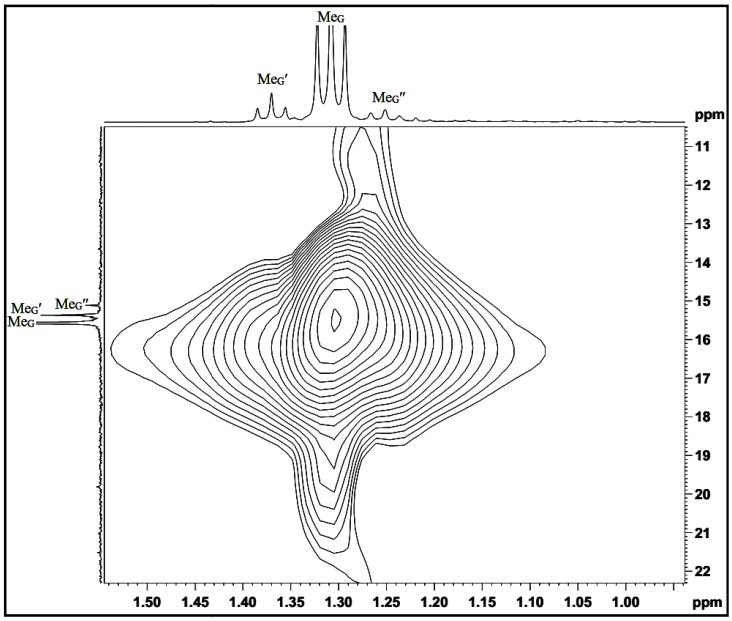


Fig. S37 HMQC data of aliphatic region during the binding kinetics study of **1a** (15 mM) with 9-EtG (90 mM) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR after 10 d.

Table S4A Selected ^{1}H NMR signals upon 9-EtG with **1a** at the starting point (t = 10 min) and after 10 d supporting various speciation.

Selected NMR singals	**Reaction mixture**							
	Starting point		**After 10 days**					
			Unreacted **1a** & 9-EtG		Adduct 1 (**3d**)		Adduct 2 (**3e**)	
	Proton no.	Chemical shifts	Proton no.	Chemical shifts	Proton no.	Chemical shifts	Proton no.	Chemical shifts
Metal complex	H6	8.94 (d)	H6	8.94 (d)	H6′	8.96 (d)	H6″	8.73 (d)
	H4	8.30 (td)	H4	8.30 (td)	H4′	8.15 (td)	H4″	8.23 (td)
	H3	7.88 (m)	H3	7.88 (m)	H3′	7.71 (d)	H3″	7.78 (d)
	H5	7.86 (m)	H5	7.86 (m)	H5′	7.31 (t)	H5″	7.41 (t)
	H7	5.01 (s)	H7	5.01 (s)	H7′	4.61 (s)	H7″	4.63 (s)
	H10	4.10 (t)	H10	4.10 (t)	H10′	4.39 (m), 4.24 (m)	H10″	4.77 (m), 4.48 (m)
	H9	3.75 (t)	H9	3.75 (t)	H9′	3.20 (m)	H9″	3.41 (m)
9-EtG	$H8_G$	7.70 (s)	$H8_G$	8.08 (s)	$H8_G$′	8.44 (s)	$H8_G$″	8.57 (s)
	NH_{2G}	6.41 (s)	NH_{2G}	6.61 (s)	NH_{2G}′	5.58 (s)	NH_{2G}″	5.64 (s)
	CH_{2G}	3.95 (m)	CH_{2G}	Merged with water	CH_{2G}′	Merged with water	CH_{2G}″	Merged with water
	Me_G	1.29 (t)	Me_G	1.32 (t)	Me_G′	1.38 (t)	Me_G″	1.27 (t)

Table S4B Selected ^{13}C NMR signals upon 9-EtG with **1a** at the starting point (t = 10 min) and after 10 d supporting various speciation.

Selected NMR singals	**Reaction mixture**							
	Starting point		After 10 days					
			Unreacted **1a** & 9-EtG		Adduct 1 (**3d**)		Adduct 2 (**3e**)	
	Carbon no.	Chemical shifts	Carbon no.	Chemical shifts	Carbon no.	Chemical shifts	Carbon no.	Chemical shifts
Metal complex	C2	159.7	C2	159.7	C2′	162.0	C2″	162.8
	C6	148.16	C6	148.16	C6′	148.18	C6″	146.4
	C4	144.3	C4	144.3	C4′	144.08	C4″	144.05
	C5	128.0	C5	128.0	C5′	126.2	C5″	126.1
	C3	125.7	C3	125.7	C3′	123.3	C3″	123.6
	C7	69.6	C7	69.6	C7′	68.0	C7″	67.5
	C9	60.4	C9	60.4	C9′	64.8	C9″	63.9
	C10	38.6	C10	38.7	C10′	40.2	C10″	40.7
9-EtG	$C6_G$	157.8	$C6_G$	156.7	$C6_G$′	155.9	$C6_G$″	Merged with $C6_G$′
	$C2_G$	153.9	$C2_G$	154.5	$C2_G$′	155.6	$C2_G$″	Merged with $C2_G$′
	$C4_G$	151.6	$C4_G$	151.2	$C4_G$′	154.9	$C4_G$″	Merged with $C4_G$′
	$C8_G$	138.4	$C8_G$	138.1	$C8_G$′	141.0	$C8_G$″	142.2
	$C5_G$	116.8	$C5_G$	113.9	$C5_G$′	113.7	$C5_G$″	113.3
	CH_{2G}	38.7	CH_{2G}	39.4	CH_{2G}′	42.6	CH_{2G}″	41.9
	Me_G	15.9	Me_G	15.6	Me_G′	15.3	Me_G″	15.1

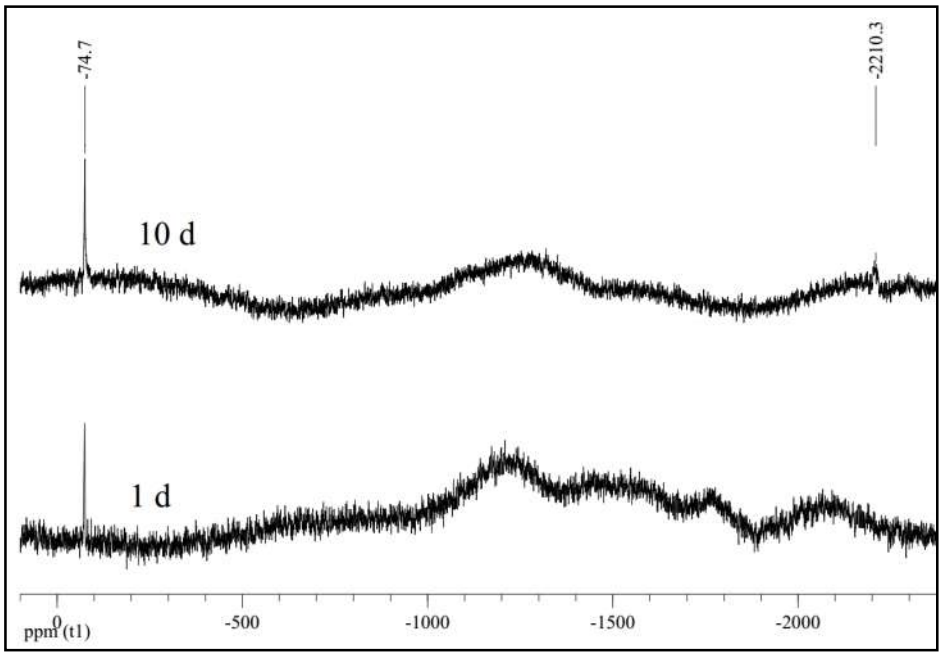


Fig. S38 Stack plot of binding kinetics study of **1a** with 9-EtG (1:6) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{195}Pt NMR. The data taken after 1 d shows no extra peak has generated except free complex (**1a**) peak at −74.7 ppm. After 10 d we find signals of 9-EtG bound complexes **3d** & **3e** at *ca.* −2210 ppm, but they are not well resolved.

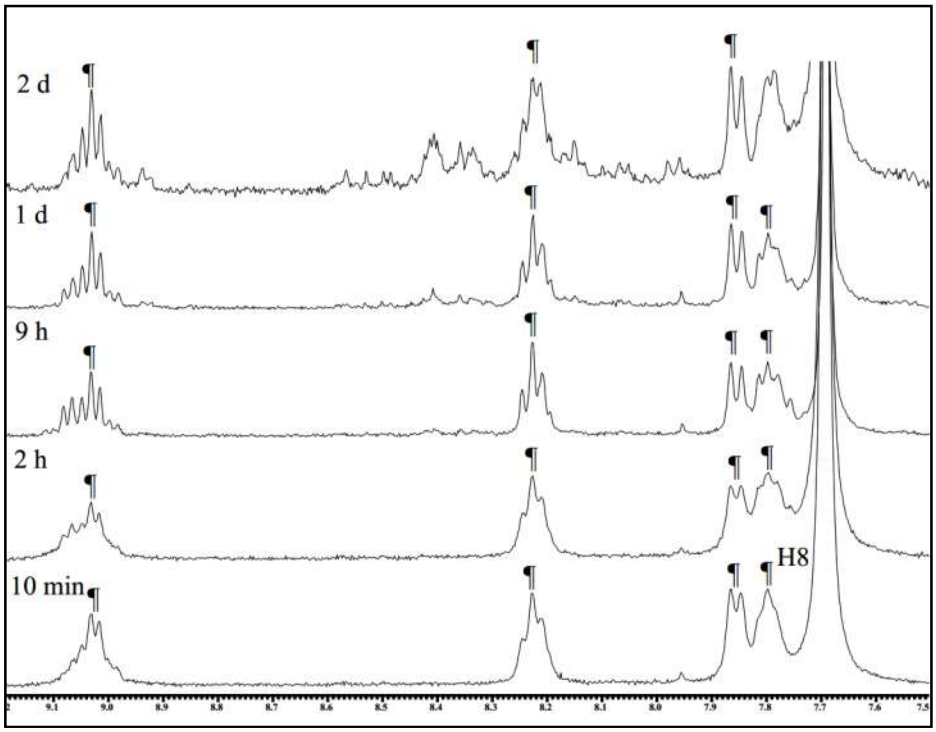


Fig. S39 Stack plot of 9-EtG binding study with **2a** by ^{1}H NMR in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture. ¶ indicates unchanged **2a**.

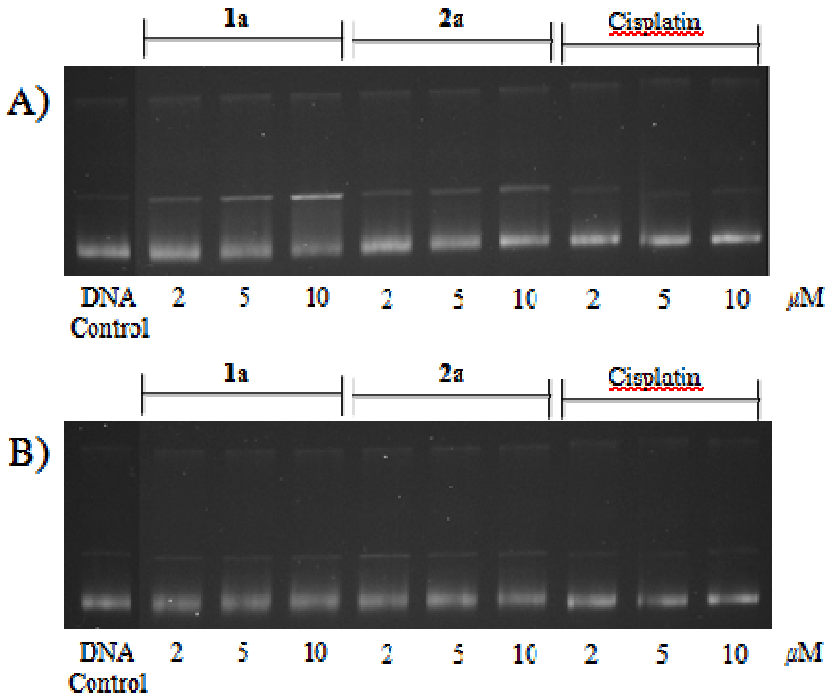


Fig. S40 Agarose gel electrophoresis of pUC19 treated with various concentrations (2, 5 & 10 μM) of **1a**, **2a** & cisplatin respectively A) without, B) with 10 equivalent of GSH for 24 h at 37 °C.

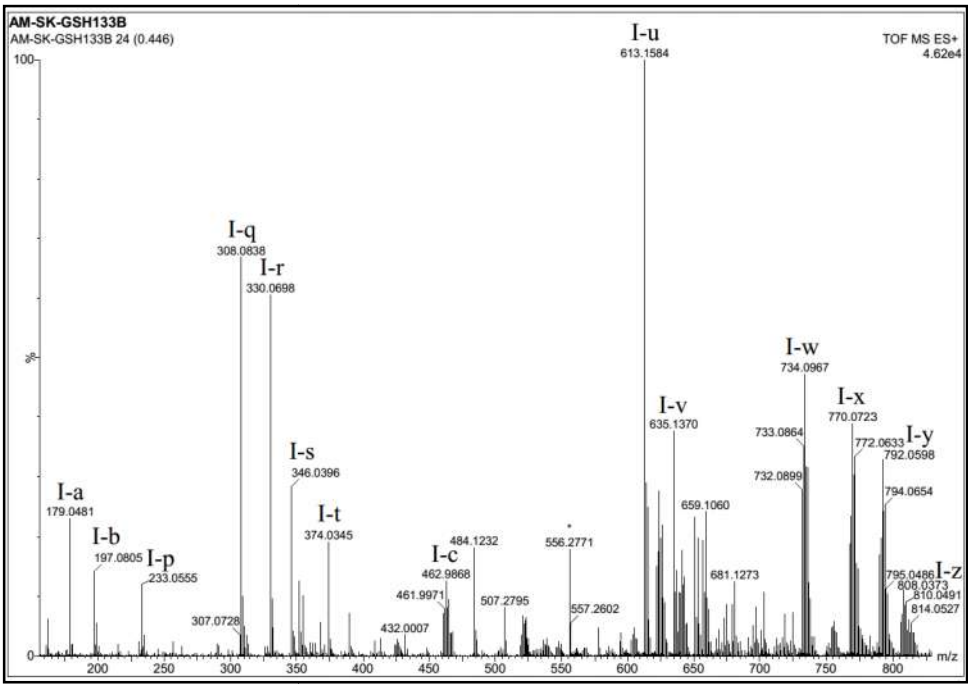


Fig. S41 ESI-MS speciation recorded during monitoring of the GSH binding study with **1a** by ^{1}H NMR after 3 h in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

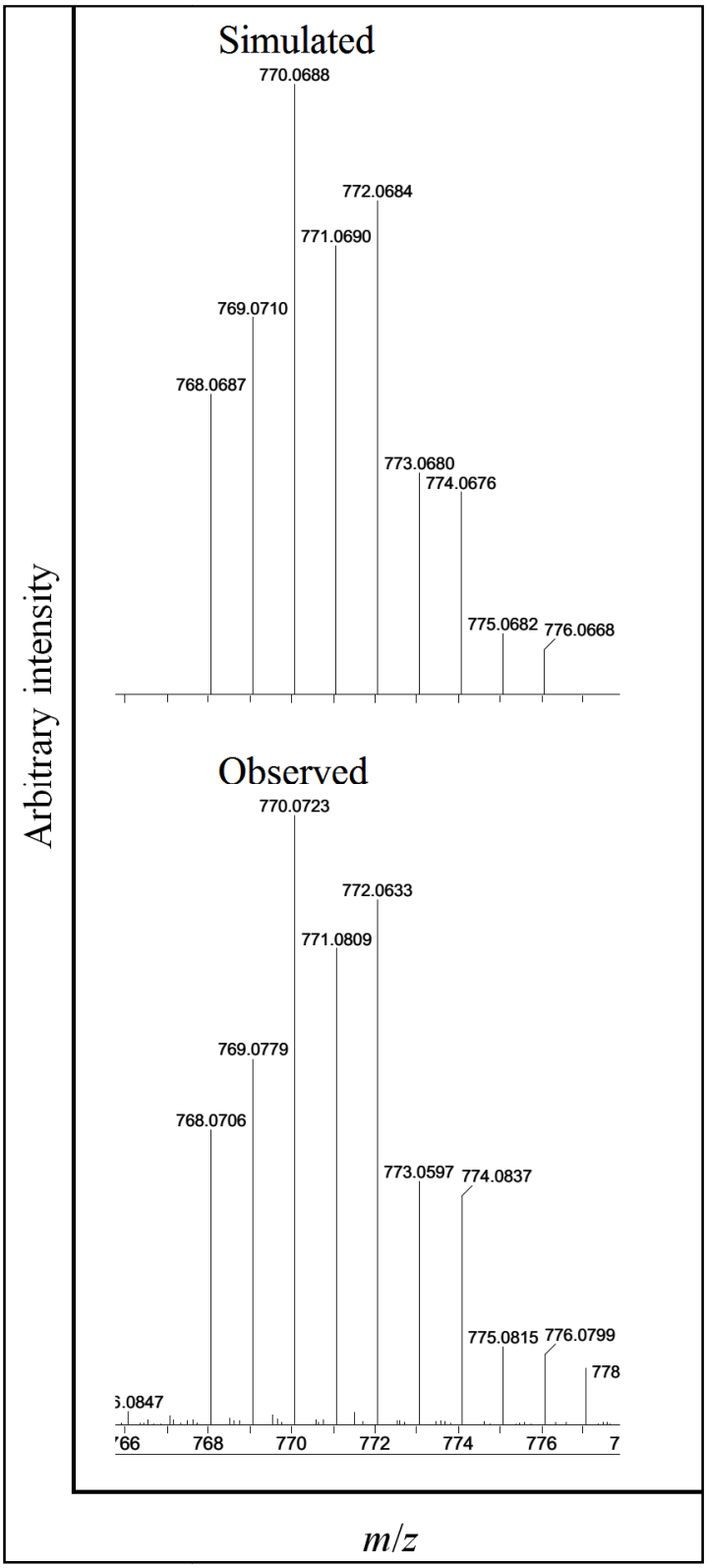


Fig. S42 Observed and simulated isotopic pattern of [**3c** (Scheme 3) + H^+]$^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^{1}H NMR after 3 h.

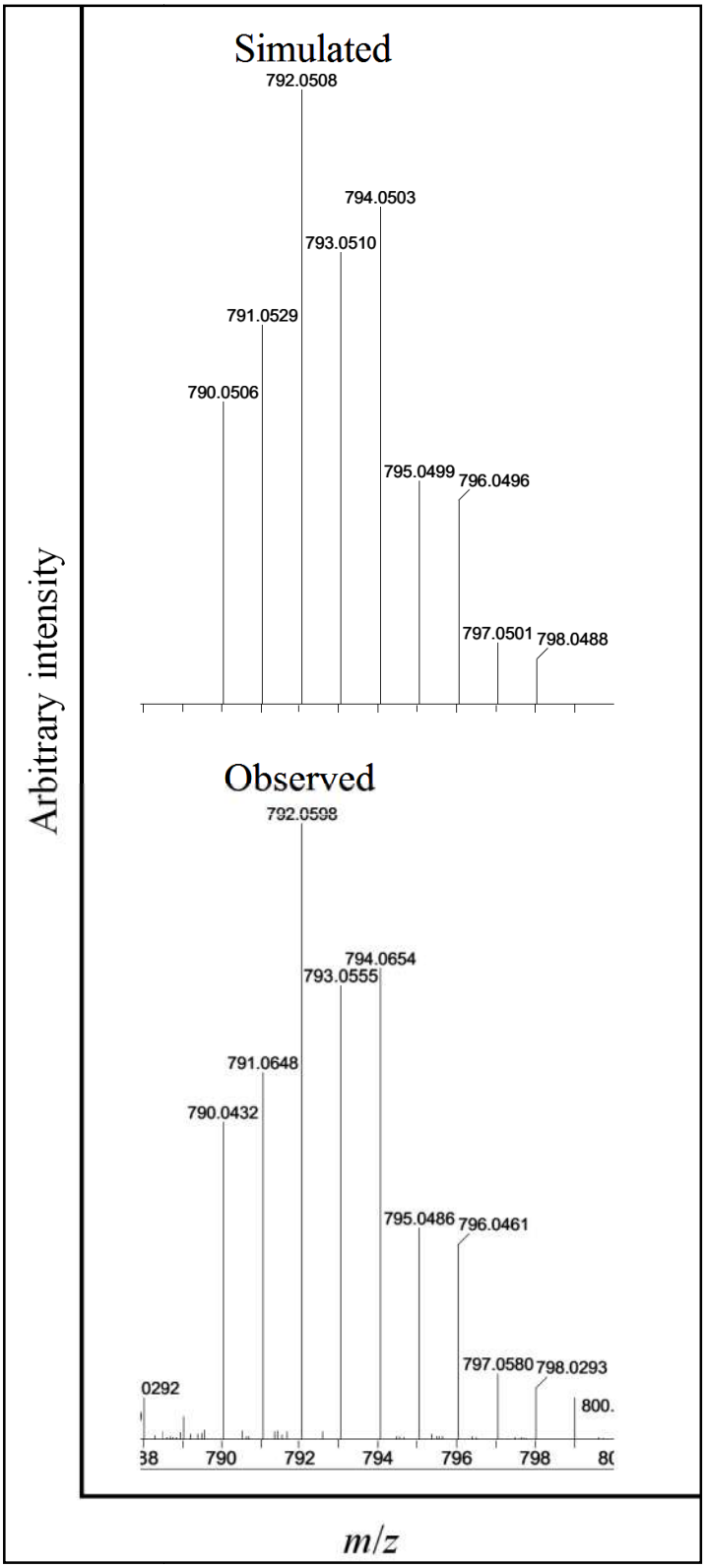


Fig. S43 Observed and simulated isotopic pattern of [**3c** (Scheme 3) + Na^{+}]$^{+}$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^{1}H NMR after 3 h.

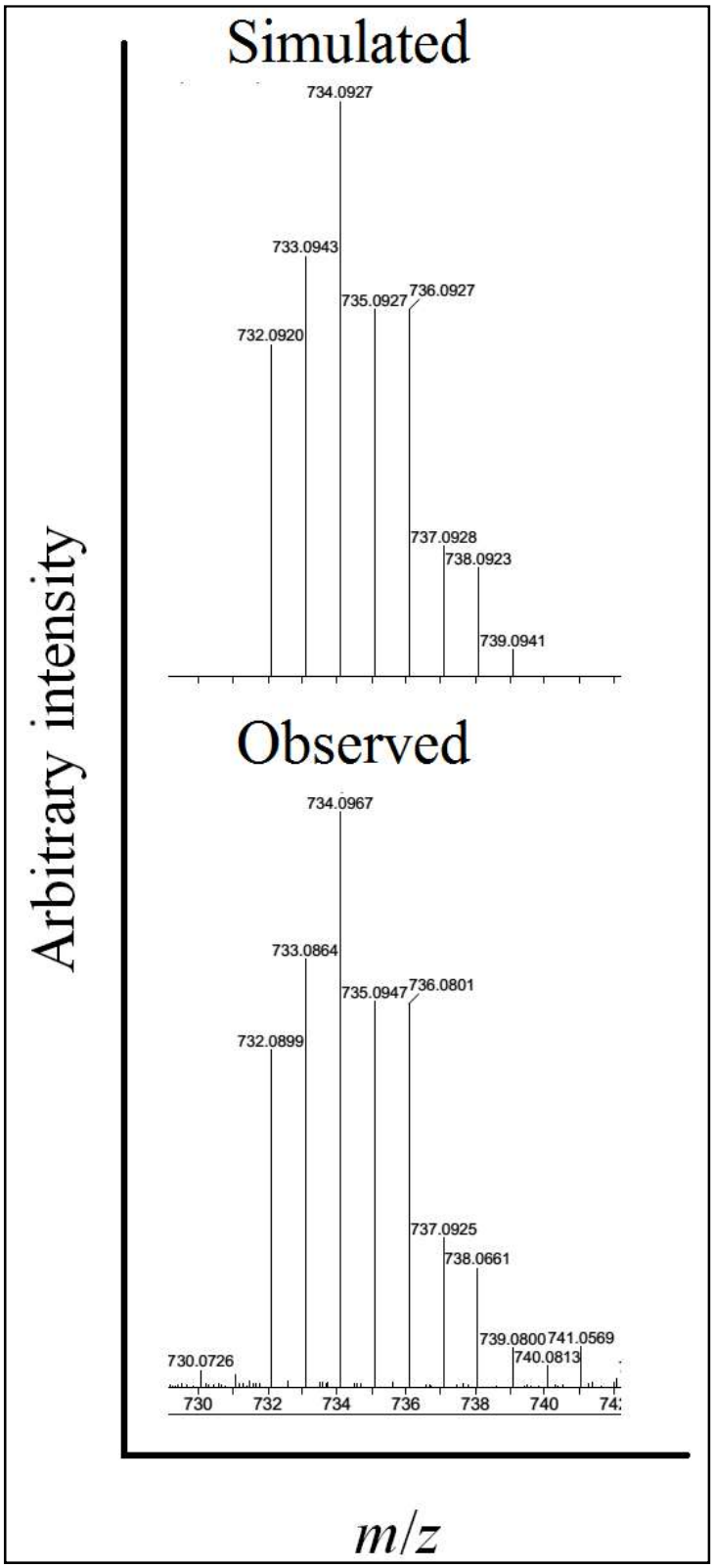


Fig. S44 Observed and simulated isotopic pattern of $[(\mathbf{3} - 2Cl^-) + (GSH - H^+)]^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^1H NMR after 3 h.

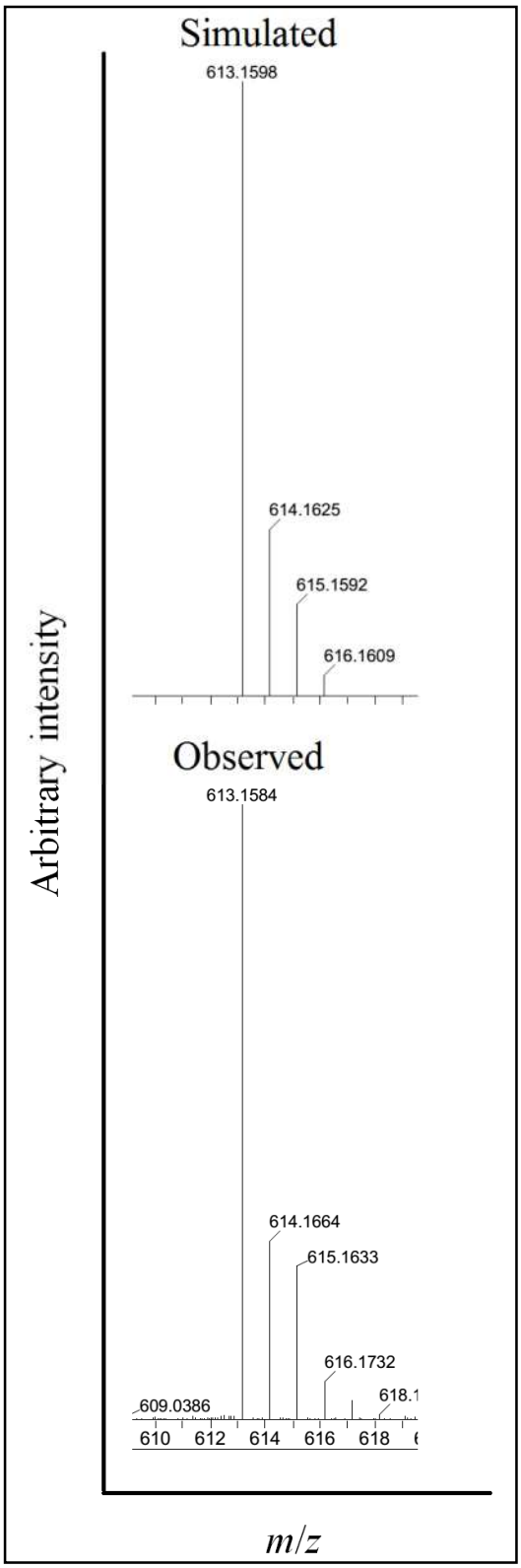


Fig. S45 Observed and simulated isotopic pattern of $[GSSG + H^+]^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^1H NMR after 3 h.

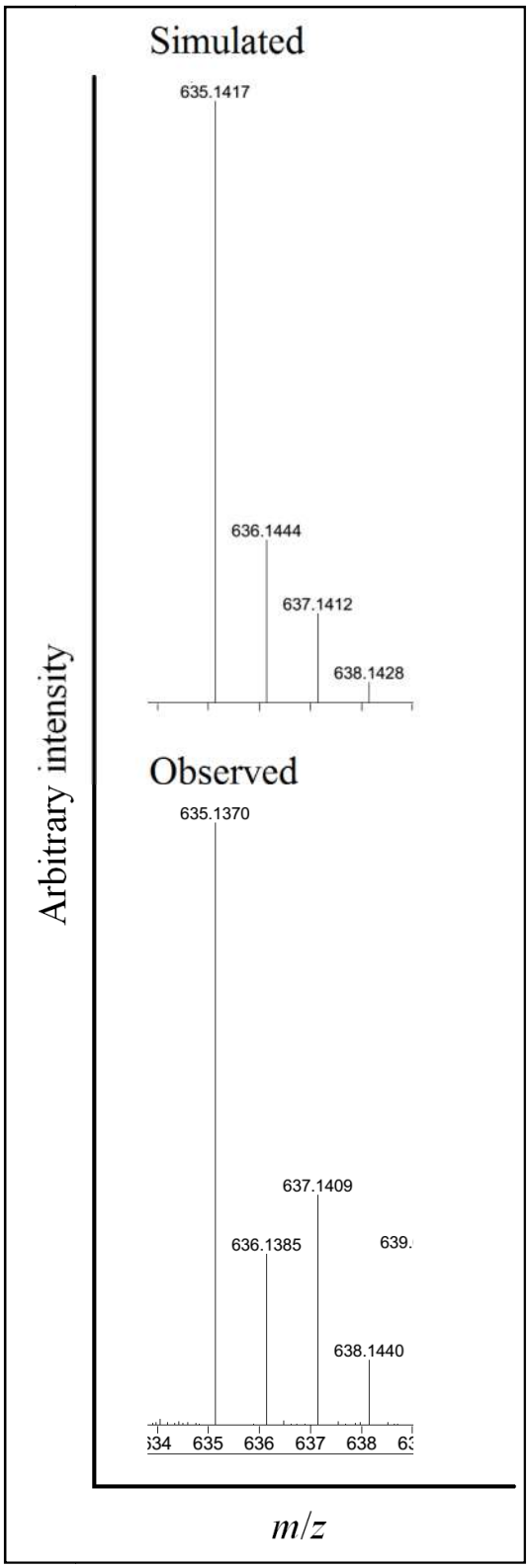


Fig. S46 Observed and simulated isotopic pattern of $[GSSG + Na^+]^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^1H NMR after 3 h.

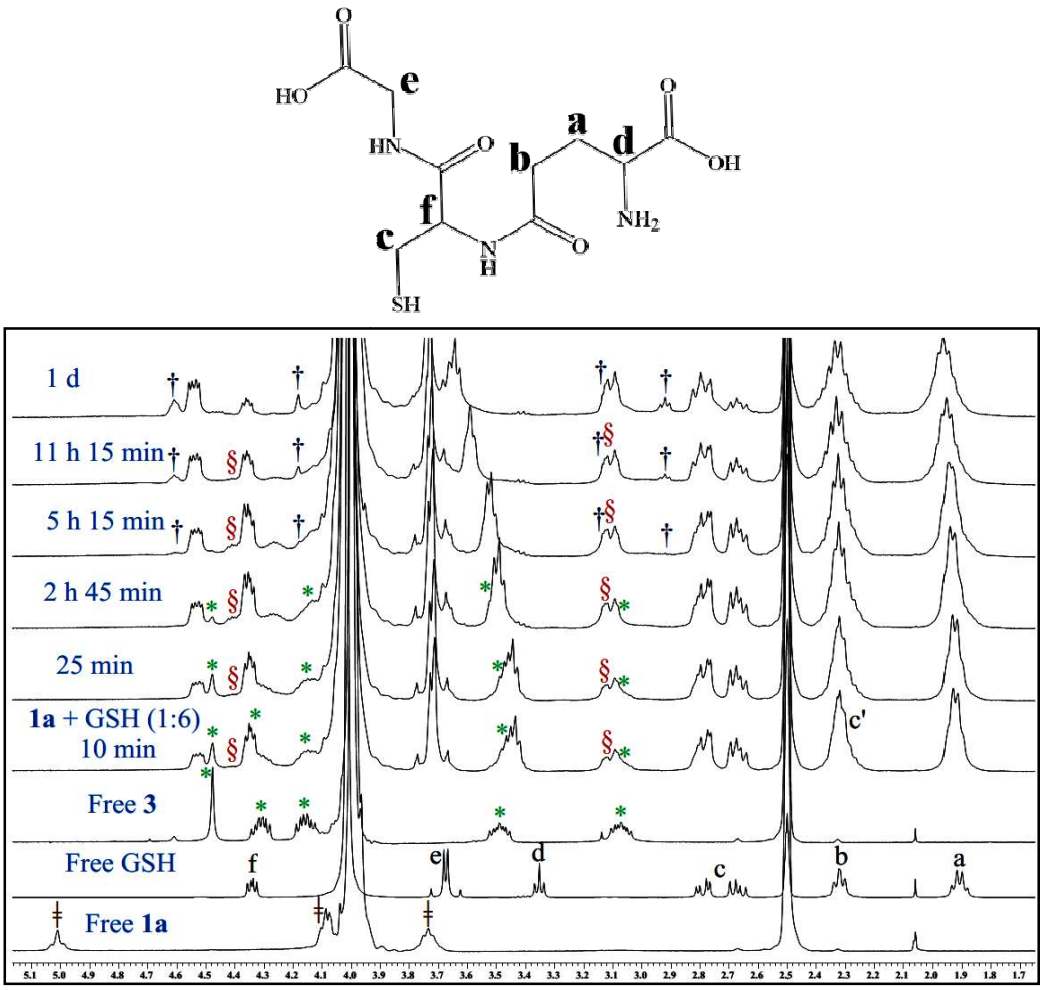


Fig. S47 Stack plot of aliphatic region during the binding kinetics study of **1a** with GSH in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR, where ǂ, *, § and † indicate the signals of intact complex **1a**, **3**, GSH bound complex **3c** and aziridinium ion **3b** respectively. 'c'proton of free GSH shifted upfield (c′) in bound complex **3c**.

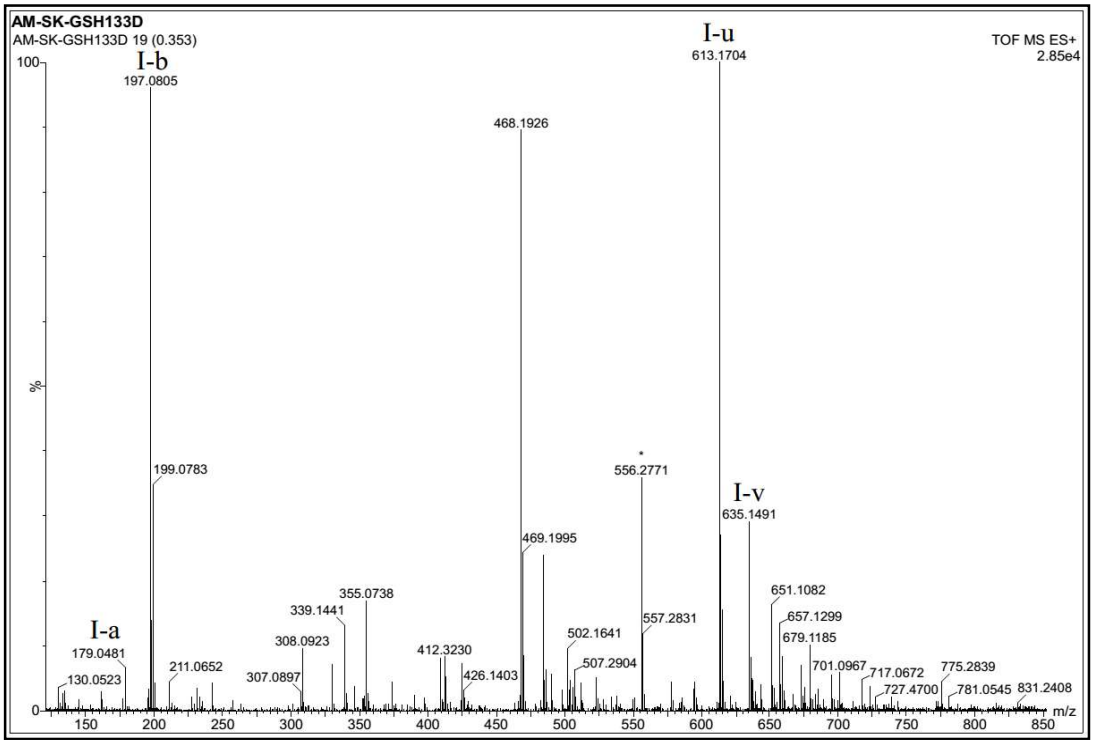


Fig. S48 ESI-MS speciation recorded during monitoring of the GSH binding study with **1a** by ^{1}H NMR after 1 d in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 mixture.

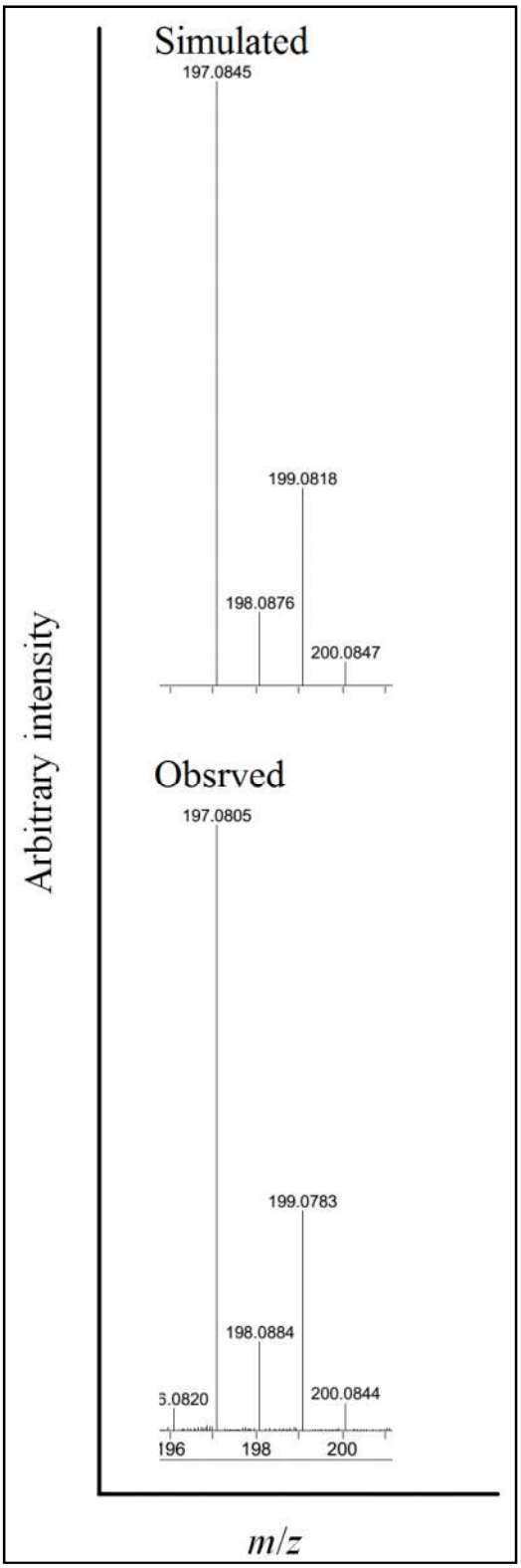


Fig. S49 Observed and simulated isotopic pattern of [**3b** (Scheme 3)] found in ESI-MS during monitoring of the GSH binding study with **1a** by ^{1}H NMR after 1 d.

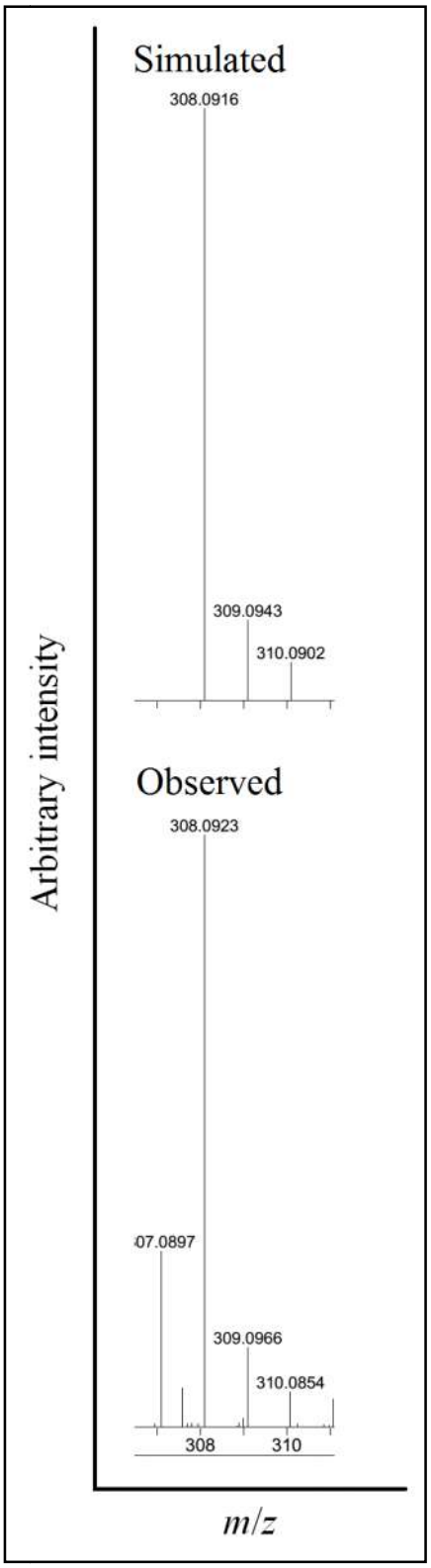


Fig. S50 Observed and simulated isotopic pattern of $[GSH + H^+]^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^1H NMR after 1 d.

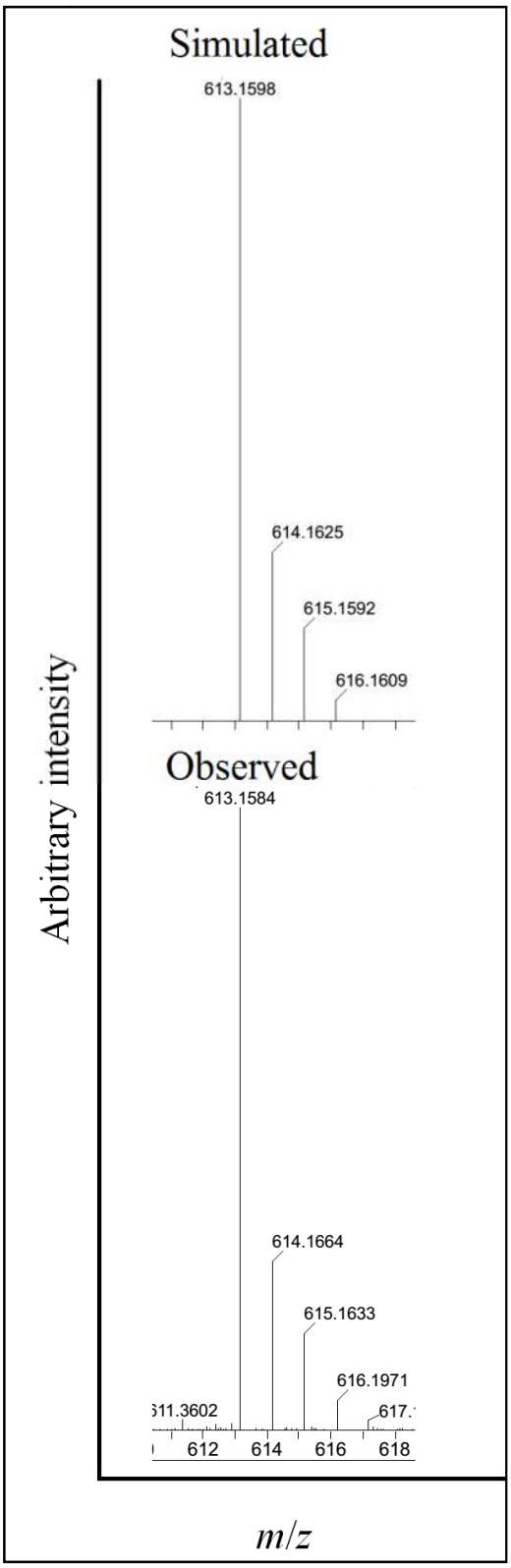


Fig. S51 Observed and simulated isotopic pattern of $[GSSG + H^+]^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^1H NMR after 1 d.

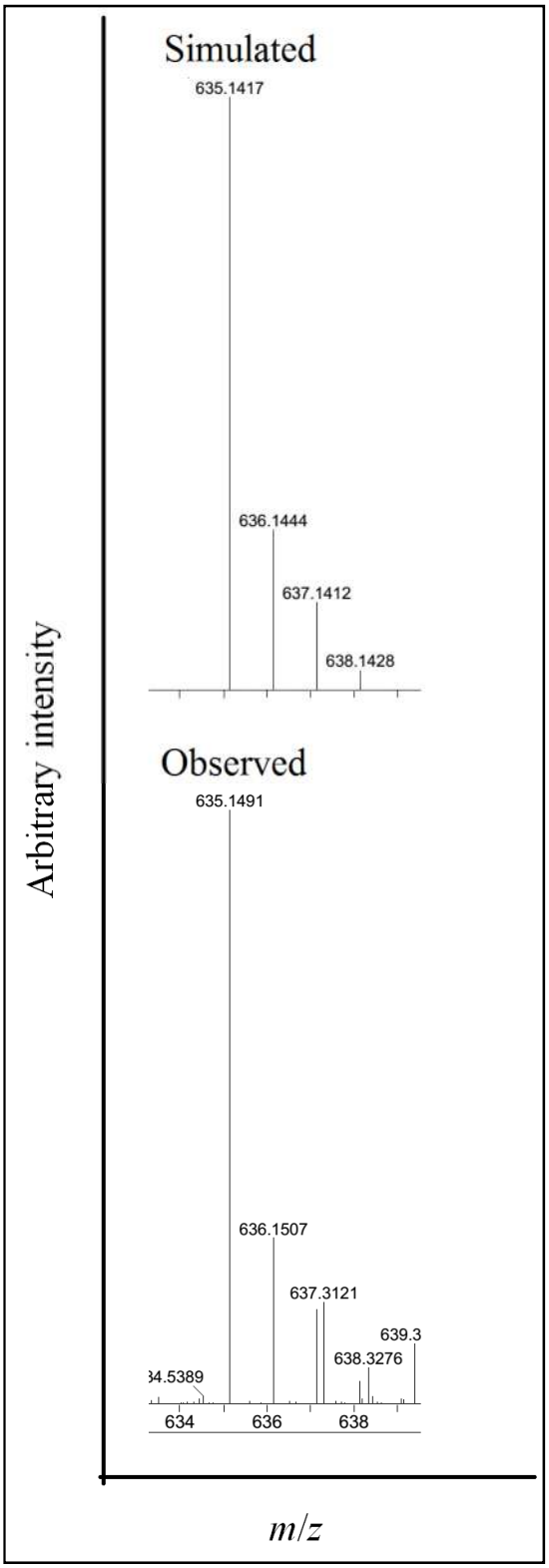


Fig. S52 Observed and simulated isotopic pattern of $[GSSG + Na^+]^+$ found in ESI-MS during monitoring of the GSH binding study with **1a** by ^1H NMR after 1 d.

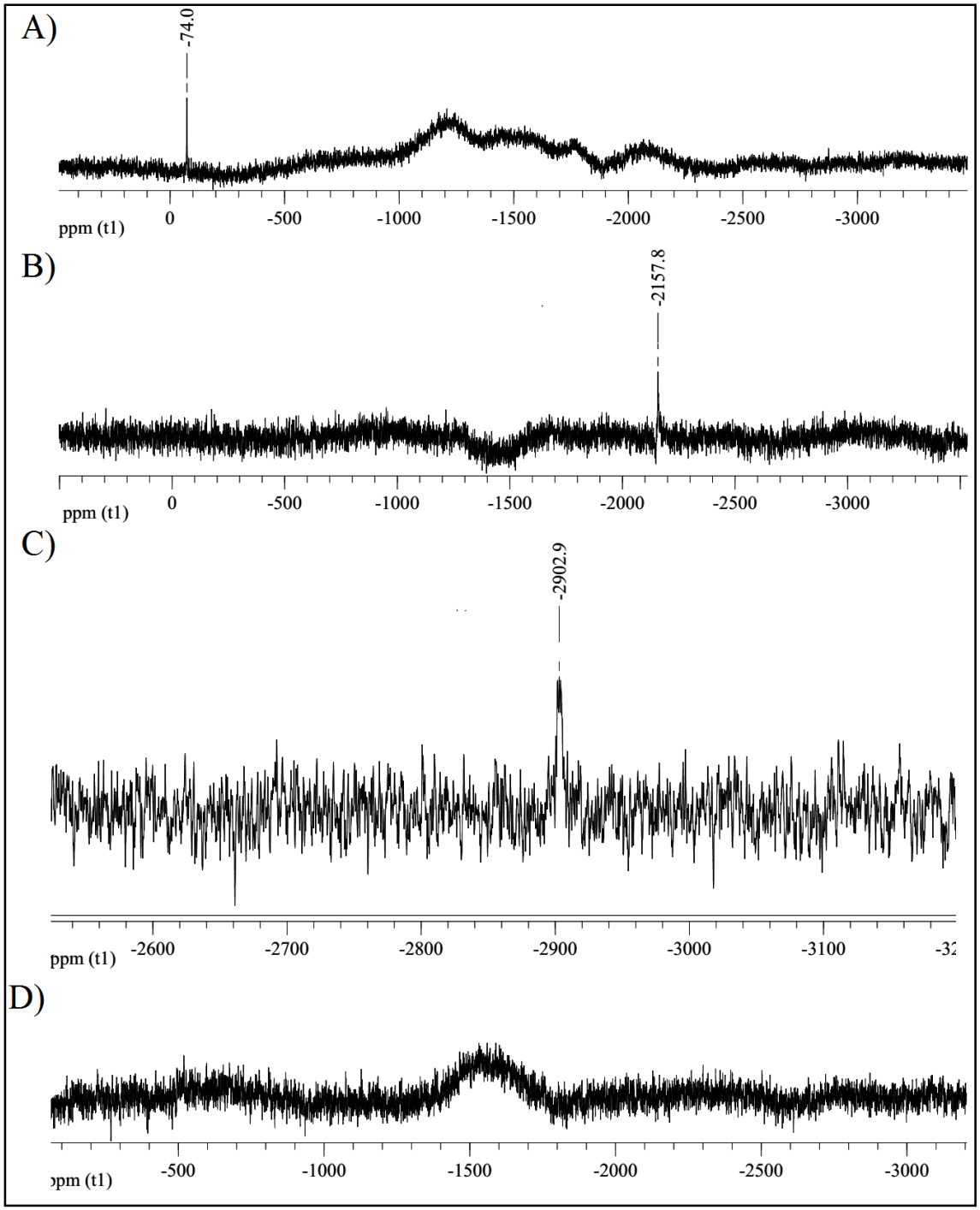


Fig. S53 Stack plot of binding kinetics study of **1a** with GSH (1:6) in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{195}Pt NMR. A) Before GSH addition, *i.e.*, free **1a**, B) after GSH addition, *i.e.*, reduced **1a** (**3**) C) after 4 h of GSH addition, *i.e.*, GSH bound **3** (**3c**) and D) after 1d where the GSH adduct (**3c**) peak vanishes.

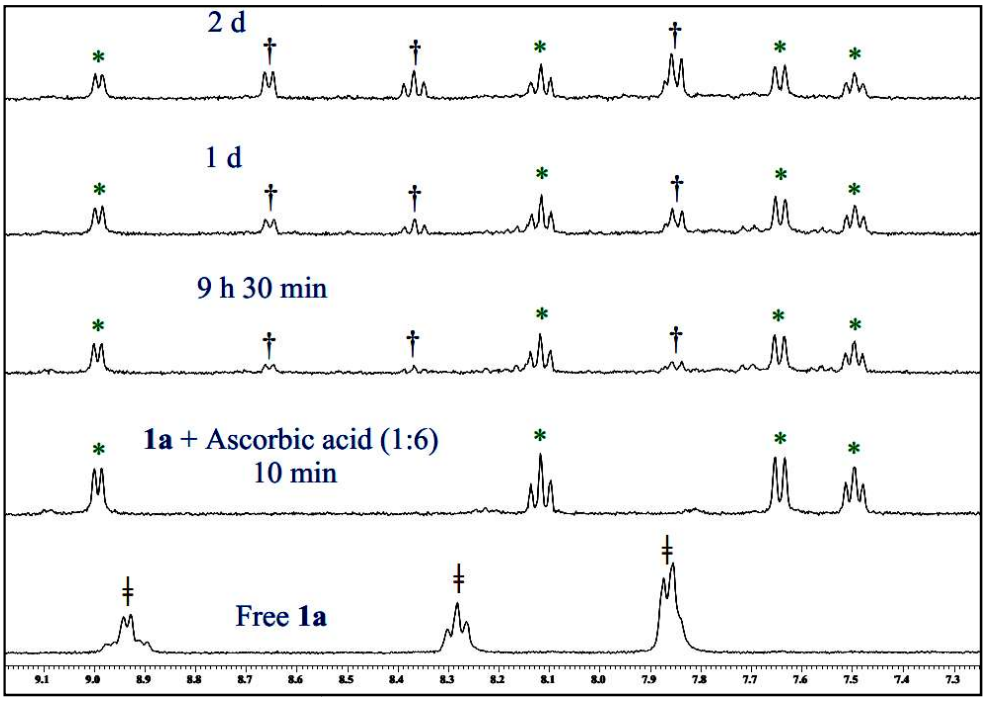


Fig. S54 Stack plot of aromatic region during the binding kinetics study of **1a** with ascorbic acid in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR, where ǂ, * and † indicate the signals of intact complex **1a**, **3** and aziridinium ion **3b** respectively.

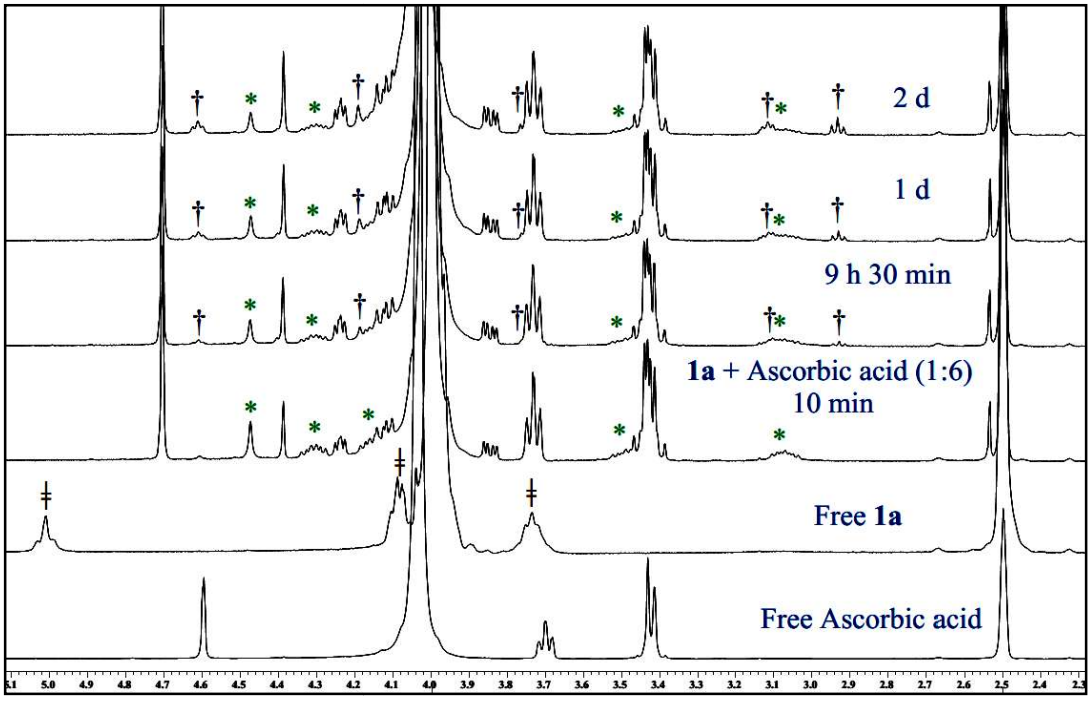


Fig. S55 Stack plot of aliphatic region during the binding kinetics study of **1a** with ascorbic acid in 20% PBS (pD 7.4, prepared in D_2O) – DMSO-d_6 by ^{1}H NMR, where ǂ, * and † indicate the signals of intact complex **1a**, **3** and aziridinium ion **3b** respectively.

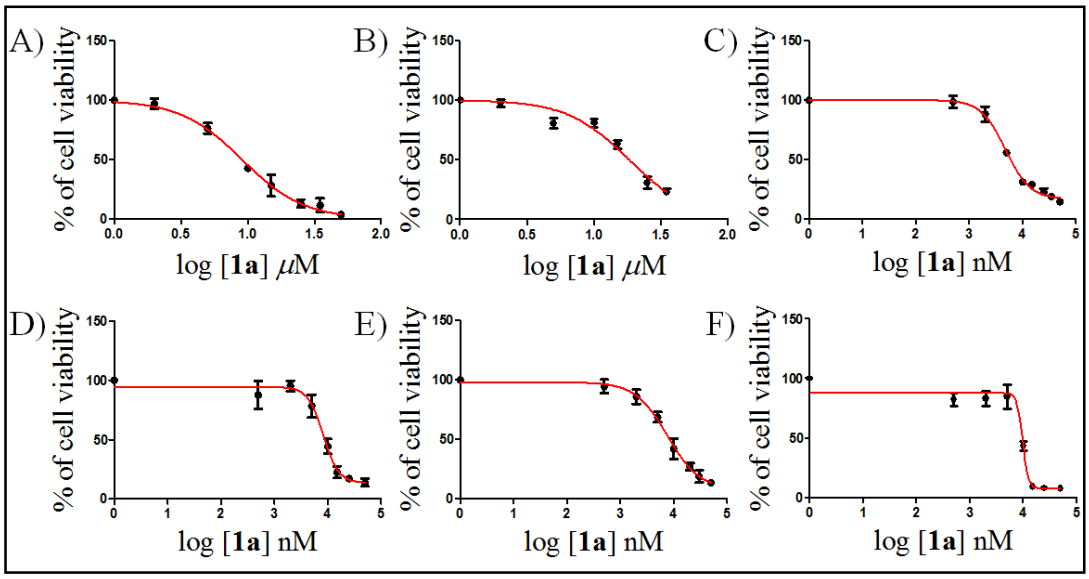


Fig. S56 Plots of cell viability (%) *vs*. log of concentrations of **1a** against A) MCF-7, B) A549, C) MIA PaCa-2, D) HepG2, E) HEK293 and NIH 3T3 cell lines after incubation for 48 h determined from MTT assays under normoxic condition. The plots provided are for one independent experiment out of the three independent experiments.

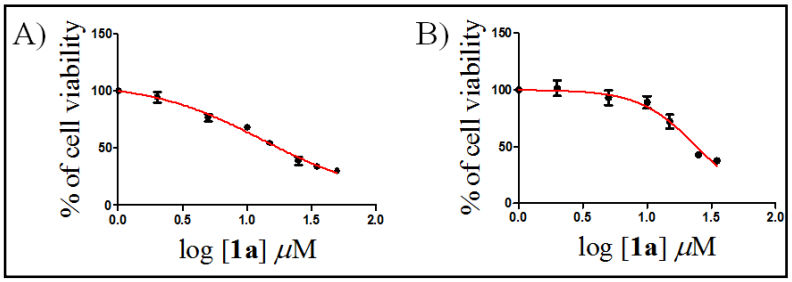


Fig. S57 Plots of cell viability (%) *vs*. log of concentrations of **1a** against A) MCF-7 and B) A549 cell lines after incubation for 48 h determined from MTT assays under normoxic condition in presence of 400 μM GSH. The plots provided are for one independent experiment out of the three independent experiments.

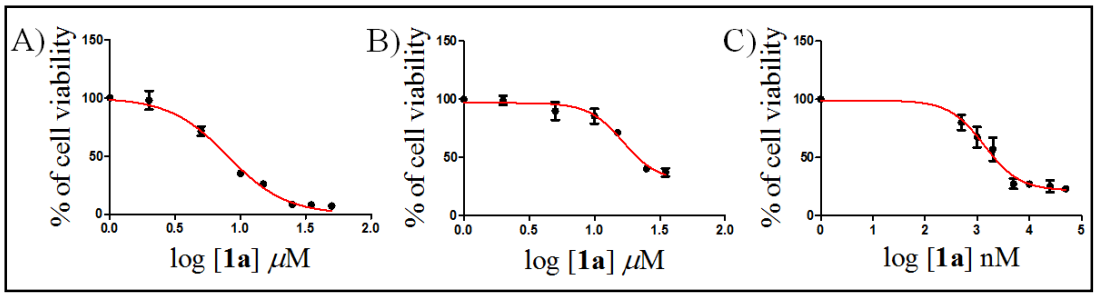


Fig. S58 Plots of cell viability (%) *vs*. log of concentrations of **1a** against A) MCF-7, B) A549 and MIA PaCa-2 cell lines after incubation for 48 h determined from MTT assays under hypoxic condition. The plots provided are for one independent experiment out of the three independent experiments.

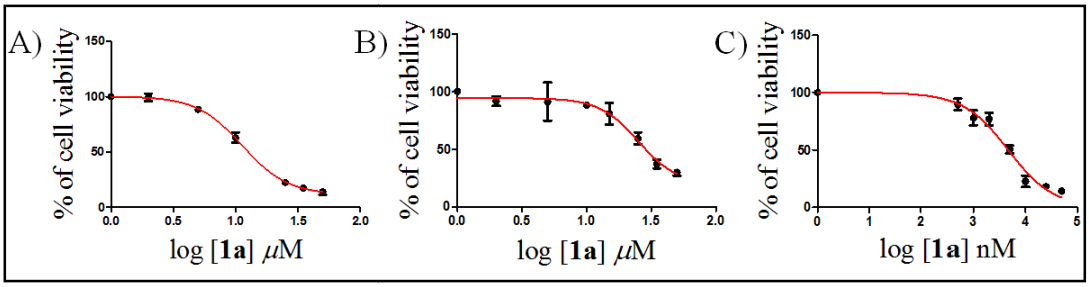


Fig. S59 Plots of cell viability (%) *vs*. log of concentrations of **1a** against A) MCF-7, B) A549 and MIA PaCa-2 cell lines after incubation for 48 h determined from MTT assays under hypoxic condition in presence of 400 μM GSH. The plots provided are for one independent experiment out of the three independent experiments.

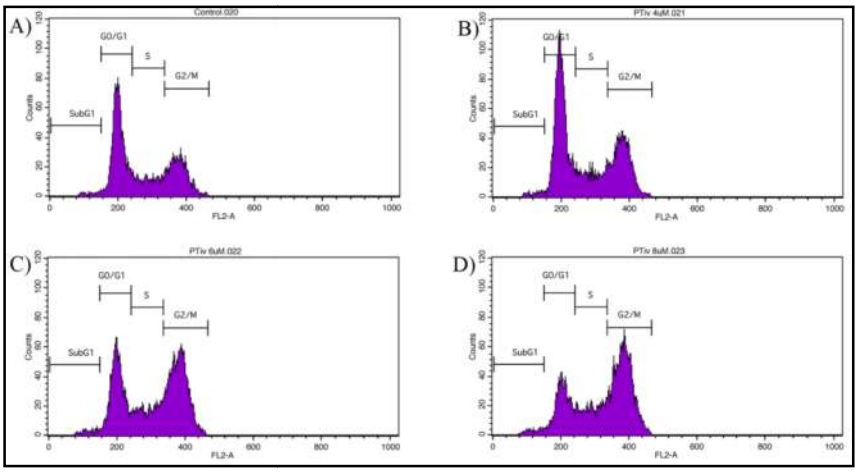


Fig. S60 Cell cycle arrest of MCF-7 treated with **1a** for 24 h. (A) DMSO control, (B) 4 μM, (C) 6 μM and D) 8 μM of **1a** treated cells. The figure represents one independent experiment.

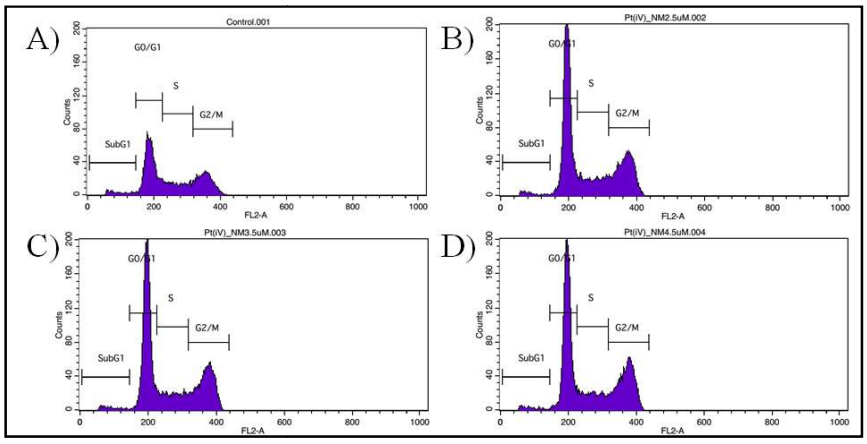


Fig. S61 Cell cycle arrest of MIA PaCa-2 treated with **1a** for 24 h. (A) DMSO control, (B) 2.5 μM, (C) 3.5 μM and D) 4.5 μM of **1a** treated cells. The figure represents one independent experiment.

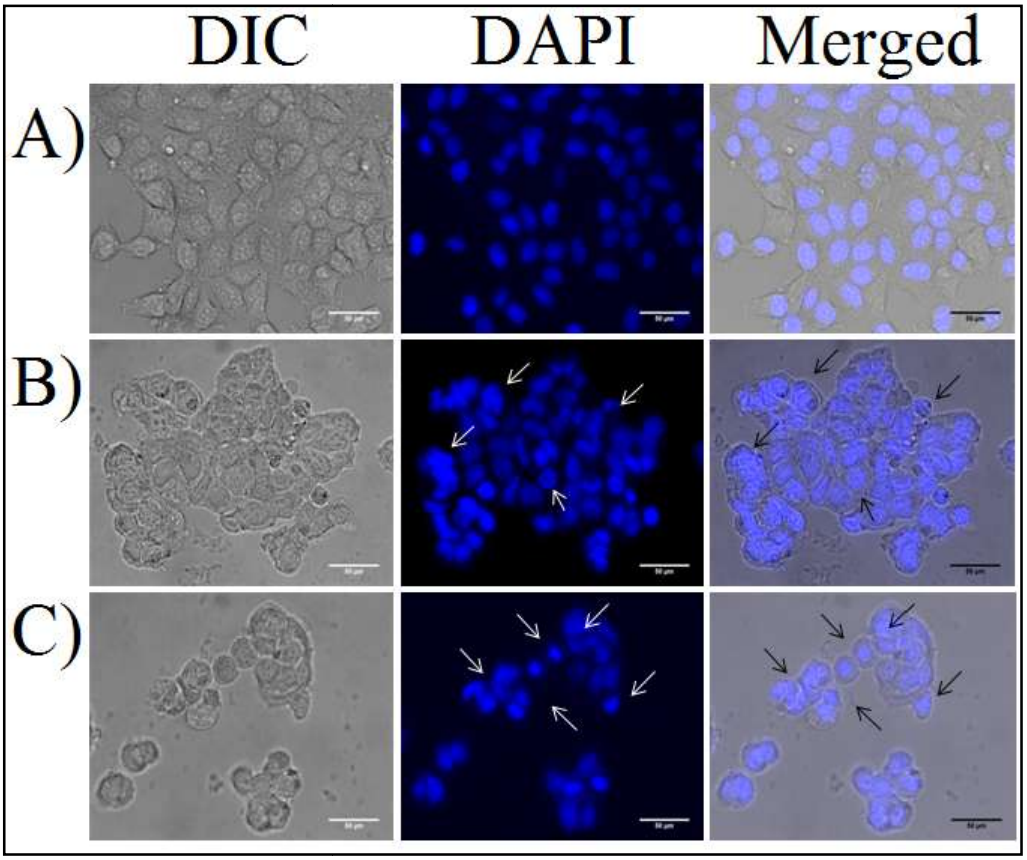


Fig. S62 Fluorescence microscopy image of MCF-7 cells after 24 h treatment with A) DMSO, B) 6 μM and C) 8 μM of **1a** respectively. Bright and dark arrows are pointing towards the nuclei with deformed morphology.

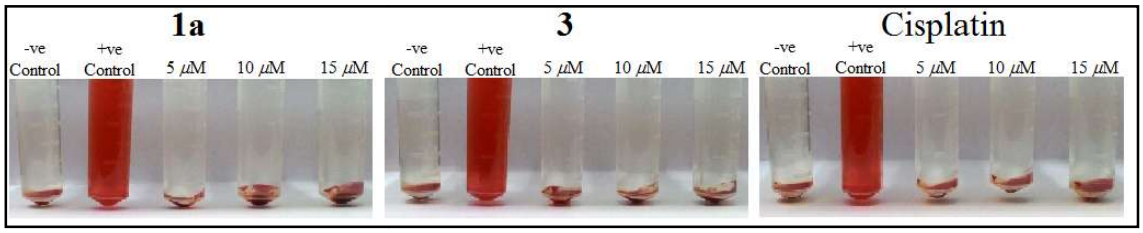


Fig. S63 Hemolysis of blood samples upon treatment with **1a**, **3** and cisplatin respectively.

Table S5 Hemolysis (%) data after 1 h of incubation with 5, 10 & 15 μM of **1a**, **3** & cisplatin

	5 μM	10 μM	15 μM
1a	0.53 ± 0.4	0.74 ± 0.6	2.04 ± 0.4
3	0.59 ± 0.4	0.52 ± 0.5	0.62 ± 1.2
Cisplatin	0.33 ± 0.1	0.36 ± 0.31	0.6 ± 0.78

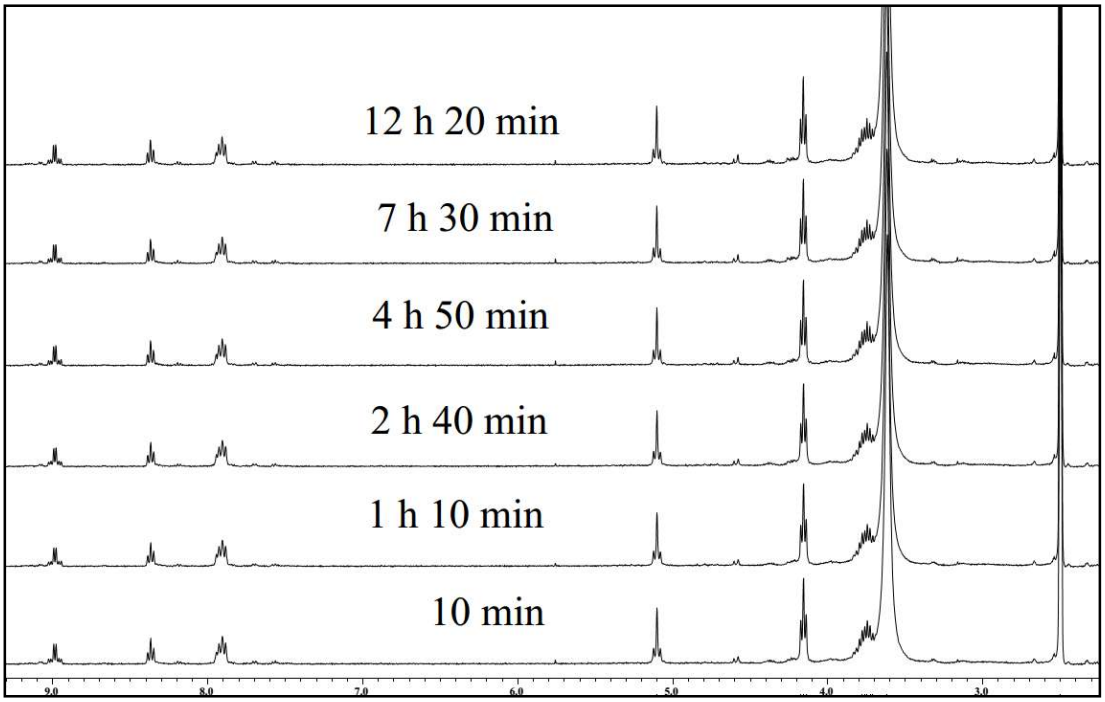


Fig. S64 Stack plot of stability kinetics study of complex **1a** in DMSO-d_6 by ^{1}H NMR.

Characterization data of complex 2: ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.06 (d, 1H, J = 6 Hz), 8.15 (td, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz, PyH4), 7.66 (d, 1H, J = 8 Hz, PyH3), 7.51 (t, 1H, J = 7 Hz, PyH5), 7.01 (br, 1H, NH), 4.79 (t, 1H, J = 5 Hz, OH), 4.40 (m, 1H, PyCH_2N), 4.25 (m, 1H, PyCH_2N), 3.79 (m, 1H, CH_2OH), 3.72 (m, 1H, CH_2OH), 3.02 (m, 1H, CH_2N), 2.84 (m, 1H, CH_2N); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.1 (PyC2), 147.2 (PyC6), 138.9 (PyC4), 124.2 (PyC5), 122.2 (PyC3), 60.6 (PyCH_2N), 58.2 (CH_2OH), 56.1 (CH_2N); ^{195}Pt NMR (107.5 MHz) δ: −2126.2; Elemental analysis calcd (%) for $C_8H_{12}Cl_2N_2OPt$: C 22.98, H 2.89, N 6.70, found: C 22.86, H 2.81, N 6.58; FT-IR (KBr, cm^{-1}): 3334 (br), 3194 (br), 1622 (s), 1566 (s), 1453 (m), 1228 (m), 676 (m).

Detailed experimental procedures

X-ray crystallography

X-ray diffraction measurement of single crystal was performed through Agilent supernova, Dual, Cu at zero, Eos diffractometer at 100(1) K using Mo X-ray source of wavelength 0.7107 Å. The processing of structure was conducted with the ShelXS[1] structure solution program using direct method and the refinement was accomplished with ShelXL[1] refinement package using least squares minimization. All the processing was executed through Olex2.[2] The non-hydrogen atoms were refined anisotropically by full matrix least-squares on F^2. Some important crystallographic refine parameters and crystal data are summarized in Table S1 and Table 1 respectively. The ORTEP diagram was processed through POV-ray with 50% probability level. Crystallographic data for the structure has been deposited at the Cambridge Crystallographic Data Centre as supplementary publication, https://deposit.ccdc.cam.ac.uk and the CCDC number is 1449897.

NMR experiments

The samples for NMR stability and binding experiments were prepared in 20% PBS (pD 7.4, prepared in D_2O) in DMSO-d_6 or 20% H_2O in DMSO-d_6. The data were recorded in JEOL ECS 400 MHz spectrophotometer. 2D NMR of 9-EtG binding kinetics was measured in Bruker Avance III 500 MHz spectrometer. DCl or NaOD was used to adjust pD to 7.4 and uncorrected pD values are reported.[3] The ESI-MS samples were prepared after taking the aliquots from NMR tubes followed by dilution in HPLC grade methanol. ^{195}Pt NMR of binding kinetics was recorded in Bruker Avance III 500 MHz spectrometer.

Interaction with plasmid DNA

Complexes of various concentrations (2, 5 & 10 μM) were incubated for 24 h at 37 °C with 4 μL of pUC19 DNA (stock concentration 30 ng μL^{-1}) in a total volume of 20 μL using 5 mM Tris-HCl (pH 7.4) containing 50 mM $NaClO_4$ and 0.5 mM EDTA. Stock solutions of complexes in DMF were used in such a way that the final DMF concentration in the reaction mixture doesn't exceed 10%. DNA control was also prepared with 10% DMF. Solutions of cisplatin were prepared in 1X PBS (pH 7.2). In the experiment with GSH, incubations were performed with 10 molar equivalent of GSH with respect to the concentration of the complexes used (i.e., 20, 50, 100 μM respectively). After incubation the whole reaction mixture was loaded in a ethidium bromide pre-stained 1% agarose gel and electrophoresis was carried for about 2 h at 80 V.

Cell lines and culture

Breast adenocarcinoma (MCF-7), lung adenocarcinoma (A549) and mouse embryonic fibroblast (NIH 3T3) were obtained from Department of Biological Sciences, IISER Kolkata (purchased from ATCC). Human hepatocellular carcinoma (HepG2), human pancreatic carcinoma (MIA PaCa-2) were obtained from NCCS (Pune, India). Human embryonic kidney cell line (HEK293) was kindly donated by Prof. S. M. Srinivasula of IISER TVM, India. MCF-7, A549, HEK293, MIA PaCa2 and NIH 3T3 cells were maintained in the logarithmic phase in Dulbecco's Modified Eagles Medium (DMEM) while HepG2 was grown on Minimum Essential Medium (MEM). Both the media were supplemented with 10% fetal bovine serum (GIBCO) and antibiotics (100 units per ml penicillin and 100 μg per ml streptomycin). Culture condition was maintained at a humid atmosphere of 95% air and 5% CO_2 at 37 °C. For Hypoxic condition oxygen was maintained at 1.5% while 5% CO_2 and other parameters were kept unaltered.

Cell viability assay

The cytotoxicity of the compounds against various cancer cell lines was evaluated based on MTT assay. Cells were seeded in 96-well microplate (Nunc) at a density of 6 $\times$ 10^3 viable cells per well in a volume of 200 μL of medium. It was subsequently incubated at 37 °C in a 5% carbon dioxide atmosphere for 48 h. After incubation, the medium was renewed with a fresh one (200 μL) followed by addition of metal complexes at appropriate concentrations. Stock solutions of metal complexes were made immediately prior to drug dilution in *ca.* 1:7 DMSO:culture media (v/v) followed by dilution with the same culture media to prepare various concentrations of solutions and added triplicate in well within 10 minutes of solution preparation so that the final DMSO concentration in well did not exceed 0.5% (The stability of **1a** in DMSO was checked up to *ca.* 12.5 h by ^{1}H NMR, Fig. S64†). The same percentage of DMSO was maintained in all types of cell based studies mentioned below. Upon 48 h of subsequent incubation, the existing drug containing media was removed followed by addition of fresh media along with 20 μL of a 1 mg mL^{-1} MTT in 1X PBS (pH 7.2). On incubation of the plates for 3 h at 37 °C in a humidified 5% CO_2 atmosphere, MTT was allowed to form formazan crystals in metabolically active cells. Finally media was removed after incubation and replaced with 200 μL of DMSO in each well

that would solubilize the formazan crystals. The absorbance for each well was recorded at 515 nm[4, 5] using a BIOTEK ELx800 plate reader.

IC_{50} values represent the drug concentration at which 50% cells are inhibited compared to control, which were calculated by fitting nonlinear curves in GraphPad Prism 5, using variable slope model constructed by plotting cell viability (%) versus log of drug concentration in μM. Each independent experiment was carried out in triplicate.

For determining IC_{50} in hypoxic condition the drugs were loaded in 96-well plate in normal atmospheric condition which took about 10 minutes before being transferred to the incubator maintained at 1.5% O_2 level. The equilibration time taken up by CO_2 incubator to reach hypoxic condition (1.5% O_2) was *ca*. 30 minutes after drug loading.

Metal accumulation study inside MCF-7 cells using ICP-MS

1.2×10^6 MCF-7 cells per 90 mm petri-dish containing 12 mL of DMEM were grown over 48 h and treated with the equitoxic (9 μM) concentration of complex solutions (**1a**, **2a**, **3** and cisplatin) for an additional 24 h. Subsequently the media was discarded and cells were washed using 1X PBS (pH 7.4). After trypsinization cells were counted accurately for each drug treated sample and 1.0×10^6 number of cells were centrifuged to form cell pellet. The cell pellets were washed twice by re-dispensing in 1X PBS (pH 7.4) followed by centrifugation. Cell pellets were then digested with 200 μL of extra pure (70% v/v) nitric acid (Sigma-Aldrich) at 100 °C for 12 h. Finally the digested cell suspension was diluted using Milli-Q water and the platinum content in the samples were analyzed on a Thermo Scientific XSERIES 2 ICP-MS instrument. Platinum standard solutions were freshly prepared before the experiment while analysis for all the samples were carried out in triplicates and the standard deviations were calculated.

Flow cytometry for cell cycle analysis

MCF-7 cells were seeded at 1.2×10^6 cells per plate in a 90 mm dia petri dish containing 12 mL of DMEM and incubated at 37 °C in a 5% carbon dioxide atmosphere for 48 h. After incubation, media was replaced with fresh media along with appropriate concentration of complex solutions and incubated for 24 h at previously mentioned culturing condition. Subsequently, drug containing media was removed and cells were harvested by trypsinization, washed twice with cold 1X PBS (pH 7.2) and fixed storing chilled 70% ethanol solution at 4 °C for 12 h. The cell pellets were re-suspended in 1X PBS solution comprising of PI (55 μg mL^{-1}) and RNase A (100 μg mL^{-1}) followed by an incubation at 37 °C for half an hour in dark for DNA staining. Finally the homogenized cell samples were analyzed in a BD Biosciences FACSCalibur flow cytometer and the resulting DNA histograms were quantified using the CellQuestPro software (BD).

DNA ladder assay for apoptosis detection

DNA ladder assay was performed in order to detect the cellular apoptosis induced by the test compound using a modified literature procedure.[6] Briefly, MCF-7 cells (1.2×10^6 per plate) were seeded in a 90 mm diameter tissue culture petri dish followed by incubation for 48 h under previously mentioned culturing condition. After incubation, media was changed and appropriate concentrations of complex solutions were added. After 24 h of additional incubation, media was collected in a tube. Cells were washed with 1X PBS (pH 7.2) and the harvested cells (after trypsinization) along with washings were collected in the same tube. The cells were washed twice with 1X PBS (pH 7.2) and centrifuged at 2500 rpm for 10 minutes at 25 °C followed by cell lysis with 500 μL of lysis buffer (20 mM Tris-HCl, 0.4 mM EDTA, 0.25% Triton-X 100, pH 8.0) after incubation at room temperature for 15 minutes. Then the lysed cells were centrifuged at 14000 rpm for 10 minutes at 4 °C and the supernatant was mixed well with 500 μL of 1:1 (v/v) mixture of phenol and chloroform followed by centrifugation again at 14000 rpm for 10 minutes at 4 °C. After that the aqueous layer was carefully taken out and mixed with 55 μL of 5 M NaCl solution and 550 μL of isopropanol. The mixture was incubated at −20 °C for overnight followed by centrifugation at 14000 rpm for 10 minutes at 4 °C and the obtained pellet was washed with 70% ice-cold ethanol and finally air dried. The dried pellet was re-suspended in 40 μL of 1X TE

buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and 8 μL of RNAse solution (150 μg mL^{-1}). After a quick centrifugation at 5000 rpm, the supernatant was loaded in 1.6% agarose gel containing ethidium bromide (1 μg mL^{-1}) and run for 3 h at 60 V in 1X TBE buffer. Untreated cells were used as controls whereas 50 bp DNA ladder was used to track the migration of bands and fragmentation sizes on the agarose gel. Visible bands on gel were observed and picture taken on exposure to UV light through gel documentation system of Bio-Rad.

JC-1 staining assay for detection of mitochondrial membrane potential change

Seeding of cells, drug treatment and final harvesting of cells after 24 h of drug exposure were carried out using same procedure followed during DNA ladder assay. The harvested cells were then collected by centrifugation at 2500 rpm at 25 °C for 5 minutes, washed with 1X PBS (pH 7.2) by subsequent centrifugation and finally the cells were mixed with 500 μL of JC-1 (10 μg mL^{-1} in 10% FBS supplemented 1X PBS). The cell suspension was subjected to 30 minutes incubation at 37 °C. The stained cells were collected by centrifugation and resuspended in 1X PBS (pH 7.2) for analysis in BD Biosciences FACSCalibur flow cytometer.

Colorimetric determination of caspase activity

Activation of Caspase was evaluated by colorimetry using Caspase Assay kit (Sigma-Aldrich). The free *p*-nitroaniline (pNA) was quantified after its cleavage from the Ac-DEVD-pNA substrate by released caspase from MCF-7 cells. It is known that the substrate Ac-DEVD-pNA is cleaved by caspase-7 or caspase-3, however activation of caspase-7 cleaves Ac-DEVD-pNA in this case since MCF-7 cells are known to be devoid of caspase-3. Briefly, 1.2×10^6 MCF-7 cells were seeded in 90 mm petri-dish and were incubated at 37 °C for 48 h. It was followed by treatment with the complex solutions for additional 24 h. After that, cells were collected and treated as per the kit manufacturer's protocol. Following 24 h incubation after addition of cell lysate, the absorbance of the released *p*NA was detected at 405 nm using ELISA plate reader. The data is graphically represented as the concentration of *p*NA released (nmol/min/ml) in y-axis and concentrations of treated drugs in x-axis. Each drug concentration has been performed in triplicate.

Optical microscopy imaging

For optical microscopic imaging, 12×10^3 number of MCF-7 cells were seeded per well in a 6-well plate over 48 h. Following the incubation, media was changed and required concentrations of the metal complex were added and further incubated for 24 h. The drug containing media were then removed and cells were fixed with 4% para formaldehyde solution in 1X PBS (pH 7.2). The fixed cells were washed with 1X PBS (pH 7.2) and incubated with DAPI (1 μg mL^{-1}) for 2 minutes. Images of MCF-7 cells were acquired using OLYMPUS IX 81 epifluorescence inverted microscope at 60X magnification. Both DIC and fluorescence microscopy images were taken and processed using OLYMPUS Cell P software.

Growth kinetics of MCF-7 under the influence of compound

In order to evaluate the growth kinetics of MCF-7 under the influence of **1a**, periodic MTT assays were performed at an interval of 24 h for 6 days. Initially cells were seeded in six 96-well microplate (Nunc) at a density of 1.5×10^3 viable cells per well in a volume of 200 μL of medium. It was subsequently incubated at 37°C in a 5% carbon dioxide atmosphere for 48 h, while one plate was removed for MTT assay at every 24 h. After 48 h of initial incubation, medium was removed and fresh medium was added (200 μL) followed by addition of metal complexes at appropriate concentrations (**1a**: 12 μM and 8 μM; cisplatin: 12 μM and 10 μM). Stock solutions were freshly prepared in DMSO (such that concentration in well did not exceed 0.5%) prior to addition of complex in well. Plates were subsequently incubated at 37°C in a 5% carbon dioxide atmosphere for 48 h. After 48 h of treatment of cells with the compound, the compound containing media was removed and replenished with fresh media to facilitate the growth of existing cells. However MTT assays were carried out

using previously described method, on each replica at every 24 h interval upto 48 h of drug removal.

Wound assay (Migration)

Wound healing assay was used as a tool to evaluate the effect of drugs against cancer cell migration.[7] Briefly, 1×10^6 cells were seeded in each well of a 6- well plate and incubated at 37 °C humidified CO_2 (5%) incubator till the formation of uniform monolayer in the plate. A sterile microtip was used to scratch across the well forming a denuded area over the cell monolayer and subsequently washed with 1X PBS (pH 7.2). Media was replaced to which sub IC_{50} concentrations (6 and 8 μM) of **1a** were added. Plates were incubated and images were captured at 12 h interval in Phase contrast microscope (OLYMPUS IX 81 epifluorescence inverted microscope). The quantification of wounded area was done using Image J software.

Hemolysis assay

The hemolysis assay was performed following standard method.[8] In a brief, blood was collected in EDTA coated vials volunteered by a healthy human after taking written permission from him (assay protocol was approved by IISER Kolkata Ethics Committee). After centrifugation at 3000 rpm for 10 minutes, erythrocytes were washed thrice with cold PBS (pH 7.4) stored at 4°C re-suspended in the same buffer to a final concentration of 20% (v/v). The reaction vial contains 2% (final concentration) cell suspension and required concentrations of compound. Different concentrations of **1a** were prepared in DMSO and diluted in PBS (pH 7.4) such that the final concentration of compound remains 5, 10 and 15 μM in the reaction vial, while the final DMSO concentration was kept at 0.2%. The vials were incubated at 37 °C for 1 h in a water bath with continuous shaking, after which the vials were centrifuged at 3000 rpm for 10 min and the supernatants were collected. Releases of hemoglobin in the supernatant were determined spectrophotometrically at 540 nm. Positive control in which complete hemolysis takes place was achieved using Triton X-100 (final concentration 0.2%). Appropriate DMSO (0.2%) containing PBS was used as negative control. The experiments were performed in triplicate and the percentages of hemolysis were calculated as follows:

Hemolysis percentage (%) = $(A_s-A_n)/(A_p-A_n) \times 100$, where A_s, A_n, A_p are absorbance of sample, negative control and positive control respectively at 540 nm.

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