Supporting Information for

Design, synthesis and biological evaluation of dihydro-2-quinolone platinum(IV) hybrids as antitumor agents displaying mitochondria injury and DNA damage mechanism

Zhifang Liu,[a] Zuojie Li,[a] Tao Du,[b] Yan Chen,[a] Qingpeng Wang,*[a] Guoshuai Li,[a] Min Liu,[a] Ning Zhang,*[a] Dacheng Li*[a,c] and Jun Han[a]

a. Institute of Biopharmaceutical Research, Liaocheng University, Liaocheng 252059, P.R. China.

b. Henkel loctite China corp.ltd, Yantai 264006, P.R. China.

c. Shandong Provincial Key Laboratory of Chemical Energy Storage and Novel Cell Technology, Liaocheng University, Liaocheng 252059, PR China.

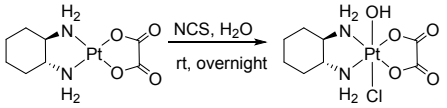
*Corresponding authors:

E-mail address: lywqp@126.com (Q. Wang), zhangning1111@126.com (N. Zhang), lidacheng62@163.com (D. Li).

Contents

1. Synthetic route of the title compounds

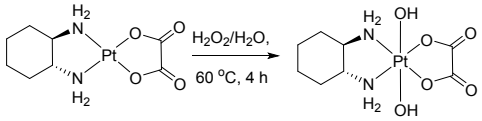
1.1 Preparation of compound O1



Oxoplatin **O1**

Oxaliplatin (1.0 g, 2.5 mmol) was suspended in distilled water 150 mL and stirred at room temperature. *N*-chloro succinimide (NCS) solution (0.392 g, 2.94 mmol) in distilled water 150 mL was added to reaction system dropwise and the resultant mixture was stirred overnight at room temperature. After that, the solid was filtrated and the solution was concentrated under vacuum. The residue was washed with ethanol and ethylether. The product was obtained as a yellow solid (1.0 g, 84%).

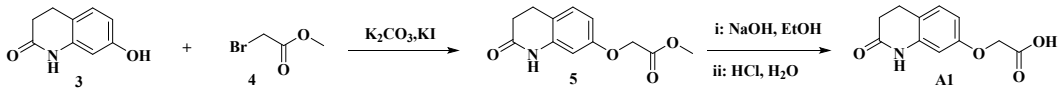
1.2 Preparation of compound O2



Oxoplatin O2

A suspension of oxaliplatin (1.0 g, 2.5 mmol) in distilled water 30 mL was stirred at room temperature. Then H_2O_2 (30%) 50 mL was added drop wise. The mixture was kept stirring for 4 h at 60 °C. Then the resultant mixture was recrystallized at 4 °C. Crude product as yellow solid was obtained after filtration. Then recrystallization in water affords pure oxoplatin as white needles (0.9 g, 79%).

1.3 Preparation of compound A1

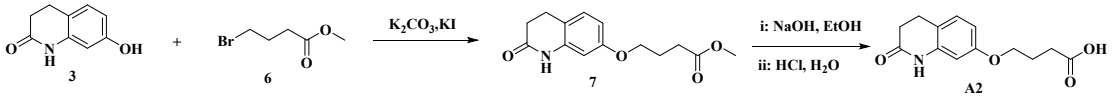


3 4 5 A1

To a flask containing DHQLO **3** (2.0 g, 12.25 mmol), K_2CO_3 (3.38 g, 24.5 mmol) and trace KI was added DMF 10 mL as solution. The suspension was stirred for 30 min, and methyl bromoacetate **4** (2.4 g, 16 mmol) was added. Then the mixture was stirred at 50 °C for 24 h. After the reaction completed, the solvent was removed under vacuum. The residue was extracted with dichloromethane (DCM), purified by column chromatography, and DHQLO ester **5** was obtained as white solide. Subsequently, compound **5** was dissolved in ethanol 100 mL, and 5% NaOH/H_2O 8.2 mL was added. The mixture was stirred for 12 h at room temperature. After the reaction completed, the resultant solution was acidified to pH = 3–4, and extracted with ethyl acetate (EA). The acid **A1** was obtained as white solid (2.2 g, 80.1%). ^{1}H

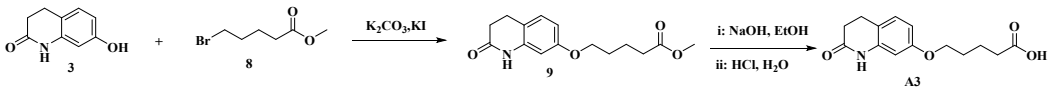
NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.41 –6.45 (m, 2H), 4.56 (s, 2H), 2.74 (t, J = 7.6 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 170.6, 157.4, 139.6, 128.8, 116.6, 107.8, 102.3, 65.0, 31.1, 24.4. MS-ESI: Calcd. for $[M+H]^+$: 222 ($M = C_{11}H_{11}NO_4$), found: 222.

1.4 Preparation of compound A2



To a flask containing DHQLO **3** (2.0 g, 12.25 mmol), K_2CO_3 (3.38 g, 24.5 mmol) and trace KI was added DMF 10 mL. The suspension was stirred for 30 min, and methyl 4-bromobutyrate **6** (2.9 g, 16 mmol) was added. Then the mixture was stirred at 50 °C for 24 h. After the reaction completed, the solvent was removed under vacuum. The residue was extracted with DCM, purified by column chromatography, and DHQLO ester **7** was obtained as white solide. Subsequently, compound **7** was dissolved in ethanol 100 mL. Then 5% NaOH/H_2O 8.2 mL was added, and the mixture was stirred for 12 h at room temperature. After the reaction completed, the resultant solution was acidified to pH = 3–4, and extracted with EA. The acid **A2** was obtained as white solid (2.5 g, 81.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 9.96 (s, 1H), 6.98 (d, J = 7.8 Hz, 1H), 6.41 – 6.45 (m, 2H), 3.86 (t, J = 6.3 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 2.43 – 2.23 (m, 4H), 1.87 – 1.91 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.5, 170.7, 158.2, 139.6, 128.8, 116.0, 108.0, 102.2, 67.0, 31.2, 30.5, 24.7, 24.4. MS-ESI: Calcd. for $[M+H]^+$: 250 ($M = C_{13}H_{15}NO_4$), found: 250.

1.5 Preparation of compound A3



To a flask containing DHQLO **3** (2.0 g, 12.25 mmol), K_2CO_3 (3.38 g, 24.5 mmol) and trace KI was added DMF 10 mL. The suspension was stirred for 30 min, and methyl 5-bromovalerate **8** (3.1 g, 16 mmol) was added. Then the mixture was stirred at 50 °C for 24 h. After the reaction completed, the solvent was removed under vacuum. The residue was extracted with DCM, purified by column chromatography, and DHQLO ester **9** was obtained as white solide. Subsequently, compound **9** was dissolved in ethanol 100 mL. Then 5% NaOH/H_2O 8.2 mL was added, and the mixture was stirred for 12 h at room temperature. After the reaction completed, the resultant solution was acidified to pH = 3–4, and extracted with EA. The acid **A3** was

obtained as white solid (2.7 g, 82.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 9.99 (s, 1H), 7.04 (d, J = 8.2 Hz, 1H), 6.51 – 6.42 (m, 2H), 3.89 (t, J = 6.0 Hz, 2H), 2.78 (t, J = 7.4 Hz, 2H), 2.42 (t, J = 7.4 Hz, 2H), 2.28 (t, J = 7.1 Hz, 2H), 1.76 – 1.58 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.8, 170.7, 158.3, 139.6, 128.8, 115.9, 107.9, 102.2, 67.5, 33.7, 31.2, 28.5, 24.4, 21.6. MS-ESI: Calcd. for $[M+H]^+$: 264 ($M = C_{14}H_{17}NO_4$), found: 264.

2. Reduction and DNA binding properties of compound 1b

The reduction and DNA binding properties of compound **1b** was tested by HPLC. The stability of compound **1b** was detected by preparing a solution of **1b** 500 μM in PBS and measured at 0.5, 4, 15 h (Figure S1). The Figure S2 depicted the influence of AsA on the reduction of compound **1b**. A solution containing compound **1b** (500 μM) and AsA (1 mM) was detected at 0.5, 4, 15 h. The emergence of acid and oxaliplatin peaks indicates the release of DHQLO and platinum(II) moieties after reduction. Finally, the solution containing compound **1b** (500 μM), 5′-GMP (3 mM) and AsA (1 mM) was tested to evaluate the degradation of compound **1b** by AsA and the following combination of the released platinum(II) moiety with 5'-GMP (Figure S3). The DNA binding competence could be demonstrated by the formation of platined-GMP (Figure S4), structure of which has been proven in our previous work[1,2].

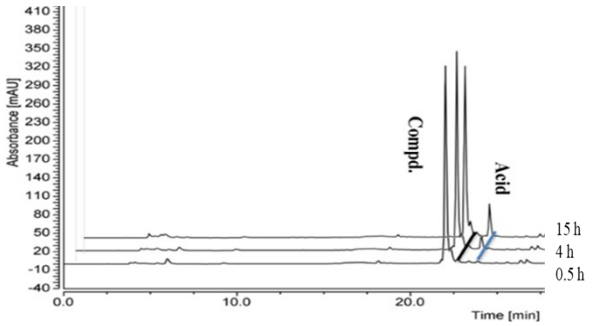


Figure S1. HPLC spectra of compound **1b** (500 μM) in PBS at 37 °C.

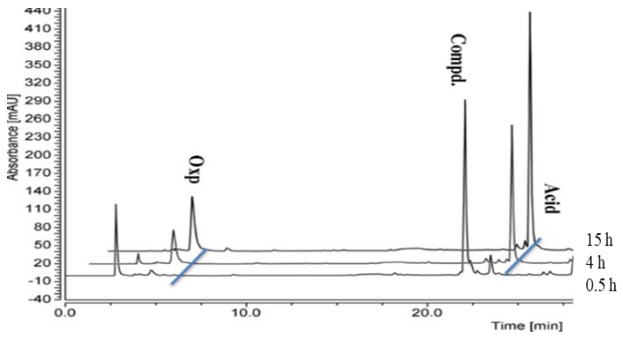


Figure S2. HPLC spectra of compound **1b** (500 μM) in the presence of AsA (1 mM) in PBS.

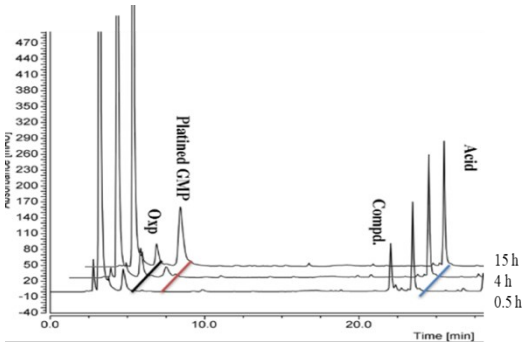


Figure S3. HPLC spectra of compound **1b** (500 μM) in the presence of AsA (1 mM) and 5'-GMP (3 mM) in PBS.

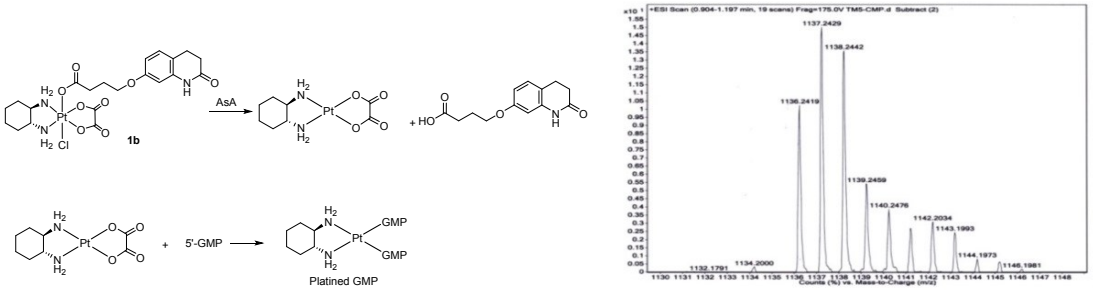


Figure S4. Reduction of **1b** and HRMS of the adduct of oxaliplatin with 5'-GMP peak (Platined-GMP)[2].

3. Apoptosis

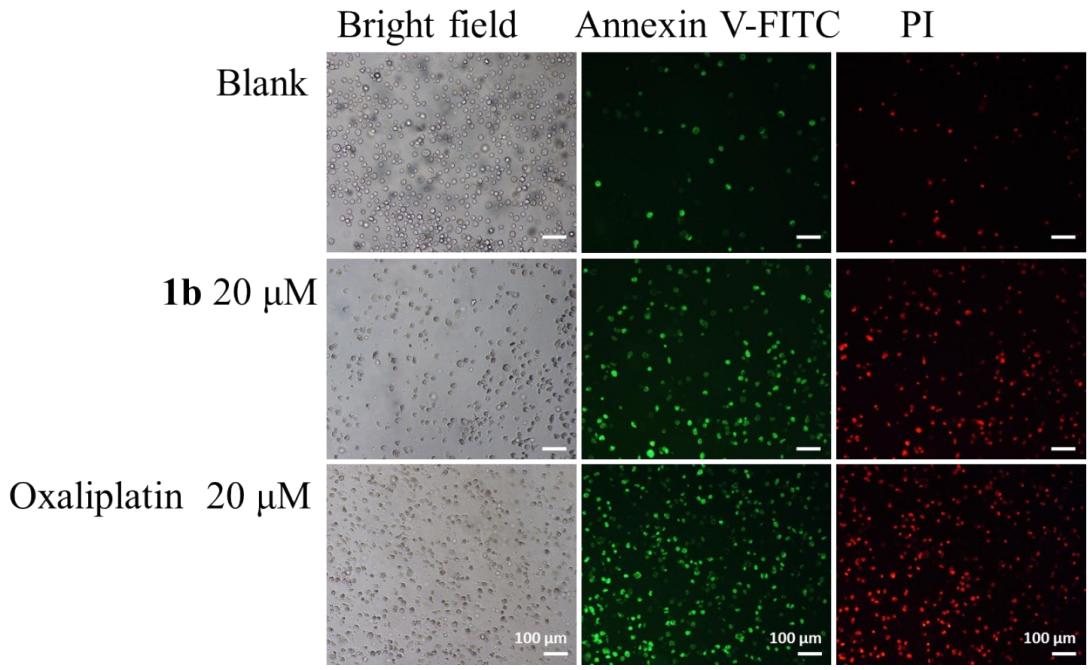


Figure S5. Fluorescence microscopic images of SKOV-3 cells treated with and without platinum complexes for 24 h at 37 °C, and stained by annexin V-FITC and PI.

4. Mitochondrial membrane depolarization

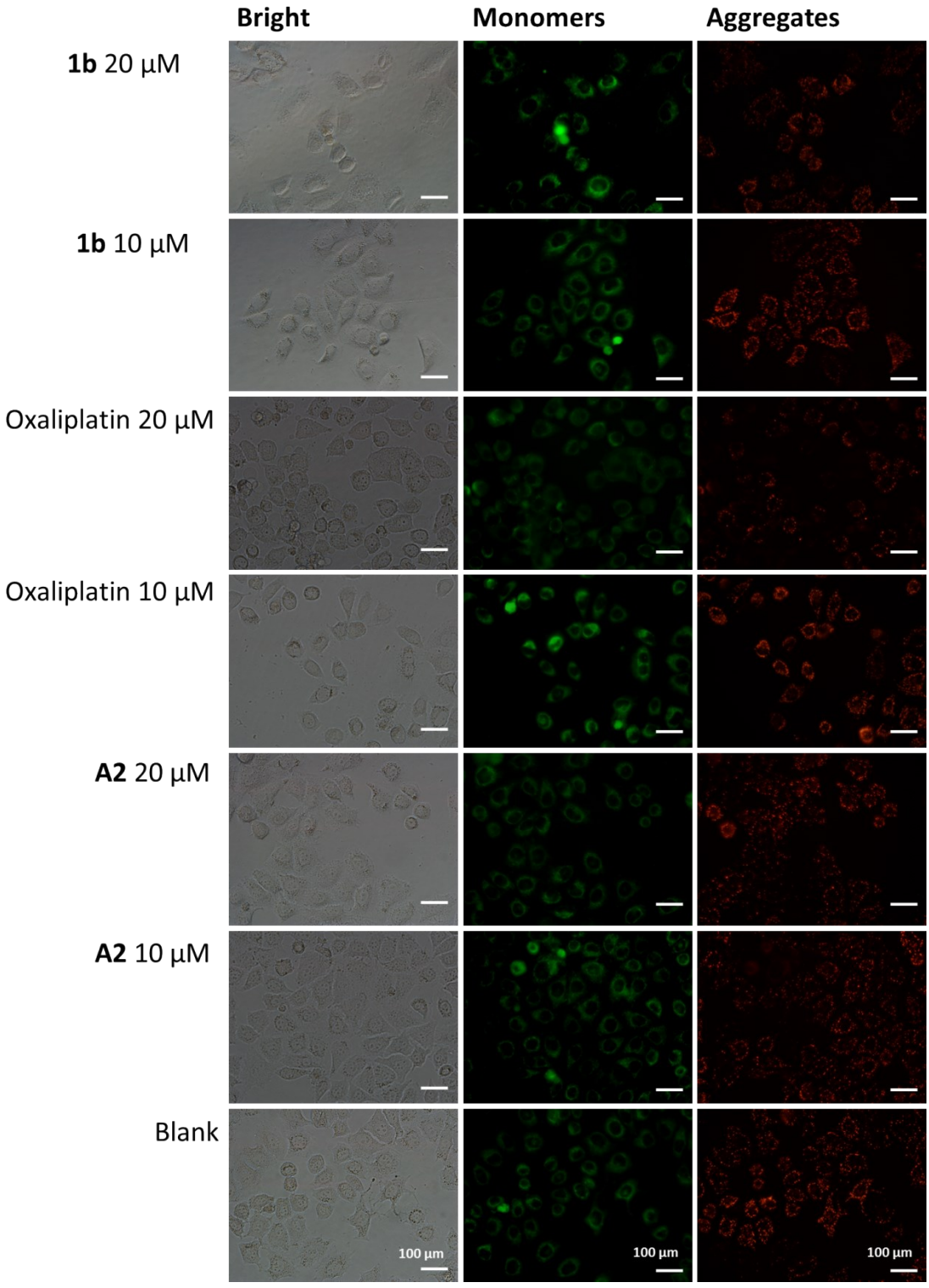


Figure S6. Fluorescence microscopic images of SKOV-3 treated with and without platinum complexes for 24 h at 37 °C, and stained by Rho 123.

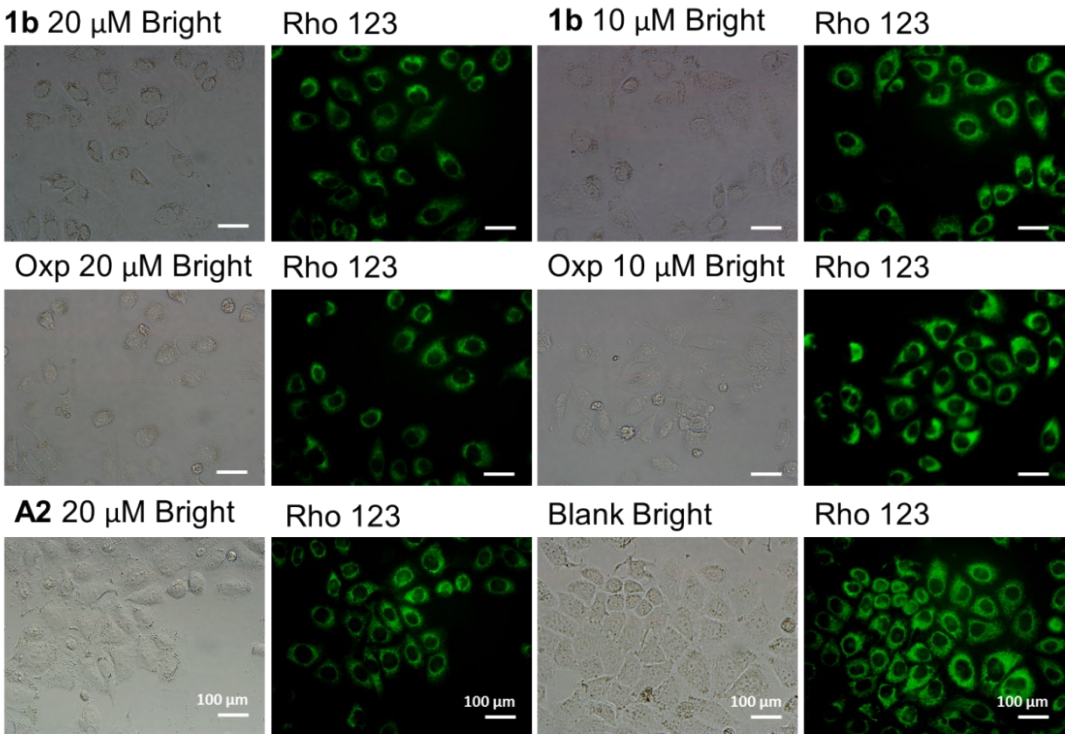


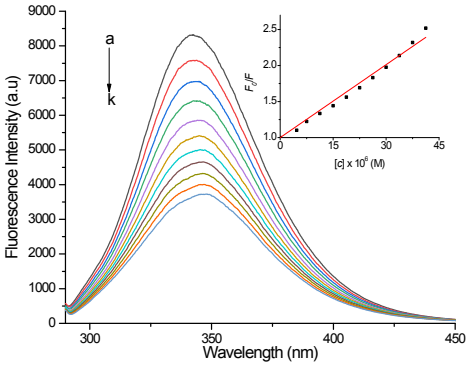
Figure S7. Fluorescence microscopic images of SKOV-3 treated with and without platinum complexes for 24 h at 37 °C, and stained by Rho 123.

5. Fluorescence analyses for binding studies with HSA

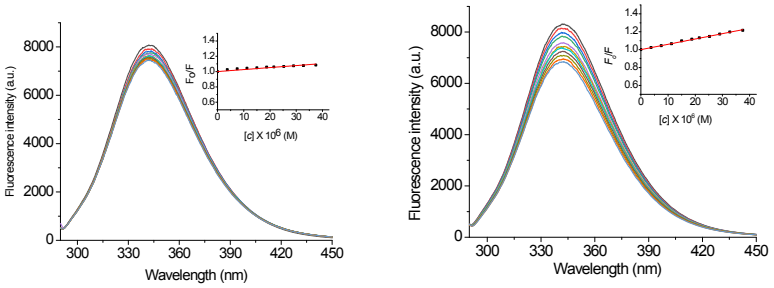
The fluorescence emission spectra of HSA, with increasing concentrations of **1b, 2b,** cisplatin and oxaliplatin at 298 K, and **1b** at 301K and 304K were tested. The quenching trend of HSA by the platinum(IV) complex is in agreement with the linear Stern-Volmer equation (eq S1). The fluorescence spectra at three different temperatures were presented as Figure 10 and S9.

$$\frac{F_0}{F} = 1 + K_{SV}[C] = 1 + K_q\tau_0[C] \quad \text{(eq S1)}$$

In eq S1, F_0 and F are the fluorescence intensities in the absence and presence of compound **1b** respectively; K_q is the quenching rate constant; τ_0 is the average lifetime of molecules in the absence of quencher and its value is about 10^{-8} s; K_{SV} is linear Stern-Volmer quenching constant; $[C]$ is the concentration of the compound. The Sterne-Volmer plots at three temperatures were drawn and given as Figure S10. The slope was obtained as K_{SV}, and the $K_q = K_{SV}/\tau_0$ was also calculated and given in Table 2 and S1.

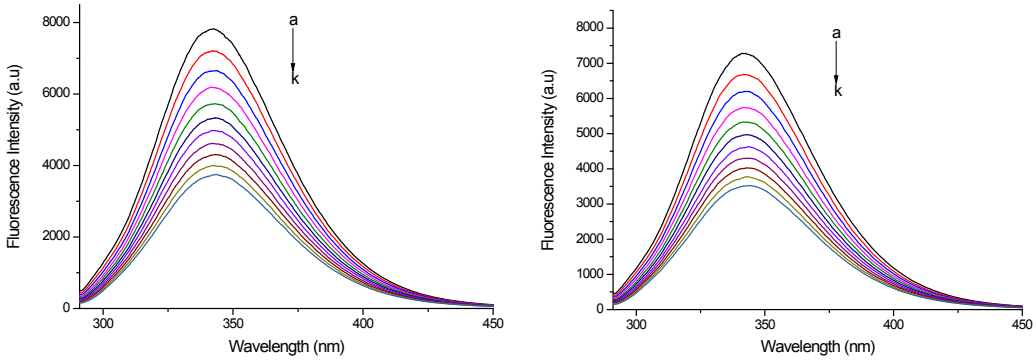


Complex **2b**



Cisplatin Oxaliplatin

Figure S8. Fluorescence spectra of HSA in the absence and presence of platinum (IV) complex **2b** (λex = 280 nm, *T* = 298 K). a–k: *c*(HSA) = 4.0 μM, *c*(complex **3**) = 0.0–37.5 μM, at increments of 3.75 μM. Inset: The Stern-Volmer plots of F_0/F versus $[C] \times 10^6$.



301K 304K

Figure S9. Fluorescence spectra of HSA in the absence and presence of platinum (IV) complex **1b** (λex = 280 nm, *T* = 301 K, 304K). a–k: *c*(HSA) = 4.0 μM, *c*(complex **1b**) = 0.0–37.5 μM, at increments of 3.75 μM.

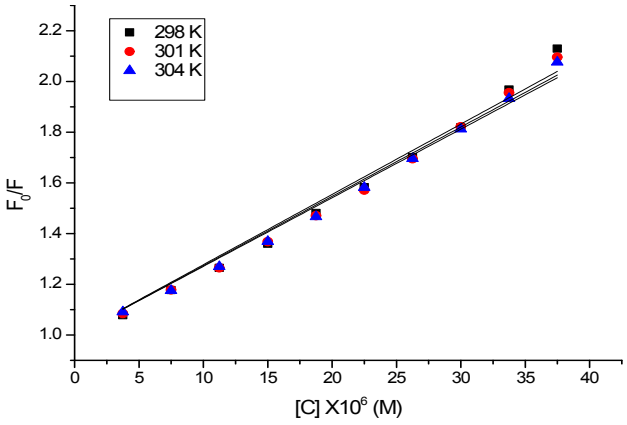


Figure S10. Sterne-Volmer plots at three different temperatures for **1b**-HSA system.

Table S1 Sterne-Volmer quenching constants for the interaction of compound **1b** with HSA at different temperatures.

T (K)	10^{-4} K_{sv} (M^{-1})	10^{-12} K_q ($M^{-1}S^{-1}$)	R[a]
298	2.77±0.06	2.77±0.06	0.9908
301	2.73±0.06	2.73±0.06	0.9927
304	2.71±0.05	2.71±0.05	0.9948

[a] R is the correlation coefficient.

For static quenching, when molecules bind independently to a set of equivalent sites on a macromolecule, the binding constant (K_b) and the number of binding sites (n) can be determined by eq S2:

$$log\frac{F_0 - F}{F} = log\ K_b + n\ log\ [C] \qquad \text{(eq S2)}$$

In eq S2, F_0 and F are the fluorescence intensities in the absence and presence of the compound respectively; [C] is the concentration of the compound; K_b is the binding constant to a site; n is the number of binding sites per HSA. The plots of log[(F_0 - F)/F] vs log[C] were drawn and shown as Figure S11. The values of K_b and n were obtained and given in Table S2.

The interaction forces between bioactive molecules and HSA include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, hydrophobic and steric contacts and so on. If the enthalpy change (ΔH) does not vary significantly over the studied temperature range, then its value and that of entropy change (ΔS) can be evaluated from the van't Hoff equation (eq S3):

$$lnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad \text{(eq S3)}$$

In eq S3, K is analogous to the associative binding constants at the corresponding temperature; R is the gas constant 8.314; ΔH is the enthalpy change; ΔS is the entropy change. The van't Hoff plots of compound **1b**-HSA system was displayed in Figure S12.

The free energy change (ΔG) was then calculated from the following equation (eq S4):

$$\Delta G = \Delta H - T\Delta S \qquad \text{(eq S4)}$$

The thermodynamic parameters ΔH, ΔG and ΔS were calculated and given in Table S2.

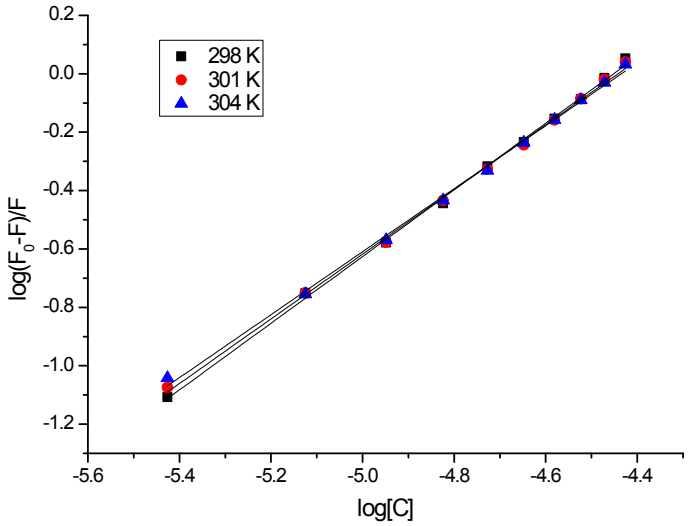


Figure S11. The plots of log $(F_0\text{-}F)/F$ vs log $[C]$ for compound **1b**-HSA system.

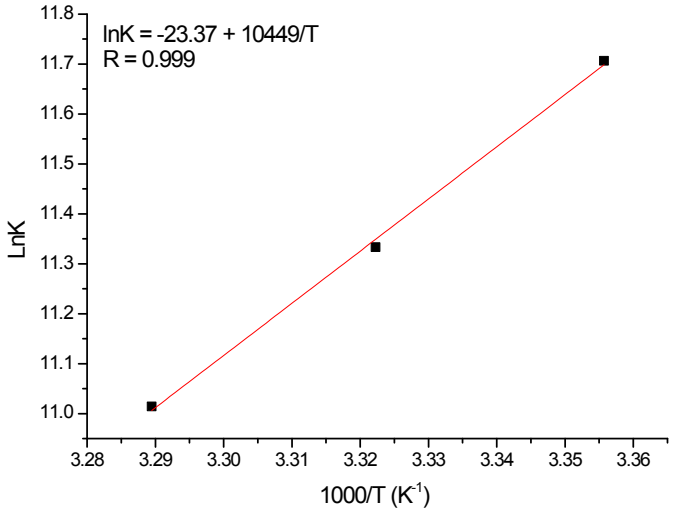
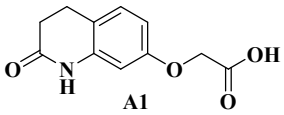


Figure S12. Van't Hoff plots of compound **1b**-HSA system.

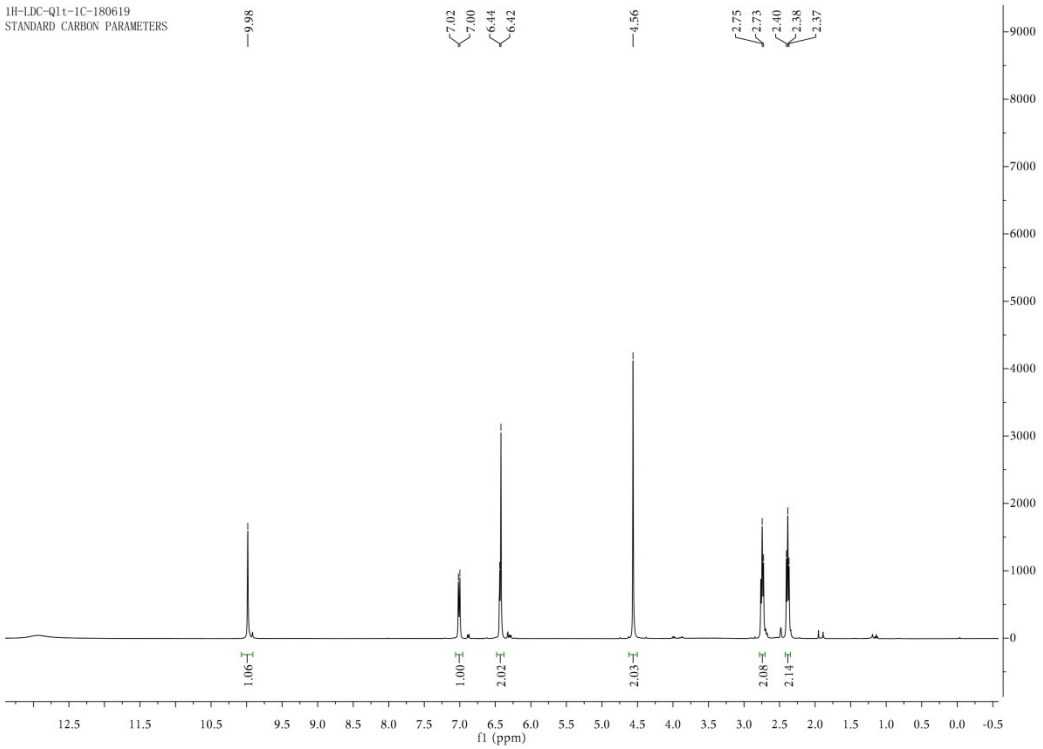
Table S2 Binding constants, sites and the thermodynamic parameters of compound **1b** with HSA at different temperatures.

T (K)	10^{-4} K_b (M^{-1})	R	n	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol K)
298	12.13±0.07	0.9994	1.14	-86.88±3.32	-28.99±0.10	-194.27±11.47
301	8.35±0.07	0.9993	1.11		-28.41±0.13	
304	6.07±0.08	0.9990	1.08		-27.82±0.16	

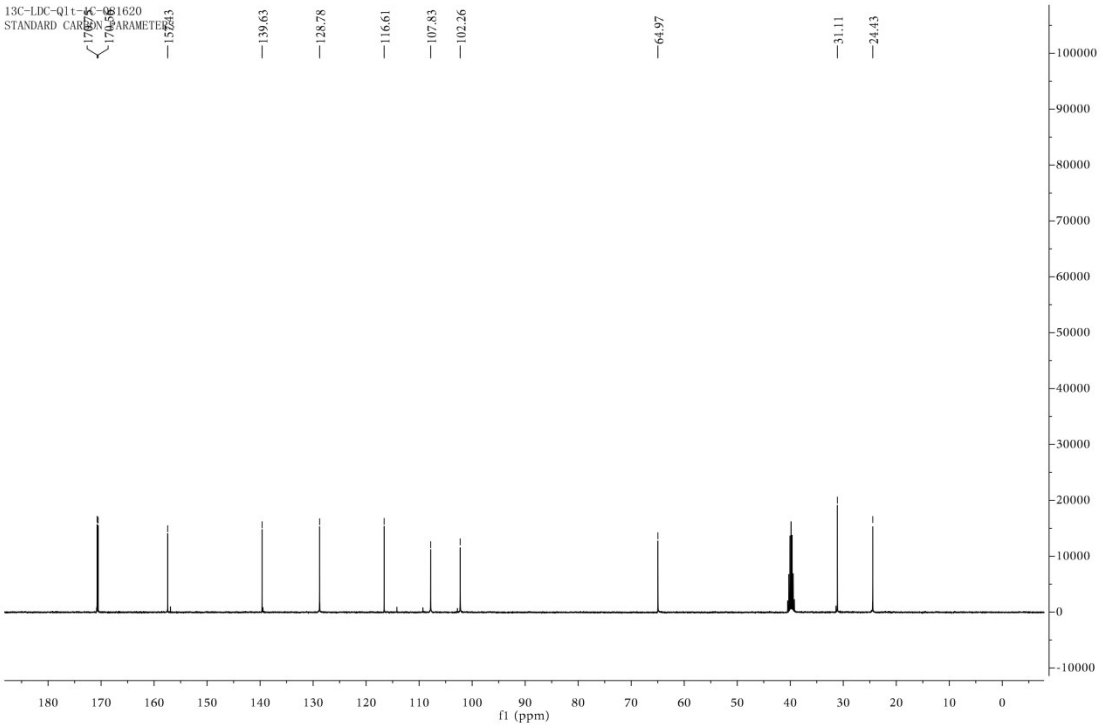
6. NMR spectra



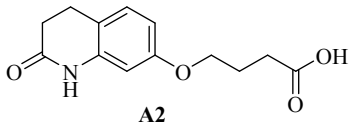
A1



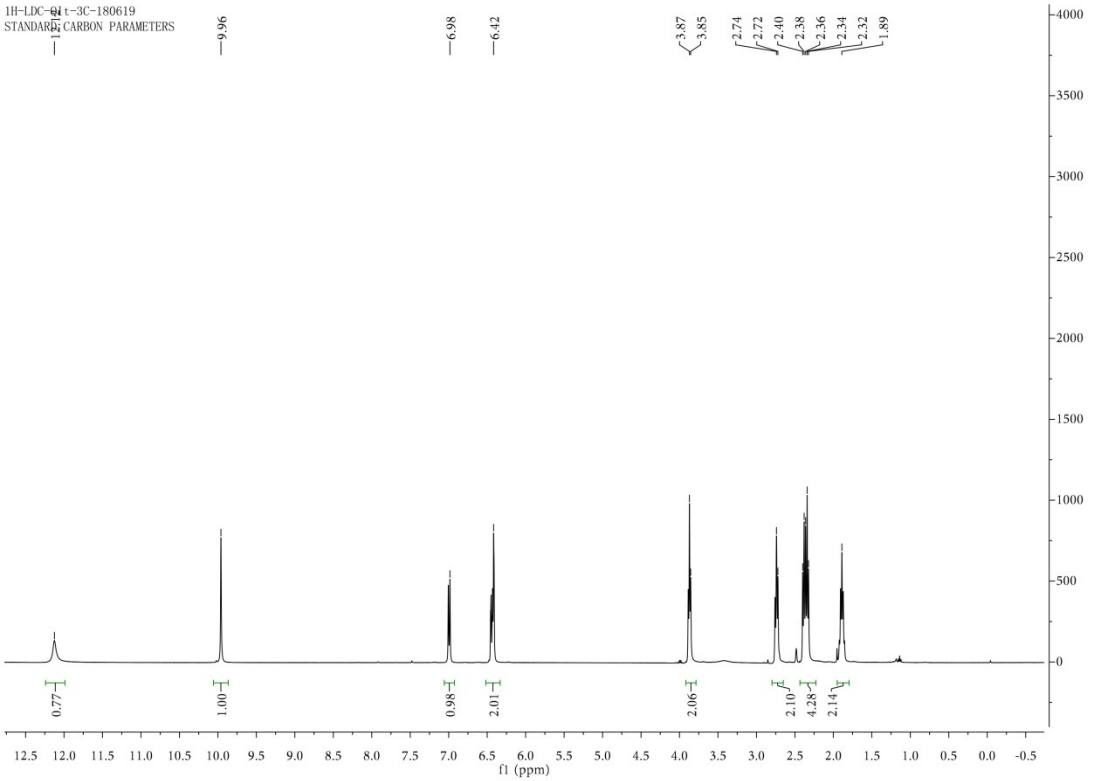
^{1}H-NMR spectrum for compound **A1**



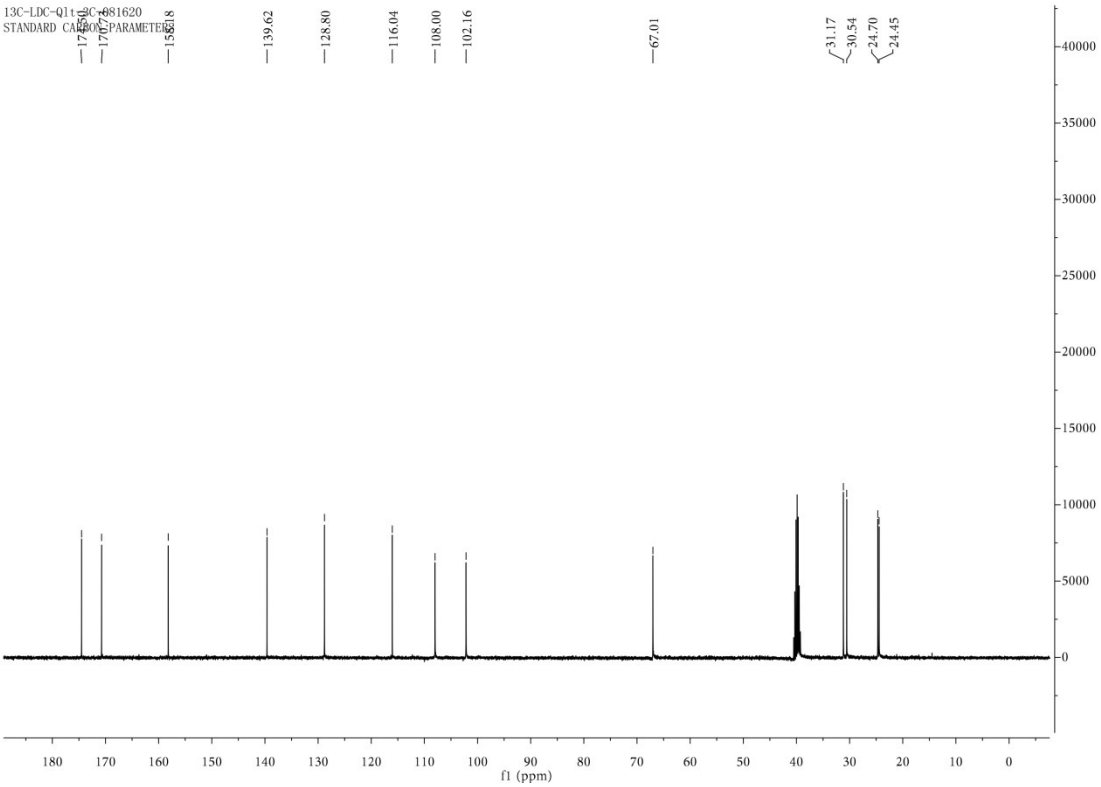
^{13}C-NMR spectrum for compound **A1**



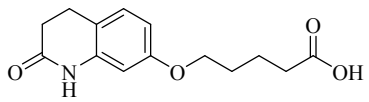
A2



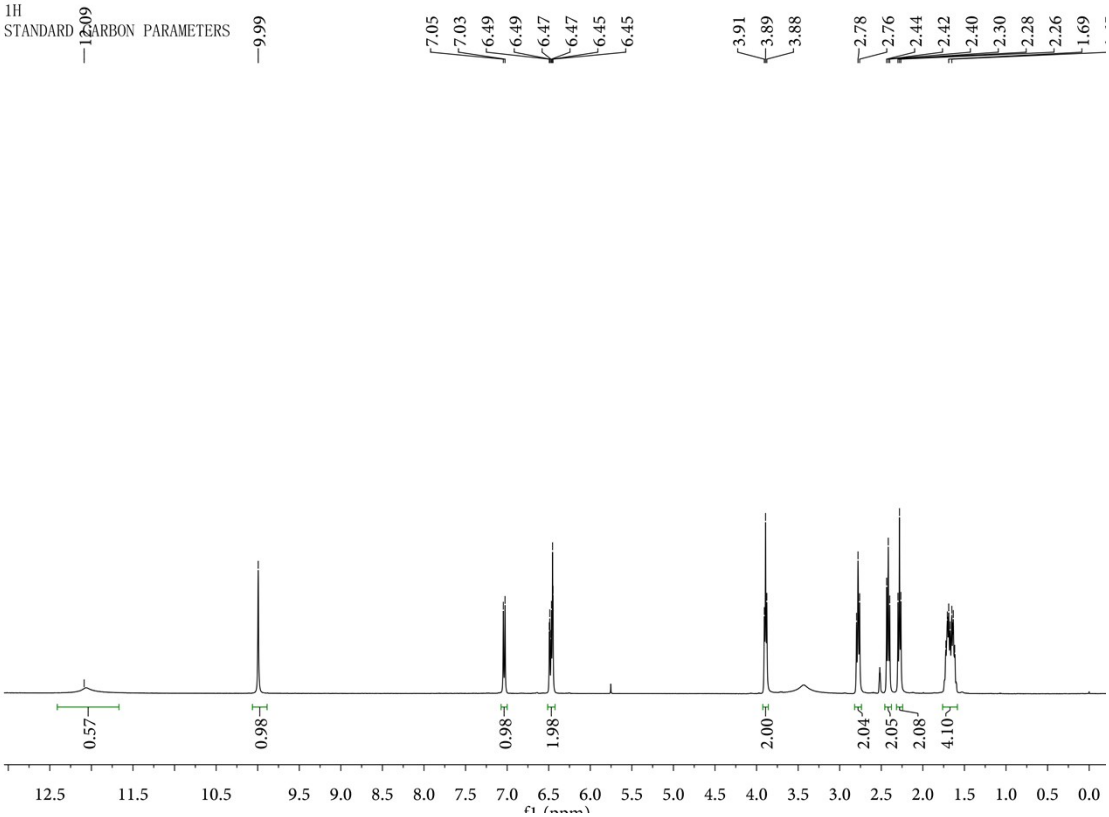
^{1}H-NMR spectrum for compound **A2**



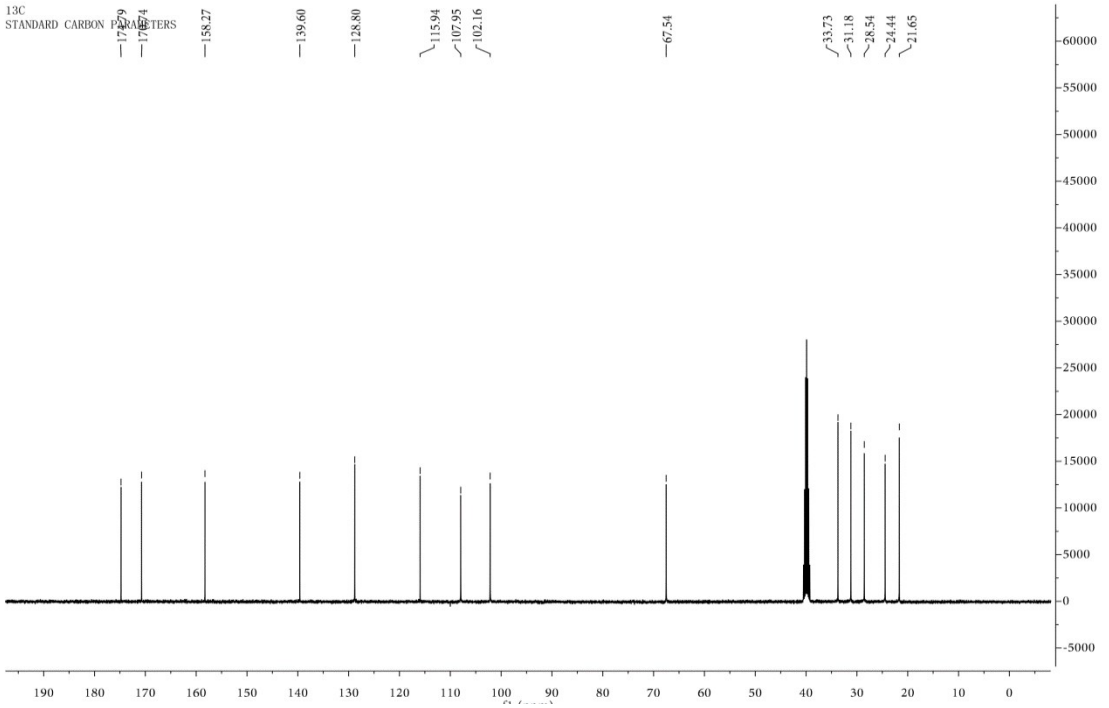
^{13}C-NMR spectrum for compound **A2**



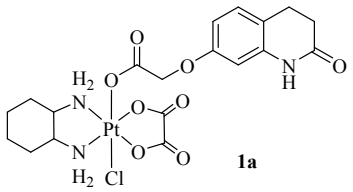
A3



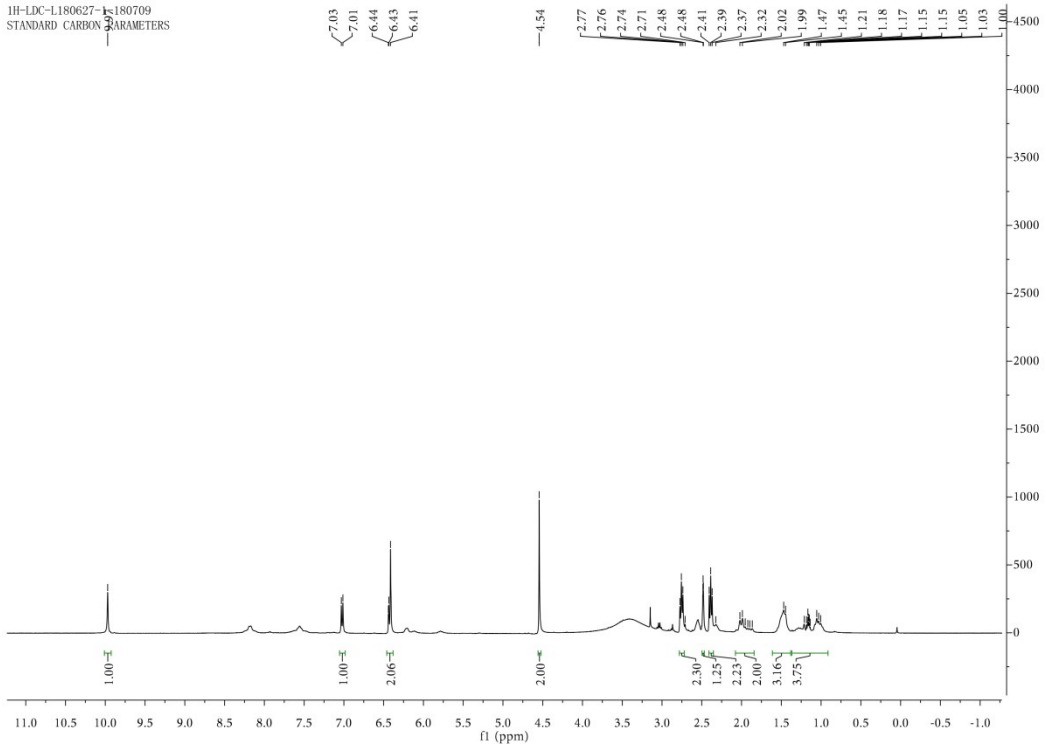
^{1}H-NMR spectrum for compound **A3**



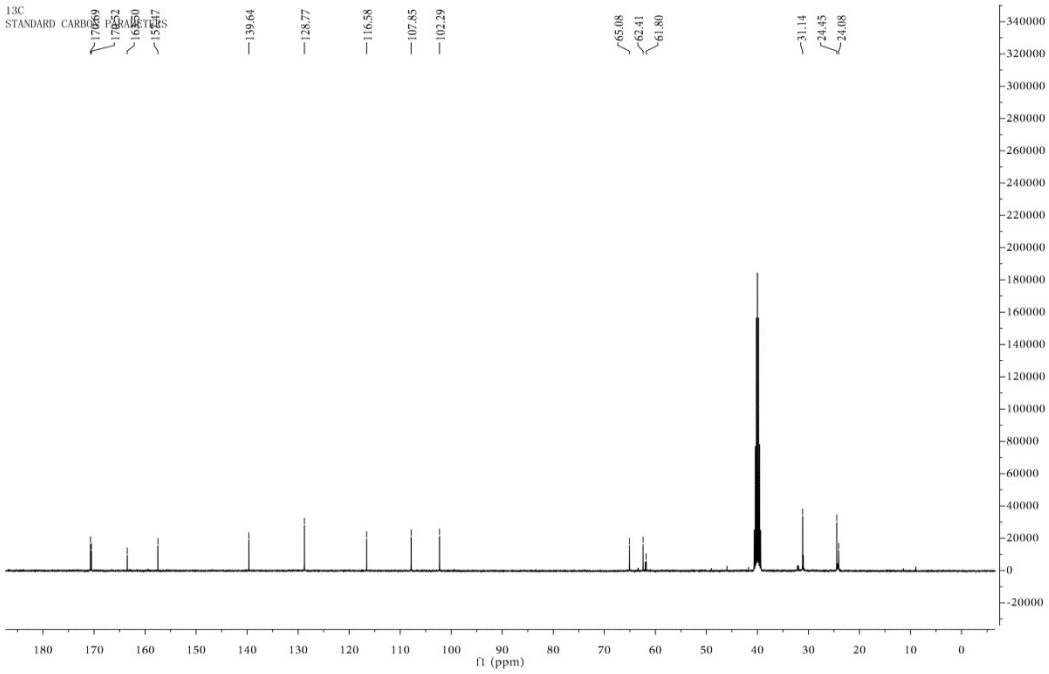
^{13}C-NMR spectrum for compound **A3**



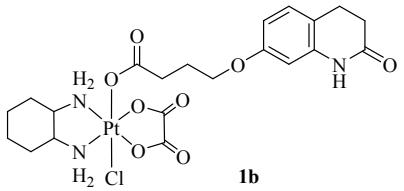
1a



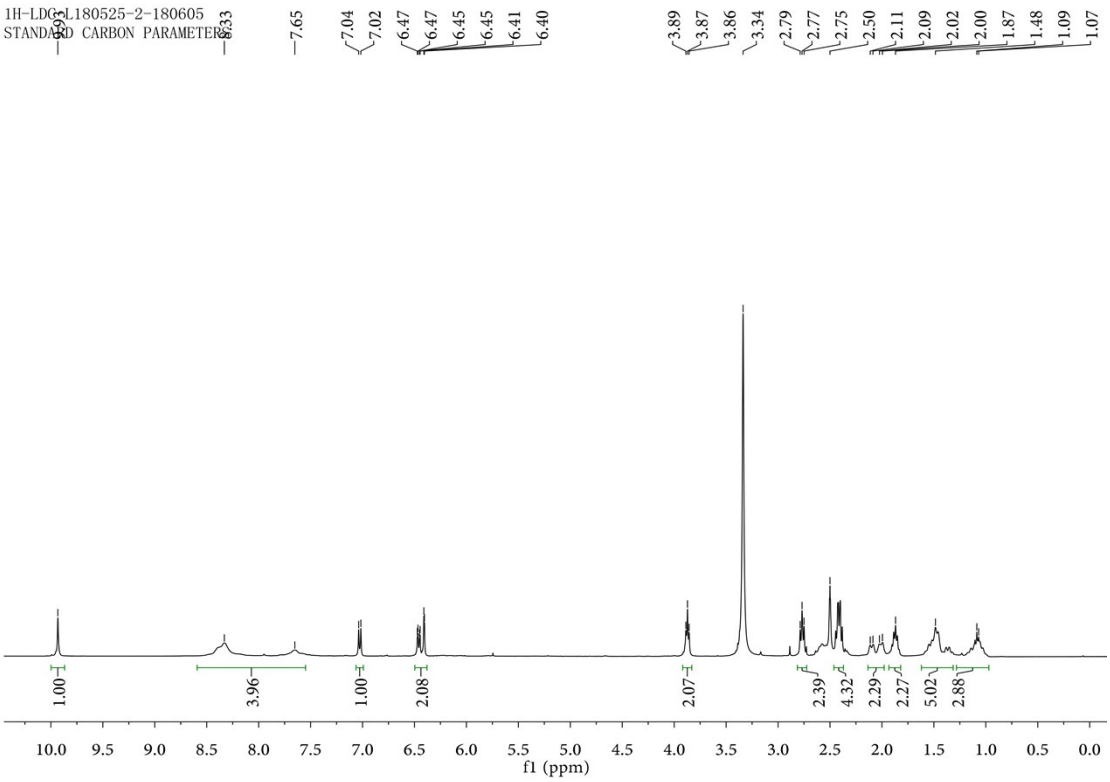
^{1}H-NMR spectrum for compound **1a**



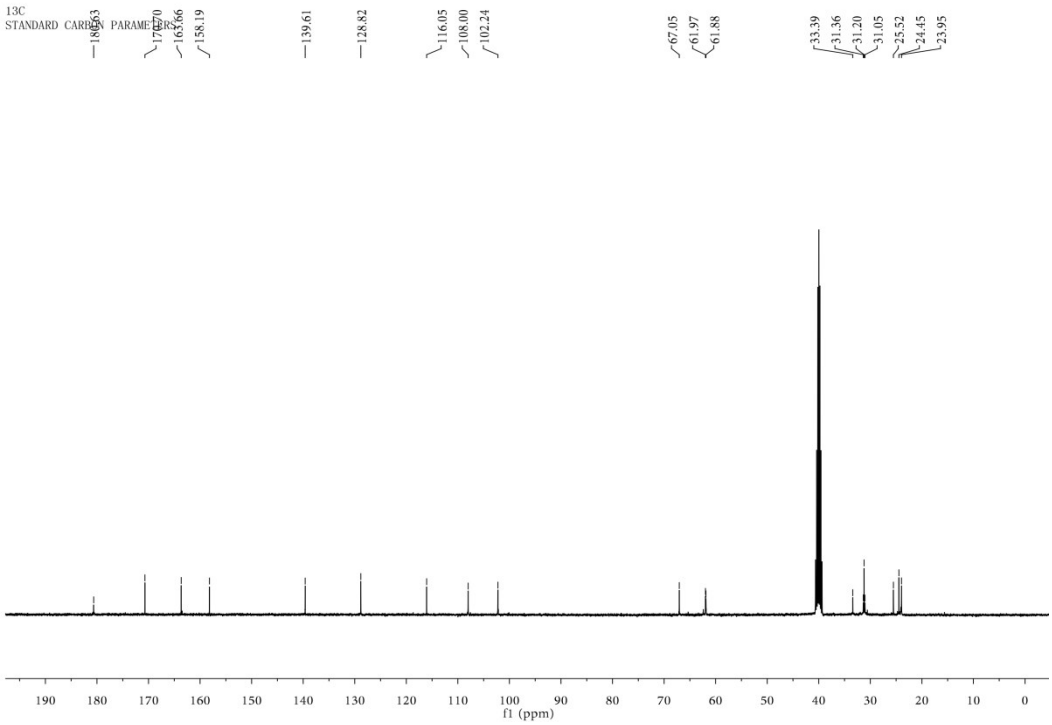
^{13}C-NMR spectrum for compound **1a**



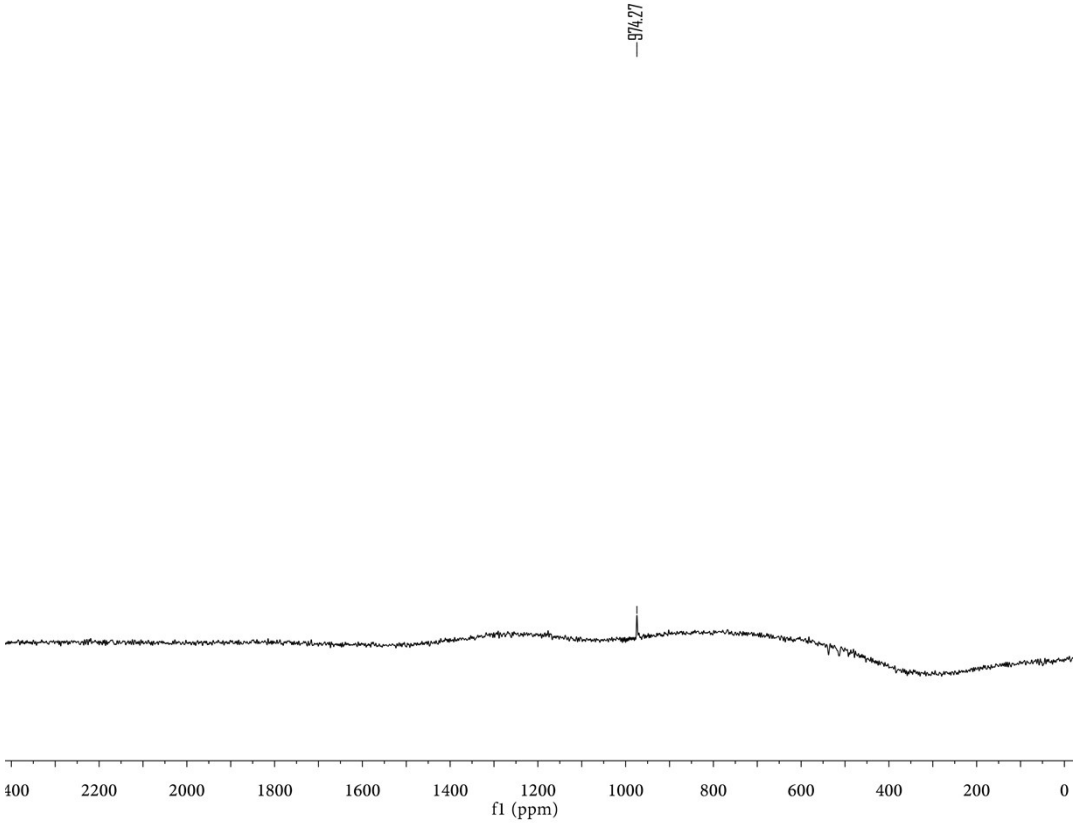
1b



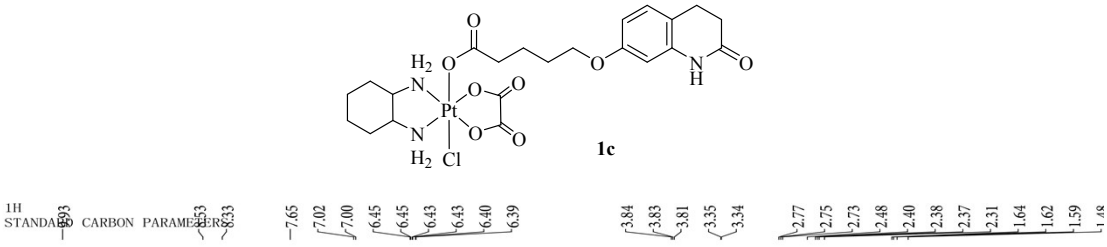
^{1}H-NMR spectrum for compound **1b**



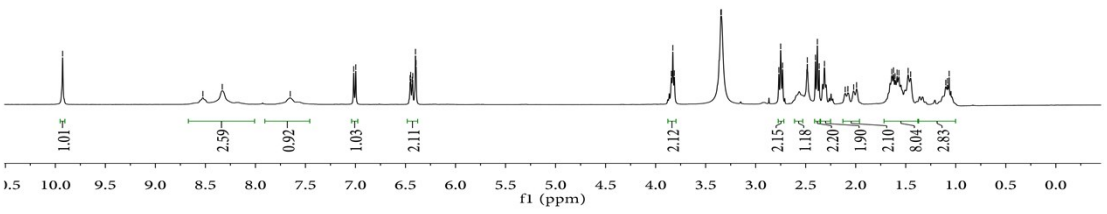
^{13}C-NMR spectrum for compound **1b**



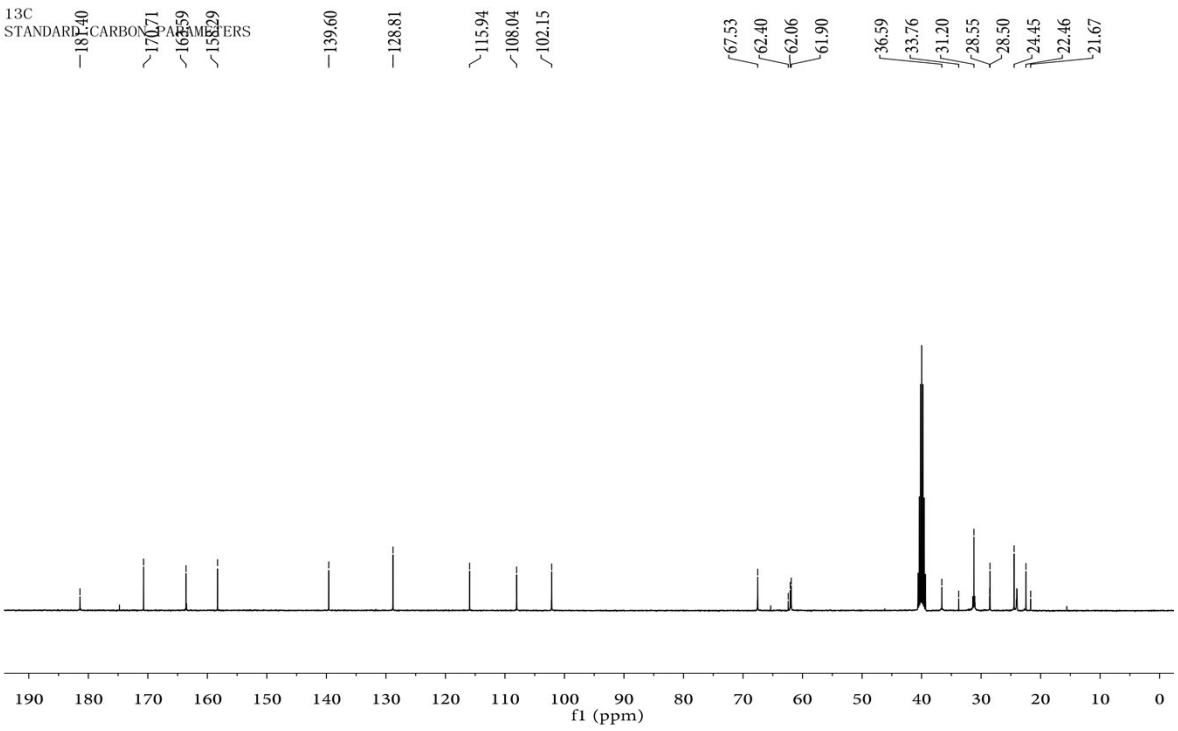
^{195}Pt-NMR spectrum for compound **1b**



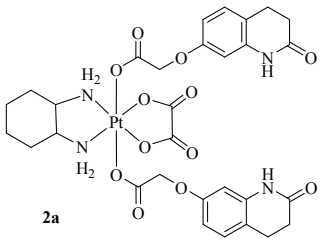
1c



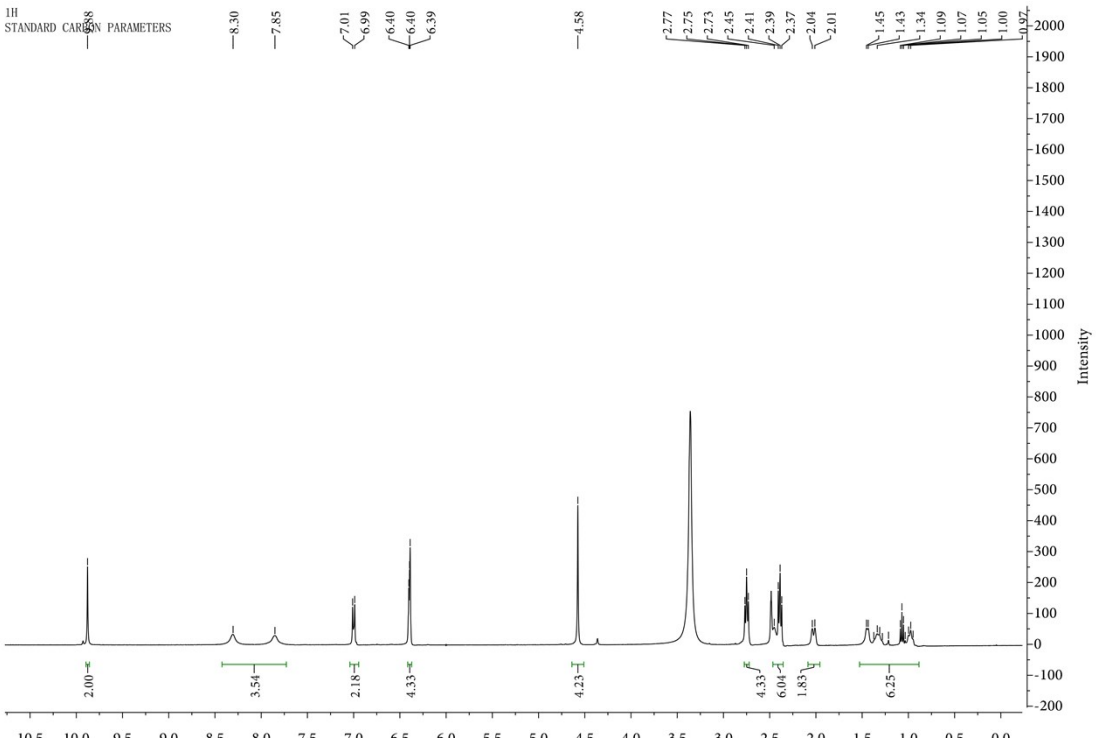
^{1}H-NMR spectrum for compound **1c**



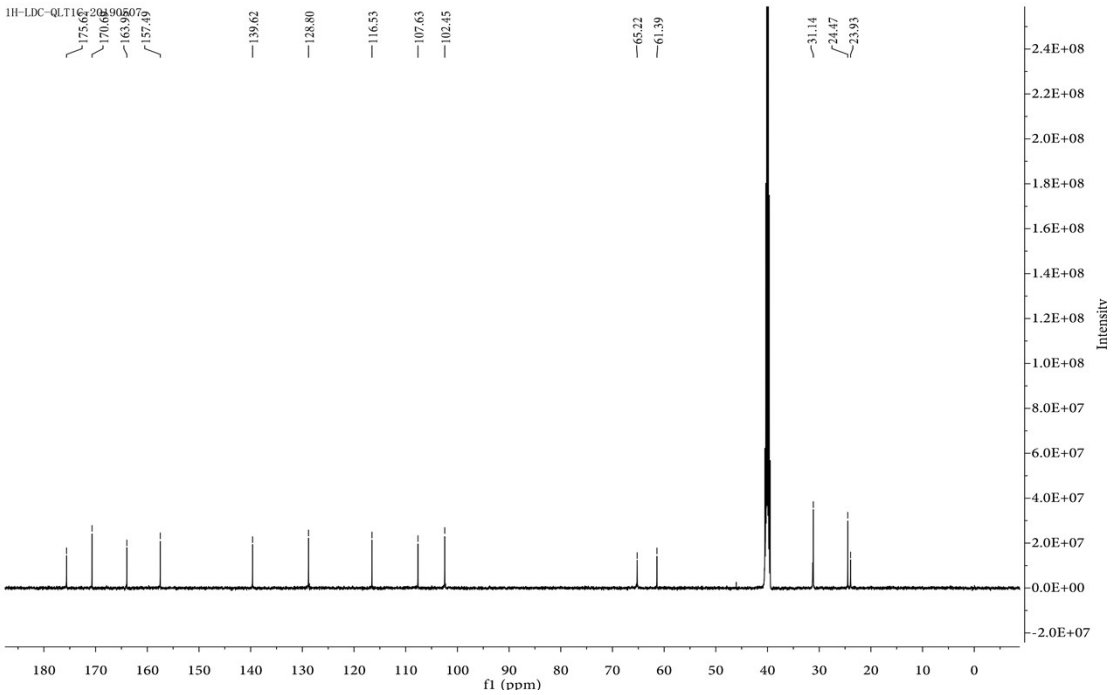
^{13}C-NMR spectrum for compound **1c**



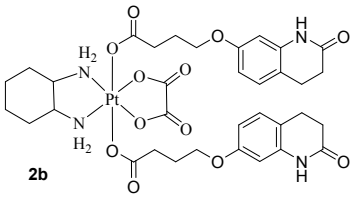
2a



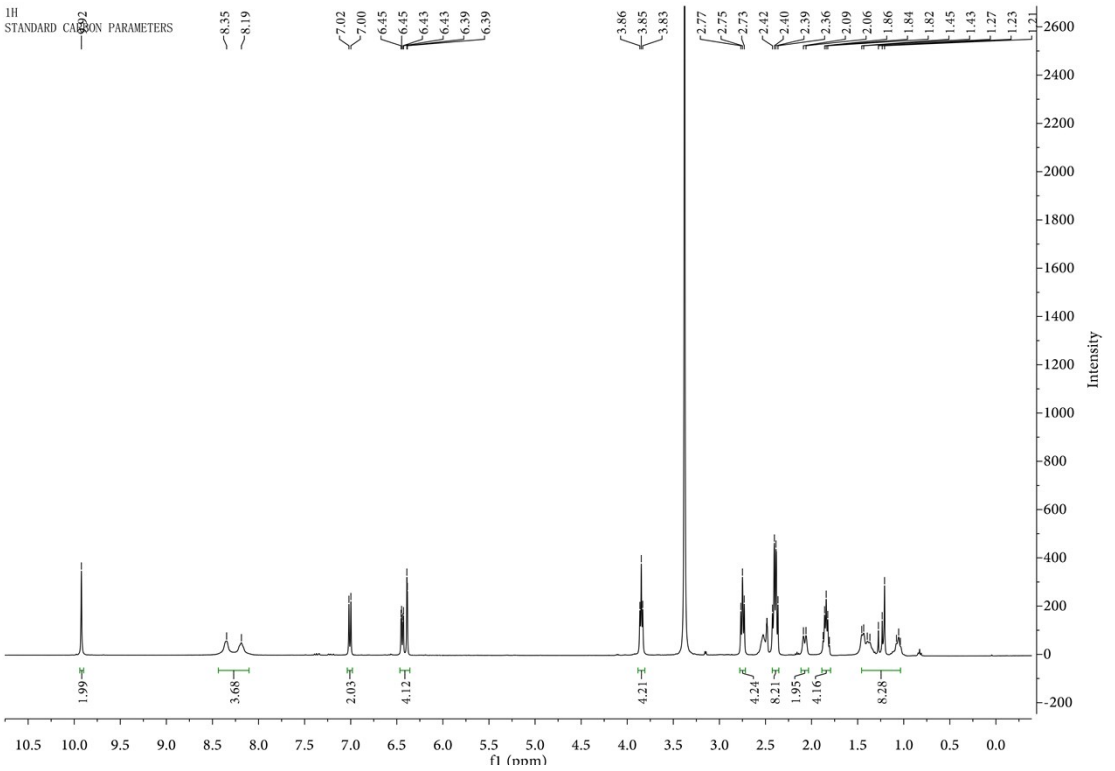
^{1}H-NMR spectrum for compound **2a**



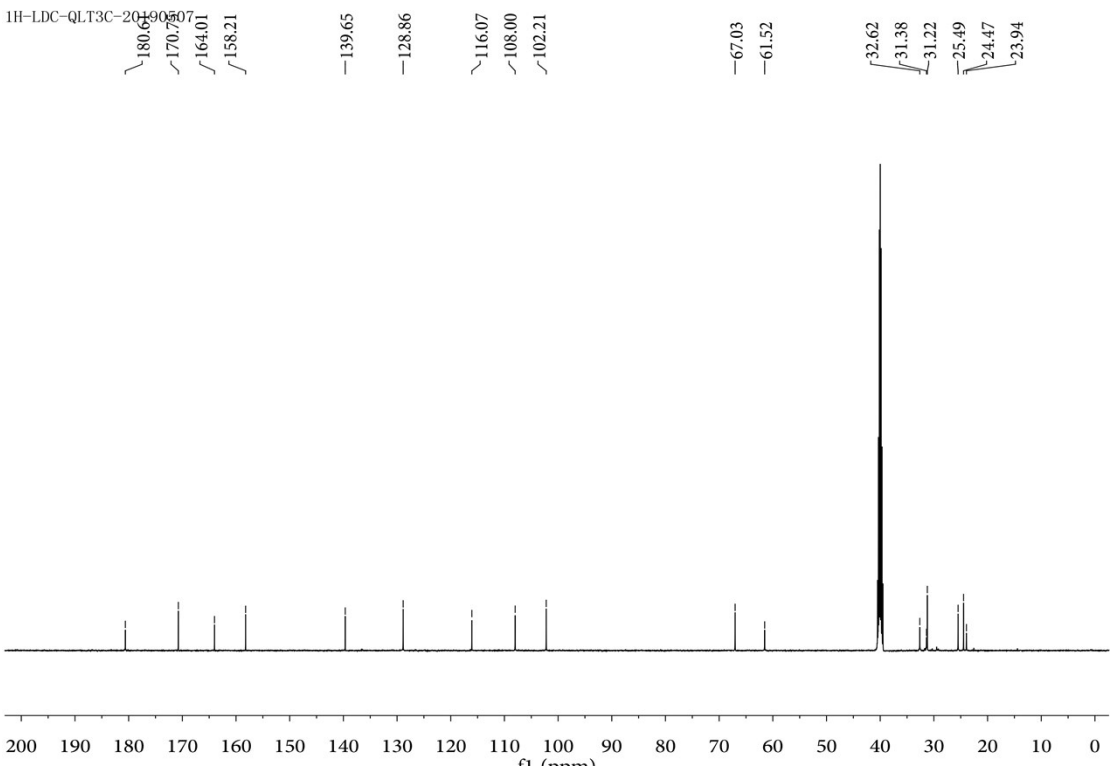
^{13}C-NMR spectrum for compound **2a**



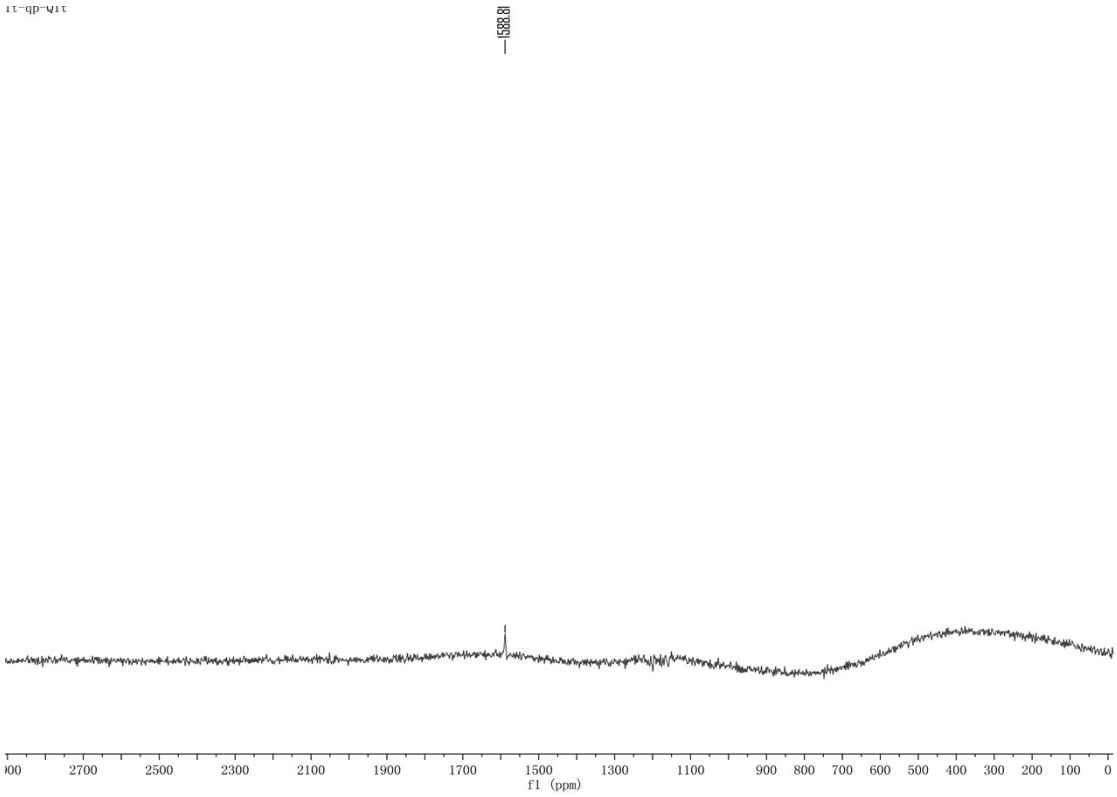
2b



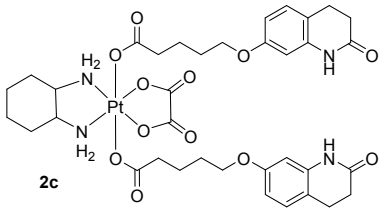
^{1}H-NMR spectrum for compound **2b**

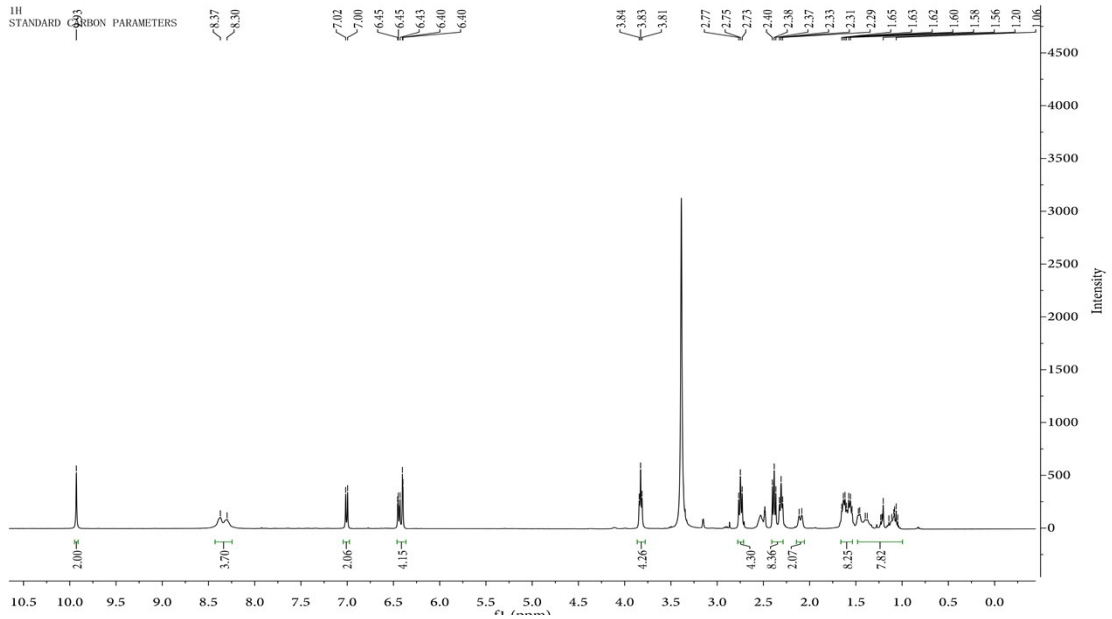


^{13}C-NMR spectrum for compound **2b**

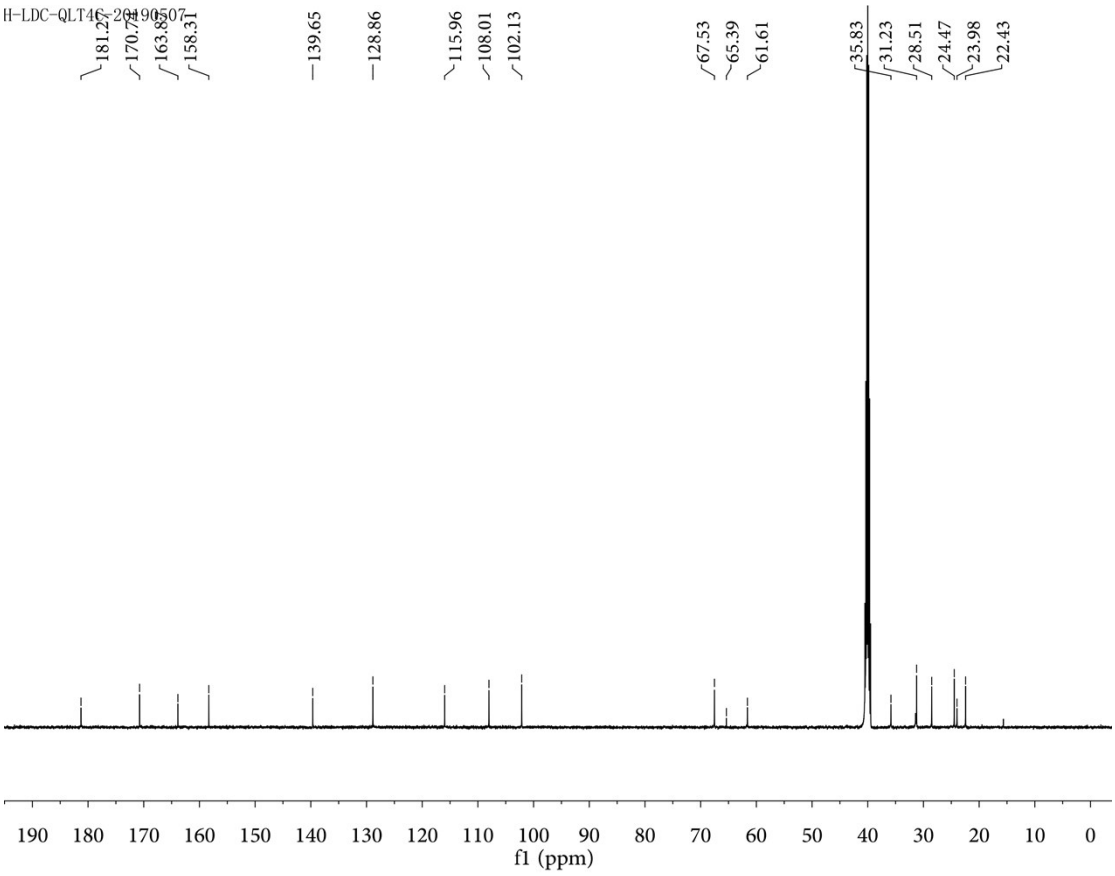


^{195}Pt-NMR spectrum for compound **2b**





^{1}H-NMR spectrum for compound **2c**



^{13}C-NMR spectrum for compound **2c**

Reference

1. Q.P. Wang, X.X. Tan, Z.F. Liu, G.S. Li, R.Y. Zhang, J.J. Wei, S.B. Wang, D.C. Li, B.Q. Wang, J. Han. Design and synthesis of a new series of low toxic naphthalimide platinum (IV) antitumor complexes with dual DNA damage mechanism. Eur. J. Phar. Sci. 124 (2018) 127–136.
2. Q.P. Wang, Z.L. Huang, J. Ma, X.L. Lu, L. Zhang, X. Wang, P. G. Wang, Design, synthesis and biological evaluation of a novel series of glycosylated platinum(IV) complexes as antitumor agents. Dalton Trans. 45 (2016) 10366–10374.