Electronic Supporting Information Materials

In vitro and *in vivo* activity of novel platinum(II) complexes with naphthalene imide derivatives inhibiting human non-small cell lung cancer cells

Guo-Bao Huang [a,1], Shan Chen [b,1], Qi-Pin Qin [a,d,*], Jin-Rong Luo [a], Ming-Xiong Tan [a,*], Zhen-Feng Wang [a], Bi-Qun Zou [c,*] and Hong Liang [d,*]

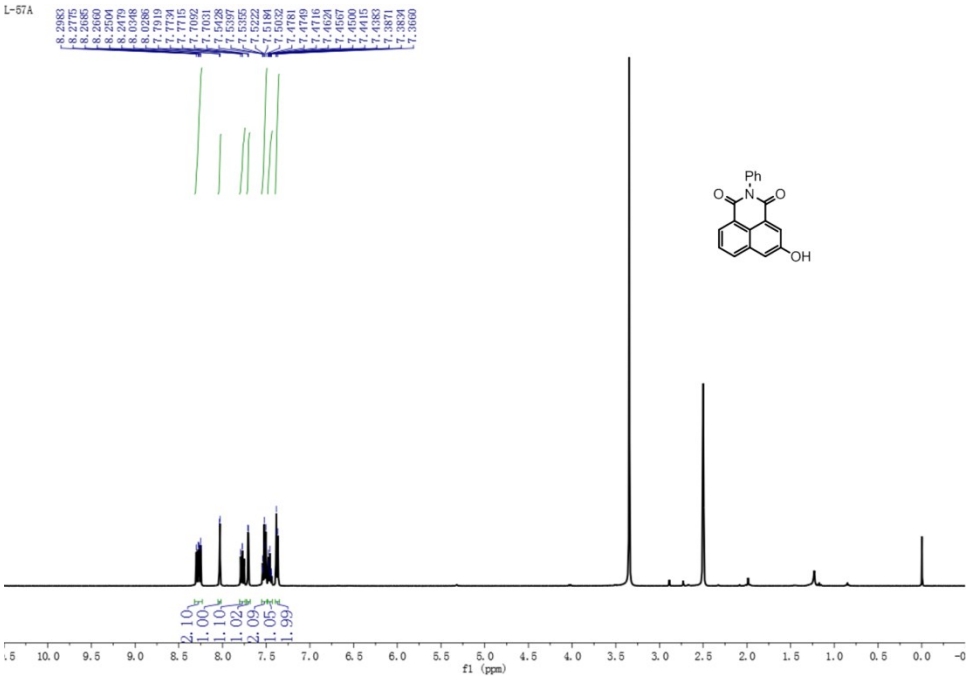

Fig. S1. ^{1}H NMR spectrum (400 MHz, DMSO, 300 K) of compound **I1**.

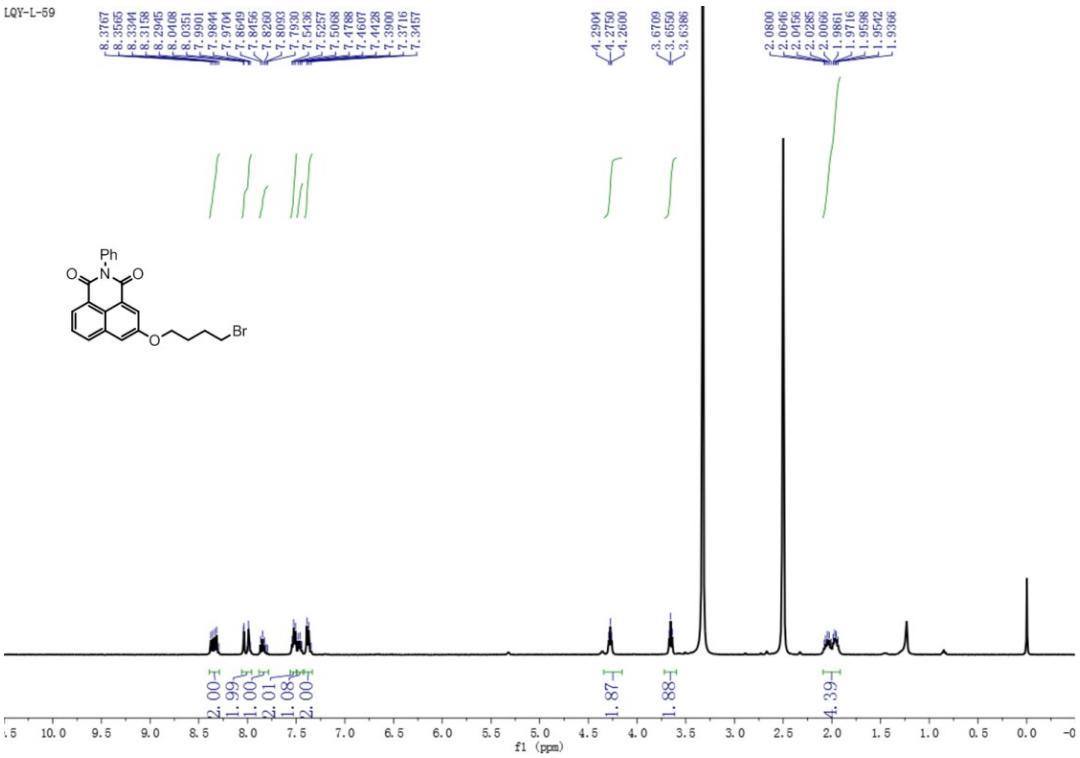

Fig. S2. ^{1}H NMR spectrum (400 MHz, DMSO, 300 K) of compound **II1**.

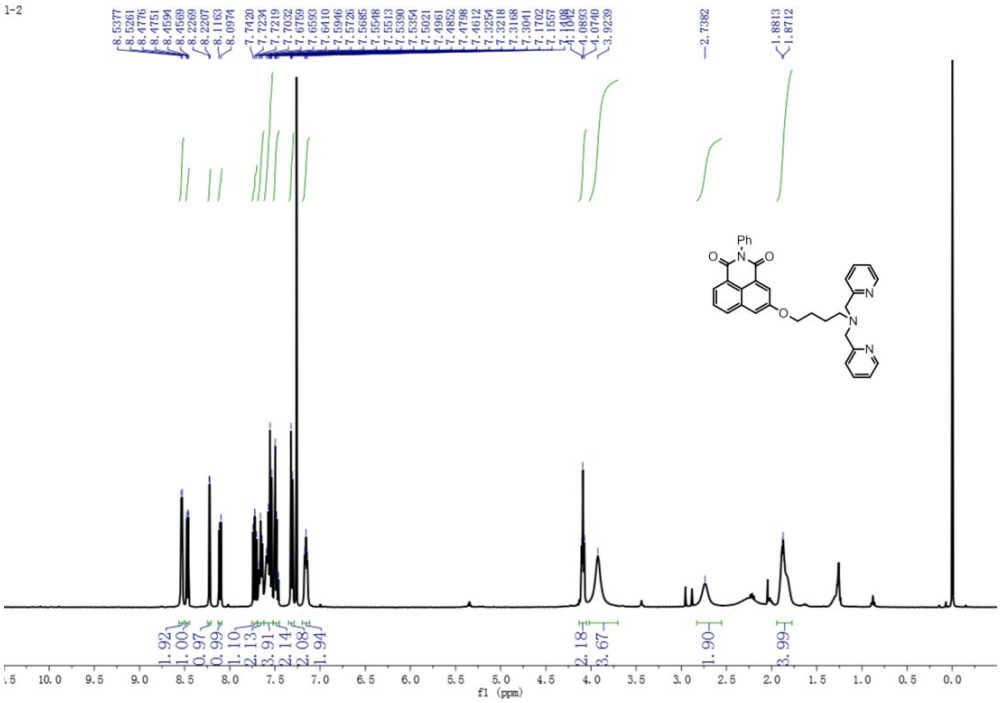

Fig. S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 300 K) of compound **L**[a].

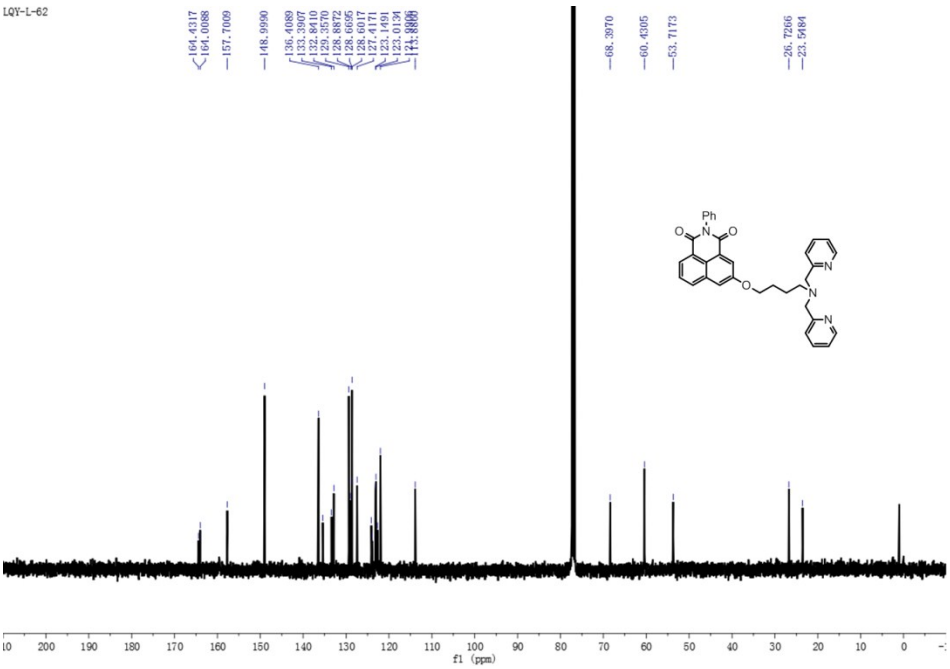

Fig. S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 300 K) of compound **L**[a].

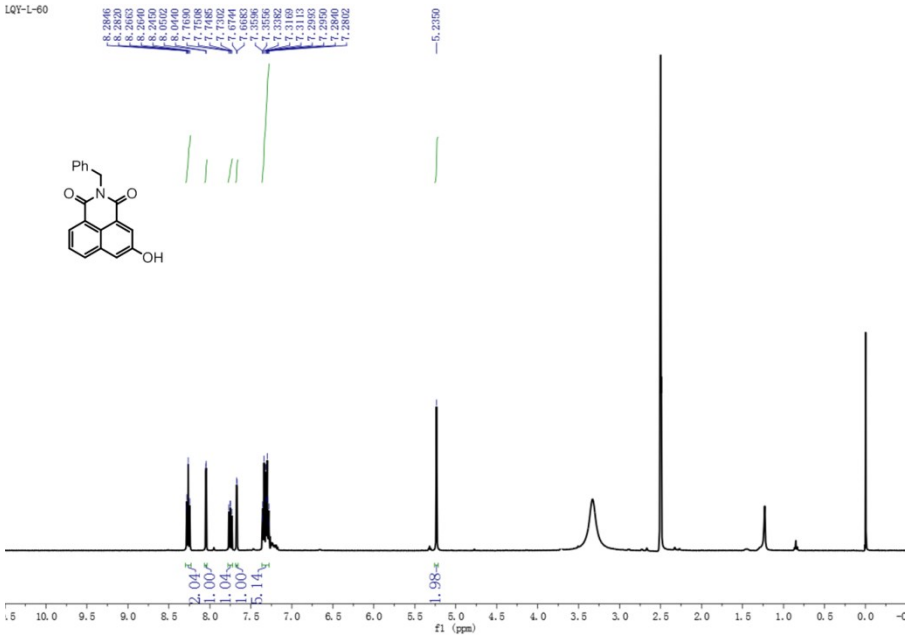

Fig. S5. ^{1}H NMR spectrum (400 MHz, DMSO, 300 K) of compound **II2**.

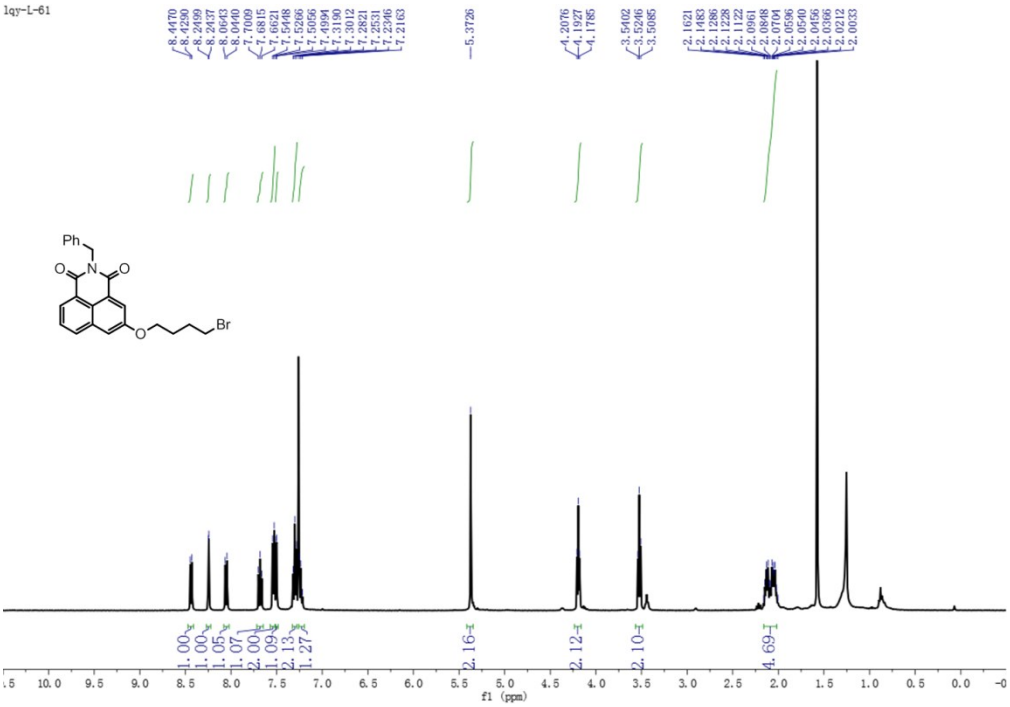

Fig. S6. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 300 K) of compound **II2**.

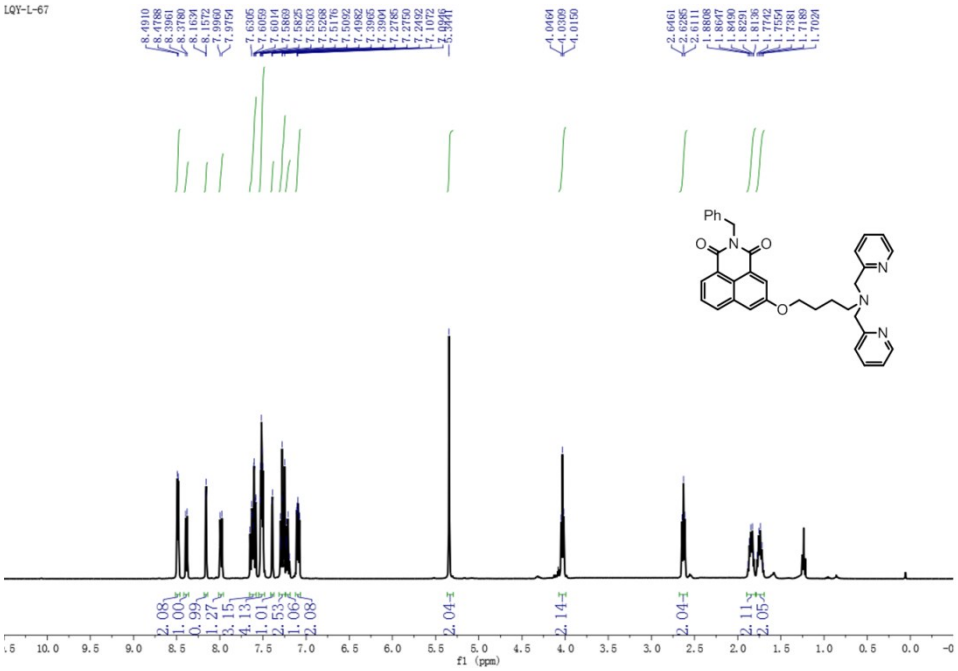

Fig. S7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 300 K) of compound **L^{b}**.

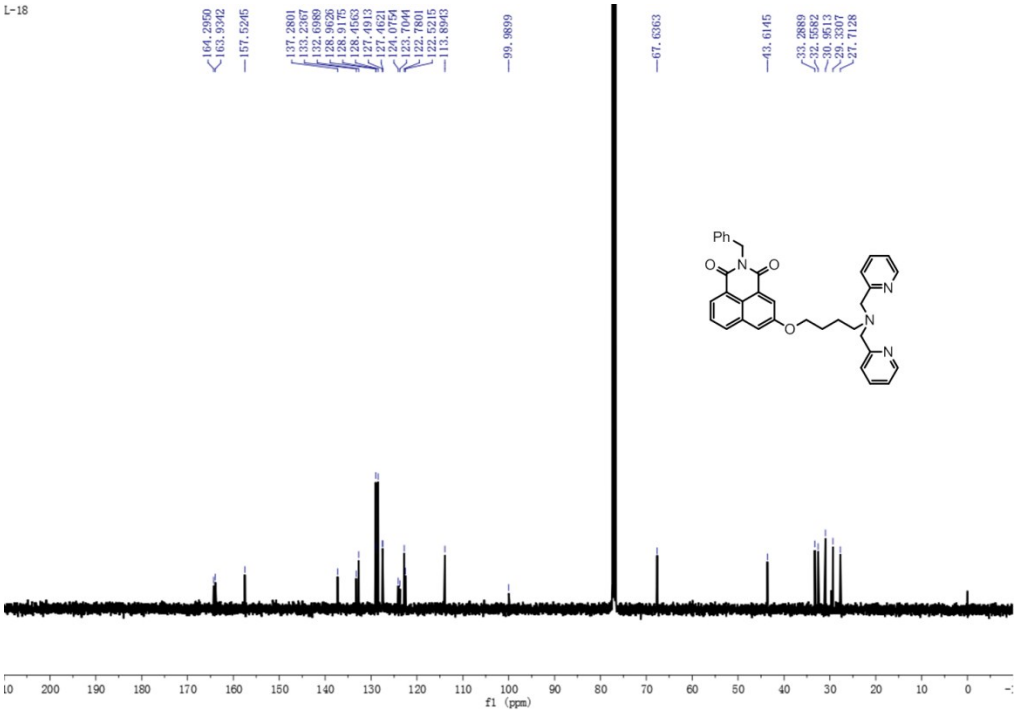

Fig. S8. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 300 K) of compound **L^{b}**.

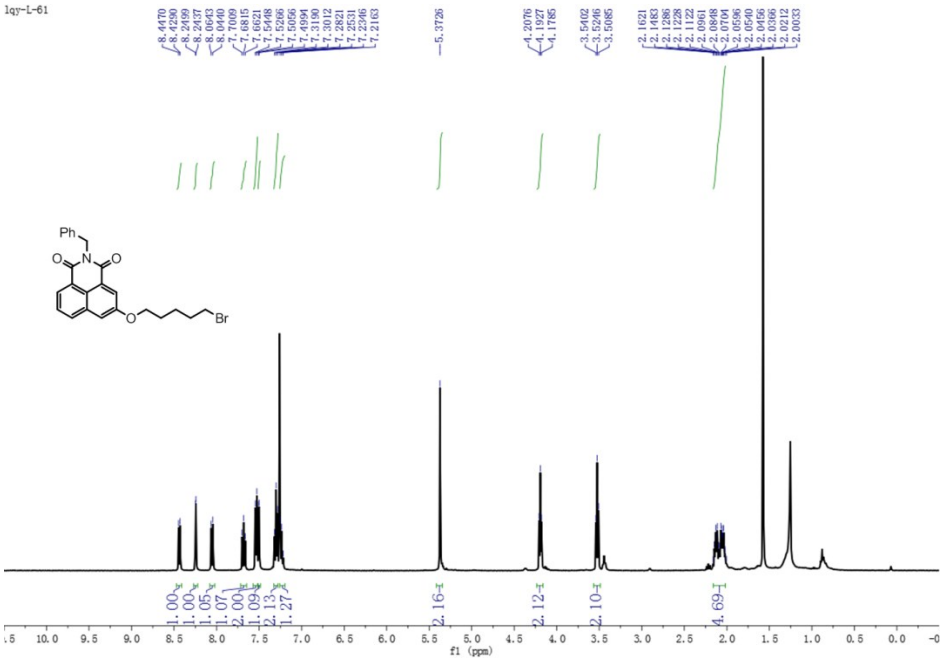

Fig. S9. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 300 K) of compound **II3**.

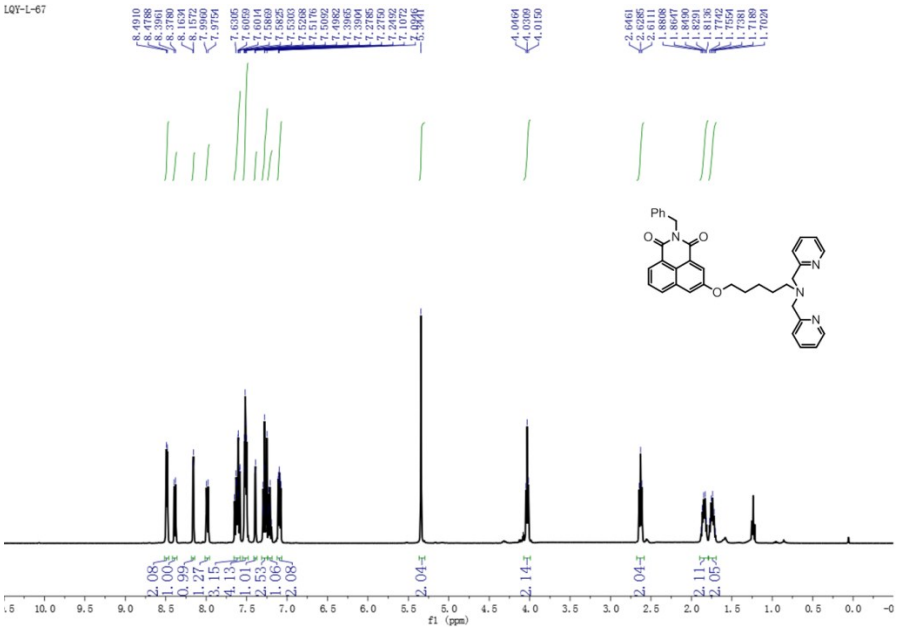

Fig. S10. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 300 K) of compound **L^{c}**.

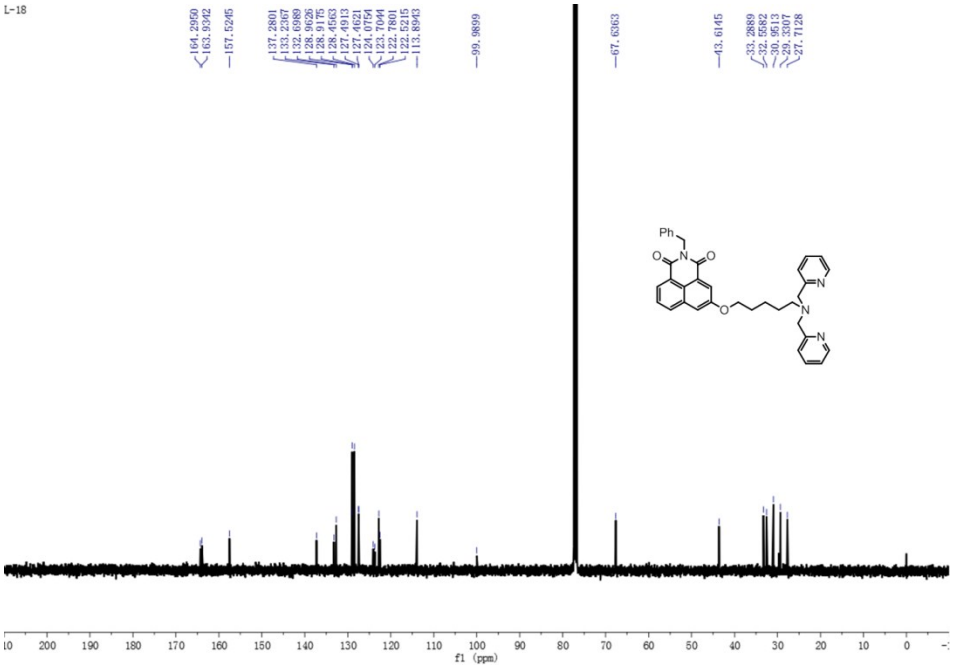

Fig. S11. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 300 K) of compound **L**^{c}.

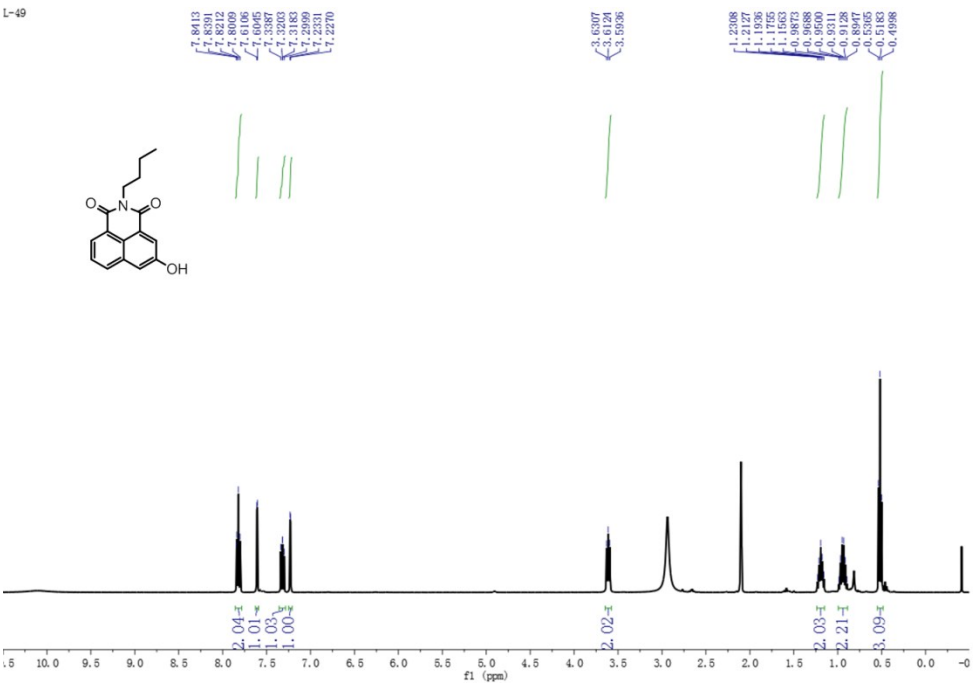

Fig. S12. ^{1}H NMR spectrum (400 MHz, D, 300 K) of compound **I3**.

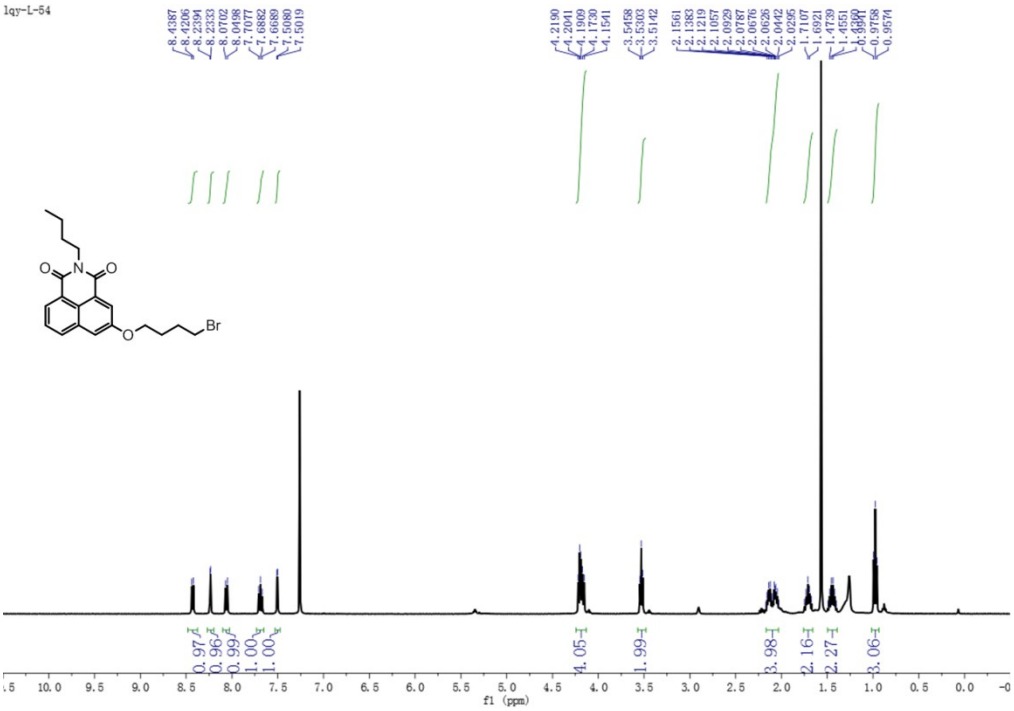

Fig. S13. ^{1}H NMR spectrum (400 MHz, D, 300 K) of compound **II4**.

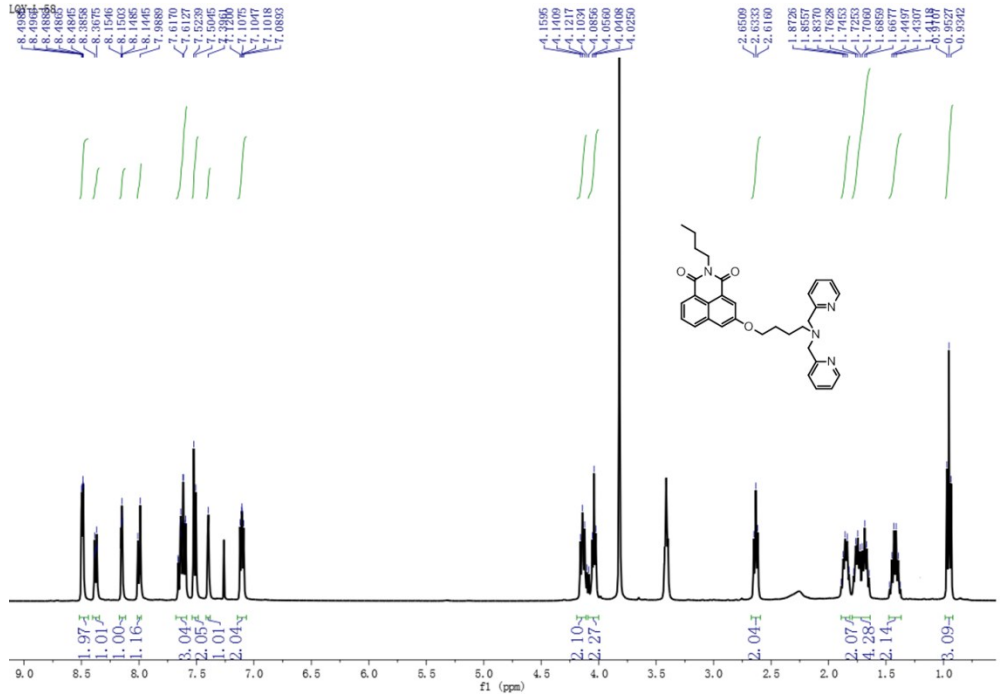

Fig. S14. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 300 K) of compound **L^{d}**.

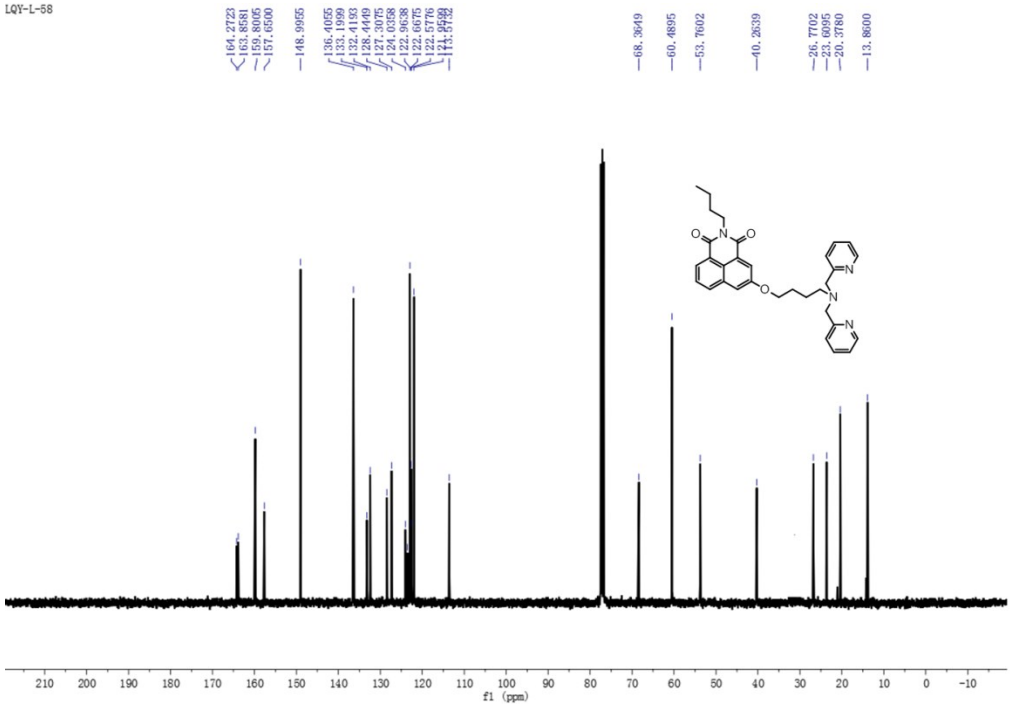

Fig. S15. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 300 K) of compound **L^{d}**.

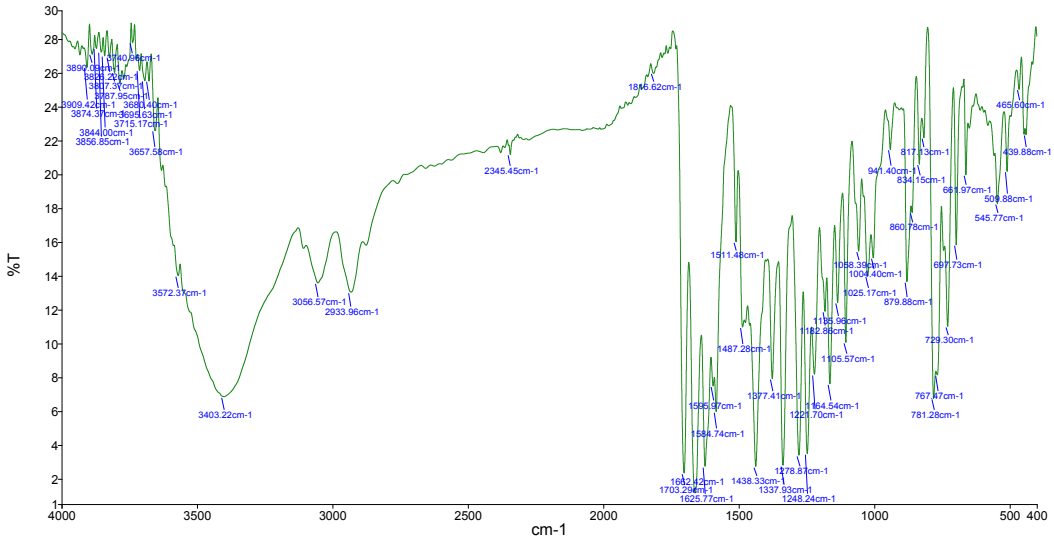

Fig. S16. IR (KBr) spectra of **1**

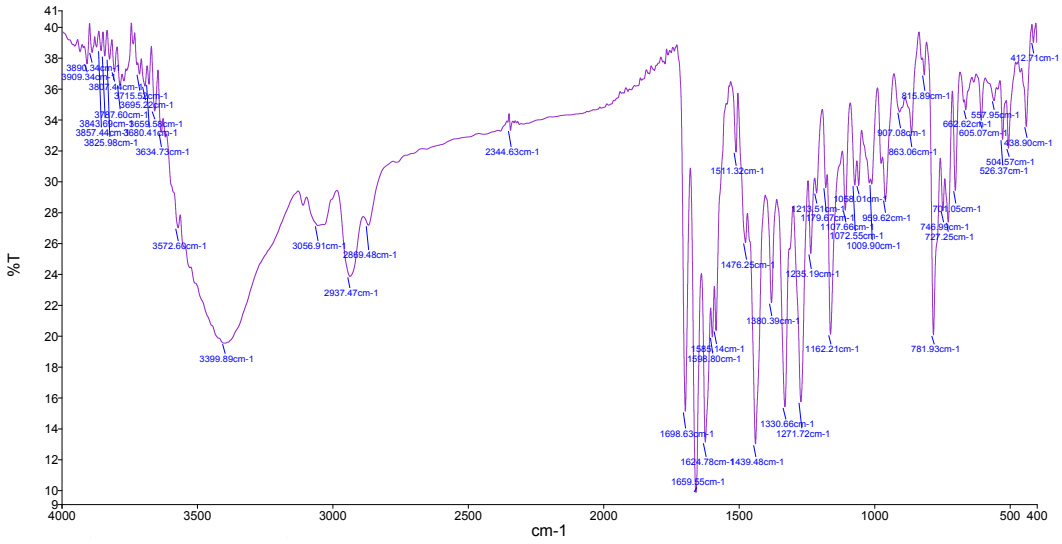

Fig. S17. IR (KBr) spectra of **2**

Fig. S18. IR (KBr) spectra of **3**

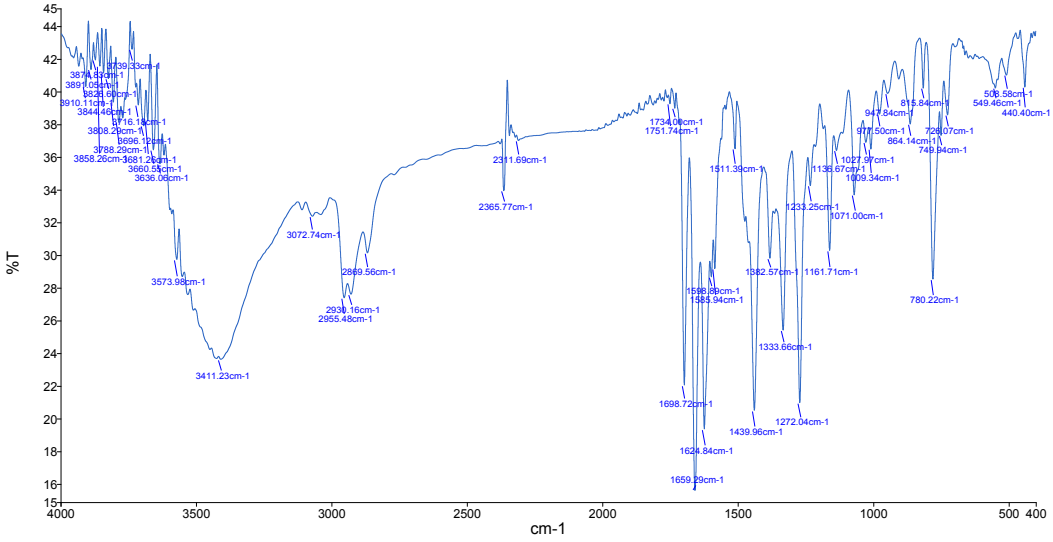

Fig. S19. IR (KBr) spectra of **4**

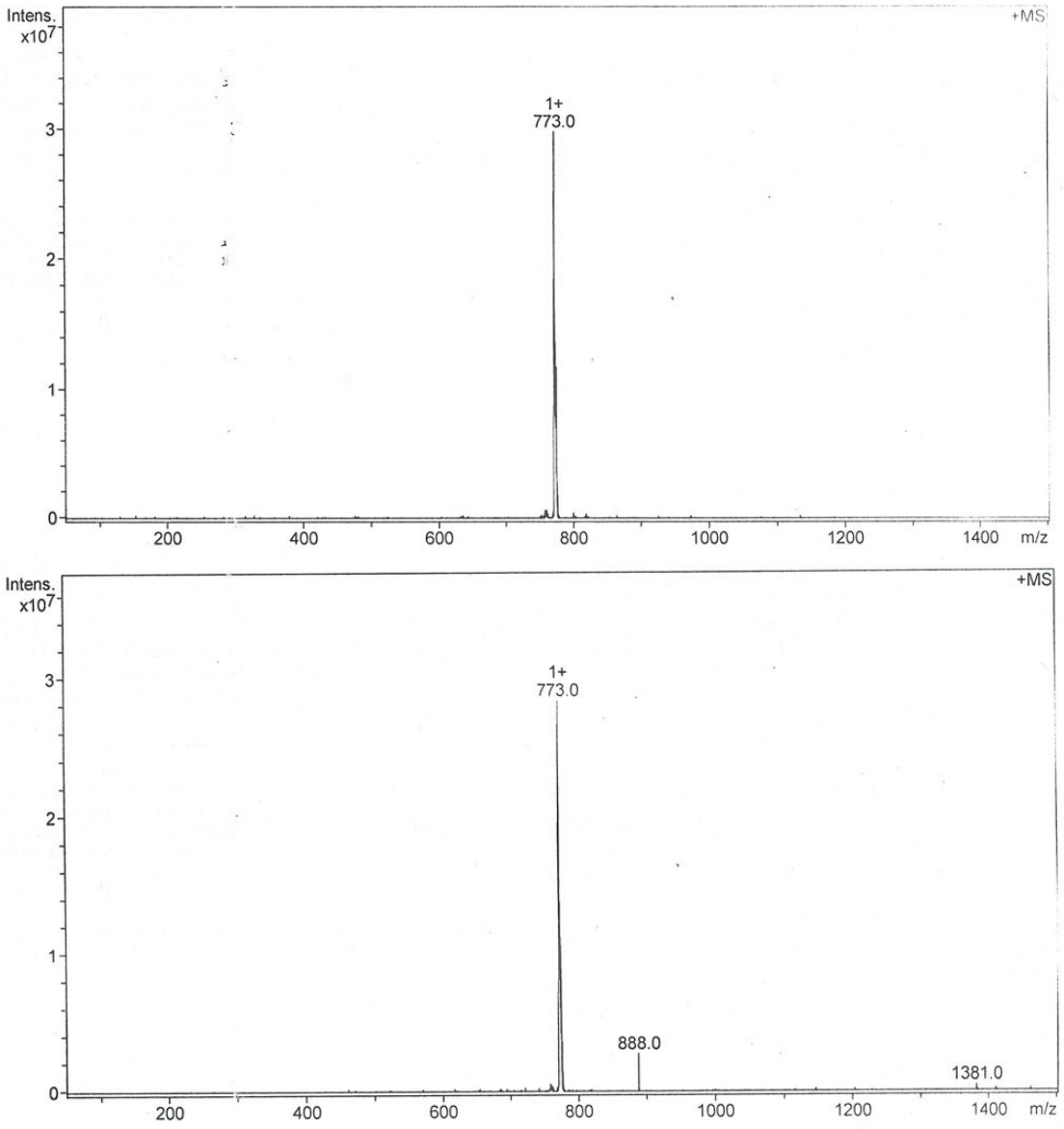

Fig. S20. The mass spectra of **1** (4.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 48 h (down), respectively.

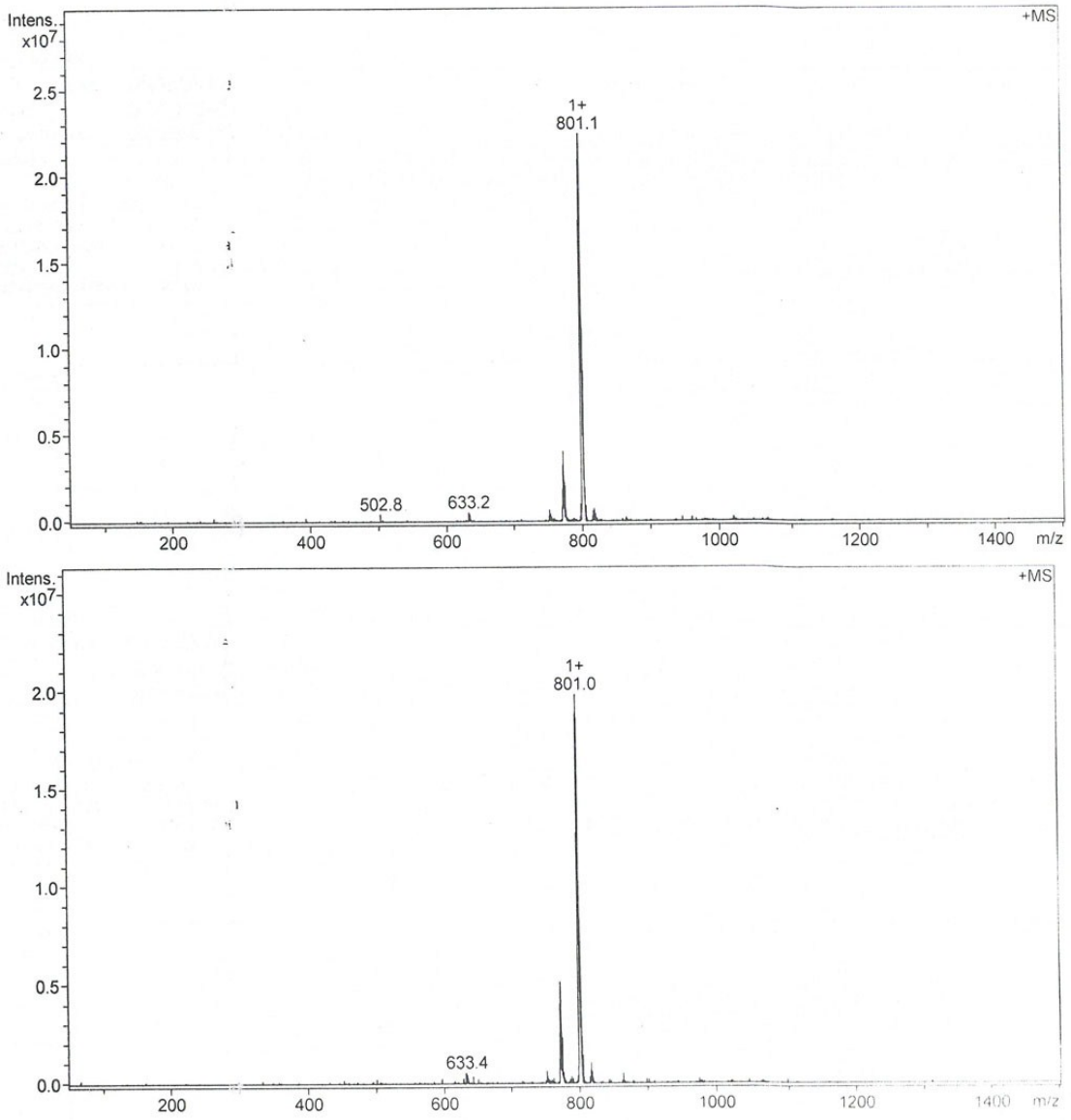

Fig. S21. The mass spectra of **2** (4.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 48 h (down), respectively.

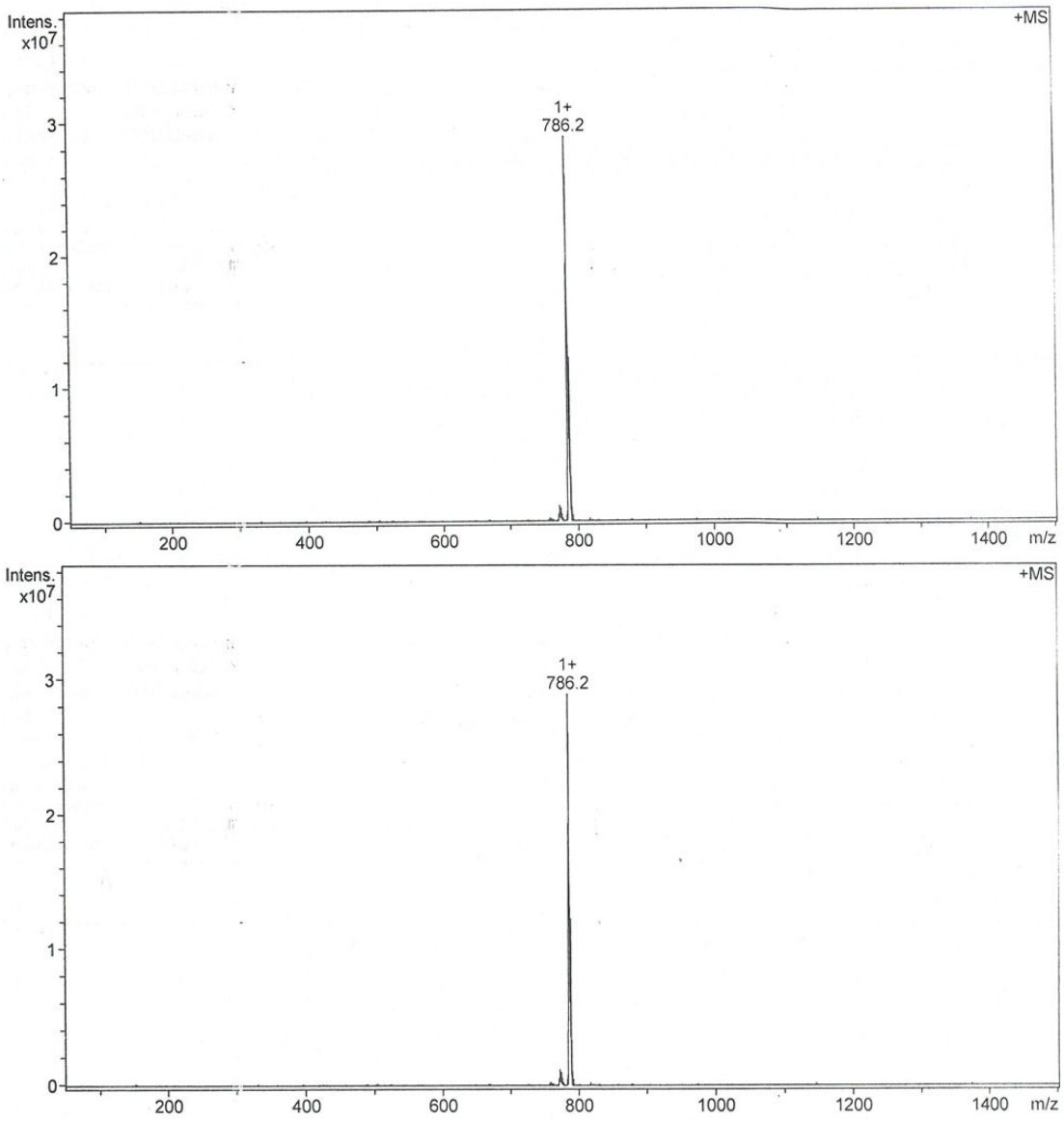

Fig. S22. The mass spectra of **3** (4.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 48 h (down), respectively.

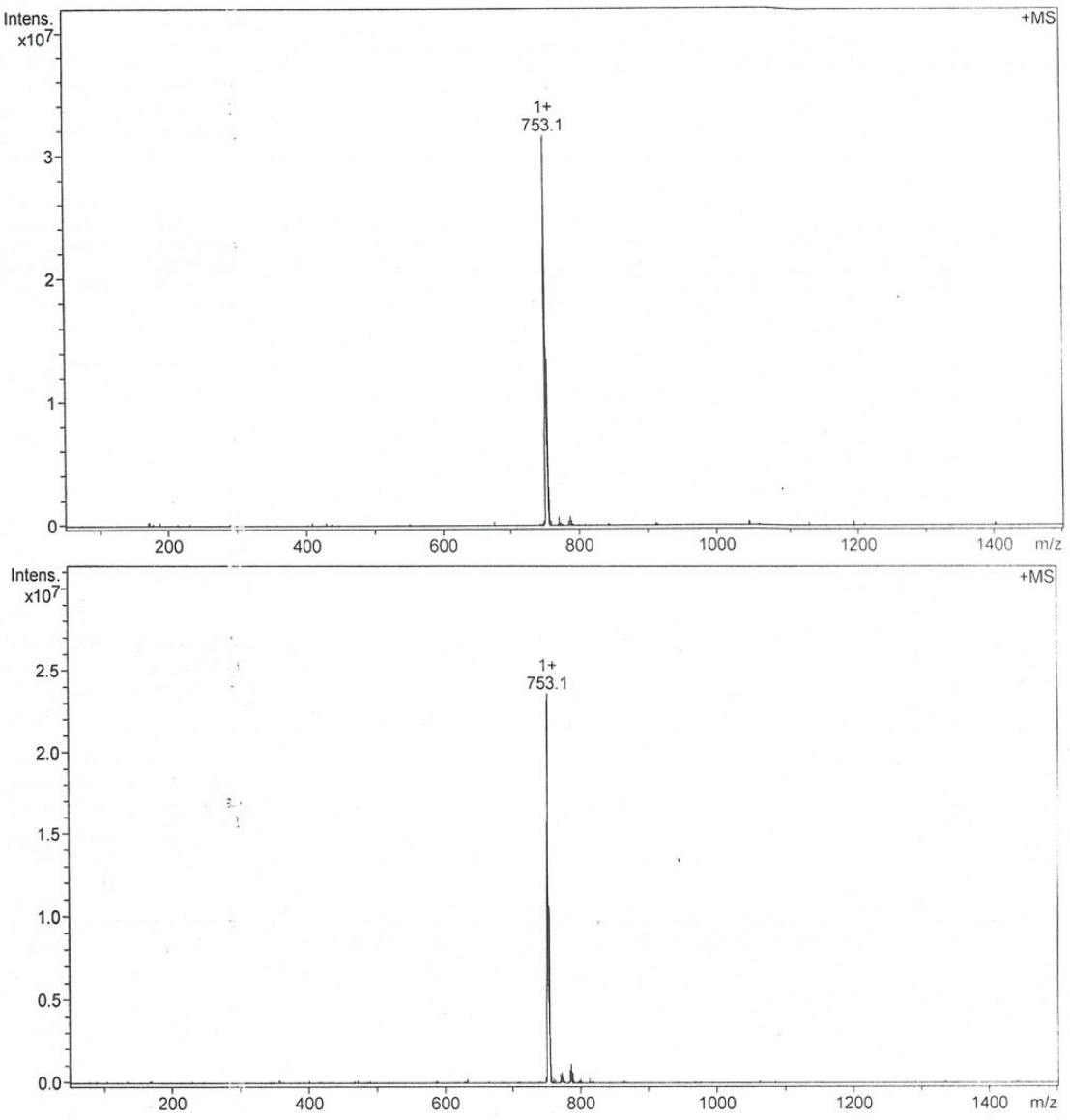

Fig. S23. The mass spectra of **4** (4.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 48 h (down), respectively.

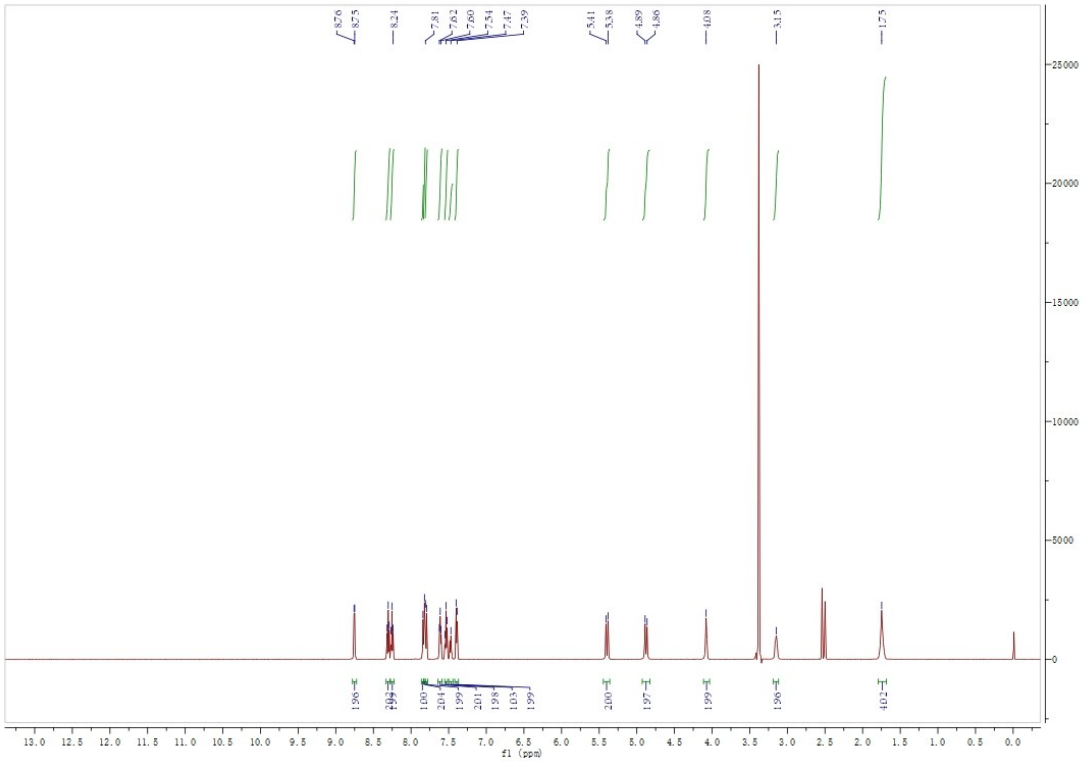

Fig. S24. ^{1}H NMR (600MHz, DMSO-d_6) for **1**

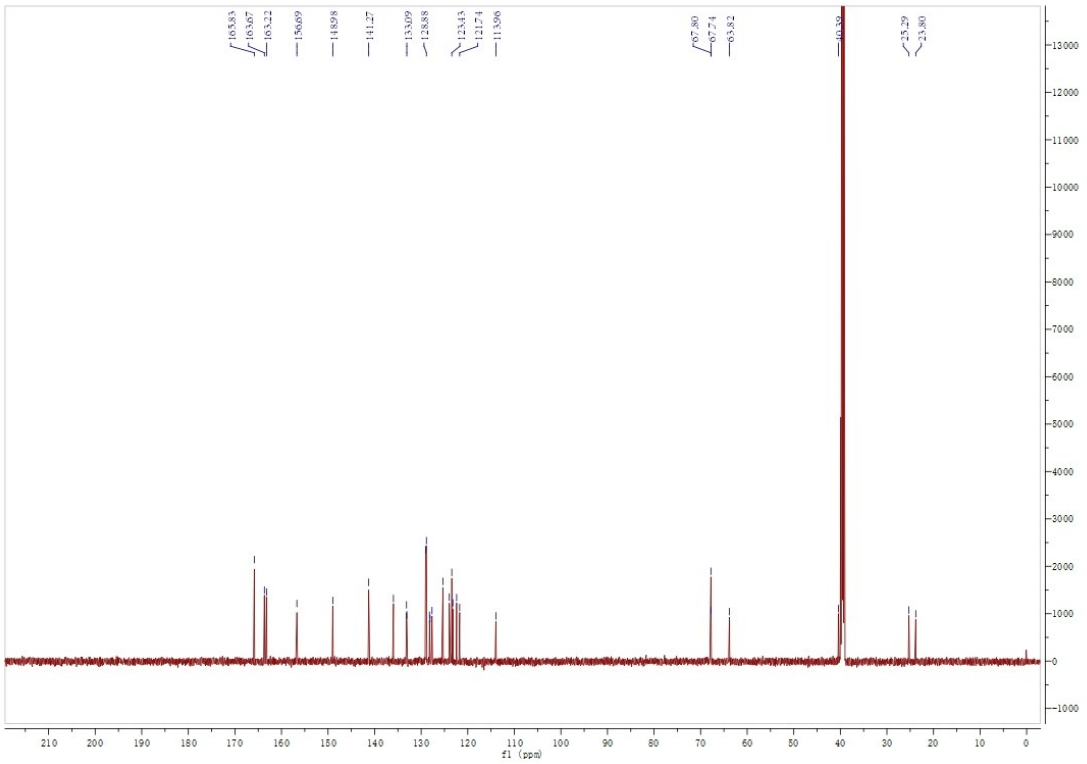

Fig. S25. ^{13}C NMR (151MHz, DMSO-d_6) for **1**

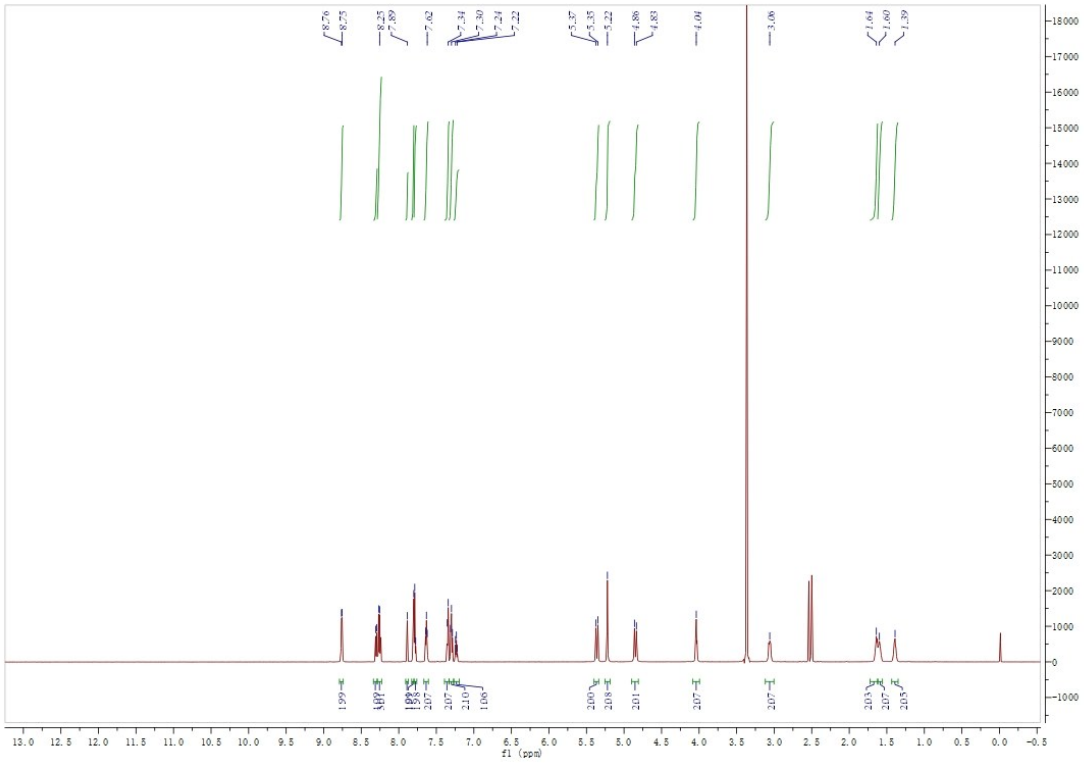

Fig. S26. ^{1}H NMR (600MHz, DMSO-d_6) for **2**

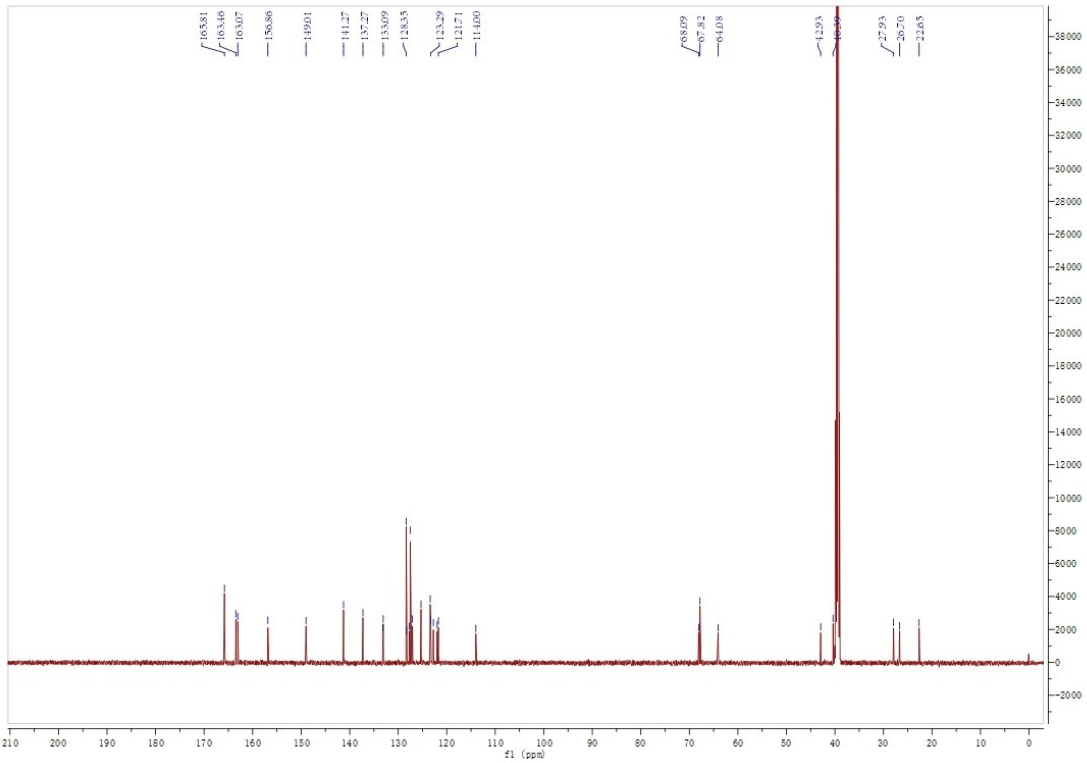

Fig. S27. ^{13}C NMR (151MHz, DMSO-d_6) for **2**

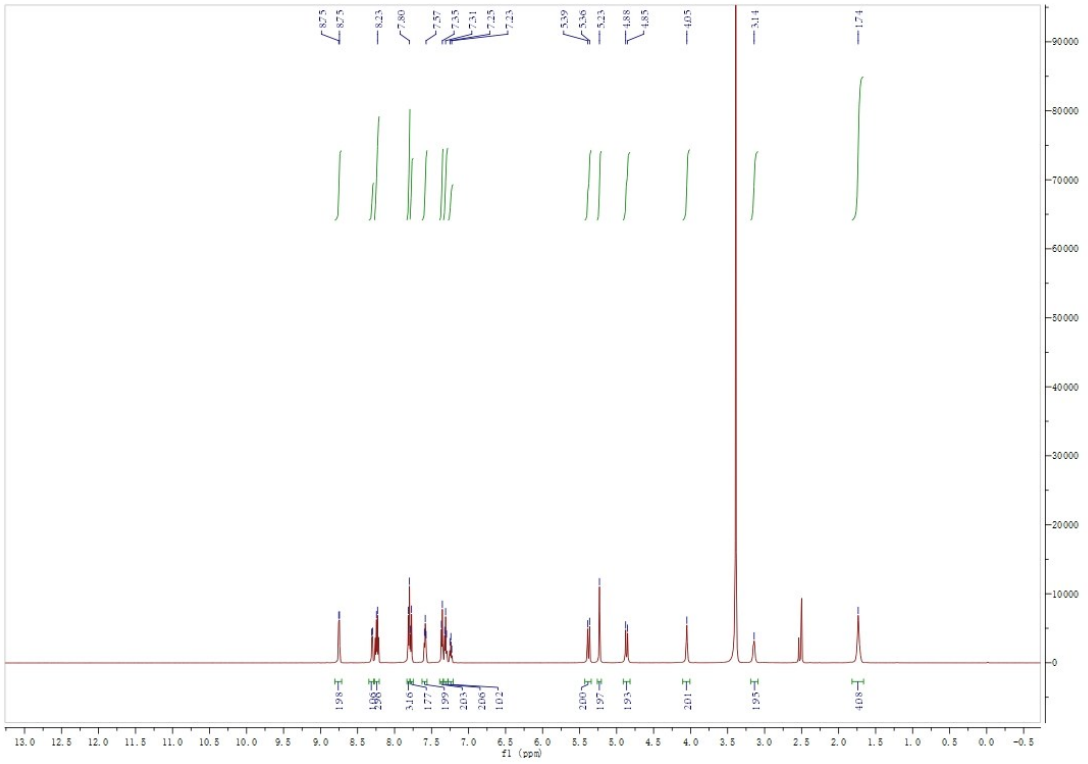

Fig. S28. ^{1}H NMR (600MHz, DMSO-d_6) for **3**

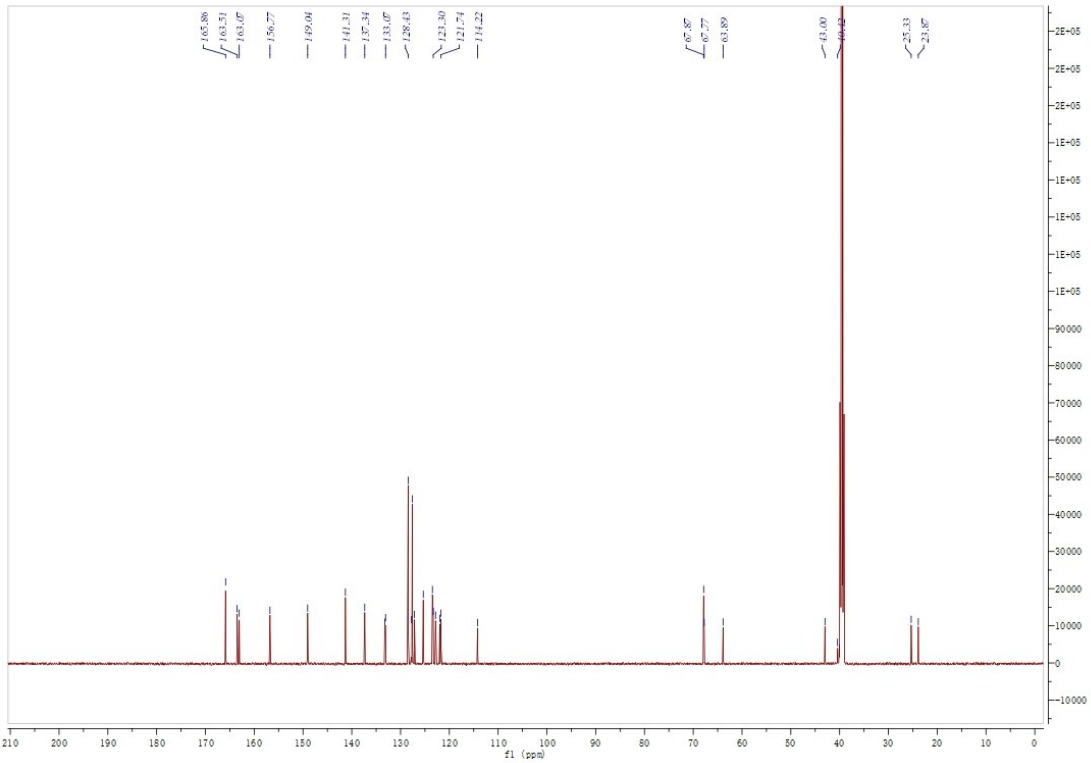

Fig. S29. ^{13}C NMR (151MHz, DMSO-d_6) for **3**

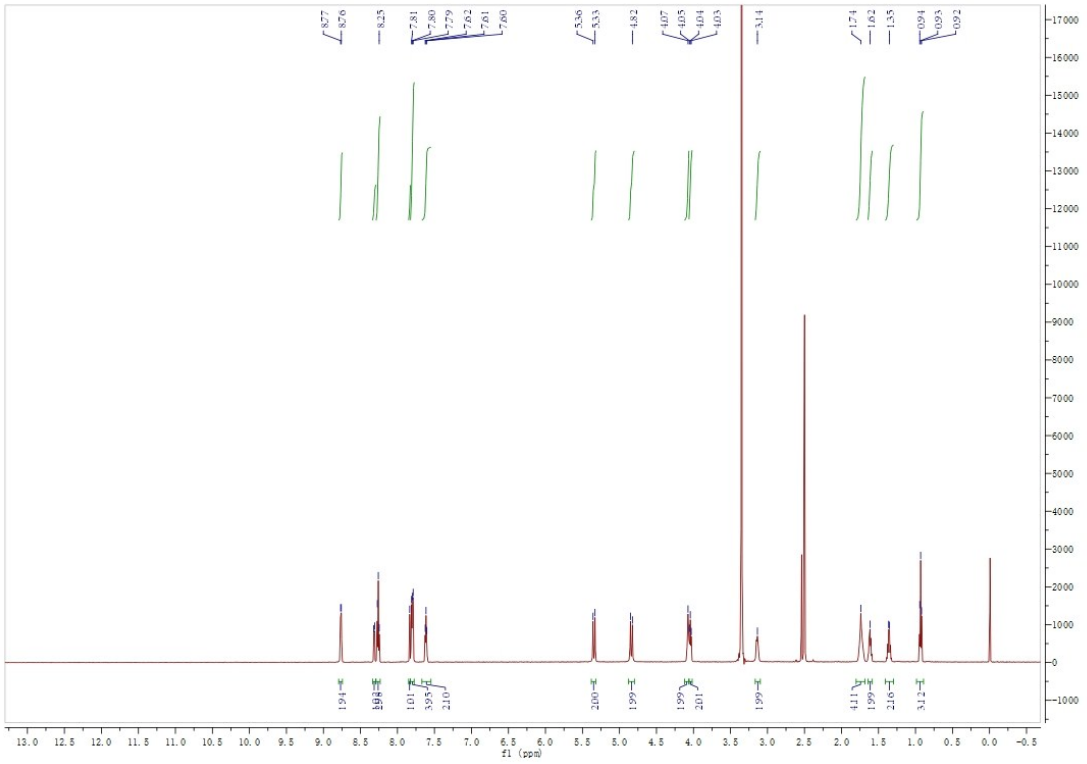

Fig. S30. ^{1}H NMR (600MHz, DMSO-d_6) for **4**

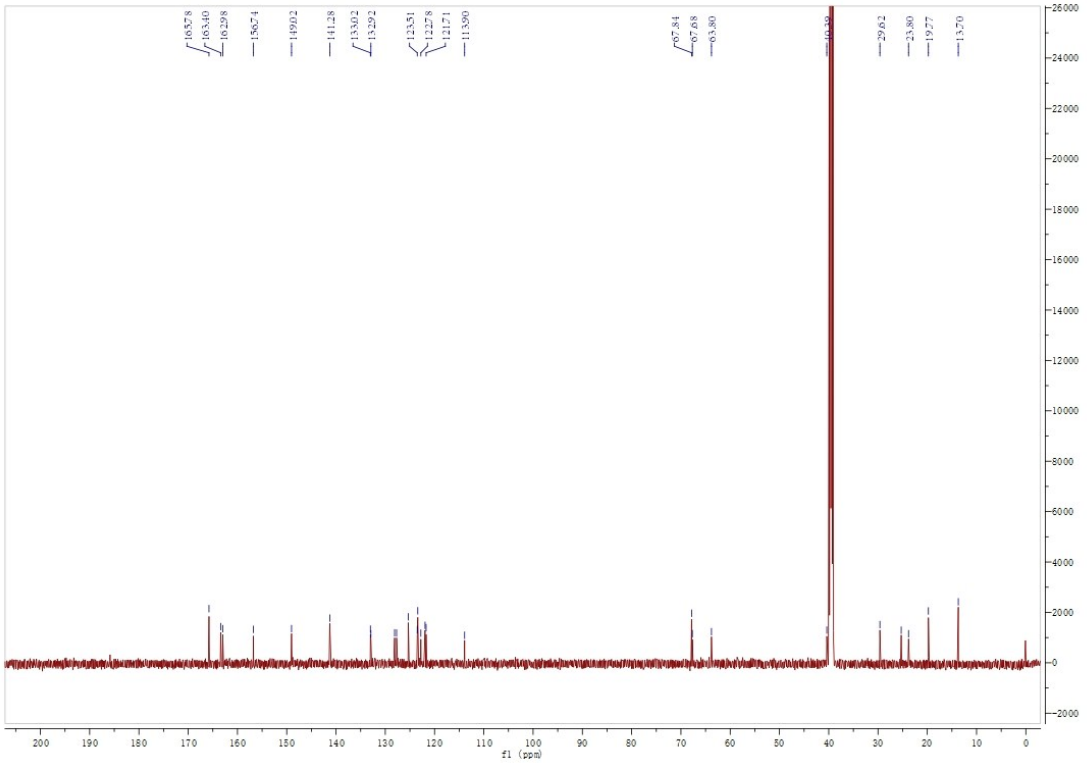

Fig. S31. ^{13}C NMR (151MHz, DMSO-d_6) for **4**

Table S1. The tumor volume in treated and non-treated mice from the date of surgery to the study end-point in the NCI-H460 xenograft model.

Group	Tumor Volume (mm^3)		T/C (%)
	(start)	(end)	
Control	72.42±12.94	1544.41±225.83	-
3 (10 mg/kg)	72.69±5.39	749.70±163.51	47.5[a]

[a] mean $p<0.05$, *p vs* vehicle control

Table S2. Average body weight in treated and non-treated mice from the date of surgery to the study end-point in the NCI-H460 xenogfart model.

Group	Body Weight (g)		RBW (%)
	(start)	(end)	(end)
Control	18.03±1.28	20.28±0.47	112.48
3 (10 mg/kg)	17.82±1.31	20.27±0.67	113.75

Table S3. In vivo anticancer activity of **3** toward NCI-H460 tumor xenograft.

Group	average tumor weight(mean ± SD g)	inhibition of tumor growth(%)
Control	1.56±0.14	-
3 (10 mg/kg)	0.92±0.19	40.7[a]

[a] mean $p<0.05$, *p vs* control.

Experimental methods

1.1 Materials

Tris, RNase A, and propidium iodide (PI) were purchased from Sigma. The antibody of c-myc, hTERT, apaf-1, cytochrome c (cyt c) and bcl-2 were purchased from Abcam. Unless otherwise stated, spectroscopic titration experiments were carried out in 10 mM Tris-HCl (pH 7.35) containing 100 mM KCl. All the cancer and normal cell lines were obtained from the Shanghai Institute for Biological Science (China). Stock solutions of the each compound (2.0×10^{-3} M) was made in DMSO, and further dilutions to working concentrations were made with corresponding buffer.

1.2 Instrumentation

Infrared spectra were obtained on a Perkine Elmer FT-IR Spectrometer. Elemental analyses (C, H, N) were carried out on a Perkin Elmer Series II CHNS/O 2400 elemental analyser. NMR spectra were recorded on a Bruker AV-500/400 NMR spectrometer. ESI-MS spectra were obtained on Thermofisher Scientific Exactive LC-MS spectrometer (ThermalElctronic, USA). MTT assay was performed on M1000 microplate reader (Tecan Trading Co. Ltd, Shanghai, China). Cell cycle and apoptosis analysis was recorded on FACS Aria II Flow Cytometer (BD Biocsiences, San Jose, USA).

1.3 Cytotoxicity assay (MTT assay)

The cell culture was maintained on RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin in 25 cm^2 culture flasks at 37 °C humidified atmosphere with 5% CO_2. All the cells to be tested in the following assays have a passage number of 3-6.

The cells 5.0×10^3 per well were seeded in triplicate in 96-well plates and incubated for 24 h at 37 °C and 5% CO_2/95% air. Then graded amounts of each compound was

added to the wells in 10 μL of FBS free culture medium and the plates were incubated in a 5% CO_2 humidified atmosphere for 48 h. Six replica wells were used as controls. Cells were grew for 12 h before treatment to reach 70% confluency and 20 μL of tested various concentrations of compounds were added to each well. The final concentration of the each tested compound were kept at 1.25, 2.5, 5, 10, 20, 40, 50, 60, 100, 150 μM, respectively. After 48 h of culture, 0.1 mg of MTT (in 20 μL of PBS) was added to each well, and cells were incubated at 37 °C for 6 h. The formed formazan crystals were then dissolved in 100 μL of DMSO and the absorbance was read by enzyme labeling instrument with 490/630 nm double wavelength measurement. The final IC_{50} values were calculated by the Bliss method (n = 5).

1.4 Cell apoptosis analysis

The apoptosis was detected by flow cytometric analysis of annexin V staining. Briefly, adherent NCI-H460 cells were harvested and suspended in the annexin-binding buffer (5×10^5 cells/mL). Then, the NCI-H460 cells after treated with **3** (0.10 μM) and **4** (18.87 μM) were incubated with annexin V-FITC and PI for 1 h at room temperature in the dark and immediately analyzed by flow cytometry. The data are presented as biparametric dot plots showing PI red fluorescence vs annexin V-FITC green fluorescence.

1.5 Telomerase activity

The telomerase extract was prepared from the NCI-H460 cells: a total of 5×10^6 NCI-H460 cells untreated or treated with **3** (0.10 μM) and **4** (18.87 μM) were pelleted, and the NCI-H460 cells were washed with 5 mL of PBS, scraped and lysed for 30 min on ice. Finally, the lysate was centrifuged at 13000 rpm for 30 min at 4 °C; the supernatant was collected and stored at −80 °C before use. The TRAP assay was performed by following previously published procedures. Telomerase extract was

prepared from NCI-H460 cells. A modified version of the TRAP assay was used. PCR was performed in a final 50 mL reaction volume composed of reaction mix (45.0 mL) containing Tris-HCl (20 mM, pH 8.0), deoxynucleotide triphosphates (50 mM), $MgCl_2$ (1.5 mM), KCl (63 mM), EGTA (1 mM), Tween-20 (0.005%), BSA (20 mg/mL), primer H21T (3.5 pmol; 5'-$G_3[T_2AG_3]_3$-3'), primer TS (18 pmol; 5'-AATCCGTCGAGCAGAGTT-3'), primer Cxext (22.5 pmol; 5'-GTGCCCTTACCCTTACCCTTACCCTAA-3'), primer NT (7.5 pmol; 5'-ATCGCTTCTCGGCCTTTT-3'), TSNT internal control (0.01 amol; 5'-ATTCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3'), Taq DNA polymerase (2.5 U), and telomerase (100 ng). Compounds or distilled water was added (5 mL). PCR was performed in an Eppendorf Master cycler equipped with a hot lid and incubated for 30 min at 30 °C, followed by 92 °C 30 s, 52 °C 30 s, and 72 °C 30 s for 30 cycles. After amplification, loading buffer (8 mL; 5×TBE buffer, 0.2% bromophenol blue, and 0.2% xylene cyanol) was added to the reaction. An aliquot (15 mL) was loaded onto a nondenaturing acrylamide gel (16%; 19:1) in 1×TBE buffer and resolved at 200 V for 1.5 h. Gels were fixed and then stained with $AgNO_3$.

1.6 Western blotting

The NCI-H460 cells after treated with **3** (0.10 μM) and **4** (18.87 μM) harvested from each well of the culture plates were lysed in 150 μL of extraction buffer consisting of 149 μL of RIPA Lysis Buffer and 1 μL PMSF (100 mM). The suspension was centrifuged at 10000 rpm at 4 °C for 10 min, and the supernatant (10 μL for each sample) was loaded onto 10% polyacrylamide gel and then transferred to a microporous polyvinylidene difluoride (PVDF) membrane. Western blotting was performed using anti-c-myc, hTERT, apaf-1, cytochrome c (cyt c), bcl-2 and β-actin antibody and horseradish peroxidase-conjugated antimouse or antirabbit secondary

antibody. Protein bands were visualized using chemiluminescence substrate.

1.7. Acute Toxicity Studies

Six-week old male and female KM mice (weight 20−22 g) were randomly divided into 3 groups (n = 6) and used to study the in vivo safety of **3**. The highest solubility of **3** in solvent (5% v/v DMSO/saline) was used as the solution, and a good practice volume (0.6 mL/20 g) by intraperitoneal injection was used. Two groups of KM mice were treated with **3** at dose 10.0 mg/kg every two day (5% v/v DMSO/saline), respectively, and one group received the same volume of solvent and used as the control. The signs of toxicity were observed, and body weight was recorded daily.

1.8 Anticancer Activity toward NCI-H460 in Vivo

The NCI-H460 cells were harvested and injected subcutaneously into the right flank of nude mice with 5×10^6 cells in 200 μL of serum-free medium. When the xenograft tumor growth to the volume about 1000 mm^3, the mice were killed and the tumor tissue were cut into about 1.5 mm^3 small pieces, and then transplanted into the right flank of female nude mice, When tumors reach a volume of 80-190 mm^3 on all mice, the mice were randomized into vehicle control and treatment groups (n=6/group), received the following treatments: (a) control, 5% v/v DMSO/saline vehicle, (b) **3** at dose 10.0 mg/kg every two day (5% v/v DMSO/saline). The tumor volumes were determined every three days by measuring length (*l*) and width (*w*) and calculating volume, tumor volume and inhibition of tumor growth were calculated using formulas 1−3:

Tumor volume: $V = (w^2\times l)/2$ (1)

The tumor relative increment rate: $T/C\ (\%) = T_{RTV}/C_{RTV}\times100\%$ (2)

inhibition of tumor growth: $IR(\%) = (W_c - W_t)/W_c\times100\%$ (3)

Where w and l mean the shorter and the longer diameter of the tumor respectively;

T_{RTV} and C_{RTV} was the RTV of treated group and control group respectively. (RTV: relative tumor volume, RTV= V_t /V_0); W_t and W_c mean the average tumor weight of complex-treated and vehicle controlled group respectively.

1.9 Statistical analysis

The experiments have been repeated from three to five times, and the results obtained were presented as means±standard deviation (SD). Significant changes were assessed by using the Student's *t* test for unpaired data, and the p values of <0.05 were considered statistically significant.