Supporting Information

Multi-Functional Biotinylated Platinum(IV)-SAHA Conjugate for Tumor-Targeted Chemotherapy

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Supplementary References

1. Instruments and Methods

1.1 Thin layer chromatography (TLC) and column chromatography

TLC was carried out on aluminum plates coated with silica gel mixed with fluorescent indicator. The purification of synthesized ligands and complexes were performed with silica gel (60-120 mesh) column chromatography.

1.2 NMR spectroscopy

^{1}H, ^{13}C and ^{195}Pt NMR spectra were acquired on a Bruker 500 MHz spectrometer in $CDCl_3$ or DMSO-d_6 at ambient temperature with tetramethylsilane (TMS) as an internal standard. NMR standards used were as follows: (^{1}H-NMR) $CDCl_3$ = 7.260 ppm; DMSO-d_6 = 2.50 ppm. (^{13}C-NMR) $CDCl_3$ = 77.00 ppm; DMSO-d_6 = 39.520 ppm. All chemical shifts (δ) are reported in ppm relative to TMS. Spin multiplicities were reported as a singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), triplet of doublets (td), doublet of doublet of doublets (ddd), multiplet (m) and broad (br) with coupling constant (J) reported in Hz.

1.3 Mass spectrometry

Electrospray ionization high resolution mass spectra (ESI-HRMS) were obtained using a Waters make ESI-MS model synapt G2 high definition mass spectrometry. Matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectral data were obtained using a Bruker made Autoflex TOF/TOF mass spectrometry. The spectra of all compounds were recorded using α-cyano-4-hydroxycinnamic acid (CHCA) as matrix.

1.4 FT-IR spectroscopy

Fourier transform-Infrared (FT-IR) spectra were measured using IR Affinity-1S (Shimadzu, Kyoto, Japan) FT-IR spectrophotometer equipped with a single reflection attenuated total reflectance (ATR) accessory. The IR spectra were recorded from 4000 to 450 cm^{-1} using a resolution of 4 cm^{-1} with 45 scans. In IR absorption spectra, the shapes and signal intensities (height) of peaks (bands) are denoted by the following abbreviations: br = broad, vs = very strong, s = strong, m = medium and w = weak.

1.5 RP-HPLC analysis

The purity of complexes was determined by analytical HPLC system (Thermo Scientific Dionex Ultimate 3000) equipped with UV-Vis detector using reversed-phase C18 column (Acclaim, Length 250 mm, internal dia. 4.6 mm, particle size 5 µm, pore size 120 Å) operating at room temperature (RT).

1.6 Determination of lipophilicity

The lipophilicity (log $P_{o/w}$) of the compounds (SAHA**, Pt(IV)-SAHA**, and **Biotin-Pt(IV)-SAHA**) were determined by the conventional flask-shaking method as reported in the literature.[1,2] Here, log $P_{o/w}$ = log (C_o/C_w) is defined as the logarithmic ratio of the compound concentration in n-octanol to that in the water phase. For this, 0.5 mg of compound was taken in 3 mL of 1:1 v/v mixture of n-octanol and water, mixed vigorously for 24 h. The mixture was then kept in a stationary state for an additional 24 h to reach saturation. The n-octanol and water phases were separated, centrifuged at 3000 rpm for 10 min and the supernatant was isolated. The concentration of complexes was then determined by UV-vis spectroscopy in both the n-octanol (C_o) and water (C_w) phases to estimate the log $P_{o/w}$ values.

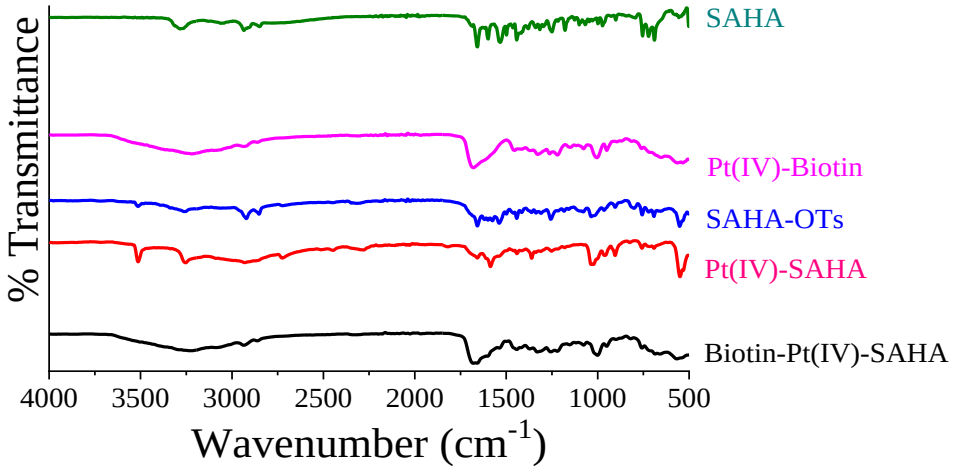

Fig. S1 IR spectra of SAHA, **Pt(IV)-Biotin**, **SAHA-OTs**, **Pt(IV)-SAHA**, **Biotin-Pt(IV)-SAHA.**

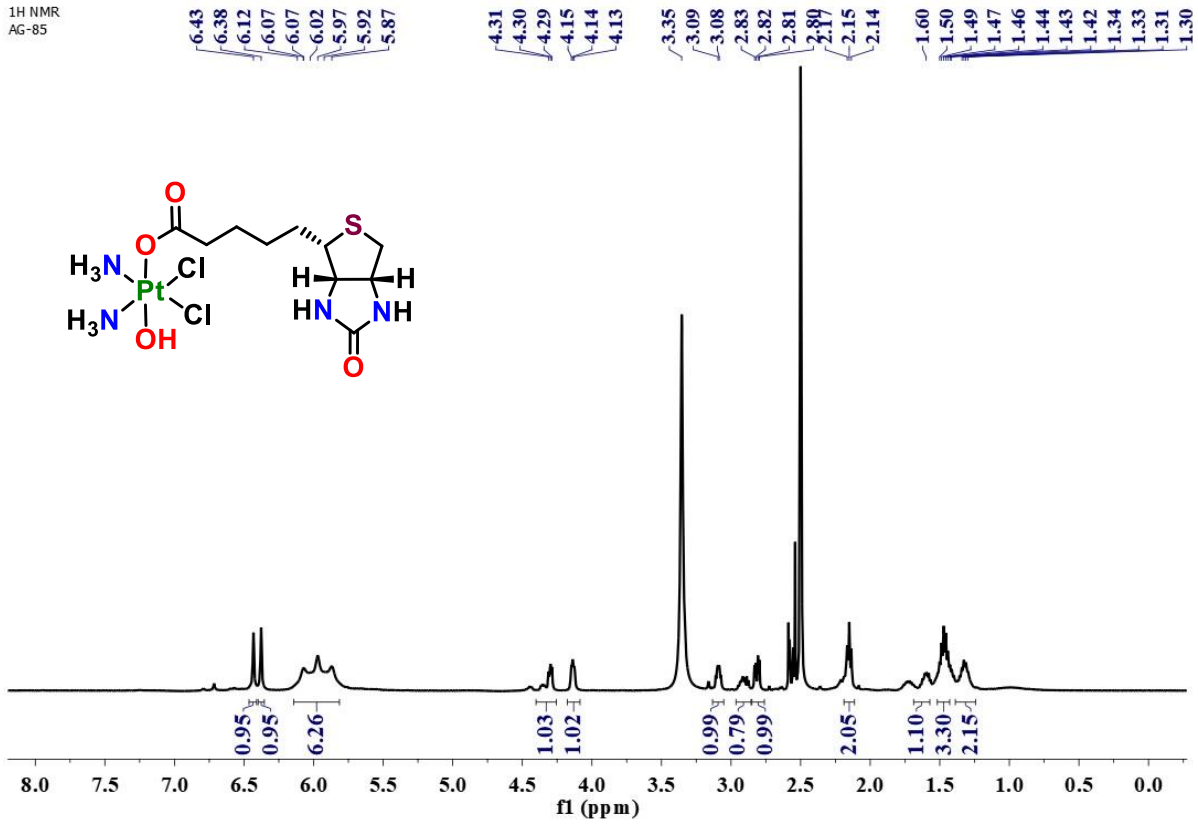

Fig. S2 ^{1}H-NMR spectrum of **Pt(IV)-Biotin** in DMSO-d_6 at 298K.

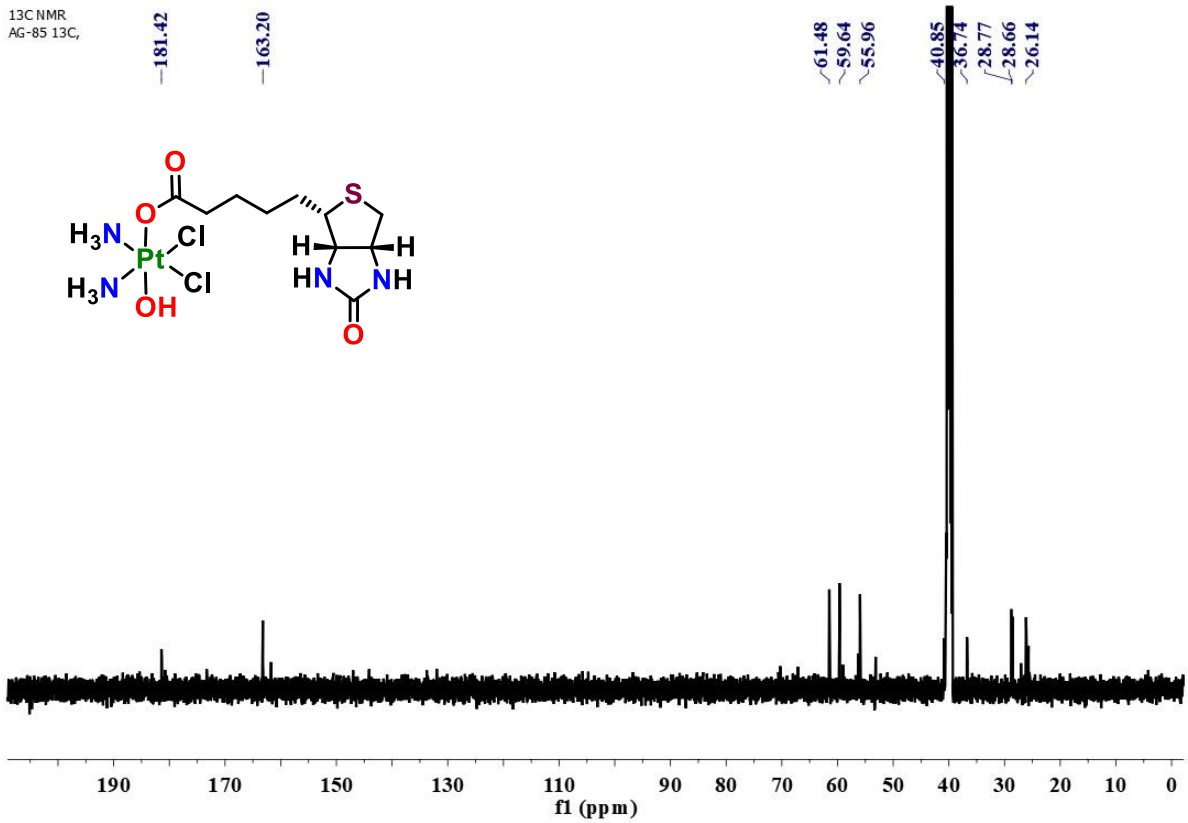

Fig. S3 ^{13}C-NMR spectrum of **Pt(IV)-Biotin** in DMSO-d_6 at 298K.

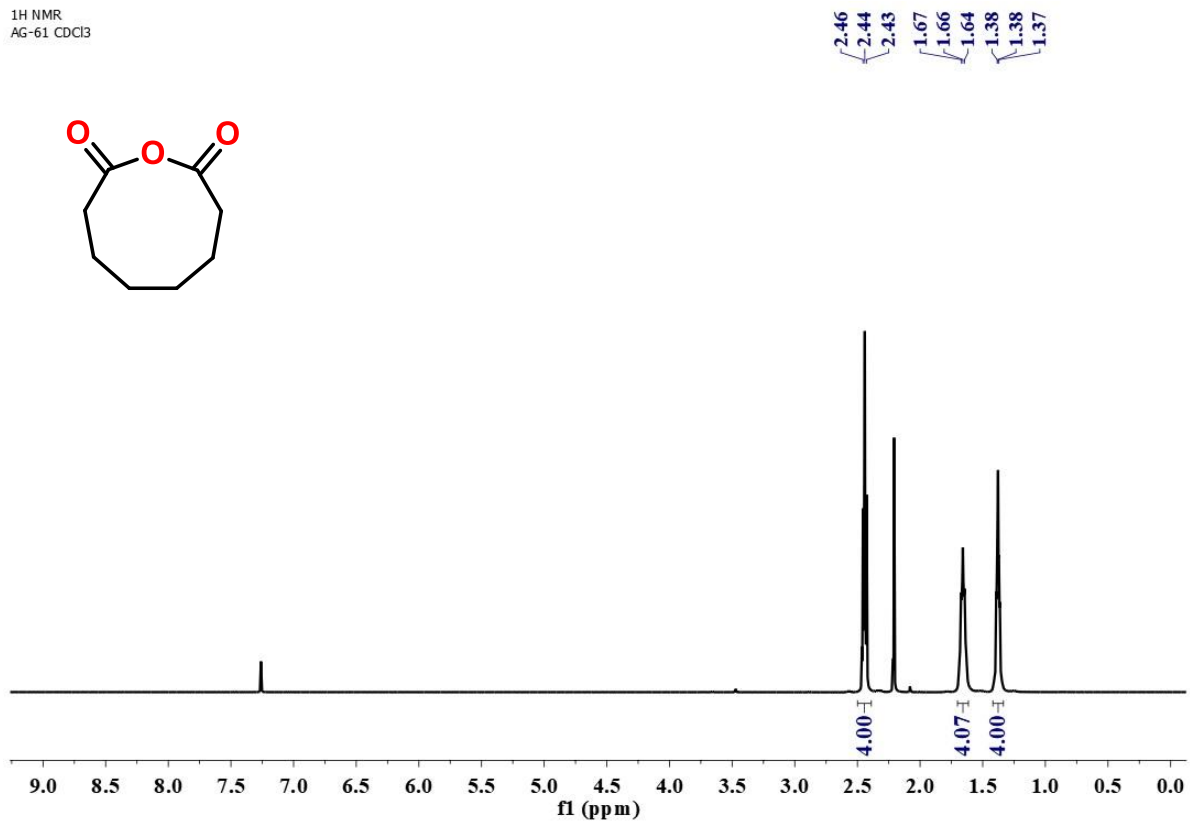

Fig. S4 ^{1}H-NMR spectrum of suberic anhydride in $CDCl_3$ at 298K.

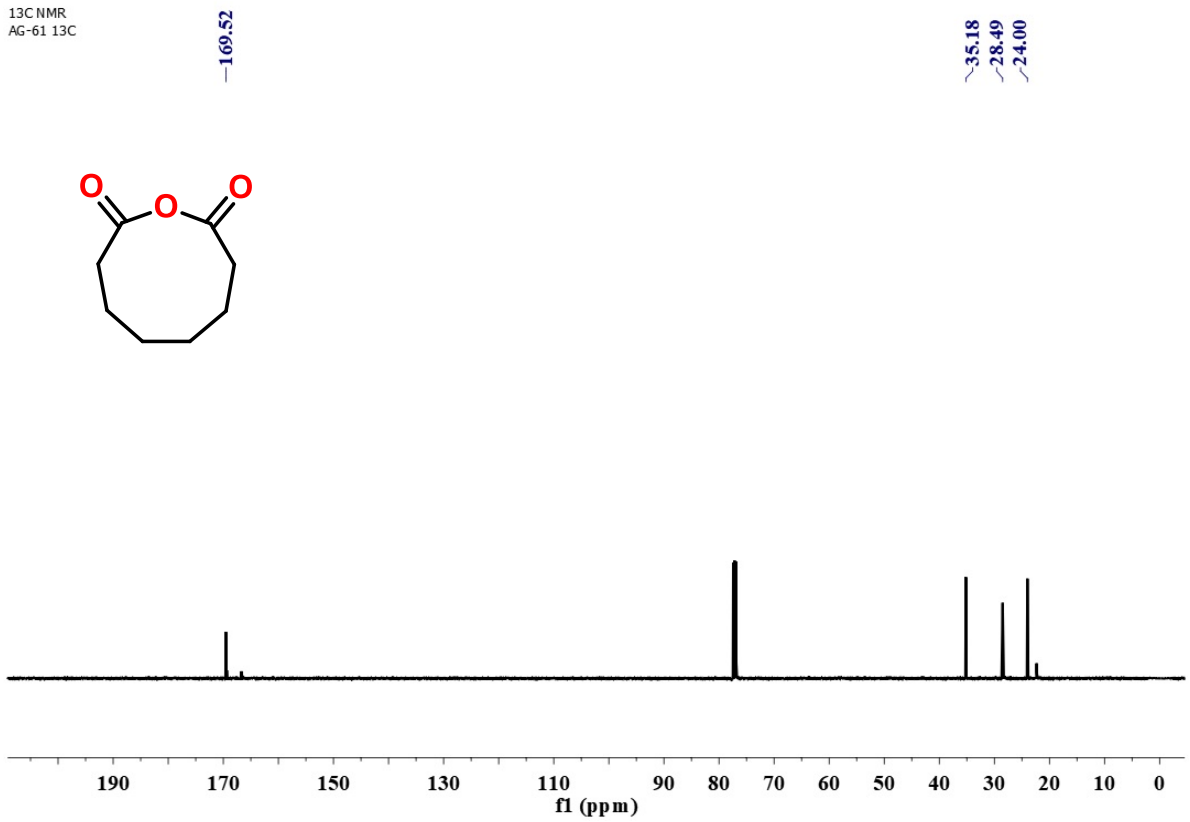

Fig. S5 ^{13}C-NMR spectrum of suberic anhydride in $CDCl_3$ at 298K.

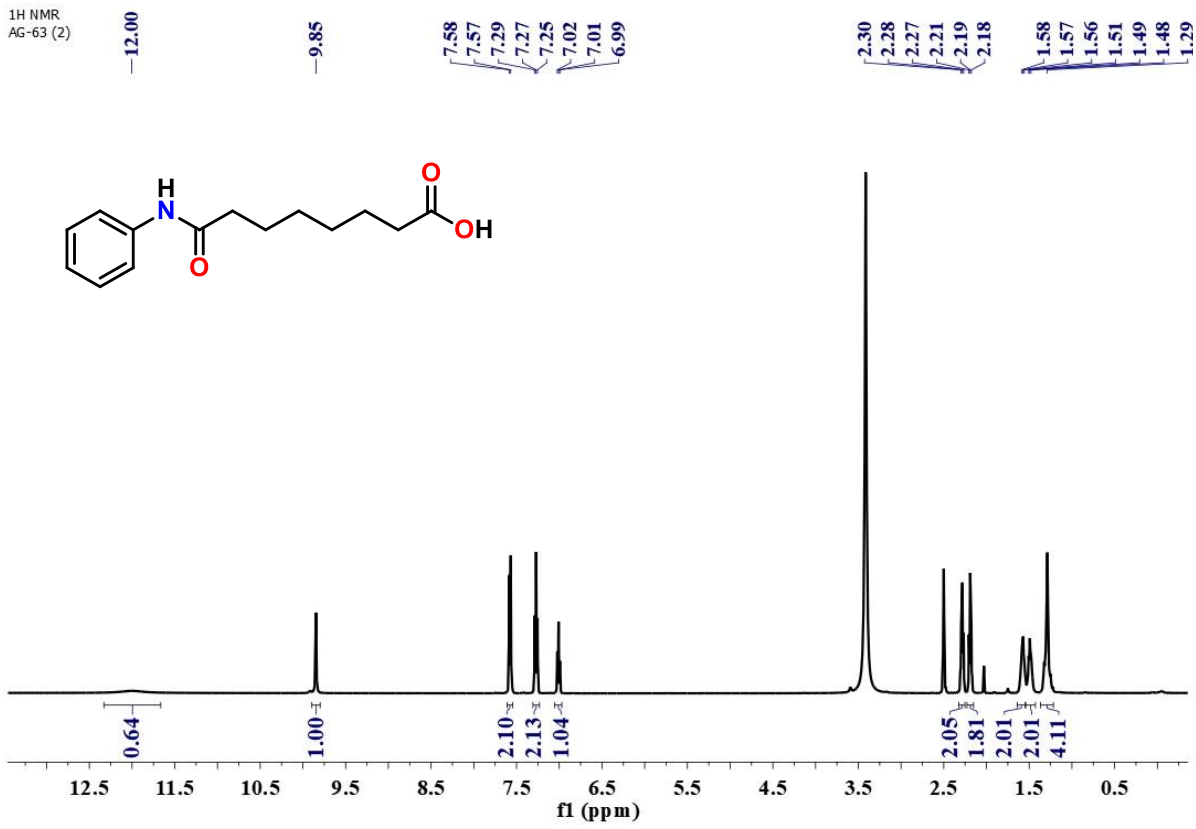

Fig. S6 ^{1}H-NMR spectrum of suberanilic acid in DMSO-d_6 at 298K.

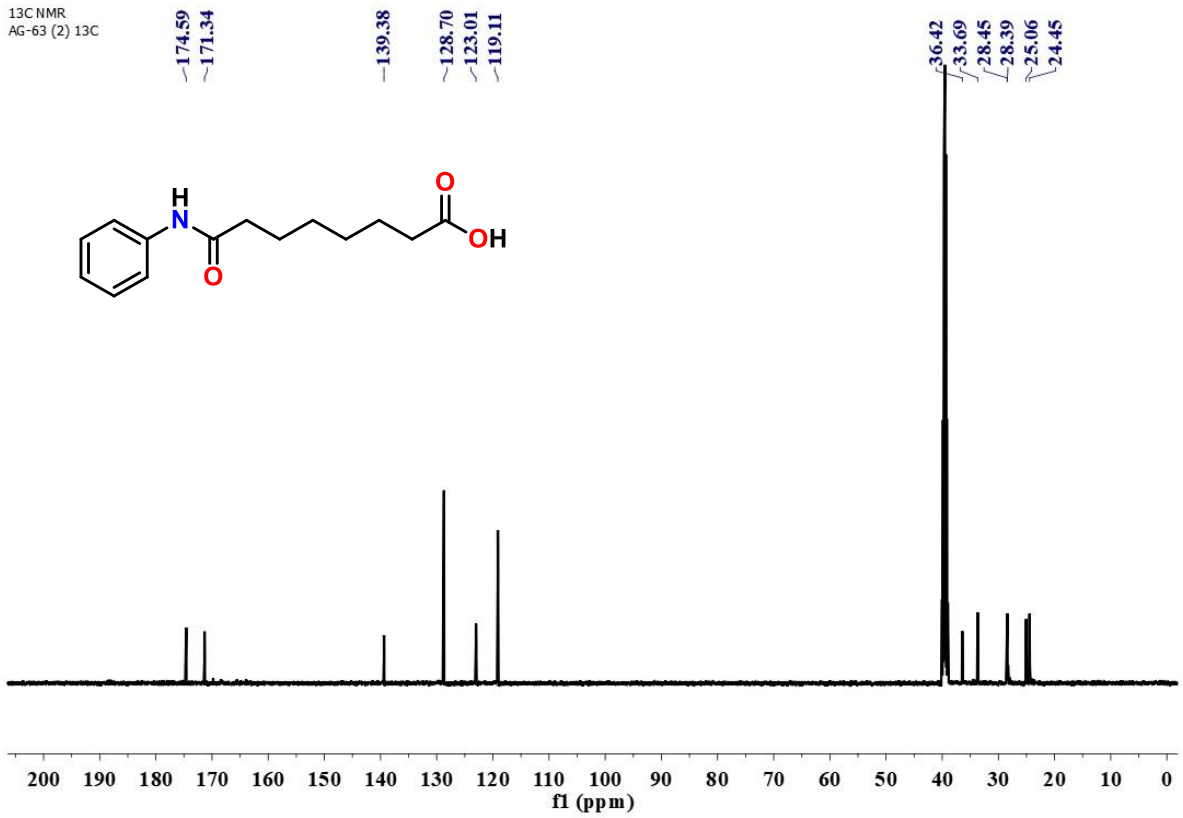

Fig. S7 ^{13}C-NMR spectrum of suberanilic acid in DMSO-d_6 at 298K.

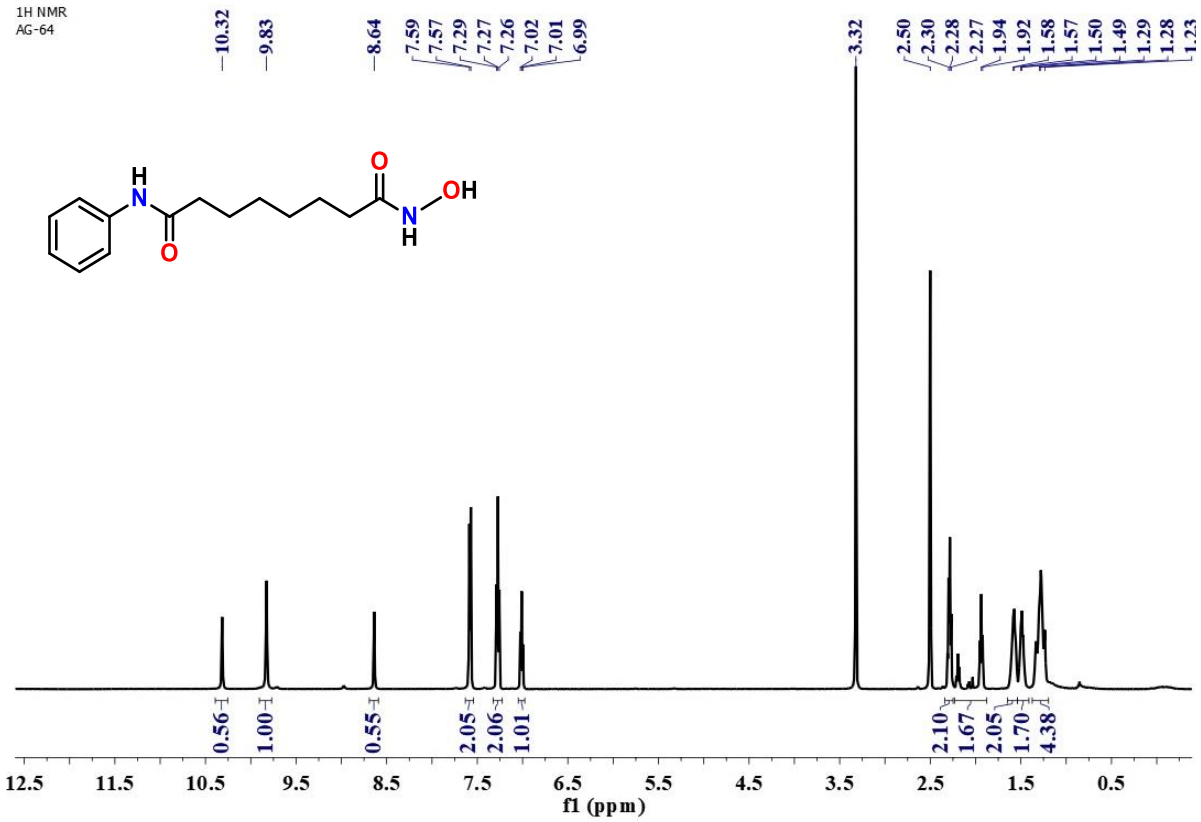

Fig. S8 ^{1}H-NMR spectrum of SAHA in DMSO-d_6 at 298K.

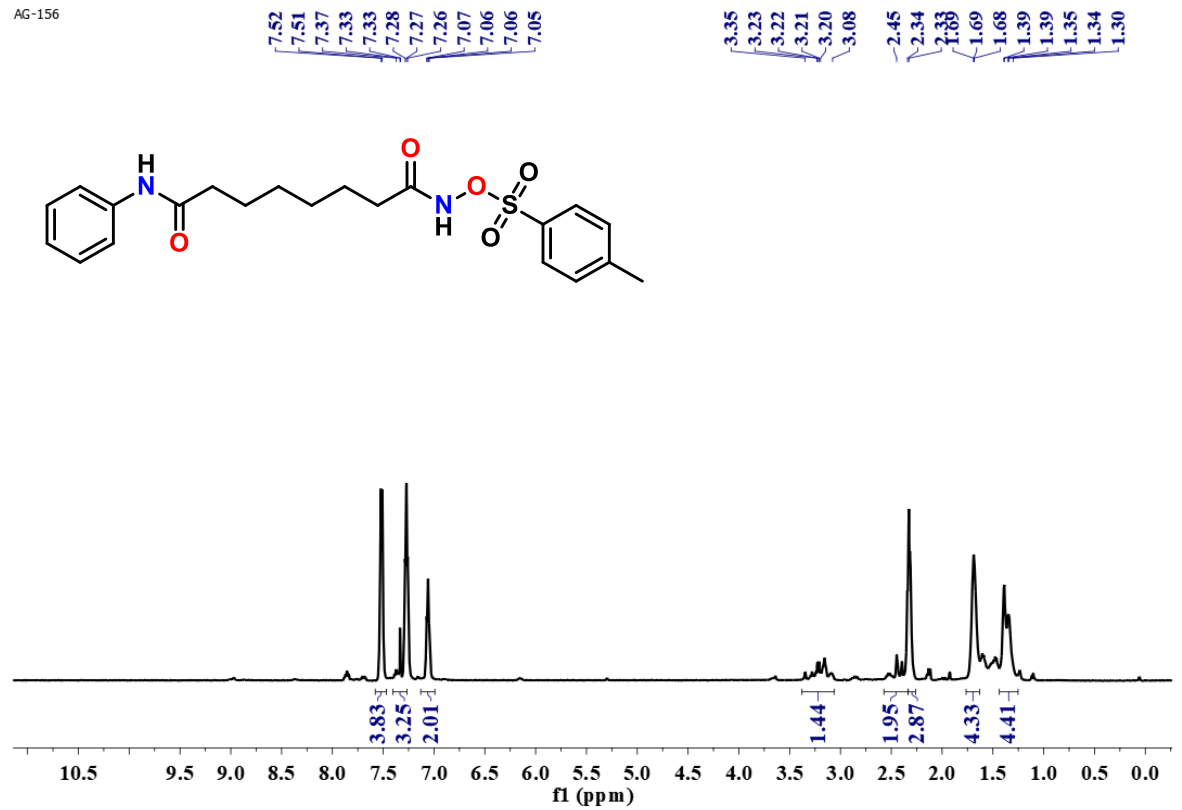

Fig. S9 ^{13}C-NMR spectrum of **SAHA-OTs** in DMSO-d_6 at 298K.

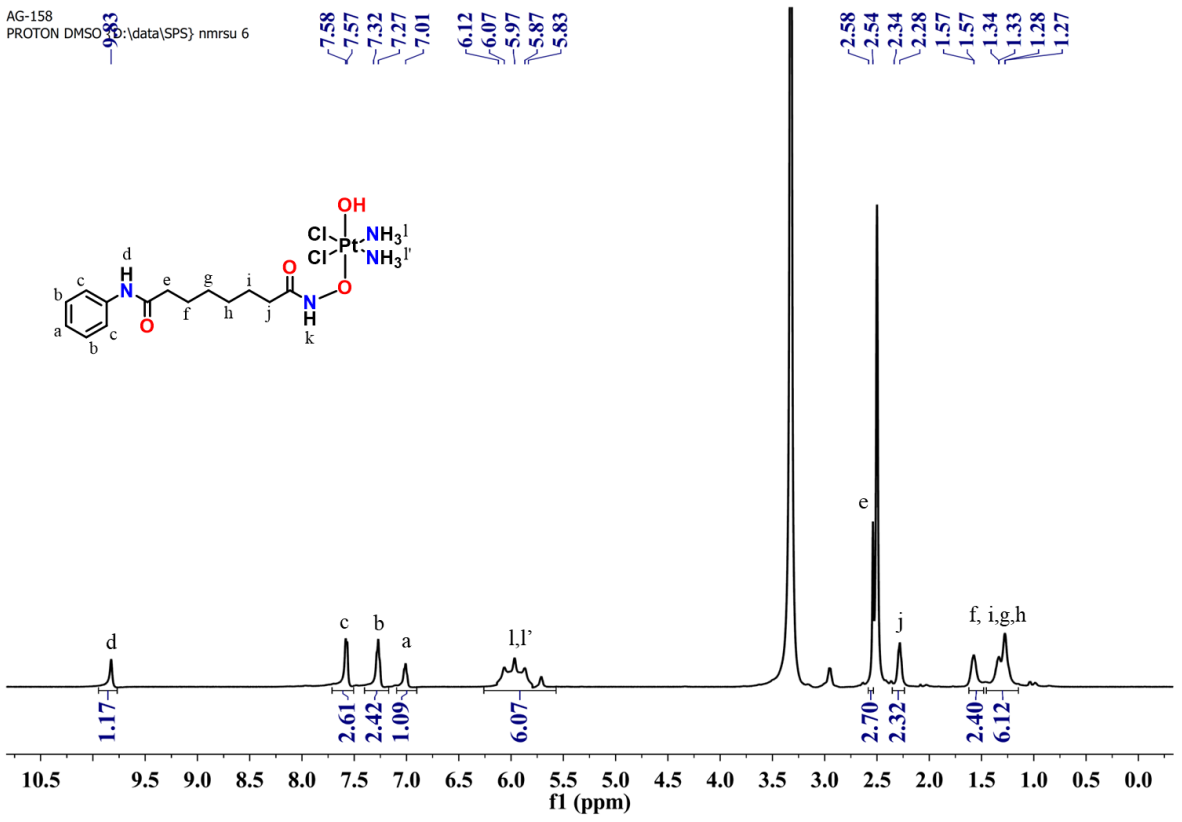

Fig. S10 ^{1}H-NMR spectrum of **Pt(IV)-SAHA** in DMSO-d_6 at 298K.

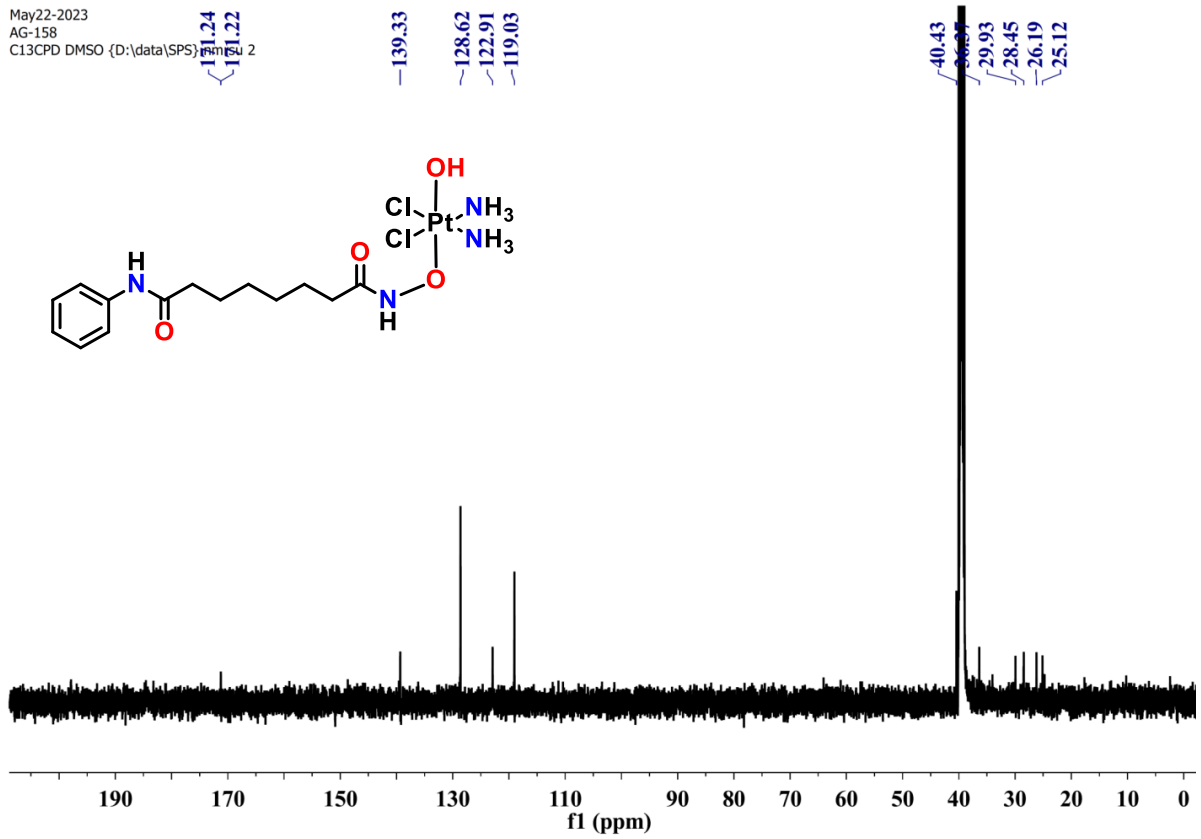

Fig. S11 ^{13}C-NMR spectrum of **Pt(IV)-SAHA** in DMSO-d_6 at 298K.

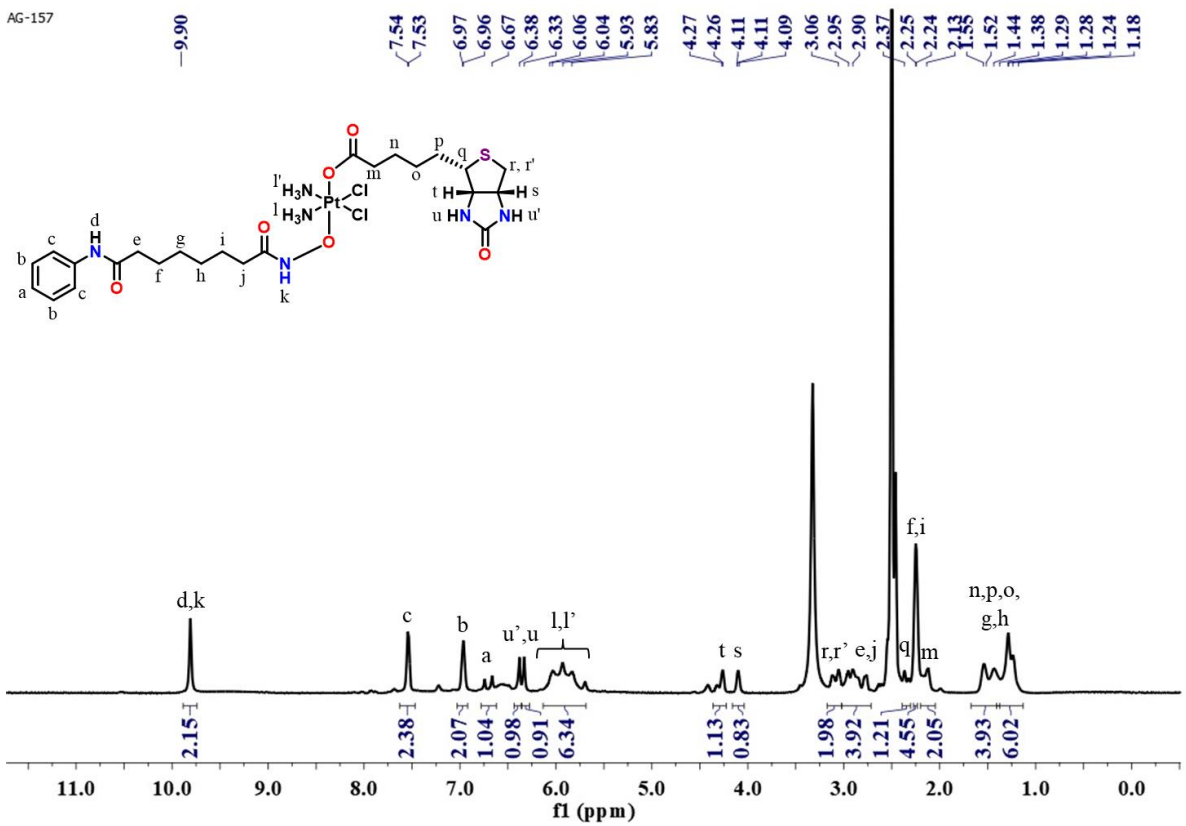

Fig. S12 ^{1}H-NMR spectrum of **Biotin-Pt(IV)-SAHA** in DMSO-d_6 at 298K.

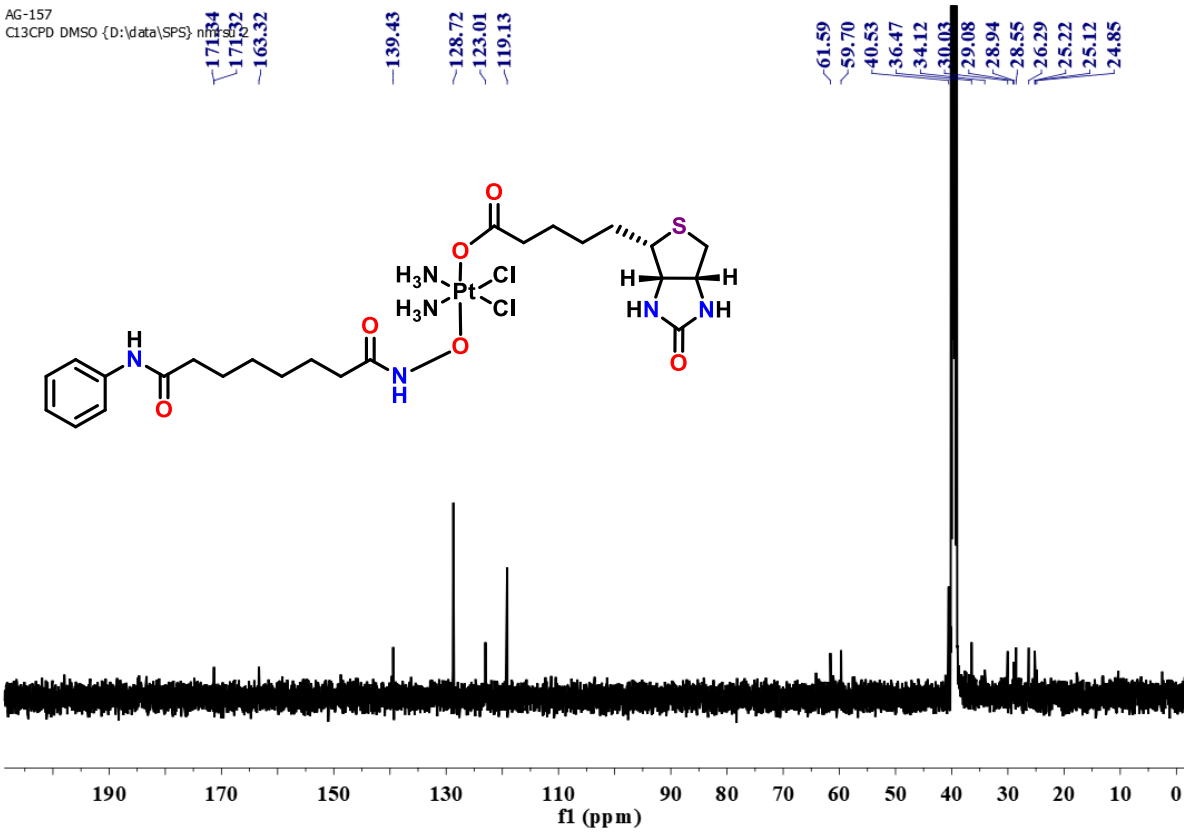

Fig. S13 ^{13}C-NMR spectrum of **Biotin-Pt(IV)-SAHA** in DMSO-d_6 at 298K.

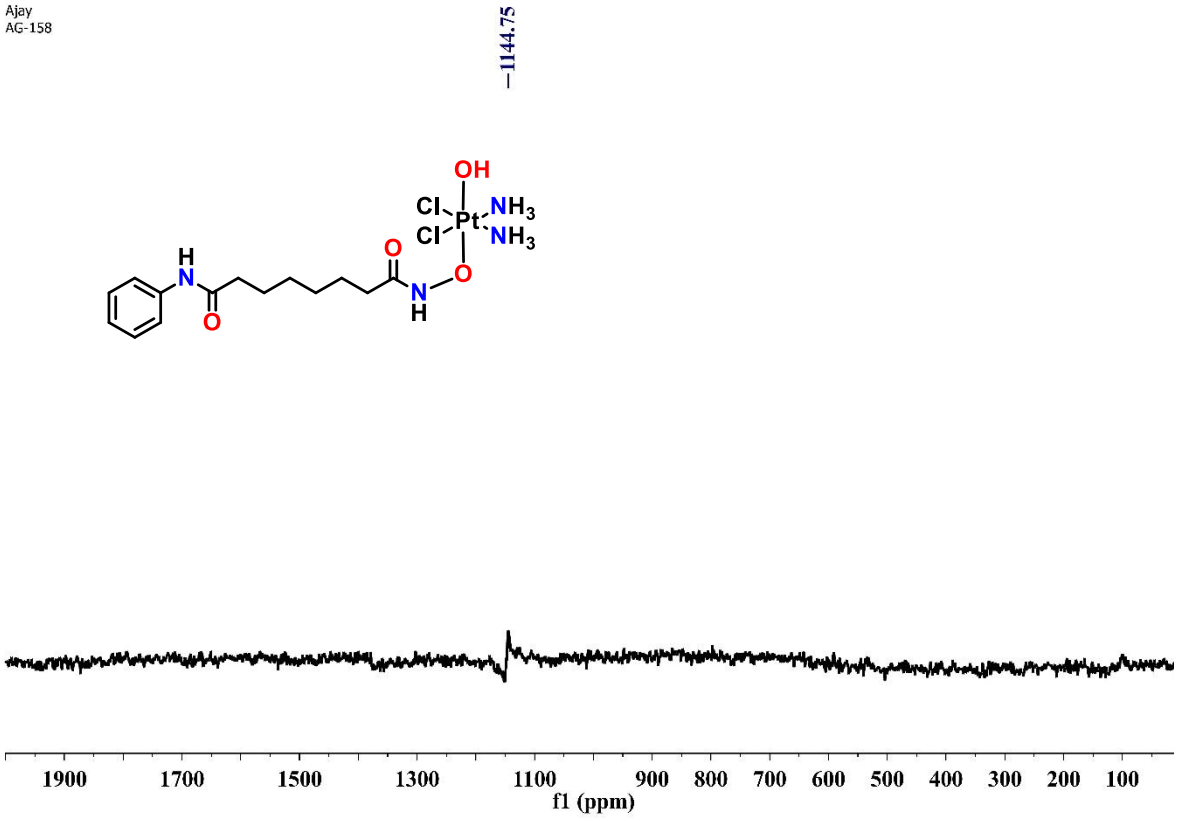

Fig. S14 ^{195}Pt-NMR spectrum of **Pt(IV)-SAHA** in DMSO-d_6 at 298K.

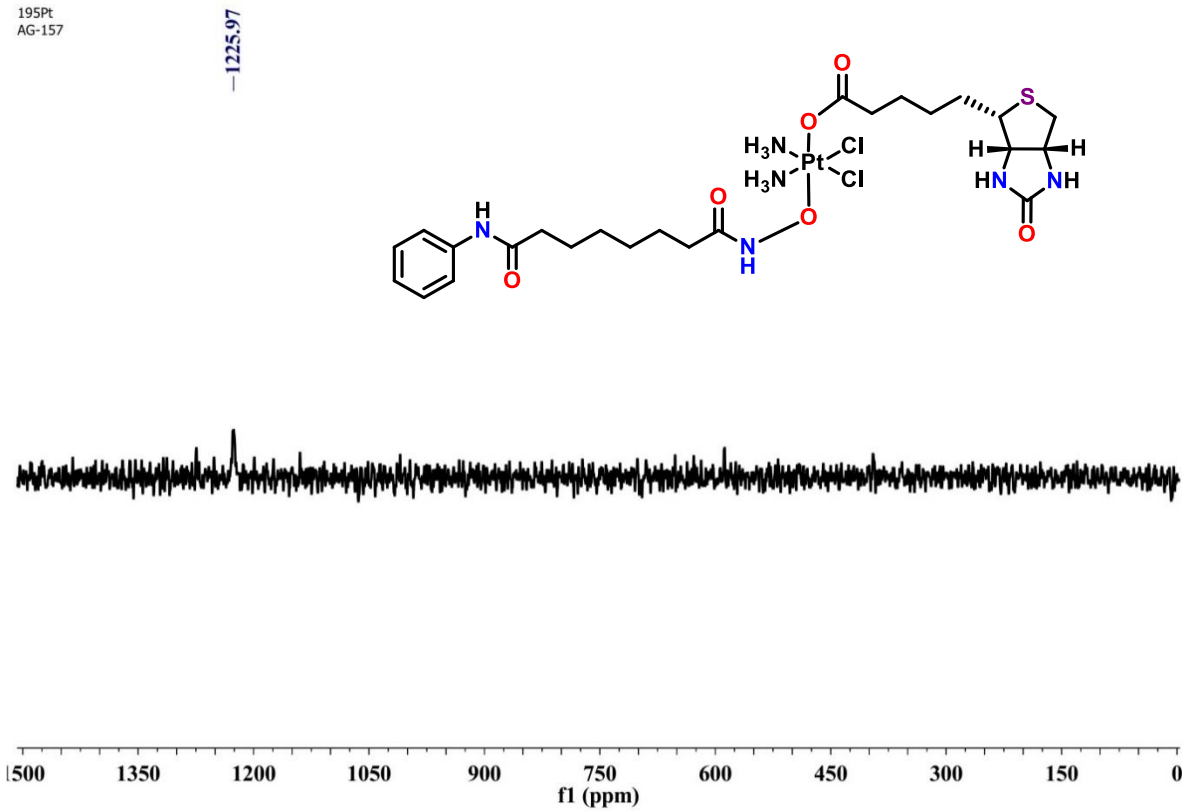

Fig. S15 ^{195}Pt-NMR spectrum of **Biotin-Pt(IV)-SAHA** in DMSO-d_6 at 298K.

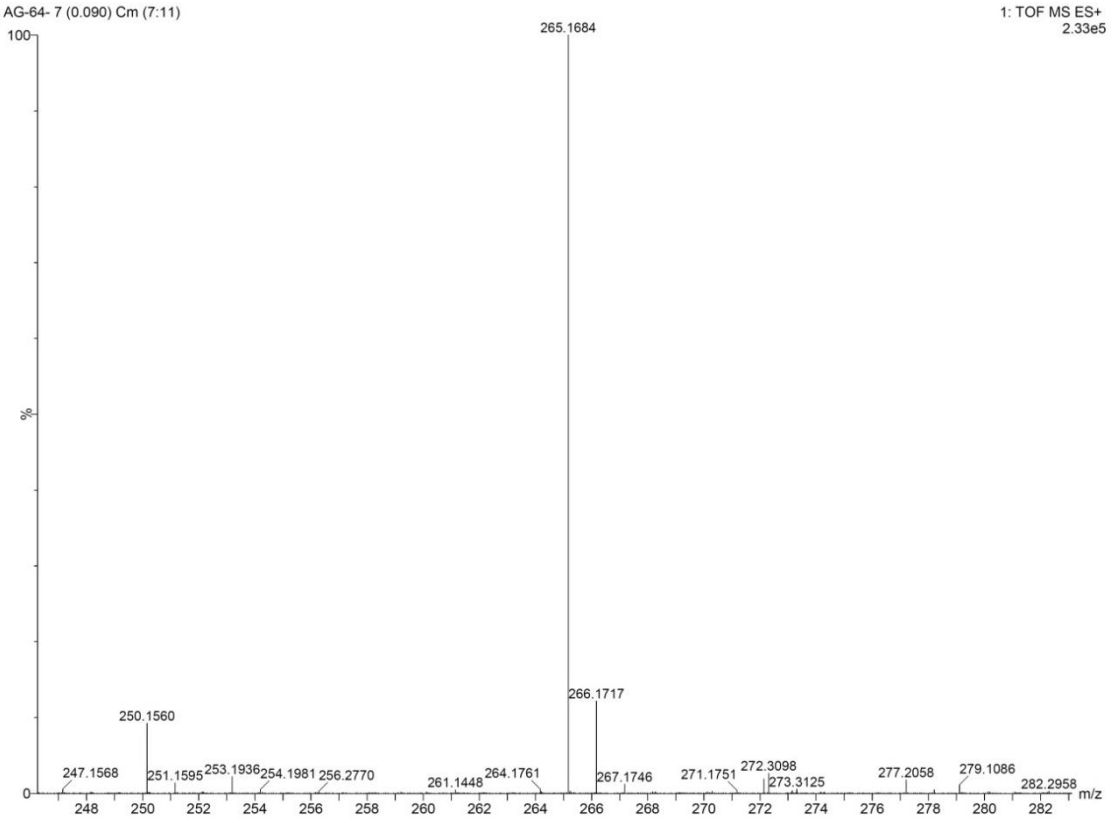

Fig. S16 Mass spectrum of SAHA in DCM/MeOH showing the peak at 265.1684 (*m/z*) assignable to $[M+H]^+$ at 298K.

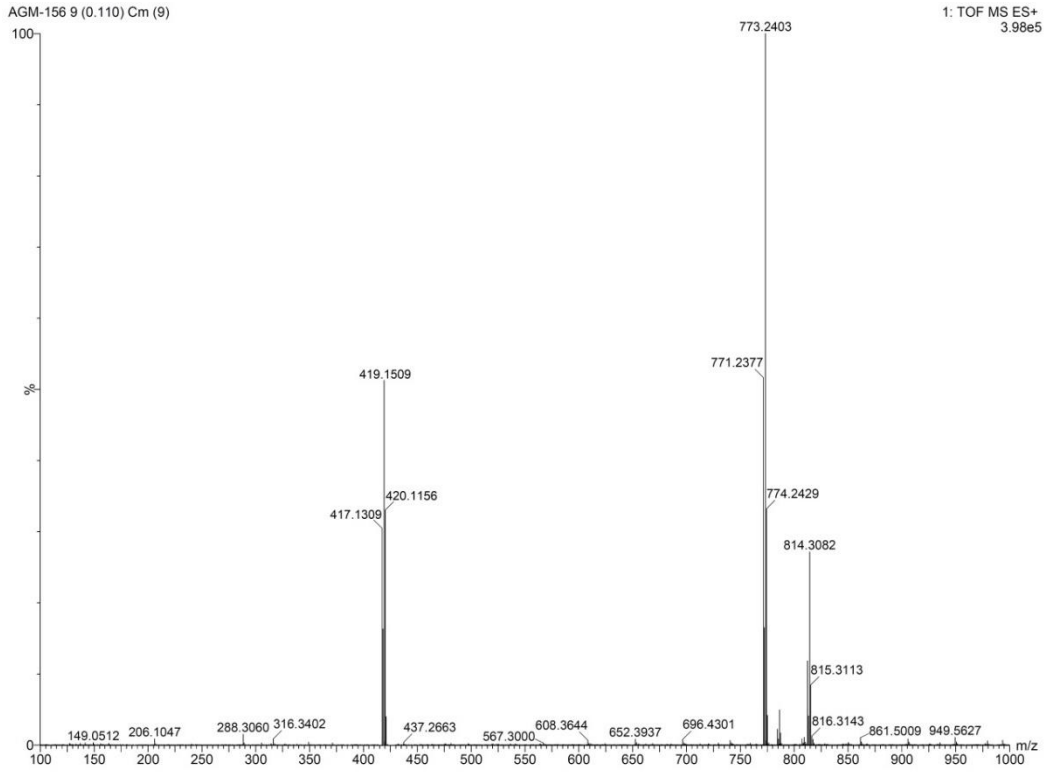

Fig. S17 Mass spectrum of **SAHA-OTs** in DCM/MeOH showing the peak at 419.1509 (*m/z*) assignable to $[M+H]^+$ at 298K.

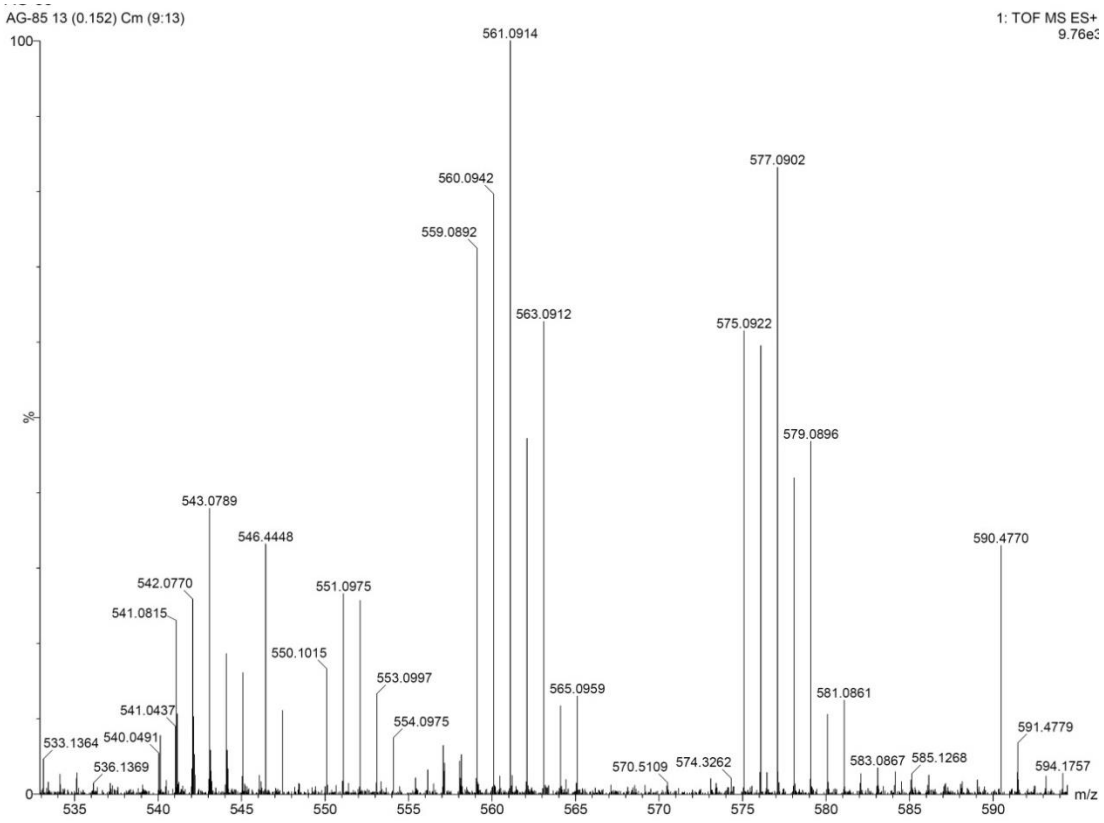

Fig. S18 Mass spectrum of **Pt(IV)-Biotin** in DCM/MeOH showing the peak at 560.0942 (*m/z*) assignable to $[M+H]^+$ at 298K.

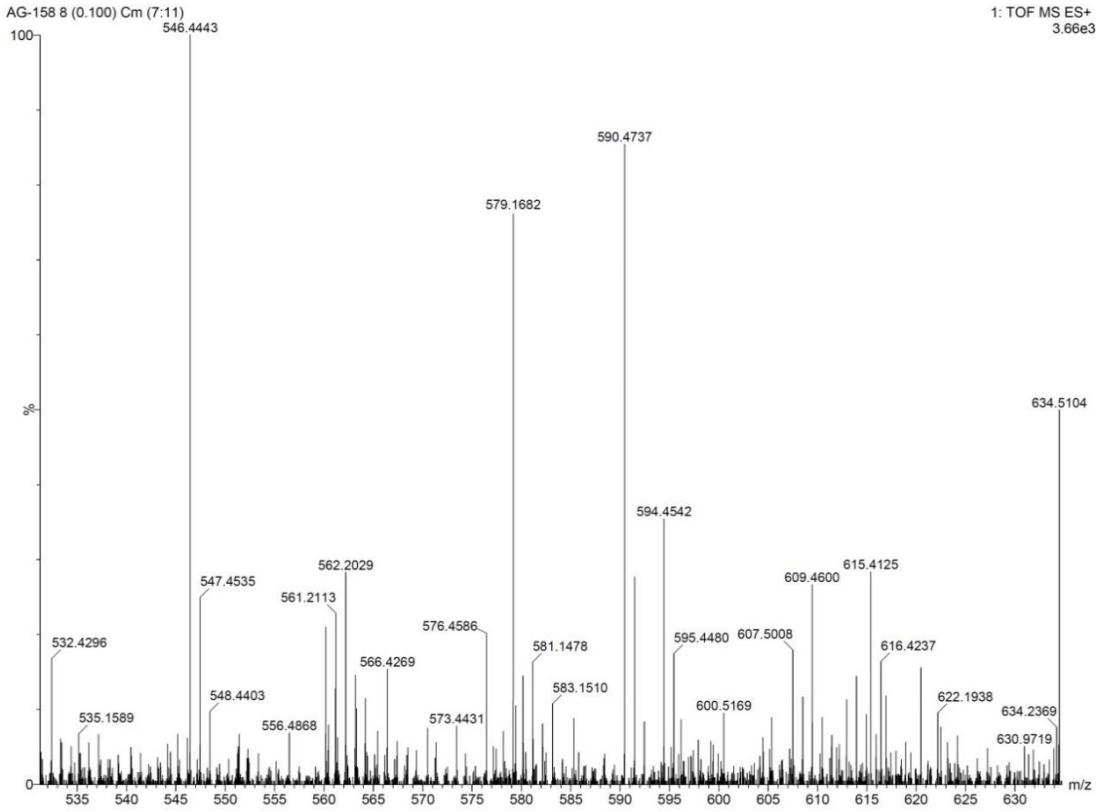

Fig. S19 Mass spectrum of **Pt(IV)-SAHA** in DCM/MeOH showing the peak at 579.1682 (*m/z*) assignable to $[M]^+$ at 298K.

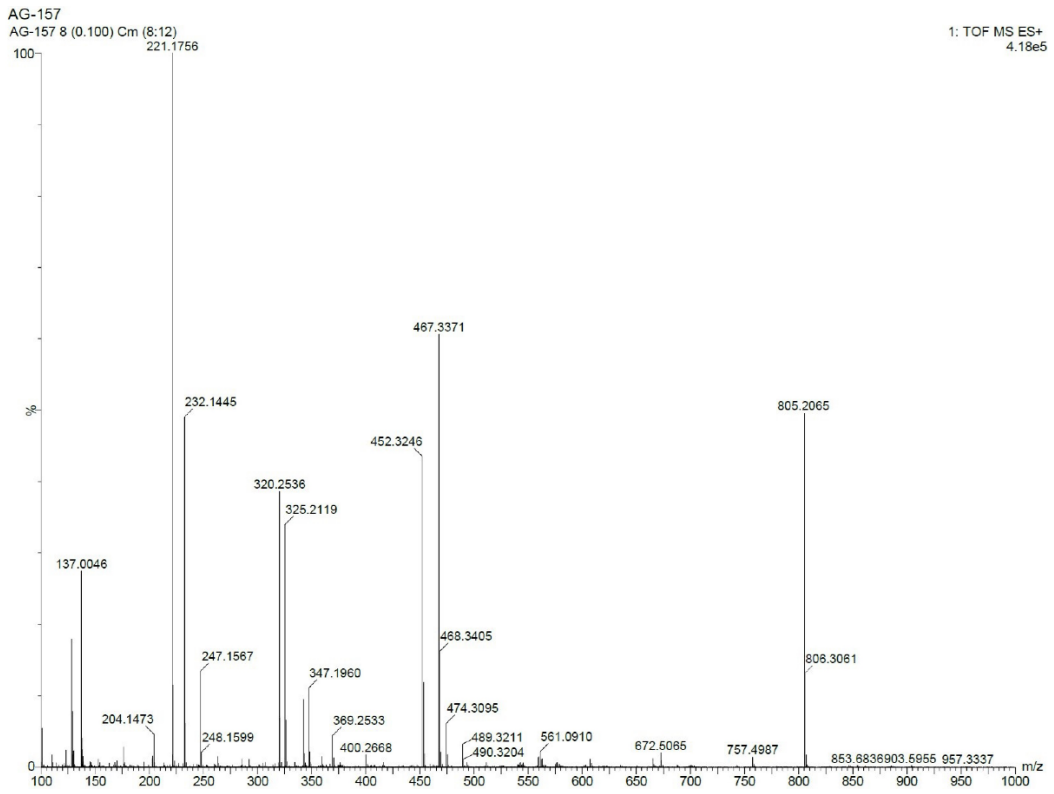

Fig. S20 Mass spectrum of **Biotin-Pt(IV)-SAHA** in DCM/MeOH showing the peak at 805.2065 (*m/z*) assignable to $[M]^+$ at 298K.

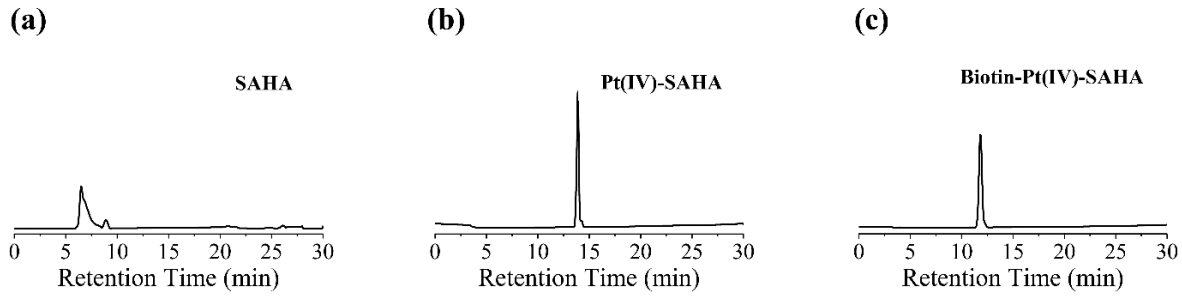

Fig. S21 HPLC chromatograms of (a) SAHA, (b) **Pt(IV)-SAHA,** and (c) **Biotin-Pt(IV)-SAHA** are showing the purity of the compounds.

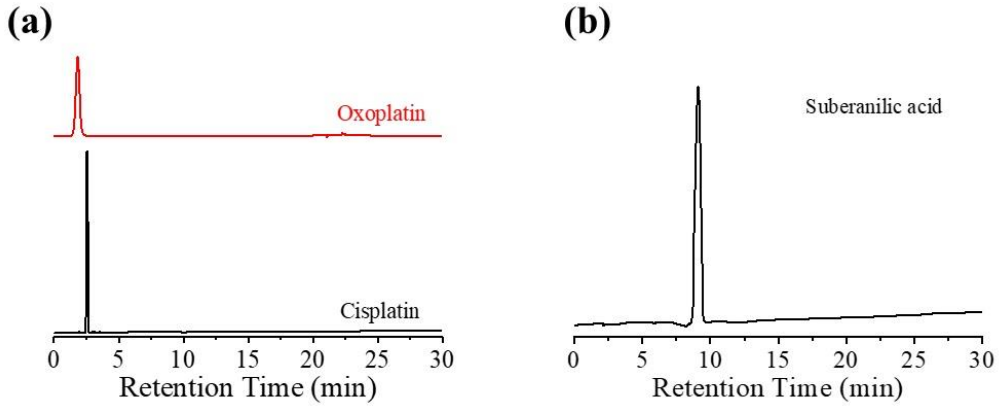

Fig. S22 HPLC chromatograms of (a) cisplatin and oxoplatin, and (b) suberanilic acid.

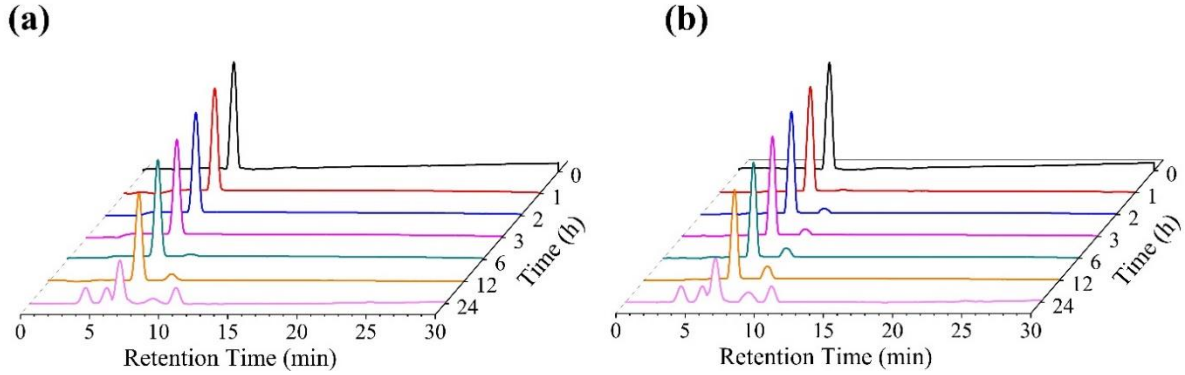

Fig. S23 Stability of SAHA (a) in PBS (5 mM, pH 7.4) and (b) in 5 mM ascorbate monitored by HPLC.

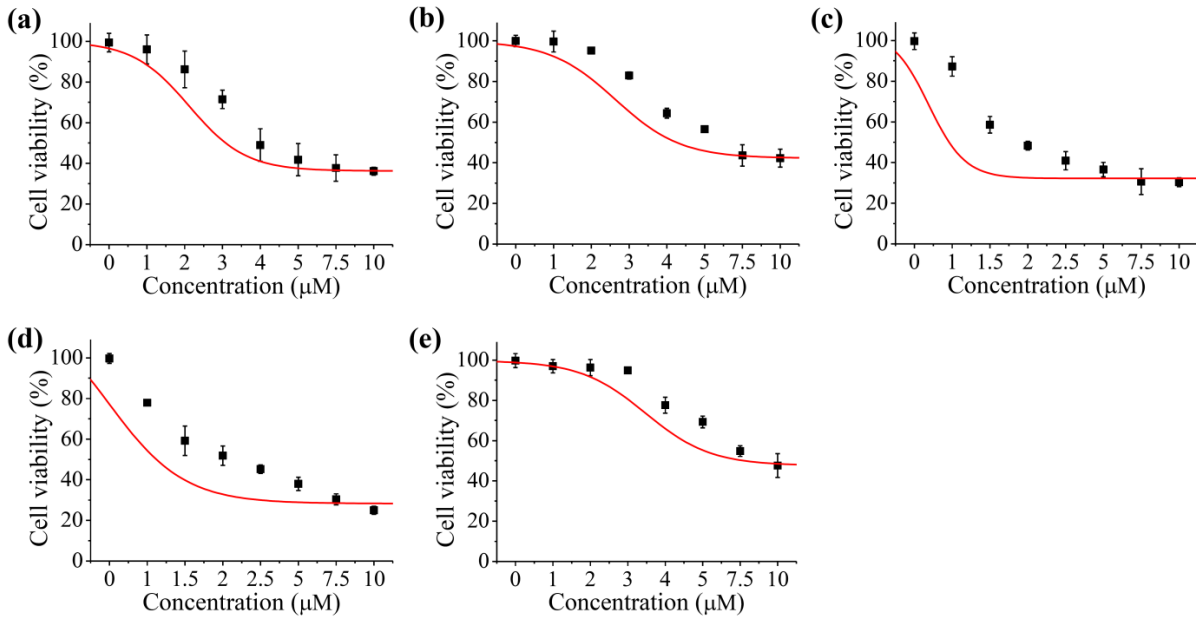

Fig. S24 Cell viability assay in MCF7 cells treated with the (a) **Pt(IV)-Biotin**, (b) **Pt(IV)-SAHA**, (c) **Biotin-Pt(IV)-SAHA**, (d) SAHA, and (e) cisplatin after 72 h incubation.

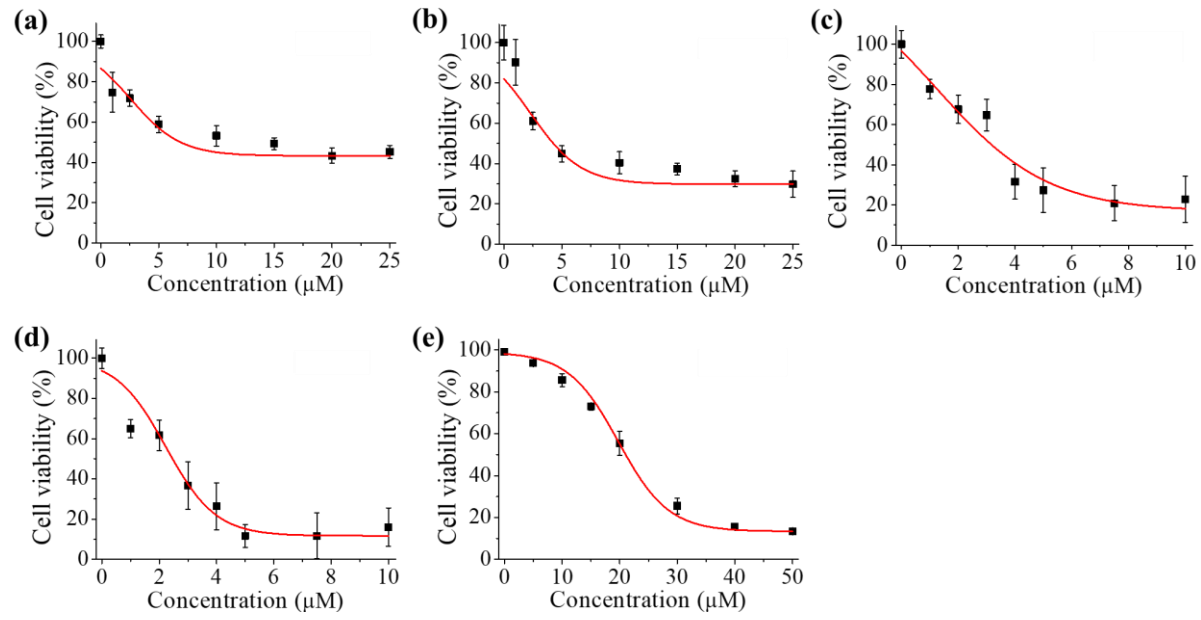

Fig. S25 Cell viability assay in A549 cells treated with the (a) **Pt(IV)-Biotin**, (b) **Pt(IV)-SAHA**, (c) **Biotin-Pt(IV)-SAHA**, (d) SAHA, and (e) cisplatin after 72 h incubation.

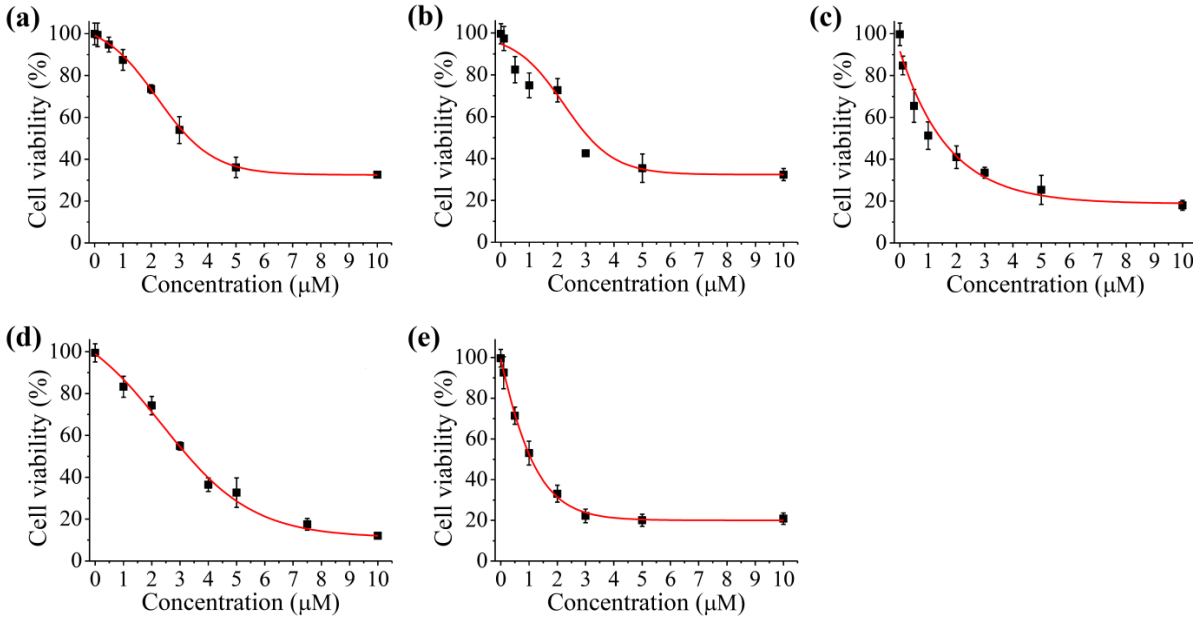

Fig. S26 Cell viability assay in A2780 cells treated with the (a) **Pt(IV)-Biotin**, (b) **Pt(IV)-SAHA**, (c) **Biotin-Pt(IV)-SAHA**, (d) SAHA, and (e) cisplatin after 72 h incubation.

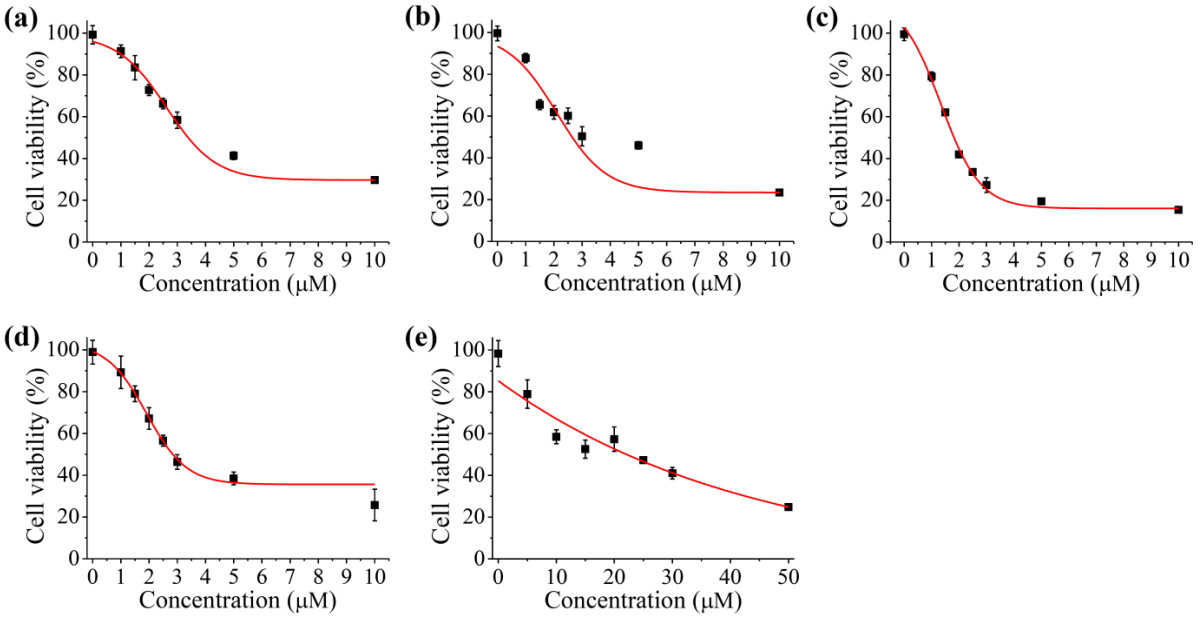

Fig. S27 Cell viability assay in A2780cisR cells treated with the (a) **Pt(IV)-Biotin**, (b) **Pt(IV)-SAHA**, (c) **Biotin-Pt(IV)-SAHA**, (d) SAHA, and (e) cisplatin after 72 h incubation.

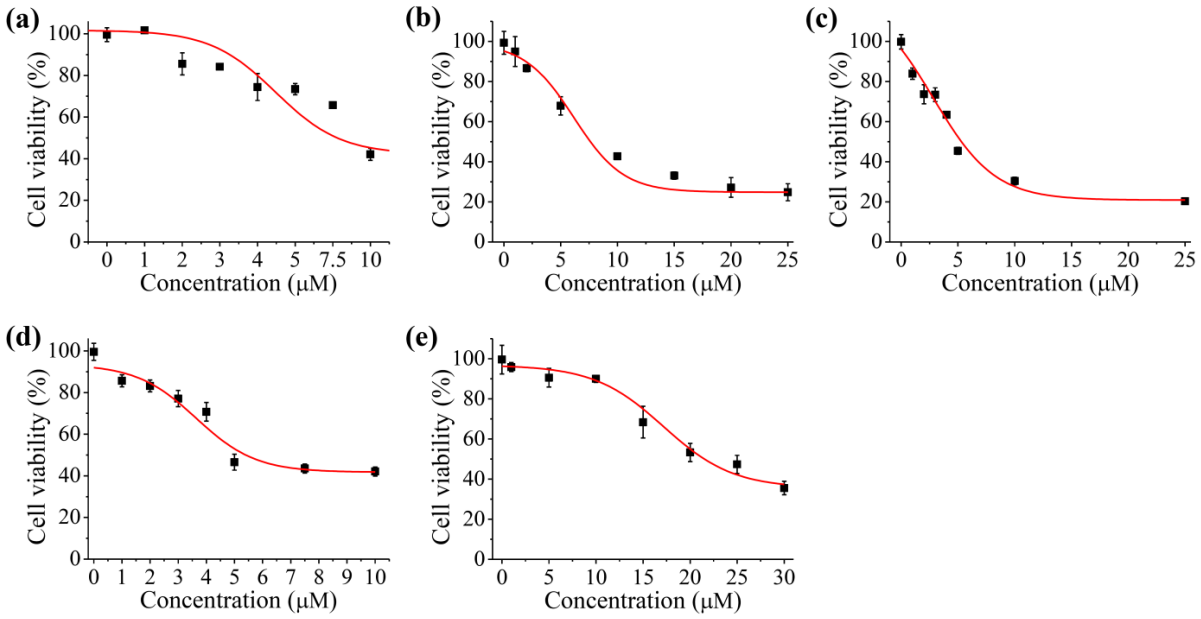

Fig. S28 Cell viability assay in HEK293 cells treated with the (a) **Pt(IV)-Biotin**, (b) **Pt(IV)-SAHA**, (c) **Biotin-Pt(IV)-SAHA**, (d) SAHA, and (e) cisplatin after 72 h incubation.

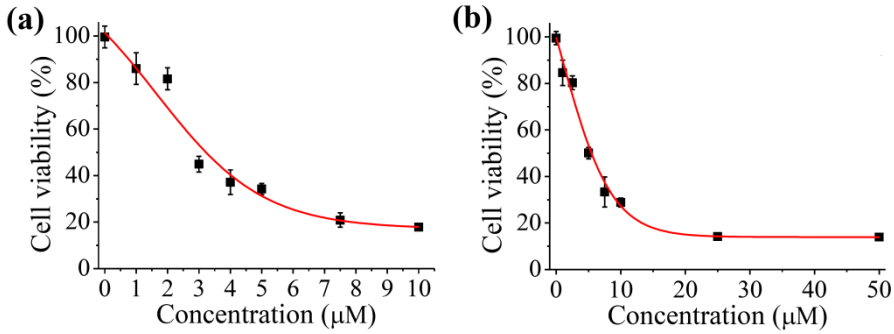

Fig. S29 Cell viability assay in A2780 cells treated with the (a) **Pt(IV)-Biotin + SAHA** at their equimolar concentration and (b) **cisplatin + SAHA** after 72 h incubation.

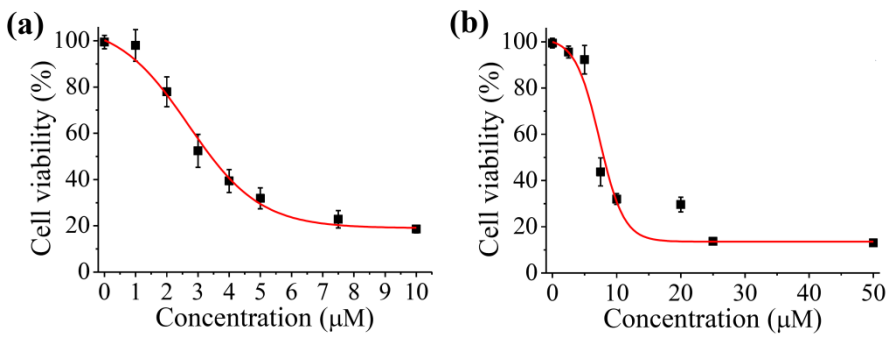

Fig. S30 Cell viability assay in A2780cisR cells treated with the (a) **Pt(IV)-Biotin + SAHA** at their equimolar concentration (b) **cisplatin + SAHA** after 72 h incubation.

References

1. A. Gupta, A. K. Pandey, T. Mondal, J. Bhattacharya and P. K. Sasmal, Multifunctional Iridium(III)-Platinum(IV) Conjugates as Potent Anticancer Theranostic Agents, *J. Med. Chem.,* 2023, **66**, 8687–8704.
2. G. Kumari, A. Gupta, R. K. Sah, A. Gautam, M. Saini, A. Gupta, A. K. Kushawaha, S. Singh and P. K. Sasmal, Development of Mitochondria Targeting AIE-Active Cyclometalated Iridium Complexes as Potent Antimalarial Agents, *Adv. Healthcare Mater.,* 2023, **12**, 2202411.