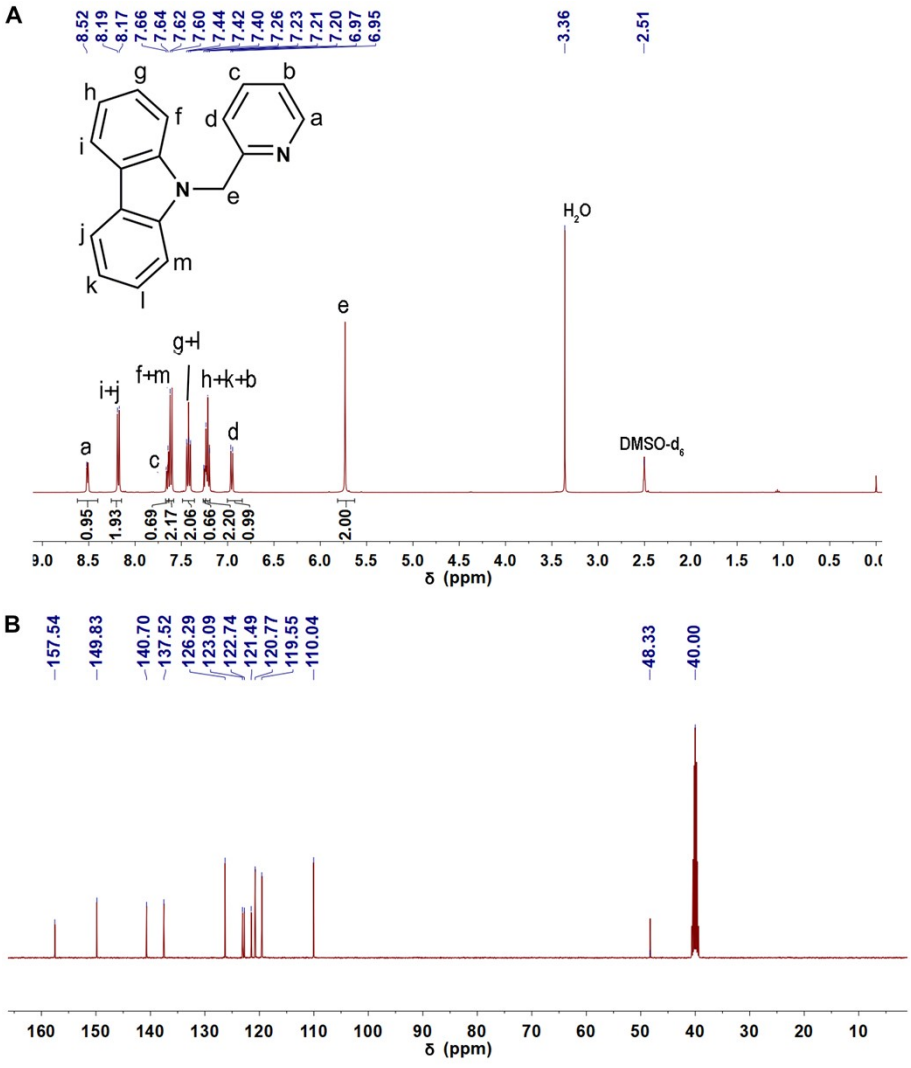
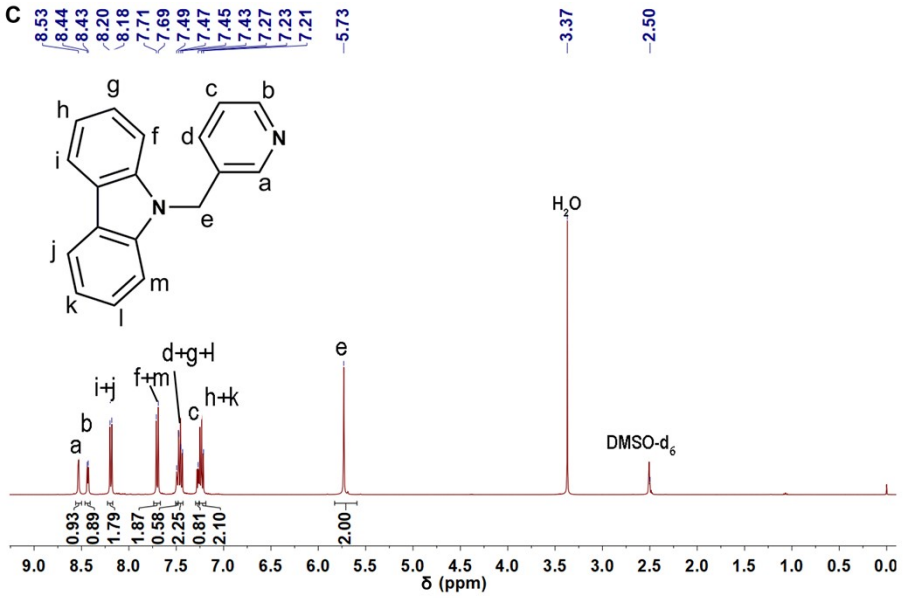
# Electronic supplementary information

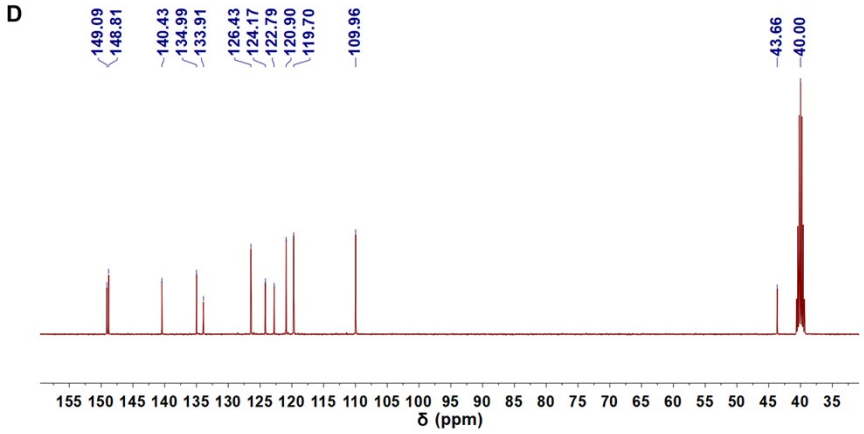
# Platinum complexes as inhibitors of DNA repair protein Ku70 and topoisomerase IIα in cancer cells

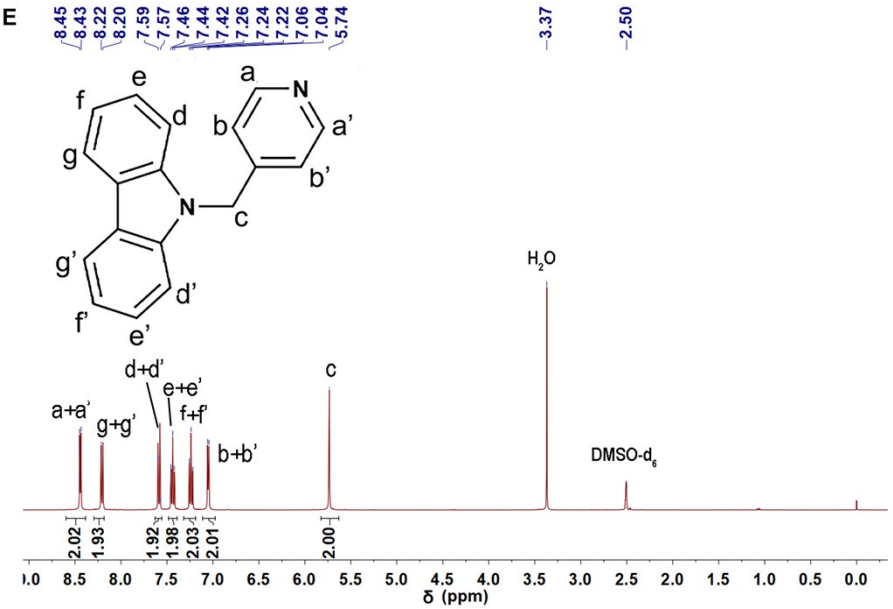
Hongmei Zhang, Ying Wang, Yanqing Wang, Qianqian Han, Honghao Yan, Tao Yang, Zijian Guo and Xiaoyong Wang*

## Supplementary figures and tables




C
8.53
8.44
8.43
8.20
8.18
7.71
7.69
7.49
7.47
7.45
7.43
7.27
7.23
7.21
5.73
3.37
2.50
g
c
b
h
f
d
N
i
a
N
e
j
m
k
l
H2O
d+g+l
e
i+j
f+m
h+k
c
b
a
DMSO-d6
0.93
0.89
1.79
1.87
0.58
2.25
0.81
2.10
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ (ppm)


D
149.09
148.81
140.43
134.99
133.91
126.43
124.17
122.79
120.90
119.70
109.96
43.66
40.00
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
δ (ppm)


E
8.45
8.43
8.22
8.20
7.59
7.57
7.46
7.44
7.42
7.26
7.24
7.22
7.06
7.04
5.74
3.37
2.50
e
a
f
d
N
b
a'
g
b'
N
c
g'
d'
f'
e'
H2O
d+d'
c
e+e'
a+a'
g+g'
f+f'
b+b'
DMSO-d6
2.02
1.93
1.92
1.98
2.03
2.01
2.00
.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
δ (ppm)

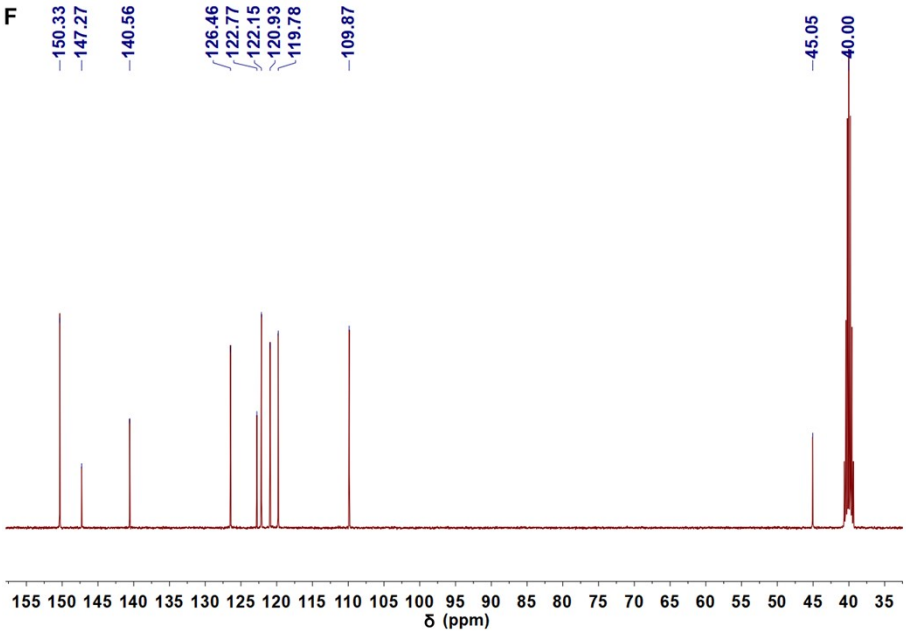
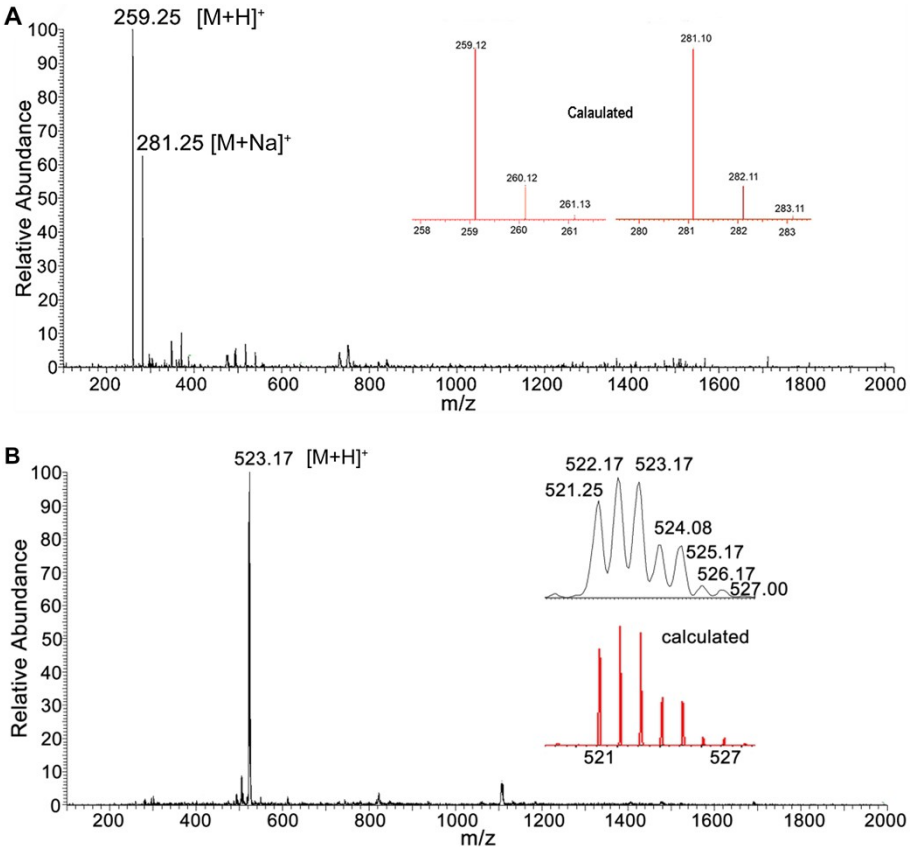



**Fig. S1** $^{1}H$ NMR (600 MHz, $D_2O$) and $^{13}C$ NMR (101 MHz, DMSO-$d_6$) spectra of N-(2-pyridylmethyl) carbazole (A, B), N-(3-pyridylmethyl) carbazole (C, D), and N-(4-pyridylmethyl) carbazole (E, F).



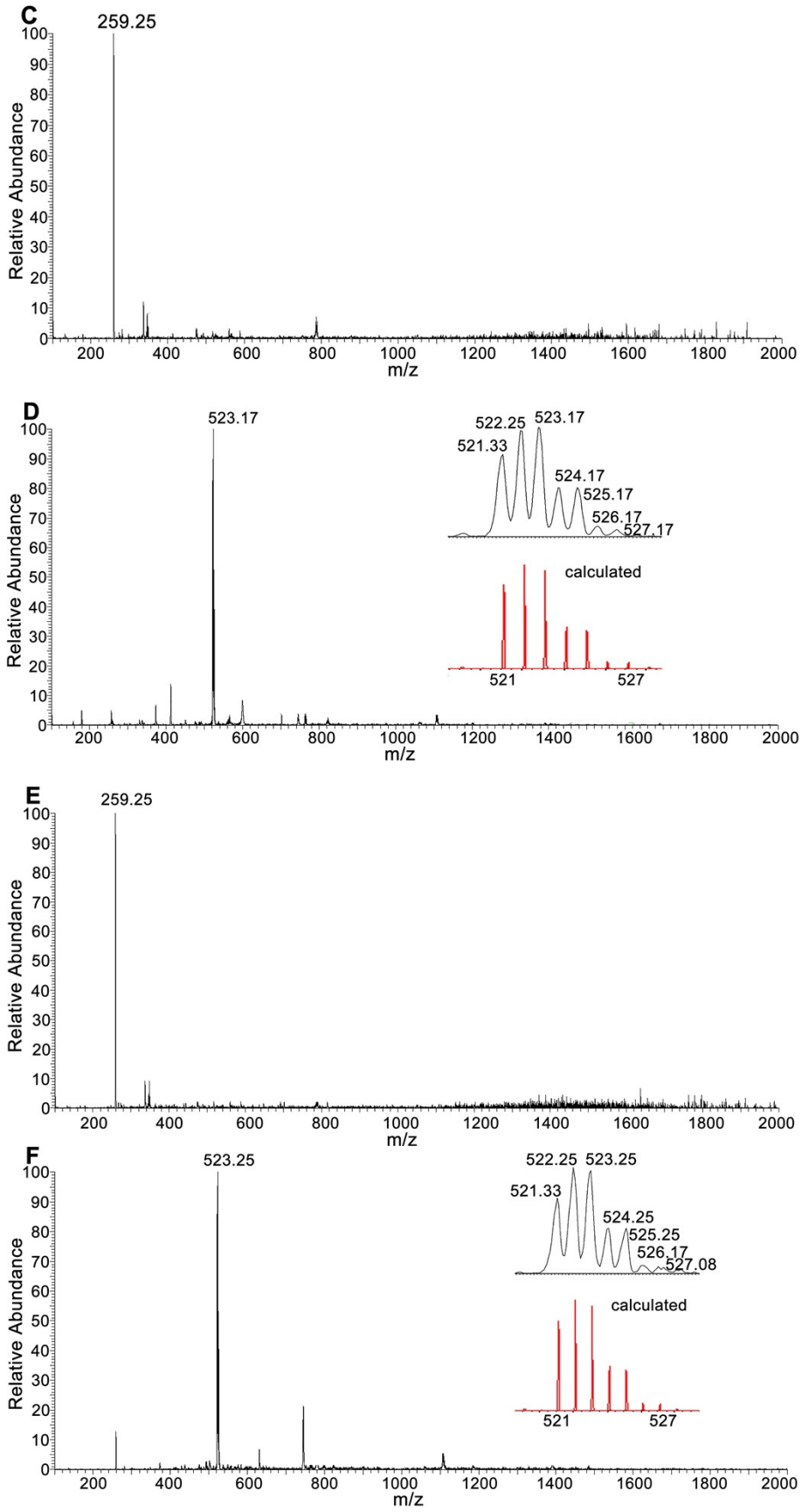
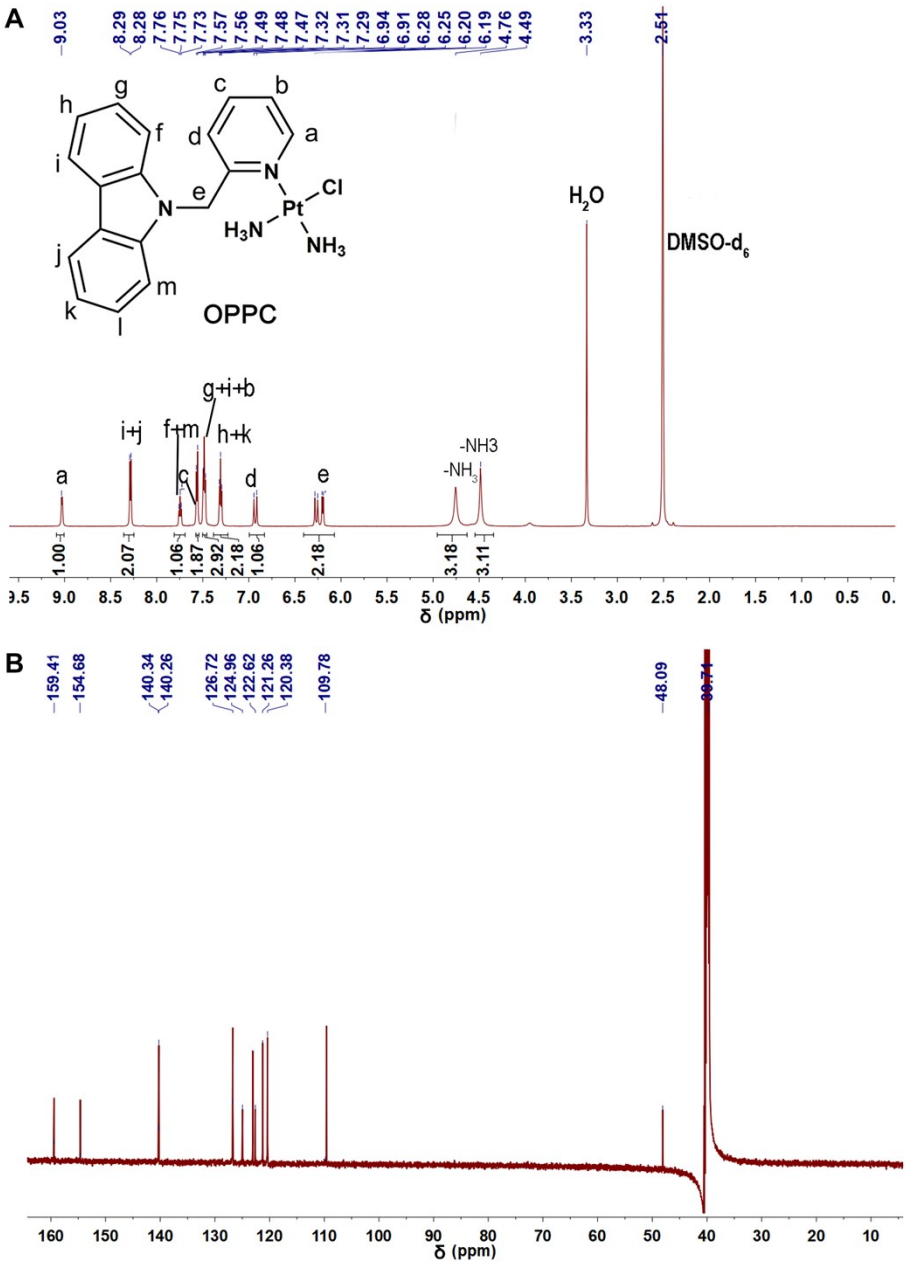
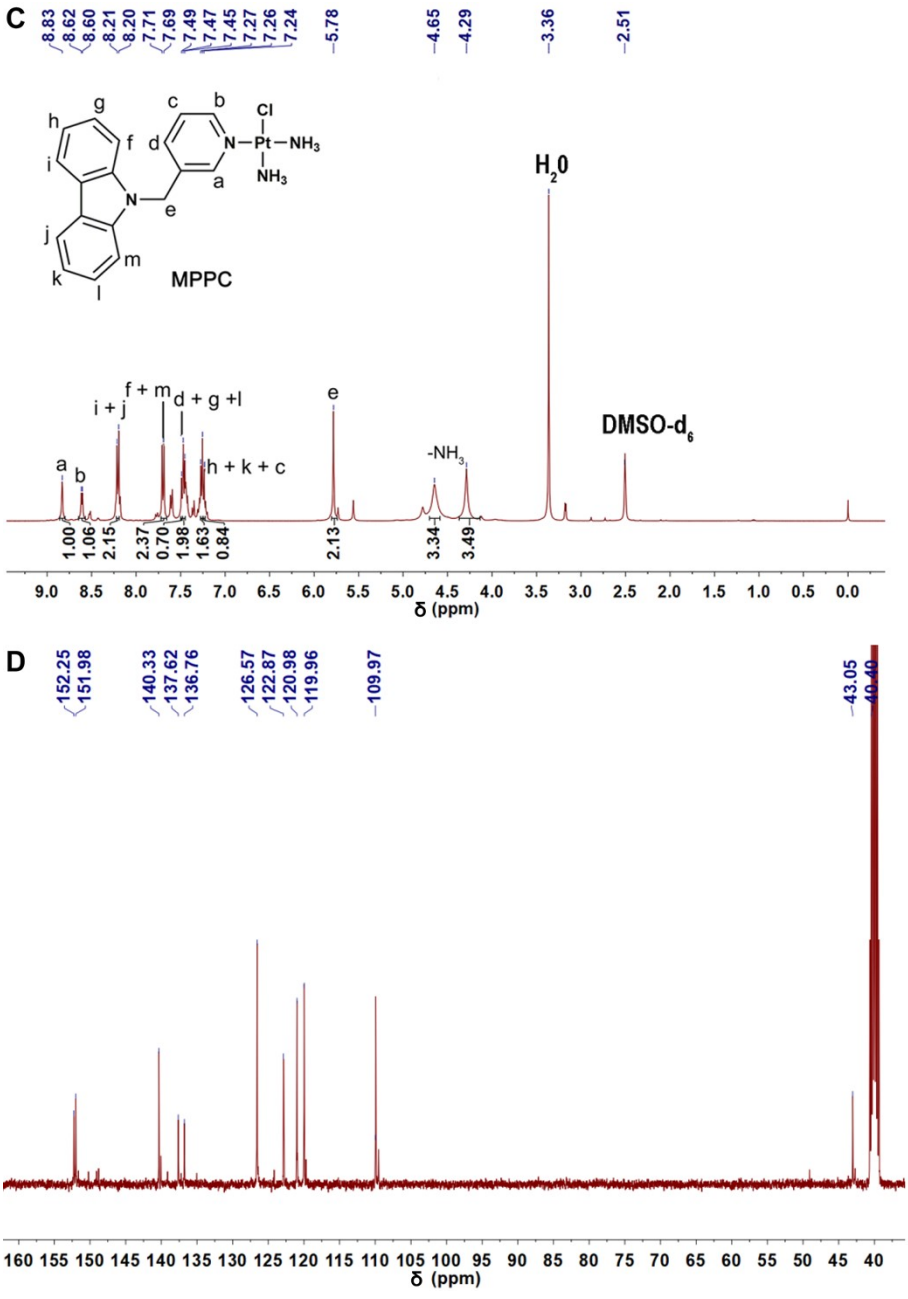

C
259.25
Relative Abundance
m/z
D
523.17
522.25
523.17
521.33
524.17
525.17
526.17
527.17
calculated
521
527
Relative Abundance
m/z
E
259.25
Relative Abundance
m/z
F
523.25
522.25
523.25
521.33
524.25
525.25
526.17
527.08
calculated
521
527
Relative Abundance
m/z

**Fig. S2** ESI-MS spectra of N-(2-pyridylmethyl) carbazole (A), OPPC (B), N-(3-pyridylmethyl) carbazole (C), MPPC (D), N-(4-pyridylmethyl) carbazole (E), and PPPC (F) in methanol.




C
8.83
8.62
8.60
8.21
8.20
7.71
7.69
7.49
7.47
7.45
7.27
7.26
7.24
5.78
4.65
4.29
3.36
2.51
MPPC
H2O
DMSO-d6
-NH3
a
b
i + j
f + m
d + g +l
h + k + c
e
1.00
1.06
2.15
2.37
0.70
1.98
1.63
0.84
2.13
3.34
3.49
δ (ppm)
D
152.25
151.98
140.33
137.62
136.76
126.57
122.87
120.98
119.96
109.97
43.05
40.40
δ (ppm)

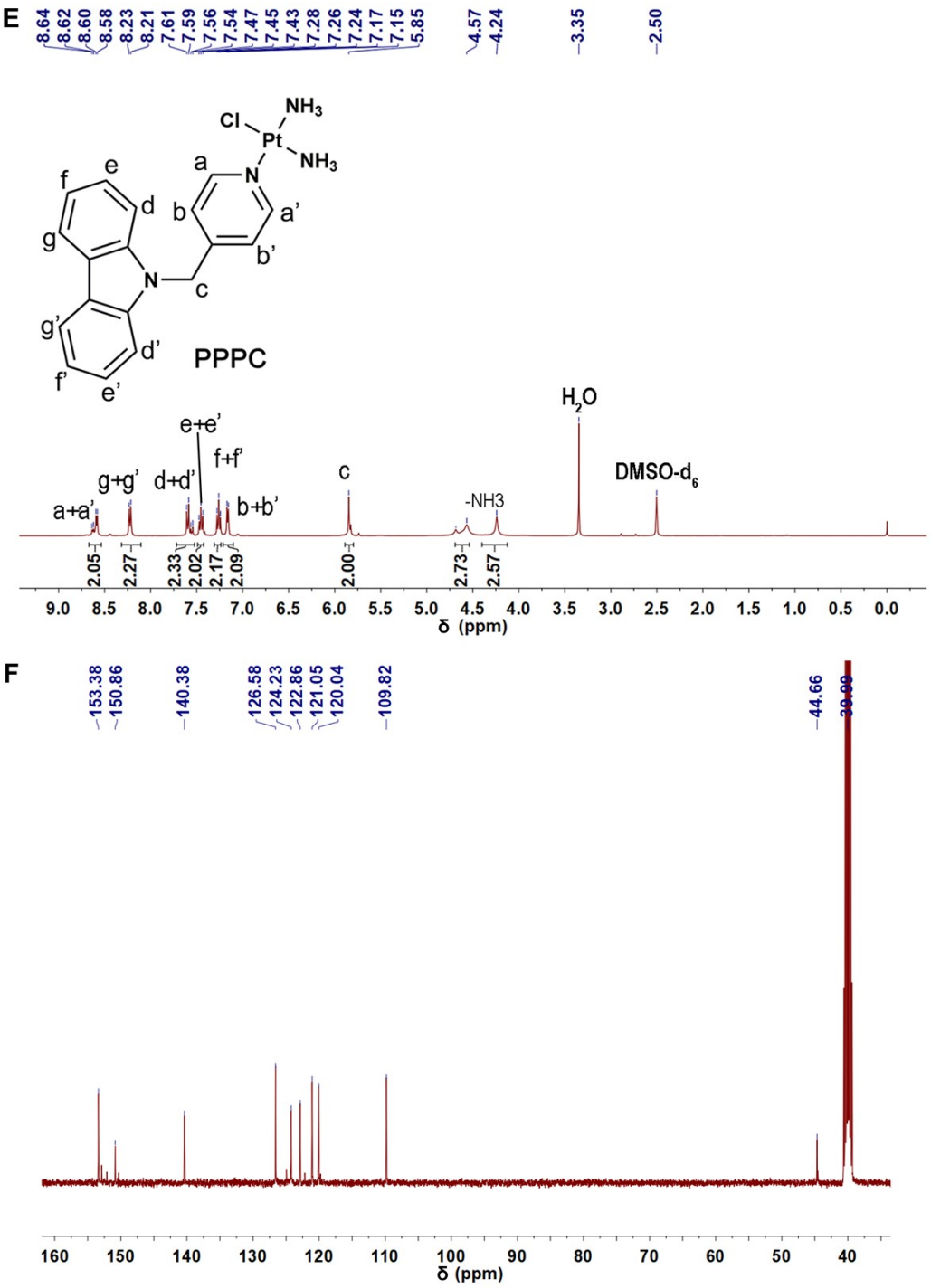



**Fig. S3** $^{1}$H NMR (600 MHz, $D_2O$) and $^{13}$C NMR (101 MHz, DMSO-$d_6$) spectra of OPPC (A, B), MPPC (C, D), and PPPC (E, F).

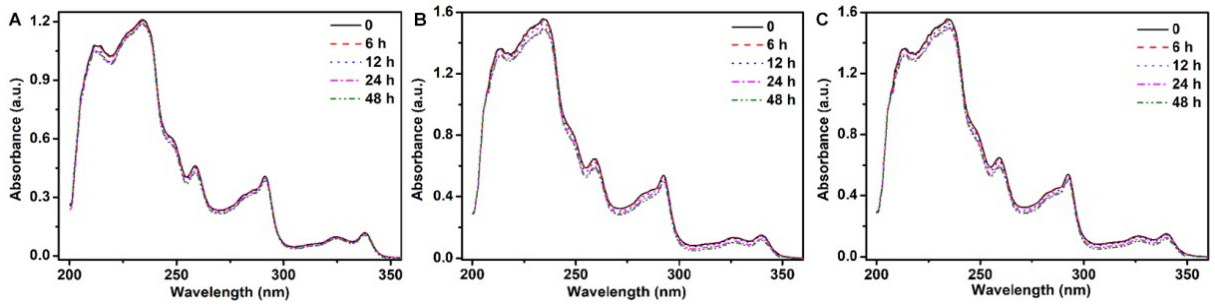
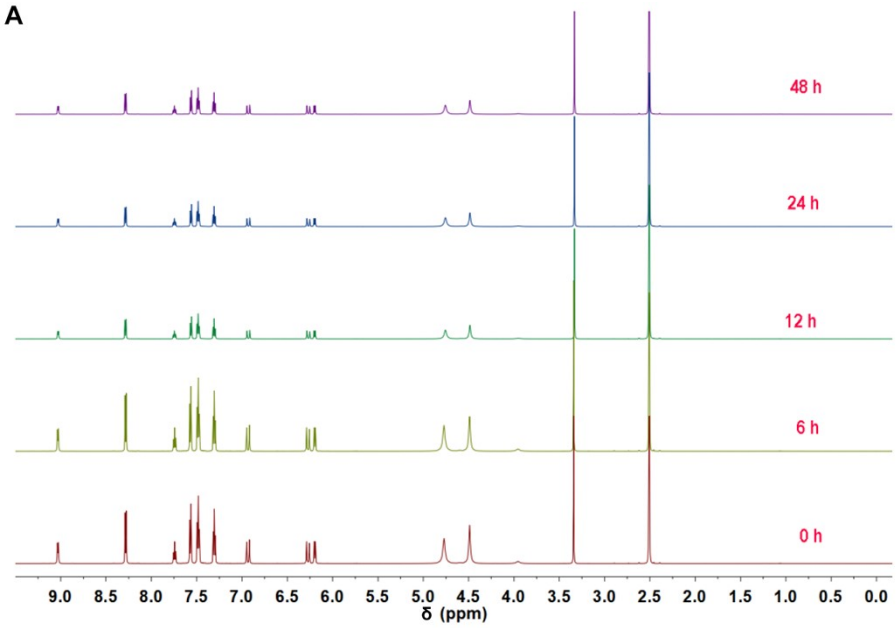



**Fig. S4** Time-dependent UV-Vis absorption spectra of OPPC (A), MPPC (B) and PPPC (C) (50 μM) in culture medium at 37 ℃ monitored at different time spans.



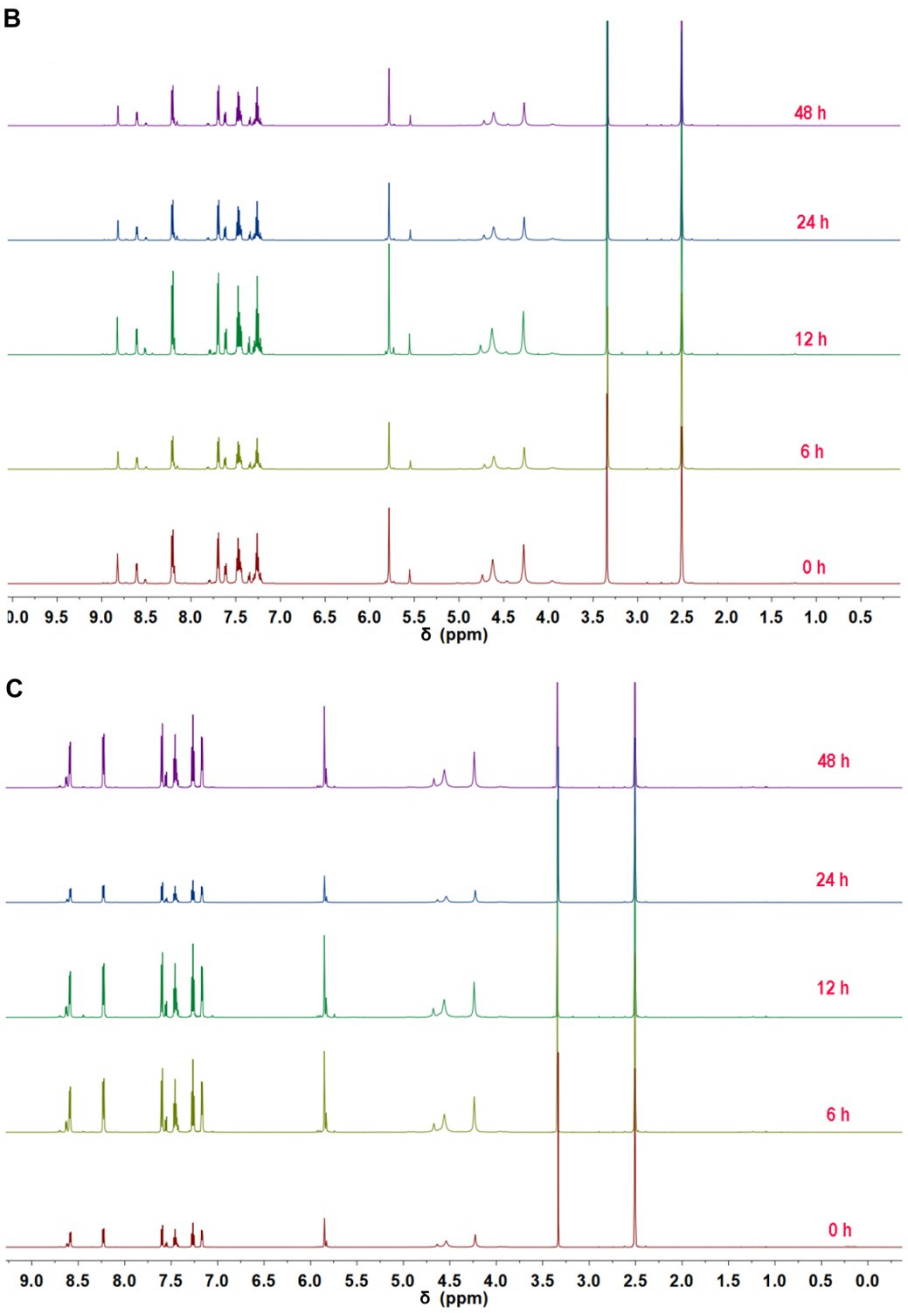



**Fig. S5.** Time-dependent $^{1}H$ NMR spectra of OPPC (A), MPPC (B), and PPPC (C) in culture medium at 37 ℃ monitored at different time spans.

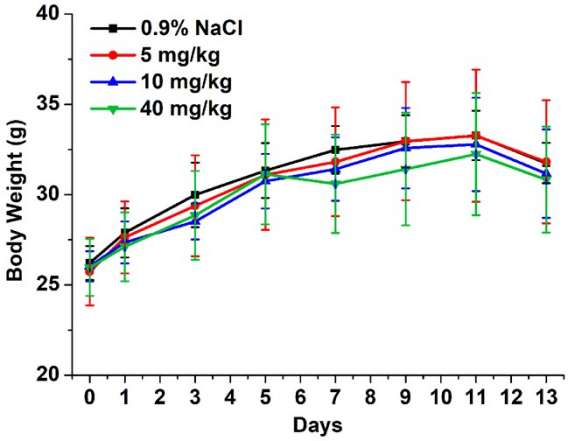



**Fig. S6** Body weight of mice treated intravenously with OPPC every two days.

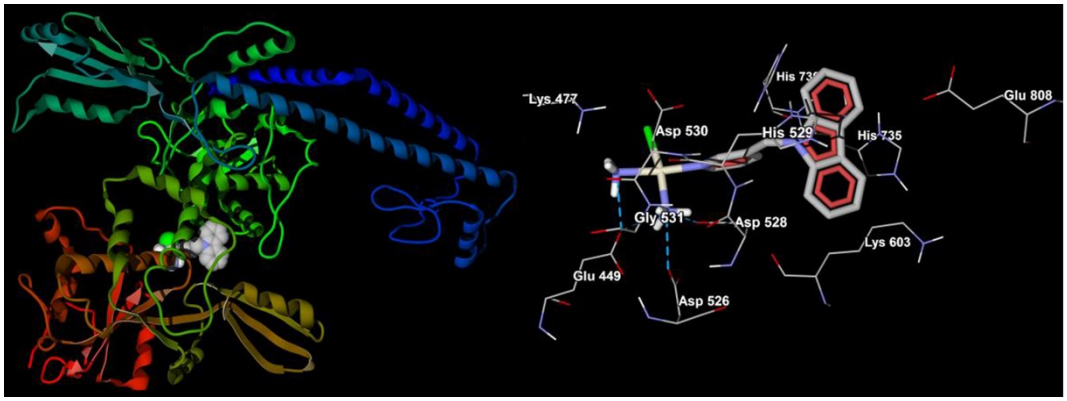



**Fig. S7** Accommodation mode of MPPC with Topo-IIα.

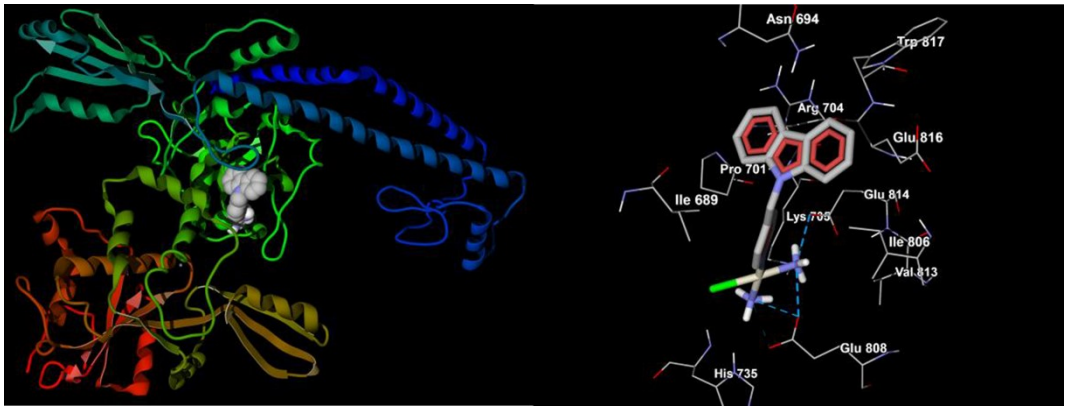



**Fig. S8** Accommodation mode of PPPC with Topo-IIα.

**Table S1** The lowest binding energy generated by the Autodock program for the docking of Top-II with OPPC, MPPC, and PPPC.

| Receptor | Ligand | ΔG (kcal/mol) | $E_{inter\text{-}mol}$ (kcal/mol) | $E_{VHD}$ (kcal/mol) | $E_{elec}$ (kcal/mol) | $E_{total}$ (kcal/mol) | $E_{torsional}$ (kcal/mol) | $E_{unbound}$ (kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| DNA | OPPC | −10.80 | −12.18 | −8.65 | −3.52 | −0.21 | +1.37 | −0.21 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MPPC | −11.10 | −12.48 | −8.26 | −4.21 | +0.28 | +1.37 | +0.28 |
| | PPPC | −11.15 | −12.52 | −7.68 | −4.85 | +0.29 | +1.37 | +0.29 |
| Topo-II | OPPC | −16.35 | −17.72 | −4.01 | −13.71 | −0.29 | +1.37 | −0.29 |
| | MPPC | −16.71 | −18.09 | −4.20 | −13.88 | +0.10 | +1.37 | +0.10 |
| | PPPC | −16.74 | −18.11 | −5.29 | −12.52 | +0.38 | +1.37 | +0.38 |

## Experimental section

**Chemicals and reagents.** Carbazole and 2-pyridinyl methyl hydrochloride was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. China. Cisplatin (CDDP) was purchased from Shandong Boyuan Pharmaceutical Co., Ltd. China. All the reagents were of analytical grade and used as received without further purification. Water was deionized and ultrafiltered by a Milli-Q apparatus (Millipore Corporation, China). Annexin V conjugated with fluorescein isothiocyanate (Annexin V-FITC) and propidium iodide (PI) were purchased from KeyGen Biotech. Co. Ltd. (Nanjing, China). Hoechst 33342 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. The antibodies used for Western blotting were purchased from Abcam. PUC19 plasmid DNA was purchased from Thermo Fisher Scientific (USA). Purified human Topo IIα enzyme, Topo IIα assay kits, and unwinding kit were purchased from TopoGen Inc. (Port Orange, FL).

**Cell lines and animals.** The human non-small-cell lung cancer A549, its CDDP-resistant counterpart A549/DDP, human ovarian cancer Caov3 and Skov3, human liver cancer HepG2, human cervical cancer HeLa, human breast cancer MCF-7 cell lines, and the human normal liver L-02 cell line were purchased from American Type Culture Collection (ATCC). Female ICR mice were purchased from the Model Animal Research Center of Nanjing University.

**Instruments.** $^{1}$H- and $^{13}$C NMR spectra were acquired on a Bruker DRX 400 MHz and 600 MHz spectrometer at 298 K. Elemental analysis was performed on an elementar vario MICRO cube elemental analyzer. Electrospray ionization mass spectra (ESI-MS) were obtained on an LCQ Fleet electrospray mass spectrometer. Circular dichroism (CD) spectra were determined by using a JASCO J-810 automatic recording spectropolarimeter (Tokyo, Japan). Fluorescence spectra were recorded on a Perkin-Elmer LS-50B fluorescence photometer. UV-Vis absorption spectra were recorded on a PerkinElmer Lambda 35 spectrophotometer. The content of Pt was determined on an inductively coupled plasma mass spectrometer (ICP-MS) using a standard

Plasma-Quad II instrument (VG Elemental, Thermo OptekCorp.). Western blotting was carried out on the Bio-Rad mini-PROTEAN tetra system and Bio-Rad Powerpack Universal. Fluorescence confocal imaging was carried out on a laser scanning confocal imaging system (Olympus TH4-200) consisting of ZEISS Laser Scanning Microscope (LSM 710) and a 20 mW-output 488 nm argon ion laser.

**Synthesis of complexes.** Ligand *N*-(2-pyridinyl methyl) carbazole was prepared as follows. 2-Chloromethylpyridine hydrochloride (5.0 g, 0.030 mol) was stirred in dry DMF (60 mL) with KOH (6.0 g) and carbazole (4.0 g, 0.024 mol) at 45 ℃ for 18 h. After filtration, the crude product was recrystallized in a hot anhydrous methanol solution, and dried under vacuum to get the white acicular crystals. Yield: 4.62 g, 75 %. $^{1}$H NMR of *N*-(2-pyridinyl methyl) carbazole (600 MHz, DMSO-$d_6$): δ (ppm) = 8.52 (s, 1 H, $CH_{2\text{-pyridine}}$), 8.19, 8.17 (d, J = 12 Hz, 2 H, $CH_{indole}$), 7.66, 7.64, (d, J=12 Hz, 1H, $CH_{2\text{-pyridine}}$), 7.62, 7.60 (d, J=12 Hz, 2 H, $CH_{indole}$), 7.44, 7.42,7.40 (t, J = 12 Hz, 2 H, $CH_{indole}$), 7.26, (s, 1 H, $CH_{2\text{-pyridine}}$), 7.23, 7.21, 7.20 (t, J = 12 Hz, 2H, $CH_{indole}$ ), 6.97, 6.95 (d, J=12 Hz, 1 H, $CH_{2\text{-pyridine}}$), 5.73 (s, 2 H, $CH_{2methylene}$). $^{13}$C NMR (600 MHz, DMSO-$d_6$): δ (ppm) = 157.54 ($C_{2\text{-pyridine}}$), 149.83 (CH, $C_{2\text{-pyridine}}$), 140.70 (CH, $C_{2\text{-pyridine}}$), 137.52 ($C_{pyrrole}$), 126.29 ($C_{pyrrole}$), 123.09 (CH, $C_{indole}$), 122.74 (CH, $C_{indole}$), 121.49 (CH, $C_{indole}$), 120.77 (CH, $C_{indole}$), 119.55 (CH, $C_{2\text{-pyridine}}$), 110.04 (CH, $C_{indole}$), 48.25 ($CH_2$, $C_{aromat}$).

The other two ligands were synthesized similarly by replacing the raw material 2-chloromethylpyridine hydrochloride with 3-chloromethylpyridine hydrochloride and 4-chloromethylpyridine hydrochloride respectively. $^{1}$H NMR of *N*-(3-pyridinyl methyl) carbazole (600 MHz, DMSO-$d_6$): δ (ppm) = 8.53 (s, 1H, $CH_{3\text{-pyridine}}$), 8.44, 8.43 (d, J = 6 Hz, 1H, $CH_{3\text{-pyridine}}$), 8.20, 8.18 (d, J = 6 Hz, 2H, $CH_{indole}$), 7.71, 7.69 (d, J = 12 Hz, 2 H, $CH_{indole}$), 7.49 (s, 1 H, $CH_{3\text{-pyridine}}$), 7.47, 7.45,7.43 (t, J=12 Hz, 2H, $CH_{indole}$), 7.27, (s, 1 H, $CH_{3\text{-pyridine}}$), 7.23, 7.21 (t, J= 12 Hz, 2 H, $CH_{indole}$), 5.73 (s, 2 H, $CH_{2methylene}$). $^{13}$C NMR (600 MHz, DMSO-$d_6$): δ (ppm) = 149.09 (CH, $C_{3\text{-pyridine}}$), 148.81 (CH, $C_{3\text{-pyridine}}$), 140.43 ($C_{pyrrole}$), 134.99 (CH, $C_{3\text{-pyridine}}$), 133.91 ($C_{3\text{-pyridine}}$), 126.43 (CH, $C_{3\text{-pyridine}}$), 124.17 ($C_{pyrrole}$), 122.79 (CH, $C_{indole}$), 120.90 (CH, $C_{indole}$), 119.70 (CH, $C_{indole}$), 109.96 (CH, $C_{indole}$), 43.66 ($CH_2$, $C_{aromat}$). $^{1}$H NMR of *N*-(4-pyridinyl methyl) carbazole (600 MHz, DMSO-$d_6$): δ (ppm) = 8.45, 8.43 (d, J =12 Hz, 2 H, $CH_{4\text{-pyridine}}$), 8.22, 8.20 (d, J = 12 Hz, 2 H, $CH_{indole}$), 7.59, 7.57 (d, J = 12 Hz, 2 H, $CH_{indole}$), 7.46, 7.44, 7.42 (t, J = 12 Hz, 2 H, $CH_{indole}$), 7.26, 7.24, 7.22 (t, J = 12 Hz, 2 H, $CH_{indole}$), 7.06, 7.04 (d, J = 6 Hz, 2 H, $CH_{4\text{-pyridine}}$), 5.74 (s, 2 H, $CH_{2methylene}$). $^{13}$C NMR (600 MHz, DMSO-$d_6$): δ (ppm) = 150.33

(CH, $C_{4\text{-pyridine}}$), 147.27 ($C_{4\text{-pyridine}}$), 140.56 ($C_{pyrrole}$), 126.46 (CH, $C_{4\text{-pyridine}}$), 122.77 ($C_{pyrrole}$), 122.15 (CH, $C_{indole}$), 120.93 (CH, $C_{indole}$), 119.78 (CH, $C_{indole}$), 109.87 (CH, $C_{indole}$), 45.05 ($CH_2$, $C_{aromat}$).

Complex OPPC was synthesized as follows. CDDP (450 mg, 1.5 mmol) and $AgNO_3$ (240 mg, 1.41 mmol) were stirred in anhydrous DMF (10 mL) overnight in the dark at 45 ℃. The light green solution containing *cis*-$[Pt(NH_3)_2Cl(DMF)]^+$ was obtained after centrifugation. *N*-(2-pyridinyl methyl) carbazole (300 mg, 1.16 mmol) was dissolved in the above solution and stirred for 48 h in the dark at 58 ℃. The resulting dark brown oily solution was filtered and evaporated. The oily substance was extracted several times with hot anhydrous methanol to clarify the extract, and then concentrated to 15 mL. A light yellow precipitate was obtained after addition of diethyl ether, which was washed with hot anhydrous methanol and diethyl ether, dried in vacuum to obtain the final product OPPC. Yield: 407.2 mg, 67%. $^1$H NMR of OPPC (600 MHz, DMSO-$d_6$): δ (ppm) = 9.03 (s, 1 H, $CH_{2\text{-pyridine}}$), 8.29, 8.28 (d, J = 12 Hz, 2 H, $CH_{indole}$), 7.76, 7.75, 7.73 (t, J = 6 Hz, 1 H, $CH_{2\text{-pyridine}}$), 7.57, 7.56 (d, J = 6 Hz, 2 H, $CH_{indole}$), 7.49 (s, $CH_{2\text{-pyridine}}$) 7.48, 7.47 (d, J = 6 Hz, 2 H, $CH_{indole}$), 7.32, 7.31, 7.29 (t, J = 6 Hz, 2 H, $CH_{indole}$), 6.94, 6.91 (d, J = 18 Hz, 1 H, $CH_{2\text{-pyridine}}$), 6.28, 6.25, 6.20, 6.19 (m, $CH_{2methylene}$), 4.76 (br, 3 H, -$NH_3$), 4.49 (br, 3 H, -$NH_3$). $^{13}$C NMR (600 MHz, DMSO-$d_6$): δ (ppm) = 159.41 ($C_{2\text{-pyridine}}$), 154.68 (CH, $C_{2\text{-pyridine}}$), 140.34 (CH, $C_{2\text{-pyridine}}$), 140.26 ($C_{Pyrrole}$), 126.72 (CH, $C_{2\text{-pyridine}}$), 124.96 ($C_{Pyrrole}$), 122.62 (CH, $C_{indole}$), 121.26 (CH, $C_{indole}$), 120.38 (CH, $C_{indole}$), 109.78 (CH, $C_{indole}$), 48.09 ($CH_2$, $C_{aromat}$). ESI-MS (positive mode, m/z) found (calcd): 523.17 (524.94). Elemental analysis determined (calcd): C, 36.02 (36.83); H, 3.65 (3.78); N, 12.13 (11.93).

MPPC and PPPC were synthesized by similar procedures except using *N*-(3-pyridinyl methyl) carbazole and *N*-(4-pyridinyl methyl) carbazole as the ligand, respectively. $^1$H NMR of MPPC (600 MHz, DMSO-$d_6$): δ (ppm) = 8.83 (s, 1H, $CH_{3\text{-pyridine}}$), 8.62, 8.60 (d, J = 12 Hz, 1 H, $CH_{3\text{-pyridine}}$), 8.21, 8.20 (d, J = 6 Hz, 2 H, $CH_{indole}$), 7.71, 7.69 (d, J = 8.0 Hz, 2 H, $CH_{indole}$), 7.49 (s, $CH_{3\text{-pyridine}}$) 7.47, 7.45 (d, J = 12 Hz, 2 H, $CH_{indole}$), 7.27, 7.26 (d, J = 6 Hz, 2 H, $CH_{indole,}$ ), 7.24 (s, 1 H, $CH_{3\text{-pyridine}}$), 5.78 (s, 2 H, $CH_{2methylene}$), 4.65 (br, 3 H, -$NH_3$), 4.29 (br, 3 H, -$NH_3$). $^{13}$C NMR (600 MHz, DMSO-$d_6$): δ (ppm) = 152.25 (CH, $C_{3\text{-pyridine}}$), 151.98 (CH, $C_{3\text{-pyridine}}$), 140.33 ($C_{pyrrole}$), 137.62 (CH, $C_{3\text{-pyridine}}$), 136.76 ($C_{3\text{-pyridine}}$), 126.57 (CH, $C_{3\text{-pyridine}}$), 122.87 ($C_{pyrrole}$), 120.98 (CH, $CH_{indole}$), 119.96 (CH, $CH_{indole}$), 109.97 (CH, $CH_{indole}$), 43.05 ($CH_2$, $C_{aromat}$). ESI-MS (positive mode, m/z) found (calcd): 523.17 (524.94). Elemental analysis determined (calcd):

C, 36.09 (36.83); H, 3.70 (3.78); N, 12.08 (11.93). $^{1}$H NMR of PPPC = (600 MHz, DMSO-$d_6$): δ (ppm) 8.64, 8.62, 8.60, 8.58 (dd, $J_1$ = 24, $J_2$ = 12 Hz, 2 H, $CH_{4\text{-pyridine}}$), 8.23 8.21 (d, J = 6 Hz, 2 H, $CH_{indole}$), 7.61, 7.59 (d, J = 12 Hz, 2 H), 7.56, 7.54 (d, J = 12.0 Hz, 2 H, $CH_{indole}$), 7.47, 7.45, 7.43 (t, J = 12 Hz, 2 H, $CH_{indole}$), 7.28, 7.26, 7.24 (t, J = 12 Hz, 2H, $CH_{indole}$), 7.17, 7.15 (d, J = 8.0 Hz, 2 H, $CH_{4\text{-pyridine}}$), 5.85 (s, 2 H, $CH_2$ $_{methylene}$), 4.57 (br, 3 H, -$NH_3$), 4.24 (br, 3 H, -$NH_3$). $^{13}$C NMR (600 MHz, DMSO-$d_6$): δ (ppm) 153.38 (CH, $C_{4\text{-pyridine}}$), 150.86 ($C_{4\text{-pyridine}}$), 140.38 ($C_{pyrrole}$), 126.58 (CH, $C_{4\text{-pyridine}}$), 124.23 ($C_{pyrrole}$), 122.86 (CH, $C_{indole}$), 121.05 (CH, $C_{indole}$), 120.04 (CH, $C_{indole}$), 109.82 (CH, $C_{indole}$), 44.66 ($CH_2$, $C_{aromat}$). ESI-MS (positive mode, m/z) found (calcd): 523.25 (524.94). Elemental analysis determined (calcd): C, 36.16 (36.83); H, 3.62 (3.78); N, 12.05 (11.93).

**Stability.** OPPC and PPPC solutions were dissolved in culture medium containing 10% FBS, while MPPC was dissolved in culture medium containing DMSO (0.1%). The solutions were incubated at 37 ℃ for 0, 6, 12, 24, and 48 h. UV-Vis absorption spectra of the solutions were determined on a Specord 200 UV-Visible. Furthermore, OPPC, PPPC, and MPPC was dissolved in culture medium containing DMSO-$d_6$, respectively. The solutions were incubated at 37 ℃ for 0, 6, 12, 24, and 48 h. $^{1}$H NMR of the solutions were determined on a Bruker DRX 600 MHz spectrometer at 298 K.

**Cell culture.** Caov3, Skov3, HepG2, HeLa, MCF-7, and L-02 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). A549 cells was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium with 10% FBS. A549/DDP cells was cultured in RPMI-1640 medium containing 10% FBS and 1000 ng $mL^{-1}$ CDDP. FBS and all culture media were from Nanjing KeyGen Biotech Co., Ltd. All cells were cultured in the medium supplemented with 100 units $mL^{-1}$ of penicillin and 100 g $mL^{-1}$ of streptomycin at 37 ℃ in a humidified atmosphere with 5% $CO_2$.

**Antiproliferative activity.** Cancer cells in logarithmic growth phase were seeded in 96-well plates at a density of 5000−8000 cells per well and incubated at 37 ℃ in a humidified incubator with 5% $CO_2$ overnight. The medium in each well was replaced with 200 μL of medium containing graded concentrations of complexes. After incubation for 48 h, MTT (20 μL, 5 mg $mL^{-1}$ in PBS) solution was added to each well, and the plates were incubated at 37 ℃ for another 4 h. The supernatant was removed and the precipitated formazan was dissolved in 200 μL

DMSO. The plates were shaken for 10 min and the optical density of each well was measured by microplate reader (Thermo Scientific Varioskan Flash) at 490 nm. Concentrations that inhibited cell growth by 50% ($IC_{50}$) after 48 h of treatment were calculated according to the ratio of ($OD_{test}$ − $OD_{blank}$)/($OD_{contol}$ − $OD_{blank}$) × 100%. The reported $IC_{50}$ values are the average of three independent experiments.

**Acute toxicity.** Female mice were purchased and housed in cages at 22 ± 2 ℃ temperature and a 12 h light-dark cycle with standard food and water available ad libitum. Different concentrations of OPPC (5, 10, 20, 40 mg $Kg^{-1}$) was dissolved in saline. The control mice were injected with identical saline (200 μL). The experiment groups were injected intravenously with solutions of OPPC in 200 μL of saline. The changes in body weight were recorded and the survival rate of the mice was calculated over a period of two weeks. All the experimental procedures on mice were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Nanjing University and were approved by the Institutional Animal Care and Use Committee of Nanjing University.

**Cell cycle analysis.** A549 cells in logarithmic growth phase were seeded in a 6-well plate and incubated at 37 ℃ in a 5% $CO_2$ humidified atmosphere overnight. The cells were incubated with the complex for 24 h, and then were collected, washed with precooled PBS, fixed in ice-cold ethanol (70%) for 12 h or stored at −20 ℃ overnight. The cells were collected by centrifugation, treated with RNase at 37 ℃ for 30 min, stained with PI at 4 ℃ for 15 min, and analyzed by flow cytometry (BD LARFortessa, USA). The cell cycle profiles were modeled using Modfit LT software (Verity Software House, Topsham, ME, USA).

**Apoptosis.** Apoptosis was detected by flow cytometry using the FITC Annexin V apoptosis detection kit I (KeyGen Biotech. Co. Ltd.). A549 cells were seeded in 6-well plates and cultured overnight at 37 ℃. After treatment with the complex for 48 h, the cells were washed twice with cold PBS and resuspended in 100 μL of 1×binding buffer at a concentration of 1 × $10^5$ cells $mL^{-1}$. The cells were stained with 5 μL of FITC Annexin V and 5 μL of PI for 30 min at room temperature in the dark. Before reading, 400 μL of 1×binding buffer was added to each tube. The cells were analyzed on a flow cytometer within 1 h.

**Cellular uptake.** A549 cells in logarithmic growth phase were seeded in a 6-well plate at a density of 2 × $10^5$ cells per well. After incubation for 24 h, the cells were treated with each

complex for 24 h. The cells were washed with PBS 2−3 times and collected by centrifugation, digested with nitric acid (100 μL) for 2 h at 95 ℃, followed by the addition of $H_2O_2$ (50 μL) at 95 ℃ for 1.5 h. The solution was diluted to 1 mL with water and the Pt content was determined by ICP-MS.

**DNA interaction.** DNA binding was tested by 1% agarose gel electrophoresis in TAE running buffer (40 mM Tris acetate, 1 mM EDTA, pH 7.4) on pUC19 plasmid DNA at 90 V for 2 h. Specifically, different concentrations of each complex were incubated with pUC19 plasmid DNA (200 ng $\mu L^{-1}$, 10 μL) in Tris-HCl buffer (50 mM Tris, 50 mM NaCl, pH 7.4). After incubation in the dark at 37 ℃ for 24 h, the reaction was quenched by adding 6×loading buffer (2 μL). The solutions were loaded onto agarose gels (1%) and subjected to electrophoresis in a TAE buffer. The gel was stained with ethidium bromide (EB, 1 μg $mL^{-1}$) and visualized under UVP gel imaging system.

The interaction mode of the complex with CT-DNA was analyzed using circular dichroism (CD) spectroscopy. CT-DNA solution was prepared with a Tris-HCl buffer (5 mM Tris, 50 mM NaCl, pH = 7.4), and the concentration of CT-DNA was determined by a nanodrop spectrophotometer at 260 nm, taking 6600 $M^{-1}$ $cm^{-1}$ as the molar absorption coefficient. Test samples were prepared by incubating CT-DNA (100 μM) with different concentrations of the complexes at 37 ℃ in the dark for 24 h. The interaction between the complex and CT-DNA in the wavelength range of 220−320 nm was detected by CD spectroscopy.

**Comet assay**. Comet assay was performed under alkaline conditions to detect DNA double strand breaks using the method previously described.[1] Briefly, A549 cells were washed with cold PBS and collected after treatment with the complexes for 24 h. The DNA damage in single cell was detected using a kit from KeyGenBiotech by gel electrophoresis according to the manufacturer's instructions. Three slides each containing 100 cells were analyzed at different concentrations. The percentage of DNA in tails was calculated using the Comet Assay Software Project (CASP, http://casplab.com/).

**Western blotting.** A549 cells ($10^7$) were treated with the complexes for 24 h and total cellular protein was isolated using RIPA lysis buffer containing protease inhibitors (Beyotime Institute of Biotechnology). The protein concentrations were quantified by the BCA method. The total protein (50 μg) from the cell lysates was resolved by SDS-PAGE and then electrophoretically

transferred onto a PVDF membrane. The membranes were blocked with 5% (w/v) BSA at room temperature for 1 h, incubated with the following primary antibodies against γ-H2AX, p-ChK1 (Ser-345), p-ChK2 (Thr-68), p53, Ku70, and Topo IIα (Abcam, Cambridge, MA, USA) in appropriate dilutions at 4 ℃ overnight. The membranes were washed with Tris buffered saline with Tween (TBST) and incubated with peroxidase-conjugated secondary antibody at room temperature for 1 h. After washing with TBST, the blots were visualized by enhanced chemiluminescence kit from Millipore.

**Immunofluorescence.** A549 cells were seeded on coverslips in 6-well plates and incubated overnight. After treatment with the complexes for 24 h, the cells were fixed with 4% paraformaldehyde at 37 ℃ for 20 min, treated with 0.2% Triton X-100 in PBS for 20 min, and blocked with 5% bovine serum albumin in PBS for 30 min. The cells were then incubated in primary antibody solution (γ-H2AX or Ku70 in 1:100 dilution) overnight at 4 ℃, and washed thrice in PBS before incubating in the dark with a FITC labeled secondary antibody for 60 min. The cells were incubated with Alexa Fluor 488-conjugated secondary antibody at 1:1000 in PBS/5% goat serum at 37 ℃ in the dark for 1 h. After washing with PBS for 3 times, the cells were counterstained with Hoechst 33342 for 10 min to visualize the nuclei. The slides were subjected to fluorescence imaging using a confocal microscope.

**Topo II-mediated kinetoplast DNA (kDNA) decatenation.** Decatenation assays were performed as follows. kDNA (120 ng) was added to the reaction buffer containing Tris-HCl (250 mM, pH 8.0), NaCl (750 mM), $MgCl_2$ (50 mM), dithiothreitol (2.5 mM), and ATP (10 mM). After addition of the complex and 1U of Topo II, the reaction solution (20 μL) was incubated at 37 ℃ for 30 min. At the end, 5×stop buffer was added and the samples were electrophoresed through a 1% agarose gel containing 1×TAE buffer at 5 V $cm^{-1}$ for 1 h. The gels were stained with EB, destained with water, and visualized by the Bio-Rad Gel-Doc XR imaging system.

**Band depletion assay.** The band depletion assay was done as described by Desai et al.[2] Briefly, A549 cells were lysed in alkaline lysis solution (200 mM NaOH, 1 mM EDTA), and the lysate was neutralized with one-tenth volume of HCl (2 M) and one-tenth volume of solution containing 10% NP40, 1 M Tris (pH 7.4), 0.1 M $MgCl_2$, 0.1 M $CaCl_2$, 10 mM DTT and 1 mM EGTA, along with 100 g $mL^{-1}$ of leupeptin, pepstatin and aprotinin. The neutralized lysate was mixed with 3×SDS sample buffer, and the lysates were separated on SDS-PAGE gels.

**Gene silencing.** A549 cells were seeded in 6-well plates and incubated for 24 h. Control miRNA was added to the transfection medium and incubated at 37 ℃ in a $CO_2$ incubator for 6 h. Loss of Topo IIα expression was achieved by using small interfering RNA (siRNA) of Topo IIα (Santa Cruz Biotech, #sc-36695, Santa Cruz, CA, USA). Cell transfection was performed using LipofectamineTM RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cells were supplemented with normal culture media and cultured at 37 ℃ with 5% $CO_2$ for up to 48 h before harvest. To determine the efficiency of gene silencing, Topo IIα protein level was determined by immunoblotting using anti-Topo IIα antibodies.

**Molecular docking.** The optimized geometrical structures of OPPC, MPPC, and PPPC were calculated by using Gaussian 09 software with DFT B3LYP/LanL2DZ base.[3] The crystal structure of Topo II (PDB, ID: 4FM9) was obtained from the Protein Data Bank.[4] Autodock 4.2.3 and AutoDock tools 1.5.4 programs was used to analysis the binding mode of Topo II with the platinum complex.[5] In the calculation, OPPC, MPPC, and PPPC was set as ligand, and Topo II was set as receptor, respectively. The even number of user-specified grid points of Topo II was set at 60 × 40 × 126, 126 × 126 × 126 and 126 × 126 × 126, respectively. The number of genetic algorithm runs, the population size, the maximum number of evals, the maximum number of generations, the maximum number of top individuals that automatically survive, the rate of gene mutation, and the rate of crossover was set at 100, 150, 25000000, 27000, 1, 0.02, and 0.8, respectively. All other parameters were default settings. After the calculation, the Molegro Molecular Viewer software was used to analysis the docked results.[6]

## References


1. H. M. Zhang, T. Yang, Y. Q. Wang, Z. Z. Zhu, Z. H. Wang, Z. J. Guo and X. Y.Wang, *Dalton Trans*. **2021**, 50, 304−310.

2. S. D. Desai, T. K. Li, A. Rodriguez-Bauman, E. H. Rubin and L. F. Liu, *Cancer Res.* **2001**, 61, 5926−5932.

3. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G.

Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, L. R. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, GAUSSIAN 09, Gaussian, Inc., Wallingford, CT, 2013.

4. T. J. Wendorff, B. H. Schmidt, P. Heslop, C. A. Austin and J. M. Berger *J. Mol. Biol.* **2012**, 424, 109–124.

5. G. M. Morris, R. Huey, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *J. Comput. Chem.* **2009**, 16, 2785–2791.

6. R. Thomsen and M. H. Christensen, *J. Med. Chem.* **2006**, 49, 3315–3321.