Electronic Supporting Information Materials

Complexes of Zn(II) with mixed tryptanthrin derivative and curcumin chelating ligands as a new promising anticancer agents

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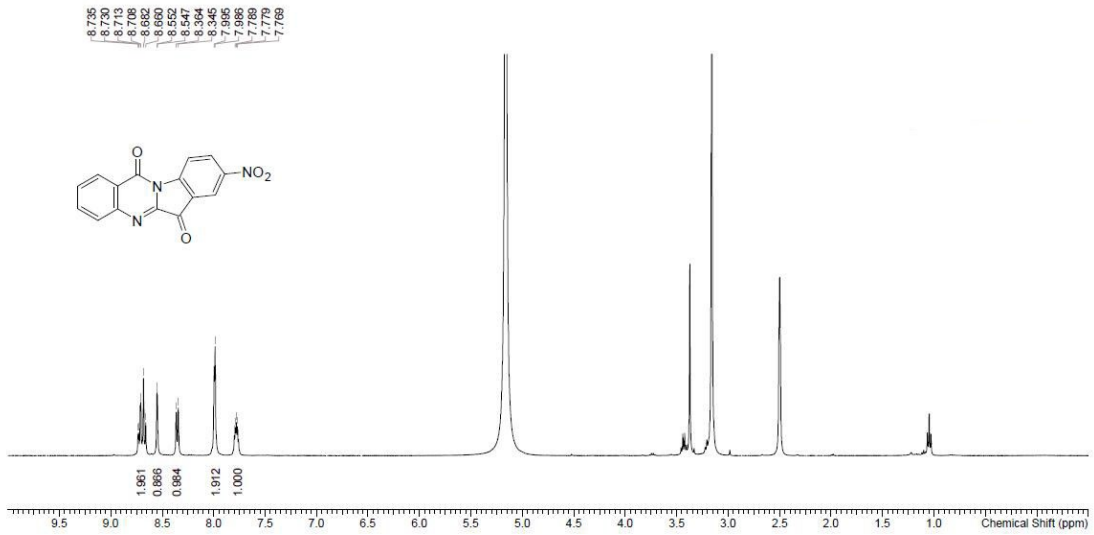

Figure S1. ^{1}H NMR (400MHz, DMSO-d_6) for **2**.

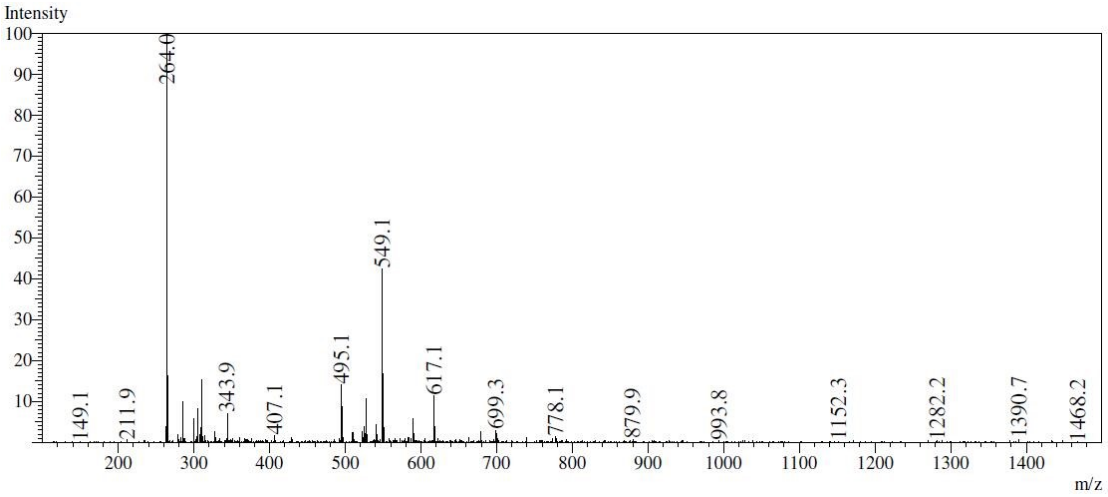

Figure S2. LC-MS spectra of **3** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

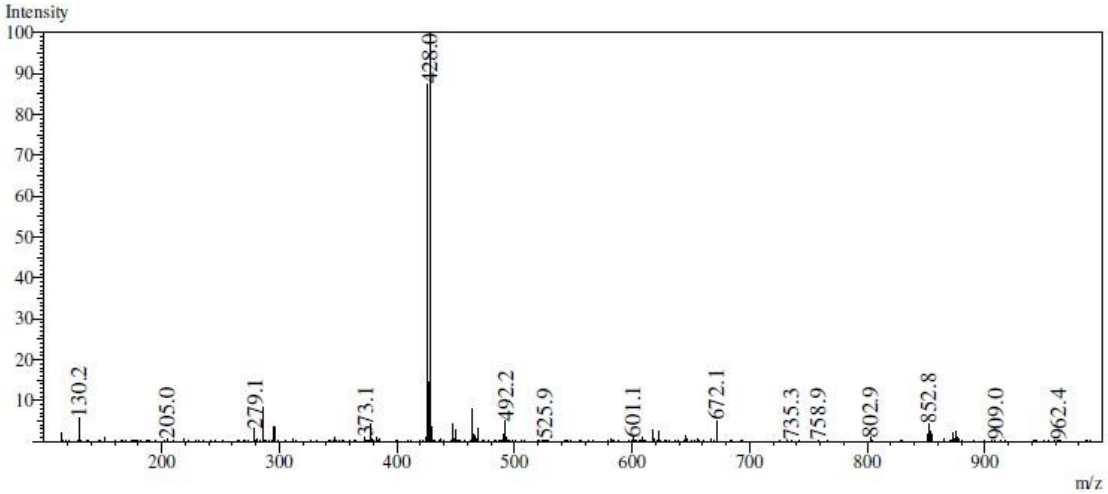

Figure S3. LC-MS spectra of **5** (2.0×10^{-5} M) in Tris-HCl buffer solution

(containing 5% DMSO) for 0 h.

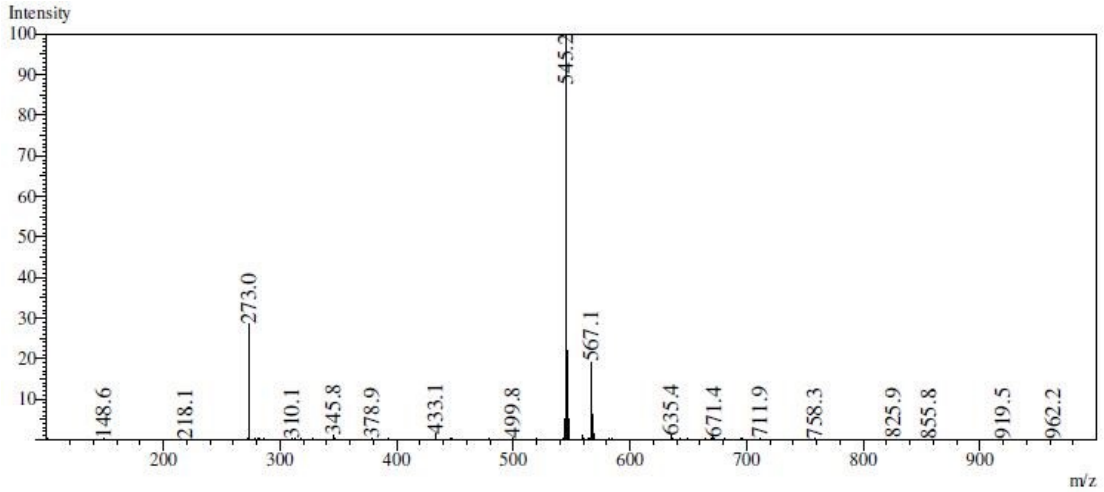

Figure S4. LC-MS spectra of **TA** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

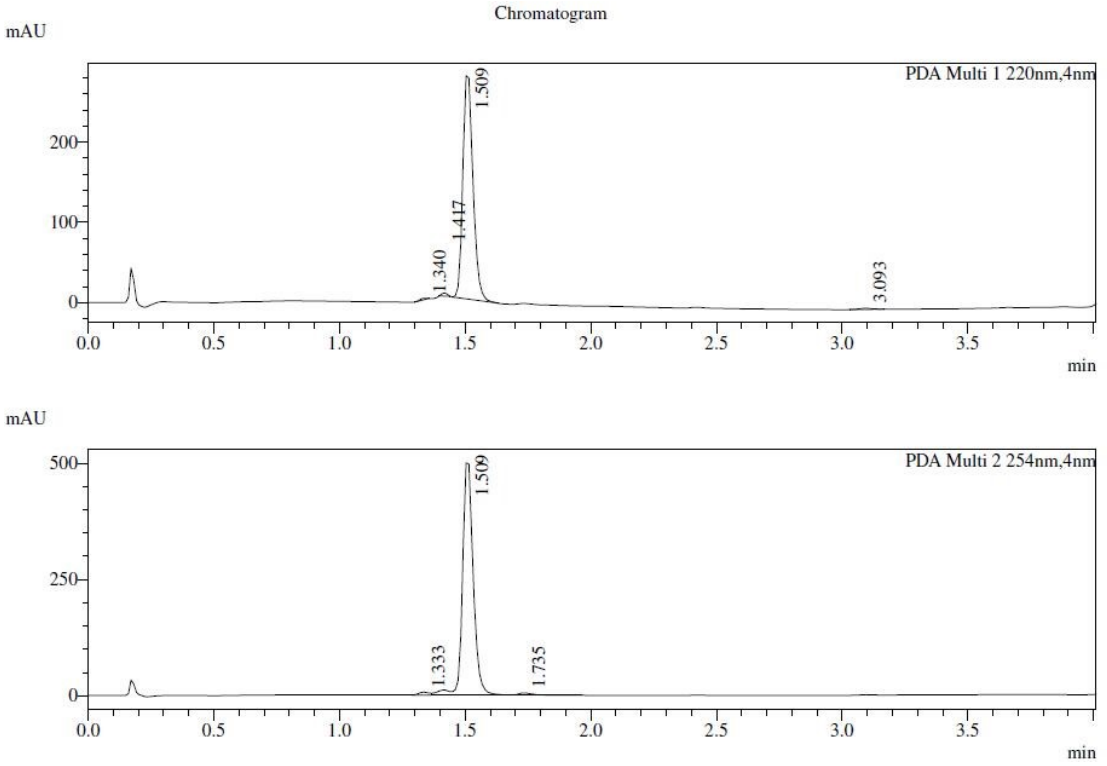

Figure S5. HPLC spectra for **TA** (2.0×10^{-5} M) in TBS (Tris-HCl buffer solution, 10 mM, pH 7.35) solution with 0 h. Column: Inertsustain C18 column (column: Phenomenex Gemini-NX C18 80×30mm×5um; mobile phase: [water (0.1%TFA)-ACN]; B%: 25%-55%). Column temperature: 37ºC.

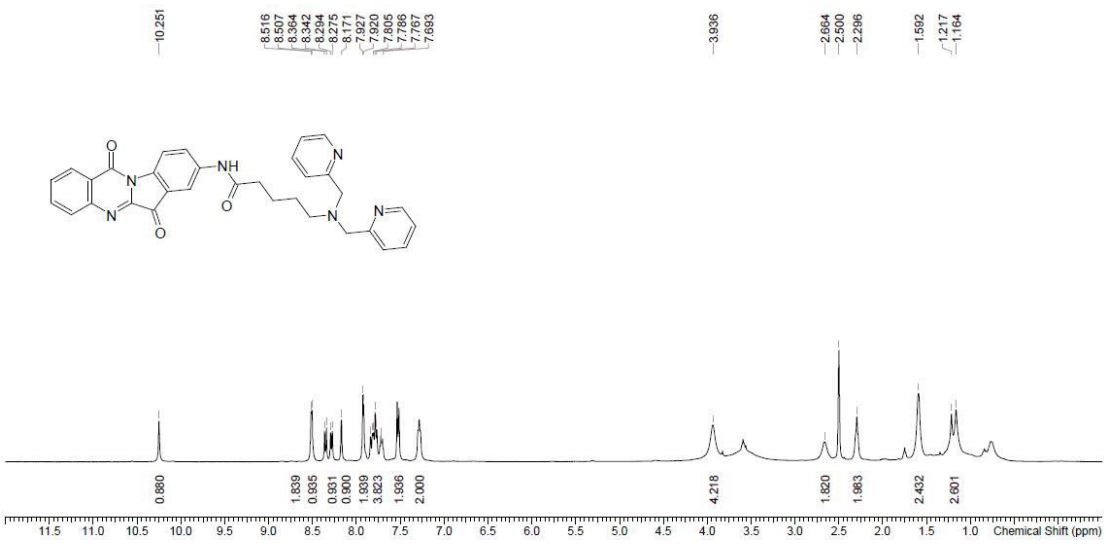

Figure S6. ^{1}H NMR (400MHz, DMSO-d_6) for **TA**.

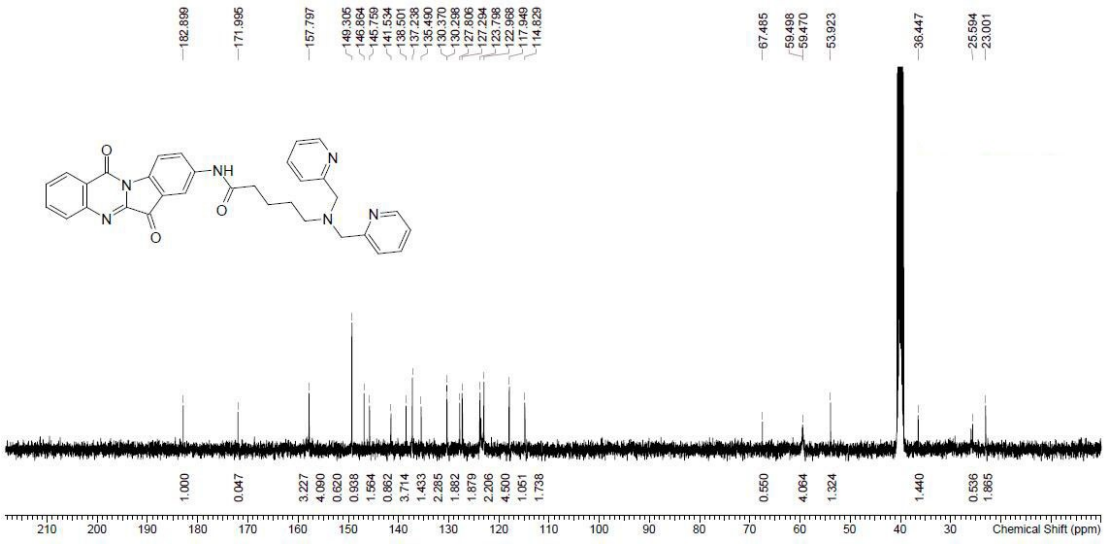

Figure S7. ^{13}C NMR (101MHz, DMSO-d_6) for **TA**.

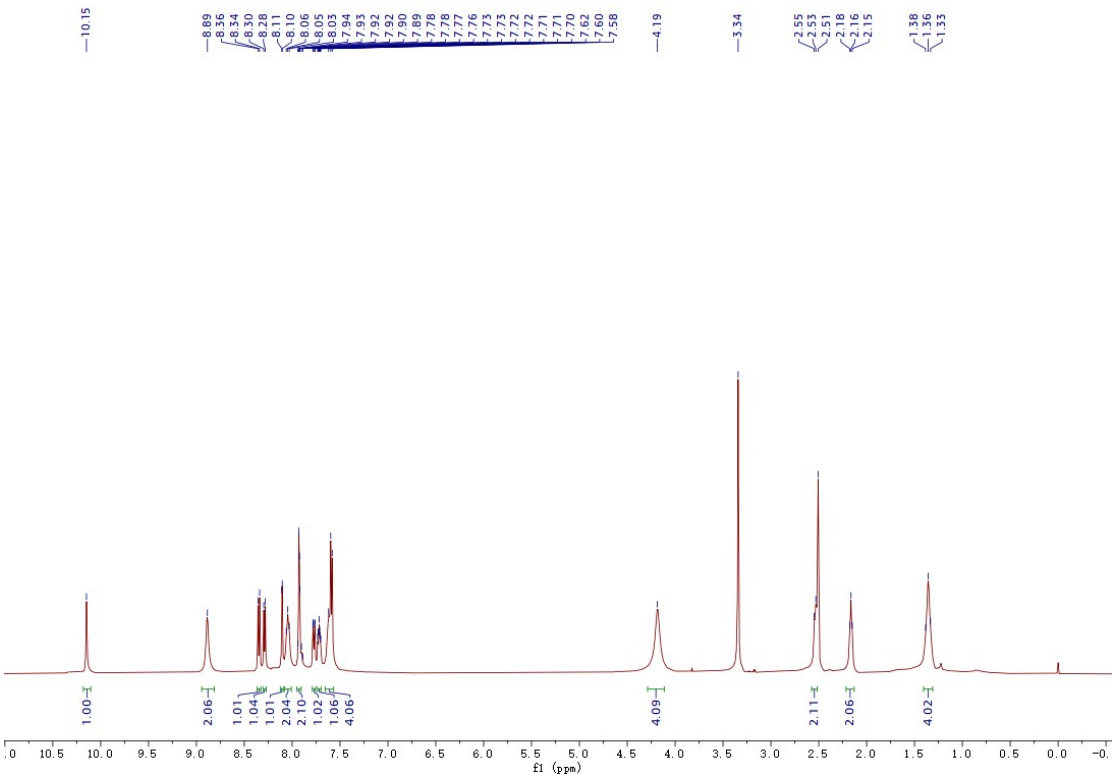

Figure S8. ^{1}H NMR (500MHz, DMSO-d_6) for **Zn(TA)**.

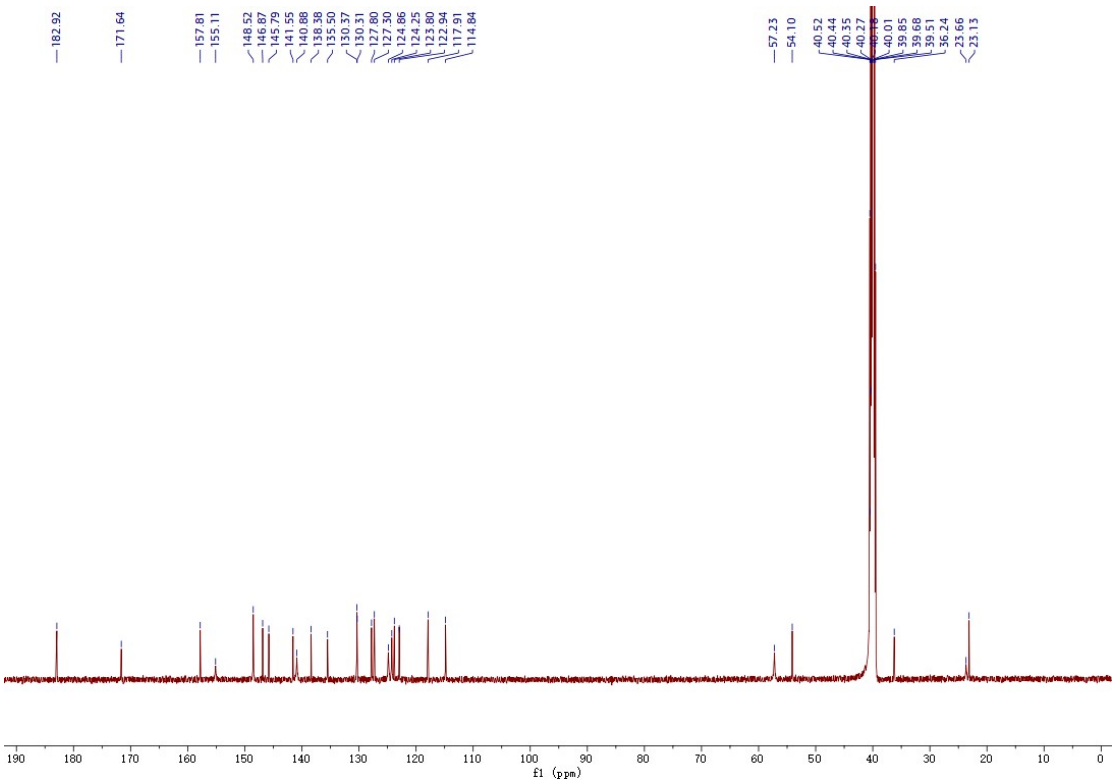

Figure S9. ^{13}C NMR (126MHz, DMSO-d_6) for **Zn(TA)**.

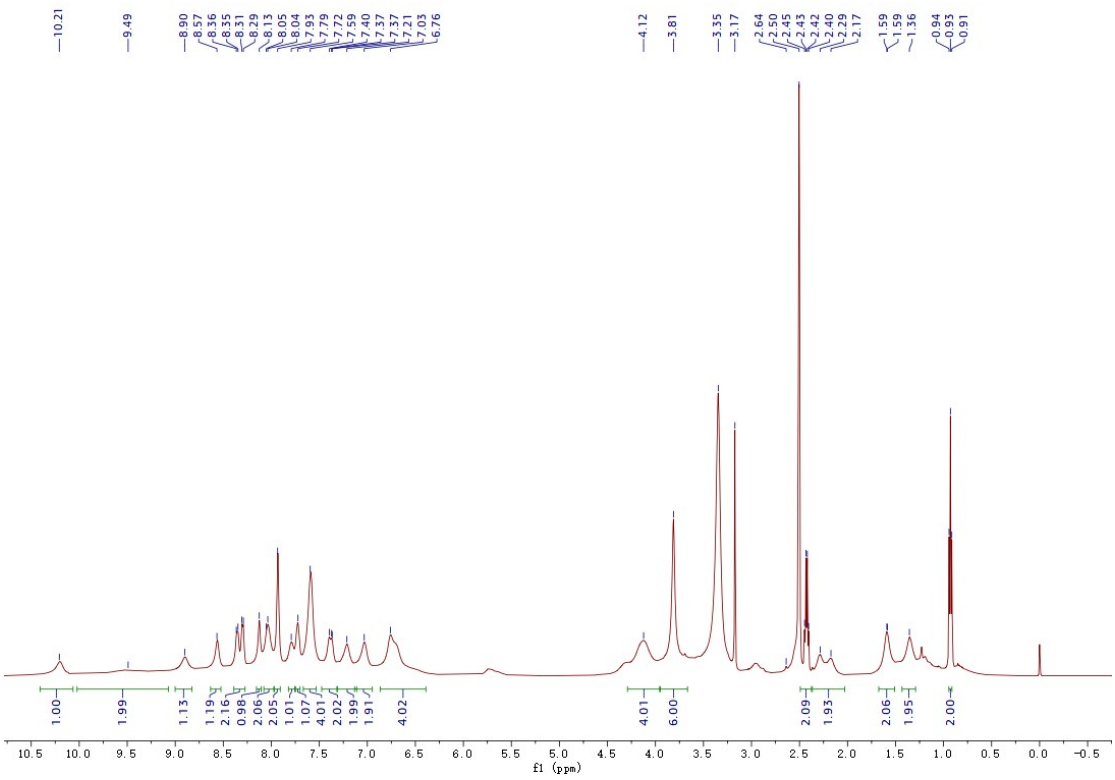

Figure S10. ^{1}H NMR (500MHz, DMSO-d_6) for **Zn(TAC)**.

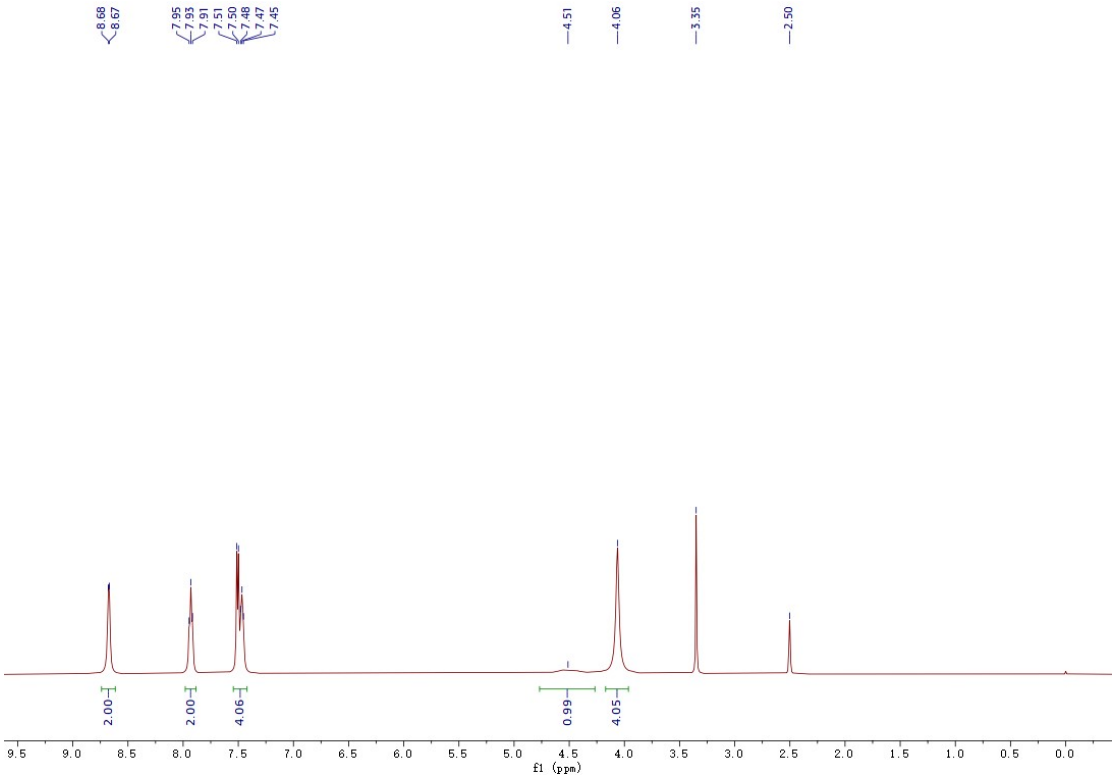

Figure S11. ^{1}H NMR (500MHz, DMSO-d_6) for **Zn(PA)**.

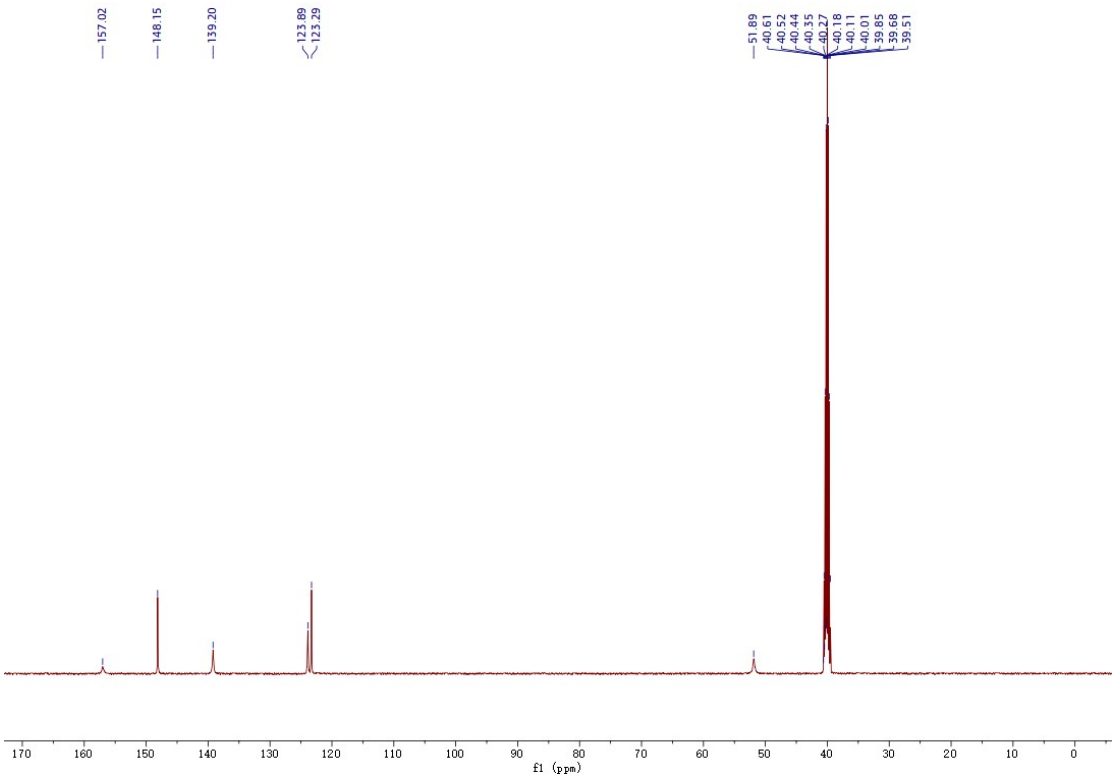

Figure S12. ^{13}C NMR (126MHz, DMSO-d_6) for **Zn(PA)**.

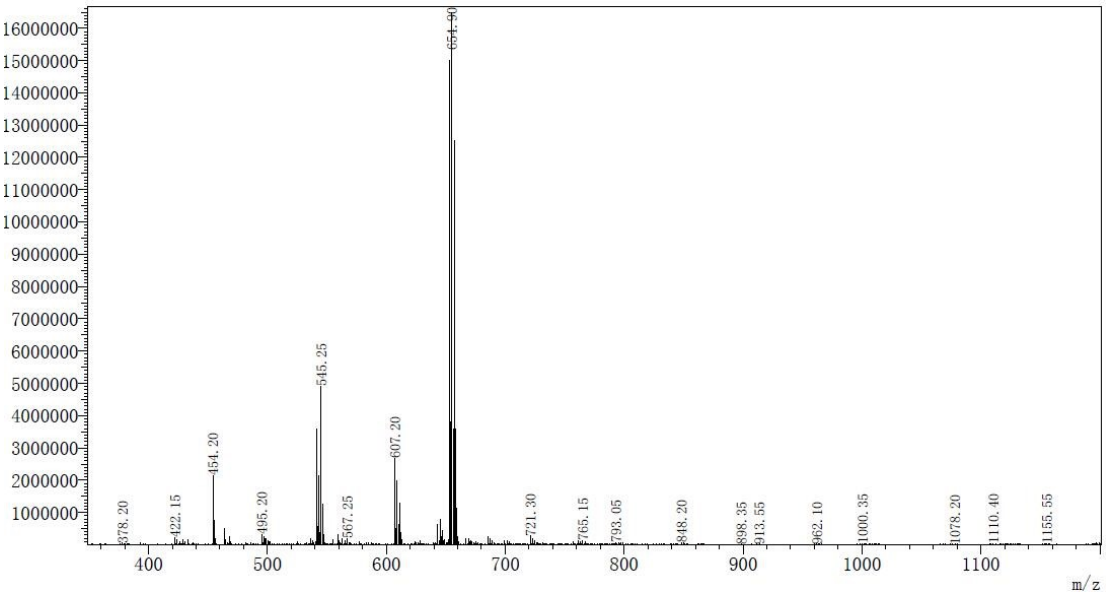

Figure S13. ESI-MS spectra of **Zn(TA)** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

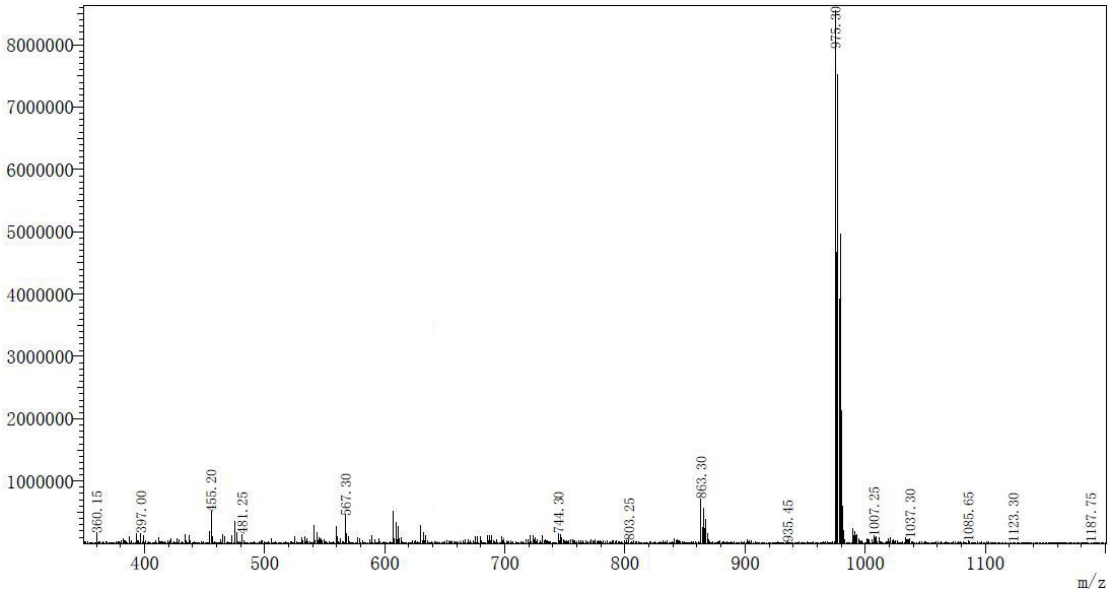

Figure S14. ESI-MS spectrum of **Zn(TAC)** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

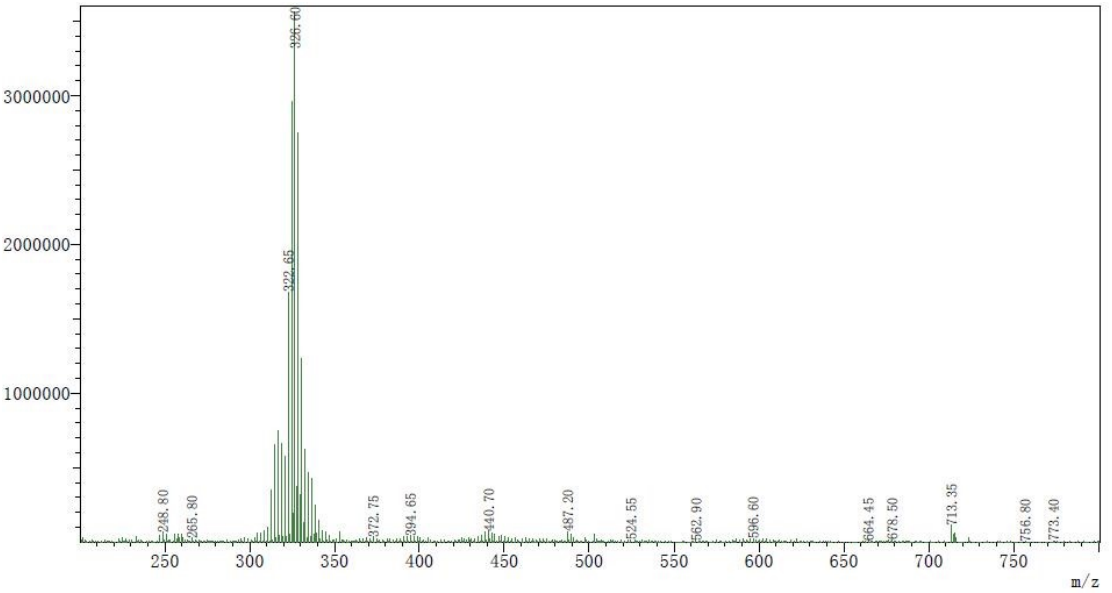

Figure S15. ESI-MS spectrum of **Zn(PA)** (2.0×10^{-5} M) in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

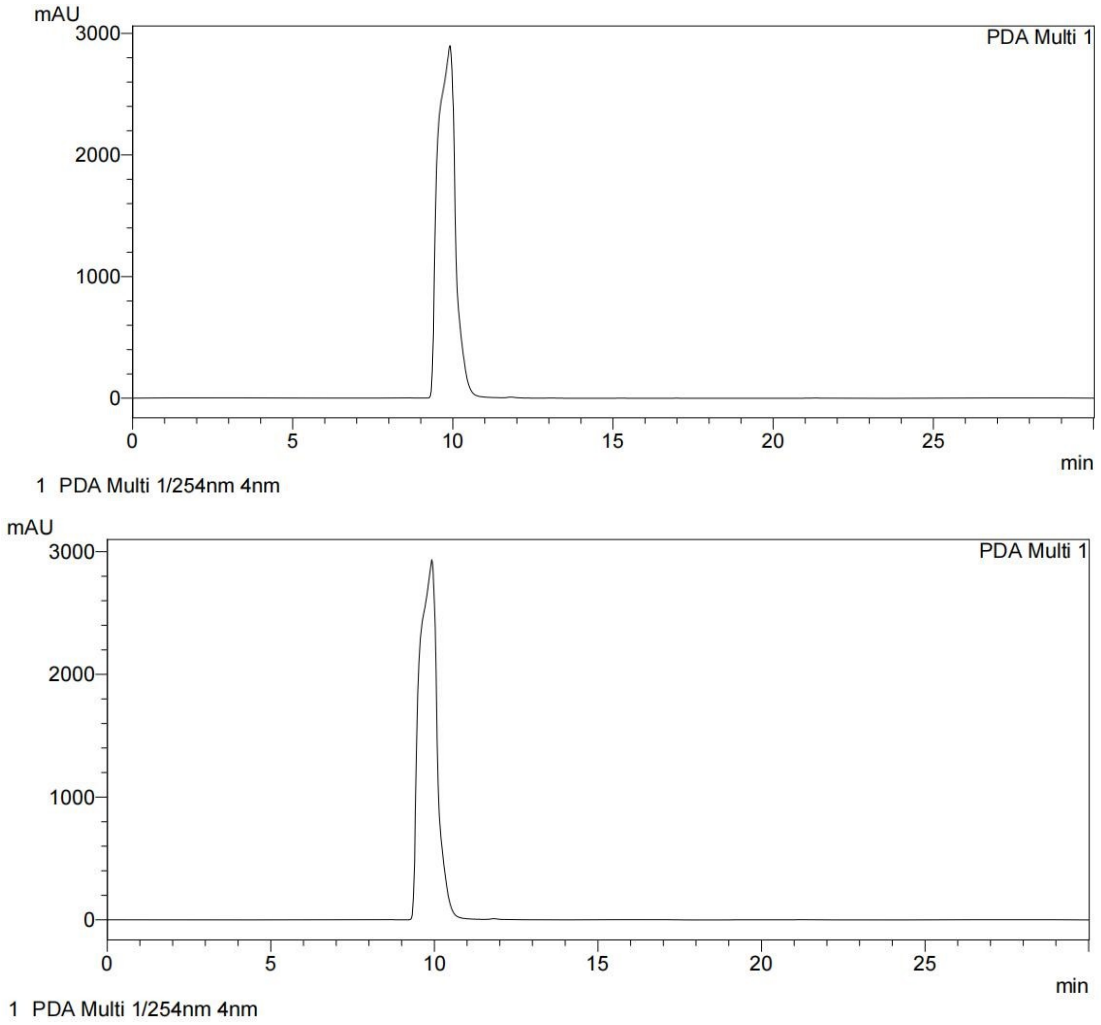

Figure S16. HPLC spectra for **Zn(TA)** (2.0×10^{-5} M) in TBS (Tris-HCl buffer solution, 10 mM, pH 7.40) solution with 0 h (up) and 24h (down), respectively. Column: Inertsustain C18 column (LC-20AT, SPD-20A HPLC COLUMN, 150mm×5.0 μm I.D.). Column temperature: 40ºC. Mobile phase: methol/H_2O containing 0.01% TFA (90:10 methol/H_2O). Flow rate: 0.6 mL/min. Injection volume: 2.0×10^{-5} M.

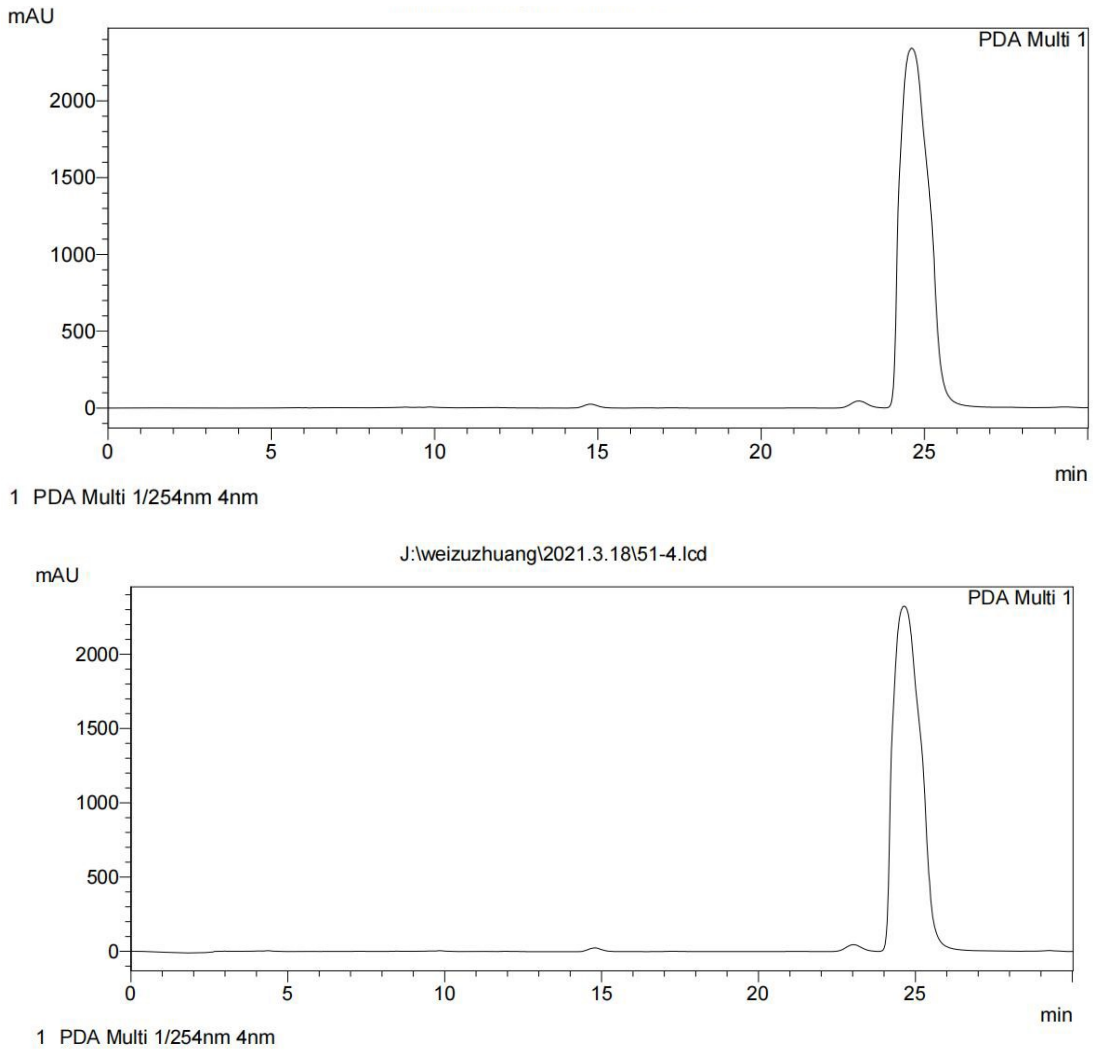

Figure S17. HPLC spectra for **Zn(TAC)** (2.0×10^{-5} M) in TBS (Tris-HCl buffer solution, 10 mM, pH 7.40) solution with 0 h (up) and 24h (down), respectively. Column: Inertsustain C18 column (LC-20AT, SPD-20A HPLC COLUMN, 150mm×5.0 μm I.D.). Column temperature: 40ºC. Mobile phase: methol/H_2O containing 0.01% TFA (90:10 methol/H_2O). Flow rate: 0.6 mL/min. Injection volume: 2.0×10^{-5} M.

Table S1. The tumor volume in treated and non-treated mice from the date of surgery to the study end point in the A549/DDP xenograft model.

	mg/kg	1day	3d			5d			7d		
		Tumor Volume (mm^3)	Tumor Volume (mm^3)	RTV	T/C%	Tumor Volume (mm^3)	RTV	T/C%	Tumor Volume (mm^3)	RTV	T/C%
control	_	92.8±8.5	168.9±20.6	1.817±0.061	100.0	326.8±64.5	3.508±0.485	100.0	509.2±65.3	5.493±0.549	100.0
Zn(TA)	2.0	91.9±10.4	162.8±8.6	1.785±0.176	96.4	287.1±45.6	3.159±0.647	87.8	405.6±35.9*	4.464±0.681*	79.7
Zn(TAC)	2.0	93.7±18.0	158.6±24.3	1.705±0.119	93.9	249.3±35.2*	2.701±0.385*	77.0	347.2±42.8**	3.789±0.640**	68.2

	mg/kg	9d			11d			13d		
		Tumor Volume (mm^3)	RTV	T/C%	Tumor Volume (mm^3)	RTV	T/C%	Tumor Volume (mm^3)	RTV	T/C%
control	_	733.3±65.1	7.963±1.065	100.0	985.8±114.7	10.676±1.370	100.0	1237.7±121.9	13.401±1.422	100.0
Zn(TA)	2.0	503.2±57.1**	5.530±0.891**	68.6	609.2±61.8**	6.683±0.920**	61.8	687.3±84.3**	7.544±1.207**	55.5
Zn(TAC)	2.0	456.5±64.0**	4.984±0.911**	62.3	554.0±56.9**	6.028±0.842**	56.2	595.3±60.6**	6.480±0.942**	48.1

	mg/kg	15d		
		Tumor Volume (mm^3)	RTV	T/C%
control	_	1520.5±177.3	16.389±1.619	100.0
Zn(TA)	2.0	717.7±85.5**	7.876±1.227**	47.2
Zn(TAC)	2.0	630.4±57.9**	6.871±1.011**	41.5

* p <0.05, ** p <0.01, p *vs* vehicle control (5.0% v/v DMSO/ saline vehicle).

Table S2. Average body weight (g) in treated and non-treated mice from the date of surgery to the study end point in the A549/DDP xenogfart model.

	mg/kg	1 d	3 d	5 d	7 d	9 d	11 d	13 d	15 d
control	_	19.9±0.5	20.1±0.5	20.2±0.5	20.4±0.5	20.5±0.5	20.7±0.5	20.8±0.5	20.9±0.6
Zn(TA)	2.0	19.8±0.6	19.9±0.6	20.0±0.6	20.2±0.6	20.3±0.6	20.5±0.6	20.7±0.7	20.8±0.6
Zn(TAC)	2.0	19.8±0.6	19.9±0.6	20.1±0.6	20.2±0.6	20.4±0.5	20.5±0.5	20.6±0.6	20.7±0.6

* p <0.05, ** p <0.01, p *vs* vehicle control (5.0% v/v DMSO/ saline vehicle).

Table S3. In vivo anticancer activity of Zn complexes toward A549/DDP tumor xenograft.

	mg/kg	average tumor weight (mean ± SD, g)	inhibition of tumor growth (%)
control	_	1.647±0.215	-
Zn(TA)	2.0	0.732±0.113**	50.5
Zn(TAC)	2.0	0.629±0.103**	57.4

* p <0.05, ** p <0.01, p *vs* vehicle control (5.0% v/v DMSO/ saline vehicle).

Methods

The other experimental methods

The other antitumor mechanism of **Zn(TA)** (3.04μM) and **Zn(TAC)** (0.14μM) were performed as our previously reported [1].

1.1 Western Blot

The A549/DDP cells were incubated with **Zn(TA)** (3.04μM) and **Zn(TAC)** (0.14μM) for 24.0 h, and then the cells 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 of PMSF (100 mM). The suspension was centrifuged at 10 000 rpm at 4 °C for 10 min, and the supernatant (10μL 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-apoptotic antibody, or anti-β-actin primary antibody and horseradish-peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody. The protein bands were visualized using chemiluminescence substrate.

1.2 Anticancer activity toward A549CDDP in vivo

The A549CDDP 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 90-100 mm^3 on all mice, the mice were randomized into vehicle control and treatment groups (n=6/group),

received the following treatments: (a) vehicle control, 5.0% v/v DMSO/ saline vehicle, (b) **Zn(TA)** at dose 2.0 mg/kg every two day (10% v/v DMSO/saline), (c) **Zn(TAC)** (2.0 mg/kg) via percutaneous injection every 2 days (q2d). 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: $T/C\ (\%) = T_{RTV}/C_{RTV} \times 100\%$ (2)

inhibition of tumor growth: $IR(\%) = (W_c - W_t)/W_c \times 100\%$ (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.

Furthermore, the A549/DDP xenograft mouse models were purchased from Changzhou Cavens Experimental Animal Co., Ltd (Jiangsu, China, Approval No. SCXK 2016-0010). The animal procedures were approved by Changzhou Cavens Experimental Animal Co., Ltd (Jiangsu, China, Approval No. SYXK (Su) 2017-0007). Further, all the experimental procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Animal experiments were approved by Changzhou Cavens Experimental Animal Co., Ltd ((Jiangsu, China).

1.3 Statistical analysis

The experiments have been repeated from three to five times, and the results obtained are presented as means ± standard deviation (SD). Significant changes were assesses by using Student's *t* test for unpaired data, and *p* values of <0.05 or <0.01 were considered significant.

References

(1) Qin, Q.-P.; Wei, Z.-Z.; Wang, Z.-F.; Huang, X.-L.; Tan, M.-X.; Zou, H.-H.; Liang, H. Imaging and therapeutic applications of Zn(II)-cryptolepine–curcumin molecular probes in cell apoptosis detection and photodynamic therapy. *Chem. Commun.* **2020**, *56*, 3999–4002.