Electronic Supporting Information Materials

Synthesis, crystal structure, cytotoxicity and action mechanism of Zn(II) and Mn(II) complexes with 4-([2,2':6',2"-terpyridin]-4'-yl)-N,N-diethylaniline as a ligand

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Table S1. Crystal data and structure refinement details for **1** and **2**.

Empirical formula	$C_{50}H_{48}Cl_2MnN_8O_8$	$C_{25}H_{24}N_6O_6Zn$
Formula weight	1014.83	569.90
Temperature/K	296.15	296.15
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$C2/c$
a / Å	19.0394(4)	11.9747(9)
b / Å	15.1400(4)	15.8152(6)
c / Å	17.3328(5)	14.1457(10)
α / °	90	90
β / °	108.185(2)	114.424(9)
γ / °	90	90
V / Å^3	4746.7(2)	2439.2(3)
Z	4	4
ρ_{calc} g/cm^3	1.4200	1.5518
μ / mm^{-1}	0.455	1.062
F(000)	2111.5	1177.8
Crystal size/mm^3	0.22 × 0.2 × 0.18	0.22 × 0.2 × 0.18
Radiation	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	5.84 to 52.74	6.32 to 52.74
Index ranges	-25 ≤ h ≤ 23, -20 ≤ k ≤ 20, -22 ≤ l ≤ 23	-15 ≤ h ≤ 14, -19 ≤ k ≤ 17, -18 ≤ l ≤ 11
Reflections collected	24898	3510
Independent reflections	9698 [R_{int} = 0.0302, R_{sigma} = 0.0407]	2103 [R_{int} = 0.0209, R_{sigma} = 0.0455]
Data/restraints/parameters	9698/0/625	2103/0/175
Goodness-of-fit on F^2	1.080	1.008
Final R indexes [I>=2σ (I)]	R_1 = 0.0672, wR_2 = 0.1939	R_1 = 0.0529, wR_2 = 0.1300
Final R indexes [all data]	R_1 = 0.0924, wR_2 = 0.2230	R_1 = 0.0653, wR_2 = 0.1467
Largest diff. peak/hole / e Å^{-3}	1.00/-0.79	0.59/-0.82

[a] $R_1 = \Sigma ||F_o| - |F_c||/\Sigma|F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Table S2 Selected bond lengths (Å) and bond angles (°) for **1** and **2**.

Bond lengths (Å) for 1							
Mn1-N1	2.252(3)	Mn1-N2	1.334(3)	Mn1-N3	2.244(3)	Mn1-N5	2.288(3)
Mn1-N6	2.190(3)	Mn1-N7	2.238(3)				
Bond angles (°) for 1							
N2-Mn1-N1	71.81(10)	N3-Mn1-N1	143.35(10)	N3-Mn1-N2	72.55(10)	N5-Mn1-N1	103.22(12)
N5-Mn1-N2	98.80(11)	N5-Mn1-N3	90.37(11)	N6-Mn1-N1	109.98(11)	N6-Mn1-N2	170.61(11)
N6-Mn1-N3	106.59(10)	N6-Mn1-N5	71.82(10)	N7-Mn1-N1	90.96(12)	N7-Mn1-N2	116.54(10)
N7-Mn1-N3	97.33(12)	N7-Mn1-N5	144.56(10)	N7-Mn1-N6	72.83(10)		
Bond lengths (Å) for 2							
Zn1-O4	2.012(3)	Zn1-O4^{1}	2.012(3)	Zn1-N2	2.051(3)	Zn1-N3	2.151(3)
Zn1-N3^{1}	2.151(3)						
Bond angles (°) for 2							
O4^{1}-Zn1-O4	85.59(17)	N2-Zn1-O4	137.20(9)	N2-Zn1-O4^{1}	137.20(9)	N3^{1}-Zn1-O4^{1}	94.42(11)
N3^{1}-Zn1-O4	106.04(11)	N3-Zn1-O4^{1}	106.04(11)	N3-Zn1-O4	94.42(11)	N3-Zn1-N2	76.07(7)
N3^{1}-Zn1-N2	76.07(7)	N3^{1}-Zn1-N3	152.13(15)	N5-O4-Zn1	112.9(2)		

Table S3. Inhibitory rates (%) of **L**, **1**, **2**, the corresponding salts and cisplatin toward four tumor cell lines and normal liver HL-7702 for 48 h.

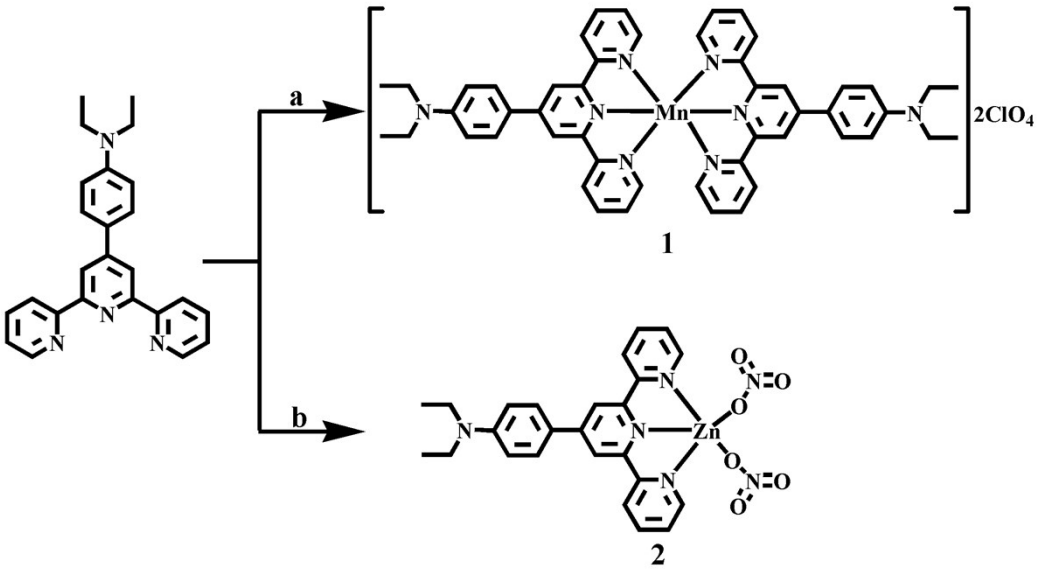
Compounds	BEL-7404	SK-OV-3	T-24	MGC80-3	HL-7702
L [a]	37.08±0.87	40.14±0.49	50.24±1.49	58.16±0.52	44.05±1.82
1 [a]	55.09±1.23	56.97±0.64	62.03±1.05	60.33±2.15	34.18±1.39
2 [a]	72.07±0.80	64.11±1.01	78.01±0.68	64.01±0.94	31.52±0.77
$Mn(ClO_4)_2{\cdot}6H_2O$ [b]	20.11±1.36	12.17±0.63	22.08±0.64	12.73±1.75	19.11±0.93
$Zn(NO_3)_2{\cdot}6H_2O$ [b]	16.02±0.57	16.15±1.98	10.89±0.37	20.37±2.49	15.88±1.56
cisplatin [a]	58.12±1.13	52.09±2.71	50.01±1.17	57.18±1.35	54.61±1.42

Results represent mean ± SD of at least five independent experiments. SD represents the standard deviation. [a]The concentration is 20 μM. [b]The concentration is 100 μM.

Table S4. IC_{50}[a] (μM) values of complexes **1** and **2** on the selected cell lines.

	BEL-7404	SK-OV-3	T-24	MGC80-3	HL-7702
L	33.19 ± 1.22	20.13 ± 0.43	12.53 ± 1.36	15.22 ± 0.59	30.82 ± 1.75
1	14.86 ± 1.29	11.53 ± 0.55	10.26 ± 1.02	12.84 ± 2.07	45.02 ± 1.33
2	5.29 ± 0.76	9.05 ± 1.09	1.28 ± 0.61	8.59 ± 0.82	51.17 ± 0.74
Cisplatin[b]	15.02 ± 1.08	15.17 ± 1.82	20.18 ± 1.07	13.02 ± 1.13	17.68 ± 1.39

[a] The IC_{50} values are presented as mean ± SD (standard error of the mean) from five independent experiments. [b] The cisplatin stock solution was prepared at a concentration of 1 mM with 0.154 M NaCl.[1]



Scheme S1. Synthetic routes for complexes **1** and **2**. Reagents: (a) $Mn(ClO_4)_2$, methanol/$CHCl_3$ (10:1), 80 ℃; (b) $Zn(NO_3)_2$, methanol/$CHCl_3$ (10:1), 80 ℃.

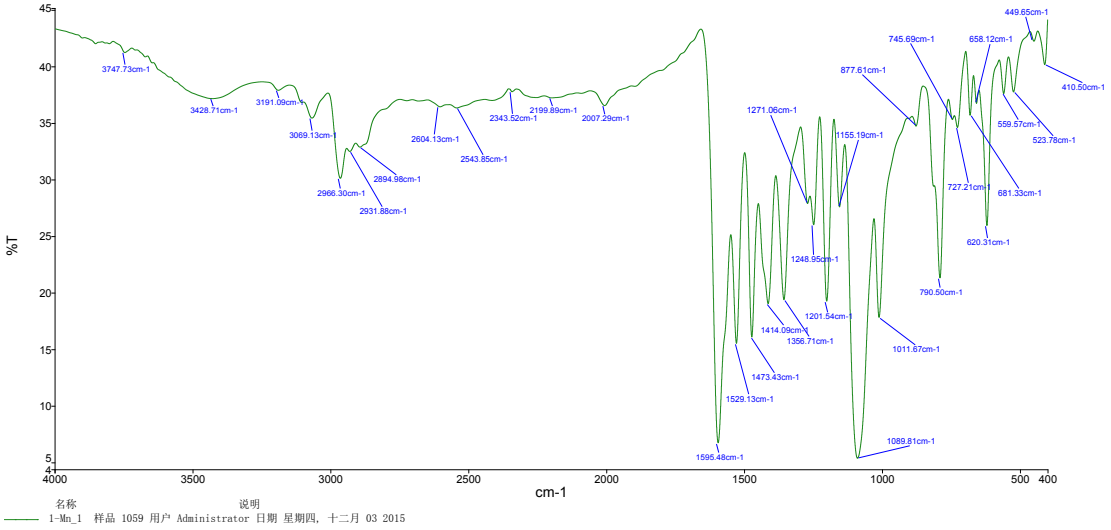


Figure S1. IR (KBr) spectra of complex **1**.

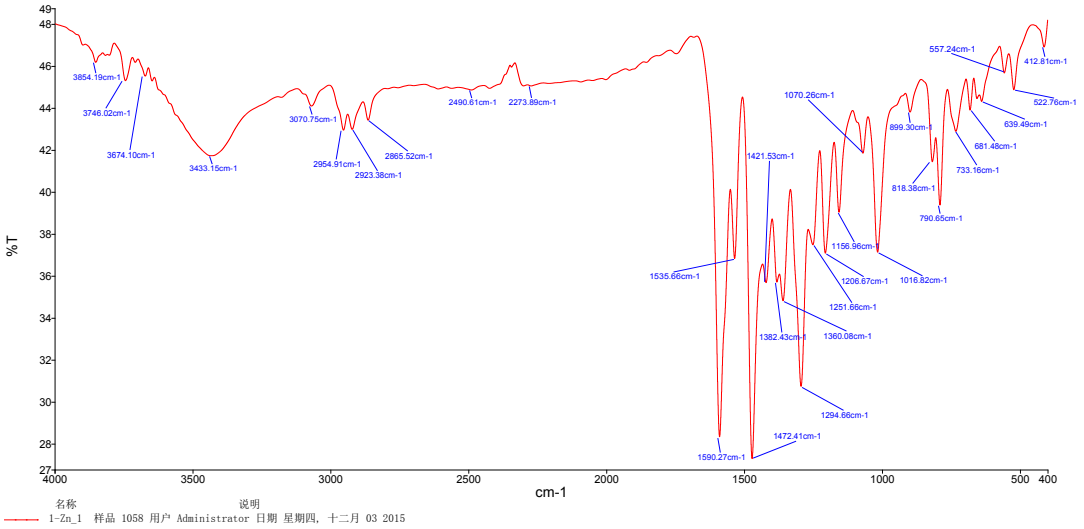


Figure S2. IR (KBr) spectra of complex **2**.

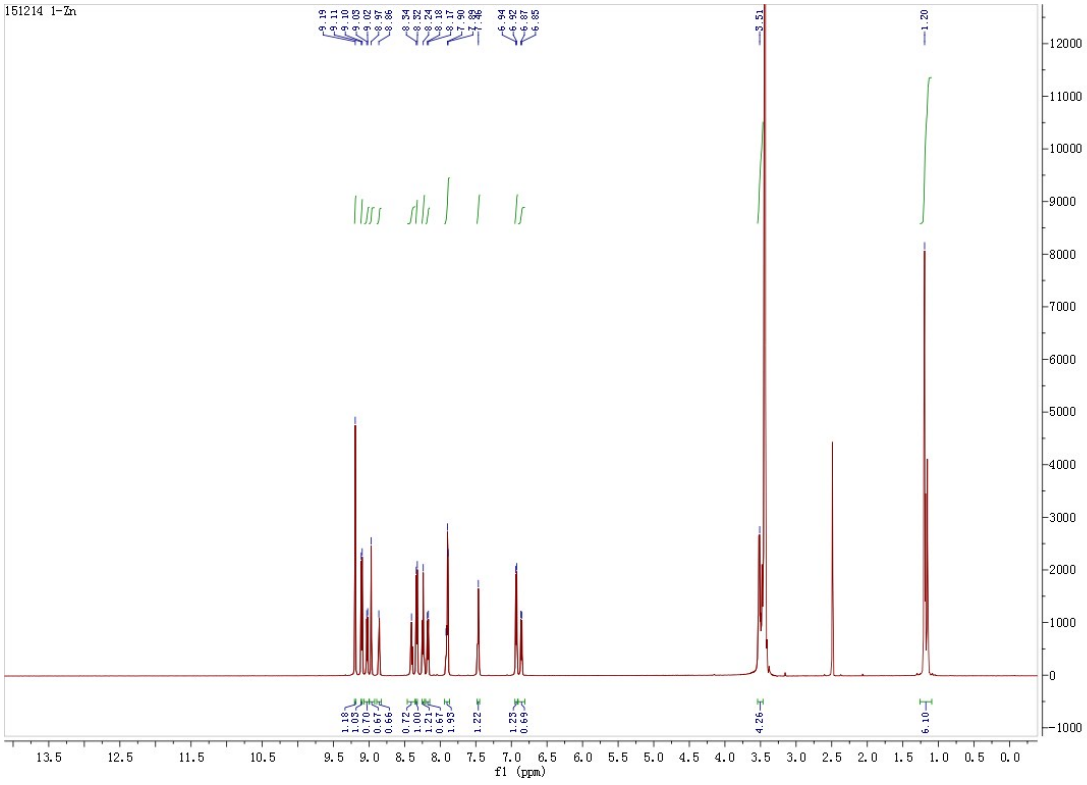


Figure S3. ^{1}H NMR (600 MHz, DMSO-d_6) for complex **2.**

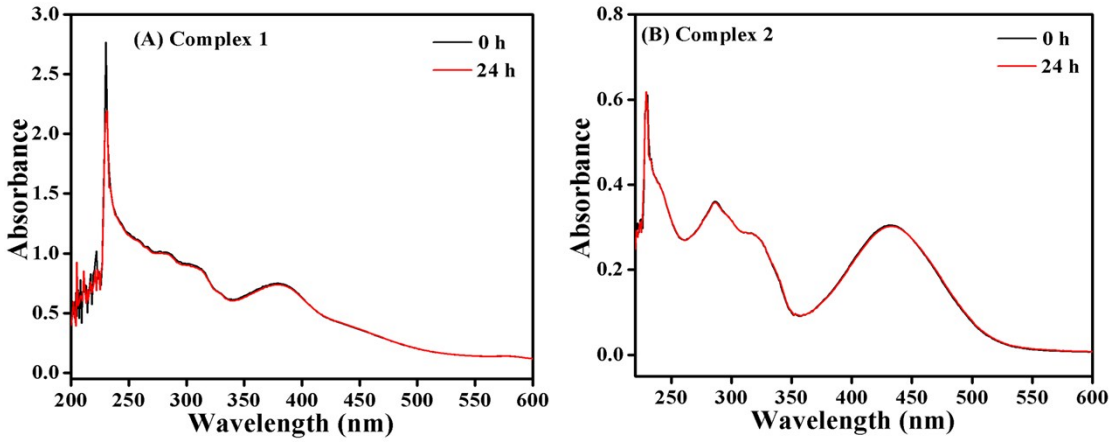


Figure S4. UV-Vis absorption spectra of complexes **1** and **2** (2.0×10^{-5} M) in Tris-HCl solution in the time course 0, and 24 h, respectively.

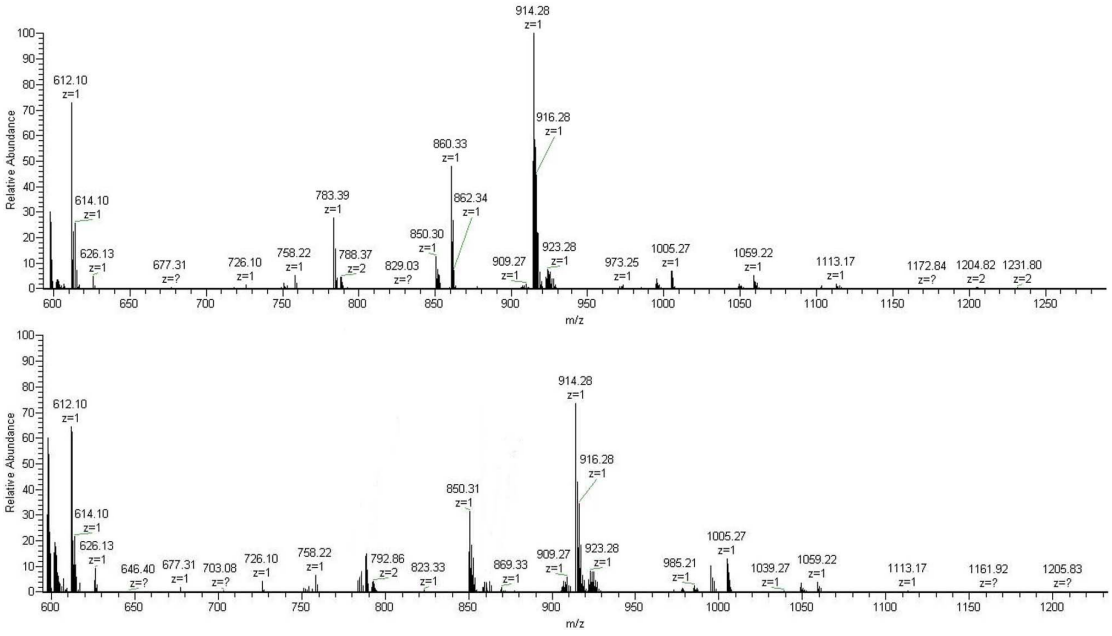


Figure S5. The mass spectra of complex **1** in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 24 h (down), respectively.

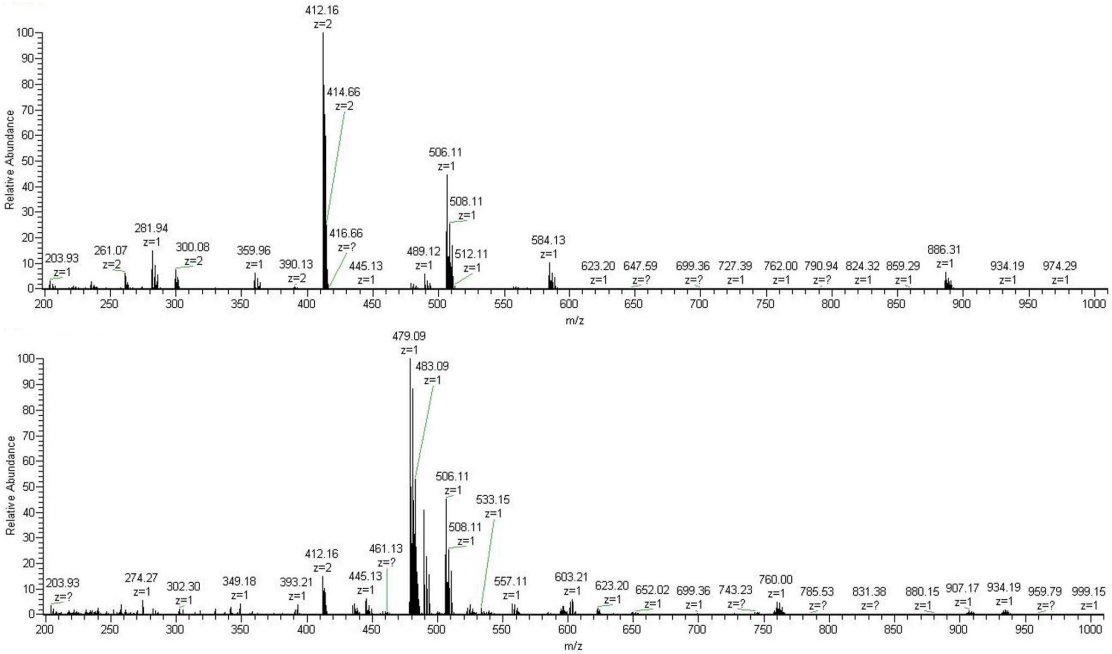


Figure S6. The mass spectra of complex **2** in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 24 h (down), respectively.

Experimental procedures

1. Organic synthesis

*1.1. Synthesis and characterization of 4-([2,2':6',2"-terpyridin]-4'-yl)-N,N-diethylaniline (**L**)*

Synthesis of 4-([2,2':6',2"-terpyridin]-4'-yl)-N,N-diethylaniline (**L**) was performed as previously reported.[2,3] The yellow product of 4-([2,2':6',2"-terpyridin]-4'-yl)-N,N-diethylaniline was prepared in methanol/$CHCl_3$ (v/v=50:1) for spectroscopic characterizations and synthesis of the metallo-complexes. The chemical and physical properties of the newly synthesized product of **L** were consistent with those reported in our previous work.[2]

*1.2. Synthesis of [Mn(**L**)$_2$]·2ClO_4 (**1**)*

$Mn(ClO_4)_2$ (0.1 mmol, 0.042 g), **L** (0.2 mmol), 1.0 mL methanol, 0.1 mL $CHCl_3$ were mixed a thick Pyrex tube (*ca.* 25 cm long). The tube was then placed in a liquid

N_2 bath to make the sample frozen. The solvent in the frozen sample was removed under vacuum while the tube was kept in the liquid N_2 bath. Afterwards, the tube was sealed and heated at 80 °C for three days. The resulting red brown crystals were used for spectroscopic characterizations. Yield: 0.0810 g, 80.0%. ESI-MS m/z: 914.28, $[M–ClO_4]^+$. IR (KBr, cm^{-1}): 3748, 3429, 3191, 3069, 2966, 2932, 2895, 2007, 1595, 1529, 1473, 1414, 1357, 1271, 1249, 1202, 1155, 1090, 1012, 878, 791, 746, 727, 681, 658, 620, 560, 524, 450, 411. Elemental analysis: theoretical mass-percentage chemical composition for $C_{50}H_{48}Cl_2MnN_8O_8$, C 59.18, H 4.77, N 11.04; experimental mass-percentage chemical composition, C 59.10, H 4.82, N 11.01.

*1.3. Synthesis of [Zn(**L**)(NO$_3$)$_2$] (**2**)*

Zn(**L**)$(NO_3)_2$ (**2**) was prepared by mixing $Zn(NO_3)_2$ (0.1 mmol), **L** (0.1 mmol), methanol (1.0 mL) and $CHCl_3$ (0.1 mL) in a thick Pyrex tube (*ca.* 25 cm long), followed by the same procedures as those described for the synthesis of $[Mn(\mathbf{L})_2]\cdot 2ClO_4$ (**1**). Yield: 0.0528 g, 93.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.19 (s, 1H), 9.10 (d, J = 8.1 Hz, 1H), 9.03 (d, J = 8.0 Hz, 1H), 8.97 (s, 1H), 8.86 (s, 1H), 8.40 (s, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.24 (s, 1H), 8.17 (d, J = 8.8 Hz, 1H), 7.90 (t, J = 8.8 Hz, 2H), 7.46 (s, 1H), 6.93 (d, J = 8.9 Hz, 1H), 6.86 (d, J = 8.9 Hz, 1H), 3.51 (s, 4H), 1.20 (s, 6H). ESI-MS *m/z*: 842.34, $[2L+Zn]^{2+}$; 506.11, $[L+Zn+NO_3]^+$. IR (KBr, cm^{-1}): 3854, 3746, 3674, 3433, 3071, 2955, 2923, 2866, 1590, 1536, 1472, 1422, 1382, 1360, 1295, 1252, 1207, 1157, 1070, 1017, 899, 818, 791, 733, 681, 639, 523, 413. Elemental analysis: theoretical mass-percentage chemical composition for $C_{25}H_{24}N_6O_6Zn$, C 52.69, H 4.24, N 14.75; experimental mass-percentage chemical composition, C 52.60, H 4.30, N 14.67.

2. X-Ray crystallography

The X-ray diffraction data collection of single crystals of complexes **1** and **2** were

performed on a SuperNova CCD area detector with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) at room temperature. The structures were determined using direct methods and refined using the SHELX-97 program.[4,5] The non-hydrogen atoms were located via successive difference Fourier synthesis. The final refinement was performed using the full-matrix least-squares method with anisotropic thermal parameters for non-hydrogen atoms on F^2. The hydrogen atoms were added theoretically on the concerned atoms. The parameters used in data collection and refinements are summarized in Tables S1−S2 together with the crystallographic data.

3. Materials, instruments, and other experimental methods

The materials, instruments, and experimental methods for the cytotoxicity assay, apoptosis analysis, cell cycle analysis, RNA extraction, reverse transcriptase–polymerase chain reaction (RT-PCR), western blot, and the transfection assay of complexes **1** and **2** were performed as reported previously.[6-8] The telomerase extract was prepared from the T-24 cells: a total of 5 × 10^6 T-24 tumor cells untreated or treated with complexes **1** and **2** were pelleted, and the cells were washed with 5 mL of PBS, scraped and lysed for 30 min on ice. Finally, the lysate was centrifuged at 13 000 rpm for 30 min at 4 °C; the supernatant was collected and stored at −80 °C before use.[6,9]. The TRAP assay was performed by following previously published procedures,[10] and the TRAP-silver staining assay of complexes **1** and **2** were performed as reported by Reed and co-workers.[9,11] Commonly, the IOD (integrated optical density) data of TRAP-silver staining assay were captured using Gel pro4.0, and the inhibitory rates of complexes **1** and **2** on telomerase could be described by the following:

$$\textbf{Inhibitory rates (\%)} = \frac{\textbf{IOD}_{\textbf{control group}} - \textbf{IOD}_{\textbf{treatment group}}}{\textbf{IOD}_{\textbf{control group}}} \times \textbf{100\%}$$

4. Statistics

The statistical analysis including the Student's *t*-test was performed using SPSS 13.0. $P \leq 0.05$ was treated as statistically significant.

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