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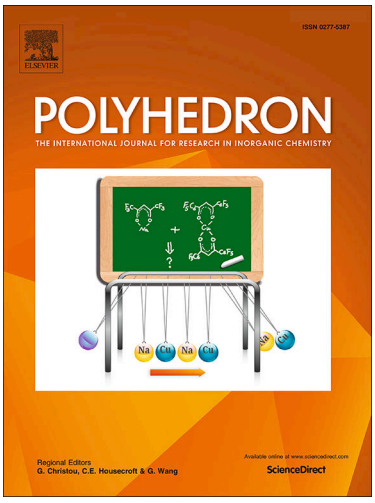

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Transition metal complexes with 6,7-dichloro-5,8-quinolinedione as mitochondria-targeted anticancer agents

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Abstract

Herein, a series of transition metal complexes, $[Zn(DQ)_2(CH_3OH)_2]$ (**1**), $[Zn(DMQ)_2(CH_3OH)_2]$ (**2**), $[Co(DQ)_2(CH_3OH)_2]$ (**3**), $[Co(DMQ)_2(CH_3OH)_2]$ (**4**), $[Ni(DQ)_2(CH_3OH)_2]$ (**5**), $[Cu(DMQ)_2(CH_3OH)_2]$ (**6**), $[Mn(DQ)_2(H_2O)_2]$ (**7**) and $[Mn(DMQ)_2(H_2O)_2]$ (**8**), containing 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands have been synthesized and characterized as potential antitumor agents. These complexes **1**−**8** exhibited evident anti-tumor activity in HeLa (cervical), MCF-7 (breast), Hep-G2 (hepatoma), T-24 (bladder), and SK-OV-3 (ovarian) human cancer cells. Interestingly, complexes **1**−**8** showed higher cytotoxicity than cisplatin against human cervical HeLa cells, and less cytotoxicity on the HL-7702 nontumorigenic cells. Mechanism studies suggested that complexes **1** and **2** arrested the cell cycle in the G1 phase and induced cancer cell death through mitochondrial dysfunction pathways. The cytotoxicity of **2** was higher than that of **1**. The different biological behavior of **1** and **2** may correlate with the presence of a 2-methyl group in 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligand. In general, our study demonstrated that Zn(II) complex **2** with 6,7-dichloro-2-methyl-5,8-quinolinedione showed high potential to be developed as a mitochondria-targeted metal antitumor agent.

Keywords: 6,7-dichloro-2-methyl-5,8-quinolinedione, Zn(II) complex, cell apoptosis, mitochondrial dysfunction

1. Introduction

Cisplatin and its analogues are widely used for the treatment of tumors.[1–24] However, the clinical success of cisplatin and its derivatives are limited by high toxicity and significant side effects.[1–25] Researchers are designing new non-platinum drugs to overcome these drawbacks. [26–36] Many transition metal complexes, such as Pt(II), Ru(II), Cu(II), Co(II), Zn(II) and Au(III) complexes, exhibit high cytotoxicity and can inhibit cancer growth by causing cell apoptosis .[26–36]

Metal ions Zn, Co, Ni, Mn, and Cu are the essential trace elements in a cell, and they represent the endogenously occurring metal ions. In the past decade, many Zn, Co, Ni, Mn, and Cu complexes have been explored.[37–65] These transition metal complexes display significant antitumor activity and potential medicinal value, and some of them have been demonstrated as promising telomerase- and G-quadruplex-targeting agents.[37–65]

5,8-Quinolinedione and its derivatives such as lavendamycin and streptonigrin exhibit antibacterial, anti-tumor, antimalarial and anti-tumor activities.[66–72] Complexes of Ru(II), Os(II), Ir(III), Pt(II) and Rh(III) with 6,7-dichloro-5,8-quinolinedione (DQ) are reported as apoptosis inhibitors.[66,73,74] To date, complexes of transition metal Zn, Co, Ni, Mn, and Cu with 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands have yet to be reported, and the biological activities of these transition metal complexes remain to be explored.

The aim of present work is to synthesize a series of new transition metal complexes, $[Zn(DQ)_2(CH_3OH)_2]$ (**1**), $[Zn(DMQ)_2(CH_3OH)_2]$ (**2**), $[Co(DQ)_2(CH_3OH)_2]$ (**3**), $[Co(DMQ)_2(CH_3OH)_2]$ (**4**), $[Ni(DQ)_2(CH_3OH)_2]$ (**5**),

$[Cu(DMQ)_2(CH_3OH)_2]$ (**6**), $[Mn(DQ)_2(H_2O)_2]$ (**7**) and $[Mn(DMQ)_2(H_2O)_2]$ (**8**), containing 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands as potential antitumor agents. The *in vitro* antiproliferative activity of **1**−**8** was investigated against HeLa (cervical), MCF-7 (breast), Hep-G2 (hepatoma), T-24 (bladder), and SK-OV-3 (ovarian) human cancer cells as well as HL-7702 nontumorigenic cells. To elucidate the possible anti-tumor mechanisms, Zn(II) complexes **1** and **2** were evaluated for mitochondrial damage (loss of $\Delta\psi_m$), effect on cell cycle distribution, ROS (reactive oxygen species) level, and initiation of mitochondria-associated events that leads to cancer cell apoptosis.

2. Experimental methods

2.1. Synthesis of DQ and DMQ ligands

The syntheses of 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands were carried out according to the reported procedures (Scheme 1).[66,73,74]

2.2. Synthesis of 1−8

The complexes **1**−**8** were prepared by treating 2.0 mmol 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands with $Zn(NO_3)_2{\cdot}6H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$ and $Mn(NO_3)_2{\cdot}6H_2O$ (1.0 mmol) in 5.0 mL CH_3OH at 80 °C for 4.0 h. The brown crystals suitable for X-ray diffraction analysis were harvested.

*Data for **1***. Yield: 70.2%. Elemental analysis calcd. (%) for $C_{20}H_{14}Cl_2N_2O_8Zn$: C 43.94, H 2.58, N 5.12; found: C 43.91, H 2.60, N 5.10. IR (KBr): 3394, 3083, 1700, 1596, 1523, 1280, 1220, 1121, 1003, 851, 684, 561, 461 cm^{-1}. ^1H NMR (500 MHz,

DMSO-d_6) δ 8.54 (d, J = 3.6 Hz, 1H), 8.40 (d, J = 7.7 Hz, 1H), 7.84 (dd, J = 7.6, 5.2 Hz, 1H), 3.35 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 180.02, 170.50, 167.96, 151.26, 148.56, 137.33, 127.96, 127.55, 113.09, 40.55, 40.38, 40.22, 40.05, 39.88, 39.72, 39.55.

Data for ***2***. Yield: 79.1%. Elemental analysis calcd. (%) for $C_{22}H_{18}Cl_2N_2O_8Zn$: C 45.98, H 3.16, N 4.87; found: C 45.97, H 3.19, N 4.86. IR (KBr): 3394, 1691, 1527, 1375, 1262, 1125, 1022, 864, 695, 674, 504, 433 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 3.35 (s, 3H), 2.70 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.08, 163.52, 148.77, 137.11, 128.12, 125.23, 113.33, 40.57, 40.40, 40.24, 40.07, 39.90, 39.74, 39.57, 24.06.

Data for ***3***. Yield: 59.8%. Elemental analysis calcd. (%) for $C_{20}H_{14}Cl_2CoN_2O_8$: C 44.47, H 2.61, N 5.19; found: C 44.49, H 2.60, N 5.17. IR (KBr): 3470, 3329, 2361, 1693, 1595, 1517, 1396, 1280, 1220, 1121, 1002, 853, 746, 683, 562, 459 cm^{-1}.

Data for ***4***. Yield: 62.4%. Elemental analysis calcd. (%) for $C_{22}H_{18}Cl_2CoN_2O_8$: C 46.50, H 3.19, N 4.93; found: C 46.48, H 3.22, N 4.92. IR (KBr): 3355, 2830, 1691, 1523, 1371, 1264, 1126, 1021, 861, 768, 694, 573, 505, 430 cm^{-1}.

Data for ***5***. Yield: 75.6%. Elemental analysis calcd. (%) for $C_{20}H_{14}Cl_2N_2CoO_8$: C 44.49, H 2.61, N 5.19; found: C 44.48, H 2.64, N 5.18. IR (KBr): 3324, 1654, 1513, 1348, 1224, 1120, 1017, 862, 675, 478 cm^{-1}.

Data for ***6***. Yield: 80.1%. Elemental analysis calcd. (%) for $C_{22}H_{18}Cl_2CuN_2O_8$: C 46.13, H 3.17, N 4.89; found: C 46.10, H 3.20, N 4.87. IR (KBr): 3481, 3065, 2938, 1698, 1620, 1521, 1408, 1253, 1126, 1021, 861, 795, 628, 436 cm^{-1}.

Data for ***7***. Yield: 59.6%. Elemental analysis calcd. (%) for $C_{18}H_{10}Cl_2MnN_2O_8$: C 42.55, H 1.98, N 5.51; found: C 42.53, H 2.00, N 5.50. IR (KBr): 3462, 2292, 1692, 1598, 1518, 1278, 1220, 1114, 1001, 852, 677, 581, 447 cm^{-1}.

Data for ***8***. Yield: 48.2%. Elemental analysis calcd. (%) for $C_{20}H_{14}Cl_2MnN_2O_8$: C 44.80, H 2.63, N 5.22; found: C 44.78, H 2.66, N 5.20. IR (KBr): 3363, 1692, 1526, 1376, 1269, 1119, 1015, 863, 706, 574, 498, 431 cm^{-1}.

2.3. The other methods

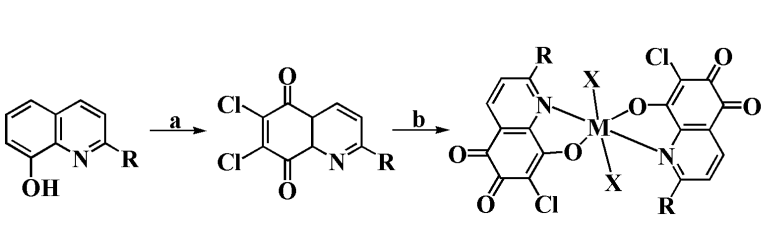
The *in vitro* antitumor activities of **1**−**8** were performed as reported by Metzler-Nolte and Lippard *et al*. [2,75−80] In addition, the experimental steps for 6,7-dichloro-5,8-quinolinedione complexes **1**−**8** were all provided in Supporting Information.

3. Results and discussion

3.1. Synthesis

The syntheses of 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands were carried out according to the reported procedures (Scheme 1).[66,73,74] Treatment of 1.0 mmol $Zn(NO_3)_2·6H_2O$, $Co(NO_3)_2·6H_2O$, $Ni(NO_3)_2·6H_2O$, $Cu(NO_3)_2·3H_2O$ and $Mn(NO_3)_2·6H_2O$ with 2.0 equivalents of 6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands, respectively, in 5.0 mL CH_3OH at 80 °C for 4.0 h led to the formation of a series of new transition metal complexes, $[Zn(DQ)_2(CH_3OH)_2]$ (**1**), $[Zn(DMQ)_2(CH_3OH)_2]$ (**2**), $[Co(DQ)_2(CH_3OH)_2]$ (**3**), $[Co(DMQ)_2(CH_3OH)_2]$ (**4**), $[Ni(DQ)_2(CH_3OH)_2]$ (**5**), $[Cu(DMQ)_2(CH_3OH)_2]$ (**6**), $[Mn(DQ)_2(H_2O)_2]$ (**7**) and $[Mn(DMQ)_2(H_2O)_2]$ (**8**) (Scheme 1). Their structures were characterized by single-crystal X-ray diffraction analysis (Tables S1−S24), elemental analysis, infrared spectroscopy, UV-Vis spectroscopy and NMR (Figures S1−S31). In addition, we showed that complexes **1**−**8** (2.0×10^{-5} mol/L) were stable in Tris-HCl buffer solution for 48 h by UV-Vis

spectroscopy (Figure S1).



1: M= Zn, R= H, X= CH_3OH;
2: M= Zn, R= CH_3, X= CH_3OH;
3: M= Co, R= H, X= CH_3OH;
4: M= Co, R= CH_3, X= CH_3OH;
5: M= Ni, R= H, X= CH_3OH;
6: M= Cu, R= CH_3, X= H_2O;
7: M= Mn, R= H, X= H_2O;
8: M= Mn, R= CH_3, X= H_2O.

Scheme 1. Synthetic routes for 6,7-dichloro-5,8-quinolinedione (DQ), 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) and their complexes **1**–**8**. (a) $NaClO_3$ (5.5 g), HCl (37.0 mL), 5.0 M NaOH solution (269.0 mL), H_2O (160.0 mL), reflux; (b) $Zn(NO_3)_2·6H_2O$, $Co(NO_3)_2·6H_2O$, $Ni(NO_3)_2·6H_2O$, $Cu(NO_3)_2·3H_2O$ or $Mn(NO_3)_2·6H_2O$, CH_3OH (5.0 mL), 80 °C, 4.0 h.

3.2. Crystal structure

As shown in Fig. 1, the Zn(II) atom is coordinated by O(1), N(1), O(4), and N(2) from the two DQ ligands, and two O atoms [O(7) and O(8)] from two CH_3OH ligands. The angle of O(1)–Zn(1)–N(1) was 80.04(8) °, while the angle of O(4)–Zn(1)–N(1) was 99.96(8) °. The coordination geometry around the Zn(II) center was octahedral with different ligating atoms (N and O atoms) from the coordination plane (Fig. 1 and Tables S1−S3). The Zn–O distances were in the range of 2.058(2)–2.192(3) Å, and the angle (°) are shown in Tables S1−S4.

Similar to Zn(II) in complex **1**, the coordination geometry around the Zn(II), Co(II), Ni(II), Cu(II) and Mn(II) atoms in complexes **2**−**8** was also distorted octahedral, as shown in Figs. 2, S26−S31. The crystallographic data and selected bond lengths (Å) are shown in Tables S1−S24, respectively.

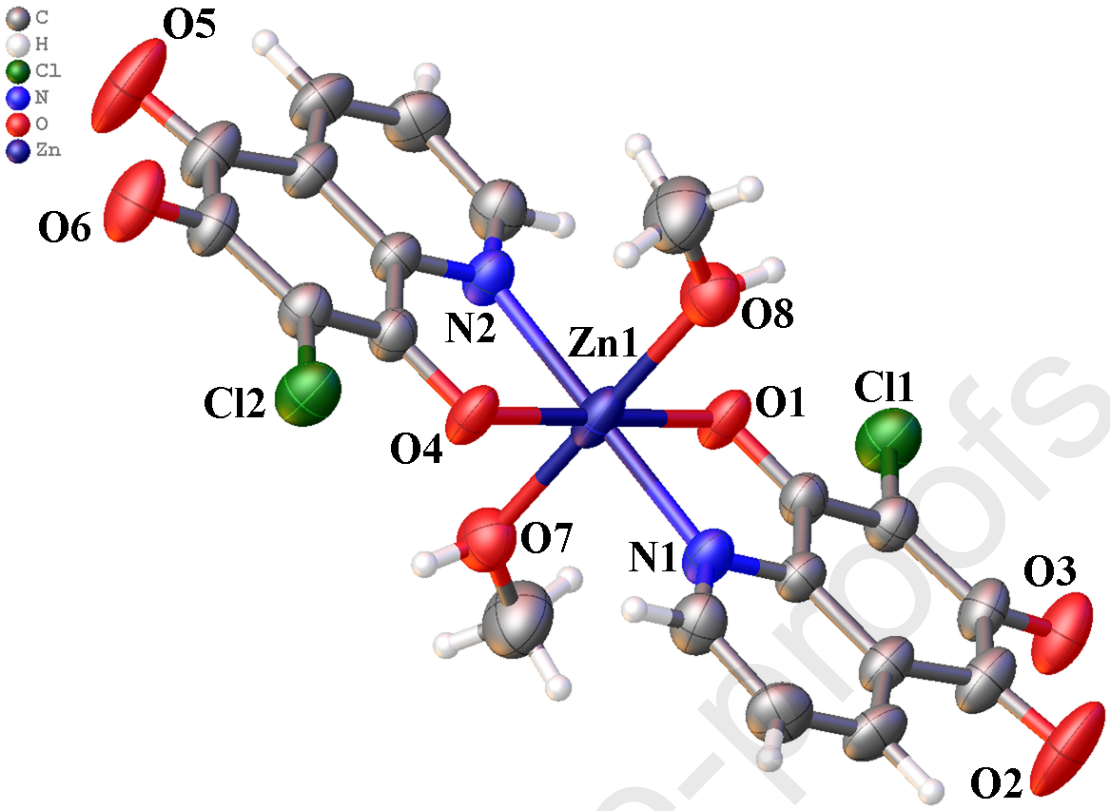


Fig. 1. Molecular structure of **1**.

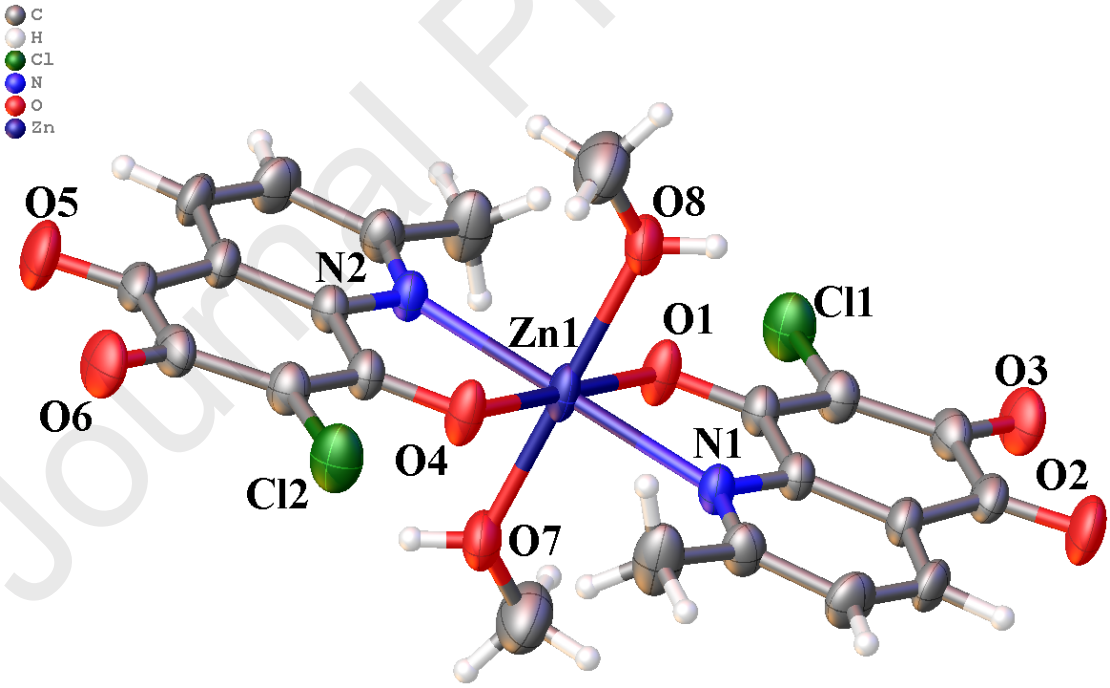


Fig. 2. Molecular structure of **2**.

3.3. *In vitro* cytotoxicity

The *in vitro* cytotoxic activities of DQ, DMQ, complexes **1**−**8**, $Zn(NO_3)_2·6H_2O$, $Co(NO_3)_2·6H_2O$, $Ni(NO_3)_2·6H_2O$, $Cu(NO_3)_2·3H_2O$, cisplatin and $Mn(NO_3)_2·6H_2O$ were evaluated against HeLa (cervical), MCF-7 (breast), Hep-G2 (hepatoma), T-24 (bladder), and SK-OV-3 (ovarian) human cancer cells as well as HL-7702 nontumorigenic cells as determined by MTT assay. As shown in Table 1, complexes **1**−**8** showed higher antitumor activity to cancer cell lines than that of DQ, DMQ, $Zn(NO_3)_2·6H_2O$, $Co(NO_3)_2·6H_2O$, $Ni(NO_3)_2·6H_2O$, $Cu(NO_3)_2·3H_2O$ and $Mn(NO_3)_2·6H_2O$. Notably, complexes **1**−**8** showed lower IC_{50} values of 1.08−10.21 μM against HeLa (cervical) cancer cells, and displayed approximately 1.2−11.8 fold higher cytotoxic activity than cisplatin (12.69 ± 0.29 μM). And their cytotoxic activities were in the following order: **2** > **1** > **6** > **4** > **3** > **8** > **7** > **5** > cisplatin > DMQ > DQ > $Zn(NO_3)_2·6H_2O$ ～$Co(NO_3)_2·6H_2O$ ～ $Ni(NO_3)_2·6H_2O$ ～ $Cu(NO_3)_2·3H_2O$ ～ $Mn(NO_3)_2·6H_2O$. The different biological behavior of **1** and **2** may correlate with the presence of a 2-methyl group in 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligand. Remarkably, complex **2** was the most cytotoxic compound in HeLa cells, with an IC_{50} value of 1.08 ± 0.15 μM, which was 1.7−16.5 times more potent than that of 6,7-dichloro-5,8-quinolinedione (DQ) Ru(II), Os(II), Ir(III), Pt(II) and Rh(III) complexes (IC_{50} > 1.8 ± 0.7 μM) [66,73,74]. In addition, complexes **1**−**8** displayed less cytotoxicity on the HL-7702 nontumorigenic cells (IC_{50} > 60.0 μM), indicating the selectivity of complexes **1**−**8** on HeLa cells.

Table 1. Cytotoxicities (IC_{50}, μM) of DQ, DMQ, complexes **1**−**8**, $Zn(NO_3)_2·6H_2O$, $Co(NO_3)_2·6H_2O$, $Ni(NO_3)_2·6H_2O$, $Cu(NO_3)_2·3H_2O$, $Mn(NO_3)_2·6H_2O$ and cisplatin toward the selected human cell lines for 24 h.

Compounds	HeLa	MCF-7	Hep-G2	T-24	SK-OV-3	HL-7702
DQ	40.15 ± 1.96	25.16 ± 1.47	28.12 ± 1.09	31.04 ± 1.07	40.15 ± 0.77	65.08 ± 1.12
DMQ	30.22 ± 0.64	18.63 ± 0.32	15.03 ± 1.00	20.17 ± 0.58	30.57 ± 1.05	63.55 ± 0.98
1	3.03 ± 0.28	3.37 ± 0.26	6.19 ± 1.49	8.45 ± 1.79	4.26 ± 0.71	70.15 ± 0.25
2	1.08 ± 0.15	1.59 ± 0.43	4.95 ± 0.82	5.26 ± 0.52	2.58 ± 0.33	72.19 ± 1.09
3	5.08 ± 1.08	6.01 ± 0.89	10.41 ± 1.09	15.99 ± 1.22	9.06 ± 2.03	65.03 ± 0.54
4	4.34 ± 0.81	5.56 ± 1.08	8.93 ± 1.02	12.97 ± 0.63	7.89 ± 1.59	64.17 ± 0.32
5	10.21 ± 0.44	12.37 ± 1.81	20.15 ± 1.02	30.00 ± 0.46	15.08 ± 0.99	69.08 ± 0.99
6	4.01 ± 1.05	4.88 ± 0.57	7.58 ± 0.66	10.56 ± 0.49	5.99 ± 0.18	60.15 ± 0.88
7	9.01 ± 1.84	9.85 ± 0.33	15.08 ± 1.64	25.87 ± 1.29	13.06 ± 1.14	65.03 ± 0.57
8	6.21 ± 0.55	8.93 ± 1.11	13.62 ± 1.00	20.39 ± 0.31	10.57 ± 0.33	80.71 ± 1.49
$Zn(NO_3)_2·6H_2O$	> 100	> 100	> 100	> 100	> 100	> 100
$Co(NO_3)_2·6H_2O$	> 100	> 100	> 100	> 100	> 100	> 100
$Ni(NO_3)_2·6H_2O$	> 100	> 100	> 100	> 100	> 100	> 100
$Cu(NO_3)_2·3H_2O$	> 100	> 100	> 100	> 100	> 100	> 100
$Mn(NO_3)_2·6H_2O$	> 100	> 100	> 100	> 100	> 100	> 100
Cisplatin	12.69 ± 0.29	12.56 ± 0.25	13.88 ± 1.03	15.02 ± 1.96	15.59 ± 1.06	18.52 ± 0.51

3.4. Cell cycle analysis

Recent studies suggested that some of the complexes can induce cell cycle arrest, and thereby induce apoptosis.[38,74–82] After treatment with **1** (3.0 μM) and **2** (1.0 μM) for 24 h (Fig. 3), the populations of G1 phase were 51.80% and 53.79% in comparison with the control cells (43.57%), suggesting that **1** (3.0 μM) and **2** (1.0 μM) caused G1 phase arrest.

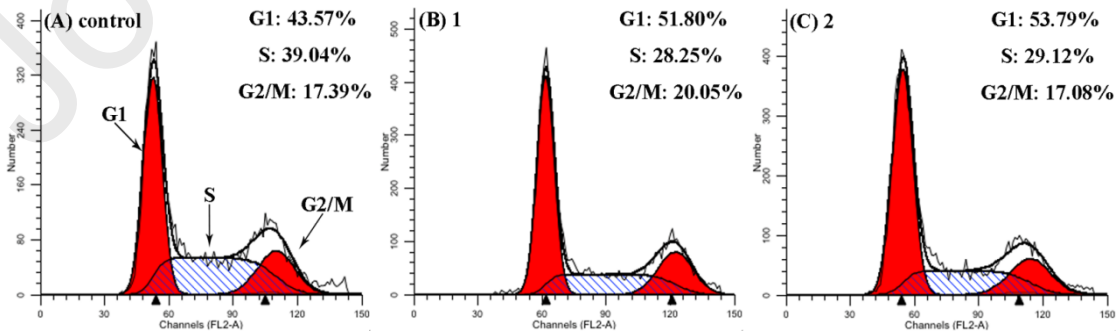


Fig. 3. Cell cycle distribution of HeLa cells (A) exposure to **1** (3.0 μM) and **2** (1.0 μM) for 24 h.

3.5. ROS level

HeLa cancer cells were incubated with **1** (3.0 μM) and **2** (1.0 μM) for 24 h to determine the effect of **1** (3.0 μM) and **2** (1.0 μM) on intracellular ROS generation. As shown in Fig. 4, **1** (3.0 μM) and **2** (1.0 μM), especially **2** (1.0 μM) can enhance the ROS levels.

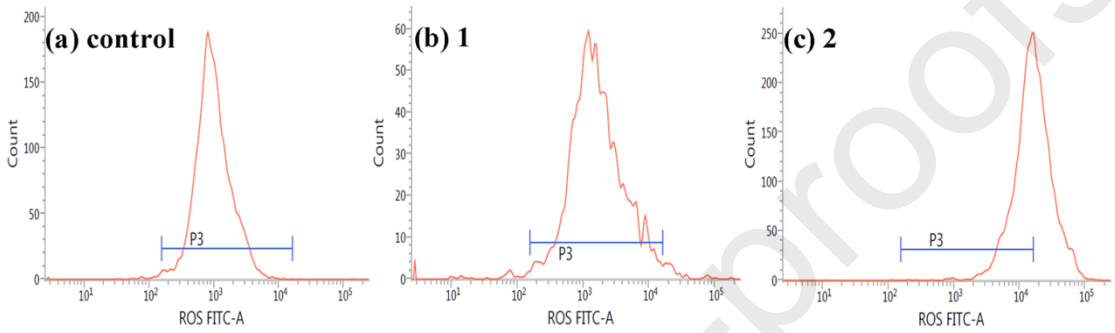


Fig. 4. Intracellular ROS was detected in HeLa cells (a) exposure to **1** (3.0 μM, b) and **2** (1.0 μM, c) for 24 h.

3.6. Mitochondrial membrane potential (MMP) loss

The ROS level is an important factor affecting the MMP expression. [77–85] Thus, to study the mechanism of **1** (3.0 μM) and **2** (1.0 μM) caused HeLa cell apoptosis, we measured the MMP by examining the fluorescence intensity of JC-1 staining. Fig. 5 revealed that **1** (3.0 μM) and **2** (1.0 μM) can induce a decrease of MMP and, thereby cause HeLa cell apoptosis. [77–85]

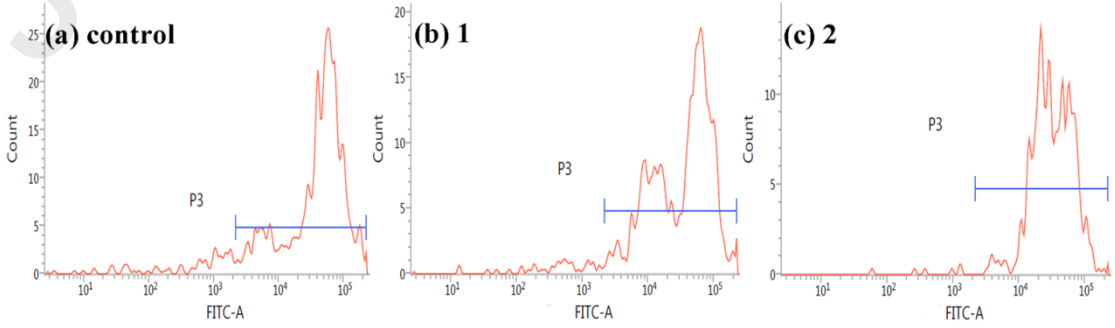


Fig. 5. The MMP level in HeLa cells (a) exposure to **1** (3.0 μM) and **2** (1.0 μM) for 24

h.

3.7. Expression of apoptosis proteins

After treatment of HeLa cells with **1** (3.0 μM) and **2** (1.0 μM) for 24 h (Figs. 6–8), the levels of cytochrome c (cyt c), apaf-1 and caspase-3/9 proteins were obviously up-regulated following the order of **2** > **1**, suggesting that **1** (3.0 μM) and **2** (1.0 μM) induced apoptosis of HeLa cells via an ROS-mediated mitochondrial dysfunction.[77–86]

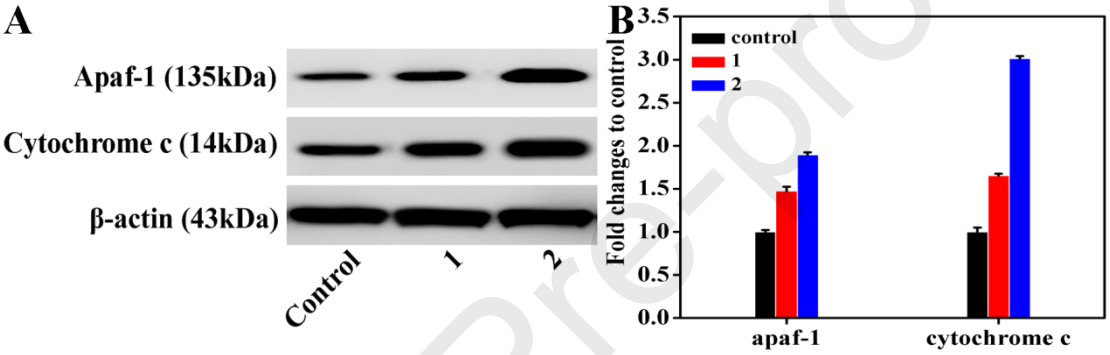


Fig. 6. The imaging (A) and expression (B) of apoptosis proteins in HeLa cells induced by **1** (3.0 μM) and **2** (1.0 μM) for 24 h.

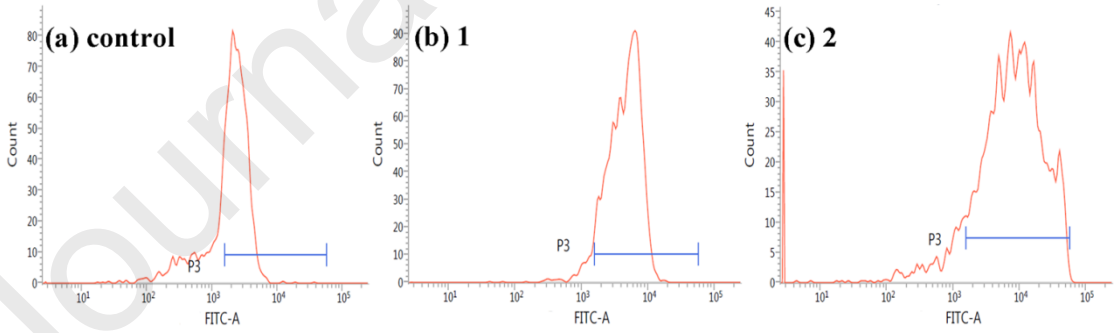


Fig. 7 The level of caspase-3 in HeLa cells (a) induced by **1** (b) and **2** (c) for 24 h.

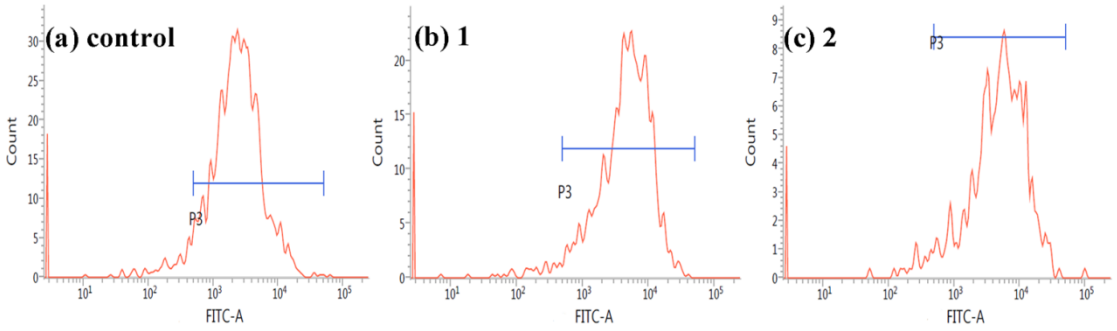


Fig. 8. The level of caspase-9 in HeLa cells (a) induced by **1** (b) and **2** (c) for 24 h.

3.8. 1 and 2 promote cell apoptosis

The ability of **1** (3.0 μM) and **2** (1.0 μM) to promote apoptosis was measured by flow cytometry. As shown in Fig. 9, the percentages (Q2 cells+Q4 cells) of the cells where **1** (3.0 μM) and **2** (1.0 μM) promoted cell apoptosis were 9.75% and 15.07%, respectively, compared with the control. Our results suggested that the intrinsic mitochondrial signal pathway was involved in **1** (3.0 μM) and **2** (1.0 μM) induced apoptosis.[87–92]

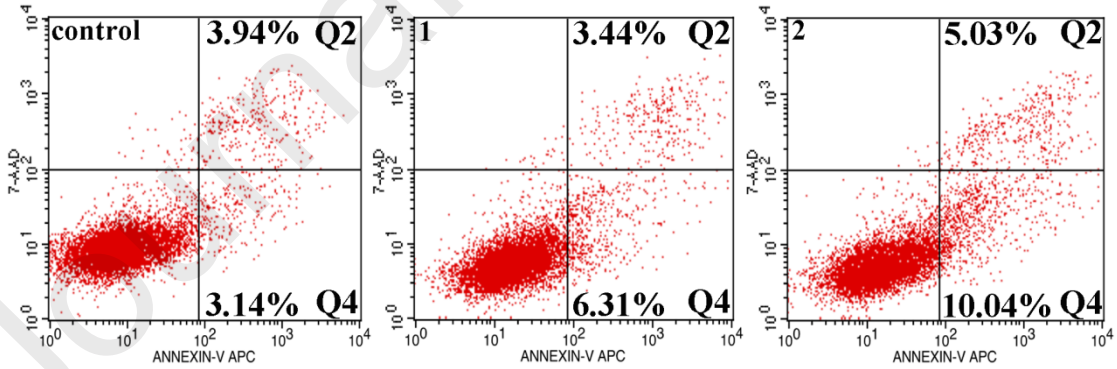


Fig. 9. The apoptosis analysis of the HeLa cells (a) treated with **1** (b) and **2** (c) for 24 h.

4. Conclusions

In conclusion, we designed and prepared eight transition metal complexes **1**–**8** with

6,7-dichloro-5,8-quinolinedione (DQ) and 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligands. Remarkably, complexes **1**−**8** showed higher cytotoxicity than cisplatin against HeLa cells, and less cytotoxicity on the HL-7702 cells. *In vitro* mechanism studies indicated that **1** and **2** induced a series of events related to mitochondrial dysfunction in HeLa cells including ROS production, loss of MMP, cell cycle arrest at G1 phase, and apoptosis in the order of **2** > **1**. The biological behavior of **1** and **2** may correlate with the presence of a 2-methyl group in 6,7-dichloro-2-methyl-5,8-quinolinedione (DMQ) ligand. Taken together, **2** is a promising candidate as a mitochondria-targeted anti-cancer agent.

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Appendix A. Supplementary material

The CCDC contains the supplementary crystallographic data for the complexes **1**−**8** were 1935961, 1935962, 1935963, 1935964, 1935965, 1936175, 1935966 and 1935967. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)

1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

References

[1] A. Gandioso, E. Shaili, A. Massaguer, G. Artigas, A. González-Cantó, J. A. Woods, P. J. Sadler, V. Marchán, Chem. Commun. 51 (2015) 9169.

[2] Y. Gothe, T. Marzo, L. Messori, N. Metzler-Nolte, Chem. Eur. J. 22 (2016) 12487.

[3] Y.-R. Zheng, K. Suntharalingam, T. C. Johnstone, S. J. Lippard, Chem. Sci. 6 (2015) 1189.

[4] D. Y. Q. Wong, J. Y. Lau, W. H. Ang, Dalton Trans. 41 (2012) 6104.

[5] J. Noh, B. Kwon, E. Han, M. Park, W. Yang, W. Cho, W. Yoo, G. Khang, D. Lee, Nat. Commun. 6 (2015) 6907.

[6] Q.-P. Qin, S.-L. Wang, M.-X. Tan, Y.-C. Liu, T. Meng, B.-Q. Zou, H. Liang, Eur. J. Med. Chem. 161 (2019) 334.

[7] Q.-P. Qin, Z.-F. Wang, S.-L. Wang, D.-M. Luo, B.-Q. Zou, P.-F. Yao, M.-X. Tan, H. Liang, Eur. J. Med. Chem. 170 (2019) 195.

[8] L. H. Hurley, Nat. Rev. Cancer 2 (2002) 188.

[9] O. Pinato, C. Musetti, C. Sissi, Metallomics 6 (2014) 380.

[10] H. Kostrhunova, J. Florian, O. Novakova, A. F. A. Peacock, P. J. Sadler, V. Brabec, J. Med. Chem. 51 (2008) 3635.

[11] Z. Liu, A. Habtemariam, A. M. Pizarro, S. A. Fletcher, A. Kisova, O. Vrana, L. Salassa, P. C. A. Bruijnincx, G. J. Clarkson, V. Brabec, P. J. Sadler, J. Med. Chem. 54 (2011) 3011.

[12] V. Milacic, D. Chen, L. Ronconi, K. R. Landis-Piwowar, D. Fregona, Q. P. Dou,

Cancer Res. 66 (2006) 10478.

[13] Q.-P. Qin, S.-L. Wang, M.-X. Tan, D.-M. Luo, Z.-F. Wang, Q.-M. Wei, X.-Y. Wu, B.-Q. Zou, Y.-C. Liu, Polyhedron 157 (2019) 219.

[14] A. Zamora, S. A. P erez, M. Rothemund, V. Rodríguez, R. Schobert, C. Janiak, J. Ruiz, Chem. Eur. J. 23 (2017) 5614.

[15] I. Eryazici, C.N. Moorefield, G. R. Newkome, Chem. Rev. 108 (2008) 1834.

[16] D. Ćoćić, S. Jovanović, S. Radisavljević, J. Korzekwa, A. Scheurer, R. Puchta, D. Baskić, D. Todorović, S. Popović, S. Matić, B. Petrović, J. Inorg. Biochem. 189 (2018) 91.

[17] S. Gama, I. Rodrigues, F. Mendes, I. C. Santos, E. Gabano, B. Klejevskaja, J. Gonzalez-Garcia, M. Ravera, R. Vilar, A. Paulo, J. Inorg. Biochem. 160 (2016) 275.

[18] P. Shi, Q. Jiang, Y. Zhao, Y. Zhang, J. Lin, L. Lin, J. Ding, Z. Guo, J. Biol. Inorg. Chem. 11 (2006) 745.

[19] G. Sava, A. Bergamo, P. J. Dyson, Dalton Trans. 40 (2011) 9069.

[20] Q. Jiang, J. Zhu, Y. Zhang, N. Xiao, Z. Guo, Biometals 22 (2009) 297.

[21] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O′Connell, P. Zanello, J. Med. Chem. 43 (2000) 3541.

[22] K. K.-W. Lo, M.-W. Louie, K. Y. Zhang, Coordin. Chem. Rev. 254 (2010) 2603.

[23] V. M. Manikandamathavan, V. Rajapandian, A. J. Freddy, T. Weyhermüller, V. Subramanian, B. U. Nair, Eur. J. Med. Chem. 57 (2012) 449.

[24] D. Lazić, A. Arsenijević, R. Puchta, Ž. D. Bugarčić, A. Rilak, Dalton Trans. 45 (2016) 4633.

[25] K. Zheng, F. Liu, X.-M. Xu, Y.-T. Li, Z.-Y. Wu, C.-W. Yan, New J. Chem. 38 (2014) 2964.

[26] S. A. Galal, K. H. Hegab, A. M. Hashem, N. S. Youssef, Eur. J. Med. Chem. 2010, 45 (2010) 5685.

[27] T. Meng, Q.-P. Qin, Z.-L. Chen, H.-H. Zou, K. Wang, F.-P. Liang, Eur. J. Med. Chem. 169 (2019) 103.

[28] Q.-P. Qin, Z.-F. Wang, X.-L. Huang, M.-X. Tan, B.-B. Shi, H. Liang, ACS Med. Chem. Lett. 10 (2019) 936.

[29] I. Kostova, Curr. Med. Chem. 13 (2006) 1085.

[30] X. Chen, L. Tang, P. Sun, Y. Qiu, G. Liang, J. Inorg. Biochem. 104 (2010) 379.

[31] I. Ott, B. Kircher, P. Schumacher, K. Schmidt, R. Gust, J. Med. Chem. 48 (2005) 622.

[32] M. A. Yakupec, Dalton Trans. (2008) 183.

[33] P. Pigeon, S. Top, A. Vessiéres, M. Huché, E. A. Hillard, E. Salomon, G. Jaouen, J. Med. Chem. 48 (2005) 2814.

[34] A. Vessiéres, S. Top, W. Beck, E. Hillard, G. Jaouen, Dalton Trans. (2006) 529.

[35] W. H. Ang, P. J. Dyson, Eur. J. Inorg. Chem. (2006) 4003.

[36] X.-D. Song, X. Kong, S.-F. He, J.-X. Chen, J. Sun, B.-B. Chen, J.-W. Zhao, Z.-W. Mao, Eur. J. Med. Chem. 138 (2017) 246.

[37] Q.-P. Qin, T. Meng, Z.-Z. Wei, C.-H. Zhang, Y.-C. Liu, H. Liang, Z.-F. Chen, Eur. J. Inorg. Chem. (2017) 1824.

[38] Q.-P. Qin, J.-L. Qin, T. Meng, W.-H. Lin, C.-H. Zhang, Z.-Z. Wei, J.-N. Chen, Y.-C. Liu, H. Liang, Z.-F. Chen, Eur. J. Med. Chem. 124 (2016) 380.

[39] K. Györyová, E. Szunyogová, J. Kovářová, D. Hudecová, D. Mudroňová, E. Juhászová, J. Therm. Anal. Calorim. 72 (2003) 587.

[40] S. Emami, S. J. Hosseinimehr, S. M. Taghdisi, S. Akhlaghpoor, Bioorg. Med. Chem. Lett. 17 (2007) 45.

[41] Q.-M. Huang, Z.-Q. Pan, P. Wang, Z.-P. Chen, X.-L. Zhang, H.-S. Xu, Bioorg. Med. Chem. Lett. 16 (2006) 3030.

[42] A. Nakayama, M. Hiromura, Y. Adachi, H. Sakurai, J. Biol. Inorg. Chem. 13 (2008) 675.

[43] H. Sakura, Y. Yoshikawa, H. Yasui, Chem. Soc. Rev. 37 (2008) 2383.

[44] M. B. Ferrari, F. Bisceglie, G. Pelosi, P. Tarasconi, R. Albertini, S. Pinelli, J. Inorg. Biochem. 87 (2001) 137.

[45] C. Bazzicalupi, A. Bencini, A. Bianchi, T. Biver, A. Boggioni, S. Bonacchi, A. Danesi, C. Giorgi, P. Gratteri, A. M. Ingrain, F. Secco, C. Sissi, B. Valtancoli, M. Venturini, Chem. Eur. J. 14 (2008) 184.

[46] P. F. Liguori, A. Valentini, M. Palma, A. Bellusci, S. Bernardini, M. Ghedini, M. L. Panno, C. Pettinari, F. Marchetti, A. Crispini, D. Pucci, Dalton Trans. 39 (2010) 4205.

[47] C. T. Liu, A. A. Neverov, R. S. Brown, J. Am. Chem. Soc. 130 (2008) 13870.

[48] T. S. B. Baul, S. Kundu, A. Linden, N. Raviprakash, S. K. Manna, M. F. C. G. da Silva, Dalton Trans. 43 (2014) 1191.

[49] S. Mandal, A. K. Rout, A. Ghosh, G. Pilet, D. Bandyopadhyay, Polyhedron 28 (2009) 3858.

[50] D. P. Singh, K. Kumar, C. Sharma, Eur. J. Med. Chem. 45 (2010) 1230.

[51] M.-X. Li, C.-L. Chen, D. Zhang, J.-Y. Niu, B.-S. Ji, Eur. J. Med. Chem. 45 (2010) 3169.

[52] M.-X. Li, L.-Z. Zhang, D. Zhang, B.-S. Ji, J.-W. Zhao, Eur. J. Med. Chem. 46 (2011) 4383.

[53] D. S. Kalinowski, P. C. Sharpe, P. V. Bernhardt, D. R. Richardson, J. Med. Chem. 50 (2007) 6212.

[54] D. R. Richardson, P. C. Sharpe, D. B. Lovejoy, D. Senaratne, D. S. Kalinowski, M. Islam, P. V. Bernhardt, J. Med. Chem. 49 (2006) 6510.

[55] R. Gust, I. Ott, D. Posselt, K. Sommer, J. Med. Chem. 47 (2004) 5837.

[56] I. Ott, K. Schmidt, B. Kircher, P. Schumacher, T. Wiglenda, R. Gust, J. Med. Chem. 48 (2005) 622.

[57] M. Belicchi-Ferrari, F. Bisceglie, C. Casoli, S. Durot, I. Morgenstern-Badarau, G. Pleosi, E. Piltti, S. Pinelli, P. Tarasconi, J. Med. Chem. 48 (2005) 1671.

[58] N. Yamamoto, A. K. Renfrew, B. J. Kim, N. S. Bryce, T. W. Hambley, J. Med. Chem. 55 (2012) 11013.

[59] B. J. Kim, T. W. Hambley, N. S. Bryce, Chem. Sci. 2 (2012) 2135.

[60] A. K. Renfrew, N. S. Bryce, T. W. Hambley, Chem. Sci. 3 (2013) 3731.

[61] J. Y.-C. Chang, G.-L. Lu, R.J. Stevenson, P. J. Brothers, G. R. Clark, K. J. Botting, D. M. Ferry, M. Tercel, W. Wilson, W. A. Denny, D. C. Ware, Inorg. Chem. 52 (2013) 7688.

[62] V. Thamilarasan, A. Jayamani, N. Sengottuvelan, Eur. J. Med. Chem. 89 (2015) 266.

[63] S. N. Georgiades, N. H. A. Karim, K. Suntharalingam, R. Vilar, Angew. Chem. Int. Ed. 2010, 49 (2010) 4020.

[64] C. Wang, Y. Li, G. Jia, Y. Liu, S. Lu, C. Li, Chem. Commun. 48 (2012) 6232.

[65] S.-T. Von, H.-L. Seng, H.-B. Lee, S.-W. Ng, Y. Kitamura, M. Chikira, C.-H. Ng, J. Biol. Inorg. Chem. 17 (2012) 57.

[66] M. Kubanik, N. Y. S. Lam, H. U. Holtkamp, T. Sohnel, R. F. Anderson, S. M. F. Jamieson, C. G. Hartinger, Chem. Commun. 54 (2018) 992.

[67] C.-K. Ryu, H.-J. Kim, Arch. Pharmacal Res., 1994, 17, 139–144.

[68] A. N. Pearce, E. W. Chia, M. V. Berridge, G. R. Clark, J. L. Harper, L. Larsen,

E. W. Maas, M. J. Page, N. B. Perry, V. L. Webb, B. R. Copp, J. Nat. Prod. 70 (2007) 936.

[69] I. A. Shaikh, F. Johnson, A. P. Grollman, J. Med. Chem. 29 (1986) 1329.

[70] D. Q. Shen, Y. Cheng, L. K. An, X. Z. Bu, Z. S. Huang, L. Q. Gu, Chin. Chem. Lett. 19 (2008) 533.

[71] Q.-M. Chen, G.-B. Yi, L.-K. An, X.-L. Feng, Acta Crystallogr. Sect. E: Struct. Rep. Online 67 (2011) o1108.

[72] M. M¸akosza, K. Wojciechowski, Chem. Rev. 104 (2004) 2631.

[73] Z. Li, J. Zhou, Y. Gan, Y. Yin, W. Zhang, J. Yang, Y. Tang, Y. Dai, J. Inorg. Biochem. 197 (2019) 110701. DOI: 10.1016/j.jinorgbio.2019.110701.

[74] T. Meng, Q.-P. Qin, Z.-L. Chen, H.-H. Zou, K. Wang, F.-P. Liang, Dalton Trans. 48 (2019) 5352.

[75] Z.-F. Chen, Q.-P. Qin, J.-L. Qin, Y.-C. Liu, K.-B. Huang, Y.-L. Li, T. Meng, G.-H. Zhang, Y. Peng, X.-J. Luo, H. Liang, J. Med. Chem. 58 (2015) 2159.

[76] A. K. P. Taggart, S.-C. Teng, V. A. Zakian, Science 297 (2002) 1023.

[77] C.-C. Zeng, S.-H. Lai, J.-H. Yao, C. Zhang, H. Yin, W. Li, B.-J. Han, Y.-J. Liu, Eur. J. Med. Chem. 122 (2016) 118.

[78] V. M. Dirsch, D. S. Antlsperger, H. Hentze, A. M. Vollmar, Leukemia 16 (2002) 74.

[79] L. Gamet-Payrastre, P. Li, S. Lumeau, G. Cassar, M. A. Dupont, S. Chevolleau, N. Gasc, J. Tulliez, F. Terce, Cancer Res. 60 (2000) 1426.

[80] J. Klucar, M. Al-Rubeai, FEBS Lett. 400 (1997) 127.

[81] Q.-P. Qin, S.-L. Wang, M.-X. Tan, Z.-F. Wang, X.-L. Huang, Q.-M. Wei, B.-B. Shi, B.-Q. Zou, H. Liang, Metallomics 10 (2018) 1160.

[82] Q.-P. Qin, B.-Q. Zou, M.-X. Tan, S.-L. Wang, Y.-C. Liu, H. Liang, New J.

Chem. 42 (2018) 15479.

[83] H.-H. Zou, L. Wang, Z.-X. Long, Q.-P. Qin, Z.-K. Song, T. Xie, S.-H. Zhang, Y.-C. Liu, B. Lin, Z.-F. Chen, Eur. J. Med. Chem. 108 (2016) 1.

[84] J. Qi, K. Qian, L. Tian, Z. Cheng, Y. Wang, New J. Chem. 42 (2018) 10226.

[85] G. Tang, Z. Nikolovska-Coleska, S. Qiu, Yang, Y. C. J. Guo, S. Wang, J. Med. Chem. 51 (2008) 717.

[86] Q.-P. Qin, Z.-F. Chen, J.-L. Qin, X.-J. He, Y.-L. Li, Y.-C. Liu, K.-B. Huang, H. Liang, Eur. J. Med. Chem. 92 (2015) 302.

[87] K. Suntharalingam, J. J. Wilson, W. Lin, S. J. Lippard, Metallomics 6 (2014) 437.

[88] J.-Q. Wang, P.-Y. Zhang, L.-N. Ji, H. Chao, J. Inorg. Biochem. 146 (2015) 89.

[89] Q. Cao, Y. Li, E. Freisinger, P. Z. Qin, R. K. O. Sigela, Z.-W. Mao, Inorg. Chem. Front. 4 (2017) 10.

[90] Q.-P. Qin, Z.-F. Wang, X.-L. Huang, M.-X. Tan, B.-Q. Zou, H. Liang, Eur. J. Med. Chem. 184 (2019) 111751.

[91] Q.-P. Qin, B.-Q. Zou, Z.-F. Wang, X.-L. Huang, Y. Zhang, M.-X. Tan, S.-L. Wang, H. Liang, Eur. J. Med. Chem. 183 (2019) 111727.

[92] Q.-P. Qin, Z.-F. Wang, X.-L. Huang, M.-X. Tan, Z.-H. Luo, S.-L. Wang, B.-Q. Zou, H. Liang, Dalton Trans. 48 (2019) 15247.

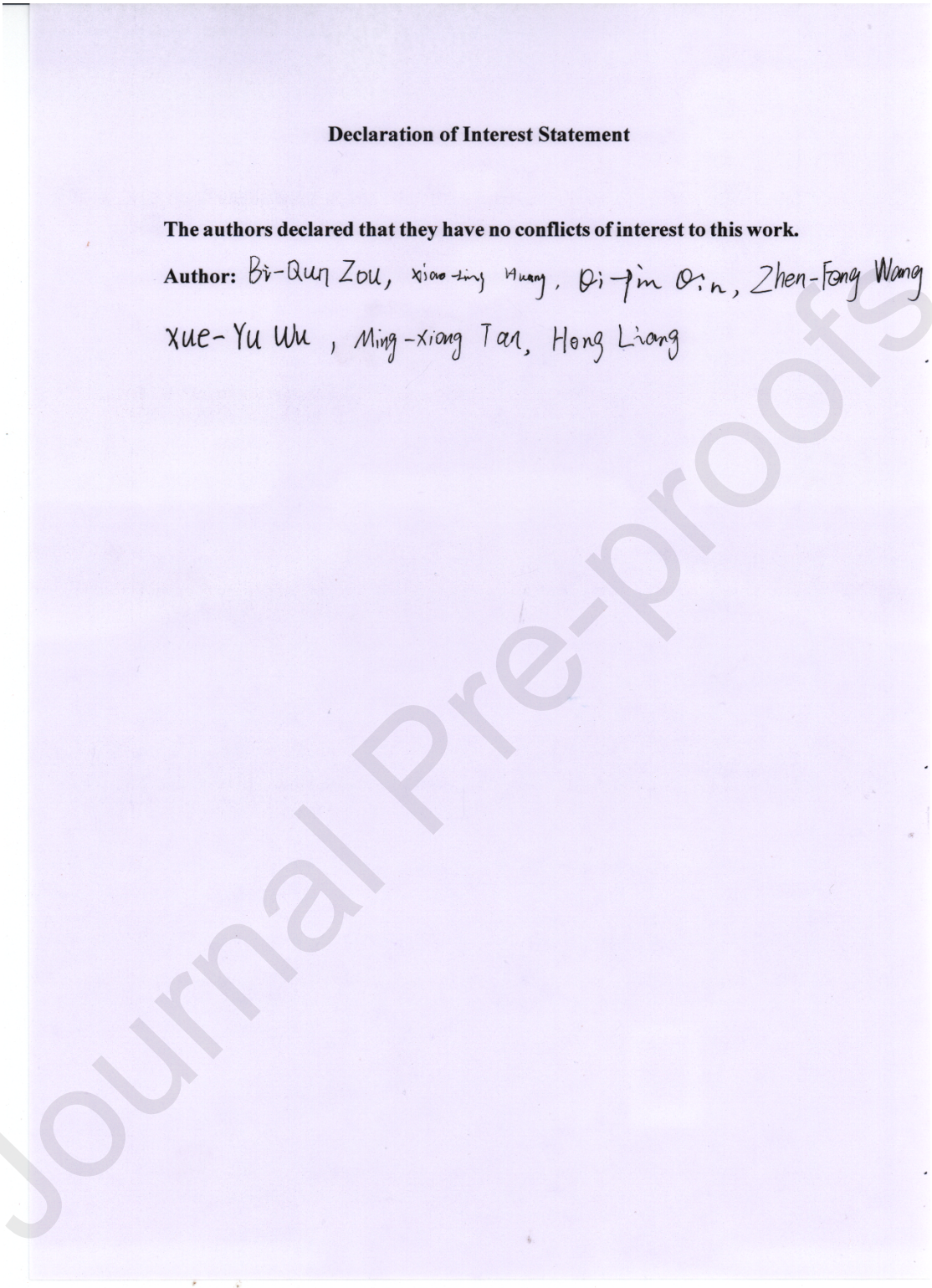
Author Contributions Section

Bi-Qun Zou and Xiao-Ling Huang conceived and designed the experiments, Bi-Qun Zou, Xiao-Ling Huang, Zhen-Feng Wang and Xue-Yu Wu synthesized chemical compounds and carried out in vitro antitumor activities of complexes **1**–**8**. Qi-Pin Qin, Ming-Xiong Tan and Hong Liang provided effective directions during the research process. Qi-Pin Qin, Ming-Xiong Tan and Hong Liang analyzed data, discussed results, wrote and commented on the paper. All authors reviewed the manuscript.

Declaration of Interest Statement

The authors declared that they have no conflicts of interest to this

work.



Declaration of Interest Statement

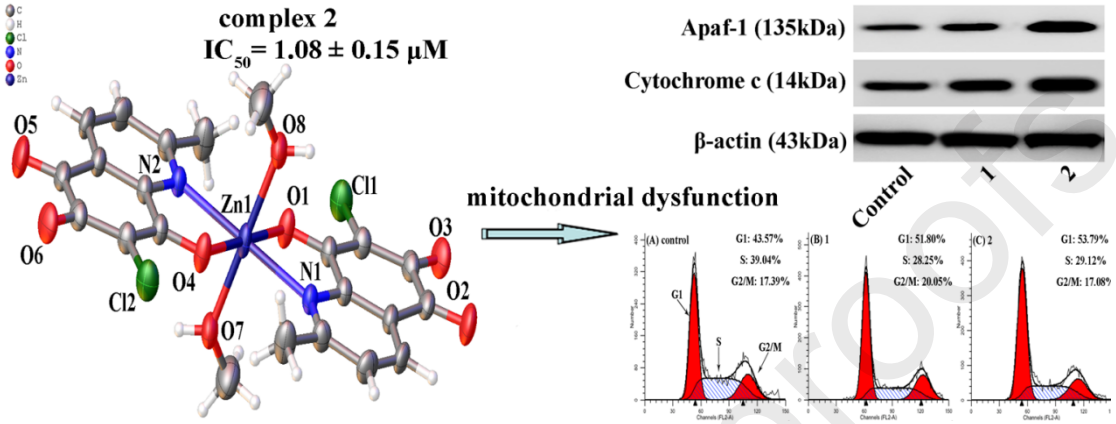
The authors declared that they have no conflicts of interest to this work.

Author: Bi-Qun Zou, Xiao-ting Huang, Qi-jin Qin, Zhen-Fang Wang Xue-Yu Wu, Ming-xiong Tan, Hong Liang

Graphical abstract

Transition metal complexes with 6,7-dichloro-5,8-quinolinedione as mitochondria-targeted anticancer agents

Bi-Qun Zou,[a,b,c] Xiao-Ling Huang,[b] Qi-Pin Qin,*[b,c] Zhen-Feng Wang,[b] Xue-Yu Wu,[b] Ming-Xiong Tan,*[b,c] Hong Liang*[c]



Complexes **1** and **2** arrested the cell cycle in the G1 phase, and inducing cancer cell death via mitochondrial dysfunction pathways was in the following order of **2** > **1**.

Highlights:

- 5,8-quinolinedione transition metal complexes **1**−**8** have been first reported.
- **1**−**8** are more selective for HeLa cells versus normal cells (HL-7702).
- **2** also caused mitochondrial dysfunction.