Bioorg. Med. Chem. 30 (2021) 115948

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Mitochondria-localizing curcumin-cryptolepine Zn(II) complexes and their antitumor activity

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ARTICLE INFO

Keywords:
Curcumin-cryptolepine
Zn(II) complexes
Cell apoptosis
Mitochondrial dysfunction

ABSTRACT

Many metal complexes are potent candidates as mitochondrial-targeting agents. In this study, four novel Zn(II) complexes, [Zn(BPQA)Cl_2] (**Zn1**), [Zn(BPQA)(Curc)]Cl (**Zn2**), [Zn(PQA)Cl_2] (**Zn3**), and [Zn(PQA)(Curc)]Cl (**Zn4**), containing *N*,*N*-*bis*(pyridin-2-ylmethyl)benzofuro[3,2-*b*]quinolin-11-amine (BPQA), N-(pyridin-2-ylmethyl)benzofuro[3,2-*b*]quinolin-11-amine (PQA), and curcumin (H-Curc) were synthesized. An MTT assay showed that **Zn1**–**Zn4** had strong anticancer activities against SK-OV-3/DDP and T-24 tumor cells with IC_{50} values of 0.03–6.19 μM. Importantly, **Zn1** and **Zn2** displayed low toxicities against normal HL-7702 cells. Mechanism experiments demonstrated that probe **Zn2** showed appreciable fluorescence in the red region of the spectrum, and substantial accumulation of **Zn2** occurred in the mitochondria after treatment, indicating increases in Ca^{2+} and reactive oxygen species levels, loss of the mitochondrial membrane potential, and consequent induction of mitochondrial dysfunction at low concentrations. In addition, the probe **Zn2** effectively (50.7%) inhibited the growth of T-24 bladder tumor cells *in vivo*. The probe **Zn2** shows potential for use in cancer therapy while retaining the H-Curc as an imaging probe.

1. Introduction

Cis-platinum and its analog are widely used as therapies in a range of cancers[1–6] but exhibit various limitations[7–13]; thus, compounds based on other metal ions, such as Zn, Co, Cu, Ru, and Ir[14–25] have attracted attention. Additionally, Zn is considered as an essential element for numerous cellular processes[26–30], and many Zn(II) compounds with strong anticancer activity have recently been reported[31–52] such as naproxen Zn(II) complexes,[27] anthracenyl-linked *bis*(pyrazolyl) methane Zn(II) complexes,[28] *ortho*-phenylendiimine Zn(II) and Cu(II) complexes,[31] 5,7-dihalo-substituted-8-quinolinoline Zn(II) and copper (II) complexes,[34] zinc-tolfenamato complexes,[36] zinc(II)-thiosemicarbazone complexes,[42] zinc *bis*(thiosemicarbazone) complexes,[43] triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) Zn(II) complexes,[28] Zn(II) *bis*(thiosemicarbazonato) complexes,[48] and zinc(II) phthalocyanine derivatives,[49] among others.

Recent studies suggested that traditional Chinese medicines composed of metal complexes have selective activity between tumor and normal cells.[16,53–61] The use of curcumin (H-Curc) and its complexes in cancer treatment has shown some success.[62] Further, the natural product cryptolepine and its Zn complexes exhibited significant effects against T-24 bladder tumor cells.[63–70] However, only two Zn complexes of cryptolepine have been reported as mitochondrial membrane-targeting drugs.[70] Additionally, a Zn complex containing *N*,*N*-*bis*(pyridin-2-ylmethyl)benzofuro[3,2-*b*]quinolin-11-amine (BPQA), *N*-(pyridin-2-ylmethyl)benzofuro[3,2-*b*]quinolin-11-amine (PQA), and H-Curc has not been reported. To exploit novel Zn(II) complexes with more extended planar cryptolepine ligands, two new cryptolepine ligands (BPQA, PQA) and four novel Zn(II) complexes, [Zn(BPQA)Cl_2] (**Zn1**), [Zn(BPQA)(Curc)]Cl (**Zn2**), [Zn(PQA)Cl_2] (**Zn3**), and [Zn(PQA)(Curc)]Cl (**Zn4**) were prepared and their biological activities were evaluated.

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https://doi.org/10.1016/j.bmc.2020.115948
Received 8 November 2020; Received in revised form 3 December 2020; Accepted 6 December 2020
Available online 19 December 2020

2. Results and discussion

2.1. Synthesis and stability

The synthesis of 11-chlorobenzofuro[3,2-*b*]quinoline (ClQ) has been reported previously.[70] In addition, the new cryptolepine BPQA and PQA ligands were synthesized via the routes shown in Scheme 1, starting from *o*-nitrobenzoic acid. Two mononuclear Zn complexes, [Zn(BPQA)Cl_2] (**Zn1**) and [Zn(PQA)Cl_2] (**Zn3**), were prepared by reacting $ZnCl_2$ with BPQA in CH_3OH (5.0 mL) at 65.0 °C for 24 h (yields: 93.6% and 80.2%, respectively). Further, **Zn1** and **Zn3** were reacted with H-Curc ligand in the presence of triethylamine (0.1 mL) and methanol (3.0 mL) at 80.0 °C for 24 h to afford [Zn(BPQA)(Curc)]Cl (**Zn2**) and [Zn(PQA)(Curc)]Cl (**Zn4**) in 88.1% and 83.9% yield (Scheme 1); their structures were determined from extensive spectral data and by elemental analyses (Figs. S1–S19). The Zn(II) centers in **Zn1**–**Zn4** were coordinated to one cryptolepine ligand, one H-Curc ligand, or one H_2O molecule, creating a distorted five-coordinate tetragonal-pyramid geometry. Further, the cryptolepine complex **Zn2** (0.03 μM) was tested for its stability in Tris-HCl buffer (10 mM, pH 7.40) by UV − vis spectroscopy. As shown in Fig. 1, the time-dependent (0 and 48 h) UV − vis spectra of these compounds indicated that the cryptolepine complex **Zn2** (0.03 μM) was stable in Tris-HCl buffer (10 mM, pH 7.40) for 48 h at room temperature.

2.2. Anticancer activity

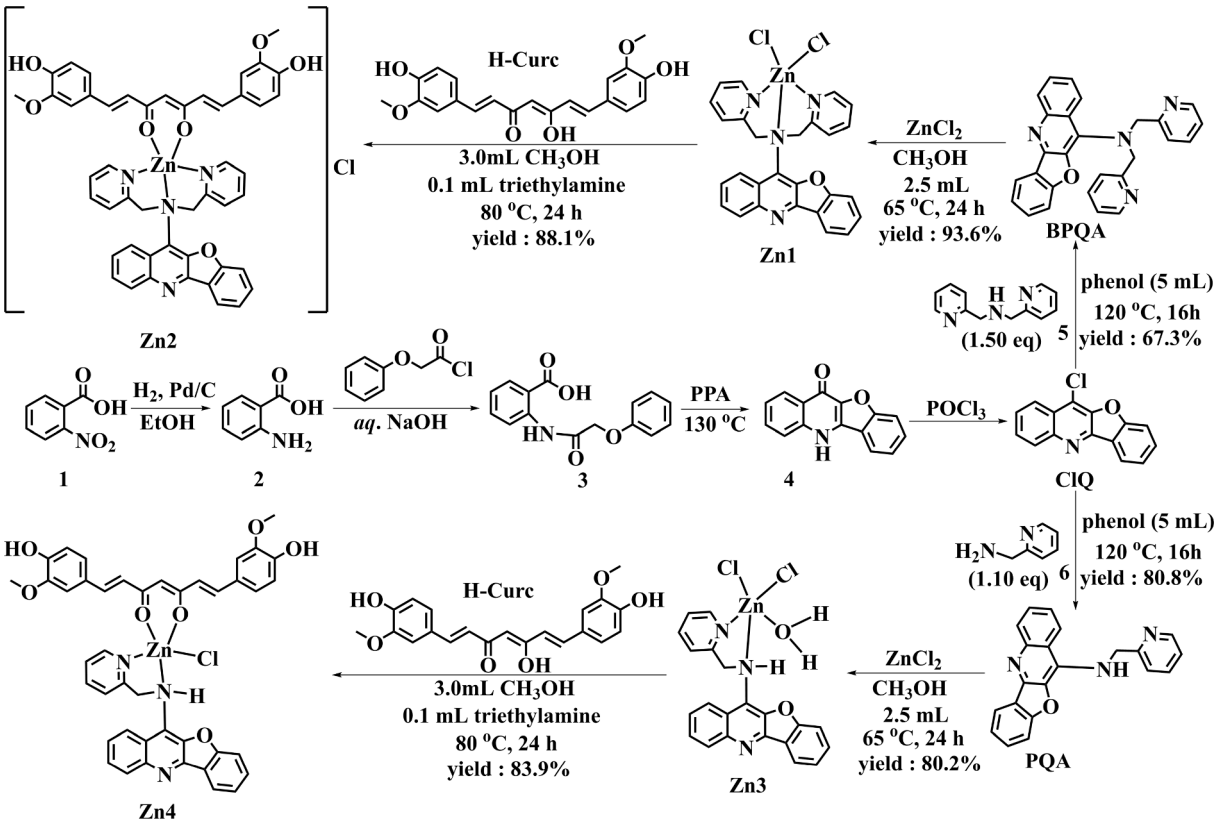
The cytotoxicity of cryptolepine compounds **Zn1** − **Zn4**, H-Curc, $ZnCl_2$, and BPQA was evaluated against the T-24, SK-OV-3/DDP, and HL-7702 cell lines by 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT) assay.[71–74] Compound **Zn2**, containing H-Curc and more extended planar cryptolepine BPQA ligands, showed the highest cytotoxicity among the tested Zn complexes **Zn1**, **Zn3**, **Zn4**, and $ZnCl_2$, and also its H-Curc, BPQA and PQA ligands against the T-24 and SK-OV-3/DDP cell lines (Table 1). For example, **Zn2** showed low IC_{50} values (0.03 ± 0.01 μM) in T-24 cells, exhibiting >3.3–1545.0-fold higher cytotoxicity than **Zn1**, **Zn3**, **Zn4**, H-Curc, PQA, and BPQA ligands, cryptolepine–H-Curc **BQ-Zn** (5.92 ± 0.35 μM),[70] and **BQCur-Zn** (1.55 ± 0.04 μM),[70] and even 610.7-fold higher cytotoxicity than cisplatin (18.32 ± 1.11 μM) and many other traditional Chinese medicines metal analogues that have been previously evaluated.[16,53–61] Complexes **Zn1** and **Zn2** exhibited obviously higher cytotoxic effects than previously reported cryptolepine–H-Curc **BQ-Zn** (5.92 ± 0.35 μM)[70] and **BQCur-Zn** (1.55 ± 0.04 μM),[70] which is correlated with the key roles of the more extended planar cryptolepine BPQA ligand. Interestingly, **Zn1** − **Zn4** showed low toxicity against normal HL-7702 cells (IC_{50} > 100 μM).

2.3. Cellular localization behaviors

Fig. 1 shows that the absorption spectra of cryptolepine complex **Zn2** (0.03 μM) were ~420.0 and 440.0 nm in Tris-HCl buffer (10 mM, pH 7.40). In addition, the emission spectra of cryptolepine complex **Zn2** (0.03 μM) displayed red emission with a maximum wavelength at approximately 600.0 nm upon excitation at 440 nm. Next, the red probe of cryptolepine complex **Zn2** (0.03 μM) was used specifically for mitochondrial membrane imaging (λ_{em} = 600 nm) upon excitation at 440 nm (Fig. 2), with the results indicating that the mitochondrial membrane potential was destroyed (Fig. 2). In contrast, complex **Zn1** (0.10 μM) showed no obvious effects, which was very similar to the results for another cryptolepine **BQ-Zn** complex.[70]

2.4. Loss of mitochondrial membrane potential ($\Delta\Psi_{MMP}$)

Mitochondria damage, particularly the loss of $\Delta\Psi_{MMP}$, is a key event in controlling the intrinsic apoptosis pathway.[57,75–79] To further evaluate the effects of cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03



Scheme 1. Synthetic route of BPQA and PQA ligand and their metal complexes **Zn1**–**Zn4**.

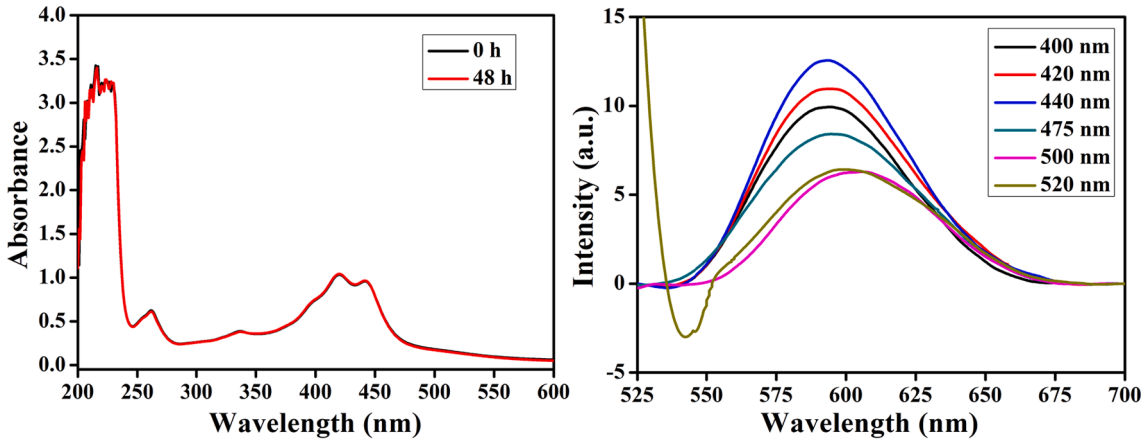


Fig. 1. (A) UV–Vis spectra and (B) emission spectra of cryptolepine complex **Zn2** (0.03 μM) in Tris-HCl buffer (10 mM, pH 7.40) at 37 °C.

Table 1
IC_{50} (μM) of cryptolepine compounds **Zn1**–**Zn4** towards three human cells.

Compounds	T-24	SK-OV-3/DDP	HL-7702
BPQA	20.13 ± 1.09	35.99 ± 1.46	>100
Zn1	0.10 ± 0.05	3.13 ± 0.59	>100
H-Curc	19.22 ± 1.17	32.68 ± 2.35	>100
Zn2	0.03 ± 0.01	1.31 ± 0.22	>100
PQA	46.35 ± 0.79	55.03 ± 0.76	>100
Zn3	2.06 ± 0.45	6.19 ± 0.52	>100
Zn4	0.14 ± 0.08	1.98 ± 0.53	>100
$ZnCl_2$	>150	>150	>150
Cisplatin	12.42 ± 0.45	69.05 ± 1.01	18.32 ± 1.11

μM) on $\Delta\Psi_{MMP}$, the fluorescence probe JC-1 was used. Green fluorescence populations of 22.69% and 37.79% were observed in the **Zn1** and **Zn2** groups, whereas this value was 6.93% in untreated T-24 cells (Fig. 3), indicating that cryptolepine complexes **Zn1** and **Zn2** induced a decrease in the $\Delta\Psi_{MMP}$ level in the order of **Zn2** > **Zn1**. These results agree with the cellular localization behaviors determined by confocal microscopy imaging of T-24 cells.

2.5. Analysis of reactive oxygen species (ROS) levels

Increases in ROS levels and loss of $\Delta\Psi_{MMP}$ are closely associated with intrinsic apoptosis in cancer cells.[57,75–79] As shown in Fig. 4, treatment of T-24 cells with the cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) led to increases in the ROS levels by 31.90% and 40.05%, respectively. Additionally, in the presence of **Zn2** (0.03 μM), with H-Curc ligand, a greater increase in ROS levels than those induced by the cryptolepine complex **Zn1** (0.10 μM) was observed. Therefore, the cryptolepine complexes **Zn1** and **Zn2** induced an increase in intracellular ROS levels in T-24 cancer cells.

2.6. Determination of intracellular [Ca^{2+}] levels

Intracellular [Ca^{2+}] levels have emerged as an important factor in many cellular processes,[76,80,81] and some metallodrugs have been shown to increase intracellular [Ca^{2+}] levels.[76,80–82] To evaluate the effects of the cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) on intracellular [Ca^{2+}] levels, T-24 cancer cells were exposed to these complexes for 24 h and then stained with Fluo-3AM. As shown in Fig. 5, intracellular [Ca^{2+}] levels in T-24 cancer cells increased steadily (~31.52% for **Zn1** and 36.48% for **Zn2**), particularly in **Zn2** (0.03 μM)-treated cells, which agrees with the above results.

2.7. Expression of apoptosis-related proteins

Mitochondria dysfunction is an important factor in cell death.[76,80–84] Upon treatment with cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM), the $\Delta\Psi_{MMP}$ was rapidly lost and ROS and [Ca^{2+}] levels were increased (Figs. 2–5), resulting in T-24 cell apoptosis. Tumor-associated mitochondrial proteins such as bcl-2, caspase-3, apaf-1, caspase-9, and

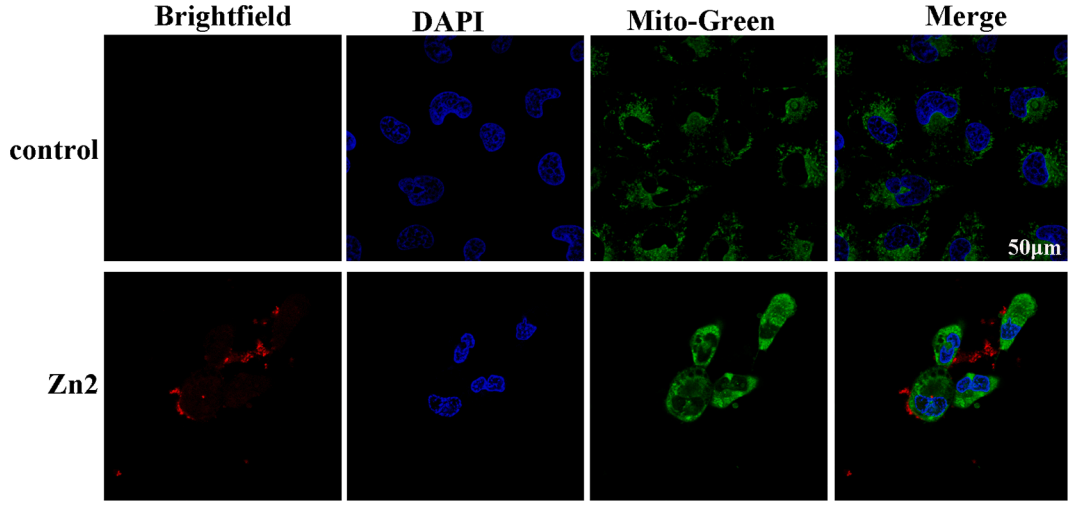


Fig. 2. Intercellular localization of cryptolepine complex **Zn2** (0.03 μM) evaluated by confocal microscopy for 24 h (λ_{ex}: 440 nm; λ_{em}: 600 nm).

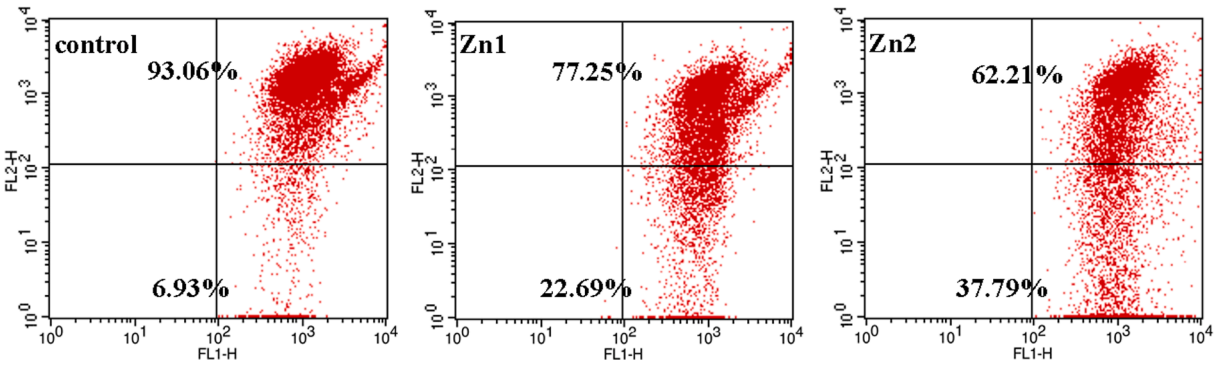


Fig. 3. Green fluorescence indicating the $\Delta\Psi_{MMP}$ level was determined by flow cytometry of T-24 cells incubated with cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) for 24 h.

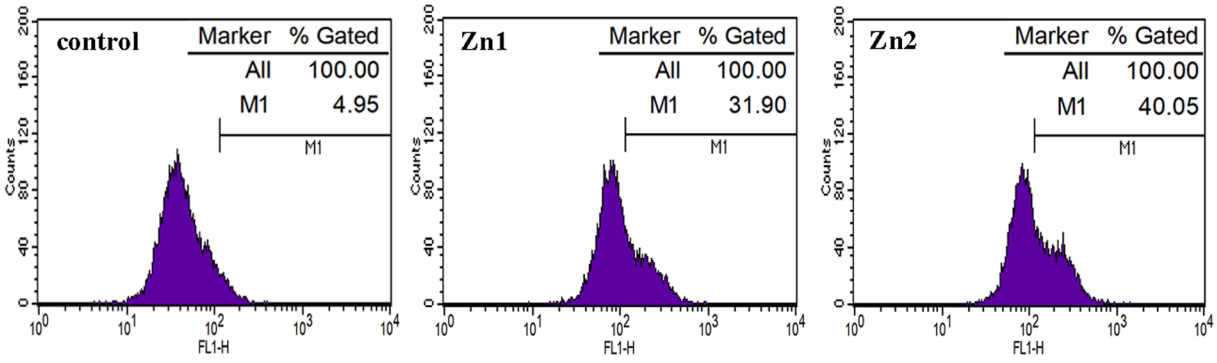


Fig. 4. Intracellular ROS in T-24 cells exposed to cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) for 24 h.

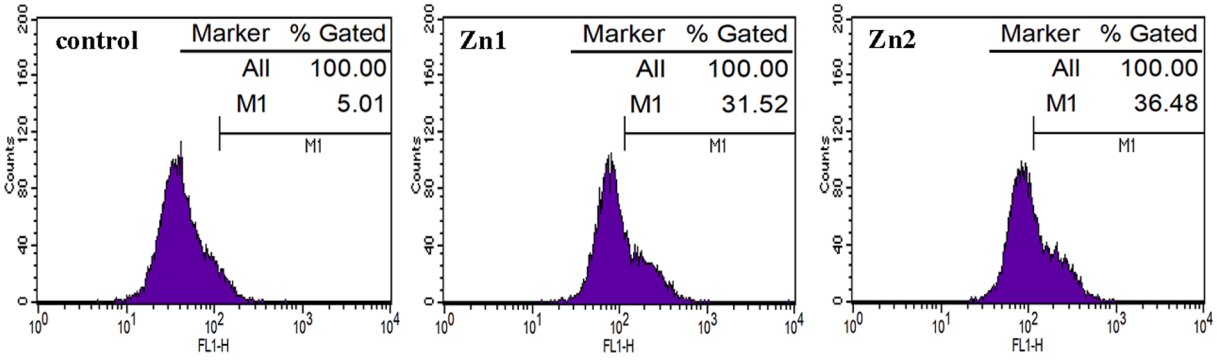


Fig. 5. Effects of cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) on intracellular [Ca^{2+}] levels in T-24 cells for 24 h.

cytochrome *c* were inhibited or activated in the mitochondria (Fig. 6), indicating that the cryptolepine complexes **Zn1** and **Zn2** induced T-24 cell apoptosis via mitochondria dysfunction.

2.8. Apoptosis

Fig. 7 shows that when T-24 cells were treated with the cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM), the percentage of apoptotic (UR + LR) cells was increased to 79.71% for **Zn1** and 95.36% for **Zn2** compared with the control (9.63%). The ability of **Zn1** and **Zn2** to induce cell apoptosis was better than that of previously reported cryptolepine-H-Curc **BQ-Zn** (~36.23%).[70] Clearly, T-24 cell apoptosis was higher with **Zn2**, as it bears one deprotonated H-Curc (H-Curc) ligand as compared with **Zn1**. Finally, nude mice bearing NCI-H460 cell xenografts are typically used to evaluate antitumor activity *in vivo*.[23,70,85–87] Compared to in control mice, there were no effects on the heart rate, body weight (Tables S1–S3), food consumption, or respiration rate in the treated mice (Tables S1–S3, Fig. 8). In addition, the cryptolepine complex **Zn2** (2.0 mg/kg) significantly reduced the cancer volume, with growth inhibition by 50.7% ($p < 0.01$) compared with 5% dimethyl sulfoxide (DMSO) in the saline group (v/v), which was greater than previously reported values for cryptolepine–H-Curc **BQ-Zn** (43.0%)[70] and cisplatin (37.1%).[23,70,85–87]

3. Conclusions

In summary, two new cryptolepine BPQA and PQA ligands and their four cryptolepine-H-Curc Zn(II) complexes **Zn1** – **Zn4** were synthesized and characterized. The MTT assay showed that cryptolepine-H-Curc **Zn1** – **Zn4** have high cytotoxic activity toward T-24 cells, with IC_{50} values of 0.10 ± 0.05, 0.03 ± 0.01, 2.06 ± 0.45, and 0.14 ± 0.08 μM, respectively. Interestingly, **Zn1** – **Zn4** displayed low toxicity against normal HL-7702 cells. Additionally, cryptolepine-H-Curc **Zn1** and **Zn2** entered the mitochondrial membrane and decreased the $\Delta\Psi_{MMP}$ and

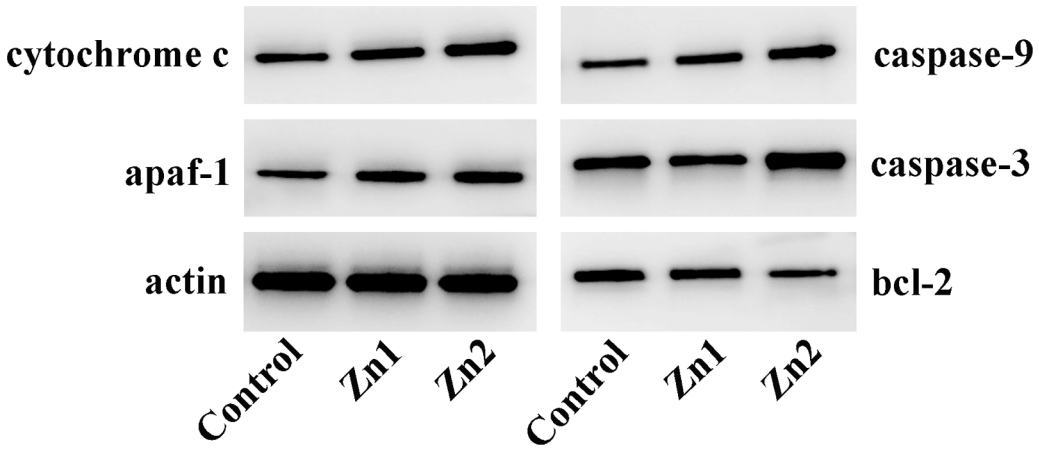


Fig. 6. Expression of five apoptosis-related proteins in T-24 cells treated with cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) for 24 h.

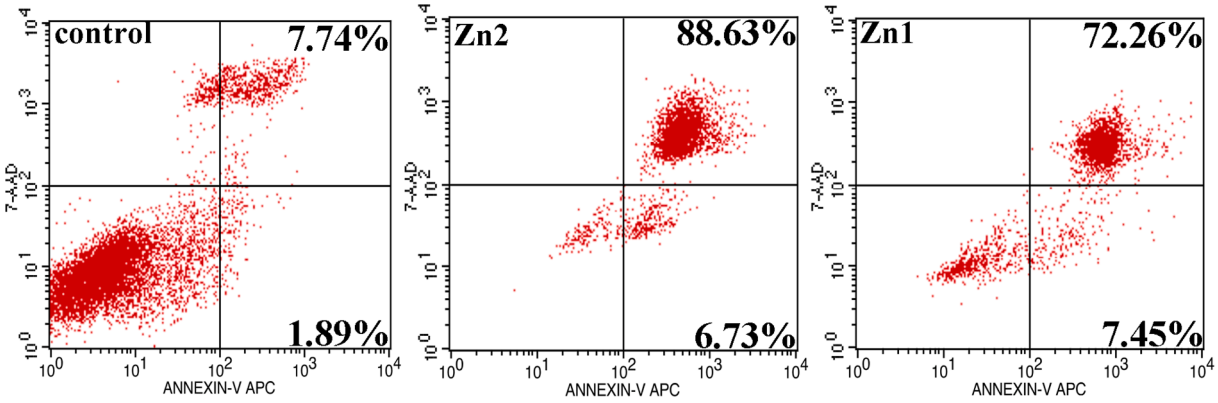


Fig. 7. T-24 cells treated with cryptolepine complexes **Zn1** (0.10 μM) and **Zn2** (0.03 μM) for 24 h and measured by flow cytometry.

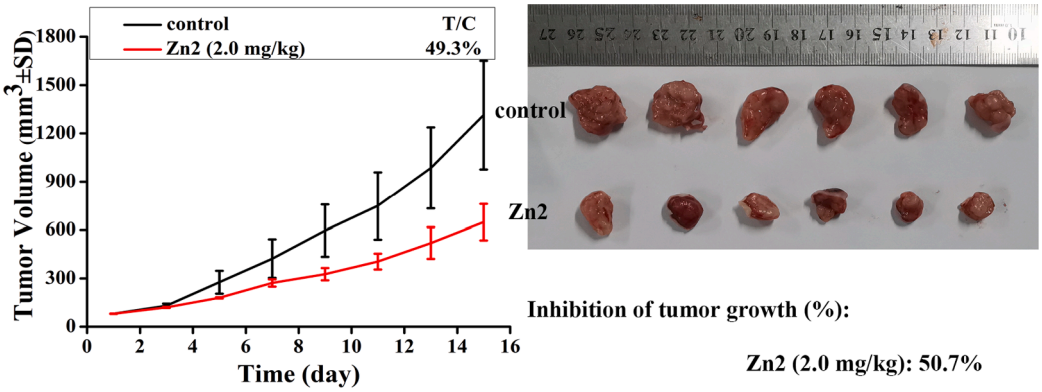


Fig. 8. Tumor volumes and image formation of T-24 xenografts after treatment with the cryptolepine complex **Zn2** (2.0 mg/kg) *via* percutaneous injection every 2 days (q2d). $p < 0.01$, compound-treated group vs. control.

increased the Ca^{2+} and ROS levels, further disturbing mitochondrial function. Further, the probe **Zn2** showed appreciable fluorescence in the red region, indicating effective (50.7%) inhibition of T-24 tumor growth *in vivo*. These results can be used to design new probe Zn drugs containing cryptolepine-H-Curc for mitochondrial targeting as anticancer drugs.

4. Experimental

4.1. Synthesis of ClQ compound

Synthesis and characterization of **ClQ** were performed as described in our previous study.[70]

4.2. Synthesis and characterization of BPQA ligand

ClQ (500 mg, 1.97 mmol, 1.00 eq) was mixed with phenol (5.00 mL) at 25 °C and stirred at 60 °C for 30 min. Compound **5** (432 mg, 2.17

mmol, 1.10 eq) was added dropwise into the mixture and stirred at 120 °C for 16 h. Thin-layer chromatography (petroleum ether: ethyl acetate = 1:1, product Rf = 0.35) indicated that the reactant was consumed completely and that two new species were formed. Finally, the mixture was added to ethyl acetate (50.0 mL) and extracted three times with aqueous NaOH (30.0 mL), followed by drying over anhydrous Na_2SO_4. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate = from 3:1 to 0:1) and evaporated to obtain the desired **BPQA** product (67.3% yield, 99.4% purity). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, J = 8.2 Hz, 1H), 8.49 (d, J = 4.9 Hz, 2H), 8.36–8.25 (m, 2H), 7.72 (t, J = 7.7 Hz, 1H), 7.60 (br dd, J = 7.5, 12.1 Hz, 2H), 7.56–7.49 (m, 3H), 7.46–7.35 (m, 3H), 7.09 (dd, J = 5.5, 6.8 Hz, 2H), 4.96 (s, 4H). ^{13}C NMR: (101 MHz, $CDCl_3$) δ 158.70, 158.23, 149.40, 147.90, 142.56, 136.60, 136.51, 130.55, 129.75, 127.93, 125.38, 124.63, 124.19, 123.42, 123.10, 122.21 (d, J = 3.7 Hz, 1C), 122.13, 112.12, 59.21. ESI-MS m/z: 417.1 $[M + H]^+$. Elemental analysis calcd (%) for $C_{27}H_{20}N_4O$: C 77.87, H 4.84, and N 13.45; found: C 77.86, H 4.86, and N 13.44.

4.3. Synthesis of [Zn(BPQA)Cl$_2$] (Zn1)

The mononuclear Zn complex [Zn(BPQA)Cl_2] (**Zn1**) was prepared by reacting $ZnCl_2$ with BPQA in CH_3OH (5.0 mL) at 65.0 °C for 24 h (yield: 93.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.65 (dd, J = 8.5, 1.4 Hz, 1H), 8.47 (d, J = 4.5 Hz, 2H), 8.22 (dd, J = 24.4, 8.0 Hz, 2H), 7.78 (ddd, J = 8.3, 6.6, 1.4 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.72–7.67 (m, 4H), 7.49 (t, J = 7.3 Hz, 1H), 7.45 (d, J = 7.8 Hz, 2H), 7.22 (dd, J = 7.5, 4.8 Hz, 2H), 4.90 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 158.49, 158.04, 149.52, 147.76, 147.36, 142.50, 137.37, 136.46, 131.63, 129.87, 128.55, 126.01, 124.76, 124.63, 124.33, 123.04, 122.98, 122.79, 122.23, 112.78, 59.01, 40.49, 40.32, 40.15, 39.99, 39.82, 39.65, 39.48. ESI-MS m/z: 514.6 $[M-Cl]^+$. Elemental analysis calcd (%) for $C_{27}H_{20}Cl_2N_4OZn$: C 58.67, H 3.65, and N 10.14; found: C 58.66, H 3.68, and N 10.13.

4.4. Preparation of [Zn(BPQA)(Curc)]Cl (Zn2)

The reactions of **Zn1** with H-Curc ligand in the presence of triethylamine (0.1 mL) and methanol (3.0 mL) at 80.0 °C for 24 h afforded [Zn(BPQA)(Curc)]Cl (**Zn2**) in 88.1% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (d, J = 8.4 Hz, 2H), 8.42 (d, J = 4.8 Hz, 3H), 8.25 (d, J = 7.6 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H), 7.79–7.64 (m, 9H), 7.52–7.39 (m, 6H), 7.28 (s, 1H), 7.20 (dd, J = 7.5, 4.8 Hz, 3H), 7.09 (d, J = 7.4 Hz, 1H), 6.79 (d, J = 8.3 Hz, 2H), 6.67 (d, J = 17.9 Hz, 2H), 4.87 (s, 6H). ESI-MS m/z: 848.95 $[M-Cl]^+$. Elemental analysis calcd (%) for $C_{48}H_{39}N_4O_7Zn$: C 67.89, H 4.63, and N 6.60; found: C 67.88, H 4.64, and N 6.58.

4.5. Synthesis and characterization of PQA ligand

ClQ (2.50 g, 9.85 mmol, 1.00 eq) was added to phenol (20.0 mL) at 25 °C. The mixture was stirred at 60 °C for 30 min and compound **6** (1.17 g, 10.8 mmol, 1.11 mL, 1.10 eq) was added dropwise into the mixture. The mixture was stirred at 120 °C for 16 h. Thin-layer chromatography (petroleum ether: ethyl acetate = 1:1, product Rf = 0.47) indicated that the reactant was consumed completely, and two new spots had formed. Finally, the mixture was added to ethyl acetate (50.0 mL) and extracted three times with aqueous NaOH (30.0 mL), pH 13, and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate = 3:1 to 1:2) and evaporated to obtain the desired product. Compound **PQA** (80.8% yield, 97.8% purity) was obtained as a light-yellow solid and evaluated by liquid chromatography-mass spectrometry, ^{1}H NMR and ^{13}C NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 4.6 Hz, 1H), 8.36 (d, J = 7.7 Hz, 1H), 8.24–8.06 (m, 2H), 7.76–7.64 (m, 2H), 7.59 (d, J = 3.7 Hz, 2H), 7.50 (dt, J = 1.1, 7.6 Hz, 1H), 7.46–7.39 (m, 2H), 7.26 (s, 1H), 6.79 (br s, 1H), 5.38 (d, J = 4.9 Hz, 2H). ^{13}C NMR: (101 MHz, $CDCl_3$) δ 158.30, 156.88, 149.08, 147.18 (d, J = 15.4 Hz, 1C), 136.76, 134.06 (d, J = 3.7 Hz, 1C), 130.29–129.35 (m, 1C), 127.92, 124.27–123.23 (m, 1C), 123.03, 122.47, 122.01 (d, J = 24.9 Hz, 1C), 120.51, 118.28, 111.88, 49.48. ESI-MS m/z: 326.1 $[M + H]^+$. Elemental analysis calcd (%) for $C_{21}H_{15}N_3O$: C 77.52, H 4.65, and N 12.91; found: C 77.51, H 4.67, and N 12.90.

4.6. Synthesis of [Zn(PQA)Cl$_2$] (Zn3)

The mononuclear **Zn3** complex was prepared by reacting $ZnCl_2$ with BPQA in CH_3OH (5.0 mL) at 65.0 °C for 24 h (yield: 80.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58–8.52 (m, 2H), 8.21 (d, J = 7.7 Hz, 1H), 8.02 (d, J = 8.5 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.74 (td, J = 7.7, 1.8 Hz, 1H), 7.70–7.65 (m, 1H), 7.65–7.57 (m, 2H), 7.47 (dq, J = 7.7, 4.3 Hz, 2H), 7.26 (dd, J = 7.4, 4.9 Hz, 1H), 5.32 (d, J = 6.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 159.55, 157.61, 149.53, 149.50, 137.49, 137.47, 132.82, 131.54, 130.02, 126.62, 124.57, 124.17, 123.13, 122.76, 122.33, 121.43, 117.79, 112.78, 50.04, 40.47, 40.30, 40.14, 39.97, 39.80, 39.64, 39.47. ESI-MS m/z: 460.1 $[M-H-(H_2O)]^-$. Elemental analysis calcd (%) for $C_{21}H_{17}Cl_2N_3OZn$: C 52.58, H 3.57, and N 8.76; found: C 52.56, H 3.60, and N 8.75.

4.7. Preparation of [Zn(PQA)(Curc)]Cl (Zn4)

In addition, the reactions of **Zn3** with H-Curc ligand in the presence of triethylamine (0.1 mL) and methanol (3.0 mL) at 80.0 °C for 24 h afforded **Zn4** in 83.9% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.44 (s, 2H), 8.52 (s, 1H), 8.49 (d, J = 8.2 Hz, 1H), 8.17 (d, J = 7.5 Hz, 1H), 8.03 (d, J = 8.2 Hz, 1H), 7.98 (s, 1H), 7.69 (t, J = 7.1 Hz, 2H), 7.60 (q, J = 8.0 Hz, 2H), 7.54–7.50 (m, 1H), 7.48–7.38 (m, 5H), 7.27 (s, 2H), 7.23–7.21 (m, 1H), 7.08 (s, 2H), 6.80 (s, 2H), 5.66 (s, 2H), 5.26 (d, J = 6.3 Hz, 2H), 2.61 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 160.33, 157.75, 149.47, 149.11, 148.99, 148.37, 147.26, 146.55, 137.30, 135.46, 133.18, 130.66, 129.53, 128.45, 127.48, 127.40, 123.93, 123.73, 123.40, 122.87, 122.72, 122.53, 121.96, 121.13, 118.55, 116.11, 112.52, 111.43, 103.59, 90.00, 56.08, 49.99, 40.49, 40.32, 40.15, 39.99, 39.82, 39.65, 39.49. ESI-MS m/z: 790.20 $[M + H]^+$. Elemental analysis calcd (%) for $C_{42}H_{34}ClN_3O_7Zn$: C 63.57, H 4.32, and N 5.30; found: C 63.55, H 4.34, and N 5.29.

4.8. Other materials and methods

The materials and methods used to prepare cryptolepine complexes **Zn1**–**Zn4** were similar to those described in our previous work[57,70,82]. In addition, the detailed experimental methods for evaluating the *in vitro* and *in vivo* anticancer activities of cryptolepine complexes **Zn1**–**Zn4** were described in the Electronic Supporting Information Materials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21867017), the basic skills improvement project for the young and middle-aged teachers in Guangxi colleges and universities (No. 2020KY14010) and the Natural Science Foundation of Guangxi (2018GXNSFBA138021).

Appendix A. Supplementary material

The supporting information contains the detailed procedures for the experimental methods and anticancer activities of BPQA, H-Curc, PQA,

$ZnCl_2$, cisplatin, **Zn1**–**Zn4**, as well as the ESI-MS, UV-Vis, IR, and NMR data, in the online version at https://doi.org/10.1016/j.bmc.2020.115948.

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