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Imaging and therapeutic applications of Zn(II)-cryptolepine-curcumin molecular probes in cell apoptosis detection and photodynamic therapy

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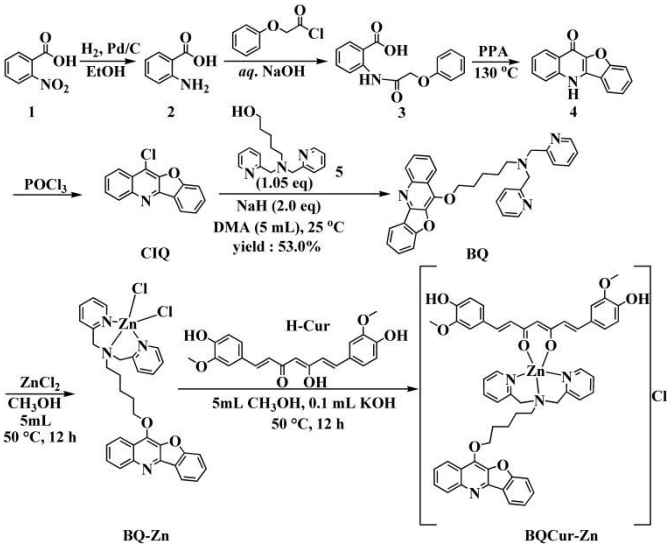
Novel red Zn(II) complex-based fluorescent probes featuring cryptolepine-curcumin derivatives, namely, [Zn(BQ)Cl$_2$] (BQ-Zn) and [Zn(BQ)(Cur)]Cl (BQCur-Zn), were developed for the simple and fluorescent label-free detection of apoptosis, an important biological process. The probes could synergistically promote mitochondrion-mediated apoptosis and enhance tumor therapeutic effects *in vitro* and *vivo*.

Pt-based medication has been successful in combating different types of malignancies.[1] However, the development of anticancer drugs, particularly cisplatin and its derivatives, is hindered by several drawbacks.[1] Thus, non-Pt-based derivatives, such as Zn(II) complexes,[2,3] have attracted increased research attention.[2,3] Zn is an essential element responsible for the activity of numerous biological systems.[2,3] A new family of Zn(II) complexes has been widely explored for application to photodynamic therapy (PDT),[3,4] cell bioimaging, and biosensing.[2–4] These complexes include quinolone-phthalocyanine Zn(II) derivatives, polyethylene glycol Zn(II) phthalocyanine complexes, and Zn(II)−thiosemicarbazone complexes.[2–4]

The modes of function of traditional Chinese medicines (TCMs) and their metal compounds in activating and/or inhibiting DNA repair, G4 DNA, caspase-3/9, telomerase, and DNA topoisomerase have recently been verified.[5–8] Curcumin (H-Cur), a natural product isolated from turmeric,[9] has emerged as an anticancer drug and antiangiogenic agent.[9] However, the clinical use of H-cur and its metal compounds as anticancer agents has yielded limited success.[9] Cryptolepine is a naturally occurring quindoline alkaloid[10] that is extensively used in antimalarial therapy.[10] The synthesis of Zn(II) complexes with mixed ligands could enhance their cytotoxicity; to date, however, the design of Zn(II) complexes with cryptolepine derivatives (BQ) or mixed H-Cur and BQ chelating ligands has not been reported.

Herein, we report the first examples of cryptolepine–H-cur Zn(II) complex-based fluorescent probes featuring H-Cur and BQ chelating ligands, namely, [Zn(BQ)Cl$_2$] (**BQ-Zn**) and [Zn(BQ)(Cur)]Cl (**BQCur-Zn**) and investigate their antiproliferative activity against human bladder (T-24) tumor cells *in vitro and vivo*.



Scheme 1. Chemical structure and synthesis routes of **BQ-Zn** and **BQCur-Zn**.

The cryptolepine ligand ([5-(benzo[4,5]furo[3,2-b]quinolin-11-yloxy)-pentyl]-bis-pyridin-2-ylmethyl-amine, BQ) was first designed and synthesized from 2-nitrobenzoic acid *via* the synthetic route shown in Scheme 1. [Zn(BQ)Cl$_2$] (**BQ-Zn**) was prepared by treating ZnCl$_2$ with BQ in 5.0 mL of CH$_3$OH at 50 °C (yield: 71.9%). The reactions between **BQ-Zn** and H-Cur were carried out by dissolution in 0.1 mL of KOH solution (0.1 M)

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and 5.0 mL of CH_3OH at a 1:1 molar ratio to obtain [Zn(BQ)(Cur)]Cl (**BQCur-Zn**) (yield: 88.3%). Characterizations and stability of BQ and its complexes **BQ-Zn** and **BQCur-Zn** are presented in Figs. S1–S18. The Zn(II) centers of **BQ-Zn** and **BQCur-Zn** exhibited a distorted five-coordinate tetragonal-pyramidal geometry (Scheme 1). To the best of our knowledge, this work is the first to synthesize Zn(II)-cryptolepine-H-cur molecular probes that can function as mitochondrion-targeting anti-cancer drugs.

Table 1. IC_{50} (μM) of **BQ-Zn** and **BQCur-Zn** toward human cells after 24.0 h of incubation

	T-24	SKDDP	MCF-7	HL-7702
BQ	25.23±1.15	62.09±1.09	20.13±1.93	>100
BQ-Zn	5.92±0.35	6.08±0.39	5.25±0.53	>100
BQCur-Zn	1.55±0.04	1.93±0.46	1.80±0.10	>100
H-Cur	20.52±1.11	28.01±1.96	19.02±1.04	>50
$ZnCl_2$	>100	>100	>100	>100
cisplatin	12.56±1.00	79.49±0.13	11.24±0.47	17.02±0.76

The toxicity of **BQ-Zn** and **BQCur-Zn** to human T-24, MCF-7, SK-OV-3/DDP (SKDDP), and HL-7702 cells (Table 1) was tested. MTT assay revealed that **BQ-Zn** and **BQCur-Zn** possess significant cytotoxicity due to the chelation of H-Cur and BQ ligands with Zn(II). As expected, **BQCur-Zn** exhibited higher cytotoxicity toward the four cancer cells, particularly T-24 cells, compared with **BQ-Zn** or cisplatin after 24.0 h and 48 h of treatment. The cytotoxicity of **BQCur-Zn** was 3.8- and 8.1-fold those of **BQ-Zn** and cisplatin, respectively. However, **BQ-Zn** and **BQCur-Zn** did not display such obvious increase in the MTT assay form 24 to 48 h (Table S1), suggesting that 24 h was chosen as incubation. In addition, **BQ-Zn** and **BQCur-Zn** showed weak cytotoxicity (IC_{50}>100 μM) to HL-7702 cells.

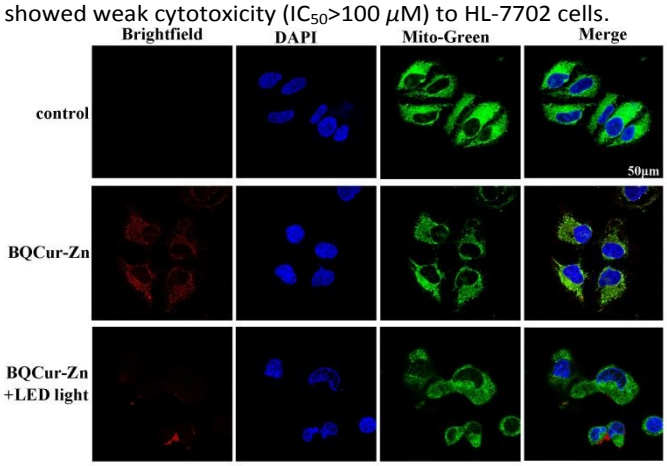

Fig. 1. Imaging of T-24 cells incubated with **BQCur-Zn** (0.26 μM, λ_{ex}= 535 nm, λ_{em}= 610–620nm) for 24.0 h and added with MitoTracker Green (100 nM, λ_{ex}= 470 nm, λ_{em} = 510–530 nm). T-24 cells were treated with **BQCur-Zn** (1.55 μM) for 4.0 h, kept in the dark or irradiated for 30.0 min with 470 nm LED light (22.5 mW/cm^2), incubated for 20.0 h in the dark.

The UV–vis spectroscopy and fluorescence titration analysis showed that **BQCur-Zn** displayed appreciable fluorescence in the red region (ca. 600±10 nm) of the visible spectrum under ambient conditions upon excitation at 470 and 535 nm (Figs. S18 and S19). Next, the cellular localization behaviors of **BQCur-Zn** (1.55 μM) and **BQ-Zn** (5.92 μM) were investigated by confocal microscopy (Zeiss 710).[1] Clear confocal microscopic images of T-24 cells treated with **BQCur-Zn** (1.55 μM) were obtained at λ_{ex} 535 nm (λ_{em}= 610–620nm); thus, the red probe may be used specifically for mitochondrial imaging (Fig. 1). Moreover, substantial accumulation of **BQCur-Zn** occurred within 24.0 h after treatment (Fig. 1), thus indicating a loss in mitochondrial membrane potential (MMP)[11] and accretion of the probe within the mitochondrial membrane (Fig. 1). In addition, **BQCur-Zn** could synergistically strengthen MMP loss and MMP was almost completely destroyed during PDT (Fig. 1). By contrast, **BQ-Zn** (5.92 μM) did not show such obvious effects. Thus, the results suggest that the toxicity mechanism of **BQCur-Zn** (1.55 μM) is distinct from that of **BQ-Zn** (5.92 μM) and other previously reported TCM metal complexes.[5–10]

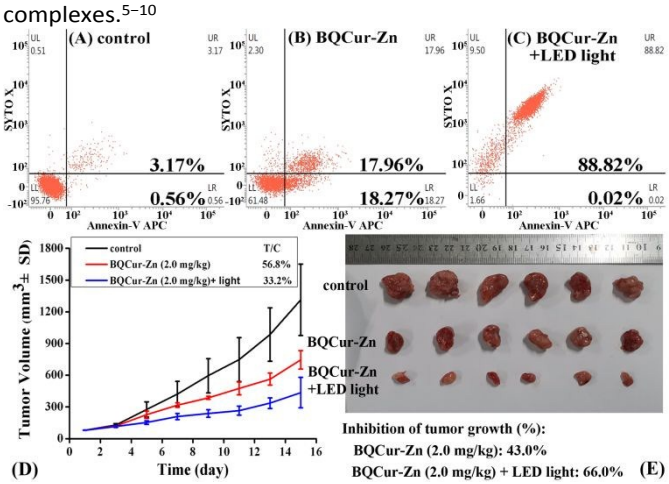

Fig. 2. BQCur-Zn induced T-24 cell apoptosis. (A) T-24 cells were treated with **BQCur-Zn** (1.55 μM) for 4.0 h, (B) kept in the dark or (C) irradiated for 30.0 min with 470 nm LED light (22.5 mW/cm^2), incubated for 20.0 h in the dark, and then stained with Annexin-V APC and SYTO X. Changes in (D) tumors and (E) images after treatment with vehicle controland **BQCur-Zn** (2.0 mg/kg) *via* percutaneous injection every 2 days (q2d). The tumor fractions were kept in the dark or irradiated for 30.0 min with 470 nm LED light (22.5 mW/cm^2) and incubated for the next injection in the dark.

After 4.0 h of incubation with different concentrations of **BQCur-Zn**, PDT was performed for 30.0 min using a LED light source (λ_{ex}= 470 nm, λ_{em}= 610–650 nm).[11i] After another 20.0 h, cell viability was measured by MTT assay. The cytotoxicity of **BQCur-Zn** to light-irradiated cells was significantly enhanced (IC_{50}= 0.03±0.01 μM) relative to that to dark-treated cells (Fig. S20). Apoptosis of T-24 cells stained with Annexin-V APC and SYTO X (Fig. 2A-C), treated with **BQCur-Zn** (1.55 μM) in the dark, and irradiated with light was visualized immediately by flow cytometry. As shown in Fig. S21 (ESI†), only light irradiation (without **BQCur-Zn** addition) showed negligible effects on the apoptosis of T-24 cells. This result is in good agreement with the low cytotoxicity of light. The population of

T-24 cell apoptosis was 88.84% in the light-irradiated group was higher than that in the dark-treated group (36.23%). Finally, we tested the efficacy of **BQCur-Zn** (2.0 mg/kg) in inhibiting tumor growth in the dark and under light irradiation using an *in vivo* animal model. **BQCur-Zn** showed greater delays in tumor growth (tumor inhibition rate, IR=66.0%) in the light-irradiated group than in the dark-treated group (IR=43.0%) and in groups treated with cisplatin (IR=37.1%) and previously reported TCM metal complexes.[5–10] We also found that model mice injected with **BQCur-Zn** do not exhibit significant body weight loss (Tables S2–S4 and Fig. 2C and D, ESI†), which suggests that **BQCur-Zn** has low systematic toxicity.

The ability of **BQCur-Zn** (1.55 μM) to confer mitochondrial dysfunction was studied by using Western blot. Upon irradiation by a LED source (λ_{ex} = 470 nm, λ_{em} = 610–650 nm)[11i] for 30 min, clear changes in apoptosis-associated proteins (e.g., bcl-2, bax, cytochrome c, apaf-1, and caspase-9/-3) relative to those in dark-treated groups were observed. This result confirms that **BQCur-Zn** (1.55 μM) can damage mitochondria more extensively under light irradiation than under dark treatment (Fig. 3).

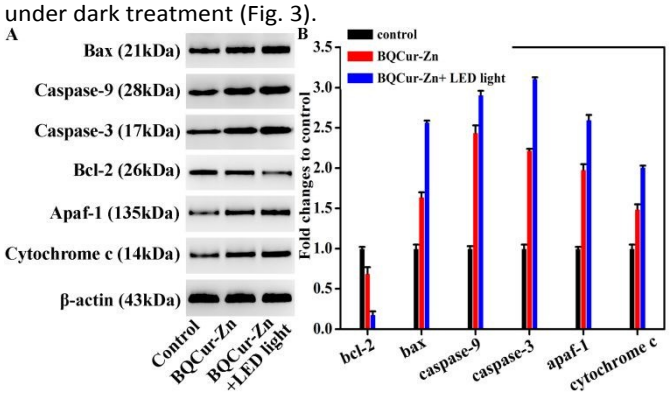

Fig. 3. Apoptosis-related protein levels in T-24 cells incubated with **BQCur-Zn**. T-24 cells were treated with **BQCur-Zn** (1.55 μM) for 4.0 h, kept in the dark or irradiated for 30.0 min with 470 nm LED light (22.5 mW/cm^2), and incubated for 20.0 h in the dark.

In summary, two new red fluorescent probes based on Zn(II) complexes with cryptolepine-H-cur derivatives and featuring mitochondrial membrane-targeting abilities were designed. Upon visible light irradiation, **BQCur-Zn** could synergistically strengthen mitochondrion-mediated apoptosis and enhance tumor therapeutic effects *in vitro* and *in vivo* during PDT. We believe that **BQCur-Zn** molecular probes have potential applications in cell apoptosis detection and PDT.

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