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An effective invasive therapeutic approach of fluoro-substituted zinc phthalocyanine derivatives as potential photosensitizer for prostate carcinoma

Tamer E. Youssef[a,b,*], **Suhailah S. Al-Jameel**[a], **Wafa M. Al-Magribi**[a]

[a] Department of Chemistry, College of Science, Imam Abdulrahman Bin Faisal University, Dammam 31441 Saudi Arabia.
[b] Basic and Applied Scientific Research Center, Imam Abdulrahman Bin Faisal University, Dammam 31441 Saudi Arabia.

*Corresponding author, e-mail: temoustafa@iau.edu.sa

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ABSTRACT: Fluoro-substituted zinc(II)phthalocyanines $(RS)_4$ZnPcs were prepared. All the structures of newly synthesized compounds was evaluated by IR and ^{1}H NMR spectral analysis. They were tested against human adenocarcinoma prostate cancer cells. A type II membrane antigen highly expressed in prostate cancer, namely prostate-specific membrane antigen, has been an attractive target for imaging and therapy. To investigate the structure-activity relationships of $(RS)_4$ZnPcs 4a–c in human adenocarcinoma prostate cancer cell model, 3 fluoro-substituted zinc(II)phthalocyanines with different terminal heteroaromatic rings have been designed and evaluated for their anti-proliferative potency *in vitro*. The detailed LD50 values of the targeted compounds were reported. Our preliminary *in vitro* studies confirm that $(RS)_4$ZnPcs 4a–c could act as an attractive photosensitizer for the early diagnosis of prostate cancer.

KEYWORDS: zinc phthalocyanine, cancer therapy, prostate cancer cell line

INTRODUCTION

Phthalocyanines (Pcs) – especially, metallophthalocyanines bearing an aluminum, zinc, indium, or silicon as a central metal atom – are excellent photosensitizers (PSs) (second generation) for photodynamic therapy (PDT) in several types of tumors [1]. They offer effective properties for an ideal PS [2]. They are absorbed in the red and near infrared regions of the visible spectrum [3]. In addition, Pcs have high photo and chemical stability [4].

Zinc phthalocyanines (ZnPcs) are valuable PSs [5–10]. When they functionalized with heterocycles units such as 4-pyridylmethyloxy and pyridyloxy groups [11, 12], adamantylethoxy zinc phthalocyanines [13], hexadecafluoro zinc phthalocyanine [14], tetracarboxy zinc phthalocyanine [15] with pentalysine peptidyl moiety (ZnPc-$(Lys)_5$) [16]. A number of cell lines [17–19] showed the efficiency of zinc phthalocyanines as photosensitizers as a result of their excellent fluorescence quantum yields [20, 21]. Recent advances in drug-delivery caused by zinc phthalocyanines are commonly used in cancer treatment with an additional benefit including the enhancement of drug-therapeutic efficiency. It enhances the pharmacological properties by altering pharmacokinetics. In addition, it improves the drug hydro-solubility and drug half-life [22]. Prostate cancer is the second highest cancer mortality in American men. There are 238 590 new cases of prostate cancer examined. Also, 720 men died due to the prostate cancer in the United States in 2013. The local radiotherapy, radical prostatectomy, chemotherapy, or hormonotherapy is used in treating localized prostate cancer [23].

Prostate-specific membrane antigen (PSMA) is a membrane-bound glycoprotein. It presents in the human prostate adenocarcinoma cell line from hormone-refractory patients [24]. In addition, PSMA is a talented target for treatment of prostate cancer [25]. Previously, Liu et al [26] reported PSMA inhibitors for targeted PDT *in vitro*. Watanabe et al [27] reported recently effective PSMA-targeted photoimmunotherapy. It targets both full antibodies and antibody fragments. Synthesized fluorinated compounds such as steroids containing 5-Fluorouracil revealed high potential therapeutic effect with implications to biological activities [28].

In this sense, zinc phthalocyanines substituted

with fluorine atoms are becoming the most appealing answer to solve chemotherapy problems such as degradation and nonspecific toxicity [29]. In addition, Chen et al [30] used a PSMA-targeted Lys-Glu-Lys urea based theragnostic agent for prostate cancer imaging and PDT. Previously, our group has described series of phthalocyanines with their antitumor activity [31–34]. In the present work, the zinc(II) phthalocyanines carrying trifluoromethyl groups have been prepared. Their biological screening results have been described.

MATERIALS AND METHODS

Materials

Fluoro-substituted zinc(II) phthalocyanines, $(RS)_4$-ZnPcs, were prepared previously from their thiophenyl phthalonitriles derivatives: 3a–c obtained from 2a–c, 4-methylthiophenol (2a), 4-(trifluoromethyl)thiophenol (2b), and 3,5-bis (trifluoromethyl)thiophenol (2c) as described by Youssef et al [34]. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from the Sigma-Aldrich Co. All the other chemicals were of analytical grade and were used without further purification.

Biological screening

In vitro assay: cell culture

In brief, all *in vitro* antitumor screening on human adenocarcinoma prostate cancer cells (American Type Culture Collection) has been performed at National Research Centre, Cairo-Egypt. Human adenocarcinoma prostate cancer cell line was obtained from America Type Culture Collection (ATCC) through VACSERA, Cairo, Egypt. Cells were cultured in Roswell Park Memorial Institute medium, RPMI-1640 (Sigma St. Lous, USA). Cells were always incubated at 36 °C in a humidified atmosphere containing 5% CO_2 and subcultured twice a week. For normal transformed cell line, a similar process was followed [35], and the raw data was filtered to remove erroneous entries.

Statistical analysis

The experiment values used in statistical analysis were means ± SD and repeated more than 3 times. A SPSS 10.0 software program (Student's unpaired two-tailed t-test) was used to calculate the differences in the mean values of the measured activities statistically. The probability values of $p < 0.01$ were statistically significant.

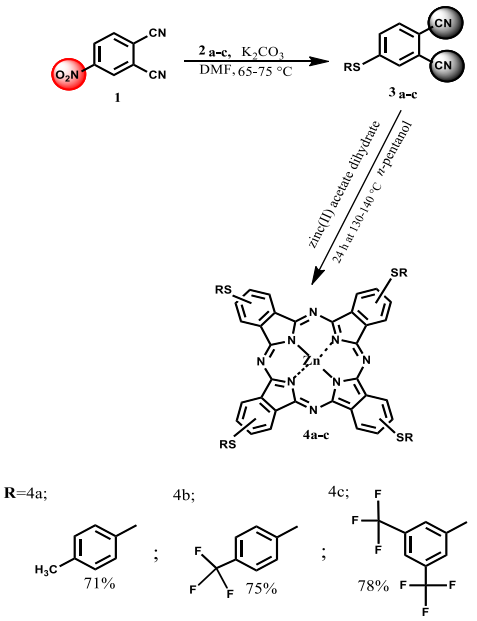

Fig. 1 Reaction pathways of zinc(II) phthalocyanines (4a–c).

RESULTS AND DICUSSION

Chemistry

Zinc(II) phthalocyanines (4a–c) were synthesized from their thiophenyl phthalonitrile derivatives (3a–c) as described previously by Youssef et al [34] with 78% of the pure phthalonitrile (3a), 70% (3b) and 62% (3c). The general synthetic scheme is shown in Fig. 1 to afford the corresponding 4a–c with 71% (4a), 75% (4b), and 78% (4c) yields.

3a–c precursors were formed with bands at ν = 2235–2233 cm^{-1} (CN) and (SH stretch) at 2595–2596 cm^{-1} indicated by the FT-IR spectra. The protons of the methyl protons of phtalonitrile 3a at δ = 1.44 (s) ppm and phenyl protons of phtalonitrile 3a at 8.31–8.42 (m) ppm were indicated with the ^{1}H NMR spectra and showed non-aggregated spectra. UV-Vis spectroscopy was performed in DMF with constant concentration at λ_{max} (nm) [(10^{-5} log ε dm^3 mol^{-1} cm^{-1})]. The characteristic Q absorption bands of zinc(II) phthalocyanines (4a–c) with extinction coefficient at around 691(5.51), 686(4.9), and 681(5.16) nm, respectively, (Fig. 2) showed that these compounds are non-aggregated under these aqueous conditions.

In vitro anti-prostate cancer

In vitro cytotoxicities of the synthesized Zn(II) phthalocyanine $(RS)_4$ZnPc derivatives were deter-

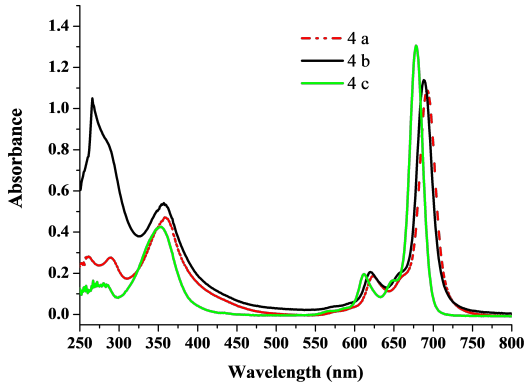

Fig. 2 UV-vis of zinc(II) phthalocyanines (4a–c).

Table 1 IC_{50}, KM of zinc(II) phthalocyanines (4a–c) against adenocarcinoma prostate cells.

Compound no.	Cytotoxicity[a] (IC_{50}, μm) PSMA
ZnPc 4a	26.2
ZnPc 4b	32.7
ZnPc 4c	7.13
Doxorubicin	7.05

[a] IC_{50}: ZnPc 50%. Values of 3 repeated experiments.

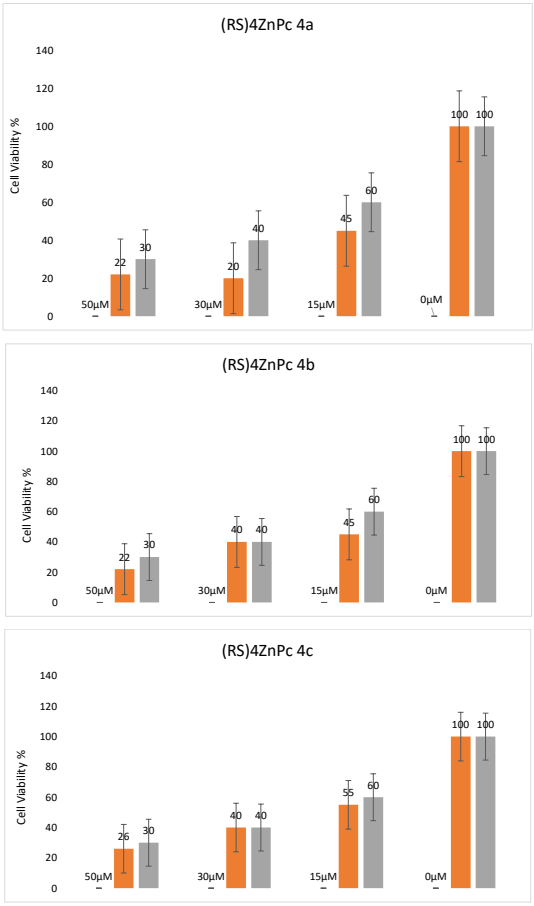

Fig. 3 The proliferation of adenocarcinoma prostate cells (orange bar) and normal cells (grey bar) at different concentrations of $(RS)_4$ZnPcs 4a–c.

mined by performing prostate cancer cell viability assays. The ability of the Zn(II) phthalocyanines to inhibit growth of human adenocarcinoma prostate cancer cell lines was measured. A doxorubicin HCl was used as a reference drug for human adenocarcinoma prostate cancer cells using MTT assay method [36, 37]. Table 1 shows the results of LC_{50} (μm) as the lethal concentration of $(RS)_4$ZnPc derivatives to cause death of 50% of the cells in 24 h.

To study the effect of zinc(II) phthalocyanines (4a–c) on tumor cell line, namely human adenocarcinoma prostate cancer cells, they were compared with normal human fibroblast healthy cells using MTT assay as shown in Fig. 3. All the tested compounds were found to have potent anti-prostate cancer activities compared to normal cells. They did not exhibit any toxicity against adenocarcinoma prostate cells in the absence of $(RS)_4$ZnPcs 4a–c. The structure-activity data acquired indicated that the presence of trifluoromethyl groups (CF_3) constitutes a promising design novel zinc(II) phthalocyanines with promising cytotoxicity. Previously results with the above method indicated that meta (trifluoromethyl) substituted zinc(II) phthalocyanine is more effective than the corresponding para compound [34]. In comparison with our work, the bis meta (trifluoromethyl) substituted zinc(II) phthalocyanine compound 4c showed higher activities compared to those of para compound 4b, and the order in the antitumor effect is 4c > 4b > 4a.

Our work describes the majority of zinc(II) phthalocyanine compounds which are typically common compounds present in most pharmaceuticals. They are intrinsically versatile and have unique physicochemical properties. They showed activity against human adenocarcinoma prostate cancer cells with IC_{50} values 26.2, 32.7 and 7.13 μm, respectively, as described in Table 1. All the tested compounds exhibited significant cytotoxicity in human adenocarcinoma prostate cancer.

Imaging has the highest sensitivity for detecting the prostate cancer, in accordance with recent observational study of 925 patients who underwent radiation therapy [38]. Few studies have described

cases of men with prostate cancer with hypogonadal serum testosterone levels (<250 ng/dl) [39]. Recent studies for chemotherapy dosing recommend the use of body surface area (BSA). Only older patients are expected to be affected significantly with more toxicity from anticancer therapies. It also tends to be under-represented in clinical trials.

Considering preliminary results and the structure – activity study, 4c that contains 8 trifluoromethyl groups (CF_3) has the most active antitumor activity against human adenocarcinoma prostate cancer cell line (7.13 μm). In case of 4a, a decrease in the potency against the human adenocarcinoma prostate cancer cell was observed due to the absence of trifluoromethyl groups. The most potent compounds (4b, and 4c) showed impressive cytotoxicity against human adenocarcinoma prostate cancer cell line. It was found 4c that was effective against human adenocarcinoma prostate cancer cell line. This current study involves *in vitro* studies because many of the *in vivo* challenges have not been completely resolved yet. We demonstrated *in vitro* that zinc(II) phthalocyanines 4a–c are effective "cell-killing" agents. They could reach regions deep in the body and be a safe clinical approach.

CONCLUSION

Zinc(II) phthalocyanines 4a–c have been synthesized and characterized. The synthesized compounds 4a–c were evaluated for *in vitro* anticancer activity. They have activity against human adenocarcinoma prostate cancer cells. The trifluoromethyl groups present at zinc(II) phtalocyanine 4c has the highest potent activity against the tested cancer cell line as shown in MTT cytotoxicity studies. The structural activity study provided good indication for cancer activity. In human adenocarcinoma prostate cancer cell line, the order in the antitumor effect is 4c > 4b > 4a. Taken together, selective enhancement of cell death in aggressive prostate cancer cell line suggests that zinc(II) phtalocyanines 4a–c are promising potential compounds. Additional research is needed on mechanism study.

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