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

Tamer E. Youssef\textsuperscript{a,b,*}, Suhailah S. Al-Jameel\textsuperscript{a}, Wafa M. Al-Magribi\textsuperscript{a}

\textsuperscript{a} Department of Chemistry, College of Science, Imam Abdulrahman Bin Faisal University, Dammam 31441 Saudi Arabia.
\textsuperscript{b} Basic and Applied Scientific Research Center, Imam Abdulrahman Bin Faisal University, Dammam 31441 Saudi Arabia.
\textsuperscript{*}Corresponding author, e-mail: temoustafa@iau.edu.sa

Received 14 Jun 2020
Accepted 30 Sep 2020

ABSTRACT: Fluoro-substituted zinc(II)phthalocyanines (RS)\textsubscript{4}ZnPcs were prepared. All the structures of newly synthesized compounds was evaluated by IR and \textsuperscript{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)\textsubscript{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 \textit{in vitro}. The detailed LD50 values of the targeted compounds were reported. Our preliminary \textit{in vitro} studies confirm that (RS)\textsubscript{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 \cite{1}. They offer effective properties for an ideal PS \cite{2}. They are absorbed in the red and near infrared regions of the visible spectrum \cite{3}. In addition, Pcs have high photo and chemical stability \cite{4}.

Zinc phthalocyanines (ZnPcs) are valuable PSs \cite{5–10}. When they functionalized with heterocycles units such as 4-pyridylmethyloxy and pyridoxy groups \cite{11,12}, adamantylethoxy zinc phthalocyanines \cite{13}, hexadecafluoro zinc phthalocyanine \cite{14}, tetracarboxy zinc phthalocyanine \cite{15} with pentalysine peptidyl moiety (ZnPc-(Lys)\textsubscript{5}) \cite{16}. A number of cell lines \cite{17–19} showed the efficiency of zinc phthalocyanines as photosensitizers as a result of their excellent fluorescence quantum yields \cite{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 \cite{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 hormone therapy is used in treating localized prostate cancer \cite{23}.

Prostate-specific membrane antigen (PSMA) is a membrane-bound glycoprotein. It presents in the human prostate adenocarcinoma cell line from hormone-refractory patients \cite{24}. In addition, PSMA is a talented target for treatment of prostate cancer \cite{25}. Previously, Liu et al \cite{26} reported PSMA inhibitors for targeted PDT \textit{in vitro}. Watanabe et al \cite{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 \cite{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. Louis, 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.

RESULTS AND DISCUSSION

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 $\nu = 2235$–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 phthalonitrile 3a at $\delta = 1.44$ (s) ppm and phenyl protons of phthalonitrile 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 $\lambda_{\text{max}}$ (nm) of $[10^{-5} \log \epsilon \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}]$. 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-
Table 1 IC\textsubscript{50}, KM of zinc(II) phthalocyanines (4a–c) against adenocarcinoma prostate cells.

| Compound no. | Cytotoxicity\textsuperscript{a} (IC\textsubscript{50}, \textmu m) PSMA |
|--------------|-------------------------------------------------|
| ZnPc 4a      | 26.2                                            |
| ZnPc 4b      | 32.7                                            |
| ZnPc 4c      | 7.13                                            |
| Doxorubicin  | 7.05                                            |

\textsuperscript{a} IC\textsubscript{50}: ZnPc 50%. Values of 3 repeated experiments.

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

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)\textsubscript{4}ZnPcs 4a–c. The structure-activity data acquired indicated that the presence of trifluoromethyl groups (CF\textsubscript{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 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\textsubscript{50} values 26.2, 32.7 and 7.13 \textmu m, 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 (CF₃) 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) phthalocyanine 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) phthalocyanines 4a-c are promising potential compounds. Additional research is needed on mechanism study.

**Acknowledgements:** The authors would like to thank Department of Pharmacognosy, National Research Center (NRC), Cairo, Egypt, for performing the anticancer activity testing and MTT cytotoxicity assay of the synthesized compounds.

### REFERENCES

1. Yoon I, Li JZ, Shim YK (2013) Advance in photosensitizers and light delivery for photodynamic therapy. *Clin Endosc* **46**, 7–13.
2. Newago M, Constantin C, Tampa M, Matei C, Lupu A, Manole E, Ion RM, Fenga C, et al (2016) Toxicological and efficacy assessment of post-transition metal (Indium) phthalocyanine for photodynamic therapy in neuroblastoma. *Oncotarget* 7, 69718–69726.
3. Gamal-Eldeen AM, Moustafa D, El-Daly SM, El-Hussieny EA (2016) Photothermal therapy mediated by gum Arabic-conjugated gold nanoparticles suppresses liver preneoplastic lesions in mice. *J Photochem Photobiol B* **163**, 47–56.
4. Soriano J, Villanueva A, Stockert JC, Cañete M (2013) *Histochem Cell Biol* **139**, 149–153.
5. Liu JY, Jiang XJ, Fong WP, Ng DK (2008) Highly phototoxic 1,4-dipegylated zinc(II) phthalocyanines. Effects of the chain length on the *in vitro* photodynamic activities. *Org Biomol Chem* **6**, 4560–4566.
6. Liu JY, Lo PC, Fong, Ng DK (2009) Effects of the number and position of the substituents on the *in vitro* photodynamic activities of glucosylated zinc(II) phthalocyanines. *Org Biomol Chem* **7**, 1583–1591.
7. Rodríguez ME, Diz VE, Arwuch J, Dicelio LE (2010) Photophysics of zinc (II) phthalocyanine polymer and gel formulation. *Photochem Photobiol* **86**, 513–519.
8. Lo PC, Zhao B, Duan W, Fong WP, Ko WH, Ng DK (2007) Synthesis and *in vitro* photodynamic activity of mono-substituted amphiphilic zinc(II) phthalocyanines. *Bioorg Med Chem Lett* **17**, 1073–1076.
9. Choi CF, Huang JD, Lo PC, Fong WP, Ng DK (2008) Glycosylated zinc(II) phthalocyanines as efficient photosensitisers for photodynamic therapy. Synthesis, physicochemical properties and in vitro photodynamic activity. *Org Biomol Chem* **6**, 2173–2181.
10. Souza JG, Gelfuso GM, Simao PS, Borges AC, López RF (2011) Iontophoretic transport of zinc phthalocyanine tetralsulfonic acid as a tool to improve drug topical delivery. *Anticancer Drugs* **22**, 783–790.
11. Scalise I, Durantini EN (2005) Synthesis, properties, and photodynamic inactivation of Escherichia coli using a cationic and a noncharged Zn(II) pyridoxophthalocyanine derivatives. *Bioorg Med Chem* **13**, 3037–3043.
12. Byrykloglu Z, Durmus M, Kantekin H (2010) Synthesis, photophysical and photochemical properties of quinoline substituted zinc(II) phthalocyanines and their quaternized derivatives. *J Photochem Photobiol A Chem* **211**, 32–41.
13. Ochoa AL, Tempesti TC, Spesia MB, Milanesio ME, Durantini EN (2011) Synthesis and photodynamic properties of adamantlylhexyloxy Zn(II) phthalocyanine derivatives in different media and in human red blood cells. *Eur J Org Chem* **50**, 280–287.
14. Allémann E, Rousseau J, Brasseur N, Kudrevich SV, Lewis K, van Lier JE (1996) Photodynamic therapy of tumours with hexadecafluoro zinc phthalocyanine formulated in PEGcoated poly(lactic) nanoparticles. *Int J Cancer* 66, 821–824.

15. Chen J, Chen H, Li Y, Wang J, Chen N (2008) Synthesis and photodynamic activity of a new type of pentalysine 2-carboxylphthalocyanine zinc. *Chem Res Chin Univ* 29, 2131–2137.

16. Chen, Z, Zhou SY, Chen JC, Deng YC, Luo ZP (2010) Synthesis and photodynamic activity of a new type of phthalocyanine-loaded dendrimer for targeted photodynamic therapy. *Chem Med Chem* 5, 890–898.

17. Wang A, Long L, Zhang C (2011) Synthesis and properties of photo-activatable phthalocyanines: a brief overview. *J Incl Phenom Macro Chem* 71, 1–24.

18. Sekkat N, Bergh H, Nyokong T, Lange N (2011) Like a bolt from the blue: Phthalocyanines in biomedical optics. *Molecules*, 17, 98–144.

19. Zorlu Y, Ermeydan MA, Dumoulin E, Ahsen V, Savoie H (2009) Glycerol and galactose substituted zinc phthalocyanines: Synthesis and photodynamic activity. *Photochem Photobiol Sci* 8, 312–319.

20. Iqbal Z, Chen J, Chen Z, Huang M (2015) Phthalocyanine-biomolecule conjugated photosensitizers for targeted photodynamic therapy and imaging. *Curr Drug Metab* 16, 816–832.

21. Taratula O, Schumann C, Naleway MA, Pang AJ, Chon KJ (2013) A multifunctional theranostic platform based on phthalocyanine-loaded dendrimer for image-guided drug delivery and photodynamic therapy. *Mol Pharmacol* 10, 3946–3952.

22. Mouo-Tynga I, Houréld NN, Abrahamse H (2013) Evaluation of cell damage induced by irradiated zincphthalocyanine-gold dendrimeric nanoparticles in a breast cancer cell line. *biomed J* 41, 254–259.

23. Samaana N, Zhongb Q, Fernandeza J, Chena G, Hussaina AM, Zhengc S, Wangb G, Chena Q-H (2014) Design, synthesis, and evaluation of novel heteroaromatic analogs of curcumin as anti-cancer agents. *Eur J Med Chem* 75, 123–131.

24. Wang X, Ma D, Olson WC, Heston WD (2011) *In vitro* and *in vivo* responses of advanced prostate tumors to PSMA ADC, an auristatin-conjugated antibody to prostate-specific membrane antigen. *Mol Cancer Ther* 10, 1728–1739.

25. Kuroda k, Liu H, Kim S, Guo M, Navarro V, Bander NH (2010) Saporin toxin-conjugated monoclonal antibody targeting prostate-specific membrane antigen has potent anticancer activity. *Prostate* 70, 1286–1292.

26. Liu T, Wu LY, Choi JK, Berkman CE (2010) Effectiveness of oral iron to manage anemia in long-term hemodialysis patients with the use of ultrapure dialyse. *Int J Oncol* 36, 777–783.

27. Watanabe R, Hanaoka H, Paik CH, Wu AM, Choyke PL, Kobayashi H (2015) Photoimmunotherapy targeting prostate-specific membrane antigen: Are antibody fragments as effective as antibodies. *J Nucl Med* 56, 140–147.

28. Kirk KL (2006) Selective fluorination in drug design and development: an overview of biochemical ratiornales. *Curr Top Med Chem* 6, 1447–1455.

29. Martins P, Jesus j, Santos S, Raposo LR, Romarodrigues C, Baptista PV, Fernandes AR (2015) Heterocyclic anticancer compounds: Recent advances and the paradigm shift towards the use of nanomedicine's tool box. *Molecules* 20, 16852–16891.

30. Chen Y, Chatterjee S, Lisok A, Minn I, Pullambhatla M, Wharram B, Wang Y, Jin J, et al (2017) A PSMA-targeted theranostic agent for photodynamic therapy. *J Photochem Photobiol B Biol* 167, 111–116.

31. Youssef TE, ALhamed YA, AL-Sharani SS, Ali KA (2014) Antitumor activity of tetra-substituted zinc phthalocyanines containing 4(3H)-quinazolinone derivatives. *Rev Chim (Bucharest)* 65, 5–11.

32. Elsharif AM, Youssef TE (2019) Synthesis of an activatable tetra-substituted nickel phthalocyanines-4(3H)-quinazolinone conjugate and its antibacterial activity. *Adv Pharmacochem Sci* 2019, ID 5964687.

33. Fadeel DA, Al-Toukhly GM, Elsharif AM, Al-Jameel SS, Mohamed HH, Youssef TE (2018) Improved photodynamic efficacy of thiophenyl sulfonated zinc phthalocyanine loaded in lipid nano-carriers for hepatocellular carcinoma cancer cells. *Photodiagnosis Photodyn Ther* 23, 25–31.

34. Al Jameel SS, Youssef TE (2018) Investigations on the antitumor activity of classical trifluorosubstituted zinc phthalocyanines derivatives. *World J Microbiol Biotechnol* 34, 1–7.

35. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 65, 55–62.

36. Barbora M, Svatopluk B, Lukas M, Jana J, Katerina LL, Hana K (2015) Phthalocyanine-mediated Photodynamic Treatment of Tumoural and Non-tumoural cell lines. *Anticancer Res* 35, 3943–3949.

37. Muehlmann LA, Ma BC, Figueiro Longo JP, de Fátima MM, Santos A, Azevedo R (2014) Aluminum-phthalocyanine chloride associated to poly(methyl vinyl ether-co-maleic anhydride) nanoparticles as a new third-generation photosensitizer for anticancer photodynamic therapy. *Int J Nanomedicine* 9, 1199–1206.

38. Fossati N, Karnes RJ, Colicchia M (2018) Impact of early salvage radiation therapy in patients with persistently elevated or rising prostate-specific antigen after radical prostatectomy. *Eur Urol* 73, 436–444.

39. Agrawal L, Arceo-Mendoza RM, Barnosky A (2016) Prevalence of hypogonadism in low-risk prostate cancer survivors. *Fed Pract* 33, 37–43.