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Improved photodynamic efficacy of thiophenyl sulfonated zinc phthalocyanine loaded in lipid nano-carriers for hepatocellular carcinoma cancer cells

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ABSTRACT

Background: The aim of the present study was to modify the structural activity of zinc(II)phthalocyanine by combining it with thiophenyl groups then loaded in lipid nano-carriers and evaluate its parameters required for the structure-activity relationship (SAR) for photodynamic therapy (PDT) of cancer.
Methods: Tetra (4–Thiophenyl) sulfonated phthalocyaninatozinc(II) (PhS·$SO_3Na)_4$ZnPc **5** was synthesized and characterized by various spectroscopic methods as a test compound. Liver hepatocellular carcinoma (HepG2) cells were treated with the synthesized (PhS·$SO_3Na)_4$ZnPc **5** derivative loaded in lipid nano carriers to understand the effect of combined compound on liver cancer cells. Furthermore, HepG2 cells were irradiated by visible red light at 60 mW/cm^2 for 20 min. The phototoxicity of (PhS·$SO_3Na)_4$ZnPc **5** after being formulated in both (L) and transfersomes (T) was investigated.
Results: Overall, the results indicate that combination of thiophenyl groups substitution, in particular in the structure of sulfonated zinc phthalocyanine is able to improve the photodynamic properties of ZnPc, and (PhS·$SO_3Na)_4$ZnPc **5** loaded in lipid nano-carriers can be a promising combined PDT treatment strategy for Liver hepatocellular carcinoma (HepG2) cells.
Conclusions: The new formulation ZnPc-lipid nano-carriers will be beneficial in the upcoming clinical trials and would enhance the inhibition of tumor growth.

1. Introduction

Photodynamic therapy (PDT) is a modality for malignant and non-malignant disorders that has attracted great interest due to its high degree of specificity and selectivity [1–3].

In the literature, Photofrin and hematoporphyrin derivatives are used as photosensitizers (PSs) in a various types of cancers [4]. Some are clinically approved by the Food and Drug Administration 'FDA' for the treatment of cancer [5]. However, they absorb light at relatively short wavelength (630 nm); in addition, they suffer many disadvantages due to their poor selectivity towards tumors and persistent skin photosensitization [6].

These disadvantages have encouraged researchers to focus on research around the synthesis and testing of Phthalocyanines (Pcs) as second generation PSs, which have been developed to overcome the problems encountered with photofrin [7], due to their intense absorption in the visible red region and higher efficacy in producing singlet oxygen [8].

Phthalocyanines i.e zinc, aluminum and silicone pthalocyanines, may induce direct destruction of the tissue, vasculature damage, inflammatory changes and/or immune response. They have proved to have a high degree of selective accumulation in the tumor tissues, so upon local irradiation, the toxic effect could be confined mainly to the tumor cells saving the healthy ones, and are superior to those typical of Photophrin [9].

Recent studies showed the efficiency of Zn(II) phthalocyanines as photosensitisers for a number of cell lines, and in vivo experiments, due to their excellent fluorescence quantum yields, and acceptable biocompatibility [10].

Recently, Youssef et al. [11] have described novel symmetrical

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series of zinc phthalocyanines with quinazolinone ring system units, in addition to another series of tetra-substituted zinc(II)phthalocyanines were also reported and were screened for their in-vitro antitumor activity on Human lung adenocarcinoma, human breast adenocarcinoma and hepatocellular carcinoma in comparison with healthy normal cells (human fibroblast cells) with some limitations [12]. These limitations of zinc phthalocyanines may be overcome by structural modifications and water-solubility, making them more suitable for PDT. So far, sulfonation of zinc phthalocyanine leads to the reduction of singlet oxygen generation [13].

New hydrophilic Pc derivatives are continually synthesized to improve and optimize their photosensitizing properties. They have better in vivo distribution and bioavailability, however, they have poor cell uptake and penetration. Moreover, hydrophilic drugs are not good candidate for transdermal or topical delivery as they can't penetrate the sctatum cornum (outer most layer of the epidermis) [14]. The pharmacokinetics of hydrophilic photosensitizers can be improved by incorporating them in vesicular nanosystems, particularly liposomes. These nanosystems can be passively accumulated in tumor cells because tumor cells are characterized by leaky vasculature and poor lymphatic drainage, a phenomenon known as enhanced retention and permeation effect (EPR effect) [15]. Liposomes composed of one or more concentric lipid bilayers, and drug molecules can be intercalated into the lipid bilayer [16].

Though liposomes are reported to be very effective as drug delivery systems, their usefulness in topical or transdermal delivery is limited as they haven't the ability to penetrate the stratum cornum effectively [17]. Incorporating an edge activator (as sodium deoxy cholate) in the liposomal membrane has proved to enhance the elasticity and deformability of the vesicles, thus enhancing their skin penetration. These elastic liposomes are called transfersomes or ultradeformable liposomes [18,19].

The present work is devoted to the successful synthesis of new thiophenyl sulfonated zinc phthalocyanine $(PhS.SO_3Na)_4ZnPc$ **5** as photosensitizer to evaluate its efficacy towards photodynamic therapy of cancer after loading in lipid nano-carriers eg.: liposomes and transfersomes. To the best of our knowledge, this is the first report aiming at modifying the structural activity of zinc(II)phthalocyanine by combining it with thiophenyl groups then loaded in lipid nano-carriers and evaluates its parameters required for the structure-activity relationship (SAR) for photodynamic therapy (PDT) of cancer.

The role of thiophenyl groups substitution in sulfonated zinc phthalocyanine $(PhS.SO_3Na)_4ZnPc$ **5** was discussed. The biological screening results obtained for the newly $(PhS.SO_3Na)_4ZnPc$ **5** showed a promising anticancer activity in vitro HEPG2 cell line.

2. Materials and methods

2.1. Chemistry

The chemicals were with the highest purity and were used without further purification. Dimethylformamide (DMF) (99.9%), 4-nitrophthalonitrile **1** (99.99%) and benzene thiol (≥98%, FG) were purchased from Sigma-Aldrich. The thiophenylphthalonitrile (**3**) was synthesized as reported in our previous work [12].Thin layer chromatography (TLC) plates 250 μm were purchased from Analtech (USA). Phospholipid from soybean oil (SPC, Mwt = 750), cholesterol 95% and Sodium deoxycholate (SDC) were purchased from Sigma-Aldrich. Chloroform, methanol, ethanol, all of analytical grade, were purchased from El Nasr Pharmaceutical Chemicals Co. (Adwic, Egypt).

2.2. Instrument and physical characterizations

The microwave oven utilized for heating was a Discover Lab Mate single-mode microwave cavity from CEM Corporation. The reactions were conducted in a 25 mL Schlenk tube, with a maximum operating temperature of 180 °C and a maximum operating pressure of 8 bar. Melting points were determined by the open capillary method and were uncorrected. Infrared spectra were recorded on a Nicolet Magna 560 spectrophotometer in the spectral range 4000–400 cm^{-1} using KBr pellets. ^1H NMR spectra were recorded using a BVT 3000 Bruker Spectro spin instrument operating at 300.13 MHz. Spectra were referenced internally to residual solvent (DMSO). UV–vis spectra were recorded using an Agilent 8453 UV–vis spectrophotometer with Dimethyl Formamide (DMF) used as solvent. Field depolarization mass spectroscopy technique (FDMS) mass spectra were recorded using a Varian MAT 711A spectrometer, operated at 70 eV for using the electron ionization technique (EIMS) and reported in mass/charge (*m/z*). Elementary analyses were performed on Carlo Erba Elemental Analyzer 1106. Purity of all synthesized compounds were checked by TLC on precoated silica gel plates utilizing chloroform/methanol in different ratios (8:2/7:3 v/v) as developing solvent system and spots were detected on exposure to UV lamp.

2.3. Synthesis of test compound

2.3.1. Synthesis of 4-Thiophenylphthalonitrile *3*

4-nitrophthalonitrile (**1**) (1 g, 5.98 mmol) was dissolved in DMF (10 mL) under nitrogen and thiophenol (**2**) (0.5 g, 4 mmol) was added, then stirred at room temperature for 30 min, an anhydrous potassium carbonate finely powdered (286 mg, 2 mmol) was added in portions during 3 h under stirring followed by stirring again at 70 °C for 24 h. The residue was poured into 100 mL cold water. It was extracted with ethyl acetate (three portions of 30 mL). The extract washed with water and dried. The crude product was recrystallized from dichloromethane to give **3** as a white crystalline solid (1.42 g, 79.5%). Mp 150–153 °C.

IR (KBr): ν = 3081(w), 3055 (s) (Ar–CH), 2955 (s), 2930 (w), 2851 (m), 2230 (CN), 1592 (C-N; C-C), 1541 (s), 1477 (s), 1440 (m), 1323 (m), 1251 (C-S-C), 1179 (s), 1133 (s), 1071 (s), 940 (s), 891 (m), 776 (m), 544 (m) cm^{-1}. ^1H NMR (DMSO-d6): σ = 8.2–8.5 (3H, m, H-arom), 8.7–8.9 (5H, m, ph) ppm. MS (EI): *m/z* (%) 236.29 (90) (M^+). Anal. Calcd. (%) for $C_{14}H_8N_2S$: C 71.16, H 3.51, N 11.75 (Found C 70.82, H 3.10, N 10.79).

2.3.2. Synthesis of tetra(4–Thiophenyl)phthalocyaninatozinc(II), $(PhS)_4ZnPc$ *4*

A solution of 4-Thiophenylphthalonitrile **3** and zinc (II) acetate tetrahydrate (1.2 g, 5 mmol) in 10 mL of dimethylaminoethanol (DMAE) was stirred for 15 min under argon atmosphere. Then, DBU (5 mL, 0.05 mmol) was added. The mixture was refluxed for 20 h at 135–140 °C. Followed by cooling at room temperature, and then precipitated with methanol (30 mL). Then the solid was filtered off, washed with water and dried under vacuum. The crude product was purified by column chromatography (silica gel, ethyl acetate/n-hexane) in different ratios (8:2/9:1 v/v) yielding **4** as a green solid (8.67 mg, 68.5%).

IR(KBr): ν = 3077–3070 (Ar-H), 2978, 1664 (C-C); 1590, 1579, 1475 (C–CH), 1419 mP h, 1416, 1258 (C-S-C), 865,753, 747,649, 527 cm^{-1}. ^1H NMR (DMSO-d6): σ = 8.3–8.6(m, 4H, Pc-H), 8.6–8.8 (m, 8H, Pc-H), 9.1–9.3 (20H, mph) ppm. UV–vis (DMF):? $?_{max}$ (nm): 689,622, 352 s h, 259 nm. MS (FD): *m/z* = 1014.59 (M^+). Elemental analysis: $C_{56}H_{36}N_8S_4Zn$, Found C 65.73, H 3.60, N 10.81. Anal. Calcd. C 66.29, H 3.58, N 11.04.

2.3.3. Synthesis of tetra (4–Thiophenyl) sulfonated phthalocyaninatozinc (II), $(PhS.SO_3Na)_4$ ZnPc *5*

Tetra (4–Thiophenyl)phthalocyaninatozinc(II) **4** (1.0 g) and 10 mL of fuming sulfuric acid (25%) were added into a quartz tube with a magnetic stirring bar. Then the quartz tube was moved into the MW reactor, and the reaction parameters of time and temperature were set up. The reaction temperature was first raised to 55 °C within 10 min and maintained for 10 min and was then raised to 120 °C in 3 min and