Antitumor Activity of Tetra-Substituted Zinc Phthalocyanines Containing 4(3H)-Quinazolinone Derivatives

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Series of tetra-substituted zinc(II)phthalocyanines (ZnPcs) bearing four 4(3H)-quinazolinone ring system units (qz) ZnPcs 4a-c have been synthesized and characterized. They were screened for their in-vitro antitumor activity on Human lung adenocarcinoma (A549), human breast adenocarcinoma (MCF-7) and Hepatocellular carcinoma (HEPG2). Preliminary study of the structure–activity relationship showed that electronic factors in the 4(3H)-quinazolinone moiety that attached to the ZnPc skeleton had a magnificent effect on the antitumor activity of the newly synthesized (qz) ZnPcs 4a-c. They showed promising anticancer activity against the tested human cancer cell lines. The detailed synthesis, characterization and biological screening data were reported.

Keywords: 4(3H)-quinazolinone, zinc(II)phthalocyanines, hetero-substituted phthalocyanines, anticancer activity, biological targeting agents.

Different series of Zinc phthalocyanines (ZnPcs) were used as valuable photosensitizers for photodynamic therapy of cancer (PDT) [1-5]. Until now, great variety of ZnPc derivatives functionalized with substituted heterocycles such as pyridoxyl, 4-pyridylmethylxyl [6] and oxyquinoline groups [7] have been offered potent PDT properties. Another derivatives of ZnPcs with multiple functional groups have been received attention as anticancer agents due to their photophysical and photochemical properties (e.g., tetracarboxylic zinc phthalocyanine[8], pentalysine peptidyl moiety(ZnPc-(Lys)5) [9], hexadecafluoro zinc phthalocyanine[10] and adamantylethoxy zinc phthalocyanines) [11].

Unfortunately, limitations of PDT of cancer have been found in previous studies [12]. It was shown that the ZnPc photosensitiser drugs require cellular oxygen to kill cancer cells via the formation of singlet oxygen. Moreover, they worked only on the area which was exposed to light. Therefore, when cancer spread in the body, it cannot be treated completely. In this regard, new hypox has been studied in the present work dealing with the combinations of chemotherapy of ZnPcs and 4(3H)-quinazolinone derivatives (which will most likely result in the enhance of their anticancer efficiency for the treatment of metastasized and localized cancers). 4(3H)-Quinazolinones are known to possess interesting drugs with diverse biological activities. They were used to modify the biological properties of several other compounds. The major effective biological activities and pharmacological properties of their derivatives include: anti-inflammatory activity [13-15], sedative [16], antimalarial [17], CNS depressant[18] analgesic [19], anticonvulsant [20], antidiabetic [21,22], antitubercular and antibacterial effects [23], antihypertensive [24], antiviral [25] and cancer chemotherapy [26-28]. The value of the 4(3H)-quinazolinone ring system derivatives as antitumor agents in drug design was also recognized [29-33]. Additionally, some researchers have reported the importance of different quinazolinone derivatives with potent antitumor activities such as, quinazolinones bearing thioureido[34] or thiazolidinone [35,36].

Earlier, Youssef et al. [37-42] have described novel symmetrically and asymmetrically Pcs with differently peripheral substitution and axial ligands for potential applications. The authors have also described the synthesis of NiPcs bearing heterocyclic moieties for pharmaceutical application [43]. In connection with a previous work and our current interest in the synthesis of organic compounds for biological evaluations [44-49]. We described herein a facile convenient synthesis of novel tetra substituted zinc phthalocyanines based on heterocyclic moiety (i.e. 4(3H)-quinazolinone ring system, (qz) ZnPcs 4a-c). To the best of our knowledge, this is the first report which aims to modify the structural activity of zinc(II)phthalocyanines by combining them with four 4(3H)-quinazolinone units and evaluates their parameters required for the structure-function relationship for cancer therapy. The biological screening results obtained for the newly (qz) ZnPcs 4a-c showed a promising anticancer activity in vitro.

Experimental part

Materials and methods

All reagents and solvents were commercial reagent grade and used without further purification. The following chemicals were purchased commercially from Aldrich and used as received: 4-nitrophthalonitrile, 2-Methyl-4(3H)-quinazolinone, 2-phenyl-4(3H)-quinazolinone and 2-mercapto-4(3H)-quinazolinone. Solvents (GR grade) from Merck (Darmstadt, Germany) were distilled. Silica gel thin-layer chromatography (TLC) plates 250 microns from Analtech (Newark, DE, USA) were used.

Physical characterizations

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

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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 sulfoxide (DMSO) used as solvent. Field desorption mass spectrometry 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.

**Typical procedure for synthesis of 4(3H)-quinazolinone-phthalonitrile precursors 3a-c**

A mixture of quinazoline derivative 1a-c (4 mg, 2.50 mmol) 1a, (5.5 mg, 2.5 mmol) 1b, (4.4 mg, 2.5 mmol) 1c and 4-nitrophthalonitrile (4 mg, 2.5 mmol) was dissolved in dry DMF (100 mL). After stirring for 20 min at room temperature, a finely grounded anhydrous K2CO3 (excess) was added and the mixture was refluxed for 24 h at 70-80°C. After cooling to room temperature, the reaction mixture was poured into ice-water. The mixture was filtered and washed with water and dried under vacuum. The crude products were purified by column chromatography (silica gel, chloroform/methanol in different ratios (8:2/7:3 v/v)) yielding 5.5 mg (76%) of the pure phthalonitrile 3a, 6.3 mg (73%) of 3b and 5.4 mg (72%) of 3c.

**Synthesis of 2-Methyl-4(3H)-quinazolinone-phthalonitrile 3a**

Prepared from 2-Methyl-4(3H)-quinazoline (1a) as a white solid; m.p. 290-292°C; IR (KBr): ν = 3070-3068 (Ar-H str), 2969, 2870 (C-H str, CH3), 2598 (SH str), 2229 (CN str), 1678 (C=O str, quz ring), 1473 (C–CH); 1441 mPh, 1409, 859, 744, 747 d(C–C), 645, 522 cm-1. 1H-NMR (DMSO-d6): δ = 7.1-7.3 (16H, m, Ar-H, quinazolinone moiety), 8.20 (1H, dd, 5-H), 8.33 (1H, d, 6-H), 8.41 (1H, s, 3-H) ppm. MO (EI): m/z = 286.29 (M+). Elemental analysis: C65H46N16O4S2, found C 66.97, H 2.87, N 18.87, Calcd. C 67.47, H 3.33, N 18.51.

**Synthesis of 2-Phenyl-4(3H)-quinazolinone-phthalonitrile 3b**

Prepared from 2-phenyl-4(3H)-quinazoline (1b) as a white solid; m.p. 310-322°C; IR (KBr): ν = 3070-3068 (Ar-H str), 2965, 2870 (C-H str, CH3), 2232 (CN str), 1677 (C=O str, quz ring), 1655 (C–C); 1584, 1571, 1479 (C–C); 1442 mPh, 1418, 856, 740, 745 d(C–C), 640, 520 cm-1. 1H-NMR (DMSO-d6): δ = 1.50 (3H, s, CH3–qz), 7.6-7.8 (4H, m, Ar-H, quinazolinone moiety), 8.20 (1H, dd, 5-H), 8.33 (1H, d, 6-H), 8.41 (1H, s, 3-H) ppm. MS (EI): m/z = 268.29 (M+). Elemental analysis: C59H45N16O4S2, found C 65.69, H 3.67, N 19.11, Calcd. C 65.32, H 3.52, N 19.57.

**Typical procedure for synthesis of 4(3H)-quinazolinone-phthalonitrile precursors 3a-c**

A solution of 4(3H)-quinazoline-phthalonitrile derivative 3a-c (5.7 mg, 2.00 mmol) 3a, (6.9 mg, 2.00 mmol) 3b, (6.0 mg, 2.00 mmol) 3c and zinc(I1) acetate dihydrate (0.1 g, 0.55 mmol) in 10 mL of n-pentanol was stirred for 10 min under argon atmosphere. Then, DBU (5 mL, 0.05 mmol) was added and the mixture was refluxed for 2 h at 130-135°C. The reaction mixture was cooled at room temperature and precipitated with methanol (25 mL). The solid was filtered and washed with water and dried under vacuum. The crude products were purified by column chromatography (silica gel, ethyl acetate/n-hexane) in different ratios (7:3 / 8:2 v/v) yielding 18 mg (74%) of the pure PcZn 4a, 21 mg (72%) of 4b, and 18 mg (71%) of 4c.

**Synthesis of Tetra [2-phenyl-4(3H)-quinazolinone-phthalocyaninatozinc(II)] (4b)**

IR (KBr): ν = 3075-3066 (Ar-H, CH2), 1672 (C=O, qz ring), 1655 (C–C); 1580, 1577, 1471 (C–C); 1411 mPh, 1410, 855, 743, 745 d(C–C), 643, 522 cm-1. 1H-NMR (DMSO-d6): δ = 1.30-1.6 (12H, m, CH2–qz), 7.3-7.6 (16H, m, Ar-H, quinazolinone moiety), 8.25 (4H, dd, 5-H), 8.53 (4H, d, 6-H), 8.60 (4H, s, 3-H) ppm. UV-Vis (DMSO): λmax (nm): 692,621, 358 sh, 290 nm. MS (FD): m/z = 1210.55 (M+). Elemental analysis: C83H69N24O4Zn, found C 66.97, H 2.87, N 18.87. Calcd. C 67.47, H 3.33, N 18.51.

**Synthesis of Tetra [2-mercapto-4(3H)-quinazolinone-phthalocyaninatozinc(II)] (4c)**

IR (KBr): ν = 3075-3065 (Ar-H, CH2), 1678 (C=O, qz ring), 1655 (C–C); 1577, 1572, 1473 (C–C); 1441 mPh, 1409, 859, 744, 747 d(C–C), 645, 520 cm-1. 1H-NMR (DMSO-d6): δ = 7.1-7.3 (16H, m, Ar-H, quinazolinone moiety), 8.00 (4H, dd, 5-H), 8.13 (4H, d, 6-H), 8.32 (4H, s, 3-H). 8.4-8.7 (20H, m, ph-qz) ppm. UV-Vis (DMSO): λmax (nm): 687,619, 355 sh, 256 nm. MS (FD): m/z = 1458.83 (M+). Elemental analysis: C83H65N24O4SH2Zn, found C 66.90, H 3.67, N 19.01, Calcd. C 67.47, H 3.33, N 18.51.

**Synthesis of Tetra [2-mercapto-4(3H)-quinazolinone-phthalocyaninatozinc(II)] (4c)**

IR (KBr): ν = 3075-3065 (Ar-H, CH2), 1678 (C=O, qz ring), 1655 (C–C); 1577, 1572, 1473 (C–C); 1441 mPh, 1409, 859, 744, 747 d(C–C), 645, 520 cm-1. 1H-NMR (DMSO-d6): δ = 3.35 (4H, s, SH-qz), 7.3-7.6 (16H, m, Ar-H, quinazolinone moiety), 8.20 (4H, dd, 5-H), 8.33 (4H, d, 6-H), 8.52 (4H, s, 3-H) ppm. UV-Vis (DMSO): λmax (nm): 679,610, 351 sh, 289 nm. MS (FD): m/z = 1282.68 (M+). Elemental analysis: C88H48N16O4Zn, found C 67.47, H 3.32, N 19.57.

**Biological screening n-vitro assay for anti-cancer activity**

The synthesized ZnPcs were supplied to the Bioassay-Cell Culture Laboratory, National Research Centre, Cairo-Egypt for in-vitro antitumor screening on Lung adenocarcinoma (A549), hepatocellular carcinoma (HePG2) and caucasian breast adenocarcinoma (MCF7) (American Type Culture Collection). Cell viability was assessed by the mitochondrial dependent reduction of 2,3-5 triphenyl tetrazolium chloride (MTT) assay.
yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [50,51].

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). HePG2 cell line was cultured in RPMI-1640 and MCF7 cell line was cultured in DMEM. Cells were plated in 96-well plates (having about 10000 cells/well). The plates are then incubated for 24 h in 37°C incubation and 5% CO₂ atmosphere before treatment with the compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO and different concentrations of the compounds under test were added to the cell monolayer. Triplicate wells were prepared for each individual concentration. Then the plate was incubated for 48 h in 37°C incubator. After the completion of compound exposure, 40 μL of MTT solution (2.5 mg/mL) was added into each well for an additional 4h. Formazan was dissolved in 200 μL (10%) Sodium Dodecyl Sulphate and the absorbance at λ = 495 nm was measured. The concentration of DMSO as a solvent for the different compounds was 0.1% in the culture medium used and was without any effect on cell growth. Cell viability at given compound concentration was calculated as the percentage of absorbance in wells with the compound-treated cells to that of vehicle control cells (100 %).

Results and discussions

Chemistry

Improved synthetic procedure for the newly Zn(II) phthalocyanines (qz)ZnPc 4a-c substituted by 4(3H)-quinazolinolone units has been described. They synthesized from their phthalonitrite derivatives 3a-c by a two-step reaction procedure depicted in scheme 1. First, a nucleophilic ipso-nitro substitution reaction of 4-nitrophthalonitrile 2 with 4(3H)-quinazolinolone derivatives 1a-c in dry DMF for 24 h at 70-80°C. Second, the cyclotetramerization reaction of 4(3H)-quinazolino-lone-phthalonitrile precursors 3a-c with Zn(II)acetate in the presence of organic base DBU in n-pentanol for 20 h at 130-135°C, afforded the corresponding (qz)ZnPcs (4a-c) with 71-74% yield. The desired phthalocyanines were separated chromatographically as a mixture of regioisomers from the reaction mixture (scheme 1). The structures of the target compounds were confirmed using IR, 1H NMR spectroscopy and mass spectra. The analyses are consistent with the predicted structures as shown in the experimental section.

The described synthetic method produced a mixture of four regioisomers with a 4(3H)-quinazolinolone units at the 2- or 3-positions of each benzene ring in the (qz)ZnPc molecule. The formation of constitutional isomers [52] and the high-dipole moment that results from the 4(3H)-quinazolinolone units at the periphery positions leads to increase the solubility of the obtained products 4a-c. The IR spectra clearly indicated the formation of 4(3H)-quinazolinolone-phthalonitriles precursors 3a-c with the appearance of absorption bands at ν= 2235-2229 cm⁻¹ (CN) and 1678-1675 C=Ostr, qz ring) and the appearance of (SH stretch) at 2598 cm⁻¹ in case of 3c. In the 1H NMR spectrum of phthalonitrile 3a the methyl protons appeared at δ= 1.50 (m) ppm, but for phthalonitrile 3a the phenyl protons appeared at 8.50 (m) and for phthalonitrile 3c the thiol protons appeared at δ = 3.30 (s) ppm, in addition to their mass spectra were consistent with the proposed structure.

Cyclotetramerization of the 4(3H)-quinazolinolone-phthalonitriles precursors 3a-c to the zinc(II)phthalocyanines (qz)ZnPcs 4a-c were confirmed by the disappearance of the sharp (CN) vibration in their IR spectra. The 1H NMR spectra of tetra-substituted zinc (II)phthalocyanines 4a-c were obtained as expected. The 1H NMR spectrum of (Ph-qz)ZnPc 4a indicated the methyl protons at δ = 1.30-1.6 ppm and the aromatic protons of Pc skeleton at 7.36 ppm. Also, the 1H NMR spectrum of (Ph-qz)ZnPc 4b indicated the aromatic protons of phenyl group at β = 8.4-8.7 ppm. In case of (SH-qz)ZnPc 4c, thiol proton appeared at δ = 3.35 ppm (see experimental part). The electronic spectra of the studied zinc(II)phthalocyanines (qz)ZnPcs 4a-c showed characteristic absorption bands in the Q band region at around 692, 687, and 679 nm, respectively, in
DMSO. The B-bands were observed at around 358, 355, and 351 nm, respectively, (fig. 1). The spectra showed monomeric behavior evidenced by a single (narrow) Q band, typical of metallated phthalocyanine complexes in DMSO [53].

In-vitro anticancer screening

Three ZnPc derivatives namely; 4a–c, were selected to be evaluated for their in vitro cytotoxic effect via the standard Doxorubicin HCl as a reference drug against a panel of three human tumor cell lines namely; hepatocellular carcinoma (HePG2), lung adenocarcinoma (A549), and breast adenocarcinoma (MCF7) using MTT assay method [62,63]. The results are presented in table 1 as LC50 (µM) which is the lethal concentration of the ZnPc which cause death of 50% of the cells in 24 h.

In case of human breast adenocarcinoma (MCF-7), the studied zinc(II)phthalocyanine (SH-qz) ZnPc 4c (IC50 = 3.65 µM) more potent than Doxorubicin (IC50 = 3.78 µM), while 4a (IC50 = 5.22 µM) and 4b (IC50 = 4.53 µM) found to be slightly less effective than the reference drug. In case of hepatocellular carcinoma (HEPG2), the studied zinc(II)phthalocyanine (SH-qz) ZnPc 4c exhibited an excellent activity with IC50 values 3.21 µM, respectively more potent than the reference drug (Doxorubicin, IC50 value 4.61 µM), while compounds 4a and 4b were found to be less effective than the reference drug with IC50 values (5.21 and 21.6 µM), respectively (table 1). The newly synthesized zinc(II) phthalocyanines (qz) ZnPcs 4a–c showed activity against human lung adenocarcinoma (A549) with IC50 values 25.6, 31.5 and 6.05 µM, respectively (table 1), less potent than the reference drug (Doxorubicin, IC50 value 2.61 µM) (fig. 2).

![Fig. 1. The absorption spectra of (qz)4ZnPcs 4a-c in DMSO](image)

![Fig. 2. Anticancer activity (IC₅₀, µM) of the synthesized zinc(II)phthalocyanines (qz)ZnPcs 4a-c against human cancer cell lines (MCF7, HEPG2 and A549)](image)

DMSO. The B-bands were observed at around 358, 355, and 351 nm, respectively, (fig. 1). The spectra showed monomeric behavior evidenced by a single (narrow) Q band, typical of metallated phthalocyanine complexes in DMSO [53].

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**Table 1**

<table>
<thead>
<tr>
<th>Compound NO.</th>
<th>Cytotoxicity a b (IC₅₀, µM)</th>
<th>MCF7</th>
<th>HEPG2</th>
<th>A549</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Me-qz)ZnPc 4a</td>
<td>5.22</td>
<td>5.21</td>
<td>25.6</td>
<td></td>
</tr>
<tr>
<td>(Ph-qz)ZnPc 4b</td>
<td>4.53</td>
<td>21.6</td>
<td>31.5</td>
<td></td>
</tr>
<tr>
<td>(SH-qz)ZnPc 4c</td>
<td>3.65</td>
<td>3.21</td>
<td>6.05</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3.78</td>
<td>4.61</td>
<td>2.61</td>
<td></td>
</tr>
</tbody>
</table>

a IC₅₀ compound concentration required to inhibit tumor cell proliferation by 50%.

b Values are means of three experiments.

Detailed interpretation of the obtained results and considering preliminary structure–activity relationship (SAR) showed that, zinc(II)phthalocyanine (SH-qz) ZnPc 4c that contains four mercapto groups (SH) attached to the 4(3H)-quinazolinone ring system at position 2 in its phthalocyanine skeleton, was the most active member in this study which revealed antitumor activity against MCF-7 and HEPG2 human tumor cell line (3.65 and 3.21 µM, respectively). Replacement of mercapto groups in zinc(II)phthalocyanine (qz) ZnPc 4c with methyl or phenyl groups as in case of 4a & 4b are associated with remarkable decrease in the potency against the two tumors cell lines. So, compound 4c showed promising anticancer activity.

Conclusions

The present study reports the successful synthesis of the title zinc(II)phthalocyanine (qz) ZnPcs 4a–c in good yields via cyclotetramerization reactions of 4(3H)-quinazolinone-phthalonitriles precursors 3a-c. The results indicated that the studied (qz) ZnPcs 4a-c possessed a broad spectrum of activity against liver and breast cancer. Preliminary study of the structure–activity relationship revealed that electronic factors in the 4(3H)-quinazolinone moiety that attached to the phthalocyanine molecule have a great effect on the antitumor activity of these Zinc(II) phthalocyanines. This proves the novelty of biological efficiency of our new (qz) 4ZnPcs 4a-c series. In MTT cytotoxicity studies, the (SH-qz) ZnPc 4c that contains 2-mercapto-4(3H)-quinazolinone group attached to the phthalocyanine molecule were found to possess potent activity against the two tested cancer cell lines. In HEPG2 and MCF-7 cell lines the antitumor effect showed in the
order $4c > 4b > 4a$. The resulted ZnPcs $4a-c$ will be subject to further study on the DNA level to know their mode of actions. In the light of above considerations and from preliminary results the future work will be focused on the synthesis of zinc(II)phthalocyanine derivatives with $4(SH)$-quinazolinone units. In addition, their application in the photodynamic therapy of cancer will be studied.

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