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Article in *Dyes and Pigments* · January 2024

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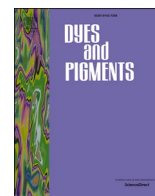


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3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy groups substituted phthalocyanines: Synthesis, characterization and their biological properties

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ARTICLE INFO

Keywords:

Antioxidant
Cytotoxicity
NO production
Phthalocyanine
Synthesis

ABSTRACT

In this study, 3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy group substituted metal-free and metallo-phthalocyanines have been synthesized. The new compounds have been characterized by a combination of several spectroscopic techniques. Using 1,1-diphenyl-2-picrylhydrazine (DPPH), OH radical scavenging, and reducing power tests, the antioxidant properties of these phthalocyanines were assessed. CoPc showed the highest antioxidant activity for DPPH assay, ZnPc and MnPc exhibited the highest and similar antioxidant activity at reducing power assay, ZnPc demonstrated the highest antioxidant activity at OH scavenging activity. By using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, cytotoxicity of phthalocyanines was evaluated, and iNOS mediated nitric oxide (NO) generation was carried out.

1. Introduction

Macrocyclic compounds, which are bioactive molecules, have been used as multifunctional building blocks to create bioactive compounds in medical fields. Due to their distinctive electrical and optical characteristics, as well as their extraordinarily high thermal and chemical stability, phthalocyanines (Pcs) are one of the favored macrocyclic molecules [1–3]. They are extensively researched in many fields such as material science and medicine, including dyes and colorants [4,5], chemical sensors [6], liquid crystal materials [7], catalyst [8–10], photodynamic therapy [11,12], antioxidant [13] and antimicrobial [14] agents. The poor solubility of Pcs limits their use in these areas. By adding different functional groups to the Pc ring or different metal cations to the cavity of the ring, their solubility can be enhanced [15, 16].

Antioxidants are natural or synthetic compounds that lessen or prevent the negative effects of free radicals. Several illnesses, including cancer, diabetes, inflammatory damage, cardiovascular disease, and neurological disorders are brought on by free radicals, which are highly reactive oxygen species [17–19]. In the pharmaceutical and food

sectors, antioxidants' toxicological and biological characteristics, detection, development, and assessment are crucial. In this context, interest in new Pc molecules with low toxicity and antioxidant activity has been increasing, and Pcs have come to the fore with these properties [20, 21]. In addition, Pcs have been found to be effective photosensitizers for the inactivation of microorganism for antimicrobial photodynamic therapy. The efficiency of the Pcs against Gram-positive bacteria has been demonstrated in earlier studies. Nevertheless, the impermeability of the bacteria's outer membrane, which is composed of a coating of lipopolysaccharides, limits its efficacy against Gram-negative bacteria [22,23].

Recently, the combined effects of photodynamic therapy and anti-cancer agents have been widely studied [24,25]. Pcs are promising second-generation photosensitizers for PDR, thanks to their favorable photophysical and photochemical properties. The suppression of macromolecular synthesis, the alteration of energy generation metabolism, and a reduction in DNA synthesis are the causes of phthalocyanines' mode of action [26].

To date, various fluorophenyl or fluoroalkyl groups substituted metallo Pcs (MPcs) have been synthesized and their properties have

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<https://doi.org/10.1016/j.dyepig.2023.111814>

Received 21 September 2023; Received in revised form 27 October 2023; Accepted 6 November 2023

Available online 10 November 2023

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been investigated [27–37]. The solubility of these compounds increases due to the high electronegative properties of the fluorine groups. This study aimed to design new antioxidant compounds. For this reason, we synthesized and characterized a novel phthalonitrile, namely 4-(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy) phthalonitrile, and its metal-free and MPc derivatives. Additionally, antioxidant, cytotoxic and NO production properties of phthalocyanines were examined.

2. Experimental

2.1. Synthesis and characterization

2.1.1. 4-(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy)phthalonitrile (1)

A mixture 2.8 g of 3,3,4,4,5,5,6,6,6-nonafluoro-hexane-1-ol (10.6 mmol) and 1.8 g of 4-nitrophthalonitrile (10.6 mmol) dissolved in 20 mL of dry dimethylformamide. Then, 2.6 g of anhydrous K_2CO_3 (19 mmol) was added to the mixture in portions. For 96 h, the reaction mixture was stirred at 65 °C in a nitrogen atmosphere. Then it was precipitated into 400 mL of the ice-water mixture after being cooled to room temperature. The precipitate was then filtered, neutralized by water washing, and dried in a vacuum. The product was purified on silica gel-packed column chromatography by using DCM as the eluent. Yield: 2.03 g (49 %). Anal. calcd. for $C_{14}H_7F_9N_2O$: C 43.09, H 1.81, N 7.18 %, found: C 43.20, H 1.80, N 7.15 %. FT-IR ν_{max} (cm^{-1}): 3088 (Ar-CH), 2984–2924 (alkyl-CH), 2237 (C \equiv N), 1604, 1309, 1224 (C–O–C), 1130, 999, 847, 723. 1H NMR (500 MHz; $CDCl_3$): δ (ppm) 7.75 (d, 1H, Ar–H), 7.31 (s, 1H, Ar–H), 7.23 (d, 1H, Ar–H), 4.39 (t, 2H, OCH_2), 2.71 (m, 2H, CH_2). ^{13}C NMR (126 MHz; $CDCl_3$): δ (ppm) 161.02 (Ar–C), 135.39 (Ar-CH), 119.61 (– CF_2), 119.22 (– CF_2), 117.63 (– CF_2), 115.44 (C \equiv N), 115.04 (Ar–C), 108.21 (– CF_3), 61.20 (OCH_2), 30.89 (CH_2). ^{19}F NMR (470 MHz, DMSO- d_6): δ (ppm) –81.05 (3F- CF_3), –112.86 (2F- CF_2), –124.26 (2F- CF_2), –126.01 (2F CF_2). MS (MALDI-TOF; matrix: CHCA): m/z 390.512 $[M]^+$, 602.837 $[M + matrix + Na]^+$.

2.1.2. 2,9/10,16/17,23/24-Tetrakis[(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy)] phthalocyanine (2)

At 80 °C, 2.08 mg (0.30 mmol) of lithium metal was dissolved in n-pentanol (4 mL). This solution was heated to 145 °C for 5 h after 200 mg (0.51 mmol) of 4-(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy)phthalonitrile (1) addition. The reaction mixture was then poured into 100 mL of water after being cooled to room temperature. This mixture was acidified with 1 mL of hydrochloric acid (HCl) to convert dilithium Pc to metal-free Pc derivative. The product was repeatedly washed with water and n-hexane before being purified on silica gel using column chromatography with tetrahydrofuran as the eluent. Yield: 0.090 g (45 %). m.p. > 200 °C. Anal. calcd. for $C_{56}H_{30}F_{36}N_8O_4$: C 43.04, H 1.93, N 7.17 %, found: C 43.18, H 1.92, N 7.19 %. FT-IR ν_{max} (cm^{-1}): 3290 (NH), 3060 (Ar-CH), 2959–2873 (alkyl CH), 1613, 1468, 1215 (C–O–C), 1095, 1020, 745. 1H NMR (500 MHz; d_6 -acetone): δ (ppm) 7.87 (m, 4H, Ar–H), 7.56 (m, 4H, Ar–H), 7.45 (m, 4H, Ar–H), 4.81 (m, 8H, OCH_2), 3.17 (m, 8H, OCH_2), –1.41 (br, 2H, NH). ^{13}C NMR (126 MHz; DMSO- d_6): δ (ppm) 178.21 (Ar–C), 169.76 (Ar–C), 166.49 (Ar–C), 158.81 (Ar–C), 142.69 (Ar–C), 141.66 (Ar–C), 130.70 (Ar-CH), 125.13 (Ar-CH), 124.41 (Ar-CH), 122.72 (CF_2), 115.99 (CF_3), 110.08 (CF_2), 56.54 (OCH_2), 35.99 (CH_2). UV–Vis (THF) λ_{max} , nm (Log ϵ): 340 (4.91), 671 (4.92), 703 (4.96). MS (MALDI-TOF): m/z 1563.004 $[M]^+$.

2.1.3. 2,9/2,9/10,16/17,23/24-Tetrakis[(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy)] phthalocyaninato zinc(II) (3)

100 mg (0.256 mmol) 4-(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy) phthalonitrile was dissolved in 2-dimethylaminoethanol (2 mL) and later 20 mg (0.109 mmol) anhydrous $Zn(CH_3COO)_2$ was added. The reaction mixture was heated to 145 °C and stirred for 24 h under a nitrogen atmosphere. The mixture precipitated in water after cooling to room temperature. The green precipitate was collected by vacuum filtration and washed with water and ethanol. The crude product was

purified using silica gel-packed column chromatography using tetrahydrofuran as eluent. Yield: 0.035 g (34 %). m.p. > 200 °C. Anal. calcd. for $C_{56}H_{28}F_{36}N_8O_4Zn$: C 41.36, H 1.74, N 6.89 %, found: C 41.22, H 1.74, N 6.90 %. FT-IR ν_{max} (cm^{-1}): 3060 (Ar-CH), 2964–2869 (alkyl CH), 1609, 1473, 1215 (C–O–C), 1128, 1005, 878, 716. 1H NMR (500 MHz; DMSO- d_6): δ (ppm) 7.78 (d, 4H, Ar–H), 7.51 (s, 4H, Ar–H), 7.39 (d, 4H, Ar–H), 5.41 (m, 8H, OCH_2), 3.14 (m, 8H, OCH_2). ^{13}C NMR (126 MHz; DMSO- d_6): δ (ppm) 172.83 (Ar–C), 163.07 (Ar–C), 155.10 (Ar–C), 143.53 (Ar–C), 135.76 (Ar-CH), 132.24 (Ar–C), 125.33 (Ar-CH), 121.17 (CF_2), 118.50 (CF_3), 109.03 (CF_2), 56.75 (OCH_2), 30.10 (CH_2). ^{19}F NMR (470 MHz, DMSO- d_6): δ (ppm) –80.50 (12F, – CF_3), –112.44 (8F, – CF_2), –123.95 (8F, – CF_2), –125.62 (8F, CF_2). UV–Vis (THF) λ_{max} , nm (Log ϵ): 348 (4.79), 675 (5.05). MS (MALDI-TOF): m/z 1626.995 $[M]^+$, 1649.543 $[M+Na]^+$.

2.1.4. 2,9/10,16/17,23/24-Tetrakis[4-(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy)] phthalocyaninato cobalt(II) (4)

Compound 4 was synthesized similarly to 3 from 1 by using 15 mg (0.116 mmol) anhydrous $CoCl_2$. Tetrahydrofuran was used as an eluent in column chromatography on silica gel to purify the product. Yield: 0.031 g (30 %). m.p. > 200 °C. Anal. calcd. for $C_{56}H_{28}F_{36}N_8O_4Co$: C 41.53, H 1.74, N 6.92 %, found: C 41.65, H 1.73, N 6.93 %. FT-IR ν_{max} (cm^{-1}): 3062 (Ar-CH), 2960–2869 (alkyl CH), 1611, 1472, 1400, 1346, 1216 (C–O–C), 1127, 1005, 877, 716. UV–Vis (THF) λ_{max} , nm (Log ϵ): 330 (4.91), 662 (5.07). MS (MALDI-TOF): m/z 1619.026 $[M]^+$.

2.1.5. 2,9/10,16/17,23/24-Tetrakis[4-(3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy)] phthalocyaninato manganese(III)chloride (5)

Compound 5 was synthesized similarly to 3 from 1 by using 14.6 mg (0.116 mmol) anhydrous $MnCl_2$. The crude product was purified using silica gel-packed column chromatography using tetrahydrofuran as eluent. Yield: 0.035 g (33 %). m.p. > 200 °C. Anal. calcd. for $C_{56}H_{28}ClF_{36}MnN_8O_4$: C 40.73, H 1.71, N 6.79 %, found: C 40.64, H 1.70, N 8.36 %. FT-IR ν_{max} (cm^{-1}): 2972–2861 (alkyl CH), 1608, 1469, 1343, 1216 (C–O–C), 1128, 1078, 877. 1H NMR (500 MHz; DMSO- d_6): δ (ppm) 7.74 (d, 4H, Ar–H), 7.36 (s, 4H, Ar–H), 7.31 (d, 4H, Ar–H), 4.47 (m, 8H, OCH_2), 2.96 (m, 8H, OCH_2). ^{13}C NMR (126 MHz; DMSO- d_6): δ (ppm) 178.72 (Ar–C), 169.24 (Ar–C), 163.19 (Ar–C), 135.73 (Ar–C), 134.96 (Ar-CH), 125.28 (Ar–C), 124.66 (Ar-CH), 120.91 (CF_2), 118.55 (CF_3), 108.76 (CF_2), 61.53 (OCH_2), 30.86 (CH_2). UV–Vis (THF) λ_{max} , nm (Log ϵ): 387 (4.77), 498 (4.26), 719 (5.07). MS (MALDI-TOF): m/z 1651.531 $[M]^+$.

2.1.6. Antioxidant activity

2.1.6.1. DPPH radical scavenging activity. Each molecule (100–500 μ g/mL) was dissolved in dimethyl sulfoxide (DMSO) and 2 mL solution was mixed with 2 mL methanolic solution of 1,1-diphenyl-2-picrylhydrazine (DPPH) at 0.1 mM concentration. The radical scavenging ability of the tested compounds was observed by measuring the decrease in UV absorbance at 517 nm after 30 min of incubation in the dark [38]. The DPPH radical scavenging activity percentage was calculated by using the following formula:

$$DPPH \text{ radical scavenging activity}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.1.6.2. Reducing power assay. Using the method in previous study, the reducing power of synthesized Pcs and the standard antioxidants was evaluated [39]. The reduction power is an indicator of the antioxidant activity of a compound. Fe^{+2} ion concentration is measured at 700 nm after the reducers convert Fe^{+3} ions to Fe^{+2} ions.

2.1.6.3. OH- scavenging assay. OH- scavenger activity of the synthesized molecules was revealed by using a procedure described before

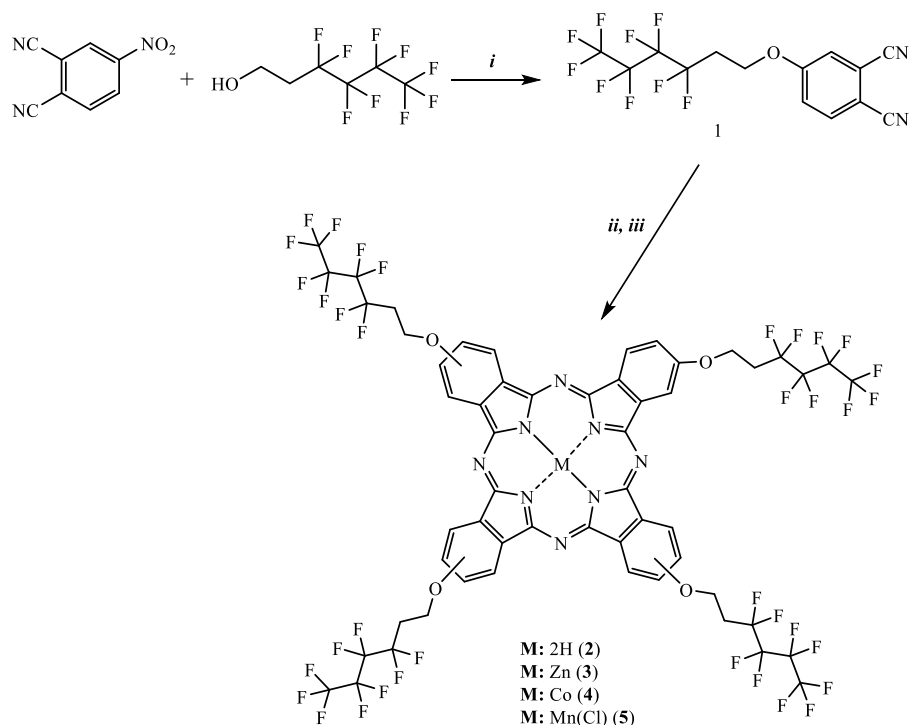


Fig. 1. Synthetic route for phthalonitrile derivative (1) and Pcs (2–5) (i: DMF, K_2CO_3 , 65 °C; ii: Lithium metal, n-pentanol, 145 °C, HCl; iii: metal salts, 2-dimethylaminoethanol, 145 °C).

[40]. OH radicals were produced using H_2O_2 and, then, the hydroxylation abilities of salicylate were investigated. The reaction mixture (3 mL) consisted of different concentrations of *Pc* molecules, along with 1 mL of $FeSO_4$ (1.5 mM), 0.7 mL of H_2O_2 (6 mM), and 0.3 mL of sodium salicylate (20 mM). The hydroxylated salicylate complex's absorbance was determined after 1 h of incubation at 37 °C.

$$\text{Scavenging rate} = [1 - (A_1 - A_2) / A_0] \times 100\%$$

where A_0 was the absorbance of the control (without Pcs) while the absorbances A_1 and A_2 corresponded to the absorbance in the presence of the *Pc* molecules and the absorbance without sodium salicylate, respectively.

2.1.6.4. Cytotoxicity assay. *Pc* samples were tested on cancerous cell lines (cervix adenocarcinoma cells (HeLa), human colon carcinoma (CaCo-2), human pancreatic carcinoma cells (PANC-1), prostatic adenocarcinoma (PC3), human breast adenocarcinoma cells (MDA-MB-231), lung carcinoma cells (A549), human breast adenocarcinoma (MCF-7), human glioblastoma cells (U87MG), mouse embryonic fibroblasts (3T3), human monocyte cells (THP-1), human lung carcinoma cells (HTB-177), human bronchioalveolar non-small cell carcinoma (CRL-5807), human squamous cell adenocarcinoma mesothelioma (CRL-5826), and murine macrophages cells (RAW 264.7)), and non-cancerous cell line (monkey kidney epithelial cells (VERO)) and healthy cell line (normal human lung fibroblasts (CCD34LU)). Cytotoxicity study was performed by using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay [41].

The cell lines were maintained in Dulbecco's modified Eagle's medium F12 (DMEM/F12), supplemented with 10 % fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 μ g/mL of streptomycin (Gibco). The cells were incubated at 37 °C in a humidified atmosphere of 5 % CO_2 . The cells were subcultured twice a week, and cells in the exponential growth phase were used in the experiments. For this purpose, all cell lines were cultivated for 24 h in 96-well microplates with an initial concentration of 1×10^5 cells/well in a humidified atmosphere with 5 %

CO_2 , at 37 °C. Then, the cultured cells were treated with different concentrations of the compounds (0.5, 5, 50 μ g/mL) followed by incubation for 48 h at 37 °C. The treatment concentration was given as μ M for pure compounds. After incubation with samples, the percentages of viable cells in each culture were assessed, and IC50 values were computed using Graph Pad Prism 5 [42].

2.1.7. Nitric oxide analysis (iNOS)

RAW 264.7 (mouse macrophages) were cultured in lipopolysaccharide (LPS) and RPMI 1640 (Roswell Park Memorial Institute (RPMI) 1640) medium with 10 % FBS (fetal bovine serum), 100 U/mL of penicillin, and 100 μ g/mL of streptomycin (Gibco) at 37 °C in a humidified atmosphere with 5 % CO_2 . Cells were seeded in 96-well plates (1×10^6 cells/mL) and incubated for 24 h for the experiment. Dilutions of the molecules (1, 10, and 100 μ g/mL) were added after inducing with LPS (5 μ g/mL), and cells were then incubated for a further 24 h at 37 °C in a humid atmosphere with 5 % CO_2 . The level of nitrite in the medium was measured using Griess reagent in supernatants. The absorbance was measured at 540 nm. In comparison to the vehicle control, the sample's percentage inhibition of nitrite generation was calculated [21,43].

3. Results and discussion

3.1. Synthesis and structural characterizations

Fig. 1 depicts the synthesis process for the phthalonitrile derivative (1) and Pcs (2–5). The precursor dinitrile compound, namely 4-(3',3',4',4',5',5',6',6',6'-nonafluoro-hexyloxy) phthalonitrile (1), was produced in dry DMF in 49 % yield by base catalyzed nucleophilic substitution reaction of 4-nitrophthalonitrile with 3,3,4,4,5,5,6,6,6-nonafluoro-hexane-1-ol. By combining compound 1 with a lithium alkoxide in this stage, cyclotetramerization was accomplished, resulting in the creation of the corresponding dilithium *Pc* (Li_2Pc) [44]. Li_2Pc was converted to metal-free *Pc* (2) by acidification with HCl. Cyclotetramerization of compound 1 with anhydrous metal salts (Zn

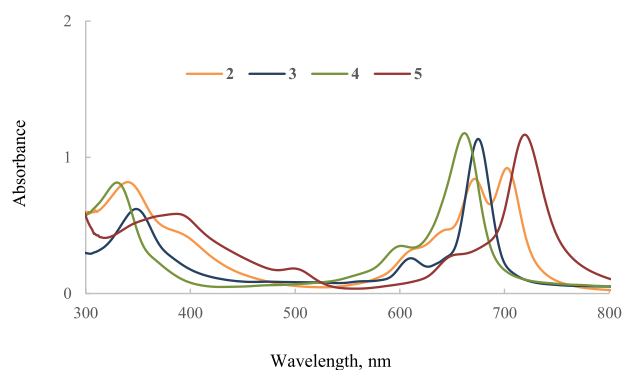


Fig. 2. UV-Vis absorption spectra of 3–5 in THF (1×10^{-5} M).

(CH_3COOH)₂, CoCl_2 , and MnCl_2) in 2-dimethylaminoethanol at 145 °C under nitrogen atmosphere led to the formation of peripherally tetra-substituted MPcs (3–5) (Fig. 1).

In this study, the solubility of 2–5 was increased, as the polarities of the solvents was also increased. All newly synthesized Pcs (2–5) exhibited good solubility in strongly polar solvents such as DMSO and DMF. Also, they could be dissolved easily in medium polar solvents such as ethyl acetate and THF, but the solubility in chloroform and dichloromethane was poor, this finding coinciding with that reported in the literature [30]. The type of central metal ions has an impact on the solubility of Pcs as well. In this study, compound 5 showed a lower solubility than those of 3 and 4 in polar solvents. The solubility of metal-free Pc was higher than its metal derivatives (3–5).

All synthesized compounds in this study were characterized using several spectroscopic methods.

Stretching vibrations for aromatic CH, aliphatic CH, $\text{C} \equiv \text{N}$, and $\text{C}-\text{O}-\text{C}$ appeared at 3088, 2984–2924, 2237, and 1224 cm^{-1} in the FT-IR spectra of compound 1 (Fig. S16). The aromatic protons showed up as a doublet, singlet, and doublet, respectively, at 7.75, 7.31, and 7.23 ppm in the ^1H NMR spectra of 1 in CDCl_3 . The CH_2 protons were seen as triplets and multiplets, respectively, at 4.39 (OCH_2) and 2.71 (CH_2) ppm (Fig. S1). The aromatic carbons atoms appeared at showed at 161.02, 135.39, 119.22, and 117.63 ppm in the ^{13}C NMR spectrum of compound 1 in CDCl_3 . The nitrile carbons were observed at 115.44 and 115.04 ppm. The aliphatic CF_2 , CF_3 and CH_2 , carbons appeared at 119.61, 119.22, 108.21, 117.63, 61.20, and 30.89 ppm, respectively (Fig. S2). The absence of strong $\text{C} \equiv \text{N}$ vibration at 2237 cm^{-1} in the FT-IR spectra of the Pc derivatives (2–5) indicated that compound 1 completed the cyclotetramerization reaction (Figs. S17–20).

Stretching vibrations of $\text{C}-\text{O}-\text{C}$, aliphatic CH, and aromatic CH were detected at 1215–1216, 2972–2861, and 3060–3062 cm^{-1} , respectively. An additional absorption band at 3290 cm^{-1} that was attributed to the NH stretching vibrations was visible in the FT-IR spectrum of metal-free Pc (2).

MS MALDI TOF measurements for compounds 1–5 were performed in THF medium. The existence of molecular peaks at m/z 390.512 $[\text{M}]^+$, $[\text{M} + \text{matrix} + \text{Na}]^+$.for 1, 1563.004 $[\text{M}]^+$.for 2, m/z 1626.995 $[\text{M}]^+$, 1649.543 $[\text{M} + \text{Na}]^+$ for 3, m/z 1619.026 $[\text{M}]^+$ for 4, and m/z 1651.531 $[\text{M}]^+$ for 5 in the mass spectra of compounds 1–5 confirmed the proposed structures (Figs. S11–S15).

Due to the mixed structural isomers of Pcs 2, 3 and 5 and the aggregation of Pcs at the concentrations utilized for NMR spectroscopy, wide peaks were seen in the ^1H NMR spectra of these compounds in CDCl_3 [27]. The aromatic, CH_2 , and OCH_2 protons appeared at 7.45–7.87, 4.81, and 3.17 ppm, respectively, in the ^1H NMR spectrum of 2 in d_6 -acetone. The inner NH protons of 2 were also recognized by a broad chemical shift at -1.41 ppm structures (Fig. S4). In the ^1H NMR spectra of compounds 3 and 5 in $\text{DMSO}-d_6$, the aromatic, CH_2 , and OCH_2

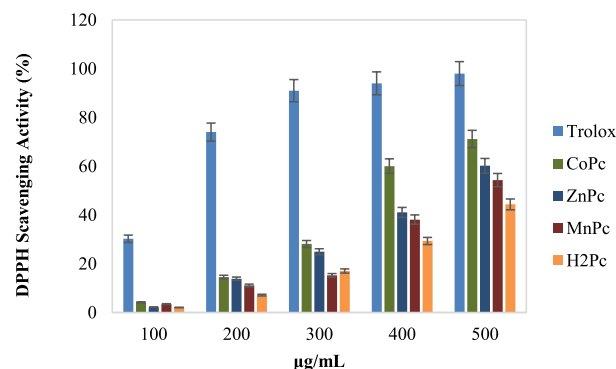


Fig. 3. Antioxidant activities of Pc molecules at different concentrations by using DPPH method (The absorbance values were converted to scavenging effects (%) and the data plotted as the means of replicate scavenging effect (%) values ± 1 S.D. ($n = 3$) against Pc concentration in μg molecule per mL reaction volume).

protons appeared at 7.78–7.39, 5.41, and 3.14 ppm for 3 and 7.74–7.31, 4.47, and 2.96 ppm for 5, respectively (Figs. S6 and S9). The paramagnetic property of CoPc prevented the detection of 4 by ^1H NMR [44]. In the ^{13}C NMR spectra of compounds 2, 3, and 5 in $\text{DMSO}-d_6$ the aromatic carbons appeared at 178.21–124.41 ppm for 2, 172.83–125.33 ppm for 3, and 178.72–124.66 ppm for 5, respectively. In the ^{19}F NMR spectra of compounds 1 and 3 in $\text{DMSO}-d_6$ the CF_3 and CF_2 fluorine atoms appeared at -81.05 – -112.86 , -124.26 , and -126.01 ppm for 1 and -80.50 – -112.44 – -123.95 , and -125.62 ppm for 3, respectively (S3 and S8).

3.1.1. Ground state electronic absorption

Fig. 2 displays the electronic absorption spectra of Pc complexes (2–5) in THF. Intense single Q-band absorptions of $\pi \rightarrow \pi^*$ transitions are present in the UV-vis absorption spectra of all MPcs (3–5) at wavelengths of 675, 662, and 719 nm, respectively.

Due to the D_{2h} symmetry, the metal-free derivative 2 produced a doublet Q band at 671 and 703 nm. The B bands of compounds 2–5 were observed at 340, 348, 330, and 387 nm, respectively. The Q-band absorptions of MPcs (3–5) increased in the following order: CoPc (4) < ZnPc (3) < MnPc (5). The Q-band of MnPc (5) was shifted by 44 and 57 nm compared to the Q-band of the CoPc (4) and ZnPc (3). Depending on the type of core metal ion, the UV-vis absorption spectra of Pcs 3–5 exhibit different behavior. Additionally, charge transfer absorption (Pc-metal, LMCT) was linked to the peak for MnPc (5) at 498 nm [28,45].

3.1.2. Antioxidant activity

The DPPH method was used to assess the antioxidant capacities of four different Pcs within the context of the investigation. Considering the metal effect they contain, the antioxidant effects of metallophthalocyanines were higher than those without metal (Fig. 3). Among the Pcs studied, CoPc (4) had the highest DPPH radical scavenging activity. These compounds' π systems, which consist of an electron density cloud above and below the internuclear axis, correspond to bonds in which atomic orbitals overlap in parallel [46]. The antioxidant functions of Pcs are closely related to the resonance in the π system [1, 47]. The list of the DPPH radical scavenging activities of Pc molecules in decreasing order is; $\text{CoPc} > \text{ZnPc} > \text{MnPc} > \text{H}_2\text{Pc}$. The mechanism of action of the MPcs is deeply related to the resonance that occurs in the π system located in these structures [1,48]. The π systems in these compounds correspond to bonds in which atomic orbitals overlap in parallel, comprising an electron density cloud above and below the internuclear axis, for example, as in the 2p orbital of nitrogen and d orbital of metal, called a pp-dp bond [49]. Pcs which have conjugated systems, readily

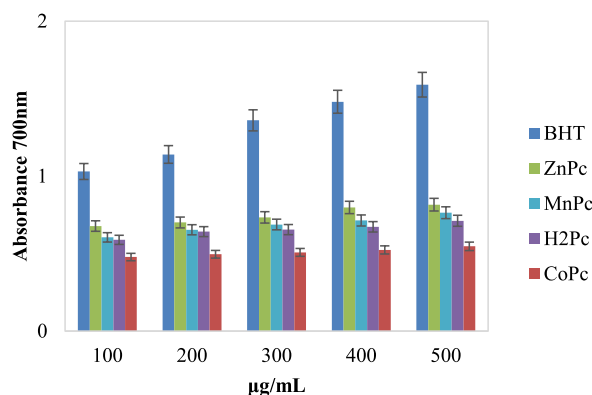


Fig. 4. Reducing power activities of the Pc molecules (The absorbance values were converted to scavenging effects (%) and the data plotted as the means of replicating scavenging effect (%) values \pm 1 S.D. ($n = 3$) against Pc concentration in μg molecule per mL reaction volume).

accept or donate electrons. Pcs containing a transition metal ion easily change their oxidation state by an electron exchange. According to literature, an oxidation number of transition metal atoms that is derived from a known d^n configuration should be specified as a physical (or spectroscopic) oxidation number (state) [50].

In a study dealing with the radical scavenging ability of newly synthesized Pc derivatives, CoPc demonstrated the strongest antioxidant activity at 50 mg/mL concentration when compared to the ZnPc [51]. In another study investigating the antioxidant properties of the metal-free, zinc, and cobalt Pcs carrying 4-methoxy-phenoxy substituents on the non-peripheral or peripheral positions, non-peripherally substituted CoPc showed the best antioxidant activity [52].

BHT showed the highest reducing activity at all tested concentrations when reducing power activities of Pcs were evaluated (Fig. 4). Among the tested molecules, ZnPc showed the strongest reducing power activity followed by MnPc > H₂Pc > CoPc.

It is seen that the metal ions in the central cavity of the synthesized Pc molecules and the substituents attached to the main skeleton of the molecules are effective on the antioxidant activity properties of metallo and metal-free Pc molecules. An evaluation of previous studies is given in Table 1.

The discovery of new antioxidant molecules in OH radical removal is important because OH radicals interact with many molecules such as sugar, amino acids, and lipids in living cells. The Fenton reaction, which uses a transition metal as a pro-oxidant in the catalytic breakdown of superoxide and hydrogen peroxide, is by far the most significant mechanism for OH production in living things [56]. According to the study performed by using synthesized molecules and positive control, ascorbic acid showed the highest radical scavenging activity against OH radical at all tested concentrations followed by ZnPc > MnPc > H₂Pc > CoPc (Fig. 5).

The antioxidant behavior of Pcs is directly related to the resonance of localized electrons. Two factors that immediately affect the π electron

Table 1
Antioxidant activities of Pc molecules from literature.

Substituent	Central metal atom	Highest activity	Concentration	Reference
(3,4,5-Trimethoxybenzyloxy)	Zn/Co/Ni	DPPH Chelating Effect	CoPc ZnPc	100 mg/mL [38]
2,6-di-tertbutylphenol	Co/Cu/Zn/Mn	DPPH	CoPc	60 mg/mL [53]
4-((4'-tert-butyl)phenoxy)phenoxy	Zn/Co/H ₂	DPPH	H ₂ Pc	500 $\mu\text{g}/\text{mL}$ [54]
2-methoxy-4-((Z)-[(4-morpholin-4-ylphenyl)imino]methyl)phenol	Zn/Cu/Co	Superoxide scvng.	CoPc	50 mg/mL [55]
		Hydroxyl Scvng.	H ₂ Pc	
		DPPH	CuPc	
		Ferric Reducing	CoPc	

density are the centrally located metal atom and connected substituent [57]. The d -electron configuration d^n is a central-atom descriptor in transition-metal complexes. For a transition metal of N valence electrons, d^n yields oxidation state as [58];

$$\text{OS} = N - n$$

Oxidation state is related to ligand and ligands are classified as "innocent" and "suspected" based on their probability of describing the oxidation state of the metal atom in the complexes [59].

3.1.3. Cytotoxic activity

Cytotoxicity activity: The cytotoxicity of Pcs was evaluated using the MTT assay. There were no toxic effects on cells in the H₂Pc and CoPc

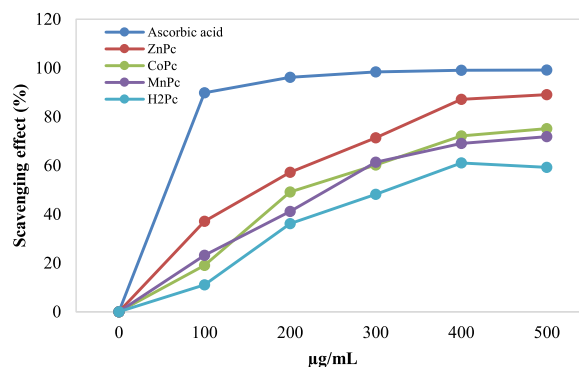


Fig. 5. OH radical scavenging activities of Pc molecules and positive control (The absorbance values were converted to scavenging effects (%) and the data plotted as the means of replicate scavenging effect (%) values \pm 1 S.D. ($n = 3$) against Pc concentration in μg molecule per mL reaction volume).

Table 2
IC₅₀ values of cytotoxic activities of cells.

Cells	IC ₅₀ ($\mu\text{g}/\text{mL}$)				
	H ₂ Pc	CoPc	MnPc	ZnPc	Dox
HeLa	–	–	0.021	0.024	0.001
3T3	–	–	–	–	0.008
A549	–	–	>0.03	0.026	0.009
CaCo-2	–	–	–	–	>0.037
PANCI	–	–	–	–	0.030
MCF-7	–	–	0.026	0.017	0.003
RAW 264.7	–	–	>0.03	>0.031	0.003
Vero	–	–	–	–	0.005
THP-1	–	–	>0.03	0.020	0.008
U87MG	–	–	–	–	0.004
MDA-MB-231	–	–	>0.03	–	0.021
PC3	–	–	–	–	0.002
CCD34LU	–	–	>0.03	>0.031	0.009
HTB-177	–	–	–	–	0.001
CRL-5807	–	–	–	–	0.009
CRL-5826	–	–	0.028	0.022	0.002

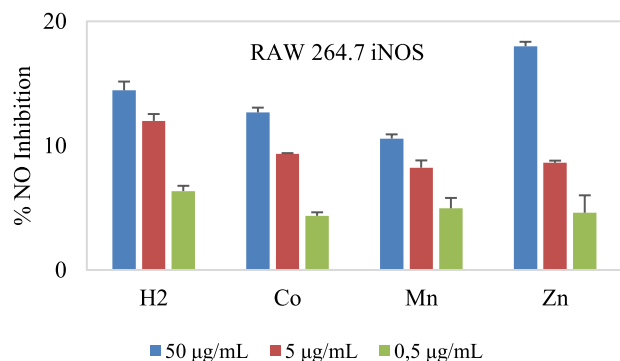


Fig. 6. LPS-induced nitrite by RAW 264.7 macrophages percent inhibition by samples.

samples (Table 2). The IC_{50} value in the MnPc sample was determined to be 0.021 μ M in HeLa, 0.028 μ M in CRL-5826, and 0.026 μ M in MCF-7 cells, respectively. Furthermore, for the ZnPc sample, the IC_{50} values were calculated to be HeLa 0.024 μ M, A549 0.026 μ M, THP-1 0.020 μ M, CRL-5826 0.022 μ M and MCF-7 0.017 μ M. Cytotoxic effects on non-cancerous CCD34LU and 3T3 cells were not determined at the dose employed. When the literature was examined, it was determined that the metal salts used in the synthesis of Pc molecules showed cytotoxic activity [60–62]. For example; in a study examining the cytotoxic activity of $MnCl_2$, its IC_{50} value on HeLa and MCF-7 cells was determined to be > 100 μ mol/L (12.58 μ g/mL) [63]. Although metal complexes exhibit cytotoxic activity, this activity decreases in Pc molecules [64,65]. The percent viability graphs of all cells are given in the electronic supplementary material (ESI) (Fig. S21 (A–R)).

Pc shows properties that provide high peroxidase-like catalytic activity, form free radicals, and inhibit the proliferation of cancer cells. When the literature was examined, it was stated that Pcs showed cytotoxic and high phototoxic effects on cancer cells [66]. MnPc and ZnPc, which were investigated as part of the study, showed minimal cytotoxicity on immune system cell lines and healthy cell lines but increased cytotoxicity on cancer cell lines. The results of this study support those of prior studies [67–70]. However, it is also crucial to investigate these compounds' phototoxicities to evaluate their potential as anticancer agents.

3.1.4. Nitric oxide analysis

The inhibitory effects of Pcs on NO expression were investigated. However, none of the samples showed any significant NO inhibition (Fig. 6).

Numerous biological functions, including cell signal transmission and cell cycle, depend on reactive oxygen species, which include hydrogen peroxide, nitric oxide, superoxide, hydroxyl radicals, and hydroxyl ions. Depending on the degree and length of exposure, these reactive molecules can cause DNA damage, oxidative stress, and cellular damage, inducing either cell survival or death pathways [71]. It is widely known that iNOS-mediated NO production suppression, which is efficient in many pathophysiological situations, can contribute to anti-inflammatory and immunoregulatory activities [72]. It was found that ZnPc had a stronger NO inhibition than other Pc molecules.

4. Conclusion

In conclusion, we have successfully synthesized and characterized peripherally 3',3',4',4',5',5',6',6'-nonafluoro-hexyloxy groups substituted phthalocyanines. In addition, we have addressed the possible use of these complexes in biological applications according to their antioxidant and cytotoxic activities. Pc molecules synthesized in

this study did not exhibit significant NO inhibition. No toxic effects on cells were seen in the H₂Pc and CoPc samples. There were no known cytotoxic effects at the dosage employed on either the cancer-free 3T3 cell line or the healthy CCD34LU cell line. All molecules showed moderate antioxidant activity all tested assays.

CRediT authorship contribution statement

Başak Sezgi Bilen: Investigation, Methodology, Writing – original draft. **Mukaddes Özçeşmeci:** Investigation, Methodology, Writing – original draft. **Mustafa Akın:** Investigation, Methodology, Writing – original draft. **Büşra Çakır:** Investigation, Methodology, Writing – original draft. **Karrar Ali Mohammed Hasan Alsakini:** Investigation, Methodology, Writing – original draft. **Ayşe Nalbantsoy:** Investigation, Methodology, Writing – original draft. **Neslihan Şaki:** Investigation, Methodology, Writing – original draft. **Esin Hamuryudan:** Investigation, Methodology, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgement

This work was supported by Research Fund of the Istanbul Technical University and Research Fund of the Kocaeli University (Project No: BAP-2022/2807).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dyepig.2023.111814>.

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