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ORIGINAL PAPERS

Synthesis and biological activity, photophysical, photochemical properties of tetra substituted magnesium phthalocyanine

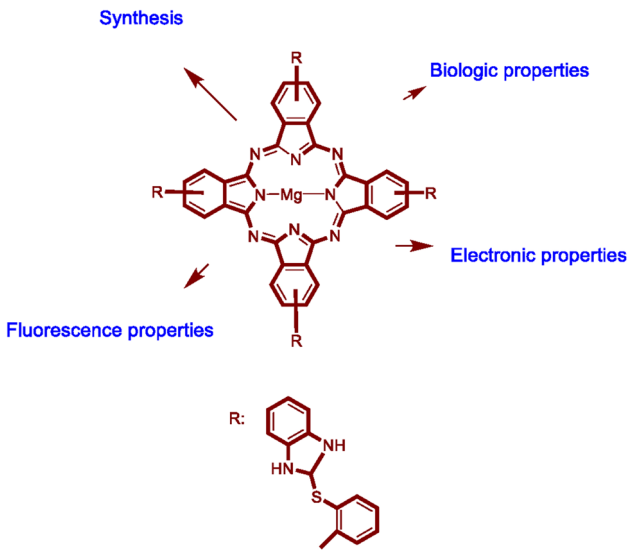
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Abstract

The compound 4-(2-((1H-benzo[d]imidazol-2-yl) thio) phenoxy) phthalonitrile was obtained from the reaction of 2-nitrophenol, 4-nitrophthalonitrile and 2-mercaptobenzimidazole. This compound was reacted with magnesium Chloride ($MgCl_2$) to yield tetrakis-[(2-((1H-benzo[d]imidazol-2-yl) thio) phenoxy) phthalocyaninato] magnesium II. New compounds were characterized by UV–vis, ^{1}H NMR, ^{13}C NMR, FTIR and Mass spectra. Electronic spectra aggregation study of magnesium phthalocyanine compound in various concentrations and diverse solvents was performed. Photoluminescence spectra of magnesium phthalocyanine in different solvents were investigated. The biological activities of **3** and **4** compounds were investigated. The results showed that **4** had excellent antioxidant and antidiabetic activities as 75.71% and 81.83%, respectively. **3** and **4** had deoxyribonucleic acid (DNA) cleavage ability and **4** caused a double-strand fracture in plasmid DNA at 100 and 200 mg/L. Both compounds showed antimicrobial activity and also **4** was more effective against pathogenic microorganisms than **3**. Photodynamic antimicrobial therapy of test compound was also more effective than without irradiation. The highest biofilm inhibition of **3** and **4** was 78.28% and 98.49% for *S. aureus* and also 73.95% and 91.13% for *P. aeruginosa*, respectively. Finally, both compounds demonstrated %100 microbial cell viability inhibition at 100 mg/L. Overall, the study suggests that both **3** and **4** have potential for further development as therapeutic agents.

Graphical Abstract



Keywords Antioxidant · Antimicrobial · DNA cleavage · Biofilm inhibition · Phthalocyanine · Synthesis

Extended author information available on the last page of the article

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1 Introduction

Phthalocyanines have the ability to form complexes with many elements in the periodic table. [1, 2]. These complex compounds are the focus of many studies due to their interesting electronic structures. Current research areas such as photodynamic therapy [3], catalyst [4], photocatalytic [5], sensor [6], photosensitizer [7], colorant [8], electrochromic [9], antioxidant [10], antibacterial [11], enzyme activity [12], dye sensitized solar cell [13] can be listed as examples.

For the applications mentioned above, it is important that phthalocyanines are soluble. In addition, the complex structure of diamagnetic elements such as Al, In, Si, Mg, Ge, Zn is preferred for photodynamic therapy applications [14, 15]. Studies showing the photophysical and chemical properties of magnesium phthalocyanine are promising for photodynamic therapy [16]. The magnesium phthalocyanine complex for these purposes is expected to show biological activity. The magnesium phthalocyanine complex is an interesting phthalocyanine derivative with its potential to have photosensitizing effects.

In this study, an original phthalonitrile derivative, which is the starting material for a phthalocyanine, and a magnesium phthalocyanine compound were synthesized and characterized. The solubility and aggregation properties of the compound were investigated. The antioxidant potential, antidiabetic, DNA cleavage, antimicrobial, photodynamic antimicrobial therapy, biofilm inhibition activities and microbial cell viability inhibition of compounds **3** and **4** were investigated.

2 Experimental

2.1 General

4-nitrophthalonitrile, 2-nitrophenol, 2-mercaptobenzimidazole, 1, 8-Diaza bicyclo [5.4.0] undec-7-ene (DBU), $MgCl_2$ potassium carbonate (K_2CO_3), dimethylformamide (DMF) chloroform($CHCl_3$), tetrahydrofuran (THF), hexanol, acetonitrile, ethyl acetate, DMF, dimethyl sulfoxide (DMSO) was obtained from Aldrich and Sigma companies. Agilent 400 MHz spectrometer, Hitachi U-2900 Spectrophotometer, LC–MS TOF electrospray ionization techniques and Thermo Scientific FT-IR spectrophotometer were used for characterization.

2.2 4-(2-((1H-benzo[d]imidazol-2-yl) thio) phenoxy) phthalonitrile (3)

2-nitrophenol **1** (0.402 g, 2.89 mmol) and 4-nitrophthalonitrile **2** (0.500 g, 2.89 mmol) were taken in 25 mL of DMF and stirred at rt. under N_2 for 30 min. Subsequently, 2-mercaptobenzimidazole ***ii*** (0.434 g, 2.89 mmol) was added to the mixture. After 15 min of stirring, K_2CO_3 (2.2 g, 16 mmol) was added within 2 h. It was stirred at room temperature for 24 h. The reaction product was precipitated on ice water (150 mL). The precipitate was filtered, neutralized by washing with distilled water. The crude product was taken into a vacuum oven at 80 °C and dried. Melting point (MP): 135–138 °C. Yield: 0.62 g. This product is soluble in acetonitrile, $CHCl_3$, ethyl acetate, THF, DMF, DMSO solvents.

IR spectrum (cm^{-1}): 3132, 3064, 2233, 1564, 1587, 1481, 1409, 1286, 1269, 1124, 1076,1006, 974, 819,750. ^{1}H NMR (400 MHz, DMSO-d_6): (δ: ppm) 12.5 (s,1H), 8.49–8.38 (m, 1H), 8.32–8.18 (m, 1H), 8.16–7.98 (m, 2H), 7.95–7.71 (m, 2H), 7.53 (t, J = 22.5 Hz, 3H), 7.33–7.16 (m, 1H), 7.13–7.04 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): (δ): 142.86, 141.90, 134.90, 133.53, 133.44, 123.26, 116.21, 115.91,115.82, 112.99 ppm. MS (ESI); (M + H) calc. for $C_{21}H_{12}N_4OS$: 368.1; found: 369.08 $[M+H]^+$.

2.3 Tetrakis-[(2-((1H-benzo[d]imidazol-2-yl) thio) phenoxy) phthalocyaninato] magnesium II (4)

100 mg of 4-(2-((1H-benzo[d]imidazol-2-yl) thio) phenoxy) phthalonitrile and 30 mg of $MgCl_2$ were heated in 3 ml of hexanol in the presence of 2 drops of DBU under N_2 at 150 °C for 16 h. After the reaction was terminated, the product, which came to room temperature, was precipitated with petroleum ether and filtered. Then the product was washed with cold and hot methanol. THF solutes were then taken. It can be dissolved in solvents such as THF, DMF, DMSO.UV–Vis (THF, 1×10^{-5} M): λmax/nm (log ε): 680 (5.08),615 (4.40),360 (4.69) nm. IR spectrum (cm^{-1}): 2950, 2850, 1666, 1598, 1479, 1408, 1307, 1269, 1226, 1143, 1089, 1041, 977, 910, 883,821,744. ^{1}H-NMR (400 MHz, DMSO-d_6): (δ): 13.07, 9.13, 8.98, 8.19, 7.61, 7.02 ppm.

2.4 DPPH radical scavenging assay

The Pc and metallo Pc' DPPH radical scavenging abilities were evaluated using previously published literature with minor changes [17]. Trolox and Ascorbic acid were utilized as positive controls while methanol as a blank. Trolox, a lipophilic compound, is effective in oil-based media, while ascorbic acid, a hydrophilic compound, is effective

in water-based media [18]. A comparison of Trolox and ascorbic acid was performed to evaluate the activity of antioxidants with different properties and to identify the most appropriate reference compound. The stock solution of compounds was diluted in various concentrations as 6.25, 12.5, 25, 50, and 100 mg L -1. A 250 µL sample and 1000 µL methanolic DPPH solution were mixed and shaken vigorously. After the solutions were incubated at 25 ºC for 30 min in a dark place, their absorbance was measured at 517 nm. If the compound behaves like the radical scavenger the DPPH solution decolorizes and the color is altered from dark purple to pale yellow. As a control, the assay mixture devoid of any compound was employed. Percent inhibition was calculated according to the formula below.

$$\text{Antioxidant Capacity (\%)} = (\text{Abs (control)} - \text{Abs (sample)}/\ \text{Abs (control)}) \times 100.$$

2.5 Antidiabetic activity

First, test compounds were incubated with alpha Amylase enzyme at 37 °C for 10 min at the concentrations of 100, 200 and 400 mg/L. Starch was then added to all test tubes and the test tubes were again incubated at 37 °C for 20 min. After 20 min of incubation, 3,5 dinitro salicylic acid (DNS) was added to each test tube and the test tubes were boiled at 100 °C for 5 min. After the tubes cooled, dilution was made and spectrophotometric measurements were made at 540 nm. The tube without test samples was used as a control and the antidiabetic activity was calculated according to the formula below.

$$\text{Antidiabetic Activity(\%)} = \left(\text{Sample}_{abs} - \text{Control}_{abs}\right)/\text{Control}_{abs} \times 100$$

2.6 DNA nuclease experiments

Gel electrophoresis is a common laboratory technique used to separate and analyze DNA, RNA, and proteins based on their charge and size. The basic principle of gel electrophoresis is to apply an electrical current to a gel matrix, which creates an electric field that allows charged molecules to migrate through the gel matrix. The gel electrophoresis technique was used to examine the DNA nuclease activity of Pc compounds according to assay with minor changes [18]. Supercoiled pBR322 plasmid DNA was incubated at 37 ºC for 90 min with increasing concentrations of test compounds (50, 100, and 200 mg L^{-1}). After that, the loading buffer was added and the compounds were loaded onto a 1.0% agarose gel and stained with EthBr in a Tris–acetic acid-EDTA buffer. Using an electrophoresis apparatus, the samples were run at 100 V for 1.5 h. Negative control was created using untreated DNA. After the electrophoresis bands were photographed by utilizing a UV illuminator.

2.7 Antimicrobial assays and photodynamic antimicrobial therapy activity

The Pc compounds were examined for their in vitro antimicrobial efficacy against Gram-positive species (including *Enterococcus faecalis* (ATCC 29212), *Enterococcus hirae* (ATCC 10541), *Staphylococcus aureus* (ATCC 25923)), and Gram-negative (including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853)), *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152), as well as fungal strains (*Candida tropicalis* (ATCC 750) and *Candida albicans* using the standard broth microdilution method. These species were grown at 37 ºC overnight before the application. The initial wells of the microplates were filled with 1024 mg/L of each of the Pc compounds under study, and the remaining wells were filled with diluted versions of the compounds. Then, each well received an inoculation of 10 µL of microorganism culture solution. On a shaker, microplates were incubated at 37 ºC for 24 h. Minimum Inhibition Concentration (MIC) data, which are described as the lowest concentration that inhibits the growth of bacteria, were used to assess antimicrobial activity after 24 h. This study was carried out in the same methodology as the antimicrobial assays section mentioned above. However, in this procedure, Pcs were exposed to LED light for 30 min prior to microbial inoculation. The results of the study were evaluated as MIC values.

2.8 Microbial cell viability inhibition test

The capacity of compounds to inhibit bacterial cell viability was tested using the strain of *E. coli* (ATCC 25922). *E. coli* was inoculated on to Nutrient Broth (NB) and grown at 37.0 ºC for one day in a shaker. After one day of incubation, *E. coli* was separated by centrifugation at 5500 rpm for 6 min. The residue of the NB medium was then rinsed off the bacterial pellet with a sterile 0.9% saline solution. After the purification step, *E. coli* strain was suspended in 10 mL of saline. The microbial cell viability experiment was conducted using this microbial suspension (2.8 × 109 CFU/mL). *E. coli* was treated at 0, 25, 50, and 100 mg L-1 concentrations of Pcs at 37 ºC for 120 min. The mixes were then diluted in a variety of ratios, inoculated on NB agar, and left to incubate for one day at 37 ºC. At the end, the colonies were counted, and the following formula was applied to calculate the microbial cell viability.

Microbial cell viability (%) = (Acontrol −Asample/ Acontrol) x 100

2.9 Biofilm inhibition activity

P. aeruginosa and *S. aureus* were applied as model microorganisms to determine the effect of compounds on biofilm inhibition. Microorganisms were added to well plates containing test compound concentrations of 0, 12.5, 25, and 50 mg L^{-1}, and the plates were then incubated at 37 ºC for three days. The wells of the plate were carefully drained after incubation and washed twice with distilled water. First, biofilms were stained with crystal violet (CV) and then incubated for 60 min. Then, CV was removed and the plates were slowly rinsed with distilled water. Ethanol was added to plates and allowed for 15 min for recovery of absorbed CV. Using a spectrophotometer, the biofilm inhibition was measured at 595 nm. The following formula was used to calculate biofilm inhibition.

$$\text{Biofilm Inhibition (\%)} = (\text{Abs(control)} - \text{Abs(sample)}/\text{Abs(control)}) \times 100$$

3 Results and discussion

3.1 characterization

Synthesis steps of magnesium II phthalocyanine and 4-(2-((1H-benzo[d]imidazol-2-yl)thio)phenoxy)phthalonitrile compounds are given in scheme 1. Magnesium II phthalocyanine was formed from the reaction of $MgCl_2$ and its phthalonitrile derivative. Magnesium II phthalocyanine compound and starting material was characterized by ^1H and ^{13}C NMR, Mass, FTIR and UV–visible spectroscopy.

When the FTIR spectrum is evaluated for compound **3**, as functional groups, aromatic protons are shown at 3132 and 3064 cm^{-1}, nitrile peak at 2233 cm^{-1} and C=C groups at 1564–1587 cm^{-1}. In addition, C–O–C groups vibrate at 1269 cm^{-1}. Unlike Compound 4, in the FTIR spectrum, the nitrile vibration appears to disappear as expected. C=C groups are observed at 1598–1479 cm^{-1}, and C–O–C vibrations are observed at 1269 cm^{-1}.

In the ^1H NMR spectrum of compound **3**, NH proton was observed at 12.5 ppm. Aromatic protons are observed in the range of 8.49–7.04 ppm (Figure S1). In the ^{13}C NMR spectrum, aromatic carbon peaks give peaks in the range of 142.86 to 112.99 ppm (Figure S2). In the ^1H NMR spectrum of compound **4**, NH proton is observed at 13.07 ppm. Aromatic protons are observed in the range of 9.13–7.02 ppm (Figure S3).

The mass spectrum of 369.08 $[M+H]^+$ is found to be consistent with the mass for compound **3**, which also supports the structure (Figure S4).

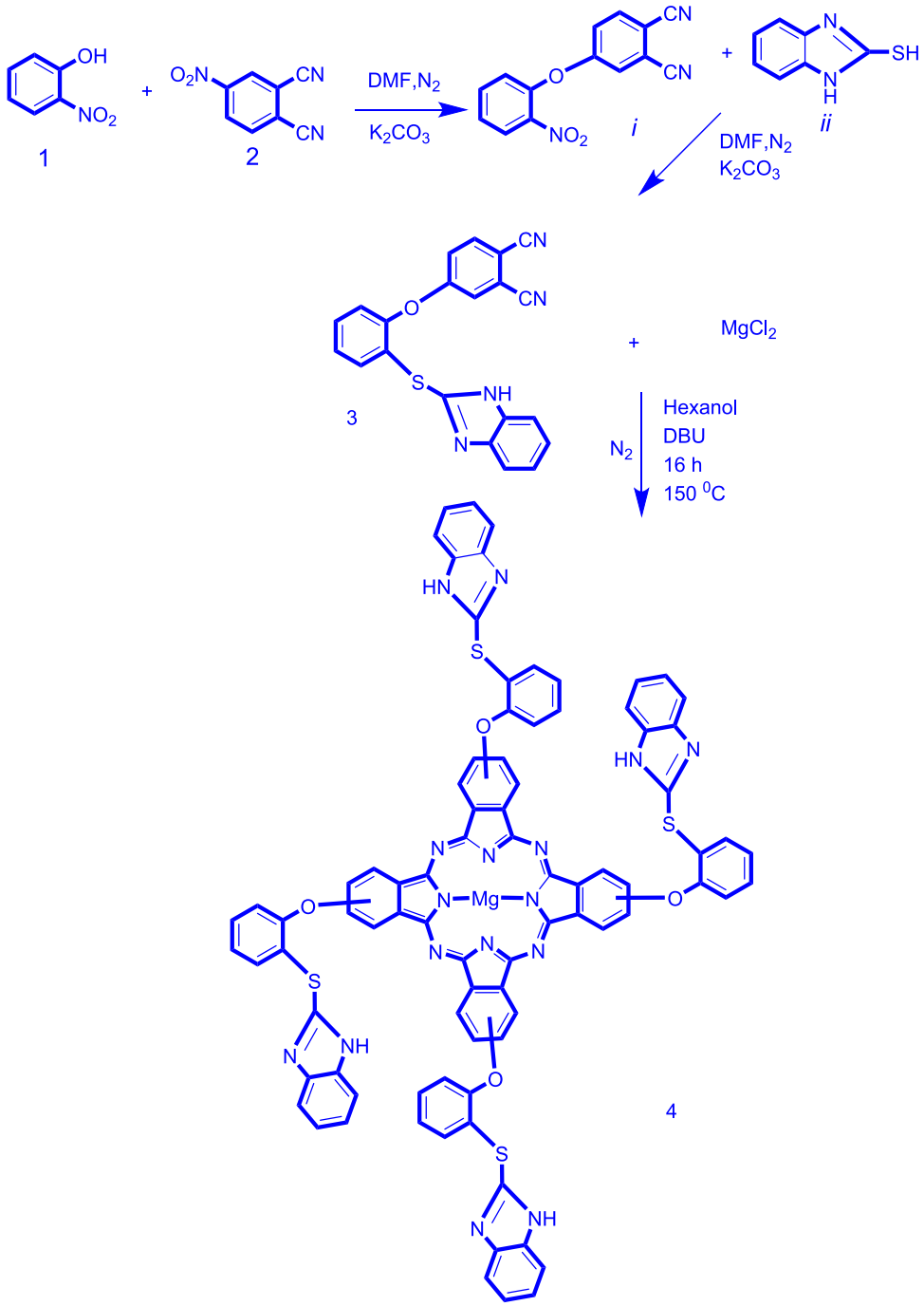
In determining the electronic properties of phthalocyanines, the UV–visible spectrum provides information about the Q and B bands specific to phthalocyanines. Here, in the UV–visible region, a Q band at 680 nm and a B band at 360 nm belonging to Magnesium phthalocyanine are observed. In addition, the band called shoulder is observed at 615 nm. The changes in the concentration of Magnesium II phthalocyanine compound in THF, DMF, DMSO are shown in the figures below (Figs. 1, 2, 3). One of the important properties sought in phthalocyanine compounds used in photodynamic therapy is the absence of aggregation. Figures 1, 2, 3 also shows the absence of aggregation (Lambert Beer law) [19, 20]. The absorption spectra of magnesium phthalocyanine compound in different solvents such as DMF, DMSO and THF are shown in Fig. 4. This also shows that the phthalocyanine compound has good solubility.

3.2 Photophysical studies

Magnesium phthalocyanine complex fluorescence spectra in THF, DMF and DMSO are shifted to red by 12, 15 and 13 nm, respectively, according to the solvents. Shift values between Stokes Absorption Q-band and fluorescence are given in Table 1. In addition, fluorescence emission, excitation and absorption spectra of this phthalocyanine in THF, DMF and DMSO solvents are given in Figs. 5, 6, 7. Fluorescence quantum yields (Φ_F) in THF, DMF and DMSO were determined as 0.36, 0.21 and 0.28, respectively. The fluorescence quantum yield values of the phthalocyanine compound were higher than the standard zinc (II) phthalocyanine [21]. When compared to similar literature studies, it is understood that the fluorescence quantum yield is at a reasonable value [22, 23]. This phthalocyanine compound exhibits high fluorescence quantum yield in different solvents, strengthening the potential of the compound to be used as a photosensitizer agent for photodynamic therapy.

3.3 Photochemical studies

In the treatment of cancer cells, singlet oxygen efficiency is an important parameter that indicates the capacity of a substance to produce singlet oxygen molecules generated by light under certain conditions. High quantum efficiency functions to convert absorbed energy into singlet oxygen production. The ability to produce singlet oxygen is a fundamental property of photosensitizers, as it exhibits cytotoxicity and damages cancer cells [24]. A photosensitizer with efficient intersystem switching has a high triplet state population and long lifetimes. This increases singlet oxygen production [25]. This phenomenon contributes significantly



Scheme 1 Schematic synthesis route of compound **3** (DMF, K_2CO_3, N_2) and compound **4** ($MgCl_2$, hexanol, N_2, 150 °C, 16 h, DBU)

to the ability of the photosensitizer to produce singlet oxygen molecules efficiently. It contributes to effectiveness in photodynamic therapy applications for cancer treatment. In photochemical study, magnesium phthalocyanine complex was dissolved in dimethyl sulfoxide (DMSO) and 1,3-diphenylisobenzofuran (DPBF) was added to it. These solutions were then exposed to light at 5 s intervals with an intensity of 7.05×10^{15} photons s^{-1} cm^{-2}. The results obtained were used to evaluate the singlet oxygen production efficiency of phthalocyanine complex (Fig. 8). The singlet oxygen yield of the compound in DMSO was found to be (Φ_Δ) = 0.13.

3.4 Antioxidant activity

Antioxidants are molecules that help protect cells from damage caused by free radicals. Free radicals can be produced by a variety of sources, including normal metabolic processes, environmental pollutants, radiation, and certain chemicals [26]. It is known that antioxidants work

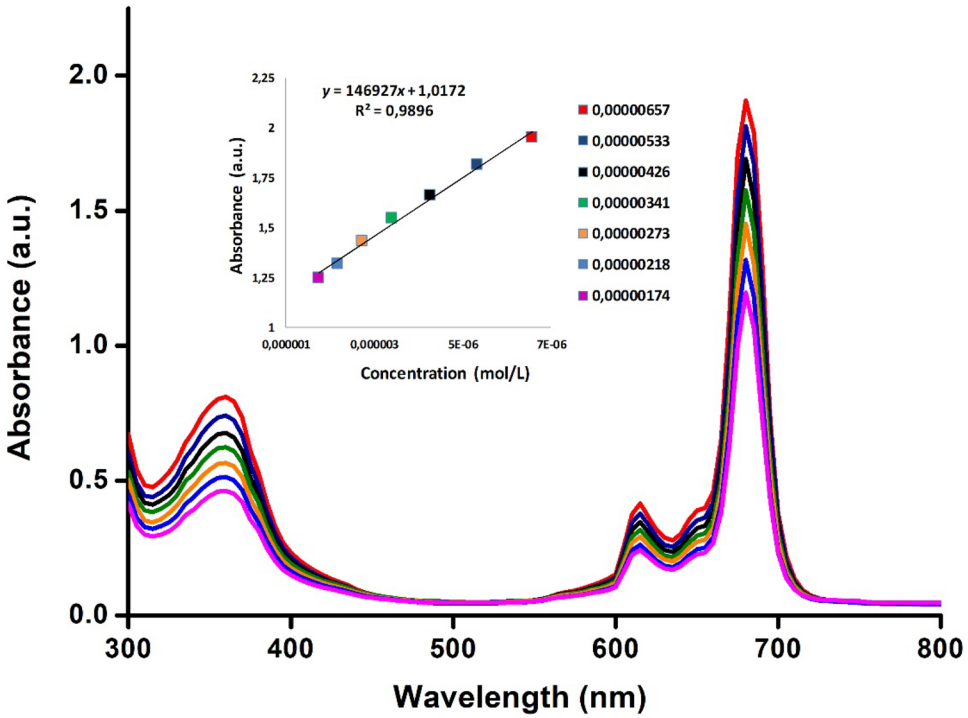


Fig. 1 Absorptions of magnesium phthalocyanine in THF at different concentrations

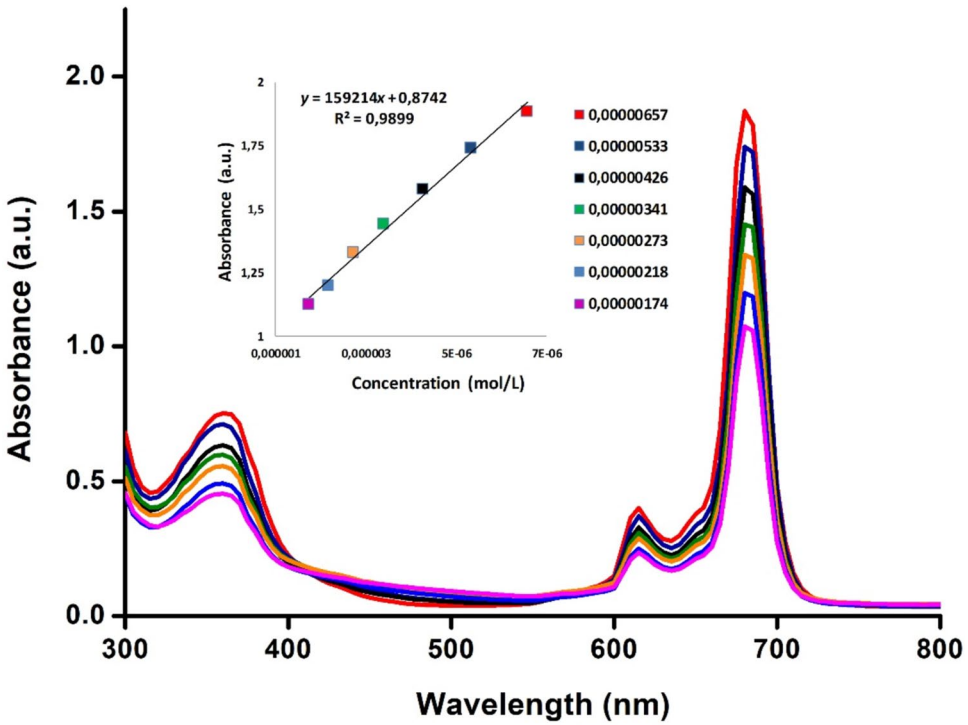


Fig. 2 The concentration dependent UV absorptions of magnesium phthalocyanine in DMF

by neutralizing free radicals in preventing them from damaging cells. They can do this by donating an electron to the free radical, which stabilizes the molecule and prevents it from reacting with other molecules in the body [27]. The antioxidant capacity of compounds **3** and **4** was evaluated using the 2,2-diphenyl-1-picryl hydrazine (DPPH) radical capture test. Both compounds demonstrated an increased scavenging ability against the DPPH radical, which showed a tendency to rise with increasing concentration (Fig. 9). The antioxidant activities of **3** and

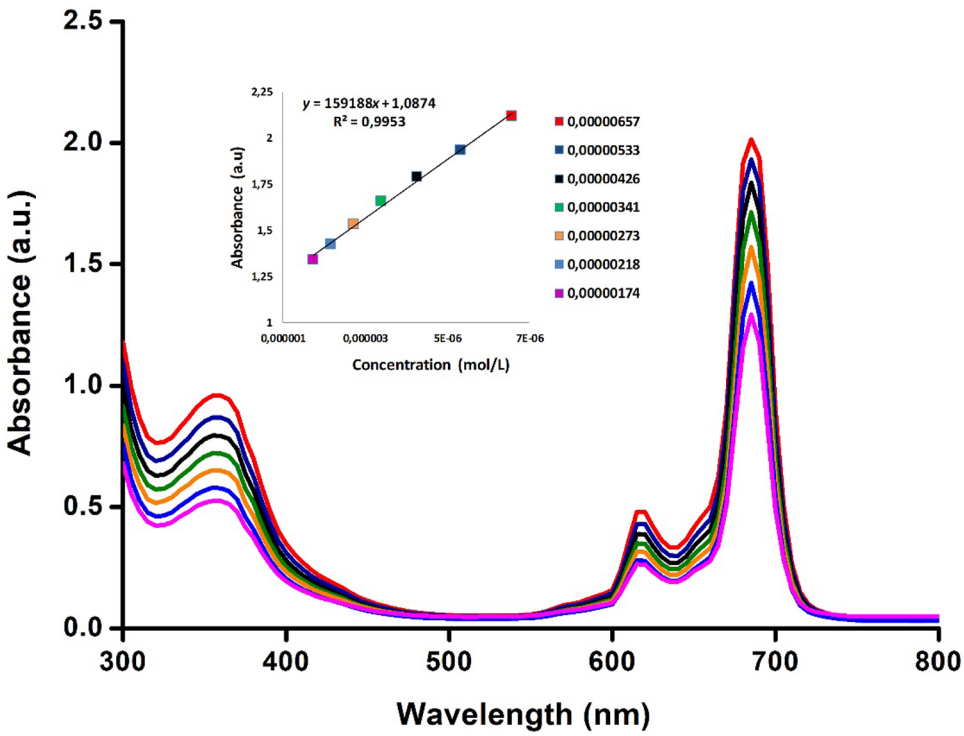


Fig. 3 Absorptions of magnesium phthalocyanine in DMSO at different concentrations

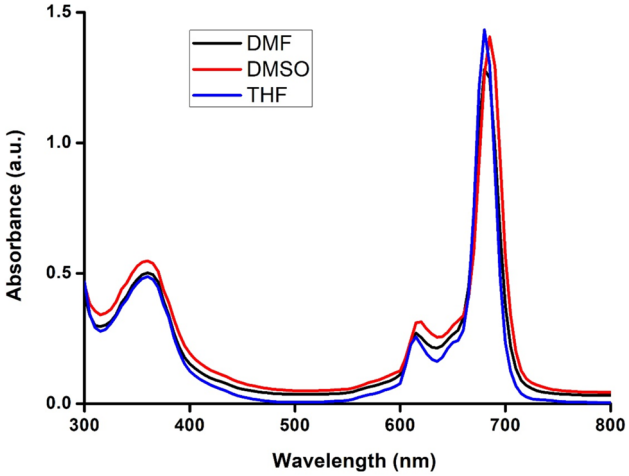


Fig. 4 UV absorptions of magnesium phthalocyanine in different solvents (1×10^{-5} M)

4 was recorded 19.95% and 37.42%, 26.41 and 42,61%, 31.82% and 46.36%, 42.55% and 59.89% at 6.25, 12.5, 25, and 50 mg/L, respectively. The highest DPPH scavenging ability of test compounds was found as 75.71% with **4** at concentration 100 mg/L. The study shows significant antioxidant properties compared to other studies that measured [28] the DPPH free radical capture capacity of compounds, and antioxidant activity was concentration dependent. Günsel et al. found that the DPPH scavenging ability of Cobalt (II) phthalocyanines was 90.33% at a concentration of 500 mg/mL [27]. In their study, Bilgiçli et al. determined the DPPH radical scavenging activities of ZnPc(3), CuPc(4) and CoPc(5) at different concentrations as 3 > 5 > 4 at the same concentrations. Among the compounds, the maximum radical scavenging activity was 37.30% for Pc compound 3 at 100 mg/mL [28]. Korkut et al. (2021) investigated the antioxidant activity of zinc (II) phthalocyanine tetranitroxide and obtained radical scavenging activity as 66.77% at 500 µg/mL [29].

Investigated the antioxidant ability of compounds and they indicated that newly synthesized compounds showed

Table 1 Fluorescence data for magnesium (II) phthalocyanine (**4**)

Compound	Solvent	Q band λmax, (nm)	Log ε	Excitation λEx, (nm)	Emission λEm, (nm)	Stokes Shift Δ Stokes, (nm)
4	THF	680	5.19	682	692	12
4	DMF	680	5.20	684	695	15
4	DMSO	685	5.19	686	698	13

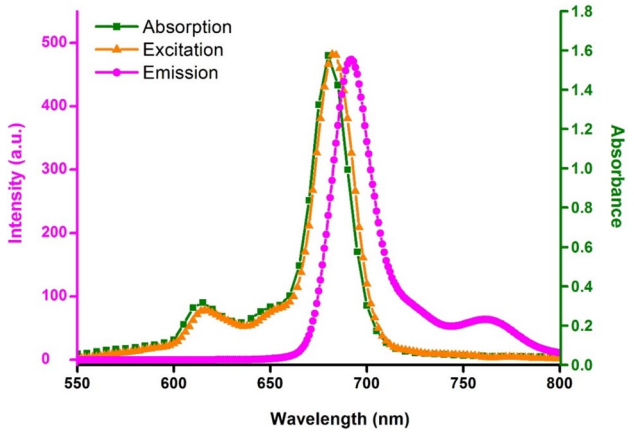


Fig. 5 Fluorescence spectra in THF

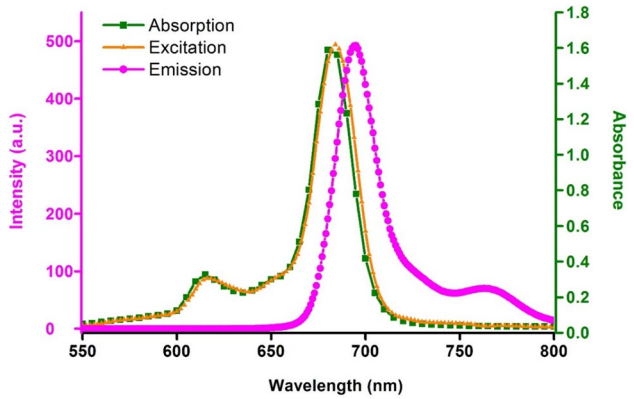


Fig. 6 Fluorescence spectra in DMF

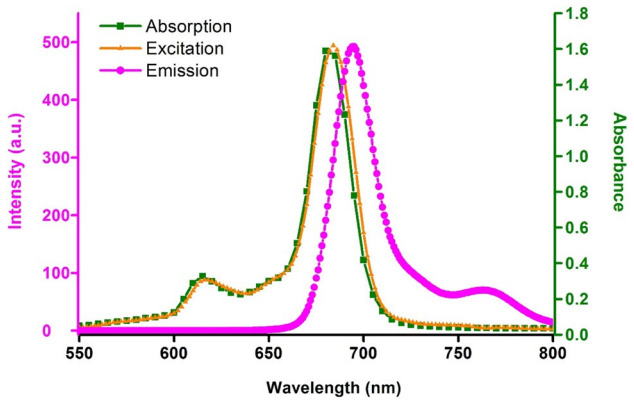


Fig. 7 Fluorescence spectra in DMSO

good antioxidant ability. It can be concluded that these new compounds can be an alternative synthetic antioxidant for humans and animals as a result of toxicological test systems.

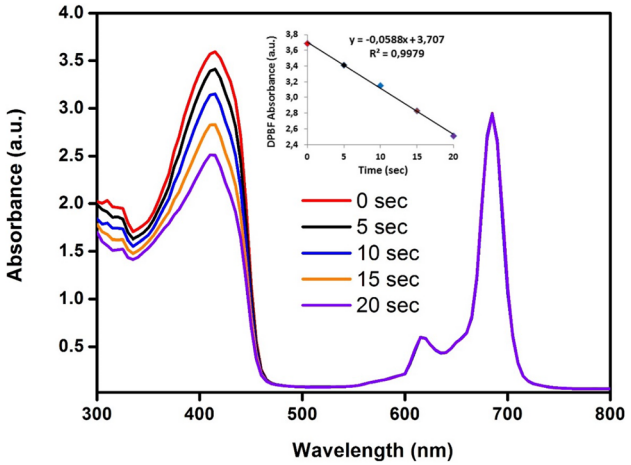


Fig. 8 Compound **4** of singlet oxygen quantum yield in 1×10^{-5} M DMSO (inset: plots of DPBF absorbance vs. time)

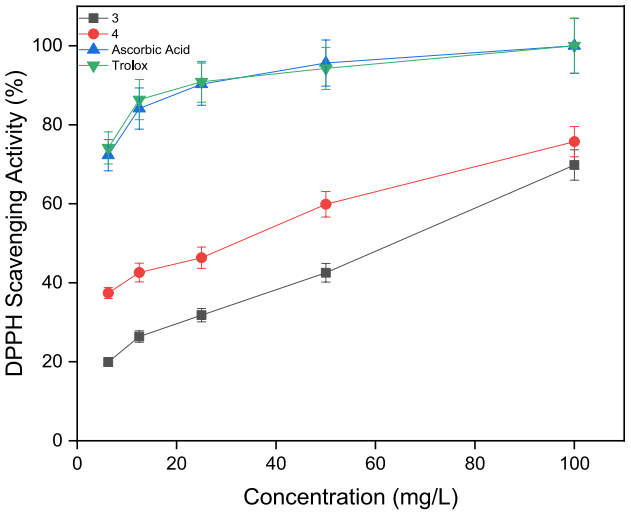


Fig. 9 DPPH Scavenging Activity

3.5 Antidiabetic activity

Characterized by high blood sugar levels, diabetes mellitus refers to a chronic medical condition. This may be due to a deficiency in insulin production or a decrease in the body's ability to use insulin. The hormone insulin is produced by the pancreas and helps regulate blood sugar. Various types of diabetes are known, such as known Type 1 and Type 2, gestational diabetes. Diabetes mellitus is the epidemic of the century, and diabetes will continue to become widespread without effective early detection methods [30]. It's important to note that diabetes medications are not a cure for diabetes, but they can help to manage the condition and prevent complications. Antidiabetic activity is a very important aspect of diabetes management.

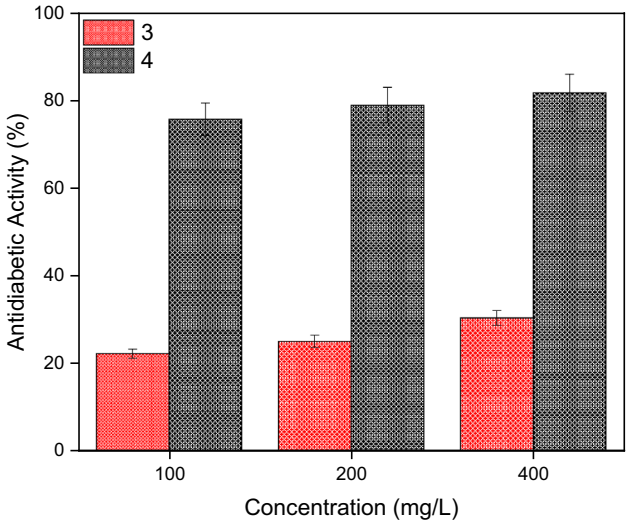


Fig. 10 Antidiabetic Activity

Antidiabetic activity refers to the potential of a substance or compound to regulate blood glucose levels by inhibiting enzymes involved in carbohydrate digestion such as α-amylase and α-glucosidase. This activity is considered to prevent postprandial increases in blood glucose levels and reduce diabetes-related complications [31]. In our study, we studied the antidiabetic activities of **3** and **4** compounds. Result is depicted in Fig. 10. As seen in Fig. 10, 4 showed higher antidiabetic activity than the **3** compound. The antidiabetic activity of **4** was recorded as 75.83%, 79% and 81.83% at 100, 200 and 400 mg/L, respectively. Gupta revealed in their study that ethyl acetate and aqueous extracts of *Terminalia bellirica* fruit had significant antioxidant and α-amylase inhibitory activity and also both extracts showed blood sugar-lowering activity in diabetic rats [32]. Rahval conducted antidiabetic studies with *Artemisia scoparia* and they noted that *A. scoparia* showed high α-glucosidase inhibitory activity [33]. Antidiabetic activities of the Pcs were also showed good agreement with these reports. There is not enough antidiabetic activity related to Pc compounds in the literature. Therefore, we think that this study will set an example for antidiabetic research studies to be conducted with Pc.

3.6 DNA cleavage activity

The DNA molecule is of vital importance for all living cells. DNA cleavage ability of organic and inorganic compounds is important, particularly for the design of anticancer and antimicrobial novel drugs nowadays. The effect of compounds on DNA cleavage ability was also investigated at 3 different concentrations (50, 100, and 200 mg/L) and *E. coli* plasmid DNA was used as target molecule. The results of DNA cleavage ability of **3** and **4** are shown in Fig. 11. In our study, DNA division activity was observed. According to our findings, **3** caused single chain fractures at 50,100 and 200 mg/L. **4** showed a single chain fracture at 50 mg/L and also it caused double strand fracture in plasmid DNA at concentrations of 100 and 200 mg/L. Uslan and Senalan reported that they investigated the interaction of novel phthalocyanines compounds with DNA molecules and they observed DNA binding and cleavage activity with new PCs [34]. Farajzadeh et al. also found that the presence of phthalocyanin complexes indicated DNA division activity [35]. The compounds are promising as chemical nuclease agents and have the potential to be used after further testing.

3.7 Antimicrobial photodynamic therapy and antimicrobial activity

The antimicrobial effect of **3** and **4** were determined by serial dilution method and result is shown in Table 2. Antimicrobial activities differ according to microorganisms. The minimum inhibition concentration (MIC) of compound **3** was found as 128 mg/L for *E. coli, L. pneumophila, E. hirae,* and *E. faecalis*; 256 mg/L for *P. aeruginosa* and *S. aureus*; 64 mg/L for *C. tropicalis* and *C. albicans*. The minimum inhibition concentration of compound **4** was found as 64 mg/L for *E. coli, P. aeruginosa, S. aureus, C. tropicalis,* and *C. albicans*; 32 mg/L for *L. pneumophila* subsp. *pneumophila*, 16 mg/L for *E. hirae, E. faecalis.* When we evaluated the results obtained from the study for **4**, it showed good antimicrobial activity against *E.*

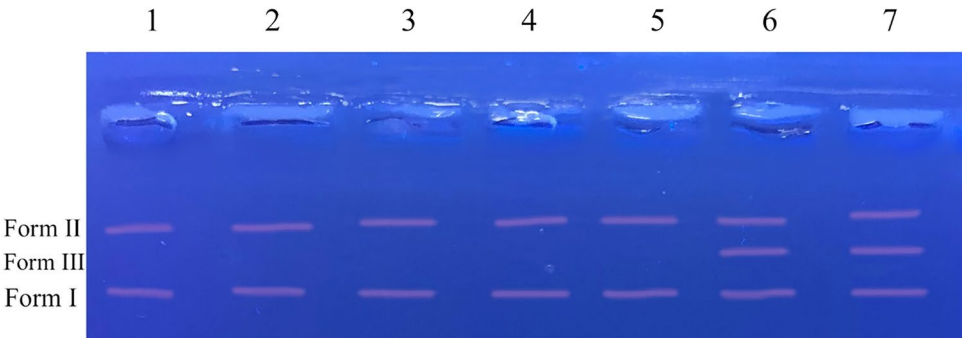


Fig. 11 DNA Cleavage activity of compounds. Lane 1, pBR 322 DNA + DMSO; Lane 2, pBR 322 DNA + 50 mg of **3**; Lane 3, pBR 322 DNA + 100 mg of **3**; Lane 4, pBR 322 DNA + 200 mg of **3**; Lane 5, pBR 322 DNA + 50 mg/L **4;** Lane 6, pBR 322 DNA + 100 mg/L **4**; Lane 7, pBR 322 DNA + 200 mg of **4**

Table 2 Antimicrobial activity

Microorganisms	MIC values (mg/L)	
	3	4
E. coli	128	64
P. aeruginosa	256	64
L. pneumophila subsp. *pneumophila*	128	32
E. hirae	128	16
E. faecalis	128	16
S. aureus	256	64
C. tropicalis	64	64
C. albicans	64	64

Table 3 Photodynamic antimicrobial Therapy

Microorganisms	MIC values (mg/L)	
	3	4
E. coli	64	16
P. aeruginosa	128	32
L. pneumophila subsp. *pneumophila*	64	8
E. hirae	64	8
E. faecalis	128	4
S. aureus	128	16
C. tropicalis	64	32
C. albicans	32	16

faecalis and *E. hirae* as MICs of 16 mg/L. The lowest antimicrobial activity for **3** was found as 256 mg/L against *P. aeruginosa* and *S. aureus*. In our study, it was determined that **4** had remarkable antibacterial activity especially for *E. hirae* and *E. faecalis*. **4** also showed more effective antimicrobial activity than the **3**. According to their in vitro study, Unluer et al. indicated that they investigated the antimicrobial ability of compounds. It was found that **3** and **4** had remarkable antimicrobial activity against *S. typhimurium* and *E. coli*. [36]. Basappa et al. synthesized and charaterized water-soluble metallophthalocyanines (MPc) [37]. Antimicrobial activiy of MPc screened against *S. aureus, K. pneumoniae, P. aureus, E. coli, A. niger,* and *C. albicans* strains. As a result, they revealed that the complexes are more active as antibacterial than antifungal. Antimicrobial photodynamic therapy (aPDT) is a type of therapy that uses a combination of a photosensitizer, light, and oxygen to selectively destroy microbial cells. aPDT has been shown to be effective against a variety of microorganisms, including bacteria, viruses, fungi, and parasites [38]. aPDT has become one of the most promising antimicrobial treatment methods in recent years. aPDT is a relatively new treatment, and more research is needed to determine its potential side effects. For compounds **3** and **4** the antimicrobial photodynamic therapy activities tested herein were also investigated. aPDT effect of **3** and **4** were determined (Table 3). Results showed that photodynamic antimicrobial therapy activities varied according to microorganisms. Photodynamic antimicrobial therapy of **3** was found as 64 mg/L for *E. coli, L. pneumophila* subsp. *pneumophila, E. hirae,* and *C. tropicalis*; 128 mg/L for *P. aeruginosa, E. faecalis,* and *S. aureus;* and 32 mg/L for *C. albicans*. aPDT of **4** was as 16 mg/L for *E. coli, S. aureus,* and *C. albicans*; 32 mg/L for *P. aeruginosa* and *C.tropicalis;* 8 mg/L for *L. pneumophila* subsp. *pneumophila* and *E. hirae*; and 4 mg/L for *E. faecalis*. **4** also displayed more effective aPDT than **3**. When we evaluated the results from the study for **4**, the best photodynamic antimicrobial therapy against *E. feacalis* was found to be 4 mg/L. Akin et al. investigated aPDT activity of sulfonated PCs in their work [39]. They found that when sulfonated PCs exposed to light, the inhibitory effect against the test microorganisms increased. George et al.) concluded in their study that cationic zinc phthalocyanin was highly phototoxic to the *E. coli* wild type upon 2 h of illumination [40, 41]. Ozturk et al. found that ZnPc had strong inhibitory effects against *C. albicans* after light irradiation [42]. In addition to Pc compounds, there have been aPDT studies on different compounds in the literatures. Zhang et al. examined photodynamic antibacterial therapy of ZnO/Bracket and it was determined that antimicrobial efficiencies of ZnO/Bracket material increased against *E. coli*, *S. mutans* and *S. aureus* strains after aPDT applications. Lee et al. noticed that TTVP was highly evident for the selective inactivation of Gram-positive bacteria via the photodynamic antibacterial pathway, implying its promising application as an effective in-vivo bactericide [43]. When Table 2 and Table 3 were compared, aPDT results were more effective than antimicrobial activity. The basic principle of aPDT is based on the activation of a photosensitizer, which reacts to light and leads to the formation of ROS, which kills microorganisms [44]. We can explain the reason why the aPDT results result in a more effective antimicrobial activity with the ROS released. The ROS can react differently to their target molecules such as proteins, enzymes, DNA etc. and this leads to microbial death. aPDT and Antimicrobial results, new compounds may be effective as antimicrobial agents for pathogenic microorganisms with further testing. Therefore, the compounds are expected to contribute to the development of new drugs.

4 Antimicrobial activity

4.1 Microbial cell viability inhibition

Microbial cell viability inhibition refers to the ability of a substance or compound to prevent the growth or survival of microorganisms, such as bacteria or fungi. Since man

has existed threatened by infections caused by pathogenic bacteria. *S. aureus* and *E. coli* are common pathogens in everyday life. They cause diseases such as diarrhea and urinary tract infection. In recent years, the fight against bacteria has become more difficult due to the growing threat of multidrug resistance and the shortage of new antibiotics in the pipeline [45]. In the current situation, it has become a necessity to develop new treatment methods for effective antibacterial treatment. Different concentrations of compounds were also tested to assess viability inhibition activity against *E. coli*. The effects of 3 and 4 on microbial cell viability of *E. coli* are shown in Fig. 12. As seen in Fig. 12, 3 and 4 show very effective microbial cell viability inhibition activity. **3** inhibited 100% of microbial cell viability at a concentration of 100 g/L, while **4** inhibited 100% of microbial cell viability at 50 mg/L and 100 mg/L. Türkan et al. indicated that they tested the antimicrobial efficiency of zinc and cobalt(II) Pc complexes and it was found that zinc and cobalt(II) Pc complexes displayed effective microbial inhibition against *E coli*. [46]. Farajzadeh et al. also examined the several biological properties of hexadeca substitute metallo Pcs [47]. Among these biological properties *E. coli* viability inhibition was also studied. They noted that the most powerful *E. coli* viability inhibition was achieved as 100% with lutetium and cobalt Pcs. As a result, newly synthesized Pcs can be used for microbial inhibition after following studies.

4.1.1 Biofilm inhibition

Biofilm is a complex community of microorganisms, such as bacteria, fungi, and algae, that adhere to surfaces and produce a protective extracellular matrix made up of proteins, carbohydrates, and DNA. The matrix protects the microorganisms from environmental stresses, antibiotics and host immune responses, making biofilms notoriously difficult to eradicate. For bacteria in the biofilm, this matrix acts as a shield and protects them from the immune response of the host cells [48]. Therefore, preventing the formation of biofilms alternative

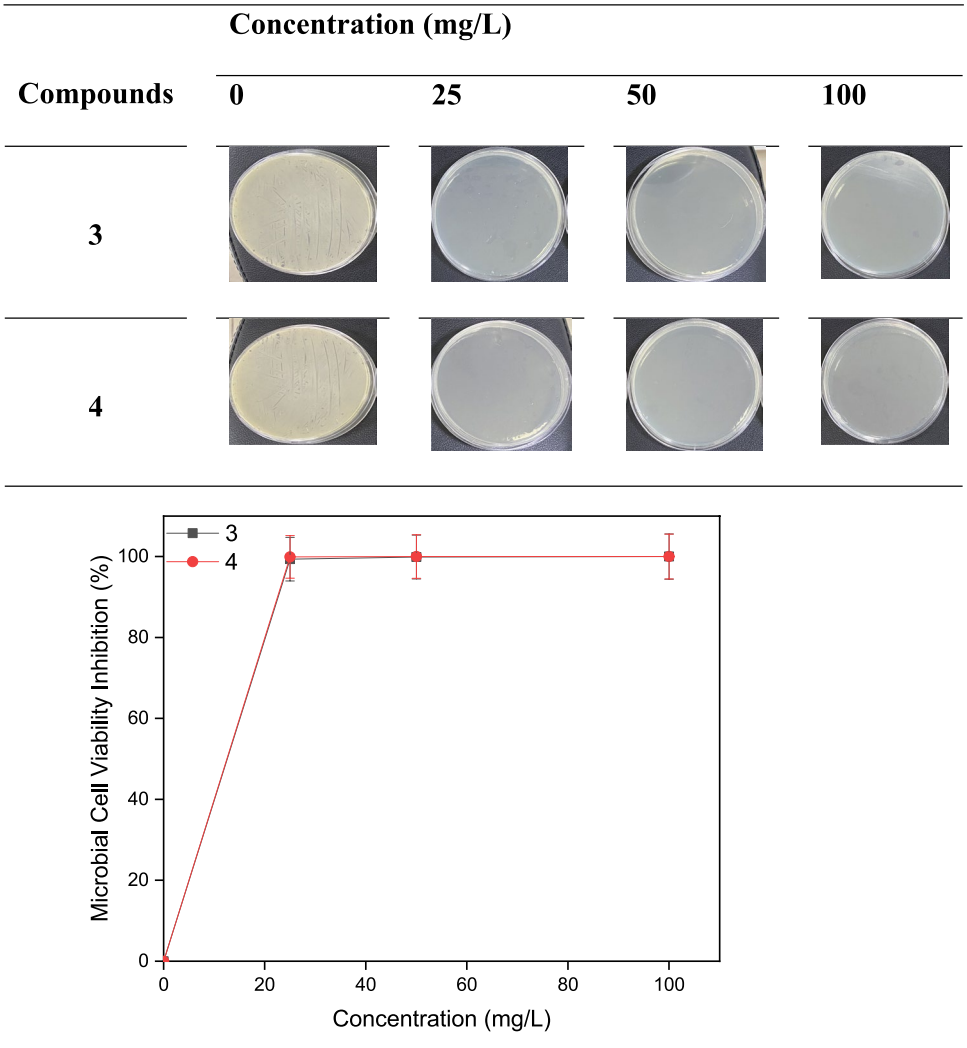


Fig. 12 Microbial Cell Viability Inhibition

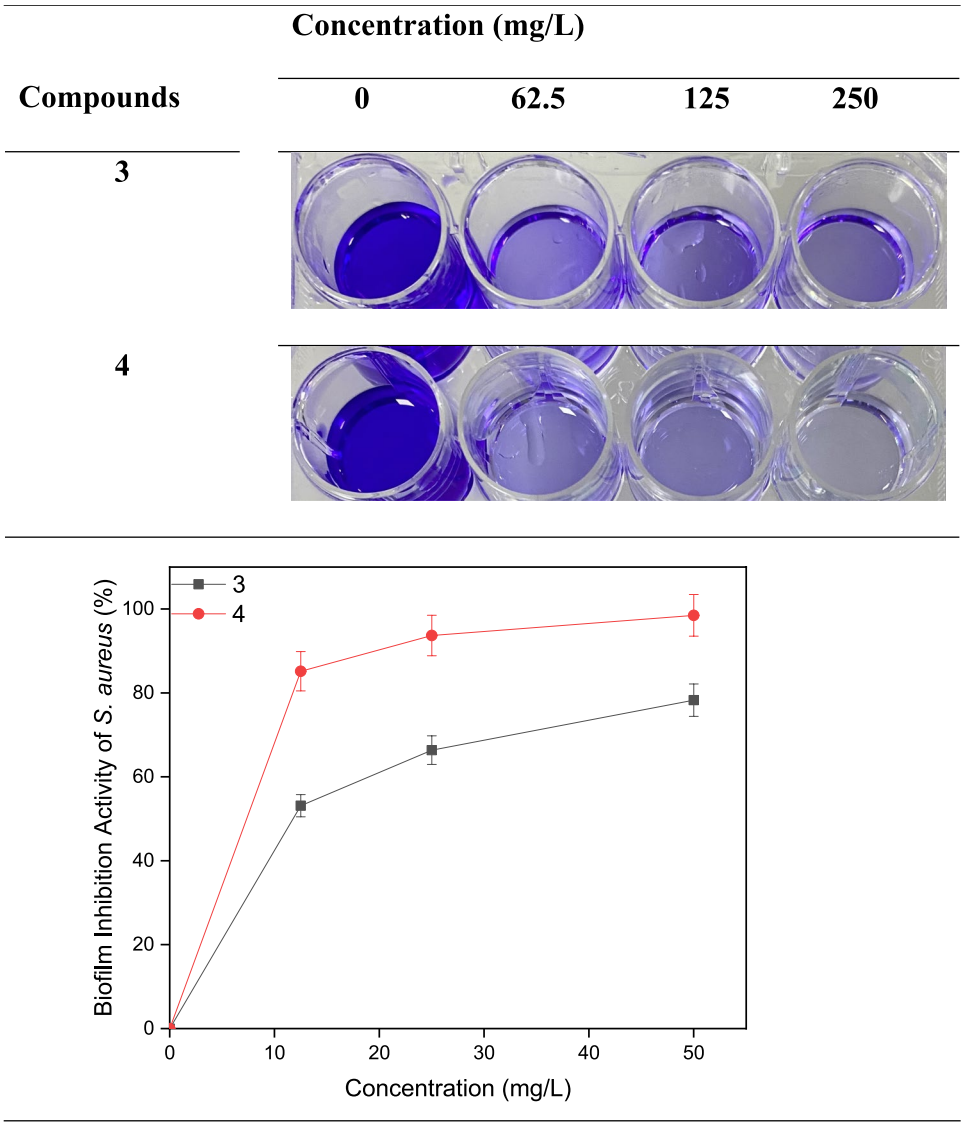


Fig. 13 Biofilm Inhibition of *S. aureus*

solutions are required. The biofilm inhibition effect of **3** and **4** compounds was tested using pathogenic *P. aerigunosa* and *S. aureus* for different concentrations (12.5, 25 and 50 mg/). The results are shown in Fig. 13 and 14. Biofilm inhibition activity was concentration-dependent. The biofilm inhibition activities of **3** against *S. aureus* and *P. aerigunosa* were 53.14%, 66.37%, and, 78.28% and 50.96%, 60.52%, and 73.95% at 12.5 mg/L, 25 mg/L, and 50 mg/L concentrations, respectively. Also, the biofilm inhibition activities of **4** against *S. aureus* and *P. aeruginosa* were determined as 85.17%, 93.68%, and 98.49% and 77.29%, 82.19%, and 91.13% at 12.5 mg/L, 25 mg/L, and 50 mg/L concentrations, respectively. When the study results are compared, it is seen that the biofilm inhibition percentages of **4** are better than **3**. Gao et al. stated that they studied biofilm inhibition of $ZnPc^{n+}$ and they found that $ZnPc^{n+}$ effectively inhibited the biofilm formation of *S. aureus*. [49] Openda et al. prepared ZnPc and InPc compounds [50]. They investigated the effect of ZnPc and InPc compounds on biofilm inhibition of *E. coli* and *S. aureus* in their study. They reported that biofilm cell survival for *E. coli* and *S. aureus* was less than 3% for *S. aureus* and less than 5% for *E coli.* Accordingly, compound **3** and **4** showed effective biofilm inhibition activity against *P. aeruginosa* and especially *S. aureus.* For various purposes, compounds **3** and **4** can be applied as biofilm inhibitors in in vivo studies.

5 Conclusion

Originally a phthalonitrile as the starting material of phthalocyanine complex and a magnesium phthalocyanine compound were synthesized. Characterization of these compounds was done by UV–vis, ^{1}H NMR, ^{13}C NMR, FTIR and Mass spectra. The aggregation behavior of the magnesium phthalocyanine

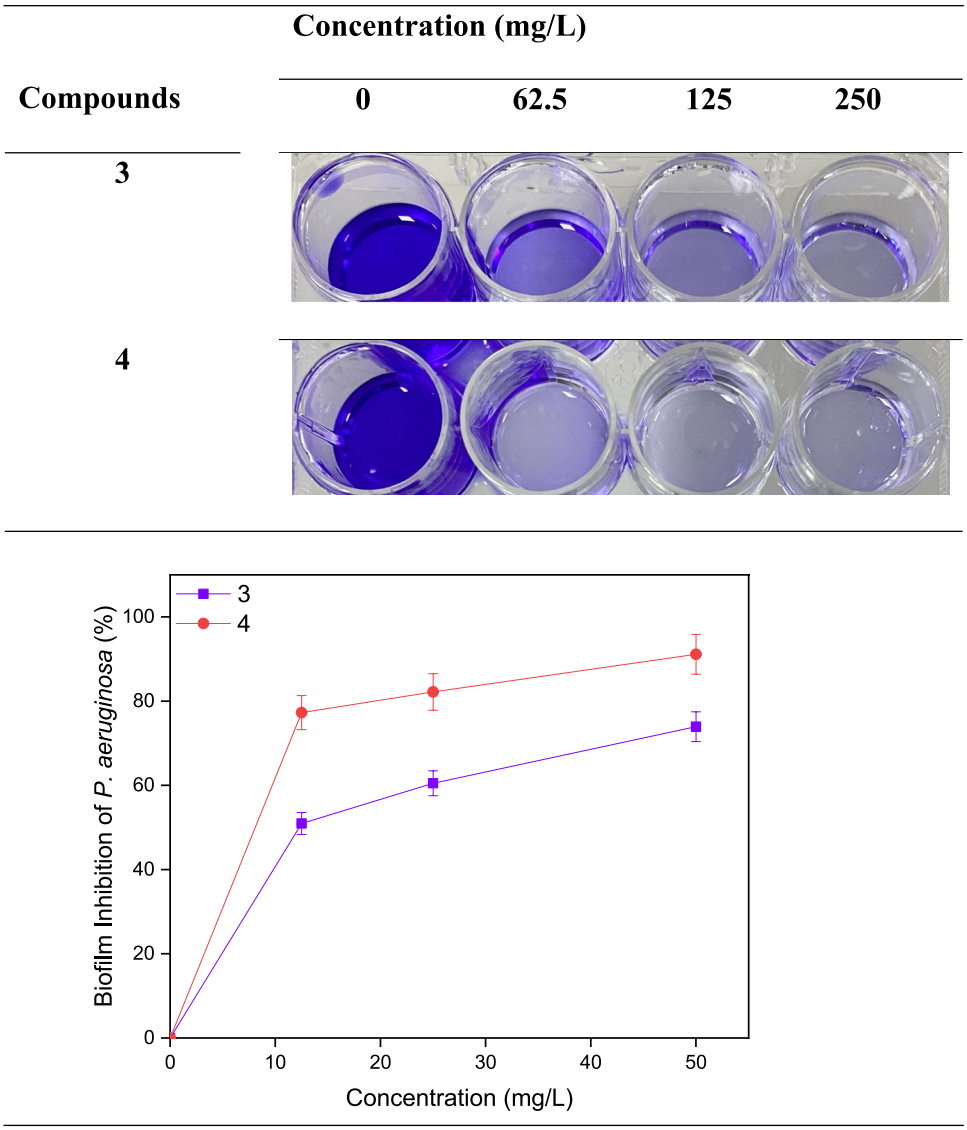


Fig. 14 Biofilm Inhibition of *P. aeruginosa*

compound was investigated in different solvents and different concentrations. It was observed that the compound did not aggregation in the working solvents. Based on the study, **3** and **4** displayed antioxidant potential through their ability to scavenge the DPPH radical and **4** showed higher antioxidant activity than **3**. The compounds also exhibited good antidiabetic activity and this property can be made them potential alternatives to Type 2 diabetes drugs. The ability of the compounds to cleavage DNA was also investigated. **3** and **4** exhibited DNA cleavage activity in plasmid DNA. Both **3** and **4** had good antimicrobial and APDT activities and also **4** demonstrated more effective antimicrobial and APDT activities against the test microorganisms than **3**. The compounds **3** and **4** also displayed effective microbial cell viability inhibition activity. The biofilm inhibition activity of **3** and **4** was also studied against *S. aureus* and *P. aeruginosa*. They caused influential anti-biofilm activity. Overall, the study suggests that **3** and **4** compounds have potential as alternative therapeutic agents for various medical conditions. Further studies are needed to determine their efficacy and safety in clinical settings.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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