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SYNTHESIS, CHARACTERIZATION, ELECTROCHEMICAL PROPERTIES OF METAL-FREE AND METALLOPHTHALOCYANINES BEARING 2-BENZYLPHENOXY SUBSTITUENTS AND INVESTIGATION OF THEIR BIOLOGICAL ACTIVITIES

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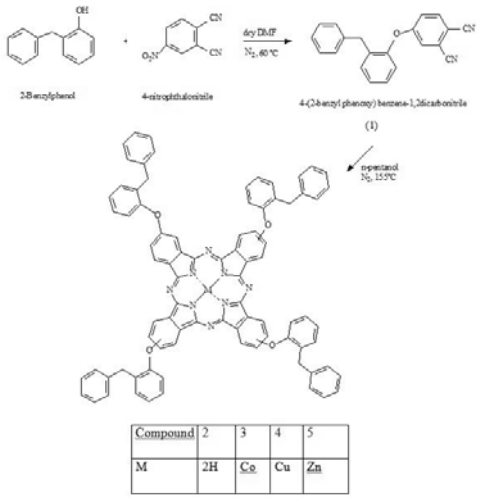
The metal-free phthalocyanine (H_2Pc) and metallophthalocyanines complexes (CoPc, CuPc and ZnPc) were synthesized by the reaction of dinitrile derivatization in dry n-pentanol by a classical method. The structures and electrochemical properties of the new compounds were characterized by using spectroscopic data, elemental analysis and electrochemical studies. Voltammetric analyses of phthalocyanines supported the proposed structure of the synthesized complexes. Antimicrobial and mutagenicity activities and DNA binding properties of the compounds were also investigated by minimum inhibitory concentration and salmonella/microsome assays and agarose gel electrophoresis. This study showed that all compounds exhibited efficient DNA-binding activity. In conclusion, non-toxic compounds may be used as effective DNA dyes for molecular biology studies and be used as anticandida drugs.



Compound	2	3	4	5
M	2H	Co	Cu	Zn

INTRODUCTION

Metal-free and metallophthalocyanines (MPcs) have been intensively investigated since their first synthesis at early last century.[1] Phthalocyanines (Pcs) are highly preferred compounds for industrial production due to their characteristics and properties. Pcs that are completely synthetic materials are used in electrophotography,[2] electrochemistry,[3] medicine,[4] optic data collection,[5] gas sensor,[6] liquid crystal,[7] laser technology[8] and the photodynamic therapy of tumors[9] as well as their classical fields. Tuning of the properties of Pcs is generally achieved through changes in the nature and bonding pattern of the substituents.[10-20] Their intense blue and green colours and stabilities towards heat, acids and bases allow them to be extensively used as pigments and dyes.[21-30] Many spectroscopic techniques such as magnetic circular dichroism (MCD), fluorescent spectroscopy and UV-vis spectroscopy, which take advantages of optical properties of PCs, have been utilized to characterize the excited states of H_2Pc and MPc.[31]

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Pcs might develop a wide range of interesting and potentially useful properties by either replacing the N-H protons in the centre of the ring by a metal ion or introducing a variety of substituent groups at the peripheral and non-peripheral sites of Pc ring.[32]

In this article, a new compound, 4-(2-benzyl phenoxy) benzene-1,2dicarbonitrile and its tetra-substituted metal free phthalocyanine (H_2Pc) and metallophthalocyanines (CoPc, CuPc, and ZnPc) are described. Also in this study, we have investigated electrochemical properties, antimicrobial acitivities, interactions with DNA and mutagenicity of the newly synthesized phthalocyanines complexes.

RESULTS AND DISCUSSION

4-(2-Benzyl phenoxy) benzene-1,2dicarbonitrile (1) was prepared as a result of the reaction between 2-benzylphenol and 4- nitrophthalonitrile at 60 ºC in dry DMF. The yield was obtained as 69 %. In the IR spectrum of compound **1**, stretching vibrations of O-H band at 3516-3240 cm^{-1} disappeared and C≡N groups were observed at 2231 cm^{-1}. ^{1}H-NMR spectrum of compound **1** indicated aromatic protons at 7.60, 7.34, 7.15, 7.01, 6.96 and aliphatic protons at 3.92 ppm. The ^{13}C NMR spectrum of compound **1** indicated the presence of nitrile carbon atoms (C≡N) in **1** at 112.68 ppm. The MS spectrum of compound **1** displayed the 314 $[M+4]^+$ parent ion peak at m/z= 310, confirming the structure.

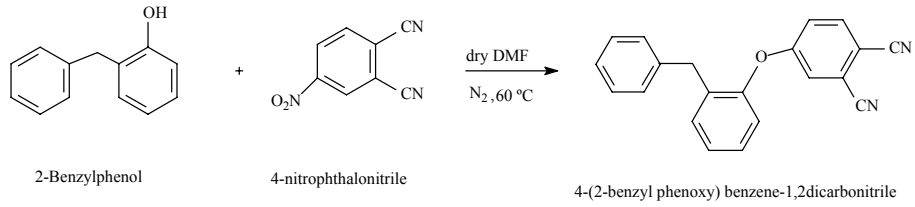
The mode of preparation of H_2Pc (2) and metallophthalocyanines (3-5) shown in scheme 1.

We synthesized H_2Pc (2) by a classical method, using n-pentanol, few drops of diazabicyclo[5.4.0]undec-7-ene (DBU) at boiling point under nitrogen gas and the yield was obtained as 28 %. Template effect of metal ions plays an important role in formation of some phthalocyanines.[33] Stretching vibration observed for starting compound **2** at 2231 cm^{-1}, which is characteristic for C≡N groups, was not observed in IR spectrum of compound **2**, however, N-H stretching vibrations were seen in phthalocyanine ring at 3290 cm^{-1}. It was concluded that these demonstrated the formation of the relevant structure as a result of cyclotetramerization. The ^{1}H-NMR spectrum of compound **2** indicated aromatic protons at 8.16, 7.34 ppm and aliphatic protons at 4.25.[34] The inner core protons N-H of this compound **2** could not be observed because of the probable strong aggregation of the molecule.[35] The ^{13}C NMR spectrum of compound **2** was confirmed by disappearance of the sharp -C≡N vibration at 112.68 ppm. The MS spectrum of compound **2** displayed the 1243 $[M]^+$ parent ion peak at m/z=1243, approving the structure.

Metallophthalocyanines **3-5** were synthesized by a classical method, using n-pentanol under nitrogen gas. The metallophthalocyanines compounds **3-5** were obtained from dry metal salts of $CoCl_2$, $CuCl_2$ and $Zn(CH_3COO)_2$. In the IR spectrum of metallophthalocyanines **3-5**, cyclotetramerization of dinitrile **1** to phthalocyanines **3, 4, 5** was confirmed by the dissappearance of the sharp -C≡N vibration at 2231 cm^{-1}. The ^{1}H-NMR spectrum of compound **5** was almost identical to that of H_2Pc (2), except for broad signals in compound **3**, from aggregation of planar phthalocyanines at the high concentration used for NMR measurements. ^{1}H-NMR spectra of compounds **3, 4** were precluded owing to their paramagnetic nature. MS spectra of compounds **3-5** showed molecular ion peaks at m/z= 1346 $[M+2Na]^+$, 1306 $[M+H]^+$, 1308 $[M+2H]^+$, respectively, confirming the proposed structure.

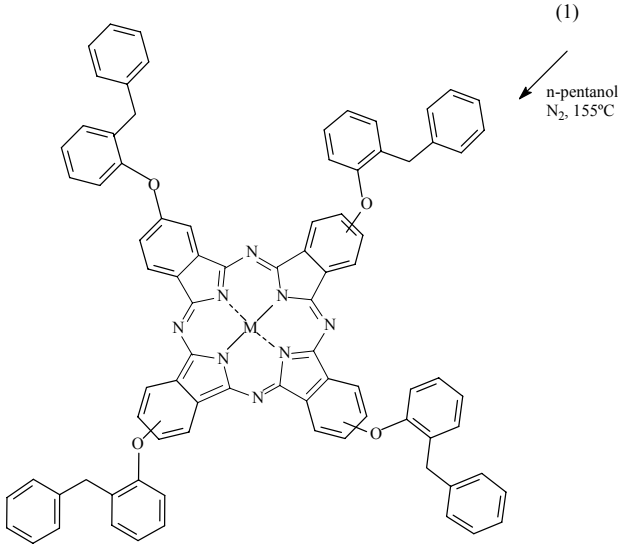
H_2Pc (2) and metallophthalocyanines (3-5) showed typical UV-visible spectra with two significant absorption bands. One of them was in the visible region at about 614-728 nm corresponding to the Q band, and the other was in the UV, approximately at 300 nm. The split Q bands, which were characteristic for metal-free phthalocyanines, were observed in compound **2** at λ_{max}= 704, 668, 644 and 608 nm (Fig. 1). These Q band absorptions showed the monomeric species with D_{2h} symmetry and due to the phthalocyanine ring related to conjugated 18π electron system. The presence of absorption band in compound **2** in the near UV region at λ_{max} = 344 nm showed Soret region B band which was ascribed to the deeper π-π* levels of LUMO transitions.[36]

The UV–vis absorption spectra of metal-lophthalocyanines **3-5** (Fig. 1) 1×10^{-5} M in chloroform showed intense Q absorption at λ_{max} = 674, 683, 682 nm, respectively with weaker absorptions at 607, 613, 615 nm, respectively. The single Q band in metallo derivatives **3-5** was characteristic. This result was typical of metal complexes of substituted and unsubstituted metallophthalocyanines with D_{4h} symmetry. B band absorptions of compounds **3-5** were observed at λ_{max} = 327, 338, 350 nm, respectively as expected.[29]



2-Benzylphenol 4-nitrophthalonitrile 4-(2-benzyl phenoxy) benzene-1,2dicarbonitrile

(1)



Compound	2	3	4	5
M	2H	Co	Cu	Zn

Scheme 1 – The synthesis of the metal-free phthalocyanine and metallophthalocyanines derivatives. Reagents and conditions (i) dry DMF, K_2CO_3, 60°C, 96 h.; (ii) n- pentanol, DBU, 155°C, (iii) dry $CoCl_2$, dry $CuCl_2$, dry $Zn(CH_3COO)_2$, n-pentanol, 155°C.

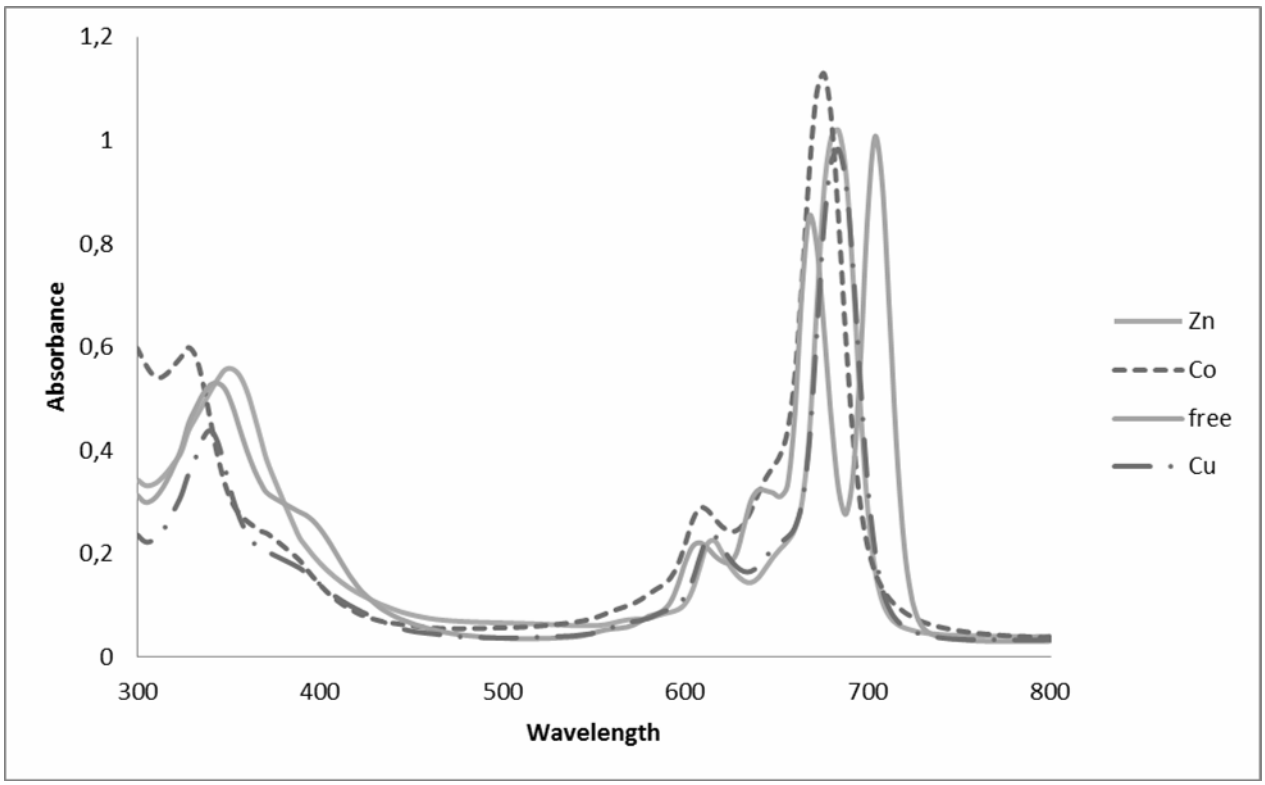


Fig. 1 – Absorption spectra of H_2Pc (2) and metallophthalocyanines (3-5) 1×10^{-5} M in chloroform.

Table 1

Voltammetric data of the phthalocyanines. All voltammetric data were given *versus* SCE

Complexes	Redox processes	[a]$E_{1/2}$	[b]ΔE_p (mV)	[c]$\Delta E_{1/2}$
H_2Pc (2)	R_1	-0.67	145	1.51
	R_2	-0.98	143	
	O_1	0.84	123	
	O_2	1.10	147	
$Cu^{II}Pc$ (4)	R_1	-0.80	118	1.57
	R_2	-1.10	134	
	O_1	0.77	90	
	O_2	0.93	106	
$Co^{II}Pc$ (3)	R_1	-0.17[d]	135	0.87
	R_2	-1.37[e]	190	
	O_1	0.70	173	
$Zn^{II}Pc$ (5)	R_1	-0.99	165	1.74
	O_1	0.75	360	

[a]: $E_{1/2}$ values ((E_{pa}+E_{pc})/2) were given *versus* SCE at 0.100 Vs^{-1} scan rate. [b]: $\Delta E_p = E_{pa}$-E_{pc}. [c]: $\Delta E_{1/2} = E_{1/2}$ (first oxidation)- $E_{1/2}$ (first reduction). [d]: This process is assigned to $[Co^{II}Pc^{2-}]/[Co^{I}Pc^{2-}]^{1-}$. [e]:This process was assigned to $[Co^{I}Pc^{2-}]^{1-}/[Co^{I}Pc^{3-}]^{2-}$.

Electrochemical studies

Voltammetric analyses of H_2-Pc (2), $Co^{II}Pc$ (3), $Cu^{II}Pc$ (4), $Zn^{II}Pc$ (5) were carried out in dichloromethane (DCM)/tetrabutylammoniumperchlorate (TBAP) electrolyte system on a Pt working electrode with (CV) and (SWV) techniques. Voltammograms of the complexes were analyzed to derive fundamental electrochemical parameters including the half-wave peak potentials ($E_{1/2}$), peak to peak potential separations (ΔE_p), the difference between the first oxidation and reduction processes ($\Delta E_{1/2}$). The results of voltammetric analyses were given in Table 1.

H_2-Pc (2) and $Cu^{II}Pc$ (4) showed similar voltammetric responses, thus CV and SWV graphs of compounds **2** and **4** were represented in Fig. 2 and Fig. 3, respectively. As shown in Fig. 2a, H_2-Pc (2) gave two reversible reduction processes, R_1 at -0.67 V (ΔE_p=145 mV), R_2 at -0.98 V (ΔE_p=143 mV) and two reversible oxidation processes O_1 at 0.84 V (ΔE_p=123 mV), O_2 at 1.10 V (ΔE_p=144 mV) within the potential window of DCM/TBAP electrolyte system. Similarly, as shown in Fig. 3a, $Cu^{II}Pc$ (4) gave two reversible reduction processes, R_1 at -0.80 V (ΔE_p=118 mV), R_2 at -1.10 V (ΔE_p=134 mV) and two reversible oxidation processes O_1 at 0.77 V (ΔE_p=90 mV), O_2 at 0.93 V (ΔE_p=106 mV). SVWs of H_2-Pc (2) and $Cu^{II}Pc$ (4) clearly supported these reversible characters of the processes, since these couples showed symmetric cathodic peaks with the same peak currents (Fig. 2b H_2-Pc (2), Fig. 3b for H_2-Pc (2)). Similar voltammetric responses were observed with different H_2-Pc (2) and $Cu^{II}Pc$ (4) in our previous papers. [38, 39]When compared with H_2Pc,[38] reduction processes of the 4-(2-benzylphenoxy) group substituted metal-free phthalocyanine (2) shifted toward the positive potentials due to the electron withdrawing effects of the substituents. Also, when compared with CuPc,[39] reduction processes of the 4-(2-benzylphenoxy) group substituted copper(II) phthalocyanine (4) shifted toward the positive potentials due to the electron withdrawing effects of the substituents.

$Co^{II}Pc$ (3) has a redox active Co^{2+} metal center. Co^{2+} ion in Pc core could give redox processes if the d orbitals were located between the HOMO and LUMO orbitals. If Co^{2+} ions gave redox processes, these processes were generally recorded at less positive and negative potentials. As shown in Fig. 4, first reduction couple of $Co^{II}Pc$ (3) shifted to less negative potential owing to the metal based character of this process. In addition to R_1 at -0.17 V, one Pc based reduction R_2 was also observed at -1.37 V. Also, $Co^{II}Pc$ (3) gave one oxidation process, O_1 at 0.70 V (ΔE_p=173 mV). Fig. 5 represented the CV and SWV responses of $Zn^{II}Pc$ (5) recorded in the cathodic and anodic potential side of the TBAP/DCM electrolyte system on a Pt working electrode. $Zn^{II}Pc$ (5) gave a ligand based on quasi-reversible reduction couple R_1 at -0.99 V (ΔE_p= 160 mV) and one irreversible oxidation process O_1 at 0.75 V (ΔE_p=360 mV) during the cathodic and anodic potential scans. Also, the peak currents increased linearly with the square root of the scan rates for scan rates ranging from 25 to 500 $mV.s^{-1}$ for phthalocyanines (Fig. 6a as an example for H_2Pc (2), Fig. 6b as an example for $Cu^{II}Pc$ (4). This linearity was confirmed by the graphic of square root of scan rate *versus* peak current (Figure 7a for H_2Pc (2) and Figure 7b for $Cu^{II}Pc$ (4)).

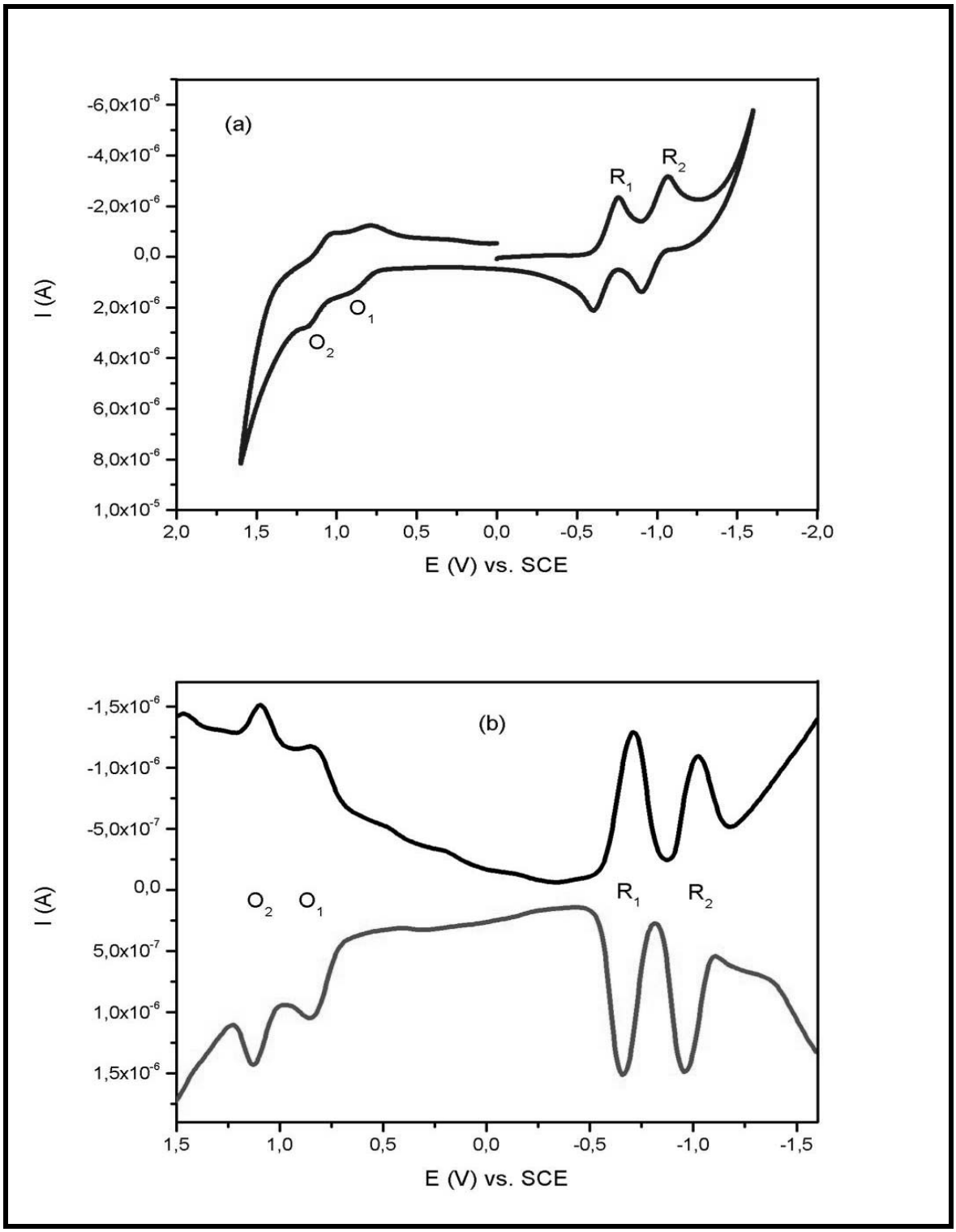


Fig. 2 – (a) CV of H_2-Pc (2). (b) SWV of H_2-Pc (2).

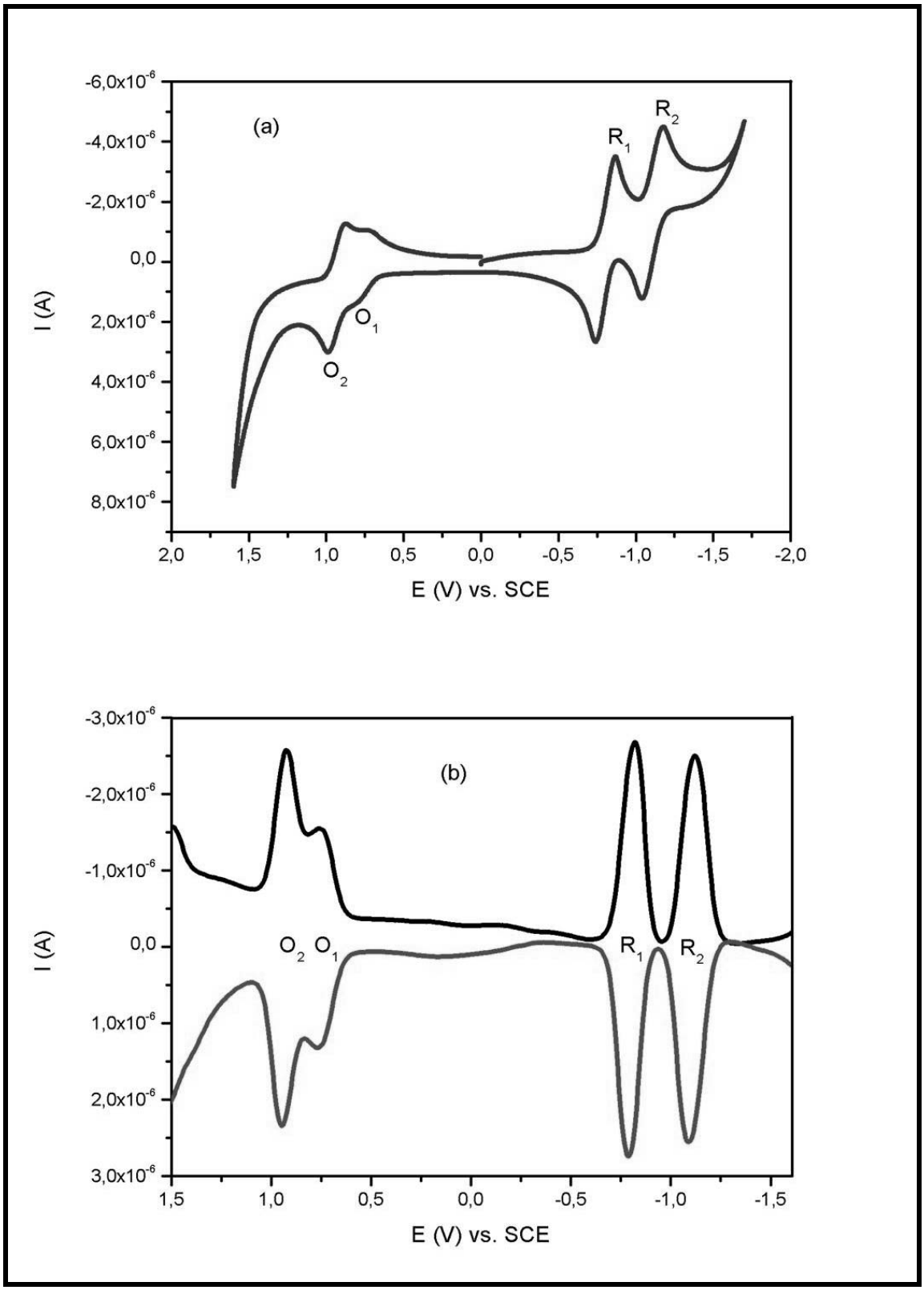


Fig. 3 – (a) CV of $Cu^{II}Pc$ (4). (b) SWV of $Cu^{II}Pc$ (4).

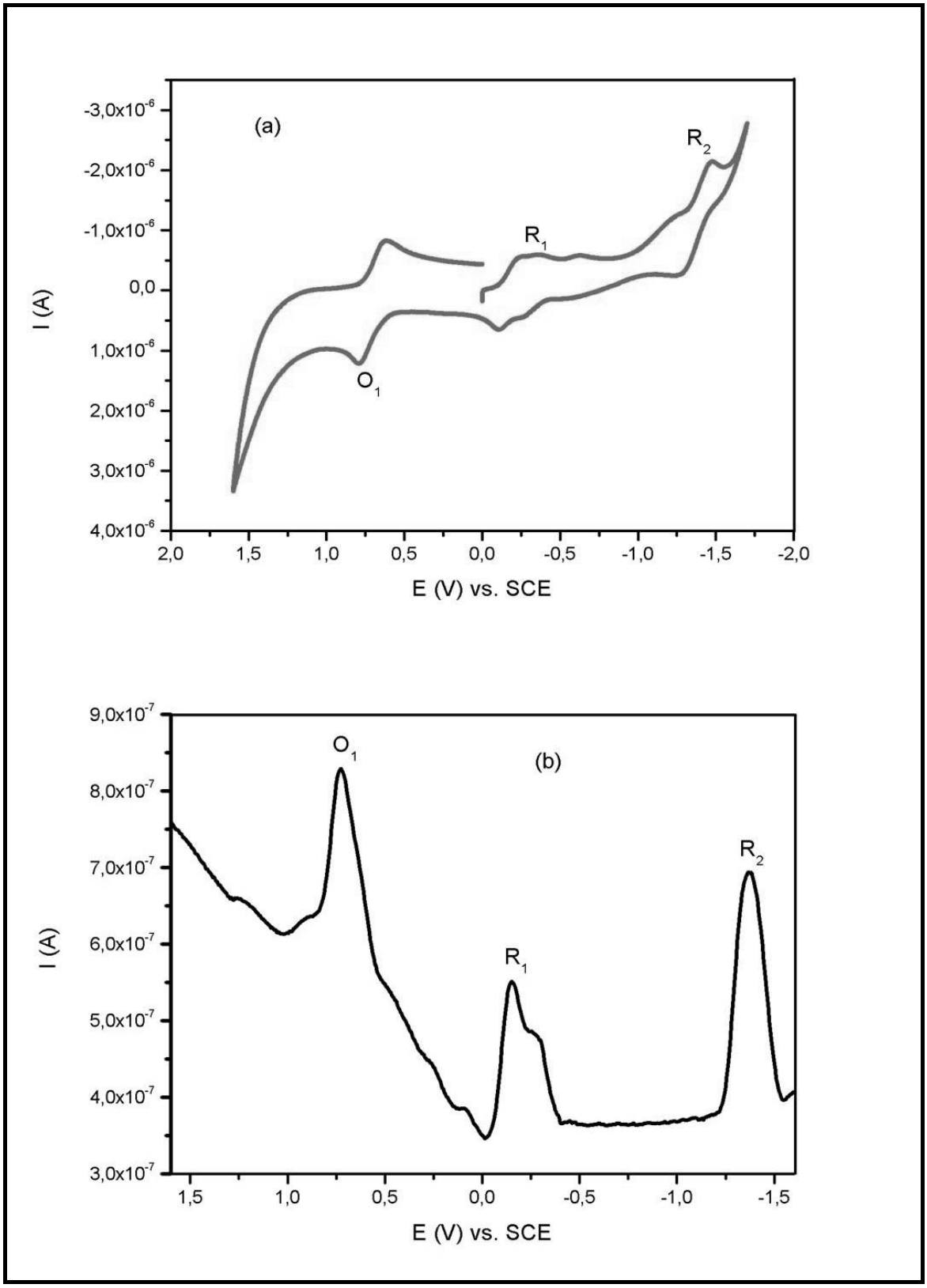


Fig. 4 – (a) CV of $Co^{II}Pc$ (3). (b) SWV of $Co^{II}Pc$ (3).

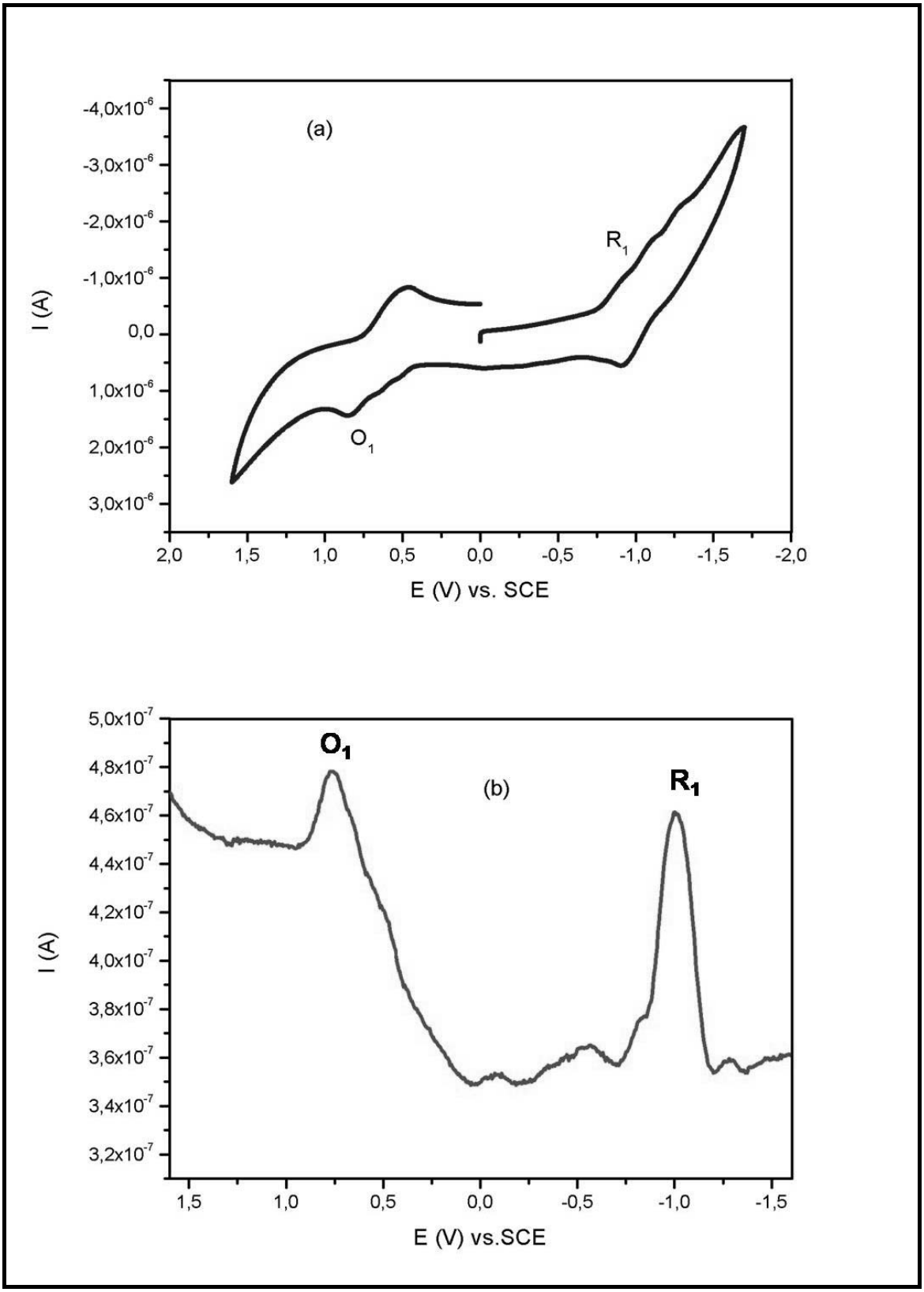


Fig. 5 – (a) CV of $Zn^{II}Pc$ (5). (b) SWV of $Zn^{II}Pc$ (5).

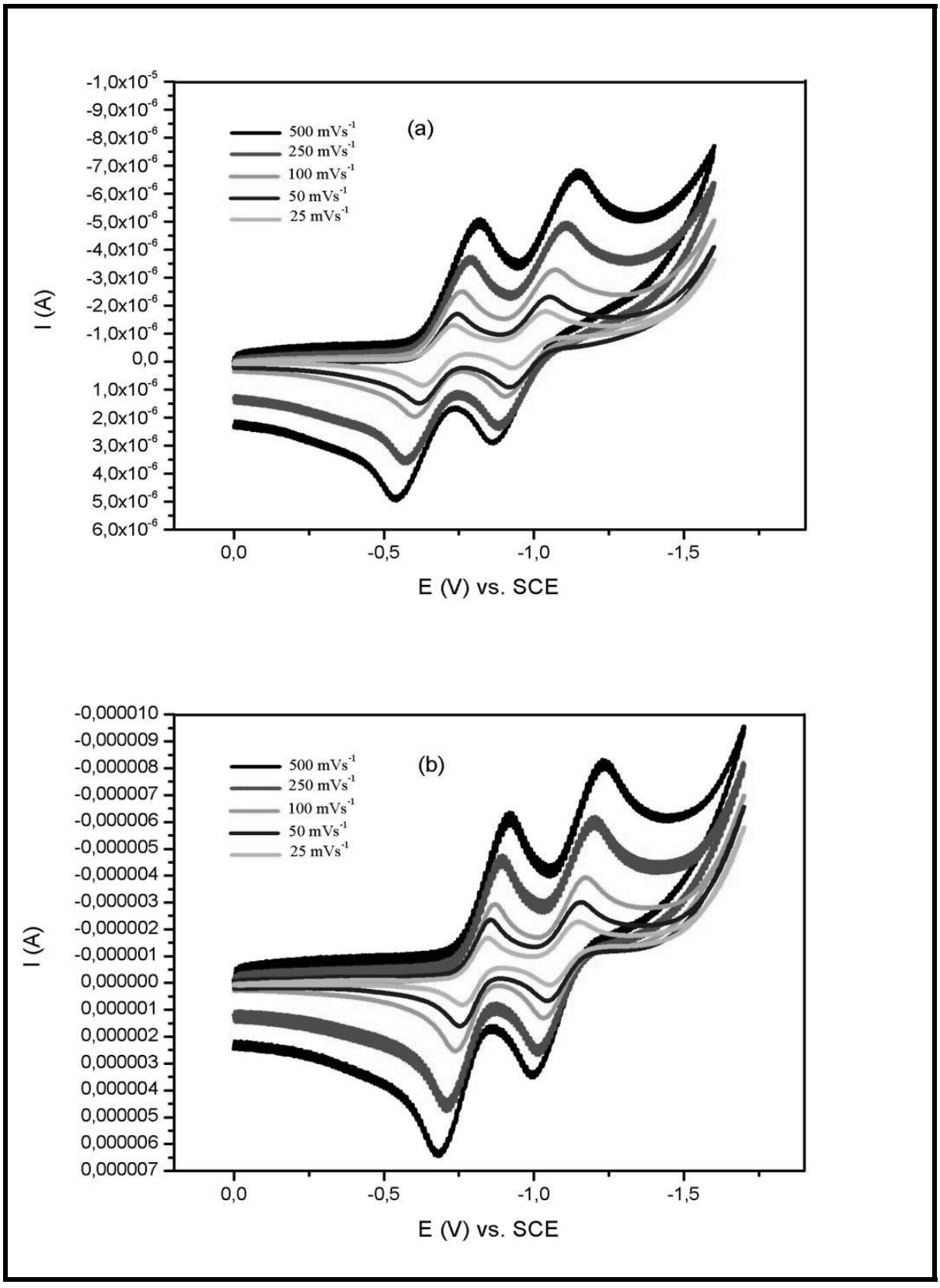


Fig. 6 – (a) CV of H_2Pc (2) at various scan rates (ranging from 25 to 500 $mV.s^{-1}$) on a Pt working electrode in DCM/TBAP. (b) CV of compound $Cu^{II}Pc$ (4) at various scan rates (ranging from 25 to 500 $mV.s^{-1}$) on a Pt working electrode in DCM/TBAP.

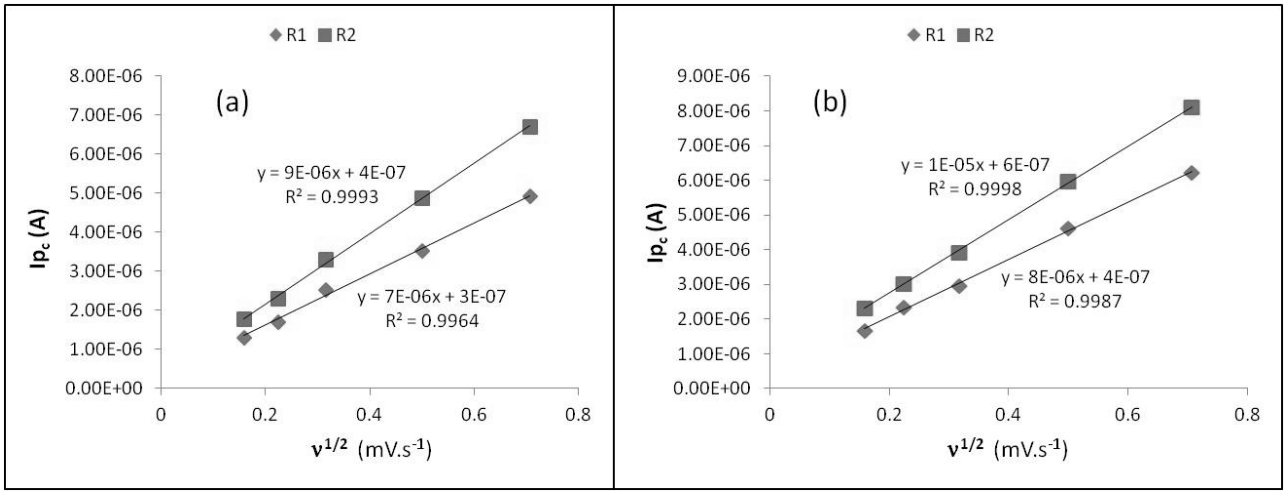


Fig. 7 – The plot of square root of scan rate *versus* peak current for (a) H_2Pc (2) and (b) $Cu^{II}Pc$ (4).

Antimicrobial Activities

Phthalocyanines and their analogues have shown great potential as photodynamic agents producing reactive oxygen species, especially in medicine.[40] However, their biocidal effects might also be employed to inhibit various undesirable organisms. The reason for performing antimicrobial activity tests in this study was to investigate the potential of these newly synthesized compounds to be used in antimicrobial drugs. All the synthesized compounds were tested against two Gram-positive and five Gram-negative bacterial strains and a yeast strain according to the literature protocol.[41] As compared with standard drug, all of the test bacteria were found to be resistant to the tested compounds. But it was observed that there were significant anticandidal activities, although not as effective as the reference compounds. MIC values for anticandidal activity were determined for all compounds, and values greater than 312 µg/mL were not given in Table 2. CuPc (4) was found to have the most effective anticandidal activity (MIC: 78 µg/mL) among the compounds tested. This might be due to its increased singlet oxygen quantum yields in the CuPc (4) compared to the Pc alone. CuPc (4) exerted toxic effect against *C. albicans* by probably increasing reactive oxygen species (ROS) levels and inhibited its development. The antifungal activity of Pcs might be due to phenol groups in the structure. The main core of phenolic compounds has been formed by at least one phenol ring, in which the hydrogen is usually replaced by a more active residue, such as hydroxyl, methyl or acetyl. The variable biological properties of the phenolics resulted from the pattern and the degree of the substitutes. Several studies in the literature have shown that phenol-containing compounds are effective antifungal agents. [42-43]

Table 2

MIC values for antimicrobial activity of compounds and antibiotics

Microorganisms	Compounds and MIC values				Antibiotics and MIC values		
	$Co^{II}Pc$ (3)	$Zn^{II}Pc$ (5)	$Cu^{II}Pc$ (4)	Nitrile Compound	Ampicillin	Amikacin	Fluconazole
Escherichia coli	-	-	-	-	7.8	0.5	N.T
Staphylococcus aureus	-	-	-	-	0.5	1	N.T
Klebsiella pneumonia	-	-	-	-	-	0.5	N.T
Yersinia pseudotuberculosis	-	-	-	-	125	31.25	N.T
Bacillus cereus	-	-	-	-	-	0.5	N.T
Acinetobacter baumannii	-	-	-	-	7.8	1	N.T
Enterobacter cloacea	-	-	-	-	-	1	N.T
Candida albicans	312	156	78	625	N.T	N.T	1.5

Minimal inhibition concentration (MIC) values were given as µg/mL and MIC values greater than 312 µg/mL were not given., **N.T:** not tested

Table 3

Mutagenicity of Co(II)Pc and Zn(II)Pc towards *S. typhimurium* TA98 and TA100 strains with and without S9

	Concentration (μg/plate)	Number of His^+ Revertants/plate			
		TA 98		TA 100	
		S9 (-)	S9 (+)	S9 (-)	S9 (+)
*Negative Control	100 μL/plate	25±4 a	34±4 a	103±6 a	128±9 a
®Positive Control		2623±54 b	2850±70 b	1948±26 b	2950±70 b
$Co^{II}Pc$ (3)	0	36±5 a	37±7 a	116±19 a	145±14 a
	500	28±6 a	57±11 a	147±7 a	186±33 a
	250	44±9 a	55±7 a	111±1 a	157±5 a
	125	34±6 a	42±4 a	138±5 a	165±7 a
*Negative Control	100 μL/plate	25±4 a	34±4 a	103±6 c	128±9 a
®Positive Control		2623±54 b	2850±70 b	1948±26 d	2950±70 b
$Zn^{II}Pc$ (5)	0	36±5 a	37±7 a	116±19 c	145±14 a
	1100	825 ±19 b	42±7 a	865±17 d	128±8 a
	550	741±21 b	48±10 a	812±14 d	130±3 a
	275	50±12 a	48±1 a	97±9 c	151±9 a

[ab] Differences between groups having the same letter in the same column are not statistically significant (ANOVA, Tamhane, $p>0.05$)
[cd] Differences between groups having the same letter in the same column are not statistically significant (ANOVA, Tukey HSD, $p>0.05$)
* Negative control: Sterile DMSO (100 μl/plate) was used as negative control for *S. typhimurium* TA98 and TA100 both in the presence and absence of S9
® Positive controls:
2-Aminofluorene (7.5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-*O*-phenylene diamine (10 μg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA98 strain.
2-Aminoanthracene (5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; Sodium azide (5 μg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA100.

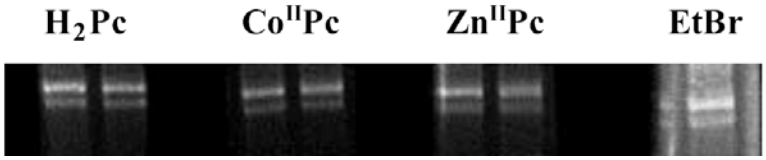


Fig. 8 – Agarose gel electrophoresis of pUC18 plasmid DNA (100 ng/uL) in presence of Pc compounds (0.5 μg/Ml). EtBr is Ethidium bromide (0.5 μg/mL).

Evaluation of Mutagenicity

To identify potential genotoxic carcinogens and germ cell mutagens, genetic toxicity testing has been routinely done. *In vitro* genotoxicity test batteries recommended by regulatory agencies to detect genotoxic carcinogens have included at least two or three test procedures, such as bacterial reverse mutation test (Ames test), mammalian cell chromosome damage test and mammalian cell mutation assay.[44] The Ames test has been known as the most accurate and commonly used procedure to detect genotoxic carcinogens which cause two classes of gene mutation, base pair substitution and small frameshift.[45] The results of the preliminary range finding tests of compounds **3** and **5** gave no toxic effect to all tester strain *S. typhimurium* TA98 and TA100 at doses of 500, 1100, 350, 500 and 750 μg/plate in the presence and absence of S9, respectively. Compounds **2** and **4** were not completely dissolved therefore they did not include exprimental setup. Based on the results of the range finding test, the doses mentioned above were determined as the highest doses. As shown in Table 3, Except for compound **5**, TA98 strain did not increase in the number of revertant colonies compared to the negative control when the bacterium was treated with compounds at 500, 1100, 350, 500 and 750 μg/plate concentrations and below both with and without metabolic activation enzymes (S9). In other words compound **3** was not found to be mutagenic for TA98 strain. Although, compound **5** did not reveal mutagenic activity for TA98 at 275 μg/plate dose, 1100 and 550 μg/plate doses were found to be mutagenic regardless of metabolic activation. Although TA98 strain increased revertant colonies when the bacterium exposed to compound **5** at doses of 1100 and 550 μg/plate in the absence of S9, the chemical did not increase the revertant colonies in the presence of S9 mix. It means that mutagenic effect of compound **5** determined in the absence of S9 was alleviated by a

metabolic activation system. Thus it may be stated from the study that except for compound **5**; compound **3** was not mutagenic in the absence of S9 mix for TA98 strain and it had no mutagenicity in the presence of S9 for TA98 strain.

The results showed that except for compound **5** at doses of 1100 and 550 µg/plate, there was no significant increase in the revertant colonies of TA100 treated with three doses of compound **3** compared with the dose of 0 µg/plate and negative control in the absence and presence of metabolic activation (Table 3). As in the TA98 strain, mutagenic effects of compound **5** chemical at the doses of 1100 and 550 µg/plate were relieved by S9 metabolic enzyme system for TA 100 strain. Other chemicals did not increase the revertant colony numbers. On the contrary the positive control substances obviously increased revertant colonies comparison with negative control. Hence, except for compound **5** (1100 and 550 µg/plate doses) all chemicals tested were found to be non mutagenic at the highest doses and lower on *S.typhimurium* TA98 and TA100 without metabolic activation, but all of them were non mutagenic with S9 both for TA98 and TA100 in the Ames Assay.

DNA Visualization

The binding of positively charged H_2Pc (2), ZnPc (5), and CoPc (3) phthalocyanines with double-stranded DNA was investigated. All compunds exihibited efficient DNA binding (Fig 8). Especially CoPc (3) might be a promising DNA staining agent due to it was not mutagenic. On the other hand, further studies on laboratory animal should be done to clarify its toxicity.

EXPERIMENTAL

Chemicals and Instruments

2-Benzylphenol and 4-nitrophthalonitrile were purchased from Aldrich. All solvents were dried and purified as described by Perrin and Armarego.[46] Acetonitrile and chloroform (Merck, spectrometric grade) were the solvents for absorption measurements. All metal perchlorates purchased from Across were of the highest quality available and vacuum dried over blue silica gel before use. The absorption spectra of the solutions were recorded using a UV mini-1240 UV Spectrophotometer. The IR spectra were recorded on a Perkin Elmer 1600 FT-IR Spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III 400 MHz NMR Spectrometer in $CDCl_3$. The chemical shifts were reported (δ) relative to Me_4Si as an internal standard. Mass spectra were measured on a Micromass Quatro LC/ULTIMA LC-MS/MS Spectrometer. The elemental analyses were performed on a Costech ECS 4010 Elemental Analyzer. Melting points were measured on an electrothermal apparatus and are uncorrected.

Sodium azide (SA), 2-aminofluorene (2-AF), dimethyl sulfoxide (DMSO), L-histidine-HCl monohydrate were obtained from Merck (KGaA Darmstadt, Germany). Other chemicals, D-glucose 6-phosphate, β-nicotinamide-adenine dinucleotide phosphate (β-NADP), D-biotin, ampicillin trihydrate, and S9 fractions of rat liver were purchased from Sigma-Aldrich (Chemie GmBH, Germany); Nutrient broth No: 2 was purchased from Oxoid (Unipath Ltd, Basingstoke Hampshire, England); 4-nitro-O-phenylenediamine (4-NPDA) was purchased from Fluka.

Synthesis

***4-(2-benzyl phenoxy) benzene-1,2dicarbonitrile* (1)**

2-Benzylphenol (2.00 g, 10.86 mmol) was dissolved in dry DMF (43 mL) and then 4- nitrophthalonitrile (1.88 g, 10.86 mmol) was added to the solution under nitrogen atmosphere. After stirring for 15 min at 25 ºC, finely ground dry K_2CO_3 (3.8 g, 27.17 mmol) was added portion-wise in 2 h. with efficient stirring. The reaction mixture was stirred under nitrogen atmosphere at 60 ºC for about 4 days. Then, the reaction mixture was poured into ice. Precipitate was filtered off, washed with water and dried in vacuum. The product was recrystalized from ethyl alcohol. Yield: 2.32 g (69 %), m.p.: 68-70 °C. Analytical calculation for, $C_{21}H_{14}N_2O$: C, 81.29; H, 4.52; N, 9.03 %. Found: C, 81.06; H, 4.25; N, 8.92. FTIR, ν_{max}/cm^{-1}: 3049(-CH_2), 3023(-CH), 2232 (C≡N), 1602, 1482, 1250, 1099, 953, 826. ^{1}H-NMR ($CDCl_3$), (δ: ppm): 7.60 (d, 1H, Ar-H), 7.34 (m, 3H, Ar-H), 7.15 (m, 3H, Ar-H), 7.01 (d, 3H,Ar-H), 6.96 (dd, 2H, Ar-H),3.92 (s, 2H, -CH_2). ^{13}C-NMR ($CDCl_3$), (δ:ppm): 161.65, 151.48, 139.11, 135.10, 132.23,128.78, 126.35, 126.78, 121.41,120.70, 117.33, 115.46, 112.68(C≡N), 108.31, 36.52(-CH_2). MS (FAB) (m/z): 314 $[M+4]^+$ ($C_{21}H_{14}N_2O$); calc: 310).

***Metal-free phthalocyanine* (2)**

A standard schlenk tube was charged with compound **1** (0.2 g, 0.65 mmol), 5 mL n-pentanol, 0.05 mL 1,8-diaza bicyclo[5.4.0]undec-7-ene(DBU) under argon atmosphere and degassed several times. The temperature of the mixture was gradually increased up to 155 ºC before it was stirred for 18 h. After cooling to room temperature, the reaction mixture was refluxed with ethyl alcohol (40 mL) to precipitate the product. Then it was washed with hot ethyl alcohol, methyl alcohol and dried *in vacuo*. The solid product was purified by preparative thin layer chromatography (TLC) using n-hexane/chloroform (1:1) solvent system. The crude product was very soluble in chloroform, acetone, ethyl acetate, THF, DMSO and DMF, but insoluble in ethyl alcohol, methyl alcohol and n-hexane. Yield: 0.055 g (28 %), m.p: > 300 °C. Analytical calculation for, $C_{84}H_{58}N_8O_4$: C, 81.09; H, 4.66; N, 9.06 %. Found: C, 80.84; H, 4.61; N, 8.82. FTIR, ν_{max}/cm^{-1}: 3290 (N-H), 3061 (-CH_2), 3026(-CH), 1603, 1449, 1224, 1007, 830. ^{1}H-NMR. ($CDCl_3$), (δ: ppm): 8.16 (m, 8H, Ar-H), 7.34 (m, 44H, Ar-H), 4.25 (dd, 8H, -CH_2). ^{13}CNMR ($CDCl_3$), (δ:ppm): 172.15, 159.35, 154.68, 146.13, 140.52, 131.47, 129.21, 128.52, 126.13, 124.57, 120.12, 108.24, 102.58, 36.30. UV-vis (chloroform)(1×10^{-5} M): λ_{max}, nm(log ε) (1×10^{-5} M): 704 (5.0), 668 (4.93), 644 (4.50), 612 (4.29), 344(4.72). MS (FAB) (m/z): 1243 $[M]^+$ ($C_{84}H_{58}N_8O_4$); calc: 1243).

General Procedure of Metallophthlaocyanines

4-(2-Benzyl phenoxy)phthalonitrile **1** (0.2 g, 0.65 mmol), and respectively anhydrous $CoCl_2$, anhydrous $CuCl_2$,

anhydrous $Zn(CH_3COO)_2$ (0.25 mmol) and n-pentanol (5 mL) were added in a standard schlenk tube under argon atmosphere and degassed several times. The temperature of the mixture was gradually increased up to 155 ºC before it was stirred for 18 h. After cooling to room temperature the reaction mixture was refluxed with ethyl alcohol (40 mL) to precipitate the product, which was filtered off and washed with water and hot ethyl alcohol, methyl alcohol then dried *in vacuo*. All compounds were soluble in chloroform, acetone, ethyl acetate, THF, DMSO and DMF, but insoluble in ethyl alcohol, methyl alcohol and n-hexane.

***Co(II) phthlaocyanine* (3)**

Compound **3** was prepared according to general procedure for metallophthalocyanines. The solid product was purified by the preparative thin layer chromatography (TLC) using n- hexane/chloroform (4:6) solvent system. Yield: 0.135 g (65 %), m.p: >300 °C. Analytical calculation for, $C_{84}H_{56}N_8O_4Co$: C, 77.53; H, 4.30; N, 8.61 %. Found: C, 77.54; H, 4.17; N, 8.01. FTIR, ν_{max}/cm^{-1}: 3058 (-CH_2), 3026 (-CH), 1599, 1449, 1228,1092, 789. UV-vis (chloroform): λ_{max}, nm(log ε) (1×10^{-5} M): 674 (5.05), 607 (4.45), 327 (4.76). MS (FAB) (m/z): 1346 $[M+2Na]^+$ ($C_{84}H_{56}N_8O_4Co$); calc: 1300).

***Cu(II) phthlaocyanine* (4)**

Compound **4** was prepared according to general procedure for metallophthalocyanines. The solid product was purified by the preparative thin layer chromatography (TLC) using n- hexane/chloroform (1:1) solvent system. Yield: 0.097 g (46 %), m.p: > 300 °C. Analytical calculation for, $C_{84}H_{56}N_8O_4Cu$: C, 77.30; H, 4.29; N, 8.58 %. Found: C, 77.64; H, 4.17; N, 8.28. FTIR, ν_{max}/cm^{-1}: 3060 (-CH_2), 3005 (CH), 1612, 1449, 1228, 1080, 875. UV-vis (chloroform): λ_{max}, nm(log ε) (1×10^{-5} M): 683 (5.0), 613 (4.37), 338 (4.64). MS (FAB) (m/z): 1306$[M+H]^+$ ($C_{84}H_{56}N_8O_4Cu$); calc: 1305).

***Zn(II) phthlaocyanine (ZnPc)* (5)**

Compound **5** was prepared according to general procedure for metallophthalocyanines The solid product was purified by the preparative thin layer chromatography (TLC) using ethyl alcohol/chloroform (1:1) solvent system. Yield: 0.050 g (24 %), m.p: > 300 °C. Analytical calculation for, $C_{84}H_{56}N_8O_4Zn$: C, 77.18; H, 4.28; N, 8.57 %. Found: C, 76.79; H, 4.03; N, 8.26. FTIR, ν_{max}/cm^{-1}: 3064 (-CH_2), 3025 (CH), 1579, 1449, 1227, 1093, 943, 695. ^{1}H-NMR. ($CDCl_3$), (δ: ppm): 7.82 (s, 4H, Ar-H),7.73 (d, 4H, Ar-H), 7.22 (m, 36H, Ar-H), 7.01 (d, 4H, Ar-H), 3.94 (s, 8H, -CH_2). ^{13}CNMR ($CDCl_3$), (δ:ppm): 167.43, 157.22, 139.50, 131.85, 129.17, 126.12, 125.39, 121.97, 110.87, 97.98, 40.14-37.43-36.27(-CH_2). UV-vis (chloroform): λ_{max}, nm(log ε) (1×10^{-5} M): 682 (5.01), 615 (4.35), 350 (4.74). MS (FAB) (m/z): 1308 $[M+2H]^+$ ($C_{84}H_{56}N_8O_4Zn$); calc: 1306).

Spectrophotometric measurements

Absorption spectra of the compounds with a concentration of $1x10^{-6}$ M in chloroform-acetonitrile solution (1:1) containing 10 molar equivalents of appropriate metal perchlorate salt were measured using 1cm long absorption cell. Stock solution of the compounds was prepared in chloroform and the solutions used for all spectrometric titrations were prepared from these solutions by dilution. Any changes in titration experiments were recorded upon addition of perchlorate of the respective metals to its chloroform solution, while the ligand concentration was kept constant ($1x10^{-6}$ M). The solution of metal perchlorates was prepared in acetonitrile. The stoichiometry of the complexes was determined by using the molar-ratio method. The stability constants were calculated according to the previously described procedure. [47]

Electrochemical Measurements

All electrochemical measurements were carried out with Gamry Interface 1000 potentiostat/galvanostat utilizing a three-electrode configuration at 25 ºC. The working electrode was a Pt disc with a surface area of 0.071 cm^2. A Pt wire was served as the counter electrode and saturated calomel electrode (SCE) was employed as the reference electrode and separated from the bulk of the solution by a double bridge. Electrochemical grade tetrabutylammonium perchlorate (TBAP) in extra pure dichloromethane (DCM) was employed as the supporting electrolyte at a concentration of 0.10 mol dm^{-3}.

Antimicrobial Activity Tests

The antimicrobial activities of newly synthesized compounds against bacterial strains and yeast isolates were determined based on a microwell dilution method [41] and minimal inhibition concentration (MIC) values (μg/mL) were calculated.

All test microorganisms were provided by the Department of Biology, Faculty of Science at Karadeniz Technical University (Trabzon, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Bacillus subtilis subsp. spizizenii* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia pseudotuberculosis* ATCC 911, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* RSKK 709, *Acinetobacter baumannii* RSKK 02026, *Enterobacter cloacea* ATCC 13047, *Candida albicans* ATCC 14053. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of compounds.

The MIC was defined as the concentration in the well containing lowest compound dose that observed no growth. Ampicillin, amikacin, and fluconazole were used as standard antibacterial and antifungal drugs, respectively. DMSO was used as solvent control.

Salmonella/Microsome Assay (Ames test)

The *Salmonella typhimurium* test strains TA98 and TA 100 were obtained from Biology Department, Faculty of Sciences Selcuk University and were tested for the presence of strain-specific markers. [48] While TA98 were used for determining the frame shift, TA100 was used to determine the base pair exchange type of mutations. The cytotoxic doses of the CoPc (500, 250, and 125 μg/plate), ZnPC (1100, 550, and 275 μg/plate), were determined. [49] The stock solutions of the test materials were dissolved in sterile dimethyl sulfoxide (DMSO) and stored at room temperature. The *S. typhimurium* strains were stored in master plates (Histidine/Biotin/ Ampicillin Agar) and before the assays one colony of the strains picked up from the master plate and incubated in nutrient broth at 37 °C for 16 h. with shaking. Positive controls were also included to ensure the performance of the tester strains: 10 μg/plate 4-NPDA for TA98 and 5 μg/plate SA for TA100 were used in the absence of S9 mix; 7.5 μg/plate 2-AF for TA98 and 5 μg/plate 2-AA for TA100 were used in the presence of S9 metabolic activation enzymes. For each series of experiments, negative controls with DMSO were included to determine the number of spontaneous revertants/plate.

The mutagenicity test was performed by mixing one of the tester strains which was cultured overnight, with the test substance in the presence and absence of S9 mixture condition. S9 mix (500 µL) (or 500 µL phosphate buffer), the test solution (100 µL) for each concentration and a cell suspension (100 µL) from an overnight culture (1-2x10^9 cells/mL) were added to 2.5 mL top agar (kept at 45 °C) and vortexed for eight seconds. The entire mixture was overlaid on the minimal agar plate. The plates were incubated at 37 °C for 72 h and then the revertant bacterial colonies on each plate were counted.[50] Both the positive and negative controls (DMSO) were maintained concurrently. Samples were tested on triplicate plates in two independent parallel experiments and the results were tabulated as the mean ± standard deviation for each condition.

DNA Visualization

DNA visualization was done according to Sambrook *et al.* (1989).[51] Agarose 0.4 grams were weighed and mixed with 40 mL 1xTAE buffer in a microwavable flask. It was incubated in a microwave for 2 minutes until the agarose is completely dissolved. The agarose solution was allowed to cool for 5 minutes to about 50 °C. Agarose was poured into a gel tray with the well comb in place. It was placed newly poured gel at room temperature for 20-30 mins, until it has completely solidified. 1 uL loading buffer was added to each pUC18 plasmid DNA (100 ng/uL). Once solidified, the agarose gel was placed into the gel box (electrophoresis unit). The gel box was filled with 1xTAE buffer until the gel is covered. All samples were carefully loaded into all the wells of the gel. The gel was run at 80-150 V until the dye line was approximately 75-80 % of the way down the gel. The power was turned off and the electrodes were disconnected from the power source, and then the gel box carefully removed from the gel. After all the pUC18 plasmid DNAs were run in the gel, each well was cut with a razor blade containing the same DNA sample and separated from each other. Separated gel fragments containing DNA samples were placed into a container filled with 50 mL of TAE running buffer. Ethidium bromide (EtBr) was added to a final concentration of 0.5 µg/mL on the control sample (EtBr binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light), and 0.5 µg/mL (phthalocyanine compounds) were added to the other gel pieces containing same pUC18 DNA and they were placed on a rocker for 20-30 mins. Using BiometraUVT-28M device that has UV light, DNA fragments were visualized and compared to each other.

CONCLUSIONS

In this work, we have demonstrated the synthesis and characterization of a new metal-free (2) and metallophthalocyanines (3-5) which contain substituted macrocyclic moieties. Structures of the new compounds were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR. Electrochemistry of properties of **2-5** were studied in solution with voltammetric measurements. Electrochemical responses of the compounds supported the proposed structure of the complexes. Since while **2, 4** and **5** gave Pc ring based redox processes, 3 gave metal based electron transfer processes in addition to the Pc based redox processes, which enriched the possible usage of the complex in various electrochemical technologies. Compound **4** exhibited good growth inhibition activity against Candida and is promising to act as a potential antimicrobial agent. Results also revealed that compound **5** showed moderate activity against Candida. Based on the results obtained, slight modifications of the structures might produce potent and potential compounds which could be used as anticandida drugs. The results of this study also suggest that compound **3** is not mutagenic, while compound **5** turned to be weak mutagens in reversion mutation assay with *Salmonella typhimurium* tester strains TA98 and TA100. Compound **3** may be a promising probe for DNA detection, however further examination for acute toxicity assay on laboratory animals must be done.

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