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ORIGINAL ARTICLE

Convenient synthesis of novel unmetalled and metallophthalocyanines bearing coumarin derivatives: synthesis, characterization, aggregation behaviors and antimicrobial activity

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Abstract Metal-free and metallophthalocyanines **3–10** carrying four coumarins group on the periphery were prepared by cyclotetramerisation of a new precursor, namely 4-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-phthalonitrile **1** and 4-(2-Oxo-2*H*-chromen-7-yloxy)-phthalonitrile **2** in the presence of the corresponding divalent metal salts (Zn (II), Cu(II) and Co(II). The aggregation behaviors of the unmetalled **3** and metallophthalocyanines **5** were investigated. The novel phthalonitrile derivatives **1–2** were synthesized by the reaction between 4-nitrophthalonitrile with 7-hydroxy-4-methylcoumarin and 7-hydroxycoumarin respectively in polar aprotic solvents (DMF or DMSO) in the presence of dry K_2CO_3 as base catalyst. The characterization of the new products involved a combination of methods including elemental analyses, IR, UV–vis, ^{1}H NMR, ^{13}C NMR and mass spectroscopies. Most of the new compounds **3–10** exhibited important antimicrobial activity.

Keywords Metallophthalocyanine · Zinc · Synthesis · Antimicrobial activity

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Introduction

Phthalocyanines have been the subject of a great deal of wide-ranging research for over 60 years. Phthalocyanines properties have interesting potential technical applications such as molecular electronics, semiconductor and electrochromic display devices, photovoltaic and solar cells, gas sensors, synthetic metals, liquid crystals, optical disks, photodynamic therapy of cancer, electrophotography, and non-linear optics [1–10].

On the other hand coumarins, comprise a very large and important family of compounds that occur widely in nature. They are found in a wide range of plants such as tonka bean, vanilla grass and cinnamon. A number of coumarin derivatives are used in the pharmaceutical industry as precursor molecules for the synthesis of many synthetic pharmaceutical compounds including anticoagulants and vitamin K antagonists, while others are used in the treatment of lymphedema [11]. Coumarin derivatives exhibit antibacterial, antifungal, anticancer, anticoagulant, anti-inflammatory [11–16]. The synthesis and characterization of Pc compounds including four peripheral 6-hydroxy-4-methylcoumarin substituents units on the periphery have been studied previously. [17].

In this regard, we report herein the synthesis of metal-free phthalocyanines (**3**,**4**) and metallophthalocyanines (**5–10**) carrying coumarins groups. The spectroscopic characterization and the electronic and aggregation behaviors of these newly synthesized complexes are also presented.

Results and discussion

The synthesis of substituted phthalonitrile derivatives is an important step in Pc synthesis. Nonperipherally substituted phthalonitrile derivatives are synthesized through reactions

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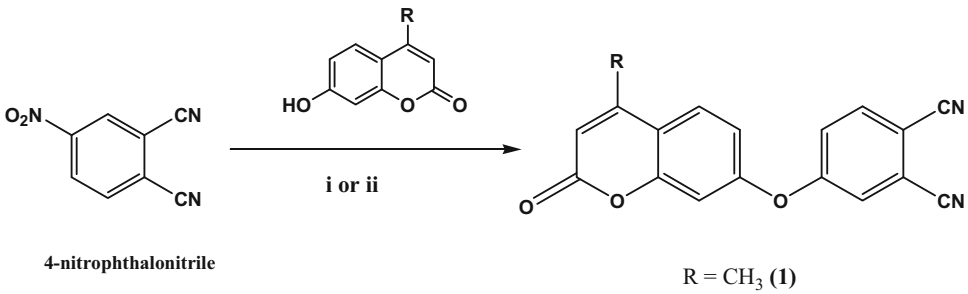
Scheme 1 protocol route for the synthesis of compounds **1–2**



4-nitrophthalonitrile

R = CH_3 **(1)**

R = H **(2)**

R = CH_3 ; **(i) :** K_2CO_3, dry DMF, r.t , 24h

R = H ; **(ii) :** K_2CO_3, DMSO, r.t , 72h

between 4-nitrophthalonitrile and *O*-, *S*-, or *N*-nucleophiles [18–23]. Using this synthetic strategy, the synthesis and characterization of metallophthalocyanines **5-10** and their precursors **1–2** are reported. The 4-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-phthalonitrile **1** was obtained from the reaction of 7-Hydroxy-4-methyl coumarin to 4-nitropphthalonitrile in the presence of K_2CO_3/DMSO under N_2 atmosphere at room temperature for 24 h. The synthesis of 4-(2-oxo-2*H*-chromen-7-yloxy)-phthalonitrile **2** was achieved in 74 % yield through base-catalyzed aromatic displacement of 4-nitrophthalonitrile with 7-hydroxycoumarin using K_2CO_3 as the base in dry DMF. The reaction was carried out at room temperature under N_2 atmosphere for 72 h (Scheme 1).

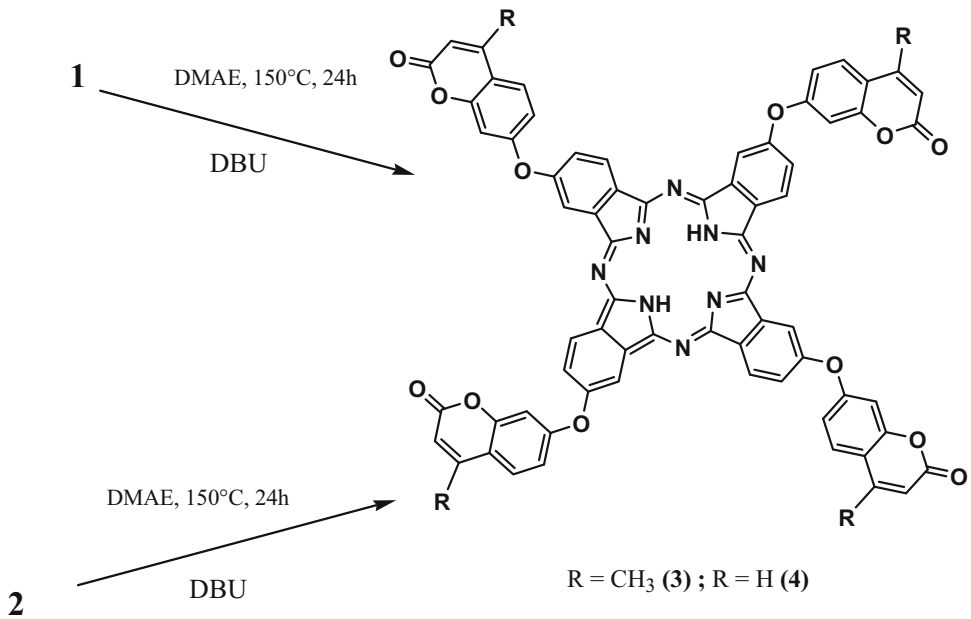
The electron-withdrawing capability of dinitrile functionalities makes 4-nitrophthalonitrile susceptible to nucleophilic attack [24, 25].

The characterization of the products **1–2** involved a combination of methods including, ^{1} H NMR, ^{13}C NMR, and NMR 2D, FT-IR, UV–vis, elemental analysis and LC–MS spectroscopy.

In the IR spectrum of compound **1**, stretching vibrations of CN groups at 2227 cm^{-1} and aromatic groups (ArCH) at 3079 cm^{-1} appeared at expected frequencies. The ^{1}H NMR spectrum of **1** in DMSO-d_6 showed signals with ranging from 7.43 to 8.11 ppm belonging to aromatic protons and $H_{2,3}$. The methyl protons appeared at 2.42 ppm. In the ^{13}C NMR spectra of **1**, the aromatic, and CH_3 carbon atoms of **1** appeared between 116.2 and 161.7 ppm and at18.5 ppm, respectively, whereas carbon atom C_2 was observed at 159.8 ppm.

^{1}H NMR spectral assignments are also supported via ^{1}H–^{13}C HMBC experiment. ^{1}H–^{13}C HMBC is more helpful to get detailed information about which protons correlate with certain carbons. From the ^{1}H–^{13}C HMBC spectra,

Scheme 2 Synthesis route for unmetalled phthalocyanine compound (**3,4**)



R = CH_3 **(3) ;** R = H **(4)**

H_3 proton at 7.43 ppm correlates with C_2 at 159.8 ppm and C_4 at 153.2 ppm. The mass spectra of phthalonitrile derivatives **1**–**2** gave the characteristic molecular ion peaks at m/z = 287.2, 301.2 respectively.

Comparison of the IR spectral data clearly indicated the formation of phthlonitrile derivative **2** by the disappearance of the phenolic OH of coumarins derivatives at 3448 cm^{-1} as expected and NO_2 band of 4-phtalonitrile at 1538–1355 cm^{-1} and appearance intense absorption band at 2225 cm^{-1} for (**2**) corresponding to the CN stretching. The characteristic carbonyl vibrations of coumarin lactone ring were observed at 1731 cm^{-1}.

Metal-free phthalocyanine compound (**3**) was synthesized by heating compound **1** in 2-*N*,*N*-dimethylaminoethanol under nitrogen atmosphere in the presence of DBU (Scheme 2).

The synthesis of the metal-free phthalocyanine compound (**4**) was accomplished by the self heating of 4-(2-oxo-2*H*-chromen-7-yloxy)-phthalonitrile **2** without metal salt at 150 °C under a N_2 atmosphere in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 24 h.

The characterization of the new products (**3**,**4**) involved a combination of methods including elemental analyses, IR, UV–vis and ^{1}H NMR spectroscopies.

The IR spectrum of metal-free phthalocyanine **3** showed a peak at 3276 cm^{-1} due to NH vibrations. The disappearance of the CN stretching vibration on the IR spectra of phthalonitrile compound **1** suggested the formation of compound phthalocyanine derivative **3**.

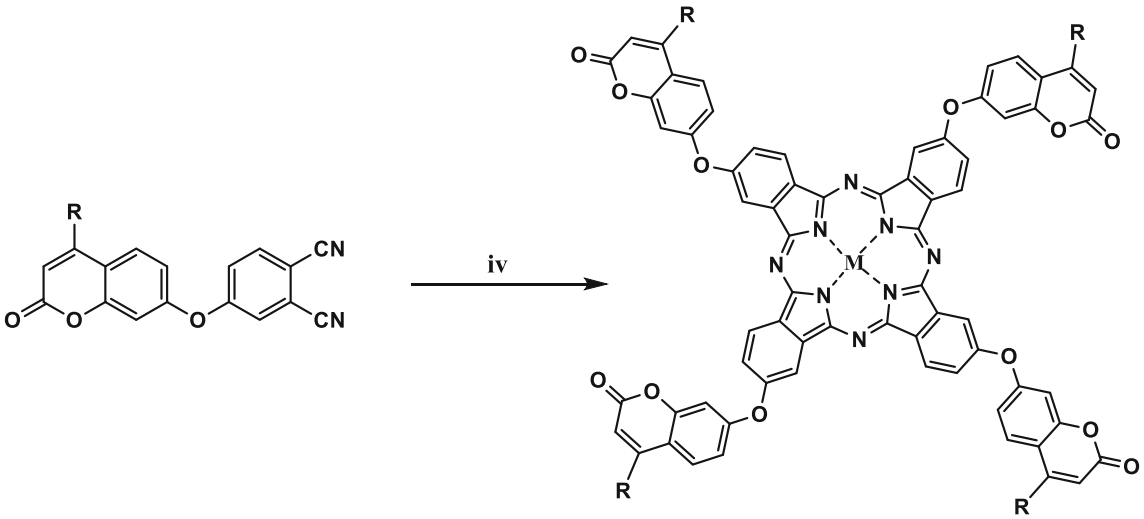
The ^{1}H NMR spectrum of compound **3** exhibited characteristic signal for methyl ($-CH_3$) at δ: 2.42, the aromatic protons appeared between 8.32 and 8.42 ppm, the typical shielding of inner core protons could not be observed due to the probable strong aggregation (especially for our molecules, due to multiplicities of the numbers of aromatic benzenes) of the molecules [26]. The elemental analysis results confirmed the structure of the desired compound **3.**

The sharp peak for the (C≡N) vibrations of phthalonitriles **2** at 2225 cm^{-1} disappeared after conversion into metal-free **4**.

Researchers generally use substituted phthalonitriles or 1,3-diimino-1*H*-isoindoles as starting materials for synthesis of Pcs [27–29]. In this work, phthalonitrile compounds (**1** and **2**) were also used as starting compounds for synthesis of the metallo-Pcs (Zn, Cu, Co) due to mild reaction conditions.

The synthesis of the metallophthalocyanines **5**–**10** was achieved by metal ion mediated cyclotetramerization reaction of the corresponding precursors **1-2** in high boiling dimethylaminoethanol (DMAE) using $Zn(ClO_4)_2.6H_2O$, $CuCl_2.2H_2O$ and $CoCl_2.6H_2O$ salts under N_2 atmosphere (Scheme 3).

Metallophthalocyanines **8**, **9**, and **10** were obtained from the reaction of phthalonitrile derivative **2** with



iv : $Zn(ClO_4)_2.6H_2O$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$, DMAE, 170°C, 24h

R	CH_3			H		
M	Zn	Co	Cu	Zn	Co	Cu
Compound	5	6	7	8	9	10

Scheme 3 Protocol synthesis of compound **5**–**10**

corresponding metal salts $Zn(ClO_4)_2{\cdot}6H_2O$ for complex **8**, $CuCl_2{\cdot}2H_2O$ for complex **10**, and $CoCl_2{\cdot}6H_2O$ for **9** in 2-(dimethylamino) ethanol at 170 °C for 24 h. The yields were considerably high (approximately 61 % for **8**, 81 % for **9** and 82 % for **10**).

The novel metallophthalocyanines are effortlessly soluble in dimethyl sulfoxide (DMSO).

R	CH_3			H		
M	Zn	Co	Cu	Zn	Co	Cu
Compound	**5**	**6**	**7**	**8**	**9**	**10**

Elemental analysis, ^{1}H NMR, IR and UV–vis spectra confirmed the proposed structures of the metallophthalocyanines **5**–**10**.

In the IR spectra of the phthalocyanines **5**–**7**, the proof of the cyclotetramerizations were the absence of the $-C\equiv N$ stretching vibration observed at 2227 cm^{-1} of the compounds **1**. The main differences between metal-free (**3**, **4**) and metallophthalocyanines (**5**–**10**) are inner core N–H stretching vibrations observed at 3276, 3418 cm^{-1} in the IR spectra, respectively. The rest of the spectra are similar to that of compounds **1**–**2**.

The ^{1}H NMR spectra of the peripherally tetra-substituted Pcs are just about the same as those of the initial compound, except for extension and small shifts of the peaks. It is expected that the broadening is due to both chemical exchange caused by aggregation-disaggregation equilibrium in $CDCl_3$ [30].

^{1}H NMR Spectrum of the phthalocyanines **5**, **8** provided the characteristic chemical shifts and confirmed the proposed structures. In that spectrum taken in DMSO-d_6 of comound **5**, aromtic protons appeared between 8.12 and 8.45 ppm. In addition to these results, elemental analysis confirmed the proposed structure. The ^{1}H NMR spectrum of **8** exhibited characteristic signals for aromatic protons between 8.38 and 8.50 ppm.

Compounds **6**, **7**, **9** and **10** have paramagnetic atoms (Co^{2+} and Cu^{2+}) in the inner core. Paramagnetic compounds would affect the magnetic shimming. For this reason, paramagnetic compounds generally are not characterized via NMR. The ^{1}H NMR spectra of the paramagnetic phthalocyanines **6**, **7**, **9** and **10** were not measured. The ^{1}H-NMR spectra of CuPc (**7**,**10**) and CoPc (**9**,**6**) were excluded due to their paramagnetic properties [31].

UV–vis electronic spectra are especially practical for identifying the structure of Pcs. Generally, for Pc complexes, UV–vis spectra show typical electronic spectra with 2 strong absorption bands known as Q and Soret bands (B) [32].

The Q bands appear at the range of 600–750 nm and consist of one sharp and intense band and two low-intense shoulders. The B bands appear at the range of 250–400 nm as a broad band. In absorption spectra of metallo-phthalocyanines, Q bands are originated from the electron transitions between the HOMO and LUMO and B bands correspond to the electron transitions between deeper levels of occupied MO and LUMO.

The UV–vis spectral data of the studied phthalocyanines **3**–**10** in DMSO were given in Table 1.

Antimicrobial activity

The synthesized compounds (**3**–**10**) were evaluated for in vitro antimicrobial activity by the well diffusion method. The antimicrobial activity of the tested compounds was presented in Table 2 by the formation of an inhibitory zone. In parallel, the MIC values were determinated in Table 3. The diameter of the zone of inhibition indicates the degree of sensitivity of the microorganisms to the compounds the well contains. It was obtained that, five compounds (**3**, **4**, **5**, **6** and **7**) present the best antimicrobial activity with a broad spectrum. The compounds **9** showed antimicrobial activity against all bacteria and fungi tested with MIC values of 0.625–5 mg/ml. The compounds **3** and **4** exhibited an important antibacterial activity against *M. luteus*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa* and antifungal activity against *C. albicans* and *C. tropicalis* with an important diameter ranging from 16 to 26 mm. The Compound **5** exhibited the highest in vitro antibacterial activity against *M. luteus* with MIC values of 0.312 mg/ml while the Compound **6** showed a moderate antibacterial activity against *M. luteus*, *S. aureus*, *L. monocytogenes*, *S. Typhimurium* and *P. aeruginosa* with MIC values ranging from 5 to 10 mg/ml.

These results show that the most synthesized compounds have an effective antimicrobial potential against food- borne pathogens and clinical microorganisms.

Aggregation behaviours

Aggregation is usually depicted as a coplanar association of rings progressing from monomer to dimer and higher order complexes. It is dependent on the concentration, nature of the solvent, nature of the substituents, complexed metal ions and temperature [33–35]. In this study, the aggregation behaviour of the metal-free **3** and metallophthalocyanine complexes **5**, are investigated in DMSO (Schemes 4, 5).

As the concentration was increased, the intensity of absorption of the Q band corresponding to monomeric

Table 1 UV–Vis data for the phthlocyanine compounds **3–10**

Compounds	Solvent	λmax (nm) (logε/dm^3 mol^{-1} cm^{-1})
3	DMSO	339 (4.729), 593 (4.535), 620 (4.633), 660 (5.005), 704 (5.066)
4	DMSO	331 (4.531), 601 (4.201), 628 (4.408), 664 (4.758), 710 (5.010)
5	DMSO	336 (5.044), 611 (4.642), 678 (5.197)
6	DMSO	342 (4.946),628 (4.706), 668 (5.060)
7	DMSO	333 (4.881), 616 (4.507), 675 (5.020)
8	DMSO	340 (5.10), 634 (4.89), 679 (5.22).
9	DMSO	350 (4.95), 612 (4.55), 679 (5.24).
10	DMSO	342 (4.79), 626 (4.67), 672 (5.07)

species also increased and there were no new bands due to the aggregated species for both of the complexes. Beer–Lambert law was obeyed in the concentrations ranging from 6×10^{-4} to 14×10^{-6}M for unmetalled **3** and metallophthalocyanines **5**. It can clearly be concluded that the phthalocyanines derivatives (**3** and **5**) did not show aggregation in DMSO for the studied concentrations.

Conclusion

In this research novel phthalonitriles and phthlocyanines were synthesized and characterized. IR, UV–vis, ^{1}H and ^{13}C-NMR, mass spectroscopy and elemental analysis were used to characterize the new compounds. All the compounds show excellent solubility in DMSO. The aggregation behaviour of the metal-free **3** and metallophthalocyanine complexes **5**, were investigated in DMSO and they did not show aggregation for concentrations ranging from 6×10^{-4} to 14×10^{-6} M. Most of the new compounds **3–10** exhibited an important antibacterial activity. The Compound **7** exhibited the highest in vitro antibacterial activity against *M. luteus* with MIC values of 0, 312 mg/ml while the Compound **8** showed a moderate antibacterial activity against *M. luteus*, *S. aureus*, *L. monocytogenes*, *and S. Typhimurium and P. aeruginosa* with MIC values ranging from 5 to 10 mg/ml.

Experimental

All reagents were obtained from Fluka and Aldrich. The purity of the products was tested in each step by TLC (SiO_2, $CHCl_3$/MeOH and THF/MeOH). Melting points were determined using an Electrothermal apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were carried on a Varian Gemini 300 (300 MHz) spectrometer using TMS as internal standard ($\delta = 0$ ppm). IR spectra were recorded on a Perkin-Elmer 398 Spectrophotometer. MS were recorded on a LC–MS–MS 8030 Shimadzu. Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.3 % of the theoretical values. The UV spectra were recorded on a Perkin Elmer Lambda 11 spectrophotometer.

Synthesis of 4-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-phthalonitrile 1

The synthesis of 2 was similar to that of **1**, except 7-hydroxy-4-methylcoumarin (1.232 g, 7 mmol) was employed instead of 7-hydroxycoumarin. The amounts of the other reagents were: 4-nitrophthalonitrile, 0.865 g (5 mmol) and anhydrous potassium carbonate, 1.035 g (7.55 mmol). Yield: (75 %). M.p = 330 °C.

FT-IR (KBr) ν cm^{-1}: 3079 (Ar–CH), 2227 (CN), 1610 (C=C), 1735 (C=O, lactone and ester), 1495 (C–O–C). ^{1}H NMR.(DMSO-d_6, 300 MHz) (δ: ppm) 2.42 (s, 3H, CH_3), 7.43-8.11 (m, 7H, $H_{arom} + H_{2,3}$).

^{13}C-NMR (DMSO-d_6, 75 MHz) (δ: ppm) 18.5(CH_3), 159.8(C_2), 153.2(C_4), 108.6(C_3), 115.4(CN), 115.7(CN), 116.2–161.7(Carom).

Calcd for $C_{18}H_{10}O_3N_2$: C, 71.52; H, 3.33; N, 9.26. Found: C, 71.5; H, 3.3; N, 9.2

MS (LCMS-MS) m/z: Calc. 302.284; Found: 302.2: m/z $[M]^+$.

Synthesis of 4-(2-Oxo-2*H*-chromen-7-yloxy)-phthalonitrile 2

4-Nitrophthalonitrile (2.25 g, 12.34 mmol) was dissolved in dry DMF (15 mL) under argon and 7-Hydroxycoumarin (2.00 g, 12.34 mmol) was added. After stirring for 15 min at room temperature, finely ground anhydrous potassium carbonate (3.67 g, 26.6 mmol) was added in portions during 2 h with efficient stirring. The reaction mixture was stirred under argon atmosphere at room temperature for 24 h after which time, the ensuing mixture was poured into 100 mL iced water and the precipitate filtered off, washed with methanol and then dried. The crude product was chromatographered over a silica gel column using a mixture of $CHCl_3$: MeOH (100:5 (v:v)) as eluent, giving a powder of 4-(2-Oxo-2*H*-chromen-7-yloxy)-phthalonitrile **1**. Finally, the pure powder was dried in a vacuum. Yield:

Table 2 Antimicrobial activity spectrum of the synthesized compound

Microrganism indicator	Compounds	Inhibition zone (mm)
Micrococcus luteus	**1**	17
LB 14110	**2**	16
	3	26
	4	26
	5	22
	6	14
	7	27
	8	18
	9	13
	10	20
Staphylococcus aureus	**1**	**12**
ATCC 6538	**2**	**11**
	3	25
	4	23
	5	–
	6	14
	7	18
	8	13
	9	14
	10	16
Listeria monocytogenes	**1**	12
ATCC 19117	**2**	10
	3	21
	4	20
	5	–
	6	13
	7	20
	8	16
	9	18
	10	14
Agrobacterium tumefaciens	**1**	13
	2	14
	3	**–**
	4	–
	5	–
	6	–
	7	19
	8	–
	9	–
	10	–
Salmonella Typhimurium	**1**	11
ATCC 14028	**2**	10
	3	12
	4	12
	5	–
	6	12
	7	19
	8	16
	9	18
	10	14
Pseudomonas aeruginosa	**1**	14
ATCC 49189	**2**	13
	3	16
	4	16
	5	–
	6	12
	7	20
	8	18
	9	16
	10	13
Candida albicans	**1**	9
	2	8
	3	22
	4	12
	5	–
	6	–
	7	10
	8	–
	9	13
	10	14
Candida tropicalis	**1**	11
R2 CIP203.	**2**	10
	3	20
	4	16
	5	–
	6	–
	7	15
	8	18
	9	14
	10	12

(74 %). M.p = 325 °C FT-IR (KBr) ν cm-1: 3075 (Ar–CH), 2225 (CN), 1602 (C=C), 1731 (C=O, lactone and ester), 1496 (C–O–C). ^{1}H NMR.(DMSO-d_6, 300 MHz) (δ: ppm) 6.47–8.18 (m, 8H, Harom + H_3). ^{13}C-NMR (DMSO-d_6, 75 MHz) (δ: ppm) 157.3(C_2), 155.4(C_4), 108.2(C_3), 115.6(CN), 115.7(CN), 109.8–160.2(Carom).

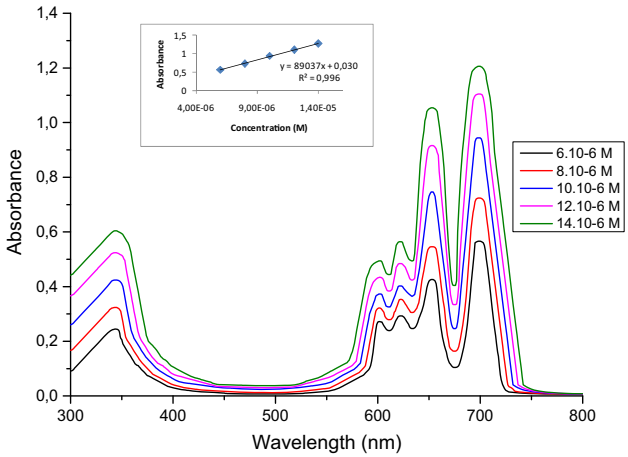
Calcd for $C_{17}H_8O_3N_2$: C, 70.83; H, 2.79; N, 9.71. Found: C, 70.80; H, 2.7; N, 9.7; MS (LC–MS) m/z: Calc. 287.2; Found: 287.2

General procedure for the synthesis of metal-free 3,4

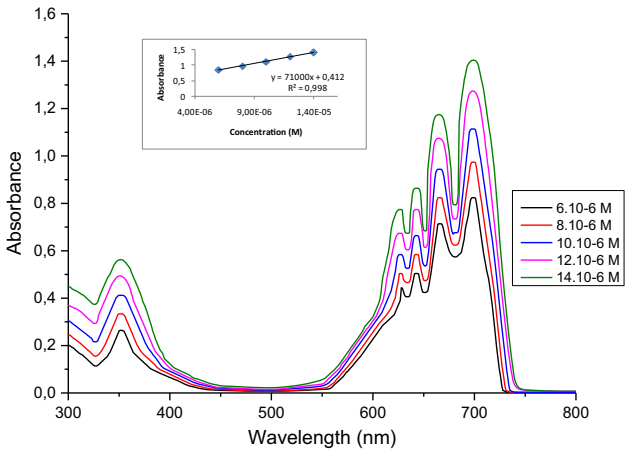
A mixture of 4-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-phthalonitrile **1** or 4-(2-Oxo-2*H*-chromen-7-yloxy)-

Table 3 Determination of the minimum inhibitory concentrations (MICs) expressed in mg/ml

Microorganism indicator	Compounds	MIC(mg/ml)
Micrococcus luteus	**3**	5
LB 14110	**4**	5
	5	0.3125
	6	10
	7	1.25
	8	4
	9	1.5
	10	8
Staphylococcus aureus	**3**	5
ATCC 6538	**4**	5
	5	–
	6	5
	7	2.5
	8	5
	9	4
	10	–
Listeria monocytogenes	**3**	2.5
ATCC 19117	**4**	5
	5	–
	6	5
	7	0.625
	8	–
	9	5
	10	3.5
Salmonella Typhimurium	**3**	5
ATCC 14028	**4**	10
	5	–
	6	10
	7	5
	8	–
	9	5
	10	8
Pseudomonas aeruginosa	**3**	5
ATCC 49189	**4**	5
	5	–
	6	10
	7	2.5
	8	5
	9	5
	10	2.2
Candida albicans	**3**	2.5
	4	5
	5	–
	6	–
	7	–
	8	–
	9	–
	10	2.5
Candida tropicalis	**3**	2.5
R2 CIP203	**4**	5
	5	–
	6	–
	7	5
	8	–
	9	2.3
	10	5



Scheme 4 The aggregation behavior of Unmetalled phthalocyanine **3** in DMSO



Scheme 5 The aggregation behavior of metallophthalocyanine **5** in DMSO

phthalonitrile **2** (0.25 g, 0.487 mmol) and catalytic amount of 1.8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 2.5 ml of DMAE was heated and stirred at 150 °C in a sealed glass tube for 24 h under N_2. After cooling to room temperature the green crude product was precipitated with ethanol, filtered and washed first with ethanol then diethyl ether and then dried in vacuo. Finally, pure metal-free phthalocyanine was obtained by column chromatography which is placed aluminum oxide using $CHCl_3$:CH_3OH (99:1) as solvent system.

Metal free Pc (3).

Yiled: 63 %

M.p = 335 °C

FT-IR (KBr, νmax, cm^{-1}): 3276 (N–H); 3071 (C–H_{arom}); 1471 (C–C); 1265 (C–N); 1602 (C=C); 1208 (C–$O_{lactone}$); 1725 (C=$O_{lactone}$).

Calc. for C68H34N8O12: C, 70.71;H, 2.96 N, 9.701Found: C, 70.7; H, 2.9, N, 9.7; ^{1}H NMR.(DMSO-d_6, 300 MHz) (*δ:* ppm): 2.42(s,12H,CH_3),8.32–8.42 (m, 28H, Harom + $H_{3,4}$).

UV/Vis (DMSO, λmax nm (log ε)): 339 (4.729), 593 (4.535), 620 (4.633), 660 (5.005), 704 (5.066).

Metal free Pc (4)

Elution solvent system: Chloroform: methanol (100:2) as eluent. Yield: 113 mg (77 %).

M.p = 315 °C

FT-IR (KBr, νmax, cm^{-1}): 3418 (N–H); 3019 (C–H_{arom}); 1489 (C–C); 1276 (C–N); 1612 (C=C); 1214 (C–$O_{lactone}$); 1731 (C=$O_{lactone}$);

Calc. for C72H42N8O12: C, 71.401; H,3.495, N, 9.252 Found: C, 71.4; H,3.4, N, 9.1; ^{1}H NMR.(DMSO-d_6, 300 MHz) (*δ:* ppm): 7.92-8.40 (m, 32H,Harom + $H_{3,4}$),

UV–vis (DMSO) λmax/nm: 331 (4.531), 601 (4.201), 628 (4.408), 664 (4.758), 710 (5.010);

General procedure for the synthesis of metal-free and metallophthalocyanines 5–10

Compounds (**1**) or (**2**) (0.24 mmol), dry *N*,*N*-dimethylaminoethanol (DMAE) (4 ml), 1,8-diazabicyclo [4.5.0]undec-7-ene (DBU) (3 drops) and (0.06 mmol) of corresponding anhydrous metal salts ($Zn(ClO_4)_2{\cdot}6H_2O$, $CuCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$ and $NiCl_2{\cdot}6H_2O$) were added in schlenk tube. The mixture was heated at reflux temperature of 170 °C for 24 h under N_2 atmosphere. Then the mixture was left for cooling at room temperature then treated with ethylacetate to precipitate the product which was then filtered off and suddenly washed with water. The green solid product was washed with hot ethanol and dried in vacuo. The raw product was purified by chromatography of silica gel column.

Zn(II)Pc (5).

Elution solvent system: chloroform: methanol (100:3) as eluent.

Yield: 45 %

M.p = 330 °C

FT-IR (KBr) ν, cm^{-1}: 3066 (C–H_{arom}); 1390 (C–C); 1280 (C–N); 1605 (C=C); 1250 (C=N); 896 (Zn–N).

Calc. for C68H32N8O12Zn: C, 67.03; H, 2.64, N, 9.19 Found: C, 67.0; H, 2.6, N 9.1; ^{1}H NMR.(DMSO-d_6, 300 MHz) (δ: ppm): 2.1(s,12H,CH_3),8.12–8.45 (m, 28H, Harom + $H_{3,4}$), UV–vis (DMSO) λmax/nm: 336 (5.044), 611 (4.642), 678 (5.197).

Co(II)Pc (6).

Elution solvent system: chloroform:methanol (100:5)as eluent.

Yield: 71 %

M.p = 335 °C

FT-IR (KBr, νmax, cm^{-1}): 3064 (C–H_{arom}); 3065 (C–H_{arom}); 1387 (C–C); 1282 (C–N); 1608 (C=C); 1254 (C=N); 894 (Co–N).

Calc. for C68H32N8O12Cu: C 67.13; H, 2.65,N 9.21Found: C 67.1; H, 2.6, N,9.2; UV–vis (DMSO) λmax/nm: 342 (4.946),628 (4.706), 668 (5.060).

Cu(II)Pc (7)

Elution solvent system: chloroform:methanol (100:5) as eluent.

Yield: 83 %

M.p = 325 °C

FT-IR (KBr, νmax, cm^{-1}): 3066 (C–H_{arom}); 1390 (C–C); 1281 (C–N); 1610 (C=C); 1250 (C=N); 898 (Cu–N).

Calc. for C72H40N8O12Cu: C 67.949; H, 3.168, N 8.805.993 Found: C 67.9; H, 3.1, N, 8.8.

UV–vis (DMSO) λmax/nm: 333 (4.881), 616 (4.507), 675 (5.020).

Zn(II)Pc (8)

Elution solvent system: chloroform: methanol (100:3) as eluent.

Yield: 61 %

M.p = 330 °C

FT-IR (KBr) ν, cm^{-1}: 1400 (C–C); 1284 (C–N); 1600 (C=C); 1550 (C=N); 896 (Zn–N).

Calc. for C72H40N8O12Zn: C, 67.850; H, 3.163, N, 8.792 Found: C, 67.8; H, 3.1, N 8.7; ^{1}H NMR.(DMSO-d_6, 300 MHz) (*δ:* ppm): 8.38-8.50 (m, 24H, Ar–H).

UV–vis (DMSO) λmax/nm: 340 (5.10), 634 (4.89), 679 (5.22).

Co(II)Pc (9). Elution solvent system: chloroform:methanol (100:2) as eluent. Yield: 114 mg (81 %). mp = 338 °C.

FT-IR (KBr) ν, cm^{-1}: 3065 (C–H_{arom}); 1406 (C–C); 1282 (C–N); 1598 (C=C); 1558 (C=N); 897 (Co–N).

Calc. for C68H32N8O12Co: C 67.38, H, 2.66, N 9.24, Found: C 67.3, H, 2.6, N 9.2. UV–vis (DMSO) λmax/nm: 350 (4.95), 612 (4.55), 679 (5.24).

Cu(II)Pc (10)

Elution solvent system: chloroform:methanol (100:5) as eluent.

Yield: (82 %)

mp = 325 °C

FT-IR (KBr) ν, cm^{-1}: 1404 (C–C); 1284 (C–N); 1604 (C=C); 1554 (C=N); 896 (Cu–N).

Calc. for: $C_{72}H_{40}N_8O_{12}Co$: C 68.196; H, 3.179, N 8.837; Found: C 68.2; H, 3.1, N, 8.8.

UV–vis (DMSO) λmax/nm: 342 (4.79), 626 (4.67), 672 (5.07).

Antimicrobial activities

Microorganisms and growth conditions

Bacterial strains: Gram-positive bacteria (*Micrococcus luteus* LB 14110, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19117 and *Agrobacterium tumefaciens*), Gram-negative bacteria (*Salmonella* Typhimurium ATCC 14028 and *Pseudomonas aeruginosa* ATCC 49189) were used as indicator microorganisms for the antibacterial activity assays. Antifungal activity was determined against the *Candida albicans* and *Candida tropicalis* R2 CIP203.

The bacterial strains using as indicator microorganisms were grown overnight in LB media under aerobic conditions and constant agitation (200 rpm) at 30 °C for *M. luteus* LB14110, *L. monocytogenes* ATCC 19117, and *A. tumefaciens*, and at 37 °C for *S. aureus* ATCC 6538, *S.* Typhimurium ATCC 14028 and *P. aeruginosa* ATCC 49189, and then diluted 1:100 in LB media and incubated for 5 h under constant agitation (200 rpm) at the appropriate temperature. *C. tropicalis* R2 CIP203 was grown in YP10 medium (10 g/l yeast extract, 10 g/l peptone, 100 g/l glucose, 15 ml of 2 g/l adenine solution) and *C. albicans* was grown in YEPD medium (10 g/l yeast extract, 20 g/l peptone, 20 g/l dextrose) at 30 °C for 48 h in an orbital incubator with shaking at 200 rpm.

Agar well diffusion method

Antimicrobial activities of the synthesized compounds were determinated by agar well-diffusion method [36]. Briefly, the synthesized compounds are allowed to diffuse out into the appropriate agar medium (LB agar medium for bacterial strains and potato dextrose agar "PDA" for fungal strains) and interact in a plate freshly seeded with a suspension of the indicators microorganisms (0.1 mL of 10^8 cells per mL). The plate was incubated at the appropriate temperature after staying at 4 °C for 2 h. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The antibacterial activity was assayed by measuring in millimeters the diameter of the inhibition zone formed around the well.

MIC determination

The antimicrobial activities of the synthesized compounds were determined by the minimum inhibitory concentration (MICs) in accordance with NCCLS guideline M7-A_6 and M38-P [37]. The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 µl. The synthesized compounds (20 mg/mL) were properly prepared in solution of dimethylsulfoxide (20 %). The inhibitory activity of each synthesized compounds was transferred to each well in order to obtain a twofold serial dilution of the original sample and to produce the concentration range of 0.0781–10 mg/mL.

To each test well 10 µL of cell suspension were added to final inoculum concentrations of 10^6 CFU/ml for each microorganisms. Positive growth control wells consisted of microorganisms only in their adequate medium. Cells suspension at the same concentration supplemented with ampicillin (50 µg/mL) was used as negative control. The plates were then covered with the sterile plate covers and incubated at the appropriate temperature of each microorganism. The MIC was defined as the lowest concentration of the synthesized compounds at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 µl of Thiazolyl Blue Tetrazolium Bromide (MTT), indicator solution (0.5 mg/ml) dissolved in sterile water were added to the wells and incubated at room temperature for 30 min.

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