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SYNTHESIS OF NOVEL ANTIBACTERIAL METAL FREE AND METALLOPHTHALOCYANINES APPENDING WITH FOUR PERIPHERAL COUMARIN DERIVATIVES AND THEIR SEPARATION OF STRUCTURAL ISOMERS

Rawdha Medyouni,[a] Naceur Hamdi,[a,b*] Anis Ben Hsouna,[b,d] Abdullah Sulaiman Al-Ayed,[b] Hussain Ali Soleiman,[b,e] Fethi Zaghrouba,[a] and Christian Bruneau[c]

[a] Higher Institute of Environmental Science and Technology, Borj-Cédria, Hammam-Lif, 2052, (University of Carthage), Tunisia

[b] Chemistry Department, College of Science and Arts at Ar-Rass, Qassim University, P. O. Box 53, Saudi Arabia

[c] Institut des Sciences Chimiques, Campus de Beaulieu, UMR6226: CNRS-Université de Rennes1, 35042 Rennes Cedex, France

[d] Biopesticides Team (LPIP), Center of Biotechnology of Sfax, P. B. 1177, 3018, Sfax, Tunisia

e Chemistry Department, Aswan Faculty of Science, Aswan University, Egypt

*Corresponding author: E-mail: naceur.hamdi@isste.rnu.tn

A**bstract** - The preparation of novel metal-free phthalocyanines and metallophthalocyanine complexes **6** and **7** (MPcs, M = Co, Zn, Cu and Mn), with four peripheral 6-hydroxy-4-methylcoumarin and 6-hydroxycoumarin substituents, were prepared by cyclotetramerization of compounds **4** and **5** with the corresponding metal salts ($Zn(OAc)_2{\cdot}2H_2O$, $Co(OAc)_2{\cdot}4H_2O$, CuCl, $Mn(OAc)_2{\cdot}4H_2O$) as a template for macrocycle formation in 2-(*N*,*N*-dimethylamino)ethanol whereby a mixture of four different structural isomers is obtained; two of these isomers, with C2''- and Cs-symmetry are isolated by HPLC and characterized by FT-IR, UV-vis, elemental analyses, ^{1}H NMR and ^{13}C NMR spectroscopies. The electronic spectra exhibit a band of coumarin identity together with characteristic bands of the phthalocyanine core. The new compounds were screened for antibacterial activity. Most of them are more active against *E. coli* and *S. aureus.*

INTRODUCTION

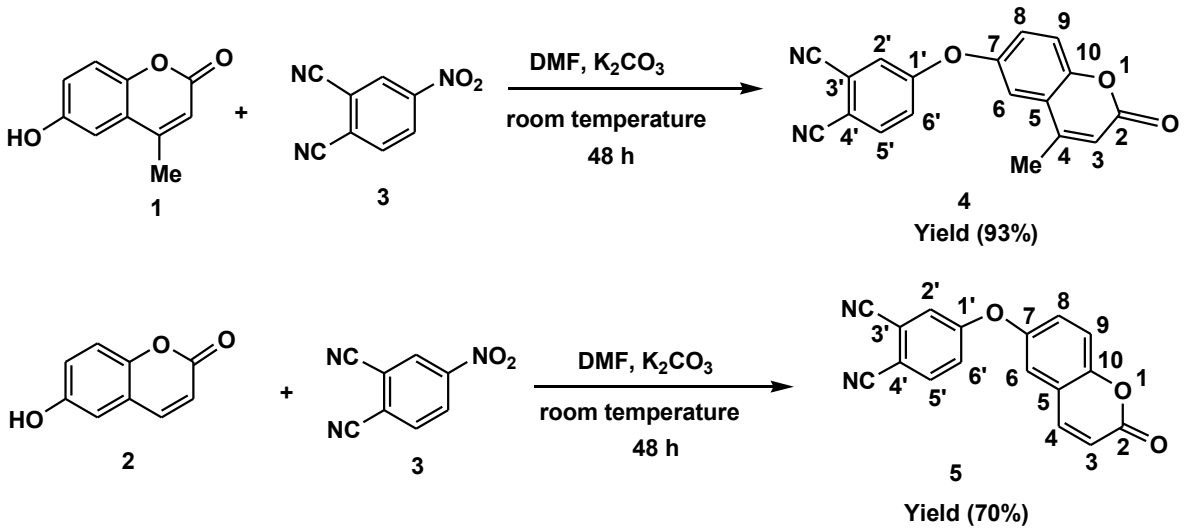
Phthalocyanines (Pcs) are an interesting class of compounds which exhibit both chemical and physical stabilities.[1-3] The Pc macrocycle can engage most metal ions in its cavity; hence scores of different metallophthalocyanines (MPcs) have been synthesized. The nature of the metal ion encapsulated within the Pc cavity plays a vital role in the properties, reactions and of course, applications of the resulting MPc. MPcs exhibit a wide range of potentialities ranging from industrial,[4,5] technological[6-8] to medical[9,10] applications. MPc derivatives in which the central metal is diamagnetic and nontransitional are photoactive, and are often employed in photosensitization and energy conversion.[11-13] Worth emphasizing is the Pcs' application as photosensitizers (PSs) in the photodynamic therapy (PDT) of tumours. The photophysics and photochemistry of InPc derivatives are well documented.[14-17] The family of functional phthalocyanines has been an interesting target for chemists for the development of further chemical reactions on phthalocyanine complexes.[18-21]

On the other hand coumarin derivatives have long been recognized to possess multiple biological activities,[22-26] especially antioxidant and anti-inflammatory activities and the coumarin unit can be found in many natural and synthetic drug molecules. Moreover, as an important class of organic heterocyclic dyes, coumarin derivatives exhibit unique photochemical and photophysical properties, which render them useful in a variety of applications such as optical brighteners, laser dyes, non-linear optical chromophores, solar energy collectors, fluorescent labels and probes in biology and medicine, as well as two-photon absorption (TPA) materials.[27-33]

In the current study, the objective is the preparation and characterization of metal free and metallophthalocyanines (Co, Zn, Cu and Mn) with four peripheral 6-hydroxy-4-methylcoumarin and 6-hydroxycoumarin substituents, with predictable biological activities. The chemical structures of the synthesized compounds were proven by FT-IR and NMR spectra. The antimicrobial activity of the synthesized compounds was evaluated against a panel of nine bacterial strains using broth microdilution methods. Results have shown that the synthesized compounds exhibited moderate to strong antimicrobial activity against the tested species.

RESULTS AND DISCUSSION

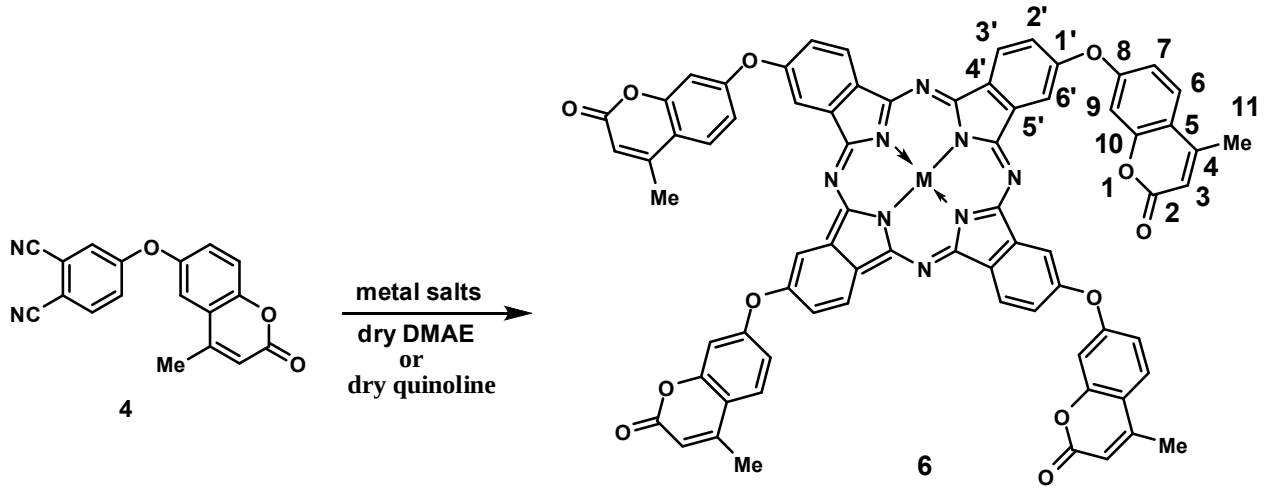
The novel 6-(3,4-dicyanophenoxy)-4-methylcoumarin **4** and 4-(2-oxo-2*H*-chromen-6-yloxy)phthalonitrile **5** were prepared by a base-catalyzed nucleophilic aromatic nitro displacement reaction between 4-nitro-1,2-dicyanobenzene, 6-hydroxy-4-methylcoumarin **1** and 6-hydroxycoumarin **2**, respectively in the presences of K_2CO_3 in dry DMF at room temperature under N_2 atmosphere for one day, and led to 68 and 93% yields of **5** and **4**, respectively (**Scheme 1)**.

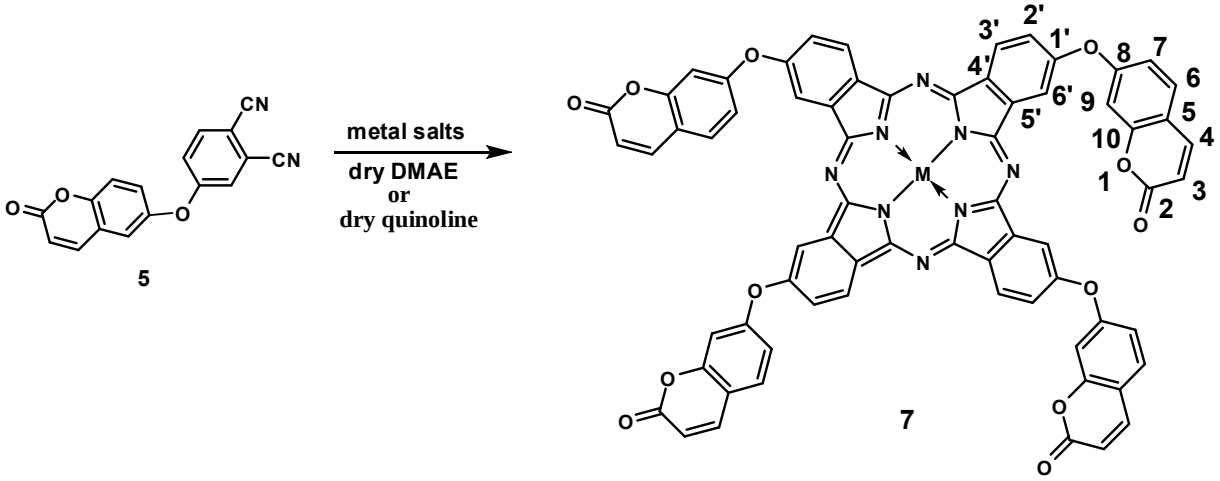


Scheme 1

The structures of the novel compounds **4** and **5** were characterized by combination of ^{1}H NMR, FT-IR, UV-vis and MS spectra data. The mass spectra of compound **4** confirmed the proposed structure by the presence of molecular ion peaks [M] at *m/z* 276. The novel metallo-Pcs **6** and **7** containing four 6-hydroxy-4-methylcoumarin or four 6-hydroxycoumarin moities were obtained respectively from the reaction of the dicyano derivatives (**4** and **5**) and the corresponding metal salts {$Zn(OAc)_2 \cdot 2H_2O$, $Co(OAc)_2 \cdot 4H_2O$, CuCl, $Mn(OAc)_2 \cdot 4H_2O$} as a template for macrocycle formation in 2-*N*,*N*-dimethylaminoethanol (DMAE) or dry quinoline at 170 °C in a sealed glass tube according to the route shown in **Scheme 2**.

The reaction gave **6** and **7** directly as mainly one isomer when examined by ^{13}C NMR spectroscopy (Table 1). But larger scale preparations of this reaction again led to **6** and **7**, only as a mixture of isomers respectively (**Scheme 3**).





Complex 6	Complex 7	Metal	Solvent	Yield % 6	Yield % 7
6b	**7b**	Zn	DMAE	95.13	64.4
6c	**7c**	Co	DMAE	94	96
6d	**7d**	Mn	DMAE	95.2	95
6e	**7e**	Cu	DMAE	96	50

Scheme 2

The yields were satisfactory and depended on the transition metal ion. The tetrasubstituted phthalocyanine products were soluble in DMF and DMSO and purified by extraction with $CHCl_3$, CH_2Cl_2, acetone, and EtOAc. Characterization of the new products **6** and **7** involved a combination of methods including ^1H and ^{13}C NMR, UV-vis and mass spectroscopy. Spectral data of the newly synthesized compounds **6** and **7** are consistent with the proposed structures.

Cyclotetramerization of the phthalonitrile derivative **4** or **5** to the metal-free phthalocyanine **6a** or **7a** was accomplished in 2-*N*,*N*-dimethylaminoethanol (DMAE) at 145 °C in sealed tube and was confirmed by the disappearance of the sharp C≡N vibration at 2333 cm^{-1}.

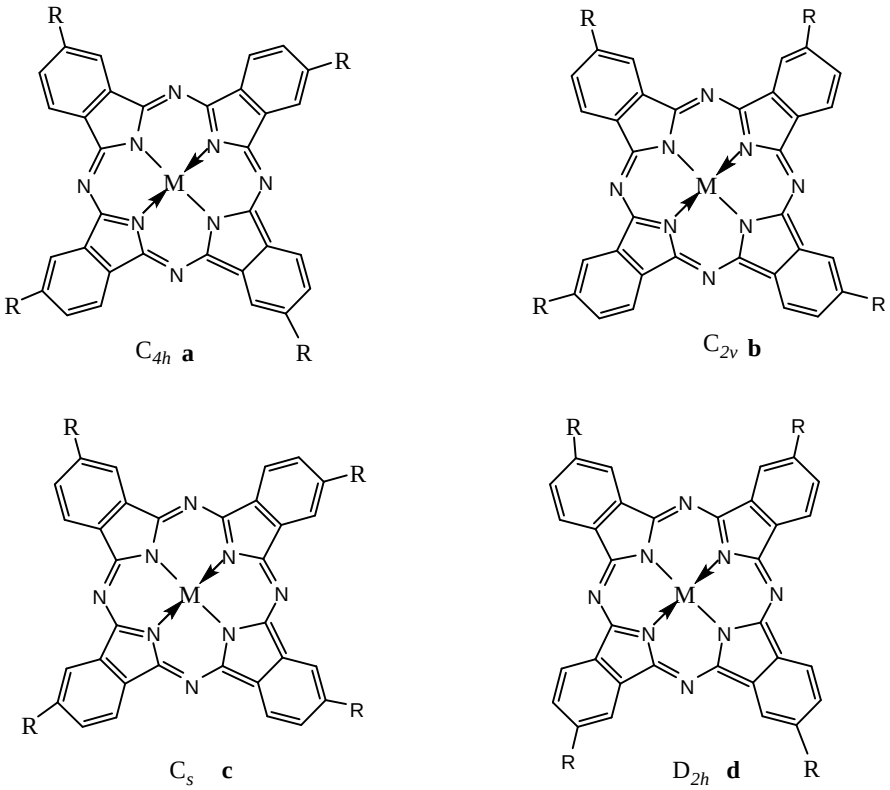
Comparison of the IR spectral data clearly indicated the formation of compounds **6a** and **7a** showing a NH stretching band due to the inner core at 3433 cm^{-1} and 3365 cm^{-1}, respectively. A strong coumarin moiety lactone C=O band at 1725 cm^{-1} was also observed.

The ^1H NMR spectroscopy proved to be a useful tool to check the structure of the synthesized compound **6a**. The ^1H NMR spectra of **6a** in DMSO-d_6 showed a characteristic signal for the H_3 proton in lactone ring at 6.4 ppm. In addition, the ^1H NMR spectrum of **6a** showed the expected pattern for coumarin moiety. In fact the two doublets at 7.60 and 8.30 ppm (J = 6.5 Hz) and two doublet of doublets at 7.30

and 7.90 ppm (J_1 = 0.3 Hz, J_2 = 0.3 Hz), were assigned to $H_{3'}$, H_6, H_8 and $H_{2'}$. The formation of the compound **6a** was further supported by recording the ^{13}C NMR spectrum.

Table 1. Shows the ^{13}C NMR chemical shifts observed for compound **6a**.

Carbon	2	3	4	5, 3'	6, 2'	7, 1'	9'	10	11	4'	5'	CO
Compound **6a**	157.5	108.4	125.1	129.1	134.1, 138.1	157.4	146.1	169.3	27.4	138.2	137.7	173.1



Scheme 3

The mixtures of isomers of phthalocyanines **6** and **7** were characterized by ^1H NMR spectra (C_6D_6). A dilute benzene solution (1.5-2.5 mg per 0.5 mL) was used to obtain good resolved NMR spectra, while high concentration showed broad signals due to aggregation. Moreover, the chemical shifts of the protons are very dependent upon the concentration of the solution. Use of $CDC1_3$ gave a badly resolved spectrum with only four broad signals for the coumarin group. $CDC1_3$ is not capable of breaking up the molecular structure completely; the molecules continue to be stacked in solution. According to **Scheme 3**, the phthalocyanine-system has four different isomers, structures **a** and **d** contain only one magnetically equivalent isoindole unit, structure **b** contains two and ***c*** four nonequivalent isoindole units, respectively. If the eight signals do not overlap one should find four signals with equal intensity for isomer **c** and two

signals with equal intensity for isomer **b,** respectively. The existence of a mixture of isomers is also confirmed by ^{13}C NMR spectra, and each ^{13}C signal appears as a split signal. Phthalocyanines **6** and **7** were prepurified by column chromatography on silica gel using $CHCl_3$ as the eluent. The separation of the isomers of **1** and **2** was attempted by HPLC. The peaks in HPLC were detected by a UV-detector in the wave region λ = 190-600 nm. This showed which peaks belong to phthalocyanines, but not to which isomer. The best indications for phthalocyanine systems are given by their UV-vis spectra in solution. The electronic absorption data of all these phthalocyanines have been measured in DMF or DMSO at a concentration of 1 x 10^{-3} mol L^{-1} (**Figure 1**, **Figure 2**).

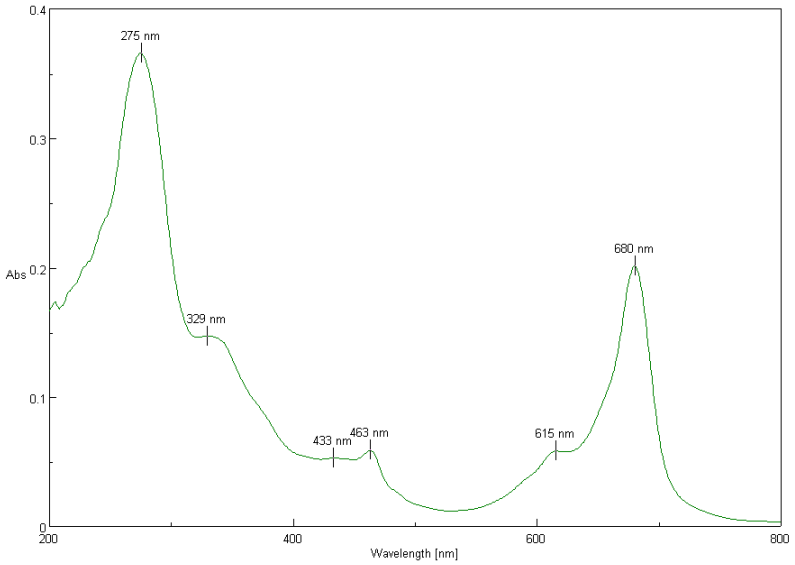


Figure 1. UV-vis spectra of **7c** (1 x 10^{-3} M) in DMF.

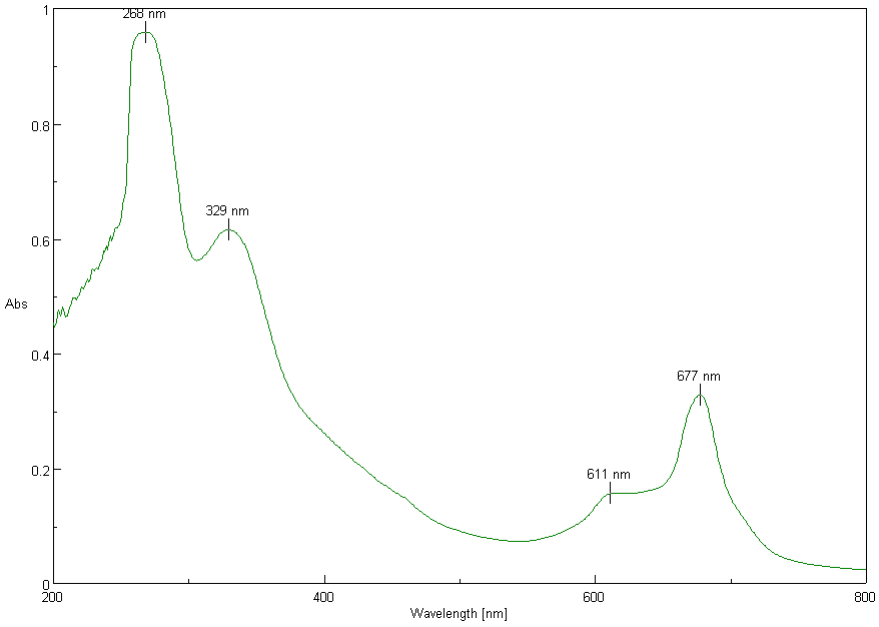


Figure 2. UV-vis spectra of **7a** (1 x 10^{-3} M) in DMF.

The UV-vis spectra of the coumarino-CoPc **7c** depended on its concentration and the B band region was observed around 338-350 nm in DMF. The phthalocyanines show typical electronic spectra with two strong absorption regions, one of them in the UV region at about 275 nm and 680 nm.

ANTIBACTERIAL ACTIVITY

All the final synthesized phthalocyanines **6** and **7** were evaluated for their *in vitro* antibacterial activities against human pathogens. The *in vitro* antibacterial activity was performed against aerobic Gram positive bacterial strains including *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880, *Listeria monocytogenes (*food isolate 2132) and Gram negative bacterium *Salmonella enterica* (food isolate), *Klebsiella pneumoniae* ATCC 10031 and *Pseudomonas aeruginosa* ATCC 9027 using serial dilution method. The results of antibacterial activity are presented in **Table 2**.

Table 2. MIC value of the phthalocyanines **6** and **7** in mg/mL.

	M. luteus	***L. monocytogenes***	***E. faecalis***	***E. coli***	***B. cereus***	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***	***S. enteritidis***
6a	-	-	-	-	-	-	-	-	-
6b	-	-	-	-	-	-	-	-	-
6c	0.625	0.625	0.625	1.25	0.625	0.312	1.25	2.5	0.625
6d	-	-	-	-	-	-	-	-	-
6e	-	-	-	-	-	-	-	-	-
7a	-	-	-	-	-	-	-	-	-
7b	-	-	-	-	-	-	-	-	-
7c	0.625	0.625	0.625	1.25	0.625	0.312	1.25	2.5	0.625
7d	-	-	-	-	-	-	-	-	-
7e	-	-	-	-	-	-	-	-	-
Genta-micin	0.625	2.5	1.25	2.5	1.25	2.5	1.2	0	2.5
DMSO 50% negative (control)	-	-	-	-	-	-	-	-	-

Abreviations: M. ***luteus: Micrococcus luteus*****; *L. monocytogenes*: *Listeria monocytogenes*****; *E. faecalis*: *Enterococcus faecalis*****; *E. coli*: *Escherichia coli*****; K.p: *Klebsiella pneumoniae*****; P.a: *Pseudomonas aeruginosa*****; *B. cereus*: *Bacillus cereus*****; *S. aureus*: *Staphylococcus aureus*****; *S. enteritidis*: *Salmonella enteritidis*****.**

We found that the activity of the synthesized compounds depends on their concentration and the strain of tested bacteria. The Gram positive bacteria were more susceptible to the antimicrobial properties of the synthesized compounds than Gram negative ones. This effect can be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram negative bacteria compared to the single membrane structure of positive ones.[34] The compound **7c** was found to be the most effective compounds against *S. aureus* with MIC value of 0.312 mg/mL. For activity against *B. cereus*, *E. faecalis* and *M. luteus* this compound exhibited a better activity. However, the synthesized compound **7c** *tested in this study*, was highly active against *L. monocytogenes* showing a lower concentration of MIC 0.625 mg/mL.

The compound **7c** was also found to inhibit the growth of clinically important Gram-negative bacteria, such as *K. pneumoniae*, *E. coli*, *P. aeruginosa* and food contaminants such as *S. enteritidis* (food isolate) with MIC value ranging between 0.625 to 2.5 mg/mL. Infections caused by these bacteria, especially those with multi-drugs resistance, are among the most difficult to treat with conventional antibiotics. In the current study, the growth of *S. aureus* was remarkably inhibited by the synthesized compound ***7c***. These results show that the synthesized compounds can be used to minimize problems of drug resistance and protect foods against multiple pathogenic bacteria. The microorganisms tested in the present investigation are large and cover the most important human pathogens known as opportunists for man and animals and causes food contamination and deterioration. The obtained results are of a great importance, particularly in the case of *B. cereus* and *S. aureus*, which are well-known for their resistance to a number of phytochemical compounds and for the production of several types of enterotoxins that causes gastroenteritis. Therefore, the phthalocyanines showed high antibacterial activities and could be considered as one of the sources of natural antibiotics for medicinal use and food anti-poisoning agents against opportunistic pathogens.

CONCLUSION

In conclusion, we have designed and synthesized in polar organic solvent soluble metallo (Zn, Co, Cu, Mn) Pc derivatives derived from phthalonitrile with four peripheral 6-hydroxy-4-methylcoumarin and 6-hydroxycoumarin substituents. The obtained phthalocyanines were characterized by standard methods (MS, ^{1}H NMR, IR and UV-vis spectral data). The antibacterial activity of the synthesized compounds was evaluated against a panel of nine bacterial strains using broth microdilution methods. Results have shown that the synthesized compounds exhibited moderate to strong antimicrobial activity against the tested species.

EXPERIMENTAL

All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. The IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrophotometer, using KBr pellets. ^{1}H and ^{13}C-NMR spectra were recorded in dimethylsulfoxide on a Varian Mercury 200 MHz spectrometer and chemical shifts were reported (d) relative to Me_4Si as internal standard. Mass spectra were measured on a DI analysis Shimadzu Qp-2010 plus spectrometer. Melting points were measured on an electrothermal apparatus and are uncorrected. Optical spectra in the UV-vis region were recorded with a UnicamUV2-100 spectrophotometer, using 1 cm pathlength cuvettes at room temperature. Elemental Analysis were performed using a Carlo Erba 1106 microanalyser at the University of Almeria in Spain.

3.1. Synthesis of 6-(3,4-dicyanophenoxy)-4-methylcoumarin (4)

6-Hydroxy-4-methylcoumarin (**1**) (3g, 17mmol) and then 4-nitrophthalonitrile (1,2-dicyano-4-nitrobenzene) (3.11 g, 17 mmol) were added with stirring to dry DMF (50 mL). After stirring for 15 min, finely ground anhydrous K_2CO_3 (2.15 g, 15.6 mmol) was added portionwise over 2 h and the ensuing mixture was stirred vigorously at room temperature for a further 28 h. The reaction mixture was then poured into water (150 mL) and the precipitate was filtered off and washed with water to yield the yellow product **4**. The slightly brown product **4** was purified by silica gel column chromatography using THF as eluent. Compound **4** was soluble in acetone, EtOAc, THF, $CHCl_3$, CH_2Cl_2, DMF and DMSO.

Yield: 93% (3.09 g); Mp 226-229 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3066-3097 (aryl, CH), 2231 (C≡N), 1762 (CO, lactone), 1598 (C=C), 1257 (Ar-O-Ar). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.02-8.07 (m, 6H, Ar-H), 7.99 (s, 1H, CH), 2.30 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 25.2 (CH_3), 160.0 (C4), 162. 1 (C2), 112.8 (C3), 116.4 (CN), 116.5 (CN), 161.1 (C1'), 120.5 (C2'), 109.1 (C3'), 121.1 (C8), 117.9 (C7), 153.9 (C6), 144.7 (C5), 155.1 (C4), 122.2 (C6'), 143.9 (C10), 109.1 (C4'), 121.1 (C9), 152.1 (C8), 156.1 (C5); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 330 (4.62), 302 (4.47). Anal. Calcd for $C_{18}H_{10}O_3N_2$: C, 71.52; H, 3.33; N, 9.27. Found: C, 71.12; H, 3.20; N, 9.11%.

Synthesis of 4-(2-oxo-2*H*-chromen-6-yloxy)phthalonitrile (5)

6-Hydroxycoumarin (**2**) (2 g, 12.34 mmol) and then 4-nitrophthalonitrile (1,2-dicyano-4-nitrobenzene) (2.25 g, 12.34 mmol) were added with stirring to dry DMF (50 mL). After stirring for 15 min, finely ground anhydrous K_2CO_3 (3.67 g, 26.6 mmol) was added portionwise over 2 h and the ensuing mixture was stirred vigorously at room temperature for a further 28 h. The reaction mixture was then poured into water (150 mL) and the precipitate was filtered off and washed with water to yield the yellow product **5**. The slightly brown product **5** was purified by silica gel column chromatography using THF as eluent. Compound **5** was soluble in acetone, EtOAc, THF, $CHCl_3$, CH_2Cl_2, DMF and DMSO.

Yield: (70%) (3.5 g); Mp 230 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3074 (aryl, CH), 2223 (C≡N), 1621 (C=C), 1248 (Ar-O-Ar); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.42 (s, 1H, C-H), 7.85-7.95 (m, 7H, H_{arom}); ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 161.2 (C2), 116.8 (C2), 117.5 (C≡N), 117.6 (C≡N), 160.5 (C1'), 119.5 (C2'), 129.2 (C3'), 142.7 (C4'), 116.4 (C5'), 129.5 (C6'), 115.4 (C3), 145.2 (C2); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 312 (4.50). Anal. Calcd for $C_{17}H_8N_2O_3$: C, 70.83; H, 2.80; N; 9.72. Found: C, 70.80; H, 2.75; N, 9.70.

3.2. Metal-free phthalocyanines 6a and 7a

6-(3,4-Dicyanophenoxy)-4-methylcoumarin (**4**) (0.2 g, 0.66 mmol) or 4-(2-oxo-2*H*-chromen-6-yloxy)-phthalonitrile (**5**) (0.5 g, 1.57 mmol) was heated with 2 mL of dry 2-(*N*,*N*-dimethylamino)ethanol in a sealed tube. The mixture was held at 145 °C for 48 h and, after cooling to room temperature, the reaction mixture was treated with dilute HCl, then filtered off and washed with water until the filtrate became neutral. The green product was purified by washing with THF, $CHCl_3$, CH_2Cl_2, MeCN, acetone, EtOAc and Et_2O, and dried. The compound is soluble in acetic acid, DMF (slightly) and DMSO (slightly). Compounds **6a** and **7a** were soluble in DMF and DMSO.

2,9,16,23-Tetrakis-(6-coumarinyloxy-4-methyl)phthalocyanine 6a: Yield: (67%) (0.135 g); Mp 290 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3433 (N-H), 3066 (aryl, CH), 2850-2918 (alkyl, CH), 1722 (CO, lactone), 1602 (C=C), 1272 (Ar-O-Ar); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.66 (s, 4H, H9), 7.46 (d, 4H, H7, J = 0.3 Hz), 7.44 (dd, 4H, H6, J_1 = 0.3 Hz, J_2 = 0.3 Hz), 7.40 (dd, 4H, H3', J_1 = 0.3 Hz, J_2 = 0.3 Hz), 7.30 (s, 4H, H6'), 7.20 (dd, 4H, H2', J_1 = 0.3 Hz, J_2 = 0.3 Hz), 6.45 (s, 4H, H3), 3.42 (s, 2H, 2NH), 2.46 (s, 12H, H_{11}); ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 157.5 (C_2), 108.4 (C_3), 125.1 (C_6), 129.1 ($C_{5,3'}$), 134.1 (C_8), 138.1 ($C_{2'}$), 157.4 ($C_{7,1'}$), 146.1 ($C_{6'}$), 169.3 (C_{10}), 27.4 (C_{11}), 138.2 ($C_{4'}$), 137.7 ($C_{5'}$), 173.1 (C4); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 679 (4.17), 631 (4.22), 328 (4.70). Anal.Calcd for $C_{72}H_{42}N_8O_{12}$: C, 71.40; H, 3.50; N, 9.25. Found: C, 70.40; H, 3.60; N, 9.20.

2,9,16,23-Tetrakis-(6-coumarinoxy)phthalocyanine 7a: Yield: (62%) (0.31 g); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3435 (N-H), 3065 (aryl CH), 2860-2920 (alkyl CH), 1725 (CO lactone), 1605 (C=C), 1275 (Ar-O-Ar); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.95 (s, 4H, H9, J = 0.3 Hz), 7.64 (dd, 4H, H6, J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.46 (dd, 4H, H3', J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.28 (dd, 4H, H2', J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.26 (dd, 4H, H7, J_1 = 0.3 Hz, J_2 = 0.0 Hz), 7.25 (s, 4H, H6'), 6.32 (s, 4H, H3'), 3.45 (s, 2H, 2NH); ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 158.4 (C_2), 109.2 (C_3), 126.2 (C_5), 128.2 ($C_{5,3'}$), 135.2 (C_6), 139.2 ($C_{2'}$), 158.5 ($C_{7,1'}$), 148.2 (C_8), 128.5 (C_{11}), 139.1 ($C_{4'}$), 138.8 ($C_{5'}$), 174.2 (C_4); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 680 (4.17), 632 (4.22), 329 (4.70). Anal. Calcd for $C_{68}H_{34}N_8O_{12}$: C, 70.71; H, 2.97; N, 9.79. Found: C, 70.70; H, 2.95; N, 9.80.

3.3. Zinc (II) phthalocyanines 6 and 7

A mixture of compound **4** (0.5 g, 1.5 mmol) or **5** (0.1 g, 0.33 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (0.086 g, 0.39 mmol for **1**; 0.018 g, 0.082 mmol for **2**) was heated at 195 °C with dry quinoline (2 mL) with stirring for 24 h. After cooling to room temperature, the reaction mixture was treated with EtOH and then filtered off and washed with water to remove unreacted $Zn(OAc)_2 \cdot 2H_2O$. The green product was purified by extraction with THF, $CHCl_3$, CH_2Cl_2, MeCN, acetone, EtOAc and Et_2O and dried.

2,9,16,23-Tetrakis(6-coumarinyloxy-4-methyl)phthalocyaninatozinc 6b: Yield: (95%) (0.5 g); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3053 (aryl, CH), 2850-2925 (alkyl, CH), 1722 (CO, lactone), 1595 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.92 (s, 4H, H9, J = 0.3 Hz), 7.82 (dd, 4H, H6, J = 6.5 Hz), 7.42 (dd, 4H, H7, J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.42 (dd, 4H, H3', J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.32 (dd, 4H, H2', J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.30 (s, 4H, H6'), 6.61 (s, 4H, H3), 2.4 (s, 12H, H_{11}); ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 156.3 (C_2), 107.2 (C_3), 132.1 ($C_{5,3'}$), 133.4 (C_6), 138.6 ($C_{2'}$), 157.4 ($C_{7,1'}$), 147.1 (C_8), 167.2 (C_{10}), 28.6 (C_{11}), 139.1 ($C_{4'}$), 138.3 ($C_{5'}$), 176.1 (C4), 131.6 ($C_{5'}$); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 688 (5.08), 617 (4.38), 340 (4.78). Anal. Calcd for $C_{72}H_{40}N_8O_{12}Zn$: C, 67.85; H, 3.16; N, 8.79. Found: C, 67.80; H, 3.20; N, 8.75.

2,9,16,23-Tetrakis(6-coumarinyloxy)phthalocyaninatozinc 7b: Yield: (64%) (0.068 g). Mp 295 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3055 (aryl, CH), 2860-2930 (alkyl, CH), 1728 (CO, lactone), 1596 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.85 (s, 1H, H9, J = 0.3 Hz), 7.82 (dd, 1H, H7, J_1 = 0.3 Hz, J_2 = 0.3 Hz), 7.65 (dd, 1H, H6, J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.55 (d, 1H, H3', J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.32 (dd, 1H, H2', J_1 = 6.5 Hz, J_2 = 6.5 Hz), 7.30 (d, 1H, H4', J = 6.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 158.3 (C_2), 108.2 (C_3), 128.2 (C_4), 133.2 ($C_{5,3'}$), 134.2 (C_6), 138.4 ($C_{2'}$), 158.3 ($C_{7,1'}$), 148.2 ($C_{9'}$), 172.3 (C_{10}), 28.5 (C_{11}), 140.1 ($C_{4'}$), 138.2 ($C_{5'}$), 178.2 (CO); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 686 (5.08), 620 (4.38), 342 (4.78). Anal. Calcd for $C_{68}H_{32}N_8O_{12}Zn$: C, 67.03; H, 2.65; N, 9.20. Found: C, 67.10; H, 2.65; N, 9.20.

3.4. Cobalt(II) phthalocyanines 6 and 7

Compound **4** (0.1 g, 0.31 mmol) or **5** (0.05 g, 0.16 mmol) and $CoCl_2 \cdot 6H_2O$ (0.018 g, 0.0078 mmol for **1**; 0.0098 g, 0.041 mmol for **2**) were heated at 155 °C with dry hexanol (2 mL), in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.05 mL) in a sealed tube with stirring for 24 h. After cooling to room temperature, the reaction mixture was treated with dilute HCl, filtered and washed with water until the filtrate became neutral in pH. The green product was purified by extraction with THF, $CHCl_3$, CH_2Cl_2, MeCN, acetone, EtOAc and Et_2O and dried. Both **6c** and **7c** were soluble in DMF and DMSO.

2,9,16,23-Tetrakis(6-coumarinyloxy-4-methyl)phthalocyaninatocobalt 6c: Yield: (94%) (0.049 g); Mp 295 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3067 (aryl, CH), 2940 (alkyl, CH), 1242 (Ar-S-Ar); UV/Vis ($CHCl_3$,

λ_{max}, nm, (ε)): 675 (4.95), 612 (4.28), 325 (4.77). Anal. Calcd for $C_{72}H_{40}N_8O_{12}Co$: C, 68.20; H, 3.18, N, 8.84. Found: C, 68.20; H, 3.10; N, 8.60.

2,9,16,23-Tetrakis(6-coumarinyloxy)phthalocyaninatocobalt 7c: Yield: (96%) (0.1 g); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3068 (aryl, CH), 2945 (alkyl, CH), 1246 (Ar-O-Ar); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 676 (4.95), 614 (4.28), 327 (4.77). Anal. Calcd for $C_{68}H_{32}N_8O_{12}Co$: C, 67.39; H, 2.66; N, 9.25. Found: C, 67.40; H, 2.60; N, 9.25.

3.5. Manganese phthalocyanines 6 and 7

Compound **4** (0.1 g, 0.31 mmol) or **5** (0.1 g, 0.33 mmol) and $MnCl_2 \cdot 6H_2O$ (0.018 g, 0.078 mmol for **4**; 0.019 g, 0.039 mmol for **5**) were heated at 155 °C with dry hexanol (2 mL), in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.05 ml) for **4**, at 195 °C with dry quinoline (2 mL) for **5** in a sealed tube with stirring for 24 h. After cooling to room temperature, the reaction mixture was treated with dilute HCl, filtered and washed with water until the filtrate became neutral in pH. The ensuing green product was purified by extraction with THF, $CHCl_3$, CH_2Cl_2, MeCN, acetone, EtOAc and Et_2O and dried. The compounds (**6e**, **7e**) were partly soluble in DMF and DMSO.

2,9,16,23-Tetrakis(6-coumarinyloxy-4-methyl)phthalocyaninato manganese 6d: Yield: (95%) (0.1 g); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3045 (aryl, CH), 2827-2910 (alkyl, CH), 1722 (CO, lactone), 1595 (C=C); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 685 (4.90), 615 (4.36), 317 (4.99). Anal. Calcd for $C_{72}H_{40}N_8O_{12}Mn$: C, 68.41; H, 3.19; N, 8.86. Found: C, 68.40; H, 3.20; N, 8.85.

2,9,16,23-Tetrakis(6-coumarinyloxy)phthalocyaninato manganese 7d: Yield: (95%) (0.1 g); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3046 (aryl, CH), 2830-2920 (alkyl, CH), 1732 (CO, lactone), 1597 (C=C); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 687 (4.90), 620 (4.36), 320 (4.99). Anal. Calcd for $C_{68}H_{32}N_8O_{12}Mn$: C, 67.61; H, 2.67; N, 9.28. Found: C, 67.60; H, 2.60; N, 9.25.

3.6. Copper (I) phthalocyanines 6 and 7

Compound **4** (0.1 g, 0.31 mmol) or **5** (0.1 g, 0.33 mmol) and anhydrous CuCl (0.0078 g, 0.078 mmol for **1**; 0.082 g, 0.082 mmol for **2**) were heated at 155 °C with dry hexanol (2 mL), in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.05 ml) for **5a**, at 195 °C with dry quinoline (2 mL) for **5b** in a sealed tube with stirring for 24 h. After cooling to room temperature, the reaction mixture was treated with dilute HCl and filtered off and then washed with water until the filtrate became neutral in pH. The green product was washed with NH_4OH (24%, 3 × 50 mL) to remove unreacted CuCl and then washed with water until the filtrate became neutral in pH. The product was purified by extraction with THF, $CHCl_3$, CH_2Cl_2, MeCN, acetone, EtOAc and Et_2O and dried. The compounds **6d** and **7d** were partially

soluble in DMF and DMSO.

2,9,16,23-Tetrakis(6-coumarinyloxy-4-methyl)phthalocyaninatocopper 6e: Yield: (96%) (0.1 g); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3066 (aryl, CH), 2850-2925 (alkyl, CH), 1714 (CO, lactone), 1595 (C=C); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)):686 (4.78), 620 (4.40), 340 (4.76). Anal. Calcd for $C_{72}H_{40}CuN_8O_{12}$: C, 67.95; H, 3.17; N, 8.80. Found: C, 67.90; H, 3.20; N, 8.80.

2,9,16,23-Tetrakis(6-coumarinyloxy)phthalocyaninatomanganese 7e: Yield: 0.052 g (50%); Mp 300 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3068 (aryl, CH), 2860-2935 (alkyl, CH), 1720 (CO, lactone), 1598 (C=C); UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 688 (4.78), 628 (4.40), 350 (4.76). Anal. Calcd for $C_{68}H_{32}CuN_8O_{12}$: C, 67.13; H, 2.65; N, 9.21. Found: C, 67.10; H, 2.60; N, 9.20.

3.7. Antibacterial activity

3.7.1. Microorganisms and growth conditions

Authentic pure cultures of bacteria were obtained from international culture collections (ATCC) and the local culture collection of the Center of Biotechnology of Sfax, Tunisia. They included Gram-positive bacteria: *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880, *Listeria monocytogenes (*food isolate 2132*)* and Gram-negative bacteria: *Salmonella enterica* (food isolate), *Klebsiella pneumoniae* ATCC 10031 and *Pseudomonas aeruginosa* ATCC 9027. The bacterial strains were cultivated in Mueller-Hinton agar (MH) (Oxoid Ltd, UK) at 37 °C except for Bacillus species which were incubated at 30 °C. Working cultures were prepared by inoculating a loop full of each test bacteria in 3 mL of Mueller-Hinton broth (MH) (Oxoid Ltd, UK) and were incubated at 37 °C for 12 h. For the test, final inoculum concentrations of 10^6 CFU/mL bacteria were used. DMSO was used as negative control.

3.7.2. Minimum inhibitory concentration measurement

Minimum inhibitory concentrations (MIC) of the synthesized compounds were determined according to the literature with minor modifications against a panel of nine microorganisms representing different species of different ecosystems. The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 µL. For susceptibility testing, 100 µL of Mueller-Hinton broth was distributed from the second to the twelfth test wells. A stock solution of the synthesized compounds was prepared by dissolving 100 µL of the tested compounds in DMSO and then adjusted to a final concentration of 50 mg/mL by Mueller-Hinton broth. The first well of the microplate was prepared by dispensing 160 µL of the growth medium and 40 µL of the synthesized compounds to reach a final concentration of 10 mg/mL and then 100 µL of scalar dilutions were transferred from the second to the ninth well. Thereafter and from each well, 10 µL of the suspension were removed and replaced by the

bacterial suspensions to final inoculum concentrations of 10^6 CFU/mL for bacteria. The final concentrations of the synthesized compounds adopted to evaluate the antimicrobial activity were 0.039 to 10 mg/mL. The 10th well was considered as positive growth control containing Mueller-Hinton media for bacterial strains, since no of the synthesized compounds was added. The plates were then covered with the sterile plate covers and incubated at 37 °C for 24 h for bacterial strains. The MIC was defined as the lowest concentration of the total essential oil at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (0.5 mg/mL) dissolved in sterile water were added to the wells and incubated at 37 °C for 30 min where microbial growth was inhibited, the solution in the well remained clear after incubation with MTT. All experiments were performed in triplicate.

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