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Synthesis, Characterization, Antioxidant Studies and *in vitro* Biological Activities of Metallophthalocyanines Bearing (4-tolylsulfonyl) Substituents

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Abstract: ***This article aims at synthesizing a new series of phthalocyanine of Zn, Co, Cu substituted by tetra-4-tolylsulfonyl, in order to investigate their spectral properties and then to testy their antioxidant, antibacterial and antifungal activities in vitro. Synthesized compounds have been characterized by elemental analysis, FT-IR, ^{1}H NMR, MALDI-MS, elemental analyses and UV–Vis spectral data. These molecules show admirable solubility in organic solvents such as THF, DMF, DMSO, Acetone and toluene, in all these solvents the behavior of accumulation was examined and showed behavior monomeric in all the solvents for the ranges of the studied concentration.. The antioxidant capacities were analyzed through radical scavenging of DPPH. Free radical scavenging capacity of phthalocyanine complexes Co (II), Cu (II) and Zn (II) are respectively 38.9%, 41.4% and 42.8% at concentration of 100 mg/L. The antimicrobial activity of compounds against seven strains, suggest are a promising antimicrobial photosensitizers for the treatment of infectious diseases.***

Keywords: Photosensitizers, Metallophthalocyanine, PACT, antibacterial, antifungal, chemotherapy, photodynamic.

1. Introduction

The emergence of microbial resistance to most of the known classes of antibiotics has led to an urgent need to identify new antimicrobial strategies [[1]–[5]].

Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans. Other fungal infections are said to be opportunistic because the causative agents cause mild or no disease in healthy individuals but may infect and cause severe disease in immunodeficient persons. The human airway is continuously open to the no sterile environment where fungal spores have the potential to reach lung tissue and produce disease. In the immuno compromised host, many fungi, including species of fungi typically considered nonpathogenic, have the potential to cause serious morbidity and mortality.

Over the last several decades the advent of the human immunodeficiency virus (HIV) epidemic and the increasing use of immunosuppressive drugs for serious medical conditions have dramatically increased the number of persons who are severely immuno compromised. In addition, the range and diversity of fungi that cause disease have broadened. Although Candida and Aspergillus species continue to be the fungal pathogens that most frequently cause invasive fungal disease in immunocompromised persons overall [[6]].

Antibiotics and antifungals at present are used within the framework of curative measures to protect the patients against fatal diseases as far as some are mortal especially for the people at risks. Now several searches in the field of the microbial pathogenesis and the appearance of new antibiotics on the market, the morbidity and the mortality bound to the pathogenic infections do not stop increasing [[7],[8]]. There is an alarming growth of the resistance in antibiotics by the pathogenic germs with a growth of the number of the deaths being caused by pathogenic germs [[9]]. It requires new efforts to discover of other alternative for to eradicate these pathogenic microbial [[10]].The use of photosensitizers and light is complementary as well as of an alternative method in the conventional method of fight against the pathogenic germs. When the light of a specific wavelength enlightened by such a photosensitizing molecule, particles with high reactivity are generated who can destroy the pathogenic microbial cells [[11]]. Previous studies brought reported a high quantum yield of the production of oxygen, a photo high toxicity when the light of specific wavelength is enlightened on them, and a big stability in the physiological conditions [[12],[13]]. Photosensitizers that are currently being studied in the fight against these microbes include porphyrins, chlorine, bacterioclorin and phthalocyanine [[14]].

Photodynamic antimicrobial chemotherapy (PACT) involves the combination of a light-sensitive dye, known as a photosensitizer (PS), and locally applied visible light [[15],[16]]. Upon illumination of the PS at one or more wavelengths corresponding to the absorption peaks, the excited molecule can react with a target (molecular oxygen or other targets within biological systems) by electron

transfer generating radical species (Type I mechanism). Alternatively, the excitation energy can be transferred from the excited triplet of the PS to triplet dioxygen forming a ground state PS and excited singlet oxygen (Type II mechanism) (Fig 1). Accumulation of such reactive species, both radical and singlet oxygen, leads to irreversible damage to the target cell.

At present, numerous PS have been developed for the targeting of localised infections and drug-resistant bacteria [[17]] and no microbial resistance to PACT has been reported so far [[18]–[19]]. Despite this, clinical applications are still relatively limited.

2. Experimental

2.1. Materials and equipments

Melting points were determined in a capillary tube and are uncorrected. The NMR spectra were recorded on a Brucker AC 300MHz spectrometer in $CdCl_3$ and $DMSO_{d6}$. Elemental analyses were determined by using Perkin-Elmer 240c elemental analyzer.Mass spectral data were collected with a BrukerAutoflex III

Smartbeam TOF/TOF Mass spectrometer.

Thin layer chromatography (TLC) was performed on precoated silica gel plates (0.25 mm, Merck). Column chromatography was performed on Merck silica gel having size (0.063-0.200 mm). ascorbic acid, BHT, DPPH. All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. Solvents were dried and purified according to standard procedure. All other materials were purchased from Aldrich and Enamine Ltd.

UV-Visible absorption spectra were recorded on Cary 2300 spectro-photometer. FT-IR: Infrared (IR) spectra were recorded on a Perkin-Elmer 65 FT-IR (ATR) instrument.

2.2 Synthesis

4-Nitrophthalonitrile was prepared according to a procedure as previously described in literature [[21]].

2.2.1. Synthesis of 4-tolylsulfonylphthalonitrile (3)

4-Nitrophthalonitrile (1.24 g, 7.2 $x10^{-3}$ mol) was dissolved in anhydrous DMF (12 mL) under nitrogen atmosphere and p-Toluenesulfinic acid sodium salt **(1)** (1.28 g,7.2 x 10^{-3} mol) was added to this solution. After stirring for 10 min, finely ground anhydrous K_2CO_3 (3 g, 21.6 10^{-3} mol) was added in portion wise over 2 h with stirring. The reaction mixture was stirred at 60 °C for 72h under nitrogen atmosphere. The product 4-(Tolyl-4-sulfonyl)-phthalonitrile was filtered off and washed with water. After drying in the desiccator the product was recrystallized from ethanol. Yield: 1.33 g (96.5%), mp 137-138°C.

FT-IR spectrum (cm^{-1}) : 3102 (Ar−H), 3075,3034 (Aliphatic C−H), 2238 (C≡N), 1670, 1591, 1492, 1478, 1447, 1387, 1330, 1272, 1214, 1185, 1154, 1122, 1085,1016, 983, 910, 875, 855,820,798,705,676,643, 631,611. ^{1}H NMR: ($CDCl_3$), (δ:ppm): 8.30 (br,s,1H), 8.25 (dd, J=1.5, 8.1 Hz 1H), 8.00 (d, J=8.1Hz, 1H), 7.66 (d, J=8.1Hz, 2H), 7.38 (d, J=8.1Hz, 2H), 2.93 (s, 3H, CH_3).

Anal calculated for $C_{15}H_{10}O_2N_2S$: C, 63.81; H, 3.57; N, 9.92; O,11,34, S, 11.35%; Found: C, 63.83; H, 3.59; N, 9.91; O,11,32, S, 11.32%. MALDI-ToF (m/z) $[M+H]^+$ calculated for $C_{15}H_{10}N_2O_2S$: 283.05, found: 283,08.

2.1.2. Synthesis of (4)-Tetra(4-Tolylsulfonyl) phthalocyanina to zinc (II) (4)

4-(Tolylsulfonyl)phthalonitrile) (3) (0.3 g, 0.97$x10^{-3}$ mol), anhydrous $Zn(CH_3COO)_2$ (0.09 g,0.48$x10^{-3}$ mol) and 0.003 L of n-pentanol were placed in a standard Schlenk tube in the presence of 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) (0.6 mL, 0.48$x10^{-3}$ mol) under a nitrogen atmosphere and held at reflux temperature for 16 h.

After cooling to room temperature, the reaction mixture was precipitated by adding it drop-wise into water/hexane (50% v/v). The crude product was collected by filtration and washed with water, hexane and ether and then dried. Purification was achieved using column chromatography on silica gel using $CHCl_3$ as eluent.

Yield: 0.132 g (49%). UV-Vis (THF) λ max (logε): 343(4,40) 684 (4,19). FT-IR: 3089 (Ar−H), 2923−2859 (Aliphatic C−H), 1603, 1593, 1489, 1395, 1300, 1184, 1147, 1084, 1039, 910, 811, 755, 745, 706, 688, 662, 604, 566,526.

Zn Pc **4** ^{1}H NMR: 8.30-7,38 (br, Ar-H) 2.93 (s, CH_3). Anal calculated for $C_{60}H_{40}O_8N_8S_4Zn$: C, 60.32; H, 3.37; N, 9.38; O,10,71, S, 10.74, Zn,5,47%; Found: C, 60.33; H, 3.39; N, 9.36; O,10,72, S, 10.73,Zn,5,46 %. MALDI-ToF (m/z) $[M+H]^+$ calculated for $C_{60}H_{40}N_8O_8S_4Zn$: 1195.66, found : 1196,68.

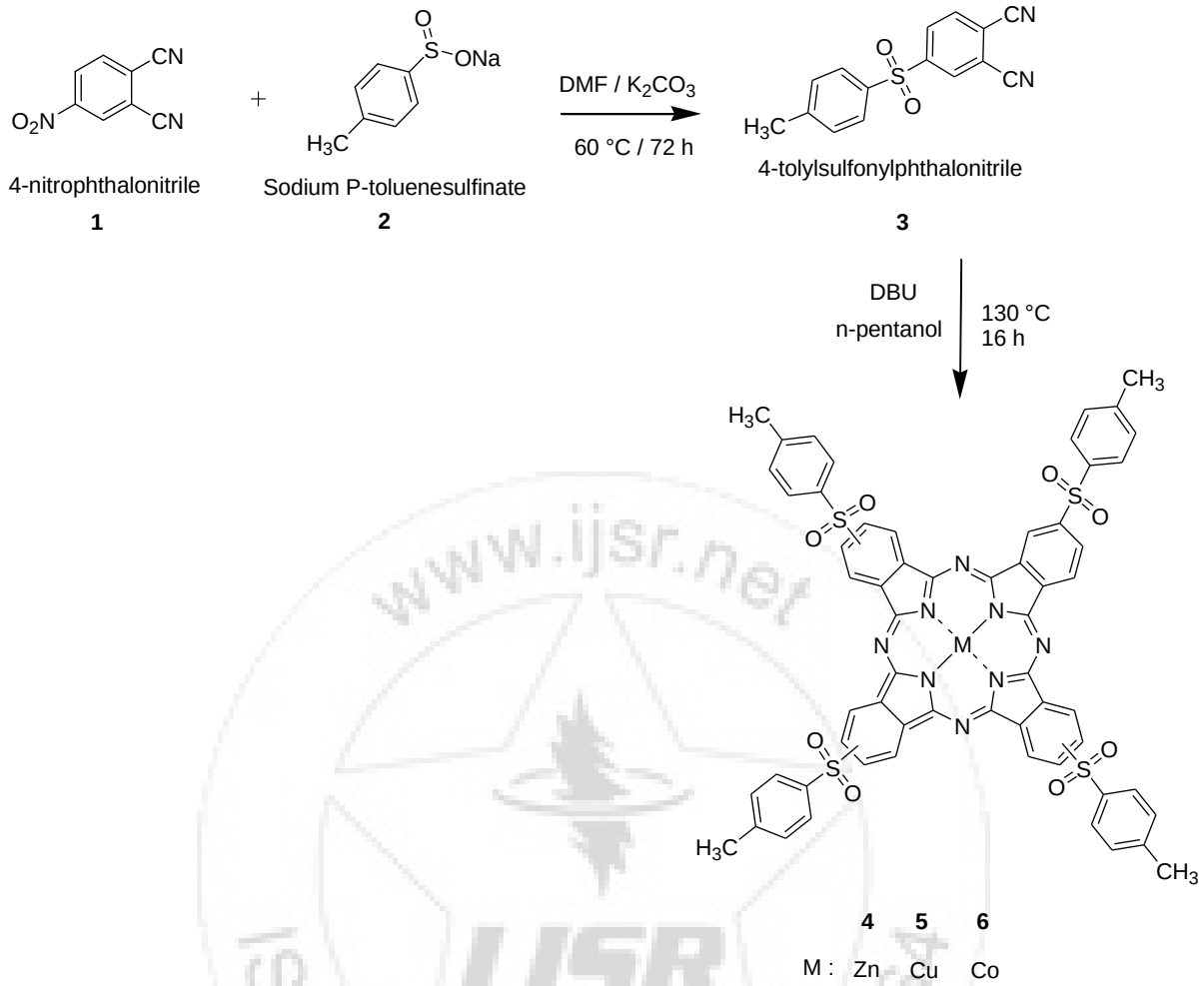
2.1.3. Synthesis of (4)-Tetra(4-Tolylsulfonyl)phthalocyaninato Copper (II) (5)

The procedure is the same with synthesis of compound 4 from 3 except using CuCl2.
The product is soluble in DMF, THF, Aceton, Toluen,CH_2Cl_2 and DMSO. The yield was 0,105 g (40%) UV-Vis (THF) λ max (logε): 341(4,39) 682 (4,18). FT-IR spectrum (cm^{-1}): 3087 (Ar−H), 2922−2860 (Aliphatic C−H), 1605, 1594, 1490, 1394, 1299, 1180, 1144, 1085, 1038, 911, 813, 756, 744, 706, 689, 663, 604, 565,528.
Anal calculated for $C_{60}H_{40}O_8N_8S_4Cu$: C, 60.42; H, 3.38; N, 9.39; O,10,73, S, 10.75, Cu,5,33%; Found: C, 60.39; H, 3.39; N, 9.38; O,10,75, S, 10.74,Cu,5,35 %. MALDI-ToF (m/z) $[M+H]^+$ calculated for $C_{60}H_{40}O_8N_8S_4Cu$: 1192,20, found:1192,11.

2.1.4. Synthesis of (4)-Tetra(4-Tolylsulfonyl)phthalocyaninato Cobalt (II) (6)

The procedure is the same with synthesis of compound 4 from 3 except using CoCl2. The product is soluble in DMF, THF, Aceton, Toluen,CH_2Cl_2 and DMSO. The yield was 0,105 (37%) UV-Vis (THF) λ max (logε): 342(4,37) 683 (4,17). FT-IR spectrum (cm^{-1}): 3088 (Ar−H), 2924−2856 (Aliphatic C−H), 1604, 1591, 1488, 1395, 1302, 1185, 1146, 1086, 1037, 910, 812, 756, 743, 705, 688, 663, 605, 566,527.

Anal calculated for $C_{60}H_{40}O_8N_8S_4Co$: C, 60.65; H, 3.39; N, 9.43; O,10,77, S, 10.79, Co,4,96%; Found: C, 60.66; H, 3.40; N, 9.43; O,10,76, S, 10.80,Co,4,95 %. MALDI-ToF (m/z) $[M+H]^+$ calculated for $C_{60}H_{40}O_8N_8S_4Co$: 1188.20, found : 1189,22.



Scheme1. The synthesis of Metallophthalocyanines 4-6

2.2 DPPH radical scavening assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging activity of all compounds was studied by apply ing the method described in literature with some modification [[22]] .

Briefly, 0,5 ml of methanol solution of the test compounds was added to 2 mL of methanolic solution of DPPH. The final concentrations of the test compounds in the reaction mixtures were 5, 10, 25, 50 and 100 mg/L. the mixture was shaken vigorously and had been in cubated for 30 min at room temperature in the dark. The decrease in absorbance of DPPH at 517 nm was mesured afterwards. A control reaction was expiremented without methanol solution of compounds. Methanol solution was used as the blank control. The DPPH scavenging activity was calculated according to the equation:

$$\boldsymbol{DPPH\ scaving\ activity}(\%) = \left(1 - \frac{\boldsymbol{Asample}}{\boldsymbol{Acontrol}}\right) \boldsymbol{X100}$$

Where A_{sample} is the absorbance in the presence of sample and $A_{control}$ is the absorbance respectively. Experimental results were compared with using ascorbic acid and BHT as standard.

Figure 2: Variation of concentration Phthaloctananine study

2.3 Antimicrobial activities

2.3.1 Antimicrobial conditions activities

In recent years, the antimicrobial effect of PDT has begun to be of great interest in view of the increasing bacterial resistance to antibiotics. The researchers proposed that antimicrobial PDT (PDTa) is a new treatment modality that can kill or inactivate certain microbial cells. [[23]].

Due to its link with biology, organic chemistry has created a scientific basis to fight the most serious infectious disease [[24]]. In this context, we have been interested in studying the biological activity of synthesized ZnPc. We have

therefore carried out various tests to highlight their antimicrobial effects.

2.3.2. Microorganisms and growth conditions:

2.3.2.1 *Bacterial Strains*

Gram-positive bacteria:
- Micrococcus luteus CIP5345
- Staphylococcus aureus Subsp CIP 4.83
- Bacillus subtilis CIP 5262

Gram-negative bacteria:
- Salmonella enterica ssp. CIP 8039
- Escherichia coli CIP 54127

2.3.2.2 *Fungal Strains:*
- Candida albicans ATCC 10231
- Aspergillus Niger ATCC 16888

The strains were used as indicator microorganisms for antibacterial activity tests. Antifungal activity was determined against Candida albicans ATCC 10231 and Aspergillus niger ATCC 16888. The bacterial strains used as indicator microorganisms were cultured Overnight in liquid LB media under aerobic conditions and constant shaking (200 rpm) at 30 ° C for M. luteus CIP5345, B.subtilis CIP 5262, and at 37 ° C for S. aureus CIP 4.83, S.Typhimurium ATCC 14028 and E.coli CIP54127, then 1: 100 diluted in LB medium and incubated for 5 h under constant agility (200 rpm) at the appropriate temperature. C. albicans was cultured in Tryptone-Soy Broth (TSB) at 30 ° C for 24 h.

2.3.3. Dissemination method of Agar Well

The agar disk diffusion method was used to determine the ability of a substance to exert an antimicrobial effect, it is also called an agar dilution technique for the evaluation of the antimicrobial activities of the compounds synthesized "Phthalocyanines" Cu(II) PC, Co(II)PC and Zn(II) PC at differents concentrations in DMSO ranging from 150 mg / L to 1250 mg / L and 20 µL were added to the disks.

The synthesized compounds can diffuse in Middle gélose agar "LB" for bacterial strains and dextrose of potatoes Agar " PDA " for the stumps of fungi. Sterile disks have was placed in each of the plates of agar which have was uniformly sowed on surface by using sterile glass balls or swabs and bacterial suspension of 10^8 UFC / ml.

The petri dish so was exposed to the light LED (red) during 1/2 hour at room temperature to be incubated in the temperature suited for every bacteria and fungi strain. After 24 hours of incubation, the resulting inhibition zones (including the diameter of the disc) will be uniformly circular because there will be a confluent growth curve. The antimicrobial activities were tested by measuring in millimeters the diameters of the inhibition.

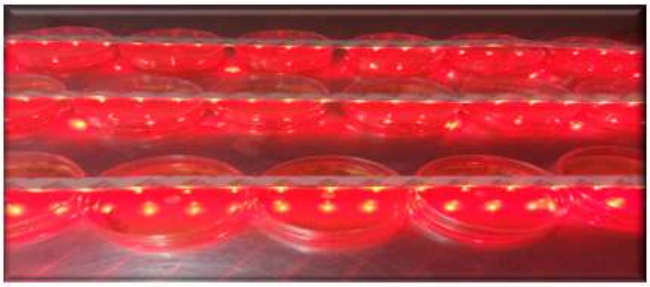


Figure 3: The petri dish so were exposed to the light LED

2.4 Statistical Analysis

Statistical study carried out by the statistical software SPSS V 19.0. All experiments were performed in triplicate, the results are expressed as mean ± SD. The results are analyzed using the one-way variance "ANOVA" and the multiple comparisons between the groups were used using the Newman-Keuls multiple comparison test at the 5% threshold and P <0.05 was considered statistically significant that P <0.01 was very significant.

3. Results and Discussion

3.1 Synthesis and Characterization

Phthalonitrile derivatives **3** obtained through the reaction of p-Toluenesulfinic acid sodium **s**alt **(1)** and 4-nitrophthalonitrile in dry DMF in the presence of dry K_2CO_3. The synthesis of phthalocyanine complexes **4-6** were achieved by healting the phthlonitrile **3** with $Zn(CH_3COO)_2$, $CoCl_2$, $CuCl_2$ metals salts under N_2 atmosphere (Sheme1). The purification of metallophthaocyanines **4-6** was carried out by column chromathography on silica gel using THF as an eluent. Characterization of the products involved a combination of methods, including IR, ^{1}H NMR, and UV-vis spectra. The proposed structures of the compounds were confirmed by the results of these analyses.

The ^{1}H NMR spectrum of **3** showed aromatic ring protons between 8.30, 8.25, 8.00, 7.66 and 7.38 ppm as multiple peaks and CH_3 protons at 2.93 ppm. The ^{1}H NMR spectrum of **3** exhibits the characteristic signals of aromatic protons at 8.30- 7.33 ppm and CH_3 protons at 2.95ppm.

The ^{1}H NMR spectrum the phthalocyanine **4** provided the characteristic chemical shifts and confirmed the proposed structures. In the spectrum taken in DMSO-d_6 aromatic protons appeared at 8.64-7.31 and CH_3 at 2.80 ppm.

^{1}H RMN measurements of the metallophtalocyanines (M: Cu, Co) were precluded due its paramagnetic nature of the metal cation. Comparison of the IR spectral data cleary indicated the formation of compound **3** by the appearance of new absorption bands. After conversion of the phthalonitrile derivate 3 into phthalocyanines **4-6**, the sharp peak for the (CN) vibrations disappeared.

The IR spectrum of **4** cleary indicated its formation by the appearance of new absorption bands at 2923 (CH_3), 2230 (CN), 1488 ($C=CH_2$), 1393-1443 (S=O). The IR spectra of

macrocyclic compounds **5-6** were very similar to the spectrum of **4** with slight shifts.

Table 1: FTIR/IR of metallophthalocyanine 4-6

	Vibration modes	νs CH3	$\nu_{c=c}$	δa_{C-H2}	δ_{C-H3}	νs S=O νa S=O	hors du plan γN-H
Compound 5 (CuPc)	number of waves (cm^{-1})	2924	1645 158	1490	1394 1442	1299 1144	911 663
Compound 6 (CoPc)	number of waves (cm^{-1})	1922	1641 156	1488	1395 1444	1302 1146	910 661
Compound 4 (ZnPc)	number of waves (cm^{-1})	2923	1644 159	1488	1393 1443	1300 1147	910 663

δ : deformation in the plane at vibrations of elongations the connection C=C aromatic. The absorption band appeared towards 1300 cm^{-1} told us to a grouping vibration **S=O**. This spectrum also showed us a band in 1393 cm^{-1}, attributed to asymmetric deformation vibration **δa** of grouping **CH3**. A band of lower absorption in 1488 cm^{-1} corresponds to the asymmetric deformation **δa** of grouping **CH_2**.

3.2 Aggregation properties

The UV-vis spectroscopy is a very useful technique which can be used to study the aggregation phenomena of phthalocyanines in both solution and solid state **[25]**. The UV-vis spectral data of the studied phthalocyanines **4-6** in the **THF** were given in **Table 1**. The UV-vis spectra of 4-6 shows the typical patterns of metallophthalocyanine complexes. They exhibited characteristic **Q** and **B** bands one in the visible region at 614-687 nm attributed to the **π-π*** transition from the highest occupied molecular orbital (HOMO) the lowest unoccupied molecular orbital (LUMO) wof the Pc^{2-} ring , and the other in the UV region 320-350 nm, arising from the deeper **π**-levels to LUMO transition.

In general, Pc aggregation is thought to reflect coplanar interactions involving macrocycle ring. These interactions occur as a result of favorable **Van der Waals** forces. **π**--stacking interactions. and solvent effects. the aggregation process of Pcs can easily be probed using electronic spectroscopy **[[26]].**

The phthalocyanine derivatives (4-6) did not show aggregation in THF at different concentrations. Beer-Lambert law was obeyed for compounds (4-6) in the concentrations ranging from **25 µM** to 1,25 µM, $1{,}25\times10^{-6}$ mol dm^{-3}, 2 ,5×10^{-6} mol dm^{-3}, 5×10^{-6} mol dm^{-3}, $7{,}5\times10^{-6}$ mol dm^{-3}, $1{,}25\times10^{-5}$ mol dm^{-3}2, 5×10^{-5} mol dm^{-3} recpectively. The fact has been verified by UV-vis investigations on solutions in the concentration 2, 5×10^{-5} -$1{,}25\times10^{-6}$ mol dm^{-3}. The graph of molar absorptivity versus concentration gave a constant value in the concentration range (Fig 4-6).

These new substituted phthalocyanines showed enhanced solubility in a number of organic solvents, e.g dichloromethane, DMSO, DMF, chloroform, toluene, etc., as excepted. Another important feature of these compounds is not to exhibit a tendency to form aggregates.

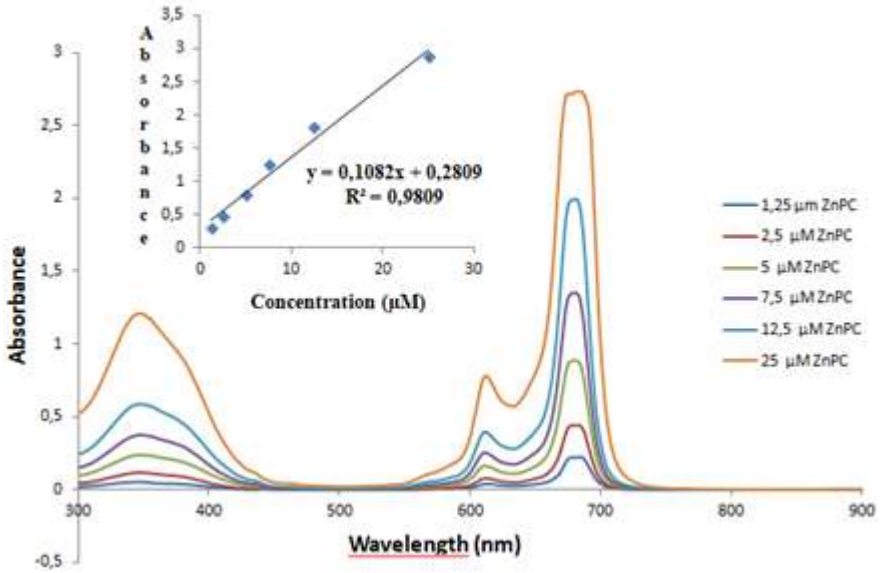


Figure 4: The aggregation behavior of phthalocyanine 4

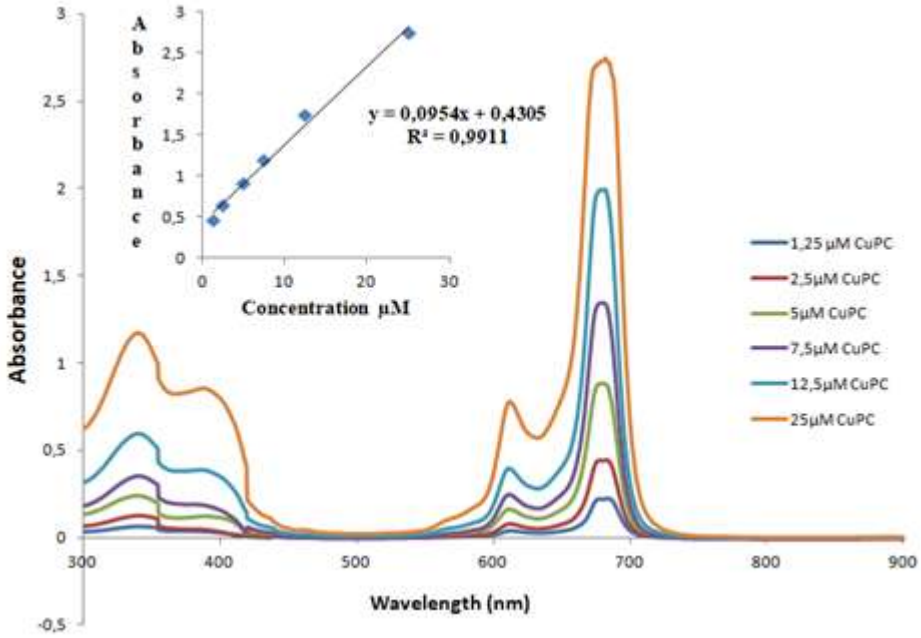


Figure 5: The aggregation behavior of phthalocyanine 5

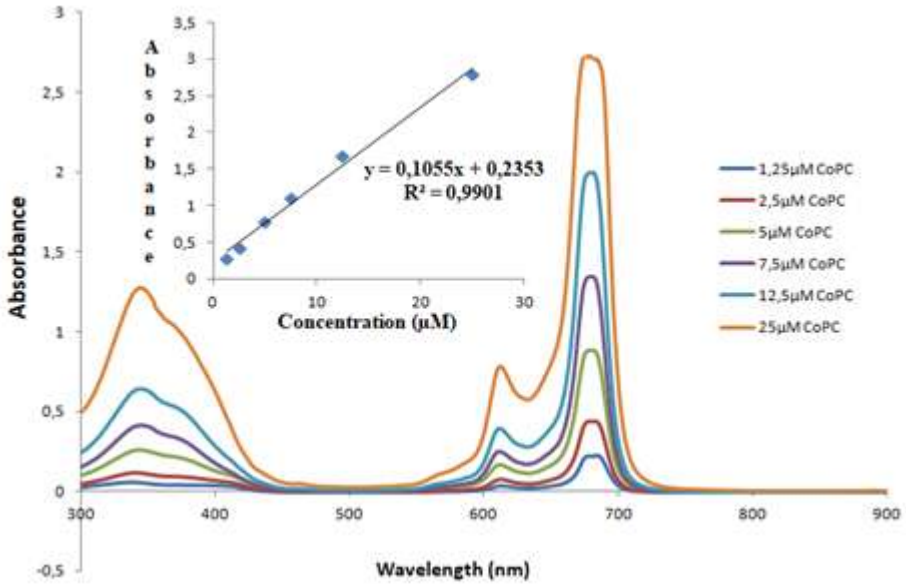


Figure 6: The aggregation behavior of phthalocyanine 6

3.3 DPPH radical scavening assay

DPPH radical scavenging activity is a mechanism of evaluating the decrease in DPPH radical absorption after exposure to radical scavengers. This activity can be used to quickly determine the antioxidant capacity [[27]]. The comparison of scavenging activity on DPPH radicals between the CH_3OH solutions of compounds can be seen in Fig 7. When the concentration of CH_3OH solution of compounds were increased, it was seen that activities were not significantly dependent concentration. The maximum DPPH scavenging activity was found to be 46,9 % with comp.4, and the minimum activity was found 40,45 % with comp.6 at 100mg/L. the result revealed that all of the CH_3OH. solutions of compounds showed low DPPH radical scavenging activity compared to ascorbic acid and BHT.

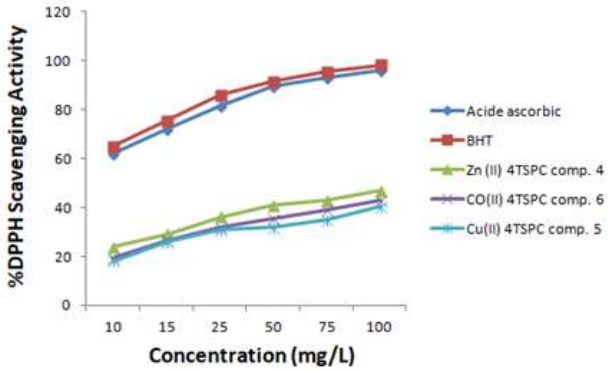


Figure 7: Radical-scavenging activity on DPPH radicals (%) of the compounds

3.4 Antimicrobial activity

In vitro preliminary screening of the antibacterial activity of 4-6 against B Subtilis CIP 5262, Micrococcus luteus CIP5345, Staphylococcus aureus Subsp CIP 4.83, Salmonella enterica ssp. CIP 8039, Escherichia coli CIP54127 and antifungal activity was determined against Candida albicans ATCC 10231 and Aspergillus Niger ATCC 16888 was studied by disk diffusion method. In this study, all compounds inhibited the growth of bacteria.

Bacteria are ranked into two main classes depending upon their response to the Gram stain which reflects differences in their morphology. Gram-negative and positive bacteria differ in the composition of their outer surface, and respond differently to antimicrobial agents. Gram-positive bacteria can easily take up molecules such as photosensitisers and can therefore be readily photoinactivated by most photosensitisers used for conventional PDT. This is not the case for Gram negative bacteria which are however relatively impermeable to neutral or anionic drugs due to their highly negatively charged surface.

Origins were used as indicator microorganisms for the tests of antibacterial activity. Antifungal activity was determined against Candida albicans ATCC 10231 and Aspergillus Niger ATCC 16888. Bacterial strains used as indicator microorganisms were cultivées Night in the circles LB settle under aerobic conditions and constant agitation (200 trs/min) in 30°C for Mr Luteus CIP5345, B Subtilis CIP 5262, and in 37°C for S. aureus CIP 4.83, S Typhymurium ATCC 14028 and E coli CIP54127, then 1: 100 diluted in environment LB and incubated during 5 hours under a constant agility (200 trs/min) in the appropriate temperature. C. Albicans was cultivated in the broth Tryptone-soya (TSB) in 30°C during 24 hours.

The phthalocyanine compounds were studied for in vitro antibacterial activity by the disk diffusion method. The antibacterial activity of the tested compounds was presented by the formation of an inhibitory zone after 24h for antibacterial and 72 h for the antifungal of incubation in **table.2-7**.

The study of the sensibility of 7 origins in the action of the Metallophthalocyanines showed clearly a variation of the diameters of inhibition which depends on the concentration of the product and on the tested origin.

The obtained results showed a significant difference between the concentrations used towards the antimicrobial origins. Indeed, the more the concentration increases, the more antimicrobial activity is important.

The spiramycin and the streptomicine revealed a higher antibacterial activity against Bacillus cereus, E.Coli, Staph aureus and Micrococcus Luteus than the tested product. However, The spiramycin showed a less important activity against Salmonella enterica than the phthalocyanine for concentrations750 and 1250.

The synthetic fungicide " fluconazole " showed an antifungal power more important than the Zn(II)4TSPC,Co(II)4TSPC and Cu(II)4TSPC against candida albicans and aspergillus Niger.

The data analysis showed that the phtalocyanine applied against Staph aureus to a concentration of 1250 gets loose significantly from other origins(stumps) .The tested product showed the antibacterial power most imortant against staph aureus .This stumps seems to be the most sensitive.

The results of the statistical analysis performed using the SPSS 19 software are shown in the table above. The study of the sensitivity of the 7 strains to the action of phthalocyanine clearly showed a variation of the diameters of inhibition which depends on the concentration of the product and the strain tested.

The results obtained showed a significant difference between the concentrations used with respect to the antimicrobial strains. Indeed, the higher the concentration, the greater the antimicrobial activity is important.

Spiramicin and streptomicin exhibited higher antibacterial activity against Bacillus cereus, E. coli, Staph aureus and Micrococcus luteus than the product tested. However, spiramicin exhibited less activity against Salmonella enterica than phthalocyanine for the 750 and 1250 concentrations.

The synthetic fungicide "fluconaazole" showed greater antifungal power than the phthalocyanine against candida albicans and aspergillus niger.

The analysis of the data showed that the phthalocyanine applied against Staph aureus at a concentration of 1250 stands out significantly from the other strains. The tested product has shown the most important antibacterial power against staph aureus. This strain seems to be the most sensitive.

This phenomenon was observed only under the conditions of irradiation, since under dark conditions did not show any activity which proves that the inhibitory activities come from the generation of singlet oxygen. But also related to another mechanism of antimicrobial action of sulfonamides that are coupled to metallophthalocyanines that possess antibacterial properties related to the inhibition of the enzyme dihydropteroate synthase (DHPS) **[[28]-[29]]**.

All the synthesized metallophthalocyanines complexes were screened in vitro for their biological activity Gram positive Staphylococcus aureus, Micrococcus luteus,Bacillus subtilis; Gram negative, Salmonella enterica ssp., Escherichia coli, two fungal strains were aspergillus niger and candida albicans with metallophthalocyanines (4-6) complexes was performed by the Agar well diffusion method **[**Error! Reference source not found.**-[31]].** The compounds were tested at different concentrations ranging from 150 to 1250 mg/L against both bacterial and fungal strains. DMSO was used as a vehicle. Streptomycin, Spiaramycin (40 µg in100µl) and Fluconazole (40 µg in100µl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37°C for antibacterial activity and 72 h at 25 °C for

antifungal activity. The results revealed that the tested compounds were considered to be modest since the values obtained were close to each other. The compounds 4 and 5 exhibited higher antimicrobial activity than compound 6 as compared with standard drugs at the same concentration. Microbial results are systematized in tables 1-3. The antibacterial and antifungal studies suggest that all synthesized metallophthalocyanine (4-6) were showed modest antibacterial and antifungal. In the case of bacteriological studies, the results were compared with the standard drug (Streptomycin and Spiramycin). Antifungal activities were compared with the standard drug (Flucanazole). All metallophthalocyanine (4-6) were showed modest against fungal species. Among the compounds 4 and 5 exhibited higher antimicrobial activity than compound 6 as compared with standard drugs.

4. Conclusion

The synthesis, characterization, aggregation, antioxidant proprieties, antibacterial and antifungal activities of new synthesized (4-tolylsulfonyl)phthalonitrile substitued metallophthalocyanine derivatives have been presented in this work the first time.
the antibacterial effect of a novel dyes have been tested through a series of bacteria. this is important in terms of biodegradation of the dyes upon use and application. These complexes has good solubility in DMF,DMSO, THF, toluene, MeOH, CHCl3.

The main goal of the work is to adress to possible use of novel phthalocyanines for biological evaluation and antioxidants applications. The maximum DPPH activity were obtained with compounds 4, 6 and 5, respectively 46,90; 43,05 and 40,45 at 100 mg/L.

This present study showed that lack of bacterial growth was observed and confirming successful bacterial growth inhibition. The maximum inhibition concentration showed with Zn(II) 4TSPC at 1250 mg/L aigainst B.subtilis, E.coli, M.luteus, S.aureus and S.entreica.

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Table 2: Antibacterial activity of compound 6 and standard antibiotics

	Antibacterial activities						
test	**Co(II) 4TSCPC**					Spiramycin	Streptomycin
Bacterial strains/concentration mg/L	150 (1)	250 (2)	400(3)	750(4)	1250(5)	400(6)	400(7)
Bacillus subtilis	6,80±0,11 g	12,20±0.2 f	17,30±0.2 e	20,90±0.23 d	25,40±0.3 b	24,67±0.3 c	32,83±0.16 a
E.coli	6,53±0,14 f	12,33±0.23 e	16,03±0.2 d	20,20±0.3 c	24,67±0.23 b	16,00±0.28 d	33,23±0,5 a
Staph. Aureus	7,03±0,14g	13,17±0.24 f	18,20±0.35e	21,60±0.3d	26,53±0.31b	24,07±0.32c	34,67±0.17a
Micrococcus Luteus	6,83±0 ,08 f	11,20±0.23e	16,33±0.38d	19,80±0.25c	23,80±0.35b	23,67±0.33b	28,83±0.14a
Salmonella enterica	7,2±015f	10,6±0.11e	14,53±0.31d	16,90±0.05c	18,50±0.26b	17,83±0.33b	25,63±0.38a

Inhibitory activity of standard drugs and Co(II) 4TSCPC against *Escherichia coli*, *Bacillus subtilis*, *Micrococcus Luteus*, *Staphylococcus aureus*, and *Salmonella enterica* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

The values (averages of three repetitions) followed by the same small letter horizontally or by the same capital letter vertically are not significantly different according to the test of the multiple comparison of Newman-Keuls at the threshold of 5 %. The results of the statistical analysis realized by means of the software SPSS 19, are represented in the table 2.

Table 3: Antifungal activity of compound 6 and standard drug

Antifungal activities						
test	**Co(II) 4TSCPC**					Flucanazole
fungal strains/concentration mg/L	150(1)	250(2)	400(3)	750(4)	1250 (5)	400(6)
Candida albicans	6,80±0.17 f	8,23±0.14 e	10,23±0.17 d	13,40±0.3 c	16,10±0.26 b	22,50±0.28 a
Aspergillus niger	6,50 f	8,07±066 e	9,50±0.28 d	12,67±0.17 c	15,80±0.17 b	20,17±0.44a

Inhibitory activity of Co(II) 4TSCPC and fluconazole drugs against Candida albicans and Aspergillus niger (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

Table 4: Antibacterial activity of compound 5 and standard antibiotics

	Antibacterial activities						
test	**Cu(II) 4TSCPC**					Spiramycin	Streptomycin
Bacterial strains/concentration mg/L	150(1)	250(2)	400(3)	750(4)	1250(5)	400(6)	400(7)
Bacillus subtilis	7,60±0.2 g	13,6±0.17 f	18,43±0.28 e	23,80±0.17 d	27,1±0.26 b	24,67±0.16 c	32,83±0.44 a
E.coli	6,70±0.2 f	12,4±0.26 e	16,27±0.26 d	21±0.5 c	26,33±0.24 b	16±0.28 d	33,23±0.5a
Staph. Aureus	7,33±0.2 g	12,33±0.16 f	17,33±0.23e	22,33±0.6d	28,03±0.14b	24,07±0.32c	34,67±0.17a
Microcus Luteus	6,50±0.32 g	10,17±0.31 f	18,03±0.08 e	21,5±0.28 d	25,4±0.32 b	23,67±0.33 c	28,83±0.14 a
Salmonella enterica	6,8±0.23f	11,2±0.23e	16,80±0.17d	20,5±0.32 b	26±0.3a	17,83±0.33c	25,63±0.38a

Inhibitory activity of standard drugs and Cu(II) 4TSCPC against *Escherichia coli*, *Bacillus subtilis*, *Micrococcus Luteus*, *Staphylococcus aureus*, and *Salmonella enterica* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE

Table 5: Antifungal activity of compound 5 and standard drug

Antifungal activities						
test	**Cu(II) 4TSCPC**					Flucanazole
fungal strains/concentration mg/L	150(1)	250(2)	400(3)	750(4)	1250(5)	400(6)
Candida albicans	7,00±0.35 f	9,00±0.15 e	12,33±0.24 d	16,00±0.28 c	19,33±0.26 b	22,50±0.28 a
Aspergillus niger	6,80±0,17f	8,63±0.18e	11,43±0.29d	15,23±0.29c	18,90±0.3b	20,17±0.44 a

Inhibitory activity of Cu(II) 4TSCPC and fluconazole drugs against Candida albicans and Aspergillus niger (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

Table 6: Antibacterial activity of compound 4 and standard antibiotics

	Antibacterial activities						
test	**Zn(II) 4TSCPC**					Spiramycin	Streptomycin
Bacterial strains/concentration mg/L	150	250	400	750	1250	400	400
Bacillus subtilis	8,47±0,5 g	14,20±0,7 f	21,93±0.6 e	26,67±0.28 c	30,33±0.76 b	24,67±0.28 d	32,83±0.76 a
E.coli	7,33±0.15 g	13,33±0.37 f	17,33±0.47 d	24,33±0.7 c	29,5±0.86 b	16±0.5 e	33,23±0.87 a
Staph. Aureus	8,27±0.25 g	14,33±0.28 f	21±0.5 e	29±0.5 c	32,5±0.5 b	24,07±0.56 d	34,67±0.3 a
Microcus Luteus	7,67±0.3 f	12,33±0.57 e	18,5±0.55 d	22,83±0.28 c	28,67±0.28 a	23,67±0.57 b	28,83±0.25 a
Salmonella enterica	7,8±0.4 f	13,0±0.5 e	18,33±0.76 d	24,67±0.28 c	29,83±0.28 a	17,83±0.57d	25,63±0.66 b

Inhibitory activity of Zn(II)4TSCPC against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Micrococcus luteus, and Salmonella enterica ssp.,(means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

Table 7: Antifungal activity of compound 4 and standard drug

Antifungal activities						
test	**Zn(II) 4TSCPC**					Flucanazole
fungal strains/concentration mg/L	150	250	400	750	1250	400
Candida albicans	7,17±0.49e	10±0.5d	14,17±0.28c	19,33±0.28b	22,17±0.76a	22,5±06a
Aspergillus niger	7,67±0.28 f	9,3±0.36 e	12,67±0.28d	17,83±0.28c	22,67±0.28a	20,17±0.76 b

Inhibitory activity of Zn(II) 4TSCPC and fluconazole drugs against Candida albicans and Aspergillus niger (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

Figure 8: Example of the antibacterial activity of metallophthalocyanine defined by disk diffusion method against E.coli and B.subtilis

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