sciendo

*Original research*

DOI:10.2478/rrlm-2020-0014

# Antimicrobial activity of different substituted meso-porphyrin derivatives

Luminița Sălăgeanu[1,2], Delia Muntean[3*], Horhat Florin George[3], Anca Lascu[2], Diana Anghel[2], Iulia Cristina Bagiu[3], Eugenia Fagadar-Cosma[2]

*1. Health Insurance House Timis, Romania*
*2. Institute of Chemistry "Coriolan Dragulescu", Timisoara, Romania*
*3. "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania*

## Abstract

*The increasing resistance against classical antibiotic treatment forces the researchers to develop novel non-toxic antimicrobial agents. The aim of this study was to determine the antimicrobial properties of seven different porphyrins having distinctive hydrophobicity/hydrophilicity: P1 meso-tetra(4-methoxy-phenyl)porphyrin, P2 Zn(II)-meso-5,10,15,20-tetrapyridylporphyrin, P3 meso-tetra(p-tolyl)porphyrin, P4 5,10,15,20-tetraphenylporphyrin; P5 (5,10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride, P6 5,10,15,20-tetrakis-(N-methyl-4-pyridyl) porphyrin-Zn(II) tetrachloride, P7 Zn(II)-5,10,15,20-meso-tetrakis-(4-aminophenyl)porphyrin. The meso-porphyrin derivatives were screened for their antimicrobial activity against six reference strains: Streptococcus pyogenes ATCC 19615, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC27853 and Candida albicans ATCC 10231. The antimicrobial activity of these samples was evaluated by the agar disk diffusion method and dilution method, with the determination of the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC). The most significant result is provided by the water-soluble P5 manifesting an obvious antimicrobial activity against Streptococcus pyogenes. On the other hand, P6 is a moderately active derivative against Streptococcus pyogenes and Escherichia coli and P7 presents moderate activity against Streptococcus pyogenes and Staphylococcus aureus. All the tested porphyrin bases, presenting hydrophobic character, have no antimicrobial activity under the investigated conditions. The common characteristics of the porphyrins that act as promising antimicrobial agents in the non-irradiated methods are: the cationic nature, the increased hydrophilicity and the presence of both amino functional groups grafted on the porphyrin ring and the coordination with Zn or phosphorus in the inner core.*

***Keywords:*** *meso-aryl porphyrins, metalloporphyrins, Gram(+) and Gram(-) bacteria, antimicrobial activity*

*Received: 9th October 2019; Accepted: 29th January 2020; Published: 13th February 2020*

## Introduction

The increasing resistance against classical antibiotic treatment forces the researchers to develop novel non-toxic antimicrobial agents capable to act efficiently and against which pathogens will not easily develop resistance (1).

Based on the well-known property of different types of porphyrin derivatives to act as photosensitizers in non-invasive photodynamic therapy

***Corresponding author**: Delia Muntean, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania. E-mail: muntean.delia@umft.ro

(PDT) of cancer (2), approaches to apply them for the developing of novel non-toxic materials able to destroy a large diversity of bacteria and fungi, is a demanded target.

Most of the natural or synthetic porphyrins are reported as inefficient on Gram(-) bacteria. Nevertheless, novel structures of water-soluble cationic porphyrins substituted in the *meso* position with hydroxyethyl, butyl or allyl functional groups as well as their metalloporphyrin derivatives with Co(II), Fe(III), Cu(II), Zn(II), Ag(I) proved to have a high capacity to inhibit both Gram(+) (*Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* MRSA) and Gram(-) (*Escherichia coli and Salmonella* spp.) bacteria (3, 4).

A higher antimicrobial capacity for *Staphylococcus aureus* was proven by Ag(I)-metalloporphyrins and a larger efficiency to both Gram(+) and Gram(-) bacteria was realized by Zn(II)-metalloporphyrins (5). Some $A_2B_2$ trans-pyridyl porphyrins, 5,15-di(pentafluorophenyl)-10,20-bis(2'/3'/4'-pyridyl)porphyrin and trans-dicationic pyridinium porphyrins, 5,15-di(pentafluorophenyl)-10,20-bis(2'/3'/4'-N-methylpyridyl) porphyrins together with their Co(II) and Zn(II) complexes manifest significant antimicrobial activities on *Staphylococcus aureus* and *Escherichia coli.* The dicationic compounds also show more increased activity than the neutral ones (6).

Tricationic porphyrins such as 5,10,15-tris-[4-(3-N,N,N-trimethylammonium-propoxy)-phenyl]-20-(4-trifluoromethylphenyl) porphyrin iodide and 5,10,15,20-tetra-(4-N,N,N-trimethylammonium-phenyl)porphyrin *p*-tosylate were also tested against *Escherichia coli* colonies under irradiation with visible light (7). It was concluded that the photodynamic inactivation capacity of the investigated tricationic porphyrins is high. Excepting the porphyrins substituted with cationic groups or administrated simultaneously with noble metal nanoparticles, Gram(-) bacteria are insensitive to all other types of porphyrin derivatives.

Zn(II), Co(II) and Mn(II) complexes of 5-(2-nitro-4,5-methylenedioxy)-phenyl-10,15,20-triphenyl-porphyrin and 5-(2-nitro-4,5-methylenedioxy)-phenyl-10, 15, 20-tri-(*p*-chlorophenyl)porphyrin were proven to be active photosensitizers against *Escherichia coli* and *Staphylococcus aureus* (8).

In order to provide biological activity, the presence of an amine function is often required in the porphyrin structure. Porphyrins bearing amino functions and glucosyl or methyl units on the *meso*-phenyl group were tested against antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus* (9). When porphyrin derivatives are substituted with at least two units of polyamines (for instance spermine or spermidine), they have increased capacity to inhibit Gram(-) bacteria without the need for a membrane destabilizing agent. In the case of Gram(+) bacteria, such as *Staphylococcus aureus*, the inactivation of bacteria is improved if glycoside groups are grafted on the amino functions already present on the *meso*-aryl-porphyrins.

Synergistic combinations of antimicrobial agents, might be the best approach in drug design for many approved pharmaceuticals to combat resistant strains (10).

Starting from the real need for the discovery of new compounds endowed with antimicrobial activity and based on our previous experience in using both porphyrins and metalloporphyrins to interact with biologically active compounds (11-15) this work aims to select some appropriate structures of porphyrins highly efficient in destroying bacteria. To achieve this purpose seven different substituted porphyrins and metalloporphyrins, having distinctive hydrophobicity/hydrophilicity, namely: *meso*-tetra(4-methoxy-phenyl) porphyrin, Zn(II)-*meso*-5,10,15,20-tetrapyridylporphyrin, *meso*-tetra(*p*-tolyl)porphyrin, 5,10,15,20-tetra

phenylporphyrin, 5,10,15,20-(tetraphenylporphinato) dichlorophosphorus (V) chloride, 5,10, 15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride and Zn(II)-5,10,15,20-*meso*-tetrakis-(4-aminophenyl)porphyrin were tested against the Gram(+) and Gram(-) bacterial strains and on *Candida albicans*.

## Material and methods

### *Reagents*

All the reagents were aquired from Sigma-Aldrich and Merck as *purum analiticum* grade. The porphyrins were previously synthesized and fully characterized as follows (Table 1): **P1** *meso*-tetra(4-methoxy-phenyl)porphyrin (16), **P2** Zn(II)-*meso*-5,10,15,20-tetrapyridylporphyrin (17), **P3** *meso*-tetra(p-tolyl)porphyrin (18), **P4** 5,10,15,20-tetraphenylporphyrin (19); **P5** (5, 10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride (20), **P6** 5,10,15,20-tetrakis-(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride (21), **P7** Zn(II)-5,10,15,20-*meso*-tetrakis-(4-aminophenyl)porphyrin (22, 23).

### *Equipment*

All UV-visible spectra were recorded on a JASCO UV- V-650 spectrometer (Japan) using standard 1 cm pass cells.

### *In vitro antimicrobial activity*

The *meso*-porphyrin derivatives were screened for their antimicrobial activity against six reference strains (ThermoScientific, USA): *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231.

The antimicrobial activity of these samples was evaluated by agar disk diffusion method and dilution method, according to the Clinical Laboratory and Standard Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the other studies (24-30).

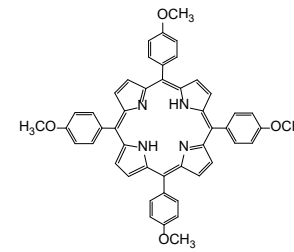
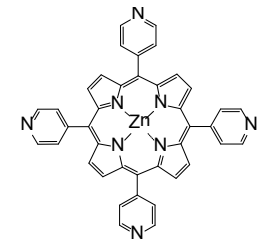
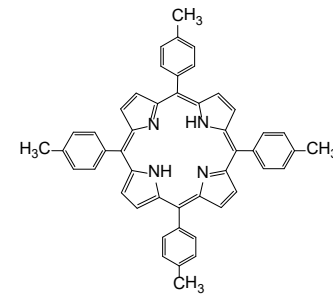
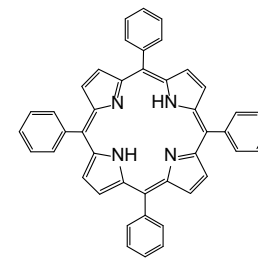
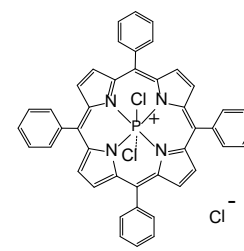
### *Disk diffusion method*

For each microorganism under study, suspensions were adjusted with physiological saline to a concentration of 0.5 Mac Farland-approx. $10^8$ CFU/mL for bacterial strains and 2 x $10^6$ CFU/mL for *Candida albicans* (CFU-colony forming units). The agar Mueller-Hinton (Sanimed, Bucharest, Romania) was inoculated with 100µL of this suspension. Ten microliters from each sample (200µg dry porphyrin/200µL DMSO) were added to a 6 mm diameter sterile blank filter disk (BioMaxima, Lublin, Poland), placed on top of the agar. The medium inoculated with the microbial suspensions was incubated at 35-37°C for 24-48h. The reading of the inhibition zones was made in millimeters, with a ruler. All tests were done in triplicate, for each microbial strain. The positive control consists of a 10 µg or 120 µg gentamycin and 25 µg fluconazole disk (BioMaxima, Lublin, Poland). As a negative control, a blank paper disk impregnated with DMSO (dimethyl sulfoxide) was used.

### *Dilution method*

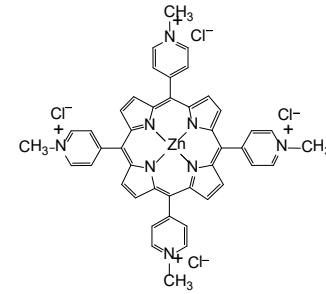
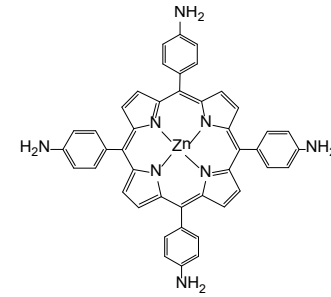
This method allows to establish the minimum inhibitory concentration (MIC). The broth dilution assay was done as recommended by the CLSI (29). The working suspension was prepared from a dilution of the standardized suspension (0.5 Mac Farland) to yield $5 \times 10^5$ CFU/mL. From the stock solutions in DMSO of the tested compounds, serial dilutions of the *meso*-porphyrin derivatives were prepared, having the following concentrations: 1, 0.5, 0.25, 0.12, 0.06, 0.03 mg /mL. In six test tubes were added 0.1 mL of each diluted solution of the investigated porphyrin, 0.4 mL Mueller Hinton broth and 0.5 mL suspension of the microorganism under study (final inoculum = $0.5 \times 10^5$ CFU/mL). After incubating the test tubes at 37°C for

**Table 1. Annotations and chemical structures of the tested porphyrins**

| Porphyrin's name | Abbreviation | Porphyrin structure |
|---|---|---|
| Meso-tetra(4-methoxy-phenyl)porphyrin | **P1** |  |
| Zn(II)-meso-5,10,15,20-tetrapyridylporphyrin | **P2** |  |
| Meso-tetra(p-tolyl)porphyrin | **P3** |  |
| 5,10,15,20-tetraphenylporphyrin | **P4** |  |
| (5,10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride | **P5** |  |

(continued on page 5)

(Table 1. Continued from page 4)

| | | |
|---|---|---|
| 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride | **P6** |  |
| Zn(II)-5,10,15,20-meso-tetrakis-(4-aminophenyl) porphyrin | **P7** |  |

24h, the MIC (the lowest concentration without visible growth) was determined. As control 0.1mL of DMSO was introduced in a tube with 0.5 mL of microbial suspension and 0.4 mL of Mueller Hinton broth.

Interpretation: the first to be read are the controls that have to be turbid (if the germs did grow). In case there is no bacterial growth, the broth in the control tube is clear and the test is invalid. If the control is appropriate, the whole procedure was performed correctly. The medium in the tubes containing the investigated porphyrins is less turbid than the control, and the smallest concentration of a substance that inhibits bacterial growth (MIC) can be identified.

To determine the minimum bactericidal concentration (MBC), a volume of 1µL from the test tubes with no visible growth was inoculated with a loop (NuovaAptaca SRL, Italy), on Columbia agar supplemented with 5% blood in order to establish the lowest concentration which killed 99.9% of the bacteria. To determine the minimum fungicidal concentration (MFC), the inoculation was performed on the Sabouraud with chloramphenicol. The plates inoculated were incubated at 35-37°C for 24-48h.

## Results

A comparison of the UV-vis behaviour of different tailored structures of porphyrins by modifying the substitution at *meso*-aryl groups to obtain some degree of hydrophilicity and to produce a bathochromic shift-moving on both the Soret and the Q Bands was a start to select the best formulation for new antibacterial compounds.

The UV-vis spectra of **P1**, **P3** and **P4** porphyrin-bases show non-aggregated *etio* type shape, characterized by sharp and intense Soret band, accompanied in each case by four Q-bands in the visible region, as shown in Figure 1. The Soret band, around 420 nm is generated by the transition from $a_{1u}(\pi)$ - $e_g^*(\pi)$ and the other four Q bands with maxima around 515, 550, 590 and 650 nm are assigned to the corresponding $a_{2u}(\pi)$ - $e_g^*(\pi)$ transitions.

In comparison to tetraphenylporphyrin **P4**, both substituted derivatives with donor electron inductive groups, namely: *meso*-tetra(*p*-tolyl)porphyrin **P3** and *meso*-tetra(4-methoxy-phenyl) porphyrin **P1** have their Soret and Q bands bathochromically shifted with 2 nm and 5 nm respectively.

As expected, the UV-vis spectra of Zn-metalloporphyrins, due to their increased $D_4h$ symmetry, are simplified to the Soret band located at around 435 nm accompanied by only two Q bands. Regarding the intensities, Zn(II)-*meso*-5,10,15,20-tetrapyridylporphyrin **P2** has the highest intensity and its large shape implies the beginning of an aggregation process in solution Figure 2.

On the other hand, the Soret band of the Zn(II)-5,10,15,20-*meso*-tetrakis-(4-aminophenyl)porphyrin **P7** has a significant shoulder at 395 nm, that is due to the presence of J-type aggregates in the organic solvent.

Two of the investigated porphyrins that are water-soluble were also compared and the 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride **P6**, that is a cationic porphyrin has all the absorption band red-shifted with 14 nm in comparison with the phosphorus containing porphyrin derivative **P5** (Figure 3).

The UV–vis spectra for all the tested porphyrins were performed in DMSO at concentrations of $10^{-5}M$, excepting **P5** and **P6** that were measured using the same concentration from double distilled water.

### *Antimicrobial activity*

The results of the investigated porphyrins are summarized in Table 2.

## Discussions

Taking into consideration that many studies have shown that porphyrins are able to inhibit the

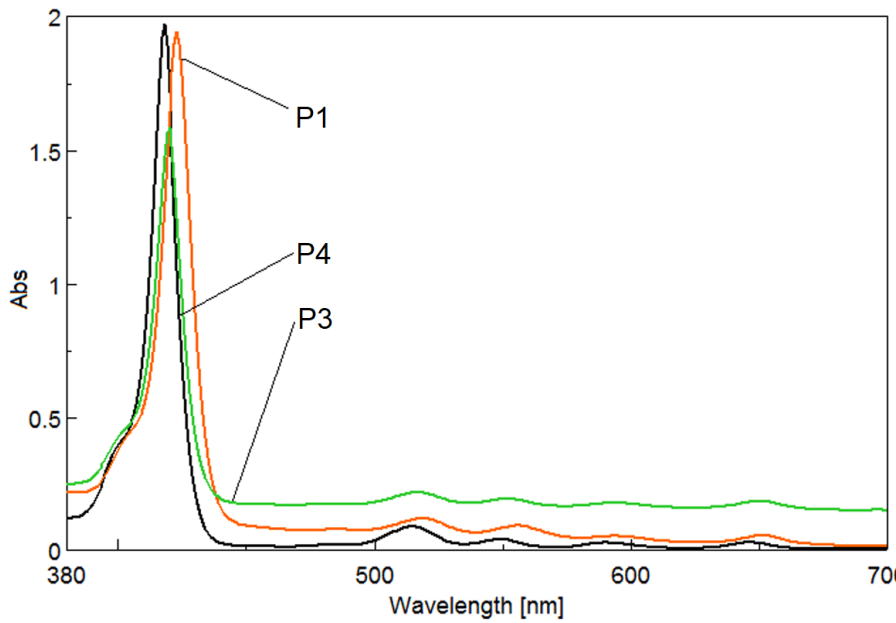



**Fig. 1. Overlapped UV-vis spectra of porphyrin bases (c=10-5M) from DMSO: P1: meso-tetra(4-methoxyphenyl)porphyrin; P3:meso-tetra(p-tolyl)porphyrin; P4: 5,10,15,20-tetraphenylporphyrin**

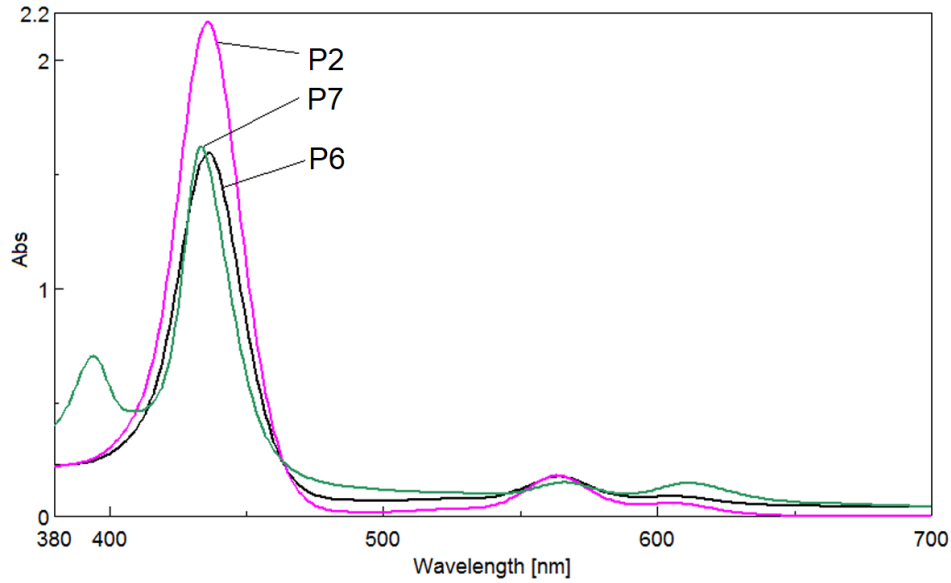



**Fig. 2. Overlapped UV-vis spectra of Zn-metalloporphyrins (c=10-5M) in DMSO: P2: Zn(II)-meso-5,10,15,20-tetrapyridylporphyrin; P6: 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride; P7: Zn(II)-5,10,15,20-meso-tetrakis-(4-aminophenyl)porphyrin**

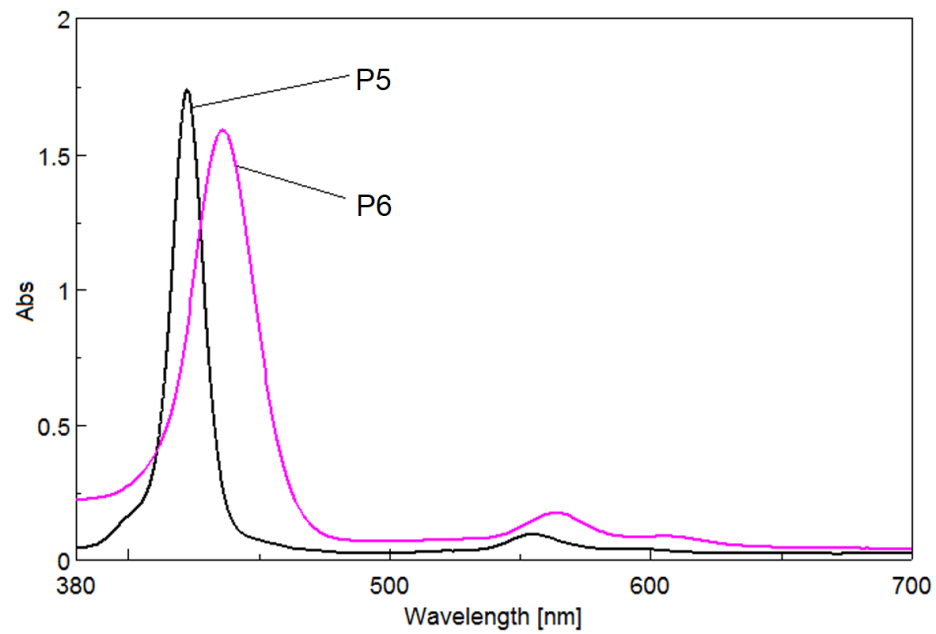



**Fig. 3. Overlapped UV-vis spectra of water-soluble porphyrins (c=10-5M) in water P5: (5,10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride and P6: 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride**

**Table 2. Antimicrobial activity**

| Porphyrin | *Candida albicans* ATCC 10231 | *Streptococcus pyogenes* ATCC 19615 | *Staphylococcus aureus* ATCC 25923 | *Escherichia coli* ATCC 25922 | *Klebsiella pneumoniae* ATCC 700603 | *Pseudomonas aeruginosa* ATCC 27853 |
|---|---|---|---|---|---|---|
| Meso-tetra(4-methoxy-phenyl)porphyrin **P1** | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm |
| Zn(II)-meso-5,10,15,20-tetrapyridylporphyrin **P2** | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm |
| Meso-tetra(p-tolyl)porphyrin **P3** | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm |
| 5,10,15,20-tetraphenylporphyrin **P4** | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm |
| (5,10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride **P5**$^{-}$ | 7 mm | 19mm MIC 0.25 mg/mL MBC 0.5 mg/mL | 14mm MIC 0.5 mg/mL MBC 0.5 mg/mL | 7 mm | 7 mm | 7 mm |
| 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride **P6** | 12 mm | 15 mm MIC 0.5 mg/mL MBC 0.5 mg/mL | 12 mm MIC 0.5 mg/mL MBC 0.5 mg/mL | 15 mm MIC 0.5 mg/mL MBC 0.5 mg/mL | 12 mm | 12 mm |
| Zn(II)-5,10,15,20-meso-tetrakis-(4-aminophenyl) porphyrin **P7** | 11 mm | 18 mm MIC 0.25 mg/mL MBC 0.5 mg/mL | 15 mm MIC 0.5 mg/mL MBC 0.5 mg/mL | 12 mm | 12 mm | 13 mm |
| Gentamycin10 µg or 120*µg | - | 20 mm* | 20 mm | 20 mm | 19 mm | 16 mm |
| Fluconazole10 µg | 29 mm | - | - | - | - | - |
| DMSO | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm | 7 mm |

growth of Gram(-) and Gram (+) bacteria under irradiation with visible light (3, 4), the challenge was to identify compounds capable to have antimicrobial activity without the need for light illumination.

As it can be easily seen (Table 2), a single por-

phyrin: (5,10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride, **P5**, a cationic derivative that is water-soluble has an obvious antimicrobial activity against *Streptococcus pyogenes.* Other worth-mentioning results are connected with an amino-substituted porphyrin, Zn(II)-5,10,15,20-*meso*-tetrakis-(4-aminophenyl)porphyrin, **P7**, that presents moderate activity against *Streptococcus pyogenes* and *Staphylococcus aureus*. The third moderately active antibacterial derivate on *Streptococcus pyogenes* and *Escherichia coli* is 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride, **P6**, again a water-soluble metallo-compound.

The common characteristics of the porphyrins that act as promising antimicrobial agents in the non-irradiated methods are: the cationic nature, the increased hydrophilicity and the presence of both amino functional groups grafted on the porphyrin ring and the coordination with Zn or phosphorus in the inner core.

Porphyrins **P1**: *meso*-tetra(4-methoxy-phenyl) porphyrin; **P3**: *meso*-tetra(*p*-tolyl)porphyrin and **P4**: 5,10,15,20-tetraphenylporphyrin, being porphyrin bases that possess electron donating groups in the *para*-phenyl positions and having a hydrophobic character (31), are inactive, as expected, against all tested Gram(-) reference strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*), Gram(+) bacterial strains (*Staphylococcus aureus, Streptococcus pyogenes)* and fungi (*Candida albicans).*

Using the diffusion and dilution method on different Gram(+): *Staphylococcus aureus*, *Streptococcus pyogenes*, and Gram(-): *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* bacterial strains and one fungal strain *Candida albicans,* a variety of porphyrin structures differentiated by their hydrophilic/hydrophobic balance, the nature of functional groups and inner core coordination were investigated.

The best results reveal that *meso*-(5,10,15,20-tetraphenylporphinato) dichlorophosphorus(V) chloride, a cationic porphyrin derivative that is water-soluble, has an obvious antimicrobial activity against a ubiquitous pathogen, *Streptococcus pyogenes*. Moderate activity against two bacterial strains *Streptococcus pyogenes* and *Staphylococcus aureus* were recorded for an amino-substituted porphyrin, Zn(II)-5,10,15,20-meso-tetrakis-(4-aminophenyl)porphyrin. A water-soluble metallo-compound, 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin-Zn(II) tetrachloride is a moderately active derivative against *Streptococcus pyogenes* and *Escherichia coli*. Similarly to other previously reported studies (32) all the tested porphyrin bases, presenting hydrophobic character, have no antimicrobial activity under the investigated conditions.

Further studies on a numerous clinical bacterial or fungal isolates are necessary to investigate and standardize the antimicrobial activity of different substituted *meso*-porphyrin derivatives. On the other hand, studies would be needed to estimate the potential toxicity of these compounds.

## Conclusions

The common characteristics of the porphyrins that act as promising antimicrobial agents in the non-irradiated methods are: the cationic nature, the increased hydrophilicity and the presence of both amino functional groups grafted on the porphyrin ring and the coordination with Zn or phosphorus in the inner core.

The perspectives in the field are unlimited. Using silica matrices that are embedded with porphyrins might be a promising substrate for improving antibacterial methods.

Synthesis of various types of water-soluble porphyrins containing S-S bond might extend the antibacterial potential over both Gram(+) and Gram(-) strains.

## Abbreviations

CFU – colony forming units
CLSI – Clinical Laboratory and Standard Institute
DMSO – dimethyl sulfoxide
EUCAST – European Committee on Antimicrobial Susceptibility Testing
MBC – minimum bactericidal concentration
MFC – minimum fungicidal concentration
MIC – minimum inhibitory concentration
MRSA – Methicillin-resistant Staphylococcus aureus
**P1** – meso-tetra(4-methoxy-phenyl) porphyrin
**P2** – Zn(II)-meso-5,10,15,20-tetrapyridylporphyrin
**P3** – meso-tetra(p-tolyl)porphyrin
**P4** – 5,10,15,20-tetraphenylporphyrin
**P5** – (5,10,15,20-tetraphenylporphinato) dichlorophosphorus (V) chloride
**P6** – 5,10,15,20-tetrakis(N-methyl-4-pyridyl) porphyrin-Zn(II) tetrachloride
**P7** – Zn(II)-5,10,15,20-meso-tetrakis-(4-aminophenyl)porphyrin
PDT – Photodynamic therapy
UV-vis – Ultraviolet visible spectroscopy

## Acknowledgements

All authors are kindly acknowledging the support received from "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania, Department of Microbiology and Virology. This work was supported by the Romanian Academy through Programme 3-Porphyrins from Institute of Chemistry "Coriolan Dragulescu".

## Author's contribution

LS – resources, writing original draft; DM – investigation, methodology, writing & editing; HFG – investigation, methodology; AL – formal analysis, validation; DA – data curation, visualization; ICB– investigation, methodology; EFC – conceptualization, methodology, supervision, writing – review & editing.

## Conflict of Interest Statement

The author reports no conflicts of interest in this work.

## References

1. Taylor P, Stapleton P, Paul Luzio J. New ways to treat bacterial infections. Drug Discov Today. 2002 Nov 1;7(21):1086-91. DOI: 10.1016/S1359-6446(02)02498-4
2. Tomé J, Neves M, Tomé A, Cavaleiro J, Soncin M, Magaraggia M et al. Synthesis and Antibacterial Activity of New Poly-S-lysine−Porphyrin Conjugates. JJ Med Chem. 2004 Dec 16;47(26):6649-52. DOI: 10.1021/jm040802v
3. Amos-Tautua B, Songca S, Oluwafemi O. Application of Porphyrins in Antibacterial Photodynamic Therapy. Molecules. 2019; 24(13):2456. DOI: 10.3390/molecules24132456
4. YuanY, Liu ZQ, Jin H, Sun S, Liu TJ, Wang X, et al. Photodynamic antimicrobial chemotherapy with the novel amino acid-porphyrin conjugate 4I: In vitro and in vivo studies. PLOS ONE, 2017,12(5): e0176529. DOI: 10.1371/journal.pone.0176529
5. Gyulkhandanyan GV, Ghazaryan RK, Paronyan MH, Ulikhanyan GI, Gyulkhandanyan AG, Sahakyan L A. Mechanisms of low-Light Therapy VII edited by Michael R. Hamblin, Juanita Anders, James D. Carroll, Proc. of SPIE. 2012;8211:821107.
6. Ramesh J, Sujatha S, Arunkumar C. Synthesis, structure, electrochemical, DNA interaction and antimicrobial studies of fluorinated trans-dicationic pyridinium porphyrins. RSC Advances. 2016;6(68):63271-85. DOI: 10.1039/C6RA09148B
7. Caminos D, Durantini E. Photodynamic inactivation of Escherichia coli immobilized on agar surfaces by a tricationic porphyrin. Bioorg Med Chem. 2006 Jun 15;14(12):4253-9. DOI: 10.1016/j.bmc.2006.01.058
8. Zhao S, Chen N, Zhang H, Zhang J, Li Z. Microwave Synthesis and Antibacterial Activity of Novel Asymmetric Porphyrin Metal Complexes from Substituted Piperonal. Chinese Journal of Applied Chemistry 2015; 32(5):542-546.

9. Sol V, Branland P, Chaleix V, Granet R, Guilloton M, Lamarche F, et al. Amino porphyrins as photo inhibitors of Gram-positive and -negative bacteria. Bioorganic & Medicinal Chemistry Letters. 2004;14(16):4207-4211. DOI: 10.1016/j.bmcl.2004.06.016
10. Krychowiak M, Kawiak A, Narajczyk M, Borowik A, Królicka A. Silver Nanoparticles Combined With Naphthoquinones as an Effective Synergistic Strategy Against Staphylococcus aureus. Front Pharmacol. 2018 Jul 26;9:816 DOI: 10.3389/fphar.2018.00816
11. Lascu A, Palade A, Birdeanu M. Procaine detection using hybrids of cobalt-metalloporphyrin with gold and silver nanoparticles. Journal of the Chemical Society of Pakistan. 2019;41(1):43-51.
12. Palade A. Comparative Diclofenac Detection For Chronic Toxicity Levels Using Water Soluble Zn-Metalloporphyrin, Gold Nanoparticles And Their Hybrid. Farmacia. 2018; 66(3):468-76. DOI: 10.31925/farmacia.2018.3.11
13. Fagadar-Cosma E, Lascu A, Shova S, Zaltariov M, Birdeanu M, Croitor L, et al. X-ray Structure Elucidation of a Pt-Metalloporphyrin and Its Application for Obtaining Sensitive AuNPs-Plasmonic Hybrids Capable of Detecting Triiodide Anions. Int J Mol Sci. 2019 Feb; 20(3): 710. DOI: 10.3390/ijms20030710
14. Alexandrova R, Kalfin R, Tudose R, Fagadar-Cosma E. Comparative cytotoxicity assays performed using a free porphyrin and its Zn(II), Co(II) and Cu() Complexes. Influence of optical and aggregation properties. Studia Universitatis Babeş-Bolyai Chemia. 2018;63(4):65-77. DOI: 10.24193/subbchem.2018.4.05
15. Lascu A, Birdeanu M, Taranu B, Fagadar-Cosma E. Hybrid Mn-Porphyrin-Nanogold Nanomaterial Applied for the Spectrophotometric Detection of β-Carotene. Journal of Chemistry. 2018; 2018:1-11. DOI: 10.1155/2018/5323561
16. Fagadar-Cosma E, Enache C, Tudose R, Armeanu I, Mosoarca E, Vlascici D, et al. UV-Vis and fluorescence spectra of meso-tetraphenylporphyrin and meso-tetrakis-(4-methoxyphenyl) porphyrin in THF and THF-water systems. The influence of pH. Revista de Chimie. 2007;58(5):451-5.
17. Fagadar-Cosma E, Enache C. Armeanu I, Fagadar-Cosma G. Comparative Investigations of the Absorption and Fluorescence Spectra of Tetrapyridylporphyrine and Zn (II) Tetrapyridylporphyrine. Dig J Nanomat Biostruct. 2007; 2:175-83.
18. Fagadar-Cosma E, Enache C, Armeanu I. Dascalu D, Fagadar-Cosma G, Vasile M, et al. The influence of pH over topography and spectroscopic properties of silica hybrid materials embedding meso-tetratolylporphyrin. Mater Res Bull. 2009; 44:426-431. DOI: 10.1016/j.materresbull.2008.04.023
19. Vlascici D, Fagadar-Cosma E, Bizerea-Spiridon O. A New Composition for Co(II)-porphyrin-based Membranes Used in Thiocyanate-selective Electrodes. Sensors. 2006; 6(8):892-900. DOI: 10.3390/s6080892
20. Fagadar-Cosma E, Badea V, Fagadar-Cosma G, Palade A, Lascu A, Fringu I, et al. Trace Oxygen Sensitive Material Based on Two Porphyrin Derivatives in a Heterodimeric Complex. Molecules. 2017; 22(10):1787. DOI: 10.3390/molecules22101787
21. Dudás Z, Fagadar-Cosma E, Len A, Románszki L, Almásy L, Vlad-Oros B, et al. Improved Optical and Morphological Properties of Vinyl-Substituted Hybrid Silica Materials Incorporating a Zn-Metalloporphyrin. Materials. 2018;11(4):565. DOI: 10.3390/ma11040565
22. Bettelheim A, White B, Raybuck S, Murray R. Electrochemical polymerization of amino-, pyrrole-, and hydroxy-substituted tetraphenylporphyrins. Inorganic Chemistry. 1987; 26(7):1009-17. DOI: 10.1021/ic00254a011
23. Araghi M, Mirkhani V, Moghadam M, Tangestaninejad S, Mohammdpoor-Baltork I. Synthesis and characterization of a new porphyrin-polyoxometalate hybrid material and investigation of its catalytic activity. Dalton Transactions. 2012;41(10):3087. DOI: 10.1039/c2dt11865c
24. Ledeţi I, Bercean V, Alexa A, Şoica C, Şuta L, Dehelean C et al. Preparation and Antibacterial Properties of Substituted 1,2,4-Triazoles. Journal of Chemistry. 2015; 2015:1-5. DOI: 10.1155/2015/879343
25. Boda FA, Mare A, Szabó ZI, Berta L, Curticapean A, Dogaru M, et al. Antibacterial activity of selected snake venoms on pathogenic bacterial strains. Rev Romana Med Lab. 2019; 27(3):305-17. DOI: 10.2478/rrlm-2019-0015
26. Muntean D, Licker M, Alexa E, Popescu I, Jianu C, Buda V, et al. Evaluation of essential oil obtained from Mentha×piperita L. against multidrug-resistant strains. Infect Drug Resist. 2019 Sep 13;12:2905-14. DOI: 10.2147/IDR.S218141
27. Corina D, Bojin F, Ambrus R, Muntean D, Soica C, Paunescu V, et al. Physico-chemical and biological

evaluation of flavonols: fisetin, quercetin and kaempferol alone and incorporated in beta cyclodextrins. Anticancer Agents Med Chem. 2017;17(4):615-26. DOI: 10.2174/1871520616666160621105306

28. Danciu C, Muntean D, Alexa E, Farcas C, Oprean C, Zupko I, et al. Phytochemical Characterization and Evaluation of the Antimicrobial, Antiproliferative and Pro-Apoptotic Potential of Ephedra alata Decne. Hydroalcoholic Extract against the MCF-7 Breast Cancer Cell Line. Molecules. 2018; 24(1):13. DOI: 10.3390/molecules24010013
29. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing 2013; Twenty third informational supplement M 100-S23.Wayne PA: CLSI.
30. EUCAST-AFST. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope W. EUCAST technical note on the EUCAST definitive document EDef 7.2: Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). Clin Microbiol Infect. 2012;18:E246-E247. DOI: 10.1111/j.1469-0691.2012.03880.x
31. Tahlan S, Kumar S, Ramasamy K, Lim S, Shah S, Mani V et al. In-silico molecular design of heterocyclic benzimidazole scaffolds as prospective anticancer agents. BMC Chemistry. 2019;13(1). DOI: 10.1186/s13065-019-0608-5
32. Tahlan S, Kumar S, Ramasamy K, Lim SM, Ali Shah SA, Mani V, et al. Design, synthesis and biological profile of heterocyclic benzimidazole analogues as prospective antimicrobial and antiproliferative agents. BMC Chemistry 2019;13(50). DOI: 10.1186/s13065-019-0567-x