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Antibacterial activity of porphyrin derivatives against multidrug-resistant bacteria

Huseyin Tasli[1], Ayse Akbiyik[2], Vildan Alptuzun[3] and Sulunay Parlar[3]
[1]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Izmir, Turkey
[2]Izmir Katip Celebi University, Faculty of Health Sciences, Fundamental of Nursing, Izmir, Turkey
[3]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Izmir, Turkey

Abstract: In this study, it was aimed to investigate the antibacterial activities of the cationic porphyrin derivatives against some multi drug resistant clinical bacterial isolates and standard strains for the development of potential antibacterial agents. In addition to the standard strains, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* clinical isolates were studied. We synthesized eight (P1-P8) cationic porphyrin derivatives. The minimum inhibitory concentrations (MIC) of these substances were determined by micro dilution method. Ciprofloxacin was used for quality control. The study was repeated three times. All porphyrin derivatives exhibited antibacterial activity at different levels according to the studied bacteria. The strongest antibacterial activity was obtained with compounds P6, P7 and P8. These compounds were found to have MIC values of <5-156µg/ml. Because of the low MIC values, it has been concluded that these synthesized porphyrin derivatives may be high-potency agents against bacteria with high resistance profile.

Keywords: Anti-bacterial agents, porphyrins, bacteria, multidrug resistance.

INTRODUCTION

Multidrug-resistant (MDR) bacterial infections have become an important problem in recent years, especially due to the inappropriate and widespread use of antibiotics (Umar *et al.,* 2015; Lippert *et al.,* 2017). Conventional antibiotic treatment of the infections caused by MDR bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia spp.* and *Proteus spp.* has become almost impossible (Yu *et al.,* 2009; Umar *et al.,* 2015; Meng *et al.,* 2015; Zeng *et al.,* 2015; Lippert *et al.,* 2017; WHO 2017). As a matter of fact, the World Health Organization (WHO) on 27 February 2017 noted that there is a need for new antimicrobials or antibiotics against human health-threatening bacteria due to the multiple drug resistance (WHO, 2017). Therefore, there is an urgent need to develop alternative treatment approaches or new generation drugs.

Because of their antimicrobial activity, porphyrin and its derivatives are among the new generation drugs investigated as potential against MDR agents (Umar *et al.,* 2015; Lippert *et al.,* 2017). Porphyrins are natural molecules and are found in the structure of biomaterials such as hemoglobin, chlorophyll and vitamin B_{12}. There are forms that can be isolated from nature and synthesized in the laboratory (Stojiljkovic *et al.,* 2001; Lippert *et al.,* 2017). Natural porphyrins are predominantly derived from protoporphyrin IX (PPIX) and can often be isolated from natural environments such as body fluids and feces of the animals, egg shells and bird feathers (Stojiljkovic *et al.,* 2001). Metalloporphyrins formed by the binding of various metal ions are important enzyme cofactors and play a role in many biological processes (Fleischer 1970; Huang *et al.,* 2000; Stojiljkovic *et al.,* 2001; Jelić *et al.,* 2012; Barona-Castaño *et al.,* 2016; Lippert *et al.,* 2017).

Investigations have shown that porphyrin analogues can easily enter bacteria (Stojiljkovic *et al.,* 2001; Carpenter *et al.,* 2012; Felgenträger *et al.,* 2013; Umar *et al.,* 2015), and exhibit antimicrobial activity due to a number of biochemical processes such as transferring electrons, catalyzing peroxidase and oxidase reactions, absorbing photons and producing reactive oxygen species. Porphyrins have been reported to be capable of damaging various intracellular components including DNA, through mechanisms such as production of reactive oxygen species, lipid per oxidation, interlocation or oxidative cleavage (Stojiljkovic *et al.,* 2001; Banerjee *et al.,* 2010; Tovmasyan *et al.,* 2013; Umar *et al.,* 2015; Lippert *et al.,* 2017). Investigations have shown that cationic porphyrins penetrate into both gram negative and gram positive bacteria more easily (Felgentrager *et al.,* 2013; Tovmasyan *et al.,* 2013; Umar *et al.,* 2015). In this study, for the development of new potential antimicrobial agents, it was aimed to investigate the synthesis and antibacterial activity of the cationic porphyrin derivatives on standard strains and some clinical bacterial isolates with multidrug-resistant. To the best of our knowledge, the antibacterial activity of porphyrin derivatives in this study was not previously reported.

**Corresponding author:* e-mail: huseyin.tasli@ege.edu.tr

MATERIALS AND METHODS

Bacteria

In the study, five clinical isolates (table 1) were used in addition to the standard strains of *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853 and *Staphylococcus aureus* ATTC 29213. The Clinical isolates were selected from anonymous isolates in bacterial stocks of a University Hospital Microbiology Laboratory. There is no need for informed consent for the use of these isolates.

Cationic porphyrin derivatives

The porphyrin derivatives (P1-P8) were prepared according to the method of Gomes *et al.* (2011). All reagents and solvents were of reagent-grade quality and obtained from commercial suppliers (Sigma, Acros and Merck). Excess of the corresponding alkyl halide (iodomethane, ethyl bromide, propyl bromide, benzyl bromide, 2-phenylethyl bromide, 3-phenylpropyl bromide, 1-bromoethanol, 3-Bromo-1-propanol) (65 mmol) was added to a suspension of 5, 10, 15, 20-tetrakis(4-pyridyl) porphyrin (120mg, 193.9µmol) in dimethylformamide (30ml). After refluxing for 1-8 h, the mixture was cooled to room temperature and the obtained precipitate was filtered and washed with diethyl ether or ethanol. The crude product was taken in acetone-water (1:1), and then filtered, and washed with acetone. After completion of the synthesis studies, the structures of the compounds were analyzed by spectroscopic methods. All melting points were determined with a capillary melting point apparatus (Stuart SMP30, Staffordshire, UK). The infrared (IR) spectra of the compounds were monitored by attenuated total reflectance (ATR) (PerkinElmer Spectrum 100 FT-IR, Waltham, MA, USA). ^{1}H NMR spectra were recorded on an Agilent 600 MHz Premium COMPACT NMR spectrometer (Santa Clara, CA, USA) by using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 as a solvent. The chemical structures of the final compounds are given in Scheme 1.

Determination of antibacterial activity

In this study, minimal inhibitory concentrations (MIC) of the compounds against clinical isolates and standard strains were evaluated by microdilution method according to Clinical & Laboratory Standards Institute (CLSI 2018). The bacterial suspension was prepared to have a final concentration of approximately $5x10^5$ colony forming unit (CFU)/ml. 50µl Mueller Hinton Broth was transferred to each well of 96-well plates. The compounds were dissolved in water, followed by two-fold serial dilutions ranging from 5000µg/mL to 5µg/mL in 96-well plates. 50 µl of bacterial suspension was added to each well of the plates, and the plates were allowed to incubate at 37°C for 16-18 hours. The MIC value was defined as the lowest compound concentration that inhibits the visible growth of a bacterium after incubation. Ciprofloxacin was used for quality control. The reliability of the test was confirmed according to the MIC limit values of the ciprofloxacin for reference strains. The experiments were repeated three times.

RESULTS

The MIC values of porphyrin derivatives and ciprofloxacin for standard bacteria and clinical isolates are given in table 2. All porphyrin derivatives exhibited antibacterial activity at different levels. When the antibacterial activities of the P1, P2 and P3 derivatives were evaluated, a 4-fold decrease in P3 MICs compared to P1 was recorded against *E. coli*-1, *P. aeruginosa*-2 and *E. coli* ATTC strain. P6, P7 and P8 showed significant antibacterial activity against both Gram (-) and Gram (+) bacteria. MIC values of them were between <5 and 156 µg/ml. According to clinical isolates, the strongest antibacterial effect was observed in the P7 compound carrying ethylphenyl side chain with MIC of 20µg/ml against the *P. aeruginosa*-1. When assessed against ATCC strains, the most effective compound (MIC <5µg/ ml) was P7 against *S. aureus* strain. Unlike P7 and P8, P6 showed very weak effect against MRSA clinical isolate. The other derivatives were evaluated as very weak against standard bacterial strains and very weak or ineffective against clinical isolates.

DISCUSSION

Numerous porphyrins with different chemical structures and properties can be isolated naturally or synthesized in the laboratory (Stojiljkovic *et al.,* 2001). Porphyrin and its derivatives, which have been reported to have significant antibacterial activity, are among the new generation agents investigated as potential against MDR bacteria (Phoenix *et al.,* 2003; Umar *et al.,* 2015; Lippert *et al.,* 2017). It has been known that cationic porphyrins penetrate both gram-negative and gram-positive bacteria more easily. In our work, the cationic porphyrin derivatives were synthesized in accordance with this situation (Scheme 1).

The porphyrin derivatives P1, P2 and P3 were obtained by adding methyl, ethyl and propyl to the side chain attached to pyridinium nitrogen (Scheme 1). When their antibacterial activities were evaluated, the chain length extended from one carbon to three carbons resulted in a partial positive effect on activity. Compared to P1, a 4-fold reduction in the MIC values of P3 against clinical isolates of *E. coli*-1, *P. aeruginosa*-2 and *E. coli* ATTC strain was recorded (table 2). Looked at other strains, there was no change in activity. P4 and P5 were obtained by adding the hydroxyl group to the terminal carbon of the ethyl and propyl chains of P2 and P3. This process did not make a positive impact in general, but led to a decrease in activity against some strains.

Table 1: Features of clinical isolates

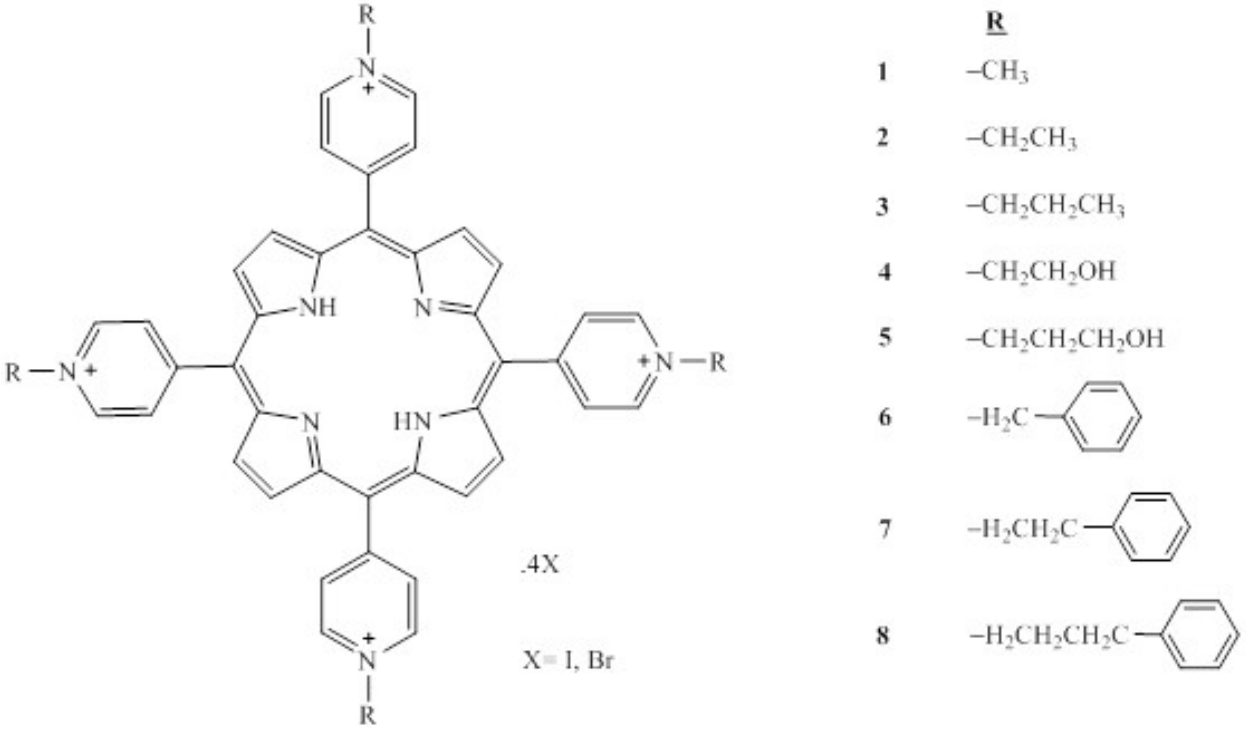
Bacteria	Resistance feature
Staphylococcus aureus	Methicillin resistant (MRSA)
Escherichia coli-1	Extended spectrum beta-lactamase positive, carbapenem sensitive
Escherichia coli-2	Carbapenem resistant
Pseudomonas aeruginosa-1	Imipenem sensitive, meropenem resistant
Pseudomonas aeruginosa-2	Carbapenem resistant

MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 2: Minimal inhibitory concentrations (µg/ml) of porphyrin derivatives and ciprofloxacin

	E. coli ATTC 25922	*E. Coli*-1 clinical isolate	*E. Coli*-2 clinical isolate	*P. aeruginosa* ATTC 27853	*P. aeruginosa*-1 clinical isolate	*P. aeruginosa*-2 clinical isolate	*S. aureus* ATTC 29213	MRSA clinical isolate
P1	313	625	1250	1250	1250	>5000	2500	2500
P2	313	625	625	1250	1250	5000	625	2500
P3	78	156	1250	1250	1250	1250	2500	2500
P4	313	313	1250	2500	1250	5000	1250	1250
P5	1250	1250	5000	2500	625	5000	2500	2500
P6	78	39	78	156	39	156	78	1250
P7	10	39	39	39	20	156	<5	39
P8	20	78	156	78	78	78	10	39
CIP	<0,016 (0,004-0,015)*	<0,016	2	0,25 (0,25-1)*	2	>16	0,125 (0,12-0,5)*	>16

*Acceptable quality control ranges of minimum inhibitory concentrations of ciprofloxacin for reference strains; MRSA (methicillin-resistant *Staphylococcus aureus*); ATTC (American Type Culture Collection); CIP (Ciprofloxacin)



Scheme 1: The chemical structures of the synthesized porphyrins (P1- P8)

The derivatives P6, P7 and P8 were synthesized by adding phenyl ring instead of hydroxyl group to the terminal carbon of the alkyl chain bound to pyridinium nitrogen (Scheme 1). The strongest antibacterial activity was obtained with these derivatives (table 2). When assessed according to clinical isolates, the strongest antibacterial effect was obtained with P7 coded derivative carrying an ethylphenyl side chain with the MIC value of 20µg/ml against *P. aeruginosa*-1 isolate. It was also found that P7 is the most effective compound against ATCC strains. The lowest MIC value (<5µg/ml) for P7 was measured against *S. aureus* strain. Antibacterial effects of the ethylphenyl bearing derivative (P7) against the *E. coli*-1, *E. coli*-2 and *P. aeruginosa*-1 isolates and the phenylpropyl bearing derivative (P8) against the *P. aeruginosa*-2 isolate were found to be stronger. In addition, both the ethylphenyl and phenylpropyl derivatives (P7, P8) against MRSA clinical isolate showed strong activity with a MIC value of 39µg/ml. The fact that adding of the phenyl ring to the side chain bound to pyridinium nitrogen leads to a significant increase in activity has shown that the phenyl structure is necessary for a stronger antibacterial effect.

Umar *et al.* (2015) reported that the porphyrin analogs they synthesized were effective against both Gram positive and Gram negative bacteria according to the disc diffusion method and that this effect was comparable to meropenem. In the study of Lippert *et al.* (2017), one of the four porphyrin derivatives they synthesized was found to have MIC values between 31.2 and 500µg/ml against different bacterial strains. In our study, the P6, P7 and P8 derivatives which we obtained strong antimicrobial activity exhibited MIC values between <5-156µg/ml against both clinical isolates and ATCC strains (except P6 MIC for MRSA). These MIC values indicate that the porphyrin derivatives we synthesize are potentially higher acting agents, especially against high-resistance profiled bacteria. Achieving low MIC values such as <5, 10, 20 µg/ml comparable to standard antibiotics has increased the importance of the porphyrin derivatives synthesized in this study. To the best of our knowledge, this study is the first to report on the antibacterial activity of these derivatives.

CONCLUSION

Porphyrin derivatives used in this study may be high-potency agents against bacteria with high resistance profile. We believe that this study will contribute to the development of clinically useful new antibacterial agents.

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