



ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Inhibitory effect of propafenone derivatives on *Pseudomonas aeruginosa* biofilm and pyocyanin production

Snežana Petrović¹, Jasmina Bašić², Zoran Mandinić³, Dragana D. Božić⁴, Marina Milenković⁴, Zorica Vujčić⁵

¹University of Belgrade, Institute for Medical Research, Centre of Research Excellence in Nutrition and Metabolism, Belgrade, Serbia;

²Medical College of Applied Sciences in Belgrade, Zemun, Serbia;

³University of Belgrade, School of Dental Medicine, Clinic for Pediatric and Preventive Dentistry, Belgrade, Serbia;

⁴University of Belgrade, Faculty of Pharmacy, Department of Microbiology and Immunology, Belgrade, Serbia;

⁵University of Belgrade, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Belgrade, Serbia

SUMMARY

Introduction/Objective Biofilm and pyocyanin production are essential components of *Pseudomonas aeruginosa* virulence and antibiotic resistance.

Our objective was to examine inhibitory effect of synthesized propafenone derivatives 3-(2-Fluorophenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5OF) and 3-(2-Trifluoromethyl-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5CF3) on biofilm and pyocyanin in *Pseudomonas aeruginosa* clinical strains.

Methods Effects were tested on nine clinical isolates and one control laboratory strain of *P. aeruginosa*. *In vitro* analysis of biofilm growing was performed by incubating bacteria (0.5 McFarland) with 5OF and 5CF3 (500–31.2 µg/ml) and measuring optical density (OD) at 570 nm. Bacteria in medium without compounds were positive control. Blank medium (an uninoculated medium without test compounds) was used as negative control. Pyocyanin production was estimated by OD at 520 nm, after bacteria incubated with 5CF3 and 5OF (250 and 500 µg/ml), treated with chloroform, and chloroform layer mixed with HCl.

Results A total of 500 µg/ml of 5OF and 5CF3 completely inhibited biofilm formation in 10/10 and 4/10 strains, respectively. A total of 250 µg/ml of 5OF and 5CF3 strongly inhibited biofilm formation in 7/10 strains, while inhibition with 125 µg/ml of 5OF and 5CF3 was moderate. Lower concentrations had almost no effect on biofilm production. Pyocyanin production was reduced to less than 40% of the control value in 6/9, and less than 50% of the control in 7/9 strains with 500 µg/ml of 5OF and 5CF3, respectively. At 250 µg/ml 5OF and 5CF3, most strains had pyocyanin production above 50% of the control value.

Conclusion Synthesized propafenone derivatives, 5OF and 5CF3, inhibited biofilms and pyocyanin production of *Pseudomonas aeruginosa* clinical strains. Presented results suggest that propafenone derivatives are potential lead-compounds for synthesis of novel antipseudomonal drugs.

Keywords: propafenone derivatives; *Pseudomonas aeruginosa*; biofilm; pyocyanin

INTRODUCTION

As an opportunistic human pathogen, *Pseudomonas aeruginosa* has evolved a number of immunoevasive strategies to impair host defense, including growing in biofilm [1, 2].

Biofilms are bacterial clusters encased in self-produced polymeric matrix attached to the epithelial surfaces or surface of medical implants. They are characterized by lower metabolic activity, increased synthesis of protective molecules, prolonged doubling time, and genetic diversity of bacterial cells, all together improving bacterial tolerance to antibiotics and survival in harsh conditions [3, 4]. Biofilm production in *Pseudomonas aeruginosa* is a well-known causative agent of antibiotic resistant infections in humans, such as pneumonia, and infections in patients with bronchiectasis and cystic fibrosis [5, 6]. Due to resistance to

phagocytosis and pronounced antibody response, those infections lead to chronic inflammation, often with a severe fatal outcome [7, 8]. Thus, there is an urgent need to develop new drugs for the treatment of *Pseudomonas aeruginosa* biofilm-associated infections. In addition, *Pseudomonas aeruginosa* pathogenicity is intimately linked to its ability to produce large variety of virulence factors, including phenazine, and most abundant pyocyanin [9, 10]. Pyocyanin is highly diffusible blue pigment, which can interact with molecular oxygen and stimulate generation of oxygen radicals, leading to redox disbalance, injury and death of host cells [11]. As virulence factor in chronic lung infection pyocyanin disrupts redox control, inhibits respiration in human cells, accelerates neutrophil apoptosis, therefore impairing host defense and favoring bacterial persistence [12, 13, 14].

Received • Примљено:

July 27, 2018

Revised • Ревизија:

July 11, 2019

Accepted • Прихваћено:

August 24, 2019

Online first: September 23, 2019

Correspondence to:

Jasmina BAŠIĆ
Medical College of Applied Sciences in Belgrade
Cara Dušana 254
11080 Zemun, Serbia
basic.jasmina23@gmail.com

Considering that ion channels are integral part of each living cell, which play a key role in cell division, proliferation, excitation, and apoptosis, modulators of ion channel activity have become important target molecules in medical chemistry [15]. Propiophenone is relatively simple compound commercially obtained from benzoic and propionic acid, it has channel-modulatory effect and serve as a precursor of numerous drugs (e.g. ephedrine, arylalkene) [16, 17, 18]. Propiophenone derivatives called propafenone are primarily known on their antiarrhythmic action, but they are also involved in treatment of many different diseases including lupus erythematosus, epilepsy, Alzheimer's disease, malaria, ebola, cancer [19–25]. In addition, recent studies have shown that analogs of propafenone exhibit antifungal activity [26]. Therefore, the molecule of propafenone has become a model of compounds used in multidrug-resistant studies [27].

Since data on antibacterial activity of propafenone derivatives are scarce, we decided to test potential antibacterial activity of 3-(2-Fluoro-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5OF) and 3-(2-Trifluoromethyl-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5CF3). Even more, because the influence of propafenone derivatives on the *Pseudomonas aeruginosa* biofilm and pyocyanin production has not yet been tested, we expanded our examinations on the influence of propafenone derivatives on expression of *Pseudomonas aeruginosa* virulence factors.

In the present study, we aimed to evaluate the inhibitory effects of ortho-fluorinated propafenone derivatives, which were synthesized in our laboratory, on biofilm and pyocyanin production in *Pseudomonas aeruginosa* clinical strains.

METHODS

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* biofilms

Test compounds

Ortho-fluorinated propafenone derivatives were synthesized at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Serbia: 5CF3: 3-(2-Trifluoromethyl-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride and 5OF: 3-(2-Fluoro-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride [28]. The structure of synthesized derivatives was spectrophotometrically analyzed at FT-IR spectrophotometer Nicolet iS10 (Thermo Fisher Scientific Inc., Waltham, MA, USA) [29].

The stock solutions of 5CF3 or 5OF (1 mg/ml) were prepared in 5% dimethyl sulfoxide (DMSO). The working solutions were prepared in trypticase soybean broth (TSB) with the addition of 1% glucose (Lab M Limited, Lancashire, UK) according to Knobloch et al. [30]. The

concentrations of working solutions of 5CF3 or 5OF were 31.2, 62.5, 125, 250, and 500 µg/ml. In previous studies, we already investigated antimicrobial effect of tested compounds in the concentration range from 500 µg/ml to 62.5 µg/ml, and the best activity was observed for 250 µg/ml and 500 µg/ml of 5OF and 5CF3 [31].

Pseudomonas aeruginosa clinical isolates

The effects of tested compounds were investigated on nine clinical isolates obtained from urine (strains 1, 2, 5, 8, 9), ear swab (strains 3, 6, 7) or sputum (strain 4) and one laboratory control strain (ATCC 27853). Bacteria were stored at -70°C in Brain Heart Infusion Broth (Lab M Limited) until needed.

Culture medium

TSB and trypticase soybean agar (Lab M Limited) were used.

Analysis

Biofilm production and quantification were performed according to protocols described by Stepanović et al. [32]. Briefly, bacteria were resuspended in saline to the density of a 0.5 McFarland standard (~10⁸ CFU/ml). In 96 microtiter plates, 180 µl of test compounds and 20 µl of bacterial suspension were added in triplicate. Bacteria incubated in medium without test compounds were used as positive control, while blank medium (uninoculated medium without test compounds) represented negative control. After incubation, which lasted 24h at 35°C, plates were washed in phosphate buffer (PBS, pH 7.2), dried, fixed with methanol, and stained with 2% crystal violet (Himedia, Mumbai, India). After washing, the color was extracted from bacteria with 96% ethanol. The OD was measured spectrophotometrically at 570 nm (ICN Flow Titertek Multiscan Plus, ICN, USA). Each experiment was repeated three times. To calculate the category of biofilm production, the optical density cut-off (ODc) was determined as three standard deviations above the mean OD of the negative control. According to the calculated results, all tested strains were categorized into four groups: OD ≤ ODc - category 0 (no biofilm production); ODc < OD ≤ 2 × ODc - category 1 (weak biofilm production); 2 × ODc < OD ≤ 4 × ODc - category 2 (moderate biofilm production), and 4 × ODc < OD - category 3 (strong biofilm production).

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* pyocyanin production

Test compounds

Ortho-fluorinated propafenone derivatives, 5CF3 and 5OF, were synthesized at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Serbia.

Working dilutions of 250 and 500 µg/ml 5CF3 and 5OF in 5% DMSO were prepared from the stock solution of 1 mg/ml in 5% DMSO. Working concentrations were chosen based on our results about 5CF3 and 5OF effect on biofilm formation, where concentrations of 250 µg/ml and 500 µg/ml appeared to have the strongest inhibitory effect.

Pseudomonas aeruginosa clinical strains

The effects of tested compounds were investigated on nine *Pseudomonas aeruginosa* clinical isolates. The sources of bacteria and storage conditions were the same as previously described in section Methods.

Culture medium

Mueller–Hinton broth for bacteria (Torlak, Belgrade, Serbia) was used (Figure 1).

Pyocyanin determination

Pyocyanin was determined as previously described by Glamočlija et al. [33]. Five milliliters of bacterial cultures in exponential phase of growth were incubated with test compounds for 24h at 37°C and then treated with 3 ml of chloroform. Separated chloroform layer was mixed with 1 ml of 0.2 M HCl. OD was measured at 520 nm [34]. Positive controls for each isolate were cultivated at the same conditions in medium without tested compounds. Values were expressed as a ratio $(OD_{520}/OD_{600}) \times 100$. Two experiments, each in triplicate, were performed. Results were calculated as the percent of the pyocyanin production compared to the positive control (expressed as $100\% \pm SD$).

Statistical analysis

Obtained data were analyzed using statistical analysis software package – SPSS Statistics Version 18.0 for Windows (SPSS Inc., Chicago, USA) and Student's t-test [35].

RESULTS

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* biofilm formation

Ortho-fluorinated propafenone derivatives, 5OF and 5CF3, inhibited production of *Pseudomonas aeruginosa* biofilms. The intensity of inhibitory effects changed in concentration dependent manner, thus, higher drug concentrations lead to stronger inhibition. The highest inhibition occurred at concentration of 500 µg/ml of both compounds. When the 5OF and 5CF3 concentrations decreased to 250 µg/ml, 125 µg/ml, 62.5 µg/ml, or 31.2 µg/ml, the inhibitory effect was also decreased. In addition, there was a variance in different isolates sensitivity to particular drug concentration. Biofilm formation was completely inhibited by 500 µg/ml of 5OF and 5CF3 in 10/10 and 4/10 strains, respectively.

In 7/10 strains, biofilm formation was strongly inhibited by 250 µg/ml 5OF and 5CF3, while inhibition with 125 µg/ml 5OF and 5CF3 was moderate. In the presence of lower 5OF and 5CF3 concentrations, 62.5 µg/ml and 31.2, 8/10 tested strains exerted strong biofilm production. Categories of biofilm production in different isolates and in the presence of various concentrations of tested compounds are presented in Table 1 and 2.

Table 1. *In vitro* effect of 5OF on the biofilm production of *Pseudomonas aeruginosa*

Parameters	5OF µg/ml					
	500	250	125	62.5	31.2	Positive control
<i>Pseudomonas aeruginosa</i> Strain number	Category of biofilm production					
1	0	1	2	2	2	2
2	0	1	1	3	3	3
3	0	0	1	2	2	2
4	0	1	1	3	3	3
5	0	1	2	3	3	3
6	0	1	2	3	3	3
7	0	2	2	3	3	3
8	0	1	2	3	3	3
9	0	1	1	2	3	3
ATCC 27853	0	0	2	3	3	3

5OF – 3-(2-fluoro-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride; Positive control – bacterial growth in medium without tested compound; 0 – no biofilm production; 1 – weak biofilm production; 2 – moderate biofilm production; 3 – strong biofilm production

Table 2. *In vitro* effect of 5CF3 on the biofilm production of *Pseudomonas aeruginosa*

Parameters	5CF3 µg/ml					
	500	250	125	62.5	31.2	Positive control
<i>Pseudomonas aeruginosa</i> Strain number	Category of biofilm production					
1	0	1	2	2	2	2
2	1	1	2	3	3	3
3	0	1	2	2	2	2
4	1	2	2	3	3	3
5	1	1	2	3	3	3
6	0	1	2	3	3	3
7	1	2	2	3	3	3
8	1	1	3	3	3	3
9	1	2	2	3	3	3
ATCC 27853	0	1	0	3	3	3

5CF3 – 3-(2-trifluoromethyl-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride; Positive control – biofilm production in medium without tested compound; 0 – no biofilm production; 1 – weak biofilm production; 2 – moderate biofilm production; 3 – strong biofilm production

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* pyocyanin production

Ortho-fluorinated propafenone derivatives, 5CF3 and 5OF, inhibited production of pyocyanin in *Pseudomonas aeruginosa*. In the presence of 500 µg/ml 5OF or 5CF3



Figure 1. *Pseudomonas aeruginosa* growth on Mueller-Hinton agar

production of pyocyanin was reduced to less than 40% of the control value in 6/9 strains, and less than 50% of the control in 7/9 strains, respectively. In the presence of 250 µg/ml 5OF or 5CF3, most strains had pyocyanin production above 50% of the control value. The difference in the sensitivity to the tested compounds among various strains was also detected. Results of inhibitory action of 5OF and 5CF3 on the pyocyanin production in *Pseudomonas aeruginosa* are expressed as the percentage of the absorbance of positive controls (presented as 100% ± SD) (Table 3).

Table 3. *In vitro* effect of ortho-fluorinated propafenone derivatives 5OF and 5CF3 on the production of pyocyanin in *Pseudomonas aeruginosa* strains

Parameters	5OF µg/ml		5CF3 µg/ml	
	500	250	500	250
<i>Pseudomonas aeruginosa</i> Strain number	Pyocyanin production as% of positive control			
1	48.6	70.5	74	79.8
2	27.1	42	36.6	79.8
3	39.3	51.8	68.5	99.4
4	33.7	31.9	34.6	54.2
5	39.4	61.1	48.3	104.9
6	34.3	49.5	35.2	46.6
7	42.7	53.4	51	56.3
8	29.8	54.2	36.9	52.4
ATCC 27853	43.6	57.6	47.9	64

5OF – 3- (2-fluoro-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride; 5CF3 – 3- (2-trifluoromethyl-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride; Positive control – pyocyanin production of each isolate in the absence of the tested compounds (100%).

DISCUSSION

Antibiotic compounds that inhibit different virulence factor, such as enterotoxins, hemolysins, biofilm, or pigments, became the focus of the present research [36]. The resistance of *Pseudomonas aeruginosa* isolates to antimicrobial drugs is largely attributed to its ability to form a biofilm and produce bacterial pigment pyocyanin [37]. In

this study, we used synthesized propafenone derivatives, 5CF3, and 5OF, to test inhibitory effect on *Pseudomonas aeruginosa* biofilm and pigment production.

Antimicrobials are generally dedicated to kill bacteria (bactericidal) or to inhibited bacterial growth (bacteriostatic). However, mostly due to frequent chromosomal mutations, *Pseudomonas aeruginosa* appeared to be extremely adaptive and acquired resistance to many antibiotics such as carbapenems, penicillins and cephalosporins. Recent efforts to develop novel class of anti-pseudomonas agents moved their focus to *Pseudomonas aeruginosa* physiology and collective behavior of bacterial population [38]. Therefore, biofilm formation and its modulation became a subject of our research interest. Our results have shown that propafenone derivative 5OF and 5CF3 significantly reduced biofilm production in all tested isolates of *Pseudomonas aeruginosa*. Previous study on propafenone compounds reported antimicrobial effect due to inhibition of ubiquitous bacterial multidrug efflux pumps [39]. Thus, by channel-blocking propafenone may decrease drug resistance and positively influence clinical outcome of *Pseudomonas aeruginosa* infections [40]. On the other hand, to the best of our knowledge, this study revealed identification of ortho-fluorinated propafenone derivatives as efficient agents that inhibit *Pseudomonas aeruginosa* biofilm formation for the first time. The inhibitory effects of both 5OF and 5CF3 were found. Numerous external factors affect biofilm formation by *Pseudomonas aeruginosa*. In addition, the type of tissue has strong impact on biofilm formation, and researchers commonly test biofilm formation of *Pseudomonas aeruginosa* from a variety of clinical sources [41]. In our study, various clinical strains showed differences in sensitivity to tested compounds, but those variations were not connected to specific bacterial source (urine, ear swab, sputum). However, we tested only nine clinical isolates (5 – urine, 3 – ear swab, 1 – sputum) and for such a small number of samples statistical data processing is not relevant.

The highest tested dose of both compounds (500 µg/ml) was the most efficient, reducing bacterial growth to the highest extent. However, when the concentration of test agents decreased bacterial growth recovered. In the present study, 5OF was more effective in reducing bacterial growth compared to 5CF3. This could be explained by higher binding affinity to bacterial transport porin in a case of monofluorinated propafenone derivatives (such as 5OF), compared to trifluoromethyl derivative (5CF3), as found in our docking studies (data not shown) [31]. Namely, biofilm formation depends on the presence of an extracellular matrix, which is a mixture of polysaccharides, proteins, and nucleic acids [extracellular DNA (eDNA)]. Matrix polysaccharides (alginate and lipopolysaccharides), which are synthesized in bacterial cytoplasm, bind to membrane transporters to be extruded out of the cell [42]. It was found that both fluorinated derivatives tested in this study briefly occupied key substrate-specific sites in the bacterial porin (Arg124). This discovery might be associated with interruption of the transport of carbohydrate compounds involved in synthesis of biofilm [43].

The blue pigment pyocyanin, chemical derivative of phenazine, is one of the most important virulence factors in *Pseudomonas aeruginosa* [44]. Pyocyanin is toxic for respiratory epithelium, it acts on the cell structure and function, disrupts normal expression of genes involved in efflux pumps, redox homeostasis and iron acquisition in human cells [45, 46, 47]. Thus, control of pyocyanin production may be a mechanism to reduce bacterial pathogenicity. Results of our study have shown that both 5OF and 5CF3 inhibited production of pyocyanin in all tested *Pseudomonas aeruginosa* isolates. The inhibitory effect was concentration dependent, higher concentrations caused stronger inhibition, while inhibitory effect decreased with lower drugs concentration. Literature survey on other drugs suggests that ortho-fluorinated propafenone derivative 5OF had significantly stronger inhibitory effect on the production of pyocyanin in *Pseudomonas aeruginosa* strains than commercial antibiotics ampicillin or streptomycin. Namely, we have shown that lower concentrations of 5OF, 500 µg/ml, and 250 µg/ml, exerted same or even enhanced inhibitory effect compared to commercial antibiotics when applied in 2–4 times higher concentration (1 mg/ml) [33, 48]. Similarly, the concentrations of 5CF3, which led to pyocyanin reduction, were within the same range as concentration of standard drugs. The observed propafenone-induced pyocyanin inhibition could be discussed in a view of recent results on pyocyanin impact on eDNA and biofilm formation [11]. It was found that pyocyanin decreases eDNA content within the *Pseudomonas aeruginosa* biofilm. Since eDNA promotes bacterial adhesion and cellular aggregation, depletion of eDNA can reduce biofilm strength and disturb protection of bacterial cells against antibiotics. Based on the

forementioned, it was assumed that reduction of pyocyanin production as detected in our study could be a model of propafenone derivatives action against *Pseudomonas aeruginosa* pathogenicity and infection.

CONCLUSION

The results of the study suggest that synthesized ortho-fluorinated propafenone derivatives inhibit biofilm and pyocyanin production in *Pseudomonas aeruginosa* clinical strains. Presented results suggest that propafenone derivatives could be considered as potential lead-compounds for synthesis of novel antipseudomonal drugs.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (OI 172041 and III 41030).

This study was done in accordance with the institutional standards of the Committee on Ethics.

This work is a part of Jasmina Bašić's PhD thesis "Examination of correlation between chemical structure, physicochemical properties and retention parameters and antimicrobial activity of newly synthesized derivatives of propiophenone," defended at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade on September 19, 2016.

Conflict of interest: None declared.

REFERENCES

- Hilker R, Munder A, Klockgether J, Losada PM, Chouvarine P, Cramer N, et al. Interclonal gradient of virulence in the *Pseudomonas aeruginosa* pangenome from disease and environment. *Environ Microbiol*. 2015;17(1):29–46.
- Streeter K, Katouli M. *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. *Infect Epidemiol Med*. 2016;2(1):25–32.
- De Kievit TR. Quorum sensing in *Pseudomonas aeruginosa* biofilms. *Environ Microbiol*. 2009;11(2):279–88.
- Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug resistant, extensively drug resistant and pandrug resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268–81.
- Gillis RJ, White KG, Choi KH, Wagner VE, Schweizer HP, Iglewski BH. Molecular basis of azithromycin-resistant *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother*. 2005;49(9):3858–67.
- Johansson EM, Cruz SA, Kolomiets E, Buts L, Kadam RU, Cacciarini M, et al. Inhibition and dispersion of *Pseudomonas aeruginosa* biofilms by glycopeptide dendrimers targeting the fucose-specific lectin LecB. *Chem Biol*. 2008;15(12):1249–57.
- Kuang Z, Hao Y, Walling BE, Jeffries JL, Ohman DE, Lau GW. *Pseudomonas aeruginosa* elastase provides an escape from phagocytosis by degrading the pulmonary surfactant protein-A. *PLoS One*. 2011;6(11):e27091.
- Pressler T, Bohmova C, Conway S, Dumcius S, Hjelte L, Høiby N, et al. Chronic *Pseudomonas aeruginosa* infection definition: Euro Care CF working group report. *J Cyst Fibros*. 2011;10 Suppl 2:S75–8.
- Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob Agents*. 2015;45(6):568–85.
- Winstanley C, O'Brien S, Brockhurst MA. *Pseudomonas aeruginosa* evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. *Trends Microbiol*. 2016;24(5):327–37.
- Das T, Manefield M. Pyocyanin promotes extracellular DNA release in *Pseudomonas aeruginosa*. *PLoS One*. 2012;7(10):e46718.
- Allen L, Dockrell DH, Patterly T, Lee DG, Cornelis P, Hellewell PG, et al. Pyocyanin production by *Pseudomonas aeruginosa* induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. *J Immunol*. 2005;174(6):3643–9.
- Lau GW, Hassett DJ, Ran H, Kong F. The role of pyocyanin in *Pseudomonas aeruginosa* infection. *Trends Mol Med*. 2004;10(12):599–606.
- Usher LR, Lawson RA, Geary I, Taylor CJ, Bingle CD, Taylor GW, et al. Induction of neutrophil apoptosis by the *Pseudomonas aeruginosa* exotoxin pyocyanin: a potential mechanism of persistent infection. *J Immunol*. 2002;168(4):1861–8.
- Bezanilla F. How membrane proteins sense voltage. *Nat Rev Mol Cell Biol*. 2008;9(4):323–32.
- Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. *Curr Clin Pharmacol*. 2010;5(1):1–29.
- Kabra, R, Chauhan N, Kumar A, Ingale P, Singh S. Efflux pumps and antimicrobial resistance: Paradoxical components in systems genomics. *Prog Biophys Mol Biol*. 2019;141:15–24.
- Jabeen I, Pleban K, Rinner U, Chiba P, Ecker GF. Structure-activity relationships, ligand efficiency, and lipophilic efficiency profiles

- of benzophenone-type inhibitors of the multidrug transporter P-glycoprotein. *J Med Chem.* 2012;55(7):3261–73.
19. Lowes DJ, Guiguemde WA, Connelly MC, Zhu F, Sigal MS, Clark JA, et al. Optimization of propafenone analogues as antimalarial leads. *J Med Chem.* 2011;54(21):7477–85.
 20. Al Hussaini M, Hammouda El, Hammouda AE. Optimizing pharmacotherapy of systemic lupus erythematosus: the pharmacist role. *Int J Clin Pharm.* 2014;36(4):684–92.
 21. Shao J, Feng G. Selective killing effect of oxytetracycline, propafenone and metazolol on A549 or HeLa cells. *Chin J Cancer Res.* 2013;25(6):662–70.
 22. Kouznetsova J, Sun W, Martínez-Romero C, Tawa G, Shinn P, Chen CZ, et al. Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. *Emerg Microbes Infect.* 2014;3(1):1–7.
 23. Banach M, Piskorska B, Borowicz-Reutt KK. Propafenone enhances the anticonvulsant action of classical antiepileptic drugs in the mouse maximal electroshock model. *Pharmacol Rep.* 2016;68(3):555–60.
 24. Ngo ST, Fang ST, Huang SH, Chou CL, Huy PDQ, Li MS, et al. Anti-arrhythmic medication propafenone a potential drug for Alzheimer's disease inhibiting aggregation of A β : in silico and in vitro studies. *J Chem Inf Model.* 2016;56(7):1344–56.
 25. Zheng WB, Li YJ, Wang Y, Yang J, Zheng CC, Huang XH, et al. Propafenone suppresses esophageal cancer proliferation through inducing mitochondrial dysfunction. *Am J Cancer Res.* 2017;7(11):2245–56.
 26. Abonia R, Garay A, Castillo J, Insuasty B, Quiroga J, Noguera M, et al. Design of Two Alternative Routes for the Synthesis of Naftifine and Analogues as Potential Antifungal Agents. *Molecules.* 2018;23(3).
 27. Chiba P, Burghofer S, Richter E, Tell B, Moser A, Ecker G. Synthesis, pharmacologic activity, and structure-activity relationships of a series of propafenone-related modulators of multidrug resistance. *J Med Chem.* 1995;38(14):2789–93.
 28. Ivković B, Vladimirov S, Novaković R, Čupić V, Heinle H, Gojković-Bukarića L. The novel phenylpropiofenone derivatives induced relaxation of isolated rat aorta. *Arzneimittelforschung.* 2012;62(7):345–50.
 29. Ivković B. Design, synthesis and biological activity of phenylpropiofenoneaminoalcoxy derivatives [dissertation]. Belgrade: Faculty of Pharmacy, University of Belgrade; 2016.
 30. Knobloch JK, Horstkotte MA, Rohde H, Kaulfers PM, Mack D. Alcoholic ingredients in skin disinfectants increase biofilm expression of *Staphylococcus epidermidis*. *J Antimicrob Chemother.* 2002;49(4):683–7.
 31. Basic J. Examination of correlation between chemical structure, physicochemical properties and retention parameters and antimicrobial activity of newly synthesized derivatives of propiofenone [dissertation]. Belgrade: Faculty of Pharmacy, University of Belgrade; 2016.
 32. Stepanović S, Vuković D, Hala V, Di Bonaventura G, Djukić S, Čirković I, et al. Quantification of biofilm microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS.* 2007;115(8):891–9.
 33. Glamočlija J, Čirić A, Nikolić M, Fernandes A, Barros L, Calhelha RC, et al. Chemical characterization and biological activity of Chaga (*Inonotus obliquus*), a medicinal mushroom. *J Ethnopharmacol.* 2015;162:323–32.
 34. Sandy SM, Foong-Yee T. Anti-quorum sensing and antimicrobial activities of some traditional Chinese medicinal plants commonly used in South-East Asia. *Mal J Microbiol.* 2012;8(1):11–20.
 35. SPSS Statistics. www.ibm.com/software/products/en/spss-statistics
 36. Escaich S. Antivirulence as a new antibacterial approach for chemotherapy. *Curr Opin Chem Biol.* 2008;12(4):400–8.
 37. Lai S, Tremblay J, Déziel E. Swarming motility: a multicellular behavior conferring antimicrobial resistance. *Environ Microbiol.* 2009;11(1):126–36.
 38. Morita Y, Tomida J, Kawamura Y. Responses of *Pseudomonas aeruginosa* to antimicrobials. *Front Microbiol.* 2014;4:422.
 39. Ramaswamy VK, Caccioto P, Mallocci G, Ruggerone P, Vargiu AV. Multidrug efflux pumps and their inhibitors characterized by computational modeling. In: Li X, Elkins CA, Zgurskaya H, editors. *Efflux-mediated drug resistance in bacteria: Mechanisms, regulation and chemical implications.* New York: Springer Verlag, Heidelberg, 2016. p 797-831.
 40. Li XZ, Nikaido H. Efflux-mediated drug resistance in bacteria: an update. *Drugs.* 2009;69(12):1555–623.
 41. Lima JLDC, Alves LR, Jacomé PRLA, Bezerra Neto JP, Maciel MAV, Morais MMC, et al. Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of isolates non biofilm-producing. *Braz J Infect Dis.* 2018;22(2):129–36.
 42. Ma L, Wang J, Wang S, Anderson EM, Lam JS, Parsek MR, et al. Synthesis of multiple *Pseudomonas aeruginosa* biofilm matrix exopolysaccharides is post-transcriptionally regulated. *Environ Microbiol.* 2012;14(8):1995–2005.
 43. Hay ID, Rehman ZU, Ghafoor A, Rehm BHA. Bacterial biosynthesis of alginates. *Chem Technol Biotechnol.* 2010;85:752–9.
 44. Britigan BE, Railsback MA, Cox CD. The *Pseudomonas aeruginosa* secretory product pyocyanin inactivates alpha1 protease inhibitor: implications for the pathogenesis of cystic fibrosis lung disease. *Infect Immun.* 1999;67(3):1207–12.
 45. Rada B, Leto TL. Pyocyanin effects on respiratory epithelium: relevance in *Pseudomonas aeruginosa* airway infections. *Trends Microbiol.* 2013;21(2):73–81.
 46. O'Malley YQ, Reszka KJ, Rasmussen GT, Abdalla MY, Denning GM, Britigan BE. The *Pseudomonas* secretory product pyocyanin inhibits catalase activity in human lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* 2003;285(5):L1077–86.
 47. Look DC, Stoll LL, Romig SA, Humlicek A, Britigan BE, Denning GM. Pyocyanin and its precursor phenazine-1-carboxylic acid increase IL-8 and intercellular adhesion molecule-1 expression in human airway epithelial cells by oxidant-dependent mechanisms. *J Immunol.* 2005;175(6):4017–23.
 48. Avdeef A, Strafford M, Block E, Balogh MP, Chambliss W, Khan I. Drug absorption in vitro model: filter-immobilized artificial membranes. 2. Studies of the permeability properties of lactones in *Piper methysticum* Forst. *Eur J Pharm Sci.* 2001;14(4):271–80.

Инхибиторни ефекат пропафенонских деривата на продукцију биофилма и пиоцијанина код бактерије *Pseudomonas aeruginosa*

Сњежана Петровић¹, Јасмина Башић², Зоран Мандинић³, Драгана Д. Божић⁴, Марина Миленковић⁴, Зорица Вујић⁵

¹Универзитет у Београду, Институт за медицинска истраживања, Центар изузетне вредности у области истраживања исхране и метаболизма, Београд, Србија;

²Висока здравствена школа струковних студија у Београду, Земун, Србија;

³Универзитет у Београду, Стоматолошки факултет, Клиника за дечју и превентивну стоматологију, Београд, Србија;

⁴Универзитет у Београду, Фармацеутски факултет, Катедра за микробиологију и имунологију, Београд, Србија;

⁵Универзитет у Београду, Фармацеутски факултет, Катедра за фармацеутску хемију, Београд, Србија

САЖЕТАК

Увод/Циљ Производња биофилма и пиоцијанина је важан фактор вируленције и антибиотске резистенције бактерије *Pseudomonas aeruginosa*.

Циљ рада је био да се испита инхибиторни ефекат синтетисаних пропафенонских деривата, 3-(2-флуоро-фенил)-1-[2-(2-хидрокси-3-пропиламино-пропокси)-фенил]-пропан-1-он-хидрохлорид (5OF) и 3-(2-трифлуорометилфенил)-1-[2-(2-хидрокси-3-пропиламино-пропокси)-фенил]-пропан-1-он-хидрохлорид (5CF3), на продукцију биофилма и пиоцијанина код клиничких изолата бактерије *Pseudomonas aeruginosa*.

Метод Ефекат пропафенонских деривата испитан је на девет клиничких изолата и једном стандардном соју бактерије *P. aeruginosa*. Утицај на продукцију биофилма испитан је *in vitro*, инкубацијом бактерија (0,5 по Макфарланду) са 5OF и 5CF3 (500–31,2 $\mu\text{g/ml}$), и мерењем оптичке густине на 570 nm. Бактерије у медијуму без испитиваних једињења су биле позитивна контрола, а сам медијум негативна контрола. Производени пиоцијанин, који је одређиван мерењем оптичке густине на 520 nm, на коинкубације бактерија са 5CF3 или 5OF (250 и 500 $\mu\text{g/ml}$), третиран је хлороформом и мешањем хлороформског слоја са HCl.

Резултати При концентрацији од 500 $\mu\text{g/ml}$ 5OF је довео до потпуне инхибиције продукције биофилма код свих испитиваних сојева (10/10). Инхибиција биофилма са 500 $\mu\text{g/ml}$ 5CF3 била је потпуна код 4/10 сојева. При концентрацији 5OF и 5CF3 од 250 $\mu\text{g/ml}$ продукција биофилма код већине испитаних изолата била је слаба, док је при концентрацији 125 $\mu\text{g/ml}$ 5OF односно 5CF3 продукција била умерена. Ниже концентрације 5OF и 5CF3 нису имале инхибиторни ефекат на формирање биофилма. У присуству 500 $\mu\text{g/ml}$ 5OF у 6/10 испитиваних сојева продукција пиоцијанина пала је на мање од 40% у односу на контролну вредност. Иста концентрација (500 $\mu\text{g/ml}$) 5CF3 снизила је продукцију пиоцијанина на мање од 50% од контроле у 7/9 сојева. При концентрацији 250 $\mu\text{g/ml}$ 5OF или 5CF3 већина сојева продуковала је пиоцијанин изнад 50% у односу на позитивну контролу.

Закључак Синтетисани пропафенонски деривати, 5OF и 5CF3, инхибирају продукцију биофилма и пиоцијанина код клиничких сојева бактерије *Pseudomonas aeruginosa*. Добијени резултати указују на то да пропафенонски деривати представљају могућа полазна једињења за синтезу нових антипсеудомонасних агенаса.

Кључне речи: пропафенонски деривати; *Pseudomonas aeruginosa*; биофилм; пиоцијанин