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Original article

ANTIBACTERIAL ACTIVITY OF 1-DODECYLPYRIDINIUM TETRAFLUOROBORATE AND ITS INCLUSION COMPLEX WITH SULFOBUTYL ETHER-β-CYCLODEXTRIN AGAINST MDR *ACINETOBACTER BAUMANNII* STRAINS

S. Rogalsky[1*], D. Hodyna[1], I. Semenyuta[1], M. Frasinyuk[1], O. Tarasyuk[1], S. Riabov[2*], L. Kobrina[2], I. Tetko[3,4], L. Metelytsia[1]

[1]V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Institute of Macromolecular Chemistry of National Academy of Sciences of Ukraine, Kyiv, Ukraine
[3]Helmholtz Munich - German Research Center for Environmental Health (GmbH), Neuherberg, Germany
[4]BIGCHEM GmbH, Unterschleißheim, Germany

*Corresponding authors: sergey.rogalsky@gmail.com, sergii.riabov@gmail.com

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Background. The bacterial pathogen *Acinetobacter baumannii* is one of the most dangerous multi-drug-resistant (MDR) microorganisms, which causes numerous bacterial infections. Nowadays, there is an urgent need for new broad-spectrum antibacterial agents with specific molecular mechanisms of action. Long-chain 1-alkylpyridinium salts are efficient cationic biocides which can inhibit enzymes involved in the biosynthesis of bacterial fatty acids. Incorporating these compounds into inclusion complexes with cyclic oligosaccharide β-cyclodextrin can reduce their relatively high acute toxicity.
Objective. The aim of this research was to develop new anti-*A. baumannii* agents based on hydrophobic 1-alkylpyridinium salt and its inclusion complex with sulfobutyl ether β-cyclodextrin (SBECD).
Methods. Hydrophobic cationic biocide 1-dodecylpyridinium tetrafluoroborate ($PyrC_{12}$-BF_4) and its inclusion complex with SBECD have been synthesized. The structure of the SBECD/$PyrC_{12}$-BF_4 complex was characterized by ^{1}H Nuclear Magnetic Resonance spectroscopy, as well as UV spectroscopy. *In vitro* antibacterial activity of the synthesized compounds was estimated against MDR clinical isolates of *A. baumannii* using standard disc diffusion method. Acute toxicity studies were performed on *Daphnia magna* model hydrobiont. Molecular docking was performed using the crystal structure of the *A. baumannii* 3-oxoacyl-[acyl-carrier-protein] reductase (FabG).
Results. The results of ^{1}H NMR study revealed the formation of an inclusion complex between SBECD and $PyrC_{12}$-BF_4. The cationic biocide demonstrated high activity against four tested antibiotic-resistant strains of *A. baumannii*, whereas the SBECD/$PyrC_{12}$-BF_4 complex was active against only two bacterial strains. Molecular docking of 1-dodecylpyridinium ligand into the active site of the *A. baumannii* (FabG) showed complex formation at an allosteric site located between subunits C, D. The acute toxicity (LC_{50}) of $PyrC_{12}$-BF_4 and its inclusion complex was found to be 0.007 and 0.033 mg/l, respectively.
Conclusions. Hydrophobic cationic biocide $PyrC_{12}$-BF_4 has high antibacterial activity against MDR *A. baumannii*. The inhibition of the active site FabG may be one of the possible mechanisms of anti-*A. baumannii* activity of the $PyrC_{12}$-BF_4. The SBECD/$PyrC_{12}$-BF_4 inclusion complex showed an almost 5-fold reduction in acute toxicity compared to $PyrC_{12}$-BF_4, while retaining activity against certain tested *A. baumannii* bacterial strains.

Keywords: cationic biocide; antibacterial activity; acute toxicity; molecular docking; β-cyclodextrin; inclusion complex.

Introduction

Long-chain quaternary ammonium compounds (QACs) are a well-known class of cationic surfactants with a broad spectrum of antimicrobial activity [1]. They have numerous industrial, medical, personal-care and household applications as cleaners, softeners, biocides, sanitizers and disinfectants [1–4]. Being amphiphilic in nature, cationic surfactants exert their biological activity mainly by inducing disintegration of bacterial membranes via electrostatic and hydrophobic interactions [5]. These properties drive the broader antimicrobial activity of QACs compared to common antibiotics, since it is difficult for bacteria to circumvent this non-specific disruption mechanism [5–7]. Thus, due to their potent activity against bacteria, fungi and viruses, QACs are widely used in clinical settings to control the spread of pathogenic microorganisms. For many years, the most commonly used QACs were benzalkonium chloride (BAC), cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC), didecyldimethylammonium chloride (DDAC) [1, 3, 8]. However, frequent use

of cationic surfactants leads to their accumulation in environment due to their chemical stability and low biodegradability, which has an extremely negative environmental impact. Moreover, the huge consumption of QACs was found to introduce multidrug resistance in some bacterial cells [6, 7, 9]. The gram-negative pathogen *Acinetobacter baumannii* is one such organism that has evolved from an accidental respiratory pathogen into a major nosocomial pathogen. Bacteria *Acinetobacter spp.* are among the six most dangerous multi-resistant ESKAPE pathogens for the human population [10]. Since 2017, the World Health Organization has included this microorganism in the list of "priority pathogens", resistant to the action of antibiotics, which pose the greatest threat to human health [11]. Nowadays, there is an urgent need in the development of new, efficient anti-*A. baumannii* agents to combat the growing threat of antimicrobial resistance. One effective solution would be the design of biocides with a specific molecular mechanism of action [12]. The enzymes involved in the biosynthesis of bacterial fatty acid (FASII) such as 3-oxoacyl-[acyl-carrier-protein] reductase (FabG) and Enoyl-ACP reductase (FabI), are important targets for new antibacterial agents [13].

Numerous studies have shown that cationic surfactants comprising heterocyclic cations with delocalized charges possess a broader range of antimicrobial activity compared to common QACs and showed strong antibiofilm activity against a panel of pathogen microorganisms [14–17]. Furthermore, 1-alkylpyridinium salts with an alkyl chain length of 12 and 14 carbon atoms showed high *in vitro* activity against clinical isolates of both gram-positive *S. aureus* and gram-negative *A. baumannii* strains [17]. Molecular docking of 1-dodecylpyridinium chloride and 1-tetradecylpyridinium bromide suggested that they can form a complex with Fab, which is a key enzyme in the biosynthesis of bacterial fatty acids. However, the high acute toxicity of long-chain 1-alkylpyridinium salts is a serious drawback limiting their practical applications [18, 19]. Recent studies have demonstrated the possibility of reducing the toxicity of cationic surfactant 1-dodecyl-3-methylimidazolium tetrafluoroborate by its complexation with cyclic oligosaccharide β-cyclodextrin (CD) [20]. The limited water solubility of cationic surfactants containing tetrafluoroborate anions makes it ideally suited for inclusion complexation with CD [20, 21]. To our knowledge, antimicrobial formulations based on inclusion complexes of long-chain 1-alkylpyridinium salts with CD have not yet been studied.

The aim of this research was to develop new anti-*A. baumannii* agents based on hydrophobic 1-alkylpyridinium salt and its inclusion complex with sulfobutyl ether β-cyclodextrin (SBECD). In particular, hydrophobic cationic biocide, 1-dodecylpyridinium tetrafluoroborate ($PyrC_{12}$-BF_4), has been synthesized. An inclusion complex of $PyrC_{12}$-BF_4 with SBECD has been prepared and characterized by spectral methods. It should be noted that SBECD has significant advantages over CD, such as high water solubility and much lower acute toxicity [22]. Comparative studies of *in vitro*, *in vivo*, and *in silico* biological activity of $PyrC_{12}$-BF_4 and $PyrC_{12}$-BF_4/SBECD complex have been carried out.

Materials and Methods

Materials. The following chemicals were used for the synthesis of cationic biocide: pyridine (99%), 1-chlorododecane (97%), hexane (98%), ethyl acetate (98%), methylene chloride (99%), tetrafluoroboric acid (48 wt.% in H_2O). All chemicals were purchased from Sigma-Aldrich and used without further purification.

Sulfobutyl ether-β-cyclodextrin sodium salt (SBECD, DS ≈ 6,5) was provided by CycloLab (Hungary). The structural formula of SBECD is shown in Fig. 1.

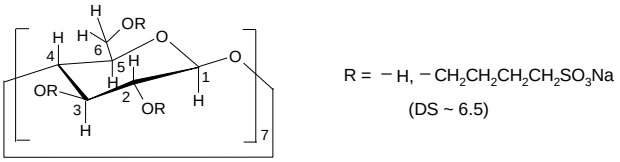
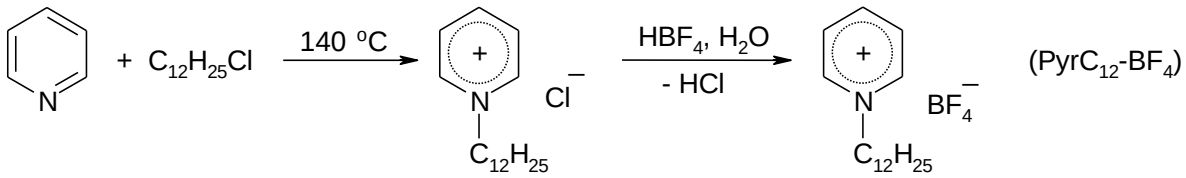


Figure 1: Chemical structure of SBECD

Synthesis of cationic biocide $PyrC_{12}$-BF_4. 1-dodecylpyridinium tetrafluoroborate ($PyrC_{12}$-BF_4) was synthesized according to the Scheme 1. The mixture of pyridine (10 g, 0.12 mol) and 1-chlorododecane (30.6 g, 0.15 mol) was stirred at 140 °C for 24 h. The obtained solid product 1-dodecylpyridinium chloride was purified by recrystallization from ethyl acetate-hexane mixture (1:2 v/v).

1-dodecylpyridinium chloride (20 g, 0.07 mol) was dissolved in water (200 ml) and tetrafluoroboric acid (20 ml) was added to the solution while stirring. The formed solid residue was extracted with methylene chloride (2 × 50 ml) and dried over sodium sulfate. The solvent was distilled off and the product was dried in vacuum at 5 mbar and 50 °C for 12 h.

1-dodecylpyridinium tetrafluoroborate ($PyrC_{12}$-BF_4).



Scheme 1: Synthesis of $PyrC_{12}$-BF_4

White solid, mp 68–69 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 6.9 Hz, 3H, CH_3), 1.14–1.41 (m, 18H, $CH_3(CH_2)_9$), 1.97 (m, 2H, NCH_2CH_2), 4.60 (t, *J* = 7.5 Hz, 2H, NCH_2), 8.03 (dd, *J* = 7.7, 6.2 Hz, 2H, C_3-H, C_5-H), 8.47 (t, *J* = 7.8 Hz, 1H, C_4-H), 8.77–8.83 (m, 2H, C_2-H, C_6-H).

^{19}F NMR (376 MHz, DMSO-d_6): δ = –148.8 (s, BF_4).

Preparation of SBECD/PyrC$_{12}$-BF$_4$ inclusion complex. 2.2 g (0.9 mmol) of SBECD was dissolved in 20 ml of deionized water followed by addition of $PyrC_{12}$-BF_4 (0.3 g, 0.9 mmol). The mixture was stirred for 12 h until a transparent solution was obtained. The water was evaporated and the obtained solid powder was dried at 130 °C for 24 h.

Spectral studies of SBECD/PyrC$_{12}$-BF$_4$ inclusion complex structure. The proton magnetic resonance (^{1}H NMR) spectra were recorded in D_2O solution on Bruker AVANCE DRX 500 spectrometer using 1,4-dioxane (δ_H = 3.75 ppm) as the internal standard. UV-spectra were recorded in H_2O solution on a Jenway 6850 spectrometer (Great Britain).

In vitro antibacterial activity. Isolates of the bacterial antibiotic-resistant strains of *A. baumannii* №1536, №871, №1355 and №725 were obtained from the collection of the Microbial Cultures Museum of the Shupyk National Healthcare University of Ukraine. The antibacterial activities of the synthesized compounds were assessed by the standard disk diffusion method on a solid Muller–Hinton nutrient medium [23]. The inoculum was prepared at a final concentration of 1×10^5 colony-forming unit (CFU) per ml using the 0.5 McFarland standard as a reference to adjust the bacterial suspensions turbidity. The 0.03 M solutions of $PyrC_{12}$-BF_4 and SBECD/$PyrC_{12}$-BF_4 complex in water were prepared. Each solution in an amount of 0.02 ml was applied to standard paper disks (6 mm), which were placed on each agar plate containing the bacterial strains. The content of the tested compounds on a disk was equivalent to 0.6 μmol. The known antibiotics Ampicillin, Carbenicillin, Cefoxitin, Ceftriaxone and Colistin were used as positive controls. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition of bacterial growth, which indicates the degree of susceptibility or resistance of *A. baumannii* against the test compounds. Clear zones of inhibition (in mm) formed around the discs were measured after 18 hour incubation period at 37 °C. Compounds with bacterial growth inhibition zones ≤15 mm were identified as inactive. All experiments were evaluated in triplicate.

In vivo acute toxicity with Daphnia magna. The acute toxicity tests of synthesized compounds on *Daphnia magna* (*D. magna)* followed the recommendations of the Organization for Economic Co-operation and Development Guideline 202 [24] using newborn organisms (2–26 h). The concentration of compounds in water ranged from 0.001 to 10 mg/l. Each compound was tested on seven daphnids placed in a 50-ml glass beaker with 30 ml of certain dose of the compound. The immobility of organisms was assessed at the 48 h of the exposure time. The determined values of 50% lethal concentration (LC_{50}) and 95% confidence intervals (CI) were statistically analyzed using the Statistica 7 program. Potassium bichromate was used as a positive control at a concentration of 1.5 mg/l (24 h). All experiments were evaluated in triplicate.

In silico molecular modeling procedure. Molecular docking was performed using the crystal structure of the *A. baumannii* 3-oxoacyl-[acyl-carrier-protein] reductase from the RCSB Protein Data Bank (PDB ID:6T65). AutoDock Tools (ADT) 1.5.6 [25] was used to prepare the protein and ligand. Subunits C and D were used for research. All hydrogen atoms were added to the protein by ADT, and then all protein atoms were renumbered using the noBondOrder method. The Gasteiger method was used to calculate the partial charges of atoms next the protein was saved in the PDBQT format. The ligand structure and its primary conformation were created by the ChemAxon Marvin Sketch 5.3.735 program [26] and saved in the mol2 format. The MOPAC2016 program [27] was applied to optimize and minimize the ligand structure. Molecular docking was performed by the AutoDock Vina 1.1.2 program [28]. The ligand docking center was the

binding site of the FabG inhibitor (PDB:6T65). A grid box of $30 \times 30 \times 30$ points with a grid step of 1.0 Å was employed. A graphic illustration of the docking results was constructed using the software Accelrys DS 4.0 [29].

Results

^{1}H NMR spectra in D_2O of $PyrC_{12}$-BF_4, SBECD, and SBECD/$PyrC_{12}$-BF_4 complex are presented in Fig. 2. The spectrum of SBECD (Fig. 2a) contains signals of sulfobutyl groups at 3.87 ppm (OCH_2), which are partially overlapped with signals of SBECD protons. The signals of $CH_2SO_3^-$ and $OCH_2CH_2CH_2CH_2SO_3^-$ groups are observed as broad peaks at 2.94 ppm (m, aprox. 11H) and 1.77 ppm (m, aprox. 22H), respectively. The signals of H_1 protons of CD are observed at 5.25–4.98 ppm (m, 7H), whereas the complex overlapping multiplets at 4.0–3.5 ppm are assigned to H_2-H_6 proton signals of CD. ^{1}H NMR spectrum of $PyrC_{12}$-BF_4 (Fig. 2b) contains signals of pyridinium ring at 8.76 ppm (m, 2H, C_2-H, C_6-H), 8.55 ppm (td, J = 7.8, 1.3 Hz, 1H, C_4-H) and 8.04 ppm (t, J = 7.0 Hz, 2H, C_3-H, C_5-H). The signals of protons of dodecyl radical are located at 0.79 ppm (t, J = 6.9 Hz, 3H, CH_3), 1.13–1.40 ppm (m, 18H, $CH_3(CH_2)_9$), 1.97 ppm (m, 2H, NCH_2CH_2) and 4.57 ppm (t, J = 7.5 Hz, 2H, NCH_2). In the ^{1}H NMR spectrum of the SBECD/$PyrC_{12}$-BF_4 inclusion complex (Fig. 2c), the signals of pyridinium ring shifted downfield to 8.90 ppm (C_2-H, C_6-H) and to 8.10 ppm (C_3-H, C_5-H). Moreover, the signals of protons of dodecyl radical shifted to a lower field value of 0.87 ppm (CH_3), 1.21–1.44 ($CH_3(CH_2)_9$), 2.05 ppm (NCH_2CH_2) and 4.64 ppm (NCH_2).

Fig. 3 shows the UV-spectra of $PyrC_{12}$-BF_4 and SBECD/$PyrC_{12}$-BF_4 inclusion complex in aqueous solution. The spectrum of pyridinium salt contains two characteristic absorption peaks at 214 and 259 nm. In the spectrum of SBECD/$PyrC_{12}$-BF_4 complex, the intensity of both pyridinium peaks, as well as their position remained unchanged.

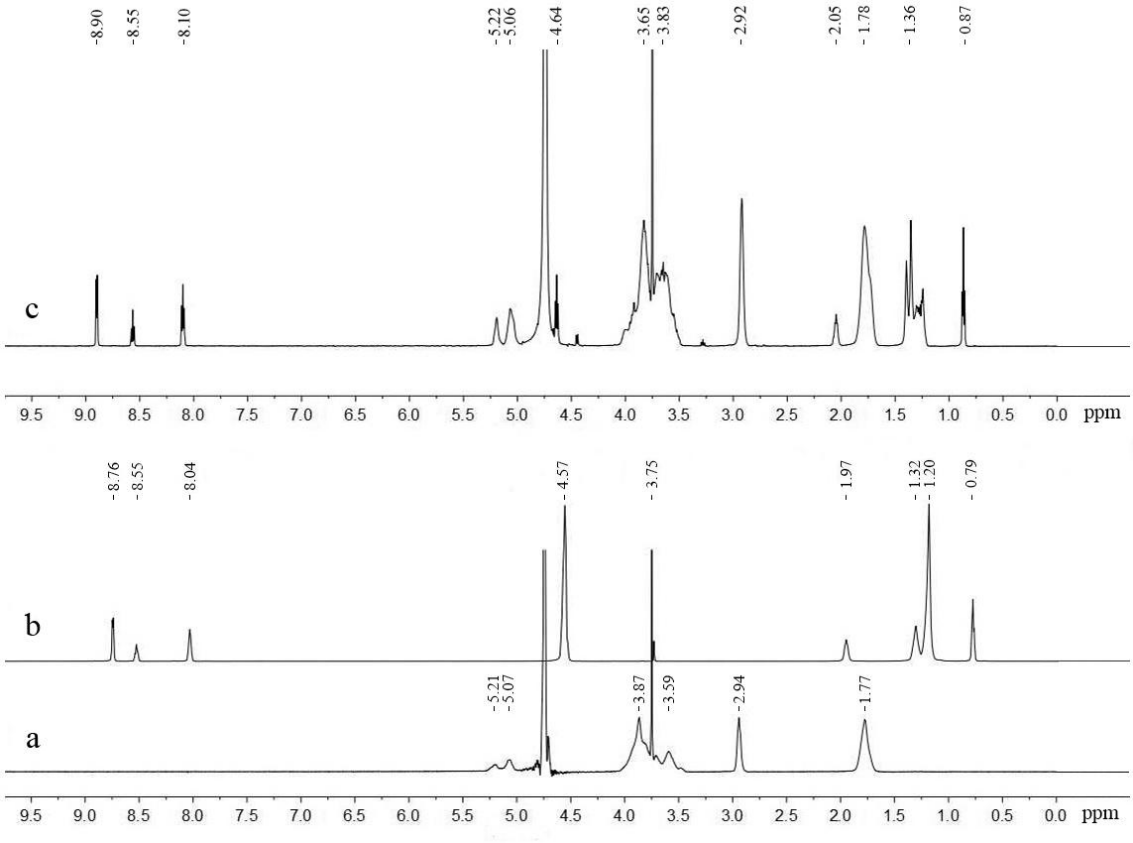


Figure 2: ^{1}H NMR spectra: (a) SBECD; (b) $PyrC_{12}BF_4$; (c) SBECD/$PyrC_{12}$-BF_4

Table 1: Anti-*A. baumannii* activity of synthesized and reference compounds

Compounds	Diameters of the inhibition zones of *A. baumannii* growth (mm)			
	№1536	№871	№1355	№725
$PyrC_{12}$-BF_4	19.7 ± 0.3	20.7 ± 0.3	25.0 ± 0.6	35.0 ± 0.6
SBECD/$PyrC_{12}$-BF_4	NA	NA	16.7 ± 0.3	26.6 ± 0.6
Ampicillin	NA	NA	NA	NA
Carbenicillin	NA	NA	NA	NA
Cefoxitin	NA	NA	NA	NA
Ceftriaxone	16.3 ± 0.3	NA	NA	NA
Colistin	NA	NA	NA	NA

Note. NA – compound is not active.

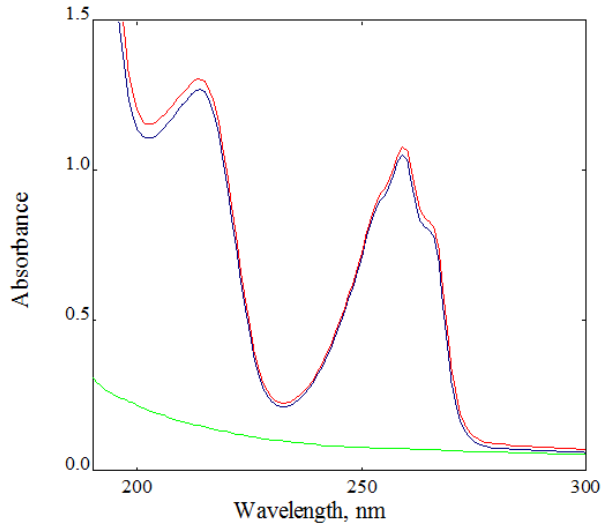


Figure 3: UV-spectra: red – $C_{12}Pyr$-BF_4 (C = 2.5·10^{-4} mol/l), blue – SBECD/$PyrC_{12}$-BF_4 (C = 2.5·10^{-4} mol/l), green – SBECD (C = 2.5·10^{-4} mol/l)

Table 1 contains the *in vitro* results of anti-*A. baumannii* activity in the presence of the studied compounds. $PyrC_{12}$-BF_4 showed high activity against all antibiotic-resistant strains in the range from 19.7 to 35.0 mm. SBECD/$PyrC_{12}$-BF_4 complex led to reduced activity (by an average of 30%) against isolate strains №1355 and №725 compared to neat cationic biocide. Moreover, no activity was detected for other two bacterial strains. It should be noted that the tested common antibiotics were also inactive against *A. baumannii* bacterial strains (Table 1).

The high anti-*A. baumannii* activity of $PyrC_{12}$-BF_4 served as the basis for *in silico* studies of its potential molecular mechanism of antibacterial action. For molecular docking, we used *A. baumannii* 3-oxoacyl-[acyl-carrier-protein] reductase PDB: 6T65. The calculated parameters result indicates the high-quality used FabG *A. baumannii* structure. The stereochemical quality of the enzyme structure is confirmed by the resolution value of the X-ray structure (2.35 Å) and Ramachandran plot analysis (Fig. 4) using the PROCHECK program [30] and the SAVES v6.0 web server. The Ramachandran plot statistics generated by PROCHECK (Fig. 4) demonstrate that 94.0% of amino acid residues are distributed in the most favored regions, 5.7% in additional allowed regions, 0.3% in generously allowed regions, and 0.0% in disallowed regions.

Initially, the quality validation of the molecular docking procedure was conducted by redocking the cocrystallized ligand ethyl 6-bromanyl-2-[(dimethylamino)methyl]-5-oxidanyl-1-phenyl-indole-3-carboxylate into the active site of FabG *A. baumannii* with calculated binding energy of –8.4 kcal/mol and RMSD value 1.33 Å for all atoms. Next, the 1-dodecylpyridinium ligand was docked into the active site of *A. baumannii* FabG (Fig. 5). The docking results show the formation of the ligand-protein complex of 1-dodecylpyridinium ligand and FabG *A. baumannii* with an estimated binding energy of –7.3 kcal/mol. The resulting complex is stabilized by a hydrogen bond (3.42 Å) with amino acid TRP103 and eight hydrophobic interactions (3.92–5.53 Å) with amino acids LEU107, LEU111, LYS112, and PHE161. Fig. 5 displays the orientation of the 1-dodecylpyridinium in the ligand-protein complex. Further analysis of the molecular docking results suggests that the ligand-FabG complex formation occurs at an allosteric site located between subunits C and D (Fig. 6). The complex is stabilized by hydrogen bonds and hydrophobic interactions. The pyridine ring in the complex is stabilized by hydrogen bonding with the amino acid TRP103 and by hydrophobic interactions with amino acids LEU107, LEU111, LYS112, and PHE161 of FabG subunit C. The aliphatic chain of 1-dodecylpyridinium cation forms two hydrophobic interactions with the PHE161 amino acid of the D subunit of the enzyme.

The results of *in vivo* testing of acute toxicity showed that *D. magna* displayed a dosage-dependent response to studied compounds (Table 2). According to commonly used hazard rating of chemical compounds [31], $PyrC_{12}$-BF_4 is classed as a supertoxic com-

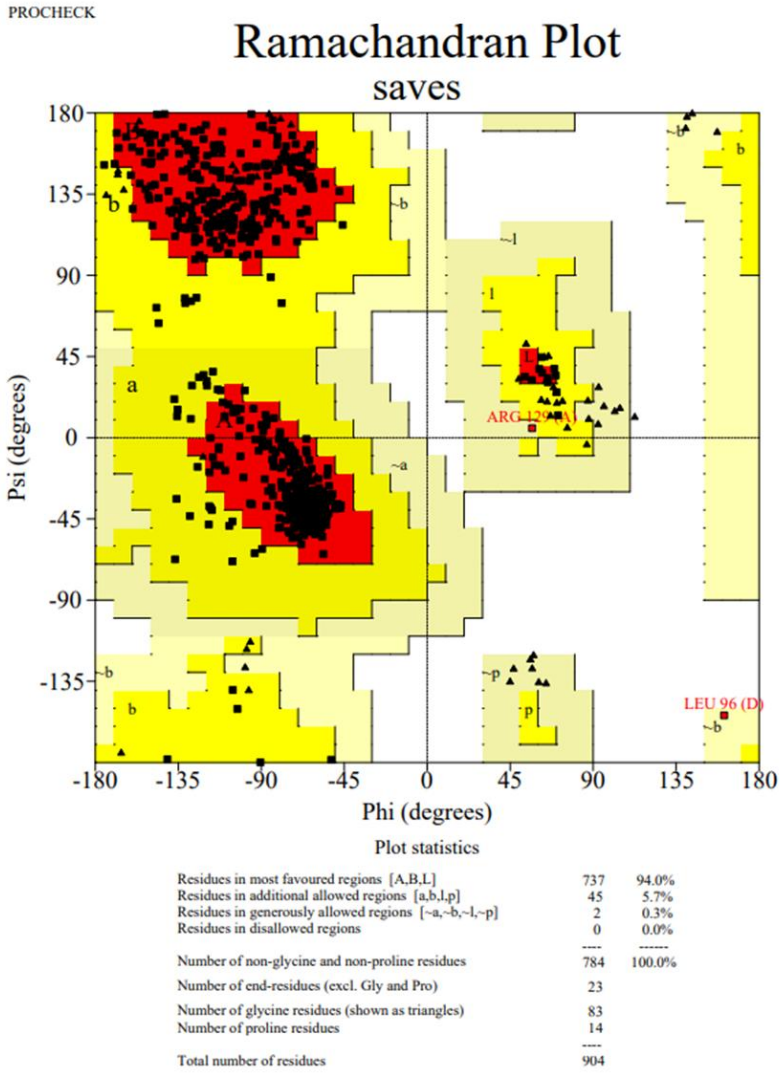


Figure 4: The Ramachandran plot analysis of the stereochemical quality of *A. baumannii* FabG structure; red – most favored regions (A, B, L); yellow – additional allowed regions (a, b, l, p); light yellow – generously allowed regions (~a, ~b, ~l, ~p); white – disallowed regions

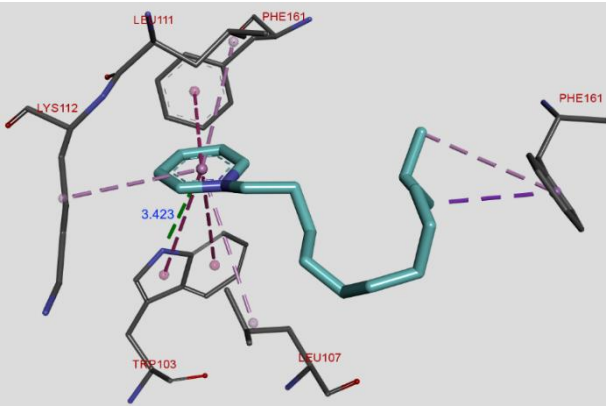


Figure 5: Molecular docking the 1-dodecylpyridinium ligand into active site of *A. baumannii* FabG; ligand is shown in blue

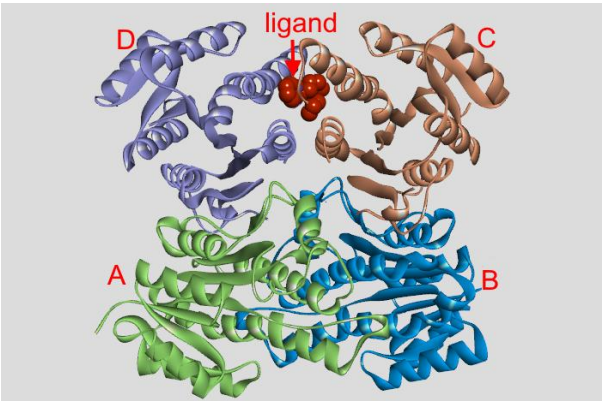


Figure 6: Cartoon of the *A. baumannii* FabG tetramer bound with the 1-dodecylpyridinium ligand located between subunits C and D; ligand shown in red

Table 2: Toxic effect of $PyrC_{12}$-BF_4 and SBECD/$PyrC_{12}$-BF_4 on the mortality (%) of *Daphnia magna*

Compound	0.001 mg/l	0.005 mg/l	0.01 mg/l	0.05 mg/l	0.1 mg/l	0.5 mg/l	1.0 mg/l
$PyrC_{12}$-BF_4	0	42	57	71	85	100	100
SBECD/$PyrC_{12}$-BF_4	0	14	28	57	71	85	100

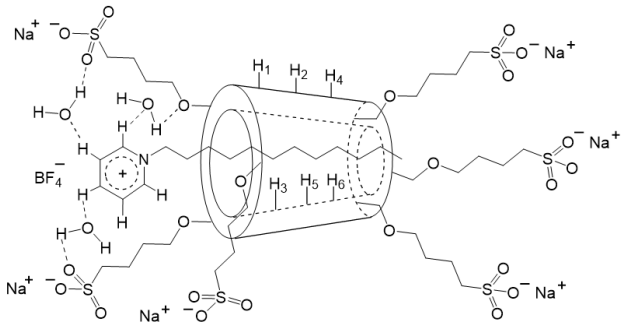
pound (category I) with LC_{50} value of 0.007 mg/l (95% CI 0.005–0.01 mg/l). SBECD/$PyrC_{12}$-BF_4 complex has an almost 5-fold greater LC_{50} value (95% CI 0.022–0.044 mg/l) and thus has a lower toxicity. According to the same rating system, it would be classed as a category II i.e., extremely toxic compound, although it is still around five times less toxic than the original compound.

Discussion

CDs are known for their ability to form water-soluble inclusion complexes with numerous hydrophobic compounds including long-chain QACs which are also called "ionic liquids" [20, 32, 33]. The aliphatic tail of the guest molecule is incorporated into the hydrophobic, cone-like inner cavity of the CD, whereas the ionic part is located outside the torus at the hydrophilic outer surface. ^{1}H NMR spectroscopy is a sensitive method that is capable of confirming the existence of inclusion complex and providing useful information on the inclusion mechanisms of CDs with guest molecules, including long-chain ionic liquids [20, 32–34].

Overall, the results of ^{1}H NMR study revealed physicochemical interactions between $PyrC_{12}$-BF_4 and SBECD. It is well known that the protons of glucopyranose units located inside the CD cavity (H-3 and H-5) undergo appreciable shielding when an inclusion complex is formed, whereas protons located outside the torus (H-1, H-2, and H-4) are relatively unaffected [20, 33]. It should be noted that the complex overlapping multiplets at 4.0–3.5 ppm in the ^{1}H NMR spectrum of SBECD (see Fig. 1a) makes it difficult to assign the H_2-H_6 proton signals of CD. However, in the spectrum of the SBECD-$PyrC_{12}$-BF_4 complex (see Fig. 1c) changes in the positions of some peaks of CD are observed. This may indirectly indicate the presence of hydrophobic interactions between alkyl radical of $PyrC_{12}$-BF_4 and protons of the inner CD cavity. The shift in the signals of protons of the dodecyl radical to a lower field value confirms their shielding when inclusion complex is formed (see Fig. 1c). The obtained data agreed with previously reported ones for the inclusion complex of CD with long-chain imidazolium-based ionic liquid [20]. However, an additional 2D NMR study is required to determine exactly which fragment of the alkyl radical is included into the CD cavity.

As for polar pyridinium cation of $PyrC_{12}$-BF_4, it is likely involved in the formation of hydrate layer located mainly outside the SBECD torus (Scheme 2). The noticeable shift in the signals of protons of pyridinium ring in the ^{1}H NMR spectrum of SBECD/$PyrC_{12}$-BF_4 inclusion complex to a lower field value (see Fig. 1c) is indicative of their physicochemical interaction with polar groups located outside the torus. Thus, hydrogen bonds mediated by water molecules may be formed between aromatic C-H protons and oxygen atoms of sulfobutyl groups (Scheme 2).



Scheme 2: Schematic drawing of SBECD/$PyrC_{12}$-BF_4 inclusion complex

In general, the mechanism of antibacterial action of cationic surfactants involves the electrostatic interaction with outermost surface of bacterial cells, which are often negatively charged and hence, they readily associate with the head groups of acidic phospholipids. The lipophilic hydrocarbon tails of the cationic amphiphilic compound then penetrate into the hydrophobic membrane core followed by deformation of membrane permeability and lethal leakage of cytoplasmic materials [5, 6, 35]. Thus, the biological activity of QACs can be attributed to the combination of several physicochemical parameters such as hydrophobicity, surface activity, adsorption at the bacterial/water interface, aqueous solubility and transport properties [15]. Although it

is generally difficult for bacteria to circumvent the non-specific disruption mechanism of action of cationic biocides, the growing rise in bacterial resistance to QACs has also been reported [1, 3, 36].

The neat pyridinium salt $PyrC_{12}$-BF_4 demonstrated high activity against all tested MDR *A. baumannii* clinical isolates, in contrast to common antibiotics, which were inactive against these bacterial strains (see Table 1). The anti-*A. baumannii* activity of $PyrC_{12}$-BF_4 is noticeably higher compared to previously studied long-chain pyridinium salts 1-dodecylpyridinium chloride and 1-tetradecylpyridinium bromide [17]. Molecular docking of this compound suggested that it can form a complex with FabG, which is an important enzyme in the biosynthesis of bacterial fatty acids and could be a promising target for potential antibacterial drugs [13, 37]. It is worth noting that the following experimental validation is needed to confirm the results of the docking study. Much like in the case of antimicrobial activity, acute toxicity of cationic surfactants is mainly determined by the length of alkyl radical. Thus, the determined acute toxicity of $PyrC_{12}$-BF_4 on *D. magna* (LC_{50} = 0.007 mg/l) is lower compared to commercial antiseptic cetylpyridinium chloride (CPC) (LC_{50} = 0.0041 mg/l) [36]. However, both compounds belong to the class of supertoxic compounds [31].

When the hydrophobic aliphatic tail of an cationic surfactant is included into the inner cavity of CD, a loss of surface activity, as well as significant changes of other physicochemical parameters of the biocide is observed [20, 21]. This also leads to the reduction in biological activity due to the reduction of free biocide in solution [20, 38]. The dynamic equilibrium between the host SBECD and the cationic biocide in aqueous solution is shifted towards the inclusion complex, which is likely stabilized by the presence of polar sulfobutyl groups in the outer hydrophilic surface of SBECD. The acute toxicity of the SBECD/$PyrC_{12}$-BF_4 inclusion complex is 5 times lower compared to that of $PyrC_{12}$-BF_4, in line with the results of similar study, in which the toxicity of another cationic biocide – 1-dodecyl-3-methylimidazolium tetrafluoroborate was reportedly lowered by 3 times after complexation with CD [20]. In the present study, a similar trend was observed for the anti-*A. baumannii* activity of $PyrC_{12}$-BF_4, which was also reduced after complexation with SBECD. Thus, it is reasonable to suggest that the SBECD could play a role as a carrier for cationic biocides which are gradually released over time.

Conclusions

The hydrophobic cationic biocide 1-dodecylpyridinium tetrafluoroborate ($PyrC_{12}$-BF_4) and its inclusion complex with sulfobutyl ether β-cyclodextrin (SBECD) have been synthesized and characterized in terms of their *in vitro* antibacterial activity against gram-negative clinical isolates of *A. baumannii*, as well as *in vivo* acute toxicity in *D. magna* hydrobionts. Moreover, *in silico* molecular docking of $PyrC_{12}$-BF_4 was performed using the crystal structure of the *A. baumannii* 3-oxoacyl-[acyl-carrier-protein] reductase (FabG).

The results of ^{1}H NMR study revealed physicochemical interactions between the C-H protons of pyridinium cations and the polar SBECD groups, as well as between the hydrophobic alkyl chain of $PyrC_{12}$-BF_4 and the inner cavity of SBECD. The cationic biocide showed high activity against four tested antibiotic-resistant strains of *A. baumannii*. The probable mechanism of the antibacterial action of the $PyrC_{12}$-BF_4 is the inhibition of *A. baumannii* FabG, which results in a stable complex with a calculated binding energy of –7.3 kcal/mol. The ligand-protein complex forms at an allosteric site located in the subunit C and D interface and is stabilized by hydrogen bonding and hydrophobic interactions involving amino acids TRP103, LEU107, LEU111, LYS112, and PHE161 of subunits C and D. Thus, the 1-dodecylpyridinium cation distorts the oligomerization interface in the FabG tetramer which participates in fatty acid biosynthesis. The complexation of $PyrC_{12}$-BF_4 with SBECD reduced its antibacterial activity, as well as its acute toxicity. Furthermore, the SBECD/$PyrC_{12}$-BF_4 inclusion complex was found to be active against only two tested *A. baumannii* strains. The acute toxicity (LC_{50}) of the cationic biocide was reduced by almost 5 times after complexation with SBECD.

Overall, the results of this study revealed the high efficacy of the long-chain pyridinium salt $PyrC_{12}$-BF_4 against *A. baumannii* MDR isolates №1536, №871, №1355, and №725 obtained from the collection of the Microbial Cultures Museum of the Shupyk National Healthcare University of Ukraine. The acute toxicity of this hydrophobic cationic biocide can be reduced by the formation of inclusion complex with SBECD. However, this approach has certain limitations since the toxicity of SBECD/$PyrC_{12}$-BF_4 complex still remains relatively high.

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Interests disclosure

The authors declare no conflict of interest.

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С. Рогальський[1], Д. Година[1], І. Семенюта[1], М. Фрасинюк[1], О. Тарасюк[1], С. Рябов[2], Л. Кобріна[2], І. Тетко[3,4], Л. Метелиця[1]

[1]Інститут біоорганічної хімії та нафтохімії ім. В.П. Кухаря НАН України, Київ, Україна
[2]Інститут хімії високомолекулярних сполук НАН України, Київ, Україна
[3]Мюнхенський центр Гельмгольца – Німецький дослідницький центр охорони навколишнього середовища та здоров'я, Нойхерберг, Німеччина
[4]BIGCHEM GmbH, Унтершляйсхайм, Німеччина

АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ 1-ДОДЕЦИЛПІРИДИНІЙ ТЕТРАФТОРБОРАТУ ТА ЙОГО КОМПЛЕКСУ ВКЛЮЧЕННЯ ІЗ СУЛЬФОБУТИЛОВИМ ЕТЕРОМ β-ЦИКЛОДЕКСТРИНУ ПРОТИ МУЛЬТИРЕЗИСТЕНТОГО ШТАМУ *ACINETOBACTER BAUMANNII*

Проблематика. Бактеріальний патоген *Acinetobacter baumannii* є одним із найнебезпечніших антибіотикорезистентних мікроорганізмів, який спричиняє численні інфекційні захворювання. Сьогодні існує гостра необхідність у розробці нових антибактеріальних агентів зі специфічним анти-*A. baumannii* механізмом дії. Довголанцюгові солі 1-алкілпіридинію належать до ефективних катіонних біоцидів, які є потенційними інгібіторами ензимів, залучених у біосинтез бактеріальних жирних кислот. Для зменшення токсичності цих сполук можуть бути використані їх синтезовані комплекси включення з циклічним олігосахаридом – β-циклодекстрином.

Мета. Метою цієї роботи було отримання нових антибактеріальних агентів, ефективних проти *A. baumannii*, на основі гідрофобної солі 1-додецилпіридинію та її комплексу включення із сульфобутиловим етером β-циклодекстрину (SBECD).

Методика реалізації. Синтезовано гідрофобний катіонний біоцид 1-додецилпіридиній тетрафторборат ($PyrC_{12}$-BF_4) та його комплекс включення з SBECD. Будову комплексу SBECD/$PyrC_{12}$-BF_4 досліджено методами протонного магнітного резонансу та спектрофотометрії. Антибактеріальну активність синтезованих сполук вивчено *in vitro* стандартним диско-дифузійним методом проти антибіотикорезистентних клінічних штамів-ізолятів *A. baumannii*. Гостру токсичність сполук визначали на прісноводному модельному гідробіонті *Daphnia magna*. Молекулярний докінг проводили за використання кристалічної структури ферменту *A. baumannii* 3-оксоацил-[ацил-носій-білок] редуктази (FabG).

Результати. Результати спектральних досліджень свідчать про утворення комплексу включення між SBECD і $PyrC_{12}$-BF_4. Катіонний біоцид проявляє активність проти чотирьох досліджених антибіотикорезистентних штамів *A. baumannii*, у той час як комплекс включення SBECD/$PyrC_{12}$-BF_4 продемонстрував активність проти двох використаних штамів *A. baumannii*. Молекулярний докінг засвідчив утворення ліганд-білкового комплексу 1-додецилпіридинієвого ліганду з активним центром FabG *A. baumannii* з очікуваною енергією зв'язку −7.3 ккал/моль. Гостра токсичність (LC_{50}) біоциду $PyrC_{12}$-BF_4 і його комплексу з SBECD становить відповідно 0,007 і 0,033 мг/л.

Висновки. Гідрофобний катіонний біоцид $PyrC_{12}$-BF_4 має високу антибактеріальну активність проти антибіотикорезистентних клінічних штамів *A. baumannii*. Одним із потенційних механізмів антибактеріальної активності сполуки проти *A. baumannii* є інгібування активного центру FabG. Комплекс включення SBECD/$PyrC_{12}$-BF_4 має в 5 разів меншу токсичність порівняно з чистим катіонним біоцидом і проявляє при цьому активність проти частини досліджених тест-культур.

Ключові слова: катіонний біоцид; антибактеріальна активність; гостра токсичність; молекулярний докінг; β-циклодекстрин; сульфобутиловий етер β-циклодекстрину; комплекс включення.