Development of novel effective agents from 1H-indolylammonium trifluoroacetates effective against conditionally pathogenic microorganisms

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Abstract

Introduction: The problem of antibiotic resistance of microorganisms is becoming more urgent in the twenty-first century. More and more pathogenic microbes are becoming resistant to two or more antibiotics. This problem has become worse into the COVID-19 pandemic. The search for new compounds with antimicrobial activity is one of the principles for overcoming the antibiotic resistance of microorganisms.

Materials and methods: Methods for the preparation, isolation, and identification of salts of 2,3,5-trimethyl-, 1,2,3,5-tetramethyl-, 2,3-dimethyl-5-methoxy-, 5-methoxy-1,2,3-trimethyl-1H-indole-6-amines and trifluoroacetic acid were developed and laboratory microbiological studies of them for antimicrobial activity were carried out. Sensitivity of the test-strains of microorganisms to the new compounds was studied. A method of serial dilutions to determine the minimal inhibitory concentration (MIC) of the compounds under study was used in the study.

Results and discussion: The compounds 5–8 showed a pronounced antibacterial activity against the test strains of microorganisms in vitro with MIC from 0.98 µg/mL to 125.0 µg/mL. The prospects for targeted synthesis of biologically active compounds which are derivatives of 1H-indolylamines with a trifluoromethyl group in the molecule were determined, and after additional studies, the compounds 5–8 may find application as water-soluble synthetic antimicrobial agents.

Conclusion: The laboratory microbiological screening of showed that they have an antimicrobial effect that exceeds the activity of the reference drug, dioxdine. The presence of molecular mechanisms predicted in silico in the spectrum of biological activity of the studied compounds, such as Pseudolysin inhibitor, OmpF inhibitor, Undecaprenyldiphosphomuramylpentapeptide beta-N-acetylglicosaminyltransferase inhibitor, UDP-epimerase inhibitor, Bacterial efflux pump inhibitor, suggests the presence of antimicrobial activity against gram-positive and gram-negative microorganisms. Trifluoroacetates 2,3,5-trimethyl-1H-indole-6-ammonium (5), 1,2,3,5-tetramethyl-1H-indole-6-ammonium (6), 2,3-dimethyl-5-methoxy-1H-indole-6-ammonium (7), 1,2,3-trimethyl-5-methoxy-1H-indole-6-ammonium (8), after additional studies, may find application as water-soluble synthetic antimicrobial agents.

Keywords

new chemical compounds, 1H-indolylamines, conditionally-pathogenic microorganisms, antimicrobial activity.
Introduction

Throughout the history of the existence of pathogenic microorganisms, the struggle against many of their representatives, both those already known and those recently identified, have been going on. The discovery of antimicrobial agents has led to the successful treatment and elimination of certain bacterial infections, but revealed the strains that are resistant to antimicrobials due to the numerous mechanisms of their antibiotic resistance (Kumarasamy et al. 2010; Parhizgari et al. 2017; Yokoyama et al. 2018).

The problem of antibiotic resistance is becoming more acute in the 21st century; a study of the mechanisms for acquiring resistance to antimicrobial agents underlies the development of new ways to combat this phenomenon (McKeegan et al. 2002; Savjani et al. 2009). Drug resistance is a growing global threat to public health that affects all major pathogens and antimicrobials (Brown and Wright 2016; Yadav et al. 2017; Obayiuwana et al. 2018; World Health Organization 2018). In the course of microbiological monitoring over recent years, the share of multiresistant strains has tended to grow, for example, methicillin-resistant S. aureus strains have a significantly higher frequency of resistance to gentamicin, clindamycin, rifampicin, tetracycline, chloramphenicol, ceftriaxone, ciprofloxacin, and erythromycin, when compared to methicillin-sensitive strains. Paeruginosa is insensitive to antipseudomonal cephalosporins – cefepime and ceftazidime, as well as piperacillin-tazobactam, imipenem, meropenem. Representatives of the Enterobacteriaceae family are resistant to three and more traditionally used antibiotics, such as cefotaxime, ceftazidime, cefepime, aztreonam, etc. (Pop-Vicas et al. 2008; Lai et al. 2014; Natan and Banin 2017; Tacconelli et al. 2018).

The uncontrolled use of antimicrobial drugs in the treatment of COVID-19-associated pneumonia has led to an unprecedented proliferation of antibiotic-resistant nosocomial strains of microorganisms.

The search for and development of new antimicrobial agents is one of the fundamental principles of overcoming the resistance of microorganisms to antibiotics.

Substituted 1H-indolylamines with a amino group in the benzene ring are known as intact compounds for the production of trifluoromethyl-substituted indolylamides. Many of the products obtained show various kinds of biological activity. So, in amides, based on substituted 1H-indol-4-ylamines and trifluoroacetocacetate, based on 1H-indole-7-amines and ethyl trifluoroacetic acid, a rather high antimicrobial activity was found (Stepanenko et al. 2018; Stepanenko et al. 2019).

In this regard, it was of interest to obtain watersoluble derivatives of 1H-indolylamines with a trifluoromethyl group in the molecule. Such compounds could be salts formed by substituted 1H-indolylamines and trifluoroacetic acid. Our out-of-experimental computer bioscreening of the structures that are salts of 2,3,5-trimethyl-, 1,2,3,5-tetramethyl-, 2,3-dimethyl-5-methoxy-, 5-methoxy-1,2,3-trimethyl-1H-indole-6-amines and trifluoroacetic acid predicts antimicrobial activity for them.

Therefore, we have developed methods for the preparation, isolation, and identification of these salts and conducted laboratory microbiological studies on their antimicrobial effects. Depending on the results of the study, the direction of development – antimicrobial chemotherapy, antiseptic or disinfectology – will be determined.

Materials and methods

Chemistry

We found that equimolecular amounts of aminoindoles 1–4 and trifluoroacetic acid in a heated benzene solution react with the formation of indolylammonium trifluoroacetates 5–8, which precipitate upon cooling (Scheme 1).

The isolated compounds are light gray, light purple crystalline substances, soluble in water. The physicochemical, spectral characteristics of the obtained new compounds are shown in Tables 1, 2.

Biological activity

In silico prediction of the spectrum of biological activity of new compounds

Computer system PASS (Prediction of Activity Spectra for Substances), version 9.1, registered in 2007, was used for predicting the biological activity of substances (Varnek 2008; Filimonov et al. 2014; Filimonov et al. 2018).

Scheme 1. Scheme for the synthesis of 1H-indol-6-ylammonium trifluoroacetates 5–8 from 1H-indol-6-ylamines 1–4 and trifluoroacetic acid.
Table 1. Physicochemical characteristics of compounds 5–8

| Compound | ** Found, % | Gross formula | Calculated, % | **Rf | T_{max} (with decomposition), °C | Yield, % |
|----------|-------------|---------------|---------------|------|-------------------------------|---------|
| Trifluoroacetate 2,3,5-trimethyl-1H-indole-6-ammonium (5) | 53.99 | C_{18}H_{18}N_{3}F_{3}O_{3} | 54.16 | 5.24 | 0.20 | >190 | 84 |
| Trifluoroacetate 1,2,3,5-tetramethyl-1H-indole-6-ammonium (6) | 55.50 | C_{18}H_{18}N_{3}F_{3}O_{3} | 55.63 | 5.67 | 0.38 | >173 | 80 |
| Trifluoroacetate 2,3-dimethyl-5-methoxy-1H-indole-6-ammonium (7) | 50.09 | C_{18}H_{18}N_{3}F_{3}O_{3} | 51.32 | 4.97 | 0.24 | >161 | 54 |
| Trifluoroacetate 5-methoxy-1,2,3-trimethyl-1H-indole-6-ammonium (8) | 52.69 | C_{18}H_{18}N_{3}F_{3}O_{3} | 52.83 | 5.38 | 0.46 | >160 | 71 |

Note: * – The amines and salts are named according to the rules of a computer program ACD/LABS IUPAC Name Generator; ** – Elemental analysis was performed on an elemental analyzer vario MICRO cube; *** – The purity of the obtained compounds was monitored, Rf was determined using TLC on Silufol UV-254 plates in the system: benzene-ethylacetate-methanol 1:1:0.1.

Table 2. Spectral characteristics of compounds 5–8

| Compound | **Spectrum NMR (DMSO-d_6), ppm. | ** UV Spectrum | ** Mass spectrum: m/z (% to T_{max}) |
|----------|---------------------------------|---------------|----------------------------------|
| 5 | 2.13 (3H, c, 3-CH_3), 2.29 (3H, c, 2-CH_2), 2.36 (3H, c, 5-CH_3), 7.26 (1H, c, H-4), 7.30 (1H, c, H-7), 9.69 (3H, c_{sh}, 6-NH_2), 10.81 (H, c, H-1) | -73.58 | 207(4.26), 233(4.52), 295(3.76) | 174(100), 173(86), 159(30), 69(53), 45(73) |
| 6 | 2.16 (3H, c, 3-CH_3), 2.31 (3H, c, 2-CH_2), 2.38 (3H, c, 5-CH_3), 3.59 (3H, c, 1-CH), 3.71 (1H, c, H-4), 3.73 (1H, c, H-7), 9.69 (3H, c_{sh}, 6-NH_2), 10.75 (H, c H-1) | -73.66 | 210(4.28), 235(4.53), 300(3.83) | 188(100), 187(71), 173(39), 69(43), 45(78), 28(61), 17(12) |
| 7 | 2.15 (3H, c, 3-CH_3), 2.29 (3H, c, 2-CH_2), 3.90 (3H, c, 5-OCH_3), 7.08 (1H, c, H-4), 7.26 (1H, c, H-7), 9.56 (3H, c_{sh}, 6-NH_2), 10.75 (H, c, H-1) | -73.56 | 207(4.42), 230(4.55), 303(4.03) | 191(18), 190(100), 176(12), 175(96), 147(69), 145(14), 69(21), 45(24), 28(11) |
| 8 | 2.18 (3H, c, 3-CH_3), 2.31 (3H, c, 2-CH_2), 3.59 (3H, c, 1-CH), 3.91 (3H, c, 5-OCH_3), 7.13 (1H, c, H-4), 7.30 (1H, c, H-7), 9.44 (3H, c_{sh}, 6-NH_2), 10.75 (H, c, H-1) | -73.63 | 213(4.63), 230(4.60), 300(3.06) | 205(15), 204(100), 190(11), 189(74), 161(47), 160(10), 69(12), 45(12), 28(8) |

Note: * – NMR spectra were recorded on a Varian Unity Inova 400 Multi-port spectrometer equipped with a 5-mm TXI probe working at 400.13 MHz for 1H resonances; ** – UV spectra were recorded on a PerkinElmer Lambda 1050 spectrophotometer using a 1 cm quartz cuvette; *** – Mass spectra were recorded on a Finnigan MAT INCOS-50 mass spectrometer with direct input of samples into an ion source at an ionization energy of 70 eV.

Antimicrobial activity of new compounds (in vitro)

During the microbiological study, the obtained compounds were used in the form of a solution (sterile water for injection was used as a solvent). The test compounds were the following: trifluoroacetate 2,3,5-trimethyl-1H-indole-6-ammonium (5), trifluoroacetate 1,2,3,5-tetramethyl-1H-indole-6-ammonium (6), trifluoroacetate 2,3-dimethyl-5-methoxy-1H-indole-6-ammonium (7), trifluoroacetate 5-methoxy-1,2,3-trimethyl-1H-indole-6-ammonium (8).

The following museum strains were used as the test microorganisms for studying the antimicrobial activity of the obtained compounds: Staphylococcus aureus 6538-P ATCC, Staphylococcus aureus 43300 ATCC (MRSA), Escherichia coli 25922 ATCC, Pseudomonas aeruginosa 27853 ATCC, and Streptococcus pyogenes 19615 ATCC. The museum strains used in this work were obtained from the collection of the Museum of Living Cultures of Federal State Budgetary Institution “Scientific Centre for Expert Evaluation of Medicinal Products (SCCEMP)” of the Ministry of Health of the Russian Federation and Becton Dickinson France S.A.S. The studied strains of microorganisms are the most frequent causative agents of infectious nonspecific human diseases, as well as the most common representatives of the nosocomial microbiota associated with diseases resulting from medical treatment. This is the reason for the choice of the test and experimental strains. The antimicrobial activity of the obtained compounds was determined by the broth serial dilution method (macrotube method) (MUK 2004; ISO 2006; EUCAST 2019; EUCAST 2021).

The antimicrobial preparation dioxidine (a derivative of di-N-hydroxyquinoline) (Biosisint PJSC, Russia, a solution for topical application, endotracheal and intravenous administrations, 10 mg/mL), widely used in medical practice, was used as a comparison drug. This drug has a high in vivo chemotherapeutic activity on model infections similar in pathogenesis to human pathological processes (purulent meningitis, pylonephritis, septicemia) and caused by strains of anaerobic bacteria that are resistant (including multiresistant) to drugs of other classes, including Pseudomonas aeruginosa strains and methicillin-resistant staphylococci. Dioxidine is characterized by a wide antibacterial spectrum with a bactericidal effect, and is also active against gram-positive and gram-negative aerobic conditionally pathogenic bacteria. The activity of dioxidine against Mycobacterium tuberculosis is shown (Padeiskaya 2011; Pipopov et al. 2013).

To assess the sensitivity of microorganisms, Mueller-Hinton broth (MHB) (HiMedia Laboratories Pvt. Limited, India) was used. The concentration of the suspension of the studied microorganism was 1.5×10^8 CFU/mL. The optical density of the bacterial suspension with a concentration...
of 1.5×10⁶ CFU/mL upon visual inspection corresponded to the McFarland turbidity standard of 0.5. A commercial turbidity standard (Sensitre, UK) was used in the work. A bacterial suspension was prepared from agar cultures. A pure culture of microorganisms grown on solid nutrient media was used to prepare the inoculum. Several same-type clearly isolated colonies were selected, which had been grown on non-selective solid nutrient media. Using the loop, a small amount of material was transferred from the tops of the colonies into a test tube with sterile saline, adjusting the inoculum density to exactly 0.5 according to the McFarland standard. Inoculum was used within 15 minutes after preparation.

The broth serial dilution method (macrotube method). Testing was carried out in a volume of 1 mL of each dilution of the test compound with a final concentration of the studied microorganism of approximately 5×10⁶ CFU/mL. MHB was poured into 0.5 mL in each tube to determine sensitivity. The number of tubes was ten, plus one for a “negative” control, that is, eleven tubes in total. A working solution of the test compound was prepared from the main solution using a liquid nutrient medium – MHB. Then the working solution in an amount of 0.5 mL, using a micropipette with a sterile tip, was introduced into the first tube containing 0.5 mL of broth. The mixture was thoroughly mixed, and, by means of a new sterile tip, 0.5 mL of the broth solution of the test compound was transferred into a second tube containing initially 0.5 mL of broth. This procedure was repeated until all the necessary dilutions were prepared. From the last tube, 0.5 mL of broth was removed. Thus, a number of test tubes were obtained with solutions of the test compound, the concentrations of which are 2 times different in the neighboring tubes. For inoculation, a standard microbial suspension was used, equal to 0.5 according to the McFarland standard, diluted 100 times in MHB, after which the concentration of the microorganism in it was approximately 10⁷ CFU/mL. Inoculum of 0.5 mL was added to each tube, containing 0.5 mL of the appropriate dilution of the test compound, and to one tube with 0.5 mL of MHB without antibiotic (negative control). The final concentration of the microorganism in each tube was approximately 5×10⁵ CFU/mL. The inoculum was introduced into the test tubes with dilutions of the test compound no later than 15–30 minutes after they had been prepared. The tubes were stopped with sterile cotton-gauze plugs, and all the control tubes, except the negative control tube, were incubated in a normal atmosphere at a temperature of 37 °C for 16–20 or 20–24 h (depending on a type of the microorganism being tested). The negative control tube was placed in a refrigerator at 4 °C, where it was stored until the results were assessed. To determine whether there was a microorganism growth, the test tubes with inoculations were examined in transmitted light. The culture growth in the presence of the test compound was compared with the reference tube (negative control) containing the original inoculum and stored in the refrigerator. The minimum inhibitory concentration (MIC) was determined by the lowest concentration of the test compound, which inhibits the visible growth of the microorganism. The experiment was carried out in four sequences.

### Results and discussion

The structure of the new compounds obtained for microbiological studies is unambiguously confirmed by an analysis of their spectral characteristics. The formation of indolylammonium salts 5–8 was confirmed by the obtained UV, ¹H NMR, ¹⁹F NMR spectra and mass spectra (Table 2).

So, the UV spectra of the obtained compounds 5–8 are characterized by three absorption bands (207.5; 233, 295 nm for salt 5, 210.5, 235, 300 nm for salt 6, 207.5, 230, 303 nm for salt 7, and 213.5, 230, 300 nm for salt 8) in contrast to the spectra of the starting aminoidoles 1–4, where there are four absorption bands. In the spectra of the compounds obtained by us, the two long-wavelength bands in the spectra of the starting amines are combined and appear as one broad long-wavelength absorption. Since absorption in the long-wave region is responsible for π-π transitions in the benzene part of the molecule, a change in their nature indicates that a change has occurred in the nature of the substitution of this ring, i.e. an amino group has converted to an ammonium group.

The ¹H NMR spectrum pattern also unambiguously confirms the formation of salts of structure 5–8. The difference between the spectra of the obtained trifluoroacetates and the spectra of the starting amines is the absence of a proton signal with an integrated intensity of two 6-NH₂ protons in the region of 4–5 ppm and the presence of a downfield much broader peak of exchange hydrogens with an integrated intensity of three protons of the ammonium NH group (9.44–9.69 ppm). In the aliphatic part of the spectra of trifluoroacetates, there are also single singlets of hydrogens of methyl groups, and in the aromatic part of the spectrum, there are signals of two protons of the benzene ring and a 1-H pyrrole fragment (for compounds 5,7). It should be noted that the values of chemical shifts of the signals of unambiguous protons towards the weak fields in the spectra of trifluoroacetates are compared with amines. Most of all, under this influence are the hydrogens of the benzol fragment, which are the closest to the positively charged ammonium group.

The presence of equivalent fluorine atoms in salt 5–8 molecules is evidenced by a single signal within the range -73.5; 73.5 (5), -73.6 (6), -73.56 (7), -73.63 (8) ppm in the HMR ¹⁹F spectra.

Under mass-spectral conditions (high temperature), trifluoroacetates 5–8 decompose to form the corresponding amine and trifluoroacetic acid. Therefore, in the spectra, there are signals of molecular ions of ¹H-indolylamines and signals of fragment ions obtained upon their splitting under conditions of electron ionization, as well as peaks of fragment particles with m/z 69, 45, 28, 17, which are formed during the decomposition of trifluoroacetic acid.
The direction of decay of molecular ions $F_7F_6$ depends on the nature of the substituent in the aromatic ring. Thus, $F_7$, $F_6$ ions (R=H, CH$_2$; R'=CH$_3$) lose a hydrogen atom or methyl radical and rearrange themselves into positively charged particles $F_7$, $F_6$, $F_5$, $F_4$, which, according to published data, have quinoline structures (Terent'ev 1979). In the case of molecular ions $F_7$, $F_6$ of aminooindoles 3, 4, the direction of decay is determined by the methoxy group. In this case, a CH$_2$ radical is cleaved from molecular ions $F_7$, $F_6$ with the formation of fragment ions $F_7$, $F_6$ which, later, with the loss of the carbon monoxide molecule, produce ions $F_5$, $F_4$ having the structure of pyrrolopyridine. This decay is characteristic of ortho-anisidines (Khmel'nitskii 1974).

An out-of-experimental prediction of the antimicrobial activity of the synthesized substituted 1H-indol-6-ylammonium trifluoroacetates 5–8 was carried out. PASS predicts that a certain compound can manifest the biological activity, but makes impossible any conclusions regarding the magnitude of the activity and the conditions of the experimental testing (dose, route of administration, biological object, gender, age, etc.), under which this activity can occur. Thus, PASS makes it possible to narrow the scope of the experimental testing in relation to specific compounds; however, any prediction must be confirmed by an experiment. According to the PASS prediction, the new derivatives have the following molecular mechanisms: Pseudolysin inhibitor is predicted with a probability of $Pa=0.467$ for compound 5, $Pa=0.433 –$ for compound 6, $Pa=0.348 –$ for compound 7, $Pa=0.428 –$ for compound 8; Omptin inhibitor with a probability of $Pa=0.314$ for compound 5, $Pa=0.394 –$ for compound 6, $Pa=0.364 –$ for compound 7, $Pa=0.381 –$ for compound 8; UDP-N-acetylglucosamine 2-epimerase inhibitor with a probability of $Pa=0.407$ for compound 5; Bacterial efflux pump inhibitor with a probability of $Pa = 0.320$ for compound 6, $Pa=0.410 –$ for compound 7 (Tables 3–6). By interacting with the molecular targets, an antimicrobial effect can be achieved.

### Table 3. The Predicted Spectrum of Biological Activity of Compound 5 (PASS) ($Pa\geq 0.3$)

| $Pa$ | $Pi$ | Activity                          |
|------|------|----------------------------------|
| 0.533 | 0.008 | Multiple sclerosis treatment      |
| 0.479 | 0.032 | Autoimmune disorders treatment   |
| 0.456 | 0.038 | HMGS2 expression enhancer         |
| 0.438 | 0.052 | Platelet derived growth factor receptor kinase inhibitor |
| 0.467 | 0.168 | Pseudolysin inhibitor             |
| 0.313 | 0.015 | Antineoplastic (sarcoma)          |
| 0.388 | 0.110 | Chloride peroxidase inhibitor     |
| 0.407 | 0.151 | UDP-N-acetylglucosamine 2-epimerase |
| 0.356 | 0.100 | Anitriarthritis                   |
| 0.318 | 0.085 | Plastoquinol-plastocyanin reductase inhibitor |
| 0.321 | 0.155 | Phosphatidylcholine-retinol O-acyltransferase inhibitor |
| 0.320 | 0.157 | Leukopoeis stimulant              |
| 0.363 | 0.217 | Aspulvinone dimethylallyltransferase inhibitor |
| 0.311 | 0.167 | Erythropoesis stimulant           |
| 0.314 | 0.192 | Omptin inhibitor                  |

The antimicrobial activity of the new compounds was studied with respect to gram-positive Staphylococcus aureus 6538-P ATCC, Staphylococcus aureus 43300 ATCC (MRSA), Streplococcus pyogenes 19615 ATCC and gram-negative Escherichia coli 25922 ATCC, Pseudomonas aeruginosa 27853 ATCC test-strains of microorganisms. Antimicrobial activity of trifluoroacetate 2,5,3-trimethyl-1H-indole-6-ylammonium (5) (Table 7): against S.aureus 6538-P ATCC MIC=1.96 µg/mL; against S.aureus 43300 ATCC (MRSA) MIC=1.96 µg/
mL; against *E. coli* 25922 ATCC – 0.98 µg/mL; against *Pseudomonas aeruginosa* 27853 ATCC – 0.98 µg/mL; against *S. pyogenes* 19615 ATCC – 0.98 µg/mL.

Antimicrobial activity of the trifluoroacetate 1,2,3,5-tetramethyl-1H-indole-6-ammonium (6) (Table 7): against *S. aureus* 6538-P ATCC MIC=7.9 µg/mL; against *S. aureus* 43300 ATCC (MRSA) MIC=7.9 µg/mL; against *E. coli* 25922 ATCC – 0.98 µg/mL; against *Pseudomonas aeruginosa* 27853 ATCC – 7.9 µg/mL; against *S. pyogenes* 19615 ATCC – 31.3 µg/mL.

Antimicrobial activity of the trifluoroacetate 2,3-dimethyl-5-methoxy-1H-indole-6-ammonium (7) (Table 7): against *S. aureus* 6538-P ATCC MIC=31.2 µg/mL; against *S. aureus* 43300 ATCC (MRSA) MIC=31.2 µg/mL; against *E. coli* 25922 ATCC – 0.98 µg/mL; against *Pseudomonas aeruginosa* 27853 ATCC – 1.96 µg/mL; against *S. pyogenes* 19615 ATCC – 0.98 µg/mL.

For dioxidine (comparison drug) against *Staphylococcus* spp. MIC=125.0–1000.0 µg/mL, against *Escherichia coli* 8.0–250.0 µg/mL, against *Pseudomonas* spp. 125.0–1000.0 µg/mL, *Streptococcus* spp. 64.0–1000.0 µg/mL (Padeiskaya 2011; Piopov et al. 2013).

### Conclusion

We continue to study for new compounds with an antimicrobial effect based on substituted 1H-indolylamines by targeted organic synthesis. Earlier, we synthesized the compounds of this series with a trifluoromethyl group in molecules, showing an effective antimicrobial activity. Previously investigated *N*-(indolyl) trifluoroacetamides 3 and 4 (Stepanenko et al. 2019) exhibited a high activity only against the gram-positive test strain of *S. aureus*. Other derivatives of substituted 6-aminoindoles (Yamashkin et al. 2020) *N*-(indolyl) trifluoroacetamides *C3, C4, X3* were highly effective against only gram-negative test strains of *E. coli* and *P. aeruginosa*. And only *N*-(indolyl) trifluoroacetamide based on substituted 6-aminoindoles *X4* showed a high antimicrobial activity against gram-positive *S. aureus* and gram-negative *E. coli* and *P. aeruginosa* test strains of microorganisms.

Following the results of the out-of-experimental screening for biological activity, new water-soluble compounds based on substituted 1H-indol-6-ylamines with a predicted antimicrobial effect were obtained. The non-experimental PASS prediction of the biological activity correlates with the revealed antimicrobial activity of the compounds under study. The set of molecular mechanisms predicted *in silico* determines the ability of the studied indolyltrifluoroacetamides and trifluoroacetates based on substituted 1H-indol-6-ylamines to suppress the growth and reproduction of test strains of the studied microorganisms.

The structure of 1H-indolylammonium trifluoroacetates, not described in the literature earlier, has been reliably proved using modern methods of physicochemical analysis.

Laboratory microbiological screening showed that they have an antimicrobial effect that exceeds the activity of the reference drug, dioxidine. The presence of molecular mechanisms predicted *in silico* in the spectrum of biological activity of the studied compounds, such as Pseudolysin inhibitor, Omptin inhibitor, Undecaprenyldiphospho-muramylpentapeptide beta-N-acetylglycosaminyltransferase inhibitor, UDP-epimerase inhibitor, Bacterial efflux pump inhibitor, suggests the presence of an antimicrobial activity against gram-positive and gram-negative microorganisms. But it must be remembered that the probability of *Pa* reflects, first of all, the similarity of the structure of molecules of a given organic compound with the structures of molecules that are most typical in the corresponding subset of “active” compounds in the training set (Varnek 2008; Filimonov et al. 2014). Therefore, as a rule, there is no direct correlation between the calculated

### Table 7. The Sensitivity of the Test Strains of Microorganisms to New Compounds 5–8 (Mueller-Hinton broth serial dilution method)

| Test-culture | *S. aureus 6538-P* ATCC | *S. aureus 43300* ATCC (MRSA) | *E. coli* 25922 ATCC | *P. aeruginosa* 27853 ATCC | *S. pyogenes* 19615 ATCC |
|--------------|--------------------------|-------------------------------|----------------------|---------------------------|-------------------------|
| Compound     | 5 | 6 | 7 | 8 | 5 | 6 | 7 | 8 | 5 | 6 | 7 | 8 | 5 | 6 | 7 | 8 | 5 | 6 | 7 | 8 |
| The concentration of a compound in the nutrient medium, µg/mL | 125.0 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 62.5 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31.3 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15.7 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7.9 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.9 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.9 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.98 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.49 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 0 | 0 | 0 | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Negative control | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- | +/- |

**Note:** “-“ – activity titer, “+++”– diffuse cloud of MHB; “++”– moderate cloud of MHB; “+”– weak cloud of MHB; “+/”– no cloud of MHB (as in the negative control); “0” – no growth when reseeding on MHA.
values of $Pa$ and the quantitative characteristics of the activity. When analyzing the PASS-predicted spectra of biological activity, it is necessary to take into account the possibilities of experimental testing. Therefore, the general recommendation is to consistently study the various predicted biological activities, from the most likely to the least likely ones.

Trifluoroacetates 2,3,5-trimethyl-1H-indole-6-ammonium (5), 1,2,3,5-tetramethyl-1H-indole-6-ammonium (6), 2,3-dimethyl-5-methoxy-1H-indole-6-ammonium (7), 1,2,3-trimethyl-5-methoxy-1H-indole-6-ammonium (8) may find application as water-soluble synthetic antimicrobial agents after additional studies.

## Conflict of interests

The authors declare no conflict of interests.

## References

- Brown ED, Wright GD (2016) Antibacterial drug discovery in the resistance era. Nature 529(7586): 336–343. https://doi.org/10.1038/nature17042 [PubMed]
- EUCAST (2019) Setting Breakpoints for New Antimicrobial Agents. www.euCAST.org/EUCAST SOP 1.3 (4 December, 2019) Previous: SOP 1.2 (3 January, 2017); SOP 1.1 (9 June, 2013). [The European Committee on Antimicrobial Susceptibility Testing – EUCAST]
- EUCAST [The European Committee in Antimicrobial Susceptibility Testing] (2021) Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021. https://www.euCAST.org/documents/rd/ [The European Committee on Antimicrobial Susceptibility Testing - EUCAST]
- Filimonov DA, Druzhilovskiy DS, Lagunin AA, Gloriozova TA, Rudik AV, Dmitriev A, Pogodin P, Poroikov VV (2018) Computer-aided prediction of biological activity spectra for chemical compounds: opportunities and limitations. Biomedical Chemistry: Research and Methods 1(1): e00004. https://doi.org/10.18097/bmcrm00004 [in Russian]
- Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskiy DS, Pogodin PV, Poroikov VV (2014) Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds 50(3): 444–457. https://doi.org/10.1007/s10593-014-1496-1
- ISO [International Organization for Standardization] (2006) Clinical Laboratory Testing and In Vitro Diagnostic Test Systems. Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices. Part 1: Reference Method for Testing the In Vitro Activity of Antimicrobial Agents Against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases. Interna Standard 20776–1. ISO, Geneva, Switzerland. 2006. [ISO STANDARDS]
- Khmel’ni’tskyi RA (1974) Mass spectrometry of indole compounds (review). Chemistry of Heterocyclic Compounds 10(3): 253–267. https://doi.org/10.1007/BF00472405
- Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Dournth SM, Giske CG, Irfan S, Krishnan P, Kumar AV, Mahajan S, Mshusaq T, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridin E, Thirunarasay MA, Turton J, Upadhyay S, Warner M, Welfare W, Livernore DM, Woodford N (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet Infectious Diseases 10(9): 597–602. https://doi.org/10.1016/S1473-3099(10)70143-2 [PubMed]
- Lai CC, Lee K, Xiao Y, Ahmad N, Veeraraghavan B, Thamlilikutkul V, Tambyah PA, Nelwan RH, Shibl AM, Wu JJ, Seto WH, Hsueh PR (2014) High burden of antimicrobial drug resistance in Asia. Journal of Global Antimicrobial Resistance 2(3): 141–147. https://doi.org/10.1016/j.jgar.2014.02.007 [PubMed]
- McKeegan KS, Borges-Walmsley MI, Walmsley AR (2002) Microbial and viral drug resistance mechanisms. Trends in Microbiology 10(10): 8–14. https://doi.org/10.1016/s0966-842x(02)02429-0 [PubMed]
- MUK [(Procedural Guideline) 4.2.1890-04] (2004) Determination of the sensitivity of microorganisms to antibacterial preparations. Clinical Microbiology and Antimicrobial Chemotherapy 6(4): 306–359. [in Russian]
- Natan M, Banin E (2017) From Nano to Micro: using nanotechnology to combat microorganisms and their multidrug resistance. FEMS Microbiology Reviews 41(3): 302–322. https://doi.org/10.1093/femsre/fux003 [PubMed]
- Obayiuwana A, Ogunjobi M, Yang M, Ibekwe M (2018) Characterization of bacterial communities and their antibiotic resistance profiles in wastewaters obtained from pharmaceutical facilities in lagos and ogun states. Nigeria International Journal of Environmental Research and Public Health 15(7): e1365. https://doi.org/10.3390/ijerph15071365 [PubMed] [PMC]
- Padeiskaya EN (2011) The antibacterial drug dioxidine: features of biological action and significance in the treatment of various forms of purulent infection. Infections and Antimicrobial Therapy 3(5): 105–155. [in Russian]
- Parhizgari N, Gouya MM, Mostafavi E (2017) Emerging and re-emerging infectious diseases in Iran. Iranian Journal of Microbiology 9(3): 122–142. [PubMed] [PMC]
- Piopov DA, Anuchina NM, Terent’ev AA, Kostik GV, Blatun LA, Rusanova EV, Aleksandrova IA, Pkhakadze Tla, Bogomolova NS, Terekhova LP (2013) Dioxidin: antimicrobial activity and prospects of clinical use at the present stage. Antibiotics and Chemotherapy [Antibiotiki i Khimioterapiia] 58(3–4): 37–42. [PubMed] [in Russian]
- Pop-Vicas A, Strom J, Stanley K, D’Agata EM (2008) Multidrug-resistant gram-negative bacteria among patients who require chronic hemodialysis. Clinical Journal of the American Society of Nephrology 3(3): 752–758. https://doi.org/10.2215/CJN.04651107 [PubMed]
- Savjani JK, Gajjar AK, Savjani KT (2009) Mechanisms of resistance: useful tool to design antibacterial agents for drug-resistant bacteria. Mini-Reviews in Medicinal Chemistry 9(2): 194–205. https://doi.org/10.2174/138955709787316038 [PubMed]
- Stepanenko IS, Yamashkin SA, Kostina YA, Batarsehva AA, Mironov MA (2018) A new group of compounds derived from 4-, 5-, 6- and 7-aminoidoles with antimicrobial activity. Research Results in Pharmacology 4(3): 17–26. https://doi.org/10.3897/rppharmacology.4.29905 [in Russian]
- Stepanenko IS, Yamashkin SA, Kot’kin AI, Yurovskaya MA (2019) Synthesis and antimicrobial activity of N-(indolyl)
trifluoroacetamides. Moscow University Chemistry Bulletin 74(5): 236–240. https://doi.org/10.3103/S0027131419050109

- Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Klyutmans J, Carmeli Y, Ouellette M, Outterson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U, Magrini N, WHO Pathogens Priority List Working Group (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases 18(3): 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3 [PubMed] [PMC]

- Terent’ev PB (1979) Mass Spectrometry in Organic Chemistry. Moscow, Graduate School, 223 pp. [in Russian]

- Varnek A (2008) Chemoinformatics Approaches to Virtual Screening. Cambridge (UK), RSC Publishing, 335 pp. https://doi.org/10.1039/9781847558879

- World Health Organization (2018) The top 10 causes of death. www.who.int/mediacentre/factsheets/fs310/en/index1.html

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