Synthesis and the Antimicrobial Activity of Salt Carbenoid Compounds

Abstract

Aim. To synthesize aliphatic and aromatic derivatives of salt carbenoid compounds of the series of imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole containing fluorophenyl, cetyl or adamantyl substituents, and study their antimicrobial (antibacterial and antifungal) activities.

Results and discussion. New derivatives of heterocyclic carbenoid salts and zwitterions based on the imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole heterocyclic systems containing fluorophenyl, cetyl or adamantyl substituents were synthesized. For this purpose, reactions of cyclization of the corresponding diimines with ethoxymethyl chloride (imidazolium salts), quaternization of the corresponding azoles with cetyl bromide or 1-adamantyl bromide in organic solvents (benzimidazolium, pyridinium and 1,3,4-oxadiazolium salts), cyclization of di(1-adamantylamino)alkanes hydrobromides with the orthoformic ester (4,5-dihydroimidazolium and tetrahydropyridinium salts) were used. Zwitterionic compounds were obtained by the reaction of the corresponding azolium salts with phenyl isothiocyanate in the presence of potassium carbonate. Some macrocyclic and adamantyl substituted heterocyclic compounds showed antifungal and antibacterial activities.

Experimental part. The structure of the compounds synthesized was proven by $^1$H and $^{13}$C NMR spectroscopy methods. The antimicrobial activity was studied out by the agar diffusion method to determine diameters of the growth inhibition zones of microorganisms (bacteria and fungi) and by the method of serial dilutions to determine the minimum inhibitory concentration and minimum bactericidal and fungicidal concentrations.

Conclusions. The synthesis of new heterocyclic carbenoid salts and zwitterions based on the imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole heterocyclic systems containing fluorophenyl, cetyl or adamantyl substituents has been performed. Compounds of macrocyclic and adamantyl heterocyclic series with antifungal and antibacterial activities have been found. 1,3-Dicetylimidazolium bromide, macrocyclic bis(decylenebenzimidazolium) bromides, azolium-N-phenylthiocarboxamides have been proven to be the most active.

Keywords: fluorooaryl, cetyl-, 1-adamantyl substituted heterocyclic salts; antimicrobial activity
Результати та їх обговорення. Синтезовано нові похідні гетероциклічних карбеноїдних солей і цвітеріонів на основі систем імідазолу, бензімідазолу, піридино, піримідино та 1,3,4-оксадіазолу, що містять флурофенілні, цетильний або адамантильний замісники. Для цього застосовано реакції циклізації відповідних діімінів діє етоксіметилхлориду (імідазолієві солі), кватернізації відповідних азолів цетибромідом або 1-адамантилбромідом в органічних розчинниках (бензімідазолів, піридінев і 1,3,4-оксадіазолів солі), циклізації гідробромідів ді(1-адамантиламіново)алканів ортоформітним естером (4,5-дігідроімідазолієві та тетрагідропіримідиневі солі). Цвітеріонні сполуки отримано реакцією відповідних азолієвих солей з фенілізотіоціанатом у присутності калій карбонату. Виявлено речовини макроциклічного й адамантагетероциклічного ряду з протигрибковою та антибактеріальною активністю. 

Експериментальна частина. Будь-якого синтезуваного сполучдя доведено методами 1Н та 13С ЯМР-спектроскопії. Антимікробну активність досліджували методом дифузії речовини в агар з визначенням діаметрів зон затримки зростання мікроорганізмів (бактерій і грибів) та методом серійних розведення із визначенням мінімальної інгібітуваної та мінімальної бактерицидної та фунгіцидної концентрації.

Висновки. Здійснено синтез нових гетероциклічних карбеноїдних солей і цвітеріонів на основі систем імідазолу, бензімідазолу, піридино, піримідино та 1,3,4-оксадіазолу, що містять флурофенілні, цетильний або адамантильний замісники. Виявлено речовини макроциклічного й адамантагетероциклічного ряду з протигрибковою та антибактеріальною активністю. Найбільш активними виявились 1,3-діцитлімідазіолів бромід, макроциклічні біс-децилен-бензімідазолій бромід, азолій-Н-фенілілікарбоксімід.

Ключові слова: флурофеніл-, цетиль-, 1-адамантилзаміщені гетероциклічні солі; антимікробна активність


Received: 11 April 2022; Revised: 25 May 2022; Accepted: 29 May 2022

Copyright © 2022, G. F. Rayenko, A. S. Avksentiev, V. Sh. Saberov, A. B. Ryabitsky, V. I. Yenya, O. Z. Komarovsky-Porokhnyavets, V. I. Lubenets, N. I. Korotkich. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0).

Funding: a grant of the National Academy of Sciences of Ukraine No. II-01-22.

Conflict of interests: the authors have no conflict of interests to declare.

### Introduction

Heterocyclic salts have recently attracted researchers’ attention with their biological activity (see, for example, a detailed review [1]). Compounds with antimicrobial, antitumor, antiprotozoal and other types of activity were found. The bactericidal activity has been determined for derivatives of ionic liquids [2–6], which are mostly imidazolium salts with the antimicrobial activity of both organic salts and carbene complexes of silver, copper(I), nickel, cobalt and palladium, and have found a particularly high activity of derivatives of adamantyl-containing 1,2,4-triazolium salts [8–12]. In the research [10], a highly active antimicrobial substance belonging to macrocyclic salts of the imidazole series has also been revealed.

This study aims to synthesize aliphatic and aromatic derivatives of a series of imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole with fluorophenyl, cetyl and adamantyl substituents and study their antimicrobial (antibacterial and antifungal) activities. It is also important to compare active carbenoïd salts and their methyl-substituted (non-carbnoïd) analogs.

### Results and discussion

1. The synthesis of imidazolium salts with cetyl and fluorophenyl substituents

A number of known antimicrobial compounds have long aliphatic substituents or fragments in their structure (e.g. 1-cetylpyridinium chloride, undecylenic acid and their derivatives). The effect of aliphatic groups on the antimicrobial activity of these compounds has not been fully elucidated though.

We have synthesized ionic compounds with cetyl substituents based on imidazole and benzimidazole, which are analogs of ionic liquids of the imidazole series. The reactions were carried out with the corresponding azoles and cetyl bromide in dioxane in the presence of sodium acetate. As a result, salts 1a and 2 were formed with the yields of 40–75% as colorless substances, which themselves might be of interest as potential biologically active compounds (Scheme 1).

The structures of salts 1a and 2 were confirmed by 1H and 13C NMR spectroscopy. Typical C–H proton signals in the region of 10.1–10.3 ppm can be found in the 1H NMR spectra of the compounds. The signals of the aliphatic fragment are observed in the region of 0.71–0.82 ppm (CH2, CH3, 1.08–1.40 ppm (CH2, 1.76–1.94 ppm (CH3, 4.20–4.47 ppm (CH2N). Resonances of imidazole...
C$_{4,5}$H protons of compound 1a are at 7.49 ppm. The $^{13}$C NMR spectra of compounds 1a and 2 contain signals of C$_2$ carbon atoms in the range of 136.5–142.6 ppm, C$_{4,5}$ atoms of the imidazole ring of compound 1a at 122.41 ppm. The resonances of aliphatic fragments are at 14.08–14.19 (CH$_3$C), 22.64–22.75 (C$_2$H$_2$C), 26.22–26.63 (C$_3$H$_2$C), 29.01–29.76 (other CH$_2$C), 31.87–31.98 (CH$_2$CN) and 47.79–49.99 ppm (CH$_2$N).

The synthesis of fluorine-containing imidazolium salts 1b,c was carried out by the reaction of glyoxal with the corresponding amines and the subsequent cyclization of the diimines 1A obtained under the action of ethoxymethyl chloride (Scheme 2). The salt yields are low (21–31%).

In the $^1$H NMR spectra of salts 1b,c the characteristic signals of C$_{4,5}$H protons at 7.87 and 8.43 ppm, and C$_2$H protons at 10.08–10.35 ppm are observed.

Thus, new imidazolium and benzimidazolium salts with cetyl groups (1a, 2) and imidazolium salts with fluorophenyl substituents (1b,c) were synthesized.

2. The synthesis of macrocyclic ionic compounds of the imidazole and benzimidazole series

In the work [10], we synthesized macrocyclic ionic compounds from imidazole, which proved to be effective as antimicrobial agents. Therefore, it was promising to synthesize related compounds, particularly from other azoles.

In this section, we describe the synthesis of macrocyclic analogs of the above carbenoids, which were obtained by quaternization of bis-azolylalkanes with dihaloalkanes. In this case, decane units were used.

Initial compounds 3A, 4A were prepared in situ by heating the corresponding benzimidazoles and imidazoles with 1,10-dibromodecane in o-dichlorobenzene followed by deprotonation of the bis-imidazolylalkane salts obtained by sodium acetate.
in acetonitrile similarly to the methods of works [10, 11]. The interaction of 1,10-bis(1-benzimidazolyl)decanes 3A with 1,10-dibromodecane in acetonitrile yielded macrocyclic salts 3a,b with the yields of 93–98 % (Scheme 3). Compound 3a crystallized well from acetonitrile. Compound 4b was similarly prepared from 2-methylimidazole in the yield of 32 %. The latter is analogous to compound 4a synthesized in the work [10]. Methyl-substituted compounds 3b and 4b are hygroscopic.

The 1H NMR spectra of compounds 3a,b, 4b contain specific resonances of aliphatic bridges in the ranges of 0.91–1.33 ppm (CH2), 1.68–1.73 ppm (CH2CN), 4.13–4.27 ppm (CH2N), signals of aromatic protons, and for 3a proton signal at 10.62 ppm (C2H). In the 13C NMR spectra of compounds 3a,b, 4b, signals of C2N carbon atoms in the region of 141.78–147.06 ppm, resonances of aliphatic units of CH2N groups at 47.35–52.22 ppm and other atoms of these units at 26.07–33.96 ppm are observed.

3. The synthesis of adamantyl-containing heterocyclic compounds

It is well known that adamantane derivatives have been proven to be effective antiviral agents, for instance the influenza A M2 ion channel protein inhibitors rimantadine and amantadine. The latter is also used as an antiparkinsonian agent inhibiting a NMDA-type glutamate receptor, increasing the dopamine release, and blocking the dopamine reuptake. Adamantyl-containing heterocyclic salts and their complexes have already been studied by the authors of the articles [8–10, 12, 13], which allowed to find new effective antimicrobial agents. In this paper, we continue our investigations aiming at synthesizing similar salt systems with the adamantane group.

Thus, we have found out that heating of 2-phenyl-1,3,4-oxadiazole with 1-adamantyl bromide in acetic acid leads to the formation of salt 5 with the yield of 40%, which is very labile under the action of even weak alkalis (potassium carbonate or acetate) and gives the product of hydrolysis of an intermediate carbene (due to the presence of a minute amount of water) — acyclic hydrazide 6 with the yield of 81% (Scheme 4).

The structure of salt 5 was confirmed by 1H NMR spectroscopy. Characteristic signals in the spectrum are the meso-proton signal C2H (11.76 ppm), as well as the resonances of CH2-protons (1.58 and 1.89 ppm) and CH-protons (2.10 ppm) of the adamantyl ring. Proton signals of aromatic nucleus are observed at 7.63 and 7.65 ppm. Characteristic signals of adamantyl (1.58, 2.00, 2.54 ppm) and formyl (9.79 ppm) protons are observed in the 1H NMR spectrum of compound 6.

A similar adamantyl derivative 7a was also obtained by heating pyidine and 1-bromoadamantane in acetic acid, followed by the ion exchange to perchlorate with the yield of 54% (Scheme 4). The subsequent exchange of a perchlorate ion to iodide gives the corresponding salt 7b.

In the 1H NMR spectrum of compound 7a proton signals of adamantyl groups at 1.75–2.30 ppm, as well as the resonance of C26H protons (9.31 ppm), C13H-protons (8.16 ppm) and C4H-proton (8.59 ppm) of the pyridinium cycle are present.
To study the antimicrobial activity, the six- and five-membered formamidinium salts (tetrahydropyrimidinium 8 and 4,5-dihydroimidazolium 9) recently described [14, 15] were also obtained by the condensation of the corresponding dibromoalkanes with 1-aminoadamantane and the subsequent cyclization of the intermediate diaminoalkanes with the orthoformic ester (Scheme 4).

Zwitterionic compounds also have an ionic structure although they do not contain external anions. Fluorine-containing zwitterion 10 (94% yield) and for comparison the known compound 11 (81% yield) were both synthesized by in situ conversion of the corresponding salts in the reaction with phenylisothiocyanate in the presence of potassium carbonate in acetonitrile at room temperature. Previously, compound 11 was also obtained by the reaction of the corresponding carbene with phenylisothiocyanate [16]. It should be noted that obtaining carbene from salt 1c is impossible due to its easy dimerization. Only in situ the approach was realized (Scheme 5).

4. The antimicrobial activity of the compounds synthesized

In this work, the antimicrobial activity of the compounds synthesized against bacterial strains of Escherichia coli 67, Staphylococcus aureus 209 P and Mycobacterium luteum VKM B-868, as well as fungi strains of Candida tenuis VKM Y-70 and Aspergillus niger VKM F-1119, was studied. The study was carried out by two methods [17, 18]: 1) the agar diffusion method to determine diameters of the growth inhibition zones of microorganisms (Method A), and 2) the serial dilutions method to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal (MBC) and fungicidal (MFC) concentrations (Method B) (see Experimental part). The activities of the compounds synthesized were compared to the activity of a known broadly used antimicrobial drug 1-cetylpyridinium chloride 12, which characteristics are given under the same conditions in the article [16], and with the activity of selected compounds 13, 14 synthesized in the work [8].

The diameters of the growth inhibition zones of microorganisms are given in Table 1. The results obtained indicate that compounds 3a, 4b, 8, 9 are among the most active in the concentration of 0.5%, but further dilution nullifies the activity. A comparison of the properties of compound 4a synthesized earlier [10] with the compounds studied, in particular, macrocyclic ones 3a,b and 4b, also shows greater activity of the former derivative 4a. The same can be said about diadamantyl-containing salts 8, 9, which...
Scheme 5. The synthesis of zwitterionic compounds 10, 11 from azolium salts

Table 1. The antimicrobial activity determined by the agar well diffusion method (Method A)

<table>
<thead>
<tr>
<th>Compound(^[a])</th>
<th>Concentration, %</th>
<th>The diameter of the growth inhibition zones(^[b]) (n = 3), mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>1b</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>1c</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>3a</td>
<td>0.5</td>
<td>10.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>3b</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>4a [10]</td>
<td>0.5</td>
<td>15.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>14.0 ± 0.3</td>
</tr>
<tr>
<td>4b</td>
<td>0.5</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>7a</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>7b</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>15.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>10.0 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>15.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>15.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>12 [10]</td>
<td>0.5</td>
<td>14.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>13 [8]</td>
<td>0.5</td>
<td>22.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>32.3 ± 0.3</td>
</tr>
<tr>
<td>14 [8]</td>
<td>0.5</td>
<td>23.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>16.0 ± 0.2</td>
</tr>
</tbody>
</table>

Notes: [a] compound 2 could not be studied due to its low solubility; [b] control values correspond to 0 mm
are less active than diadamantyl-containing salts 13, 14 (Figure) synthesized in [8].

Table 2 shows the data of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the compounds synthesized against the bacterial strains determined by the method of serial dilutions (Method B).

As one can see from Table 2, for most compounds the antibacterial activity is low or absent in the concentrations studied. But macrocyclic compounds 3a,b have good indicators of both MIC and MBC (not more than 62.5 μg mL⁻¹).

The activity of compound 3a, for which the MIC reaches 7.8 μg mL⁻¹, and MBC 15.6 μg mL⁻¹ on the culture of M. luteum, is particularly high. For compound 11, the MIC observed is 7.8 μg mL⁻¹.

It should be noted that in most cases, a high activity of the compounds studied is observed for only one culture – M. luteum. For comparison, the activity of compound 4a [10] previously synthesized is much higher (MIC and MBC reaches 3.9 μg mL⁻¹ against the E. coli and M. luteum cultures). Compared to the activity of compound 12 (MIC 3.9 and 7.8 μg mL⁻¹ and MBC 7.8 and 15.6 μg mL⁻¹ on the cultures of S. aureus and M. luteum, respectively), the related imidazolium salt 1a showed a substantially lower antimicrobial action.

Table 3 shows similar indicators of MIC and MFC determined by the Method B on the cultures of fungi C. tenuis and A. niger.

As can be seen from these data, a sufficiently high activity is observed for compound 1a on the C. tenuis culture (MIC 15.6 μg mL⁻¹, MFC 31.2 μg mL⁻¹), however, these values indicate a slightly lower fungicidal effect than that for pyridinium salt 12 (MIC 3.9 μg mL⁻¹, MFC 7.8 μg mL⁻¹) and especially for macrocyclic salt 4a (MIC 1.9 μg mL⁻¹, MFC 3.9 μg mL⁻¹). The culture of C. tenuis is more sensitive to the action of carbenoid compound 3a (MIC 7.8 μg mL⁻¹, MFC 15.6 μg mL⁻¹) compared to that of non-carbenoid compound 3b (MIC 62.5 μg mL⁻¹ against the culture of C. tenuis and MIC 62.5 μg mL⁻¹ against the culture of A. niger).

![Figure. The known compounds with a significant antimicrobial activity](image-url)

Table 2. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)[a][b] of the compounds determined by the serial dilutions method (Method B)

<table>
<thead>
<tr>
<th>Compound[c]</th>
<th>Bacteria cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli 67</td>
</tr>
<tr>
<td></td>
<td>MIC, μg mL⁻¹</td>
</tr>
<tr>
<td>1a</td>
<td>+</td>
</tr>
<tr>
<td>1b</td>
<td>+</td>
</tr>
<tr>
<td>1c</td>
<td>+</td>
</tr>
<tr>
<td>3a</td>
<td>+</td>
</tr>
<tr>
<td>3b</td>
<td>+</td>
</tr>
<tr>
<td>4a [10]</td>
<td>3.9</td>
</tr>
<tr>
<td>4b</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>7a</td>
<td>+</td>
</tr>
<tr>
<td>7b</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>125.0</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>12 [10]</td>
<td>31.2</td>
</tr>
</tbody>
</table>

Notes: [a] “+” means no antibacterial effect was observed in the concentrations studied (growth of the microorganisms); [b] control values correspond to “+”; [c] Compound 2 could not be tested due to its low solubility; [d] no indicator of bactericidal effect was found in the concentrations studied.
Zwitterion 10 also noticeably inhibits the growth of *A. niger* (MIC 62.5 μg mL⁻¹).

Thus, we have found new compounds 1a, 3a,b, 10, 11 with the antimicrobial activity, which can be used as a basis for new improved series of compounds for biological research.

### Conclusions

The synthesis of new heterocyclic carbenediimide salts and zwitterions based on the imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole heterocyclic systems containing fluoro-phenyl, cetyl or adamantyl substituents has been performed. Compounds of macrocyclic and adamantyl heterocyclic series with antifungal and antibacterial activities have been found. 1,3-Dicetylimidazolium bromide, macrocyclic *bis*(decelylbenzimidazolium) bromides, azolium-N-phenylthiocarboximides have been proven to be the most active.

### Experimental part

¹H NMR and ¹³C NMR spectra were recorded using a Bruker Avance II 400 spectrometer (400 MHz for ¹H NMR and 100 MHz for ¹³C NMR spectra) in DMSO-*d₆* or CDCl₃ solution. The ¹H NMR and ¹³C NMR chemical shifts are reported relative to tetramethylsilane (TMS) (solution). To assess purity of the compounds synthesized, thin-layer chromatography was performed on silica gel with chloroform or the mixture of chloroform and methanol (10:1) as an eluent, followed by development with iodine. Melting points were measured on a Boethius chair (Nagema, Germany). The elemental analysis was performed in the analytical laboratory of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine. Commercial solvents and reagents were used in the syntheses, except specially indicated cases.

**1,3-Dicetylimidazolium bromide (1a)**

The mixture of imidazole (0.68 g, 10 mmol, 1.0 equiv) and hexadecyl bromide (7.32 g, 24 mmol, 2.4 equiv) in anhydrous dioxane (3 mL) was stirred at 100 °C for 1 h. Then anhydrous sodium acetate (0.821 g, 10 mmol, 1.0 equiv) was added to the solution and stirred at 100 °C for 16.5 h. The precipitate of inorganic salts was filtered off. The solution was heated to boiling and cooled to room temperature. A colorless precipitate formed was filtered off, washed with hexane and dried.

**Yield – 4.47 g (75%).** M. p. 65°C. Anal. Calcd for *C*₂₅₅*H*₄₀*Br*N₂, %: C 70.32; H 11.63; Br 13.37; N 4.69. Found, %: C 70.40; H 11.65; Br 13.29; N 4.67. ¹H NMR (400 MHz, CDCl₃), δ, ppm: 0.71 (6H, s, 2 × CH₃); 1.08 (52H, m, 26 × CH₂); 1.76 (4H, s, 2 × CH₂CN); 4.20 (4H, s, 2 × CH₂N); 7.49 (2H, s, C₆¹₁H₄); 10.07 (1H, s, CH=NH). ¹³C NMR (100 MHz, CDCl₃), δ, ppm: 14.08 (CH₃); 22.64 (C₆H₄); 26.22 (C₆H₄); 29.01 (C₆H₄); 29.32 (C₆H₄); 29.40 (C₆H₄); 29.51 (C₆H₄); 29.60, 29.62, 29.63, 29.66 (C₆³¹₁H₄); 30.32 (CH₂CCN);
1,3-Bis(2,3,4-trifluorophenyl)imidazolium chloride (1b)

**Step 1.** \(N,N'\)-Bis(2,3,4-trifluorophenyl)glyoxalidimine. The solution of 2,3,4-trifluoromethyline (4.9 g, 33.3 mmol) and 40% glyoxal solution (4.83 g, 33.3 mmol) in 20 mL of isopropyl alcohol was stirred at room temperature for 7 days. The solvent was evaporated, and the resulting residue containing diimine \(1\text{A}\) was used without purification in the next step.

**Step 2.** The cyclization reaction. Anhydrous zinc chloride (4.09 g, 30 mmol) and ethoxymethyl chloride (5.67 g, 60 mmol) were added to the solution of diimine \(1\text{A}\) obtained in the previous step, in chloroform (50 mL) and stirred at room temperature for 3 days. The solution was evaporated, and the organic salt was extracted with hot water (100 mL). The water solution was evaporated to a small volume. A colorless precipitate formed was filtered off and dried.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.

**Step 2.** The cyclization reaction. Anhydrous zinc chloride (4.09 g, 30 mmol) and ethoxymethyl chloride (5.67 g, 60 mmol) were added to the solution of diimine \(1\text{A}\) obtained in the previous step, in chloroform (50 mL) and stirred at room temperature for 3 days. The solution was evaporated, and the organic salt was extracted with hot water (100 mL). The water solution was evaporated to a small volume. A colorless precipitate formed was filtered off and dried.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.

**Step 2.** The cyclization reaction. Anhydrous zinc chloride (4.09 g, 30 mmol) and ethoxymethyl chloride (5.67 g, 60 mmol) were added to the solution of diimine \(1\text{A}\) obtained in the previous step, in chloroform (50 mL) and stirred at room temperature for 3 days. The solution was evaporated, and the organic salt was extracted with hot water (100 mL). The water solution was evaporated to a small volume. A colorless precipitate formed was filtered off and dried.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.

**Step 2.** The cyclization reaction. Anhydrous zinc chloride (4.09 g, 30 mmol) and ethoxymethyl chloride (5.67 g, 60 mmol) were added to the solution of diimine \(1\text{A}\) obtained in the previous step, in chloroform (50 mL) and stirred at room temperature for 3 days. The solution was evaporated, and the organic salt was extracted with hot water (100 mL). The water solution was evaporated to a small volume. A colorless precipitate formed was filtered off and dried.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%.
47.35 (CH₃N); 113.33 (C₄₋₇, Ar); 127.12 (C₅₋₆, Ar); 131.11 (ipso-C, Ar); 141.78 (C₂N).

1,3-Bis(1,10-decyl-2-methylbenzimidazolium) bromide (3b)

The solution of 1,10-bis(2-methylbenzimidazol-1-yl)decane of type 3A obtained from 2-methylbenzimidazole (1.64 g, 12.46 mmol) and 1,10-dibromodecane (1.87 g, 6.23 mmol), similarly to the preparation of salt 3a, was washed by hexane (15 mL) threefold, another portion of 1,10-dibromodecane (1.87 g, 6.23 mmol) in acetonitrile (8 mL) was added and refluxed for 8 h. Then another portion of acetonitrile (10 mL) was added, and the solution was refluxed for 24 h. The mother liquor was evaporated, and a colorless solid residue was dried.

Yield – 4.29 g (98%). M. p. 167–170°C. Anal. Calcd for C₃₀H₅₂Br₂N₄: %: C 61.54; H 7.75; Br 22.74; N 7.2. Found, %: C 61.52; H 7.76; Br 22.74; N 7.29.

1H NMR (CDCl₃, 400 MHz), δ, ppm: 1.01–1.49 m (24H, 12CH₂); 1.81 (6H, s, 2CH₃); 3.06 (8H, s, 4CH₂CN); 4.44 (8H, s, 4CH₂N); 7.50–7.80 (4H, m, ArH); 7.80–8.04 (4H, m, ArH).

13C NMR (100 MHz, CDCl₃), δ, ppm: 31.14, 33.67, 33.96, 33.04 (CH₂C+CH₂C); 52.22 (CH₃N); 118.58; 131.93; 136.23; 147.06 (C₃N).

1,3-Bis(1,10-decyl-2-methylimidazolium) bromide (4b)

The solution of 1,10-bis(2-methylimidazol-1-yl)decane of type 4A obtained from 2-methylimidazole (1.02 g, 12.46 mmol) and 1,10-dibromodecane (1.87 g, 6.23 mmol) similarly to the preparation of salt 3A was washed by hexane (15 mL) threefold, another portion of 1,10-dibromodecane (1.87 g, 6.23 mmol) in acetonitrile (8 mL) was added, and the mixture obtained was refluxed for 8 h. The resulting solution was evaporated to dryness, and an oily colorless residue was dried and solidified while standing.


1H NMR (CDCl₃, 400 MHz), δ, ppm: 1.20 (10H, m, 5×CH₂); 1.25 (6H, m, 3×CH₃); 1.75 (8H, s, 4CH₂); 2.66 (6H, s, 2×CH₂); 3.17 (8H, s, 4CH₂CN); 4.13 (8H, s, 4CH₂N); 7.60 (4H, s, C₅₋₆H). 13C NMR (100 MHz, CDCl₃), δ, ppm: 9.87 (CH₂); 25.78, 28.47, 28.72, 29.40 (CH₂); 48.18 (CH₃N); 121.39 (C₄₋₇); 142.63 (C₃N).

4-(1-Adamantyl)-2-phenyl-1,3,4-oxadiazolium bromide (5)

The solution of 2-phenyl-1,3,4-oxadiazole (2.93 g, 20 mmol) and 1-bromoadamantane (4.73 g, 22 mmol) in glacial acetic acid (3 mL) was stirred at 120°C for 1 day. The mixture of methyl tert-butyl ether/acetic acid (10:1) (10 mL) was added to the solution, and a colorless precipitate formed was filtered off and dried.

Yield – 2.9 g (40%). M. p. > 250°C. Anal. Calcd for C₁₉H₂₁BrN₂O: %: C 59.84; H 5.86; Br 22.12; N 7.75. Found, %: C 59.72; H 5.88; Br 22.20; N 7.77. 1H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.58 (6H, m, CH₃Ad); 1.89 (6H, m, CH₂Ad); 2.10 (3H, m, CH Ad); 7.55 (2H, dd, J₁ = 7.6 Hz, J₂ = 7.6 Hz, ArH); 7.65 (1H, dd, J₁ = 7.6 Hz, J₂ = 7.6 Hz, ArH); 7.95 (2H, d, J = 7.6 Hz), 11.76 (1H, s, C₃H₄N).

1-(1-Adamantyl)-1-formyl-2-benzoylhydrazine (6)

Anhydrous potassium carbonate (0.70 g, 1.94 mmol) was added to a solution of salt 5 (0.3 g, 0.83 mmol) in acetonitrile (2 mL) and stirred at 35–40°C for 12 h. The solution was filtered from inorganic substances and evaporated to dryness to give a colorless compound 6.

Yield – 0.2 g (81%). M. p. 154–156°C. Anal. Calcd for C₁₄H₂₂N₂O₅: %: C 72.46; H 7.43; N 9.39. Found, %: C 72.38; H 7.40; N 9.50. 1H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.48–1.66 (12H, m, CH₂Ad); 2.00 (3H, m, CH Ad); 7.46 (2H, dd, J₁ = 7.2 Hz, J₂ = 7.2 Hz, ArH); 7.50 (1H, dd, J₁ = 7.2 Hz, J₂ = 7.2 Hz, ArH); 7.84 (2H, d, J = 7.2 Hz, ArH); 9.79 (1H, s, CHO), NH (in exchange).

1-(1-Adamantyl)pyridinium perchlorate (7a)

Anhydrous pyridine (0.8 mL, 10 mmol) was added to a suspension of 1-bromoadamantane (2.15 g, 10 mmol) in acetic acid (2 mL). The mixture was heated at 140°C for 24 h under the nitrogen atmosphere and cooled to room temperature. Acetic acid was extracted with hexane, the precipitate was triturated with hexane and then with methyl tert-butyl ether. The precipitate (2.12 g, 72%) of bromide 7A was filtered off, dissolved by heating in water (5 mL), and filtered after the treatment with activated carbon. The excess of sodium perchlorate (1.47 g, 12 mmol) was added to the hot solution. After cooling, a colorless precipitate was filtered off and dried.

Yield – 1.59 g (54%). M. p. 238–240°C. Anal. Calcd for C₁₇H₂₀ClN₂O₆: %: C 57.42; H 6.42; Cl 11.30; N 4.46. Found, %: C 57.35; H 6.40; Cl 11.41; N 4.44. 1H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.75 (6H, s, CH₂Ad); 2.30 (9H, s, CH₃+CH Ad); 8.16 (2H, dd, J₁ = 7.2 Hz, J₂ = 7.2 Hz, C₅H₄N⁺); 8.59 (1H, dd, J₁ = 7.2 Hz, J₂ = 7.2 Hz, C₅H₄N⁺); 9.31 (2H, d, J = 7.2 Hz, C₆H₅N⁺).

1-(1-Adamantyl)pyridinium iodide (7b)

The salt was obtained by the exchange of ions from perchlorate 7a and potassium iodide in acetone.
Yield – 94%. M. p. 249–250°C. Anal. Calcd for C_19_H_23_IN: %; C 52.80; H 5.91; I 37.19; N 4.10. Found: %; C 52.87; H 5.90; I 37.10; N 4.13. The compound has similar spectral characteristics to perchlorate 7a.

1,3-Bis(2-trifluoromethylphenyl)imidazolium-2-(N-phenylthiocarboximide) (10)

The mixture of 1,3-bis(2-trifluoromethylphenyl)imidazolium perchlorate (1c) (0.30 g, 0.66 mmol) and anhydrous potassium carbonate (0.182 g, 1.32 mmol, 2 equiv) in anhydrous acetonitrile (3 mL) was stirred at room temperature under the nitrogen atmosphere for 10–15 min, phenyl isothiocyanate (0.08 mL, 0.66 mmol, 1 equiv) was then added, and the mixture was stirred at room temperature for 20 h. The precipitate of inorganic salts was filtered off and washed with hot anhydrous acetonitrile. The solution of the residue was triturated off, washed with hexane and dried.

Yield – 0.29 g (91%). M. p. 165°C. Anal. Calcd for C_{24}H_{22}F_6N_3S: %; C 58.66; H 3.08; N 8.55; S 6.52. Found: %; C 58.85; H 3.01; N 8.49; S 6.46.

1H NMR (CDCl_3, 400 MHz), δ, ppm: 6.41 (2H, s, C_6H_4^{tBu}); 6.73 (1H, t, J = 6.4 Hz, ArH); 6.96 (2H, t, J = 6.4 Hz, ArH); 7.53–7.94 (10H, m, ArH).

13C NMR (100 MHz, CDCl_3), δ, ppm: 120.81; 121.50; 123.60; 126.33; 126.97; 127.57; 130.10; 130.82; 132.04; 132.53; 147.71 (ipso-C, PhN); 150.89 (C=N); 162.77; 163.79 (N=). Found, %: C 52.87; Н 5.90; I 37.10; N 4.13. The compound has similar spectral characteristics to perchlorate 7a.

Method A. 0.5% and 0.1% solutions of the test substances in DMSO were prepared and introduced to the culture medium. The antimicrobial activity of the compounds synthesized was studied on test bacteria cultures of Escherichia coli 67, Staphylococcus aureus 209 P and Mycobacterium luteum VKM B-868 and fungi Candida tenuis VKM Y-70 and Aspergillus niger VKM F-1119 by the agar diffusion method on a solid nutrient medium – meat-peptone agar (MPA) for bacterial strains and wort agar (WA) for fungi. The microbial load was 10⁶ colony-forming units (CFU) in 1 mL. The 0.5 McFarland standard test of turbidity was used to make the bacterial suspension. Counting of cells (spores) of fungi was carried out in the Goryaev’s chamber. The duration of incubation of bacteria was 24 h at 35°C, fungi – 48–72 h at 28–30°C. The degree of the activity of the compounds studied was assessed by the diameters of the growth inhibition zones for test cultures of microorganisms, assuming that at a diameter of 11–15 mm a microorganism is insensitive to the drug, it is sensitive at 16–25 mm, and is highly sensitive at > 25 mm. Each experiment was repeated thrice.

Method B. The minimum inhibitory (MIC), bactericidal (MBC) and fungicidal (MFC) concentrations were determined by the serial dilution method in a liquid nutrient medium. The initial solution of a substance was prepared in DMSO in the concentration of 10000 μg mL⁻¹. The solution was then two-fold serially diluted with DMSO, and 0.1 mL of each dilution was then transferred to tubes and diluted to the volume of 1 mL with the nutrient medium reaching a concentration of the substance from 0.9 to 500 μg mL⁻¹. The meat peptone broth was used as a nutrient medium for bacteria and the untouched beer wort of 6–8°Blg – for fungi. Bacterial and fungal inocula were sown in the culture medium (the microbial load – 10⁶ CFU in 1 mL). The seeded tubes were kept in a thermostat at the appropriate temperature (37°C – for the bacterial strains; 30°C – for fungal strains) for 24–72 h. The results were evaluated for the presence or absence of growth of microorganisms, the visual inspection was performed in transmitted light, comparing the degree
of microbial turbidity of the nutrient medium with the “negative control”.

To determine the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) from tubes, in which the medium solutions were visually transparent, 0.02 mL of the medium was taken and applied to a sterile MPA (for bacterial strains) or WA (for fungal strains) in sterile Petri dishes incubated in a thermostat. The results were evaluated for testing bacteria in 24 h, for testing fungi in 48–72 h. In the absence of growth of the microorganism colonies on the incubated Petri dishes, MBC or MFC of the test substance was determined. Each experiment was repeated thrice.

**Acknowledgements**

The authors gratefully thank the National Academy of Sciences of Ukraine for financial support (grant II-01-22).

**References**


Information about the authors:

Gennady F. Rayenko, Ph.D. in Chemistry, Senior Researcher, Department of Chemistry of Heterocyclic Compounds, Deputy Director of L. M. Litvinenko Institute of Physical Organic and Coal Chemistry of the National Academy of Sciences of Ukraine; https://orcid.org/0000-0002-3694-536X.

Olexandr S. Avksentiev, Engineer of the 1st category, Laboratory of Chemistry of Stable Carbenes, Institute of Organic Chemistry of the National Academy of Sciences of Ukraine.

Vagiz Sh. Saberov, Ph.D. in Chemistry, Senior Researcher, Laboratory of Chemistry of Stable Carbenes, Institute of Organic Chemistry of the National Academy of Sciences of Ukraine; https://orcid.org/0000-0003-1309-3309.

Alexey B. Ryabitsky, Manager of NMR Spectroscopy, Life Chemicals Inc.; https://orcid.org/0000-0002-7323-1390.

Vasil I. Yenya, Ph.D. in Chemistry, Researcher, Laboratory of Chemistry of Stable Carbenes, Institute of Organic Chemistry of the National Academy of Sciences of Ukraine; https://orcid.org/0000-0002-2151-2561.

Vira I. Lubenets, D.Sc. in Chemistry, Professor of the Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University; https://orcid.org/0000-0001-6189-0084.

Olena Z. Komarovska-Porokhnyavets, Ph.D. in Chemistry, Associate Professor of the Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University; https://orcid.org/0000-0003-2439-481X.

Nikolai I. Korotkikh (corresponding author), D.Sc. in Chemistry, Professor, Head of the Laboratory of Chemistry of Stable Carbenes, Institute of Organic Chemistry of the National Academy of Sciences of Ukraine; https://orcid.org/0000-0003-0774-6588; e-mail for correspondence: nkorotkikh@ua.fm.