Journal of Organic and Pharmaceutical Chemistry

Advanced Research

OPEN ACCESS

Freely available online on
http://ophcj.nuph.edu.ua

UDC 547.78+547.79

G. F. Rayenko[1], O. S. Avksentiev[2], V. Sh. Saberov[2], A. B. Ryabitsky[3], V. I. Yenya[1,2], O. Z. Komarovska-Porokhnyavets[4], V. I. Lubenets[4], N. I. Korotkikh[1,2]

[1] L. M. Litvinenko Institute of Physical Organic and Coal Chemistry of the National Academy of Sciences of Ukraine, 50, Kharkiv road, Kyiv, 02160, Ukraine
[2] Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, 5, Murmanskaya str., Kyiv, 02660, Ukraine
[3] Life Chemicals Inc., 5, Murmanskaya str., Kyiv, 02660, Ukraine
[4] Lviv Polytechnic National University, 12, Bandera str., Lviv, 79013, Ukraine

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-phenylthiocarboximides have been proven to be the most active.

Keywords: fluoroaryl, cetyl-, 1-adamantyl substituted heterocyclic salts; antimicrobial activity

Г. Ф. Раєнко[1], О. С. Авксентьєв[2], В. Ш. Сабєров[2], О. Б. Рябицький[3], В. І. Єня[1,2], О. З. Комаровська-Порохнявець[4], В. І. Лубенець[4], М. І. Коротких[1,2]

[1] *Інститут фізико-органічної хімії і вуглехімії ім. Л. М. Литвиненка Національної академії наук України, Харківське шосе, 50, м. Київ, 02160, Україна*
[2] *Інститут органічної хімії Національної академії наук України, вул. Мурманська, 5, м. Київ, 02660, Україна*
[3] *ТОВ НВП І. Ф. Лаб, вул. Мурманська, 5, м. Київ, 02660, Україна*
[4] *Національний університет «Львівська політехніка», вул. Бандери, 12, м. Львів, 79013, Україна*

Синтез і антимікробна активність сольових карбеноїдних сполук

Анотація

Мета. Синтезувати аліфатичні й ароматичні похідні сольових карбеноїдних сполук ряду імідазолу, бензімідазолу, піридину, піримідину та 1,3,4-оксадіазолу, що містять флуорофенільні, цетильний або адамантильний замісники, та дослідити їхню антимікробну (антибактеріальну й протигрибкову) активність.

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Результати та їх обговорення. Синтезовано нові похідні гетероциклічних карбеноїдних солей і цвітеріонів на основі систем імідазолу, бензімідазолу, піридину, піримідину та 1,3,4-оксадіазолу, що містять флуорофенільні, цетильний або адамантильний замісники. Для цього застосовано реакції циклізації відповідних діімінів дією етоксиметилхлориду (імідазолієві солі), кватернізації відповідних азолів цетилбромідом або 1-адамантилбромідом в органічних розчинниках (бензімідазолієві, піридинієві та 1,3,4-оксадіазолієві солі), циклізації гідробромідів ді(1-адамантиламіно)-алканів ортоформіатним естером (4,5-дигідроімідазолієві та тетрагідропіримідинієві солі). Цвітеріонні сполуки отримано реакцією відповідних азолієвих солей з фенілізотіоціанатом у присутності калій карбонату. Виявлено речовини макроциклічного й адамантилгетероциклічного ряду з протигрибковою та антибактеріальною активністю.
Експериментальна частина. Будову синтезованих сполук доведено методами ^{1}H та ^{13}C ЯМР-спектроскопії. Антимікробну активність досліджували методом дифузії речовини в агар з визначенням діаметрів зон затримки зростання мікроорганізмів (бактерій і грибів) та методом серійних розведень із визначенням мінімальної інгібувальної та мінімальних бактерицидної і фунгіцидної концентрацій.
Висновки. Здійснено синтез нових гетероциклічних карбеноїдних солей і цвітеріонів на основі систем імідазолу, бензімідазолу, піридину, піримідину та 1,3,4-оксадіазолу, що містять флуорофенільні, цетильний або адамантильний замісники. Виявлено речовини макроциклічного й адамантилгетероциклічного ряду з протигрибковою та антибактеріальною активністю. Найбільш активними виявилися 1,3-дицетилімідазолій бромід, макроциклічні *біс*-децилен-бензімідазолій броміди, азолій-N-фенілтіокарбоксіміди.
Ключові слова: флуороарил-, цетил-, 1-адамантилзаміщені гетероциклічні солі; антимікробна активність

Citation: Rayenko, G. F.; Avksentiev, A. S.; Saberov, V. Sh.; Ryabitsky, A. B.; Yenya, V. I.; Komarovska-Porokhnyavets, O. Z.; Lubenets, V. I.; Korotkikh, N. I. Synthesis and the antimicrobial activity of salt carbenoid compounds. *Journal of Organic and Pharmaceutical Chemistry* **2022**, *20* (2), 14–26.
https://doi.org/10.24959/ophcj.22.258880
Received: *11 April 2022*; **Revised:** *25 May 2022*; **Accepted:** *29 May 2022*

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 one long aliphatic N-substituent. Oligomeric imidazolium salts with the antimicrobial activity are described in the works [1, 7]. The authors of the article have been studying the antimicrobial activity of both organic salts and carbene complexes of silver, copper(I), nickel, cobalt and palladium, and have found a particularly highly active 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 carbenoid salts and their methyl-substituted (non-carbenoid) analogs.

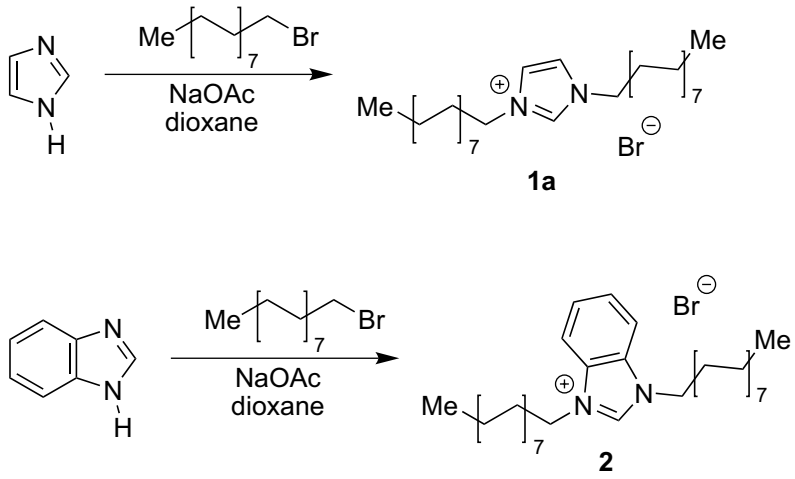
■ Results and discussion

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 ^{1}H and ^{13}C NMR spectroscopy. Typical C^{2}H proton signals in the region of 10.1–10.3 ppm can be found in the ^{1}H NMR spectra of the compounds. The signals of the aliphatic fragment are observed in the region of 0.71–0.82 ppm (CH_3C), 1.08–1.40 ppm (CH_2C), 1.76–1.94 ppm (CH_2CN), 4.20–4.47 ppm (CH_2N). Resonances of imidazole



Scheme 1. The synthesis of cetyl substituted ionic compounds **1a** and **2**

$C^{4,5}H$ 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_3C), 22.64–22.75 (C^2H_2C), 26.22–26.63 (C^3H_2C), 29.01–29.76 (other CH_2C), 31.87–31.98 (CH_2CN) and 47.79–49.99 ppm (CH_2N).

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 ^1H NMR spectra of salts **1b,c** the characteristic signals of $C^{4,5}H$ protons at 7.87 and 8.43 ppm, and C^2H protons at 10.08–10.35 ppm are observed.

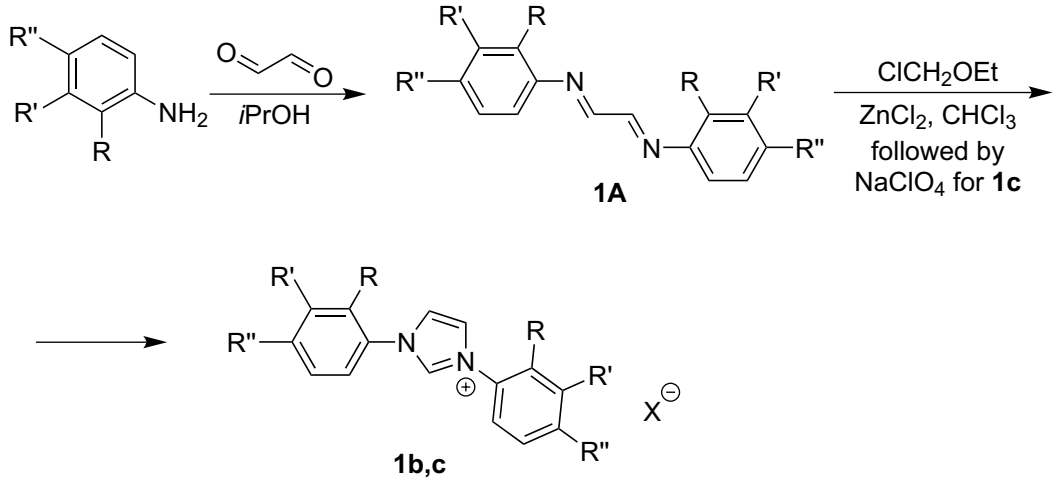
Thus, new imidazolium and benzimidazolium salts with cetyl groups (**1a, 2**) and imidazolium salts with fluorophenyl substituents (**1b,c**) were synthesized.

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.

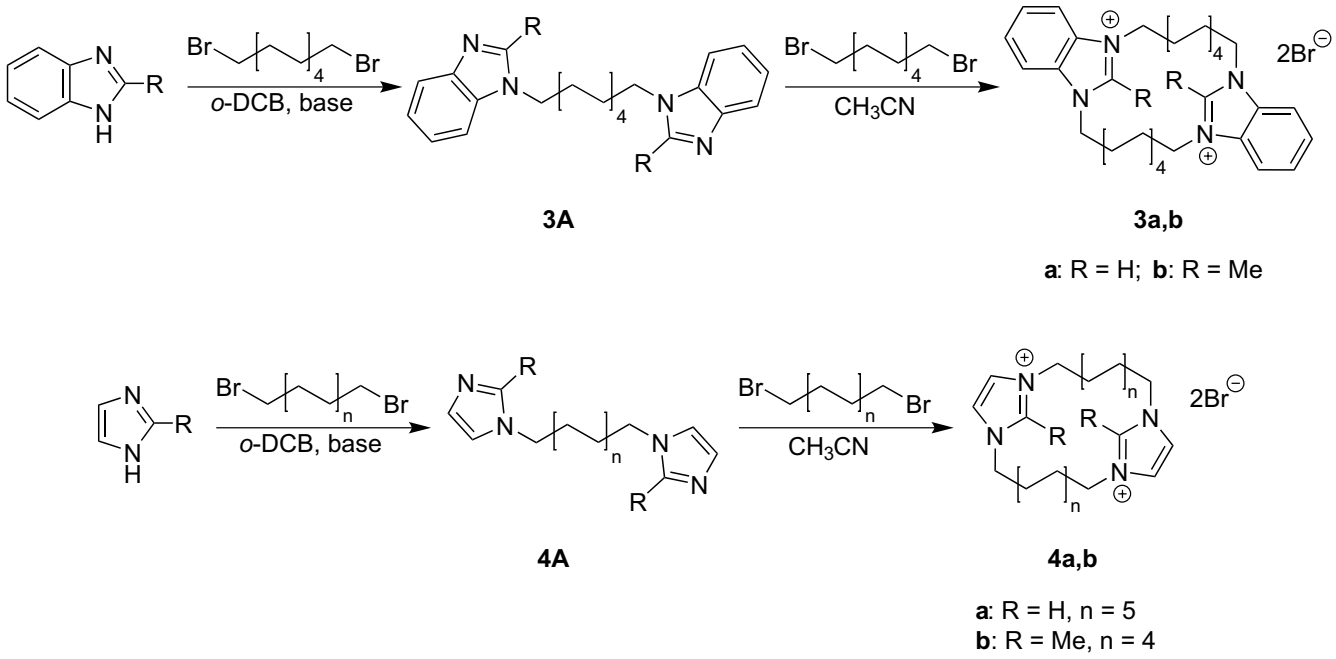
Initial compounds **3A**, **4A** were prepared *in situ* by heating the corresponding benzimidazoles and imidazoles with 1,10-dibromodecane in *o*-dichlorobenzene followed by deprotonation of the *bis*-imidazolylalkane salts obtained by sodium acetate



b: R = R' = R'' = F, X = Cl;
c: R = CF_3, R' = R'' = H; X = ClO_4

Scheme 2. The synthesis of fluorine-containing imidazolium salts

ISSN 2308-8303 (Print) / 2518-1548 (Online)



Scheme 3. Formation of macrocyclic salts **3a,b** and **4a,b**

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 ^{1}H NMR spectra of compounds **3a,b**, **4b** contain specific resonances of aliphatic bridges in the ranges of 0.91–1.33 ppm (CH_2C), 1.68–1.73 ppm (CH_2CN), 4.13–4.27 ppm (CH_2N), signals of aromatic protons, and for **3a** proton signal at 10.62 ppm (C^2H). In the ^{13}C NMR spectra of compounds **3a,b**, **4b**, signals of C^2N carbon atoms in the region of 141.78–147.06 ppm, resonances of aliphatic units of CH_2N 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

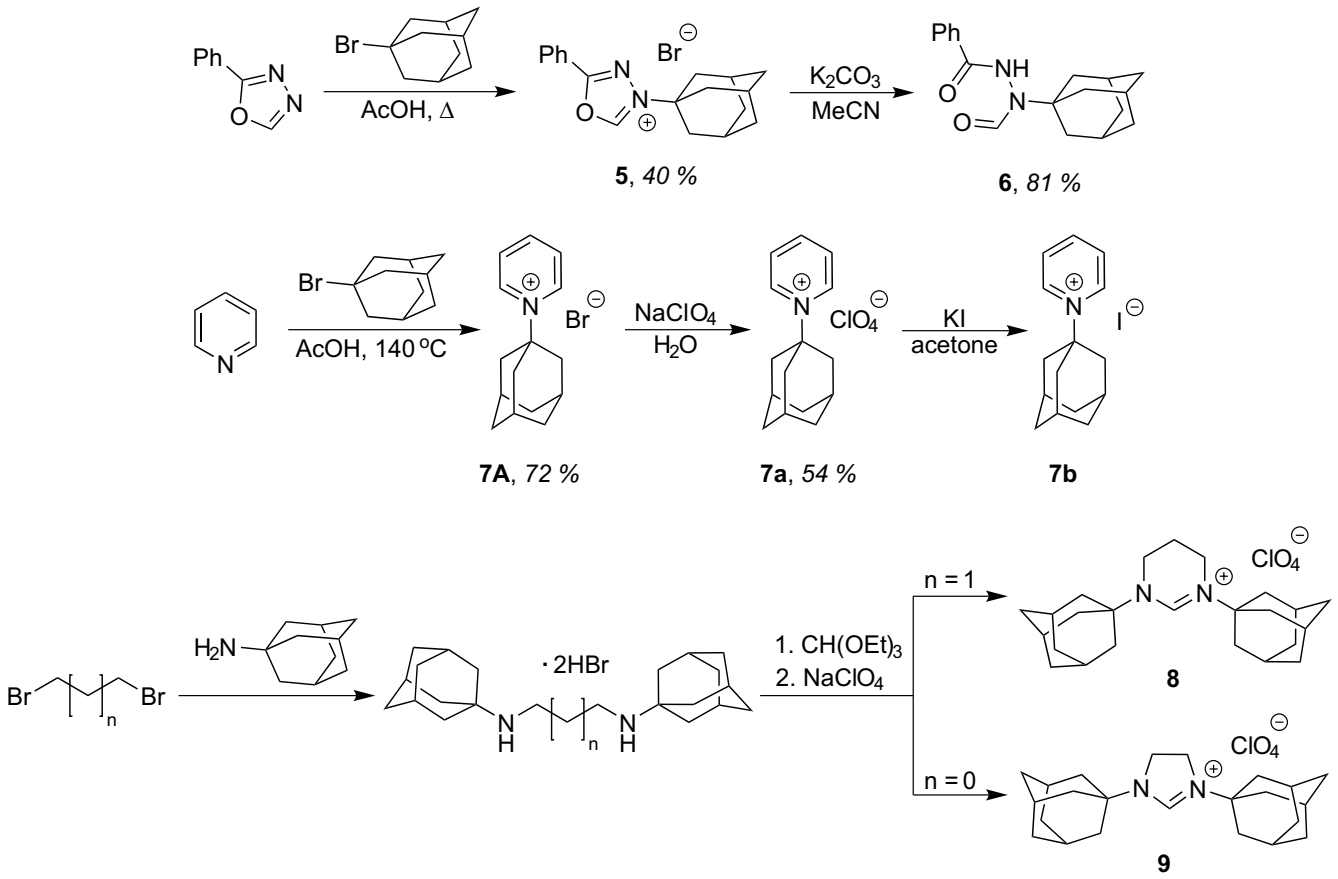
It is well known that adamantane derivatives have been proven to be effective antiviral agents, for instance the influenza A M2 ion channel protein inhibitors rimantadine and amantadine. The latter is also used as an antiparkinsonian agent inhibiting a NMDA-type glutamate receptor, increasing the dopamine 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 ^{1}H NMR spectroscopy. Characteristic signals in the spectrum are the *meso*-proton signal C^2H (11.76 ppm), as well as the resonances of CH_2-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 ^{1}H NMR spectrum of compound **6**.

A similar adamantyl derivative **7a** was also obtained by heating pyridine 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 ^{1}H NMR spectrum of compound **7a** proton signals of adamantyl groups at 1.75–2.30 ppm, as well as the resonance of $C^{2,6}H$ protons (9.31 ppm), $C^{3,5}H$-protons (8.16 ppm) and C^4H-proton (8.59 ppm) of the pyridinium cycle are present.



Scheme 4. Synthetic approaches to the adamantyl-containing salts

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).

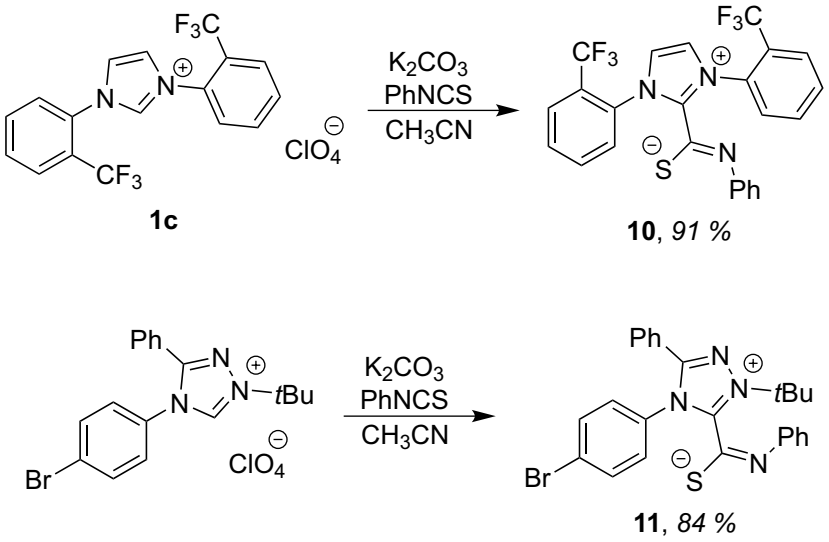
Zwitterionic compounds also have an ionic structure although they do not contain external anions. Fluorine-containing zwitterion **10** (94% yield) and for comparison the known compound **11** (81% yield) were both synthesized by *in situ* conversion of the corresponding salts in the reaction with phenylisothiocyanate 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

ISSN 2308-8303 (Print) / 2518-1548 (Online)

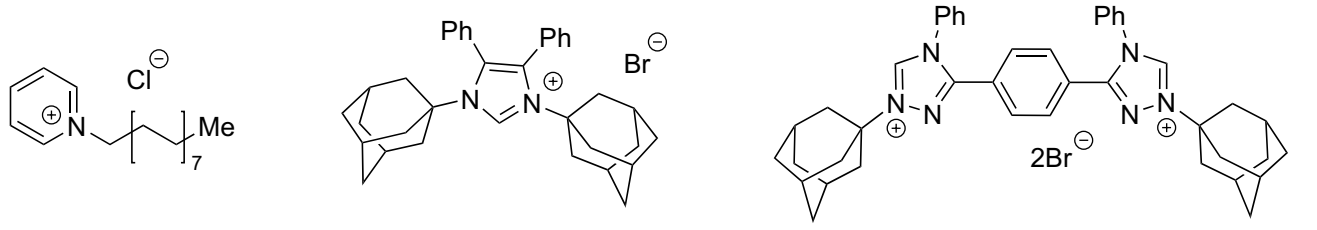


Scheme 5. The synthesis of zwitterionic compounds **10**, **11** from azolium salts

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

Compound[a]	Concentration, %	The diameter of the growth inhibition zones[b] (n = 3), mm				
		E. coli 67	*S. aureus* 209 P	*M. luteum* VKM B-868	*C. tenuis* VKM Y-70	*A. niger* VKM F-1119
1a	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
1b	0.5	0	0	0	0	8.0 ± 0.2
	0.1	0	0	0	0	0
1c	0.5	0	0	0	0	7.0 ± 0.1
	0.1	0	0	0	0	0
3a	0.5	0	10.0 ± 0.3	10.0 ± 0.1	0	10.0 ± 0.1
	0.1	0	0	0	0	0
3b	0.5	0	0	0	0	7.0 ± 0.2
	0.1	0	0	0	0	0
4a [10]	0.5	15.4 ± 0.4	21.4 ± 0.2	23.0 ± 0.3	18.0 ± 0.2	9.7 ± 0.2
	0.1	7.0 ± 0.1	15.0 ± 0.2	14.0 ± 0.3	12.0 ± 0.2	6.0 ± 0.1
4b	0.5	8.4 ± 0.2	10.0 ± 0.2	15.0 ± 0.4	10.0 ± 0.2	7.0 ± 0.2
	0.1	0	0	0	0	0
5	0.5	0	0	0	0	10.0 ± 0.1
	0.1	0	0	0	0	0
7a	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
7b	0.5	0	0	0	0	8.0 ± 0.2
	0.1	0	0	0	0	0
8	0.5	0	15.0 ± 0.3	12.0 ± 0.2	0	10.0 ± 0.1
	0.1	0	0	0	0	0
9	0.5	10.0 ± 0.2	15.0 ± 0.4	15.0 ± 0.2	0	0
	0.1	0	0	0	0	0
10	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
11	0.5	0	0	0	0	7.0 ± 0.1
	0.1	0	0	0	0	0
12 [10]	0.5	0	0	14.4 ± 0.3	0	10.0 ± 0.2
	0.1	0	0	12.0 ± 0.2	0	7.0 ± 0.1
13 [8]	0.5	0	22.3 ± 0.3	39.3 ± 0.2	0	15.6 ± 0.3
	0.1	0	19.6 ± 0.2	32.3 ± 0.3	0	10.0 ± 0.1
14 [8]	0.5	11.3 ± 0.2	23.6 ± 0.4	35.6 ± 0.2	0	0
	0.1	0	16.0 ± 0.2	24.0 ± 0.3	0	0

Notes: [a] compound **2** could not be studied due to its low solubility; [b] control values correspond to 0 mm



12 **13** **14**

Figure. The known compounds with a significant antimicrobial activity

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 31.2 μg mL^{-1}, MFC 62.5 μg mL^{-1} against the culture of *C. tenuis* and MIC 62.5 μg mL^{-1} against the culture of *A. niger*).

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

Compound[c]	Bacteria cultures					
	E. coli 67		*S. aureus* 209 P		*M. luteum* VKM B-868	
	MIC, μg mL^{-1}	MBC, μg mL^{-1}	MIC, μg mL^{-1}	MBC, μg mL^{-1}	MIC, μg mL^{-1}	MBC, μg mL^{-1}
1a	+	+	250.0	500.0	250.0	500.0
1b	+	+	+	+	250.0	500.0
1c	+	+	250.0	500.0	125.0	500.0
3a	+	+	+	+	7.8	15.6
3b	+	+	+	+	31.2	62.5
4a [10]	3.9	3.9	31.2	62.5	3.9	3.9
4b	+	+	+	+	250.0	500.0
5	+	+	+	+	+	+
7a	+	+	+	+	250.0	500.0
7b	+	+	250.0	500.0	250.0	500.0
8	+	+	31.2	62.5	31.2	62.5
9	125.0	250.0	31.2	62.5	15.6	62.5
10	+	+	+	+	+	+
11	+	+	+	+	7.8	N[d]
12 [10]	31.2	125	3.9	7.8	7.8	15.6

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

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Table 3. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC)[a],[b] of the compounds determined by method of serial dilutions (*Method B*)

Compound[c]	Fungi cultures			
	C. tenuis VKM Y-70		*A. niger* VKM F-1119	
	MIC, μg mL^{-1}	MFC, μg mL^{-1}	MIC, μg mL^{-1}	MFC, μg mL^{-1}
1a	15.6	31.2	+	+
1b	+	+	250.0	500.0
1c	125.0	250.0	500.0	N[d]
3a	7.8	15.6	125.0	250.0
3b	31.2	62.5	62.5	250.0
4a [10]	1.9	3.9	3.9	62.5
4b	500.0	N	500.0	N
5	+	+	500.0	N
7a	+	+	+	+
7b	250.0	500.0	250.0	N
8	250.0	500.0	500.0	N
9	125.0	250.0	+	+
10	+	+	+	+
11	+	+	62.5	N
12 [10]	3.9	7.8	7.8	62.5

Notes: [a] "+" means no antifungal effect was observed in the concentrations studied (growth of the microorganisms was observed); [b] control values correspond to "+"; [c] compound **2** could not be tested due to its low solubility; [d] no indicator of fungicidal 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 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-phenylthiocarboximides have been proven to be the most active.

Experimental part

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance II 400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectra) in DMSO-d_6 or $CDCl_3$ solution. The ^{1}H NMR and ^{13}C 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 $C_{35}H_{69}BrN_2$, %: 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. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.71 (6H, s, 2×CH_3C); 1.08 (52H, m, 26×CH_2C); 1.76 (4H, s, 2×CH_2CN); 4.20 (4H, s, 2×CH_2N); 7.49 (2H, s, C^{4,5}H$_{Im}$); 10.07 (1H, s, C^{2}HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 14.08 (CH_3C); 22.64 ($C^{2}H_2$C); 26.22 ($C^{3}H_2$C); 29.01 ($C^{4}H_2$C); 29.32 ($C^{5}H_2$C); 29.40 ($C^{6}H_2$C); 29.51 ($C^{7}H_2$C); 29.60, 29.62, 29.63, 29.66 ($C^{8\text{-}13}H_2$C); 30.32 (CH_2CCN);

31.87 (CH_2CN); 49.99 (CH_2N); 122.41 ($C^{4,5}_{Im}$); 136.48 (C^2N).

1,3-*Bis*(2,3,4-trifluorophenyl)imidazolium chloride (1b)

Step 1. N,N'-Bis(2,3,4-trifluorophenyl)glyoxaldiimine. The solution of 2,3,4-trifluoroaniline (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 **1A** 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 **1A** 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%. M. p. 244–246°C (water). Anal. Calcd for $C_{15}H_7ClF_6N_2$, %: C 49.40; H 1.93; Cl 9.72; N 7.68. Found, %: C 49.52; H 1.89; Cl 9.69; N 7.63. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 7.58–7.65 (2H, m, ArH); 7.92–7.97 (2H, m, ArH); 8.43 (2H, s, $C^{4,5}H$); 10.35 (1H, s, C^2HN).

1,3-*Bis*(2-trifluoromethylphenyl)imidazolium perchlorate (1c)

Step 1. N,N'-Bis(2-trifluoromethylphenyl)glyoxaldiimine. 40% Solution of glyoxal (4.85 g, 33.4 mmol) was added to the solution of 2-trifluoromethylaniline (4.66 g, 28.89 mmol) in isopropyl alcohol (18 mL). The mixture was stirred for 5 days at room temperature, the solution was evaporated and the resulting residue containing diimine **1A** was used without purification for the synthesis of salt **1c**.

Step 2. The cyclization reaction. Anhydrous zinc chloride (1.98 g, 14.5 mmol) and ethoxymethyl chloride (1.88 g, 21.7 mmol) were added successively to a solution of diimine **1A** in chloroform (40 mL), and the mixture was stirred at room temperature for 3 days. The solution was evaporated. The residue was extracted with hot water. The excess of sodium perchlorate was added to the water solution, and the colorless precipitate was filtered off.

Yield – 2.66 g (50% based on the starting aniline). M. p. 255–257°C (water). Anal. Calcd for $C_{17}H_{11}ClF_6N_2O_4$, %: C 44.71; H 2.43; Cl 7.76; N 6.13. Found, %: C 44.65; H 2.44; Cl 7.81; N 6.17. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 7.84–7.90 (3H, m, ArH); 7.92–7.95 (3H, m, ArH); 8.08–8.10 (2H, m, ArH); 8.14 (2H, d, J = 8.0 Hz, $C^{4,5}HN$); 10.08 (1H, s, C^2HN).

1,3-Dicetylbenzimidazolium bromide (2)

The mixture of benzimidazole (1.18 g, 10 mmol), cetyl bromide (6.41 g, 21 mmol) and sodium acetate (0.82 g, 10 mmol) in anhydrous dioxane (4 mL) was stirred at 100°C for 4 h. The solution was filtered from the inorganic precipitate in a hot state and evaporated to give colorless salt **2**, which was recrystallized from acetonitrile.

Yield – 2.6 g (40%). M. p. 116–118°C (acetonitrile). Anal. Calcd for $C_{39}H_{71}BrN_2$, %: C 72.30; H 11.05; Br 12.33; N 4.32. Found, %: C 72.42; H 11.03; Br 12.26; N 4.29. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.82 (6H, t, J = 6.8 Hz, 2×CH_3C); 1.18–1.20 (48H, m, 24×CH_2C); 1.24–1.40 (4H, m, 2×CH_2C); 1.94 (4H, t, J = 6.4 Hz, 2×CH_2CN); 4.47 (4H, t, J = 6.8 Hz, 2×CH_2N); 7.61–7.64 and 7.68–7.71 (4H, m, ArH); 11.31 (1H, s, C^2HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 14.19 (CH_3C); 22.75 (CH_2C); 26.63 (CH_2C); 29.13; 29.42; 29.46; 29.58; 29.62; 29.66; 29.72; 29.76 (CH_2C); 31.98 (CH_2CN); 47.79 (CH_2N); 113.21 (*ipso*-C); 127.20 ($C^{5,6}$); 131.28 ($C^{4,7}$) (Ar); 142.64 (C^2).

1,3-*Bis*(1,10-decylenebenzimidazolium) bromide (3a)

The solution of benzimidazole (1.50 g, 12.72 mmol) and 1,10-dibromodecane (1.91 g, 6.36 mmol) in *o*-dichlorobenzene (4 mL) was stirred at 130°C for 8 h, then anhydrous sodium acetate (1.04 g, 12, 72 mmol) was added, and the stirring was continued under the same conditions for 4 h. A precipitate was filtered off, the mother liquor containing 1,10-di(benzimidazol-1-yl)decane of type **3A** with the additional portion of 1,10-dibromodecane (1.91 g, 6.36 mmol) was stirred at 130°C for 8 h. Then acetonitrile (10 mL) was added, and the solution was refluxed for 24 h. A colorless precipitate was filtered off, washed with acetonitrile and hexane, dried and recrystallized from acetonitrile.

Yield – 4.0 g (93%). M. p. 122–124°C (acetonitrile). Anal. Calcd for $C_{34}H_{50}Br_2N_4$, %: C 60.54; H 7.47; Br 23.69; N 8.31. Found, %: C 60.68; H 7.41; Br 23.58; N 8.34. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 0.91 (10H, m, 5×CH_2C); 1.01 (14H, m, 7×CH_2C); 1.68 (8H, s, 4×CH_2CN); 4.27 (8H, s, 4×CH_2N); 7.34 (4H, s, $ArH^{5,6}$); 7.55 (4H, s, $ArH^{4,7}$); 10.62 (2H, s, C^2HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 26.07, 28.49, 28.76, 29.94 (CH_2C, CH_3C);

ISSN 2308-8303 (Print) / 2518-1548 (Online)

47.35 (CH_2N); 113.33 ($C^{4,7}$, Ar); 127.12 ($C^{5,6}$, Ar); 131.11 (*ipso*-C, Ar); 141.78 (C^2N).

1,3-*Bis*(1,10-decylene-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 $C_{36}H_{54}Br_2N_4$, %: C 61.54; H 7.75; Br 22.74; N 7.97. Found, %: C 61.64; H 7.76; Br 22.69; N 7.92. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 1.01–1.49 m (24H, $12CH_2C$); 1.81 (6H, s, $2\times CH_3C$); 3.06 (8H, s, $4\times CH_2CN$); 4.47 (8H, s, $4\times CH_2N$); 7.50–7.80 (4H, m, ArH); 7.80–8.04 (4H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 31.14, 33.67, 33.96, 33.04 (CH_2C+CH_3C); 52.22 (CH_2N); 118.58; 131.93; 136.23; 147.06 (C^2N).

1,3-*Bis*(1,10-decylene-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.

Yield – 1.20 g (32%). M. p. 124–127°C. Anal. Calcd for $C_{28}H_{50}Br_2N_4$, %: C 55.82; H 8.36; Br 26.52; N 9.30. Found, %: 55.88; H 8.32; Br 26.60; N 9.20. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 1.20 (10H, m, $5\times CH_2C$); 1.25 (6H, m, $3\times CH_2C$); 1.73 (8H, m, $4\times CH_2C$); 2.66 (6H, s, $2\times CH_3C$); 3.17 (8H, s, $4\times CH_2CN$); 4.13 (8H, s, $4\times CH_2N$); 7.60 (4H, s, $C^{4,5}HN$). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 9.87 (CH_3C); 25.78, 28.47, 28.72, 29.40 (CH_2C); 48.18 (CH_2N); 121.39 ($C^{4,5}$); 142.63 (C^2N).

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 $C_{18}H_{21}BrN_2O$, %: 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. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.58 (6H, m, CH_2 Ad); 1.89 (6H, m, CH_2 Ad); 2.10 (3H, m, CH Ad); 7.55 (2H, dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, ArH); 7.65 (1H, dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, ArH); 7.95 (2H, d, J = 7.6 Hz), 11.76 (1H, s, C^5HN).

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 $C_{18}H_{22}N_2O_2$, %: C 72.46; H 7.43; N 9.39. Found, %: C 72.38; H 7.40; N 9.50. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.48–1.66 (12H, m, CH_2 Ad); 2.00 (3H, m, CH Ad); 7.46 (2H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, ArH); 7.50 (1H, dd, J_1 = 7.2 Hz, J_2 = 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 $C_{15}H_{20}ClNO_4$, %: 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. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.75 (6H, s, CH_2 Ad); 2.30 (9H, s, CH_2+CH Ad); 8.16 (2H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, $C^{3,5}H_{pyr}$); 8.59 (1H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, C^4H_{pyr}); 9.31 (2H, d, J = 7.2 Hz, $C^{2,6}H_{pyr}$).

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 $C_{15}H_{20}IN$, %: 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 mother liquor was evaporated *in vacuo*, the residue was triturated with hexane. A pale yellow precipitate was filtered off, washed with hexane and dried.

Yield – 0.29 g (91%). M. p. 165°C. Anal. Calcd for $C_{24}H_{15}F_6N_3S$, %: 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. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 6.41 (2H, s, $C^{4,5}H_{Im}$); 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). ^{13}C 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^2N); 162.77; 163.79 (N*C*S).

1-*tert*-Butyl-3-phenyl-4-(4-bromophenyl)-1,2,4-triazolium-5-(N-phenylthiocarboximide) (11)

A mixture of 1-*tert*-butyl-4-(4-bromophenyl)-3-phenyl-1,2,4-triazolium perchlorate (0.30 g, 0.66 mmol) and anhydrous potassium carbonate (0.192 g, 1.39 mmol, 2.11 equiv) in anhydrous acetonitrile (3 mL) was stirred under nitrogen atmosphere at room temperature for 10 min, and then phenyl isothiocyanate (0.08 mL, 0.66 mmol, 1 equiv) was added. The mixture was additionally stirred at room temperature for 4 h. A precipitate of inorganic salts was filtered off and washed with anhydrous acetonitrile. The filtrate was evaporated *in vacuo*, the solid residue was triturated with hexane. A pale yellow precipitate was filtered off, washed with hexane and dried.

Yield – 0.27 g (84%). M. p. 198–199°C (benzene). Anal. Calcd for $C_{25}H_{23}BrN_4S$, %: C 61.10; H 4.72; Br 16.26; N 11.40; S 6.52. Found, %: C 61.31; H 4.54; Br 16.22; N 11.44; S 6.49. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 2.00 (9H, s, *t*Bu); 7.02–7.57 (14H, m, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz), δ, ppm: 28.7 (CH_3C, *t*Bu); 67.4 (*ipso*-C, *t*Bu); 121.4; 123.7; 129.2; 128.6; 128.8; 129.1; 131.1; 131.9; 123.1; 125.4; 132.8; 149.9; 150.6; 150.0 (C^3_{triaz}), 166.5 (N*C*S).

Procedures for assessing the antimicrobial activities of the compounds synthesized

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 10^9 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 – 10^6 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

ISSN 2308-8303 (Print) / 2518-1548 (Online)

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

1. Gravel, J.; Schmitzer, A. R. Imidazolium and benzimidazolium-containing compounds: from simple toxic salts to highly bioactive drugs. *Org. Biomol. Chem.* **2017,** *15* (5), 1051–1071. https://doi.org/10.1039/C6OB02293F.
2. Pernak, J.; Sobaszkiewicz, K.; Mirska, I. Anti-microbial activities of ionic liquids. *Green Chem.* **2003,** *5* (1), 52–56. https://doi.org/10.1039/B207543C.
3. Garcia, M. T.; Ribosa, I.; Perez, L.; Manresa, A.; Comelles, F. Aggregation Behavior and Antimicrobial Activity of Ester-Functionalized Imidazolium- and Pyridinium-Based Ionic Liquids in Aqueous Solution. *Langmuir* **2013,** *29* (8), 2536–2545. https://doi.org/10.1021/la304752e.
4. Cancemi, P.; Buttacavoli, M.; D'Anna, F.; Feo, S.; Fontana, R. M.; Noto, R.; Sutera, A.; Vitale, P.; Gallo, G. The effects of structural changes on the anti-microbial and anti-proliferative activities of diimidazolium salts. *New J. Chem.* **2017,** *41* (9), 3574–3585. https://doi.org/10.1039/C6NJ03904A.
5. Achar, G.; Agarwal, P.; Brinda, K. N.; Małecki, J. G.; Keri, R. S.; Budagumpi, S. Ether and coumarin–functionalized (benz)imidazolium salts and their silver(I)–N–heterocyclic carbene complexes: Synthesis, characterization, crystal structures and antimicrobial studies. *J. Organomet. Chem.* **2018,** *854*, 64–75. https://doi.org/10.1016/j.jorganchem.2017.11.005.
6. Turkyilmaz, M.; Uluçam, G.; Aktaş, Ş.; Okan, S. E. Synthesis and characterization of new N-heterocyclic carbene ligands: 1,3-Bis(acetamide)imidazol-3-ium bromide and 3-(acetamide)-1-(3-aminopropyl)-1*H*-imidazol-3-ium bromide. *J. Mol. Struct.* **2017,** *1136*, 263–270. https://doi.org/10.1016/j.molstruc.2017.02.022.
7. Liu, L.; Huang, Y.; Riduan, S. N.; Gao, S.; Yang, Y.; Fan, W.; Zhang, Y. Main-chain imidazolium oligomer material as a selective biomimetic antimicrobial agent. *Biomaterials* **2012,** *33* (33), 8625–8631. https://doi.org/10.1016/j.biomaterials.2012.08.006.
8. Korotkikh, N. I.; Shvaika, O. P.; Kiselyov, A. V.; Knishevitsky, A. V.; Glinyana, N. V.; Marichev, K. A.; Novikov, V. P.; Lubenets, V. I.; Iskiv, O. P.; Moskalenko, N. I.; Komarovska-Porokhnyavets, O. Z. Antymikrobna aktyvnist proto- ta metalo-karbenovykh spoluk riadu azoliv ta azyniv [Antimicrobial activity of proto- and metal-carbene compounds of the azole and azine series, *in Ukrainian*]. *Visnyk Natsionalnoho universytetu "Lvivska politekhnika"* **2008**, 622, 3–6.
9. Kiselyov, A. V.; Knishevitsky, A. V.; Rayenko, G. F.; Korotkikh, N. I.; Pekhtereva, T. M.; Shvaika, O. P.; Klimnyuk, S. I.; Pokryshko, O. V.; Gryschuk, B. D. Synthesis and antimicrobial activity of some aromatic derivatives of azoles. *Journal of Organic and Pharmaceutical Chemistry* **2009**, *7* (1), 59–63.
10. Marichev, K. A.; Glinyana, N. V.; Korotkikh, N. I.; Shvaika, O. P.; Kiselyov, A. V.; Knishevitsky, A. V.; Dudarenko, G. V.; Komarovska-Porokhnyavets, O. Z. Novikov, V. P.; Lubenets, V. I. Antimicrobial activity of proto- and metal-carbene compounds of azoles and pyridine. *Journal of Organic and Pharmaceutical Chemistry* **2011**, *9* (3), 72–79.
11. Marichev, K. O. Syntez i vlastyvosti novykh typiv karbenoidiv azolnoho riadu. Makrotsyklichni i polimerni systemy [Synthesis and properties of new types of azole carbenoids. Macrocyclic and polymer systems, *in Ukrainian*]. Ph.D. Thesis, L. M. Litvinenko Institute of Physical Organic and Coal Chemistry of the National Academy of Sciences of Ukraine, Donetsk, 2012.
12. Saberov, V. Sh.; Marichev, K.A.; Korotkikh, N. I.; Shvaika, O. P.; Rodik, R.V.; Drapaylo, A.B.; Pekhtereva, T.M.; Komarovska-Porokhnyavets, O. Z.; Lubenets, V. I.; Novikov, V.P. Synthesis and antimicrobial activity of precarbene and metal-carbene compounds of the imidazole series. *Journal of Organic and Pharmaceutical Chemistry* **2014**, *12* (2), 36–43. https://doi.org/10.24959/ophcj.14.790.
13. Glinyana, N. V.; Saberov, V. Sh.; Knishevitsky, A. V.; Korotkikh, N. I.; Shvaika, O. P.; Papayanina, O. S.; Komarovska-Porokhnyavets, O. Z.; Lubenets, V. I.; Novikov, V. P. Synthesis and antimicrobial activity of metal-carbene compounds of the 1,2,4-triazole series. *Journal of Organic and Pharmaceutical Chemistry* **2014**, *12* (2), 44–52. https://doi.org/10.24959/ophcj.14.793.
14. Korotkikh, N. I.; Rayenko, G. F.; Saberov, V. Sh.; Derevenets, M. O. 1-Adamantylzamishcheni tsyklichni formamidyniievi soli [1-Adamantyl substituted cyclic formamidinium salts, *in Ukrainian*]. Ukraine patent 141798, April 27, 2020.
15. Korotkikh, N. I.; Rayenko, G. F.; Saberov, V. Sh.; Derevenets, M. O. Sposib syntezu alifatychnykh pokhidnykh tsyklichnykh formamidyniievykh solei [Method for the preparation of aliphatic derivatives of cyclic formamidinium salts, *in Ukrainian*]. Ukraine patent 141799, April 27, 2020.
16. Glinyanaya, N. V.; Rayenko, G. F.; Korotkikh, N. I.; Rusanov, E. B.; Ryabitsky, A. B.; Shvaika, O. P. Halogen-containing heteroaromatic carbenes of the 1,2,4-triazole series and their transformations. *RSC Adv.* **2021,** *11* (49), 30841–30848. https://doi.org/10.1039/D1RA04337D.
17. Pro zatverdzhennia metodychnykh vkazivok "Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ" [Guidlines "Determination of the susceptibility of microorganisms to antibacterial drugs", *in Ukrainian*]. https://zakon.rada.gov.ua/rada/show/v0167282-07#Text (accessed February 11, 2022), Ministry of Health of Ukraine, Order #167, 05.04.2007.
18. Harkavenko, T. O.; Gorbatyuk, O. I.; Kozytska, T. G.; Andriyashchuk, V. O.; Harkavenko, V. M.; Dybkova, S. M.; Azirkin, I. B. *Metodychni vkazivky shchodo vyznachennya chutlyvosti mikroorhanizmiv do antybakterial'nykh preparativ* [Guidelines for determining the susceptibility of microorganisms to antimicrobial drugs, *in Ukrainian*]; State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise: Kyiv, 2021.

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.

ISSN 2308-8303 (Print) / 2518-1548 (Online)