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**Chemistry**

# SYNTHESIS, ANTIBACTERIAL, ANTIFUNGAL, AND ANTIOXIDANT ACTIVITY OF NEW (BENZO)IMIDAZO [2,1-*B*][1,3] THIAZINE QUATERNARY SALTS

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**Abstract.** A series of quaternary salts – 1-phenacyl(4-chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo [4,5] imidazo [2,1-*b*][1,3] thiazinium bromides **4a-f** were obtained. Their structures were rigorously proven by the methods of $^{1}$H NMR, $^{13}$C NMR spectroscopy, and chromatography-mass spectrometry. All obtained compounds were tested in *in vitro* experiments for antibacterial, antifungal, and antioxidant activity. Bioscreening results showed that 1-(4-chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo [4,5] imidazo [2,1-*b*][1,3] thiazinium bromide **4f** exhibits the highest antibacterial activity against the strain of gram-positive bacteria *Staphylococcus aureus* ATCC 25923 at a concentration of 125 μg/mL, and salts **4a, c, f** – against strains of gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 at a concentration of 62.5 μg/mL. In turn, salt **4c** demonstrated the best rate of radical inhibition at the level of 69.3 %.

**Keywords:** (benzo)imidazo [2,1-*b*][1,3] thiazines, acetophenones, quaternary salts, antibacterial, antifungal, antioxidant activity.

## 1. Introduction

The trends of modern fine organic synthesis are closely related to the implementation of approaches to the creation of new heterocyclic structures, which are known to contain several biophoric fragments. An attractive option for solving such a problem is the construction of hybrid molecules by effective combination of biopromising molecular platforms. A prominent place among the latter is occupied by azole-azine systems[1–5], which include functional imidazo [2,1-*b*][1,3] thiazine derivatives that are suitable for the construction of hybrid structures. Among the representatives of this type of compounds, substances with antitumor[6], antiviral[7], antioxidant[8], antifungal[9], and antiparasitic[10] effects, cytotoxic effects on various types of F2408 and 5RP7 cells[11] were found. The imidazothiazine ring is also an important pharmacophore fragment of inhibitors of *Mycobacterium tuberculosis* (MIC 16 μg/mL)[12–14], inhibitors of the cannabinoid-activated protein GPR18[15], and reversible inhibitors of TbAdoMetDC of African trypanosomiasis[16].

Recently, we developed effective methods for the synthesis of a series of new derivatives of the azole-azine type, 6,7-dihydro-5*H*-imidazo [2,1-*b*][1,3] thiazines and their benzoannelated analogs incorporating pyridinyloxy- or 1,2,3-triazole pharmacophore fragments. Hybrid imidazo-thiazine-pyridine and imidazo-thiazine-triazole compounds obtained for the first time showed moderate antibacterial, antifungal, and anti-inflammatory effects in experimental *in vitro* studies[17–19]. The analysis of literature sources clearly showed that acetophenone derivatives are no less interesting biologically active compounds which are characterized by diverse biological activity, *e.g.*, methyl-substituted 2-hydroxyacetophenones and 4-alkoxyacetophenones inhibit *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* at a minimum inhibitor concentration of 12.5 μg/mL[20–22], acetophenone benzoylhydrazones and acetylphenylpyrrolidinediones have shown promising antioxidant and cytotoxic activity[23, 24], and 4-(2-propylthio) acetophenone oxime and α-(phenylselanyl)acetophenones are potential antidepressants and anti-inflammatory agents[25, 26]. A preparative method of producing the active ingredient of the broad-spectrum fungicide Tilt was developed based on substituted acetophenones. Thus, it seemed reasonable to focus our efforts on some convenient synthetic transfor-

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mations of functionalized derivatives of imidazo [2,1-*b*] thiazine systems. In particular, the combination of such pharmacophores as the imidazo [2,1-*b*][1,3] thiazine framework and the phenacyl fragment can be considered as an effective option for the construction of new hybrid structures with a potential biological effect. Therefore, the purpose of this work was to obtain new acetylphenyl-substituted (benzo)imidazo [2,1-*b*] thiazines and to study their biological activity.

## 2. Experimental

### 2.1. Materials and Methods

Chemical reagents for this study were provided by the Enamine Ltd. All reagents were chemically pure and used without further purification. The solvents were purified according to standard procedures.

$^{1}$H and $^{13}$C NMR spectra were recorded by a Varian VXR-400 spectrometer (400 and 126 MHz, respectively) in pulsed Fourier mode in DMSO-$d_6$ and in $CDCl_3$, the internal standard TMS. Mass spectra were recorded by an Agilent LC/MSD SL instrument, Zorbax SB-C18 column, 4.6 × 15 mm, 1.8 μm (PN 82(c)75-932), DMSO-$d_6$ solvent, electrospray ionization at atmospheric pressure. Elemental analysis was performed by a PerkinElmer CHN Analyzer 2400 series in the analytical laboratory of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine. Melting points were determined on a Kofler bench and are uncorrected.

### 2.2. General Procedure for the Synthesis of Quaternary Salts of 3-Hydroxy-3,4-Dihydro-2H-(Benz)Imidazo [2,1-b][1,3] Thiazine 4a-f

A mixture of imidazothiazine **1a-c** (1.0 mmol) and phenacyl bromide **2a,b** (1.2 mmol) in 1 mL of DMF was heated at 423 K for 30 min. The reaction mixture was cooled; the formed precipitate was filtered off, washed with DMF (0.5 mL), diethyl ether (1 mL), and dried. Salts **4a-f** were obtained as white and yellow powders.

#### 2.2.1. 1-Phenacyl-6-hydroxy-6,7-dihydro-5*H*-imidazo [2,1-*b*][1,3] thiazinium bromide 4a

Yield: 73 %, mp 537–538 K. Crystallize from ether. $^{1}$H NMR spectrum, DMSO-$d_6$, δ, ppm (*J*, Hz): 3.56–3.60 m (2H, $SCH_2$), 4.23–4.34 m (2H, $NCH_2$), 4.56–4.59 m (1H, CH), 5.94–5.96 m (3H, $CH_2$+OH), 7.71–7.74 m (3H, $2CH_{imidazol.}$+Ar), 7.80–7.82 m (2H, Ar), 8.09–8.12 m (2H, Ar). $^{13}$C NMR spectrum, DMSO-$d_6$, δ, ppm: 32.4 ($C^7$), 52.1 ($C^5$), 54.3 ($CH_2$), 58.5 ($C^6$), 123.4 ($C^2$), 123.9 ($C^3$), 129.6, 130.8, 132.7, 140.0 ($C_{arom.}$), 142.3 ($C^{8a}$), 190.2 (C=O). Mass spectrum, *m/z*: 276 $[M+H]^+$. Found, %: C 47.37; H 4.46; N 7.75. $C_{14}H_{16}BrN_2O_2S$. Calculated, %: C 47.19; H 4.49; N 7.86.

#### 2.2.2. 1-Phenacyl-2,3-diphenyl-6-hydroxy-6,7-dihydro-5*H*-imidazo [2,1-*b*][1,3] thiazinium bromide 4b

Yield: 55 %, mp 448–449 K. Crystallize from ether. $^{1}$H NMR spectrum, DMSO-$d_6$, δ, ppm (*J*, Hz): 3.61–3.66 m (2H, $SCH_2$), 3.88–3.94 m (1H, $NCH_2$), 4.24–4.29 m (1H, $NCH_2$), 4.61–4.65 m (1H, CH), 5.79–5.82 m (2H, $CH_2$), 5.97 s (1H, OH), 7.42–7.49 m (6H, Ar), 7.63–7.66 m (4H, Ar), 7.95–8.05 m (5H, Ar). $^{13}$C NMR spectrum, DMSO-$d_6$, δ, ppm: 32.4 ($C^7$), 50.9 ($C^5$), 51.6 ($CH_2$), 58.1 ($C^6$), 124.7 ($C^2$), 125.0 ($C^3$), 128.3, 128.9, 129.0, 129.2, 129.6, 130.1, 130.3, 130.4, 130.6, 131.0, 131.4, 132.3 ($C_{arom.}$), 142.7 ($C^{8a}$), 190.2 (C=O). Mass spectrum, *m/z*: 428 $[M+H]^+$. Found, %: C 61.70; H 4.69; N 5.41. $C_{26}H_{24}BrN_2O_2S$. Calculated, %: C 61.41; H 4.72; N 5.51.

#### 2.2.3. 1-Phenacyl-6-hydroxy-6,7-dihydro-5*H*-benzo[4,5]imidazo[2,1-*b*][1,3]thiazinium bromide 4c

Yield: 87 %, mp 526–527 K. Crystallize from ether. $^{1}$H NMR spectrum, DMSO-$d_6$, δ, ppm (*J*, Hz): 3.53–3.57 m (1H, $SCH_2$), 3.69–3.72 m (1H, $SCH_2$), 3.44–3.47 m (1H, $NCH_2$), 4.58–4.61 m (1H, $NCH_2$), 4.73–4.75 m (1H, CH), 6.08 s (1H, OH), 6.27–6.38 m (2H, $CH_2$), 7.56–7.63 m (2H, Ar), 7.66–7.69 m (2H, Ar), 7.79–7.83 m (1H, Ar), 7.94–8.19 m (4H, Ar). $^{13}$C NMR spectrum, DMSO-$d_6$, δ, ppm: 33.1 ($C^2$), 49.5 ($C^4$), 52.0 ($CH_2$), 58.2 ($C^3$), 112.2 ($C^6$), 112.5 ($C^9$), 125.9 ($C^7$), 126.5 ($C^8$), 129.2 ($C^{5a}$), 129.5 ($C^{9a}$), 132.3, 132.9, 134.1, 135.3 ($C_{arom.}$), 151.6 ($C^{8a}$), 190.0 (C=O). Mass spectrum, *m/z*: 326 $[M+H]^+$. Found, %: C 53.42; H 4.39; N 7.04. $C_{18}H_{18}BrN_2O_2S$. Calculated, %: C 53.20; H 4.43; N 6.90.

#### 2.2.4. 1-(4-Chlorophenacyl)-6-hydroxy-6,7-dihydro-5H-imidazo[2,1-b][1,3]thiazinium bromide 4d

Yield: 78 %, mp 532–533 K. Crystallize from ether. $^{1}$H NMR spectrum, DMSO-$d_6$, δ, ppm (*J*, Hz): 3.55–3.59 m (2H, $SCH_2$), 4.23–4.28 m (2H, $NCH_2$), 4.56–4.58 m (1H, CH), 5.93–5.96 m (3H, $CH_2$+OH), 7.70–7.74 m (3H, $2CH_{imidazol.}$+Ar), 7.80–7.83 m (1H, Ar), 8.09–8.11 m (2H, Ar). $^{13}$C NMR spectrum, DMSO-$d_6$, δ, ppm: 32.3 ($C^7$), 52.1 ($C^5$), 54.2 ($CH_2$), 58.5 ($C^6$), 123.4 ($C^2$), 123.9 ($C^3$), 129.7, 130.8, 132.7, 140.0 ($C_{arom.}$), 142.3 ($C^{8a}$), 190.2 (C=O). Mass spectrum, *m/z*: 310 $[M+H]^+$. Found, %: C 43.27; H 3.80; N 7.25. $C_{14}H_{15}ClBrN_2O_2S$. Calculated, %: C 43.02; H 3.84; N 7.17.

### 2.2.5. 1-(4-Chlorophenacyl)-2,3-diphenyl-6-hydroxy-6,7-dihydro-5H-imidazo [2,1-b][1,3] thiazinium bromide 4e

Yield: 53 %, mp 442–443 K Crystallize from ether. $^{1}$H NMR spectrum, DMSO-$d_6$, δ, ppm (*J*, Hz): 3.61–3.66 m (2H, $SCH_2$), 3.88–3.96 m (1H, $NCH_2$), 4.25–4.31 m (1H, $NCH_2$), 4.62–4.64 m (1H, CH), 5.79–5.81 m (2H, $CH_2$), 5.99 s (1H, OH), 7.44–7.49 m (6H, Ar), 7.64–7.66 m (4H, Ar), 7.95–8.04 m (4H, Ar). $^{13}$C NMR spectrum, DMSO-$d_6$, δ, ppm: 32.1 ($C^7$), 50.4 ($C^5$), 51.8 ($CH_2$), 58.2 ($C^6$), 124.5 ($C^2$), 124.6 ($C^3$), 128.3, 128.7, 129.1, 129.2, 129.5, 130.1, 130.2, 130.3, 130.6, 131.1, 131.8, 132.1 ($C_{arom.}$), 142.5 ($C^{8a}$), 190.1 (C=O). Mass spectrum, *m/z*: 462 $[M+H]^+$. Found, %: C 57.77; H 4.20; N 5.29. $C_{26}H_{23}ClBrN_2O_2S$. Calculated, %: C 57.56; H 4.24; N 5.16.

### 2.2.6. 1-(4-Chlorophenacyl)-6-hydroxy-6,7-dihydro-5H-benzo[4,5]imidazo[2,1-b][1,3]thiazinium bromide 4f

Yield: 81 %, mp 500–501 K. Crystallize from ether. $^{1}$H NMR spectrum, DMSO-$d_6$, δ, ppm (*J*, Hz): 3.53–3.57 m (1H, $SCH_2$), 3.69–3.72 m (1H, $SCH_2$), 4.43–4.47 m (1H, $NCH_2$), 4.56–4.60 m (1H, $NCH_2$), 4.73–4.76 m (1H, CH), 6.06 s (1H, OH), 6.26–6.36 m (2H, $CH_2$), 7.56–7.63 m (2H, Ar), 7.75–7.77 m (2H, Ar), 7.92–7.98 m (2H, Ar), 8.17–8.19 m (2H, Ar). $^{13}$C NMR spectrum, DMSO-$d_6$, δ, ppm: 32.5 ($C^2$), 49.1 ($C^4$), 51.4 ($CH_2$), 57.7 ($C^3$), 111.7 ($C^6$), 112.0 ($C^9$), 125.4 ($C^7$), 126.0 ($C^8$), 129.2 ($C^{5a}$), 130.6 ($C^{9a}$), 131.8, 132.3, 132.4, 139.7 ($C_{arom.}$), 151.1 ($C^{8a}$), 189.7 (C=O). Mass spectrum, *m/z*: 360 $[M+H]^+$. Found, %: C 48.86; H 3.90; N 6.20. $C_{18}H_{17}ClBrN_2O_2S$. Calculated, %: C 49.09; H 3.86; N 6.36.

## 2.3. Antimicrobial Activity

The study of the antibacterial and antifungal effect of obtained compounds involved the micromethod of two-fold serial dilutions in a liquid nutrient medium[27]. Museum strains of bacteria and fungi of the Department of Microbiology and Virology of the Bukovinian State Medical University and I. Mechnikov Institute of Microbiology and Immunology of National Academy of medical sciences of Ukraine were used for the research. The minimum bacteriostatic (MBsC) and bactericidal (MBcC) or fungistatic (MFsC) and fungicidal (MFcC) concentrations of imidazothiazinium salts **4a-f** were determined in relation to reference bacterial strains (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10702, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* 410) and fungi of the genus (*Aspergillus niger* K 9, *Candida albicans* ATCC 885/653). 0.05 mL of a 4-hour culture of microorganisms was added to sterile 96-well polystyrene tablets (for fungi, 103 CFU/mL was used in Sabouraud liquid medium, and for bacteria, 1 mL of meat-peptone broth contained 105 CFU/mL). A suspension of the studied microorganisms (inoculum) was prepared from a day-old culture. Several isolated colonies of the same type were selected with a culture loop, a small amount of material was transferred to a test tube with a sterile physiological solution, and using a DEN-1 Biosan densitometer, a microorganism suspension of 1.5 × 108 CFU/mL was obtained which is comparable to the McFarland turbidity standard of 0.5. Then, no later than 15 minutes, the necessary working microbial suspension was obtained by tenfold dilutions in the nutrient medium. Solutions of the studied compounds **4a-f** for the micromethod of serial dilutions were prepared at a concentration of 1000 μg/mL, using DMSO as a solvent. The starting working solutions were stored at a temperature not higher than 20 °C. 0.05 mL of the matrix solution of the test substance was added to the first well, after mixing, 0.05 mL was transferred to the following well of the first row, thus obtaining a dilution from 500 μg/mL to 0.24 μg/mL. After that, the tablets with bacterial cultures were placed in a humid chamber in a thermostat at 37 °C and incubated for 24 hours (for fungi, at 28 °C for 48 hours, respectively).

The lowest concentration of the investigated substance in the presence of which no culture growth was observed was taken as the bacteriostatic or fungistatic concentration (MBsC, MFsC). The minimum bactericidal or fungicidal concentrations (MBcC, MFcC) were determined by sowing the contents of the wells of the tablet with dilutions onto the appropriate dense nutrient media (meat-peptone agar for bacteria, dense Sabouraud medium for yeast-like fungi). The minimum bactericidal (fungicidal) concentration of the studied compounds was established according to the growth results in the appropriate media. All experiments were accompanied by appropriate control tests (control of the medium for sterility, culture growth in a medium without a compound, culture growth in a medium with a solvent). To obtain reliable results, the experiments were performed three times with each concentration of the compound and the studied culture of microorganisms.

## 2.4. Antioxidant Activity (DPPH Analysis)

Antioxidant activity of the synthesized compounds was evaluated by the analysis of inhibition of diphenylpicrylhydrazide (DPPH) radicals according to the technique in[28]. 1 mL of DPPH solution (8 mg/100 mL) was added to the methanol solutions of the studied compound and of ascorbic acid as a standard and left at room temperature in a dark place for 1 hour. The absorbance value was determined at 517 nm using a UV-

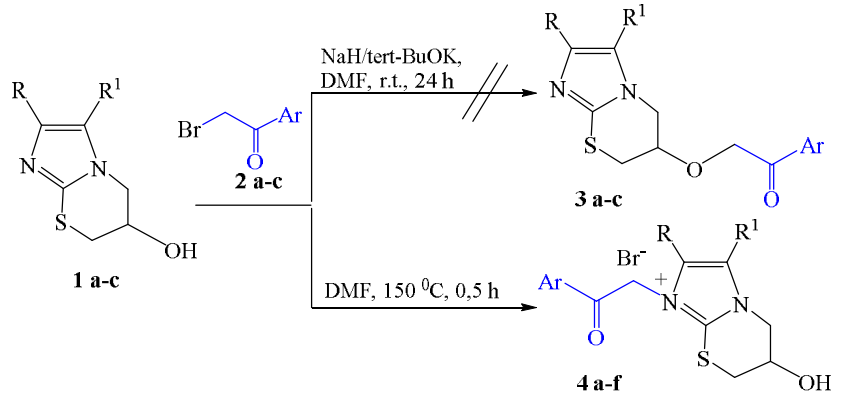
1800 spectrophotometer (Shimadzu, Japan). Each sample was analyzed in triplicate. The inhibition percentage was calculated relative to the standard:

$$I\,\% = ((A_{blank} - (A_{sample+DPPH} - A_{sample}))/ A_{blank} \cdot 100\,\%,$$

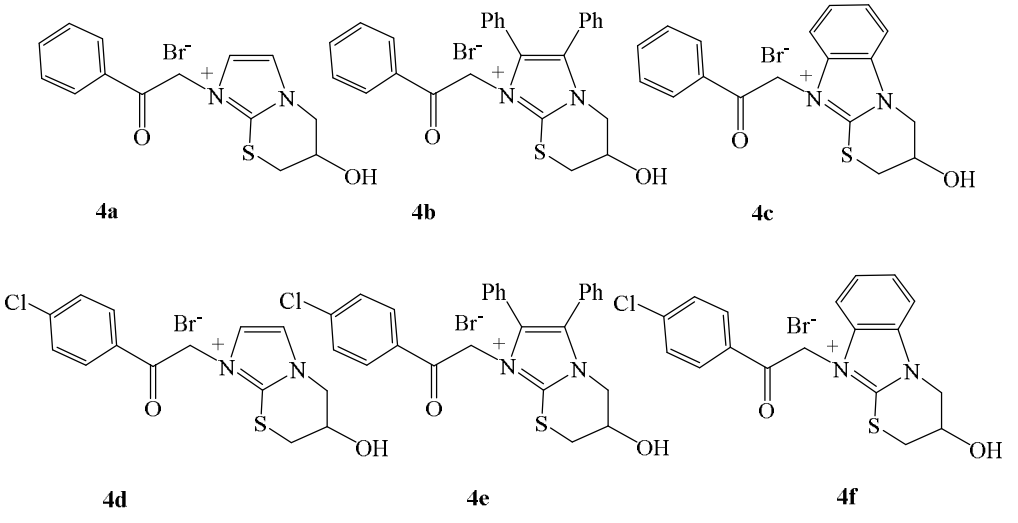
where $A_{blank}$ is the absorption of the standard; $A_{sample+DPPH}$ is the absorption of the studied compound after 60 min of incubation with DPPH solution; $A_{sample}$ is the absorption of the studied compound without DPPH solution.

## 3. Results and Discussion

One of the powerful tools for structural modification of heterocyclic compounds is the alkylation reaction of basic scaffolds, which allows significant diversification their chemical variety. Hydroxy(benzo) imidazo [2,1-*b*][1,3] thiazines **1a-c** were chosen as starting materials for the synthesis of imidazothiazines functionalized in the hydrogenated thiazine cycle, prone to further post-functionalization (Scheme). These compounds were obtained by the reaction of (benz) imidazoline thiones with 2-chloromethyloxirane by modified methods.[17] Previously, it was shown on the example of 6-hydroxy-2-nitro-6,7-dihydro-5*H*-imidazo [2,1-*b*][1,3] thiazines that in a DMF solution in the presence of NaH, they are smoothly alkylated by benzyl halides on the oxygen atom with the formation of respective benzyloxy derivatives, among which compounds with antituberculosis[29] and antitrypanosomal activity[14] were found. That is why it seemed advisable to test 6-hydroxyimidazo [2,1-*b*][1,3] thiazines **1a,b,** and their benzoannelated analogue **1c** in a reaction with the functional alkylating reagent bromomethylenearylketone **2a** to synthesize O-alkylation products **3a-c**. The reaction in dry DMF in the presence of a base at room temperature for 24 h failed, however, to isolate the target compounds. No interaction at all occurred when *tert*-BuOK was used as a base, and the use of NaH led to the destruction of the thiazine nucleus.




**1-3**: R = R$^1$ = H (**a**); R = R$^1$ = Ph (**b**); RR$^1$ = (CH=CH)$_2$ (**c**);
**2**: Ar = Ph (**a**); Ar = 4-ClC$_6$H$_4$ (**b**)




**Scheme.** Alkylation reaction of 3-hydroxy-3,4-dihydro-2H-(benz)imidazo [2,1-b][1,3] thiazines **1a-c**

**Table.** Antimicrobial activity of 1-phenacyl(4-chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo[4,5]imidazo[2,1-*b*][1,3]thiazinium bromides 4a-f

| Compound | Gram-positive bacteria | | | | Gram-negative bacteria | | | | | | Fungi | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus ATCC 25923* | | *B. cereus ATCC 10702* | | *E. coli ATCC 25922* | | *P. aeruginosa ATCC 27853* | | *P. mirabilis 410 (BSMU)* | | *C. albicans ATCC 885/653* | | *A. niger K9* | |
| | MBsC | MBcC | MBsC | MBcC | MBsC | MBcC | MBsC | MBcC | MBsC | MBcC | MFsC | MFcC | MFsC | MFcC |
| 4a | 125 | 500 | 125 | 250 | 62.5 | 125 | 62.5 | 62.5 | 125 | 125 | 62.5 | 125 | 125 | 250 |
| 4b | 125 | 500 | 125 | 250 | 62.5 | 125 | 62.5 | 125 | 125 | 125 | 62.5 | 125 | 125 | 250 |
| 4c | 125 | 500 | 125 | 250 | 62.5 | 125 | 62.5 | 62.5 | 125 | 125 | 62.5 | 125 | 125 | 250 |
| 4d | 125 | 250 | 125 | 250 | 62.5 | 125 | 62.5 | 125 | 125 | 125 | 62.5 | 125 | 125 | 250 |
| 4e | 125 | 500 | 125 | 250 | 62.5 | 125 | 62.5 | 125 | 125 | 125 | 62.5 | 125 | 125 | 250 |
| 4f | 125 | 125 | 125 | 250 | 62.5 | 125 | 62.5 | 62.5 | 125 | 125 | 62.5 | 125 | 125 | 250 |
| (DMSO)* | + | | + | | + | | + | | + | | + | | + | |
| Control ** | 0.48 | 0.97 | 0.97 | 1.95 | 1.95 | 3.9 | 31.25 | 31.25 | 15.62 | 31.25 | 3.9 | 7.81 | 7.81 | 7.81 |

* The growth of microorganisms is observed

** Dekasan (decamethoxine solution 0.2 mg/mL) produced by “Yuria-Pharm”.

Studying the reactions of dioxyno [2,3-*f*][1,3] thiazino [3,2-*a*] benzimidazole with benzyl halides or halomethyl ketones, Orlov *et al.*[30] discovered the possibility of formation of the corresponding imidazothiazinium salts at elevated temperatures. It seemed thus appropriate to investigate imidazo [2,1-*b*][1,3] thiazines **1a-c** with two pronounced nucleophilic centers (N1 atom and OH group) in this type of transformation. It was established that these compounds react with bromoacetophenones **2a,b** when heated to 150°C in DMF in the absence of a base with the formation of quaternary salts, 1-phenacyl(4-chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo [4,5] imidazo [2,1-*b*][1,3] thiazinium bromides **4a-f**. The obtained result is evidence that, under such conditions, the alkylation of the more nucleophilic N1 atom of the imidazothiazole ring takes place, without any transformation involving the hydroxyl group.

The structure of the synthesized compounds was confirmed by the results of measurements of $^{1}$H NMR, $^{13}$C NMR, and chromatography-mass spectrometry. For instance, imidazothiazine derivatives **4a,d** show the signals of the benzene ring protons, which are identified in the range of 7.80–8.12 ppm, and for diphenyl substituted **4b,e** and their benzo analogues **4c,f,** these overlay the signals of phenylene protons. The diproton quartet of the endocyclic methylene group for compounds with imidazo [2,1-*b*][1,3] thiazine and 2,3-diphenylimidazo [2,1-*b*][1,3] thiazine scaffolds is identified at 5.79–5.96 ppm; for benzimidazo [2,1-*b*][1,3] thiazine derivatives it is shifted to a weak field and appears at 6.26–6.38 ppm, which reliably confirms the presence of a phenacyl substituent in the structure of the newly synthesized salts.

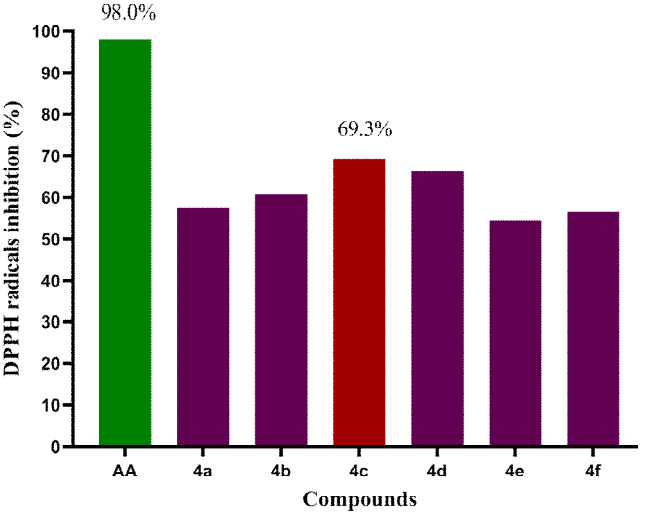
**Study of antimicrobial activity**

The results of screening studies showed that the tested compounds exhibit moderate antimicrobial activity with MBsC and MFsC ranging from 62.5 to 125 μg/mL. It was established that derivatives **4a-f** have an inhibitory effect on strains of bacteria *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and fungi *C. albicans* ATCC 885/653 at a concentration of 62.5 μg/mL, and the growth of bacteria such as *S. aureus* ATCC 25923, *B. cereus* ATCC 10702 and *A. niger* K 9 fungi are inhibited at a concentration of 125 μg/mL. The antimicrobial activity of the tested compounds does not significantly depend on the nature of the substituents in position 1.

It should be noted that the greatest inhibitory activity was observed for the 4-chlorophenacyl-substituted derivative **4f** against gram-positive bacteria *S. aureus* ATCC 25923 at a concentration of 125 μg/mL, and the salts with imidazothiazine **4a,c** and benzimidazothiazine **4f** nuclei against strains of gram-negative bacteria *P. aeruginosa* ATCC 27853 at a concentration of 62.5 μg/mL. At the same time, the presence of two bulky phenyl substituents in the imidazole cycle of the synthesized compounds **4b,e** contributes to a decrease in antibacterial activity against both gram-positive and gram-negative bacteria (Table).

**Study of antioxidant activity**

The synthesized compounds were evaluated for their ability to inhibit 1,1-diphenyl-2-picrylhydrazyl (DRPH) radicals[26]. Performed experimental studies included assessment of DPPH radical scavenging activity by derivatives **4a-f** (methanol solution, measurement after 60 min) at a concentration of 5 mM. This approach allows a quick search for potential hit compounds, saving time and quantities of substances. Ascorbic acid was used as a standard. Results of the screening of radical scavenging activity of 1-phenacyl(4-chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo[4,5] imidazo [2,1-*b*][1,3] thiazinium bromides **4a-f** that are presented in Figure demonstrate inhibition of DPPH radicals in the range from 54.5 to 69.3 %. The hit compound **4c** is worth noting, which showed the best rate of radical inhibition and is of interest for in-depth pharmacological research aimed at designing potential synthetic antioxidants.




Inhibition of DPPH radicals by salts **4a-f** at a concentration of 5 mM. Ascorbic acid was used as a standard (green). The highest antioxidant activity is compound **4c** (red)

## 4. Conclusions

Characteristics of the 6-hydroxy-6,7-dihydro-5*H*-(benzo)imidazo [2,1-*b*][1,3] thiazines and bromomethylenearyl ketones interaction were studied. The reaction in DMF without the presence of a base leads to quaternary salts, 1-phenacyl(4-chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo [4,5] imidazo [2,1-*b*][1,3] thiazinium bromides **4a-f**, which were investigated for antibacterial,

antifungal, and antioxidant activity. The highest antibacterial activity is shown by 1-(4chlorophenacyl)-6-hydroxy-6,7-dihydro-5*H*-benzo [4,5] imidazo [2,1-*b*][1,3] thiazinium bromide **4f** in relation to the strain of gram-positive bacteria *S. aureus* ATCC 25923 at a concentration of 125 μg/mL, as well as salts **4a, c, f** in relation to the strain of gram-negative bacteria *P. aeruginosa* ATCC 27853 at a concentration of 62.5 μg/mL. Additionally, the bromide **4c** demonstrated the best rate of free radical inhibition at the level of 69.3 %.

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## СИНТЕЗ, АНТИБАКТЕРІАЛЬНА, ПРОТИГРИБКОВА Й АНТИОКСИДАНТНА АКТИВНІСТЬ НОВИХ (БЕНЗО)ІМІДАЗО [2,1-B][1,3] ТІАЗИНОВИХ ЧЕТВЕРТИННИХ СОЛЕЙ

***Анотація.*** *Реакція 6-гідрокси-6,7-дигідро-2H-(бенз) імідазо [2,1-b][1,3] тіазинів з галогеновмісними ацетофенонами дала серію четвертинних солей 1-фенацил(4-хлорфенацил)-6-гідрокси-6,7-дигідро-5H-бензо[4,5] імідазо [2,1-b][1,3] тіазинію броміди* ***4a-f****, структуру яких чітко доведено методами* $^{1}$*H ЯМР,* $^{13}$*C ЯМР та хроматомас-спектрометрії. Усі отримані сполуки перевірено в експериментах in vitro на антибактеріальну, протигрибкову й антиоксидантну активність. Результати біоскринінгу показали, що 1-(4-хлорфенацил)-6-гідрокси-6,7-дигідро-5H-бензо [4,5] імідазо [2,1-b][1,3] тіазинію бромід* ***4f*** *демонструє найвищу антибактеріальну дію, активність щодо штаму грампозитивних бактерій Staphylococcus aureus ATCC 25923 у концентрації 125 мкг/мл, а солі* ***4a, c, f*** *щодо штамів грамнегативних бактерій Pseudomonas aeruginosa ATCC 27853 у концентрації 62,5 мкг/мл. Найкращий показник як інгібітор вільних радикалів продемонструвала сіль* ***4c*** *на рівні 69,3 %.*

***Ключові слова:*** *(бензо)імідазо [2,1-b][1,3] тіазини, ацетофенони, четвертинні солі, антибактеріальна, протигрибкова, антиоксидантна активність.*