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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 3-ARYLAMINOMETHYL-1-(2-OXO-2-ARYLETHYL)-6,7,8,9-TETRAHYDRO-*5H*-[1,2,4]TRIAZOLO[4,3-A] AZEPIN-1-IUM BROMIDES AND ARYL-(4-R^1-PHENYL-5,6,7,8-TETRAHYDRO-2,2A,8A-TRIAZACYCLOPENTA[*CD*]AZULEN-1-YLMETHYL)-AMINES

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The aim *of this work is to develop methods of synthesis of 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides and aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-ylmethyl)-amines and to study their antimicrobial activity against strains of gram-positive and gram-negative bacteria as well as yeast fungi.*

Materials and methods. *^{1}H NMR spectra were recorded on Bruker 400 spectrometer operating at frequency of 400 MHz. Antimicrobial activity of the compounds synthesized was evaluated by their minimum inhibitory concentration (MIC) values.*

Results and discussion. *The interaction of 3-arylaminomethyl-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepines with substituted phenacyl bromides produced novel 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides. The latter when refluxed in 10 % solution of NaOH gave aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-ylmethyl)-amines. The study of antimicrobial activity of the compounds obtained allowed to find derivatives which are active against C. albicans and S. aureus strains. Among the compounds tested 3-[(4^1-bromophenylamino)-methyl]-1-[2-(4-methoxyphenyl)-2-oxoethyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide* ***5cd*** *appeared to be more active than the reference drug Cefixime and displayed close antimicrobial activity as the antibiotic Linezolid.*

Conclusions. *It was found out that derivatives of 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides display broad spectrum of antimicrobial activity and are able to inhibit growth of both bacteria and fungi. S. aureus and C. albicans turned out to be the most sensitive strains to the compounds tested, MIC was in the range of 6.2-25.0 μg/mL. Gram-negative strains of microorganisms were less sensitive to the compounds evaluated and* ***5fa*** *was the most active derivative displaying antimicrobial activity at the concentration of 50.0 μg/mL. Antimicrobial activity of triazoloazepinium bromide derivatives was similar to that one of Linezolid and Fluconazole reference drugs and more pronounced than the activity of Cefixime.*

Hence, the data gathered evidence the feasibility of further study of the antimicrobial properties of the most active compounds in in vivo experiments aiming at assessment of the prospects for the creation of new effective and safe antimicrobial drugs based on them

Keywords: *3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides, antibacterial activity, in vitro tests, minimum inhibitory concentration*

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1. Introduction

Unreasonable usage of antimicrobial drugs leads to appearance and spread of microorganism strains which are resistant to antimicrobials [1, 2]. In addition to antimicrobial resistance spreading, existing drugs also have some drawbacks. Some of them display narrow spectrum of antimicrobial activity, unsatisfactory pharmacokinetic, high rate of side effects etc [3, 4]. These facts are the reasons that medicine is in urgent need of novel antimicrobial substances [5, 6]

The core of *5H*-[1,2,4]triazolo[4,3-a]azepine is a part of many compounds possessing analgesic, anxiolytic, anti-inflammatory and antitumor activities Additionally, structurally close to them 3-biphenyl-3*H*-imidazo[1,2-*a*]azepin-1-ium bromides display antibacterial and antifungal activity [7].

These compounds can be prepared by the condensation of 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-*a*]azepines with substituted phenacyl bromides in ethyl acetate.

The aim of this work is to develop methods of synthesis of 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides and aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-tri-

azacyclopenta[*cd*]azulen-1-ylmethyl)-amines and to study their antimicrobial activity against strains of gram-positive and gram-negative bacteria as well as yeast fungi. Previously one compound of 5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[*cd*]azulene series has been synthesized and tested to possess antitumor activity. Experiments carried out revealed its potency to inhibit the growth of cancerous leukemia cells of CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226 lines [8].

2. Planning (methodology) of the research

For planning research, the following algorithm of actions was developed:

I stage. Synthesis of selected series of compounds and establishment of its chemical characteristics.

II stage. Establishment of the minimum inhibitory concentration (MIC) of 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a] azepin-1-ium bromides and aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[*cd*]azulen-1-ylmethyl)-amines against bacteria and fungi.

In order to obtain the target 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides as the objects for further antibacterial studies, starting 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-*a*]azepines were first synthesized according to the method [9]. Aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[*cd*]azulen-1-ylmethyl)-amines were obtained by refluxing above mentioned *5H*-[1,2,4]triazolo[4,3-*a*]azepin-1-ium bromides in 10 % NaOH.

Antimicrobial activity screening of the compounds synthesized was performed by measuring their minimum inhibitory concentration (MIC) values.

3. Materials and methods

Chemical experiments. ^{1}H NMR spectra were recorded on a Bruker 400 (Germany) spectrometer in DMSO-d_6 solutions. ^{13}C NMR spectra on a Varian Mercury-400 100 MHz in DMSO-d_6 using tetramethylsilane as an internal standard. Chemical shifts were reported in ppm units using δ scale. The mass spectra were recorded on an Agilent 1200 LC/MSD SL instrument (Santa Clara, CA, USA). Elemental analysis was performed on a EuroVector EA-3000 instrument. Melting points were determined on a Kofler bench. All solvents and reagents were commercially available.

3-Arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines **3a-f** contain three nitrogen atoms which could be alkylated (two nitrogen atoms of the triazole moiety and one exocyclic nitrogen atom), thus three different products or their mixtures can be formed. Nevertheless, NaOH promoted cyclization of the alkylated products **5** into aryl-(4-R^1-phenyl)-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[*cd*]azulen-1-ylmethyl)-amines **6** unambiguously proves that the alkylation of amines **3a-f** proceeds only on the nitrogen atom at the position 1 of the heterocyclic system. Antimicrobial activity of the compounds synthesized was evaluated by their minimum inhibitory concentration (MIC) values.

3-Arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines (3 a-f) were prepared according to the known method [15] by the interaction of of 7-methoxy-3,4,5,6-tetrahydro-2*H*-azepine **1** with (4-R-phenylamino)-acetic acid hydrazide (**2 a-f**).

Synthesis of 3-arylaminomethyl-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-a]azepines quaternary salts (5bb,cd,dc,db,ea,eb,fa,fc)

A mixture of 3-arylaminomethyl-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepines **3a-f** (0.01 mol) and appropriate alkylating reagent **4a-e** (0.01 mol) was refluxed for 2 h in 80 mL of ethyl acetate and left overnight at room temperature. The obtained solid products were collected by filtration, washed with ethyl acetate, and recrystallized from the appropriate solvent.

3-[(4^1-Chlorophenylamino)-methyl]-1-[2-(4-chlorophenyl)-2-oxoethyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide (5bb). Yield 2.02 g (79 %). M.p.=145–147 °C (from propanol-2). Anal. Calcd. for $C_{22}H_{23}BrCl_2N_4O$. %: N 9.85. Found, %: N 9.71. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.80-1.97 (m, 6H, $(CH_2)_3$), 3.32 (m, 2H, 9-CH_2), 4.65 (m, 4H, NH$\underline{CH_2}$+5-CH_2), 6.46 (s, 2H, $COCH_2$), 6.74 (m, 1H, NH), 6.71 and 7.02 (d-d, 4H, C_6H_4, *J*=8.8 Hz), 7.85 and 8.17 (d-d, 4H, C_6H_4, *J*=8.0 Hz). MS m/z: 431.1 [M+].

3-[(4^1-Bromophenylamino)-methyl]-1-[2-(4-methoxyphenyl)-2-oxoethyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide (5cd). Yield 2.12 g (77 %). M.p.=204–205 °C (from ethanol). Anal. Calcd. for $C_{23}H_{26}Br_2N_4O_2$. %: N 10.2. Found, %: N 10.4. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.80–1.98 (m, 6H, $(CH_2)_3$), 3.24 (m, 2H, 9-CH_2), 3.92 (s, 3H, OCH_3), 4.59 (m, 2H, 5-CH_2), 4.65 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.32 (s, 2H, $COCH_2$), 6.70 (m, 1H, NH), 6.67 and 7.15 (d-d, 4H, C_6H_4, *J*=8.9 Hz), 7.07 and 8.07 (d-d, 4H, C_6H_4, *J*=8.8 Hz). MS m/z: 471.1 [M+].

***1-[2-(4-Chlorophenyl)-2-oxoethyl]-3-[(4^1-methoxyphenylamino)-methyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide* (*5db*)**. Yield 1.72 g (67 %). M.p.=175–176 °C (from propanol-2). Anal. Calcd. for $C_{23}H_{26}BrClN_4O_2$. %: N 11.1. Found, %: N 11.0. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.69–1.86 (m, 6H, $(CH_2)_3$), 3.26 (m, 2H, 9-CH_2), 3.64 (s, 3H, OCH_3), 4.46 (m, 2H, 5-CH_2), 4.60 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.00 (m, 1H, NH), 6.36 (s, 2H, $COCH_2$), 6.65 and 6.74 (d-d, 4H, C_6H_4, *J*=8.7 Hz), 7.71 and 8.10 (d-d, 4H, C_6H_4, *J*=8.4 Hz). MS m/z: 427.0 [M+].

***1-[2-(4-Bromophenyl)-2-oxoethyl]-3-[(4^1-methoxyphenylamino)-methyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide* (*5dc*)**. Yield 2.23 g (81 %). M.p.=211–212 Anal. Calcd. for $C_{23}H_{26}Br_2N_4O_2$. %: N 10.2. Found, %: N 10.3. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.69–1.86 (m, 6H, $(CH_2)_3$), 3.26 (m, 2H, 9-CH_2), 3.64 (s, 3H, OCH_3), 4.46 (m, 2H, 5-CH_2), 4.60 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.09 (m, 1H, NH), 6.38 (s, 2H, $COCH_2$), 6.66 and 7.72 (d-d, 4H, C_6H_4, *J*=9.0 Hz), 7.88 and 8.00 (d-d, 4H, C_6H_4, *J*=8.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.8, 23.7, 26.1, 28.8, 35.6, 39.0, 43.1, 52.6, 55.6, 112.3, 114.3, 126.1, 132.2, 134.7, 138.6, 142.2, 150.7, 155.0, 164.7, 188.4. MS m/z: 471.2 [M+].

***3-(p-Tolylaminomethyl)-1-(2-oxo-2-phenylethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides* (*5ea*)**. Yield 1.55 g (68 %). M.p.=198–199 °C (from propanol-2). Anal. Calcd. for $C_{23}H_{27}BrN_4O$. %: N 12.3. Found, %: N 12.4. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.70–1.86 (m, 6H, $(CH_2)_3$), 2.14 (s, 3H, CH_3), 3.23 (m, 2H, 9-CH_2), 4.46 (m, 2H, 5-CH_2), 4.63 (d, 2H, NH$\underline{CH_2}$, *J*=4.4 Hz), 6.25 (m, 1H, NH), 6.38 (s, 2H, $COCH_2$), 6.61 and 6.90 (d-d, 4H, C_6H_4, *J*=8.1 Hz), 7.64 - 8.08 (m, 5H, C_6H_5). MS m/z: 376.4 [M+].

***1-[2-(4-Chlorophenyl)-2-oxoethyl]-3-(p-tolylaminomethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide* (*5eb*)**. Yield 1.74 g (71 %). M.p.=219–221 °C (from ethanol). Anal. Calcd. for $C_{23}H_{27}BrClN_4O$. %: N 11.4. Found, %: N 11.3. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.70–1.86 (m, 6H, $(CH_2)_3$), 2.15 (s, 3H, CH_3), 3.22 (m, 2H, 9-CH_2), 4.43 (m, 2H, 5-CH_2), 4.62 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.19 (m, 1H, NH), 6.35 (s, 2H, $COCH_2$), 6.60 and 6.90 (d-d, 4H, C_6H_4, *J*=8.4 Hz), 7.73 and 8.07 (d-d, 4H, C_6H_4, *J*=8.0 Hz). MS m/z: 411.0 [M+].

3-[(3^1-Chloro-2^1-methylphenylamino)-methyl]-1-(2-oxo-2-phenylethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide (5fa). Yield 3.87 g (79 %). M.p.= 225–226 °C (from ethanol). Anal. Calc. for $C_{23}H_{26}BrClN_4O$, %: N 11.4. Found, %: N 11.2. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.76 – 1.91 (m, 6H, $(CH_2)_3$), 2.21 (s, 3H, CH_3), 3.27 (m, 2H, 9-CH_2), 4.53 (m, 2H, 5-CH_2), 4.65 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.13 (m, 1H, NH), 6.43 (s, 2H, $COCH_2$), 6.65 - 8.10 (m, 8H, C_6H_5+C_6H_3).

***1-[2-(4^1-Bromophenyl)-2-oxoethyl]-3-[3^1-chloro-2^1-methylphenylamino)-methyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-1-ium bromide* (*5fc*)**. Yield 2.45 g (86 %). M.p.=210–211 °C (from ethanol). Anal. Calcd. for $C_{23}H_{25}Br_2ClN_4O$. %: N 9.85. Found, %: N 9.71. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.73–1.89 (m, 6H, $(CH_2)_3$), 2.19 (s, 3H, CH_3), 3.24 (m, 2H, 9-CH_2), 4.51 (m, 2H, 5-CH_2), 4.71 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.09 (m, 1H, NH), 6.36 (s, 2H, $COCH_2$), 6.62 - 7.00 (m, 3H, C_6H_3), 7.85 and 8.01 (d-d, 4H, C_6H_4, *J*=8.9 Hz).

***(4^1-Chlorophenyl)-(4-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-ylmethyl)-amine* (*6ba*) and *(4^1-bromophenyl)-(4-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-ylmethyl)-amine* (*6ca*)** were obtained by the method described in the work [10].

Aryl-(4R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacycloenta[cd]azulen-ylmethyl)-amines (6ab,ba,cd).

A mixture of (0.01 mol) 3-arylaminomethyl-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-a]azepines quaternary salts (**5bb,cd,dc,db,ea,eb,fa,fc**) and 30 mL 10 % solution of NaOH was refluxed for 2 h and left overnight at room temperature. The obtained solid products were collected by filtration, washed with water, and recrystallized from the appropriate solvent.

***[4-(4-Chlorophenyl)-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-ylmethyl]-phenylamine* (*6ab*)**. Yield 1.74 g (57 %). M.p.=204–206 C (from propanol-2). Anal. Calcd. for $C_{22}H_{21}ClN_4$. %: N 14.9. Found, %: N 14.7. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.93–2.02 (m, 4H, $(CH_2)_2$), 2.74 (m, 2H, CH_2), 4.03 (m, 2H, CH_2), 4.40 (d, 2H, NH$\underline{CH_2}$, *J*=4.3 Hz), 6.77 (t, 1H, NH, *J*=4.3 Hz), 6.55–7.12 (m, 5H, C_6H_5), 7.25 (s, 1H, CH), 7.38 and 7.46 (d-d, 4H, C_6H_4, *J*=8.8 Hz).

***[4-(4-Bromophenyl)-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-ylmethyl]-(4^1-methoxyphenyl)-amine* (*6dc*)**.

Yield 1.35 g (60 %). M.p.=172–174 °C (from benzene). Anal. Calcd. for $C_{23}H_{23}BrN_4O$. %: N 12.4. Found, %: N 12.5. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 1.92–2.01 (m, 4H, $(CH_2)_2$), 2.74 (m, 2H, CH_2), 3.63 (s, 3H, OCH_3), 4.03 (m, 2H, CH_2), 4.34 (d, 2H, NH$\underline{CH_2}$, *J*=5.6 Hz), 5.86 (t, 1H, NH, *J*=5.6 Hz), 6.67 and 6.75 (d-d, 4H, C_6H_4, *J*=9.2), 7.25 (s, 1H, CH), 7.30 and 7.51 (d-d, 4H, C_6H_4, *J*=8.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.8, 25.5, 26.8, 45.0, 46.4, 55.6, 75.8, 113.3, 114.3, 117.6, 121.1, 124.3, 128.2, 130.8, 136.4, 139.9, 145.0, 149.0, 155.0. MS m/z: 452.2 [M+].

Antimicrobial activity screening

All compounds were dissolved in DMSO to give stock solutions with concentration of 10 mg/mL, and aliquots were diluted in water and 5 μL dispensed into empty 384-well plates in duplicates for each strain. Once cells were added to the plates, this gave a final compound concentration of 32 μg/mL, or in case of a serial dilution assay compound concentrations from 50 to 0.78 μg/mL, in both cases with a maximum DMSO concentration of 0.3 %, which does not show antimicrobial activity and does not affect the results of microbiological screening.

Antimicrobial activity of the compounds was evaluated by their MIC values, which were determined by known approaches [11, 12]. In the research gram-positive bacterial strains of methicillin-susceptible *Staphylococcus aureus* (ATCC 25923 (MSSA)), methicillin-resistant *Staphylococcus aureus* (ATCC 43300 (*MRSA*)), gram-negative bacterial strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) as well as yeast fungi of *Candida albicans* (NCTC 885/653), *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (H99 ATCC 208821) were used. Selected reference strains of microorganisms are recommended for the evaluation of antimicrobial activity according to the guidelines of CLSI. The suspension of microorganisms (the microbial load was 1–2×10^5 colony-forming units per 1 mL (bacteria) or 1–2×10^4 fungi units per 1 mL (yeast fungi)) was transferred into the solutions of the compounds tested (in concentrations of 0.78–50 g/mL and 32 g/mL). The mixtures containing bacterial cultures were kept in thermostat for 18–24 h at 35–37 °C, and the mixtures containing the culture with yeast fungi were kept in thermostat for 24–48 h at 30–32 °C.

The inhibition level of the bacterial test-cultures growth treated with a specific concentration of the compounds tested was determined by measuring the optical density at wavelength of 600 nm (OD_{600}) using a multifunctional monochromator-based microplate reader Tecan M1000 Pro. The percentage of inhibition was calculated for each well using values obtained for negative control wells (containing only growth medium) and for positive control wells (containing bacterial/fungi inoculum suspension without the compounds (reference drugs) tested).

The inhibition level of *C. albicans* strain growth treated with the compounds tested was determined by measuring the optical density at wavelength of 530 nm (OD_{530}). The inhibition level of *C. neoformans* strain growth was determined by measuring the difference in optical density at wavelengths of 600 and 570 nm ($OD_{600-570}$) after adding resazurin solution (to its final concentration of 0.001 %) and incubation of the solutions at 35 °C for 2 h. The optical density of the mixtures containing fungi cultures was measured using multi-mode microplate reader Biotek Synergy HTX. The percentage of fungi growth inhibition was calculated in the same way as for the bacterial cultures [13, 14].

Negative growth inhibition values (Table 2) indicate lower growth rate (lower OD_{600} values) as compared to the negative control wells (inhibition level is set to 0 %). The growth rate of all bacterial and fungal strains was in the range of +/–10 % which is within normal growth rate distribution stated for bacteria/fungi [15, 16].

4. Results

The key intermediates – 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines (**3 a-f)** were synthesized by condensation of 7-methoxy-3,4,5,6-tetrahydro-2*H*-azepine **1** with (4-R-phenylamino)-acetic acid hydrazide (**2 a-f)**. Interaction of 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines (**3 a-f)** with substituted phenacyl bromides **4** in ethyl acetate resulted in 3-arylaminomethyl-1-(2-oxo-2-aryl-ethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides **5.** The latter when refluxed in 10 % solution of NaOH gave aryl-(4R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacycloenta[*cd*]azulen-ylmethyl)-amines. **6**. Compounds **3-6** are crystalline solids (Fig. 1).

Antimicrobial activity of 3-arylaminomethyl-1-(2-oxo-2-aryl-ethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides **5** was measured against gram-positive (*S. aureus* ATCC 25923) and gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacterial strains as well as yeasts fungi (*Candida albicans* NCTC 885/653). The results of antimicrobial activity study are given in the Table 1.

Compounds **3a-f** and **6ab,ba,ca,cd** were also tested to possess antimicrobial activity against bacterial strains of *S. aureus* (ATCC 43300), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (ATCC 700603), *A. baumannii* (ATCC 19606) and fungal strains of *C. albicans* (ATCC 90028), *C. neoformans* (ATCC 208821). The compounds were assessed in concentration of 32 μg/ml. The values of the strains growth inhibition are given in the Table 2.

The tests were initially carried out using compounds **3**, **6** in concentration of 32 μg/mL in duplicates in order to find active derivatives. Next, to determine MIC values preliminary assays were followed by a dose response test, using 8 double dilution concentrations of the compounds in duplicates.

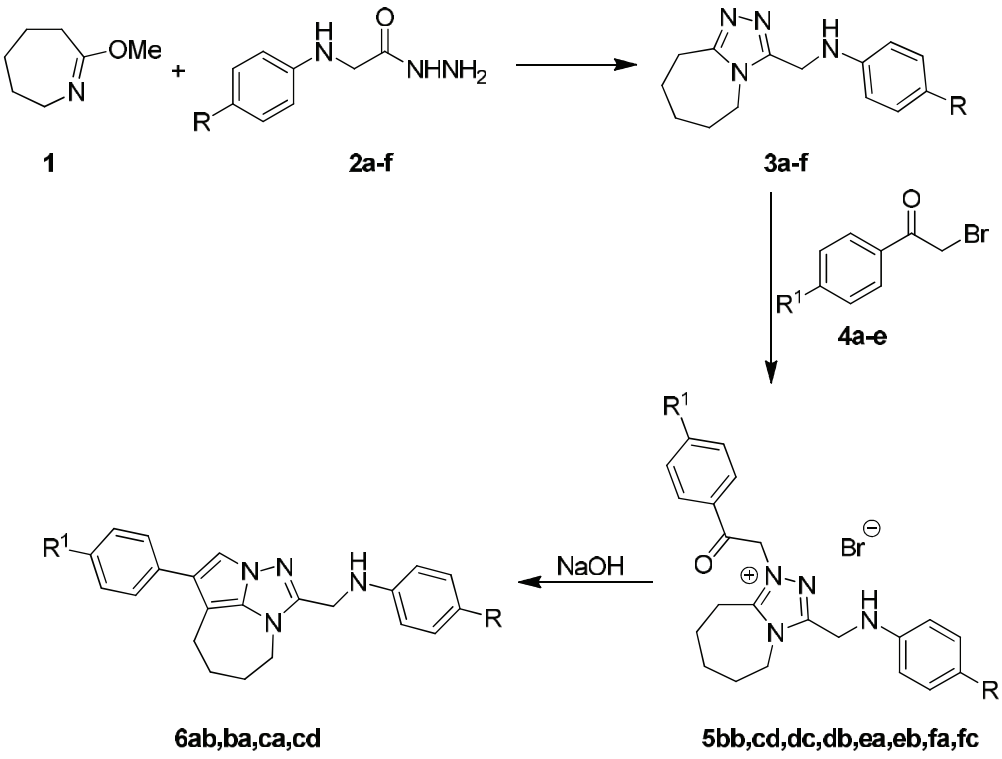

Fig. 1. Synthetic routes to 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines **3 a-f**, 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides **5** and aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[*cd*]azulen-1-ylmethyl)-amines **6**

Table 1

Antimicrobial activity of 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides **5bb,cd,db,dc,ea,eb,fa,fc**

Compounds	R	R^1	MIC, μg/mL			
			C. albicans NCTC 885/653	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
5bb	Cl	Cl	6.2	12.5	>50.0	>50.0
5cd	Br	OCH_3	25.0	6.2	>50.0	>50.0
5db	OCH_3	Cl	>50.0	>50.0	>50.0	>50.0
5dc	OCH_3	Br	50.0	>50.0	>50.0	>50.0
5ea	CH_3	H	>50.0	>50.0	>50.0	>50.0
5eb	CH_3	Cl	25.0	12.5	>50.0	>50.0
5fa	$2CH_3$,3Cl	H	>50.0	12.5	50.0	>50.0
5fc	$2CH_3$,3Cl	Br	12.5	12.5	>50.0	>50.0
Linezolid			not active	8.0	not active	not active
Cefixime			not active	8.0–32.0	0.25–1.0	not active
Fluconazole			1.0	not active	not active	not active

Table 2

Growth inhibition (%) of the bacterial and fungal strains under influence of 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines **3a-f** and aryl-(4-R^1-phenyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[*cd*]azulen-1-yl methyl)-amines **6ab,ba,ca,cd** in concentration of 32 μg/mL

Compounds	OD_{600}					OD_{530}	$OD_{600-570}$
	Sa	**Ec**	**Kp**	**Pa**	**Ab**	Ca	Cn
3a R=H	0.63	–8.6	4.13	11.29	10.15	–0.87	–38.16
3b R=Cl	18.8	2.56	12.23	12.3	16.01	6.05	–37.4
3e R=CH_3	10.99	–2.97	10.57	13.42	6.86	4.53	–34.32
6ba R=Cl, R^1=H	3.01	–9.71	–4.28	7.59	–2.7	4.76	–50.2
6dc R=OCH_3, R^1=Br	3.24	–14.42	–7.85	5.56	19.87	5.6	–38.04
6ca R=Br, R^1=H	15.34	–14.24	–8.25	5.08	–19.07	7.82	–55.82
6ab R=H, R^1=Cl	6.17	–7.44	1.86	8.97	3.4	1.84	–18.63

Note: Sa – S. aureus (MRSA) ATCC 43300; Ec – E. coli ATCC 25922; Kp – K. pneumoniae ATCC 700603; Ab – A. baumannii ATCC 19606; Pa – P. aeruginosa ATCC 27853; Ca – C. albicans ATCC 90028; Cn – C. neoformans H99 ATCC 208821; OD – optical density (measured at a specific wavelength)

5. Discussion

The obtained data indicate that six compounds (**5bb**, **5fc**, **5cd**, **5eb**, and **5fa**) display antimicrobial activity against gram-positive bacteria (*S. aureus*). The compounds are active in concentration range of 6.2–25.0 μg/mL, and derivative **5cd** appeared to be the most active. It is worth noting that antistaphylococcal activity of the compounds mentioned was similar to the activity of Linezolid and more pronounced than the activity of Cefixime [17, 18].

At the same time the compounds tested had no activity against gram-negative bacteria. Only compound **5fa** inhibited growth of *E. coli* strain in concentration of 50.0 g/mL. It was also established that all derivatives are inactive against *P. aeruginosa* and inferior to the reference drug Cefixime [19, 20].

Investigation of antifungal properties of the compounds evidence that four out of nine derivatives (**5bb, 5fc, 5cd** and **5eb**) inhibited the growth of *C. albicans* in concentrations of 6.2–25.0 g/mL. The most active derivative **5bb** is of the same level of activity as antifungal drug Fluconazole [21, 22]. The study of antibacterial and antifungal activities of 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides **5** demonstrate that representatives of this class of compounds may have either single (antibacterial or antifungal) or polyvalent (antibacterial and antifungal) activity. The level of inhibiting effect and the antimicrobial spectrum of the compounds synthesized are clear evidences of prospects to create new antimicrobial drugs based on them.

Study limitations. Because serial dilution is performed in a stepwise manner, it requires a more extended period. Prepared environments must be deployed immediately, with no storage capability. It is limiting the efficiency of the method.

Prospects for further research. The further investigation of antimicrobial properties of the most active derivatives **5bb** and **5cd** seems to be promising way of finding and creating novel effective antimicrobial drugs.

6. Conclusion

A series of new 3-arylaminomethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepines, 3-arylaminomethyl-1-(2-oxo-2-arylethyl)-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-1-ium bromides and aryl-(4-R^1-phe-

nyl-5,6,7,8-tetrahydro-2,2a,8a-triazacyclopenta[cd]azulen-1-yl methyl)-amines has been synthesized.

Experiments carried out revealed the compounds synthesized to possess broad spectrum of antimicrobial activity (compounds **5fa, 5bb, 5fc, 5cd** та **5eb**) inhibiting the growth of bacterial and fungal strains.

Conflict of interests

The authors declare that they have no conflict of interests.

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