СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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The synthesis, spectral properties and the biological activity of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione derivatives

Aim. To synthesize the series of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione, to study their spectral properties and antibacterial activity.

Materials and methods. The methods of organic synthesis, instrumental methods of organic compounds analysis, as well as the agar diffusion method were used.

Results and discussion. By the interaction of 3-arymethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with arenesulfonyl cholrides in the presence of triethylamine the series of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione was obtained. For the compounds containing the fragments of 1-sulfonylamido-(2,4)- and 3,4-difluorobenzene the ^{1}H-^{1}H coupling constants in their ^{1}H{^{19}F}-NMR fluorine decoupled spectra, as well as the ^{19}F-^{19}F coupling constants in the ^{19}F{^{1}H}-NMR proton decoupled spectra were measured. The antimicrobial activity screening showed that the growth of such bacterial strains as *Staphylococcus aureus* and *Bacillus subtilis* was inhibited by the compounds of the series obtained.

Conclusions. It has been found that the interaction of 3-arymethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with arenesulfonyl cholrides is an effective way for the synthesis of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with the promising biological activity against the strains of gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. Among 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione derivatives 3-(3-methylbenzyl)-7-(toluene-4-sulfonyl)-1,3,7-triazaspiro[4.4]nonane-2,4-dione exhibited the highest activity.

Key words: *hydantoin; sulfonamides; pyrrolidone; antibacterial agents*

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Синтез, спектральні характеристики та біологічна активність похідних 7-аренсульфоніл-3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діону

Мета роботи – синтез ряду 7-аренсульфоніл-3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діонів, дослідження їх спектральних характеристик та антибактеріальної активності.

Матеріали та методи. Методи органічного синтезу, інструментальні методи встановлення будови органічних сполук, метод дифузії в агар.

Результати та їх обговорення. При взаємодії 3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діонів з аренсульфохлоридами в присутності триетиламіну було отримано ряд похідних 7-аренсульфоніл-3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діону. Для сполук, що містять 1-сульфаніламідо-(2,4)- та 3,4-дифлуоробензенові фрагменти, виміряні константи спін-спінової взаємодії Гідроген-Гідроген у ^{1}H{^{19}F}-ЯМР-спектрах з пригніченням взаємодії Гідроген-Флуор, а також константи спін-спінової взаємодії Флуор-Флуор у ^{19}F{^{1}H} ЯМР-спектрах. Дані мікробіологічного скринінгу показують, що грампозитивні бактерії, такі як *Staphylococcus aureus* та *Bacillus subtilis* є чутливими до сполук досліджуваного ряду.

Висновки. Встановлено, що взаємодія 3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діонів з аренсульфохлоридами є ефективним методом синтезу 7-аренсульфоніл-3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діонів, які проявляють перспективну біологічну активність по відношенню до штамів грампозитивних бактерій *Staphylococcus aureus* та *Bacillus subtilis*. Найбільшу активність в ряду заміщених 7-аренсульфоніл-3-арилметил-1,3,7-триазаспіро[4.4]нонан-2,4-діонів проявив 3-(3-метилбензил)-7-(толуен-4-сульфоніл)-1,3,7-триазаспіро[4.4]нонан-2,4-діон.

Ключові слова: *гідантоїн; сульфаміди; піролідон; антибактеріальні засоби*

К. Ю. Кроленко, С. В. Власов, И. А. Журавель, Т. П. Осолодченко

Синтез, спектральные характеристики и биологическая активность производных 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4]нонан-2,4-диона

Цель работы – синтез ряда 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4]нонан-2,4-дионов, исследование их спектральных характеристик и антибактериальной активности.

Материалы и методы. Методы органического синтеза, инструментальные методы определения структуры органических соединений, метод диффузии в агар.

Результаты и их обсуждение. При взаимодействии 3-арилметил-1,3,7-триазаспиро[4.4]нонан-2,4-дионов с аренсульфохлоридами в присутствии триэтиламина был получен ряд производных 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4]нонан-2,4-диона. Для соединений, содержащих 1-сульфаниламидо-(2,4)- и 3,4-дифторбензольные фрагменты, измерены константы спин-спинового взаимодействия водород-водород в $^{1}H\{^{19}F\}$-ЯМР-спектрах с подавлением взаимодействия водород-фтор, а также константы спин-спинового взаимодействия фтор-фтор в $^{19}F\{^{1}H\}$ ЯМР-спектрах. Данные микробиологического скрининга показывают, что грамположительные бактерии, такие как *Staphylococcus aureus* и *Bacillus subtilis*, чувствительны к соединениям исследуемого ряда.

Выводы. Установлено, что взаимодействие 3-арилметил-1,3,7-триазаспиро[4.4]нонан-2,4-дионов с аренсульфохлоридами является эффективным методом синтеза 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4] нонан-2,4-дионов, которые проявляют перспективную противомикробную активность по отношению к штаммам грамположительных бактерий *Staphylococcus aureus* и *Bacillus subtilis*. Наибольшую активность в ряду замещенных 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4]нонан-2,4-дионов проявил 3-(3-метилбензил)-7-(толуол-4-сульфонил)-1,3,7-триазаспиро[4.4]нонан-2,4-дион.

***Ключевые слова**: гидантоин; сульфамиды; пирролидон; антибактериальные средства*

As it was earlier reported the substituted 3-arymethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones, their 7-benzoyl derivatives [1], and unsymmetrical ureas on their basis [2] showed the antibacterial effect against gram-positive, gram-negative bacteria and fungi. The antibacterial activity is also typical for the compounds with the $ArSO_2NRR'$ fragment [3, 4].

In recent years the fluorine-containing molecules are of a great interest to scientists [5-8] since regardless of its larger atomic radii fluorine causes less steric complications for receptor binding rather than the hydrogen atom [9]. Therefore, obtaining of the novel fluorine-containing compounds is a promising way for developing effective antibacterial drugs, that is why we decided to make the combination of the 3-arymethyl-1,3,7-triazaspiro[4.4] nonane-2,4-dione moiety with the sulfonamide fragment the objects for further studies of spectral characteristics and the antibacterial activity.

Materials and Methods

Chemical Part

All solvents and reagents were obtained from the commercial sources. ^{1}H, ^{13}C and ^{19}F NMR-spectra were recorded with a Bruker 170 Avance 500 spectrometer at 500 MHz for NMR ^{1}H-spectra, at 125 MHz for NMR ^{13}C-spectra and 376 MHz for NMR ^{19}F-spectra; the solvent was DMSO-d_6; TMS was used as an internal standard for ^{1}H, ^{13}C and $CFCl_3$ for ^{19}F-spectra. Chromato-mass spectra were recorded using an Agilent 1100 HPLC device equipped with a diode matrix detector and a mass-spectrometer (Agilent LC-MSD SL), and the Zorbax SB-C18 column (4.6×15 mm) with atmospheric pressure chemical ionization (APCI) was used for the analyses. The TLC was performed on the aluminium plates covered with Merck, Kiesgel 60 F-254. The melting points were measured with a Kofler melting point apparatus and were not corrected.

The substituted 3-arymethyl-1,3,7-triazaspiro[4.4] nonane-2,4-diones (1.1-1.4) were obtained by the previously reported methods [5].

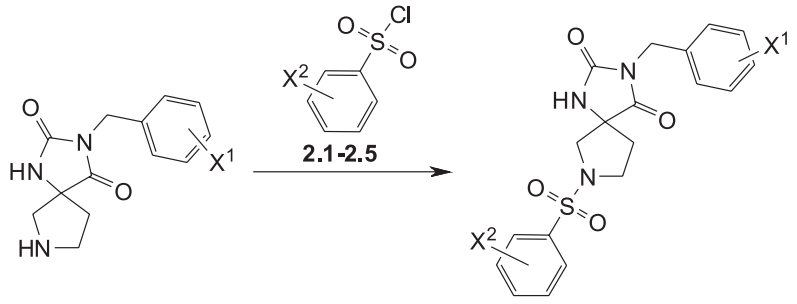
The general method for the synthesis of the substituted 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione (3.1-3.13). To the solution of 3-arymethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione (1.2 mmole) **1.1-1.4** in 10 ml of 1,4-dioxane add 0.17 ml (1.25 mmole) of triethylamine and 1.2 mmole of the corresponding arenesulfonyl chloride (**2.1-2.5**), heat the reaction mixture for 3 h at 80°C while stirring. Then cool it to room temperature and dilute with 50 ml of water, mix for 30 min. Purify the precipitate obtained by crystallization from ethanol.

Microbiological Experiment

According to the WHO recommendations to assess the activity of the compounds tested the following strains of microorganisms were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2785 3, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 653/885. The inoculum suspension was prepared using a Densi-La-Meter apparatus (PLIVA-Lachema, Czech Republic; the wavelength of 540 nm). The suspension was prepared according to the instruction for the apparatus and the Information Letter about innovation in the healthcare system, No.163 – 2006 "Standardization of microbial suspension" [10-12]. The cultures were synchronized under the low temperature conditions (4°C). The density of the inoculum was 107 cells per 1 ml of the medium and was determined by comparing with McFarland standard [13]. The 18-24 hour old culture of microorganisms was used for the test. Mueller-Hinton agar was applied for bacteria. The strain of *Candida albicans* was cultivated using Sabouraud agar. The experiment was carried out using the agar "well" diffusion method [14]. The compounds studied were introduced as 0.3 ml of the DMSO solution (with the concentration of 100 μg/ml) aliquots. The standards were introduced as the solution in DMSO (30 μg/lm) for Metronidazole and as the water solution (30 μg/lm) for Synthomycine. The antibacterial activity was assessed by measuring the growth inhibition zones for each microorganism.

Results and Discussion

To obtain the target novel 3-arymethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones modified with the sulfonamide fragment the synthesis of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones **3** series was performed. The synthesis of compounds **3** was carried out by the interaction of the secondary spiroamines obtained **1.1-1.4** by the methods previously reported [1] with the substituted arenesulfonyl chlorides **2.1-2.5**



1.1 X^1=H; **1.2** X^1=4Me; **1.3** X^1=2,5diMe; **1.4** X^1=3Me; **2.1** X^2=H; **2.2** X^2=2,4diF; **2.3** X^2=3Cl; **2.4** X^2=3,4diF; **2.5** X^2=4Me; **3.1** X^1=H, X^2=H; **3.2** X^1=4Me, X^2=2,4diF; **3.3** X^1=4Me, X^2=H; **3.4** X^1=4Me, X^2=3Cl; **3.5** X^1=4Me, X^2=3,4diF; **3.6** X^1=2,5diMe, X^2=H; **3.7** X^1=2,5diMe, X^2=2,4diF; **3.8** X^1=2,5diMe, X^2=3Cl; **3.9** X^1=2,5diMe, X^2=4Me; **3.10** X^1=3Me, X^2=H; **3.11** X^1=3Me, X^2=4Me; **3.12** X^1=3Me, X^2=3,4diF; **3.13** X^1=H, X^2=4Me

Scheme

in the 1,4-dioxane medium. Compounds **3.1-3.13** were isolated after recrystallization from ethanol as white crystalline solids (Scheme).

In the ^{1}H and ^{19}F NMR-spectra of the fluorine-containing samples (**3.2**, **3.5**, **3.7**, **3.12**) obtained the multiplicity of the proton signals for difluorobenzenesulfonyl amide fragment were unclear to be interpreted correctly. Such situation was caused by the presence of two magnetically and chemically non-equivalent fluorine atoms, which impeded the spectra because of splitting the protons signals. For resolution of the spectra for the compounds with 2,4- and 3,4-difluorobenzenesulfonyl amide fragments (**3.2** and **3.5**, respectively) the proton or fluorine decoupled spectra ^{1}H{^{19}F} and ^{19}F{^{1}H} were measured for the adequate interpretation of the proton spectral signals.

In the ^{1}H{^{19}F}NMR-spectrum of compound **3.2** with the fluorine decoupling three signals in the region of aromatic proton resonance with the equal integral intensity were observed. In the spectra the proton signal in position 5 was observed as a doublet of doublets (dd) at 7.35 ppm due to the interaction with the protons in positions 6 and 3 with the constants of 8.8 and 2.4 Hz, respectively. Thus, the signal of the proton in position 3 was observed at 7.65 ppm as a doublet with the constant of 2.4 Hz, while the doublet signal at 7.92 ppm (J = 8.8 Hz) was the signal of the proton in position 6.

Similarly, the decoupling from protons simplifies the interpretation of ^{19}F NMR-spectrum of compound **3.2**. Two non-equivalent fluorine atoms in the molecule obtained interacted with the coupling constant equal to 12.5 Hz. The presence of such strong electron-acceptor as the sulfonamide group shifted the signal of the conjugated fluorine atom down-field, so the doublet at -101.74 ppm was the signal of the fluorine atom in position 4, and the signal at -103.13 ppm was produced with the fluorine atom in position 2.

The successful interpretation and the coupling constants measurement for the compound with the 3,4-difluorobenzenesulfon amide fragment **3.5** was achieved by detection of ^{1}H{^{19}F} and ^{19}F{^{1}H} NMR-spectra. The normal ^{1}H and ^{19}F NMR-spectra of compound **3.5** contained the unclear picture of multiplet signals.

In the ^{1}H{^{19}F} NMR-spectrum there was the doublet of doublets at 7.73 ppm produced by the proton in position 6, for this signal the first coupling constant was 8.7 Hz; it was caused by the interaction with the proton in position 5, while the second constant (J = 1.9 Hz) resulted from the interaction with the proton in position 2. The doublet at 7.77 ppm (J = 8.7 Hz) was a signal of the proton in position 5, and the doublet at 7.98 (J = 1.9 Hz) was the signal of the proton in position 2. The characteristic difference in intensity of the doublet components (the "roof effect") indicates the magnetic similarity of protons in positions 5 and 6.

The fluorine atoms in the 3,4-difluorobenzenesulfon amide fragment gave two doublets with the spin-spin coupling constant of 21.8 Hz, being 9.3 Hz more than the constant for the 2,4-difluorosubstituted fragment.

Concerning ^{13}C NMR-spectra of compounds **3.2** and **3.5** the signals of carbon atoms were also split because of the presence of the fluorine atoms in the aromatic ring. For example, the signal of the carbon atom in position 3 of the 1-sulfonylamido-2,4-difluorobenzene fragment for compound **3.2** was observed as a triplet at 106.9 ppm with the constant of 26.5 Hz due to its interaction with two fluorine atoms in *ortho*-positions. The carbon atoms in positions 2 and 4 of the same fragment were observed as a doublet of doublets at 161.0 ppm (J_1 = 256.3 Hz, J_2 = 13.1 Hz) and 165.8 (J_1 = 254.7 Hz, J_2 = 12.0 Hz), respectively, where the first constant was caused by the *ipso*-carbon-fluorine interaction and the second one was the result of the *meta*-carbon-fluorine interaction. Similarly, the signals of the carbon atoms in positions 5, 6 and 1 were observed as three doublets at 112.97 (J = 19.4 Hz), 133.56 (J = 11.2 Hz) and 122.09 (J = 15.4 Hz) ppm; it was obvious that the *meta*-constant for carbon-fluorine (the carbon atom in position 6) was less than the *ortho*-constants for the carbon atoms in positions 5 and 1.

Thus, using the NMR-spectroscopic experiential techniques the coupling constants for ^{1}H-^{1}H and ^{19}F-^{19}F were

Table 1

The ^{1}H, ^{13}C, ^{19}F NMR-, LC-MS-spectral data and the melting points for the derivatives of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione **3**

Compounds	M.p., °C	^{1}H NMR, δ, ppm (*J*, Hz)	^{13}C and ^{19}F NMR, δ, ppm(*J*, Hz)	$[M+H]^{+}$
1	2	3	4	5
3.1	162-163	2.09-1.89 (m, 2H); 3.51-3.26 (m, 4H); 4.49 (s, 2H); 7.20 (d, *J* = 7.4, 2H); 7.25 (t, *J* = 7.2, 1H); 7.32 (t, *J* = 7.3, 2H); 7.66 (t, *J* = 7.6, 2H); 7.74 (t, *J* = 7.3, 1H); 7.83 (d, *J* = 7.6, 2H); 8.83 (s, 1H)	35.9; 41.8; 47.6; 55.9; 65.9; 127.7; 127.9; 128.0; 129.1; 130.0; 133.9; 136.2; 137.0; 155.9; 174.4	386.0
3.2	180-181	2.05 (dt, *J* = 12.8, 6.4, 1H); 2.16 (dt, *J* = 14.7, 7.5, 1H); 2.26 (s, 3H); 3.59-3.34 (m, 4H); 4.44 (s, 2H); 7.09 (d, *J* = 7.8, 2H); 7.12 (d, *J* = 7.9, 2H); 7.32 (t, *J* = 8.4, 1H); 7.61 (t, *J* = 9.0, 1H); 7.89 (dd, *J* = 14.7, 8.2, 1H); 8.80 (s, 1H)	21.2; 35.8; 41.6; 47.2; 55.1; 65.6; 106.9 (t, *J* = 26.5); 112.9 (d, *J* = 19.4); 122.0 (d, *J* = 15.4); 127.8; 129.6; 133.5 (d, *J* = 11.2); 134.0; 137.1; 155.9; 161.0 (dd, *J* = 256.3, 13.1); 165.8 (dd, *J* = 254.7, 12.0); 174.4 ^{19}F NMR: -103.11 (dd, *J* = 20.3, 10.7); -101.74 (dt, *J* = 16.7, 8.2)	436.0
3.3	150-151	2.12-1.79 (m, 2H); 2.25 (s, 3H); 3.52-3.18 (m, 4H); 4.43 (s, 2H); 7.08 (d, *J* = 7.8, 2H); 7.11 (d, *J* = 7.2, 2H); 7.66 (t, *J* = 7.0, 2H); 7.75 (t, *J* = 7.0, 1H); 7.82 (d, *J* = 7.9, 2H); 8.80 (s, 1H)	21.2; 35.9; 41.6; 47.6; 55.9; 65.8; 127.8; 128.0; 129.6; 130.0; 133.9; 134.0; 136.2; 137.1; 155.9; 174.4	400.0
3.4	162-163	1.98 (dt, *J* = 12.8, 6.3, 1H); 2.06 (dt, *J* = 15.0, 7.6, 1H); 2.26 (s, 3H); 3.52-3.34 (m, 4H); 4.43 (s, 2H); 7.09 (d, *J* = 8.0, 2H); 7.12 (d, *J* = 8.1, 2H); 7.68 (t, *J* = 8.2, 1H); 7.86-7.75 (m, 3H); 8.78 (s, 1H)	21.2; 35.8; 41.6; 47.6; 55.7; 65.7; 126.7; 127.5; 127.8; 129.6; 132.0; 133.9; 134.0; 134.8; 137.1; 138.2; 155.9; 174.4	434.0
3.5	170-171	1.98 (dt, *J* = 12.9, 6.4, 1H); 2.09 (dt, *J* = 14.9, 9.5, 1H); 2.26 (s, 3H); 3.38-3.51 (m, 2H); 3.33 (t, *J* = 5.4, 2H); 4.43 (s, 2H); 7.08 (d, *J* = 7.9, 2H); 7.11 (d, *J* = 7.9, 2H); 7.73 (dd, *J* = 15.5, 6.9, 2H); 7.95 (t, *J* = 8.2, 1H); 8.75 (s, 1H)	21.2; 35.8; 41.6; 47.6; 55.6; 65.7; 118.0 (d, *J* = 19.7); 119.3 (d, *J* = 18.3); 126.0; 127.8; 129.6; 133.5; 134.0; 137.1; 150.1 (dd, *J* = 251.5, 13.3); 153.1 (dd, *J* = 253.8, 12.5); 155.9; 174.3 ^{19}F NMR: -135.23 – -135.69 (m); -131.20 – -131.55 (m)	436.0
3.6	131-132	2.11-1.93 (m, 2H); 2.21 (s, 3H); 2.23 (s, 3H); 3.37-3.51 (m, 2H); 3.36 (s, 2H); 4.42 (s, 2H); 6.78 (s, 1H); 6.95 (d, *J* = 7.5, 1H); 7.03 (d, *J* = 7.5, 1H); 7.66 (t, *J* = 7.5, 2H); 7.74 (t, *J* = 7.3, 1H); 7.83 (d, *J* = 7.5, 2H); 8.84 (s, 1H)	18.8; 21.2; 36.0; 47.6; 55.9; 65.8; 66.9; 128.0; 128.4; 130.0; 130.6; 132.8; 133.9; 134.5; 135.3; 136.2; 156.0; 174.5	414.2
3.7	146-147	2.14-2.02 (m, 1H); 2.21 (s, 4H); 2.24 (s, 3H); 3.60-3.39 (m, 4H); 4.43 (s, 2H); 6.79 (s, 1H); 6.97 (s, 1H); 7.03 (d, *J* = 7.3, 1H); 7.31 (s, 1H); 7.90 (d, *J* = 6.0, 1H); 8.82 (s, 1H)	18.8; 21.2; 35.9; 47.2; 55.1; 65.6; 106.9 (t, *J* = 26.7); 112.9 (d, *J* = 25.0); 122.1 (d, *J* = 10.9); 128.0; 128.4; 130.6; 132.8; 133.5 (d, *J* = 10.3); 134.5; 135.3; 156.0; 160.0 (dd, *J* = 256.2, 13.8); 165.8 (dd, *J* = 254.4, 12.0); 174.6 ^{19}F NMR: -103.1 (dd, *J* = 20.1, 11.1); -101.7 (dt, *J* = 17.2, 8.6)	450.0

measured, and it helped to interpret properly the spectra of compounds **3.2** and **3.5** containing 1-sulfonylamido-(2,4)- and 3,4-difluorobenzene fragments, being typical for many biologically active substances [15-17]. The data obtained may be used for interpretation of the similar spectra of more complex molecules. The ^{1}H, ^{13}C, ^{19}F NMR, LC-MS-spectral data for compounds **3** and the melting points are listed in Tab. 1.

The screening data of the antimicrobial activity for compounds **3** showed the sensitivity of almost all bacterial strains for all of the samples studied. The highest activity was revealed by 3-(3-methylbenzyl)-7-(toluene-4-sulfonyl)-1,3,7-triazaspiro[4.4]nonane-2,4-dione (**3.11**) against the strain of *Bacillus subtilis*. In this case, similarly to the aryl amides of 3-arymethyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-caroxylic acids previously reported [2], the tendency of the antimicrobial activity increase for the compound containing methyl substituents in the aromatic ring is typical of the novel 7-arenesulfonyl derivatives. The highly active samples **3.8** and **3.9** also confirm such correlation. The results of the antimicrobial activity screening for compounds **3.1-3.13** are given in Tab. 2.

Continuation of Table 1

1	2	3	4	5
3.8	179-180	2.03 (dt, *J* = 12.7, 6.3, 1H); 2.09 (dt, *J* = 19.2, 6.1, 1H); 2.21 (s, 3H); 2.24 (s, 3H); 3.54-3.36 (m, 4H); 4.42 (s, 2H); 6.79 (s, 1H); 6.95 (d, *J* = 7.4, 1H); 7.03 (d, *J* = 7.6, 1H); 7.68 (t, *J* = 7.8, 1H); 7.82 (dd, *J* = 15.6, 4.8, 3H); 8.82 (s, 1H)	18.8; 21.2; 35.9; 47.7; 55.7; 65.7; 126.7; 127.5; 128.0; 128.4; 130.6; 132.0; 132.8; 133.9; 134.4; 134.8; 135.3; 138.3; 156.0; 174.5	448.2
3.9	154-155	1.99 (dt, *J* = 11.9, 5.9, 1H); 2.12-2.02 (m, 1H); 2.21 (s, 3H); 2.25 (s, 3H); 2.42 (s, 3H); 3.51-3.28 (m, 4H); 4.44 (s, 2H); 6.81 (s, 1H); 6.96 (d, *J* = 7.1, 1H); 7.03 (d, *J* = 7.3, 1H); 7.45 (d, *J* = 7.5, 2H); 7.71 (d, *J* = 7.6, 2H); 8.75 (s, 1H)	18.8; 21.2; 21.6; 36.0; 47.6; 56.0; 65.9; 128.0; 128.1; 128.4; 130.5; 130.6; 132.8; 133.3; 134.5; 135.3; 144.3; 156.0; 174.5	428.2
3.10	129-130	2.08-1.87 (m, 2H); 2.26 (s, 3H); 3.52-3.33 (m, 4H); 4.44 (s, 2H); 6.97 (d, *J* = 7.5, 1H); 7.00 (s, 1H); 7.06 (d, *J* = 7.4, 1H); 7.19 (t, *J* = 7.5, 1H); 7.66 (t, *J* = 7.5, 2H); 7.79-7.69 (m, 1H); 7.82 (d, *J* = 8.1, 2H); 8.82 (s, 1H)	21.5; 35.9; 41.8; 47.6; 55.9; 65.8; 124.8; 128.0; 128.3; 128.6; 129.0; 130.0; 133.9; 136.1; 136.9; 138.2; 155.9; 174.4	400.2
3.11	176-177	1.95 (dt, *J* = 13.0, 6.5, 1H); 2.02 (dt, *J* = 15.3, 7.7, 1H); 2.26 (s, 3H); 2.41 (s, 3H); 3.33 (s, 2H); 3.47-3.34 (m, 2H); 4.44 (s, 2H); 6.97 (d, *J* = 7.6, 1H); 7.01 (s, 1H); 7.07 (d, *J* = 7.4, 1H); 7.19 (t, *J* = 7.5, 1H); 7.46 (d, *J* = 7.9, 2H); 7.70 (d, *J* = 8.1, 2H); 8.82 (s, 1H)	21.5; 21.6; 35.9; 41.8; 47.6; 56.0; 65.9; 124.8; 128.1; 128.3; 128.6; 129.0; 130.5; 133.2; 136.9; 138.2; 144.3; 155.9; 174.4	414.0
3.12	152-153	2.00 (dt, *J* = 12.8, 6.4, 1H); 2.10 (dt, *J* = 14.9, 9.3, 1H); 2.27 (s, 3H); 3.50-3.34 (m, 4H); 4.44 (s, 2H); 6.97 (d, *J* = 7.5, 1H); 7.01 (s, 1H); 7.07 (d, *J* = 7.4, 1H); 7.20 (t, *J* = 7.5, 1H); 7.80-7.64 (m, 2H); 7.95 (t, *J* = 8.0, 1H); 8.77 (s, 1H)	21.5; 35.8; 41.8; 47.6; 55.6; 65.7; 118.0 (d, *J* = 19.5); 119.3 (d, *J* = 18.5); 124.8; 126.0; 128.4; 128.6; 129.0; 133.6; 136.9; 138.2; 150.1 (dd, *J* = 251.4, 13.3); 153.1 (dd, *J* = 253.6, 12.5); 155.9; 174.4 ^{19}F NMR:-135.31 – -135.63 (m); -131.18 – -131.52 (m)	436.2
3.13	187-188	1.95 (dt, *J* = 12.7, 6.3, 1H); 2.07-1.99 (m, 1H); 2.41 (s, 3H); 3.51-3.32 (m, 4H); 4.49 (s, 2H); 7.19 (d, *J* = 7.5, 2H); 7.26 (t, *J* = 7.2, 1H); 7.32 (t, *J*= 7.3, 2H); 7.46 (d, *J* = 8.0, 2H); 7.70 (d, *J* = 8.1, 2H); 8.84 (s, 1H)	21.6; 35.9; 41.8; 47.6; 56.0; 65.9; 127.7; 127.9; 128.1; 129.1; 130.5; 133.2; 137.0; 144.3; 155.9; 174.5	400.2

Table 2

The antimicrobial activity of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione derivatives 3

Compounds	Diameter of the growth inhibition zones*, mm					
	Gram-positive bacteria		Gram-negative bacteria			Fungi
	S. a.	B. c.	E. c.	P. v.	P. a.	C. a.**
Metronidazole	14	16	14	0	0	14
Synthomycine	14	17	17	17	17	0
3.1	18	19	15	18	17	18
3.2	16	20	16	17	17	19
3.3	19	19	17	18	16	19
3.4	15	16	15	17	15	17
3.5	18	18	16	17	16	18
3.6	16	18	15	18	15	17
3.7	17	19	17	19	17	19
3.8	22	22	20	18	18	20
3.9	20	20	18	19	17	20
3.10	18	19	17	17	17	20
3.11	18	23	16	17	18	21
3.12	17	21	16	17	18	18
3.13	17	20	17	17	18	19

Notes: * – The average value for three experiments; ** – Test-strains are listed in the experimental part.

CONCLUSIONS

The reaction of 3-arymethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with arenesulfonyl chlorides has been shown to be an effective method for obtaining of a variety of the substituted derivatives of 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione as compounds with the promising potent biological activity. The ^{1}H-^{1}H and ^{19}F-^{19}F spin-spin coupling constants measured for the compounds with 1-sulfonylamido-(2,4)- and 3,4-difluorobenzene fragments may be used for interpretation of the spectra of more complex compounds with the same substituents. The screening data of the antimicrobial activity have shown that the compounds obtained are active against *Staphylococcus aureus* and *Bacillus subtilis*, and 3-(3-methylbenzyl)-7-(toluene-4-sulfonyl)-1,3,7-triazaspiro[4.4]nonane-2,4-dione is the most active. The tendency of the antimicrobial activity increase for the compound containing methyl substituents in the aromatic ring is typical for the novel 7-arenesulfonyl-3-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione series.

Conflicts of Interest: authors have no conflict of interest to declare.

REFERENCES

1. An Efficient Synthesis of 1,3,7-Triazaspiro[4.4]Nonane-2,4-Dione Derivatives and Antimicrobial Activity Thereof / K. Yu. Krolenko, O. V. Silin, S. V. Vlasov et al. // Chem. Heterocycl. Comp. – № 51. – P. 472–477. doi: 10.1007/s10593-015-1721-6.
2. The synthesis and the antimicrobial activity of the substituted aryl amides of 3-arylmethyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylic acids / K. Yu. Krolenko, S. V. Vlasov, O. D. Vlasova, I. O. Zhuravel // News of Pharmacy. – 2016. – № 4 (88). – P. 17–21.
3. Inhibition of carbonic anhydrases from the extremophilic bacteria Sulfurihydrogenibium yellostonense (SspCA) and S. azorense (SazCA) with a new series of sulfonamides incorporating aroylhydrazone-, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazinyl- or 2-(cyanophenylmethylene)-1,3,4-thiadiazol-3(2H)-yl moieties / A. Alafeefy, H. Abdel-Aziz, D. Vullo et al. // Biorg. Med. Chem. – 2014. – № 22. – P. 141–147. doi: 10.1016/j.bmc.2013.11.042.
4. Synthesis and characterization of new N-(4-(4-chloro-1H-imidazol-1-yl)-3-methoxyphenyl)amide/sulfonamide derivatives as possible antimicrobial and antitubercular agents / P. Ranjith, R. Pakkath, K. Haridas, S. Kumari // Eur. J. Med. Chem. – 2014. – № 71. – P. 354–365. doi: 10.1016/j.ejmech.2013.11.002.
5. Fluorine in medicinal chemistry / S. Purser, P. Moore, S. Swallow, V. Gouverneur // Chem. Soc. Rev. – 2008. – № 37. – P. 320–330. doi: 10.1039/b610213c.
6. Fluorine in pharmaceutical industry: fluorine-containing drugs introduced to the market in the last decade (2001–2011) / J. Wang, M. Sánchez-Roselló, J. Aceña et al. // Chem. Rev. – 2013. – № 114. – P. 2432–2506. doi: 10.1021/cr4002879.
7. Kenneth, L. K. Fluorine in medicinal chemistry: recent therapeutic applications of fluorinated small molecules / Kirk Kenneth L. // J. Fluorine Chem. – 2006. – № 127. – P. 1013–1029. doi: 10.1016/j.jfluchem.2006.06.007.
8. Park, B. Metabolism of fluorine-containing drugs / B. Park, N. Kitteringham, P. O'Neill // Annu. Rev. Pharmacool. Toxicol. – 2001. – № 41. – P. 443–470. doi: 10.1146/annurev.pharmtox.41.1.443.
9. O'Hagan, D. Some influences of fluorine in bioorganic chemistry / D. O'Hagan, H. Rzepa // Chem. Commun. – 1997. – № 7. – P. 645–652. doi: 10.1039/a604140j.
10. Бактеріологічний контроль поживних середовищ [Текст]: інформаційний лист / МОЗ України № 05. 4. 1 / 1670. – К., 2000. – 4 с.
11. Волянський, Ю. Л. Вивчення специфічної активності протимікробних лікарських засобів : метод. рек. МОЗ України / Ю. Л. Волянський, І. С. Гриценко, В. П. Широбоков та ін.; ДФЦ МОЗ України. – К., 2004. – 38 с.
12. American Society for Microbiology. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology. – Washington, 2005. – P. 236.
13. McFarland, J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines / Joseph McFarland // J. Am. Med. Assoc. – 1907. – № 49. – P. 1176–1178. doi: 10.1001/jama.1907.25320140022001f.
14. Well diffusion for antifungal susceptibility testing / S. Magaldi, S. Mata-Essayag, C. De Capriles et al. // Int. J. Infect. Dis. – 2004. – № 8. – P. 39–45. doi: 10.1016/j.ijid.2003.03.002.
15. A general approach to indole-7-yl derivatives of isoxazole, oxadiazole, thiadiazole and pyrazole / A. Polozov, G. Hategan, H. Cao et al. // Tetrahedron Lett. – 2010. – № 51. – P. 575–578. doi: 10.1016/j.tetlet.2009.11.073.
16. Design, synthesis and biological evaluation of novel 4-alkynyl-quinoline derivatives as PI3K/mTOR dual inhibitors / X. Lv, H. Ying, X. Ma et al. // Eur. J. Med. Chem. – 2015. – № 99. – P. 36–50. doi: 10.1016/j.ejmech.2015.05.025.
17. Synthesis, biological evaluation of new oxazolidino-sulfonamides as potential antimicrobial agents / A. Kamal, P. Swapna, R. Shetti та ін. // Eur. J. Med. Chem. – 2013. – № 62. – P. 661–669. doi: 10.1016/j.ejmech.2013.01.034.

REFERENCES

1. Krolenko, K. Y., Silin, O. V., Vlasov, S. V., Zhuravel, I. O., & Kovalenko, S. M. (2015). An Efficient Synthesis of 1,3,7-Triazaspiro[4.4]Nonane-2,4-Dione Derivatives and Antimicrobial Activity Thereof. *Chemistry of Heterocyclic Compounds, 51 (5),* 472–477. doi: 10.1007/s10593-015-1721-6.
2. Krolenko, K. Yu., Vlasov, S. V., Vlasova, O. D., Zhuravel, I. O. (2016). The synthesis and the antimicrobial activity of the substituted aryl amides of 3-arylmethyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylic acids. *News of Pharmacy, 4 (88)*, 17–21.
3. Alafeefy, A. M., Abdel-Aziz, H. A., Vullo, D., Al-Tamimi, A.-M. S., Al-Jaber, N. A., Capasso, C., Supuran, C. T. (2014). Inhibition of carbonic anhydrases from the extremophilic bacteria Sulfurihydrogenibium yellostonense (SspCA) and S. azorense (SazCA) with a new series of sulfonamides incorporating aroylhydrazone-, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazinyl- or 2-(cyanophenylmethylene)-1,3,4-thiadiazol-3(2H)-yl moieties. *Bioorganic & Medicinal Chemistry, 22 (1)*, 141–147. doi: 10.1016/j.bmc.2013.11.042.
4. Ranjith, P. K., Pakkath, R., Haridas, K. R., Kumari, S. N. (2014). Synthesis and characterization of new N-(4-(4-chloro-1H-imidazol-1-yl)-3-methoxyphenyl)amide/sulfonamide derivatives as possible antimicrobial and antitubercular agents. *European Journal of Medicinal Chemistry, 71, 354–365*. doi: 10.1016/j.ejmech.2013.11.002.

5. Purser, S., Moore, P. R., Swallow, S., Gouverneur, V. (2008). Fluorine in medicinal chemistry. *Chem. Soc. Rev., 37 (2)*, 320–330. doi: 10.1039/b610213c.
6. Wang, J., Sánchez-Roselló, M., Aceña, J. L., del Pozo, C., Sorochinsky, A. E., Fustero et al. (2014). Fluorine in Pharmaceutical Industry: Fluorine-Containing Drugs Introduced to the Market in the Last Decade (2001–2011). *Chemical Reviews, 114 (4)*, 2432–2506. doi: 10.1021/cr4002879.
7. Kirk, K. L. (2006). Fluorine in medicinal chemistry: Recent therapeutic applications of fluorinated small molecules. *Journal of Fluorine Chemistry, 127 (8)*, 1013–1029. doi: 10.1016/j.jfluchem.2006.06.007.
8. Park, B. K., Kitteringham, N. R., O'Neill, P. M. (2001). Metabolism of fluorine-containing drugs. *Annual Review of Pharmacology and Toxicology, 41 (1)*, 443–470. doi: 10.1146/annurev.pharmtox.41.1.443.
9. O'Hagan, D., Rzepa, H. S. (1997). Some influences of fluorine in bioorganic chemistry. *Chemical Communications, (7)*, 645–652. doi: 10.1039/a604140j.
10. MOZ Ukrainy. (2000). *Bakteriolohichnyi kontrol' pozhyvnykh seredovyshch*. Informatsiinyi lyst № 05. 4. 1 / 1670. Kyiv, 4.
11. Volians'kyi, Yu. L., Hrytsenko, I. S., Shyrobokov, V. P. et al. (2004). *Vyvchennia spetsyfichnoi aktyvnosti protymikrobnykh likars'kykh zasobiv*. Kyiv, 38.
12. American Society for Microbiology. (2005). Manual of Antimicrobial Susceptibility Testing. *American Society for Microbiology*. Washington, 236.
13. McFarland, J. (1907). The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Jama: The Journal of the American Medical Association, XLIX (14)*, 1176–1178. doi: 10.1001/jama.1907.25320140022001f.
14. Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M., Olaizola, C., Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases, 8 (1)*, 39–45. doi: 10.1016/j.ijid.2003.03.002.
15. Polozov, A. M., Hategan, G., Cao, H., Kiselyov, A. S., Zeller, W., Singh, J. (2010). A general approach to indole-7-yl derivatives of isoxazole, oxadiazole, thiadiazole and pyrazole. *Tetrahedron Letters, 51 (4)*, 575–578. doi: 10.1016/j.tetlet.2009.11.073.
16. Lv, X., Ying, H., Ma, X., Qiu, N., Wu, P., Yang, B., Hu, Y. (2015). Design, synthesis and biological evaluation of novel 4-alkynyl-quinoline derivatives as PI3K/mTOR dual inhibitors. *European Journal of Medicinal Chemistry, 99*, 36–50. doi: 10.1016/j.ejmech.2015.05.025.
17. Kamal, A., Swapna, P., Shetti, R. V. C. R. N. C., Shaik, A. B., Narasimha Rao, M. P., Gupta, S. (2013). Synthesis, biological evaluation of new oxazolidino-sulfonamides as potential antimicrobial agents. *European Journal of Medicinal Chemistry, 62*, 661–669. doi: 10.1016/j.ejmech.2013.01.034.

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