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-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with arenesulfonyl chlorides 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 $^{19}F$-NMR fluorine decoupled spectra, as well as the $^{19}F$-$^{1}H$ coupling constants in the $^{1}HNMR$ 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-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with arenesulfonyl chlorides 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
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]ионана-2,4-диоксов с аренсульфохлоридами в присутствии триэтиламина был получен ряд производных 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4]ионана-2,4-диокса. Для соединений, содержащих 1-сульфаниламидо-(2,4)- и 3,4-дифторбензиловые фрагменты, измерены константы спин-спинового взаимодействия водород-водород в $^{1}H$-ЯМР-спектрах с подавлением взаимодействия водород-фтор, а также константы спин-спинового взаимодействия фтор-фтор в $^{19}F$-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]ионана-2,4-диоксов с аренсульфохлоридами является эффективным методом синтеза 7-аренсульфонил-3-арилметил-1,3,7-триазаспиро[4.4]ионана-2,4-диоксов, 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]ионан-2,4-диокнов проявил 3-(3-метилбензил)-7-(тогоп-4-сульфонил)-1,3,7-триазаспиро[4.4]ионан-2,4-дион.

Ключевые слова: гидантоин; сульфамиды; пирролидон; антибактериальные средства

As it was earlier reported the substituted 3-arylmethyl-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$_2$NR$'$R 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-arylmethyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones 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. $^1H$, $^{13}C$ and $^{19}F$ NMR-spectra were recorded with a Bruker 170 Avance 500 spectrometer at 500 MHz for NMR $^1H$-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 $^1H$, $^{13}C$ and CFC$_3$ for $^{19}F$-spectra. Chromat-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-arylmethyl-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-arylmethyl-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 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/ml) for Metronidazole and as the water solution (30 µg/ml) for Synthomyicine. The antibacterial activity was assessed by measuring the growth inhibition zones for each microorganism.

Results and Discussion

To obtain the target novel 3-arylmethyl-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.
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 difluorobenzensulfonyl 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-difluorobenzensulfonyl 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 26.5 Hz due to its interaction with 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 resulted from the interaction with the proton in position 2.

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 ($J = 254.7$ 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 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{$^{19}$F} and $^{19}$F{$^1$H} were
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 measured, and it helped to interpret properly the spectra of compounds 3.2 and 3.5 containing 1-sulfonylamidoo-(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-arylmethyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylic acids previously reported [12], 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.

| Compounds | M.p., °C | $^1$H NMR, δ, ppm (J, Hz) | $^{13}$C and $^{19}$F NMR, δ, ppm(J, Hz) | [M+H]$^+$ |
|-----------|----------|---------------------------|----------------------------------------|----------|
| 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; 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 | 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 | 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; 141.2 | 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 (t, 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 (dd, 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 | 450.0 |
Continuation of Table 1

| 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.** |
| Metronidazol | 14 | 16 | 14 | 0 | 0 | 14 |
| Synthomycin | 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 | 16 | 17 |
| 3.5       | 18 | 18 | 17 | 16 | 17 | 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 |

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

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

The reaction of 3-aryl methyl-1,3,7-triazaspiro[4.4]nonane-2,4-diones with arenesulfonyl chlorides has shown to be an effective method for obtaining a variety of the substituted derivatives of 7-arenesulfonyl-3-aryl methyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione as compounds with the promising potent biological activity. The 1H- and 19F 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-aryl methyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione series.

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

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