The synthesis and the antimicrobial activity of $N'$-substituted 5-amino-4-arylsulfonyl-3-$N$-phenylaminopyrazoles

This article is continuation of the development of methods for the synthesis of small molecules based on the structure of 5-aminopyrazole. The synthesis and the antimicrobial activity for a series of new $N'$-substituted 5-amino-4-arylsulfonyl-3-$N$-phenylaminopyrazoles have been described.

**Aim.** To synthesize derivatives of 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles and study their antimicrobial and antifungal properties.

**Materials and methods.** The methods of organic synthesis, instrumental methods of organic compounds analysis and methods of microbiological screening were used.

**Results and discussion.** 5-Amino-4-arylsulfonyl-3-phenylaminopyrazoles were prepared by the reaction of arylsulfonylacetonitriles with isothiocyanates in the presence of NaOH and CH$_3$I with further cyclization with hydrazine hydrate. The reaction of this compounds with $N$-arylchloroacetamides finished a series of $N1$-substituted 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles. The antibacterial and antifungal properties of the compounds synthesized were studied. Some of the compounds obtained appeared to be potent inhibitors for several pathogenic bacterial and fungal lines.

**Conclusions.** The synthetic scheme for obtaining of $N'$-substituted 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles, which can be used for creation of a library of compounds for in vitro antimicrobial screening, has been proposed. Some of the compounds synthesized are of certain interest as potential pharmaceutical agents and can be used to develop new antifungal agents.

**Key words:** 5-aminopyrazole; synthesis; antimicrobial activity; antifungal activity
Various substituted aminopyrazoles are an important scaffold for medical chemistry and present in the core of many pharmacological agents. For example, Cefotizone is a cephalosporin antibiotic of the 5th generation [1], Tozasertib is a pan-Aurora inhibitor [2]. Polysubstituted derivatives of pyrazole can act as antibacterial [3-6], anti-inflammatory [3, 7, 8], cytostatic [2, 9, 10], anesthetic [11], anticancer [12-15], antiepileptic agents [16-18] and as insecticides [19, 20].

These examples illustrate the ongoing interest toward new small molecules with the pyrazole ring and have prompted us to explore the synthetic route of chemical modification of 3,5-diaminopyrazole, which can serve as a promising source of bioactive molecules.

**Experimental Part**

All reagents and solvents were obtained from the commercial sources. Elemental analysis was performed on a Euro EA-3000 apparatus. Melting points were obtained by a Buchi B-520 device. The NMR-spectra were recorded with a Bruker 170 Avance spectrometer at 200 MHz, 500 MHz (DMSO-d₆); TMS was used as an internal standard; chemical shifts were reported in ppm. LC/MS spectra were recorded with a PE SCIEX API 150EX liquid chromatograph equipped with a UV detector (215 and 254 nm). Elution started with water and ended with acetonitrile/water (95 : 5, v/v); a linear gradient at the flow rate of 0.15 mL/min and the cycle time of 25 min was used. According to LC/MS data all compounds synthesized had purity > 95 %. The TLC was performed on the aluminum plates covered with a silica gel (Merck, Kieselgel 60 F-254).

**The general procedure for 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles (1.1-1.2).** To the solution of sodium hydroxide (85 mmol, 3.4 g) in water (100 mL) add the mixture of the corresponding arylsulfonylacetonitrile (80 mmol) and phenylisothiocyanate (85 mmol, 11.47 g) in dioxane (150 mL) and stir at room temperature. After that add methyl iodide (85 mmol, 5.3 g), and stir for 3 h at room temperature. Dilute the reaction mixture with cool water (200 mL). Filter the precipitate formed and wash with water and propanol-water mixture (1:1). Add hydrazine hydrate (66 mmol, 3.3 mL) and few drops of triethylamine to the corresponding N,S-acetale (60 mmol) in propanol-water mixture (180 mL) and reflux for 3 h. After that dilute the solution with water (150 mL). Filter the precipitate formed and wash with water and propanol-water mixture (1:1).

5-Amino-4-phenylsulfonyl-3-phenylaminopyrazole 1.1. Yield – 91 %. M. p. – 196 °C. ¹H NMR δ: 6.10 (s, 2H, NH), 6.75 (t, 1H, NH), 7.18 (t, 2H, Ar-H), 7.71 (m, 6H, Ar-H), 7.95 (d, 2H, Ar-H), 11.30 (s, 1H, NH).

5-Amino-4-(4′-methylphenyl)sulfonyl-3-phenylaminopyrazole 1.2. Yield – 96 %. M. p. – 206-07 °C. ¹H NMR δ: 2.29 (s, 3H, CH₃), 6.08 (s, 2H, NH), 6.75 (t, 1H, NH), 7.21 (t, 2H, Ar-H), 7.33 (d, 2H, Ar-H), 7.49 (m, 3H, Ar-H), 7.86 (d, 2H, Ar-H), 11.27 (s, 1H, NH).

**The general procedure for N′-alkylation 5-amino-4-(4′-methylphenyl)sulfonyl-3-N′-phenylaminopyrazole.** To the mixture of 5-amino-4-(4′-methylphenyl)sulfonyl-3-N′-phenylaminopyrazole (5 mmol, 1.64 g) and K₂CO₃ (15 mmol, 0.87 g) in DMF (50 mL) add the appropriate N-arylcloacetamide (6 mmol). Stir the reaction mixture at 80 °C for 1 h, then cooled to room temperature. Add water (150 mL), filter the precipitate formed, and crystallize from the mixture of ethanol – DMF (1:1).

**Microbiological experiment**

The antibacterial and antifungal activity was determined in vitro by the double dilution method in the Anti bacterial Agents Laboratory of Mechnikov Institute of Microbiology and Immunology. As a microbial model a set of clinical and reference strains of microorganisms such as Escherichia coli ATCC 25922 (F-50), Staphylococcus aureus ATCC 25923 (F-49), Bacillus anthracoides...
ATCC 1312, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 885-653 were used. As a standard of the antibacterial action Palinium and Nevigramon were chosen due to their significant activity against most gram-negative and gram-positive bacteria. Moreover, they are widely used in antimicrobial therapy. As a standard of the antifungal action Fluconazolum was used.

**Results and discussion**

A convenient and effective synthetic scheme for preparation \(N^1\)-substituted 4-alkyl/arylsulfonyl-5-amino-3-alkylthiopyrazoles and their acylation products [5] was developed. The key procedures, which led to the target compounds, were the synthesis of the corresponding \(S,S\)-acetals and their cyclization with hydrazine hydrate. In this paper, using the similar synthetic scheme, a new series of 4-arylsulfonyl derivatives of 5-aminopyrazoles with 3-arylamino fragment was obtained. For this purpose arylisothiocyanates were used at the first stage of interaction, and as intermediates \(N,S\)-acetals were prepared. The reaction of arylsulfonylecyanitiles 1.1-1.2 with phenylisothiocyanate in the presence of NaOH in the presence of K₂CO₃ at 80 °C led to \(N^1\)-substituted pyrazoles 3.1-3.11. The yields of the target products depended on the nature of substitutes in aryl fragments. Recrystallization from propanole-2 resulted in 3.1-3.11 as light yellow residues with 61-88 % yields (Tab. 1).

Tab. 2 shows MIC (minimal inhibitory concentration, mg/mL) values of \(N^1\)-substituted 5-amino-4-(4'-methylphenyl)sulfonyl-3-phenylaminopyrazoles 3.1-3.11. Most compounds analyzed were active \textit{in vitro} experiments. Thus, eight compounds demonstrated a significant activity against one or more strains of gram-negative and
### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>R²</th>
<th>Yield, %</th>
<th>M. p., °C</th>
<th>¹H NMR δ, ppm (DMSO, 200 MHz)</th>
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<tr>
<td>3.1</td>
<td>H</td>
<td>61</td>
<td>278-80</td>
<td>2.30 (s, 3H, CH₃), 4.65 (s, 2H, CH₂), 6.42 (s, 2H, NH₂), 6.79 (t, 1H, Ar-H), 7.02 (t, 1H, Ar-H), 7.17-7.35 (m, 5H, Ar-H), 7.25 (s, 1H, Ar-H), 7.50 (m 5H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.17 (s, 1H, NH)</td>
</tr>
<tr>
<td>3.2</td>
<td>2-F</td>
<td>78</td>
<td>271-72</td>
<td>2.32 (s, 3H, CH₃), 4.69 (s, 2H, CH₂), 6.43 (s, 2H, NH₂), 6.80 (t, 1H, Ar-H), 7.18 (m, 4H, Ar-H), 7.34 (d, 2H, Ar-H), 7.51 (s+m, 5H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.25 (s, 1H, NH)</td>
</tr>
<tr>
<td>3.3</td>
<td>2,4-diF</td>
<td>82</td>
<td>264-66</td>
<td>2.30 (s, 3H, CH₃), 4.69 (s, 2H, CH₂), 6.43 (s, 2H, NH₂), 6.80 (t, 1H, Ar-H), 7.18 (m, 4H, Ar-H), 7.34 (d, 2H, Ar-H), 7.51 (s+m, 5H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.25 (s, 1H, NH)</td>
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<td>3.4</td>
<td>2-Cl-4-F</td>
<td>70</td>
<td>&gt; 300</td>
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<tr>
<td>3.5</td>
<td>2-Me-5-F</td>
<td>80</td>
<td>291-92</td>
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<tr>
<td>3.6</td>
<td>4-i-Pr</td>
<td>68</td>
<td>218-19</td>
<td>1.10 (d, 6H, 2CH₃), 2.29 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 4.62 (s, 2H, NH₂), 6.80 (t, 1H, Ar-H), 7.16 (m, 4H, Ar-H), 7.35 (d, 2H, Ar-H), 7.48 (m, 5H, NH+Ar-H), 7.89 (d, 2H, Ar-H), 10.05 (s, 1H, NH)</td>
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<td>269-70</td>
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<td>3.8</td>
<td>3,5-diMe</td>
<td>81</td>
<td>248</td>
<td>1.10 (d, 6H, 2CH₃), 2.29 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 4.62 (s, 2H, NH₂), 6.80 (t, 1H, Ar-H), 7.16 (m, 4H, Ar-H), 7.35 (d, 2H, Ar-H), 7.48 (m, 5H, NH+Ar-H), 7.89 (d, 2H, Ar-H), 10.05 (s, 1H, NH)</td>
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<td>3.9</td>
<td>3-OMe</td>
<td>74</td>
<td>231-33</td>
<td>2.28 (s, 3H, CH₃), 3.67 (s, 3H, OCH₃), 4.65 (s, 2H, CH₂), 6.24 (s, 2H, NH₂), 6.61 (dd, 1H, Ar-H), 6.81 (t, 1H, Ar-H), 7.04 (dd, 1H, Ar-H), 7.17 (t, 3H, Ar-H), 7.32 (m, 1H, Ar-H), 7.47 (s, 3H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.20 (s, 1H, NH)</td>
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<td>79</td>
<td>&gt; 300</td>
<td>1.13 (t, 3H, CH₂CH₃), 2.27 (s, 3H, CH₃), 3.90 (q, 2H, CH₂CH₃), 4.61 (s, 2H, CH₂), 6.42 (s, 2H, NH₂), 6.61 (dd, 1H, Ar-H), 6.80 (t, 1H, Ar-H), 7.04 (dd, 1H, Ar-H), 7.17 (t, 3H, Ar-H), 7.32 (m, 1H, Ar-H), 7.47 (s, 3H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.01 (s, 1H, NH)</td>
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<tr>
<td>3.11</td>
<td>3,4-diOMe</td>
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<td>256-57</td>
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</tbody>
</table>

The antibacterial and antifungal activity of N¹-substituted 5-amino-4-(4’-methylphenyl)sulfonyl-3-phenylaminopyrazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Staphylococcus aureus ATCC 25923</th>
<th>Esherichia coli ATCC 25922</th>
<th>Pseudomonas aeruginosa ATCC 27853</th>
<th>Proteus vulgaris ATCC 4636</th>
<th>Bacillis anthracoides ATCC 1312</th>
<th>Candida albicans ATCC 885-653</th>
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<td>100</td>
<td>100</td>
<td>12,5</td>
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<tr>
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<td>25</td>
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<td>100</td>
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<td>12,5</td>
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<td>50</td>
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<td>50</td>
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<td>100</td>
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<td>100</td>
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<td>3.8</td>
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<td>100</td>
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<td>12,5</td>
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<td>100</td>
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<td>12,5</td>
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<tr>
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<td>100</td>
<td>50</td>
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<tr>
<td>Palinum</td>
<td>6,25</td>
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<td>12,5</td>
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<tr>
<td>Nevigramon</td>
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<td>6,25</td>
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<tr>
<td>Fluconazolum</td>
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</table>
gram-positive bacteria with MIC value of 25.0 mg/mL. Four compounds were active against E. coli; three of them revealed the same efficacy as Palinin (MIC 25 mg/mL), two compounds (3.4, 3.7) exhibited the efficacy inhibiting of E. coli and St. aureus. However, all compounds synthesized were practically inactive against Pr. vulgaris and B. anthracoides.

Most derivatives of pyrazole analyzed were active against C. albicans: six of them revealed better efficacy than the standard antifungal drug, Fluconazolom. The most active antifungal compounds were 3.3, 3.4, 3.8 and 3.10 (MIC 12.5 mg/mL).

One can assume that synthesized compounds have a broad potential of the pharmacological activity especially as antifungal agents; and development of the methods of synthesis and the study of pharmaceutical properties of these compounds are topical directions of pharmaceutical and medical chemistry.

CONCLUSIONS

The synthetic scheme for obtaining of N'-substituted 5-amino-4-aryl sulfonyl-3-phenylaminopyrazoles, can be used for creation of a library of compounds for in vitro antimicrobial screening, has been proposed. Some of the compounds synthesized are of certain interest as potential pharmaceutical agents and can be used to develop new antifungal agents.

Conflict of Interests: authors have no conflict of interests to declare.

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