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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 6-PHENYL-1H-PYRROLO[3,4-*c*]PYRIDINE-1,3-DIONE DERIVATIVES

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Abstract: The new pyrrolo[3,4-*c*]pyridines have been synthesized. 4-Methyl-6-phenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3-dione (**1**) was the key intermediate for the synthesis of the novel derivatives of various chemical structures. In the first step, the pyrrolo[3,4-*c*]pyridine-1,3-dione **1** was alkylated to the corresponding *N*-alkyl-4-methyl-6-phenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3-dione derivatives **2a-f**. The Mannich bases **3a-j** were synthesized by treating pyrrolo[3,4-*c*]pyridine-1,3-dione **1** with appropriate amines and formaldehyde. Hydrolysis of ester **2a** gave the appropriate acid **5**. Next, amides **4a-e** have been obtained. The structures of the new compounds were confirmed by elemental analysis, IR and NMR spectra. The antitumor and antimicrobial activities *in vitro* of the obtained derivatives were examined. Mannich bases **3c** and **3g** showed activity against *C. albicans* and *S. aureus*.

Keywords: pyrrolo[3,4-*c*]pyridine-1,3-dione, Mannich bases, antiproliferative activity *in vitro*, antimicrobial activity *in vitro*

Pyrrolo[3,4-*c*]pyridine is one of the six structural isomers of the bicyclic ring system containing pyrrole moiety condensed with a pyridine scaffold.

Based on the review (1), biological investigations have shown that pyrrolo[3,4-*c*]pyridine derivatives have a wide spectrum of action. Most of them have been studied as analgesic and sedative agents (2-7). Pyrrolo[3,4-*c*]pyridines can be used in the treatment of the nervous (8, 9) and immune (10, 11) systems diseases. Anticancer (10-12), antituberculostatic (13), antiviral (14) activities also have been found. The broad spectrum of biological activity of pyrrolo[3,4-*c*]pyridine derivatives is the main reason for preparation of the new compounds containing this scaffold.

The aim of this work was to synthesize the new pyrrolo[3,4-*c*]pyridine derivatives with potential biological activity. In the previous paper (15) the way of synthesizing 6-phenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3-diones was determined. As a continuation of that research the new derivatives have been synthesized by a modification of the substituent in the position 2 of the pyrrolo[3,4-*c*]pyridine scaffold. The substrate in this study was 4-methyl-6-phenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3-dione (**1**) (15). In the first step, two series of novel derivatives have been synthesized: *N*-alkyl-4-methyl-6-phenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3-diones **2a-f** and Mannich bases **3a-j**. Next, the substrate was ester **2a**. Acid **5** and the appropriate amides **4a-e** were formed. All derivatives were evaluated for their antiproliferative activity *in vitro*.

Some reviews (16, 17) showed that Mannich bases possess various biological properties. A number of Mannich bases have been examined as antibacterial and antifungal agents (17). Therefore, new obtained Mannich bases **3a-j** were tested for antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Enterococcus faecalis* and *Candida albicans*.

EXPERIMENTAL

Chemistry

Melting points were measured in open glass capillaries with a MEL-TEMP apparatus (Barnstead

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International, Dubuque, IO, USA) and were uncorrected. The new products were analyzed using a Perkin Elmer 2400 analyzer (Waltham, MA, USA). IR spectra were performed on the Thermo Scientific Nicolet iS50 FT-IR spectrometer (Thermo Fisher Scientific Inc., USA) or on a Specord M80 Spectometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with a Bruker Avance ARX-300 MHz spectrometer (Bruker Analytic, Karlsruhe, Germany) with TMS as the internal standard. The course of the reactions and the purity of the compounds were checked by TLC, using aluminum sheet silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany). The chemicals for the syntheses were purchased from Chempur (Piekary Sl., Poland), Alfa Aesar (Karlsruhe, Germany), and Lancaster (Frankfurt am Main, Germany). Compounds **1** and **2a-d** were prepared according to the methods presented in the previous papers (15, 20).

General procedure for the synthesis of *N*-alkyl-4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione derivatives (2e-f)

To a solution of 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione **1** (0.01 mol) in anhydrous *N*,*N*-dimethylformamide (100 mL), sodium hydride (0.01 mol) was added. The mixture was stirred at room temperature for 2 h. To obtained sodium salt, iodomethane (0.01 mol), or bromoethane (0.01 mol) was dropped. The mixture was stirred at room temperature for 4 h, and next, it was diluted with ice-water. The obtained solid was filtered, dried and recrystallized.

2,4-Dimethyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (2e)

Yield 2.04 g (81%), beige solid, crystallized from methanol, m.p. 198-199°C. IR (KBr, cm^{-1}): 1780, 1720 (C=O), 750 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.81 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 7.50-7.52 (m, 3H, arom.), 8.19-8.25 (m, 3H, arom.). Analysis: calcd. for $C_{15}H_{12}N_2O_2$ (252.08): C, 71.38; H, 4.80; N, 11.13%; found: C, 70.99; H, 4.68; N, 11.28%.

2-Ethyl-4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (2f)

Yield 2.07 g (78%), beige solid, crystallized from methanol, m.p. 161-163°C. IR (KBr, cm^{-1}): 1780, 1720 (C=O), 720 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.18 (t, *J* = 6.9 Hz, 3H, CH_3), 2.84 (s, 3H, CH_3), 3.62 (q, *J* = 7.2 Hz, 2H, CH_2), 7.52-7.53 (m, 3H, arom.), 8.20-8.23 (m, 3H, arom.). Analysis: calcd. for $C_{16}H_{14}N_2O_2$ (266.29): C, 72.20; H, 5.33; N, 10.50%; found: C, 72.51; H, 5.14; N, 10.23%.

General procedure for the synthesis of Mannich bases (3a-j)

To a solution of 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione **1** (0.01 mol) in ethanol (20 mL), formaldehyde (0.02 mol) and the appropriate amine (0.02 mol) were added. The mixture was heated under reflux with stirring for 2-4 h. After cooling, the obtained solid was filtered, dried and recrystallized.

2-[(Dibutylamino)methyl]-4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (3a)

Yield 2.64 g (70%), white solid, crystallized from ethanol, m.p. 89-91°C. IR (KBr, cm^{-1}): 2950 (CH), 1776, 1720 (C=O), 1092 (C-N), 752 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 0.86-0.90 (m, 6H, CH_3), 1.27-1.47 (m, 8H, CH_2), 2.45-2.50 (m, 4H, CH_2), 2.83 (s, 3H, CH_3), 4.51 (s, 2H, CH_2), 7.50-7.55 (m, 3H, arom.), 8.21-8.25 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 13.83 (2C), 19.80 (2C), 20.94, 29.43 (2C), 51.09 (2C), 56.57, 111.08, 121.05, 127.45 (2C), 128.91 (2C), 130.58, 137.10, 141.36, 155.55, 161.03, 167.99, 169.19. Analysis: calcd. for $C_{23}H_{29}N_3O_2$ (379.49): C, 70.80; H, 6.39; N, 13.12%; found: C, 70.42; H, 6.70; N, 13.05%.

2-[(4-Chloroanilino)methyl]-4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (3b)

Yield 3.10 g (82%), white solid, crystallized from ethanol, m.p. 205-207°C. IR (KBr, cm^{-1}): 3300 (NH), 1768, 1716 (C=O), 1096 (C-N), 748 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.84 (s, 3H, CH_3), 5.14 (d, *J* = 6.6 Hz, 2H, CH_2), 6.04 (t, *J* = 6.5 Hz, 1H, NH), 6.64-6.69 (m, 1H, arom.), 7.07-7.09 (m, 1H, arom.), 7.14-7.20 (m, 1H, arom.), 7.25-7.27 (m, 1H, arom.), 7.50-7.55 (m, 3H, arom.), 8.22-8.28 (m, 3H, arom.). Analysis: calcd. for $C_{21}H_{16}ClN_3O_2$ (377.82): C, 66.80; H, 4.31; N, 11.12%; found: C, 67.07; H, 4.20; N, 11.37%.

2-[(2-Chloroanilino)methyl]-4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (3c)

Yield 2.78 g (73%), beige solid, crystallized from ethanol, m.p. 185-186°C. IR (KBr, cm^{-1}): 3450 (NH), 1760, 1720 (C=O), 1100 (C-N), 750 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.83 (s, 3H, CH_3), 5.14 (d, *J* = 6.9 Hz, 2H, CH_2), 6.01-6.06 (t, *J* = 6.8 Hz, 1H, NH), 6.66-6.69 (m, 1H, arom.), 7.09-7.28 (m, 3H, arom.), 7.49-7.51 (m, 3H,

arom.), 8.15-8.23 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 20.94, 47.07, 111.19, 111.86, 118.38, 118.41, 120.86, 127.45 (2C), 127.99, 128.88 (2C), 129.29, 130.61, 136.99, 141.19, 141.51, 155.69, 161.12, 166.66, 167.89. Analysis: calcd. for $C_{21}H_{16}ClN_3O_2$ (377.82): C, 66.80; H, 4.31; N, 11.12%; found: C, 66.78; H, 4.24; N, 11.08%.

2-[(4-Ethoxyanilino)methyl]-4-methyl-6-phenyl-pyrrolo[3,4-c]pyridine-1,3-dione (3d)

Yield 2.52 g (65%), yellow solid, crystallized from ethanol, m.p. 155-156°C. IR (KBr, cm^{-1}): 3400 (NH), 1776, 1712 (C=O), 1244 (CO), 1080 (C-N), 748 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.25-1.30 (t, *J* = 6.9 Hz, 3H, CH_3), 2.84 (s, 3H, CH_3), 3.91 (q, *J* = 6.4 Hz, 2H, CH_2), 4.83 (d, *J* = 6.9 Hz, 2H, CH_2), 5.22-5.24 (m, 1H, NH), 6.76-6.82 (m, 2H, arom.), 7.06-7.09 (m, 2H, arom.), 7.52-7.54 (m, 3H, arom.), 8.17-8.25 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 14.97, 20.94, 54.83, 61.69, 63.07, 81.92, 111.20, 114.80, 117.04, 120.98, 127.49 (2C), 128.93 (2C), 130.65, 137.03, 140.02, 141.31, 152.39, 155.73, 161.17, 166.82, 168.10. Analysis: calcd. for $C_{23}H_{21}N_3O_3$ (387.43): C, 71.33; H, 5.49; N, 10.81%; found: C, 71.68; H, 5.09; N, 10.55%.

2-(Anilinomethyl)-4-methyl-6-phenyl-pyrrolo[3,4-c]pyridine-1,3-dione (3e)

Yield 2.96 g (86%), beige solid, crystallized from ethanol, m.p. 134-136°C. IR (KBr, cm^{-1}): 3410 (NH), 1770, 1710 (C=O), 1076 (C-N), 744, 696 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.83 (s, 3H, CH_3), 5.02 (d, *J* = 6.9 Hz, 2H, CH_2), 6.62-6.66 (m, 1H, NH), 6.83-6.86 (m, 2H, arom.), 7.09-7.23 (m, 3H, arom.), 7.49-7.51 (m, 3H, arom.), 8.20-8.23 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 20.94, 47.25, 111.19, 112.43 (2C), 114.52, 117.20, 120.85, 127.47 (2C), 128.92 (2C), 128.99, 130.61, 137.03, 141.24, 146.05, 155.66, 161.14, 166.74, 168.01. Analysis: calcd. for $C_{21}H_{17}N_3O_2$ (343.38): C, 73.53; H, 5.01; N, 12.22%; found: C, 73.87; H, 4.61; N, 12.38%.

2-[(4-Benzylpiperazin-1-yl)methyl]-4-methyl-6-phenyl-pyrrolo[3,4-c]pyridine-1,3-dione (3f)

Yield 3.15 g (74%), yellow solid, crystallized from ethanol, m.p. 155-157°C. IR (KBr, cm^{-1}): 1716 (C=O), 1168, 1144 (C-N), 760, 748 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.50-2.56 (m, 8H, CH_2), 2.84 (s, 3H, CH_3), 3.41 (s, 2H, CH_2), 4.48 (s, 2H, CH_2), 7.24-7.27 (m, 5H, arom.), 7.53-7.55 (m, 3H, arom.), 8.23-8.26 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 20.97, 49.93 (2C), 52.39 (2C), 59.14, 61.88, 111.16, 126.79, 127.50 (2C), 128.05 (2C), 128.63 (2C), 128.96 (2C), 130.59 (2C), 137.19, 138.17, 141.37, 155.67, 161.07, 167.63, 168.80. Analysis: calcd. for $C_{26}H_{26}N_4O_2$ (426.51): C, 73.27; H, 6.14; N, 13.11%; found: C, 73.33; H, 6.01; N, 12.90%.

4-Methyl-2-(morpholinomethyl)-6-phenyl-pyrrolo[3,4-c]pyridine-1,3-dione (3g)

Yield 2.43 g (72%), beige solid, crystallized from ethanol, m.p. 148-150°C. IR (KBr, cm^{-1}): 1772, 1716 (C=O), 1340, 1276 (CO), 1160, 1104 (C-N), 760 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.52 (s, 4H, CH_2), 2.83 (s, 3H, CH_3), 3.54 (s, 4H, CH_2), 4.44 (s, 2H, CH_2), 7.52-7.55 (m, 3H, arom.), 8.21-8.27 (m, 3H, arom.). Analysis: calcd. for $C_{19}H_{19}N_3O_3$ (337.37): C, 67.64; H, 5.68 N, 12.46%; found: C, 67.69; H, 5.65; N, 12.82%.

4-Methyl-6-phenyl-2-(1-piperidylmethyl)pyrrolo[3,4-c]pyridine-1,3-dione (3h)

Yield 2.12 g (63%), yellow solid, crystallized from ethanol, m.p. 170-172°C. IR (KBr, cm^{-1}): 1776, 1716 (C=O), 1176, 1072 (C-N), 756 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.25-1.31 (m, 2H, CH_2), 1.43-1.51 (m, 4H, CH_2), 2.86 (s, 3H, CH_3), 3.32 (s, 4H, CH_2), 4.44 (s, 2H, CH_2), 7.54-7.56 (m, 3H, arom.), 8.24-8.29 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 20.96, 23.28, 25.42 (2C), 51.16 (2C), 60.19, 111.16, 121.08, 127.51 (2C), 128.96 (2C), 130.59, 137.19, 141.38, 155.63, 161.09, 167.69, 168.89. Analysis: calcd. for $C_{20}H_{21}N_3O_2$ (337.37): C, 71.62; H, 6.31 N, 12.53%; found: C, 71.23; H, 6.00; N, 12.93%.

4-Methyl-2-[(4-methylpiperazin-1-yl)methyl]-6-phenyl-pyrrolo[3,4-c]pyridine-1,3-dione (3i)

Yield 2.08 g (59%), yellow solid, crystallized from ethanol, m.p. 169-171°C. IR (KBr, cm^{-1}): 1776, 1716 (C=O), 1176, 1148, 1132, 1072 (C-N), 756 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.10 (s, 3H, CH_3), 2.25-2.75 (m, 4H, CH_2), 2.48-2.53 (m, 4H, CH_2), 2.84 (s, 3H, CH_3), 4.45 (s, 2H, CH_2), 7.52-7.55 (m, 3H, arom.), 8.20-8.28 (m, 3H, arom.). Analysis: calcd. for $C_{20}H_{22}N_4O_2$ (350.41): C, 68.55; H, 6.33; N, 15.99%; found: C, 68.50; H, 6.02; N, 16.09%.

4-Methyl-6-phenyl-2-[(4-phenylpiperazin-1-yl)methyl]pyrrolo[3,4-c]pyridine-1,3-dione (3j)

Yield 2.86 g (69%), beige solid, crystallized from ethanol, m.p. 163-165°C. IR (KBr, cm^{-1}): 1772, 1716 (C=O), 1168, 1112 (C-N), 756, 696 (CH

arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.65-2.75 (m, 4H, CH_2), 2.86 (s, 3H, CH_3), 3.11-3.15 (m, 4H, CH_2), 4.55 (s, 2H, CH_2), 6.74-6.77 (m, 1H, arom.), 6.87-6.90 (m, 2H, arom.), 7.14-7.20 (m, 2H, arom.), 7.53-7.55 (m, 3H, arom.), 8.22-8.28 (m, 3H, arom.). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 20.98, 48.21 (2C), 49.90 (2C), 59.15, 111.17, 115.52 (2C), 118.85, 122.01, 162.50, 127.501 (2C), 128.84 (2C), 128.96 (2C), 130.60, 137.17, 141.37, 150.98, 155.68, 167.64, 168.82. Analysis: calcd. for $C_{25}H_{24}N_4O_2$ (412.48): C, 70.27; H, 6.04; N, 13.66%; found: C, 70.63; H, 5.66; N, 13.38%.

General procedure for the synthesis of amides (4a-e)

To a solution of ethyl 2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridine-2-yl)acetate (**2a**) (0.01 mol) in anhydrous toluene (20 mL) the appropriate amine (0.02 mol) and catalytic amount of sodium methoxide were added. The mixture was stirred at room temperature (comp. **4a-b**) or at 60°C (comp. **4c-e**) for 2-4 days. After cooling, the mixture was washed with 5% HCl solution. The obtained solid was filtered, dried and recrystallized.

***N*-Isopropyl-2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetamide (4a)**

Yield 1.96 g (58%), white solid, crystallized from ethanol, m.p. 205-207°C. IR (KBr, cm^{-1}): 3310 (NH), 1760, 1650, 1630 (C=O), 680 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.01-1.14 (m, 6H, CH_3), 2.62 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 3.97-3.99 (m, 1H, CH), 7.49-7.51 (m, 3H, phenyl), 8.10-8.13 (m, 3H, phenyl) 8.77 (s, 1H, NH). Analysis: calcd. for $C_{19}H_{19}N_3O_3$ (337.37): C, 67.60; H, 5.75; N, 12.53%; found: C, 67.53; H, 6.06; N, 12.36%.

***N*-Ethyl-2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetamide (4b)**

Yield 1.82 g (56%), white solid, crystallized from ethanol, m.p. 143-145°C. IR (KBr, cm^{-1}): 3450 (NH), 1760, 1710, 1630, (C=O), 750 (CH arom.).^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.33-1.37 (t, *J* = 7.0 Hz, 3H, CH_3), 3.05 (s, 3H, CH_3), 3.92 (s, 2H, CH_2), 4.36-4.38 (q, *J* = 6.9 Hz, 2H, CH_2), 7.51-7.54 (m, 3H, arom.), 8.17-8.19 (m, 3H, arom.) 10.50 (s, 1H, NH). Analysis: calcd. for $C_{18}H_{17}N_3O_3$ (323.34): C, 66.90; H, 5.32; N, 13.02%; found: C, 66.98; H, 5.16; N, 12.64%.

***N*-Benzyl-2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetamide (4c)**

Yield 2.64 g (68%), white solid, crystallized from ethanol, m.p. 222-224°C. IR (KBr, cm^{-1}): 3300 (NH), 1650, 1580 (C=O), 650 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.32 (s, 3H, CH_3), 4.40-4.46 (m, 4H, CH_2), 7.26-7.51 (m, 8H, arom.), 7.90 (s, 1H, arom.), 8.10-8.12 (m, 2H, arom.), 9.01 (s, 1H, NH). Analysis: calcd. for $C_{23}H_{19}N_3O_3$ (385.42): C, 71.73; H, 5.05; N, 10.92%; found: C, 71.57; H, 4.89; N, 11.03%.

4-Methyl-2-(2-morpholino-2-oxo-ethyl)-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (4d)

Yield 2.36 g (65%), white solid, crystallized from ethanol, m.p. 191-193°C. IR (KBr, cm^{-1}): 1720, 1680, (C=O), 1240 (CO), 1120 (C-N), 740 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.86 (s, 3H, CH_3), 3.44-3.49 (m, 2H, CH_2), 3.59-3.67 (m, 6H, CH_2), 4.57 (s, 2H, CH_2), 7.54-7.56 (m, 3H, arom.), 8.27-8.30 (m, 3H, arom.). Analysis: calcd. for $C_{20}H_{19}N_3O_4$ (365.38): C, 65.73; H, 5.20; N, 11.51%; found: C, 65.44; H, 5.19; N, 11.19%.

4-Methyl-2-[2-oxo-2-(1-piperidyl)ethyl]-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (4e)

Yield 2.21 g (61%), white solid, crystallized from ethanol, m.p. 186-188°C. IR (KBr, cm^{-1}): 1720, 1650, (C=O), 1250 (CO), 1100 (C-N), 740 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.45-1.61 (m, 4H, CH_2), 2.87 (s, 3H, CH_3), 3.32-3.51 (m, 6H, CH_2), 4.53 (s, 2H, CH_2), 7.54-7.56 (m, 3H, arom.), 8.30-8.31 (m, 3H, arom.). Analysis: calcd. for $C_{21}H_{21}N_3O_3$ (363.41): C, 69.42; H, 5.78; N, 11.57%; found: C, 69.35; H, 6.09; N, 11.45%.

2-(4-Methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridin-2-yl)acetic acid (5)

The solution of ester **2a** (0.01 mol) in 100 mL of the mixture of HCl/CH_3COOH (1 : 2) was refluxed for 5 h. Next, 0.1 M sodium hydroxide was added to the mixture to pH = 3-4. The precipitate was filtered, dried and crystallized. Yield 2.17 g (73%), white solid, crystallized from ethanol, m.p. 186-188°C. IR (KBr, cm^{-1}): 3470 (OH), 1720, 1620, (C=O), 1400 (OH), 1250 (CO), 750 (CH arom.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.85 (s, 3H, CH_3), 4.34 (s, 2H, CH_2), 7.53-7.55 (m, 3H, arom.), 8.26-8.30 (m, 3H, arom.), 13.35 (s, 1H, COOH). Analysis: calcd. for $C_{16}H_{12}N_2O_4$ (296.28): C, 64.92; H, 4.14; N, 9.52%; found: C, 65.06; H, 4.05; N, 9.64%.

***In vitro* antimicrobial assay**

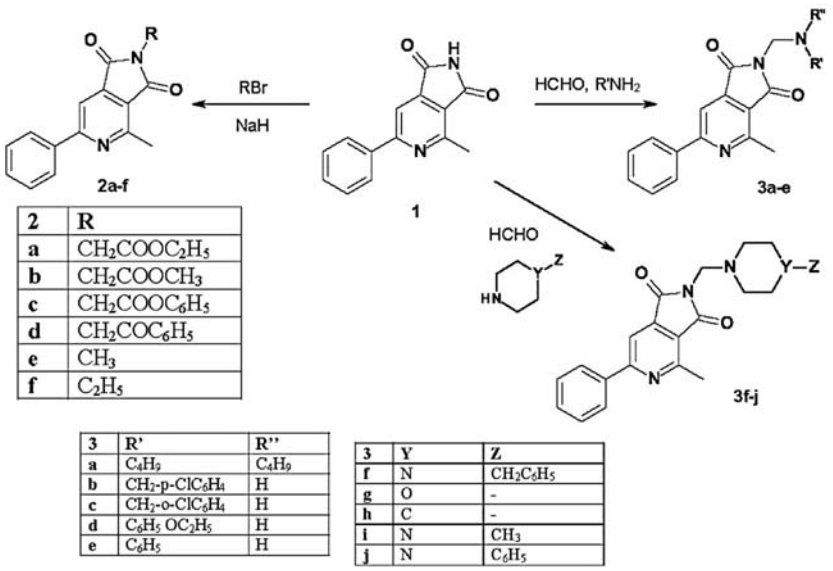
Culturing and preparation of microbial strains

The following microbial strains from ATCC cultures were used for experimental purposes: *Staphylococcus aureus* 6538; *Pseudomonas aerugi-*

nosa 15442; *Enterococcus faecalis* 29212; *Candida albicans* 10231. Strains cultured on appropriate agar plates (Columbia Agar Plate for *S. aureus*; Sabouraud Plate for *C. albicans*; McConkey Agar Plate for *P. aeruginosa*) were transferred to liquid Tryptic Soya Broth with exception of *E. faecalis*, which was transferred to rich Brain-Heart Infusion Broth. Strains were incubated for 24 h/37°C. After incubation time, strains were diluted to the density of 10^5 cfu using densitometer and serial dilution method. The microbial dilutions were introduced to wells of 96-well plates containing solutions of DMSO-solved compounds. Final concentration of DMSO in wells was 1%, whereas the final concentrations of compounds tested were 100, 50, 25, 12.5 mg/L with exception of **2a** compound which the highest final concentration to achieve was 50 mg/L. The test plates were incubated for 18 h/37°C in plate shaker to provide appropriate oxygenation and growth conditions.

Spectrometric assessment

After incubation time, the plates were introduced to spectrometer. For bacterial strains, wavelength of 580 nm was used, whereas for fungus *Candida albicans*, the wavelength applied was 530

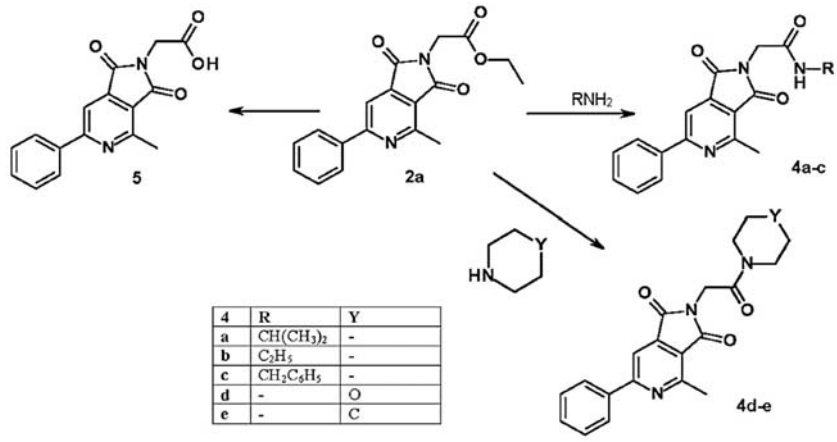


2	R
a	$CH_2COOC_2H_5$
b	CH_2COOCH_3
c	$CH_2COOC_6H_5$
d	$CH_2COC_6H_5$
e	CH_3
f	C_2H_5

3	R'	R''
a	C_4H_9	C_4H_9
b	CH_2-p-ClC_6H_4	H
c	CH_2-o-ClC_6H_4	H
d	C_6H_5 OC_2H_5	H
e	C_6H_5	H

3	Y	Z
f	N	$CH_2C_6H_5$
g	O	-
h	C	-
i	N	CH_3
j	N	C_6H_5

Figure 1. Synthesis of 4-methyl-6-phenyl-1H-pyrrolo[3,4-*c*]pyridine-1,3-dione derivatives



4	R	Y
a	$CH(CH_3)_2$	-
b	C_2H_5	-
c	$CH_2C_6H_5$	-
d	-	O
e	-	C

Figure 2. Synthesis of ethyl 2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-c]pyridin-2-yl)acetate derivatives

nm. The absorbance values were recorded. The microbial solutions prepared in the manner described above but with no compound applied served as control of experiment and their absorbance values were considered 100% of possible microbial growth. The aqueous chlorhexidine gluconate solution (of 100 mg/L final concentration) served as positive control of experiment. This compound possesses well-documented activity against all microbial strains used in this work (18). All measurements were performed in triplicate.

Statistical analysis

To compare differences in ability of microbial growth reduction displayed by compounds analyzed, Kruskal-Wallis test with *post-hoc* Dunn's analysis was performed. The differences were considered statistically significant if p value was < 0.05.

***In vitro* antiproliferative assay**

Human leukemia cells MV-4-11 (1×10^4 cells per well) were seeded in 96-well plates (Corning, New York, United States) 24 h before application of compounds. Before usage, the compounds were dissolved in DMSO to the concentration of 10 mg/mL and subsequently diluted in culture medium to reach the required concentrations (ranging from 0.1 to 100 µg/mL). The assay was performed after exposure to varying concentrations of tested compounds for 72 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed as described previously (19). The results were calculated as an inhibitory concentration 50 (IC_{50}) - the concentration of the tested compounds which inhibits proliferation of 50% of the cell population. IC_{50} values for each individual experiment were estimated (20) and mean values ± SD are presented. The compounds in each concentration were tested in triplicate in a single experiment, which was made at least in 3 repetitions.

RESULTS AND DISCUSSION

Chemistry

The obtained earlier 4-methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (**1**) was alkylated with iodomethane or bromoethane (Fig. 1), according to the method described in the previous work (21). The new *N*-alkyl pyrrolo[3,4-*c*]pyridine derivatives **2e-f** were isolated with very good yield (78-81%). IR

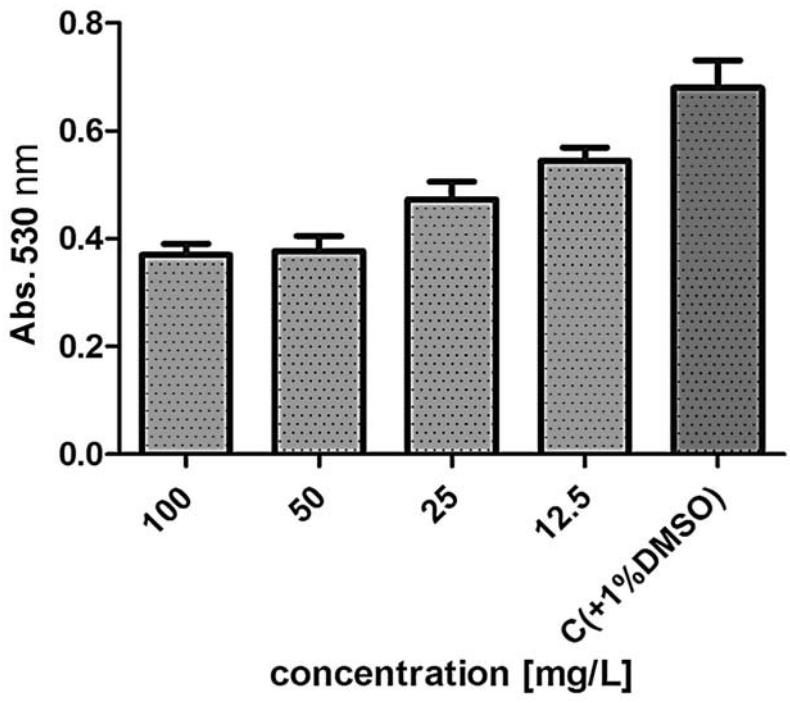


Figure 3. Comparison of *C. albicans* growth in the different concentrations of **3c** compound. The C(+1%DMSO) is a control sample (fungus incubated in medium supplemented with 1% DMSO but without **3c** compound). The value of absorbance of this sample reflects potential of *C. albicans* to growth in conditions undisturbed by **3c** compound

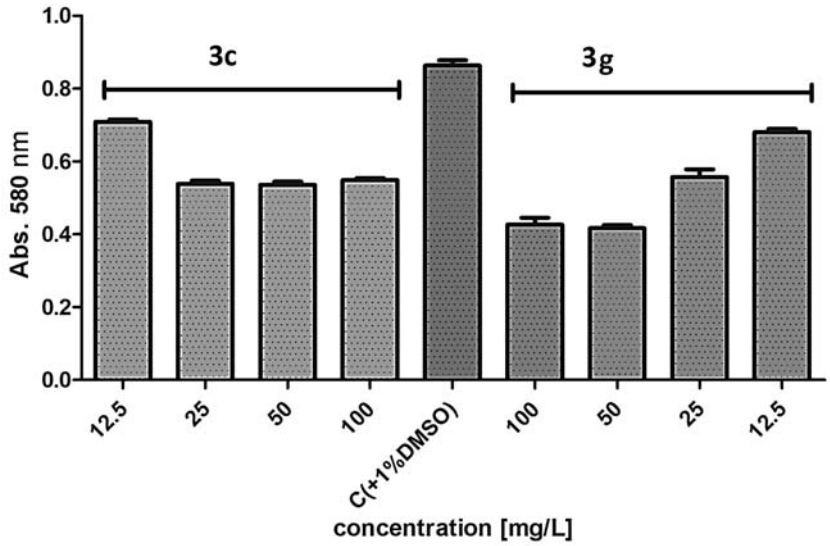


Figure 4. Comparison of *S. aureus* growth in the different concentrations of **3c** and **3g** compounds. The C(+1% DMSO) is a control sample (*Staphylococcus* incubated in medium supplemented with 1% DMSO but without **3c** or **3g** compound). The value of absorbance of this sample reflects potential of *C. albicans* to growth in conditions undisturbed by compounds analyzed

spectra of the obtained compounds **2e-f** displayed absorption bands within the range ν = 3000-3400 cm^{-1} characteristic for the NH. ^{1}H NMR spectra contain three-protons singlet at δ = 3.03 ppm for the methyl group of compound **2e** and three-protons triplet and two-protons quarted for ethyl group of compound **2f**, instead of one-proton singlet of NH, which was observed for imide **1**. 4-Methyl-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione (**1**) was used as the substrate for the Mannich condensation with formaldehyde and selected primary and secondary amines. The reactions were carried out in boiling ethanol. In ^{1}H NMR spectra of Mannich bases from primary amines **3b-e**, two-proton doublets at δ = 4.82-5.14 ppm characteristic for $–CH_2$- group, were observed. In case of Mannich bases **3a,f-j**, obtained with secondary amines, two-proton singlets at δ = 4.44-4.55 ppm have been present and the absence of signal for NH was observed. In the next stage, methyl 2-(4-methyl-1,3-dioxo-6-phenyl-pyrrolo[3,4-*c*]pyridine-2-yl)acetate **(2a)** was used as the substrate for aminolysis and hydrolysis. The appropriate amides **4a-e** have been synthesized in the reaction of ester **2a** with selected amines: ethylamine, isopropylamine, benzylamine, morpholine and piperidine (Fig. 2). Catalytic amount of sodium methoxide have been used to promote the amidation of ester **2a** (22). IR spectra of secondary amides **4a-c** contain, among other absorption bands, those characteristic for the NH in the range ν = 3300-3450 cm^{-1}. Characteristic signals for the protons of amides **4a-e** are found in the aliphatic part of the ^{1}H NMR spectrum. Hydrolysis of ester **2a** was carried out in a boiling aqueous solution of HCl/CH_3COOH and gave the appropriate acid **5** (Fig. 2). IR spectrum contains band at ν = 3470 cm^{-1} for COOH. In the ^{1}H NMR spectra of obtained acid **5**, two signals disappear: a triplet due to the CH_3 group and a quartet due to the CH_2 group, which were observed in the ^{1}H NMR spectra of ester **2a**. The appearance of the singlet at δ = 13.35 ppm indicates the proton of carboxylic group of the acid **5**.

Eighteen new derivatives **2e-f**, **3a-j**, **4a-e**, **5** were obtained from the syntheses described here. These compounds may be used as starting material for further chemical modifications in order to obtain biologically active derivatives.

In vitro antimicrobial assay

Among the tested compounds, **3g** and **3c** displayed antimicrobial activity against *S. aureus*. The last of compounds mentioned, exhibited activity against *C. albicans* also (Figs. 3, 4).

The 100 and 50 mg/L concentrations of **3c** compound were able to reduce *C. albicans* growth in statistically significant manner ($p < 0.05$, Kruskal-Wallis test). The observed reduction was 45.61% and 44.56% for 100 and 50 mg/L concentrations, respectively (Fig. 3). Compound **3c** was also able to partially reduce growth of *S. aureus*,

Table 1. *In vitro* percent of growth inhibition of MV-4-11 cells caused by the selected compounds.

Compound (100 µg/mL)	Growth inhibition of MV-4-11
1	50.77 ± 10.16%
2c	27.62 ± 6.75%
2f	41.41 ± 4.48%
4a	37.40 ± 8.51%
5	44.65 ± 7.29%

however observed trends were not statistically significant ($p > 0.05$). Contrary, use of compound **3g** correlated with statistically significant reduction of staphylococcal growth (Fig. 4).

Application of 100 and 50 mg/L concentrations of **3g** compound led to reduction of staphylococcal growth by 50.52 and 51.72%, respectively. The use of chlorhexidine gluconate (positive control of experiment) in 100 and 50 mg/L concentration led to 100% of reduction of microbial count.

***In vitro* antiproliferative assay**

The synthesized compounds were screened for their antiproliferative activity using human biphenotypic B myelomonocytic leukemia cells - MV-4-11. For five compounds: **1**, **2c**, **2f**, **4a**, **5** antitumor activity was expressed only as the percentage of growth inhibition of the treated cells in the highest concentration used (100 µg/mL (Table 1). The activity of compounds **2a,b**, **2d,e**, **4b-e**, **3a-j,** was higher with IC_{50} value ranged between 19-70 µg/mL (Table 2). The antiproliferative activity of other tested derivatives was at very low level. The most active were obtained Mannich bases, however they showed no activity at a level that would qualify them for research on other cell lines.

CONCLUSIONS

As the continuation of the previous research (15, 20), some novel pyrrolo[3,4-*c*]pyridine derivatives have been synthesized. Eighteen new compounds were isolated and their structures were confirmed by IR, NMR spectra and elemental analysis. The new synthesized derivatives and also some of prepared earlier compounds (**1, 2a-d**) were screened for their antimicrobial and anticancer activity *in vitro*. Two Mannich bases **3c** and **3g** showed the moderate activity against *S. aureus*. Compound **3c** reduces *C. albicans* growth in statistically significant manner. Among the tested compounds only the Mannich bases showed antiproliferative activity *in vitro* on MV4-11 human leukemia cell line.

Table 2. The antiproliferative activity *in vitro* of the tested compounds against the MV-4-11 cell line.

Compound	IC_{50} (µg/mL)
2a	62.9 ± 3.11
2b	59.49 ± 25.5
2d	57.79 ± 17.77
2e	48.01 ± 4.19
3a	25.39 ± 3.6
3b	33.74 ± 10.52
3c	60.37 ± 25.68
3d	19.54 ± 1.77
3e	21.8 ± 0.92
3f	25.06 ± 2.12
3g	26.41 ± 3.57
3h	32.2 ± 0.96
3i	29.1 ± 3.22
3j	26.54 ± 1.35
4b	70.4 ± 10.84
4c	31.86 ± 1.1
4d	67.66 ± 21.01
4e	55.28 ± 13.7
Cisplatin	0.17 ± 0.05

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