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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW SULFONAMIDE ISOXAZOLO[5,4-*b*]PYRIDINE DERIVATIVES

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Abstract: A series of novel sulfonamide isoxazolo[5,4-b]pyridines were synthesized. The substrates for their synthesis were 3-aminoisoxazolo[5,4-b]pyridine and selected aryl sulfonic chlorides, chlorosulfonic acid and selected amines. Reactions were carried out using the classical and microwave methods. Selected compounds were tested towards antibacterial and antiproliferative activity. The structure of the obtained new derivatives was determined by elemental analysis and acquired IR and ^{1}H NMR spectra. Among the tested compounds: N-isoxazolo[5,4-*b*]pyridine-3-yl-benzenesulfonamide (**2**) and N-isoxazolo[5,4-*b*]pyridine-3-yl-4-methylbenzene-sulfonamide (**5**) showed antimicrobial activity towards *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) at doses: 125, 250 and 500 μg. Both compounds showed a 50% inhibition of proliferation of breast carcinoma cell line MCF7 at concentrations of 152.56 μg/mL and 160 161.08 ĕg/mL, respectively.

Key words: isoxazolopyridines, microwave synthesis, antimicrobial, antiproliferative activity

The presented work is a continuation of our studies on the synthesis of new isoxazolopyridine derivatives. The derivatives obtained by our team, namely isoxazolo[5,4-b]pyridine and isoxazolo[4,5-b]pyridine showed antiproliferative activity (1-3). Based on a literature review it could be stated that the isoxazolo[5,4-b]pyridine derivatives constitute a broad-spectrum group of biologically active compounds. Many studies have confirmed their anti-inflammatory (4, 5), hypotensive (6), analgesic (7), antisclerotic (8) anticonvulsant (9), and psychotropic (10, 11) activity. In contrast, antimicrobial activity has been observed in the group of isoxazolopyridine carboxylic derivatives (12), and sulfonamide derivatives have shown anti-inflammatory activity (4). Sulfonamides are a group of compounds that are used as antibacterial (Sulfanilamidum derivatives), antidiabetic (Tolbutamidum), diuretic (Furosemidum), analgesic (Celecoxibum), anti-inflammatory (Sulfasalazinum) and neuroleptic (Thiotixenum) drugs.

Derivatives of the heterocyclic systems with a sulfonamide moiety, being a subject of research, exhibited different biological activity: antitumor (4, 13-17), antiparasitic (18), anti-inflammatory (19), antidiabetic (20, 21), antiallergic (16), neuroleptic (22) and antibacterial (23-25). The sulfonamide derivative of indole (Indisulam) (26) and sulfonamide pyridine derivative (ABT 51) (27) - cell cycle inhibitors, in clinical trials have shown antitumor activity against a broad spectrum of cancer cells, including those resistant to other conventional chemotherapy.

Due to the emergence of bacterial resistance to antibiotics, there is still a need to develop new effective antibacterial drugs. We were interested to find whether the new sulfonamide derivatives of isoxazolo[5,4-b]pyridine show antibacterial and antiproliferative activity. In the presented paper, the conventional and microwave synthesis of new sulfonamide derivatives of isoxazolo[5,4-b]pyridine and

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the preliminary results of their biological studies are presented.

EXPERIMENTAL

Chemistry

Melting points were determined with a Boethius apparatus and are uncorrected. Elemental analyses were performed on a Perkin Elmer 2400 analyzer (Waltham, MA, USA), and the results are within ± 0.4% of the theoretical values obtained for the new compounds. Infrared (IR) spectra were recorded with a Specord M80 spectrophotometer (Zeiss/Analytic Jena, Germany) for KBr pellets. Hydrogen-1 NMR (^{1}H NMR) spectra were recorded with a Bruker Avance ARX-300 instrument (Bruker Analytic, Karlsruhe, Germany) using DMSO-d_6 or $CDCl_3$ as internal standards. Chemical shifts are reported in ppm from the internal tetramethylsilane reference. The progress of the reaction and the purity of the compounds were monitored using thin layer chromatography (TLC) on analytical silica gel plates (Merck F_{254}, Darmstadt, Germany). Microwave-assisted synthesis was performed in a laboratory microwave RM 800PC reactor (Plazmatronika, Wrocław, Poland). Water was purified using an Aquadem SDF-Ion exchanger system (TKA, Thermo Scientific). All chemicals and reagents for the synthesis were obtained from Alfa Aeser (Karlsruhe, Germany), Lancaster Synthesis (Morecambe, England), and Chempur (Piekary Śląskie, Poland).

Synthesis of N-isoxazolo[5,4-b]pyridin-3-yl-benzenesulfonamide (2), 4-bromo-N-isoxazolo[5,4-b]pyridin-3-yl-benzenesulfonamide (3), 4-chloro-N-isoxazolo[5,4-b]pyridin-3-yl-benzenesulfonamide (4), N-isoxazolo[5,4-b]pyridin-3-yl-4-methylbenzenesulfonamide (5), N-isoxazolo[5,4-b]pyridin-3-yl-4-methoxybenzenesulfonamide (6)

Conventional conditions (general procedure A)

To a solution of 3-aminoisoxazolo[5,4-b]pyridine (**1**) (1.35 g, 0.01 mol) (1) in 100 mL of tetrahydrofurane, a few drops of anhydrous pyridine and 0.01 mol of the appropriate arylsulfonyl chlorides: benzene-, 4-bromobenzene-, 4-chlorobenzene-, 4-methyl- and 4-methoxybenzenesulfonyl chlorides were added. The reaction mixture was heated under reflux for 6 h. The solvent was evaporated under vacuum and the residue obtained was triturated with water, filtered, dried and crystallized from ethanol.

Microwave conditions (general procedure B)

To a solution of 3-aminoisoxazolo[5,4-b]pyridine (**1**) (1.35 g, 0.01 mol) in 100 mL of tetrahydrofurane, a few drops of anhydrous pyridine and 0.01 mol of the appropriate arylsulfonyl chlorides: benzene-, 4-bromobenzene-, 4-chlorobenzene-, p-toluene- and 4-methoxybenzenesulfonyl chlorides were added. The reaction mixture was heated under reflux while being stirred in the microwave reactor in an aluminum bath at 60-65°C for 15 min (3 × 5 min with 5 min breaks) at microwave power P = 240 W. The solvent was evaporated under vacuum and the residue obtained was triturated with water, filtered, dried and crystallized from ethanol.

N-Isoxazolo[5,4-b]pyridin-3-yl-benzenesulfonamide (2)

Yield 70% (A), 75% (B); m.p. 188-189°C. IR (KBr, cm^{-1}): 2900-3300 (NH,CH), 1600, 1580, 1450 (C=C, C=N), 1330, 1160 (SO_2); ^{1}H NMR ($CDCl_3$, δ, ppm): 7.35 (dd, 1H, H-5), 7.52-7.94 (m, 6H, H-4 + Ph), 8.68 (dd, 1H, H-6), 10.82 (s, br, 1H, NH). Analysis: calcd. for $C_{12}H_9N_3O_3S$ (275.28): C 52.36, H 3.30, N 15.26%; found: C 52.27, H 3.05, N 15.15%.

4-Bromo-N-isoxazolo[5,4-b]pyridin-3-yl-benzenesulfonamide (3)

Yield 60% (A), 75% (B); m.p. 211-212°C; IR (KBr, cm^{-1}): 2790–3150 (NH, CH), 1610, 1500, 1450 (C=C, C=N), 1350, 1175 (SO_2), 1070 (p-ArBr); ^{1}H NMR ($CDCl_3$, δ, ppm): 7.42 (dd, 1H, H-5), 7.73–7.94 (m, 6H, H-4 + Ph), 8.72 (dd, 1H, H-6), 10.93 (s, br, 1H, NH). Analysis: calcd. for $C_{12}H_8BrN_3O_3S$ (354.18): C 40.69, H 2.28, N 11.86%; found: C 40.53, H 2.21, N 11.65%.

4-Chloro-N-isoxazolo[5,4-b]pyridin-3-yl-benzenesulfonamide (4)

Yield 60% (A), 75% (B); m.p. 210–212°C; IR (KBr, cm^{-1}): 3250 (NH, CH), 1610, 1500, 1450 (C=C, C=N), 1350, 1165 (SO_2); ^{1}H NMR ($CDCl_3$, δ, ppm): 7.42 (dd, 1H, H-5), 7.73–7.94 (m, 6H, H-4 + Ph), 8.72 (dd, 1H, H-6), 10.93 (s, br, 1H, NH). Analysis: calcd. for $C_{12}H_8ClN_3O_3S$ (309.73): C 46.54, H 2.60, N 13.57%; found: C 46.25, H 2.72, N 13.36%.

N-Isoxazolo[5,4-b]pyridin-3-yl-4-methylbenzenesulfonamide (5)

Yield 66% (A), 72% (B); m.p. 172–174°C; IR (KBr, cm^{-1}): 3100 (NH, CH), 1600, 1510, 1450 (C=C, C=N), 1310, 1160 (SO_2), 780 (CH_3); ^{1}H NMR ($CDCl_3$, δ, ppm): 2.48 (s, 3H, CH_3), 7.34 (m, 3H, H-5 + Ph), 7.77 (m, 3H, H-4 + Ph), 8.67 (dd, 1H, H-6), 10.83 (s, br, 1H, NH). Analysis: calcd. for $C_{13}H_{11}N_3O_3S$ (289.31): C 53.97, H 3.83, N 14.52%; found: C 53.65, H 3.52, N 14.35%.

N-Isoxazolo[5,4-b]pyridin-3-yl-4-methoxybenzenesulfonamide (6)

Yield 63% (A), 76% (B); m.p. 195–197°C; IR (KBr, cm^{-1}): 3150 (NH, CH), 1610, 1500, 1450 (C=C, C=N), 1350, 1170 (SO_2), 1270 (ArC-O-C), 780 (CH_3); ^1H NMR ($CDCl_3$, δ, ppm): 3.91 (s, 3H, CH_3), 6.96 (m, 2H, Ph), 7.37 (dd, 1H, H-5), 7.81 (m, 3H, H-4 + Ph), 8.67 (dd, 1H, H-6), 10.96 (s, br, 1H, NH). Analysis: calcd. for $C_{13}H_{11}N_3O_4S$ (305.31): C 51.14, H 3.63, N 13.76%; found: C 51.02, H 3.57, N 13.37%.

Synthesis of N-(isoxazolo[5,4-b]pyridin-3-yl-)-N-(phenylsulfonyl)benzenesulfonamide (7), 4-bromo-N-[(4-bromophenyl)sulfonyl]-N-(isoxazolo[5,4-b]pyridin-3-yl-)benzenesulfonamide (8), 4-chloro-N-[(4-chlorophenyl)sulfonyl]-N-(isoxazolo[5,4-b]pyridin-3-yl-)benzenesulfonamide (9), 4-methyl-N-[(4-methylphenyl)sulfonyl]-N-(isoxazolo[5,4-b]pyridin-3-yl-)benzenesulfonamide (10)

Conventional conditions (general procedure A)

To a solution of 3-aminoisoxazolo[5,4-b]pyridine (**1**) (1.35 g, 0.01 mol) in 100 mL of tetrahydrofurane, a few drops of anhydrous pyridine and 0.02 mol of the appropriate arylsulfonyl chlorides: benzene-, 4-bromobenzene-, 4-chlorobenzene- and 4-methylbenzenesulfonyl chlorides were added. The reaction mixture was heated under reflux for 6 h. The solvent was evaporated under vacuum and the residue obtained was triturated with water, filtered, dried and recrystallized from ethanol.

Microwave conditions (**g**eneral procedure B)

To a solution of 3-aminoisoxazolo[5,4-b]pyridine (**1**) (1.35 g, 0.01 mol) in 100 mL of tetrahydrofurane, a few drops of anhydrous pyridine and 0.02 mol of the appropriate arylsulfonyl chlorides: benzene-, 4-bromobenzene-, 4-chlorobenzene- and 4-methylbenzenesulfonyl chlorides were added. The reaction mixture was heated under reflux while being stirred in the microwave reactor in an aluminum bath at 60-65°C for 15 min (3 × 5 min with 5 min breaks) at microwave power P = 240 W. The solvent was evaporated under vacuum and the residue obtained was triturated with water, filtered, dried and recrystallized from ethanol.

N-(Isoxazolo[5,4-b]pyridin-3-yl-)-N-(phenylsulfonyl)benzenesulfonamide (7)

Yield 70% (A), 82% (B); m.p. 189-191°C; IR (KBr, cm^{-1}): 3100 (NH, CH), 1600, 1500, 1450 (C=C, C=N), 1170 (SO_2 N=); ^1H NMR ($CDCl_3$, δ, ppm): 7.36 (m, 3H, H-5 + Ph), 7.52–7.94 (m, 6H, H-4 + Ph), 8.69 (dd, 1H, H-6). Analysis: calcd. for $C_{18}H_{13}N_3O_5S_2$ (415.44): C 52.04, H 3.15, N 10.11%; found: C 52.03, H 3.22, N 10.39%.

4-Bromo-N-[(4-bromophenyl)sulfonyl]-N-(isoxazolo[5,4-b]pyridin-3-yl-)benzenesulfonamide (8)

Yield 62% (A), 70% (B); m.p. 229-230°C; IR (KBr, cm^{-1}): 3100 (NH, CH), 1600, 1500, 1450 (C=C, C=N), 1170 (SO_2N=), 1070 (p-ArBr); ^1H NMR ($CDCl_3$, δ, ppm): 7.41 (dd, 1H, H-5), 7.69-7.80 (m, 6H, H-4 + Ph), 8.72 (dd, 1H, H-6). Analysis: calcd. for $C_{18}H_{11}Br_2\ N_3\ O_5S_2$ (573.23): C 37.72, H 1.93, N 7.33%; found: C 37.61, H 1.97, N 7.42%.

4-Chloro-N-[(4-chlorophenyl)sulfonyl]-N-(isoxazolo[5,4-b]pyridin-3-yl-)benzenesulfonamide (9)

Yield 60% (A), 67% (B); m.p. 208-209°C; IR (KBr, cm^{-1}): 3120 (NH, CH), 1610, 1500, 1450 (C=C, C=N), 1350, 1170 (SO_2N), 1070 (p-ArCl); ^1H NMR ($CDCl_3$, δ, ppm): 6.97 (m, 2H, Ph), 7.38 (dd, 1H, H-5), 7.82 (m, 3H, H-4 + Ph), 8.68 (dd, 1H, H-6). Analysis: calcd. for $C_{18}\ H_{11}Cl_2N_3O_5S_2$ (484.33): C 44.64, H 2.29, N 8.68%; found: C 44.33, H 2.27, N 8.65%.

4-Methyl-N-[(4-methylphenyl)sulfonyl]-N-(isoxazolo[5,4-b]pyridin-3-yl-)benzenesulfonamide (10)

Yield 68% (A), 75% (B); m.p. 199-201°C; IR (KBr, cm^{-1}): 3100 (NH, CH), 1600, 1500, 1450 (C=C, C=N), 1170 (SO_2N=), 825 (p-Ar-CH_3); ^1H NMR ($CDCl_3$, δ, ppm): 2.48 (s, 3H, CH_3), 7.36 (m, 5H, H-5 + Ph), 7.70 (m, 5H, H-4 + Ph), 8.67 (dd, 1H, H-6). Analysis: calcd. for $C_{20}H_{17}N_3O_5S_2$ (443.49): C 54.17, H 3.86, N 9.47%; found: C 54.10, H 3.75, N 9.37%.

Synthesis of N-isoxazolo[5,4-b]pyridin-3-ylbenzamide (11), 4-bromo-N-isoxazolo[5,4-b]pyridin-3-ylbenzamide (12) and 4-chloro-N-isoxazolo[5,4-b]pyridin-3-ylbenzamide (13)

Conventional conditions (general procedure A)

To a solution of 3-aminoisoxazolo[5,4-b]pyridine (1.35 g, 0.01 mol) (**1**) in tetrahydrofurane (100 mL) a few drops of pyridine and benzoyl chloride or 4-bromobenzoyl chloride or 4-chlorobenzoyl chloride was added. The reaction mixture was heated under reflux for 10 h. The solvent was then removed in vacuum and the residue obtained was triturated with water, filtered, dried and recrystallized from ethanol.

Microwave conditions (**g**eneral procedure B)

To a solution of 3-aminoisoxazolo[5,4-b]pyridine (**1**) (1.35 g, 0.01 mol) in tetrahydrofurane (100 mL) a few drops of pyridine and benzoyl chloride or 4-bromobenzoyl chloride or 4-chlorobenzoyl chloride was added. The reaction mixture was heated under reflux while being stirred in the microwave reactor in an aluminum bath at 60-65°C for 15 min. (3 × 5 min. with 5 min. breaks) at microwave power P = 240 W. The solvent was removed under reduced pressure. The residue obtained was triturated with water, filtered, dried and crystallized from ethanol.

N-Isoxazolo[5,4-b]pyridin-3-ylbenzamide (**11**)

Yield 70% (A), 75% (B); m.p. 213-214°C; IR (KBr, cm^{-1}): 2880–3100 (NH, CH), 1680, 1560 (NHCO), 1600, 1500, 1450 (C=C, C=N); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.37 (dd, 1H, H-5), 7.49–7.55 (m, 5H, Ph), 8.15 (dd, 1H, H-4), 8.43 (dd, 1H, H-6), 12.40 (s, br, 1H, NH). Analysis: calcd. for $C_{13}H_9N_3O_2$ (239.23): C 65.27, H 3.79, N 17.56%; found: C 65.27, H 3.94, N 17.95%.

4-Bromo-N-isoxazolo[5,4-b]pyridin-3-ylbenzamide (**12**)

Yield 63% (A), 70% (B); m.p. 263-266°C; IR (KBr, cm^{-1}): 2800–3100 (NH, CH), 1670, 1570, 1420 (NHCO), 1620, 1520, 1420 (C=C, C=N), 1065, p-Ar-Br); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.40 (dd, 1H, H-5), 7.66 (dd, 1H, H-4), 7.63–7.86 (m, 2H, Ph), 8.03–8.07 (m, 2H, Ph), 8.27 (dd, 1H, H-6), 10.21 (s, br, 1H, NH). Analysis: calcd. for $C_{13}H_8BrN_3O_2$ (318.13): C 49.08, H 2.53, N 13.21%; found: C 48.29, H 2.43, N 12.92%.

4-Chloro-N-isoxazolo[5,4-b]pyridin-3-ylbenzamide (**13**) (1)

Yield 65% (A), 70% (B); m.p. 278-280°C; IR (KBr, cm^{-1}): 2780–3080 (NH,CH), 1680, 1560 (NHCO), 1610, 1500, 1450 (C=C, C=N), 1090 (p-Ar-Cl). ^{1}H NMR ($CDCl_3$, δ, ppm): 6.48 (dd, 1H, H-5), 7.60 (dd, 1H, H-4), 7.65–7.87 (m, 2H, Ph), 8.05–8.09 (m, 2H, Ph), 8.27 (dd, 1H, H-6), 10.20 (s, br, 1H, NH). Analysis: calcd. for $C_{13}H_8ClN_3O_2$ (273.68): C 57.05, H 2.95, N 15.35%; found: C 56.94, H 2.95, N 15.12%.

Synthesis of 3-(isoxazolo[5,4-b]pyridin-3-ylcarbamoyl)benzenesulfonyl chloride (**14**), **2-bromo-5-(isoxazolo[5,4-b]pyridin-3-ylcarbamoyl)benzenesulfonyl chloride** (**15**) **and 2-chloro-5-(isoxazolo[5,4-b]pyridin-3-ylcarbamoyl)benzenesulfonyl chloride** (**16**)

Conventional conditions (general procedure A)

N-Isoxazolo[5,4-b]pyridin-3-ylbenzamide (**11**) or 4-bromo-N-isoxazolo[5,4-b]pyridine-3-ylbenzamide (**12**) or 4-chloro-N-isoxazolo[5,4-b]pyridine-3-ylbenzamide (**13**) (0.01 mol) was taken in a two-necked flask with a dropping funnel and reflux condenser with $CaCl_2$ guard tube. Chlorosulfonic acid (0.01 mol) was added through a dropping funnel in a small portion with occasional shaking. The reaction mixture was heated in a water bath for 1 h, cooled and poured onto crushed ice. The separated solid was filtered off, washed with water, dried, and the crude products were used for the subsequent synthesis.

Synthesis of 4-bromo-3-[(3-methoxyphenyl)sulfamoyl]-N-isoxazolo[5,4-b]pyridine-3-yl)benzamide (**17**), **4-bromo-3-[(4-methoxyphenyl)sulfamoyl]-N-isoxazolo[5,4-b]pyridine-3-yl)benzamide** (**18**) and **4-chloro-3-[(3-trifluoromethylphenyl)sulfamoyl]-N-isoxazolo[5,4-b]pyridine-3-yl)benzamide** (**19**)

Conventional conditions (general procedure)

To a mixture of the appropriate sulfonyl chlorides: 3-(isoxazolo[5,4-b]pyridin-3-ylcarbamoyl)benzenesulfonyl chloride (**14**), 2-bromo-5-(isoxazolo[5,4-b]pyridin-3-ylcarbamoyl)benzenesulfonyl chloride (**15**) and 2-chloro-5-(isoxazolo[5,4-b]pyridin-3-ylcarbamoyl)benzenesulfonyl chloride (**16**) in DMF and equimolar amounts of the appropriate aromatic amines: 3-methoxy-, 4-methoxy- or 3-(trifluoromethyl)aniline were added. The mixture reaction was stirred for about 3-5 h and heated under reflux for 1 h. The solvent was removed under reduced pressure and the residue was triturated with water and filtered. The obtained solid was dried and crystallized from ethanol.

4-Bromo-3-[(3-methoxyphenyl)sulfamoyl]-N-isoxazolo[5,4-b]pyridin-3-yl)benzamide (**17**)

Yield 59%; m.p. 279-281°C; IR (KBr, cm^{-1}): 2700–3100 (NH, CH), 1670, 1570 (NHCO), 1620, 1530, 1400 (C=C, C=N), 1380, 1140 (SO_2NH), 1240 (C-O-C), 1070 (p-Ar-Br). ^{1}H NMR ($CDCl_3$, δ, ppm): 3.91 (s, 3H, CH_3), 6.40 (dd, 1H, H-5), 7.65 (dd, 1H, H-4), 7.83–8.08 (m, 7H, 2×Ph), 8.28 (dd, 1H, H-6), 10.21 (s, br, 1H, NH), 12.22 (s, 1H, NH). Analysis: calcd. for $C_{20}H_{15}BrN_4O_5S$ (503.33): C 47.73, H 3.00, N 11.13%; found: C 47.53, H 3.12, N 11.04%.

4-Bromo-3-[(4-methoxyphenyl)sulfamoyl]-N-isoxazolo[5,4-b]pyridin-3-yl)benzamide (**18**)

Yield 65%; m.p. 269-271°C; IR (KBr, cm^{-1}): 2900–3100 (NH, CH), 1670, 1570 (NHCO), 1620,

1520, 1400 (C=C, C=N), 1380, 1140 (SO_2NH), 1240 (C-O-C), 1070 (p-Ar-Br). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.91 (s, 3H, CH_3), 6.41 (dd, 1H, H-5), 7.65 (dd, 1H, H-4), 7.83–8.03 (m, 7H, 2×Ph), 8.30 (dd, 1H, H-6), 10.20 (s, br, 1H, NH), 12.21 (s, 1H, NH). Analysis: calcd. for $C_{20}H_{15}BrN_4O_5S$ (503.33): C 47.73, H 3.00, N 11.13%; found: C 47.48 H 3.02, N 11.21%.

4-Chloro-3-[(3-trifluoromethylphenyl)sulfamoyl]-N-isoxazolo[5,4-b]pyridine-3-yl)benzamide (**19**)

Yield 70%; m.p. 259-261°C; IR (KBr, cm^{-1}): 3100 (NH, CH), 1670, 1570 (NHCO), 1620, 1520, 1400 (C=C, C=N), 1380, 1140 (SO_2NH), 1090 (p-Ar-Cl). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.42 (dd, 1H, H-5), 7.67 (dd, 1H, H-4), 7.84–8.00 (m, 7H, 2×Ph), 8.30 (dd, 1H, H-6), 10.20 (s, br, 1H, NH), 12.20 (s, 1H, NH). Analysis: calcd. for $C_{20}H_{12}Cl\ F_3N_4O_4S$ (496.85): C 48.35, H 2.43, N 11.28%; found: C 48.25, H 2.35, N 11.26%.

Biology

***In vitro* antibacterial activity**

Antibacterial activity was tested against four strains: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6033) and two clinical isolates: *Salmonella enterica* and *Klebsiella pneumoniae*. All bacterial strains were obtained from the Polish Collection of Microorganisms (PCM). Screening tests were performed by the disc diffusion method on Mueller-Hinton agar, according to the recommendations of the Clinical and Laboratory Standards Institute (29), with sulfanilamide and sulfadiazine as references. Tested compounds were dissolved in DMSO and applied on Whatman GF/F glass microfibre 5 mm sterile disks. The size of the inhibition zone was determined after 20 h of incubation at 37°C.

MIC (minimum inhibitory concentration) values were determined for strain selected in the screening test using the serial dilutions method with rezazurine as a marker of bacterial growth (30).

***In vitro* cytotoxicity**

Toxicity of compounds **2** and **5** was tested using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) proliferation assay on breast cancer cell line (MCF7). Levofloxacin was used as a reference.

RRSULTS AND DISCUSSION

Chemistry

The synthesis of the new sulfonamide derivatives of isoxazolo[5,4-b]pyridine is presented in Figure 1. In the first step, 3-aminoisoxazolo[5,4-b]pyridine (**1**) (1) was subjected to arylsulfonation reaction using selected arylsulfonyl chlorides: benzenesulfonyl, 4-bromo-, 4-chloro-, 4-methyl- and 4-metoxybenzenesulfonyl chloride in tetrahydrofuran in the presence of a catalytic amount of anhydrous pyridine. As a result of these condensation reactions, appropriately substituted phenyl-, 4-chloro-, 4-bromo-, 4-methyl- and N-isoxazolo[5,4-b]pyridin-3-yl-4-methoxybenzenesulfonamides (**2**-**6**) were obtained. Reactions were carried out in a conventional way through 6 h heating in a heating mantle. For comparison, the reaction mixtures were heated in a Plazmatronika RM 800 microwave reactor at 60-65° C in 3 cycles for 5 min with 5 min intervals using microwave power of P = 240 W.

The heating time for the reaction mixtures in the microwave reactor was much shorter compared to the heating in the heating mantle. Reaction yield was higher by 35% on average when microwave radiation was applied. The structures of the obtained new derivatives were determined by elemental analysis and spectral spectra. In the IR spectra of **2**-**6** derivatives two characteristic absorption bands of secondary sulfonamide moiety in the range between 1160 and 1350 cm^{-1} were observed. In the IR spectrum for the N-isoxazolo[5,4-b]pyridin-3-yl-4-

Table 1. Bacterial strains used in this work.

	Bacteria	PCM number	ATCC number
1.	*Escherichia coli*	PCM 2057	ATCC 25922
2.	*Staphylococcus aeureus*	PCM 2054	ATCC 25923
3.	*Pseudomonas aeruginosa*	PCM 2058	ATCC 27853
4.	*Bacillus subtilis*	PCM 2021	ATCC 6033
5.	*Salmonella enterica*	PCM 2565	ATCC 13311
6.	*Klebsiella pneumonie*	clinical strain	

Table 2. The antimicrobial activity of isoxazole derivatives[5,4-b]pyridines.

Compound	dose [µg/disc]	*E. coli* d [mm]	*P. aeruginosa* d [mm]	*B. subtilis* d [mm]	*S. aureus* d [mm]
2	500	22	31	no activity	no activity
	250	6	13	not tested	not tested
3	500	no activity	no activity	no activity	no activity
5	500	22	32	no activity	no activity
	250	6	14	not tested	not tested
6	500	no activity	no activity	no activity	no activity
17	250	no activity	no activity	no activity	no activity
18	440	no activity	no activity	no activity	no activity
Sulfanilamide	500	no activity	no activity	25	no activity
Sulfadiazine	500	14	no activity	22	12

methoxybenzenesulfonamide (**6**) an absorption band representing an ether moiety at 1270 cm^{-1} is also observed.

In the ^1H NMR spectra of compounds **2**-**6**, signals of aromatic protons of pyridine and phenyl ring are observed in the range of δ = 7.3-8.6 ppm. In the field of low intensity, single signals at δ = 10.8 ppm corresponding to the NH proton of $–SO_2NH-$ moiety are present. For comparison, the abovementioned reactions were carried out *via* a two-stage process. After a quick heating of the reaction mixture in a heating mantle, in order to dissolve the reactants in the solvent, further heating was performed in a microwave reactor at 50-60°C in three cycles for 3 min with 5 min. intervals. The applied microwave power was P = 160 W. The resulting reaction yields were higher by 5% on average compared to yields obtained *via* a one-stage method.

However, after adaptation of the excess of selected aryl sulfonyl chlorides in the above reactions disulfonamides isoxazolo[5,4-b]pyridine **7**-**10** derivatives presented in Figure 1 were extracted. In the IR spectra of these compounds, an intense absorption bands at 1170 cm^{-1} characteristic for the secondary sulfonamides moiety (= NSO_2-) were observed.

In the second stage of the study, 3-aminoisoxazolo[5,4-b]pyridine (**1**) was the substrate for the synthesis of 4-bromo-, 4-chloro-N-(isoxazolo[5,4-b]pyridin-3-yl)benzamide and N-(isoxazolo[5,4-b]pyridin-3-yl)benzamide **11**-**13**, which were previously obtained using the classical method (1), and for comparison their syntheses were also done using the microwave method.

Subsequently, the obtained amides **11-13** were subjected to sulfonation reaction with sulfonic acid yielding the appropriate sulfochlorides **14**-**16**. The extracted sulfochlorides were subjected to amonolysis with selected amines: 3- and 4-methoxyaniline and 3-trifluoromethylaniline, in the presence of catalytic amount of triethylamine yielding the appropriate sulfonamide derivatives presented as structures **17**-**19**. In the IR spectra of the compounds two characteristic absorption bands for sulfonamides are observed at 1160 and 1370 cm^{-1}. However, in the spectra of compounds **17**-**19,** except for signals from aromatic protons in the range of δ = 6.5-8.5 ppm, additional signals of the phenyl rings appeared in the range of δ = 7.5-8.24 ppm. In the spectra of compounds **17**-**18,** in the magnetic field of high intensity, at δ = 3.9 ppm signals of the methoxy protons are observed.

Antibacterial activity

In order to assess the activity of the synthesized compounds, series of disk diffusion test were performed. The assays were performed as described in the material and methods section, with four strain types. In the first test, all compounds were administered at a dose of 500 µg per disc. In the case of compounds **17** and **18** doses were reduced to 440 µg and 250 µg, respectively. Reduced doses were caused by poor solubility. For strains which showed sensitivity to the tested compound, doses in the range from 20 to 250 µg/disc were examined, and all tests were done in triplicate. Surprisingly, compounds **2** and **5** inhibit the growth of strains of *P. aeruginosa* and *E. coli*. A dose of 500 µg/disc of **2** gave the inhibition zone of 31 mm **5** of 32 mm on *P. aeruginosa* plate and 22 mm for both compounds on the *E. coli* plate. Lower doses gave smaller inhibition zones. All results are summarized in Table 2.

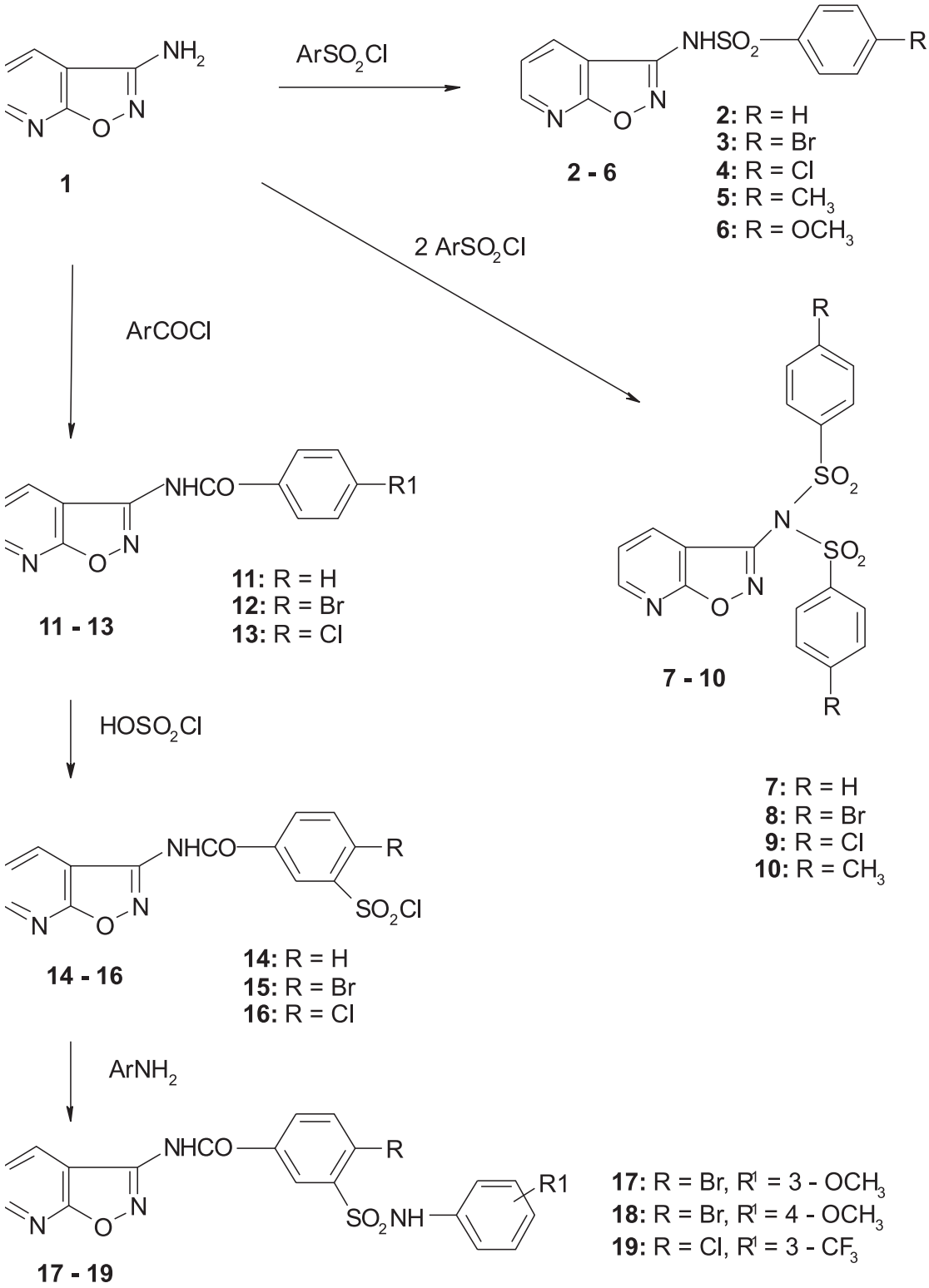

Figure 1. Synthesis of sulfonamides of isoxazolo[5,4-b]pyridines

Administration of 125 μg on disc caused a 6 mm slower growth zone on the *P. aeruginosa* plate. Because the effect on the growth of *E. coli* was visible but explicitly weaker, we also tested other *Enterobacteriaceae* bacteria: *Salmonella enterica* and *Klebsiella pneumoniae*, but we did not observe any inhibition of growth (data not shown). We also determined a minimal inhibitory concentration (MIC) for **2** and **5** in tests against *P. aeruginosa*. The values obtained are **2** – 47 μg/mL, **5** – 44 μg/mL.

As shown, the tested compounds **2** and **5** are characterized by antibacterial activity against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, do not show such activity against representatives of Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and interestingly, are inactive against other tested Gram-negative bacteria *Salmonella enterica* and *Klebsiella pneumoniae*. Such differences in activity against bacteria of the same taxonomic family indicate a high specificity of these compounds against the bacteria *Pseudomonas*. A number of antibacterial compounds specific against Gram-positive bacteria is known while there are few factors that selectively inhibit the growth of Gram-negative bacteria. In medicine, particularly valuable compounds specifically inhibit bacterial narrow taxon and do not affect the species closely related. Comparison of the effects of all the tested antimicrobial compounds allow to suspect that a key problem limiting the activity of the resulting compounds is the solubility. Further studies are needed to explain the mechanism of action and base of the compound specificity.

MTT cytotoxicity activity

Levofloxacin has proven anti-*Pseudomonas* activity and is administered as a drug of choice in humans; its cellular toxicity must therefore be negligible in the human cell line. With the antibacterial activity of **2** and **5** and their potential use in pharmaceutical practice shown in this work, it was necessary to demonstrate that they also lack any substantial cellular toxicity in humans. Here we show that after 48 h of incubation, the IC_{50} of **2** was 190.67 μg/mL; **5** – 163.35 μg/mL while levofloxacin showed IC_{50} at 131.9 μg/mL in the MCF7 cell line. After 72 h of incubation, the IC_{50} of **2** was 152.56 μg/mL, **5** - 161.08 μg/mL, while IC_{50} of levofloxacin was at the level of 114.92 μg/mL. Both tested compounds showed even lower antiproliferative activity in the MCF7 cell line than the levofloxacin control.

CONCLUSIONS

The use of microwave radiation in conducted syntheses allowed to obtain products with a higher yield and in shorter time. Compounds **2** and **5** are not cytotoxic, as they do not exert adverse effects on the cell cycle of the human MCF7 cell line. The compounds tested are potentially of higher therapeutical value in anti-*Pseudomonas* antibiotic treatment than commercially available specifics since they show a high level of specificity against the *Pseudomonas* strains and a lower cytotoxicity than levofloxacin with the MTT test in the MCF7 cell line. The problem is their poor solubility in water. Perhaps an appropriate solvent composition or other additives would allow the use of the presented compounds in medicine.

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