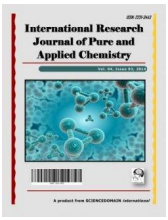

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Synthesis and *In vitro* Antimicrobial Activity of New Schiff Bases of 1,3,4-thiadiazole and 1,2,4-triazole

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Authors' contributions

This work was carried out in collaboration between all authors. Author ŁP design the study, performed the synthesis of new Schiff bases, analyzed the spectral data of obtained compounds and wrote the first draft of the manuscript excluding the in vitro antimicrobial section. Authors MM, PP, KP, MB and MC helped with the synthesis of new Schiff bases. Author AB performed the in vitro antimicrobial assays for synthesized compounds and wrote the antimicrobial section of this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study presents the synthesis, spectral analysis and *in vitro* antimicrobial evaluation of a new series of Schiff bases of 1,3,4-thiadiazole and 1,2,4-triazole (**1**-**10**). The structure of obtained compounds was confirmed by spectral analysis (IR, ^{1}H NMR and ^{13}C NMR) and elemental analysis. All synthesized compounds were screened for their *in vitro* antimicrobial activities using the broth microdilution method against a panel of bacterial strains including Gram-positive bacteria, Gram-negative bacteria and yeasts belonging to *Candida*s pp.

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Keywords: Schiff bases; 1,3,4-thiadiazole derivatives; 1,2,4-triazole derivatives; antibacterial activity; antifungal activity; MIC.

1. INTRODUCTION

Since last few decades, there is tremendous growth of research in the synthesis of nitrogen and sulfur containing heterocyclic derivatives because of their utility in various applications. Schiff bases, named after Hugo Schiff, are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. Structurally, a Schiff base, also known as imine or azomethine, is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C=O) has been replaced by an imine or azomethine group [1-4].

Schiff bases are some of the most widely used organic compounds and have attracted great and growing interest in chemistry and biology for many years due to its facile synthesis and wide applications [1-4]. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilisers [5]. Schiff bases bearing aryl groups or heterocyclic residues have also been shown to exhibit a broad range of biological activities, including, antibacterial [5-7], antifungal [8-10], antiproliferative [11,12], and antiviral properties [13,14].

Similarly, the occurrence of 1,3,4-thiadiazole or 1,2,4-triazole system in numerous biologically active molecules has been recognized to possess activities such as analgesic [15], antibacterial [16-18], antifungal [19,20], anti-inflammatory [21], antitumor [22,23] and antitubercular [24].

Keeping in view of the above facts and in continuation of our search on biologically potent molecules, we herein report the synthesis and *in vitro* evaluation as antimicrobial agents new Schiff bases derived from 1,3,4-thiadiazole and 1,2,4-triazole derivatives.

2. EXPERIMENTAL DETAILS

2.1 Chemistry

All reagents were purchased from Sigma-Aldrich (Munich, Germany) and Merck Co. (Darmstadt, Germany) and used without further purification. Melting points were determined in Fisher-Johns blocks (Fisher Scientific, Germany) and presented without any corrections. The IR spectra (v, cm^{-1}) were recorded in KBr tablets using a Specord IR-75 spectrophotometer (VEB Carl Zeiss, Jena, Germany). The ^{1}H NMR spectra were recorded on a BrukerAvance 300 apparatus (BrukerBioSpin GmbH, Germany) in DMSO-d_6 with TMS as internal standard. The ^{13}C NMR spectra were recorded on a Bruker Avance 300 apparatus. Chemical shifts are given in ppm (δ-scale). The purity of obtained compounds was checked by TLC on aluminium oxide 60 F254 plates (Merck Co. USA), in a $CHCl_3/C_2H_5OH$ (10:1, v/v) solvent system. The spots were detected by exposure to a UV lamp at 254 nm. Elemental analyses of the obtained compounds was performed for C, H, N on AMZ 851 CHX analyser (PG, Gdańsk, Poland). The maximum percentage differences between calculated and found values for each element were within the error and amounted to ± 0.4%.

2.1.1 Synthesis of new Schiff base derivatives (1-10)

To a suspension of 2-amino-5-methylsulfanyl-1,3,4-thiadiazole (**A**) or 3,5-diamino-4*H*-1,2,4-triazole (**B**) (10 mmol) in ethanol (25 mL), an equimolar of various substituted aromatic aldehydes (10 mmol) was added. The suspension was heated until clear solution was obtained. Then few drops of glacial acetic acid were added as a catalyst. The solution was refluxed for 4 hrs. After the completion of the reaction, the solution was cooled to room temperature. The obtained precipitate was filtered off and crystallized from ethanol.

1-(2-fluorophenyl)-*N*-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]methanimine (**1**)

Yield: 88.2%; grayish solid; m.p.: 112-114ºC. IR (KBr), v (cm^{-1}): 3080 (CH aromatic), 3055, 1452 (CH aliphatic), 1618 (C=N), 1395 (C-N), 654 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.64 (s, 3H, CH_3), 7.26-7.29 (m, 2H, ArH), 7.46-7.53 (m, 1H, ArH), 8.15-8.20 (m, 1H, ArH), 8.99 (s, 1H, =CH); ^{13}C NMR (DMSO) δ (ppm) = 18.0 (CH_3), 117.1, 124.7, 125.6, 131.6, 135.3 ($5C_{ar}$), 149.8 ($C_{thiadiazole}$), 159.4 (=CH), 161.9 (Car), 174.7 ($C_{thiadiazole}$). Analysis for $C_{10}H_8FN_3S_2$ (253.32) Calculated: C: 47.41%, H: 3.18%, N: 16.59%, Found: C: 47.49%, H: 3.12%, N: 16.63%.

1-(3-fluorophenyl)-*N*-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]methanimine (**2**)

Yield: 69.2%; grey solid; m.p.: 117-119ºC. IR (KBr), ν (cm^{-1}): 3069 (CH aromatic), 3059, 1459 (CH aliphatic), 1629 (C=N), 1409 (C-N), 648 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.65 (s, 3H, CH_3), 7.21-7.24 (m, 2H, ArH), 7.60-7.66 (m, 1H, ArH), 7.82-7.91 (m, 1H, ArH), 8.82 (s, 1H, =CH); ^{13}C NMR (DMSO) δ (ppm) = 18.1 (CH_3), 116.9, 118.7, 124.7, 129.8, 138.8 ($5C_{ar}$), 149.8 ($C_{thiadiazole}$), 163.52 (=CH), 163.7 (C_{ar}), 174.7 ($C_{thiadiazole}$). Analysis for $C_{10}H_8FN_3S_2$ (253.32) Calculated: C: 47.41%, H: 3.18%, N: 16.59%, Found: C: 47.45%, H: 3.21%, N: 16.56%.

1-(4-fluorophenyl)-*N*-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]methanimine (**3**)

Yield: 70.1%; yellowish solid; m.p.: 132-134ºC. IR (KBr), ν (cm^{-1}): 3076 (CH aromatic), 3046, 1448 (CH aliphatic), 1633 (C=N), 1401 (C-N), 656 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.57 (s, 3H, CH_3), 7.07-7.11 (m, 2H, ArH), 7.57-7.59 (m, 1H, ArH), 8.90 (s, 1H, =CH); ^{13}C NMR (DMSO) δ (ppm) = 18.0 (CH_3), 115.4, 131.7, 132.7 ($5C_{ar}$), 149.8 ($C_{thiadiazole}$), 164.8 (=CH), 165.3 (C_{ar}), 174.7 ($C_{thiadiazole}$). Analysis for $C_{10}H_8FN_3S_2$ (253.32) Calculated: C: 47.41%, H: 3.18%, N: 16.59%, Found: C: 47.38%, H: 3.22%, N: 16.52%.

4-bromo-2-[{[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]imino}methyl]phenol (**4**)

Yield: 97.0%; yellow solid; m.p.: 126-128ºC. IR (KBr), ν (cm^{-1}): 3595 (OH), 3046 (CH aromatic), 3038, 1455 (CH aliphatic), 1622 (C=N), 1406 (C-N), 644 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.79 (s, 3H, CH_3), 6.98-7.01 (m, 1H, ArH), 7.63-7.72 (m, 1H, ArH), 8.01-8.03 (m, 1H, ArH), 9.02 (s, 1H, =CH), 11.36 (s, 1H, OH); ^{13}C NMR (DMSO) δ (ppm) = 18.1 (CH_3), 112.2, 120.1, 120.9, 133.7, 136.4 ($5C_{ar}$), 149.8 ($C_{thiadiazole}$), 159.9 (C_{ar}), 164.7 (=CH), 174.7 ($C_{thiadiazole}$). Analysis for $C_{10}H_8BrN_3OS_2$ (330.22) Calculated: C: 36.37%, H: 2.44%, N: 12.72%, Found: C: 36.42%, H: 2.41%, N: 12.76%.

1-(2-chloro-6-fluorophenyl)-*N*-[5-(methylsulfanyl)-1,3,4-thiadiazol-2-yl]methanimine (**5**)

Yield: 72.9%; whitish solid; m.p.: 119-122ºC. IR (KBr), ν (cm^{-1}): 3059 (CH aromatic), 3047, 1457 (CH aliphatic), 1631 (C=N), 1412 (C-N), 650 (C-S).^{1}H NMR (DMSO-d_6) δ (ppm) = 2.66 (s, 3H, CH_3), 7.02-7.05 (m, 1H, ArH), 7.29-7.55 (m, 2H, ArH), 8.79 (s, 1H, =CH); ^{13}C NMR (DMSO) δ (ppm) = 18.1 (CH_3), 115.7, 125.5, 126.7, 135.3, 137.8 ($5C_{ar}$), 149.1 (=CH), 149.8 ($C_{thiadiazole}$), 160.4 (C_{ar}), 174.7 ($C_{thiadiazole}$). Analysis for $C_{10}H_7ClFN_3S_2$ (287.76) Calculated: C: 41.74%, H: 2.45%, N: 14.60%, Found: C: 41.78%, H: 2.41%, N: 14.65%.

N,N'-4*H*-1,2,4-triazole-3,5-diylbis[1-(2-fluorophenyl)methanimine] (**6**)

Yield: 67.0%; yellowish solid; m.p.: 224-226ºC. IR (KBr), ν (cm^{-1}): 3055 (CH aromatic), 3030, 1460 (CH aliphatic), 1594 (C=N), 1596 (NH), 1396 (C-N).^{1}H NMR (DMSO-d_6) δ (ppm) = 6.20-6.22 (m, 2H, ArH), 7.40-7.47 (m, 1H, ArH), 7.77-7.85 (m, 4H, ArH), 9.07 (s, 2H, 2x =CH), 12.14 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 117.1, 124.7, 125.6, 131.6, 135.3 ($10C_{ar}$), 161.6 ($2C_{triazole}$), 151.9 ($2C_{ar}$), 163.0 (2x =CH). Analysis for $C_{16}H_{11}F_2N_5$ (311.29) Calculated: C: 61.73%, H: 3.56%, N: 22.50%, Found: C: 61.78%, H: 3.51%, N: 22.58%.

N,N'-4*H*-1,2,4-triazole-3,5-diylbis[1-(3-fluorophenyl)methanimine] (**7**)

Yield: 55.3%; yellowish solid; m.p.: 270-272ºC. IR (KBr), ν (cm^{-1}): 3038 (CH aromatic), 3021, 1448 (CH aliphatic), 1611 (C=N), 1600 (NH), 1410 (C-N).^{1}H NMR (DMSO-d_6) δ (ppm) = 6.20-6.22 (m, 2H, ArH), 7.40-7.47 (m, 1H, ArH), 7.50-7.66 (m, 1H, ArH), 7.77-7.85 (m, 4H, ArH), 9.06 (s, 2H, 2x =CH), 12.11 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =116.9, 118.7, 124.7, 129.8, 138.8 ($10C_{ar}$), 161.6 ($2C_{triazole}$), 163.7 ($2C_{ar}$), 165.7 (2x =CH). Analysis for $C_{16}H_{11}F_2N_5$ (311.29) Calculated: C: 61.73%, H: 3.56%, N: 22.50%, Found: C: 61.76%, H: 3.54%, N: 22.46%.

N,N'-4*H*-1,2,4-triazole-3,5-diylbis[1-(4-fluorophenyl)methanimine] (**8**)

Yield: 69.1%; yellow solid; m.p.: 264-266ºC. IR (KBr), ν (cm^{-1}): 3060 (CH aromatic), 3025, 1458 (CH aliphatic), 1618 (C=N), 1598 (NH), 1408 (C-N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 6.20-6.22 (m, 2H, ArH), 7.32-7.50 (m, 4H, ArH), 7.98-8.09 (m, 2H, ArH), 9.06 (s, 2H, 2x =CH), 12.09 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 115.4, 119.2, 131.7, 132.7, 137.9 ($10C_{ar}$), 161.6 ($2C_{triazole}$), 165.3 ($2C_{ar}$), 166.7 (2x =CH). Analysis for $C_{16}H_{11}F_2N_5$ (311.29) Calculated: C: 61.73%, H: 3.56%, N: 22.50%, Found: C: 61.69%, H: 3.60%, N: 22.53%.

N,N'-4*H*-1,2,4-triazole-3,5-diylbis[1-(2-hydroxy-5-bromophenyl)methanimine] (**9**)

Yield: 73.0%; yellow solid; m.p.: 242-244ºC. IR (KBr), ν (cm^{-1}): 3609 (OH), 3040 (CH aromatic) 3035, 1441 (CH aliphatic), 1633 (C=N), 1615 (NH), 1404 (C-N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 6.98-7.07 (m, 2H, ArH), 7.57-7.68 (m, 2H, ArH), 8.01-8.12 (m, 2H, ArH), 9.51 (s, 2H, 2x =CH), 12.24 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 112.5, 120.1, 120.9, 133.7, 136.4, 159.9 ($12C_{ar}$), 161.6 ($2C_{triazole}$), 167.5 (2x =CH). Analysis for $C_{16}H_{11}Br_2N_5O_2$ (465.10) Calculated: C: 41.32%, H: 2.38%, N: 15.06%, Found: C: 41.37%, H: 2.41%, N: 15.02%.

N,*N'*-4*H*-1,2,4-triazole-3,5-diylbis[1-(2-chloro-6-fluorophenyl)methanimine] (**10**)

Yield: 85.2%; yellow solid; m.p.: 252-254ºC. IR (KBr), ν (cm^{-1}): 3046 (CH aromatic), 3026, 1442 (CH aliphatic), 1610 (C=N), 1605 (NH), 1412 (C-N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 7.03-7.05 (m, 2H, ArH), 7.65-7.69 (m, 2H, ArH), 8.09-8.12 (m, 2H, ArH), 9.49 (s, 2H, 2x =CH), 12.22 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 115.7, 125.5, 126.7, 135.3, 137.8 ($10C_{ar}$), 149.0 (2 x =CH), 160.4 ($2C_{ar}$), 161.6 ($2C_{triazole}$). Analysis for $C_{16}H_9Cl_2F_2N_5$ (380.18) Calculated: C: 50.55%, H: 2.39%, N: 18.42%, Found: C: 50.61%, H: 2.37%, N: 18.46%.

2.1 Microbiology

2.2.1*In vitro* antimicrobial assay

The examined compounds (**1**-**10**): were *in vitro* screened for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [25] and Clinical and Laboratory Standards Institute guidelines [26] against a panel of reference strains of 19 microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus pyogenes* ATCC 19615, *Streptococcus mutans* ATCC 25175), Gram-negative bacteria (*Bordetella bronchiseptica* ATCC 4617, *Escherichia coli* ATCC 3521, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027) and fungi belonging to yeasts (*Candida albicans* ATCC 2091, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019). These microorganisms came from American Type Culture Collection (ATCC), routinely used for the evaluation of antimicrobials. All the used microbial cultures were first subcultured on nutrient agar or Sabouraud agar at 35°C for 18-24 hrs or 30°C for 24-48 hrs for bacteria and fungi, respectively.

The surface of Mueller-Hinton agar or Mueller-Hinton agar with 5% sheep blood (for bacteria) and RPMI 1640 with MOPS (for fungi) were inoculated with the suspensions of bacterial or fungal species. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of McFarland standard scale 0.5 - approximately 1.5 x 108 CFU (Colony Forming Units)/ml for bacteria and 0.5 McFarland standard scale – approximately 5 x 105 CFU/ml) for fungi.

Samples containing 5 mg, 1 mg and 0.5 mg of tested compounds **1**-**10** were dissolved in 1 ml dimethyl sulphoxide (DMSO). Next 50 µl of the tested compound was dropped into the wells (d = 6 mm) on the mentioned above agar media. The agar plates were preincubated at room temperature for 1h, next they were incubated at 37°C for 24hrs and 30°C for 48 hrs for bacteria and fungi, respectively. After the incubation period, the zones of growth inhibition were measured and average values were calculated. The wells containing DMSO without the tested compound was used as controls.

Furthermore, bacterial and fungal suspensions were put onto Petri dishes with solid media containing 1 mg/ml of tested compounds **1**-**10** followed incubation at 37°C for 24 hrs and 30°C for 48hrs for bacteria and fungi, respectively. The inhibition of microbial growth was judged by comparison with a control culture prepared without any sample tested. Ciprofloxacin, vancomycin or fluconazole (Sigma) were used as a reference antibacterial or antifungal compounds, respectively.

Subsequently MIC (Minimal Inhibitory Concentration) of the compounds was examined by the microdilution broth method, using their two-fold dilutions in Mueller-Hinton broth or Mueller-Hinton broth with 5% sheep blood (for bacteria) and RPMI 1640 broth with MOPS (for fungi) prepared in 96-well polystyrene plates. Final concentrations of the compounds ranged from 1000 to 0.488 µg/ml. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with

an optical density of 0.5 McFarland standard. Next 2 µl of each bacterial or fungal suspension was added per each well containing 200 µl broth and various concentrations of the examined compounds. After incubation (37°C, 24 hrs) the MIC was assessed spectrophotometrically as the lowest concentration of the samples showing complete bacterial or fungal growth inhibition. Appropriate DMSO, growth and sterile controls were carried out. The medium with no tested substances was used as control.

The MBC (Minimal Bactericidal Concentration) or MFC (Minimal Fungicidal Concentration) are defined as the lowest concentration of the compounds that is required to kill a particular bacterial or fungal species. MBC/MFC was determined by removing 20 µl of the culture using for MIC determinations from each well and spotting onto appropriate agar medium. The plates were incubated for 37ºC for 24 hrs and 30°C for 48hrs for bacteria and fungi, respectively. The lowest compounds concentrations with no visible growth observed was assessed as a bactericidal/fungicidal concentration [27].

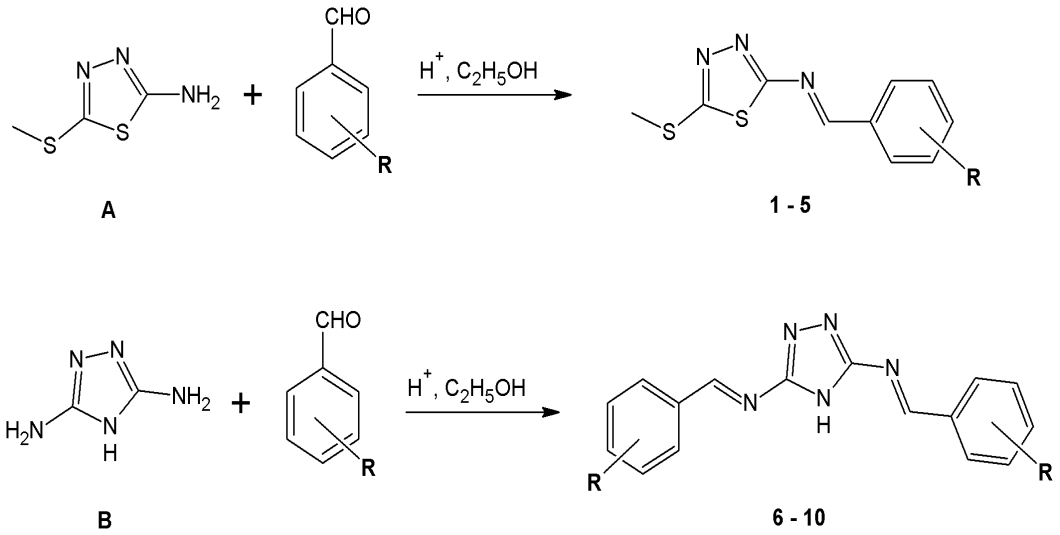
In this study, no bioactivity was defined as a MIC > 1000 µg/ml, mild bioactivity as a MIC in the range 501 – 1000 µg/ml, moderate bioactivity with MIC from 126 to 500 µg/ml, good bioactivity as a MIC in the range 26–125 µg/ml, strong bioactivity with MIC between 10 and 25 µg/ml and very strong bioactivity as a MIC < 10 µg/ml [28].

The MBC/MIC or MFC/MIC ratios were calculated in order to determine bactericidal/fungicidal (MBC/MIC ≤ 4, MFC/MIC ≤ 4) or bacteriostatic/fungistatic (MBC/MIC > 4, MFC/MIC > 4) effect of the tested compounds is shown in Table 1.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The aim of this study was to obtain new Schiff base derivatives (**1**-**10**) incorporating the 1,3,4-thiadiazole and 1,2,4-triazole moiety as antimicrobial agents. New compounds (**1**-**10**) were obtained by the condensation reaction of 2-amino-5-methylsulfanyl-1,3,4-thiadiazole (**A**) or 3,5-diamino-4*H*-1,2,4-triazole (**B**) with various aromatic aldehydes (Scheme 1.). The method used for synthesis of new compounds (**1**-**10**) was based on similar syntheses described earlier [29, 30] with some modifications. In our case as a catalyst we used glacial acetic acid instead of concentrated sulfuric acid or benzyl triethylammonium chloride (BTEAC) [29] and polyethylene glycol 400 (PEG-400) [30].



R = 2-F (**1**, **6**), 3-F (**2**, **7**), 4-F (**3**, **8**), 2-OH-5-Br (**4**, **9**), 2-Cl-6-F (**5**, **10**)

Scheme 1. Synthetic pathway leading to new Schiff base derivatives (1-10)

Table 1. The activity data of tested compounds expressed as MIC (MBC) [µg/ml] against the reference strains of bacteria and fungi

Species		MIC (MBC/MFC) [µg/ml] of the tested compounds								CIP/VA* /FLU**
		2	3	4	5	6	8	9	10	
Gram-positive bacteria	*Staphylococcus aureus* ATCC 25923	-	-	62.5 (250)	62.5 (500)	500 (>1000)	-	-	-	0.488
	Staphylococcus aureus ATCC 6538	-	-	125 (500)	500 (1000)	-	-	-	-	0.244
	Staphylococcus epidermidis ATCC 12228	500 (>1000)	-	62.5 (125)	250 (500)	1000 (>1000)	-	-	-	0.122
	Micrococcus luteus ATCC 10240	1000 (>1000)	-	125 (125)	1000 (>1000)	-	-	-	-	0.976
	Bacillus subtilis ATCC 6633	1000 (1000)	-	62.5 (250)	500 (1000)	500 (>1000)	-	-	-	0.031
	Bacillus cereus ATCC 10876	500 (>1000)	-	125 (>1000)	1000 (>1000)	-	-	-	-	0.061
	Streptococcus pneumoniae ATCC 49619	-	-	500 (1000)	-	-	-	-	-	0.244*
	Streptococcus pyogenes ATCC 19615	-	-	125 (500)	-	-	-	-	-	0.244*
	Streptococcus mutans ATCC25175	-	-	250 (500)	-	1000 (>1000)	-	-	-	0.976*
Gram-negative bacteria	*Bordetella bronchiseptica* ATCC 4617	-	-	62.5 (250)	-	-	-	-	-	0.976
	Escherichia coli ATCC 3521	-	-	250 (500)	-	-	-	-	-	0.015
	Escherichia coli ATCC 25922	-	-	250 (500)	-	-	-	-	-	0.004
	Klebsiell apneumoniae ATCC 13883	-	-	500 (500)	-	-	-	-	-	0.122
	Proteus mirabilis ATCC 12453	-	-	500 (1000)	-	-	-	-	-	0.030
	Salmonella typhimurium ATCC 14028	-	-	250 (500)	-	-	-	-	-	0.061
	Pseudomonas aeruginosa ATCC 9027	-	-	1000 (>1000)	-	-	-	-	-	0.488
Fungi	*Candida albicans* ATCC 2091	1000 (>1000)	250 (250)	31.25 (62.5)	250 (500)	-	1000 (>1000)	250 (1000)	250 (500)	0.244**
	Candida albicans ATCC 10231	1000 (>1000)	250 (500)	31.25 (125)	250 (500)	-	-	250 (1000)	250 (1000)	0.976**
	Candida parapsilosis ATCC 22019	500 (>1000)	125 (1000)	62.5 (250)	125 (1000)	-	1000 (>1000)	250 (1000)	250 (1000)	1.953**

The standard antibiotics used as positive controls: ciprofloxacin (CIP) or vancomycin (VA) for bacteria and fluconazole (FLU**) for fungi.*

Obtained compounds (**1**-**10**) are stable solids at room temperature and their spectral data (IR, ^{1}H NMR and ^{13}C NMR) and elemental analysis is in full agreement with the proposed structures. The IR spectra of synthesized compounds **1**-**10** confirmed the presence of appropriate functional groups in obtained derivatives. The ^{1}H NMR spectra of the derivatives **1**-**10** showed one proton singlet signal typical for the =CH group in the δ 8.79 – 9.51 ppm range and one proton singlet signal for the NH group at δ 12.09 – 12.24 ppm (compounds **6**-**10**), what confirmed the successful formation of the desired products. The ^{13}C NMR spectra of compounds **1**-**10** also confirmed the presence of =CH group in the range δ 149.1 – 167.5 ppm. All aromatic and aliphatic signals in the ^{1}H NMR and ^{13}C NMR spectra for all of synthesized compounds were observed at expected regions.

Synthesized compounds (**1**-**10**) were subjected to *in vitro* antimicrobial assays to define their biological activity.

3.2 Microbiology

Using preliminary agar dilution method we showed that, the tested compounds **1**, **2**, **3**, **5**, **6**, **8**-**10** had no influence on the growth of the reference strains of bacteria from *Enterobacteriaceae* family and *Pseudomonas aeruginosa*, belonging to Gram-negative bacteria, even at maximal concentration used (2000 µg/ml) (Table 1).

The results of our study indicated that among the examined compounds **1**-**10**, the widest spectrum of antibacterial activity and the highest activity possessed compound **4** against all tested strains of bacteria both Gram-positive and Gram-negative or yeasts belonging to *Candida* spp. This compound indicated good bactericidal activity against reference strains of staphylococci (MIC = 62.5 - 125 µg/ml), micrococci (MIC = 125 µg/ml), *Bacillus* spp. ATCC, *Streptococcus pyogenes* ATCC 19615 and *Bordetella bronchiseptica* ATCC 4617 (MIC = 62.5 - 125 µg/ml, MBC = 250 - 500 µg/ml, MBC/MIC = 1 - 4). The bactericidal activity to remaining streptococci was moderate (MIC = 250 - 500 µg/ml, MBC = 500 - 1000 µg/ml, MBC/MIC = 2). The compound **4**, as the only, indicated also moderate bactericidal activity against rod-shaped bacteria belonging to *Enterobacteriaceae* family (*Escherichia coli* ATCC 3521, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATTC 12453 and *Salmonella typhimurium* ATCC 14028) (MIC = 250 - 500 µg/ml, MBC = 500 - 1000 µg/ml, MBC/MIC = 1 - 2) and *P. aeruginosa* ATCC 9027 (MIC = 1000 µg/ml, MBC > 1000 µg/ml) (Table 1).

The compound **5** showed a different effect against staphylococci, streptococci and *Bacillus* spp. (MIC = 62.5 - 1000 µg/ml, MBC = 500 - 1000 µg/ml) and lower activity or no activity to remaining bacteria. The compound **2** indicated activity only against *S. epidermidis* ATTC 12228, *M. luteus* ATCC 10240 and *Bacillus* spp. The minimal bactericidal concentrations values ranged from 500 to 1000 µg/ml and MBC > 1000 µg/ml (Table 1.).

From the 1,2,4-triazole derivatives only compound **6** showed some activity against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATTC 6633 and *Streptococcus mutans* ATTC 25175 (MIC = 500 - 1000 µg/ml, MBC => 1000 µg/ml) or had no activity. The compounds **1**, **3**, **7**, **8**, **9** and **10** had no influence on the growth of any tested bacteria (Table 1.).

Moreover, compounds **2**-**5**, **8**-**10** indicated activity against all reference *Candida* spp. strains. These yeasts were especially sensitive to compound **4**, which showed good fungicidal activity (MIC = 31.25 - 62.5 µg/ml, MFC/MIC = 2 – 4). The compounds **3**, **5**, **9**, **10** inhibited their growth with MIC = 125 - 250 µg/ml (MFC/MIC = 1 - 8). The lowest activity against *Candida* spp. strains showed compounds**2** and **8** (MIC = 500 - 1000 µg/ml and MFC > 1000 µg/ml) in contrast to compounds **1**, **6** and **7** which exhibited no activity (Table 1).

4. CONCLUSION

In this paper we synthesized a new series of Schiff base derivatives (**1**-**10**) by the condensation reaction of 2-amino-5-methylsulfanyl-1,3,4-thiadiazole (**A**) or 3,5-diamino-4*H*-1,2,4-triazole (**B**) with various aromatic aldehydes. The structure of obtained compounds was confirmed by spectral methods and elemental analysis. All synthesized derivatives were *in vitro* screened for their antimicrobial activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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