DR ŁUKASZ POPIOŁEK (Orcid ID : 0000-0002-3250-3257)

DR ANNA BIERNASIUK (Orcid ID : 0000-0002-2844-9876)

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Synthesis of promising antimicrobial agents: hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid

Łukasz Popiołek[1]*, Bernadetta Rysz[1], Anna Biernasiuk[2], Monika Wujec[1]

[1] Department of Organic Chemistry, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, 4A Chodźki Street, 20-093 Lublin, Poland;

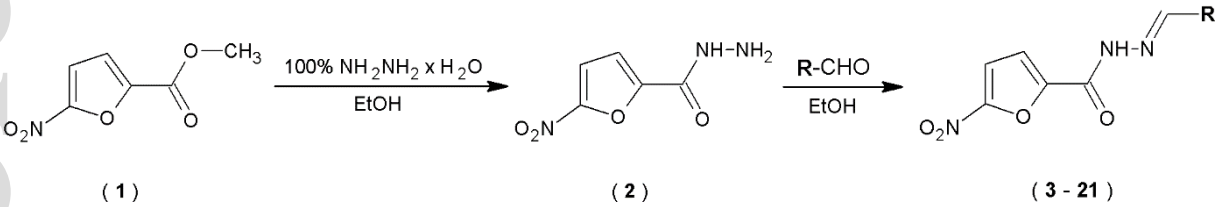
[2] Department of Pharmaceutical Microbiology, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, 1 Chodźki Street, 20-093 Lublin, Poland;

*corresponding author: lukasz.popiolek@umlub.pl

ABSTRACT

In this research we synthesized and evaluated for *in vitro* antimicrobial activity a new series of hydrazide-hydrazones obtained from 5-nitrofuran-2-carboxylic acid. New compounds were identified and characterized by spectral methods (^{1}H NMR and ^{13}C NMR). All tested hydrazide-hydrazones proved to be promising antimicrobial agents. Antimicrobial and antifungal activity of new derivatives of 5-nitrofuran-2-carboxylic acid revealed in many cases to be higher than the activity of reference substances (nitrofurantoin, cefuroxime and ampicillin).

GRAPHICAL ABSTRACT



1. INTRODUCTION

Discovery of novel antimicrobial agents represents an up to-date research topic due to the increasing number of microbial strains resistant to commonly used chemotherapeutics (Coates, 2002). Based on the above fact, there is a considerable need for discovery of new compounds which will act as antimicrobial agents (Coates, 2002; Moellering, 2011).

A significant part among thousands of new biologically active compounds synthesized each year by medicinal chemists around the world belongs to the group of hydrazide-hydrazones (Rollas, 2007; Bala, 2013; Popiołek, 2017A). This is due to the fact that this group of compounds possesses very interesting and broad spectrum of biological properties (Rollas, 2007; Bala, 2013; Popiołek, 2017), which include antimicrobial (Rollas, 2002; Metwally, 2006; Backes, 2014; Manikandan, 2017; Zha, 2017), anticancer (Kumar, 2012; Nasr, 2014, 2018; Nikolova-Mladenova, 2017; Tantak, 2017; Sreenivasulu, 2019), anti-inflammatory (Sondhi, 2006; Gökçe, 2009; Moldovan, 2011), anticonvulsant (Dehestani, 2018), and analgesic activity (Navidpour, 2014).

In our previous research a series of hydrazide-hydrazones showed very promising antimicrobial and antifungal activities (Popiołek, 2016A, 2016B, 2017B, 2017C, 2018A). For instance, a series of nitrofurazone analogues containing hydrazide-hydrazone moiety synthesized by our group displayed, depending on the compounds and bacterial or fungal strains, higher antimicrobial activity than ciprofloxacin, nitrofurantoin or cefuroxime, which were used as reference substances (Popiołek, 2017C). In that research we used different hydrazides of carboxylic acids and condensed them with 5-nitro-2-furaldehyde (Popiołek, 2017C). In our current research we decided to modify previous research and we used 5-nitrofuran-2-carboxylic acid hydrazide and condensed it with various aliphatic and aromatic aldehydes. The data obtained allow us to investigate the influence of different substituents on the antimicrobial activity of hydrazide-hydrazones. In addition to this, in our earlier study hydrazide-hydrazones of 3-hydroxy-2-naphthoic acid also displayed significant anticancer activity against human renal cell adenocarcinoma (769-P) and

human hepatocellular carcinoma (HepG2) and proved to be safe towards normal reference cell lines (rat cardiomyocytes - H9c2 and green monkey kidney cells - GMK), which is very important in designing novel antimicrobial agents (Popiołek, 2018B).

Motivated by the above facts and findings and in continuation of our ongoing efforts towards with the searching for antimicrobial agents, in this research we have designed, synthesized and *in vitro* evaluated for antimicrobial activity a new series of hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid.

2. MATERIALS AND METHODS

2.1 General

Reagents and solvent used in this research were purchased from Sigma-Aldrich (Munich, Germany) and Merck Co. (Darmstadt, Germany) and were used without further purification. Melting points were determined on Fisher-Johns blocks melting point apparatus (Fisher Scientific, Germany) and left uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on the Bruker Avance 300 apparatus (Bruker BioSpin GmbH, Germany) in DMSO-d_6 with TMS as the internal standard. Chemical shifts are reported in ppm (δ) with the use of TMS as the standard reference. The coupling constants (*J*) are given in Hertz. The progress of the reaction and purity of the obtained compounds were monitored by TLC using pre-coated aluminum sheet 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 the UV lamp at 254 nm. The elemental analysis of the obtained compounds was carried out with the AMZ 851 CHX analyser (PG, Gdańsk, Poland). The results of elemental analysis (C, H, N) were within ± 0.4% of the calculated values.

2.2 Synthesis of 5-nitrofuran-2-carboxylic acid hydrazide (**2**)

This compound was synthesized according to the procedure described earlier (Ahmad, 2016). In two-neck round-bottom flask equipped with condenser, thermometer and magnetic stirrer 0.002 mole (0.34g) of methyl ester of 5-nitrofuran-2-carboxylic acid was placed and 5 mL of anhydrous ethanol was added. Subsequently, the flask was equipped with ice bath and the content was stirred for 15 minutes, until the ester was dissolved. Next 100 µl of 100% hydrazine hydrate was added and the content was stirred for 30 minutes. The temperature of the reaction mixture should be

around 0°C. After that formed precipitate was filtered off and dried. Obtained compound was re-crystallized from ethanol.

CAS Number: 5469-78-3; Yield: 73%; M.p.: 160ºC; ^{1}H NMR (DMSO-d_6) δ (ppm): 4.71 (s, 2H, NH_2), 7.36-7.37 (d, 1H, ArH, J = 3 Hz), 7.74-7.76 (d, 1H, ArH, J = 6 Hz), 10.21 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 113.9, 115.2, 148.2, 152.0 ($4C_{ar}$), 156.1 (C=O); For $C_5H_5N_3O_4$ (171.11) Calculated: C%: 35.10, H%: 2.95, N%: 24.56; Found: C%: 35.22, H%: 2.93, N%: 24.61.

2.3 Synthesis of hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid (**3** – **21**)

0.0012 Mole (0.2g) of hydrazide of 5-nitrofuran-2-carboxylic acid (**2**) and 5 mL of ethanol (96%) was placed in a round bottom flask. Hydrazide was heated under reflux until it completely dissolved in ethanol. Then 0.00132 mole of appropriate aldehyde was added and the content of the flask was heated under reflux for 2 hours. After that, the solution was allowed to cool and the formed precipitate was filtered off and re-crystallized from ethanol.

N-(2-methylpropylidene)-5-nitrofuran-2-carbohydrazide (**3**)

Yield: 10%; M.p.: 163°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.07-1.09 (d, 6H, 2xCH_3, J = 6 Hz), 2.55-2.59 (m, 1H, CH), 7.49-7.50 (d, 1H, ArH, J = 3 Hz), 7.72-7.74 (d, 1H, ArH, J = 6 Hz), 7.78-7.79 (d, 1H, =CH, J = 3 Hz), 11.80 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 19.9 (2xCH_3), 31.6 (CH), 113.8, 116.7, 147.7, 152.1 (4C_{ar}), 152.9 (=CH), 159.3 (C=O); For $C_9H_{11}N_3O_4$ (225.20) Calculated: C%: 48.00, H%: 4.92, N%: 18.66; Found: C%: 48.10, H%: 4.91, N%: 18.71.

N-(2,2-dimethylpropylidene)-5-nitrofuran-2-carbohydrazide (**4**)

Yield: 72%; M.p.: 184°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (s, 9H, 3xCH_3), 7.48-7.50 (d, 1H, ArH, J = 6 Hz), 7.74 (s, 1H, =CH), 7.77-7.79 (d, 1H, ArH, J = 6 Hz), 11.76 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 27.5 (3xCH_3), 35.29 ($C_{t\text{-}butyl}$), 113.3, 121.1, 146.6, 152.2 (4C_{ar}), 156.6 (=CH), 161.8 (C=O); For $C_{10}H_{13}N_3O_4$ (239.23) Calculated: C%: 50.21, H%: 5.48, N%: 17.56; Found: C%: 50.34, H%: 5.47, N%: 17.51.

N-[3-(methylsulphanyl)propylidene]-5-nitrofuran-2-carbohydrazide (**5**)

Yield: 47%; M.p.: 126°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 2.09 (s, 3H, CH_3), 2.57-2.61 (t, 2H, CH_2, J = 6 Hz), 2.68-2.74 (q, 2H, CH_2, J = 6 Hz), 7.50-7.51 (d, 1H, ArH, J = 3 Hz), 7.78-7.79 (d, 1H, ArH, J = 3 Hz), 7.79-7.83 (t, 1H, =CH, J = 9 Hz, J = 3 Hz), 11.95 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.6 (SCH_3), 30.3 (CH_2), 33.6 (CH_2), 115.4, 123.6, 138.2, 150.1 (4C_{ar}), 154.8 (=CH), 156.2 (C=O); For $C_9H_{11}N_3O_4S$ (257.27) Calculated: C%: 42.02, H%: 4.31, N%: 16.33; Found: C%: 41.96, H%: 4.30, N%: 16.29.

5-nitro-*N*-(pentylidene)furan-2-carbohydrazide (**6**)

Yield: 14%; M.p.: 140°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.88-0.93 (t, 3H, CH_3, J = 6 Hz, J = 9 Hz), 1.28-1.40 (m, 2H, CH_2), 1.43-1.53 (m, 2H, CH_2), 2.25-2.32 (q, 2H, CH_2, J = 9 Hz), 7.48-7.50 (d, 1H, ArH, J = 6 Hz), 7.77-7.79 (t, 1H, =CH, J = 6 Hz), 7.78-7.80 (d, 1H, ArH, J = 6 Hz), 11.85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.2 (CH_3), 22.2 (CH_2), 28.5 (CH_2), 32.2 (CH_2), 113.3, 116.8, 146.6, 147.7 (4C_{ar}), 152.8 (=CH), 155.1 (C=O); For $C_{10}H_{13}N_3O_4$ (239.23) Calculated: C%: 50.21, H%: 5.48, N%: 17.56; Found: C%: 50.35, H%: 5.46, N%: 17.51.

N-(hexylidene)-5-nitrofuran-2-carbohydrazide (**7**)

Yield: 37%; M.p.: 130°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.86-0.90 (t, 3H, CH_3, J = 6 Hz), 1.28-1.33 (m, 4H, 2xCH_2), 1.45-1.55 (m, 2H, CH_2), 2.24-2.32 (q, 2H, CH_2, J = 9 Hz), 7.48-7.49 (d, 1H, ArH, J = 3 Hz), 7.77-7.78 (d, 1H, ArH, J = 3 Hz), 7.79-7.80 (t, 1H, =CH, J = 3 Hz), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.0 (CH_3), 22.9 (CH_2), 27.2 (CH_2), 30.0 (CH_2), 31.0 (CH_2), 115.4, 123.6, 150.1, 154.8 (4C_{ar}), 156.2 (=CH), 163.3 (C=O); For $C_{11}H_{15}N_3O_4$ (253.25) Calculated: C%: 52.17, H%: 5.97, N%: 16.59; Found: C%: 51.25, H%: 5.95, N%: 16.60.

N-(heptylidene)-5-nitrofuran-2-carbohydrazide (**8**)

Yield: 45%; M.p.: 138°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.85-0.89 (t, 3H, CH_3, J = 6 Hz), 1.26-1.34 (m, 6H, 3xCH_2), 1.47-1.51 (m, 2H, CH_2), 2.24-2.31 (q, 2H, CH_2, J = 9 Hz), 7.48-7.50 (d, 1H, ArH, J = 6 Hz), 7.76-7.79 (t, 1H, =CH, J = 6 Hz, J = 3 Hz), 7.78-7.79 (d, 1H, ArH, J = 3 Hz), 11.84 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.4 (CH_3), 22.5 (CH_2), 26.3 (CH_2), 28.8 (CH_2), 31.5 (CH_2), 32.5 (CH_2), 113.9, 116.8, 147.7, 152.2 (4C_{ar}), 152.9 (=CH), 155.1 (C=O); For $C_{12}H_{17}N_3O_4$ (267.28) Calculated: C%: 53.92, H%: 6.41, N%: 15.72; Found: C%: 53.79, H%: 6.42, N%: 15.66.

5-nitro-*N*-(octylidene)furan-2-carbohydrazide (**9**)

Yield: 19%; M.p.: 124°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.84-0.89 (t, 3H, CH_3, J = 9 Hz, J = 6 Hz), 1.27-1.30 (m, 8H, 4xCH_2), 1.47-1.52 (m, 2H, CH_2), 2.24-2.31 (q, 2H, CH_2, J = 9 Hz), 7.48-7.50 (d, 1H, ArH, J = 6 Hz), 7.76-7.79 (t, 1H, =CH, J = 6 Hz, J = 3 Hz), 7.78-7.79 (d, 1H, ArH, J = 3 Hz), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.4 (CH_3), 22.5 (CH_2), 26.4 (CH_2), 28.9 (CH_2), 29.1 (CH_2), 31.7 (CH_2), 32.5 (CH_2), 113.9, 116.8, 147.7, 152.2 (4C_{ar}), 152.9 (=CH), 155.1 (C=O); For $C_{13}H_{19}N_3O_4$ (281.31) Calculated: C%: 55.50, H%: 6.81, N%: 14.94; Found: C%: 55.68, H%: 6.79, N%: 14.96.

5-nitro-*N*-(nonylidene)furan-2-carbohydrazide (**10**)

Yield: 24%; M.p.: 120°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.84-0.88 (t, 3H, CH_3, J = 6 Hz), 1.26-1.32 (m, 10H, 5xCH_2), 1.47-1.52 (m, 2H, CH_2), 2.24-2.31 (q, 2H, CH_2, J = 6 Hz, J = 9 Hz), 7.48-7.49 (d, 1H, ArH, J = 3 Hz), 7.76-7.79 (t, 1H, =CH, J = 6 Hz, J = 3 Hz), 7.78-7.89 (d, 1H, ArH, J = 3 Hz), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.5 (CH_3), 22.6 (CH_2), 26.4 (CH_2), 27.6 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 31.7 (CH_2), 32.5 (CH_2), 113.9, 116.8, 147.7, 152.2 (4C_{ar}),

152.9 (=CH), 155.1 (C=O); For $C_{14}H_{21}N_3O_4$ (295.33) Calculated: C%: 56.94, H%: 7.17, N%: 14.23; Found: C%: 56.98, H%: 7.16, N%: 14.26.

N-[(2-bromophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**11**)

CAS Number: 1243683-04-6; Yield: 81%; M.p.: 244-245°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.42-7.47 (t, 1H, ArH, J = 6 Hz, J = 9 Hz), 7.57-7.59 (d, 1H, ArH, J = 6 Hz), 7.65-7.68 (m, 1H, ArH), 7.75-7.77 (d, 1H, ArH, J = 6 Hz), 7.81-7.82 (d, 1H, ArH, J = 3 Hz), 7.92-7.94 (m, 1H, ArH), 8.46 (s, 1H, =CH), 12.42 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 113.9, 117.3, 122.7, 126.9, 129.9, 131.6, 133.5, 136.8, 147.6, 148.3 (10C_{ar}), 152.3 (=CH), 153.5 (C=O); For $C_{12}H_8BrN_3O_4$ (338.11) Calculated: C%: 42.63, H%: 2.38, N%: 12.43; Found: C%: 42.59, H%: 2.37, N%: 12.46.

N-[(3-bromophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**12**)

CAS Number: 1243683-04-6; Yield: 91%; M.p.: 173-175°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.57-7.58 (d, 1H, ArH, J = 3 Hz), 7.66-7.73 (m, 4H, ArH), 7.81-7.82 (d, 1H, ArH, J = 3 Hz), 8.47 (s, 1H, =CH), 12.34 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 115.4, 123.6, 123.9, 125.7, 129.7, 129.9, 130.6, 136.4, 150.1, 154.8 (10C_{ar}), 156.2 (=CH), 160.1 (C=O); For $C_{12}H_8BrN_3O_4$ (338.11) Calculated: C%: 42.63, H%: 2.38, N%: 12.43; Found: C%: 42.69, H%: 2,38, N%: 12.40.

N-[(4-bromophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**13**)

CAS Number: 913480-93-0; Yield: 68%; M.p.: 204°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.30-7.33 (d, 2H, ArH, J = 9 Hz), 7.50-7.55 (m, 1H, ArH), 7.57-7.59 (d, 1H, ArH, J = 6 Hz), 7.81-7.83 (d, 1H, ArH, J = 6 Hz), 7.94-7.98 (m, 1H, ArH), 8.75 (s, 1H, =CH), 12.37 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 113.9, 116.6, 117.4, 125.5, 126.9, 142.9, 147.4, 152.3 (10C_{ar}), 153.3 (=CH), 160.7 (C=O); For $C_{12}H_8BrN_3O_4$ (338.11) Calculated: C%: 42.63, H%: 2.38, N%: 12.43; Found: C%: 42.59, H%: 2.38, N%: 12.39.

N-[(2-chlorophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**14**)

Yield: 79%; M.p.: 240°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.45-7.50 (m, 2H, ArH), 7.55-7.56 (m, 1H, ArH), 7.58-7.60 (d, 1H, ArH, J = 6 Hz), 7.82-7.83 (d, 1H, ArH, J = 3 Hz), 8.02-8.05 (m, 1H, ArH), 8.91 (s, 1H, =CH), 12.50 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 113.4, 113.9, 117.3, 121.6, 129.5, 133.3, 135.5, 147.5, 148.8, 152.3 (10C_{ar}), 153.3 (=CH), 156.9 (C=O); For

$C_{12}H_8ClN_3O_4$ (293.66) Calculated: C%: 49.08, H%: 2.75, N%: 14.31; Found: C%: 49.15, H%: 2,74, N%: 14,33.

N-[(3-chlorophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**15**)
CAS Number: 125273-90-7; Yield: 67%; M.p.: 158-160°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.38-7.44 (m, 1H, ArH), 7.47-7.52 (m, 1H, ArH), 7.59-7.60 (d, 1H, ArH, J = 3 Hz), 7.71-7.74 (m, 1H, ArH), 7.82-7.83 (d, 1H, ArH, J = 3 Hz), 8.00-8.03 (m, 1H, ArH), 8.86 (s, 1H, =CH), 12.51 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 113.9, 117.5, 124.3, 127.9, 128.7, 132.7, 133.1, 133.7, 147.4, 148.3 (10C_{ar}), 152.3 (=CH), 153.3 (C=O); For $C_{12}H_8ClN_3O_4$ (293.66) Calculated: C%: 49.08, H%: 2.75, N%: 14.31; Found: C%: 49.11, H%: 2.75, N%: 14.28.

N-[(4-chlorophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**16**)
CAS Number: 29448-95-1; Yield: 87%; M.p.: 204°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.54-7.56 (d, 2H, ArH, J = 6 Hz), 7.56-7.58 (d, 1H, ArH, J = 6 Hz), 7.77-7.80 (d, 2H, ArH, J = 9 Hz), 7.81-7.82 (d, 1H, ArH, J = 3 Hz), 8.49 (s, 1H, =CH), 12.32 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 115.3, 123.6, 129.1, 129.4, 134.1, 135.1, 150.2, 154.8 (10C_{ar}), 156.3 (=CH), 161.3 (C=O); For $C_{12}H_8ClN_3O_4$ (293.66) Calculated: C%: 49.08, H%: 2.75, N%: 14.31; Found: C%: 49.01, H%: 2.74, N%: 14.27.

N-[(2-fluorophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**17**)
CAS Number: 125273-98-5; Yield: 83%; M.p.: 208°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28-7.35 (m, 2H, ArH), 7.52-7.54 (d, 1H, ArH, J = 6 Hz), 7.55-7.59 (m, 2H, ArH), 7.81-7.82 (d, 1H, ArH, J = 3Hz), 8.50 (s, 1H, =CH), 12.36 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 115.4, 117.3, 123.5, 124.5, 125.3, 129.4, 130.8, 150.1, 154.8 (9C_{ar}), 156.2 (=CH), 160.4 (C_{ar}), 167.2 (C=O); For $C_{12}H_8FN_3O_4$ (277.21) Calculated: C%: 51.99, H%: 2.91, N%: 15.16; Found: C%: 52.05, H%: 2.90, N%: 15.10.

N-[(3-fluorophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**18**)
CAS Number: 125273-99-6; Yield: 93%; M.p.: 192-194°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.30-7.35 (t, 2H, ArH, J = 6 Hz, J = 9 Hz), 7.56-7.57 (d, 1H, ArH, J = 3 Hz), 7.80-7.81 (d, 1H, ArH, J = 3 Hz), 7.82-7.85 (m, 2H, ArH), 8.50 (s, 1H, =CH), 12.27 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 114.2, 115.4, 116.9, 121.9, 123.6, 130.0, 137.0, 150.2, 154.8 (9C_{ar}), 156.1 (=CH), 162.1

(C_{ar}), 162.7 (C=O); For $C_{12}H_8FN_3O_4$ (277.21) Calculated: C%: 51.99, H%: 2.91, N%: 15.16; Found: C%: 52.12, H%: 2.91, N%: 15.12.

N-[(4-fluorophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**19**)

CAS Number: 125274-00-2; Yield: 71%; M.p.: 222-223°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.19-7.24 (m, 2H, ArH), 7.46-7.52 (m, 1H, ArH), 7.58-7.60 (d, 1H, ArH, *J* = 6 Hz), 7.82-7.84 (d, 1H, ArH, *J* = 6 Hz), 7.93-7.97 (m, 1H, ArH), 8.73 (s, 1H, =CH), 12.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 113.9, 117.4, 127.4, 129.1, 132.7, 135.9, 140.2, 147.5 (10C_{ar}), 152.7 (=CH), 153.3 (C=O); For $C_{12}H_8FN_3O_4$ (277.21) Calculated: C%: 51.99, H%: 2.91, N%: 15.16; Found: C%: 51.89, H%: 2.90, N%: 15.19.

N-[(2-iodophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**20**)

Yield: 71%; M.p.: 206-208°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.26-7.31 (t, 2H, ArH, *J* = 6 Hz, *J* = 9 Hz), 7.58-7.59 (d, 1H, ArH, *J* = 3 Hz), 7.74-7.77 (d, 1H, ArH, *J* = 9 Hz), 7.81-7.84 (m, 2H, ArH) 8.42 (s, 1H, =CH), 12.37 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 103.3, 115.4, 123.5, 127.1, 128.2, 129.2, 130.3, 140.0, 150.2, 154.8 (10C_{ar}), 156.1 (=CH), 166.3 (C=O); For $C_{12}H_8IN_3O_4$ (385.11) Calculated: C%: 37.42, H%: 2.09, N%: 10.91; Found: C%: 37.39, H%: 2.08, N%: 10.93.

N-[(3-iodophenyl)methylidene]-5-nitrofuran-2-carbohydrazide (**21**)

Yield: 76%; M.p.: 222°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.05-7.09 (t, 1H, ArH, *J* = 6 Hz), 7.44-7.46 (d, 1H, ArH, *J* = 6 Hz), 7.56-7.57 (d, 1H, ArH, *J* = 3 Hz), 7.69-7.71 (d, 1H, ArH, *J* = 6 Hz), 7.84-7.85 (d, 1H, ArH, *J* = 3 Hz), 8.00-8.03 (m, 1H, ArH), 8.45 (s, 1H, =CH), 10.94 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 99.3, 115.4, 123.5, 126.4, 130.3, 134.3, 134.9, 137.9, 150.2, 154.7 (10C_{ar}), 156.1 (=CH), 161.4 (C=O); For $C_{12}H_8IN_3O_4$ (385.11) Calculated: C%: 37.42, H%: 2.09, N%: 10.91; Found: C%: 37.50, H%: 2.09, N%: 10.89.

2.4 In vitro antimicrobial assay

The examined compounds **2 – 21** were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST discussion document E. Dis 5.1) and Clinical and Laboratory Standards Institute (CLSI M27-S4) guidelines against a panel of reference

and clinical or saprophytic strains of microorganisms including Gram-positive bacteria, Gram-negative bacteria and fungi belonging to yeasts. Detailed antimicrobial assays procedures were described by our group earlier (Popiołek, 2015).

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 a MIC from 126 to 500 μg/ml, good bioactivity as a MIC in the range 26 – 125 μg/ml, strong bioactivity with a MIC between 10 and 25 μg/ml and very strong bioactivity as a MIC < 10 μg/ml. 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 (Wiegand, 2008; O'Donnell, 2010).

3. RESULTS AND DISCUSSION

3.1 Chemistry

The main aim of this research was the synthesis, chemical structure identification and *in vitro* antimicrobial activity evaluation of new hydrazide-hydrazones. As a starting material for the synthesis of new compounds we used methyl ester of 5-nitrofuran-2-carboxylic acid (**1**), which was converted to an appropriate hydrazide (**2**) in the reaction with 100% hydrazine hydrate. New compounds were synthesized on the basis of the condensation reaction of hydrazide of 5-nitrofuran-2-carboxylic acid (**2**) with diverse aliphatic and aromatic aldehydes (Scheme 1.). Among nineteen hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid seven compounds are registered in CAS database (**12**, **13**, **15 - 19**). The rest of the compounds (**3-11**, **14**, **20**, **21**) are new in the literature and their chemical structures, physio-chemical properties and biological activity have not been reported so far. Chemical structures of all synthesized compounds were confirmed by spectral methods (^{1}H NMR and ^{13}C NMR).

In the case of the synthesis of hydrazide of 5-nitrofuran-2-carboxylic acid (**2**) on the ^{1}H NMR spectrum we observed characteristic signals which correspond to hydrazide moiety [-(C=O)-$NHNH_2$]. For the NH group we found singlet peak at δ 10.21 ppm and for the NH_2 group singlet peak appeared at δ 4.71 ppm. On ^{13}C NMR spectrum signal for carbonyl group was found at δ 156.1 ppm.

Hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid (**3-21**) possessed the following characteristic signals on the ^{1}H NMR spectra, which confirmed the successful conduction of condensation reactions: singlet for the NH group in the range of 10.94 – 12.54 ppm and signal for

the =CH group at δ 7.74 – 8.91 ppm. Signals for other aliphatic and aromatic fragments of synthesized molecules were observed at expected values of chemical shift. In case of ^{13}C NMR spectra signals for =CH and C=O groups appeared in the range of δ 152.3 – 156.6 ppm and 153.5 – 166.3 ppm, respectively.

3.2 In vitro antimicrobial assays

Our data presented in Table 1. indicated that newly synthesized compounds **2 – 21** showed interesting antimicrobial activity. Those substances exhibited comparable antibacterial effect against all reference Gram-positive bacteria. Their minimal inhibitory concentrations (MICs) were 0.24 – 500 μg/ml and minimal bactericidal concentrations (MBCs) ranged from 0.48 μg/ml to >1000 μg/ml. Compounds **12 – 20** exhibited the highest activity towards them (MIC = 0.48 – 500 μg/ml and MBC = 0.98 μg/ml – >1000 μg/ml). The most sensitive were staphylococci to **12 – 20**. Those were *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 6538, and from other bacteria – *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 10876. Compounds **12 – 20** showed very strong or strong bactericidal activity against the above bacteria (MIC = 0.48 – 15.62 μg/ml and MBC = 0.98 – 62.5 μg/ml, MBC/MIC = 1 – 4). Additionally, *S. epidermidis* ATCC 12228 was also very susceptible to hydrazide of 5-nitrofuran-2-carboxylic acid **2** and hydrazide-hydrazones **3 – 9** and **11** with (MIC = 0.24 – 7.81 μg/ml, MBC = 0.48 – 31.25 μg/ml). These compounds showed also bactericidal effect (MBC/MIC = 1 – 4) towards reference *S. epidermidis*. Among Gram-positive bacteria, *Micrococcus luteus* ATCC 10240 (MIC = 125 – 500 μg/ml, MBC = 250 – > 1000 μg/ml) was the least sensitive to substances **2 – 21**.

Among newly synthesized hydrazide-hydrazones, compounds **3 – 7** showed also good, strong and even very strong bactericidal activity against Gram-negative bacteria from *Enterobacteriaceae* family, with MIC = 7.81 – 62.5 μg/ml, MBC = 15.62 – 125 μg/ml and MBC/MIC ≤ 4. The activity of other substances towards *Enterobacteriaceae,* namely **8 – 10**, **14 – 16** and **19 – 20** was lower (MIC = 125 – 500 μg/ml, MBC = 125 – > 1000 μg/ml). Moreover, some of them: **11 – 13**, **17 – 18** and **21** were not active against these bacteria. In turn, the compounds (except substances **11** with 2-bromophenyl and **19** with 4-fluorophenyl substituent) showed especially mild or moderate effects towards *Bordetella bronchiseptica* ATCC 4617. Furthermore, hydrazide **2** and hydrazide-hydrazone with pentylidene substituent **6** exhibited some activity towards *Pseudomonas aeruginosa* ATCC 9027 (MIC = 500 – 1000 μg/ml, MBC = 500 – > 1000 μg/ml) and these microorganisms were the least sensitive to tested compounds.

New hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid **3 – 21** indicated also some anticandidal activity. The values of MICs of compound **20**, which contains the 2-iodophenyl substituent, were the highest (from 7.81 μg/ml to 15.62 μg/ml), indicating its strong or very strong activity against all reference *Candida* spp. Additionally, minimal fungicidal concentrations

(MFCs) of compound **20** ranged from 15.62 μg/ml to 31.25 μg/ml and this compound had fungicidal effect (MFC/MIC = 1 – 2). In the case of remaining compounds, antifungal activity was varied (MIC = 15.62 – 1000 μg/ml, MFC = 15.62 – >1000 μg/ml). Moreover, some of the compounds, had no any activity. This applies in particular to compounds **11**, **12** and **21** with 2-bromophenyl, 3-bromophenyl and 3-iodophenyl substituents, respectively. All reference strains were insensitive to them.

In comparison to the ampicillin, which was used as the reference substance the activity against *Bacillus subtilis* ATCC 6633 was the same as that of ampicillin (MIC = 62.5 μg/ml) for compounds **3**, **7** and **9** (MIC = 62.5 μg/ml). Hydrazide **2** and hydrazide-hydrazones **4** and **11** displayed two times higher activity (MIC = 31.25 μg/ml), hydrazide-hydrazones **6** and **19** were sixteen times more active (MIC = 3.91 μg/ml), whereas hydrazide-hydrazones with halogensubstitued phenyl **12** – **18** were thirty two times more active (MIC = 1.95 μg/ml) than ampicillin against this bacterium (MIC = 62.5 μg/ml). Compound **20** with 2-iodophenyl substituent was the most active. Its MIC value against *Bacillus subtilis* ATCC 6633 (MIC = 0.48 μg/ml) was 130 times lower than for ampicillin.

Antibacterial activity of hydrazide-hydrazone **7** against *Staphylococcus epidermidis* ATCC 12228 was the same as the activity of cefuroxime (MIC = 0.24 μg/ml). Towards *Bacillus subtilis* ATCC 6633 compounds **6** and **19** (MIC = 3.91 μg/ml), which contain pentylidene and 4-fluorophenyl substituents were four times more active than cefuroxime (MIC = 15.62 μg/ml), compounds **12** – **18** (MIC = 1.95 μg/ml) were eight times more active, whereas hydrazide-hydrazone **20** showed the highest activity and was thirty two times more active (MIC = 0.48 μg/ml). When comparing the activity towards *Bacillus cereus* ATCC 10876, substances with pentylidene **6**, 4-bromophenyl **13** and 4-fluorophenyl **19** substituents showed the activity (MIC = 31.25 μg/ml) equal to the activity of cefuroxime against this bacterium (MIC = 31.25 μg/ml). The MIC values of hydrazide-hydrazones with phenyl substituent substituted with chlorine at *meta*-position **15** and *para*- position **16** (MIC = 15.62 μg/ml) were two times lower than the MIC of cefuroxime and for hydrazide-hydrazone with 2-iodophenyl **20** it was four times lower (MIC = 7.81 μg/ml).

The activity of synthesized hydrazide **2** and hydrazide-hydrazones **3** – **21** in comparison with the activity of nitrofurantoin is as follows: against *Staphylococcus aureus* ATCC 6538 hydrazide **2** and hydrazide-hydrazones **6** and **12** – **18** showed the same MIC values as nitrofurantoin (MIC = 15.62 μg/ml) against this bacterium. Compound with 4-chlorophenyl substituent **16** was two times

more active (MIC = 7.81 μg/ml), hydrazide-hydrazone with 4-fluorophenyl substituent **19** was four time more active (MIC = 3.91 μg/ml) and the compound substituted with 2-iodophenyl **20** was eight times more active (MIC = 1.95 μg/ml) than nitrofurantoin. The MIC values of synthesized derivatives of 5-nitrofuran-2-carboxylic acid for hydrazide-hydrazones **11**, **13**, **15**, **16**, **17** and **18** (MIC = 7.81 μg/ml) against *Staphylococcus aureus* ATCC 43300 were equal to the MIC value of nitrofurantoin (MIC = 7.81 μg/ml). The compounds substituted with halogen at *ortho*- position **14** (chlorine) and **20** (iodine) (MIC = 3.91 μg/ml) were two times more potent than nitrofurantoin towards this bacterium. Towards *Staphylococcus epidermidis* ATCC 12228 hydrazide of 5-nitrofuran-2-carboxylic acid **2** and hydrazide-hydrazones **3**, **4**, **5**, **8** and **17** displayed activity equal to nitrofurantoin (MIC = 3.91 μg/ml), whereas compounds **11**, **15**, **18** and **21** (MIC = 1.95 μg/ml) were two times more potent than nitrofurantoin (MIC = 3.91 μg/ml) against this bacterium. The MIC values for compounds **6**, **13**, **14**, **16** (MIC = 0.98 μg/ml) and for compounds **12**, **19**, **20** (MIC = 0.48 μg/ml) were four and eight times lower, respectively, than the MIC values for nitrofurantoin (MIC = 3.91 μg/ml). Hydrazide-hydrazone with hexylidene moiety **7** (MIC = 0.24 μg/ml) proved to be the most active and showed the activity sixteen times higher than nitrofurantoin. In the case of the activity against *Bacillus subtilis* ATCC 6633 compounds **6** (substituted with pentylidene substituent) and **19** (substituted with 4-fluorophenyl) were active at the same level as nitrofurantoin (MIC = 3.91 μg/ml). Hydrazide-hydrazones with halogensubstituted phenyl **12 – 18** (MIC = 1.95 μg/ml) were two times more active, and compound **20** substituted with 2-iodophenyl (MIC = 0.48 μg/ml) was almost eight times more active than nitrofurantoin (MIC = 3.91 μg/ml) on the basis of their MIC values. Compound **20** showed also activity at the same level in comparison to the nitrofurantoin towards *Bacillus cereus* ATCC 10876 (MIC = 7.81 μg/ml). Hydrazide **2** showed activity equal to nitrofurantoin (MIC = 125 μg/ml) against Gram-negative strain *Bordetella bronchiseptica* ATCC 4617, whereas hydrazide-hydrazones with alkyl chain **6** (pentylidene) and **7** (hexylidene) (MIC = 7.81 μg/ml) were two times more active towards *Klebsiella pneumoniae* ATCC 13883 than nitrofurantoin (MIC = 15.62 μg/ml). Towards *Proteus mirabilis* ATCC 12453 compounds with short substituents **3**, **4**, **5** were as active as nitrofurantoin (MIC = 62.5 μg/ml), hydrazide **2** had its MIC values two times lower (MIC = 31.25 μg/ml) and compounds **6** and **7** had their MIC values four times lower (MIC = 15.62 μg/ml) than nitrofurantoin (MIC = 62.5 μg/ml). In comparison to nitrofurantoin compounds **3**, **4**, **5** against *Salmonella typhimurium* ATCC 14028 showed activity equal to nitrofurantoin (MIC = 31.25 μg/ml), hydrazide **2** and hydrazide-hydrazone **7** were two times more

active (MIC = 15.62 μg/ml) and compound **6** was four times more active (MIC = 7.81 μg/ml). The activity against *Escherichia coli* ATCC 25922 for compounds **2** and **6** was equal to the activity of nitrofurantoin (MIC = 7.81 μg/ml).

4. CONCLUSIONS

In this research we synthesized and tested a new series of hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid for *in vitro* bioactivity. Our antimicrobial activity assay results indicated that the newly synthesized compounds **3** - **21** showed high antibacterial activity, especially against Gram-positive bacteria. In the case of Gram-negative bacteria, the most active were compounds **3 – 7**. In turn, **20** was particularly effective against fungi belonging to yeasts from *Candida* spp. The hydrazide-hydrazones which contain alkyl chains as substituents (**3 – 10**) showed higher activity against Gram-negative bacteria than hydrazones with aryl substituents (**11 – 21**), with the exception of heptylidene (**8**), octylidene (**9**) and nonylidene (**10**) substituents. Whereas hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid with halogensubstituted phenyl as substituents (**11 – 21**) inhibited the growth of Gram-positive bacterial strains and fungal *Candida* spp. species more efficiently than hydrazones with alkyl chains substituents (**3 – 10**). Performed structure-activity analysis suggests that synthesized hydrazide-hydrazones may be regarded as promising antimicrobial agents what specifically applies to hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid with short alkyl chains and halogensubstituted phenyl at *ortho*-position, especially with iodine atom.

CONFILCT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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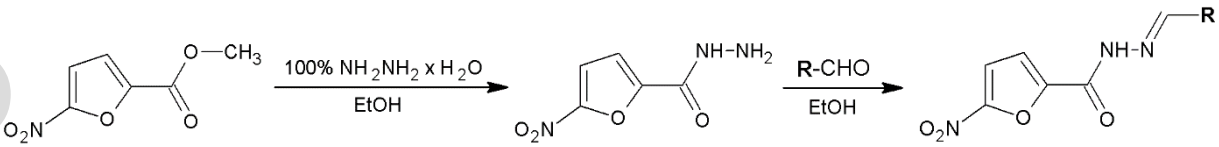
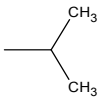
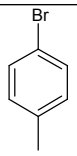
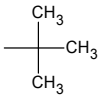
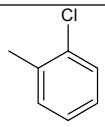
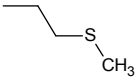
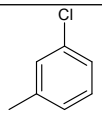
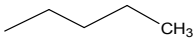
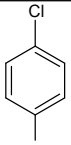
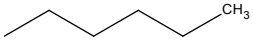
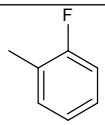
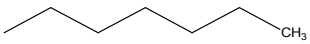
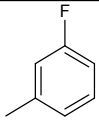
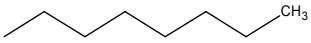
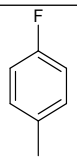
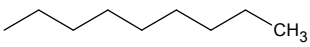
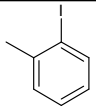
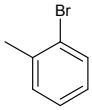
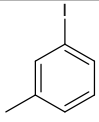
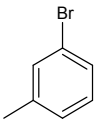


(**1**) (**2**) (**3 - 21**)

Compound number	R	Yield (%)	Compound number	R	Yield (%)
3		10	**13**		68
4		72	**14**		79
5		47	**15**		67
6		14	**16**		87
7		37	**17**		83
8		45	**18**		93
9		19	**19**		71
10		24	**20**		71
11		81	**21**		76

12		91

Scheme 1. Synthesis of new hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid

Table 1. The activity data of compounds 2 – 21 expressed as MIC (MBC or MFC) [µg/ml] and {MBC/MIC or MFC/MIC} against the reference strains of bacteria and fungi

	Species	MIC (MBC or MFC) [µg/ml] and {MBC/MIC or MFC/MIC} of the tested compounds																			CIP/ NY*	NIT	CFX	APC
		2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**				
Gram-positive bacteria	*Staphylococcus aureus* ATCC 25923	62.5 (62.5) {**1**}	62.5 (125) {**2**}	62.5 (125) {**2**}	125 (125) {**1**}	62.5 (62.5) {**1**}	125 (125) {**1**}	250 (500) {**2**}	250 (250) {**1**}	250 (250) {**1**}	125 (500) {**4**}	250 (1000) {**4**}	62.5 (62.5) {**1**}	62.5 (125) {**2**}	31.25 (62.5) {**2**}	31.25 (125) {**4**}	62.5 (62.5) {**1**}	31.25 (62.5) {**2**}	62.5 (62.5) {**1**}	31.25 (62.5) {**2**}	250 (>1000) {>4}	0.48 (0.48)	15.62 (15.62)	0.49	nd
	Staphylococcus aureus ATCC 6538	15.62 (31.25) {**2**}	62.5 (62.5) {**1**}	31.25 (62.5) {**2**}	31.25 (62.5) {**2**}	15.62 (31.25) {**2**}	31.25 (62.5) {**2**}	62.5 (250) {**4**}	62.5 (125) {**2**}	125 (250) {**2**}	31.25 (62.5) {**2**}	15.62 (31.25) {**2**}	15.62 (15.62) {**1**}	15.62 (15.62) {**1**}	15.62 (62.5) {**4**}	7.81 (15.62) {**2**}	15.62 (31.25) {**2**}	15.62 (15.62) {**1**}	3.91 (15.62) {**4**}	1.95 (3.91) {**2**}	125 (>1000) {>8}	0.24 (0.24)	15.62 (15.62)	0.98	nd
	Staphylococcus aureus ATCC 43300	31.25 (31.25) {**1**}	62.5 (62.5) {**1**}	15.62 (31.25) {**2**}	62.5 (62.5) {**1**}	15.62 (31.25) {**2**}	15.62 (62.5) {**4**}	125 (250) {**2**}	125 (125) {**1**}	250 (250) {**1**}	7.81 (7.81) {**1**}	15.62 (62.5) {**4**}	7.81 (7.81) {**1**}	3.91 (7.81) {**2**}	7.81 (15.62) {**2**}	7.81 (7.81) {**1**}	7.81 (15.62) {**2**}	7.81 (15.62) {**2**}	15.62 (31.25) {**2**}	3.91 (7.81) {**2**}	250 (1000) {**4**}	0.24 (0.24)	7.81 (15.62)	nd	nd
	Staphylococcus epidermidis ATCC 12228	3.91 (3.91) {**1**}	3.91 (3.91) {**1**}	3.91 (7.81) {**2**}	3.91 (15.62) {**4**}	0.98 (1.95) {**2**}	0.24 (0.48) {**2**}	3.91 (15.62) {**4**}	7.81 (31.25) {**4**}	31.25 (31.25) {**1**}	1.95 (7.81) {**4**}	0.48 (1.95) {**4**}	0.98 (3.91) {**4**}	0.98 (3.91) {**4**}	1.95 (7.81) {**4**}	0.98 (3.91) {**4**}	3.91 (7.81) {**2**}	1.95 (7.81) {**4**}	0.48 (1.95) {**4**}	0.48 (1.95) {**4**}	1.95 (3.91) {**2**}	0.12 (0.12)	3.91 (7.81)	0.24	nd
	Micrococcus luteus ATCC 10240	125 (250) {>2}	250 (500) {>2}	250 (500) {>2}	250 (500) {>2}	125 (250) {>2}	250 (500) {>2}	500 (500) {**1**}	500 (1000) {**4**}	500 (>1000) {>2}	250 (>1000) {>4}	125 (>1000) {>8}	125 (>1000) {>8}	31.25 (>1000) {>32}	500 (1000) {**2**}	500 (>1000) {>2}	125 (>1000) {>8}	500 (>1000) {>2}	250 (>1000) {>4}	250 (>1000) {>4}	500 (>1000) {>2}	0.98 (1.98)	62.5 (62.5)	0.98	nd
	Bacillus subtilis ATCC 6633	31.25 (62.5) {**2**}	62.5 (62.5) {**1**}	31.25 (62.5) {**2**}	125 (125) {**1**}	3.91 (7.81) {**2**}	62.5 (62.5) {**1**}	125 (125) {**1**}	62.5 (62.5) {**1**}	125 (125) {**1**}	31.25 (31.25) {**1**}	1.95 (3.91) {**2**}	1.95 (3.91) {**2**}	1.95 (3.91) {**2**}	1.95 (3.91) {**2**}	1.95 (7.81) {**4**}	1.95 (3.91) {**2**}	1.95 (3.91) {**2**}	3.91 (7.81) {**2**}	0.48 (0.98) {**2**}	250 (>1000) {>4}	0.03 (0.03)	3.91 (3.91)	15.62	62.5
	Bacillus cereus ATCC 10876	62.5 (62.5) {**1**}	125 (250) {**2**}	62.5 (125) {**2**}	125 (500) {**4**}	31.25 (62.5) {**2**}	125 (125) {**1**}	250 (250) {**1**}	125 (250) {**2**}	62.5 (250) {**4**}	125 (250) {**2**}	125 (>1000) {>8}	31.25 (250) {8}	62.5 (62.5) {**1**}	15.62 (62.5) {**4**}	15.62 (31.25) {**2**}	62.5 (125) {**2**}	62.5 (125) {**2**}	31.25 (125) {**4**}	7.81 (15.62) {**2**}	250 (>1000) {>4}	0.06 (0.12)	7.81 (15.62)	31.25	nd
	Bordetella bronchiseptica ATCC 4617	125 (250) {**2**}	250 (500) {**2**}	250 (500) {**2**}	250 (1000) {**4**}	250 (500) {**2**}	500 (1000) {**2**}	1000 (>1000) {>1}	500 (>1000) {>2}	1000 (>1000) {>1}	-	1000 (>1000) {>1}	1000 (>1000) {>1}	250 (>1000) {>4}	1000 (>1000) {>1}	1000 (>1000) {>1}	1000 (>1000) {>1}	1000 (>1000) {>1}	-	250 (>1000) {>4}	1000 (>1000) {>1}	0.98 (0.98)	125 (>1000)	nd	nd
	Klebsiella pneumoniae ATCC 13883	31.25 (31.25) {**1**}	31.25 (31.25) {**1**}	31.25 (62.5) {**2**}	62.5 (62.5) {**1**}	7.81 (15.62) {**2**}	7.81 (15.62) {**2**}	250 (1000) {**4**}	250 (250) {**1**}	500 (500) {**1**}	-	-	-	500 (1000) {**2**}	500 (500) {**1**}	125 (>1000) {>8}	-	-	250 (250) {**1**}	500 (1000) {**2**}	-	0.12 (0.12)	15.62 (31.25)	nd	nd
	Proteus mirabilis ATCC 12453	31.25 (62.5) {**2**}	62.5 (62.5) {**1**}	62.5 (125) {**2**}	62.5 (125) {**2**}	15.62 (31.25) {**2**}	15.62 (31.25) {**2**}	250 (1000) {**4**}	250 (500) {**2**}	500 (1000) {**2**}	-	-	-	500 (1000) {**2**}	500 (500) {**1**}	125 (>1000) {>8}	-	-	250 (500) {**2**}	500 (500) {**1**}	-	0.03 (0.03)	62.5 (125)	nd	nd
	Salmonella typhimurium ATCC 14028	15.62 (31.25) {**2**}	31.25 (125) {**4**}	31.25 (125) {**4**}	31.25 (62.5) {**2**}	7.81 (31.25 {**4**}	15.62 (62.5) {**4**}	250 (500) {**2**}	125 (250) {**2**}	500 (500) {**1**}	-	-	-	500 (1000) {**2**}	250 (1000) {**4**}	250 (>1000) {>4}	-	-	250 (250) {**1**}	500 (1000) {**2**}	-	0.06 (0.06)	31.25 (62.5)	nd	nd
	Escherichia coli ATCC 25922	7.81 (15.62) {**2**}	31.25 (125.5) {**4**}	31.25 (125) {**4**}	31.25 (31.25) {**1**}	7.81 (15.62) {**2**}	31.25 (31.25) {**1**}	125 (125) {**1**}	125 (125) {**1**}	500 (500) {**1**}	-	-	-	500 (500) {**1**}	500 (1000) {**2**}	500 (>1000) {>2}	-	-	125 (250) {**2**}	500 (1000) {**2**}	-	0.004 (0.004)	7.81 (15.62)	nd	nd
	Pseudomonas aeruginosa	500 (500)	-	-	-	1000 (>1000)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.48 (0.98)	-	nd	nd

	ATCC 9027	{**1**}				{>1}																		
Fungi	*Candida albicans* ATCC 2091	62.5 (250) {**4**}	1000 (>1000) {>1}	125 (500) {**2**]	250 (500) {**2**}	62.5 (250) {**4**}	125 (250) {**2**}	62.5 (250) {4}	125 (1000) {**4**}	250 (>1000) {>4}	-	-	125 (>1000) {>8}	500 (>1000) {>2}	15.62 (250) {16}	15.62 (500) {32}	500 (>1000) {>2}	125 (>1000) {>8}	31.25 (250) {8}	15.62 (15.62) {**1**}	-	0.24* (0.24)	na	na	na
	Candida albicans ATCC 10231	125 (500) {**4**}	1000 (>1000) {>1}	250 (1000) {**4**}	250 (1000) {**4**}	125 (500) {**4**}	250 (500) {**2**}	250 (250) {**1**}	125 (500) {**4**}	500 (>1000) {>2}	-	-	62.5 (250) {**4**}	-	31.25 (250) {8}	15.62 (15.62) {**1**}	-	250 (>1000) {>4}	31.25 (250) {8}	7.81 (15.62) {**2**}	-	0.48* (0.48)	na	na	na
	Candida parapsilosis ATCC 22019	1000 (>1000) {>1}	-	1000 (>1000) {>1}	1000 (>1000) {>1}	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {**2**}	-	-	1000 (>1000) {>1}	-	500 (>1000) {>2}	-	-	-	250 (>1000) {>4}	15.62 (31.25) {**2**}	-	0.24* (0.48)	na	na	na
	Candida glabrata ATCC 90030	1000 (>1000) {>1}	-	1000 (>1000) {>1}	1000 (>1000) {>1}	250 (500) {**2**}	500 (500) {**1**}	500 (>1000) {>2}	500 (>1000) {>2}	-	-	-	-	250 (>1000) {>4}	500 (>1000) {>2}	-	1000 (>1000) {>1}	-	250 (>1000) {>4}	7.81 (15.62) {**2**}	-	0.24* (0.48)	na	na	na
	Candida krusei ATCC 14243	500 (1000) {**2**}	1000 (>1000) {>1}	1000 (>1000) {>1}	500 (>1000) {>2}	250 (500) {**2**}	250 (500) {**2**}	500 (>1000) {>2}	250 (>1000) {>4}	-	-	-	250 (>1000) {>4}	250 (>1000) {>4}	62.5 (1000) {16}	15.62 (31.25) {**2**}	1000 (>1000) {>1}	31.25 (>1000) {>32}	15.62 (500) {32}	15.62 (15.62) {**1**}	-	0.24* (0.24)	na	na	na

"-" – no activity; nd – not determined; na – not applicable; The standard chemotherapeutics used as positive controls: ciprofloxacin (CIP), nitrofurantoin (NIT), cefuroxime (CFX), ampicillin (APC) for bacteria and nystatin (NY*) for fungi. Compounds with bactericidal effect (MBC/MIC ≤ 4) or fungicidal effect (MFC/MIC ≤ 4), MBC/MIC or MFC/MIC in these cases is bolded. The standard antibiotics used as positive controls: ciprofloxacin (CIP) for bacteria and nystatin (NY*) for fungi; "-" no bioactivity