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Design, synthesis and *in vitro* antimicrobial activity of hydrazide-hydrazones of 2-substituted acetic acid

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KEYWORDS

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ABSTRACT

In this study 30 hydrazide-hydrazones of phenylacetic (**3**-**10**) and hydroxyacetic acid (**11**-**32**) were synthesized by the condensation reaction of appropriate 2-substituted acetic acid hydrazide with different aromatic aldehydes. The obtained compounds were characterized by spectral data and evaluated *in vitro* for their potential antimicrobial activities against a panel of reference strains of microorganisms, including Gram-positive bacteria, Gram-negative bacteria and fungi belonging to the *Candida* spp. The results from our antimicrobial assays indicated that among synthesized compounds **3**-**32**, especially compounds **6**, **14** and **26** showed high bactericidal activity (MIC = 0.488 – 7.81 μg/ml) against reference Gram-positive bacteria and in some cases their activity was even better than that of commonly used antibiotics, like cefuroxime or ampicillin.

INTRODUCTION

The treatment of bacterial and fungal infectious diseases remains a challenging problem because of the increasing number of multi-drug resistant pathogens. Despite the fact that a wide variety of drugs are being used in dealing with infections, still there is a need for the development of new effective antibacterial and antifungal agents [1].

Hydrazide-hydrazone compounds are not only intermediates in synthesis and drug development but they are also very effective organic compounds on their own [2, 3]. It is well known from the literature that this class of compounds possesses diverse biological and pharmacological properties, such as antiviral [4], anti-inflammatory [5, 6], antiamoebic [7], antiprotozoal [8] and anticancer activities [9-12]. The hydrazide-hydrazone derivatives also play an essential role as antimicrobial agents by virtue of their highly reactive azomethine group (-NH-N=CH-) in their structure [13-19].

Isoniazid (isonicotinic acid hydrazide) is characterized by a very high antitubercular activity towards *Mycobacterium tuberculosis* [20, 21]. Maccari et al. and Coelho et al. synthesized new isoniazid hydrazide-hydrazones by reacting isonicotinic acid hydrazide with various aldehydes and ketones [22, 23]. Antimicrobial activity assays revealed that these compounds had significant inhibitory activity against various strains of *M. tuberculosis*, including isoniazid-resistant strains [22, 23]. Buu-Hoi et al. synthesized some hydrazide-hydrazones with antitubercular activity that were reported to have lower toxicity than hydrazides because of the blockage of NH_2 group [24].

Recently we reported interesting results concerning the synthesis and antibacterial activity of hydrazide-hydrazones [25-27]. It is worth to stress that in our previous research hydrazide-hydrazones of substituted benzoic acids showed significant activity against Gram-postitive bacteria [28]. Especially *N*-[((2-hydroxy-3,5-diiodophenyl)methylidene]-3-methoxybenzhydrazide displayed activity much higher than commonly used chemotherapeutics against *Bacillus* spp. [28]. The above mentioned findings further support the growing importance of the synthesis of hydrazide-hydrazones derivatives.

Encouraged by above facts and in continuation of our work in describing novel biologically active compounds, we hereby report the synthesis, spectral studies and antimicrobial evaluation of new hydrazide-hydrazone derivatives of 2-substituted acetic acids.

MATERIALS AND METHODS

Chemistry

All required chemicals and solvents were purchased from Sigma-Aldrich (Munich, Germany) and Merck Co. (Darmstadt, Germany) and used without further purification. Melting points were determined with Fisher-Johns blocks (Fisher Scientific, Germany) and are 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. The chemical shifts are expressed on the δ (ppm) scale with the use of TMS as the standard reference. The coupling constants (*J*) are given in Hertz. Spin multiplet are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and b (broad). The progress of the reaction and purity of obtained compounds were monitored by TLC using precoated 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 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. ClogP values of synthesized compounds were calculated using HyperChem^{TM} 8.0.8 [29].

Preparation of 2-phenylacetic acid hydrazide (**1**)

The compound was prepared according to a procedure from the literature [30].

CAS Registry Number: 937-39-3. Analytical and spectral data is consistent with that reported in the literature [30]. Yield: 83%; m.p.: 116-117°C. ClogP = 0.80; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.49 (s, 2H, CH_2), 3.76 (s, 2H, NH_2), 7.29-7.43 (m, 5H, ArH), 8.19 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 40.1 (CH_2), 128.4, 129.2, 129.4, 134.8 ($6C_{ar}$), 173.9 (C=O). Analysis for $C_8H_{10}N_2O$ (150.18) Calculated: C: 63.98%, H: 6.71%, N: 18.65%; Found: C: 63.94%, H: 6.73%, N: 18.70%.

Preparation of 2-hydroxyacetic acid hydrazide (**2**)

The compound was prepared according to a procedure from the literature [31].

CAS Registry Number: 3530-14-1. Analytical and spectral data is consistent with those reported in the literature [31]. Yield: 63%; m.p.: 122-123°C. ClogP = -1.60. ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.90 (s, 2H, NH_2), 4.21 (s, 2H, CH_2), 5.30 (s, 1H, OH), 8.84 (s, 1H,

NH); ^{13}C NMR (DMSO) δ (ppm) = 61.2 (CH_2), 171.0 (C=O). Analysis for $C_2H_6N_2O_2$ (90.08) Calculated: C: 26.67%, H: 6.71%, N: 31.10%; Found: C: 26.71%, H: 6.68%, N: 31.14%.

Preparation of hydrazide-hydrazones (3-32)

General procedure

The solution of 0.01 mole of adequate 2-substituted acetic acid hydrazide (**1** or **2**) and appropriate substituted aromatic aldehyde (0.011 mole) was heated under reflux for 3h. After that it was cooled and the formed precipitate was filtered off and recrystallized from ethanol.

2-phenyl-*N*-[1*H*-pyrrol-2-ylmethylidene]acethydrazide (**3**)

CAS Registry Number: 67973-81-3. Yield: 80%; m.p.: 210-212°C; ClogP = 2.13; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.98 (s, 2H, CH_2), 6.10-6.14 (m, 2H, ArH), 6.41-6.45 (m, 2H, ArH), 6.94-6.97 (m, 1H, ArH), 7.23-7.35 (m, 2H, ArH), 7.83 (s, 1H, ArH), 8.06 (s, 1H, =CH), 11.07 (s, 1H, NH), 11.27 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 40.9 (CH_2), 112.85. 120.8, 128.4, 129.2, 129.4, 134.8 ($8C_{ar}$), 135.0 (=CH), 144.9, 148.3 ($2C_{ar}$), 168.3 (C=O). Analysis for $C_{13}H_{13}N_3O$ (227.26) Calculated: C: 68.70%, H: 5.77%, N: 18.49%; Found: C: 68.81%, H: 5.73%, N: 18.53%.

N-[(5-chlorofuran-2-yl)methylidene]-2-phenylacethydrazide (**4**)

CAS Registry Number: 406702-39-4. Yield: 80%; m.p.: 150-152°C; ClogP = 2.44; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.91 (s, 2H, CH_2), 6.64-6.66 (m, 2H, ArH), 6.97-6.98 (m, 2H, ArH), 7.28-7.33 (m, 2H, ArH), 7.81 (s, 1H, ArH), 8.03 (s, 1H, =CH), 11.40 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 27.5 (CH_2), 109.8, 115.7, 116.3, 126.9, 128.4, 128.8, 129.5, 130.0 ($8C_{ar}$), 135.9 (=CH), 149.6, 167.1 ($2C_{ar}$), 172.7 (C=O). Analysis for $C_{13}H_{11}ClN_2O_2$ (262.69) Calculated: C: 59.44%, H: 4.22%, N: 10.66%; Found: C: 59.49%, H: 4.26%, N: 10.62%.

N-[(5-iodofuran-2-yl)methylidene]-2-phenylacethydrazide (**5**)

CAS Registry Number: 343592-07-4. Yield: 79%; m.p.: 157-159°C; ClogP = 2.74; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.91 (s, 2H, CH_2), 6.81-6.85 (m, 2H, ArH), 7.25-7.33 (m, 4H, ArH), 7.81 (s, 1H, ArH), 8.00 (s, 1H, =CH), 11.38 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 28.1 (CH_2), 115.2, 116.7, 126.9, 128.6, 128.8, 129.5, 130.0 ($7C_{ar}$), 136.0 (=CH), 154.6, 154.7, 167.1 ($3C_{ar}$), 172.6 (C=O). Analysis for $C_{13}H_{11}IN_2O_2$ (354.14) Calculated: C: 44.09%, H: 3.13%, N: 7.91%; Found: C: 44.12%, H: 3.15%, N: 7.88%.

N-[(5-nitrofuran-2-yl)methylidene]-2-phenylacethydrazide (**6**)

CAS Registry Number: 324577-71-1. Yield: 93%; m.p.: 186-188°C; ClogP = 0.27; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.96 (s, 2H, CH_2), 7.21-7.36 (m, 4H, ArH), 7.77-7.81 (m, 2H, ArH), 7.94 (s, 1H, ArH), 8.19 (s, 1H, =CH), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 27.9 (CH_2), 115.3, 126.9, 127.2, 128.7, 128.9, 129.6, 130.1, 131.4 ($8C_{ar}$), 135.6 (=CH), 152.2, 168.3 ($2C_{ar}$), 173.2 (C=O). Analysis for $C_{13}H_{11}N_3O_4$ (273.24) Calculated: C: 57.14%, H: 4.06%, N: 15.38%; Found: C: 57.19%, H: 4.02%, N: 15.42%.

N-[(2-bromo-5-fluorophenyl)methylidene]-2-phenylacethydrazide (**7**)

Yield: 58%; m.p.: 161-163°C; ClogP = 4.29; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.02 (s, 2H, CH_2), 7.20-7.63 (m, 4H, ArH), 7.71-7.73 (m, 1H, ArH), 7.74-7.77 (m, 2H, ArH), 8.29-8.30 (d, 1H, ArH, *J* = 3 Hz), 8.53 (s, 1H, =CH), 11.69 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 27.6 (CH_2), 118.0, 118.3, 118.8, 119.2, 126.8, 127.2, 128.8, 129.6, 129.9 ($10C_{ar}$), 135.5 (=CH), 136.1, 167.3 ($2C_{ar}$), 173.1 (C=O). Analysis for $C_{15}H_{12}BrFN_2O$ (335.17) Calculated: C: 53.75%, H: 3.61%, N: 8.36%; Found: C: 53.81%, H: 3.60%, N: 8.39%.

N-[(2-chloro-3-methoxyphenyl)methylidene]-2-phenylacethydrazide (**8**)

Yield: 95%; m.p.: 197-199°C; ClogP = 3.62; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.88 (s, 3H, CH_3), 3.99 (s, 2H, CH_2), 7.18-7.35 (m, 4H, ArH), 7.37-7.39 (m, 1H, ArH), 7.50-7.53 (m, 1H, ArH), 7.58-7.61 (m, 2H, ArH), 8.42 (s, 1H, =CH), 11.60 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 27.9 (CH_2), 56.8 (CH_3), 113.4, 121.5, 124.1, 126.8, 128.4, 129.2, 129.4, 135.0 ($11C_{ar}$), 150.00 (=CH), 157.6 (C_{ar}), 168.3 (C=O). Analysis for $C_{16}H_{15}ClN_2O_2$ (302.76) Calculated: C: 63.47%, H: 4.99%, N: 9.25%; Found: C: 63.51%, H: 4.97%, N: 9.28%.

N-[(2-chloro-5-nitrophenyl)methylidene]-2-phenylacethydrazide (**9**)

CAS Registry Number: 349457-86-9. Yield: 90%; m.p.: 193-195°C; ClogP = -1.53; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.03 (s, 2H, CH_2), 7.20-7.29 (m, 1H, ArH), 7.31-7.34 (m, 1H, ArH), 7.81-7.82 (d, 1H, ArH, *J* = 3 Hz), 7.84-7.85 (d, 1H, ArH, *J* = 3 Hz), 8.20-8.25 (m, 2H, ArH), 8.38 (s, 1H, =CH), 8.62-8.63 (d, 1H, ArH, *J* = 3 Hz), 8.66-8.67 (d, 1H, ArH, *J* = 3 Hz), 11.79 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 27.8 (CH_2), 122.0, 124.7, 128.2, 129.2, 129.4, 134.7, 135.5, 139.1, 145.9 ($10C_{ar}$), 148.2 (=CH), 155.1, 159.4 ($2C_{ar}$), 168.3 (C=O). Analysis for $C_{15}H_{12}ClN_3O_3$ (317.73) Calculated: C: 56.70%, H: 3.81%, N: 13.23%; Found: C: 56.74%, H: 3.83%, N: 13.20%.

N-[(4-hydroxy-3,5-dimethoxyphenyl)methylidene]-2-phenylacethydrazide (**10**)

CAS Registry Number: 341973-80-6. Yield: 91%; m.p.: 156-158°C; ClogP = 2.57; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.79 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 6.94 (s, 2H, ArH), 7.18-7.33 (m, 4H, ArH), 7.85 (s, 1H, ArH), 8.09 (s, 1H, =CH), 8.87 (s, 1H, OH), 11.29 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 28.9 (CH_2), 56.8 (2xCH_3), 107.4, 128.1, 128.4, 129.2, 129.4, 134.8, 137.4 (10C_{ar}), 147.0 (=CH), 148.6 (2C_{ar}), 168.3 (C=O). Analysis for $C_{17}H_{18}N_2O_4$ (314.34) Calculated: C: 64.96%, H: 5.77%, N: 8.91%; Found: C: 64.99%, H: 5.74%, N: 8.94%.

2-hydroxy-*N*-[1*H*-pyrrol-2-ylmethylidene]acethydrazide (**11**)

Yield: 46%; m.p.: 186-188°C; ClogP = -0.27; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.92 (s, 2H, CH_2), 5.49 (s, 1H, OH), 6.10-6.13 (m, 1H, ArH), 6.39-6.41 (m, 1H, ArH), 6.87-6.90 (m, 1H, ArH), 8.20 (s, 1H, =CH), 10.90 (s, 1H, NH), 11.45 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.7 (CH_2), 109.6, 113.6, 122.8, 127.5 (4C_{ar}), 140.9 (=CH), 168.0 (C=O). Analysis for $C_7H_9N_3O_2$ (167.16) Calculated: C: 50.29%, H: 5.43%, N: 25.14%; Found: C: 50.33%, H: 5.41%, N: 25.18%.

N-[(5-chlorofuran-2-yl)methylidene]-2-hydroxyacethydrazide (**12**)

CAS Registry Number: 1883135-55-4. Yield: 47%; m.p.: 150-152°C; ClogP = 0.04; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.97 (s, 2H, CH_2), 5.63 (s, 1H, OH), 6.65-6.66 (d, 1H, ArH, *J* = 3 Hz), 6.95-6.96 (d, 1H, ArH, *J* = 3 Hz), 8.22 (s, 1H, =CH), 11.31 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.7 (CH_2), 109.9, 116.1, 136.6, 137.6 (4C_{ar}), 149.7 (=CH), 168.9 (C=O). Analysis for $C_7H_7ClN_2O_3$ (202.59) Calculated: C: 41.50%, H: 3.48%, N: 13.83%; Found: C: 41.54%, H: 3.49%, N: 13.85%.

2-hydroxy-*N*-[(5-iodofuran-2-yl)methylidene]acethydrazide (**13**)

Yield: 67%; m.p.: 177-179°C; ClogP = 0.34; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.97 (s, 2H, CH_2), 5.63 (s, 1H, OH), 6.79-6.80 (d, 1H, ArH, *J* = 3 Hz), 6.83-6.85 (m, 1H, ArH), 8.20 (s, 1H, =CH), 11.21 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.7 (CH_2), 96.4, 116.5, 122.7, 136.4 (4C_{ar}), 154.8 (=CH), 168.9 (C=O). Analysis for $C_7H_7IN_2O_3$ (294.05) Calculated: C: 28.59%, H: 2.40%, N: 9.53%; Found: C: 28.65%, H: 2.38%, N: 9.56%.

2-hydroxy-*N*-[(5-nitrofuran-2-yl)methylidene]acethydrazide (**14**)

CAS Registry Number: 89792-34-7. Yield: 96%; m.p.: 200-202°C; ClogP = -2.13; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.03 (s, 2H, CH_2), 5.72 (s, 1H, OH), 7.21-7.22 (d, 1H, ArH, *J* = 3 Hz), 7.78-7.79 (d, 1H, ArH, *J* = 3 Hz), 8.38 (s, 1H, =CH), 11.73 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.9 (CH_2), 114.8, 116.2, 135.3, 151.7 ($4C_{ar}$), 153.1 (=CH), 168.7 (C=O). Analysis for $C_7H_7N_3O_5$ (213.15) Calculated: C: 39.44%, H: 3.31%, N: 19.71%; Found: C: 39.49%, H: 3.29%, N: 19.75%.

N-[(2-bromo-5-fluorophenyl)methylidene]-2-hydroxyacethydrazide (**15**)

Yield: 70%; m.p.: 187-189°C; ClogP = 1.89; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.02 (s, 2H, CH_2), 5.64 (s, 1H, OH), 7.21-7.30 (m, 1H, ArH), 7.61-7.68 (m, 1H, ArH), 7.70-7.76 (m, 1H, ArH), 8.74 (s, 1H, =CH), 11.63 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.8 (CH_2), 118.5, 119.5, 135.4, 140.9 ($4C_{ar}$), 145.2 (=CH), 160.3, 163.5 ($2C_{ar}$), 169.3 (C=O). Analysis for $C_9H_8BrFN_2O_2$ (275.07) Calculated: C: 39.30%, H: 2.93%, N: 10.18%; Found: C: 39.35%, H: 2.90%, N: 10.21%.

N-[(2-chloro-3-methoxyphenyl)methylidene]-2-hydroxyacethydrazide (**16**)

Yield: 77%; m.p.: 165-167°C; ClogP = 1.22; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.88 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 5.61 (s, 1H, OH), 7.16-7.20 (m, 1H, ArH), 7.31-7.38 (m, 1H, ArH), 7.48-7.55 (m, 1H, ArH), 8.83 (s, 1H, =CH), 11.58 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 56.8 (CH_3), 61.7 (CH_2), 113.9, 118.7, 121.8, 133.4, 144.0 ($5C_{ar}$), 144.2 (=CH), 155.4 (C_{ar}), 169.1 (C=O). Analysis for $C_{10}H_{11}ClN_2O_3$ (242.66) Calculated: C: 49.50%, H: 4.57%, N: 11.54%; Found: C: 49.50%, H: 4.59%, N: 11.52%.

N-[(2-chloro-5-nitrophenyl)methylidene]-2-hydroxyacethydrazide (**17**)

Yield: 95%; m.p.: 232-233°C; ClogP = -0.5; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.05 (s, 2H, CH_2), 5.70 (s, 1H, OH), 7.81-7.84 (d, 1H, ArH, *J* = 9 Hz), 8.19-8.25 (m, 1H, ArH), 8.64-8.65 (d, 1H, ArH, *J* = 9 Hz), 8.87 (s, 1H, =CH), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.8 (CH_2), 121.5, 125.6, 132.0, 133.7, 137.8, 142.0 ($6C_{ar}$), 147.1 (=CH), 169.5 (C=O). Analysis for $C_9H_8ClN_3O_4$ (257.63) Calculated: C: 41.96%, H: 3.13%, N: 16.31%; Found: C: 41.93%, H: 3.14%, N: 16.35%.

2-hydroxy-*N*-[(4-hydroxy-3,5-dimethoxyphenyl)methylidene]acethydrazide (**18**)
Yield: 61%; m.p.: 112-114°C; ClogP = -0.26; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.79 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 5.57 (s, 1H, OH), 6.91-6.92 (d, 2H, ArH, *J* = 3 Hz), 8.24 (s, 1H, =CH), 8.86 (s, 1H, OH), 11.04 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 56.5 (2xCH_3), 61.7 (CH_2), 104.7, 104.9, 124.8, 125.0, 138.3, 142. (6C_{ar}), 148.5 (=CH), 168.4 (C=O). Analysis for $C_{11}H_{14}N_2O_5$ (254.24) Calculated: C: 51.97%, H: 5.55%, N: 11.02%; Found: C: 51.95%, H: 5.57%, N: 11.06%.

N-[(3-chloro-4-methoxyphenyl)methylidene]-2-hydroxyacethydrazide (**19**)
Yield: 85%; m.p.: 231-232°C. ClogP = 1.22. ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.90 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 5.59 (s, 1H, OH), 7.18-7.24 (m, 1H, ArH), 7.56-7.62 (m, 1H, ArH), 7.73-7.76 (m, 1H, ArH), 8.30 (s, 1H, =CH), 11.20 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 56.7 (CH_3), 61.7 (CH_2), 113.4, 122.0, 128.0, 128.2, 128.4, 142.1 (6C_{ar}), 146.4 (=CH), 168.7 (C=O). Analysis for $C_{10}H_{11}ClN_2O_3$ (242.66) Calculated: C: 49.50%, H: 4.57%, N: 11.54%; Found: C: 49.55%, H: 4.55%, N: 11.58%.

N-[(3,5-dibromo-4-hydroxyphenyl)methylidene]-2-hydroxyacethydrazide (**20**)
Yield: 94%; m.p.: 258-260°C; ClogP = 2.26; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.98 (s, 2H, CH_2), 5.59 (s, 1H, OH), 7.82-7.83 (d, 2H, ArH, *J* = 3 Hz), 8.22 (s, 1H, =CH), 11.29 (s, 1H, OH), 11.40 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.7 (CH_2), 112.7, 129.3, 130.8, 131.0, 140.9 (5C_{ar}), 144.9 (=CH), 152.6 (C_{ar}), 168.8 (C=O). Analysis for $C_9H_8Br_2N_2O_3$ (351.98) Calculated: C: 30.71%, H: 2.29%, N: 7.96%; Found: C: 30.76%, H: 2.27%, N: 7.99%.

N-[(3-bromo-4-methoxyphenyl)methylidene]-2-hydroxyacethydrazide (**21**)
Yield: 94%; m.p.: 237-239°C; ClogP = 1.50; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.89 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 5.58 (s, 1H, OH), 7.14-7.20 (t, 1H, ArH, *J* = 9 Hz), 7.59-7.65 (m 1H, ArH), 7.87-7.89 (m, 1H, ArH), 8.29 (s, 1H, =CH), 11.20 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 56.9 (CH_3), 61.7 (CH_2), 111.6, 113.3, 128.7, 128.9, 130.9, 131.2 (6C_{ar}), 146.2 (=CH), 168.7 (C=O). Analysis for $C_{10}H_{11}BrN_2O_3$ (287.11) Calculated: C: 41.83%, H: 3.86%, N: 9.76%; Found: C: 41.87%, H: 3.84%, N: 9.79%.

N-[(3-bromo-4-hydroxyphenyl)methylidene]-2-hydroxyacethydrazide (**22**)
Yield: 40%; m.p.: 190-192°C; ClogP = 1.46; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.96 (s, 2H, CH_2), 5.57 (s, 1H, OH), 6.97-7.01 (m, 1H, ArH), 7.45-7.50 (m, 1H, ArH), 7.76-7.82 (m, 1H, ArH), 8.24 (s, 1H, =CH), 10.77 (s, 1H, OH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.7 (CH_2), 110.2, 116.9, 127.3, 127.5, 128.1, 131.7 ($6C_{ar}$), 146.6 (=CH), 168.6 (C=O). Analysis for $C_9H_9BrN_2O_3$ (273.08) Calculated: C: 39.58%, H: 3.32%, N: 10.26%; Found: C: 39.63%, H: 3.30%, N: 10.29%.

N-[(2-bromo-3-hydroxy-4-methoxyphenyl)methylidene]-2-hydroxyacethydrazide (**23**)
Yield: 65%; m.p.: 216-218°C; ClogP = 1.25; ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.87 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 5.56 (s, 1H, OH), 7.03-7.09 (m, 1H, ArH), 7.35-7.44 (m, 1H, ArH), 8.68 (s, 1H, =CH), 9.65 (s, 1H, OH), 11.43 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 56.7 (CH_3), 61.7 (CH_2), 111.4, 112.3, 118.1, 126.5, 143.1, 144.2 ($6C_{ar}$), 147.1 (=CH), 168.7 (C=O). Analysis for $C_{10}H_{11}BrN_2O_4$ (303.11) Calculated: C: 39.62%, H: 3.66%, N: 9.24%; Found: C: 39.66%, H: 3.64%, N: 9.26%.

N-[(2,3-difluorophenyl)methylidene]-2-hydroxyacethydrazide (**24**)
Yield: 51%; m.p.: 179-181°C; ClogP = 1.24; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.02 (s, 2H, CH_2), 5.64 (s, 1H, OH), 7.76-7.90 (m, 2H, ArH), 8.02-8.05 (m, 1H, ArH), 8.47 (s, 1H, =CH), 11.48 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.7 (CH_2), 118.8, 122.0, 124.7, 124.8, 125.6, 135.5 ($6C_{ar}$), 139.6 (=CH), 169.1 (C=O). Analysis for $C_9H_8F_2N_2O_2$ (214.17) Calculated: C: 50.47%, H: 3.77%, N: 13.08%; Found: C: 50.54%, H: 3.79%, N: 13.05%.

2-hydroxy-*N*-{[4-(trifluoromethyl)phenyl]methylidene}acethydrazide (**25**)
Yield: 74%; m.p.: 142-144°C; ClogP = 1.84; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.01 (s, 2H, CH_2), 5.90 (s, 1H, OH), 7.51-7.53 (d, 2H, ArH, J = 6 Hz), 7.65-7.67 (d, 2H, ArH, J = 6 Hz), 8.41 (s, 1H, =CH), 10.18 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.9 (CH_2), 124.5, 127.2, 129.5, 130.2, 135.9 ($6C_{ar}$), 148.6 (=CH), 168.7 (C=O). Analysis for $C_{10}H_9F_3N_2O_2$ (246.18) Calculated: C: 48.79%, H: 3.68%, N: 11.30%; Found: C: 48.83%, H: 3.70%, N: 11.33%.

2-hydroxy-*N*-[(2-hydroxy-3,5-diiodophenyl)methylidene]acethydrazide (**26**)
Yield: 94%; m.p.: 248-250°C; ClogP = 2.76; ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.06 (s, 2H, CH_2), 5.72 (s, 1H, OH), 7.77-7.78 (d, 1H, ArH, J = 3 Hz), 8.03-8.04 (d, 1H, ArH, J = 3 Hz),

8.42 (s, 1H, =CH), 11.99 (s, 1H, OH), 12.90 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.5 (CH_2), 82.5, 88.2, 120.7, 139.2, 146.8 ($5C_{ar}$), 147.6 (=CH), 157.0 (C_{ar}), 169.2 (C=O). Analysis for $C_9H_8I_2N_2O_3$ (445.98) Calculated: C: 24.24%, H: 1.81%, N: 6.28%; Found: C: 24.28%, H: 1.80%, N: 6.30%.

N-[(3-ethoxy-2-hydroxyphenyl)methylidene]-2-hydroxyacethydrazide (**27**)
Yield: 97%; m.p.: 207-209°C; ClogP = 0.76; ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.32-1.36 (t, 3H, CH_3, J = 6 Hz), 4.00-4.08 (q, 2H, CH_2, J = 9 Hz, J = 6 Hz), 4.03 (s, 2H, CH_2), 5.65 (s, 1H, OH), 6.77-6.85 (m, 1H, ArH), 6.96-7.05 (m, 1H, ArH), 7.18-7.20 (d, 1H, ArH, J = 6 Hz), 8.59 (s, 1H, =CH), 11.13 (s, 1H, OH), 11.50 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 13.8 (CH_3), 61.9 (CH_2), 64.5 (CH_2), 118.7, 120.1, 120.4, 122.2, 146.9, 147.9 ($6C_{ar}$), 149.4 (=CH), 168.7 (C=O). Analysis for $C_{11}H_{14}N_2O_4$ (238.24) Calculated: C: 55.46%, H: 5.92%, N: 11.76%; Found: C: 55.51%, H: 5.90%, N: 11.79%.

N-[(3-ethoxy-4-methoxyphenyl)methylidene]-2-hydroxyacethydrazide (**28**)
Yield: 61%; m.p.: 172-174°C; ClogP = 0.79; ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.33-1.37 (t, 3H, CH_3, J = 6 Hz), 3.80 (s, 3H, CH_3), 3.98 (s, 2H, CH_2), 4.01-4.08 (q, 2H, CH_2, J = 6 Hz, J = 9 Hz), 5.58 (s, 1H, OH), 6.97-7.02 (m, 1H, ArH), 7.12-7.14 (m, 1H, ArH), 7.23-7.26 (m, 1H, ArH), 8.28 (s, 1H, =CH), 11.08 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 13.8 (CH_3), 56.8 (CH_3), 61.9 (CH_2), 64.5 (CH_2), 111.9, 114.5, 121.8, 128.9 ($4C_{ar}$), 147.9 (=CH), 149.2, 151.7 ($2C_{ar}$), 168.7 (C=O). Analysis for $C_{12}H_{16}N_2O_4$ (252.27) Calculated: C: 57.13%, H: 6.39%, N: 11.10%; Found: C: 57.18%, H: 6.37%, N: 11.07%.

2-hydroxy-*N*-[(4-propoxyphenyl)methylidene]acethydrazide (**29**)
Yield: 81%; m.p.: 122-124°C; ClogP = 1.52; ^{1}H NMR (DMSO-d_6) δ (ppm) = 0.96-1.00 (t, 3H, CH_3, J = 6 Hz), 1.68-1.81 (m, 2H, CH_2), 3.94-3.98 (t, 2H, CH_2, J = 6 Hz), 4.00 (s, 2H, CH_2), 5.58 (s, 1H, OH), 6.96-7.06 (m, 2H, ArH), 7.55-7.60 (m, 2H, ArH), 8.65 (s, 1H, =CH), 11.06 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 10.6 (CH_3), 21.4 (CH_2), 61.9 (CH_2), 71.7 (CH_2), 115.1, 127.4, 128.8 ($5C_{ar}$), 148.6 (=CH), 159.5 (C_{ar}), 167.7 (C=O). Analysis for $C_{12}H_{16}N_2O_3$ (236.27) Calculated: C: 61.00%, H: 6.83%, N: 11.86%; Found: C: 61.05%, H: 6.85%, N: 11.84%.

2-hydroxy-*N*-{[4-(pyrrolidin-1-yl)phenyl]methylidene}acethydrazide (**30**)
Yield: 81%; m.p.: 256-258°C; ClogP = 1.55; ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.94-19.8 (m, 4H, 2x$CH_{2\text{-pyrrolidine}}$), 3.26-3.29 (m, 4H, 2x$CH_{2\text{-pyrrolidine}}$), 3.93 (s, 2H, CH_2), 5.53 (s, 1H, OH), 6.53-6.58 (m, 2H, ArH), 7.41-7.48 (m, 2H, ArH), 8.20 (s, 1H, =CH), 11.09 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 25.4 (2$C_{\text{pyrrolidine}}$), 47.7 (2$C_{\text{pyrrolidine}}$), 61.7 (CH_2), 111.9, 121.4, 128.6, 128.9, 144.9 (5C_{ar}), 148.9 (=CH), 149.3 (C_{ar}), 167.9 (C=O). Analysis for $C_{13}H_{17}N_3O_2$ (247.29) Calculated: C: 63.14%, H: 6.93%, N: 16.99%; Found: C: 63.17%, H: 6.91%, N: 17.02%.

2-hydroxy-*N*-{[4-(piperidin-1-yl)phenyl]methylidene}acethydrazide (**31**)
Yield: 76%; m.p.: 160-162°C; ClogP = 1.28; ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.53-1.58 (m, 6H, 3x$CH_{2\text{-piperidine}}$), 3.23-3.25 (m, 4H, 2x$CH_{2\text{-piperidine}}$), 3.94 (s, 2H, CH_2), 5.55 (s, 1H, OH), 6.91-6.99 (m, 2H, ArH), 7.43-7.49 (m, 2H, ArH), 8.23 (s, 1H, =CH), 10.93 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 23.4, 25.1, 49.7 (5$C_{\text{piperidine}}$), 61.9 (CH_2), 110.8, 124.6, 129.7 (5C_{ar}), 148.6 (=CH), 152.5 (C_{ar}), 168.7 (C=O). Analysis for $C_{14}H_{19}N_3O_2$ (261.32) Calculated: C: 64.35%, H: 7.33%, N: 16.08%; Found: C: 64.39%, H: 7.37%, N: 16.11%.

2-hydroxy-*N*-[quinolin-2-ylmethylidene]acethydrazide (**32**)
Yield: 87%; m.p.: 219-221°C. ClogP = 1.45. ^{1}H NMR (DMSO-d_6) δ (ppm) = 4.16 (CH_2), 5.73 (s, 1H, OH), 7.61-7.66 (m, 1H, ArH), 7.71-7.88 (m, 1H, ArH), 7.99-8.14 (m, 3H, ArH), 8.38-8.42 (m, 1H, ArH), 8.57 (s, 1H, =CH), 11.73 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) = 61.9 (CH_2), 117.9, 126.9, 127.6, 128.0, 129.7, 130.1, 136.3, 141.8 (8C_{ar}), 143.4 (=CH), 151.5 (C_{ar}), 168.7 (C=O). Analysis for $C_{12}H_{11}N_3O_2$ (229.23) Calculated: C: 62.87%, H: 4.84%, N: 18.33%; Found: C: 62.92%, H: 4.83%, N: 18.30%.

Microbiology

***In vitro* antimicrobial assay**

The examined compounds **3-32** were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [32] and Clinical and Laboratory Standards Institute guidelines [33] against a panel of reference and clinical or saprophytic strains of microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876,

Micrococcus luteus ATCC 10240), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Bordetella bronchiseptica* ATCC 4617, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027) and fungi belonging to yeasts (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019).

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 [34, 35]. 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 [36].

All the experiments were repeated three times (n = 3) and representative data are presented. The detailed procedure for the *in vitro* antimicrobial assay is presented in Supplementary Materials.

The statistical analysis of obtained antimicrobial assays results is presented in Table 1S. in Supplementary Materials. Minimal Inhibitory Concentration (MIC) values in this table are presented as 'Mode MIC' - most frequent value in the data set of MIC values and 'Mean MIC ± SE' - standard error (SE) is the standard deviation of the sampling distribution of the mean of MIC values.

RESULTS AND DISCUSSION

Chemistry

The main aim of this study was to synthesize new hydrazide-hydrazones (**3**-**32**) and to investigate theirs *in vitro* antimicrobial activities. The synthesis of hydrazide intermediate (**1**, **2**) and target compounds (**3**-**32**) was performed according to the reactions outlined in Scheme 1.

In this study the hydrazides of phenylacetic acid (**1**) and glycolic acid (**2**) were obtained by the reaction of commercially available ethyl esters with hydrazine hydrate [30, 31]. Proton assignments in ^{1}H NMR spectra for compounds **1** and **2** showed signals at δ 3.76 ppm (**1**), 3.90 ppm (**2**) for NH_2 group and δ 8.19 ppm (**1**), 8.84 ppm (**2**) for NH group. Compounds **1** and **2** showed also the disappearance of the characteristic signals for the ethyl group, which also successfully confirmed the formation of appropriate hydrazides (**1**, **2**). The treatment of

obtained hydrazides (**1**, **2**) with appropriate substituted aldehydes yielded the final products (**3-32**).

The elemental analysis was performed to confirm the purity of compounds. The compounds (**3-32**) were characterized by ^{1}H NMR and ^{13}C NMR spectral studies. The ^{1}H NMR spectra of all hydrazide-hydrazones (**3-32**) confirmed the proposed structures. In all the compounds the NH peaks of hydrazone appeared at δ 10.18 – 11.83 ppm. The peak for =CH group was observed at δ 8.00 – 8.87 ppm, confirming the formation of final products. In the ^{13}C NMR spectra the peaks for carbonyl carbon (C=O) were present in the range δ 168.0 – 173.2 ppm (Scheme 1.). The signals for other aliphatic and aromatic residues in ^{1}H NMR and ^{13}C NMR appeared in their respective range. Moreover the elemental analyses were consistent with their assigned structures. All obtained physico-chemical data for synthesized compounds is given in Experimental Section.

Among the synthesized derivatives (**3-32**) only compound **3** [37], **6** [38] and **14** [39] have any reference in the literature. Structures of compounds **4**, **5**, **9**, **10** and **12** are known, but there are no references reporting their use, synthesis and physico-chemical characterization. In addition to this our 22 synthesized hydrazide-hydrazones: **7**, **8**, **11**, **13** and **15-32** are new and their structures and synthesis have not been reported in the literature so far.

In vitro antimicrobial activity against a panel of reference strains

The results of our study indicated that among the examined compounds **3-10** (hydrazide-hydrazones of phenylacetic acid) only compound **6** had inhibitory effect on the growth of reference strains of microorganisms. On the basis of minimal inhibitory concentration (MIC) values presented in Table 1, obtained by the broth microdilution method, it was shown that tested compound **6** exhibited very strong activity against all tested reference Gram-positive bacteria with MIC = 0.488 – 7.81 µg/ml and MBC = 0.488 – 15.62 µg/ml. This compound showed bactericidal effect towards these bacteria (MBC/MIC = 1 – 4). The bacteria belonging to *B. subtilis* ATTC 6633 were the most sensitive to this compound (MIC = MBC = 0.488 µg/ml, MBC/MIC = 1). It is worth stressing that the activity of compound **6** against *B. subtilis* ATTC 6633 was 32 times higher than the activity of cefuroxime (MIC = 15.63 µg/ml), whereas the activity against *B. cereus* ATCC 10876 was 8 times higher than activity of cefuroxime (MIC = 31.25 µg/ml) and 16 times higher than the activity of ampicillin (MIC = 62.5 µg/ml).

The tested compound **6** was found also to be active against reference Gram-negative bacteria (*Bordetella* spp. ATTC, *Pseudomonas* spp. ATTC and rod-shaped bacteria of the family *Enterobacteriaceae*) with MIC = 31.25 – 62.5 μg/ml, MBC = 31.25 – 250 μg/ml and bactericidal effect (MBC/MIC = 1 – 4). Among them, *Escherichia coli* ATTC 25922 was especially susceptible to compound **6** (MIC = MBC = 31.25 μg/ml, MBC/MIC = 1) (Table 1). It is worth to add that according to the study recently performed by Palace-Berl et al. *N*-[(5-nitrofuran-2-yl)methylidene]-2-phenylacethydrazide (**6**) shows also promising activity against *Trypanosoma cruzi* in comparison to benznidazole [38].

The results of our study indicated that among the examined hydrazide-hydrazones of hydroxybenzoic acid (**11**-**32**), substance **14** showed the widest spectrum of antimicrobial activity against all tested reference bacteria and yeasts. This compound showed very strong bactericidal effect towards *Staphylococcus* spp. ATTC and *Bacillus* spp. ATTC (MIC = 1.95 – 7.81 μg/ml and MBC = 3.91 – 15.62 μg/ml). The activity of this compound against *B. subtilis* ATCC 6633 (MIC = 7.81 μg/ml) was 2 times higher than the activity of cefuroxime against this bacterium (MIC = 15.63 μg/ml). In the case of *B. cereus* ATCC 10876 the values of MIC of compound **14** (MIC = 7.81 μg/ml) were 4 times lower than the MIC of cefuroxime (MIC = 31.25 μg/ml) and 8 times lower than the MIC of ampicillin (MIC = 62.5 μg/ml). In both cases this compound showed the bactericidal effect (MBC/MIC = 1 – 2).

In turn, minimum concentration of **14**, which inhibited growth of *M. luteus* ATTC 10240 was 62.5 μg/ml and MBC = 125 μg/ml. Gram-negative rods from *Enterobacteriaceae* family were also susceptible to this substance at concentrations from 7.81 μg/ml (*S. typhimurium* ATTC 14028 and *E. coli* ATTC 25922) to 62.5 μg/ml (*P. mirabilis* ATTC 12453). In addition, compound **14** possessed mild bioactivity against *P. aeruginosa* ATTC 9027 and yeasts belonging to *Candida* spp. ATTC (MIC = 1000 μg/ml) (Table 1). It is worth to mention that according to Hossack 2-hydroxy-*N*-[(5-nitrofuran-2-yl)methylidene]acethydrazide (**14**) shows also antibacterial activity against *Proteus vulgaris* urinary tract infections in rats comparable to nitrofurantoin [39].

Compound **26** exhibited very high inhibitory effect on the growth of all tested reference strains of Gram-positive bacteria with MIC = 3.91 – 7.81 μg/ml and MBC = 7.81 – 125 μg/ml. This compound showed very strong activity towards these bacteria with bactericidal effect against them, with the exception of micrococci (bacteriostatic activity – MBC/MIC = 16). Compound **26** showed also good bioactivity towards reference yeasts *Candida* spp. (MIC = 125 μg/ml, MBC = 500 – 1000 μg/ml and MBC/MIC = 4 – 8) (Table 1).

Moreover, compounds **16** and **20** exhibited good or moderate and **22** – moderate or mild activity towards all reference Gram-positive bacteria. The remaining newly synthesized compounds had some inhibitory effect on the growth of reference strains of bacteria and yeasts or were inactive against these microorganisms (Table 1).

The influence of substituents on the antimicrobial activity of synthesized compounds (**3-32**) is presented in Figure 1.

We would like to underline that strong activity of three synthesized compounds, in some cases even better than that of commonly used antibiotics, against Gram-positive is crucial due to the fact that these bacteria are common causes of severe infections. This significant activity also presents an opportunity for searching new hydrazide-hydrazone analogues as potential antimicrobial agents.

CONCLUSIONS

In this study 30 hydrazide hydrazones of phenylacetic (**3-10**) and hydroxyacetic acid (**11-32**) were synthesized and evaluated *in vitro* for their potential antimicrobial activities. Our results indicated that among newly synthesized compounds **3-32**, especially compounds **6**, **14** and **26** showed high bactericidal activity against reference bacteria. These substances exhibited very strong or strong bactericidal effect towards Gram-positive and Gram-negative bacteria, respectively. Thus, these hydrazide-hydrazones may be regarded as precursor compounds for searching for new hydrazide-hydrazones showing significant antimicrobial activity.

CONFILCT OF INTEREST

The authors declare no conflict of interest.

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Compound No	**R_1**	**R_2**	**Compound No**	**R_1**	**R_2**
3	C_6H_5	pyrrol-2-yl	**18**	OH	4-OH-3,5-diOCH_3-Ph
4	C_6H_5	5-chlorofuran-2-yl	**19**	OH	3-Cl-4-OCH_3-Ph
5	C_6H_5	5-iodofuran-2-yl	**20**	OH	2,3-diBr-4-OH-Ph
6	C_6H_5	5-nitrofuran-2-yl	**21**	OH	3-Br-4-OCH_3-Ph
7	C_6H_5	2-Br-5-F-Ph	**22**	OH	3-Br-4-OH-Ph
8	C_6H_5	2-Cl-3-OCH_3-Ph	**23**	OH	2-Br-3-OH-4-OCH_3-Ph
9	C_6H_5	2-Cl-5-NO_2-Ph	**24**	OH	2,3-diF-Ph
10	C_6H_5	4-OH-3,5-diOCH_3-Ph	**25**	OH	4-CF_3-Ph
11	OH	pyrrol-2-yl	**26**	OH	2-OH-3,5diI-Ph
12	OH	5-chlorofuran-2-yl	**27**	OH	3-OC_2H_5-2-OH-Ph
13	OH	5-iodofuran-2-yl	**28**	OH	3-OC_2H_5-4-OCH_3-Ph
14	OH	5-nitrofuran-2-yl	**29**	OH	4-OC_3H_7-Ph
15	OH	2-Br-5-F-Ph	**30**	OH	4-pyrrolidinyl-Ph
16	OH	2-Cl-3-OCH_3-Ph	**31**	OH	4-piperidinyl-Ph
17	OH	2-Cl-5-NO_2-Ph	**32**	OH	quinolin-2-yl

Scheme 1. Synthesis route to new hydrazide-hydrazone derivatives (**3**-**32**).

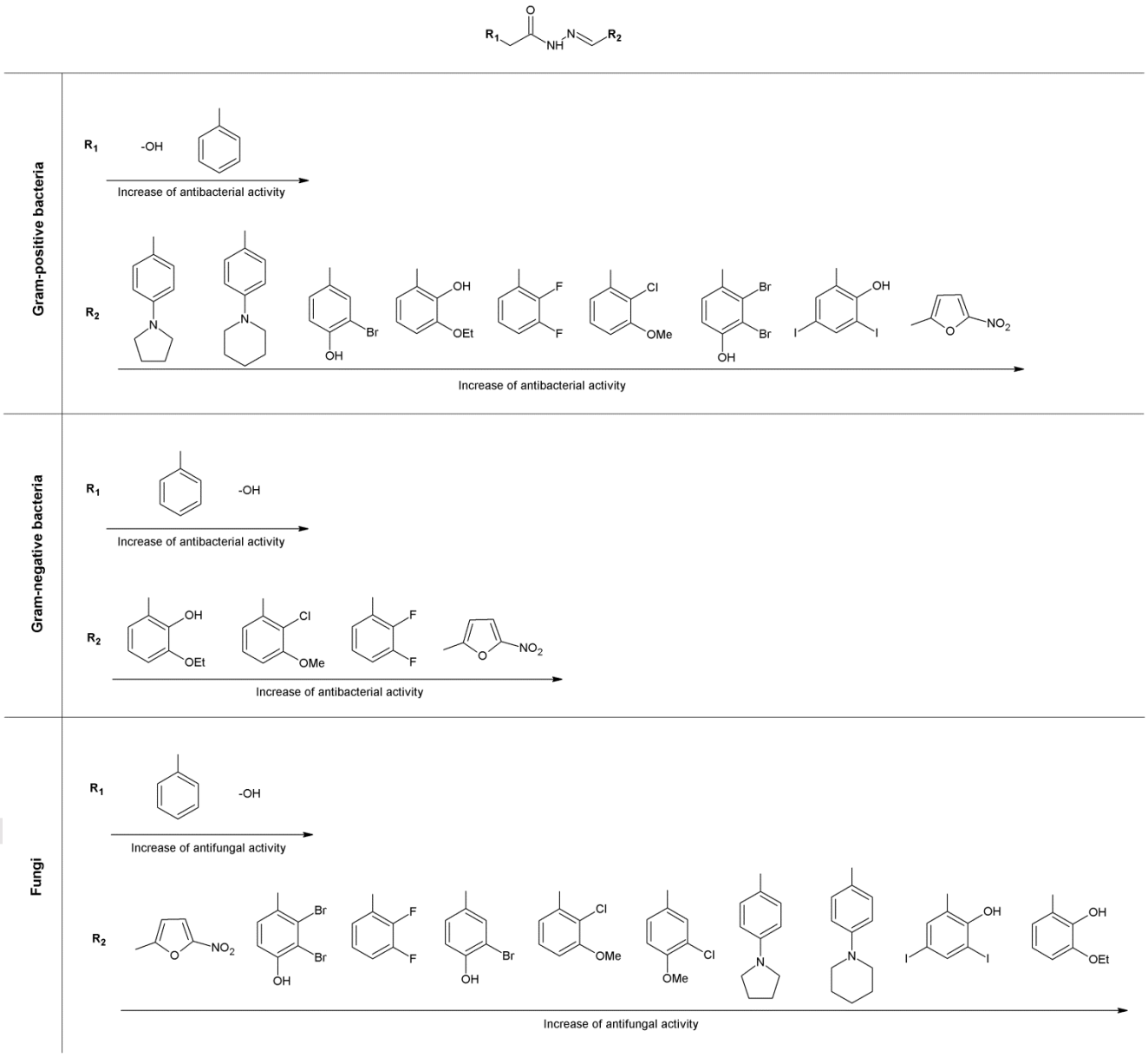
Figure 1. The influence of substituents on the antimicrobial activity of hydrazide-hydrazone derivatives.

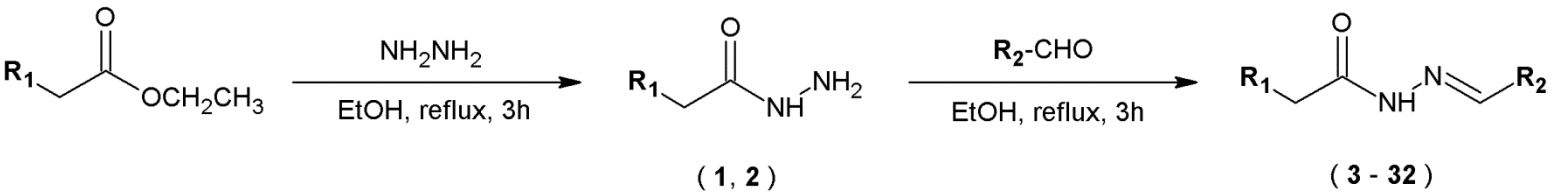
Table 1. The activity data of hydrazide-hydrazones expressed as MIC (MBC/MFC) [µg/ml] against the reference strains of bacteria and fungi.

Species		MIC (MBC/MFC) [µg/ml] of the tested compounds														
		6	**14**	**16**	**19**	**20**	**22**	**24**	**26**	**27**	**30**	**31**	**CIP**	**CFX**	**APC**	**FLU**
Gram-positive bacteria	*Staphylococcus aureus* ATCC 25923	**1.95 (3.91)**	**7.81 (7.81)**	**125 (250)**	-	31.25 (500)	500 (>1000)	1000 (>1000)	**7.81 (7.81)**	-	1000 (>1000)	-	0.488	0.49	nd	na
	Staphylococcus aureus ATCC 6538	**7.81 (15.62)**	**7.81 (7.81)**	**125 (500)**	-	**31.25 (125)**	1000 (>1000)	-	**7.81 (7.81)**	-	-	-	0.244	0.98	nd	na
	Staphylococcus aureus ATCC 43300	**3.91 (3.91)**	**3.91 (7.81)**	**250 (500)**	-	**62.5 (250)**	1000 (>1000)	-	**7.81 (15.62)**	-	-	-	0.244	nd	nd	na
	Staphylococcus epidermidis ATCC 12228	**1.95 (1.95)**	**1.95 (3.91)**	500 (>1000)	-	**250 (500)**	1000 (>1000)	-	**3.91 (7.81)**	1000 (>1000)	-	-	0.122	0.24	nd	na
	Micrococcus luteus ATCC 10240	**7.81 (15.62)**	**62.5 (125)**	500 (>1000)	-	500 (>1000)	1000 (>1000)	-	7.81 (125)	125 (>1000)	-	-	0.976	0.98	nd	na
	Bacillus subtilis ATCC 6633	**0.488 (0.488)**	**7.81 (7.81)**	**62.5 (125)**	-	**250 (500)**	1000 (1000)	**500 (1000)**	**7.81 (7.81)**	-	-	500 (>1000)	0.031	15.63	nd	na
	Bacillus cereus ATCC 10876	**3.91 (15.62)**	**7.81 (15.62)**	**62.5 (125)**	-	**500 (1000)**	1000 (>1000)	**500 (500)**	**7.81 (7.81)**	-	-	-	0.061	31.25	62.5	na
Gram-negative bacteria	*Bordetella bronchiseptica* ATCC 4617	**62.5 (125)**	**62.5 (250)**	-	-	-	-	1000 (>1000)	-	500 (>1000)	-	-	0.976	nd	nd	na
	Klebsiella pneumoniae ATCC 13883	**31.25 (62.5)**	**15.62 (31.25)**	-	-	-	-	1000 (>1000)	-	-	-	-	0.122	nd	nd	na
	Proteus	**31.25**	**62.5**	-	-	-	-	-	-	-	-	-	0.030	nd	nd	na

	mirabilis ATCC 12453	**(62.5)**	**(62.5)**													
	Salmonella typhimurium ATCC 14028	**31.25 (62.5)**	**7.81 (7.81)**	-	-	-	-	1000 (>1000)	-	-	-	-	0.061	nd	nd	na
	Escherichia coli ATCC 25922	**31.25 (31.25)**	**7.81 (7.81)**	250 (>1000)	-	-	-	**500 (500)**	-	-	-	-	0.004	nd	nd	na
	Pseudomonas aeruginosa ATCC 9027	**62.5 (250)**	1000 (>1000)	-	-	-	-	-	-	-	-	-	0.488	nd	nd	na
Fungi	*Candida albicans* ATCC 10231	1000 (>1000)	-	1000 (>1000)	500 (>1000)	1000 (>1000)	1000 (>1000)	1000 (>1000)	125 (1000)	**125 (250)**	500 (>1000)	500 (>1000)	na	na	na	0.976
	Candida parapsilosis ATCC 22019	-	1000 (>1000)	1000 (>1000)	500 (>1000)	-	1000 (>1000)	1000 (>1000)	**125 (500)**	250 (500)	250 (>1000)	**250 (1000)**	na	na	na	1.953

The standard compounds used as positive control for bacteria: CPX – Ciprofloxacin; CFX – Cefuroxime; APC – Ampicillin and for fungi: FLU – Fluconazole.
na - not applicable; nd – not determined; '-' – no activity; MIC – Minimal Inhibitory Concentration; MBC – Minimal Bactericidal Concentration; Compounds with bactericidal effect (MBC/MIC ≤ 4) are marked in bold.





(**1**, **2**) (**3 - 32**)