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Novel Derivatives of 4-Methyl-1,2,3-Thiadiazole-5-Carboxylic Acid Hydrazide: Synthesis, Lipophilicity, and In Vitro Antimicrobial Activity Screening

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Abstract: Bacterial infections, especially those caused by strains resistant to commonly used antibiotics and chemotherapeutics, are still a current threat to public health. Therefore, the search for new molecules with potential antimicrobial activity is an important research goal. In this article, we present the synthesis and evaluation of the in vitro antimicrobial activity of a series of 15 new derivatives of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid. The potential antimicrobial effect of the new compounds was observed mainly against Gram-positive bacteria. Compound **15**, with the 5-nitro-2-furoyl moiety, showed the highest bioactivity: minimum inhibitory concentration (MIC) = 1.95–15.62 μg/mL and minimum bactericidal concentration (MBC)/MIC = 1–4 μg/mL.

Keywords: 1,2,3-thiadiazole derivatives; antimicrobial activity; hydrazide–hydrazones; lipophilicity; organic synthesis

Citation: Paruch, K.; Popiołek, Ł.; Biernasiuk, A.; Berecka-Rycerz, A.; Malm, A.; Gumieniczek, A.; Wujec, M. Novel Derivatives of 4-Methyl-1,2,3-Thiadiazole-5-Carboxylic Acid Hydrazide: Synthesis, Lipophilicity, and In Vitro Antimicrobial Activity Screening. *Appl. Sci.* **2021**, *11*, 1180. https://doi.org/10.3390/app11031180

Academic Editor: Lucjusz Zaprutko
Received: 10 January 2021
Accepted: 25 January 2021
Published: 27 January 2021

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1. Introduction

Antibiotics have been one of the most dynamically developing groups of medicines of the last decade [1]. The first use of an antibiotic (penicillin) in the early 20th century became a landmark in treating infections. Thanks to this, it was possible to reduce the mortality and the risk of postinfectious complications [2]. Unfortunately, as a result of the common use of penicillin, a strain of *Staphylococcus aureus* bacterium appeared, which produced the penicillinase enzyme, giving it resistance to penicillin [3]. In response to this fact, newer antibiotics were introduced as treatments. Additionally, strains of bacteria resistant to these new antibacterial agents were also isolated [4]. This was an important signal that the golden age of antibiotics would not last forever. This century has seen that the problem of antibiotic resistance poses a real threat to patients and global public health [5]. Alarm pathogens are particularly dangerous due to therapeutic limitations. These include multidrug-resistant pathogens (MDR), extended resistance pathogens (XDR), as well as pathogens resistant to all available antibacterial drugs (PDR) [6,7]. This group includes extremely virulent strains of methicillin-resistant *S. aureus* (MRSA) [3,8,9]. This etiological agent is thought to be one of the most common causes of life-threatening infections in palliative care facilities and during inpatient treatment [10]. This problem has been noticed by key institutions and global and European public health organizations [1]. The EAAD (European Antibiotic Awareness Day) and the WAAW

(World Antibiotic Awareness Week) information campaigns, organized by the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO), aim to raise awareness of global antimicrobial resistance and encourage general public healthcare professionals to follow best practices to avoid the further emergence and spread of drug-resistant infections [11].

Among chemotherapeutics, we can find compounds which contain the hydrazide–hydrazone moiety in their chemical structure (i.e., nitrofurazone, furazolidone, and nitrofurantoin) [12] (Figure 1). As can be seen from the literature review, the compounds with an azomethine group (–NH–N=CH–) show a significant and broad spectrum of bioactivity, mainly antibacterial [13–20], antifungal [21], antitubercular [22], antimycobacterial [23], anticancer [24–27], anti-inflammatory [28], anticonvulsant [29], and antiviral activity [30]. Several previously published articles by our research team proved that hydrazide–hydrazones can exhibit significant antimicrobial and anticancer activity [12,31–35]. The method of synthesis that allowed us to obtain hydrazide–hydrazones with good yields was described in our previous articles: the condensation of hydrazide with an appropriate aldehyde in an ethyl alcohol environment [32,34,35].

The 1,2,3-thiadiazole moiety also has great potential for designing new molecules with potential antimicrobial activity [36,37]. In the work of Shin et al., the biological activities of a series of new 1β-methylcarbapenems were compared. Among the tested compounds, the 1,2,3-thiadiazole derivative showed the strongest antibacterial activity and advanced pharmacokinetics in rats compared with other five-membered and six-membered derivatives [37].

We assumed that the combination of these two pharmacophores—the hydrazide–hydrazone and 1,2,3-thiadiazole moieties—could have a beneficial biological effect.

Based on the results obtained so far and on literature reports on the biological potential of hydrazide–hydrazones, in this study, we synthesized novel compounds in order to obtain substances with significant antimicrobial activity.

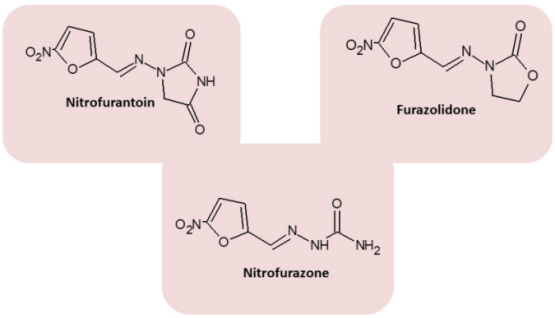


Figure 1. Commonly used antimicrobial agents with hydrazide–hydrazone scaffold.

2. Materials and Methods

2.1. Chemistry

The Procedure of the Synthesis 4-Methyl-1,2,3-Thiadiazole-5-Carbohydrazide Derivatives (**2–16**)

New derivatives of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide (**2–16**) were synthesized according to literature findings [31,32,34]. Hydrazide (**1**) (0.01 mol) was placed in a round-bottomed flask and 15 mL of ethanol (96%) was added. After dissolving the hydrazide (**1**) in ethanol, appropriate substituted aldehyde (0.01 mol) was added. The solution was heated under reflux for 3 h. After that, it was allowed to cool and was

placed in a refrigerator for 24 h. The formed precipitate was filtered off and recrystallized from ethanol.

Detailed physicochemical properties of new derivatives of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide are presented in the Supplementary Materials (**2**–**16**).

2.2. Microbiology

The in vitro bioactivity screening of compounds **2**–**16** (Table 1) was performed according to the procedure described earlier by our group [31,32,34], as well as by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute guidelines [38,39]. In microbiology assays, we used a panel of reference and clinical or saprophytic strains of microorganisms from the American Type Culture Collection (ATCC). All the experiments were repeated three times and representative data are presented. All the stock solutions of the tested compounds were dissolved in DMSO.

Table 1. The antimicrobial activity assay results for compounds **2–16**.

Species/Compound No.		MIC (MBC or MFC) (μg/mL) and {MBC/MIC or MFC/MIC} Compounds and Reference Substances												
		2	**3**	**5**	**6**	**8**	**9**	**14**	**15**	**16**	**CIP/NY ***	**NIT**	**CFX**	**APC**
Gram-positive bacteria	*Staphylococcus aureus* ATCC 25923	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {>1}	-	500 (>1000) {>2}	-	1000 (>1000) {>1}	7.81 (7.81) {**1**}	500 (>1000) {>2}	0.48 (0.48)	15.62 (15.62)	0.49	nd
	Staphylococcus aureus ATCC 6538	1000 (>1000) {>1}	1000 (>1000) {>1}	1000 (>1000) {>1}	-	1000 (>1000) {>1}	-	-	1.95 (3.91) {**2**}	250 (>1000) {>4}	0.24 (0.24)	15.62 (15.62)	0.98	nd
	Staphylococcus aureus ATCC 43300	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {>1}	-	500 (>1000) {>2}	-	-	3.91 (3.91) {**1**}	250 (>1000) {>4}	0.24 (0.24)	7.81 (15.62)	nd	nd
	Staphylococcus aureus ATCC 29213	500 (>1000) {>2}	500 (>1000) {>2}	1000 (>1000) {>1}	-	500 (>1000) {>2}	-	1000 (>1000) {>1}	3.91 (7.81) {**2**}	1000 (>1000) {>1}	0.48 (0.48)	nd	nd	nd
	Staphylococcus epidermidis ATCC 12228	500 (>1000) {>2}	500 (>1000) {>2}	1000 (>1000) {>1}	-	500 (>1000) {>2}	1000 (>1000) {>1}	1000 (>1000) {>1}	1.95 (3.91) {**2**}	500 (>1000) {>2}	0.12 (0.12)	3.91 (7.81)	0.24	nd
	Enterococcus faecalis ATCC 29212	500 (>1000) {>2}	1000 (>1000) {>1}	1000 (>1000) {>1}	-	500 (>1000) {>2}	-	-	15.62 (62.5) {**4**}	1000 (>1000) {>1}	0.98 (1.95)	nd	nd	nd
	Micrococcus luteus ATCC 10240	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {>1}	250 (>1000) {>4}	250 (>1000) {>2}	250 (>1000) {>2}	1000 (>1000) {>1}	7.81 (31.25) {**4**}	250 (>1000) {>4}	0.98 (1.95)	62.5 (62.5)	0.98	nd
	Bacillus subtilis ATCC 6633	500 (>1000) {>2}	500 (>1000) {>2}	500 (>1000) {>1}	-	500 (>1000) {>2}	-	250 (>1000) {>4}	3.91 (3.91) {**1**}	1000 (>1000) {>1}	0.03 (0.03)	3.91 (3.91)	15.62	62.5
	Bacillus cereus ATCC 10876	1000 (>1000) {>1}	500 (>1000) {>2}	1000 (>1000) {>1}	1000 (>1000) {>1}	1000 (>1000) {>1}	-	1000 (>1000) {>1}	7.81 (31.25) {**4**}	500 (>1000) {>2}	0.06 (0.12)	7.81 (15.62)	31.25	nd
Gram-negative bacteria	*Bordetella bronchiseptica* ATCC 4617	1000 (>1000) {>1}	-	-	-	-	-	-	1000 (>1000) {>1}	-	0.98 (0.98)	125 (>1000)	nd	nd

	Klebsiella pneumoniae ATCC 13883	-	-	-	-	-	-	-	500 (>1000) {>2}	-	0.12 (0.24)	15.62 (31.25)	nd	nd
	Proteus mirabilis ATCC 12453	-	-	-	-	-	-	-	500 (>1000) {>2}	-	0.03 (0.03)	62.5 (125)	nd	nd
	Salmonella typhimurium ATCC 14028	-	-	-	-	-	-	-	500 (>1000) {>2}	-	0.06 (0.06)	31.25 (62.5)	nd	nd
	Escherichia coli ATCC 25922	-	-	-	-	-	-	-	125 (>1000) {>1}	-	0.004 (0.008)	7.81 (15.62)	nd	nd
	Pseudomonas aeruginosa ATCC 9027	-	-	-	-	-	-	-	-	-	0.48 (0.98)	-	nd	nd
Fungi	*Candida albicans* ATCC 2091	-	1000 (>1000) {>1}	-	-	1000 (>1000) {>1}	-	-	-	1000 (>1000) {>1}	0.24 * (0.24)	na	na	na
	Candida albicans ATCC 10231	1000 (>1000) {>1}	1000 (>1000) {>1}	1000 (>1000) {>1}	-	1000 (>1000) {>1}	-	-	1000 (>1000) {>1}	1000 (>1000) {>1}	0.48 * (0.48)	na	na	na
	Candida parapsilosis ATCC 22019	1000 (>1000) {>1}	500 (>1000) {>2}	1000 (>1000) {>1}	-	500 (>1000) {>2}	-	-	1000 (>1000) {>1}	500 (>1000) {>2}	0.24 * (0.48)	na	na	na
	Candida glabrata ATCC 90030	-	1000 (>1000) {>1}	-	-	-	-	-	-	-	0.24 * (0.48)	na	na	na
	Candida krusei ATCC 14243	-	-	-	-	1000 (>1000) {>1}	-	-	-	-	0.24 * (0.24)	na	na	na

"–" no activity; nd, not determined; na, not applicable; MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; MFC = minimum fungicidal concentration. The standard chemotherapeutics used as positive controls: ciprofloxacin (CIP), nitrofurantoin (NIT), cefuroxime (CFX), and ampicillin (APC) for bacteria and nystatin (NY *) for fungi. Compounds with bactericidal effect (MBC/MIC ≤ 4) or fungicidal effect (MFC/MIC ≤ 4) are bolded. No bioactivity—MIC > 1000 μg/mL; mild bioactivity—MIC = 501–1000 μg/mL; moderate bioactivity—MIC = 126–500 μg/mL; good bioactivity—MIC = 26–125 μg/mL; strong bioactivity—MIC = 10–25 μg/mL; very strong bioactivity—MIC < 10 μg/mL.

2.3. Lipophilicity

The experimental lipophilicity of new derivatives of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide (**2–6**, **8–16**) (Tables 2 and 3) was determined with the use of reversed-phase thin-layer chromatography. The detailed procedure is presented in the Supplementary Materials.

Table 2. The R_{M0} values of the synthesized 4-methyl-1,2,3-thiadiazole-5-carbooxylic acid derivatives (**2–6** and **8–16**).

Compound No.	R_{M0}	S	r^2	φ
Acetone–water				
2	3.04	−0.04	0.9969	71.65
3	3.31	−0.05	0.9937	71.41
4	3.33	−0.05	0.9908	71.81
5	3.12	−0.05	0.9933	68.74
6	2.54	−0.04	0.9974	65.19
8	2.98	−0.04	0.9937	68.59
9	3.45	−0.05	0.9932	70.94
10	2.87	−0.04	0.9926	65.99
11	2.20	−0.04	0.9909	62.60
12	2.48	−0.04	0.9613	69.78
13	3.05	−0.04	0.9891	69.24
14	1.77	−0.03	0.9147	65.78
15	1.60	−0.03	0.9765	59.34
16	3.03	−0.04	0.9942	68.79
Acetonitrile–water				
2	2.15	−0.03	0.9968	74.34
3	2.11	−0.03	0.9939	74.16
4	2.07	−0.03	0.9971	75.29
5	1.70	−0.02	0.9888	69.37
6	1.81	−0.03	0.9933	56.23
8	1.91	−0.03	0.9892	68.36
9	2.14	−0.03	0.9971	70.08
10	2.12	−0.03	0.9862	64.32
11	1.70	−0.03	0.9956	56.05
12	1.92	−0.03	0.9955	69.76
13	1.96	−0.03	0.9970	71.49
14	1.96	−0.04	0.9447	66.23
15	0.89	−0.02	0.9643	52.19
16	1.85	−0.03	0.9862	68.01
1,4-Dioxane–water				
2	3.08	−0.05	0.9900	66.87
3	3.01	−0.05	0.9831	64.51
4	3.09	−0.05	0.9973	65.47

5	2.87	−0.05	0.9785	62.08
6	1.97	−0.04	0.9873	55.08
8	2.63	−0.04	0.9858	64.05
9	3.00	−0.05	0.9978	63.29
10	2.70	−0.05	0.9824	59.91
11	2.02	−0.04	0.9812	55.25
12	2.80	−0.05	0.9830	62.22
13	3.01	−0.05	0.9724	64.55
14	1.83	−0.03	0.9433	55.45
15	1.30	−0.03	0.9867	50.08
16	2.72	−0.04	0.9806	62.01
		Methanol–water		
2	3.94	−0.05	0.9672	84.91
3	3.88	−0.05	0.9778	86.13
4	3.54	−0.04	0.9792	88.16
5	3.71	−0.05	0.9849	82.17
6	2.88	−0.04	0.9915	77.01
8	3.47	−0.04	0.9796	82.13
9	3.87	−0.04	0.9826	84.45
10	3.58	−0.05	0.9834	81.74
11	3.20	−0.04	0.9821	77.20
12	3.79	−0.05	0.9878	83.11
13	3.85	−0.04	0.9621	85.94
14	3.18	−0.04	0.9717	81.44
15	2.26	−0.03	0.9456	71.85
16	3.51	−0.04	0.9729	81.72

φ is the amount of organic modifier in the mobile phase, R_{M0} and *S* are the intercept and slope of the linear calibration equation, and *r* is the correlation coefficient.

Table 3. The log P_{EXP} values of the synthesized 4-methyl-1,2,3-thiadiazole-5-carbooxylic acid derivatives (**2**–**6** and **8**–**16**).

Compound No.	Log $P_{acetone}$	Log $P_{acetonitrile}$	Log $P_{1,4\text{-dioxane}}$	Log $P_{methanol}$
2	3.55	3.10	3.44	4.09
3	3.89	3.02	3.37	4.02
4	3.90	2.90	3.45	3.66
5	3.64	1.96	3.25	3.84
6	2.92	2.23	2.44	2.93
8	3.47	2.48	3.03	3.57
9	4.05	3.09	3.37	4.01
10	3.33	3.02	3.09	3.70
11	2.49	1.94	2.49	3.28
12	2.84	2.52	3.18	3.92
13	3.55	2.62	3.38	4.00

14	1.96	2.62	2.31	3.25
15	1.74	−0.11	1.84	2.24
16	3.53	2.34	3.11	3.63

3. Results

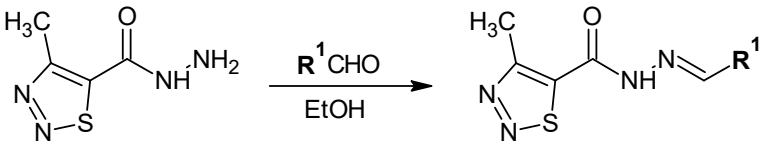
3.1. Chemistry

For the purpose of this research, we synthesized 15 new hydrazide–hydrazones of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (**2–16**) (Scheme 1). Novel compounds (**2–16**) were obtained in good yields (57–98%). Synthesized substances are stable solids and can be dissolved in DMSO at room temperature. The successful synthesis of novel hydrazide–hydrazones was confirmed by elemental analysis and the FT-IR, ^{1}H NMR, and ^{13}C NMR spectra.

Novel derivatives of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (**2–16**) on the ^{1}H NMR spectra possessed the following characteristic signals: The singlet signal for the NH group was in the range of 12.10–12.87 ppm and the singlet signal for the =CH group was at δ 8.06–8.63 ppm, which confirmed the successful conduction of condensation reactions. Signals for other fragments of synthesized molecules (**2–16**) were observed at expected values of chemical shift.

On the ^{13}C NMR spectra for compounds (**2–16**), signals for =CH and C=O groups were observed in the range of δ 135.59–146.67 ppm and 163.31–164.61 ppm, respectively. Additionally, on the FT-IR spectra, we observed characteristic signals for =CH and C=O groups at expected regions.

Synthesized substances (**2–16**) were subjected to in vitro antimicrobial bioassays and to lipophilicity analysis (**2–6**, **8–16**).



1 **2-16**

Compound No.	R¹	Compound No	R¹
2	2-ClC_6H_4	**10**	3-I-4-OH-5-$OCH_3C_6H_2$
3	3-ClC_6H_4	**11**	2-Cl-6-$NO_2C_6H_3$
4	4-ClC_6H_4	**12**	2,3-di$OCH_3C_6H_3$
5	2-FC_6H_4	**13**	2,4-di$OCH_3C_6H_3$
6	3-FC_6H_4	**14**	3,4-di$OCH_3C_6H_3$
7	4-FC_6H_4	**15**	5-nitrofuran-2-yl
8	3-OC_2H_5-4-OHC_6H_3	**16**	1*H*-pyrrol-2-yl
9	2-Br-6-OHC_6H_3		

Scheme 1. Synthesis scheme of novel derivatives of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide.

3.2. Microbiology

The results of the performed tests, as indicated in Table 1, show the potential antimicrobial activity of some of the obtained compounds, namely, **2**, **3**, **5**, **6**, **8**, **9**, and **14–16**, against tested microorganisms. In contrast, for compounds **4**, **7**, **10**, **11**, **12**, and **13**, no antimicrobial activity was found.

Among the microorganisms that were taken into account in the evaluation of the activity of these substances, Gram-positive bacteria turned out to be the most sensitive. The tested compounds showed a similar effect to them, with the exception of hydrazide–

hydrazone **15**. This compound had particular effectiveness. The minimum inhibitory concentration (MIC) of substance **15** which inhibited bacterial growth ranged from 1.95 μg/mL (for some *Staphylococcus* spp.) to 15.62 μg/mL (for *Enterococcus faecalis* ATCC 29212). This proves the strong or very strong activity of this substance against these microorganisms. The minimum bactericidal concentration (MBC) values of hydrazide–hydrazone **15**, which showed a lethal effect against Gram-positive bacteria, were in the range of 3.91–62.5 μg/mL. The MBC/MIC values for this hydrazide–hydrazone were within the range of 1–4, which indicates the lethal effect of this compound. The activity of compound **15** against the *Staphylococcus aureus* strains ATCC 25923 and ATCC 43300 was twofold greater than the reference compound—nitrofurantoin. On the other hand, against the *S. aureus* ATCC 6538 strain, the activity of this substance was seven times higher than for nitrofurantoin. Compound **15** also showed good activity in comparison to nitrofurantoin against strains of *S. epidermidis* ATCC 12228 and *Micrococcus luteus* ATCC 10240—two and eight times greater, respectively.

The remaining substances (**2**, **3**, **5**, **8**, and **16**) inhibited the growth of the bacterial strains at the concentrations of 250–1000 μg/mL. The minimum bactericidal concentrations that killed tested microbes were >1000 μg/mL. Compounds **6**, **9**, and **14** displayed similar ranges of activity against some Gram-positive bacteria (*Staphylococcus* spp., *Bacillus subtilis* ATCC 6633, and *M. luteus* ATCC 10240), or not at all.

Among Gram-negative bacteria, the antimicrobial effect was only demonstrated by compound **15** (MIC = 125–1000 μg/mL, MBC > 1000 μg/mL). *Escherichia coli* ATCC 25922 was the most sensitive strain against this substance (MIC = 125 μg/mL). In contrast, *Pseudomonas aeruginosa* ATCC 9027 did not have any sensitivity. Compound **2** was also found to have a weak effect against *Bordetella bronchiseptica* ATCC 4617. No antibacterial effect was observed for the remaining Gram-negative rods. In relation to yeast-like fungi of the genus *Candida*, a slight antifungal effect of compounds **2**, **3**, **5**, **8**, **15**, and **16** was demonstrated (weak or moderate antimycotic effect). The minimum fungicidal concentration (MFC) values of the tested substances exceeded 1000 μg/mL. The *Candida parapsilosis* ATCC 22019 strain was the most sensitive to the tested substances (**2**, **3**, **5**, **8**, **15**, and **16**). The remaining compounds (**4**, **6**, **7**, and **9**–**14**) did not show an inhibitory effect on the growth of the fungi in the tested concentration ranges.

3.3. Lipophilicity

It is well known that chromatographic methods allow for establishing the experimental lipophilicity. In this research, the standardization procedure with six reference substances covering the range of lipophilicity of 0.46–3.80 was used. As a result, their log P values were highly correlated with their R_{M0} values in four organic modifiers, namely, acetone, acetonitrile, 1,4-dioxane, and methanol solvent systems, and appropriate calibration curves for further lipophilicity study were obtained:

(1) acetone: $\log P_{EXP} = 1.250R_{M0} - 0.256$; $r^2 = 0.7265$
(2) acetonitrile: $\log P_{EXP} = 2.563R_{M0} - 2.402$; $r^2 = 0.8984$
(3) 1,4-dioxane: $\log P_{EXP} = 0.902R_{M0} + 0.662$; $r^2 = 0.9457$
(4) methanol: $\log P_{EXP} = 1.101R_{M0} - 0.242$; $r^2 = 0.9821$

The obtained coefficients of determination (r^2) for the mentioned equations were above 0.89 for three organic modifiers (i.e., acetonitrile, 1,4-dioxane, and methanol). Moreover, the r^2 values were rather high (>0.72) for all organic modifiers used, including acetone. Similarly, for six reference compounds, the correlations between the R_F and R_{M0} values for **2**–**6** and **8**–**16** were sufficiently high ($r^2 \geq 0.91$) for all solvents used. In addition, even better correlations (i.e., $r^2 \geq 0.98$ for 12 or 11 compounds) were obtained for acetonitrile, acetone, and 1,4-dioxane solvent systems, providing accuracy for further lipophilicity determination (Table 2). Experimental lipophilicity ($\log P_{EXP}$) of the synthesized 14 hydrazide–hydrazones (**2**–**6** and **8**–**16**) was calculated on the basis of the above calibration equations and respective R_{M0} values (Table 3). Generally, the highest $\log P_{EXP}$

values were calculated for the methanol–water solvent systems, except for compounds **4** and **13**, while the lowest values were obtained for acetonitrile, except for compound **14**. As far as more detailed differences were concerned, the highest log P_{EXP} values were calculated for compounds **2–4** containing monochloro(phenyl) substituents in *ortho-*, *meta-*, or *para*-positions. This was observed for all solvent systems used in our experiments. Thus, it can be stated that the position of the chloride atom in a phenyl ring did not affect the lipophilicity of these three derivatives (**2–4**). On the contrary, clearly seen differences were observed for dimethoxyphenyl-substituted hydrazide–hydrazones (compounds **12–14**). Between them, compound **13**, with a 2,4-dimethoxyphenyl substituent, showed the highest log P values for all solvent systems used in our experiments, illustrating the importance of positional isomerism in the lipophilicity of these compounds. At the same time, two chlorophenyl substituted derivatives (i.e., **2** and **3**) showed higher log P values than similar fluorophenyl-substituted compounds (**5** and **6**). When compounds **8** and **10** were compared, the 4-hydroxy-3-iodo-5-methoxyphenyl-substituted hydrazide–hydrazone (**10**) was found to be more lipophilic than compound **8** with a 4-hydroxy-5-ethoxyphenyl substituent. This was observed for almost all solvent systems used in the present study (i.e., for the mixtures with acetonitrile, 1,4-dioxane, and methanol). Bearing in mind all the tested hydrazide–hydrazones (**2–6** and **8–16**), the lowest lipophilicity was shown for compound **15** substituted with the 5-nitro-2-furyl moiety.

4. Discussion

Condensation reaction of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide (**1**) with appropriate aldehydes allowed us to obtain a series of new hydrazide–hydrazone derivatives (**2–16**). The reaction efficiency was in the range of 57–98%, what can be considered as satisfactory. The most active compound was the hydrazide–hydrazone numbered **15**, which had a 5-nitro-2-furyl moiety in its structure (i.e., a moiety analogous to a group of medicines, such as nitrofurazone, furazolidone, and nitrofurantoin). Due to this, it can be concluded that this fragment of compound **15** is essential for its bioactivity. It can also be seen that compounds **2**, **3**, **5**, and **6**, with the halogen atom in the *ortho-* and *meta*-position of the phenyl ring, had greater antimicrobial activity in comparison to compounds **4** and **7** with the halogen atom in the *para*-position, which did not show any activity.

5. Conclusions

In this research, we designed, synthesized, and analyzed 15 novel hydrazide–hydrazones (**2–16**) and evaluated them for their in vitro antimicrobial potential and lipophilicity.

The performed activity assays of the hydrazide–hydrazones showed that the best activity was attributed to substance **15** with the 5-nitro-2-furyl moiety, which showed activity especially against all tested Gram-positive bacterial strains. Based on the lipophilicity measured in methanol, because in this case the correlation coefficient is the highest, it can be concluded that despite the differences in the structure of Gram-negative bacteria and Gram-positive bacteria, the most desirable lipophilicity is within the limits of 2.25 (compound **15**). Above this value, activity decreases. On the other hand, lipophilicity in the range of 3.63–4.09 causes activity against Gram-positive bacteria (compounds **2**, **3**, **5**, and **16**).

Supplementary Materials: The following are available online at www.mdpi.com/2076-3417/11/3/1180/s1.

Author Contributions: K.P. participated in the design of the study, performed the synthesis of new hydrazide-hydrazones, participated in the analysis of the spectral data of obtained compounds, and wrote the first draft of the manuscript, with the exception of antimicrobial activity and lipophilicity sections. K.P. also revised critically the final version of manuscript. Ł.P. participated in the design of the study and in the analysis of the spectral data of obtained compounds and wrote the

associated section of the manuscript. A.B. performed the antimicrobial activity analysis of the obtained compounds and wrote the associated section of the manuscript. A.B.-R. performed the lipophilicity analysis of the obtained compounds and wrote the associated section of the manuscript. A.M. supervised and was involved in the conception of the antimicrobial activity analysis, helped with interpretation of antimicrobial activity data of synthesized compounds. A.G. supervised and was involved in the conception of the lipophilicity analysis, helped with interpretation of lipophilicity data of synthesized compounds. M.W. participated in the discussion section of the manuscript and revised critically the final version of manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article or supplementary material.

Conflicts of Interest: The authors declare no conflict of interest.

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