



Original article

Synthesis and investigation of antimicrobial activities of nitrofurazone analogues containing hydrazide-hydrazone moiety

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ABSTRACT

In this research we synthesized and tested for *in vitro* antimicrobial activity 21 nitrofurazone analogues. The compounds we obtained were identified on the basis of ¹H NMR and ¹³C NMR spectroscopy. The *in vitro* screening of antimicrobial properties of synthesized compounds revealed a wide spectrum of antimicrobial activity. Compounds **28**, **29**, **32–43**, and **45–48** showed very high bactericidal effect towards *Staphylococcus* spp. ATTC and *Bacillus* spp. ATTC (MIC = 0.002–7.81 µg/ml and MBC = 0.002–31.25 µg/ml). The levels of activity of several compounds were far better than those of nitrofurantoin, ciprofloxacin or cefuroxime.

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1. Introduction

The increasing number of cases of multidrug-resistant infections difficult to diagnose and treat pose a major concern to public health care. To overcome these problems, developing new and safe antimicrobial agents with better effectiveness is required (Coates et al., 2002). One of several routes to find new chemotherapeutic agents is to modify the chemical structure of existing medicines which could result in broadening the spectrum of their activity and reducing their toxicity to human body (Moellering, 2011). In our research we decided to synthesize nitrofurazone analogues, because nitrofurazone is important antibacterial agent (McCalla et al., 1970) and in its structure we found the hydrazide-hydrazone moiety which is of our interest due to its promising biological activity (Fig. 1) (Rollas and Küçükgül, 2007; Bala et al., 2013).

Recently we have published interesting results concerning antibacterial activity of hydrazide-hydrazone derivatives (Popiołek et al., 2014, 2016a,b; Popiołek and Biernasiuk, 2016a,b). Hydrazide-hydrazones of 3-methoxybenzoic acid showed signifi-

cant antibacterial activity against Gram-positive bacterial strains, especially against *Bacillus* spp. ATTC (Popiołek and Biernasiuk, 2016a). In addition to this we have reported that hydrazide-hydrazones of 2-substituted acetic acid displayed potent bactericidal activity against Gram-positive and Gram-negative bacterial strains (Popiołek and Biernasiuk, 2016b).

It is worth to add that beside antibacterial activity (Küçükgül et al., 2002, 2003; Özkay et al., 2010; Deep et al., 2010; Rasras et al., 2010; Kumar et al., 2011; Rutkauskas et al., 2013; Pieczonka et al., 2013; Cukurovali and Yilmaz, 2014; Satyanarayana et al., 2014; Morjan et al., 2014; Rambabu et al., 2015), hydrazide-hydrazone derivatives have attracted much attention thanks to their usability as intermediates in organic synthesis (Rollas and Küçükgül, 2007; Bala et al., 2013) and they display a wide spectrum of such interesting biological properties as antifungal (Loncle et al., 2004; Backes et al., 2014), antitubercular (Koçyiğit-Kaymakçioğlu et al., 2006, 2009; Pavan et al., 2010; Velezheva et al., 2016), antiviral (Şenkardes et al., 2016), anticancer (Kumar et al., 2012; Çıkla et al., 2013; Wardakhan et al., 2013; Nasr et al., 2014; Küçükgül et al., 2015; He et al., 2016; Mukherjee et al., 2016), anti-inflammatory (Moldovan et al., 2011) and analgesic activity (Mohareb et al., 2010).

Based on the afore mentioned facts, and in an attempt to find new potent antimicrobial agents thanks to this research we synthesized and evaluated for their *in vitro* antimicrobial activity 21 analogues of nitrofurazone and we discovered that they showed very high bactericidal activity, particularly against *Staphylococcus* spp. ATTC and *Bacillus* spp. ATTC.

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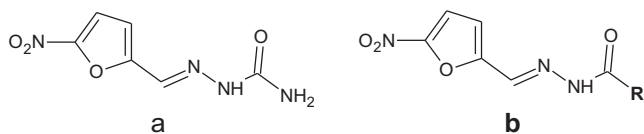


Fig. 1. Chemical structure of nitrofurazone (A) and synthesized analogues (B).

2. Materials and methods

2.1. Chemistry

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 ^1H NMR and ^{13}C NMR spectra were recorded on the BRUKER AVANCE 300 apparatus (Bruker BioSpin GmbH, Germany) in $\text{DMSO-}d_6$ 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 obtained compounds were monitored by TLC, using pre-coated aluminum sheet 60 F254 plates (Merck Co. USA), in a $\text{CHCl}_3/\text{C}_2\text{H}_5\text{OH}$ (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 $\pm 0.4\%$ of the calculated values.

2.1.1. Preparation of carboxylic acid hydrazides (9–13, 20)

The compounds **11**, **12**, **13** were prepared using the procedures reported earlier (Popiołek et al., 2016b). Compound **9**, **10**, **20** were synthesized by following procedure: 0.01 mol of appropriate ethyl ester of carboxylic acid was dissolved in ethanol and heated under reflux with 0.011 mol of 100% hydrazine monohydrate for 2 h. After that the solution was cooled to room temperature and the precipitate formed. Then it was filtered off, dried and recrystallized from ethanol.

Physico-chemical and spectral data of compounds **9–13**, **20** are presented in [Supplementary Materials](#).

2.1.2. Preparation of nitrofurazone analogues (28–48)

2.1.2.1. General procedure. 0.01 mol of previously obtained carboxylic acid hydrazides (**9–13**, **20**) or commercially available hydrazides (**7**, **8**, **14–19**, **21–27**) were dissolved in 10–20 ml of ethanol and then 0.011 mol of 5-nitro-2-furaldehyde was added. The mixture was heated under reflux for 3 h. After that the solution was allowed to cool at room temperature and then was placed in refrigerator for 12 h. Subsequently the precipitation created was filtered off and recrystallized from ethanol.

Physico-chemical and spectral data of compounds **28–48** are presented in [Supplementary Materials](#).

2.2. Microbiology

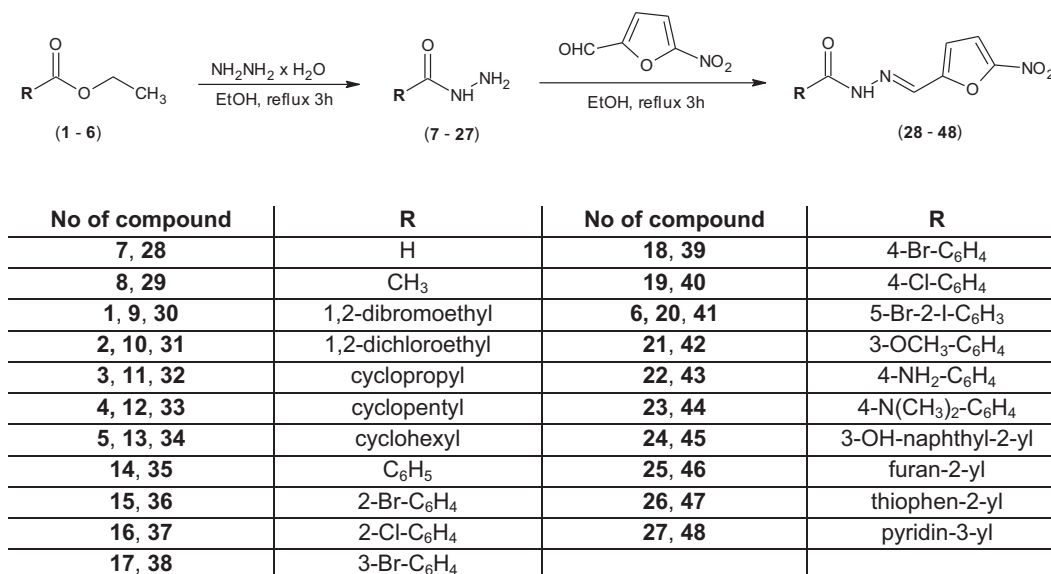
2.2.1. In vitro antimicrobial assay

The examined compounds were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST discussion document E. Dis 5.1, 2003) and Clinical and Laboratory Standards Institute guidelines (M27-S4, 2012).

In this research a panel of reference and clinical or saprophytic strains of microorganisms was used. This included 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).

The antimicrobial assays were performed like in our previous research concerning *in vitro* screening of hydrazide-hydrazone derivatives (Popiołek and Biernasiuk, 2016a,b). Nitrofurantoin, ciprofloxacin, and cefuroxime (Sigma-Aldrich) were used as reference antibacterial compounds. Fluconazole (Sigma-Aldrich) was used as reference antifungal positive control. All the experiments were repeated three times and representative data were presented.

The MBC/MIC or MFC/MIC ratios were used to determine bactericidal/fungicidal ($\text{MBC/MIC} \leq 4$, $\text{MFC/MIC} \leq 4$) or bacteriostatic/fungistatic ($\text{MBC/MIC} > 4$, $\text{MFC/MIC} > 4$) effect of the tested compounds (Wiegand et al., 2008).



Scheme 1. Synthesis pathway to nitrofurazone analogues (28–48).

Table 1A

The activity data expressed as MIC (MBC or MFC) [$\mu\text{g/ml}$] and {MBC/MIC or MFC/MIC ratio} against the reference strains of microorganisms. The standard chemotherapeutics agents: nitrofurantoin (NIT), ciprofloxacin (CIP), cefuroxime (CFX) and fluconazole (FLU) were used as positive control.

Species		MIC (MBC or MFC) [$\mu\text{g/ml}$] and {MBC/MIC or MFC/MIC ratio} of the tested compounds															
		28	29	30	31	32	33	34	35	36	37	38	NIT	CIP	CFX	FLU	
Gram-positive bacteria	<i>Staphylococcus aureus</i>	7.81	7.81	15.62	31.25	3.91	1.95	1.95	3.91	3.91	3.91	0.061	15.62	0.488	0.49	na	
	ATCC 25923	{1}	{1}	{8}	{8}	{2}	{2}	{8}	{2}	{1}	{2}	{128}					
	<i>Staphylococcus aureus</i>	7.81	7.81	31.25	31.25	3.91	3.91	0.98	3.91	1.95	7.81	0.98	15.62	0.244	0.98	na	
	ATCC 6538	{1}	{1}	{2}	{2}	{2}	{1}	{4}	{2}	{2}	{2}	{2}					
	<i>Staphylococcus aureus</i>	0.98	1.95	7.81	7.81	0.98	0.244	0.244	1.95	0.488	7.81	0.122	7.81	0.244	nd	na	
	ATCC 43300	{4}	{2}	{2}	{2}	{2}	{2}	{2}	{2}	{2}	{2}	{2}					
	<i>Staphylococcus epidermidis</i>	0.98	0.98	3.91	3.91	0.061	0.061	0.244	0.48	0.061	0.244	0.002	3.91	0.122	0.24	na	
	ATCC 12228	{2}	{2}	{4}	{4}	{4}	{2}	{2}	{2}	{2}	{1}	{1}					
	<i>Micrococcus luteus</i>	62.5	62.5	250	62.5	62.5	500	250	250	62.5	1000 (>1000)	125	62.5	0.976	0.98	na	
	ATCC 10240	{2}	{4}	{4}	{8}	{1}	{>2}	{>4}	{>4}	{2}	{2}	{1}					
	<i>Bacillus subtilis</i>	0.98	1.95	3.91	3.91	0.002	0.244	0.98	1.95	3.91	1.95	0.244	3.91	0.031	15.63	na	
	ATCC 6633	{1}	{1}	{2}	{2}	{1}	{32}	{4}	{1}	{1}	{1}	{4}					
	<i>Bacillus cereus</i>	7.81	7.81	31.25	62.5	0.98	3.91	0.98	3.91	1.95	1.95	0.488	7.81	0.061	31.25	na	
	ATCC 10876	{1}	{1}	{1}	{2}	{4}	{2}	{16}	{1}	{2}	{1}	{2}					
	Gram-negative bacteria	<i>Bordetella bronchiseptica</i>	31.25	62.5	125	62.5	500	500	-	-	125	-	1000	125	0.976	nd	na
ATCC 4617		{125}	{500}	{500}	{250}	{>1000}	{>1000}			{1000}		{>1000}	{>1000}				
<i>Klebsiella pneumoniae</i> ATCC 13883		7.81	7.81	125	125	31.25	1000	-	-	125	-	-	15.62	0.122	nd	na	
		{15.62}	{7.81}	{250}	{125}	{>1000}	{>1000}			{500}			{31.25}				
<i>Proteus mirabilis</i>		31.25	62.5	500	250	250	250	-	-	62.5	-	-	62.5	0.030	nd	na	
ATCC 12453		{62.5}	{125}	{1000}	{500}	{>1000}	{>1000}			{250}			{125}				
<i>Salmonella typhimurium</i>		7.81	7.81	125	62.5	0.98	500	-	-	62.5	-	-	31.25	0.061	nd	na	
ATCC 14028		{7.81}	{7.81}	{500}	{125}	{7.81}	{>1000}			{250}			{62.5}				
<i>Escherichia coli</i>		7.81	3.91	125	62.5	0.98	1000	-	-	31.25	-	-	7.81	0.004	nd	na	
ATCC 25922		{7.81}	{7.81}	{250}	{125}	{1.95}	{>1000}			{31.25}			{15.62}				
<i>Pseudomonas aeruginosa</i>		500	-	250	62.5	-	-	-	-	-	-	-	-	0.488	nd	na	
ATCC 9027		{1000}		{500}	{125}												
Fungi		<i>Candida albicans</i>	125	500	125	62.5	-	500	125	250	31.25	125	1000	na	na	na	0.98
		ATCC 10231	{250}	{>1000}	{500}	{125}		{>1000}	{>1000}	{>1000}	{62.5}	{250}	{>1000}				
			{2}		{4}	{2}		{>2}	{>8}	{>4}	{2}	{2}	{>1}				
	<i>Candida parapsilosis</i>	250	1000	125	62.5	-	1000	500	500	125	1000	1000	na	na	na	1.95	
ATCC 22019	{500}	{>1000}	{1000}	{250}		{>1000}	{>1000}	{>1000}	{1000}	{>1000}	{>1000}						
	{2}	{>1}	{8}	{4}		{>1}	{>2}	{>2}	{8}	{>1}	{>1}						

na - not applicable; nd - not determined; '-' - no activity; MIC - Minimal Inhibitory Concentration; MBC - Minimal Bactericidal Concentration; MFC - Minimal Fungicidal Concentration; Compounds with bactericidal effect (MBC/MIC \leq 4) or fungicidal effect (MFC/MIC \leq 4) are marked in bold.

Table 1B

The activity data expressed as MIC (MBC or MFC) [$\mu\text{g/ml}$] and {MBC/MIC or MFC/MIC ratio} against the reference strains of microorganisms. The standard chemotherapeutics agents: nitrofurantoin (NIT), ciprofloxacin (CIP), cefuroxime (CFX) and fluconazole (FLU) were used as positive control.

Species		MIC (MBC or MFC) [$\mu\text{g/ml}$] and {MBC/MIC or MFC/MIC ratio} of the tested compounds											NIT	CIP	CFX	FLU
		39	40	41	42	43	44	45	46	47	48					
Gram-positive bacteria	<i>Staphylococcus aureus</i>	7.81 (31.25)	3.91 (7.81)	3.91 (7.81)	3.91 (3.91)	3.91 (3.91)	500 (>1000)	0.98 (1.95)	3.91 (7.81)	7.81 (15.62)	3.91 (3.91)	15.62 (15.62)	0.488	0.49	na	
	ATCC 25923	{4}	{2}	{2}	{1}	{1}	{>2}	{2}	{2}	{2}	{1}					
	<i>Staphylococcus aureus</i>	7.81 (31.25)	0.488 (0.98)	3.91 (3.91)	3.91 (3.91)	3.91 (3.91)	500 (>1000)	0.48 (1.95)	3.91 (7.81)	7.81 (15.62)	3.91 (3.91)	15.62 (15.62)	0.244	0.98	na	
	ATCC 6538	{4}	{2}	{1}	{1}	{1}	{>2}	{4}	{2}	{2}	{1}					
	<i>Staphylococcus aureus</i>	3.91 (15.62)	3.91 (3.91)	0.98 (3.91)	1.95 (1.95)	1.95 (1.95)	62.5 (125)	0.48 (0.98)	0.98 (1.95)	0.98 (1.95)	1.95 (3.91)	7.81 (15.62)	0.244	nd	na	
	ATCC 43300	{4}	{1}	{4}	{1}	{1}	{2}	{2}	{2}	{2}	{2}					
	<i>Staphylococcus epidermidis</i>	0.031 (0.122)	0.015 (0.061)	0.24 (0.48)	0.48 (0.48)	0.24 (0.24)	3.91 (62.5)	0.12 (0.24)	0.98 (0.98)	0.98 (1.95)	0.98 (1.95)	3.91 (7.81)	0.122	0.24	na	
	ATCC 12228	{4}	{4}	{2}	{1}	{1}	{16}	{2}	{1}	{2}	{2}					
	<i>Micrococcus luteus</i>	-	-	-	250 (>1000)	125 (>1000)	500 (>1000)	125 (>1000)	125 (>1000)	500 (>1000)	31.25 (62.5)	62.5 (62.5)	0.976	0.98	na	
	ATCC 10240				{>4}	{>8}	{>2}	{>8}	{>8}	{>2}	{2}					
	<i>Bacillus subtilis</i>	7.81 (15.62)	0.244 (0.244)	0.98 (1.95)	1.95 (1.95)	0.98 (0.98)	62.5 (1000)	0.12 (0.48)	0.98 (0.98)	0.98 (0.98)	1.95 (3.91)	3.91 (3.91)	0.031	15.63	na	
	ATCC 6633	{2}	{1}	{2}	{1}	{1}	{16}	{4}	{1}	{1}	{2}					
	<i>Bacillus cereus</i>	3.91 (31.25)	0.98 (7.81)	1.95 (1.95)	0.98 (0.98)	0.98 (0.98)	31.25 (>1000)	0.98 (1.95)	3.91 (7.81)	1.95 (1.95)	7.81 (7.81)	7.81 (15.62)	0.061	31.25	na	
	ATCC 10876	{8}	{8}	{1}	{1}	{1}	{>32}	{2}	{1}	{1}	{1}					
Gram-negative bacteria	<i>Bordetella bronchiseptica</i>	1000 (>1000)	-	-	-	-	-	-	-	15.62 (125)	125 (>1000)	0.976	nd	na		
	ATCC 4617	{>1}								{8}						
	<i>Klebsiella pneumoniae</i> ATCC 13883	-	-	-	-	-	-	-	1000 (>1000)	-	62.5 (62.5)	15.62 (31.25)	0.122	nd	na	
	<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	1000 (>1000)	-	31.25 (62.5)	62.5 (125)	0.030	nd	na	
	ATCC 12453								{>1}	{2}						
	<i>Salmonella typhimurium</i>	-	-	-	-	-	-	-	125 (>1000)	-	31.25 (31.25)	31.25 (62.5)	0.061	nd	na	
	ATCC 14028								{>8}	{1}						
	<i>Escherichia coli</i>	1000 (>1000)	-	250 (250)	-	1000 (>1000)	-	-	15.62 (>1000)	-	31.25 (125)	7.81 (15.62)	0.004	nd	na	
	ATCC 25922	{>1}		{1}		{>1}			{>64}	{4}						
	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	0.488	nd	na	
	ATCC 9027															
Fungi	<i>Candida albicans</i>	-	-	-	-	-	-	-	-	250 (>1000)	na	na	na	0.98		
	ATCC 10231									{>4}						
	<i>Candida parapsilosis</i>	1000 (>1000)	1000 (>1000)	-	-	-	-	-	-	-	na	na	na	1.95		
	ATCC 22019	{>1}	{>1}													

na - not applicable; nd - not determined; '-' - no activity; MIC - Minimal Inhibitory Concentration; MBC - Minimal Bactericidal Concentration; MFC - Minimal Fungicidal Concentration; Compounds with bactericidal effect (MBC/MIC \leq 4) or fungicidal effect (MFC/MIC \leq 4) are marked in bold.

3. Results and discussion

3.1. Chemistry

Nitrofurazone analogues analyzed in this study were obtained in the condensation reaction of appropriate carboxylic acid hydrazides (**7–27**) with 5-nitro-2-furaldehyde. The reactions were performed by heating substrates under reflux for 3 h. In the case of the synthesis of nitrofurazone analogues **28**, **29**, **35–40**, **42–48** commercially available hydrazides of carboxylic acids (**7**, **8**, **14–19**, **21–27**) were used. Whereas for the synthesis of nitrofurazone analogues (**30–34**, **41**) we initially conducted the synthesis of hydrazides (**9–13**, **20**) by the reaction of appropriate ethyl esters (**1–6**) with hydrazine monohydrate.

Chemical structures of synthesized compounds were confirmed on the basis of ^1H NMR and ^{13}C NMR spectroscopy. The spectra of the compounds we obtained gave satisfactory results and confirmed the formation of expected products. In the ^1H NMR spectra

of compounds (**28–48**) two singlet signals for =CH and NH groups appeared at δ 7.92–8.74 ppm and δ 10.95–12.40 ppm, respectively. As for the ^{13}C NMR spectra, signals for =CH group were found in the range of δ 151.6–152.8 ppm, and for the carbonyl group (C=O) at δ 160.1–174.9 ppm. Signals for other aliphatic and aromatic groups in compounds (**28–48**) were observed at expected regions. Reactions conducted in this study were performed according to the steps presented in the Scheme 1.

3.2. In vitro antimicrobial assay

The results of our study indicated that examined compounds (**28–48**) exhibited a wide spectrum of antimicrobial activity against tested reference bacteria and yeasts (Tables 1A and 1B). Among these compounds, **28**, **29**, **32–43**, and **45–48** showed very strong, mainly bactericidal effect towards *Staphylococcus* spp. ATCC and *Bacillus* spp. ATCC (MIC = 0.002–7.81 $\mu\text{g/ml}$ and MBC = 0.002–

31.25 µg/ml). Substances **38** and **45** were especially potent because of MIC < 1 µg/ml (0.002–0.98 µg/ml) against these bacteria.

Staphylococcus epidermidis ATCC 12228 was the most sensitive to all compounds, while *Micrococcus luteus* ATCC 10240 was the least susceptible. The minimum inhibitory concentrations (MIC) of nitrofurazone analogues (with the exception of inactive **39**, **40** and **41**), which inhibited the growth of micrococci and killed them (MBC) ranged from 31.25 µg/ml to 1000 µg/ml and from 62.5 µg/ml to >1000 µg/ml, respectively.

The bioactivity of compounds **30**, **31** and **44** against Gram-positive bacteria was lower (MIC = 3.91–500 µg/ml and MBC = 7.81 to >1000 µg/ml).

Some of the compounds showed activity towards Gram-negative bacteria. The all reference rods from *Enterobacteriaceae* family were susceptible to compounds **28–33**, **36** and **48** at concentrations from 0.98 µg/ml (*S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922 against **32**) to 500 µg/ml (*P. mirabilis* ATCC 12453 against **30**). The other compounds indicated mainly mild bioactivity or had no effect towards these bacteria. Among the studied compounds, derivatives **28**, **30**, and **31** exhibited also some activity against *P. aeruginosa* ATCC 9027 (MIC = 62.5–500 µg/ml and MBC = 125–1000 µg/ml). The same substances (**28**, **30**, and **31**) showed simultaneously the widest spectrum of antimicrobial activity against all tested reference Gram-positive and Gram-negative bacteria and fungi.

The activity of compounds **28**, **29**, **32–43**, and **45–48** was from two to two thousand times higher, depending on the compounds and bacterial strains, in comparison with the activity of nitrofurantoin (Table S1 in Supplementary Materials). It is worth to mention especially compounds **32** and **38**, which showed almost two thousand times higher activity than nitrofurantoin against *Bacillus subtilis* ATCC 6633 and *Staphylococcus epidermidis* ATCC 12228, respectively.

Compounds **32**, **33**, **36**, **38**, **39** and **40** showed from 2 to 61 times better activity than ciprofloxacin on the basis of MIC values. Especially, compound **38** can be considered as the best analogue because its MIC value was 61 times lower than the MIC of ciprofloxacin against *Staphylococcus epidermidis* ATCC 12228 (Table S2 in Supplementary Materials).

The antibacterial activity of all tested compounds (**28–48**) against Gram-positive bacteria was also in some cases higher, depending on the compounds and bacterial strains, than the activity of cefuroxime (Table S3 in Supplementary Materials). Especially it is worth to mention compound **32** whose MIC value against *Bacillus subtilis* ATCC 6633 was almost 8000 times lower than the MIC of cefuroxime.

Moreover **30**, **31** and **36** possessed good fungicidal or fungistatic bioactivity against yeasts belonging to *Candida* spp. ATCC with MIC = 31.25–125 µg/ml and MFC = 125–1000 µg/ml and it was higher than that of fluconazole used as the reference substance. The remaining newly synthesized compounds were less active or inactive towards reference fungi (Tables 1A and 1B).

4. Conclusions

In our research we synthesized and evaluated a series of 21 nitrofurazone analogues for *in vitro* antimicrobial activity. All synthesized compounds have been identified by means of ¹H NMR and ¹³C NMR spectroscopy, and subjected to *in vitro* antimicrobial assays. Our antimicrobial screening results revealed that several synthesized compounds possessed very high bactericidal activity, mainly against Gram-positive bacteria. It is worth to stress that in many cases the activity of obtained derivatives was far better than the activity of commonly used chemotherapeutic agents.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jsps.2017.05.006>.

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