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Synthesis and in vitro Anti-Bacterial Activity of 2-(5-Nitro-2-heteroaryl)-1,3,4-Thiadiazole Derivatives

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Abstract

A new series of 2-(5-nitro-2-heteroaryl)-1,3,4-thiadiazole derivatives, including nitro furan, nitro thiophene and nitro imidazole, were synthesized and screened *in vitro* for their inhibitory activity against eight bacterial strains. The results showed that most of the synthesized compounds were active against Gram-positive bacteria, determined by MIC method. Among them, compounds **6a**, **6b** and **6d** exhibited strong anti-bacterial effects against the Gram-positive bacteria. The oxidation of synthesized compounds to sulfinyl and sulfonyl bearing analogues did not improve the activity.

Keywords*:* 1,3,4-Thiadiazole; Antibacterial activity; Synthesis.

Introduction

Excessive and inappropriate use of antibiotics without the prescription led to antibiotic resistance crisis, endangering the health of millions of people worldwide. This problem is rising to dangerously high levels in all parts of the world, threated the abilities to treat common infectious disease. Antibiotic resistance resulted in higher medical costs, prolonged hospital stays and increased mortality. The lack of new drug development by pharmaceutical companies due to the reduced economic incentives has worsened this issue. Therefore, continuous efforts to implement new policies, renew research efforts, and pursue steps to manage the crisis are greatly needed. [1, 2]. In order to discover novel antibiotic agents, the combination of moieties/blocks of known antimicrobial agents has found its place between medicinal chemists and considered as a plausible alternative. All efforts in this field are directed to improve the drug potency and find a new class of antibiotics.

Among different forms of thiadiazole, 1,3,4-thiadiazole and its derivatives continue to be of great interest to a large number of researchers. The

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thiadiazole moiety is prevalent in biologically active compounds, exhibiting diverse activities involving anticonvulsant, anti-oxidant, anti-inflammatory, anti-neoplastic, anti-tubercular, anti-helicobacter pylori, and anti-fungal activity [3-7]. For example, Cefazolin (CFZL) and Cefazedone (CFZD) are 1,3,4-thiadiazole-containing antibiotics, used for the treatment of bacterial infections [8-10]. Cefazolin was patented in 1967 and came into commercial use in 1971. It is on the World Health Organization's List of Essential Medicines, which lists the most effective and safe medicines needed in a health system. The wholesale cost in the developing world is about US$1.20 to US$1.41 per day. 1,3,4-thiadiazoles are associated with diverse biocidal activities probably by the virtue of a toxophoric –N=C-S- grouping. Furthermore, the introduction of different groups at C-5 of thiadiazole nucleus involving benzyl- or 4-substituted benzyl and adamantly moiety enhanced the antibacterial and antifungal activity of the compound.

5-Nitroheteroaryl nucleus, including thiophene, furan and imidazole are the most important building blocks in bioactive agents [11-15]. The anti-bacterial effects of these systems have been assessed in recently synthesized compounds, containing 1,3,4-thiadiazole core with varied substituents at the C2-position of the thiadiazole ring [16]. The anti-microbial activities of nitroimidazole, nitrothiophene and nitrofuran have been shown against *amoeba* and *aerobic microbes*. Nitrofurans have been appeared in antibacterial drugs including furazidin, furazolidone and nitrofurantoin, applied for the treatment of urinary tract infections, diarrhea and enteritis in humans [17,18]. Regarding various reports concerning the antibacterial activities and special features of 5-nitroheteroaryl and 1,3,4-thiadiazoles, we decided to combine these moieties to synthesize [2-(5-nitro-2-furyl), 2-(5-nitro-2-thienyl) and 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)]-1,3,4-thiadiazole derivatives and then evaluated for their antibacterial activities by using MIC method.

Materials and Methods

Chemistry

In the present research, the required chemical substances, reagents and solvents were obtained from the reputable companies, Sigma-Aldrich and Merck. Electro-thermal IA-9100 capillary devices were used to calculate melting point, and there was no need to modify. A shimadzu 470 spectrograph using potassium bromide (KBr) disk was recruited to obtain IR spectra. A Bruker 80 MHz (Bruker Bioscience, Billerica, MA, USA), in deutrated solvents such as $CDCl_3$. recorded ^{1}H-NMR spectra, and the chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as an interval standard.

2-(5-Nitro-2-furyl)-5-(n-butylthio)-1,3,4-thiadiazole (6a)

To a mixture of 2-mercapto-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**4a**, 1 mmol) [22] and *n*-butyl bromide (1.5 mmol) in dimethyl formamide (20 ml), K_2CO_3 (1 mmol) was added portionwise and the mixture was stirred for 72 h at room temperature. Upon completion, the solvent was removed and the crude mixture was extracted with $CHCl_3$ (3×10ml). The yellow solid was obtained after recrystallization from ethanol-water. Yield: 87 %; mp 100-102 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3410, 1504, 1338. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.42 (d, 1H, J = 3.7 Hz), 7.29 (d, 1H, J = 3.7 Hz), 3.40 (t, 2H, S-CH_2, J = 7.0 Hz), 1.53-1.56 (m, 4H, S-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.97 (t, 3H, CH_3, J = 6.2 Hz).

2-(1-Methyl-5-nitro-1H-imidazol-2-yl)-5-(n-butylthio)-1,3,4-thiadiazole (6b)

Yield: 81 %; m.p. 87-88 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3375, 1512, 1335. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 8.06 (s, 1H, H_4-imidazole), 4.54 (s, 3H, N-CH_3), 3.31 (t, 2H, S-CH_2, J = 7.3 Hz), 1.49-1.53 (m, 4H, S-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.97 (t, 3H, CH_3, J = 6.1 Hz).

2-(5-Nitro-2-thienyl)-5-(n-butylthio)-1,3,4-thiadizaole (6c)

Yield: 64 %; m.p. 119-121 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3010, 1513, 1328. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.86 (d, 1H, J = 4.1 Hz), 7.25 (d, 1H, J = 4.1 Hz), 3.40 (t, 2H, S-CH_2, J = 7.5 Hz), 1.58-1.61 (m, 4H, S-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.96 (t, 3H, CH_3, J = 6.4 Hz).

2-(5-Nitro-2-furyl)-5-(n-pentylthio)-1,3,4-thiadiazole (6d)

Yield: 91 %; m.p. 72-74 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3431, 1504, 1350. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.42 (d, 1H, J = 3.8 Hz), 7.26 (d, 1H, J = 3.8 Hz), 3.30 (t, 2H, S-CH_2, J = 7.4 Hz), 1.40-1.45 (m, 6H, S-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.92 (t, 3H, CH_3, J = 7.0 Hz).

2-(1-Methyl-5-nitro-1H-imidazole-2-yl)-5-(n-pentylthio)-1,3,4-thiadiazole (6e)

Yield: 78 %; m.p. 112-114 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3345, 1523, 1334. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 8.07 (s, 1H, H_4-imidazole), 4.55 (s, 3H, N-CH_3), 3.40 (t, 2H, S-CH_2, J = 7.3 Hz), 1.39-1.45 (m, 6H, S-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$), 0.93 (t, 3H, CH_3, J = 6.8 Hz).

2-(5-Nitro-2-thienyl)-5-(n-pentylthio)-1,3,4-thiadiazole (6f)

Yield: 64 %; mp 157-159 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3130, 1506, 1420. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.87 (d, 1H, J = 3.5 Hz), 7.36 (d, 1H, J = 3.5 Hz), 3.40 (t, 2H, S-CH_2, J = 7.0 Hz), 1.38-141 (m, 6H, S-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$), 0.92 (t, 3H, CH_3, J = 7.0 Hz).

2-(1-Methyl-5-nitro-1H-imidazol-2-yl)-5-(n-butylsulfinyl)-1,3,4-thiadiazole (7a)

$NaHCO_3$ (0.35 mmol) was added to the mixture of compound **6b** (0.35 mmol) in CH_2Cl_2 (10 ml) and cooled to 0 ^{o}C. Then, *meta*-chloroperoxybenzoic acid (MCPBA) (60%, 0.35 mmol) was added to the mixture and the reaction was continued at this temperature for another 12 h. The reaction was extracted with $CHCl_3$ (3×10ml) and the crude was recrystallized from ethanol-water. Yield: 62 %; mp 99-101 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3152, 1529, 1356, 1088. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 8.10 (s, 1H, H_4-imidazole), 4.58 (s, 3H, N-CH_3), 3.30 (t, 2H, SO-CH_2, J = 7.1 Hz), 1.35-1.28 (m, 4H, SO-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.97 (t, 3H, CH_3, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 172.4, 152.9, 150.9, 140.2, 129.3, 53.7, 32.6, 25.0, 21.1, 13.5.

MS(m/z): 315 (M^+), 244, 210, 184, 131, 105, 71, 57, 48.

2-(5-Nitro-2-thienyl)-5-(n-butylsulfinyl)-1,3,4-thiadiazole (7b)

As expressed for **7a**, this compound was achieved from **6c**. Yield: 48 %; mp 109-111 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3050, 1504, 1330, 1050. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.93 (d, 1H, J = 4.0 Hz), 7.51 (d, 1H, J = 4.0 Hz), 3.45-3.20 (m, 2H, SO-CH_2), 2.02-1.27 (m, 4H, SO-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 1.00 (t, 3H, CH_3, J = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 172.1, 160.2, 151.2, 142.3, 129.2, 126.9, 53.7, 25.2, 21.1, 13.5. MS(m/z): 317 (M^+), 185, 131, 105, 73, 57, 48.

2-(1-Methyl-5-nitro-1H-imidazole-2-yl)-5-(n-pentylsulfinyl)-1,3,4-thiadiazole (7c)

Yield: 56 %; mp 91-93 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3136, 1520, 1353, 1049. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 8.10 (s, 1H, H_4-imidazole), 4.57 (s, 3H, N-CH_3), 3.45 (t, 2H, SO-CH_2, J = 7.5 Hz), 1.30 (m, 6H, SO-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-), 0.91 (t, 3H, CH_3, J = 7.3 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 172.4, 152.9, 150.9, 140.2, 129.3, 54.2, 32.7, 30.0, 23.1, 22.8, 14.2. MS(m/z): 329 (M^+), 210, 195, 185, 131, 119, 105, 71, 57, 48.

2-(5-Nitro-2-furyl)-5-(n-pentylsulfinyl)-1,3,4-thiadiazole (7d)

Yield: 66 %; mp 106 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3136, 1523, 1369, 1046. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.47-7.43 (m, 2H, H_4, H_3-furan), 3.19 (t, 2H, SO-CH_2, J = 7.6 Hz), 1.31-1.28 (m, 6H, SO-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$), 0.91 (t, 3H, CH_3, J = 7.2 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 171.5, 160.6, 153.4, 146.5, 115.2, 113.4, 54.2, 30.1, 23.2, 22.8, 14.1. MS(m/z): 315 (M^+), 210, 195, 169, 145, 119, 105, 71, 57, 48.

2-(5-Nitro-2-furyl)-5-(n-butylsulfonyl)-1,3,4-thiadiazole (8a)

$NaHCO_3$ (1.12 mmol) was added to the mixture of compound **6a** (0.35 mmol) in CH_2Cl_2 (10 ml) and cooled to 0 ^{o}C. Then, MCPBA (60%, 1.2 mmol) was added to the mixture and the reaction was continued at this temperature for another 72 h. The reaction was extracted with CHCl3 (3×10ml) and the crude was recrystallized from ethanol-water. Yield: 54 %; mp 106-108 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3100, 1539, 1353, 1150. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.51 (d, 1H, J = 3.5 Hz), 7.29 (d, 1H, J = 3.5 Hz), 3.50 (t, 2H, SO_2-CH_2, J = 7.0 Hz), 1.40-1.37 (m, 4H, SO_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.97 (t, 3H, CH_3, J = 6.6 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 162.8, 160.6, 153.4, 146.5, 115.4, 115.2, 53.8, 26.6, 20.7, 13.9. MS (m/z): 317 (M^+), 259, 195, 179, 138, 121, 64, 58, 43.

2-(5-Nitro-2-thienyl)-5-(n-butylsulfonyl)-1,3,4-thiadiazole (8b)

Yield: 68 %; mp 120-122 ^{o}C; IR (KBr, cm^{-1}) ν_{max}: 3125, 1510, 1334, 1160. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.92 (d, 1H, J = 4.0 Hz), 7.53 (d, 1H, J = 4.0 Hz), 3.67 (t, 2H, SO_2-CH_2, J = 7.0 Hz), 1.75-1.72 (m, 4H, SO_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$), 0.97 (t, 3H, CH_3, J = 6.6 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 162.1, 161.3, 151.2, 142.8, 129.1, 128.7, 53.6, 26.6, 20.7, 13.9. MS (m/z): 332 (M^+), 211, 179, 153, 121, 64, 58, 43.

2-(5-Nitro-2-furyl)-5-(n-pentylsulfonyl)-1,3,4-thiadiazole (8c)

As expressed for **8a**, this composition was obtained from **6d**. Yield: 73 %; mp 122 ^{o}C; IR (KBr, cm^{-1}) ν $_{max}$: 2940, 1532, 1350, 1170. ^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.50 (d, 1H, J = 3.0 Hz), 7.32 (d, 1H, J = 3.0 Hz), 3.48 (t, 2H, SO_2-CH_2, J = 7.0 Hz), 1.28-1.26 (m, 6H, SO_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$), 0.91 (t, 3H, CH_3, J = 7.5 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 162.8, 160.6, 153.4, 146.5, 115.4, 115.2, 54.0, 29.7, 24.3, 22.1, 14.0. MS (m/z): 331 (M^+), 195, 135, 121, 71, 64, 57, 43.

2-(5-Nitro-2-thienyl)-5-(n-pentylsulfonyl)-1,3,4-thia-

diazole (8d)

Yield: 67 %; mp 146 °C; IR (KBr, cm^{-1}) ν_{max}: 3062, 1490, 1319, 1150 .^{1}H-NMR ($CDCl_3$, 80 MHz) δ (ppm): 7.97 (d, 1H, J = 4.8 Hz), 7.55 (d, 1H, J = 4.8 Hz), 3.60 (t, 2H, SO_2-CH_2, J = 6.9 Hz), 1.45-1.39 (m, 6H, SO_2-CH_2-CH_2-CH_2-CH_2), 0.92 (t, 3H, CH_3, J = 7.3 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 162.1, 161.2, 151.2, 142.8, 129.2, 128.6, 54.1, 29.6, 24.4, 22.1, 14.1. MS (m/z): 347 (M^+), 193, 211, 153, 135, 71, 64, 57, 43.

Pharmacology

Anti-microbial activity

Minimal inhibitory concentration (MIC) was measured to assess anti-microbial effect of the synthesized compounds using Mueller-hinton agar medium. 6.4 mg of each study compounds was dissolved in the minimum amount of DMSO (dimethyl sulfoxide) and distilled water added up to 10 ml. Eight serial dilutions were created up to half from this solution; and for preparing the concentrations ranging from 0.5 to 64 µg/ml, 2 ml of them was poured on 18 ml Muller-Hinton agar medium at 50 °C. The 24 h. bacterial suspensions were provided equal to optical density of 0.5 McFarland Standard solution (0.08-0.1), and five µl after diluting by 0.1 was inoculated to each plate that incubated for 24 h. at 37 °C. There were also negative and positive controls for DMSO to omit the effect of it, as well as confirm the microbial suspension purity and contamination.

Bacterial strains

The 24h bacterial cultures of *Staphylococcus epidermidis (S.epidermidis, Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumonia), Serratia marcescens (S. marcescens), Streptococcus pyogenes (S. pyogenes), Micrococcus luteus (M. luteus)* were used to assess *in vitro* antibacterial activity of the compounds (Table 1).

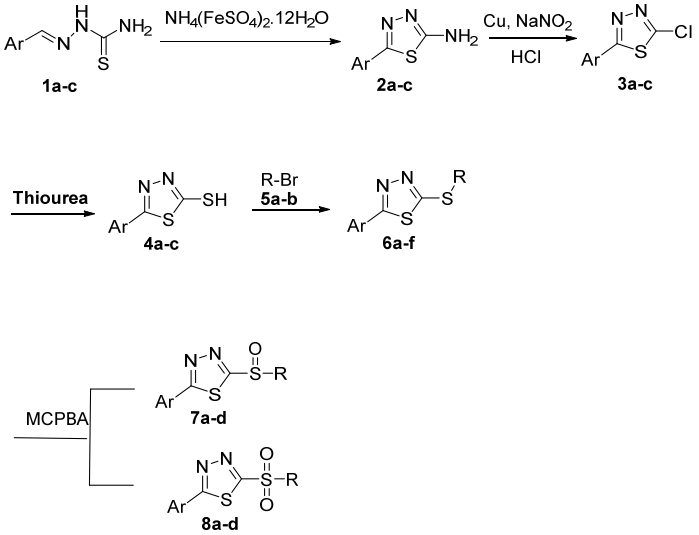
Results and Discussion

Chemistry

As outlined in Scheme 1, arylthiosemicarbazones (**1a-c**), prepared according to previously reported method, underwent cyclization by the action of ammonium ferric sulfate to produce 2-amino-5-

Table 1. The study bacterial strains along with Persian Type Culture Collection (PTCC) code

Bacterial strain	PTCC
Staphylococcus epidermidis (S.epidermidis)	1114
Bacillus subtilis (B. subtilis)	1023
Escherichia coli (E. coli)	1330
Pseudomonas aeruginosa (P. aeruginosa)	1074
Klebsiella pneumoniae (K. pneumonia)	1053
Serratia marcescens (S. marcescens)	1621
Streptococcus pyogenes (S. pyogenes)	1447
Micrococcus luteus (M. luteus)	1110



Scheme 1. Synthesis of **6a-f**, **7a-d** and **8a-d**

(het)aryl-1,3,4-thiadiazoles (**2a-c**). Diazotization by using copper powder in hydrochloric acid gave 2-heteroaryl-5-chloro-1,3,4-thiadiazoles (**3a-c**) [19-22] which was converted to **4a-c** upon the reaction with thiourea in refluxing ethanol. S-Alkylation reaction with *n*-butyl- and *n*-pentyl bromide in dimethyl formamide (DMF) gave **(5a-b**), which upon oxidation afforded sulfinyl and sulfonyl derivatives. By utilizing 1 and 4 equivalent *meta*-chloroperoxybenzoic acid as an oxidant, the desired compounds **7a-d** and **8a-d** were obtained, respectively.

Antibacterial assay

The antibacterial effects of the synthesized compounds were evaluated against Gram-positive and Gram-negative strains, using MIC assay. The results were compared with ciprofloxacin as the standard drug (Table 2, 3). The importance of 5-nitroheterocycles including furan, thiophene and *N*-methyl imidazole as anti-Gram-positive agents has been reported in the literature by our research team. In addition, the antibacterial activity of different heterocyclic cores, like oxazolidinone and 7-piperazinylquinolones was also

Table 2. Anti-microbial effect of synthesized compounds against Gram-positive bacteria using MIC (µg/mL)

			Gram-positive			
Compound	**R**	**Ar**	***S.epidermidis***	***B. subtilis***	***S. pyogenes***	***M. luteus***
6a	butyl	5-nitro-furan-2-yl	≤0.5	≤0.5	0.5<MIC≤1	8<MIC≤16
6b	butyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	≤0.5	≤0.5	0.5<MIC≤1	32<MIC≤64
6c	butyl	5-nitro-thiophen-2-yl	16<MIC≤32	>64	0.5<MIC≤1	>64
6d	pentyl	5-nitro-furan-2-yl	≤0.5	≤0.5	≤0.5	0.5<MIC≤1
6e	pentyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	8<MIC≤16	8<MIC≤16	4<MIC≤8	8<MIC≤16
6f	pentyl	5-nitro-thiophen-2-yl	1<MIC≤2	>64	>64	>64
7a	butyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	>64	8<MIC≤16	4<MIC≤8	8<MIC≤16
7b	butyl	5-nitro-thiophen-2-yl	16<MIC≤32	8<MIC≤16	0.5<MIC≤1	>64
7c	pentyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	>64	16<MIC≤32	16<MIC≤32	16<MIC≤32
7d	pentyl	5-nitro-furan-2-yl	>64	2<MIC≤4	32<MIC≤64	32<MIC≤64
8a	butyl	5-nitro-furan-2-yl	>64	16<MIC≤32	32<MIC≤64	4<MIC≤8
8b	butyl	5-nitro-thiophen-2-yl	8<MIC≤16	>64	32<MIC≤64	>64
8c	pentyl	5-nitro-furan-2-yl	16<MIC≤32	8<MIC≤16	>64	8<MIC≤16
8d	pentyl	5-nitro-thiophen-2-yl	4<MIC≤8	>64	32<MIC≤64	16<MIC≤32
Ciprofloxacin	-	-	0.5<MIC≤1	0.25<MIC≤0.5	32<MIC	8<MIC≤16

Table 3. Anti-microbial effect of synthesized compounds against Gram-negative bacteria using MIC (µg/mL)

			Gram-negative			
Compound	**R**	**Ar**	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	***S. marcescens***
6a	butyl	5-nitro-furan-2-yl	>64	>64	>64	>64
6b	butyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	>64	>64	>64	>64
6c	butyl	5-nitro-thiophen-2-yl	>64	>64	>64	>64
6d	pentyl	5-nitro-furan-2-yl	>64	>64	>64	>64
6e	pentyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	>64	>64	>64	>64
6f	pentyl	5-nitro-thiophen-2-yl	>64	0.5<MIC≤1	>64	>64
7a	butyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	>64	>64	>64	>64
7b	butyl	5-nitro-thiophen-2-yl	32<MIC≤64	>64	>64	>64
7c	pentyl	1-methyl-5-nitro-1*H*-imidazole-2-yl	>64	>64	>64	>64
7d	pentyl	5-nitro-furan-2-yl	>64	>64	>64	>64
8a	butyl	5-nitro-furan-2-yl	>64	>64	>64	>64
8b	butyl	5-nitro-thiophen-2-yl	>64	4<MIC≤8	>64	32<MIC≤64
8c	pentyl	5-nitro-furan-2-yl	>64	>64	16<MIC≤32	>64
8d	pentyl	5-nitro-thiophen-2-yl	32<MIC≤64	>64	>64	>64
Ciprofloxacin	-	-	1<MIC≤2	0.25<MIC≤0.5	≤0.125	≤0.125

examined and reported [23, 24]. In these reports, nitrofuran analogues exhibited more potent inhibitory activities compared to other heterocyclic counterparts. Interestingly, similar results were obtained in this study, proved us that working on this heterocyclic core is a right way to generate novel antibacterial agents. None of compounds showed superior antibacterial activity compared to those of ciprofloxacin. Compounds **6a**, **6b** and **6d** had strong antibacterial effects against the Gram-positive bacteria in comparison with positive control. In general, no or weak effects on Gram-negative bacteria were observed, whereas compound **6f** had an average anti *P. aeruginosa* activity. The lengthening of alkyl chain did not improve the activity of **6a-f.**

The oxidation of thioalkyl side chain led to weak activity against Gram-positive and Gram-negative bacteria. According to the findings, the antibacterial activity was decreased in sulfinyl containing derivatives. Of compounds containing 5-nitro-2-furyl core, the highest antibacterial effect on Gram-positive bacteria was found in compound **6d** with *n*-Pentyl moiety (Table 2). In addition, the maximum antibacterial activity between thioalkyl bearing side chain derivatives was related to 5-nitro-2-furyl containing derivatives.

Conclusions

Based on *in vitro* assessing antibacterial effects of novel synthesized series of 5-nitro hetero aryl-1,3,4-thiadizole derivatives, the synthesized compounds showed the highest resistant on Gram-negative bacteria. Among these, the best antibacterial activity against Gram-positive bacteria was observed in the compound **6d** bearing *n*-Pentyl residue. Regarding the good activity of nitrofuran containing compounds in this report and weak inhibitory activities, we believe more structural modifications are necessary to achieve more potent compounds.

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