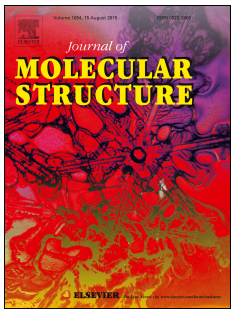

New 1,3,4-thiadiazoles based on thiophene-2-carboxylic acid: Synthesis, characterization, and antimicrobial activities

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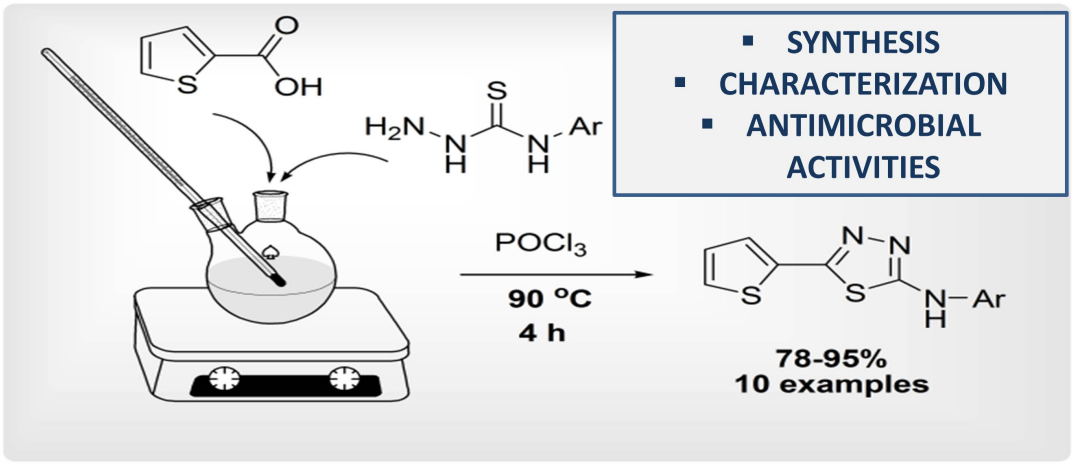
SYNTHESIS
CHARACTERIZATION
ANTIMICROBIAL ACTIVITIES
POCl3
90 °C
4 h
78-95%
10 examples

New 1,3,4-Thiadiazoles Based on Thiophene-2-Carboxylic Acid: Synthesis, Characterization, and Antimicrobial Activities

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Abstract

Novel 1,3,4-thiadiazole derivatives were prepared by cyclization reaction of thiophene-2-carboxylic acid with *N*-arylthiosemicarbazides and $POCl_3$. The antibacterial activities of the synthesized compounds were tested against Gram-negative bacteria (*Salmonella enteritidis, Salmonella typhimurium, Enterobacter aerogenes, Salmonella infantis, Salmonella kentucky, and Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, and Enterococcus durans*), and the fungus *Candida albicans* using the disk diffusion method. Selected 1,3,4-thiadiazole derivatives exhibited effective antimicrobial activity against *Staphylococcus aureus*, *C. albicans*, *S. typhimurium*, *Enterobacter aerogenes*, and *S. kentucky*. Therefore, 1,3,4-thiadiazole derivatives can be considered as bioactive agents for pharmacological and medicinal applications. The synthesized compounds were characterized by using IR, ^{1}H NMR, and ^{13}C NMR spectroscopies and elemental analysis.

Keywords: thiophene-2-carboxylic acid, 1,3,4-thiadiazoles, *N*-arylthiosemicarbazides, antimicrobial activity, spectroscopic methods.

1. Introduction

Heterocyclic chemistry is one of the most active fields of synthetic organic chemistry research. Thiazoles, thiadiazoles, indoles, oxadiazoles, and pyrroles are important classes of heterocyclic compounds due to their interesting biological and synthetic applications [1-3]. Thiadiazole is a heterocyclic compound containing both nitrogen and sulfur atoms as part of the five-membered aromatic ring. There are four isomeric forms of thiadiazole: 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole; and 1,3,4-thiadiazole. The thermally most stable among these four isomers is 1,3,4-thiadiazole [4].

1,3,4-Thiadiazoles have been widely studied for their biological and therapeutic activities with the following reported properties; antileishmanial [5], antimicrobial [6,7], antifungal [8,9], antibacterial [8,10], analgesic [11], anticonvulsant [12,13], antioxidant [14], anti-inflammatory [15], antipsychotic [16], antitumor [17,18], antidepressive [19], antihypertensive [20], antiviral [21], antihistamine [22], and antitubercular [23]. Additionally, 1,3,4-thiadiazoles are broadly used in agricultural applications, such as in pesticides, herbicides, fungicides, insecticides, and bactericides [23-25].

In this study, *N*-aryl-1,3,4-thiadiazole derivatives were prepared through the reaction of thiophene-2-carboxylic acid, *N*-arylthiosemicarbazides, and phosphorous oxychloride ($POCl_3$). The synthesized thiadiazoles were characterized (IR, NMR, and elemental analysis) and studied as antimicrobial agents against 10 microorganisms.

2. Experimental

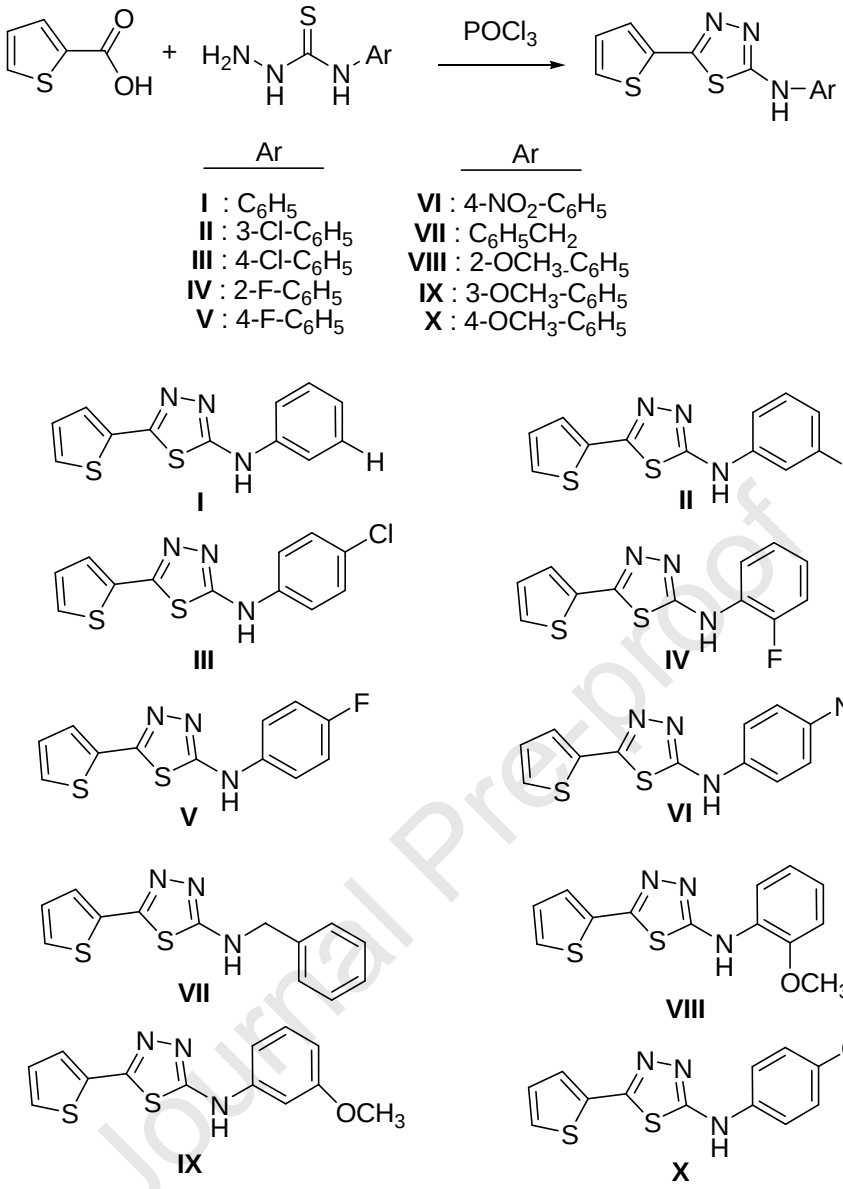
2.1. Measurement and reagents

All starting materials and solvents were purchased from Aldrich, Carlo Erba, Acrõs Organics, or Merck Chemical Company and used without further purification. Melting points

(uncorrected) were measured on a Stuart SMP 30 electrothermal apparatus. The elemental analysis was performed on CHNS-932 (LECO). IR spectra were recorded on Bruker Alpha FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a JEOL-ECS 400 MHz spectrometer using tetramethylsilane as an internal standard. The splitting patterns are indicated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), td (triplet of doublet) and m (multiplied).

2.2. Synthesis

The general procedure for thiadiazole synthesis was as follows: The mixture of 2-thiophenecarboxylic acid (n mol) and *N*-arylthiosemicarbazide derivatives (n mol) was chilled in a refrigerator and phosphorous oxychloride (3n mol) was added drop-wise by stirring. Then, refluxing was continued 90^{o}C for 4 h. After completion of the reaction, the mixture was cooled to room temperature, poured into ice-cold water with stirring, and then neutralized with ammonia. The precipitated product was filtered, washed with water, and crystallized in a suitable solvent. Novel thiadiazole derivatives were synthesized according to the method [6] illustrated in Scheme 1.



Scheme 1. Synthetic overview of 1,3,4-thiadiazole formation.

Synthesis of *N*-phenyl-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (I): Brown solid. Yield: 88%. m.p. 70–72 °C. IR (KBr) υ_{max}/cm^{-1}: 3360.4 (stretching, –NH), 3099.9 ($Ar_{benzene}$, C–H), 2981.6 ($Ar_{thiophene}$, C–H), 1600.1 (thiadiazole, C=N), 709.2 (C–S–C). ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 10.48 (s, 1H, –NH), 7.68-7.66 (1H, d, *J* = 4.9 Hz, thiophene), 7.60-7.58 (2H, d, *J* = 7.9 Hz, ArH), 7.55-7.50 (1H, d, *J* = 4.0 Hz, thiophene), 7.34-7.30 (2H, t, *J* = 7.9 Hz, ArH), 7.14-7.12 (1H, t, *J* = 4.3 Hz, thiophene), 7.00-6.96 (1H, t, *J* = 7.3 Hz, ArH) ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 164.1, 152.4, 140.9, 132.9, 129.7, 129.1, 128.7, 122.7,

118.1, 117.3. Anal. Calcd. for $C_{12}H_9N_3S_2$ (%): C, 55.58; H, 3.50; N, 16.20; found: C, 55.33; H, 3.45; N, 15.99.

Synthesis of *N*-(3'-chlorophenyl-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (II): White solid. Yield: 84%. m.p. 166–168 °C. IR (KBr) υ_{max}/cm^{-1} : 3185.0 (stretching, –NH), 3052.3 ($Ar_{benzene}$, C–H), 2878.8 ($Ar_{thiophene}$, C–H), 1593.8 (thiadiazole, C=N), 716.0 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 10.69 (s, 1H, –NH), 7.87 (s, 1H, ArH), 7.70-7.68 (1H, d, *J* = 4.9 Hz, thiophene), 7.57-7.56 (1H, d, *J* = 3.7 Hz, thiophene), 7.41-7.39 (1H, dd, *J* = 7.3, 1.2 Hz, ArH), 7.35-7.31 (1H, t, *J* = 8.2 Hz, ArH), 7.15-7.13 (1H, t, *J* = 4.0 Hz, thiophene), 7.04-7.01 (1H, dd, *J* = 7.3, 1.8 Hz, ArH). ^{13}C NMR (DMSO-d_6) δ/ppm: 163.59, 153.15, 142.19, 134.07, 132.70, 131.26, 129.69, 129.41, 128.78, 122.19, 117.46, 116.51. Anal. Calcd. for $C_{12}H_8N_3S_2Cl$ (%): C, 49.06; H, 2.74; N, 14.30; found: C, 48.74; H, 2.88; N, 14.19.

Synthesis of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (III): White solid. Yield: 86%. m.p. 223 °C. IR (KBr) υ_{max}/cm^{-1} : 3250.3 (stretching, –NH), 3036.8 ($Ar_{benzene}$, C–H), 2981.3 ($Ar_{thiophene}$, C–H), 1596.3 (thiadiazole, C=N), 690.2 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 8.24 (1H, s, –NH), 7.67-7.66 (1H, d, *J* = 5.0 Hz, thiophene), 7.63-7.61 (2H, d, *J* = 8.7 Hz, ArH), 7.53-7.52 (1H, d, *J* = 3.7 Hz, thiophene), 7.37-7.35 (2H, d, *J* = 8.7 Hz, ArH), 7.14-7.12 (1H, t, *J* = 4.7 Hz, thiophene). ^{13}C NMR (DMSO-d_6) δ/ppm: 163.8, 152.9, 152.6, 139.8, 132.6, 129.5, 129.3, 128.8, 126.1, 119.6. Anal. Calcd. for $C_{12}H_8N_3S_2Cl$ (%): C, 49.06; H, 2.74; N, 14.30; found: C, 48.95; H, 2.69; N, 14.17.

Synthesis of *N*-(2'-fluorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (IV): Yellow solid. Yield: 78%. m.p. 108 °C. IR (KBr) υ_{max}/cm^{-1}: 3434.6 (stretching, –NH), 3124.3 ($Ar_{benzene}$, C–H), 2981.9 ($Ar_{thiophene}$, C–H), 1622.1 (thiadiazole, C=N), 613.2 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 10.29 (1H, s, –NH), 8.33-8.29 (1H, t, *J* = 8.2 Hz, J_{HF} = 8.1 Hz, ArH), 7.67-7.66 (1H, d, *J* = 5.0 Hz, thiophene), 7.53-7.52 (1H, d, *J* = 3.2 Hz, thiophene),

7.27-7.16 (2H, m, ArH), 7.14-7.12 (1H, t, *J* = 4.2 Hz, thiophene), 7.06-7.00 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.2, 153.7, 153.6, 151.3, 132.9, 132.2, 130.6, 129.4, 129.2, 129.1, 128.8, 125.4, 125.3, 123.9, 123.8, 121.2, 115.9, 115.7. Anal. Calcd. for $C_{12}H_8N_3S_2F$ (%): C, 51.97; H, 2.91; N, 15.15; found: C, 51.88; H, 2.88; N, 14.99.

Synthesis of *N*-(4'-fluorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (V): White solid. Yield: 82%, mp: 175–178 °C. IR (KBr) υ_{max}/cm^{-1} : 3259.7 (stretching, –NH), 3164.6 ($Ar_{benzene}$, C–H), 2876.3 ($Ar_{thiophene}$, C–H), 1587.5 (thiadiazole, C=N), 631.7 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 10.49 (s, 1H, NH), 7.68-7.66 (1H, d, *J* = 4.9 Hz, thiophene), 7.63-7.59 (2H, d, *J* = 4.3 Hz, J_{HF} = 3.4 Hz, ArH), 7.53-7.51 (1H, d, *J* = 3.7 Hz, thiophene), 7.12-7.19 (3H, m, thiophene(1H) and ArH (2H, *J* = 9.2 Hz, J_{HF} = 9.1 Hz)). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.20, 159.11, 157.08, 156.74, 152.41, 137.45, 132.89, 129.38, 129.15, 128.73, 119.86, 119.77, 116.35, 116.12. Anal. Calcd. for $C_{12}H_8N_3S_2F$ (%): C, 51.97; H, 2.91; N, 15.15; found: C, 51.73; H, 2.87; N, 14.93.

Synthesis of *N*-(4'-nitrophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (VI): Yellow solid. Yield: 95%. m.p. 237–240 °C. IR (KBr) υ_{max}/cm^{-1} : 3168.9 (stretching, –NH), 3044.4 ($Ar_{benzene}$, C–H), 2913.4 ($Ar_{thiophene}$, C–H), 1583.3 (thiadiazole, C=N), 687.3 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 11.21 (s, 1H, -NH), 8.22-8.20 (2H, d, *J* = 8.5 Hz, ArH), 7.80-7.78 (2H, d, *J* = 9.2 Hz, ArH), 7.71-7.70 (1H, d, *J* = 4.9 Hz, thiophene), 7.59-7.58 (1H, d, *J* = 3.1 Hz, thiophene), 7.15-7.13 (1H, t, *J* = 4.0 Hz, thiophene). ^{13}C NMR (DMSO-d_6) δ/ppm: 162.9, 154.5, 146.6, 141.5, 132.5, 130.1, 129.8, 128.8, 126.0, 117.6. Anal. Calcd. for $C_{12}H_8N_4O_2S_2$ (%): C, 47.36; H, 2.65; N, 18.41; found: C, 46.98; H, 2.76; N, 18.44.

Synthesis of *N*-benzyl-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (VII): Yellow solid. Yield: 90%. m.p. 95 °C; IR (KBr) υ_{max}/cm^{-1} : 3188.0 (stretching, –NH), 3080.0 ($Ar_{benzene}$, C–H), 2981.7 ($Ar_{thiophene}$, C–H), 1571.7 (thiadiazole, C=N), 693.1 (C–S–C). ^{1}H NMR (DMSO-

d_6) δ/ppm: 8.42-8.39 (1H, t, *J* = 5.5 Hz, -NH), 7.59-7.58 (1H, d, *J* = 4.9 Hz, thiophene), 7.38-7.37 (1H, d, *J* = 4.2 Hz, thiophene), 7.35-7.29 (4H, m, ArH), 7.25-7.22 (1H, m, ArH), 7.09-7.07 (1H, t, *J* = 4.3 Hz, thiophene), 4.49-4.48 (2H, d, *J* = 5.5 Hz, $-CH_2$). ^{13}C NMR (DMSO-d_6) δ/ppm: 168.50, 151.04, 138.95, 133.52, 128.95, 128.55, 128.31, 128.28, 128.10, 127.74, 48.61. Anal. Calcd. for $C_{13}H_{11}N_3S_2$ (%): C, 57.12; H, 4.06; N, 15.37; found: C, 56.97; H, 3.98; N, 15.16.

Synthesis of *N*-(2'-methoxyhenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (VIII): Yellow solid. Yield: 87%. m.p. 68–70 °C. IR (KBr) υ_{max}/cm^{-1} : 3187.1 (stretching, –NH), 3082.5 ($Ar_{benzene}$, C–H), 2928.3 ($Ar_{thiophene}$, C–H), 1569.5 (thiadiazole, C=N), 694.8 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 9.85 (1H, s, –NH), 8.24-8.22 (1H, d, *J* = 8.5 Hz, ArH), 7.64-7.62 (1H, d, *J* = 4.9 Hz, thiophene), 7.48-7.46 (1H, d, *J* = 3.7 Hz, thiophene), 7.13-7.10 (1H, t, *J* = 4.3 Hz, thiophene), 7.03-6.91 (3H, m, ArH), 3.83 (3H, $-OCH_3$). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.7, 153.0, 148.9, 133.1, 129.9, 129.0, 128.9, 128.7, 123.6, 121.2, 119.7, 111.6, 56.3. Anal. Calcd. for $C_{13}H_{11}N_3OS_2$ (%): C, 53.96; H, 3.83; N, 14.52; found: C, 53.88; H, 3.75; N, 14.28.

Synthesis of *N*-(3'-methoxyhenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**IX**): White–yellow solid. Yield: 89%. m.p. 201–204 °C. IR (KBr) υ_{max}/cm^{-1} : 3267.0 (stretching, –NH), 3062.8 ($Ar_{benzene}$, C–H), 2960.7 ($Ar_{thiophene}$, C–H), 1578.3 (thiadiazole, C=N), 699.7 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 10.49 (s, 1H, -NH), 7.68-7.66 (1H, d, *J* = 4.9 Hz, thiophene), 7.53-7.52 (1H, d, *J* = 3.1 Hz, thiophene), 7.31 (1H, s, ArH), 7.24-7.20 (1H, t, *J* = 8.2 Hz, ArH), 7.15-7.12 (1H, t, *J* = 4.2, thiophene), 7.07-7.05 (1H, dd, *J* = 7.9, 1.8 Hz, ArH), 6.58-6.56 (1H, dd, *J* = 8.2, 2.1 Hz, ArH), 3.72 (3H, s, $-OCH_3$). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.0, 160.5, 152.5, 142.1, 132.9, 130.5, 129.4, 129.2, 128.7, 110.7, 108.1, 104.1, 55.6. Anal. Calcd. for $C_{13}H_{11}N_3OS_2$ (%): C, 53.96; H, 3.83; N, 14.52; found: C, 53.74; H, 3.78; N, 14.60.

Synthesis of *N*-(4'-methoxyhenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (X): Yellow solid. Yield: 93%. m.p. 181–184 °C. IR (KBr) υ_{max}/cm^{-1} : 3241.3 (stretching, –NH), 3111.3 ($Ar_{benzene}$, C–H), 2981.9 ($Ar_{thiophene}$, C–H), 1588.2 (thiadiazole, C=N), 698.5 (C–S–C). ^{1}H NMR (DMSO-d_6) δ/ppm: 10.27 (1H, s, –NH), 7.64-7.62 (1H, dd, *J* = 5.0, 0.9 Hz, thiophene), 7.51-7.47 (2H, d, J = 8.2 Hz, ArH), 7.47-7.45 (1H, d, *J* = 3.7 Hz, thiophene), 7.13-7.10 (1H, t, *J* = 4.3 Hz, thiophene), 6.92-6.88 (2H, d, *J* = 8.2 Hz, ArH), 3.69 (3H, –OCH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.9, 155.3, 151.4, 134.4, 133.1, 129.0, 128.8, 128.7, 120.1, 114.9, 55.8. Anal. Calcd. for $C_{13}H_{11}N_3OS_2$ (%): C, 53.96; H, 3.83; N, 14.52; found: C, 53.90; H, 3.75; N, 14.73.

2.3. Antimicrobial Activity Testing

2.3.1. Microorganism Strains

Gram negative (*S. enteritidis*, *S. typhimurium*, *Enterobacter aerogenes*, *S. infantis*, *S. kentucky*, and *E. coli*) and Gram positive (*Staphylococcus aureus* (ATCC 25923), *B. subtilis*, and *Enterococcus durans*) bacteria were selected for antibacterial activity testing. The fungus *C. albicans* (ATCC 26555) was selected for antifungal activity testing. Streptomycin and ketoconazole were used as reference clinical standards for the antibacterial and antifungal activities, respectively. Most strains were ATCC, DSMZ, and SL type standard ones. Other strains, which have no standard ID information, were isolated from food samples and identified in the Department of Biology at Ankara University.

2.3.2. Disc Diffusion Method

The disk diffusion method published by Andrews was used for antimicrobial activity testing [26]. Petri dishes (90 mm diameter) containing 25 mL of Mueller–Hinton Agar were used [27,28]. The newly synthesized 1,3,4-thiadiazole derivatives were dissolved in ethanol to

make a final concentration of 5 mg/mL. Thereafter, 30 µg/mL, 50 µg/mL, and 80 µg/mL samples were loaded on empty sterile antibiotic disks. The disks were maintained at room temperature for 24 h in aseptic conditions. Microorganism suspensions (0.5 McFarland) were inoculated on the surfaces of the Mueller–Hinton Agar plates and kept in aseptic conditions for 2–3 min before application to the disks [29]. After 24 h, the zone of inhibition, including the disk, was measured. The zone diameters were measured in millimeters. The disc diffusion assay was performed in triplicate. Compounds **III**, **VI**, and **IX** were not tested because they could not be dissolved in solvent.

3. Results and Discussion

3.1. Spectral characterization

Physical data, IR, ^{1}H NMR, and ^{13}C NMR of the synthesized compounds are presented in Tables 1–4, respectively.

Table 1. Physical characterization data for synthesized 1,3,4-thiadiazoles.

Compounds	-Ar	IUPAC name	Yield %	Melting Point (°C)	Elemental Analysis (Theoretical/Found)
I	-C_6H_5	*N*-(phenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	88	70–72	(%): C, 55.58; H, 3.50; N,16.20 (%): C, 55.33; H, 3.45; N, 15.99
II	3-Cl-C_6H_5	*N*-(3'-chlorophenyl)-5 (thiophen-2-yl)-1,3,4 thiadiazol-2-amine	84	166–168	(%): C, 49.06; H, 2.74; N,14.30 (%): C, 48.74; H, 2.88; N, 14.19
III	4-Cl-C_6H_5	*N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	86	223	(%): C, 49.06; H, 2.74; N,14.30 (%): C, 48.95; H, 2.69; N, 14.17
IV	2-F-C_6H_5	*N*-(2'-fluorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	78	108	(%): C, 51.97; H, 2.91; N,15.15 (%): C, 51.88; H, 2.88; N, 14.99
V	4-F-C_6H_5	*N*-(4'-fluorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	82	175–178	(%): C, 51.97; H, 2.91; N,15.15 (%): C, 51.73; H, 2.87; N, 14.93
VI	4-NO_2-C_6H_5	*N*-(4'-nitrophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	95	237–240	(%): C, 47.36; H, 2.65; N,18.41 (%): C, 46.98; H, 2.76; N, 18.44
VII	$C_6H_5CH_2$	*N*-(benzyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	90	95	(%): C, 57.12; H, 4.06; N,15.37 (%): C, 56.97; H, 3.98; N, 15.16
VIII	2-OCH_3-C_6H_5	*N*-(2'-methoxyhenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	87	68–70	(%): C, 53.96; H, 3.83; N,14.52 (%): C, 53.88; H, 3.75; N, 14.28
IX	3-OCH_3-	*N*-(3'-methoxyhenyl)-5-	89	201–204	(%): C, 53.96; H, 3.83; N,14.52

	C_6H_5	(thiophen-2-yl)-1,3,4-thiadiazol-2-amine			(%): C, 53.74; H, 3.78; N, 14.60
X	4-OCH_3-C_6H_5	*N*-(4'-methoxyhenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine	93	181–184	(%): C, 53.96; H, 3.83; N,14.52 (%): C, 53.90; H, 3.75; N, 14.73

3.2. Interpretation of the IR Spectra

IR spectra were obtained between 4000 and 200 cm^{-1} on a Bruker Alpha FT-IR spectrophotometer. At the end of the reaction, the carbonyl group absorption on the starting material (carboxylic acid) was neither observed near 1700 cm^{-1} nor the OH stretching band observed at 3500–2700 cm^{-1}. Furthermore, the asymmetric and symmetric stretching bands of the *N*-arylthiosemicarbazides amino group (–NH_2) were not observed at 3500–3200 cm^{-1}. Instead, new absorptions for the amine group (–NH) stretching vibrations were observed at 3434.6–3168.9 cm^{-1}, absorptions for –C=N group on the thiadiazole ring were observed at 1622.1–1569.5 cm^{-1}, the –C-N group absorptions were observed at 1292.3-1194.7cm^{-1}, and C–S–C vibrations from the thiadiazole ring were observed at 716.0–613.2 cm^{-1} for the synthesized compounds (**I–X**). The other remarkable very strong vibrational bands were in the spectrum of compounds **II-X** resulting from the Ar–Cl, Ar-F, -NO_2, -CH_2, -C-O functions, respectively. Ar-Cl absorption bands of compounds **II** and **III** were observed at 866.2 and 871.3 cm^{-1}. Ar-F absorption bands were observed at 845.9 and 827.3 cm^{-1} for compounds **IV** and **V**. The asymmetric and symmetric stretching bands of the nitro group (–NO_2) were observed at around 1431.9 and 1304.5 cm^{-1} for compound **VI**. The -CH_2 absorption band of compound **VII** was observed at 2888.7 cm^{-1}. The –C-O group absorption bands of compounds **VIII-X** were observed at 1245.1, 1234.7, and 1226.6 cm^{-1}, respectively. These observations provided key evidence for thiadiazole formation. These data are consistent with the values of earlier reported for similar compounds [1,6,12,30]. Table 2 lists the key IR absorptions of the synthesized thiadiazole, and a representative IR spectrum of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**III**) is presented in Figure 1.

Table 2. Key IR absorption frequencies of the synthesized compounds (**I–X**).

Compounds	$\upsilon_{(-NH)\text{-stretching}}$	$\upsilon_{C\text{-}H\ (Aromatic)}$	$\upsilon_{C\text{-}H\ (Ar\text{-}thiophene)}$	$\upsilon_{C=N\ (thiadiazole)}$	$\upsilon_{C\text{-}N}$	$\upsilon_{C\text{-}S\text{-}C}$
I	3360.4	3099.9	2981.6	1600.1	1255.3	709.2
II	3185.0	3052.3	2878.8	1593.8	1259.0	716.0
III	3250.3	3036.8	2981.3	1596.3	1241.2	690.2
IV	3434.6	3124.3	2981.9	1546.4	1249.9	613.2
V	3259.7	3164.6	2876.3	1587.5	1258.8	631.7
VI	3168.9	3044.4	2913.4	1583.3	1194.7	687.3
VII	3187.1	3082.5	2928.3	1569.5	1259.5	694.8
VIII	3207.8	3102.3	2981.8	1599.9	1292.3	699.3
IX	3267.0	3062.8	2960.7	1578.3	1250.4	699.7
X	3241.3	3111.3	2981.9	1588.2	1278.3	698.5

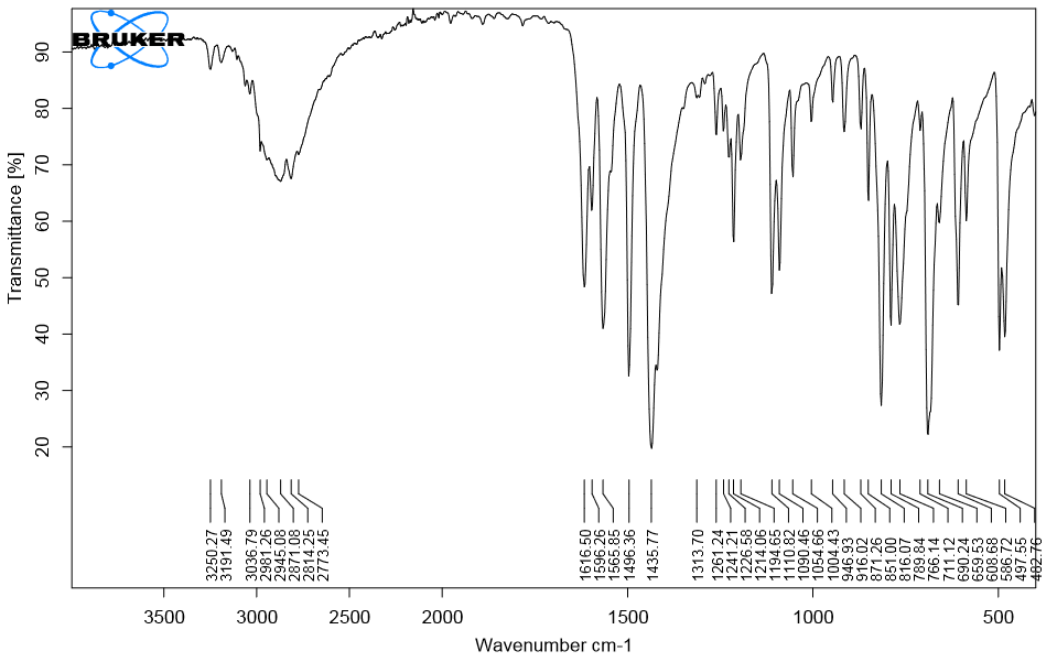


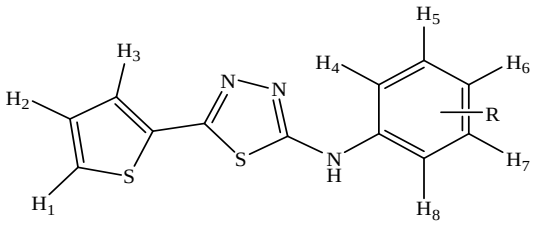
Figure 1. IR spectrum of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**III**).

3.3. Interpretation of the ^{1}H NMR Spectra

The ^{1}H NMR spectra of the synthesized compounds were recorded in DMSO-d_6 and the chemical shifts are presented in Table 3. For compounds **I-X**, aromatic proton signals of the

benzene ring were observed between 8.33 and 6.56 ppm, and aromatic proton signals of the thiophene region were observed between 7.71 and 7.07 ppm. In compounds (**VIII**, **IX**, and **X**), –OCH_3 proton signals were observed as a singlet in the range of 3.83–3.69 ppm. The –NH proton signals were observed as singlets in the range of 11.21-8.24 ppm for all the compounds. For compound **VII**, the methylene group proton signal was detected as a doublet at 4.49-4.48 (2H, d, *J* = 5.5 Hz) ppm; the –NH proton signals were observed as a triplet 8.42-8.39 (1H, t, *J* = 5.5 Hz) A representative ^{1}H NMR spectrum of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**III**) is presented in Figure 2. As seen in compound **III**, while aromatic protons (H4-H8) of benzene ring were observed at 7.63-7.35 ppm, aromatic protons (H1-H3) of thiophene ring were observed at 7.67-7.12 ppm. The H4 and H8 proton coupled to the H5 and H7 proton and detected doublet peaks at 7.63-7.61 (*J* = 8.7 Hz) ppm, respectively. The H5 and H7 proton coupled to the H4 and H8 proton and detected doublet peaks at 7.37-7.35 (*J* = 8.7 Hz) ppm, respectively. The H1 proton coupled to the H2 proton and detected doublet peaks at 7.67-7.66 (*J* = 5.0 Hz) ppm. The H2 proton coupled to the H1 and H3 proton and detected triplet peaks at 7.14-7.12 (*J* = 4.7 Hz) ppm. The H3 proton coupled to the H2 proton and detected doublet peaks at 7.53-7.52 (*J* = 3.7 Hz) ppm. The amino group (–NH) proton signal showed as a singlet in the range of 8.24 ppm. DMSO-d_6 and water in DMSO (HOD, H_2O) signals were seen around at 2.50 (quintet) and 3.30 (variable, based on the solvent and its concentration) ppm, respectively [31]. These results are consistent with the values of earlier reported for similar compounds [1,6,12, 30]. Moreover, selected compounds' IR, ^{1}H NMR, and ^{13}C NMR spectra were given as supplementary material.

Table 3. ^{1}H NMR data of compounds for **I–X**, (δ/ppm).



Comp.	Aromatic protons (benzene)			Aromatic protons (thiophene)			-OCH_3 or -CH_2	-NH
	H4;H8	H5; H7	H6	H1	H2	H3		
I	7.60-7.58 (2H, d, *J* = 7.9 Hz)	7.34-7.30 (2H, t, *J* = 7.9 Hz)	7.00-6.96 (1H, t, *J* = 7.3 Hz)	7.68-7.66 (1H, d, *J* = 4.9 Hz)	7.14-7.12 (1H, t, *J* = 4.3 Hz)	7.55-7.50 (1H, d, *J* = 4.0 Hz)	-	10.48
II	7.87 (1H, s) 7.04-7.01 (1H, dd, *J* = 7.3, 1.8 Hz)	7.35-7.31 (1H t, *J* = 8.2 Hz)	7.41-7.39 (1H, dd, *J* = 7.3, 1.2 Hz)	7.70-7.68 (1H, d, *J* = 4.9 Hz)	7.15-7.13 (1H, t, *J* = 4.0 Hz)	7.57-7.56 (1H, d, *J* = 3.7 Hz)	-	10.69
III	7.63-7.61 (2H, d, *J* = 8.7 Hz)	7.37-7.35 (2H, d, *J* = 8.7 Hz)	-	7.67-7.66 (1H, d, *J* = 5.0 Hz)	7.14-7.12 (1H, t, *J* = 4.7 Hz)	7.53-7.52 (1H, d, *J* = 3.7 Hz)	-	8.24
IV	8.33-8.29 (1H, t, *J* = 8.2 Hz, J_{HF} = 8.1 Hz), 7.06-7.00 (1H, m), 7.27-7.16 (2H, m)			7.67-7.66 (1H, d, *J* = 5.0 Hz)	7.14-7.12 (1H, t, *J* = 4.2 Hz)	7.53-7.52 (1H, d, *J* = 3.2 Hz)	-	10.29
V	7.63-7.59 (2H, d, *J* = 4.3 Hz, J_{HF} = 3.4 Hz)	7.13-7.19 (2H, m, *J* = 9.2 Hz, J_{HF} = 9.1 Hz)	-	7.68-7.66 (1H, d, *J* = 4.9 Hz)	7.12-7.13 (1H, m)	7.53-7.51 (1H, d, *J* = 3.7 Hz)	-	10.49
VI	7.80-7.78 (2H, d, *J* = 9.2 Hz, ArH)	8.22-8.20 (2H, d, *J* = 8.5 Hz, ArH),	-	7.71-7.70 (1H, d, *J* = 4.9 Hz)	7.15-7.13 (1H, t, *J* = 4.0 Hz)	7.59-7.58 (1H, d, *J* = 3.1 Hz)	-	11.21
VII	7.35-7.29 (4H, m)		7.25-7.22(1H, m)	7.59-7.58 (1H, d, *J* = 4.9 Hz)	7.09-7.07 (1H, t, *J* = 4.3 Hz)	7.38-7.37 (1H, d, *J* = 4.2 Hz)	4.49-4.48	8.42-8.39
VIII	8.24-8.22 (1H, d, *J* = 8.5 Hz), 7.03-6.91 (3H, m, ArH),			7.64-7.62 (1H, d, *J* = 4.9 Hz)	7.13-7.10 (1H, t, *J* = 4.3 Hz)	7.48-7.46 (1H, d, *J* = 3.7 Hz)	3.83	9.85
IX	7.31 (1H, s) 7.24-7.20 (1H, t, *J* = 8.2 Hz)	6.58-6.56 (1H, dd, *J* = 8.2, 2.1 Hz)	7.07-7.05 (1H, dd, *J* = 7.9, 1.8 Hz)	7.68-7.66 (1H, d, *J* = 4.9 Hz)	7.15-7.12 (1H, t, *J* = 4.2 Hz)	7.53-7.52 (1H, d, *J* = 3.1 Hz)	3.72	10.49
X	7.47-7.51 (2H, d, *J* = 8.2 Hz)	6.88-6.92 (2H, d, *J* = 8.2 Hz)	-	7.64-7.62 (1H, dd, *J* = 5.0, 0.9 Hz)	7.13-7.10 (1H, t, *J* = 4.3 Hz)	7.45-7.47 (1H, d, *J* = 3.7 Hz)	3.69	10.27

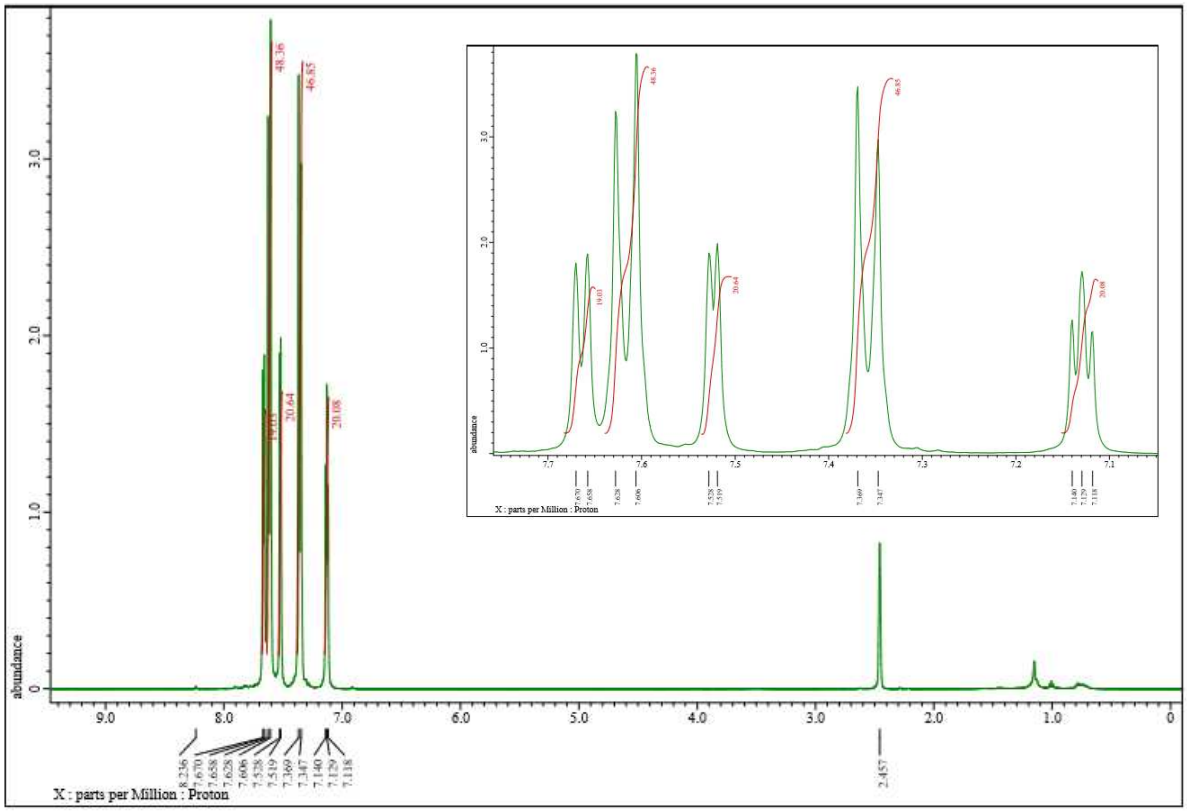


Figure 2. ^{1}H NMR spectrum of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**III**).

3.4. Interpretation of the ^{13}C NMR Spectra

The ^{13}C NMR spectra of the synthesized compounds were recorded in DMSO-d_6 and the chemical shifts are presented in Table 4. The aromatic C signals from the benzene ring (C7–C12) were observed between 157.1 and 104.1 ppm for all compounds (**I–X**), those from the thiophene ring (C1–C4) were observed between 152.4 and 121.2 ppm, and from the 1,3,4-thiadiazole ring (C5 and C6) were observed between 168.5 and 151.0 ppm.

In the presence of electronegative elements (–N, -O, –Cl, and –F), the resonances of the carbon atoms shifted down-field (high values of δ). Conversely, the carbon atoms resonated up-field (low values of δ) due to the increase in electron density in the presence of electropositive atoms (Li, Si). In compounds **III**, **V** (two carbons), **VI**, and **X**, the C10 carbons resonated at 152.6; 157.1, 156.7; 151.4; and 146.6 ppm, respectively. As for, the

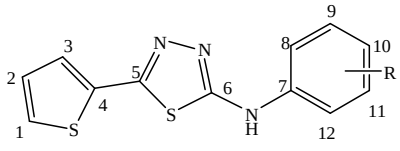
effect of different groups are seen in the compounds **III**, **V**, **VI**, and **X**, where the signals of the *para* carbons are shifted upfield by the 4-Cl, 4-F, 4-NO_2, and 4-OCH_3 groups, relative to the signals of benzene (**I**, 128.5 ppm). Additionally, in compounds **IV** and **V**, the C atoms (for C7-C12) were also split into doublets due to interacting with the atomic nucleus of F.

The C6 signal was down-field compared to the C5 due to the decrease in the electron density from multiple inductive effects (surrounded by heteroatoms). For all compounds (**I–X**) the C6 was observed between 168.5 and 162.9 ppm, whereas C5 was observed between 159.1 and151.0 ppm.

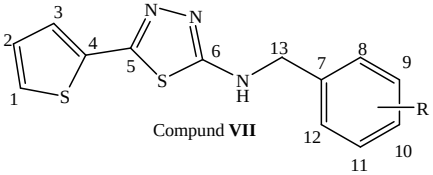
In compounds (**VIII**, **IX,** and **X**), the methoxy carbon atoms (–OCH_3) resonated at 56.3, 55.6, and 55.8 ppm, respectively. In compounds (**VIII**, **IX,** and **X**), the C7-C12 carbon atoms were affected by both methoxy (–OCH_3) and amino (-NH, C7) groups. Therefore, the shifts induced by these substituents were added, wherein the signals of the carbons (C7-C12) were observed up-field or down-field shifts relative to *ortho*, *meta*,and *para* position of these groups.

For compound **VII**, the methylene C13 was detected at 48.6 ppm. These data consistent with the values of earlier reported for similar compounds [1,6,12,30,32]. A representative ^{13}C NMR spectrum of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**III**) is presented in Figure 3.

Table 4. ^{13}C NMR data of compounds **I–X**, (δ/ppm)



Compounds **I-X** except for **VII**



Compund **VII**

R	H	3-Cl	4-Cl	2-F	4-F	4-NO_2	H	2-OCH_3	3-OCH_3	4-OCH_3
	I	**II**	**III**	**IV**[a]	**V**[a]	**VI**	**VII**	**VIII**	**IX**	**X**
C1	132.9	131.3	132.6	132.9	128.7	128.8	133.5	129.9	130.5	133.1
C2	129.1	128.8	128.8	130.6	137.5	130.1	128.6	128.9	128.7	128.8
C3	122.7	122.2	126.1	121.2	132.9	129.8	127.7	128.7	129.2	128.7
C4	140.9	132.7	129.3	132.2	152.4	132.5	138.9	129.0	129.4	129.0
C5	152.4	153.1	152.9	151.3	159.1	154.5	151.0	153.0	152.5	155.3
C6	164.1	163.6	163.8	164.2	164.2	162.9	168.5	164.7	164.0	164.9

C7	129.7	134.1	139.8	123.9 123.8 *J* = 4.5 Hz	129.4 129.2 *J* = 12.0 Hz	141.5	128.1	133.1	142.1	134.4
C8	117.3	129.4	129.5	129.4 129.2 *J* = 8 Hz	116.4 116.1 *J* = 23.0 Hz	117.6	128.2	123.6	110.7	114.9
C9	118.1	129.7	119.6	125.4 125.3 *J* = 16 Hz	119.9 119.8 *J* = 8.6 Hz	126.0	128.9	119.7	132.9	120.1
C10	128.7	117.5	152.6	129.1 128.8 *J* = 26 Hz	157.1 156.7 *J* = 234.5 Hz	146.6	128.3	121.2	108.1	151.4
C11	118.1	142.2	119.6	115.9 115.7 *J* = 34 Hz	119.9 119.8 *J* = 8.6 Hz	126.0	128.9	111.6	160.5	120.1
C12	117.3	116.5	129.5	153.7 153.6 *J* = 227.5 Hz	116.4 116.1 *J* = 23.0 Hz	117.6	128.2	148.9	104.1	114.9
C13	-	-	-	-	-	-	48.6	-	-	-
R	-	-	-	-	-	-	-	56.3	55.6	55.8

[a] In compounds **IV** (different six C) and **V** (different four C), C7-C12 atoms were observed split into doublets by interacting with the atomic nucleus of F.

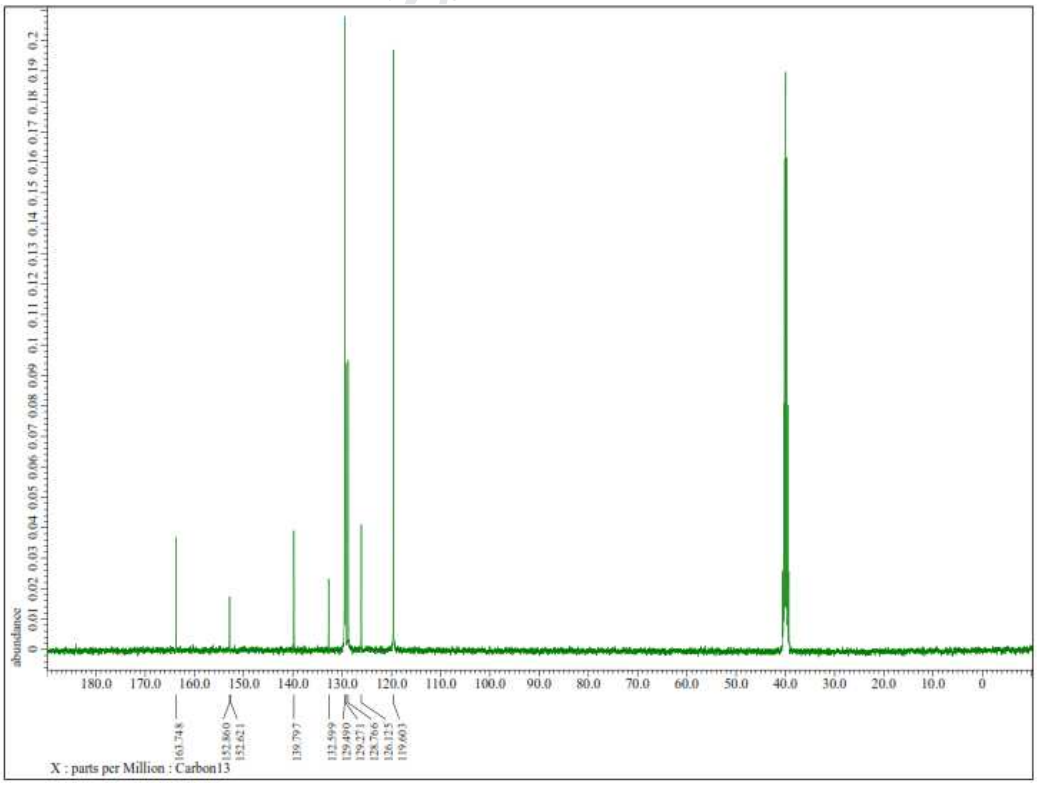


Figure 3. ^{13}C NMR spectrum of *N*-(4'-chlorophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**III**).

3.5. Antimicrobial Activity

In vitro antimicrobial activity tests of the synthesized 1,3,4-thiadiazoles were performed against nine different bacteria (*Salmonella enteritidis, Staphylococcus aureus, Salmonella typhimurium, Enterobacter aerogenes, Salmonella infantis, Bacillus subtilis, Salmonella kentucky, Escherichia coli,* and *Enterococcus durans*) and one fungus (*Candida albicans*) using the disk diffusion method. The antimicrobial activities were measured with zone inhibition in mm and the results were presented in Table 5. Compounds **V** and **VIII** revealed antifungal activity against *C. albicans,* with inhibition zones of 7 and 8 mm, respectively. Compared to standard of Ketoconazole (13 mm), compounds **V** and **VIII** were shown moderate antifungal activity. All the tested compounds (compounds **III**, **VI**, and **IX** could not be tested due to solubility issues) revealed antibacterial activity against *Staphylococcus aureus*. Compound **II** has the best antibacterial activity against *Staphylococcus aureus* with inhibition zones of 14 mm at 80 µg/mL. Compared to standard of Streptomycin (18 mm), compounds **V**, **VII** and **X** have shown good antibacterial activities, while compounds **I**, **II**, **IV**, and **VIII** have exhibited moderate antibacterial activities. Compound **V** demonstrated good inhibition against *Staphylococcus aureus* at 50 and 80 µg/mL. The order of the antimicrobial activity against *Staphylococcus aureus* is as follows: **V**=**VII**>**X**>**I**>**II**>**IV**>**VIII** (30 µg/mL), **V**>**VII**>**X**>**I**>**II**>**VIII**=**IV** (50 µg/mL), and **II**>**V**>**VII**>**X**>**I**>**IV**>**VIII** (80 µg/mL).

Compound **II** reported antibacterial activity against *S. typhimurium* with an inhibition zone of 7 mm. Compound **VII** revealed antibacterial activity against *Enterobacter aerogenes* with inhibition zones of 8, 9, and 9 mm using 30, 50, and 80 µg/mL, respectively. Compounds **VIII** and **X** indicated antibacterial activity against *S. kentucky* with inhibition zones from 7 to 9 mm. Nevertheless, none of the tested compounds demonstrated antibacterial activity against

the remaining five bacterial strains (*S. enteritidis, S. infantis, B. subtilis, E. coli,* and *Enterococcus durans*).

The synthesized 1,3,4-thiadiazoles presented differing degrees of antimicrobial activity. All of the testable compounds presented significant antibacterial activity against *Staphylococcus aureus.* Selected compounds presented antimicrobial activity against *C. albicans, S. typhimurium, Enterobacter aerogenes,* and *S. kentucky*; however, none of the compounds presented antimicrobial activity against *S. enteritidis, S. infantis, B. subtilis, E. coli,* and *Enterococcus durans.* These results corroborate those of Hussain et al [33]. They synthesized novel 4-amino-2-{5-[(4-substitutedphenyl)amino]-1,3,4-thiadiazole-2-yl} phenol derivatives and found them to display antibacterial activity against *E. coli* (Gram negative) and *Staphylococcus aureus* (Gram-positive) bacteria. Moreover, Gür and co-workers studied 1,3,4□thiadiazole moiety and their antimicrobial activities [34]. They found antifungal activity against *C. albicans*, antibacterial activities against *Staphylococcus aureus* (Gram-positive), *and Enterobacter aerogenes* (Gram negative). Bhat and co-workers studied antimicrobial activities of 3-(1,3,4-thiadiazole-2-yl)quinoline derivatives [35]. They found antibacterial activities against *S. typhimurium* (Gram negative).

Table 5. Antimicrobial activity results of compounds **I**, **II**, **IV**, **V**, **VII**, **VIII**, and **X**.

	Microorganisms, zone of inhibition (mm)										
Comp.	µg/mL	*S. enteritidis*	*C. albicans*	*Stap. aureus*	*S. Typhimur.*	*E. aerogenes*	*S. infants*	*B. subtilis*	*S. kentucky*	*E. coli*	*E. durans*
I	30	-	-	10	-	-	-	-	-	-	-
	50	-	-	10	-	-	-	-	8	-	-
	80	-	-	10	-	-	-	-	-	-	-
II	30	-	-	8	7	-	-	-	-	-	-
	50	-	-	9	7	-	-	-	-	-	-
	80	-	-	14	7	-	-	-	-	-	-
IV	30	-	-	7	-	-	-	-	-	-	-
	50	-	-	8	-	-	-	-	-	-	-

	80	-	-	9	-	-	-	-	-	-	-
V	30	-	7	12	-	-	-	-	-	-	-
	50	-	7	13	-	-	-	-	-	-	-
	80	-	8	13	-	-	-	-	-	-	-
VII	30	-	-	12	-	8	-	-	-	-	-
	50	-	-	12	-	9	-	-	-	-	-
	80	-	-	12	-	9	-	-	-	-	-
VIII	30	-	7	-	-	-	-	-	9	-	-
	50	-	7	8	-	-	-	-	9	-	-
	80	-	7	8	-	-	-	-	9	-	-
X	30	-	-	11	-	-	-	-	7	-	-
	50	-	-	11	-	-	-	-	7	-	-
	80	-	-	11	-	-	-	-	7	-	-
Strept.	80	5	NA	18	12	15	6	2	14	-	5
Ketoco.	80	NA	13	NA	NA	NA	NA	NA	NA	NA	NA

Strep. : Streptomycin *Ketoco.: Ketoconazole* NA = not applicable

Note: Compounds **III**, **VI,** and **IX** could not be studied due to solubility issues

4. Conclusions

In this study, novel 1,3,4-thiadiazole compounds were synthesized from the reaction of thiophene-2-carboxylic acid and *N*-arylthiosemicarbazides in the presence of phosphorous oxychloride at reflux. The synthesized compounds were characterized by using IR, ^{1}H NMR, and ^{13}C NMR spectroscopies and elemental analysis. The resulting 1,3,4-thiadiazole derivatives were prepared in excellent yields (78–95%) and tested for the antimicrobial activity. Selected compounds demonstrated activity against *Staphylococcus aureus, C. albicans, S. typhimurium, Enterobacter aerogenes,* and *S. kentucky*. It was seen that the 1,3,4-thiadiazole compounds have antibacterial activity against *Staphylococcus aureus, S. typhimurium, Enterobacter aerogenes,* and *S. Kentucky,* along with antifungal activity against *C. albicans*. Compound **II** is the best antibacterial activity against *Staphylococcus aureus* (gram-positive). Compounds **V**, **VII** and **X** were shown good antibacterial activities against *Staphylococcus aureus.*

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Supplementary Material

IR, ^{1}H NMR, and ^{13}C NMR, spectra of the compounds are given in the supporting information.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Highlights

- New 1,3,4-thiadiazoles based on thiophene-2-carboxylic acid were synthesized.
- The structures were determined using spectroscopic methods.
- The synthesized compounds were examined for antimicrobial activities.
- Some 1,3,4-thiadiazoles had effective antimicrobial activity against *S. aureus*, *S. typhimurium*, *E. aerogenes*, *S. kentucky*, and *C. albicans*.

Author Contribution Statement

Halit Muğlu: Supervision, Methodology, Project administration, Resources.

Hasan Yakan: Conceptualization, Methodology, Writing- Original draft preparation, Writing-Review & Editing.

Hanan Almabrok Shouaib: Investigation, Methodology, Formal analysis.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: