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Papers

SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT, ANTIBACTERIAL AND ENZYME INHIBITOR EVALUATION OF NEW FURAN-TETHERED 1,3,4-THIADIAZOLES**

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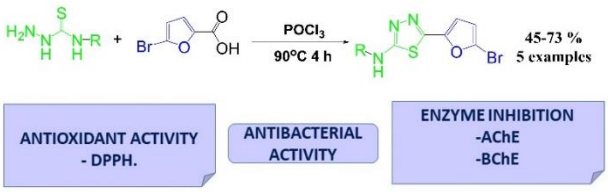
Novel furan-tethered 1,3,4-thiadiazoles (**1-5**) were prepared and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. Compounds, in general, showed antibacterial effects on selected bacteria, including *K. pneumoniae*, *E. faecium*, S. *epidermidis*, *S. macrescens*, *B. subtilis*, *E. coli*, and *S. kentucky*. Besides, none of the compounds had effects on *P. aeruginosa*. *In vitro* antioxidant activity determination was carried out using the DPPH method, and results of the synthesized compounds were found to be close to each other showing lower antioxidant activity than the standard used (ascorbic acid). *In vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition of the synthesized compounds was performed using Ellman's spectrophotometry method. The compounds showed inhibition with IC_{50} values ranging from 27.39 to 45.71 µM for AChE and 13.35 to 115.17 µM for BChE. Among the synthesized derivatives, compound **2** exhibited the strongest inhibitory effect against both AChE, and BChE.



INTRODUCTION

Thiadiazole is an aromatic five-membered hetero-ring compound with one sulfur and two nitrogen atoms. 1,3,4-thiadiazole is one of the four isomeric forms of thiadiazole and is thermally the most stable among these isomers.[1-3] 1,3,4-thiadiazoles have been reported to be obtained through various synthetic methods such as cyclization of thiosemicarbazides,[4] thiosemicarbazones,[5] aldehyde methylthio (thiocarbonyl) hydrazones[6] or acylhydrazines.[7]

1,3,4-thiadiazole moieties composed of the azole group have wide applications in

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agricultural, biological, medicinal, and materials chemistry.[8-12] They have shown a wide range of activities such as antiviral, antimicrobial, antioxidant, anti-inflammatory, antidepressant, antileishmanial, antiproliferative, analgesic, anticonvulsant, anticancer, and antitubercular.[13-27]

In the recent years, the 1,3,4-thiadiazole moieties have attracted immense attention owing to their notable antioxidant characteristics, such as harm to oxidative stress in living organisms, increased healthy longevity, and prevention of DNA oxidation.[28,29] Therefore, novel derivatives have been synthesized with more powerful effects.

Recently, several 1,3,4-thiadiazoles have been shown to possess antimicrobial activity against various microorganisms.[4,15,30,31] However, antibiotic-resistant bacterial strains revealed the need of syntheses and exploration of novel, more confident, harmless, and powerful antimicrobial reagents.[32]

Alzheimer's disease (AD) is an irreversible neurological illness that causes an age-related gradual decline in cognitive abilities, diminished ability to perform fundamental daily tasks, and personality characteristic variations. AD physiopathology is complicated, involving environmental and genetic variables.[33] The cholinergic hypothesis, which is the oldest of the ideas presented, targets acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).[34-36] The loss of neurons and axons that occurs with the development of AD produces a drop in acetylcholine (ACh) levels. It becomes progressively harder to sustain nerve impulse continuity at low neurotransmitter concentrations. A vital strategy for increasing ACh levels is to inhibit the activity of the AChE and BChE enzymes that degrade ACh. Increase in ACh levels caused by AChE inhibition have been proven to ameliorate cognitive impairment in the early stages of AD.[35] It is also known that BChE is closely related to AChE.[37] The structural difference between them is that the choline subregion of AChE includes much more aromatic amino acids than that of BChE.[38] AChE activity steadily diminishes while BChE activity increases with AD, and BChE then establishes a compensatory mechanism for ACh metabolism. As a result, the regulation of ACh may be increasingly under BChE control, and dual inhibitors may outperform potent effects.[39] Currently, the only cholinesterase inhibitors utilized in clinical trials for treating AD are rivastigmine, donepezil, and galantamine.[40] In recent years, 1,3,4-thiadiazole derivatives have gained considerable attention for their potential in addressing neurodegenerative disorders, particularly Alzheimer's disease (AD).[41-44] Emerging evidence supports the potential of 1,3,4-thiadiazole derivatives as dual inhibitors of AChE and BChE. For instance, older synthesized imidazothiadiazole derivatives that showed significant inhibition of both enzymes, supported by *in vitro* data and molecular docking studies.[44] Similarly, reported thiadiazole compounds with potent cholinesterase inhibitory activity, exhibiting IC_{50} values as low as 0.986 µM, highlighting their promise as AD therapeutics.[42] Moreover, developed sulfonamide-linked thiadiazoles with dual inhibition properties and neuroprotective potential.[43] These circumstances underscore the need for effective and novel AChE and BChE inhibitors to develop new treatments for AD.

Because of the above reasons, the objectives of the present investigation were (a) to obtain novel furan-tethered 1,3,4-thiadiazole derivatives; (b) to elucidate the structures of the compounds using FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopic methods and elemental analysis; (c) to assess *in vitro* antioxidant (DPPH), antibacterial and cholinesterase (AChE and BChE) inhibitory properties.

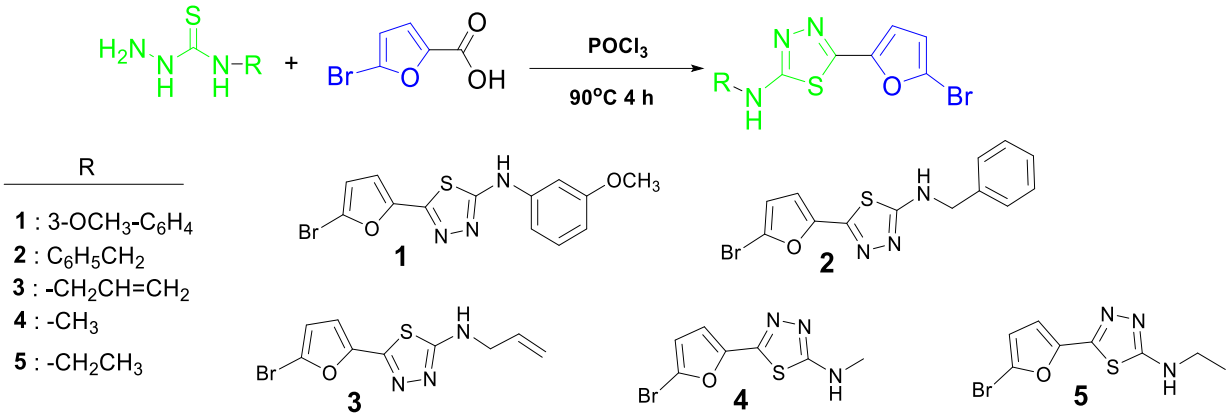
EXPERIMENTAL

Reagents

Chemicals utilized in this study were procured from Carlo Erba, Aldrich, Acros Organics, or Merck and used without further purification. The melting points were determined using an electrothermal apparatus of the Stuart SMP 30 type. Elemental analyses were accomplished using CHNS-932 (LECO). A Bruker Alpha FT-IR spectrophotometer was used for Fourier transform infrared (FTIR) spectra with ATR mode. ^{1}H NMR (400 Mhz) and ^{13}C NMR (101 Mhz) spectra were performed on DMSO-d_6 using a JEOL-ECS 400 MHz spectrometer. Tetramethylsilane was used as an internal standard.

Synthesis Procedure

The mixture of 5-bromo-2-furoic acid (3 mmol) and *N*-aryl/allyl/alkyl thiosemicarbazide derivatives (3 mmol) was cooled in a refrigerator and phosphorus oxychloride (9 mmol) was added dropwise under stirring. The mixture was refluxed at 90 °C for 4 h. After the reaction was finished, the mixture was allowed to cool to room temperature before being stirred in ice-cold water and neutralized with ammonia. After filtering and washing with water, the precipitated product crystallized in an appropriate solvent (Acetic acid, Acetonitrile, Chloroform, DMF, DMSO, THF). Synthesis of these thiadiazoles was accomplished using this method[45] shown in Scheme 1.



Scheme 1 – Synthesis of furan-tethered 1,3,4-thiadiazole derivatives **1-5**

Antioxidant activity assay

For determination of the antioxidant activity of the synthesised compounds (**1-5**), we used the DPPH method previously reported.[46,47] Briefly, 200 µM stock solutions of the synthesised compounds were prepared in DMSO. Subsequently, five different volumes were taken from the stock solutions (10, 25, 50, 100, and 250 µL) and 3 mL DPPH stock solution, sufficient ethanol was added up to a total of 5 mL. The concentrations of the compounds added to the previously prepared DPPH solution were used in the range of 0.4–10 µM. The prepared mixtures were allowed to stand in the dark for 30 min at room temperature and then read against a blank at 517 nm. The percentage inhibition of the antioxidant activity was calculated using the following equation (1)[48]:

$$\text{inhibition (\%)} = [(A_0 - A_1) / A_0 \times 100] \quad (1)$$

where A_0 is the control absorbance (blank, no compound) and A_1 is the compound absorbance. The IC_{50} value is a typical measurement used to assess antioxidant activity. The antiradical activity, which is known as the IC_{50} (mg/mL), is represented by the quantity of antioxidants necessary to lower the initial DPPH concentration by 50%.[49]

Antibacterial activity testing

Microorganism strains

Antibacterial efficacy of the compounds was evaluated against five Gram-negative bacteria; *Pseudomonas aeruginosa* (DSMZ 50071), *Klebsiella pneumoniae* (food isolate), *Escherichia coli* (food isolate), *Serratia marcescens* (clinical isolate), *Salmonella kentucky* (food isolate), and three Gram-positive bacteria; *Enterococcus faecium* (food isolate), *Bacillus subtilis* (DSMZ 1971), and *Staphylococcus epidermidis* (DSMZ 20044). All of the bacteria were from the Microbiology Laboratory's culture collection at Kastamonu University's Department of Biology.

Preparation of chemical compounds

To prepare the stock solutions, 24 mg of each compound were dissolved in 1 mL of dimethyl sulfoxide (DMSO). Because DMSO damages living cells, the in-test concentration was established at 1%.[50]

Preparation of the inocula

Similar colonies of the bacteria used in the study were collected using a sterile loop and placed in a 0.9% sterile saline solution after being cultured for 24 hours at 37 °C. The McFarland standard of 0.5 was applied to the turbidities.[51]

Minimum inhibition concentration (MIC) test

MIC test were determined via the broth dilution technique in accordance with established protocols reported in prior studies.[52, 53] In a 96-well plate, serial 2-fold dilutions were performed, yielding a concentration range of 0.234 to 120 µg/mL. The MIC value was determined as the lowest extract concentration necessary to prevent any discernible bacterial growth.[54, 55] All assays were carried out in triplicate, and the results are presented as mean values.

Minimum bactericidal concentration (MBC) test

The MBC test shows the lowest growth-inhibiting antimicrobial agent level of the MIC test. And it also complements the MIC test, in which MBC shows the lowest level of antimicrobial agents that cause microbial death under certain conditions. Subculturing the contents of non-turbid MIC test wells onto agar plates was used to determine the MBC values.[56]

AChE and BChE inhibition assay

Using a modified version of Ellman's spectrophotometric method, the inhibitory activities of the compounds against AChE and BChE were determined.[57] Cholinesterase activity tests were carried out using bovine serum BChE and electric eel AChE (Type-VI-S, Sigma) enzymes. Acetylthiocholine iodide and butyrylthiocholine iodide were used as reaction substrates. The cholinesterase activity was measured using 5,5'-dithio-*bis* (2-nitrobenzoic) acid (DTNB). The reaction system was composed of at least five different concentrations of each thiadiazole derivative, 200 µL buffer (0.1 M, pH 7.8: Tris-HCl buffer for the AChE assay, and phosphate buffer for the BChE assay), 100 µL DTNB (2 mM), 100 µL acetylthiocholine iodide/*S*-butyrylthiocholine iodide (2 mM), and 5/3 µL of AChE/BChE solution (0.28 units/mL for the AChE assay and 0.32 units/mL for the BChE assay). After that, 100/100 µL of acetylthiocholine iodide/butyryl thiocholine iodide was added to start each reaction. The hydrolysis of acetylthiocholine iodide/ butyrylthiocholine iodide was predicated on creating the yellow 5-thio-2-nitrobenzoate anion in a UV-spectrophotometer at 412 nm as a result of the interaction of DTNB with thiocholines catalyzed by the enzymes.

RESULT AND DISCUSSION

Physical data

Table 1 presents the physical data of the compounds mentioned in Scheme 1. The compounds were accomplished from 5-bromo-2-furoic acid and thiosemicarbazide derivatives with good yields.

Table 1

Physical data and elemental analysis

Entry	Compound's Names	M. P. (ºC)	Yields (%)	Color	Calculated/Found		
					C%	H%	N%
1	5-(5-bromofuran-2-yl)-*N*-(3-methoxyphenyl)-1,3,4-thiadiazol-2-amine	175–176	53	Light Brown	44.33/44.08	2.86/2.93	11.93/11.87
2	*N*-benzyl-5-(5-bromofuran-2-yl)-1,3,4-thiadiazol-2-amine	137–138	73	Light Brown	46.44/46.35	3.00/3.10	12.50/12.39
3	*N*-allyl-5-(5-bromofuran-2-yl)-1,3,4-thiadiazol-2-amine	97–98	45	Cream	37.78/37.64	2.82/2.91	14.69/14.56
4	5-(5-bromofuran-2-yl)-*N*-methyl-1,3,4-thiadiazol-2-amine	123–124	60	Cream	33.32/33.19	2.33/2.40	16.16/16.11
5	5-(5-bromofuran-2-yl)-*N*-ethyl-1,3,4-thiadiazol-2-amine	125–126	49	Light Brown	35.05/35.20	2.94/3.00	15.33/15.29

Vibrational frequencies

Table 2 illustrates the IR vibrations of the synthesized compounds. At FT-IR spectra of the synthesized compounds, the carboxylic acid (–COOH) signal of the initial material was not seen at 3550–2750 cm^{-1}. Furthermore, the amino group's asymmetric and symmetric stretching bands ($–NH_2$) did not appear at 3400–3150 cm^{-1}. These findings showed that the reaction was successful as predicted. For all

compounds (**1-5**), characteristic peaks corresponding to the amino group (–NH) were observed in the range of 3213–3136 cm^{-1}. The C=N stretching vibrations associated with the thiadiazole ring appeared between 1610 and 1534 cm^{-1}, while C–N were detected in the range of 1276–1255 cm^{-1} (see Figs. S1–S4 for supporting information). Additionally, signals corresponding to C–O stretching of the aryl ring were recorded between 1136 and 1092 cm^{-1}. In the case of compound **1**, the absorption band for the –NH group was observed at 3136 cm^{-1}, as illustrated in Fig. 1. Aromatic C–H stretching vibrations were detected in the region of 3066–2946 cm^{-1}, whereas aliphatic C–H stretching was noted between 2854 and 2818 cm^{-1}. The C=N vibrations were detected at 1608 and 1584 cm^{-1}. A distinct absorption for the C–S bond was found at 672 cm^{-1}, while the C–N stretching vibration appeared at 1255 cm^{-1}. The C–O stretching of the aryl group was observed at 1113 cm^{-1}, and the C–Br stretching vibration was recorded at 654 cm^{-1}. The frequency values of the compound were almost the same as those of similar compounds.[4, 45, 58, 59]

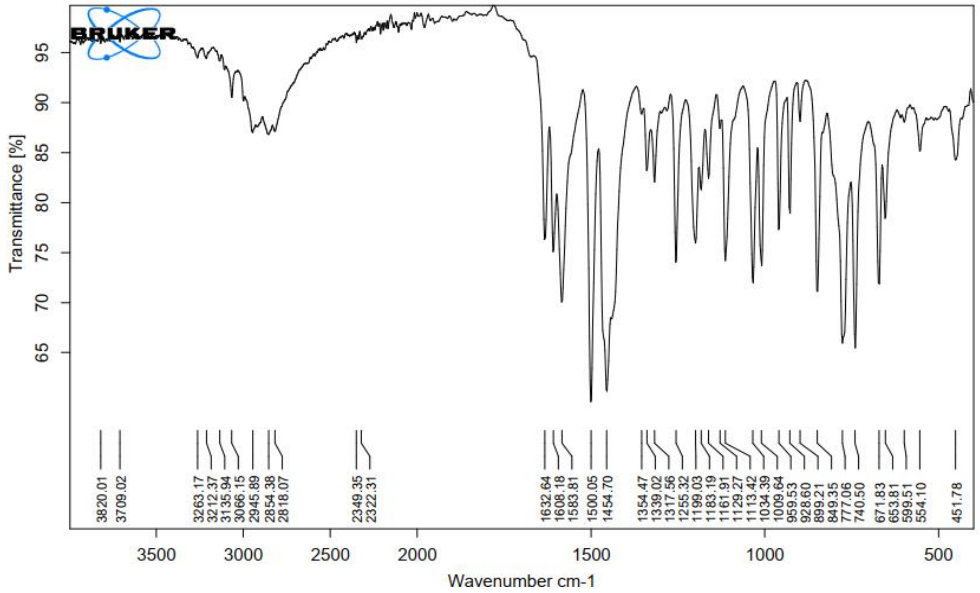


Fig. 1 – The IR spectrum of compound **1** (ATR).

Table 2

FT-IR values of the compounds (with ATR mode, cm^{-1})

Comp.	-NH	Ar CH	C=N	C–N	C–O	C–S	C–Br	Spec. Vib.
1	3136	3066–2946	1608–1584	1255	1113	672	654	Aliph. CH: 2854-2818
2	3213	3088–3027	1578–1555	1276	1093	657	612	Aliph. CH: 2927-2872
3	3178	3134–3079	1610–1549	1270	1127	672	644	Aliph. CH: 2971-2930
4	3212	3138–3111	1582–1558	1260	1131	672	655	Aliph. CH: 2933-2789
5	3210	3122–2973	1555–1534	1259	1136	652	593	Aliph. CH: 2927-2884

NMR spectral interpretations

The ^{1}H NMR spectra of the synthesized compounds were taken in DMSO-d_6 as a solvent, and the chemical shifts are given in Table 3 (see Figs. S5–S8 for supporting information).

For all compounds (**1-5**), the proton signal of –NH was detected as a singlet at 10.61–7.99 ppm, the furan ring proton signals (H1 and H2) were resonated between 7.18 and 6.77 ppm. In compounds **1** and **6**, the phenyl ring aromatic proton signals (H3-H7) were observed at 7.40–6.62 ppm as shown Table 3. For compound **1**, the proton signal of –NH was detected as a singlet at 10.61 ppm. The methoxy (OCH_3) group peak on the aryl ring was observed as a singlet at 3.77 ppm. The aromatic proton signals of the furan ring (H1 and H2) were

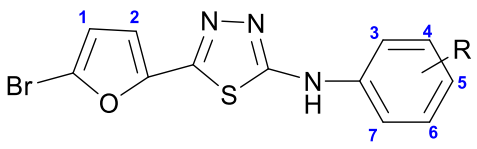
detected between 7.18 and 6.84 ppm (Fig. 2). The H1 proton coupled to the H2 showed a doublet peak at 6.85-6.84 ppm. The H2 proton coupled to the H1 exhibited a doublet peak at 7.18–7.17 ppm. The aromatic proton signals of the aryl ring (H3–H7) were observed between 7.35 and 6.62 ppm (Fig. 2). The H3 proton signal was detected as a singlet at 7.35 ppm. The H5 proton coupled to the H6 was detected as a doublet peak at 7.14–7.12 ppm. The H6 proton coupled to the H5 and H7 proton and detected as a triplet peak at 7.30–7.26 ppm. The H7 proton coupled to the H6 and H5 proton and detected as a doublet of doublet peaks at 6.64–6.62 ppm. These data match the values that were previously published for similar compounds in the literature.[4,45,59–61]

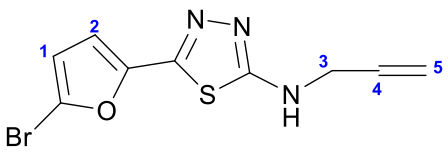
The ^{13}C NMR spectral data for each compound, recorded in DMSO-d_6, are summarized in Table 4 (see Figs. S9-S12 for supporting information).

As shown in Fig. 3, the ^{13}C NMR spectra of compound **1** revealed 13 distinct resonances that were in good agreement with the suggested structure. In compound **1**, the carbon signals of the furan ring (C1–C4) were detected between 147.4 and 113.5 ppm. The C1–C3 carbon atoms were observed at 124.5, 113.5, and 115.0 ppm, respectively. An electron-negative oxygen atom caused the C4 carbon atom to shift down-field (high values, δ) at 147.4 ppm, which was observed. The C5 and C6 carbon atoms of the 1,3,4-thiadiazole ring were detected at 147.5 and 164.1 ppm, respectively. At the same time, the C6 carbon is shifted down-field (high values, δ) due to the presence of both the electronegative sulfur atom and an amino group (NH), and the C5 carbon is shifted due to the presence of the electronegative sulfur atom. The carbon signals of the aryl ring (C7-C12) were also observed at 141.9, 104.1, 160.5, 108.2, 130.5, and 110.5 ppm, respectively. The C7 carbon is shifted down-field (high values, δ) at 141.9 ppm due to the amino group (NH) presence. The C9 carbon is shifted down-field (high values, δ) at 160.5 ppm due to the presence of the methoxy (OCH_3) group. The carbon atom of the methoxy (OCH_3) group on the aryl ring was observed at 55.5 ppm. For all compounds (**1-5**), the C5 and C6 carbon atoms of the 1,3,4-thiadiazole ring were detected at 147.9–147.5 and 169.4–164.1 ppm. The C4 carbon atom was observed at 147.4–145.6 ppm. The data obtained as a result of this analysis are in agreement with the results of other similar compounds previously reported in the literature.[1–3, 45,62]

Table 3

^{1}H NMR values of the compounds (δ, ppm, in DMSO-d_6, 400 Mhz)





Comp.	H1	H2	NH	H3	H4	H5	H6	H7	R
1	6.85–6.84 d	7.18–7.17 d	10.61 s	7.35 s	–	7.14–7.12 d	7.30–7.26 t	6.64–6.62 dd	OCH_3:3.77 s
2	6.79–6.78 d	7.02–7.01 d	8.56–8.55 t			7.40–7.29, 5H,m			CH_2:4.55 d
3	6.79–6.78 d	7.02–7.01 d	8.25–8.23 t	3.97–3.96 t	5.98–5.88 m	5.30–5.16 dd	–	–	–
4	6.79–6.78 d	7.02–7.01 d	7.99 q	2.93 d	–	–	–	–	–
5	6.78–6.77 d	7.01–7.00 d	8.06 t	3.35–3.32 p	1.21–1.18 t	–	–	–	–

s (singlet), d (doublet), dd (doublet of doublets), t (triplet), td (triplet of doublet), and m (multiplet).

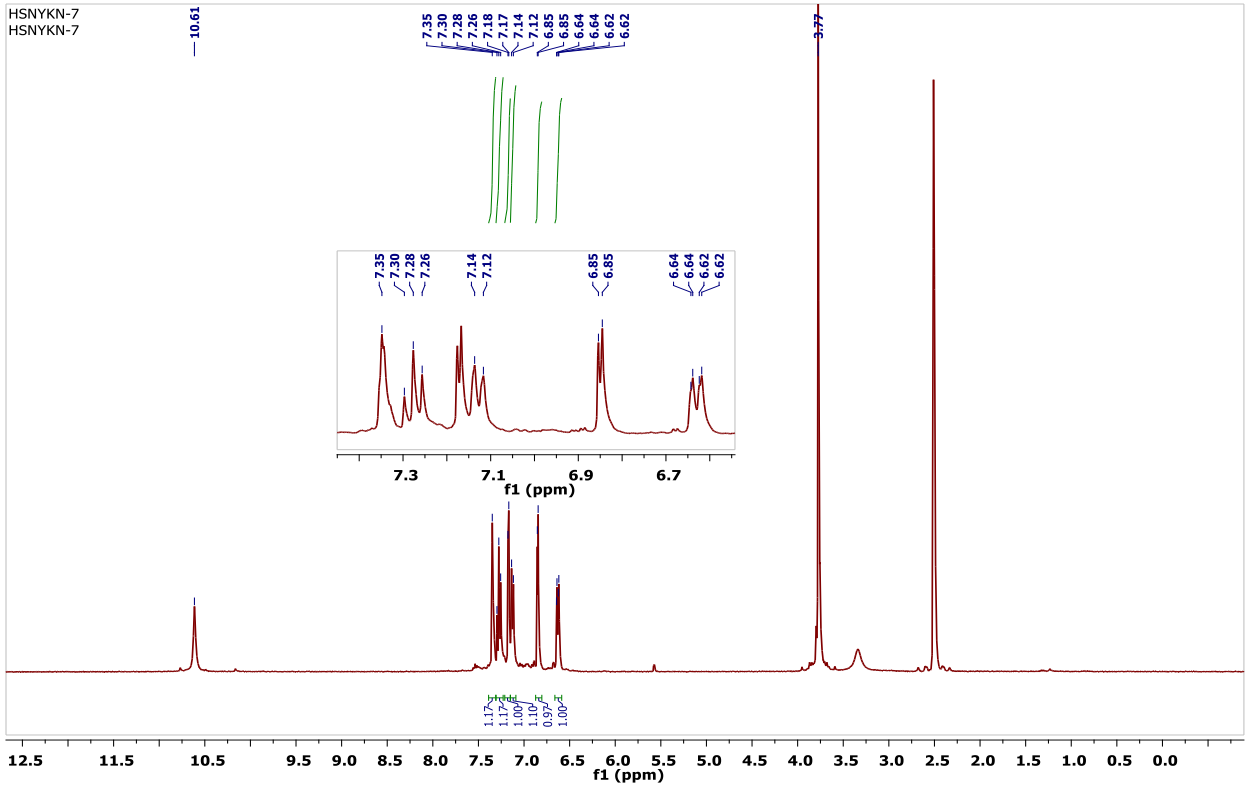


Fig. 2 – ^{1}H NMR spectrum of compound **1** (in DMSO-d_6).

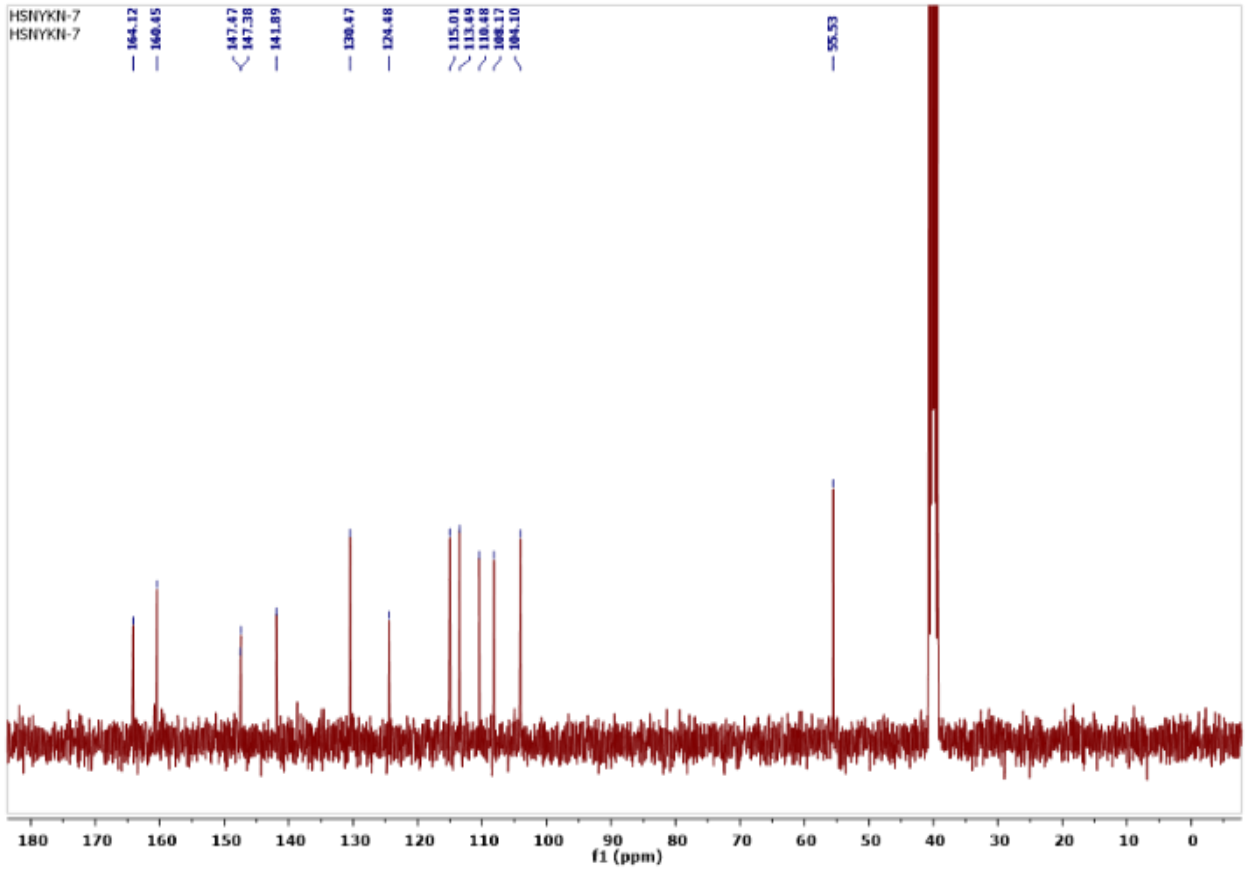
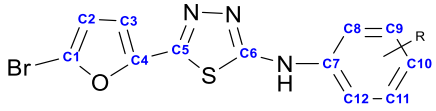
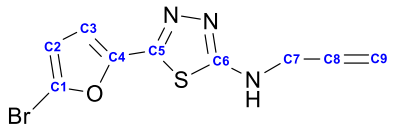


Fig. 3 – ^{13}C NMR spectrum of compound **1** (in DMSO-d_6)

Table 4

^{13}C NMR values of the compounds (δ, ppm, in DMSO-d$_6$, 101 Mhz)





C/Compound	**1**	**2**	**3**	**4**	**5**
C1	124.5	123.7	123.7	123.7	123.7
C2	113.5	112.4	112.4	112.4	112.3
C3	115.0	114.8	114.8	114.8	114.8
C4	147.4	146.2	146.0	145.7	145.6
C5	147.5	147.8	147.9	147.7	147.8
C6	164.1	168.6	168.7	169.4	168.5
C7	141.9	138.7	47.4	31.9	40.3
C8	104.1	128.1	134.6	-	14.6
C9	160.5	128.9	116.9	-	-
C10	108.2	127.7	-	-	-
C11	130.5	128.1	-	-	-
C12	110.5	128.9	-	-	-
R	55.5	CH_2:48.7	-	-	-

Antioxidant activity

The DPPH radical scavenging technique was utilized to assess the antioxidant activity tests using ascorbic acid as a reference. As per Fig. 4, the inhibition percentages of ascorbic acid and synthesized compounds are given by their concentration. Antioxidant inhibition percentages changed directly to the concentration increase for all molecules. Of the compounds showing very close values concerning their antioxidant activities, compound **4** exhibited the highest percentage of inhibition among other compounds. Compound **4** showed the highest percentage inhibition at all concentration.

In addition, the IC_{50} values of the synthesized compounds were calculated, expressing the antioxidant activity required to reduce DPPH concentration by 50%.[49] The IC_{50} values for compounds **1-5** and ascorbic acid are summarized in Table 5. The IC_{50} value calculated for ascorbic acid was 5.289 ± 0.02 µM. The antioxidant activities of the compounds followed this order **4** > **2** > **5** > **3** > **1**, from highest to lowest. It was observed that the IC_{50} values of all of the synthesized molecules (**1-5**) varied in the range of 9.308-17.525 µM, and thus their antioxidant activities were very close to each other. When we compare the free radical scavenging activity of the compounds, the compound having methyl group, one of the functional groups -R connected to the *N*-group in the *N*-(R)-5-(5-bromofuran-2-yl)-1,3,4-thiadiazol-2-amine molecule showed the highest antioxidant activity. Especially as in compounds **1**

and **2**, functional atoms/groups (3-OCH_3, -CH_2) attached to the NH-phenyl structure were effective in differentiating the antioxidant activities of the molecule according to the positions and kinds of the atoms/groups to which it was attached. As mentioned in previous studies, 1,3,4-thiadiazol compounds appear to have antioxidant activity using DPPH assay.[14, 63-65]

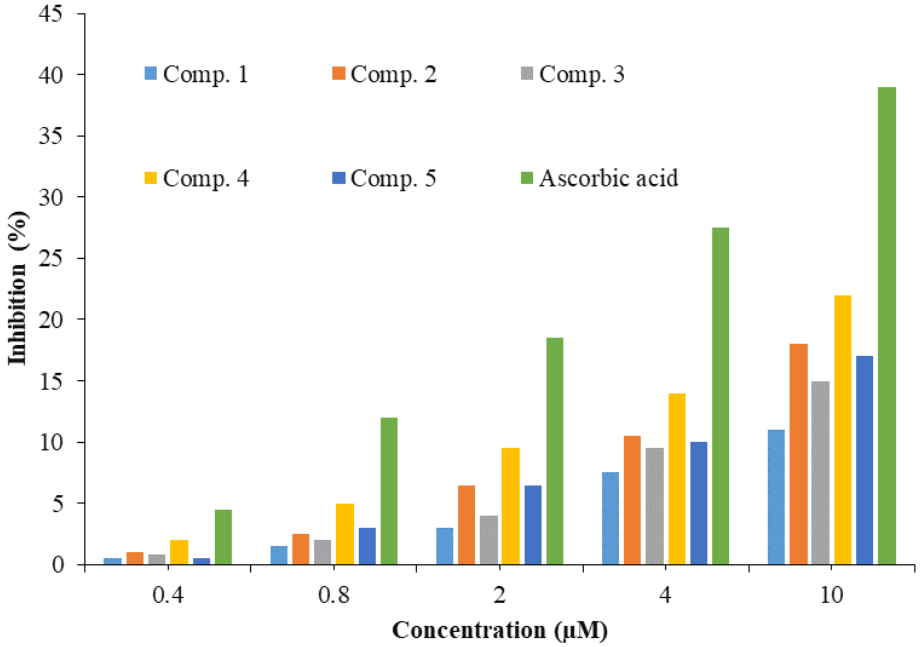


Fig. 4 – Change in percentage of inhibition calculated by the DPPH method for compounds **1-5** and ascorbic acid.

Table 5

IC_{50} values of ascorbic acid and all compounds (**1**–**5**) calculated using the DPPH method

Compounds	DPPH radical scavenging activity IC_{50} (µM)*	R^2
1	17.525 ± 0.15	0.946
2	10.909 ± 0.06	0.945
3	13.086 ± 0.17	0.938
4	9.308 ± 0.04	0.973
5	12.502 ± 0.09	0.926
Ascorbic acid	5.289 ± 0.02	0.987

* IC_{50} = concentration (µM) indicating 50% DPPH radical inhibition. Values are expressed as mean (n = 3).

Antibacterial activity assessments

According to the in vitro antibacterial activity results for the synthesized 1,3,4-thiadiazole compounds, it was determined that these compounds did not show antibacterial activity against *P. aeruginosa* (Table 6). It is previously stated that if MBC/MIC value is lower than 4, the compound of interest is accepted to have a bactericidal activity. Where this value is equal to or higher than 4, the type of activity is proposed to be bacteriostatic.[66, 67]

Compounds **1** and **4** showed the highest antibacterial activity for *K. pneumoniae* with a MIC value of 30 µg/mL. Compound **5** presented antibacterial activity against *K. pneumoniae* with a MIC value of 60 µg/mL, while compounds **2** and **3** with a MIC value of 120 µg/mL. According to the MBC test for *K. pneumoniae*, compounds **1**, **2**, **3**, and **5** showed bactericidal activity as the MBC/MIC values were lower than 4. Compound **4** presented bacteriostatic activity for *K. pneumoniae*.

The MIC test results showed that all compounds except for **4** presented antibacterial activity on *E.*

faecium. Compound **1** had the highest antibacterial activity on *E. faecium* with a MIC value of 15 μg/mL. Compound **5** showed antibacterial activity with MIC values of 30 μg/mL, while compounds **2** and **3** had a MIC value of 60 μg/mL. According to the MBC/MIC test, compounds **3** and **5** presented bactericidal activity. The results regarding *E. faecium* showed that compound **5** is quite promising. Moreover, it is unclear if compounds **1** and **2** have bactericidal or bacteriostatic activities due to the lack of MBC values.

Compounds **1**, **3**, and **4** presented an antibacterial activity against *B. subtilis*. Compound **3** showed the highest activity with a MIC value of 7.5 μg/mL. In addition, compound **4** had MIC values of 15 μg/mL. Compound **1** showed antibacterial activity with a MIC value of 120 μg/mL. Moreover, compounds **2** and **5** did not present antibacterial activity against *B. subtilis*. Besides, the MBC values of **1**, **3**, and **4** have not been found, so it is not possible to determine whether these compounds have bactericidal or bacteriostatic activities.

According to the MIC test; compounds **3** and **4** exhibited a low antibacterial activity against *S. macrescens* with a MIC value of 120 μg/mL, while other compounds did not show any effect. Only compound **1** are observed to present antibacterial activity against *S. kentucky* with MIC values of 120 μg/mL. In contrast, compounds **1** and **4** had an activity on *E. coli* and compound **1** on *S. epidermidis* with the same MIC values. Furthermore, the MBC values for all compounds have not been determined against *B. subtilis*, *S. macrescens*, *S. kentucky*, *E. coli*, and *S. epidermidis*, so it is not possible to determine whether the compounds' activities are bactericidal or bacteriostatic.

Table 6

MIC and MBC values of the synthesized compounds (μg/mL)

Bacteria	**Activity**	**1**	**2**	**3**	**4**	**5**
K. pneumoniae	MIC	30	120	120	30	60
	MBC	30	120	120	120	60
E. faecium	MIC	15	60	60	–	30
	MBC	–	–	60		30
B. subtilis	MIC	120	–	7.5	15	–
	MBC	–		–	–	
S. marcescens	MIC	–	–	120	120	–
	MBC			–	–	
S. kentucky	MIC	120	–	–	–	–
	MBC	–				
E. coli	MIC	120	–	–	120	–
	MBC	–			–	
S. epidermidis	MIC	120	–	–	–	–
	MBC	–				
P. aeruginosa	MIC	–	–	–	–	–
	MBC					

Önkol *et al.*[68] synthesized 1,2,4-triazole and 1,3,4-thiadiazole derivatives and screened their antimicrobial activities against selected microorganisms with a concentration range of 1024 and 2 μg/mL. However, none of the compounds had antibacterial activity against *P. aeruginosa* at any concentration.[68] Similarly, we observed no activity for *P. aeruginosa* for any of our compounds. They reported that some of the compounds showed no activity against *B. subtilis* at any concentration, while some presented antibacterial activity *against B. subtilis* with MIC values between 32 and 512 μg/mL. In our study, compounds **1**, **3**, and **4** showed antibacterial activity against *B. subtilis* with MIC values between 7.5 and 120 μg/mL. In their study, the compounds did not show activity against *E. coli* at any concentration, but our compounds (**1** and **4**) showed activity with a MIC value of 120 μg/mL. Although this may mean that our compounds have more effective activity against *B. subtilis* and *E. coli*, the difference in the activities is possibly related to the difference in the strains used in these studies.

Moshafi *et al.*[69] reported that antibacterial activity was observed for 2-(5-nitro-2-furyl)-5-(*n*-butylthio)-1,3,4-thiadiazole and 2-(1-methyl-5-nitro-H1-imidazole-2-yl)-5-(*n*-butylthio)-1,3,4-thiadiazole against *E. coli*.[69] In our study, compounds **1** and **4** showed antibacterial activity for *E. coli* with MIC values of 120 μg/mL. In the same

study, some compounds showed no activity against *B. subtilis* at any concentration, while some showed antibacterial activity against *B. subtilis* with MIC values between 0.5 and 16 μg/mL. In our study, compounds **1**, **3**, and **4** presented antibacterial activity against *B. subtilis* with MIC values between 120, 7.5, and 15 μg/mL, respectively. In the same study, 2-(1-methyl-5-nitro-H1-imidazol-2-yl)-5-(*n*-butylthio)-1,3,4-thiadiazole had antibacterial activity against *K. pneumoniae* with MIC values between 0.5 and 64 μg/mL. In our study, all compounds showed antibacterial activity against K. *pneumoniae* with 30, 60 and 120 μg/mL MIC values. Additionally, in their study, 2-(1-methyl-5-nitro-H1-imidazol2-yl)-5-(*n*-butylthio)-1,3,4-thiadiazole presented antibacterial activity against *S. marcescens* with MIC values between 0.5 and 64 μg/mL. In our study, compounds **2** and **3** showed antibacterial activity against *S. marcescens* with MIC values of 120 μg/mL. However, the differences between the MIC values in these two studies should be considered when comparing the differences between the strains.

In another study, [70] synthesized 1,3,4-thiadiazole derivatives and examined the antibacterial activities of these compounds against some bacteria in a concentration range between 12.5 and 100 μg/mL. For bis (2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide, the MIC value against *E. coli* was 250 μg/mL.[70] In our study for *E. coli*, compounds **1** and **4** showed antibacterial activity with MIC values of 120 μg/mL. Additionally, they reported that the MIC for 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide was less than 250 μg/mL against *E. faecium*. In our study, compounds **1**, **2**, **3**, and **5** had MIC values between 15 and 60 μg/mL. In addition, they calculated the MIC value of 2-amide-1,3,4-thiadiazole less than 250 μg/mL against *B. subtilis*. However, we obtained those values between 7.5 and 120 μg/mL for compounds **1**, **3**, and **4**.

AChE and BChE inhibition

In vitro inhibitory effects of synthesized novel *N*-aryl/alkyl-1,3,4-thiadiazole derivatives on AChE and BChE enzyme activities were analyzed, and the calculated IC_{50} values were shown in Table 7. We provide here the inhibitory effects of *N*-substituted-1,3,4-thiadiazole derivatives (**1-5**) on the cholinesterase activity of AChE and BChE, using AChI, and BChI as substrates, respectively. All produced compounds inhibited AChE at the micromolar level. As for BChE, micromolar inhibitions were also observed for the derivatives except for **1**. Compound **2** showed a better inhibitory effect on AChE with IC_{50} values of 27.39 μM, and **2** exhibited the highest effects against BChE with IC_{50} values of 13.35 μM. Other compounds also exhibited similar AChE inhibitory activity with IC_{50} values ranging from 29.78 to 45.71 μM, but moderate BChE inhibitory activity ranging from 52.20 to 115.17 μM. However, compound **1** had no inhibition on BChE activity.

Taha *et al.*[71] reported the synthesis and AChE&BChE inhibition effects of indole-based thiadiazole derivatives. Similar to the findings of our study, IC_{50} values of their derivatives ranged from 0.17 to 33.10 μM against AChE and from 0.30 to 37.60 μM against BChE. Moreover, they reported that the most powerful analogues have *para*, *ortho* and *meta*-fluoro substitutes.[71]

Table 7

IC_{50} values of the synthesized compounds for AChE and BChE

Compounds	**IC_{50} (μM)**	
	AChE	**BChE**
1	29.78±0.04	N.D.
2	27.39±0.01	13.35±0.02
3	45.71±0.02	115.17±0.02
4	38.56±0.02	90.40±0.03
5	38.54±0.02	52.20±0.02
Rivastigmine[72]	501±0.04	19.95±0.01
Tacrine[73]	18.46±0.03	2.50±0.03

The anticholinesterase (AChE and BChE) activity evaluation of acridine derivatives containing 1,3,4-thiadiazole moiety synthesized by Lotfi *et al.*[74] reported that all derivatives could

inhibit both enzymes and had significantly more inhibition effect against AChE than BChE. The IC_{50} values for AChE and BChE of these derivatives ranged from 0.002 to 1.78 μM and 0.031 to 13.12 μM, respectively, with stronger inhibition effects than our findings.[74] In another study containing 1,3,4-thiadiazole derivatives, good to moderate inhibitory activities were observed with IC_{50} values ranging from 128.42 to 0.06 μM for AChE and >500 to 0.29 μM for BChE. It was also reported that a thiadiazole derivative with a Br substituent in the *ortho*-position exhibited the most potent inhibitory effect against AChE and BChE, with IC_{50} values of 0.06 and 0.29 μM, respectively.[75] Similar results were reported by Skrzypek et al.[76] on (1,3,4-thiadiazol-2-yl)benzene-1,3-diol based compounds in vitro AChE and BChE inhibitor effect, with IC_{50} values ranging from >500 to 0.053 μM and from >500 to 0.105 μM, respectively.[76] Similar results were obtained in a study on chalcone derivatives by Arslan *et al.*[77]

CONCLUSIONS

A new series of the synthesis of furan-tethered 1,3,4-thiadiazole derivatives using various *N*-substituted-thiosemicarbazide derivatives and characterization with spectroscopic methods was achieved in this study. The synthesized derivatives exhibited variable antioxidant and antibacterial activities against *B. subtilis*, *K. pneumoniae*, and *E. faecium*. The antioxidant activities of the compounds decreased in the following order **4** > **2** > **5** > **3** > **1**. Compound **4** showed the highest antioxidant activity, while compound **3** exhibited the highest antibacterial activity against *B. subtilis*, and compound **1** presented relatively high antibacterial activity against *E. faecium*. Moreover, not all compounds showed antibacterial activity against *P. aeruginosa*. The same of synthesized compounds also showed potential as a source of antibacterial agents against the same tested strains. Additionally, the synthesized compounds exhibited inhibitory properties against AChE and BChE enzymes, with compound **2** exhibiting the highest inhibitory property against AChE and BChE.

The results suggest that the variations in the structure of the furan-tethered 1,3,4-thiadiazole derivatives significantly influenced their biological activities. The structure-activity relationships indicate that specific functional groups and their positions on the phenyl ring are crucial for enhancing antioxidant and antibacterial properties. These findings highlight the promising pharmacological profiles of the synthesized derivatives, which could serve as lead compounds in the design of novel antioxidant and antibacterial agents. Further studies, including *in vivo* testing and additional mechanistic studies, are needed to evaluate the therapeutic potential and safety of these compounds.

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