Scientific paper

New Bis-1,3,4-Thiadiazoles Based on Fumaric Acid: Preparation, Structure Elucidation, Antibacterial Activities, and Quantum-Chemical Studies

Halit Muğlu,[1,*] Hasan Yakan,[2] Ghaith Alabed Ibrayke Elefkhakry,[1] Ergin Murat Altuner,[3,*] and M. Serdar Çavuş[4]

[1] *Department of Chemistry, Kastamonu University, Kastamonu, Turkey*

[2] *Department of Chemistry Education, Ondokuz Mayıs University, Samsun, Turkey*

[3] *Department of Biology, Kastamonu University, Kastamonu, Turkey*

[4] *Biomedical Engineering Department, Kastamonu University, Kastamonu, Turkey*

* *Corresponding author: E-mail: hmuglu@kastamonu.edu.tr ergin.murat.altuner@gmail.com*

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Abstract

New bis-1,3,4-thiadiazoles **1**–**7** were obtained by the reaction of fumaric acid and *N*-(alkyl/aryl/cyclic)thiosemicarbazides in the presence of phosphorous oxychloride. The structures of all compounds were elucidated by FT-IR, ^{1}H NMR, and ^{13}C NMR and elemental analysis. Antibacterial activity of the compounds was studied for eight selected bacteria. Compounds **2**–**7** exhibited effect on *Klebsiella pneumoniae*. However, none of the compounds effect on *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Salmonella enterica* serovar *Kentucky*, *Serratia marcescens*. Self-consistent reaction force (SCRF) calculations were performed in DMSO medium to examine solvent energies using CPCM and SMD models. 6-31G(d) and 6-311++G(2d,2p) basis sets were used for DFT calculations. Besides electronic parameters such as electronegativity, electrophilicity and spectroscopic examinations of the compounds, QTAIM, local electron affinities, and Fukui analyses were also performed. Theoretical approaches supporting the experimental observations revealed that compounds containing aromatic and cyclic groups exhibit stronger antibacterial behavior than compounds containing aliphatic groups.

Keywords: Bis-1,3,4-thiadiazoles; fumaric acid; antibacterial activity; spectroscopic methods; DFT calculations.

1. Introduction

Heterocyclic compounds represent a very significant part of organic chemistry. They have an extensive scope of medicinal, biological, and synthetic applications.[1] Thiadiazoles have both one sulphur and two nitrogen atoms which form aromatic five-membered heterocyclic ring compounds. Thiadiazoles have four isomeric forms, 1,3,4-thiadiazole is one of them, being thermally the most stable among these isomers.[1h]

1,3,4-Thiadiazoles exhibit various applications in medicinal, biological, agricultural, and materials chemistry such as antioxidant,[2] antiviral,[3] antifungal,[4] antimicrobial,[5] analgecis,[6] antidepressant,[7] antileishmanial,[8] anticonvulsant,[9] anti-inflammatory,[10] antitubercular,[11] anticancer,[12] and antiproliferative activities.[13]

Despite the fact that an extensive number of chemotherapeutics and antibiotics exist, the appearance of new and old antibiotic-resistant bacterial strains have shown a need to consider both the synthesis and exploration of novel more harmless, powerful, and confident antimicrobial agents.[14]

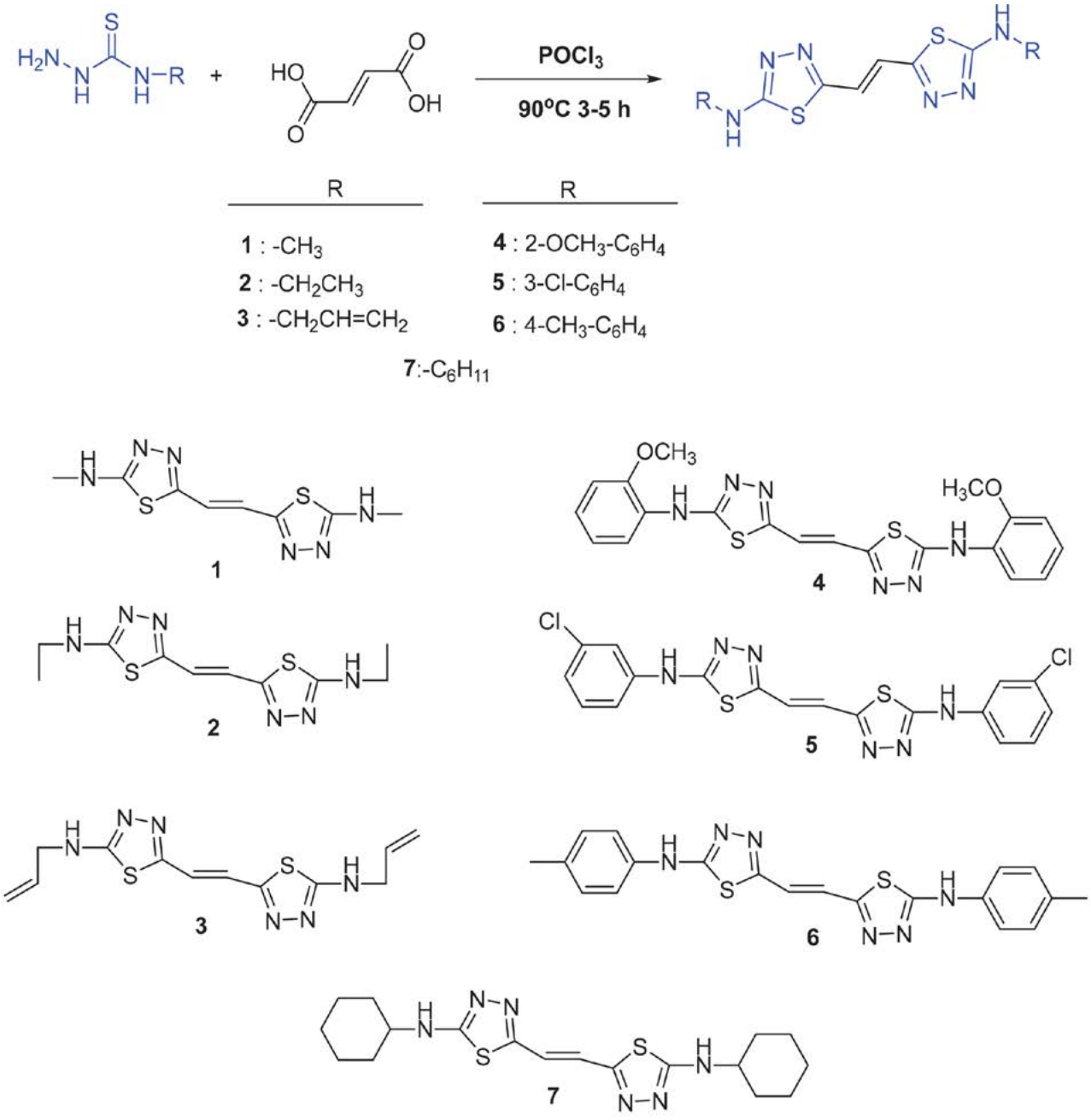
In the paper, new bis-1,3,4-thiadiazole derivatives were obtained from the reaction of fumaric acid, *N*-(alkyl/aryl/cyclic)thiosemicarbazides, and phosphorous oxychloride ($POCl_3$). The structures of the compounds were determined by using FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopic methods and elemental analysis. The antibacterial activity of the compounds was investigated against several Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus epidermidis*, and *Enterococcus*

faecium) and Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica* serovar *Kentucky*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) by minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) tests. Moreover, optimized molecular structures and spatial conformations of the compounds were investigated by theoretical studies using the DFT method; experimental spectroscopic data were also supported by the calculation results. Some electronic parameters of the compounds were calculated and used to establish the structure-activity relationship.

2. Experimental

2. 1. Measurement and Reagents

Melting points were determined on a Stuart SMP 30 electrothermal apparatus. Eurovector EA3000-Single device was utilized for elemental analysis. A Bruker Alpha FT-IR spectrophotometer were used to obtain Fourier transform infrared (FT-IR) spectra. ^{1}H NMR and ^{13}C NMR spectra were taken in DMSO-d_6 on a Bruker Avance DPX-400 spectrophotometer (400 MHz and 101 MHz, respectively) spectrometer using tetramethylsilane as an internal standard. The splitting patterns are indicated as



Scheme 1. Structures of compounds **1**–**7** and synthetic route to them.

s (singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplet), td (triplet of doublet) and m (multiplet). All chemical reagents were purchased from Aldrich, Carlo Erba, Acros Organics, or Merck Chemical Company and used without further purification.

2. 2. Synthesis of the Compounds 1–7

The mixture of fumaric acid (*n* mol) and *N*-(alkyl/aryl/cyclic)thiosemicarbazide derivatives (2*n* mol) was chilled in a refrigerator and phosphorous oxychloride (3*n* mol) was added drop-wise during stirring. Then, refluxing was continued at 90 ºC for 3–5 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. These new bis-1,3,4-thiadiazoles were prepared according to the procedure described in the literature.[1b] They were obtained in good yield (61–93%) as shown in Scheme 1.

2. 3. Antibacterial Activity Testing

Bacteria Strains

The antibacterial activities of the synthesized compounds were determined against three Gram-positive (*Bacillus subtilis* DSMZ 1971, *Enterococcus faecium* (food isolate), and *Staphylococcus epidermidis* DSMZ 20044) bacteria, and five Gram-negative (*Escherichia coli* (food isolate), *Klebsiella pneumoniae* (food isolate), *Pseudomonas aeruginosa* DSMZ 50071, *Salmonella enterica* serovar *Kentucky* (food isolate), and *Serratia marcescens* (clinical isolate)). All the bacteria were obtained from the culture collection of Kastamonu University, Department of Biology, Microbiology Laboratory.

Preparation of Chemical Compounds

The stock solutions were prepared by dissolving 24 mg of each compound **1**–**7** in 1 mL of dimethyl sulfoxide (DMSO) (Merck). Since DMSO is toxic for living cells, the in-test DMSO concentration was adjusted as 1%.[15]

Preparation of the Inocula

The bacteria used in the study were incubated at 37 °C for 24 hours and similar colonies were collected by a sterile loop, transferred into 0.9% sterile saline solution, and the turbidities were adjusted to 0.5 McFarland standard.[16]

Minimum Inhibition Concentration (MIC) Test

MIC test was applied as a broth dilution test as previously defined in previous studies.[17] Serial 2-fold dilutions were done in a 96-well plate and a concentration range of 0.234–120 μg/mL was obtained. The MIC value was determined as the lowest concentration of extract inhibiting any visible bacterial growth.[18] All tests were studied in triplicate.

Minimum Bactericidal Concentration (MBC) Test

The MBC test is complementary to the MIC test, where the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth, and the MBC demonstrates the lowest level of antimicrobial agent that causes microbial death. The MBC values were determined by sub-culturing the contents of non-turbid MIC test wells to agar plates. The MBC is identified as the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by ≥99.9%.[19]

Controls

1% DMSO was used as a negative control, where gentamicin (GEN), tobramycin (TOB), and ciprofloxacin (CPFX) were used as positive controls.

2. 4. Computational Procedure

The molecular structure of the compound in the ground state (in vacuum) was obtained from the optimization calculations performed using the B3LYP/6-311++g(2d,2p) level of theory by the Kohn–Sham density functional theory (KS-DFT).[20] All calculations were performed using Gaussian 09 software[21] without any symmetry restrictions. The optimized state geometries of the compounds with minimum energy correspond to the global minimum energy points on the potential energy surface, that is, no imaginary frequencies are present in the IR calculations. Moreover, solvent effects were studied using solvation model based on density (SMD) and conductor-like polarizable continuum model (CPCM), at the same level of theory, to calculate the properties of compounds in solution.

^{1}H and ^{13}C NMR chemical shifts calculations were performed using Gauge-independent atomic orbital (GIAO) method in dimethyl sulfoxide (DMSO) phase, in accordance with the experiments. Relative chemical shift values were obtained by subtracting (31.8149 and 183.737 ppm for ^{1}H and ^{13}C NMR, respectively) from the absolute chemical shielding of tetramethylsilane (TMS), which was also calculated at B3LYP/6-311++g(2d,2p) level of theory.

The electronic parameters of the compounds were obtained from the calculations performed at the level of B3LYP/6-311++g(2d,2p) in the gas phase. Global chemical reactivity parameters such as HOMO-LUMO energy gap (E_g), Chemical hardness (**η**), electronegativity (**χ**), and electrophilic index (**ω**) were calculated using frontier molecular orbital (FMO) energy eigenvalues. The calculations of the Fukui functions were performed at the B3LY-

P/6-31g(d) level, and the electrophilic and nucleophilic attack regions were analyzed by visualizing the data. In addition, the reactivity and behavior of the compounds in different environments were estimated by FMO calculations and prominent descriptors were reported. Besides the population analysis of natural bond orbitals (NBOs), intramolecular interactions were studied through topological properties using Bader's quantum theory of atoms in molecules (QTAIM) approach[22] and were also used to calculate electron charge distributions on compounds. Ring critical points (RCPs) of charge density distribution and bond critical points (BCPs) of bonded atoms were determined by QTAIM analyses performed using Multiwfn software,[23] and interaction region indicator (IRI) calculations were performed to visualize intramolecular interactions.

3. Results and Discussion

3. 1. Physical Data

All the compounds are new. In Table 1, the physical data, melting points, yields, and elemental analysis of these compounds are presented.

Table 1. Physical data and elemental analysis results of the compounds

Comp.	Compound's Names	M. P. (°C)	Yields% (Mass, g)	Colour	Calculated/Found C%	H%	N%
1	(*E*)-*N*-methy-5-(2-(5-(methylamino)-1,3,4-thiadiazol-2-yl)vinyl)-1,3,4-thiadiazol-2-amine	65 240–241	Brown (0.206)	37.78/	3.96/ 37.69	33.04/ 4.05	 32.89
2	(*E*)-*N*-ethyl-5-(2-(5-(ethylamino)-1,3,4-thiadiazol-2-yl)vinyl)-1,3,4-thiadiazol-2-amine	>300	61 (0.296)	Dark Brown	42.53/ 42.45	5.00/ 4.90	29.76/ 29.67
3	(*E*)-*N*-allyl-5-(2-(5-(allylamino)-1,3,4-thiadiazol-2-yl) vinyl)-1,3,4-thiadiazol-2-amine	>300	73 (0.289)	Cream	47.04/ 46.94	4.61/ 4.71	27.43/ 27.35
4	(*E*)-*N*-(2-methoxyphenyl)-5-(2- (5-(2-methoxyphenylamino)-1,3,4-thiadiazol-2-yl)vinyl)-1,3,4-tiadiazol-2-amine	175–176 (0.404)	87	Dark Yellow 54.72	54.78/ 4.08	4.14/ 19.21	19.16/
5	(*E*)-*N*-(3-chlorophenyl)-5-(2-(5-(3-chlorophenylamino)-1,3,4-thiadiazol-2-yl)vinyl)-1,3,4-thiadiazol-2-amine	168–169	75 (0.289)	Dark Cream	48.33/ 48.26	2.70/ 2.75	18.79 18.64
6	(*E*)-5-(2-(5-(*p*-toluidin)-1,3,4-thiadiazol-2-yl)vinyl)-*N*-*p*-tolyl-1,3,4-thiadiazol-2-amine	198-199	88 (0.462)	Dark Yellow	59.09/ 59.17	4.46/ 4.41	20.67/ 20.88
7	(*E*)-*N*-cyclohexyl-5-(2-(5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)vinyl)-1,3,4-tihadiazol-2-amine	>300	93 (0.457)	Cream	55.35/ 55.43	6.71/ 6.59	21.52/ 21.46

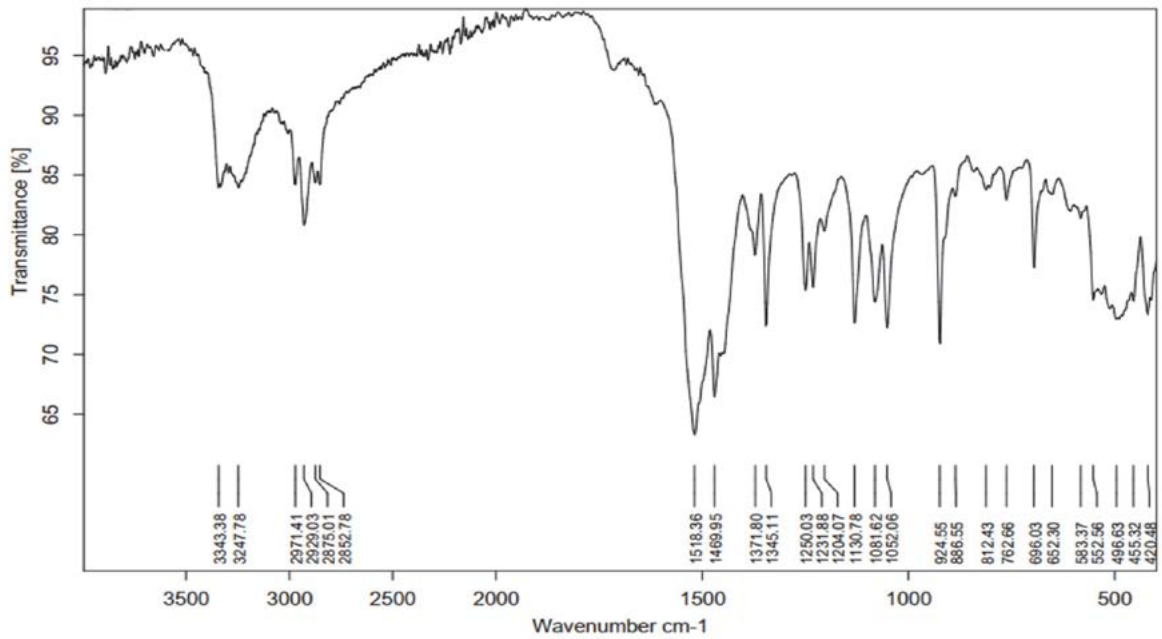


Figure 1. IR spectrum of compound **2**

3. 2. Vibrational Frequencies

In the FT-IR spectrum of the synthesized compounds, the fumaric acid (–COOH) signal of the starting material was not observed near 3500–2700 cm^{-1}. Furthermore, the asymmetric and symmetric stretching bands of the amino group ($–NH_2$) were not observed at 3450–3200 cm^{-1}. These results pointed out a successful reaction, as expected. For all compounds, the peaks of the amino group (–NH) were showed between at 3248–3195 cm^{-1}; the –C=N stretching vibrations of thiadiazole ring were observed between at 1635 and 1518 cm^{-1}; the –C–N stretching vibrations were shown between 1174–1083 cm^{-1}; the –C–S signals were observed between 721–634 cm^{-1}. For compound **2**, the band of the amino group (–NH) was shown at 3248 cm^{-1} as shown in Figure 1. The aliphatic CH stretching vibrations were observed at 2929–2853 cm^{-1}. The –C=N stretching vibrations of thiadiazole ring appeared at 1518 cm^{-1}. The –C–N stretching vibration appeared at 1131 cm^{-1}; the –C–S signal was observed at 696 cm^{-1}. These values provided significant proofs for the products formation. These observations are consistent with values published previously for similar compounds.[1b,5b,24] IR vibrations for the synthesized compounds are presented in Table 2.

3. 3. ^{1}H NMR Spectral Interpretations

The ^{1}H NMR spectra of the synthesized compounds were measured in DMSO-d_6 as the solvent and the chemi-

Table 2. Experimental and calculated FT-IR values of the compounds **1**–**7** (cm^{-1}).

	C.	–NH	Ar. CH	C=N	C–N	C–S	Spec. Vib.
Experimental	**1**	3199	–	1586–1555	1153	634	CH(*): 2924–2847
	2	3248	–	1518	1131	696	CH(*): 2929–2853
	3	3219	–	1591–1532	1142	697	CH(*): 2928–2871
	4	3248	3075–3002	1635–1601	1174	654	CH(*): 2939–2837 -C-O : 1114
	5	3247	3092–3061	1631–1585	1170	721	-C-Cl : 994
	6	3195	3061–2992	1635–1604	1083	714	CH(*): 2920–2874
	7	3230	–	1611–1560	1120	696	CH(*): 2926–2852
Calculated	**1**	3645.81	–	1518.64, 1510.03	1040.41	685.41	CH(*): 3140.07–3030.96
	2	3622.25	–	1511.22, 1506.33	1060.03	685.91	CH(*): 3113.31–3016.33
	3	3619.58	–	1508.94, 1498.25	1054.43	686.96	CH(*): 3222.01–3019.94
	4	3622.59	3239.37–3195.42	1513.02, 1500.08	1266.40	689.77	CH(*): 3142.48–3024.14 -C-O : 1051.00
	5	3642.73	3250.32–3166.74	1513.73, 1500.88	1256.08	688.46	-C-Cl : 907.16
	6	3642.51	3239.59–3151.08	1512.38, 1501.75	1252.28	687.50	CH(*): 3106.15–3028.97
	7	3625.36	–	1511.35, 1509.87	1087.40	684.87	CH(*): 3097.13–3011.82

C.: Compounds, (*) : Aliphatic CH.

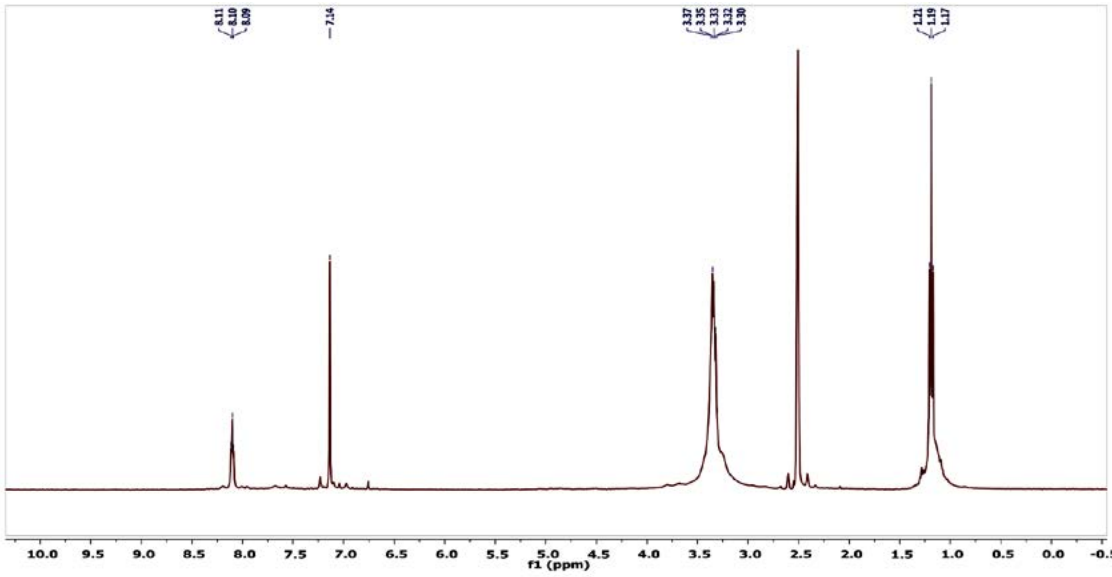
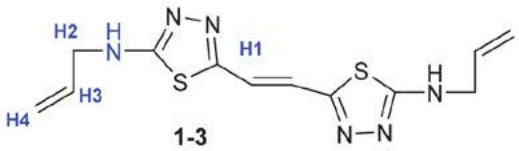
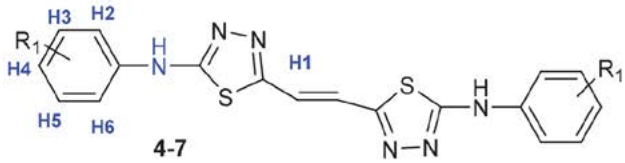


Figure 2. ^{1}H NMR spectrum of compound **2**.

Table 3. Experimental and calculated ^{1}H NMR values of the compounds (**δ**, ppm, in DMSO–d_6)



R: CH_3, CH_3CH_2, CH_2=CH-CH_2



R: 2-OCH_3, 3-Cl, 4-CH_3, C_6H_{11}

	C.	NH	H1	H2	H3	H4	H5	H6
Experimental	**1**	8.06–8.03 (q)	7.15 (s)	2.93–2.92 (d)	–	–	–	–
	2	8.11–8.09 (t)	7.14 (s)	3.97–3.28 (p)	1.21–1.17 (t)	–	–	–
	3	8.33–8.30 (t)	7.14 (s)	3.97–3.88 (t)	5.97–5.87 (m)	5.29–5.15 (dd)	–	–
	4	10.05 (s)	7.36 (s)	OCH_3: 3.89 (s)	8.31–8.29 (m)	7.09–7.04 (m)		7.01–6.97 (m)
	5	10.52 (s)	7.50–7.48 (d)	7.94 (s)	–	7.41–7.37 (dd)		7.10–7.08 (d)
	6	10.22 (s)	7.46 (s)	6.84–6.80 (d)	7.14–6.99 (d)	CH_3: 2.51 (s)	7.14–6.99 (d)	6.84–6.80 (d)
	7	7.93–7.91 (d)	6.74 (s)	1.95–1.03, (m), Cyclic 11H				
Calculated	**1**	5.09	7.76	3.29–3.02	–	–	–	–
	2	4.79	7.76	3.68–3.41	1.41–1.26	–	–	–
	3	4.66	7.79	4.69, 3.79	6.51	5.82–5.63	–	–
	4	8.19	7.99	4.38, 3.97	7.30	7.46	7.42	9.03
	5	7.37	8.05	9.18	–	7.38	7.71	7.16
	6	7.25	7.98	8.94	7.61	CH_3: 2.63–2.15	7.70	7.25
	7	4.66	7.72	2.14–1.29, Cyclic 11H				

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

cal shifts are shown in Table 3. For compound **2**, the proton signal of –NH coupled to the CH_2 proton was detected as a triplet at 8.11–8.09 ppm. The alkyenic (CH=C) H1 proton was shown as a singlet at 7.14 ppm. The H2 (CH_2) proton coupled to the H3 and NH protons was observed as a pentet at 3.37–3.30 ppm. The H3 (CH_3) proton coupled to the H2 protons was observed as a triplet at 1.21–1.17 ppm as shown in Figure 2. DMSO-d_6 and water in DMSO (HOD, H_2O) signals are shown around at 2.00, 2.50 (quintet) and 3.30 (variable, based on the solvent and its concentration) ppm, respectively.[25] These data are consistent with the values of those reported earlier for similar compounds.[5b,26]

3. 4. ^{13}C NMR Spectral Interpretations

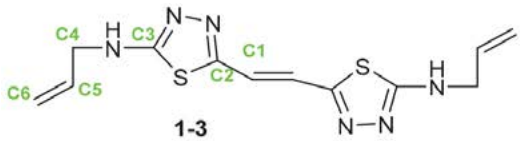
The ^{13}C NMR spectra of all compounds were measured in DMSO-d_6 and the chemical shifts are shown in Table 4. The ^{13}C NMR spectrum of the compound **2** showed 5 different resonances in good agreement with the proposed structure as shown in Figure 3. In compound **2**, the carbon signals of thiadiazole ring (C2 and C3) were detected at 155.4 and 169.0 ppm, respectively. The C3 carbon atom is shifted down-field (high values, δ) due to the presence of electron-negative nitrogen atom (NH group). The alkyenic C1 carbon atom (CH=C) was observed at 124.6 ppm. While the C4 (CH_2) carbon atom was detected at 40.1 ppm, the C5 (CH_3) resonated at 14.6 ppm. The C4 (CH_2) carbon atom is shifted down-field (high values, δ) as does the C3 carbon atom due to the presence of electron-egative nitrogen atom (NH group).

For compounds **1**–**7**, the alkyenic C1 carbon atom (CH=C) was observed between 125.7 and 120.8 ppm. While the C2 carbon signals of thiadiazole ring resonated a between 157.2 and 152.4 ppm, the C3 resonated between 169.6 and 164.3 ppm. For compound **3**, the C4 carbon atom (CH_2) was observed at 47.8 ppm. The alkyenic C5 and C6 carbon atoms (CH=CH_2) were detected at 134.6 and 117.3 ppm, respectively. In compounds **4**–**6**, the aromatic carbons (C4–C9) were observed at 149.1–111.7, 142.0–116.7, and 138.8–117.9 ppm, respectively. The methoxy (–OCH_3) signal was detected at 56.4 ppm for compound **4**, the methyl (CH_3) group was observed at 20.8 ppm for compound **6**. In compound **7**, the cyclic carbons (C4–C9) were observed between 53.9 and 24.7 ppm. These spectroscopic data are consistent with the values reported previously for similar compounds in the literature.[5b,26a,b,27]

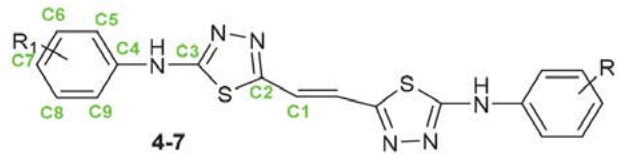
3. 5. Antibacterial Activity Assessments

In vitro antibacterial activity results for the synthesized 1,3,4-thiadiazole compounds are given in Table 5. The antibacterial activities of bis-1,3,4-thiadiazole compounds **1**–**7** were determined against three Gram-positive (*S. epidermidis*, *B. subtilis*, and *E. faecium*) and Gram-negative (*S. enterica* serovar *Kentucky*, *E. coli*, *K. pneumoniae*, *S. macrescens*, and *P. aeruginosa*) bacteria by two sequen-

Table 4. Experimental and calculated 13C NMR values of the compounds (δ, ppm, in DMSO–d6)



R: CH_3, CH_3CH_2, CH_2=CH-CH_2



R: 2-OCH_3, 3-Cl, 4-CH_3, C_6H_{11}

	Comp.	C1	C2	C3	C4	C5	C6	C7	C8	C9	R_1
Experimental	**1**	124.9	155.7	169.6	32.2	–	–	–	–	–	–
	2	124.6	155.4	169.0	40.1	14.6	–	–	–	–	–
	3	124.9	155.8	168.5	47.8	134.6	117.3	–	–	–	–
	4	125.2	157.1	165.3	129.7	149.1	111.7	123.7	121.0	120.0	56.4
	5	125.7	157.2	164.3	142.0	117.6	133.9	122.5	131.2	116.7	–
	6	121.2	154.8	165.4	138.8	117.9	129.9	130.2	130.2	117.9	20.8
	7	120.8	152.4	169.4	53.9	32.5	24.7	25.7	24.7	32.5	–
Calculated	**1**	122.7	164.4	178.7	32.6	–	–	–	–	–	–
	2	122.8	164.3	178.2	44.1	14.8	–	–	–	–	–
	3	123.1	164.9	178.3	52.6	143.9	125.6	–	–	–	–
	4	123.3	165.0	173.3	134.9	154.0	113.4	127.2	125.4	121.8	57.6
	5	123.7	165.7	173.8	147.6	122.0	149.4	127.5	136.2	120.6	–
	6	123.1	164.8	173.5	144.1	121.9	136.2	140.0	135.6	122.5	22.3
	7	122.1	163.6	177.6	53.6	29.0	23.8	22.5	23.6	33.2	–

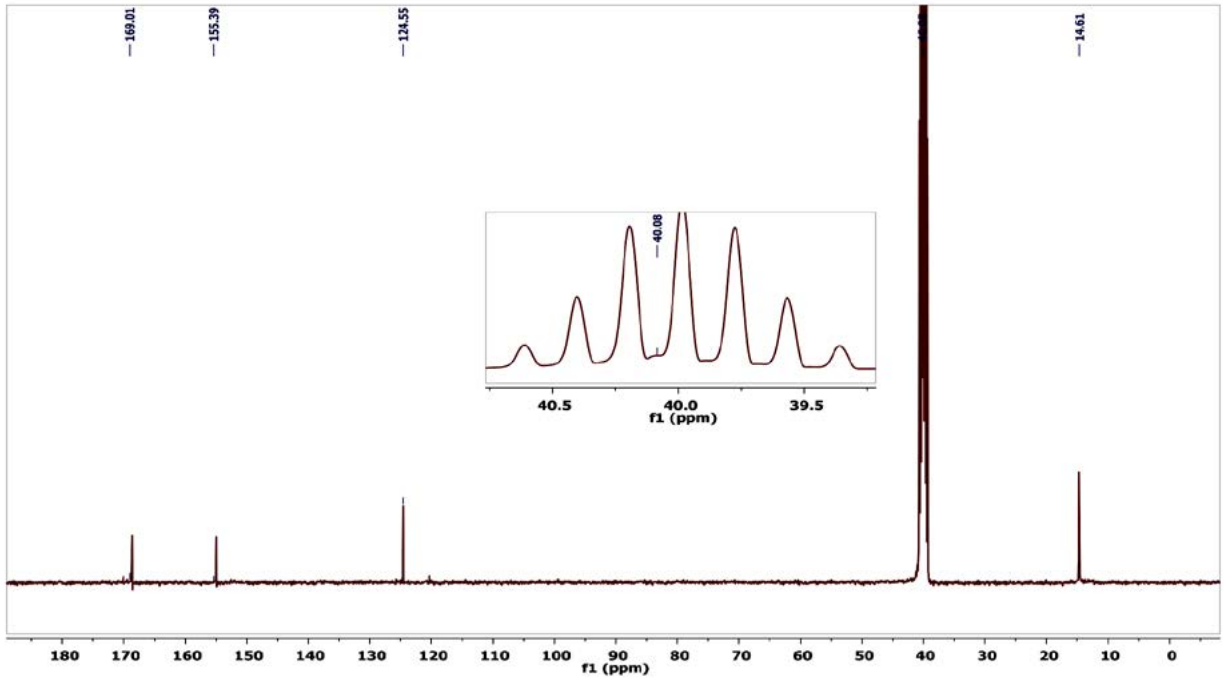


Figure 3. ^{13}C NMR spectrum of compound **2**.

tial tests, the first being the minimum inhibitory concentration (MIC) and the second the minimum bactericidal concentration (MBC) test.

The results of the antibacterial screening given in Table 5 show that none of the compounds **1**–**7** had activity against *P. aeruginosa*, *S. epidermidis*, *S. enterica* serovar *Kentucky*, and *S. macrescens*. All compounds except **1** showed an effect on Gram-negative *K. pneumoniae*. Compounds **2** and **3** showed antibacterial activity at MIC concentrations of 2.75 mg/L. The rest of compounds **4**, **5**, **6**, and **7** presented antibacterial activity with MIC concentrations of 1.375 mg/L.

Table 5. MIC and MBC values of the synthesized compounds (mg/L).

Bacteria	Activity	1	2	3	4	5	6	7	GEN	TOB	CPFX
S. enterica serovar *Kentucky*	MIC	–	–	–	–	–	–	–	2.500	2.500	0.078
	MBC	–	–	–	–	–	–	–			
E. coli	MIC–	–	–	5.5	–	–	–	5.5	10.00	10.00	–
	MBC	–	–	5.5	–	–	–	–			
K. pneumoniae	MIC	–	2.75	2.75	1.375	1.375	1.375	1.375	0.078	0.078	–
	MBC		5.5	5.5	5.5	5.5	5.5	5.5			
S. macrescens	MIC	–	–	–	–	–	–	–	–	–	–
	MBC	–	–	–	–	–	–	–			
P. aeruginosa	MIC	–	–	–	–	–	–	–	10.00	10.00	1.250
	MBC	–	–	–	–	–	–	–			
S. epidermidis	MIC	–	–	–	–	–	–	–	–	–	–
	MBC	–	–	–	–	–	–	–			
B. subtilis	MIC	–	–	–	2.75	5.5	2.75	2.75	1.250	2.500	–
	MBC	–	–	–	5.5		–	5.5			
E. faecium	MIC	–	–	5.5	–	5.5	5.5	5.5	–	0.625	0.156
	MBC	–	–	–	–	5.5	5.5	–			

GEN: gentamycin, TOB: tobramycin, CPFX: ciprofloxacin.

The activity type of any compound is decided by looking at the MBC/MIC ratio. If the MBC/MIC ratio is lower than 4 the activity of this compound is accepted as bactericidal, otherwise the activity is decided to be bacteriostatic.[28]

The MBC test applied after the MIC test for *K. pneumoniae* shows that compounds **2** and **3** presented a bactericidal activity because MBC/MIC is lower than 4. Compounds **4**, **5**, **6** and **7** showed bacteriostatic activity because MBC/MIC is equal to 4.

MIC test results demonstrated that compounds **3** and **7** have antibacterial activity on Gram-negative *E. coli* at concentrations of 5.5 mg/L. According to the MBC test, compound **3** exhibited a bactericidal activity, but it is not possible to conclude whether the activity of compound **7** is bactericidal or bacteriostatic. Because the MBC of the compound could not be determined, thus MBC/MIC ratio cannot be calculated.

Compounds **4**, **6**, and **7** show antibacterial activity on Gram-negative *B. subtilis* at 2.75 mg/L, and 5.5 mg/L for compound **5**. MBC test shows that compounds **4** and **7** possess bactericidal activity because MBC/MIC ratio is lower than 4. On the other hand, since the MBC of compounds **5** and **6** could not be determined, it is not possible to conclude whether the activities of these two compounds are bactericidal or bacteriostatic.

Compounds **3**, **5**, **6**, and **7** exhibited antibacterial activity on Gram-negative *E. faecium* at concentrations of 5.5 mg/L for each. MBC test shows that compounds **5** and **6** have bactericidal activities because MBC/MIC ratios are lower than 4. On the other hand, since the MBC of compounds **3** and **7** could not be determined, it is not possible to conclude whether the activities of these two compounds are bactericidal or bacteriostatic.

Rauckyte *et al.*[29] synthesized some 1,3,4-thiadiazole derivatives and investigated 12.5, 25.0, 50.0, and 100 g/L concentrations of these compounds for their antibacterial activity against selected bacterial strains. As a result, they observed that the MIC value against *E. coli* for 2-acetamide-1,3,4-thiadiazol-5-sulfonamide was 12.5 g/L. In our study, 1,3,4-thiadiazole derivatives (compounds **3** and **7**) showed antibacterial activity against *E. coli* with MIC values of 0.0055 g/L. It was seen that our compounds showed higher antibacterial activity against *E. coli*, compared to the compounds synthesized by Rauckyte *et al.*.[29] But these two results cannot be compared since the *E. coli* strains were not the same.

The MIC value against *E. faecium* for 2-acetamide-1,3,4-thiadiazol-5-sulfonamide was 12.5 g/L as determind by Rauckyte *et al.*[29] In our study, compounds **3**, **5**, **6**, and **7** demonstrated antibacterial activities with MIC values of 0.0055 g/L. This shows that our compounds have higher antibacterial activity against *E. faecium*, but since the strains used in these studies were different it is not suitable to compare these results.

The MIC value against *B. subtilis* for 2-acetamide-1,3,4-thiadiazol-5-sulfonamide has shown no activity as determined by Rauckyte *et al.*[29] In the study conducted for *B. subtilis*, compounds **4**, **5**, **6**, and **7** showed antibacterial activity with MIC values either 0.00275 or 0.0055 g/L. This indicates that the compounds show higher antibacterial activity against *B. subtilis*, again it is not convenient to compare the results as the *B. subtilis* strains used in these studies were different.

Moshafi *et al.*[30] synthesized 5-nitro-2-furyl and 5-nitro-2-imidazolyl derivatives of 1,3,4-thiadiazole and tested their antibacterial activity against *S. epidermidis* PTCC 1114, *B. subtilis* PTCC 1023, *Streptococcus pyogenes* PTCC 1447, *Micrococcus luteus* PTCC 1110, *E. coli* PTCC 1330, *P. aeruginosa* PTCC 1074, *K. pneumoniae* PTCC 1053, *S. marcescens* PTCC 1621 with a concentration range of 0.5, 1, 2, 4, 8, 16, 32 and 64 mg/L. As a result, they observed that the activity of the compounds were ranging between 0.50 and 64.0 mg/L. For example, the MIC for 2-(1-methyl-5-nitro-*H*1-imidazol-2-yl)-5-(*n*-pentylsulfinyl)-1,3,4-thiadiazole against *K. pneumoniae* was obtained to be 0.50–64.0 mg/L In addition, the same MIC value was observed for 2-(5-nitro-2-furyl)-5-(*n*-butylthio)-1,3,4-thiadiazole against *E. coli*. Also, 2-(5-nitro-2-furyl)-5-(*n*-butylsulfonyl)-1,3,4-thiadiazole presented a MIC value of 0.50–16.0 mg/L against *B. subtilis*. In our study, compounds **2**, **3**, **4**, **5**, **6**, and **7** showed antibacterial activity against *K. pneumoniae* with MIC values of 2.75 and 1.375 mg/L. Again, compounds **3** and **7** presented MIC values of 5.5 mg/L against *E. coli*. In addition, compounds **4**, **5**, **6**, and **7** showed antibacterial activity with MIC values either 2.75 or 5.5 g/L against *B. subtilis*. These results indicate that our compounds usually showed higher antibacterial activity against *K. pneumoniae*, *E. coli*, and *B. subtilis*. However, these differences may be due to the use of different strains.

3. 6. Theoretical Analysis

Although electronic parameters such as the kinetic stability or chemical hardness of a molecule, hence its E_g, have a role on the probability of a chemical reaction to take place, the unpredictability of the steps of the reactions with living organisms such as bacteria causes these electronic parameters to be interpreted with hypothetical approaches specific to the relevant experiment in a way that supports the experimental data. It is known that HOMOs are the parameters that determine the capacity to donate electrons and LUMOs to accept electrons. The HOMO-LUMO gap represents the range in the binding energy spectrum in which the most probable excitations can occur, and indeed there is an inverse relationship between the HOMO-LUMO energy gap and the probability of excitations, i.e. the smaller the E_g means that the excitation will occur more easily. Furthermore, E_g can be a useful tool to study (or predict) the stability and chemical reactivity of a molecule, taking into account its sensitive dependence on many factors such as environment (reaction medium), molecular conformation, temperature, conjugation effects, intra- and intermolecular interactions. The small HOMO-LUMO gap of a molecule results in a lower kinetic stability, meaning higher chemical reactivity or lower chemical hardness. In this context, although not a very strong approximation, among synthesized compounds, compounds **4**–**7** can be expected to exhibit higher chemical reactivity than compounds **1**–**3** (see Figure 4b). The calculations show that the compounds can be classified in three groups: the first group, compounds **1**–**3** containing aliphatic structures attached to –NH; the second group, compounds **4**–**6** containing aromatic structures attached to –NH; and the third group, compound **7** with cyclic structure. The antibacterial ac-

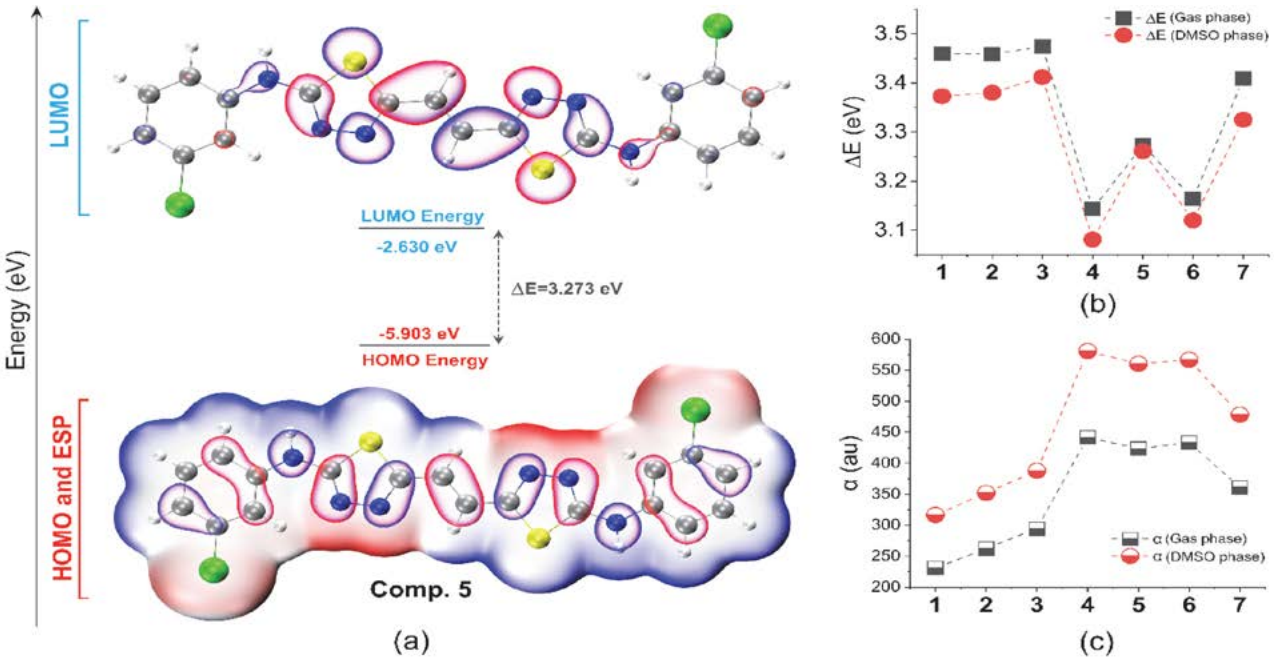


Figure 4. (a) HOMO-ESP and LUMO maps of compound **5**; (b) HOMO-LUMO energy gap values of the compounds calculated in gas and DMSO phase; (c) Polarizability values of the compounds calculated in gas phase and DMSO phase (by 6-311++g(2d,2p) basis set).

tivities of the first group compounds appear to be weak, where compound **1** shows no activity, but in this group, the antibacterial activity increases partially as the aliphatic component grows, but aliphatic components do not seem to have a significant effect on the E_g values of the compounds (see Figure 4b). It can be said that aromatic and cyclic structures reduce the E_g values of the compounds and increase the antibacterial activity. On the other hand, the fact that compound **7** has a higher E_g value (3.409 eV) than the compounds in the second group (3.143, 3.273, and 3.164 eV for compounds **4**–**6**, respectively), but is more sensitive to bacterial diversity, strengthens the opinion that cyclic group is a stronger factor for antibacterial activity than aromatic structures. In addition, the fact that the E_g values obtained from the calculations in the DMSO phase are lower than those in the gas phase (see Figure 4b) leads to the expectation that the compounds will show higher activity in the solution. At this point, the solubility of the compounds in DMSO is another parameter that affects the reactions. As it is known, the solvation energy is the difference of the total energies of the compound in the solvent and vacuum environment and depends on the nature of both the solvent and the compound (and the substituted groups). Solvation energies of the first group compounds were calculated to be lower than for those in the other groups (–16.40, –17.54, and –16.42 kcal/mol for compounds **1**–**3**, respectively), which indicates that the first group compounds have poor solubility in DMSO. It was observed that the antibacterial properties of the second and third group compounds with high solubility or high solvation energies (between –18.76 kcal/mol and –22.82 kcal/mol) were higher (see Supplementary file, Tables S1, S2 for all calculated electronic data).

The interactions of the compounds with bacteria occur electrostatically in the first place, and in the later stages, the intramolecular interactions are involved in determining the coordination of the reaction. At this point, although molecular polarizability is defined as the response of electron distribution on a molecule to an external static electric field, it is an important parameter because the intermolecular interaction energy can be expressed in terms of polarization and dispersion contributions. The polarizability of the first group compounds to which the aliphatic groups are attached was calculated to be lower (230.778, 262.483, and 294.367 a.u. for compounds **1**–**3**, respectively) than that of the other compounds containing aromatic (441.604, 423.492, and 433.128 a.u. for compounds **4**–**6**, respectively) and cyclic (361.137 a.u. for compound **7**) structures (see Figure 4c). In addition, compound **1**, which does not exhibit antibacterial properties, has the lowest polarizability. It was observed that the compounds with the highest polarizability were those with aromatic rings. Calculations performed in the DMSO phase show that the polarizibity values of the compounds increase proportionally in this phase. Although the dipole moments and polarizabilities of the compounds contribute to the activation of reactive sites in intermolecular interactions, the conformational degrees of freedom of the compounds are another strong factor. π-Conjugation between thiadiazole groups reduces the conformational degrees of freedom of the compounds, and the reduction of the effect of intramolecular interactions on conformations increases the probability of the compounds to be exposed to steric effects during the reaction. Moreover, intramolecular interactions of aliphatic, aromatic or cyclic structures become a variable of the degree of freedom as a limiting parameter of the conformational orientations of the compounds. Since intramolecular interactions change the electron density distributions of the reactive sites, they can cause the reaction to occur easily and quickly, and vice versa. The average root mean square (RMS) errors of the superimpositions of the gas and DMSO phase conformations of the compounds were 0.057, 0.042, and 0.044 Å for the first group compounds, respectively; 0.019, 0.016, and 0.019 Å for the second group of compounds, respectively; for com-

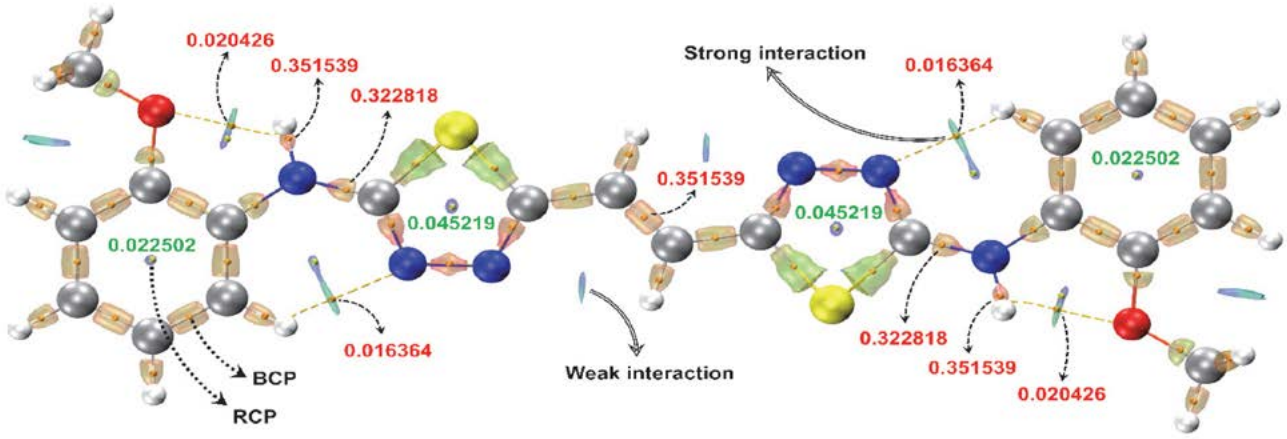


Figure 5. IRI surfaces of compound **5** and QTAIM data: Electron density (e/bohr^3) in BCP and RCP; RCP: Ring critical point, BCP: Bond critical point.

pound **7** with a cyclic group, it was calculated as 0.175 Å. Cyclohexyl group does not show resonance effects due to its absence of π-conjugation, and the larger RMS error of interaction with DMSO than that of other compounds indicates a higher conformational freedom than in the other compounds. This property of cyclohexyl ring may mean that compound **7** can perform a high electrophilic attack as well as less exposure to the steric effect. Moreover, the cyclohexyl group pumps electrons to the thidiazole region, and in addition to being exposed to nucleophilic attacks by molecular structures (or atoms) with high electronegativity, it also supports the electrophilic attack of the thiadiazole region. The IRI surfaces and QTAIM data of compound **4** are given in Figure 5 as a quantitative visualization of intramolecular interactions (see Supplementary Figure S19 for all compounds).

The growth of electron donor aliphatic groups in the first group compounds caused an increase in electron density towards the central thiadiazole structures. In addition, in terms of the change of electron distribution on the compounds, both the conformational and electronic properties of aliphatic, aromatic and cyclic groups are among the factors that determine the degree of electrophilic attack (or nucleophilic attack) of the compounds. Figure 6 shows the electrophilic and nucleophilic attack region maps of compound **6** (see Supplementary Figure S20 for all compounds). The methyl group pumps electrons to the phenyl ring inductively and exhibits *ortho-para* directing behavior. An increase in electron density is observed both on the carbon atom to which it is attached and on the *para* carbon atom of the phenyl ring. A similar situation is observed in the methoxy substituted compound **4** exhibiting both strong mesomeric and weak inductive effect. In compound **5**, which has an electron-withdrawing chlorine substituent from the ring with an inductive effect, a result close to the effect of the methoxy group was observed. In addition, intramolecular interaction of –NH with phenolic groups and electron donation to the phenyl ring with strong mesomeric effect strengthens the electrophilic attacks of compounds **4**–**6**. In this context, it can be said that the second group compounds can perform electrophilic attack, which is partially stronger than the cyclic compound **7**, but much stronger than the first group compounds. The nucleophilic attack regions of the compounds are predominantly concentrated around the alkenylic group (–C=C–) and the thiadiazole carbon atom to which this group is attached.

Considering whether the compounds show antibacterial action properties in terms of electrophilic and nucleophilic attacks, we can say that the reaction with *K. pneumoniae, B. subtilis,* and *E. faecium* bacteria occurs through electrophilic attacks, because for all of the compounds, the nucleophilic attack sites are on the thiadiazole and alkenylic groups; if the reactions had occurred through nucleophilic attacks, similar reaction rates would be expected for each bacterial species, but this was not observed for other bacterial species. At this point, the inability of the nucleophilic attack regions to perform the desired reaction may be due to the π-conjugation of these regions forcing the molecular structure to be planar and the aliphatic, aromatic and cyclic groups creating a steric effect. Moreover, the inability of the compounds to show activity on *S. enterica* serovar *Kentucky*, *S. macrescens*, *P. aeruginosa*, and *S. epidermidis* species may be due to the fact that these bacterial species react with nucleophilic rather than electrophilic attacks.

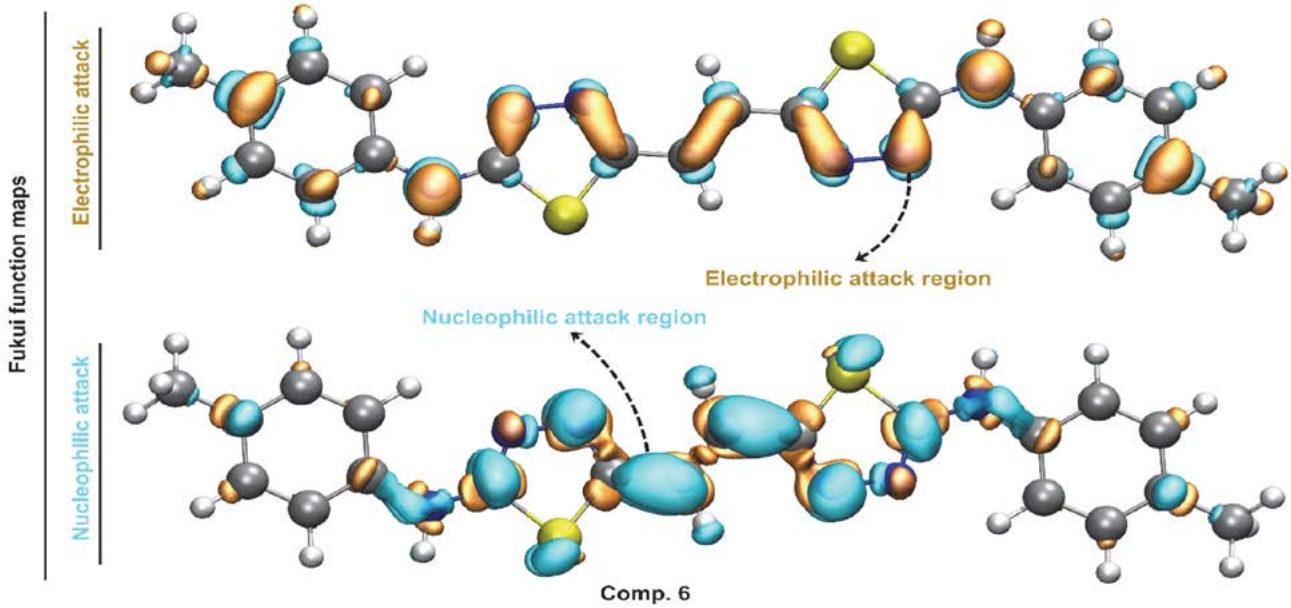


Figure 6. Electrophilic and nucleophilic attack sites maps of compound **6** (by 6-31g(d) basis set).

4. Conclusions

In this study, seven new bis-1,3,4-thiadiazoles **1–7** were obtained in excellent yields (61–93%) starting from fumaric acid. The synthesized compounds were characterized with FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods and elemental analysis. The conformations of the compounds and some electronic parameters were calculated. Experimental spectroscopic data were supported by DFT calculations.

As a result of experimental MIC procedures, thiadiazoles were found to have antimicrobial activity against *K. pneumoniae*, *E. coli*, *B. subtilis*, and *E. faecium*. We also evaluated the antibacterial activity of newly synthesized compounds with the MBC test. As a result of the MBC test, it was observed that the activity of compounds **2–7** on *K. pneumoniae*, compounds **5** and **6** on *E. faecium*, compounds **4** and **7** on *B. subtilis*, and compound **3** on *E. coli* were bactericidal. None of the compounds was found to have activity against *P. aeruginosa*, *S. epidermidis*, *S. enterica* serovar *Kentucky*, and *S. macrescens*.

Although the calculations cannot give definite answers in determining the antibacterial properties of the compounds, the fact that the antibacterial experiments give very close results each time reveals the existence of dominant variables that determine the results of the reactions. In this study, it was investigated whether there is a relationship between the antibacterial properties of the compounds and electronic data, electrophilic and nucleophilic attack sites. Although the HOMO-LUMO gap is a quantity related to the reactivity of the compounds, single molecular calculations cannot determine the conformational orientations caused by intermolecular interactions in the reaction medium of the compounds and the changes in electronic data caused by these orientations, that is, the E_g values change directly depending on the intermolecular interactions (hence their conformation) of the compounds during the reaction process, which prevents E_g from being a quantitative determinant of the reactions. However, it is also possible to make some predictions, albeit rough, for compounds that are similar derivatives of each other and in which only the substituents change. Especially in molecules with less conformational freedom, the effect of substituted groups can be analyzed with more accurate results. In this context, electronic data such as E_g, polarizability, solvent effects and Fukui function maps calculated for compounds **1–7** can be helpful in determining the reactivity and possible electrophilic/nucleophilic attack properties of the compounds, albeit partially. Although it is undeniable that more data is needed for the results of unknown variables in the reaction environment with bacteria, it was also observed in calculations that compounds with aromatic and cyclic groups are more reactive than compounds containing aliphatic groups, in accordance with experimental data.

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Author contribution statement

Halit Muğlu: Supervision, Methodology, Synthesis, Writing-Review. **Hasan Yakan**: Conceptualization, Methodology, Structure Characterization, Writing-Review. **Ghaith Alabed Ibrayke Elefkhakry**: Synthesis, Investigation, Methodology. **Ergin Murat Altuner:** Antibacterial Activities Assay, Methodology, Writing-Review. **M. Serdar Çavuş:** Theoretical Calculations, Writing–Review.

Declaration of competing interest

The authors declare that they have no conflict of interest. This study was not supported by any organization .

Supplementary Material

All spectra (FT-IR, ^{1}H NMR and ^{13}C NMR) of the compounds are presented in the supporting information.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Povzetek

Serijo novih bis-1,3,4-tiadiazolov **1**–**7** smo pripravili z reakcijo med fumarno kislino in *N*-(alkil/aril/ciklo)tiosemikarbazidi v prisotnosti fosforjevega oksiklorida. Strukture vseh spojin smo določili z FT-IR, ^{1}H NMR in ^{13}C NMR ter z elementno analizo. Antibakterijsko aktivnost vseh spojin smo proučili na osmih izbranih bakterijah. Spojine **2**–**7** izkazujejo učinek na *Klebsiella pneumoniae*. Vendar pa nobena izmed spojin ni učinkovala na *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Salmonella enterica* serovar *Kentucky*, in *Serratia marcescens*. SCRF ("self-consistent reaction force") izračune smo izvajali v DMSO kot mediju z namenom ugotoviti energije solvatacij s pomočjo CPCM in SMD modelov. Pri DFT izračunih smo uporabili bazne sete 6-31G(d) and 6-311++G(2d,2p). Poleg elektronskih parametrov smo raziskali tudi elektronegativnost, elektrofilnost in spektroskopske lastnosti spojin, QTAIM, lokalne elektronske afinitete ter izvedli Fukuijevo analizo. Teoretični rezultati podpirajo eksperimentalna opažanja ter nakazujejo, da spojine, ki vsebujejo aromatske ali ciklične skupine, izkazujejo močnejše antibakterijsko delovanje kot spojine, ki vsebujejo alifatske skupine.