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Novel Bis-1,3,4-Thiadiazoles Derivatives: Synthesis, Spectroscopic Characterization, DFT Calculations and Evaluation of their Antimicrobial and Antioxidant Activities

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ABSTRACT

Two new, bis-1,3,4-thiadiazoles derivatives (**I** and **II**), were prepared by cyclization reaction of oxalic acid with *N*-alkyl/allyl thiosemicarbazides and phosphorous oxychloride ($POCl_3$). Then the newly prepared products screened for their antimicrobial and antioxidant activities. The biological activity results shown that tested compounds exhibited effective antibacterial activity against six different bacteria. However, the compound **II** demonstrated greater $ABTS^{\cdot+}$ scavenging ability. The characterization of the synthesized molecules was done by FT-IR, ^1H NMR, ^{13}C NMR spectroscopic methods and elemental analysis. Moreover, the experimental FT-IR and NMR spectra of the molecules were compared with the results calculated at the cc-pvtz, 6-311g(2d,2p), and 6-311++g(2d,2p) levels of theory. The effect of substituted groups on the spectral and electronic properties of the compounds was investigated. NCI and QTAIM analyses were performed to examine the effects of allyl group and intramolecular interactions on σ and π bonds. How the N-H bonds of the substituted groups affect the bond degrees was investigated using Fuzzy, Laplacian and Mayer approaches, and the relationship of the data with the antioxidant properties of the compounds was examined. In addition, the relationship between bond stretching force constant and intrinsic bond strength index, electron density, and delocalization index for some bonds was revealed.

Keywords: Bis-thiadiazoles, Biological activity, DFT, IBSI, Force constant (FC).

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Introduction

Thiadiazoles are five-membered heterocyclic compounds consisting of two nitrogen and one sulfur atom in the ring. Compounds having thiadiazole moiety possess medicinal characteristics and unique biological activity due to their strong aromaticity. When different functional groups that interact with biological receptors are added to this ring, moiety compounds with extraordinary properties are obtained. Thiadiazoles and their derivatives have various biological and medicinal characteristics such as antimicrobial [1], anticancer [2], antibacterial, antiviral [3], antifungal [4] antitubercular [5], antihypertensive [6], anticonvulsant [7], diuretic [8], antioxidant [9] properties. They are also used for various applications such as pesticides, herbicides, and insecticides in the agriculture field [10].

Accordingly, we have synthesized two new bis-1,3,4-thiadiazoles derivatives and investigated their antimicrobial and antioxidant characteristics. *In vitro* antimicrobial activity tests on the synthesized two new compounds were performed against six different bacteria and two fungi using the microdilution method (MIC). The results of the biological activity analyses reveal that these synthesized two new compounds show effective antibacterial activity against *B. subtilis*, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Besides, the antioxidant activities of these molecules were explored by employing ABTS radical scavenging method.

In the theoretical approaches, DFT calculations of the compounds were made first and the data were compared with the experimental results. Subsequently, the effects of intramolecular interactions on IR and NMR spectra were revealed. Quantum theory of the atom in the molecule (QTAIM) [11, 12] and non-covalent interaction (NCI) [13] analyses were performed to examine the relationship between intramolecular interactions, bond orders, and spectral data, depending on the electron charge distribution on the bonds. Furthermore, the relationship between properties such as bond length, Laplacian bond order (LBO), Fuzzy bond order (FBO), Mayer bond order (MBO) and bond stretching force constant (FC), Intrinsic bond strength index (IBSI), electron density (Rho) at the bond critical point (BCP), and DI were analyzed.

Materials and Methods

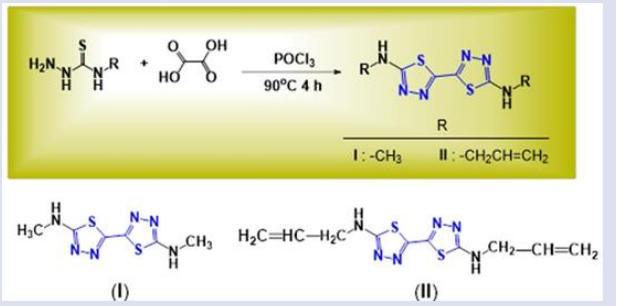
Instrumentation

All the reagents and solvents were acquired from Sigma Aldrich or Merck Chemical Company and were used without further purification. The melting points were

determined using a Stuart SMP30 apparatus. Eurovector EA3000 elemental analyser was used to define elemental analysis. Infrared spectra were recorded with a Bruker Alpha Fourier transform FT-IR spectrometer. ^{1}H and ^{13}C-NMR spectra were registered using a Bruker Avance DPX-400 spectrophotometer (400 and 101 MHz) in DMSO-d_6.

General Synthesis Procedure for Novel bis- 1,3,4-thiadiazoles (I and II)

The mixture of oxalic acid (n mol) and N-alkyl/allyl thiosemicarbazide (2n mol) was chilled in a refrigerator and phosphorous oxychloride (3n mol) was added drop-wise by stirring. Then, refluxing was continued 90 °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 final product was filtered, washed with water, and crystallized in a suitable solvent. These novel thiadiazoles were prepared according to indicated the prodecure [14] in Scheme 1.



Scheme 1. Synthetic route for new bis- 1,3,4-thiadiazoles compounds.

Detection of Antimicrobial Activity

The antimicrobial activities were tested against the following eight microorganisms including Gram-staining-positive (*B. subtilis* ATCC 6633; *S. aureus* ATCC 25923; *E. faecalis* ATCC 29212), Gram-staining-negative (*E. coli* ATCC 25922; *K. pneumoniae* ATCC 70060; *P. aeruginosa* ATCC 27853) bacteria and fungi (*A. niger* ATCC 16404; *C. albicans* ATCC 1023). The antimicrobial screening activity was determined in 96-well microplates by microdilution method (MIC) [15]. Synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) at the appropriate concentration. The cultures were obtained from nutrient broth for all the bacterial strains after 24 h of incubation at 37 °C. Fungi were maintained in nutrient broth after incubation for 24 h at 28 °C. Bacterial and fungi cells were homogenized in nutrient broth. The turbidity of bacterial and fungi suspensions was set at a concentration of approximately 10^6 cells/ml. Only inoculated broth was used as controls. 100 mL suspension of each microorganism and 100 mL suspension of compound tested were added into the wells. The microplate with no growth of microorganism was recorded to represent the MIC enounced in µg/mL.

Measurements of Antioxidant Activity

$ABTS^{\cdot+}$ radical scavenging activity of the samples were measured spectrophotometrically at 734 nm on the results of the oxidation of 2,2-azinobis-(3-ethylbenzothiozine-6-sulfonic acid) ammonium salt ($ABTS^{2-}$) with of peroxydisulphate based on form $ABTS^{\cdot+}$ radical and butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ) and α-tocopherol standards were used [16, 17]. IC_{50} (µg/mL) values of the results were calculated.

Theoretical Processes

The ground state optimized geometries of the compounds and the calculations of the electronic parameters of these geometries were performed using the Gaussian 09 software package [18]. Becke three-parameter hybrid functional combined with Lee-Yang-Parr correlation functional (B3LYP) was used in the Kohn-Sham DFT [19, 20] calculations. Calculations were performed at the cc-pvtz, 6-311g(2d,2p), and 6-311++g(2d,2p) levels of the theory without any geometry restrictions. The optimized geometries with minimum energy correspond to the actual minimum points on the potential energy surface, and no imaginary frequencies were observed in the calculations.

Frontier molecular orbital (FMO) energy eigenvalues and chemical reactivity parameters such as HOMO-LUMO energy gap, chemical hardness, electronegativity, electrophilic index depending on these eigenvalues were also calculated in the gas phase using the aforementioned basis sets.

IR calculations were performed in the gas phase for all three basis sets mentioned. NMR calculations were performed with the Gauge-Including Atomic Orbital (GIAO) approach, using conductor-like polarizable continuum model (CPCM) in the DMSO phase in accordance with the experiment. The calculated TMS values of ^{1}H and ^{13}C NMR for cc-pvtz, 6-311g(2d,2p), and 6-311++g(2d,2p) basis sets are 31.7450 and 184.4735; 31.8181 and 183.8257; 31.8149 and 183.7737, respectively.

QTAIM analyses, NCIs, electron density distributions, FBO and LBO, and IBSI [21, 22] calculations of the certain bonds were performed using Multiwfn [23] software. Bond stretching force constant (FC) were calculated separately with the mentioned three basis sets using Forcegen [24] software.

Results and Discussion

Analytical Data

Table 1. Analytical data for the synthesized compounds.

Entry	Compound's Names	M. P. (°C)	Yields (%)	Colour	Calculated/Found C%	H%	N%
I	*N5,N5'*-dimethyl-[2,2'-bi(1,3,4-thiadiazole)]-5,5'-diamine	>320	72	Light Yellow	31.57/31.69	3.53/3.55	36.81/36.76
II	*N5,N5'*-diallyl-[2,2'-bi(1,3,4-thiadiazole)]-5,5'-diamine	230-232	83	Yellow	42.84/42.93	4.31/4.34	29.98/29.92

FT-IR Spectral Analysis

In the IR spectra data of the compounds, the corresponds to carboxylic acid (-COOH) absorption bands of the starting material were not observed at approximately 3500-2800 cm^{-1}. Furthermore, asymmetric and symmetric stretching absorption bands of the NH_2 group were not appeared around 3400–3150 cm^{-1}. For compounds I and II, the peaks of the -NH were showed at 3188 and 3189 cm^{-1}, the -C=N stretching frequencies of thiadiazole ring were observed at 1556 and 1576 cm^{-1}; the -C–N stretching frequencies were showed at 1158 and 1143 cm^{-1}; the -C–S signals of aryl ring were observed at 697 and 691 cm^{-1}, respectively as shown in Figure 1. These frequency values of the target compounds were nearly in accordance with the literature [25-27]. The IR stretching vibration frequencies of the compounds are summarized in Table 2.

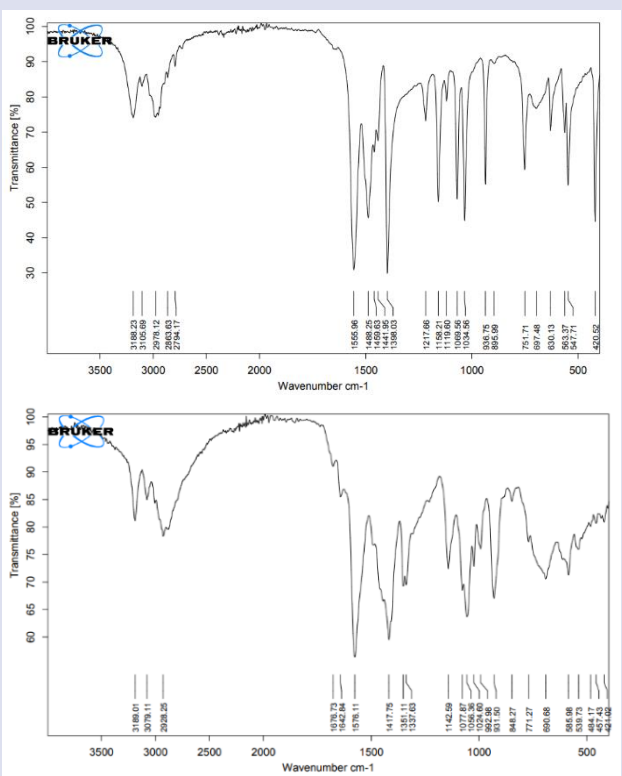


Figure 1. IR spectra of the compounds.

Table 2. Experimental and calculated IR values of the target compounds (cm^{-1}).

		Comp.	-NH	Aliph. CH	C=N	C-N	C-S
Exp.		I	3188	2978- 2863	1556	1158	697
		II	3189	2928-2827	1576	1143	691
Calculated	BS1	I	3647.7	3131.4-3021.9	1476.7	1560.4	731.8
		II	3621.9	3216.2-3007.8	1476.9	1537.2	730.5
	BS2	I	3641.3	3139.8-3026.1	1478.0	1558.1	727.5
		II	3621.1	3218.7-3014.3	1478.2	1534.0	725.9
	BS3	I	3649.1	3140.0-3032.5	1472.7	1553.5	728.1
		II	3621.8	3221.3-3020.2	1472.7	1526.9	726.8

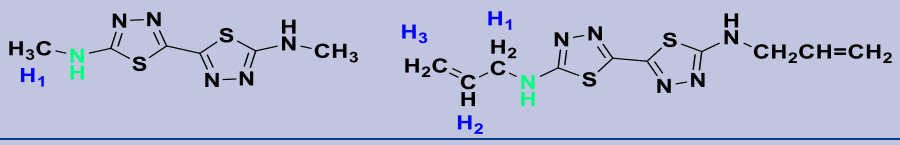
BS1: B3lyp/cc-pvtz, BS2: B3lyp/6-311g(2d,2p), BS3: B3lyp/6 311++g(2d,2p).

H NMR Spectral Analysis

The ^1H NMR spectra of the compounds are given in Figure 2. For compound I, the proton signal of –NH was detected as a quartet at 8.13-8.09 ppm. The methoxy group (OCH_3) peak on the aryl ring was resonated as a doublet at 2.95-2.94 ppm. For compound II, the proton

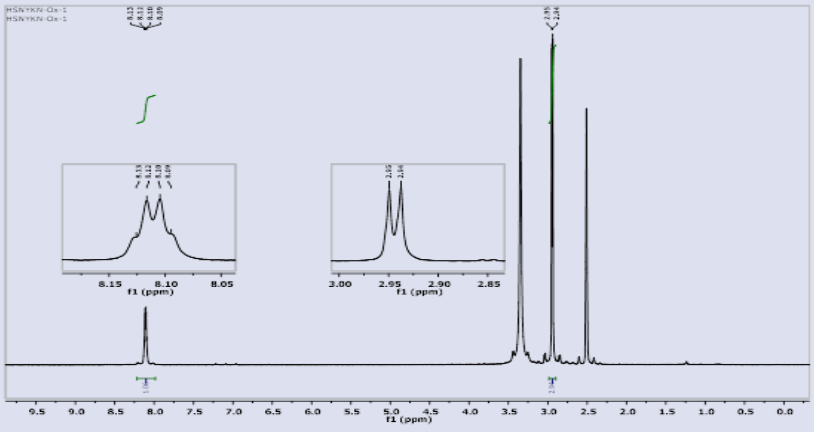
signal of –NH was detected as a triplet at 8.37-8.35 ppm. The methylene (–N-CH_2) group peak on the aryl ring was observed as a triplet at 4.00-3.97 ppm. The methine (–C-CH=) group peak on the aryl ring was observed as a multiplet at 5.96-5.89 ppm. The methylene (–C=CH_2) group peak on the aryl ring was appeared as a multiplet at 5.30-5.14 ppm. DMSO-d_6 and water in DMSO (HOD, H_2O) signals are shown around at 2.00, 2.50 (quintet) and 3.30 ppm, respectively [28]. These observed are compatible with the literature [25-27]. The chemical shift values are presented in Table 3.

Table 3. ^{1}H NMR values of the compounds (δ, ppm, in nDMSO-d_6).

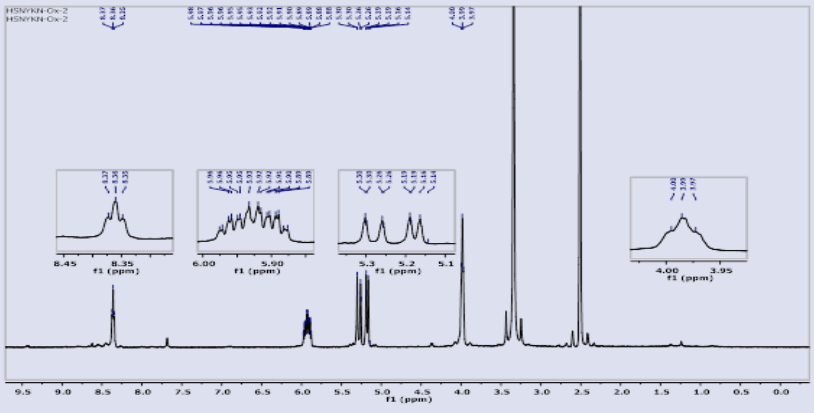


Exp.		Comp.	NH	H1	H2	H3
		I	8.13-8.09 (q)	2.95-2.94 (d)	-	-
		II	8.37-8.35 (t)	4.00-3.97 (t)	5.96-5.89 (m)	5.30-5.14 (m)
Calculated	BS1	I	5.17	3.17, 3.01	-	-
		II	4.75	4.76, 3.78	6.41	5.75, 5.58
	BS2	I	4.97	3.23-2.92	-	-
		II	4.60	4.73, 3.71	6.37	5.73, 5.58
	BS3	I	5.19	3.27-3.03	-	-
		II	4.76	4.69, 3.82	6.52	5.81, 5,64

d: doublet, t: triplet, q: quartet, m: multiplied.
BS1: B3lyp/cc-pvtz, BS2: B3lyp/6-311g(2d,2p), BS3: B3lyp/6-311++g(2d,2p).



(I)



(II)

Figure 2. ^{1}H NMR spectra of compounds I and II.

^{13}C NMR Spectral Analysis

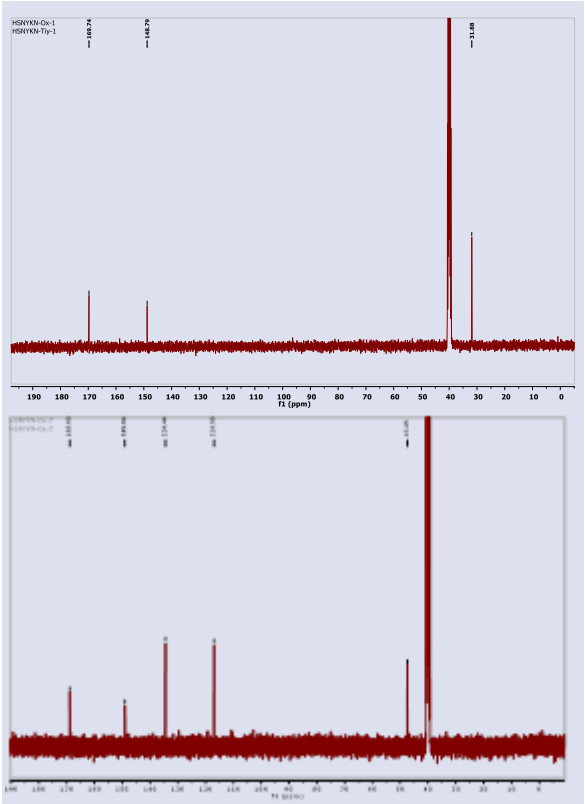


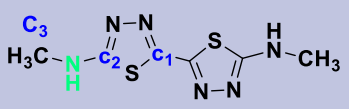
Figure 3. ^{13}C NMR spectra of compounds I and II.

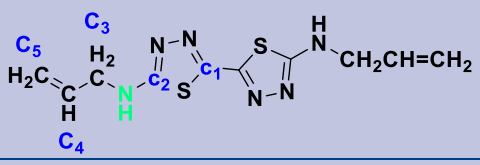
The ^{13}C NMR spectra of the compounds I and II recorded in DMSO-d_6 showed 3 and 5 different carbon atoms resonances respectively, in good agreement with the proposed structure as shown in Figure 3. The chemical shift results are given in Table 4.

In compound I, the carbon signals (C1 and C2) of thiadiazole ring were detected at 148.79 and 169.74 ppm, respectively. The C2 carbon (C2-NH) is shifted down-field (high values, δ) at 169.74 ppm due to the presence of amino group (NH). The carbon atom of methyl (N-CH_3) group on the aryl ring was observed at 31.88 ppm.

For compound II, the carbon signals (C1 and C2) of thiadiazole ring were detected at 149.04 and 168.90 ppm, respectively. The C2 carbon (C1-NH) is shifted down-field (high values, δ) at 168.90 ppm due to the presence of amino group (NH). The carbon atom of methylene (N-CH_2) group on the aryl ring was observed at 47.45 ppm. The carbon atom of methine group (-CH=) was detected at 134.44 ppm. The methylene (–C=CH_2) carbon atom was resonated at 116.99 ppm. These spectroscopic data are in agreement with values previously reported in the literature [25].

Table 4. ^{13}C NMR values of the title compounds (δ, ppm, in DMSO-d_6)





		Comp.	C_1	C_2	C_3	C_4	C_5
Exp.		I	148.79	169.74	31.88	-	-
		II	149.04	168.90	47.45	134.44	116.99
Calculated	BS1	I	158.99	175.84	32.62	-	-
		II	159.52	175.83	52.41	142.67	124.91
	BS2	I	157.97	175.55	32.58	-	-
		II	158.27	175.04	52.22	142.19	125.02
	BS3	I	160.08	177.47	32.67	-	-
		II	160.64	177.30	52.59	143.81	125.88

Antimicrobial Evaluation

Antimicrobial activity tests of the compounds were performed against six different bacteria and two fungi using the microdilution method (MIC). In this study based on serial dilution, MIC value for DMSO used as a control was found to be > 4000 µg/mL. When the effect of the one compound on bacteria is examined, it is seen that MIC values are 1000 µg/ml for *B.subtilis*, 1000 µg/ml for *S.aureus*, 500 µg/ml for *E. faecalis*, 500 µg/ml for *E. coli*, 1000 µg/ml for *K. pneumonia* and 1000 µg/ml for *P.aeruginosa*. Likewise, when the effect of the second compound on bacteria is examined, it is seen that MIC values are 250 µg/ml for *B.subtilis*, 500 µg/ml for *S.aureus*, 500 µg/ml for *E. faecalis*, 125 µg/ml for *E. coli*, 250 µg/ml for *K. pneumonia* and 1000 µg/ml for *P.aeruginosa*. Two new compounds synthesized did not show antifungal activity against *A. niger* and *C. albicans*. The MIC values were showed in Table 5.

Table 5. The minimum inhibition concentrations (MIC's) of the tested compounds.

Sample	Minimum inhibition concentration (µg/mL)							
	Gram-staining-positive			*Gram-staining-negative*			*Fungi*	
	B. subtilis	*S. aureus*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. niger*	*C. albicans*
Compound I	1000	500	500	500	1000	1000	-	-
Compound II	250	500	500	125	250	1000	-	-

Compound I: *N5,N5'*-dimethyl-[2,2'-bi(1,3,4-thiadiazole)]-5,5'-diamine;
Compound II: *N5,N5'*-diallyl-[2,2'-bi(1,3,4-thiadiazole)]-5,5'-diamine.

In 2009 Demirbaş et al. [29] conducted a similar study. They synthesized some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and found them to moderate activity against all the tested microorganisms (*E. coli*, *Y. pseudotuberculosis, P. aeruginosa, E. faecalis, S. aureus, B. cereus*) except *C. tropicalis* and *C. albicans*. Muglu et al. (2018) [14] synthesized new 1,3,4-thiadiazole compound and all the tested compounds showed good antimicrobial activities against *S. aureus*.

Antioxidant Evaluation

The antioxidant activities of the samples (I and II) were determined through the $ABTS^{\cdot+}$ scavenging activity test. In the present study, the $ABTS^{\cdot+}$ scavenging activity results of the tested compounds have shown in Table 6. The compound II (IC_{50} value of 68.93±0.79 µg/mL) was found to be the most active with comparison to compound I. It was even found to have activity close to the α-tocopherol standard. This effect may be owing to its role of the allyl group ($-CH_2CH=CH_2$) and activating the bithiadiazole rings [30]. In compound I, the methyl group ($-CH_3$) at position 2,2' of the bis- 1,3,4-thiadiazole nucleus is substituted whereas in II this has an allyl group. This indicates that the allyl group on the bithiadiazole rings enhances the activity. As result, the $ABTS^{\cdot+}$ scavenging activity was significantly ameliorated when the methyl groups were replaced by allyl groups. According to the IC_{50} values, the antioxidant capacities of the tested compounds were observed lower than that of the standards products. The $ABTS^{\cdot+}$ scavenging effect of the title compounds and standards decreased in the following order: TBHQ> BHA> α-tocopherol> II> I.

Table 6. IC_{50} values for the compounds.

Compounds	ABTS radical scavenging activity, IC_{50}^{*} (µg/mL)
Compound I	103.26±1.39
Compound II	68.93±0.79
BHA	33.91±2.09
TBHQ	32.09±2.02
α -tokoferol	64.86±3.28

Values are expressed as means (n = 3).
*IC_{50} = the concentration (µg /mL) exhibiting 50% inhibition of ABTS radical.

These results further demonstrated that the structure of the products and electronic effects of groups/substituents in the structures plays an important role of antioxidant activity [31]

Theoretical Calculations

Calculations reveal that the allyl group decreases the E_{HOMO} and E_{LUMO} energies of the compound while increasing the energy gap $\Delta E=E_{LUMO}-E_{HOMO}$ (Figure 4).

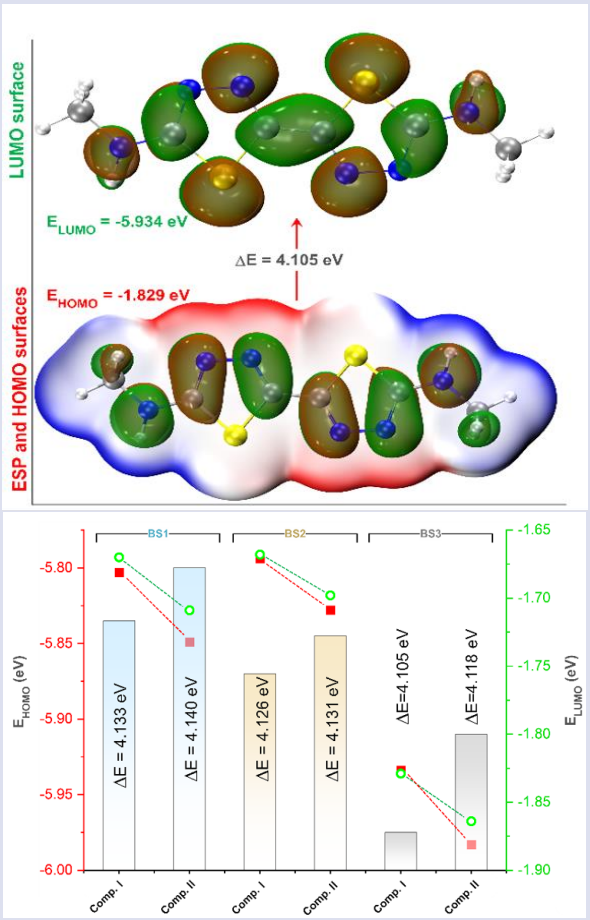


Figure 4. HOMO-ESP and LUMO surface of comp. I (BS3). The graph shows the HOMO-LUMO energy eigenvalues and Energy gap, ΔE, values of the compounds calculated by the basis sets BS1, BS2, and BS3.

In this context, we can say that compound I is more reactive. In parallel, the chemical hardness of compound I is lower.Contrary to these data, compound II was both more electronegative and had higher electrophilic index (Figure 5). However, there are many variables that dominate chemical reactions, and such static variables of the whole molecular structure can be partially helpful in predicting reaction mechanisms, but it can be misleading

to determine the reactivity of a compound in a particular reaction by considering these values. Certain reaction mechanisms are directly related to specific local regions of the compounds. For example, the antioxidant behavior of synthesized thiadiazole derivative compounds is closely dependent on both the conformative and electronic properties of the N–H bond. Although these are not the only parameters that determine the antioxidant reactions, an analysis of the static and dynamic parameters of the N–H bond is a useful method to predict the antioxidant property of the compound. Intramolecular interactions are among the factors that affect bond dissociation energy, and in polyatomic molecules, treating two atoms in a particular bond in isolation from other bonds and atoms can yield false results. In addition, bond strengths (or bond force constants) are strongly dependent on bond order of the bonded atoms and molecular conformation, which affects intramolecular interactions and thus charge distribution. NCI analysis shows that the non-covalent interaction of the allyl moiety with the thiadiazole region is stronger than that of the methyl group (Figure 6). This may result in compound II retaining its current conformation more than compound I. In addition, both the allyl group and intramolecular interactions affected the electronic data of the bonds in the reactive region in parallel in terms of IBSI, FC, Rho, and DI variables.

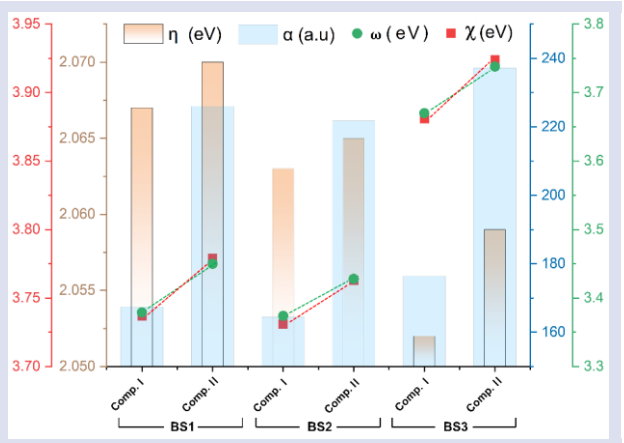
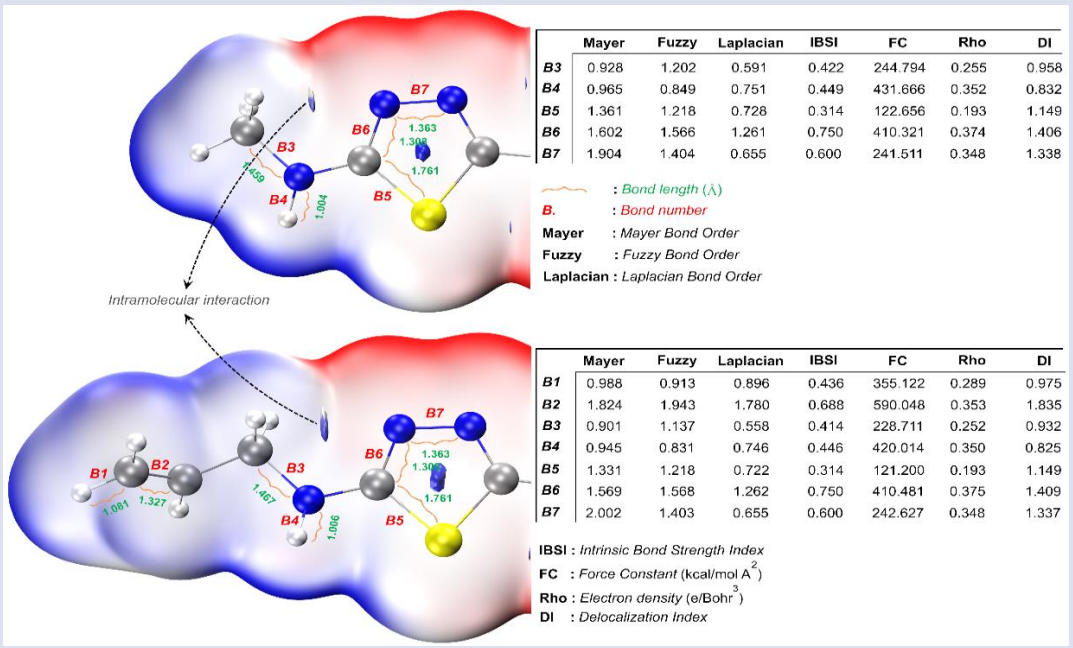


Figure 5. Chemical hardness (η), Electronegativity (χ), Polarizability (α), and Electrophilic index (**ω**) values of compounds, calculated by BS1, BS2, and BS3.

The decrease in the charge density at BCP of the N–H bond resulted in a weakening of the delocalization on the bond and thus an increase in the polarization coefficient of the N hybridization from 0.8351 to 0.8358 ($\sigma_{NH} = 0.8351(sp^{2.95})_N + 0.5501(s)_H$ for compound I, and $\sigma_{NH} = 0.8358(sp^{2.99})_N + 0.5490(s)_H$ for compound II, calculated by BS3 in the NBO analysis).



	Mayer	Fuzzy	Laplacian	IBSI	FC	Rho	DI
B3	0.928	1.202	0.591	0.422	244.794	0.255	0.958
B4	0.965	0.849	0.751	0.449	431.666	0.352	0.832
B5	1.361	1.218	0.728	0.314	122.656	0.193	1.149
B6	1.602	1.566	1.261	0.750	410.321	0.374	1.406
B7	1.904	1.404	0.655	0.600	241.511	0.348	1.338

	Mayer	Fuzzy	Laplacian	IBSI	FC	Rho	DI
B1	0.988	0.913	0.896	0.436	355.122	0.289	0.975
B2	1.824	1.943	1.780	0.688	590.048	0.353	1.835
B3	0.901	1.137	0.558	0.414	228.711	0.252	0.932
B4	0.945	0.831	0.746	0.446	420.014	0.350	0.825
B5	1.331	1.218	0.722	0.314	121.200	0.193	1.149
B6	1.569	1.568	1.262	0.750	410.481	0.375	1.409
B7	2.002	1.403	0.655	0.600	242.627	0.348	1.337

Figure 6. NCI, QTAIM, and some electronic data of the compounds I and II., calculated by 6-311++g(2d,2p).

Although the electronic variables considered for compound II were generally compatible with each other for the bonds of interest, the FBO and DI values did not change in parallel with the other variables, especially in the N–H and C–S bonds (Figure 7). FC values of sigma bonds B1, B3, and B5 were calculated lower than FC of electronegative atom groups and π bond (H_2C=CH–), but there is no linear relationship between FCs of σ and π bonds.

Although the prediction of antioxidant properties of the target compounds has difficulties due to the dynamic nature of the reactions, the bond stretching FC data reveal that compound II will exhibit higher antioxidant properties. It has been determined that the Allyl group weakens the N–H bond (the bond length also increased from 1.004 to 1.006 Å), that is, it causes bond strength reducing effects and therefore reduces the bond dissociation energy.

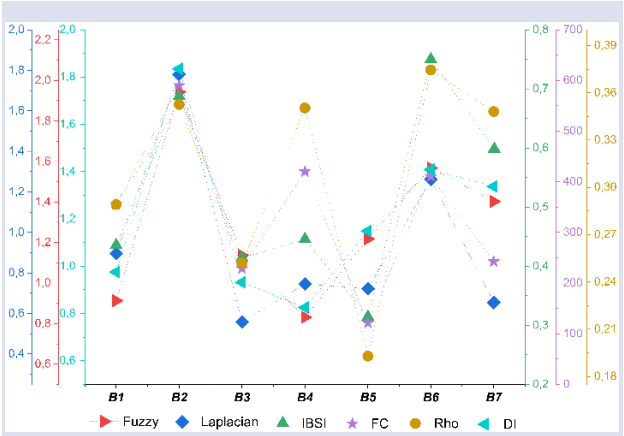


Figure 7. FBO, LBO, IBSI, FC, Rho, and DI values of the compound II, calculated by BS3.

Furthermore, it was observed that the electron density of the N–H bond, which plays an active role in antioxidant reactions, in BCP decreased (0.352 *e*/Bohr^3 for compound I, 0.350 *e*/Bohr^3 for compound II), and accordingly, both IBSI and bond stretching FC values also decreased (IBSI and FC values for compound I were calculated as 0.449 and 431,666 kcal/mol A^2, respectively; 0.446 and 420.014 kcal/mol A^2 for compound II). It can be said that this decrease in the bond strength and force constant of N–H bond results in easier breakage of the bond and therefore an increase in antioxidant properties. It is also a useful tool as the degree of a bond is usually related to the number of electrons forming the bond, and hence the bond strength; and overall, greater proportionality between bond strength and LBO emerged compared to the Fuzzy and Mayer approaches. MBO calculations increased the degree of =N–N= bond above 2 by adding the diffuse functions to the calculations. Especially adding the diffuse functions to the calculations may give wrong results in terms of MBO calculations. Moreover, there is a close relationship between IBSI and bond stretching FC, and it can be predicted that these parameters can be used to describe the chemical reactivity of compounds with predictable reactive sites.

Conclusions

In this study, new bis- 1,3,4-thiadiazole compounds were prepared in excellent yields of 72–83%. The compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. In the next step, the compounds screened for their *in vitro* for their antimicrobial and antioxidant activities. The results of microbial activity studies show that the two synthesized compound exhibited effective antibacterial activity against *B. subtilis*, *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Among the tested molecules, compound **II** exhibited greater ABTS$^{\cdot+}$ radical scavenging ability. This effect can be explained with in presence of the allyl group in the structures. Therefore, the new two synthesized compounds can be considered as bioactive substances for pharmacological and medical applications.

Although HOMO-LUMO energy eigenvalues of molecules and parameters such as energy gap, chemical hardness, and electronegativity obtained from these eigenvalues are useful, they are insufficient to explain the reaction mechanisms in which local regions are reactive because they are obtained from the molecular orbital approach. Calculations on selected bonds of compounds reveal that parameter such as LBO, IBSI, and bond stretching FC can be helpful tools in understanding antioxidant reaction mechanisms. An inverse relationship was observed between the IBSI and bond stretching FC values of the N–H bond and its reactivity in antioxidant reactions, that is, the IBSI and FC values of the N–H bond of the compound decreased while the antioxidant property increased. QTAIM analysis plays an especially important role in correlating the charge densities (Rho) of the bonds in the BCP and the DI data with the strength of the bond. By which atomic groups the bonds are formed affects the way these analyses are used. LBO calculations yielded more consistent results for bonds with electronegative atoms, and a close relationship was also observed between LBO, IBSI and FC.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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