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SYNTHESIS, SPECTROSCOPIC STUDIES AND ANTIOXIDANT ACTIVITIES OF NEW 2-BENZYLIDENE-N-PHENYLHYDRAZINE-1-CARBOTHIOAMIDE DERIVATIVES

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ABSTRACT

Nowadays, thiosemicarbazone based schiff bases, which are the focus of attention due to their differential properties, are being synthesized in many studies and new aromatic structure added compounds are of significant interest in drug design and biochemistry. In this study, new thiosemicarbazide schiff bases containing different substituted groups were synthesized. The structure of new compound derivatives was characterized by proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR), fourier transform infrared spectroscopy (FTIR) and elemental analyses. In addition, radical quenching capacities of newly synthesized compounds were investigated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method. IC_{50} values were calculated for the compounds and it was found that the antioxidant activities of the compounds changed in the order of 2>5>1>4>3. These results indicated that the presence of methoxy substituted groups that provide electrons to the aromatic ring structures of schiff bases would have positive effects on the antioxidant activities of the compounds.

Keywords: Schiff Base, Carbothioamide, IR Spectroscopy, ^{13}CNMR, DPPH Scavenging Method.

1 INTRODUCTION

Thiosemicarbazones are formed by the synthesis reaction between aldehyde and thiosemicarbazide, along with the addition of alkyloxy chain ester linkage groups in the first step [1]. Thiosemicarbazone ligands and transition metal complexes are of significant interest today due to their potential pharmacological properties and different stereochemical roles [2,3]. In one of the studies in this field, Hernandez and colleagues synthesized benzaldehyde

thiosemicarbazone derivatives and palladium (ii) and platinum (ii) complexes and evaluated the in vitro antitumor activities of these compounds. As a result of the study, they found that the synthesized complexes were more cytotoxic than their ligands with IC50 values in the range of 0.07–3.67 μM [4]. As a similar example, organometallic aromatic compounds attached to thiosemicarbazones can show a strong affinity towards sulfur and nitrogen-containing biomolecules such as amino acids (cysteines and methionines), peptides (glutathione) and proteins (metallothionein) [5].

Thiosemicarbazones, an important class of Schiff bases, have attracted significant attention for many years due to their biological activities and coordination chemistry properties [6-8]. Some of these biological activities can be listed as antiparasitic [9], potential anti-trypanosomal agents [10], antitumor activities [11], nuclear medicine [12] and anticancer activities [13]. In addition to these biological activities, thiosemicarbazone compounds also stand out with their antioxidant properties [14]. In recent years, thio/carbohydrazone derivatives have been synthesized and these compounds containing many functional groups have been investigated thanks to their antioxidant properties that reduce the damage of oxidative stress in living organisms and prevent DNA oxidation [15]. Hamedifar and coworkers synthesized 10 new carbothioamide molecule derivatives. These molecules inhibited tyrosinase enzyme and showed strong activities in antioxidant tests with DPPH [16].

The main purpose of this study is to synthesize new thiosemicarbazone compounds containing 2-benzylidene-n-phenylhydrazine-1-carbothioamide and to reveal their antioxidant properties. For this purpose, the synthesized compounds were characterized by elemental analyses, FTIR and NMR analyses. Then, the antioxidant properties of the synthesized compounds were investigated by DPPH method.

2 MATERIAL AND METHOD

This study, which we conducted in two parts, covers synthesis and antioxidant measurements. All solvents were used at analytical grade and all chemicals were supplied by Merck and Sigma-Aldrich Co. LLC. Synthesis studies, antioxidant determinations, elemental analyses and FTIR analyses were carried out at Kastamonu University Central Research Laboratory. FTIR analyses of solid samples in a short time under room conditions were performed using the Bruker Alpha FTIR device. 1H and 13C NMR analyses were carried out at Tokat Gaziosmanpaşa University Scientific, Technological Research and Application Center. NMR measurements were made on solutions of synthesized compounds prepared in dimethyl

sulfoxide (DMSO-d6) using JEOL ECX-400 (400 MHz) device. Antioxidant measurements were measured with the help of SHIMADZU UVmini-1240 UV-Visible spectrophotometer (Schimadzu Corp., Kyoto, Japan).

2.1 Synthesis of Thiosemicarbazone Derivatives (1–5)

2.1.1 Step 1: Synthesis of Thiosemicarbazides

First, various aromatic isothiocyanates (10 mmol) and hydrazine monohydrate (10 mmol) were separately dissolved in 96% pure ethanol (20 mL) and cooled in an ice bath (0–5 °C) for 15 min. Then, the isothiocyanate solution was added dropwise to the hydrazine solution under strong magnetic stirring. The mixture was stirred in an ice bath for another 120 min and then left in a refrigerator at 0–4 °C overnight. The white-cream precipitate that formed was vacuum filtered, washed with cold alcohol and dried in air. The obtained crude product was recrystallized with hot ethanol to obtain pure thiosemicarbazide derivatives (Figure 1) [17].

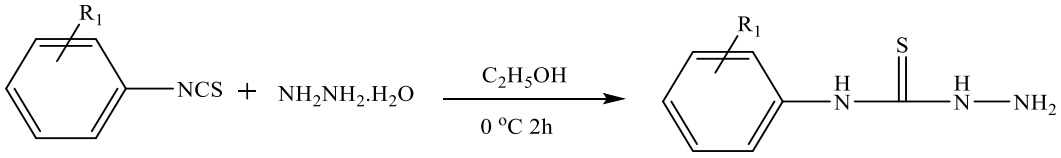
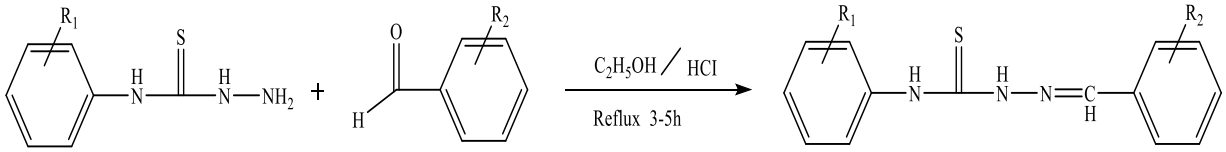


Figure 1. Thiosemicarbazide derivatives synthesis

2.1.2 Step 1: Synthesis of Thiosemicarbazones

Pure thiosemicarbazide derivatives (2 mmol), appropriate benzaldehyde derivatives (2 mmol) and a drop of concentrated HCl were dissolved in 96% ethanol (20 mL) and reacted under reflux conditions at 78 °C for 3–5 h. The reaction time was monitored by thin layer chromatography (TLC). When the reaction was complete, the mixture was cooled to room temperature and the solid product formed was vacuum filtered and dried. Purification was performed by recrystallization from ethanol. The products were obtained in yields ranging from 60% to 86% and their structures are shown in Figure 2 [17].

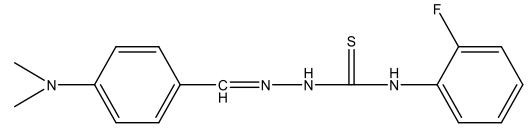
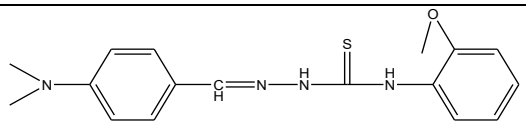
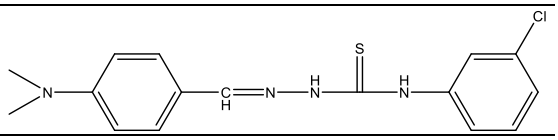
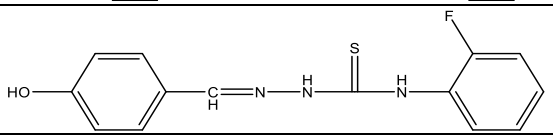
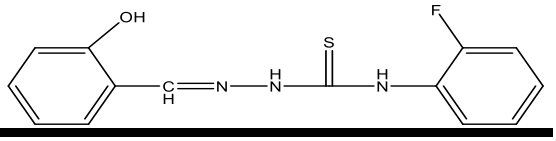


R_1: 1,2,3 -N(CH_3); 4) 4-OH; 5) 2-OH R_2:1,4,5) 2-F; 2) 2-OCH_3; 3) 3-CI

Figure 2. Synthesis reaction of carbothioamide derivatives

The names and molecular structures of the compounds synthesized in the study are given in Table 1.

Table 1. Names and structures of the compounds synthesized in the study

No	Compound name	Molecular structure
1	2-(4-(dimethylamino)benzylidene)-N-(2-fluorophenyl)hydrazine-1-carbothioamide	
2	2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide	
3	2-(4-(dimethylamino)benzylidene)-N-(3-choloro)hydrazine-1-carbothioamide	
4	N-(2-fluorophenyl)-2-(4-hydroxybenzylidene)hydrazine-1-carbothioamide	
5	N-(2-fluorophenyl)-2-(2-hydroxybenzylidene)hydrazine-1-carbothioamide	

2.2 DPPH Radical Scavenging Application

The DPPH radical quenching method, which is frequently used to determine antioxidant activities, is based on the principle that its solution in ethanol shows maximum absorbance at 515-517 nm wavelength.

In this study, we tried to evaluate the DPPH radical quenching activities of the compounds we synthesized, their solutions (2×10^{-4} M stock solution) in 25 mL of DMSO by observing absorbance changes. For this purpose, different volumes of these stock solutions (15 µL, 30 µL, 60 µL, 120 µL, 180 µL) were taken in 6 mL of DPPH mixture and their absorbances at 517 nm were recorded after 30 minutes of incubation. Thus, radical quenching activities were calculated as percent inhibition with the following formula:

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

Where A_0 is the absorbance of the control solution (without antioxidant) and A_1 is the absorbance of the sample solution (when antioxidant was added) [18]. In addition, the IC_{50} (mg/mL) value used to interpret the antiradical activity was calculated. Thus, the antioxidant

activities of the molecules could be compared by comparing the amount of antioxidant required to reduce the DPPH radical concentration by 50% [19-21].

3 RESULTS AND DISCUSSION

3.1 Characterization of Carbotioamide Compounds

FTIR, ^{1}H-NMR and ^{13}C-NMR analyses were performed for the synthesized Carbothioamide compounds and the spectroscopic analysis spectra for Compound 2 are exemplified in Figure 3, Figure 4 and Figure 5, respectively.

In the synthesized compounds, the FTIR voltages of the characteristic groups C-O, C-N, C=N, C=S belonging to the Thiosemicarbazide group and the aromatic ring in the synthesized carbothioamide structure are given in Table 2. When the FTIR results for Compound 2 (2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide) were examined, the N-H stretching vibration band was seen at 3290 cm^{-1}, the C-H stretching bands of the aromatic ring were in the range of 3139-2980 cm^{-1}, the C-H stretching bands of aliphatic group were seen as a separate band in the range of 2892-2813 cm^{-1}, the C=N and C-N bands belonging to the thiosemicarbazide group were seen at 1606 cm^{-1} and 1271 cm^{-1}, respectively. In addition, the C=S stretching bands belonging to the thiosemicarbazide group were observed at 1440 cm-1. The C-O band in the methoxy structure was found only in Compound 2 at 1178 cm^{-1}. In other compounds, specific C-F and C-Cl bands were observed at 1097-1101 cm^{-1} and 1056 cm^{-1}, respectively. The -OH band was observed at 3317 and 3386 cm^{-1} only for Compound 4 and Compound 5, as expected.

Table 2. Frequency values of FTIR (cm^{-1}, υ_{max}) spectra of characteristic groups of synthesized compounds

Compound ID	-OH	-NH	Ar.CH	Aliph.CH	C=N	C=S	C-N	C–O	Spec. Vib.
1	---	3302	3136-2975	2884-2794	1602	1443	1281	-	C-F:1101
2	---	3290	3139-2980	2892-2813	1606	1440	1271	1178	---
3	---	3261	3138-2986	2906-2814	1584	1428	1270	-	C-CI:1056
4	3317	3256	2980	---	1599	1398	1278	-	C-F:1097
5	3386	3155	2992	---	1603	1402	1280	-	C-F:1100

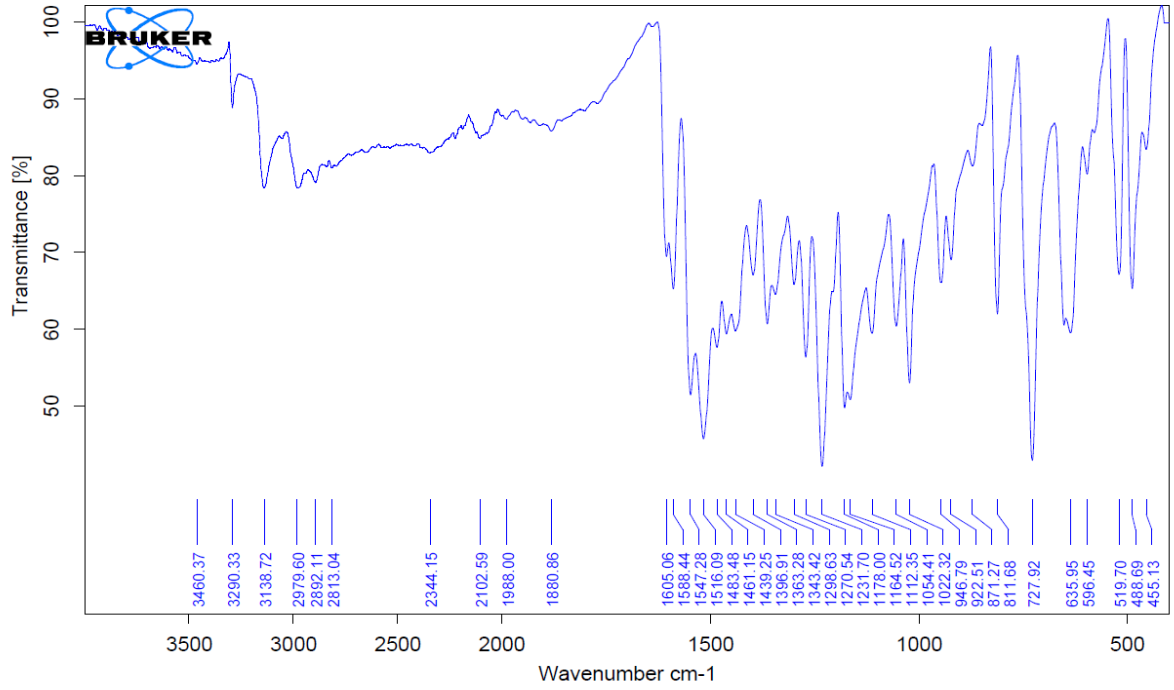


Figure 3. IR spectrum of the Compound 2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide

^{1}H-NMR proton spectra of DMSO solutions of the synthesized compounds were obtained with a Bruker NMR spectrometer at 400 MHz. ^{1}H-NMR results of 2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide compound are given in Figure 4. The common structure of all newly synthesized compounds is that the protons belonging to the N-H groups are singlet in the range of 11.92-11.73 ppm. N-H group bound to aromatic structure showed singlet in the range of 9.97-9.80 ppm. Proton (H8) belonging to CH=N group showed singlet in the range of 8.49-8.04 ppm for the synthesized compounds. For Compound 2, methoxy proton (R1) bound to ring structure gave singlet at 3.92 ppm. For Compound 1, Compound 2 and Compound 3, the protons belonging to the methyl structure attached to the other ring gave singlets at 2.98, 2.99 and 2.99 ppm, respectively. In all synthesized compounds belonging to aromatic rings, the protons H1-H5 and H9-H13 gave doublets between 8.13-7.14 and 7.21-6.68 ppm, respectively.

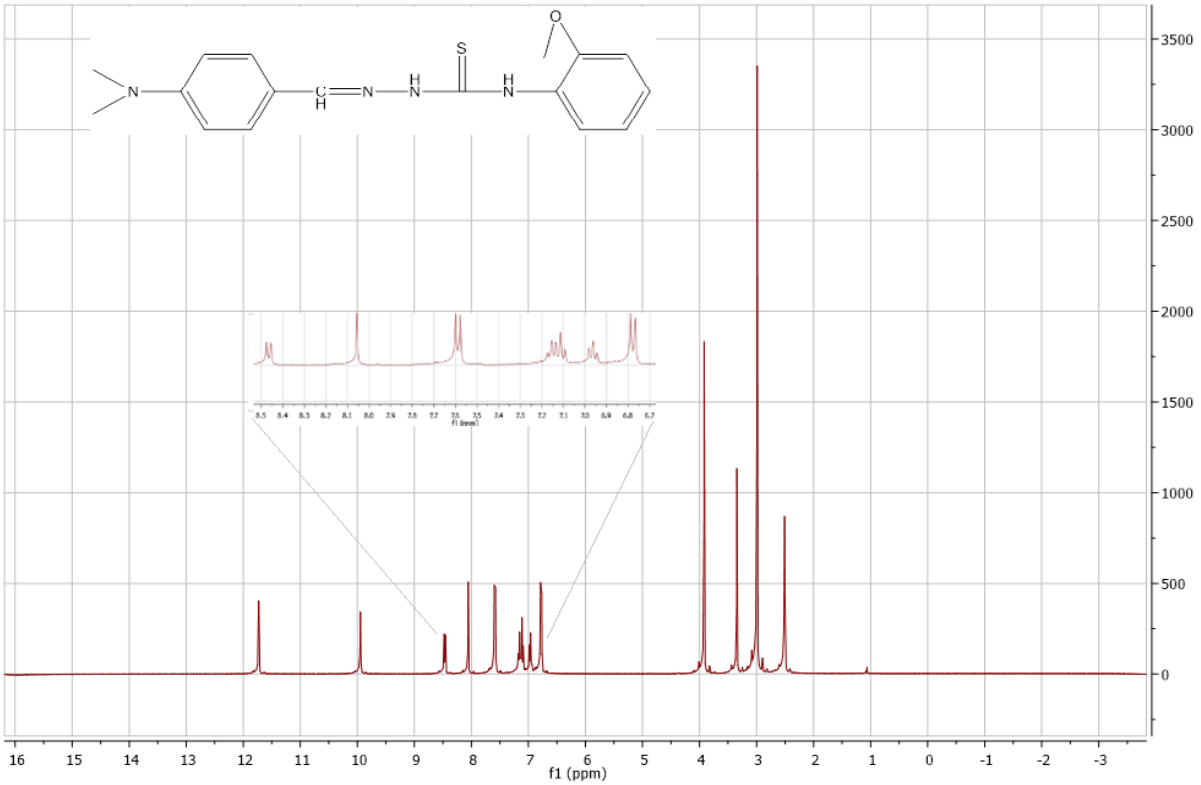
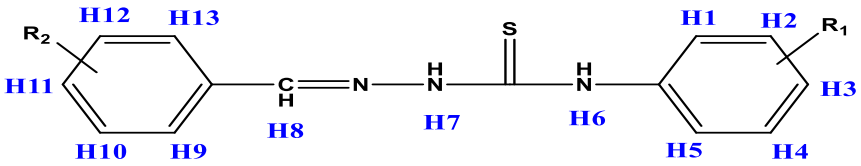


Figure 4. [1]H-NMR spectrum of the Compound 2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide

Table 3. Peak data for 1H-NMR spectra of characteristic groups of synthesized compounds (400 MHz, DMSO-d6, ppm)



Compounds ID	H7, NH	H6, NH-Ar	H8, CH=N	H1-H5	R1	R2	H9-H13
1	11.74 (s,1H)	9.80 (s,1H)	8.04 (s,1H)	7.68 – 7.66 (d, 2H, H5), 7.63 – 7.59 (m, 3H, H2), 7.32 – 7.28 (m, 3H, H3, H4)	-	CH_3:2.98 (s,6H)	6.74-6.72 (d, J = 8.7 Hz, 4H, H9,H10)
2	11.73 (s,1H)	9.95 (s,1H)	8.18 (s,1H)	8.13 (d, J = 15.5 Hz, 2H, H5), 8.10 (m, 3H, H2), 7.17- 7.14 (m, 3H, H3,H4)	OCH_3: 3.92 (s,3H)	CH_3: 2.99 (s,6H)	6.68 (d, J = 8.1 Hz, 4H, H9, H10)
3	11.73 (s,1H)	9.92 (s,1H)	8.05 (s,1H)	7.75 (dd, J = 39.8, 10.1 Hz, 1H, H5), 7.63 (d, J = 7.5 Hz, 2H, H1), 7.38 –7.24 (t, J = 8.1 Hz, 3H, H3,H4)	-	CH_3: 2.99 (s,6H)	6.73 (d, J = 8.8 Hz, 4H, H9,H10)
4	11.82 (s,1H)	9.96 (s,1H)	8.19 (s,1H)	7.99 (d, J = 16.7 Hz, 1H, H5), 7.72 (d, J = 8.5 Hz, 2H, H2), 7.49 – 7.16 (m, 3H, H3,H4)	-	OH: 9.86 (s,1H)	6.72 (d, J = 8.0 Hz, 4H, H9,H10),
5	11.92 (s,1H)	9.97 (s,1H)	8.49 (s,1H)	8.07 (d, J = 7.5 Hz, 2H, H5), 7.71 (d, J = 7.8 Hz, 2H, H2), 7.66 – 7.23 (m, 3H, H3,H4),	-	OH: 9.83 (s,1H)	7.21 (d, J = 7.7 Hz, 4H, H12), 7.23 – 6.10 (m, 3H, H9), 6.75 – 6.10 (m, 4H, H11, H10)

The ^{13}C-NMR results of the 2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide compound are given as an example in Figure 5. The characteristic carbon peaks of all synthesized molecules are shown in the Table 4. Accordingly, the C peaks belonging to the C=S group in all compounds were found in the range of 174.12-177.19 ppm and the C peaks belonging to the C=N group were found in the range of 140.39-144.95 ppm. In addition, peaks were found in the range of 130.51-115.93 ppm for the C1-C6 carbons in the aromatic ring groups attached to the thiosemicarbazide group in the compounds. The peak belonging to the carbon in the methoxy group for Compound 2 was observed at 56.53 ppm. Peaks belonging to the carbons in the dimethyl amine structure attached to the aromatic ring in Compound 1, Compound 2 and Compound 3 were observed at 40.63, 40.41 and 40.21 ppm, respectively.

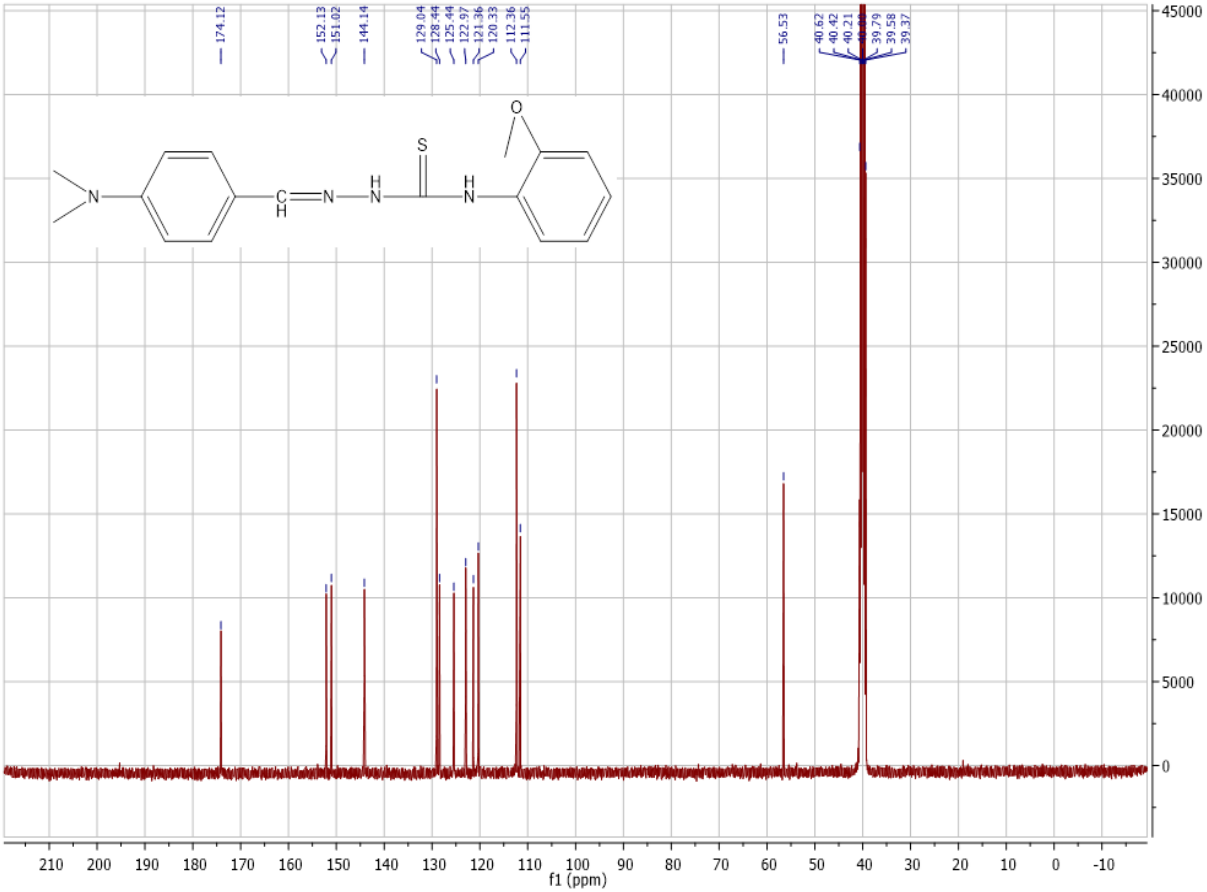
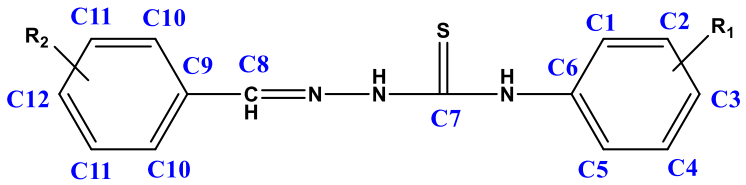


Figure 5. ^{13}C-NMR spectrum of 2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide

Table 4. Peak data for ^{13}C-NMR spectra of characteristic groups of synthesized compounds (DMSO-d_6, ppm)



Compounds ID	Ar, (C1-C6)	OCH_3	CH_3	C9	C10	C11	C12	C=S	C=N
1	129.42 (C6), 128.10-128.13 (C2), 127.86-127.75 (C4), 124.38-124.35 (C3), 121.59-116.13 (C5), 115.93-112.12 (C1).	-	40.63	130.23	156.31	152.05	158.75	176.67	144.57
2	125.44 (C6), 122.97 (C2), 121.36 (C4), 120.33 (C3), 112.36 (C5), 111.56 (C1).	56.53	40.41	129.04	128.44	151.02	152.13	174.12	144.14
3	129.62 (C6), 125.17 (C2), 125.05 (C4), 124.21 (C3), 121.48 (C5), 112.09 (C1).	-	40.21	132.56	129.97	149.21	152.08	175.12	144.95
4	129.86 (C6), 128.43-128.35 (C2), 127.84-127.67 (C4), 125.41-124.42 (C3), 124.38-116.17 (C5), 116.03-115.97 (C1).	-	-	130.52	158.89	156.59	159.97	177.15	143.90
5	130.51-128.46 (C6), 128.40-127.78 (C2), 127.66-127.29 (C4), 124.44-120.71 (C3), 119.70-116.55 (C5), 116.19-115.99 (C1).	-	-	131.85	157.01	156.21	158.68	177.19	140.39

In addition, elemental data of 5 new carbothioamide compounds were determined experimentally and theoretically and are shown in Table 5. Some physicochemical properties of these elements are shown in Table 6.

Table 5. Experimental and calculated elemental analysis of synthesized compounds

Compounds	Theoretical			Experimental		
	C%	H%	N%	C%	H%	N%
1	60.74	5.42	17.71	58.70	4.95	17.12
2	62.17	6.14	17.06	60.85	5.57	16.74
3	57.73	5.15	16.83	56.80	4.86	16.32
4	58.12	4.18	14.52	56.96	3.95	13.92
5	58.12	4.18	14.52	57.35	3.86	13.84

Table 6. Physicochemical parameters of 5 new carbothioamide compounds

Compounds Name	MW (g)	MP (°C)	Yield (%)	Solubility in DMSO	Colour
2-(4-(dimethylamino)benzylidene)-N-(2-fluorophenyl)hydrazine-1-carbothioamide	316.4	181-182	83	+	Red
2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide	328.4	199-200	86	+	Yellow
2-(4-(dimethylamino)benzylidene)-N-(3-choloro)hydrazine-1-carbothioamide	332.9	170-171	80	+	Pink
N-(2-fluorophenyl)-2-(4-hydroxybenzylidene)hydrazine-1-carbothioamide	389.3	187-188	60	+	Light pink
N-(2-fluorophenyl)-2-(2-hydroxybenzylidene)hydrazine-1-carbothioamide	389.3	172-173	73	+	White

3.2 Investigation of Antioxidant Activities

In this study, ascorbic acid was used as the standard antioxidant. The antioxidant activities of the synthesized molecules were calculated in the concentration range of 0.5 – 6.0 µM and the (%) inhibition data are given in Figure 6. Accordingly, inhibition values (%) showed a linear increase with concentration. It was found that the synthesized molecules showed much lower inhibition percentage against the standard antioxidant.

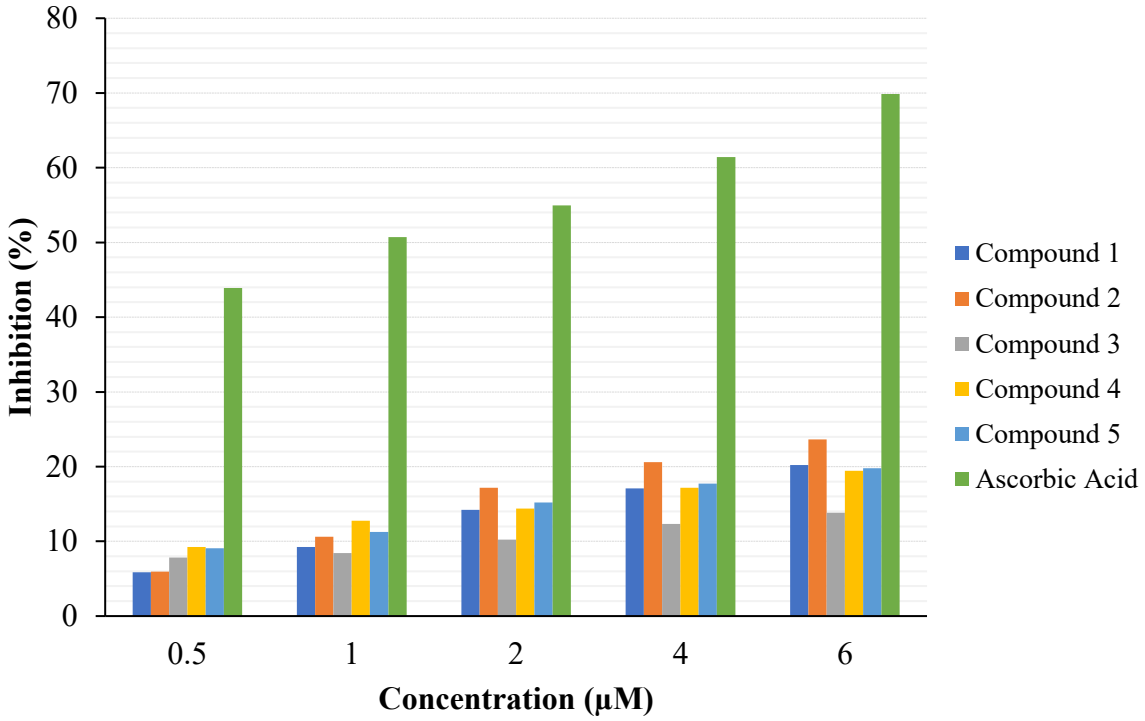


Figure 6. % inhibition graph of 5 new carbothioamide compounds

In addition, antioxidant activities of 5 new synthesized carbothioamide compounds were compared by calculating IC_{50} (mg/mL) values. Linear regression equations of ascorbic acid and 5 new compounds and R^2 values and IC_{50} values of these equations are shown in Table 4.

Table 4. IC_{50} values of ascorbic acid and 5 new carbothioamide compounds calculated by DPPH method

Compounds	Linear Concentration Equation (y=ax±b)	IC_{50} (µM)	R^2
1	y = 3.54x + 4.96	17.83±0.45	0.905
2	y = 2.42x + 6.78	14.30±0.42	0.870
3	y = 1.10x + 7.56	38.45±0.93	0.977
4	y = 1.64x + 10.16	24.18±0.51	0.914
5	y = 2.79x + 6.24	15.70±0.43	0.988
Ascorbic Acid	y = 6.27x + 37.37	2.02±0.05	0.988

Considering the IC_{50} values, the DPPH radical reducing capacity of the synthesized 5 new carbothioamide compounds is quite low compared to ascorbic acid (2.02±0.05 μM). The small IC_{50} values for these new compounds are an indication of their ability to quench free radicals at the same rate even at lower concentrations. Therefore, it is seen that these compounds exhibit stronger antioxidant activity. In this study, the IC_{50} values of the newly synthesized 5 carbothioamide compounds followed the order 3>4>1>5>2. These data showed that Compound 3 exhibited the lowest and Compound 2 the highest antioxidant activity.

The common feature of compounds 1, 5 and 4 is that they have electronegative F atoms on the ring attached to the N-(2-fluorophenyl)hydrazine-1-carbothioamide structure, i.e. thiosemicarbazone structures. In contrast, groups such as -F and -Cl are Ain and colleagues found that the 18 thiocarbohydrazone derivatives they synthesized had strong antioxidant properties in DPPH-like tests and also had low cell toxicity [22].

The free radical scavenging potential of the carbothioamide derivatives we synthesized in this study can change significantly due to the effect of various electron-donating or electron-withdrawing substituents in their structures. For example, the $-N(CH_3)_2$ group in 2-(4-(dimethylamino)benzylidene)-N-(2-fluorophenyl)hydrazine-1-carbothioamide and similar compounds, as a strong electron-donating group, transfers electrons from the aromatic ring to the π-system via resonance, increasing the delocalization of radical centers and thus strengthening free radical stabilization [23]. Likewise, the $-OCH_3$ group supports antioxidant activity by increasing electron density through the mesomeric effect [24] (Santos et al., 2016; Wang et al., 2017).

As in the study of Rajan et al., 2001, in Compound 2, the $–OCH_3$ group substituted to the aromatic ring provided the highest antioxidant activity of this compound since it could provide electrons to the ring [25]. As reported by Gudipati et al., 2011, it was thought that the electronegative fluorine atom substituted to the aromatic ring attached to the carbothioamide group may be responsible for the lower antioxidant activity [26]. In contrast, groups such as -F and -Cl are electron-withdrawing, and especially the fluorine atom can stabilize radical intermediates through intramolecular hydrogen bonds due to its small size and high electronegativity [27]. The -Cl group at the 3-position can suppress the antioxidant behavior to some extent by reducing the electron density of the aromatic system with both inductive and weak mesomeric effects. In addition, the -OH groups located in 2- or 4-hydroxybenzylidene derivatives provide phenolic structures that can neutralize free radicals through direct hydrogen donation, a well-known antioxidant mechanism [28]. With the joint effect of all these groups, the antioxidant activities of the compounds are determined by both electron density changes and resonance stabilization of radical intermediate species.

4 CONCLUSION AND SUGGESTIONS

Thiosemicarbazone-linked aromatic compounds continue to be the subject of fields such as biochemical applications, medicine and pharmaceutical applications, thanks to their various biological effects such as antioxidant activities and outstanding pharmacological properties. In this study, a new carbothioamide compound containing thiosemicarbazone-based aromatic group was synthesized and its antioxidant properties were found. Newly synthesized carbothioamide compounds showed DPPH radical quenching activity in the range of 6.0–23.5% at concentrations of 0.5–6.0 µM. The highest activity was observed in 2-(4-(dimethylamino)benzylidene)-N-(2-methoxy)hydrazine-1-carbothioamide with 23.5% inhibition rate. Although the IC_{50} values of these compounds at concentrations of 0.5–6.0 µM were higher than the IC_{50} value of ascorbic acid in the same concentration range, it was determined that they exhibited significant antioxidant effect. As a result, it was observed that methoxy and hydroxy groups attached to the aromatic ring have significant contributions in this activity. These results indicate that thiosemicarbazone derivatives can be made more effective by structural modifications in improving their antioxidant potential. In the future, examining the effects of such compounds on different radical species, investigating their biological activities at cell culture level and evaluating their pharmacokinetic profiles will be guiding in terms of biomedical applications. In addition, deepening the structure-activity relationships with molecular modeling and in silico analyses may pave the way for new targeted compound designs.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

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