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New 5-methylisatin-thiosemicarbazones: preparation, spectroscopic study and antioxidant properties

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Abstract

New isatin-thiosemicarbazone (1–6) were obtained from various 5-methylisatin and various isothiocyanates with good yields and efficient methods. The structures of the synthesized compounds were clarified by FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques and elemental analysis. The antioxidant activity properties of the compounds were investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and total reducing power determinations. In our total reducing power study, where we chose Trolox as the standard antioxidant, it was found that the reducing powers of compounds containing different substituent groups and positions were close to each other and lower than Trolox. In the study using butylated hydroxytoluene (BHT) as a reference antioxidant, DPPH antiradical scavenging capacities of the synthesized compounds were determined. The free radical scavenging effects of the synthesized compounds were compared with the IC_{50} values obtained from the concentration equations and were found in the order BHT > 6 > 3 > 5 > 1 > 2 > 4. Structural and electronic properties of the compounds were examined by density functional theory (DFT) calculations and their relationship with the antioxidant properties of the compounds was discussed. DFT calculations played an important role in determining how the electronic properties of the synthesized compounds, especially in the presence of different substituents and in the ortho and meta positions, and accordingly the antioxidant properties of the compounds, changed. Experimental and theoretical results confirmed that methoxy-containing structures in the synthesized compounds exhibit higher antioxidant effects compared to halogen-containing structures, regardless of their position.

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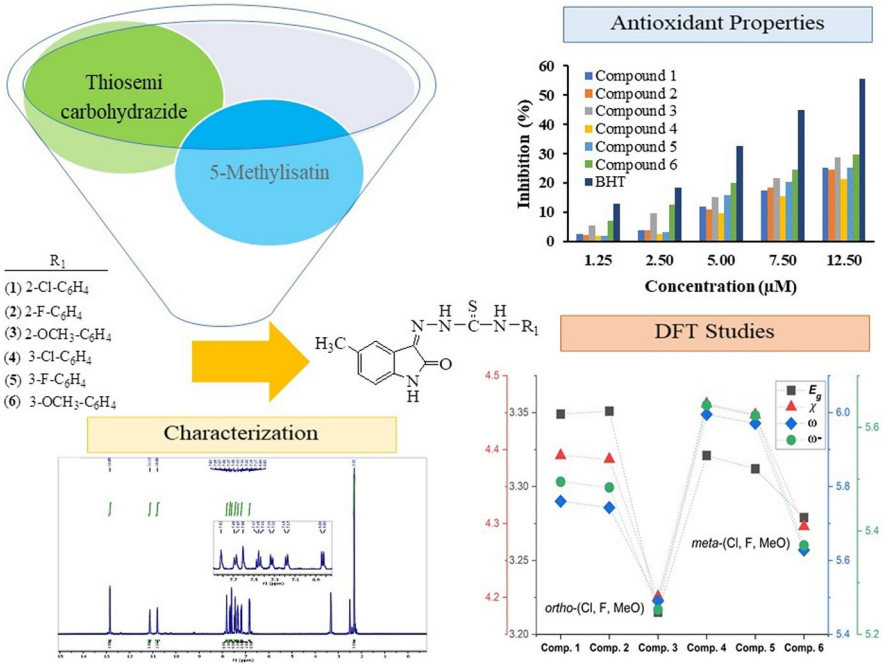
Graphical abstract



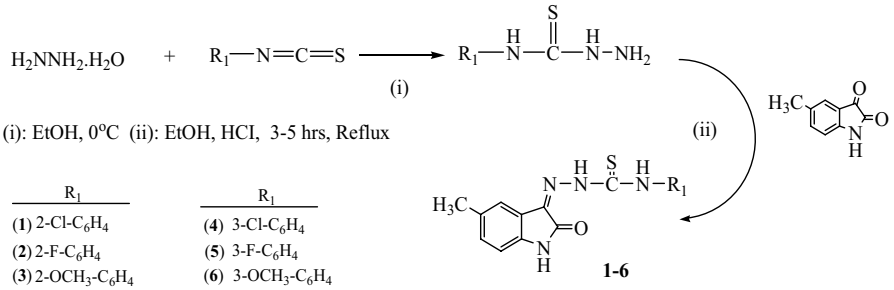
Keywords Thiosemicarbazones · 5-Methylisatin · Antioxidant activity · Spectroscopic techniques

Introduction

Isatin (1H-indole-2,3-dione) composes a substantial class of heterocyclic structures found in plant and human tissue, is the indole-based derivatives. They have various medicinal and biological properties such as antibacterial [1], antioxidant [2–4], antifungal [5], antitubercular [6], anticancer [7], and anti-HIV [8].

Thiosemicarbazones have –NH–C(=S) NH–N=bond, are a substantial class of synthetic organic chemistry. They are also used as versatile intermediates for synthesizing a great number of moieties. Thiosemicarbazones have several biological and medicinal properties such as anticancer [9], anticonvulsant [10], antituberculosis [11], urease inhibitory activity [12], antimicrobial [13], antioxidant agents [14–16], antibacterial [17], and antiviral [18].

Thiosemicarbazone-containing compounds are a well-known class of ligands with a wide range of biological activities [19]. It has been reported that these molecules, which have been synthesized and tested in large numbers so far, show good antioxidant properties [20]. Previous results have shown that thiosemicarbazones and isatin-formed derivatives have not been sufficiently studied so far. Therefore, in



Scheme 1 Synthetic route for the obtained molecules (1–6)

this study, it was aimed to elucidate their antioxidant properties by synthesizing new thiosemicarbazones and isatin compounds. For this purpose, condensation reaction of 5-methylisatin and various isothiocyanates was carried out under reflux in ethanol medium and a series of new 5-methylisatin-thiosemicarbazones were obtained. New molecular structures were elucidated by Fourier transform infrared (FT-IR), proton and carbon nuclear magnetic resonance (^{1}H NMR & ^{13}C NMR) and elemental analysis methods. Additionally, the spectral and electronic properties of the compounds were analyzed by DFT calculations. In particular, the effects of substituents on the antioxidant properties of the compounds were discussed.

Experimental

Synthesis of 5-methylisatin-thiosemicarbazone compounds (1–6)

6.0 mmol of hydrazine monohydrate solution and 6.0 mmol of isothiocyanate molecule species were added dropwise into 20 mL of ethanol while cooling in an ice bath and stirred vigorously for 30 min. After this mixture was kept in the refrigerator for 24 h, the resulting precipitate was filtered. The filtrate was dried and washed with ethanol to obtain thiosemicarbohydrazide [21–23]. The formed thiosemicarbohydrazide (3.0 mmol) was added to 20 mL of ethanol with 3.0 mmol of 5-methyl isatin and one drop of dilute HCl solution and stirred under reflux for 3–5 h. The solid obtained at the end of the reaction was filtered, washed and dried in an oven. Thus, the synthesized compounds were prepared with a yield of (55–85%) via the reaction shown in Scheme 1 according to the previously reported procedure [24] with minor modifications. The detailed knowledge about measurement and reagents is given in supplemental material.

Antioxidant activity measurements

All new compounds were screened for their in vitro antioxidant activities. In order to investigate the antioxidant properties of the synthesized compounds, reducing power determination and total antioxidant capacity determination were carried out.

Total reducing capacity using the potassium ferricyanide reduction method

The reducing capacity of a compound can serve as an important indicator of its potential antioxidant activity [25]. The basis of the ferricyanide method is based on the interaction of $[Fe(CN)_6]^{3-}$ with antioxidants and its reduction to $[Fe(CN)_6]^{4-}$, and the formation of $[Fe(CN)_6]^{4-}$ with the addition of Fe(III) in the medium forms a Prussian blue charge-transfer complex [26].

$$Fe(CN)_6^{3-} + AOx \rightarrow Fe(CN)_6^{4-} + AOx \text{ (oxidized state)} \quad (1)$$

$$Fe(CN)_6^{4-} + Fe^{3+} \rightarrow Fe\left[Fe(CN)_6\right]^- \quad (2)$$

In this study, 1 mL of the solutions of the synthesized compounds in dimethyl sulfoxide (DMSO) (0.25–125 µM) was taken and mixed with 2.5 mL of 0.2 M phosphate buffer (pH = 6.5). Then, 2.5 mL of potassium ferricyanide (1 g/100 mL) was added and these mixtures were incubated at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to the mixtures and they were centrifuged at 3000 rpm at room temperature for 10 min. The obtained supernatant was taken and first 2.5 mL of H_2O and then 0.5 mL of ferric chloride (1%) were added. The mixture was incubated at 37 °C for 10 min and then the absorbances were measured at 700 nm [27, 28].

Free radical scavenging activity

One of the widely used and accepted models to evaluate the free radical scavenging activity is the DPPH method [29, 30]. First of all, in order to achieve this method, solutions of the synthesized compounds were prepared at concentrations of 1.25, 2.50, 5.00, 7.50, 12.5 µM in DMSO. Then, antioxidant activity measurements were performed by incubating the solutions prepared with 1,1-diphenyl-2-picrylhydrazil stock solution in ethanol for 30 min at room temperature. The absorbance measurements of the prepared solutions were measured at 517 nm and the percentage of radical scavenging activity of the samples was calculated by the following formula [31]:

$$\text{Inhibition } (\%) = \left[(A0 - A1)/A0\right] \times 100 \quad (3)$$

Accordingly; A0 represents the absorbance of the solution without the compound, and A1 represents the absorbance of the sample solution containing the synthesized compounds [32]. Then, the IC50 parameter, which helps us rank the antioxidant activities of the compounds and is a measure of the 50% inhibition capacity, was determined from the $y = mx + c$ formula curve obtained using the inhibition (%)-concentration graph [33].

Theoretical computational measurements

The calculations were performed using the ORCA 5.0.3 software [34] with density functional theory (DFT) [35, 36]. The computations utilized Becke's 3-parameter exchange functional (B3) combined with the Lee–Yang–Parr correlation functional (LYP), as the B3LYP functional. Additionally, the DEF2-TZVPP basis set was employed along with D3BJ dispersion correction. These calculations were conducted without any symmetry constraints on the compounds. The optimized structures of the compounds represent the true minimum energy. This was demonstrated by the absence of imaginary frequencies in the IR vibrational calculations conducted in the gas phase.

Electronic parameters were obtained with the help of same level gas phase calculations. Frontier molecular orbital (FMO) energy eigenvalue data were then used to calculate global chemical reactivity parameters such as chemical hardness, electronegativity and electrophilic index. Further investigation into the relationship between antioxidant activity and molecular structure involved calculating different global reactivity parameters, such as HOMO–LUMO gap (E_g), electronegativity (χ), global hardness (η), electrophilicity (ω), electrodonating power (ω^-), and electroaccepting power (ω^+).

Relative chemical shift values were determined by deducting the absolute chemical shielding of tetramethylsilane (TMS) calculated at the B3LYP/DEF2-TZVPP level (31.698 ppm for ^{1}H NMR and 183.951 ppm for ^{13}C NMR), after re-optimizing the compounds in the DMSO phase and employing the CPCM-SCRF procedure to model solvent effects.

Results and discussion

The physical properties, yields, melting points and elemental analysis results of the synthesized compounds are given in Tables 1 and 2.

FTIR vibration frequencies

The FTIR spectrum results of the molecules confirmed that the symmetric and unsymmetric stretching bands belonging to amino groups (NH_2) were not observed at 3500–3350 cm^{-1}. Also, the –C=N stretching bands belonging to imine group were observed at 1626–1603 cm^{-1}, indicating that a successful reaction took place as expected. As an example of FTIR spectra of compounds, the spectrum of Compound **3** is presented in Fig. 1. For compound **3**, C=S vibration was characteristically given at 1476 cm^{-1} and S=C–N vibration at 1314 cm^{-1}.

For all compounds, while the –NH stretching vibrations of isatin ring were observed at 3302–3230 cm^{-1}, the–NH stretching vibrations of thiosemicarbazone region were detected at 3218–3126 cm^{-1}, respectively (see Figs. S1–S6).

Table 1 Physical properties of the synthesized compounds

Comp	Structure Name	M. P. (°C)	Yield (%)	Colour
1	*N*-(2-chlorophenyl)-2-(5-methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide	249–250	85	Rose-light purple
2	*N*-(2-fluorophenyl)-2-(5-methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide	246–247	58	Rose-light purple
3	*N*-(2-methoxyphenyl)-2-(5-methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide	239–241	83	Rose-light purple
4	*N*-(3-chlorophenyl)-2-(5-methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide	233–235	62	Rose-light purple
5	*N*-(3-fluorophenyl)-2-(5-methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide	225–227	55	Light reddish- brick
6	*N*-(3-methoxyphenyl)-2-(5-methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide	194–195	74	Light reddish- brick

Table 2 Elemental analysis results of the compounds

Comp	Molecular mass (g/mol)	Molecular formula	Calculated			Experimental		
			C (%)	H (%)	N (%)	C (%)	H (%)	N (%)
1	344.8	$C_{16}H_{13}ClN_4OS$	55.73	3.80	16.25	55.66	3.89	16.32
2	328.4	$C_{16}H_{13}FN_4OS$	58.53	3.99	17.06	58.41	4.04	17.13
3	340.4	$C_{17}H_{16}N_4O_2S$	59.98	4.74	16.46	60.09	4.78	16.37
4	344.8	$C_{16}H_{13}ClN_4OS$	55.73	3.80	16.25	55.65	3.85	16.22
5	328.4	$C_{16}H_{13}FN_4OS$	58.53	3.99	17.06	58.41	3.87	17.12
6	340.4	$C_{17}H_{16}N_4O_2S$	59.98	4.74	16.46	59.91	4.73	16.50

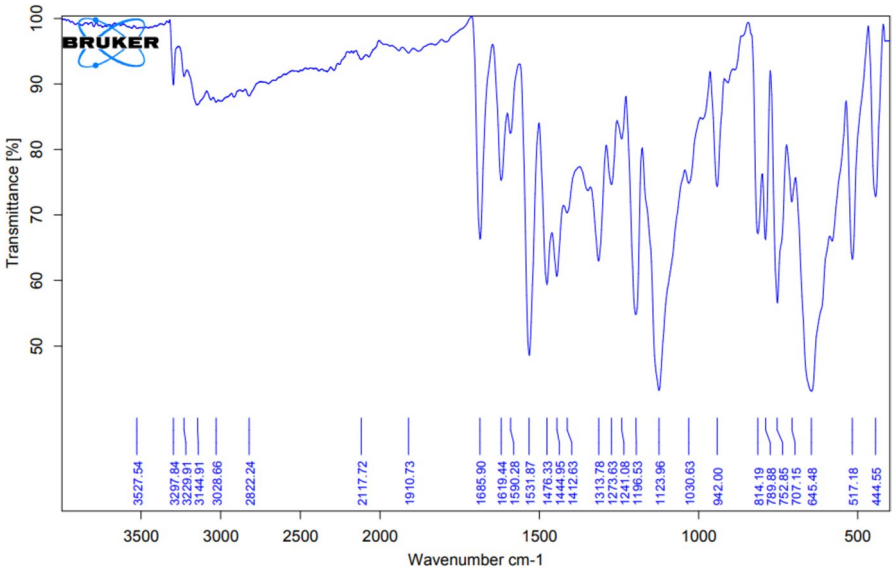


Fig. 1 FTIR spectrum of compound 3

Stretching vibrations of the –C = O structure were observed at 1693–1680 cm^{-1}, the vibration signal of the –C = S structure at 1483–1473 cm^{-1} and the stretching vibrations of the –C–N structure at 1316–1308 cm^{-1}.

For compounds **1** and **4**, stretching vibrations of –C–Cl structure were observed at 939 and 1027 cm^{-1}, respectively. For compounds **2** and **5**, vibrations of –C–F structure were detected at 1202 and 1205 cm^{-1}, respectively. For compounds **3** and **6**, –C–O structure showed stretching vibrations at 1124 and 1154 cm^{-1}, respectively. These FTIR spectra showed significant evidence that the molecules were synthesized correctly, and FTIR data for all molecules are presented in Table 3. These observations are agreement with values published formerly for similar compounds [14, 16, 37, 38].

Table 3 Experimental and calculated IR vibration frequencies of the compounds (cm^{-1})

	Comp	ν_{NH} (ist)	ν_{NH} (tcrb)	$\nu_{C=O}$ (ist)	$\nu_{C=N}$	$\nu_{C=S}$	$\nu_{S=C-N}$ ν_{ArC-N}	Subs
Experimental	1	3267	3201	1688	1621	1482	1310	C–Cl:939
	2	3230	3174	1685	1626	1473	1308	C–F:1202
	3	3297	3145	1686	1619	1476	1314	C–O:1124
	4	3287	3218	1680	1605	1480	1313	C–Cl:1027
	5	3301	3126	1683	1603	1478	1314	C–F:1205
	6	3302	3176	1693	1610	1483	1316	C–O:1154
Calculated	1	3640.26	3472.81, 3425.02	1757.27	1617.57	1413.17	1382.65, 1263.62	C–Cl: 1052.32
	2	3640.21	3505.13, 3427.73	1757.59	1618.65	1414.10	1390.54, 1274.11	C–F: 1217.31
	3	3640.73	3498.98, 3428.56	1756.01	1612.99	1415.64	1398.10, 1272.55	O–CH_3: 1057.68
	4	3640.23	3507.01, 3425.38	1757.83	1617.05	1412.72	1385.63, 1258.52	C–Cl: 1122.70
	5	3640.08	3509.31, 3425.93	1757.49	1617.22	1413.61	1385.89, 1289.12	C–F: 1285.76
	6	3640.60	3510.62, 3429.46	1756.86	1615.06	1414.59	1391.22, 1309.64	C–O: 1081.44

Subs.: Substituents, Comp.: Compounds

Examination of ^{1}H NMR results

The synthesized compounds were dissolved in DMSO-d^6 and ^{1}H NMR spectra were obtained. The chemical shift values for the compounds are shown in Table 4. DMSO-d^6 signals were seen around 2.00, 2.55 ppm (quintet) and the signals of water impurity (HOD, H_2O) in DMSO were seen around 3.40 ppm [39]. For the synthesized compounds, the –NH proton signals of the isatin ring were detected as a singlet at 11.18–11.11 ppm. The –N^{1}H and –N^{2}H proton signals of the thiosemicarbazone region in the compounds appeared as a singlet at 10.86–10.42 and 12.85–12.76 ppm, respectively (see Figs. S7–S12).

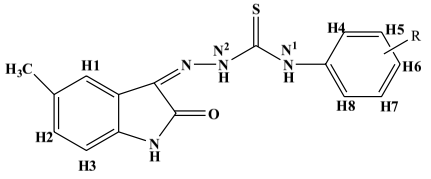
For all compounds, the aromatic proton signals of the phenyl ring (H5–H8) were observed at 7.82–6.85 ppm. The ^{1}H NMR spectrum results obtained for compound **3** are shown in Fig. 2. In compound **3**, the H5 and H8 protons coupled to the H6 and H7 proton and showed a doublets of doublet peak at 7.18–7.13 ppm; proton H6 bound to protons H5 and H7 showed a triplet peak at 7.31–7.27 (J = 7.8 Hz) ppm. Proton H7 bound to protons H6 and H8 showed a triplet peak at 7.03–6.99 (J = 7.6 Hz) ppm. For the whole compound, aromatic proton signals of isatin ring (H1–H3) were detected between 7.61 and 6.81 ppm. For compound **3**, proton H1 appeared as a singlet peak at 7.49 ppm. Proton H2 bound to proton H3 and a doublet peak was observed at 7.83–7.81 (J = 7.6 Hz) ppm. The H3 proton bound to the H2 proton and a doublet peak was detected at 6.84–6.82 (J = 7.9 Hz) ppm. For compound **3**, the proton signals of methoxy (–OCH_3) and methyl (–CH_3) groups were observed as a singlet at 3.87 and 2.31 ppm, respectively. These consequences are consistent with values those for similar compounds in the literature [3, 14, 16, 38].

Examination of ^{13}C NMR results

The synthesized compounds were dissolved in DMSO-d^6 and ^{13}C NMR spectra were obtained. The chemical shift values of the compounds are summarized in Table 5. In all compounds (**1–6**), the characteristic –C = O (C7) peaks belonging to the isatin ring were observed at 163.2–160.9 ppm. The characteristic –C = N (C8) peaks belonging to the imine structure resonated between 140.9 and 140.7 ppm. The characteristic –C = S (C9) peaks belonging to the thiosemicarbazone moiety in the compounds were observed at 178.4–176.5 ppm (see Figs. S13–S18). The aromatic carbon signals attached to the isatin ring (C1–C6) were detected between 133.3 and 111.4 ppm. The aromatic carbon signals of the phenyl ring (C10–C15) were found at 163.3–111.3 ppm. When the ^{13}C NMR spectrum for compound **3** is examined, it is seen that there are 15 different resonance carbon atoms compatible with the target structure (Fig. 3). The methoxy (–OCH_3) and methyl (–CH_3) carbon atoms of compound **3** showed resonance at 56.3 and 21.1 ppm, respectively. In addition, the C10 and C15 carbon atoms of compounds **2** and **5** were separated into doublets by interaction with the F atom nucleus.

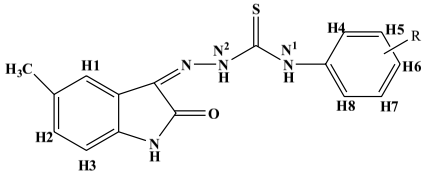
In addition, the presence of 2-Cl (136.6 ppm), 2-F (159.0 ppm), 2-OCH_3 (153.3 ppm), 3-Cl (140.4 ppm), 3-F (163.3 ppm) and 3-OCH_3 (159.7 ppm) atoms/

Table 4 Experimental and calculated ^{1}H NMR values of the compounds (δ, ppm)



	Comp	=N–N^{2}H	isatin NH	–N^{1}H–Ar	isatin H	ArH	–CH_3/OCH_3
Experimental	1	12.84 (s, 1H)	11.11 (s, 1H)	10.74 (s, 1H)	7.55 (s, 1H), 7.20–7.18 (d, *J* = 7.9 Hz, 1H), 6.85–6.83 (d, *J* = 7.8 Hz, 1H)	7.61–7.59 (d, *J* = 7.6 Hz, 2H), 7.46–7.37 (dt, *J* = 19.6, 7.5 Hz), 2H)	2.31 (s, 3H)
	2	12.85 (s, 1H)	11.15 (s, 1H)	10.69 (s, 1H)	7.55 (s, 1H) 7.19–7.17 (d, *J* = 7.9 Hz, 1H), 6.84–6.82 (d, *J* = 7.9 Hz, 1H)	7.53–7.51 (m, 1H), 7.42–7.26 (m, 3H)	2.30 (s, 3H)
	3	12.76 (s, 1H)	11.15 (s, 1H)	10.42 (s, 1H)	7.49 (s, 1H), 7.83–7.81 (d, *J* = 7.6 Hz, 1H), 6.84–6.82 (d, *J* = 7.9 Hz, 1H)	7.31–7.27 (t, *J* = 7.8 Hz, 1H), 7.18–7.13 (dd, *J* = 12.7, 8.1 Hz, 2H), 7.03–6.99 (t, *J* = 7.6 Hz, 1H)	3.87 (s, 3H, OCH_3) 2.31 (s, 3H)
	4	12.85 (s, 1H)	11.13 (s, 1H)	10.80 (s, 1H)	7.60 (s, 1H), 7.19–7.17 (d, *J* = 7.8 Hz, 1H), 6.84–6.82 (d, *J* = 7.9 Hz, 1H)	7.82 (s, 1H), 7.69–6.67 (d, *J* = 7.8 Hz, 1H), 7.47–7.43 (t, *J* = 8.0 Hz, 1H), 7.34–7.32 (d, *J* = 7.9 Hz, 1H)	2.32 (s, 3H)
	5	12.85 (s, 1H)	11.18 (s, 1H)	10.86 (s, 1H)	7.61 (s, 1H), 7.20–7.18 (d, *J* = 7.8 Hz, 1H), 6.85–6.83 (d, *J* = 7.9 Hz, 1H)	7.65 (s, 1H), 7.53–7.51 (d, *J* = 8.0 Hz, 1H), 7.49–7.44 (dd, 1H), 7.14–7.10 (t, *J* = 8.3 Hz, 1H)	2.31 (s, 3H)

Table 4 (continued)



	Comp	$=N–N^2H$	isatin NH	$–N^1H–Ar$	isatin H	ArH	$–CH_3/OCH_3$
	6	12.79 (s, 1H)	11.16 (s, 1H)	10.74 (s, 1H)	7.61 (s, 1H), 7.18–7.16 (d, $J=7.9$ Hz, 1H), 6.83–6.81 (d, $J=7.9$ Hz, 1H)	7.36–7.31 (m, 2H), 7.26–7.24 (d, $J=8.2$ Hz, 1H), 6.87–6.85 (m, 1H)	3.79 (s, 3H, OCH_3), 2.31 (s, 3H)
Calculated	1	13.22	7.66	11.23	8.06 (H1), 7.64 (H2), 7.19 (H3)	8.04 (H5), 7.66 (H6), 7.83 (H7), 10.39 (H8)	2.22–2.67 (3H)
	2	13.06	7.65	11.00	8.06 (H1), 7.64 (H2), 7.19 (H3)	7.77 (H5), 7.67 (H6), 7.71 (H7), 10.08 (H8)	2.23–2.69 (3H)
	3	13.06	7.65	11.27	8.11 (H1), 7.64 (H2), 7.19 (H3)	7.48 (H5), 7.67 (H6), 7.44 (H7), 10.04 (H8)	4.14–4.67 (3H, OCH_3) 2.25–2.72 (3H, CH_3)
	4	13.16	7.65	10.53	8.08 (H1), 7.64 (H2), 7.19 (H3)	7.67 (H4), 7.63 (H6), 7.87 (H7), 9.97 (H8)	2.25–2.71 (3H)
	5	13.14	7.65	10.57	8.09 (H1), 7.64 (H2), 7.19 (H3)	7.38 (H4), 7.38 (H6), 7.91 (H7), 9.86 (H8)	2.24–2.70 (3H)
	6	13.05	7.64	10.48	8.07 (H1), 7.63 (H2), 7.19 (H3)	7.14 (H4), 7.18 (H6), 7.85 (H7), 9.52 (H8)	3.94–4.23 (3H, OCH_3) 2.26–2.71 (3H, CH_3)

Comp.: Compounds

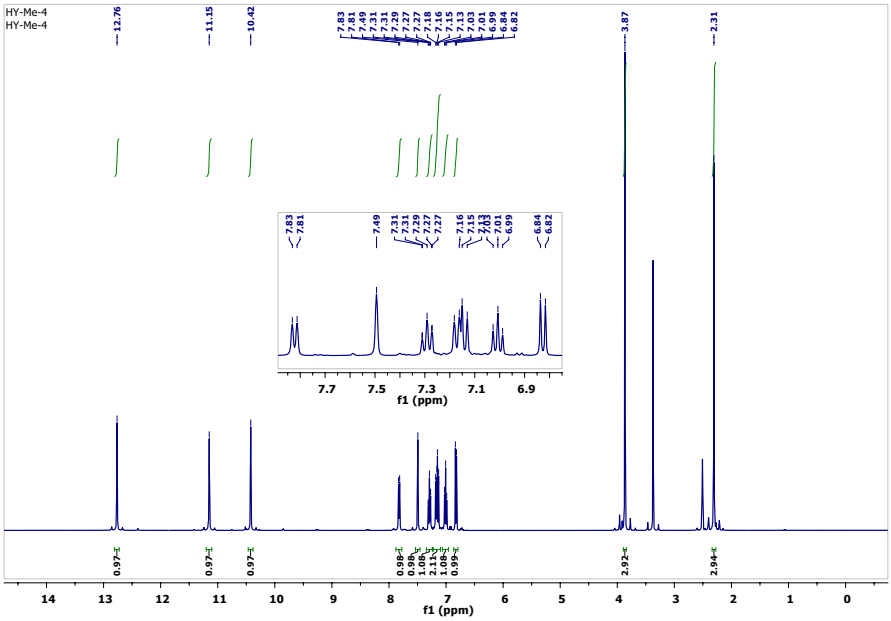


Fig. 2 ^{1}H NMR spectrum of compound 3

groups caused the carbon signals to appear in lower ranges compared to the benzene signal (128.5 ppm), i.e., high δ values in all compounds. These data are in agreement with the data that reported similar compounds [3, 14, 16, 38, 40].

Evaluation of antioxidant activity

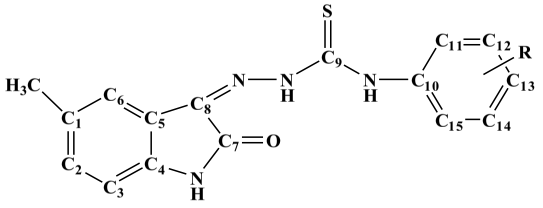
Previously, using similar methods, antioxidant activity properties of biochemically important preparations or many newly synthesized compounds were exemplified by capacity reduction and radical scavenging mechanisms such as prevention of radical chain formation, decomposition of peroxides, binding of catalysts and prevention of continuous hydrogen abstraction [41]. In this study, total antioxidant capacities of newly synthesized compounds were interpreted using reducing power tests and DPPH radical quenching methods.

Total reducing power assay

In the determination of reducing power, the presence of reductants in the solution reduces the Fe^{3+}/ferricyanide complex to ferrous form and turns the yellow test solution into green–blue color. Thus, this can be monitored by absorbance measurement at 700 nm [42].

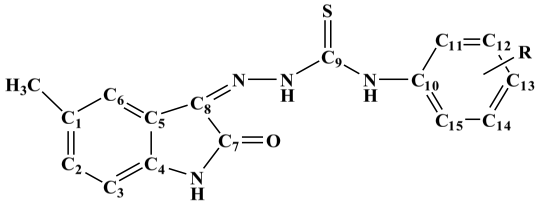
Figure 4 shows the absorbance values of reducing power of new compounds dissolved in DMSO measured using potassium ferricyanide reduction method and

Table 5 Experimental and calculated ^{13}C NMR data of compounds 1–8, (δ/ppm)



		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	Subs.
Experimental	1	131.9	130.8	120.3	132.4	111.4	129.3	163.2	140.9	178.0	133.2	136.6	128.0	130.1	122.1	131.8	21.1[a]
	2	133.2	131.9	120.4	132.4	122.1	130.5	163.2	140.9	178.4	127.0126.9	159.0156.5	116.5116.3	129.5 129.4	111.4 111.3	124.9 124.8	21.1[a]
	3	131.9	132.8	120.3	132.4	111.4	127.9	163.2	140.8	176.5	127.5	153.3	112.2	120.5	126.6	121.8	21.1[a] 56.3[b]
	4	131.9	130.4	120.3	132.5	111.4	126.2	163.2	140.9	176.7	133.3	124.4	140.4	125.4	132.9	122.3	21.1[a]
	5	133.3	131.9	120.3	132.5	111.4	122.3	160.9	140.9	176.7	140.7140.6	130.4130.3	163.3163.2	121.7 121.6	112.8 112.5	113.2 112.9	21.1[a]
	6	131.9	132.9	120.4	132.3	111.5	122.3	163.2	140.7	176.5	140.0	111.9	159.7	118.0	129.6	111.3	21.1[a] 55.7[b]

Table 5 (continued)



		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	Subs.
Calculated	1	142.3	139.5	115.6	148.1	127.7	128.1	169.3	137.6	183.1	143.4	136.9	136.8	131.5	133.9	123.6	22.5[a]
	2	142.3	139.4	115.6	148.1	127.7	127.9	169.3	137.3	183.5	134.7	163.9	120.9	131.4	130.7	123.5	21.1[a]
	3	142.2	139.1	115.5	147.8	127.9	127.7	169.3	136.8	181.7	134.9	157.9	114.7	131.4	124.9	122.1	22.6[a] 58.5[b]
	4	142.3	139.4	115.6	148.1	127.7	127.8	169.3	137.4	182.9	148.4	126.2	149.0	130.9	136.9	120.3	22.6[a]
	5	142.4	139.4	115.6	148.0	127.7	127.9	169.3	137.3	182.9	148.9	112.3	174.6	116.9	137.6	118.0	22.6[a]
	6	142.2	139.1	115.6	147.9	127.9	127.6	169.3	136.9	182.5	148.2	113.2	169.9	111.7	136.7	113.5	22.6[a] 58.1[b]

Subs.: Substituents

[a]CH_3, [b]OCH_3

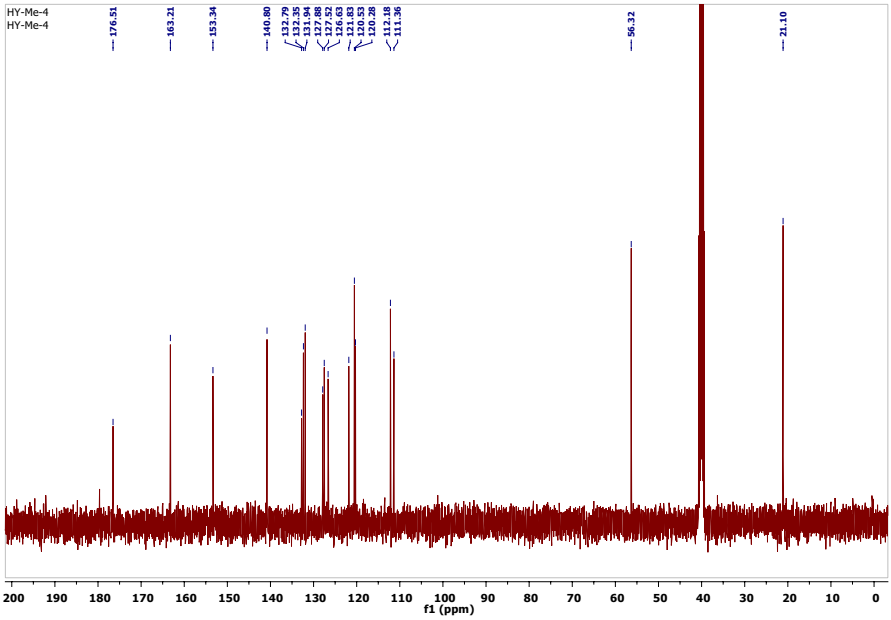


Fig. 3 ^{13}C NMR spectrum of compound 3

prepared at different concentrations (0.02, 0.40, 2.40, 5.30, 10.30 µM). Increasing absorbance showed that reducing power of compounds at different concentrations increased and the results were compared with Trolox used as standard. Compounds showed lower reducing power than Trolox used as standard at all concentrations. As can be seen from Fig. 4, compounds exhibited similar reducing power to each other. The highest increase was observed in compound **1** depending on increasing concentration. In this study, methoxy substituted compounds **6** and **3** showed higher reducing power compared to other electronegative F and Cl atom substituted compounds except compound **1**. In the study conducted by Singhal et al. [43] on the synthesis of Chalconesemicarbazone derivatives, it was reported that methoxy substituent attached to the aldehyde group also supports antioxidant activity. Previous studies have mentioned that hydroxyl and methoxy groups of aldehyde structures exhibit high reducing activity [44, 45]. Based on the observed results, the reducing power of the compounds synthesized in this study, both those containing different substituent groups and those containing substituent groups at different positions with the same group, were found to be very close to each other.

DPPH radical scavenging activity assay

In order to interpret the antioxidant properties of the synthesized compounds, firstly the (%) inhibition values showing the antioxidant capacities of each compound at different concentrations were calculated with the help of DPPH radical-compound

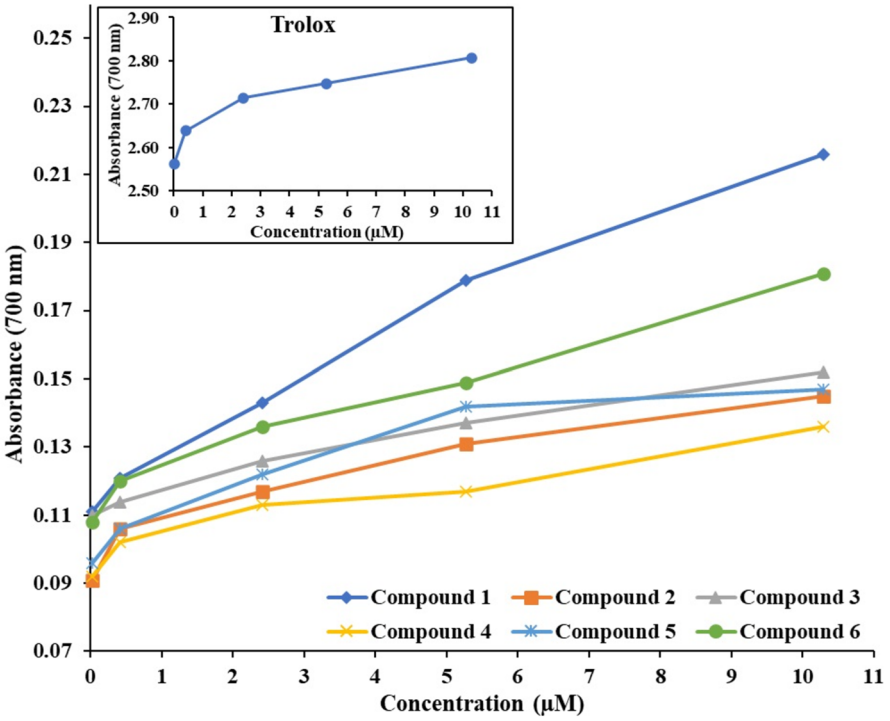


Fig. 4 Reducing power assays of the synthesized compounds measured at different concentrations (0.02, 0.40, 2.40, 5.30, 10.30 µM)

absorbance values. In this study, the percentage inhibition changes depending on the concentration of butylated hydroxytoluene (BHT) and the synthesized compounds that we used to compare their antioxidant properties are given in Fig. 5. When the DPPH free radical scavenging activity changes were examined, the synthesized compounds **3**, **5**, and **6** showed a more regular increase depending on the concentration. Compounds **1**, **2**, and **4** showed a very low percentage of inhibition at the first 2 concentrations compared to the standard. These inhibition changes, which differ from molecule to molecule, vary according to the position and structure of the thiosemicarbazone-bound substituents rather than the isatin groups.

In antioxidant capacity determinations, half maximal antioxidant concentration (IC_{50}) values, which is a quantitative measure of the amount of antioxidant required to inhibit a certain amount of radicals by 50%, were calculated. In order to more clearly reveal the differences between the antioxidant capacities of the synthesized molecules, IC_{50} values obtained from the concentration equations for BHT and molecule samples are given in Table 6. Accordingly, it was found that the free radical scavenging effects of the molecules varied in the order of BHT > 6 > 3 > 5 > 1 > 2 > 4.

Naik et al. reported that, similar to our study, the antioxidant activity of substituted anilines rather than the isatin structure increased significantly. They found that molecules containing electron-donating methoxy substituents showed greater antioxidant activity. especially in comparison with butylated hydroxyanisole (BHA)

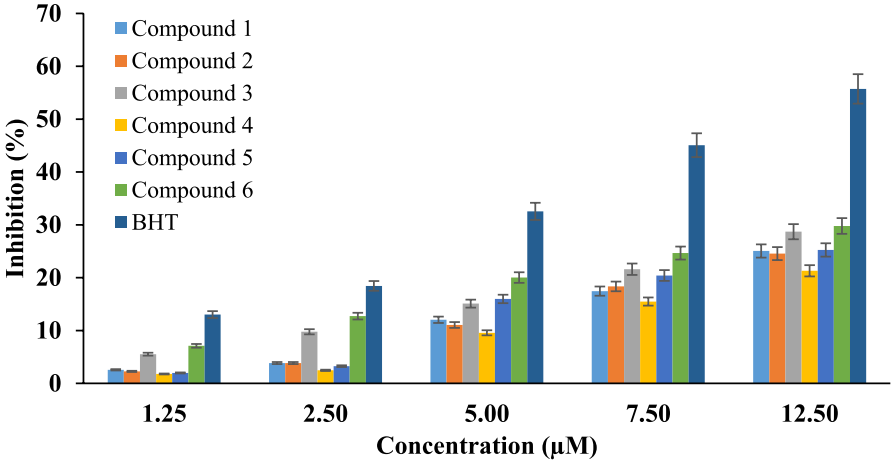


Fig. 5 Percent of inhibition calculated by the DPPH method for BHT and compounds 1–6. (Error bars represent standard deviations for n = 3 and indicate that the observed experimental effect is significant with 95% accuracy.)

[46]. In this study, it was also observed that methoxy substituted structures showed higher antioxidant activity than the others, but showed much lower antioxidant activity than the standard BHT. Groups attached to thiocarbohydrazone structures have important roles on antioxidant activity, as Pakravan et al. summarized earlier and Božić et al. mentioned in their work on synthesizing carbohydrazones compounds [15, 47]. It was reported by G. Kiran et al. [3] that especially halogen-containing systems significantly increased antioxidant activity. Similarly, halogen substituted groups were used in this study and their antioxidant effects were observed. At the same time, methoxy substituted structures were used in this study. As in the study of Bakır and Lawag [48] it was found that compounds with –OMe structures exhibited better antioxidant activity in this study. When the different substituents attached to the thiosemicarbazide structures in the *ortho* position in compounds **1**, **2**, and **3** were examined, it was observed that the antioxidant activity changed in the order –OMe > F > Cl. This order was also found for compounds **4**, **5**, and **6** attached to the ring in the thiosemicarbazide structure at the *meta* position. As in reducing power, the lowest antioxidant activity was exhibited by compound 4 with 3-Cl substitution.

DFT analysis

The compounds can be examined under 2 groups according to their *ortho*- and *meta*- Cl, F, and MeO substituents, respectively. Although the effects of substituents on the antioxidant properties of compounds depend on many internal and external factors such as intermolecular-intramolecular interactions and solvent effects, it is also obvious that the electronic properties of the compounds will affect their chemical behavior. HOMO–LUMO surfaces of compounds vary depending on the positions of the substituents (Fig. 6; see Supplementary Fig. S19 for all). As seen from both inhibition percentage data and IC_{50} values,

Table 6 Percent inhibition (%) and IC_{50} values for BHT and synthesized compounds 1–6

Concentration (μM)	Inhibition (%)						
	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	BHT
1.25	2.56 ± 0.01	2.27 ± 0.01	5.52 ± 0.02	1.78 ± 0.01	1.97 ± 0.01	7.10 ± 0.04	13.02 ± 0.06
2.50	3.85 ± 0.01	3.85 ± 0.01	9.76 ± 0.04	2.47 ± 0.01	3.25 ± 0.01	12.72 ± 0.06	18.44 ± 0.08
5.00	12.03 ± 0.05	11.05 ± 0.05	15.09 ± 0.06	9.57 ± 0.04	15.98 ± 0.06	20.02 ± 0.06	32.54 ± 0.21
7.50	17.46 ± 0.07	18.34 ± 0.08	21.60 ± 0.09	15.48 ± 0.06	20.41 ± 0.09	24.65 ± 0.10	45.07 ± 0.27
12.50	25.05 ± 0.10	24.56 ± 0.10	28.70 ± 0.11	21.30 ± 0.09	25.25 ± 0.10	29.78 ± 0.12	55.72 ± 0.33
DPPH scavenging activity IC_{50} (μM)*	23.91 ± 0.06	23.96 ± 0.06	22.31 ± 0.04	27.35 ± 0.06	22.52 ± 0.04	21.74 ± 0.04	10.12 ± 0.02
Concentration Equality (y = ax + b)	2.08x + 0.22	2.09x + 0.02	2.05x + 4.37	1.85x − 0.50	2.18x + 0.82	1.95x + 7.65	3.90x + 10.53
R^2	0.976	0.967	0.978	0.966	0.888	0.92	0.956

*IC_{50} = the concentration (μM) exhibiting 50% inhibition of DPPH radical

Values are expressed as means (n = 3)

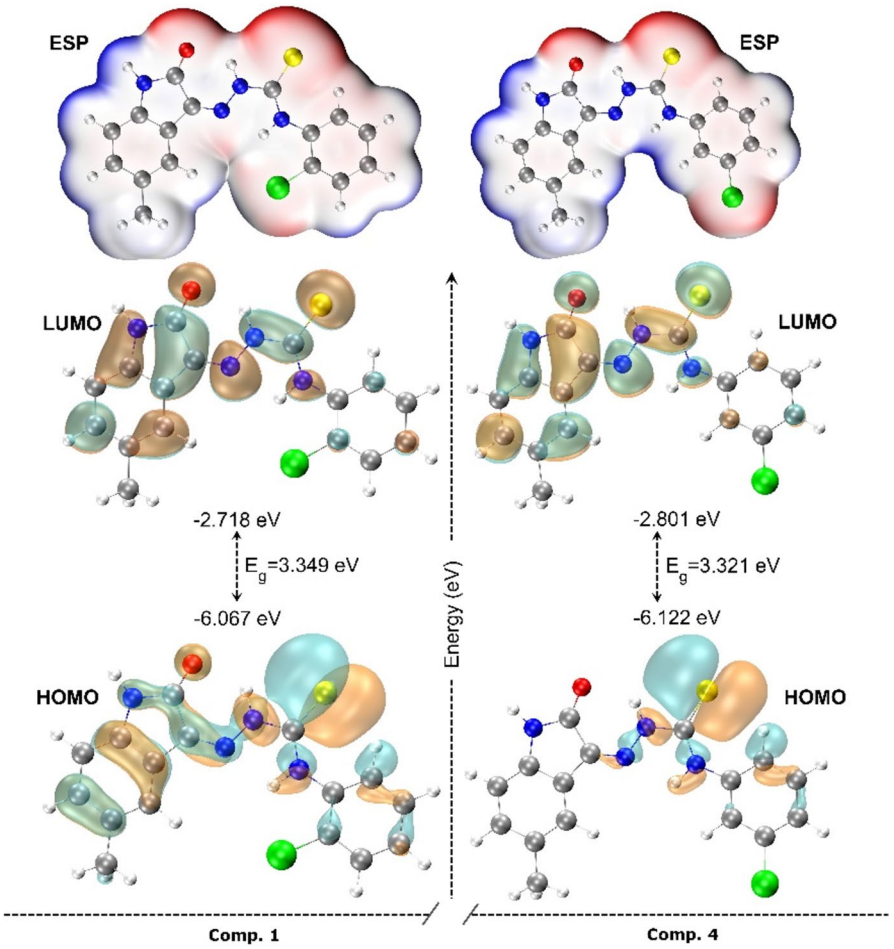


Fig. 6 HOMO–LUMO and ESP maps of the compounds 1 and 4

ortho/*meta*-MeO substituted compounds **3** and **6** exhibited high antioxidant activity. While the LUMO distributions in these compounds do not differ much from each other, it has been observed that in the *ortho* position of MeO, HOMOs are concentrated on the benzene ring and thiourea region, whereas in the *meta* position, they are generally concentrated on the thiourea region. ESP surfaces also indicate that the *ortho*-MeO substituent contributes more to intramolecular interactions. The effects of chlorine and fluorine atoms on HOMO are more obvious than their effects on LUMO. Although the *meta*-F substituent had a similar effect to *meta*-MeO in terms of the distribution of HOMOs, it enhanced a wide HOMO distribution in the isatin-thiourea region when in the *ortho* position. It has been observed that the chlorine atom has an effect on HOMO-LUMOs similar to that of the fluorine atom in the *ortho* and *meta* positions. It is quite difficult to compare the effects of electron-withdrawing fluorine and chlorine substituents on the

HOMO–LUMO surfaces of compounds (ESP-HOMO–LUMO surface maps are very similar). Essentially, MeO is considered an electron donor group, while fluorine and chlorine are members of the electron-withdrawing group. While methoxy has a strong electron-donating and reactivity-increasing effect in the *ortho* position, these effects are weak in the *meta* position, but it is clear that MeO increases the antioxidant effect of the compounds more than fluorine and chlorine, both in the *ortho* and *meta* positions.

The electronic properties of compounds **1**–**3** and **4**–**6**, according to the *ortho* and *meta* positions of the substituents, revealed similar data within their groups (Fig. 7). While the E_g values of *ortho* substituted compounds **1**–**3** were calculated as 3.349, 3.351, and 3.215 eV, respectively (the effect of MeO is pronounced), the E_g values of *meta* substituted compounds **4**–**6** were similarly calculated as 3.321, 3.312, and 3.279 eV, respectively. A low E_g value indicates high reaction ability, but their evaluation is a little more difficult due to the contribution of *ortho* substituents to intramolecular interactions. For example, for *meta*-(Cl, F, MeO) substituted compounds, the lowest E_g value was calculated for MeO substituted compound **6** and the highest for Cl substituted compound **4** (see Supplementary Table S1). These results are also consistent with the IC_{50} values of compounds **4**–**6**. However, for compounds **1**–**3**, while the lowest E_g value of MeO substituted compound **3** is compatible with the experimental antioxidant effect data, the same is not true for the effects of fluorine and chlorine substitutes, but it can be said that they cause effects close to each other. Moreover, meta-substituted compounds exhibit similar inhibition effects across all concentrations, while the influence of ortho substituents on the compound's inhibition varies with concentration, which can be attributed in part to intramolecular effects.

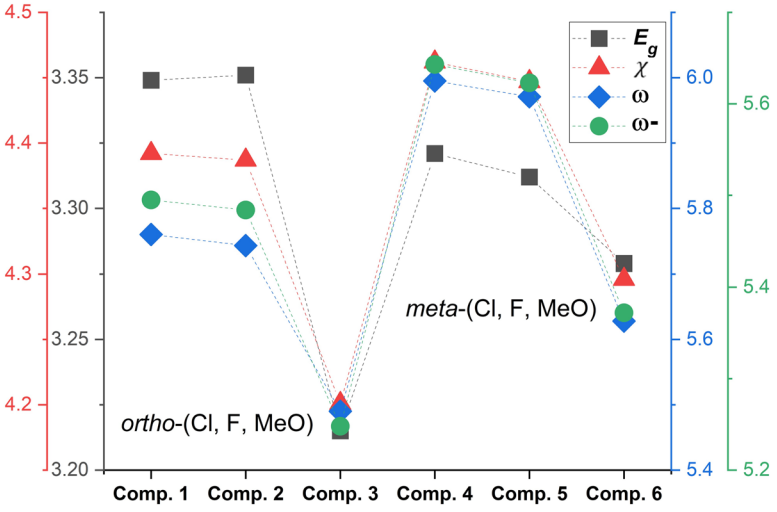


Fig. 7 Some calculated electronic data of the compounds, in eV unit, (E_g: Energy gap, χ: Electronegativity, ω: Electrophilic index, ω.⁻: Electrodonating power)

Conclusions

In this paper, new 5-methylisatin-thiosemicarbazone compounds have been achieved with good yields of 55–85%. The molecular structures of the newly synthesized compounds were characterized using spectroscopic techniques such as FT–IR, ^{1}H NMR, ^{13}C NMR and elemental analysis methods. The in vitro antioxidant activities of these molecules were observed using the DPPH free radical scavenging process and the total reducing power method. IC_{50} values of the samples were found to vary between 8.44 and 10.66 µM. The results in this study clearly revealed that different substituents were effective on the antioxidant activity in carbothioamide compounds formed with thiosemicarbazone structures. In the synthesized compounds, methoxy-containing structures showed the highest DPPH free radical scavenging effect compared to halogen-containing structures, regardless of which position they were attached to. DFT calculations reveal some findings by evaluating the effects of the electronic properties of compounds on antioxidant activity. It was observed that compounds with MeO substituted generally had lower E_g values, indicating higher reactivity. Intramolecular effects, especially at ortho substituents, affected the inhibition effects of compounds at different concentrations. While meta-substituted compounds showed more consistent inhibition effects depending on concentrations, the inhibition effect varied in ortho-substituted compounds. These conclusions highlight that electronic properties, especially the presence and position of substituents, play an important role in determining the antioxidant properties of compounds.

Author contributions statement

Bakır T: Conceptualization, methodology, formal analysis, writing-original draft preparation, investigation, resources, software, writing-review and editing.

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Author contributions Temel Kan BAKIR (T.B.) carried out the synthesis, analysis and characterization stages of the work. The examination of experimental and theoretical data and the preparation of the draft article were done by T.B. T.B. wrote the main manuscript text and prepared figures (1-6) and tables (1-6) and reviewed the manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The author declares that he has no conflict of interest.

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