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Preparation, characterization, antioxidant properties of novel Schiff bases including 5-chloroisatin-thiocarbohydrazone

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Abstract

Thanks to its pharmaceutical properties, the Schiff group of isatin has recently had a wide range of uses. In this study, seven new Schiff bases of isatin and its derivatives were prepared from thiocarbohydrazide, isatin and substituted aldehydes in the presence of ethanol under reflux. The chemical structures of the products were confirmed by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The in vitro antioxidant features of all the compounds were evaluated by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging method. The antioxidant effect was evaluated separately in two different stages: monosubstituted products synthesized with aldehyde groups and disubstituted products bound with isatin group. Accordingly, the compounds **3** and **4** showed the highest antioxidant activity and the isatin group suppressed the antioxidant effect of the disubstituted Schiff bases products.

Keywords Thiocarbohydrazone · Schiff bases · 5-chloroisatin · DPPH · Antioxidant evaluation

Introduction

Schiff bases compounds are including structure of –C=N– (azomethine group). They are generally prepared from the condensation of primary amines and active carbonyl groups. Schiff bases are important class of compounds in pharmaceutical and biological field [1–4]. Schiff bases of isatin are known to possess a wide range of pharmacological properties including antibacterial [5–9], anticonvulsant [10, 11], anti-HIV and antifungal activity [3, 6, 7, 9, 12, 13]. They display broad-spectrum pharmacological and biological properties such as antitumor [14–16], antimicrobial

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and antioxidant [5, 17, 18], cytotoxicity, anti-influenza virus and antiviral activity [19–21].

The production of reactive oxygen species (ROS) increases with the passage of time and causes many physiological disorders in the human body, such as cardiovascular diseases. High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells [22]. Minimizing oxidative damage may be an important approach in the prevention or treatment of these diseases [23].

Schiff bases and metal complexes may play an important role in exhibiting antioxidant properties. In a recent study on thymol and carvacrol Schiff base derivatives at a concentration of 5 μg/mL, antioxidant activity was observed with 60–90% inhibition [24]. In addition, 2-oxoquinoline-3-carbaldehyde Schiff bases have been reported to be excellent antioxidant agents and their activities show a comparable level of antioxidant activity to ascorbic acid used as standard [25]. The antioxidant activities of Schiff bases and metal complexes derived from methoxylated cinnamyl aldehydes from natural phenyl propene have recently been investigated and their inhibition properties demonstrated [26].

In previous studies, it has been reported that isatin-thiocarbohydrazone derivatives exhibit a number of chemotherapeutic activities and are intended to be used as synthetic antioxidants to protect against reactive oxygen species and suppress cell damage in recent years [27, 28]. In light of the above literature, we aimed to synthesize the isatin analogs obtained by combining thiocarbohydrazone with different functionalized aldehydes in the development of new antioxidant agents. Thus in this study, we have reported the effect of isatin on antioxidant activity in disubstituted thiocarbohydrazone products.

Materials and methods

Measurement and reagents

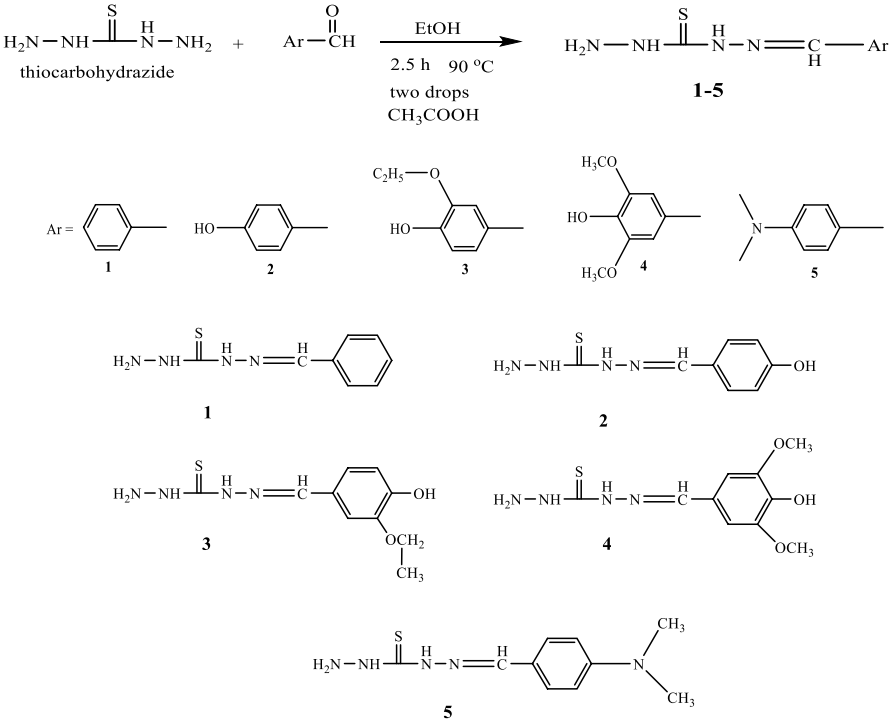
5-chloroisatin, thiocarbohydrazide and aldehydes are provided from Sigma-Aldrich Co. LLC. All solvents were used in analytical purity. Deionized purity water was used in each step. Melting points were recorded using Stuart Melting Point 30 apparatus and uncorrected. Elemental analyses and FT-IR analyses were performed in Kastamonu University Central Research Laboratory. The elemental analysis was conducted by using Eurovector EA3000-Single. Bruker Alpha FT-IR spectrometer was used for infrared spectra. ^{1}H NMR and ^{13}C NMR analyses were performed in Bolu Abant University Central Research Laboratory. ^{1}H and ^{13}C NMR spectra were taken on JEOL ECX-400 (400 MHz) in DMSO-d_6 spectrophotometer. Absorbances were measured by SHIMADZU UVmini-1240 UV–visible spectrophotometer (Shimadzu Corp., Kyoto, Japan manufactures) using a pair of equivalent quartz cuvettes of 1 cm thickness at 517 nm.

Synthesis of monosubstituted products (1–5)

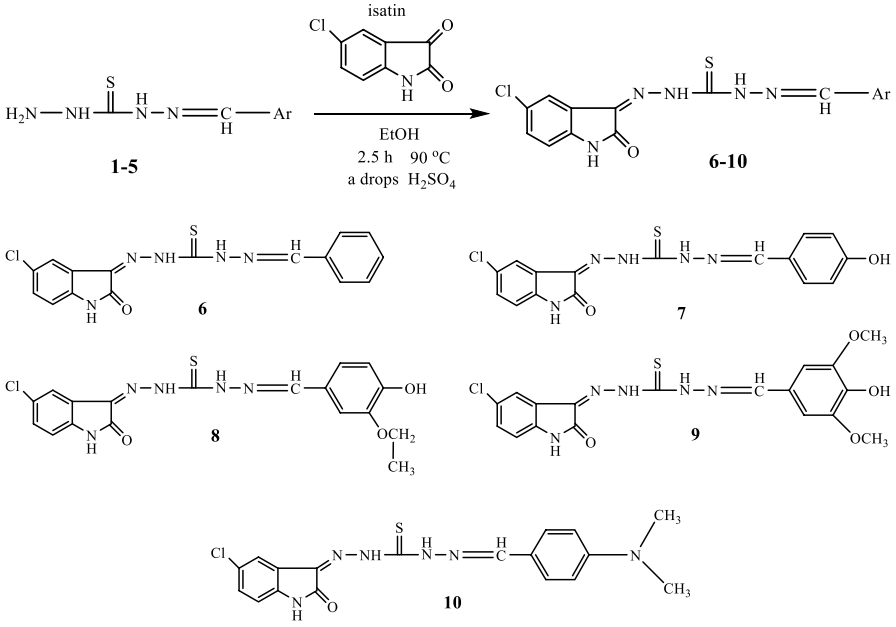
Substituted aldehydes (2.50 mmol), thiocarbohydrazide (3.00 mmol) in 20 mL of absolute ethanol and two drops of acetic acid were added to into a (100 mL) round-bottom flask and reaction mixture refluxed 2.5 h at 90 °C. The solid product so formed was filtered and washed with hot ethanol (75%) and then dried. The synthesis reaction of monosubstituted products is shown in Scheme 1. Two of the synthesized monosubstituted products (**3**–**4**) were new.

Synthesis of disubstituted products (6–10)

20 mL of absolute ethanol and one drop of sulfuric acid was added to the mixture of equimolar monosubstituted thiocarbohydrazide (0.70 mmol) and isatin (0.70 mmol) and then refluxed for 2.5 h at 90 °C in 100 mL round-bottom flask. The solid product so formed was filtered and washed with hot ethanol and then dried. All newly synthesized disubstituted products and synthesis reaction are given in Scheme 2.



Scheme 1 General synthesis of monosubstituted products



Scheme 2 General synthesis of disubstituted products

Antioxidant activity measurements

To evaluate the antioxidant activity of the newly synthesized compounds, a solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was first prepared. For this, 4.75×10^{-5} mol of DPPH was dissolved in 250 mL of ethanol and used as a stock at a concentration of 1.90×10^{-4} M. Then, solutions of different concentrations of synthesized compounds (0.25 µM, 0.50 µM, 1.00 µM, 2.50 µM, 5.00 µM) were prepared in dimethyl sulfoxide (DMSO).

Blank [Ethanol + DPPH) System Solution Preparation

This solution was prepared as follows: 4 mL (stock 1.90×10^{-4} M) DPPH) + 1 mL ethanol; total volume = 5 mL reaction mixture.

Example [Compound solution + Ethanol + DPPH) system solution preparation

This solution was prepared as follows: 4 mL (stock 1.90×10^{-4} M) DPPH) + X mL compound solution + (1−X) mL ethanol; total volume = 5 mL reaction mixture.

Then, the different concentrations of DPPH calibration solutions prepared with ethanol were incubated in the dark for 30 min at room temperature and absorbance at 517 nm against the blanket of ethanol was recorded. The percentage radical damping activity was calculated by the following formula:

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

where A_0 is the absorbance of control solution (no antioxidant added) and A_1 is the absorbance of sample solution (when antioxidant) [29]. The amount of antioxidant

required to reduce DPPH concentration by 50% is a commonly used parameter to measure antioxidant activity and is referred to as IC_{50}. The IC_{50} value was determined from the formula curve "$y=mx+c$" obtained from the inhibition (%)—concentration plot for synthesized compounds [30].

Results and discussion

Physical data

In this study, a total of seven new compounds were synthesized: two monosubstituted (**3**–**4**) and five disubstituted (**6**–**10**) products. The current experimental results for the compound's name, physical data, yields and melting points are summarized in Table 1, and elemental analyses are given in Table 2.

Vibrational frequencies

In the FT-IR spectrum of the synthesized compounds, the aldehyde group (–CHO two bands) signal of the monosubstituent thiocarbohydrazone was not observed near 2750–2650 cm^{-1}. Furthermore, the asymmetric and symmetric stretching bands of the amino group ($–NH_2$) were not appeared at 3600–3200 cm^{-1} except for compounds 3 and 4. These results indicated a successful reaction as expected.

Moreover, general structures of the synthesized compounds are shown in Fig. 1. For compound **3**, the asymmetric and symmetric stretching bands of the carbohydrazide amino group ($–N^3H_2$) were observed at 3145 and 3120 cm^{-1} as shown in Fig. 2. The NH_2 bond is under OH bond for compound **4**. The bands 3183, 3295 and 3315, 3290 cm^{-1} were assigned to amino groups ($–N^1H$, $–N^2H$) stretching in compounds **3** and **4** of thiocarbohydrazide, respectively. For compounds **3** and **4**, the OH and aromatic CH vibration bands were observed at 3315, 3030 and 3233, 3001 cm^{-1}, respectively. In compounds **6**–**10**, the –NH (isatin) stretching vibrations were appeared at between 3187 and 3083 cm^{-1}. For compounds **6**–**10**, the C=S signals of thiocarbohydrazide region were observed at between 1388 and 1328 cm^{-1}. The C=O signals of isatin ring were observed at between 1704 and 1687 cm^{-1}, for compounds **6**–**10**. In compounds **6**–**10**, the –C=N stretching vibrations were appeared at 1621 and 1575 cm^{-1}. For compounds **6**–**10**, the C–Cl signals of isatin ring were observed at between 892 and 817 cm^{-1}. In compounds **7**–**9**, the –C–O signals of phenyl ring were observed at between 1074 and 1059 cm^{-1} as shown in Fig. 3. These frequency values of all the synthesized compounds were nearly in agreement with the same similar compounds [3, 19, 31]. The IR peaks of the compounds are given in Table 3.

^{1}H NMR spectra

The ^{1}H NMR spectra of the synthesized compounds were detected in DMSO-d_6 as solvent and showed general scheme for ^{1}H NMR spectra interpretations in Fig. 4.

Table 1 The data for yields and melting points for the synthesized compounds

Comp.	Name	Color	Yield (%)	*m.p.* (°C)	Lit. yield (%)	Lit. *m.p.* (°C)
1	1-Benzylidene thiocarbonohydrazone	White	75	184	85 [18]	183–185
2	1-(4-Hydroxybenzylidene) thiocarbonohydrazone	Light cream	75	214	63 [19]	210
3	1-(3-Ethoxy-4-hydroxybenzylidene) thiocarbonohydrazone	Light yellow	77	206	A	A
4	1-(3,5-Dimethoxy-4-hydroxybenzylidene) thiocarbonohydrazone	Light yellowish cream	80	226	A	A
5	1-(4-(Dimethylamino)benzylidene) thiocarbonohydrazone	Yellowish green	55	178	61 [20]	178–180
6	1-Benzylidene-5-(6-chloro-2-oxoindolin-3-ylidene)thiocarbonohydrazone	Light brown	57	234	A	A
7	1-(4-Hydroxybenzylidene)-5-(6-chloro-2-oxoindolin-3-ylidene)thiocarbonohydrazone	Light orange	83	254	A	A
8	1-(4-Hydroxy-3-ethoxybenzylidene)-5-(6-chloro-2-oxoindolin-3-ylidene)thiocarbonohydrazone	Pale yellow	76	248	A	A
9	1-(4-Hydroxy-3,5-dimethoxybenzylidene)-5-(6-chloro-2-oxoindolin-3-ylidene) thiocarbonohydrazone	Yellowish brown	91	267	A	A
10	1-(4-(Dimethylamino)benzylidene)-5-(6-chloro-2-oxoindolin-3-ylidene)thiocarbonohydrazone	Dark brown	51	238	A	A

A, Novel compounds

Table 2 The results for elemental analysis for the synthesized compounds

Compound	Calculated %			Found %		
	C	H	N	C	H	N
3	47.185	5.549	22.019	46.417	5.447	21.387
4	44.392	5.220	20.716	42.926	5.131	20.375
6	53.654	3.380	19.561	52.769	3.219	18.998
7	51.358	3.236	18.724	50.829	3.193	18.312
8	51.687	3.859	16.750	50.730	3.813	16.374
9	49.781	3.717	16.133	48.153	3.676	15.897
10	53.876	4.274	20.952	51.973	4.236	20.615

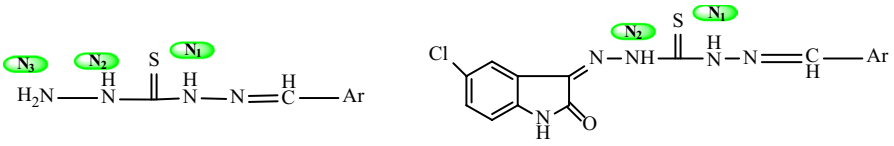


Fig. 1 General structures of the synthesized compounds

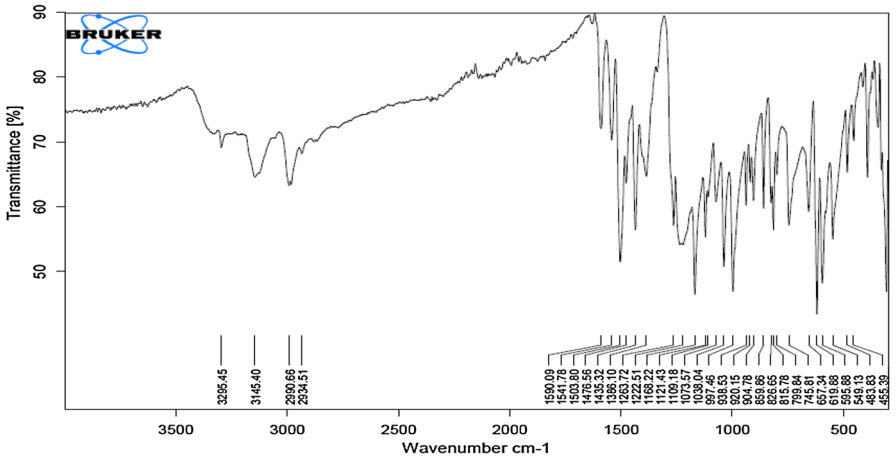


Fig. 2 FT-IR spectrum of compound **3**

For **3**, the aromatic proton signals of aryl ring (H1, H4 and H5) were observed between 7.45 and 6.74 ppm (Fig. 5). The H1 proton coupled to the H5 proton and showed doublet peaks at 7.45–7.44 ppm. The H5 proton coupled to the H4 and H1 proton and observed doublet of doublet peaks at 7.01–6.98 ppm. The H4 proton coupled to the H5 proton and detected doublet peaks at 6.76–6.74 ppm. The signal of imin (–CH=N) was observed as a singlet at 7.86 ppm. The proton signals of carbohydrazide regions occurred from $-N^1H$, $-N^2H$ and $-N^3H_2$. These amino peaks were observed as a singlet at 9.53, 11.06 and 4.75 ppm, respectively.

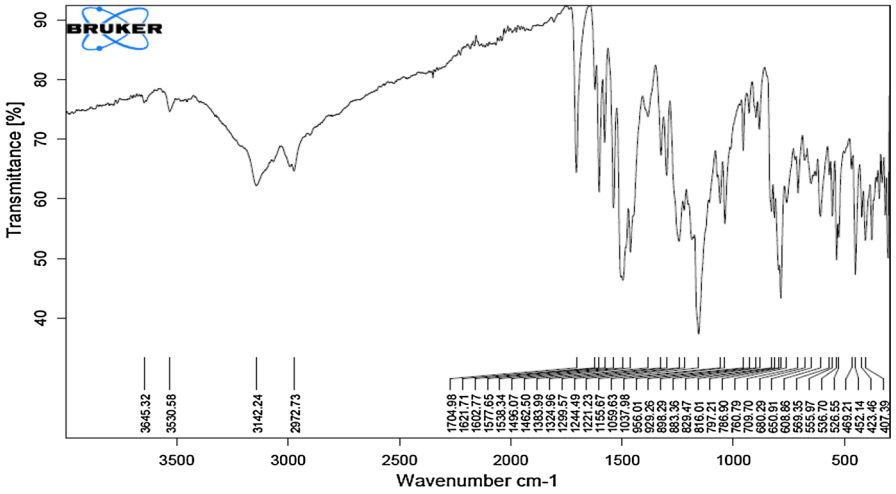


Fig. 3 FT-IR spectrum of compound **7**

Table 3 Characteristic IR bands for the compounds (cm^{-1})

Comp.	$\nu^{1}_{N\text{-}H}$	$\nu^{2}_{N\text{-}H}$	$\nu^{3}_{N\text{-}H}$	$\nu_{C\text{–}H\ (Ar.)}$	$\nu_{C=S}$	$\nu_{C=N}$	$\nu_{C\text{–}N}$	ν_{OH}	ν_{NH} (ist)	$\nu_{C\text{–}Cl}$	$\nu_{C=O}$ (ist)
Exp.											
3	3183	3295	3145 3120	3030	1386	1590	1168	3315	–	–	–
4	3290	3315	*	3001	1367	1584	1176	3233	–	–	–
6	3477	3595	–	3035	1379	1586	1199	–	3123	817	1698
7	3530	3645	–	3035	1384	1578	1221	3142	3187	883	1704
8	3315	3423	–	3035	1382	1580	1203	3274	3083	883	1703
9	3374	3481	–	2983	1383	1584	1199	3286	3129	883	1697
10	3421	3584	–	2993	1364	1597	1185	3144	3183	882	1698

ist Isatin

*NH band is under OH band

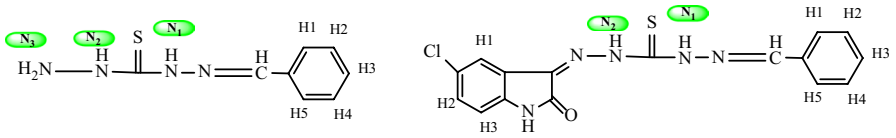


Fig. 4 General scheme for ^{1}H NMR spectra interpretations

The hydroxyl group (–OH) proton signals were detected as a singlet at 9.15 ppm. The methyl group ($–CH_3$) proton signals were detected as a triplet in the range of 1.33–1.29 ppm. The methylene group ($–OCH_2$) proton signals were detected as a quartet in the range of 4.09–4.04 ppm. In compound **7**, the aromatic proton

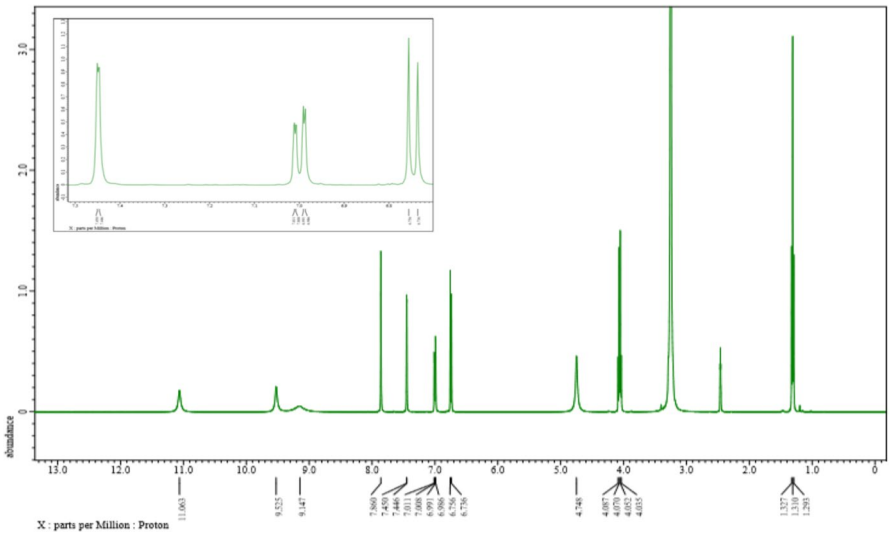


Fig. 5 [1]H NMR spectrum of compound **3**

signals of aryl ring (H1, H4 and H5) were observed between 7.30 and 6.95 ppm (Fig. 6). The H1 proton showed a singlet peaks at 7.30–7.29 ppm. The H4 proton coupled to the H5 proton and observed doublet peaks at 7.24–7.22 ppm. The H5 proton coupled to the H4 proton and detected doublet peaks at 6.97–6.95 ppm. The signal of imin (–CH=N) was observed as a singlet at 8.04 ppm. The proton

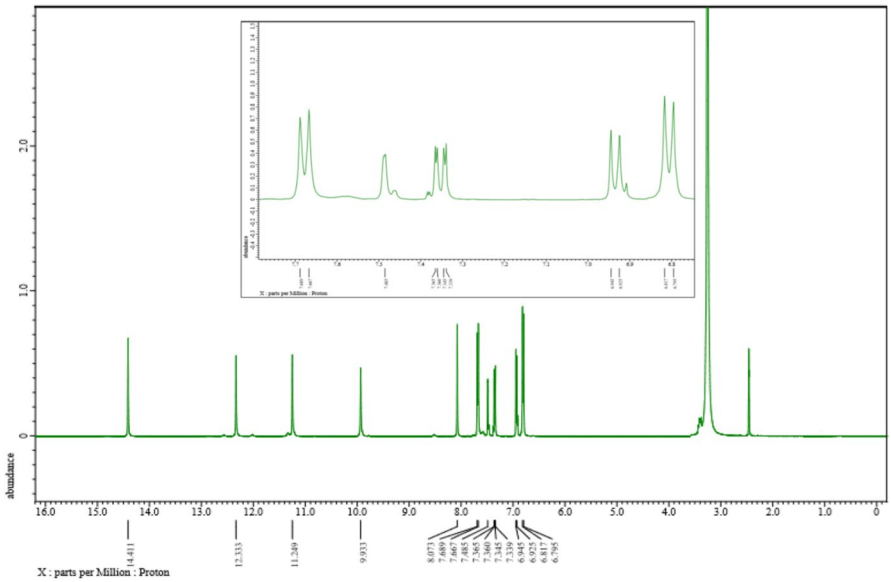


Fig. 6 [1]H NMR spectrum of compound **7**

signals of carbohydrazide regions occurred from $–N^1H$ and $–N^2H$. These amino peaks were observed as a singlet at 11.26 and 12.34 ppm, respectively. The hydroxyl group (–OH) proton signals were detected as a singlet at 8.95 ppm. The methoxy group ($–OCH_3$) proton signals were detected as a singlet at 3.81 ppm. The amino (–NH) proton signals of isatin region were detected as a singlet in the range of 14.40 ppm. The aromatic proton signals of isatin ring (H1, H2 and H3) were observed between 7.49 and 6.91 ppm. The H1 proton coupled to the H2 proton and showed doublet peaks at 7.49–7.47 ppm. The H2 proton coupled to the H3 and H1 proton and observed doublet of doublet peaks at 7.37–7.34 ppm. The H3 proton coupled to the H2 proton and detected doublet peaks at 6.93–6.91 ppm. 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 [32]. These data are consistent with the values of earlier reported for similar compounds [3, 17, 31, 33]. The proton chemical shift values of the synthesized compounds are given in Table 4.

^{13}C NMR spectra

The ^{13}C NMR spectra of all compounds were obtained in DMSO-d_6 and showed general scheme for ^{13}C NMR spectra interpretations in Fig. 7. Also, the chemical shifts are shown in Table 5. The ^{13}C NMR spectrum of the compound **3** showed ten different resonances in good agreement with the proposed structure as shown in Fig. 8. In compound **3**, methyl substituted aromatic carbon ($–CH_3$) was observed at 15.5 ppm, $–OCH_2$ signal was observed at 64.8 ppm, and –C=S signal of carbohydrazide region was detected at 177.2 ppm. The characteristic –CH=N (imin) peak was observed at 143.6 ppm. The aromatic carbons (C1–C6) were also observed at 126.3, 112.1, 149.8, 147.8, 116.2 and 123.0 ppm, respectively. The resonances of the C3 and C4 carbon atoms shifted downfield due to the presence of electron-withdrawing groups $–OC_2H_5$ and –OH, respectively. The ^{13}C NMR spectrum of the compound **7** showed 17 different resonances in good agreement with the proposed structure in Fig. 9. In compound **7**, methyl substituted aromatic carbon ($–OCH_3$) was observed at 56.1 ppm, and –C=S signal of carbohydrazide region was detected at 175.6 ppm. The characteristic –CH=N (imin) peak was observed at 147.4 ppm. The carbonyl atom (–C=O) of isatin region was observed at 163.0 ppm. The –C=N atom of isatin ring was appeared at 131.1 ppm. The aromatic carbons (C1–C6) of aryl region were also observed at 127.0, 112.6, 145.8, 150.9, 113.0 and 126.8 ppm, respectively. The resonances of the C3 and C4 carbon atoms shifted downfield due to the presence of electron-withdrawing groups –OH and $–OCH_3$, respectively. The aromatic carbons (C1–C6) of isatin region were also observed at 137.1, 121.1, 122.5, 141.5, 114.5 and 132.0 ppm, respectively. The resonances of the C1 and C4 carbon atoms shifted downfield due to the presence of electron-withdrawing groups –Cl and –NH, respectively. These spectroscopic data are consistent with the values of previously reported similar compounds and the literature [3, 17, 31, 33]. The carbon chemical shift values of the synthesized compounds are given in Table 5.

Table 4 ^{1}H NMR (δ, ppm, in DMSO-d_6) values related to synthesized compounds

Comp.	–CH=N	–N^{2}H–N	=N–N^{1}H	–N^{3}H$_2$	–Ar H	–OH	–CH$_2$	–CH$_3$	–OCH$_3$	İsatin ArH	İsatin NH
Exp.											
3	7.86	11.06	9.53	4.75	7.45–7.44 (1H) 7.01–6.98 (1H) 6.76–6.74 (1H)	9.15	4.09–4.04	1.33–1.29	x	x	x
4	7.86	11.04	9.59	4.77	7.03 (2H)	8.60	x	x	3.77	x	x
6	8.17	12.49	11.27	x	7.46–7.34 (1H) 6.94–6.92 (4H)	x	x	x	x	7.85–7.80 (2H) 7.49 (1H)	14.48
7	8.07	12.33	11.25	x	7.69–7.67 (2H) 6.82–6.80 (2H)	9.93	x	x	x	7.49 (1H) 7.36–7.34 (1H) 6.95–6.93 (1H)	14.41
8	8.03	12.36	11.18	x	7.59 (1H) 7.11–7.08 (1H) 6.81–6.79 (1H)	9.39	4.18–4.1	1.39–1.35	x	7.49–7.48 (1H) 7.37–7.34 (1H) 6.95–6.93 (1H)	14.46
9	8.03	12.45	11.22	x	7.18 (2H)	8.81	x	x	3.84	7.49–7.48 (1H) 7.37–7.34 (1H) 6.94–6.92 (1H)	14.51
10	8.03	12.25	11.19	x	7.64–7.62 (2H) 6.70–6.68 (2H)	x	x	2.93	x	7.48 (1H) 7.36–7.34 (1H) 6.95–6.89 (1H)	14.36

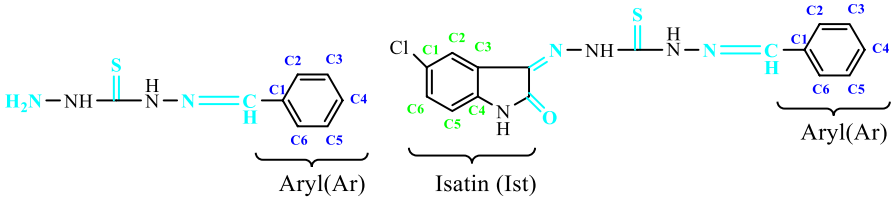


Fig. 7 General scheme for ^{13}C NMR spectra interpretations

Antioxidant activity assays

It may be assumed that the antioxidant activities of the molecules examined are related to their hydrogen losing capacity/abilities, that is, structurally stable radical formation after the compounds interact with the DPPH free radical [34]. In this study, DPPH radical scavenging method was applied and gallic acid which is a water-soluble antioxidant is used as standard. Absorbance against different concentrations of DPPH was graphed before starting the study, and the calibration equation obtained from the graph was found to be $y = 7.58 \times 10^3$c-0.01 ($R^2 = 0.989$). This calibration equation was used to control calculated percent inhibition of gallic acid and synthesized compounds.

The products synthesized in this study were named as monosubstituted and disubstituted product. Firstly, the Schiff bases we created with aldehyde derivatives were given as monosubstituted products. The percent change in the antioxidant inhibition concentration of the compounds obtained is given in Fig. 10a for monosubstituted products (compounds **1**–**5**). All compounds showed a steady increase in direct proportion to the concentration increase. Compounds **3** and **4** of the monosubstituted products exhibited the highest percentages of inhibition.

The disubstituted products formed by monosubstituted products with 5-chloroisatin were compared among themselves in Fig. 10B (disubstituted products **6**–**10**). As with the monosubstituted products, the increase in concentration and percentage inhibition is regular for the end products **1**, **2**, **3** and **4**. Compounds **3**, **4** and **5** showed a higher percent inhibition increase with increasing concentration. These results show that, similar to that of Bozic et al., monosubstituent-containing thiocarbohydrazone molecules are more active than the corresponding disubstituted thiocarbohydrazone derivatives in the DPPH radical scavenging process; this indicates the importance of the –NH–NH_2 group to the higher efficacy of antioxidant activities [34].

The IC_{50} value is a commonly used parameter for measuring antioxidant activity and refers to the antiradical activity required to reduce the DPPH concentration by 50% [32]. In this study, linear regression equations with DPPH method and IC_{50} values are summarized in Table 6 with monosubstituted products **1**–**5** and disubstituted products **6**–**10** and with DPPH method. In addition, gallic acid calibration equation was obtained in order to compare the free radical scavenging effects of gallic acid and synthesized molecules.

Table 5 ^{13}C NMR (δ, ppm, in DMSO-d_6) values related to synthesized compounds

Comp.	C=S	–CH=N	Ar C1	Ar C2	Ar C3	Ar C4	Ar C5	Ar C6	CH_2	CH_3	C=N	C=O	Ist C1	Ist C2	Ist C3	Ist C4	Ist C5	Ist C6
Exp.																		
3	177.2	143.6	126.3	112.1	149.8	147.8	116.2	123.0	64.8	15.5	x	x	x	x	x	x	x	x
4	176.5	138.4	125.1	106.3	148.5	143.9	148.5	106.3	x	56.5	x	x	x	x	x	x	x	x
6	176.2	141.5	134.3	129.6	128.1	127.0	128.1	129.6	x	x	145.6	163.1	132.1	120.6	122.4	137.3	113.1	131.2
7	176.0	141.3	120.7	128.1	129.4	145.3	129.4	128.1	x	x	137.3	162.9	132.0	122.4	127.3	134.1	113.1	131.2
8	175.5	141.2	123.8	113.1	147.9	150.4	115.9	125.5	64.2	14.8	127.2	162.9	136.8	120.6	122.5	145.6	110.7	131.0
9	175.5	149.1	124.6	106.1	149.1	139.3	145.5	106.1	x	56.6	127.2	162.8	136.8	120.6	122.6	141.3	113.3	131.1
10	175.5	145.5	130.9	124.2	106.0	148.7	106.0	124.2	x	56.3	136.8	162.9	139.3	120.6	122.3	141.0	112.9	127.0

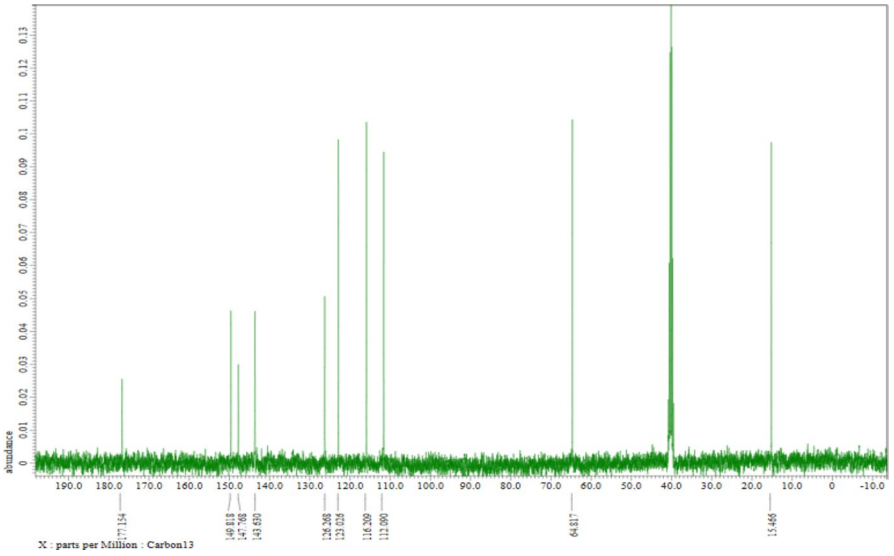


Fig. 8 ^{13}C NMR spectrum of compound **3**

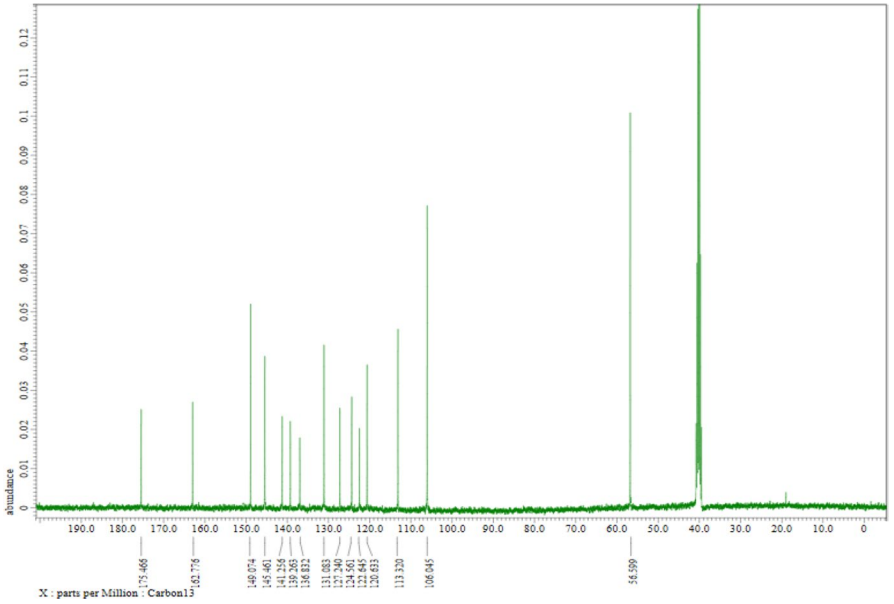


Fig. 9 ^{13}C NMR spectrum of compound **9**

IC_{50} values calculated by DPPH method were found to be IC_{50}: 9773 μM for gallic acid. Accordingly, IC_{50} values for monosubstituted products followed the order of **5 > 1 > 2 > 3 > 4**. This order was found as **6 > 7 > 9 > 8 > 10** for the

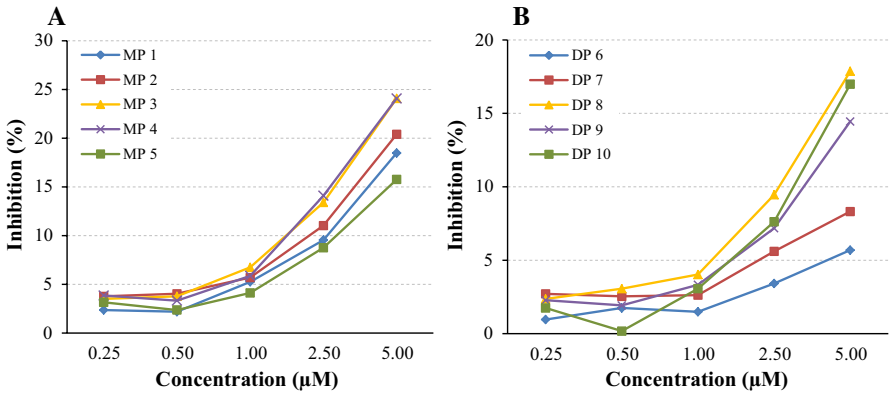


Fig. 10 Percent inhibition of radical scavenging activity calculated by the DPPH method for the monosubstituted products (MP 1–5) (**a**) and disubstituted products (DP 6–10) (**b**) at different concentrations

Table 6 The linear regression equation and IC_{50} values of gallic acid, the compounds **1–10** by DPPH method

Compounds	Concentration equation (0.25–5.0) × 10^{-6} M	R^2	IC_{50} (μM)
Gallic acid	$y = 4.975x + 1.374$	0.999	9.773
Monosubstituted products			
1	$y = 3.443x + 1.195$	0.992	14.173
2	$y = 3.569x + 2.382$	0.997	13.343
3	$y = 4.411x + 2.138$	0.998	10.852
4	$y = 4.490x + 1.938$	0.989	10.704
5	$y = 2.823x + 1.609$	0.987	17.145
Disubstituted products			
6	$y = 0.969x + 0.868$	0.971	50.677
7	$y = 1.284x + 1.985$	0.961	37.386
8	$y = 3.306x + 1.240$	0.997	14.750
9	$y = 2.661x + 0.909$	0.990	18.450
10	$y = 3.419x - 0.405$	0.976	14.744

disubstituted products. When IC_{50} values are taken into consideration, it can be said that if the value is smaller, then the antioxidant activity is greater. This means it can scavenging the same amount of free radicals even at low concentrations, and these substances show stronger antioxidant activity. According to these results, compound **4** showed the highest antioxidant activity among monosubstituted and compound **10** showed the highest antioxidant activity among the disubstituted products. In addition, compounds **8** and **9** showed higher antioxidant activity than other disubstituted compounds, as in monosubstituent analogs. This result confirms that the electron-donating methoxy substituent in compounds containing phenolic structure, as mentioned in previous studies, increases

the stability and thus antioxidant activity of the radical [35–37]. None of these compounds showed antioxidant activity as high as gallic acid which is a natural antioxidant.

Conclusion

We have synthesized seven new Schiff bases bearing isatin-thiocarbohydrazone in the presence of ethanol under reflux: Two are monosubstituted thiocarbohydrazone products (3–4) and five are disubstituted thiocarbohydrazone products (6–10). They were synthesized with good yields of 51–91%. All the products were characterized by ^{1}H NMR, IR and elemental analyses. The in vitro antioxidant features of all the compounds were evaluated by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging method. Compounds **3** and **4** of the monosubstituted products exhibited the highest percentages of inhibition.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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