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Preparation, Characterization, and Antioxidant Features of Some New Schiff Bases Derived from Isatins and Hydrazine

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ABSTRACT: New isatins based on Schiff bases were prepared from hydrazine, 5-substituted isatins, and aldehydes under reflux in ethanol. The structures of the synthesized compounds were elucidated by using IR, ^{13}C NMR, ^{1}H NMR spectroscopy and elemental analysis. All the compounds were tested *in vitro* antioxidant activity with free radical scavenging method using by 1,1-diphenyl-2-picryl hydrazyl (DPPH). The results were given with calculated IC_{50} values.

Keywords: Schiff bases, isatin, DPPH, antioxidant features, spectroscopic method.

Hidrazin ve Isatinlerden Elde Edilen Bazı Yeni Schiff Bazlarının Sentezi, Karakterizasyonu ve Antioksidan Özellikleri

ÖZET: Yeni schiff bazlı isatinler, hidrazin, 5-sübstitütentli isatinler ve çeşitli aldehitlerin etil alkolde geri soğutucu altındaki tepkimesi sonucu elde edilmiştir. Sentezlenen bileşiklerin yapısı IR, ^{13}C NMR, ^{1}H NMR spektroskopik veriler ve elementel analiz sonuçlar ile aydınlatılmıştır. Sentezlenen bileşiklerin antioksidan aktivite testleri 2,2-difenil-1-pikril hidrazil (DPPH) radikal giderme yöntemi ile yapılmıştır. Sonuçlar, hesaplanan IC_{50} (yarı maksimum inhibitör derişim) değerleri ile verilmiştir.

Anahtar Kelimeler: Schiff bazı, isatin, DPPH, antioksidan özellikler, spektroskopik yöntem.

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INTRODUCTION

Isatins are a significant group of organic compounds owing to their importance potential application in medicinal chemistry and biological activity such as anti-bacterial (Chohan et al., 2004), anti-viral (Jarrahpour et al., 2007; Abbas et al., 2013), anti-fungal (Sinha et al., 2008), anti-oxidant (Andreani et al., 2010; Naik et al., 2011; Premanathan et al., 2012; Kiran et al., 2013) anti-convulsant (Sridhar et al., 2002), anti-tubercular (Aboul-Fadl and Bin-Jubair, 2010; Liang et al., 2014; Gabr et al., 2017), anti-HIV (Pandeya et al., 2000; Bal et al., 2005).

Schiff bases of bearing isatin are known to have a wide range of pharmacological and biological properties. They were reported as anti-convulsant (Verma et al., 2004), anti-bacterial (Chohan et al., 2004; Jarrahpour et al., 2007), anti-viral (Jarrahpour et al., 2007; Abbas et al., 2013), anti-oxidant (Kiran et al., 2012; Bakır et al., 2018) , anti-HIV (Pandeya et al., 2000; Bal et al., 2005) and anti-fungal activity (Pandeya et al., 2000; Chohan et al., 2004; Jarrahpour et al., 2007).

We studied that new isatins based on Schiff bases were prepared two stages. First, hydrazine and 5-substituted isatins react with to give intermediate product under reflux in ethanol. Second, intermediate product and aldehydes react with to give isatins based on Schiff bases in the presence of a drop HCl under reflux in ethanol. They were determined by using IR, ^{1}H NMR, ^{13}C NMR spectroscopy, elemental analysis. All the compounds were tested by 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical trapping method for *in vitro* antioxidant activity. The results were given with calculated IC_{50} values.

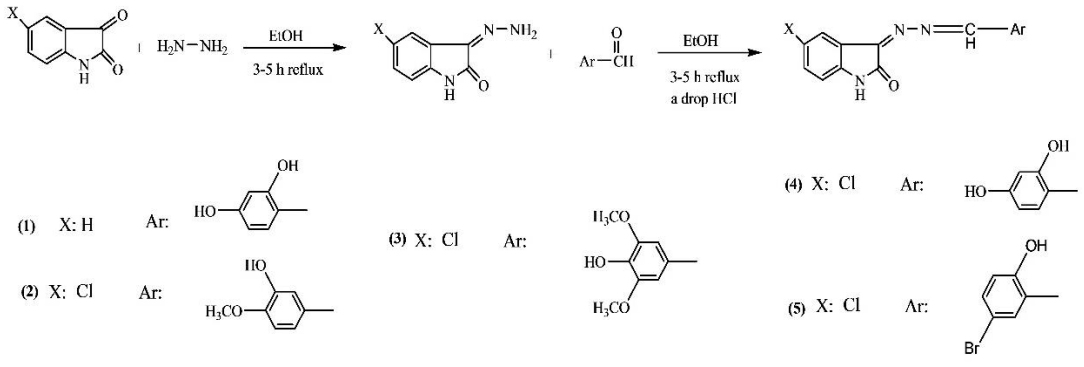
MATERIALS AND METHODS

Measurement and Reagents

All reagents and solvents were used without further purification and purchased from Aldrich, Sigma, or Merck Chemical Company. The solvents were spectroscopic grade. The elemental analysis was measured on Eurovector EA3000-Single. Melting points were determined using Stuart Melting Point 30 apparatus. IR spectra were taken on the Bruker Alpha FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were taken on JEOL ECX-400 (400 MHz) in DMSO-d_6 spectrophotometer. Absorption measurements were recorded with SHIMADZU UV Pharmaspec 1700 spectrophotometer.

Synthesis 5-Substituted Isatin-Hydrazones Based on Schiff Bases (1-5)

5-Substituted isatins (5 mmol) and hydrazine (5 mmol) in ethanol-water mixture (3:1, 40 mL) were stirred and refluxed for 3-5 h. Then, the reaction mixture reacts with equimolar amount of various aldehydes in a drop HCl as catalyst in the presence of ethanol (40 mL) for 3-5 h. After finishing reaction, the mixture was filtered and washed with ethanol. The formed solid was isolated and dried to give the products **1-5** as shown in Scheme 1.



Scheme 1. General synthesis of 5-substituted isatin based on Schiff bases

Antioxidant Activity

DPPH was used in ethanol at a concentration of 55 μM so as to evaluate the antioxidant activity of the synthesized compounds. Stock solutions of the compounds were prepared in DMSO to 250 μM. To the previously prepared (4 mL) DPPH solution were added compound solutions of different concentrations (0.25, 0.50, 1.00, 2.50, 5.00 μM) and enough ethanol to a total of 5 mL. This mixture could stand in a dark room at room temperature for 30 minutes and then read at 517 nm against a blank (Blois 1958; Manjula et al., 2015).

The percentage inhibition of the free radical concentration for the sample compounds was calculated and compared to the standard Trolox. Radical scavenging activity was expressed as a percentage of inhibition and calculated using formulas:

Radical scavenging activity (%) = $[(A_o-A_1) / A_o \times 100]$

Where A_o is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound (Naik et al., 2011; Meral and Doğan, 2012).

In addition, IC_{50} values were calculated from the calibration curve. The IC_{50} value is defined as the concentration of the test compound required to obtain half maximum inhibition, and the low IC_{50} value indicates more antioxidant activity (Frankel et al., 2000).

RESULTS AND DISCUSSION

Physical Data

All the synthesized compounds were new. The current experimental results for the physical data, melting points, yields, and elemental analyses are summarized in Tables 1 and 2.

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

Compounds	Mol. Weight (g mol^{-1})	Solubility	Melting Point (ºC)	Yields (%)
1	329.74	DMSO (+)	>350	62
2	359.77	DMSO (+)	258-259	80
3	281.27	DMSO (+)	276-278	60
4	315.72	DMSO (+)	>350	65
5	378.61	DMSO (+)	293-295	90

Table 2. The results for elemental analysis for the synthesized compounds

Compounds	Calculated			Experimental		
	N %	C %	H %	(N) %	(C) %	(H) %
1	12.74	58.28	3.67	12.62	57.66	3.56
2	11.68	56.76	3.92	10.91	55.56	3.75
3	14.94	64.05	3.94	13.98	64.20	3.79
4	13.31	57.07	3.19	13.21	56.28	2.98
5	12.21	52.35	2.93	11.85	51.65	2.78

Vibrational Frequencies

At the end of the reaction, the aldehyde group (-CHO) signal was not observed near 2750-2650 cm^{-1}. Also, the asymmetric and symmetric stretching bands of the amino group ($-NH_2$) were not appeared at 3600–3200 cm^{-1}. These results indicated a successful reaction as expected. In compound **2**, the -OH peak of aryl region was observed at 3357 cm^{-1}, the -NH stretching vibration of isatin ring was observed at 3237 cm^{-1}; the C=O signal of isatin ring was observed at 1721 cm^{-1}, the -C=N stretching vibration was appeared at 1591 cm^{-1}; the -C-N stretching vibration was observed at 1179 cm^{-1}; the -C-O signal of aryl ring was observed at 1068 cm^{-1}, the -C-Cl signal was observed at 894 cm^{-1} as shown in

Figure 1. Furthermore, IR peaks of the compounds are given in the Table 3. These frequency values of the synthesized compounds are agreement with the similar compounds (Pandeya et al., 2000; Jarrahpour et al., 2007; Bekircan and Bektas, 2008).

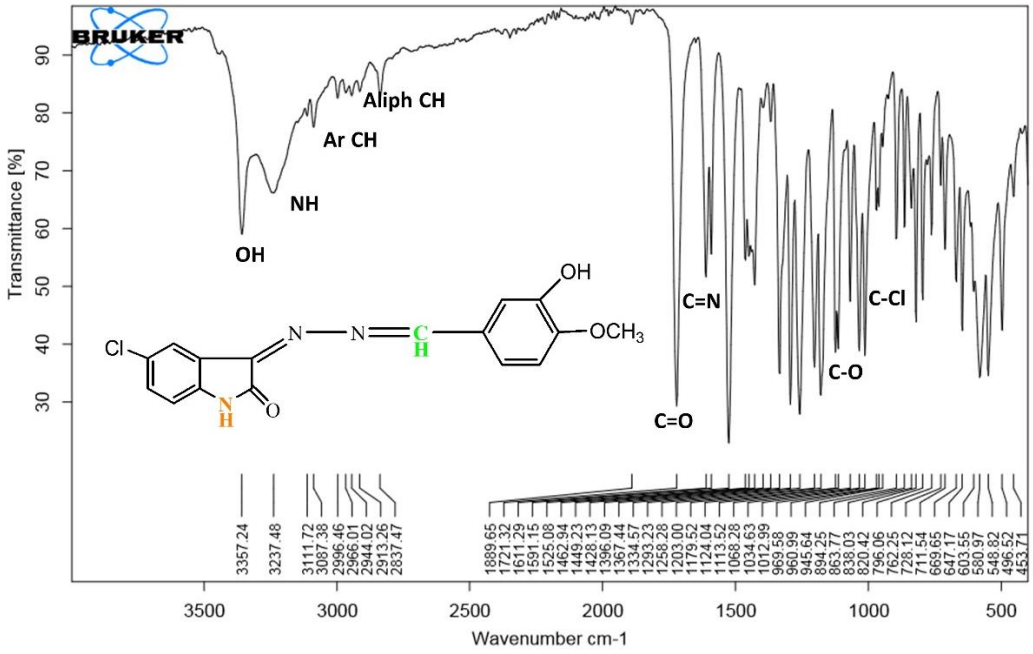


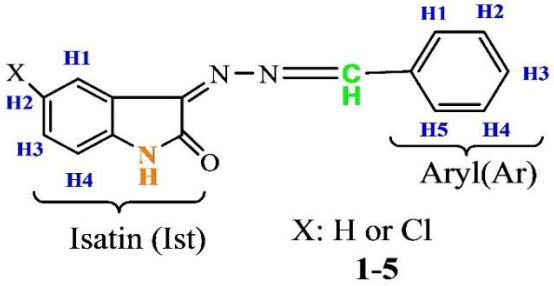
Figure 1. FT-IR spectrum of compound **2**

Table 3. Experimental FT-IR values of the compounds (cm^{-1})

Comp.	-OH	-NH	Ar CH	Aliph CH	C=O	C=N	C-O	C-Cl
1	3312	3241	3082-3014	2976-2856	1713	1542	1081	887
2	3357	3237	3111-3087	2966-2837	1721	1591	1068	894
3	3302	3242	3096-3007	2972-2844	1738	1532	1074	893
4	3332	3136	3078-3012	2966-2842	1720	1571	1073	857
5	3313	3239	3092-3016	2981-2869	1734	1616	1071	895

^{1}H NMR Spectral Interpretation

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 spectral interpretations in Scheme 2.



Scheme 2. General scheme for ^{1}H NMR spectral interpretations

For compound **5**, the H5 aromatic proton signal of aryl ring was observed as a singlet at 7.76 ppm. The H3 proton of aryl ring coupled to the H2 and H5 proton and observed doublet of doublets peaks at

6.88 ppm. The H2 proton of aryl ring coupled to the H3 proton and showed doublet peaks at 6.90 ppm. The signal of imin (-CH=N) was observed as a singlet at 8.89 ppm. The -NH signal of isatin was observed as a singlet at 11.09 ppm. The -OH signal of aryl ring was observed as a singlet at 7.85 ppm. The aromatic protons signals of isatin ring (H1-H4) were observed between 7.17 and 7.49 ppm. The H1 proton of isatin was observed singlet peak at 7.17 ppm. The H3 proton of isatin coupled to the H1 and H4 proton and observed doublet of doublets peaks at 7.49 ppm. The H4 proton of isatin coupled to the H3 proton and showed doublet peaks at 7.44 ppm as shown in Figure 2. DMSO-d_6 and water in DMSO (HOD, H2O) signals are shown around at 2.00, 2.50 (quintet) and 3.30 (variable, based on the solvent and its concentration) ppm, respectively (Fleming and Williams, 1966). These data are consistent with the values of earlier reported for similar compounds (Pandeya et al., 2000; Jarrahpour et al., 2007; Bekircan and Bektas, 2008). Proton chemical shift values of the synthesized compounds are given in the Table 4.

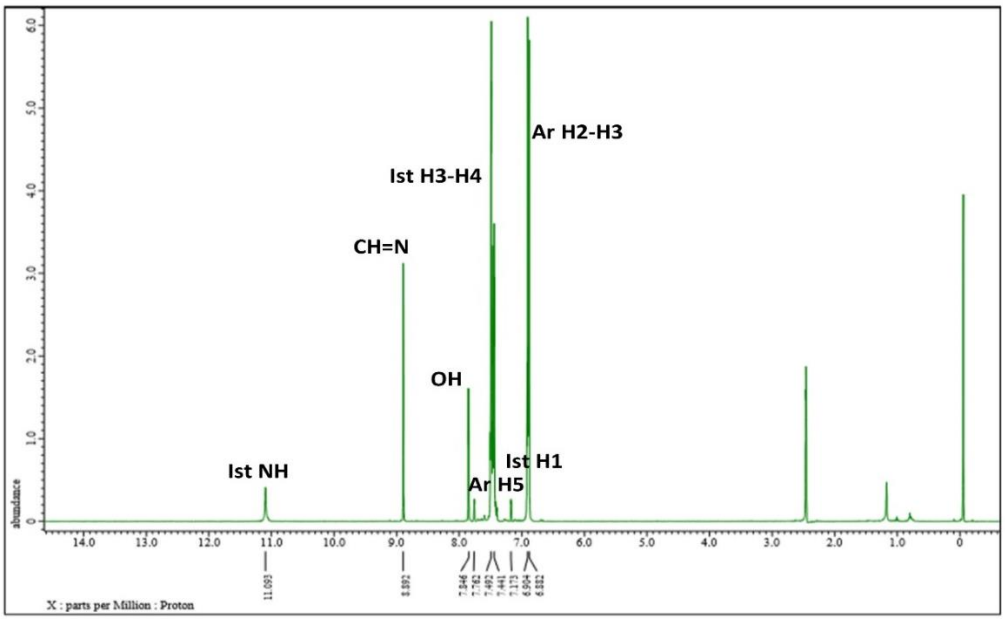


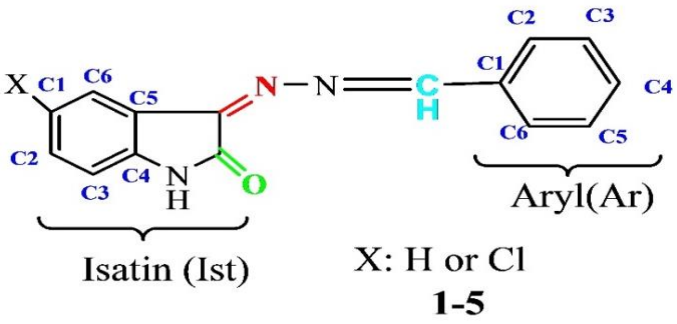
Figure 2. ^{1}H NMR spectrum of compound **5**

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

Comp.	CH=N	Ar H1	Ar H2	Ar H3	Ar H4	Ar H5	Ist NH	Ist H1	Ist H2	Ist H3	Ist H4	-OH	OCH_3
1	9.88	-	8.88	-	7.73	8.02	10.97	6.99	6.87	6.40	7.46	10.89 10.78	-
2	8.76	6.87	-	-	7.08	7.40	9.72	8.55	-	7.34	7.96	7.86	3.83
3	8.59	8.10	-	-	-	8.10	11.10	7.27	-	6.88	7.45	7.75	3.83
4	9.87	-	7.38	-	6.88	6.45	10.97	6.84	-	7.46	7.68	8.86 8.01	-
5	8.89	-	6.90	6.88	-	7.76	11.09	7.17	-	7.49	7.44	7.85	-

^{13}C NMR Spectral Interpretation

The ^{13}C NMR spectra of the synthesized compounds were detected in DMSO-d_6 as solvent and showed general scheme the spectral interpretations in Scheme 3.



Scheme 3. General scheme for ^{13}C NMR spectral interpretations

For compound **2**, the -C=O signal of isatin region was detected at 165.0 ppm. The characteristic -CH=N (imin) peak was observed at 144.1 ppm. The characteristic -C=N peak of isatin ring was observed at 150.4 ppm. The methoxy (-OCH_3) peak was detected at 56.3 ppm as shown in Figure 3. The aromatic carbons (C1-C6) of aryl ring were observed at 126.5, 112.9, 164.6, 152.6, 112.6, and 128.5 ppm, respectively. The resonances of the C3 and C4 carbon atoms shifted down-field due to the presence of electron-withdrawing groups –OCH_3 and -OH, respectively. The aromatic carbons (C1-C6) of isatin region were also observed at 147.6, 124.1, 126.3, 133.3, 118.3, and 114.2 ppm, respectively. The resonances of the C1 and C4 carbon atoms shifted down-field due to the presence of electron-withdrawing groups -Cl and -NH, respectively. These data are consistent with the values of earlier reported for similar compounds (Jarrahpour et al., 2007; Bekircan and Bektas, 2008; Özkınalı et al., 2018). The carbon chemical shift values of the synthesized compounds are given in the Table 5.

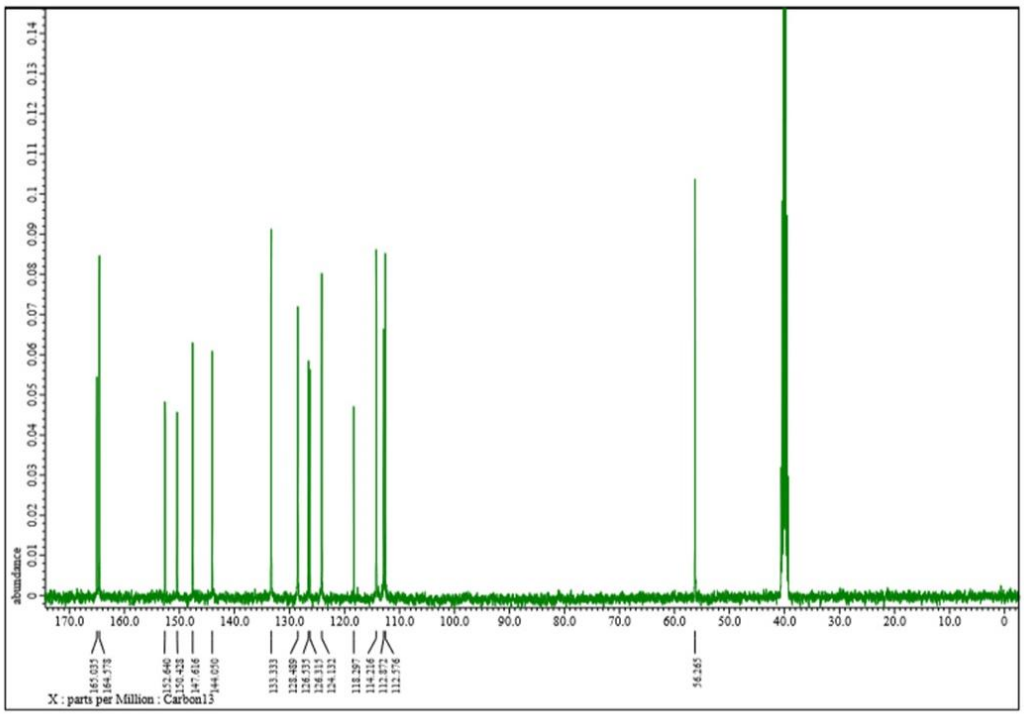


Figure 3. ^{13}C NMR spectrum of compound **2**

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

Comp.	Ist C1	Ist C2	Ist C3	Ist C4	Ist C5	Ist C6	Ist C=N	Ist C=O	CH=N	Ar C1	Ar C2	Ar C3	Ar C4	Ar C5	Ar C6
1	145.1	131.9	128.7	134.9	111.8	116.1	150.8	165.5	145.4	134.0	163.9	102.8	161.6	109.5	123.2
2	147.6	124.1	126.3	133.3	118.3	114.2	150.4	165.0	144.1	126.5	112.9	164.6	152.6	112.6	128.5
3	140.9	126.6	128.5	134.7	117.5	124.2	146.3	165.5	144.8	133.3	106.8	164.7	148.6	163.7	113.2
4	137.8	127.8	126.1	131.8	112.8	111.6	149.9	165.3	143.9	133.0	164.6	103.1	161.9	109.7	118.3
5	136.0	128.5	126.6	134.5	113.2	111.1	146.3	163.8	144.8	132.1	161.3	119.5	117.6	158.2	120.9

Evaluation of Antioxidant Activity

Many biochemical reactions involve the production of reactive oxygen species (ROS) that cause cell damage and are controlled by the antioxidant defense system in the body (Kiran et al., 2012). This situation is protected by many antioxidant molecules and antioxidant enzymes. In recent years, many studies have shown that isatin derivative molecules exhibit synthetic antioxidant properties (Kiran et al., 2012; Andreani et al., 2010).

An antioxidant is defined as any substance that will delay or prevent the oxidation of the substrate, even when present at much lower concentrations than the oxidizable substrate (Wanasundara and Shahidi, 2005; Brewer, 2011; Shahidi, 2015; Uruk and Kahraman, 2017). In this study, IC_{50} values were calculated at the end of DPPH analysis for the synthesized compounds and Trolox as shown in Table 6. The IC_{50} values of all test compounds were between 21.78 and 24.82 μM. Although compounds **1** and **2** had a slightly stronger antioxidant activity against DPPH radicals, the others exhibited very low activity compared to a strong H donor such as Trolox in Figure 4. Compound **2** had the strongest antioxidant activity among the synthesized compounds.

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

Compounds	DPPH activity IC_{50} (μM)
1	21.83
2	21.78
3	24.82
4	24.44
5	24.64
Trolox	16.09

Kiran and co-workers studied antioxidant properties of isatin based on Schiff bases and IC_{50} values were measured between 26.56 and 57.40 μM with DPPH method (Kiran et al., 2012). These values exhibited antioxidant activity lower than that of the present studies. The other study of Kiran and co-workers antioxidant properties of isatin derivatives and IC_{50} values were measured at between 20.56 and 58.70 μM with DPPH method (Kiran et al., 2013). In this study, IC_{50} values were measured between 21.78 and 24.82 μM with DPPH method. The results of this work showed that the synthesized compounds are remarkably scavenged by the DPPH free radical. These values corroborate the values of formerly reported similar compounds.

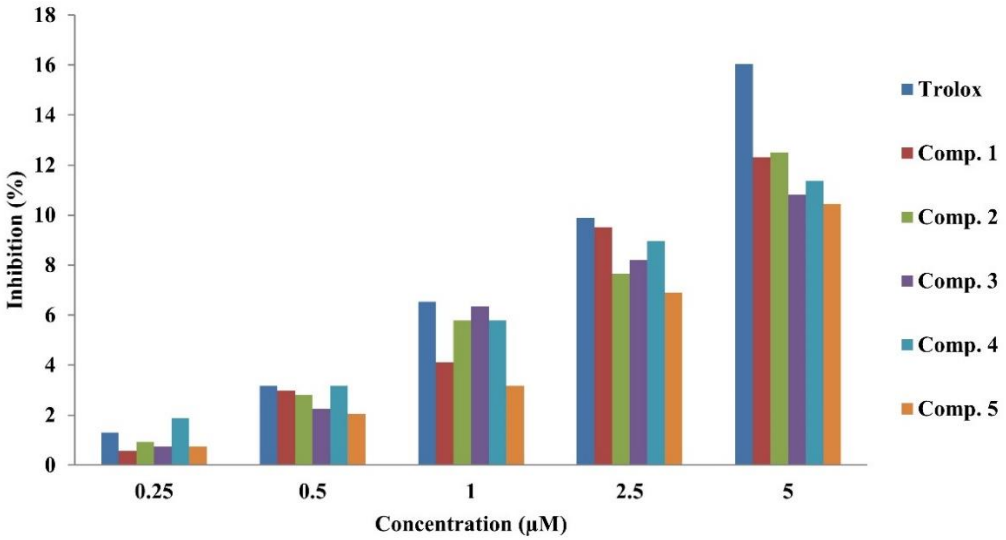


Figure 4. Variation of percent inhibition calculated by the DPPH method for Trolox and the compounds at different concentrations.

CONCLUSION

Some new isatin-hydrazones based on Schiff bases were synthesized with good yields of 60-90%. All the compounds were elucidated by ^{1}H NMR, ^{13}C NMR, IR spectroscopy and elemental analyses. The *in vitro* antioxidant properties of the synthesized compounds were measured by the DPPH free radical trapping method and compared to the standard Trolox. Although compound **2** had the strongest antioxidant activity among the synthesized compounds, it had a lower antioxidant activity against DPPH radicals according to the standard Trolox.

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