Research on Chemical Intermediates
https://doi.org/10.1007/s11164-020-04185-w

Novel Schiff bases derived from isothiocyanates: synthesis, characterization, and antioxidant activity

Hasan Yakan[1]

Received: 8 April 2020 / Accepted: 11 June 2020

Abstract

A series of novel thiosemicarbazones including Schiff bases were synthesized by treatment of various aryl-substituted aldehydes with thiosemicarbazides in ethanol containing one drop of hydrochloric acid at reflux for 3–5 h. For this, thiosemicarbazides were obtained from hydrazine monohydrate and isothiocyanates in cold dry ethanol at 0 °C for 1 h. FT-IR, ^{1}H NMR, ^{13}C NMR, and LC–MS/MS spectroscopic methods and elemental analysis were used to characterize the identification of the synthesized products. The in vitro antioxidant activity of these compounds was tested by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical trapping method. All of the synthesized compounds showed lower antioxidant activity than the ascorbic acid standard and followed the sequence **I** > **VII** > **X** > **VI** > **IV** > **IX** > **XI** > **II** > **V** > **III** > **VIII**.

Keywords Schiff bases · Isothiocyanates · Thiosemicarbohydrazides · Antioxidant activity · Spectroscopic elucidation

Introduction

Schiff bases are a significant class of organic and inorganic chemistry, which have –CH=N– bond. Schiff bases have shown an extensive spectrum of biological activities and medicinal properties such as antibacterial [1], antimicrobial [2], antifungal [3, 4], antioxidant [5, 6], antiviral [3, 7], anti-HIV [8], antitubercular [9], anticancer [10], antiinflammatory agents [11], and anticonvulsant [12] activity.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11164-020-04185-w) contains supplementary material, which is available to authorized users.

✉ Hasan Yakan
hasany@omu.edu.tr

[1] Department of Mathematics and Science Education, Faculty of Education, Ondokuz Mayıs University, Samsun, Turkey

Published online: 20 June 2020

Compounds with the structure of –NH–C(=S)NH–N= are known as thiosemicarbazones, which are important class of compounds in chemistry, biological, and pharmaceutical field. Thiosemicarbazones have showed biological and medicinal applications including antimicrobial [13], antifungal [14], antibacterial [14, 15], antioxidant [16–18], anti-HIV [19], anticonvulsant [13, 20], antiviral [21], antituberculosis [22], anticancer [23], enzymatic inhibition [14, 24], and cytotoxicity [13, 14] properties.

The importance of free radicals and reactive oxygen species (ROS) in the pathogenicity of diverse diseases such as inflammatory, metabolic disorders, cellular aging, reperfusion damage, and cancer has attracted considerable attention [25, 26]. Therefore, antioxidants have been reported to play an important role in protecting humans against many mortal diseases.

In view of these proofs, thiosemicarbazones including Schiff bases are significant which not only possess biological activities, but also in medicinal applications. In this paper, a new series of novel thiosemicarbazones including Schiff bases were synthesized. The chemical structure of the products was characterized by using methods FT-IR, ^{1}H NMR, ^{13}C NMR, and LC–MS/MS spectroscopy and elemental analysis. These compounds were performed antioxidant activity in vitro by using DPPH free radical scavenging method.

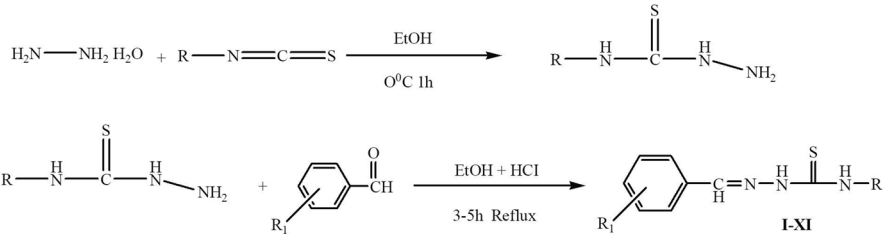
Experimental section

Instruments and reagents

All the chemical materials were bought in Sigma-Aldrich or Merck Chemical Company and were used without further purification. The solvent was spectroscopic grade. Melting points were measured using a Stuart SMP 30 melting point apparatus and were uncorrected. The elemental analysis was performed on a Eurovector EA3000-Single. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra (in DMSO-d_6) were taken at 25 °C using a Bruker Avance DPX-400 spectrometer operating at 400 MHz and 100 MHz, respectively. The splitting patterns are indicated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplied). Absorption measurements were recorded with a Shimadzu UVM-1240 UV–visible spectrophotometer. Liquid chromatography—tandem mass spectrometry (LC–MS/MS) measurements were recorded with a Shimadzu LCMS-8030 Plus spectrophotometer.

Synthesis of novel thiosemicarbazones including Schiff bases (I–XI)

Thiosemicarbazides (0.6 mmol), various aryl-substituted aldehydes (0.6 mmol), and one drop of HCl were added to aqueous EtOH (30 mL), and the mixture was refluxed at 78 °C for 3–5 h. The obtained precipitate was filtered and washed, and then, the formed colored solid was isolated and dried in air. The products were obtained good yields (63–83%) as shown in Scheme 1.



R	R_1	Compound
3-F-C_6H_5	3-OC_2H_5-4-OH	**I**
	3-OH-4-OCH_3	**II**
	2-OH-5-Br	**III**
	4-OH	**IV**
	2,4-diOH	**V**
2-F-C_6H_5	3-OC_2H_5-4-OH	**VI**
	3-OH-4-OCH_3	**VII**
	2-OH-5-Br	**VIII**
	3-OCH_3-4-OH	**IX**
-CH_3	3-OC_2H_5-4-OH	**X**
	3-OH-4-OCH_3	**XI**

Scheme 1 Synthetic path for the novel thiosemicarbazones including Schiff bases (**I**–**XI**)

Antioxidant activity measurements by using DPPH method

The DPPH method is a method showing that the ability of compounds to lose hydrogen and structurally stable radicals is associated with higher cleaning activity of the molecule.

Antioxidant activity determinations of compounds synthesized using DPPH free radical cleaning method were performed. For this, Brand-Williams et al. [27] method implementation was used with minor changes. The stock solutions of the synthesized compounds were prepared to be 250 µM in DMSO and mixed with 3 mL DPPH (250 µM) solution in different volumes (10–200 µL). Sufficient ethanol was added until the total solution volume was 5 mL. The prepared mixtures were kept in a dark room for 30 min and read against the blanks to compare with a sample without sample at 517 nm. Antioxidant activity was calculated using the equation shown below as a percentage of inhibition [28]:

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

Here, A_0 is the absorbance of the control (empty, without compound) and A_1 is the absorbance of the compound. With the help of the inhibition values obtained, IC_{50} (µM) values were calculated and used to evaluate antioxidant activity [29].

Results and discussion

Physical data

All the synthesized compounds are new. In Table 1, the physical data, melting points, yields, LC–MS/MS results, and elemental analysis of these compounds are presented.

Vibrational frequencies

In the FT-IR spectra, the signal of the aldehyde group (–CHO, two bands) of the starting material was not observed at 2780–2660 cm^{-1}. Moreover, the symmetric and asymmetric stretching bands of the amino group ($–NH_2$) did not show at 3520–3250 cm^{-1}. Instead, new absorptions for the –C=N stretching bands of imine group were observed at 1599–1522 cm^{-1}. These results indicated a successful reaction, as expected. For all compounds (**I**–**XI**), the –OH stretching vibrations were observed in the range of 3574–3376 cm^{-1}, the amino group (=N–NH and –NH–Ar) stretching vibrations were observed in the range of 3395–3284 and 3299–3116 cm^{-1}, aromatic and aliphatic C–H stretching vibrations were observed in the range of 3168–3017 and 2995–2962 cm^{-1}, –C=N characteristic absorptions of imine group were observed between 1595 and 1539 cm^{-1}, the –C=S signal of semicarbazide region was observed in the range of 1474–1352 cm^{-1}, the –C–N group absorptions were observed at 1333–1207 cm^{-1}, the –C–O stretching vibration was appeared in the range of 1222–1094 cm^{-1}, and the Ar–F stretching vibration was appeared at 1172–1044 cm^{-1}, except for compounds **X** and **XI**. Ar–Br absorption bands of compounds **III** and **IX** were observed at 629 and 591 cm^{-1} (see Figures S7 and S22 in supplementary material).

In compound **IV**, the –OH stretching vibration was observed at 3574 cm^{-1}, while the =N–NH (amino) stretching vibration was observed at 3308 cm^{-1}; the –NH–Ar (amino) stretching vibration was observed at 3145 cm^{-1}. The –C=N stretching vibration was observed at 1592 cm^{-1}; the –C=S signal of semicarbazide region was observed at 1434 cm^{-1}; the –C–N stretching vibration was appeared at 1277 cm^{-1}; the –C–O stretching vibration was appeared at 1220 cm^{-1}; the –C–F stretching vibration was appeared at 1067 cm^{-1} as shown in Fig. 1. These values provided significant proofs for the products formation. IR values of all compounds are listed in Table 2, and spectra of them are given in supplementary material. These observations are consistent with values published formerly for similar compounds [17, 18, 30, 31].

Interpretation of the ^{1}H NMR spectra

The ^{1}H NMR spectra of the synthesized compounds were detected in DMSO-d_6 as solvent. For compounds **I**–**IX**, the –NH peak (=N–NH) was observed as a singlet at 11.97–11.72 ppm, and the –NH signal (–NH–Ar) was detected as a singlet

Table 1 Physical data, LC–MS/MS results, and elemental analysis results of the novel thiosemicarbazones including Schiff bases

Comp	Mol. formula	Mol. weight (g/mol)	LC–MS/MS	m.p. (°C)	Yield %	Calculated			Experimental		
						C%	H%	N%	(C)%	(H)%	(N)%
I	$C_{16}H_{16}FN_3O_2S$	333.09	334.10 $[M+H]^+$	186–187	71	57.64	4.84	12.60	57.49	4.87	12.57
II	$C_{15}H_{14}FN_3O_2S$	319.08	320.05 $[M+H]^+$	174–175	63	56.41	4.42	13.16	56.18	4.37	13.11
III	$C_{14}H_{11}BrFN_3OS$	366.98	366.00 $[M-H]^+$	172–173	81	45.67	3.01	11.41	45.55	2.99	11.37
IV	$C_{14}H_{12}FN_3OS$	289.07	290.05 $[M+H]^+$	171–172	65	58.12	4.18	14.52	58.26	4.10	14.44
V	$C_{14}H_{12}FN_3O_2S$	305.06	306.05 $[M+H]^+$	225–227	68	55.07	3.96	13.76	54.98	3.98	13.71
VI	$C_{16}H_{16}FN_3O_2S$	333.09	334.10 $[M+H]^+$	187–188	71	57.64	4.84	12.60	57.55	4.87	12.63
VII	$C_{15}H_{14}FN_3O_2S$	319.08	320.05 $[M+H]^+$	183–184	73	56.41	4.42	13.16	56.33	4.40	13.13
VIII	$C_{14}H_{11}BrFN_3OS$	366.98	366.00 $[M-H]^+$	264–265	66	45.67	3.01	11.41	45.74	3.04	11.44
IX	$C_{15}H_{14}FN_3O_2S$	319.08	318.10 $[M-H]^+$	178–179	69	56.41	4.42	13.16	56.32	4.37	13.12
X	$C_{11}H_{15}N_3O_2S$	253.09	252.05 $[M-H]^+$	146–147	83	52.15	5.97	16.59	51.96	5.93	16.55
XI	$C_{10}H_{13}N_3O_2S$	239.07	240.00 $[M+H]^+$	224–225	77	50.19	5.48	17.56	50.27	5.43	17.48

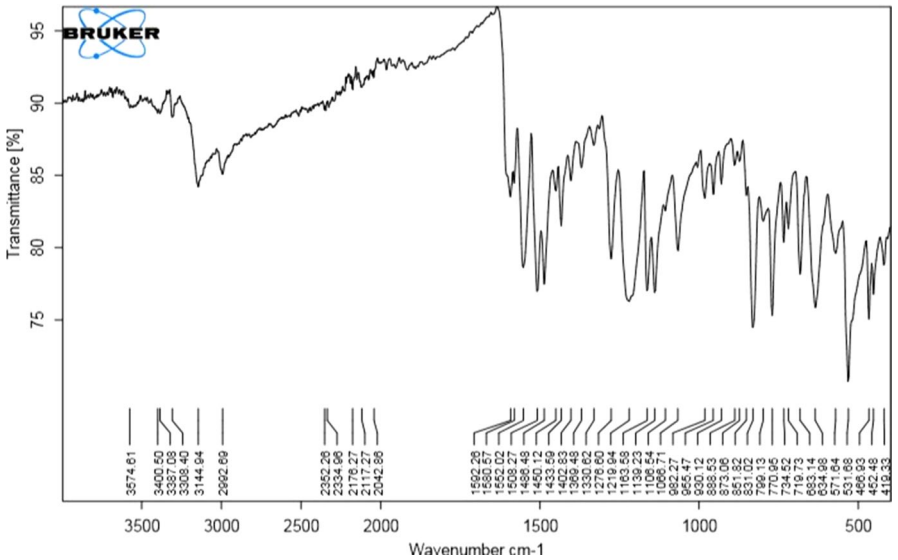


Fig. 1 IR spectrum of compound **IV**

Table 2 FT-IR values of the compounds (cm^{-1})

Compound	OH	=N–NH	–NH–Ar	C=N	C=S	C–N	C–O	C–F
I	3554	3395	3299	1595	1405	1213	1173	1140
II	3465	3323	3148	1575	1400	1272	1220	1134
III	3376	3285	3116	1539	1474	1281	1097	1048
IV	3574	3308	3145	1592	1434	1277	1220	1067
V	3378	3284	3156	1583	1447	1270	1144	1073
VI	3527	3292	3135	1589	1458	1221	1094	1044
VII	3494	3290	3145	1592	1427	1265	1222	1172
VIII	3489	3378	3141	1562	1461	1263	1195	1078
IX	3517	3289	3146	1592	1427	1333	1222	1172
X	3525	3363	3150	1552	1375	1298	1112	–
XI	3446	3318	3171	1547	1352	1207	1120	–

at 11.40–9.82 ppm. The signal of imine (–CH=N) was observed as a singlet at 10.24–8.03 ppm. The –OH proton signal was observed as a singlet in the range of 10.36–9.06 ppm. In ^{1}H NMR spectrum of compound **IV**, while the –NH peak (=N–NH) was observed as a singlet at 11.79 ppm, the –NH signal (–NH–Ar) was detected as a singlet at 10.05 ppm. The signal of imine (–CH=N) was observed as a singlet at 8.08 ppm. The –OH (H1) proton signal was observed as a singlet in the range of 9.96 ppm as shown in Fig. 2. The H2 and H5 proton coupled to the H3 and H4 proton and detected doublet peaks at 6.83–6.81 ppm, respectively. The H3 and H4 proton coupled to the H2 and H5 proton and detected doublet peaks at 7.75–7.72

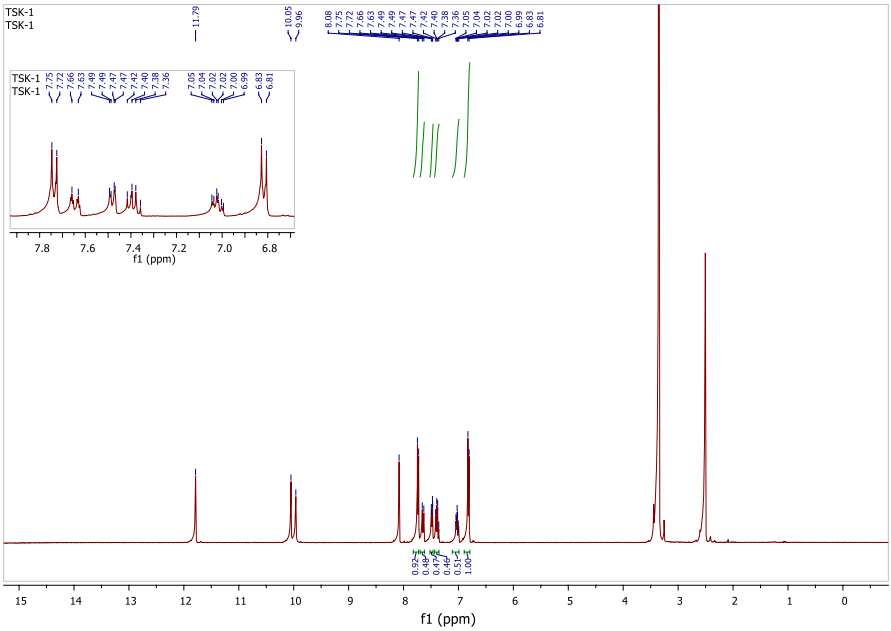
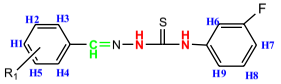


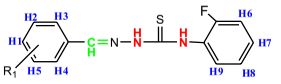
Fig. 2 ^{1}H NMR spectrum of compound **IV**

ppm, respectively. The H6 proton coupled to the H7 proton and was observed as a doublet peak at 7.66–7.63 ppm. The H7 proton coupled to the H6 and H8 proton showed doublet of doublets peaks at 7.49–7.47 ppm. The H8 proton coupled to the H6, H7, and H8 proton showed doublets of triplet peaks at 7.05–6.99 ppm. The H9 proton coupled to the H7 and H8 proton showed doublet of doublets peaks at 7.42–7.36 ppm. Additionally, while the proton atoms of ethoxy ($–OCH_2CH_3$) group were detected around at 4.11–4.06 (quartet, 2H) and 1.37–1.32 (triplet, 3H) ppm for compounds **I**, **VI**, and **X**, the methoxy ($–OCH_3$) signal was observed as a singlet peak around at 3.83 ppm for compounds **II**, **VII**, **IX**, and **XI** as shown in Table 3 (see also Figs. 5, 17, 20, and 29). In compounds **I**, **VI**, and **X**, the CH_3 proton of ethoxy coupled to OCH_2 protons showed as triplet peaks at 1.37–1.32 ppm, whereas CH_2 proton of ethoxy coupled to CH_3 protons was resonated as quartet peaks at 4.11–4.06 ppm (see Figures S2, S14, and S26 in supplementary material). The proton of CH_3 coupled to NH proton was detected as doublet peaks at 3.03–3.02 and 3.01–2.99 ppm, whereas NH proton coupled to CH_3 protons showed quartet peaks at 8.37–8.36 and 8.40–8.38 ppm for compounds **X** and **XI** (see Figures S26 and S29 in supplementary material). DMSO-d_6 and water in DMSO (HOD, H_2O) signals were shown about at 2.00, 2.50 (quintet), and 3.30 (variable, depending on the solvent and its concentration) ppm, respectively [32]. These data are in agreement with the values of previously reported for similar compounds [17, 18, 30, 31]. It is apparent that structure of the obtained compounds and electronic effects of groups/substituents in structures plays a significant role to chemical shift value. Electron-donating groups (OH, NH_2, OCH_3, CH_3, etc.) and electron-withdrawing groups (NO_2, CN, CF_3,

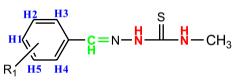
Table 3 ^{1}H NMR (δ, ppm, in DMSO-d_6) values related to synthesized compounds*



I–V



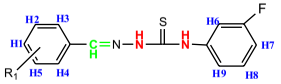
VI–IX



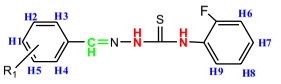
X–XI

Comp	C=N–NH	C=S–NH	CH=N	H1	H2	H3	H4	H5	H6	H7	H8	H9
I	11.80 s	10.04 s	8.03 s	9.49 –OH s	4.12–4.07 q (2H) 1.37–1.33 t (3H)	7.21–7.19 d	7.64–7.61 dd	6.84–6.82 D	7.50 s	7.46–7.37 m	7.06–7.01 t	7.46–7.37 m
II	11.80 s	10.08 s	8.05 s	3.83 s OCH_3	9.11 –OH s	7.65–7.62 d	7.02–6.96 m		7.20–7.18 d	7.47–7.38 m		
III	11.97 s	10.84 s	10.24 s	8.43–8.33 d	6.86–6.84 dd	10.36 –OH s	7.08–7.03 d	–	7.57–7.36 m			
IV	11.79 s	10.05 s	8.08 s	9.96 –OH s	6.83–6.81 d	7.75–7.72 d	7.75–7.72 d	6.83–6.81 d	7.66–7.63 d	7.49–7.47 dd	7.05–6.99 td	7.42–7.36 dd
V	11.72 s	11.40 s	9.83 s	10.21 –OH s	8.78 s	10.00 –OH s	7.89–7.87 d	7.02–6.98 d	8.38 s	6.41–6.28 m	7.48–7.36 m	7.67–7.64 dd

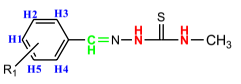
Table 3 (continued)



I–V



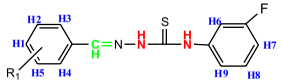
VI–IX



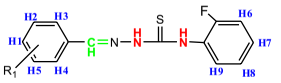
X–XI

Comp	C=N–NH	C=S–NH	CH=N	H1	H2	H3	H4	H5	H6	H7	H8	H9
VI	11.81 s	9.82 s	8.04 s	9.47 –OH s	4.11–4.05 q (2H) 1.36–1.32 t (3H)	7.51 s	7.16–7.13 d	6.83–6.81 d	7.35–7.21 m		7.57–7.54 t	7.35–7.21 m
VII	11.83 s	9.83 s	8.05 s	3.83 s OCH_3	9.55 –OH s	7.52 s	7.17–7.14 d	6.82–6.80 d	7.58–7.52 dd	7.35–7.21 m		
VIII	11.96 s	10.34 s	8.95 s	8.41–8.32 d	6.97–6.84 dd	10.03 –OH s	7.91–7.90 d	–	7.56–7.23 m			
IX	11.83 s	9.83 s	8.05 s	9.54 –OH	3.83 s OCH_3	7.52 s	7.17–7.15 d	6.82–6.80 d	7.58–7.54 dd	7.35–7.23 m		
X[a]	11.31 s	8.37–8.36 q	7.93 s	9.41 –OH s	4.11–4.06 q (2H) 1.37–1.33 t (3H)	7.40 s	7.11–7.08 d	6.82–6.80 d	–	–	–	–

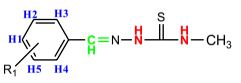
Table 3 (continued)



I–V



VI–IX



X–XI

Comp	C=N–NH	C=S–NH	CH=N	H1	H2	H3	H4	H5	H6	H7	H8	H9
XI[b]	11.31 s	8.40–8.38 q	7.91 s	3.81 s OCH_3	9.06 –OH s	7.31–7.30 d	7.11–7.09 dd	6.95–6.93 d	–	–	–	–

s singlet, *d* doublet, *dd* doublet of doublet, *dt* doublet of triplet, *t* triplet, *td* triplet of doublet, *m* multiplied

[a]CH_3: 3.03–3.02 (d)

[b]CH_3: 3.01–2.99 (d)

F, Cl, Br, etc.) have affected chemical shift value with mesomeric (or resonance), inductive effect [33]. Moreover, the position of these groups/substituents in the molecules plays a major role as well. Proton chemical shift values of the synthesized compounds are given in Table 3. Moreover, all tested compounds' IR, ^{1}H NMR, and ^{13}C NMR spectra were given in supplementary material (Figures S1–S30).

Interpretation of the ^{13}C NMR spectra

The ^{13}C NMR spectra of all compounds were obtained in DMSO-d_6 as solvent. The ^{13}C NMR spectrum of the compound **IV** showed 14 different resonances in good agreement with the proposed structure as shown in Fig. 3. For compounds **I**–**XI**, the –C=S (C8) signals of the thiosemicarbohydrazide region were detected between 177.9 and 175.2 ppm, and the –C=N peaks were observed in the range of 144.6–138.5 ppm. In compounds **I**–**V**, the C11 carbon atoms were detected at 163.3–160.1 ppm, shifted downfield (high values, δ) due to the presence of fluorine atom (*m*-F) (see Figures S3, S6, S9, and S12 in supplementary material). The C10 carbon atoms were detected at 161.2–156.6 ppm, shifted downfield (high values, δ) due to the presence of fluorine atom (*o*-F) for compounds **VI**–**IX**. Furthermore, the C9–C14 atoms were also split into doublets due to interacting with the atomic nucleus of F except for compounds **X** and **XI**. The C1 carbon atoms were observed at 147.6–163.2 ppm, shifted downfield (high values, δ) due to the presence of hydroxyl or methoxy group (–OH or –OCH_3) except for compounds **III**–**VIII**.

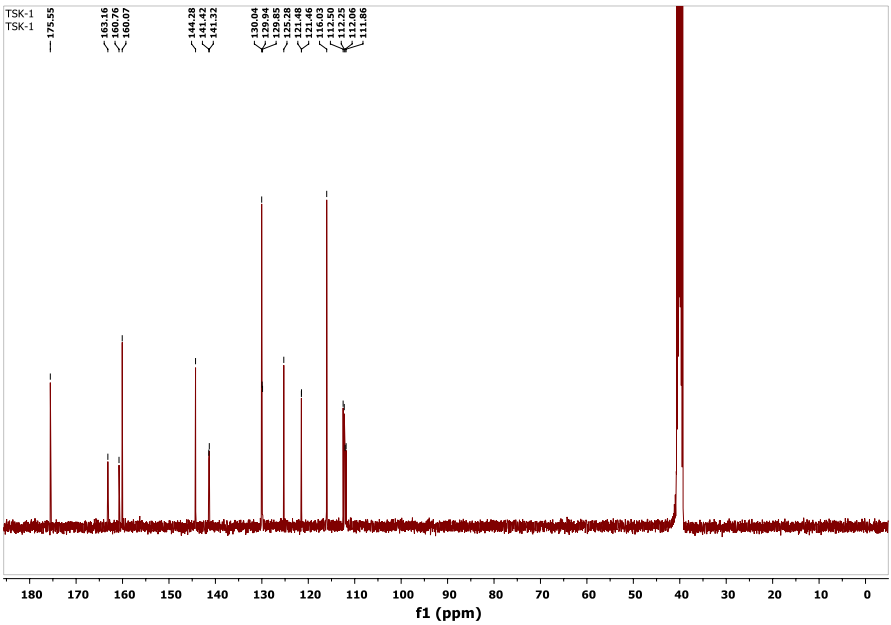


Fig. 3 ^{13}C NMR spectrum of compound **IV**

The C2 carbon atoms were observed at 147.1–149.9 ppm, shifted downfield (high values, δ) due to the presence of hydroxyl, methoxy, or ethoxy group (–OH, $–OCH_3$, or $–OC_2H_5$) except for compounds **III**–**V**, **VIII**. The C3 carbon atoms were observed at 156.4, 158.8, and 156.3 ppm, shifted downfield (high values, δ) due to the presence of hydroxyl group (–OH) for compounds **III**, **V**, and **VIII**, respectively (see Figures S9, S12 and S21 in supplementary material).

In ^{1}H NMR spectrum of compound **IV**, the characteristic –C=N (C7) peak was observed at 144.3 ppm; the –C=S (C8) signal of the thiosemicarbohydrazide region was detected at 175.6 ppm. The carbons (C1–C6) of the aldehyde ring were observed at 163.2, 116.0, 130.0, 125.3, 130.0, and 116.0 ppm, respectively. The C1 carbon atom resonated downfield (high value, δ) due to the presence of hydroxy (–OH) group. Moreover, equivalent-symmetry carbon atoms (C2 with C6, C3 with C5) had the same chemical environment and gave the same signal. The aromatic carbons (C9–C14) of aryl-thiosemicarbohydrazone ring were observed at between 141.4 and 111.9 ppm. The aromatic carbons (C9–C14) were observed at 141.4, 141.3; 112.5, 112.3; 160.8, 160.1; 112.1, 111.9; 129.9, 129.8; 121.5; and 121.4 ppm, respectively. The C11 (160.8, 160.1 ppm) carbon atom was shifted downfield (high values, δ) due to the presence of fluorine atom. Additionally, while the carbon atoms of ethoxy ($–OCH_2CH_3$) group were detected around at 64.5 and 15.2 ppm for compounds **I**, **VI**, and **X**, the methoxy ($–OCH_3$) signal was observed around at 56.3 ppm for compounds **II**, **VII**, **IX**, and **XI**. These results are suitable with the values published previously for similar compounds [17, 18, 30, 31]. The carbon chemical shift values of the synthesized compounds are given in Table 4 (see also supplementary material).

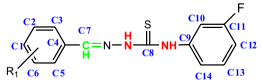
Evaluation of antioxidant activity

Ascorbic acid was used as standard for antioxidant activity evaluations. Inhibition percentages of ascorbic acid and synthesized compounds calculated against concentration are given in Fig. 4.

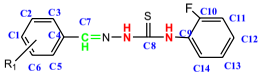
In this study, antioxidant inhibition percentages for the 11 different molecules we synthesized showed a regular increase in direct proportion to the increase in concentration. Among them, especially compounds **I**, **VII**, and **X**, although low against standard antioxidants, exhibited the highest inhibition rates among other compounds. Compound **VI** showed a lower percent inhibition in the concentration ranges of (0.5–2.5) μM, but showed a lower increase in concentrations of 5–10 μM and showed a stable state in percent inhibition. IC_{50} values were also calculated to evaluate antioxidant activities. In this study, IC_{50} values for ascorbic acid and compound are summarized in Table 5. Accordingly, the calculated IC_{50} value for ascorbic acid was 10.50 ± 0.01 μM. IC_{50} values for the newly synthesized compounds followed the order **I** > **VII** > **X** > **VI** > **IV** > **IX** > **XI** > **II** > **V** > **III** > **VIII**.

It is possible to compare free radical scavenging activity between different molecules by knowing that antioxidant activity is inversely proportional to increase in IC_{50} values. According to these results, *p*-hydroxybenzaldehyde-derived compounds containing methoxy and ethoxy groups at different positions

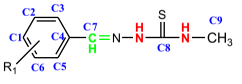
Table 4 ^{13}C NMR (δ, ppm, in DMSO-d_6) values related to synthesized compounds



I–V



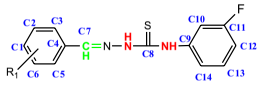
VI–IX



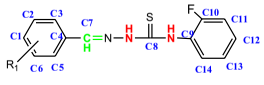
X–XI

Comp	C1	C2	C3	C4	C5	C6	C7 C=N	C8 C=S	C9	C10	C11	C12	C13	C14	R_1
I	147.6	149.9	112.2	125.6	123.1	115.9	144.6	175.6	141.5 141.4	112.9 112.6	163.3 161.1	112.1 112.0	130.0 129.9	121.9 121.8	64.6 15.2
II	150.4	147.2	112.5	127.2	113.9	112.3	144.3	175.7	141.4 141.3	112.1 112.0	163.2 160.8	111.9 111.8	129.9 129.8	121.5 121.3	56.1
III	134.1	118.7	156.4	123.0	129.0	111.6	138.9	176.3	141.4 141.3	113.4 113.2	163.2 160.8	112.5 112.3	130.0 129.9	122.4 122.3	–
IV	163.2	116.0	130.0	125.3	130.0	116.0	144.3	175.6	141.4 141.3	112.5 112.3	160.8 160.1	112.1 111.9	129.9 129.8	121.5 121.4	–
V	161.3	129.9	158.8	121.2	133.4	129.1	141.9	175.2	141.6 141.4	108.7 108.3	162.5 162.2	102.9 102.8	112.1 112.0	111.9 111.8	–
VI	147.7	149.9	111.6	130.8	125.8	115.9	144.3	177.2	123.2 123.1	159.0 156.6	116.2 116.0	127.8 127.7	124.5 124.4	128.6 128.5	64.4 15.2
VII	149.6	148.6	114.2	123.1	115.8	110.4	144.2	177.2	124.5 124.4	159.1 156.6	116.2 116.0	128.5 128.4	125.8 125.7	130.7 130.6	56.4
VIII	135.9	127.7	156.3	128.8	133.9	121.1	138.5	177.9	116.3 116.1	161.2 158.2	111.6 111.1	124.5 123.2	119.5 118.7	132.0 131.1	–
IX	148.6	149.6	110.3	130.7	125.8	115.8	144.3	177.2	123.4 123.1	159.1 156.6	116.2 116.0	127.8 127.7	124.5 124.4	128.6 128.5	56.3
X[a]	147.6	149.5	111.6	126.1	122.5	115.9	142.9	177.8	–	–	–	–	–	–	64.5 15.2

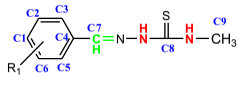
Table 4 (continued)



I–V



VI–IX



X–XI

Comp	C1	C2	C3	C4	C5	C6	C7 C=N	C8 C=S	C9	C10	C11	C12	C13	C14	R_1
XI[b]	150.0	147.1	113.8	128.1	121.1	112.5	143.0	177.9	–	–	–	–	–	–	56.4

[a]C=S–NH–CH_3: 31.3 ppm

[b]C=S–NH–CH_3: 31.5 ppm

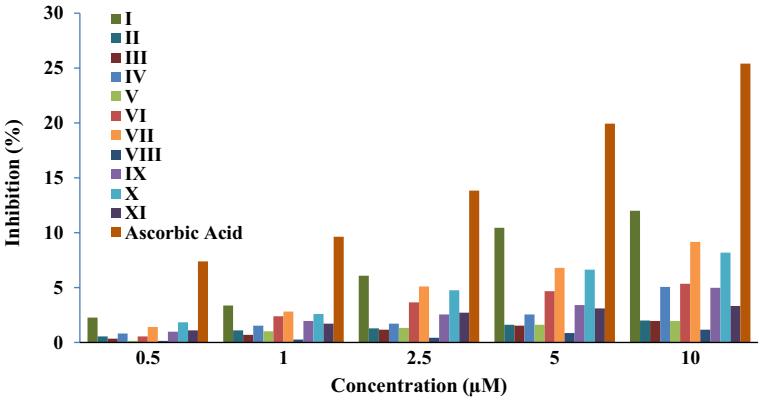


Fig. 4 Percentage change of inhibition calculated by DPPH method for ascorbic acid and synthesized compounds

Table 5 IC_{50} values for the synthesized compounds

Compounds	DPPH scavenging activity IC_{50} (µM)[a]
I	19.24 ± 0.01
II	118.06 ± 0.09
III	146.51 ± 0.16
IV	53.17 ± 0.05
V	123.05 ± 0.15
VI	42.32 ± 0.03
VII	26.04 ± 0.02
VIII	191.06 ± 0.23
IX	53.19 ± 0.05
X	29.99 ± 0.01
XI	84.96 ± 0.12
Ascorbic acid	16.50 ± 0.01

Values are expressed as means ($n = 3$)

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

showed the highest antioxidant activity. However, synthesized structures (**III** and **VIII**) possessing bromine atom exhibited lower antioxidant activity. Furthermore, as Muğlu et al. [34] mentioned in their study, the presence of thio group is effective in showing antioxidant properties of compounds. In particular as mentioned in the studies of Bakır and Lawag [5], the presence of electron-donating methoxy and ethoxy structures in the compounds containing phenolic structure (**I**, **VII**, **X**, **VI**, **IX**, **XI**, **II**) contributed to the increase in the antioxidant activity of the compound.

Conclusions

In this paper, novel thiosemicarbazones including Schiff bases have been prepared with good yields of 63–83%. All the products were characterized by methods with FT-IR, ^{1}H NMR, ^{13}C NMR, and LC–MS/MS spectroscopy and elemental analyses. The in vitro antioxidant activity of the synthesized compounds was tested by the DPPH free radical scavenging method. Compound **I** of among the tested compounds exhibited the most satisfactory antioxidant activity against the DPPH radical. IC_{50} values of the thiosemicarbazones including Schiff bases ranged from 19.24 to 191.06 μM.

Acknowledgements I would like to thank the Scientific Technological Research and Applications Center (Gübitam) and Dr. Ömer Faruk Ensari for taking the NMR spectra.

Compliance with ethical standards

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

References

1. A. Goszczyńska, H. Kwiecień, K. Fijałkowski. Med. Chem. Res. **24**, 3561 (2015)
2. A. Cinarli, D. Gürbüz, A. Tavman, A.S. Birteksöz. Bull. Chem. Soc. Ethiop. **25**, 407 (2011)
3. A. Jarrahpour, D. Khalili, E. De Clercq, C. Salmi, J. Brunel, Molecules **12**, 1720 (2007)
4. S. Bharti, G. Nath, R. Tilak, S. Singh, Eur. J. Med. Chem. **45**, 651 (2010)
5. T.K. Bakır, J.B. Lawag, Res. Chem. Intermed. **46**, 2541 (2020)
6. Y. Zhang, Y. Fang, H. Liang, H. Wang, K. Hu, X. Liu, X. Yi, Y. Peng, Bioorg. Med. Chem. Lett. **23**, 107 (2013)
7. K.S. Kumar, S. Ganguly, R. Veerasamy, E. De Clercq, Eur. J. Med. Chem. **45**, 5474 (2010)
8. S. Pandeya, D. Sriram, G. Nath, E. De Clercq, Pharm. Acta Helv. **74**, 11 (1999)
9. M.J. Hearn, M.H. Cynamon, M.F. Chen, R. Coppins, J. Davis, H.J.-O. Kang, A. Noble, B. Tu-Sekine, M.S. Terrot, D. Trombino, Eur. J. Med. Chem. **44**, 4169 (2009)
10. S.M. Emam, I.E. El Sayed, M.I. Ayad, H.M. Hathout, J. Mol. Struct. **1146**, 600 (2017)
11. B. Mohan Sahoo, S. Chandra Dinda, B.V. Ravi Kumar, J. Panda, P.S. Brahmkshatriya, Lett. Drug Des. Discov. **11**, 82 (2014)
12. S.K. Sridhar, S.N. Pandeya, J.P. Stables, A. Ramesh, Eur. J. Pharm. Sci. **16**, 129 (2002)
13. M.M. Aly, Y.A. Mohamed, K.A. El-Bayouki, W.M. Basyouni, S.Y. Abbas, Eur. J. Med. Chem. **45**, 3365 (2010)
14. H. Pervez, M.S. Iqbal, M.Y. Tahir, F.H. Nasim, M.I. Choudhary, K.M. Khan, J. Enzyme Inhib. Med. Chem. **23**, 848 (2008)
15. H. Govender, C. Mocktar, H.M. Kumalo, N.A. Koorbanally, Phosphorus Sulfur Relat. Elem. **194**, 1074 (2019)
16. S. Ghosh, A.K. Misra, G. Bhatia, M. Khan, A. Khanna, Bioorg. Med. Chem. Lett. **19**, 386 (2009)
17. M. Bingul, E. Şenkuytu, M.F. Saglam, M. Boga, H. Kandemir, I.F. Sengul, Res. Chem. Intermed. **45**, 4487 (2019)
18. H. Muğlu, Res. Chem. Intermed. **46**, 2083 (2020)
19. T.R. Bal, B. Anand, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett. **15**, 4451 (2005)
20. A. Kshirsagar, M.P. Toraskar, V.M. Kulkarni, S. Dhanashire, V. Kadam. Int. J. Chem. Tech. Res **1**, 696 (2009)
21. C. Shipman Jr., S.H. Smith, J.C. Drach, D.L. Klayman, Antimicrob. Agents Chemother. **19**, 682 (1981)
22. N. Solak, S. Rollas, Arkivoc **xii**, 173 (2006)

23. W. Hu, W. Zhou, C. Xia, X. Wen, Bioorg. Med. Chem. Lett. **16**, 2213 (2006)
24. H. Pervez, N. Manzoor, M. Yaqub, A. Khan, K.M. Khan, F-u-H Nasim, M.I. Choudhary, Lett. Drug Des. Discov. **7**, 102 (2010)
25. B. Halliwell, J.M. Gutteridge, *Free radicals in biology and medicine* (Oxford University Press, Oxford, 2015)
26. Y.K. Tyagi, A. Kumar, H.G. Raj, P. Vohra, G. Gupta, R. Kumari, P. Kumar, R.K. Gupta, Eur. J. Med. Chem. **40**, 413 (2005)
27. W. Brand-Williams, M.-E. Cuvelier, C. Berset, LWT-Food. Sci. Technol. **28**, 25 (1995)
28. N. Naik, H.V. Kumar, P.B. Vidyashree, J. Pharm. Res. **4**, 2686 (2011)
29. E.N. Frankel, A.S. Meyer, J. Sci. Food Agric. **80**, 1925 (2000)
30. K.H.D. Reddy, S.-M. Lee, K. Seshaiah, R.K. Babu, J. Serb. Chem. Soc. **78**, 229 (2013)
31. P. Tarasconi, S. Capacchi, G. Pelosi, M. Cornia, R. Albertini, A. Bonati, P.P. Dall'Aglio, P. Lunghi, S. Pinelli, Bioorg. Med. Chem. **8**, 157 (2000)
32. D. Williams, I. Fleming, *Spectroscopic methods in organic chemistry* (McGraw Hill Book Co. Ltd, Maidenhead, 1973)
33. C. Hansch, A. Leo, R. Taft, Chem. Rev. **91**, 165 (1991)
34. H. Muğlu, M.S. Çavuş, T. Bakır, H. Yakan, J. Mol. Struct. **1196**, 819 (2019)