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Synthesis, characterization, and antioxidant activity of some new N^4-arylsubstituted-5-methoxyisatin-β-thiosemicarbazone derivatives

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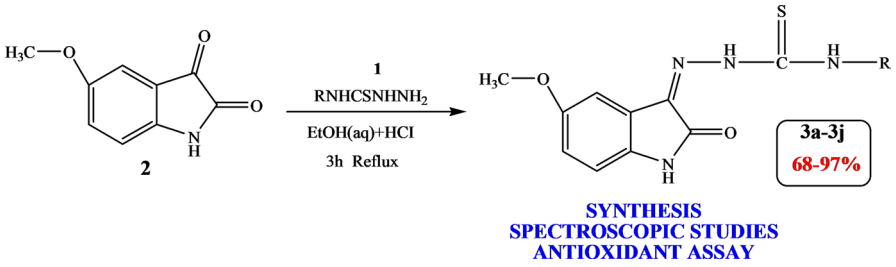
Abstract

Firstly, thiosemicarbazides were prepared by the reaction of hydrazine monohydrate with isothiocyanates in cold dry ethanol at 0 °C for 1 h. After that, new isatin-β-thiosemicarbazones were synthesized by treatment of 5-methoxyisatin with thiosemicarbazides in aqueous ethanol containing one drop of hydrochloric acid at reflux for 3 h. The chemical structures of products were confirmed by means of IR, ^{1}H NMR, and ^{13}C NMR data and by elemental analysis. All the synthesized compounds were evaluated for their antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging method. The synthesized molecules showed lower antioxidant activity than the standard Trolox (8.757 µM). IC_{50} values of the newly synthesized isatin-β-thiosemicarbazone derivatives ranged from 12.455 to 73.471 µM.

Graphic abstract



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

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Introduction

Thiosemicarbazones are a significant class of synthetic compounds and have a variety of pharmacological and biological applications [1–6]. They have revealed wide spectrum of activities which includes anticancer [5, 7], anti-HIV [8, 9], anticonvulsant [10, 11], antimalarial [12, 13] anti-inflammatory [14], enzymatic inhibition [3, 4, 15], anti-viral [16], antioxidant [17], antifungal [18], and antibacterial [18, 19].

Isatin derivatives have shown an extensive spectrum of biological activities such as anti-bacterial [20], anti-viral [21], antifungal [20], antimicrobial [22, 23], antioxidant [24, 25], anti-tubercular [26], anti-HIV [8, 27], and anticonvulsant [28].

Free radicals are produced under certain environmental conditions and during normal cellular functions in the body. Many articles have reported potential beneficial effects of different free radical scavenging antioxidant polyphenols against neoplastic diseases in human biology [24]. Schiff bases have been investigated as antioxidants by many researchers because of their ability to scavenge free radicals [29].

In this work, isatin-β-thiosemicarbazones including halogen, methoxy, methyl, and nitro groups were synthesized and characterized by using methods such as IR, ^{1}H NMR, and ^{13}C NMR spectroscopy and by elemental analysis. The synthesized compounds were determined antioxidant activity in vitro by using DPPH free radical scavenging method.

Experimental section

Measurement and reagents

All starting materials and solvent were purchased from Aldrich, Sigma, or Merck Chemical Company and were used without further purification. The solvent was spectroscopic grade. The elemental analysis was performed on a CHNS-932 (LECO). Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL ECX-400 (400 MHz) in DMSO-d_6 spectrophotometer. The splitting patterns are indicated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplied). Melting points were measured using a Gallenkamp melting point apparatus and were uncorrected. Absorption measurements were recorded with a SHIMADZU UVM-1240 UV–visible spectrophotometer.

Synthesis of substituted thiosemicarbazides (1a–j)

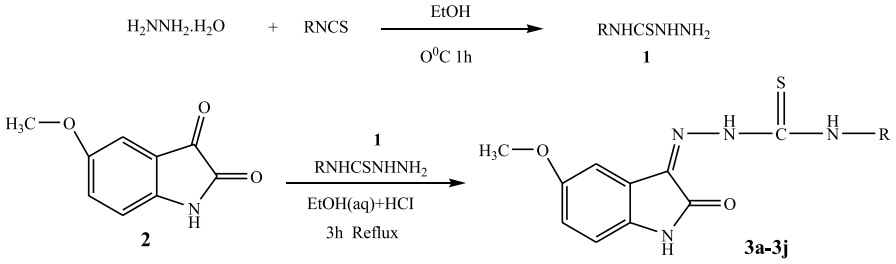
To a solution of hydrazine monohydrate (5 mmol) in ethanol (10 mL), a suspension of an appropriate isothiocyanate (5 mmol) in ethanol (10 mL) was added

dropwise with vigorous stirring and cooling in an ice bath. The mixture was allowed to stand overnight. The solid product so formed was filtered, dried, and crystallized from ethanol to give product.

Synthesis of N^4-substituted 5-methoxyisatin-β-thiosemicarbazones (3a–j) N^4-substituted thiosemicarbazide (**1a–j**) (0.8 mmol), 5-methoxyisatin (0.8 mmol), and one drop of HCl were added to aqueous EtOH (20 mL), and the mixture was refluxed at 78 °C for 3 h. The obtained precipitate was filtered and washed with EtOH to afford pure desired products or recrystallized from ethanol. Then the formed color solid was isolated and dried in air. The products were obtained good yields (68–97%) as shown in Scheme 1.

N^4-2-methoxyphenyl-5-methoxyisatin-β-thiosemicarbazone (3a) Yield: 87%, m.p.: 218–220 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3207, υ(–C=N–NH and –NH–Ar carbazide) 3137, 3092, υ(Ar C–H) 3043–2995, υ(C=O) 1687, υ(C=N) 1544, υ(C=S) 1487, υ(C–N) 1182; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.71 (s, NH ist), 11.01 (s, NH), 10.39 (s, NH), 7.67–7.65 (d, 1H, J = 7.9 Hz), 7.28–7.23 (m, 2H, ArH), 7.10–7.08 (d, 1H, J = 7.9 Hz), 6.98–6.94 (t, 1H, J = 7.6 Hz), 6.92–6.89 (dd, 1H, J = 8.5, 2.4 Hz, ArH), 6.83–6.81 (d, 1H, J = 8.5 Hz, ArH), 3.79 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 177.07 (C=S), 163.24 (C=O), 155.84 (C–O), 153.90 (C–O), 136.79 (C=N), 132.97, 128.35, 127.55, 127.51, 121.13, 120.65, 118.15, 112.47, 112.34, 106.65, 56.28 (OCH_3), 56.08 (OCH_3); Elemental Analysis Calcd: C, 57.29; H, 4.53; N, 15.72. Found: C, 56.84; H, 4.61; N, 15.34.

N^4-2-fluorophenyl-5-methoxyisatin-β-thiosemicarbazone (3b) Yield: 82%, m.p.: 234–235 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3309, υ(–C=N–NH and –NH–Ar carbazide) 3147, 3096, υ(Ar C–H) 3040–3005, υ(C=O) 1686, υ(C=N) 1536, υ(C=S)



3a R=2-$H_3COC_6H_4$
3b R=2-FC_6H_4
3c R=4-$H_3COC_6H_4$
3d R=3-FC_6H_4
3e R=4-$O_2NC_6H_4$
3f R=2,4-$CI_2C_6H_3$
3g R=-$CH_2CH_2C_6H_4$
3h R=2-$CH_3C_6H_4$
3i R=4-FC_6H_4
3j R=3-ClC_6H_4

Scheme 1 Synthetic route for the 5-methoxyisatin-β-thiosemicarbazone derivatives (**3a**–**3j**)

1479, υ(C–N) 1193, υ(C–F) 906; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.81 (s, NH ist), 11.02 (s, NH), 10.64 (s, NH), 7.47–7.43 (t, 1H, J = 7.3 Hz), 7.39–7.34 (m, 1H, ArH), 7.32–7.30 (d, 2H, J = 7.9 Hz), 7.27–7.21 (m, 1H, J = 7.7 Hz, ArH), 6.92–6.89 (dd, 1H, J = 8.5, 2.4 Hz, ArH), 6.83–6.81 (d, 1H, J = 8.5 Hz, ArH), 3.71 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 178.54, 163.29, 159.11, 156.64, 155.86, 136.82, 133.51, 130.80, 129.74, 129.66, 126.98, 126.86, 124.99, 124.96, 121.27, 121.16, 118.23, 116.65, 116.45, 112.47, 106.91, 56.11; Elemental Analysis Calcd: C, 55.81; H, 3.81; N, 16.27. Found: C, 55.44; H, 3.79; N, 16.05.

N^4-4-methoxyphenyl-5-methoxyisatin-β-thiosemicarbazone (3c) Yield: 85%, m.p.: 214–215 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3318, υ(–C=N–NH and –NH–Ar carbazide) 3188, 3132, υ(Ar C–H) 3070–3032, υ(C=O) 1662, υ(C=N) 1576, υ(C=S) 1475, υ(C–N) 1174; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.69 (s, NH ist), 11.00 (s, NH), 10.64 (s, NH), 7.42–7.39 (d, 2H, J = 9.2 Hz, ArH), 7.36–7.35 (d, 1H, J = 1.8 Hz,), 6.94–6.92 (d, 2H, J = 9.2 Hz, ArH), 6.91–6.88 (dd, 1H, J = 8.5, 2.4 Hz), 6.82–6.80 (d, 1H, J = 8.5 Hz), 3.73 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 177.14, 163.34, 158.00, 155.85, 136.61, 132.61, 131.58, 127.95, 121.01, 117.74, 114.13, 112.36, 106.91, 56.14, 55.81; Elemental Analysis Calcd: C, 57.29; H, 4.53; N, 15.72. Found: C, 56.88; H, 4.39; N, 15.60.

N^4-3-fluorophenyl-5-methoxyisatin-β-thiosemicarbazone (3d) Yield: 80%, m.p.: 226–227 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3339, υ(–C=N–NH and –NH–Ar carbazide) 3184, 3127, υ(Ar C–H) 3072–3035, υ(C=O) 1680, υ(C=N) 1514, υ(C=S) 1474, υ(C–N) 1178, υ(C–F) 900; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.81 (s, NH ist), 11.03 (s, NH), 10.79(s, NH), 7.58–7.54 (dd, 1H, J = 11.0, 2.4 Hz, ArH), 7.47–7.38 (m, 2H, ArH), 7.36–7.35 (d, 1H, J = 2.4 Hz,), 7.10–7.05 (m, 1H, ArH), 6.92–6.90 (dd, 1H, J = 8.5, 2.4 Hz), 6.82–6.80 (d, 1H, J = 8.5 Hz), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 176.78 (C=S), 163.35, 160.94, 155.88, 155.79, 140.66, 140.54, 136.80, 133.46, 130.52, 130.43, 122.01, 121.98, 121.11, 118.13, 113.40, 113.19, 113.06, 112.82, 112.43, 107.29, 56.18; Elemental Analysis Calcd: C, 55.81; H, 3.81; N, 16.27. Found: C, 55.33; H, 3.81; N, 16.15.

N^4-4-nitrophenyl-5-methoxyisatin-β-thiosemicarbazone (3e) Yield: 87%, m.p.: 247–248 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3307, υ(–C=N–NH and –NH–Ar carbazide) 3246, 3218, υ(Ar C–H) 3079–3014, υ(C=O) 1697, υ(C=N) 1542, υ(C=S) 1466, υ(C–N) 1154, $\upsilon(NO_2)$: 1438–1326 (as, ss); ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.96 (s, NH ist), 11.06 (s, NH), 11.01 (s, NH), 8.25–8.23 (d, 2H, J = 8.5 Hz, ArH), 8.04–8.01 (d, 2H, J = 9.2 Hz, ArH), 7.35–7.34 (d, 1H, J = 2.4 Hz), 6.93–6.91 (dd, 1H, J = 8.5, 2.4 Hz), 6.83–6.81 (d, 1H, J = 8.5 Hz), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: δ 176.89, 163.06, 156.02, 145.18, 144.66, 136.97, 134.07, 125.42, 124.49, 120.51, 118.31, 112.50, 107.44, 56.07; Elemental Analysis Calcd: C, 51.75; H, 3.53; N, 18.86. Found: C, 50.90; H, 3.42; N, 18.65.

N^4-2,4-dichlorophenyl-5-methoxyisatin-β-thiosemicarbazone (3f) Yield: 97%, m.p.: 249–250 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3265, υ(–C=N–NH and

–NH–Ar carbazide) 3172, 3074, υ(Ar C–H) 3032–2994, υ(C=O) 1689, υ(C=N) 1535, υ(C=S) 1453, υ(C–N) 1162, υ(C–Cl) 856; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.84 (s, NH ist), 11.03 (s, NH), 10.73 (s, NH), 7.74–7.73 (d, 1H, J = 1.8 Hz, ArH), 7.55–7.53 (d, 1H, J = 8.5 Hz, ArH), 7.49–7.46 (dd, 1H, J = 8.5, 2.4 Hz, ArH), 7.29–7.28 (d, 1H, J = 2.4 Hz), 6.93–6.90 (dd, 1H, J = 8.5, 2.4 Hz), 6.83–6.81 (d, 1H, J = 8.5 Hz), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 178.29, 163.31, 155.77, 136.83, 135.96, 133.68, 133.43, 133.03, 132.48, 129.70, 128.10, 121.02, 118.32, 112.62, 106.74, 56.13; Elemental Analysis Calcd: C, 48.62; H, 3.06; N, 14.18. Found: C, 47.91; H, 2.99; N, 14.03.

N^4-2-phenylethyl-5-methoxyisatin-β-thiosemicarbazone (3g) Yield: 86%, m.p.: 179–180 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3369, υ(–C=N–NH and –NH–Ar carbazide) 3235, 3207, υ(Ar C–H) 3062–3022, υ(C=O) 1680, υ(C=N) 1515, υ(C=S) 1475, υ(C–N) 1137; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.58 (s, NH ist), 10.97 (s, NH), 9.28 (t, J = 5.8 Hz, 1H, NH–CH_2), 7.30–7.23 (m, 4H, ArH), 7.20–7.19 (d, 1H, J = 2.4 Hz), 7.18–7.16 (t, 1H, J = 6.4 Hz, ArH), 6.91–6.88 (dd, 1H, J = 8.2, 2.7 Hz), 6.81–6.79 (d, 1H, J = 8.5 Hz), 3.78 (dd, 2H, J = 15.6, 5.8 Hz, N–CH_2), 3.73 (s, 3H, OCH_3), 2.93 (t, 2H, J = 7.9 Hz, –CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 177.56, 163.29, 155.82, 139.14, 136.39, 132.63, 129.13, 129.02, 126.55, 121.53, 117.48, 112.47, 106.19, 56.17, 45.96, 34.43; Elemental Analysis Calcd: C,61.00; H, 5.12; N, 15.81. Found: C, 59.80; H, 5.01; N, 15.64.

N^4-2-methylphenyl-5-methoxyisatin-β-thiosemicarbazone (3h) Yield: 84%, m.p.: 223–224 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3311, υ(–C=N–NH and –NH–Ar carbazide) 3229, 3173, υ(Ar C–H) 3065–2992, υ(C=O) 1736, υ(C=N) 1586, υ(C=S) 1480, υ(C–N) 1165; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.70 (s, NH ist), 11.01 (s, NH), 10.64 (s, NH), 7.35–7.34 (d, 1H, J = 1.8 Hz), 7.30–7.28 (m, 1H, J = 2.4 Hz, ArH), 7.24–7.22 (d, 3H, J = 9.2 Hz, ArH), 6.91–6.88 (dd, 1H, J = 8.5, 2.4 Hz), 6.83–6.80 (d, 1H, J = 8.5 Hz), 3.71 (s, 3H, OCH_3), 2.21 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 177.78, 163.30, 155.72, 137.91, 136.71, 136.13, 132.94, 130.89, 129.07, 128.00, 126.83, 121.31, 118.11, 112.48, 106.84, 56.33, 18.20; Elemental Analysis Calcd: C, 59.98; H, 4.74; N, 16.46. Found: C, 59.43; H, 4.67; N, 16.38.

N^4-4-fluorophenyl-5-methoxyisatin-β-thiosemicarbazone (3i) Yield: 68%, m.p.: 235–236 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3334, υ(–C=N–NH and –NH–Ar carbazide) 3194, 3103, υ(Ar C–H) 3069–2995, υ(C=O) 1693, υ(C=N) 1527, υ(C=S) 1450, υ(C–N) 1151, υ(C–F) 936; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.76 (s, NH ist), 11.02 (s, NH), 10.74 (s, NH), 7.57–7.54 (q, 2H, J = 4.5 Hz, ArH), 7.35–7.34 (d, 1H, J = 2.4 Hz), 7.24–7.20 (t, 2H, J = 8.9 Hz, ArH), 6.92–6.89 (dd, 1H, J = 8.2, 2.7 Hz), 6.82–6.80 (d, 1H, J = 8.5 Hz), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 177.33, 163.35, 161.83, 159.41, 155.86, 136.75, 135.32, 135.29, 133.20, 128.74, 128.65, 121.21, 118.06, 115.80, 115.57, 112.39, 107.13, 56.16; Elemental Analysis Calcd: C, 55.81; H, 3.81; N, 16.27. Found: C, 55.37; H, 3.67; N, 16.13.

N^4-3-chlorophenyl-5-methoxyisatin-β-thiosemicarbazone (3j) Yield: 73%, m.p.: 211–212 °C, IR (KBr, cm^{-1}): υ(–NH ist) 3332, υ(–C=N–NH and –NH–Ar carbazide) 3299, 3220, υ(Ar C–H) 3032–2992, υ(C=O) 1688, υ(C=N) 1537, υ(C=S) 1476, υ(C–N) 1146, υ(C–Cl) 856; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 12.82 (s, NH), 11.04 (s, NH), 10.79 (s, NH), 7.74 (s, 1H, ArH), 7.61–7.59 (d, 1H, J = 7.9 Hz, ArH), 7.43–7.39 (t, 1H, J = 7.9 Hz, ArH), 7.36–7.35 (d, 1H, J = 1.8 Hz), 7.31–7.29 (dd, 1H, J = 7.9, 1.2 Hz, ArH), 6.93–6.91 (dd, 1H, J = 8.2, 2.1 Hz), 6.83–6.81 (d, 1H, J = 8.5 Hz), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 176.88, 163.24, 155.77, 140.32, 136.84, 133.50, 133.01, 130.53, 126.45, 125.76, 124.77, 121.13, 118.15, 112.44, 107.27, 56.20; Elemental Analysis Calcd: C, 53.26; H, 3.63; N, 15.53. Found: C, 53.15; H, 3.54; N, 15.35.

Antioxidant activity measurements by using DPPH method

Antioxidant activity of newly synthesized compounds was determined by the DPPH radical scavenging activity analysis. For this, free radical scavenging ability was evaluated according to the method of Brand-Williams et al. [30] with slight modification.

In order to evaluate the antioxidant activity of the products, stock solutions of the compounds were prepared in DMSO to 250 µM. Compounds were dissolved in DMSO and diluted to five different concentrations. We used DPPH in ethanol at a concentration of 50 µM. To the previously prepared (5 mL), DPPH solution were added compound solutions of different concentrations (5 µM, 10 µM, 20 µM, 50 µM, and 100 µM) and sufficient ethanol to total 6 mL. This mixture was allowed to stand in a dark room at room temperature for 30 min and then was read at 517 nm against a blank. Radical scavenging activity was expressed as a percentage of inhibition and calculated using the formula:

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

where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The results were expressed as IC_{50} values (the test concentration required to sweep 50% free radicals) [31].

Results and discussion

Physical data

Ten of 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 Physical data of the 5-methoxyisatin-β-thiosemicarbazone derivatives (**3a**–**3j**)

Entry	Compound code	R	Molecular formula	Molecular weight	m.p. (°C)	Yield %
1	**3a**	2-$OCH_3C_6H_4$	$C_{17}H_{16}N_4O_3S$	356.41	218–220	87
2	**3b**	2-FC_6H_4	$C_{16}H_{13}FN_4O_2S$	344.37	234–235	82
3	**3c**	4-$OCH_3C_6H_4$	$C_{17}H_{16}N_4O_3S$	356.41	214–215	85
4	**3d**	3-FC_6H_4	$C_{16}H_{13}FN_4O_2S$	344.37	226–227	80
5	**3e**	4-$NO_2C_6H_4$	$C_{16}H_{13}N_5O_4S$	371.38	247–248	87
6	**3f**	2,4-ClC_6H_3	$C_{16}H_{12}Cl_2N_4O_2S$	395.27	249–250	97
7	**3g**	$–CH_2CH_2C_6H_5$	$C_{18}H_{18}N_4O_2S$	354.43	179–180	86
8	**3h**	2-$CH_3C_6H_4$	$C_{17}H_{16}N_4O_2S$	340.41	223–224	84
9	**3i**	4-FC_6H_4	$C_{16}H_{13}FN_4O_2S$	344.37	235–236	68
10	**3j**	3-ClC_6H_4	$C_{16}H_{13}ClN_4O_2S$	360.82	211–212	73

Table 2 Results of elemental analysis for the compounds

Compounds	Calculated			Experimental		
	C%	H%	N%	(C)%	(H)%	(N)%
3a	57.29	4.53	15.72	56.84	4.61	15.34
3b	55.81	3.81	16.27	55.44	3.79	16.05
3c	57.29	4.53	15.72	56.88	4.39	15.60
3d	55.81	3.81	16.27	55.33	3.81	16.15
3e	51.75	3.53	18.86	50.90	3.42	18.65
3f	48.62	3.06	14.18	47.91	2.99	14.03
3g	61.00	5.12	15.81	59.80	5.01	15.64
3h	59.98	4.74	16.46	59.43	4.67	16.38
3i	55.81	3.81	16.27	55.37	3.67	16.13
3j	53.26	3.63	15.53	53.15	3.54	15.35

Vibrational frequencies

In the FT-IR spectrum of the synthesized compounds, the asymmetric and symmetric stretching bands of the amino group ($–NH_2$) did not observe at 3600–3200 cm^{-1}, as expected. In compound **3e**, while the –NH (isatin) stretching vibration was observed at 3307 cm^{-1}, the –NH (semicarbazone) stretching vibrations were observed at 3246 and 3218 cm^{-1}. The C=O signal of isatin ring was observed at 1697 cm^{-1}, and the –C=N stretching vibration was observed at 1542 cm^{-1}; the C=S signal of isatin ring was observed at 1466 cm^{-1}, and the –C–N stretching vibration was appeared at 1154 cm^{-1}; the asymmetric and symmetric stretching bands of the nitro group ($–NO_2$) were observed at around 1438 and 1326 cm^{-1}, as shown in Fig. 1. These observations provided key evidence for the products formation. These data are consistent with values reported previously

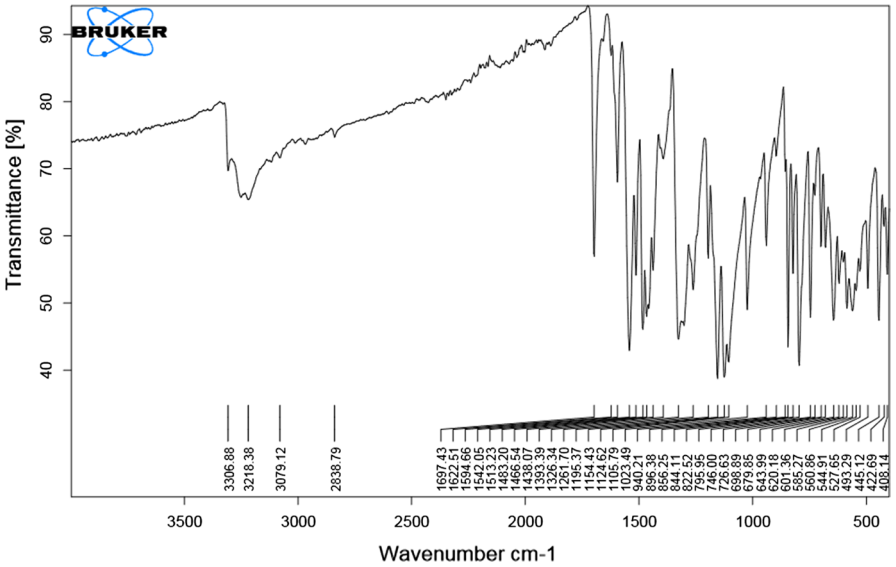
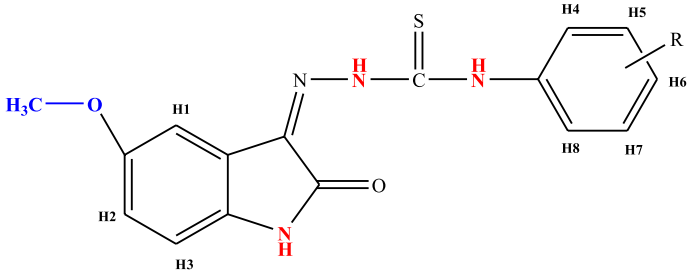


Fig. 1 IR spectrum of compound **3e**

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

Comp.	–NH (ist)	–NH (chz)	–NH–Ar (chz)	Ar CH	C=O	C=N	C=S	C–N	Spec. vib.
3a	3207	3137	3092	3043–2995	1687	1544	1478	1182	–
3b	3309	3147	3096	3040–3005	1686	1536	1479	1193	C–F: 906
3c	3318	3188	3132	3070–3032	1662	1576	1475	1174	–
3d	3339	3184	3127	3072–3035	1680	1514	1474	1178	C–F: 900
3e	3307	3246	3218	3079–3014	1697	1542	1466	1154	NO_2: 1438–1326$_{(as, ss)}$
3f	3265	3172	3074	3032–2994	1689	1535	1453	1162	C–Cl: 856
3g	3369	3235	3207	3062–3022	1680	1515	1475	1137	–
3h	3311	3229	3173	3065–2992	1736	1586	1480	1165	–
3i	3334	3194	3103	3069–2995	1693	1527	1450	1151	C–F: 936
3j	3332	3299	3220	3032–2992	1688	1537	1476	1146	C–Cl: 856

chz carbohydrazide, *ist* isatin, *as* asymmetric stretching, *ss* symmetric stretching



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

for similar compounds [2, 6, 32]. The IR peaks of the compounds are given in Table 3.

Interpretation of the ^{1}H NMR spectra

The ^{1}H NMR spectra of the synthesized compounds were detected in DMSO-d_6 as solvent. The general scheme for ^{1}H NMR spectral interpretations is shown in Scheme 2.

For compound **3e**, while the –NH peak of isatin was observed as a singlet at 12.96 ppm, the –NH signals of semicarbazone were detected as singlets at 11.06 and 11.01 ppm. The proton signals (H1–H2–H3) of isatin ring were observed

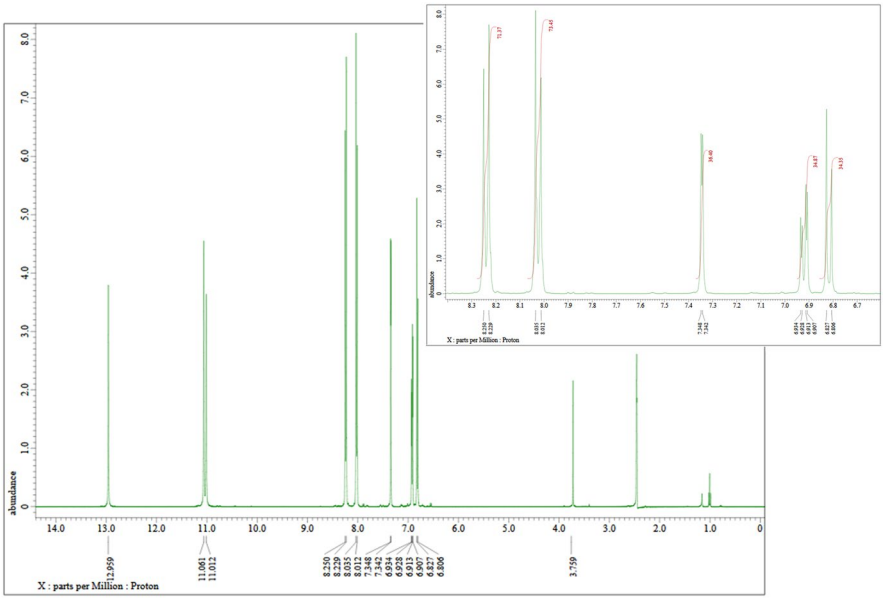


Fig. 2 ^{1}H NMR spectrum of compound **3e**

between 7.35 and 6.81 ppm (Fig. 2). The H1 proton was observed as doublet peak at 7.35–7.34 ($J = 2.4$ Hz) ppm. The H2 proton coupled to the H3 and H1 proton showed doublet of doublets peaks at 6.93–6.91 ($J = 8.5$, 2.4 Hz) ppm. The H3 proton coupled to the H2 proton and detected doublet peaks at 6.83–6.81 ($J = 8.5$ Hz) ppm. The aromatic protons (H4–H8) of benzene ring were observed at 8.25–8.01 ppm. The H4 and H8 proton coupled to the H5 and H7 proton and detected doublet peaks at 8.04–8.01 ($J = 8.5$ Hz) ppm, respectively. The H5 and H7 proton coupled to the H4 and H8 proton and detected doublet peaks at 8.25–8.23 ($J = 9.2$ Hz) ppm, respectively. The methoxy group (–OCH_3) proton signal was observed as a singlet in the range of 3.72 ppm. DMSO-d_6 and water in DMSO (HOD, H_2O) signals were shown around at 2.00, 2.50 (quintet), and 3.30 (variable, based on the solvent and its concentration) ppm, respectively [33]. These results are consistent with the values of formerly reported for similar compounds [2, 6, 32]. Proton chemical shift values of the compounds are presented in Table 4. Moreover, selected compounds' IR, ^{1}H NMR, and ^{13}C NMR spectra were given as supplementary material.

Interpretation of the ^{13}C NMR spectra

The ^{13}C NMR spectra of all compounds were obtained in DMSO-d_6 and are shown in the general scheme for ^{13}C NMR spectral interpretations in Scheme 3.

The ^{13}C NMR spectrum of compound 3e showed 14 different resonances in good agreement with the proposed structure as shown in Fig. 3. In compound **3e**, the –C=S (C9) signal of the carbohydrazide region was detected at 176.89 ppm. The characteristic –C=N (C8) and –C=O (C7) peaks of the isatin ring were observed at 136.97 and 163.06 ppm, respectively. The carbons (C1–C6) of the isatin ring were observed at 156.02, 112.50, 120.51, 134.07, 118.31, and 107.44 ppm, respectively. The C1 carbons atom shifted downfield (high values of δ) due to the presence of methoxy group. The aromatic carbons (C10–C15) of aryl ring were observed at between 145.18 and 124.49 ppm. The C13 (145.18 ppm) carbon atom was shifted downfield (high values of δ) due to the presence of nitro group. The methoxy (–OCH_3) peak were detected at 56.07 ppm. Additionally, the C atoms (for C10–C15) were also split into doublets due to interacting with the atomic nucleus of F for compounds **3b**, **3d**, and **3i**. These data are consistent with the values reported earlier for similar compounds [2, 6, 32]. The carbon chemical shift values of the synthesized compounds are given in Table 5 (see Supplementary Material).

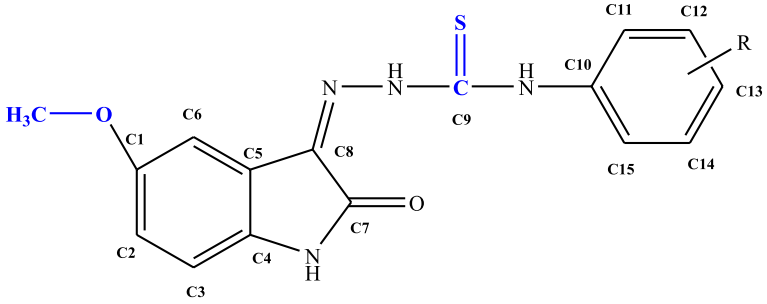
Evaluation of antioxidant activity

Due to concentration of Trolox and synthesized compounds, percent inhibition changes are given in Fig. 4. Accordingly, all the compounds produced a free radical scavenging which increased with concentration. Others compounds except for 3e showed a lower inhibition increase depending on concentration.

An antioxidant is defined as any substance that delays or prevents oxidation of a substrate, even at low concentrations as compared to an oxidizable substrate [34]. Antioxidant activity of all synthesized compounds performed using DPPH method

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

Comp.	–NH ist	=N–NH	–NH–Ar	Ist H1	Ist H2	Ist H3	Ar H4	Ar H5	Ar H6	Ar H7	Ar H8	OCH_3	*R*
3a	12.71	11.01	10.39	7.10–7.08	6.98–6.94	7.67–7.65	7.28–7.23		6.92–6.89	6.83–6.81	–	3.79	3.71
3b	12.81	11.02	10.64	7.32–7.30	7.47–7.43	7.32–7.30	–	6.83–6.81	6.92–6.89	7.27–7.21	7.39–7.34	3.71	–
3c	12.69	11.00	10.64	7.36–7.35	6.91–6.88	6.82–6.80	6.94–6.92	7.42–7.39	–	7.42–7.39	6.94–6.92	3.73	3.71
3d	12.81	11.03	10.79	7.36–7.35	6.92–6.90	6.82–6.80	7.10–7.05	–	7.47–7.38		7.58–7.54	3.72	–
3e	12.96	11.06	11.01	7.35–7.34	6.93–6.91	6.83–6.81	8.04–8.01	8.25–8.23	–	8.25–8.23	8.04–8.01	3.72	–
3f	12.84	11.03	10.73	7.29–7.28	6.93–6.90	6.83–6.81	–	7.74–7.73	–	7.49–7.46	7.55–7.53	3.72	–
3g	12.58	10.97	9.28	7.20–7.19	6.91–6.88	6.81–6.79	7.30–7.23		7.18–7.16	7.30–7.23		3.73	CH_2: 3.78 2.93
3h	12.70	11.01	10.64	7.35–7.34	6.91–6.88	6.83–6.80	–	7.30–7.28	7.24–7.22			3.71	2.21
3i	12.76	11.02	10.74	7.35–7.34	6.92–6.89	6.82–6.80	7.24–7.20	7.57–7.54	–	7.57–7.54	7.24–7.20	3.72	–
3j	12.82	11.04	10.79	7.36–7.35	6.93–6.91	6.83–6.81	7.74	–	7.61–7.59	7.43–7.39	7.31–7.29	3.72	–



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

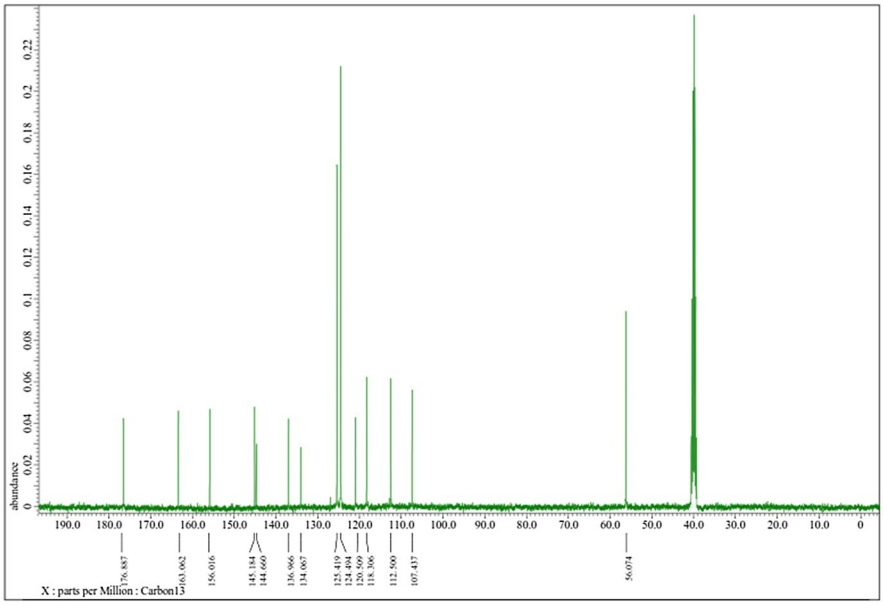


Fig. 3 ^{13}C NMR spectrum of compound **3e**

was expressed in IC_{50} values in Table 6. IC_{50} values of all the compounds were between 12.455 and 73.471 µM. Of all the test compounds, compound 3e had the most potential antioxidant activity against the DPPH radical. In particular, it has been found that the antioxidant activities of the test compounds are provided by the presence of electron-withdrawing groups on the aromatic ring [35].

As in the structure of the substituents in the carbohydrazone, the structure of the substituents in the thiocarbohydrazone plays an important role for antioxidant activity [36]. As previously reported by Kiran et al. [37] with novel bis-isatin carbohydrazone derivatives, halogen-bonded aromatic structures were found to be responsible for antioxidant activity. In addition, researchers have reported that the compound containing the nitro group (electron-withdrawing group) in

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

Comp.	C1	C2	C3	C4	C5	C6	C7 C=O	C8 C=N	C9 C=S	C10	C11	C12	C13	C14	C15	OCH_3
3a	155.84	112.47	121.13	132.97	118.15	106.65	163.24	136.79	177.07	127.55	128.35	120.65	127.51	112.34	153.90	56.28
																56.08
3b	159.11	112.47	130.80	133.51	118.23	106.91	163.29	136.82	178.54	129.74	156.64	121.27	126.98	116.65	124.99	56.11
										129.66	155.86	121.16	126.86	116.45	124.96	
3c	155.85	112.36	121.01	132.61	117.74	106.91	163.34	136.61	177.14	127.95	131.58	114.13	158.00	114.13	131.58	56.14
																55.81
3d	160.94	112.43	121.11	133.46	118.13	107.29	163.35	136.80	176.78	130.52	122.01	155.88	140.66	113.40	113.06	56.18
										130.43	121.98	155.79	140.54	113.19	112.82	
3e	156.02	112.50	120.51	134.07	118.31	107.44	163.06	136.97	176.89	144.66	125.42	124.49	145.18	124.49	125.42	56.07
3f	155.77	112.62	121.02	133.43	118.32	106.74	163.31	136.83	178.29	133.03	135.96	132.48	133.68	129.70	128.10	56.13
3g	155.82	112.47	121.53	139.14	117.48	106.19	163.29	136.39	177.56	132.63	129.02	126.55	129.13	126.55	129.02	56.17
																45.96
																34.43
3h	155.72	112.48	121.31	136.71	118.11	106.84	163.30	137.91	177.78	129.07	136.13	126.83	132.94	128.00	130.89	56.33
																18.20
3i	155.86	112.39	121.21	133.20	118.06	107.13	163.35	136.75	177.33	128.74	135.32	115.80	161.83	115.80	135.32	56.16
										128.65	135.29	115.57	159.41	115.57	135.29	
3j	155.77	112.44	121.13	133.50	118.15	107.27	163.24	136.84	176.88	133.01	126.45	140.32	124.77	130.53	125.76	56.20

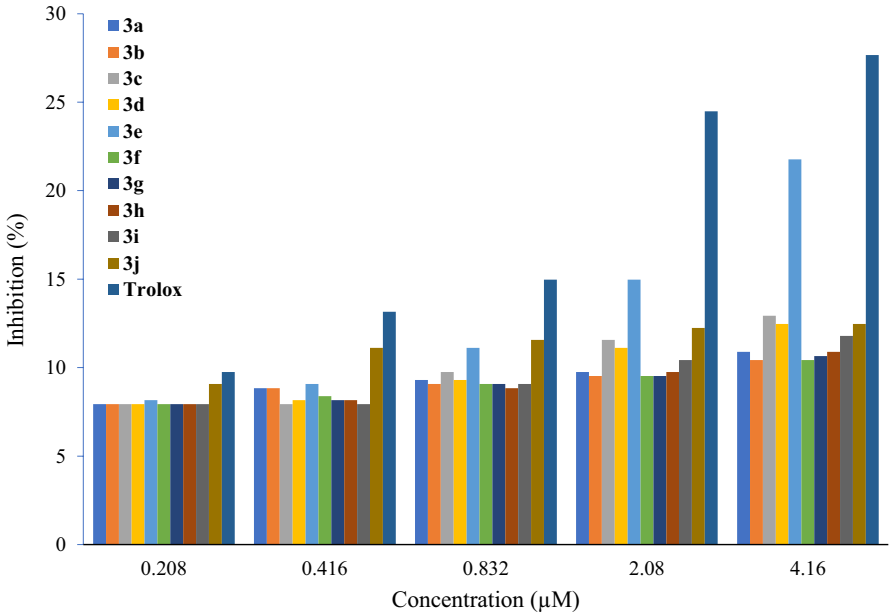


Fig. 4 Percent of inhibition calculated by the DPPH method for Trolox and compounds **3a–3j** at different concentrations

Table 6 IC_{50} values of Trolox and compounds **3a–3j**

Compounds	IC_{50} (μM)[a]
Trolox	8.757 ± 0.07
3a	66.178 ± 0.11
3b	79.927 ± 0.13
3c	32.626 ± 0.09
3d	36.258 ± 0.09
3e	12.455 ± 0.06
3f	73.471 ± 0.12
3g	64.704 ± 0.10
3h	57.545 ± 0.09
3i	42.269 ± 0.06
3j	64.478 ± 0.06

[a]IC_{50} = the concentration (μM) values are expressed in mean ± SD ($n = 3$)

the synthesized compounds shows more antioxidant activity than those containing substituted bromine, fluorine, and chlorine [31, 38–40]. In parallel with these studies, **3e** molecule showed more antioxidant activity than other electron-withdrawing substituted halogen groups. In this study, it was seen that the substituted nitro group structures of thiosemicarbazone molecules attached to methoxy isatin

exhibited active antioxidant properties, unlike the molecules synthesized with thiosemicarbazone-free isatin group by Khan et al. [41].

Conclusions

In this study, new 5-methoxyisatin-β-thiosemicarbazone derivatives have been obtained with excellent yields of 68–97%. All the synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR, and IR and by elemental analyses. The in vitro antioxidant activity of the compounds was determined by the DPPH free radical scavenging method. IC_{50} values of the newly synthesized isatin-β-thiosemicarbazone derivatives ranged from 12.455 to 73.471 μM. Compound 3e of among the test compounds showed the most acceptable antioxidant activity against the DPPH radical.

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References

1. M.C. Liu, T.S. Lin, J.G. Cory, A.H. Cory, A.C. Sartorelli, J. Med. Chem. **39**, 2586 (1996)
2. 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)
3. G.K. Kumar, G.E. Chavarria, A.K. Charlton-Sevcik, W.M. Arispe, M.T. MacDonough, T.E. Strecker, S.E. Chen, B.G. Siim, D.J. Chaplin, M.L. Trawick, Bioorg. Med. Chem. Lett. **20**, 1415 (2010)
4. N. Fujii, J.P. Mallari, E.J. Hansell, Z. Mackey, P. Doyle, Y. Zhou, J. Gut, P.J. Rosenthal, J.H. McKerrow, R.K. Guy, Bioorg. Med. Chem. Lett. **15**, 121 (2005)
5. W.X. Hu, W. Zhou, C.N. Xia, X. Wen, Bioorg. Med. Chem. Lett. **16**, 2213 (2006)
6. M.S. Almutairi, A.S. Zakaria, P.P. Ignasius, R.I. Al-Wabli, I.H. Joe, M.I. Attia, J. Mol. Struct. **1153**, 333 (2018)
7. Z. Bakherad, M. Safavi, A. Fassihi, H. Sadeghi-Aliabadi, M. Bakherad, H. Rastegar, J.B. Ghasemi, S. Sepehri, L. Saghaie, M. Mahdavi, Res. Chem. Intermed. **45**, 2827 (2019)
8. T.R. Bal, B. Anand, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett. **15**, 4451 (2005)
9. D. Banerjee, P. Yogeeswari, P. Bhat, A. Thomas, M. Srividya, D. Sriram, Eur. J. Med. Chem. **46**, 106 (2011)
10. J. Dimmock, J. McColl, S. Wonko, R. Thayer, D. Hancock, Eur. J. Med. Chem. **26**, 529 (1991)
11. A. Kshirsagar, M.P. Toraskar, V.M. Kulkarni, S. Dhanashire, V. Kadam, Int. J. Chem. Tech. Res **1**, 696 (2009)
12. D.L. Klayman, J.P. Scovill, J.F. Bartosevich, C.J. Mason, J. Med. Chem. **22**, 1367 (1979)
13. R.B. de Oliveira, E.M. de Souza-Fagundes, R.P. Soares, A.A. Andrade, A.U. Krettli, C.L. Zani, Eur. J. Med. Chem. **43**, 1983 (2008)
14. I.H. Hall, S. Chen, K. Rajendran, D. West, Appl. Organomet. Chem. **10**, 485 (1996)
15. 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)
16. C. Shipman Jr., S.H. Smith, J.C. Drach, D.L. Klayman, Antimicrob. Agents Chemother. **19**, 682 (1981)
17. M. Bingul, E. Şenkuytu, M.F. Saglam, M. Boga, H. Kandemir, I.F. Sengul, Res. Chem. Intermed. **45**, 4487 (2019)
18. H. Pervez, M.S. Iqbal, M.Y. Tahir, F.U.H. Nasim, M.I. Choudhary, K.M. Khan, J. Enzyme Inhib. Med. Chem. **23**, 848 (2008)
19. H. Govender, C. Mocktar, H.M. Kumalo, N.A. Koorbanally, Phosphorus Sulfur Silicon Relat. Elem. **194**, 1074 (2019)

20. Z.H. Chohan, H. Pervez, A. Rauf, K.M. Khan, C.T. Supuran, J. Enzyme Inhib. Med. Chem. **19**, 417 (2004)
21. S.Y. Abbas, A.A. Farag, Y.A. Ammar, A.A. Atrees, A.F. Mohamed, A.A. El-Henawy, Monatsh. Chem. **144**, 1725 (2013)
22. S. Dilber, M. Saban, A. Gelineo, L. Arsenijević, M. Bogavac, S. Pavlov, Pharmazie **45**, 800 (1990)
23. A. Patel, S. Bari, G. Talele, J. Patel, M. Sarangapani, Iran. J. Pharm. Sci. **5**, 249 (2010)
24. A. Andreani, S. Burnelli, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, L. Varoli, M.A. Cremonini, G. Placucci, Eur. J. Med. Chem. **45**, 1374 (2010)
25. J. Haribabu, G. Subhashree, S. Saranya, K. Gomathi, R. Karvembu, D. Gayathri, J. Mol. Struct. **1110**, 185 (2016)
26. T. Aboul-Fadl, F.A. Bin-Jubair, Int. J. Res. Pharm. Sci. **1**, 113 (2010)
27. D. Sriram, P. Yogeeswari, K. Meena, Pharmazie **61**, 274 (2006)
28. S.N. Pandeya, A.S. Raja, J.P. Stables, J. Pharm. Pharm. Sci. **5**, 266 (2002)
29. K. Tadele, J. Pharm. Med. Res. **3**, 73 (2017)
30. W. Brand-Williams, M.-E. Cuvelier, C. Berset, Lwt-Food Sci. Technol. **28**, 25 (1995)
31. N. Naik, H. Vijay Kumar, P.B. Vidyashree, J. Pharm. Res. **4**, 2686 (2011)
32. H.P. Ebrahimi, J.S. Hadi, T.A. Alsalim, T.S. Ghali, Z. Bolandnazar, Spectrochim Acta A Mol. Biomol. Spectrosc. **137**, 1067 (2015)
33. D. Williams, I. Fleming, *Spectroscopic Methods in Organic Chemistry* (McGraw-Hill, New York, 1996)
34. F. Shahidi, *Handbook of Antioxidants for Food Preservation* (Elsevier, Amsterdam, 2015), pp. 1–14
35. G. Kiran, T. Maneshwar, Y. Rajeshwar, M. Sarangapani, J. Chem. (2013). https://doi.org/10.1155/2013/192039
36. A. Božić, N. Filipović, I. Novakovic, S. Bjelogrlić, J. Nikolić, S. Drmanić, A. Marinković, J. Serb. Chem. Soc. **82**, 5 (2017)
37. G. Kiran, M. Sarangapani, T. Gouthami, A.R. Narsimha Reddy, Toxicol. Environ. Chem. **95**, 367 (2013)
38. P. Pakravan, S. Kashanian, M.M. Khodaei, F.J. Harding, Pharmacol. Rep. **65**, 313 (2013)
39. H. Sudhamani, S.K. Thaslim Basha, S. Adam, B. Vijay Bhaskar, C. Naga Raju, Monatsh. Chem. **148**, 1525 (2017)
40. M. AlGhorbania, N.D. Rekhab, V. Lakshmi Ranganathaa, T. Prashantha, T. Veerabasappagowdab, S.A. Khanuma, Russ. J. Bioorg. Chem. **41**, 554 (2015)
41. K.M. Khan, M. Khan, N. Ambreen, F. Rahim, B. Muhammad, S. Ali, S.M. Haider, S. Perveen, M. Choudhary, J. Pharm. Res **4**, 10 (2011)