

# Synthesis, Structural Characterization, and Biological Evaluation of Some Hydrazone Compounds as Potential Antioxidant Agents

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**Abstract**—Antioxidants are sufficiently stable molecules that neutralize free radicals by electron transfer and thereby reduce their damaging capacity. Hydrazones, which are frequently used in the discovery studies of new antioxidant agents, are bioactive compounds with very significant utilization areas because of their various biological and clinical applications. In this study, in vitro antioxidant activities of the previously synthesized hydrazone compounds against various free radicals were examined, one by one. It was determined that among the synthesized compounds (**II–VIII**), especially the compound (**II**) had a significant effect ( $IC_{50} = 4.4 \pm 0.04 \mu\text{g/mL}$  for DPPH,  $IC_{50} = 1.8 \pm 0.07 \mu\text{g/mL}$  for ABTS and  $A_{0.5} = 2.2 \pm 0.02 \mu\text{g/mL}$  for CUPRAC) on the prevention of free radical-induced oxidative stress, as better results were obtained than the standard substance. All synthesized compounds showed good antioxidant effects compared to BHA in the CUPRAC method. The obtained results demonstrate that most of the synthesized molecules are promising antioxidant agents.

**Keywords:** free radicals, hydrazones, antioxidant agent, antioxidant activity

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## INTRODUCTION

Free radicals are short-lived, unstable, with very low molecular weight and highly active atoms or molecules that contain one or more unpaired electrons in their atomic orbital. Since free radicals are very unstable and highly reactive structures, molecules can easily take up an electron or share an electron. Therefore, they show both a reducing and oxidizing behavior. They attack essential macromolecules in the body, leading to cell damage and homeostatic degradation. Free radicals damage biological molecules in the membranes of cells, such as cell nuclei, DNA, proteins, carbohydrates, and lipids. In these processes, reactive oxygen species such as hydrogen peroxide, hydroxyl radical, superoxide anion, peroxynitrite radical and peroxy radical are formed [1]. Oxidative damage caused by free radicals on biomolecules is defined as oxidative stress [2]. Cells and tissues have a system called antioxidants [3] that inhibit radical products and reactions. It is molecules that reduce or prevent the oxidation of a substrate at low concentrations, mainly due to its free radical scavenging properties, thus fighting against the oxidation created by free

radicals. The low molecular weight antioxidants can safely interact with free radicals and terminate the chain reaction before important molecules are damaged [3, 4]. These substances taken with foods are important in preventing various health problems such as cancer and cardiovascular diseases and delaying the aging process [5, 6]. Therefore, obtaining these molecules synthetically or from natural sources is of great importance.

The design and synthesis studies of new bioactive molecules possessing biological activities such as antioxidant, anticancer, anti-Alzheimer, etc., have attracted significant attention in recent years [7–10]. Especially, medicinal chemists are constantly synthesizing new molecules and are studying their biological activities in the various fields extensively. They are trying to identify the molecules that have the potential to be used as drug candidates [8, 11]. For this, much research has been carried out in this field by using a large number of molecules belonging to different classes of organic compounds. The studies on this subject, which are still in the research and development stage, have been going on [12, 13].

Hydrazones are among the organic compounds whose biological activities are mainly investigated [14,

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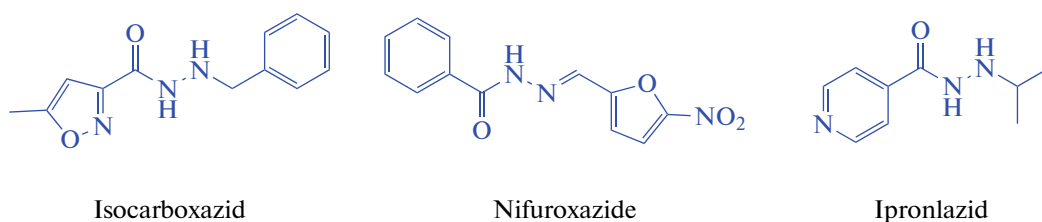


Fig. 1. Hydrazone and hydrazide derivatives used as drugs

15]. These compounds are easily obtained by the condensation reaction of the hydrazides with aldehydes and ketones in various organic solvents. They are considered as one of the most important classes of organic compounds extensively employed for the design and discovery of bioactive agents in medicinal chemistry due to their broad spectrum of biological activities [14, 16]. In recent years, many researchers have synthesized them as target molecules in their studies [17]. Consequently, many biologically active hydrazone compounds possessing the various electron-donor and electron-withdrawing functional groups on their molecular scaffolds have been prepared up to now [16, 18, 19]. The vast majority of these hydrazone compounds in the experimental studies have been determined to show antimicrobial [20], antidepressant [21], anti-inflammatory [22], anticancer [20], and antitubercular activities [23], etc. Apart from their biological properties listed above, it has been put forth in the literature survey that many of these compounds have also demonstrated antioxidant activities [20, 24–29]. In addition to these, many hydrazide-hydrazone derivatives have been synthesized and employed as drugs so far. Examples of these are drugs such as isocarboxazid, nifuroxazide, and iproniazid (Fig. 1) [30].

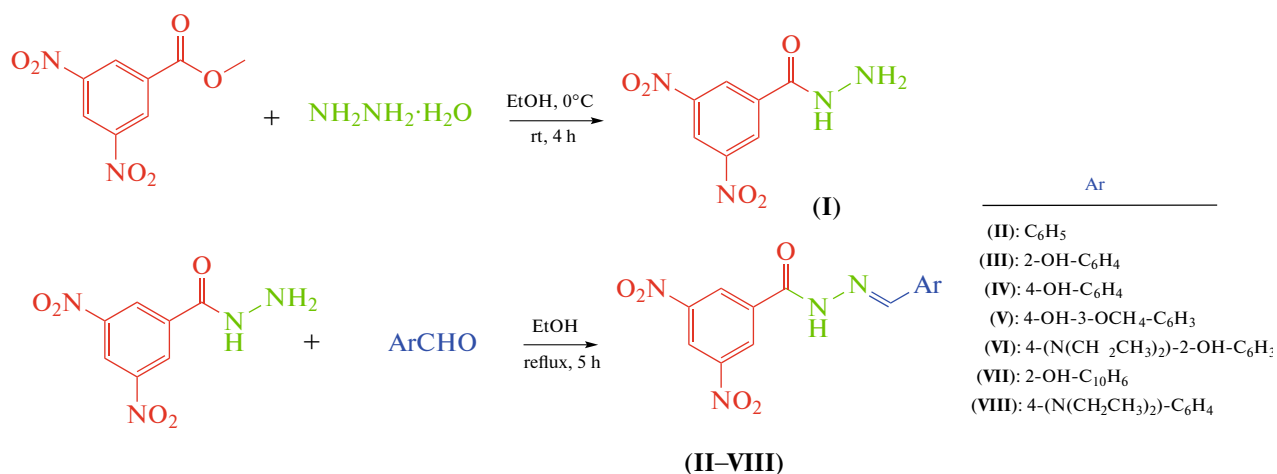
In the light of the information given above, the current study has been conducted to contribute to the ongoing studies on the design and the discovery of newer and safer antioxidant agents. It has been determined in the literature research that the compounds

employed in this study have been used in different studies until now. Still, the lack of antioxidant activity studies encouraged us to realize this study. So, the antioxidant activity of these compounds have been investigated for the first time by us. This situation encouraged us to realize this study. Herein, we have reported synthesis, characterization, and biological evaluation of some hydrazone compounds (II–VIII) derived from 3,5-dinitrobenzohydrazide (I) as effective antioxidant agents. All previously-synthesized hydrazone compounds were re-characterized by employing elemental analysis and some spectroscopic techniques such as FT-IR,  $^1\text{H}$ - and  $^{13}\text{C}$  NMR.

## RESULTS AND DISCUSSION

### Synthesis and Structural Analysis

The synthetic approaches for the synthesis of target molecules were demonstrated in Scheme 1. In this study, hydrazide (I) and seven hydrazone compounds (II–VIII) were re-synthesized in high purity and good yields (76–89%) in two steps according to the procedures reported in the literature [31–36]. The molecular structures of hydrazide (I) and hydrazone derivatives (II–VIII) synthesized were characterized by specific properties (molecular weight, melting point, color, and yield) and spectral characteristics (elemental analysis, IR,  $^1\text{H}$ - and  $^{13}\text{C}$  NMR) are presented Tables 1 and 2, respectively.



Scheme 1. Synthesis pathway of hydrazide (I) and hydrazone compounds (II–VIII).

**Table 1.** The specific properties of all synthesized compounds (I–VIII)

Comp.	Molecular formula	Molecular weight, g/mol	Melting point, °C	Color	% Yield
(I)	C <sub>7</sub> H <sub>6</sub> N <sub>4</sub> O <sub>5</sub>	226.15	156–157 (lit. [31] 153–154)	Yellowish solid	83
(II)	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> O <sub>5</sub>	314.26	268–269 (lit. [32] 262)	White solid	86
(III)	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> O <sub>6</sub>	330.26	240–241 (lit. [33] 238)	Yellow solid	81
(IV)	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> O <sub>6</sub>	330.26	272–273 (lit. [35] 270–274)	Yellow solid	76
(V)	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>7</sub>	360.28	308–309	Orange solid	79
(VI)	C <sub>18</sub> H <sub>19</sub> N <sub>5</sub> O <sub>6</sub>	401.38	266–267	Red solid	84
(VII)	C <sub>18</sub> H <sub>12</sub> N <sub>4</sub> O <sub>6</sub>	380.32	319 (lit. [33] 240)	Yellow solid	89
(VIII)	C <sub>16</sub> H <sub>15</sub> N <sub>5</sub> O <sub>5</sub>	357.33	284–285	Red solid	80

In the FT-IR spectra of hydrazide (I), the absorption peak of the N–H band and carbonyl stretching (C=O) were observed at 3352–3279 and 1644 cm<sup>-1</sup>, respectively. When FT-IR spectra of all hydrazone compounds (II–VIII) were examined, N-H and C=N absorption bands, which are good evidence of the hydrazone compounds (–NH–N=CH–) was formed, were detected in the 3249–3100 and 1625–1590 cm<sup>-1</sup> regions, respectively. A specific strong band in the 1685–1650 cm<sup>-1</sup> region of C=O confirmed the hydrazone property of all compounds [37]. Additionally, asymmetric and symmetric stretching bands of NO<sub>2</sub> group in hydrazone compounds were determined as 1539–1519 and 1341–1334 cm<sup>-1</sup>, respectively [38]. The chemical structure of the hydrazide compound (I) was confirmed by the presence of 10.52 ppm (–CONH–) and 4.76 ppm (–NH<sub>2</sub>) signals for hydrazide protons (–CONHNH<sub>2</sub>) according to the <sup>1</sup>H NMR spectrum. In the <sup>1</sup>H NMR spectra of the hydrazone compounds (II–VIII), the proton belonging to NH group resonated as a singlet at 12.73–12.14 ppm. Another evidence that hydrazone compounds were synthesized was the disappearance of the proton peaks of free amino group of the hydrazide compound. The proton peaks belonging to the azomethine group were observed to resonate at 9.22–8.69 ppm and singlet as expected in all seven compounds. Also, the signal of aromatic protons was detected in the expected region. <sup>13</sup>C NMR spectrum of hydrazone molecules was found that C=O carbon in the carbonyl group resonated at 160.32–157.86 ppm, the aromatic carbons resonated in the range of 159.06–96.76 ppm. Resonance of the carbon atom of –CH=N (azomethine) in the range of 154.94–152.72 ppm was found to be compatible with the literature data [28].

#### Antioxidant Activity Results

While the hydrazone compounds (II–VIII) synthesized in this study are used for different purposes, their antioxidant activity has not been evaluated

before. There have been many studies in the literature on antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory and neuraminidase inhibitory effects of these compounds [39, 40]. However, no study has been found on the antioxidant activity of these compounds up to now. Our study fills a gap in the literature in this respect. All obtained molecules were easily synthesized with pretty good yields. Their molecular structures were identified by utilizing elemental analysis and the various spectroscopic techniques such as FT-IR, <sup>1</sup>H and <sup>13</sup>C NMR. In this study, three methods (ABTS, DPPH, and CUPRAC) were employed to determine the antioxidant capacities of the target molecules. Butylated hydroxyanisole (BHA) was utilized as a positive control. IC<sub>50</sub> and A<sub>0.5</sub> values of synthesized molecules with BHA are presented in Table 3.

DPPH method is one of the most widely employed spectrophotometric methods in antioxidant activity measurements [41]. The interaction of DPPH reagent with all substances in the mixture, even the weakest antioxidants, and reaction with both lipophilic and hydrophilic antioxidants is the advantage of this method [42]. Hydrazones are known to have many biological activities [43, 44]. The result we obtained in this study also supports this consideration. Compound (I) has exhibited good activity (IC<sub>50</sub> = 9.2 ± 0.01 µg/mL) than both BHA (IC<sub>50</sub> = 49.0 ± 0.09 µg/mL) and other compounds (except compound (II)) in DPPH test system. Compounds (II) (IC<sub>50</sub> = 4.4.0 ± 0.04 µg/mL) and (VI) (IC<sub>50</sub> = 25.1 ± 0.30 µg/mL) have also exhibited good activity than BHA (IC<sub>50</sub> = 49.0.0 ± 0.09 µg/mL), respectively. Activity ordering of compounds is determined as (II) > (VI) > BHA > (VIII) > (III) > (IV) > (V) > (VII) in the DPPH test system. Figure 2 represents the inhibition percentage (I%) of different concentrations of the molecules. The high activities of the compounds (II) and (VI) have been exhibited striking activity even at low concentrations (Table 3).

ABTS radical is soluble in both aqueous and organic solvents and thus can be used to measure the

**Table 2.** Molecular structures and spectral data of all of the synthesized compounds (I–VIII)

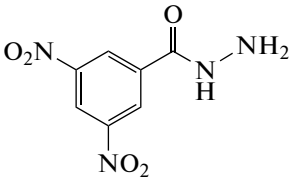
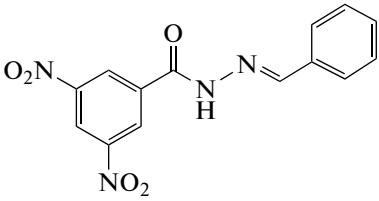
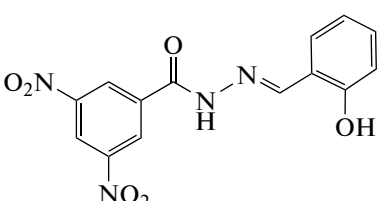
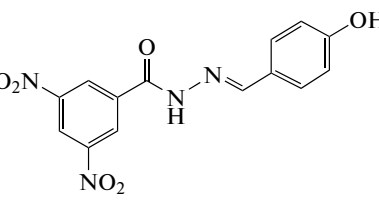
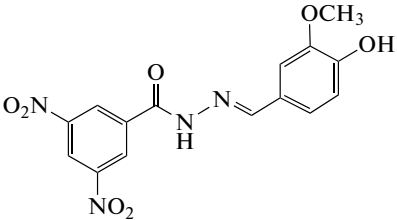
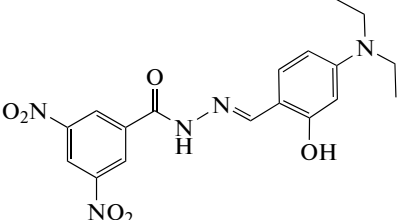
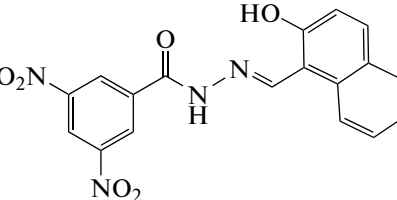
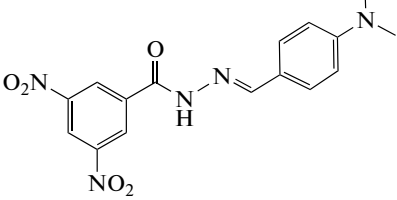
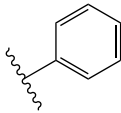
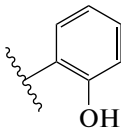
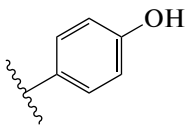
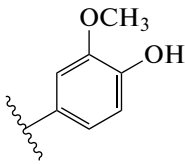
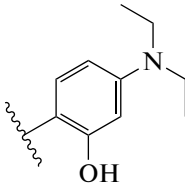
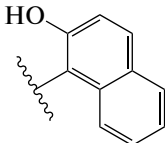
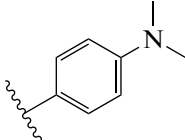
Comp.	Chemical Structures	Spectral data [FT-IR (ATR) $\nu_{\max}$ $\text{cm}^{-1}$ ] and $^1\text{H}$ NMR/ $^{13}\text{C}$ NMR (ppm) $\delta$ ; CHN analysis]
(I)	 <p>3,5-Dinitrobenzohydrazide [31]</p>	<p>FT-IR (ATR) <math>\nu_{\max}</math> <math>\text{cm}^{-1}</math>: 3352, 3279 (N–H), 3088 (C–H), 1644 (C=O), 1530 (NO<sub>2</sub>, asymmetric), 1340 (NO<sub>2</sub>, symmetric) (Fig. S1). <math>^1\text{H}</math> NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 10.52 (s, 1H, CONH), 9.02 (d, <math>J</math> = 2.0 Hz, 2H, ArH), 8.94 (t, <math>J</math> = 2.1 Hz, 1H, ArH), 4.76 (s, 2H, NH<sub>2</sub>) (Fig. S2). <math>^{13}\text{C}</math> NMR (100 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 161.77 (C=O), 148.63, 136.37, 127.63, 121.10 (ArC) (Fig. S3). CHN analysis: C, 37.18; H, 2.67; N, 24.77%. Found: C, 37.40; H, 2.70; N, 24.34%</p>
(II)	 <p><i>N'</i>-Benzylidene-3,5-dinitrobenzohydrazide [32]</p>	<p>FT-IR (ATR) <math>\nu_{\max}</math> <math>\text{cm}^{-1}</math>: 3176 (N–H), 3094 (C–H), 2874 (C–H), 1661 (C=O), 1625 (C=N), 1528 (NO<sub>2</sub>, asymmetric), 1334 (NO<sub>2</sub>, symmetric) (Fig. S4). <math>^1\text{H}</math> NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 12.43 (s, 1H, NH), 9.14 (d, <math>J</math> = 2.0 Hz, 2H, ArH), 9.01 (t, <math>J</math> = 2.0 Hz, 1H, ArH), 8.52 (s, 1H, N=CH), 7.79 (dd, <math>J</math> = 6.4, 2.8 Hz, 2H, ArH), 7.50 (dd, <math>J</math> = 5.2, 2.0 Hz, 3H, ArH) (Fig. S5). <math>^{13}\text{C}</math> NMR (100 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 159.50 (C=O), 150.12 (C=N), 148.65, 136.40, 134.29, 131.05, 129.39, 128.37, 127.83, 121.75 (ArC) (Fig. S6). CHN analysis: C, 53.51; H, 3.21; N, 17.83%. Found: C, 53.66; H, 3.31; N, 17.88%</p>
(III)	 <p><i>N'</i>-(2-Hydroxybenzylidene)-3,5-dinitrobenzohydrazide [33]</p>	<p>FT-IR (ATR) <math>\nu_{\max}</math> <math>\text{cm}^{-1}</math>: 3329 (O–H), 3177 (N–H), 3096 (C–H), 2874 (C–H), 1685 (C=O), 1613 (C=N), 1527 (NO<sub>2</sub>, asymmetric), 1339 (NO<sub>2</sub>, symmetric) (Fig. S7). <math>^1\text{H}</math> NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 12.59 (s, 1H, NH), 10.93 (s, 1H, OH), 9.15 (d, <math>J</math> = 2.0 Hz, 2H, ArH), 8.99 (t, <math>J</math> = 2.0 Hz, 1H, ArH), 8.73 (s, 1H, N=CH), 7.64 (d, <math>J</math> = 6.8 Hz, 1H, ArH), 7.36–7.30 (m, 1H, ArH), 6.94 (d, <math>J</math> = 7.6 Hz, 2H, ArH) (Fig. S8). <math>^{13}\text{C}</math> NMR (100 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 159.11 (C=O), 149.44 (C=N), 157.86, 148.61, 135.93, 132.36, 129.23, 128.31, 121.73, 119.90, 119.15, 116.84 (ArC) (Fig. S9). CHN analysis: C, 50.92; H, 3.05; N, 16.97%. Found: C, 50.94; H, 3.06; N, 16.91%</p>
(IV)	 <p><i>N'</i>-(4-Hydroxybenzylidene)-3,5-dinitrobenzohydrazide [34]</p>	<p>FT-IR (ATR) <math>\nu_{\max}</math> <math>\text{cm}^{-1}</math>: 3326 (O–H), 3172 (N–H), 3100 (C–H), 2876 (C–H), 1658 (C=O), 1597 (C=N), 1527 (NO<sub>2</sub>, asymmetric), 1340 (NO<sub>2</sub>, symmetric) (Fig. S10). <math>^1\text{H}</math> NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 12.22 (s, 1H, NH), 10.01 (s, 1H, OH), 9.12 (d, <math>J</math> = 2.0 Hz, 2H, ArH), 8.99 (t, <math>J</math> = 2.0 Hz, 1H, ArH), 8.40 (s, 1H, N=CH), 7.61 (d, <math>J</math> = 8.8 Hz, 2H, ArH), 6.86 (d, <math>J</math> = 8.4 Hz, 2H, ArH) (Fig. S11). <math>^{13}\text{C}</math> NMR (100 MHz, DMSO-<i>d</i><sub>6</sub>, ppm): <math>\delta</math> 160.32 (C=O), 150.35 (C=N), 159.06, 148.60, 136.58, 129.65, 128.24, 125.24, 121.52, 116.21 (ArC) (Fig. S12). CHN analysis: C, 50.92; H, 3.05; N, 16.97%. Found: C, 50.94; H, 3.08; N, 16.94%</p>

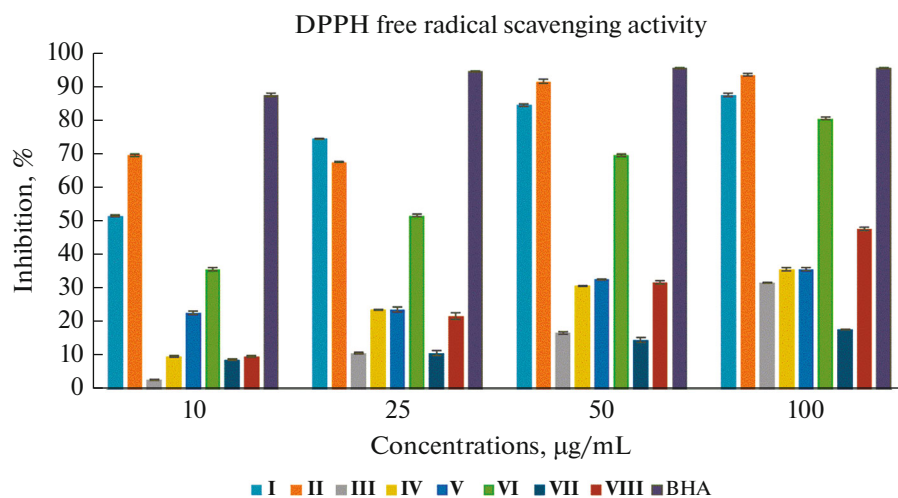
Table 2. (Contd.)

Comp.	Chemical Structures	Spectral data [FT-IR (ATR) $\nu_{\max}$ $\text{cm}^{-1}$ ] and $^1\text{H}$ NMR/ $^{13}\text{C}$ NMR (ppm) $\delta$ ; CHN analysis]
(V)	 <p><i>N'</i>-(4-Hydroxy-3-methoxybenzylidene)-3,5-dinitrobenzohydrazide [35]</p>	FT-IR (ATR) $\nu_{\max}$ $\text{cm}^{-1}$ : 3334 (O–H), 3249 (N–H), 3091 (C–H), 2977, 2859 (C–H), 1673 (C=O), 1597 (C=N), 1528 (NO <sub>2</sub> , asymmetric), 1334 (NO <sub>2</sub> , symmetric) (Fig. S13). $^1\text{H}$ NMR (400 MHz, DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 12.21 (s, 1H, NH), 9.62 (s, 1H, OH), 9.10 (d, $J$ = 2.0 Hz, 2H, ArH), 8.97 (t, $J$ = 2.0 Hz, 1H, ArH), 8.37 (s, 1H, N=CH), 7.32 (d, $J$ = 1.2 Hz, 1H, ArH), 7.12 (dd, $J$ = 8.0, 1.6 Hz, 1H, ArH), 6.84 (d, $J$ = 8.0 Hz, 1H, ArH), 3.85 (s, 3H, OCH <sub>3</sub> ) (Fig. S14). $^{13}\text{C}$ NMR (100 MHz, DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 159.20 (C=O), 150.66 (C=N), 149.95, 148.66, 136.63, 130.77, 128.27, 127.64, 125.66, 123.16, 115.94, 109.53 (ArC), 56.05 (OCH <sub>3</sub> ) (Fig. S15). CHN analysis: C, 50.01; H, 3.36; N, 15.55%. Found: C, 50.11; H, 3.33; N, 15.51%
(VI)	 <p><i>N'</i>-(4-(Diethylamino)-2-hydroxybenzylidene)-3,5-dinitrobenzohydrazide</p>	FT-IR (ATR) $\nu_{\max}$ $\text{cm}^{-1}$ : 3428 (O–H), 3294 (N–H), 3096 (C–H), 2972, 2873 (C–H), 1681 (C=O), 1624 (C=N), 1519 (NO <sub>2</sub> , asymmetric), 1334 (NO <sub>2</sub> , symmetric) (Fig. S16). $^1\text{H}$ NMR (400 MHz, DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 12.36 (s, 1H, NH), 11.14 (s, 1H, OH), 9.13 (d, $J$ = 2.0 Hz, 2H, ArH), 8.99 (t, $J$ = 2.0 Hz, 1H, ArH), 8.51 (s, 1H, N=CH), 7.29 (d, $J$ = 8.8 Hz, 1H, ArH), 6.30 (dd, $J$ = 8.8, 2.4 Hz, 1H, ArH), 6.14 (d, $J$ = 2.0 Hz, 1H, ArH), 3.39 (q, 4H, N(CH <sub>2</sub> ) <sub>2</sub> ), 1.12 (t, 6H, (CH <sub>3</sub> ) <sub>2</sub> ) (Fig. S17). $^{13}\text{C}$ NMR (100 MHz, DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 160.18 (C=O), 151.43 (C=N), 158.46, 151.01, 148.62, 136.27, 131.86, 128.16, 121.49, 106.69, 104.34, 97.76 (ArC), 44.30 N(CH <sub>2</sub> ) <sub>2</sub> , 12.99 (CH <sub>3</sub> ) <sub>2</sub> (Fig. S18). CHN analysis: C, 53.86; H, 4.77; N, 17.45%. Found: C, 53.91; H, 4.70; N, 17.49%
(VII)	 <p><i>N'</i>-((2-Hydroxynaphthalen-1-yl)methylene)-3,5-dinitrobenzohydrazide [33]</p>	FT-IR (ATR) $\nu_{\max}$ $\text{cm}^{-1}$ : 3182 (N–H), 3100 (N–H), 3015 (C–H), 2852 (C–H), 1651 (C=O), 1621 (C=N), 1539 (NO <sub>2</sub> , asymmetric), 1341 (NO <sub>2</sub> , symmetric) (Fig. S19). $^1\text{H}$ NMR (400 MHz, DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 12.73 (s, 1H, NH), 12.38 (s, 1H, OH), 9.50 (s, 1H, N=CH), 9.18 (br.s, 2H, ArH), 9.03 (br.s, 1H, ArH), 8.45 (d, $J$ = 8.4 Hz, 1H, naphthyl–H), 7.97 (d, $J$ = 8.8 Hz, 1H, naphthyl–H), 7.92 (d, $J$ = 7.6 Hz, 1H, naphthyl–H), 7.64 (t, $J$ = 7.2 Hz, 1H, naphthyl–H), 7.44 (t, $J$ = 7.2 Hz, 1H, naphthyl–H), 7.26 (d, $J$ = 8.8 Hz, 1H, naphthyl–H) (Fig. S20). $^{13}\text{C}$ NMR (100 MHz, CDCl <sub>3</sub> and DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 158.90 (C=O), 149.14 (C=N), 148.83, 136.21, 133.60, 132.21, 129.94, 129.35, 128.35, 128.25, 128.16, 123.95, 121.67, 121.25, 119.26, 108.92 (ArC) (Fig. S21). CHN analysis C <sub>18</sub> H <sub>12</sub> N <sub>4</sub> O <sub>6</sub> : C, 56.85; H, 3.18; N, 14.73%. Found: C, 56.81; H, 3.21; N, 14.66%
(VIII)	 <p><i>N'</i>-(4-(Dimethylamino)benzylidene)-3,5-dinitrobenzohydrazide [36]</p>	Red solid. Yield: 80%. Mp 284–285°C. FT-IR (ATR) $\nu_{\max}$ $\text{cm}^{-1}$ : 3198 (N–H), 3064 (C–H), 2852, 2814 (C–H), 1650 (C=O), 1590 (C=N), 1522 (NO <sub>2</sub> , asymmetric), 1335 (NO <sub>2</sub> , symmetric) (Fig. S22). $^1\text{H}$ NMR (400 MHz, DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 12.14 (s, 1H, NH), 9.12 (d, $J$ = 2.0 Hz, 2H, ArH), 8.99 (t, $J$ = 2.0 Hz, 1H), 8.37 (s, 1H, N=CH), 7.59 (d, $J$ = 8.8 Hz, 2H, ArH), 6.78 (d, $J$ = 8.8 Hz, 2H, ArH), 3.00 (s, 6H, N(CH <sub>3</sub> ) <sub>2</sub> ) (Fig. S23). $^{13}\text{C}$ NMR (100 MHz, CDCl <sub>3</sub> and DMSO- <i>d</i> <sub>6</sub> , ppm): $\delta$ 158.97 (C=O), 151.06 (C=N), 152.39, 148.72, 136.99, 129.27, 128.20, 121.56, 121.42, 112.30 (ArC), 40.81 (N(CH <sub>3</sub> ) <sub>2</sub> ) (Fig. S24). CHN analysis: C, 53.78; H, 4.23; N, 19.60%. Found: C, 53.56; H, 4.42; N, 19.85%

**Table 3.** The antioxidant activity of all synthesized compounds (I–VIII)

Comp.	Ar	DPPH IC <sub>50</sub> , µg/mL	ABTS IC <sub>50</sub> , µg/mL	CUPRAC A <sub>0.5</sub> , µg/mL
(I)	—	9.2 ± 0.01	3.4 ± 0.03	4.3 ± 0.08
(II)		4.4 ± 0.04	1.8 ± 0.07	2.2 ± 0.02
(III)		157.1 ± 0.10	12.0 ± 0.06	11.8 ± 0.08
(IV)		188.2 ± 0.10	46.8 ± 0.02	10.4 ± 0.01
(V)		194.3 ± 0.08	6.0 ± 0.06	24.1 ± 0.04
(VI)		25.1 ± 0.30	8.4 ± 0.20	17.2 ± 0.16
(VII)		282.2 ± 0.06	5.7 ± 0.23	18.6 ± 0.03
(VIII)		101.1 ± 0.7	19.0 ± 0.08	7.3 ± 0.12
BHA*	—	49.0 ± 0.09	2.1 ± 0.01	26.0 ± 0.20

Results are given as means ± sd. \* Positive control.



**Fig. 2.** Inhibition percentage (1%) of different concentrations of the samples in DPPH test system. Results were expressed as means and standard deviation.

antioxidant capacity of lipophilic and hydrophilic compounds. Compound (II) exhibited excellent activity ( $IC_{50} = 1.8 \pm 0.07 \mu\text{g/mL}$ ) than other compounds in the ABTS test system and the DPPH test system. When we compare the compounds (VII) ( $IC_{50} = 5.7 \pm 0.23 \mu\text{g/mL}$ ) and (V) ( $IC_{50} = 6.0 \pm 0.06 \mu\text{g/mL}$ ) to the BHA ( $IC_{50} = 2.1 \pm 0.01 \mu\text{g/mL}$ ), we see that they exhibit a substantial activity. Activity ranking of the compounds has been identified as (II) > BHA > (VII) > (V) > (VI) > (III) > (VIII) > (IV) in the ABTS test system, and compound (II) again has excellent activity even than BHA.

CUPRAC reagent is stable, inexpensive, easily available, and responsive to hydrophilic and lipophilic antioxidants. In this method, the results were interpreted as absorbance values different from ABTS and DPPH methods, and the absorbance 0.5 was calculated as  $\mu\text{g/mL}$ . In this assay, all compounds presented excellent activity than BHA ( $A_{0.5} = 26.0 \pm 0.20 \mu\text{g/mL}$ ). Then, again compounds (II) ( $A_{0.5} = 2.2 \pm 0.02 \mu\text{g/mL}$ ) and (VIII) ( $A_{0.5} = 7.3 \pm 0.12 \mu\text{g/mL}$ ) presented better activity than others. Activity ordering of compounds was determined as (II) > (VIII) > (IV) > (III) > (VI) > (VII) > (V) > BHA in the CUPRAC test system.

It should be kept in mind that because the action mechanism of each test system is different, a sample exhibiting low activity in one test may show high activity in the other. Therefore, it is essential to test more than one method in antioxidant activity determination studies. The synthesized hydrazone/hydrazone compounds exhibited different levels of antioxidant activity. Considering that a low  $IC_{50}$  value indicates high antioxidant activity, we see that compound (II) in this study has excellent activity in all three test methods (Table 3). Comparing the compounds according to all

test systems, shows that the order of activity differs. For example, we know that compound (V) exhibits relatively low activity in the DPPH and CUPRAC test systems but high activity in the ABTS test system. This reveals the necessity of using more than one test system in the antioxidant activity determination studies, as mentioned above.

In the literature, hydrazone compounds are frequently employed in the discovery studies of molecules with antioxidant activity. In one of the studies to discover new antioxidant agents, Bozkurt et al., [28] synthesized a series of hydrazone derivatives and determined that some of the synthesized compounds exhibited higher activity for the lipid peroxidation inhibitory activity in the  $\beta$ -carotene/linoleic acid assay. In DPPH free scavenging activity and the cation radical scavenging activity in  $ABTS^{+\cdot}$  activity, compound (IIb) was found to be more active with  $IC_{50} = 4.13 \pm 0.54 \mu\text{M}$ . It was determined that, the  $A_{0.5}$  values of all synthesized compounds were better than  $\alpha$ -tocopherol in the CUPRAC reduced power assay. In another study using hydrazone derivatives, Sicak et al., [29] synthesized a series of fluorine-containing chiral hydrazone-hydrazone derivatives as new antioxidant agents and found that compounds (V) ( $IC_{50} = 2.2 \pm 0.0 \mu\text{M}$  for CUPRAC), (IX) ( $IC_{50} = 1.2 \pm 0.4 \mu\text{M}$  for  $ABTS^{+\cdot}$ ), and (X) ( $IC_{50} = 1.8 \pm 0.1 \mu\text{M}$  for  $ABTS^{+\cdot}$ ) indicated higher activity than BHT and  $\alpha$ -tocopherol employed as positive standards. Some of the synthesized compounds in the literature observed to have better antioxidant activity than standard compounds. When the results of our study were compared with the literature, it was determined that the antioxidant capacity of the synthesized compound (II) was good.

## EXPERIMENTAL

### Materials and Instrumentation

All commercially available chemicals and the standards employed for the biological assays and synthesis of target molecules were procured from commercial suppliers such as Aldrich and Merck Chemical companies. All these chemicals purchased were of analytical grade and were employed without further purification. Infrared spectra were recorded on an Agilent Cary 630 spectrophotometer with ATR in the scanning range of 4000–400  $\text{cm}^{-1}$ . Elemental analysis was established by employing a Thermo Scientific Flash 2000 elemental analyzer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{DMSO}-d_6$  solutions on a Bruker AVANCE III 400 MHz spectrometer using tetramethylsilane as the internal reference at 400 MHz and 100 MHz, respectively. Melting points were measured by employing a Barnstead IA9100 Electrothermal Digital Melting Points Apparatus.

**Synthesis of compound (I).** A solution of methyl 3,5-dinitrobenzoate (10 mmol, 2.2614 g) was added to a 100 mL round-bottom flask containing ethanol (40 mL) at  $0^\circ\text{C}$ . 80% hydrazine hydrate (30 mmol, 1.82 mL) was added dropwise to this stirred solution. The resultant mixture was stirred for 4 h at RT. Upon completion of the reaction, the precipitated product was filtered and rinsed thoroughly with 20 mL of ethanol and 20 mL of diethyl ether. The product acquired was employed without further purification [31].

**Synthesis of target molecules (II–VIII).** A solution of an appropriate aldehyde (1 mmol) in 20 mL ethanol was added to the stirred solution of compound (I) (1 mmol) in the molar ratio (1 : 1) in 20 mL of ethanol as solvent. The reaction mixture obtained was refluxed with vigorous stirring for 5 h and then cooled down to ambient temperature, and the resulting residue was removed by filtration, dried in air, and purified by crystallizing with ethanol to afford a pure product.

**Antioxidant assays.** According to literature, the antioxidant activity of all synthesized molecules (I–VIII) were determined by three different methods: ABTS cation radical decolorization, DPPH free radical scavenging activity, and cupric reducing antioxidant capacity (CUPRAC) assay. BHA was employed as the positive control. The percentage inhibition and half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) were calculated. All experiments were conducted in triplicate.

**DPPH free radical scavenging activity assay.** DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in methanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. DPPH radical scavenging activities of all synthesized compounds (I–VIII) were measured by a spectrophotometric method. 4 mL of 0.004% DPPH solution in methanol

and 1 mL of different concentrations of samples were incubated 30 min in the dark at RT. After the incubation spectrophotometric measure was performed at 517 nm [45]. Inhibition percentage ( $I\%$ ) of the samples was calculated according to the following equivalent, and  $\text{IC}_{50}$  values of samples were calculated.

$$I\% = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100,$$

$A_{\text{blank}}$  refers to the absorption of the tube that includes all reagents except sample, and  $A_{\text{sample}}$  refers to the absorption of the sample tubes.

**ABTS cation radical decolorisation assay.** The ABTS assay measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase, as compared with BHA. The ABTS is generated by reacting with a strong oxidizing agent with the ABTS salt. The inhibition of decolorization percent of ABTS cation radical of all synthesized compounds (I–VIII) in this study were determined the inhibition percentage as a function of time and concentration. Preparation of  $\text{ABTS}^{\cdot+}$  (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) solution was initiated by 7 mM ABTS solution in water. Adding 2.45 mM potassium persulfate produced  $\text{ABTS}^{\cdot+}$  solution after incubation of in the dark for 16 hours. The solution was diluted with water to an absorbance of 0.7 at 734 nm. 4 mL of  $\text{ABTS}^{\cdot+}$  solution and 1 mL of different concentrations of samples were incubated for 30 min. in dark at RT. After the incubation spectrophotometric measure was conducted at 734 nm [46]. Inhibition percentage ( $I\%$ ) of samples was calculated according to the following equivalent and  $\text{IC}_{50}$  values of samples.

$$I\% = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100,$$

$A_{\text{blank}}$  refers to the absorption of the tube that includes all reagents except sample, and  $A_{\text{sample}}$  refers to the absorption of the sample tubes.

**Cupric reducing antioxidant capacity (CUPRAC) assay.** This method consists of reducing of  $\text{Cu(II)}$ -neocuproine into its colored form  $\text{Cu(I)}$ -neocuproine chelate in the presence of antioxidant molecules. For this, 1 mL of  $1 \times 10^{-2}$  M copper (II) chloride, 1 mL of  $7.5 \times 10^{-3}$  M neocuproine (2,9-dimethyl-1,10-phenanthroline), 1 mL of ammonium acetate buffer (pH 7), 1.1 mL sample in different concentrations were incubated for 30 min and read against blank at 450 nm [47].  $A_{0.5}$  value was calculated for each sample.

## CONCLUSIONS

In this research, seven hydrazone derivatives have been synthesized and their antioxidant activity was evaluated by three different methods. In DPPH free radical scavenging assay, compounds (I) ( $\text{IC}_{50} = 9.2 \pm 0.01 \mu\text{g/mL}$ ), (II) ( $\text{IC}_{50} = 4.4 \pm 0.04 \mu\text{g/mL}$ ) and (VI) ( $\text{IC}_{50} = 25.01 \pm 0.30 \mu\text{g/mL}$ ) were found to have good antioxidant activity than BHA ( $\text{IC}_{50} = 49.0 \pm$



0.09  $\mu\text{g}/\text{mL}$ ). Compound (II) in the series was determined to have excellent antioxidant capacity. In ABTS cation radical scavenging assay, compound (II) ( $\text{IC}_{50} = 1.8 \pm 0.07 \mu\text{g}/\text{mL}$ ) was found to have good antioxidant activity than BHA ( $\text{IC}_{50} = 2.1.0 \pm 0.01 \mu\text{g}/\text{mL}$ ). Apart from this compound (II), it can be said that compounds (I) ( $\text{IC}_{50} = 3.4 \pm 0.03 \mu\text{g}/\text{mL}$ ), (V) ( $\text{IC}_{50} = 6.0 \pm 0.06 \mu\text{g}/\text{mL}$ ), (VI) ( $\text{IC}_{50} = 8.4 \pm 0.20 \mu\text{g}/\text{mL}$ ) and (VII) ( $\text{IC}_{50} = 5.7 \pm 0.23 \mu\text{g}/\text{mL}$ ) show antioxidant activity close to BHA. In the CUPRAC assay, it was determined that all tested molecules showed cupric reducing antioxidant activity, and it was determined that all the compounds screened had excellent antioxidant potential than BHA. Among these tested compounds, compound (II) ( $A_{0.5} = 2.2 \pm 0.02 \mu\text{M}$ ) was the most potent antioxidant molecule. In conclusion, when our results are compared with the literature, it could be said that some of the synthesized compounds in this study may have promising properties as antioxidant agent.

#### COMPLIANCE WITH ETHICAL STANDARDS

The article contains no studies involving humans or animals as subjects of the study.

#### Conflict of Interests

The authors declare they have no conflicts of interest.

#### SUPPLEMENTARY INFORMATION

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