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Synthesis and Characterization of Novel Hydrazone Based Anti-mutagenic and Antioxidative Agents

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ABSTRACT

The present study was designed to produce novel hydrazine and evaluate their biological properties including antioxidant, antityrosinase and antimutagenic. 4-allyloxybenzoyl hydrazine **(1)** reacts with 5-acetyl-1,3-dimethyl barbituric acid **(2)** and 2-Isonitrosoacetophenone **(3)** in the presence of acetic acid as a catalyst to produce the hydrazone derivatives **4** and **5** in high yields respectively. The new hydrazone derivatives **4** and **5** have been fully characterized by using multinuclear NMR (^{1}H, ^{13}C) spectroscopy and elemental analysis. The compounds **4** and **5** were studied for their antioxidant and tyrosinase enzyme inhibition activity. In addition the mutagenic and antimutagenic activities were evaluated by Ames *Salmonella*/ microsome mutagenicity test. The results showed that both of compounds exhibited significant antioxidative and antimutagenic activity and compound **5** has shown moderate tyrosinase inhibition activity. This study suggested that these compounds could be considered as novel bioactive agents in pharmaceutical area.

INTRODUCTION

There are several biologically active organic compounds present significant application in the bioorganic chemistry. Most of the common features of these compounds have several donor atoms such as nitrogen, sulphur or oxygen which interact to biological material *in vivo* or *in vitro* environments (Wang *et al.,* 2010; Chugunova *et al.,* 2014; Glinma *et al.,* 2015). Oximes and barbiturates have been known for a long time as highly biologically active compounds (Laxmi *et al.,* 2012; Angelusiu *et al.,* 2010; Savini *et al.,* 2002). Besides on behalf of their biological importance, hydrazone derivatives containing barbiturate or oxime moiety possess diverse pharmacological activities. These types compounds were named by hydrazone-barbiturates and hydrazone-oximes which are typically act as tridentate, bidentate or monodentate compounds reacting through the oxygen or imine nitrogens depending on the reaction conditions (Neumann *et al.,* 2014; Giziroglu *et al.,* 2013; Gup *et al.,* 2006). Antioxidant and antimutagenic effects of organic compounds are important parameters due to their prevention and treatment capabilities on several diseases (Spada *et al.,* 2008; Parvathy *et al.,* 2010; Salleh *et al.,* 2014). Interests in search for new antioxidants and antimutagens have grown dramatically over past years because of their significant biological, industrial and economic impact. In this context, several researchers investigated novel compounds for use as antioxidative and antimutagenic food or drug additives (Siddaiah *et al.,* 2007; Parvathy *et al.,* 2009).

Survey of literature revealed that there are several reports on antimicrobial, antitubercular, anticonvulsant, antitumor and anti-inflammatory activities concerning the structures of such molecules with hydrazone, barbiturate and oxime groups (Abele *et al.,* 2010; Neumann *et al.,* 2014; Zheng *et al.,* 2010; Zaragoza-Dörwald, F. 2012). Interestingly, although over the years, too many compounds including hydrazone-barbiturates and hydrazone-oximes moiety have been synthesized, there are very few examples mentioned about antioxidant activity, tyrosinase inhibition activity and mutagenic or antimutagenic potential (Giziroglu *et al.,* 2013; Khan *et al.,* 2011; Zaragoza-Dörwald, F. 2012). Due to the limited information, we selected antioxidant, antimutagenic and tyrosinase inhibitory properties of new hydrozane derivatives as biological activity parameters.

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In this study we aimed to synthesize new hydrazone derivatives containing barbiturate and oxime moiety by bringing together 4-allyloxybenzoyl hydrazine (**1)** and 5-acetyl-1,3-dimethyl barbituric acid **(2)** or 2-Isonitrosoacetophenone **(3).** These compounds are fully characterized by multinuclear NMR (^{1}H, ^{13}C) spectroscopy and elemental analysis. Antioxidant potentials of the compounds **4** and **5** were determined by eight assays including total antioxidant, reducing power, radical scavenging and metal chelating activity. We also investigate their tyrosinase enzyme inhibition activity because of its relation with antioxidant properties including chelating activity in biochemical applications (Woo and Je 2013). The screening of their mutagenic and antimutagenic activities was investigated by Ames *Salmonella*/microsome mutagenicity test (Maron and Ames 1983).

EXPERIMENTAL

General

Commercially available reagents were used without further purification. 4-allyloxybenzoyl hydrazine was prepared by refluxing ethyl 4-allyloxybenzoate (10 mmol) with hydrazine hydrate (2.5 mL) for 4 h (Shanker *et al.,* 2013; Komurcu *et al.*, 1995). 1,3-dimethyl-5-acetyl-barbituric acid (2) was prepared according to the reported procedures (Jursic *et al.,* 2011). Melting points were measured with an Electro thermal 9200 melting point apparatus and the values are uncorrected. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker Ultrashield 400 Plus NMR spectrometer. Chemical shifts are reported in ppm downfield from Me_4Si and were referenced to solvent peaks. Elemental analyses were done on a Leco-932.

Synthesis

1,3-dimethyl-5-acetyl barbituric acid 4-Allyloxybenzoyl hydrazone (4)

A solution of 4-allyloxybenzoyl hydrazine (1) (1.92 g, 10 mmol) in ethanol (50 mL) was added to a ethanol (30 ml) suspension of 1,3-dimethyl-5-acetyl barbituric acid (2) (1.98 g,10 mmol) at room temperature. After stirring for 5 min at the same temperature, catalytic amount glacial acetic acid was added to the reaction mixture and refluxed for 12 h. After cooling, the white solid precipitate was filtered and washed with ethanol. Recrystallization from DMSO gave white crystals. (2.4 g, 72%). mp 220 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.72 (s, 1H); 7.80 (s, 1H); 7.74(d, J_{HH} = 6.9 Hz, 2H); 6.94 (d, J_{HH} = 6.9 Hz, 1H); 5.98 (m, 1H,); 5.36 (d, J_{HH} = 5.8 Hz, 1H), 5.28 (d, J_{HH} = 5.8 Hz, 1H); 3.28 (s, 3H); 3.26 (s, 3H); 2.71 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.1, 165.8, 154.0, 151.8, 135.4, 132.3, 128.4, 118.0, 115.9, 87.8, 69.5, 66.0, 28.1, 17.8 ppm. Anal. Calcd for $C_{18}H_{20}N_4O_5$: C 58.06, H 5.41, N 15.05 found C 58.19, H 5.45, N 15.58.

2-isonitrosoacetophenone 4-Allyloxybenzoyl hydrazone (5)

A solution of 4-allyloxybenzoyl hydrazine (1) (1.92 g, 10 mmol) in ethanol (50 mL) was added to a ethanol (20 ml) suspension of 2-isonitrosoacetophenone (3) (1.49 g, 10 mmol) at room temperature. After stirring for 5 min at the same temperature, catalytic amount glacial acetic acid was added to the reaction mixture and refluxed for 6 h. After cooling, the yellow solid precipitate was filtered and washed with ethanol. Recrystallization from ethanol gave light yellow crystals. (2.4 g, 72%). mp 185-190 ^{o}C (dec). ^{1}H NMR (400 MHz, DMSO-d6): δ 13.18 (s, 1H); 12.63 (s, 1H); 8.51 (s, 1H); 7.87 (d, J_{HH} = 7.8 Hz, 2H); 7.75-7.44 (m, 5 H); 7.07 (d, J_{HH} = 7.8 Hz, 1H); 6.07 (m, 1H,), 5.44 (d, J_{HH} = 5.3 Hz, 1H), 5.28 (d, J_{HH} = 5.3 Hz, 1H) ppm. ^{13}C NMR (100 MHz, DMSO-d6): δ = 161.3, 145.2, 136.3, 133.2, 129.5, 129.2, 128.5, 127.0, 124.9, 124.7, 117.9, 114.8, 68.4 ppm. Anal. Calcd for $C_{18}H_{17}N_3O_3$: C 66.86, H 5.30, N 13.00 found C 66.99, H 5.43, N 13.47.

Antioxidant and Tyrosinase inhibition activity

Tyrosinase inhibition activity

Tyrosinase inhibitory activity was measured using a modified dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate, as previously reported (Zengin *et al*., 2014a). Sample solution (25 µL) was mixed with tyrosinase solution (40 µL) and phosphate buffer (100 µL, pH 6.8) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of L-DOPA (40 µL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (tyrosinase) solution. The sample and blank absorbance were read at 492 nm after 10 min incubation at 25 °C. Absorbance of the blank was subtracted from that of the sample.

Total antioxidant activity by phosphomolybdenum method

Total antioxidant activity of the samples was evaluated by phosphomolybdenum method (Giziroglu *et al*., 2013). Sample solution (0.2 mL) was combined with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance was read at 695 nm after 90 min incubation at 95 °C.

Radical scavenging activity

The effects of the samples on 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS [2,2 Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid)] cation, nitric oxide ($^{\cdot}$NO) and superoxide anion ($O_2^{\cdot-}$) radicals were estimated according to the procedure in literature.

Effect of the samples on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were estimated according to a method reported by Sarikurkcu (2011) and Giziroglu *et al*. (2013). Sample solution (1 mL) was added to a 4 mL of 0.004% methanol solution of DPPH. Sample absorbance was read at 517 nm after 30 min incubation at room temperature in dark.

Scavenging activities of the samples on ABTS cation radical was measured according to the method of Zengin *et al*. (2014a). Briefly, $ABTS^{\cdot+}$ radical cation was produced directly by

reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12-16 h in dark at the room temperature. Prior to beginning the assay, ABTS solution was diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Sample solution (1 mL) was added to ABTS solution (2 mL) and mixed. Sample absorbance was read at 734 nm after 30 min incubation at room temperature.

Superoxide anion radical scavenging activity was carried out in riboflavin-light-nitroblue tetrazolium (NBT) system (Sarikurkcu *et al*., 2014). Sample solution (0.25 mL) was added to reaction mixture containing riboflavin (0.1 mL, 0.1 mg/mL), ethylenediaminetetraacetic acid (EDTA) (0.1 mL, 12 mM), NBT (0.05 mL, 1 mg/mL), phosphate buffer (1 mL, 50 mM, pH 7.8) and 1-butanol (0.5 mL). The reaction mixture was illuminated for 10 min at room temperature and the sample absorbance was read at 560 nm. Un-illuminated reaction mixture was used as a blank. Absorbance of the blank was subtracted from that of the sample

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which can be measured by Griess reaction (Sarikurkcu *et al*., 2014). Sample solution (0.5 mL) was mixed with sodium nitroprusside (0.5 mL, 5 mM) in phosphate buffer (0.2 M, pH 7.4) and incubated for 150 min at room temperature. Similarly, a blank was prepared by adding sample solution (0.5 mL) to phosphate buffer (0.5 mL). Diluted Griess reagent (1 mL, 1:1) was added to the incubated sample and allowed to stand for 30 min. The sample and blank absorbance were read at 548 nm. Absorbance of the blank was subtracted from that of the sample.

Reducing power

The reducing power was investigated using cupric ion reducing (CUPRAC) and ferric reducing antioxidant power (FRAP) methods, as previously described in the literature.

The cupric ion reducing activity (CUPRAC) was determined according to the method of Zengin *et al*. (2014b). Sample solution (0.5 mL) was added to a premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH_4Ac buffer (1 mL, 1 M, pH 7.0). Similarly, a blank was prepared by adding sample solution (0.5 mL) to a premixed reaction mixture (3 mL) without $CuCl_2$. Then, the sample and blank absorbance were read at 450 nm after 30 min incubation at room temperature. Absorbance of the blank was subtracted from that of the sample.

FRAP assay was carried out as described by Zengin *et al*. (2014b). Sample solution (0.1 mL) was added to a premixed FRAP reagent (2 mL) containing acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10:1:1 (v/v/v). Then, the sample absorbance was read at 593 nm after 30 min incubation at room temperature.

Metal chelating activity on ferrous ions

Metal chelating activity on ferrous ions was determined by the method described by (Zengin *et al*. (2014b). Briefly, sample solution (2 mL) was added to $FeCl_2$ solution (0.05 mL, 2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Similarly, a blank was prepared by adding sample solution (2 mL) to $FeCl_2$ solution (0.05 mL, 2 mM) and water (0.2 mL) without ferrozine. Then, the sample and blank absorbance were read at 562 nm after 10 min incubation at room temperature. Absorbance of the blank was subtracted from that of the sample

Mutagenic and Antimutagenic activity

Bacterial strains

S. typhimurium TA98 and *S. typhimurium* TA100 were used for the mutagenity and antimutagenity tests. The strains were analyzed according to the procedure in literature. (Mortelmans and Zieger 2000; Oh *et al.*, 2008; Basgedik *et al.*, 2011)

Mutagenic and antimutagenic activity

Viability assays and determination of test concentrations

Cytotoxic dose of the compounds **4** and **5** were determined by the method of Mortelmans and Zeiger (2000). The toxicity of the compounds **4** and **5** toward *S. typhimurium* TA98 and TA100 were estimated according to the procedure in literature (Santana- Rios *et al.*, 2001; Yu *et al*., 2001; Gulluce *et al.*, 2001).

Mutagenicity and antimutagenicity tests

In this study, the plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays as previously described in the literature (Maron and Ames, 1983). The known mutagens 4-nitro-o-phenylenediamine (4-NPD) 3 µg/plate) for *S. typhimurium* TA98 and sodium azide (NaN_3) (8 µg/plate) for *S. typhimurium* TA100 were used as positive controls and Dimethyl sulfoxide was used as a negative control in mutagenicity and antimutagenicity tests.

The mutagenic activity assay conducted with TA98 and TA100 strains of *S. typhimurium*, according to literatures with 100 µl test compounds at different concentrations (5, 0.5, 0.05 mg/plate for **4** and 2, 0.2, 0.02 mg/plate for **5**) (Gulluce *et al.*, 2010).

The antimutagenic assay conducted with the same strains, 100 µl of the overnight bacterial culture, 100 µl mutagen, 100 µl test compounds at different concentrations (5, 0.5, 0.05 mg/plate for **4** and 2, 0.2, 0.02 mg/plate for **5**) (Gulluce *et al.*, 2011).

The plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays (Maron and Ames, 1983). For the mutagenicity and antimutagenity assays, calculations were done according to methods in literature (Ikken *et al*., 1999; Negi *et al*., 2003; Evandri *et al*., 2005; Gulluce *et al.*, 2011).

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (mean ± SD). Differences between the values were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test (α=0.05). All the analyses were carried out by using SPSS v22.0 software.

RESULTS AND DISCUSSION

Synthesis and spectroscopic studies

The new hydrazone derivatives **4** and **5** were prepared by reaction of 4-allyloxybenzoyl hydrazine 1 with 5-acetyl-1,3-dimethyl barbituric acid **2** and 2-isonitrosoacetophenone **3** in the presence of EtOH along with catalytic amount of acetic acid (Fig. 1).

The compound **4** and **5** are soluble in DMF, DMSO, $CHCl_3$, Ethanol however, it is insoluble in water and other common organic solvents such as toluene, hexane, pentane, diethyl ether etc.

The new barbiturate derivative **4** and **5** are nonhydroscopic and characterized by ^1H, and ^{13}C NMR spectroscopy and elemental analysis. According to literature reports, unlike compound **5**, compound **4** may exist in three tautomeric forms as shown in Fig. 2 (DaSilva *et al.*, 2003; Giziroglu *et al.*, 2013).

To decide which tautomeric form of the compound 4 in the solution, we checked the ^{13}C NMR spectra. The ^{13}C NMR spectra of **4** in $CDCl_3$ display two peaks belonging to -NH-C=C carbons at 87.8 and 118.0 ppm reveals that this compound exist in tautomeric form(II) in the solution (DaSilva *et al*., 2003; Giziroglu *et al.*, 2013). Another distinctive signal in ^{13}C NMR varies from 115.9 to 154.0 ppm indicates aromatic and allylic CH carbons. The ^1H NMR spectra of compound **4** show signals at 2.71, 3.26 and 3.28 ppm can be attributed to $-CH_3$ protons. The characteristic allyl protons are observed at 5.28, 5.36 and 5.98 ppm. The NH proton bound to carbonyl of the compound **4** was observed at 13.72 whereas the NH proton bound alkenyl group appeared at the higher field at 7.80 ppm.

Examination of ^1H NMR spectra of compound **5** in DMSO-d6 solution revealed that the chemical shift of the oxime and amidic protons have characteristic values at 13.18 and 12.63 ppm as broad signals, respectively. The allylic protons are observed at 5.28, 5.44 and 6.07 ppm. The ^{13}C NMR spectra of **5** in DMSO-d6 solution exhibit that the chemical shift of the amidic carbonyl carbons have characteristic values at 161.3. The peaks at 68.4 attributed to carbon of -C-O-Ar. Another ^{13}C NMR signals varies from 114.8 to 145.2 ppm indicates aromatic and allylic -CH carbons.^1H NMR spectras for 4 and 5 are shown in Fig. 3.

All of spectral results were confirmed by elemental analysis, and the molecular formula was determined as $C_{18}H_{20}N_4O_5$ for **4** and $C_{18}H_{17}N_3O_3$ for **5**.

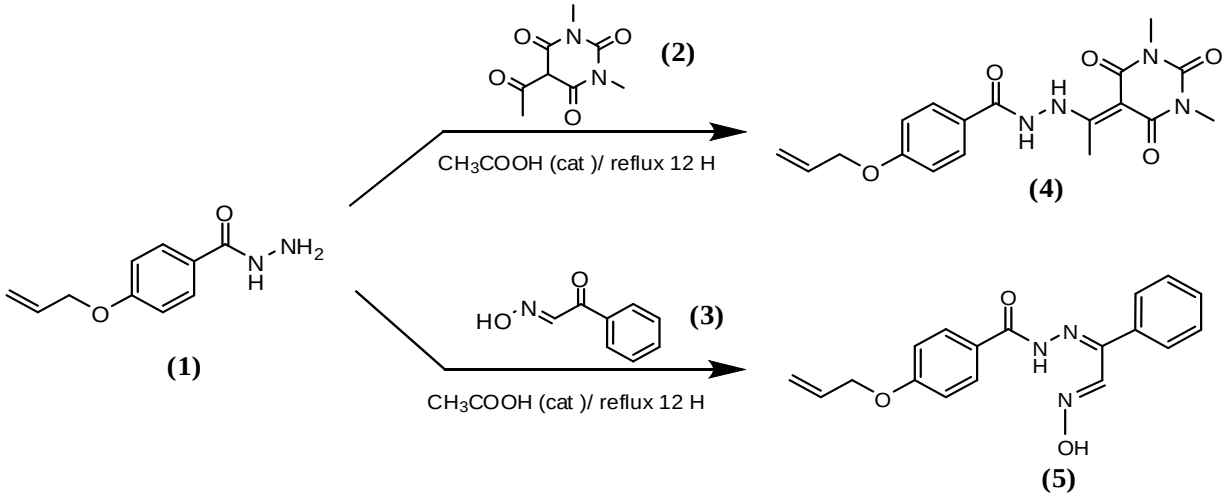

Fig. 1: Synthesis of compounds **4** and **5.**

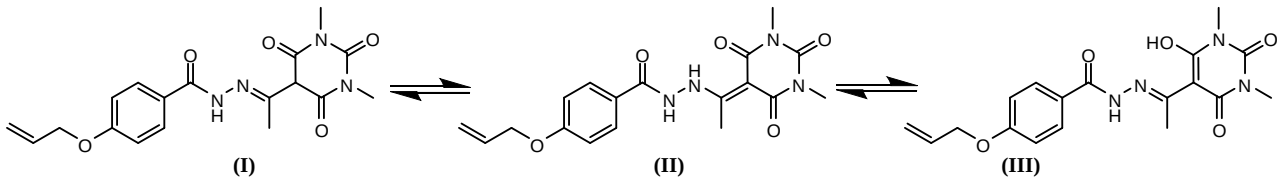

Fig. 2: Tautomeric forms of compound **4.**

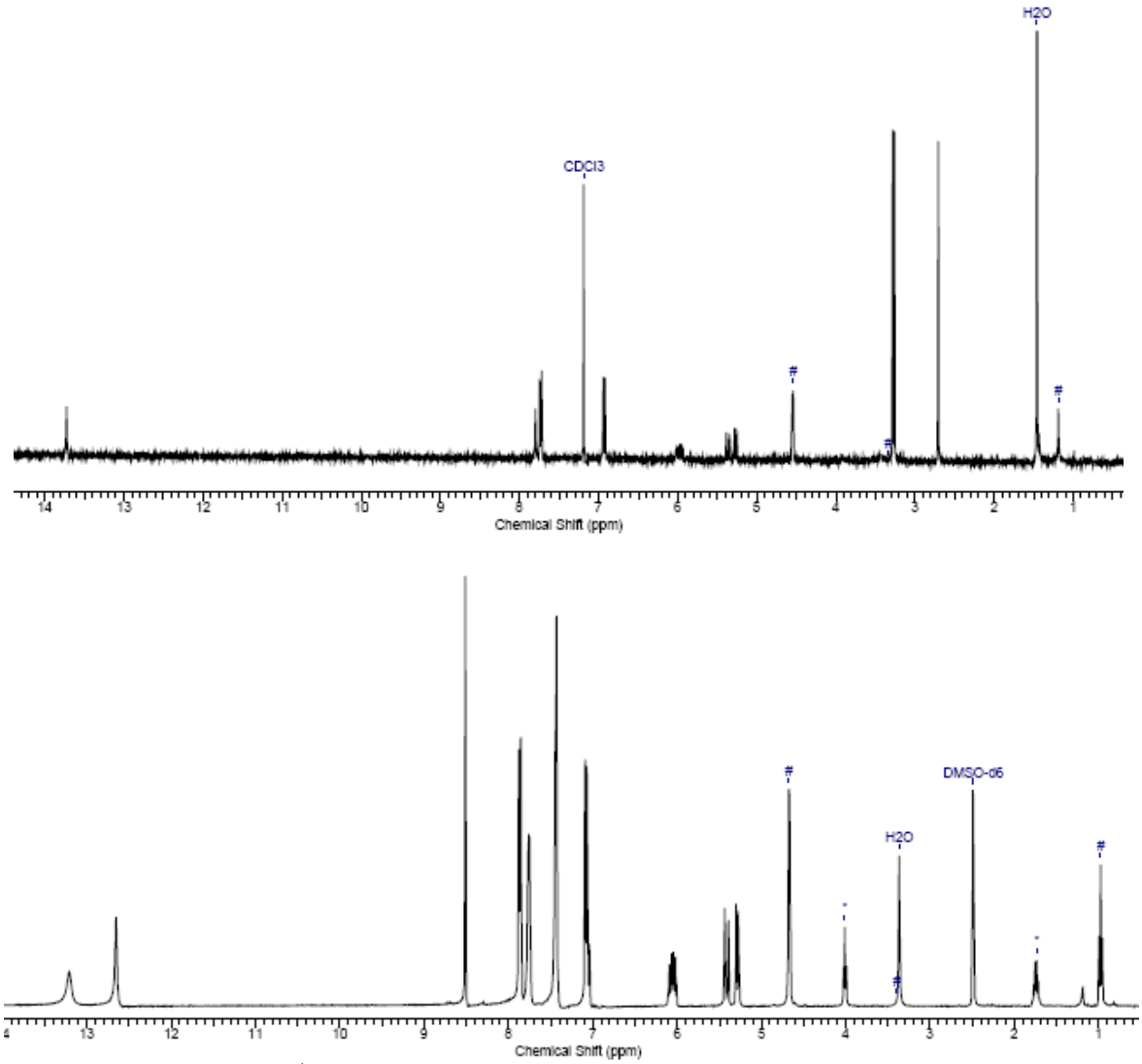

Fig. 3: ^{1}H NMR spectras for **4 (a)** and **5 (b)**, (#): ethanol (*): solvent impurities.

Antioxidant and Tyrosinase inhibition activity

To explore the antioxidant activity of compounds **4** and **5**, we used eight different test method namely 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS [2,2 Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid)] cation, nitric oxide ($^{\cdot}$NO) and superoxide anion ($O_2^{\cdot-}$) radicals, cupric ion reducing (CUPRAC), ferric reducing antioxidant power (FRAP) and total antioxidant activity phosphomolybdenum methods. Four different radicals (DPPH, ABTS, NO radical, O_2 radical) were used for define of radical-scavenging activity of compounds **4** and **5**. The basis of test methods is that the antioxidants react with the free radical and changes their absorbencies. The degree of the changing in absorbance indicates the scavenging potential of the samples. Usually the radical scavenging activity was dependent on concentration and was increased with amount of the sample. The results were presented in **Table 1** and it is important to note that the lower IC_{50} value of sample indicate greater radical scavenging. We would like to note that the compound **4** and **5** with IC_{50} values 4.99 and 6.81 mM respectively greater than those of trolox (7.29 mM) for superoxide anion radical scavenging activity. The Tyrosinase enzyme inhibition analysis showed us the compound **5** display good inhibition activity against mushroom tyrosinase. The tyrosinase inhibition of the synthesized compounds were spectrophotometrically measured and the results are expressed as kojic acid equivalents (Table 1) The compound **5** which has IC_{50} value of 3.90 for tyrosinase inhibition has also more effective metal chelating activity among the both of compounds. The results showed us the oxime and imine groups cause increase in inhibitory activity. Therefore we can conclude that the inhibition mechanism could be explained that copper ions in the tyrosinase enzyme were complexed by oxime and imine groups. In the other words oxime group could be oxidized by tyrosinase enzyme. Total antioxidant activities reflect the capacity of a nonenzymatic, antioxidant defense system. The phosphomolybdenum test system is based on the reduction of molybdenum by the antioxidative compounds following the forming of a green molybdenum (V) complex, which show strong absorbance at 695 nm. Total antioxidant capacity of sample is presented as equivalents of ascorbic acid (Table 2).

Table 1: Inhibition concentration of the samples and standards for radical scavenging, metal chelating and tyrosinase enzyme inhibitory activities[a].

Samples	DPPH radical*	ABTS radical cation*	Nitric oxide radical*	Superoxide anion radical*	Metal chelating**	Tyrosinase inhibition***
4	5.64±0.01y	3.34±0.03y	na[c]	4.99±0.01y	14.13±0.64x	na
5	51.38±8.96x	4.21±0.11x	9.07±0.69x	6.81±0.37x	6.50±1.06y	3.90±0.25x
Standards						
Trolox	0.39±0.01y	0.25±0.03z	4.93±0.01y	7.29±0.23x	-	-
EDTA[b]	-	-	-	-	0.11±0.01z	-
Kojic acid	-	-	-	-	-	0.61±0.01y

[a]Data marked with different letters within the same column indicate significant difference statistically ($p < 0.05$). [b]EDTA, disodium edentate. [c]na, not active. * Inhibition concentration (IC_{50}: mM) at which 50% of the radicals were scavenged. ** Inhibition concentration (IC_{50}: mM) at which 50% of the Fe^{2+}-ferrozine complex were inhibited. *** Inhibition concentration (IC_{50}: mM) at which 50% of the tyrosinase enzyme were inhibited.

Table 2: Effective concentration of the samples and standards for reducing power and total antioxidant activity phosphomolybdenum method[a]

Samples	Total antioxidant activity	Reducing power	
	Phosphomolybdenum*	CUPRAC*	FRAP*
4	5.08±0.57x	3.39±0.16x	2.69±0.32x
5	1.31±0.02y	1.02±0.08y	4.10±0.66x
Standards			
Trolox	0.16±0.01y	0.21±0.01z	0.18±0.01y

[a]Data marked with different letters within the same column indicate significant difference statistically ($p < 0.05$). * Effective concentration (EC_{50}: mM) at which the absorbance was 0.5 for reducing power and total antioxidant activity.

Table 3: The antimutagenicity assay results of the comppounds **4** and **5** for *S. typhimurium* TA98 and TA100 bacterial strains.

Test items	Concentration (mg/plate)	Number of revertants			
		TA98		TA100	
		Mean± S. error	Inhibition (%)	Mean± S. error	Inhibition (%)
Compound **4**	5	53.66± 3.78[a]	74.37	9.4± 2.4	95.33
	0.5	57.5± 2.88	72.49	18± 5.52	91.05
	0.05	168.4± 7.95	19.43	157.2± 7.19	21.8
Negative control		5.66± 1.52[a]		10.33± 2.51	
*4-NPD**	3	209± 4.58		-	
*NaN_3**	8	-		201± 6.24	
Compound **5**	2	41.2± 15.46	80.29	386± 138.59	31.92
	0.2	69.8± 16.14	66.61	409.5± 3.53	27.77
	0.02	105.4± 15.66	49.57	466± 89.28	17.81
Negative control		5.66± 1.52[a]		39.5± 9.19	
*4-NPD**	3	209± 4.58		-	
*NaN_3**	8	-		567± 104.65	

*4-NPD and NaN_3 were used as positive controls for S. *typhimurium* TA98 and TA100 strains, respectively.
[a]Values expressed are means ± S.D. of three parallel measurements. The regression analysis was carried out in Microsoft Excel between percent inhibition of mutagenicity and log values of concentrations of the samples.

According to test results compound **4** has better activity than compound **5**. When we look at the reducing power assay, we used two different test system namely cupric ion reducing (CUPRAC) and ferric reducing antioxidant power (FRAP). In these methods reduction of metal ions can be monitored by calculating the absorption changes. Therefore, the absorption changes are entirely based on the total reducing power of the electron donating antioxidants present in the reaction mixture. As it can be seen in Table 2, CUPRAC result followed the same tendency as those obtained using the phosphomolybdenum total antioxidant capacity. Surprisingly there is no correlation of FRAP test with phosphomolybdenum total antioxidant capacity.

Mutagenic and antimutagenic properties

The hydrazone derivatives **4** and **5** which were tested at three different concentrations, including 5, 0.5, 0.05 mg/plate for **4** and 2, 0.2, 0.02 mg/plate for **5**, did not exhibit any mutagenic effect in the mutagenicity assays performed with *S. typhimurium* TA98 and TA100. The possible antimutagenic potential of the compounds **4** and **5** were examined against 4-NPD and NaN_3 in *S. typhimurium* TA 98 and TA 100, respectively. The results were evaluated by using standard plate incorporation method and summarized in **Table 3.** In the antimutagenicity assays performed with TA98 and TA100 strains, the compound **5** exhibited strong antimutagenic effects at 2, 0.2 and 0.02 mg/plate concentrations. The compound **5** also exhibited moderate antimutagenic effects at 2 and 0.2 mg/plate concentrations on TA100 strain. The strongest antimutagenic activity was observed at 2 mg/ plate concentration against *S. typhimurium* TA98 strain but the 0.02 mg/plate concentration of **5** didn't exhibit any antimutagenic effect against *S. typhimurium* TA100. The compound **4** showed antimutagenic effects at 5 and 0.5 mg/plate concentrations. The strongest antimutagenic activity was observed at 5 mg/ plate concentration against *S. typhimurium* TA100 strain.

CONCLUSION

In this report we synthesized and characterized of new hydrazone based antimutagenic and antioxidative compounds. For the structural characterization various spectroscopic techniques were used. These compounds are new examples of aroylhydrazone derivatives which are known as biologically active compounds. For the first time we explored mutagenic and antimutagenic activity of a member of these kinds of compounds and it has shown significant antimutagenic activity. In addition we studied their tyrosinase inhibition activity and antioxidative properties *in vitro* by several assay systems. The modifications of the aroyl hydrazone moiety to increase of water solubility as well as their several biological activities are under active investigation.

REFERENCES

Abele E, Abele R, Golomba L, Visnevska J, Beresneva T, Rubina K and Lukevics E. Oximes of six-membered heterocyclic compounds with two and three heteroatoms. II. Reactions ans Biological activity. Chemistry of Heterocyclic Compounds, 2010; 46; 905-930.

Angelusiu MV, Barbuceanu S-F, Draghici C and Almajan GL. New Cu(II), Co(II), Ni(II) complexes with aroyl-hydrazone based ligand. Synthesis, spectroscopic characterization and in vitro antibacterial evaluation. European Journal of Medicinal Chemistry, 2010; 45: 2055-2062.

Basgedik B, Ugur A and Sarac N. Antimicrobial, antioxidant, antimutagenic activities, and phenolic compounds of Iris germanica. Industrial Crops and Products, 2011; 61: 526-530

Chugunova EA, Voloshina AD, Mukhamatdinova RE, Serkov IV, Proshin AN, Gibadullina EM, Burilov AR, Kulik NV, Zobov VV, Krivolapov DB, Dobrynin AB and Goumont R. The Study of the Biological Activity of Amino-Substituted Benzofuroxans. Letters in Drug Design & Discovery, 2014; 11; 502-512

Da Silva ET and Lima ELS. Reaction of 1,3-dimethyl-5-acetyl-barbituric acid (DAB) with primary amines. Access to intermediates for selectively protected spermidines. Tetrahedron Letters, 2003; 44; 3621-3624.

Evandri MG, Battinelli L, Daniele C, Mastrangelo S, Bolle P, Mazzanti G. The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. Food Chem. Toxicol, 2005; 43; 1381-1387.

Giziroglu E, Aygun M, Sarikurkcu C, Kazar D, Orhan N, Firinci E, Soyleyici HC and Gokcen C. Synthesis, characterization and antioxidant activity of new dibasic tridentate ligands: X-ray crystal structures of DMSO adducts of 1,3-dimethyl-5-acetyl-barbituric acid o-hydroxybenzoyl hydrazone copper(II) complex. Inorganic Chemistry Communications, 2013; 36; 199-205

Glinma B, Gbaguidi F, Kassahein UC, Kpoviessi SDS, Houngbeme A, Houngue HD, Accrombessi GC, Poupaert JH. Synthesis and trypanocidal activity of salicylhydrazones and p-tosylhydrazones of S-(+)-carvone and arylketones on African trypanosomiasis. J App Pharm Sci, 2015; 5 (06): 001-007.

Gulluce M, Agar G, Baris O, Karadayi M, Orhan F and Sahin F. Mutagenic and Antimutagenic Effects of Hexane Extract of some Astragalus Species Grown in the Eastern Anatolia Region of Turkey. Phytotherapy Research, 2010; 24: 1014-1018

Gulluce M, Agar G, Aslan A, Karadayi M, Bozari S and Orhan F. Protective effects of methanol extracts from Cladonia rangiformis and Umbilicaria vellea against known mutagens sodium azide and 9-aminoacridine. Toxicology and Industrial Health, 2011; 27: 675-682

Gup, R. and Giziroglu E, Metal complexes and solvent extraction properties of isonitrosoacetophenone 2-aminobenzoylhydrazone. Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy, 2006; 65(3-4); 719-726.

Ikken Y, Morales P, Maetinez A, Marin ML, Haza AI, Cambero MI. Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evulated by the Ames test. J. Agric. Food Chem, 1999; 47; 3257-3264.

Jursic BS and Neumann DM. Preparation of 5-formyl- and 5-acetylbarbituric acids, including the corresponding Schiff bases and phenylhydrazones. Tetrahedron Letters, 2011; 42; 8435-8439.

Khan KM, Khan M, Ali M, Taha M, Hameed A, Ali S, Perveen S and Choudhary MI. Synthesis and DPPH Radical Scavenging Activity of 5-Arylidene-N,N-Dimethylbarbiturates. Medicinal Chemistry, 2011; 7; 231-236.

Komurcu SG, Rollas S, Ulgen M, Gorrod, JW, Cevikbas A. Evaluation of some arylhydrazones of p-aminobenzoic acid hydrazide as antimicrobial agents and their in vitro microsomal metabolism Boll.Chim.Farm, 1995; 134; 375-379.

Laxmi SV, Janardhan B, Rajitha B, Raghavaiah P and Srinivas P. Synthesis, single crystal X-ray studies and antimicrobial activities of novel Indole barbiturates. Medicinal Chemistry Research, 2012; 21:2896-2901

Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. Mutat. Res, 1983; 113(3-4); 173-215.

Maron DM and Ames BN. Revised methods for the Salmonella mutageniticty test. Mutation Research, 1983; 113; 173-215

Mortelmans K, Zeiger E. The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res, 2000; 455(1-2); 29-60.

Negi PS, Jayaprakash GK, Jena BS. Antioxidant and antimutagenic activities of pomegranate peel extracts. Food Chem, 2003; 80(3); 393-397.

Neumann DM, Cammarata A, Backes G, Palmer GE and Jursic BS. Synthesis and antifungal activity of substituted 2,4,6-pyrimidinetrione carbaldehyde hydrazones. Bioorganic & Medicinal Chemistry, 2014; 22:813-826

Oh HT, Kim SH, Choi HJ, Chung MJ, Ham SS. Antioxidative and antimutagenic activities of 70% ethanol extract from masou salmon (*Oncorhynchus masou*). Toxicol. In Vitro, 2008; 22; 1484-1488.

Parvathy KS, Negi PS and Srinivas P. Antioxidant, antimutagenic and antibacterial activities of curcumin-beta-diglucoside. Food Chemistry, 2009; 115:265-271.

Parvathy KS, Negi PS and Srinivas P. Curcumin-amino acid conjugates: Synthesis, antioxidant and antimutagenic attributes. Food Chemistry, 2010; 120: 523-530.

Salleh WMNHWS, Ahmad F, Yen KH., Antioxidant and Anti-tyrosinase Activities from Piper officinarum C.DC (Piperaceae). J App Pharm Sci, 2014; 4 (05): 087-091.

Santana-Rios G, Orner GA, Amantana A, Prowost C, Wu SY, Dashwood RH. Potent antimutagenic activity of white tea in the *Salmonella* assay. Mutat. Res, 2001; 495; 61-74.

Sarikurkcu C. Antioxidant activities of solvent extracts from endemic Cyclamen mirabile Hildebr. tubers and leaves. Afr J Biotechnol, 2011; 10; 831-839.

Sarikurkcu C, Uren MC, Tepe B, Cengiz M, Kocak MS. Phenolic content, enzyme inhibitory and antioxidative activity potentials of Phlomis nissolii and P. pungens var. pungens. Ind Crop Prod, 2014; 62; 333-340.

Savini L, Chiasserini L, Gaeta A and Pellerano C. Synthesis and anti-tubercular evaluation of 4-quinolylhydrazones. Bioorganic & Medicinal Chemistry, 2002; 10:2193-2198.

Shanker G, Prehm M and Tschierske C. Laterally connected bent-core dimers and bent-core-rod couples with nematic liquid crystalline phases. Journal of Materials Chemistry, 2012; 22;168-174.

Siddaiah V, Maheswara M, Rao CV, Venkateswarlu S and Subbaraju GV. Synthesis, structural revision, and antioxidant activities of antimutagenic homoisoflavonoids from Hoffmanosseggia intricata. Bioorganic & Medicinal Chemistry Letters, 2007; 17:1288-1290.

Spada PDS, Nunes de Souza GG, Bortolini GV, Henriques JAP and Salvador M Antioxidant, mutagenic, and antimutagenic activity of frozen fruits. Journal of Medicinal Food, 2008; 11:144-151

Wang XL, Wan K and Zhou CH. Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial

and antifungal activities. European Journal of Medicinal Chemistry, 2010; 45: 4631-4639

Woo J-Y and Je J-Y. Antioxidant and tyrosinase inhibitory activities of a novel chitosan-phloroglucinol conjugate. International Journal of Food Science and Technology, 2013; 48; 1172-1178.

Yu Z, Xu M, Santana-Rios G, Shen R, Izquierdo-Pulido M, Williams DE, Dashwood RH. A comparison of whole wheat, refi ned wheat and wheat bran as inhibitors of heterocyclic amines in the *Salmonella* mutagenicity assay and in the rat colonic abberant crypt focus assay. Food Chem. Toxicol, 2001; 39; 655-665.

Zaragoza-Dörwald, F. 2012. Hydrazines, Acylhydrazines, and Hydrazones, in Lead Optimization for Medicinal Chemists: Pharmacokinetic Properties of Functional Groups and Organic Compounds, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R. Sideritis galatica Bornm.: A source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. J Funct Foods, 2014a; 11; 538-547.

Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R, Ceylan O. A comprehensive study on phytochemical characterization of Haplophyllum myrtifolium Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, skin diseases and type II diabetes. Ind Crop Prod, 2014b; 53; 244-251.

Zheng L-W, Li Y, Ge D, Zhao B-X, Liu Y-R, Lv H-S, Ding J and Miao J-Y. Synthesis of novel oxime-containing pyrazole derivatives and discovery of regulators for apoptosis and autophagy in A549 lung cancer cells. Bioorganic & Medicinal Chemistry Letters, 2010; 20; 4766-4770.

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