Synthesis and study of antioxidant and antimicrobial activityof imidazole derivatives

Maryam Ghazvinia*[a], Fatemeh Sheikholeslami-Farahani[b], Masoomeh Salimifard[c] and Ali Akbar Eslami[d]

[a]Department of Chemistry, Payame Noor university, P. O Box:3971189451, Tehran, Iran.
[b]Department of Chemistry, Firoozkooh Branch, Islamic Azad University, Firoozkooh, Iran
[c]Danayan Fara Kimiya (DFK)company, Gheysar AminpourBlv., Shahin Shahr, Isfahan, Iran
[d]Department of Chemistry, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.

Abstract: In the current research, imidazole derivatives are synthesized via a new process via four component reactions of trichloroacetonitrile, amides, alkyl bromides and amino acids catalyzed by zinc oxide nanoparticles (ZnO-NPs) as a simple and recyclable catalyst in water at room temperature. Also, the antioxidation property of some prepared imidazole is investigated by employing trapping diphenyl-picrylhydrazine (DPPH) radical and the ferric reduction experiment. Among the investigated compounds, compounds 5b exhibits good results with butylated hydroxytoluene (BHT) and 2-tert-butylhydroquinone (TBHQ) as standard antioxidants. In addition, the Gram positive and negative bacteria disk diffusion research is used for the confirmation of antimicrobial power of some prepared imidazoles. The achieved outcomes of disk diffusion experiment showed that these compounds resisted the growth of bacteria. Our procedure has a few benefits relative to the reported method such as good rate of reaction, high efficiency, simple removal of catalyst from mixture of reaction. In the yield of the product, ZnO-NPs show a satisfactory recyclable activity.
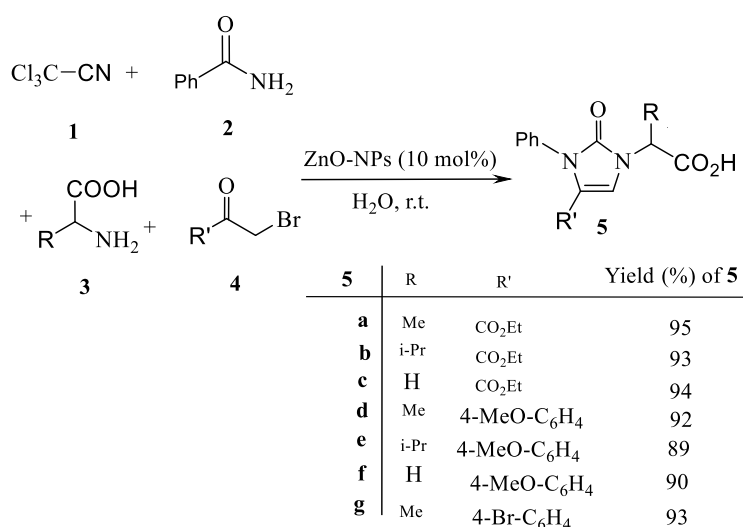
Introduction

Introduction

One of the best procedures for the preparation of intricate compounds by employing simple reagents is multicomponent reactions (MCRs) [1-5]. The compounds prepared by using MCRs are beneficial for synthetic and medicinal chemists [6-8]. Furthermore, resources keeping and reducing costs of the used procedure are carried out by the green process. Utilizing eco-friendly and ecologically solvents rather than harmful organic solvents and economical starting materials are important points for the development of green preparation of organic compounds [9].

Recently, there has been an enormous emphasis on the green and sustainable chemistry, where high importance has been given to the development of novel and eco-friendly methodologies, which can decrease or remove the utilization, and make use of unsafe industrial wastes [10].Among the solvents, water is the best green, accessible and low-cost solvent for increasing the velocity of organic process for water-insoluble starting materials [11]. Also, all organic products precipitate in water, remain in the solution and could be extracted by filtration [12]. Imidazole nucleus is the most interesting heterocycle that is broadly found in natural products and biologically active molecules. In addition, heterocyclic compounds with imidazole core have a very important place in medicinal chemistry and play a chief role in the eradication of several types of

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*Corresponding author:E-mail: Maryam_1547@yahoo.com

diseases. New derivatives of imidazole for medicinal fields are being energetically developed universally.Imidazole derivatives show various biological and pharmacological activities such as herbicidesand fungicides. So, the synthesis of imidazole derivatives is a significant subject in modern organic chemistry. Some of the organic reaction should be performed in the presence of a catalyst. Thus, one of the optimization factors is the determination of nanocatalyst and its amount.15Some of the organic reactions exhibit high efficiency and choose better nanocatalysts. ZnO nanoparticles because of their low price and non-poisonous properties are utilized in the organic reactions [13]. The bacterial resistance to drugs creates substantial problems in the process of many infectious diseases. Hence, finding a new method to eliminate these pathogens is essential. Therefore, the previous investigation emphasised on the antibacterial property of the newly synthesized compounds. Investigation of the antioxidant ability of some synthesized compounds is another subject in this research. Usually, the compounds, which have an antioxidant ability due to their reductive properties and chemical structure, remove the negative effect of free radicals and are used as transitional metal chelators. Also, these compounds could avoid or lessen the symptoms of many diseases, such as cardiovascular diseases, inflammatory bowel syndrome, cancer, ageing, and Alzheimer's [14]. In continuation of our attempts to expand new synthetic procedure for main organic compounds with biological activity [15, 16], in this work, we synthesized new derivatives of imidazole **5** from the reaction of trichloroacetonitrile **1**, amides **2**, alkyl bromides **4** and amino acids **3** using ZnO-NPs in the water at room temperature (Scheme 1).

5	R	R'	Yield (%) of 5
a	Me	CO_2Et	95
b	i-Pr	CO_2Et	93
c	H	CO_2Et	94
d	Me	$4\text{-MeO-C}_6\text{H}_4$	92
e	i-Pr	$4\text{-MeO-C}_6\text{H}_4$	89
f	H	$4\text{-MeO-C}_6\text{H}_4$	90
g	Me	$4\text{-Br-C}_6\text{H}_4$	93

Scheme 1. Preparation of imidazole derivatives of **5**

Also, antioxidant and antimicrobial activity of the synthesized imidazole was investigated and as shown, compound 5b exhibits good antioxidant activity and the outcomes of the disk diffusion experiment showed that this compound avoided the growth of bacterial.

Result & Discussion

Chemistry

In this research work, we described the preparation of imidazole derivatives **5** via an effective four component reaction of trichloroacetonitrile **1**, amides **2**, aminoacides **3**, alkyl bromides **4** and catalytic amount of ZnO NPs in aqueous medium at normal temperature in good efficiency (Scheme **1**). In the beginning step of this research work, the condensation process of trichloroacetonitrile **1**, benzamide **2**, alanine **3a** and ethyl bromopyruvate **4a** was utilized as a selected process for finding the best reaction conditions for the synthesis of **5a**. The synthesis of compound 5a was not proceeded without catalyst (Table 1, entry 1) and very

complex mixture in the absence of cataltst. For this reason, Fe_3O_4-MNPs (10 mol%) as catalyst was poured in the mixture of the sample reaction. The yield of **5a** was 56% (Table 1, entry 23). For better evaluation of the catalyst effect, several catalysts such as ZnO-nanoparticles, CuO-anoparticles, TiO_2-nanoparticles, Et_3N, K₂CO3, pyridine and KF/Clinoptinolite nanoparticles were applied in the model reaction. As a result, these outcomes displayed that the ZnO nanoparticles are the best catalyst for the sample reaction. Then, all reactions were performed with 10 mo% of ZnO nanoparticles as catalysts. By raising the amount of catalyst from 10-20%, no considerable change in the yields of reaction was observed and 10 mol% of catalyst was selected as an optimum amount.

Another factor for obtaining the optimization conditions is the investigation of temperature effect on the yield of the reaction. So, with raising the reaction temperature to 80 oC, no change was observed in 5a (Table 1, entry 7). As expected, in the optimum conditions, compound 5a was produced in 95% yield as excellent yield (Table 1, entry 22). Selecting the best solvent is another important factor in the determination of optimization conditiosn. For this reason, selecting the best solvent is performed by examing the effects of solvents on the yield of sample reaction by adding 10 mol% of ZnO nanoparticles and room temperature. The outcomes tabulated in Table 3 exhibited that H_2O is better than other solvents in the reaction sample.

Table 1. Effect of solvent, catalyst and temperature on the condensation reaction of compound **5a**

Entry	Catalyst[a]	Solvent[b]	Temp.[c]	Yield (%)[d]
1	-	-	r.t.	----
2	-	-	80	----
3	-	H_2O	r.t.	10
4	-	CH_3CN	r.t.	10
5	-	Toluene	r.t.	12
6	Et_3N	CH_3CN	r.t.	45
7	Et_3N	CH_3CN	100	45
8	Et_3N	H_2O	r.t.	58
9	Et_3N	H_2O	100	58
10	Et_3N	---	r.t.	25
11	Pyridine	CH_3CN	r.t.	75
12	Pyridine	CH_3CN	100	75
13	Pyridine	H_2O	r.t.	80
14	Pyridine	H_2O	100	82
15	K_2CO_3	CH_3CN	100	45
16	K_2CO_3	H_2O	100	15
17	KF/CP (NPs)	CH_3CN	r.t.	65
18	KF/CP (NPs)	H_2O	r.t.	80
19	TiO_2-NPs	CH_3CN	r.t.	25
20	TiO_2-NPs	H_2O	r.t.	35
21	ZnO-NPs	CH_3CN	r.t.	83
22	**ZnO-NPs**	**H_2O**	**r.t.**	**95**
23	Fe3O4-MNPs	H2O	r.t.	56

For reusing the selected catalyst, it should be removed from the mixture of reaction, washed and dried after completion of the reaction. As shown in the Table 2, the catalyst could be used four times for the synthesis of compound 5a as a reaction sample.

Table 2. Reused catalyst activity for the production of compound **5a**

Run	% Yield[a]
1st	95
2nd	95
3rd	93
4nd	93
5nd	87

Using spectroscopy methods such as IR, ^{1}H NMR, ^{13}C NMR, and mass spectral information the structure of synthesized imidazole **5** was confirmed. Among the prepared imidazoles, we investigated the spectral data of imidazole **5a** as a sample. The ^{1}H NMR spectral data of 5a revealed one singlet for methin protons at 7.68 ppm and one broad singlet for COOH proton at 10.87 ppm. Three carbonyl group of 5a in the 13C NMR spectral data was seen at 162.3, 167.6, 171.2 ppm. Also, the usual C=O bands were seen in the IR spectrum of 5a. Even if there is no data about the details of the

mechanism for the preparation of imidazole **5**, the recommended mechanisms are described in the Scheme 2. First, trichloroacetonitrile **1**, amides **2** and ZnO nanoparticles were mixed and isocyanate **6** was produced. Intermediate **7** as nucleophile was produced from the reaction of isocyanate 6 with aminoacid 3. Then, intermediate 7 is combined with alkyl bromides 4 to produce intermediate 8. The creation of intermediate

9 was carried out by intermolecular cyclization of intermediate 8. In the end, by the elimination of water of intermediate 9, imidazole 5 was produced (Scheme 2). The main benefits of our method are employing few nanocatalysts, high yield of product, short time of reaction and simple separation of product from a mixture of reaction.
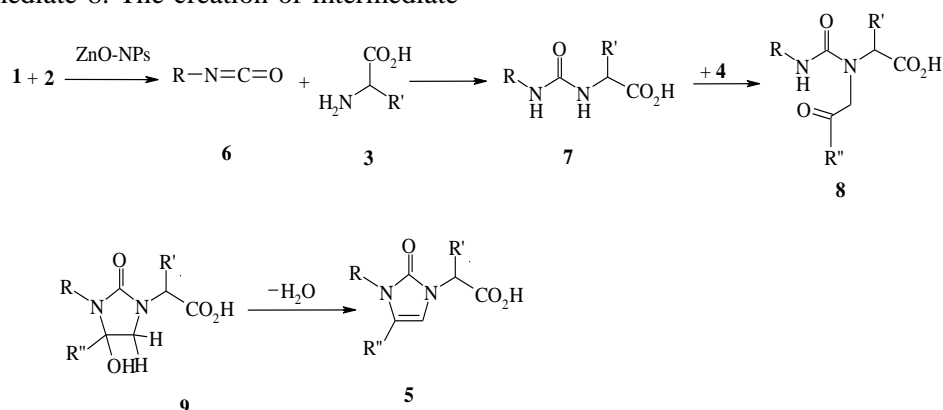
Scheme 2. Plausible mechanism for the preparation of **5**.

Antioxidant ability for the evaluation of benzazepins by utilizing free radical of DPPH

Trapping of DPPH radical test is generally employed due to the antioxidant capacity or strength of compounds for obtaining selected imidazole free radical and investigating their percentage of inhibition of oxidation in foods and biological structures. In these evaluations, antioxidant capacity of the synthesized compounds was determined by taking hydrogen atom or one electron by DPPH radical. The antioxidant ability of the synthesized compounds depends on their percentage of DPPH radical free trapping. The electron or hydrogen donating power of compounds **5a, 5b, 5d** and **5f** to the radical of DPPH determined their antioxidant ability. The radical of DPPH absorption was decreased from 517 nm when one electron or hydrogen was reduced from the antioxidant.

In this work, the ability of imidazole **5a, 5b, 5d** and **5f** as antioxidant was evaluated in relation to the BHT and TBHQ as standard and prepared antioxidant with different concentrations. Overall, the power of DPPH trapping was obtained as TBHQ>BHT>**5b>5a>5f>5d** (Figure 1).As seen in Figure 5, the novel prepared imidazole in all concentrations exhibits a good activity relative to BHT and TBHQ. In the prepared imidazole, compound 5b showed very good radical trapping activity compared to BHT and TBHQ as standard antioxidants.

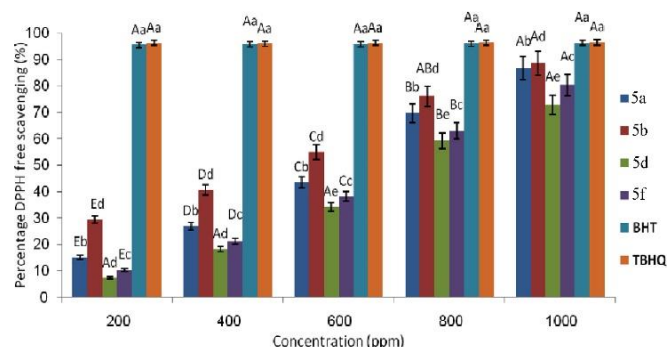**Figure 1**. The activity of imidazole **5a, 5b, 5d** and **5f** for radical scavenging

The potential of synthesized imidazoles to reduce Ferric ions (Fe^{3+})

The ability of some synthesized imidazole such as **5a, 5b, 5d** and **5f** to reduce ferric ions (Fe^{3+}) is calculated based on the quantity of reduction of Fe^{3+}/ferricyanide to the Fe^{2+}/ ferrous at 700 nm [40].As seen in Figure 2, compound 5b showed good ability than BHT and TBHQ as standard antioxidants. The reducing activity trend of the samples was as follows: TBHQ>BHT>**5b>5a>5f>5d**. The outcomes were displayed in Figure 2.

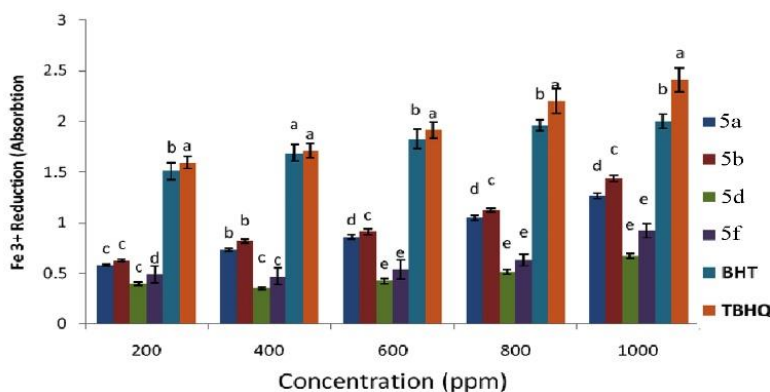**Figure 2.** Antioxidant power of compounds **5a, 5b, 5d** and **5f** basis in reducing ferric ions (Fe^{3+})

Evaluation of the antibacterial ability of some prepared imidazole

The evaluation of antimicrobial activity of some prepared imidazole performed in a comparison between our synthesized imidazole activity with Streptomycin and Gentamicin as the standard drug. The outcomes are tabulated in Table 3. The type of bacteria and concentration of compounds are two effective factor in the diameter of the inhibition zone. As shown in Table 3, the antimicrobial ability of the synthesized imidazole **5b, 5c, 5e** and **5g** were showing good activity in the presence of Gram positive bacteria and Gram negative bacteria. So the maximum effect of prepared imidazole was on Escherichia coli.

Table 3. The antibacterial ability of the some prepared imidazole

Compounds	Staphylococcus aureus (+)	Bacillus cereus (+)	Escherichia coli(-)	Klebsiella pneumoniae (-)
5a	8	10	9	---
5b	17	20	21	18
5c	18	22	23	17
5d	---	5	10	6
5e	15	21	23	20
5f	7	7	8	----
5g	15	21	24	20
Streptomycin	16	24	25	23
Gentamicin	19	23	24	21

Conclusion

In summary, imdazole derivatives were produced in excellent yield from the reaction of trichloroacetonitrile, amides, alkyl bromides and amino acids using ZnO-NPs in water at room temperature. In addition, the power of synthesized imidazole as antioxidant was determined by radical trapping of DPPH and the power of reducing ferric was analyzed. The tested imidazoles display good radical trapping of DPPH but exhibited moderate FRAP relative to BHT and TBHQ as synthetic antioxidants. The outcomes of disk diffusion experiment exhibit that imidazole **5b, 5c, 5e** and **5g** avoided the bacterial growth. The superiorities of this procedure are environmental, high yield of product and low amounts of catalyst and short time of reaction.

Experimental

General

In this research, all chemicals are purchased from Fluka (Buchs, Switzerland) and employed without any purification. For measuring infrared spectroscopy and melting point, a Shimadzu IR-460 spectrometer and Electrothermal 9100 apparatus are utilized, respectively. BRUKER DRX-400 AVANCE spectrometer is used for giving the 1H, and 13C-NMR spectra at 400.1 and 100 MHz, respectively. For recording mass spectra, A FINNIGAN-MAT 8430 spectrometer with an ionization potential of 70 eV was utilized. The scanning electron microscopy (SEM) employing a Holland Philips XL30 microscope was used for the determination of ZnO nanocomposites morphology. X-ray diffraction (XRD) analysis at room

temperature using a Holland Philips Xpert X-ray powder diffractometer, with CuKα radiation (λ=0.15406 nm), with 2θ ranging from 20 to 80° was employed for the characterization of crystalline structure of Fe3O4/CuO nanocomposites. Scherrer's formula; D= 0.9λ/β cosθ was employed for calculating the average crystallite size where D is the diameter of the nanoparticles, λ (CuKα) =1.5406 Å and β is the full-width at half-maximum of the diffraction lines.[38]

Preparation of nanoparticles of ZnO (ZnO-NPs)

Preparation of ZnO-nanoparticles was performed by using a procedure that was reported in the literature.[38]

Common process for generation of compounds 5

The trichloroacetonitrile **1** (2 mmol) and amides **2** (2 mmol) were mixed with ZnO-NPs (10 mol%) in water (5 mL). After 45 min, amino acids **3** (2 mmol) was added to the previous mixture at room temperature. After 30 mins α-haloketones **4** (2 mmol) was added to the mixture and stirred for 3 h. After 3 h, the reaction is completed and TLC confirms the progress of the reaction. At last, the solid residue was collected by filtration and cleaned with EtOAC to remove ZnO-NPs and after evaporating solvent and washing solid with Et₂O, compounds 5 was afforded as pure product.

2-[4-(Ethoxycarbonyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]propanoic acid(5a).

Pale yellow powder, m.p. 168–170 ℃. IR (KBr) (vmax/cm-1): 3100–3590 (COOH), 1742, 1725, 1678, 1625, 1287, 1129 cm-1. 1H NMR: 1.36 (t, 3H, 3J = 7.4, CH3), 1.87 (d, 3H, 3J = 7.3, CH3), 4.28 (q, 2H, 3J = 7.4, OCH2), 6.36 (q, 1H, 3J = 7.3, CH), 7.36 (t, 2H, 3J = 7.6, 2CH), 7.48 (t, 1H, 3J = 7.6, CH), 7.68 (s, 1H, CH), 8.27 (d, 2H, 3J = 7.6, 2CH), 10.87 (s br., 1H, COOH). 13C NMR: 14.5 (CH3), 15.7 (CH3), 56.4 (CH), 61.8 (OCH2), 121.7 (CH), 128.3 (2CH), 129.2 (2CH), 130.2 (CH), 132.7 (C), 136.7 (C), 162.3 (C=O), 167.6 (C=O), 171.2 (C=O). EI-MS, m/z (%): 304 (M+, 11), 276 (81), 77 (100), 73 (43), 45 (100). Anal. Calcd for C15H16N2O5 (304.3): C, 59.21; H, 5.30; N, 9.21. Found: C, 59.36; H, 5.43; N, 9.38.

2-[4-(Ethoxycarbonyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]-3-methyl butanoic acid(5b).

Cream powder, m.p. 178-180℃. IR (KBr) (vmax/cm-1): 2950–3620 (COOH), 1743, 1727, 1695, 1638, 1275, 1136 cm-1. 1H NMR: 1.02 (d, 3H, 3J = 7.3, CH3), 1.35 (d, 3H, 3J = 7.3, CH3), 1.37 (t, 3H, 3J = 7.4, CH3), 3.26 (m, 1H, CH), 4.26 (q, 2H, 3J = 7.4, OCH2), 6.12 (1H, d, 3J = 7.8, CH), 7.52 (t, 2H, 3J = 7.7, 2CH), 7.64 (t, 1H, 3J = 7.7, CH), 7.75 (s, 1H, CH), 8.25 (d, 2H, 3J = 7.6, 2CH), 10.86 (s br., 1H, COOH). 13C NMR: 13.8 (CH3), 19.2 (CH3), 19.8 (CH3), 33.7 (CH), 61.8 (OCH2), 73.5 (CH), 121.7 (CH), 128.3 (2CH), 128.8 (2CH), 130.4 (CH), 132.7 (C), 136.5 (C), 161.2 (C=O), 173.7 (C=O), 175.3 (C=O). EI-MS, m/z (%): 332 (M+, 15), 303 (29), 276 (59), 77 (100), 77 (38), 45 (100). Anal. Calcd for C17H20N2O5 (332.35): C, 61.44; H, 6.07; N, 8.43. Found: C, 61.62; H, 6.24; N, 8.62.

2-[4-(Ethoxycarbonyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]acetic acid (5c).

Pale yellow powder, m.p. 121-123 ℃. IR (KBr) (vmax/cm-1): 3080-3595 (COOH), 1738, 1726, 1687, 1647, 1295, 1156 cm-1. 1H NMR: 1.32 (t, 3H, 3J = 7.4, CH3), 4.28 (q, 2H, 3J = 7.4, CH2), 5.64 (s br, 2 H, CH2), 7.38 (t, 2H, 3J = 7.6, 2CH), 7.56 (t, 1H, 3J = 7.6, CH), 7.62 (s, 1H, CH), 8.26 (d, 2H, 3J = 7.6, 2CH), 10.87 (s br., 1H, COOH). 13C NMR: 14.2 (CH3), 48.7 (CH2), 61.6 (OCH2), 121.5 (CH), 129.2 (2CH), 129.8 (CH), 130.4 (2CH), 133.0 (C), 137.3 (C), 162.8 (C=O), 169.7 (C=O), 174.1 (C=O). EI-MS, m/z (%): 290 (M+, 10), 276 (52), 261(23), 77 (100), 77 (34), 45 (100). Anal. Calcd for C14H14N2O5 (290.27): C, 57.93; H, 4.86; N, 9.65. Found: C, 58.16; H, 4.97; N, 9.78.

2-[4-(4-methoxyphenyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]propanoic acid (5d).

White powder, m.p. 189-190 ℃. IR (KBr) (vmax/cm-1): 2860–3250 (COOH), 1740, 1697, 1625, 1387, 1252 cm-1. 1H NMR: 1.78 (d, 3H, 3J = 7.3, CH3), 3.86 (s, 3H, OCH3), 4.89 (q, 1H, 3J = 7.3, CH), 6.56 (s, 1H, CH), 7.12 (d, 2H, 3J = 7.8, 2CH), 7.33 (d, 2H, 3J = 7.8, 2CH), 7.36-7.52 (m, 3H, 3CH), 8.25 (d, 2H, 3J = 7.6, 2 CH), 10.98 (s br, 1H, COOH). 13C NMR: 14.2 (CH3), 55.6 (OCH3), 56.8 (CH), 107.2 (CH), 115.4 (2 CH), 122.3 (C), 128.5 (2CH), 130.2 (2CH), 131.6 (2CH), 132.5 (CH), 136.7 (C), 138.4 (C), 139.6 (C), 158.3 (C), 172.6 (C=O), 173.5 (C=O). EI-MS, m/z (%): 338 (M+, 10), 310 (45), 105 (100), 77 (31), 73 (48), 45 (18). Anal. Calcd for C19H18N2O4 (338.36): C, 67.45; H, 5.36; N, 8.28. Found: C, 67.62; H, 5.54; N, 8.45.

2-[4-(4-methoxyphenyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]-3-methyl butanoic acid (5e).

White powder, m.p. 201-203℃. IR (KBr) (vmax/cm-1): 2860-3325 (COOH), 1738, 1689, 1634,

1386, 1294 cm-1. 1H NMR: 1.02 (d, 3H, 3J = 7.2, CH3), 1.14 (d, 3H, 3J = 7.2, CH3), 3.25 (m, 1H, CH), 3.89 (s, 3H, OCH3), 4.38 (d, 1H, 3J = 7.3, CH), 6.68 (s, 1H, CH), 7.12 (d, 2H, 3J = 7.8, 2CH), 7.38 (d, 2H, 3J = 7.8, 2CH), 7.52 (t, 2H, 3J = 7.6, 2CH), 7.58 (t, 1H, 3J = 7.6, 2CH), 8.14 (d, 2H, 3J = 7.6, 2CH), 10.76 (s br., 1H, COOH). 13C NMR: 18.7 (CH3), 21.2 (CH3), 28.3 (CH), 55.6 (OCH3), 72.3 (CH), 107.6 (CH), 114.8 (2CH), 122.4 (C), 129.5 (2CH), 130.2 (2CH), 131.7 (2CH), 133.3 (CH), 135.6 (C), 136.4 (C), 138.3 (C), 157.2 (C), 173.4 (C=O). EI-MS, m/z (%): 366 (M+, 15), 310 (39), 105 (100), 77 (86). Anal. Calcd for C21H22N2O4 (366.41): C, 68.84; H, 6.05; N, 7.65. Found: C, 68.96; H, 6.22; N, 7.83.

*2-[4-(4-methoxyphenyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]acetic acid (**5f**).*

White crystals, m.p. 162-164 ºC. IR (KBr) (vmax/cm-1): 2863–3300 (COOH), 1742, 1694, 1637, 1378, 1296 cm-1. 1H NMR: 3.78 (s, 3H, OCH3), 4.78 (s, 2H, CH2), 7.038 (s, 1H, CH), 7.14 (d, 2H, 3J = 7.8, 2CH), 7.36 (d, 2H, 3J = 7.8, 2CH), 7.47-7.58 (m, 3H, 3CH), 8.23 (d, 2H, 3J = 7.6, 2CH), 11.25 (s br., 1H, COOH). 13C NMR: 48.7 (CH2), 56.4 (OCH3), 107.6 (CH), 115.8 (2CH), 123.5 (C), 129.7 (2CH), 130.4 (2CH), 131.7 (2CH), 132.6 (CH), 137.6 (C), 138.5 (C), 140.2 (C), 157.3 (C), 173.6 (C=O). EI-MS, m/z (%): 324 (M+, 15), 310 (35), 105 (100), 77 (28), 45 (68). Anal. Calcd for C18H16N2O4 (324.33): C, 66.66; H, 4.97; N, 8.64. Found: C, 66.82; H, 5.22; N, 8.85.

*2-[4-(4-bromophenyl)-2-oxo-3-phenyl-2,3-dihydro-1H-imidazol-1-yl]propanoic acid (**5g**).*

Pale yellow crystals, m.p. 198-200 ºC. IR (KBr) (vmax/cm-1): 2960–3375 (COOH), 1738, 1714, 1648, 1487, 1367, 1298 cm-1. 1H NMR: 1.56 (d, 3H, 3J = 7.2, CH3), 4.69 (q, 1H, 3J = 7.2, CH), 6.63 (s, 1H, CH), 7.34-7.42 (m, 3H, 3CH), 7.45 (d, 2H, 3J = 7.8, 2CH), 7.56 (d, 2H, 3J = 7.8, 2CH), 8.15 (d, 2H, 3J = 7.6, 2CH), 11.23 (s br., 1H, COOH). 13C NMR: 14.8 (CH3), 57.4 (CH), 109.3 (CH), 124.6 (C), 129.5 (2CH), 130.4 (2CH), 130.8 (C), 132.7 (2CH), 133.2 (C), 133.6 (2CH), 137.8 (C), 138.6 (C), 166.4 (C=N), 173.5 (C=O). EI-MS, m/z (%): 388 (M++2, 10), 386 (M+, 10), 358 (31), 360 (31), 105 (100), 77 (64). Anal. Calcd for C18H15BrN2O3 (387.23): C, 55.83; H, 3.90; N, 7.23. Found: C, 55.98; H, 4.12; N, 7.42.

Determination of antioxidant activity using radical trapping test by DPPH

The radical trapping experiment by DPPH was employed for evaluation of antioxidant ability of some generated compounds such as **5a, 5b, 5d** and **5f** as per the procedure of Shimada et al [17]. For achieving this purpose, different concentrations (200–1000 ppm) of compounds **5a, 5b, 5d** and **5f** were added to DPPH methanolic solution (1 mmol/L) with an equal volume. The mixture was mixed for 30 min at ambient temperature and after this time it was put in a gloomy space and the mixture absorbance was recorded at 517 nm. The compounds **5a, 5b, 5d** and **5f** were exchanged with methanol (3 mL) in the standard type. The standard antioxidants in this experiment are Butylated hydroxytoluene (BHT) and 2-tertbutylhydroquinone (TBHQ). By using Yen and Duh [18] formula, the percentage of inhibition for the radical of DPPH was measured.

Evaluation of reducing ability of the synthesized compounds

The ability of reducing iron (III) was evaluated for compounds **5a, 5b, 5d** and **5f** using the method by Yildirim et al.[19] For this purpose, the samples (1 mL), potassium ferricyanide (K3Fe(CN)6; 2.5 mL, 10g/L) and buffer of phosphate (2.5 mL, 0.2 mol/L, pH 6.6) were combined together and sustained for 30 min at 50 oC. Then, to the previous solution trichloroacetic acid (2.5 mL, 10% w/v) was added and centrifuged for 10 min. In the end, the supernatant (2.5 mL), distilled water (2.5 mL) and FeCl3 (0.5 mL, 1 g/L) were mixed together and at 700 nm the absorbance of the samples was measured. The higher reducing power was attributed to higher absorbance. For accuracy, each calculation was performed three times. The SPSS software version 18.0 by running one way study of variance (ANOVA) was used for data analyzation of compounds that confirmed variation of samples and control. Separation of mean with the important quantity of 95% ($P < 0.05$) was performed by Duncan multiple range experiments.

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