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ORIGINAL PAPER

The synthesis of imidazoles and evaluation of their antioxidant and antifungal activities

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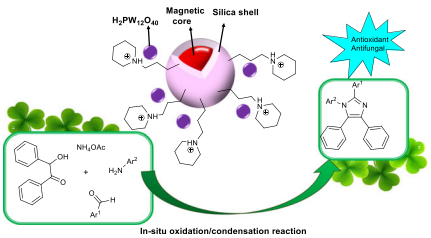
Abstract

Tri- and tetra-substituted imidazole compounds were synthesized through in situ oxidation–condensation in the presence of catalytic amount of $H_2PW_{12}O_{40}^-$ loaded on the ionic liquid-functionalized magnetic nanoparticles. Then, the antioxidant and antifungal activities of the new imidazoles were evaluated. The effectiveness of the samples as DPPH radical scavengers was confirmed by the measured IC_{50} values and thiophenyl-containing product showed the best IC_{50} of 0.12 when compared to the standard ascorbic acid. Moreover, all compounds have antifungal activity against *Fusarium oxysporum*.

Graphical Abstract



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Keywords Imidazole · Antioxidant · Fungicide · Phosphotungstic acid · Magnetic properties

Introduction

Through physiological and metabolic processes and food products, free radicals and partially reduced oxygen-containing compounds are produced. These species are toxic since they oxidize the vital biomolecules (proteins, lipids, nucleic acids, sugars) leading to tissue injury, DNA damaging, and various diseases such as arthritis, cancer, and Alzheimer's disease [1]. Antioxidants are commonly organic compounds which by preventing the oxidation of biomolecules can inhibit DNA mutations, oxidative stress, and other forms of cell damage. Antioxidant activity of organic compounds is usually evaluated using a spectrophotometer with regard to the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. This method is straightforward, cost-effective, and rapid to find the non-enzymatic antioxidants [2].

1.35 Million people die annually as a result of fungal infection. Among the pathogens and parasites, fungi are the ones that endanger the health of humans and other creatures. Some antifungal compounds are produced naturally although resistance to these compounds occurs

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correspondingly. Therefore, development of new antifungal agents is inevitable [3].

Imidazole is a nitrogen-rich heterocyclic compound which derivatives are applied in different fields of study including sensors [4], catalysis [5–7] and electrocatalysis [8], drug discovery [9–13], producing fuel cells [14], and conducting polymers [15]. Due to the importance of imidazole compounds, an optimized methodology is essential for their synthesis [16–21]. Therefore, according to the significance of imidazole compounds, herein we used a new phosphotungstate-based catalyst for the preparation of tri- and tetra-substituted imidazole compounds. Then, the antioxidant and antifungal activities of the produced imidazoles are investigated and discussed.

Results and discussion

Magnetite is a perfect metal oxide support, easy to make, having a very active surface for immobilization of metals, silica, and ligands, which can be separated by an external magnet after the reaction, thus converting it to a more sustainable catalyst [22]. Therefore, we selected magnetic nanoparticles (MNPs) as the core of the catalyst to make an easily separable heterogeneous catalyst. Then, the surface of MNPs was covered with silica and subsequently, the surface of silica was modified with a new type of ionic liquid including a piperidinium moiety which is different from the traditional types containing the imidazolium cations [23, 24]. Consequently, the synthesis of imidazole compounds was catalyzed by $H_2PW_{12}O_{40}^-$ loaded on the ionic liquid-functionalized magnetic nanoparticles (see ESI for the preparation and characterization of the catalyst). The oxidizing nature of catalyst was tested through the multicomponent reaction of benzoin, aldehyde, aniline derivative, and ammonium acetate. As it is evident, benzoin is oxidized to benzil in the presence of $H_3PW_{12}O_{40}$ loaded on the catalyst surface and then, benzil follows the reactions. The reaction of benzoin (**1**), ammonium acetate (**2**) and 4-fluorobenzaldehyde was selected as the model reaction, and its condition was modified in the presence of the mentioned catalyst (Table 1). Among the tested conditions as shown in entries 1–4 of Table 1, refluxing in a solvent was better than the solvent-free condition. Protic solvent (EtOH) plays a significant role in the formation of the product since the used catalyst can exchange proton to activate the starting materials. Therefore, refluxing in EtOH was selected as the best condition for this reaction in the presence of the catalyst. To examine the effect of $H_2PW_{12}O_{40}^-$ and ionic liquid phase on this reaction, synthesis of **4a** was tested in the presence of naked MNPs and silica-coated MNPs under modified conditions (entries 5 and 6, respectively). The results demonstrated that both of them affect this reaction but not efficiently. Low reaction yield after 3 h in the absence of the catalyst displays the vital role of catalyst in this reaction. To study the generality of this reaction, various tri- and tetra-substituted imidazole derivatives (Scheme 1 and 2) were synthesized in the presence of $H_3PW_{12}O_{40}/Fe_3O_4@SiO_2$–Pr–Pi (Table 2). The high yield of products proves the efficiency of the prepared catalyst. It is essential to mention that separation of the catalyst by the use of an external super magnet is the main advantage of this procedure.

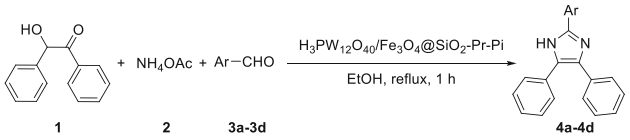
The proposed mechanism is shown in Scheme 3. Initially, the oxygen atom of aldehyde carbonyl group is protonated by the catalyst and then, the released ammonia from ammonium acetate attacks the activated carbonyl group. The amine **5** is added to the formed imine **7** to gain intermediate **8**. Afterward, the latter is condensed with the activated benzil **9** (which is produced through oxidation of benzoin **1** by the $H_2PW_{12}O_{40}^-$, following protonation), and the final product is obtained after dehydration and aromatization of intermediate **10**.

Antioxidant activity of tetra-substituted imidazoles

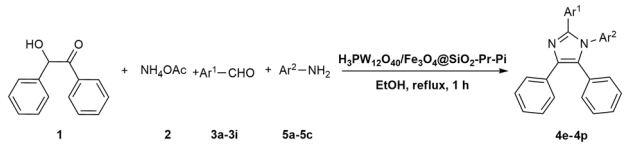
In this research, all new imidazoles were investigated for antioxidant activity and all of them showed lower scavenging activity than that of ascorbic acid as a standard antioxidant except the sample **4n**. The radical scavenging activity of the new imidazoles decreased in the following

Table 1 Optimizing the reaction conditions or the synthesis of imidazole **4a**

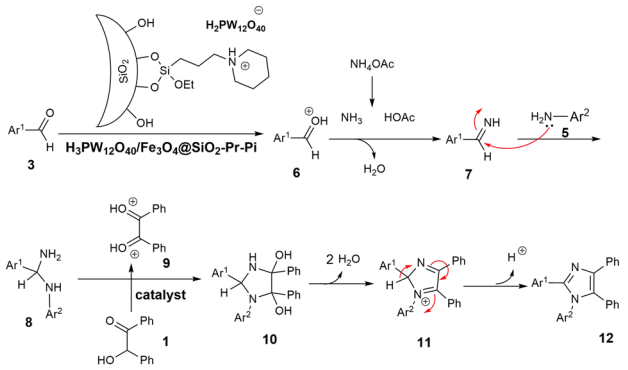
Entry	Catalyst	Solvent	Condition	Time/h	Yield/%
1	$H_3PW_{12}O_{40}/Fe_3O_4@SiO_2$–Pr–Pi	–	30 °C	3.5	70
2	$H_3PW_{12}O_{40}/Fe_3O_4@SiO_2$–Pr–Pi	CH_3CN	Reflux	1.5	70
3	$H_3PW_{12}O_{40}/Fe_3O_4@SiO_2$–Pr–Pi	THF	Reflux	1.5	60
4	$H_3PW_{12}O_{40}/Fe_3O_4@SiO_2$–Pr–Pi	EtOH	Reflux	1	95
5	Fe_3O_4	EtOH	r.t.	3	50
6	$Fe_3O_4@SiO_2$	EtOH	60 °C	3	70
7	$Fe_3O_4@SiO_2$–Pr–Pi	EtOH	Reflux	2	75
8	–	EtOH	Reflux	3	30



Scheme 1 .



Scheme 2 .



Scheme 3 .

order: **4n** > ascorbic acid > **4 m** > **4p** > **4o** > **4c** > **4d** (Table 3). The higher antioxidant activity is reflected in a lower IC_{50}. On the other hand, the effectiveness of the samples as DPPH radical scavengers was confirmed by the measured IC_{50} values (Fig. 1).

Antifungal activity

Evaluation of new antifungal agents through in vitro susceptibility testing can help establish guidelines for the potential clinical application of new the rapiers. *Fusarium* is an essential genus of fungal pathogens, responsible for devastating diseases such as cereal scab, which has epidemic levels [30]. Antifungal activity of the new compounds was evaluated here against *Fusarium oxysporum* through mycelial growth inhibition using a microscale-amended-medium assay (Table 4). Our results showed that all compounds have antifungal activity against *Fusarium oxysporum*, especially the samples **4d**, **4c**, and **4o**. So they may be considered as promissory antifungal agents.

Conclusion

In this paper, the synthesis of tri- and tetra-substituted imidazole derivatives was investigated in the presence of $H_2PW_{12}O_{40}^-$ loaded on the magnetic nanoparticle-supported ionic liquid phase. Simple preparation and also easy isolation of this catalyst from the reaction mixture using an external magnet are its advantages. The new imidazole compounds were also analyzed for their antioxidant properties by DPPH free radical scavenging assay. Also, the antifungal activity of the synthesized compounds was assessed by the cultivation of the fungus *Fusarium oxysporum* on potato dextrose agar medium containing them. The results showed that these compounds exhibited ranges of biological activities.

Table 2 $H_3PW_{12}O_{40}$/Fe_3O_4@SiO_2–Pr–Pi-catalyzed synthesis of tri- and tetra-substituted imidazoles

Entry	No.	Ar^1	Ar^2	Yield/%	M.p./°C	Lit. m.p./°C
1	**4a**	4-F-C_6H_4	H	95	252–253	250–251 [25]
2	**4b**	4-Cl-C_6H_4	H	95	260–261	262–264 [25]
3	**4c**	4-OMe-C_6H_4	H	90	220–221	228–231 [25]
4	**4d**	4-Me-C_6H_4	H	96	232–233	230–232 [25]
5	**4e**	4-Cl-C_6H_4	4-Me-C_6H_4	98	223–225	224–226 [26]
6	**4f**	4-Cl-C_6H_4	4-Cl-C_6H_4	95	209–210	209–211 [27]
7	**4g**	4-Me-C_6H_4	4-Cl-C_6H_4	95	219–220	221–223 [26]
8	**4h**	4-Me-C_6H_4	4-Br-C_6H_4	96	226–227	228–230 [28]
9	**4i**	4-OMe-C_6H_4	4-Br-C_6H_4	97	220–223	220–222 [28]
10	**4j**	4-Biphenyl	4-Br-C_6H_4	97	280–281	281–283 [28]
11	**4k**	2-Naphthyl	4-Br-C_6H_4	96	253–254	253–255 [28]
12	**4l**	2,4-Cl_2-C_6H_3	4-Cl-C_6H_4	95	244–247	246–248 [28]
13	**4m**	Thiophen-2-yl	C_6H_5	95	248–249	247–251 [29]
14	**4n**	Thiophen-2-yl	4-Me-C_6H_4	92	201–203	–
15	**4o**	Thiophen-2-yl	4-OMe-C_6H_4	93	189–191	–
16	**4p**	Thiophen-2-yl	4-Cl-C_6H_4	90	216–218	–

Table 3 Antiradical activity (%) of the new imidazole compounds and ascorbic acid as standard

Sample	Concentration/mg cm^{-3}					Average
	0.2	0.4	0.6	0.8	1	
4 m	$75.35^{c} \pm 0.40$	$75.63^{c} \pm 2.7$	$79.31^{b} \pm 0.04$	$81.54^{b} \pm 1.33$	$85.11^{a} \pm 1.06$	79.38
4n	$81.13^{c} \pm 1.3$	$82.85^{bc} \pm 1.6$	$85.58^{b} \pm 2.7$	$88.84^{a} \pm 0.011$	$88.67^{a} \pm 0.08$	85.41
4p	$74.71^{b} \pm 1.7$	$71.27^{b} \pm 0.72$	$72.31^{b} \pm 0.12$	$82.85^{a} \pm 0.7$	$83.34^{a} \pm 1.03$	77.11
4d	$61.67^{c} \pm 0.20$	$61.13^{c} \pm 0.72$	$61.71^{c} \pm 0.24$	$65.31^{b} \pm 1.57$	$69.58^{a} \pm 0.14$	63.88
4c	$70.37^{b} \pm 0.5$	$71.22^{b} \pm 0.06$	$70.88^{b} \pm 0.47$	$72.91^{a} \pm 0.05$	$74.02^{a} \pm 1.3$	71.88
4o	$67.76^{b} \pm 0.39$	$68^{b} \pm 0.06$	$67.55^{b} \pm 0.48$	$69.58^{a} \pm 0.05$	$70.69^{a} \pm 1.3$	75.69
Ascorbic acid	$80.84^{a} \pm 0.80$	$81.84^{a} \pm 1.70$	$81.21^{a} \pm 1.40$	$80.80^{a} \pm 2.30$	$80.16^{a} \pm 1.90$	80.61

The experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($p \leq 0.05$)

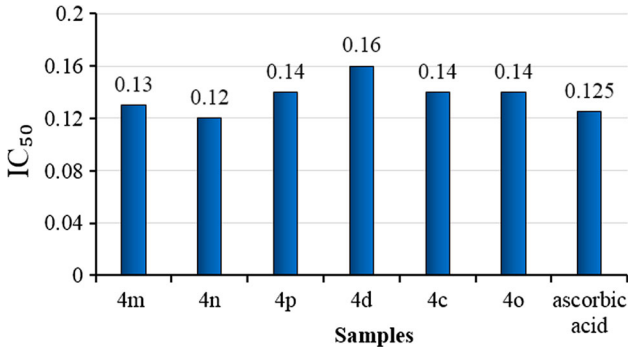


Fig. 1 Comparison of antioxidant activity (IC_{50} values) of the samples and ascorbic acid as standard

Experimental

All chemicals were commercially available substances purchased from Merck and Fluka Companies and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively (Bruker Avance). A Perkin-Elmer GX FT-IR spectrometer was used for Fourier transform infrared (FT-IR) spectra. Elemental analyses (CHN) were obtained by the use of an Elemental Combustion System 4010. Thermo-gravimetric analyses (TGA) were performed on a PYRIS DIAMOND. The images of scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analyses were done on a FESEM-SIGMA (Germany) instrument. A ZEISS-EM3200 instrument was used to capture transmission electron microscopy (TEM) images. Brunauer–Emmett–Teller (BET) analysis was performed on a BELSORP instrument.

Preparation of piperidine-modified silica-coated magnetic nanoparticles (Fe_3O_4@SiO_2–Pr–Pi)

The silica-coated magnetic Fe_3O_4 nanoparticles were prepared through the method published before [31, 32]. Afterward, 3 g Fe_3O_4@SiO_2 and (3-chloropropyl)triethoxysilane (10 mmol) in 80 cm^3 of dry toluene were refluxed under nitrogen for 12 h. The treated Fe_3O_4@SiO_2–Pr–Cl was filtered, washed well with toluene and diethyl ether, and dried at 80 °C for 6 h under reduced pressure. To prepare piperidine-modified magnetic nanoparticles (Fe_3O_4@SiO_2–Pr–Pi), first 1.0 g Fe_3O_4@SiO_2–Pr–Cl was dispersed in a mixture of 50 cm^3 dry toluene and 3 cm^3 piperidine for 30 min. Then, the reaction mixture was refluxed for 72 h. The solid phase was filtered off, washed with toluene and ethanol, and dried at 60 °C in a vacuum.

Loading the $H_3PW_{12}O_{40}$ on the Fe_3O_4@SiO_2–Pr–Pi nanoparticles

Fe_3O_4@SiO_2–Pr–Pi (0.5 g) and 1 g $H_3PW_{12}O_{40}$ were dispersed in 70 cm^3 methanol and the mixture was refluxed for 24 h under N_2 atmosphere. Then, the solid was separated using an external super magnet and washed

Table 4 Antifungal activity (%) of the new imidazole compounds

Concentration/ppm	Sample					
	4 m	**4n**	**4p**	**4d**	**4c**	**4o**
200	$41^{f} \pm 1.11$	$49^{e} \pm 1.3$	$30^{g} \pm 1.6$	$53^{d} \pm 1.8$	$61^{c} \pm 1.7$	$72^{b} \pm 1.2$

The experiment was performed in triplicate and expressed as mean ± SD. Values in the row with different superscripts are significantly different ($p \leq 0.05$)

thoroughly with methanol using a Soxhlet apparatus and then dried for characterization (see ESI).

General procedure for the preparation of tri-substituted imidazoles 4a–4d

A mixture of 0.21 g benzoin (**1**, 1 mmol), the corresponding aldehyde **3** (1 mmol), and 0.12 g ammonium acetate (**2**, 1.5 mmol) dissolved in 3 cm^3 EtOH was heated in the presence of 2 mg of the magnetic catalyst under reflux condition. The progress of the reaction was monitored by thin layer chromatography (TLC) with a tank of EtOAc and *n*-hexane (ratio of 2:5). After completion of the reaction, the catalyst was separated using an external magnet, and the residue was dissolved in an excess amount of hot EtOH to obtain a clear solution and then to gain pure crystals of the product. The obtained products were analyzed by melting points, FT-IR and NMR spectra.

General procedure for the preparation of tetra-substituted imidazoles 4e–4p

A mixture of 0.21 g benzoin (**1**, 1 mmol), the corresponding aldehyde **3** (1 mmol), amine **5** (1 mmol), and 0.12 g ammonium acetate (**2**, 1.5 mmol) dissolved in 3 cm^3 EtOH was heated in the presence of 2 mg of the magnetic catalyst under reflux condition. The progress of the reaction was monitored by thin layer chromatography (TLC) with a tank of EtOAc and *n*-hexane (ration of 2:5). The workup process was same as the procedure mentioned before.

4,5-Diphenyl-2-(thiophen-2-yl)-1-(p-tolyl)-1H-imidazole (4n, $C_{26}H_{20}N_2S$) White solid; m.p.: 201–203 °C; FT-IR (KBr): $\bar{\nu} = 3433, 3059, 2917, 1600, 1512, 1226\ cm^{-1}$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 2.30$ (s, 3H, $-CH_3$), 6.49–7.44 (m, 17H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 125.2, 126.2, 126.4, 127, 127.4, 128.1, 128.4, 128.7, 129.4, 129.9, 130, 131, 131.2, 132.9, 133.4, 134, 136.7, 139, 141.4$ ppm.

1-(4-Methoxyphenyl)-4,5-diphenyl-2-(thiophen-2-yl)-1H-imidazole (4o, $C_{26}H_{20}N_2OS$) White solid; m.p.: 189–191 °C; FT-IR (KBr): $\bar{\nu} = 3104, 3064, 2929, 1607, 1512, 1254\ cm^{-1}$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.75$ (s, 3H, OCH_3), 6.55–7.51 (m, 17H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.3, 114.4, 125.2, 126.2, 126.4, 127, 127.4, 128.1, 128.4, 128.5, 130.1, 130.2, 131, 131.4, 132.9, 134.1, 136.6, 141.6, 159.5$ ppm.

1-(4-Chlorophenyl)-4,5-diphenyl-2-(thiophen-2-yl)-1H-imidazole (4p, $C_{25}H_{17}ClN_2S$) White solid; m.p.: 216–218 °C; FT-IR (KBr): $\bar{\nu} = 3430, 3055, 1600, 1580, 1490\ cm^{-1}$; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 6.58–7.54$ (m, 17H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 125.6, 126.2, 126.6, 127.3, 127.5, 128.1, 128.5, 128.6, 129.4, 129.7, 131, 131.2, 132.5, 133.8, 134.1, 135, 136.8, 141.3$ ppm.

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