Iranian Journal of Organic Chemistry Vol. 16, No. 4(2024) 3919-3928

Synthesis and Study of antioxidant activity of pyrazolopyrimidins employing $ZnO@Fe_3O_4$ nanoparticles

Mahboubeh Ghasemian Dazmiri[a*], Sayyed Narges Hosseini[b], Narges Ghasemi[c]

[a]*Department of Chemistry,Facualty of Chemistry, University of Mazandaran, Babolsar, Iran.*
[b]*Department of Chemistry,TarbiatModaresUniversity, Tehran, Iran.*
[c]*National Petrochemical Company (NPC), petrochemical Research and Technology Company, Arak Center, Iran*

Received: September 2024; Revised: September 2024; Accepted: October 2024

Abstract: The preparation of pyrazolo pyrimidinone derivatives were performed by using five component reactions of phthalaldehyde, cyanomethylamine, electron deficient acetylenic compounds, isocyanate, hydrazine and catalytic amounts of ZnO@Fe3O4-magnetic nanoparticles as a high performance catalyst under ultrasonic conditions at ambient temperature in aquause media at room temperature. It should be mentioned this catalyst was prepared using Spinacia oleracea water extract. In addition, for investigation of antioxidant ability radical trapping by DPPH and reducing power of ferric ion experiments was performed. As a result, synthesized compounds show excellent radical trapping by DPPH and good reducing ability of ferric ion. The current procedure has the benefits for instance excellent yield of reaction, green media and easy separation of product and catalyst.

Keywords: ZnO@Fe3O4-magnetic NPs, Ability of oxidation, pyrazolo pyrimidinone, DPPH radical trapping, Cyano methylamine.

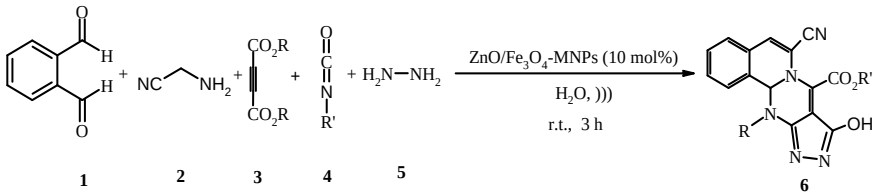
Introduction

Recently a diversity of procedures and mechanisms has been developed based on green chemistry or sonochemistry [1]. Sonochemistry as an original and valuable method has attracted increasing interest in accelerating organic reactions [2-5]. This procedure can be very efficient and is applicable to a broad variety of practical synthesis. Luche and coworkers have carried out a number of investigations, which provided the basis for using sonochemistry in organic synthesis [6-9]. The significant features of the ultrasound approach in organic reactions are improvement of reaction rates, formation of pure products with high yields and easier process. This method is also considered as a help in terms of energy protection and waste decreasing when compared with traditional methods [10, 11].

Recently, multicomponent reactions (MCRs) are more interesting type of reaction due to mixing three or more reactants in one-pot and generating one product [12-18] and economically useful and environmentally secure than to multi-step methods. MCRs are very important in the synthesis of new drugs and agrochemicals [19-26]. In the multistep reaction generally due to multiple stages of separation of product, generate large amounts of waste that often involve the employ of pricey, poisonous or unsafe solvents in all stage.The one-pot generation of compounds with small heterocycle display of different and intricate compounds with little heterocycles show a powerful method in synthetic chemistry [27].The creation of reactions with extremely economical condition, low synthetic process and short time is a chief topic in advanced synthetic chemistry. In addition carrying out synthesis of organic compounds in water

*Corresponding author email: pmgh@yahoo.com

media is very interesting because of water is cheap solvent, more available with high amounts. For the reactions that starting compounds aren't solved in water, the rate of reaction improves. Separation of products in these reactions is very easy because of products aren't solved in water and separated by employing filtration [28-30]. Pyrimidinones due to having broad range of biological activities [34] such as antibacterial, antiviral, antihypertensive, and antitumor effects and their effect as calcium channel blockers [32] are very interesting compounds which have a chief place in natural and synthetic organic chemistry. Moreover, some bioactive alkaloids containing the pyrimidine unit which are isolated from sea sources, exhibit anti-HIV ability [33]. Sketching and employing suitable catalyst increases the way to green chemistry. In the field of catalyst one of the optimizations factor is expansion of using nanocatalyst. In the presence of nanocatalyst, some organic reactions have excellent yields and selectivity of product than to usual sized [34, 35]. In this research investigation of antioxidant ability for some of the synthesized compounds is performed. Frequently compounds with antioxidant ability, eliminate the negative property of free radicals and utilize as transitional metals chelators. This result is due to their reducing properties and chemical structure. Also, these compounds could be avoid or decrease many sicknesses such as cardiovascular, inflammatory bowel syndrome, cancer, ageing, and Alzheimer [36-38]. In continuation of our challenges to develop new synthetic process for significant organic compounds with biological ability [39-47], we do the generation of new pyrazozlo pyrimidinones **6** employing the reaction of phthalaldehyde**1**, cyano methyl amine **2**, electron deficient acetylenic compounds **3**, isocyanates **4**, hydrazine **5** and ZnO@Fe_3O_4-MNPs as catalyst using ultrasonic conditions at ambient temperature in water as green media in excellent yields (Scheme 1).



6	R	R'	Yield % of 6
a	Me	C_6H_5	90
b	Me	4-NO_2-C_6H_4	87
c	Me	4-Br-C_6H_4	90
d	Me	3-Br-C_6H_4	90
e	Me	4-MeO-C_6H_4	95
f	Et	4-MeO-C_6H_4	95

Scheme 1: Synthesis of compounds **6**

Result and discussion

Chemistry

In this research pyrazozlo pyrimidinones **6** was synthesized in excellent yields by employing the reaction of phthalaldehyde**1**, cyano methyl amine **2**, electron deficient acetylenic compounds **3**, isocyanates **4**, hydrazine **5** and ZnO@Fe_3O_4-MNPs as catalyst using ultrasonic conditions at ambient temperature in water as green media (Scheme 1). In the starting stage of this work, for obtaining the best situations for preparation of **6e**, the reaction of phthalaldehyde**1**, cyano methylamine **2**, dimethyl acetylenedicarboxylate**3a**, 4-methoxy phenylisocyante**4e** and hydrazine **5** under ultrasonic conditions was utilized as a model reaction to reach the best conditions (Table 1). By altering the catalyst, amount of catalyst and temperature of reaction could obtain the best conditions for preparation of compound **6e**. The reaction mixture is very busy in absence of catalyst. This reaction was not performed without any catalyst even after 10 h and very busy mixture in absence of catalyst (Table 1, entry 1). For this reason, 10 mol% catalyst such as ZnO-NPs was added to the mixture of reaction. The yield of compound **6e** was 75% after 5 h (Table 1, entry 16). For investigation the catalytic effect on this reaction, several catalyst such as ZnO-nanorods, CuO-NPs, Al_2O_3, Et_3N, Fe_3O_4-MNPs, Fe_3O_4/ZnO and TiO_2-NPs were tested in this reaction. Therefore, these outcomes exhibited the Fe_3O_4/ZnO-MNPs is the best catalyst for this reaction. Then, the reaction was done in the presence of 10 mo% of Fe_3O_4/ZnO-MNPs as catalyst. By increasing the amount of catalyst from 10-30%, didn't seen any considerable

change in the yields of reaction. Also, by increasing the reaction temperature to 80 °C the yield of reaction wasn't increased (entry 2, Table 1). Thus, to find out the best amount of catalyst, (10–30 mol%) of Fe_3O_4/ZnO-MNC were used in sample reaction (synthesis of **6e**). The outcomes exhibited 10 mol% of catalyst is sufficient for prepare a good yield of **6e**.

Table 1: Selecting the best conditions for generation of **6e**

Entry	Catalyst[a]	Solvent[b]	Temp.[c]	Time[d]	Yield (%)[e]
1	-	-	r.t.	15	----
2	-	-	80	20	----
3	-	H_2O	r.t.	15	5
4	-	CH_3CN	r.t.	15	5
5	-	Toluene	r.t.	15	10
6	Et_3N	CH_3CN	r.t.	8	45
7	Et_3N	CH_3CN	80	8	47
8	Et_3N	H_2O	r.t.	5	68
10	TiO_2-NPs	CH_3CN	r.t.	10	25
11	TiO_2-NPs	H_2O	r.t.	6	35
12	CuO-NPs	CH_3CN	r.t.	8	48
13	CuO-NPs	H_2O	r.t.	5	50
14	Fe_3O_4/ZnO	CH_3CN	r.t.	3	92
15	**Fe_3O_4/ZnO**	**H_2O**	**r.t.**	**3**	**95**

By changing the amounts of catalyst from 10 to 25%, wasn't seen significant change in the yield of compound **6e**. Therefore, the best amount of Fe_3O_4/ZnO MNPs as catalyst is 10 mol%.

For confirmation the reusability of the catalyst, we separated the catalyst by external magnet and use again in the sample reaction (the preparation of **6e**). The results displayed that the catalyst can be recycled five times with any losing of ability. For the recycling of catalyst, after completion of reaction, the catalyst separated from mixture of reaction by external magnet and cleaned by water and then dried at room temperature for 24 h and utilized for the next catalytic cycle. The Fe_3O_4/ZnO-MNPs was prepared by using *Spinacia oleracea* water extract as green media. The *Spinacia oleracea* water extract acts as the moderate base source material in the formation of Fe_3O_4/ZnO-MNPs. The scanning electron microscopy images (SEM) Figure 1 and X-ray diffraction patterns (XRD) Figure 2 utilized for determination and confirmation of the structure and particle size of *SO*- Fe_3O_4/ZnO-MNCs.

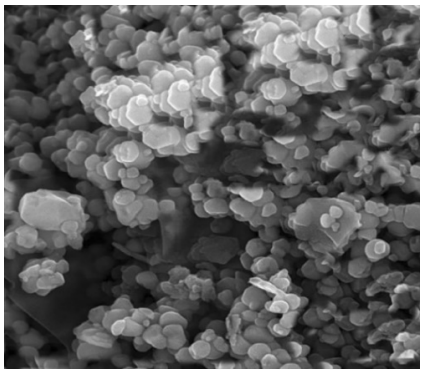


Figure 1.SEM of Fe_3O_4/ZnO-MNPs

The equation of Debye–Scherrer's ($D=K\lambda/\beta\cos\theta$) was used for calculating the *SO*-Fe_3O_4/ZnO-MNPs particles size which has been found to be 35 nm.

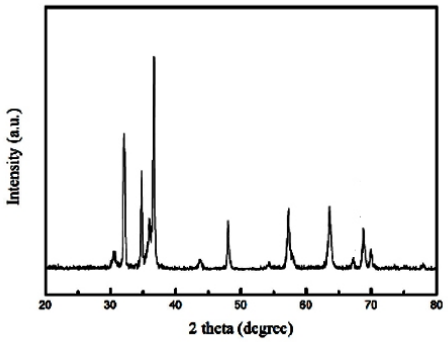


Figure 2. XRD image of Fe_3O_4/ZnO-MNPs

The EDX technique was used for confirmation of the synthesized Fe_3O_4/ZnO-MNPs elemental analysis (Figure 3). As shown in Figure 3, Zn, Fe and O peaks of *SO*-Fe_3O_4/ZnO-MNPs indicate a successful synthesis. Also, the presence of carbon peak in the EDX spectrum indicates the presence of organic compounds at the nanoscale.

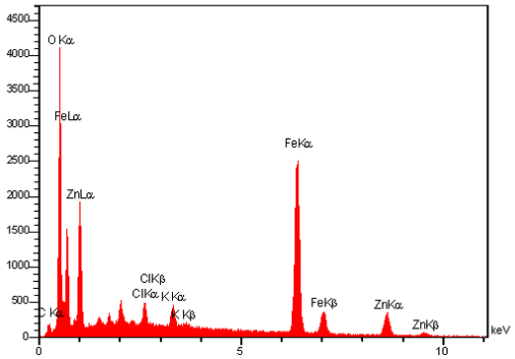


Figure 3. EDX image of green *SO*-Fe_3O_4/ZnO-MNPs

Transmission electron microscopy (TEM), was used to achieving a apparent size, form and structural image of the nanoparticles (Figure 4). Transmission electron microscope image reveals the size of the synthesized Fe_3O_4/ZnO-MNPs to be less than 40 nm.

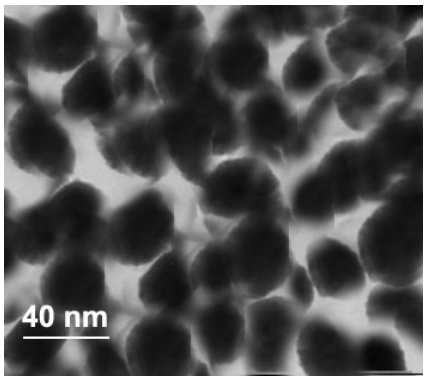


Figure 4. TEM image of Fe_3O_4/ZnO-MNPs

After determination, the optimum condition for generation of compound **6a-6h** (Scheme 1), the structure of these compounds was confirmed by using ^{1}H NMR, ^{13}C NMR, IR and mass spectra that were consistent with the suggested structure. The ^{1}HNMR spectrum of **6b** display one singlet at 3.82 ppm for methoxy protons, two singlet at 6.52 and 6.87 ppm for methin and methylene proton respectively, one singlet at 11.62 ppm for hydroxy proton along with resonance for aromatic moiety. The resonance of CN and carbonyl groups in ^{13}C NMR spectrum of **6b** display at 108.3 and 163.5 ppm respectively. The mechanism of performing these reactions recommended in Scheme 2. Initially, phthalaldehyde**1**and cyanomethyl amine **2** reacted in the presence of green Fe_3O_4/ZnO-MNPs and generated isoquinoline derivatives **7**. Then, activated acetyleniccompounds**3** reacts with isoquinoline**7** and produce intermediate **8**. Intermediate **8** with negative charge attack to isocyanate **4** in the presence of Fe_3O_4/ZnO-MNPs and produce intermediate **9**. Interrmolecular cyclization of intermediate **9**leads to the product **10**. Hydrazine 5 reacts with carbonyl group of **10** in the presence of catalyst andinterrmolecular cyclization of intermediate **10** by elimination of ROH leads to the product **6** (Scheme 2)**.**

The synthesis of pyrazozlo pyrimidinones 6 is investigated in two procedures. In first procedure, pyrimidinone derivatives 10 was separated from mixture of reaction and then reacted with hydrazine 5 in the presence of Fe3O4/ZnO-MNPs under similar conditions. In second procedure, all of starting materials react in one pot and compounds 6 were synthesize without separation of intermediate 10 under similar conditions. The results show the yield of reaction in two procedures different completely. As shown in results, yields of reactions in the second procedure are higher than those in the first procedure that is one of the advantages of multi-component reactions. In multi step reactions, the yield of final product due to separation of some intermediate is low.

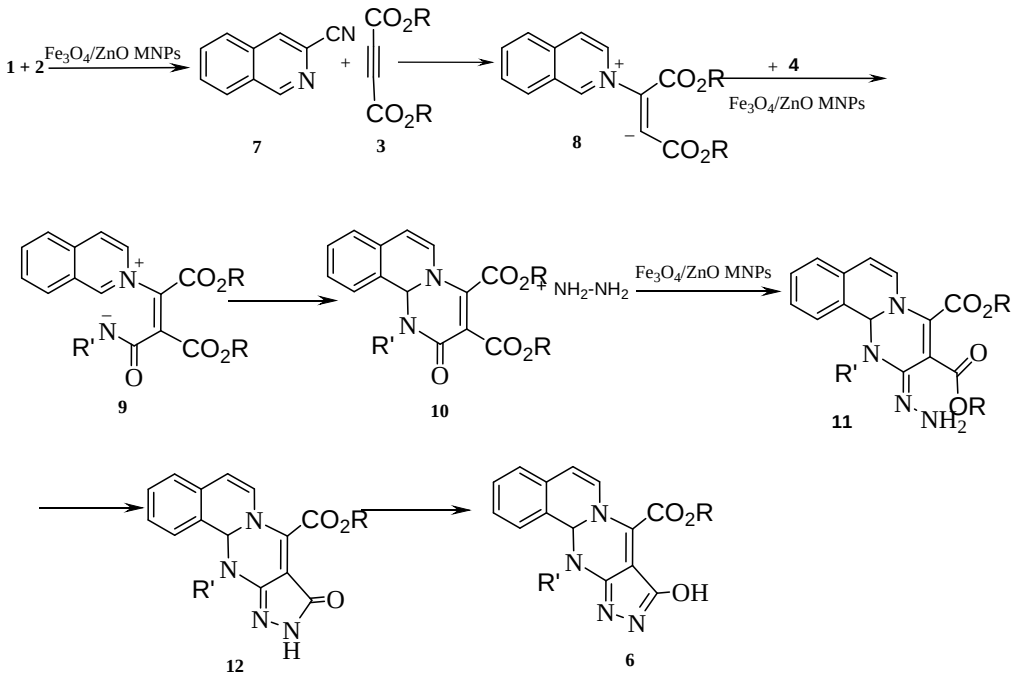
Diphenyl-2-picrylhydrazyl (DPPH) utilizing for evaluation of antioxidant ability

DPPH radical trapping experiment is generally employed for the approval of antioxidant ability or power of compounds to get free radicals of some synthezied compounds and antioxidant property of them in foods and biological structures [35, 51]. In these experiment, taking one electron or the hydrogen atom of synthezied compounds was performed by DPPH radical and show an valuation of antioxidant capacity basis of free radical trapping. The electron or hydrogen donating power of compounds **6a-6d**to the DPPH radical determined the antioxidant ability of them. The absorption of DPPH radical was decreased from 517 nm when give one electron or hydrogen from antioxidant or a radical typs.

In this research, the antioxidant ability or power of compounds **6a-6d**for taking free radicals was compared to synthesized antioxidant such as BHT and TBHQ at different concentrations. Overall, the power of DPPH

trapping was obtained TBHQ>BHT>**6d**>**6b**>**6c**>**6a** (Figure 5).

As shown in Figure 5, the new synthesized compounds in all concentrations have moderate distinctions than to BHT and TBHQ. Among selected synthezied compounds, **6d** was shown excellent radical trapping activity relative to standards.



Scheme 2 suggested mechanism for preparation of **6.**

As shown in Figure 5, the new synthesized compounds in all concentrations have moderate distinctions than to BHT and TBHQ. Among selected synthezied compounds, **6d** was shown excellent radical trapping activity relative to standards

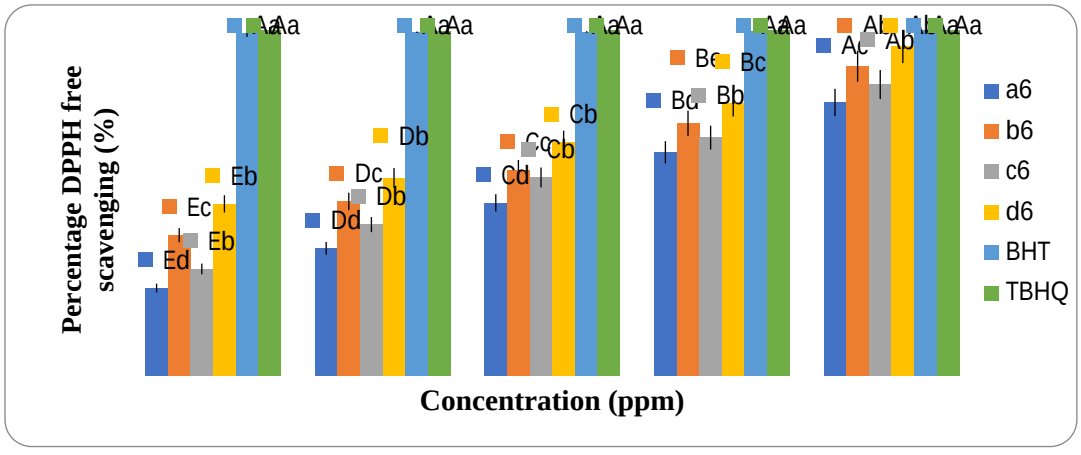


Figure 5. Radical trapping activity (RSA) of compounds **6a-6d**

The potential of synthesiaedcopmpounds by Ferric ions (Fe^{3+}) reducing

The ability of reducing ferric ions (Fe^{3+}) by some synthesized compounds such as **6a-6d** are calculated by the quantity of Fe^{3+}/ferricyanide reduced to the Fe^{2+}/ ferrous at 700 nm [48]. As shown in Figure 6, in this test, compound **6d** was shown good reducing ability than to standard antioxidants such as BHT and TBHQ. The reducing activity trend of the samples was as follows: TBHQ>BHT>**6d**>**6a**>**6b**>**6c**. The outcomes are displayed in Figure 6.

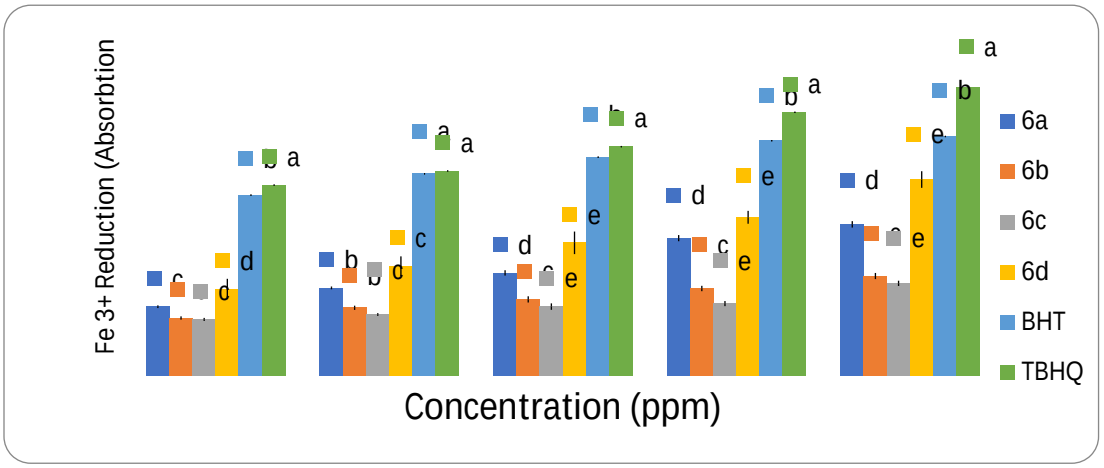


Figure 6. Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **6a-6d**.

In summary, functionalized pyrimidinones are prepared in good to excellent yield using five component reactions of phthalaldehyde, methylamine, electron deficient acetylenic compounds, trichloroacetonitrile, amides and catalytic amounts of ZnO@Fe_3O_4-MNPs under ultrasonic conditions at ambient temperature in water as green media. In addition, compound **6d** was displayed a excellent radical trapping ability and good reducing activity relative to standards (BHT and TBHQ). The advantages of our procedure than to other reported articles are green reaction conditions; employ a low amount of nanocatalyst, high yield, short time of reaction and easy separation of catalyst and product from the mixture of reactions and synthesis of nanocatalyst using water extract of *Spinacia oleracea*as green media which are in good agreement with some principles of green chemistry. Also, we know the isoquinoline convert to *N*-oxide in the presence of air and yield of reaction was low. For this reason, in this procedure isoquinoline is produced *in situe* and used in the reactions.

Experimental

All of starting materials, solvents and other chemicals are bought from Fluka (Buchs, Switzerland) and utilize with any purification. Electrothermal 9100 device are used for measuring the melting points and Shimadzu IR-460 spectrometer are employed for giving IR spectra. BRUKER DRX-500 AVANCE spectrometer at 500 and 125 MHz was employed for giving the ^{1}H, and ^{13}C-NMR spectra. A FINNIGAN-MAT 8430 spectrometer with an ionization potential of 70 eV was utilized for recording mass spectra. The Holland Philips XL30 microscope was used for giving scanning electron microscopy (SEM) imagedeterminationofZnO/Fe_3O_4-MNCsmorphology. A Holland Philips Xpert X-raypowderdiffractometer, withCuK□radiation (λ=0.15406 nm) and 2θ ranging from 20 to 80°, wasemployedforX-raydiffraction (XRD) analysis at roomtemperature and characterization of crystalline structure of ZnO/Fe_3O_4-MNCs. 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 (CuK_α) =1.5406 **Å** and *β*isthe full-width at half-maximumofthediffractionlines.

Green synthesis of Fe_3O_4/ZnO-MNC

Fe_3O_4 magnetic nanoparticles (MNPs) were synthesized according to previously reported methods [46]. 4 mmol $Fe_2(SO_4)_3.4H_2O$ and 2 mmol $FeSO_4.7H_2O$ were dissolved in 20 mL of *Spinacia oleracea* water extract in an ultrasonic bath at 80 °C. 5 mL of (ammonia: *Spinacia oleracea* water extract (0.1:3) was added to the warm solutions and mixed for 2 h. The mixture of reaction cooled to ambient temperature and

the resulting precipitate separated by filtration. All procedures carried out under nitrogen gas. For synthesis of Fe_3O_4/ZnO-MNCs, 0.1 g synthesized Fe_3O_4-MNPswas dispersed in 50 mL of *Spinacia oleracea* water extract and sonicated for 30 min. Then 0.03 M $Zn(NO_3)_2$ solutions (10 mL) was added to previous solution to obtain mixed Fe_3O_4-MNPs, $Zn(OH)_2$ colloids at room temperature. By using centrifuge, the colloid was separated, dried and calcinated at 400 ◦C for 5 h. The product was separated using external magnetic field and cleaned by washing with water and ethanol several times after cooling at r.t to afford the Fe_3O_4/ZnO-MNCs.

Typical procedure for generation of pyrimidinone derivatives 6

To a stirred mixture of phthalaldehyde**1** (2 mmol) and cyano methylamine **2** (2 mmol) was added dialkylacetylene dicarboxylates **3** (2 mmol)in the presence ofZnO/Fe_3O_4 MNC (10 mol%) after 45 min in water (5 mL) at room temperature under ultrasonic conditions. Isocyanate **4** (2 mmol) was added to mixture gently after 20 min and hydrazine **5** was added finally after 25 min in the presence of ZnO/Fe_3O_4 MNC (10 mol%) (10 mol%). After 3 h, the reaction is completed and progress of the reaction is confirmed by TLC. Finally, the catalyst was separated by external magnet and the solid residue was collected by filtration and the residue was purified by column chromatography (5:1 hexane/EtOAc) to afforded pure title compounds.

Methyl 9-hydroxy-12-phenyl-12, 12a-dihydropyrazolopyrimido[2,1-a]isoquinoline-8-carboxylate(6a).

Pale yellow powder, mp 127-129°C, yield 90%, IR (KBr) (ν_{max}/cm^{-1}): 2195, 1735, 1696, 1585, 1478 and 1184 cm^{-1}. ^{1}H-NMR: δ 3.85 (3 H, s, MeO), 6.48 (1 H, s, CH), 6.85 (1 H, s, CH), 7.54 (2 H, t, ^3J = 7.2, 2 CH), 7.63 (1 H, t, ^3J = 7.2, CH), 7.72 (1 H, t, ^3J = 7.2 Hz, CH), 7.78 (1 H, t, ^3J = 7.2 Hz, CH), 7.94 (1 H, d, ^3J = 7.5 Hz, CH), 8.06 (2 H, d, ^3J = 7.3, 2 CH), 8.73 (1 H, d, ^3J = 7.5 Hz, CH), 11.32 (1 H, s, OH) ppm. ^{13}C-NMR: δ 52.6 (MeO), 73.2 (CH), 108.2 (CH), 109.2 (CN), 112.6 (C), 114.3 (C), 122.3 (CH), 128.2 (CH), 129.2 (CH), 129.7 (CH), 130.4 (CH), 131.2 (2 CH), 133.8 (2 CH), 134.2 (C), 138.4 (C), 144.2 (C), 145.3 (C), 146.7 (C), 156.3 (C), 162.6 (C=O) ppm. Anal. Calcd for $C_{22}H_{15}N_5O_3$ (397.39): C, 66.49; H, 3.80; N, 17.62 found: C, 66.63; H, 3.96; N, 17.83.

Methyl 9-hydroxy-12-(4-nitrophenyl)-12, 12a-dihydropyrazolopyrimido[2,1-a]isoquinoline-8-carboxylate(6b).

Yellow crystals, mp 154-156°C, yield 87%. IR (KBr) (ν_{max}/cm^{-1}): 2198, 1732, 1658, 1587, 1489, 1365, 1258 and 1157 cm^{-1}. ^{1}H-NMR: δ3.82 (3 H, s, MeO), 6.52 (1 H, s, CH), 6.87 (1 H, s, CH), 7.58 (2 H, d, ^3J = 7.6, 2 CH), 7.67 (1 H, t, ^3J = 7.6 Hz, CH), 7.72 (1 H, t, ^3J = 7.6 Hz, CH), 7.86 (1 H, d, ^3J =7.8 Hz, CH), 8.02 (1 H, d, ^3J = 7.8 Hz, CH), 8.26 (2 H, d, ^3J = 7.8, 2 CH), 11.62 (1 H, s, OH) ppm. ^{13}C-NMR: δ 53.3 (MeO), 74.6 (CH), 107.2 (CH), 108.3 (CN), 108.8 (C), 109.4 (C), 125.2 (2 CH), 127.2 (2 CH), 128.3 (CH), 128.7 (CH), 129.4 (CH), 131.2 (CH), 134.2 (C), 138.2 (C), 139.4 (C), 145.2 (C), 146.7 (C), 149.2 (C), 156.7 (C), 163.5 (C=O). Anal. Calcd for $C_{22}H_{14}N_6O_5$ (442.38): C, 59.73; H, 3.19; N, 19.00 found: C, 59.92; H, 3.34; N, 19.23.

Methyl 9-hydroxy-12-(4-bromoophenyl)-12, 12a-dihydropyrazolopyrimido[2,1-a]isoquinoline-8-carboxylate (6c).

Orange crystals, mp 166-168°C, yield 90%. IR (KBr) (ν_{max}/cm^{-1}): 2189, 1727, 1657, 1478. 1356, 1286 and 1295 cm^{-1}. ^{1}H-NMR: δ 3.85 (3 H, s, MeO), 6.38 (1 H, s, CH), 6.92 (1 H, s, CH), 7.14 (2 H, d, ^3J = 7.8, 2 CH), 7.55 (2 H, d, ^3J = 7.8, 2 CH), 7.63 (1 H, t, ^3J = 7.6 Hz, CH), 7.68 (1 H, t, ^3J = 7.6 Hz, CH), 7.82 (1 H, d, ^3J = 7.6 Hz, CH), 8.35 (1 H, d, ^3J = 7.6 Hz, CH), 10.85 (1 H, s, OH) ppm. ^{13}C-NMR: δ 52.4 (MeO), 73.8 (CH), 108.3 (CH), 109.4 (CN), 113.2 (CH), 114.5 (CH), 117.2 (CH), 128.2 (CH), 128.6 (CH), 129.4 (CH), 133.4 (2 CH), 134.2 (C), 134.8 (2 CH), 139.2 (C), 145.5 (C), 146.3 (C), 148.2 (C), 157.2 (C), 162.6 (C=O). Anal. Calcd for $C_{22}H_{14}BrN_5O_3$ (476.28): C, 55.48; H, 2.96; N, 16.78 found: C, 55.63; H, 3.18; N, 16.94.

Methyl 9-hydroxy-12-(3-bromophenyl)-12, 12a-dihydropyrazolopyrimido[2,1-a]isoquinoline-8-carboxylate (6d).

White powder, yield: (90%); m.p. 169-171°C. IR(KBr)(ν_{max}/cm^{-1}): 2185, 1723, 1699, 1523, 1489, 1358 and 1124 cm^{-1}. ^{1}H-NMR: δ 3.87 (3 H, s, MeO), 6.42 (1 H, s, CH), 6.87 (1 H, s, CH), 7.23 (1 H, d, ^3J = 7.8, CH), 7.32 (1 H, s, CH), 7.43 (1 H, t, ^3J = 7.8 Hz, CH), 7.58 (1 H, d, ^3J = 7.8 Hz, CH), 7.63 (1 H, t, ^3J = 7.5 Hz, CH), 7.75 (1 H, t, ^3J = 7.5 Hz, CH), 7.82 (1 H, d, ^3J = 7.5 Hz, CH), 7.87 (1 H, d, ^3J = 7.5 Hz, CH), 11.35 (1 H, s, OH) ppm. ^{13}C-NMR: δ 52.6 (MeO), 74.5 (CH), 108.4 (CH), 109.2 (CN), 113.2 (C), 114.5 (C), 122.3 (C), 123.2 (CH), 127.4 (CH), 128.5 (CH), 129.2 (CH), 129.8 (CH), 130.4 (CH), 131.2 (CH), 132.6 (CH), 134.2 (C), 139.4

(C), 145.2 (C), 146.3 (C), 146.7 (C), 158.2 (C), 161.8 (C=O). Anal. Calcd for $C_{22}H_{14}BrN_5O_3$ (476.28): C, 55.48; H, 2.96; N, 16.78 found: C, 55.62; H, 3.15; N, 16.92.

Methyl 9-hydroxy-12-(4-methoxyphenyl)-12, 12a-dihydropyrazolopyrimido[2,1-a]isoquinoline-8-carboxylate (6e).

Yellow powder, mp 136-138°C, yield 95%, IR (KBr) (ν_{max}/cm^{-1}): 2198, 1724, 1664, 1574, 1387 and 1247 cm^{-1}. ^{1}H-NMR: δ 3.75 (3 H, s,MeO), 3.87 (3 H, s,MeO), 6.56 (1 H, s, CH), 6.92 (1 H, s, CH), 7.08 (2 H, d, ^3J = 7.6 Hz, 2 CH), 7.24 (2 H, d, $^3J_{HH}$ = 7.6 Hz, 2 CH), 7.63 (1 H, t, ^3J = 7.8 Hz, CH), 7.72 (1 H, t, $^3J_{HH}$ = 7.8 Hz,CH), 7.78 (1 H, d, $^3J_{HH}$ = 7.8 Hz, CH), 7.86 (1 H, d, $^3J_{HH}$ = 7.8 Hz, CH), 12.04 (1 H, s, OH) ppm. ^{13}C-NMR: δ 53.2 (MeO), 55.7 (MeO), 76.8 (CH), 107.4 (CH), 108.3 (C), 113.4 (C), 114.3 (C), 115.4 (2 CH), 127.2 (CH), 128.4 (CH), 129.2 (CH), 129.8 (CH), 134.5 (2 CH), 134.8 (C), 139.2 (C), 140.2 (C), 145.3 (C), 146.7 (C), 155.2 (C), 158.6 (C), 163.5 (C=O) ppm. Anal. Calcd for $C_{23}H_{17}N_5O_4$ (427.41): C, 64.63; H, 4.01; N, 16.39 found: C, 64.82; H, 4.18; N, 16.54.

Ethyl 9-hydroxy-12-(4-methoxyphenyl)-12, 12a-dihydropyrazolopyrimido[2,1-a]isoquinoline-8-carboxylate (6f).

Yellow powder, mp 143-145°C, yield 95%, IR (KBr) (ν_{max}/cm^{-1}): 2196, 1725, 1695, 1578, 1385 and 1293 cm^{-1}. ^{1}H-NMR: δ 1.37 (3 H, t, ^3J = 7.4 Hz, Me), 3.86 (3H, s, MeO), 4.27 (2 H, q, ^3J = 7.4 Hz, OCH_2), 6.63 (1 H, s, CH), 6.94 (1 H, s, CH), 7.12 (2 H, d, ^3J = 7.6 Hz, 2 CH), 7.28 (2 H, d, $^3J_{HH}$ = 7.6 Hz, 2 CH), 7.65 (1 H, t, ^3J = 7.8 Hz, CH), 7.74 (1 H, t, $^3J_{HH}$ = 7.8 Hz,CH), 7.83 (1 H, d, $^3J_{HH}$ = 7.8 Hz, CH), 7.92 (1 H, d, $^3J_{HH}$ = 7.8 Hz, CH), 11.86 (1 H, s, OH) ppm. ^{13}C-NMR: δ 14.2 (Me), 55.8 (MeO), 62.4 (OCH_2), 75.6 (CH), 107.8 (CH), 109.2 (C), 112.6 (C), 113.8 (C), 116.4 (2 CH), 127.6 (CH), 128.5 (CH), 129.7 (CH), 130.4 (CH), 133.6 (2 CH), 134.2 (C), 139.5 (C), 141.4 (C), 145.6 (C), 147.2 (C), 156.8 (C), 159.3 (C), 163.8 (C=O) ppm. Anal. Calcd for $C_{24}H_{19}N_5O_4$ (441.44): C, 65.30; H, 4.44; N, 15.86 found: C, 65.43; H, 4.62; N, 16.03.

Determination of antioxidant activity using radical trapping test by (DPPH)

The radical trapping experiment by DPPH was employed for valuation of antioxidant ability for some generated compounds such as **6a-6d** as indicated by Shimada et al [48] procedure. For achieving to this purpose, different concentrations (200–1000 ppm) of compounds **6a-6d** were added to DPPH methanolic solution (1 mmol/L) with an equal volume. The mixtures were mixed for 30 min at ambient temperature and after this time puted in a dark room. Then, the mixture absorbance was calculated and recorded at 517 nm. The compounds **6a-6d** was exchanged with methanol (3 mL) in the standard type. The standard antioxidant such as Butylated hydroxytoluene (BHT) and 2-tertbutylhydroquinone (TBHQ) were employed as standard control sample. The percentage inhibition of the DPPH radical was measured using Yen and Duh [49] formula.

Evaluation of reducing ability for synthesized compounds

The ability of reducing iron (III) was evaluated for the compounds **6a-6d** using Yildirim et al. method [50]. For this purpose, the samples (1 mL), phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$; 2.5 mL, 10g/L) were combined together and sustained for 30 min at 50 oC. Then, trichloroacetic acid (2.5 mL, 10% w/v) was added to the previous solution and centrifuged for 10 min. In the end, the supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 1 g/L) and the samples absorbance was computed at 700 nm. The higher reducing power was attributed to higher absorbance. For accuracy of calculating, each calculation was performed in three times. The SPSS software version 18.0 was used for data analyzation of compounds by running one way analysis of variance (ANOVA) that confirmed variation in the mean value of samples and control. All removing were done by Duncan multiple range tests employing the importance level of 95% ($P < 0.05$).

Acknowledgeent

We gratefully acknowledge from Science and Research Branch, Islamic Azad University, Tehran, Iran because of spiritual support.

References

[1] Kantam, M. L.; Rajasekhar, C. V.; Gopikrishna, G.; Reddy, K. R.; Choudary, B. M. *Tetrahedron Lett.*, **2006**, *47*, 5965.
[2] Liu, Y. Q.; Li, L. H.; Yang, L.; Li, H. Y. *Chem. Papers*, **2010**, *64*, 533.
[3] Meciarova, M.; Polackova, V.; Toma, S. *Chem. Papers*, **2002**, *56*, 208.
[4] Meciarova, M.; Toma, S.; Babiak, P. *Chem. Papers*, **2004**, *58*, 104.

[5] Tabatabaeian, K.; Mamaghani, M.; Mahmoodi, N. O.; Khorshidi, A. *Catal. Commun.* **2008**, *9*, 416.
[6] M. Meciarova, S. Toma, J. L. Luche, *Ultrasonics Sonochem.* **2001**, *8*, 119.
[7] M. Vinatoru, E. Bartha, F. Badea, J. L. Luche, *Ultrasonics Sonochem.*, **1998**, *5*, 27.
[8] T. Ando, T. Kimura, M. Fujita, J. M. Leveque, J. L. Luche, *Tetrahedron Lett.*, **2001**, *42*, 6865.
[9] N. Cabello, P. Cintas, J. L. Luche, *Ultrasonics Sonochem.* **2003**, *10*, 25.
[10] V. Kumar, A. Sharma, M. Sharma, U. K. Sharma, A. K. Sinha, *Tetrahedron*, **2007**, *63*, 9718.
[11] A. K. Sinha, A. Sharma, B. P. Joshi, *Tetrahedron*, **2007**, *63*, 960.
[12] Dömling, A. *Comb. Chem. High Throughput Scr.* **1998**, *1*, 1.
[13] Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.***2000**, *39*, 3169.
[14] Weber, L. *Drug Discovery Today*, **2002**, *7*, 143.
[15] Zhu, J., Bienayme, H., Eds.; Wiley-VCH: Weinheim, **2005**.
[16] Wipf, P.; Kendall, C. *Chem. Eur. J.* **2002**, *8*, 1779.
[17] Balme, G.; Bossharth, E.; Monteiro, N. *Eur. J. Org. Chem.***2003**, 4101.
[18] Jacobi von Wangelin, A.; Neumann, H.; Gordes, D.; Klaus, S.; Strubing, D.; Beller, M. *Chem.–Eur. J.* **2003**, *9*, 4286.
[19] (a) Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.***2000**, *39*, 3168. (b) Ugi, I.; Dömling, A. *Endeavour* **1994**, *18*, 115. (c) Heck, S.; Dömling, A. *Synlett*, **2000**, 424.
[20] (a) Ganem, B. *Acc. Chem. Res.* **2009**, *42*, 463; (b) Dömling, A.; Ugi, I. *Angew. Chem. Int. Ed.* **2000**, *39*, 3169.
[21] (a) Shaabani, A.; Maleki, A.; Rezayan, A. H.; Sarvary, A. J. *Mol. Divers.***2011**, *15*, 41; (b) Altug, C.; Burnett, A. K.; Caner, E.; D€ur€ust, Y.; Elliott, M. C.; Glanville, R. P. J.; Guy, C.; Westwell, A. D. *Tetrahedron*, **2011**, *67*, 9522.
[22] Rostami-Charati, F.; Hajinasiri, R.; Sayyed Alangi, S. Z.; Afshari Sharif Abad, S. *Chem. Papers*, **2016**, *70*, 907.
[23] Sajjadi-Ghotbabadi, H.; Javanshir, Sh.; Rostami-Charati, F.; *Catal. Lett.* **2016**,*146*, 338.
[24] Soleimani, A.; Asadi, J.; Rostami-Charati, F.; Gharaei R. C*omb. Chem .High Throughput Scr.* **2015**, 18, 505.
[25] Rostami-Charati, F.; Hossaini, Z. S.; Sheikholeslami-Farahani, F.; Azizi, Z.; Siadati. S. A. *Comb. Chem. High Throughput Scr.*.**2015**, *18*, 872.
[26] (a) Elinson, M. N.; Ilovaisky, A. I.; Merkulova, V. M.; Belyakov, P. A.; Chizhov, A. O. *Tetrahedron,* **2010**, *66*, 4043; (b) Dekamin, M. G.; Mokhtari, Z. *Tetrahedron,* **2012**, *68*, 922; (c) Dekamin, M. G.; Mokhtari, Z.; Karimi, Z. *Sci. Iran. Trans. C: Chem. Chem. Eng*. **2011**, *18*, 1356.
[27] Weber, L. *Curr. Med. Chem.***2002**, *9*, 2085.
[28] (a) Chanda, A.; Fokin, V. V. *Chem. Rev.* **2009**, *109*, 725.
[29] Butler, R. N.; Coyne, A. G. *Chem. Rev.* **2010**, *110*, 6302.
[30] Simon, M. O.; Li, C. *J. Chem. Soc. Rev.* **2012**, *41*, 1415.
[31] Dennis, R.; Romulo, F. S. C.; Sergio, A. A.; Marcelo, G. M. D.; Angelode, F.; Ronaldo, A. P.; Luciana, K. K.; Marcia, A. A.; Joao Ernesto, C. *Bioorg. Chem.* **2006**, *34*, 173.
[32] Kappe, C. O. Biologically active dihydropyrimidones of the Biginelli-type a literature survey. *Eur. J. Med. Chem.***2000**, *35*, 1043.
[33] Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; Brosse, C. D.; Mai, S.; Trunch, A.; Faulkner, D. J.; Carte, B.; Breen, A. L.; Hertzberg, R. P.; Johnson, R. K.; Westley, J. W.; Pttos, B. C. M. *J. Org. Chem.***1995**, *60*, 1182
[34] Rostamizadeh, S.; Nojavan, M.; Aryan, R.; Isapoor, E.; Azad, M. *J. Mol. Catal. A: Chem.* **2013**, *374–375*, 102.
[35] Beydoun, D.; Amal, R.; Low, G.; McEvoy, S. *J. Nanopart. Res.* **1999**, *1*, 439.
[36] (a) Halliwell, B. *Free Rad.l Res.***1999**, *31*, 261; (b) Ahmadi, F.; Kadivar, M.; Shahedi, M. *Food Chem.***2007**, *105*, 57.
[37] Babizhayev, M. A.; Deyev, A. I.; Yermakovea, V. N.; Brikman, I.V.; Bours, J. *Drugs* **2004**, *5*, 125.
[38] Liu, L.; Meydani, M. *Nutr. Rev.* 2002, *60*, 368.
[39] Rajabi, M.; Hossaini, Z. S.; Khalilzadeh, M. A.; Datta, Sh.; Halder, M.; Mousa, Sh. A. *J. Photochem. Photobiol. B: Biol*. **2015**, 148, 66.
[40] Yavari, I.; Sabbaghan, M.; Hossaini, Z. S. *Chemical Monthly***, 2008**, *139*, 625.
[41] Yavari, I.; Sabbaghan, M.; Hossaini, Z. S. *Synlett*, **2008,** 1153.
[42] Yavari, I.; Hossaini, Z. S.; Sabbaghan, M.; Ghazanfarpour-Darjani, M. *Tetrahedron,* **2007**, *63*, 9423.
[43] Yavari, I.; Sabbaghan, M.; Hossaini, Z. S.; Ghazanfarpour-Darjani, M. *Helvetica Chimica Acta*, **2008**, *91*, 1144.
[44] Rostami-Charati, F. *Chin. Chem. Lett.*, **2014**,169.

[45] Rostami-Charati, F.; Hossaini , Z. S.; Khalilzadeh, M. A.;Jafaryan,H. *J. Heteroc. Chem.* ***2012***, *49*, 217.
[46] Hajinasiri, R.; Hossaini, Z. S.; Rostami-Charati, F. *Heteroatom Chem.* ***2011***, *22*, 625.
[47] Rostami Charati,F.; Hossaini, Z. S.; Hosseini-Tabatabaei**,** M. R. *Phosph., Sulf. Sil. Rel. Elem. A.* **2011**, 186, 1443.
[48] Shimada, K.; Fujikawa, K.; Yahara, N.T. *J. Agri. Food Chem.***1992**, *40*, 945.
[49] Yen, G.C.; Duh, P.D. *J. Agri. Food Chem.***1994**, *42*, 629.
[50] Yildirim, A.; Mavi, A.; Kara, A. A.; *J. Agri. Food Chem.***2001**, *49*, 4083
[51] (a) Halliwell, B. *Free Radical Res.***1999**, *31*, 261; (b) Ahmadi, F.; Kadivar, M.; Shahedi, M. *Food Chem.***2007**, *105*, 57.