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Green synthesis of pyrazols derivatives and investigation of antioxidant activity

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Abstract: In this research Fe_3O_4 MNPs was used for the synthesis of pyrazolotriazole derivatives via multicomponent condensation of aromatic aldehyde, malononitrile, diethyl hydrazine-1,2-dicarboxylate and ammonium acetate in water at room temperature. 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 because of having NH_2 group.The current procedure has the benefits for instance excellent yield of reaction, green media and easy separation of product and catalyst.

Keywords: Diethyl hydrazine-1,2-Dicarboxylate, Malononitrile, Pyrazolotriazole, Fe_3O_4 MNPs.

Introduction

Substituted 1,2,4-triazoles and their derivatives are key skeletons of many biologically active moleculers and important organic compounds, and they exhibit wide applications in pesticides, medicines, functional materials and organocatalysts [1-3]. In addition, a number of natural products contain a 1,2,4- triazole motif [4, 5]. Owing to their important properties and applications, various methods for synthesis of 1,2,4-triazole derivatives have been developed [6, 7]. N-heterocycles constitute a very important class of compounds. In particular, 1,2,4-triazolpyrimidine derivatives include a large number of natural products, pharmaceuticals, and functional materials [8].

Multicomponent reactions (MCRs) are generally defined as reactions where more than two starting materials react to form a product. Generally, there are three different possible classification schemes of MCRs according to reaction mechanism, components involved, or intrinsic variability [9].

The development of new MCRs is an interesting research topic in applied areas of organic, medicinal, and pharmaceutical chemistry [10]. MCRs have attracted considerable interest owing to their exceptional synthetic efficiency. Hundreds of MCRs have recently been described. These reactions play a pivotal role in the synthesis of natural and unnatural products because of their importance of therapeutic and pharmacological uses. *N*-Heterocycles receive considerable attention in the literature as a consequence of their exciting biological properties and their role as pharmacophores [11]. In this work Fe_3O_4 MNPs has a good catalyst for the synthesis of pyrazolotriazole derivatives **5** via four component reaction between aromatic aldehydes **1**, malononitrile **2**, diethyl hydrazine-1,2-dicarboxylate **3** and ammonium acetate **4** in water at room temperature (Scheme **1**) .

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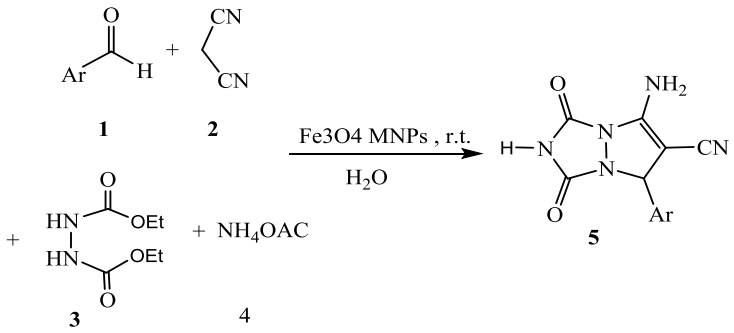

Entry	Ar	Time(min)	Yield[b] (%)
5a	4-NO_2-C_6H_4	35	92
5b	2-Cl-C_6H_4	40	86
5c	C_6H_5	40	90
5d	4-Cl-C_6H_4	40	95
5e	2-NO_2-C_6H_4	40	85
5f	4-Br-C_6H_4	40	90
5g	4-Me-C_6H_4	45	82

Scheme 1: Synthesis of pyrazoles

Results and discussion

At first, for the optimization of the reaction conditions, the model reaction was carried out by using diethyl hydrazine-1,2-dicarboxylate, 4-chlorobenzaldehyde, malononitrile and ammonium acetate under various conditions. The reaction was conducted in various solvents using Fe_3O_4 MNPs as a catalyst under several solvent and also under solvent-free conditions. By altering the catalyst, amount of catalyst and temperature of reaction could obtain the best conditions for preparation of compound **5a**. The reaction mixture have very low rate in absence of catalyst. This reaction have very low yield without catalyst even after 15 h (Table 1, entry 1). For this reason, 10 mol% catalysts such as ZnO-NPs were added to the mixture of reaction. The yield of compound **5a** was 78% after 3 h (Table 1, entry 12). For investigation the catalytic effect on this reaction, several catalyst such as ZnO-nanorods, CuO-NPs, KF/CP NPs, Cu@KF/CP NPs, Et_3N, Fe_3O_4-MNPs, Fe_3O_4/ZnO and TiO_2-NPs were tested in this reaction. As shown in Table 1, the Fe_3O_4-MNPs are the best catalyst for this reaction. Then, for determination best amount of catalyst the sample reaction was performed in the presence of 0.02-0.05 g of Fe_3O_4 MNPs as catalyst. By increasing the amount of catalyst from 0.02 g, didn't seen any considerable change in the yields of reaction. Therefore, the best amount of Fe_3O_4 MNPs as catalyst is 0. 02 g. Also, by increasing the reaction temperature to 100 ^oC the yield of reaction wasn't increased (entry 2, Table 1) and room temperature is selected as reaction temperature. For confirmation the reusability of the catalyst, we separated the catalyst from mixture of reaction, washed by water and then dried at room temperature for 24 h and utilized for the next catalytic cycle. The results displayed that the catalyst can be reused four times with any losing of ability (Table 2). This procedure for synthesis of pyrazolotriazole derivatives is very easy and yields of product are excellent. Also, the employing catalyst is cheap and separation of it very easy. Time of reaction of short and price of starting materials and catalyst is inexpensive. In reported literature most of procedure is very difficult conditions for the synthesis of these derivatives.

The Fe_3O_4 MNP catalyst was prepared according to a literature procedure.The analysis structure and size distribution of the nanoparticles was performed by scanning electron microscopy (SEM, Fig. 1) and X-ray diffraction (XRD, Fig. 2) methods. The average

crystalline size (*D*) for Fe_3O_4 MNPs was calculated based on peak with the strongest intensity using the Debye–Scherrer equation ($D = K\lambda/\beta\cos\theta$); where β is full-width at half-maximum or half-width in radians and *K* is the so-called shape factor (0.89), θ is Bragg's diffraction angle, and λ is the X-ray wavelength used (1.5406 Å for CuK_α line). Particles size of Fe_3O_4 MNPs has been found to be 21–23 nm.

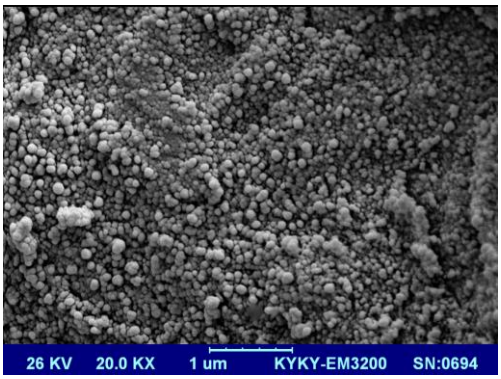

Figure 1. SEM image of Fe_3O_4 nanoparticles

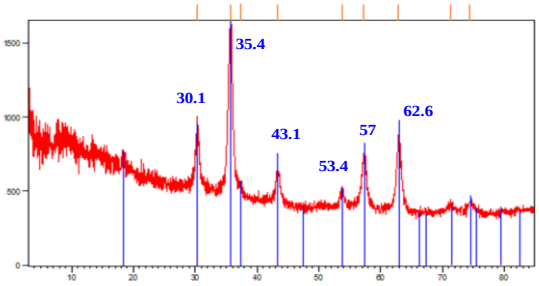

Figure 2. XRD spectra of Fe_3O_4 nanoparticles

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.[56, 57] 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 **5a-5d** 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 **5a-5d** 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>**5b**>**5d**>**5c**>**5a** (Figure 3).

As shown in Figure 3, the new synthesized compounds in all concentrations have moderate distinctions than to BHT and TBHQ. Among selected synthezied compounds, **5b** was shown excellent radical trapping activity relative to standards (BHT and TBHQ).

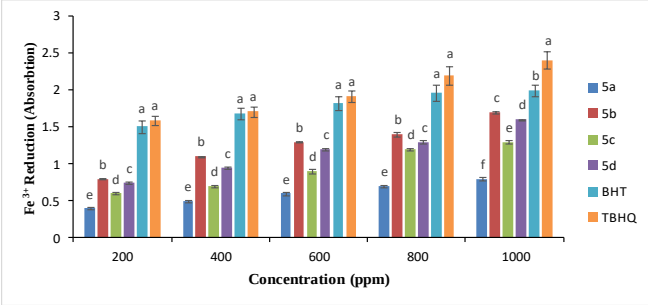

Figure 3. Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **5a-5d**.

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

The ability of reducing ferric ions (Fe^{3+}) by some synthesized compounds such as **5a-5d** are calculated by the quantity of $Fe^{3+/}$ferricyanide reduced to the Fe^{2+}/ferrous at 700 nm. As shown in Figure 7 in this test, compound **5b** 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>**5b**>**5d**>**5c**>**5a**. The outcomes are displayed in Figure 4.

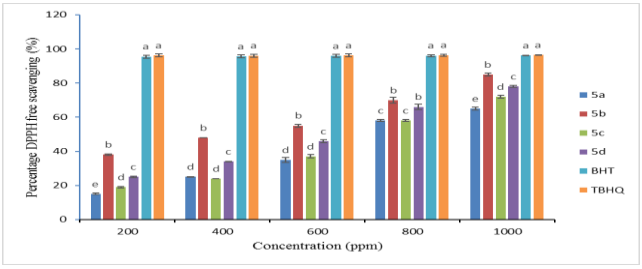

Figure 4. Radical trapping activity (RSA) of compounds **5a-5d.**

Experimental

IR spectra (KBr medium) were recorded on a Shimadzu IR-460 spectrometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker DRX-500

AVANCE spectrometer at 500 and 125 MHz, respectively, in $CDCl_3$ using TMS as internal standard. Mass spectra (electron impact ionization) were recorded on a Finnigan MAT 8430 spectrometer operating at an ionization potential 70 eV. Elemental analyses were performed using a Heraeus CHN–O-Rapid analyzer. All chemicals used in this work were purchased from Fluka and were used without further purification. The morphology of Fe_3O_4 nanoparticles was characterized by SEM using a Holland Philips XL30 microscope. Crystalline structure of Fe_3O_4 MNPs was characterized by XRD analysis at room temperature using a Holland Philips Xpert X-ray powder diffractometer.

Preparation of Fe_3O_4 MNPs:

$FeCl_3 \cdot 6H_2O$ (10.8 g, 40 mmol) and $FeCl_2 \cdot 4H_2O$ (4.0 g, 20 mmol) were dissolved in deionized water (100 ml), degassed with N_2 for 15 min and heated to 80°C. A solution of NH_4OH (32%, 15 ml) was then added dropwise, and the precipitated solid was separated after 15 min by a magnet and washed with NaCl solution (0.1 mol, 100 ml). The MNP formation was confirmed by XRD and SEM.

Typical procedure for the synthesis of pyrazolotriazoles:

A mixture of malononitrile (1.0 mmol), aromatic aldehyde (1.0 mmol), diethyl hydrazine-1,2-dicarboxylate (1.0 mmol), and ammonium acetate (1.0 mmol), and Fe_3O_4 MNPs (0.02 gr) was poured in the water and mixed for 35-45 min. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. The solid residue was dissolved in water to separate the catalyst and wash with diethylether. By recrystallization from ethanol, pure products were obtained.

7-amino-5-(4-chlorophenyl)-1,2,3,5-tetrahydro-1,3-dioxo-2-phenylpyrazolo[1,2-a][1,2,4]triazole-6-carbonitrile 5a:

White powder mp >223 ºC, yield 95%, IR (KBr) (ν_{max}, cm^{-1}):1731, 1712 (CO ester), ^1H NMR (500 MH_Z, DMSO-d_6): δ = 6.03 (1H, s, CH), 7.35-8.35 (11H, m, *H*-Ar and NH_2). ^{13}C NMR (125.8 MH_Z, DMSO-d_6): δ = 61.7, 63.6, ,116.4, 124.2, 126.7, 128.4, 128.8, 129.2, 129.7, 130.3, 131.0, 131.8, 146.3, 148.0, 150. 7,154. Analyses: Calcd. for $C_{18}H_{12}ClN_5O_2$: C, 59.11; H, 3.31;N, 19.15 Found: C, 59.34; H, 3.12;N, 19.36 %.

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 **5a-5d** as indicated by Shimada et al [58] procedure. For achieving to this purpose, different concentrations (200–1000 ppm) of compounds **5a-5d** 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 **5a-5d** 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 [59] formula.

Evaluation of reducing ability for synthesized compounds:

The ability of reducing iron (III) was evaluated for the compounds **5a-5d** using Yildirim et al. method.[60] 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% ($P < 0.05$).

Conclusion

In conclusion, we have demonstrated that Fe_3O_4 MNPs can be used as catalyst for efficient synthesis of pyrazolotriazole derivatives in water and room temperature. Moreover, the cheapness, easy availability of the reagent, high yield, purity of the products easy and clean workup in comparison with reported catalysts are some important advantages

presented in this work. Easy workup, green conditions, and reusability of catalyst make this method an attractive procedure among other approaches.

Acknowledgement

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