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Folic acid-supported Fe_3O_4 magnetic nanoparticles as a new, highly effective heterogeneous biocatalyst for the synthesis of 3,4-dihydropyrimidine thiones and their *in vitro* investigation as antibacterial active agents

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ABSTRACT

Folic acid–coated Fe_3O_4 magnetic nanoparticles (Fe_3O_4@FA) with core-shell structure as a new, benign, efficient and recoverable biocatalyst was synthesized without any complementary linker using co-precipitation method. The prepared catalyst efficiency was evaluated in the one-pot synthesis of various 3,4-dihydropyrimidine thiones via multi-component coupling reaction of aromatic aldehydes, methyl acetoacetate and thiourea under solvent–free conditions. The new catalyst was simply separated with the assistance of an external magnet and applied five times without considerable decrease in its activity. To characterize the structure of new catalyst, various techniques such as Fourier transform infrared (FT-IR), Scanning electron microscopy (SEM), Vibration sample magnetometer (VSM), X-ray diffraction (XRD), energy dispersive X-ray analysis (EDAX) and UV-Vis spectroscopy were utilized. Several advantages of this green protocol including high yield of product, short reaction times, simple workup, absence of hazardous solvents, high catalytic activity, and excellent recoverability and reusability of the nanocatalyst make it more economic rather than other procedures. *In vitro* antibacterial activity of 3,4-dihydropyrimidine derivatives was studied against a series of pathogenic microorganisms using agar well diffusion procedure.

Keywords*: Antibacterial activity, Folic acid, Agar well diffusion, Magnetic heterogeneous biocatalyst, Multi-component reaction, Solvent-free condition.*

1. INTRODUCTION

Nanomaterials due to their interesting confidants such as utility in various precincts containing nanocatalysis [1], magnetite-supported catalysis[2-4], nanoelectronics [5-8], integrated catalysis [9], magnetic resonance imaging [10,11], drug delivery system [12,13], cancer treatment through hyperthermia [14,15], and etc. [16-20] have procured extensive regard. Magnetic nanoparticles (MNPs) because of their good chemical resistance, facile synthesis and surface modification, high surface area, easy work-up and separation, low toxicity has been employed as a superb kind of catalyst support [21]. The surface modification of iron oxide nanoparticles, because of their aggregation and problem in dispersion in the organic phase is very substantial in many applications. It is noticeable that the heterogeneous catalysis in compared with homogeneous catalysis, due to easy reusability and handling has received significant consideration [22-25].

Modification of magnetic nanoparticles with vitamins, have procured remarkable attention, because of minimize disparate kind of difficulties such as using a mineral acid catalyst (HCl, $HClO_4$) and a solid acid catalyst (clays, zeolites) [26, 27]. Multicomponent reactions (MCRs) due to having some superiorities such as simple procedure, great atom economy, short reaction time , excellent selectivity and yield, and low cost, minimized waste generation have become potent tools in organic and medicinal chemistry [28-32] and obtained superb attention recently [33]. One of these MCRs is Biginelli condensation to prepare 3,4-dihydropyrimidine thione analogues. It is worth to nothing that, the heterocycles with the pyrimidine core are a class of compounds that represent variant biological activities like antitumor, anti-inflammatory, antihypertensive, potassium channel antagonists, calcium channel antagonists anti-HIV, antiviral, anti-malarial, antibacterial and antifungal [34-50]. Therefore, because of pharmacological and medicine activity significance of these heterocyclic compounds, variant methods have been developed to prepare these compounds under ultrasound irradiation , heating and microwave conditions [51-53] using kind of catalysts such as Bronsted acids, Lewis acids, ionic liquids, graphite and heterogeneous nanocatalysts [54-65]. However, some of these procedures suffer from one or more constraints like prolonged reaction temperature and time, low efficiency of the favorable product, monotonous work-up, toxicity, and large quantity and poor recoverability and reusability of the catalyst. Accordingly, there are still a greet requirement to develop a clean method and employing benign catalysts with considerable recyclability at the end of the reaction content.

In this research, we develop a green protocol to the synthesis a new Fe_3O_4 nanoparticle-supported folic acid (MNPs-FA) and evaluate its application as an efficacious, non-toxic and magnetically recoverable heterogeneous catalyst for the preparation of substituted 3,4-dihydropyrimidine thiones via the one-pot three-component reaction of thiourea, β-ketoester and varied aromatic aldehydes under solvent-free conditions with

excellent yield. Also, all synthesized 3,4-dihydropyrimidines were studied for their in vitro antibacterial activity.

2. EXPERIMENTAL SECTION

Iron (II) chloride, Iron (III) chloride, ammonium hydroxide solution (NH_4OH), folic acid, different aldehydes, methyl acetoacetate, thiourea and all solvents were purchased from the Sigma-Aldrich company and applied without extra purification. To study the FT-IR spectra of prepared samples, Perkin-Elmer in the region 400-4000 cm^{-1} using pressed potassium bromide discs was employed. Melting points were recorded on Electrothermal 9200 apparatus and are not corrected. ^{1}H-NMR and ^{13}C-NMR spectra were accomplished with Bruker Advance 400Hz spectrometer in DMSO-d6 and using TMS as an internal standard. UV-Vis spectra were performed on Shimadzue 1800 UV. The X-ray diffraction pattern (XRD) of catalyst was taken out on X'PertPro (Cu kα radiation, λ=0.15405 nm) in the range of 2Θ = 20°-80°.To study magnetization property of nanocatalyst, BHV-S5 vibrating sample magnetometer (VSM), with varying magnetic field from -10000 to 10000 was applied. The nanocatalyst morphology and component was measured on Hituch S4160 scanning electron microscopy. Thin layer chromatography (TLC) was accomplished on UV active aluminum backed plates of silica gel (TLC silica gel 60 F254).

Preparation of Fe_3O_4 MNPs: $FeCl_3.6H_2O$ (6 mmol), FeCl2.4H2O (3 mmol), were dissolved in 150 ml distilled water under nitrogen atmosphere and vigorous mixing. Thereupon, tween 80 (1.5 ml) was added to the resultant orange solution and the mixture was mixed and heated at 80-85˚C for about 1 h under vigorous stirring. Ammonium hydroxide solution (15 ml) was added dropwise to the resulting mixture to get a black suspension. The resulting suspension was refluxed at 80-85˚C for about 6h. Eventually, nanoparticles were separated with aid a magnet, washed with distilled water to get the pH 7 and dried at 25 ˚C in vacuum for 24 h.

Preparation of folic acid coated Fe_3O_4 MNPs : Folic acid with a specified concentration in ethanol (50 ml, 5 mg/ml) was added to the suspension of magnetite nanoparticles (0.6 g in 20 ml of ethanol)**.** The mixture stirred for 24 h at room temperature. Eventually, nanoparticles were separated using the magnet, washed with ethanol and finally dried at 25˚C in vacuum for 12 h.

Typical Procedure for the preparation of 3, 4-Dihydropyrimidine -2(1H) thiones: MNPs- Folic acid (0.05 gr) as a nanocatalyst were surcharged to a mixture of methyl acetoacetate (1 mmol), thiourea (1.5 mmol) and aromatic aldehyde (1 mmol). The mixture was heated on an oil bath at 100 °C for approximately 90 min. After fulfillment of the reaction, the resulting solidified mixture was diluted with 5 ml hot ethanol and the nanocatalyst was separated with assistance an external magnet. The obtained liquid was slowly heated to evaporate the solvent and the solid product was washed with distilled water, recrystallized from the ethanol and dried at 50 °C under vacuum for 3h. The result related to spectra information for novel samples are indexed below.

Methyl-6-methyl-4-(5-hydroxymethyl-2-furyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4l): IR (KBr): ν_{max}: 3402 , 3354, 3125, 2811, 1697, 1611, 1097cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6,): δ= 1.45 (s, 3H, CH_3), 3.05 (s, 1H, OH), 3.87 (s, 3H, OCH_3), 4.47 (s, 2H, CH_2OH), 5.35 (s, 1H, H-4), 6.24(d, 1H , Ar-H), 6.66 (d, 1H, Ar-H), 8.49 (s, 1H, NH), 8.84 (s, 1H, NH) ppm; ^{13}C NMR(100 MHz, DMSO-d_6): δ= 170.3, 160.8, 152.6, 149.3, 146.5, 111.7, 105.8, 103.2, 54.9, 52.7, 49.3, 16.8 ppm.

Methyl-6-methyl-4-(5-methyl-2-theinyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4m): IR (KBr): ν_{max}: 3341, 3094, 2854, 1711, 1617, 1078 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.57 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 3.58 (s, 3H, OCH_3), 4.96 (s, 1H, H-4), 6.58(d, 1H , Ar-H), 6.69 (d, 1H , Ar-H), 8.57 (s, 1H, NH), 8.84 (s, 1H, NH) ppm; ^{13}C NMR(100 MHz, DMSO-d_6): δ= 171.8, 161.4, 151.5, 135.5, 131.7, 129.1, 125.4, 107.3, 54.1, 50.7, 16.8, 16.1 ppm.

Methyl-6-methyl-4-(3-methyl-2-theinyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4n): IR (KBr): ν_{max}: 3366, 3154, 2896, 1706, 1604, 1095cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.63 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 5.14 (s, 1H, H-4), 6.62(d, 1H , Ar-H), 6.87 (d, 1H, Ar-H), 8.32 (s, 1H, NH), 8.76 (s, 1H, NH) ppm; ^{13}C NMR(100 MHz, DMSO-d_6): δ= 169.4, 159.7, 150.8, 136.4, 134.9, 121.1, 103.5, 53.7, 49.8, 17.1, 14.3 ppm.

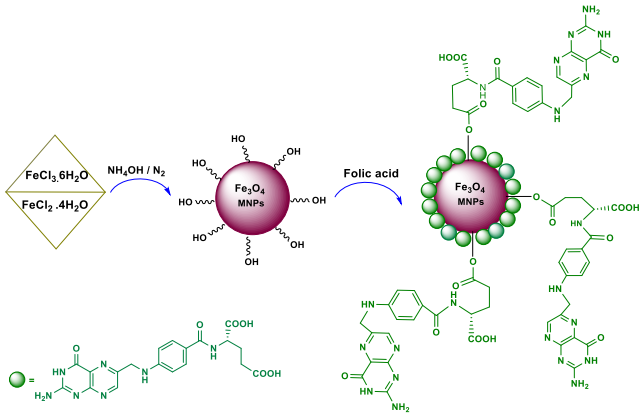
Methyl-6-methyl-4-(4-pyridyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4o): IR (KBr): ν_{max}: 3395, 3097, 2884, 1704, 1587, 1106 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.66 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 5.31 (s, 1H, H-4), 7.57 (d, 2H, Ar-H), 8.76(d, 2H, Ar-H) ,8.95 (s, 1H, NH), 9.42 (s, 1H, NH) ppm; ^{13}C NMR(100 MHz, DMSO-d_6): δ= 170.8, 160.1, 151.9, 145.6, 141.3, 125.4, 102.8, 57.3, 51.6, 17.4 ppm.

3. RESULTS SECTION

Folic acid magnetic nanoparticles core shell composite was synthesized using the co-precipitation of Fe^{3+} and Fe^{2+} ions in attendance of folic acid (Scheme 1). MNPs-FA nanocatalyst structure was characterized using FT-IR, XRD, VSM, SEM, EDS, and UV-Vis techniques.

The FT-IR spectra of FA-conjugated Fe_3O_4 MNPs, Fe_3O_4 and folic acid are exhibited in Fig. 1. As can be seen from the FT-IR spectra of Fe_3O_4 in Fig. 1a, the obvious peak at about 633 cm^{-1} is concerned to the Fe-O stretching bond that confirms the formation of magnetite core. Also, the emerged peaks at 3393 cm^{-1} and 1622 cm^{-1} \pertain to the O-H group that can be concerned to the absorbed water molecules [66]. The FT-IR spectra of folic acid (Fig**.** 1b), demonstrates stretching vibration of C=O at 1691 cm^{-1}, O-H and N-H groups peak at the region of 2500-3500 cm^{-1}.

According to Fig. 1c, the FT-IR spectra of Fe_3O_4@ folic acid MNPs are exactly different from the Fe_3O_4 and bare folic acid. The specific peak at 633 cm^{-1}, is attributed to the Fe-O stretching absorption bond. In addition, the band at 1701 cm^{-1} is due to the COOFe group that confirms the complexation between carboxylate moiety of folic acid group and ions on the surface of Fe_3O_4.



Scheme 1. Preparation of FA-Fe_3O_4 MNPs.

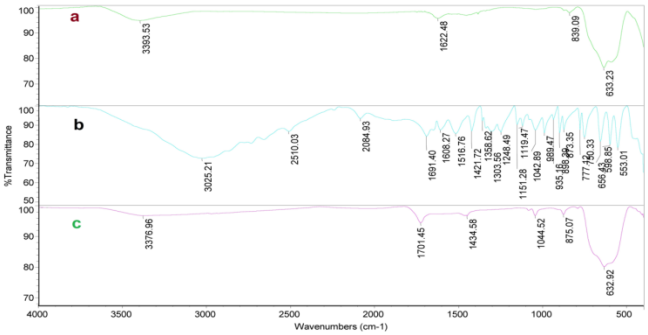


Figure 1. FT-IR spectra of (a) Fe_3O_4 MNPs, (b) free folic acid, (c) MNPs-Folic acid.

Beside the FT-IR technique, to assess the conjugated folic acid on the Fe_3O_4 MNPs, UV-Vis spectrophotometer was applied. Folic acid coated MNPs and free folic acid was analyzed in the region of 200-600 nm. The apparent absorbance of free folic acid and folic acid-loaded MNPs became visible at 284 nm. Regarding to the obtained results, it can be figured out that Fe_3O_4-MNPs was successfully immobilized with folic acid (Fig. 2). To calculate the quantity of folic acid coated MNPs, specific concentrations of folic acid were prepared and the absorbance of the sample was compared with them. Finally, the quantity of folic acid coated MNPs were found approximately 0.625 mmol per gram.

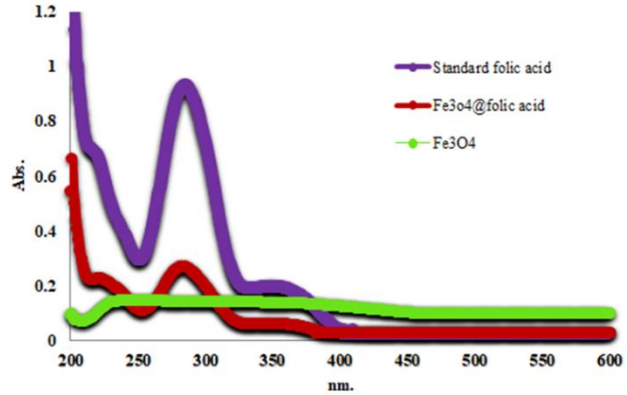


Figure 2. UV-Vis absorbance of free folic acid, MNP-Folic acid and Fe_3O_4 MNPs.

To figure out the morphology and particle size of the catalyst, scanning electron microscopy (SEM) was utilized (Fig. 3). SEM image illustrates, the folic acid coated MNPs possess spherical shape with nano dimension range from 7 to 17 nm. The components of the nanocatalyst were performed using energy dispersive spectrometer (Fig. 4). According to the Fig. 4, presence of Fe, O, C and N signals confirm that folic acid successfully was capped on the surface of Fe3O4 magnetite nanoparticles.

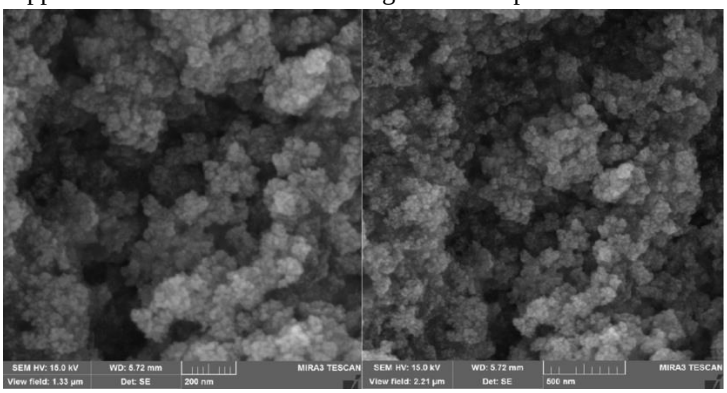


Figure 3. SEM images of MNP-Folic acid.

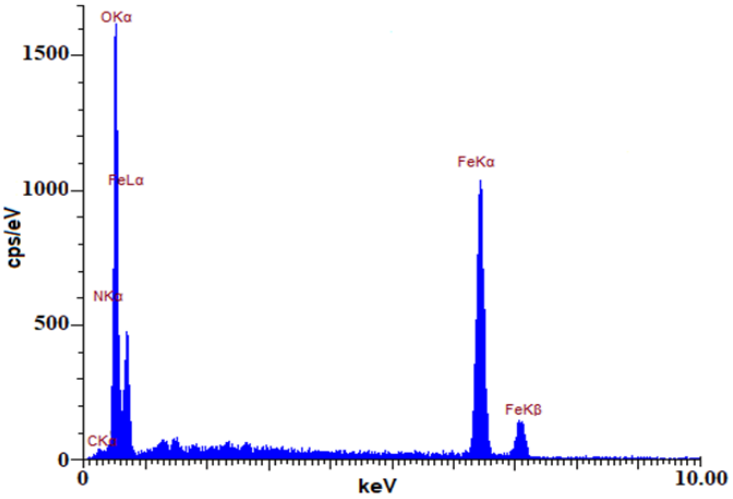


Figure 4. EDAX spectra of MNP-Folic acid.

X-ray diffraction technique was applied to check the crystalline structure of folic acid-conjugated MNPs (Fig. 5). XRD pattern of Fe_3O_4@FA MNPs was gathered in the region 2Θ= 20-80º. Pursuant to Fig. 5, the distinct peaks that were located at 30.4º, 35.8º, 43.4º, 53.7º, 57.3º, 63.0º, 74.4º are corresponded to the crystalline planes (220), (311), (400), (422), (511), (440) and (533) for spinel ferrite structure, respectively (Fe_3O_4, reference Jcpds no, 82-1533). The average crystalline size of FA-coated Fe_3O_4 MNPs was evaluated by the Scherer's equation ($D=k\lambda/\beta\cos\Theta$); where D is average crystalline size, λ is the X-ray wavelength, k is a constant (equal to 0.94), β is the full-width at half-maximum (FWHM)expressed in radians and Θ is the Bragg angle expressed in degree. The size of FA- conjugated MNPs obtained from this equation was found to be approximately 9 nm.

Vibration sample magnetometer (VSM) for FA-coated Fe_3O_4 MNPs is displayed in Fig.6. As can be observed in Fig. 6, the saturation magnetization (Ms) related to Fe_3O_4@FA MNPs is 56 emu/g which this magnetization value is less than the uncoated Fe_3O_4 MNPs (88 emu/g) [67, 68]. This reduction in the saturation magnetization value in Fe_3O_4@FA rather than the bulk Fe_3O_4

related to diamagnetic organic material loading (folic acid) on the surface of Fe_3O_4 MNPs. Fe_3O_4@FA show superparamagnetic feature because no hysteresis is observed in the sample. Also the magnetic properties of FA- capped MNPs were figured out by external magnet that confirmed the modified nanoparticles had an excellent magnetic responsibility (Fig.6, right insert).

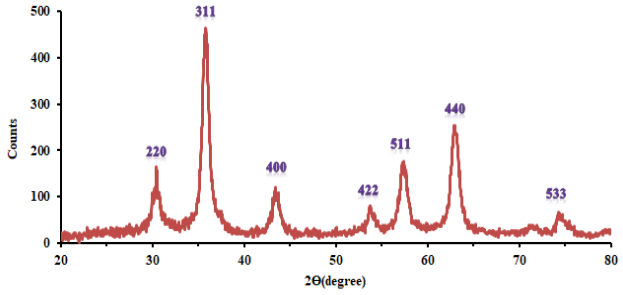


Figure 5. XRD diffraction pattern of MNP-Folic acid.

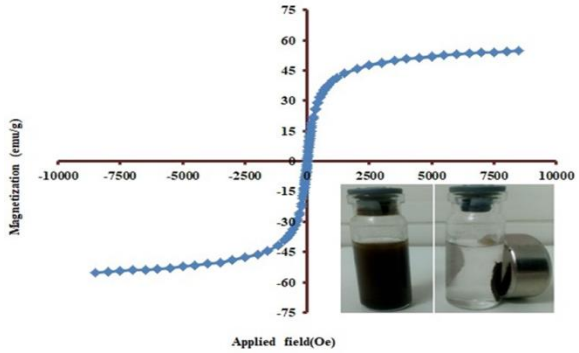


Figure 6. The magnetic diagram of MNPs-Folic acid. Right inset: the picture exhibits the catalyst was dispersed in liquid and captured by the outer magnet.

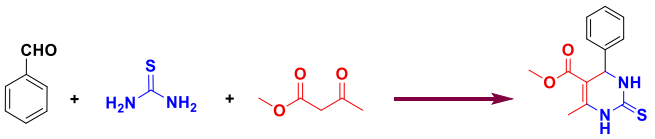
The catalytic activity of Fe_3O_4@FA MNPs was measured in the three-component Biginelli reaction to prepare various 3, 4-dihydropyrimidine thiones (Scheme 2). We first examined the reaction in the presence of benzaldehyde (1 mmol), methyl acetoacetate (1 mmol), thiourea (1.5 mmol) and Fe_3O_4@FA MNPs (100 mg) in the presence of ethanol under reflux condition for a determined time (as demonstrated by TLC).

Scheme 2. Synthesis of 3,4-dihydropyrimidine-2-(1H)-thiones.

Afterwards, diverse catalysts, different solvents, solvent–free conditions. Various amount of catalyst in order to optimize the reaction conditions were applied (Table1). In the first step, the efficiency of new nanocatalyst was compared with different catalysts and the results are shown in Table1. It was observed that the Fe_3O_4@FA MNPs were more efficacious rather than other catalysts (entries 1-5). It should be noted out that in the absence of a catalyst, no product was received. Next, we examined the Biginelli reaction in different solvents and solvent-free condition at 100 ˚C. According to Table 1(entry 10), the best result detected under solvent-free reaction conditions. Finally, in order to optimize the quantity of Fe_3O_4@FA MNPs, various amounts of FA-coated MNPs was applied and the optimum quantity of the catalyst was detected to be 50 mg.

Accordance with optimized results and to figure out the effect of the magnetic nanocatalyst, several 3, 4-dihydropyrimidine thiones were synthesized and the respective results are represented in Table 2.

Table 1. Optimization experiments for the one-pot three component reaction of benzaldehyde (1mmol), thiourea (1.5mmol) and methyl acetoacetate (1mmol) in the presence MNP-Folic acid under different condition.

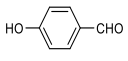
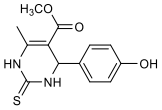
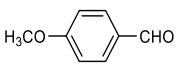
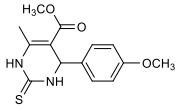


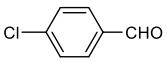
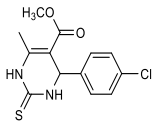
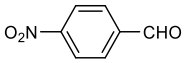
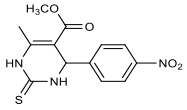
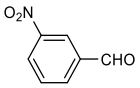
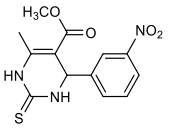
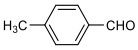
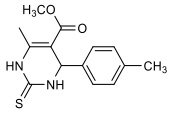
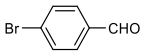
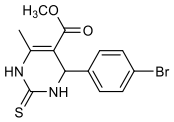
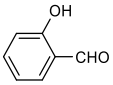
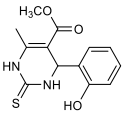
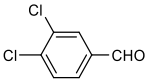
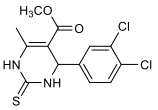
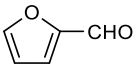
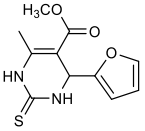
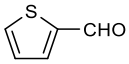
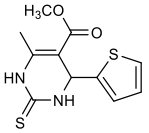
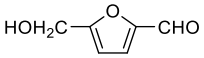
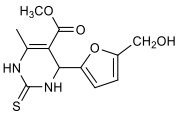
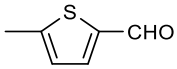
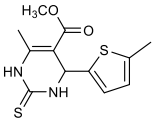
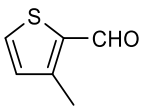
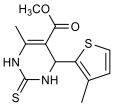
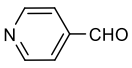
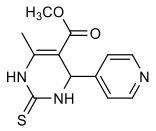
Entry	Catalyst (g)	Solvent	Condition	Time (min)	Yield (%)[a]
1	Fe_3O_4 MNPs (0.2)	EtOH	Reflux	600	0
2	HCl (0.3)	EtOH	Reflux	480	25
3	Silica sulfonic acid (0.1)	EtOH	Reflux	240	67
4	Proline (0.1)	EtOH	Reflux	300	54
5	MNP-FA (0.1)	EtOH	Reflux	180	83
6	-	EtOH	Reflux	900	0
7	MNP-FA (0.1)	CH_3CN	Reflux	180	61
8	MNP-FA (0.1)	MeOH	Reflux	180	74
9	MNP-FA (0.1)	Water	Reflux	180	0
10	MNP-FA (0.1)	-	100 °C	90	91
11	MNP-FA (0.075)	-	100 °C	90	91
12	MNP-FA (0.05)	-	100 °C	90	91
13	MNP-FA (0.025)	-	100 °C	90	37

[a] Isolated yields

The reaction of thiourea, methyl acetoacetate with different kind of aldehydes containing electron-withdrawing, electron-donating and heteroaromatic aldehydes gave the favorable 3, 4-dihydropyrimidine thiones derivatives in excellent yield.

Table 2. Synthesis of 3,4-dihydropyrimidine-2-(1H)-thiones using MNP-Folic acid under Solvent-free condition.

Entry	Aldehyde	Product		Yield (%)[a]	M.P. (°C)	
					Found	**Reported [Lit.]**
1			**4a**	90	208-210	210-211 [69]
2			**4b**	93	177-179	178-180 [70]

3			**4c**	91	183-185	180-183 [71]
4			**4d**	91	204-206	205-207 [69]
5			**4e**	89	248-249	250-252 [69]
6			**4f**	92	154-155	156-158 [72]
7			**4g**	87	181-182	180-181 [73]
8			**4h**	87	213-214	209- 213 [73]
9			**4i**	88	205-207	204-206 [73]
10			**4j**	91	251-254	252-254 [73]
11			**4k**	91	228-230	230-232 [72]
12			**4l**	89	241-244	-
13			**4m**	90	257-259	-
14			**4n**	89	217-219	-
15			**4o**	88	234-236	-

[a]Isolated yields

The reusability of folic acid–coated magnetic nanoparticles was examined using frequent runs in the Biginelli reaction of thiourea (1.5 mmol), benzaldehyde (1mmol) and methyl acetoacetate (1mmol) in solvent-free condition for 90 min. To reuse the catalyst after each cycle, ethanol as a green solvent was added to the reaction mixture and the catalyst was isolated by an external magnet and washed different times with ethanol and dried at room temperature. On the basis of Fig. 7, nanocatalyst can be reused five times without substantial loss of catalytic activity. Unfortunately, using the catalyst more than five times decrease its catalytic activity that might be due to the chemical poising of the surface of the Fe_3O_4@FA MNPs [74].

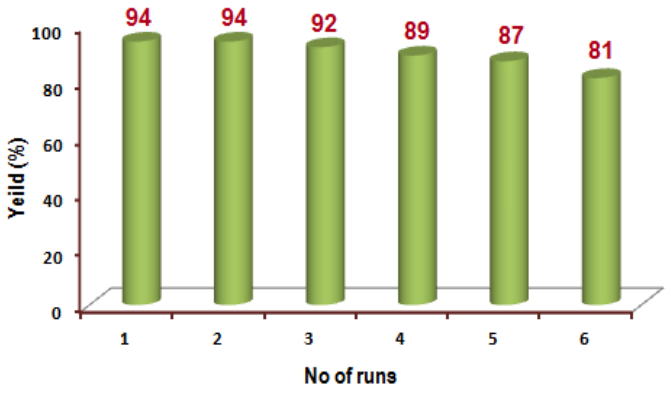


Figure 7. Recyclability of MNP-Folic acid in the reaction of benzaldehdye (1 mmol), methyl acetoacetate (1 mmol), thiourea (1.5 mmol) and catalyst (0.05 g) under solvent-free conditions.

Antibacterial activity studies. The in vitro antibacterial activity of 3,4-dihydropyrimidine derivatives figured out against Gram-positive (staphylococcus aureus ATTC NO. 6538, Staphylococcus epidermidis ATCC NO. 12228) and Gram-negative bacterial strains (*Pseudomonas aeruginosa* ATTC NO. 9027, Escherichia coli ATCC NO. 8739) using agar well diffusion technique [75, 76]. Bacterial strains were cultured individually on a plate of Mueller-Hinton agar and incubated for 18-24 h at 37 ºC. Sterile buffer phosphate was used to make 1.5×10^8 cfu/ml suspensions of each examined bacterial strain and all obtained suspensions were controlled with 0.5 McFarland standard. All sample and ciprofloxacin as a positive control were dissolved in DMSO and to verify testing conditions, DMSO was performed as a negative control drug standard. A sufficient amount of sterile Muller-Hinton agar (approximately 20 ml) and 100 μl of suspensions of each bacterial were mixed and poured into sterile petri dishes and allowed to solidify. The wells were pierced in the culture plates, filled alternate with the test solution, DMSO, ciprofloxacin and incubated 24 h at 37 °C. Finally, inhibition zone around each well was measured. The results are presented in Table 3. The results of antibacterial activity show clearly that higher antibacterial activity is related to 4j-o compounds against tested Gram-positive and Gram-negative bacterial strains. It is noticeable that all compounds showed better antibacterial activity rather than ciprofloxacin.

Table 3. Antimicrobial activity of compounds 4a-o based on agar well diffusion technique (inhibition zone/mm)

Entry	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus (ATCC NO. 6538)	*S. epidermidis* (ATCC NO. 12228)	*P. aeruginosa* (ATCC NO. 9027)	*E. coli* (ATCC NO. 8739)
4a	23	25	25	24
4b	23	25	25	23
4c	22	25	25	23
4d	24	27	24	23
4e	25	27	26	26
4f	23	25	26	23
4g	24	25	27	25
4h	23	26	24	25
4i	24	26	24	25
4j	26	30	30	28
4k	28	28	28	29
4l	29	30	28	29
4m	26	30	28	27
4n	26	32	30	27
4o	29	32	30	29
Ciprofloxacin	22	25	24	22

4. CONCLUSIONS

We have successfully synthesized the affective, recoverable and superparamagnetic folic acid-coated nanocomposite as a catalyst using an easy one-pot synthesis based on co-precipitation technique and characterized by FT-IR, SEM, VSM, XRD, EDXA and UV-Vis techniques. This recoverable heterogeneous catalyst provides a facile strategy to the synthesis a diversity of heterocycles under benign conditions. The performance of new magnetic nanocatalyst was examined in one–pot three-component reaction to produce 3,4-dihydropyrimidine thiones under solvent–free conditions. Fe_3O_4@FA MNPs have picked out as a desired catalyst because of some advantages such as superb recoverability and reusability, simple preparation and eco- friendly. It is noticeable that the nanocatalyst is stable under the reaction condition and can be easily isolated by using a magnet and reused 5 times without any considerable decrease in catalytic activity. The in vitro antibacterial activity of compounds 4a-o was

evaluated and the results showed better antibacterial activity for all compounds in compared with ciprofloxacin drug standard.

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6. ACKNOWLEDGEMENTS

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