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Chitosan-attached nano-Fe_3O_4 as a superior and retrievable heterogeneous catalyst for the synthesis of benzopyranophenazines using chitosan-attached nano-Fe_3O_4

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Abstract: A simple and rapid method for the preparation of benzopyranophenazines is presented, involving a one-pot four-component reaction of hydroxynaphthoquinone, *o*-phenylenediamine, benzaldehydes, and malononitrile with nano-Fe_3O_4@chitosan as an efficient heterogeneous solid acid catalyst under reflux conditions in ethanol. The catalyst is characterized by powder X-ray diffraction (XRD), scanning electron microscopy (SEM), magnetic susceptibility measurements, energy-dispersive X-ray spectroscopy (EDS), and Fourier transform infrared (FT-IR) spectroscopy. Atom economy, high catalytic activity, a wide range of products, excellent yields in short reaction times, and low catalyst loading are some of the important features of this method.

Keywords: catalytic activity; chitosan; nano-Fe_3O_4; one-pot reaction; pyranophenazines.

1 Introduction

Phenazines possess many important biological properties, including antitumor [1], antimicrobacterial [2], antiproliferative [3], antibiotic [4], antifungal [5], and anti-inflammatory [6]. Some phenazines isolated from *Streptomyces* (a marine bacterium) have been described with biological significance (Fig. 1) [7–10]. Finding effective methods for the synthesis of phenazines through multicomponent reactions (MCRs) is a significant area of research in organic and medicinal chemistry. Recently, there have been reports on the synthesis of phenazines using *p*-TSA (*para*-toluenesulfonic acid) [11], glacial acetic acid [12], 1,4-diazabicyclo[2.2.2]octane (DABCO) [13, 14], thiourea-based organocatalysts [15], caffeine [16], theophylline [17], L-proline [18], 1-butyl-3-methylimidazolium hydroxide ([Bmim]OH) [19], Et_3N [20], pyridine [21], and oxalic acid [22]. However, some of the reported methods suffer from drawbacks such as long reaction times, generation of a large amount of waste, unpleasant reaction conditions, and use of toxic and nonreusable catalysts. There remains a need, therefore, for new, efficient, and mild approaches to obtain such products in high yields. Magnetic materials have established themselves as a special group of heterogeneous catalysts owing to their diverse applications in syntheses and catalysis [23, 24]. To overcome the drawback of the separation of the catalyst, nanomagnetic materials have emerged as recoverable and retrievable catalysts. The surface of magnetic nanoparticles (MNPs) can be functionalized simply through convenient surface modifications to enable the loading of a variety of required functionalities [25–27]. Here we reported the use of nano-Fe_3O_4@chitosan as an efficient catalyst for the preparation of benzopyranophenazines by a one-pot four-component reaction of hydroxynaphthoquinone, *o*-phenylenediamine, benzaldehydes, and malononitrile under reflux conditions in ethanol (Scheme 1).

2 Results and discussion

Figure 1 shows the powder X-ray diffraction (XRD) pattern of nano-Fe_3O_4@chitosan. The pattern agrees well with the reported pattern for Fe_3O_4 (JCPDS No. 75-0449). The crystallite size of nano-Fe_3O_4@chitosan, as calculated by the Scherrer equation, is 15–20 nm, which is in good agreement with the results obtained by scanning electron microscopy (SEM; see below), which was used to determine the particle size and morphology. The statistics of the results from the SEM images clearly demonstrate that the average size of nano-Fe_3O_4@chitosan is 10–25 nm (Fig. 2).

Figure S1 shows the Fourier transform infrared (FT-IR) spectrum of nano-Fe_3O_4@chitosan. It displays a broad band at about 3423 cm^{-1}, which corresponds to the stretching vibrations of O–H and N–H groups. Peaks appearing at 2923 and 2855 cm^{-1} are characteristic of C–H stretching vibrations. The band at 1590 cm^{-1} is assigned to

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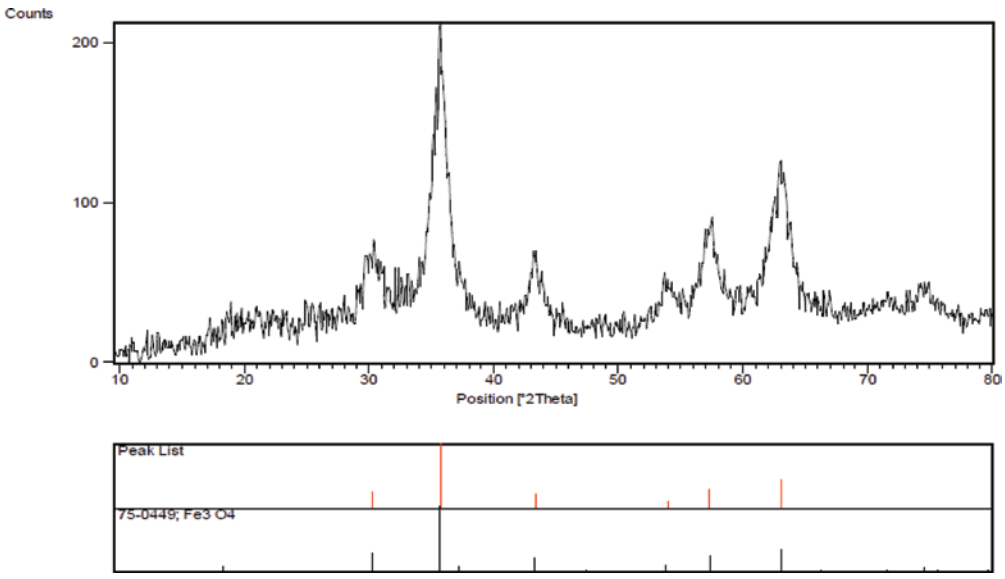
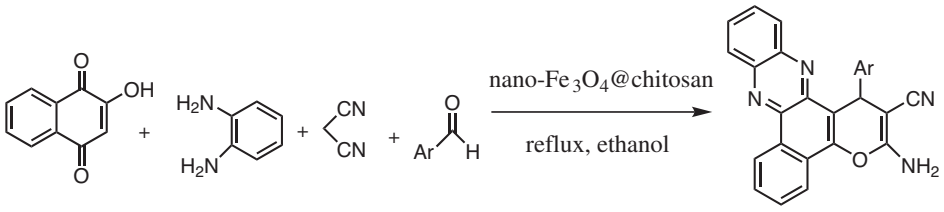


Fig. 1: XRD patterns of nano-Fe_3O_4@chitosan.



Scheme 1: Synthesis of benzopyranophenazines using nano-Fe_3O_4@chitosan.

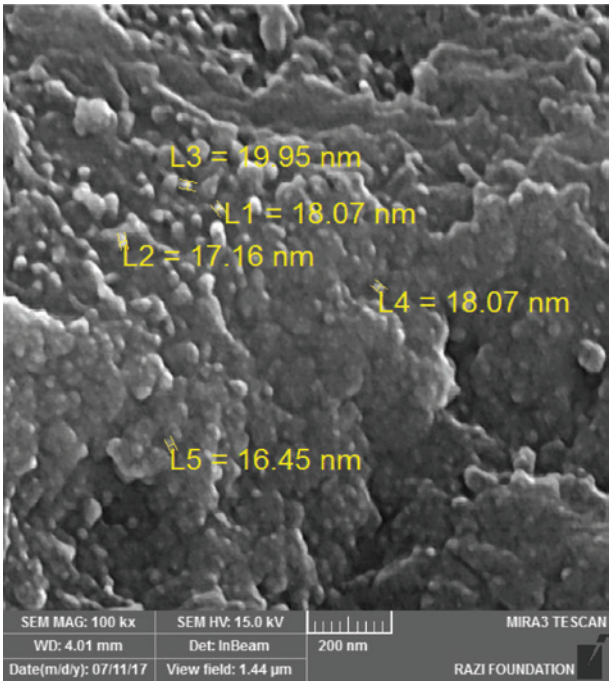


Fig. 2: SEM photograph of nano-Fe_3O_4@chitosan.

N–H bending vibration, and those at 1375–1450 cm^{-1} are assigned to C–H bending vibration in chitosan. The bands at 1069 and 628 cm^{-1} are assigned to the stretching vibrations of C–O and Fe–O, respectively.

In order to investigate the size distribution of the nanocatalysts, DLS (dynamic light scattering) measurements of the nanoparticles were carried out (Fig. 3). The dispersion for the DLS analysis (2.5 g nanocatalyst in 50 mL ethanol) was carried out using an ultrasonic bath (60 W) for 30 min.

The magnetic properties of the nanoparticles were characterized using a vibrating sample magnetometer (VSM). Magnetic measurements show that nano-Fe_3O_4 and nano-Fe_3O_4@chitosan have saturation magnetization values of 48.5 and 22.32 emu g^{-1}, respectively (Fig. 4). These results demonstrate that the magnetization decreases by coating and functionalization.

The EDS (energy-dispersive X-ray spectroscopy) spectrum of nano-Fe_3O_4@chitosan (Fig. 5) shows that the elemental composition consists of carbon, oxygen, iron, and nitrogen.

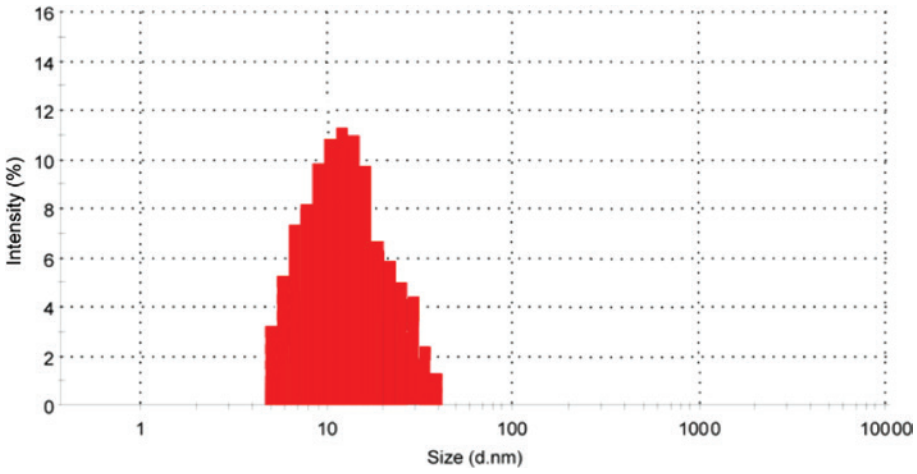


Fig. 3: DLS of nano-Fe_3O_4@chitosan.

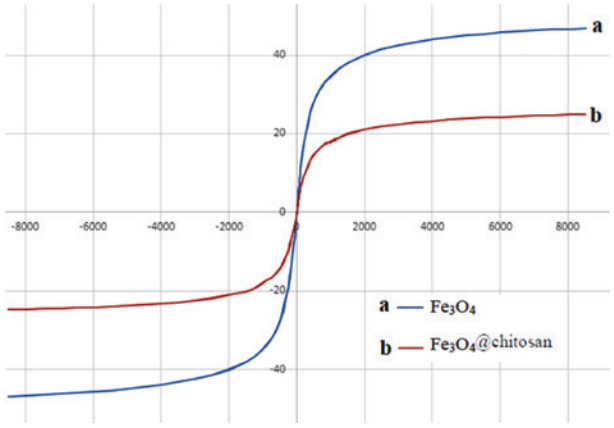


Fig. 4: VSM curve of (a) nano-Fe_3O_4 and (b) nano-Fe_3O_4@chitosan.

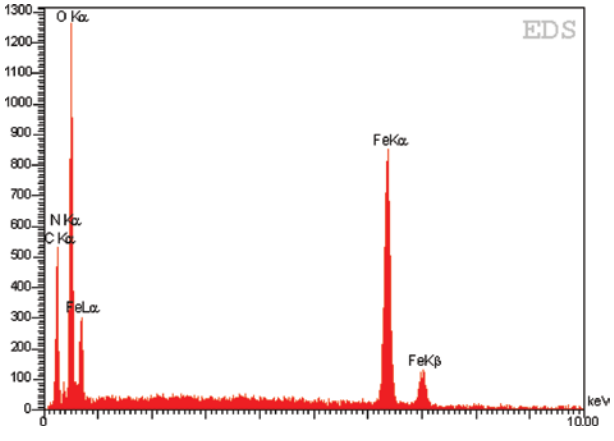


Fig. 5: EDS of nano-Fe_3O_4@chitosan.

Initially we studied and optimized the different reaction parameters for the preparation of benzopyranophenazine by the condensation reaction of hydroxynaphthoquinone, *o*-phenylenediamine, 4-chlorobenzaldehyde, and malononitrile as a model reaction. To obtain the ideal reaction conditions for the synthesis of compound **5b**, we studied other catalysts and solvents, as shown in Table 1. Screening of diverse catalysts such as Et_3N, imidazole, *p*-TSA, nano-Fe_3O_4, chitosan, and nano-Fe_3O_4@chitosan revealed nano-Fe_3O_4@chitosan as the most effective catalyst to perform this reaction under reflux conditions in ethanol. From further studies on the catalyst loading, we found that the yield of compound **5b** remained almost the same when 10 mg of nano-Fe_3O_4@chitosan was used (Table 1). Using lower catalyst loadings (8 mg) afforded **5b** in 88% yield.

The above results show that the present catalytic method is extendable to a wide variety of substrates to

Table 1: Optimization of the reaction conditions.[a]

Entry	Solvent (reflux)	Catalyst	Time (min)	Yield[b] (%)
1	EtOH	–	600	Trace
2	EtOH	Et_3N (10 mol.%)	400	45
3	EtOH	Imidazole (10 mol.%)	400	40
5	EtOH	*p*-TSA (10 mol.%)	200	58
6	EtOH	Fe_3O_4 (8 mol.%)	250	48
7	EtOH	Chitosan (12 mg)	200	58
8	H_2O	Nano-Fe_3O_4@chitosan (8 mg)	150	68
9	CH_2Cl_2	Nano-Fe_3O_4@chitosan (8 mg)	150	60
10	CH_3CN	Nano-Fe_3O_4@chitosan (8 mg)	120	75
11	EtOH	Nano-Fe_3O_4@chitosan (8 mg)	80	86
12	EtOH	Nano-Fe_3O_4@chitosan (10 mg)	80	90
13	EtOH	Nano-Fe_3O_4@chitosan (12 mg)	80	90

[a]Reaction conditions: 2-hydroxynaphthalene-1,4-dione (1 mmol), *o*-phenylenediamine (1 mmol), 4-chlorobenzaldehyde (1 mmol), and malononitrile (1.5 mmol) as a model reaction. [b]Isolated yields.

create a wide library of benzopyranophenazines. From the above observation, it is important to mention that electron-withdrawing groups increase the reaction rate and give better yields than electron-donating groups (Table 2).

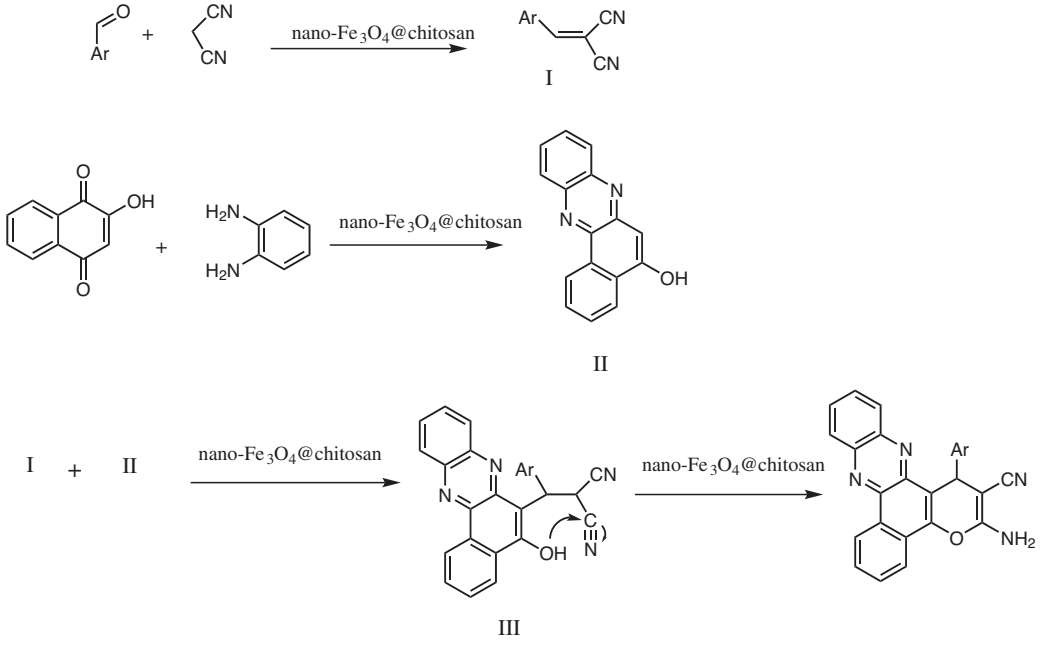
We investigated the reusability of nano-Fe_3O_4@chitosan as the catalyst for the preparation of the product **5b**. We found that product yields reduced only to a small extent on each reuse (run 1, 90%; run 2, 90%; run 3, 90%; run 4, 89%; run 5, 89%, run 6, 88%). After completion of the reaction, the nanocatalyst could be easily separated using an external magnet. The catalyst was washed four times with ethanol and dried at room temperature for 24 h before reuse.

A proposed mechanism for the synthesis of benzopyranophenazines using nano-Fe_3O_4@chitosan is shown in Scheme 2: (i) The initial condensation of hydroxynaphthoquinone with *o*-phenylenediamine affords the intermediate **I**. (ii) A Knoevenagel condensation of malononitrile and benzaldehydes forms the intermediate **II**. (iii) The Michael addition of intermediate **I** with intermediate **II** gives the intermediate **III**, which in subsequent cyclization and tautomerism affords the corresponding product. In this mechanism, the surface atoms of nano-Fe_3O_4@chitosan activates the C=O and C≡N groups for better reaction with the nucleophiles. This proposed mechanism is supported by other reports in the literature [15, 19, 29]. Nanoparticles have good catalytic activity owing to their large surface area and number of active sites. However, the activity of the catalysts is also influenced by the acid-base properties and many other factors such as the geometric structure (particularly pore structure), the distribution of sites, and the polarity of the surface sites [30]. The free hydroxyl and amino groups distributed on the surface of chitosan supported by nano-Fe_3O_4 are believed to activate the substrates predominantly through hydrogen bonding [29].

Table 2: Synthesis of benzopyranophenazine derivatives.

Entry	R	Product	Time (min)	Yield[a] (%)	m.p. (°C) found (reported[b])
1	H	**5a**	90	86	297–300 (298–300)
2	4-Cl	**5b**	80	90	290–292 (288–290)
3	2-Cl	**5c**	85	88	299–302 (301–303)
4	4-Br	**5d**	80	90	282–284 (283–285)
5	4-F	**5e**	80	92	273–276 (274–276)
6	3-NO_2	**5f**	80	90	277–281 (278–279)
7	4-NO_2	**5g**	80	94	280–282 (281–283)
8	4-CN	**5h**	85	86	288–290
9	4-NMe_2	**5i**	100	78	261–263 (261–263)
10	4-Me	**5j**	100	80	293–295 (293–294)
11	2-OMe	**5k**	100	82	268–270 (270–272)
12	3-OMe	**5l**	100	80	239–241 (240–242)
13	4-OMe	**5m**	100	78	268–269
14	2,4-Dichloro	**5n**	80	92	306–309 (308–310)

[a]Isolated yields. [b]All reported values are from the literature [28].



Scheme 2: Proposed mechanism for the synthesis of benzopyranophenazines.

3 Experimental section

Reagent grade chemicals were purchased from Sigma-Aldrich or Merck and were used without further purification. Chitosan with an average molecular weight of 290 000 Da was purchased from Sigma-Aldrich. The products were isolated and characterized by physical and spectral data. NMR spectra were obtained on a Bruker Avance 400 MHz spectrometer (^{1}H NMR at 400 Hz, ^{13}C NMR at 100 Hz) in DMSO-d_6 using trimethylsilane (TMS) as the internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in hertz (Hz). FT-IR spectra were recorded with KBr pellets by a Nicolet Magna 550 IR spectrometer. CHN compositions were measured using a Carlo ERBA Model EA 1108 analyzer. Powder XRD was carried out on a Philips X'pert diffractometer with monochromatized Cu$K\alpha$ radiation ($\lambda = 1.5406$ Å). The morphology of the products was visualized by SEM (MIRA3). DLS was accomplished with a Malvern instrument (Malvern Zetasizer). Magnetic properties of the magnetite nanoparticles were measured using a VSM (Meghnatis Daghigh Kavir Co., Kashan Kavir, Iran) at room temperature.

3.1 Preparation of nano-Fe_3O_4@chitosan

Our purpose was to synthesize a magnetic nanocatalyst with a mass ratio of chitosan to nano-Fe_3O_4 (g/g) = 2:1. In order to achieve this, 1 g of chitosan and 0.43 g (2.1 mmol) of $FeCl_2{\cdot}4H_2O$ and 1.17 g of $FeCl_3{\cdot}6\ H_2O$ (2×2.1 mmol) were placed in a 100-mL round bottom flask, and 40 mL of 0.4 M HCl was added to them under stirring at room temperature. The mixture was stirred until complete dissolution. Then 400 mL of NH_3 (0.7 M) was added to the mixture under argon gas for 20 min. The formed nanocatalyst was filtered and washed with H_2O and dried in an oven at 70°C.

3.2 General procedure for the synthesis of benzopyranophenazines

Nano-Fe_3O_4@chitosan (10 mg) was added to a mixture of hydroxynaphthoquinone (1 mmol) and *o*-phenylenediamine (1 mmol) in EtOH (5 mL) at room temperature under stirring. After 5 min, the aldehydes (1 mmol) and malononitrile (1.5 mmol) were added and the mixture was refluxed for the appropriate amount of time (Table 2). The progress of the reaction was monitored by thin-layer chromatography (TLC; EtOAc/*n*-hexane 2:1), and the mixture was cooled to room temperature. After completion of the reaction, the nanocatalyst was easily separated using an external permanent magnet. The solvent was evaporated and the solid obtained was filtered and washed with EtOH and water. The pure products were characterized by comparison of their physical data (melting points, IR, and ^{1}H NMR) with those of known compounds in the literature. The FT-IR and ^{1}H NMR spectra of selected compounds **5** are given as supplementary material (available online).

3.2.1 3-Amino-1-(4-cyano-phenyl)-1*H*-benzo[*a*]pyrano[2,3-*c*]phenazine-2-carbonitrile (5h)

Yellow solid. m.p. 288–290°C. FT-IR (KBr): ν (cm^{-1}) = 3322, 3176, 3045, 2831, 2182, 2139, 1644, 1622, 1584, 1483, 1455, 1444, 1392, 1383, 1355, 1337, 1292, 1256, 1160. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 5.42 (s, 1H, CH), 7.23 (s, 2H, NH_2), 7.38 (d, J = 8.0 Hz, 2H, Ar-H), 7.42 (d, J = 8.0 Hz, 2H, Ar-H), 7.83–8.08 (m, 4H, Ar-H), 8.12–8.15 (m, 1H, Ar-H), 8.17–8.22 (m, 1H, Ar-H), 8.42 (d, 1H, J = 7.6 Hz, Ar-H), 9.17 (d, 1H, J = 7.2 Hz, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6: δ (ppm) = 37.3, 57.9, 113.8, 115.3, 118.3, 122.1, 124.3, 125.5, 126.3, 127.8, 128.2, 128.6, 129.0, 129.2, 130.1, 130.3, 130.6, 130.8, 139.9, 140.1, 140.7, 141.4, 145.6, 146.5, 159.5. Analysis for $C_{27}H_{15}N_5O$: calcd. C76.22, H 3.55, N 16.46; found C 76.18, H 3.43, N 16.35.

3.2.2 3-Amino-1-(4-methoxy-phenyl)-1*H*-benzo[*a*]pyrano[2,3-*c*]phenazine-2-carbonitrile (5m)

Yellow solid. m.p. 268–269°C. IR (KBr): ν (cm^{-1}) = 3315, 3174, 3048, 2829, 2180, 1652, 1620, 1585, 1487, 1465, 1450, 1394, 1384, 1350, 1330, 1293, 1258, 1163. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 3.84 (s, 3H, OCH_3), 5.83 (s, 1H, CH), 6.65 (d, 2H, J = 7.6 Hz, Ar–H), 6.90 (d, 2H, J = 7.6 Hz, Ar–H), 7.35 (s, 2H, NH_2), 7.85–7.93 (m, 4H, Ar-H), 7.98–8.40 (m, 3H), 9.10 (d, 1H, J = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 37.5, 55.2, 58.3, 112.1, 115.2, 115.5, 120.2, 120.4, 121.4, 125.2, 127.0, 129.1, 129.3, 129.7, 130.1, 130.5, 130.8, 130.9, 140.3, 141.2, 141.9, 146.4, 147.3, 159.4, 160.5. Analysis for $C_{27}H_{18}N_4O_2$: calcd. C 75.34, H 4.21, N 13.02; found C 75.25, H 4.15, N 12.93.

4 Conclusion

We have developed a straightforward and efficient method for the preparation of benzopyranophenazines using nano-Fe_3O_4@chitosan as an efficient, heterogeneous, acidic, solid catalyst. The method offers several advantages including the rapid synthesis of potentially

pharmaceutically useful heterocyclic molecules, the use of easily available starting compounds, high yields, short reaction times, reusability of the catalyst, and low amounts of the catalyst.

5 Supporting information

The FT-IR spectrum of nano-Fe_3O_4@chitosan (Fig. S1) as well as copies of the FT-IR and ^1H NMR spectra of selected compounds **5** are given as supplementary material available online (DOI: 10.1515/znb-2019-0091).

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