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# Preparation of α-chitin-based nanocomposite as an effective biocatalyst for microwave aided domino reaction

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ABSTRACT

In this paper, chitin (Ch) was extracted by an optimized method from cuttlebone of the Persian Gulf cuttlefish (Sepiidae, Cephalopoda). The extracted chitin was characterized by Fourier-transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD) and thermal gravimetric analysis (TGA) which showed that the extracted chitin was in alpha form. The degree of N-acetylation (DA) and degree of substitution (DS) of α-chitin were calculated using titration method and FTIR spectroscopy and found to be 80–82% and 19.57 respectively. The α-Chitin was used as biomolecules for the preparation of nanostructured Ch/ZnO via a hydrothermal method. The obtained nanocomposite was characterized using FT-IR, XRD, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX) analysis. The antimicrobial aspect of Ch/ZnO nanocomposite was previously proposed. In this paper, attempt was made to add the catalytic feature to these traits. For this purpose, the nanostructured Ch/ZnO was used as reusable nanocatalyst in the green and efficient synthesis of Benzo[a]pyrano(2,3-c)phenazine derivatives thru a four components microwave aided domino reaction. Eco-friendly, easy work up and separation of the nanostructured catalyst are some of the highlighted features this protocol.

## 1. Introduction

Current tendencies toward sustainable development reveal the importance of use of renewable biopolymers as catalyst supports. Due to their unique structures and functional groups, polysaccharides were used as supports for metal catalysts. Chitin, polymer extracted from crustacean shells, is a natural amino polysaccharide. It is a linear polymer resembling to cellulose by the β-(1→4) glycosidic bond between the constituent units (Fig. 1). Accordingly, "chitin may be described as cellulose with one hydroxyl group on each monomer replaced with an acetyl amine group" (Rui et al., 2008). Based on different polymer chains formation, chitin exists in three know forms such as α, β and γ. In both α and β chitin, the sheets of neighboring chitin chains contract hydrogen bonds between them, forming linear fibers of very high resistance. The forms α and β differ only in the fact that in α-chitin the chain clusters are consecutively antiparallel, though in β-chitin they are parallel (Petrov et al., 2012). Since chitin is stable in the range of 260–400 °C, (Ding et al., 2016) it can be used as a polymer unit in various hydrothermal synthesis methods. The presence of the acetamide groups gives it biological functions such as biodegradability and biocompatibility, which makes chitin a functional material of great importance. So, the conversion of natural chitin resource into valuable composites is of foremost interest. As a result, in recent years, research on the development of new chitin-based functional materials has received considerable attention (Noguchi et al., 2019).

The nucleation and growth of ZnO crystals can be influenced by the application of biomolecules such as polypeptides proteins and also polysaccharides which can cause the formation of ZnO with controllable morphology and unique structural and physicochemical properties under mild conditions (Tomizaki et al., 2012) (Waltz et al., 2012). As far as we know, there are only a few publications on the combination of chitin with ZnO, (Kumar et al., 2013) and none with α-chitin. So, in the first part of this work, chitin was extracted from cuttlebone by modified method, the extracted chitin was then used to prepare Ch/ZnO *via* a hydrothermal method. Since the antimicrobial activity of Ch/ZnO nanocomposite was previously reported, (Wysokowski et al., 2013) the study of the catalytic activity of Ch/ZnO was the main objective of our research. For this purpose, the synthesis of Benzo[a]pyrano(2,3-c)phenazine derivatives which comprise two bio-active heterocyclic cores phenazines (I) and chromenes (II) was pursued (Fig. 2a).

Phenazine (I) which exists in natural and synthetic products, shows a variety of biological functions, including antimalarial (Wang et al., 2011) antibacterial, antiparasitic (Kour et al., 2013) fungicidal, (Saluja et al.,

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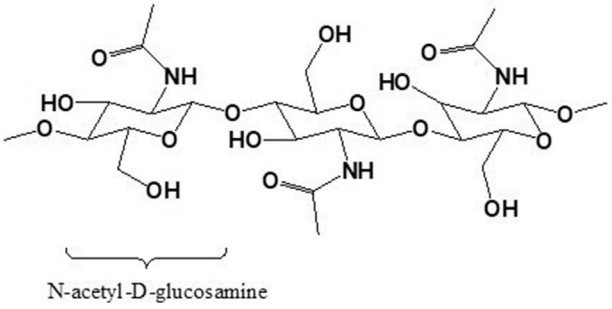



**Fig. 1.** Chemical structure of chitin.

2014) antitumor (Khurana et al., 2012) and antiplatelet (Shaterian and Mohammadnia, 2013) activities. In addition, chromenes (II) as an important class of compounds, widely present in plants, including edible vegetables and fruits (Curini et al., 2006) also exhibit remarkable effects as pharmaceuticals, (Yu et al., 2003) including antifungal (Tangmouo et al., 2006) and antimicrobial activity (Abd-el-aziz et al., 2004). However compounds incorporating both phenazine and chromene motifs which contains four pharmacophore features namely, hydrogen bond acceptor (HBA), hydrogen donor (HBD), hydrophobic (H) and aromatic ring (AR) have rarely been described (Fig. 2b).

Herein we wish to report the modified extraction of chitin from cuttlebone "the sophisticated buoyancy device of cuttlefish", (Checa, Cartwright, Sánchez-almazo and Andrade, 2015) which was characterized as α-chitin revealed by FT-IR, XRD and DSC analysis. Afterward, the nanostructured Ch/ZnO was prepared *via* hydrothermal method to be subsequently used as reusable nano catalyst in the green and efficient synthesis of Benzo[a]pyrano(2,3-c)phenazine derivatives thru a four components dominoreaction of 2-hydroxynaphthalene-1,4-dione, O-phenylenediamine, aldehydes, and malononitrile in the presence of Ch/ZnO as the catalyst under microwave irradiation (Scheme 1).

## 2. Materials and methods

### 2.1. Materials

All reagents were purchased from Merck and Aldrich and used without further purification. Cuttlebone were purchased from grocery at Tajrish (Tehran, Iran). Double distilled water was used throughout the experiment. The Ch/ZnO was prepared through the hydrothermal method according to previously reported procedures (Kumar et al., 2013). All yields refer to isolated products after purification.

The NMR spectra were recorded on a Bruker Avance DPX 500 MHz instrument. The spectra were measured in DMSO-*d6* relative to TMS (0.00 ppm). IR spectra were recorded on a Shimadzu (FT-IR 400s) spectrophotometer. Melting points were determined with an electro thermal 9100 apparatus. Microwave irradiation was carried out with Milestone, Microsynth model. The DSC analyses were conducted with a TA-A1 instrument (Pishtaz Engineering Co.) under a protective nitrogen gas atmosphere. Accurately weighed dry material was placed in an aluminum cup and hermetically sealed. The measurements were carried out from 25 to 350 °C under nitrogen at a scanning rate of 10 °C/min.

### 2.2. Chittin extraction

Several techniques to extract chitin from different sources have been reported. The most common method is referred to as the chemical procedure. The chemical method for isolation of chitin from the waste involves various major steps: extraction of protein matter in alkaline medium (deproteinization) and it is traditionally done by treating the waste with aqueous solutions of NaOH. Elimination of inorganic matter

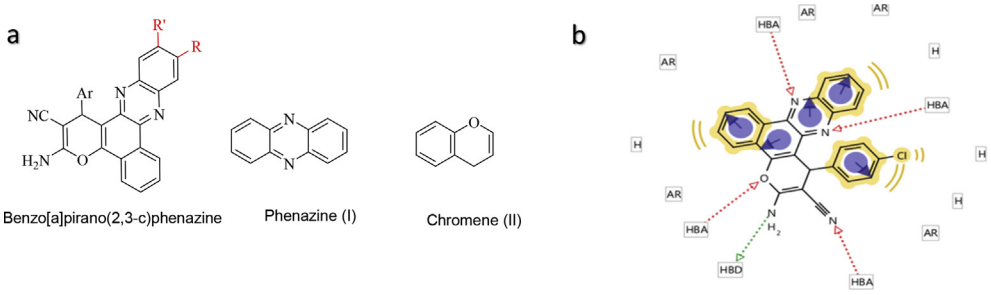
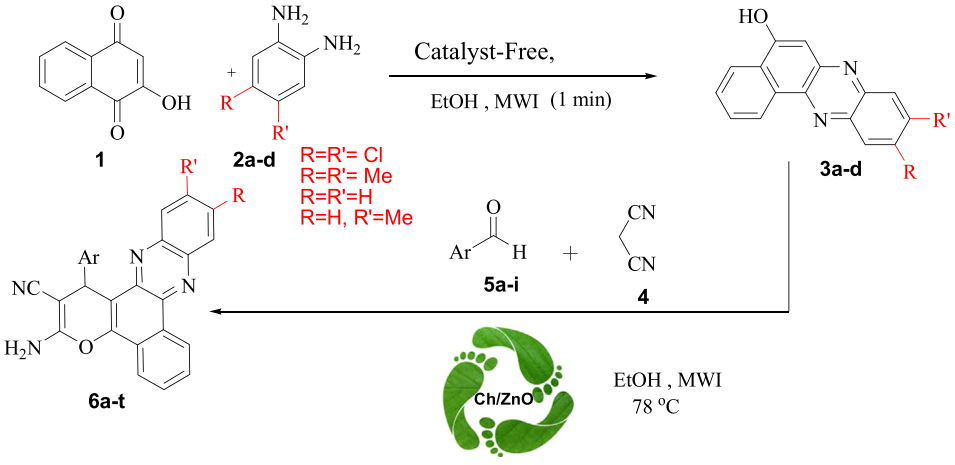



**Fig. 2.** a) Benzo[a]pyrano(2,3-c)phenazine comprising two bio-active heterocyclic cores phenazines (I) and chromenes (II), b) Pharmacophoric features of Benzo[a]pyrano(2,3-c)phenazine generated by LigandScout 3.12 colored as follows: HBD (green), HBA (red), H (mustard) and AR (blue).




**Scheme 1.** Synthesis of Benzo[a]pyrano[2,3-c]phenazine Derivatives.

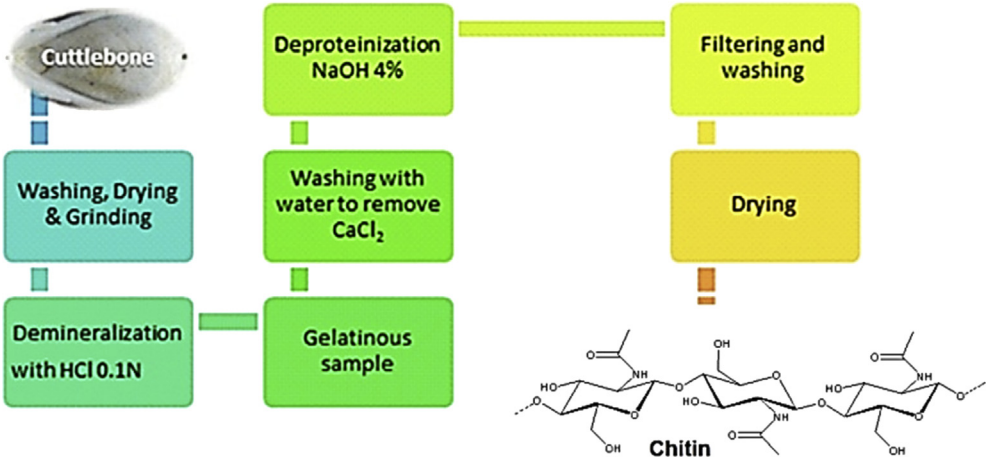



**Fig. 3.** Complete process for extraction of chitin from cuttlebon.

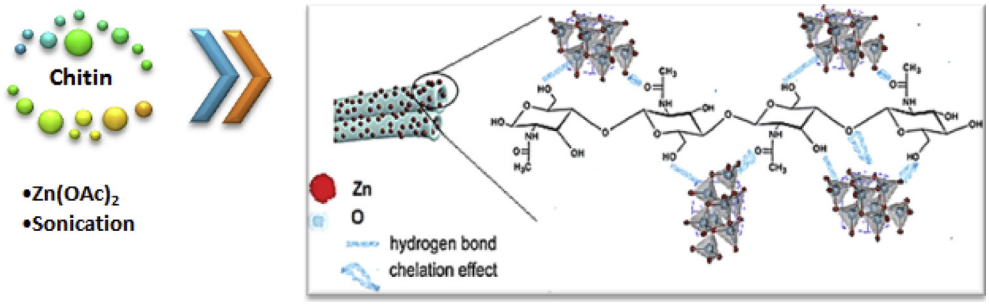



**Fig. 4.** Overall process for preparation of Ch/ZnO.

($CaCO_3$) in dilute acidic medium (demineralization), which is accomplished by using HCl and finally bleaching in dilute NaOCl. The resulting chitin by our extraction method was colorless and no bleaching step with sodium hypochlorite (NaClO) was required (Fig. 3).

### 2.3. Isolation of α-chitin from cuttlebone

At first, the hard shell was separated from cuttlebone. Then some of the split cuttlebone were placed into the beaker. Then a dilute solution of hydrochloric acid (6N) was added slowly to the split cuttlebone. After 48 h the gelatinous sample was isolated and rinsed three times with distilled water. The sample was poured into a beaker, and covered with 4% NaOH for 24 h, after that isolated and washed three times with distilled water up to pH 8. The extracted chitin was dried in an oven at 70 °C.

### 2.4. Preparation of Ch/ZnO

Ch/ZnO nanocomposites were prepared by hydrothermal process. The overall process was shown in Fig. 4. Extracted chitin (0.14g) and 20 mL of aqueous solution of $Zn(CH_3COOH)_2.2H_2O$ (0.0625 M) was transferred in a beaker. The mixture was sonicated for 15 min at room temperature (40W). Afterward, a solution sodium hydroxide (1 M) was added to mixture until the pH reached 13.7 and the sonication was pursued for 15 more minutes at room temperature. The solution was then transferred into an autoclave (250 mL) and placed in an oven at 70 °C for 3 h. The Obtained product was washed with distilled water several time and then dried.

## 3. Result and discussion

### 3.1. Characterization of chitin and Ch/ZnO nanocomposite

The FTIR spectra of extracted chitin was presented in Fig. 5. The high crystallization of the samples leads to the appearance of a series of absorption peaks. The frequency of the carbonyl C=O regions of the amides (between 1600 and 1500 $cm^{-1}$) is of great importance because it makes

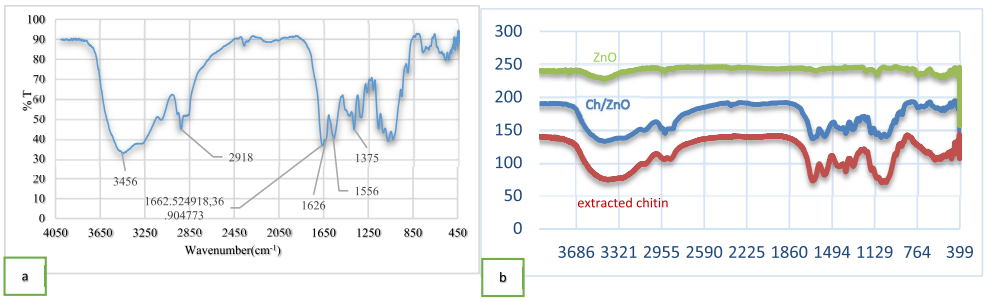



**Fig. 5.** FT-IR spectrum of a) extracted chitin, b) ZnO (green); Ch/ZnO (blue) and extracted chitin (red).

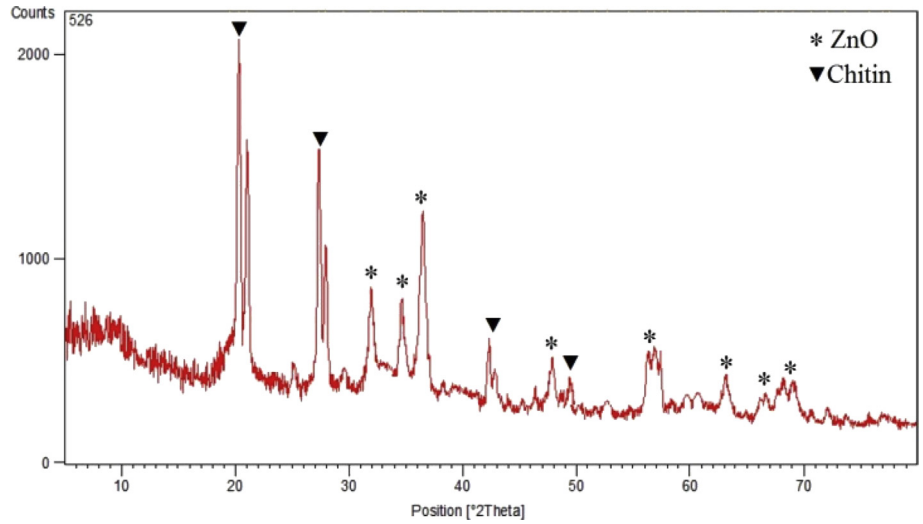



**Fig. 6.** XRD pattern of Ch/ZnO.

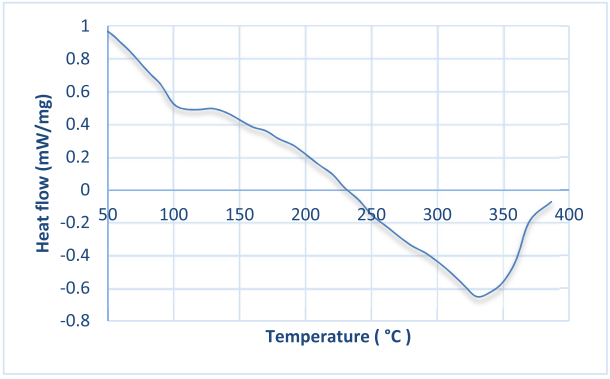



**Fig. 7.** DSC thermogram of extracted chitin.

it possible to distinguish α- and β-chitin. Chitin shows two peaks of amide I and II. The band corresponding to amide I is divided into two peaks at 1656 and 1620 $cm^{-1}$ for α-chitin or in a single peak at 1626 $cm^{-1}$ for β-chitin. The amide II band is unique and is 1556 $cm^{-1}$ for α-chitin and 1560 $cm^{-1}$ for β-chitin. The peak at 1660 $cm^{-1}$ is due to the hydrogen bonds between the C = O groups and the N–H groups of the neighboring chains; the absorption peak at 1621 $cm^{-1}$ can be explained by the presence of a hydrogen bond of the C = O group with the hydroxymethyl group of neighboring chitin residue in the same chain. The presence of two peaks of amide I of α-chitin is discussed in the literature. These results show that, the chitin extracted from cuttlebone is in form of α−chitin. The infrared spectrum also makes it possible to estimate the purity of the sample. The obtained chitin is pure due to the absence of the peak at 1540 $cm^{-1}$ which may indicate the presence of protein impurities.

The XRD pattern of Ch/ZnO is presented in Fig. 6. As can be seen from the figure, all diffraction peaks were consistent with the values of the standard (JCPDS Card no. 36–1451), and, the nanowires showed a pure hexagonal ZnO structure with high crystallinity. The peaks at $2\theta \approx 31.64^{o}$, $34.46^{o}$ and $36.43^{o}$ 47.90°, 56.52°, 63.25°, 66.31°, 68.21°, and

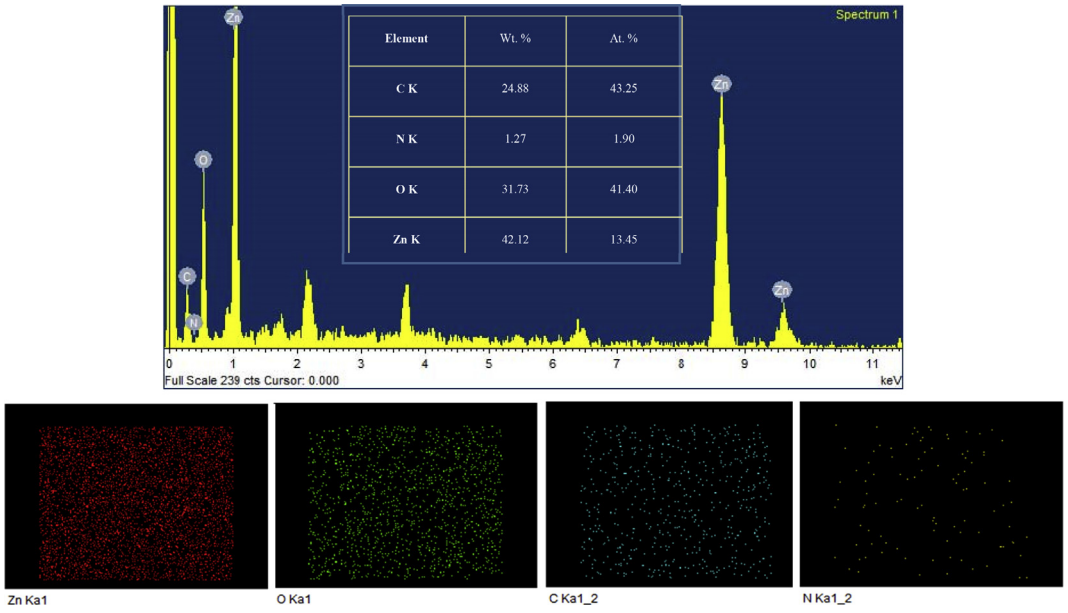



**Fig.8.** EDX pattern of Ch/ZnO, elemental compositions and color mapping of Ch/ZnO.

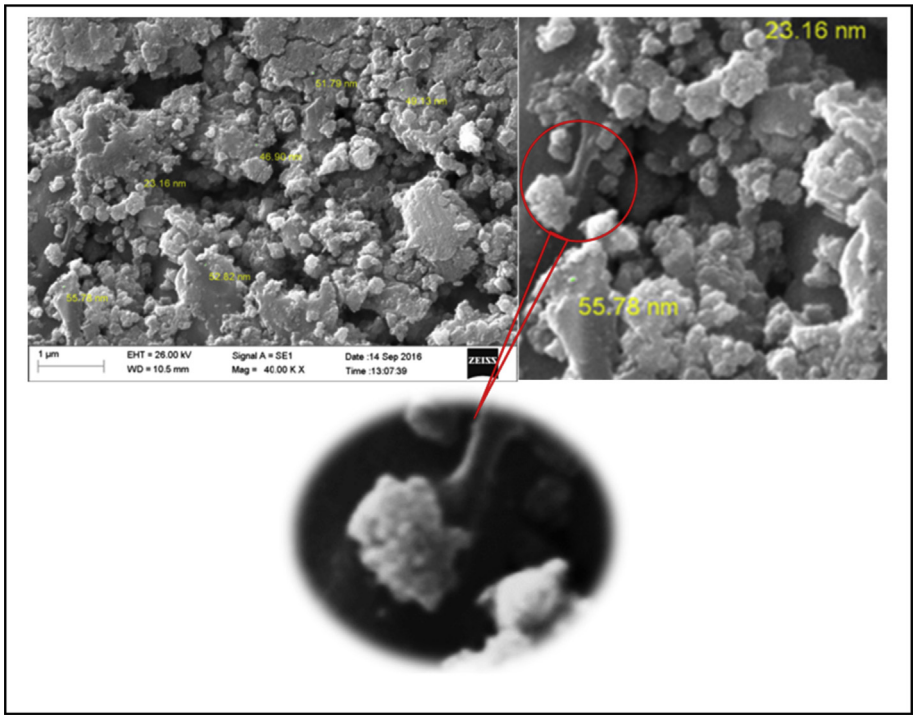



**Fig. 9.** SEM image of Ch/ZnO.

69.03° were attributed to (100), (002), (101), (102), (110), (103), (200), (112), and (201) of ZnO planes, respectively, indicating that the samples were polycrystalline wurtzite structure. The sharp crystalline reflections at 2θ around 20–21° and 27–28° and minor reflections at higher 2θ can be observed and attribute to α-chitin.

The Differential Scanning Calorimetry (DSC) analysis was applied to extracted chitin and the thermal degradation values are depicted in Fig. 7. The DSC curves of extracted chitin, shows that chitin has a high exothermic peak at 330 °C, suggested that the crystalline structure is very rigid and confirm the form α-Chitin because of intersheet and intrasheet hydrogen bonding.

The EDX pattern shown in Fig. 8 also confirmed the presence of ZnO on the cellulosic surface of PS by displaying the peaks for carbon, nitrogen, oxygen, and zinc.

The morphology of Ch/ZnO was investigated by using SEM and the images are presented in Fig. 9. The ZnO NPs consist of clusters of small grains on cellulose stem.

### 3.2. Determination of the degree of acetylation (DA) and degree substitution (DS)

#### 3.2.1. Determination of the DA and DS by titration

Chitin is mainly characterized by its degree of acetylation (DA), that is to say the percentage of acetylated units. These parameters modulate, for example, the charge density of the polymer, and therefore its solubility and its biofunctionalities. The degree of acetylation of extracted chitin was measured by acid-base titration method (Diop et al., 2011) consisting of complete basic hydrolysis of the ester linkages and titration of the excess alkali. In this method, 0.11 g of extracted chitin was added to 5.5 mL of 75% ethanol solution (v/v). The dispersion was stirred for 30 min at 50 °C. After being cooled to room temperature, 4.4 mL of a solution of 0.5N KOH was added to the solution and stirred for 72 h to achieve saponification at room temperature. The excess of alkali in the solution was titrated with 0.5N HCl using phenolphthalein as an indicator. Degree of acetylation (DA) was calculated according to the following Eq. (1)

$$\%\ \mathrm{DA}_{\ \mathrm{Total}} = \frac{(V_0 - V_s)\ \times N\ HCl\ \times 0.043\ \times 100}{M_s} \tag{1}$$

Here $V_O$ is the mL of 0.5N HCl used to titrate blank; $V_s$ is the mL of 0.5N HCl used to titrate sample; N is the normality of used HCl; $M_s$ is the sample amount as dry substance (g); 43 is the formula mass of the acetyl group. Degree of substitution (DS) was calculated according to the following Eq. (2):

$$\mathrm{DS} = \frac{MW\ chitin\ \times \%DA\ (Total)}{(mass\ of\ acetyl\ group \times 100) - (mass\ of\ acetyl\ group - mass\ of\ hydrogen) \times \%DA\ (Total)} \tag{2}$$

$$\%\ \mathrm{DA}_{\ \mathrm{Total}} = \frac{4.2 \times 0.5\ \times 0.043\ \times 100}{0.11}$$

$$\%\ \mathrm{DA}_{\ \mathrm{Total}}\ =\ 82.09$$

$$\mathrm{DS} = \frac{203.19\ \times \%DA\ (Total)}{(43 \times 100) - (43 - 1) \times \%DA\ (Total)}$$

where 203.19 is the molecular weight of chitin monomer unit, 43 is the formula mass of the acetyl group, and 1 is the atomic mass of hydrogen.

$$\mathrm{DS} = \frac{203.19\ \times 82.09}{(43 \times 100) - (42) \times 82.09}\ \ \mathrm{DS}\ =\ 19.57$$

**Table 1**
Determination of the DA using infrared spectroscopy based on the absorption band ratios.

| $A_M/A_R$ | % DA |
|---|---|
| $A_{1655}/A_{3450}$ | 80 |
| $A_{1554}/A_{3450}$ | 82 |

**Table 2**
Optimization of catalyst and catalyst loading for preparation 3-amino-2-cyano-1-(4-chlorophenyl)-1H-benzo[a]pyrano[2,3-c]phenazine.

| Entry | Catalyst | Loading(mg) | Time(min) | Yield (%) |
|---|---|---|---|---|
| 1 | - | - | 7 | Trace |
| 2 | Ch/ZnO | 1 | 7 | 22 |
| 3 | Ch/ZnO | 5 | 7 | 39 |
| 4 | Ch/ZnO | 10 | 5 | 45 |
| 5 | Ch/ZnO | 15 | 5 | 76 |
| 6 | Ch/ZnO | 20 | 5 | 93 |
| 7 | Ch/ZnO | 25 | 5 | 93 |
| 8 | Chitin | 20 | 5 | Trace |
| 9 | ZnO | 20 | 5 | 71 |

### 3.2.2. Determination of the DA using infrared spectroscopy

FTIR spectroscopy is also used to determine the degree of acetylation of chitin and chitosan. The principle is based on the absorption band ratios (AM/AR), where AM is the intensity of the characteristic band of N-acetylation, which is a measure of the N-acetyl or amine content, and AR is the intensity of a reference band that does not change with different DA values. (Kasaai, 2010) According to the studies described in literature (Moore and Roberts, 1980) (Domszy and Roberts, 1985) (Baxter et al., 1992) (Roberts, 1992), several specific signal ratios are proposed for determining DA for chitin and chitosan. For the extracted chitin, the DA reached nearly 80–82 % with the absorption band ratios A1655/A3450 and A1554/A3450 (Table 1).

### 3.3. General procedure for the synthesis of functionalized benzo[a]pyrano[2,3-c]phenazine derivatives under microwave conditions

A mixture of O-phenylenediamine (1 mmol, 0.108 g) and 2-hydroxynaphthalene-1,4-dione (1 mmol, 0.147 g) was placed in a round-bottom flask and subjected to microwave irradiation for 1 min. Then, a mixture of 4-chlorobenzaldehyde (1 mmol, 0.140g), malononitrile (1 mmol, 0.066g) and Ch/ZnO (20mg) in EtOH (2 mL) was added to the flask and

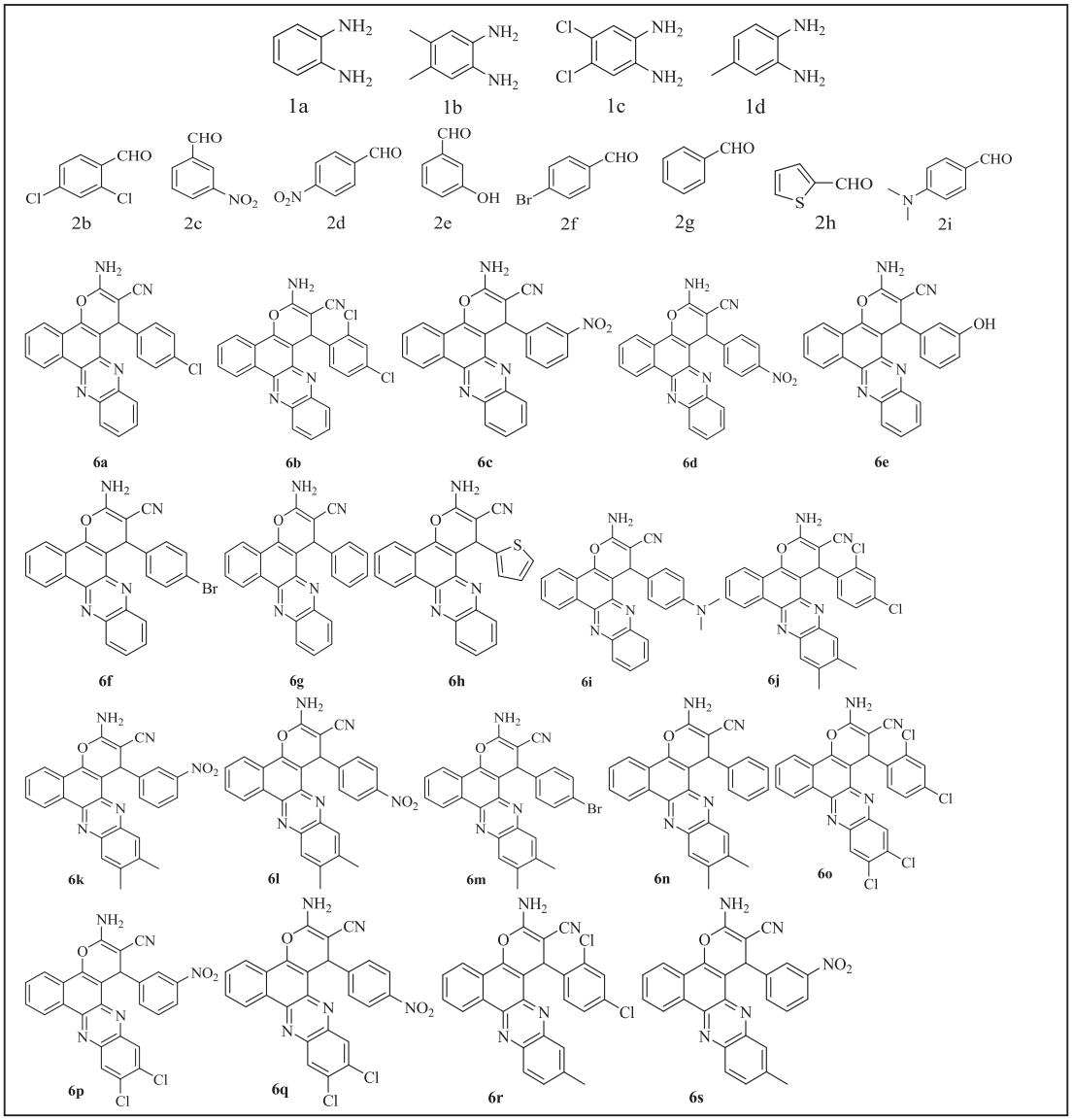



**Fig. 10.** Aromatic diamines (**1a-d**), aromatic aldehydes (**2b-g**) and products (**6a-s**).

was heated for 4 min at 78 °C under microwave irradiation. Completion of the reaction was monitored by TLC. Upon completion, the reaction mixture was dissolved in dimethyl sulfoxide (DMSO) and the catalyst was separated by filtration. The solvent was removedusing a rotary evaporator, the product was recrystallized to obtain the pure yellow solid.

### 3.4. Selected spectral data for new compounds

**(6r):** 3-Amino-1-(2,4-dichloro-phenyl)-11-methyl-1H-benzo[a]pyrano[2,3-c]phenazine-2- carbonitrile.

Yellow solid, m.p.: >300 °C; IR (KBr, ν, cm -1): 3485, 3342, 3174, 2182, 1659, 1625, 1594, 1497, 1471, 1410, 1392, 1343, 1322, 1295, 1267, 1207, 1166, 1099, 1062, 1023, 876, 763.

1H NMR (400 MHz, DMSO-d6) (δ, ppm): 9.06 (dd, 1H, J1 = 1.2 Hz, J2 = 7.6 Hz, Ar–H), 8.36 (dd, 1H, J1 = 0.8 Hz, J2 = 8.8 Hz, Ar–H), 7.99–7.89 (m, 2H, Ar–H), 7.81 (s, 1H, Ar–H), 7.55 (d, 1H, J = 2.0 Hz, Ar–H), 7.50 (s, 1H, Ar–H), 7.40 (s, 2H, NH2), 7.13–7.07 (m, 2H, Ar–H), 5.75 (s, 1H, CH), 2.44 (s, 3H, CH3)

**(6s):** 3-Amino-11-methyl-1-(3-nitro-phenyl)-1H-benzo[a]pyrano [2,3-c]phenazine-2-carbo nitrile.

Yellow solid, m.p.: >300 °C; IR (KBr, ν, $cm^{-1}$): 3481, 3336, 3210, 2199, 1712, 1658, 1614, 1593, 1533, 1473, 1392, 1345, 1293, 1265, 1207, 1163, 1105, 1055, 875, 766, 727.

$^1$H NMR (400 MHz, DMSO-*d6*) (δ, ppm): 9.04–9.05 (m, 1H, Ar–H), 8.34–8.32 (d, 1H, *J* = 6.3 Hz, Ar–H), 7.78–7.95 (m, 3H, Ar–H), 7.78 (s, 1H, Ar–H), 7.50–7.66 (m, 3H, Ar–H), 7.34 (s, 1H, Ar–H), 7.08 (s, 2H, $NH_2$), 5.66 (s, 1H, CH), 2.54 (s, 3H, $CH_3$).

### 3.5. Synthesis of benzo[a]pyrano(2,3-c)phenazine derivatives

At the onset of this methodology, several test reactions were carried out and the effects of solvent, temperature, catalyst, and catalyst loading were and optimized for four-component reaction of 2-hydroxynaphthalene-1,4-dione (1) with O-phenylenediamine (2) 4-chlorobenzaldehyde (4a) and malononitrile (5) as a model reaction for the synthesis of benzo[a]pyrano(2,3-c)phenazine in the presence of heterogeneous Ch/ZnO bio nanocatalyst (Scheme 1). The results revealed that the first step of reaction i.e. the Knoevenagel condensation occurs in the absence of any catalyst under MW irradiation in 1 min. The presence of catalyst is crucial in the second step (Table 2, entry 1) and the best amount of catalyst is proven to be20 mg (Table 1, entry 6). The results also shown the remarkable synergistic effect of Chitin and ZnO (Table 2, entry 8, 9).

In order to examine the scope and generality of this procedure, a series of benzo[a]pyrano(2,3-c)phenazine derivatives (see Fig. 10) were synthesized under the optimized conditions (Table 3) using different aromatic diamines and aromatic aldehydes (see Fig. 10).

The mechanism shown in Fig. 11 can be proposed for the synthesis of Benzo[a]pyrano(2,3-c)phenazine derivatives. 6*H*-benzo[a]phenazin-5-one (**3**) in tautomeric equilibrium with benzo[a]phenazin-5-ol (**3′**) was obtained after the nucleophilic attack of o-phenylenediamine (**2a**) to 2-hydroxynaphthalene-1,4-dione (**1**) followed by dehydration. Subsequent Michael addition of 6*H*-benzo[a]phenazine-5-ol (**3'**) to benzylidenemalononitrile (**i**), produced *via* Knoevenagel condensation of aryl aldehydes (**4**) with malononitrile (**5**) catalyzed by Ch/ZnO, provided the desired products (**6**) after cyclization of intermediate (**ii**).

**Table 3**
Synthesis of benzo[a]pyrano[2,3-c]- phenazine derivatives under Microwave Irradiation.

| Entry | Diamines | Aldehyde | Products | Time (min) | Yield (%) | M.p. (°C) |
|---|---|---|---|---|---|---|
| 1 | 1,2- Phenylenediamine**(1a)** | 2,4-Dichlorobenzaldehyde**(2b)** | **6a** | 5 | 93 | 291–292 |
| 2 | 1,2- Phenylenediamine**(1a)** | 2,4-Dichlorobenzaldehyde**(2b)** | **6b** | 4 | 95 | >300 |
| 3 | 1,2- Phenylenediamine**(1a)** | 3-Nitrobenzaldehyde**(2c)** | **6c** | 4 | 91 | 280–282 |
| 4 | 1,2- Phenylenediamine**(1a)** | 4-Nitrobenzaldehyde**(2d)** | **6d** | 6 | 89 | 284–285 |
| 5 | 1,2- Phenylenediamine**(1a)** | 3-Hydroxybenzaldehyde**(2e)** | **6e** | 6 | 84 | 289–290 |
| 6 | 1,2- Phenylenediamine**(1a)** | 4-Bromobenzaldehyde**(2f)** | **6f** | 5 | 92 | 282–285 |
| 7 | 1,2- Phenylenediamine**(1a)** | Benzaldehyde**(2g)** | **6g** | 5 | 90 | 299–301 |
| 8 | 1,2- Phenylenediamine**(1a)** | 2-Thiophenecarboxaldehyde**(2h)** | **6h** | 5 | 86 | 259–261 |
| 9 | 1,2-Phenylenediamine**(1a)** | 4-(dimethylamino)benzaldehyde**(2i)** | **6i** | 5 | 89 | 260–262 |
| 10 | 4,5-Dimethyl-1,2-phenylenediamine**(1b)** | 2,4-Dichlorobenzaldehyde**(2b)** | **6j** | 6 | 82 | >300 |
| 11 | 4,5-Dimethyl-1,2-phenylenediamine**(1b)** | 3-Nitrobenzaldehyde**(2c)** | **6k** | 6 | 82 | 277–279 |
| 12 | 4,5-Dimethyl-1,2-phenylenediamine**(1b)** | 4-Nitrobenzaldehyde**(2d)** | **6l** | 6 | 83 | 298–301 |
| 13 | 4,5-Dimethyl-1,2-phenylenediamine**(1b)** | 4-Bromobenzaldehyde**(2f)** | **6m** | 6 | 87 | 295–297 |
| 14 | 4,5-Dimethyl-1,2-phenylenediamine**(1b)** | Benzaldehyde**(2g)** | **6n** | 7 | 84 | >300 |
| 15 | 4,5-Dichloro-1,2-phenylenediamine**(1c)** | 2,4-Dichlorobenzaldehyde**(2b)** | **6o** | 5 | 80 | >300 |
| 16 | 4,5-Dichloro-1,2-phenylenediamine**(1c)** | 3-Nitrobenzaldehyde**(2c)** | **6p** | 5 | 81 | 280–281 |
| 17 | 4,5-Dichloro-1,2-phenylenediamine**(1c)** | 4-Nitrobenzaldehyde**(2d)** | **6q** | 5 | 83 | >300 |
| 18* | 4-Methyl-1,2-phenylenediamine**(1d)** | 2,4-Dichlorobenzaldehyde**(2b)** | **6r** | 5 | 91 | >300 |
| 19* | 4-Methyl-1,2-phenylenediamine**(1d)** | 3-Nitrobenzaldehyde**(2c)** | **6s** | 4 | 86 | >300 |

* New derivatives.

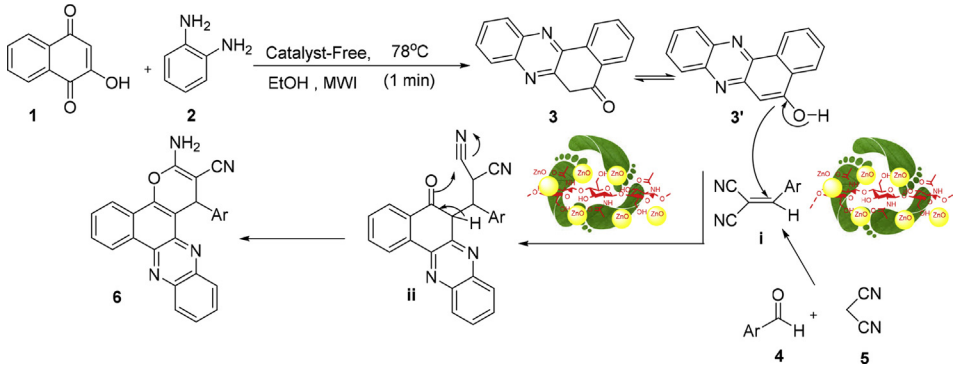



**Fig. 11.** Shown is a proposed mechanism for the synthesis of Benzo[a]pyrano(2,3-c)phenazine derivatives.

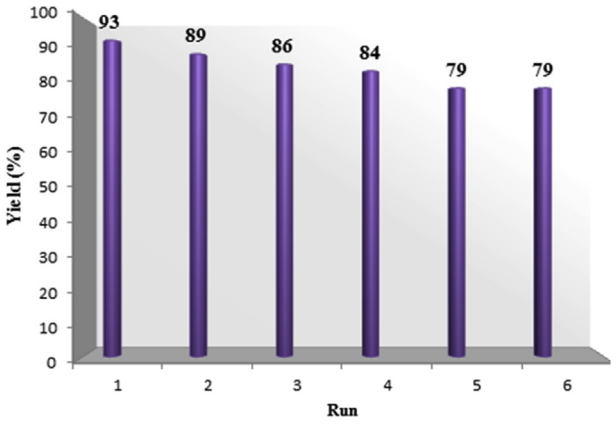



**Fig. 12.** The recyclability of Ch/ZnO.

### 3.6. Recycling performance

One of the advantages of heterogeneous catalysts is the possibility of easy separation from the reaction mixture and reusability in catalytic system. In this regard, recyclability of Ch/ZnO was studied in model reaction. After completion of the reaction, the catalyst was recovered by an external magnet and washed several times with EtOH and EtOAc, respectively and dried at 50 °C. The recycled catalyst was reused for next run. Although some decrease in catalytic activity was observed after six runs (Fig. 12) this can be mostly accredited to inaccuracies during filtration and collecting the catalyst that may cause a reduction in the initial amount of used catalyst.

### 3.7. Photophysical studies

The optical properties of the new phenazine derivatives **6m, 6s** and **6r** were measured by fluorescence spectroscopy in DMSO at room temperature (Fig. 13). The fluorescence studies were conducted at an excitation wavelength of 350 nm. The results shown that all new derivatives are fluorescent in solution. The **6s** and **6r** compounds show similar fluorescence spectra in the visible region from 457-485 nm, and the maximum emission was observed at 471 nm. The electronic effects of the electron-attracting nitro substituent group on the aromatic aldehyde have a great influence on the fluorescence property of compound 6m, and the fluorescence emission of phenazine framework was red-shifted and weakened, (Khurana et al., 2012) compared to 6s and 6r which their intensity are 8 times stronger than **6**m.

### 3.8. Comparisons with other reports

In order to evaluate the ability and efficiency of this method and the prepared catalyst, a comparison has been made with the previous reported and published procedures for the synthesis of Benzo[a] pyrano(2,3-c)phenazine derivatives. As the data from Table 4 show, this method, compared to the other methods, has advantages such as short time, high yield, the use of a safer solvent and a mild catalyst, with an easy method of preparation and in accordance with the principles of green chemistry.

This comparison was made on 2-hydroxynaphthalene-1,4-dione with O-phenylenediamine, 4-chlorobenzaldehyde, and malononitrile as the model reaction.

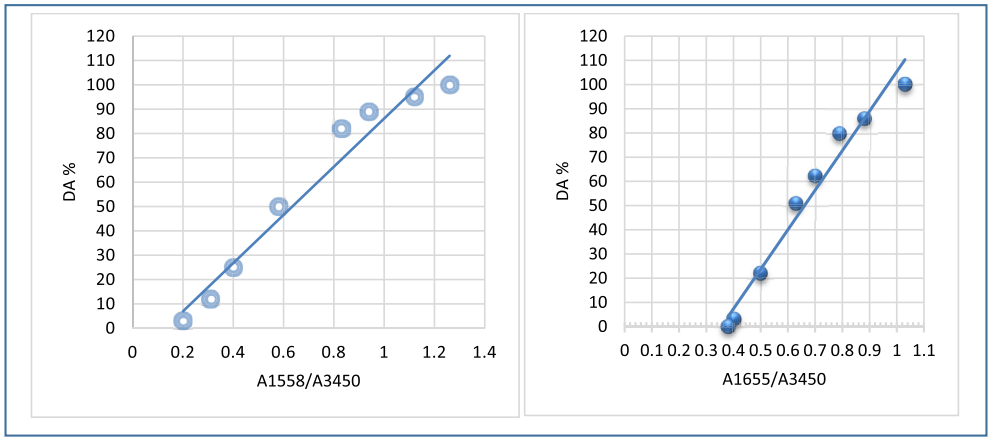



**Fig. 13.** Emission spectra of compounds 6m, 6r and 6s in DMSO, 20 °C.

**Table 4**
The comparison of Ch/ZnO nanocomposite as catalyst for the synthesis benzo[a]pyrano(2,3-c)phenazine derivatives with other catalysis.

| Entry | Cat. & loading | Solvent | Condition | Time (min) | Yield (%) | Reference |
|---|---|---|---|---|---|---|
| 1 | Acetic acid (5% mol) | $H_2O$ | MW | 15 | 89 | (Wang et al., 2011) |
| 2 | PEG 400 (10% mol) | EtOH | Reflux | 120 | 92 | (Khurana et al., 2012) |
| 3 | Ionic Liquid (5% mol) | $H_2O$ | Reflux | 10 | 96 | (Shaterian and Mohammadnia, 2013) |
| 4 | **Ch/ZnO (20 Mg)** | **EtOH** | **MW** | **5** | **93** | **This work** |
| 5 | [Co (TPPABr)]CBr (20 Mg) | EtOH | Reflux | 45 | 91 | (Dashteh et al., 2019) |
| 6 | $Fe_3O_4$@$SiO_2$-TCT-Theophylline (1.1% mol) | EtOH | Reflux | 20 | 95 | (Esmaeilpour et al., 2018) |
| 7 | Caffeine (20%mol) | - | MW | 10 | 90 | (Chaudhary and Khurana, 2018) |

Bold signifies the present work.

## 4. Conclusion

In this study chitin was extracted via an optimized method from cuttlebone of the Persian Gulf cuttlefish and characterized. The results from FT-IR, XRD and TGA showed that the extracted chitin was in alpha form. The DA and DS were determined by using titration method and FTIR spectroscopy and found to be 80–82% and 19.57 respectively. These properties are valuable for biomedical applications.

The thermo-stable α-chitin was used as a low cost natural template for the preparation of nanostructured Ch/ZnO *via* a hydrothermal method. The catalytic performance of Ch/ZnO was investigated for the first time in the synthesis of 3-amino-12-methyl-1-*H*-benzo[a]pyrano [2,3-c] phenazine-2-carbonitrile under microwave condition. The environmental sustainability, recyclability, alteration of waste to value-added catalyst, easy work up and separation of the nanostructured catalyst are some of the highlighted features this protocol.

## Declarations

### *Author contribution statement*

Shahrzad Javanshir, Shiva Molaei, Sarvenaz Abolghasem: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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### *Competing interest statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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