RESEARCH PAPER

Cu/ZnO@GO Nanocomposite: A Recyclable and Benign Catalyst in Aqua-Mediated Synthesis of Spiro-Fused Oxindole-Quinazolinone Derivatives and Evaluation of Antioxidant Activity

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ABSTRACT

In this study, a Cu/ZnO@GO nanocomposite was applied as a novel heterogeneous green catalyst for the synthesis of a series of spiro-fused oxindole-quinazolinones by a three-component reaction of isatins, urea, and cyclohexanones. Numerous factors, such as timing and temperature, different solvents, and catalysts, have been examined in order to achieve the optimal processing conditions. An aqueous extract from the Petasites hybridus rhizome was used to directly create the mesoporous Cu/ZnO@GO nanocomposite utilizing a green method without a template. The Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive X-ray spectrometry (EDS) have been utilized to the characterization of the produced nanocatalyst. In comparison to conventional catalysts, the aforementioned catalyst promotes the conversion of the starting material to the target product with a good yield, lower process energy, high efficiency, and recyclability, according to the obtained results. This study represents a significant advancement in the creation of high-efficiency green catalysts in this field and offers an argument for extending the role of nanocatalyst-based approaches for the sustainable synthesis of spirooxindole derivatives. Additionally, it makes use of an effective combination of Cu/ZnO@GO nanocomposite as a useful heterogeneous catalyst and water as a green solvent. The catalyst can be recovered and reused for at least four runs without noticeable influence on the product yields. The antioxidant properties of several of the generated compounds were also examined. The test compounds indicate potential antioxidant properties.

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INTRODUCTION

The spirooxindole moiety is probably one of the most well-known heterocycles due to its extensive application in medical fields. Many commercially available drug molecules contain spirooxindole as the main structural unit [1, 2]. Compounds containing the spirooxindole exhibit a broad spectrum of biological activities, including antioxidant [3], antimicrobial [4], antiviral [5], anticancer [6, 7], and antimalarial effects [8]. Fig. 1 shows some of the spirooxindole-bearing compounds known for their biological significance [9].

A literature screening reveals that the use of nanostructured materials as prospective heterogeneous catalysts is one of the key and desired ways for chemists to drive sustainable and environmentally friendly chemical processes [10]. Graphite material-derived nanostructures are intriguing materials with numerous technological applications in electrochemistry, photocatalysis, and water purification [11-13]. Graphene has been found to be an excellent catalyst carrier because of its high specific surface area pore volume, well-organized layered structure, superior chemical stability, and cost-effectiveness [14].

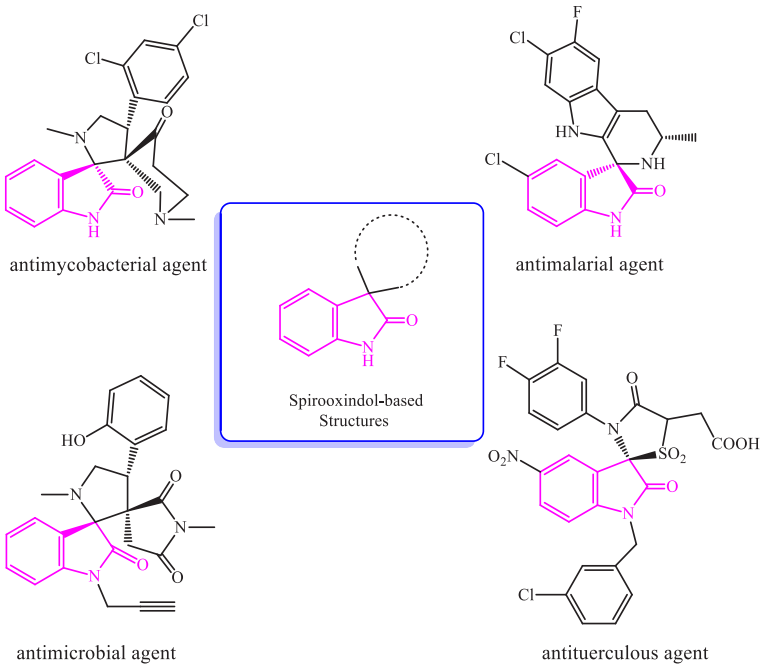


Fig. 1. Exemplary instances of bioactive compounds bearing the spirooxindole structures.

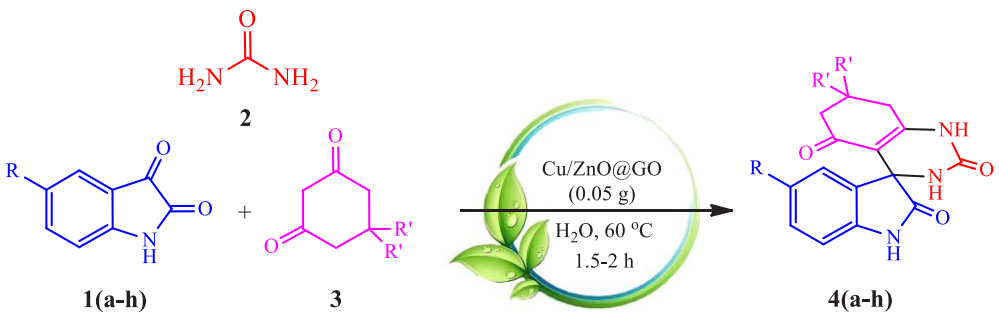


Fig. 2. Cyclocondensation reaction of isatins, urea, and cyclohexanones using Cu/ZnO@GO nanocomposite in water.

The key paradigm of green chemistry is accomplished by decreasing waste, as well as the utilization of renewable raw materials, green solvents, and efficiently recoverable catalysts [15]. In organic reactions, water provides the most favorable alternatives for process efficiency when utilized in heterogeneous catalysis [16].

Typically, the antioxidant compounds can principally neutralize free radicals and thus retard or avert cell harm. These substances have been used to combat oxidative stress, which is a factor in several illnesses, such as cancer, Alzheimer's disease, atherosclerosis, and chronic obstructive pulmonary disease. Recently, there has been a growing interest in developing effective synthesis of antioxidant compounds due to their many benefits, such as anti-aging and anti-inflammatory properties [17].

Considering our expertise with developing nanomaterial catalysis in heterocyclic chemistry [18-20], we herein report the development of a new superior method for the synthesis of a series of spiro[3,4']1,3-dihydro-2*H*-indol-2-one-4',6',7',8'-tetrahydro-2',5'(1'*H*,3'*H*)-quinazoline-diones (4). That was achieved by a three-component reaction of isatins (1), urea (2), and cyclohexanones (3) using Cu/ZnO@GO nanocomposite as a catalyst at room temperature in water (Fig. 2). This study aims to explore a new, green, and mild method for synthesizing target compounds and investigate their application as a new class of antioxidant candida, despite our prior publication on a practical technique under solvent-free conditions [21].

MATERIALS AND METHODS

Chemical reagents and solvents were purchased from Merck and Fluka Companies and utilized without additional purification. IR spectra were recorded on an ABB FT-IR (FTLA 2000) spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 AVANCE at 500 and 125 MHz, respectively, in DMSO-d_6 as the solvent and TMS as the internal standard. Elemental analyses were performed on a Foss-Heraeus *CHN*-O-rapid analyzer. X-ray diffraction (XRD) was recorded on a Bruker AXS instrument employing CuKα X-ray radiation. Scanning electron microscopy (SEM) was captured with a TESCAN VEGA3 coupled to an energy-dispersive X-ray (EDS) detection system.

General procedure for the synthesis of Cu/ZnO@GO Nanocomposite

In this work, the nanocatalyst was prepared using water extracted from the *Petasites hybridus rhizome* as green media, which has a stabilizing and reducing agent role in the synthesis of nanocatalysts in high yield. To achieve the *Petasites hybridus rhizome* medium, 10 g of dried and powdered plant is stirred in 100 ˚C of deionized water for 1 hour. After this time, the mixture is brought to room temperature and filtered. The extract is stored in the refrigerator to prevent mold [22]. For solution preparation, a mixture of zinc acetate (1.5 g, $Zn(OAc)_2$) and $CuCl_2$ (1.5 g) was added to 5 mL of water-based extract derived from the rhizome of *Petasites hybridus*. After heating the mixture to 200 ˚C in a round-bottom flask, the solution was agitated for 1 hour. After the process is fully completed, it is imperative to cool the solution until it reaches room temperature. Upon attaining the requisite temperature, the mixture underwent spinning at 7000 rpm for about 10 min to eliminate residual components. Following the separation of the Cu/ZnO nanoparticles, they were rinsed with a mixture of a 50% ethanol/water solution and subsequently air-dried for 24 h at ambient temperature. Graphene oxide (0.1 g, GO) along with 0.1 g of Cu/ZnO nanoparticles was thoroughly dispersed in a 10 mL portion of water-based extract obtained from *Petasites hybridus* rhizomes, and it was stirred at 200 ˚C over a period of 60 min. Subsequently, the resultant Cu/ZnO@GO was passed through a filtration process and extensively rinsed using a 1:1 mixture of ethanol and water solution to obtain the Cu/ZnO@GO nanocomposite with a high yield [23].

General procedure for the preparation of compounds 4a-h

A mixture of isatins 1 (1 mmol), 1,3-cyclohexanediones 2 (1 mmol), urea (3, 1 mmol), and Cu/ZnO@GO (0.05 g) in H_2O (10 mL) was stirred at 60 ˚C temperature for an appropriate time (Table 2). The reaction's advancement was observed *via* TLC employing a 3:2 ethyl acetate/n-hexane mixture as the eluent. Upon completion of the reaction, the reaction mixture was filtered, and the solid obtained was dissolved in hot ethanol (3 mL). The catalyst was removed for reusing by centrifuging and washing with ethanol and then oven-drying for several hours. The pure white

title product was obtained by cooling the ethanol solution to room temperature, diluting it with 1 mL H_2O and allowing it to crystallize.

Spiro[3,4′]1,3-dihydro-2H-indol-2-one-4′,6′,7′,8′-tetrahydro-2′,5′(1′H,3′H)-quinazoline-dione (4a)

White powder; yield: 0.255 g (90%), m.p. 342-344 ˚C (lit. >300 ˚C [18]). IR (KBr): ν_{max} = 3379, 3348, 3201, 1709, 1645, 1629, 1525 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.20 (2 H, m, CH_2), 3.03 (2 H, m, CH_2), 3.49 (2 H, m, CH_2), 6.95–7.53 (4 H, m, HAr), 7.68 (1 H, s, NH), 8.21 (1 H, s, NH), 9.03 (1 H, s, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 21.9, 27.1, 36.8, 82.5, 106.1, 114.3, 116.7, 120.4, 121.5, 123.2, 139.0, 157.7, 159.1, 165.3, 190.6 ppm. Analysis calcd for $C_{15}H_{13}N_3O_3$ (283.29): C 63.60, H 4.62, N 14.83; found C 63.78, H 4.85, N 14.96%.

Spiro[3,4′]1,3-dihydro-5-methoxy-2H-indol-2-one-7′,7′-dimethyl-4′,6′,7′,8′-tetrahydro-2′,5′(1′H,3′H)-quinazoline-dione (4g)

White powder; yield: 0.314 g (92%), m.p. 235-237 ˚C (lit. >300 ˚C [18]). IR (KBr): ν_{max} = 3380, 3290, 3152, 2898, 1723, 1665, 1627, 1529 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.93 (3 H, s, CH_3), 1.05 (3 H, s, CH_3), 2.11 (2 H, m, CH_2), 2.39 (2 H, m, CH_2), 3.75 (3 H, s, OCH_3), 7.09-7.22 (3 H, m, HAr), 7.66 (1 H, s, NH), 9.37 (1 H, s, NH), 10.11 (1 H, s, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 28.5, 30.4, 37.5, 50.1, 56.7, 78.5, 111.7, 114.6, 116.8, 117.4, 123.9, 148.3, 152.0, 158.5, 160.1, 170.1, 192.4 ppm. Analysis calcd for $C_{18}H_{19}N_3O_4$ (341.36): C 63.33, H 5.61, N 12.31; found C 63.14, H 5.48, N 12.13%.

Study of antioxidant activity utilizing DPPH radical trapping test

The antioxidant activity of the produced compounds was tested by measuring the scavenging capacity of free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) for each molecule, applying previously reported techniques [24, 25]. To do this, we added different concentrations (200–1000 ppm) of specific products in dimethyl sulfoxide (DMSO) to the control solution, which comprised two milliliters of DPPH and two milliliters of methanol. The mixture was immediately mixed for 1 min and then kept in the dark at ambient temperature for 30 min. At 517 nm, the residual DPPH absorbance was then measured. The experiment included a few well-known radical scavengers as positive controls, including butylated hydroxytoluene (BHT) and 2-tert-butylhydroquinone (TBHQ). The antioxidant activity (AA) as DPPH percentage inhibition owing to the investigated substances was estimated using an equation given by Yen et al. [26].

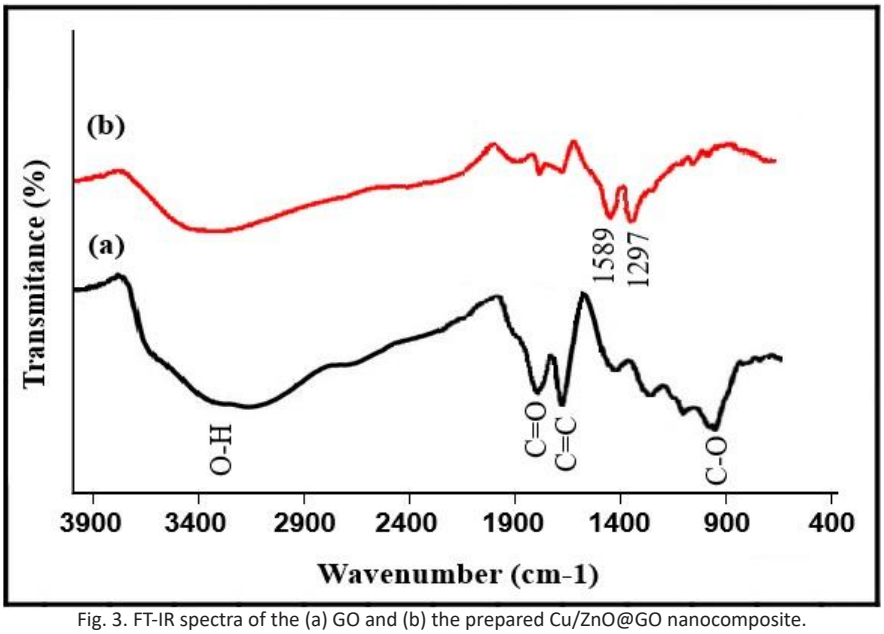


Fig. 3. FT-IR spectra of the (a) GO and (b) the prepared Cu/ZnO@GO nanocomposite.

RESULTS AND DISCUSSION

The main aim of this research, as shown in Fig. 2, is to develop the catalytic applications of the Cu/ZnO@GO nanocomposite for the environmentally friendly synthesis of a series of spiro-fused oxindole-quinazolinones. In this regard, the Cu/ZnO@GO nanocomposite was first prepared using a green approach without a template. The structural correctness of the synthesized nanocomposite was then assessed using several techniques, including Fourier transformed infrared absorption spectroscopy (FT-IR), X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), and energy dispersive X-ray spectrometry (EDS). The FT-IR spectra of the separated pure objects, GO and Cu/ZnO@GO nanostructures, are illustrated in Fig. 3. Distinct peaks at 3330 cm^{-1} and 3337 cm^{-1} are attributed to the stretching vibrations of OH bonds in water molecules integrated inside the produced particles. The characteristic absorption peaks of graphene at 1745, 1650, and 1027 cm^{-1} are related to the stretching bonds of C=O, C=C, and the deformation bond of C-O, respectively. In the FT-IR spectra of the Cu/ZnO@GO nanocomposite, the characteristic signals at 1297 and 1589 cm^{-1} correspond to metal-oxide vibrational modes, specifically Zn-O and Cu-O stretching.

Fig. 4 displays the XRD spectra of the separated

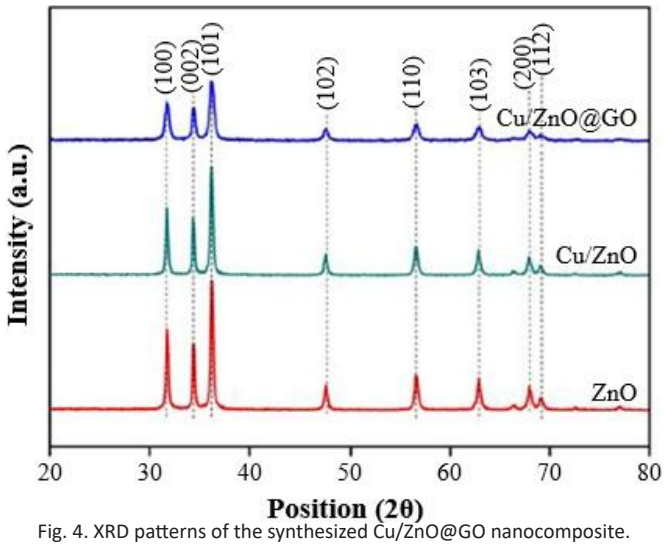


Fig. 4. XRD patterns of the synthesized Cu/ZnO@GO nanocomposite.

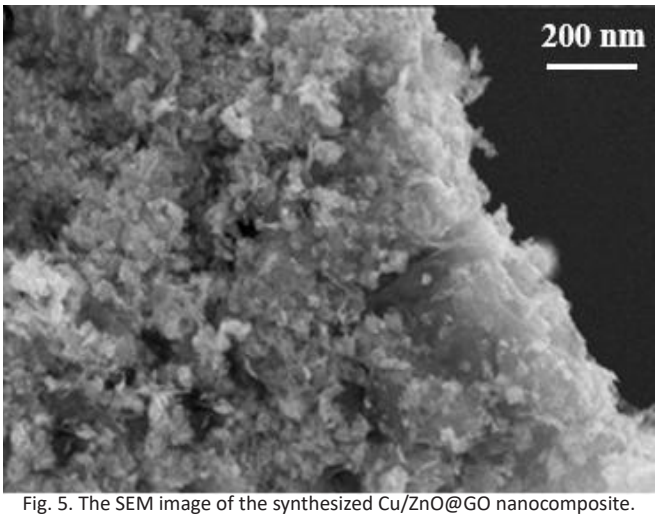


Fig. 5. The SEM image of the synthesized Cu/ZnO@GO nanocomposite.

pure components, ZnO, ZnO@GO, and Cu/ZnO@GO nanostructures. The gradual deposition of Cu and ZnO nanoparticles onto the GO sheets explains why the Cu/ZnO@GO particles lack a clear structure. According to JCPDS No. 01-082-9744, the nanocomposite's XRD pattern precisely matches the ZnO-related peaks with the hexagonal wurtzite structure. Additionally, the Cu peaks match the typical powder diffraction card with the number JCPDS No. 01-087-0717 on it. Moreover, Fig. 4 shows that the peak intensities corresponding to the ZnO principal planes (100), (002), and (101) decrease with the addition of Cu.

The SEM image was employed to analyze the shape of the synthesized Cu/ZnO@GO nanocomposite, which provided obvious proof of its effective construction (Fig. 5). Image reveal that there is a homogeneous dispersion on the surface of the catalyst, which may be successful in its catalytic application. EDS analysis used to identify the chemical composition of the nanocomposite is shown in Fig. 6. This spectrum includes the identifiable signals for the Cu, Zn, and O elements. The weight proportion of the necessary nanocomposite components signifies successful creation.

Achieving a certain path for the synthesis mentioned above was pursued by optimizing the conditions whose results are illustrated in Table 1. This table shows the determination of the reaction

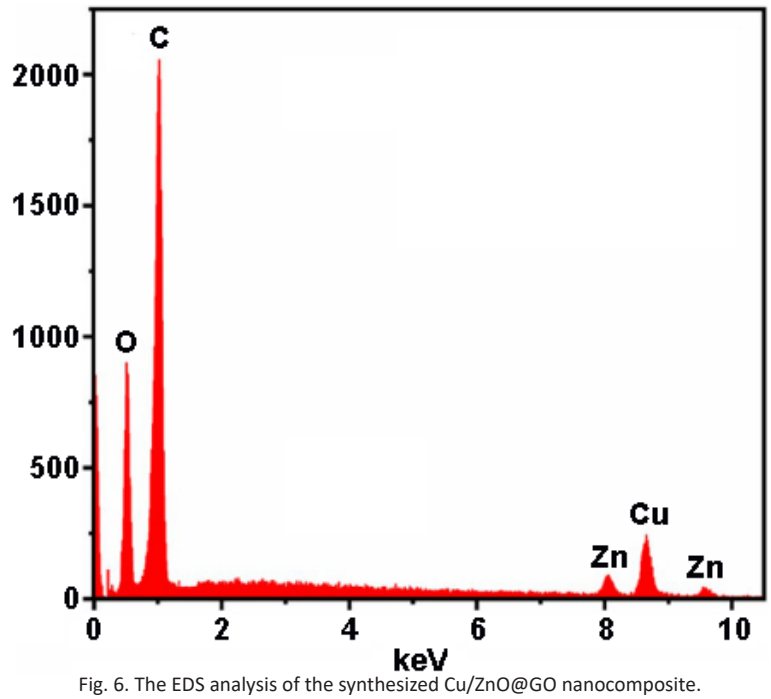
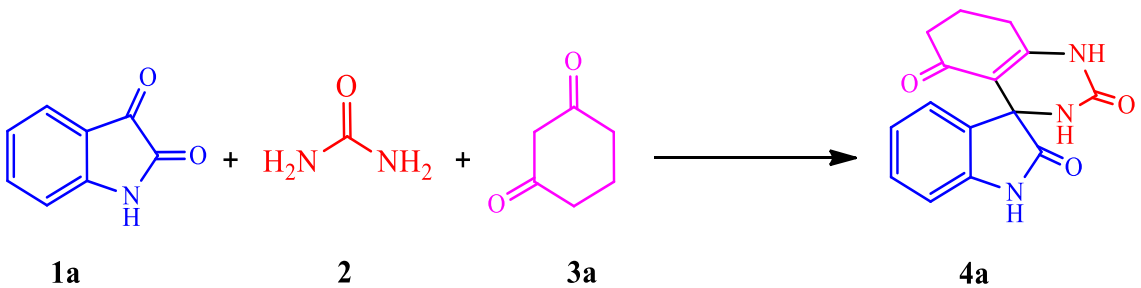


Fig. 6. The EDS analysis of the synthesized Cu/ZnO@GO nanocomposite.



1a **2** **3a** **4a**

Fig. 7. Synthesis of spiro[3,4′]1,3-dihydro-2*H*-indol-2-one-4′,6′,7′,8′-tetrahydro-2′,5′(1′*H*,3′*H*)-quinazoline-dione (4a) under various conditions.

environment conditions, the amount of catalyst, and the appropriate temperature through a model reaction of isatin (1a), urea (2), and cyclohexanone (3a) for the synthesis of specific product 4a (Fig. 7). Firstly, the crucial role of catalyst was investigated, and it was found that performing the reaction in the absence of any added catalyst led to achieving a low conversion (33%) even after 12 h in water at reflux (Table 1, entry 1). Further, in the evaluation and comparison of the catalytic activity of *p*-TsOH, DAHP, and Cu/ZnO@GO nanocomposite, it was revealed that the Cu/ZnO@GO nanocomposite was the best among those tested (Table 1, entries 2-4). Moreover, 0.05 g of nanocatalyst was determined as the reasonable value for the target reaction to complete after 2 h at 60 ˚C in water (Table 1, entry 4 vs. entries 5 & 6). We then set out to explore the effectiveness of temperature on the reaction. Notably, no excellent effect was observed when the reaction was accomplished using 0.05 g of the nanocatalyst in water under reflux (Table 1, entry 4 vs. entry 7). Finally, the influence of the reaction media in the model reaction was investigated using various solvents such as EtOH, CH_3CN, CH_2Cl_2, DMF, versus H_2O. Water was selected as a superior reaction medium for this reaction in the presence of 0.05 g of Cu/ZnO@GO at 60 ˚C (Table 1, entry 4 vs. entries 8-11). Generally, such reaction conditions can provide a green, safe, and gentle route.

After establishing the optimal conditions for the aforementioned reaction, we looked into the substrate scope of the reaction by selecting a number of 5-substituted isatins that were tested in the reaction with urea and 1,3-cyclohexanedione or dimedone to produce the target compounds in 1.5–2 hours in high yields (Table 2).

Furthermore, we studied the catalyst

Table 1. Optimization of reaction conditions for preparation of spiro[3,4′]1,3-dihydro-2*H*-indol-2-one-4′,6′,7′,8′-tetrahydro-2′,5′(1′*H*,3′*H*)-quinazoline-dione (4a).

Entry	Conditions	Catalyst (g)	Time (h)	Yield (%)[d]
1	H_2O (Reflux)	-----	12	33
2	H_2O (60 ˚C)	*p*-TsOH[b] (15 mol%)	6	60
3	H_2O (60 ˚C)	DAHP[c] (15 mol%)	6	75
4	H_2O (60 ˚C)	Cu/ZnO@GO (0.05)	2	90
5	H_2O (60 ˚C)	Cu/ZnO@GO (0.04)	2	81
6	H_2O (60 ˚C)	Cu/ZnO@GO (0.07)	2	90
7	H_2O (Reflux)	Cu/ZnO@GO (0.05)	1.5	92
8	EtOH (60 ˚C)	Cu/ZnO@GO (0.05)	2	78
9	CH_3CN (60 ˚C)	Cu/ZnO@GO (0.05)	3	81
10	CH_2Cl_2 (60 ˚C)	Cu/ZnO@GO (0.05)	4	75
11	DMF[a] (60 ˚C)	Cu/ZnO@GO (0.05)	5	70

[a]DMF: Dimethyl formamide.
[b]*p*-TsOH: *p*-Toluenesulfonic acid.
[c]DAHP: Diammonium hydrogen phosphate.
[d]Isolated yield.

Table 2. Cu/ZnO@GO nanocomposite catalyzed syntheses of spiro[3,4′]1,3-dihydro-2*H*-indol-2-one-4′,6′,7′,8′-tetrahydro-2′,5′(1′*H*,3′*H*)-quinazoline-diones 4a-h.

Product	R	R'	Time (h)	Yield (%)[a,b]	M.p (˚C)	
					Obsd.	Lit.
4a	H	H	2	90	342-344	>300 [21]
4b	Br	H	2	88	354-355	>300 [21]
4c	OCH_3	H	2	90	230-232	>300 [21]
4d	NO_2	H	1.5	94	356-358	>300 [21]
4e	H	CH_3	1.5	91	351-353	>300 [21]
4f	Br	CH_3	1.5	90	368-370	>300 [21]
4g	OCH_3	CH_3	2	92	235-236	>300 [21]
4h	NO_2	CH_3	1.5	94	363-365	>300 [21]

[a]Yields refer to those of pure isolated products characterized by IR, ^{1}H NMR and ^{13}C NMR spectral data and by elemental analyses.

recyclability in the synthesis of product 4a. After completion of the reaction, the catalyst was successfully recovered in a simple manner as described in the general procedure and afforded completion of the subsequent reactions in the comparable reaction time and product yield during the first four runs, as that for the fresh (product yields: 90 (fresh), 88, 87, 85, and 82%, respectively).

A probable reaction mechanism for the present reaction is given in Fig. 8. It is proposed that via coordinating through acidic sites, the nanocatalyst may activate the carbonyl group of isatin 1. In order to get alkene 7 via intermediate 6, it was

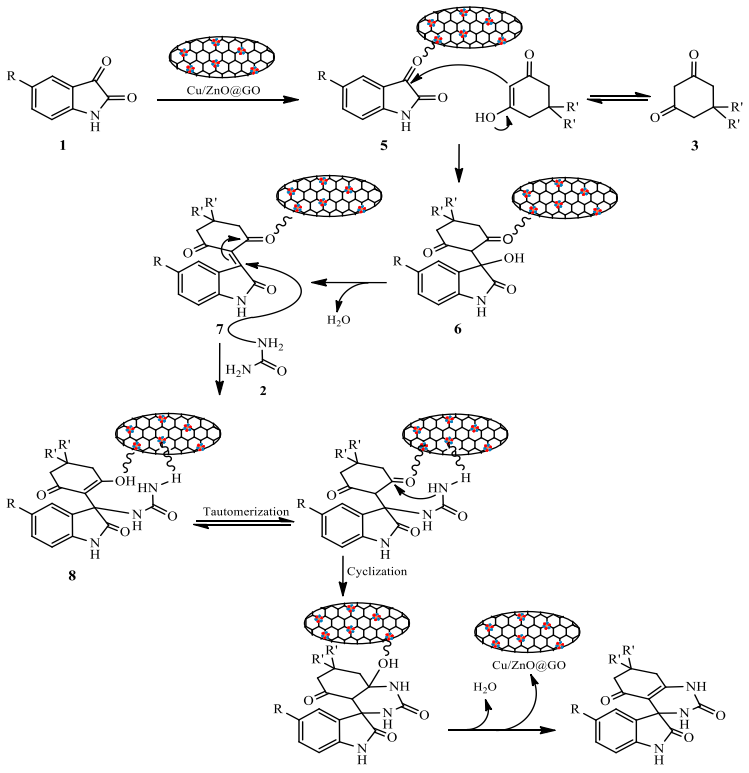


Fig. 8. Proposed mechanism for the synthesis of compound 4.

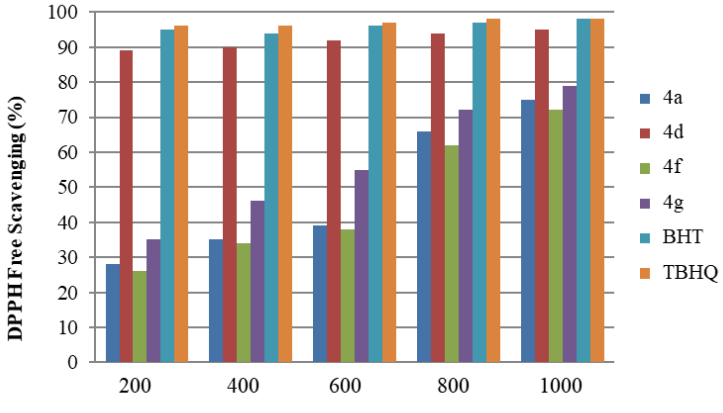


Fig. 9. Radical scavenging activities (RSA) of selected products compared with BHT as well as TBHQ as a standard antioxidant.

then suggested that this intermediate go through a straight Knoevenagel reaction with enolized 1,3-cyclohexanedione 2. Intermediate 8 is produced by the subsequent Michael-type addition of urea 3 to alkene 7. Further intramolecular cyclization of 8 in the presence of nanocomposite forms product 4, following dehydration of cyclized intermediate 9.

Aantioxidant evaluations

The chosen products, such as 4a, 4d, 4f, and 4g, were examined in this investigation for their capacity to capture free radicals in comparison to butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) at different concentrations. The findings were graded from highest to lowest activity as follows: TBHQ>BHT≥4d>4g>4a≈4f. The effectiveness of DPPH trapping in the studied substance and standards was generally equivalent. At a dosage of 1000 ppm, all test chemicals generally exhibit potential radical scavenging in comparison to BHT and TBHQ (Fig. 9).

CONCLUSION

The current study provides a sustainable and eco-friendly synthesis of a series of spiro-fused oxindole-quinazolinones employing a Cu/ZnO@GO nanocomposite as a superior and reusable heterogeneous catalyst. The catalyst that was exhibited was made without a template and in an environmentally friendly manner. One of the main characteristics of the technology that is being discussed is the simultaneous presence of several biologically active cores within the produced products. Melting point, FT-IR, ^{1}HNMR, ^{13}C-NMR, and elemental analysis were among the methods used to assess and validate the target products. The present protocol has its benefits, including achieving higher reaction yields within a significantly shorter reaction time, the use of a green, reusable heterogeneous nanocatalyst, and paving the route for sustainable synthesis of spirooxindole derivatives. Furthermore, our investigation into the antioxidant capacity of several items displayed promising results. In the field of spirooxindole chemistry, this work is therefore a significant step toward the creation of a high-efficiency Cu/ZnO@GO nanocomposite catalyst that may be utilized for the synthesis of kinase inhibitors, particularly for the identification of anticancer drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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