Dalton Transactions

An international journal of inorganic chemistry

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NiO nanocomposites/rGO as heterogeneous catalysis for imidazole scaffolds with their applications in inhibiting DNA binding activity

Gyanendra Kumar, Navin Kumar Mogha, Manish Kumar, Subodh, and Dhanraj T. Masram* Department of Chemistry, University of Delhi, Delhi-110007, India

Abstract

Herein, we report a facile approach to synthesize a new highly versatile heterogeneous catalyst by spontaneous aerial oxidation based on nickel oxide nanocomposites immobilized over the surface-functionalized reduced graphene oxide sheet. Reduced graphene oxide/NiO nanocomposites (rGO-NiO-NCs) is used as highly efficient, cost-effective, reusable, selective, and eco-friendly nano-catalyst without losing any activity even after five reaction cycle. Nickel loading on the rGO/NiO nanocomposites is found to be 3.3 At% which contributes to the effective and efficient use of rGO-NiO-NCs as nano-catalyst for the synthesis of imidazole derivatives. Consequently, a series of imidazole derivatives were synthesized, catalyzed by rGO-NiO-NCs, in 60 min with high yields (86 to 96 %) under green conditions. Furthermore, the present synthetic methodology is used for the synthesis of highly aromatic imidazole derivatives (B1-B3) whose calf thymus-DNA binding affinity suggesting their superior inhibition ability to the displace of ethidium bromide (EB), which is further confirmed by molecular docking. Additionally, green chemistry matrix of the synthesized reaction is found to be very close to ideal values such as Carbon efficiency (82.32 %), E-factor (0.51), Atom economy (77.86 %), Process mass intensity (1.51), and Reaction mass efficiency (66.14 %).

Keywords: Graphene oxide; Heterogeneous catalyst; Trisubstituted imidazoles; Nickel oxide Nanocomposites, Molecular docking

*Corresponding author: E-mail; *dhanraj_masram27@rediff mail.com*; Tel: +91-11-27666646-161.

1. Introduction

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Multicomponent reactions (MCRs) have an important status among organic and medicinal chemistry for their advantageous applications in single pot synthesis of complex organic molecules from easily available substrates with a high degree of atom economy. ^{1–3} Currently, MCRs are used for the rapid synthesis of complex molecular structures in particular heterocycles, ⁴ polymers, ⁵ macrocyclic compounds, ⁶ and natural products. ⁷ Along with these applications MCRs, approaches are also used for the Pharmaceutical industry, drug discovery and development programs ⁸ for the synthesis of 2,4,5-trisubstituted imidazoles.

2,4,5-Trisubstituted imidazoles exhibit several pharmacological and biological properties, therefore, occupying a significant place in the field of synthetic, pharmaceutical and natural organic chemistry. ^{9,10} The N-heterocyclic family of 2,4,5-Trisubstituted imidazoles have extended applications in different realms such as material chemistry, ¹¹ polymer chemistry, ¹² agro-chemicals, ¹³ cosmetics, ¹⁴ photosensitive compounds for photography ¹⁵ and anti-corrosion material for some transition metals. ¹⁶ Consequently, synthetic methodologies for 2,4,5-trisubstituted imidazoles preparation are very important and therefore, need to develop on a regular basis.

Synthesis of 2,4,5-trisubstituted imidazoles and their derivatives usually involve a three-component condensation reaction of a 1,2-diketone/hydroxy ketone with several aldehydes in presence of ammonium acetate. Accordingly, a large number of catalysts such as ceric ammonium nitrate (CAN), ¹⁷ poly(4-vinyl pyridinium butane sulfonic acid) hydrogen sulfate, ¹⁸ ZrO₂-supported β -cyclodextrin (ZrO₂- β -CD), ¹⁹ nanocopperferrite, ²⁰ silica gel/NaHSO₄ or HClO₄–SiO₂, silica gel/zeolite HY, BF₃·SiO₂ heteropolyacids, silica-supported sulfuric acid, NiCl₂·6H₂O, InCl₃·3H₂O, Al₂O₃, ionic liquids, molecular iodine or proline are used to synthesize these substituted imidazoles with improved yield. ²¹ Though these particular synthetic procedures are appropriate for some synthetic conditions but most of them have one or more than one shortcoming, for example, costly reagents and catalysts, longer reaction times, no or low selectivity, difficult post-reaction work-up, and use of large quantity of catalysts, which ultimately results in toxic waste generation in large amounts. To overcome these difficulties, an urgent need of some highly efficient cost-effective heterogeneous catalysis with green synthesis methodology is required.

Graphene Oxide, being an exclusive 2-D carbon material has a tremendous demand in the development of heterogeneous catalysts and other composite materials owing to its amazing chemical, physical and electrical characteristics. ²² The presence of oxygencontaining functional groups over the graphene sheets helps in functionalization of GO by

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various precursors such as polymers, silylating agents, metals and metal oxide nanoparticles and other surfactants. ²³ Furthermore, only a few active groups on GO surface can react with epoxy matrix, significant in weak interfacial interactions between GO sheets and matrix. These difficulties can be addressed by functionalizing GO with some big or small molecules and polymers. The surface functionalization was found to noticeably expand the compatibility and dispersibility of GO sheets.^{24–27} A suitable surface modification enhances the stability of the material by modifying the surface area, selectivity while minimizing aggregation and toxicity. These polyfunctional materials immobilized with nanoparticles show a wide range of applications in pharmaceuticals and chemical industry as catalysts, drug delivery, sensors, charge storage, and photocatalyst. ²⁸

After taking account of all these factors, it would be very necessary to use a functionalized rGO template for metal nanoparticle or nanocomposites immobilization and sought their application in heterogeneous catalysis. Consequently, we report the synthesis of NiO nanocomposites immobilized over the surface-functionalized reduced graphene oxide by spontaneous aerial oxidation. ²⁹ Significance of such surface modification is the availability of more binding sites for NiO nanocomposites and it provides abundant surface exposure for catalysis in comparison to other support materials. The rGO-NiO-NCs nano-catalyst is employed as a stable, efficient, recyclable heterogeneous catalyst for high yield synthesis of 2,4,5-trisubstituted imidazoles *via* MCRs (Scheme 1). Authors calculated green chemistry matrix of the synthesized imidazole reaction and it is found to be much close to the ideal values such as Carbon efficiency, E-factor, Atom economy, Process mass intensity, Reaction mass efficiency Furthermore, their B-DNA (1BNA dodecamer) of calf thymus inhibition studies were also performed to estimate their binding efficiencies, which is validated by molecular docking.



Scheme 1. Schematic illustration for the synthesis of reduced graphene oxide nickel oxide nanocomposites (rGO-NiO-NCs).

2. Results and discussion

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2.1. Structural Characterization of rGO-NiO- Nanocomposites

The powder X-Ray Diffraction (XRD) pattern of GO, rGO-NH₂, and rGO-NiO-NCs can be seen in Fig. 1(a). XRD pattern of the GO, rGO-NH₂ and rGO-NiO-NCs show the two characteristic peaks of plane $\langle 001 \rangle$ and $\langle 100 \rangle$ of GO at $2\theta = 10.3^{\circ}$ and 42.12° respectively. ³⁰ While a broad peak at $2\theta = 23.5^{\circ}$, which can be attributed to the peak for SiO₂ and partial reduction of GO to rGO. The broad diffraction peak of rGO-NiO-NCs indicates that the synthesized composite is almost amorphous in nature. Though no peaks for NiO are obtained which might be due to the less loading of the NiO.



Fig. 1. (a) Powder XRD patterns and **(b)** Raman spectra of (i) GO, (ii) rGO-NH₂, (iii) rGO-NiO-NCs nanocatalyst.

Raman spectroscopy used for the determination of ordered and disordered crystal structures of graphitic materials. The Raman spectra of GO, rGO-NH₂, rGO-NiO-NCs is shown in Fig. 1(b). The Raman spectra of rGO-NiO-NCs and other intermediates show two characteristic peaks at around 1347, and 1604 cm⁻¹, ³¹ which were associated with the well-denoted D band and G bands, respectively. The G band refers to the zone center E2g phonon, corresponding to the carbon sp² vibrations of the domains in the plane. ³² The D band is related to the out-ofvibrational breathing plane modes deriving from k-point phonon of A_{1g} symmetry. ³³ The values of I_D/I_G were found to be 0.83, 0.84, and 0.86 for GO, rGO-NH₂, and rGO-NiO-NCs respectively. The gradually increase in the I_D/I_G ratio is indicative of increasing disorder in the graphene oxide lattice due to its functionalization with SiO_2 and NiO.

X-ray photoelectron spectroscopy analysis was also performed to determine the composition of the synthesized rGO-NiO-NCs material given in (Fig. 2). XPS full scan spectrum of the nanocomposite is shown in Fig. 2(f).

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Fig. 2(a) shows XPS spectrum of C1s having two peaks at binding energy 282.72 and 284.21 eV which can be assigned to C-O and C-C, respectively. **Fig. 2(b)** shows XPS spectrum of N1s, peak at 397.81 eV correspond for N=C, indicating to the formation of Schiff base whereas another peak at 399.54 eV attributes for N-H bond. XPS spectrum of O1s contains a peak at 530.27 eV represents bonding between O²⁻ ion and Ni and shown in **Fig. 2(c)**, hence the presence of NiO is confirmed. Functionalization with SiO₂ can be determined by the presence of a peak at 100.40 eV, in the XPS spectrum of Si2p indicates for Si-O bond, shown in **Fig. 2(d)**. XPS spectrum of Ni2p, shown in **Fig. 2(e)** contains two peaks at 854.96 and 873.07 eV corresponds to the binding energy of Ni2p_{3/2} and Ni2p_{1/2}, respectively. ³¹ Whereas two small peaks for Ni2p_{3/2} and Ni2p_{1/2} satellites observed at 860.15 and 879.03 eV respectively.

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Fig. 2. The XPS spectra of rGO-NiO-NCs nano-catalyst for (a) carbon, (b) nitrogen (c) oxygen, (d) silicon, (e) nickel, and (f) full-range spectra of elements.

After that, the nickel content is also confirmed by inductive coupled plasma optical emission spectroscopy (ICP-OES) and the nickel loading was detected to be 1.56 mmolg⁻¹.

2.2. Morphological Characteristics of rGO-NiO-Nanocomposites

The morphological characterization of rGO-NiO-NCs is performed by FESEM and HRTEM, herein by **Fig. 3(a)** and **(b)** depicts the FESEM images of rGO-NiO-NCs showing NiO nanocomposites are attached to the wrinkled surface of rGO under different magnifications.



Fig. 3. (a) and **(b)** FESEM micrographs, while **(c)** HRTEM micrograph of rGO-NiO-NCs, and **(d)** FESEM image of the recycled of rGO-NiO-NCs nano-catalyst.

The inset image in Fig. 3(b) illustrates the single NiO nanocomposites consisting of several nanosized NiO nanoparticles. Furthermore, HRTEM micrographs in Fig. 3(c) exhibited twisted nanosheets of rGO with attached NiO nanocomposites, while the inset shows the Selected Area Electron Diffraction (SAED) pattern for the rGO-NiO-NCs nano-catalyst, confirming the presence of different planes of the NiO in the nano-catalyst. The elemental percentage obtained from energy-dispersive X-ray (EDX) analysis of rGO-NiO-NCs nano-catalyst indicating about 3.3 atomic percent of nickel element present in nano-catalyst. While Recycled catalyst is shown in Fig. 3(d), here no significant change in morphology of the nano-catalyst is observed establishing the reusability potential of rGO-NiO-NCs nano-catalyst.

A detailed chemical analysis of the rGO-NiO-NCs is provided by EDX elemental mapping **Fig.4 (a-e)** and EDX pattern **Fig. 4 (f)**. The GO is mainly composed of carbon and oxygen. The loading of NiO nanocomposites on functionalized rGO changed the elemental

composition with the introduction of nickel, nitrogen, oxygen, and silicon, which are core elements of the rGO-NiO-NCs apart from carbon and oxygen. Furthermore, EDX elemental mapping is performed to understand the distribution of the NiO nanocomposites on functionalized rGO. The elemental mapping indicated the variable distribution of Ni, N, O, C and Si over the surface of rGO.



Fig. 4. **(a-e)** Elemental mapping of different elements and **(f)** EDX pattern obtained of rGO-NiO-NCs nano-catalyst.

2.3. Optimization of 2, 4, 5-trisubstituted-imidazoles

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To get the best results from the nano-catalyst it is very necessary to optimize the type of solvent, temperature, time and the amount of catalyst which has been used during the optimize reaction. The benchmark reaction of benzil (0.5 mmol), benzaldehyde (0.5 mmol), and ammonium acetate (1 mmol) was used as a model reaction for optimization of reaction conditions (**Table 1**), we further proceeded our examination by testing the possibility of the reaction using a series of polar and apolar solvents such as methanol, toluene, acetonitrile, and ethanol, water in highly regiocontrolled fashion, affording the conforming imidazole derivatives. In conclusion, the reaction in ethanol medium afforded the desired target molecule within an excellent yield at 1 h (**Fig. 5**). The rest of the solvents gave less yield or no desired product formation was observed, whereas the nitrogen source NH₄OAc was exchanged with NH₄OH and the only H₂O byproduct was formed. Additionally, the reaction performed at room temperature had no impact on the construction of the chosen product. Hence, we optimize the reaction using temperature (50-55 °C) and 55 °C was found to be the optimum temperature for imidazole derivatives synthesis. In product, increment yield is observed with increase in amount of nano-catalyst. Afterward, no products were observed

when the reaction was performed in the absence of nano-catalyst. Moreover, aromatic aldehydes with both activating and deactivating substituted groups such as -OH, -OMe, -Cl, -Br, and -NO₂ reacted to afford the corresponding imidazole products almost equally in high yields (**Table 2**). Furthermore, the electron releasing (-OMe) and electron-withdrawing (-Br) groups at the meta position of the aromatic ring gave less yield as compare to para and orthosubstituted aromatic aldehydes (Entry A5 and A9). Ortho-substituted benzyl alcohols participated well in the synthesis of the corresponding products in good to excellent yield in spite of the steric factor (Entry A3). It was found that the reaction **11**^b shows the best-case scenario in greener solvent with 25 mg of rGO-NiO-NCs nano-catalyst as compare to others.



Fig. 5. rGO-NiO-NCs catalyzed the synthesis of 2, 4, 5-trisubstituted imidazole derivatives.

Entry	Solvent	Temperature	Catalyst	Time	Yield
		(°C)	(mg)	(min)	(%) ^c
1	EtOH	50	0	120	-
2	EtOH	60	5	120	58
3	EtOH	50	5	60	67
4	EtOH	50	10	55	80
5	Toluene	50	10	80	45
6	Solvent-free	50	15	60	50
7	Solvent-free	50	25	60	60
8	Solvent-free	120	25	60	62
9	Water	50	25	60	Trace
10	EtOH	50	15	60	80
11 ^b	EtOH	55	25	60	96
12	CH ₃ CN	50	25	70	47
13	МеОН	50	25	50	92

Table	1: Optimization	of the	reaction	conditions ^a	for	access	of	imidazole	derivative	via
rGO-N	iO-NCs									

14	EtOH	RT	25	60	59
15	EtOH	120	25	60	70
aReaction	conditions: Benzil	(0.5 mmol), Ber	nzaldehyde (0.5 mm	nol), NH ₄ OAc (1	mmol), solvents (5 ml)
^c Isolated	Yields, RT= room te	mperature			

Later, the optimized reaction conditions are utilized to synthesize different derivatives of the 2,4,5-trisubstituted imidazoles using several substituted benzaldehydes (**Table 2**). Yields obtained in these reactions are found to be satisfactorily high without any significant change, where electron-withdrawing and electron releasing groups at the meta position of the aromatic ring gave less yield as compare to ortho and para-substituted aromatic aldehydes.

 Table 2: Synthesis of 2,4,5-trisubstituted imidazoles catalyzed by rGO-NiO-NCs nanocatalyst.

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Entry	Aldehydes	Time (min)	Yield* (%)	TON [#]
A1	СНО	60	93	23.84
A2	но-Сно	60	88	22.56
A3	СНО	60	96	24.61
A4	Сно	60	94	24.10
A5	СНО	60	86	22.56
A6	СІ—	60	91	23.33
A7		60	92	23.58
A8	н₃со-√Сно	60	89	22.82



*Isolated yields, #TON is the number of moles of product per mol of catalyst.

Even with highly aromatic aldehydes (**Table 3**) such as 1-naphthaldehyde, 2-naphthaldehyde and anthracene-9-carbaldehyde product obtained in very high yields indicating catalyst is free from any electronic interference for its activity electron-withdrawing derivative or highly aromatic substituents do not affect the efficiency of rGO-NiO-NCs nanocatalyst.

Entry	Aldehydes	Time(min)	Yield* (%)	TON
B1	СНО	60	94	24.10
B2	СНО	60	96	24.61
B3	СНО	60	91	23.33

 Table 3: Synthesis of highly aromatic 2,4,5-trisubstituted imidazoles catalyzed by rGO-NiO-Nanocomposites.

*Isolated yields.

3. DNA binding Study

The *in vitro* competitive inhibition study of the compounds (**B1-B3**) with Ethidium bromide (EB) against the calf thymus DNA was investigated *via* fluorescence spectroscopy technique. EB is a fluorescent dye, emits intense fluorescence in the presence of DNA due to it have strong intercalation between the double helix base pairs. Results indicate that B1, B2, and B3

show better competition against EB. ³⁴ The extent of fluorescence quenching of EB bound to DNA can be used to determine the extent of binding between the compound and DNA. The emission spectra of EB bound DNA complex has been recorded in the absence as well as the presence of the increasing amounts of the compounds (**Fig. 6**). After the addition of compounds, a significant decrease in the intensity of the emission band is observed indicating the binding of the compound to DNA grooves by displacing EB. The quenching efficiency of the compounds has been calculated using the *Stern-Volmer equation* (1). ³⁵



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Fig. 6. Fluorescence emission spectra of EB bound DNA complex in the absence as well as the presence of an increasing concentration of compounds images of (a) B1, (b) B2 (c) B3.

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The arrows represent the change of intensity during increasing amounts of compounds B1-B3.

Here, I_0 and I are the fluorescence intensities in the absence as well as the presence of the quencher, [Q] is the concentration of the quencher (B1-B3) and K_{sv} is the linear *Stern-Volmer* quenching constant which is obtained by the slop of the plots $\frac{I_0}{I}$ Vs [Q].

The K_{sv} values calculated for the compounds are $0.0281 \pm 0.0049 \text{ M}^{-1}$ for B1, $0.0148 \pm 0.0015 \text{ M}^{-1}$ for B2 and $0.0049 \pm 0.0052 \text{ M}^{-1}$ for B3 and these values showed that they can be bind tightly to the CT DNA *via* interclative binding mode. In conclusion, **B1** compound provides an excellent binding affinity with the highest K_{sv} value ($0.0281 \pm 0.0049 \text{ M}^{-1}$) toward CT DNA among all other compounds.

4. Molecular Docking Analysis

The allosteric molecular docking studies of B1, B2, B3 and ethidium bromide with B-DNA dodecamer of calf thymus were performed to find the potent inhibitor by applying the Lamarckian genetic algorithm. ³⁶ (**Fig. 7**), depicts the molecular docking results obtained, for B1, B2, B3, and ethidium bromide. The binding energy value for all compounds is given in (**Table 4**). The binding energy values for the B1, B2, B3 and ethidium bromide are -8.3, -7.4, -7.8, and -7.0 KJ/mol respectively. The result indicates that B1 has a potent one among all. All molecules almost targeted the minor grooves of the B-DNA. B1, B2, and B3 more strongly bind to the B-DNA compare to ethidium bromide. The H-bond length in case of B1 is 2.91Å, for B2 is 2.09 Å and for B3 is 2.93 Å respectively. Docking results successfully corroborate the experimental result.



Fig. 7. Molecular docking result images of **(a)** B1, **(b)** B2 **(c)** B3 and **(d)** Ethidium bromide (EB) with B-DNA showing binding of the molecules in the minor groove. Where the color showing of B-DNA Green = Cytosine, Purple = Guanine, Red = Adenine, Sky Thymine. **Table 4:** Docking score of all four compounds

Compounds	Binding Score	Root mean square deviation (RMSD) of		
		docking		
<i>B1</i>	-8.3	RMSD (1b.)	RMSD (u.b.)	
<i>B2</i>	-7.4	0.00	0.00	
<i>B3</i>	-7.8	0.00	0.00	
EB	-7.0	0.00	0.00	

(1.b. = lower bound u.b. = upper bound)

Table 5: Comparison of results with different types of catalysts for the synthesis of 2,4,5-trisubstituted-imidazole.

Entry	Reagents	Conditions	Time (min)	*Yield (%)	Ref.
1	SSA	Water/Reflux	240	70	37
2	Yb(OTf) ₃	HOAc	60	92	38

3	InCl ₃ .3H ₂ O	MeOH/rt	500	82	39
4	Polymer-ZnCl ₂	EtOH	240	96	40
5	L – proline	MeOH/60 °C	540	90	21
6	$NiCl_2{\cdot}6H_2O/Al_2O_3$	Reflux/EtOH	90	89	41
7	NiCl ₂ .6H ₂ O	EtOH/60 °C	60	85	-
8	rGO-NiO-NCs	EtOH/55°C	60	96	This work

4.1. Recyclability of the rGO-NiO-NCs nanocatalyst

Potential of catalyst depends upon its stability and recyclability which can be assessed by reusing catalysts many times and observing the change in the yields. In the present study, rGO-NiO-Nanocomposites nanocatalyst is recovered by centrifugation followed by multiple washing with ethanol and ethyl acetate before using it in successive reaction.



Fig. 8. Recyclability of the rGO-NiO-NCs nanocomposites.

The recovered catalyst accomplished the respective transformation for five times without any substantial loss in activity for 2,4,5-trisubstituted-imidazoles synthesis. Recyclability is an important part of the heterogeneous catalysts and the results were shown in (**Fig. 8**). Since heterogeneous catalysis requires direct contact between the reactants and the catalytic surface. The area of active surface goes at the top of the list and found high yields. The conversions of nanocomposites were yields decreased at the 5th cycle of the catalyst and yield obtained is only a loss of 86%. This may be due to the loss of Surface functionalization and or which belonged to physical adsorption rather than covalent bonding. On the other hand, it may be also due to the reduction of catalyst activity. There was a small decrease in the yield of the first to second run. However, conversion of yield was observed to show a significant decrease in the third run, and up to 5th run. The images of FESEM (**Fig. 3d**) and FTIR spectra are given in (**Fig. S1** (**d**)) also indicate the stability of the catalyst.

The green chemistry metrics calculated for entry (A7) under the optimized condition. It is evident from **Table 6**, the green chemistry metrics are close to ideal values. ⁴² Additionally, the expectation that is environmental improvements and quantifying technical can make the profits of new technologies more tangible, perceptible and adoption of green chemistry technologies in industrial and pharmaceuticals.

S.No.	GC matrices	Ideal value	Calculated for (A7)
1	Carbon efficiency	100	82.32 %
2	E-factor	0	0.51
3	Atom economy	100	77.86 %
4	Process mass intensity	1	1.51
5	Reaction mass efficiency	100	66.14 %

Fable 6 : Calculation of gree	n chemistry matrix	for compound A7.
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4.2. Proposed Mechanism

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Fig. 9, illustrates the plausible mechanism for the synthesis of the 2,4,5-trisubstituted imidazoles catalyzed by rGO-NiO-NCs nano-catalyst compare from previous literature.^{43,44} Firstly, the reaction proceeds through the diamine intermediate which is formed by the C=O activation of an aldehyde on the surface to increase the polarity of the carbonyl group due to the rGO-NiO-NCs. Similarly, condensation of a diamine with benzil followed by dehydration and then 1-5 hydrogen shift to form the desired product. The presence of electron-withdrawing groups (-NO₂) afforded the corresponding 2,4,5-trisubstituted imidazoles in quicker reaction time with higher yields. A comparison of similar work with other already reported literature is also provided in (**Table 5**). In conclusion, the present work is much superior in terms of the yields obtained and reaction conditions including lesser reaction time and higher yields at 55 °C in a green solvent.



Fig. 9. The plausible mechanism for the synthesis of the 2,4,5-trisubstituted imidazoles catalyzed by rGO-NiO-NCs nanocomposites.

5. Conclusion

The authors have reported the synthesis, characterization, and applications of new rGO-NiO-NCs nano-catalyst used for the synthesis of trisubstituted imidazoles. The NiO-NCs supported over the surface-functionalized reduced graphene oxide sheet demonstrated excellent catalytic property with improved yields under the green solvent. rGO-NiO-NCs nano-catalyst has shown a high level of catalytic activity, with shorter reaction time, stability, recyclability with green chemistry matrices in a cost-effective manner. rGO-NiO-NCs catalyzed highly aromatic aldehydes to their corresponding trisubstituted imidazoles unhindered manner with high yields. Further, the synthesized of highly aromatic 2,4,5-trisubstituted imidazoles such as 2-(naphthalen-1-yl)-4,5-diphenyl-1H-imidazole, 2- (anthracen-9-yl)-4,5-diphenyl-1H-imidazole, and 2-(naphthalen-2-yl)-4,5-diphenyl-1H-imidazole were subjected to study the competitive DNA binding activity against ethidium bromide from its binding position, which is successfully corroborated by molecular

docking. Results indicate that rGO-NiO-NCs nano-catalyst can be used for the synthesis of imidazole scaffolds of industrial and pharmaceutical applications.

6. Experimental section

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6.1. Materials & Methods

Detail of material used and instrumentation employed for the characterization and it provided in *supporting information* (1).

6.2. Synthesis of Graphene oxide (GO)

Graphene oxide was synthesized using improved hummers method to the previous reports, $^{45-47}$ with some minor modifications. Typically, to a 9:1 solution of concentrated sulfuric acid and phosphoric acid (90:10 ml), graphite powder (1.0 g) was added. After stirring for 20 min, potassium permanganate (6.0 g) was slowly added to the reaction mixture, creating a slight exothermal, and stirred for 12 h at 55 °C. The reaction mixture was cooled to room temperature and transferred into crushed-ice (500 ml), followed by addition of 30 % hydrogen peroxide (3 ml) and stirred for 1 h, yellow-brown precipitate obtained was separated using centrifugation for 15 min at 6000 rpm, and washed with deionized water (400 ml × 3), HCl (200 ml × 3) to remove metal ions, sulfate, and chloride ions, ethanol (200 ml × 3), diethyl ether (100 ml × 2). Finally, the solid product was vacuum dried in vacuum at 40 °C for 10 h, obtaining brownish graphene oxide (GO) powder form.

6.3. Synthesis of functionalized graphene oxide (rGO-NH₂ and rGO-Schiff-base)

Graphene oxide (1.0 g) was well dispersed in 40 ml of ethanol by ultrasonication for 45 min, Afterwards, 3-aminopropyltriethoxysilane (2.3 mmol) was added and the mixture was refluxed at 80 °C for 24 h in an inert atmosphere. After the stipulated time, the reaction mixture was allowed to cool at room temperature, centrifuged at 4500 rpm for 10 min, and the resultant black solid material (rGO-NH₂) obtained was washed by ethanol solution (three times) to remove the unreacted 3-APTES moieties. rGO-NH₂ was dried in an oven at 55 °C for 12 h.

0.5 g of rGO-NH₂ was dispersed in 20 ml ethanol by ultrasonication for 30 min followed by the addition of 2-hydroxy-1-naphthaldehyde (20 ml). The reaction mixture was refluxed for 4 h at 60 °C, and solid product (rGO-Schiff-base) was filtered, washed with hot ethanol and dried in vacuum.

6.4. Synthesis of nickel oxide nanocomposites on functionalized reduced graphene oxide (rGO-NiO-NCs)

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rGO-Schiff-base (0.5 g) was dispersed in ethanol and used ultrasonicated for 15 min then added to 10 ml solution of NiCl₂.6H₂O followed by vigorous stirring for 12 h to complete spontaneous aerial oxidation, the solid product was separated by centrifugation at 5000 rpm for 5 min and washed with ethanol solution (3 times). The black solid material (rGO-NiO-NCs) nanocomposites were obtained after drying under vacuum for 12 h.

6.5. Synthesis of 2, 4, 5-trisubstituted-imidazole derivatives

The synthesis of trisubstituted imidazole derivatives was carried out by a simple procedure in which the mixture of benzaldehyde (0.5 mmol), benzil (0.5 mmol), NH₄OAc (1 mmol), in presence of 20 ml of ethanol solution was heated at 55 °C for 60 min in the presence of catalyst (rGO-NiO-NCs) (25 mg). The reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction, the catalyst was separated by centrifugation, and the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was collected dried over anhydrous Na₂SO₄ and concentrated on vacuum evaporator. The product was further purified by recrystallization from ethanol. Different substituted benzaldehydes were subjected to this reaction in the presence of rGO-NiO-Nanocomposites in order to obtained corresponding trisubstituted-imidazole derivatives. Afterward, these particular reaction conditions were quantified for their suitability and efficiency with highly aromatic reactants such as 1-naphthaldehyde, 2-naphthaldehyde, and anthracene-9-carbaldehyde for the synthesis of corresponding trisubstituted imidazole derivatives derivatives shown in (**Table 2 & 3**).

6.6. DNA binding studies

The competitive binding experiments were carried out in the buffer (150 μ M NaCl and 15 μ M trisodium citrate at pH 7.2) by EB bound DNA solution and changing the concentrations of the compounds. The fluorescence spectra of EB bound DNA were measured using an excitation wavelength of 490 nm and the emission range was set between 500 to 800 nm. The stock solution of EB bound DNA complex was prepared by adding 60 μ M EB and 90 μ M CT-DNA in buffer solution and incubated for 30 min at 25 °C before being used for titrations. The quenching ability of the compounds was explored by slowly adding a changing amount of the compounds into the EB-DNA complex.

6.7. Molecular docking

Molecular docking uses the atomic label interaction to define the interaction between the small drug molecule and macromolecules like protein and nucleic acids. Molecular docking

was performed by Autodock Vina. Where the x-center, y-center, and z-center were set as 15.24, 21.41, 10.13 respectively. The search box size was set as 17.15, 21.52 and 40.29 for corresponding axis x, y, and z. The exhaustiveness was set to 8. The post dock modeling was performed *via* Bio-via Discovery Studio 2019. The H-bond length is labeled in the red color.

Supplementary Materials

Supplementary material includes general experimental details and methods, UV-vis and FTIR of the catalysts, Spectral data of ¹H NMR, ¹³C NMR and green matric calculation.

Conflict of interest

The author declares no conflicts of interest.

Acknowledgment

Authors are thankful to the Head, Department of Chemistry, University of Delhi for providing access to University Science Instrumentation Centre (USIC). Authors also thankful to Sophisticated Analytical Instrument Facility of All India Institute of Medical Sciences (SAIF)-AIIMS, New Delhi, to access the TEM facility. Mr. Gyanendra Kumar, Mr. Manish Kumar and Mr. Subodh are grateful to University Grants Commission (UGC-SRF) for providing financial support.

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Graphical Abstract



The NiO nanocomposites immobilized on the surface-functionalized reduced graphene oxide (rGO) sheet. This nano-catalyst has been an excellent catalytic activity, recyclability, and high yields for the synthesis of trisubstituted-imidazoles with their applications in inhibiting DNA binding activity and molecular docking analysis.