Final Progress Report
(July 2012 - December 2015)

MAJOR RESEARCH PROJECT

"Clean synthesis and bioactivity evaluation of newer benzimidazole compounds"

[F.No. 41-302/2012, 13/07/12]

ज्ञान-विज्ञान विमुक्तये

UNIVERSITY GRANTS COMMISSION, NEW DELHI

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Final Report of the work done on the Major Research Project (July 2012 - December 2015)

Objectives of the Project:

- Synthesis of newer benzimidazole compounds through greener approaches.
- Antimicrobial, antioxidant and anticancer activity evaluation of synthesized benzimidazole compounds.

Objective I

Facile one-pot clean synthesis of 2-substituted and 1, 2-disubstituted benzimidazoles using bismuth nitrate as a greener catalyst

1. Introduction

At the beginning of this century, green chemistry has attracted a considerable importance in the development of environmentally benign routes to numerous materials. Green chemistry mainly emphasizes towards the pollution prevention through eco-friendly design of chemical products and processes.[1] The development of greener methodologies for syntheses of heterocyclic compounds is still a stimulating task in the field of organic synthesis. Among the heterocycles, benzimidazole derivatives are the important class of nitrogen containing heterocycles with a wide range of medicinal properties such as serotoninergic 5-HT3 and 5-HT4 receptors in the CNS,[2] antihistamine,[3] anticancer,[4,5] antibacterial,[6] antifungal,[7] anti-inflammatory, antianalgesic,[8] antioxidant,[9] antidiabetic,[10] selective neuropeptide YY1 receptor antagonists,[11] antimalerial, antitubercular,[12] antiulcer,[13] etc. where moiety plays the role of 'Master Key'.[14] Therefore, it is an imperative anchor for development of new therapeutic drugs, as illustrated and supported by some commercial benzimidazole products in Fig. 1.

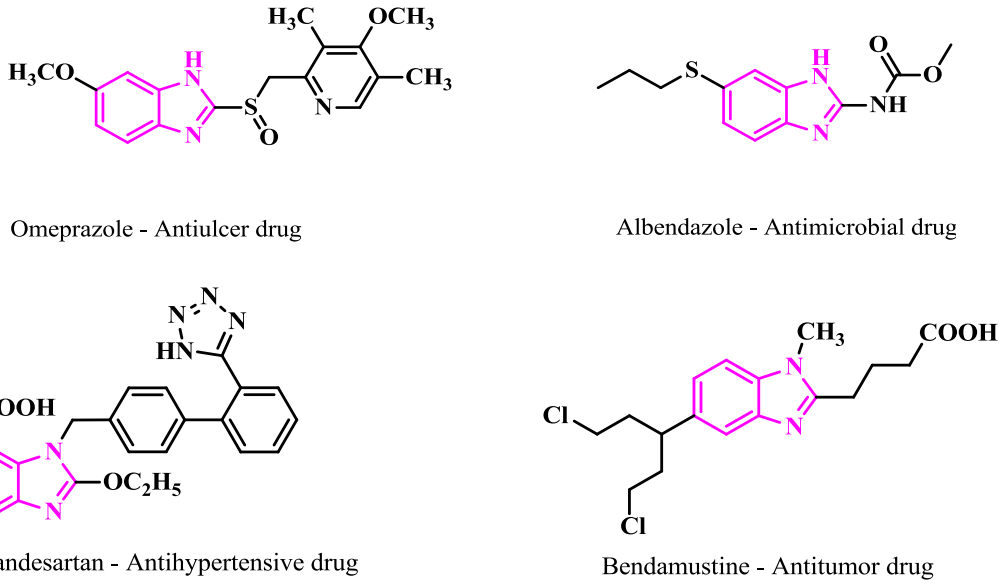


Fig. 1. Benzimidazole containing important commercial drugs.

Generally, the synthesis of benzimidazole involves the reaction of *o*-phenylenediamine either with carboxylic acids, carboxaldehydes or their derivatives (chlorides, nitriles, and orthoesters) under strongly acidic conditions with high temperature,[15] Furthermore, Cascade reactions of *o*-haloaniline with amidine hydrochlorides[16] and intramolecular palladium-catalyzed aryl amination are alternative ways for synthesis of benzimidazole.[17,18] A variety of catalysts are reported in the benzimidazole synthesis, such as $FeCl_3$-doped polyaniline nanoparticles,[19] solvent free $SiO_2/ZnCl_2$,[20] cobalt (II) chloride hexahydrate,[21] $[Sm(OTf)_3]$,[22] $[In(OTf)_3]$,[23] sodium metabisulfite,[24] silphox[$POCl_3$-n $(SiO_2)_n$],[25] potassium persulfate-$CuSO_4$,[26] indion 190 resin,[27] ammonium acetate,[28] thiamine hydrochloride,[29] SDS micelles, DBSA, $Fe_3O_4@SiO_2@(NH_4)_6$-Mo_7O_{24} magnetic core-shell nanocomposite, boron trifluoride etherate ($BF_3.OEt_2$), Cu-nanoparticles/SiO_2, LiBr,[30] *etc.*

At present, bismuth (III) compounds have recently attracted much attention in organic transformations due to their high acidity, thermal stability, low toxicity, low cost, and good

stability [31], Furthermore, bismuth nitrate is reported as an eco-friendly nitrating agent for selective nitration of organic compounds.[32,33] Current literature reveals that bismuth nitrate has been utilized as an effective catalyst in the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones,[34] guanidylation of N-benzoylthioureas,[35] synthesis of coumarins,[36] Paal–Knorr synthesis of pyrroles,[37] chemoselective synthesis of acylals[38] *etc.*

Nevertheless, most of the aforesaid methods of benzimidazole synthesis have disadvantages like, use of expensive reagents and catalysts, harsh reaction conditions and long reaction time *etc.* Moreover, several of these reactions have been reported at higher temperatures which are not accepted as environmentally friendly. Therefore, the search continues for a better catalyst to synthesize benzimidazoles in term of operational simplicity. To address this problem, in our present research investigation, we wish to report bismuth nitrate as an efficient catalyst for synthesis of 2-substituted benzimidazoles and 1,2-disubstituted benzimidazoles. Very interestingly, herein, we revealed that a change of substituent, specifically a replacement of C_3 or C_4 hydrogen by either hydroxyl or methoxy group on the aldehyde unit, dramatically influences the course of the reaction.

2. Experimental

2.1. Chemicals and instruments

All chemicals and materials are procured from S. D. fine chemicals Ltd. and Spectrochem Chemicals Pvt. Ltd. and used without further purification. Melting points were determined with open capillary method and are uncorrected. IR spectra were recorded in KBr on Shimadzu IR Affinity-1 FT-IR spectrophotometer and ^{1}H NMR spectra were recorded on a Bruker Avance II 300 and 400 MHz NMR spectrophotometer in $CDCl_3$/DMSO using TMS as internal standard.

Mass spectra were recorded on Waters, Q-Tof Micromass (LCMS) spectrometer and Varian Inc. 410 Prostar Binary LC with 500 Mass Spectrophotometer.

2.2. General procedure for the synthesis of 2-substituted benzimidazoles and 1, 2-disubstituted benzimidazoles

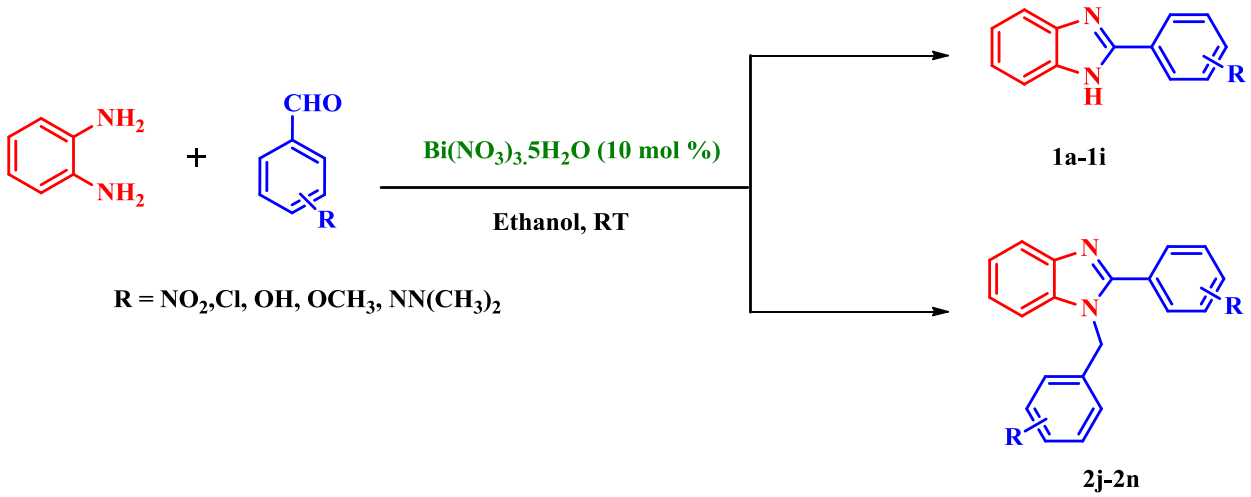
A mixture containing *o*-phenylenediamine (1 mmol), ethanol (5 mL), and bismuth nitrate (10 mol %) was taken in a round-bottom flask. To this mixture, a solution of aldehyde 1.1 mmol for the synthesis of 2-substituted benzimidazoles and 2.1 mmol for the synthesis of 1,2 disubstituted benzimidazole in ethanol (5 mL) was added dropwise with stirring and stirring was continued until the completion of reaction at room temperature. After completion of the reaction (monitored by TLC, Hexane: Ethyl acetate), the reaction mixture was poured into crushed ice to give solid product, which was filtered, washed with water and dried. The crude product was recrystallized from ethanol to afford pure 2-substituted benzimidazoles or 1,2-disubstituted benzimidazoles in good to better yields. Spectroscopic data for all the synthesized compounds are depicted in supplementary data, which is in harmony with the structures.

3. Results and discussion

3.1. Optimization of reaction conditions

In present work, we have reported a simple and environmentally benign, one pot procedure for the synthesis of 2-substituted benzimidazoles and 1,2-disubstituted benzimidazoles. In this study, we examined the synthesis of 2-substituted benzimidazoles by the reaction of *o*-phenylenediamine with 4-nitrobenzaldehyde using bismuth nitrate as catalyst in ethanol at room temperature. The reaction was completed within 60 min. to give the 2-(4-nitrophenyl)-1*H*-benzimidazole as a product with quantitative yield (Scheme 1). Encouraged by this result, we

studied different parameters of reaction and the obtained results are summarized in Table 1, 2, & 3. In order to find out optimum reaction condition for the synthesis of 2-substituted benzimidazole, a separate study with different catalysts and solvents was performed (Table 1 & 2). Bismuth nitrate was found to be an efficient catalyst for synthesis of 2-substituted benzimidazoles over the other catalyst studied (Table 1).



Scheme 1. Bismuth nitrate mediated synthesis of 2-substituted benzimidazole derivatives.

3.2. Effect of catalyst and solvent

In order to study the role of catalyst, a controlled reaction was performed using *o*-phenylenediamine with 4-nitrobenzaldehyde in ethanol without catalyst. The percentages of products formed under controlled condition and with the different catalysts are summarized in Table 1. The results revealed that, under the controlled condition the percentage of products were less even after 8 h in most of the cases, whereas in the presence of bismuth nitrate the obtained yield was highest within a short reaction period of 1 h only. In order to study the effect of solvents over the oxidative coupling of 4-nitrobenzaldehyde with *o*-phenylenediamine, we carried out the reaction in different solvents. Less polar and aprotic solvents like dichloromethane, toluene and tetrahydrofuran were found to be unsuitable for the reactions,

whereas, more polar and protic solvents like ethanol, glycerol, polyethylene glycol (PEG), etc., were appropriate solvents to afford the higher yields (Table 2). Amidst, in order to get more insides about the amount of catalyst required for the efficient conversion, we preformed the experiments by using different percentage loading of catalyst in ethanol at room temperature. The experimentation revealed that 10 mol % loading of catalyst afforded the desired products with highest yield just within 1 h (Table 3). Thus, it was found that bismuth nitrate plays a benign role of accelerator promoting the time and cost effective formation of product.

Table 1 Effect of catalyst on synthesis of 2-(4-nitrophenyl) benzimidazole[a&b]

Entry	Catalyst	Temp (^{0}C)	Time (h)	Yield (%)[c]
1	None	RT	8	45
2	None	78-80	7	60
3	L- Proline	RT	8	52
4	**$Bi(NO_3)_3.5H_2O$**	**RT**	**1**	**96**
5	$ZrO(NO_3).H_2O$	78-80	4	61
6	1,10-Phenanthroline	RT	4	56
7	1,10-Phenanthroline	78-80	5	70
8	$Cd(NO_3).5H_2O$	RT	4	67
9	$Cd(NO_3).5H_2O$	78-80	4	74
10	$Ba(NO_3)_2$	RT	5	65
11	$CsNO_3$	RT	8	58
12	$Pb(NO_3)_2$	RT	8	71
13	$Ca(NO_3)_2.4H_2O$	RT	8	58

[a]Reaction condition: *o*-Phenylenediamine (1mmol), 4-nitrobenzaldehyde (1.1 mmol), and Catalyst (10 mol%).[b]Solvent : Ethanol (10 ml). [c]Isolated yield.

Table 2 Effect of solvent on synthesis of 2-(4-nitrophenyl)benzimidazole[a]

Entry	Solvent	Temp	Time (h)	Yield (%)[b]
1	Solvent free	RT	1	49
2	THF	RT	4	21
3	Acetonitrile	RT	2	63
4	DMF	RT	2	65
5	**Ethanol**	**RT**	**1**	**96**
6	Methanol	RT	1	76
7	Dichloromethane	RT	5	58

8	Glycerol	RT	3	76
9	Glycerol	90 ^{0}c	4	78
10	Toluene	RT	6	40
11	PEG – 400	RT	7	81
12	PEG – 400	90 ^{0}c	3	82

[a]Reaction condition: *o*-Phenylenediamine (1 mmol), 4-nitrobenzaldehyde (1.1 mmol), and Catalyst : Bismuth nitrate (10 mol %), [b]Isolated yield

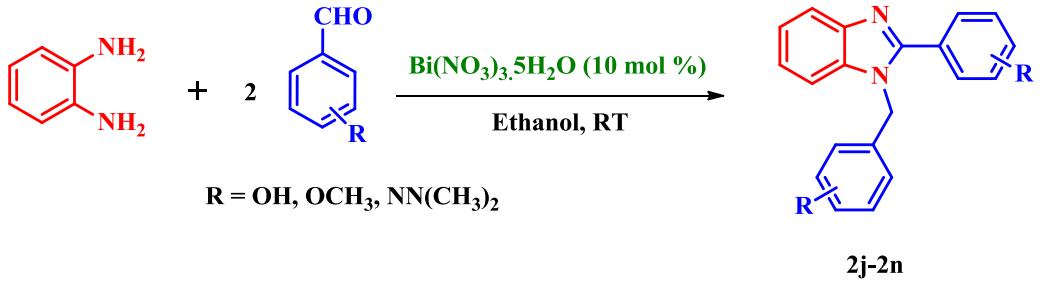
Table 3 Optimization of amount of catalyst for synthesis of 2-(4-nitrophenyl) benzimidazole

Entry	Catalyst loading (mol %)	Time (h)	Yield (%)[b]
1	5	1	84
2	**10**	**1**	**96**
3	15	1	91
4	20	1	94

Table 4 Synthesis of benzimidazole derivatives from *o*-phenylenediamine and aldehydes.

Entry	R	Product	Time (min.)	Yield (%)[b]	M.P. (^{o}C)		Ref
					Found	Reported	
1.	4-NO_2-C_6H_4	**1a**	60	96	314-316	317	[21]
2.	3-NO_2-C_6H_4	**1b**	90	97	144-146	144	[21]
3.	2-NO_2-C_6H_4	**1c**	60	95	264	260-263	[25]
4.	2-Cl-C_6H_4	**1d**	55	76	234	234	[25]
5.	4-Cl-C_6H_4	**1e**	60	84	290	292	[29]
6.	3-Pyridinyl	**1f**	90	73	246	241-243	[26]
7.	3-Indolyl	**1g**	60	85	222-224	225	[26]
8.	2, 5 $(OCH_3)_2$-C_6H_3	**1h**	60	86	218-220	--	--
9.	2-OH-C_6H_4	**1i**	90	85	242	242	[24]
10.	3-OH-C_6H_4	**2j**	300	49	250-252	255-257	[39]
11.	4-OH-C_6H_4	**2k**	300	50	210-212	222	[39]
12.	4-OH,3-OCH_3-C_6H_3	**2l**	300	48	214-216	190-192	[39]
13.	3,4 $(OCH_3)_2$-C_6H_3	**2m**	300	48	168-170	170-172	[39]
14.	4-$(CH_3)_2N$-C_6H_4	**2n**	300	42	172-174	165-167	[40]

[a]Reaction condition: *o*-Phenylenediamine (1mmol), 4-nitrobenzaldehyde (1.1 mmol), and Catalyst : Bismuth Nitrate (10 mol%). [b]Isolated yield



Scheme 2. Bismuth nitrate mediated synthesis of 1, 2-disubstituted benzimidazole derivatives

Table 5 Synthesis of 1, 2 disubstituted benzimidazole derivatives from *o*-phenylenediamine and aldehydes

Entry	R	Product	Time (min.)	Yield (%)[b]	M.P. (°C)		Ref
					Found	Reported	
10.	3-OH-C_6H_4	2j	60	90	250-252	255-257	[39]
11.	4-OH-C_6H_4	2k	65	81	210-212	222	[39]
12.	4-OH,3-OCH_3-C_6H_3	2l	75	91	214-216	190-192	[39]
13.	3,4$(OCH_3)_2$-C_6H_3	2m	60	84	168-170	170-172	[39]
14.	4-$(CH_3)_2N$-C_6H_4	2n	60	85	172-174	165-167	[40]

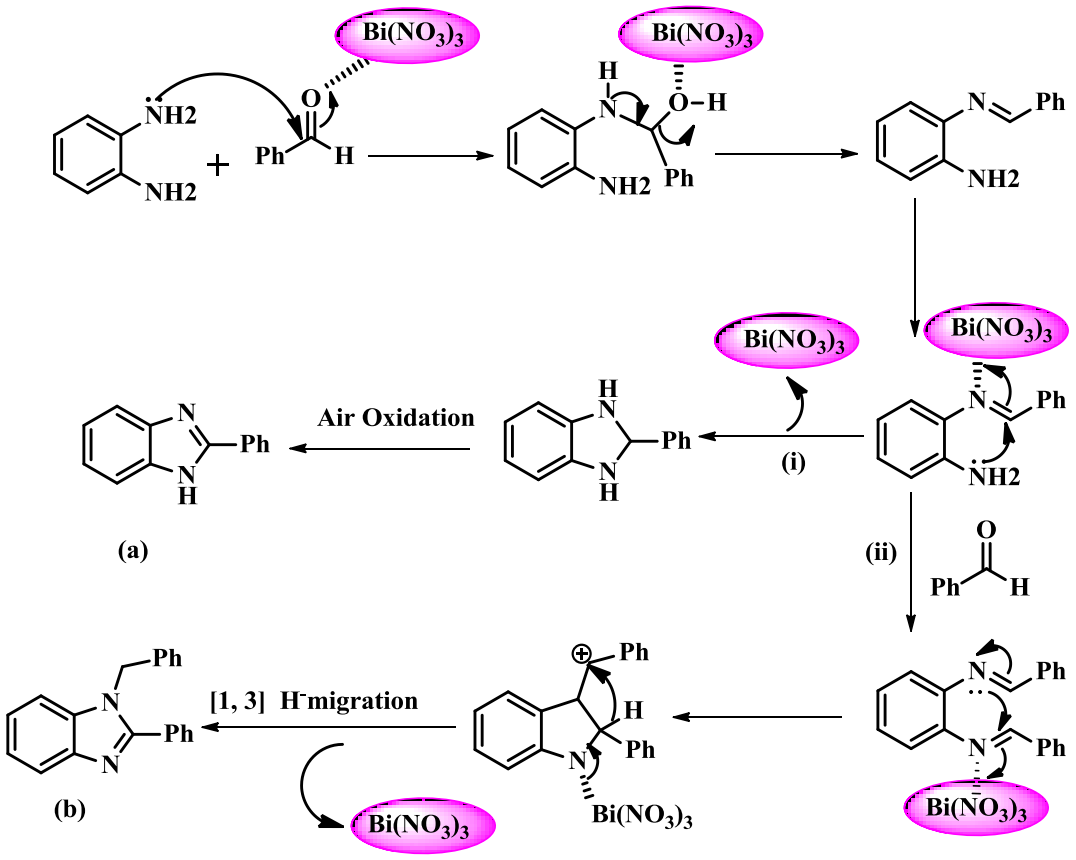
[a]Reaction condition: *o*-Phenylenediamine (1mmol), 4-nitrobenzaldehyde (2.1 mmol), and Catalyst : Bismuth Nitrate (10 mol%), [b]Isolated yield.

Under the same optimized reaction conditions, i.e. by using 10 mol % bismuth nitrate as a catalyst in ethanol at room temperature, we performed the reactions of variety of aromatic and heteroaromatic aldehydes with *o*-phenylenediamine to give quantitative yields of products (Table 4). In several reactions, dialdimines were formed as byproducts (1-5 %) which were separated during recrystallization. Several functional groups such as Cl, Br, NO_2, OCH_3 and sensitive heterocyclic molecules like indole-3-carboxaldehyde and pyridyl-3-carboxaldehyde were compatible with the reaction conditions. All the synthesized compounds were isolated, purified and characterized by FT-IR, ^{1}HNMR, and LC-MS spectroscopic techniques. In ^{1}HNMR analysis, the compound (2n) shows the two sharp singlets for -NCH_3. This is due to conjugation of

unpaired electrons on the nitrogen atom with aromatic π electrons and this conjugation makes a partial double bond between ipso carbon and nitrogen atom.

From the above data, it was observed that the all reactions would have offered the expected corresponding 2-substituted benzimidazoles, but surprisingly the reactions with aromatic aldehydes carrying either hydroxyl or methoxy groups at meta or para positions, resulted in the formation of 1,2-disubstituted benzimidazoles. As per the optimized conditions, we have used *o*-Phenylenediamine: aldehydes (1:1.1 mmol), and found that, still after 5 h *o*-phenylenediamine was unreacted whereas other precursor i.e. aldehyde gets completely consumed. After isolation and purification of product it was observed that the obtained products was 1,2-disubstituted benzimidazoles and not expected 2-substituted benzimidazole. With this observation, we planned to set the reactions with two equivalents of aldehydes and one equivalent of *o*-phenylenediamine under the optimized conditions, where, 1,2-disubstituted benzimidazoles was obtained as major product with excellent yields (Table 5, Scheme 2).

3.3. Plausible mechanism



Scheme 3. Plausible mechanism for synthesis of (i) 2-substituted benzimidazoles (a) and (ii) 1,2-disubstituted benzimidazoles (b).

4. Conclusion

In summary, bismuth nitrate played a crucial role of mild and efficient green catalyst for the syntheses of 2-substituted and 1,2-disubstituted benzimidazoles in quantitative yields from *o*-phenylenediamine and a wide variety of aldehydes. This methodology followed column chromatography-free protocol availing the abolishment of large amount of organic solvents. A one pot synthesis with short reaction time; use of inexpensive, non-toxic, and easily available catalyst; easy isolation of products with higher yields are the key leads of the methodology

executed herein. Thus, this work furthered a green, time as well as cost effective route to researcher's further dealing with these scaffolds in future.

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Outcome of Research:

- Facile one-pot clean synthesis of benzimidazole motifs: Exploration on bismuth nitrate accelerated subtle catalysis, Vilas N. Mahire and Pramod P. Mahulikar* ***Chin. Chem. Lett. 2015, 26, 983–987*** (IF = 1.587) (Research article accepted with Cover page)

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Original article

Facile one-pot clean synthesis of benzimidazole motifs: Exploration on bismuth nitrate accelerated subtle catalysis

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ABSTRACT

In the present letter, an efficient, clean and one-pot synthesis of 2-substituted benzimidazole and 1,2-disubstituted benzimidazole derivatives has been explored by reacting *o*-phenylenediamine with aromatic aldehydes using bismuth nitrate as a catalyst in ethanol at ambient temperature. This methodology avails with faster reactions, excellent yield, mild reaction conditions, use of inexpensive and non-toxic catalyst compared to literature reported hitherto.

Objectives II & III

Synthesis and bioactivity evaluation of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one using Silane@TiO_2 as a reusable nanocatalyst

1. Introduction

In recent years, nano-catalysis has been utilized as an alternative approach for the improvement in rates, yields and workability of many significant organic reactions. This also leads to a search for or design of new efficient affordable catalyst for specific applications in synthetic chemistry.[1] Nanoparticles have large surface area and due to this they get strongly agglomerated and hence their surface modification is an alternative way to produce new hybrid nanoparticles having virtuous properties.[2,3] The covalent attachment of functionalities on to the surface of nanoparticles enhances their newer properties which either increases the interaction or decrease the aggregation of nanoparticles which lead to various purposes to wider applications.[4-7] Nano materials have wide applications in science and technology such as medicinal, cosmetics and environmental purification etc. Titanium dioxide belongs to the family of transition metal oxides and is considered as an ideal photocatalyst because of its merits such as low cost, nontoxicity, high stability, optical and electronic properties etc. Furthermore, its band gap is sufficient to initiate a variety of organic reactions.[8,9]

Multi-component reactions are becoming the most significant synthetic tool for synthesis and design of new libraries of molecules of pharmaceutical interest through single step reaction.[10-12] In modern organic synthesis, combination of green chemistry and multi-component reactions will lead to generation of structural complexity in a single step from three or more reactants with greater efficiency and atom economy.[13-16] Therefore, the design of efficient multi-component reactions offers an enduring challenge for synthetic organic chemists.

In the development of several new drugs, the fused heterocyclic scaffolds with nitrogen and oxygen atoms play an important role in medicinal chemistry. Benzimidazole derivatives are important pharmacophores and considered as a privileged structures in medicinal chemistry.[17-18] Benzimidazole derivatives have been reported to possess diverse biological activities such as antihistamine, antitumor, antibacterial, antifungal and antioxidant[19] etc. Various substituted benzimidazoles are known to have diverse biological activities and among them 2-substituted benzimidazoles are found to be more potent.[20] Furthermore, the chromene[2,3-d]pyrimidine derivatives occupy an important place in the realm of natural and synthetic organic chemistry because of their biological and pharmacological activities such as antioxidant, antimicrobial,[21] antitumor,[22] antitubercular,[23] analgesic,[24] inflammatory[25] etc.

To the best of our knowledge only Heravi *et al*. and Mazhukina *et al*. have reported the synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one derivatives.[26] However, there are no other reports on the synthesis of these benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-ones. This inspired us to continue our work on the multi-component synthesis under environmentally benign approach. In this current article, we wish to report synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one derivatives by a reaction of 4-hydroxycoumarin (**1**) with aromatic aldehydes (**2a-q**) and 2-aminobenzimidazole (**3**), in the presence of catalytic amount of silane@TiO_2 nanoparticles as an efficient catalyst (Scheme 2). Among the synthesized compounds most of the compounds showed comparable antibacterial and antioxidant activities. However, the synthesized compounds were found to be inactive against human cancer cell line HL60 and MCF7.

2. Experimental

2.1 Material and Methods

All chemicals purchased from Sigma-Aldrich and S. D. fine chemicals Ltd. were used without further purification. All solvents and chemicals obtained from S. D. fine chemicals used were of reagent grade. Melting points of all synthesized compounds were taken in open capillary tubes and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on 0.2 mm precoated silica-gel 60 F254 plates (E. Merck). The spots were detected under ultraviolet (UV) light. The IR spectra (KBr) were recorded on Shimadzu IR Affinity-1 FT-IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a FT-NMR Cryo-magnet Bruker Avance-II spectrometer at 400 and 100 MHz respectively using DMSO-d_6 as solvent and the chemical shift values are recorded on the δ scale and coupling constant (*J*) values are in hertz (Hz). Mass spectra were recorded on Waters, Q-Tof Micromass (LCMS) spectrometer. TGA data were obtained with Perkin Elmer instrument at the temperature range of 28-800 ^{o}C at a constant heating rate of 10 ^{o}C/min in the nitrogen atmosphere (20 mL/min). X-ray diffractograms (XRD) of the catalyst were recorded in the range of 10-80 on a Bruker X-ray diffractometer with Ni-filtered Cu Kα radiation at a wavelength of 1.54060 Å. The FE-SEM analysis of the catalyst was performed on a Hitachi S-4800 scanning electron microscope (Japan) with accelerating voltage of 30 Kv. The EDX analysis of catalyst was performed by using Bruker EDX flash detector mode 5030. The Transmission Electron Microscopy of the catalyst was performed on PHILIPS CM200 of operating voltages of 20-200 kv, Resolution 2.4 A^{o} and DPPH radical scavenging assay was performed on Shimadzu UV mini-1240 UV-Vis Spectrophotometer.

2.2 Preparation of Silane@TiO_2 nanoparticles

The TiO_2 nanoparticles were synthesized by sol-gel method[27] and then treated for surface modification. The surface modification of TiO_2 was achieved by reacting TiO_2 nanoparticles with

vinyltriethoxysilane.[28] Typically, TiO_2 nanoparticles (1 g) were dispersed in acetone (10 mL) and the mixture was sonicated for 5-6 minutes. Simultaneously, vinyltriethoxysilane (0.5 mL) solution was prepared in acetone (10 mL) and then added slowly into TiO_2 solution under vigorous stirring provided by a high speed disperser and the mixture was stirred for an additional 20 minutes. After the modification of TiO_2, acetone was removed by evaporation. The silane@TiO_2 nanoparticles were washed with ethanol three times for the removal of physically adsorbed vinyltriethoxysilane. Finally, the silane@TiO_2 powder was dried at 110 ^oC for 2 h in oven. The schematic representation for the synthesis of silane@TiO_2 is summarized in Scheme 1.

2.3 General procedure for synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one

In a 50 mL round-bottom flask, a mixture of 4-hydrocoumarin **1** (2 mmol, 0.32 g), aldehyde **2a-q** (2 mmol), 2-aminobenzimidazole **3** (2 mmol, 0.27 g) and 10 mol % of silane@TiO_2 catalyst in ethanol (5 mL) was heated at reflux for specified time (Table 3). After completion of the reaction (monitored by TLC), the reaction mixture was filtered and then the solid residue was dissolved in DMF (5 mL), followed by centrifugation of the mixture for 5-10 min at 2000-3000 rpm to remove the catalyst. The organic solution was then poured into cold water (15 mL), filtered, washed with ethanol and dried to afford the pure products. (The residual catalyst was washed with ethanol and dried in oven at 110 ^oC for 2-3 h and reused)

2.4 Biological characterization of synthesized compounds

2.4.1 DPPH free radical scavenging assay

The scavenging of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical by chemically synthesized seventeen compounds were analysed. One millilitre of 0.2 mM DPPH reagent prepared in methanol were added in tubes containing 0.8 mL of each compound (1 mg mL^{-1} in

DMSO) and the mixture was allowed to stand for 30 min in the dark at room temperature. Similarly, same protocol was performed for L-ascorbic acid as a standard antioxidant. The absorbance of the resulting mixture was measured at 517 nm using UV-Vis spectrophotometer. The control was prepared by adding only DMSO to DPPH reagent and the analysis followed as described above. The % scavenging activity was determined as follows.

$$\text{Radical scavenging activity (\%)} = [(A_0-A_1)]/A_0\times 100$$

Where, A_0 is the absorption of the control (blank, only DMSO) and A_1 is the absorption of the compound.

2.4.2 Antibacterial evaluation

The antibacterial activity of the synthesized compounds was studied by disc diffusion method. Sterilized filter paper discs (5 mm diameter) impregnated with a solutions of the test compounds in DMSO (200, 400, 600, 800 and 1000 μg/mL) were placed on an agar plate seeded with the appropriate test organisms. Gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) were used as test organisms. Streptomycin was used as standard antibacterial agent while DMSO was used as a control. The plates were incubated at 37 ^{o}C for 24 h and zone of inhibitions (dia, mm) shown by compounds were measured.

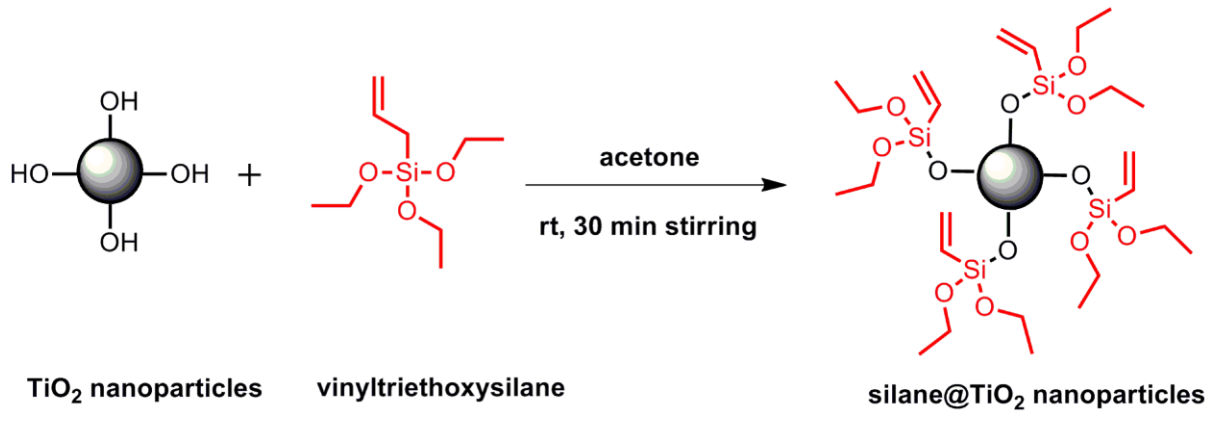
2.4.3 SRB assay procedure

The cell lines (HL60, MCF7) were grown in RPMI 1640 medium containing 10 % fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 μL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in dimethyl

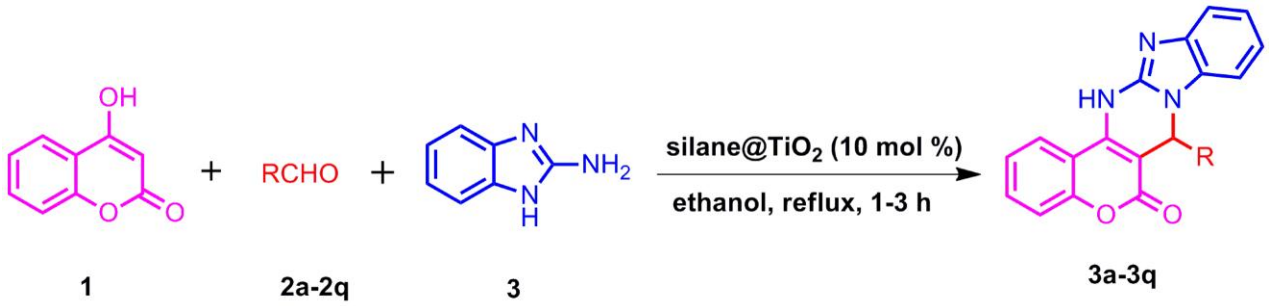
sulphoxide (DMSO) to prepare stock of 10^{-2} concentration. At the time of experiment four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. The cell growth at absence of any test material was considered 100% and in turn growth inhibition was calculated. GI_{50} values were determined by regression analysis.

3. Results and discussion



Scheme 1. Schematic route for the synthesis of the silane@TiO_2 nanoparticles.



Scheme 2. Synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one using silane@TiO_2 nanoparticles.

3.1 FT-IR analysis of the catalyst

The surface modification of TiO_2 nanoparticles was evaluated by FT-IR spectroscopy. The Figure 1 shows the FT-IR spectrum of TiO_2 (a), silane@TiO_2 (b) and vinyltriethoxysilane (c). In the spectrum 1a, the hydroxyl groups present on the surface of nano TiO_2 exhibit absorption bands at 3348 and 3453 cm^{-1} respectively, which were found to be absent in the spectrum 1b. This indicates the absence of hydroxyl groups on the surface of silane@TiO_2, as ethoxy groups of vinyltriethoxy silane get condensed with hydroxyl groups present on TiO_2 surface. The bands at 1100 cm^{-1} and 760 cm^{-1} are assigned to the symmetrical and asymmetrical stretching of the Si-O bonds, respectively. The bands appearing at 1400 cm^{-1} also substantiate the presence of Si-C bond of vinyltriethoxy silane. The absorption band at 2990 cm^{-1} is attributed to symmetrical and asymmetrical stretchings of $–CH_2$ and $–CH_3$ groups. Thus, FT-IR spectra confirm the successful surface modification of TiO_2 nanoparticles.

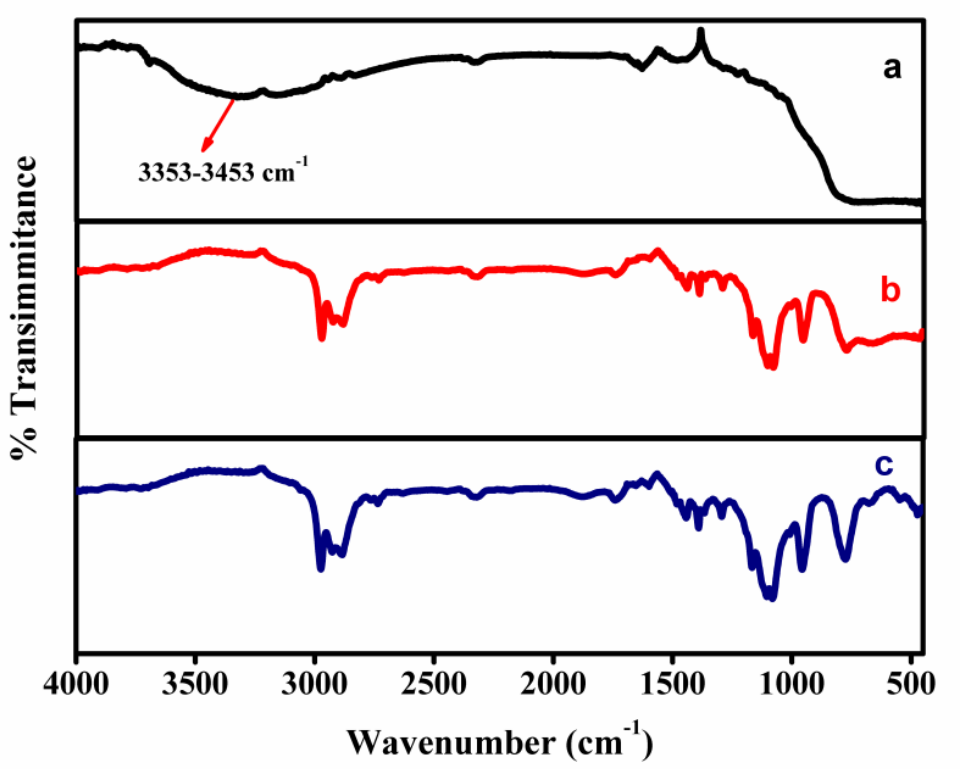


Figure 1. FT-IR spectrum of (a) TiO_2, (b) silane@TiO_2, (c) vinyltriethoxysilane.

3.2 XRD spectral analysis

Figure 2 shows the XRD patterns of the TiO_2 and silane@TiO_2 nanoparticles. The peaks appearing at 101, 111, 112, 210, 121, 212, 032, 114, 024 are for the crystalline portion of TiO_2. The patterns allow the comparison with similar peaks appeared in case of silane@TiO_2 compared to the TiO_2 nanoparticles, at 2θ values corresponding to the planes 101, 112, 200, 211, 204, 220, 301. Therefore, it is satisfying to observe that the modification of TiO_2 does not change the crystalline structure of silane@TiO_2. The XRD pattern of silane@TiO_2 shows broad peak at 2θ =15-25^o indicates the amorphous nature of silica.[29] The crystalline size of most intense peak of silane@TiO_2 nanoparticles, determined from the half-width of the diffraction using the Debye-Scherrer equation, is approximately 5.4 nm. The Sherrer's equation is $D=K\lambda/\beta\cos\theta$, where D is

the crystallite size, λ is wavelength of the radiation, θ is the Bragg's angle and β is the full width at half maximum.

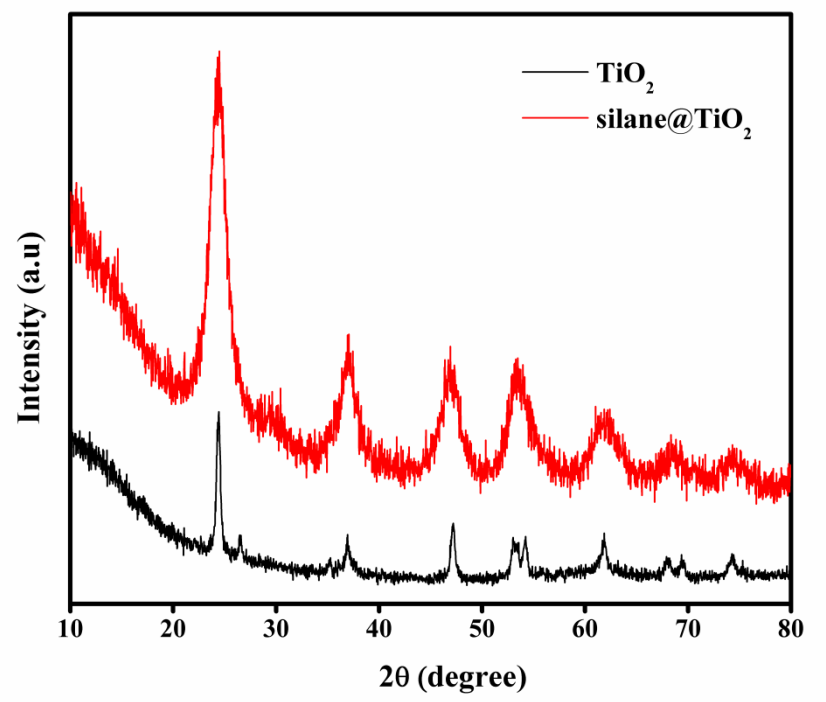


Figure 2. XRD pattern of TiO_2 and silane@TiO_2 nanoparticles.

3.3 FE-SEM and EDX analysis of the catalyst

FE-SEM analysis is another useful tool for the analysis of the surface morphology of a catalyst. The FE-SEM images of TiO_2 and silane@TiO_2 nanoparticles are shown in Figure 3a-b. Notably, the particles are seen to be in the nano range (< 100 nm). Figure 3c show the EDX spectrum of silane@TiO_2 nanoparticles in which the characteristic peaks of Si and C are observed, which confirm the conversion of TiO_2 into silane@TiO_2 nanoparticles.

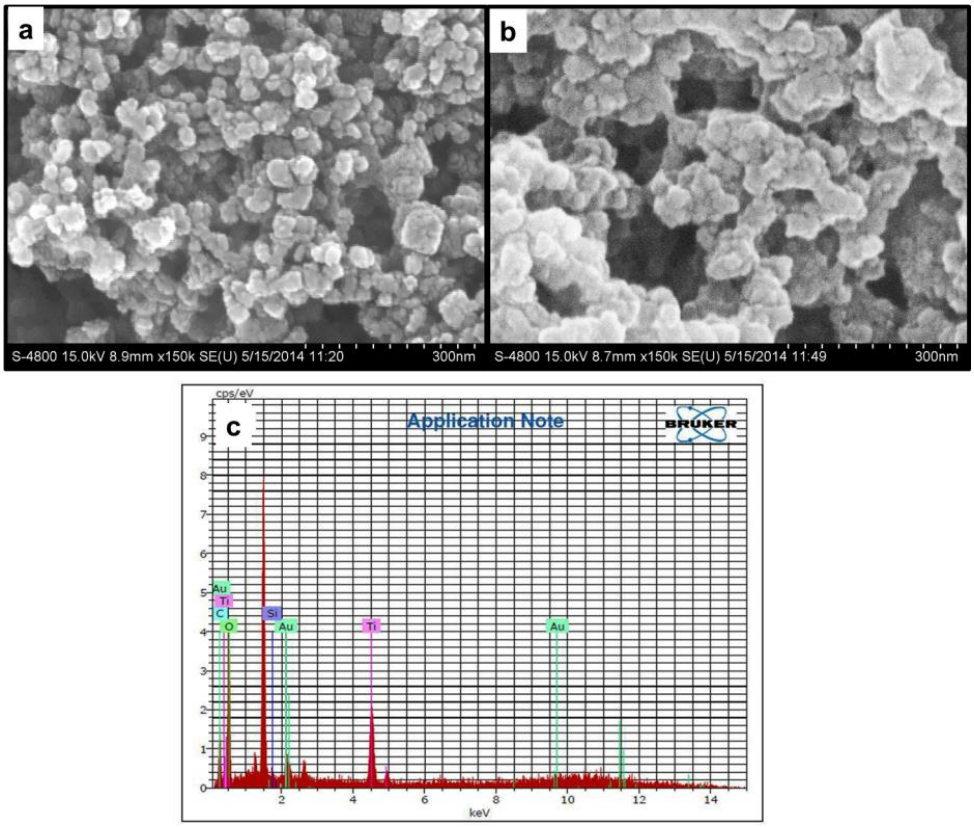


Figure 3. (a, b) FE-SEM images of TiO_2 and silane@TiO_2 nanoparticles, (c) EDX spectrum of silane@TiO_2 nanoparticles.

3.4 TG analysis of the catalyst

The thermal properties of TiO_2 and silane@TiO_2 nanoparticles were investigated using thermo gravimetric analysis (TGA) and the observed result are shown in Figure 4. The TGA curve (a) shows slight weight loss (4.83 %) probably due to vaporization of water or other volatile impurities that physically adsorbed at the particle surface. The TiO_2 nanoparticles were relatively stable in air and merely somewhat decomposed. The TGA curve (b) shows two step degradation in that the first weight loss started from 28-165 ^oC (10.69 %) and second weight loss began in the range of 165-762 ^oC (7.61 %). The silane@TiO_2 nanoparticles showed two steps weight loss which is attributed to dehydration of the water absorbed by nanoparticles and oxidative thermal decomposition of the grafted vinyltriethoxy silane on the TiO_2 nanoparticles.

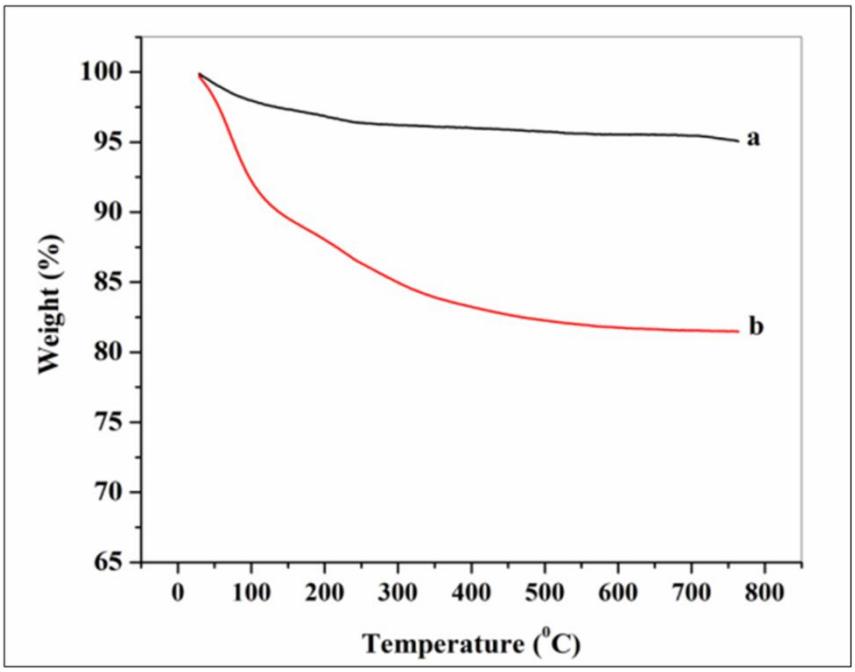


Figure 4. TGA thermograms of (a) TiO_2 and (b) silane@TiO_2 nanoparticles under nitrogen atmosphere at a heating rate of 10 ^oC/min.

3.5 TEM analysis

The morphology of the nanoparticles of TiO_2 and silane@TiO_2 (Fig. 5a, b) was studied using transmission electron microscopy (TEM). From TEM images it is observed that, the TiO_2 nanoparticles have spherical morphology with an average particle size of about 8 nm, while the silane@TiO_2 nanoparticles show distorted spherical morphology with the average particle size of about 9 nm. The TEM images thus indicate that the synthesized nanoparticles have nearly same crystalline character as that of TiO_2. The selected area electron diffraction (SAED) image of silane@TiO_2 nanoparticles (Fig. 5c) show ring patterns, confirming the crystalline nature.

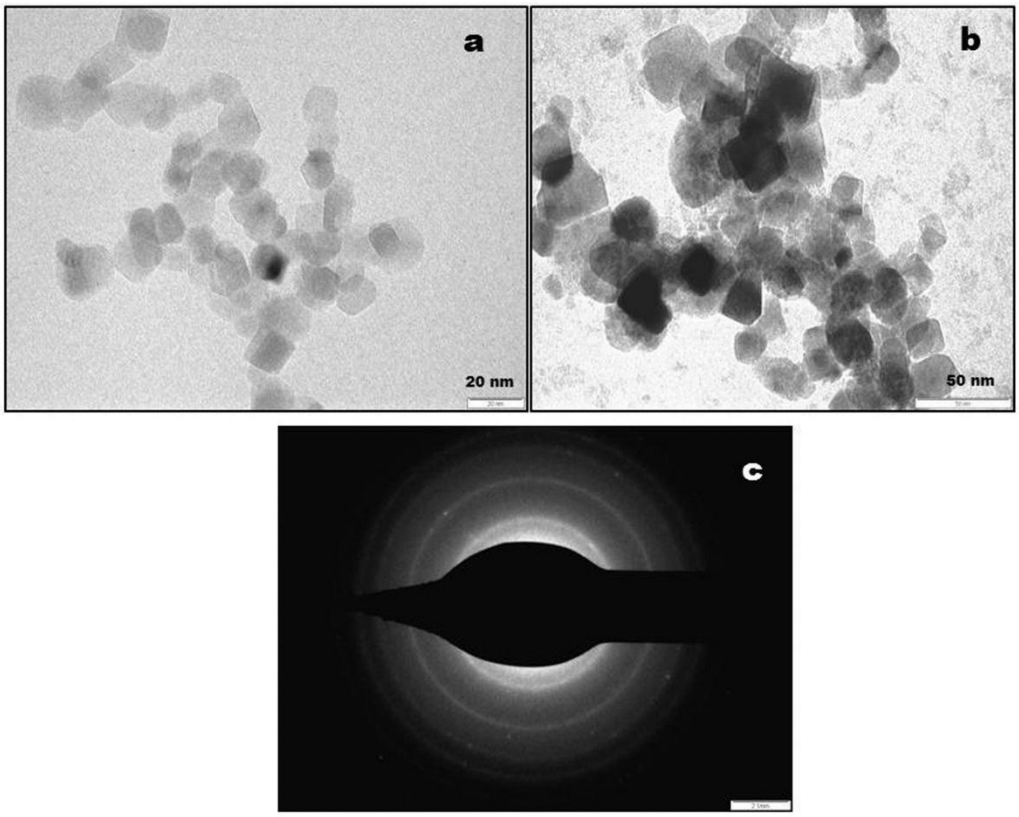
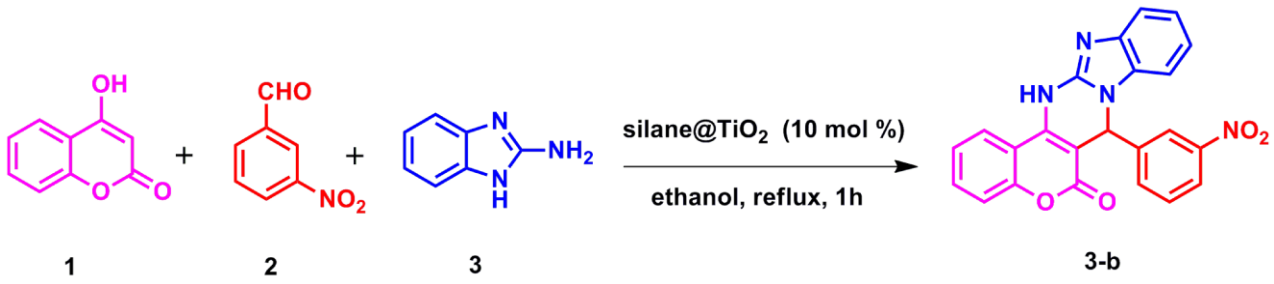


Figure 5. (a, b) TEM images TiO_2 and silane@TiO_2 nanoparticles. (c) Selected area electron diffraction pattern of silane@TiO_2 nanoparticles.

3.6 Optimization of reaction conditions



Scheme 3 Reaction of 4-hydroxycoumarin (**1**), 3-nitrobenzaldehyde (**2**), and 2 aminobenzimidazole (**3**) in the presence of silane@TiO_2 nanoparticles.

We began our study from the reaction of 4-hydroxycoumarin **1** (2 mmol) with 3-nitrobenzaldehyde **2** (2 mmol), and 2-aminobenzimidazole **3** (2 mmol) as the model starting

material in the presence of 5 mol % silane@TiO_2 nanoparticles in ethanol (5 mL) at 78-80 ^oC for 1 h to give the desired product **3b** in 75 % yield. Encouraged by this result, we further moved to study and optimize the best reaction conditions by using different amounts of silane@TiO_2 nanoparticles. The model reaction was stirred under same reaction conditions in the absence of silane@TiO_2 nanoparticles which yielded the target product **3b** in 33% yield only even after 8 h (Table 1, entry 1). Moreover, an increase in the amount (0 mol % to 10 mol %) of silane@TiO_2 nanoparticles decreased the reaction time and increased the yield of the product. (Table 1, entries 1–4). However, the yield did not increased when excess amount (15 mol %) of silane@TiO_2 nanoparticles were used under the similar reaction conditions. Therefore, 10 mol % of silane@TiO_2 nanoparticles were efficiently utilized for this multi-component reaction. Again, the model reaction was chosen for examining the effect of solvent on rate of reaction (Table 2). The low yield of the target product **3b** was obtained when the mixture was refluxed for 6 h in the presence of 10 mol % of silane@TiO_2 nanoparticles in acetonitrile, toluene, THF, DMF etc. (Table 2, entries 1, 2, 3, 7). The reaction using ethanol as the solvent gave the corresponding product **3b** in high yield (81 %) with short reaction time (Table 2, entry 6), However, when the model reaction was carried at room temperature in ethanol (Table 2, entry 5), even after 12 h only 30 % yield was obtained. From these experimentations, it is clear that reaction could not proceed smoothly at room temperature and hence refluxing under ethanol was preferred. Here, ethanol was chosen as a reaction medium for all further reactions since it is accepted as a green solvent. Therefore, the best and the most optimum reaction condition for the target reaction is 10 mol % of silane@TiO_2 nanoparticles as the catalyst in ethanol at reflux temperature. To study the recyclability and catalytic activity of the catalyst, the silane@TiO_2 nanoparticles (10 mol %) were used for the model reaction (Table 1). The silane@TiO_2 nanoparticles can be recycled and

reused as a catalyst for at least three times without significant loss in catalytical activity for this multi-component reaction.

To realize the efficiency and limitations of the catalyst in this multi-component reaction, we applied the optimized reaction conditions of 10 mol % silane@TiO_2 nanoparticles in ethanol at reflux temperature to a series of aldehydes (Table 3). The aromatic and hetero-aromatic aldehydes could react smoothly to give the corresponding benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one in good yields (Table 3, entries 1–17). Most significantly, aromatic aldehydes carrying either electron-donating or electron-withdrawing substituents including hydroxyl group and methoxy group could react efficiently to give the corresponding products.

Table 1. Effect of catalyst loading on the model reaction[a]

Entry	Catalyst (mol %)	Time (h)	Yield of 3b[b] (%)
1	0	8	33
2	5	1	75
3[c]	**10**	**1**	**81,79,75**
4	15	1	80

[a]Conditions: 4-hydroxycoumarin (2 mmol), 3-nitrobenzaldehyde (2 mmol), 2-aminobenzimidazole (2 mmol) and silane@TiO_2 nanoparticles (10 mol %), solvent (5 mL). [b]Isolated yields. [c]Catalyst was recycled for three times.

3.6.1 Effect of different reaction media

Table 2. Reaction of 4-hydroxycoumarin (**1**), 3-nitrobenzaldehyde (**2**), and 2 aminobenzimidazole (**3**) in different solvents[a].

Entry	Solvent	Temp (^oC)	Time (h)	Yield of 3b[b] (%)
1	Acetonitrile	80	6	47
2	Toluene	100	6	36

3	THF	Reflux	6	40
4	Acetone	Reflux	2	60
5	Ethanol	RT	12	30
6	**Ethanol**	**78-80**	**1**	**81**
7	DMF	120	6	53
8	DCM	Reflux	5	46

[a]Conditions: 4-hydroxycoumarin (2 mmol), 3-nitrobenzaldehyde (2 mmol), 2-aminobenzimidazole (2 mmol), and, silane@TiO_2 nanoparticles (10 mol %), solvent (5 mL). [b]Isolated yields.

Table 3. Synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one using silane@TiO_2 nanoparticles[a].

Entry	**Ar**	**Product**	**Time (h)**	**Yield (%)[c]**	**M.P. (^oC)**
1	2-NO_2-C_6H_4	**3a**	1	75	230-232
2	3-NO_2-C_6H_4	**3b**	1	81	246-248
3	4-NO_2-C_6H_4	**3c**	1	82	208-210
4	2-Pyridinyl	**3d**	2	90	228-230
5	3-Pyridinyl	**3e**	2	83	>250 (dec.)
6	4-OCH_3-C_6H_4	**3f**	2	70	180
7	4-CH_3-C_6H_4	**3g**	2	90	202-204
8	4-Cl-C_6H_4	**3h**	2	91	218-220
9	3,4-$(OCH_3)_2$-C_6H_3	**3i**	1	91	180
10	4-F-C_6H_4	**3j**	1	91	230-232
11	3-Cl-C_6H_4	**3k**	1	86	238-240
12	3-OH-C_6H_4	**3l**	2	85	>250 (dec.)
13	4-OH-C_6H_4	**3m**	2	89	246-248
14	4-OH, 3,5-$(OCH_3)_2$-C_6H_2	**3n**	3	75	208-210
15	3-OH, 4-OCH_3-C_6H_3	**3o**	2	71	182-184

16	1-Naphthyl	**3p**	3	96	234-236
17	4-OH, 3-OCH_3-C_6H_3	**3q**	2	89	166-168

[a]General reaction conditions: 4-hydroxycoumarin **1** (2 mmol), aldehydes **2a-2q** (2 mmol), 2-aminobenzimidazole **3** (2 mmol), and silane@TiO_2 nanoparticles (10 mol %), ethanol (5 mL). [b]All products were characterized by IR, ^1H and ^{13}C NMR and mass spectrometry. [c]Isolated yield

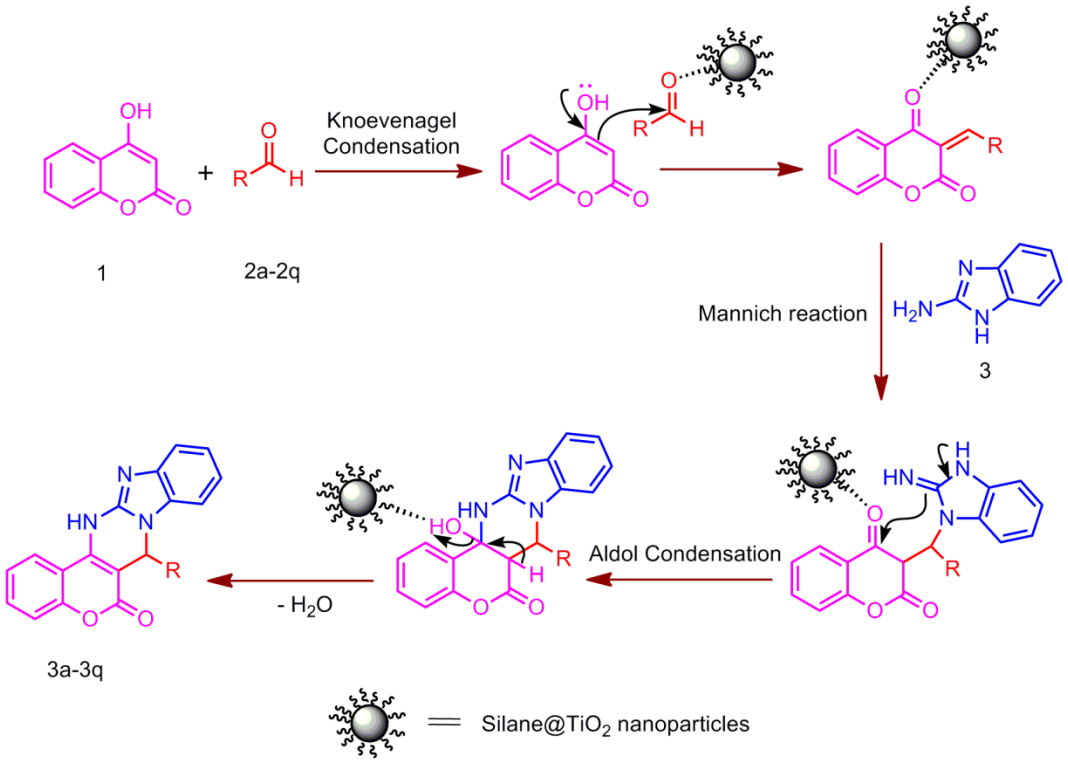


Figure 6. Plausible mechanism for synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one.

3.7 Biological evaluation

3.7.1 DPPH Radical scavenging assay

Antioxidant activity of compounds is related to their electron or hydrogen radical donating ability to DPPH radical, so that they become stable diamagnetic molecules. The DPPH is a stable free radical and it accepts an electron or hydrogen radical to become as a stable diamagnetic molecule.[30] The reduction competency of DPPH radicals was determined by a

decrease in their absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of the reaction between antioxidant molecules and radical progresses, which resulted in the scavenging of the radical by hydrogen donation.

The electronic absorption spectra of solutions of compounds (3a-q) were measured in DMSO. All synthesized compounds were screened for their antioxidant activity by measuring their scavenging ability towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The disappearance of DPPH was measured spectrophotometrically at 517 nm using ascorbic acid as standard. The results are shown in Figure 7. It can be concluded from Figure 7 that four compounds **3i**, **3n**, **3o** and **3q** showed comparable radical scavenging activity in comparison with standard ascorbic acid, whereas other compounds **3b**, **3e**, **3f**, **3g**, **3h**, **3l** and **3p** showed lower scavenging activity.

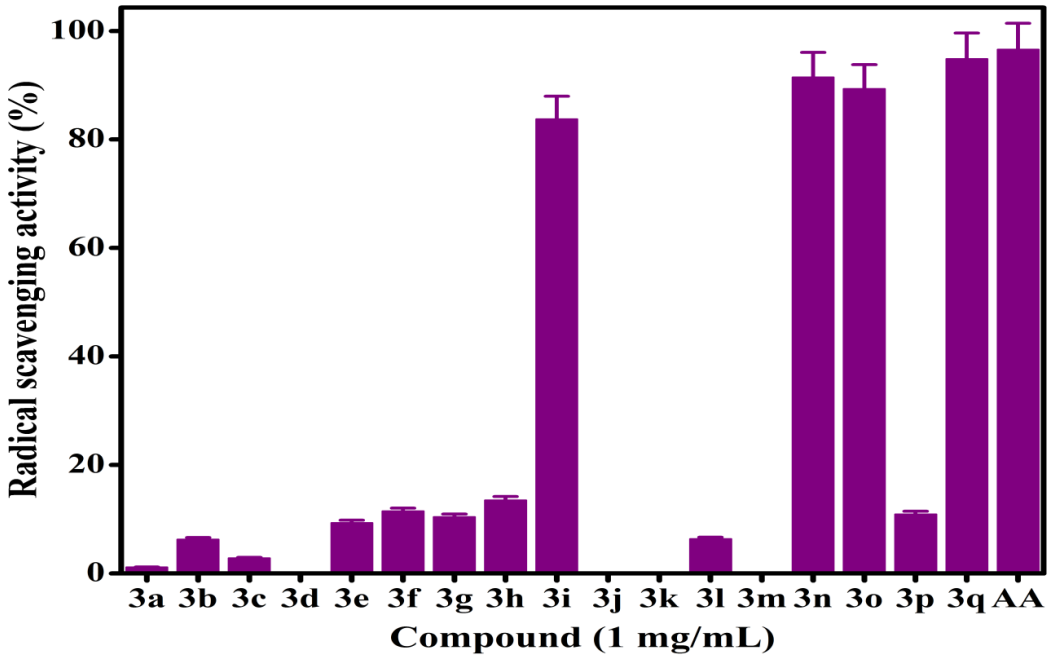


Figure 7. DPPH radical scavenging assay of synthesized compounds (3a-3q).

3.7.2 Antibacterial Activity

All the synthesized compounds were screened for their *in vitro* antibacterial activity against gram-positive bacteria (*Staphylococcus aureus* ATCC-29213) and gram-negative bacteria (*Escherichia coli* ATCC-8739). A disc diffusion method was used for the determination of antibacterial activity using streptomycin as reference drug. The five concentrations of test compounds i.e., 200, 400, 600, 800, 1000 µg/mL were chosen for evaluation. The results were noted for each tested compound as the average diameter (mm) of inhibition zone of bacterial growth around the discs (Fig. 8, 9).

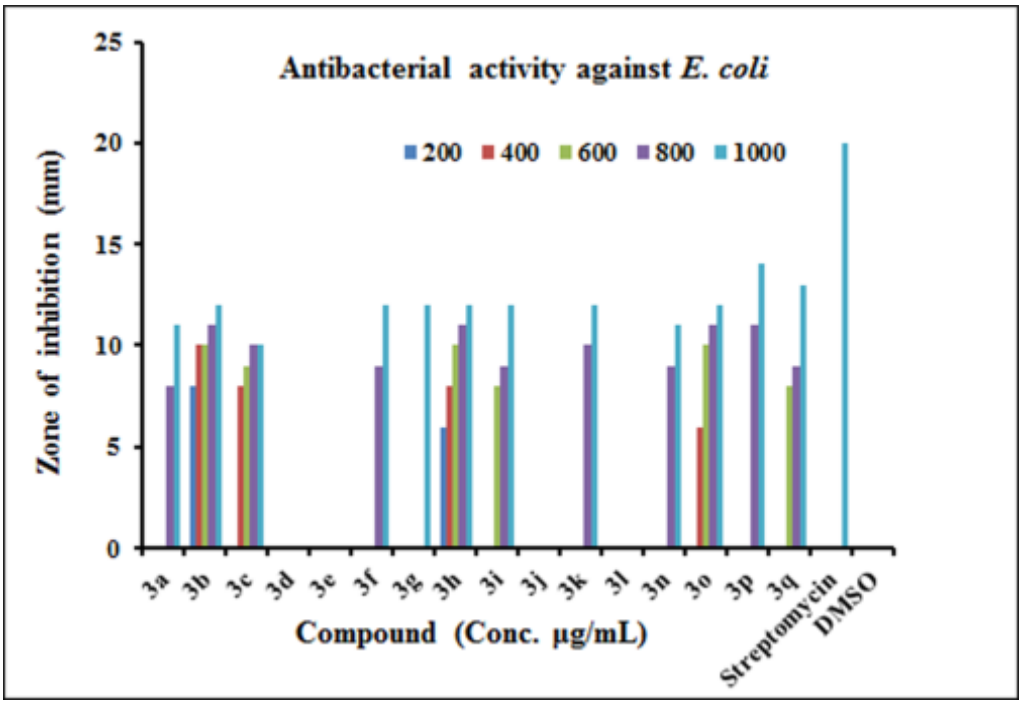


Fig. 8 Antibacterial activity of compounds (3a-3q) against *Escherichia coli*.

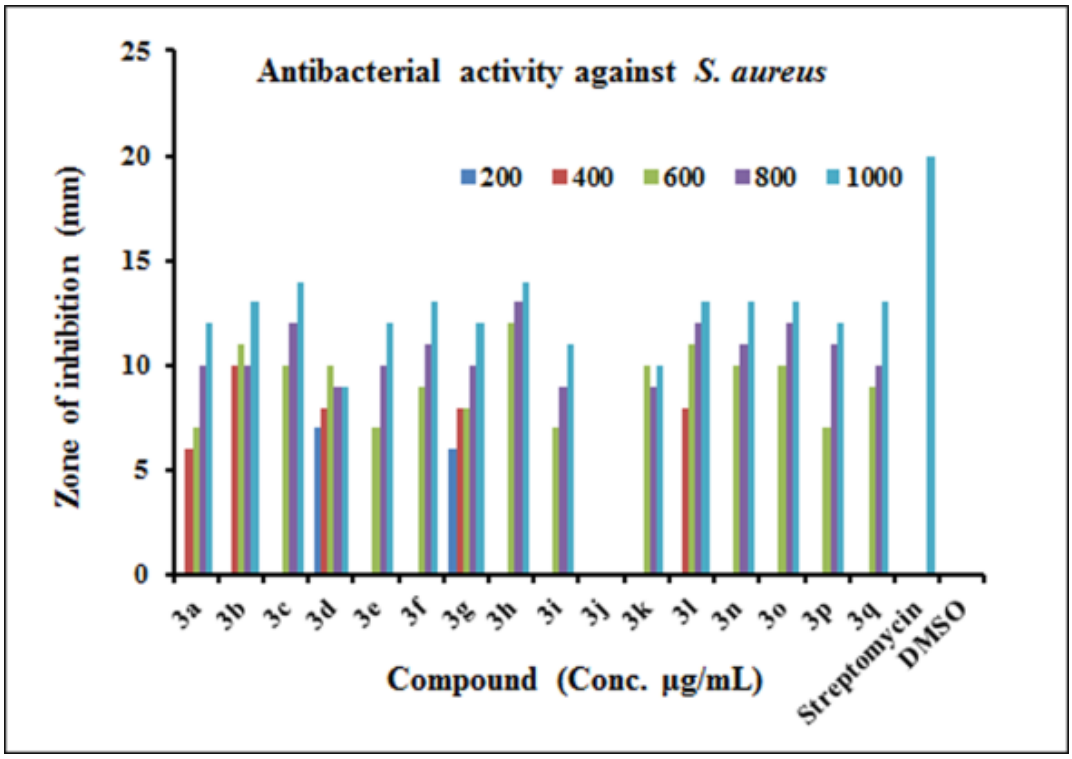


Fig. 9 Antibacterial activity of compounds (3a-3q) against *Staphylococcus aureus*.

3.7.3 *In-vitro* anticancer activity

Anti-cancer screening against cell line HL-60 (Human leukemia cell line), MCF7 (Human breast cell line)

Total 15 synthesized derivatives of benzo[4,5]imidazo[1,2 a]chromeno[4,3-d]pyrimidin-6-one were tested for their *in-vitro* anticancer potential against two human cancer cell lines; Human leukemia cell line (HL60) and Human breast cell line (MCF7). The 48 h continuous drug exposure protocol was followed using sulforhodamine B (SRB) protein assay.[31] The viability of cells was determined for the tested compounds at four different concentrations 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M using DMSO as solvent. The *in-vitro* anticancer activity are expressed as GI_{50} [µM], the concentration of compound resulting in a 50 % reduction in the net protein increase. Adriamycin (Doxorubicin) was used as a positive control drug. The results of

anticancer screening are summarized in Table 4 and growth curves showing activity of tested of substituted benzo[4,5]imidazo[1,2 a]chromeno[4,3-d]pyrimidin-6-one (3a-3q) against HL60 and MCF7 cell lines in comparison with the standard Adriamycin are shown in Figure S2 and S3 (Supplementary information).

Table 4 *In vitro* anticancer activity of substituted benzo[4,5]imidazo[1,2 a]chromeno[4,3-d]pyrimidin-6-one (3a-3q) against HL-60 and MCF7 cell line[a]

Compound	**LC_{50}[b]**		**TGI[c]**		**GI_{50}[d]**	
	HL60	MCF7	HL60	MCF7	HL60	MCF7
3a	NE	>100	NE	>100	NE	>100
3b	NE	>100	NE	>100	NE	79.2
3c	NE	>100	NE	>100	NE	98.6
3d	NE	>100	NE	>100	NE	87.6
3e	NE	>100	NE	>100	NE	>100
3f	NE	>100	NE	>100	NE	>100
3h	NE	>100	NE	>100	NE	98.5
3i	NE	>100	NE	>100	NE	>100
3j	NE	>100	NE	>100	NE	>100
3k	NE	>100	NE	>100	NE	>100
3l	NE	>100	NE	>100	NE	>100
3m	NE	>100	NE	>100	NE	>100
3n	NE	>100	NE	>100	NE	76.2
3o	NE	>100	NE	>100	NE	>100
3q	NE	>100	NE	>100	NE	92.2
ADR	<0.1	>100	<0.1	56.2	<0.1	<0.1

[a]Concentration in μM, [b]LC_{50}: drug molar concentration causing 50 % cell death, [c]TGI: drug molar concentration resulting in total growth inhibition, [d]GI_{50}: drug molar concentration causing 50 % cell growth inhibition. NE: Not evaluable data. Experiment need to be repeated using different set of drug concentration.

4. Conclusion

In summary, we have developed an efficient and environmentally benign method for the synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one using silane@TiO_2 nanoparticles as heterogeneous catalyst. The current methodology avails with potentials such as clean and mild reaction conditions, short reaction time, and excellent yield of products. The present work adheres to the concept of green chemistry and provides a synthetic strategy for chemists to opt and confront researchers towards multi-component reactions via use of nanocatalysts. Among the synthesized compounds most of the compounds showed comparable antibacterial and antioxidant activities. However, the synthesized compounds were found to be inactive against human cancer cell line HL60 and MCF7.

References

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Outcome of Research:

- Silane@TiO_2 nanoparticles: An efficient and reusable catalyst for facile synthesis of biologically active benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one derivatives, Vilas N. Mahire, Vijay E. Patel, Ashok B. Chaudhari, Vikas V. Gite, and Pramod P. Mahulikar* ***J. of Chem. Sci***. 2016, 128, 671-679

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Silane@TiO_2 nanoparticles-driven expeditious synthesis of biologically active benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one scaffolds: A green approach

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Abstract. A simple, efficient and environmentally benign protocol has been developed for the synthesis of substituted benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one by a reaction of 4-hydroxycoumarin, aldehydes and 2-aminobenzimidazole using silane@TiO_2 nanoparticles as heterogeneous catalyst under reflux condition in ethanol. The surface modification of TiO_2 nanoparticles was confirmed by using FT-IR, FE-SEM, EDX, XRD and TEM analyses. Furthermore, the stability of the catalyst was evaluated by thermal gravimetric analysis (TGA). Some advantages of this method are high yield of products, short reaction time; recyclability of the catalyst and column chromatography-free protocol. The synthesized compounds were screened for their *in vitro* antioxidant activity and most of the compounds exhibited remarkable antioxidant activity.

Keywords. 2-aminobenzimidazole; 4-hydroxycoumarin, aldehydes; silane@TiO_2 nanoparticles; benzo[4,5] imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one.

Objectives IV & V

DES mediated facile synthesis of benzimidazoquinazolinone motifs and their antioxidant potency evaluation

1. Introduction

In green chemistry, considerable attention is focused on the development of clean, high yielding and environmentally friendly chemical processes and technologies.[1] From the last two decades, an ionic liquid has gained enormous attention from the scientific community for their various applications.[2] The ionic liquids are always highlighted by their high price, poor biodegradability, biocompatibility, toxicity and sustainability in the environment,[3] therefore, now days, deep eutectic solvents (DESs) are better alternative to ionic liquids (ILs).[4] Generally, DESs are the mixtures of quaternary ammonium salt such as choline chloride with hydrogen bond donor like urea, carboxylic acid and amide. DESs are environmentally benign, cheap, non-toxic and biodegradable solvents.[5] The negligible vapour pressure, nonflammability, non-reactiveness with water and synthesis from readily available raw materials makes them promising alternative to ionic liquids (ILs).[6, 7]

Over the past decades, multicomponent reactions (MCR's) also have gained significant importance from the medicinal chemistry as a tool for the synthesis of a wide variety of biologically relevant scaffolds for the generation of libraries of compounds.[8] Furthermore, MCR's with environmentally benign solvents like DES is one of the most suitable strategies for green chemical process and developing the libraries of medicinal scaffolds.[9-11] Quinazolines and condensed quinazolines occupy prominent position in realm of medicinal chemistry because of their diverse medicinal properties such as anticancer,[12] histamine H1-receptor blockers,[13] analgesic, anti-inflammatory, antibacterial,[14] anti-hypertensive agents,[15] etc. The general method for synthesis of benzimidazoquinazolinone involves the reaction of 2-aminobenzimidazole,

aldehydes and 1, 3-cyclohexadione or dimedone. In the literature, catalysts such as silica gel,[16] *p*-TsOH.H_2O,[17] I_2,[18] $H_6P_2W_{18}O_{62}$.$18H_2O$,[19] and NH_2SO_3H,[20] and solvents like DMF,[21,22] and ionic liquids [$bmim^+$][BF_4^-] [23] etc., are reported for the synthesis of benzimidazoquinazolinone. However, some of these methods are associated with limitations such as use of toxic solvent, drastic reaction conditions, long reaction time and low yield of products. In current article, in order to develop the greener methodology, we wish to report catalyst free approach for synthesis of benzimidazoquinazolinones using deep eutectic solvent (choline chloride: glycerol). Figure 1 shows the important bioactive drugs containing benzimidazole and pyrimidine as core moiety in their structures. The synthetic strategies for the synthesis of DES and the target derivatives (4a-p) are depicted in Scheme 1 & 2, respectively.

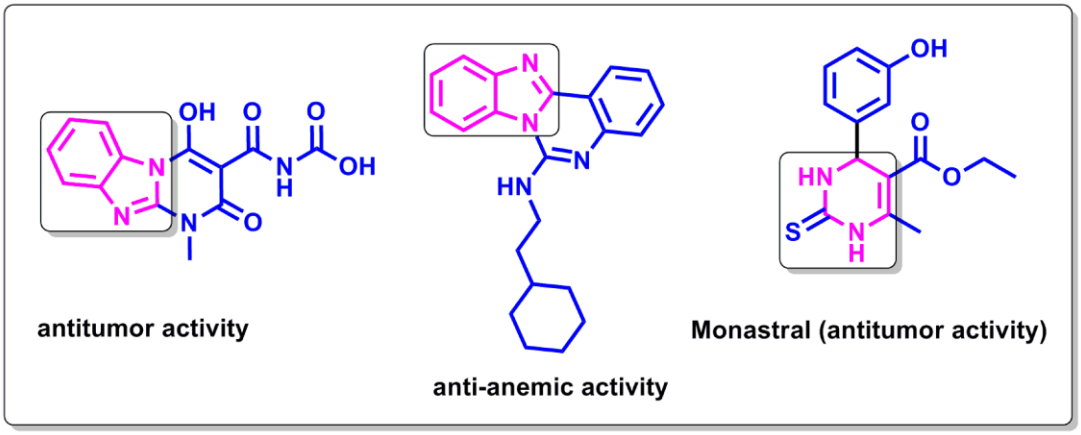


Figure 1 Benzimidazole and pyrimidine ring containing important bioactive molecules.

2. Experimental

2.1 Materials and Methods

All the solvents and reagents were purchased from commercial suppliers, Sigma-Aldrich, S. D. fine chemicals Ltd, Spectrochem India Ltd., and used without further purification. The progress of each reaction was monitored by thin layer chromatography (TLC) on 0.2 mm precoated silica-gel 60 F254 plates (Merck, Germany) and locating the spots using UV light as

the visualizing agent. Melting points were obtained by an open capillary method and are uncorrected. The IR spectra (KBr) were recorded on Shimadzu FT-IR-8400 Spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a FT-NMR Bruker Avance-II Spectrometer at 400 and 100 MHz, respectively using DMSO-d_6 as solvent. The mass spectra were recorded on Waters, Q-Tof Micromass (LCMS) spectrometer and Agilent Technologies 1100 series mass spectrometer in ESI mode. The antioxidant assay was performed on Shimadzu UV mini-1240 and Agilent Technologies Cary 60 UV-Vis Spectrophotometers.

2.2 General procedure for preparation of deep eutectic solvent (DES)

Choline chloride (100 g, 0.71 mol) and glycerol (131.9 g, 1.43 mol) were placed in a 500 mL round bottom flask and stirred at 80 ^{o}C. After 15 to 20 min, a homogenous colorless liquid solution being formed; this was cooled to room temperature and used in reactions without any purification (Scheme 1).

2.3 General procedure for synthesis of benzimidazoquinazolinone scaffolds

In a 50 mL round-bottom flask, a mixture of 2-aminobenzimidazole **1** (2 mmol), aldehyde **2a-p** (2 mmol), 1, 3-cyclohexadione **3** (2 mmol) and deep eutectic solvent (5 mL) was stirred at 80 ^{o}C for specified time (scheme 2, Table 2). After completion of reaction, (monitor by TLC), the reaction mixture was cooled to room temperature and then poured into crushed ice to get solid product, which was filtered, dried and recrystallized from ethanol to afford the pure product.

2.4 Experimental protocol for antioxidant activity

2.4.1. DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method described by Wu *et al.* was used to assay the free radical scavenging ability of benzimidazoquinazolinone

derivatives (4a-4p) with modifications.[24] One millilitre of 0.2 mM DPPH reagent prepared in methanol was added in tubes containing 0.8 ml of each compound (1 mg mL^{-1} in DMSO) and the mixture was allowed to stand for 30 min in the dark at room temperature. Similarly, same protocol was performed for L-ascorbic acid as a standard antioxidant. The absorbance of the resulting mixture was measured at 517 nm using UV-Vis spectrophotometer. The control was prepared by adding only DMSO to DPPH reagent and the analysis performed as described above. The % scavenging activity was determined as follows.

$$\text{Radical scavenging activity (\%)} = [(A_0-A_1)/A_0]\times 100$$

Where, A_0 is the absorption of the control (blank, only DMSO) and A_1 is the absorption of the compounds (4a-4p).

2.4.2 Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP ability of the compounds was determined by the protocol described by Udayaprakash *et al*. with modifications.[25] One millilitre of compound (1 mg mL^{-1}), 2.5 ml phosphate buffer (0.1 M, pH 7) and 1 % potassium ferricyanide (2.5 mL) were mixed and incubated at 50±2 ^{o}C for 30 min. To the solution, 2.5 mL of 10 % trichloroacetic acid was added and centrifuged at 7000 rpm for 10 min. Distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ were added to 2.5 mL supernatant. The absorbance of the solution was measured at 700 nm using UV-Vis Spectrophotometer. In all experiments, L-ascorbic acid was used as standard. The percentage of reduction of the compound as compared to standard was calculated by following formula.

$$\text{Percentage (\%) of reduction power} = [1 – (1 – As / Ac)] \times 100$$

Where Ac is the absorption of standard at maximum concentration tested and As is the absorption of the compound.

2.4.3 ABTS activity

The ABTS ability of the compounds was determined by the protocol described by Udayaprakash *et al*. with modifications.[25] A solution of 7 mM ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and potassium persulphate (2.45 mM) was prepared and incubated in the dark for 14 h, after which the solution was diluted with absolute ethanol till the absorbance reached 0.7±0.001 at 734 nm. In a fresh test tube, one millilitre of diluted solution was mixed with 100 µL of compound (1 mg mL^{-1}) and after 5 min the absorbance was measured at 734 nm. α-Tocopherol was used as standard. The percentage reduction (I %) against ABTS was calculated by using following equation;

$$\text{Percentage reduction (I \%)} = [(A_0-A_1)/A_0]\times 100$$

Where A_0 is the absorption of the control (blank, only DMSO) and A_1 is the absorption of the compound.

2.4.4 Metal chelating activity

The metal chelating ability of the synthesized compounds (4a-p) was determined by the protocol described by Nagulendran *et al*. with modifications.[26] Ferrous chloride (50 µL of 2 mM) was added into the test tubes containing 400 µL of compound (1 mg mL^{-1}). The reaction was started by adding ferrozine (200 µL of 5 mM) and the final volume of the mixture was adjusted to 4.0 mL using absolute ethanol. The mixture was shaken and incubated at RT for 10 min. To determine the ferrous chelating activity of compounds, the absorbance of the mixture was measured on UV-Vis spectrophotometer at 562 nm. α-Tocopherol was used as standard. The

percentage of inhibition of ferrozine-Fe^{2+} complex formation (I %) was calculated by using following equation.

$$\text{Radical scavenging activity (I \%)} = [(A_0\text{-}A_1)/A_0]\times 100$$

Where A_0 is the absorption of the control (blank, only DMSO) and A_1 is the absorption of the compound.

3. Results and Discussion

Our group is constantly working on synthesis and bioactivity evaluation of benzimidazole compounds and development of greener synthetic approaches.[27] In current article, we wish to report catalyst free synthesis of benzimidazoquinazolinone derivatives using choline chloride: glycerol (DES) as an efficient, recyclable solvent. Glycerol is cheap, renewable, non-flammable and biocompatible liquid and choline chloride (quaternary ammonium salt) is a member of vitamin B family and serves as a dietary supplement of animal feeds. Therefore, we decided to prepare DES (Scheme 1) from these two components (1:2 ratios) and study its applications as solvent for greener organic synthesis.

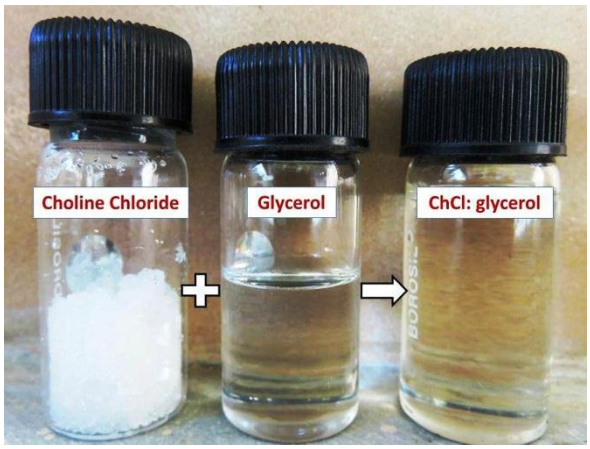
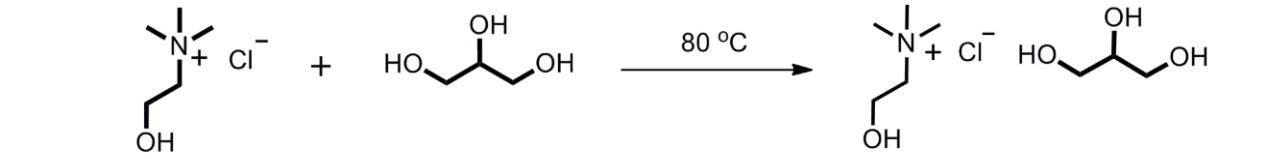
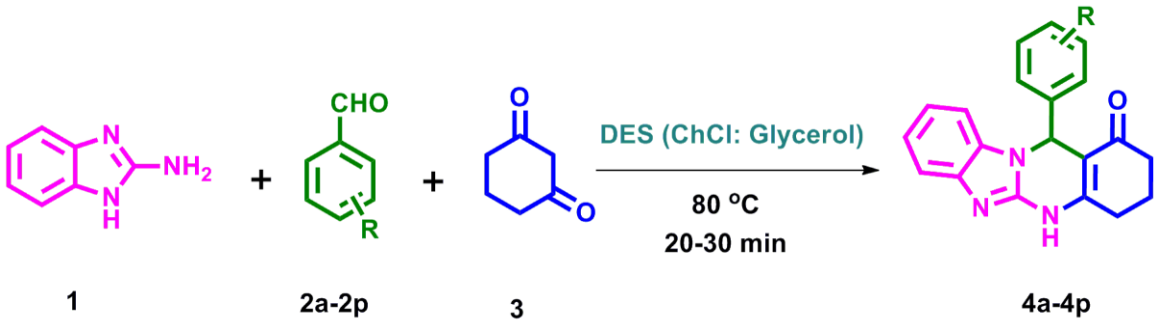


Figure 2 Photograph showing preparation of deep eutectic solvent (ChCl: glycerol).



Scheme 1 Preparation of deep eutectic solvent (ChCl: glycerol).



Scheme 2 General scheme for synthesis of benzimidazoquinazolinones in ChCl: glycerol.

3.1 Optimization of reaction conditions

The model reaction of 2-aminobenzimidazole, 3-nitrobenzaldehyde and 1,3-cyclohexadione in ChCl: glycerol at 80 ^{o}C was carried out for the synthesis of benzimidazoquinazolinone derivatives. The reaction was completed within 30 min to give the (3-nitrophenyl) benzimidazoquinazolinone **4b** as a product with quantitative yield (Table 1). Encouraged by this result and hence the effect of various solvents on rate of reaction was studied on model reaction. Solvents like ethanol, acetonitrile, DMF, chloroform, THF and glycerol etc, were used for optimization of reaction condition (Table 1). The ChCl: glycerol was found to be an efficient solvent for the synthesis of benzimidazoquinazolinone derivatives. However, the reactions using deep eutectic solvents like ChCl: Urea and ChCl: Oxalic acid gave unsatisfactory results (Table 1). The model reaction was performed at ambient temperature but incomplete conversion of reactant into product was observed. The model reaction at 80 ^{o}C afforded 91 % yield of **4b** within 30 min. To realize the efficiency and limitations of the DES in this multi-

component reaction, we applied the optimized reaction conditions to a series of aldehydes (Table 2). The aldehydes containing electron donating as well electron withdrawing groups such as hydroxyl group, methoxy group and nitro group could react efficiently to give the corresponding products with good to quantitative yield (Table 2, entries 1-16). Furthermore, the efficiency of DES was evaluated by recycling it for next reaction. The decrease in the yield was observed after second cycle, may be due to the decrease in attractive forces within DES. Thus, it was found that ChCl: glycerol plays a benign role of accelerator for promoting the time and cost effective formation of product. All the synthesized compounds were isolated, purified and characterized by FT-IR, ^{1}H NMR, and Mass spectrometric techniques.

Table 1 Reaction of 2-aminobenzimidazole **1**, 3-nitrobenzaldehyde **2b** and 1, 3 cyclohexadione **3** in different solvents[a]

Entry	Solvent	Temp (^{o}C)	Time (min)	Yield of 4b[b] (%)
1	Neat	80	180	31
2	Ethanol	Reflux	60	33
3	Acetonitrile	Reflux	180	56
4	DMF	120 ^{o}C	180	75
5	Chloroform	Reflux	240	70
6	THF	Reflux	240	57
7	Glycerol	90	180	77
8	ChCl: Glycerol	RT	180	39
9	ChCl: Glycerol	60	180	74
10[c]	ChCl: Glycerol	80	30	91,74,63
11	ChCl: Urea	80	120	41
12	ChCl: Oxalic acid	80	120	42

[a]Conditions: 2-aminobenzimidazole 1 (2 mmol), 3-nitrobenzaldehyde 2b (2 mmol), 1, 3-cyclohexadione (2 mmol) and DES (5 mL). [b]Isolated yields. [c]DES was reused three times.

Table 2 Synthesis of benzimidazoquinazolinones in deep eutectic solvent[a].

Entry	R	Product	Time (min)	Yield (%)[b]	M.P. (°C)		Ref.
					Found	Reported	
1	2-$NO_2C_6H_4$	4a	30	52	>300	>300	-
2	3-$NO_2C_6H_4$	4b	30	91	>300	>300	[28]
3	4-$NO_2C_6H_4$	4c	30	78	212	-	
4	4-$CH_3C_6H_4$	4d	30	84	>300	>300	[28]
5	4-$OCH_3C_6H_4$	4e	20	81	>300	>300	[28]
6	4-OH, 3-$OCH_3C_6H_3$	4f	20	71	>300	-	
7	3-OH, 4-$OCH_3C_6H_3$	4g	30	81	238-240	-	
8	1-Naphthyl	4h	30	85	>300	-	
9	4-BrC_6H_4	4i	30	78	>300	-	
10	2,3,4-$(OCH_3)_3C_6H_2$	4j	30	63	248-250	-	
11	4-$SCH_3C_6H_4$	4k	20	78	>300	-	
12	C_6H_5	4l	20	74	>300	>300	[28]
13	2,5-$(OCH_3)_2C_6H_3$	4m	30	89	260 (dec.)	-	
14	3,4-$(OCH_3)_2C_6H_3$	4n	30	75	>300	-	
15	4-OHC_6H_4	4o	30	85	>300	-	
16	3,4-$(OH)_2C_6H_3$	4p	45	60	262 (dec.)	-	

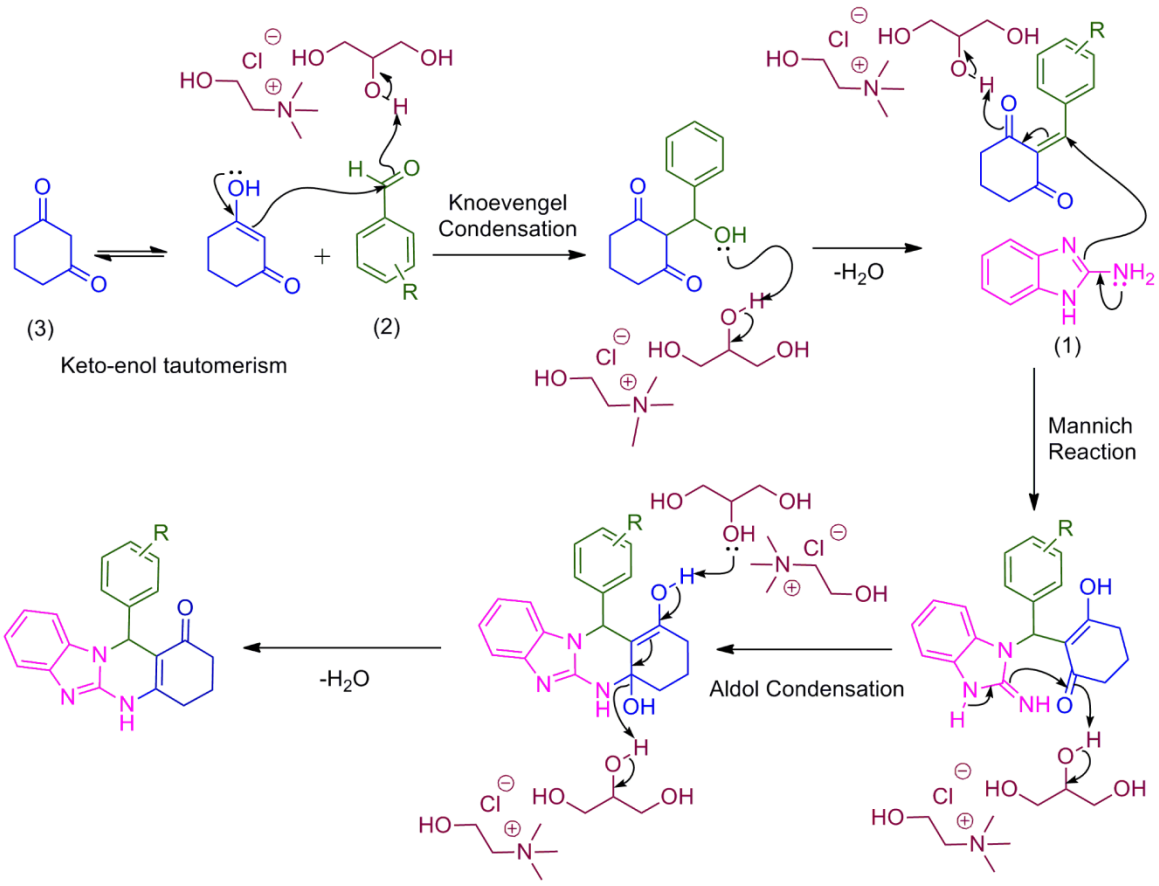
[a]General reaction conditions: 2-aminobenzimidazole 1 (2 mmol), aldehydes 2a-2p (2 mmol), 1,3-cyclohexadione 3 (2 mmol), and ChCl: glycerol (5 mL). [b]All products were characterized by IR, ^1H and ^{13}C NMR and mass spectrometry. [c]Isolated yield.

Table 3 Comparison of ChCl: glycerol with some reported methods.

Entry	Catalyst	Condition	Time (min)	Solvent	Yield (%)	
1	NH_2SO_3H	Reflux	20	CH_3CN	90	[20]
2	I_2	Reflux	10	CH_3CN	84	[18]
3	Ionic Liquid	90 °C	360	--	84	[23]
4		Reflux	5	DMF	58	[21]
5	-	MW	5	DMF	89	[22]
6	-	Reflux	360	DMF	65	[22]
7[a & b]	**-**	**80 °C**	**30**	**ChCl: Glycerol**	**91**	**This work**

[a]General reaction conditions: 2-aminobenzimidazole 1 (2 mmol), 3-nitrobenzaldehyde 2b (2 mmol), 1,3-cyclohexadione 3 (2 mmol), and DES (5 mL). [b]Isolated yield.

Table 3 shows the comparison of efficiency of ChCl: glycerol (DES) with the reported methods. It is clear that the reported methodologies are associated with issues such as long reaction time, use of hazardous solvents and reflux conditions. These conditions are abiding the green chemistry protocol. Hence, it is worthy to mention that, the present work overcomes the drawbacks of the reported methods.



Scheme 3 Plausible mechanism for synthesis of benzimidazoquinazolinone derivatives.

3.2 Antioxidant study

The measurement of antioxidant capacity is a difficult task because antioxidants can act by many different mechanisms, such as hydrogen donating, electron donating, free-radical

scavenging and chelating metal ions which can initiate free-radical reactions. Therefore a single test for antioxidant capacity does not adequate.[29]

3.2.1 DPPH radical scavenging activity

DPPH free radical scavenging is the regularly used method to evaluate the antioxidant property of natural or synthetic compounds. This method is based on DPPH reaction with hydrogen donors to produce stable product causing change in a color from purple to faint yellow.[30] DPPH radical scavenging activity of benzimidazoquinazolinone derivatives (4a-4p) are depicted in Figure 3. L-Ascorbic acid was used as the positive control, while the compounds such as 4a, 4b, 4d, 4f, 4g and 4i showed excellent DPPH radical scavenging activity.

3.2.2 Ferric reducing antioxidant power (FRAP) assay

The benzimidazoquinazolinone derivatives (4a-4p) were evaluated for ferric reducing antioxidant power (FRAP). Reducing power assay monitors the electron donating capacity of reducing agents (i.e. antioxidants) causes the reduction of the ferricyanide complex (Fe^{3+}) to the ferrous (Fe^{2+}) ions, thereby generating chromogenic complex.[31] The absorbance was measured at 700 nm of the resultant blue-green colored solution proportional to the amount of Fe^{2+} in the mixture. The standard L-ascorbic acid showed 100 % reducing ability. Compounds such as 4a, 4f and 4g showed moderate ferric reducing capacity, while 4p exhibited excellent activity (Figure 3).

3.2.3 ABTS assay

In this study, the transformations for the $ABTS^{\bullet+}$ radical cation scavenging capacity of benzimidazoquinazolinone derivatives (4a-p) was evaluated. Stable radicals of ABTS were generated when ABTS was mixed with potassium persulphate and incubated under dark conditions and estimated by spectrophotometrically at 734 nm is the characteristic wavelength

for the ABTS radicals.[32] Compounds such as 4f and 4g showed comparable radical scavenging ability, while 4a, 4b and 4c reflected better activity. α-tocopherol was used as standard.

3.2.4 Metal chelating ability

In this assay, the complex formation between Fe^{2+} and ferrozine was studied. The decrease in violet color intensity is observed, when the metal chelating compound is present in the solution. The diminishing of ferrous ions by the benzimidazoquinazolinones provides protection against oxidative destruction of free radicals. The percentage of iron chelating ability of α-tocopherol and benzimidazoquinazolinones is shown in Figure 3. Benzimidazoquinazolinone compounds 4i, 4m, 4n, 4o and 4p showed significant metal chelating capacity when compared with standard α-tocopherol, while compounds 4e and 4j exhibited moderate metal chelation activity.

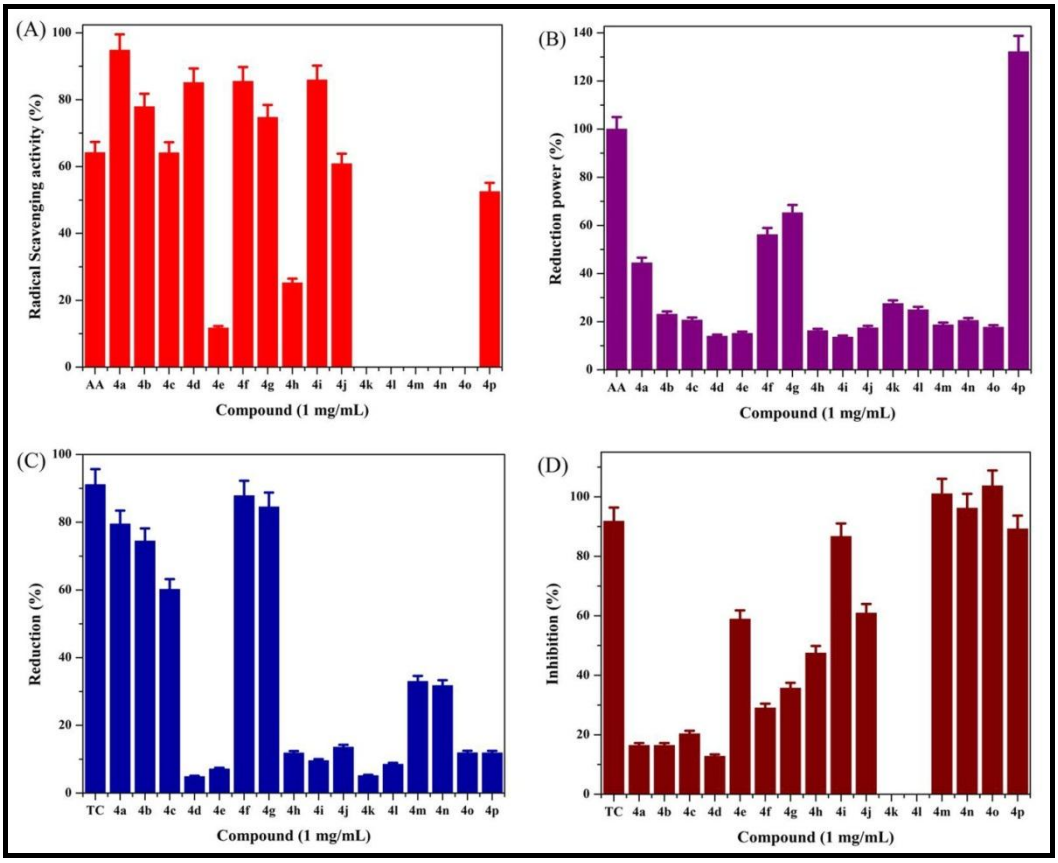


Figure 3 *In vitro* antioxidant activity of benzimidazoquinazolinones, (A) DPPH radical scavenging assay, (B) Ferric reducing antioxidant power (FRAP) assay, (C) ABTS and (D) Metal chelation assay (where AA= Ascorbic acid, TC= α-Tocopherol).

4. Conclusion

In summary, we have developed catalyst free protocol for environmentally benign synthesis of benzimidazoquinazolinones using ChCl: glycerol as solvent. The novelty of this work is use of non-toxic, low cost and environmentally benign solvent. The methodology avails with potentials such as clean and mild reaction conditions, short reaction time and quantitative yield of products. Further it is noteworthy to mention that, amongst the synthesized compounds, most of the compounds showed acceptable antioxidant activity.

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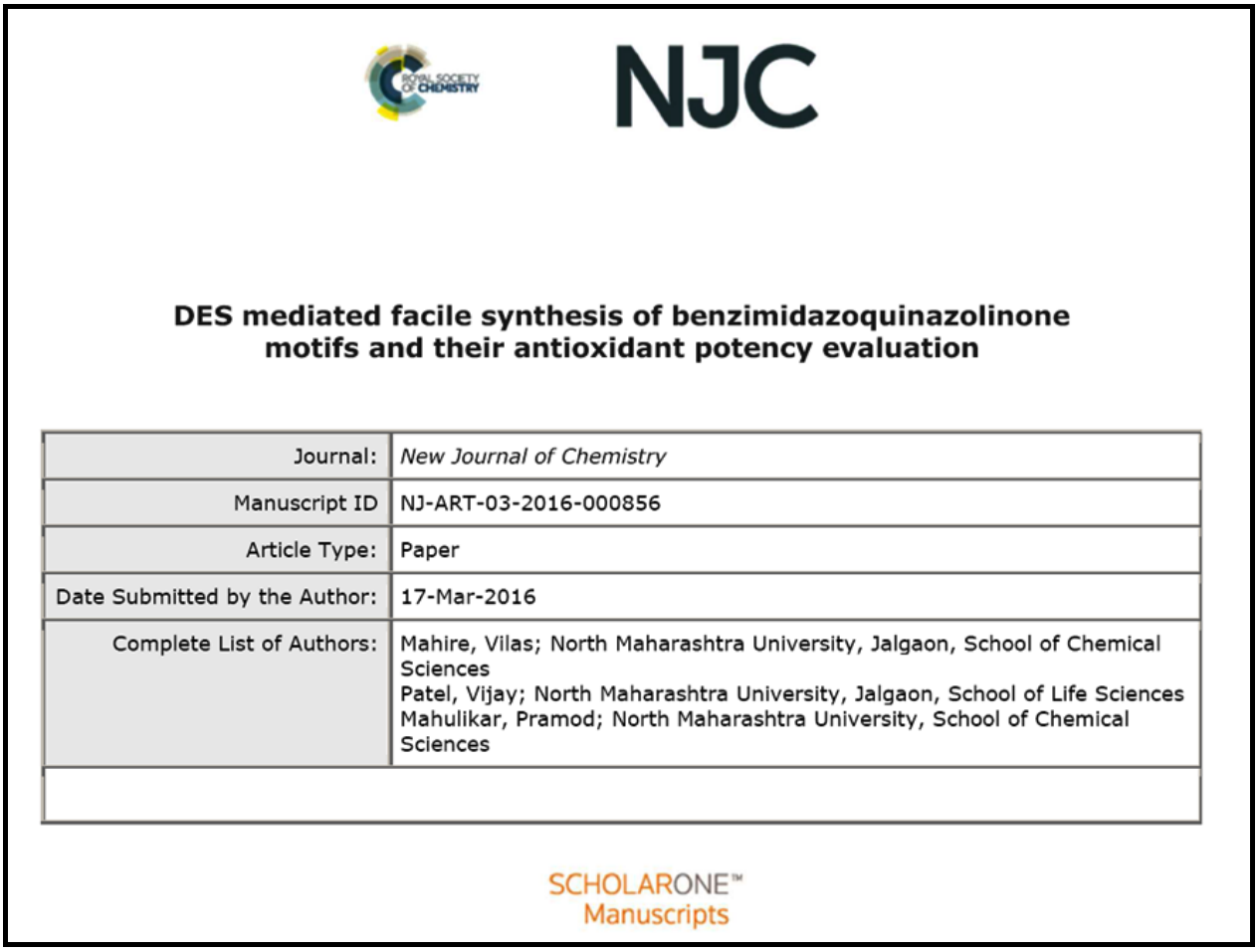
Outcome of Research:

- Deep eutectic solvent mediated facile synthesis of benzimidazoquinazolinone motifs and their antioxidant evaluation, Vilas N. Mahire, Vijay E. Patel and Pramod P. Mahulikar*, **New Journal of Chemistry.** (Submitted IF=3.084)



NJC

DES mediated facile synthesis of benzimidazoquinazolinone motifs and their antioxidant potency evaluation

Journal:	*New Journal of Chemistry*
Manuscript ID	NJ-ART-03-2016-000856
Article Type:	Paper
Date Submitted by the Author:	17-Mar-2016
Complete List of Authors:	Mahire, Vilas; North Maharashtra University, Jalgaon, School of Chemical Sciences Patel, Vijay; North Maharashtra University, Jalgaon, School of Life Sciences Mahulikar, Pramod; North Maharashtra University, School of Chemical Sciences

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Manuscripts

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1.	Name of the Principal Investigator	Prof. P. P. Mahulikar
2.	Dept. of Principal Investigator University/College	School of Chemical Sciences, North Maharashtra University, Jalgaon- 425001, (Maharashtra)
3.	UGC approval Letter No. and Date	F.No. 41-302/2012 (SR), dated13/07/12
4.	Title of the Research Project	"Clean synthesis and bioactivity evaluation of newer benzimidazole compounds"
5.	Effective date of starting the project	01/07/2012
6.	a. Period of Expenditure	01st July 2012 to 31st December 2015
	b. Details of Expenditure	

S.No.	Item	Amount Approved (Rs.)	Amount Received (Rs.)	Expenditure Incurred (Rs.)
i.	Books & Journals	30,000.00	30,000.00	29,934.00
ii.	Project Fellow Salary	4,89,067.00	4,40,160.00	4,39,674.00
iii.	HRA to Project Fellow	48,907.00	44,016.00	44,016.00
iv.	Chemicals & Glassware	2,50,000.00	2,25,000.00	1,93,317.00
v.	Contingency	30,000.00	27,000.00	29,982.00
vi.	Hiring Services	40,000.00	36,000.00	49,120.00
vii.	Field Work/Travel	20,000.00	18,000.00	11,615.00
viii.	Overhead Charges	84,800.00	84,800.00	84,800.00
	Total	**9,92,774.00**	**9,04,976.00**	**8,82,458.00**

c . Staff
Date of Appointment **12th September 2012**

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment@revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of **Rs. 9,04,976/-** (Nine Lakh Four Thousand Nine Hundred Seventy Six Rupees only) received from the University Grants Commission under the scheme of support for Major Research Project entitled **"Clean synthesis and bioactivity evaluation of newer benzimidazole compounds"** vide UGC letter No. **F.No. 41-302/2012 (SR), dated13/07/12** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

18/04/16

Prof. P. P. Mahulikar
Principal Investigator
School of Chemical Sciences

Prof. P. P. MAHULIKAR
DIRECTOR
School of Chemical Sciences
NMU, Jalgaon-425001

Prof. A. M. Mahajan
Registrar
North Maharashtra University, Jalgaon

18/4/16

REGISTRAR
North Maharashtra University
Jalgaon

Annexure – V

ज्ञान-विज्ञान विमुक्तये

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate

It is certified that the grant of **Rs. 9,92,774/- (Rupees Nine lakh Ninty Two Thousand Seven Hundred Seventy Four only)** has been allocated from University Grants Commission and grant of **Rs. 9,04,976/- (Rupees Nine Lakh Four Thousand Nine Hundred Seventy Six only)** received under the scheme of support for Major Research Project entitled as **"Clean synthesis and bioactivity evaluation of newer benzimidazole compounds"** vide UGC letter No. F.No. 41-302/2012 (SR), dated 13/07/12 from which **Rs. 8,82,458/- (Rupees Eight Lakh Eighty Two Thousand Four Hundred Fifty Eight only)** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Prof. P. P. Mahulikar
Principal Investigator
School of Chemical Sciences

Dr. B. D. Karhad
Finance & Accounts Officer
North Maharashtra University, Jalgaon

Finance & Accounts Officer
North Maharashtra University
Jalgaon-425001,(M.S.)

Prof. A. M. Mahajan
Registrar
North Maharashtra University, Jalgaon

REGISTRAR
North Maharashtra University
Jalgaon

Signed But Rs. 8,82,458/-
For RATAN CHANDAK & CO.
Chartered Accountants

Signature of Chartered Accountant
with seal and registration No.
Prior to the statutory auditors

(K. K. Mundada)
Partner
M.No. 102492
10/5/16

Ratan Chandak & Co. Firm No. 108696W Chartered Accountants

NORTH MAHARASHTRA UNIVERSITY JALGAON-425001

Annexure –VIII

ज्ञान-विज्ञान विमुक्तये

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Final Report of the work done on the Major Research Project
(Report to be submitted within 6 weeks after completion of each year)

1.	Project report No.	Final Report
2.	UGC Reference	F. No. 41-302/2012 (SR), dated13/07/12
3.	Period of report:	from 01 July 2012 to 31 December 2015
4.	(a) Name of the Principal Investigator:	**Prof. P. P. Mahulikar**
5.	(b) Deptt: (c) University/College where work has progressed	School of Chemical Sciences, North Maharashtra University, Jalgaon- 425001, (Maharashtra)
6.	Effective date of starting of the project:	**01/07/2012**
7.	Grant approved and expenditure incurred during the period of the report:	
	a. Total amount approved b. Total amount received c. Total expenditure	**Rs. 9,92,774/-** **Rs. 9,04,976/-** **Rs. 8,82,458/-**
i.	Brief objective of the project	1. Synthesis of newer benzimidazole compounds through greener approaches. 2. Evaluation of bioactivity of the synthesized benzimidazole compounds.
ii.	Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication.	The research work is carried out as per the plan and objectives of the project. The detailed progress report for three years is attached separately. The outcome of the project has been published in the international journals of repute. 1. Vilas N. Mahire, Pramod P. Mahulikar, Facile one-pot clean synthesis of benzimidazole motifs: Exploration on bismuth nitrate accelerated subtle catalysis, **Chin. Chem. Lett. 2015, 26, 983-987 (IF 1.587)**

		2. Vilas N. Mahire, Vijay E. Patel, Ashok B. Chaudhari, Vikas V. Gite and Pramod P. Mahulikar, Silane@TiO2 nanoparticles-driven expeditious synthesis of biologically active benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one scaffolds: A green approach, Journal of Chemical Sciences, Vilas N. Mahire, Vijay E. Patel and Pramod P. Mahulikar, **J. Chem. Sci. 2016, 128, 671-679 (IF=1.197)** 3. DES mediated facile synthesis of benzimidazoquinazolinone motifs and their antioxidant potency evaluation, **New J. Chem.** (Manuscript Submitted, **IF= 3.084**)
iii.	Has the progress been according to original plan of work and towards achieving the objective? if not, state reasons,	Yes. Project work is in progress according to original plan of work and objectives. The Project work is being carried out as per the plan and schedule given in the proposal.
iv.	Please indicate the difficulties, if any, experienced in implementing the project	No difficulties were observed in the implementation project.
v.	If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet. **N. A.**	
vi.	If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission. **YES.**	
vii.	Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as	
	(a) Manpower trained	Mr. Vilas Nana Mahire (Project Fellow)
	(b) Ph. D. awarded	Project Fellow registered for Ph.D. degree.
	(c) Publication of results	Papers Published: 02 Paper submitted: 01 Under preparation: 01 (the copies of the published papers are attached separately in the progress report)
	(d) other impact, if any (Activities of project staff during the period of report) • Poster Presentation, National Science Day Celebration (NSD-2016) under the theme of Make in India: Science and Technology (To raise Public Appreciation on Scientific issues for the development of Nation), School of Chemical Sciences, North Maharashtra University, Jalgaon, March 11, 2016 **(Second Prize)**.	

- Oral Presentation, Greener synthesis using silane@TiO_2 nanoparticles, UGC sponsored National Conference on Chemical Synthesis of Nanomaterials and their Applications in Environment, G. T. Patil Arts, Commerce & Science College, Nandurbar, February 09, 2016.
- Poster Presentation, 10^{th} Maharashtra State Level Avishkar 2015, Savitribai Phule Pune University, Pune, January 10-12, 2016.
- Poster Presentation, 34^{th} Annual Conference, Indian Council of Chemists, Uka Tarsadia University, Bardoli, Surat, December 26-28, 2015.
- Poster Presentation, University Level "Avishkar 2015", North Maharashtra University, Jalgaon, December 22-13, 2015 (**Second Prize**).
- Poster Presentation in RSC-Symposium on Frontiers of Advances in Chemistry and Technology-2015,mSchool of Chemical Sciences, North Maharashtra University, Jalgaon, December 11-12, 2015 **(Best Poster Presentation Award).**
- Poster Presentation in International Conference on New Horizons in Synthetic and Material Chemistry (ICSMC-2015), Department of Chemistry, University of Mumbai, Mumbai, November 26-18, 2015
- Poster Presentation in NMU Campus Level Avishkar 2015, North Maharashtra University, November 22, 2015.
- Oral Presentation in Summer School 2015, Graduate School of Advance Technology and Science, **Tokushima University, Tokushima, Japan**, August 07, 2015 **(Best Oral Presentation Award).**
- Poster Presentation in International Conference on Functional Materials@Nanoscale: Concerns and Challenges (ICFMNCC-2015). Rayat Shikshan Sanstha's Karmaveer Bhaurao Patil Mahavidyalaya, Pandharpur, Dist-Solapur, March 9-11, 2015.
- Participated in One day National level Seminar on Innovative Concepts and Methodologies in Pharmaceutical Research, H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist-Dhule (Maharashtra), March 04, 2015.
- Poster Presentation, National Conference on Recent Trends in Environmental Protection 2015, Smt. P. K. Kotecha Mahila Mahavidyalaya, Bhusawal, February 12-13, 2015 **(Best Poster Presentation Award).**
- Poster Presentation in State Level Avishkar-2014, Maharashtra Animal & Fishery Sciences University, January 21-23, 2015.
- Poster Presentation in 3^{rd} Global Sustainable Biotech Congress 2014, North Maharashtra University, Jalgaon, December 01-05, 2014.
- Poster Presentation in University Level Avishkar 2014, North Maharashtra University, Jalgaon, October 17-18, 2014 **(First Prize).**
- Poster Presentation in NMU Campus Level Avishkar, North Maharashtra University, Jalgaon, October 07, 2014.

- Poster Presentation in 3rd International Conference on "Chemistry for Sustainable Development: Indian Perspective" Indian Council of Chemists, **Dubai & Abu Dhabi,** June 10-14, 2014
- Poster presentation, International Conference on Global Opportunities for Latest Developments in Chemistry and Technology-2014, February 06-08, 2014 **(Best Poster Presentation Award).**
- Participated in Vibrant Gujarat National Education Summit 2014, Mahatma Mandir, Gandhinagar, Gujarat, January 10-11, 2014.
- Poster Presentation, University level Avishkar 2013, North Maharashtra University, Jalgaon, October 18-19, 2013.
- Poster Presentation, NMU Campus Avishkar 2013, North Maharashtra University, Jalgaon, October 05, 2013.
- Participated in First National Conference on Innovation in Chemistry- Laboratory to Society, School of Chemical Sciences, North Maharashtra University, Jalgaon, March 11, 2013.
- Poster Presentation in University Industry Summit organized by North Maharashtra University, Jalgaon, Rotary Club Aurangabad, Chamber of Marathwada Industries and Agriculture and Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, February 16, 2013.
- Poster Presentation in State Inter University Research Convention, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri, January 07-09, 2013.
- Poster Presentation in University Level Avishkar 2012, North Maharashtra University, Jalgaon, December 13-14, 2012 **(First Prize).**
- Participated in Short Course on Microwave Assisted Organic Synthesis, Indian Institute of Technology, Powai, Mumbai, October 31, 2012.

18/4/2016

Prof. P. P. Mahulikar
Principal Investigator
School of Chemical sciences

Prof. P. P. MAHULIKAR
DIRECTOR
School of Chemical Sciences
NMU, Jalgaon-425001

SCHOOL OF CHEMICAL SCIENCES
NMU JALGAON

Prof. A. M. Mahajan
Registrar
North Maharashtra University, Jalgaon

REGISTRAR
North Maharashtra University
Jalgaon

NORTH MAHARASHTRA UNIVERSITY JALGAON

Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	**Title of the Project:** "Clean synthesis and bioactivity evaluation of newer benzimidazole compounds"
2.	**Name and address of the Principal Investigator:** Prof. P. P. Mahulikar School of Chemical Sciences, North Maharashtra University, Jalgaon- 425001, (Maharashtra)
3.	**Name and Address of the Institution:** School of Chemical Sciences, North Maharashtra University, Jalgaon- 425001, (Maharashtra)
4.	**UGC approval letter no. and date:** F.No. 41-302/2012 (SR), dated13/07/12
5.	**Date of Implementation :** 01/07/2012
6.	**Tenure of the Project:** 01st July 2012 to 31st December 2015
7.	**Total grant allocated:** 9,92,774.00
8.	**Total grant received:** 9,04,976.00
9.	**Final expenditure:** 8,82,458.00
10.	**Title of the Project:** "Clean synthesis and bioactivity evaluation of newer benzimidazole compounds"
11.	**Objectives of the Project:** 3. Synthesis of newer benzimidazole compounds through greener approaches. 4. Bioactivity evaluation of synthesized benzimidazole compounds.
12.	**Whether objectives were achieved (give details)** The project entitled "Clean synthesis and bioactivity evaluation of newer benzimidazole compounds" is basically base on two main objectives - Development of greener alternative methods for the synthesis of benzimidazole compounds - Bioactivity evaluation of synthesized compounds **Objective I:** Synthesis of 2-substituted and 1,2 disubstituted benzimidazole compounds is achieved by using

	bismuth nitrate as an green catalyst. **Objective II:** Synthesis of benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one derivatives using Silane@TiO_2 nanocatalyst. **Objective III:** Synthesized compounds were screened for their antioxidant, antimicrobial and anticancer activities. Most of the compounds exhibit excellent antioxidant and antibacterial properties. However, the synthesized compounds were found to be inactive against human cancer cell line Blood Cancer (Cell line HL60) and Breast Cancer (Cell line MCF7). **Objective IV:** Applicationn of Deep Eutectic Solvent for the synthesis of biologically active benzimidazoquinazolinone derivatives. The ChCl: glycerol (DES) is found to be efficient and recyclable solvent for the synthesis of diversified molecules. **Objective V:** The synthesized benzimidazoquinazolinone derivatives were evaluated for their antioxidant properties using DPPH radical scavenging, Ferric reducing antioxidant power (FRAP), ABTS, Metal chelating ability assay. Most of the compounds showed good to comparable antioxidant activity.
13.	**Achievements from the Project** The research work is carried out as per the plan and objectives of the project. The detailed progress report for three years is attached separately. The outcome of the project has been published in the international journals of repute. 4. Vilas N. Mahire, Pramod P. Mahulikar, Facile one-pot clean synthesis of benzimidazole motifs: Exploration on bismuth nitrate accelerated subtle catalysis, **Chin. Chem. Lett. 2015, 26, 983-987 (IF 1.587)** 5. Vilas N. Mahire, Vijay E. Patel, Ashok B. Chaudhari, Vikas V. Gite and Pramod P. Mahulikar, Silane@TiO2 nanoparticles-driven expeditious synthesis of biologically active benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one scaffolds: A green approach, Journal of Chemical Sciences, Vilas N. Mahire, Vijay E. Patel and Pramod P. Mahulikar, **J. Chem. Sci. 2016, 128, 671-679 (IF=1.197)** DES mediated facile synthesis of benzimidazoquinazolinone motifs and their antioxidant potency evaluation, **New J. Chem.** (Manuscript Submitted, **IF= 3.084**)
14.	**Summary of the findings in 500 words)** 1. Synthesis of 2-substituted and 1,2 disubstituted benzimidazole compounds is achieved by

	using bismuth nitrate as an green catalyst. 2. The synthesized Benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one derivatives are shows good to moderate antioxidant as well as antimicrobial activity. 3. The synthesized benzimidazoquinazolinone derivatives were evaluated for their antioxidant properties using DPPH radical scavenging, Ferric reducing antioxidant power, ABTS, Metal chelating ability assay. Most of the compounds showed good to comparable antioxidant activity
15.	**Contribution to the society (give details)** The theme of project is Green/Clean/Environmentally benign chemistry and hence emphasizes on the minimization of environmental pollution and hazards. The developed synthetic methods under this project work are safer and ecofriendly than the reported and conventional methods. Some of the synthesized compounds exhibited good to comparable bioactivities (antioxidant and antibacterial activities).
16.	**Whether any Ph.D. enrolled/produced out of the project** Mr. Vilas N. Mahire (Project Fellow) has been enrolled for the Ph.D. program at School of Chemical Sciences, North Maharashtra University, Jalgaon. The research work is in progress according to the objectives.
17.	**No. of publications out of the Project (please attach)** Paper Published : 02 Paper Submitted : 01 Under Preparation : 01 The copies of the published papers are attached with the report.

Prof. P. P. Mahulikar
Principal Investigator
School of Chemical Sciences

Prof. P. P. MAHULIKAR
DIRECTOR
School of Chemical Sciences
NMU, Jalgaon-425001

Prof. A. M. Mahajan
Registrar
North Maharashtra University, Jalgaon

REGISTRAR
North Maharashtra University
Jalgaon

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journal homepage: www.elsevier.com/locate/cclet

Original article

Facile one-pot clean synthesis of benzimidazole motifs: Exploration on bismuth nitrate accelerated subtle catalysis

Vilas N. Mahire, Pramod P. Mahulikar *

School of Chemical Sciences, North Maharashtra University, Jalgaon 425 001, Maharashtra, India

ARTICLE INFO

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Benzimidazole
o-Phenylenediamine
Aldehydes
Bismuth nitrate
Green chemistry

ABSTRACT

In the present letter, an efficient, clean and one-pot synthesis of 2-substituted benzimidazole and 1,2-disubstituted benzimidazole derivatives has been explored by reacting *o*-phenylenediamine with aromatic aldehydes using bismuth nitrate as a catalyst in ethanol at ambient temperature. This methodology avails with faster reactions, excellent yield, mild reaction conditions, use of inexpensive and non-toxic catalyst compared to literature reported hitherto.

1. Introduction

At the beginning of this century, green chemistry has attracted a considerable importance in the development of environmentally benign routes to numerous materials. Green chemistry mainly emphasizes towards the pollution prevention through eco-friendly design of chemical products and processes [1]. The development of greener methodologies for syntheses of heterocyclic compounds is still a stimulating task in the field of organic synthesis. Among the heterocycles, benzimidazole derivatives are the important class of nitrogen containing heterocycles with a wide range of medicinal properties such as serotoninergic 5-HT3 and 5-HT4 receptors in the CNS [2], antihistamine [3], anticancer [4,5], antibacterial [6], antifungal [7], anti-inflammatory, antianalgesic [8], antioxidant [9], antidiabetic [10], selective neuropeptide YY1 receptor antagonists [11], antimalerial, antitubercular [12], antiulcer [13], *etc.* where moiety plays the role of 'Master Key' [14]. Therefore, it is an imperative anchor for development of new therapeutic drugs, as illustrated and supported by some commercial benzimidazole products in Fig. 1.

Generally, the synthesis of benzimidazole involves the reaction of *o*-phenylenediamine either with carboxylic acids, carboxaldehydes or their derivatives (chlorides, nitriles, and orthoesters) under strongly acidic conditions with high temperature [15], Furthermore, Cascade reactions of *o*-haloaniline with amidine hydrochlorides [16] and intramolecular palladium-catalyzed aryl amination are alternative ways for synthesis of benzimidazole [17,18]. A variety of catalysts are reported in the benzimidazole synthesis, such as $FeCl_3$-doped polyaniline nanoparticles [19], solvent free $SiO_2/ZnCl_2$ [20], cobalt (II) chloride hexahydrate [21], $[Sm(OTf)_3]$ [22], $[In(OTf)_3]$ [23], sodium metabisulfite [24], silphox$[POCl_{3-n}\ (SiO_2)_n]$ [25], potassium persulfate-$CuSO_4$ [26], indion 190 resin [27], ammonium acetate [28], thiamine hydrochloride [29], SDS micelles, DBSA, $Fe_3O_4@SiO_2@(NH_4)_6$–Mo_7O_{24} magnetic core-shell nanocomposite, boron trifluoride etherate ($BF_3.OEt_2$), Cu-nanoparticles/SiO_2, LiBr [30], *etc.*

At present, bismuth (III) compounds have recently attracted much attention in organic transformations due to their high acidity, thermal stability, low toxicity, low cost, and good stability [31]. Furthermore, bismuth nitrate is reported as an eco-friendly nitrating agent for selective nitration of organic compounds [32,33]. Current literature reveals that bismuth nitrate has been utilized as an effective catalyst in the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones [34], guanidylation of *N*-benzoylthioureas [35], synthesis of coumarins [36], Paal–Knorr synthesis of pyrroles [37], chemoselective synthesis of acylals [38], *etc.*

Nevertheless, most of the aforesaid methods of benzimidazole synthesis have disadvantages like, use of expensive reagents and catalysts, harsh reaction conditions and long reaction time, *etc.* Moreover, several of these reactions have been reported at higher

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Silane@TiO_2 nanoparticles-driven expeditious synthesis of biologically active benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one scaffolds: A green approach

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Abstract. A simple, efficient and environmentally benign protocol has been developed for the synthesis of substituted benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one by a reaction of 4-hydroxycoumarin, aldehydes and 2-aminobenzimidazole using silane@TiO_2 nanoparticles as heterogeneous catalyst under reflux condition in ethanol. The surface modification of TiO_2 nanoparticles was confirmed by using FT-IR, FE-SEM, EDX, XRD and TEM analyses. Furthermore, the stability of the catalyst was evaluated by thermal gravimetric analysis (TGA). Some advantages of this method are high yield of products, short reaction time; recyclability of the catalyst and column chromatography-free protocol. The synthesized compounds were screened for their *in vitro* antioxidant activity and most of the compounds exhibited remarkable antioxidant activity.

Keywords. 2-aminobenzimidazole; 4-hydroxycoumarin, aldehydes; silane@TiO_2 nanoparticles; benzo[4,5]imidazo[1,2-a]chromeno[4,3-d]pyrimidin-6-one.

1. Introduction

In recent years, nano-catalysis has been utilized as an alternative approach for the improvement in rates, yields and workability of many significant organic reactions. This also leads to a search for or design of new efficient affordable catalysts for specific applications in synthetic chemistry.[1] Nanoparticles have large surface area and due to this they get strongly agglomerated and hence their surface modification is an alternative way to produce new hybrid nanoparticles having virtuous properties.[2,3] The covalent attachment of functionalities on to the surface of nanoparticles enhances their newer properties which either increases the interaction or decrease the aggregation of nanoparticles which lead to various purposes to wider applications.[4–7] Nano materials have wide applications in science and technology such as medicinal, cosmetics and environmental purification, etc. Titanium dioxide belongs to the family of transition metal oxides and is considered as an ideal photocatalyst because of its merits such as low cost, nontoxicity, high stability, optical and electronic properties, etc. Furthermore, its band gap is sufficient to initiate a variety of organic reactions.[8,9]

Multi-component reactions are becoming the most significant synthetic tool for synthesis and design of new libraries of molecules of pharmaceutical interest through single step reactions.[10–12] In modern organic synthesis, combination of green chemistry and multi-component reactions will lead to generation of molecules with structural complexity in a single step from three or more reactants with greater efficiency and atom economy.[13–16] Therefore, the design of efficient multi-component reactions offers an enduring challenge for synthetic organic chemists.

In the development of several new drugs, the fused heterocyclic scaffolds with nitrogen and oxygen atoms play an important role in medicinal chemistry. Benzimidazole derivatives are important pharmacophores and considered as privileged structures in medicinal chemistry.[17,18] Benzimidazole derivatives have been reported to possess diverse biological activities such as antihistamine, antitumor, antibacterial, antifungal and antioxidant,[19] etc. Various substituted benzimidazoles are known to have diverse biological activities and among them 2-substituted benzimidazoles are found to be more potent.[20] Furthermore, the chromene[2,3-d]pyrimidine derivatives occupy an important place in the realm of natural and synthetic organic chemistry because of their biological and pharmacological activities

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