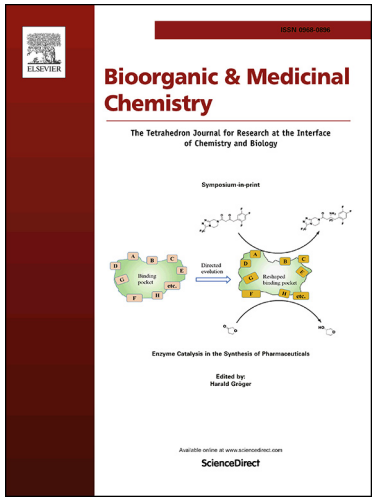
Accepted Manuscript



A reusable magnetic nickel nanoparticle based catalyst for the aqueous synthesis of diverse heterocycles and their evaluation as potential anti-bacterial agent

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A reusable magnetic nickel nanoparticle based catalyst for the aqueous synthesis of diverse heterocycles and their evaluation as potential anti-bacterial agent

Deboshikha Bhattacharjee,[a] Sanjoy Kumar Sheet,[a] Snehardinarayan Khatua,[a] Koel Biswas,[b] Sataram Joshi[b] and Bekington Myrboh[a*]

[a]*Centre for Advanced Studies in Chemistry, Department of Chemistry, North-Eastern Hill University , Mawlai Shillong -793022 India.*

[b]*Microbiology Laboratory, Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong – 793022, India*

Abstract

A library of biologically important heterocycles, viz. pyrazolyl pyrimidine-triones, bis(heterocyclyl)methanes were succesfully synthesised by the condensation of barbituric acid, pyrazolone with an aldehyde and dimedone/4-hydoxy coumarin with various substituted aldehydes in aqueous medium at room temperature catalysed by nickel nanoparticles which proved to be an efficient magnetically recyclable catalyst. The method is simple, eco-friendly and gave excellent yields of the products without taking recourse to column chromatographic separation procedures. Computational method was employed to elucidate the selective formation of uncyclised product in reaction course. The biological activity of the synthesized compounds were investigated and the results demonstrated profound antibacterial activity.

1. Introduction

The discovery of multi-component reaction has led the synthesis of library of diverse heterocycles in a simple, efficient and concerted steps minimising the by-products and maximising the atom economy in a more environment friendly manner. Keeping in mind the green principles of chemistry, the use of harmful volatile organic solvents have been avoided and in lieu of hazardous solvents many alternative reaction media have been introduced.[1] One such reaction medium is the 'universal solvent' i.e. water as it is the safest and most abundant substance on our planet.[2] Besides as a solvent, water tends to enhance the reaction rate due to its polarity, hydrophobic packing and hydrogen bonding.[3] The search for a catalyst which is nontoxic, affordable, easy to recycle and having high catalytic efficiency is an ongoing

process. Thus, the development of multi-component reaction in safer reaction medium using a catalyst having the above mentioned properties is highly desirable.

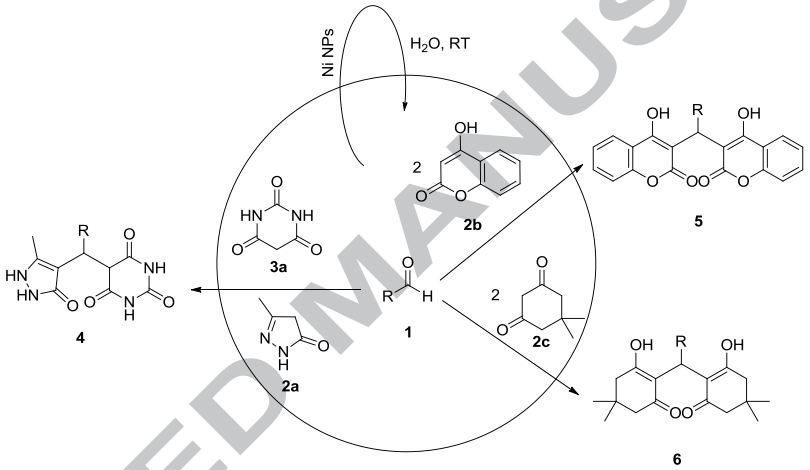
The application of nanoparticles as catalysts in synthetic chemistry has gained tremendous interest in recent years.[4] Lately nickel nanoparticles have been the focus of attention because of their unique properties and potential applications in magnetism,[5] energy technology,6 electronics,[7] and biomedicine.[8] In comparison with the other metals however, nickel nanoparticles have found less application in catalysis with only few reports of their application in organic reactions.[9]

Barbiturates are important medicinal scaffolds which demonstrate inhibition against protein kinase C (PKC)[10] and found presence in CNS drugs like sedative, anxiolytic, anesthetic, antirpileptic and anticonvulsant.[11-13] In similar manner pyrazolones are also well known inhibitors of numerous oncogenic signal transduction kinases such as RTK c-Met, ALK, VEGFR-2 TGF bR1, and HAT.[14] Moreover, the pyrazolone nucleus are core structural component of compounds having antipyretic, anti-inflammatory, antimicrobial, analgesic, antifungal, and antiviral activity.[15-19] The zwitterionic compounds on the other hand, are excellent options for drug designing and delivery because of their specific protein absorption, evasion of quick recognition by immune system, slow blood clearance from the body and excellent water solubility.[20] Inspite of their biological significance however, not many reports are available in the literature which describes the synthesis of pyrazolyl pyrimidinetriones derivatives in its zwitterionic form except for those by Bihani *et al.*[20] On the other hand, bis(heterocyclyl)methanes which are also building blocks in natural porphyrins[21] are studied extensively because of their diverse biological and pharmacological activities.[22] A number of methods have been developed for their synthesis using different catalyst like molecular iodine,[23] Zn(proline)$_2$,[24] surfactants,[25] ionic liquids,[26] $MnCl_2$,[27] DBU,[28] TiO_2/SO_4^{2-},[29] choline hydroxide,[30] TBAB,[31] nanomaterial,[32] $Mn(pbdo)_2Cl_2$/MCM-41,[33] $Zr(DP)_2$[34] by conventional method as well as by microwave irradiation. The common drawbacks like longer reaction time, high temperature, tedious work-up, non-recyclability of catalyst and use of hazardous organic solvents and expensive reagents still plague many of these methods. As part of our continuing effort in the of development of greener synthetic methodology,[35] we decided to employ nickel nanoparticles as a catalyst in the three component reactions involving barbituric acid, an aldehyde, pyrazolone and an aldehyde with dimedone/4-hydroxy coumarins respectively for generating heterocycles having potential biological activity. We report herein result of our effort.

2. Results and discussion

2.1 Chemistry

Thus, when aldehyde **1** (2.1 mmol), pyrazolone **2a** (2.0 mmol) and barbituric acid **3a** (2.0 mmol) are reacted at room temperature in aqueous medium in presence of Nickel nanoparticles (NPs) we were gratified to note that the three component reaction yielded pyrazolyl pyrimidinetriones. Similarly when pyrazolone and barbituric acid are replaced by 4-hydoxy coumarin **2b** / dimedone **2c** (4 mmol) and condensed with an aldehyde **1** (2.1 mmol) bis(heterocyclyl) methanes are formed under similar reaction condition (Scheme 1).



Scheme 1. Synthesis of pyrazolyl pyrimidinetriones, bis(4-hydroxy-2H-chromen-2-one) and bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) derivatives

2.1.1 Preparation of Nickel nanoparticles and its Characterization

Nickel nanoparticles were synthesized by the chemical reduction method as per the reported procedure.[36] To a solution of nickel chloride hexahydrate in water hydrazine hydrate was added followed by addition of NaOH to maintain the alkalinity. The reaction was stirred at 60^{o}C till a black precipitate was formed. To ensure the complete conversion, the reaction mixture was allowed to stir for another 2 hrs at 60^{o}C. The precipitate thus formed was filtered, washed several times with ethanol and then dried overnight in a hot air oven at 80^{o}C. The nanoparticles exhibited magnetic properties and were used as such to catalyse our reactions. The reaction involved in the synthesis of nanoparticles (Nps) is generally represented as

$$2\,Ni^{2+} + N_2H_4 + 4OH^- \xrightarrow{60\ ^{o}C} 2Ni + N_2 + H_2O$$

The synthesised dried powder was characterized using transmission electron microscope (TEM), scanning electron microscope (SEM) energy-dispersive X-ray (EDX) analysis and powder x-ray diffraction (PXRD).

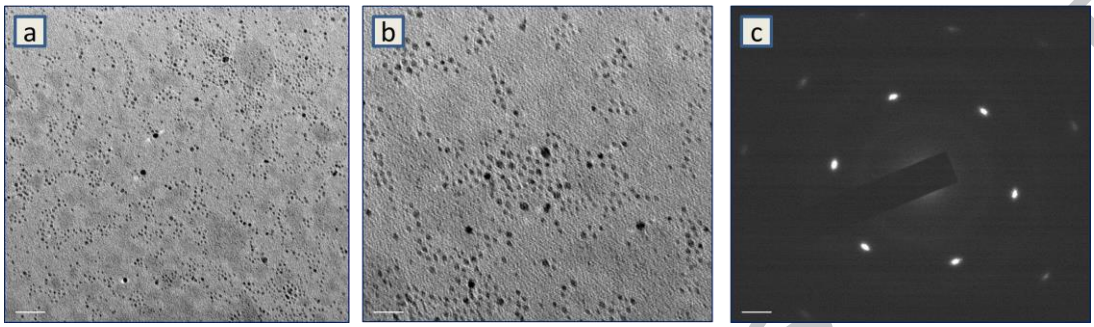


Fig.1. (a,b) TEM images of Ni NPs. The scale bar in the TEM image is 100 nm and 50 nm respectively. (c) SAED image at 21 nm.

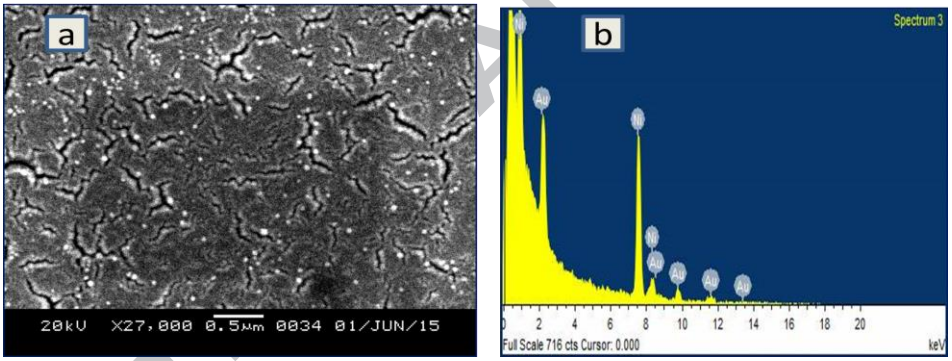


Fig.2 (a) SEM image of Ni Nps (b) EDAX Image of Ni Nps.

From the TEM (Fig. 1) and SEM (Fig. 2) image it is clear that the prepared nickel nanoparticles are nearly spherical in shape has an average size of 10–20 nm. The presence of some larger particles must be due to aggregation or overlapping of smaller particles. The EDAX data (Fig.2b) of Ni nanoparticles shows the presence of Ni peak with no trace of oxygen, thus confirming the nanoparticle is composed of nickel only.

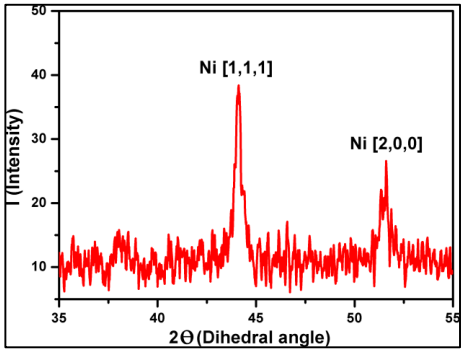


Fig.3. Powder XRD of Ni NPs showing shows characteristic dihedral angles at 44.12°, 51.60°

The powder XRD pattern of the nickel nanoparticles (Fig. 3) shows characteristic dihedral angles at 44.12°, 51.60° corresponding to Nickel (0) nanoparticles. No obvious peak of nickel oxide or hydroxide was detected.

The FTIR spectroscopy of nickel nanoparticles were measured between 400 and 4000 cm^{-1} showed peaks at 3440, 1633, 1384, 1110,752, 601 cm^{-1} (Fig.4).

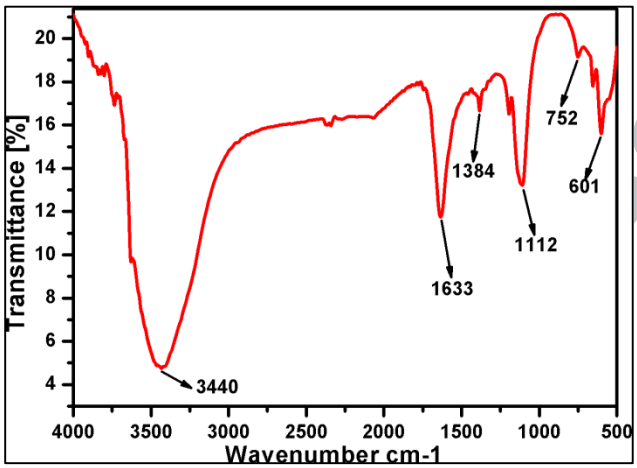


Fig. 4. FTIR spectrum of Ni NPs

Thereafter the successful synthesis and characterisation of Ni Nps, the as synthesised nanoparticles were employed as a catalyst in the multi-component reaction under various reaction condition. At first, a neat reaction of 4-chloro benzaldehyde **1a** (2.1 mmol), pyrazolone **2a** (2.0 mmol), barbituric acid **3a** (2.0 mmol) ran in the presence of catalytic amount of Ni nanoparticles which did not show any product formation. The subsequent reactions were then carried out in different solvent systems as shown in Fig.5. It was observed that the reactions carried out in ACN and DMF showed some product formation after 12 hrs. When the solvent system was changed to ethanol, the yield of the product increased dramatically to 75% in 6 hrs. The yield of the reaction further increased marginally in ethanol: water (1:1) solvent system but the best result was obtained when reaction was carried out in water affording 92% yield of the product in 3 hrs. Next, the catalyst loading was varied and we found that 25mg/mmol of the catalyst is the required amount for optimum yield of the product (Fig.6). When the reaction was carried out at room temperature, in absence of the catalyst no product formation was observed. After prolonged heating the reaction mixture for 16 hrs 70 % consumption of starting material was observed along with desired product formation.

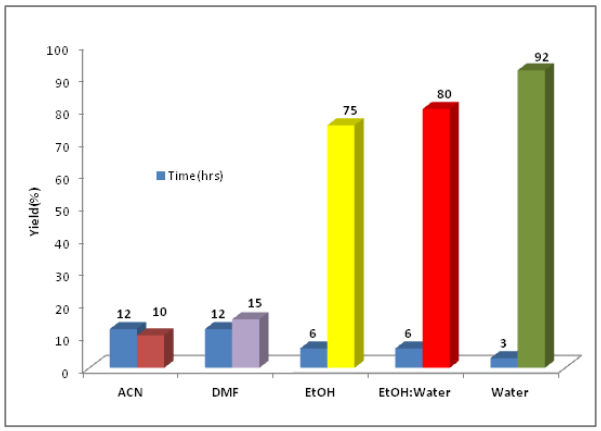


Fig.5. Effect of solvent on the rate of the reaction and the yield of the product

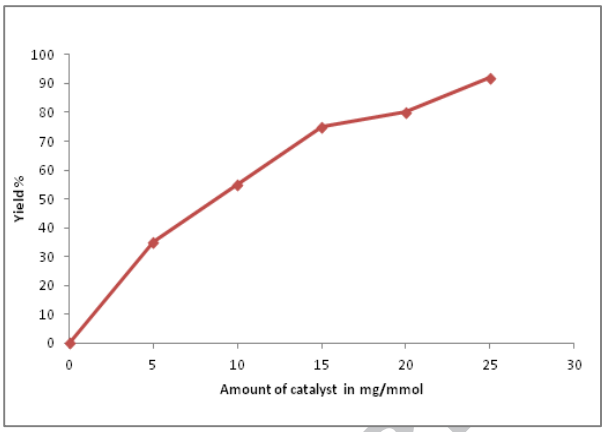
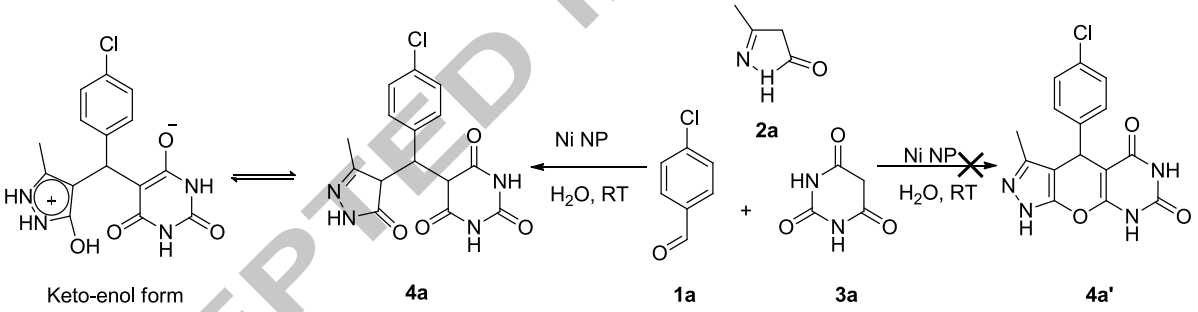


Fig.6. Effect of Catalyst loading

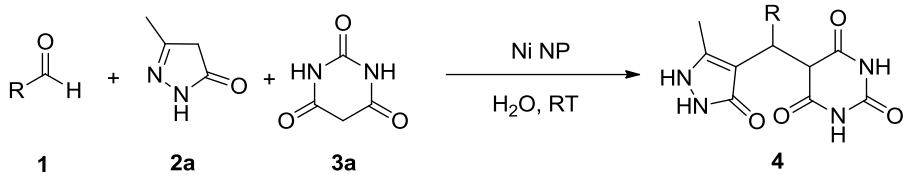
The structure of the compound **4a** formed was confirmed by ^{1}H, ^{13}C NMR, LC-MS, IR analyses and by single crystal XRD. The single crystal data of **4a** (Fig.7a) revealed that contrary to our expectation, the uncyclised product (Scheme 2) was formed. This can be attributed to the fact that both barbiturate and pyrazolone can exist in keto and enol forms to give zwitterionic moieties in which one hydrogen atom of the barbiturate is transferred of to pyrazolone imparting negative and positive charge respectively.[20]

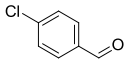
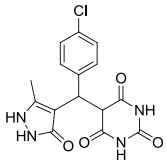
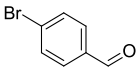
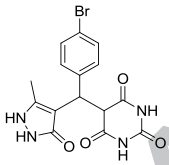
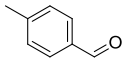
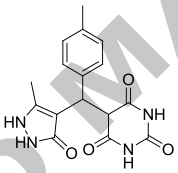
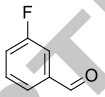
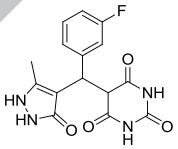
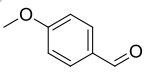
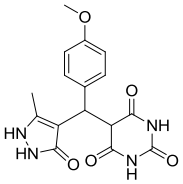
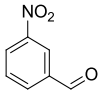
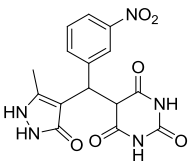
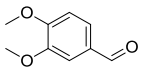
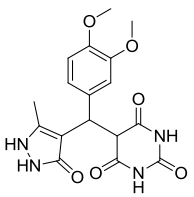


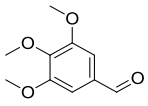
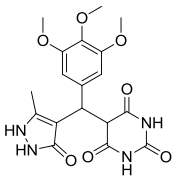
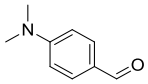
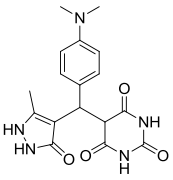
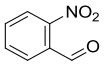
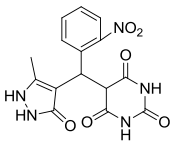
Scheme 2. The product formation **4a**

After the optimisation of the reaction was achieved, the generality of the method was studied by carrying out the reactions with differently substituted aromatic aldehydes. All the reactions proceeded smoothly to give the desired products in good to excellent yields with high purity. The presence of electron withdrawing or releasing substituents in the ortho-meta and para-positions do not seem to influence the reaction. All the pyrazolyl pyrimidinetriones derivatives (**4a–4k,** Table 1) were obtained in pure form by simple filtration and recrystallisation without purification by column chromatography and were characterised by ^{1}H, ^{13}CNMR, Mass spectral and IR analyses.

Table 1. Ni Nps catalyzed synthesis of pyrazol-4-yl-methyl-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione derivatives



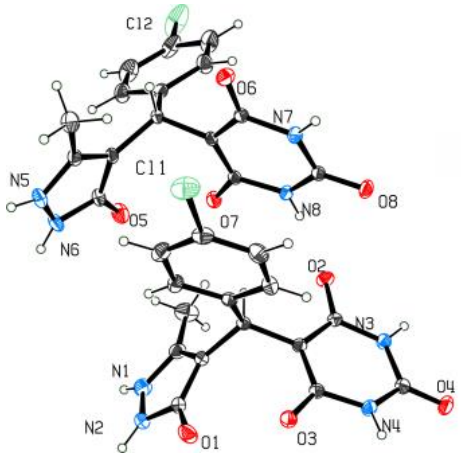
Entry	Aldehyde	Product (4)[a]	Time (hrs)	Yield[b]/ (%)
4a			3	92
4b			3	90
4c			3	94
4d			3	88
4e			3	93
4f			3	90
4g			3	91

4h			3	89
4i			3	85
4k			3	83

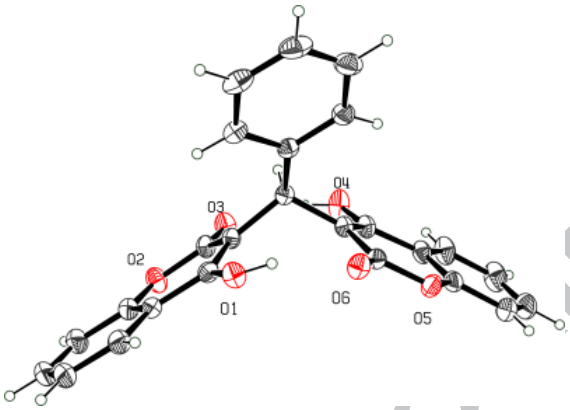
[a] Products were characterized by ^{1}H & ^{13}C NMR Mass and IR analyses. Reaction condition: aldehyde (2.1 mmol), pyrazolone (2.0 mmol), barbituric acid (2.0 mmol), Ni Nps (25mg/mmol), Solvent H_2O, room temperature

[b] Isolated yields

Encouraged by these results, the reactions involving active methylene compounds such as dimedone and 4-hydroxy coumarins with substituted aldehydes were also studied. The same optimised reaction condition was employed for the synthesis of bis(4-hydroxy-2*H*-chromen-2-one) and bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) derivatives. As expected the desired products were formed in each case irrespective of different positional substitution with excellent yield and purity. Even hetero and aliphatic substituted aldehydes were explored and to our amaze, the substituent resulted in good yield. The products obtained **(5a-5k, 6a-6k)** were purified by simple filtration, washing with water and crystallisation from ethanol. All the synthesised products (Table 2) are fully characterized by ^{1}HNMR, ^{13}CNMR, IR, and Mass spectral analyses. The derivatives **5j** and **6a** were characterised by single crystal x-ray analysis (Fig. 7b and 7c).



(a)



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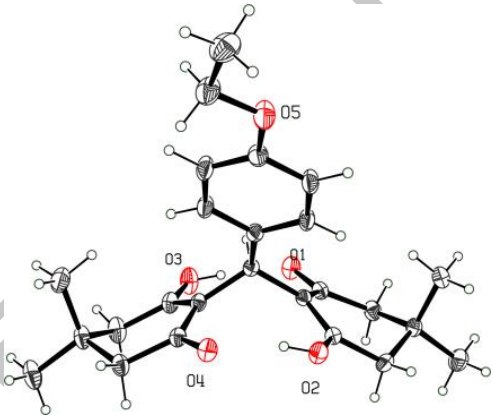
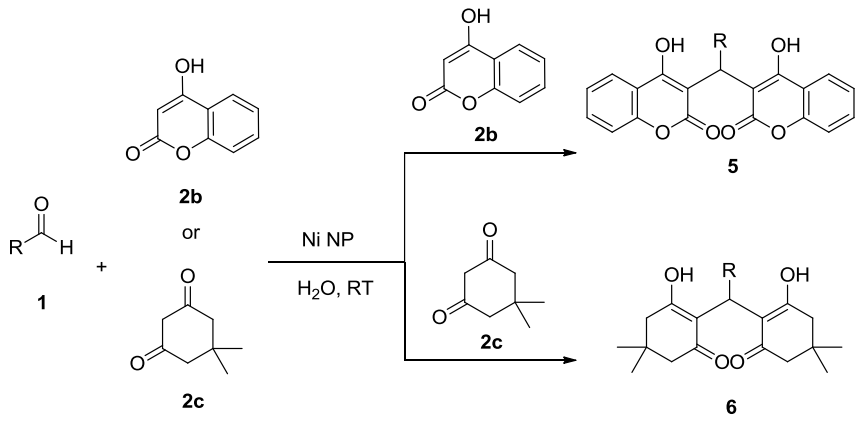
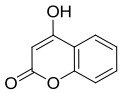
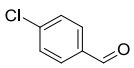
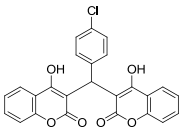
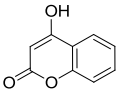
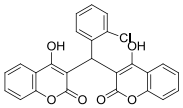
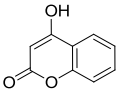
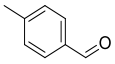
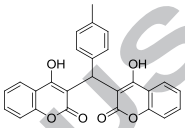
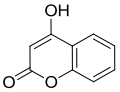
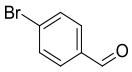
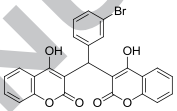
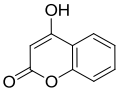
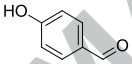
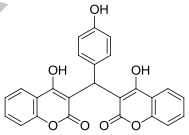
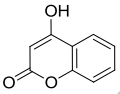
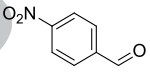
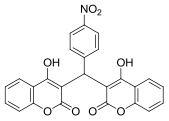
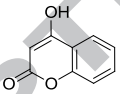
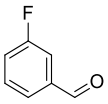
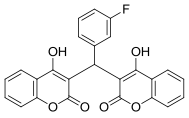
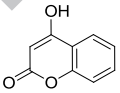
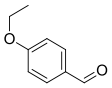
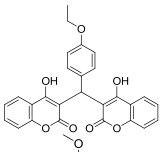
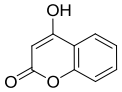
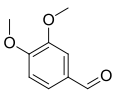
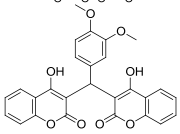
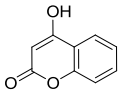
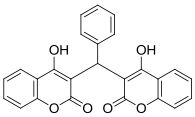
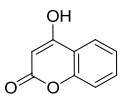
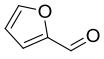
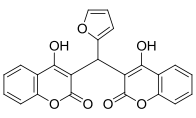
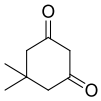
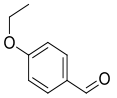
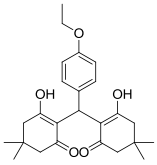


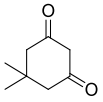
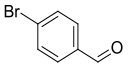
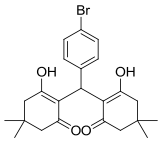
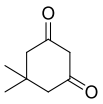
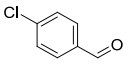
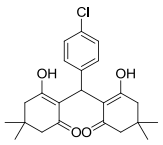
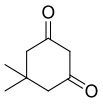
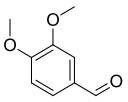
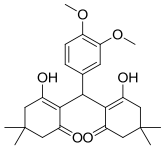
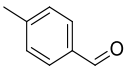
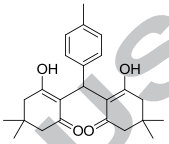
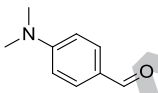
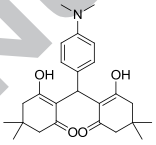
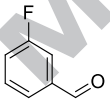
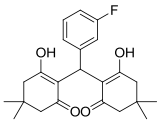
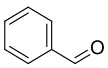
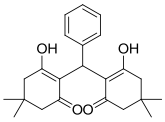
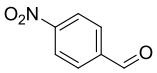
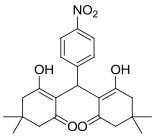
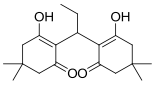
(c)

Fig. 7. ORTEP plots of compound **4a, 5j and 6a** with 30% thermal ellipsoid probability.

Table 2. Ni Nps catalyzed synthesis of bis(4-hydroxy-2*H*-chromen-2-one) and bis(3-hydroxy-5,5-dimethylcyclohex-2- enone) derivatives



Entry	Active methylene compound	Aldehyde	Product (5/6)[a]	Time (hrs)	Yield[b]/ (%)
5a				3	94
5b				3	86
5c				3	90
5d				3	89
5e				3	87
5f				3	92
5g				3	89
5h				3	93
5i				3	91
5j				3	92
5k				3	86
6a				3	93

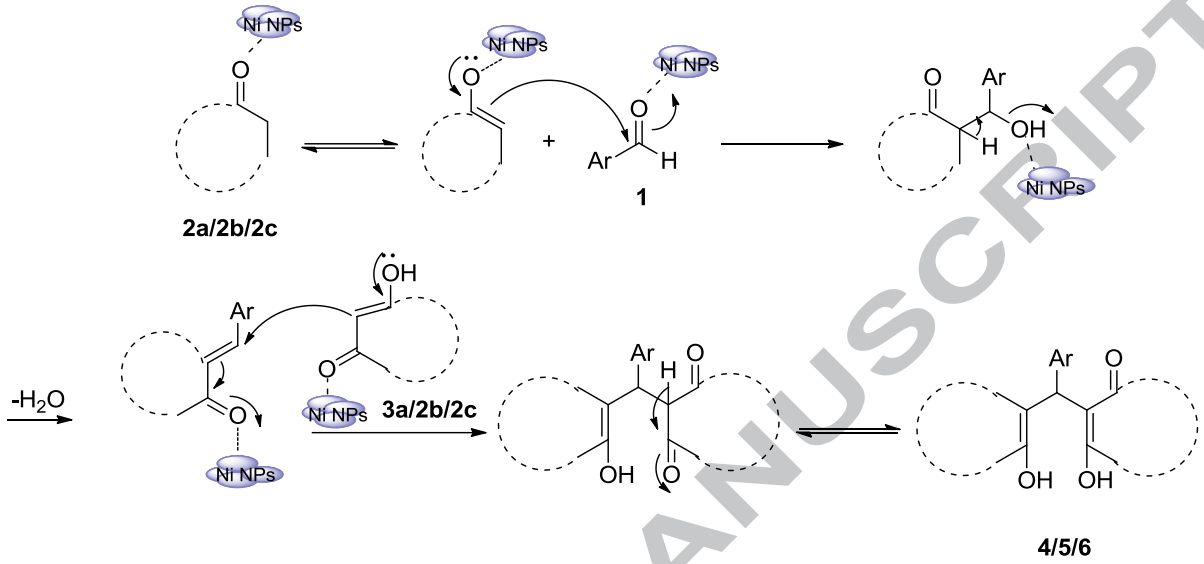
6b				3	88
6c				3	90
6d				3	85
6f				3	91
6g				3	88
6h				3	86
6i				3	90
6j				3	89
6k				3	80

[a] Products were characterized by ^{1}H & ^{13}C NMR Mass and IR analyses. Reaction condition: aldehyde (2.1 mmol), Active methylene compounds (4.0 mmol), Ni Nps (25 mg/mol), Solvent H_2O , room temperature

[b] Isolated yields

The plausible mechanism is depicted in Scheme 3. The reaction presumably proceeds through the initial activation of the carbonyl carbon atom of both active methylene compound and aldehyde by co-ordination with the Ni nanoparticle which increases the electrophilicity of the carbonyl carbon atom.[32c] Nucleophilic attack of the enolized pyrazolone / 4-hydroxy coumarin / dimedone at the electrophilic carbon centre of the aldehydes takes place to form

Knoevenagel product followed by intermolecular cyclization with active methylene group (barbituric acid/4-hydroxy coumarin/dimedone), dehydration and tautomerisation afforded the final product **4/5/6**.



Scheme 3. Plausible mechanism for the formation of the product **4, 5, 6**

The reusability of the catalyst was studied by performing many runs of the model reaction for the synthesis of **4a** using recycled Ni nanoparticles, and to our delight it was found that the yield of the desired product **4a** was not affected much till the 4th cycle (Fig.8). The decrease in the yield can be due to the fact that the nanoparticles might have aggregated which is evident from the TEM images (Fig. S4. In supporting information). The nanoparticles were recovered magnetically by a simple magnet from the reaction mixture. Then washed with acetone and millipore water for several times and dried in oven. After complete drying the recovered nanoparticles were reused as an efficient catalyst.

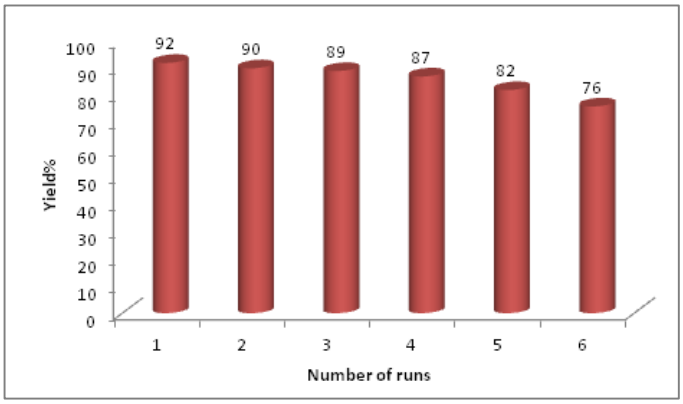


Fig.8. Reusability of the Ni nanoparticles for the synthesis of **4a**

2.2 Theoretical studies

Density functional theory (DFT) calculations [37] were performed with the Gaussian 09 program package[38] for compound **6a** to explicate the selective formation of uncyclised product over cyclised one. The geometry optimizations of cyclised and uncyclised product was performed using Becke's three-parameter exchange functional[39] and the nonlocal correlation functional of Lee, Yang, and Parr (B3LYP) in conjunction with the 6-31G(d,p) basis set. The energy profile diagram reveals that uncyclised product **6a** is energetically favourable compare to cyclised product (**6a′**) as the total energy of **6a′** is significantly higher than **6a** (Fig. S5. In supporting information). This considerable energy difference (ΔE= -20.6518 Kcal/mol) indisputably suggests the exclusive formation of uncyclised products.

2.3 Biological Studies

The pyrazolyl pyrimidinetriones, bis(4-hydroxy-2*H*-chromen-2-one) and bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) derivatives are known to have significant biological activity. All the synthesized compounds were therefore screened for their antibacterial activity. The antibacterial activities, given by the inhibition zone of all the synthesized compounds **4(a-k), 5(a-k), 6(a-k)** against five common pathogens which include spoilage organisms viz. *Escherichia coli, Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Salmonella enterica* are shown in (Fig. S6, Fig. S7, Fig. S8 In supporting information) respectively. Compound **4(a-k)** and **5(a-k)** exhibited distinctly high activity against the tested Gram positive and Gram negative organisms. Compounds **6b** and **6j** however showed mild activity against few of the organisms tested.

Pyrazolyl pyrimidinetriones and bis(4-hydroxy-2*H*-chromen-2-one) derivatives which showed the best antimicrobial activity using the diffusion method were further tested by the dilution method to determine the Minimum Inhibitory Concentration (MIC) (Table 3) Compound **4a, 4d, 4g, 4h** showed the best activity with MIC = 0.39 mg/mL against *K. pneumoniae* and **4j** with MIC = 0.39 mg/mL against *B. cereus.*

Compounds **4c, 4e, 4i** with the range of MIC = 3.12 mg/ml and 6.25 mg/mL showed antimicrobial activity against *B.cereus* and *K. pneumoniae* respectively at a relatively high concentration. Compounds **5a, 5b, 5j** exhibited better activity with MIC = 0.19 mg/mL against *K. pneumoniae* while **5a, 5j** with MIC = 0.39 mg/mL and 0.19 mg/ml against *B.*

cereus respectively. From the above results it can be concluded that compounds **4b, 4d, 4g, 4j**, **5a, 5c, 5j** are potential antibacterial agents for pharmaceutical application.

Table 3. Minimal inhibitory concentration (MIC) pyrazolyl pyrimidinetriones **4(a-k)**, bis(4-hydroxy-2*H*-chromen-2-one) **5(a-k)** against two tested reference strains

Compound Number	***B. cereus***	***K. pneumoniae***	**Compound Number**	***B. cereus***	***K. pneumoniae***
	MIC* (mg/ml)	**MIC* (mg/ml)**		**MIC* (mg/ml)**	**MIC* (mg/ml)**
4a	1.56 ± 0.03	0.39 ± 0.22	**5a**	0.39 ± 0.22	0.19 ± 0.11
4b	0.78 ± 0	0.78 ± 0.22	**5b**	3.12 ± 0	0.19 ± 0.05
4c	6.25 ± 1.8	3.12 ± 0	**5c**	0.39 ± 0	0.39 ± 0.11
4d	0.78 ± 0.45	0.39 ± 0	**5d**	3.12 ± 1.8	0.39 ± 0.11
4e	6.25 ± 1.8	3.12 ± 0.90	**5e**	6.25 ± 0	3.12 ± 1.8
4f	1.56 ± 0	0.78 ± 0.22	**5f**	0.78 ± 0.45	0.39 ± 0.22
4g	0.78 ± 0.45	0.39 ± 0	**5g**	0.78 ± 0	0.39 ± 0.11
4h	1.56 ± 0.90	0.39 ± 0.22	**5h**	3.12 ± 0.90	0.78 ± 0
4i	3.12 ± 0	3.12 ± 0.90	**5i**	0.78 ± 0.22	0.78 ± 0.45
4j	0.39 ± 0.22	0.78 ± 0.45	**5j**	0.19 ± 0.05	0.19 ± 0
4k	6.25 ± 1.8	3.12 ± 0.90	**5k**	3.12 ± 1.8	0.78 ± 0.45
Chloramph-enicol	0.09 ± 0	0.09 ± 0	**Chloramph-enicol**	0.09 ± 0	0.09 ± 0

*MIC values are mean ± SD of three replicates

3. Conclusions

A novel and efficient three-component condensation reaction of aryl aldehydes, and C–H-activated compounds has been developed for the synthesis of diverse heterocycles. In general, improvements in the rates and product yields are observed when the reaction is carried out in the presence of Ni NPs at room temperature. The DFT calculations were done for structure elucidation. The catalyst can be recovered magnetically and reused up to four successive runs without much noticeable change in the product yield. The protocol is eco-friendly, needs no chromatographic methods of purification. In addition the antibacterial assay was performed and it showed that pyarazolo pyrimidone **(4)** and bis(4-hydroxy-2*H*-chromen-2-one) **(5)** were found to be potential antibacterial agent, while bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) **(6)** did not show much effect on the bacterial growth of inhibition.

4. Experimental

4.1 General Information

All commercially available chemicals and reagents were purchased from Sigma Aldrich, Merck and were used without further purification. Purity of the products were confirmed by infrared (IR), ^{1}H-NMR, ^{13}C-NMR and mass spectra. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr pellets on a Perkin Elmer Spectrum 400 FTIR instrument, and the frequencies are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance II-400 spectrometer in DMSO-d_6/$CDCl_3$ (Chemical shifts in δ with TMS as internal standard). Mass spectral data were obtained with a WATERS (ZQ–4000) mass spectrometer. Elemental analysis was done using CHN-OS analyser model: Perkin Elmer 2400 series II. All reactions were monitored by thin layer chromatography (TLC) using precoated aluminum sheets (silica gel 60 F_{254} 0.2-mm thickness). Powder XRD analysis was conducted with an X'Pert Pro instrument. The TEM images were captured using a transmission electron microscope of JEM-2100 make, 200 kV (JEOL) and SEM and EDS imaging were carried out with scanning electron microscope of JSM-6360 (JEOL) make.

4.1.1 Synthesis of Nickel Nanoparticles

Nickel chloride hexahydrate (1.138 gm) was dissolved in 100 ml deionized water. To this solution 1.2ml of hydrazine hydrate, which acts as a reducing agent was added followed by addition of 1 ml of 1 M NaOH to maintain the alkalinity during the course of reaction. The reaction was stirred in a capped vessel in a heating magnetic stirrer at 60°C till black precipitate was formed. To ensure the complete conversion, the reaction mixture was allowed to stir for another 2 hrs at 60°C. The precipitate thus formed was filtered, washed several times with ethanol and then dried overnight in a hot air oven at 80°C.

4.1.2 General procedure for synthesis of compound pyrazolyl pyrimidinetriones (4a-4k)

A mixture of aldehyde **1** (2.1 mmol), pyrazolone **2a** (2.0 mmol) and barbituric acid **3a** (2.0 mmol) in 5 mL milliprore water was stirred at room temperature for appropriate time in the presence of Ni nanoparticles (25mg/mmol). The completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was filtered and then washed with water. The residue was recrystallised from 5 mL ethanol to give the pure product.

4.1.3 General procedure for synthesis of compound bis(4-hydroxy-2*H*-chromen-2-one) (5a-5k) and bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (6a-6k)

A mixture of aldehyde **1** (2.1 mmol), 4-hydroxy coumarin **2b**/ dimedone **2c** (4.0 mmol) in 5 mL milliprore water was stirred at room temperature for appropriate time in the presence of Ni nanoparticles (25mg/mmol). The completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was filtered and then washed with water. The residue was recrystallised from 5 mL ethanol to give the pure product.

4.2 Material and Methods for biological studies

To determine the activity of the supernatant, agar well-diffusion method was used. Reference strains, *Streptococcus pyogenes* MTCC 1925, *Enterococcus faecalis* MTCC 2729, *E.coli* MTCC 730, *Klebsiella pneumoniae* MTCC 109 and *Bacillus cereus* MTCC 430 used for the study were obtained from Institute of Microbial Technology (Microbial Type Culture Collection Centre, Chandigarh, India).

Muller Hilton Agar (HiMedia, Mumbai, India) plates were swabbed with bacterial cell suspension of the indicator strains and clinical pathogens adjusted to 1.5 x 10^8 CFU/ml. 5 mm diameter wells were cut in the agar plate using a sterile cork borer. 100 µl aliquot of each compound was added to each well and the plates were incubated at 37ºC for 24 hr. The broad-spectrum antibiotic chloramphenicol (30 mcg) was used as a positive control and DMSO solution was used as a negative control. After incubation, zones of inhibition around the wells were measured manually. All observations were made in triplicates.

Determination of minimum inhibitory concentration (MIC): MIC of pyrazolyl pyrimidinetriones **4(a-k)**, bis(4-hydroxy-2*H*-chromen-2-one) **5(a-k)** derivatives were performed in triplicate using broth microdilution method in 96 multi-well microtiter plates. 0.5 McFarland standard suspension of the reference strains were inoculated in Muller-Hilton broth (HiMedia, India). 50 µl of the inoculum was added to appropriate wells containing 50 µl of pyrazolyl pyrimidinetriones **4(a-k)**, bis(4-hydroxy-2*H*-chromen-2-one) **5(a-k)**. The final concentrations of the **4(a-k)** and **5(a-k)** in the range 0.024 mg/ml - 400 mg/ml (in DMSO) was used to evaluate the antibacterial activity. The 96 well plates were then sealed with parafilm and incubated in aseptic condition at 37 ˚C for 18 h. After incubation, 50 µl of 0.5% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) (Sigma, India) was added to the wells and the plates were incubated for another hour in dark. The colorless tetrazolium salt gets reduced to a red colored compound by biologically active organisms. Therefore, the inhibition of growth can be detected when the solution in the well remains clear after the incubation with TTC. The lowest concentration of each compound showing no visible growth was recorded as the MIC.

4.3 X-ray Crystallography

The X-ray data of **4a, 5j** and **6a** were collected at 293 K with a Agilent Xcalibur (Eos, Gemini) diffractometer using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). The data was collected and reduced in CrysAlis PRO (Agilent, 2011) software and cell refinement was done in CrysAlis PRO software.[40] The absorption was corrected by SCALE3 ABSPACK multi-scan method in CrysAlisPro.[40]The structures were solved by direct methods using the program SHELXS-2014[41] and refined by full matrix least-squares calculations (F^2) by using the SHELXL-2014[41] software. All non-H atoms were refined anisotropically against F^2 for all reflections. All hydrogen atoms were placed at their calculated positions and refined isotropically. ORTEP image of **4a, 5j, 6a** is shown in Fig. 6

and the details of data collection and refinements are given in Table S1, S2 and S3 in electronic supplementary information. The .cif files for **4a**, **5j** and **6a** were deposited with the Cambridge Crystallographic Data Centre, and the following code was allocated: CCDC-1562450, CCDC: 1562452 and CCDC: 1562451. This data can be obtained free of charge *via* the Internet: www.ccdc.cam.ac.uk/ data_request/cif.

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Conflict of interest

The authors have declared no conflict of interest.

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HIGHLIGHTS

- Magnetically recoverable & reusable catalyst easily prepared and utilised
- Aqueous Media reaction devoid of toxic reagent
- Non-chromatographic method of purification for synthesised compounds
- Number of prepared derivatives found to have antimicrobial activity