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Development of new methodology for the synthesis of enamino analogues employing by 'Ferric ammonium nitrate'/'Nano Copper ferrite' catalyst and evaluation of brine shrimp lethality bioassay

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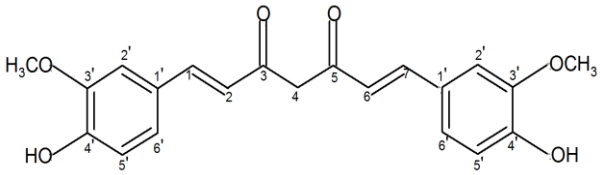
Abstract: The synthesis of enamino carbonyl compounds, enamino curcumin analogues using Ferric (III) Ammonium Nitrate/Nano Copper Ferrite catalyst. The title compounds were screened for Brine shrimp Lethality bioassay. β-Enamino carbonyl compounds and esters are the important and valuable synthones for the construction of bio-active heterocycles such as pyrazoles, oxazoles, quinolines, dibenzodiazepines, pyridinones, tetrahydrobenzoxazines, tetronic acids and tetrahydro phenanthridines. They have been used for the preparation of different important antibacterial, anti inflammatory, anticonvulsant and antitumour agents. They are also important precursors for the synthesis of 3-amino sugar derivatives, azo compounds, β-amino ketones, hexahydroazulenes and indolizidine alkaloids. The following scheme explains the synthesis of β-Enamino curcumin compounds(IV and V) which are potent anticancer activity.

Keywords: β-Enamino Curcumin compounds, Nano Copper ferrite, Ferric ammonium nitrate

INTRODUCTION

Based on early research conducted with cell cultures and animal models, pilot and clinical trials indicate curcumin may have potential as a therapeutic agent in diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis, as well as certain types of cancer. Animal studies have shown curcumin is rapidly metabolized, conjugated in the liver, and excreted in the feces, therefore having limited systemic bioavailability. A 40 mg/kg intravenous dose of curcumin given to rats resulted in complete plasma clearance at one hour postdose. An oral dose of 500 mg/kg given to rats resulted in a peak plasma concentration of only 1.8 ng/mL, with the major metabolites identified being curcumin sulfate and curcumin glucuronide. Data on the pharmacokinetics, metabolites, and systemic bioavailability of curcumin in humans, mainly conducted on cancer patients, are inconclusive.



CURCUMIN-(I)

In the other side, β-Enamino carbonyl compounds and esters are the important and valuable synthones for the construction of bio-active heterocycles such as pyrazoles, oxazoles, quinolines, dibenzodiazepines, pyridinones, tetrahydrobenzoxazines, tetronic acids and tetrahydro phenanthridines.[1-5] They have been used for the preparation of different important antibacterial,[6] anti inflammatory,[7] anticonvulsant[8] and antitumour agents.[9] They are also important precursors for the synthesis of 3-amino sugar derivatives,[10] azo compounds,[11] β-amino ketones,[12] hexahydroazulenes[13] and indolizidine alkaloids.[14] Thus it is highly desirable to review on these compounds.

The versatility of enaminones is in great part due to their promptness to both electrophilic and nucleophilic attack.[15] For this reason, they have been used in the synthesis of various heterocycles and natural products[16].

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Enamine of β-dicarbonyl compounds and their chemistry has been reviewed.[1, 17] These enaminones have demonstrated a potential as multipurpose synthetic intermediates in organic synthesis,[18-22] in the pharmaceutical development[19-22] and in heterocyclic synthesis.[19] The most important and straight forward method involves the direct condensation of β–dicarbonyl compounds with amines at reflux in an aromatic solvent with azeotropic removal of water.[23,24]. Based upon these avalible examples we prepared β-enamino curcumin analogs in the root of Green synthesis promoted by Ferric Ammonium Nitrate and Copper Nano ferrite catalysts with a good yield at less expensive and less time. In this chapter, we present the synthesis of curcumin analogs along with their structural characterization as well as study of their Brine shrimp lethality test.

Synthesis of β-Enamino curcumin analogues:

In this present work a new methodology was developed by the employing 5% FAN in presence of nano copper ferrite as catalyst for the synthesis of enamino 1,3 - dicarbonyl and curcumin analogues.

Preparation of the nano catalyst:

The catalyst was synthesized by citrate gel precursor method. Copper (II) nitrate and iron (III) nitrate were taken in stoichiometric proportions and minimum amount of deionized water was added to produce clear cationic solution. Citric acid solution was then prepared in stoichiometric ratio. Aqueous solutions with 1:1 molar ratio of metal ion solutions were mixed and citric acid was added in equimolar ratio to the above mixed metal ion solution. pH was adjusted to 7 by adding ammonia solution. The aqueous mixture was kept for stirring to form a highly viscous gel. The gel was then heated gradually up to 90◦C to evolve reddish brown gases and became dried gel which was finally treated at 350◦C for 1 h to observe whether the dry gel burnt out in self propagating manner to form loose powder. The finely powdered particles were calcinated at 600◦C. The powder was then characterized. XRD studies were carried out to the above nano ferrite and XRD spectrum is presented in Fig.1. From the XRD data, describes that the synthesized powder has nano size crystalline. The SEM studies are carried out on the copper ferrite sample at 600◦C, and it is presented in Fig.2. The TEM image was recorded and presented in Fig.3.

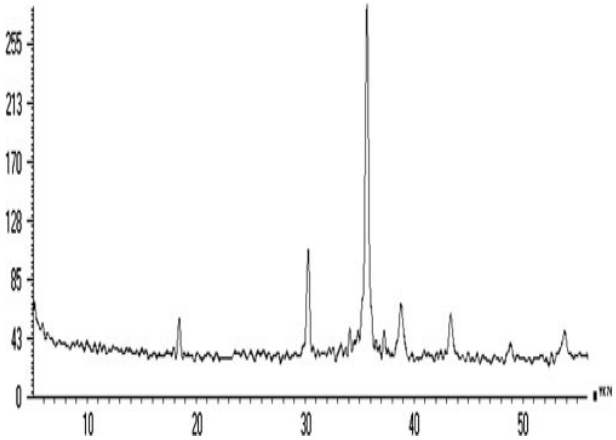

Fig.1: XRD spectrum of $CuFe_2O_4$ at 600 °C

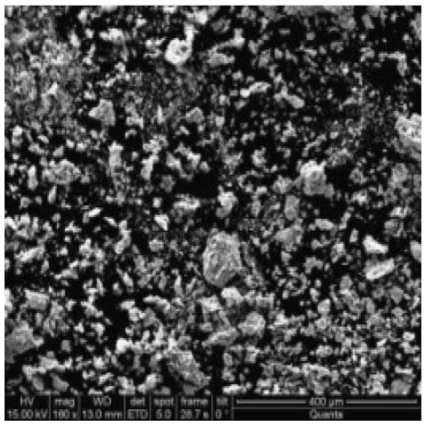

Fig.2: SEM image $CuFe_2O_4$

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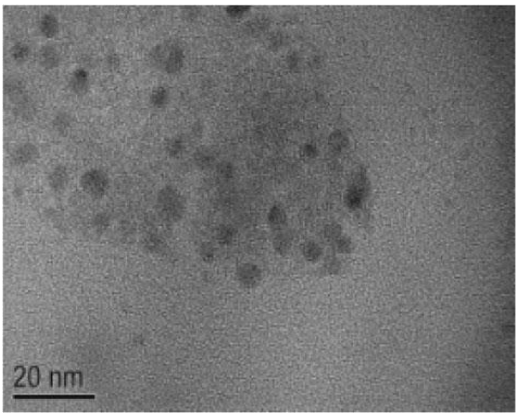
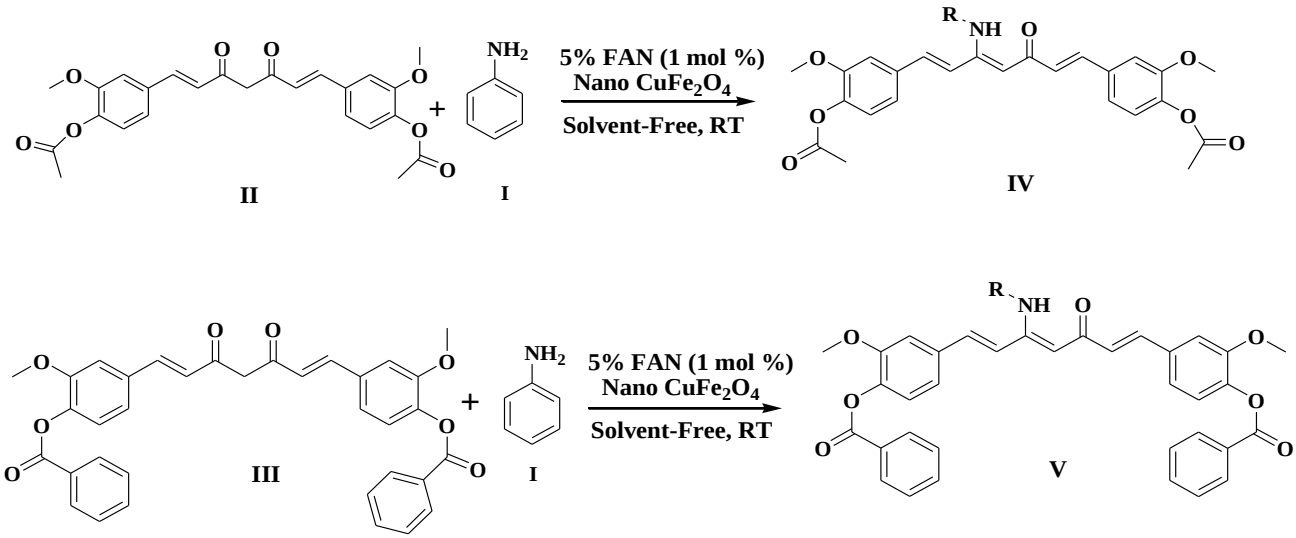


Fig.3: TEM image of $CuFe_2O$

Synthesis of enamino derivatives of curcumin analogues using FAN / Copper Nano Ferrite as Catalyst.

GENERAL PROCEDURE

A mixture of primary amine (I) (1 mmol) and Acetyl curcumin (II) (1 mmol)/ Benzoyl curcumin (III) was stirred at room temperature in the presence of 5% of ferric(III) ammonium nitrate solution (1 mol %) for an appropriate time (10-12 min) and copper nano ferrite(1 mol %) progress of reaction was monitored by TLC, and the reaction mixture was then filtered to separate the catalyst and the filtrate was quenched with a few drops of water and the product was extracted with dichloromethane and the solvent was removed under reduced pressure. Further purification was attained by column chromatography, a colourless crystalline compounds **IV&V** were formed and recrystallized from ethanol. The pure compound was then characterized. Spectral data of the synthesized compounds are given below, and the synthetic scheme is depicted in **Scheme-1.**



Scheme-1: Synthesis of β – enamino curcumin compounds (IV, V)

Synthesis of Enamino Benzoyl curcumin (V):
Compound (III) was further treated with equivi molor of aniline in the presence of FAN and nano copper ferrite to yield enamino Benzoyl curcumin (**V**), the product was isolated by column chromatography and finally recrystallized from ethanol.

Characterization of compound (V):
The compound(V) was characterized by using HPLC, IR, ^{1}HNMR and Mass. The purity of the **compound (V**) was checked by HPLC. The mobile phase was Acetonitrile. The flow rate was found to be 1 mL / Min. Retention time was 5.621 min. The purity of the compound(V) is 98.51%.

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Compound (V) was further characterized by IR, ^{1}HNMR and Mass. The IR spectrum of **compound (V**) was recorded analyzed as follows**.**

IR (KBr, Cm^{-1}): 1020 Cm^{-1} (-OCH_3 Str) , 1282 Cm^{-1} (O=C-O- Str), 1439, 1500, 1520, 1580 Cm^{-1} (Aro- C=C Str), 1620 (-CH_2- Str), 1719 Cm^{-1} (C=O Str), 2960 Cm^{-1} (-CH_3 Str), 3045 Cm^{-1} (-HC=CH -Str), 3115 Cm^{-1} (Ar –CH Str), 3501 Cm^{-1} (-NH Str).

^{1}**HNMR** (90 MH_Z, DMSO) of compound (V) was recorded
δ 3.1 (s, - $COCH_3$), 3.8 (s, - OCH_3), 4.4 (s, C=C-H), 6.8 – 7.6 (Ar –H), 12.1 (s, N-H).

Mass (ESI): 652.8 [M+1]
Based on the above spectral data the structure of **compound (V)** was confirmed.

Evaluation of Brine Shrimp Lethality Assay of curcumin analogues (II,IV,V) Cytotoxicity of Enamino Curcumin Analogues:
After confirm the structures of the **compounds (II, IV** and **V)**, it was proposed to investigate the Brine Shrimp Lethality Test of these compounds. The Brine Shrimp Lethality Test was carried out in collaboration with Laila Nutraceuticals Research and development centre, Vijayawada, Andhra Pradesh.

With reference Launainson J.L. et al[25] and Krishnaraju et. Al[26] To Evaluation of cytotoxic activity of test **compounds (II,IV** and **V)** by Brine Shrimp Lethality Test . It was explain in the following procedure. The Chemicals & Reagents were used as NaCl, NaOH, DMSO. The Standard drug is Podophyllotoxin.

Solutions preparation:
Brine Solution: 38 gm of NaCl was weighed and dissolved in 1000mL of distilled water and autoclaved at 15 lbs pressure for 15-20 min and after starilization pH was adjusted to 8.5 by using 1M NaOH solution.

Hatching of shrimps: 100mg of Artemia salina eggs was added to 200 mL of sterile brine solution and aerated for 38h.

Text compounds (II, IV and **V) preparation:** Appropriate dilutions of test compounds (2.5 µg/mL to 100 µg/mL) were prepared.

Experimental Method:
Step-1 : 5 mL of sterile brine solution was pipetted into each test tube and from that 100 µL was taken out.
Step-2: 100 µL of different concentrations of the **compound** (II, IV and V) were added to the test tube with control in triplicates and the solution was vortexed thoroughly.
Step-3:10 shrimps were added to each test tube and the surviving larvae of test and control test tubes were observed after 24h. Replicas were maintained for accurate results.

Calculation: Percentage lethality was determined by comparing the mean surviving larvae of **compounds** (II, IV & V) and control tubes. The ED_{50} values were obtained using fenny probed analysis software at 95% confidence limits from observed data.

The cytotoxic activity of curcumin analogues**(**compounds II, IV and V) was measured by **ED_{50}** as well as **LCL** and **UCL** on brine Shrimp at different concentrations (2.5µg/mL–100µg/mL) with reference to control drug podophyllotoxin were presented in Table 1.

TABLE.1

UCL = Upper confidence limit, LCL = Lower confidence limit

Test items	ED_{50} (µg/mL)	Degree (s) of freedom	UCL	LCL
Acetyl curcumin(II)	52.98	4.459	64.38	43.99
Acetyl Enamino Curcumin (IV)	86.92	2.023	85.63	51.68
Benzoyl Enamino Curcumin(v)	82.98	3.639	84.23	53.59
Podophyllotoxin	2.99	1.747	3.83	1.91

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CONCLUSION

According to above Brine Shrimp Lethality Assay of curcumin analogues Cytotoxicity of Enamino Curcumin Analogues were moderately used for cytotoxicity. Furthur research about curcumin analogues are under the process.

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