Selective synthesis of 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione using copper oxide nanoparticles for potential inhibitors of β-ketoacyl-[acyl-carrier-protein] synthase III of Mycobacterium tuberculosis

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Received 12 October 2015; accepted (revised) 10 May 2016

A simple and selective synthesis of 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione derivatives has been investigated by multicomponent reaction of aromatic aldehydes, 4-hydroxycoumarin and dimedone using copper oxide nanoparticles. All the synthesised coumarin derivatives have been screened for anti-tubercular activity. The virtual analysis of the synthesised derivatives have also been carried out against β-ketoacyl-[acyl-carrier-protein] synthase III. The inhibition of the β-ketoacyl-[acyl-carrier-protein] synthase III is confirmed from the docking study. The experimental results have shown that the compound 10,10-dimethyl-7-(3-nitrophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)dione (MIC 6.25 µg/mL) is a good anti-tubercular agent and as good as standard streptomycin drug based on minimum inhibitory concentration.

Keywords: Multicomponent reaction, copper oxide nanoparticles, β-ketoacyl-[acyl-carrier-protein] synthase III, docking analysis

Along with the sub-saharan African countries, India has a most prominent burden of communicable diseases like TB and Malaria. According to a recent report of WHO, 1/3 of the world population is infected by TB and more than 80% cases are from the 22 high-burden countries like India\textsuperscript{1}. Now-a-days, the problem of the tuberculosis is becoming more critical with the emergence of multi drug resistant (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) tuberculosis\textsuperscript{2}. The TDR tuberculosis is the most lethal form of tuberculosis due to the resistance of the organism towards all drugs presently in clinical use\textsuperscript{3}. Moreover, proper treatment of tuberculosis is another issue of concern in India, due to socio-economical status of the Indian population\textsuperscript{4}. The presently used anti-tuberculosis drugs target dynamically growing bacteria in cell processes such as chromosome replication, cell wall biogenesis, protein synthesis and the resistance towards them is more lethal in diabetics and AIDS patients\textsuperscript{5}. In case of tuberculosis, the β-ketoacyl-[acyl-carrier-protein] synthase-III enzyme plays a vital role as a crucial link between the fatty acid synthase-I and fatty acid synthase-II pathways which results in the synthesis of the mycolic acids. The β-ketoacyl-[acyl-carrier-protein] is a key enzyme involved in the cell wall biosynthesis in mycobacteria. This enzyme is involved in fatty acid biosynthesis (FabH) that occurs in plants and bacteria. The enzyme of FAS-I and FAS-II are not found in mammals. The selective inhibition of these enzymes is a prime requirement to distinguish the potent anti tuberculosis agents with relatively low toxicity towards human beings\textsuperscript{6,7}. The significance of the inhibition of β-ketoacyl-acyl carrier protein synthase-III which can act as attractive target for the antimycobacterial agents and pharmacophoric model for inhibitors of β-ketoacyl-acyl carrier protein synthase-III of \textit{Staphylococcus aureus}. β-Ketoacyl-acyl carrier protein synthase-III plays an essential and regulatory role in bacterial fatty acid synthesis. It represents a promising target for design of novel antibacterial compounds which act as potential drugs\textsuperscript{8}. Based on the recent findings and our previous experience we have tried to design a novel class of anti tubercular agents with their possible action on β-ketoacyl-acyl carrier protein synthase-III of mycobacterium\textsuperscript{9}. 
A number of synthetic methodologies including multistep synthesis are reported for the designing of new bioactive heterocycles over the last few decades. Among these methodologies, multicomponent reactions (MCRs) have gained a vital importance in medicinal, organic, pharmaceutical, agrochemicals and combinatorial chemistry. The MCR strategies involve three or more than three reactants in a single reaction vessel to form a new desired product which pertains to structural unit of all components. The stringent molecules derived from these types of diversity generating reactions have been successfully employed for establishing several biologically active agents. Since the last decade, industrial and academic researchers have applied these powerful MCRs strategies as one of the most efficient and cost-effective tools for combinatorial synthesis.

Coumarin is an important group of oxygen containing heterocycles which is deeply acknowledged in the contemporary organic chemistry. Both naturally and synthetically obtained coumarin derivatives are used as additives in foods, cosmetics, optical brightener and also posses properties of several analytical reagents. They have several types of pharmacological properties such as antibacterial, anticancer, anticoagulant, anti-HIV, antioxidant and spasmylocic activities.

Several methods have been reported for synthesis of coumarin derivatives till date with their own merits and demerits. Basically, these derivatives have been synthesized by three component condensation of 4-hydroxycoumarin, aromatic aldehydes and active methylene compounds like malononitrile and Meldrum’s acid to form a 3,4-dihydropyrano[γ]chromene and tetrahydrobezo[b]pyran derivatives. The catalysts like HPA, TMG, DAHP, TBAB, DBU, [Bmim]OH, hexamethylditin, MgO (Ref 31), K2PO4 (Ref 32), ZnO nanoparticle, SDS, Na2SeO4 (Ref 35), trisodium citrate, S-proline, NaBr (Ref 38), hexamethyltetramine, MgO (Ref 31), K3PO4 (Ref 32), ZnO nanoparticle, SDS, Na2SeO4 (Ref 35), trisodium citrate, S-proline, NaBr (Ref 38), Ruthenium(III) (Ref 39), K2CO3 under microwave irradiation, and Fermented baker’s yeast are reported in the literature for synthesis of substituted coumarin derivatives. One of the most attractive synthetic strategies favoured by organic chemists is the use of metal oxide nanoparticles as catalyst in a wide range of organic synthetic methodologies.

Among these, copper oxide nanoparticles have been extensively used in organic transformation due to their high catalytic efficiency, large surface to volume ratio and recyclability in many organic transformations.

To the best of our knowledge, there are very few reports on three component reaction of 4-hydroxycoumarin, aromatic aldehydes and cyclic 1,3-dicarbonyl compounds to form dihydrochromeno derivatives. One of the major shortcomings of the reported methods is side formation of 5a along with the desired product 4a (Scheme I).

Although a long term problem associated with the TB therapy and our previous experience in related area, herein we introduce our investigation in the selective synthesis of 10,11-dihydropyrano[4,3-b]chromene-6,8(7H,9H)-dione derivatives without the formation of unwanted 3,3'-arylmethylene-bis-4-hydroxycoumarin by using copper oxide nanoparticles and their anti-tuberculosis activity (Scheme I).
Results and Discussion

Chemistry

In the beginning as a trial experiment, 4-hydroxycoumarin (1 mmol), 3-nitrobenzaldehyde (1 mmol) and dimedone (1 mmol) were stirred in 5 mL water without any catalyst at reflux condition. A sticky mass was observed after several hours of reflux which unfortunately indicated formation of the intermediate Knoevenagel product monitored by thin layer chromatography [TLC] (Entry-8, Table I). In the pursuit of suitable catalytical conditions for this selective transformation, we employed a variety of acid and base catalysts such as PTSA, trisodium citrate, triethyl amine, diamonium hydrogen phosphate (DAHP) and sodium dodecyl sulphate (SDS) for synthesis of 10,10-dimethyl-7-(3-nitrophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione (Table II, Entry-1). Further, to assess the solvent effects in order to establish appropriate reaction conditions, we screened different catalyst with several solvent systems depicted in Table I. It clearly suggests that the copper oxide nanoparticles are proved to be dominating over the other employed catalyst and solvent systems. Eventually, the protocol was set using 4-hydroxy coumarin, aromatic aldehyde and dimedone in presence of catalytic amount (0.08 g) of Copper oxide nanoparticles in ethanol at reflux temperature to obtain selectively 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione in good to excellent yield (Table I).

These positive results encouraged us to ensure the applicability of current protocol to get a new library of 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione derivatives using optimised conditions with various aromatic aldehydes bearing electron withdrawing and donating group. This optimised result shows that this new protocol is very useful for synthesis of target molecule giving

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehydes</th>
<th>Products</th>
<th>Time (h)</th>
<th>m.p. (°C)</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>4-HO</td>
<td>4a</td>
<td>15</td>
<td>(256-257)</td>
<td>84</td>
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<tr>
<td>2</td>
<td>2-HO</td>
<td>4b</td>
<td>10</td>
<td>(210-211)</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>3-HO</td>
<td>4c</td>
<td>11</td>
<td>(270-271)</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>4-HO</td>
<td>4d</td>
<td>04</td>
<td>258-262</td>
<td>83</td>
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<tr>
<td>5</td>
<td>5-HO</td>
<td>4e</td>
<td>09</td>
<td>226-227</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>6-HO</td>
<td>4f</td>
<td>09</td>
<td>240-243</td>
<td>78</td>
</tr>
<tr>
<td>7</td>
<td>7-HO</td>
<td>4g</td>
<td>10</td>
<td>190-192</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>8-HO</td>
<td>4h</td>
<td>08</td>
<td>220-222</td>
<td>78</td>
</tr>
<tr>
<td>9</td>
<td>9-HO</td>
<td>4i</td>
<td>09</td>
<td>235-240</td>
<td>85</td>
</tr>
</tbody>
</table>

Table I — Study of solvent and catalytic effect in synthesis of 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Condition</th>
<th>Time</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PTSA</td>
<td>(Reflux) Ethanol</td>
<td>Up to 20hrs.</td>
<td>55% No product formed</td>
</tr>
<tr>
<td>2</td>
<td>Triethyl amine</td>
<td>(Reflux) Ethanol</td>
<td>Up to 25hrs.</td>
<td>45%</td>
</tr>
<tr>
<td>3</td>
<td>Trisodium citrate</td>
<td>(Reflux) Ethanol</td>
<td>Up to 20hrs</td>
<td>35% This work</td>
</tr>
<tr>
<td>4</td>
<td>Without catalyst</td>
<td>(Reflux) Ethanol</td>
<td>12-20hrs</td>
<td>No product formed</td>
</tr>
<tr>
<td>5</td>
<td>CuO nanoparticle</td>
<td>(Reflux) Ethanol</td>
<td>Up to 20hrs</td>
<td>Sticky mass No product formed</td>
</tr>
<tr>
<td>6</td>
<td>SDS</td>
<td>(Reflux) Water</td>
<td>Up to 20hrs</td>
<td>No product formed</td>
</tr>
<tr>
<td>7</td>
<td>Without catalyst</td>
<td>(Reflux) Water</td>
<td>Up to 20hrs</td>
<td>Sticky mass No product formed</td>
</tr>
<tr>
<td>8</td>
<td>Without catalyst</td>
<td>RT (Water)</td>
<td>Up to 20hrs</td>
<td>40%</td>
</tr>
<tr>
<td>9</td>
<td>PTSA</td>
<td>RT (Ethanol)</td>
<td>Up to 20hrs</td>
<td>40%</td>
</tr>
<tr>
<td>10</td>
<td>CuO nanoparticle</td>
<td>RT (Ethanol)</td>
<td>Up to 20hrs</td>
<td>40%</td>
</tr>
<tr>
<td>11</td>
<td>DAHP</td>
<td>RT (Water: Ethanol) (50:50)</td>
<td>Up to 20hrs</td>
<td>45%</td>
</tr>
<tr>
<td>12</td>
<td>DAHP</td>
<td>Reflux (Water: Ethanol) (30:70)</td>
<td>Up to 20hrs</td>
<td>55%</td>
</tr>
<tr>
<td>13</td>
<td>Trisodium citrate</td>
<td>Reflux (Water: Ethanol) (50:50)</td>
<td>Up to 20hrs</td>
<td>40%</td>
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</tbody>
</table>
excellent yield of the product. The results are shown in Table II. The previously reported compounds are confirmed by IR and $^1$H NMR and the newly synthesized compounds are characterised by IR, $^1$H and $^{13}$C NMR and mass spectra.

The possible mechanism for the selective formation of 4a using CuO nanoparticles has been shown in Scheme II. The initial step is formation of the intermediate 5 by Knoevenagel condensation of 4-hydroxy coumarin 1 and aromatic aldehyde 2. The formed Knoevenagel product and dimedone 3 undergoes Michael type addition to form an intermediate 6 which subsequently undergoes cyclization followed by intramolecular dehydration to form a desired product 4a. The isolated products were interpreted on the basis of their relevant spectroscopic data without any further purification. The simple experimental procedure, easy isolation of product and selectivity are the noteworthy aspects for this protocol (Scheme II).

**Anti-tuberculosis activity**

6,8(7H,9H)-dione derivatives (4a-4i) were evaluated for their antituberculosis activity against *Mycobacterium tuberculosis* strain. Minimum Inhibition Concentration (MIC) was determined by BACTEC radiometric method using Microplate Alamar Blue assay (MABA). All the compounds were screened against MTB H$_37$Rv with the concentration range from 6.5 to 50 µg/mL. The present anti-tuberculosis drugs such as pyrazinamide, ciprofloxacin and streptomycin were used as reference drugs for the study. All screened compounds show good anti-tuberculosis results but interestingly compound 10,10-dimethyl-7-(3-nitrophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)dione (Table II, entry 1) is highly active against MTB with MIC of only 6.25 µg/mL. This experimental result of the said compound resembles with the MIC of the standard streptomycin drug. This is the key finding of this research article (Figure 1).

**Molecular Docking analysis**

The virtual screening is the computational methodology in which the macro molecular and micro molecular interactions are checked. Virtual analysis of the synthesised derivatives were carried out using

![Scheme II — Mechanistic pathway of the reaction](image-url)
protein structure of β-ketoacyl-[acyl-carrier-protein] synthase-III obtained from the www.rcsb.org which is a key enzyme involved in the cell wall synthesis of M. tuberculosis. Docking analysis of the synthesised derivatives were carried out in the biopredictda module of the V life MDS 4.3. All the synthesised molecules were docked in the similar binding site of β-ketoacyl-[acyl-carrier-protein] synthase-III. The most active compound 10,10-dimethyl-7-(3-nitrophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)dione showed characteristic interactions like hydrogen bonding ARG249 (1.5 Å), ARG36 (1.8 Å) (Green Colour) and hydrophobic interaction with GLY209, PRO210, VAL212, PHE213, ASN247, ILE250 (Figure 2).

10,10-dimethyl-7-(4-nitrophenyl)-10,11-dihydrochromeno [4,3-b]chromene-6,8(7H,9H)-dione is another active compound which showed hydrogen bond interaction with ARG249 (1.5 Å) and hydrophobic interaction with ARG36, THR37, GLY152 GLY209, PRO210, VAL212, PHE213, ASN247, ILE250. In latter molecule, due to electron withdrawing effect of nitro group the ring becomes flat which results in the unavailability of the ring towards the hydrophobic interaction.

**Prediction of ADME properties**

Drug likeness plays important role in the drug discovery process. The new chemical entities with desirable pharmacokinetic properties might act as good drug candidates. The compounds which do not violate Lipinsky rule are potential drug candidates. All the molecules synthesised are found to be good drug candidates. Percent oral absorption of all molecules were also calculated and all the molecules were shown to have good absorption ranging from 70 to 90% (Table III).

**Experimental Section**

**Catalyst preparation**

The CuO (II) nanoparticles (NPs) were prepared by the co-precipitation method\(^\text{38}\). The morphological, structural and optical properties of CuO nanoparticles
(NPs) were studied by using scanning electron microscope (SEM, Figure 1) and powder X-ray diffraction (PXRD, Figure 2). The matching of PXRD pattern with standard (JCPDS #45-0937) reveals the phase pure monoclinic structured CuO (II) nanoparticles (NPs). The nanocrystalline nature of CuO powder also clarified by calculating the crystallite size for (84.25) reflection by using the Scherrer equation \( D = \frac{K \lambda}{\beta \cos \theta} \). The SEM analysis of CuO nanoparticles carried out at different magnification shows the agglomeration nature (Figure 3 and Figure 4).

**Synthesis and characterisation**

Melting points were observed in an open capillary and are uncorrected. The desired structures of all of the compounds were confirmed from their relevant spectral data. IR spectra (KBr) were recorded on a Perkin-Elmer Spectrum 100 FT-IR instrument. The \(^1\)H and \(^{13}\)C NMR spectra were obtained (DMSO-\(d_6\)) or (CDCl\(_3\)) on a Bruker (300 MHz) NMR instrument using TMS as an internal standard. The XRD was determined by Bruker D\(_2\)-Phaser and SEM was performed by JSM-670IF (Japan) instruments respectively. The progress of reaction was checked by thin layer chromatography (TLC) using silica gel G (LR). All commercially available chemicals were purchase from Sigma-Aldrich and used without purification.

**Molecular Docking Analysis**

To explore the interactions of the compounds, we carried out binding simulations using biopredicta module of Vlife MDS 4.3 suite. Crystal structures of \(\beta\)-ketoacyl-[acyl-carrier-protein] synthase III (PDB code: 1YWF) was utilized for the docking simulations which was downloaded from the free protein database at www.rcsb.org.

**Prediction of ADME properties**

A computational study of titled compounds was performed for prediction of ADME properties. Polar surface area (TPSA), Log P, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms were calculated using Molinspiration online property calculation toolkit and Vlife MDS 4.3 and Absorption (%ABS) was predicted and calculated by:

\[
% \text{ABS} = 109 \times (0.345 \times \text{TPSA}) \quad (\text{Ref 50})
\]

<table>
<thead>
<tr>
<th>No. of Moles</th>
<th>TPSA</th>
<th>LogP</th>
<th>% Oral Absorption</th>
</tr>
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<td>102.34</td>
<td>4.773</td>
<td>73.69</td>
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<td>56.516</td>
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</tr>
<tr>
<td>4</td>
<td>85.978</td>
<td>4.178</td>
<td>79.33</td>
</tr>
<tr>
<td>5</td>
<td>56.516</td>
<td>4.738</td>
<td>89.50</td>
</tr>
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</tr>
<tr>
<td>8</td>
<td>76.744</td>
<td>4.359</td>
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</tr>
<tr>
<td>9</td>
<td>102.34</td>
<td>4.773</td>
<td>73.81</td>
</tr>
</tbody>
</table>

Figure 3 — X-ray diffraction (XRD) of CuO nanoparticles

Figure 4 — Scanning Electron Microscope (SEM) of CuO nanoparticles
General procedure for synthesis of 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione derivative
In the general reaction for synthesis of selective dihydrochromeno derivatives, an equimolar mixture of 4-hydroxy coumarin (0.165 g,1 mmol), aromatic aldehyde (0.150 g, 1 mmol), dimedone (0.140 g, 1 mmol) and Cu-O nanoparticles (0.08 g) in catalytic amount were dissolve in 5.0 mL ethanol and the solution was vigorously stirred at reflux temperature. The progress of reaction was monitored by thin layer chromatography (TLC). On completion of reaction the crude product obtained was filtered and washed with ethanol. The product was purified by recrystallization from ethanol to afford the pure desired product.

The spectral data of products are given as follows.

10,10-Dimethyl-7-(3-nitrophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione, 4a:
White solid. m.p.240-45°C. (Lit. 256-57°C)\(^\text{\textsuperscript{45}}\). IR (KBr): 1719 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.12 (s, 3H, -CH\(_3\)), 1.62 (s, 3H, -CH\(_3\)), 2.30-2.33 (q, 2H, -CH\(_2\)), 2.73-2.77 (q, 2H, -CH\(_2\)), 5.07 (s, 1H, -CH), 7.34-7.43 (m, \(J = 9\) Hz, Ar-H), 7.45-7.50 (m, 1H, \(J = 9\) Hz, Ar-H), 7.59-7.65 (m, 1H, \(J = 6\) Hz, Ar-H), 7.90-7.94 (m, 2H, \(J = 3\) Hz, Ar-H), 8.04-8.08 (m, 2H, \(J = 3\) Hz, Ar-H).

10,10-Dimethyl-7-(4-nitrophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione, 4b:
White solid. m.p.202-205°C. (Lit. 210-211°C)\(^\text{\textsuperscript{45}}\). IR (KBr): 1716, 1663 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.10 (s, 3H, -CH\(_3\)), 1.20 (s, 3H, -CH\(_3\)), 2.24-2.39 (q, 2H, -CH\(_2\)), 2.73 (s, 2H, -CH\(_2\)), 5.06 (s, 1H, -CH), 7.35-7.43 (q, 2H, \(J = 9\) Hz, Ar-H), 7.56-7.63 (t, 3H, \(J = 9\) Hz Ar-H), 7.89-7.92 (m, 1H, \(J = 9\) Hz, Ar-H), 8.12-8.15 (d, 2H, \(J = 9\) Hz, Ar-H).

10,10-Dimethyl-7-(4-chlorophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione, 4c:
White solid. m.p.245-50°C. (Lit. 270-271°C)\(^\text{\textsuperscript{45}}\). IR (KBr): 1662, 1719 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.10 (s, 3H, -CH\(_3\)), 1.18 (s, 3H, -CH\(_3\)), 2.30-2.32 (m, 2H, -CH\(_2\)), 2.69-2.70 (m, 2H, -CH\(_2\)), 4.94 (s, 1H, -CH), 5.80-5.84 (m, 4H, \(J = 9\) Hz, Ar-H), 7.37-7.40 (m, 4H, \(J = 9\) Hz, Ar-H), 5.77-5.78 (m, 1H, \(J = 9\) Hz, Ar-H), 7.86-7.89 (dd, 1H, \(J = 9\) Hz, Ar-H).

10,10-Dimethyl-7-(4-hydroxy-3-methoxyphenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione, 4d:
White solid. m.p.258-62°C. (Lit. 271-72°C)\(^\text{\textsuperscript{45}}\). IR (KBr): 1662, 1723 cm\(^{-1}\); \(^1\)H NMR (300 MHz,CDCl\(_3\)): \(\delta\) 1.12 (s, 3H, -CH\(_3\)), 1.18 (s, 3H, -CH\(_3\)), 2.31-2.32 (d, 2H, -CH\(_2\)), 2.69-2.70 (d, 2H, -CH\(_2\)), 3.93 (s, 3H, -OCH\(_3\)), 4.89 (s, 1H, -CH), 6.62-6.65 (m, 1H, \(J = 3\) Hz, Ar-H), 6.75-6.78 (d, 1H, \(J = 3\) Hz, Ar-H), 7.14 (s, 1H, -OH), 7.28-7.34 (m, 3H, \(J = 3\) Hz, Ar-H), 7.36-7.39 (m, 1H, \(J = 3\) Hz, Ar-H), 7.55-7.60 (m, 1H, \(J = 6\) Hz, Ar-H).

10,10-Dimethyl-7-(theiophen-2-yl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione, 4e:
Grey solid. m.p.226-27°C. (Lit. 215-18°C)\(^\text{\textsuperscript{45}}\). IR (KBr): 1664, 1715 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.19 (s, 3H, -CH\(_3\)), 1.58 (s, 3H, -CH\(_3\)), 2.37 (s, 2H, -CH\(_2\)), 2.68-2.77 (m, 2H, -CH\(_2\)), 5.37 (s, 1H, -CH), 6.88-6.91 (m, 1H, \(J = 3\) Hz, Ar-H), 7.09-7.12 (m, 2H, \(J = 3\) Hz, Ar-H), 7.34-7.36 (m, 2H, \(J = 3\) Hz, Ar-H), 7.37-7.39 (m, 1H, \(J = 6\) Hz, Ar-H), 7.56-7.62 (m, 1H, \(J = 3\) Hz, Ar-H).

10,10-Dimethyl-7-(2-naphthophenyl)-10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione, 4f:
White solid. m.p.240-43°C. IR (KBr): 1667, 1722 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.06 (s,3H, -CH\(_3\)), 1.18 (s,3H, -CH\(_3\)), 2.17-2.33 (q,2H, -CH\(_2\)), 2.74-2.75 (s,2H, -CH\(_2\)), 5.78 (s,1H, -CH), 3.73-3.76 (m, 2H, \(J = 9\) Hz, Ar-H), 7.38-7.41 (m, 1H, \(J = 9\) Hz, Ar-H), 7.48-7.52 (m, 1H, \(J = 6\) Hz, Ar-H), 7.55-7.60 (m, 1H, \(J = 6\) Hz, Ar-H), 7.65-7.70 (m, 2H, \(J = 9\) Hz, Ar-H), 7.78-7.81 (m, 1H, \(J = 9\) Hz, Ar-H), 7.92-7.95 (m, 1H, \(J = 6\) Hz, Ar-H), 8.94 (m, 1H, Ar-H); \(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 27.51, 29.12, 32.33, 40.88, 50.57, 107.88, 113.78, 116.46, 116.94, 122.39, 124.22, 124.85, 125.81, 126.25, 128.02, 128.18, 131.80, 132.13, 133.57, 152.48, 153.80, 160.66, 161.66, 196.15; MS: m/z 422. Anal. Calcd for C\(_{28}\)H\(_{32}\)O\(_4\): C, 79.72; H, 7.70; O, 15.08. Found: C, 79.10; H, 7.65; O, 15.01%.
White solid. m.p.220-22°C. IR (KBr): 1657, 3365 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.01 (s, 3H, -CH₃), 1.10 (s, 3H, -CH₃), 2.12-2.28 (q, 2H, -CH₂), 2.67 (s, 2H, -CH₂), 4.63 (s, 1H, CH), 6.57-6.60 (d, 2H, J = 9 Hz, Ar-H), 7.01-7.04 (d, 2H, J = 6 Hz, Ar-H), 7.26-7.38 (m, 2H, J = 9 Hz, Ar-H), 7.53-7.56 (t, 1H, J = 6 Hz, Ar-H), 7.83-7.86 (d, 1H, J = 6 Hz, Ar-H), 7.92 (s, 1H, -OH); ¹³C NMR (75 MHz, CDCl₃): δ 27.34, 29.08, 32.33, 32.41, 50.69, 106.74, 113.67, 114.87, 115.23, 116.71, 122.79, 124.73, 129.58, 132.61, 133.56, 152.34, 153.77, 156.49, 160.41, 162.25, 196.06; MS: m/z 388. Anal. Calcd for C₂₅H₂₆O₅: C, 74.10; H, 5.15; O, 22.95%. Found: C, 74.10; H, 5.15; O, 20.51%.

4.50; N, 20.51; O, 22.95. Found: C, 69.01; H, 4.59; N, 3.36; O, 23.00. Found: C, 69.06; H, 4.59; N, 3.36; O, 23.00. Found: C, 69.01; H, 4.50; N, 20.51; O, 22.95%.

Conclusion

In conclusion, we have developed a convenient method for the selective synthesis of 10,11-dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione derivatives via the use of recyclable and heterogeneous copper oxide nanoparticles. Among the tested compounds for their anti-tubercular activity, 4a is found to be a potential molecule against H₃⁷Rv with MIC value 6.25 µg/mL which compares to standard Streptomycin. The synthesized 4b also showed good antioxidant activities. This experimental observation will help in the future for a further development of new drugs for the benefit of the society against MDR, XDR and TDR tuberculosis.

Acknowledgments

One of the authors (PVA) is thankful to University Grants Commission, New Delhi for sanctioning a major project [F. No. 39-786/2010(SR)] and another author (KTP) thanks Shivaji University authorities for providing financial support in the form of DRF.

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