Synthesis of chromene-2-thiones and in vitro evaluation of their antifungal and antibacterial activities

Nepram Sushuma Devi, Thokchom Prasanta Singh, Nonibala Khumanthem & Okram Mukherjee Singh

*Department of Chemistry, Manipur University, Canchipur 795 003, India
bDepartment of Life Science, Manipur University, Canchipur 795 003, India
E-mail: ok_mukherjee@yahoo.co.in

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Cyclocondensation of β-oxodithioesters with substituted 2-hydroxy benzaldehydes in presence of copper(II)chloride as the catalyst yields 3-alkanoyl/aroyl/ heteroaroyl-2H-chromene-2-thiones (87–95% yields). These reactions are carried out under solvent-free conditions. All the synthesized compounds have been evaluated for their antifungal and antibacterial activities using tube dilution technique. Only five compounds are found exhibiting good antifungal and antibacterial activities.

Keywords: β-Oxodithioester, cupric chloride, coumarin, solvent free, antimicrobial activity

Coumarins, which are derivatives of benzo-2-pyrones or chromen-2-ones, are widely distributed in various species of plants. Coumarins have attracted intensive attention of chemists due to their potential for biological activities such as antifungal, antihypertensive, antioxidant, anti-inflammatory, and antimicrobial activity. They also possess steroid sulfatase inhibitory, lipid-lowering, anticarcinogenic, acaricidal, potential anticonvulsant and analgesic activities. Certain coumarins also inhibit acetylcholinesterase (involved in Alzheimer’s disease), aromatase (breast cancer therapy), and squalene-hopenecyclase (cholesterol lowering and antitrypanosomal drugs). They are also used in liquid crystal displays, information systems and as insecticides, rodenticides in pharmaceuticals and as printing inks, paints and as synthetic rubber in industrial products. Certain coumarins are reported to behave as potent anti-HIV agents. Prompted by these literature reports, it was decided to synthesise novel coumarins by simple methods and evaluation of their antimicrobial activities.

Note

Results and Discussion

Chemistry

Diverse approaches have been used to synthesize numerous artificial coumarins. Classical routes to coumarins involve mostly Pechmann and Knoevenagel condensation reactions. To improve the yields of products by these classical reactions, several catalytic systems and reaction conditions have been introduced. Versatile coumarin syntheses could be achieved through organopalladium intermediates. Efficient syntheses of a variety of 3-substituted coumarins via Wittig reaction in room-temperature ionic liquids have been reported. Recently, there have been reports on the facile synthesis of coumarinyl isothiocyanate from amino coumarin. A simple, efficient, and green protocol for synthesis of coumarin-3-carboxylic acids was also reported via Knoevenagel condensation of Meldrum’s acid with ortho-hydroxyaryl aldehydes in ionic liquids. The synthesis of certain 3-substituted-2H-chromene-2-thiones by using SnCl2 as catalyst has been recently reported. However, the reported synthetic procedure has certain limitations such as longer reaction time, lower yields and use of hazardous reagent such as piperidine. Keeping in mind the themes of green chemistry principles, the synthesis of such bioactive compounds has been explored further using easily accessible, non toxic and milder Lewis acid catalyst systems. CuCl2 was found to be the best catalyst, giving the highest yield of the product under a short duration of 1 h. Also, the antifungal and antibacterial properties of such compounds have been evaluated.

As depicted in Scheme I, the condensation reaction of β-oxodithioester 1a (2.5 mmol) with 2-hydroxy benzaldehyde 2a (2.5 mmol) was initially studied by heating at a temperature of 90°C in the presence of catalytic amount of CuCl2.2H2O (10 mol %) without using any solvent. After 1 h, TLC shows complete conversion of the starting materials to 3-benzoyl-2H-chromene-2-thione (3a) (Table I, entry 1). It was purified by recrystallization from hot ethanol to get yellow crystals having m.p.170–71°C. The structure of compound 3a was confirmed from the spectral and analytical data. The presence of the IR absorption bands at 1662 cm⁻¹ indicates the presence of the...
carbonyl functional group, which is further confirmed by the $^{13}$C NMR peak at $\delta$ 192.3. $^1$H NMR data exactly confirms the presence of ten aromatic protons as multiplets at the range of $\delta$ 7.37-7.97. The mass spectrum and also the C, H, N analytical data as shown in the Experimental Section supports the structure of 3a.

The same process was successfully extended to afford coumarins 3b-o by treating different $\beta$-oxodithioesters 1b-g and variously substituted 2-hydroxy benzaldehydes 2a-c under the same catalytic conditions (Scheme I). Almost all the reactions proceed smoothly within a short duration and yielding the desired products in good to excellent yields. The results are summarized in Table I. The structure of the newly synthesized compounds 3a-o was confirmed by melting point, elemental analysis, MS, FT-IR, NMR ($^1$H and $^{13}$C) spectroscopic data.

A plausible mechanism for the reaction can be postulated based on the literature as shown in the following Scheme II. Substituted/unsubstituted ortho hydroxy benzaldehyde 2 initially condenses with dithioester 1 in presence of cupric chloride, generating a copper complex A. The thienolate form undergoes intramolecular ring cyclisation through a new C-C bond with the electrophilic carbon atom of B to generate the intermediate C. Finally C is transformed to the desired product 3 by dehydration.

**Biology**

The newly synthesized compounds were subjected to in vitro antimicrobial screening using the tube dilution technique. The minimum inhibitory concentrations (MICs) of the tested compounds were determined as described with minor modifications. The MIC is defined as the minimum concentration of compound required to inhibit 99% of bacterial growth, and the MIC values of the synthesized compounds along with the standard drugs. All the fifteen compounds were screened against fungal strains *Curvularia oryzae* (MTCC 2605) and *Fusarium oxysporum* (MTCC 278) for their antifungal, and gram-positive bacteria *Bacillus amyloliquefaciens* (MTCC 610) and *Bacillus cereus* (MTCC 1307) for their antimicrobial activities. Ten compounds show poor activity with insignificant MIC values. Only five compounds 3a, 3b, 3g, 3j and 3m had MICs ranging from moderate to good activity (Table II). Thus, the antibacterial and antifungal activities of the five compounds have been selectively analyzed.

The drug Ampicillin trihydrate was used as standard drug and it exerted an antibacterial activity of MIC, 50 µg mL$^{-1}$ against *B. amyloliquefaciens* and MIC of 30 µg mL$^{-1}$ against *B. cereus*. Compound 3b with MIC of 25 µg mL$^{-1}$ possessed excellent antibacterial activity, followed by compound 3a (MIC, 30 µg mL$^{-1}$). Compounds 3g and 3j (MIC, 100 µg mL$^{-1}$) possessed moderately good antibacterial activity. Weak antibacterial activity was expressed by compound 3m as the MIC value was found to be 200 µg mL$^{-1}$. The compounds 3b and 3a were found to be more potent than the standard drug as the MICs were less than that of the standard.

Fluconazole was used as the standard drug for evaluating the antifungal activity against the test fungi *C. oryzae* and *F. oxysporum*. Fluconazole had MIC value against the test fungi of MIC of 40 µg mL$^{-1}$ against *C. oryzae* and 30 µg mL$^{-1}$ against *F. oxysporum*. Compounds 3b and 3a possessed excellent antifungal activity as their MICs value were 10 and 25 µg mL$^{-1}$ respectively. Compounds 3g and 3j with moderately good antifungal activity and weak antifungal activity was expressed by compound 3m (MIC of 200 µg mL$^{-1}$). The newly synthesized compounds 3a and 3b showed very good antifungal activity as the MIC value was less than that of the standard drug fluconazole (Table II).
Table I — CuCl₂ catalyzed preparation of coumarins under solvent-free conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹ (1)</th>
<th>R²/R³ (2)</th>
<th>Coumarin (3)</th>
<th>m.p. (°C)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="" alt="1(a)" /></td>
<td><img src="" alt="1(a)" /></td>
<td><img src="" alt="1(a)" /></td>
<td>170-71</td>
<td>93</td>
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<tr>
<td>2.</td>
<td><img src="" alt="2(b)" /></td>
<td><img src="" alt="2(a)" /></td>
<td><img src="" alt="2(b)" /></td>
<td>187-89</td>
<td>95</td>
</tr>
<tr>
<td>3.</td>
<td><img src="" alt="3(c)" /></td>
<td><img src="" alt="3(a)" /></td>
<td><img src="" alt="3(c)" /></td>
<td>185-86</td>
<td>90</td>
</tr>
<tr>
<td>4.</td>
<td><img src="" alt="4(d)" /></td>
<td><img src="" alt="4(a)" /></td>
<td><img src="" alt="4(d)" /></td>
<td>172-73</td>
<td>95</td>
</tr>
<tr>
<td>5.</td>
<td><img src="" alt="5(e)" /></td>
<td><img src="" alt="5(a)" /></td>
<td><img src="" alt="5(e)" /></td>
<td>178-79</td>
<td>91</td>
</tr>
<tr>
<td>6.</td>
<td><img src="" alt="6(f)" /></td>
<td><img src="" alt="6(a)" /></td>
<td><img src="" alt="6(f)" /></td>
<td>179-81</td>
<td>87</td>
</tr>
<tr>
<td>7.</td>
<td><img src="" alt="7(a)" /></td>
<td><img src="" alt="7(b)" /></td>
<td><img src="" alt="7(g)" /></td>
<td>142-43</td>
<td>93</td>
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<tr>
<td>8.</td>
<td><img src="" alt="8(b)" /></td>
<td><img src="" alt="8(b)" /></td>
<td><img src="" alt="8(h)" /></td>
<td>140-42</td>
<td>91</td>
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</table>

— Contd
**Experimental Section**

**Chemistry**

Melting points are uncorrected and were determined in capillary tubes on an apparatus containing silicon oil. The IR spectra were recorded on a Perkin Elmer 983 spectrometer in KBr pellets with absorption given in cm$^{-1}$. $^1$H and $^{13}$C NMR spectra were recorded respectively on a Varian EM-

### Table I — CuCl$_2$ catalyzed preparation of coumarins under solvent-free conditions — Contd

<table>
<thead>
<tr>
<th>Entry</th>
<th>$R^1$ (1)</th>
<th>$R^2$ $R^3$ (2)</th>
<th>Coumarin (3)</th>
<th>m.p. (°C)</th>
<th>Yield$^b$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.</td>
<td><img src="image" alt="c" /></td>
<td>$R^2=H$, $R^3=OCH_3$ (b)</td>
<td><img src="image" alt="d" /></td>
<td>175-76</td>
<td>95</td>
</tr>
<tr>
<td>10.</td>
<td><img src="image" alt="e" /></td>
<td>$R^2=H$, $R^3=OCH_3$ (b)</td>
<td><img src="image" alt="f" /></td>
<td>163-65</td>
<td>92</td>
</tr>
<tr>
<td>11.</td>
<td><img src="image" alt="g" /></td>
<td>$R^2=H$, $R^3=OCH_3$ (b)</td>
<td><img src="image" alt="h" /></td>
<td>145-46</td>
<td>89</td>
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<tr>
<td>12.</td>
<td><img src="image" alt="i" /></td>
<td>$R^2=H$, $R^3=OCH_3$ (b)</td>
<td><img src="image" alt="j" /></td>
<td>165-67</td>
<td>87</td>
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<tr>
<td>13.</td>
<td><img src="image" alt="k" /></td>
<td>$R^2=Br$, $R^3=H$ (c)</td>
<td><img src="image" alt="l" /></td>
<td>180-81</td>
<td>91</td>
</tr>
<tr>
<td>14.</td>
<td><img src="image" alt="m" /></td>
<td>$R^2=Br$, $R^3=H$ (c)</td>
<td><img src="image" alt="n" /></td>
<td>208-09</td>
<td>87</td>
</tr>
<tr>
<td>15.</td>
<td><img src="image" alt="o" /></td>
<td>$R^2=Br$, $R^3=H$ (c)</td>
<td><img src="image" alt="p" /></td>
<td>195-96</td>
<td>89</td>
</tr>
</tbody>
</table>

$^a$ Reaction conditions: 1 (2.5 mmol), 2 (2.5 mmol), CuCl$_2$ (10 mol %), 90°C, 1.0-2.0 h.

$^b$ Isolated yield
390 (300 MHz and 75.5 MHz) spectrometer. The chemical shifts (δ, ppm) and the coupling constants (Hz) are reported in the standard fashion with reference to internal tetramethyl silane (TMS). The MS spectra were recorded on a Jeol JMSD-300 mass spectrometer. Elemental analyses were performed on a Carlo Erba’s 108 microanalyzer.

**General procedure for the synthesis of 3-alkanoyl/aroyl/heteroaroyl-2H-chromene-2-thiones, 3a-o**

Salicylaldehyde/substituted salicylaldehyde 2 (2.5 mmol), β-oxodithioester 1 (2.5 mmol), and CuCl₂·2H₂O (10 mol %) were heated at 90°C with stirring for 1-2 h. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, water was added, and the product was extracted with ethyl acetate. The crude products were purified and separated by column chromatography over silica gel, using increasing amounts of ethyl acetate in hexanes as eluent.

**3-Benzoyl-2H-chromene-2-thione, 3a.** Yellow crystals. ¹H NMR (300 MHz, CDCl₃): δ 7.37-7.42 (m, 1H), 7.45-7.56 (m, 3H), 7.59-7.66 (m, 3H), 7.68-7.71 (m, 1H), 7.94-7.96 (m, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ 116.7, 119.9, 125.9, 128.6, 128.7, 129.6,

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**Table II — Antimicrobial activity of prepared coumarins**

<table>
<thead>
<tr>
<th>Compd</th>
<th>Anti-fungal activity (MIC) µg mL⁻¹</th>
<th>Anti-bacterial activity (MIC) µg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. oryzae</td>
<td>F. oxysporum</td>
</tr>
<tr>
<td>3a</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>3b</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3g</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3j</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>3m</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Ampicillin trihydrate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flucanozole</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>
7.58-7.60 (m, 4H), 7.83 (d, 1H), 8.19 (d, 1H), 8.29-8.35 (m, 1H), 8.51 (s, 1H), 8.81-8.85 (m, 1H); 13C NMR (75.5 MHz, CDCl3): δ 20.5, 113.9, 119.6, 128.4, 129.8, 131.1, 134.5, 156.3, 191.7, 193.7; IR (KBr): 1225, 1600, 1657, 3023, 3067 cm⁻¹; MS: m/z 204 (M⁺). Anal. Calcd for C₁₅H₁₄O₂S: C, 64.69; H, 3.95; S, 15.70. Found: C, 64.78; H, 4.08; S, 15.70%.

3-(4-Acetylpyridine)-2H-chromene-2-thione, 3f. Yellow crystals. ¹H NMR (300 MHz, CDCl3): δ 6.70-6.75 (m, 4H), 7.35-7.40 (m, 4H), 7.45-7.50 (m, 4H), 8.05-8.10 (m, 4H), 8.65-8.75 (m, 2H), 9.00-9.10 (m, 1H); ¹³C NMR (75.5 MHz, CDCl3): δ 125.8, 129.5, 129.8, 131.1, 134.5, 156.3, 191.7, 193.7; IR (KBr): 1225, 1600, 1657, 3023, 3067 cm⁻¹; MS: m/z 204 (M⁺). Anal. Calcd for C₁₅H₁₄O₂S: C, 64.69; H, 3.95; S, 15.70. Found: C, 64.78; H, 4.08; S, 15.70%.

3-Benzoyl-8-methoxy-2H-chromene-2-thione, 3g. Yellow crystals. ¹H NMR (300 MHz, CDCl3): δ 3.80 (s, 3H), 3.92 (s, 3H), 3.95 (s, 3H), 4.30; S: C, 68.44; H, 4.32; S, 9.85%.

3-(4-Chlorobenzoyl)-2H-chromene-2-thione, 3h. Yellow crystals. ¹H NMR (300 MHz, CDCl3): δ 3.92 (s, 3H), 4.77 (s, 3H), 7.66 (s, 1H), 7.83-7.93 (m, 2H), 8.19-8.29 (m, 2H); ¹³C NMR (75.5 MHz, CDCl3): δ 65.3, 114.1, 114.2, 119.5, 120.7, 125.8, 128.6, 132.3, 133.9, 139.8, 146.8, 146.9, 147.1, 191.2, 191.7; IR (KBr): 1274, 1593, 1651, 1664, 3051 cm⁻¹; MS: m/z 326 (M⁺). Anal. Calcd for C₁₅H₁₄O₂S: C, 66.24; H, 4.32; S, 9.82. Found: C, 66.25; H, 4.31; S, 9.85%.

3-(4-Methoxybenzoyl)-8-methoxy-2H-chromene-2-thione, 3i. Yellow crystals. ¹H NMR (300 MHz, CDCl3): δ 3.95 (s, 3H), 7.07-7.13 (m, 2H), 7.19-7.26 (m, 1H), 7.36 (d, J = 5.4 Hz, 2H), 7.57 (s, 1H), 7.75 (d, J = 3.6 Hz, 2H); ¹³C NMR (75.5 MHz, CDCl3): δ 56.3, 114.7, 119.7, 120.6, 125.9, 129.1, 130.9, 134.1, 139.0, 140.3, 146.8, 147.1, 191.2, 191.7; IR (KBr): 1235, 1605, 1654, 3045, 3060 cm⁻¹; MS: m/z 330 (M⁺). Anal. Calcd for C₁₅H₁₁ClO₃S: C, 61.73; H, 3.35; S, 9.69. Found: C, 61.75; H, 3.31; S, 9.65%.

3-(4-Methylbenzoyl)-8-methoxy-2H-chromene-2-thione, 3j. Yellow crystals. ¹H NMR (300 MHz, CDCl3): δ 2.43 (s, 3H), 2.45-2.50 (m, 2H), 6.57-6.62 (m, 2H), 7.25-7.30 (m, 2H), 7.31-7.36 (m, 2H), 7.45-7.50 (m, 2H), 7.51-7.55 (m, 2H), 7.61-7.65 (m, 2H), 7.77-7.82 (m, 2H), 8.19-8.24 (m, 2H); ¹³C NMR (75.5 MHz, CDCl3): δ 20.5, 113.9, 119.6, 128.4, 128.6, 129.8, 131.1, 134.5, 156.3, 191.7, 193.7; IR (KBr): 1225, 1600, 1657, 3023, 3067 cm⁻¹; MS: m/z 204 (M⁺). Anal. Calcd for C₁₅H₁₄O₂S: C, 64.69; H, 3.95; S, 15.70. Found: C, 64.78; H, 4.08; S, 15.70%.
3(2-Acetylthiophene)-8-methoxy-2H-chromene-2-thione, 3l. Yellow crystals. 1H NMR (300 MHz, CDCl3): δ 3.83 (s, 3H), 6.74-6.79 (m, 2H), 7.06-7.09 (t, 1H), 7.25 (s, 1H), 7.40-7.42 (m, 1H), 7.64 (d, J = 3.9 Hz, 1H), 7.83 (d, J = 2.7 Hz, 1H); 13C NMR (75.5 MHz, CDCl3): δ 56.1, 111.5, 119.7, 120.2, 121.2, 125.1, 128.1, 134.7, 135.5, 138.7, 143.3, 144.7, 146.4, 185.6, 192.8; MS: m/z 302 (M+). Anal. Calcld for C13H10O3S: C, 59.58; H, 3.33; S, 9.29. Found: C, 59.55; H, 3.31; S, 21.18%.

3-Benzoyl-6-bromo-2H-chromene-2-thione, 3o. Yellow crystals. 1H NMR (300 MHz, CDCl3): δ 7.19 (s, 1H), 7.33-7.43 (m, 3H), 7.53-7.56 (m, 1H), 7.64-7.69 (m, 2H), 7.85 (d, J = 5.4 Hz, 2H); 13C NMR (75.5 MHz, CDCl3): δ 118.3, 118.6, 121.4, 128.8, 129.6, 130.6, 131.7, 134.2, 135.4, 135.9, 140.1, 155.8, 191.8, 192.8; IR (KBr): 1234, 1587, 1662, 3064 cm⁻¹; MS: m/z 345 (M+). Anal. Calcld for C16H10BrO3S: C, 50.62; H, 2.12; S, 8.45. Found: C, 50.67; H, 2.63; S, 9.29. Found: C, 55.69; H, 2.61; S, 9.31%.

3-(4-Methoxybenzoyl)-6-bromo-2H-chromene-2-thione, 3m. Yellow crystals. 1H NMR (300 MHz, CDCl3): δ 3.8 (s, 3H), 6.87 (d, J = 6.6 Hz, 2H), 7.33 (d, J = 6.6 Hz, 1H), 7.40 (s, 1H), 7.26-7.67 (m, 2H), 7.73-7.87 (m, 5H); 13C NMR (75.5 MHz, CDCl3): δ 55.6, 114.2, 118.3, 118.5, 121.5, 128.3, 130.5, 131.3, 132.2, 135.7, 140.4, 155.7, 164.5, 190.3, 193.0; IR (KBr): 1236, 1593, 1643, 3051 cm⁻¹; MS: m/z 375 (M+). Anal. Calcld for C16H10BrO3S: C, 54.41; H, 2.95; S, 8.55. Found: C, 54.45; H, 2.93; S, 8.57%.

3-(Chlorobenzoyl)-6-bromo-2H-chromene-2-thione, 3n. Yellow crystals. 1H NMR (300 MHz, CDCl3): δ 7.41-7.55 (m, 3H), 7.73-7.87 (m, 5H); 13C NMR (75.5 MHz, CDCl3): δ 118.4, 118.7, 121.3, 129.1, 129.2, 130.7, 130.9, 132.1, 133.8, 136.1, 139.7, 155.8, 190.6, 192.7; IR (KBr): 1234, 1587, 1662, 3053 cm⁻¹; MS: m/z 377 (M+). Anal. Calcld for C16H8BrClO4S: C, 50.62; H, 2.12; S, 8.45. Found: C, 50.67; H, 2.09; S, 8.43%.

Biology

For antimicrobial activity determination, the following microorganisms were employed: fungal strains Curvularia oryzae (MTCC 2605), Fusarium oxysporum (MTCC 278), and gram-positive bacteria Bacillus amyloliquefaciens (MTCC 610) and Bacillus cereus (MTCC 1307). These were purchased from Institute of Microbial Technology (IMTECH) Chandigarh, India. The fungal and bacterial cultures were maintained on potato dextrose agar (PDA) and Mueller Hinton agar (MHA), respectively and preserved at 4°C. Media components were purchased from Hi Media, Mumbai, India. All the chemicals used were of analytical grade.

Antimicrobial evaluation

Ampicillin trihydrate and fluconazole were used as standard antibacterial and antifungal agents respectively. Antibacterial activities of the test compounds were carried out in Mueller-Hinton medium. 20 mL of sterilized medium was dispensed in each borosilicate glass test tube (150×20 mm). The test compounds were first dissolved in acetone and then added to the tube in order to attain final drug concentrations of 500, 200, 100, 50, 25 and 10 µg/mL. Inoculum of standard suspension (1× 10⁶ cells mL⁻¹) was added to the tubes containing different test concentration. For the antifungal activity, the experiment was carried out in potato dextrose agar medium. The microbial inoculum size used for antifungal activity was 10⁶ cells mL⁻¹. The tubes were incubated at 37°C for 48 hr and then examined for the presence and absence of growth of the organism. The lowest concentration, which showed no visible growth was taken as an end point minimum inhibitory concentration (MIC).

Conclusion

In conclusion, cupric chloride catalysed synthesis of coumarins has been successfully demonstrated by condensation of β-oxodithioester with salicylaldehyde under solvent free conditions. The newly synthesized compound 3a and 3b exerted promising antifungal and antibacterial activity as they are more potent than the standard drugs ampicillin trihydrate and fluconazole against the fungi C. oryzae, F. oxysporum and bacteria B. amyloliquefaciens and B. cereus.

Acknowledgements

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References

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NOTES