Synthesis and In Vitro Antimicrobial Screening of 3-Acetyl-4-Hydroxycoumarin Hydrazones

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Abstract
We report the organic syntheses of some new 3-acetyl-4-hydroxycoumarin hydrazones with different acid hydrazides. The structures of the synthesized compounds have been established on the basis of physical and spectral data. They show a prominent absorption of -(C=N-) in FTIR a common peak of 4-OH at 15.90 ppm in the form of broad singlet. The antibacterial activity of synthesized compound is compared to antibacterial activity of the standard antibiotics streptomycin. All the synthesized compounds are moderately active when compared with standard streptomycin.

Keywords
3-Acetyl-4-hydroxycoumarin, acid hydrazides, Antibacterial and Schiff Base.

INTRODUCTION
Coumarins have been established as a well known naturally occurring heterocyclic compounds isolated from various plants. They belong to the family of lactones having 1-benzopyran-2-one system that can be isolated from plants as well as can be carried out in the laboratory¹. Coumarin is versatile pharmacophore which exhibits a wide variety of biological activities like antibacterial²-³ and antimicrobial⁴. Coumarins class of compounds, which occupy a special role in nature. They belong to the flavonoid class of plant secondary metabolite, which have been found to exhibit a variety of biological activities, usually associated with low toxicity and have raised considerable interest because of their potential beneficial effects on human health⁵. Coumarins substituted in the pyrone ring include 4-hydroxycoumarin⁶. The synthetic compound, warfarin, belongs to this coumarin subtype. Coumarin is water insoluble; however, 4-hydroxy substitution confers weakly acidic properties to the molecule that makes it water soluble under slightly alkaline conditions. The coumarin structure is derived from cinnamic acid via orthoxygenation,
trans-Cis isomerisation of the side chain double bond, and lactonisation\textsuperscript{7}.

The synthesis of coumarin (2-oxo-2H-chromene) derivatives has attracted considerable attention of organic and medicinal chemists due to its wide usage in food additives, fragrances, pharmaceuticals, and agrochemicals. Furthermore, the pharmacological and biochemical properties as well as therapeutic applications of coumarins depend upon the pattern of substitution\textsuperscript{8}. Coumarin derivatives have been reported for anticoagulant, anti-inflammatory\textsuperscript{9}, antibacterial\textsuperscript{10–19}, anti-HIV, antioxidant\textsuperscript{20}, antiallergic, anticancer\textsuperscript{21} and ant-proliferative and antiviral\textsuperscript{22} activities, like anti-inflammatory, anti microbial, anti-tumour, anti-HIV, hericidal, fungicidal\textsuperscript{23} and CNS stimulant\textsuperscript{24} activities. It was found that when one biodynamic heterocyclic system was coupled with another heterocyclic system, enhanced biological activity was produced.

The present work is aimed at synthesizing the hydrazones of 3-acetyl,4-hydroxy coumarin with three acid hydrazides such as 4-hydroxy benzoic acid hydrazide, Indol-3-acetic acid hydrazide and isoniazide in the form of Schiff bases. An in-vitro antibacterial activity was also performed on the synthesized compounds against Gram +ve (\textit{Staphylococcus aureus}) and Gram-ve species (\textit{Escherichia coli}).

\begin{equation}
\begin{array}{c}
\text{OH} \\
\text{POCl}_3 \\
\text{Gluacial} \\
\text{CH}_3\text{COOH} \\
\text{OH} \\
\text{O} \\
\text{3-Acetyl,4-hydroxy coumarin (3AC)}
\end{array} + \begin{array}{c}
\text{H}_2\text{N} \\
\text{Ar} \\
\text{3-Acetyl,4-hydroxy coumarin Schaff Base}
\end{array}
\end{equation}

\textbf{Scheme 1: General Method for the Synthesis of 3-Acetyl,4-hydroxy coumarin Hydrazone}

To 100 mg of 3-acetyl,4-hydroxy coumarin in Round bottom flask and dissolved it in the 15ml methanol, 2-3 drops of trifluoroacetic acid (TFA) was added as a catalyst. An equivalent amount of respective acid hydrazide or sulphur drugs were added to the above solution and the reaction mixture was stirred at for 2-3 hours at room temperature. The reaction was monitored by TLC. After the reaction was complete, the reaction mixture was poured in ice and the formed precipitate was filtered through Whatmann filter paper No. 42. The solid obtained was dried under IR lamp and recrystallized from ethanol.

Bacterial Strain-Strains of Escherichia coli, Staphylococcus aureus were grown in Muller Hinton broth overnight at 37°C. Overnight cultures were diluted with MHB and were measured spectrometrically at 620nm.

\textbf{EXPERIMENTAL PROCEDURE}

Solvents for synthesis were reagent grade and used as obtained. The starting materials such as 4-hydroxy coumarin, 4-hydroxy benzoic acid hydrazide, Indol-3-acetic acid hydrazide, and isoniazide were obtained from Sigma-Aldrich chemicals and Glacial acetic acid, POCl\textsubscript{3}, Trichloroacetic acid, Piperidine, aceton, methanol, ethanol and dichloromethane were obtained from SD-FCL Chemical Limited, Mumbai, India. All compounds were routinely checked by TLC on silica gel G plates using petroleum ether/ethyl acetate (7:3; 6:4; 5:5 by V/V) as solvent system and the developed plates were visualized by UV light and iodine vapours.

3-Acetyl-4-hydroxy coumarin (3AC) was synthesized from 4-hydroxy coumarin as explained by Kozeta et al\textsuperscript{25}. In a 100ml flask were mixed 4g (25mmol) of 4-Hydroxy coumarin with 8ml (133.3mmol) acetic acid and 3ml POCl\textsubscript{3} as catalyst. The mixture was refluxed at 250°C for about 3.5 hours on sand bath until a wine-red crystalline precipitate was formed. The flask was placed into the ice bath until the precipitate was formed. After the vacuum filtration the product was air-dried and recrystallized from ethanol. The recrystallization from ethanol gave a white brown product of 3AC compound at 90% yield. Melting point 136°C. The Schiff’s bases were synthesized by condensing 3-acetyl,4-hydroxy coumarin with different acid hydrazides and sulphur drugs as explained by Anees Pangal et al\textsuperscript{26} (Scheme 1).

\textbf{RESULTS AND DISCUSSIONS}

Melting points of the synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus and are uncorrected. The H\textsuperscript{1}-NMR spectra were obtained on a 500 MHz from Savitribai Phule Pune University, Pune. IR spectra
were recorded by “FT-IR Jasco” spectrometer at our centre. The structures of the synthesized compounds have been established on the basis of physical and spectral data. They show a prominent absorption of -(C=N-) in FTIR. It also shows a common peak of 4-OH at 15.90 ppm in the form of broad singlet. The detailed physical and spectral properties are summarized in table 1. All the synthesized compounds are moderately active against Gram +ve and Gram –ve bacteria. From the results of activity, graphs are plotted as concentration against OD. From various graphs *E. coli* showed maximum inhibition at 20-40µg/ml range in **SB2** compound concentration and **S. aureus** showed maximum activity at 40-60 µg/ml range in **SB3** compound. Similarly, minimum inhibition in *E. coli* was observed at 20-40 µg/ml concentration in **SB1** compound and with the same compound minimum inhibition was observed in the range of 60-80 µg/ml range for *S. aureus*. All the synthesized compounds are moderately active when compared with standard streptomycin (Figure 1).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Code for Schiff Base</th>
<th>Ar</th>
<th>Complete Structure</th>
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<tbody>
<tr>
<td>1</td>
<td>SB1</td>
<td>O</td>
<td><img src="image" alt="Structure SB1" /></td>
</tr>
<tr>
<td>2</td>
<td>SB2</td>
<td>O</td>
<td><img src="image" alt="Structure SB2" /></td>
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<tr>
<td>3</td>
<td>SB3</td>
<td>O</td>
<td><img src="image" alt="Structure SB3" /></td>
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<tbody>
<tr>
<td>1</td>
<td>SB1</td>
<td><img src="image" alt="Structure SB1" /></td>
<td>266-218 White</td>
<td>79</td>
<td>FTIR (cm⁻¹):1095(C-O), 1647.56 (-C=N), 3265.86 (-NH), 3081.69(-OH), 2958.27 (-CH), 1660.41 (-C=O), 1057.03 (-N-N), 1500 to 1600 (Aromatic region). H¹-NMR (d-DMSO) (δ, ppm): 2.75 (s, 3H, -COCH₃), 11.5 (s, 1H, -NH), 15.70 (bs, H, -OH), 10.30(s, 1H, -OH), 6.91(d, 2H), 7.82 (d, 2H), 7.30 (m, 2H), 7.65 (dt, 1H), 7.90 (dd, 1H)</td>
</tr>
<tr>
<td>2</td>
<td>SB2</td>
<td><img src="image" alt="Structure SB2" /></td>
<td>206-208 Orange</td>
<td>85</td>
<td>FTIR (cm⁻¹):1095(C-O), 1647.56 (-C=N), 3265.86 (-NH), 3081.69(-OH), 2958.27 (-CH), 1660.41 (-C=O), 1057.03 (-N-N), 1500 to 1600 (Aromatic region).</td>
</tr>
</tbody>
</table>
CONCLUSION
We reported the synthesis and structural characterization of three new hydrazones of 3-acetyl-4-hydroxy coumarin. All the synthesized compounds show effective antibacterial activity. From various graphs, *E. coli* showed maximum inhibition at 20-40 µg/ml range in SB2 compound and *S. aureus* showed maximum activity at 40-60 µg/ml range in SB3 compound. Similarly, minimum inhibition in *E. coli* was observed at 20-40 µg/ml concentration in SB1 compound and with the same compound minimum inhibition was observed in the range of 60-80 µg/ml range for *S. aureus*. All the synthesized compounds are moderately active when compared with standard streptomycin. The shown activity can be accounted for the presence of polar interactions in the respective hydrazide. As the synthesized Schiff bases are antibacterial, study of their other biological activities is of further interest.

Table 1: Detailed physical and spectral properties

<table>
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<tr>
<th>Compound</th>
<th>Formula</th>
<th>Physical Property</th>
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<tr>
<td>SB1</td>
<td>3</td>
<td>White</td>
</tr>
<tr>
<td>SB2</td>
<td>230-232</td>
<td>White</td>
</tr>
<tr>
<td>SB3</td>
<td>70</td>
<td>White</td>
</tr>
</tbody>
</table>

**H\(^1\)-NMR** (*d*-DMSO) (δ, ppm):
2.75 (s, 3H, -COCH\(_3\)), 8.85 (s, 1H, -NH), 15.70 (bs, H, -OH), 7.90 (3, 3H), 7.82 (d, 2H), 7.30 (m, 2H), 7.65 (dt, 1H).

**FTIR** (cm\(^{-1}\)):
1095(C-O), 1647.56 (-C=N), 3265.86 (-NH), 2958.27 (-CH), 1660.41 (-C=O), 1057.03 (-N-N), 1500 to 1600 (Aromatic region).

**H\(^1\)-NMR** (*d*-DMSO) (δ, ppm):
2.75 (s, 3H, -COCH\(_3\)), 3.80 (s, 2H, -CH\(_2\)), 11.6 (s, 1H, -NH), 15.90 (bs, 1H, -OH), 7.28 (m, 4H), 7.02 (t, 1H, indolic H), 7.63 (m, 2H), 7.95 (dt, 1H), 7.90 (dd, 1H), 11.00 (s, 1H, indolic –NH)
AKNOWLEDGMENT

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