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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.

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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 laboratory[1].Coumarin is versatile pharmacophore which exhibits a wide variety of biological activities like antibacterial[2-3] and antimicrobial[4].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 health[5].

Coumarins substituted in the pyrone ring include 4-hydroxycoumarin[6].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 orthohydroxylation,

trans-Cis isomerisation of the side chain double bond, and lactonisation[7].

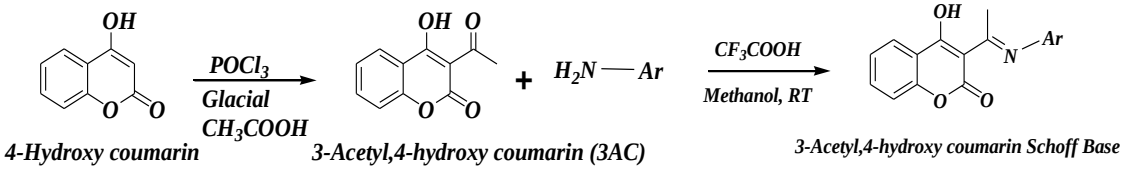
The synthesis of coumarin (2-oxo-2H-chromene) derivatives has attracted considerable attention of organic and medicinal chemists due to its wide usage in food additives, fragrances, pharmaceuticals, and agrochemicals. Furthermore, the pharmacological and biochemical properties as well as therapeutic applications of coumarins depend upon the pattern of substitution[8].Coumarin derivatives have been reported for anticoagulant, anti-inflammatory[9], antibacterial[10-19], anti-HIV, antioxidant[20], antiallergic, anticancer[21] and ant-proliferative and antiviral[22] activities, like anti inflammatory, anti microbial, anti-tumour, anti-HIV, herbicidal, fungicidal[23] and CNS stimulant[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 (***Staphylococcus aureus***) and Gram-ve species (***Escherichia coli***).

EXPERIMENTAL PROCEDURE

Solvents for synthesis were reagent grade and used as obtained. The starting materials such as 4-hydroxycoumarin, 4-hydroxy benzoic acid hydrazide, Indol-3-acetic acid hydrazide, and isoniazide were obtained from Sigma-Aldrich chemicals and Glacial acetic acid, $POCl_3$, Trichloroacetic acid, Piperidine, acetone, 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-hydroxycoumarin (3AC) was synthesized from 4-hydroxycoumarin as explained by Kozeta et al[25]. In a 100ml flask were mixed 4g (25mmol) of 4-Hydroxy coumarin with 8ml (133.3mmol) acetic acid and 3ml $POCl_3$ as catalyst. The mixture was refluxed at 250ºC 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-hydroxycoumarin with different acid hydrazides and sulpha drugs as explained by Anees Pangal et al[26](**Scheme 1)**.



4-Hydroxy coumarin *3-Acetyl,4-hydroxy coumarin (3AC)* *3-Acetyl,4-hydroxy coumarin Schoff Base*

Scheme 1: General Method for the Synthesis of 3-Acetyl,4-hydroxycoumarin Hydrazone

To 100 mg of 3-acetyl,4-hydroxycoumarin 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 sulpha 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.

Antibacterial activity is done using broth micro dilution assay using 96 micro titer plates. Different concentration of compounds (20, 40, 60, 80, 100µg/ml) were used. Transferring 100µl of given compound and 100µl of inoculum broth in each well. Organism is used as positive control and sterile MHB as blank. Microtiter plate is incubated at 37˚C overnight. After incubation plate were read by ELISA reader. Graph was plotted for concentration versus absorbance.

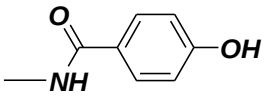
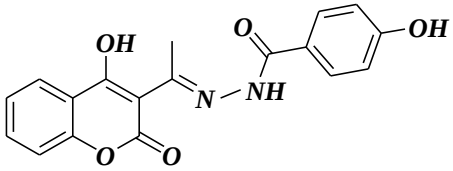
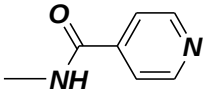
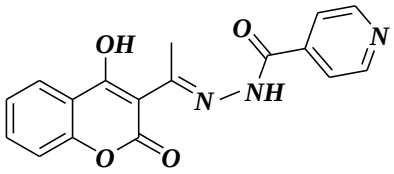
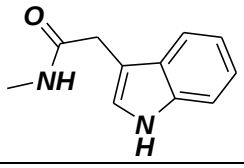
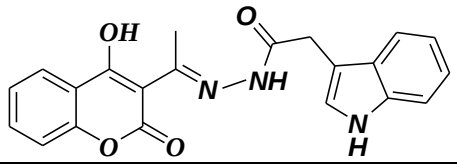
RESULTS AND DISCUSSIONS

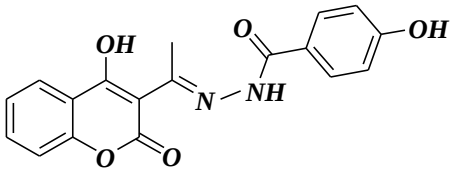
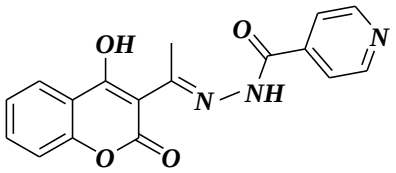
Melting points of the synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus and are uncorrected. The H^1-NMR spectra were obtained on a 500 MHz from Savitribai Phule Pune University, Pune. IR spectra

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)**.

Sr. No.	Code for Schiff Base	Ar	Complete Structure
1	SB1		
2	SB2		
3	SB3		

Sr. No.	Code	Structure of Hydrazones	M. P. °C & Colour	% Yield	Spectral Properties
1	SB1		266-218 White	79	**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). **H^1-NMR** (*d-DMSO*) (δ, ppm): 2.75 (s, 3H, -$COCH_3$), 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)
2	SB2		206-208 Orange	85	**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).

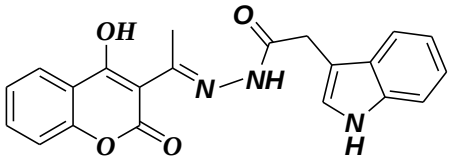
3	SB3		230-232 White	70	**H^1-NMR** (*d-DMSO*) (δ, ppm): 2.75 (s, 3H, -$COCH_3$), 8.85 (s, 1H, -NH), 15.70 (bs, H, -OH), 7.90 (3, 3H), 7.82 (d, 2H), 7.30 (m, 2H), 7.65 (dt, 1H). **FTIR (cm^{-1}):**1095(C-O), 1647.56 (-C=N), 3265.86 (-NH), 3081.69(-OH), 2958.27 (-CH), 1660.41 (-C=O), 1057.03 (-N-N), 1500 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)

Table 1: Detailed physical and spectral properties

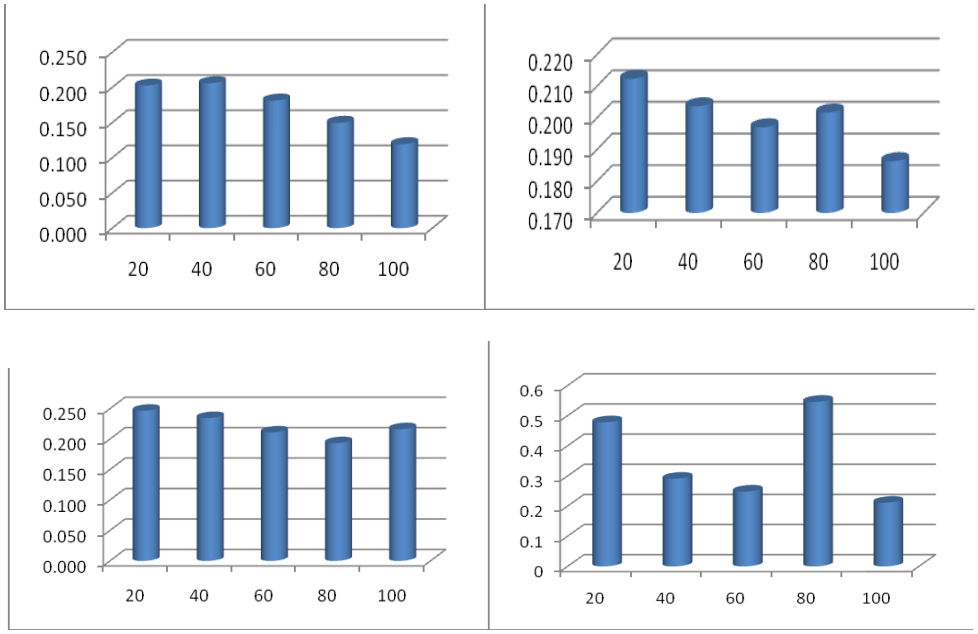


Figure 1: Some results of antibacterial activity

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 20-40µg/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.

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