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# 6-AMINO-7-HYDROXY-4-METHYLCOUMARIN HYDRAZONES: SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY

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## ABSTRACT

New hydrazones, AMC1 and AMC2 were synthesized from 6-Amino-7-hydroxy-4-methylcoumarin with two heterocyclic aldehyde and ketone. The structures of the synthesized compounds were established on the basis of physical and spectral data. They shows a prominent absorption of -(C=N-) in FTIR. Antibacterial activity of these compounds was performed on Gram +ve and Gram –ve bacteria. Both were found active against Gram +ve and Gram –ve bacteria with the Minimum Inhibitory Concentration (MIC) of 50μgmL$^{-1}$. A survey of existing literature revealed that there are no reports describing the synthesis of such hydrazones.

**KEYWORDS:** Hydrazone, 6-Amino-7-hydroxy-4-methylcoumarin, 7-hydroxy-4-methylcoumarin, Antibacterial.

## INTRODUCTION

Coumarins have been synthesized 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.[1] Coumarins have attracted considerable attention due to their wide spectrum of pharmacological and biological activities[2] as anti-coagulant[3], antitumor[4], antifungal[5], antiviral[6], antibacterial[7] and CNS stimulant.[8] Hydrazones and substituted hydrazones are pharmacologically active and show anticancer and antibacterial activity the presence of azomethine group.[9] Coumarin represents the core structure for many pharmaceutical compounds which have beneficial effects on human health. Furthermore, the

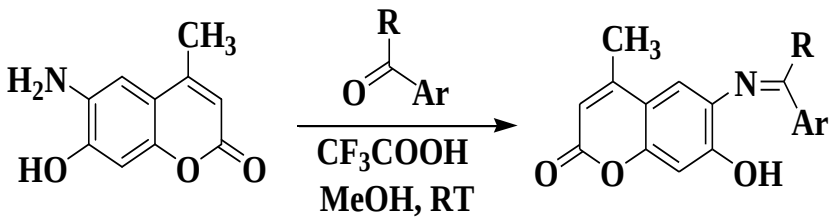
pharmacological properties of coumarins depend upon the pattern of substitution and are reported to possess many pharmacological activities.[10]

In the present work synthesized the hydrazones of 6-Amino-7-hydroxy-4-methylcoumarin hydrazones with two heterocyclic aldehyde and ketone. The synthesized compounds were characterized by FTIR and $^1$HNMR. An *in-vitro* antibacterial activity was also performed on the synthesized compounds against Gram +ve ***(Staphylococcus aureus)*** and two Gram –ve species (***Pseudomonas aeroginosa***).

## EXPERIMENTAL PROCEDURE

Solvents for synthesis were reagent grade and used as obtained. The starting materials such as resorcinol, 3-acetyl indol and indol-3-carboxaldehyde were obtained from Sigma-Aldrich chemicals and glacial acetic acid, trichloroacetic acid, ethylacetoacetate, Piperidine, $H_2SO_4$, $HNO_3$, $SnCl_2.2H_2O$, 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 as solvent system and the developed plates were visualized by UV light and iodine vapours.

The starting compound 6-Amino-7-hydroxy-4-methylcoumarin was synthesized in four steps as explained by Radwan et al.[11] Finally, the target compounds **AMC1** and **AMC2** were obtained by combining with 3-acetyl indol and indol-3-carboxaldehyde using triflouroacetic acid as catalyst in methanol (Figure 1).[12]

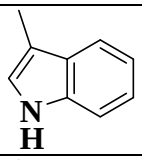
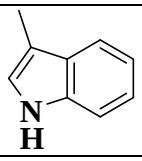



*6-Amino-7-hydroxy-4-methylcoumarin(V)*

*6-Amino-7-hydroxy-4-methyl-coumarin hydrazones(AMC1 and AMC2)*

**Figure 1: General Scheme for the synthesis of 6-amino-7-hydroxy-4-methylcoumarin hydrazone (AMC1 and AMC2**

***Where-***

| Sr. No. | Code | Ar | R |
|---|---|---|---|
| 1 | AMC1 |  | -$CH_3$ |
| 2 | AMC2 |  | -H |

**Antibacterial Activity**

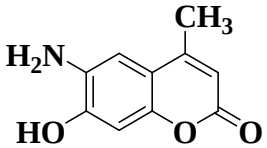
The *in vitro* antibacerial activity was performed according to procedure explained by Arpit et al.[13] A standardize inoculums were inoculated with the help of a sterile cotton swab on the surface of the agar plate. Disc of antimicrobial agents were placed on the surface of agar plate. The plates were incubated at 37°C for 24 hours and susceptibility is determined on the basis of zone of inhibition. A standard and control strain was also tested for comparison. The diameter of the zone of growth inhibition around each disc were measured and compared with zones of inhibition of standard and control.

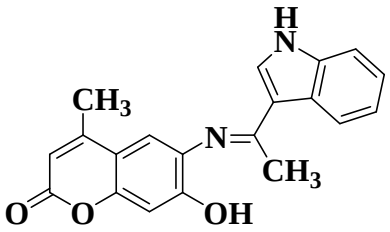
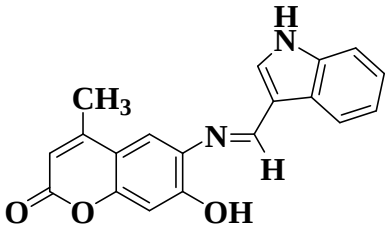
**RESULTS AND DISCUSSIONS**

Melting points of the synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus and are uncorrected. The H1-NMR spectra were obtained on a 500 MHz from NCL, 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 shows a prominent absorption of -(C=N-) in FTIR. It also shows a common peak indolic -NH at 11.9 and 12.14 ppm in the form of singlet. The detailed physical and spectral properties are summarized in **table-1**.

**Table 1: Spectral Characterisation of 6-amino-7-hydroxy-4-methylcoumarin hydrazones (AMC1 and AMC2)**

| Sr. No. | Code | Structure of Hydrazones | M. P. °C, Colour and % Yield | Spectral Properties |
|---|---|---|---|---|
| 1 | 6-Amino-7-hydroxy-4-methylcoumarin |  | 273-274 Yellow 70%[14] | - |

| | | | | |
|---|---|---|---|---|
| **2** | **AMC1** |  | **198-200 White 90%** | **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,): 1.52 (s, 3H, -CH3), 2.51 (s, 3H, -CH3), 11.9 (s, 1H, -NH),5.50 (s, 1H), 5.70 (s, 1H, -OH), 7.18-7.23(m, 4H), 7.47 (d, 1H), 8.18 (d, 1H), 8.30 (d, 1H) |
| **3** | **AMC2** |  | **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). **H$^1$-NMR** (*d-DMSO*): (δ, ppm,): 1.90 (s, 3H, -$CH_3$), 12.14 (s, 1H, -NH), 5.50 (s, 1H), 5.70 (s, 1H, -OH), 7.18-7.23 (m, 4H), 7.47 (d, 61H), 8.18 (d, 1H), 8.30 (d, 1H), 7.50 (s, 1H) |

## ANTIBACTERIAL DISCUSSION

The chemically synthesized compounds were tested for antimicrobial activity. Strains of both Gram positive and Gram negative bacteria were used for experimentation. Amoxicillin (25 µg/mL$^{-1}$) was used as standard which showed a zone of inhibition of 8mm. The compounds were serially diluted and different dilutions were tested against three organisms such as *Staphylococcus aureus* and *Pseudomonas aeroginosa*. The tested compounds such as AMC1 and AMC2 showed a good activity against both Gram positive and Gram negative bacteria. The results are more promising against Gram positive organisms. The disc diffusion method was used for determining the antibacterial activity of the compounds and the results obtained were summarized in following table-

**Table-2: Results of Antibacterial Activity**

| Organisms | *Conc.* (µg/mL$^{-1}$) | AMC1 | AMC2 |
|---|---|---|---|
| ***Staphylococcos aureus*** | **25** | **-** | **-** |
| | **50** | **6** | **7** |
| | **75** | **7** | **7** |
| | **100** | **9** | **12** |
| | **125** | **10** | **10** |
| | **150** | **12** | **14** |
| ***Pseudomonas aeroginosa*** | **25** | **-** | **-** |
| | **50** | **5** | **6** |
| | **75** | **5** | **7** |

| | | | |
|---|---|---|---|
| | **100** | **6** | **9** |
| | **125** | **7** | **10** |
| | **150** | **8** | **12** |

The results clearly indicate that the compounds have selective action on Gram negative organisms. The basic difference between Gram positive and Gram negative organism lies in cell wall organization. The compounds seem to interfere or inhibit the cell wall organization or maybe they do not allow the synthesis of cell wall or one of its components in Gram positive bacteria. Also the zone of inhibition seen around the antibiotic disc seems to increase in diameter with the increase in concentration of the drug.

## CONCLUSION

We reported the synthesis and structural characterization of 6-Amino-7-hydroxy-4-methylcoumarin hydrazones. All compounds show effective antibacterial activity and are moderately active when compared with standard Amoxicillin. The Minimum Inhibitory Concentration (MIC) of AMC1 and AMC2 is 50 $\mu g \cdot mL^{-1}$ for Gram +ve and Gram –ve bacteria whereas Since the compounds show activity against both Gram +ve and –ve bacteria, all the compounds can be screened for other enteric organisms which are known pathogens like *Salmonella*, *Pseudomonas*, *Vibrio* etc. Also Antifungal, Antiviral, Cytotoxic and anti-inflammatory activities can also be carried out.

## ACKNOWLEDGEMENT

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## REFERENCES

1. N Batra; S Batra; A Pareek; BP Nagori. *Int. Res. Journal of Pharm.*, 2013; 3(7): 24-29.
2. A Pangal; M Gazge; V. Mane; JA Shaikh. *IJPRBS*, 2013; 2(6): 168-194.
3. S Gummudavelly; YS Ranganath; S Bhasker; N Rajkumar. *Asian J. Research Chem*, 2009; 2(1): 46-48.
4. VR Rajeshwar; K Srimanth; PV Kumar. *Indian Journal of Heterocyclic chemistry*, 2004; 14: 141-144.
5. GR Madhavan; B Vadla; M Bejugam; R Chakrabarti. *Bioorganic and Medicinal Chemistry Letters.*, 2003; 13(4): 2547-2551.
6. ZM Nofal; MI El-Zahar; SS Abd El-Karim. *Molecules*, 2000; 5(2): 99-113.

7. VV Mulwad; JM Shirodkhar. *Indian Journal of Chemistry*, 2002; 41B: 1263-1267.
8. RM Pilpa; SA Robson; VA Villarea; ML Wong; M Phillips; RT Clubb. *J. Biol. Chem*., 2009; 9, 284(2): 1166-76.
9. H Kaur; S Kumar; I Singh; A Kumar. *International Journal of Pharma Sciences and Research (IJPSR)*, 2010; 1(1): 58-65.
10. A Behrami; I Krasniqi. *Jrnl of Chemical and Pharm Res*, 2012; 4(5): 2495-2500.
11. R El-Haggar; RI Al-Wabli. *Molecules*, 2015; 20: 5374-5391.
12. A Pangal; K Ahmed; S Shaikh. *Int. Res. Journal of Pharm*., 2013; 4(8): 172-176.
13. AD Shah; DJ Raval; VP Jatakiya; DJ Sen; R Badmanaban. *Int. J. Drug Dev. & Res.*, 2012; 4(2): 205-215.