Synthesis and Characterization of New Schiff Bases Containing Coumarin Derivatives and Study Their Antimicrobial and Antioxidant Activities

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Citation

Abstract
New Schiff bases were synthesized by the condensation of aryl/hetero aromatic aldehydes with different amines under conventional conditions and characterized by IR, ¹HNMR and LC-MS spectral methods. The synthesized compounds have been screened for antimicrobial activity. The free radical scavenging activity have been determined by measuring Their interaction with the stable free radical DPPH and all prepared compounds have shown encouraging antioxidant activities.

1. Introduction

Azomethine group (-C=O-) containing compounds typically known as Schiff bases have been synthesized by the condensation of primary amines with active carbonyls. Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antioxidant, antibacterial,1-6 antifungal3-6 and antitumor activity.7-

Derivatives have been of great interest because of their role in natural and synthetic organic chemistry.

Many products which contain a coumarin subunit exhibit biological activity such as molluscicides, anthelmintic, hypnotic, insecticidal activity and some are serving as anticoagulant agents16. So coumarins containing a Schiff base are expected to have enhanced antitumor and other biological activities. The chemical structures of the newly synthesized complexes were confirmed. The microbial activities of all synthesized compounds and their in vitro antioxidant activities were also investigated. The prepared compounds were characterized by IR, ¹HNMR and LC-MS spectral methods.

2. Materials and Methods

2.1. Chemicals and Instruments Required

The used chemicals are of analytical grade i.e. resorcinol, ethylacetoacetate, conc.
H_{2}SO_{4}, ortho aminophenol, Para xylene, hydroxyl amine, benzene, ethanol, conc. HCl. All the melting points were determined in open capillaries, using Boitut melting point apparatus. The IR spectra of the compounds were recorded on Shimadzu IR Affinity FTIR spectrophotometer using KBr discs and the values are expressed in cm\(^{-1}\). The 1H NMR spectra of compounds were recorded on Bruker Avance II 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

### 2.2. Experimental Section

Resorcinol treated with ethylacetacetate in the presence of sulphuric acid at 10 °C yielded 7-Hydroxy-4-methyl-chromen-2-one (1).\(^{11}\) Treating compound 1 in dry DMF with ethyl chloroacetate in the presence of K\(_2\)CO\(_3\) at 80 °C for 10 hours yielded ethyl 2-[(4-methyl-2-oxo-chromen-7-yloxy)acetate (2) in 82 % yield, whereas in literature the conversion of 1 into 2 was reported under the refluxing conditions for 10 hrs in dry acetone with 40 % yield. Much variation in yield percentage was not observed the conversion of 1 into 2 is also effected by using anhydrous sodium bismutate as catalyst instead of K\(_2\)CO\(_3\) (Scheme 1).

Compound 2 was heated with hydrazine hydrate in ethanol on a water bath for 1 hour to obtain 2-[(4-methyl-2-oxo-chromen-7-yloxy)acetohydrazide (3). The 2-[(4-Methyl-2-oxo-chromen-7-yloxy)-N′(substitutedmethylene)acetohydrazides (4) synthesized by the condensation of 2-[(4-Methyl-2-oxo-chromen-7-yloxy)acetohydrazide (3) with different aryl/hetero aromatic aldehydes and characterized through the spectral data (Scheme 2).

The synthesis of 4 was optimized by varying the solvents, reaction time, temperature and the results are given in Table 1. It was found that the yield was up to 83.4 % when the reaction mixture was refluxed for 30 min. in a chloroform/methanol mixture.

### Table 1. Yield of 4a at different reaction conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Temp. (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>30</td>
<td>Reflux</td>
<td>65.6</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>60</td>
<td>Reflux</td>
<td>77.5</td>
</tr>
<tr>
<td>3</td>
<td>CHCl + Methanol</td>
<td>20</td>
<td>Reflux</td>
<td>82.6</td>
</tr>
<tr>
<td>4</td>
<td>CHCl + Methanol</td>
<td>30</td>
<td>Reflux</td>
<td>83.4</td>
</tr>
<tr>
<td>5</td>
<td>CHCl + Methanol</td>
<td>60</td>
<td>Reflux</td>
<td>86.2</td>
</tr>
</tbody>
</table>

**Preparation of Ethyl 2-[(4-Methyl-2-oxo-chromen-7-yloxy)acetate (2)**

(a) Conventional method using K\(_2\)CO\(_3\), Method A. To a solution of 7-Hydroxy-4-methyl-chromen-2-one 1 in dry DMF, anhydrous potassium carbonate (1.0 molar equiv) and ethyl chloro acetate (1.0 molar equiv) were added. The resultant mixture was stirred at 80 °C for 10h, cooled and then the reaction mixture was added to a large amount of water. The precipitate solid was filtered, washed with excess of water. The crude product was purified by crystallization from ethanol. The yield of product was 81-82 %
Preparation of 2-[(4-Methyl-2-oxo-chromen-7-yl)oxy]acetohydrazide (3)

Ethyl 2-[(4-methyl-2-oxo-chromen-7-yl)oxy]acetate (3, 0.01 mole) in ethanol (20 mL) was stirred at room temperature for 20 min. To this mixture hydrazine hydrate (0.014 mole) was added. The resultant mixture was stirred at room temperature for 15 min, and the solid filtered with a glass funnel. The residue was dried and then desiccated to afford a crystalline powder. The powder was recrystallized from chloroform/methanol and gave colorless needles. The yield of product was 88-90 %. M.p. 202-204 °C; IR (KBr) ν: 3331(NH₂), 3082(CH), 2958, 1731(C=O), 1675(-C=C-), cm⁻¹; ¹H-NMR (400 MHz, DMSO, ppm), δ = 9.41 (1H, s, NH), 7.69 (1H, d, 6-H), 7.00 (1H, dd, 5-H), 6.70 (1H, d, 8-H), 6.21 (1H, s, 3-H), 4.61 (2H, s, NH₂), 4.35 (2H, s, OCH₂), 2.39 (3H, s, CH₃); LC-MS: m/z 249.0 (M+1).

Synthesis of 2-[(4-Methyl-2-oxo-chromen-7-yl)oxy]-N’-(substituted methylene)acetohydrazides (4)

Conventional. A mixture of compound 3 (0.01 mole) in chloroform/methanol (1:1) mixture (30 mL), aryl / hetero aromatic aldehyde (0.01 mole) and 1 mL of glacial acetic acid was refluxed on a water bath for 60 minutes. The mixture was allowed to cool, and then the separated solid was filtered, washed with excess of methanol.

N,N’-[1,4-phenylenedi(methylidene)]bis[2-[(4-methyl-2-oxo-chromen-8-yl)oxy]acetohydrazide]

Spectral characterization: IR (KBr) ν: at 1635 cm⁻¹ (C=N), 1650 cm⁻¹ (lactone C=O), 1780 cm⁻¹ (C=O), 3435 cm⁻¹ (-NH) and 1621 cm⁻¹ (CH=CH); ¹H-NMR (400 MHz, DMSO, ppm), δ = 8.31 (1H, S, HC=N), 8.32 (1H, s, NH), 7.72 (aromatic protons), 2.40 (3H, s, CH₃), 4.8 (2H, S, CH₂).

N-(2-methoxybenzylidene)-2-[(4-methyl-2-oxo-chromen-8-yl)oxy]acetohydrazide [5]

Spectral characterization: IR (KBr) ν: at 1610 cm⁻¹ (C=N), 1654 cm⁻¹ (lactone C=O), 1775 cm⁻¹ (C=O), 3440 cm⁻¹ (-NH) and 1620 cm⁻¹ (CH=CH); ¹H-NMR (400 MHz, DMSO, ppm), δ = 8.12 (1H, S, HC=N), 8.20 (1H, s, NH), 7.88 (aromatic protons), 4.81 (1H, s, OCH₃), 2.40 (3H, s, CH₂), 4.77 (2H, d, CH₂) 4.87 (2H, S, CH₂).

N-[2-(dimethylamino)benzylidene]-2-[(4-methyl-2-oxo-chromen-8-yl)oxy]acetohydrazide [6]

Spectral characterization: IR (KBr) ν: at 1612 cm⁻¹ (C=N), 1648 cm⁻¹ (lactone C=O), 1779 cm⁻¹ (C=O), 3440 cm⁻¹ (-NH) and 1620 cm⁻¹ (CH=CH); ¹H-NMR (400 MHz, DMSO, ppm), δ = 8.45 (1H, S, HC=N), 8.35 (1H, s, NH), 7.50 (aromatic protons), 2.33 (3H, s, CH₂) 4.88 (2H, S, CH₂).

N-(2-hydroxy-3-methoxybenzylidene)-2-[(4-methyl-2-oxo-chromen-8-yl)oxy]acetohydrazide[7]
Spectral characterization: IR (KBr) ν: at 1618 cm⁻¹ (C=N), 1658 cm⁻¹ (lactone C=O), 1782 cm⁻¹ (C=O) 1620 cm⁻¹ (CH=CH) 3447 cm⁻¹ (-NH), and 3470 cm⁻¹ (OH); ¹H-NMR (400 MHz, DMSO, ppm, δ) = 8.09 (1H, s, HC=N), 8.4 (1H, s, NH), 7.56 (aromatic protons), 4.86 (1H, s, OCH₃), 2.40 (3H, s, CH₃), 11.8 ppm (1H, broad OH), 4.91 (2H, S, CH₂).

N-(2-hydroxybenzylidene)-2-[(4-methyl-2-oxo-chromen-8-yl)oxy]acetohydrazide[8]

Spectral characterization: IR (KBr) ν: at 1622 cm⁻¹ (C=N), 1643 cm⁻¹ (lactone C=O), 1788 cm⁻¹ (O=CH), 3467 cm⁻¹ (OH) and 3450 cm⁻¹ (-NH), ¹H-NMR (400 MHz, DMSO, ppm), δ = 8.67 (1H, s, HC=N), 8.45 (1H, s, NH), 7.80 (aromatic protons), 2.40 (3H, s, CH₃), 11.9 ppm (1H, broad OH), 4.5 (2H, S, CH₂).

2.3. Antibacterial Studies

Condensation products of some active group with coumarin-derivatives, (Rabarova et al, Lacova et al [8] found to possess antimicrobial Staphylococcus aureus, as Gram-positive bacteria. Nutrient agar plates were seeded using 0.1 of overnight cultures. Cylindrical plugs were removed from the agar plates using a sterile cork borer and 100 µL of the tested compound (50µg/ml, 100µg/ml EtOH) were added to the well in triplicates. Blank solvent was used as control. Plates inoculated with tested bacteria were incubated at 37°C, while those of Fungi were incubated at 30°C. Results were taken after 24 h of incubation and were recorded as average diameter of inhibition zone in mm. All the newly synthesized compounds were subjected to antimicrobial screening by in vitro cup plate technique, using positive controls Nystatine.

Compounds I showed remarkable activity towards the gram positive bacteria Staphylococcus and gram negative Pseudomonas, Salmonella sp and E.Coli but Klebsiella sp not yet effect with this comp while compound III(100µg) appear to have remarkable activity of negative and positive gram bacteria.

The Gram Negative bacteria Klebsiella sp proved to be sensitive toward compound III (50µg). All prepared compounds showed very good activity toward the tested strains Staphylococcus aureus, Compound III proved to be the most active broad spectrum antimicrobial agents in this study. In conclusion this study revealed that the heterocyclic system bearing coumarin moiety could be useful as template for future, development through modification or derivatization to design a more potent antimicrobial agents.

Table 2. Antibacterial activity of compounds(I,III)

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>Sal</th>
<th>K</th>
<th>Ps</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33</td>
<td>33</td>
<td>32</td>
<td>26</td>
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<td>III</td>
<td>40</td>
<td>39</td>
<td>31</td>
<td>26</td>
<td>25</td>
</tr>
</tbody>
</table>

Gram Negative bacteria: Escherichia coli, Pseudomonas aeruginosa, Klebsiella sp. Salmonella sp.
Gram positive bacteria: Staphylococcus aureus
2.4. Radical Scavenging Activity

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical assay provides an easy and rapid way to is a product of the reaction between DPPH• and an antioxidant:

\[(AH) \cdot + \text{AH} \rightarrow \text{DPPH} + A^* \tag{1}\]

The reversibility of the reaction is evaluated by adding DPPHH at the end of the reaction. If there is an increase in the percentage of remaining DPPH• at the plateau, the reaction is reversible, otherwise it is a complete reaction. DPPH was used as stable free radical electron acceptors or hydrogen radical to become a stable diamagnetic molecule [9]. DPPH• is a stable free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis. [10]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The graph was plotted with percentage scavenging effects on the y-axis and concentration (µg/mL.) on the x-axis. A solution of corresponding coumarin derivative in DMSO (0.75 mL 0.2 mM solution) was added to a DMSO solution of DPPH radical (0.75 mL 0.2 mM solution), so that the final concentration of DPPH radical and the synthesized compound in a solution was 0.1 mM. The mixture was shaken and left at room temperature. After 30 min the absorbance at 517 nm was determined and the scavenging activity was calculated according to the Eq. (1). Ascorbic acid was used as a reference compound which take the first column and take 90 % DPPH radical scavenging activity.

![Graph showing radical scavenging activity of compounds (I-VI)](image)

Fig. 1. Radical scavenging percent of coumarin derivatives

Results of antioxidant activity show that compound 6 exhibited the highest free radical scavenging activity (65 %), which was comparable to that of standard ascorbic acid (90%), while antioxidant activity of compound 3 exhibited the lowest free radical scavenging activity (65 %).

3. Results and Discussion

The characterization data of compounds are given in the Experimental section. All the newly synthesized compounds gave satisfactory analyses for the proposed structures, which were confirmed on the basis of Their IR and 1 H-NMR spectral data. The IR spectra of these compounds showed moderately strong bands around 3600 cm⁻¹ - 3400 cm⁻¹, 1600-1650 cm⁻¹, characteristic of the–OH, NH₂, and C=O respectively., a characteristic signal due to the H¹ – NMR spectra of compounds contains coumarin signals in the range of 6 = 6.4–7.8 ppm, The signals due to the aromatic protons appeared as multiplets in the range of 6 = 7–8 ppm, the -OH– protons. The signal due to the NH protons appeared at 5.8. According to Fig. 1. Compounds 1 showed remarkable activity towards the gram positive bacteria Staphylococcus and gram negative Pseudomonas, Salmonella sp and E.Coli but Klebsiella sp not yet effect with this comps while compound III(100µg) appear to have remarkable activity of negative and positive gram bacteria .the best DPPH scavengers were found to be compounds 1 and 6 90 % and 69 % DPPH radical scavenging activity.

4. Conclusions

To conclude, the modified procedure for the conversion of 1 into 2 with dry DMF at 80 oC instead of dry acetone resulted with improved yield of 82 %.The new Schiff base have been synthesized by reaction of 2-[(4-Methyl-2-oxo-chromen-7-yl)oxy]acetohydrazide (3) with aryl/hetero aromatic aldehydes under conventional methods. The compounds 1 and 3were tested for antibacterial activity by disc diffusion method, showing moderate to potent inhibition. we have successfully demonstrated the antioxidant evaluation by DPPH free radical scavenging.

References
