SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NOVEL 4-HYDROXY COUMARIN DERIVATIVES BEARING AZO MOIETY

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ABSTRACT
The objective of this study was to investigate in vitro antimicrobial activity of ten 4-hydroxycoumarin derivatives bearing azo group at C-3 position. Some new derivatives of coumarin dyes 3(a-j) were prepared by the coupling of 4-hydroxycoumarin with derivatives diazonium salts, which were obtained by diazotization of aniline derivatives in presence of sodium nitrite and concentrated HCl. All the synthesized derivative compounds were characterized by elementary analysis, IR, $^1$H NMR and further screened individually to their preliminary in-vitro antimicrobial activity against four human pathogenic bacteria viz., E.coli, S.aureas, S. typhi and Pseudomonas species. Antibacterial activity of each compound was compared with standard Ciprofloxacin. In-vitro antibacterial activity results revealed that all the synthesized compounds inhibited the growth of E.coli and S. aureus as compared to other bacterial pathogens except 3f; but all the products were ineffective against P. aeruginosa. The compounds like 3b, 3c and 3h have excellent activity against all bacterial species.

Keywords: 4-Hydroxy coumarin, Derivative of aniline, diazotization, Antibacterial activity.

INTRODUCTION
Azo dyes are the largest and the most popular group of dyes showing the full palette of colours. Azo dyes compounds are containing –N=N– group as a characteristic chromophore, and mainly obtained in diazotization and coupling reaction. As per literature survey, it was found that azo dyes have been most widely used in different application fields, such as dying textile fibres, biomedical studies and advanced in organic synthesis as well as shows variety of interesting biological activities including antibacterial and pesticide activities. Synthesis of azo dyes involves diazotization of a primary aromatic amine followed by coupling with one or more nucleophiles. Amino, hydroxy and active methylene groups are commonly used coupling compounts. In pharmaceutical, azo linkage was used to protect drug from undesirable reaction such as prontosil was found to protect against streptococcal infections in mice and inactive on bacterial cultures. 4-Hydroxycoumarin is a structurally Benz[α]pyrone derivative which contains hydroxyl group in fourth position of coumarin. The chemistry and pharmacological action of 4-hydroxy coumarin have great interest to medicinal chemistry because of its derivative possess various biological activity such as anticoagulant, antibacterial, antifungal, anticancer, antiinflammatory, antiviral, antioxidant, and analgesic. The azo dye sulphonamides antibacterial drugs were the first effective chemotherapeutic agents that could be used systemically for the cure of bacterial infection in humans. The above observations prompted us to synthesize the title compounds with the presumption that introduction of Azo group in 4-hydroxy coumarin would produce new compounds with significant antibacterial activity.

EXPERIMENTAL
The chemicals used in the present studies were of synthetic grade, Merck company Ltd. The products were characterized by IR and $^1$H NMR. The melting points were determined by open capillary method and found to be uncorrected. The IR spectra were recorded on Perkin-Elmer spectrum FTIR instrument in the form of KBr pellet. The $^1$H NMR spectra were measured in CDCl$_3$ solutions on a Brucker 400 MHz
spectrometer using TMS as an internal reference (δ ppm). The purity of compounds was checked by TLC. Elemental (C, H, N) analysis indicated that the calculated and found values were within the acceptable limits (±0.4%).

**General synthesis procedure of 4-hydroxy-3-(substituted phenyl diazenyl)-2H-chromen-2-one compounds (3a-j)**

Ten different individual substituted aromatic amines were mixed with 2.5 mL conc. HCl and 2.5 mL (4 N) cold solution of NaNO2 was added with the frequent stirring. The temperature of the reaction was maintained up to 0-5°C. Diazonium salt solution prepared above and was added drop wise to the alkaline solution of 4-hydroxy coumarin. The reaction mixture stirred for 10-20 minutes maintaining the temperature 5-10°C. The colored products obtained were filtered, washed with water and finally the products were dried. The entire product individually recrystallized from 50% ethanol.

**Table -1: Characterization of synthesized Azo compounds by IR & ¹HNMR Spectral data**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Aryl substituent</th>
<th>IR(cm⁻¹)</th>
<th>¹HNMR(δ)</th>
<th>Recrystallized by</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>Phenyl</td>
<td>3000(Ar-H), 1615 (C=C of pyrone), 1555(N=N), 1720(C=O str, α pyrone), 730(C-H out plane, mono disub).</td>
<td>7.42- 7.88(m,9H,Ar-H), 17.07(s, 1H, enolic OH).</td>
<td>50% ethanol</td>
</tr>
<tr>
<td>3b</td>
<td>4-methoxyphenyl</td>
<td>1616 (C=C of pyrone), 1557(N=N), 1720(C=O str, α pyrone) 1220, (C-O str of Ar-OCH₃), 825(C-H out plane, Para disub phenyl).</td>
<td>6.99-7.90(m, 8H, Ar-), 3.83(d, 3H, -OCH₃), 17.07(s, 1H, enolic OH).</td>
<td>50% ethanol</td>
</tr>
<tr>
<td>3c</td>
<td>4-chlorophenyl</td>
<td>1615 (C=C of pyrone), 1555(N=N), 1717(C=O, str, α pyrone), 772(Ar C- Cl str) 835(C-H def Para disub phenyl).</td>
<td>7.27-7.84(m, 8H, Ar-H), 17.07(s, 1H, enolic OH).</td>
<td>50% ethanol</td>
</tr>
<tr>
<td>3d</td>
<td>4-bromophenyl</td>
<td>1620 (C=C of pyrone), 1558(N=N), 1715(C=O str, α pyrene), 695(Ar C- Br str) 835(C-H out plane para disub phenyl).</td>
<td>7.27-7.84(m, 8H, Ar-H), 17.07(s, 1H, enolic OH).</td>
<td>50% ethanol</td>
</tr>
<tr>
<td>3e</td>
<td>4-hydroxy phenyl</td>
<td>1625 (C=C of pyrone), 1558(N=N), 3610(phenolic-OH), 1715(C=O, str, α pyrene).</td>
<td>6.82-7.84(m, 8H, Ar-H), 17.07(s, 1H, enolic OH), 9.86(s, 1H,phenolic-OH).</td>
<td>50% ethanol</td>
</tr>
<tr>
<td>3f</td>
<td>2-carboxyphenyl</td>
<td>1620 (C=C of pyrone), 1560(N=N), 1720(C=O str, α pyrene) 3400(Ar C=C COOH), 1400(C-O str).</td>
<td>7.42-8.21(m,8H,Ar-H), 12.82(m, 1H, carboxylic-OH).</td>
<td>Ethanol+DMF</td>
</tr>
<tr>
<td>3g</td>
<td>4-nitrophenyl</td>
<td>2890(Ar-H), 1558(N=N), 1720(C=O, str, α pyrene), 1520, 1350(NO₃ str).</td>
<td>7.18-8.10(m, 8H, Ar-H), 17.07(s, 1H, enolic OH).</td>
<td>50% ethanol</td>
</tr>
<tr>
<td>3h</td>
<td>4-sulphamidophenyl</td>
<td>2900(Ar-H), 1555(N=N), 1720(C=O, str,α pyrene), 1320(sym str-SO₂), 1149 (asym str-SO₃) 3200, 3260(N-H str, SO₂NH₂), 902(S-N str).</td>
<td>7.42-7.86(m,8H,Ar-H), 17.07(s, 1H, enolic OH), 7.39(s, 2H,-NH₂).</td>
<td>Ethanol+DMF</td>
</tr>
</tbody>
</table>
4-HYDROXY COUMARIN DERIVATIVES

RESULTS AND DISCUSSION

A series of 4-hydroxy-3-(substituted phenyldiazenyl)-2H-chromen-2-one (3a-i) were synthesized by coupling of diazonium salt of aniline derivatives 2(a-j) with 4-hydroxycoumarin in presence of sodium hydroxide (Scheme-1). The coupling mechanism of organic reaction is summarized in the scheme. The crude products re-crystallized from 50% ethanol while some compounds from mixture of ethanol and DMF. All prepared compounds were physically characterized by IR (cm⁻¹) and HNMR (chemical shift δ) as mentioned in Table-1 and 2. 4-hydroxy coumarin nucleus is containing active hydrogen group at C₃
position which on attacked by strong $N_2^+$electrophiles to produce 3-azosubstitutedcoumarin. The following peaks are confirmed the structure of 3-azosubsituted of 4-hydroxy coumarin; the peaks at 1570-1560cm$^{-1}$, 1620-1615cm$^{-1}$,1720-1715cm$^{-1}$ in FTIR are the groups of $-\text{N=N}$-, $-\text{C=C}$-aromatic str and $\text{C=O}$ str respectively. The compound 3h shows IR peak at 1360-1306 cm$^{-1}$, 1154-1149 cm$^{-1}$ for N-H (str) and N-H (def) NH$_2$of SO$_2$NH$_2$ respectively. The NH proton of the SO$_2$NH$_2$ group observed NMR at $\delta$7.00-7.39 ppm as singlet. The $^1$HNMR spectra showed board singlet at $\delta$ 17.07, which suggests the presence of enolic OH in all the prepared compounds. The enolic –OH group of all the compounds were chemically detected by the treatment with FeCl$_3$ solution, which gives characteristic colour.

Table-3: Antimicrobial properties of the synthesized Expressed as zone of Inhibition

<table>
<thead>
<tr>
<th>Compounds (conc.100 µg/ml)</th>
<th>Micro-organism &amp; Zone of Inhibition(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.coli</td>
</tr>
<tr>
<td>3a</td>
<td>14</td>
</tr>
<tr>
<td>3b</td>
<td>19</td>
</tr>
<tr>
<td>3c</td>
<td>18</td>
</tr>
<tr>
<td>3d</td>
<td>15</td>
</tr>
<tr>
<td>3e</td>
<td>15</td>
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<tr>
<td>3f</td>
<td>-----</td>
</tr>
<tr>
<td>3g</td>
<td>16</td>
</tr>
<tr>
<td>3h</td>
<td>18</td>
</tr>
<tr>
<td>3i</td>
<td>12</td>
</tr>
<tr>
<td>3j</td>
<td>15</td>
</tr>
<tr>
<td>DMSO</td>
<td>-----</td>
</tr>
<tr>
<td>Ciprofloxacin (conc.100 µg/ml)</td>
<td>20</td>
</tr>
</tbody>
</table>

-----:- No Antimicrobial growth

**Antibacterial activity**

Antibacterial activity results of ten 3(a-j) derivatives of azo coumarin dyes are shown in Table-3. The results of these studies revealed that all the synthesized compounds exhibited tremendous inhibition of *E.coli* and *S. aureus* when compared to other bacterial strains except 3f. The compounds 3a, 3d and 3e were found to moderate to weak activities against all bacterial stains but all the products were ineffective against *P. aeruginosa*. Due to the presence of Cl, $-\text{OCH}_3$, $-\text{NO}_2$, SO$_2$NH$_2$ as structural substitution at para position of phenyl ring, the compounds like 3b, 3c, 3g & 3h have an excellent activity against all bacterial strains. But the products, like 3a, 3d, and 3e were found to moderate to weak activities against all bacterial pathogens.

**Resonance structural Hybrids of coupled azocoumarin**

**CONCLUSION**

The present research work involves the synthesis of novel 3-substituted azocoumarin derivative to explore their antimicrobial activity. All the compounds were recrystallized and structurally interpreted by IR and
Compounds 3b, 3c, 3g and 3h were exhibited excellent antibacterial activity against *E. coli* and *S. aureus*, whereas the synthesized compounds (3a, 3i) were found to be moderate to weak activity. Hence, it is concluded that there is a scope for further study in developing some lead compounds for the treatment of bacterial and fungal diseases.

\[
\text{Ar-NH}_2
\]

1(a-j)

\[
\text{NaNO}_2/\text{Conc.HCl/O}^0
\]

Diazotisation 40-50mins

\[
\text{HO}
\]

\[
\text{Ar-N=N-Cl}
\]

1(a-j)

\[
\text{NaOH,O}^0/30\text{mins}
\]

2(a-j)

\[
\text{HC}_1
\]

Diazotisation 3(a-j)

Ar: phenyl, 4-methoxy phenyl, 4-chloro phenyl, 4-bromophenyl, 4-hydroxy phenyl, 2-carboxy phenyl, 4-nitro phenyl, 4-sulphamido phenyl, 3-bromo 4-methyl phenyl, napthyl.

Formation of diazonium salt, generation of electrophile species

\[
\text{N} \equiv \text{N} + \text{Ar}
\]

Coupling Reaction of diazonium electrophile with 4-hydroxy coumarin

\[
\text{N} \equiv \text{N} \text{OH}
\]

Mechanism of coupling reaction synthetic scheme

**ACKNOWLEDGEMENTS**

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**REFERENCES**