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# SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF COUMARIN GLYOXALS

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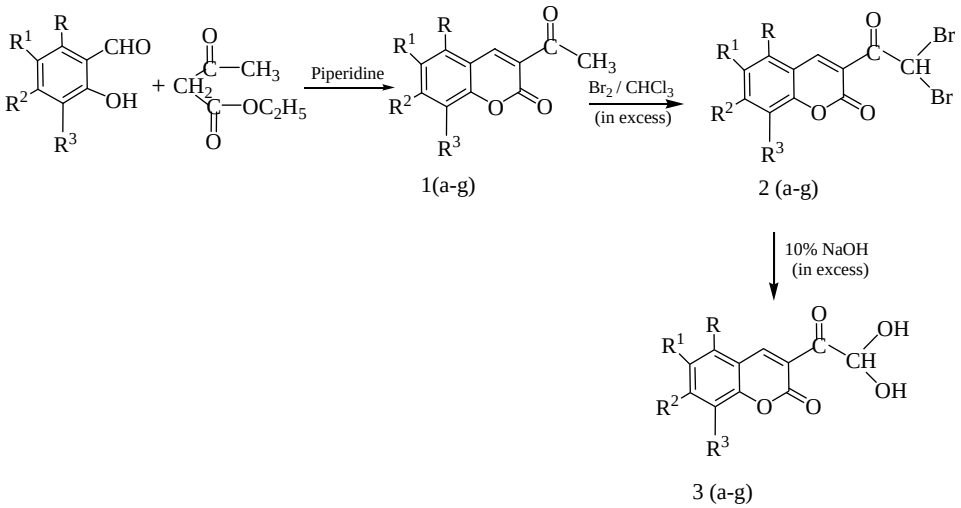
## ABSTRACT
Bromination of 3-acetylcoumarins with excess of bromine to yield 3-ω-gemdibromoacetyl coumarins. In sodium hydroxide solution (10%), 3-ω-gemdibromoacetyl coumarins loses two bromine atoms to yield 3-ω-gemdihydroxyacetyl coumarins. The newly synthesised compounds established by IR, $^1$H NMR and mass spectral studies. All the title compounds have been subjected to *in vitro* antibacterial testing against two pathogenic stains. Among the tested compounds **3c** displayed significant antibacterial activity and rest of them are moderate to least activity.

**KEYWORDS:** 3-acetylcoumarins, 3-ω-gemdibromoacetyl coumarins, 3-ω- gemdihydroxyacetyl coumarins and Antibacterial.

## INTRODUCTION
A number of aromatic glyoxals.[1] have been found to be highly active against New castle disease virus and influenza virus in embryonated eggs.[2]In general the glyoxals were highly active, most of the exceptions being compounds that were quite insoluble in water or were too toxic to allow an adequate dose. In general the sodium bisulphate addition compounds were active than the glyoxal hydrates. This may be due to partly to the significant antiviral activity of sodium bisulphate itself but is probably mostly due to increased solubility. The most of the glyoxal acetyls are used as intermediates.

3-ω-gemdibromoacetyl coumarins which are obtained by the bromination of 3-acetylcoumarins[3,4] using excess of bromine in chloroform. Since 3-ω-gemdibromoacetyl coumarins[5] has two bromine atoms on the same carbon atom which is susceptible to nucleophilic attack. In 10% sodium hydroxide solution 3-ω-gemdibromoacetyl coumarins **2(a-g)** loses two bromine atoms to yield 3-ω-gemdihydroxyacetyl coumarins **3(a-g)** in good yields.

**Scheme**




1(a-g) 2 (a-g)

3 (a-g)

| Compound 3 | a | b | c | d | e | f | g |
|---|---|---|---|---|---|---|---|
| R | H | H | H | H | H | Benzo | H |
| $R^1$ | Br | Cl | Br | -CH3 | H | | H |
| $R^2$ | H | H | H | H | H | H | -Benzo |
| $R^3$ | H | H | Br | H | -OCH3 | H | |

## RESULTS AND DISCUSSION

IR spectrum of 6-bromo-3-ω-gemdihydroxyacetyl coumarin **(3a, $R^1$= 6-Br)** exhibited two carbonyl stretching vibration at 1743 $cm^{-1}$ and 1700 $cm^{-1}$ due to ketocarbonyl and characteristic lactone carbonyl of the coumarin respectively whereas stretching vibrations of =C-H, C=C and C-O-C were observed at 2923 $cm^{-1}$, 1596 $cm^{-1}$ and 1104 $cm^{-1}$ respectively. A broad band between 3475-3360 $cm^{-1}$ due to hydroxyl group. The $^1$H NMR Spectrum displayed singlet at δ 5.17 δ due to CO-CH and broad singlet at δ 4.31 due to two gemdihydroxy group, ($D_2O$ exchanged), whereas doublet at δ 7.23 (d, J=8.7 Hz, 1H) due to $C_8$-H of Coumarin, peak at δ 7.62, (dd $J_m$= 2.1 Hz, $J_{o\ and\ m}$ 8.7 Hz and 1.9 Hz, 1H) due to $C_7$-H of coumarin and $C_5$-H at δ 7.70 (d, J=2.1 Hz, 1H). The $C_4$-H of coumarin found to resonate at δ7.89 (s, 1H).

## EXPERIMENTAL

### Instrumentation

Melting points were determined with open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison, WI, USA) as KBr discs. 1H NMR spectra were recorded on Bruker 400 MHz Spectrometer using $CDCl_3$ and DMSO-d6 as solvents and TMS as internal standard. All chemical shifts were reported as δ values (ppm). Mass spectra were recorded using Shimadzu GCMSQP2010S. The elemental analysis were carried out using Hereaus CHN rapid analyser. Reaction progress was checked by TLC and purity of the compounds were checked by other analytical methods.

### General Procedure

### Preparation of 3-acetyl coumarins (1a-g).

### (General procedure)

Equimolar quantities of different salicylaldehydes and distilled ethylacetoacetate were taken in a conical flask and kept for 10 hours in the presence of catalytic amount of piperidine. The resulting yellow coloured solid was repeatedly washed with cold ethanol, dried to yield colourless 3-acetyl coumarins and used for next step without further purification.

### Preparation of 3-ω-gem-dibromoacetyl coumarins (2a-g).

### (General procedure)

Bromine (0.02 mol) in 2 ml chloroform was added to substituted 3-acetyl coumarins (0.1 mol) in dry chloroform drop wise with stirring for 5 hours. Reaction mixture was warmed to expel HBr gas, cooled and separated solid was filtered, dried and recrystallised from chloroform.

### Synthesis of 3-ω-gem-dihydroxyacetyl coumarins (3a-g)

### (General procedure)

To 2 gm of substituted 3-ω-gemdibromoacetyl coumarins, sodium hydroxide solution (10 ml of 10%) was added at room temperature and kept overnight. The resulting red colored clear solution was filtered and acidified with dil. HCl. Separated solid was filtered washed with excess of water dried and recrystallised from ethanol and dioxan mixture.

### Spectral Data

**6-bromo-3-ω-gemdihydroxyacetyl coumarin(3a)**

Red coloured solid; yield 67%; m.p 163 ºC; **IR** (KBr) $cm^{-1}$, 3475-3360 (br,-OH), 1743 (C=O,keto), 1700(C=O,lactone),1104(C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, *δ*): 5.17 (s, 1H,-COCH), 7.23 (d, J = 8.7 Hz, 1H $C_8$-H), 7.62 (dd, $J_m$ =2.1 Hz, $J_o$=8.7 Hz,1H, $C_7$-H), 7.70 (d, $J_m$ =2.1 Hz, 1H, $C_5$-H) and 4.31 (br, 2H-OH, -OH, $D_2O$-exchanged), 7.89 (s, 1H, $C_4$-H of coumarin).**GC/MS** *m/z*: 399 observed. Anal.Calcd. for $C_{11}H_7O_5Br$; C,44.29; H,2.34; Found: C,44.25; H, 2.32.

**6-chloro-3-ω-gemdihydroxyacetyl coumarin(3b)**

Red coloured solid; yield 60%; 200 m.p ºC; **IR** (KBr) $cm^{-1}$, 3426 (-OH), 1736(C=O,keto), 1702(C=O,lactone), 1114(C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, *δ* ): 5.18 (s, 1H-COCH), 7.28 (d, J= 8.7 Hz, $J_o$=6.9 Hz, 1H, 1H, $C_8$-H), 7.48 (dd, $J_m$ =1.8 and 2.1 Hz $C_7$-H) 7.52 (d, J=2.4 Hz, 1H, $C_5$-H) 7.85 (s, 1H, $C_4$-H of coum.) and 4.50(br, 2H-OH, $D_2O$-exchanged).**GC/MS** *m/z*:255 observed. Anal.Calcd. for $C_{11}H_7O_5Cl$; C,51.87; H,2.75; Found: C,51; H,2.73.

**6,8-bromo-3-ω-gemdihydroxyacetyl coumarin(3c).**

Red coloured solid; yield 72 %; m.p 215 ºC; **IR** (KBr) $cm^{-1}$, 3475 (-OH), 1743 (C=O,keto), 1700 (C=O,lactone), 1104 (C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, *δ* ): 5.18 (s, 1H-COCH), 7.68 (d, J= 2.1 Hz, 1H, $C_7$-H), 7.86 (d, J =2.4 Hz, 1H $C_5$-H), 7.89 (s, 1H, $C_4$-H of coumarin) and 4.40(br, 2H-OH, $D_2O$-exchange).**GC/MS** *m/z*:377 observed. Anal.Calcd.for $C_{11}H_6O_5Br_2$; C,34.93; H,1.58; Found: C,34.90; H,1.56.

**6-methyl-3-ω-gemdihydroxyacetyl coumarin(3d).**

Red coloured solid; yield 59%; m.p 145 ºC; **IR** (KBr) $cm^{-1}$, 3345 (br,-OH), 1732 (C=O,keto), 1710(C=O,lactone),1114(C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, *δ*): 2.44(s,3H –6CH3), 5.19 (s, 1H,-COCH), 7.26 (d, J = 8.7 Hz, 1H $C_8$-H), 7.60 (dd, $J_m$ =2.1 Hz, $J_o$=8.7 Hz,1H, $C_7$-H), 7.69 (d, $J_m$ =2.1 Hz, 1H, $C_5$-H) and 4.30 (br, 2H-OH, -OH, $D_2O$-exchanged), 7.90 (s, 1H, $C_4$-H of coumarin). **GC/MS**

*m/z*: 235 observed. Anal.Calcd.for $C_{12}H_{10}O_5$; C,61.54; H,4.30; Found: C,61.52; H,4.32.

**8-methoxy-3-ω-gemdihydroxyacetyl coumarin(3e).**
Red coloured solid; yield 62 %; 152 m.p ºC; **IR** (KBr) $cm^{-1}$(-OH), (C=O,keto),(C=O,lactone), (C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, $\delta$): 3.91 (s, 3H OCH3), 5.20 (S, 1H, -COCH), 7.25-7.50(m, Ar-H) and 8.20 (s, 1H, C4-H), 4.39 (br, 2H-OH, -OH, $D_2O$-exchanged).**GC/MS** *m/z*: 251observed. Anal.Calcd. for $C_{12}H_{10}O_6$; C,57.60; H,4.03,; Found: C,5758; H,4.00.

**5,6-benzo-3-ω-gemdihydroxyacetyl coumarin(3f).**
Red coloured solid; yield 60 %; m.p 188 ºC; **IR** (KBr, $cm^{-1}$), 3451 (-OH), 1734 (C=O,keto), 1710 (C=O,lactone), 1114 (C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, $\delta$ ); 5.10 (s, 1H-COCH),7.68-8.65 (M,6H,Ar-H), 9.10 (1H,C4-H), 4.32 (br,2H, D2O exchanged).**GC/MS** *m/z*: 271observed. Anal.Calcd. for $C_{15}H_{10}O_5$; C,66.66; H,3.70; Found: C,66.64; H,3.68.

**7,8-benzo-3-ω-gemdihydroxyacetyl coumarin(3g).**
Red coloured solid; yield 70 %; m.p 195 ºC; **IR** (KBr) $cm^{-1}$, 3427 (-OH),1740 (C=O,keto), 1693(C=O,lactone), 1103(C-O-C). **$^1$H NMR** (CDCl3+DMSO-d6, 400 MHz, TMS, $\delta$ ): 5.19 (s, 1H-COCH), 7.58 (d, J= 5.4 Hz, 1H, $C_7$-H), 7.64 (t, J = 4.5 Hz, 1H, $C_9$-H) 7.576 (t, J = 4.5 Hz, 1H, $C_8$-H) 8.06 (d, J = 4.8, 1H, $C_{10}$-H), 8.19(d, J=5.4 Hz, 1H, $C_6$-H), 8.48 (d, J =5.1 Hz, 1H ($C_5$-H), 8.89 (S, 1H, $C_4$-H and 3.40 (br, 2H-OH $D_2O$-exchanged).**GC/MS** *m/z*: 271 observed. Anal.Calcd.for $C_{15}H_{10}O_5$; C,66.66; H,3.70; Found: C,66.64; H,3.68.

**Antibacterial activity**
The agar disc-diffusion method[6] was used for the screening of *in vitro* antibacterial activity. The antibacterial activity of the synthesized compounds **3(a-g)** were screened against *Staphylococcus Aureus* and *Escherichia Coli* using nutrient agar medium.. The minimum inhibitory concentration (MIC) as carried out using micro dilution susceptibility method[7]. Ciprofloxacin was used as a standard antibacterial drug. The observed data on the antibacterial activity of compounds and control drugs are given in **Table-1.** The investigation of antibacterial screening revealed that some of the newly synthesized compounds showed moderate to good inhibition at 13-100 µg/mL in DMSO. Amongst all the compounds **3c** showed excellent antibacterial activity against *S-Aureus* and *E-Coli* (MIC: 13µg/mL). Remaining compounds displayed moderate to least activity against both bacteria.

**Table 1: Antibacterial activity of compounds 3(a-g).**

| Sl. No. | Compound | Bacterial Stains (Gram + ve and - ve), In µg/mL | |
|---|---|---|---|
| | | ***S. Aureus*** | ***E. Coli*** |
| 1 | 3a | 17 | 17 |
| 2 | 3b | 20 | 20 |
| 3 | 3c | 13 | 13 |
| 4 | 3d | 100 | 100 |
| 5 | 3e | 50 | 50 |
| 6 | 3f | 75 | 75 |
| 7 | 3g | 75 | 75 |
| **Ciprofloxacine** | | **3.25** | **3.25** |

## CONCLUSION

In conclusion, we have described method for the synthesis of 3-ω-gemdihydroxyacetyl coumarins.The acetyl coumarins are converted into 3-ω-gemdibromoacetyl coumarins this leads to the formation of 3-ω-gemdihydroxyacetyl coumarins in 10% sodium hydroxide solution. All the title compounds were subjected to *in vitro* antibacterial testing against two pathogenic strains. Among the tested compounds, **3c** showed significant antibacterial activity against both *Aureus* and *Escherichia Coli* with MIC 13µg/mL, further understanding the mechanism of biological action are still required in order to fully develop of these compounds as potent antibacterial drugs.

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