WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 3, 1574-1589 Research Article ISSN 2278 – 4357

# SYNTHESIS, CHARACTERIZATION AND ANTI BACTERIAL ACTIVITY OF COUMADIN DERIVATIVES

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Article Received on
16 Jan. 2019,

Revised on 05 Feb. 2019,
Accepted on 26 Feb. 2019

DOI: 10.20959/wjpps20193-13372

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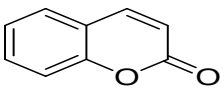
## ABSTRACT

A number of natural and synthetic coumarin derivatives have been reported to exert notably antimicrobial activity. Starting from 7-hydroxy-4-methyl coumarin which was prepared via pechmann condensation, some coumarin derivatives were synthesized by mannich reactions. The synthesized compounds were 8-((dimethylamino)methyl-7-hydroxy-4-methyl coumarin, 8-((diethylamino)methyl-7-hydroxy-4-methyl coumarin, 7-hydroxy-4-methyl-8-(piperidin-1-yl methyl)-coumarin,7-hydroxy-4-methyl-8-(morpholinomethyl)-coumarin, 8-((dimethylamino)methyl-7-ethoxy-4-methyl coumarin, 8-((diethylamino)methyl-7-ethoxy-4-methyl coumarin, 7-ethoxy-4-methyl-8-(morpholino methyl)-coumarin. The prepared compounds were evaluated for their antimicrobial activity against gram positive bacteria *Bacillus subtilis, Staphylcoccus aureus and* Gram negative bacteria *Pseudomonas aeruginosa, Escherichia coli.*

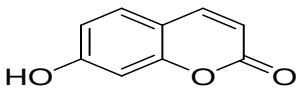
## GENERAL INTRODUCTION

### A brief overview of coumarins and their anti bacterial activity

Compounds containing the coumarin moiety (2*H*-1-benzopyran-2-one) 1 constitute an important class of heterocycles, many examples of which are found in nature. Coumarin itself was first isolated in 1822 from the tonka bean[1]. Coumarin and its derivatives have been isolated from sweet clover, bison grass and woodruff.



1
coumarin




2
umbelliferone

Coumarins can be divided into four sub-types: i) Simple coumarins which are hydroxylated, alkoxylated or alkylated on the benzene ring (*e.g.* umbelliferone 2)[2,3] ii)Furanocoumarins, which contain a five-membered furan ring attached to the coumarin moiety and which are sub-divided into the linear furanocoumarins (*e.g.* xanthotoxin 3)and the angular furanocoumarins (*e.g.* angeligin 4)[2,8] iii) Pyranocoumarins, containing six-membered ring attached to the coumarin moiety (*e.g.* seselin 5 and xanthyletin 6)[2,9] and iv) Coumarins with substituents in the pyrone ring (*e.g.* warfarin 7).

Many compounds containing a coumarin moiety have also been found to exhibit useful and multi-biological activities, including anti-inflammatory[4], anticonvulsant[5], antiviral[6], antimicrobial[7] and anti-HIV[9] properties. Warfarin & Dicoumarol are the naturally occurring coumarins.

**Reactivity of coumarins**

Coumarin and its derivatives are highly reactive. Because the coumarin moiety is aliphatic, it is likely to undergo ring-opening at the acyl centre or conjugate addition at the carbon-carbon double bond[18,19]. The carbonyl lactone group of the coumarin nucleus characterized by an IR absorption band at 1700 $cm^{-1}$[20]. The presence of a methyl group at C-4 or C-6 makes the coumarin nucleus more reactive, and can result in the coumarin nucleus undergoing halogenation as well as condensation with the aldehydes.[20] Carbon-6 on the aromatic ring can undergo electrophilic attack, *e.g.* sulfonation or Friedel-crafts acylation leading to the formation 6-substituted derivatives. Electrophilic attack at C-3 can only occur under forcing conditions unless a C-4 substituent, such as a hydroxyl group directs the incoming group to the C-3 position.[21,22] The nucleophilic attack on the coumarin nucleus depends on the nature of the nucleophile. Thus, strong nucleophiles, such as secondary amines, are likely to open the ring through attack at the acyl centre, whereas weak nucleophiles attack the C-4 position (*e.g.* bromination).

A methyl substituent on the coumarin nucleus may react differently, depending on the position of attachment. For example, a methyl group attached to C-6 or C-4 is more reactive than methyl groups at the C-3 or C-5 positions.[21,23] A methyl substituent on the pyran moiety of coumarin appears to be stable under bromination conditions whereas bromination is observed on the 7-methyl group attached to the benzene ring.

## MATERIAL AND METHODS

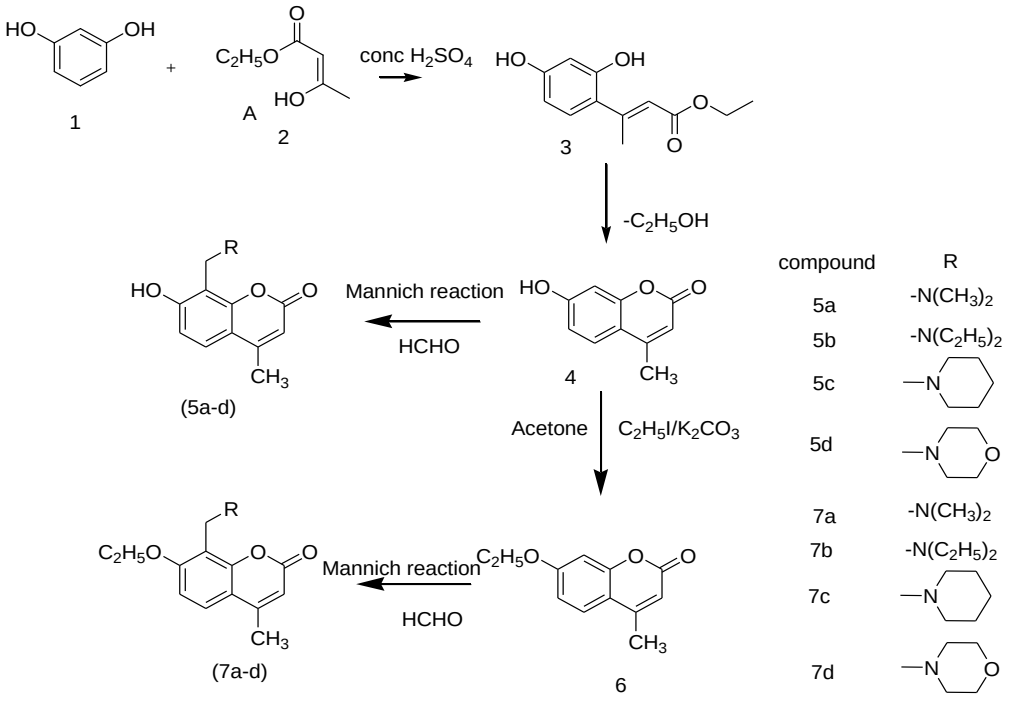
All the chemicals (stating materials, solvents, reagents) used in this work are laboratory grade. Proton Magnetic Resonance Spectra was recorded on AVANCE 300 MHz spectrophotometer using Tri Methyl Silane(TMS) as internal standard. Chemical shifts were expressed in ppm units downfield from TMS. Melting points were recorded on BTI melting point apparatus, using one-end open capillary.

IR spectra were scanned on SCHIMADZU spectrometer with Potassium Bromide Disc. All the reactions were monitored by analytical Thin Layer Chromatography (TLC) using E-merc 0.25 Silica gel plate. Visualization was accomplished with UV light (256nm) and Iodine chamber. Mass Spectrum was recorded on ESI-MS.

The solvents and reagents were purified and dried according to the procedure given in the Vogel's Text Book for Practical Organic Chemistry.

### Synthesis and characterisation of mannich bases of 7-hydroxy, 4-methyl coumarin

As a part of the present investigation on novel coumarin derivatives, it has been planned to synthesise a new series of mannich bases of coumarin derivatives. i.e., 7-hydroxy/7-ethoxy-4-methyl coumarins using different amines such as dimethyl amine, diethyl amine, piperidine, morpholine as per out lined in scheme.




scheme

**Synthesis of 7-Hydroxy 4-Methyl Coumarin[4]**

Resorcinol, ethyl aceto acetate have been taken as starting materials and converted into 7-hydroxyl,4- methyl coumarin in presence of concentrated sulfuric acid by pechmann condensation. The product obtained has been identified by data of the literature. The reaction of resorcinol with ethyl acetoacetate in presence of concentrated sulfuric acid has yielded 7-hydroxy, 4- methyl coumarin .M.P. 182$^{o}$C

As the IR spectrum of the compound (in KBr) has showed characteristic peaks (in $cm^{-1}$) at 1664 $cm^{-1}$(C=O), 3490 $cm^{-1}$(OH), 1267 $cm^{-1}$ (C=O), 2950 $cm^{-1}$(aliphatic C-H) which indicates the 7$^{th}$ hydroxy and 4$^{th}$ methyl substitution on coumarin moiety. Molecular formula-$C_{10}H_8O_3$, Molecular weight-176, Yield - 50.6 %.

**Synthesis of mannich bases of 7- hydroxy/7-ethoxy 4-methyl coumarins[4/6]**

7- hydroxy, 4-methyl coumarin has been subjected to mannich condensation with secondary amines in presence of paraformaldehyde , ethanol and concentrated hydrochloric acid. The reaction conditions yielded a single product. The product has been characterized as 8-amino alkyl, 7- hydroxy,4- methyl coumarin.

For example a 7- hydroxy, 4- methyl coumarin with dimethyl amine on refluxing in the presence of paraformaldehyde in ethanol and concentrated hydrochloric acid, has yielded a product. Purification of the product by recrystallisation from chloroform yielded a colourless crystalline solid, M.P. 134-138$^{o}$C.

The IR spectrum of above compound (in KBr) has showed characteristic peaks (in $cm^{-1}$) at: 2920.23$cm^{-1}$ ($CH_2$ stretching), 1367.53 $cm^{-1}$(C-N stretching), and 1718.58 $cm^{-1}$ (δ – lactone stretching) which indicates 8- (dimethyl amino) methyl substitution at 8$^{th}$ position has occurred.

**Synthesis of 7- ethoxy 4- methyl coumarin[6]**

7-hydroxy 4-methyl coumarin has been taken as starting material and converted into 7–ethoxy, 4- methyl coumarin on reaction with ethyl iodide and potassium carbonate in acetone. The product obtained has been identified by physical and spectral data. The reaction of 7-hydroxy,4- methyl coumarin with ethyl iodide and potassium carbonate in acetone has yielded 7- ethoxy, 4- methyl coumarin. M.P. 114-116$^{o}$C.

The IR spectrum of the compound (in KBr) has showed characteristic peaks (in $cm^{-1}$) at: 2819.93cm-1(C-O stretching) indicates the substitution at $7^{th}$ position in place of hydrogen atom has occurred.

**Synthesis and characterisation of mannich bases of 7- ethoxy,4- methyl coumarin**

As a part of the present investigation on novel coumarin derivatives, it has been planned to synthesise a new series of mannich bases of coumarins. i.e., 7-ethoxy-4- methyl coumarin by using different amines such as dimethyl amine, diethyl amine, piperidine, morpholine as per the synthetic route outlined in scheme.

7-ethoxy-4-methyl coumarin has been subjected to mannich condensation with secondary amine in presence of paraformaldehyde, ethanol and concentrated hydrochloric acid. The reaction conditions yielded a single product. The product has been characterized as 8-(dialkyl amino)- methyl 7-ethoxy- 4- methyl coumarin.

For example a 7 ethoxy 4- methyl coumarin with dimethyl amine on refluxing in the presence of paraformaldehyde in ethanol and concentrated hydrochloric acid, has yielded a product. Purification of the product by recrystallsation from chloroform yielded a colourless crystalline solid M.P. 110-112$^{o}$C.

The IR spectrum of above compound (in KBr) has showed characteristic peaks (in $cm^{-1}$) at: 2941.44 cm-1 (-$CH_2$- stretching), 3431.36 cm-1 (O-H stretching), 1327.03 $cm^{-1}$ C-N stretching, 1743.65 cm-1 (δ-lactone) which indicates (dimethyl amino)methyl substitution at $8^{th}$ position has occurred.

**Synthesis and characterisation of 7–hydroxy, 4- methyl coumarin[4]**

Concentrated sulphuric acid (50 ml) was taken in a three necked round bottomed flask (250 ml) and cooled to 10$^{o}$C. Powdered resorcinol (5g, 0.04 M) dissolved in freshly distilled ethyl aceto acetate (6.8 ml, 0.05M) was taken in a dropping funnel. The mixture of resorcinol and ethyl acetoacetate was added dropwise to cold conc $H_2SO_4$ solution with constant stirring. The temperature was maintained always below 10$^{o}$ C. After adding all the portions of the resorcinol and ethyl aceto acetate mixture, the solution was kept aside for 12 hrs, without further cooling. Then the reaction mixture was poured into crushed ice (150g). The precipitate obtained was filtered and dried. The dried product was dissolved in NaOH solution (10%) then acidified with dilute $H_2SO_4$ till the solution was acidic to litmus. This

solution was kept aside for 10 min. the formed precipitate was washed with water and recrystallised from the ethanol. Yield - 50.6 %, M.P. 180-190$^{o}$C, FTIR -1743 cm$^{-1}$(C=O δ-lactone stretching), 3529 cm$^{-1}$(OH stretching), 1265 cm$^{-1}$ (C=O stretching), 2960 cm$^{-1}$(aliphatic C-H stretching) 1481, 1450, 1431 cm$^{-1}$(aromatic ring C=C stretching). Fig.1, H$^{1}$ NMR (DMSO) δ 2.3 (S, CH3), δ 9.8(S, OH), δ 6.3-7(m, Ar-H). Molecular formula- $C_{10}H_8O_3$-176.

**Synthesis and chaaracterisation of mannich bases of coumarin derivatives 8-(( dimethyl amino) methyl) 7- hydroxy 4- methyl coumarin (5a)**

In a 250-ml three necked round-bottomed flask attached to a reflux condenser are placed 3 g (0.5 mole) of 7-hydroxy-4-methyl coumarin7 ml (0.65 mole) of dimethylamine, and 1 g (0.22 mole) of paraformaldehyde. After the addition of 0.04 ml of concentrated hydrochloric acid in 8 ml. of 95% ethanol, the mixture is refluxed on a water bath for 2 hours. The yellowish solution is filtered, if it is not clear, and is transferred to a 1, l- wide-mouthed Erlenmeyer flask. Subsequently, the solvents were removed by distillation, and the solid mass obtained was washed with water. It was dried in vacuum and re-crystallized from methanol. Yield 2 g (68–72%) and melts at 138–141°C.

Further derivatives are obtained by mannich reactions of 7-hydroxy-4-methyl coumarin with diethyl amine, piperidine and morpholine, dimethyl amine, diethyl amine, piperidine and morpholine are used as amines for mannich bases of 7- hydroxy- 4- methyl coumarin.

FTIR: 2937 cm$^{-1}$ ($CH_2$ stretching), 3608 cm$^{-1}$(OH stretching), 1219 cm$^{-1}$(C-O stretching), 1319 cm$^{-1}$(tertiary nitrogen atom stretching), 1716 cm$^{-1}$ (C-O δ - lactone stretching), 813,796,840 cm-1 (aromatic ring deformation), 1444,1458,1496 cm-1(aromatic ring C=C stretching) fig.2.,

$^{1}$H NMR (CDCl3) δ 2.43 (s, 3H-CH3 at position 4), δ 8.03(s, 1H-OH at position 7), δ 6.27-7.46(d,d 2H Ar-H), δ 2.27,2.27 (s,s 6H- dimethyl amine), δ 3.62 (s, 2H-CH2- at position 6).fig.11. M.P:134-138$^{o}$C. Molecular Weight 233g.

The mass spectrum of the compound showed the molecular ion at m/z 234 agreeing to the mass number of its assigned structure, fig.12.

**8-(( diethyl amino) methyl)7- hydroxy 4- methyl coumarin (5b)**

FTIR: 2939.52 cm-1 (-$CH_2$- stretching), 3568.3 cm-1(OH stretching), 1219.01 $cm^1$ (CO stretching), 1319.31 cm-1 (tertiary nitrogen atom stretching), 1744.06 $cm^1$ (C-O δ lactone), 796,813,840 cm-1(aromatic ring C-H deformation), 1444, 1458, 1498 cm-1 (aromatic ring C=C stretching) fig.3, M.P 123-125$^oC$, Molecular Formula $C_{15}H_{19}O_3N$, Molecular Weight 261g.

**7-hydroxy-4-methyl-8-(piperdin-1-yl methyl) coumarin (5c)**

FTIR: 2933.73 $cm^{-1}$ (-CH2- stretching), 3527.8 $cm^{-1}$ (OH stretching), 1344.38 $cm^1$ (tertiary nitrogen atom stretching), 1734 $cm^{-1}$ (C-O δ lactone), 810,827,842 $cm^{-1}$( aromatic ring C-H deformation), 1384,1442,1477 $cm^{-1}$ (aromatic ring C=C stretching) fig.4., M.P. 137-139$^oC$, Molecular Formula $C_{16}H_{19}O_3N$, Molecular Weight 273g.

**7-hydroxy-4-methyl-8-(morpholino-methyl),coumarin (5d)**

FTIR: 2920.23 $cm^{-1}$( (-CH2-) stretching), 3412.08 $cm^{-1}$( OH stretching), 1220.94 $cm^{-1}$( CO stretching), 1367.53 cm-1(tertiary nitrogen atom stretching), 1718.58 $cm^{-1}$( C-O δ-lactone stretching), 802,827,842 $cm^{-1}$( aromatic ring C-H deformation), 1527,1597,1571 $cm^{-1}$ (aromatic ring C=C stretching). Fig.5., M.P. 136-138$^oC$, Molecular Formula $C_{16}H_{17}O_4N$, Molecular Weight 287g.

**Synthesis and characterisation of mannich bases of 7 –ethoxy 4-methyl coumarin (6)**

To the 0.005 moles of coumarin 0.005 m of ethyl iodide was added in a three necked round bottomed flask add 20 ml of acetone to the above mixture. Reflux for 3 hrs. after refluxing take the reaction mixture and evaporated. White colour powder of 7 ethoxy 4 methyl coumarin was obtained of 2g (90%). Molecular Formula $C_{12}$ $H_{12}O_3$ , Molecular Weight 204.22g, M.P. 113-114$^oC$.

FTIR: 3529.73 $cm^{-1}$ (OH stretching), 2941.44 $cm^{-1}$($CH_3$ stretching), 1205.51 $cm^{-1}$ (C-O stretching), 1743.65 $cm^{-1}$ (C-O δ-lactone stretching), 1265.30 $cm^{-1}$ (C=O stretching), 2819.93 $cm^{-1}$ ($CH_3$-O stretching), 804,850,856 $cm^{-1}$ (Aromatic ring C-H deformation), 1431,1450,1481 $cm^{-1}$ (aromatic ring C=C stretching).fig.6.

**8((dimethyl amino) methyl ) 7 ethoxy 4 methyl coumarin(7a)**

In a 250-ml.3 necked round-bottomed flask attached to a reflux condenser are placed 3 g (0.5 mole) of 7-ethoxy 4- methyl coumarin, 7 ml (0.65 mole) of dimethylamine, and 1 g (0.22 mole) of paraformaldehyde. After the addition of 0.04 ml of concentrated hydrochloric acid in 8 ml of 95% ethanol, the mixture is refluxed on a water bath for 2 hours. The yellowish solution is filtered, if it is not clear, and is transferred to a 1-l wide-mouthed Erlenmeyer flask.

Subsequently, the solvents were removed by distillation, and the solid mass obtained was washed with water. It was dried in vacuum and re-crystallized from methanol.product weighs1.6 g. (70%) and melts at 128–131°C; Furthur derivatives are obtained by mannich reactions of 7 ethoxy 4 methyl coumarin with diethyl amine, piperidine and morpholine . dimethyl amine, diethyl amine, piperidine and morpholine are used as amines for mannich bases of 7- ethoxyl 4- methyl coumarin.

FTIR: 2941.44 $cm^{-1}$ (-$CH_2$- stretching), 3431.36 $cm^{-1}$(O-H stretching), 1327.03 $cm^{-1}$(tertiary nitrogen atom stretching), 1743.65 $cm^{-1}$( C-O δ-lactone stretching), 802,823,850 $cm^{-1}$(aromatic ring C-H deformation), 1431,1500,1543 $cm^{-1}$ (aromatic ring C=C stretching). Fig.7. M.P. 126-128$^oC$, Molecular Formula $C_{15}H_{19}O_3N$, Molecular Weight259g.

**8((diethyl amino) methyl ) 7 ethoxy 4 methyl coumarin (7b)**

FTIR: 2941.44 $cm^{-1}$((-$CH_2$-) stretching), 3529.73 $cm^{-1}$ (O-H stretching), 1265.30 $cm^{-1}$(C-O stretching), 1327.03 $cm^{-1}$ (tertiary nitrogen atom stretching), 1743.65 $cm^{-1}$ (C-O δ lactone stretching),804,850,792 $cm^{-1}$(Aromatic ring C-H deformation), 1431,1460,1500 $cm^{-1}$(aromatic ring C=C stretching).fig.8. M.P. 124-126$^oC$, Molecular Formula $C_{15}H_{23}O_3N$, Molecular Weight 289g.

**7- ethoxy 4- methyl-8-(piperdin-1-yl methyl) coumarin(7c)**

FTIR: 2933.73 $cm^{-1}$ (-$CH_2$- stretching), 3527.80 $cm^{-1}$(O-H stretching), 1220.94 $cm^{-1}$(C-O stretching), 1344.38 $cm^{-1}$(tertiary nitrogen atom stretching), 1744.06 $cm^{-1}$(C-O δ-lactone stretching), 810,842,798 $cm^{-1}$(Aromatic ring C-H deformation), 1442,1477,1529 $cm^{-1}$(aromatic ring C=C stretching).fig.9., M.P. 132-134$^oC$, Molecular Formula $C_{18}H_{23}O_3N$, Molecular Weight 301g.

**7- ethoxy 4-methyl-8-(morpholino methyl) coumarin(7d)**

FTIR: 2960.73 $cm^{-1}$((-$CH_2$-) stretching), 3529.73 $cm^{-1}$(O-H stretching), 1265.30 $cm^{-1}$ (C-O stretching), 1325.10 $cm^{-1}$(tertiary nitrogen atom stretching), 1743.65 $cm^{-1}$(C-O δ-lactone stretching), 802,850,792 $cm^{-1}$(aromatic ring C-H deformation), 1431,1481,1388 $cm^{-1}$(aromatic ring C=C stretching).fig.10., M.P.126-130$^oC$, molecular formula $C_{18}H_{21}O_4N$, Molecular Weight 315g.

## BIOLOGICAL ACTIVITY

### Preparation of test compounds for anti microbial activity

100,75,50,25 and 10µg/ml of synthesized test compounds were dissolved in DMSO and used for the test.

### Micro Organisms used

The following two gram positive and gram negative micro organisms were used for determining anti microbial activity. All the cultures were procured from MTCC, Chandigarh and sub cultured on nutrient agar fortnightly (Table-1).

**Table 1: List of Gram positive and Gram negative micro organisms used in the test.**

| S.No | Gram positive bacteria | Gram Negative bacteria |
|---|---|---|
| 1. | *Bacillus subtilis* | *Pseudomonas aeruginosa* |
| 2. | *Staphylococcus aureus* | *Escherichia coli* |

### Preparation of media

All the above cultures were inoculated in pre sterilized, nutrient broth and incubated for 24hr at 37$^oC$. 24 hr cultures of the above micro organisms were used for the assay.

### Anti microbial activity of the test compounds against the above micro organisms using agar diffusion method

Anti microbial activity of the test compounds against the above micro organisms was checked by agar gel diffusion method. 0.1ml of the culture was seeded in 25ml of molten nutrient agar, mixed and poured in sterile petriplate and allowed to solidify. The wells were bored with 8mm borer in the seeded agar. 0.1 ml of test compound was added in each well. The plates were kept aside for 10 min. After 10 min the plates were incubated at 37$^oC$ for 24hr.

**Measurement of zone of inhibition**

After 24 hr the zone of inhibition in the plates was measured in mm using a Antibiotic zone reader. The experiments were repeated in triplicate and the mean value is represented in the results.

**Table-2: Zone of inhibition of synthesized compounds in mm on gram positive bacteria.**

| Compound | *Bacillus subtilis* | | | | | *Staphylococcus aureus* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc(µg/ml) | **10** | **25** | **50** | **75** | **100** | **10** | **25** | **50** | **75** | **100** |
| 5a | 0.9 | 2.1 | 3.4 | 4.1 | 5.4 | 1.1 | 2.1 | 3.1 | 6.1 | 7.2 |
| 5b | 0.8 | 2.3 | 3.2 | 4.4 | 6.3 | 2.0 | 3.4 | 4.0 | 5.5 | 7.4 |
| 5c | 0.7 | 2.4 | 3.5 | 5.3 | 7.2 | 1.8 | 2.3 | 3.2 | 5.4 | 7.5 |
| 5d | 1.9 | 2.1 | 3.6 | 4.2 | 8.1 | 1.4 | 2.5 | 4.5 | 6.1 | 7.9 |
| 7a | 0.6 | 1.9 | 3.1 | 4.5 | 5.3 | 1.3 | 2.7 | 4.1 | 4.8 | 7.4 |
| 7b | 0.7 | 2.2 | 4.0 | 5.1 | 8.4 | 2.5 | 3.5 | 4.2 | 5.9 | 5.9 |
| 7c | 0.8 | 2.3 | 8.2 | 11.0 | 13.0 | 3.1 | 5.1 | 7.1 | 7.6 | 11.0 |
| 7d | 1.0 | 2.1 | 3.4 | 4.3 | 6.2 | 1.3 | 6.3 | 7.2 | 8.3 | 10.4 |
| Gentamycin(10µg/ml) | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 |

**Table 3: Zone of inhibition of synthesized compounds in mm on gram negative bacteria.**

| Compound | *Pseudomonas aeruginosa* | | | | | *Escherichia coli* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc (µg/ml) | 10 | 25 | 50 | 75 | 100 | 10 | 25 | 50 | 75 | 100 |
| 5a | | | | | | 2.1 | 3.1 | 4.6 | 5.1 | 7.1 |
| 5b | | | | | | 1.9 | 4.0 | 5.3 | 5.2 | 8.9 |
| 5c | | | | | | 2.4 | 3.3 | 4.1 | 4.4 | 8.1 |
| 5d | | | | | | 3.2 | 4.1 | 5.2 | 4.2 | 5.3 |
| 7a | | | | | | 2.1 | 3.7 | 4.4 | 4.6 | 6.1 |
| 7b | | | | | | 3.1 | 4.2 | 5.1 | 4.3 | 5.2 |
| 7c | 4.5 | 6.2 | 8.1 | 7.2 | 9.3 | 1.9 | 3.2 | 4.5 | 5.4 | 8.3 |
| 7d | | | | | | 4.0 | 5.1 | 5.6 | 6.1 | 7.4 |
| Gentamycin( 10µg/ml) | 11.5 | 11.5 | 11.5 | 11.5 | 11.5 | 11.7 | 11.7 | 11.7 | 11.7 | 11.7 |

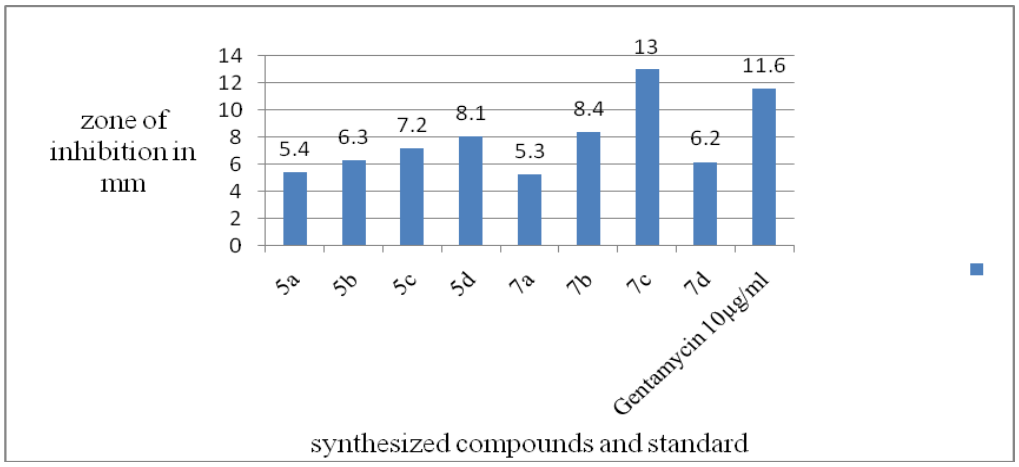



**Fig.13. Anti bacterial activity of synthesized compounds against *Bacillus subtilis* at a concentration of 100 µg/ml.**

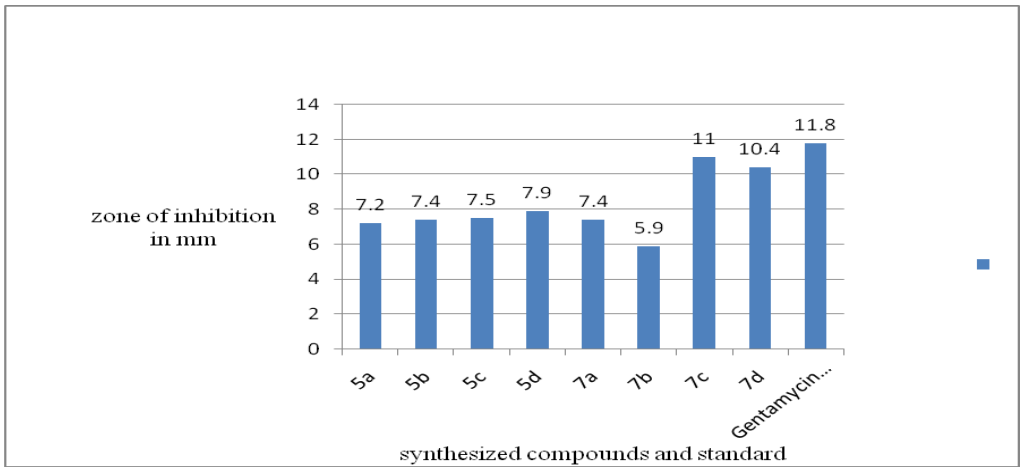



**Fig.14: Anti bacterial activity of synthesized compounds against S*taphylococcus aureus* at a concentration of 100 µg/ml.**

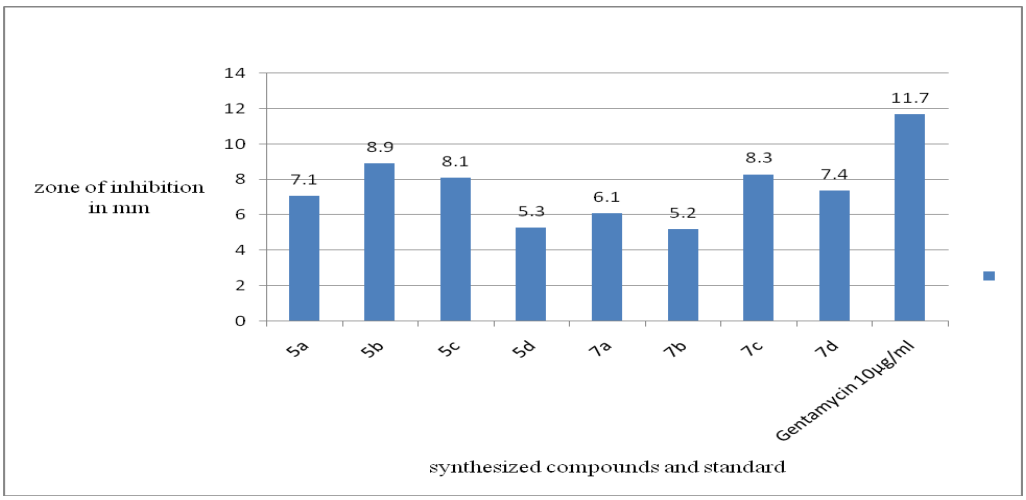



**Fig.15: Anti bacterial activity of synthesized compounds against *Escherichia coli* at a concentration of 100 µg/ml.**

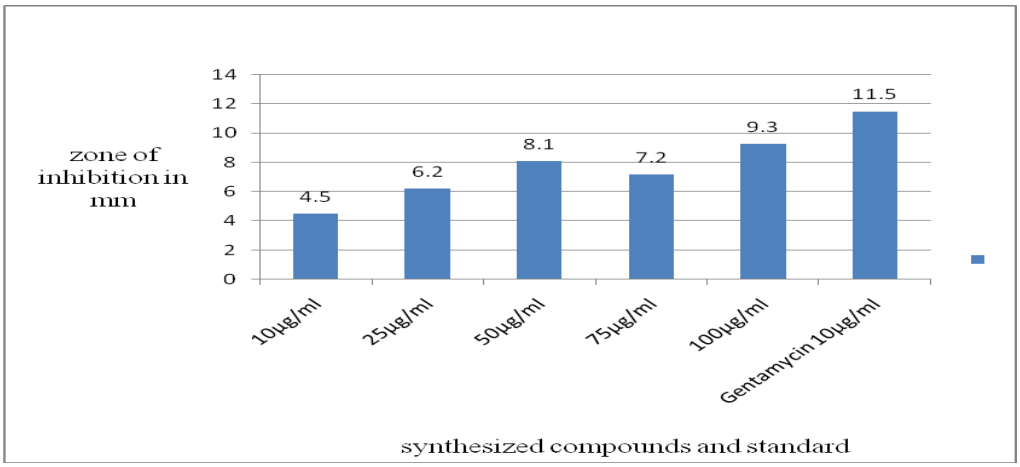



**Fig.16: Anti bacterial activity of 7c compound against *Pseudomonas aeruginosa* at all concentrations.**

## RESULTS AND DISCUSSIONS

- In order to prepare a variety of coumarin derivatives, the 7-hydroxy-4-methyl coumarin was selected as the starting compound.
- As depicted in Scheme, 7-hydroxy-4-methyl coumarin was synthesized via Pechmann reaction by the condensation between resorcinol and ethyl acetoacetate in the presence of concentrated sulfuric acid.
- As depicted in scheme, 7-ethoxy-4-methyl coumarin was also synthesized from ethyl iodide and 7-hydroxy-4-methyl coumarin in presence of potassium carbonate and acetone as solvent. The synthesized 7-hydroxy-4-methyl coumarin , 7-ethoxy-4-methyl coumarins were subjected to mannich reaction using secondary amines like dimethyl amine, diethyl amine, piperidine, and morpholine.
- Resulted title compounds were then evaluated for their anti bacterial activity using cup plate method against gram positive, gram negative bacteria.
- The synthesized compounds are 8-(dimethyl amino)methyl,7-hydroxy-4-methyl coumarin, 8-(diethyl amino)methyl,7-hydroxy-4-methyl coumarin, 8-(-1-methyl piperdinyl)-7- hydroxy-4-methyl coumarin , 8-(1-methyl morpholyl), 7-hydroxy-4-methyl coumarin, 8- (dimethyl amino)methyl 7-ethoxy-4-methyl coumarin, 8-(diethyl amino)ethyl-7-ethoxy-4- methyl coumarin, 8-(1-methyl piperdinyl) 7-ethoxy-4-methyl coumarin, 8-(1-methyl morpholyl) 7-ethoxyl-4- methyl coumarin.

### Anti bacterial activity

The results reported in Table: 2 and 3 indicate that the title compounds have moderate to significant antibacterial activity when compared to the reference standard. However, among the compounds tested compounds '7c' has been found to be superior in their antibacterial activity particularly against gram positive and gram negative bacteria employed at 10-100µg/ml.

The variation in zone of inhibition was found to be in the range of 5.4 and 13.0 mm against *Bacillus subtilis*, 5.9 to 11.0 mm against *Staphylococcus aureus*, 4.5 to 9.3mm against *Pseudomonas aeruginosa* and 5.2 to 8.9mm against *Escherichia coli.*

From the present investigation it was found that

1. All the compounds were found to have moderate to significant antibacterial activity except on *Pseudomonas aeruginosa* when compared to the standard.

2. The incorporation of ethoxy group at 7-position on the 7-hydroxy,4methyl coumarin ring system increased the antibacterial activity.
3. Introduction of heterocyclic ring system like N-methyl piperidyl (7c) and N-methyl morpholinyl(7d) substitution at 8-position on the 7-hydroxy,4-methyl coumarin ring system further increased the antibacterial activity.
4. Among all the titled compounds mannich bases of 7-ethoxy, 8-methyl piperidyl 4-methyl coumarin has demonstrated maximal antibacterial activity.

## CONCLUSION

A new series of mannich bases of 7-hydroxy / 7-ethoxy-4-methyl coumarins were prepared in good yields with convenient route.

The anti bacterial activity of the prepared compounds was tested against gram positive and gram negative bacteria. All the compounds were found to have moderate to significant antibacterial activity except on *Pseudomonas aeruginosa* when compared to the standard. The variation in the zone of inhibition of the test compounds was in the range of 5.4 to 13.0 mm against *Bacillus subtilis,* 5.9 to 11.0 mm against *Staphylococcus aureus,* 4.5 to 9.3mm against *Pseudomonas aeruginosa* and 5.2 to 8.9 mm against *Escherichia coli* at 100µg/ml of concentration.

From the present investigation it was found that

1. The incorporation of ethoxy group at 7-position on the 7-hydroxy,4methyl coumarin ring system increased the antibacterial activity.
2. Introduction of heterocyclic ring system like N-methyl piperidyl (7c) and N-methyl morpholinyl(7d) substitution at 8-position on the 7-hydroxy,4-methyl coumarin ring system further increased the antibacterial activity.
3. Among all the titled compounds mannich bases of 7-ethoxy, 8-methyl piperidyl 4-methyl coumarin has demonstrated maximal antibacterial activity.

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