# Synthesis and Antimicrobial Evaluation of Coumarin Derivatives

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## ABSTRACT

Synthesis of the coumarin dimers was attained from the coupling of the p-hydroxy- modified benzaldehyde with two equivalent of 4-hydroxycoumarin under reflux. The target compounds were screened for their antibacterial activity against Escherichia coli (E.*coli*), Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA), Enterococcus species (Esp.), Streptococcus pyogenes (SP), and Streptococcus pneumoniae (SPn). Certain compounds showed good or moderate antimicrobial activity on SA and SP.

**Keywords**: Coumarin, Coupling, Antibacterial activity.

# 香豆素衍生物之合成及抗菌活性評估

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## 摘　　要

本研究合成一系列具有抑菌潛能之香豆素衍生物，合成法是利用偶合反應將被修飾過的對位氫氧基苯甲醛 (*p*-hydroxy-modified benzaldehyde) 所產生之衍生物與二當量的 4-氫氧基香豆素 (4-hydroxycoumarin) 進行偶合反應後，得到最終目標產物。目標產物針對大腸桿菌、金黃色葡萄球菌、綠膿桿菌、腸球菌、化膿性鏈球菌、肺炎鏈球菌等六種細菌進行抑制菌落生長活性試驗。抗菌試驗結果顯示，一些化合物對金黃色葡萄球菌、化膿性鏈球菌具有好或中等程度的抑制效果。

**關鍵字：**香豆素，偶合反應，抗菌活性

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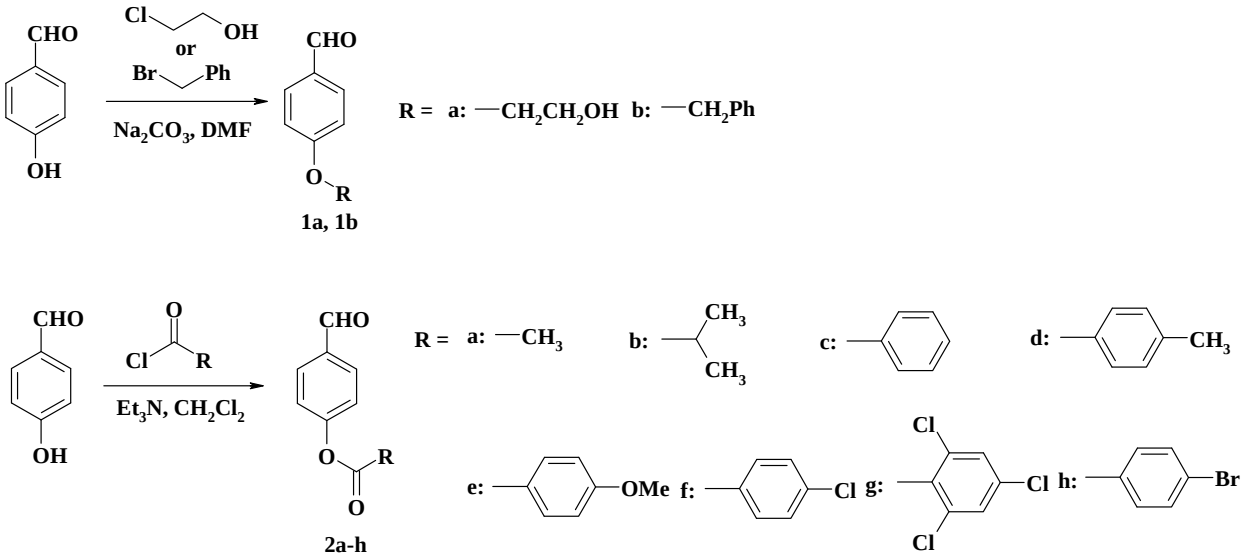
# I. INTRODUCTION

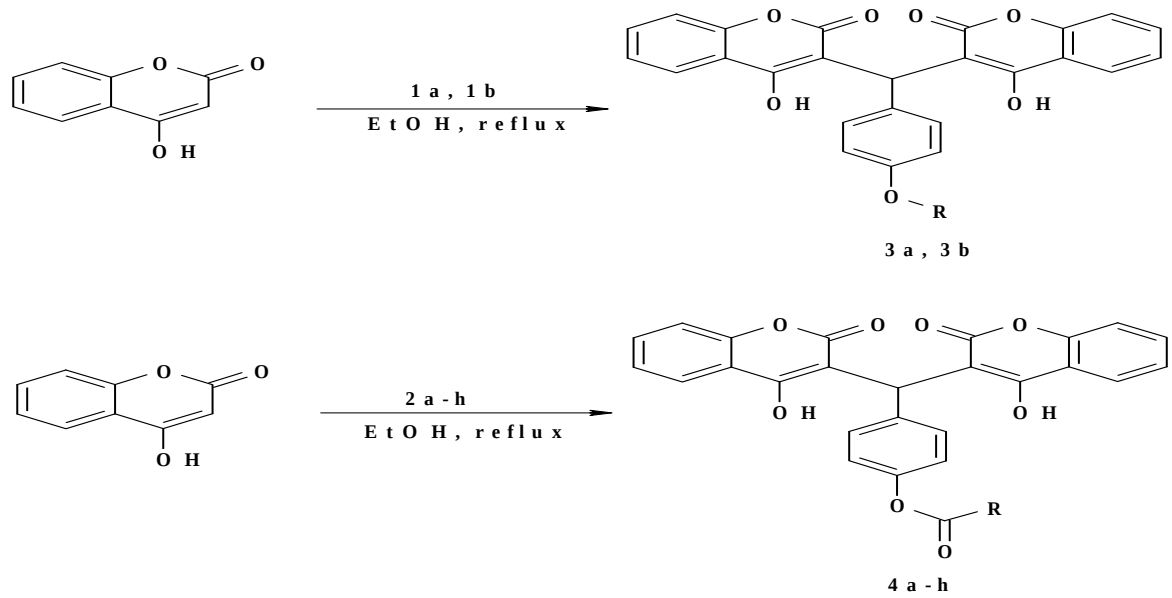
Bacterial diseases such as staphyl-ococcal, hemolytic streptococcus, gonorrhea, syphilis, gangrene and bacterial endocarditic appeared horrifying effects on the people who were infected. They suffered from painful headache, fever, paralysis and degeneration of internal organs to unbearable death. Until the discovery and use of antibiotics, doctors had no vital tool in the battle against these terribly overwhelming bacterial diseases. The most widely used antibiotic of all is penicillin, which has easily saved many lives since it entered wide use during World War II. Since then, antimicrobial agents, used to treat bacterial infections, have been one of the most prescribed agents in the world and their clinical use has saved innumerable millions of lives. With the development of new bacterial strains resistant to many currently available antibiotic treatments, there is increasing interest in the discovery of novel antibacterial agents.

Coumarins have attracted intense interest in recent years because of their diverse pharmacology properties [1]. A lot of coumarins have been identified from natural sources, especially green plants. The very long association of plant coumarins with various animal species and other organisms throughout evolution may account for the extraordinary rang of biochemical and pharmacological activities. Coumarins have important effects in enzyme inhibitors [2-4], and precursors of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, as well as defense against infection. The coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic [5]. Antibacterial activeties of coumarins have been broadly investigated [6]. However, few studies of antimicrobial properties of synthetic simple coumarin derivatives have been performed. In addition, new attention for coumarin containing antibiotics such as novobiocin, clorobiocin [7] and coumermycin A created by a number of Streptomyces species has been aroused by the finding that these antibiotics are effective inhibitors of bacterial DNA gyrase and topoisomerase [8]. Therefore, it is worthwhile to study antimicrobial effect of newly synthesized coumarin derivatives. In the present work, the synthesis of coumarin derivatives was performed and the inhibitory activity of these derivatives on several microorganisms was achieved.

**Scheme 1**



**Scheme 2**




# II. RESULTS AND DISCUSSION

## 2.1 Chemistry

The synthetic route to the target compounds 3a, 3b and 4a-h are outlined in Scheme 1-2. Starting 4-Hydroxybenzaldehy- de was transformed to their corresponding intermediates 4-(2-hydroxyethoxy)benzaldehyde (1a), 4-benzyl-oxybenzaldehyde (1b) and 4-substituted aromatic aldehydes (2a-h) by the nucleophilic reaction of 4-hydroxy- benzaldehyde with 2-chloroethanol, benzyl bromide and substituted acid chloride respectively. Target compounds was prepared by the condensation of two equivalent of 4-hydroxycoumarin coupling with key intermediates in ethanol under reflux. All the structures of target compounds were established by 1H-NMR, MS spectroscopy and elementary analysis.

## 2.2 Antibacterial Activity

In this study, the in vitro disc diffusion susceptibility test [9-11] of the prepared coumarin derivatives against Escherichia coli (E.coli), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Enterococcus species* (Esp.), *Streptococcus pyogenes* (SP), and *Streptococcus pneumoniae* (SPn) was examined. Table 1 lists the inhibitory activity results. All compounds showed no inhibitory activity against *E.coli*, PA, Esp and SPn. Over ten compounds, three compounds **4a**, **4b**, and **4c** exerted good inhibitory effects against SA with inhibition zone over 10 mm at concentration of 50 g/disc. At the same concentration on bacterial SA, four compounds **3a**, **4d**, **4e**, **4f**, and **4h** showed moderate activity with inhibition zone range from 7.7 to 8.7 mm. Among ten compounds, only three compounds **3a**, **4a,** and **4b** possessed moderate antimicrobial action against SP with inhibition zone range from 8.0 to 8.2 mm. The present study reports the antimicrobial activity of ten coumarin compounds. The preliminary results suggested that our coumarin derivatives generally contain good or moderate antibacterial activity on SA and SP and their activities depend on the position of substituents. It is suggested that 3,3'-(4-hydroxybenzylidene)bis-4-hydroxycoumarin ske-leton are essential for activity. Replacement of 4-hydroxybenzylidene moiety on the skeleton with 4-acyloxybenzylidene may enhance the antimicrobial properties. In conclusion, we have prepared ten coumarin derivatives. Their inhibitory activity against SA and SP would be useful to make some correlations of available data which would help the researchers in discovering and developing of new active compounds used in coumarin drug design.

# III. EXPERIMENTAL

## 3.1 General Experimental Procedures

Melting points were taken on a BUCHI 530 apparatus and are uncorrected. Merck Art No.

105554 plates percolated with Silica gel 60 containing fluorescent indicator were used for thin-layer chromatography, and Silica gel 60 (Merck Art No 109385, 230-400 mesh) was employed for column chromatography. Evaporations were carried out at < 50 °C using a rotary evaporator at reduced pressure (water aspirator). $^{1}$H- and $^{13}$C-NMR spectra were obtained with a Varian 300 NMR spectrometer at 300 and 75 MHz, respectively. Where necessary, deuterium exchange experiments were used to obtained proton shift assignments. Mass spectra were recorded on a JEOL J.M.S-300 spectro- photometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of P2O5 for at least 12 h unless otherwise specified. Elemental analyses were obtained using a Perkin-Elmer 2400 Elemental Analyzer. **4-(2-Hydroxy-ethoxy)benzaldehyde (1a).** The reaction mixture of 2-chloroethanol (3.3 g, 41 mmol), 4-hydroxybenzaldehyde (5.0 g, 41 mmol), sodium carbonate (5.9 g, 56 mmol) and DMF (15 ml) was heated under reflux for 24 h. After cooling, the mixture was poured into water. Extraction with ethyl acetate, drying with magnesium sulfate the mixture was concentrated under reduced pressure. Column chromatography of

Table 1. Antimicrobial Activity of compound 3-4 at 50μg/disc

| Compounds. | *E.coli* | SA | PA | Esp | SP | SPn |
|---|---|---|---|---|---|---|
| **3a** | N | N | N | N | 7.7±0.58 | N |
| **3b** | N | 7.7±0.58 | N | N | N | N |
| **4a** | N | 10.3±0.58 | N | N | 8.0±1.00 | N |
| **4b** | N | 10.3±1.00 | N | N | 8.2±0.29 | N |
| **4c** | N | 11.3±0.58 | N | N | +/- | N |
| **4d** | N | 7.7±0.58 | N | N | +/- | N |
| **4e** | N | 8.7±0.58 | N | N | +/- | N |
| **4f** | N | 7.7±0.58 | N | N | N | N |
| **4g** | N | N | N | N | N | N |
| **4h** | N | 7.7±0.58 | N | N | N | N |
| **Blank paper discs** | N | N | N | N | N | N |
| **Getamicin** (10μg/disc) | 19-26 | 19-27 | 16-21 | – | – | – |
| **Getamicin** (120μg/disc) | – | – | – | ≧10 | – | – |
| **Vancomycin** (30μg/disc) | – | – | – | – | 17 | – |
| **Penicillin** (10μg/disc) | – | – | – | – | – | 30 |

*Escherichia coli* (*E.coli*), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Enterococcus* species (Esp.), *Streptococcus pyogenes* (SP), and *Streptococcus pneumoniae* (SPn) Diameter of the zone of inhibition (in mm). N indicates no inhibition zone and +/- show little activity was observed.

the residue on silica gel with 1 : 1 n-hexane / EtOAc as eluent gave 1a (4.4 g, 65 %) as a light brown oil. Rf 0.45 (n-hexane / EtOAc = 1 / 1). 1H-NMR ($CDCl_3$) δ: 3.79-4.25 (m, 4H, $CH_2$), 6.96 (d, 2H, J = 58.5 Hz), 7.77 (d, 2H, J = 58.5 Hz), 9.81 (s, 1H, CHO).

**4-(Benzyloxy)benzaldehyde (1b).** The reaction mixture of benzyl bromide (1.05 g, 6.1 mmol), 4-hydroxybenzaldehyde (0.75 g, 6.1 mmol), sodium carbonate (5.9 g, 56 mmol) and DMF (15 ml) was heated under reflux for 24 h. After cooling, the mixture was poured into water. Extraction with ethyl acetate, drying with magnesium sulfate the mixture was concentrated under reduced pressure. Recrystallization of the residue with Ethanol obtained **1b** (1.12 g, 86 %) as a white crystal. $R_f$ 0.52 (*n*-Hexane / EtOAc = 1 / 1 ). mp: 70-72 °C. $^1$H-NMR ($CDCl_3$) δ: 5.16 (s, 2H, $CH_2O$), 7.08 (d, 2H, $J$ = 8.5 Hz, $H_3$, $H_5$), 7.38-7.43 (m, 5H, Ar-H), 7.84 (d, 2H, $J$ = 8.5 Hz, $H_2$, $H_6$), 9.89 (s, 1H, CHO).

**Preparation of 4-arylcarbonyoxybez- adehyde (2a-h). General Procedure:** Acid chloride (2.8 g; 20 mmol) was added to a solution of 4-hydroxy-benzaldehyde (1.24 g, 10 mmol) and triethyl- amine (3 ml) in dry dichloride (20 ml) under ice bath. The reaction mixture was stirred at room temperature for 8 h. Extraction with ethyl acetate, drying with magnesium sulfate the mixture was concentrated under reduced pressure. Column chromatography of the residue on silica gel with 1 : 1 *n*-hexane / EtOAc as eluent gave **2a** (1.45 g, 64 %) as a yellow crystal. **2b-h** were obtained under the same condition: 4-Acetoxybenz- aldehyde (**2a**), 4-[(Isobutyryl)oxy]benzaldehy- de(**2b**), Benzoic Acid 4-Formylphenyl Ester (**2c**), 4-Methylbenzoic Acid 4-Formyl phenyl Ester (**2d**), 4-Methoxybenzoic Acid 4-Formyl- phenyl Ester (**2e**), 4-Chlorobenzoic Acid 4-Formylphenyl Ester (**2f**), 4-[(2,4,6-Tri- chlorobenzoyl)oxy]benz-aldehyde (**2g**), 4-Bromobenzoic Acid 4-Formyl-phenyl Ester (**2h**). These ester compounds were identified by melting point and $^1$H-NMR. Data were in agreement with literature values [3].

**Condensation of Substituted Aromatic Aldehydes with 4-Hydroxycoumarin (3a, 3b, 4a-h). General Procedure**: The reaction mixture of 4-hydroxycoumarin (2.2 equi- valent), aromatic aldehyde (1 equivalent) in ethanol was heated under reflux for 48 h. The mixture was concentrated under reduced pressure to furnished product. Pure products were obtained under recrystalliztion with certain solvents.

**3,3'-[4-(2-Hydroxyethoxy)benzylidene]bis(4-hyd roxy-coumarin) (3a)**: Yield 64 %. $R_f$ 0.16 (n-hexane / EtOAc = 1 / 4). mp: 176-177 °C. $^1$H-NMR (DMSO-$d_6$) δ: 3.69, 3.91 (m, 2H each, $OCH_2CH_2OH$), 6.28 (s, 1H, CH), 6.78-7.91 (m, 12H, Ar-H). MS m/z: 472 ($M^+$). *Anal.* Calcd for $C_{27}H_{20}O_8$: C, 68.64; H, 4.27. Found: C, 68.49; H, 4.51.

**3,3'-[4-(Benzyloxy)benzylidene]bis-4-hydroxyco umarin (3b)**: Yield 63 %. $R_f$ 0.38 (hexane / ethyl acetate = 1 / 4). mp: 180-181 °C. $^1$H-NMR (DMSO-$d_6$) δ: (ppm) 5.05 (s, 2H, $CH_2$) 6.28 (s, 1H, CH) 6.86-7.90 (m, 15H, Ar-H), MS m/z: 518 ($M^+$). *Anal*. Calcd for $C_{32}H_{22}O_7$: C, 74.12; H,4.28. Found: C, 74.12; H, 4.28.

**3,3'-[4-Acetoxy-benzylidene]bis-4-hydroxycoum arin (4a)**: Yield: 64 %. $R_f$ 0.25 ( hexane / ethyl acetate = 1 / 4). mp: 221-223 °C. $^1$H-NMR (DMSO-$d_6$) δ: 2.25 (s, 1H, $CH_3$) 6.34 (s, 1H, CH) 6.96-7.92 (m, 12H, Ar-H). MS m/z: 470 ($M^+$). *Anal.* Calcd for $C_{27}H_{18}O_8$:C, 68.94; H, 3.86 Found: C, 69.0; H, 3.85.

**3,3'-{4-[(Isobutyryl)oxy]-benzylidene}bis-4-hydr oxy-coumarin (4b)**: Yield: 43 %. $R_f$ 0.19 (hexane / ethyl acetate = 1 / 1). mp:171-174 °C. $^1$H-NMR (DMSO- $d_6$) δ: 1.22 (s, 6H, $CH_3$), 3.18 (s, 1H, CH), 6.31 (s, 1H, CH), 6.92-7.89 (m, 12H, Ar-H). MS m/z : 498 ($M^+$). *Anal.* Calcd for $C_{29}H_{22}O_8$: C, 69.88; H, 4.45. Found: C, 69.88; H, 4.45.

**3,3'-[4-(Benzoyloxy)benzylidene]bis(4-hydroxy-c oumarin) (4c)**: Yield 43 %. $R_f$ 0.43 (*n*-hexane / EtOAc = 1 / 4). mp: 200-201 °C. $^1$H-NMR (DMSO-$d_6$) δ: 6.37 (s, 1H, CH), 7.12-8.14 (m, 17H, Ar-H). MS *m*/*z*: 532 ($M^+$). *Anal.* Calcd for $C_{32}H_{20}O_8$: C, 72.18; H, 3.79. Found: C, 72.16; H, 3.65.

**3,3'-[4-(4-Methylbenzoyloxy)benzylidene]bis(4-h ydroxy-coumarin) (4d)**: Yield 64 %. $R_f$ 0.26 (*n*-Hexane / EtOAc = 1 / 4). mp: 164-165 °C. $^1$H-NMR (DMSO-$d_6$) δ: 2.50 (s, 3H, $CH_3$), 6.32 (s, 1H, CH), 7.09-8.02 (m, 16H, Ar-H). MS *m*/*z*: 546 ($M^+$). *Anal.* Calcd for $C_{33}H_{22}O_8$: C, 72.52; H, 4.06. Found: C, 72.59; H, 4.40.

**3,3'-[4-(4-Methoxbenzoyloxy)benzylidene]bis(4-hydroxy-coumarin) (4e)**: Yield 60 %. $R_f$ 0.48 (*n*-hexane / EtOAc = 1 / 4). mp: 159-160 °C. $^1$H-NMR (DMSO-$d_6$) δ: 3.85 (s, 3H, $CH_3$), 6.37 (s, 1H, CH), 7.08-8.05 (m, 16H, Ar-H). MS *m*/*z*: 562 ($M^+$). *Anal.* Calcd for $C_{33}H_{22}O_9$: C, 70.46; H, 3.94. Found: C, 70.28; H, 4.16.

**3,3'-[4-(4-Chlorobenzoyloxy)benzylidene]bis(4-h ydroxy-coumarin) (4f)**: Yield 70 %. $R_f$ 0.21 (*n*-hexane / EtOAc = 1 / 4). mp: 147-148 °C. $^1$H-NMR (DMSO-$d_6$) δ: 6.41 (s, 1H, CH), 7.14-8.12 (m, 16H, Ar-H). MS *m*/*z*: 567 ($M^+$). *Anal.* Calcd for $C_{32}H_{19}ClO_8$: C, 67.79; H, 3.38. Found: C, 67.49; H, 3.61.

**3,3'-[4-[(2,4,6-Trichlorobenzoyl)oxy-benzylidene ]bis-4-hydroxycoumarin (4g)**: Yield 56 %. $R_f$ 0.32 (hexane / ethyl acetate = 1 / 1). mp: 150-152 °C. $^1$H-NMR (DMSO-$d_6$) δ: 6.11 (s, 1H, CH), 7.24-8.11 ( m, 14H, Ar-H ). MS m/z:634 ($M^+$). ***Anal.*** Calcd for $C_{22}H_{17}Cl_3O_8$: C, 60.45; H, 2.69 Found C, 59.85; H, 2.88.

**3,3'-[4-(4-Bromobenzoyloxy)benzylidene]bis(4-h ydroxy-coumarin) (4h)**: Yield 38 %. $R_f$ 0.18 (*n*-hexane / EtOAc = 1 / 4). mp: 159-160 °C. $^1$H-NMR (DMSO-$d_6$) δ: 6.38 (s, 1H, CH), 7.14-8.06 (m, H, Ar-H). MS *m*/*z*: 611 ($M^+$). ***Anal.*** Calcd for $C_{32}H_{19}BrO_8$: C, 62.86; H, 3.13. Found: C, 63.02; H, 3.44.

### 3.2 Antibacterial Assay

Disk diffusion (Kirby-Bauer) is one of the most commonly used antimicrobial susceptibility testing (AST) methods among diagnostic laboratories [12].

Growth inhibitory activity of the target compounds were tested against six strains (*Escherichia coli*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Enterococcus species*; *Streptococcus pyogenes*; *Streptococcus pneumoniae*). These microorganisms were provided from the Microbiology Laboratory Culture Collection, Department of pathology, Tri-service General Hospital, Taipei, Taiwan. Bacteria were incubated at 37°C for 24h in nutrient broth (Difco). The disk diffusion test was performed on each bacterial strain using unsupplemented Mueller-Hinton agar (Becton Dickinson Microbiology System, Cocleysville, Md). With the Kirby-Bauer method, an activity growing broth culture is diluted until the turbidity matches that of a MacFarland 0.5 $BaSO_4$ standard (ca. $10^8$ colony-forming units [CFU]/ml). 15 ml of muellerr hinton agar (MHA, Oxoid) and sabouraud dextrose agar (SDA) (sterilized in a flask and cooled to 40-50 °C) were homogenously distributed onto the sterilized Petri dishes. Sterilized blank paper discs (BD, USA) 6 mm in diameter were saturated with 50μg of tested compounds per disc, then placed onto agar plates which had previously been inoculated with the above microorganisms. Sterilized blank paper discs were used negative control and standard paper discs treated with gentamycin, vancomycin and penicillin (BD, USA) saturated antibiotics were used as positive controls. Following incubation for 16 to 18h at 37 °C, inhibition zones appearing around the discs were measured and recorded in mm.

## IV. CONCLUSION

Ten new chemically coumarin analogues have been synthesized. Among these easily available biscoumarin, all the target compounds were found to be active molecules antibacterial activity, especially against *Staphyococcus aureus* and *Streptococcus pyogenes*. These compounds may prove useful in structure–activity relationship studies on bacterial inhibitors and may ultimately lead to new classes of potent therapeutic agents. Further detailed studies of the biological activity of these compounds will reveal their full potential.

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