



Synthesis and Evaluation of Novel 4-hydroxycoumarin Derivatives as Potential Anti-microbial Agents

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ABSTRACT

Coumarins are well known for their significant biological potential against several onsets. A series of novel 4-hydroxycoumarin substituted derivatives were synthesized and screened for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacterial strains. The zone inhibition was observed against 10 μ L against different for each compound. The outcomes of the study showed that out of 10 synthesized compounds, compound **4a**, **4b**, **4h**, and **4j** showed most significant inhibitory potential against different microbial strains. The zone of inhibition for compound **4a** and **4b** was found as 6.36 ± 0.162 , 5.60 ± 0.049 , 3.61 ± 0.176 , 5.64 ± 0.021 and 7.29 ± 0.339 , 5.53 ± 0.459 , 3.35 ± 0.226 , 5.55 ± 0.042 mm while compound **4h** and **4j** exhibited 7.10 ± 0.544 , 5.11 ± 0.183 , 3.95 ± 0.226 , 4.94 ± 0.494 and 6.46 ± 1.725 , 4.53 ± 0.261 , 3.83 ± 0.791 , 5.40 ± 0.049 mm, respectively.

Keywords: Coumarin, 4-aminocoumarin derivatives, Spectral analysis, Antibacterial activity.

INTRODUCTION

Inflammation is a complex phenomenon accompanied by interrelationships between humoral and cellular reactions induced by the diversity of inflammatory mediators such as toll like receptors (TLRs), interleukins (ILs), etc. The symptoms of the inflammation indicate many onsets within the body's system. Although it is still a challenging endeavors to cop inflammation and its biological neutralization. It is critical need for better-tolerated and more efficient drugs which evade the suffering volunteers from such complications.¹ Moreover, the recent past years envisaged exploring the medicinal

system concerning a better approach to treat many life-threatening infections caused by multi-drug resistant for *Gram-positive* (+ve) and *Gram-negative* (-ve) pathogenic bacteria.² Instead of antibiotic-resistant drugs, researchers are still engaged to generate the potential drug which not only resists the microorganism survivability but also obviates the infections which cause significant morbidity and mortality in a living organism. Although several clinical reports have been published concerning today's emergence of antibiotics resistance.^{3,4}

Coumarins or 2H-1-benzopyran-2-one are aromatic organic compounds that consist of a



large class of phenolic compounds that occurred naturally or were derived by fusion of benzene and α -pyrone rings.⁵ As per the scientific reports, more than 1300 coumarins have been acknowledged as secondary metabolites from natural resources.⁶ Coumarins and their derived products are potentially acting as an anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, etc.⁷ The coumarin molecule has been accompanied by its unique anti-edema and anti-inflammatory activities. Thus, coumarin derivatives could be potentially active in the treatment of all high protein edemas.¹ In addition, it is suggested to have a critical need for the development of new and novel coumarins derivatives against the upstream infection of microorganisms especially for *Gram-positive* and *Gram-negative* bacterias as coumarins are usually associated with low toxicity and have raised considerable interest because of their potential benefits on human health.⁸ Furthermore, it has been scientifically validated that a series of novel Schiff's base- accustomed coumarins compounds play a significant role against various diseases even acts as anti-inflammatory agents.

Taking all these facts into consideration, the present prospects of the study is associated to target base synthesis of several coumarin derivatives by using Schiff's base. In addition, all the synthesized compounds were evaluated for their antibacterial and anti-inflammatory activity using in-silico, *In-vitro*, and in-vivo approaches. The target of our study is mainly associated to replace the drugs causing antibacterial resistance and development of newly antibacterial agent which may be facilitated as an effective and economic antibacterial drug.

MATERIAL AND METHOD

Reagents, instruments and measurements

All reagents, solvents, and catalysts were of analytical grade and used directly. The melting point was determined in open glass capillary tubes and incorporated accordingly. The completion of reactions was monitored by TLC using silica gel-G coated glass plates and visualization was done by using iodine/UV lamp. IR spectra were recorded on Bruker alpha-T spectrophotometer using A.T.R. technique. ¹H NMR spectra were recorded on a Bruker Advance II 400 NMR spectrometer in CDCl₃ as the solvent and TMS as an internal standard.

Microbial strains such as *S. aureus* (MTCC No. 3161), *B. subtilis* (MTCC No. 441), *E. coli* (MTCC NO. 1687) and *P. aeruginosa* (MTCC NO. 424) were collected from the institutional lab.

Chemistry

The synthesis process was completed in four steps as shown in Fig. 1. Compound 1a was prepared via amination of 4-hydroxycoumarin with ammonium acetate. Further, this compound was treated with ethylchloroacetate in presence of KOH/ K₂CO₃ yielding N-alkylated product (2a). The reaction of this product with hydrazine hydrate yielded desired hydrazide compound (3a). Schiff base derivatives (4a-4j) were obtained by reaction of 3a with different aromatic aldehydes in presence of glacial acetic acid in alcohol. The process for the synthesis of coumarin derivatives involves four steps as follows.

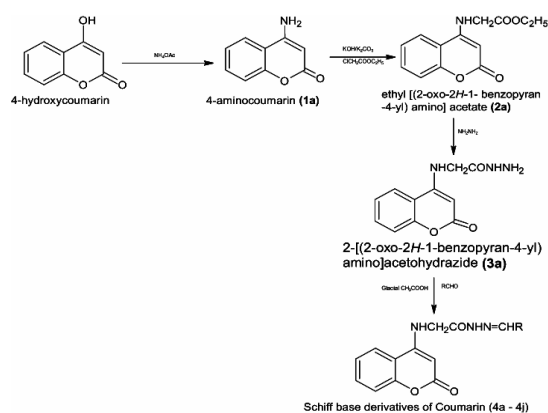


Fig. 1. Schematic representation of reaction process for the synthesis of coumarin derivatives

Synthesis of 4-aminocoumarin (1a)

During the process for the synthesis of 4-aminocoumarin (1a), a mixture of 4-hydroxycoumarin (0.01mol) and ammonium acetate (0.01mol) was stirred at 1600 for 3 h in DMF. After completion of the reaction, the content was cooled at room temperature and then poured into water and stirred for 10 minute. The completion of the reaction was checked by TLC. The solid was collected by filtration, washed, recrystallized from appropriate solvent and dried.

Synthesis of ethyl [(2-oxo-2H-1-benzopyran-4-yl) amino] acetate (2a)

4-aminocoumarin, 1a (0.01mol) was treated with ethylchloroacetate (0.01mol) in the presence of KOH/K₂CO₃ and solvent (70 mL). The solution was refluxed for about 3 hours. After that the solution

was cooled and poured to cold water, the precipitate was filtered off, washed with water, and dried to yield N-substituted compound **2a**.

Synthesis of 2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (**3a**)

Compound **2a** (0.01 mol) was reacted with hydrazine hydrate (0.01 mol) in presence of ethanol and refluxed for 3-4 hours. The reaction mixture was cooled and poured into cold water under vigorous stirring. The crude products obtained were filtered off, dried and recrystallized by using an appropriate solvent to yield desired compound **3a**.

General procedure for the synthesis of Schiff bases (**4a-4j**)

Compound **3a** was reacted with a different aromatic aldehyde in the presence of glacial acetic acid in alcohol and refluxed for 3-4 hours. After completion of the reaction (checked by TLC), the reaction mixture was cooled and poured into cold water with continuous stirring. The solid thus obtained was filtered, dried and recrystallized from appropriate solvent to yield corresponding Schiff bases (**4a-4j**). Further, the percentage yield and melting point of the entire synthesized compound were determined. The purity of the compounds was checked by TLC using appropriate solvent systems and the structures of all the derivatives were validated by mass spectroscopic IR, and ¹H-NMR data.⁹⁻¹²

The schematic representation of the process in chemistry involved in the synthesis of coumarin derivatives is summarised in Figure 1.

2-[(2-oxo-2H-1-benzopyran-4-yl)amino]-N'-(E)-phenylmethylidene]acetohydrazide (**4a**)

MS (ESI) m/z: 322.14 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3413.71, 3381.52 (=N, -NH), 1758.31 (C=C/C=N), 1698.03 (C=O), 1437.62, 1228.44 (C-O-C and C-H), 1158.11, 1037.32, 815 (aliphatic amines aromatic C-H vibrations). ¹H-NMR (CD₃OD, 500MHz): δ 10.164, 8.753, 8.121 (3H, 3s, 2NH, CH), 7.934 (1H, m, Ar, CH-5), 7.703, 7.389 (3H, m, Ar, CH-2, 6 & 7), 7.162 (3H, m, Ar, CH-3, 4, 5, benzylidene), 6.936 (2H, m, Ar, CH-6, 7), 4.472, 3.813 (3H, 2s, Ar, CH-3, CH₂).

N'-(E)-(4-chlorophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (**4b**)

MS (ESI) m/z: 356.08 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3531.05, 3419.72, 2912.95 cm^{-1} (=N, -NH,

CH₂), 1697.37 (C=O) 1438.28, 1228.44 (C-O-C and C-H), 1158.12, 1037.32 and 702.37 (aliphatic amines, aromatic C-H and C-Cl vibrations). ¹H-NMR (CD₃OD, 500MHz): δ 10.182, 8.953 and 8.321 (3H, 3s, 2NH, CH), 7.9742 (1H, m, Ar, CH-5), 7.751 and 7.412 (3H m, Ar, CH-2, 6 & 7), 7.184 and 6.927 (4H, m, Ar, CH-6, 8 & 3, 5), 4.292 and 3.733 (3H, 2s, Ar, CH-3, CH₂).

N'-[(E)-(3-chlorophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (**4c**)

MS (ESI) m/z: 356.29 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3447.96, 3385.34 and 1731.06 (=N, -NH and C=C/C=N), 1665.03 (C=O), 1445.23 and 1392.58 (C-O-C and mono-substituted alkynes), 1107.23 and 725.03 (aliphatic amines and C-Cl vibrations). ¹H-NMR (CD₃OD, 500MHz): δ 10.173, 8.413 and 8.281 (3H, 3s, 2NH, CH), 8.142 (1H, m, Ar, CH-5), 6.891 and 6.732 represents for seven protons (7H, 2m, Ar, CH-6, 7, 8 of coumarin ring and CH-2, 3, 4, 6 of benzylidene ring), 4.912 and 3.973 (3H, 2s, Ar, CH-3, CH₂).

N'-[(E)-(3-nitrophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (**4d**)

MS (ESI) m/z: 337.01 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3521.88, 3458.01 and 1701.43 (=N, -NH and C=C/C=N) 1659.72 (C=O), 1479.05 (C-O-C), 1112.95, 1069.28 and 768.25 (aromatic and aliphatic amines and C-H scissoring vibrations). ¹H-NMR (CD₃OD, 500MHz): δ 10.113, 8.793 and 8.423 (3H, 3s, 2NH, CH), 8.232 (1H, m, Ar, CH-5), 7.613 (1H, m, Ar, CH-7), 7.191 and 6.692 (6H, 2m, Ar, CH-6, 8 of coumarin ring and CH-2, 3, 4, 6 of benzylidene ring), 5.284, 4.752 and 3.973 (5H, 3s, NH₂, CH-3, CH₂).

N'-[(E)-(4-hydroxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (**4e**)

MS (ESI) m/z: 337.98 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3587.15, 3421.49, 3188.25 and 1754.59 (=N/-NH, -OH, -CH₂ C=C/C=N), 1665.21 (C=O), 1601.83, 1495.24, 1443.47, 1339.62 (diketones, C-O-C, aromatic C-H/mono-substituted alkynes), 1062.91, and 735.38 (aromatic and aliphatic amines and scissoring vibrations). ¹H-NMR (CD₃OD, 500MHz): δ 10.113, 9.431, 8.769 and 8.459 (4H, 4s, 2NH, OH, CH), 8.213 (1H, m, Ar, CH-5), 7.729 (3H, 2m, J=7.12 Hz, Ar, CH-2, 6 and 7), δ 7.213 (2H, 1m, Ar, CH-6, 8), 6.711 (2H, d, J=1.84, Ar, CH-3, 5), 4.892 and 4.073 (3H, 2s, CH-3, CH₂).

***N'*-(*E*)-(3,4-dihydroxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (4f)**

MS (ESI) *m/z*: 353.24 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3585.17, 3412.94, 3179.27 and 1745.95 (=N/NH, -OH, $-\text{CH}_2\text{C}=\text{C}=\text{N}$), 1661.25 (C=O) 1603.81, 1495.42, 1443.47, 1339.67 (diketones, C-O-C, aromatic C-H and mono-substituted alkynes), 1062.19, 1012.78 and 735.38 (aromatic and aliphatic amines and scissoring vibrations). $^1\text{H-NMR}$ (CD₃OD, 500MHz): δ 10.113, 9.431, 8.769 and 8.459 (5H, 3s, 2NH, 2OH, CH), 8.231, (1H, m, Ar, CH-5), 7.782 (1H, m, Ar, CH-7), 7.313 (2H, m, Ar, CH-6, 8), 6.811 and 6.412 (3H, 2d, J=1.98, 6.97, Ar, CH-2, 3, 6 of dihydroxybenzylidene ring), δ 4.892 and 4.062 (3H, 2s, CH-3, CH₂).

***N'*-(*E*)-[4-(dimethylamino)phenyl]methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (4g)**

MS (ESI) *m/z*: 364.27 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3432.89, 3349.72, 3250.02, 2991.10 and 1724.11 cm^{-1} (=N/NH, $-\text{CH}_3/\text{CH}_2$, C=C/C=N), 1663.92 (C=O), 1619.05, 1467.72 and 1112.02 (diketones, C-O-C, aromatic and C-H stretching), 1053.29, 912.32 and 629.18 (aromatic and aliphatic amines and acetylenic vibrations). $^1\text{H-NMR}$ (CD₃OD, 500MHz): δ 10.113, 8.769 and 8.459 (3H, 3s, 2NH, CH), 8.225 (1H, m, Ar, CH-5), 7.631 (3H, m, Ar, CH-6, 7, 8; coumarin), 6.983, 6.427 (4H, 2m, Ar, CH-2, 3, 5, 6; benzylidene), 4.892, 3.762 and 3.056 (9H, 3s, Ar, CH-3, CH₂, 2CH₃).

***N'*-(*E*)-(3,4,5-trimethoxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (4h)**

MS (ESI) *m/z*: 412.36 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3476.58, 3409.95, 3276.82, 3052.47, and 1901.93 (=N/NH, $-\text{CH}_3$, C=N), 1681.46 (C=O), 1583.47, 1457.17 and 1349.83 (diketones, C-O-C, aromatic and mono-substitution), 1247.26 (carbonyls), 1093.44 and 897.14 (aliphatic amines and scissoring vibrations). $^1\text{H-NMR}$ (CD₃OD, 500MHz): δ 10.102, 8.769 and 8.459 (3H, 3s, 2NH, CH), 8.236 (1H, 2d, J=7.32 Hz, Ar, CH-5), 7.632 (1H, m, Ar, CH-7), 7.023 (2H, m, Ar, CH-6, 7) 6.318 (2H, s, Ar, CH-2, 6), 4.892, 3.762 3.502, and 3.253 (12H, 4s, Ar, CH-3, CH₂, 3CH₃).

***N'*-(*E*)-(3,4-dimethoxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide(4i)**

MS (ESI) *m/z*: 382.78 (M+1); (FTIR, ν_{\max} ,

cm^{-1}); 3479.02, 3388.74, 3239.98, 3057.12 and 1903.49 (=N/NH, $-\text{CH}_3/\text{CH}_2$, C=N), 1735.15 (C=O), 1607.7, 1547.83, 1512.42, 1482.97 and 1269.23 (C-O-C, aromatic C-H and mono-substitution/carbonyls), 1079.32, 981.07 and 907.35 (aromatic, aliphatic amines and scissoring vibrations). $^1\text{H-NMR}$ (CD₃OD, 500MHz): δ 10.102, 8.769 and 8.459 (3H, 3s, 2NH, CH), 8.236 (1H, m, Ar, CH-5), 7.622 (1H, m, Ar, CH-7), 7.323 (1H, d, J= 1.12, Ar, CH-2), 7.023 (2H, m, Ar, CH-6, 7), 6.632 and 6.286 (2H, 2d, J=1.87, 1.73, Ar, CH-5, 6), 4.892 and 3.573 (3H, 2s, Ar, CH-3, CH₂), 3.253 (6H, d, J=7.01, 2CH₃).

***N'*-(*E*)-(4-bromophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide (4j)**

MS (ESI) *m/z*: 401.11 (M+1); (FTIR, ν_{\max} , cm^{-1}); 3401.82, 3357.48, 3049.95 and 1752.29 (=N/NH, CH₂, C=C/C=N), 1653.54 (C=O), 1607.82, 1501.29, 1459.48, 1385.47 and 1209.25 (diketones, C-O-C, aromatic C-H and mono-substitution/carbonyls group), 1098.39, 1025.07, 907.59 and 785.48 (aromatic, aliphatic amines, C-Br and scissoring vibrations). $^1\text{H-NMR}$ (CD₃OD, 500MHz): δ 10.102, 8.769 and 8.459 (3H, 3s, 2NH, CH), 8.236 (1H, 2d, J=7.32 Hz, Ar, CH-5), 7.613 (5H, m, Ar, CH-7, 2, 3, 5, 6), 7.192 (2H, m, Ar, CH-6, 8), 4.892 and 4.073 (3H, 2s, CH-3, CH₂). The structures of synthesized compounds with the melting point, percentage yield, and TLC retardation factor are summarized in Table 1.

Antibacterial activity

In-vitro antibacterial activity of the screened coumarin derivatives compounds was performed through zone inhibition assay/well-diffusion assay against *S. aureus*, *B. subtilis* (Gram-positive) and *E. coli*, *P. aeureginosa* (Gram-negative) bacterial strain using the standard protocol with some modification.¹³ In brief, the bacterial inoculum was evenly spread over the surface of the agar Petri dishes plate using a sterile cotton swab. Thereafter, four holes of 5 mm diameter were made using a sterile tip. 10 μL of drug solution (1 mg/mL) was added for two wells and the next two wells were treated with 10 μL of autoclaved double-distilled water as a control treatment. Plates were incubated for 72 h under aerobic conditions and at 37°C temperature. The antimicrobial effect was determined by the clear zone in the agar, which was measured after the completion of treatment.

Table 1: Chemical structures/IUPAC name and physicochemical characters of synthesized compounds

Sr.No.	Structure	Name	m.p.(°C)	Yield%	R _f value
4a		2-[(2-oxo-2H-1-benzopyran-4-yl)amino]-N'[(E)-phenylmethylidene]acetohydrazide	219-221	73.29	0.85
4b		N'[(E)-(4-chlorophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	198-200	65.17	0.74
4c		N'[(E)-(3-chlorophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	235-237	75.86	0.6 5
4d		N'[(E)-(3-nitrophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	180-182	80.29	0.69
4e		N'[(E)-(4-hydroxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	220-222	75.44	0.77
4f		N'[(E)-(3,4-dihydroxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	197-199	63.19	0.41
4g		N'[(E)-[4-(dimethylamino)phenyl]methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	200-201	73.15	0.59
4h		N'[(E)-(3,4,5-trimethoxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	231-233	74.86	0.63
4i		N'[(E)-(3,4-dimethoxyphenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	185-187	78.19	0.72
4j		N'[(E)-(4-bromophenyl)methylidene]-2-[(2-oxo-2H-1-benzopyran-4-yl)amino]acetohydrazide	206-208	77.47	0.67

RESULTS AND DISCUSSION

Schiff bases are the ketone or aldehyde organic compounds which are widely used in synthesis of many organic compounds including coumarins. Although the classical synthesis mainly involves condensation of carbonyl compounds and used for highly electrophilic carbonyl and nucleophilic amine compounds.¹⁴ In our study, several coumarin derivatives were synthesized through Schiff base reaction. During the synthesis process, the reaction was characteristically monitored by thin-layer chromatography (TLC) to determine purity of compounds. The percentage yield and melting points

of each synthesized compound were determined successfully. The resulted outcomes of the study have been summarized in Table 1.

Antibacterial activity of compounds

Zone inhibition assay or agar disk-diffusion method is one of the most widely and economic methods used for many clinical microbiology laboratories for routine evaluation of antimicrobial testing. In this method, generally agar plates are inoculated with the testing bacterial strain and potency of the tested compound is evaluated by diffusing the test sample into the prepared disk. Furthermore, after a period of incubation, zone of

inhibition against the bacterial growth is evaluated, that reveals the potency of tested compound.¹⁵ In our study, *In-vitro* antibacterial activity of the screened coumarin derivatives compounds was performed through zone inhibition assay/well-diffusion assay against *S. aureus*, *B. subtilis* (*Gram-positive*) and *E. coli*, *P. aeureginosa* (*Gram-negative*) bacterial strain. The inhibitory action to bacterial strain was considered with the direct proportion of obtained zone observed in the agar plate. The resulted outcomes suggest that compounds 4a, 4b, 4h, and 4j showed potential inhibitory effects against *S. aureus* while compounds 4a, 4b, 4h, and 4i

showed potential inhibitory effects against *B. subtilis*. Moreover, 4a, 4b, 4g, 4h, 4i and 4j showed good potential inhibitory effects against the survivability of *E. coli* while compounds 4a, 4b, 4h, and 4j showed potential inhibitory effects against *P. aeureginosa* survivability. Ceftriaxone and erythromycin were used as a positive control to determine the correlative effect of synthesized compounds. Out of 10 compounds, compounds 4a, 4b, 4h, and 4j showed significant inhibitory potential even against all microbial strains. The average inhibitory effect by all the ten compounds and the standard drug has been summarized in Table 2.

Table 2: Antibacterial activity of synthesized compounds against *S. aureus*, *B. subtilis* and *E. coli*, *P. aeureginosa* using well diffusion assay or zone inhibition assay

Sr. No.	Compound name	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeureginosa</i>
1	4a	6.36 ± 0.162	5.60 ± 0.049	3.61 ± 0.176	5.64 ± 0.021
2	4b	7.29 ± 0.339	5.53 ± 0.459	3.35 ± 0.226	5.55 ± 0.042
3	4c	5.37 ± 0.368	4.25 ± 0.228	2.09 ± 0.266	4.28 ± 0.258
4	4d	4.28 ± 0.279	3.27 ± 0.238	3.01 ± 0.237	3.96 ± 0.245
5	4e	3.57 ± 0.115	3.01 ± 0.023	2.58 ± 0.138	2.99 ± 0.247
6	4f	3.98 ± 0.019	2.98 ± 0.038	2.95 ± 0.438	3.01 ± 0.543
7	4g	5.62 ± 0.862	3.55 ± 1.944	3.82 ± 0.014	5.92 ± 0.650
8	4h	7.10 ± 0.544	5.11 ± 0.183	3.95 ± 0.226	4.94 ± 0.494
9	4i	5.89 ± 0.098	4.85 ± 0.993	3.99 ± 0.286	4.27 ± 0.118
10	4j	6.46 ± 1.725	4.53 ± 0.261	3.83 ± 0.791	5.40 ± 0.049
11	Ceftriaxone	6.35 ± 0.216	5.37 ± 0.227	6.81 ± 0.286	6.02 ± 0.176
12	Erythromycin	6.29 ± 0.123	6.37 ± 0.851	6.69 ± 0.248	6.31 ± 0.284

Previous studies have been reported that coumarin and its derivatives are the potential candidates which show strong antibacterial activity. A study conducted by Singh *et al.*, 2015 reported that coumarin derivatives showed potent antimicrobial activities against seven of the nine microbial strains examined in the study. The inhibitory potential was expressed in form of MIC values ranging between 1.09 and 25 µg/mL and was the most active compound of the series.¹⁶ Furthermore, many reported studies strongly emphasized to have the strong antibacterial potential of coumarins.^{17,18} In a study conducted by Chattha *et al.*, evaluated antibacterial activity of coumarins -3-acetic acid derivatives and the reported that some of coumarins -3-acetic acid derivatives were found to have potential inhibitory effect against several *Gram-positive* and negative bacterial strains.¹⁹ Our study clearly demonstrate the potential antimicrobial compounds which would work against the drugs seems to have antibacterial resistant. Moreover, the study demonstrate an silent answer to the continual demand for new antibiotics for the unceasing emergence of antibiotic-resistant strains and the growing interest in the substitution of synthetic antibiotic.

CONCLUSION

In this study, ten coumarins 4-aminocoumarin derivatives were synthesized, characterized and evaluated for their antibacterial activity, successfully. The coumarins 4-aminocoumarin derivatives showed significant inhibitory potential against the *Gram-positive* and negative bacterial strains. Furthermore, compound 4a, 4b, 4h, and 4j showed significant inhibitory potential even against all microbial strains. These analogues are chemically tractable and hence provide ample prospects for further adaptation to obtain potent antimicrobial agents the antibacterial resistant drugs.

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Conflict of interest

The authors declare no conflict of interest

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