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Design, Synthesis and Evaluation of coumarin derivatives as antimicrobial activity

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ABSTRACT

Coumarin exhibit a wide range of activities like antimicrobial,antiviral,antifungal,anti-inflammatory activity, anticancer, antimalarial It is a group of organic compound which contain aromatic ring and a pyrone nucleus Coumarins name has been derived from 'Coumarou', the vernacular call of tonka bean (Dipteryx odorata Willd, Fabaceae), from which coumarin, it became remote in 1820. Coumarinor 2H-chromen-2-one is a fragrant organic chemical compound with formulation $C_9H_6O_2$. Its molecule can be defined as a benzene molecule Present study includes synthesis of various analogues of coumarin and their potential for anti-microbial activity.Present work emphasizes on Design and synthesis of coumarin derivatives, Characterization of synthesized coumarin derivatives, Evaluation of synthesized compounds for anti-microbial activity.

KEYWORD: *vernacular cell, pyrone, coumarin derivatives, dipteryx odorata, antimmicrobial*

INTRODUCTION

Coumarins name has been derived from 'Coumarou', the vernacular call of tonka bean (Dipteryx odorata Willd, Fabaceae), from which coumarin, it became remote in 1820. Coumarinor 2H-chromen-2-one is a fragrant organic chemical compound with formulation $C_9H_6O_2$. Its molecule can be defined as a benzene molecule, with two adjoining hydrogen atoms mostly replaced by using a lactone-like chain -(CH)=(CH)−(C=O)−O−, forming a 2nd 6-membered heterocycle that usually shares two carbons with the benzene ring. It may be located in the benzopyrone chemical category, and also as lactone. [1] Coumarin is a crystalline and colourless solid with a candy smell similar to the fragrance of vanilla and a bitter flavor.[2] It is present in number of plants, wherein it could also function as a chemical defense against the predators. Where it inhibits the synthesis of vitamin K, a similar compound is used in the prescriptions is warfarin drug as an anticoagulant, which inhibits blood clot formation, deep vein thrombosis, and pulmonary embolism There are four main coumarin sub-types: [5-7]

Simple coumarins, Furanocoumarins, Pyranocoumarins and Pyrone-substituted coumarin. The

Coumarins was first isolated in 1822 and then synthesized in 1868. Food and Drug Administration banned them in 1950s as they were suspected and categorised as category 1 hepatotoxin and carcinogen, this data was based on the test performed with animal. However, this could require a revision until the next animal records were received. Numbers of coumarin derivatives were supposed to possess tumoristatic, immune-stimulatory and anticoagulant activities.

MATERIAL AND METHOD

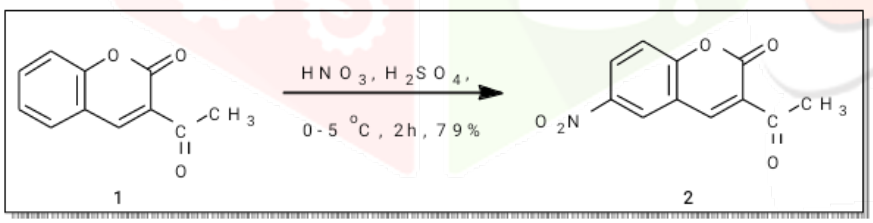
CHEMICALS AND INSTRUMENTS

The chemicals which are used in this are 2-oxo-2*H*-1-benzopyran-3-carboxylic acid, Sulfuric acid, Tetra hydro furan (THF), Nitric acid, Methanol, Diethyl ether, Dichloromethane, Tri ethylamine, N, N-dimethyl formamide, Hexanes, Palladium on Carbon (Pd/C), Hydrochloric Acid, Sodium Sulphate, Acetyl chloride, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5b]pyridinium3oxidhexafluorophosphate, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium (HATU)

The IR Spectra of compounds recorded. The 1H NMR of compounds is recorded and The evaluation of synthesized compounds has done by the Agar diffusion method. Agar diffusion method is of three types; Agar cup method, Paper disc method and Agar ditch method. In present work, Agar cup method was used. Tobramycin was used as reference drugs.

1 Series 1: 3-acetyl-6-(substituted amino)-2H-chromen-2-one derivatives

Step- 1

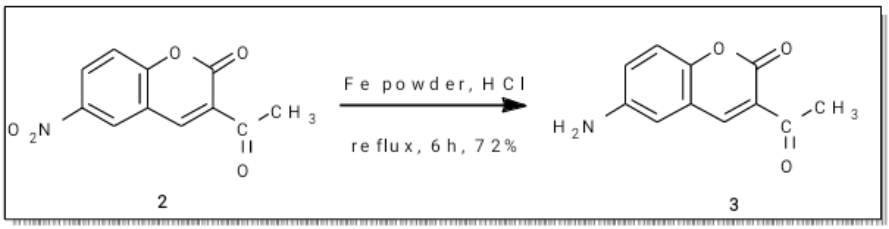


Synthesis of 3-acetyl-6-nitro-2H-chromen-2-one (2): A mixture of 3-acetyl coumarin **1** (0.188 gm, 1 mmole) and concentrated sulphuric acid (1.10 ml) was stirred at 0 °C for 15 minutes. Then a mixture of concentrated nitric acid (0.06 ml, d 1.4) and sulphuric acid (0.2 ml, 98%) was added. The temperature was kept at 0-5 °C during the period of addition, and the mixture was then continuously stirred for 2 hours at 5 °C. Then the mixture was poured into a conical flask contained 25 ml of an ice water. Solid precipitated out of the water layer, filtered and dried, then purified by silica gel column chromatography (petroleum ether/benzene 1:1 v/v) to isolate the compound 3-acetyl-6-nitro-2H-chromen-2-one **2** (yield 79%).

^{1}H NMR (400 MHz, DMSO-d_6) : δ 2.45 (3H, s), 7.44 (1H, dd, J = 7.9, 0.4 Hz), 8.51 (1H, dd, J = 7.9, 1.9 Hz), 8.78 (1H, s), 8.84 (1H, dd, J = 1.9, 0.4 Hz). MS (ESI) calcd for $C_{18}H_{13}NO_4$ $[M+1]^+$ 308.08, found m/z

308.10

Step-2

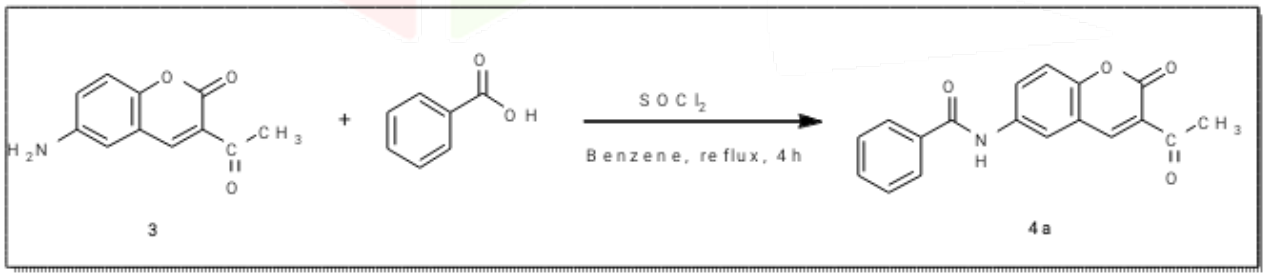


4.2 Synthesis of 3-acetyl-6-amino-2H-chromen-2-one (3): A mixture of compound **2** 3-acetyl-6-nitro-2H-chromen-2-one (0.233 g, 1 mmol 3.14 gm, 0.01 mole) with Iron powder (0.296 g), concentrated hydrochloric acid (1.11 ml) and ethanol (10 ml) was refluxed for 6 hours. Then cool the reaction mixture, the precipitate formed was filtered off, washed with a copious amount of water and dried. Crude product was purified by recrystallization with ethanol and compound 3-acetyl-6-amino-2H-chromen-2-one **3** formed yield (72 %).

^{1}H NMR: δ 2.44 (3H, s), 6.77 (1H, dd, *J* = 8.5, 1.7 Hz), 7.20 (1H, dd, *J* = 8.5, 0.5 Hz), 7.51 (1H, dd, *J* = 1.7, 0.5 Hz), 8.65 (1H, s). MS (ESI) calcd for $C_{11}H_9NO_3$ $[M+1]^+$ 226.04, found m/z 226.17

Step- 3 Synthesis of Coumarin derivatives through the various benzoic acids

Synthesis of Compound- 4a

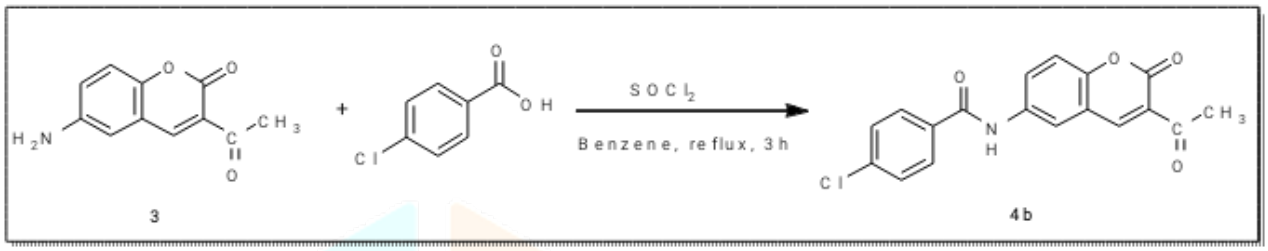


Synthesis of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)benzamide (4a): To a mixture of 3-acetyl-6-aminocoumarin (0.203 g, 1 mmol) and benzoic acid (0.122 g, 1 mmol) was added thionyl chloride (0.141 g, 1.2 mmol, 1.2 equiv) and benzene (1.5 ml) and refluxed for 2 hr. Then the mixture was poured into a conical flask contained 25 ml of an ice water. Solid precipitated out of the water layer, filtered and dissolved in 1.25 ml of 2N sodium hydroxide solution and filtered again. The solution was acidified with 2N hydrochloric acid, filtered by Buchner funnel and washed with a copious amount of cold water and concentrated in vacuo to give the crude product which was purified by re-crystallization from

ethanol and gave compound *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)benzamide **(4a)** (yield 65%).

3314(N-H str), 3082 (C-H str), 3056(aromatic C-H str), 1744 (lactone ester), 1693 (CONH amide), 1680 (C=O str), 1537 (C=C aromatic), 1424 (C-O-C str). ^{1}H NMR: δ 2.51 (3H, s), 8.32-8.49 (2H, 7.46 (dd, *J* = 8.6, 1.5 Hz), 7.43 (dd, *J* = 8.6, 0.5 Hz)), 7.53-7.63 (3H, m), 7.58 (dd, *J* = 8.5, 7.5 Hz)), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 8.00 (2H, dd, *J* = 8.5, 1.9 Hz), 8.71 (1H, s). MS (ESI) calcd for $C_{18}H_{13}NO_4$ $[M+Na]^+$ 330.07, found m/z 330.18.

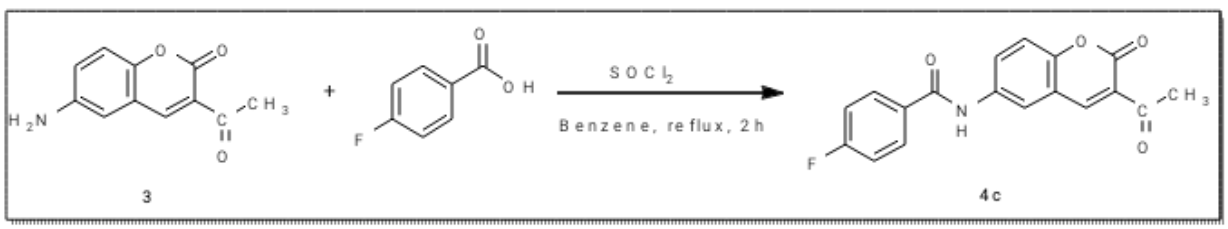
Synthesis of Compound- 4b



Synthesis of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-chlorobenzamide (4b): To a mixture of 3-acetyl-6-aminocoumarin (0.203 g, 1 mmol) and 4-chlorobenzoic acid (0.156 g, 1 mmol) was added thionyl chloride (0.141 g, 1.2 mmol, 1.2 equiv) and benzene (1.5 ml) and refluxed for 2 hr. Then the mixture was poured into a conical flask contained 25 ml of an ice water. Solid precipitated out of the water layer, filtered and dissolved in 1.25 ml of 2N sodium hydroxide solution and filtered again. The solution was acidified with 2N hydrochloric acid, filtered by Buchner funnel and washed with a copious amount of cold water and concentrated in vacuo to give the crude product which was purified by re-crystallization from ethanol and gave compound *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-chlorobenzamide **(4b)** (yield 68%).

3320(N-H str), 3050 (C-H str), 3067(aromatic C-H str), 1766 (lactone ester), 1681 (CONH amide), 1686 (C=O str), 1528 (C=C aromatic), 1421 (C-O-C str), 800 (C-Cl). ^{1}H NMR: δ 2.54 (3H, s), 8.63 (2H, s), 7.43 (dd, *J* = 8.6, 0.5 Hz)), 7.53 (2H, dd, *J* = 8.7, 1.4 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 7.75 (2H, dd, *J* = 8.7, 1.8 Hz), 8.71 (1H, s). MS (ESI) calcd for $C_{18}H_{12}ClNO_4$ $[M+Na]^+$ 364.03, found m/z 364.19

Synthesis of Compound- 4c

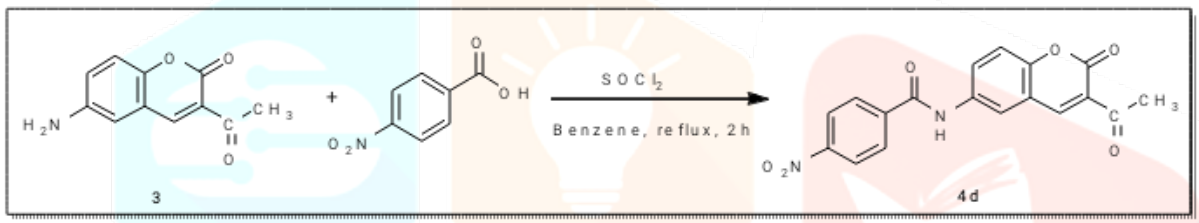


Synthesis of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-fluorobenzamide (4c): To a mixture of 3-acetyl-6-aminocoumarin (0.203 g, 1 mmol) and 4-fluorobenzoic acid (0.140 g, 1 mmol) was added thionyl chloride (0.141 g, 1.2 mmol, 1.2 equiv) and benzene (1.5 ml) and refluxed for 2 hr. Then the mixture was poured into a conical flask contained 25 ml of an ice water. Solid precipitated out of the water

layer, filtered and dissolved in 1.25 ml of 2N sodium hydroxide solution and filtered again. The solution was acidified with 2N hydrochloric acid, filtered by Buchner funnel and washed with a copious amount of cold water and concentrated in vacuo to give the crude product which was purified by re-crystallization from ethanol and gave compound *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-fluorobenzamide **(4c)** (yield 71%).

3330(N-H str), 3056 (C-H str), 3047(aromatic C-H str), 1746 (lactone ester), 1689 (CONH amide), 1680 (C=O str), 1538 (C=C aromatic), 1429 (C-O-C str), 1155 (C-F). ^{1}H NMR: δ 2.16 (3H, s), 7.67-7.70 (4H, 7.46 (dd, *J* = 8.6, 1.5 Hz), 7.47 (dd, *J* = 8.7, 1.1 Hz), 7.43 (dd, *J* = 8.6, 0.5 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 7.90 (2H, dd, *J* = 8.7, 1.8 Hz), 8.71 (1H, s). MS (ESI) calcd for $C_{18}H_{12}FNO_4$ $[M+Na]^+$ 348.06, found m/z 348.22

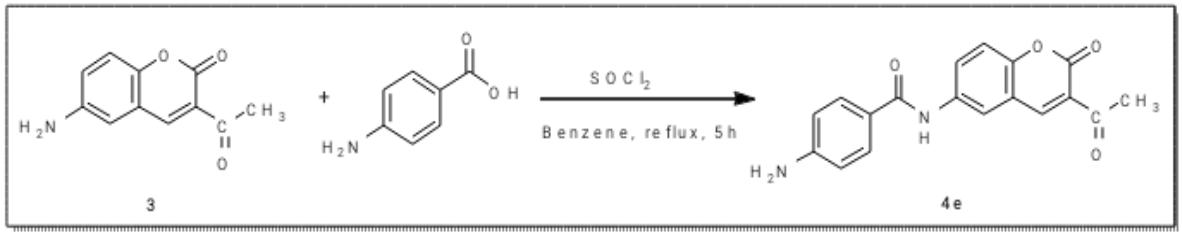
Synthesis of Compound- 4d



Synthesis of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-nitrobenzamide (4d): To a mixture of 3-acetyl-6-aminocoumarin (0.203 g, 1 mmol) and 4-nitrobenzoic acid (0.167 g, 1 mmol) was added thionyl chloride (0.141 g, 1.2 mmol, 1.2 equiv) and benzene (1.5 ml) and refluxed for 2 hr. Then the mixture was poured into a conical flask contained 25 ml of an ice water. Solid precipitated out of the water layer, filtered and dissolved in 1.25 ml of 2N sodium hydroxide solution and filtered again. The solution was acidified with 2N hydrochloric acid, filtered by Buchner funnel and washed with a copious amount of cold water and concentrated in vacuo to give the crude product which was purified by re-crystallization from ethanol and gave compound *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-nitrobenzamide **(4d)** (yield 66%).

3342 (N-H str), 3060 (C-H str), 3052 (aromatic C-H str), 1748 (lactone ester), 1692 (CONH amide), 1689 (C=O str), 1530 (C=C aromatic), 1516 (NO_2), 1436 (C-O-C str), 1355 (NO_2). ^{1}H NMR: δ 2.46 (3H, s), 8.30-8.19 (2H, 7.46 (dd, *J* = 8.6, 1.5 Hz), 7.43 (dd, *J* = 8.6, 0.5 Hz)), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 7.89 (2H, dd, *J* = 8.6, 1.4 Hz), 8.14 (2H, dd, *J* = 8.6, 1.8 Hz), 8.71 (1H, s). MS (ESI) calcd for $C_{18}H_{12}N_2O_6$ $[M+Na]^+$ 375.05, found m/z 375.23

Synthesis of Compound- 4e

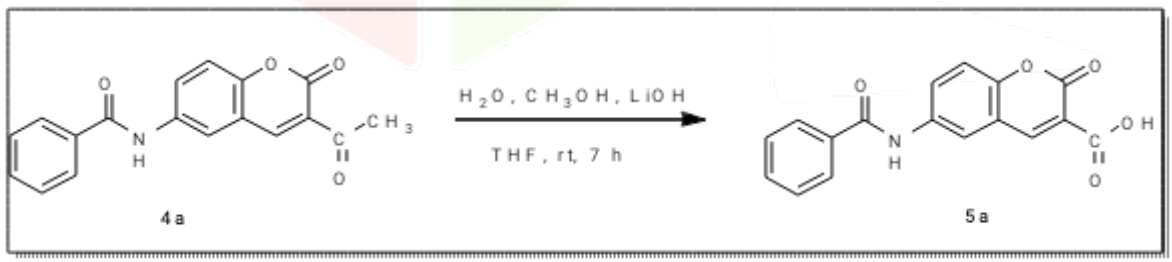


Synthesis of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-aminobenzamide (4e): To a mixture of 3-acetyl-6-aminocoumarin (0.203 g, 1 mmol) and 4-aminobenzoic acid (0.137 g, 1 mmol) was added thionyl chloride (0.141 g, 1.2 mmol, 1.2 equiv) and benzene (1.5 ml) and refluxed for 2 hr. Then the mixture was poured into a conical flask contained 25 ml of an ice water. Solid precipitated out of the water layer, filtered and dissolved in 1.25 ml of 2N sodium hydroxide solution and filtered again. The solution was acidified with 2N hydrochloric acid, filtered by Buchner funnel and washed with a copious amount of cold water and concentrated in vacuo to give the crude product which was purified by re-crystallization from ethanol and gave compound *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-aminobenzamide **(4e)** (yield 64%).

3352 (N-H str), 3053 (C-H str), 3050 (aromatic C-H str), 1745 (lactone ester), 1686 (CONH amide), 1678 (C=O str), 1605 (NH_2 bending), 1537 (C=C aromatic), 1436 (C-O-C str). ^{1}H NMR: δ 2.96 (3H, s), 8.87 (2H, dd, *J* = 8.6, 1.1 Hz), 7.40-7.48 (2H, dd, *J* = 8.6, 1.5 Hz), 7.42 (dd, *J* = 8.6, 0.5 Hz), 7.55 (2H, dd, *J* = 8.6, 1.7 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 8.71 (1H, s). MS (ESI) calcd for $C_{18}H_{14}N_2O_4$ $[M+Na]^+$ 345.08, found m/z 345.30

Series 2: Hydrolysis of Compounds 4 derivatives

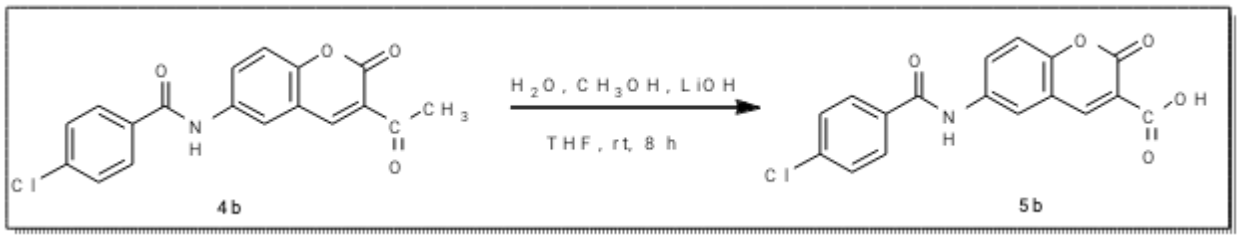
Synthesis of Compound- 5a



Synthesis of 6-benzamido-2-oxo-2H-chromene-3-carboxylic acid (5a): The previously synthesized molecule of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)benzamide **(4a)** (0.307 g , 1 mmol) was mixed with mixtures of water (1.5 ml), methanol (1.5 ml) and THF (1.5 ml) then added LiOH monohydrate (1.13 g, 3.69 mmol) and reaction mixture were stirred at RT for 6 hr. When all starting materials were consumed reaction mass acidified with 3M HCl solution until 2.0 pH was reached and extracted with ethyl acetate. Organic layer was dried over sodium sulfate and distilled out under vaccum to get crude product which was purified by silica gel chromatography (hexane: ethylacetate 8:2 v/v) and gave compound 6-benzamido-2-oxo-2H-chromene-3-carboxylic acid **(5a)** (yield 58%).

3314(N-H str), 3056(aromatic C-H str), 2841(carboxylic acid OH str), 1744 (lactone ester), 1693 (CONH amide), 1537 (C=C aromatic), 1424 (C-O-C str).^{1}H NMR: δ 7.40-7.48 (2H, 8.10(dd, *J* = 8.6, 1.5 Hz), 7.42 (dd, *J* = 8.6, 0.5 Hz), 7.53-7.63 (3H, m), 7.58 (dd, *J* = 8.5, 7.5 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 8.00 (2H, dd, *J* = 8.5, 1.9 Hz), 8.81 (1H, s). MS (ESI) calcd for $C_{17}H_{11}NO_5$ $[M+1]^+$ 310.07, found m/z 310.18

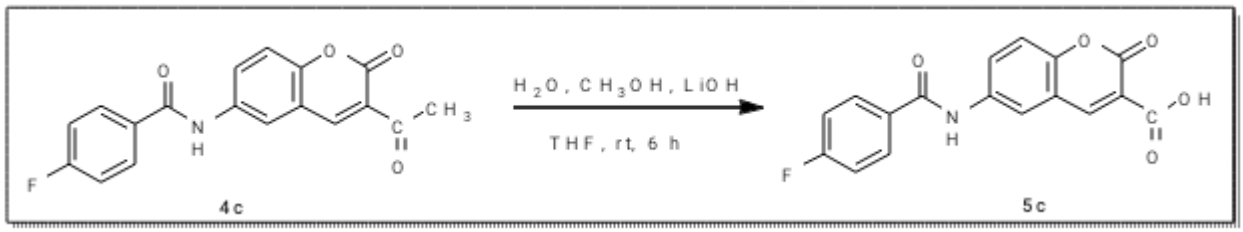
Synthesis of Compound- 5b



Synthesis of 6-(4-chlorobenzamido)-2-oxo-2H-chromene-3-carboxylic acid (5b): The previously synthesized molecule of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-chlorobenzamide **(4b)** (0.341 g, 1 mmol) was mixed with mixtures of water (1.5 ml), methanol (1.5 ml) and THF (1.5 ml) then added LiOH monohydrate (1.26 g, 3.69 mmol) and reaction mixture were stirred at rt for 6 hr. When all starting materials were consumed reaction mass acidified with 3M HCl solution until 2.0 pH was reached and extracted with ethyl acetate. Organic layer was dried over sodium sulfate and distilled out under vacuum to get crude product which was purified by silica gel chromatography (hexane: ethylacetate 8:2 v/v) and gave compound 6-(4-chlorobenzamido)-2-oxo-2H-chromene-3-carboxylic acid **(5b)** (yield 56 %)

3391 (Amide-NH), 3055 (Aromatic-CH), 2596 (Carboxylic acid-OH) 1760 (lactone ester), 1669 (Amide CO), 1618(Amide-NH), 1573 (Aromatic C-C), 800 (C-Cl). ^{1}H NMR: δ 8.40-8.48 (2H, 7.45 (dd, *J* = 8.6, 1.5 Hz), 7.42 (dd, *J* = 8.6, 0.5 Hz), 7.53 (2H, dd, *J* = 8.7, 1.4 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 7.71 (2H, dd, *J* = 8.7, 1.8 Hz), 8.81 (1H, s). MS (ESI) calcd for wes$C_{17}H_{10}ClNO_5$ $[M+1]^+$ 344.03, found m/z 344.22

Synthesis of Compound- 5c

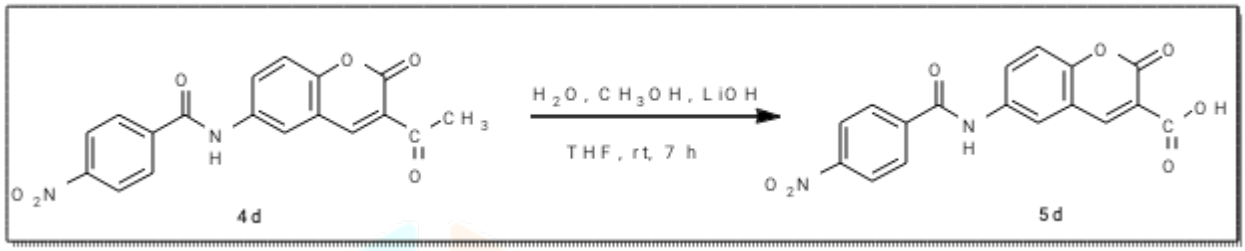


Synthesis of 6-(4-fluorobenzamido)-2-oxo-2H-chromene-3-carboxylic acid (5c): The previously synthesized molecule of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-fluorobenzamide **(4c)** (0.325 g ,1 mmol) was mixed with mixtures of water (1.5 ml), methanol (1.5 ml) and THF (1.5 ml) then added LiOH monohydrate (1.20 g, 3.69 mmol) and reaction mixture were stirred at room temp for 6 hr. When all starting materials were consumed reaction mass acidified with 3M HCl solution until 2.0 pH was reached and extracted with ethyl acetate. Organic layer was dried over sodium sulfate and distilled out under vacuum to get crude product which was purified by silica gel chromatography

(hexane:ethylacetate 8:2 v/v) and gave compound 6-(4-fluorobenzamido)-2-oxo-2H-chromene-3-carboxylic acid **(5c)** (yield 61%).

3387 (Amide-NH), 3050 (Aromatic-CH), 2591 (Carboxylic acid-OH) 1765 (lactone ester), 1672 (Amide CO), 1622(Amide-NH), 1567 (Aromatic C-C), 1155 (C-F). ^{1}H NMR: δ 8.10-7.90 (4H, 7.45 (dd, *J* = 8.6, 1.5 Hz), 7.47 (dd, *J* = 8.7, 1.1 Hz), 7.42 (dd, *J* = 8.6, 0.5 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 7.90 (2H, dd, *J* = 8.7, 1.8 Hz), 8.81 (1H, s). MS (ESI) calcd for $C_{17}H_{10}FNO_5$ $[M+1]^+$ 328.06, found m/z 328.40

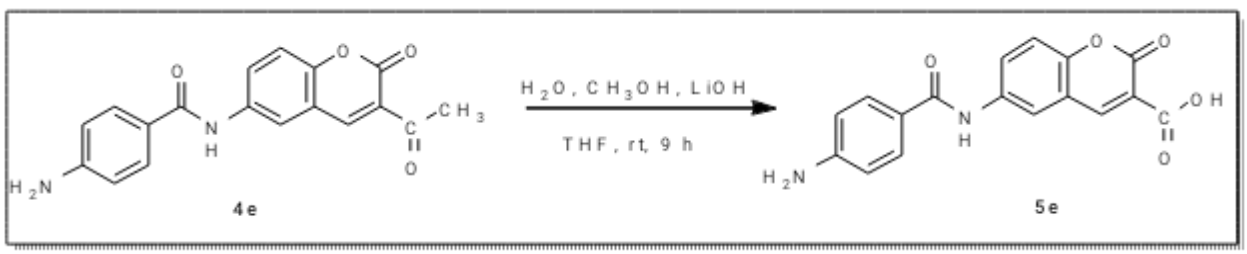
Synthesis of Compound- 5d



Synthesis of 6-(4-nitrobenzamido)-2-oxo-2H-chromene-3-carboxylic acid (5d): The previously synthesized molecule of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-nitrobenzamide **(4d)** (0.352 g ,1 mmol) was mixed with mixtures of water (1.5 ml), methanol (1.5 ml) and THF (1.5 ml) then added LiOH monohydrate (1.30 g, 3.69 mmol) and reaction mixture were stirred at room temp for 6 hr. When all starting materials were consumed reaction mass acidified with 3M HCl solution until 2.0 pH was reached and extracted with ethyl acetate. Organic layer was dried over sodium sulfate and distilled out under vacuum to get crude product which was purified by silica gel chromatography (hexane: ethylacetate 8:2 v/v) and gave compound 6-(4-nitrobenzamido)-2-oxo-2H-chromene-3-carboxylic acid **(5d)** (yield- 57%).

3390(Amide-NH), 3058 (Aromatic-CH), 2591 (Carboxylic acid-OH) 1756 (lactone ester), 1678 (Amide CO), 1631 (Amide-NH), 1561 (Aromatic C-C), 1520 (NO_2), 1361 (NO_2). ^{1}H NMR: δ 7.40-7.49 (2H, dd, *J* = 8.6, 1.4 Hz), 7.43 (dd, *J* = 8.6, 0.5 Hz), 7.64 (1H, dd, *J* = 1.4, 0.5 Hz), 7.89 (2H, dd, *J* = 8.6, 1.4 Hz), 8.14 (2H, dd, *J* = 8.6, 1.8 Hz), 8.81 (1H, s). MS (ESI) calcd for $C_{17}H_{10}N_2O_7$ $[M+1]^+$ 355.05, found m/z 355.34

Synthesis of Compound- 5e



Synthesis of 6-(4-aminobenzamido)-2-oxo-2H-chromene-3-carboxylic acid (5e): The previously synthesized molecule of *N*-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-aminobenzamide **(4e)** (0.322 g ,1 mmol) was mixed with mixtures of water (1.5 ml), methanol (1.5 ml) and THF (1.5 ml) then added LiOH monohydrate (1.19 g,

3.69 mmol) and reaction mixture were stirred at room temp for 6 hr. When all starting materials were consumed reaction mass acidified with 3M HCl solution until 2.0 pH was reached and extracted with ethyl acetate. Organic layer was dried over sodium sulfate and distilled out under vaccume to get crude product which was purified by silica gel chromatography (hexane: ethylacetate 8:2 v/v) and gave compound 6-(4-aminobenzamido)-2-oxo-2H-chromene-3-carboxylic acid **(5e)** (yield-55%).

3384(Amide-NH), 3048 (Aromatic-CH), 2590 (Carboxylic acid-OH) 1751 (lactone ester), 1668 (Amide CO), 1633 (Amide-NH), 1610 (NH_2 bending), 1560 (Aromatic C-C). ^{1}H NMR: δ 8.39-8.18 (2H, dd, *J* = 8.6, 1.5 Hz), 7.42 (dd, *J* = 8.6, 0.5 Hz), 7.55 (2H, dd, *J* = 8.6, 1.7 Hz), 7.64 (1H, dd, *J* = 1.5, 0.5 Hz), 8.83 (1H, s). MS (ESI) calcd for $C_{17}H_{12}N_2O_5$ $[M+1]^+$ 325.08, found m/z 325.32.

Antimicrobial evaluation

Following bacteria were selected for *in-vitro* study.

1). Gram-positive: *Bacillus subtilis* ATCC 14579 and *Staphylococcus aureus* ATCC 25923

2). Gram-negative: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922

Culture medium preparation:

Culture medium is prepared by using Peptone (1.0 gm), NaCl (0.5 gm), Meat extracts (0.3 gm) and Agar (2.0 gm). All ingredient except agar was dissolved in distilled water (100 ml) and pH of medium was adjusted to 7.6 then after agar was added to the medium and dispensed in 25 ml quantity in different test tubes. The test tubes were plugged by cotton-wool and sterilized at 121.5°C for 20 minutes.

Antibacterial susceptibility testing: Nutrient agar broth was inoculated with 0.5 ml of culture medium which was 24 hour old and mixed well by shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set for some time and then the “cups” was made by punching into the agar surface with a sterile cup borer. Into this “cups” test solution was added by previously sterilized micropipette. The plates were noted. The antibacterial activity of all synthesized compounds was studied at 1000 ppm concentration *in-vitro* and calculated in percentage (%) of inhibition, shown in Table 1 and 2.

The percentage inhibition for bacteria was calculated after five days using the given formula.

$$\text{Percentage of inhibition} = 100\left(1 - \frac{Y}{X}\right)$$

Where

X = Area of colony in control plate.

Y = Area of colony in test plate.

The percentage (%) data are shown in ***table 6 and 7*** *as follows;*

- *(+) = Small clearing zone (<50%), slightly active,*
- *(++) = Medium clearing zone (51-55%), moderately active,*
- *(+++) = Large clearing zone (56-60%), highly active and,*
- *(++++) = Very large clearing zone (>60%), very high activity.*

Table 6: Antibacterial activity of compounds **(4a-e)**

Compounds (4a-e)	% Zone of Inhibition			
	Gram +Ve		Gram –Ve	
	Bacillus Subtilis	*Staphylococcus Aureus*	*Pseudomonas aeruginosa*	*E-Coli*
4a	+	++	+	++
4b	++	+	++	++
4c	+++	+	+++	++
4d	++	+++	++	+++
4e	++	++	++	++
Tobramycin	++++	+++	++++	++++

Table 7: Antibacterial activity of compounds **(5a-e)**

Compounds (5a-e)	% Zone of Inhibition			
	Gram +Ve		Gram –Ve	
	Bacillus subtilis	*Staphylococcus Aureus*	*Pseudomonas aeruginosa*	*E-Coli*
5a	++	++	++	++
5b	+	+	++	++
5c	++	++++	+++	++++
5d	++++	+++	++++	+++
5e	++	+++	++	++
Tobramycin	++++	+++	++++	++++

The compounds tested for antibacterial activity show percentage (%) of zone of inhibition of bacterial growth of Gram-positive and Gram-negative bacteria. For antibacterial study, 4 compounds of **Series 1** (**4a-e**) and 4 compounds of **Series 2** (**5a-e**) were tested for bacterial strain Gram-positive bacterial strains *B.subtilis* and *S.aureus* and Gram-negative bacterial strains *P. aeruginosa*, and *E.Coli*.

In **Series 1** Compound **4d** were found promising activity against bacterial strain *S.aureus* and *E.Coli* In **Series 2,** compound **5d** were found promising activity against bacterial strain *B.subtilis* and *P. aeruginosa*

CONCLUSION:

The compound 6-(4-nitrobenzamido)-2-oxo-2H-chromene-3-carboxylic acid, N-(3-acetyl-2-oxo-2H-chromen-6-yl)-4-nitrobenzamide shows maximum activity as antimicrobial and further research needed

to establish the molecular mechanism.

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