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Original Article

SYNTHESIS AND CHARACTERIZATION OF NEW COUMARIN DERIVATIVES CONTAINING VARIOUS MOIETIES WITH ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Objective: The purpose of this research is to evaluate the antibacterial activity of different moieties (Schiff bases, chalcones, hydrazones and hydrazinyl thiazole) derivatives, inserted at carbon 8 of 7-hydroxy-4-methyl coumarin, using *in vitro,* serial broth dilution method.

Methods: A series of new coumarin derivatives, including (Schiff bases, chalcones, hydrazones and hydrazinyl thiazole), were prepared from 7-hydroxy-4-methyl coumarin, by insertion of the formyl group, at carbon number 8 using Duff reaction. The structure of the new synthesized derivatives elucidated and confirmed utilizing the corresponding analytical and spectroscopic data; including FT-IR, ^{13}C-NMR, and mass spectroscopy. All new coumarin derivatives have been screened for their preliminary antibacterial activity, by serial broth dilution method against two **G**ram-positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus hemolyticus*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*).

Results: All the synthesized compounds have been found to exhibit considerable antibacterial activity *in vitro*. Among all the derivatives, compound (5a), showed the highest rate of inhibition, against (*Escherichia coli*), while compound (6a), showed the greatest anti-bacterial activity against (*Staphylococcus hemolyticus*), each with minimum inhibitory concentration of (25µg/ml), and the highest MIC of 200 µg/ml for compound 2, against. *Klebsiella pneumonia*e.

Conclusion: Our results displayed a substantial preliminary antibacterial activity of the new coumarin moieties, especially some hydrazones and chalcones at C8 of the coumarin nucleus, against Gram-positive and a Gram-negative bacteria with distinguished MIC.

Keywords: Coumarin**,** Duff reaction**,** Chalcones, Hydrazones, *Schiff* base**,** Antibacterial activity.

INTRODUCTION

Coumarin itself is a natural product found in some plant sources, including the sweet clover and Tonka bean. Some coumarin derivatives have been found to be largely distributed in the plant kingdom [1]. It has an aromatic odor often flatteringly associated with the sweet fragrance of freshly mown hay; therefore, it was used as a flavoring agent [2]. The coumarins are of great interest due to their biological properties [3]. In actual, their physiological, bacteriostatic, and anti-tumor activity, makes these interesting compounds attractive for further backbone derivatization and screening as novel therapeutic agents [4]. Coumarin and its derivatives have been found to possess antimicrobial [5], antiviral [6], anti-inflammatory [7], antioxidant [8], anti-cancer [9], anticoagulant and enzyme inhibition activities [10].

Hence in the light of wide applications of coumarin derivatives, it was planned to synthesize new series of coumarin derivatives at C8 by using Duff reaction and preliminary screen for their antibacterial activity.

MATERIALS AND METHODS

The melting points were determined, by open capillary tubes on a Stuart/SMP3 melting point apparatus version 5.0 and were used uncorrected. The IR spectra were recorded, on a Perkin-Elmer FT-IR spectrometer using KBr disc. ^{13}C-NMR spectra, were recorded on a Bruker FT-NMR spectrophotometer-500 MHz, in SAIF (sophisticated analytical instrument facility), a research center in India/Chennai, in DMSO-d_6 and $CDCl_3$ solvent, using TMS, (tetra methyl silane) as an internal standard and the values are expressed in ppm (part per million). The mass spectra were recorded using Agilent Technology (HP), GC/MS model 5973 network mass selective detector. The purity of the synthesized compounds and the progress of the reaction was determined by Thin Layer Chromatography on aluminum silica gel 60 F_{254} (Merck) detected by UV (ultraviolet) light (254 nm). All the chemicals purchased were of analytical grade and were used without further purification unless otherwise stated.

Synthesis of 7-hydroxy-4-methyl coumarin 1 [11]

A solution of (0.01 mole, 1.1 g) resorcinol and (0.01 mole, 1.3 ml) ethyl acetoacetate was added drop wise over a period of 30 min, with continuous stirring to (10 ml) of concentrated sulfuric acid in an ice bath so that the temperature of the mixture did not rise above 10 ˚C. The reaction mixture was kept at room temperature for 3 hrs, and then poured with vigorous stirring into a mixture of ice and water. The precipitate was filtered off and washed with water, then after drying, recrystallized using ethanol to get the pure product.

Creamy colored needles; yield: 78%; m. p. 181-183 ˚C; IR (KBr, cm^{-1}): 3155(υ OH), 1789 (υ C=O), 1599 (υ C=C).

Synthesis of 8-formyl-7-hydroxy-4-methyl coumarin 2 [12]

A mixture of 7-hydroxy-4-methyl-coumarin (0.025 moles,5.1 g) and Hexamethylenetetramine (0.07 moles,9.8 g) in glacial acetic acid (40 ml) was heated at 85-90 C˚ on a water bath for 7 hrs The hexamine adduct so formed was hydrolyzed with 20% HCl (75 ml), and the mixture was heated for another 30 min. After cooling, the reaction mixture was extracted with diethyl ether (50 ml) twice, and the ether layer was evaporated by using a rotary evaporator. The yellow colored crystals obtained which was recrystallized from ethanol.

Pale yellow colored crystals; yield: 22%; m. p. 177-179 ˚C; IR (KBr, cm^{-1}): 3437 (υ OH), 1745 (υ C=O, lactone), 1644 (υ C=O, aldehyde), 1594 (υ C=C); ^{13}C-NMR ($CDCl_3$, δ ppm): 193.4 (C=O aldehyde), 165.3 (C=O lactone), 159.2, 156.1, 152.7, 132.9, 114.3, 112.1, 111.9, 108.7 (C $_{\text{aromatic and alkene}}$),18.9 ($CH_3$); MS *m/z* (%): 204 (96) [M]$^+$, 176 (84), 175 (91), 147 (68), 143 (11),130 (17).

Synthesis of aromatic amine Schiff bases 3a-c [13]

To a solution of (0.01 mole) compound (2) in (25 ml) absolute ethanol, an appropriate aromatic amine, (0.01 mole) of aniline and (0.005 mole) of 4-amino benzoic acid and 4-Nitro aniline), was added with continuous stirring, and then was refluxed for a period

of 30 min, the precipitate formed was filtered, dried and recrystallized from ethanol.

Aniline schiff base 3a

light orange colored precipitate; yield: 91%; m. p. 168-170 ˚C; IR (KBr, cm^{-1}): 3450 (υ OH), 3080(υ C-H, aromatic), 1736 (υ C=O, lactone), 1618 (υ C=N), 1577 (υ C=C); ^{13}C-NMR ($CDCl_3$, δ ppm): 166.8 (C=O lactone), 160.3 (C=N), 156.4, 154.5, 153.3, 146.4, 129.5, 129.3, 127.7, 121.3, 115.1, 110.9,, 106.8 ($C_{aromatic\ and\ alkene}$), 18.9 ($CH_3$); MS *m/z* (%): 280 (89) [M+1], 202 (43).

4-amino benzoic acid Schiff base 3b

Orange colored precipitate; yield: 89%; m. p. 241-243˚C; IR (KBr, cm^{-1}): 3420 (υ OH),3075 (υ C-H aromatic),1723 (υ C=O, lactone), 1700 (υ C=O, carboxylic acid), 1616 (υ C=N), 1578 (υ C=C); MS *m/z* (%): 323 (65) $[M]^+$, 295 (52), 278 (17), 202 (11), 175 (21).

4-Nitro aniline Schiff base 3c

Intense orange colored precipitate; yield: 90%; m. p. 264-266 ˚C; IR (KBr, cm^{-1}): 3460 (υ OH), 3090(υ C-H aromatic),1744 (υ C=O, lactone), 1615 (υ C=N), 1570 (υ C=C); ^{13}C-NMR ($CDCl_3$, δ ppm): 165.4 (C=O lactone), 165.3 (C=N), 159.8, 154.6, 153.1, 152.9, 146.5, 132.9, 126.3, 125.2, 122.2, 114.5, 114.3, 113.3, 112.1 ($C_{aromatic\ and\ alkene}$), 18.9 ($CH_3$); MS *m/z* (%): 324 (78) $[M]^+$, 275 (11), 254 (34), 215 (61).

Synthesis of amino acid Schiff bases 4a-c [14]

To a homogeneous solution of (0.01 mole) amino acid,(glycine, alanine and phenylalanine) and (0.01 mole,0.4g) sodium hydroxide in (20 ml) absolute ethanol; (0.01 mole, 2.04 g) of compound (2) which was also dissolved in absolute ethanol, was added drop by drop with continuous stirring. After 2-3 min. of the addition, 20% of the mixture was evaporated, and (1 ml) of acetic acid was added, the mixture was left at room temperature for 2-3 hrs and the precipitate was collected by filtration, washed with cold ethanol, dried and recrystallized from methanol.

Glycine Schiff base 4a

Faint yellow colored precipitate; yield: 72%; m. p. 207-209 ˚C (decomposed); IR (KBr, cm^{-1}): 3421 (υ OH), 3070 (υ C-H aromatic), 1724 (υ C=O, lactone), 1650 (υ C=O, carboxylic acid), 1582 (υ C=N), 1531 (υ C=C); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.8 (C=O carboxylic acid), 172.4 (C=O lactone), 162.8 (C=N), 159.5, 157.2, 154.9, 132.1, 120.6, 106.3, 106.2, 102.7 ($C_{aromatic\ and\ alkene}$), 53.5 ($CH_2$), 18.1 ($CH_3$)); MS *m/z* (%): 262 (82) [M+1], 216 (78).

Alanine Schiff base 4b

Yellow colored precipitate; yield: 67%; m. p. 149-151 ˚C; IR (KBr, cm^{-1}): 3430 (υ OH), 1712 (υ C=O, lactone), 1642 (υ C=O, carboxylic acid), 1581 (υ C=N), 1520 (υ C=C); ^{13}C-NMR (DMSO-d_6, δ ppm): 178.8 (C=O carboxylic acid), 175.9 (C=O lactone), 163 (C=N), 157.8, 157.2, 154.9, 132.1, 120.6, 106.3, 106.3, 102.8 ($C_{aromatic\ and\ alkene}$), 50.5 (CH), 18.2 and 16.1 (CH_3); MS *m/z* (%): 276 (91) [M+1], 230 (22).

Phenyl alanine Schiff base 4c

Intense yellow colored precipitate; yield: 53%; m. p. 231-233 ˚C (decomposed); IR (KBr, cm^{-1}): 3434 (υ OH), 3080 (υ C-H aromatic), 1716 (υ C=O, lactone), 1630 (υ C=O, carboxylic acid), 1583 (υ C=N), 1503 (υ C=C).

Synthesis of chalcones 5a-c [15]

A mixture of compound (2), (0.01 mole, 2.04 g) and an appropriate aromatic ketones: [(0.02 mole), acetophenone, 2-hydroxy acetophenone, and (0.01 mole) dehydro acetic acid] was dissolved in (25 ml) absolute ethanol and placed in a 100 ml round-bottom flask equipped with a magnetic stirrer in an ice bath (0-10 ˚C). Then sodium hydroxide solution (10 ml of 10 %) was added dropwise to the reaction mixture with vigorous stirring for 30 min. until the solution became turbid. The reaction temperature was maintained between 20-25 ˚C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hrs the reaction mixture was left to stand for overnight in the refrigerator. The precipitate obtained was filtered, washed with cold water, and recrystallized from ethanol.

Acetophenone chalcone 5a

Greenish yellow colored precipitate; yield: 70%; m. p. 170-172 ˚C; IR (KBr, cm^{-1}): 3394 (υ OH), 3080 (υ C-H aromatic) 1719 (υ C=O, lactone), 1684 (υ C=O, α,β unsaturated ketone), 1640 (υ C=C alkene), 1584 (υ C=C aromatic).

2-Hydroxy acetophenone chalcone 5b

Greenish yellow colored precipitate; yield: 64%; m. p. 187-189 ˚C; IR (KBr, cm^{-1}): 3420 (υ OH), 1719(υ C=O, lactone), 1684 (υ C=O, α,β unsaturated ketone), 1640 (υ C=C alkene), 1585 (υ C=C aromatic); ^{13}C-NMR (DMSO-d_6, δ ppm): 190.7 (C=O α,β unsaturated ketone), 178.2 (C=O lactone), 164.3, 157, 155.9, 131.4, 121.6, 111.5, 106.4, 105.3 ($C_{aromatic\ and\ alkene}$), 18.3 ($CH_3$); MS *m/z* (%): 322 (91) $[M]^+$, 201 (82), 175 (56).

Dehydroacetic acid chalcone 5c

Greenish yellow colored precipitate; yield: 36%; m. p. 207-209 ˚C; IR (KBr, cm^{-1}): 3393 (υ OH), 1716 (υ C=O, lactone), 1685 (υ C=O, α,β unsaturated ketone), 1640 (υ C=C alkene), 1585 (υ C=C aromatic); ^{13}C-NMR (DMSO-d_6, δ ppm): 190.9 (C=O α,β unsaturated ketone), 178.3 (C=O lactone), 165, 164.4, 157.2, 156.1 ($C_{aromatic}$), 131.6 (C=C $_{alkene}$), 121.6, 111.6, 106.5, 105.4 ($C_{aromatic\ and\ alkene}$), 37.8 ($CH_3$), 18.3 ($CH_3$); MS *m/z* (%): 354 (82) $[M]^+$, 201 (95), 175 (43).

Synthesis of hydrazone derivatives 6a-c [16, 17]

Compound (2), (0.01 mole) and (0.01 mole) of hydrazine derivatives: (hydrazine hydrate 99%, 2,4-dinitrophenyl hydrazine, and thiosemicarbazide) was refluxed in a round bottom flask for 1hr in the presence of absolute ethanol as a solvent, the obtained precipitate was collected by filtration, dried and recrystallized from ethanol.

8-(hydrazonomethyl)-7-hydroxy-4-methyl coumarin 6a

Creamy colored precipitate; yield: 91%; m. p. 273-275 ˚C; IR (KBr, cm^{-1}): 3381 (υ OH), 3295 and 3218 (υ NH_2), 1695 (υ C=O, lactone), 1605 (υ C=N), 1579 (υ C=C aromatic).

8-((2-(2,4-dinitrophenyl) hydrazono) methyl)-7-hydroxy-4-methyl coumarin 6b

Intense orange colored precipitate; yield: 88%; m. p. 287-295 ˚C (decomposed.); IR (KBr, cm^{-1}): 3420 (υ OH), 3231 (υ N-H), 1736 (υ C=O, lactone), 1607 (υ C=N), 1570 (υ C=C aromatic); MS *m/z* (%): 384 (94) $[M]^+$, 367 (52), 323 (40), 202 (88), 188 (19), 175 (44).

2-((7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl) methylene) hydrazinecarbothioamide 6c

Faint yellow colored precipitate; yield: 93%; m. p. 279-285˚C (decomposed); IR (KBr, cm^{-1}): 3400 (υ OH), 3390 and 3283 (υ NH_2), 3185 (υ N-H), 1727 (υ C=O, lactone), 1599 (υ C=N), 1465 (υ C=C aromatic); ^{13}C-NMR ((DMSO-d_6, δ ppm): 178.3 (C=S), 164.1 (C=O lactone), 160 (C=N), 159.7, 159.2, 154.1, 153.9, 133.7, 121.7, 113.6, 112.5($C_{aromatic\ and\ alkene}$), 111.2, 55.5 (C-N), 18.8 (CH_3); MS *m/z* (%): 277 (88) $[M]^+$, 202 (76), 188 (57), 175 (70).

Synthesis of 2-(2-((7-hydroxy-4-methyl-2-oxo-2H-chromen-8yl) methylene) hydrazinyl) thiazol-4 (5H)-one 7 [18, 19]

A mixture of compounds (6c), (0.01 mole) and chloroacetic acid (0.01 mole) in glacial acetic acid (30 ml) containing anhydrous sodium acetate (0.04 mole) was refluxed for 17 hrs. (Monitored by TLC). The reaction mixture was cooled, and the obtained precipitate was filtered off, dried and recrystallized from ethanol.

Pale yellow colored precipitate; yield: 83%; m. p. 321-330 (dec.) ˚C; IR (KBr, cm^{-1}): 3446 (υ OH), 3300 (υ N-H), 1731 (υ C=O, lactone), 1687 (υ C=O, amide), 1624 (υ C=N), 1555 (υ C=C aromatic); ^{13}C-NMR (DMSO-d_6, δ ppm): 174.2 (C=O amide), 166.6 (C=O lactone), 161.9 (C=N), 159.8 ($C_{aromatic}$ attached to OH group), 159.5 (C=N), 154.2, 153.4, 129.4, 116.6, 113.7,, 112.4 ($C_{aromatic\ and\ alkene}$), 34.1 (C-S), 18.7 ($CH_3$); MS *m/z* (%): 317 (70) $[M]^+$, 202 (84), 175 (34), 160 (51).

Antibacterial activity

The new coumarin derivatives were screened for their preliminary antibacterial activity by using macro-broth dilution method**.**

A series of 6 to 9 broth tubes containing 1 ml Muller Hinton autoclaved and cooled then inoculated with (50 µl) bacterial inoculum of bacterial suspension at McFarland turbidity of 0.5. Then a defined concentration of coumarin derivatives is added to each broth of a serial doubling concentration, except the negative control in which the solvent (DMSO) was added and a positive control with bacterial inoculum without adding any derivatives. After those broths are incubated aerobically at 37 ˚C for 24 hrs, the minimum inhibitory concentration (MIC) is determined by visual observation, as the lowest concentration that inhibits bacterial growth and appears as a clear broth tube.

RESULTS AND DISCUSSION

Chemistry

The coumarin derivatives, (Scheme-1 and Scheme-2), have been synthesized from 7-hydroxy-4-methyl coumarin, by inserting the formyl group, (COH) at the carbon 8 by Duff reaction, or (hexamine aromatic formulation) to afford 8-formyl-7-hydroxy-4-methyl coumarin, (2) which is characterized by an IR band of C=O stretching of aldehyde displayed at 1644 cm^{-1}, and ^{13}C-NMR peak, at 193.4 ppm.

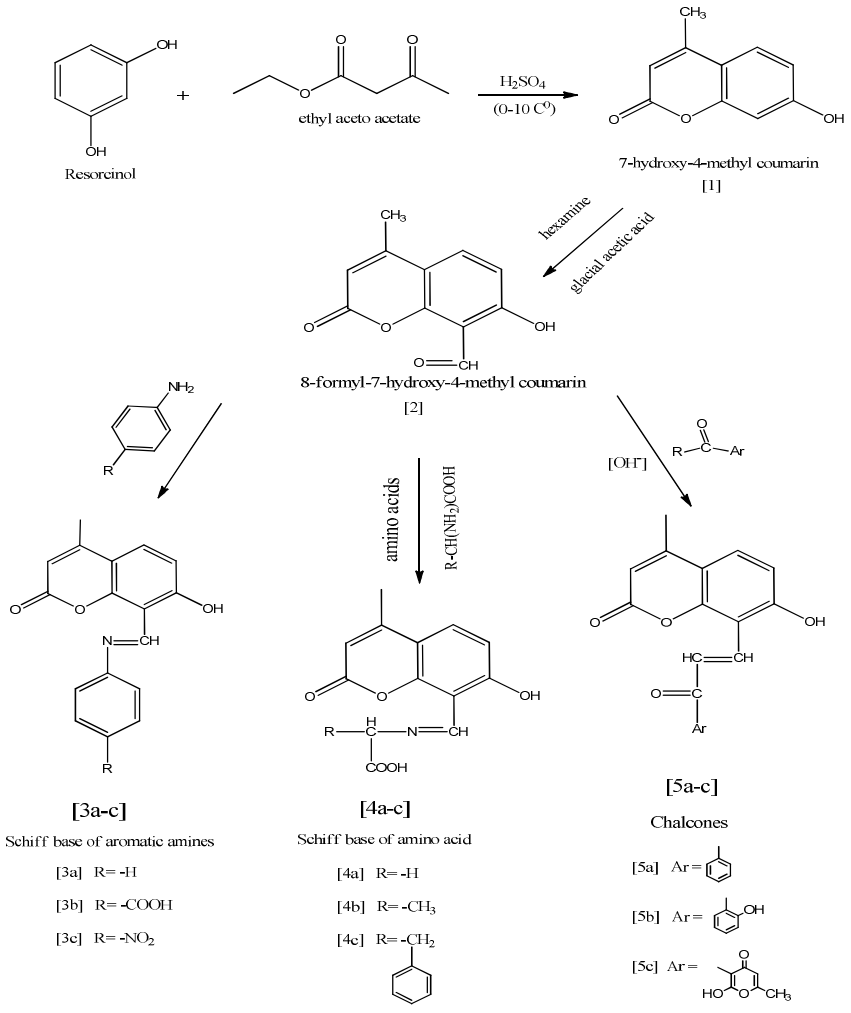
The Schiff bases of aromatic amines (3a-c) have been synthesized in a good yield by condensation of compound (2) with different aromatic amines in absolute ethanol, as a solvent. The C=N stretching band of Schiff bases displayed in the IR region (1615-1618 cm^{-1}) and at (160-165 ppm) in ^{13}C-NMR. The amino acid Schiff bases [4a-c] were prepared by reaction of a compound (2) with different amino acids in a basic medium, using alcoholic sodium hydroxide solution. The C=N stretching of amino acid Schiff bases has been characterized in the IR region (1619-1623 cm^{-1}) and ^{13}C-NMR analysis displayed at (162-163 ppm).

The chalcone derivatives (5a-c) were synthesized, by aldol condensation reaction; in which the compound (2) was reacted with different aromatic ketones at a temperature not exceeding 25 ˚C using 10% sodium hydroxide solution.

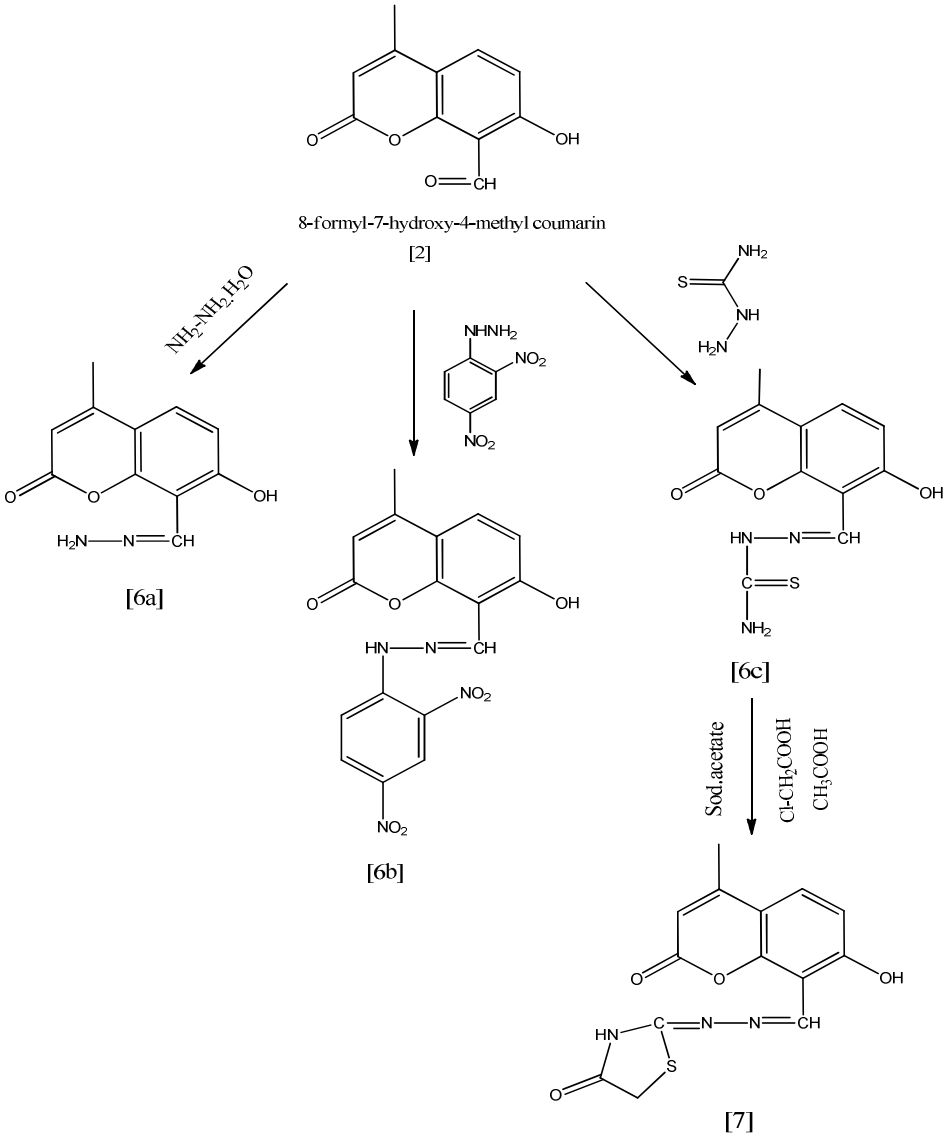
The characteristic bands of C=O stretching of α, β unsaturated ketone appeared IR in the range of (1684-1685 cm^{-1}) with reduced frequency due to the conjugation. While in ^{13}C-NMR the C=O of α, β unsaturated ketone displayed in the region of (190 ppm).

Hydrazone derivatives of coumarin (6a-c) were prepared by refluxing compound (2) with different hydrazines in absolute ethanol as a solvent. The C=N stretching of hydrazone derivatives has been distinguished by IR spectroscopy in the range of (1599-1607 cm^{-1}) and at (160-161 ppm) in ^{13}C-NMR.

Compound (7) which is a hydrazinyl thiazole derivative of coumarin was prepared by reaction of compound (6c) with chloroacetic acid in glacial acetic acid as a solvent, using anhydrous sodium acetate. It is characterized by the following IR bands of C=O stretching of lactone at 1731 cm^{-1}, C=O stretching of amide at 1687 cm^{-1}, and C=N stretching at 1624 cm^{-1}. ^{13}C-NMR showed 174.2 ppm (C=O amide), 166.6 ppm (C=O lactone), and 161.9 ppm (C=N), and the mass spectroscopy displayed a peak at m/z: 317 [M].



Scheme 1: Synthesis of coumarin derivatives (1-5c)



Scheme 2: Synthesis of coumarin derivatives (6a-7)

Antibacterial activity

In this study, the tube dilution technique is performed for preliminary screening the anti-bacterial activity of new coumarin derivatives, against two Gram-positive (G^{+ve}) bacteria, *Staphylococcus epidermidis,* and *Staphylococcus hemolyticus*, and two Gram-negative (G^{-ve}) bacteria, *Escherichia coli,* and *Klebsiella pneumoniae* to determine their MIC *in vitro.* Results are listed in table 1.

Table 1: It shows the antibacterial activity (Mean MIC values in µg/ml) of coumarin derivatives

Compound No.	*Staphylococcus epidermidis* (G^{+ve}) µg/ml	*Staphylococcus hemolyticus* (G^{+ve}) µg/ml	*Escherichia coli* (G^{-ve}) µg/ml	*Klebsiella pneumoniae* (G^{-ve}) µg/ml
2	100	100	150	200
3a	100	75	100	150
3b	150	100	100	150
3c	100	75	150	150
4a	75	100	100	150
4b	50	100	100	150
4c	50	75	75	150
5a	75	75	25	100
5b	50	50	75	150
5c	100	100	100	150
6a	50	25	50	150
6b	75	75	100	100
6c	100	100	100	150
7	50	50	100	100

C-, Negative control, solvent (DMSO),-indicates (no inhibition) and C+, a positive control indicates a broth without adding any coumarin derivative.

From the results illustrated in Table.1, it is clear that all the synthesized coumarin derivatives have displayed antibacterial activity against both Gram-positive and Gram-negative bacteria *in vitro*, the MIC ranges between 25-200 µg/ml, in which they varies according to the derivatives and bacteria. The lowest concentration that demonstrated no visible growth was taken as an end point, known the minimum inhibitory concentration (MIC).

The lowest MIC is 25 µg/ml for both compounds (5a) against *Escherichia coli* and 6a against *Staphylococcus hemolyticus*, and the highest MIC of 200 µg/ml for compound (2), against *Klebsiella pneumonia*.

Also compound (7) exhibited moderate antibacterial activity towards *Staphylococcus epidermidis* and *Staphylococcus hemolyticus*, with (MIC of 50 µg/ml).

It's worth to mention that the previous report [13] indicated that some of the Schiff bases of triazole derivatives, inserted at C8 of the coumarin nucleus, demonstrated a remarkable antifungal property when compared towards the antibacterial activity, using a cup plate method.

Also Sudhir S Sawant *et al.* [20] demonstrated an enhanced antimicrobial activity of some complexes of Co (II), Ni (II), Cu (II) and Zn (II) with Schiff bases, using a tube dilution technique, which was attributed to the complex formation action with all metal ions.

Our results demonstrated that the new chalcones (bearing a phenyl group), have a promising potent antibacterial activity against Gram-negative bacteria in addition to the considerable antibacterial activity exhibited by the hydrazone, and the cyclized hydrazinyl thiazole derivatives, against Gram–positive bacteria.

Efforts are under progress in the evaluation of these derivatives *in vivo* studies, especially their analgesic and anti-inflammatory properties and the results will be published later.

CONCLUSION

In this study, some new coumarin derivatives have been synthesized from 7-hydroxy-4-methyl coumarin, by applying Duff reaction to insert the formyl group at carbon 8 of coumarin nucleus. The synthesized compounds have been screened for their preliminary antibacterial activities, by determining their minimum inhibitory concentration (MIC), by serial broth dilution method, in which they demonstrated considerable antibacterial activities, against both Gram-positive and Gram-negative bacteria, with varying ranges of MIC.

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CONFLICT OF INTERESTS

Declared None

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