ORIGINAL ARTICLE

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Synthesis and biological evaluation some new of 7-hydroxy-4-methylcoumarin derivative

Aniruddh Dev Singh[1*], Sushil Kumar[2], Sarika Saini[3], Harsh Prabha[4]

[1]Department of Pharmaceutical Sciences, Glocal School of Pharmacy, Glocal University, Saharanpur, Uttar Pradesh, India, [2]Department of Pharmaceutical Sciences, IFTM University, Moradabad, Uttar Pradesh, India, [3]Department of Pharmaceutical Sciences, Smt. Tarawati Institute of Bio-Medical and Allied Sciences, Roorkee, Uttarakhand, India, [4]Department of Agriculture, Doon College of Education, Sunderpur, Saharanpur

ABSTRACT

7-hydroxy-4-methylcoumarin derivatives were synthesized. The title compounds (M1-M5) were obtained by the reaction of 7-hydroxy-4-methylcoumarin with ethyl chloroacetate in the presence of potassium carbonate in acetone afforded ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetate, which react with hydrazine hydrate in ethanol afforded 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide, on further reaction with various acetophenones in the presence of glacial acetic acid in absolute alcohol gave 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-substituted acetohydrazides (M1-M5). The structures of target compounds (M1-M5) were established on the basis of infrared and ^{1}H-nuclear magnetic resonance spectral analysis. Target compounds (M1-M5) were screened for their antimicrobial activity and showing significant antimicrobial activity as compared to standard drug.

Key words: 7-hydroxy-4-methylcoumarin, acetophenones, schiff base, acetohydrazides, antimicrobial activity

INTRODUCTION

Coumarin

Coumarin is a fragrant chemical compound in the benzopyrone chemical class, found in many *plants*, notably in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), sweet woodruff (*Galium odoratum*), mullein (*Verbascum* spp.), sweet grass (*Hierochloe odorata*), cassia cinnamon (*Cinnamomum aromaticum*), melilot (*Melilotus* ssp.), *Panicum clandestinum* or "Deers Tongue", and sweet clover (*Fabaceae* spp.).[1] Coumarins have been synthesized by several routes including Pechmann,[2] Perkin,[3] Knoevenagel synthesis, and biological evaluation some new of 7-hydroxy-4-methylcoumarin derivatives,[4] Reformatsky,[5] and Wittig[6] reactions. Coumarins also exhibit anticoagulant activity and some coumarin drugs are widely used as anticoagulants - warfarin and acenocoumarol.[7-10] These investigations have revealed their potentials as versatile biodynamic agent for example 3-heteroaryl substituted coumarins and benzocoumarins of potential interest as pharmaceutical and photochromic dyes.[11,12] Similarly, aromatic chalcones and heteroaromatic chalcones synthesized from 3-acetylcoumarin with aromatic and heteroaromatic aldehyde exhibit high potency as antibacterial agent.[13]

Schiff Bases

Schiff base (or azomethine), named after Hugo Schiff, is a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group but not hydrogen. Schiff bases are of general formula $R^1R^2N=R^3$.

Where, R^3 is an aryl or alkyl group that makes the Schiff base a stable imine.

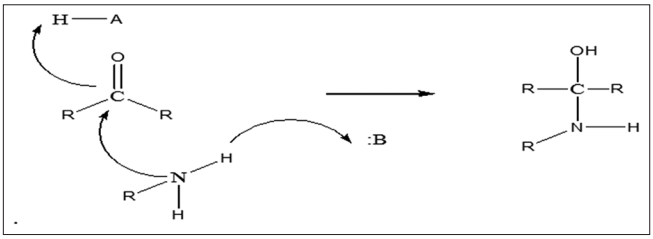
Schiff base can be synthesized from an aromatic amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by a dehydration to generate an imine.[14]

In recent years, there has been an increased interest in the chemistry of Schiff bases because of their biological significances. Various Schiff bases have been reported to possess significant antimicrobial, analgesic, and anti-inflammatory activity.

Mechanism of Schiff Base

The electrophilic carbon atoms of aldehydes and ketones can be targets of nucleophilic attack by amines. The end result of this reaction is a compound in which the C=O double bond is replaced by a C=N double bond. This type of compound is known as an imine or Schiff base.

Mechanistically, the formation of an imine involves two steps. First, the amine nitrogen acts as a nucleophile, attacking the carbonyl carbon. This is closely analogous to hemiacetal and hemiketal formation.



Address for correspondence:
Aniruddh Dev Singh, Glocal School of Pharmacy, Glocal University, Saharanpur, Uttar Pradesh, India. E-mail: anirudh@theglocaluniversity.in

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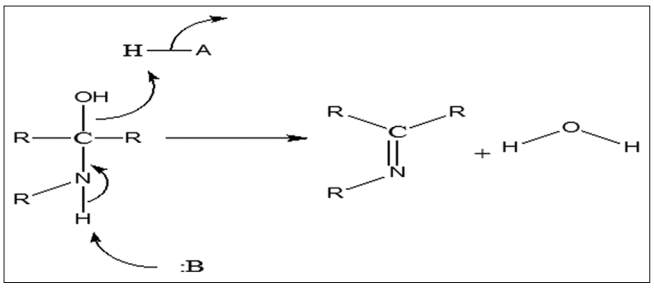
Based on your knowledge of the mechanism of acetal and ketal formation, you might expect that the next step would be attack by a second amine to form a compound with a carbon bound to two amine groups – the nitrogen version of a ketal. Instead, what happens next is that the nitrogen is deprotonated, and the electrons from this N-H bond "push" the oxygen off of the carbon, leaving us with a C=N double bond (an imine), and a displaced water molecule.



The conversion of an imine back to an aldehyde or ketone is a hydrolysis, and mechanistically is simply the reverse of imine formation.[15]

OBJECTIVE

Literature review revel that 7-hydroxy-4-methylcoumarin derivatives are safer and more potent antimicrobial compounds. In view of these observations, we have decided to synthesize some new 7-hydroxy-4-methylcoumarin derivatives and evaluate for their antimicrobial activity.

PLAN OF WORK

Chemical Studies

Scheme of synthesis

Step I: Synthesis of ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate.

Step II: Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide.

Step III: Synthesis of various 7-hydroxy-4-methylcoumarin derivatives.

a. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-phenylethylidene) acetohydrazide (M-1).
b. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-bromophenyl)ethylidene) acetohydrazide (M-2).
c. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)ethylidene) acetohydrazide (M-3).
d. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene) acetohydrazide (M-4).
e. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-hydroxyphenyl)ethylidene) acetohydrazide (M-5).

Physicochemical Studies

Confirmation of the structures of the compounds by

a. Physical constants.
b. Thin-layer chromatography (TLC).
c. Rotational and vibrational spectra (infrared [IR] spectroscopy).
d. Nuclear magnetic resonance (NMR) spectra (NMR spectroscopy).

Biological Studies

Antimicrobial activity.

MATERIALS AND METHODS

- Reagents and solvents: Most of the solvents used were of L.R. grade and purified before the use in different reactions. Chemicals used were obtained from Central Drug House Pvt., Ltd. (CDH) Table 1. All the glass wares used were of borosil glass work Ltd. As presented in Table 2.
- Equipment: The melting points of the synthesized compounds as well as intermediates were determined by open capillary methods and were uncorrected.
- The purity of the compounds was routinely checked by TLC using iodine vapors for detection of the spots.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the sovent}}$$

- The IR spectra of the synthesized compounds were recorded in potassium bromide discs and on Fourier transform-IR (FT-IR) spectrophotometer Model-8300 of Shimadzu, at Punjab University, Chandigarh.
- The NMR spectra of the synthesized compounds were recorded in dimethyl sulfoxide (DMSO) using Model AV-300 Broke Jeol at 300 MHz spectrophotometer, at Punjab University, Chandigarh.

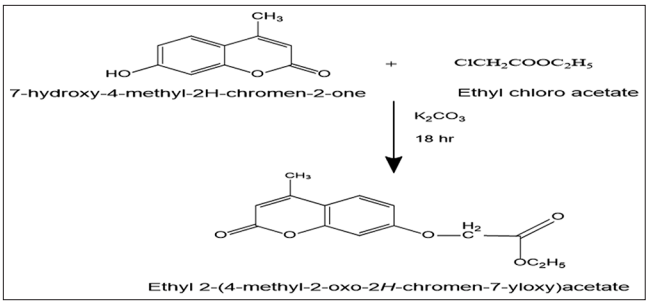
Materials

Chemical Studies

1. Synthesis of ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate.
2. Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide.
3. Synthesis of various 7-hydroxy-4-methylcoumarin derivatives.

a. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-phenylethylidene) acetohydrazide (M-1).
b. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-bromophenyl)ethylidene) acetohydrazide (M-2).
c. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)ethylidene) acetohydrazide (M-3).
d. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene) acetohydrazide (M-4).
e. 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-hydroxyphenyl)ethylidene) acetohydrazide (M-5).

Synthesis of ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate



Procedure

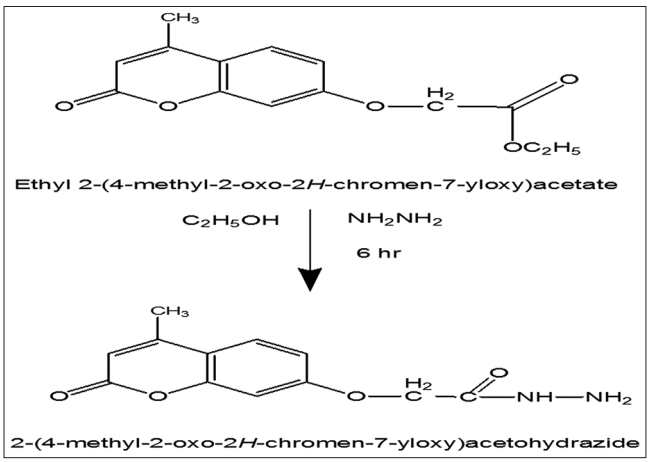
A mixture of 7-hydroxy-4-methylcoumarin (0.01 mole), ethyl chloroacetate (0.01 mole), and anhydrous K_2CO_3 (0.01 mole) in

dry acetone was refluxed on a water bath for 24 h. The mixture was then filtered and solvent was removed under reduced pressure. The resulting solid was recrystallized from ethanol to afford ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate.

Physical parameters

a. Percentage yield: 90%.
b. Melting point: 90–92°C.
c. Rf value: 0.71.
d. Mobile phase: Ethyl acetate: Hexane (1:1).

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide



Procedure

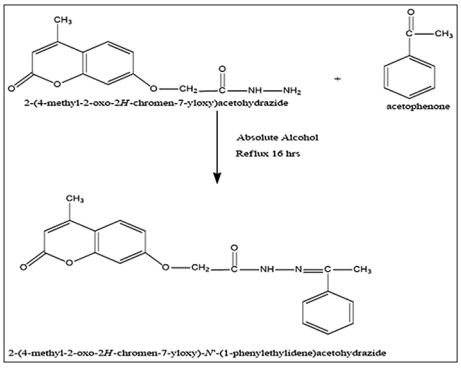
A mixture of ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate (0.01 mole) and hydrazine hydrate (0.02 mole) in ethanol was refluxed on a water bath for 6 h. After cooling, the solid that separated was washed with water, dried and recrystallized from ethanol. Needle-shaped crystals of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide were obtained.

Physical parameters

a. Percentage yield: 85%.
b. Melting point: 190–192°C.
c. R_f value: 0.63.
d. Mobile phase: Ethyl acetate: Methanol (1:1).

Synthesis of 7-hydroxy-4-methylcoumarin derivatives

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-phenylethylidene) acetohydrazide (M-1)



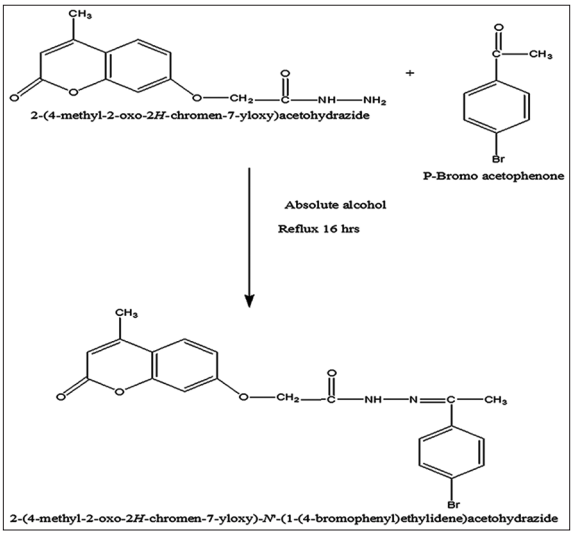
Procedure

A mixture of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mole), acetophenone (0.0008 mole) and 2–3 drops of glacial acetic acid in absolute alcohol (60 ml), was refluxed on water bath for 16 h. The reaction mixture was poured into the crushed ice. Filter, dried, and recrystallized from ethanol to afford the compound (M-1).

Physical parameters

a Percentage yield: 81%.
b Melting point: 160–165°C.
c R_f value: 0.20.
d obile phase: Ethyl acetate: Hexane (1:1).

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-bromophenyl)ethylidene) acetohydrazide (M-2)



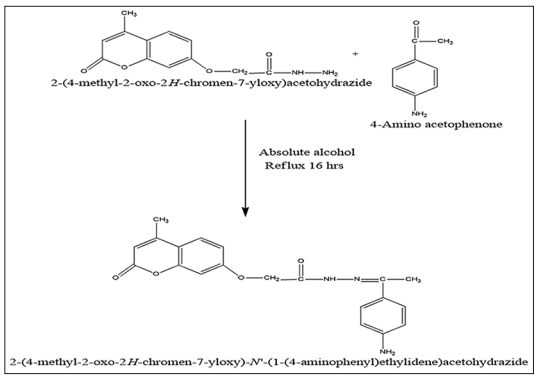
Procedure

A mixture of compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mole), p-bromoacetophenone (0.0008 mole) and 2–3 drops of glacial acetic acid in absolute alcohol (60 ml), was refluxed on water bath for 16 h. The reaction mixture was poured into the crushed ice. Filter, dried, and recrystallized from ethanol to afford the compound (M-2).

Physical parameters

a. Percentage yield: 80%.
b. Melting point: 220–225°C.
c. R_f value: 0.40.
d. Mobile phase: Ethyl acetate: Hexane (1:1).

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)ethylidene) acetohydrazide (M-3)



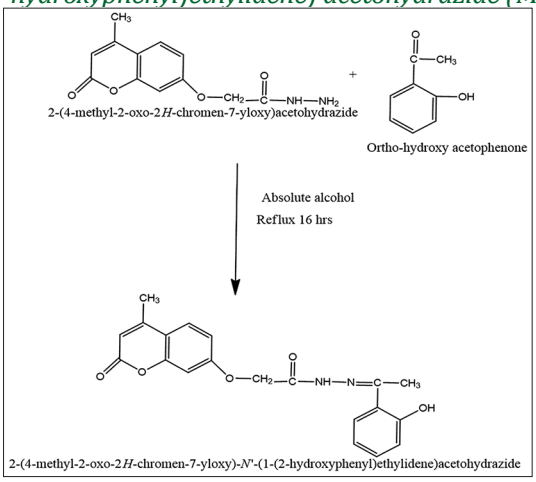
Procedure

A mixture of compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mole), 4-aminoacetophenone (0.0008 mole) and 2-3 drops of glacial acetic acid in absolute alcohol (60ml), was refluxed on water bath for 16 h. The reaction mixture was poured into the crushed ice. Filter, dried, and recrystallized from ethanol to afford the compound (M-3).

Physical parameters

a. Percentage yield: 74%
b. Melting point: 213–218°C
c. R_f value: 0.31
d. Mobile phase: Ethyl acetate: Hexane (1:1).

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene) acetohydrazide (M-4)



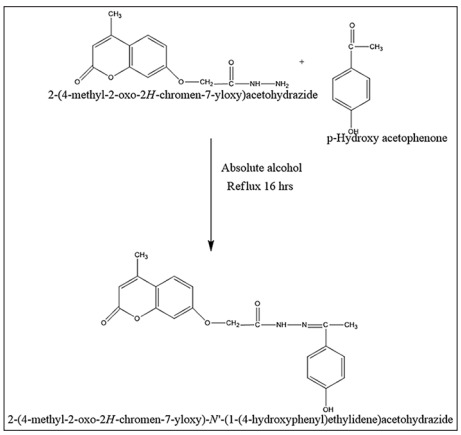
Procedure

A mixture of compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mole), o-hydroxyacetophenone (0.0008mole) and 2-3 drops of glacial acetic acid in absolute alcohol (60ml), was refluxed on water bath for 16 h. The reaction mixture was poured into the crushed ice. Filter, dried, and recrystallized from ethanol to afford the compound (M-4).

Physical parameters

a. Percentage yield:79%
b. Melting Point: 262–265°C
c. R_f value: 0.24
d. Mobile Phase: Ethyl acetate: Hexane (1:1).

2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-hydroxyphenyl)ethylidene) acetohydrazide (M-5)



Procedure

A mixture of compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mole), p-hydroxyacetophenone (0.0008 mole) and 2–3 drops of glacial acetic acid in absolute alcohol (60 ml), was refluxed on water bath for 16 h. The reaction mixture was poured into the crushed ice. Filter, dried, and recrystallized from ethanol to afford the compound (M-5).

Physical parameters

a. Percentage yield: 76%
b. Melting point: 225–230°C
c. Rf value: 0.31
d. Mobile phase: Ethyl acetate: Hexane (1:1).

Biological Evaluation

Antimicrobial activity[16-19]

The potential of microorganisms is tremendous as far as their beneficial effects are concerned. The increased resistance of microorganisms to various drugs is also cause of alarm. Several research and development units are therefore in search of such drugs which shall be susceptible to different microorganisms and also be active against a broad spectrum of microbes. Earlier studies have shown that coumarin possess antibacterial activity. Hence, an effort was made to check the antibacterial activity of the synthesized compounds. Important factors for the antimicrobial activity are size of the inoculum, metabolic state of organisms, pH, temperature, and duration of interaction, concentration of the inhibitor and presence of interfering substances. There are two official methods for determining antimicrobial activity.

1. Paper disk plate technique (disk diffusion method).
2. Tube-dilution technique (broth microdilution technique).

Determination of antibacterial activity

To determine the antimicrobial activity of synthesized compounds, zone of inhibition (a clear area) is measured around the disk which indicates that the organism was inhibited by the drug, which diffused into the agar from the disk. To determine zone of inhibition, paper disk plate technique was used.

Disc diffusion technique (determination of zone of inhibition)

Preparation of solution of synthesized compound

Accurately weighed 25 mg of each synthesized compound was transferred to different 100 ml volumetric flasks. These compounds were then dissolved in 2 ml. DMSO and volumes in each flask were made up to 100 ml with sterile distilled water. These suspension (each having concentration 250 µg/ml) was used as stock suspension. 2 ml and 4 ml of these suspensions were transferred into two 10 ml volumetric flasks and further dilutions were made up to 10 ml mark with sterile distilled water. The final suspension contained 50 and 100 µg of each compound per ml of the suspension.

Preparation of stock solution of standard drug (ciprofloxacin)

Ciprofloxacin (25 mg) was accurately weighed and transferred into 100 ml volumetric flask. The drug was dissolved in 2 ml. DMSO and diluted up to 100 ml with sterile distilled water. The final solution contained 250 µg/ml of the standard drug

ciprofloxacin. This solution in volume of 2 ml and 4 ml were transferred into two 10 ml volumetric flasks and further dilutions were made up to 10 ml mark with sterile distilled water. The final solutions contained 50 and 100 µg ciprofloxacin per ml of the solution.

Sterilization of glasswares

Petri dishes, pipettes, and culture tubes were washed with distilled water, dried in oven, packed in brown paper, and then autoclaved at 15 lb /inch^2 pressure (121°C) for 15 min.

Test strains

Bacillus subtilis (MTCC-441)
Escherichia coli (ESS 2231)

Composition of media

Nutrient agar was used for the purpose which contains the constituents as presented in Table 3.

Procedure for preparation of media

Peptone (5 g), beef extract (5 g), and sodium chloride (2.5 g) (all of biological grades) were weighed and dissolved in 400 ml of distilled water in a 500 ml volumetric flask and warmed. 10 g agar was dissolved in 50 ml of warm distilled water. The two solutions were mixed and the volume in volumetric flask was made up to 500 ml with warm distilled water. This nutrient agar media was sterilized in an autoclave at 15 lb /inch^2 pressure (121°C) for 15 min.

METHODOLOGY

Following steps were followed for the determination of antibacterial activity of synthesized compounds:

- Laminar airflow bench was swapped with 70 % alcohol and UV lamp was switched on. After 30 min, the UV lamp was switched off.
- All the reagents, media, inoculum, and glassware were placed in laminar airflow bench and aseptic condition was maintained.
- The plates were inoculated within minutes of the preparation of suspension so that the density did not change. A sterile cotton swab over was dipped into the suspension and the medium was inoculated by even streaking of the swab over the entire surface of the plate in three directions. After the inoculum had dried, the drug solution was poured on the disk, and then, disk was placed on the agar plate with the help of forceps. After inoculation at 37°C for 48 h, the zone of inhibition was measured using mm scale.
- Negative controlled plate - in this plate, only nutrient agar medium was poured, i.e., it did not contain drug dilution and inoculum.
- Positive controlled plate - in this plate, nutrient agar medium was poured, and after its solidification, inoculum was spreaded over the surface of culture plate. However, this Petri plate did not contain drug solution.

Table 1: Chemicals

Name	Specification	Manufacturer/ supplier
7-hydroxy-4-methylcoumarin	LR Grade	CDH Lab. Reagent
Ethyl chloroacetate	LR Grade	SD Fine Chem Ltd.
Hydrazine hydrate	LR Grade	CDH Lab. Reagent
Glacial acetic acid	LR Grade	CDH Lab. Reagent
Potassium carbonate	LR Grade	CDH Lab. Reagent
Acetophenone	LR Grade	CDH Lab. Reagent
p-bromoacetophenone	LR Grade	HIMEDIA Lab. Reagent
4-aminoacetophenone	LR Grade	CDH Lab. Reagent
o-hydroxyacetophenone	LR Grade	CDH Analytical Reagent
p-hydroxyacetophenone	LR Grade	HIMEDIA Lab. Reagent
Acetone	LR Grade	CDH Lab. Reagent
Ethyl acetate	LR Grade	CDH Lab. Reagent
Ethanol	LR Grade	Loba Chemie Ltd.
Methanol	LR Grade	CDH Lab. Reagent
Dimethyl sulfoxide	LR Grade	CDH Lab. Reagent
Hexane	LR Grade	CDH Lab. Reagent

RESULTS

Chemical Analysis

Several of 7-hydroxy-4-methylcoumarin derivatives were prepared according to scheme. Percentage yields of all the compounds were calculated and tabulated in Table 4. Their physical constants and thin layer chromatography primarily confirmed purity of the synthesized compounds. Melting points and R_f values are also given in the Table 4.

Structures of synthesized compounds were confirmed by IR and ^{1}H-NMR spectroscopy. IR spectra were obtained by preparing KBr pellets on shimadzu FT-IR spectrophotometer 8300 and are expressed in terms of wave number (cm^{-1}) as presented in (Table 5-9).

Table 2: Glass wares

Name	Specification	Manufacturer/ supplier
Beakers (ml)	100, 250, 500, 1000	Borosil Glass Works Ltd.,
RBF (one, two, and three neck) (ml)	250, 500	Borosil Glass Works Ltd.,
Conical flask (ml)	100, 250	Borosil Glass Works Ltd.,
Volumetric flask (ml)	100, 250	Borosil Glass Works Ltd.,
Funnel (mm)	100 (diameter)	Borosil Glass Works Ltd.,
Pipettes (ml)	1, 2,10	Borosil Glass Works Ltd.,
Test tubes (ml)	10	Borosil Glass Works Ltd.,
Reflux condenser (mm)	200 (length of jacket)	Borosil Glass Works Ltd.,
Distillation unit	1.5 L/H (output capacity)	Borosil Glass Works Ltd.,
Magnetic stirrer	-	-
Thermometer	-	-

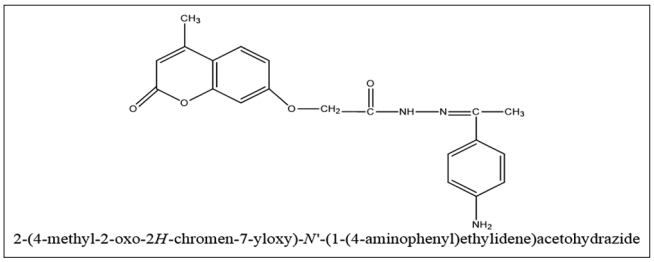
Table 3: Composition of nutrient agar media

Peptone (g)	10
Beef extract (g)	10
NaCl (g)	5
Agar (g)	20
Distilled water (ml)	Up to 1000

The ^{1}H-NMR spectra of the synthesized compounds were recorded in DMSO, using AV-300 BROKE JEOL at 300MHz spectrophotometer as presented in (Table 10-14).

IR and NMR spectra

IR of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-phenylethylidene) acetohydrazide (M-1)



2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*'-(1-(4-aminophenyl)ethylidene)acetohydrazide

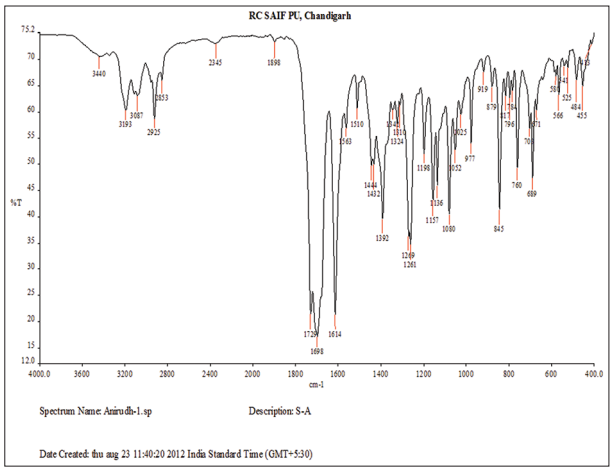


Table 4: Physical constants of 7-hydroxy-4-methylcoumarin derivatives

Compound code	M.P. (C) uncorrected	Yield (%)	R_f value*
M-1	160-165	80	0.20
M-2	220-225	81	0.40
M-3	213-218	74	0.31
M-4	262-265	79	0.24
M-5	225-230	76	0.31

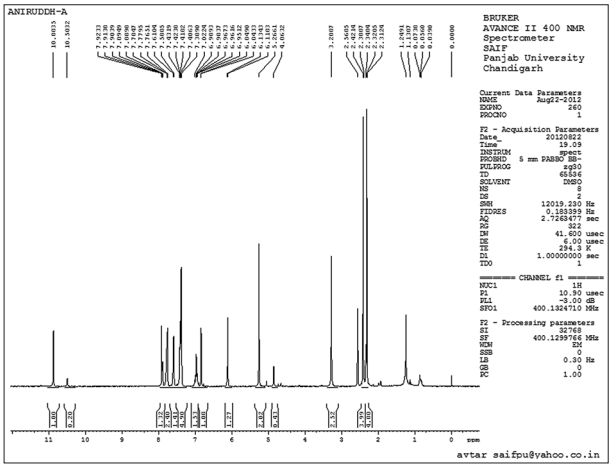
*Mobile Phase: Ethyl acetate:Hexane (1:1)

Table 5: IR spectra value of M-1

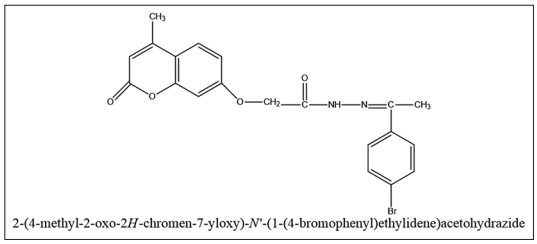
ν (cm^{-1})	Functional group assignment
3193	N-H str.
3027	C-H str. Aromatic
2925	C-H str. Aliphatic
1729	C=O str.
1698	C=O str.
1614	C=C str. aromatic
1563	C=N str.
1392	C-N str.
1080	C-O str. in C-O-C

IR: Infrared

NMR spectra of (M-1)



IR Spectra of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-bromophenyl)ethylidene) acetohydrazide (M-2)



2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*'-(1-(4-bromophenyl)ethylidene)acetohydrazide

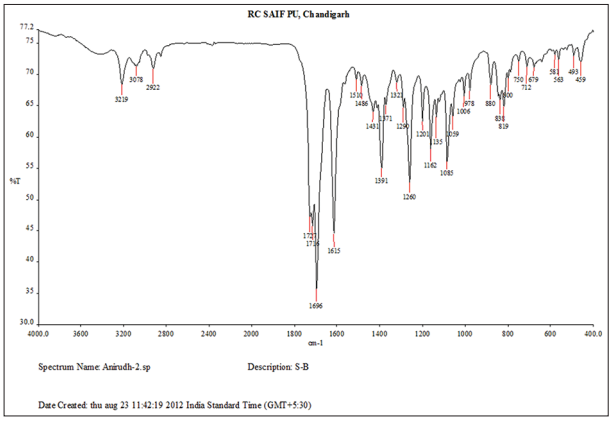
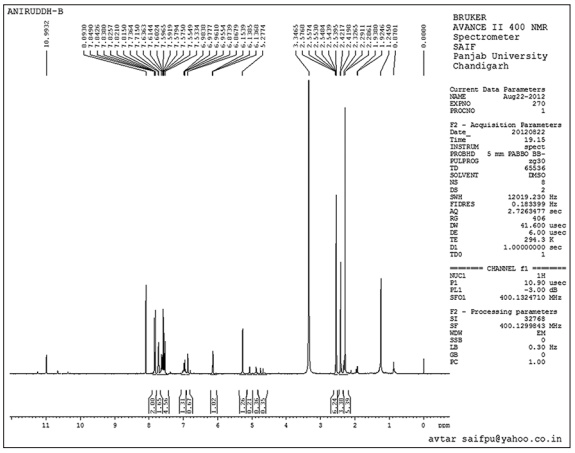


Table 6: NMR spectra value of M-1

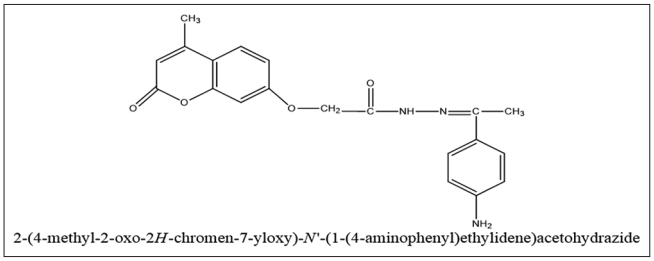
Chemical shift(δ) (ppm)	Number of protons	Inferences
10.88	1H	-NH
6.11–7.92	9 H	Aromatic
5.26	2H	$-OCH_2$
3.28	3H	CH_3 attached to coumarin ring
2.34	3H	CH_3

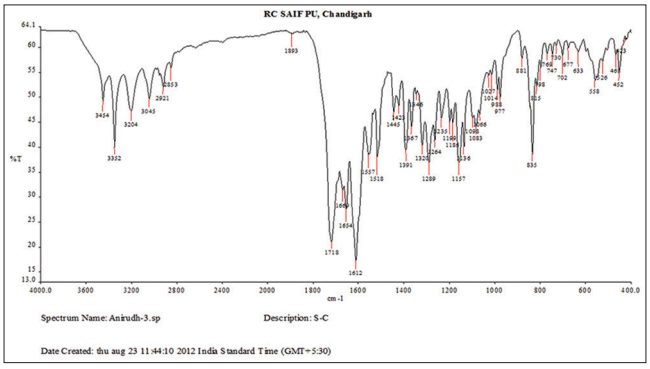
NMR: Nuclear magnetic resonance

NMR spectra of M-2



IR Spectra of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)ethylidene) acetohydrazide (M-3)



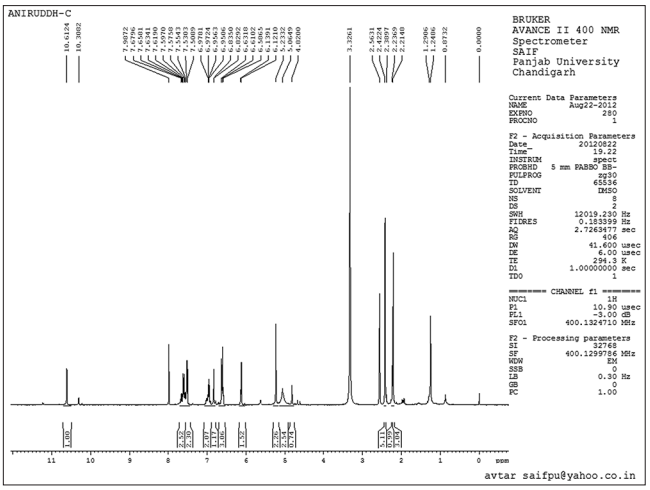


NMR spectra of M-3

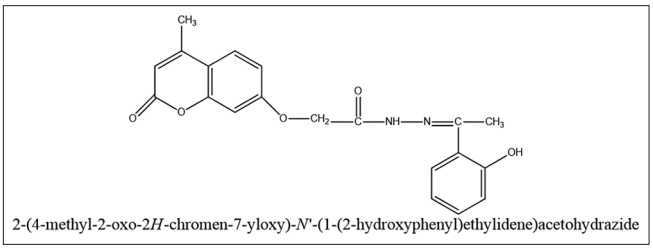
Table 7: IR spectra value (M-2)

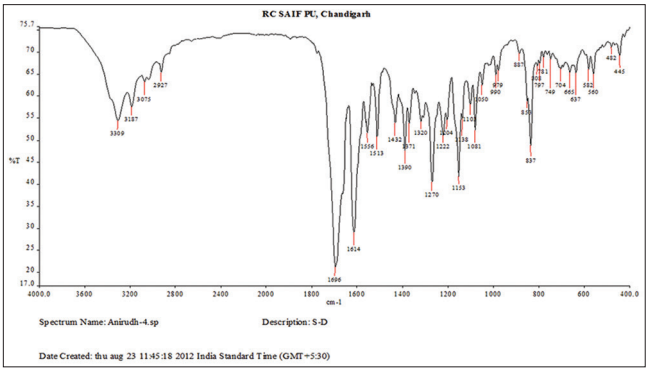
ν (cm $^{-1}$)	Functional group assignment
3219	N-H str.
3078	C-H str. Aromatic
2922	C-H str. Aliphatic
1727	C=O str.
1696	C=O str.
1615	C=C str. aromatic
1510	C=N str.
1391	C-N str.
1085	C-O str. in C-O-C

IR: Infrared



IR Spectra of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene) acetohydrazide (M-4)



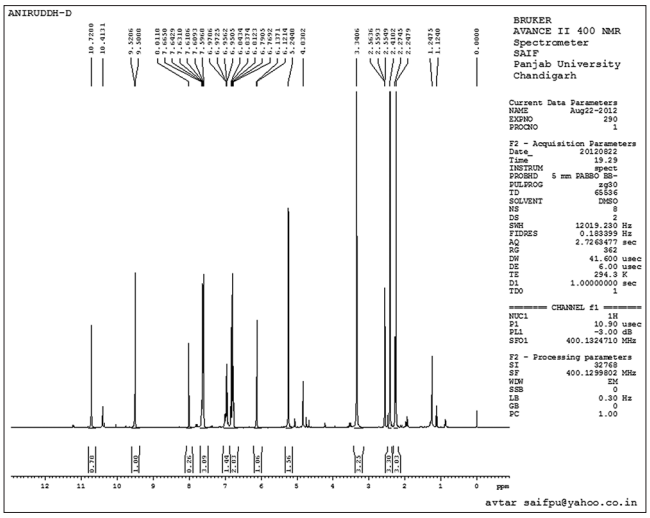


NMR spectra of M-4

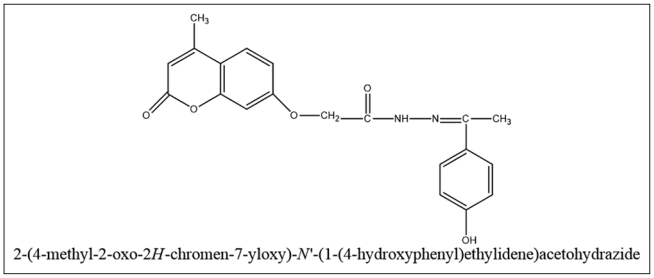
Table 8: NMR spectra value (M-2)

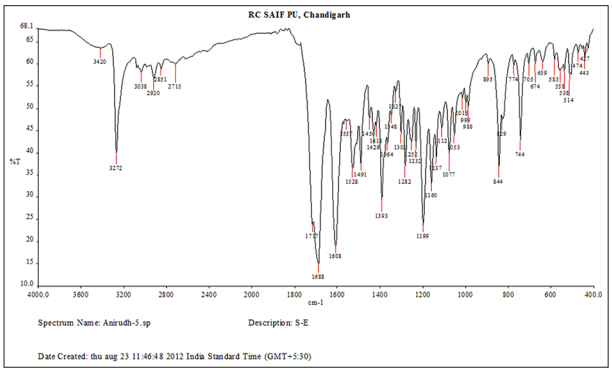
Chemical shift (δ) (ppm)	Number of protons	Inferences
10.99	1H	-NH
6.13-8.09	8 H	Aromatic
5.27	2H	-OCH_2
3.34	3H	CH_3 attached to coumarin ring
2.42	3H	CH_3

NMR: Nuclear magnetic resonance



IR Spectra of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-4-hydroxyphenyl)ethylidene) acetohydrazide (M-5)



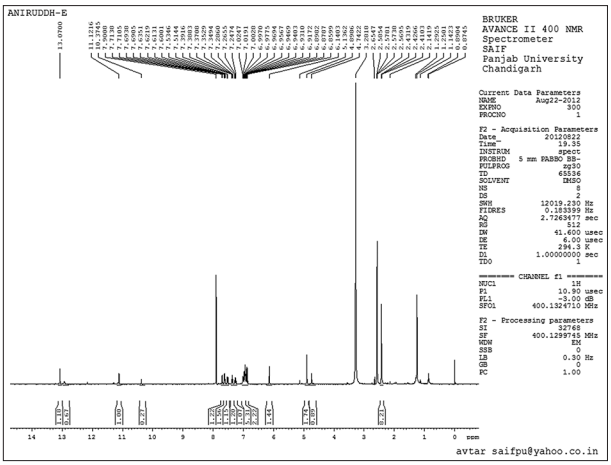


NMR spectra of M-5

Table 9: IR spectra value (M-3)

ν (cm^{-1})	Functional group assignment
3352	N-H str.
3045	C-H str. Aromatic
2921	C-H str. Aliphatic
1718	C=O str.
1669	C=O str.
1612	C=C str. aromatic
1518	C=N str.
1391	C-N str.
1083	C-O str. in C-O-C

IR: Infrared



Antimicrobial Activity

All the target compounds were evaluated for their *in vitro* antibacterial activity against both Gram-positive (*B. subtilis*) and Gram-negative bacteria (*E. coli*) using ciprofloxacin as

Table 10: NMR spectra value of M-3

Chemical shift (δ) (ppm)	Number of protons	Inferences
10.61	1H	-NH
6.12–7.98	8 H	Aromatic
5.06	2 H	NH_2
5.23	2H	$-OCH_2$
3.32	3H	CH_3 attached to coumarin ring
2.23	3H	CH_3

NMR: Nuclear magnetic resonance

Table 11: IR spectra value (M-4)

ν (cm^{-1})	Functional group assignment
3309	N-H str.
3075	C-H str. Aromatic
2927	C-H str. Aliphatic
1696	C=O str.
1614	C=O str.
1556	C=C str. aromatic
1513	C=N str.
1390	C-N str.
1081	C-O str. in C-O-C

IR: Infrared

Table 12: NMR spectra value (M-4)

Chemical shift (δ) (ppm)	Number of protons	Inferences
10.72	1H	-OH
10.41	1 H	-NH
6.12-8.01	8 H	Aromatic
5.24	2H	$-OCH_2$
3.34	3H	CH_3 attached to coumarin ring
2.41	3H	CH_3

NMR: Nuclear magnetic resonance

standard drugs in concentration of 50 μg/ml and 100 μg/ml. Disc diffusion method was used for the determination of the preliminary antibacterial activity. The results were recorded for each tested compound is shown in Tables 15 and 16 and Figures 1 and 2.

Table 13: IR spectra value (M-5)

ν (cm^{-1})	Functional group assignment
3272	N-H str.
3038	C-H str. Aromatic
2920	C-H str. Aliphatic
1717	C=O str.
1688	C=O str.
1608	C=C str. aromatic
1528	C=N str.
1393	C-N str.
1077	C-O str. in C-O-C

IR: Infrared

Table 14: NMR spectra value (M-5)

Chemical shift (δ) (ppm)	Number of protons	Inferences
13.07	1H	-OH
11.12	1 H	-NH
6.14–7.62	8 H	Aromatic
4.89	2H	$-OCH_2$
3.28	3H	CH_3 attached to coumarin ring
2.41	3H	CH_3

NMR: Nuclear magnetic resonance

Table 15: Antibacterial activity data of synthesized compounds against *E. coli*

Compounds	Zone of inhibition in mm	
	E. coli (ESS 2231)	
	100 μg	50 μg
Control	-	-
Ciprofloxacin	25	22
M-1	11	8
M-2	18	13
M-3	14	9
M-4	16	12
M-5	19	17

E. coli: Escherichia coli

Table 16: Antibacterial activity data of synthesized compounds against B. subtilis

Compounds	Zone of inhibition in mm	
	B. Subtilis (ACC-132)	
	100 μg	50 μg
Control	-	-
Ciprofloxacin	24	20
M-1	11	9
M-2	15	11
M-3	10	6
M-4	13	7
M-5	16	11

B. subtilis: Bacillus subtilis

Results of Antibacterial Activity

The results of antibacterial activity were shown that the synthesized compounds exhibited mild-to-moderate antibacterial activity against (Gram-positive) *B. subtilis* (MTCC-441) and (Gram-negative) *E. coli* (ESS 2231) at concentration of 100 μg/ml by disc diffusion method as compared to ciprofloxacin. The compound M-2 showed potent activity against both *E. coli* and *B. subtilis*. The solvent control, i.e., dimethyl formamide did not show any activity.

DISCUSSION

Literature review revels that 7-hydroxy-4-methylcoumarin derivatives possess diverse type of biological activity including antimicrobial and anti-inflammatory activity. In view of these observations, we synthesized some new 7-hydroxy-4-methylcoumarin derivatives and evaluated for their antimicrobial activity.

In the synthesis, the compound ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate was obtained by reacting 7-hydroxy-4-methyl-2H-chromen-2-one with ethyl chloroacetate in the presence of potassium carbonate in acetone. The ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate was reacted with hydrazine hydrate in ethanol to afford 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide which further reacted with acetophenones in the presence of glacial acetic acid in absolute

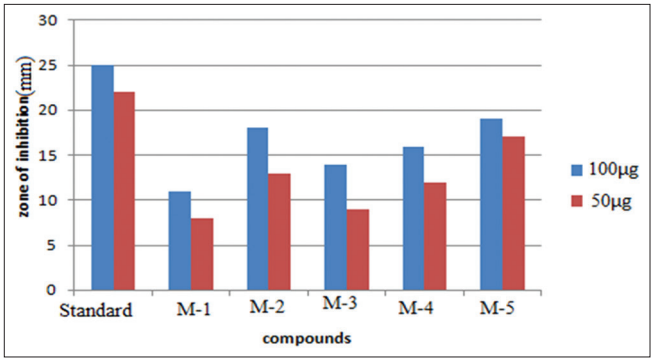


Figure 1: Antibacterial activity of synthesized compounds against *Escherichia coli*

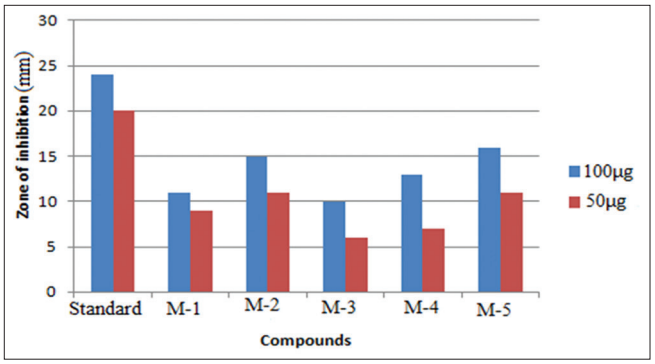


Figure 2: Antibacterial activity of synthesized compounds against *Bacillus subtilis*

alcohol to afford various coumarin derivatives, namely, M-1, M-2, M-3, M-4, and M-5. The structures of target compounds were established on the basis of IR and ^{1}H-NMR spectral studies. The purity and homogeneity of all compounds were confirmed by their sharp melting point and TLC. All the above results positively confirmed the formation of the synthesized compounds and hence correctness of the anticipated structures drawn for synthesized compounds. All the synthesized compounds have been tested for antibacterial activity by disk diffusion method. Most of the targeted compounds showed significant activity against both strain as compared to ciprofloxacin. Among the compound M-5 showed maximum zone of inhibition 19 mm against *E. coli* (ESS 2231) and 16 mm against *B. subtilis* (MT).

REFERENCES

1. Monga PK, Sharma D, Dubey A. Corporative study of microwave and conventional synthesis and pharmacological activity of coumarin. E-international Sci Res J 2012;4:1749-2094.
2. Abramov RS, Bankoort DL. New tetrazolic azodyes linked to (thio) barbiturate and electron rich aromatic as potential. J Phys Chem 1993;31:23.
3. Noroozi-Pesyan N, Khalaf J, Malekpoor Z. Derivatizing reagent- kit using a single coumarin fluorophor. Prog Color Colorants Coat 2009;2:61-70.
4. Takadate A, Masuda T, Murata C, Isobe A, Shinohara T, Irikura M, *et al*. Synthesis and characterization of new azo coumarin dyes. Anal Sci 1997;13:753.
5. Brafola G, Fringuelli F, Piermatti O, Pizzo F. Simple and efficient one-pot preparation 3- substituted coumarin in water. E-international Sci J Chem 1996;43:1257.
6. Campbell HA, Link K. Studies on the hemorrhagic sweet clover disease. IV. The isolation and crystallization. J Biol Soc 1941;21:138.
7. Mazumder A, Wang S, Neamati N, Nicklaus M, Sunder S, Chen J, *et al*. Antiretroviral agents as inhibitors of both human immunodeficiency virus type 1 integrase and protease. J Med Chem 1996;39:2472-81.
8. Kathuria A, Gupta A, Priya N, Singh P, Raj HG, Prasad AR, *et al*. Specificities of calreticulin transacetylase to acetoxy derivatives of 3-alkyl-4-methylcoumarins: Effect on the activation of nitric oxide synthase. Bioorg Med Chem 2009;17:1550.
9. Musicki B, Periers AM, Laurin P, Ferroud D, Benedetti Y, Lachaud S, *et al*. Improved antibacterial activities of coumarin antibiotics bearing 5′,5′-dialkylnoviose: Biological activity of RU79115. Bioorg Med Chem Lett 2000;10:1695-9.
10. Inoue Y, Kondo H, Taguchi M, Jinbo Y, Sakamoto F, Tsukamoto G. Synthesis and antibacterial activity of thiazolopyrazine-incorporated tetracyclic quinolone antibacterials. J Med Chem 1994;37:586-92.
11. Rajitha B, Naveen KV, Someshwar P, Venu MJ, Reddy PN, *et al*. Di pyridine copper chloride catalysed coumarin synthesisvia pechmann condensation under conventional heating and microwave irradiation. Online J Org Chem 2006;12:23-7.
12. Martinz PR. Ionic liquid and microwave-assisted organic synthesis: A green and synergic couple. J Mex Chem Soc 2007;51:252-64.
13. Al-Zaydi KM. Microwave assisted synthesis, part 1: Rapid solventless synthesis of 3-substituted coumarins and benzocoumarins by microwave irradiation of the corresponding enaminones. Molecules 2003;8:541-55.
14. International Union of Pure and Applied Chemistry (Schiff Base). Compendium of Chemical Terminology. 2nd ed. Internet ed. (The "Goldbook"). Online Corrected Version: (2006)-Schiff Bases. Oxford: International union of Pure and Applied Chemistry (Schiff Base); 1997.
15. Hernández-Molina R, Mederos A. Acyclic and Macrocyclic Schiff Base Ligands in Comprehensive Coordination Chemistry II. Vol. 2. New York: Pergamon Press; 2003. p. 411-46.
16. Sahu SK. synthesis characterization and biological activity of 2-methyl-3-aminoquinazolin-4(3H)-one Schiff bases. J Braz Chem Soc 2008;1:5-22.
17. Alagarsamy V, Murugananthan G, Venkateshperumal R. Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-methyl-3-substituted quinazolin-4-(3H)-ones. Biol Pharm Bull 2003;26:1711-4.
18. Winter CA, Risley EA, Nuss GW, Ratheesh M, Helen A. *A. carrageenin*- induced edema in hind paw of rat as anassay for antinflammatory drug. J Biotechnol 1962;6:1209-11.
19. Pelczar MJ, Chan EC, Kreig NR. A Text Book of Microbiology. 5th ed. Chennai: Oriental Longman Publications; 1993. p. 133-49.