WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 547-560. Research article ISSN 2277– 7105

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 7 HYDROXY 4 METHYL COUMARIN DERIVATIVES AS ANTI-INFLAMMATORY & ANTIMICROBIAL AGENTS.

Dr. Mane B. Y. and Garud S. B.*

Padmashri Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar.
Post - MIDC, Vilad Ghat, Ahmednagar- 414111. (MS), India.

Article Received on
10 December 2017,
Revised on 31 Dec. 2017,
Accepted on 21 Jan. 2018
DOI: 10.20959/wjpr20183-10590

***Corresponding Author**
Garud S. B.
Padmashri Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar. Post - MIDC, Vilad Ghat, Ahmednagar- 414111. (MS), India.

ABSTRACT

Coumarin nucleus derivatives shows various activities like anticoagulant, antimicrobial, anti-inflammatory, analgesic, antioxidant, anti cancer, antiviral, antimalarial etc. The anti-inflammatory and antimicrobial activities are most widely studied. The anti-inflammatory activity is due to the inhibition of COX enzymes. But this results in the damage of mucosa. So it is necessary to develop and evaluate the drugs which will selectively give anti-inflammatory activity without side effects. In the present study ten novel coumarin derivatives are synthesized and pharmacologically evaluated for anti inflammatory & antimicrobial activities & are characterized for IR & NMR spectra. Anti inflammatory activity is tested by carageenan induced rat paw edema model & antimicrobial activity is tested against E. Coli & S. aureus, by cup plate method. The result of present investigation was excellent and compounds showed significant inhibition against carageenan induced rat paw edema model and exhibited antimicrobial activity against S. aureus & E. coli. The compounds 4d showed the excellent activity. Also compounds 4e & 4h showed moderate anti inflammatory activity. Also various compounds showed significant antimicrobial activity.

KEYWORDS: 7 hydroxy 4 methyl coumarin, anti-inflammatory, antimicrobial.

INTRODUCTION

Balaji PN reported antimicrobial activity with the series of various substituted Schiff base compounds of 7 hydroxy 4 methyl coumarin. Venkatesh Mutalik reported antibacterial activity on S.aureus, E.coli & antifungal activity on A. niger & C. Albicans.

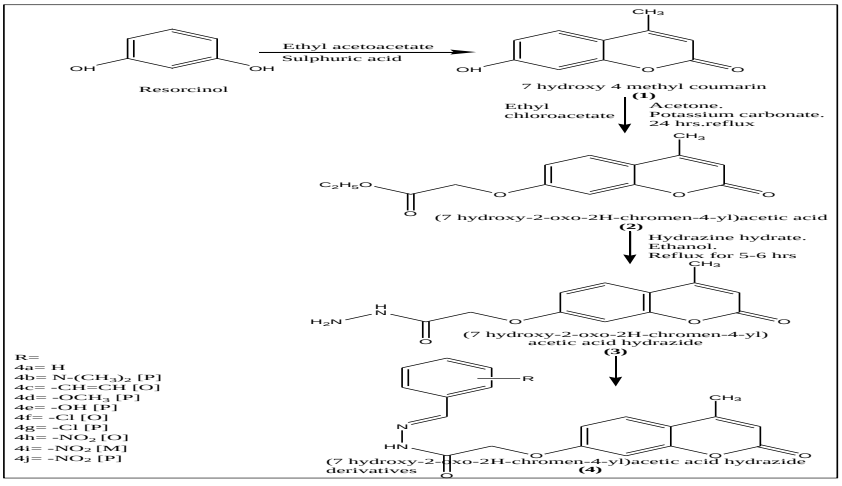
Coumarin is a Benzopyrone nucleus. Various substituted coumarins shows various activities like anticoagulant activity, bacteriostatic activity, but most widely reported activities are anti inflammatory and anticancer activities. Present Study was conducted to synthesize coumarin Schiff bases, to evaluate against anti inflammatory & antimicrobial activity. The specific objectives of present work are as follows -

1. To establish the scheme of proposed compound on the basis of literature survey.
2. To achieve the synthesis of series of substituted (7hydroxy - 2-oxo - 2H-chromen - 4-yl) acetic acid hydrazide derivatives.
3. To establish the structure of synthesized compounds by IR, NMR & Mass spectroscopy.
4. Pharmacological evaluation of synthesized compounds for anti inflammatory and antimicrobial activity.

MATERIAL AND METHOD

The melting point of synthesized compounds were determined by open capillary method which are uncorrected, the synthesized compounds are characterized & identified by elemental analysis. IR spectra were recorded on a JASCO FT-IR 4100 spectrophotometer using KBR powder technique. Some selected compounds were subjected to NMR spectra. Data were recorded on Bruker 400 MHZ in CDcl3 using TMS as an internal standard and FAB - mass for structural confirmation.

Scheme



Procedure -Synthesis of 7 hydroxy 4 methyl coumarin(1)

In two necked 500ml round bottom flask take 250 ml conc. Sulphuric acid and keep into ice bath until the temperature of solution becomes 0 to 10 ºC. After solution becomes ice cold, to this add solution of resorcinol 33gms (0.01moles) and 35ml (0.01moles) of ethylacetoacetate,

dropwise for two hrs. After completion of addition the reaction mixture was added into crushed ice. The yellowish solid separated out which was filtered off. The product was dried and recrystallized from ethanol.

Procedure for the preparation of ethyl 2-(2-oxo-4-methyl-2H-chromen-7-yloxy) acetate. (2)

A mixture of compound 1(3.7gm, 0.014moles), unhydrous potassium carbonate (1.93 gm, 0.014 moles) and ethyl chloro acetate (1.7 ml, 0.014moles) in dry acetone was refluxed for 24hrs.After the reaction has finished, the mixture was poured on ice/water. The solid obtained was filtered off and recrystallized from ethanol.

Procedure for synthesis of (7 hydroxy - 2oxo-2H-chromen - yl) acetic acid hydrazide. (3)

In 500 ml R.B.F. take 20 ml of absolute ethanol. To this add 7 gm (0.026mole) of (7-hydroxy - 2oxo - 2H-chromen - 4-yl)acetic acid and 1.3ml (0.026 mole) of hydrazine hydrate. The resultant reaction mixture was refluxed for 5-6 hrs. The reaction mixture was added in to ice cold water. The solid separates out which was filtered and dried and recrystallized from ethanol.

General procedure for synthesis of (7 - hydroxy - 2-oxo-2H-chromen - 4-yl) acetic acid hydrazide derivatives. (4-a to 4-j)

In the two necked R.B.F. take 20 ml absolute ethanol. To this add 1 gm (0.001mole) of (7-hydroxy - 2-oxo - 2H-chromen - 4-yl acetic acid hydrazide. To this solution add equimolar amount of different aromatic aldehyde and drop glacial acetic acid. The resultant reaction mixture was poured into the ice cold water. The solid separated out was filtered and dried. The solid was recrystallized into ethanol. Thin Layer Chromatography (TLC)- Thin Layer Chromatography is performed to monitor the completion of reaction.

Anti inflammatory Activity

-Selection of experimental animals

Healthy albino rats of weight 180 to 250 gm were used for the evaluation of anti inflammatory activity.

-Laboratory condition

The rats were housed comfortably in a group of six in single clean polypropylene cages with a metal frame lid on the top.

-Food and water

All the animals had free access to water and standard pelletized laboratory animal diet ad libitum.

Procedure (Carageenan induced acute paw edema model)

(1) The animals were weighted, numbered and divided into twelve groups of six each & mark was made on the ankle joint of each rat, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume.

(2) 10 ml /kg body weight of normal saline (0.9%Nacl) was injected to the control group & 15 mg /kg of Ibuprofen was injected to standard group. The test compounds (100mg /kg) were injected to the remaining test group.

(3) After one hour 0.1ml of 1%(w/v) carageenan in normal saline solution was injected to the plantar region of left paw of control group, standard group and the test group animals

(4) Paw volume up to the ankle joint was measured in drug treated & untreated groups after carageenan challenge at 120min. Using a plethysmometer.

(5) The edema was expressed as an increase in the volume of paw, and the percentage of inhibition for each rat and each group was obtained as follows -

Percentage of inhibition =Vc-Vt /Vc *100

Vc =the mean increase in the paw thickness in the control group of rats.

Vt=the mean increase in the paw thickness in the rats treated with test compounds.

Antimicrobial Activity

- Diluted microorganisms of Gram +ve&Gram - ve, both the strains were selected.

-S. aureus (Gram+ve) &E.coli (Gram - ve) were taken to test the synthesized compounds against.

-Cup plate method was selected for the testing of compounds.

Procedure (Cup plate method)

(1) The antibacterial activity of newly synthesized coumarins was conducted against Gram positive bacteria i. e. Staphylococcus aureus & Gram negative bacteria i. e. Escherichia coli by using cup plate method. Amoxicillin was employed as reference standard to compare the result. Nutrient broth was used for the preparation of inoculation of the bacteria and nutrient agar was used for the screening methods.

(2) Each compound (5 mg) was dissolved in dimethylsulphoxide (DMSO) (5 ml) at a concentration of 1000 µgm /ml.

(3) Amoxicillin solution was also prepared at a concentration of 1000 µgm /ml in sterilized distilled water.

(4) All the compounds were tested at a concentration of 0.05 ml (50 µgm) & 0.1 ml (100 µgm) level and DMSO used as a control.

(5) The solutions of each test compound, control and reference standard (0.05 & 0.1 ml) were added separately in the cups and the plates were kept undisturbed for at least two hrs in refrigerator to allow diffusion of the solution properly into the nutrient agar medium.

(6) petridish were subsequently incubated at 37±1°C for 24 hrs. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

RESULTS

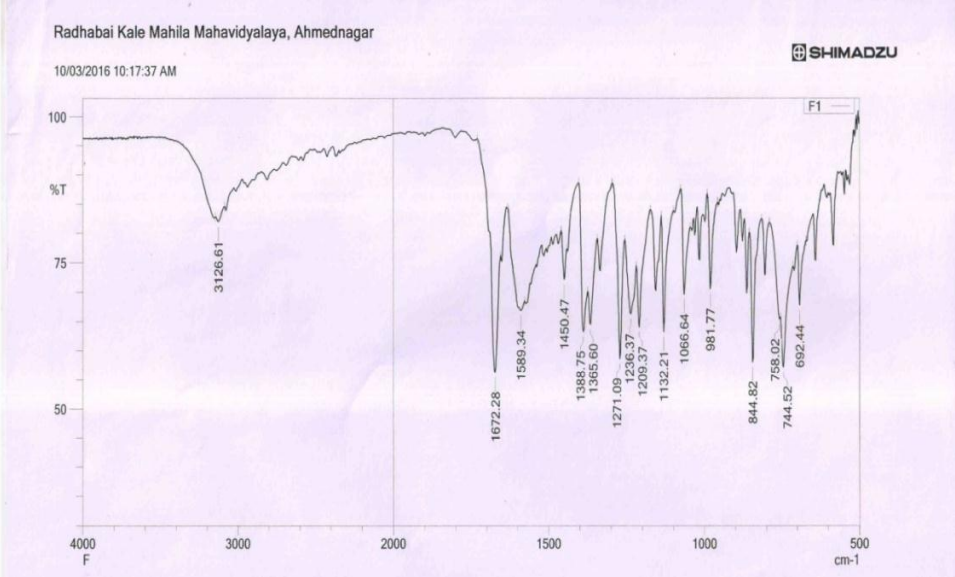
Table 1 and 2 gives the physicochemical properties of the various intermediate and synthesized compounds respectively.

Table 1: Physicochemical properties of Intermediates.

Compound	Molecular formula	Molecular weight	% Yield	Melting point(°C)	R_f value
1	$C_{10}H_8O_3$	176	82.42	72	0.43
2	$C_{14}H_{14}O_5$	262	69	193	0.59
3	$C_{12}H_{12}O_4N_2$	248	62	203	0.52

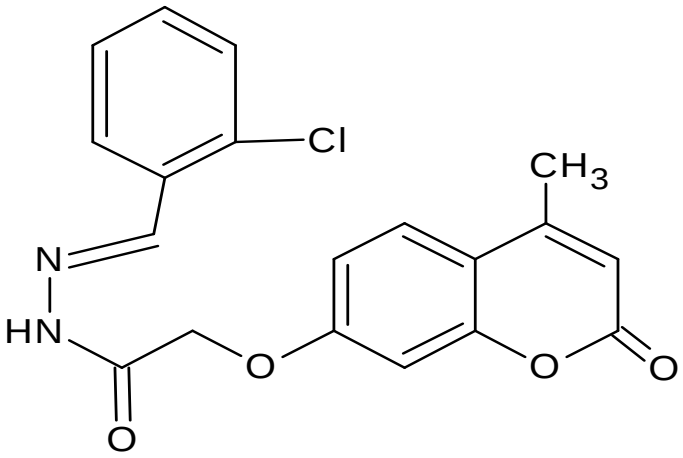
Table 2: Physicochemical properties of the title compounds.

Comp-ound	Molecular formula	Ph-CHO	Mole.weight	% Yield	Melt.po-int (°C)	R_f-value
4a	$C_{19}H_{16}O_4N_2$	Benzaldehyde	336	63	201	0.62
4b	$C_{21}H_{21}O_4N_3$	P-dimethylamino benzaldehyde.	379	60	199	0.53
4c	$C_{21}H_{18}O_4N_2$	Cinnamaldehyde.	362	48	213	0.61
4d	$C_{20}H_{18}O_5N_2$	p-anisaldehyde.	366	52	209	0.58
4e	$C_{18}H_{16}O_5N_2$	4 hydroxy benzaldehyde.	340	63	215	0.68
4f	$C_{19}H_{15}O_4N_2Cl$	2 chloro benzaldehyde.	370	70	213	0.57
4g	$C_{19}H_{15}O_4N_2Cl$	p- chloro benzaldehyde.	370	63	211	0.59
4h	$C_{19}H_{15}O_6N_2$	2 nitro benzaldehyde	381	74	203	0.57
4i	$C_{19}H_{15}O_6N_2$	3 nitro benzaldehyde.	381	60	209	0.53
4j	$C_{19}H_{15}O_6N_2$	p-nitro benzaldehyde.	381	61	213	0.64



IR Interpretation results- IR interpretation data of various compounds is given below.

Comp.4f

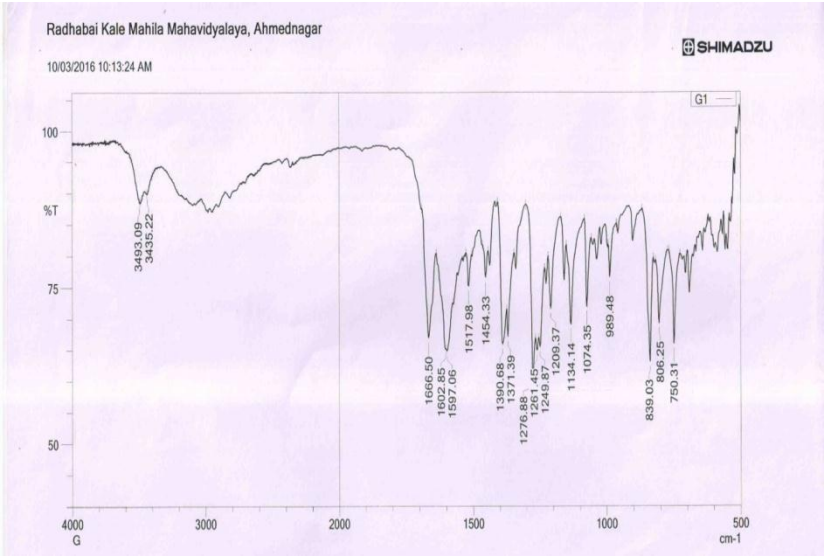


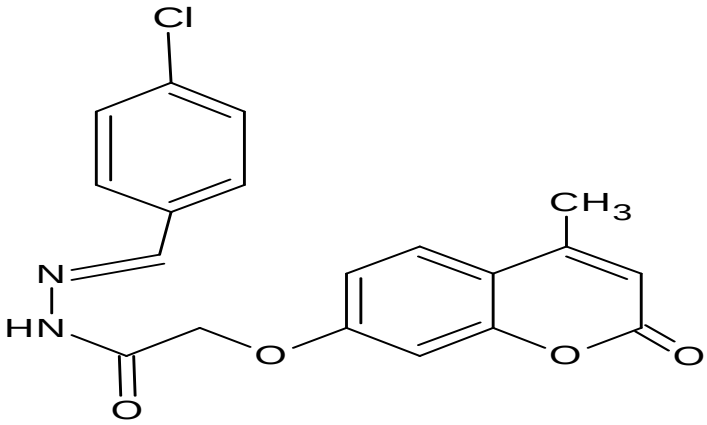
N'-2 chlorobenzylidene - 2-(2-oxo - 4-methyl - 2H-chromen - 7-yloxy) acetohydrazide.

Table 3:

Sr.No.	Frequency cm^{-1}	Functional Group
1	3126.61	=C-H (Alkene)
2	1672.28	>C=O (Ketone)
3	1589.34	-C=C-(Aromaticring)
4	1450.47,981.77	$-CH_3$ (Alkane)
5	1365.60,1209.37	-C-O- (Ether)

Comp.4g-



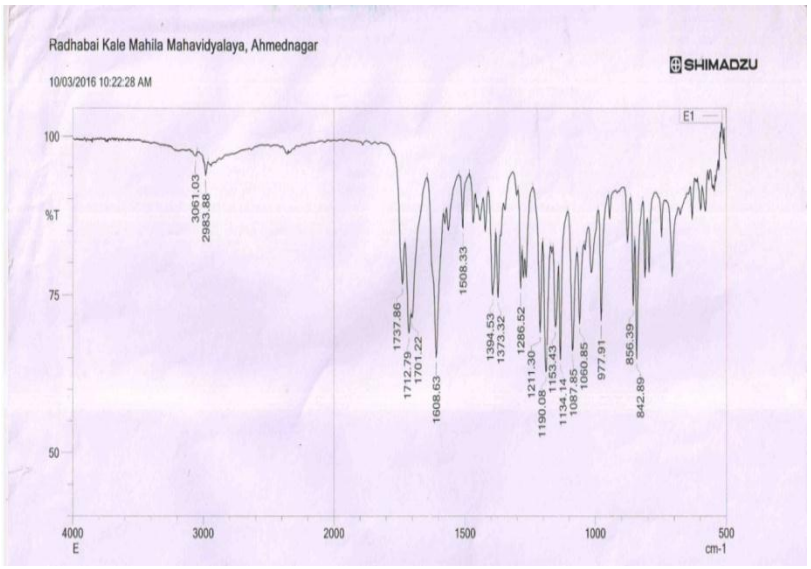


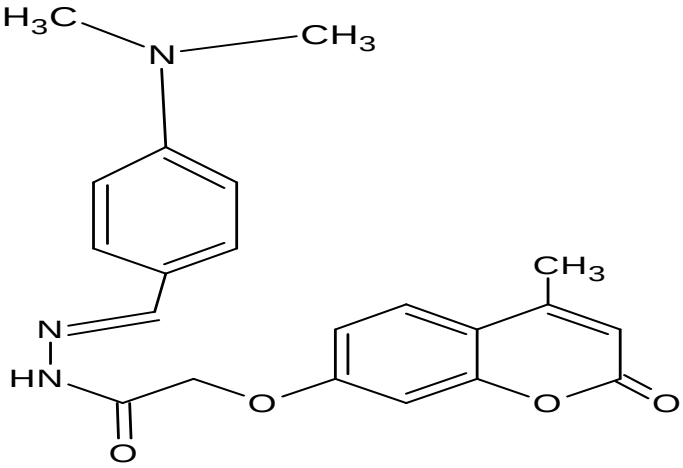
N'-p chlorobenzylidene - 2-(2-oxo - 4-methyl - 2H-chromen - 7-yloxy) acetohydrazide.

Table 4:

Sr.No.	Frequency cm^{-1}	Functional Group
1	3439.09	=C-H (Alkene)
2	1666.50	>C=O (Ketone)
3	1602.85,1597.06,1517.98	-C=C- (Aromatic ring)
4	1454.33	$-CH_3$ (Alkane)
5	1249.87	-C-O- (Ether)
6	1390.68	-NO2 (Nitro group)
7	1276.88	-C-N< (Amine)

Comp.4b-



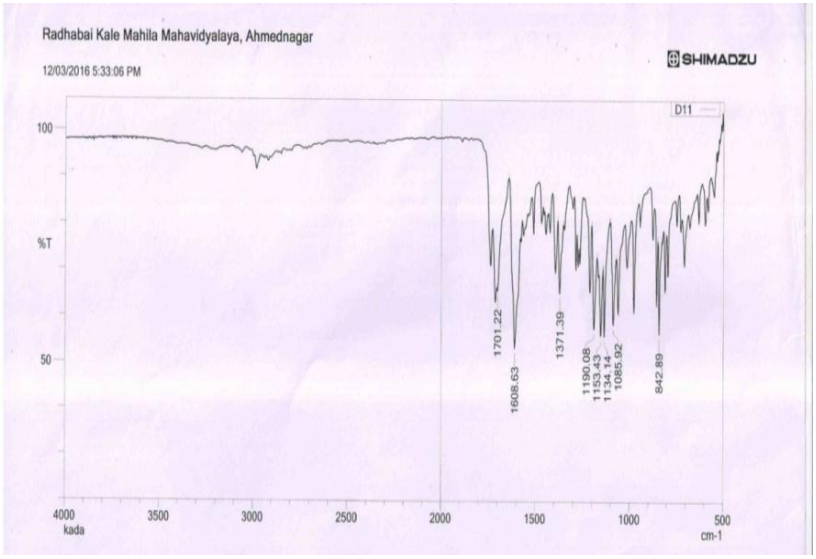


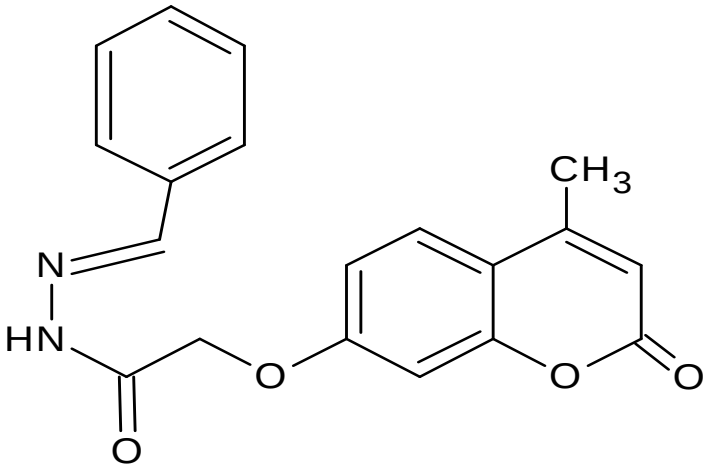
N'-p dimethyl amine benzylidene 2-(2-oxo - 4-methyl - 2H-chromen- 7-yloxy) acetohydrazide.

Table 5:

Sr.No.	Frequency cm^{-1}	Functional Group
1	3061.03	=C-H (Alkene)
2	1712.79	>C=O (Ketone)
3	1508.33	-C=C- (Aromatic ring)
4	1190.08,1087.85	$-CH_3$ (Alkane)
5	1211.30	-C-O- (Ether)
6	1286.52	-C-N< (Amine)

Comp.4a-



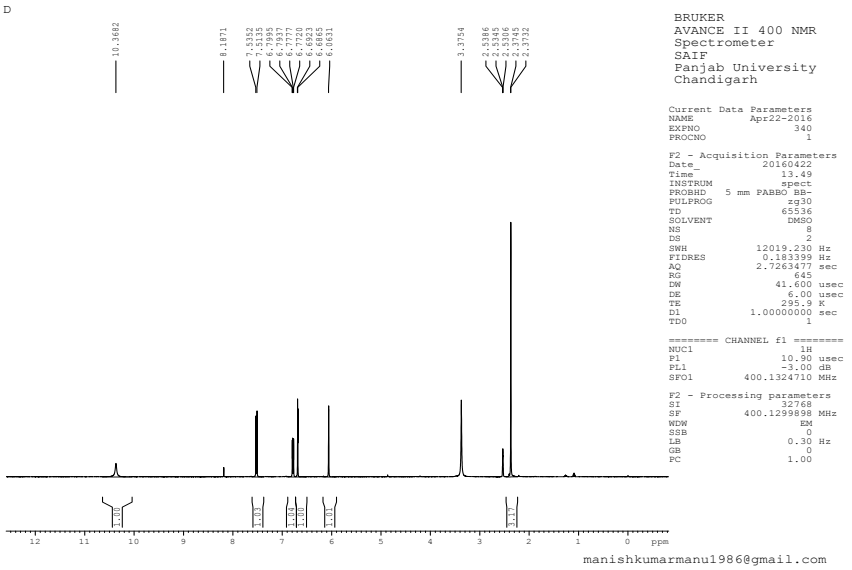


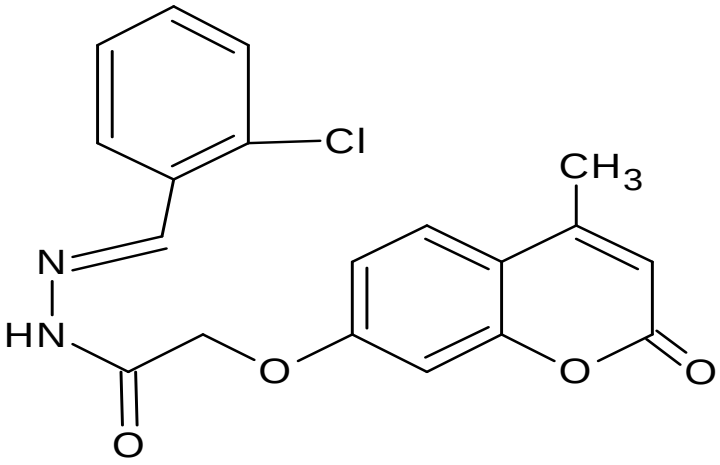
N'-benzylidene - 2-(2-oxo - 4-methyl - 2H-chromen - 7-yloxy) acetohydrazide.

Table 6:

Sr.No.	Frequency cm^{-1}	Functional Group
1	1701.22	>C=O (Ketone)
2	1608.63	-C=C- (Aromatic ring)
3	1371.39	$-CH_3$ (Alkane)
4	1153.43	-C-O- (Ether)
5	1190.08	-C-N< (Amine)

NMR Results of Compound 4f.





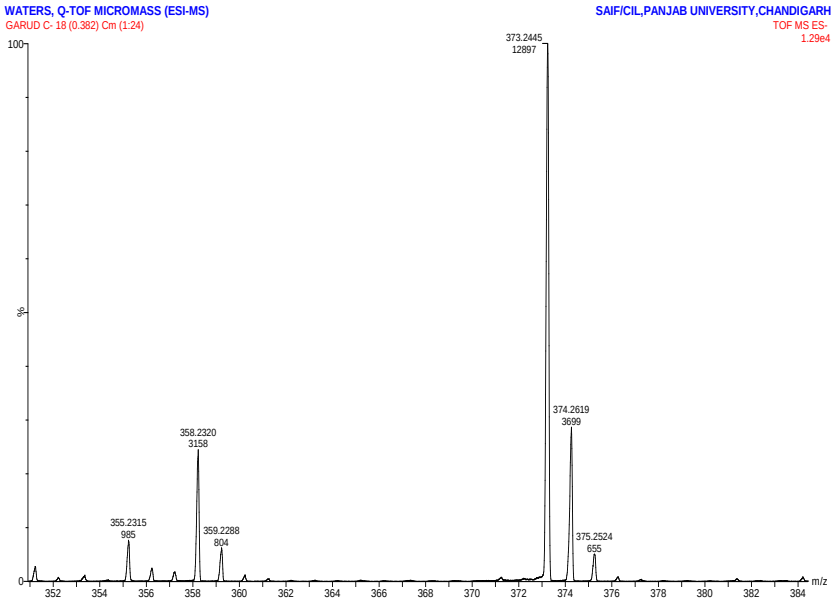
N'-2 chlorobenzylidene - 2-(2-oxo - 4-methyl - 2H-chromen - 7-yloxy) acetohydrazide.

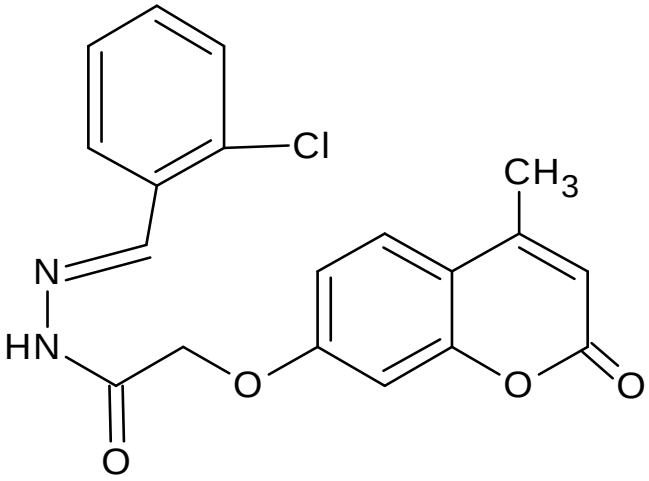
Table 7:

Sr.No.	Delta Value	Proton
1	7.5	Benzilidene
2	8.1	Secondary amine
3	2.5	Methylene group
4	6.6,6.7	Aromatic ring
5	3.3	Methyl group

NMR result of comp 4f is interpreted in table no. 7.

LC-MS Result of compound 4f.





N'-2 chlorobenzylidene - 2-(2-oxo - 4-methyl - 2H-chromen - 7-yloxy) acetohydrazide.

Table 8:

Compound	Percentage	Molecular wt.	m/z ratio
4f	17.7	370.7864	373.2445

The result of Liquid Chromatography - Mass Spectroscopy is mentioned in table no. Table no. 8

RESULT AND DISCUSSION

Table 9: Results of anti-inflammatory activity of the compounds.

Group	Dose	Oedema± S.E.M.	Percentage inhibition
Control	Normal Saline Solution 10ml/kg	3.28±0.02887	-
Standard	Ibuprofen 15mg/kg	0.24±0.01424	92.68
4a	100mg/kg	2.078±0.03124	36.64
4b	100mg/kg	3.23±0.03124	-
4c	100mg/kg	2.42±0.03464	26.21
4d	100mg/kg	0.427±0.023	86.98
4e	100mg/kg	1.531±0.02892	53.32
4f	100mg/kg	2.60±0.03468	20.73
4g	100mg/kg	3.088±0.02892	5.85
4h	100mg/kg	1.38±0.03464	57.92
4i	100mg/kg	3.001±0.02893	8.50
4j	100mg/kg	2.60± 0.03120	20.73

The results of anti-inflammatory activity are given in table no. 9. Among all the synthesized compounds, compound 4d exhibited excellent anti-inflammatory activity, with percent inhibition 86.98 as compared to standard.

Table 10: Results of the antimicrobial activity of the compounds.

Sr. No.	Compound	Zone of inhibition against Gram +ve bacteria.(mm) (S.aureus)		Zone of inhibition against Gram -ve bacteria.(mm) (E.coli)	
		50µgm/ml	100µgm/ml	50µgm/ml	100µgm/ml
1	4a	17	18	14	16
2	4b	16	18	12	13
3	4c	19	20	16	14
4	4d	15	17	14	15
5	4e	08	12	13	16
6	4f	16	18	12	17
7	4g	15	19	14	15
8	4h	17	18	09	15
9	4i	14	16	14	17
10	4j	10	16	12	15
11	Standard	23	27	21	24

As it is mentioned in the above table no. 10, among all the synthesized compounds, compounds 4a, 4b, 4c, 4f, 4h showed significant antimicrobial activity against Gram +ve bacteria and compounds 4a,4c,4g,4i showed significant antimicrobial activity against Gram -ve bacteria in concentration of 50 µgm/ml.

Compounds 4c, 4g exhibited significant antimicrobial activity, while 4a, 4b, 4g exhibited moderate antimicrobial activity, against Gram +ve bacteria and Compounds 4a,4f,4i,4e exhibited significant antimicrobial activity, while 4c,4d,4g,4h,4j exhibited moderate antimicrobial activity, against Gram –ve bacteria in concentration of 100 µgm/ml.

CONCLUSION

The synthesized compounds are identified by spectral data and compounds shows significant to moderate anti-inflammatory activity, some compounds shows prominent antimicrobial activity, based on this the further studies will be done in future.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Chemistry, University of Pune, SAIF Panjab University, Chandigarh for NMR & LC-MS spectral data, Radhabai Kale Mahila Mahavidyalaya Ahmednagar for IR data and the Principal, Padmashri Dr Vithalrao Vikhe Patil Foundation's college of pharmacy, Ahmednagar for providing laboratory facilities.

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