



# SYNTHESIS AND BIOLOGICAL EVALUATION OF (7-HYDROXY-2-OXO-2H-CHROMEN-4-YL) ACETIC ACID HYDRAZIDE DERIVATIVES USED AS A POTENT BIOLOGICAL AGENTS

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## ABSTRACT

The purpose of this research was to development of new potent bioactive molecule with less toxic, safer and easy available. Modern therapeutic is based on scientific observation supported by systematic assessment of activity of drug is simulated and clinical condition. The integrity of the drug molecule, optimization of biological effect, uniform and consistent availability of drug from the dosage.

In the present investigation an attempt is carried out for the synthesis of (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazide (V) from (7-hydroxy-2-oxo-2H-chromen-yl) acetic acid ethyl ester (IV) and to carry out their biological activity. Various Phenols like resorcinol (I); m-cresol; has been condensed with ethylacetoacetate (II) in presence of concentrated sulphuric acid to form 7-hydroxy-4-methylcoumarin (III) by pechmann reaction.

Further these (7-hydroxy-2-oxo-2H-cromen-4-yl) acetic acid hydrazide (V) condensed with various Schiff's base. Also ethyl [(8-amino-4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate have been condensed with fluoroaniline, 4-chlorodinitrobenzene, 4-chlorobenzonitrile, dibromoethane and dibromopropane. Hydrazide derivatives were synthesized to increase Log P value by increasing microbial intracellular concentration and to decrease microbial resistance. The newly synthesized compounds were tested for its antimicrobial, analgesic and antiinflammatory activity. The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, <sup>1</sup>H NMR and mass spectral data.

**Keywords:** Antimicrobial, Analgesic and Antiinflammatory activity, Coumarin and Microbial intracellular concentration.

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## INTRODUCTION

A number of natural and synthetic coumarin (2-oxo-2H-chromen) derivatives have been reported to exert notably antimicrobial<sup>1-4</sup>, analgesic<sup>5-6</sup> and anti-inflammatory<sup>7-8</sup> activity. Moreover; the antibiotic novobiocin belongs to the hydroxy coumarin series. On the other hand, a large number of hydrazides have been reported to possess antibacterial<sup>9</sup>, antifungal<sup>10</sup>, antitumor<sup>11</sup>, antitubercular<sup>12</sup>, antiviral<sup>13</sup> and other biological activities. Presently there are a number of drugs used clinically. In view of these, a project was undertaken to synthesize a new series of (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazide by conventional method and to evaluate the new compounds for their pharmacological activity.

The title compounds were screened for antimicrobial activity by cup plate method<sup>14</sup>, analgesic activity studies were carried out by acetic acid induced writhing method<sup>15</sup> and anti-inflammatory 'Carrageenan induced oedema test'<sup>16</sup>. Synthesis of title compounds was shown in Scheme 1 and 2. The physical constants, yield and analytical data of (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazide derivatives.

## EXPERIMENTAL

The melting points were determined in open capillary tube using Precision melting point apparatus and uncorrected. Thin-layer chromatography was performed with fluorescent silica gel plates HF<sub>254</sub> (Merck),

plates were viewed under UV 254 and 265 light. Infrared spectra's ( $\nu\text{-cm}^{-1}$ ) were recorded on a Shmadzu FT-IR 4000; using KBr disks.  $^1\text{H-NMR}$  spectra were recorded on Bruker Spectrophotometer at 300MHz frequency in  $\text{CDCl}_3$  as well as DMSO using TMS as internal standard reference. Peaks are reported in ppm downfield of TMS. Mass spectra were recorded on 'GCMS-QP2010s' instrument by direct injection method.

### 7-Hydroxy-4-methyl coumarin (III)

In two necked 500ml round bottom flask take 250 ml conc.  $\text{H}_2\text{SO}_4$  and keep into ice bath until the temperature of solution becomes  $0\text{-}10^\circ\text{C}$ . after solution becomes ice cold to this add solution of resorcinol (I), 33 gm(0.01moles) and 35 ml (0.01moles) of ethylacetoacetate(II) dropwise for two hrs after completion of addition the solution stirred for one hr at room temperature and this reaction mixture Was stirred for 16 hrs at room temperature after this the reaction mixture was added into crushed ice. The yellowish solid separated out which was filtered off and the solid was dissolved into the 5% sodium hydroxide solution. And made acidic with 2M  $\text{H}_2\text{SO}_4$  the resultant solid separated was filtered and wash with ice cold water.

### (7-Hydroxy-2-oxo-2H-chromen-4-yl) acetic acid(IV)

In two necked 500 ml R.B.F. take 50-60ml dry DMF. To this add 10gms 7-hydroxy-4-yl coumarin (III), and ethylchloroacetate 6.8 ml (0.056moles) and anhydrous pot. Carbonate 7.7 gms. The resultant mixt was stirred for 9-10 hrs at  $80^\circ\text{C}$ . Then after completion of reaction which monitored by taking TLC the reaction mixture was filtered and pour into large amount of water. The solid separated was filtered and wash with water .the solid was dried and recrystlised from ethanol. The melting point of solid was found to be  $212^\circ\text{C}\text{-}214^\circ\text{C}$ .

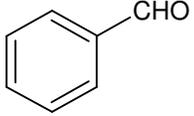
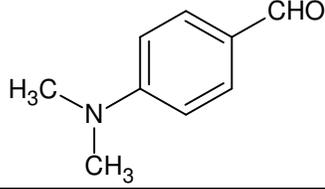
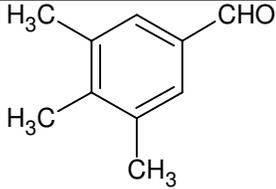
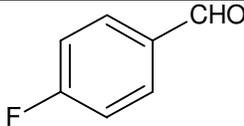
### (7-Hydroxy-2oxo-2H-chromen-yl) acetic acid hydrazide(V)

In 500ml R.B.F take 20ml of absolute ethanol to this add 7gms (0.026mole) of (7-hydroxy-2oxo-2H-chromen-4-yl) acetic acid (IV) and 1.3ml(0.026mole) of hydrazine hydrate. The resultant reaction mixture was refluxed for 5-6hrs .after completion of reaction which monitored by taking TLC the reaction mixt was added into ice-cold water. The solid separates out which was filtered and dried and recrystlised from ethanol.

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

In two necked R.B.F. take 20ml absolute ethanol to this add 1gm (0.00mole) of (7-hydroxy-2oxo-2H-chromen-4-yl)acetic acid hydrazide (V). To this solution add equimolar amount of different aromatic aldehyde and drop glacial acetic acid. The resultant reaction mixt was pour into the ice-cold water. The solid separated was filtered and dried. The solid was recrystlised into ethanol.

Table-1:Different aryl aldehyde attaches with the hydrazide-hydrazone.

S.No.	Compound Code	R-CHO	Sr.No.	Compound Code	R-CHO
1	VIa		6	VI f	
2	VIb		7	VIg	

3	VIc		8	VIh	
4	VI d		9	VI i	
5	VI e		10	VI j	

Table-2: Some characterizations of the compounds

Compound Code	Molecular formula	M.P. (°C)	R <sub>f</sub> Value	% Yield	LC-Mass	IR (KBr cm <sup>-1</sup> )	NMR (δ ppm)
VI a	C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>4</sub>	213	0.65	67	-	3325,3227,3169,2920, 1722,1599,1153	-
VI b	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	218	0.45	64	377	3325,3227,2920, 1722,1650,1599,1153	2.40(s,3H,,CH <sub>3</sub> ) , 3.81(t,1HCH,benzopyran ring) ,6.11(d,2H ,CH <sub>2</sub> ), 6.20 -7.45(m,8H,Ar-CH),10.52(s,1H,NH)
VI c	C <sub>19</sub> H <sub>15</sub> N <sub>3</sub> O <sub>6</sub>	210	0.65	74	-	3325,3227,3169,1722, 1599,1337,1153	-
VI d	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	198	0.53	71	-	3325,3227,3169,2960, 1722,1620, 1599,1153	-
VI e	C <sub>17</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub>	207	0.42	67	-	3325,3227,3169,1722, 1632,1599,1153	-
VI f	C <sub>21</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub>	210	0.45	65	377	3325,3227,3169,1722, 1599,1153,813	2.42 (s,3H,CH <sub>3</sub> ), 4.37(d,1H,CH of ring), 6.22(s,2H ,CH <sub>2</sub> ), 11.36(s,1H,NH), 6.44 - 8.15(m,7H,Ar-CH)
VI g	C <sub>19</sub> H <sub>15</sub> FN <sub>2</sub> O <sub>4</sub>	216	0.56	65	-	-	--
VI h	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub>	214	0.41	78	-	-	-
VI i	C <sub>19</sub> H <sub>15</sub> BrN <sub>2</sub> O <sub>4</sub>	219	0.55	66	-	-	-
VI j	C <sub>21</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	212	0.47	73	-	-	-

## RESULTS AND DISSCUSION

From the literature survey it reveals that the coumarin have been reported for number of pharmacological activities and some molecules have shown significant activities and some compounds shows moderate and good activities. Here we have synthesized some novel (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazide derivatives analogues and screened them for their antibacterial, anti-inflammatory, analgesic activities.

The purity and homogeneity of the synthesized compounds were preliminary checked by their physical constant. The final compounds were found to be soluble in organic solvents. These compounds were characterized by spectral studies for structural elucidation and studies showed satisfactory results.

### Biological Agents

All the newly synthesized novel (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazidewere assayed in vitro for their antibacterial activity against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria), Amoxicillin was used as the standard antibacterial agents, anti-inflammatory activity by ‘Carrageenan induced oedema test using ibuprofen as a standard and analgesic activities by acetic acid induced writhing using ibuprofen as the standard.

The obtained results revealed that the nature of substituent and substitution pattern on the coumarin ring may have a considerable impact on the antibacterial, anti-inflammatory and analgesic activities of the synthesized compounds.

### Antibacterial Activity

Synthesized compounds were evaluated for antibacterial activity by cup-plate diffusion method using *Staphylococcus aureus* and *Escherichia coli*. The amoxicillin were used as standard drug for antibacterial activity. Test compounds and standard drug were used as at the concentration of 100ug/0.01ml. The zones of inhibition of compounds were recorded after incubation of 24 hr at 37°C.

Compounds VIa and VIb exhibited good activity against both bacteria *Staphylococcus aureus* and *Escherichia coli*, Whereas Compounds VIj and VIk showed moderate activity against both bacteria and remaining compounds displayed weak antibacterial activity.

Table-3: Antibacterial activity of newly synthesized compounds [VI a-j].  
Note: Standard (S) – Amoxicillin; Control (C) – DMSO

S. No.	Compound	Zone of inhibition (in mm)	
		<i>E.coli</i>	<i>S.aureus</i>
1	VI a	16	17
2	VI b	17	16
3	VI c	11	09
4	VI d	08	10
5	VI e	10	12
6	VI f	11	09
7	VI g	11	13
8	VI h	12	12
9	VI i	13	15
10	VI j	13	14
11	S	23	21
12	C	08	08

### Anti-inflammatory activity

Synthesized compounds were screened for their anti-inflammatory activity by ‘Carrageenan induced oedema test using ibuprofen as a standard compound. 1% Carrageenan produced increase in paw volume (oedema) of all the animals of various groups.

The onset action was evident from 1 hour in various test groups. The compounds VIc, VIj and VIk showed significant anti-inflammatory activity, other compounds are less active. When compared with standard.

Table-4: Anti-inflammatory activity of newly synthesized compounds [VI a-j].  
Note: N=6; One way ANOVA followed by multiple Tukey's comparison test. \* P≤0.05; \*\* P≤0.01; \*\*\*P≤0.001 when compared with control

S. No	Compound	Dosage	The increase in Paw volume (ml)	% inhibition (mean)
1	VIa	20 mg/kg	0.32±0.01**	47.54
2	VIb	20 mg/kg	0.37±0.02*	39.34
3	VIc	20 mg/kg	0.25±0.01***	59.01
4	VI d	20 mg/kg	0.47±0.01	22.95
5	VIe	20 mg/kg	0.38±0.02**	37.70
6	VI f	20 mg/kg	0.29±0.01***	52.45
7	VI g	20 mg/kg	0.34±0.02**	44.26
8	VI h	20 mg/kg	0.41±0.01	32.78
9	VI i	20 mg/kg	0.39±0.01	36.06
10	VI j	20 mg/kg	0.24±0.02***	60.65
11	Ibuprofen	5mg/kg	0.14±0.01***	75.90

#### Analgesic activity

Synthesized compounds were also screened for their by acetic acid induced writhing using ibuprofen as the standard. The compounds VIa, VIj, VI f and VI e, showed significant analgesic activity while remaining other less active. When their compared with standard.

Table-5: Analgesic activity of newly synthesized compounds [VI a-j].  
Note: N=6; One way ANOVA followed by multiple Tukey's comparison test \* P≤0.05; \*\* P≤0.01; \*\*\*P≤0.001 when compared with standard.

S.No.	Derivatives	Dosage	Number of writhings in 10 minutes (mean ± SEM)	% Inhibition
1	VIa	20 mg/kg	32.33 ± 2.30***	59.68
2	VIb	20 mg/kg	45.5 ± 1.88**	42.83
3	VIc	20 mg/kg	52.66 ± 3.77	33.84
4	VI d	20 mg/kg	49.16 ± 1.01*	38.24
5	VIe	20 mg/kg	38.33 ± 2.30**	51.84
6	VI f	20 mg/kg	35.5 ± 1.88***	55.40
7	VI g	20 mg/kg	42.66 ± 3.77**	46.40
8	VI h	20 mg/kg	57.50 ± 2.57	27.76
9	VI i	20 mg/kg	33.66 ± 3.47***	57.78
10	VI j	20 mg/kg	40.51 ± 2.57**	49.10
11	Pentazocine	5 mg/kg	21.66 ± 1.06***	72.78

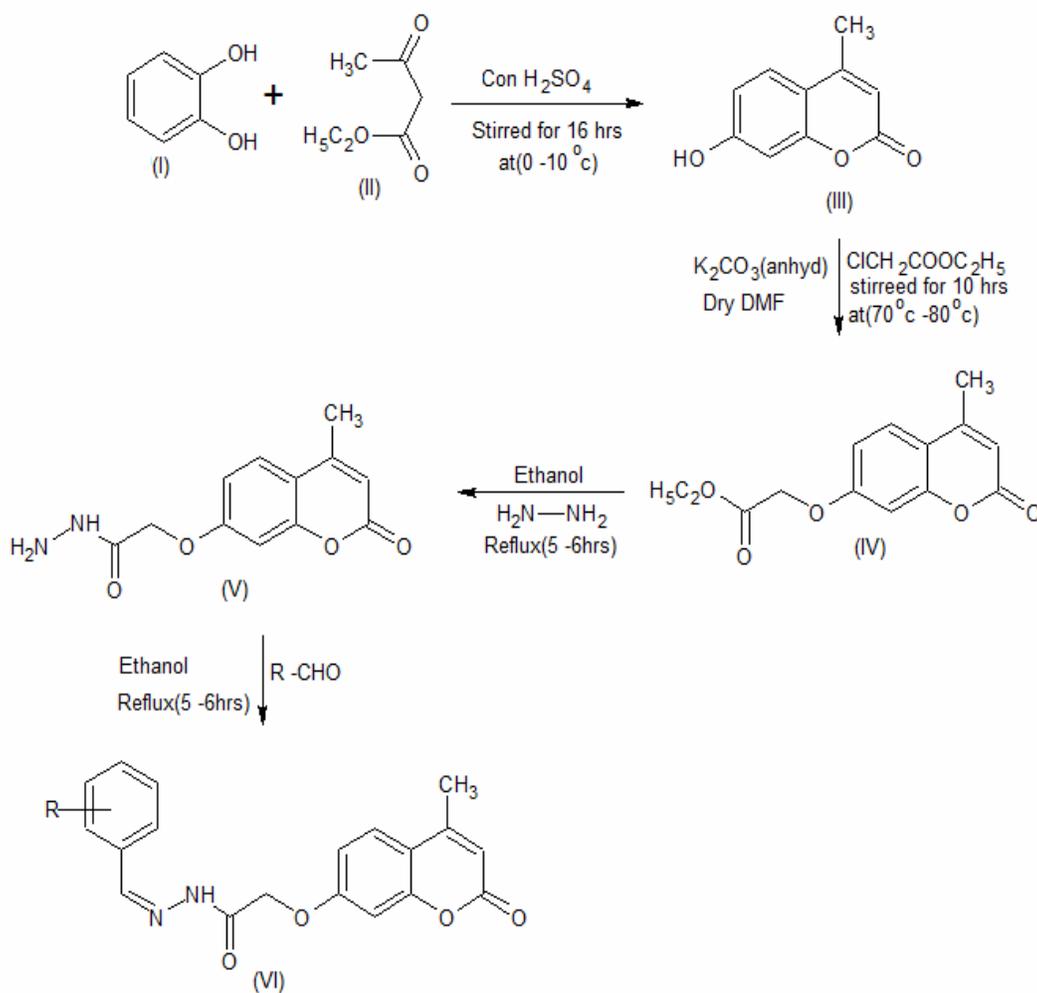
### CONCLUSION

From the data of the Table no. and of antibacterial, anti-inflammatory and analgesic activity, it is clearly concluded that the synthesized compounds are promisingly significant, good antimicrobial agents and anti-fungal agents.

The substituted benzofuran moieties are already known for different biological activities. Here we have synthesized some novel benzofuran analogues combining with different substituted aromatic and hetero cyclic amine ring system with view to get a good antibacterial, anti-inflammatory and analgesic activity with less toxic effects.

As per the results of screening it is clearly indicated that the compounds of the scheme have shown good antibacterial, anti-inflammatory and analgesic activity equipotent with the standard drugs.

From the above results one can establish that the synthesized substituted coumarin can be rich source for the exploitation. Therefore, in search of new generation of the active compounds, it may be worthwhile to explore the possibility in this area or by making or introducing different functional groups or secondary amines or by cyclization as substitutions. Which may results into better pharmacological agents.



Scheme-1

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