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Anti-inflammatory activity of newly synthesised N-[4′-Oxo-2′-(substituted Aryl/ Heteryl)-Thiazolidin-3′-yl]-3-Carboxamido-2H-Chromen-2-one derivatives

V.N. Indulatha, N. Gopal[1], B. Jayakar [2]**

****Department of Pharmaceutical Chemistry, Periyar College of Pharmaceutical Sciences, Tiruchirapalli, Tamilnadu, India.**

[1] Department of Pharmaceutical Chemistry, Balaji Institute of Pharmaceutical Sciences, Warangal, Andhra Pradesh, India.

[2] Vinayaka Missions College of Pharmacy, Salem, Tamilnadu, India.

*****Corres. author: indhumpharm@yahoo.co.in***
Phone no: +91- 99524 01083

Abstract: Coumarins, an old class of compounds, are naturally occurring benzopyrone derivatives. The titled compounds of *N*-[2-(2-substituted aryl / heteryl)-4-oxo-1,3-thiazolidin-3-yl]-2-oxo-2*H*-chromene-3-Carboxamide derivatives (IV-a-m) totally thirteen compounds were prepared from benzylidine derivative of coumarin -3-carbohydrazide (II) which were derived from 3-carbethoxy coumarin derivative by Knoevenagal condensation method. All the Newly synthesized coumarin substituted with thiazolidinones was evaluated for their anti inflammatory activity. Screening of all the synthesized coumarin derivatives by Hind paw oedema method using the standard drug used is Aspirin and test Compounds were give at a dose of 100 mg/kg (bw).
Keywords: Coumarin, Anti inflammatory activity, Carboxamide,Hind paw oedema method, Thiazolidinones.

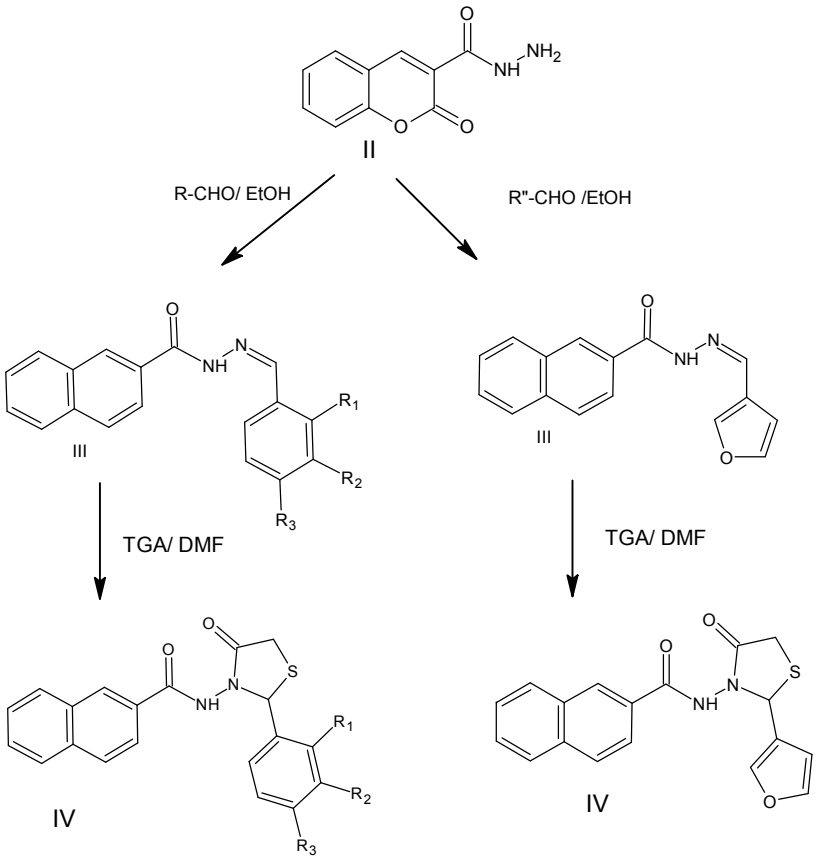
Introduction

Coumarin (1, 2-benzopyrone) the parent molecule of coumarin derivatives, is the simplest compound of a large class of naturally occurring phenolic substances made of fused benzene and α-pyrone ring. A lot of coumarins have been identified from natural sources, especially green plants. Most of the synthesized ones were not to possess notable activity than the warfarin as anticoagulant, due to the position of substitution the biological profile varies in their activity too. In later 1950's this problem was overcome by altering the substitution at various position results activation and inactivation equally.[1]Most of the derivatives of Coumarin were attempted at 4 and 7 and found that the pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. Coumarins have attracted intense interest in recent years because of

their diverse pharmacological properties at third position. [2] The investigation of coumarin compounds revealed that a wide spectrum of medicinal plant extracts that were in use as early as 1000 A.D.[3] contains a high content of coumarins.

In this work Coumarins are synthesized by Knoevenagal condensation.[4] Most of coumarins and substituted with thiazolidinones and azetidinones were possess various biological activities like antibacterial, analgesic, anticonvulsant, antitubercular activity. β lactone of coumarin possess strong antibacterial activity and Aryl-3-alkyl-4- thiazolidinones have been found to be most active as psychomotor anticonvulsants and Barbiturate potentiating agents from our previous work. [5-8] The targeted compounds of *N*-[2-(2-substituted aryl / heteryl)-4-oxo-1, 3-thiazolidin-3-yl]-2-oxo-2*H*-chromene-3-Carboxamide (IV-a-m) were prepared through the initially synthesize from N-(2-substituted benzylidene)-3-carbohydrazide-2H-Chromen-2-one (III a-m). In the first step of this scheme Salicylaldehyde and diethyl malonate condensed in presence of piperidine and ethanol to form its coumarin-3- carboxylate by the method known as Knoevenagal condensation method[9] and it was converted into its corresponding coumarin acid hydrazide (II) derivative by treating with hydrazine hydrate in ethanol. Free amino group of coumarin hydrazide were converted into Aldamino group substituted with benzylidine derivative.

Scheme for the Synthesis of Coumarin Derivatives



R=Aromatic aldehyde

R"=Heteryl aldehyde

R_1=Cl,OH,NO_2

R_2=OH,OCH_3

R_3=Cl,OH,OCH_3,$N(CH_3)_2$,$N(C_2H_5)_2$

1. Chemistry:

General Procedure

Step I: Synthesis of 3-(Carbethoxy)-2H-chromen-2-one

To a solution of salicylaldehyde (0.01 mole; 2.04 gms) in absolute ethanol (25ml) was added diethyl malonate (0.01 mol; 1.41 gms), piperidine (0.01 mol; 0.50 ml) and few drops of glacial acetic acid (0.01 mol; 0.50 ml) . The reaction mixture was refluxed for 2 hrs, cooled to room temperature and poured onto crushed ice. A product which separated was filtered and washed with ice cold aq. 50% ethanol and air dried. It was crystallized form ethanol to give yellow colored crystalline compound I, m.p.: 175-177^0 C (Yield 65.72 %) which was TLC pure [Benzene and acetone (9:1)]

Step II: Synthesis of 3-(carbohydrazide)-2H-chromen-2-one

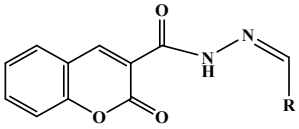
3-(Carbethoxy)-2H-chromen-2-one (I; 0.10 mol; 2.04 gms) was refluxed for 3 hrs with hydrazine hydrate (0.12 mol; 1.41 gms) in absolute ethanol (25 ml). The reaction mixture was cooled to room temperature. Upon cooling the solution clusters of colorless crystalline compound deposited, which was filtered, and crystallized from methanol to give colorless crystalline compound II which was TLC pure [Benzene and acetone (9:1)] m.p.: 182-184 ^{0} C (Yield: 72.23 %).

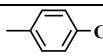
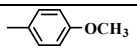
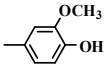
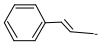
Step III: Synthesis of N- (substituted benzylidene) 3-carbohydrazide -2H-chromen-2-one derivatives (III): A mixture of II and substituted aromatic aldehydes was dissolved in ethanol and refluxed for 4 hrs. The reaction mixture was cooled to room temperature and poured onto crushed ice. A product (III a-m), which separated was filtered, washed with water and crystallized from suitable solvent to give TLC pure crystals (Benzene: Acetone 9:1).

Step IV: Synthesis of N- [4'- oxo -2'- (substituted aryl / heteryl thiazolidin -2- one) -3- carboxamido-2H-Chromen-2-one- (IV): A mixture of III and thioglycolic acid in presence of Aluminium trichloride was refluxed for 5 hrs in an oil bath. The reaction mixture was cooled and triturated with 10% sodium carbonate solution. A solid mass, which separated out was filtered, washed several time with water and crystallized from ethanol to give TLC pure compounds IV a-m. The physical parameters were measured and the collected physical data of the newly synthesized coumarin derivatives were tabulated in Table no 1 and 2.

All the newly synthesized compounds of N- [4'- oxo - 2'- (substituted aryl / heteryl thiazolidin - 2 - one) - 3- Carboxamido - 2H - Chromen - 2-one derivatives were recrystallised and checked their purity by TLC method using benzene: acetone (90:10).The structures of the newly synthesized compounds were established by ^{1}H NMR and IR Spectra.

Table No.:1 Physical Data of N- (Substituted benzylidene) -3- carbohydrazide -2H- Chromen -2- one derivatives (III a-m)



Compound Code	Substituent (R)	Molecular Formula	M. Wt.	Melting Point (°C)	R_f Value	% Yield
III a		$C_{19}H_{13}ClN_2O_4S$	400.83552	189-191	0.5230	72.23
III b		$C_{20}H_{16}N_2O_5S$	396.41644	199-201	0.4603	87.70
III c		$C_{19}H_{14}N_2O_5S$	382.38986	182-184	0.5142	80.35
III d		$C_{19}H_{14}N_2O_4S$	366.39046	196-200	0.8275	75.75
III e		$C_{19}H_{17}N_3O_3$	335.35658	234-237	0.4482	82.43
III f		$C_{20}H_{16}N_2O_5S$	412.41584	212-214	0.5846	88.49
III g		$C_{20}H_{19}N_3O_3$	349.383	278-280	0.5416	72.83

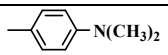
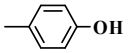
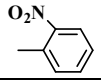
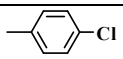
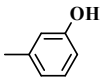
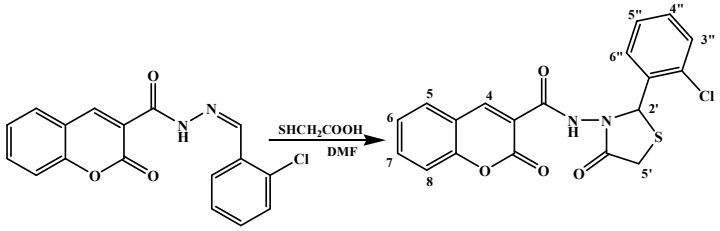
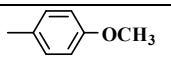
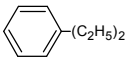
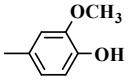
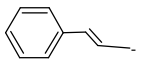
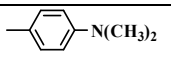
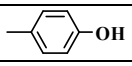
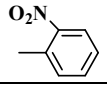
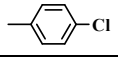
III h		$C_{21}H_{19}N_3O_4S$	409.45826	202-204	0.7058	91.10
III i		$C_{20}H_{14}N_2O_4S$	382.38986	191-193	0.5689	87.22
III j		$C_{21}H_{19}N_3O_4S$	411.38802	162-164	0.5322	87.87
III k		$C_{19}H_{14}N_2O_5S$	400.83552	196-198	0.5846	84.47
III l		$C_{19}H_{15}N_3O_4S$	356.35258	146-149	0.5901	70.80
III m		$C_{19}H_{13}ClN_2O_4S$	382.38986	176-178	0.4843	61.71

Table No.: 2 Synthesis of N- [(Substituted) – thiazolidin - 3' – yl] – 3 – carboxamido - 2H - chromen -2-one (IV a-m)



Compound Code	Substituent (R)	Molecular Formula	M. Wt.	Melting Point (°C)	R_f Value	% Yield
IV a		$C_{19}H_{13}ClN_20_4S$	400.84	221-223	0.6666	69.34
IV b		$C_{20}H_{16}N_20_5S$	396.42	141-143	0.6393	73.98
IV c		$C_{19}H_{14}N_20_5S$	382.39	291-293	0.5892	90.76
IV d		$C_{19}H_{14}N_20_4S$	366.39	282-284	0.4828	83.86
IV e		$C_{23}H_{23}N_3O_4S$	437.51	301-303	0.6904	67.88
IV f		$C_{20}H_{16}N_20_6S$	412.42	286-287	0.5344	82.20
IV g		$C_{20}H_{14}N_2O_4S$	378.40	210-214	0.6393	66.77
IV h		$C_{21}H_{19}N_30_4S$	409.46	274-276	0.5600	94.03
IV i		$C_{19}H_{14}N_20_5S$	382.39	298-300	0.5000	78.60
IV j		$C_{19}H_{13}N_30_6S$	411.39	221-223	0.4745	83.49
IV k		$C_{19}H_{13}ClN_20_4S$	400.84	231-233	0.5849	71.29
IV l		$C_{17}H_{12}N_20_5S$	356.35	192-194	0.3958	85.97
IV m		$C_{19}H_{14}N_20_5S$	382.39	211-213	0.4561	71.39

2. Pharmacology

Investigations with animals in the experimental section that the research followed by the national ethical standards for the care and use of laboratory animals and was approved by the Institutional Ethical Animal experimentation Committee (IEAC).

Anti-Inflammatory Activity[10]

Animals: The studies were carried out on healthy Wister rats weighing between 150-200 gms. The rats were grouped and maintained under standard laboratory conditions with natural light dark cycle. They were fed on standard pellet diet and water ad libitum.

Test compounds, their doses and routs of administration: Carrageenan (0.1 ml of 1% w/c carrageenan suspension in carboxy methyl cellulose) was injected in the sub plantar region of the rats. The standard drug used is Aspirin and test Compounds III $_{a-m}$, IV $_{a-m}$, were giving at a dose of 100 mg/kg (bw). All the test compounds and standard drugs were administered intraperitoneally in the form of 0.5 % CMC.

Experimental procedure: Overnight fasted animas (rats) were weighed and grouped in batches of six each. An identification mark was made with the help of a permanent marker on the left hind paw of each animal near tibia tarsus junction to ensure constant paw volume. The initial paw volume of each rat was measured by mercury displacement method. Test compounds and standard drugs were administered intraperitoneally. After 30 minutes of drug administration, 0.1 ml of 1% of carrageenan was injected in the plantar region of the left hind paw and immediately, paw volume was measured using plythesmograph and also at 60, 120 and 180 minutes reading were recorded.

Thus the edema volume in control group (V_c) and edema volume in groups treated with test compounds (V_t) were measured and the percentage of inhibition of edema was calculated using the given formula and the results were tabulated in Table No.:3-4.

$$\% \text{ Protection} = 100 \quad X \quad \frac{1 - V_t}{V_c}$$

RESULTS AND DISCUSSION

Experimental

1.0. Chemistry:

The reagents / chemicals / solvents used during the course of these studies were obtained from Merck (India), SD Fine and CDH Laboratories and were of the laboratory grade. The solvents were purified by distillation before their use. The solvent systems used for Thin Layer Chromatography were given in the experimental procedure. Silica Gel G used for TLC was CDH brand. Iodine chamber and UV lamps were used for visualization of TLC spots. Whatmann filter paper (No. 1, England) was used for filtration (vacuum or ordinary).

The H^1 NMR spectra were recorded either on 300 MHz or 400 MHz instruments. IR spectra were recorded in Perkin Elmer Company. Melting points of all the compounds were recorded in liquid paraffin bath in open capillary tubes and are uncorrected.

2.0. Pharmacological screening:

2.1. Anti-Inflammatory Activity by Rat Paw Edema Method

Inflammation is a tissue reaction to infection, irritation or foreign substance. The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants. Substances such as carrageenan, formalin, bradykinin, histamine, 5-hydroxy tryptamine, mustard or egg white, when injected in the dorsum of the foot or rats produced acute paw edema within few minutes of the injection. Carrageenan induced paw edema is most commonly used method in experimental pharmacology.

Statistically Analysis: All the obtained results were expressed by Mean ± SEM for each group. Statistical analysis was performed by One way Analysis of Variance (ANNOVA) followed by Dunnet's Test. All compounds showed significant difference when compared with control in all dose levels ($p<0.001$).

All the newly synthesized N-(substituted benzylidene)-3-carbohydrazide-2H-Chromen-2-ones (III $_{a-c, d, f, h-m}$), N-[4'-oxo-2'-(substituted aryl / heteryl)-thiazolidin-3'-yl]-3-carboxamido -2H-chromen-2-ones(IV $_{a-b, d, f, h-m}$), 3-[5'-Thioxo-4',5'-dihydro-1',3',4'-oxadiazol-2'yl-2H-Chromen-2-one (VI) and 3-[5'-(Substituted phenyl) -1',3',4'-oxadiazol-2'yl-2H-Chromen-2-one derivatives (VII $_{a-e}$) were tested for anti-inflammatory activity at a dose of 100 mg/kg (bw) and compared with Standard drug Aspirin [100 mg/kg

(bw)]. The paw volume was measured by using plythesmograph at 60, 120 & 180 min. The data of 1 hr and 2 hrs was subjected to statistical analysis by One-way Analysis of Variance (ANOVA) followed by Dunnet's test.

N - (Substituted benzylidene) - 3 - carbohydrazide - 2H – Chromen – 2 - ones (III $_{a-m}$)

All the test compounds were showed 50 to 60 % inhibition of edema after 3 hrs from the time of administration, while compound III $_{b,}$ III $_{c}$, III $_{f}$ and III $_{k}$ showed less activity even after 3 hrs at a dose of 100 mg/kg (bw). Standard drug Aspirin showed 70.75 % of inhibition after 1 hr and 69.17 % inhibition of edema after 2 hrs from the time of administration. Among the test compounds compound III $_{l}$ & III $_{a}$ showed 55.45 and 51.44 % inhibition of edema respectively after 1 hr with a pValue of less than 0.001 ($p<0.001$) indicating that these two compounds are statistically highly significant from the standard drug aspirin. Test compounds III $_{b}$, III $_{c}$ and III $_{K}$ showed very less percentage of inhibition of edema (10 to 20 %) after 1 hr from the time of administration, where as remaining compounds exhibited 39 to 47 % of inhibition and considered these compounds are moderate in their action when compared to the standard drug Aspirin (70.75 %) .

Compound III $_{l}$ found to be quite superior in its percentage of inhibition of edema after 2 hrs from the time of administration (60.47 %) when compared with the standard drug aspirin (69.17 %) with the pValue of less than 0.05 ($p < 0.05$) indicating that they are statistically significant different from aspirin.

Compound III $_{a}$ and III $_{d}$ showed 58.80 & 54.32 % inhibition of edema respectively after 2 hrs from the time of administration and their p Values were less than 0.01 indicating that they are statistically slightly significant and these compounds could be considered to be good in controlling the inflammation in comparison to standard drug aspirin. Compounds III $_{j}$ and III $_{m}$ showed same percentage of inhibition (49.71%) of edema after 2 hrs from the time of administration with p Value of $p<0.001$ indicating that statistically highly significant. All the test compounds showed almost same level of percentage of inhibition after 3 hrs from the time of administration when compared to the inhibition of edema after 2 hrs, while standard drug aspirin showed a decrease in inhibition of edema after 3 hrs from the time of administration.

In conclusion, almost all the test compounds exhibited a moderate anti-inflammatory activity while compound III $_{l}$ showed a good anti-inflammatory activity in comparison to the standard drug Aspirin. Compound III $_{b}$, III $_{c}$, III $_{f}$ and III $_{k}$ were not to posses anti-inflammatory activity even at higher doses also.

N-[4'- oxo - 2' - (substituted aryl/ heteryl) - thiazolidin-3'-yl] – 3 - carboxamido- 2H-chromen-2-ones (IV $_{a-m}$)

The newly synthesized N-[4'-oxo-2'-(substituted aryl / heteryl)-thiazolidin-3'-yl]-3-carboxamido-2H-chromen-2-ones (IV $_{a-m}$) exhibited the maximum inhibition of edema after 2 hrs from the time of administration and the percentage inhibition is decreased after 3 hrs from the time of administration. After 1 hr from the time of administration, the standard drug Aspirin showed 69.11 % of inhibition of edema and in the test compounds, compound IV $_{l}$ showed maximum inhibition of edema 57.03 % with a p Value of less than 0.05 ($P<0.05$) indicating that it is statistically significant different from aspirin. It could be considered as good in controlling the inflammation as standard drug, followed by compound IV $_{f}$ showed 57.03 % of inhibition of edema and p Value is $p<0.01$ indicating that statistically slightly significant. Compound IV $_{m}$ exhibited 52.14 % of inhibition of edema after 1 hr from the time of administration and remaining all compounds did not show any activity after 1 hr from the time of administration.

Table No.: 3: Anti-Inflammatory Activity of N- (Substituted Benzylidene) - 3 - Carbohydrazide - 2H - Chromen -2- One Derivatives (III $_{a-m}$)

Compound No	After 1 hour		After 2 hours		After 3 hours	
	Mean±S.D.	% Inhibition	Mean±S.D.	% Inhibition	Mean±S.D.	% Inhibition
III $_a$	6.44±0.96	51.44*	6.81±0.98**	58.80	9.19±0.62	54.94
III $_b$	10.33±1.03	22.10	11.58±0.73	29.97	12.93±0.82	36.57
III $_c$	11.87±0.75	10.49	13.30±0.78	19.52	14.32±1.16	29.77
III $_d$	7.03±0.64	47.03	7.55±1.36**	54.32	9.59±0.57	52.94
III $_e$	7.16±0.54	41.34	8.41±0.73	47.58	10.17±0.35	50.71
III $_f$	10.91±0.43	17.77	13.41±1.34	18.89	15.58±0.73	23.59
III $_g$	5.89±0.48	54.89*	6.51±0.78***	60.01	7.48±0.81	64.05
III $_h$	7.76±1.10	41.48	8.62±0.93	47.83	10.09±0.84	50.52
III $_i$	8.05±0.89	39.33	8.62±0.49	47.83	9.75±0.79	52.17
III $_j$	7.19±1.00	45.77	8.31±1.18*	49.71	9.76±0.45	52.12
III $_k$	11.41±1.35	13.97	11.91±0.75	27.96	13.41±1.00	34.22
III $_l$	5.91±0.75	55.45*	6.54±1.08***	60.47	7.21±0.80	64.62
III $_m$	7.03±0.86	47.03	8.31±1.18*	49.71	8.59±1.09	57.84
Control	13.27±0.39	-	16.53±0.93	-	20.38±0.45	-
Aspirin	3.88±0.76	70.75	5.10±0.83	69.17	8.37±1.38	58.95

* p< 0.001; ** p < 0.01; *** p < 0.05; when compared against Aspirin

Table No.: 4: Anti-Inflammatory Activity of N- [4'- Oxo -2'- (Substituted Aryl / Heteryl) – Thiazolidin - 3' - yl] – 3 – Carboxamido - 2H –Chromen – 2 - One Derivatives (IV $_{a-m}$)

Compound No	After 1 hour		After 2 hours		After 3 hours	
	Mean±S.D.	% Inhibition	Mean±S.D.	% Inhibition	Mean±S.D.	% Inhibition
IV $_a$	7.81±0.99	42.76	6.48±1.51**	60.18	9.15±0.98	51.33
IV $_b$	12.41±0.85	9.02	13.59±0.82	16.40	15.79±0.97	15.99
IV $_c$	7.55±0.88	43.01	9.01±0.45	43.01	10.11±0.47	42.01
IV $_d$	8.25±0.79	39.53	8.59±1.08	47.17	11.22±1.23	40.31
IV $_e$	9.47 ±0.66	32.24	9.04±0.12	43.02	10.14±0.78	43.01
IV $_f$	5.861±0.98	57.03**	6.761±1.07**	58.43	12.22±0.78	34.99
IV $_g$	12.01±0.85	9.01	13.47±0.12	16.10	15.52±0.14	15.71
IV $_h$	9.18±0.95	32.67	9.26±0.84	43.08	10.39±0.82	44.75
IV $_i$	9.80±0.87	28.14	10.71±1.00	34.13	11.84±1.04	37.02
IV $_j$	11.80±1.02	13.49	6.54±0.95*	59.77	14.54±0.82	22.63
IV $_k$	7.66±1.04	43.82	9.181±0.91	43.52	10.72±0.85	42.97
IV $_l$	5.62±1.00	58.77***	6.97±0.92*	57.15	10.34±0.80	45.00
IV $_m$	6.53±0.87	52.14*	6.76±1.0 *	58.43	9.00±0.64	52.15
Control	13.61±0.89	-	16.26±0.97	-	18.80±0.52	-
Aspirin	4.21±0.75	69.11	4.10±0.85	74.81	8.87±1.00	52.83

* p< 0.001; ** p < 0.01; *** p < 0.05; when compared against Aspirin

Conclusion:

Compound IV $_a$, IV $_f$, IV $_j$, IV $_l$ and IV $_m$ exhibited 57 - 60% of inhibition of edema after 2 hrs from the time of administration, while the standard drug aspirin showed 74.81 % inhibition of edema. Among the synthesized compounds, compound IV $_a$ exhibited 60.18 % inhibition of edema after 2 hrs from the time of administration and found to be quite superior in activity and is statistically slightly significant ($p<0.01$), followed by compound IV $_f$ and IV $_m$ which are statistically highly significant ($p<0.001$) when compared with the standard drug. Both these compounds exhibited 58.43 % inhibition of edema after 2 hrs from the time of administration and considered to be good anti-inflammatory action when compared to the Aspirin. Compounds IV $_d$, IV $_h$, and IV $_k$ showed 47.17, 43.08 and 43.52 % respectively, inhibition of edema after 2 hrs from the time of administration and considered to be moderate in their action, where as compound IV $_b$ showed 16.40 % inhibition of edema and this compound were not showed any increase or decrease in the activity even after 3 hrs from the time of administration. Hence, compound IV $_b$ could be considered as devoid of anti-inflammatory activity. The values were considered pValue of <0.05, <0.01, < 0.001 to be statistically significant, slightly significant and highly significant respectively.

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