Research Article

Synthesis and pharmacological evaluation of schiff bases of 7-amino-4-methyl coumarins as novel anti-inflammatory agents

R. Jaya Krishna, Abhishek Pandagale, P. M. Ronad, S. S. Honnalli, Bhrigu Kumar Das, Pramod C. Gadad[*]

KLE College of Pharmacy, Hubli, Karnataka, India (580031)

Abstract

Objective: The present investigation involves the synthesis and examination of anti-inflammatory activity of schiff bases of 7-amino-4-methyl-coumarins in different experimentally induced *in-vivo* inflammatory models. **Materials and methods:** The compounds were synthesized by reaction of 7-amino-4-methyl-coumarin with ethyl chloroformate to obtain 7-urethane-4-methyl-benzopyran-2-one. This was further condensed with hydrazine hydrate to obtain N-(4-methyl-2-oxo-2H-chromen-7-yl) hydrazine carboxamide, which was refluxed with substituted aromatic aldehydes to form schiff base (IVa-c). The structure of the newly synthesized compounds was confirmed by spectral data. The *in-vivo* anti-inflammatory activity of synthetic compounds was carried out using acute anti-inflammatory models (Histamine, Carrageenan and Formalin induced paw oedema) and sub-acute anti-inflammatory model (Cotton pellet granuloma). **Results and conclusion:** The results showed that treatment with the coumarin derivatives (50 mg/kg) significantly inhibit the acute and sub-acute anti-inflammatory models when compared with the control treated rats. The present study suggests that the coumarin derivatives possess anti-inflammatory activity in different experimentally induced inflammatory models.

Keywords: Coumarin derivatives; anti-inflammatory; inflammatory mediators; diclofenac sodium

Introduction

Inflammation usually a life preserving phenomena is a local response of mammalian tissue to infection, irritation or any foreign substance. It is a complex body defense process for elimination or minimizing the spread of injurious agents. The classic signs of inflammation are local redness, swelling, heat, pain and loss of function. It is characterized by immune cell invasion, local release of cytokines and sometimes accompanied by functional or structural damage of the invaded tissue. It is regulated by a cascade of inflammatory mediators such as histamine, bradykinin, nitric oxide (NO), prostaglandin E_2 (PGE_2), cytokines, necrosis factor-α (TNF-α) and interleukin-6 (IL-6); playing a crucial role in acute and chronic inflammation (Fujiwara and Kobayashi, 2005; Ferrero-Miliani et al., 2007; Mitchell and Cotran, 2000).

An inflammatory response occurs in three distinct temporal phases, each apparently mediated by different mechanism: Acute inflammation characterized by transient local vasodilation, increased capillary permeability which represent the early body reaction and it is of short duration. Sub-acute inflammation characterized by infiltration of leukocytes and phagocytic cells and chronic inflammation during which the tissue degeneration and fibrosis occur (Pountos et al., 2011).

Throughout the world rise of inflammatory disease as arthritis, allergic rhinitis, eczema and asthma are treated with conventional anti-inflammatory drugs and NSAIDs. Despite their widespread use, NSAIDs are often associated with severe side effects; the most common being gastrointestinal bleeding (Gupta et al., 2005). Coumarins are one of the groups of compounds that have been reported to possess various effects on inflammation, nociception and pyrexia. From previous studies it has been shown that natural products containing coumarins as active constituent have been

Address for Corresponding Author:

Dr. Pramod C. Gadad

Department of Pharmacology, KLE College of Pharmacy, Vidyanagar, Hubli, Karnataka, India (580031)

E-mail: gadadpramod@gmail.com

reported for anti-inflammatory, analgesic and antipyretic activities (Gupta et al., 2005; Arul et al., 2005). Hence, it is important to develop novel derivative of anti-inflammatory agents with minimal adverse effects. Some chemically synthesized coumarin derivatives including novel benzopyranone congeners have also been reported for their anti-inflammatory, analgesic and antipyretic activities (Eissa et al., 2009). Some bi heterocyclic coumarin derivatives (Ghate et al., 2005), 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (Khode et al., 2009), 6-aminomethyl derivatives of benzopyran-4-one (Hasan et al., 2009) are reported to have anti-inflammatory and analgesic activities. Further novel pyrimidine derivatives of coumarin have been reported for its anti-inflammatory and antipyretic activities (Keri et al., 2010).

Hence, in the present study we investigated the anti-inflammatory effect of some novel coumarin derivatives viz. 1-(2-hydroxybenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7-yl) semicarbazide (referred as compound I), 1-(4-chlorobenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7-yl) semicarbazide (referred as compound II) and 1-(2-nitrobenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7-yl) semicarbazide (referred as compound III) in different *in-vivo* experimental inflammatory models.

Materials and methods

Animals

Male albino rats of wistar strain weighing 150-200 g were procured from the animal house of Venkateshwar Enterprises, Bangalore, Karnataka for this study. The animals were acclimatized for one week in standard polypropylene cages and maintained at $27^oC \pm 2^oC$ under 12 h dark and light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum* was provided. Ethical clearance for the use of animals was obtained from the institutional animal ethics committee prior to the beginning of the project work.

Chemicals and reagents

Histamine, Carrageenan, Formalin were purchased from Himedia Ltd. Mumbai. Marketed product of Diclofenac sodium was purchased from Korten Pharmaceutical Pvt. Ltd. Mumbai. All the other chemicals (solvents and reagents) used in this study were of analytical grade obtained from S.D. Fine chemicals.

Chemistry

The melting points were determined by open capillaries on a Thermonik melting point apparatus and are uncorrected. The IR spectra were recorded on a Thermo nicolet FTIR-200 spectrometer, using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II, 400 MHz, spectrometer in $CDCl_3$ using TMS as an internal standard and the values are expressed in δ ppm. The mass spectra were recorded using

GCMS-QP 2010 Shimaduzu (Cipla Laboratory, Mumbai).

Synthesis: (Rama Ganesh et al., 2010; Ronad et al., 2008; Robinson, 1990; Pretka and Wilmington, 1961)

1. Synthesis of N-(4-methyl-2-oxo-2*H*-chromene-7-yl) hydrazine carboxamide [III]:

An intimate mixture of 7-carbethoxyamino-4-methylcoumarin (1.9 g, 0.01 mmol) and hydrazine hydrate (99%) (2 ml, 0.02 mmol) in absolute methanol on water bath was allowed for 5.0 hrs. Completion of reaction was monitored by TLC. After cooling, the reaction mixture was poured into ice-cold water. The solid thus separated was collected by filtration, dried and re-crystallized from ethanol. Rf: 0.48; Solvent system: Chloroform: Acetone (4:1); M.P.170-175^{0}C; yield: 60%.

IR(KBr) cm^{-1}: 3268.07-3336.13 (-NH$_2$); 1692.4 (>C=O stretching 2-α-Pyrone); 1619.61 (NH-C=O of amide).

2. Synthesis of 2-benzylidene-N-(4-methyl-2-oxo-2*H*-chromen-7-yl) hydrazine carboxamide derivatives [IV] (Figure I):

A mixture of N-(4-methyl-2-oxo-2H-chromene-7-yl) hydrazine carboxamide (1.5 g, 0085 mmol) and substituted aromatic aldehyde (0.017 mmol) in 25 ml of absolute alcohol and 0.5 ml of acetic anhydride was refluxed for 6 h, then, the solvent was removed under reduced pressure. The resulting crude schiff' base was washed with cold water and re-crystallized by using appropriate solvents. The purity of the compounds was confirmed by TLC using silica gel G as stationary phase and mobile phase used is ethylacetate:cyclohexane (1:2).

1-(2-hydroxybenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7-yl) semicarbazide (IVa):

IR (KBr) cm^{-1}: 1235.46 (C-O); 1573.05 (>C=C); 1578.17 (C=N); 1618.22 (CONH); 1684.67 (>C=O of 2-α-pyrone); 2983.60, 2981.35 (Ar-CH); 3335.43 and 3264.19 (NHCONH stretching).

^{1}**H NMR (DMSO) 400Mz δ ppm:** 1.2 (s,3H,4-CH$_3$); 6.2 (s,1H,C$_3$-H); 7.4-7.6 (m,7H,Ar-H); 7.9(s,1H,CH=N); 9.8 (s, 2H, >CONH).

1-(4-cholorobenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7-yl) semicarbazide (IVb):

IR (KBr) cm^{-1}: 757.41 (C-Cl); 1235.46 (C-O); 1578.17 (C=N); 1618.43 (CONH); 1684.67 (>C=O of 2-α-pyrone); 2981.35 (Ar-CH); 3264.77 and 3337.73(NHCONH stretching).

^{1}**H NMR (DMSO) 400Mz δ ppm:** 1.3 (s,3H,4-CH$_3$); 6.1 (s,1H,C$_3$-H); 7.4-7.6 (m,7H,Ar-H); 8.02 (s,1H,CH=N); 9.8 (s, 2H, >CONH).

1-(2-nitrobenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7-yl) semicarbazide (IVc):

IR (KBr) cm^{-1}: 1232.57 (C-O); 1574.52 (C=N); 1620.34(CONH); 1690.39 (>C=O of 2-α-pyrone); 3264.22 and 3334.49 (NHCONH stretching).

^{13}C NMR (DMSO) 400Mz δ ppm: 179.9 (4-CH$_3$); 111.7 (C$_3$ of 2-α-pyrone); 104.48-153.78 (12 Ar-C); 160.06 (>C=O of 2-α-pyrone).

Experimental protocol

Rats were divided into 5 groups with 6 animals in each group. Group I served as control animals and was treated with 1% w/v sodium CMC *p.o.* Group II served as standard with Diclofenac sodium (10 mg/ml/kg, *p.o.*). Group III-V animals were treated with the test compound I, II and III (50 mg/5ml/kg body weight, *p.o.*).

Acute anti-inflammatory models

Histamine induced hind paw oedema (Gupta et al., 2005)

For the determination of anti-inflammatory effect, the histamine induced paw oedema model was employed. Thirty minutes after the oral administration of the control, standard and test compound, each rat was injected with a freshly prepared 0.1 ml of 1 mg/ml histamine in normal saline into the subplantar region of the right hind paw. The paw volume was measured by means of a volume displacement technique using a digital plethysmometer before and 0.5, 1, 2, 3, 4 and 5 h after induction of inflammation. The percentage (%) inhibition of oedema is calculated as:

Percentage inhibition: $[V_c-V_t/V_c] \times 100$

V_c= Oedema volume/paw thickness of control animals.

V_t= Oedema volume/paw thickness of treated animals.

Carrageenan induced hind paw oedema (Winter et al., 1962)

Oedema was induced by injecting 0.1 ml of 1% carrageenan suspension according to Winter *et. al*., 1962. The treatment, grouping of the animals and percentage of inhibition remain similar to that of the histamine induced inflammatory model except the inducing agent.

Formalin induced hind paw oedema (Kalkhambkar et al., 2008)

The model as employed was treated in the same way as in above model except that formalin (0.1 ml of 1% v/v) was used as oedematogenic agent. The other details remain similar as mentioned in the above model.

Sub-acute anti-inflammatory models

Cotton pellet granuloma (Vogel and Gang, 2008)

The model as adopted for sub-acute inflammation was induced by cotton wool. The adsorbent cotton wool used was cut into pieces weighing 10 ± 1 mg, made into pellets and was sterilized (120ºC; 2 h). Prior to implantation the animal was anaesthetized, abdomen was shaved cleanly, swabbed with 70 % ethanol and two pieces of sterilized cotton pellets were implanted subcutaneously, one on each side of the abdomen. The test drugs were administered once daily throughout the experimental period of 7 days. On the 8th day, rats were anaesthetized and pellets were dissected and dried at 60ºC for 18 h, weighed after cooling. The mean weight of the cotton pellets of the control as well as of the test group was calculated. The results are expressed as percentage inhibition of granuloma, calculated as:

Percentage inhibition: $[Wt_c-Wt_t/Wt_c] \times 100$

Wt_c= Granuloma weight of control animals.

Wt_t= Granuloma weight of treated animals.

Statistical analysis

All data are expressed as Mean ± Standard Deviation (S.D). The statistical significance was analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. For statistical analysis, data was computed by using Graph Pad Prism Software version 5.0.

Results

Acute anti-inflammatory models

Effect of coumarin derivatives on histamine induced hind paw oedema volumes and percentage inhibition: Table 1 shows the status of paw oedema volumes and percentage inhibition of the control, standard and test compound in experimental animals. There was a gradual increase in oedema paw volumes in control group showing its maximum value at 3 h. The standard (Diclofenac sodium) and test compound groups (50 mg/kg) showed significant decrease in paw volume when compared with control group. The test compound group II and III significantly inhibited ($p<0.001$) the histamine induced hind paw oedema formation at 30 min when compared with the control group.

Effect of coumarin derivatives on carrageenan induced hind paw oedema volumes and percentage inhibition: The changes in the level of paw oedema volumes and percentage inhibition of the control, standard and test compound in experimental animals are illustrated in table 2. Animals administered with the standard (Diclofenac sodium) and test compound groups (50 mg/kg) showed significant decrease in paw volume when compared with control group. The test compound II and III significantly inhibited ($p<0.001$) the carrageenan induced hind paw oedema formation from 2 to 5 h when compared with the control group.

Effect of coumarin derivatives on formalin induced hind paw oedema volumes and percentage inhibition: Activity for the changes in the level of paw oedema volumes
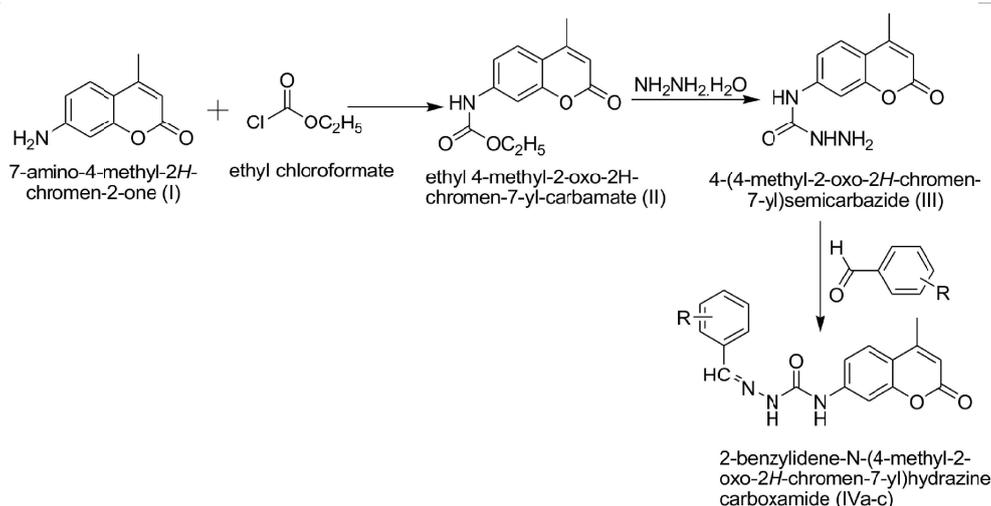
Figure 1. Synthetic route for coumarin derivatives

Table 1. Effect of coumarin derivatives on Histamine induced hind paw oedema volumes and percentage inhibition

Treatment	Dose	Rat hind paw volume change in ml (Mean ± SD) and % inhibition					
		30min	1st h	2nd h	3rd h	4th h	5th h
Histamine control	3ml/kg	0.53±0.06	0.57±0.06	0.60±0.05	0.67±0.04	0.59±0.06	0.58±0.07
Diclofenac sodium	10mg/kg	0.29±0.06***	0.46±0.03**	0.44±0.04***	0.41±0.05***	0.39±0.03***	0.40±0.05***
		(45.28%)	(19.28%)	(26.66%)	(38.80%)	(33.89%)	(31.03%)
Compound-I	50mg/kg	0.43±0.03*,###	0.51±0.02	0.49± 0.06*	0.54±0.05**	0.45±0.05***	0.44±0.07*
		(18.86%)	(10.52%)	(18.33%)	(19.40%)	(23.72%)	(24.13%)
Compound-II	50mg/kg	0.38±0.05***,#	0.49±0.06	0.50±0.05*	0.55±0.07*,##	0.48±0.04**,#	0.42±0.06**
		(28.30%)	(14.03%)	(16.66%)	(17.9%)	(18.64%)	(27.58%)
Compound-III	50mg/kg	0.35±0.05***	0.48±0.05	0.46±0.06**	0.52±0.06**,#	0.43±0.03***	0.41±0.09**
		(33.96%)	(15.78%)	(23.33%)	(22.38%)	(27.11%)	(29.31%)

Values are expressed as Mean ± S.D (n=6). Comparisons are made between: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with histamine control, ### $p < 0.001$, ## $p < 0.01$, # $p < 0.05$ when compared with diclofenac sodium.

Table 2. Effect of coumarin derivatives on Carrageenan induced hind paw oedema volumes and percentage inhibition.

Treatment	Dose	Rat hind paw volume change in ml (Mean ± SD) and % inhibition					
		30min	1st h	2nd h	3rd h	4th h	5th h
Carrageenan control	3ml/kg	0.91±0.09	1.04±0.09	1.20±0.07	1.46±0.08	1.36±0.07	1.33±0.07
Diclofenac sodium	10mg/kg	0.58±0.04***	0.73±0.06***	0.89±0.03***	0.93±0.04***	0.85±0.02***	0.82±0.04***
		(36.26%)	(28.80%)	(25.83%)	(36.30%)	(37.50%)	(38.34%)
Compound-I	50mg/kg	0.88±0.04###	0.96±0.02###	1.11±0.13###	1.14±0.13***,##	1.17±0.10***,###	1.19±0.07**,###
		(3.29%)	(7.69%)	(7.50%)	(16.17%)	(19.86%)	(10.52%)
Compound-II	50mg/kg	0.87±0.07###	0.91±0.06*,###	0.94±0.06***	1.01±0.10***	1.03±0.06***,###	1.11±0.05***,###
		(4.39%)	(12.50%)	(21.66%)	(30.82%)	(24.26%)	(16.54%)
Compound-III	50mg/kg	0.84±0.05###	0.90±0.07**,###	0.93±0.06***	0.96±0.06***	1.00±0.04***,##	0.99±0.08***,##
		(7.69%)	(13.46%)	(22.50%)	(34.24%)	(26.47%)	(25.56%)

Values are expressed as Mean ± S.D (n=6). Comparisons are made between: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with carrageenan control, ### $p < 0.001$, ## $p < 0.01$ when compared with diclofenac sodium.

and percentage inhibition was statistically significant for the treatment group when compared to control group (table 3). Significant reduction ($p<0.001$) in formalin induced hind paw oedema was observed in the test compound group II and III from 2 to 5 h when compared with the control group.

Sub-acute anti-inflammatory models

Effect of coumarin derivatives on cotton pellet granuloma: The effect of coumarin derivatives and diclofenac sodium on the proliferative phase of inflammation (wet, dry and transudative weight) has been summarized in table 4. The percentage of inhibition of compound III and diclofenac sodium (wet weight) was found to be 57.60 and 58.50 % ($p<0.001$) respectively. Similar observations were made for dried and transudative weight as well. The inhibition of inflammation by compound III and diclofenac sodium were as 59.55 and 64.01 % ($p<0.001$) respectively.

Discussion

In this study, acute and sub-acute anti-inflammatory *in-vivo* models were assessed for evaluating the effect of coumarin derivatives to be used in the treatment of inflammatory disorders. They are the suitable test procedure being employed, which has been commonly used to screen the antiedematous effect of any drug. Coumarins constitute one of the major classes of naturally occurring compounds, and interest in its chemistry continues unabated because of its usefulness as biologically active agents.

The inflammatory process consists of a series of events that can be elicited by numerous stimuli. Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms: An acute phase, characterized by local vasodilation and increased capillary permeability mediated by the release of serotonin and histamine (Winter et al., 1962; Crunkhorn and Meacock, 1971), an sub-acute phase, characterized by infiltration of leukocytes and phagocytic cells which is mediated by the release of prostaglandins, protease and lysosome (Vinegar et al., 1987a) and a chronic proliferative phase in which tissue degeneration and fibrosis

Table 3. Effect of coumarin derivatives on Formalin induced hind paw oedema volumes and percentage inhibition.

Treatment	Dose	Rat hind paw volume change in ml (Mean ± SD) and % inhibition					
		30min	1st h	2nd h	3rd h	4th h	5th h
Formalin control	3ml/kg	0.73±0.05	0.83±0.04	0.89±0.05	0.99±0.07	1.07±0.06	1.19±0.04
Diclofenac sodium	10mg/kg	0.33±0.03***	0.50±0.04***	0.67±0.05***	0.71±0.03***	0.64±0.07***	0.64±0.08***
		(54.79%)	(39.75%)	(24.71%)	(28.28%)	(40.80%)	(46.21%)
Compound-I	50mg/kg	0.71±0.05###	0.75±0.04*,###	0.81±0.03###	0.89±0.04***,##	0.90±0.06***,###	0.97±0.05**,###
		(2.73%)	(9.63%)	(8.98%)	(10.10%)	(15.88%)	(18.48%)
Compound-II	50mg/kg	0.72 ±0.03###	0.76±0.06#	0.79±0.03***	0.84±0.04***	0.90±0.04***,###	0.98±0.06***,###
		(1.36%)	(8.43%)	(11.23%)	(15.15%)	(15.88%)	(17.64%)
Compound-III	50mg/kg	0.65±0.07###	0.68±0.05#	0.73±0.05***	0.77±0.05***	0.83±0.03***,##	0.82±0.05***,##
		(10.95%)	(18.07%)	(17.97%)	(22.22%)	(22.42%)	(31.09%)

Values are expressed as Mean ± S.D (n=6). Comparisons are made between: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with formalin control, ### $p < 0.001$, ## $p < 0.01$, # $p < 0.05$ when compared with diclofenac sodium.

Table 4. Effect of coumarin derivatives on Cotton pellet granuloma in experimental rats

Treatment	Dose	Wet weight (mg)	% of inhibition	Dry weight (mg)	% of inhibition	Transudative weight	% of inhibition
Control	3ml/kg	183.8+7.26	--	76. 23±2.80	--	107.57±6.50	--
Diclofenac sodium	10mg/kg	76.27 ±4.53***	58.50	27.43 ± 2.78***	64.01	48.84±6.037***	54.63
Compound-I	50 mg/kg	103.6± 11.52***,###	43.63	45.97± 4.22***,##	39.69	57.65±8.966***	46.39
Compound-II	50 mg/kg	94.12 ±3.77***,###	48.79	42.67± 6.68***,##	44.02	54.78±6.21***	52.13
Compound-III	50 mg/kg	77.92 ±2.02 ***	57.60	30.83 ±6.30***	59.55	47.08±6.20***	56.19

Values are expressed as Mean ± S.D (n=6). Comparisons are made between: *** $p < 0.001$ when compared with control, ### $p < 0.001$, ## $p < 0.01$ when compared with diclofenac sodium.

occur (Winter et al., 1962). Acute inflammation is marked by three major components as changes in vascular caliber leading to increased blood flow, structural changes of microvasculature causing leakage of blood vessels and extravasation of leucocytes leading to their accumulation and activation at the site of injury.

As discussed above, histamine a basic amine is a potent vasodilator as well as an important mediator of inflammation which enhance the vascular permeability. They act with prostaglandins (PGs) to induce oedema (Linardi et al., 2000). In this work, the reference drug diclofenac sodium significantly decreased paw volume until the end of 5 h compared to control group. However, coumarin derivatives differed in their response as they were not successful at 1 h, but they did produce anti-inflammatory effects at 30 min and later from 2nd hour until the end of the study when compared with the control. This change in their response indicates that the compounds although successfully inhibiting the release of histamine from the mast cells and other inflammatory mediators in the beginning, might not be effective in inhibiting the release of PGs. Therefore, it can be inferred that the inhibitory effect of coumarin derivatives may be due to anti-histaminic activity.

Carrageenan induced paw oedema in rats is commonly used for acute inflammation and is believed to be bi-phasic (Vinegar et al., 1969b). The first phase is the resultant of the concurrent release of early mediators, such as histamine, serotonin, and kinins (Crunkhorn and Meacock, 1971) and the second phase is explained by an increased production of PGs, oxygen-free radicals, and inducible cyclo-oxygenase (COX-2) (Gepdiremen et al., 2004). In our study, reference drug diclofenac sodium significantly decreased paw volume from the beginning to end of the study at 5 h compared to control rats. On the contrary, coumarin compound II and III showed marginal anti-inflammatory activity at 1 h and highly significant activity only at 2nd hour. However, during the late phases from 2nd hour to until the end of the study coumarin derivatives successfully inhibited the inflammation due to carrageenan. The effect in the late phase of inflammation may be due to inhibition of COX-2 and PGs. These observations are in confirmation with the results obtained by using histamine as a phlogistic agent. Hence, it can be inferred that the inhibitory effect of coumarin derivatives on carrageenan-induced paw oedema may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

Formalin induced paw oedema closely resembles human arthritis (Greenwald, 1991). In the formalin induced rat paw inflammation model, coumarin derivative compound II and III as well as reference drug diclofenac sodium significantly decreased paw volume from 2nd hour until termination of the study at 5 h. It is a known fact that formalin releases bradykinins and other cytokines at the later phases of inflammatory reaction (Lee and Jeong, 2002). These results suggest that the test compounds are effective in blocking the release of these inflammatory mediators.

Inflammatory granuloma is a typical feature of an established sub-acute/chronic inflammatory process (Olajide et al., 2000). The cotton pellet method has been widely employed to evaluate the transudative, exudative and proliferative components of sub-acute/chronic inflammation. This is done so as the dried weight of the cotton pellet correlates well with the amount of granulomatous tissue (Swingle and Shideman, 1972). In cotton pellet induced granuloma, coumarin derivatives and reference drug diclofenac sodium were significantly effective and showed the inhibition of granuloma formation. In this study, all the three coumarin derivatives decreased both wet and dry weight of the cotton pellets compared to control groups. This may be due to their ability to reduce the number of fibroblasts and synthesis of collagen and mucopolysaccharide, which constitute part of proliferation stage of inflammatory response causing tissue remodeling.

Conclusion

The results of the present findings support the use of coumarin derivatives for the management of inflammatory conditions as assayed by different acute and sub-acute anti-inflammatory models. The synthesized compounds were characterized by spectral studies, which include IR, ^{1}H NMR and ^{13}C NMR. The characteristic peak at 1685 cm^{-1} α-pyrone and that of schiff base at 1577cm^{-1} in IR confirmed the formation of the targeted compounds. Further, the reaction was ascertained by detailed ^{1}HNMR study of the products. A peak at δ 7.8-7.1 is characteristic for aromatic protons and also singlet at δ 7.9 is of CH of imine linkage shows the formation of Schiff base. It can be concluded that coumarin derivatives 1-(2-nitrobenzylidene)-4-(4-methyl-2-oxo-2H-chromen-7yl) semicarbazide (compound III) shows promising anti-inflammatory activity. However, other derivatives need further evaluation as the results are not consistent in all the models of inflammation. Further studies are needed to elucidate the detail mechanism of pathways involved in the anti-inflammatory activity.

Acknowledgement

Conflict of interest statement

The authors of this publication declared that there is no conflict of interest.

References

Arul V, Miyazaki S, Dhananjayan R. 2005. Studies on the anti-inflammatory, antipyretic and analgesic properties

of the leaves of *Aegle marmelos* Corr. Journal of Ethnopharmacology, 96(1-2):159-163.

Crunkhorn P, Meacock SCR. 1971. Mediators of the inflammation inducedin the rat paw by carrageenan. British Journal of Pharmacology, 42(3):392-402.

Eissa AA, Farag NA, Soliman GA. 2009. Synthesis, biological evaluation and docking studies of novel benzopyranone congeners for their expected activity as anti-inflammatory, analgesic and antipyretic agents. Bioorganic & Medicinal Chemistry, 17(14):5059-5070.

Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clinical & Experimental Immunology, 147(2):227-235.

Fujiwara N, Kobayashi K. 2005. Macrophages in inflammation. Current Drug Targets - Inflammation & Allergy, 4(3):281-286.

Gepdiremen A, Mshvildadze V, Suleyman H, Elias R. 2004. Acute and chronic anti-inflammatory effects of *Hedera cochica* in rats. Journal of Ethnopharmacology, 94(1):191-195.

Ghate M, Kusanur RA, Kulkarni MV. 2005. Synthesis and *in-vivo* analgesic and anti-inflammatory activity of some bi heterocyclic coumarin derivatives. European Journal of Medicinal Chemistry, 40(9):882-887.

Greenwald RA. 1991. Animal model for the evaluation of arthritic drugs. Methods and findings in experimental and clinical pharmacology, 13(2):75-83.

Gupta M, Mazumder UK, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti BB. 2005. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. Journal of Ethnopharmacology, 98(3):267-273.

Hasan SM, Alam MM, Husain A, Khanna S, Akhtar M, Zaman MS. 2009. Synthesis of 6-aminomethyl derivatives of benzopyran-4-one with dual biological properties: Anti-inflammatory, analgesic and antimicrobial. European Journal of Medicinal Chemistry, 44(12):4896-4903.

Kalkhambkar RG, Kulkarni GM, Kamanavalli CM, Premkumar N, Asdaq SM, Sun CM. 2008. Synthesis and biological activities of some new fluorinated coumarins and 1-aza coumarins. European Journal of Medicinal Chemistry, 43(10):2178-2188.

Keri RS, Hosamani KM, Shingalapur RV, Hugar MH. 2010. Analgesic, anti-pyretic and DNA cleavage studies of novel pyrimidine derivatives of coumarin moiety. European Journal of Medicinal Chemistry, 45(6):2597-2605.

Khode S, Maddi V, Aragade P, Palkar M, Ronad PK, Thippeswamy AHM, *et al*. 2009. Synthesis and pharmacological evaluation of novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-

inflammatory and analgesic agents. European Journal of Medicinal Chemistry, 44(4):1682-1688.

Lee IO, Jeong YS. 2002. Effects of different concentrations of formalin on paw edema and pain behaviors in rats. Journal of Korean Medical Science, 17(1):81-85.

Linardi A, Costa SK, da Silva GR, Antunes E. 2000. Involvement of kinins, mast cells and sensory neurons in the plasma exudation and paw oedema induced by staphylococcal enterotoxin B in the mouse. European Journal of Pharmacology, 399(2-3):235-242.

Mitchell RN, Cotran RS. 2000. In; Robinsons Basic Pathology, 7th edition, pp. 33, Harcourt (India) Pvt Ltd., New Delhi.

Olajide OA, Awe SO, Makinde JM, Ekhelar AI, Olusola A, Morebise O. 2000. Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. Journal of Ethnopharmacology, 71(1-2):179-186.

Pountos I, Georgouli T, Bird H, Giannoudis PV. 2011. Nonsteroidal anti-inflammatory drugs: prostaglandins, indications, and side effects. International Journal of Interferon, Cytokine and Mediator Research, 3:19-27.

Pretka JE, Wilmington Del. 1961. 4-substituted-7-carboalkoxyamino coumarins. United States Patent Office, Patent No 3, 008, 969.

Rama Ganesh CK, Bodke YD, Venkatesh KB. 2010. Synthesis and biological evaluation of some innovative coumarins containing thiazolidin-4-one ring. NOPR: Indian Journal of Chemistry, 49B:1151-1154.

Robinson R. 1990. 7-amino-4-methyl-coumarin-3-carboxyalkyl derivatives and fluorescent conjugate there of United States Patent Office, Patent No 4, 956, 480.

Ronad PM, Dharbamalla S, Hunshal R, Maddi V. 2008. Synthesis of novel substituted 7-(Benzylideneamino)-4-methyl-2H-chromen-2-one derivatives as anti-inflammatory and analgesic agents. Arch Pharm (Weinheim), 341(11):696-700.

Swingle K, Shideman FE. 1972. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. Journal of Pharmacology and Experimental Therapeutics, 183(1):226-234.

Vinegar R, Truax JF, Selph JL, Johnston R, Venable AL, McKenzie KK. 1987a. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Federation Proceedings, 46(1):118-126.

Vinegar R, Schreiber W, Hugo R. 1969b. Biphasic development of carrageenan oedema in rats. Journal of Pharmacology and Experimental Therapeutics, 166(1):96-103.

Vogel GH, Gang W. 2008. Psychotropic and neurotropic activity. In drug discovery and evolution, 5th edition, pp. 577-585, Springer-verlag berlein heiderberg, Germany.

Winter CA, Risley EA, Nuss GW. 1962. Carrageenan Induced oedema in Hind paws of the rats as an assay of anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine, 111:544-547.