ANTIOXIDANT ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF SOME SELECTED HETEROARYL SUBSTITUTED COUMARINS

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Summary

Significant amounts of Coumarins, with benzopyrone ring units, are frequently present in plants. Simple coumarins and analogues are a large class of compounds with a wide spectrum of biological activity. Several series of coumarin derivatives were synthesized and five of the 5-(substituted phenyl)-1-phenyl- 2-pyrozoline-3″-yl) 6-halogen substituted coumarins were tested for *in vivo* analgesic and anti-inflammatory studies. The compounds were selected on the basis of log P values and *in vitro* radical scavenging activities. Anti-inflammatory activity was determined by carrageenan induced rat paw edema and analgesic activity was assessed by inhibition of hyperalgesia produced by carrageenan induced inflammation in the same rats by measuring the paw withdrawal latency. All compounds tested pharmacologically showed good analgesic and anti-inflammatory activity in Wistar rats. The radical scavenging property may be one of the probable reasons for their analgesic and anti-inflammatory activity.

Key words: Analgesic, Anti-inflammatory, Antioxidant, Coumarins

Introduction

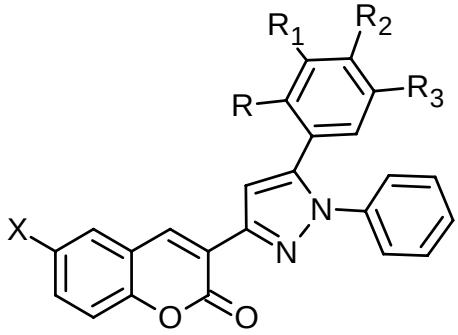
Coumarins are a class of compounds with benzopyrone ring system, occur in plants. The plant extracts containing coumarin-related heterocycles are employed as herbal remedies in traditional systems of medicine, have now been extensively studied for their biological activities. Simple coumarins and analogues are a large class of compounds exhibiting a wide spectrum of pharmacological activities such as anticoagulant, antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-hyperlipidemic, HIV protease inhibitor and anticancer (1-3) actions. Coumarins have showed anti-tumour activity *in vivo*, which is believed to be due to its metabolites (e.g. 7-hydroxycoumarin). Coumarin-3-sulfonamides and carboxamides were reported to exhibit selective cytotoxicity against mammalian cancer cell lines. Some of them target topoisomerase. 7-hydroxycoumarin inhibits cyclin- D_1 over-expression in many types of cancer. Esculetin inhibits growth and cell cycle progression by inducing arrest of the G_1 phase in HL-60 leukaemia cells, resulting from the inhibition of retinoblastoma protein phosphorylation (4-5). Other spectrum of enzymatic targets for synthetic coumarins includes inhibition of aromatase (6-7), monoamino oxidase (8-9), acetylcholinesterase (10).

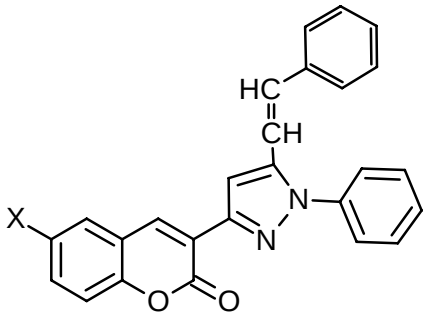
The C_4-substituted aryloxymethyl, arylaminomethyl, and dichloroacetamidomethyl coumarins, along with the corresponding 1-azacoumarins, have been demonstrated to be potential anti-microbial and anti-inflammatory agents (11-12). Coumarin and carbostyrils have also been found to act as anti-bacterial via the DNA gyrase pathway. Some of them bind to non-quinolone regions of the DNA gyrase (13). To expand the structural diversity of synthetic courmarins for biological functions, attempts have also been made to attach a chloramphenicol side chain at C-3 position of coumarin (1).

Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognised as dual inhibitors of lipoxygenase (LOX) and cycloxygenase (COX) enzymic systems (14-15). Coumarins with phenolic hydroxyl groups scavenge reactive oxygen species and suppress inflammation, edema and pain. Moreover coumarin suppresses the fatty acid hydroperoxy intermediates, especially 5-hydroxy-6,8,11,14-eicosatetraenoate (5-HETE) and 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) in the arachidonic acid pathway of inflammation. They also inhibit neutrophil-dependent superoxide anion generation (16).

The number of biological activities explored out of this system has eventually led to the development and design of newer substituted coumarins. Our team effort in the past to synthesis many coumarin series is evident for the biological activity (17-23). Among them, 5-(substituted phenyl)-1-phenyl-2-pyrozoline-3″-yl) 6-halogen substituted compounds have been selected for the *in vivo* animal studies (table 1) on the basis of log P value (within the range of 0.253 to 0.835)and the *in vitro* radical scavenging activity reported previously by us (23).

Tables 1: Chemical structures of test compounds; 5-(substituted phenyl)-1-phenyl-2-pyrozoline-3″-yl) 6-halogen substituted coumarins





Compound	X	R	R_1	R_2	R_3
SA 3	Cl	H	H	OCH_3	H
BSJ 3	H	H	H	OCH_3	H
BSJA1	Br	H	H	$N(CH_3)_2$	H

Compound	*X*
BSJ 4	H
BSJA 4	Br

Materials and Methods

Chemicals

Chemicals used for the experiments were all AR grade obtained from Loba Chemicals. Diphenyl Pecryl Hydrazyl (DPPH $^{\bullet}$), carrageenan and aspirin were obtained from Sigma chemicals. 1.0 mmol of DPPH and all the investigational compound solutions were prepared in methanol for testing antioxidant activity. For testing anti-inflammatory activity the standard drug and test compounds were made into suspension with 0.5 % carboxy-methyl cellulose (CMC).

Animals

In-bred Wistar rats maintained under controlled conditions of temperature (23 ± 2°C) and humidity (50 ± 5%) and a 12-hour light–dark cycle were used for the experiment. The animals were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat food and water *ad libitum*. The studies were conducted with the prior approval of Institutional Animal Ethics Committee.

Antioxidant activity

Antioxidant activity was determined by DPPH radical scavenging method as described earlier (24). Different aliquots of test compound (10 to 1000µg/ml) in methanol were mixed with 1 mM of methanolic DPPH solution to a final volume of 2.0 ml, incubated for 20 minutes at room temperature and absorbance measured at 517nm. Blank was carried out in the same manner without the drug and ascorbic acid was taken as the standard. The experiment was performed in triplicate and the percentage radical scavenging was determined as [(Blank – Test)/ Blank] x 100. IC_{50} was calculated using the Microsoft excel.

Anti-inflammatory and analgesic activity

Studies were carried out in in-bred Wistar rats. The anti-inflammatory and analgesic activities were assessed by carrageenan induced paw edema and pain due to inflammation was assessed by the paw volume and paw withdrawal response respectively as described earlier (25).

Animal were divided into seven groups of six animals each. Group I received 0.5% CMC (vehicle), group-II received aspirin at a dose of 100 mg/kg per oral as the control and the standard drug. Animals in the remaining groups from III to VII, received the test compounds SA-3, BSJ-3, BSJ-4, BSJA-1 and BSJA-4 respectively, at a dose of 100mg/kg per oral. 0.1ml of 1% W/V carrageenan was injected into the subplantar region of left hind paw to all animals receiving standard and test compounds. The hind paw volume was measured using plethysmograph (Inco India) at different time intervals of 0, 30, 60, 120 and 180 min. The percentage increase in mean paw volume at different time intervals was compared with respective control and standard.

The inflammation induced pain (hyperalgesia) was assessed by thermally evoked paw withdrawal latency. A commercially available water bath maintained at temperature 47 ± 0.3°C was used. Basal paw withdrawal latencies (pre-carrageenan) from the thermal source was established three times for each paw, at an interval of 5 min. Paw withdrawal latency (in seconds) was observed as a violent or jerky movement. A cut-off time of 15 sec. was imposed to avoid any thermal injury to paw. Carrageenan treated (post-carrageenan) paw withdrawal latencies were recorded at 0, 30 and 60 min. Data analysed by ANOVA using Graphpad prism.

Results and discussion

All test compounds selected showed $DPPH^{\bullet}$ radical scavenging activity. The IC_{50} values of investigational compounds ranged from 60 to 280µg/ml and fell short of standard ascorbic acid values as shown in the table 2.

All the compounds showed significant anti-inflammatory activity compared to the carrageenan induced group I as shown in the table 3. Carrageenan is strong chemical for release of inflammatory mediators. It activates the COX / LOX pathways of inflammation. Also several oxygen derived free radical will be released during such inflammation. The tested compounds may show its activity by radical scavenging ability as it was evident from in vitro DPPH radical scavenging activity. The anti-inflammatory activity of compounds SA-3, BSJ-3 and BSJ-4 was comparable to that of aspirin as there was no significant difference between the treated groups and aspirin. The anti-inflammatory activity showed up to 180 min indicating fairly long duration of action.

There was significant difference in each treated group compared to that of pre-carrageenan mean paw withdrawal latency (table 4). The analgesic activity was significant compared to that of carrageenan treated hyperalgesic rats. Further the test compounds SA-3, BSJ-3 and BSJ-4 showed statistically significant activity to that of aspirin. The suppression of free radical mediated intermediates of pain could be one of the probable mechanisms of action of these test compounds.

Table 2: DPPH radical scavenging assay

Compound	IC_{50} (µg/ml)
Ascorbic acid	10.00
SA-3	67.39
BSJ-3	60.97
BSJ-4	135.71
BSJA-1	277.43
BSJA-4	203.82

Table 3: Anti-inflammatory Activity: Effect of various compounds on carrageenan induced rat paw edema

Group n=6	Treatment (100 mg/ kg)	**% Increase in mean paw volume**			
		30 min	**60 min**	**120 min**	**180 min**
I	5% CMC	13.00 ± 0.3	15.33 ± 0.5	16.52 ± 0.4	18.50 ± 0.5
II	Aspirin	10.58± 0.3[a]	11.3 ± 0.1	12.08 ± 0.1	12.83 ± 0.2[a]
III	SA-3	10.08± 0.3[a]	10.41 ± 0.2[a]	13.16 ± 0.6[a]	14.66 ± 0.7[a]
IV	BSJ-3	9.33 ± 0.1[a]	11.25 ± 0.2[a]	12.66 ± 0.3[a]	14.33 ±0.3[a]
V	BSJ-4	9.58 ± 0.4[a]	9.16 ± 0.4[a]	12.00 ± 0.1[a]	13.25 ± 0.3[a]
VI	BSJA-1	11.33±0.3[b]	11.08±0.7	15.33 ± 0.7[b]	16.16 ± 0.7[a, b]
VII	BSJA-4	12.75 ± 0.3	12.91 ± 0.2[a]	13.50±0.3[a, b]	15.50 ± 0.1[a, b]

a: $p<0.05$; compared to the control group I
b: $p<0.05$; compared to the aspirin treated group II

Table 4: Analgesic activity: hind paw withdrawal latency (time in sec)

Group n=6	Treatment (100 mg/kg)	**Mean paw withdrawal latency (time in sec)**		
		0 min (pre-carrageenan)	**After 30 min**	**After 60 min**
I	5% CMC	3.32 ± 0.5	3.66 ± 0.4	4.33 ± 0.9
II	Aspirin	4.81 ± 0.8	9.83 ± 1.1[a]	12.66± 0.7[a]
III	SA-3	4.22 ± 0.9	7.33 ± 1.2[a]	8.50 ± 0.8[a]
IV	BSJ-3	3.21 ± 0.5	6.03 ± 0.3[a]	7.60 ± 0.2[a]
V	BSJ-4	3.24 ± 1.1	7.44 ± 1.4[a]	8.66 ± 0.2[a]
VI	BSJA-1	4.20 ± 1.1	4.50±1.1[a, b]	5.16 ± 1.0[a, b]
VII	BSJA-4	4.33 ± 1.3	4.83±1.7[a, b]	5.66 ± 1.2[a, b]

a: $p<0.05$; compared to the control group I
b: $p<0.05$; compared to the aspirin treated group II

Conclusions

All tested compounds namely, SA-3, BSJ-3, BSJ-4, BSJA-1 and BSJA-4 showed significant anti-inflammatory and analgesic activity. The analgesic and anti-inflammatory activity of these compounds may be attributed to their radical scavenging ability. The COX /LOX inhibition could be another mechanism of action of these compounds. Further the role of these test compounds in suppression of inflammatory mediators in arachidonic acid pathway or the signaling of inflammatory pathways need to be thoroughly investigated.

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