International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME NOVEL DIARYLSULFONYLUREA-CHALCONE HYBRIDS IN CARRAGEENAN–INDUCED PAW EDEMA IN RATS

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ABSTRACT

A series of some novel diarylsulfonylurea-chalcone hybrids synthesized in our earlier study as potential 5-lipoxygenase inhibitors were now subjected for the anti-inflammatory activity by using carrageenan induced rat paw edema method. The compounds **4o**, **4q**, **4r**, **4t** and **4y** were selected for the study; the rationale behind the selection of these compounds is mainly due to their potential inhibitory activity against 5-lipoxygenase enzyme. Among the compounds tested **4r** and **4o** have been displayed remarkable percentage reduction of paw volume in comparison with standard drug Aceclofenac.

Key words: anti-inflammatory, diarylsulfonylurea-chalcone hybrids, Aceclofenac

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. The inflammatory process is invariably characterized by a production of prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor (PAF) and by a release of chemicals from tissues and migrating cells. Carrageenan-induced local inflammation is commonly used to evaluate non-steroidal anti-inflammatory drugs (NSAID). It appears that the onset of the carrageenan local inflammation has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radical, as well as to the release of other neutrophil-derived mediators [1-3]. In the recent past, the use of non steroidal anti-inflammatory drugs (NSAIDs) for treatment of inflammation has gained a significant momentum, the anti-inflammatory activity was mediated by blocking the metabolism of arachidonic acid through the inhibition of 5-LO and thereby production of prostaglandins [4, 5]. Keeping this in view, we have synthesized a series of some novel diarylsulfonylurea-chalcone hybrids as novel class of 5-lipoxygenase inhibitors. As a part of extended research programme the compounds with remarkable 5-lipoxygenase activity such as **4o**, **4q**, **4r**, **4t** and **4y** which earlier reported from our research work [6]. have been subjected for acute toxicity and anti-inflammatory activities (carrageenan induced rat paw edema method) in standard animal models.

MATERIALS AND METHODS

Animals: Albino wistar rats (150-200g) of either sex were used. The animals housed under standard laboratory conditions maintained at 25±1^{o}C and under 12/12 hour light/dark cycle and fed with standard pellet diet and water *ad libitum*. The experimental protocols were approved by

Institutional Animal Ethics Committee (Regd No: 517/PO/C/2001/CPCSEA).

Acute toxicity study: Acute toxicity of diarylsulfonylurea derivatives were determined in albino wistar rats with the staircase method. Each group of 6 animals was fasted for 24 hour prior to the administration of the test compounds. The test compounds, **4o**, **4q**, **4r**, **4t** and **4y** were administered orally in doses up to 2000 mg/kg by suspending in 1 % C.M.C solution and were kept under observation for a period of 24 hour.

Experimental models for testing anti-inflammatory activity: Acute inflammatory condition is produced in the animals by adapting the following methods [7]. a) Carrageenan-induced pedal inflammation, b) Egg-white induced pedal inflammation and c) Dextrin-induced pedal inflammation. Chronic inflammatory condition is produced in the animals by adapting the following methods.

Formaldehyde- induced pedal inflammation

a) Implantation of cotton pellets
b) Granular pouch
c) Tuberculin sensitivity
d) Fred's adjuvant

Instruments used to measure the paw oedema are,

a) Plethysmograph
b) Zeitlin's apparatus

Procedure: The compounds were tested for anti-inflammatory activity by Carrageenan induced rat paw oedema model [8]. employing Zeitlin's apparatus to measure the paw thickness.

MATERIALS

All the materials used for this experiment are of analytical grade. Carrageenan was procured from Hi-media. Sodium CMC (E. Merck), Saline (Core health care) was purchased from the local supplier. Aceclofenac sample was the gift sample from Jagsonpal, New Delhi.

Preparation of sodium CMC suspension: Stock suspension of sodium CMC was prepared by triturating 1g of sodium- CMC in 100 mL of distilled water and used for suspending the test compounds and standard drug.

Preparation of Carrageenan suspension: 1% Suspension of Carrageenan sodium salt was prepared by sprinkling 100 mg of Carrageenan powder in 10 mL of saline (0.9% NaCl) solution and set aside to soak for 1 h. A homogenous suspension was then obtained by thorough mixing with a magnetic stirrer.

EXPERIMENTAL PROCEDURE

Inflammation was induced by injecting 0.05 mL of 1% Carrageenan suspension subcutaneously into the sub plantar region of the right hind paw and 0.05 mL of saline was injected into the sub plantar region of the left hind paw [9]. for all groups. One hour prior to Carrageenan injection, the groups III to VII treated with diarylsulfonylurea-chalcone hybrids **4o**, **4q**, **4r**, **4t** and **4y** (10 mg/kg). 1% sodium CMC gel (1mL/kg), was given to group-I used as Carrageenan treated control and the standard drug Aceclofenac (2 mg/kg) was administered to group-II. All the doses were administered orally. Anti-inflammatory activity was evaluated by measuring Carrageenan induced paw oedema [10].

Measurement of paw thickness: The thickness of the both paws of each rat was measured before Carrageenan injection and after Carrageenan injection at time intervals 0.5, 1, 2, 3, 4 and 6 hr using Zeitlin's constant load lever method [11]. consisting of a graduated micrometer combined with a constant loaded lever system to magnify the small changes in paw thickness during the course of the experiment. The percent increase of paw oedema thickness [12]. was determined at 0.5, 1, 2, 3, 4 and 6 hrs after induction of inflammation.

Percentage increase in paw thickness = $\frac{Y_t - Y_0}{Y_0}$ X 100

Where

Y_t = paw thickness at the time't' hours (After injection)

Y_o = paw thickness at the time '0' hours (Before injection)

The percent inhibition of paw oedema thickness is calculated using the formula,

Percentage inhibition = $\left[1 - \frac{Y_t}{Y_c}\right]$ X 100

Where,

Y_t = Average increase in paw thickness in groups tested with test compounds

Y_c = Average increase in paw thickness in control

The results and statistical analysis of anti-inflammatory activity of Aceclofenac and the compounds tested are shown in **Table 1.**

Anti-inflammatory activity: The results of anti-inflammatory activity revealed that the compounds

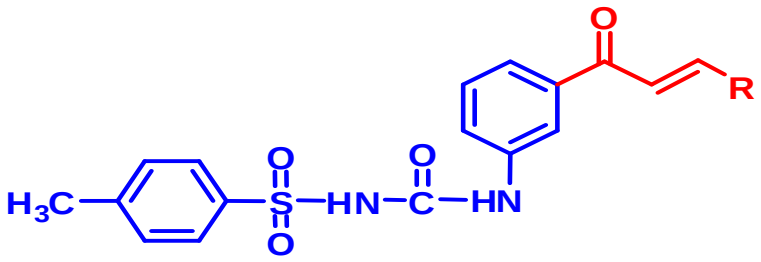
4o, 4q, 4r, 4t and **4y** exhibited considerable activity, but not at identical dose (10 mg/kg) when compared with reference standard Aceclofenac (2 mg/kg). In addition, it was found that diarylsulfonylurea-chalcone hybrids **4r** and **4o** showed maximum activity and this may be due to the presence of 2,4-dichlorophenyl and 4-fluorophenyl moieties as ring-B of diarylsulfonylurea-chalcone hybrid respectively. Moreover, it was also observed that the compounds **4y, 4q** and **4t** carrying anthracen-9-yl, 4-chlorophenyl and 4-bromophenyl as ring-B of diarylsulfonylurea-chalcone hybrid respectively, showed remarkable activity. The low toxicity of synthesized compounds was evident from the observation that there was no mortality in rat at doses up to 2000 mg/kg.

Statistical Analysis: Results are expressed as mean ± SD. The statistical analysis was performed by One Way Analyses of Varience (ANOVA) followed by Dunnet's t-test. The $p<0.05$ was considered as statistically significant.

CONCLUSION

In examination, the five diarylsulfonylurea-chalcone hybrids such as **4o**, **4q**, **4r**, **4t** and **4y** screened for their anti-inflammatory activity by using Carrageenan induced rat paw oedema method and the results revealed the positive contribution of di halogen substitution on the phenyl ring B of α,β-unsaturated ketone towards the observed anti-inflammatory activity. From the present investigation the proposed mechanism of the observed anti-inflammatory activity of diarylsulfonylurea-chalcone hybrids may be due to the inhibition of 5-lipoxygenase enzyme activity in a leukotriene biosynthetic pathway.

Table 1. Anti-inflammatory activity of diarylsulfonylurea-chalcone hybrids **4o**, **4q**, **4r**, **4t, 4y**.



Code	R	% Inhibition ± SEM at various time intervals					
		0.5 h	1.0 h	2.0 h	3.0 h	4.0 h	6.0 h
4o	4-FC_6H_4	15.23 ± 0.75*	24.07 ± 0.89	46.47 ± 1.45*	78.91 ± 1.33*	89.84 ± 1.88	93.80 ± 2.53
4q	4-ClC_6H_4	12.90 ± 0.69*	13.21 ± 0.69*	43.89 ± 1.85*	63.55 ± 2.03	80.65 ± 2.32*	98.35 ± 2.98
4r	2,4-diClC_6H_3	25.29 ± 0.78*	36.67 ± 0.99*	69.57 ± 1.65*	88.97 ± 1.66*	98.38 ± 2.01	98.38 ± 1.99
4t	4-BrC_6H_4	29.42 ± 1.23*	29.79 ± 1.32	37.84 ± 1.52*	68.31 ± 1.98	86.21 ± 2.05*	98.26 ± 2.56
4y	Anthracen-9-yl	15.88 ± 0.77*	36.47 ± 0.92*	62.14 ± 1.35	83.45 ± 1.81*	95.10 ± 2.95	98.40 ± 3.25
Aceclofenac		20.26 ± 0.90	23.95 ± 0.97	58.00 ± 1.52	67.93 ± 1.68	97.09 ± 1.97	99.98 ± 2.00

All values are represented as mean±SEM (n=6). *P<0.01 compared to reference standard Aceclofenac. Student's t-test. Dosage: Aceclofenac (2 mg/kg) and test compounds (10 mg/kg) body weight of rat.

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