

Research article**ANTI-INFLAMMATORY, ANALGESIC AND ANTI ULCER ACTIVITY OF CERTAIN THIAZOLIDINONES**

*TARANALLI A.D., THIMMAIAH N.V., SRINIVAS S., SARAVANAN E. Department of Pharmacology, . L. E. S's College of Pharmacy, Nehrunagar, Belgaum-590 010, Karnataka, India.

BHAT A.R. Department of Pharmachemistry, K. L. E. S's College of Pharmacy, Nehrunagar, Belgaum-590 010, Karnataka, India. E-mail: ashok_taranalli@yahoo.co.in

ABSTRACT

The thiazolidine-4-one derivatives were synthesized from sulphanilamide and evaluated for anti-inflammatory, analgesic and anti-ulcer activity. Anti-inflammatory activity was investigated by carrageenin-induced paw edema test and analgesic activity by acetic acid induced writhing and rat caudal immersion method. Anti-ulcer activity was investigated by pylorus ligation ulcer model. All the compounds showed significant anti-inflammatory, analgesic and anti-ulcer activity at 100 mg/kg b.w. The nimesulide was used as standard drug for comparison. The unsubstituted compound TH-1 and with substitution at R1-CH₃ in TH-3 showed potential activity. However substitution of functional group at R2-OCH₃ in TH-2, R2-CH₃, R3-CH₃ and R4-NH₂ in TH-6 did not produce any potential activity.

KEY WORDS Thiazolidinones, Anti-inflammatory, Analgesic, Antiulcer.

INTRODUCTION

Inflammation is defined as a tissue directed response to noxious and injurious external and internal stimuli, which is predominantly mediated by arachidonic acid metabolites. In the early 1990s two groups independently detected the existence of two cyclooxygenase COX-1 and COX-2.¹ While the inhibition of COX-2 activity led to anti-inflammatory activity, COX-1 inhibition led to ulcerogenic activity. Compounds containing a simple thiazole nucleus and many thiazole derivatives were found to have biological and commercial interest. Thiazolidinones exhibit antifungal, antibacterial, anticonvulsant, anthelmintic, anti-tubercular, antioxidant and calcium antagonistic activity.^{2, 3} Therefore present study was planned to investigate the thiazolidine-4-ones for their anti-inflammatory, analgesic and antiulcer activities.

MATERIALS AND METHODS**Pharmacological Screening:****Animals**

Albino Wistar rats of either sex weighing 150-200g were used. Animals

were housed in groups of six per cage at a temperature of 25±1°C and relative humidity of 45 – 55%. A 12:12 hour dark: light cycle was followed during the experiments. Animals had free access to food and water, however, food but not water was withdrawn six hours before and during the experiments. The animals were obtained from the Central Animal House of J. N. Medical College, Belgaum (India). The Institutional Animal Ethical Committee approved the protocol of the study.

Drugs

The drugs synthesized in the Chemistry laboratory of K. L. E. S's College of pharmacy, Belgaum, were used. The drugs were coded as TH-1, TH-2, TH-3 and TH-6 (derivatives of thiazolidine-4-one). The Standard drug (Nimesulide) was used obtained from Lincoln Pharmaceuticals ltd. Ahmedabad.

Toxicity Studies

The acute toxicity study was done as per the OECD guidelines (407). The compounds were administered orally in different doses, where 24h toxicity was recorded to identify the toxic does. The

Table 1- Substitutions in structure

Sl. No.	Compound Code	R (R)	R' (R1)	R'' (R2)	R''' (R3)
01.	TH-1	H	H	H	H
02.	TH-2	H	OCH ₃	H	H
03.	TH-3	H	CH ₃	H	H
04.	TH-6	H	CH ₃	CH ₃	NH ₂

Table 2: Effect of Thiazolidinone Derivatives on Carrageenan induced paw edema

Compound Code	Dose (mg/kg)	Mean Paw Volume at different time intervals		Percentage Inhibition of edema volume	
		1 st h	3 rd h	1 st h	3 rd h
Control	0.5% CMC	1.22±0.037	1.00±0.031	0.0%	0.0%
Standard (Nimesulide)	50	0.82±0.037**	0.50±0.031**	32.78%	50.00%
TH-1	100	0.60±0.070**	0.42±0.058**	50.81%	58.00%
TH-2	100	0.82±0.037**	0.54±0.024**	32.78%	46.00%
TH-3	100	0.70±0.031**	0.44±0.024**	42.62%	56.00%
TH-6	100	0.76±0.024**	0.56±0.024**	37.70%	44.00%

N=6, Values are Mean±SEM **P<0.01(significant), values are compared with control group.

Table 1: Analgesic activity of Thiazolidinones in acetic acid induced writhing in mice.

Compound Code	Dose (mg/kg)	Writhing Response	Percentage Inhibition
Control	0.5% CMC	59.80±1.65	0.0%
Standard (Nimesulide)	50	31.20±1.42**	47.82%
TH-1	100	24.80±1.56**	58.52%
TH-2	100	27.40±1.63**	54.18%
TH-3	100	39.00±1.70**	34.78%
TH-6	100	23.20±0.86**	61.20%

N=6, Values are Mean±SEM **P<0.01(significant) values are compared with control group

doses of the test compounds were then toxicity as 100 mg/kg for evaluation.

Anti-Inflammatory Activity:

The anti inflammatory activity was studied using Carrageenan Induced Paw Edema test⁴. All the test compounds namely TH-1, TH-2, TH-3 and TH-6 were administered 100mg/kg body weight based upon their acute toxicity studies and nimesulide 50mg/kg b.w. was used as standard. The test compounds were administered orally to the rats suspended in 0.5% carboxymethyl cellulose (CMC). The control animals received 0.5% CMC.

fixed on the basis of their acute Thirty minutes after drug administration, 0.1ml of 1% carrageenan (Sigma) in normal saline solution was injected into the sub plantar region of one of the hind paws. The paw edema volume was recorded using a plethysmometer (UGO Basile, Italy) at different time intervals

Analgesic Activity:

The Analgesic Activity was studied using acetic acid induced writhing and rat caudal immersion method.^{5,6}

i) Writhing Test:

In Acetic acid induced writhing test, the test compounds, standard and vehicle were administered orally to the mice at 100mg/kg b.w. concentration and 30 min later 0.6% acetic acid solution (10ml/kg b.w.) was injected intraperitoneally. Nimesulide (50mg/kg) was used as standard. The number of writhes induced in each mouse was observed for 10 minutes period starting 10 minutes after injection of acetic acid. The analgesic activity was expressed in terms of percentage inhibition of writhes produced by acetic acid.

ii) Rat Caudal Immersion Method:

In rat caudal immersion method, the test compounds, standard and vehicle as given above were administered orally at a concentration of 100mg/kg b.w. The reaction time for withdrawal of tail was recorded after 60 minutes from the administration of test compounds. It was determined by immersing the tail up to the caudal portion (5cm from the tip) in hot water ($55 \pm 0.5^\circ\text{C}$) and by noting the time taken to withdraw the tail clearly out of water. Observations were made at an interval of 30, 60, and 180 minutes after the initial reading.

Anti-ulcer activity:

Anti-ulcer activity studied using pylorus ligation ulcer model.^{6, 7} Male rats weighing between 140-175g were selected and divided into six groups consisting of six animals each and were fasted over night. One group received 0.5% CMC. The second group received Ranitidine (50mg/kg) and other groups received test compounds (100 mg/kg) by oral route 30min prior to pyloric ligation. Animals were scarified 4 hrs later and the stomach was opened to collect the gastric contents. The gastric contents were centrifuged at 1000 rpm for 10 min and supernatant was

collected. The gastric pH was determined by using pH meter and ulcer score were recorded.

Statistical Analysis

The Statistical analysis was performed by using One Way ANOVA followed by Dunnet's "t" Test. and the $P < 0.01$ was taken as significant.

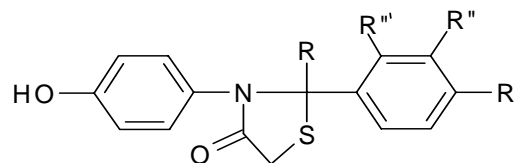
Structure:

FIG. 1. General Structures of Compounds TH1-TH6

RESULTS

All the four compounds TH-1, TH-2, TH-3 and TH-6 produced significant inhibition of edema in carrageenan induced paw edema model at a dose of 100mg/kg b.w. These compounds had an increased significance ($P < 0.01$) at various time intervals and showed maximum activity (58%, 46%, 56%, and 44% respectively) at 3rd hour. The compounds TH-1 (58%) and TH-3 (56%) showed maximum inhibition as compared to standard nimesulide (50%). All the test compounds showed significant analgesic activity in acetic acid induced writhing and in caudal immersion method. In acetic acid writhing, TH-1 (58.52%) and TH-6 (61.20%) significantly reduced the number of writhes in mice as compared to standard. In caudal immersion method all the compounds showed maximum activity at 3rd hour. In pylorus ligation method, all the compounds at the tested dose level exhibited varying degree of antiulcer activity. The compounds TH-1 and TH-3 exhibited good gastro protective action as indicated by their low ulcer score and low pH.

Table 3: Analgesic activity of Thiazolidinones in rat caudal immersion method

Comp. code	Dose (mg/kg)	Caudal immersion in rats (in sec.) at various time intervals				Percentage inhibition			
		½ hr	1 st hr	2 nd hr	3 rd hr	½ hr	1 st hr	2 nd hr	3 rd hr
Control	0.5% CMC	3.400 ±0.5099	3.400 ±0.5099	3.400 ±0.5099	3.400 ±0.5099	0.0%	0.0%	0.0%	0.0%
Standard	50	4.600 ±0.2449	7.200 ±0.3742**	8.600 ±0.2449**	9.800 ±0.2000**	26.08%	52.77%	60.46%	65.30%
TH-1	100	4.800 ±0.3742	9.000 ±0.3162**	9.600 ±0.2449**	10.00 ±0.0**	29.167%	62.22%	64.58%	66.00%
TH-2	100	4.600 ±0.5099	6.400 ±0.2449**	7.400 ±0.2449**	7.800 ±0.5831**	26.08%	46.87%	54.04%	56.41%
TH-3	100	5.200* ±0.3742	7.000 ±0.4472**	8.600 ±0.2449**	10.00 ±0.0**	34.61%	51.42%	60.46%	66.00%
TH-6	100	4.800 ±0.3742	7.600 ±0.2449**	8.600 ±0.2449**	10.00 ±0.0**	29.16%	55.26%	60.46%	66.00%

N=6, Values are Mean±SEM **P<0.01(significant), values are compared with control group.

Table 4: Effect of Thiazolidinone derivatives on pylorus ligation induced ulceration in rats.

Sl. No.	Compound Code	Dose (mg/kg)	Anti-ulcer Activity in pylorus ligated rats		
			pH	Ulcer Score	Percentage inhibition of Ulcer
1.	Control	0.5% CMC	0.9800±0.03742	9.300±0.2550	00.00%
2.	Standard	50	3.200±0.08367**	2.600±0.2915**	72.04%
3.	TH-1	100	1.580±0.03742**	4.600±0.1871**	50.53%
4.	TH-2	100	2.100±0.03162**	7.500±0.3536**	19.35%
5.	TH-3	100	3.140±0.05099**	3.800±0.2550**	59.13%
6.	TH-6	100	2.420±0.03742**	7.000±0.2739**	24.73%

N=6, Values are Mean±SEM **P<0.01(significant), values are compared with control group.

DISCUSSION

In the present study, four derivatives of thiazolidine-4-one synthesized (TH-1, TH-2, TH-3 and TH-4) from sulphanilamide, a well known anti-bacterial agent were evaluated for anti-inflammatory, analgesic and anti-ulcer activities using various models. In the compounds synthesized, free amine group was replaced by thiazolidine-4-one with

functional group substitution at R1, R2, R3 and R4. All these compounds showed significant anti-inflammatory, analgesic and anti-ulcer activity. The unsubstituted compound TH1 and with substitution at R1-CH3 in TH3 showed potential activity. However substitution of functional group at R2-OCH3 in TH-2, R2-CH3, R3-CH3 and R4-NH2 in TH-6 does not show potential activity.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. F. V. Manvi, Principal, K. L. E. S's College of Pharmacy, Belgaum and Chairman, Board of Management, K. L. E. Society, Belgaum, Karnataka for providing all the facilities to carry out this work.

REFERENCES

1. Silverstein FE, Faish G, Goldstein JL, Simon LS, Pinus T, Whelton R, et. al. Gastrointestinal toxicity with celecoxib vs non-steroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis. *J Am Med Ass* 2000; 284:1247-55.
2. Singh PS, Parmar SS, Krishna Raman, Stenberg VI. Chemistry and biological activity of thiazolidinones. *Chem Rev* 1981;81(2):175-203
3. Tatsukya Kato, Tomokazu Ozaki, Kazuhiko Tamura, Yoshiyuki Suzuki, Michitaka Akima, Nobuhiro et. al. 1. Novel calcium antagonist with both calcium overload inhibition and antioxidant activity. 2. Structure-activity relationships of thiazolidinone derivatives. *J Med Chem* 1999;42:3134-3146
4. Kumar P, Knaus EE. Synthesis and anti-inflammatory activity of N-substituted dihydropyridylacetic acid, ester and amides. *Drug Design Discov* 1987; 2:145-49.
5. Fukawa K, Kawana O, Hibi M, Misaka N., Ohba S, Hatanaka, Y. Methods for evaluation of analgesic agents in rats. *J Pharmacol Methods* 1980; 4:251-59.
6. Vogel HG, Editors. *Drug Discovery and Evaluation - Pharmacological Assays*. 2nd Edn. Berlin, New York: Springer Verlag; 2002. P.759-867.
7. Subudhi BB, Bhatta D, Panda P, Misra P, Pradhan D. Synthesis and antiulcer activity of 2-(1'Iminothioimido Substituted)-1'-Substituted Phenyl benzoic acids. *Indian J Pharm Sci* 2008;70(3):381-83.