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Pharmacological evaluation of thiazolidinone derivatives for anti-ulcer and antibacterial activities

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ABSTRACT

The newly synthesized thiazolidinone derivatives were evaluated for anti-ulcer and antibacterial activities. The anti-ulcer activity of thiazolidinone derivatives was screened by pylorus ligation and ethanol-induced ulcer methods in the doses of 20 and 30mg/kg. All the four test compounds showed significant percentage preventive ulcer ratio when compared to standard. The antibacterial activity was screened by agar cup plate method and the test compounds have also shown significant activity against gram-positive and gram-negative organisms compared to their standards.

Key words: Anti-ulcer, anti-bacterial, and thiazolidinone.

INTRODUCTION

Thiazolidinones were found to exhibit many biological activities such as antimicrobial[1], local anesthetic[2,3], anticonvulsant[4], antitubercular[5-8], CNS depressant[9-10], anti-inflammatory[11], antitumor[12], and antihypertensive actions[13]. The reports of the antimicrobial activities of thiazolidinones prompted us to take up the study in detail. The research work has been carried out by comprising thiazolidinone moiety for anti-ulcer and antibacterial activities. The synthesized compounds were taken for pharmacological activities.

MATERIAL AND METHODS

Wistar strain albino rats of either sex weighing 200-250 g were kept in the department animal house and maintained under standard environmental conditions and was fed with standard pellet diet and water *ad libitum.* The experiments were performed followed by approval from Institutional Animal Ehical Committee.

Acute toxicity studies

Acute toxicity studies were carried out following OECD guidelines[14] and were found to be safe up to 500mg/kg body weight in albino Wistar rats.

Anti-ulcer activity

The anti-ulcer activity was studied by using two different ulcer models, Pylorus ligation induced ulcers and Ethanol induced ulcers.

Pylorus ligation induced ulcer method

The rats were divided into ten groups of six each. The group I served as control group and group II served as standard, which received ranitidine 50mg/kg and the remaining groups received the test compounds in the doses of 20 and 30 mg/kg orally 1 h prior to pyloric ligation.

Pyloric ligation was performed as described by Shay H. *et.al*[15]. Animals were fasted for 36 h prior to surgical procedure and kept in raised mesh-bottomed cages to avoid coprophagy. Under ether anesthesia a midline abdominal incision was made. A ligation was made at the junction of pylorus part of stomach and duodenum part of small intestine, care being taken that neither damage to blood supply nor traction on the pylorus. The stomach was then replaced carefully and abdominal valve closed by interrupted sutures. The animals were deprived of both food and water during the postoperative period and were sacrificed at the end of 19-20 h after the operation. The stomach was dissected out as a whole by passing a ligature at the esophageal end.

The stomach was separated from the surrounding tissues and organs and thus brought as a whole along with its contents and drained into a graduated centrifuge tube. The contents were subjected to centrifugation at 3000 rpm for 10 min. The supernatant volume was subjected to determine the free and total acidity by titration with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator. The stomach was opened along the greater curvature and pinned on a cork plate. The inner gastric surface was examined for gastric lesions using magnifying lens.

Mucosal lesions were counted and the severity of lesions was recorded with the following scores as 0 - no ulcer, 0.5 – redness, 1 - superficial ulcers, 2 - deep ulcers, and 3 - Perforated ulcers.

The ulcer index was calculated using the formula

$U_I = U_N + Us + U_P \times 10^{-1}$

U_N = Average number of ulcers per animal

U_S = Average of severity score

U_P = Percentage of animals with ulcer

The % Preventive ratio of ulcer was calculated using the formula.

$$\% \text{ Preventive Ratio of ulcer} = \frac{U_c - U_t}{U_c} \times 100$$

Where,

U_t = Ulcer index of test group

U_c = Ulcer index of control group.

Acidity was calculated by using the formula.

$$\text{Acidity (meq/L/100g)} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1}$$

Ethanol induced ulcers

The ethanol induced ulcer method was performed as described by Astudillo Luis[16]. Adult albino rats (200-250g) of either sex were selected and fasted for 24 h with free access to water before the experiment. They were divided into ten groups of six rats each. The group I served as control and group II served as standard, which received omeprazole at the dose of 20 mg/kg orally. The remaining groups received test compounds at the dose of 20 and 30 mg/kg orally. After 1 h, 1 ml of 99% ethanol was administered to each animal by oral route. Animals were killed at 1 hour after ethanol administration by deep ether anesthesia. The stomach was removed and opened along the greater curvature and pinned on a cork plate. The inner gastric mucosal surface was examined for gastric lesions using magnifying lens.

Anti-bacterial activity

The method described by Hugo and Russel[17] was employed for screening anti-bacterial activity of the test compounds at a concentration of 50 and 100 µg/ml using DMSO as a solvent against *S. aureus, B. subtilis, P. aeruginosa, and E. coli.* The zone of inhibition of each strain was recorded. The activity has been compared with known standard streptomycin for gram-negative organisms and procaine penicillin for gram-positive organisms at a concentration of 50 and 100 µg/ml.

Statistical analysis

The values are expressed as mean ± SEM. The data were analysed by One-way analysis (ANOVA) followed by Dunnet's t-test considering $P < 0.05$ as the level of significance.

RESULTS

Anti-ulcer Activity

Pylorus ligation induced ulcers in rats

Pylorus ligation for 19 h resulted in increase in accumulation of gastric volume, free acidity, total acidity and ulcer index. The test compounds produced significant reduction in gastric volume, free acidity, total acidity and ulcer index when compared to the standard drug ranitidine Table 1.

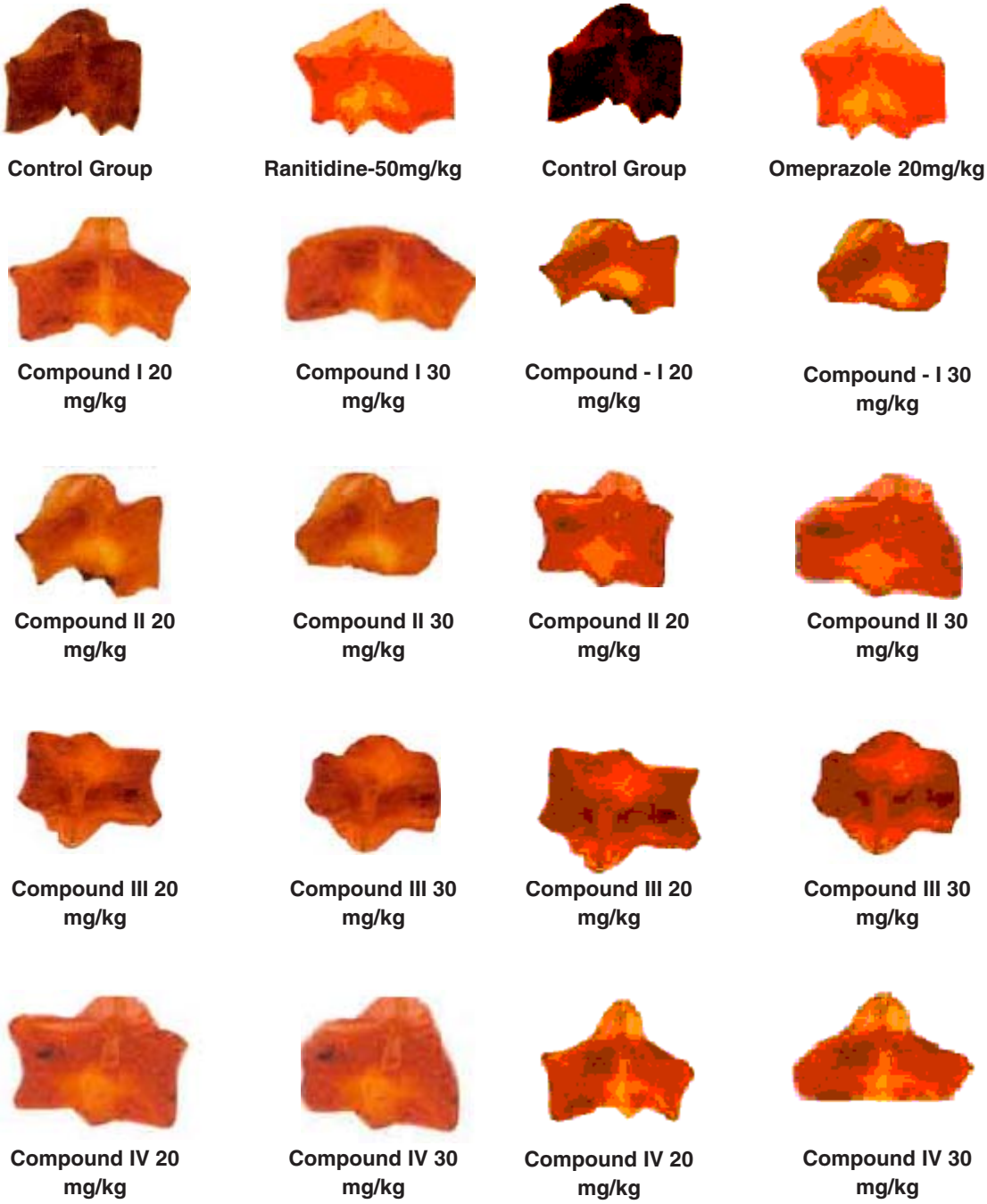

Fig. 1: Photograph showing ant-ulcer activity by pylorus ligastion ulcer model

Fig. 2: Photograph showing ant-ulcer activity by ethanol induced ulcer model

Ethanol induced ulcers in rats

Animals treated with test compounds at 20 and 30mg/kg exhibited significant (P<0.01) protection against ethanol induced mucosal injury when compared to standard drug omeprazole. Table 2

Antibacterial activity

The anti-bacterial activity of the test compounds was screened against *S. aureus, B. subtilis, P. aeruginosa, and E. coli* at a concentration of 50 and 100 1µg/ml by agar cup plate method. The standard drugs used for antibacterial screening were streptomycin for gram-negative organisms and procaine penicillin for gram-positive organisms. All the test compounds have shown significant activity Table.3.

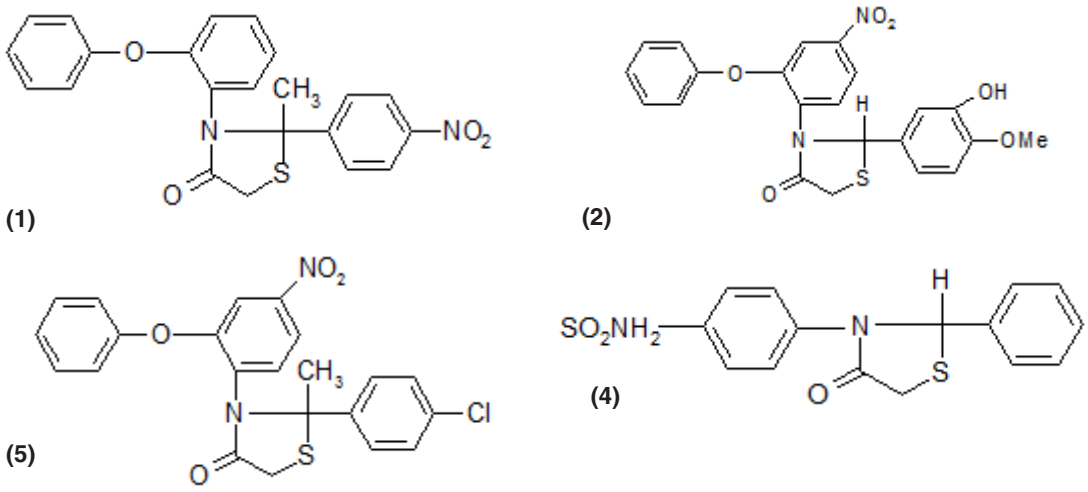

Fig. 3. Structures of the test compounds

Table 1: Results of test compounds on pylorus ligation induced ulcers in rats

Compd.	Dose (mg/kg)	Gastric volume	Free acidity meq/L	Total acidity meq/L	Ucler index	% Preventive Ratio
Control		9.8 ± 0.21	48.6 ± 4.20	102 ± 70.0	3.75 ± 0.25	0.00
Ranitidine	50	**5.93 ± 0.13	**32.2 ± 3.0	**58.8 ± 3.6	**0.83 ± 0.21	78.00
I	20	**6.11 ± 0.27	37.0 ± 3.1	**65.8 ± 3.5	**1.33 ± 0.21	65.00
	30	**6.81 ± 0.26	38.0 ± 2.0	**62.1 ± 3.5	**1.25 ± 0.21	67.00
II	20	**6.93 ± 0.22	38.5 ± 1.8	**62.1 ± 1.7	**1.75 ± 0.11	53.00
	30	**6.76 ± 0.43	*35.1 ± 2.5	**58.6 ± 2.3	**1.41 ± 0.15	62.00
III	20	**6.53 ± 0.40	45.8 ± 2.2	91.0 ± 3.6	*2.83 ± 0.16	25.00
	30	8.65 ± 0.16	48.1 ± 1.5	**68.3 ± 5.9	3.0 ± 0.18	20.00
IV	20	**6.26 ± 0.18	25.3 ± 2.8	**54.3 ± 3.3	**1.66 ± 0.21	56.00
	30	**6.35 ± 0.24	**25.8 ± 2.9	**51.3 ± 4.9	**1.58 ± 0.15	58.00

Values are expressed as mean ± SEM. n = 6; *p<0.05, ** p<. 01 compared to control.

Table 2: Results of test compounds on ethanol induced ulcers in rats

Compound	Dose (mg/kg)	Ulcer Index (Mean ±SEM)	% Preventive Ratio
Control		3.83±0.21	0.00%
Omeprazole	20	**1.0±0.18	74.00%
I	20	**1.75±0.21	54.00%
	30	**1.33±0.21	65.00%
II	20	**1.66±0.21	57.00%
	30	**1.58±0.15	59.00%
III	20	**2.75±0.21	28.00%
	30	**2.83±0.16	26.00%
IV	20	**1.58±0.23	59.00%
	30	**1.58±0.15	59.00%

Values are expressed as mean ± SEM. n = 6; *p<0.05, ** p<. 01 compared to control.

Table 3: Results of antibacterial activity of the test compounds

Compound	Zone of Inhibition (mm)							
	Gram Positive				Gram Negative			
	S.aureus		S. albus		E. coli		P. aerugenosa	
	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg
I	14	13	14	13	9	13	8	18
II	13	20	16	22	15	20	13	22
III	10	12	11	14	10	12	11	9
IV	13	16	16	19	12	16	18	19
Procaine Penicillin	20	22	22	30	-	-	-	-
Streptomycin	-	-	-	-	20	22	22	32

Blank: DMSO - Resistant
Readings are average of three plates

DISCUSSION

All the test compounds were useful in reducing gastric volume, free acidity, total acidity and ulcer index when compared to standard by pylorus ligation method. The thiazolidinones containing diphenyl ether substituent on the compounds I - III and sulphonamide substituent on the compound IV was found to be important for the biological activity.

In Ethanol induced ulcer method necrotizing agents such as ethanol, when given intragastrically to rats produce severe gastric hemorrhagic erosions characterized by multiple hemorrhagic red patches of different sizes along with longitudinal axis of glandular stomach. Animals treated with omeprazole showed significant protection against ethanol induced mucosal injury when compared with control group; similarly all the

test compounds exhibited significant reduction in ulcer index.

All the test compounds showed significant antibacterial activity against both gram positive and gram-negative organisms when compared to the standard. Further research work is necessary to find out the possible useful moiety.

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