EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY AND ULCEROGENIC LIABILITY OF 5-(3-CHLORO-1-BENZOTHIEN -2-YL)-4-PHENYL-4H-1,2,4-TRIAZOLE-3-THIOL

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ABSTRACT

The aim of this study was to evaluate the anti-inflammatory and the ulcerogenic activity of 5-(3-chloro-1-benzothien -2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol. The anti-inflammatory effect of the compound was determined by comparing it with a standard drug (naproxen). The anti-inflammatory effects were studied by using carrageenan-induced paw edema method and Cotton pellet induced granuloma in rats and the ulcerogenic liability was assessed and compared with a standard drug. The results showed that the compound had an obvious anti-inflammatory effect and the activity is comparable to that of the standard drug with less ulcerogenic effects.

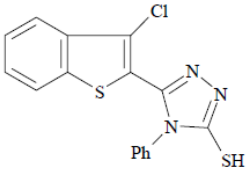
INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain and inflammation. It forms a distinct group with different chemical structures sharing similar effects , which are characterized by their ability to relief pain , fever , and inflammatory disorders.[1] It is used as analgesic, anti-inflammatory, antipyretic, antiplatelet, prolongation of gestation and labour, patency of ductus arteriosus, primary dysmenorrhea[2], and in cancer[3]. These drugs are responsible for a large number of adverse drug reactions which range from effects on GIT to effects on liver , kidney, spleen , CNS , eyes , lungs , pancreas, skin, blood and bone marrow. [2] ,[4] These compounds act by inhibition of the enzyme cyclooxygenase (COX), thus preventing prostaglandin synthesis [5]. They have greater selectivity to inhibit COX-1 (constitutively expressed and providing cytoprotection in the gastrointestinal tract and necessary for normal platelet aggregation and renal function) than COX-2 (inducible by inflammatory stimuli) [6-9]. Consequently, long-term therapy with non-selective NSAIDs may cause gastrointestinal complications ranging from stomach irritation to life-threatening gastrointestinal ulceration and bleeding [10] and the patients who use NSAIDs on a chronic basis have about three times greater relative risk for serious adverse gastro intestinal events compared to the population of non-user.[11],[12]

MATERIALS AND METHODS

Chemical Synthesis of 5-(3-chloro-1-benzothien -2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol:

The chemical synthesis of this compound was made according to the method reported in A. Hussain K. Sharba et al ; mp 231-233°C (lit. mp 231°C) [13]



Anti-inflammatory activity:

***Carrageenan-induced rat paw edema*:**[14] Anti-inflammatory activity was carried out using carrageenan-induced rat paw edema. The rats weighing between 150-200 g were selected randomly. Animals were divided into different groups with six animal in each group. Group I rats were used as control and each animal in this group received dimethyl sulfoxide (DMSO) i.p. only. Group II received naproxen (standard drug) dissolved in DMSO (50 mg/kg i.p.) [15] , while group IIIa, IIIb, IIIc received the synthesized compound (10 mg, 15 mg, and 20 mg /kg i.p.) respectively 30 min before inducing inflammation with the injection of fresh carrageenan. The rat hind paw edema was used as a model of acute inflammation. Acute inflammation of the hind paw was induced in each of the rats by injecting carrageenan (0.1 mL (3%)/kg) into the sub plantar surface of the right hind paw. Linear diameter of the injected paw was measured (with a screw gauze) for 4 h at 60 min intervals after the administration of the phlogistic agent. Increases in the linear diameter of the right hind paws were taken as indicators of paw edema..Percentage inhibition of the edema was calculated from the formula:

% inhibition = (C0–Ct/C0)×100

Where C0 is the average inflammation (hind paw edema) of the Group I (control) at a given time; and Ct is the average inflammation of the (Group II) or (Group III) at the same time.

***Cotton pellet induced granuloma in rats*:** The animals were divided into three groups of six animals in each group. Cotton pellets weighing 50 ± 2 mg were autoclaved and implanted subcutaneously into the back of each rat. The first group served as control and received the vehicle only (sodium CMC). Second group of animals were administered with standard drug naproxen (50mg/kg). The animals of third group were treated with the synthesized compound (20 mg/kg). All the doses were administered orally for seven days. On the 8th day, the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60^{o}C weighed and compared with control. [16] Percentage inhibition of the granuloma was calculated from the formula:

% inhibition = (Wc–Wd/Wc)×100.

Where Wc is the average weight of the granuloma of the Group I (control); and Wd is the the average weight of the granuloma of (Group II) or (Group III)].

Ulcerogenic liability:

The ulcerogenic liability was determined in rats following the previously reported standard method [17,18] .Rats of either sex (pregnant female rats were excluded) weighing 150-200 g were divided into three groups of five animals each. The first group (group I) was considered as control and receive normal saline only. Group II receive naproxen (standard) 50 mg/kg and group III receive the tested compound 20

mg/kg. The animals were fasted 18 hours before drug administration. Naproxen and the tested compounds were suspended in saline solution and were administered orally for three successive days to fasted rats. One hour after the last dose, the animals were sacrificed by cervical dislocation and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens (10 x) for the presence of lesions and erosions. The stress lesion was defined as a round or linear mucosal black or red defect [19] and the ulcer index was defined as the sum of lengths & widths of all ulcers for each stomach. While inhibition percentage of peptic ulceration was calculated by the following formula: Inhibition % =(UIc-UIt/UIc)x100%. Where UIc: Ulcer index of control group, UIt: Ulcer index of treatment group [20]

RESULTS AND DISCUSSION

Anti-inflammatory activity was carried out for the synthesized compounds using carrageenan-induced rat paw edema method and cotton pellet induce granuloma in rats method Carrageenan-induced paw edema in rat and cotton pellet induce granuloma in rats method has been accepted as a useful tool for investigating anti-inflammatory agents. It is suggested that there are biphasic effects in carrageenan-induced edema. The early hyperaemia results from the release of histamine and serotonin, the delayed phase of carrageenan-induced edema results mainly from the potentiating effects of bradykinin on mediator release, and also from prostaglandins which produce edema after the mobilization of leukocytes [21]. The edema was reached its highest thickness 2 h after the application of the stimuli. The synthesized compound shows a dose dependant potential anti-inflammatory activity as compared to the standard drug as shown in table (1).

Table (1) *Carrageenan-induced rat paw edema as a measure for acute inflammation.*

Groups	**Change in paw diameter (in mm) after drug administration**				**Percentage inhibition of edema**			
	1h	**2h**	**3h**	**4h**	**1h**	**2h**	**3h**	**4h**
I	0.71±0.04	0.76±0.03	0.71±0.05	0.61±0.03				
II	0.43±0.02**	0.44±0.05**	0.45±0.06**	0.41±0.04*	39	42	37	33
IIIa	0.58±0.02*	0.60±0.03	0.57±0.07*	0.49±0.05	18	21	20	20
IIIb	0.52±0.05*	0.55±0.07*	0.51±0.03*	0.48±0.04	27	28	28	21
IIIc	0.46±0.05**	0.51±0.04*	0.48±0.05**	0.47±0.02*	35	33	32	23

*P<0.05; **P<0.01.

Chronic inflammation occurs by means of the development of proliferative cells. Anti inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides. [22] The synthesized compound showed significant anti-inflammatory activity in cotton pellet induced granuloma and have a potential activity to delay the formation of chronic inflammatory conditions, which

reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during
granuloma tissue formation. The tested compound shows good chronic anti inflammatory effect as compared with naproxen as shown in Table (2).

Table (2): Cotton pellet induced granuloma in rats

Groups	Weight of granuloma (mg)	Percent inhibition
Group I (Control)	188.67 ±1.2	0%
Group II Standard	88.83 ±1.72	53.3%
Group III Synthesized comp.	106 ± 2.26	44.4%

It has been reported in the literature that lower ulcerogenic activity of compounds is associated with the 1,2,4- triazole moiety. The results of biological studies showed that 1,2,4-triazole derivative of NSAIDs have anti inflammatory and analgesic effect with minimum ulcerogenic and lipid peroxidase activities. It increase the COX-2 selectivity of the compound resulting in less effect on COX-1[23].Thus, this study shows that the tested compound has inhibited the induction of gastric mucosal lesions as shown in Table (3).

Table (3): Ulcerogenic liability of the tested compound

Group	Ulcer Area (mm).	% ulcer induction
Group I	15.32 ±4.23	Control
Group II	121.21 ±18.1	87.3%
Group III	73.81 ±14.53	79.3%

تقييم التأثير المضاد للالتهابات و التأثير المقرح ل 5-(3-كلورو-1-بنزوثين-2-يل)-4-فنيل-4ه -1,2,4-ترايزول-3-ثايول

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الخلاصة

الغرض من هذه الدراسة هو لتقييم التأثير المضاد للالتهابات و التأثير المقرح ل 5-(3-كلورو-1-بنزوثين-2-يل)-4-فنيل-4ه -1,2,4-ترايزول-3-ثايول. إن التأثير المضاد للالتهاب للمركب تم تحديده عن طريق مقارنته مع عقار قياسي (نابروكسين) و تم دراسة هذا التأثير باستخدام الكاراجينان المحفز لورم القدم و قطع القطن المحفزة للورم الحبيبي في الجرذان. أما التأثير المقرح فتم تقييمه و مقارنته مع العقار القياسي. أظهرت النتائج إن المركب له تأثير مضاد للالتهابات واضح مقارب للعقار القياسي و بتأثير مقرح اقل.

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