ANTI-ULCEROGENIC EFFECT OF 2-(PYRIMIDINYLSULFINYL) BENZIMIDAZOLE DERIVATIVE AGAINST DIFFERENT ULCEROGENIC AGENTS IN RATS

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Summary

 The present study was designed to investigate the effect of 2-(pyrimidinylsulfinyl) benzimidazole derivative against experimentally induced gastric ulcer. The ulcer score against various ulcer inducing agents viz., ethanol/HCl, acetylsalicylic acid and indomethacin screening model in Wistar rats .The various degrees of inhibitions by 2-(pyrimidinylsulfinyl) benzimidazole derivative (50-200 mg/kg/p.o) were statistically significant ($p < 0.001$). The effect of several synthesized compound was comparable to that of the standard drugs used. The mechanism can be attributed to decrease in gastric acid secretary activity along with strengthening of mucosal defensive mechanism by prostaglandin synthesis and inhibiting the leukotriene synthesis. Thus, 2-(pyrimidinylsulfinyl) benzimidazole derivative exhibited a good antiulcer activity

Keywords: Benzimidazole, 2-(pyrimidinylsulfinyl) benzimidazole, Anti-ulcer.

Introduction

Peptic ulcer disease is common, affecting millions of people yearly. The principle causes of peptic ulcer are infection by *Helicobacter pylori* and administration of NSAIDs (nonsteroidal anti-inflammatory drugs). *Helicobacter pylori* is the commonest cause of peptic ulceration, but only 15% of infected people develop an ulcer in their lifetime. Peptic ulcers developed due to an imbalance between aggressive factors (*H. pylori*, NSAIDs, gastric acid) and protective factors (mucin, bicarbonate and prostaglandins) leading to an interruption in the mucosal integrity [1].Although exact etiology of the disease is not known, an imbalance between defensive mechanisms (gastric mucosal integrity) and aggressive factors (gastric acid secretion)   result in peptic ulcer. Available information supports a central role of *Helicobacter pylori* [2, 3].
Consequently, reduction of gastric acid production as well as reinforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease.
Prostaglandins are synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Also, there is evidence

concerning the participation of reactive oxygen species in the etiology and pathophysiology of ulcer [4].Non-judicious use of alcohol, NSAIDs and even bile reflux can also lead to mucosal ulceration. NSAIDs are used worldwide for the treatment of pain, rheumatic, cardiovascular diseases, and more recently, for the prevention of colon cancer and Alzheimer's disease [5]. It is known that stress, alcohol, steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are some of the factors that increased ulcer risk [6]. A number of products have been used for the treatment of gastric ulcers such as antacids, proton pump inhibitors or antihistaminics [7]. A multiple mechanism action of drugs and formulations are effective in healing experimentally induced gastric ulcers [8].

Azinazoles including pyrido-, pyrimido-, and triazinobenzimidazole heterocycles have played a pivotal role in the development of effective pharmacophores [9].Anti-ulcerogenic activity exhibited by some benzimidazole synthesis [10].
However, the aim of this study was to design a series of new 2-(pyrimidinylsulfinyl) benzimidazole derivative (**A**) as interesting bioactive analogues of benzimidazole and its derivative and the evaluation of the anti-ulcerogenic properties of these derivative.
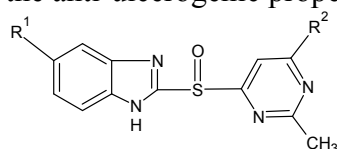
2-[(2-methyl,6-alkylpyrimidin-4-yl)sulfinyl]-5-alkyl-1*H*-benzimidazole

(A)

Materials and methods
Chemical synthesis: Synthesis of 2-(pyrimidinylsulfinyl) benzimidazole derivatives (BD).
Preparation of 2-[(2, 6-alkylpyrimidin-4-yl) sulfinyl] 5-alkyl-1h-benzimidazole.

A solution of m-chloroperbenzoic acid 0.30 g (1.75 mol) was added drop wise to solution of 2-[(2 methyl,6-alkylpyrimidin-4-yl) sulfanyl]-5-alkyl-1H-benzimidazole in 35 ml of CH_2Cl_2 at 0 °C. The reaction mixture was stirred at the same temperature for an hour. The solution was washed with 10% Na_2CO_3 solution and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography.2-[(2 alkyl,6-alkylpyrimidin-4-yl) sulfinyl]-5-alkyl-1H-benzimidazole was obtained in 89% yield.
The structure of the compound was established by recording its IR, NMR spectra.

2-[(2-Methyl,6-alkylpyrimidin-4-yl)sulfanyl]
-5-alkyl-1*H*-benzimidazole

m-chloro
perbenzoic acid

2-[(2-Methyl,6-alkylpyrimidin-4-yl)sulfinyl]-5-alkyl-1*H*-
benzimidazole
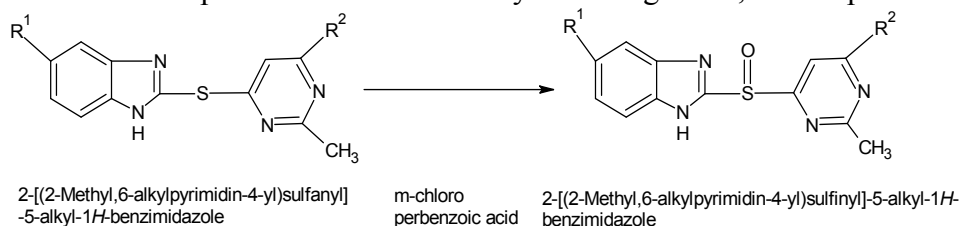
Animals
Male Wistar rats weighing 200±20g were selected. All animals were maintained in standard propylene cages and with free access to standard diet and continuous water supply. The animals were housed at room temperature (20±2 °C) on a normal day-night cycle (0600 h to 1800 h). All animal experimentation was carried out after approval of the protocol by the Institutional Ethical Committee of Nagpur University.

Chemicals

All reagents used were of high-grade purity. Acetylsalicylic acid and indomethacin were procured from Sigma-Aldrich Corporation USA, whereas cimetidine was of M/s Cadila pharmaceutical Company India. All the chemicals used in the other solutions were of analytical grade.

Toxicity studies

Toxicity studies were carried out on normal healthy rats. The 2-(pyrimidinylsulfinyl) benzimidazole derivative (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes.

Experimental models

Ethanol/HCl-induced gastric ulceration in rats

The experiment was performed according to the Mizui and Dotuchi method with slight modifications [11]. In brief 24 h fasted rats in the weight range of 180–200 g with free access to water were treated with different doses of 2-(pyrimidinylsulfinyl) benzimidazole derivative (50 mg to 200 mg/kg/oral). One group was kept as vehicle control and another group treated with cimetidine, a standard drug. One hour after the drug administration the animals received 1.5 ml of a mixture of 70% alcohol+5% HCl as ulcerogenic agent. The animals were sacrificed 4 h after the ulcerogenic dose of ethanol/HCl mixture by cervical dislocation. The stomach removed, cut opened along the greater curvature washed with normal saline and observed for the severity of the ulcers according to the scale mentioned above. The gastric fluid was collected for the determination of its volume and pH.

Acetylsalicylic acid (ASA)-induced gastric ulcer

Gastric ulcerations were induced experimentally in male Wistar rats according to the Asano method [12].Following a 24 h fasting with water *ad libitum*, rats were dosed orally with different doses of 2-(pyrimidinylsulfinyl) benzimidazole derivative (BD), cimetidine and vehicle (Tween 80). One hour later 300mg/kg per oral of ASA was administered. The animals were sacrificed 4 h after ASA dosing; stomach was removed and observed for percent protection of ulcerative lesions.

Indomethacin-induced gastric ulcer

Male Wistar rats (180–200 g) were deprived of food for 24 h with free access to water prior to the experiment. The animals received oral doses of 2-(pyrimidinylsulfinyl) benzimidazole derivative (BD), cimetidine and vehicle, following 1 h before administration of 20 mg/kg per oral of indomethacin. Six hour after indomethacin administration the animals were sacrificed, their stomach removed and examined for ulcer protection [12].

Ulcer score: the numbers of ulcers were counted using magnifying lenses. Each ulcer was then measured with a vernier calliper to assess the diameter. Ulcer index was determined by scoring method of Suzuki et al. (1976).The percent protection with each test drug dose was also calculated by the following formula [13]:

$$\% \text{ Protection} = \frac{(\text{UI control} - \text{UI treated})}{(\text{UI control})} \times 100,$$

where UI stands for ulcer index.

Statistical analysis

Statistical analysis was carried out by using Graph-Pad Instat statistical package (Graphpad Software Inc.). Values are expressed as mean±S.E.M. For multiple comparisons, one way ANOVA was used followed by Tukey's post hoc test. p value <0.05 was considered to be significant.

Results

Ethanol/HCl-induced gastric ulceration

Administration of different doses of 2-(pyrimidinylsulfinyl) benzimidazole derivative (BD) shows significant (p < 0.001) changes in the ulcer index in ethanol/HCl-induced rats. The ulcerated control group showed the ulcer index 11 ± 3.75, and the maximum numbers of ulcers were of the ulcer score 5 and 10, and also a number of perforated ulcers (Score 25). However, the standard drug cimetidine (100 mg/kg) exhibited maximum reduction in ulcer index compared to the control animals (Table.1).

Acetylsalicylic acid (ASA)-induced gastric ulceration

Aspirin treatment resulted in the production of gastric lesions in glandular segment of the stomach. Oral administration of BD at different dose levels inhibited the appearance of gastric lesions in a dose dependent manner. Significant anti-ulcer effect started at a dose of 200 mg/kg with an inhibition as compared to the control group. Similarly, cimetidine shows significant (p<0.001) decrease in ulcer production (Table 1).

Indomethacin-induced gastric ulceration

Oral administration BD pretreatment on indomethacin-induced gastric ulceration showed a dose dependent reduction in ulcer index in pretreated groups relative to control. The reduction was statistically significant (P<0.01) compared to control (Table 1). The effect was comparable to that of the standard drug, cimetidine.

Table. 1 Anti-ulcerogenic effect of BD against different ulcerogenic agents in rats

Treatment and dose	Different ulcer-inducing agents		
	Ethanol/HCl	ASA	Indomethacin
Control	$11.56 \pm 3.75^{@}$	$5.80 \pm 1.72^{@}$	$4.8 \pm 0.5^{@}$
Cimetidine (100 mg/kg)	$7.45 \pm 0.25^{***}$	$2.78 \pm 0.80^{***}$	$3.0 \pm 0.4^{***}$
BD (50 mg/kg)	$9.14 \pm 2.25^{*}$	$4.35 \pm 0.68^{*}$	$3.9 \pm 0.2^{*}$
BD (100 mg/kg)	8.24 ± 1.75	$4.25 \pm 0.70^{*}$	$3.6 \pm 0.4^{**}$
BD (200 mg/kg)	$7.68 \pm 2.75^{***}$	$2.98 \pm 0.36^{***}$	$3.2 \pm 0.8^{***}$

Values are expressed as mean±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group.
p- value   # p<0.001 ,***p<0.001, **p<0.01, *p<0.05 (One-way ANOVA followed by Tukey's post hoc test).

Discussion

The 2-(pyrimidinylsulfinyl) benzimidazole compound was successfully prepared by developed process and further purified using different solvents the compound were recrystalized by using ethanol and checked the purity by thin layer chromatographic techniques.

The antiulcer activity of BD was evaluated using ethanol/HCl, ASA and indomethacin–induced ulcer models. The anti-ulcer effect was evident by the significant inhibitory effect depicted in Table 1 in comparison to vehicle control and standard drug cimetidine. Various mechanisms have been thought to be involved in the ulcer production in different experimental models [14]. Hence, it is not possible to propose a single mechanism for anti-

ulcer effect to a particular drug. Ethanol-induced gastric lesion formation may be due to stress in gastric blood flow that contributes to the development of the hemorrhage and necrotic aspects of tissue injury [15].HCl further deepens the necrosis and increase tissue injury. It was observed in this study that the BD reduced significantly ethanol/HCl- induced ulcer. The significant inhibitory effect of BD in ASA and indomethacin-induced ulceration further support the antiulcer effect of 2-(pyrimidinylsulfinyl) benzimidazole derivative. The ulceration induced by ASA is attributed mainly due to the biosynthesis of cytoprotective prostaglandin resulting in overproduction of leukotriene and other products of 5-lipoxygenase pathway [16]. These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions, and drop in the transmucosal potential difference and induce the formation of erosions and ulcers [17]. These results indicate a possible local increase in synthesis of cytoprotective prostaglandin, inhibition of leukotriene and gastric mucosal permeability to H^+ and Na^+ ions by BD. Indomethacin, a known ulcerogen especially in an empty stomach [18] causes ulcer mostly on the glandular (mucosal) part of the stomach [19,20].The BD was observed to significantly reduce mucosal damage in the indomethacin–induced ulcer model, suggesting the possible effect of BD and involvement of prostaglandin in the anti ulcer effect of BD.

Conclusion

In conclusion, it is demonstrated that, the anti-ulcer data of BD signifies that it might be either acting by increasing the gastric mucosal resistance, local synthesis of cytoprotective prostaglandins and/or inhibiting the leukotriene synthesis. Hence the above result shows that 2-(pyrimidinylsulfinyl) benzimidazole derivative have a definite antiulcer activity with lesser side effect. The encouraging results showed may lead to the development of novel antiulcer drug if explored further.

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