

SYNTHESIS AND STUDY OF SOME NOVEL BENZIMIDAZOLE ANALOGS AS POTENTIAL ANTIULCER AGENTS

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ABSTRACT:

The present investigation is concerned with synthesis of new substituted benzimidazole derivatives **4a-4f** with the objective of discovering novel and potent antiulcer agent. The structures of all the synthesized compounds were characterized by spectral and elemental analysis. The synthesized compounds were screened for their antiulcer activity at the doses of 10 and 20 mg / kg p. o. The compound **4d** showed highest antiulcer activity.

**Keyword:** Synthesis, Benzimidazole, Piperazine, H<sup>+</sup>/K<sup>+</sup>ATPase, Antiulcer

1. Introduction

Gastric acid has been known to play an important role in many physiological processes, such as digestion, sterilization of food and absorption of calcium and iron.<sup>1</sup> The awareness of its central role in the etiology of peptic ulcer disease (PUD) and gastroesophageal reflux disease (GERD) has grown over the years. Peptic ulcer disease (PUD) occurs when gastric acid and other digestive juices erode the inner lining of the stomach or the first part of the small intestine. This inner lining is a complex mucous barrier that protects the gastrointestinal (GI) lining from the corrosive acids and other chemicals that the stomach produces to digest food. When the barrier breaks down, the lining is exposed to the destructive potential of the digestive juices. For many years, scientists believed that acidic foods and stress caused peptic ulcers and treated the ulcers with surgery and medications to lessen the symptoms. However, research conducted in the 1980s revealed that the most common cause of peptic ulcers is infection by bacteria called *Helicobacter pylori*,<sup>2,3</sup> which can survive in the acidic environment of the stomach. Such bacteria can produce a change in the mucous barrier, which results in ulcers. Several other risk factors associated with recurrence of PUD includes cigarette smoking, chronic consumption of ulcerogenic drugs like NSAID, consumption of alcohol for prolonged periods, age, emotional stress and family history.<sup>4,6</sup> Benzimidazole sulfinyl methyl pyridine is a well established class of H<sup>+</sup>/K<sup>+</sup>ATPase inhibitors, therapeutically useful in the treatment of acute and chronic ulcer conditions.<sup>1</sup> In the past few years, research for

new antiulcer agents has focused on numerous structural patterns of benzimidazole sulfinyl methyl pyridine moiety by substitution on the benzimidazole ring, methyl sulfinyl chain and pyridine.<sup>2</sup>

In view of these reports, it was thought worthwhile to synthesize and investigate the compounds in which the benzimidazole derivatives have been linked with the piperazine moiety.<sup>7</sup> On the basis of the above mentioned reports, the present work is concerned with the synthesis of different novel 2-[[4-(substituted phenyl) piperazine-1-yl] methyl sulfinyl]-1H-benzo (d) imidazole derivatives with the objective of discovering novel antiulcer agents.

2. Experimental

Melting points were determined with Lab line melting point apparatus and are uncorrected. The reactions were monitored by thin layer chromatography (TLC) using silica gel-G (benzene:ethanol, 1:5). Infra-Red spectra were recorded on a Shimadzu 8400-s spectrophotometer using KBr pellets. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were recorded on a Version 300 MHz spectrometer (CDCl<sub>3</sub>, δppm) with TMS as an internal standard. MS spectra were recorded on Shimadzu QP5050 spectrophotometer. Elemental analysis (C, H, N) were performed on a FLASH EA 1112 analyzer and were within ± 0.4 of the theoretical value.

**2.1 General Procedure for synthesis of 2-[[4-(substituted phenyl) piperazine-1-yl] methyl thio]-1H-benzo (d) imidazole (3a-f):** 5-methoxy 2-mercapto benzimidazole (0.01 mole) refluxed

with 4-substituted phenyl 2-chloromethyl piperazine 1-yl (0.01 mole) in four times dioxane with dry potassium carbonate for 24 hrs. Reaction mixture was monitored by TLC and dumped in ice cold water. The mixture was extracted with dichloromethane and dried on sodium sulphate. The dichloromethane was distilled out. Product was filtered, recrystallized from ethanol.

3a. IR (KBr): 1580  $\text{cm}^{-1}$  (C=C Ar), 2945  $\text{cm}^{-1}$  (C-H alkyl), 1610  $\text{cm}^{-1}$  (C=N), 1530  $\text{cm}^{-1}$  (C-N), 3240  $\text{cm}^{-1}$  (N-H), 748  $\text{cm}^{-1}$  (C-S-C), 1264 (C-O-C).

**2.2 General Procedure for synthesis of 2-[[4-(substituted phenyl) piperazine-1-yl] methyl sulfinyl]-1H-benzo (d) imidazole (4a-f):** To a solution of appropriate 2-[[4-(substituted phenyl) piperazine-1-yl] methyl thio]-1H-benzo (d) imidazole (3a-f) (0.002mol) in dichloromethane (50ml), hydrogen peroxide (30%w/v, 0.3ml, 0.002mol,) in acetic acid (10 ml) was added dropwise in reaction mixture. The mixture was heated at 60°C under stirring for 3-5 hr. The solvent was then removed under reduced pressure. The residue was poured in ice cold water. The semisolid product was obtained. Product was purified by column chromatography. The structures of all the products have been ascertained by their spectral data and elemental analysis. All the products were in semisolid state with the percentage yield in between 70-75%

(4a) IR (KBr)  $\text{cm}^{-1}$ : 3264 (N-H stretching), 3145 (C-H aromatic), 2932 (C-H alkyl stretching), 1585 (C-N stretching), 1245 (C-O-C stretching), 1020 (S=O stretching), 775 (C-S-C stretching).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ ppm: 1.1 (s, 3H,  $\text{CH}_3$ ), 2.3 (t, 4H, H, 2, 6, piperazine), 2.5 (t, 4H, H, 3, 5, piperazine), 3.9 (s, 3H,  $\text{OCH}_3$ ), 5.4 (s, 2H,  $\text{CH}_2\text{S}$ ), 6.2-7.8 (m, 7H, ArH), 14.1 (s, 1H, NH).

MS(m/z)384[M]<sup>+</sup>, Calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{S}$ : C, 62.50; H, 6.25; N, 14.58. Found: C, 62.61; H, 6.45; N, 14.45.

(4b) IR (KBr)  $\text{cm}^{-1}$ : 3315 (N-H stretching), 3148 (C-H aromatic), 2945 (C-H alkyl stretching), 1580 (C-N stretching), 1230 (C-O-C stretching), 1010 (S=O stretching), 790 (C-S-C stretching).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ ppm: 2.4 (t, 4H, H, 2, 6, piperazine), 2.6 (t, 4H, H, 3, 5, piperazine), 3.8 (s, 3H,  $\text{OCH}_3$  phenyl), 3.9 (s, 3H,  $\text{OCH}_3$  benzimidazole), 5.4 (s, 2H,  $\text{CH}_2\text{S}$ ), 6.4-7.8 (m, 7H, ArH), 14.3 (s, 1H, NH).

MS(m/z)400[M]<sup>+</sup>, Calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_3\text{S}$ : C, 60; H, 6; N, 14. Found: C, 60.04; H, 6.08; N, 14.11.

(4c) IR (KBr)  $\text{cm}^{-1}$ : 3260 (N-H stretching), 3142 (C-H aromatic), 2930 (C-H alkyl stretching),

1590 (C-N stretching), 1241 (C-O-C stretching), 1025 (S=O stretching), 776 (C-S-C stretching).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ ppm: 1.2 (s, 3H,  $\text{CH}_3$ ), 2.4 (t, 4H, H, 2, 6, piperazine), 2.5 (t, 4H, H, 3, 5, piperazine), 3.9 (s, 3H,  $\text{OCH}_3$ ), 5.5 (s, 2H,  $\text{CH}_2\text{S}$ ), 6.6-7.8 (m, 7H, ArH), 14.3 (s, 1H, NH).

MS(m/z)384[M]<sup>+</sup>, Calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{S}$ : C, 58.47; H, 4.66; N, 11.86. Found: C, 58.60; H, 4.76; N, 11.82.

(4d) IR (KBr)  $\text{cm}^{-1}$ : 3305 (N-H stretching), 3140 (C-H aromatic), 2933 (C-H alkyl stretching), 1578 (C-N stretching), 1255 (C-O-C stretching), 1030 (S=O stretching), 775 (C-S-C stretching).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ ppm: 2.3 (t, 4H, H, 2, 6, piperazine), 2.6 (t, 4H, H, 3, 5, piperazine), 3.7 (s, 3H,  $\text{OCH}_3$  phenyl), 3.9 (s, 3H,  $\text{OCH}_3$  benzimidazole), 5.6 (s, 2H,  $\text{CH}_2\text{S}$ ), 6.9-7.8 (m, 7H, ArH), 14.2 (s, 1H, NH).

MS(m/z)400[M]<sup>+</sup>, Calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_3\text{S}$ : C, 60; H, 6; N, 14. Found: C, 60.08; H, 6.05; N, 14.18.

(4e) IR (KBr)  $\text{cm}^{-1}$ : 3315 (N-H stretching), 3155 (C-H aromatic), 2944 (C-H alkyl stretching), 1580 (C-N stretching), 1242 (C-O-C stretching), 1035 (S=O stretching), 776 (C-S-C stretching).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ ppm: 2.5 (t, 4H, H, 2, 6, piperazine), 2.6 (t, 4H, H, 3, 5, piperazine), 3.8 (s, 3H,  $\text{OCH}_3$ ), 5.5 (s, 2H,  $\text{CH}_2\text{S}$ ), 6.9-7.6 (m, 7H, ArH), 14.1 (s, 1H, NH).

MS(m/z)405[M]<sup>+</sup>, Calcd for  $\text{C}_{19}\text{H}_{21}\text{N}_4\text{O}_2\text{S}\text{Cl}$ : C, 56.29; H, 5.18; N, 13.82. Found: C, 58.42; H, 5.22; N, 13.86.

(4f) IR (KBr)  $\text{cm}^{-1}$ : 3264 (N-H stretching), 3145 (C-H aromatic), 2932 (C-H alkyl stretching), 1585 (C-N stretching), 1245 (C-O-C stretching), 1020 (S=O stretching), 775 (C-S-C stretching).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ ppm: 2.2 (t, 4H, H, 2, 6, piperazine), 2.6 (t, 4H, H, 3, 5, piperazine), 3.8 (s, 3H,  $\text{OCH}_3$ ), 5.4 (s, 2H,  $\text{CH}_2\text{S}$ ), 6.8-7.7 (m, 7H, ArH), 14.2 (s, 1H, NH).

MS(m/z)388[M]<sup>+</sup>, Calcd for  $\text{C}_{19}\text{H}_{21}\text{N}_4\text{O}_2\text{SF}$ : C, 58.76; H, 5.41; N, 14.43. Found: C, 58.92; H, 5.62; N, 14.40.

**2. 3. Acute toxicity (LD<sub>50</sub>):** Healthy adult albino mice (20–25 g) of either sex, starved overnight were subjected to acute toxicity studies as per guidelines (AOT No. 425) suggested by Organization for Economic Co-operation and Development (OECD) 2001. The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles for any lethality or death for next 48 h. at a dose of 2000 mg / kg b. wt. p. o. did not produce any toxicity.

**2.4. Antiulcer Studies**

### 2.4.1 Pylorus ligation induced gastric ulcer:

The different doses were screened for antiulcer activity by pylorus ligation in shay rat method using Omeprazole as standard.<sup>8</sup> Wistar albino rats of either sex (150-200g) were kept in the departmental animal house at room temperature 25-30°C. (Institute Animal Ethical Committee No. 651 / 02 / BC / CPCSEA of R. C. Patel College of Pharmacy, Shirpur, and were as per CPCSEA guidelines.) Rats were divided into various groups. Omeprazole (10 and 20 mg/kg) and pure compounds **4a-f** (10 and 20 mg/kg), were suspended in 1% suspension of CMC (Carboxy methyl cellulose) in distilled water and administered by oral route. The animals were fasted for 48 h prior the experiment, but had free access to water. After the fasting period, the animals were given the drug samples p.o, 1 h prior the ligation. Thereafter, the rats were anaesthetized with anesthetic ether. An incision of 1 cm length in the abdomen just below the sternum was made. The stomach was exposed. A thread was passed around the pyloric sphincter and a light knot was applied to it taking due care that no blood vessel was tied along the knot. Then incision was closed by stitching the abdominal wall by a thread. The underlying skin was cleaned of any bleeding etc. An antiseptic cream was applied over the wound. Thereafter, the animal was kept in a separate cage and allowed to recover. Four hours later these animals were sacrificed and the stomach of each of the animals was isolated and cut open through its greater curvature. Its contents emptied in to

graduated test tube, volume was recorded. pH was measured by pH meter. Ulcer score were observed and ulcer index were calculated by One Way ANOVA analysis and results were compared with control by student's t test.

### 3. Results and Discussion

All the newly compounds **4a-f** were synthesized as shown in Scheme 1 and tested in vivo in order to evaluate their antiulcer activity. These compounds were screened for their antiulcer activity at a dose of 10 and 20 mg / kg p. o. exhibited good antiulcer activity as shown in Table 1. The characteristic feature of this series is substituted phenyl piperazine moiety linked by sulfinyl methyl at second position of benzimidazole nucleus. It was observed that compound **4d** showed maximum antiulcer activity. Piperazine substituted with 4-methoxy phenyl showed good antiulcer activity may be due to electron donating nature of methoxy group. These compounds may be resembling the action of the proton pump inhibitors i.e. omeprazole and omeprazole like drugs which irreversibly bind to the gastric proton pump on the parietal cell membrane, inhibiting the release of hydrogen ions from the parietal cells into the lumen of gastric glands and henceforth in the stomach. However, it is very difficult at this stage to explain the exact mechanism of action of these novel benzimidazole analogs and extensive studies are required further to understand their specific mechanism of action.

Table 1

Comp. No.	R	Pylorus ligated antiulcer activity					
		Ulcer index $\pm$ SEM		PH of gastric juice $\pm$ SEM		Volume of gastric juice(ml) $\pm$ SEM	
		10mg	20mg	10mg	20mg	10mg	20mg
4a	2-CH <sub>3</sub>	3.5 $\pm$ 0.1581	2.2 $\pm$ 0.1304***	3.2 $\pm$ 0.07071	4.2 $\pm$ 0.08945***	7.4 $\pm$ 0.114***	5.0 $\pm$ 0.08718**
4b	2-OCH <sub>3</sub>	2.9 $\pm$ 0.1811*	2.1 $\pm$ 0.07071***	4.3 $\pm$ 0.07071***	4.9 $\pm$ 0.07072***	6.5 $\pm$ 0.1241	4.5 $\pm$ 0.05099***
4c	4-CH <sub>3</sub>	3.2 $\pm$ 0.3	2.1 $\pm$ 0.08124***	3.5 $\pm$ 0.1703*	4.2 $\pm$ 0.1949**	7.2 $\pm$ 0.05832**	5.5 $\pm$ 0.07071*
4d	4-OCH <sub>3</sub>	2.6 $\pm$ 0.1871**	1.6 $\pm$ 0.07071***	5.3 $\pm$ 0.08366***	6.1 $\pm$ 0.1703***	6.0 $\pm$ 0.07071	4.0 $\pm$ 0.1***
4e	4-Cl	4.0 $\pm$ 0.2236	2.8 $\pm$ 0.07071**	2.8 $\pm$ 0.07071	3.5 $\pm$ 0.1703*	9.0 $\pm$ 0.07071***	6.5 $\pm$ 0.04473
4f	4-F	3.8 $\pm$ 0.255	2.5 $\pm$ 0.07071**	3.0 $\pm$ 0.07071	3.8 $\pm$ 0.0707**	9.0 $\pm$ 0.07071***	6.2 $\pm$ 0.07072
Omeprazole		0.7 $\pm$ 0.255***	0 $\pm$ 0***	7.0 $\pm$ 0.1703***	7.02 $\pm$ 0.102***	1.92 $\pm$ 0.2035***	1.34 $\pm$ 0.14***
Control		4 $\pm$ 0.3162		2.86 $\pm$ 0.20		6.1 $\pm$ 0.2191	

n=5, Values are expressed as mean  $\pm$  SEM ; \* P < 0.05 compared to control group (Student's t test)

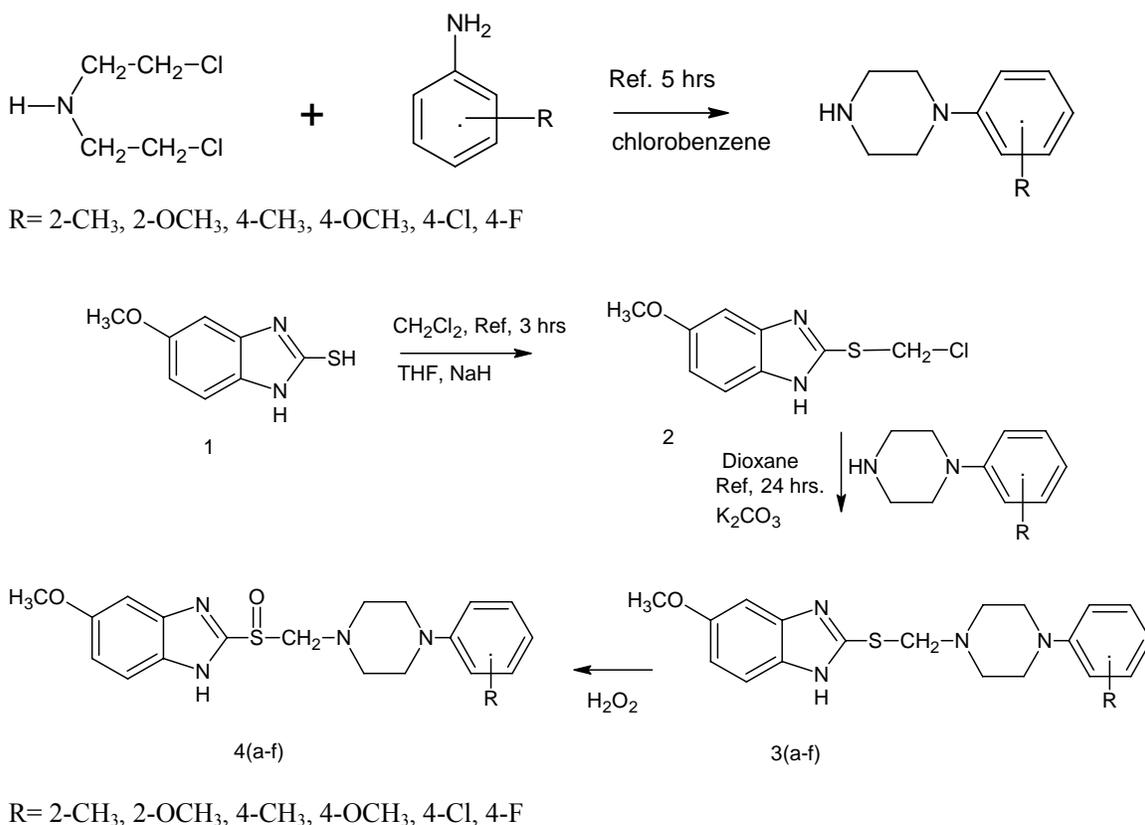
\*\* P < 0.01 compared to control group (Student's t test); \*\*\* P < 0.001 compared to control group (Student's t test)

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**Scheme-1**