Synthesis and Pharmacological Evaluation of Novel Benzimidazole Derivatives as Antiulcer and H+ K+ ATPase Inhibitor

Khan Farhan R.1* and Mohd. Saleemuddin Farooqui2

1Patal Dhamal Wadwani College of Pharmacy Yavatmal, Maharashtra, India.
2Shree Sainath College of Pharmacy, Dawalameti, Amravati Road, Nagpur, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

ABSTRACT

Aims: To Synthesis the novel substituted benzimidazole derivatives and screened Pharmacologically as antiulcer and H+ K+ ATPase inhibitor.

Study Design: Study design as series of substituted benzimidazole derivatives prepared by three different reaction scheme and further evaluated pharmacologically in desired activity.

Place and Duration of Study: In Patal Dhamal Wadwani College of Pharmacy Yavatmal, Maharashtra, India, between July 2014 to August 2017.

Methodology: A series of new 2-[(substituted-pyrimidin-4-yl) sulfinyl]-1H-benzimidazole (54a-54i) were synthesized by the condensation of O-phenylenediamine, KOH and CS2 resulting potassium 1H-benzimidazole 2-thiolate further treated with glacial acetic acid gives 1H-benzimidazole 2-thiol (2-mercaptopbenzimidazole). 4-Chloro 2 Methyl, 6-Alklypyrimidine- 4-ol prepared by ethanimidamide and alkyl acetoacetate gives 2-Methyl, 6-Alklypyrimidine-4-ol by chlorination using thionyl chloride 4-Chloro 2 Methyl, 6-Alklypyrimidine- 4-ol was obtained. further reacted with

*Corresponding author: E-mail: frk01234@rediffmail.com;
1. INTRODUCTION

Benzimidazoles are high melting compounds. The parent compound melts at 170°C. Benzimidazoles are commonly soluble in polar solvents and sparingly soluble in nonpolar solvents. The introduction of polar substituents on different positions of benzimidazole ring increases their solubility in nonpolar solvents e.g. 2 methyl benzimidazoles is soluble in ether. Similarly, the introduction of polar groups in ring increases solubility in polar solvents.

A peptic ulcer is one of the most important problems worldwide and also causes high-rate morbidity. Over the past few years, a substantial increase was seen in peptic ulcer incidence all over the world. Gastric ulcer is defined as a break in the lining of mucosa and various factors that influence gastric mucosal barrier such as NSAIDs drugs, hydrochloric acid, smoking of Cigarette, pepsin, and H. pylori cause an ulcer. Gastric acid hypersecretion is a pathological condition, which arises due to excessive secretion of hydrochloric acid from the parietal cells of gastric mucosa through the proton pumping H+ K+ ATPase. The instantaneous cause of peptic ulceration is digestion of the mucosa by pepsin and acid of the gastric juice. So far, the sequence of events leading to this is unidentified. The severe pain and irritation in the upper abdomen are symptoms of peptic ulcer. If a peptic ulcer is not treated appropriately, it may lead to perforations in the wall of the gastrointestinal tract. In the treatment of gastric and duodenal ulcer disease inhibition of gastric acid, secretion has been proven to be a powerful therapeutic principle.

Proton pump inhibitors (PPIs) category of therapeutic agents for most acid-related diseases, including, gastroesophageal reflux diseases, peptic ulcer diseases, and acute gastrointestinal bleeding. Benzimidazole type agents contribute significantly to these agents like omeprazole and pantoprazole. 1H Benzimidazole exhibits remarkable basic characteristics because of its nitrogen content, hence the active substance of several drugs. According to various studies, benzimidazoles structures were present in several classes of drugs. This research study was aimed to develop antiulcer and antisecretory agents from different benzimidazole derivatives.

2. MATERIALS AND METHODS

The chemicals used in the present research project work were purchased from Loba, Merck, and Fisher scientific chemicals. The melting point of the synthesized compounds was determined in an open capillary using the LABHOSP melting point apparatus. The purity and homogeneity of compounds were ascertained by sharp melting points and thin-layer chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates using Butanol: Ethanol: Water (9:1:1) solvent system. Visualization was done in a UV light chamber at 254 nm, iodine chamber.
infrared spectra for the synthesized compounds were recorded using SHIMADZU-FTIR 8400 spectrophotometer using potassium bromide pellet technique and sodium chloride cells for liquid samples. H1NMR spectra of the synthesized compounds were taken using Bruker ACF-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. The Mass spectra were recorded using SHIMADZU GCMS-8040 mass spectrometer. The structures were confirmed by spectral studies like FT-IR, NMR, and Mass Spectroscopy. Chemical shift is recorded in parts per million (ppm, δ) and the signals are described as (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), and m (multiplet). All the synthesized compounds were characterized based on the mass profile of respective compounds.

3. SYNTHESIS OF COMPOUNDS

The synthesis of compounds is illustrated in Scheme 1, 2, 3 and 4. Details are described as follows.

![Reaction scheme 1 of the synthesized compound](image)

**Table 1. List of synthesized compound (54a-54i)**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Compound code</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>54a</td>
<td>H</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>2.</td>
<td>54b</td>
<td>H</td>
<td>( \text{C}_2\text{H}_5 )</td>
</tr>
<tr>
<td>3.</td>
<td>54c</td>
<td>H</td>
<td>( \text{C}_3\text{H}_7 )</td>
</tr>
<tr>
<td>4.</td>
<td>54d</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>5.</td>
<td>54e</td>
<td>( \text{CH}_3 )</td>
<td>( \text{C}_2\text{H}_5 )</td>
</tr>
<tr>
<td>6.</td>
<td>54f</td>
<td>( \text{CH}_3 )</td>
<td>( \text{C}_3\text{H}_7 )</td>
</tr>
<tr>
<td>7.</td>
<td>54g</td>
<td>( \text{NO}_2 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>8.</td>
<td>54h</td>
<td>( \text{NO}_2 )</td>
<td>( \text{C}_2\text{H}_5 )</td>
</tr>
<tr>
<td>9.</td>
<td>54i</td>
<td>( \text{NO}_2 )</td>
<td>( \text{C}_3\text{H}_7 )</td>
</tr>
</tbody>
</table>
3.1 Scheme 1

3.1.1 Synthesis of benzimidazole 2-thiolate

O-phenylenediamine 10.8g(0.1mol), 5.65 gm (0.1 mol) of potassium hydroxide and 7.67 gm (0.1 mol,6.19ml) of carbon disulphide, 100 mL of 95% ethanol and 15 mL of water in 500 ml of round bottom flask were heated under reflux for 3 hours. 1.15 gm. Charcoal was added cautiously and further heated for next 10 min; Charcoal was removed by filtration. The filtrate was heated to 60-70°C and allowed to cool at room temperature. The product was collected on a Buckner funnel.
and dried overnight. The dried product was recrystallized from ethanol, the yellowish white crystals of potassium 1H-benzimidazole 2-thiolate were collected.

3.1.2 Synthesis of benzimidazole 2-thiol

Potassium 1H-benzimidazole 2-thiolate was mixed with 300 mL of water and 25 mL of acetic acid stirred for another hour at 60-70 °C. The crude product of 1H-benzimidazole 2-thiol starts separating as glistening white crystal on cooling, then kept in refrigerator for 3 hours for complete crystallization.

3.2 Scheme 2

3.2.1 Synthesis of substituted pyrimidine

3.2.1.1 Synthesis of 2-methyl, 6-alkylpyrimidine-4-ol

In 250 ml round bottom flask 5.8 g (0.1 mol) of ethanimidamide and 10.80 ml (0.1 mol) alkyl acetate were placed then sodium ethoxide was added and refluxed for 2-3 hours. Reaction mixture filtered and 2-Methyl 6-pyrimidine-4-ol was collected as solid.

3.2.1.2 Synthesis of 4-chloro 2-methyl 6-alkylpyrimidine-4-ol

In 250 ml round bottom flask 0.1 mol of 2,6 alkylpyrimidine-4-ol and 10 ml (0.1 mol) thionyl Chloride was placed followed by few pieces of porcelain and refluxed for 2-3 hours. The reaction mixture was filtered and solid product of 4-Chloro 2-methyl 6 Alkyl pyrimidine-4-ol was collected.

3.3 Scheme 3

3.3.1 Synthesis of 2-(Pyrimidinylsulfinyl) benzimidazol derivatives

3.3.1.1 Synthesis of 2-[(2, 6-alkylpyrimidin-4-yl) sulfinyl] 5-alkyl-1H benzimidazole

Sodium hydroxide 3.9 g (0.1 mol) was slowly added over 5 minutes to a stirred solution of 2-mercaptopbenzimidazole 0.29 g (1.4 mol) in ethanol 20 ml. 4-chloro 2-methyl 6 alkylpyrimidine-4-ol was slowly added to the 2-mercaptopbenzimidazole solution at 0°C, and stirred for 12 hours at room temperature. After the solvent was removed under reduced pressure, the residue was poured into 10% NaHCO₃ solution and extracted with ethyl acetate. The organic layer was dried over MgSO₄, and concentrated. The desired coupling product was obtained as a semi-solid.

3.3.1.2 Synthesis of 2-[(2,6-alkylpyrimidin-4-yl) Sulfinyl] 5-alkyl-1H benzimidazole

m-chloroperbenzoic acid 0.30 g (1.75 mol) solution was added drop wise to solution of 2-[(2 methyl, 6-alkylpyrimidin-4-yl) sulfinyl]-5-alkyl-1H-benzimidazole in 35 ml of dichloromethane (CH₂Cl₂) at 0 °C. The reaction mixture was stirred at the same temperature for an hour. The solution was washed with 10% sodium bicarbonate (NaHCO₃) solution and dried over magnesium sulfate (MgSO₄). After removal of the solvent, the residue was purified by column chromatography. 2-[(2alkyl,6-alkylpyrimidin-4-yl)sulfinyl]-5-alkyl-1H-benzimidazole was obtained. The structure of final compounds was characterized by IR, NMR and Mass spectral techniques.

4. PHARMACOLOGICAL EVALUATION

Acute oral toxicity and LD50 determination the Organization for Economic Co-operation and Development (OECD) guideline 423 (ATCM - Acute Oral Toxicity Classic Method) was followed. In accordance with OECD Test Guideline, nulliparous and of non-pregnant female Albino rats weighing between 150 and 220 g, weighing having age 8–12 weeks were randomly selected. Animal were divided into 10 group. Each group contained 6 mice, starved for 24 hours with water ad libitum prior to test. On the day of the experiment, animals were administered with 25mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg, and 200 mg/kg of test drug and its derivatives and kept in plastic cages. The animals were then observed continuously for 3 hours for general behavioral, neurological, autonomic profiles, and mortality on every 30 min for the next 3 hours and finally for the next 24 hours or a total of 14 days. One group was used as control receiving only 2% tween-80 solution. The LD50 of all the derivatives was found >200mg/kg.

4.1 Antiulcer Activity

Antiulcer activity was performed by Aspirin-induced gastric ulcer model, where ulceration induced by Acetylsalicylic acid (ASA) drug [21].

4.1.1 Procedure

Albino rats of either sex weighing between (150-200 g) were divided into 11 groups each group contain six animals (n=6) and drugs/vehicle was
administered as, Group I was served as normal control given with vehicle (2 ml/kg tween 80) only. Group II (Pantoprazole 25 mg/kg body weight) with standard drug, and groups III - XI were treated with test groups (Comp. 54a-54i, dose: 200mg/kg). The animals were then fasted (with free access to water) for a period of 24 hours so as to ensure complete gastric emptying and a steady state gastric acid secretion. The 24 h fasted animals were again administered with the drugs/vehicle on the morning of the experiment. Sixty minutes after administration of the drugs/vehicle, Acetylsalicylic acid (ASA) was administered in a dose of 300 mg/kg body weight orally to all the animals. Food was withheld for a duration of 5 more hours. Animals were then respectfully sacrificed by an overdose of anesthetic ether.

The abdomen was opened and a ligature was placed around the esophagus close to the diaphragm. The stomach was excised, the contents are drained in a centrifuge tube. Stomach was opened along the greater curvature the and pinned on a cork plate. The mucosa was examined for ulcers microscopically with the help of hand lens (10x). Ulcer index UI is calculated:

\[
UI = UN + US + UP \times 10^{-1}
\]

- **UN**= average of number of ulcers per animal
- **US** = average of severity score
- **UP** = percentage of animals with ulcers

Normal colorations 0.0, Red coloration 0.5, Spot ulcer 1.0
Hemorrhagic stress 1.5, Deep ulcer 2.0, Perforation 3.0

Ulcer index the number of ulcers counted by using magnifying glass. Ulcer severity score given in above table. Ulcer index and acidity of the gastric content of treated animals are compared with controls. Using various doses, dose-response curves can be established for ulcer formation and gastric acid secretion. Ulcer index and acidity of the gastric content of treated animals are compared with controls. The % inhibition of ulcer were calculated by formula,

\[
\text{Percentage inhibition of ulcer} = \frac{\{UI \text{ control group} - UI \text{ test group} / UI \text{ control group}\} \times 100}
\]

### 4.2 Antisecretory Activity [22]

The gastric H+, K+ ATPase is responsible for the transport of HCl in the parietal cell of the gastric mucosa through membrane by H+ for K+ exchange catalyzed by ATP driven phosphorylation/dephosphorylation. It catalyzes an electroneutral exchange of cytoplasmic protons for extra cytoplasmic potassium, there is activation of a K+ and perhaps a Cl conductance in the pump membrane which allows K to access the extra-cytoplasmic face of the pump, enabling dephosphorylation and recycling of the pump.

4.2.1 Determination of gastric H⁺/K⁺-ATPase activity [23]

Antisecretory activity was performed in fasting Albino rats (non-pregnant female) of weighing between (150-200 g). The animals were divided into 11 groups. Each group contain 6 animals. Group I was served as normal control given with vehicle only. Group II with standard drug, and groups III- IX were treated with test groups (Comp. 54a-54i, dose: 200mg/kg). After 30 min acetylsalicylic acid was administered at a dose of 300 mg/kg, and after 6 hours rats were respectfully sacrificed by using anesthetic ether and their stomachs were dissected out for determination of gastric lesions, washed in warm water. the upper two fifths of the stomach form the rumen with squamous epithelium and possess little protective mechanisms against the corrosive action of gastric juice. Below a limiting ridge, in the glandular portion of the stomach, the protective mechanisms are better in the mucosa of the medium two fifths of the stomach than in the lowest part, forming the antrum. Therefore, lesions occur mainly in the rumen and in the antrum. The mucosal surface was then gently scraped with a blunt surface to collect the adherent mucus.

4.2.2 Collection of gastric juice

Gastric juice was collected into centrifuge tubes and centrifuged at 1000 rpm for 10min and volume was noted. The pH of the gastric juice was recorded by pH meter and the gastric content is subjected for analysis of total acidity.

4.2.3 Assays of H⁺/K⁺-ATPase activity

4.2.3.1 Determination of total acidity in gastric juice [24]

One milliliter of gastric juice was pipetted out into a 100 ml conical flask. 2-3 drops of Topfer’s reagent were added and the solution was titrated against 0.01 N NaOH. Then 2-3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge occurred.
Because of the presence of single hydrogen, the hydrogen couple with two more hydrogen.

5 ppm and 7.3 ppm of pyrimidine ring. The sharp multiplet peak integration at 2.33 indicating the presence of amid group.

Similarly, compound showed the presence of broad 1H multiplet at δ 7.76 -7.64, for the proton of NH present in the benzimidazole ring in the compound along with doublet at δ 7.25 indicate ArH aromatic pyrimidine moiety as expected to attached with substituted benzene ring. Two singlets were observed at δ 2.63(s,1H) and 2.50(s,1H) each integrating for two protons of methyl groups attached with pyrimidine rings. Compound shows double doublet at δ 7.25 indicate aromatic pyrimidine moiety as expected to attached with substituted benzene ring. Mass spectrum displayed a molecular ion peak at m/z 272 (M + 1)^+ displayed a molecular ion peak at m/z 272 (M + 1)^+ and molecular ion peak at m/z 301.1(M + 1)^+.

4.2.5 Statistical analysis

The results were expressed as % inhibition ± S.E.M. and analyzed by one-way ANOVA followed by Dunnett’s test. P < 0.05 was considered statistically significant.

5. RESULTS

<table>
<thead>
<tr>
<th>Code</th>
<th>Compound name</th>
<th>IR (KBr)cm⁻¹/1H NMR (CDCl₃, δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54a</td>
<td>2-[2-(2,6- dimethylpyrimidin-4-yl)sulfinyl]-1H-benzimidazole</td>
<td>IR (KBr, v, cm⁻¹): 3480 (N-H str.), 3060 (Ar-C-H str.), 1650 (C=O str.), 1590 (N-H bend), 1270,1305 (C-N Str.),1483(C=C stretch), 680,825(C-H bending, aromatic substitution); 1HNMR(500MHz,Choloroform-d) δ 7.76-7.64(m,1H),7.25(dd,J=5.6,3.4 Hz,1H) 2.63(s,1H),2.50(s,1H) ; Mass : m/z 272 (M+) Analytical data Calculated for C₁₇H₁₂N₂O₂S, C (57.34%) H (4.44%) N (20.57%) O (5.88%) S (11.77%)</td>
</tr>
<tr>
<td>54b</td>
<td>2-[6-ethyl-2- methylpyrimidin-4-yl)sulfinyl]-1H-benzimidazole</td>
<td>IR (KBr, v, cm⁻¹): 3485 (N-H str.), 3050 (Ar-C-H str.), 1597 (N-H bend), 1470, 1398 (C=C str. 830,690 (C-H bend), 1HNMR(500MHz,Choloroform-d) δ 7.72-7.60(m,1H),7.21(dd,J=5.6,3.4 Hz,1H) 2.67(s,1H)1.19(t, J=6.7Hz,1H) ;Mass : m/z 272 (M+) Analytical data Calculated for C₁₇H₁₂N₂O₂S, C (57.34%) H (4.44%) N (20.57%) O (5.88%) S (11.20%)</td>
</tr>
<tr>
<td>54c</td>
<td>2-[2-methyl-6- propylpyrimidin-4-yl)sulfinyl]-1H-benzimidazole</td>
<td>IR (KBr, v, cm⁻¹): 3254 (N-H str.), 3048 (C-H str.), 3275 (N-H str.), 1640 (C-O str.), 1584 (N-H bend), 810,870 (C-H bend), 1HNMR(500MHz,Choloroform-d) δ 7.74-7.66(m,2H),7.63(dd,J=5.5,3.4Hz,1H)7.21(dd,J=5.6,3.4Hz,2H)2.88(t, J=7.9Hz,2H),2.50(s,3H)1.82(dd,J=7.9,6.5,1.3Hz,2H)0.98(t, J=6.6Hz,3H) ; Mass : m/z 301 (M+) Analytical data Calculated for C₁₇H₁₄N₂O₂S, C (59.98%) H (5.37%) N (18.65%) O (5.33%) S (10.67%)</td>
</tr>
<tr>
<td>54d</td>
<td>2-[2,6- dimethylpyrimidin</td>
<td>IR (KBr, v, cm⁻¹): 3480 (N-H str.), 3052 (C-H str.), 1650 (C=C str.), 1228,1295 (C-N str.), 1390 (C-H bend)</td>
</tr>
</tbody>
</table>
54a 2-[6-ethyl-2-methylpyrimidin-4-y]sulfanyl-5-methyl-1H-benzimidazole

54b 2-[6-propylpyrimidin-2-y]sulfanyl-5-methyl-1H-benzimidazole

54c 2-[6-dimethylpyrimidin-4-y]sulfanyl-5-nitro-1H-benzimidazole

54d 2-[6-ethyl-2-Methylpyrimidin-4-y]sulfanyl-5-nitro-1H-benzimidazole

54e 2-[6-ethyl-2-methylpyrimidin-4-y]sulfanyl-5-methyl-1H-benzimidazole

54f 2-[2,6-dimethylpyrimidin-4-y]sulfanyl-5-nitro-1H-benzimidazole

54g 2-[6-ethyl-2-Methylpyrimidin-4-y]sulfanyl-5-nitro-1H-benzimidazole

54h 2-[6-ethyl-2-Methylpyrimidin-4-y]sulfanyl-5-nitro-1H-benzimidazole

54i 2-[2-methyl-6-propylpyrimidin-4-y]sulfanyl-5-nitro-1H-benzimidazole

6. DISCUSSION

6.1 Anti-ulcer Activity

All the newly synthesized benzimidazole containing substitution as pyrimidinyl sulfanyl (54a-f) and substitution as pyrimidinyl-sulfanyl-nitro-benzimidazole (54g-h) were tested for anti-ulcer activity at a dose of 200 mg/kg. All the test compounds were showed 30 to 70% inhibition of Ulcer. It was found that the compound 54a (45.20%), 54d (41.20%) and 54h (37.10%) shows lowest antiulcer activity. While the compound 54b (15.99 %) and 54e (52.68%) shows slightly significant antiulcer activity compared to standard and 54c (74.03%), 54f (72.87%) and 54i (75.15%) shows highly significant antiulcer activity compared to standard drug.

Results revealed that compound 54c, 54f and 54i as indicated by their low ulcer score 0.58±0.08, 0.75±0.11 and 0.58±0.33 respectively, while total ulcer index found 4.50, 4.70 and 4.40 respectively for these compounds.
It was interesting to note that the compound 54c (2-[(2-methyl-6-propylpyrimidin-4-yl)sulfinyl]-1Hbenzimidazole) with propyl substitution on pyrimidinyl-sulfinyl-nitro-benzimidazole ring showed highly significant activity (74.03 %), similar response was studied by synthesizing compounds 54f (5-methyl-2-[(2-methyl-6-propylpyrimidin-4-yl)sulfinyl]-1H-benzimidazole) (72.87%) and 54i (2-[(2,6-dimethylpyrimidin-4-yl)sulfinyl]-5-nitro-1Hbenzimidazole) (75.15%) having propyl substitution on pyrimidinyl ring attach to benzimidazole. The results are shown in Table 3 and Fig. 4, 5 & 6.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Group specification</th>
<th>Number of ulcer</th>
<th>Ulcer score</th>
<th>Total ulcer index</th>
<th>% inhibition of ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Ecospirin)</td>
<td>4.83 ± 0.54</td>
<td>2.5 ± 0.22</td>
<td>17.33</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Standard (Pantoprazole)</td>
<td>0.33 ± 0.21</td>
<td>0.5 ± 0.12</td>
<td>4.13</td>
<td>76.16</td>
</tr>
<tr>
<td>3</td>
<td>54a</td>
<td>3.33 ± 0.35</td>
<td>2.16 ± 0.15</td>
<td>9.50</td>
<td>45.20</td>
</tr>
<tr>
<td>4</td>
<td>54b</td>
<td>2.16 ± 0.52</td>
<td>1.83 ± 0.10</td>
<td>8.32</td>
<td>51.99</td>
</tr>
<tr>
<td>5</td>
<td>54c</td>
<td>0.66 ± 0.20</td>
<td>0.58 ± 0.08</td>
<td>4.50</td>
<td>74.03</td>
</tr>
<tr>
<td>6</td>
<td>54d</td>
<td>3.01 ± 0.77</td>
<td>2.02 ± 0.23</td>
<td>10.08</td>
<td>41.83</td>
</tr>
<tr>
<td>7</td>
<td>54e</td>
<td>1.80 ± 0.24</td>
<td>1.75 ± 0.21</td>
<td>8.20</td>
<td>52.68</td>
</tr>
<tr>
<td>8</td>
<td>54f</td>
<td>0.55 ± 0.22</td>
<td>0.75 ± 0.11</td>
<td>4.70</td>
<td>72.87</td>
</tr>
<tr>
<td>9</td>
<td>54g</td>
<td>0.62 ± 0.21</td>
<td>0.84 ± 0.04</td>
<td>5.10</td>
<td>70.57</td>
</tr>
<tr>
<td>10</td>
<td>54h</td>
<td>1.16 ± 0.40</td>
<td>1.41 ± 0.51</td>
<td>11.02</td>
<td>37.10</td>
</tr>
<tr>
<td>11</td>
<td>54i</td>
<td>0.55 ± 0.35</td>
<td>0.58 ± 0.33</td>
<td>4.40</td>
<td>75.15</td>
</tr>
</tbody>
</table>

Results are means ± SE of the numbers of animals in parenthesis; different from control group (ANOVA, and dunnnet’s test p < 0.05; p < 0.01); dose of STD and test group 200mg/kg body weight; No. of animal in group (n) = 6; values are the mean ± SEM, table follows one way ANOVA.

Fig. 4. Excised Stomach View; A & B - Induced ulceration after dosing Acetylsalicylic acid (ASA); C - Ulceration after dosing Pantoprazole; D - Ulceration after dosing Benzimidazole derivative.
Fig. 5. Anti-ulcer activity of synthesized compounds (54a-54i) observed in stomach’s opened greater curvature view.

Table 4. Anti-secretory activity of synthesized compounds (54a-54i)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Total acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CTL Drug (Ecospirin)</td>
<td>4.76±0.12</td>
<td>2.23±0.14</td>
<td>93.23±1.89</td>
</tr>
<tr>
<td>2</td>
<td>STD Drug (Pantoprazole)</td>
<td>3.19±0.07</td>
<td>5.12±0.03</td>
<td>32.73±1.07</td>
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<tr>
<td>3</td>
<td>54a</td>
<td>2.89±0.05</td>
<td>3.63±0.15</td>
<td>47.70±1.12</td>
</tr>
<tr>
<td>4</td>
<td>54b</td>
<td>3.12±0.05</td>
<td>3.98±0.09</td>
<td>54.13±1.23</td>
</tr>
<tr>
<td>5</td>
<td>54c</td>
<td>4.02±0.20</td>
<td>4.13±0.03</td>
<td>36.25±1.10</td>
</tr>
<tr>
<td>6</td>
<td>54d</td>
<td>4.82±0.04</td>
<td>4.25±0.17</td>
<td>35.21±1.21</td>
</tr>
<tr>
<td>7</td>
<td>54e</td>
<td>5.02±0.06</td>
<td>3.01±0.05</td>
<td>68.15±1.45</td>
</tr>
<tr>
<td>8</td>
<td>54f</td>
<td>4.08±0.10</td>
<td>4.45±0.03</td>
<td>37.35±1.10</td>
</tr>
<tr>
<td>9</td>
<td>54g</td>
<td>3.37±0.11</td>
<td>4.65±0.08</td>
<td>38.20±1.07</td>
</tr>
<tr>
<td>10</td>
<td>54h</td>
<td>5.17±0.07</td>
<td>3.42±0.03</td>
<td>82.21±1.68</td>
</tr>
<tr>
<td>11</td>
<td>54i</td>
<td>5.32±0.12</td>
<td>3.21±0.02</td>
<td>75.15±0.15</td>
</tr>
</tbody>
</table>

Results are means ± SE of the numbers of animals in parenthesis; different from control group (ANOVA, and Dunnett’s test p < 0.05; p < 0.01).

Fig. 6. Antiulcer activity of synthesized compounds (54a-54i)
- Synthesized compound
- Pantoprazole (Standard)
- Ecospirin (Control)
6.2 Antisecretory Activity

All the test compounds (54a-i) were showed 39 to 88 % total acidity. It was found that the compound 54e (47.05 %), 54h (38.92%) and 54i (42.66%) shows lowest antisecretory activity. While the compound 54a (68.08 %) and 54b (59.25%) shows slightly significant secretory activity and 54c (88.88 %), 54d (91.03 %), 54f (86.48%) and 54g (84.21%) shows highly significant antisecretory activity when compare to standard drug.

Results revealed that compound 54c, 54d, 54f and 54g as indicated by their pH 4.13± 0.03, 4.25 ± 0.17, 4.45± 0.03 and 4.65± 0.08 respectively when compare to standard drug pH 5.12± 0.03. It was interesting to note that the compound 54c (88.88 %) (2-[(2-methyl-6-propylpyrimidin-4-yl)sulfinyl]-1Hbenzimidazole), 54d (91.03%) (2-[(2,6-dimethylpyrimidin-4-yl)sulfinyl]-5-methyl-1Hbenzimidazole) with methyl propyl and dimethyl propyl substitution on pyrimidinyl-sulfinyl-benzimidazole ring showed highly significant activity (74.03 %), similar response was studied by synthesizing compounds 54f ( 5-methyl-2-[(2- methyl-6-propylpyrimidin-4-yl)sulfinyl]-1H-benzimidazole) (86.48%) and 54g ( 5-methyl-2-[(2- methyl-6-propylpyrimidin-4-yl)sulfinyl]-1H-benzimidazole) (84.21%) having dimethyl and propyl substitution on pyrimidinyl ring attach to benzimidazole. The results are shown in Table 4 and Fig. 7.

7. CONCLUSION

The literature surveys are done earlier concluded that among all benzimidazole derivatives 2-substituted benzimidazole shows more potent and efficient pharmacological activities, hence their synthesis and design became the potential area of research. Similarly, it was observed that substitution in benzimidazole moiety can cause an extensive enhancement in biological activities, and these modifications can be used as potential therapeutic agents in the future. It encouraged the researchers the designing more efficient, potent, and novel benzimidazole derivatives. Our research work provokes to work for the development of different derivatives of 2-(pyrimidinyl-sulfinyl) benzimidazole as the antulcer and antisecretory agent which have lesser side effect and better action than few of marketed drugs. The encouraging results showed may lead to the development of novel drugs if explored further.

CONSENT
It’s not applicable.

ETHICAL APPROVAL

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of College, constituted by the guidelines of the Committee for Control and Supervision of Experiment on Animals (CPCSEA), Government of India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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