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Amino acid derivatives of 2-Mercaptobenzimidazoles suppress cytokines at the site of inflammation and block gastric H^+/K^+ ATPase

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Abstract: Routinely used anti-inflammatory drugs are associated with off-target effects such as cyclooxygenase (COX)-1 inhibition and gastric ulcers. The aim of this study is to examine the anti-inflammatory potential and gastroprotective effects of synthetic amino acid derivatives of 2-mercaptobenzimidazole (MBAA1, MBAA2, MBAA3, MBAA4 and MBAA5). The results showed that compound MBAA5 possess a potential anti-inflammatory action by inhibition of 15-LOX and COX-2. MBAA5 also attenuated the pro-inflammatory cytokines and mediators (TNF-α, IL-1β and COX-2) in rat hind paw in carrageenan-induced inflammatory model of rat. 2-mercaptobenzimidazole derivative, MBAA5 also inhibited gastric H^+/K^+ ATPase and demonstrated a better selectivity index for COX-2 (SI 27.17) in comparison to celecoxib (SI 41.43). Molecular docking studies predicted the binding interactions of the synthesized compounds with retrieved target proteins of H^+/K^+ ATPase, COX-1, COX-2, and 15-LOX. The results of *in silico* and molecular docking analysis of amino acid derivatives of 2-mercaptobenzimidazoles further explained their pharmacological activities. Moreover, these compounds presented better antimicrobial activity against three clinical isolates of *Helicobacter pylori*. Together, our findings suggested that these synthetic 2-mercaptobenzimidazole derivatives are safer therapeutic candidates for inflammation.

Keywords: Benzimidazoles, Inflammation, H^+/K^+ATPase, COX-1/COX-2, *Helicobacter pylori*

INTRODUCTION

The process of inflammation is physiologically beneficial as well as pathologically stressful at the same time, leading to undesired and unnecessary effects (Saied *et al*., 2021). As inflammation is a multifaceted response and with the involvement of different molecular mediators and cells, it helps the tissue to regain its homeostasis (Rathinam and Chan, 2018). NSAIDs are mostly prescribed for the symptomatic management of fever, pain/inflammation (Kelleni, 2021). Although these drugs have found their way as a conventional therapeutic approach against tissue injuries and infections associated with inflammation, however, they are non-selective for both isoforms of cyclooxygenases (COXs) enzymes (a.COX-1 and b.COX-2) (Woo and Hyun, 2017). Frequent use of NSAIDs in chronic pain has been associated with gastrointestinal (GI) irritation leading to various GI complications such as bleeding and ulcers (Rayado *et al*., 2018). The COX-2 selective NSAIDs have been associated with cardiac effects such as congestive heart failure (CHF) and myocardial infarction (MI) (Tariq *et al*., 2018). The Clinical importance of benzimidazole's drugs is evident against pain/inflammation and gastrointestinal diseases (Kaur and Silakari, 2018). For instance, omeprazole is the first therapeutically used benzimidazole, has anti-inflammatory and anti-oxidant potential as well as its gastroprotective action (Noor *et al*., 2017). Previously reported that substituted mercaptobenzimidazoles are robust inhibitors of H^+/K^+ ATPase (Ali *et al*., 2018). Taking into consideration this multidimensional pharmacological potential of benzimidazole (Choudhary *et al*., 2021), we have designed this study to investigate the amino acid conjugated derivatives of 2-mercaptobenzimidazole. Novel 2-mercaptobezimidazole derivatives were chosen based on its previously reported anti-inflammatory potential (Khan *et al*., 2020) (fig. 1). The present study will demonstrate whether these novel 2-mercaptobenzimidazole derivatives exerts their anti-Inflammatory role with a better gastric safety profile. The outcome of this study will also provide an insight about underlying mechanism for gastroprotective effects of these derivatives.

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MATERIALS AND METHODS

Materials

Carrageenan was purchased from Sigma Aldrich and diluted in normal saline (0.9% NaCl) before use. Three clinical isolates of *Helicobacter pylori* were obtained from Care Endoscopy Clinics and Labs (Rawalpindi, Pakistan). COX-1/COX-2 inhibition assay Kit (Cat. #. 560131, Cayman Chemical, USA), H^+/K^+ ATPase enzyme inhibition assay kit (Cat. #. E-BC-K122-S; Elabscience USA) were purchased from Cayman. Amino acid derivatives of 2-mercaptobenzimidazoles (MBAA1, MBAA2, MBAA3, MBAA4, MBAA5) were obtained at the Department of Pharmaceutical Chemistry, Riphah International University, Islamabad. Adult Sprague-Dawley rats of either sex, having 220-350 g in weight, were taken from the animal house of RIPS, Riphah International University, Islamabad, Pakistan. Animals were housed 4-5/cage in a temperature and humidity-controlled environment (Temp: 22 ± 1ºC; Relative humidity (RH): 50-60%) with 12 hours light/dark cycle. There was free access to food and water for animals. Experiments were approved by the RIPS ethical Committee, Islamabad, Pakistan (Ref No. REC/RIPS/2020/010).

Anti-Helicobacter pylori activity

2-mercaptobenzimidazole derivatives were assessed for antibacterial activity against *H. Pylori* by disc diffusion method (Okinczyc *et al*., 2020). Three strains of *H. Pylori* were received from the patients with gastric ulcer, under consent, at the Care Endoscopy Clinics and Labs (Rawalpindi, Pakistan). Biopsies were placed in modified Campy Thio-medium (Adinortey *et al*., 2018). Isolates were identified by morphology and urease test kit. The 2-mercaptobenzimdazole derivatives with different concentrations (0.5, 1, 2, 4, 8, 16, 32 µg/disk) were loaded on disks and placed on an agar plate (Mueller Hinton). Following an incubation period of three to five days at 37ºC, the inhibition zones for every disk were measured.

Determination of cytokines levels in rat hind paw

Inflammatory cytokines, COX-2, IL-1β and TNF-α in paw tissues of rat were measured as per previously reported method (Karim *et al*., 2019). Briefly, 1% (w / v) carrageenan (100µL) was administered on the subplantar surface of the hind paw. Amino acid derivatives of 2-mercaptobenizmidazoles (MBAA1, MBAA2, MBAA3, MBAA4, MBAA5) were dissolved (3% dimethyl sulfoxide (DMSO); 1.5% Tween-80) in normal saline. The rats received 2-mercaptobenizmidazoles at the dose of 10mg/kg by Intraperitoneal injection (5ml/Kg), 30 min before carrageenan injection. Rats in the negative control group, received the same volume of vehicle only. Animals in positive control group received celecoxib (10 mg / kg) 30 min before carrageenan administration. Rats were then sacrificed 5 h later and tissues of the rat's hind paw were collected by cutting the paw at the level of the calcaneus bone. These rat paw tissues were preserved by dipping in liquefied nitrogen gas (N_2) and finally stored at -80ºC. At the time of experiments, tissues were homogenized in 200µL of phosphate buffer (10% w/v, 0.1M PBS, pH 7.4) and were centrifuged at 13500g for 25-35 min at 4-6ºC. Finally, supernatants were collected, and the levels of cytokines were measured by standard curves (0-1000 ng/mL) as per commercial guidelines of Enzyme linked immunosorbent assay (ELISA) kits.

Inhibition of Cyclooxygenase (COX-1 and COX-2) enzymes

Cyclooxygenase inhibition was analysed for amino acid derivatives of 2-mercaptobenzimdazoles against COX-1 and COX-2, using COX inhibition kits (Abdelgawad *et al*., 2017). 10µL of Cyclooxygenase enzyme (COX-1/COX-2), 150µL of the buffer assay, 10µL of heme solution, and different concentrations (30µL of 0.01-300µg/mL in 3% v/v DMSO) of the test compounds were added into ninety-six (96) well plate. Plate was vigorously stirred for 20s followed by incubation at 25ºC for 15-20 min. To start the reaction, 10µL (10µM) of substrate solution (arachidonic acid) was added. After stirring, the plate was incubated at 25ºC for 10min, and absorbance was calculated at a wavelength of 412nm by using a Multiskan™ microplate reader. Concentration of test compounds (MBAA1, MBAA2, MBAA3, MBAA4, MBAA5) that afford fifty percent inhibition (IC_{50}, µM) of COX-1/COX-2 was calculated by using GraphPad prism(6.0).

Inhibition of Lipoxyggenase (15-LOX) enzyme

15-LOX enzyme assay was determined by the following literature method (Amin *et al*., 2020). Briefly, an enzyme solution of 15-LOX (167 U/ml) was synthesized in borate buffer with 134 µM of linoleic acid (substrate). Test compounds were dissolved in DMSO (3%, v/v) at different concentrations (0.01-300µg/mL) and 30 µL of this solution was added to the enzyme solution (20µL). DMSO (3%, v/v) alone was used as negative control while quercetin (30µL, 0.01-300µg/mL) was a positive control for 15-LOX inhibition. The mixture was incubated for 90s and absorbance was recorded at 234nm. Concentration of test compounds that afford fifty percent inhibition of 15-LOX (IC_{50}, µM) was determined by using GraphPad prism(6.0).

In Vivo gastric H^+/K^+ ATPase inhibition assay

The inhibitory effect of 2-mercaptobenzimidazoles on rat gastric H^+/K^+ ATPase was determined by calorimetric analysis (Miyazaki *et al*., 2018). The commercially available H^+/K^+ ATPase assay kit was used for analysis. Briefly, the Hydrogen-Potassium (H^+/K^+) ATPase assay was evaluated by quantifying the inorganic phosphate (Pi) released by hydrolysis of adenosine triphosphate (ATP). Sprague Dawley rats were distributed in three groups

(n=3). 96% ethanol was administered (5mL/kg, per oral) for induction of gastric ulcers. Normal saline (5mL/kg) was administered in the saline group. 10 mg/kg (per oral) of test compounds (MBAA1, MBAA2, MBAA3, MBAA4, MBAA5) were given to the animals in treatment groups. For positive control, the standard drug omeprazole was used. 1-hour post-treatment, rats were sacrificed, stomach was excised, and homogenate of rat gastric mucosal tissue was prepared in normal saline and centrifugation was don at 13500g for 1 hr. Finally, supernatant was collected, and the release of Pi was quantified at 660 nm *via* a UV-spectrophotometer. Every 1 µM of Pi produced from the decomposition of 1 mg of ATP, per hr, is defined as 1 U of ATPase activity (1µM Pi/mg ATP/hr). Therefore, results are presented as units of ATPase activity against different test compounds and control groups.

Molecular docking analysis

Structures (Three-dimensional or 3D) of COX-1 from *Ovis aries,* COX-2 from *Homo sapiens*, 15-LOX from *Homo sapiens*, and H^+/K^+ ATPase from *Sus scrofa* were taken from (www.rcsb.org) Protein-Databank (PDB) freeware with PDB-IDs of 5U6X, 5KIR, 4NRE and 5YLU, respectively. These retrieved structures of proteins were further prepared for docking utilizing Autodock-Tools software. After energy minimization, these structures were saved in the required format (pdbqt). The Ramachandran and hydrophobicity graphs of these structures were also retrieved from PDB. The structural information of target proteins was retrieved by VADAR 1.8 (Shah and Kim, 2021). Amino acid derivatives of 2-mercaptobenzimidazoles were drawn by ACD/Chem Sketch freeware saved in mol files, that were furthered modified by discovery studio for energy minimization and saved in pdb format. Finally, virtual screening tool (PyRx) was used for molecular docking of all the synthesized ligands against different prepared proteins COX-1, COX-2, 15-LOX and H^+/K^+ ATPase (Imran *et al*., 2021).

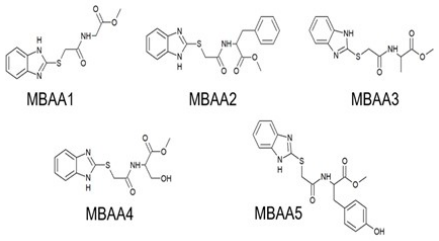
STATISTICAL ANALYSIS

Data was analyzed using Graph Pad Prism software (6.0) and was shown as the mean ± standard error mean (SEM). IC_{50} was calculated through linear regression between concentration and percent (%) inhibition by using Graph Pad Prism(6.0). The data was analyzed by one-way ANOVA followed by Tukey's post hoc test. A value of $p<0.05$ was considered as statistically significant.

RESULTS

Antibacterial assay against Helicobacter pylori

2-mercaptobenzimidazole "MBAA1, MBAA2, MBAA3, MBAA4, MBAA5" was assessed for anti-bacterial activity against three clinical isolates of *H. pylori* by disk diffusion method. Average diameters of inhibition zones for 2-mercaptobeznimdazoles at different doses (0.5-32 µg/ disk) are summarized in fig. 2. Metronidazole was used as a positive control. At all seven doses, the derivatives of 2-mercaptobenzimidazole have shown potential anti-*H. pylori* activity against three different clinical isolates (fig. 2). Compounds MBAA5 exhibited antibacterial activity insignificantly different from Metronidazole.



Methyl {2-[(1H-benzimidazol-2-yl)sulfanyl]acetamido}acetate **(MBAA1)**
Methyl 2-{2-[(1H-benzimidazol-2-yl)sulfanyl]acetamido}-3-phenylpropanoate **(MBAA2)**
Methyl 2-{2-[(1H-benzimidazol-2-yl)sulfanyl]acetamido}propanoate **(MBAA3)**
Methyl 2-{2-[(1H-benzimidazol-2-yl)sulfanyl]acetamido}-3-hydroxypropanoate **(MBAA4)**
Methyl 2-{2-[(1H-benzimidazol-2-yl)sulfanyl]acetamido}-3-(4-hydroxyphenyl)propanoate **(MBAA5)**

Fig. 1: Structures of amino acid derivatives of 2-mercaptobenzimidazoles.

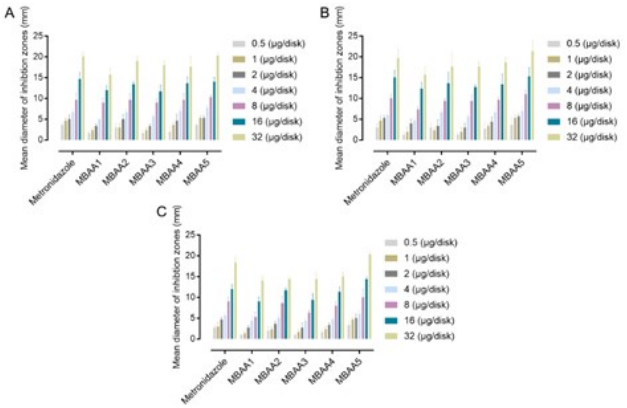


Fig. 2: Bar graphs showing the average diameter of the zone of inhibition (mm) (Mean ± SEM (n =3). produced by synthesized compounds (MBAA1, MBAA2, MBAA3, MBAA4, MBAA5) and metronidazole against three different clinical isolates (A= isolate no.1, B= isolate no. 2, C= isolate no. 3) of *H. Pylori.*

Biochemical analysis of cytokines and inflammatory mediators

The expression of inflammatory cytokines and mediators (COX-2, IL-1β and TNF-α) in hind paw tissues was measured in celecoxib and test compounds administered to rats. There was a significant increase in the IL-1β expression in the control group as compared to the celecoxib treated group after the injection of carrageenan (fig. 3). It was observed that compounds MBAA4 and MBAA5 substantially and significantly (***$p<0.001$) reduced the IL-1β expression in rat hind paw as compared to celecoxib treatment. The compounds MBAA1, MBAA2 and MBAA3 also decreased the expression of IL-1β in rat paw tissue, as compared to the celecoxib group, but not as strong as observed with MBAA4 and

MBAA5 (fig. 3). Carrageenan administration in the hind paw of rat also increased expression of TNF-α in the control group in comparison to the celecoxib group as presented in fig. 3. TNF-α expression was lowered in all treated groups, however, a significant reduction was observed with compounds MBAA4 and MBAA5 (fig. 4B). Similarly, compounds MBAA4 and MBAA5 significantly (***p<0.001) suppressed the carrageenan-induced COX-2 expression in rat paw tissue. Slight suppression of COX-2 (*p<0.05) was observed with compounds MBAA1 and MBAA3 (fig. 3). These results explain the anti-inflammatory potential of 2-mercaptobenzimidazoles, based on their ability to inhibit the expression these cytokines.

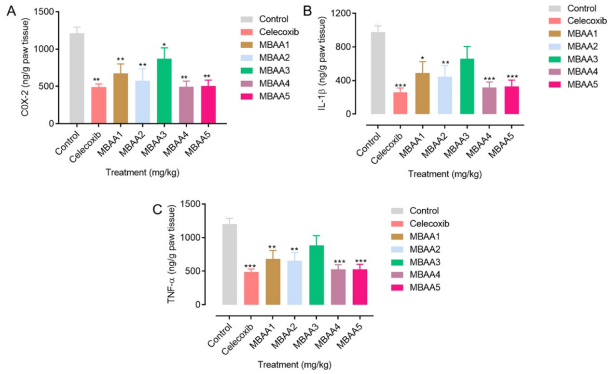


Fig. 3: Effect of amino acid derivatives of 2-mercaptobenzimidazoles on IL-1β (A), TNF-α (B) and COX-2 (C) expression (Mean ± SEM; n=6) in carrageenan induced inflammation in rat hind paw. **p< 0.01, ***p<0.001 vs control group.

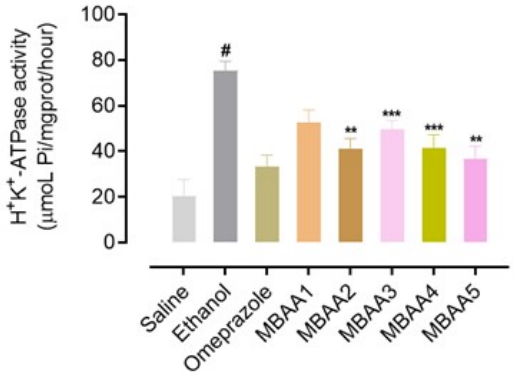


Fig. 4: Effect of 2-mercaptobenzimidazoles hybrids (MBAA1, MBAA2, MBAA3, MBAA4, MBAA5 (mean ± SEM) in rat gastric H+-K+ ATPase inhibitory assay (n =6). **p<0.01, ***p<0.001 vs ethanol group. while #p<0.01, relative to saline.

In Vivo H^+/K^+ ATPase activity

The H^+/K^+ ATPase assay for amino acid derivatives of 2-mercaptobenzimidazole "MBAA1, MBAA2, MBAA3, MBAA4 and MBAA5" was determined by commercially available colorimetric assay kits. The inhibition activity (units: µmol.Pi/mgprot/h) by the compounds is summarized in fig. 4. Omeprazole was used as positive control. The results revealed that compound MBAA5 showed potential H^+-K^+ ATPase activity among five compounds with a value of 36.82±3.11 units. Standard drug omeprazole has shown H^+-K^+ ATPase activity value of 33.22±2.89.

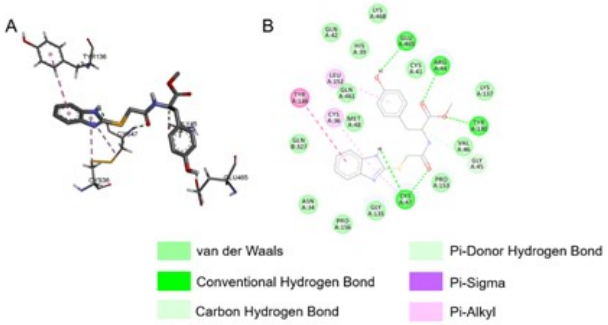


Fig. 5: Presentation MBAA5 with COX-2 (PDB-ID:5KIR), 3D(A) and 2D(B).

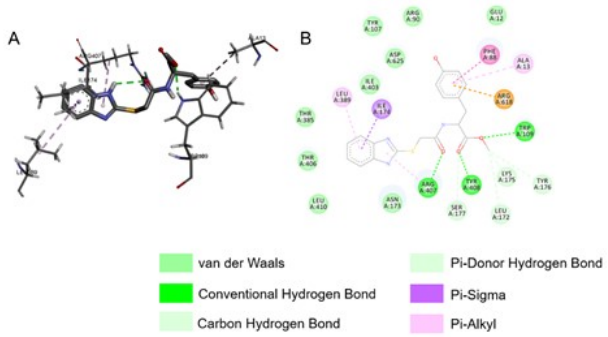


Fig. 6: Presentation of MBAA5 with 15-LOX (PDB-ID: 4NRE), 3D(A) and 2D(B)

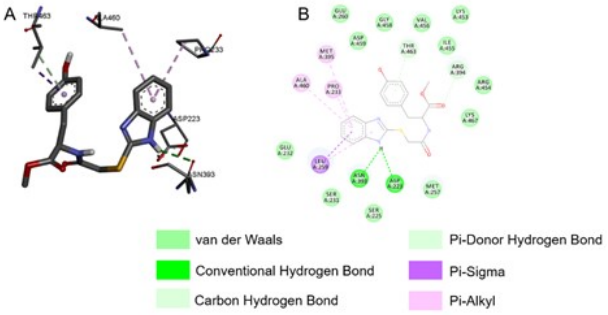


Fig. 7: Presentation of MBAA5 with H^+/K^+ ATPase (PDB-ID: 5YLU), 3D(A) and 2D(B)

In vitro inhibition assays of 15-LOX. COX-1 and COX-2

2-mercaptobenzimidazole derivatives presented remarkable enzyme inhibition of 15- LOX, COX-1, and COX-2 as presented in table 1. 0.01 to 300µM concentration was utilized for the computation of IC_{50} values of test compounds. The Selectivity index (SI) of COX-2 for each compound was calculated and presented in table 1. Among these, MBAA5 has shown better COX-2 SI = 27.17 as compared to celecoxib COX-2 SI = 41.37. Furthermore, 2-mercaptobenzimidazole derivatives were

Table 1: IC_{50} (μM) values of 15-LOX, COX-1 and COX-2.

Compound	15-LOX IC_{50} (μM)	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	Selectivity index (COX-2 SI)
MBAA1	96.56	21.72	1.852	11.73
MBAA2	64.39	23.68	1.425	16.62
MBAA3	91.13	20.42	2.222	9.19
MBAA4	77.5	22.19	1.496	14.83
MBAA5	57.41	25.46	0.9372	27.17
Celecoxib	-	27.79	0.6718	41.37
Quercetin	45.32	-	-	-

Table 2: Binding affinities of ligands for COX-1, COX-2, 15-LOX and H^+/K^+ ATPase

Compound	Highest binding affinity (kcal/mol) (ΔG)			
	COX-1	COX-2	15-LOX	H^+/K^+ ATPase
MBAA1	-7.3	-7.3	-7.1	-6.1
MBAA2	-8.6	-8.3	-8.5	-7.0
MBAA3	-7.1	-7.7	-7.4	-5.9
MBAA4	-7.5	-7.2	-7.3	-6.2
MBAA5	-8.5	-9.1	-9.3	-7.4
Mofezolac	-9.4	-	-	-
Celecoxib	-	-10.1	-9.9	-
Omperazol	-	-	-	-6.9

also tested for the 15-LOX enzyme of orange peel. Among the synthesized 2-mercaptobenzimidazole derivatives, MBAA5 has shown results comparable to quercetin, as standard reference inhibitor (IC_{50}=7.41 μM).

Docking studies

COX-1(PDB-ID:5U6X) consisted of 107 residues (9%) β-sheets, 447 residues (40%) helices, 156 residues (14%) turns and 552 residues (49%) coils, from a total of 1106 residues. The R-value of COX-1 (PDB-ID: 5U6X) appeared to be 0.234 and resolution existed as 2.93Å. Dimensions of unit cell were seen to be; a= 181.463, b= 181.463and c= 103.5. Further, the amino acid residues (99.15%) were in the allowed regions in the Ramachandran (R) plot. COX-2(PDB-ID:5KIR) have 102 residues (9%) of β-sheets, 539 residues (49%) of coils, 459 residues (41%) helices and144 residues (13%) turns from total of 1102 residues. The R-value of COX-2(PDB-ID: 5KIR) shown to be 0.220 and the resolution was obsereved as 2.70 Å. The dimensions of unit cell were detected to be; a = 126.989, b =149.422 and c=185.055. Amino acid residues (98.0%) were in the permissible regions of R-plot. 15-LOX(PDB-ID:4NRE) contained 138 residues (20%) β sheets, 259 residues (38%) coils, 278 residues (41%) helices, and 168 residues (24%) turns from a total of 675 residues. The R-value of 15-LOX(PDB-ID:4NRE) was 0.203 and the resolution was 2.63 Å. The dimension of cell were; a =155.794, b= 155.794 and c = 263.219. 97.62% of amino acids were in permisible region of R- plot. H^+/K^+ ATPase (PDB-ID:5YLU) was composed of 516 residues (41%) helices, 444 residues (36%) coils, , 184 residues (14%) turns and 273 residues (22%) β-sheets from total of 1234 residues. The R-value of H^+/K^+ ATPase (PDB-ID: 5YLU) was seen to be 0.288 and the resolution was observed as 2.80 Å. The dimension of unit cell was noted to be: a = 104.82, b = 104.82 and c = 367.08. 98.70% amino acid residues were in the allowed regions of R-plot. To evaluate the affinity of the target protein and the best-docked pose complex of docked ligand, E-value (kcal/mol) was used. Binding affinities of ligands with target protein are presented in table 2).

In COX-2 (PDB-ID: 5KIR), the MBAA5 has made best stable complex (E-value = -9.1 kcal/mol) as depicted in fig. 5. MBAA4 also displayed a promising binding affinity (E-value = -8.4 kcal/mol).

MBAA5 also presented the highest E-value (-9.3 kcal/mol) with 15-LOX target protein (PDB-ID: 4NRE), MBAA2 showed good binding attraction with15-LOX target protein (PDB-ID:4NRE) (E-value = -8.5 kcal/mol). The binding interaction of MBAA5 with 15-LOX is presented in fig. 6.

In, MBAA5 exhibited stable complex and displayed good binding affinity value (-7.4 kcal/mol). Binding of H^+/K^+ ATPase (PDB-ID: 5YLU) with MBAA5 are presented in fig. 7. MBAA2 has also shown a good binding attraction with E-value of -7.0 kcal/mol.

DISCUSSION

Cytokines are important inflammatory mediators produced against the inflammatory stimulus. COX-2 is induced in response to these cytokines and participates in

the synthesis of various mediators that mediate pain (Matsuka *et al*., 2020). Benzimidazole derivatives have been reported to have a strong effect in the decrease of expression of pro-inflammatory cytokines in the previously reported studies (Imran *et al*., 2021). The present study supported this argument, and synthesized benzimidazole amino acid conjugates reduced the expression of cytokines through their anti-inflammatory action. Compound MBAA4 and MBAA5 significantly reduced the pro-inflammatory cytokine's expression in hind paw tissues of rats as compared to the control group of celecoxib. The significant reduction in the expression of these mediators further supported the potential of synthesized benzimidazole derivatives as strong anti-inflammatory (Nandha *et al*., 2018). Recently, COX-2 and 15-LOX dual inhibition have emerged as potential targets for treating inflammation with less GI side effects (Alaaeddine *et al*., 2020). *In vitro* anti-inflammatory studies of the 2-mercaptobenzimidazole derivatives revealed that synthesized benzimidazole derivatives have dual inhibition of 15-LOX and COX-2. Further, synthesized benzimidazoles showed better SI for COX-2. Among these, MBAA5 has shown higher COX-2 SI (27.17), as compared to celecoxib SI (41.37). At para position of compound MBAA5, presence of hydroxyl group in the aromatic ring might enhance potency and selectivity of this molecule. So, the initial structure activity relationship could be assumed that phenyl moiety is required for high potency and selectivity. The gastric H^+-K^+ ATPase that exports hydrogen ion and generate a highly acidic environment (Clarke, 2018), hence an important target for acid suppression and gastroprotection (Sun *et al*., 2017). Results of in *vivo* gastric H^+-K^+ ATPase inhibitory assay has demonstrated that compound MBAA5 exhibited strong inhibition of H^+K^+ ATPase comparable to the control drug omeprazole. It was suggested that benzimidazole derivatives, are converted into reactive sulfhydryl (SH) reagents with gastric acid, and they may attach to the H^+/K^+ ATPase through cysteine residue (CYS813 or CYS822) (Jana *et al*., 2019). This leads to the decreased production of hydrochloric acid in the gastric mucosa and demonstrates favorable anti-ulcer effects (Strand *et al*., 2017). Results of above-mentioned pharmacological activities were further validated by *in silico* studies. MBAA5 exhibited antibacterial activity near to metronidazole against *H. pylori*. This indicates the importance of benzimidazole derivatives in the development of antibacterial agents against *H. pylori* for gastroprotective effects (Zaman *et al*., 2019).

CONCLUSION

In conclusion, our findings demonstrated that 2-mercaptobenzimidazole derivatives were found to attenuate inflammation and ameliorated gastrotoxicity *via* COX-2 selectivity, H^+/K^+ ATPase inhibition and anti-*H. pylori* activity. MBAA4 and MBAA5 also demonstrated an active reduction in the cytokine's levels in rat hind paw. Amino acid derivative of 2-mercaptobenzimidazoles were also able to bind with COX-2 with higher affinity than COX-1, indicating less potential for the off-target side effect of gastric ulcers. Our results suggest that these derivatives of 2-mercaptobenzimidazoles have strong anti-inflammatory effect by 15-LOX and COX-2 inhibition. However, extensive study is still requisite to explain the underlying gastroprotective mechanisms of MBAA1-MBAA5.

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