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Design, synthesis and pharmacological evaluation of omeprazole-like agents with anti-inflammatory activity

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ABSTRACT

A new series of novel benzimidazole derivatives containing substituted pyrid-2-yl moiety and polyhydroxy sugar conjugated to the *N*-benzimidazole moiety has been synthesized and evaluated as orally bioavailable anti-inflammatory agents with anti-ulcerogenic activity. The anti-inflammatory and anti-ulcerogenic activities of these compounds were compared to diclofenac and omeprazole, respectively. In carrageenan-induced paw oedema assay, 2-methyl-*N*-((3,4-dimethoxypyridin-2-yl)methyl)-1*H*-benzimidazol-5-amine (**12d**) and 1-(1,2,3,5-tetrahydroxy-α-D-mannofuranose)-5-(((3,4-dimethoxypyridin-2yl)methyl)amino)-2-methyl-1*H*-benzimidazole (**15d**) displayed dose-dependent anti-inflammatory activities by decreasing the inflammation by 62% and 72%, respectively which is comparable to that of diclofenac (73%). In contrast to diclofenac, the anti-inflammatory activity of these compounds was not only free from any side effects on the gastric mucosa but also showed significant anti-ulcerogenic activity in rat pyloric ligation and ethanol-induced gastric ulcer models similar to that of omeprazole. Together, these findings suggest that **12d** and **15d** are potent anti-inflammatory agents with concurrent anti-ulcerogenic activity and support its clinical promise as a component of therapeutic strategies for inflammation, for which the gastric side effects are always a major limitation.

1. Introduction

Gastrointestinal toxicity is the most common adverse effect of the currently available non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, and naproxen. Such adverse effects are manifested by dyspepsia, ulcers, or bleeding.[1] The gastrointestinal damage from NSAIDs is generally attributed to two factors; first, the local irritation by carboxylic acid group present in many NSAIDs (topical effect); second, the decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining gastrointestinal integrity and homeostasis.[2] The pharmacology of NSAIDs is linked to the inhibition of prostaglandin biosynthesis from arachidonic acid by inhibiting cyclooxygenases.[3] Therefore, patients treated with NSAIDs for long periods of time, may suffer from noticeable gastrointestinal toxicity. Consequently, synthetic approaches based on chemical mimicking NSAIDs have been taken with the aim of improving its safety profile. The concurrent use of NSAIDs with gastric proton pump enzyme (H^+/K^+-ATPase) inhibitors represents a major approach to minimize such adverse effects.[4,5] Many pharmaceutical companies have spent considerable efforts in the identification of irreversible and reversible inhibitors of the H^+/K^+-ATPase. Substances belonging to the class of irreversible inhibitors are called proton pump inhibitors (PPIs)[6–8] such as omeprazole, lansoprazole, pantoprazole, rabeprazole and esomeprazole. PPIs are widely used as acid inhibitory agents for the treatment of disorders related to gastric acid secretion.[9,10]

Many of PPIs are benzimidazole derivatives[11,12] which consist of two fragments of benzimidazole and pyridine. These PPIs act as prodrugs owing to protonation of the pyridine ring under the gastrointestinal acid environment, resulting in a chemical rearrangement which forms sulfenic acid then sulfonamide by dehydration. The active enzyme inhibitor is either the sulfenic acid or the sulfonamide which reacts with cysteine residues of the H^+/K^+-ATP enzyme (Fig. 1).[13–15] Omeprazole, a benzimidazole proton pump inhibitor, has anti-inflammatory and antioxidant properties besides its ability to stimulate gastric mucus secretion.[16] Benzimidazole and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities including anti-inflammatory and analgesic.[17] The unique

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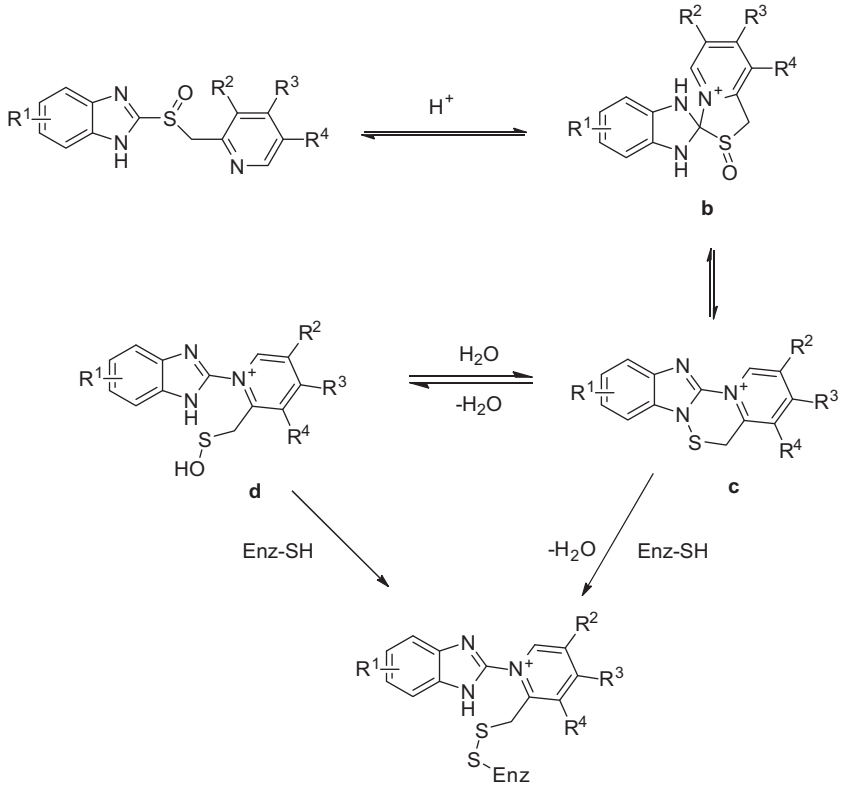


Figure 1. Mechanism of acid transformation of 2-[(2-pyridylmethyl)sulfinyl]benzimidazole derivatives. First, the gastric acidity causes protonation of the pyridine ring into intermediate, **b**. Then, a chemical rearrangement forms sulfenic acid **c**, which forms sulfonamide **d** by dehydration. The active enzyme inhibitor is either the sulfenic acid **c** or the sulfonamide **d** which reacts with cysteine of the H^+/K^+-ATP enzyme.[13–15]

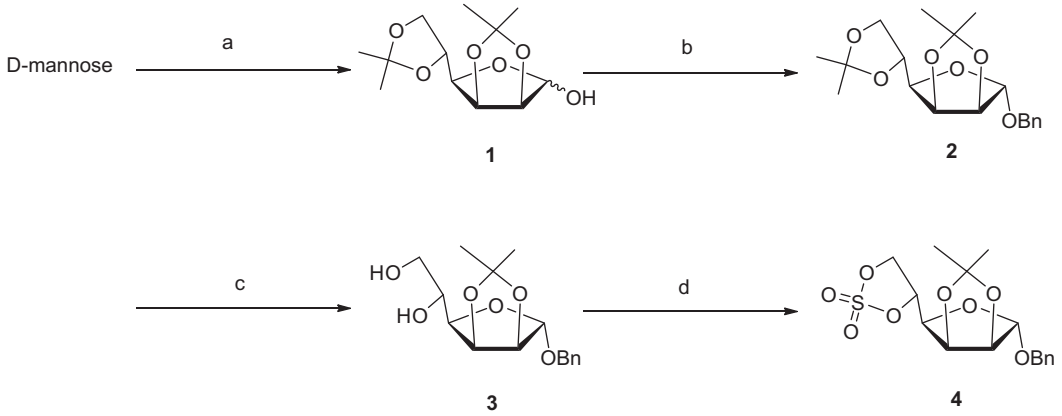
structural features and pharmaceutical activities of benzimidazoles have encouraged us to synthesize novel orally bioavailable 2-methyl-N-substituted benzimidazole sugar conjugates and study its anti-inflammatory and anti-ulcerogenic activities.

2. Results and discussion

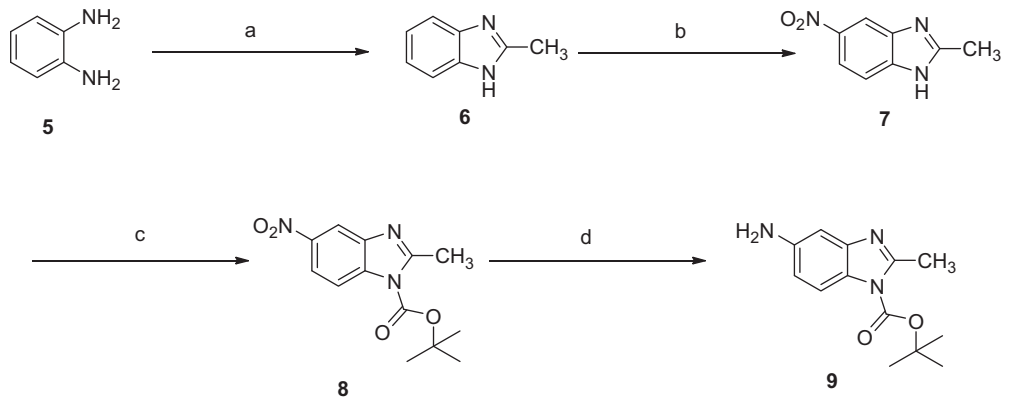
2.1. Chemistry

2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (**1**) was prepared by the reaction of D-mannose with acetone in the presence of catalytic amount of concentrated sulfuric acid.[18–20] Benzylation of 2,3:5,6-di-*O*-isopropylidene-D-mannose (**1**) using benzyl bromide and sodium hydride afforded a mixture of anomers **2α**:**2β** (6.8:1), which was separated by column chromatography. Only the α-isomer was isolated by crystallization using ethyl acetate/hexane, the β-anomer was not considered.[21,22]

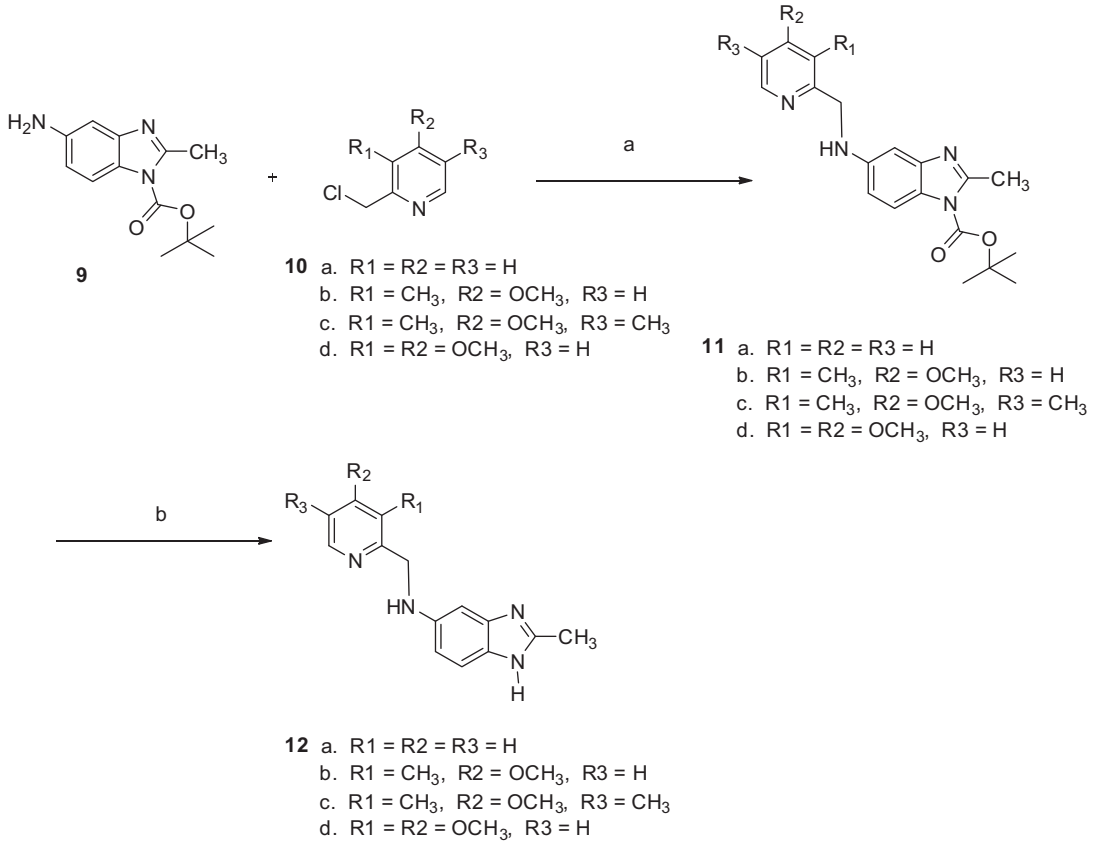
Selective acid catalyzed hydrolysis of **2** gave diol **3**.[21] Cyclic sulfate **4** was obtained by the treatment of compound **3** with thionyl chloride, $RuCl_3{\cdot}3H_2O$ and $NaIO_4$ in anhyd CCl_4. All products were purified by column chromatography and the structure of the synthesized compounds was determined by ^1H, ^{13}C NMR (Scheme 1). Cyclic sulfate **4** is at least as reactive as epoxides and its reactivity is the result of its ring strain which may be due to angle strain and the partial double bond character between the ring oxygen and sulfur atom. In addition, the use of cyclic sulfate in the synthesis



Scheme 1. Preparation of cyclic sulfate. Reagents and conditions: (a) acetone, H_2SO_4, Na_2CO_3, rt, 75%; (b) NaH, THF, benzyl bromide, rt, 86%; (c) 80% acetic acid, reflux, 89%; (d) thionyl chloride, CCl_4, $RuCl_3{\cdot}3H_2O$, $NaIO_4$, rt, 91%.



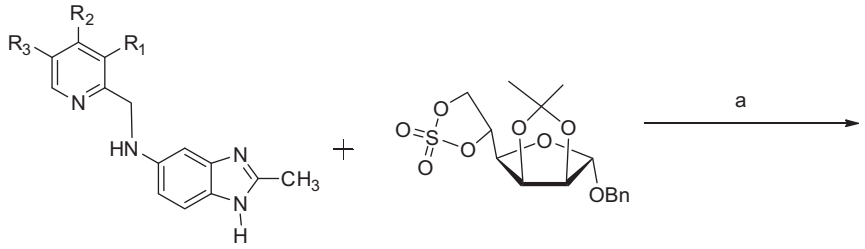
Scheme 2. Preparation of cyclic *N*-Boc benzimidazole derivative. Reagents and conditions: (a) 4 N HCl, CH_3COOH, reflux, 68%; (b) H_2SO_4/HNO_3, ammonia, rt, 58%; (c) Boc anhydride, $NaHCO_3$, methanol, rt, 78%; (d) Pd/C, acetic acid, methanol, H_2 gas, rt, 75%.



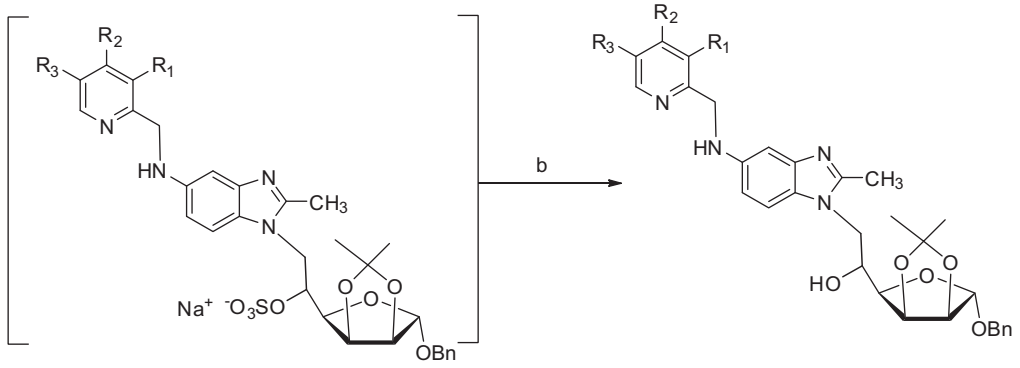
Scheme 3. Preparation of deprotected benzimidazole derivatives carrying substituted pyridine moiety. Reagents and conditions: (a) K_2CO_3, KI, acetone, ethanol, reflux, **11a**: 45%, **11b**: 52%, **11c**: 42%, **11d**: 59%; (b) trifluoroacetic acid, CH_2Cl_2, rt, **12a**: 98%, **12b**: 97%, **12c**: 98%, **12d**: 98%.

produced only a single substituted product. 2-Methyl-1*H*-benzimidazole (**6**) and 2-methyl-5-nitrobenzimidazole (**7**) were prepared as reported before.[23–25]

N-Boc-protected 2-methyl-5-nitro-1*H*-benzimidazole (**8**) was obtained by the reaction of compound **7** with Boc anhydride in the presence of sodium bicarbonate in methanol. The hydrogenation of the 5-nitro group in compound **8** using Pd on charcoal, acetic acid and methanol gave 1-(*tert*-butoxycarbonyl)-2-methyl-5-amino-1*H*-benzimidazole (**9**) in 75% yield (Scheme 2). The structure of **8** and **9** were confirmed by NMR and mass spectra. Condensation of 2-chloromethyl pyridine hydrochloride (**10a–d**) with 1-(*tert*-butoxycarbonyl)-2-methyl-5-amino-1*H*-benzimidazole (**9**) by stirring in acetone/ethanol containing K_2CO_3/KI at 60 °C for about 8 h yielded **11a–d** (Scheme 3). The structure of newly synthesized benzimidazole derivatives **11a–d** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and the elemental analysis. The desired deprotected benzimidazole derivatives (**12a–d**) were obtained by the treatment of *t*-Boc protected benzimidazole derivatives **11a–d** with trifluoroacetic acid in CH_2Cl_2 and the mixture was stirred at room temperature for 18 h (see spectral results in Section 4). The ring opening of cyclic sulfate **4** with deprotected benzimidazole derivatives **12a–d** was performed in tetrahydrofuran (THF) using NaH as a base. The obtained 2-sulfate ester derivatives **13a–d** (not isolated) were subsequently hydrolyzed to the hydroxy compounds **14a–d** by the treatment with concentrated sulfuric acid and water. Hydrogenolysis of **14a–d** with palladium on carbon

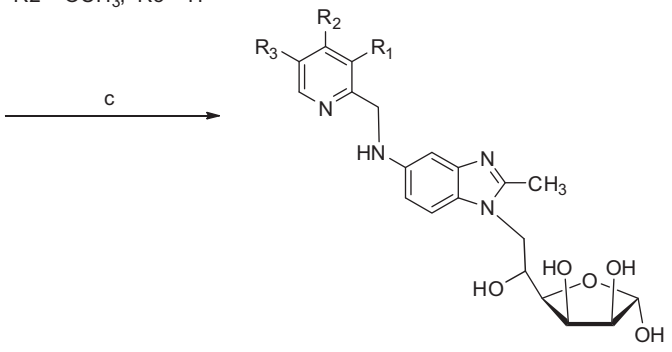


12 a. R1 = R2 = R3 = H
b. R1 = CH_3, R2 = OCH_3, R3 = H
c. R1 = CH_3, R2 = OCH_3, R3 = CH_3
d. R1 = R2 = OCH_3, R3 = H



13 a. R1 = R2 = R3 = H
b. R1 = CH_3, R2 = OCH_3, R3 = H
c. R1 = CH_3, R2 = OCH_3, R3 = CH_3
d. R1 = R2 = OCH_3, R3 = H

14 a. R1 = R2 = R3 = H
b. R1 = CH_3, R2 = OCH_3, R3 = H
c. R1 = CH_3, R2 = OCH_3, R3 = CH_3
d. R1 = R2 = OCH_3, R3 = H



15 a. R1 = R2 = R3 = H
b. R1 = CH_3, R2 = OCH_3, R3 = H
c. R1 = CH_3, R2 = OCH_3, R3 = CH_3
d. R1 = R2 = OCH_3, R3 = H

Scheme 4. Preparation of target compounds. Reagents and conditions: (a) NaH, THF, rt; (b) H_2SO_4, water, rt, $NaHCO_3$, **14a**: 58%, **14b**: 72%, **14c**: 60%, **14d**: 56%; (c) Pd/C, acetic acid, ethyl acetate/methanol, H_2, trifluoroacetic acid/water, rt, **15a**: 67%, **15b**: 60%, **15c**: 73%, **15d**: 76%.

in aqueous acetic acid followed by treatment with TFA/water mixture produced **15a–d** in a good yield (Scheme 4). The structures of **15a–d** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and the elemental analysis data which revealed the absence of both isopropylidene protons and the benzyl group (see spectral results in Section 4).

2.2. Biological evaluation

2.2.1. Anti-inflammatory activity

The anti-inflammatory activity of all the newly synthesized compounds were tested in vivo using carrageenan-induced paw oedema model in rats. Rats received test compounds in three different doses; 10, 20 and 30 mg/kg by oral gavage. Most of the test compounds reduced paw oedema in a dose- and time-dependent manners. Compounds **12a–d** exhibited moderate anti-inflammatory activity compared to diclofenac (Table 1).

Compound **12d**, at 20 and 30 mg/kg, resulted in a dose-dependent reduction of carrageenan-induced oedema by 57% and 61%, respectively after 3 h of inflammation induction. Compounds **12a**, **12b**, **12c** at the same doses showed a moderate anti-inflammatory activity. The relatively high anti-inflammatory activity of compound **12d** may be due to the presence of the electron-donating methoxy group at 3- and 4-positions in pyrid-2-yl compounds while bulky substituents such as methyl and methoxy groups at the 3-, 5- and 4-positions in pyridine reduce the activity. At the

Table 1
Effect of compounds **12a–d** on carrageenan-induced paw oedema in rats

Treatment	Dose (mg/kg)	Oedema (ml) (mean ± SEM)			% Inhibition after 3 h
		60 min	120 min	180 min	
Control	—	0.90 ± 0.03	1.13 ± 0.04	0.76 ± 0.03	—
12a	10	0.97 ± 0.01	1.08 ± 0.08	0.69 ± 0.01	10
	20	0.90 ± 0.01	1.08 ± 0.08	0.52 ± 0.08*	32
	30	0.87 ± 0.01	1.03 ± 0.05	0.42 ± 0.04**	45
12b	10	0.70 ± 0.01**	0.90 ± 0.02**	0.68 ± 0.01	11
	20	0.69 ± 0.01**	0.69 ± 0.03**	0.37 ± 0.03**	52
	30	0.64 ± 0.01**	0.65 ± 0.03**	0.34 ± 0.01**	55
12c	10	0.70 ± 0.02**	0.88 ± 0.02**	0.69 ± 0.01	10
	20	0.69 ± 0.01**	0.67 ± 0.03**	0.39 ± 0.04**	49
	30	0.64 ± 0.01**	0.62 ± 0.02**	0.36 ± 0.02**	53
12d	10	0.70 ± 0.02**	0.88 ± 0.02**	0.65 ± 0.01*	15
	20	0.70 ± 0.01**	0.69 ± 0.03**	0.33 ± 0.03**	57
	30	0.65 ± 0.01**	0.65 ± 0.03**	0.30 ± 0.01**	61
Diclofenac	20	0.38 ± 0.01**	0.35 ± 0.01**	0.20 ± 0.02**	74

Each value represents mean ± SEM ($n = 6$).
* $p < 0.05$ when compared to control.
** $p < 0.01$ when compared to control.

Table 2
Effect of compounds **15a–d** on carrageenan-induced paw oedema in rats

Treatment	Dose (mg/kg)	Oedema (ml) (mean ± SEM)			% Inhibition after 3 h
		60 min	120 min	180 min	
Control	—	0.90 ± 0.03	1.13 ± 0.04	0.76 ± 0.03	—
15a	10	0.69 ± 0.02*	0.89 ± 0.02*	0.70 ± 0.04	8
	20	0.68 ± 0.03*	0.69 ± 0.03*	0.40 ± 0.03*	47
	30	0.63 ± 0.02*	0.64 ± 0.03*	0.37 ± 0.01*	52
15b	10	0.68 ± 0.02*	0.88 ± 0.06*	0.60 ± 0.01*	22
	20	0.68 ± 0.03*	0.68 ± 0.01*	0.36 ± 0.03*	53
	30	0.63 ± 0.01*	0.64 ± 0.01*	0.32 ± 0.03*	58
15c	10	0.61 ± 0.01*	0.64 ± 0.01*	0.49 ± 0.01*	36
	20	0.50 ± 0.01*	0.48 ± 0.01*	0.38 ± 0.01*	50
	30	0.49 ± 0.01*	0.43 ± 0.01*	0.34 ± 0.01*	56
15d	10	0.53 ± 0.01*	0.52 ± 0.01*	0.45 ± 0.01*	41
	20	0.44 ± 0.01*	0.40 ± 0.01*	0.29 ± 0.01*	62
	30	0.41 ± 0.01*	0.38 ± 0.01*	0.21 ± 0.03*	72
Diclofenac	20	0.38 ± 0.01*	0.35 ± 0.01*	0.20 ± 0.02*	73

Each value represents mean ± SEM ($n = 6$).
* $p < 0.01$ when compared to control.

same time, methyl and methoxy groups at the 3- and 4-positions can improve the activity.

Compounds **15a–d**, at 10, 20 and 30 mg/kg doses, displayed more significant anti-inflammatory activity than compounds **15a–d** (Table 2). Among the **15a–d** series, compound **15d** exhibited the most potent anti-inflammatory activity with 62% and 72% inhibition of inflammation at 20 and 30 mg/kg doses, respectively which was comparable to that of diclofenac sodium (73%). On the other hand, compound **15a** showed relatively poor anti-inflammatory activity. The high potency of the compound **15d** could be attributed to the presence of electron-donating methoxy groups at 3- and 4-positions of pyrid-2-yl moiety and it could be also dependent on the polyhydroxy sugar conjugated to the *N*-benzimidazole moiety. To conclude, the present pharmacological investigation showed that compounds **12d** and **15d** possess a potential anti-inflammatory activity, which was evidenced by significant reduction of carrageenan-induced paw oedema in rats.

2.2.2. Anti-ulcerogenic activity

Gastrointestinal ulcer is a common disorder that affects millions of people worldwide. The imbalance between aggressive factors (gastric juice and pepsin) and protective factors (mucosal blood flow, bicarbonate secretion, mucosal integrality, cell regeneration, prostaglandins and other hormones) is considered the major mechanism for the induction of gastric ulcer. Most of the used drugs for the treatment of gastric ulcer inhibit the acid secretion, protect the mucosa, and inhibit the *Helicobacter pylori*.

Here we used two different experimental models to investigate the protective effect of test compounds on gastric ulcer namely, alcohol-induced gastric ulcer in mice and pylorus ligation-induced gastric ulcer in rats. Alcohol can cause the lesion of gastric mucosa by reinforcement of the aggressive factors while weaken the protective factors. Similarly, pylorus ligation leads to the accumulation of gastric juice and enzymes in the stomach causing mucosal injury. In both models of gastric ulcer, the newly synthesized compounds showed dose-dependent preventive and therapeutic actions on the gastric mucosa as indicated by decreased ulcer size (Table 3). The new compounds significantly decreased the gastric acid secretion and inhibited H^+/K^+-ATPase in a profile similar to that of omeprazole (Table 4).

In alcohol induced gastric ulcer, polyhydroxy sugar conjugated to the *N*-benzimidazole (**15a–d**) produced a significant reduction of the length of gastric lesions compared to control group while compound **12d** exhibited mild anti-ulcer effect. This weaker activity of **12d** may be due to the absence of sugar moiety attached to benzimidazole. Compounds **15b–d** were more effective than **15a** which could be due to the presence of electron donating group in the pyridine moiety (Table 3).

Table 3
Effect of test compounds on the ulcer index

Treatment	Dose (mg/kg)	Length of lesion (mm)	Inhibition ratio (%)
Control	—	126.0 ± 2.5	—
12d	3.6	88 ± 4.5*	30.0
	7.2	67 ± 4.5*	46.8
15a	3.6	68 ± 4.5*	46.0
	7.2	58 ± 2.9*	54.0
15b	3.6	53 ± 4.7*	57.9
	7.2	42 ± 4.5*	66.6
15c	3.6	47 ± 3.9*	62.6
	7.2	30 ± 2.5*	76.2
15d	3.6	49 ± 4.9*	61.0
	7.2	34 ± 4.6*	73.0
Omeprazole	3.6	40 ± 5.0*	68.3

Values are expressed as mean ± SD (n = 6).
* p <0.01 compared to control group.

In pylorus ligation-induced gastric ulcer model, compound **12d** exhibited a mild inhibition of the activity of both H^+/K^+-ATPase and pepsin and a mild reduction of the volume of gastric juice. This relatively weak anti-ulcer activity may be due to the absence of sugar moiety attached with benzimidazole. In the contrary, the compounds with polyhydroxy sugar conjugated to the *N*-benzimidazole (**15a–d**) displayed potent inhibition of the activity of H^+/K^+-ATPase and pepsin in addition to potent inhibition of the gastric juice production in a manner similar to that of omeprazole (Table 4).

3. Conclusions

In this work, we have prepared a series of benzimidazole sugar conjugates carrying substituted pyrid-2-yl moiety, **15a–d**, from 2-methyl-N-(substituted-pyridin-2-ylmethyl)-1*H*-benzimidazol-5-amine, **12a–d,** and cyclic sulphate benzyl 2,3-*O*-isopropylidene-5,6-*O*-sulfuryl-α-D-mannofuranoside **4**. The newly synthesized *N*-benzimidazole derivatives were found to possess potent anti-inflammatory and anti-ulcerogenic activities. The results showed that compounds **12d** and **15d** possess a potential anti-inflammatory effect, which was evidenced by the significant reduction of the inflammation in paw oedema model. The potency of the compound **15d** is attributed to the presence of the electron-donating methoxy groups at 3- and 4-positions in pyrid-2-yl moiety also the potent anti-inflammatory activity of compound **15d** is dependent on the linked-position of the polyhydroxy sugar conjugated to the *N*-benzimidazole moiety. In addition, the results showed that newly synthesized compound **12d** which lacks the sugar moiety attached to benzimidazole exhibited mild anti-ulcer effect on ulcer induced by alcohol. However, **15a–d** exhibited remarkable anti-ulcer effect on ulcer induced by alcohol which may be due to the sugar moiety attached with benzimidazole. Compounds **15b–d** were more effective than **15a** which may be due to the presence of electron donating group in the pyridine moiety and polyhydroxy sugar conjugated to the *N*-benzimidazole. In conclusion, our results suggest that **12d** and **15d** have potent anti-inflammatory activity comparable to that of diclofenac and this activity is not only free from gastrointestinal toxicity, but also exhibits a potent anti-ulcerogenic effects comparable to that of omeprazole. These findings support clinical promise of both **12d** and **15d** as components of therapeutic strategies for inflammation, for which the gastric side effects are always a major limitation.

4. Experimental

4.1. Chemistry

All chemicals were purchased from common commercial suppliers and used without further purification. Melting points (mp) were determined on a Gallenkamp melting point apparatus and were uncorrected; IR spectra (KBr disks) were recorded on Bruker Vector 22 instrument. ^1H and ^{13}C NMR spectra were recorded on a Jeol ECX-spectrometer at 300 MHz, respectively, in $CDCl_3$ as a solvent and TMS as an internal standard at the National Research Center. Mass spectra were recorded on Thermo Finnigan LCQ Advantage spectrometer in ESI mode, I Spray Voltage 4.8 kV. Microanalyses were performed at the Micro analytical Center of Cairo University. All reactions were performed under positive pressure of inert atmosphere. Thin layer chromatography (TLC) was performed on silica gel 60 F254 plastic plates (E. Merck, layer thickness 0.2 mm). Detection was achieved by treatment either with a solution of 20 g of ammonium molybdate and 0.4 g of cerium (IV) sulfate in 400 ml of 10% H_2SO_4 or with 15% H_2SO_4, and heating at 150 °C.

4.1.1. 2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranose (1)[18–20]

Concd sulfuric acid (14 ml) was added to a solution of D-mannose (20 g, 0.11 mol) in anhydrous acetone (30 ml) and the mixture was stirred at rt for 4 h then neutralized with satd Na_2CO_3 solution. The reaction mixture was filtered and the filtrate was extracted using ethyl acetate (3 × 100 ml). The organic layers were evaporated and concentrated to dryness under reduced pressure gave **1** (21.6 g, 75%) as white crystals mp 121–122 °C. ^1H NMR (300 MHz, $CDCl_3$ δ 1.31 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.90 (br s, 1H), 4.05 (m, 2H), 4.16 (dd,

Table 4
Effect of test compounds on pylorus ligation-induced gastric ulcer

Treatment	Dose (mg/kg)	The activity of H^+/K^+-ATPase (μmol Pi/h/mg prot)	Peptic activity μg tyrosine/ml/4 h	The volume of gastric juice (ml)	The pH value of gastric juice
Sham	—	5.60 ± 0.5**	2.60 ± 0.75*	1.10 ± 0.64**	3.88 ± 0.42*
Control	—	11.44 ± 1.5**	5.70 ± 2.40	5.96 ± 0.80	2.60 ± 0.55
12d	3.6	7.91 ± 1.0**	5.02 ± 1.11	2.98 ± 0.42**	3.64 ± 1.12*
	7.2	6.88 ± 1.15**	4.74 ± 1.15	2.60 ± 0.51**	4.01 ± 1.75**
15a	3.6	6.91 ± 1.0**	4.72 ± 1.11	2.40 ± 0.42**	4.04 ± 1.12*
	7.2	6.02 ± 1.15**	4.44 ± 1.15	2.30 ± 0.51**	4.41 ± 1.75**
15b	3.6	6.78 ± 1.2**	4.62 ± 1.11	2.31 ± 0.40**	4.10 ± 1.12*
	7.2	6.00 ± 1.12**	4.34 ± 1.15	2.24 ± 0.31**	4.31 ± 1.75**
15c	3.6	7.01 ± 1.14**	4.80 ± 1.01	2.48 ± 0.66**	4.14 ± 1.62*
	7.2	6.62 ± 1.00**	4.56 ± 1.20	2.38 ± 0.50**	4.39 ± 1.21**
15d	3.6	6.88 ± 1.21**	4.79 ± 1.24	2.56 ± 0.20**	4.53 ± 1.32*
	7.2	6.34 ± 1.25**	4.54 ± 1.05	2.39 ± 0.41**	4.73 ± 1.35**
Omeprazole	3.6	7.10 ± 1.02**	4.92 ± 1.22	2.64 ± 1.00**	4.26 ± 0.92**

Values are expressed as mean ± SD (n = 6).
* p <0.05 compared to control group.
** p <0.01 compared to control group.

J = 3.6, 7.1 Hz, 1H), 4.39 (ddd, J = 5.4, 5.5, 7.1 Hz, 1H), 4.59 (d, J = 5.9 Hz, 1H), 4.79 (dd, J = 3.6, 5.9 Hz, 1H), 5.36 (d, J = 3.6 Hz, 1H).

4.1.2. Benzyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (2)[21,22]

To a solution of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (11 g, 0.042 mmol) in THF (5 ml), a suspension of 60% NaH (0.24 g, 10 mmol) was added at 0 °C, and the mixture was stirred for 40 min. To the mixture, benzyl bromide (0.51 g, 3 mmol) was added and the reaction mixture was stirred overnight and concentrated by evaporation. A mixture of MeOH (30 ml) and water (100 ml) was added to the residue. The aqueous phase was extracted with EtOAc and dried (Na_2SO_4). The organic layer was concentrated under reduced pressure and the residue was purified by crystallization using ethyl acetate/hexane to provide **2α** (12.7 g, 86%) as white crystals, mp 81–82 °C. ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.34 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.93 (m, 1H), 4.18–4.47 (m, 3H), 4.50–4.65 (2d, J = 12.0 Hz, 2H, OCH_2Ph), 4.75–4.88 (m, 2H), 5.11 (s, 1H, H-l), 7.26 (m, 5H, Ar).

4.1.3. Benzyl 2,3-*O*-isopropylidene-α-D-mannofuranose (3)[21]

A solution of **2** (1.2 g, 4.3 mmol) was dissolved in 80% acetic acid (100 ml), and the reaction mixture was refluxed for 3 h. The solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate/methanol, 20:1) to provide **3** (0.94 g, 89%) as white crystals, mp 81–82 °C. ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.36 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.90–3.40 (br s, 2H, 2OH), 3.65 (m, 2H), 3.80 (d, J = 7.0 Hz, 2H), 4.00 (m, 2H), 4.49–4.65 (2d, J = 11.0 Hz, 2H, OCH_2Ph), 5.10 (s, 1H, H-l), 7.30 (m, 5H, Ar).

4.1.4. Benzyl 2,3-*O*-isopropylidene-5,6-*O*-sulfuryl-α-D-mannofuranoside (4)

The diol **3** (15.4 g, 50 mmol) was dissolved in anhyd CCl_4 (50 ml) then thionyl chloride (4.4 ml, 60 mmol) was added. The mixture was refluxed for 30 min then the solution was cooled to 0 °C and diluted with MeCN (50 ml). $RuCl_3{\cdot}3H_2O$ (8 mg, 0.03 mmol), $NaIO_4$ (16 g, 75 mmol) and H_2O (50 ml) were added to the mixture. This mixture was kept at rt for 1 h and then Et_2O (400 ml) was added. The organic phase was separated, washed successively with H_2O (200 ml), satd $NaHCO_3$ (2 × 200 ml), dried ($MgSO_4$) and concentrated. The product was purified by column chromatography using (petroleum ether/diethyl ether, 9:1) gave **4** (16.8 g, 91%) as white solid, mp 106 °C. ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.20 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 4.22 (dd, J = 9.6, 3.5 Hz, 1H), 4.40–4.51 (m, 4H), 4.61–4.81 (m, 3H), 5.06 (s, 1H, H-l), 7.26 (m, 5H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$ δ 26.6, 69.9, 72.8, 79.3, 81.1, 84.3, 85.7, 105.3, 120.0, 128.2, 128.1, 129.0, 138.1 ppm.

4.1.5. 2-Methyl-1*H*-benzimidazole (6)[23–25]

A mixture of *o*-phenylenediamine dihydrochloride (3.24 g, 0.03 mol) in (20 ml) 4 N HCl and (0.09 mol) of acetic acid was refluxed for 4 h. The cooled reaction mixture was rendered distinctly basic by gradual addition of concd ammonia solution. The precipitate was collected and recrystallized from 10% aqueous ethanol gave **6** (2.69 g, 68%) as white solid, mp 173–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.51 (s, 3H, CH_3), 5.01 (s, 1H, NH), 7.22–7.59 (m, 4H, Ar-H).

4.1.6. 2-Methyl-5-nitro-1*H*-benzimidazole (7)[23–25]

Concd sulfuric acid (10 ml) was added with stirring to 2-methyl-1*H*-benzimidazole (0.66 g, 0.005 mol) then a nitrating mixture made from HNO_3 (0.36 ml) and concd sulfuric acid (0.4 ml) was cautiously added dropwise. After stirring for 2 h, compound **7** was isolated by pouring on ice-water then neutralizing with aqueous ammonia (0.51 g, 58%) as white solid, mp 223–225 °C. ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.57 (s, 3H, CH_3), 4.71 (s, 1H, NH), 7.51 (d, J = 8.8 Hz, 1H, H7), 8.09 (d, J = 8.8 Hz, 1H, H6), 8.24 (s, 1H, H4).

4.1.7. 1-(*tert*-Butoxycarbonyl)-2-methyl-5-nitro-1*H*-benzimidazole (8)

A mixture of 2-methyl-5-nitro-1*H*-benzimidazole (0.7 g, 4 mmol), $(Boc)_2O$ (4.4 mol) and $NaHCO_3$ (4.4 mol) in methanol (30 ml) was stirred at rt for 24 h. The solid was filtered then the solvent was evaporated and purified by column chromatography (petroleum ether/ethyl acetate, 10:1) gave **8** (0.86 g, 78%) as white solid, mp 134–136 °C. ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.43 (s, 9H, $3CH_3$), 2.50 (s, 3H, CH_3), 7.61 (d, J = 9 Hz, 1H, H7), 8.02 (d, J = 9 Hz, 1H, H6), 8.34 (s, 1H, H4). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.9, 28.6, 84.1, 84.3, 85.7, 113.3, 116.8, 119.0, 137.0, 138.2, 140.1, 145.0, 148.1 ppm. MS (EI) m/z (%): 277.11 (20, M+), 176 (100, M-101). Anal. Calcd for $C_{13}H_{15}N_3O_4$: C, 56.31; H, 5.45; N, 15.15. Found: C, 56.50; H, 5.27; N, 15.10.

4.1.8. 1-(*tert*-Butoxycarbonyl)-2-methyl-5-amino-1*H*-benzimidazole (9)

Pd on charcoal 10% (8 mg) was added to a solution of 1-(*tert*-butoxycarbonyl)-2-methyl-5-nitro-1*H*-benzimidazole (**8**) (0.55 g, 2 mmol) and acetic acid (0.2 ml) in methanol (20 ml) then the mixture was hydrogenated at 3.4 bar and stirred at rt for 24 h. The reaction mixture was filtered on Celite. All organic layers were concentrated to dryness under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 1:1) gave **9** (0.37 g, 75%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.36 (s, 9H, 3 CH_3), 2.59 (s, 3H, CH_3), 5.71 (br s, 2H), 7.51 (d, J = 8.2 Hz, 1H, H7), 7.92 (d, J = 8.2 Hz, 1H, H6), 8.20 (s, 1H, H4). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.5, 28.0, 83.0, 97.9, 113.0, 117.8, 119.8, 139.1, 140.8, 145.1, 150.1 ppm. MS (EI) m/z (%): 247.13 (14, M+), 146 (100, M-101). Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 63.00; H, 6.81; N, 16.78.

4.1.9. General procedure for the synthesis of (11a–d)

2-Chloromethyl pyridine hydrochloride (0.8 g, 5.0 mmol), 2-chloromethyl-4-methoxy-3-methylpyridine hydrochloride (1.0 g, 5.0 mmol), 2-chloromethyl-4-methoxy-3,5-dimethylpyridine hydrochloride (1.1 g, 5.0 mmol), 2-chloromethyl-3,4-dimethoxypyridine hydrochloride (1.12 g, 5.0 mmol), K_2CO_3 (1.1 g, 8.0 mmol) and KI (1.32 g, 8 mmol) were added to a magnetically stirred solution of **9** (1.24 g, 5.0 mmol) in a mixture of acetone (30 ml) and ethanol (30 ml). The reaction mixture was stirred constantly at 60 °C for about 8 h. When the reaction was finished, as monitored by TLC, water (100 ml) was added. Then the reaction mixture was extracted with DCM (30 ml × 4). The organic phase was combined and concentrated under reduced pressure gave crude compounds **11a–d**.

4.1.9.1. 1-(*tert*-Butoxycarbonyl)-2-methyl-5-((pyridine-2-ylmethyl)amino)-1*H*-benzimidazole (11a). Pale yellow solid, (0.76 g, 45%); mp: 276–279 °C. FT-IR ν (cm^{-1}): 3394 (NH), 1689 (C=O), 1624 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.36 (s, 9H, 3 CH_3), 2.59 (s, 3H, CH_3), 4.20 (s, 1H, NH), 4.61 (d, J = 10.1 Hz, 1H), 4.76 (d, J = 10.1 Hz, 1H), 7.48–7.94 (m, 5H), 8.20 (s, 1H), 8.27 (d, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 15.2, 29.4, 48.7, 82.7, 102.9, 116.2, 117.4, 120.6, 121.5, 123.1, 138.9, 140.5, 141.7, 143.6, 150.6, 153.9, 162.9 ppm. MS (EI) m/z (%): 338.17 (20, M+), 237 (100, M-101), 145 (80, M-193). Anal. Calcd for $C_{19}H_{22}N_4O_2$: C, 67.44; H, 6.55; N, 16.56. Found: C, 67.24; H, 6.65; N, 16.60.

4.1.9.2. 1-(*tert*-Butoxycarbonyl)-2-methyl-5-(((4-methoxy-3-methylpyridin-2-yl)methyl)amino)-1*H*-benzimidazole (11b). Yellow solid, (1.0 g, 52%); mp: 231–233 °C. FT-IR ν (cm^{-1}): 3400 (NH), 1682 (C=O), 1625 (C=N). ^{1}H NMR (300 MHz,

$CDCl_3$ δ 1.36 (s, 9H, 3 CH_3), 2.34 (s, 3H), 2.51 (s, 3H, CH_3), 3.75 (s, 3H, CH_3), 4.29 (s, 1H, NH), 4.64 (d, J = 10.3 Hz, 1H), 4.78 (d, J = 10.3 Hz, 1H), 7.50–7.75 (m, 3H), 8.23 (s, 1H), 8.37 (d, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 11.4, 15.7, 28.8, 46.8, 56.9, 82.9, 101.9, 105.4112.5, 116.8, 118.1, 119.9, 121.5, 137.8, 139.3, 143.6, 153.9, 160.8, 165.1 ppm. MS (EI) m/z (%): 382.20 (12, M+), 281 (100, M-101), 145 (56, M-237). Anal. Calcd for $C_{21}H_{26}N_4O_3$: C, 65.95; H, 6.85; N, 14.65. Found: C, 66.01; H, 6.78; N, 14.55.

4.1.9.3. 1-(*tert*-Butoxycarbonyl)-2-methyl-5-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)amino)-1*H*-benzimidazole (11c).

White solid, (0.83 g, 42%); mp: 289–291 °C. FT-IR ν (cm^{-1}): 3396 (NH), 1694 (C=O), 1635 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.35 (s, 9H, 3 CH_3), 2.41 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 4.35 (s, 1H, NH), 4.69 (d, J = 11.3 Hz, 1H), 4.79 (d, J = 11.3 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 8.27 (s, 1H), 8.35 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 11.6, 14.8, 15.8, 28.3, 45.9, 60.8, 83.1, 102.2, 111.5, 115.0, 115.7, 116.5, 119.1, 136.9, 140.1, 141.6, 148.5, 150.9, 159.1, 164.9 ppm. MS (EI) m/z (%): 396.22 (25, M+), 295 (100, M-101), 145 (72, M-237). Anal. Calcd for $C_{22}H_{28}N_4O_3$: C, 66.64; H, 7.12; N, 14.13. Found: C, 66.54; H, 7.31; N, 14.11.

4.1.9.4. 1-(*tert*-Butoxycarbonyl)-2-methyl-5-(((3,4-dimethoxypyridin-2-yl)methyl)amino)-1*H*-benzimidazole (11d).

White solid, (1.18 g, 59%); mp 239–241 °C. FT-IR ν (cm^{-1}): 3390 (NH), 1687 (C=O), 1632 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.36 (s, 9H, 3 CH_3), 2.39 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.39 (s, 1H, NH), 4.79 (d, J = 11.0 Hz, 1H), 4.82 (d, J = 11.0 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 6.5 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 8.27 (s, 1H), 8.35 (d, J = 6.5 Hz 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.6, 28.9, 43.9, 57.7, 60.9, 83.8, 102.7, 106.7, 115.9, 116.4, 119.2, 137.9, 139.1, 141.9, 142.5, 143.1, 144.2, 150.8, 159.2 ppm. MS (EI) m/z (%): 398.20 (12, M+), 297 (100, M-101), 145 (60, M-253). Anal. Calcd for $C_{21}H_{26}N_4O_4$: C, 63.30; H, 6.58; N, 14.06. Found: C, 63.44; H, 6.39; N, 14.12.

4.1.10. General procedure for the synthesis of (12a–d)

The *t*-Boc protected benzimidazole derivatives **11a** (4.0 g, 9.0 mmol), **11b** (3.4 g, 9.0 mmol), **11c** (3.6 g, 9.0 mmol) or **11d** (3.58 g, 9.0 mmol) and trifluoroacetic acid (0.46 g, 4 mmol) in CH_2Cl_2 (50 ml) were stirred at room temperature for 18 h. The solvents were removed by rotary evaporation and the residue was recrystallized from acetonitrile to provide (98%) of the desired deprotected benzimidazole derivatives (**12a–d**).

4.1.10.1. 2-Methyl-*N*-(pyridin-2-ylmethyl)-1*H*-benzimidazol-5-amine (12a).

White solid, (2.76 g, 98%); mp: 296–298 °C. FT-IR ν (cm^{-1}): 3410 (NH), 1624 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.30 (s, 3H, CH_3), 4.60 (s, 1H, NH), 4.68 (d, J = 8.9 Hz, 1H), 4.77 (d, J = 8.9 Hz, 1H), 7.48–7.94 (m, 5H), 8.20 (s, 1H), 8.15–8.24 (m, 2H), 11.7 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.2, 47.7, 101.8, 115.8, 117.6, 120.6, 121.4, 123.9131.8, 137.9, 140.4, 149.6, 152.9, 158.9 ppm. MS (EI) m/z (%): 338.12 (100, M+), 145 (60, M-193). Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.57; H, 5.92; N, 23.51. Found: C, 70.52; H, 5.82; N, 23.41.

4.1.10.2. 2-Methyl-*N*-((4-methoxy-3-methylpyridin-2-yl)methyl)-1*H*-benzimidazol-5-amine (12b).

Yellow oil, (2.4 g, 97%). FT-IR ν (cm^{-1}): 3410 (NH), 1628 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 4.59 (s, 1H, NH), 4.74 (d, J = 9.3 Hz, 1H), 4.79 (d, J = 9.3 Hz, 1H), 7.51–7.70 (m, 3H), 8.22 (s, 1H), 8.37 (d, 1H), 12.11 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$ δ 10.8, 14.7, 46.3, 56.6, 101.7, 104.8, 111.5, 115.8, 117.1, 123.5, 131.4, 137.8, 147.6, 152.9, 161.8, 164.8 ppm. MS (EI) m/z (%): 282.15 (100, M+), 145 (70, M-137). Anal. Calcd for $C_{16}H_{18}N_4O$: C, 68.06; H, 6.43; N, 19.84. Found: C, 68.16; H, 6.33; N, 19.81.

4.1.10.3. 2-Methyl-*N*-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-1*H*-benzimidazol-5-amine (12c).

Colorless oil, (2.6 g, 98%). FT-IR ν (cm^{-1}): 3394 (NH), 1634 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.31 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 4.68 (d, J = 10.2 Hz, 1H), 4.73 (s, 1H, NH), 4.82 (d, J = 10.2 Hz, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 8.26 (s, 1H), 8.34 (s, 1H), 12.20 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$ δ 11.0, 14.6, 15.8, 45.6, 60.4, 101.2, 111.0, 115.8, 116.2, 116.8, 123.1, 131.8, 137.9, 148.2, 152.8, 158.1, 165.9 ppm. MS (EI) m/z (%): 296.16 (100, M+), 145 (52, M-151). Anal. Calcd for $C_{17}H_{20}N_4O$: C, 68.89; H, 6.80; N, 18.90. Found: C, 68.79; H, 6.70; N, 18.98.

4.1.10.4. 2-Methyl-*N*-((3,4-dimethoxypyridin-2-yl)methyl)-1*H*-benzimidazol-5-amine (12d).

Yellow oil (2.6 g, 98%). FT-IR ν (cm^{-1}): 3423 (NH), 1630 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.28 (s, 3H, CH_3), 3.60 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 4.75 (d, J = 10.2 Hz, 1H), 4.80 (s, 1H, NH), 4.85 (d, J = 10.2 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 8.27 (s, 1H), 8.32 (d, J = 6.8 Hz 1H), 11.90 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.0, 42.9, 56.3, 60.8, 101.9, 105.8, 115.6, 116.8, 123.8, 131.8, 137.8, 141.3, 142.8, 143.8, 152.6, 157.6 ppm. MS (EI) m/z (%): 298.14 (100, M+), 145 (60, M-153). Anal. Calcd for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.33; H, 6.19; N, 18.70.

4.1.11. General procedure for the synthesis of (14a–d)

To an ice-cold suspension of 60% NaH (65 mmol) in THF (130 ml), benzimidazole derivatives **12a** (1.5 g, 6.5 mmol), **12b** (1.8 g, 6.5 mmol), **12c** (1.9 g, 6.5 mmol) or **12d** (1.92 g, 6.5 mmol) was added dropwise. The reaction mixture was stirred for 1 h then cyclic sulfate **4** (2.8 g, 7.5 mmol) was added. The reaction mixture was stirred overnight. Cleavage was carried out by the addition of concd H_2SO_4 (3.6 ml) and water (1.3 ml). After stirring for 1 h at room temperature, the reaction mixture was neutralized by adding saturated $NaHCO_3$, and washed with $CHCl_3$ (2 × 20 ml). The organic layers were collected, dried with anhydrous Na_2SO_4, and evaporated. The oil obtained was purified by column chromatography gave **14a–d**.

4.1.11.1. 1-(5-Hydroxy benzyl-2,3-*O*-isopropylidene-α-D-mannofuranose)-5-((pyridin-2-ylmethyl)amino)-2-methyl-1*H*-benzimidazole (14a).

Colorless oil, (2.0 g, 58%); FT-IR ν (cm^{-1}): 3450 (OH), 3390 (NH), 1630 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.30 (br s, 1H, OH) 4.20 (m, 1H), 4.47 (d, J = 11.8 Hz, 2H), 4.58 (s, 1H, NH), 4.61–4.68 (m, 4H),4.71 (d, J = 8.9 Hz, 1H), 4.77 (d, J = 8.9 Hz, 1H), 4.92 (dd, J = 3.6, 3.6 Hz, 1H), 5.09 (s, 1H), 7.28 (m, 5H), 7.48–7.94 (m, 4H), 8.20 (s, 1H), 8.15–8.24 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.7, 26.5, 47.6, 47.8, 69.0, 71.4, 80.2, 80.4, 84.7, 101.8, 104.9, 113.4, 115.8, 120.9, 121.1, 121.4, 127.5, 127.7, 128.0, 128.4, 128.7, 131.8, 133.4, 137.3, 137.9, 139.4, 148.8, 149.3, 158.7 ppm. Anal. Calcd for $C_{30}H_{34}N_4O_5$: C, 67.91; H, 6.46; N, 10.56. Found: C, 67.78; H, 6.36; N, 10.50.

4.1.11.2. 1-(5-Hydroxy benzyl-2,3-*O*-isopropylidene-α-D-mannofuranose)-5-(((4-methoxy-3-methylpyridin-2-yl)methyl)amino)-2-methyl-1*H*-benzimidazole (14b).

Colorless oil, (2.7 g, 72%); FT-IR ν (cm^{-1}): 3435 (OH), 3384 (NH), 1635 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.36 (br s, 1H, OH), 3.68 (s, 3H, OCH_3), 4.22 (m, 1H), 4.44 (d, J = 11.3 Hz, 2H), 4.50 (s, 1H, NH), 4.58–4.69 (m, 4H), 4.73 (d, J = 8.3 Hz, 1H), 4.80 (d, J = 8.3 Hz,

1H), 4.90 (dd, J = 3.6, 3.6 Hz, 1H), 5.13 (s, 1H), 7.32 (m, 5H), 7.44–7.74 (m, 3H), 8.20 (s, 1H), 8.37 (d, J = 5.8, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 10.5, 14.2, 27.2, 46.0, 49.8, 57.0, 67.6, 71.0, 80.6, 83.4, 84.9, 102.0, 103.8, 104.6, 111.6, 113.7, 115.6, 121.2, 121.4, 127.5127.7, 128.2, 128.4, 131.5, 133.2, 137.9, 138.1, 148.0, 149.6, 159.7, 165.1 ppm. Anal. Calcd for $C_{32}H_{38}N_4O_6$: C, 66.88; H, 6.67; N, 9.75. Found: C, 66.77; H, 6.80; N, 9.65.

4.1.11.3. 1-(5-Hydroxy benzyl-2,3-*O*-isopropylidene-α-D-mannofuranose)-5-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)amino)-2-methyl-1*H*-benzimidazole (14c). Colorless oil, (2.3 g, 60%); FT-IR ν (cm^{-1}): 3420 (OH), 3380 (NH), 1630 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.28 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.41 (br s, 1H, OH), 3.81 (s, 3H, OCH_3), 4.20 (m, 1H), 4.44 (d, J = 11.6 Hz, 2H), 4.53 (s, 1H, NH), 4.58–4.69 (m, 4H), 4.74 (d, J = 8.5 Hz, 1H), 4.81 (d, J = 8.5 Hz, 1H), 4.94 (dd, J = 3.6, 3.6 Hz, 1H), 5.15 (s, 1H), 7.30 (m, 5H), 7.60 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 8.22 (s, 1H), 8.31 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 10.8, 14.6, 15.8, 26.2, 45.8, 49.8, 50.6, 60.1, 67.3, 71.9, 80.2, 83.4, 85.2, 101.9, 104.8, 111.5, 113.6, 115.2, 115.9, 121.0, 127.9, 128.1, 128.2, 128.4, 131.9, 133.6, 137.9, 138.4, 148.2, 149.6, 159.7, 164.1 ppm. Anal. Calcd for $C_{33}H_{34}N_4O_6$: C, 67.33; H, 6.85; N, 9.52. Found: C, 67.47; H, 6.80; N, 9.40.

4.1.11.4. 1-(5-Hydroxy benzyl-2,3-*O*-isopropylidene-α-D-mannofuranose)-5-(((3,4,dimethoxypyridin-2-yl)methyl)amino)-2-methyl-1*H*-benzimidazole (14d). Colorless oil, (2.1 g, 56%); FT-IR ν (cm^{-1}): 3428 (OH), 3386 (NH), 1635 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 1.29 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.49 (br s, 1H, OH), 3.61 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.16 (m, 1H), 4.40 (d, J = 11.0 Hz, 2H), 4.60 (s, 1H, NH), 4.63–4.70 (m, 4H), 4.79 (d, J = 8.4 Hz, 1H), 4.86 (d, J = 8.4 Hz, 1H), 4.90 (dd, J = 3.5 Hz, 1H), 5.10 (s, 1H), 7.35 (m, 5H), 7.58 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 6.2 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 8.23 (s, 1H), 8.30 (d, J = 6.2 Hz 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.9, 26.9, 42.8, 50.4, 57.1, 61.4, 67.6, 72.9, 80.2, 83.7, 85.2, 101.6, 105.6, 105.9, 113.0, 115.9, 122.0, 128.9, 129.0, 129.1, 129.2, 129.4, 132.9, 134.6, 137.8, 138.9, 142.1, 143.0, 143.9, 149.2, 157.2 ppm. Anal. Calcd for $C_{32}H_{38}N_4O_7$: C, 65.07; H, 6.48; N, 9.49. Found: C, 65.19; H, 6.28; N, 9.40.

4.1.12. General procedure for the synthesis of (15a–d)

Pd on charcoal 10% (10 mg) was added to a solution of **14a** (1.1 g, 2.0 mmol), **14b** (1.15 g, 2.0 mmol), **14c** (1.17 g, 2.0 mmol) or **14d** (1.18 g, 2.0 mmol) and acetic acid (0.2 ml) in ethyl acetate/methanol mixture, 4:1 (20 ml), then the mixture was hydrogenated and stirred at rt for 2 days. The reaction mixture was filtered on Celite. The organic layer was concentrated to dryness under reduced pressure. Then, 1:1 mixture of trifluoroacetic acid/water (5 ml) was added with stirring at room temperature for 24 h. The solvents were evaporated and the residue was evaporated to afford **15a–d**.

4.1.12.1. 1-(1,2,3,5-Tetrahydroxy-α-D-mannofuranose)-5-((pyridin-2ylmethyl)amino)-2-methyl-1*H*-benzimidazole (15a). Colorless oil, (0.56 g, 67%); FT-IR ν (cm^{-1}): 3450–3400 (OH), 3380 (NH), 1635 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.50 (s, 3H, CH_3), 3.64 (m, 1H), 3.84 (m, 2H), 3.91 (m, 1H), 4.29 (m, 1H), 4.71 (m, 2H), 5.41 (s, 1H), 5.60 (d, J = 11.8 Hz, 1H), 5.73 (d, J = 11.8 Hz, 1H), 7.20 (m, 2H), 7.30–7.52 (m, 2H), 7.48–7.94 (m, 4H), 8.20 (s, 1H), 8.15–8.24 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 14.2, 47.8, 51.8, 67.0, 75.4, 77.2, 84.9, 101.7, 103.1, 113.8, 115.0, 121.0, 121.8, 131.6, 133.8, 138.3, 139.4, 148.8, 151.3, 156.7 ppm. Anal. Calcd for $C_{20}H_{24}N_4O_5$: C, 59.99; H, 6.04; N, 13.99. Found: C, 60.09; H, 6.17; N, 13.10.

4.1.12.2. 1-(1,2,3,5-Tetrahydroxy-α-D-mannofuranose)-5-(((4-methoxy-3-methylpyridin-2-yl)methyl)amino)-2-methyl-1*H*-benzimidazole (15b). Yellow oil, (0.53 g, 60%); FT-IR ν (cm^{-1}): 3455–3410 (OH), 3395 (NH), 1630 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.41 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.60 (s, 3H, OCH_3), 3.65 (m, 3H), 3.80 (m, 3H), 3.98 (m, 2H), 4.39 (m, 1H), 4.81 (m, 2H), 5.42 (s, 1H), 5.66 (d, J = 11.8 Hz, 1H), 5.78 (d, J = 11.8 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 8.27 (s, 1H), 8.32 (d, J = 6.8 Hz 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 11.0, 14.8, 45.8, 51.0, 56.0, 66.4, 75.2, 77.0, 84.9, 101.9, 103.0, 104.6, 111.8, 113.0, 115.0, 131.3, 133.0, 137.3, 148.0, 149.3, 160.0, 162.9 ppm. Anal. Calcd for $C_{22}H_{28}N_4O_6$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.40; H, 6.25; N, 12.69.

4.1.12.3. 1-(1,2,3,5-Tetrahydroxy-α-D-mannofuranose)-5-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)amino)-2-methyl-1*H*-benzimidazole (15c). Pale yellow oil, (0.66 g, 73%); FT-IR ν (cm^{-1}): 3450–3415 (OH), 3400 (NH), 1631 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.30 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 3.75 (m, 3H), 3.81 (m, 3H), 3.98 (m, 2H), 4.40 (m, 1H), 4.80 (m, 2H), 5.40 (s, 1H), 5.56 (d, J = 10.7 Hz, 1H), 5.77 (d, J = 10.7 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.73 (d, J = 8.3 Hz, 1H), 8.23 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 10.7, 14.9, 15.4, 45.0, 50.0, 60.1, 65.4, 74.8, 78.1, 85.9, 102.8, 104.3, 112.7, 114.1, 116.8, 117.0, 132.7, 136.9, 147.5, 150.9, 156.7, 160.1 ppm. Anal. Calcd for $C_{23}H_{30}N_4O_6$: C, 60.25; H, 6.59; N, 12.22. Found: C, 60.37; H, 6.70; N, 12.12.

4.1.12.4. 1-(1,2,3,5-Tetrahydroxy-α-D-mannofuranose)-5-(((3,4-dimethoxypyridin-2-yl)methyl)amino)-2-methyl-1*H*-benzimidazole (15d). Pale yellow oil, (0.7 g, 76%); FT-IR ν (cm^{-1}): 3458–3410 (OH), 3405 (NH), 1633 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$ δ 2.41 (s, 3H, CH_3), 3.60 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.70 (m, 3H), 3.80 (m, 3H), 3.93 (m, 2H), 4.42 (m, 1H), 4.69 (m, 2H), 5.36 (s, 1H), 5.60 (d, J = 11.7 Hz, 1H), 5.76 (d, J = 11.7 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.61 (d, J = 6.0 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 8.20 (s, 1H), 8.31 (d, J = 6.0 Hz 1H). ^{13}C NMR (75 MHz, $CDCl_3$ δ 15.9, 43.0, 52.0, 57.5, 61.1, 67.4, 76.7, 78.6, 86.3, 101.0, 104.9, 105.8, 113.7, 115.1, 132.0, 134.0, 138.9, 142.8, 144,0, 145.1, 151.5, 155.8 ppm. Anal. Calcd for $C_{22}H_{28}N_4O_7$: C, 57.38; H, 6.13; N, 12.17. Found: C, 57.51; H, 6.03; N, 12.10.

5. Pharmacological assay

5.1. Animals

Sprague-Dawley strain rats (120–130 g) or Swiss albino mice (20–25 g) obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt and group-housed under conditions of constant photoperiod (12-h light/dark cycle) with ad libitum access to food and water. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985).

5.2. Anti-inflammatory activity

The newly synthesized compounds were screened for anti-inflammatory activity using the carageenan-induced paw oedema assay in rats. Their activity was compared with diclofenac sodium. Rates were randomized into groups (n = 6) that received test compounds at doses of 10, 20 or 30 mg/kg or diclofenac sodium (20 mg/kg) and control groups received saline by oral gavage. Thirty minutes post treatment, paw oedema was induced by sub plantar injection of 100 μl of 1% sterile carageenan in saline into

the right hind paw as mentioned before.[26] Contralateral paw received an equal volume of saline. Paw volume was determined using a plethysmometer (Ugo Basile, Milan, Italy) before and at 1, 2, and 3 h after carageenan injection. The difference between the initial and subsequent paw volume represents the actual oedema volume. The percent inhibition of inflammation was calculated as following:

$$\text{Percentage of inhibition of oedema} = (1 - V_t/V_c)100$$

where V_t and V_c are the oedema volume in the drug-treated and control groups, respectively.

5.3. Anti-ulcerogenic activity

5.3.1. Ethanol-induced gastric ulcers

Ethanol-induced gastric ulcers model was performed as described before.[27] Briefly, mice were randomized into groups ($n = 6$) that received test compounds (3.6 and 7.2 mg/kg), omeprazole (3.6 mg/kg) or vehicle (carboxy methyl cellulose solution 0.25%, w/v) for 3 days. Animals were fasted for 24 h before the intragastric administration of 90% alcohol (10 ml/kg). The animals were sacrificed by cervical dislocation under ether anesthesia 1 h post alcohol administration. The stomach was taken out and cut open along the greater curvature, length of each lesion was measured under anatomical microscopic.

5.3.2. Pylorus ligation-induced gastric ulcers

Pylorus ligation-induced gastric ulcers model was induced according to the procedure mentioned before.[28] In summary, rats were randomized into groups ($n = 6$) that received test compounds (3.6 and 7.2 mg/kg), omeprazole (3.6 mg/kg) or vehicle (carboxy methyl cellulose solution 0.25%, w/v) for 3 days by oral gavage. On the third day and after 24 h of animal fasting with free access to water, animals were anaesthetized using pentobarbitone (35 mg/kg, ip), then the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply, the stomach was placed carefully and the abdomen wall was closed in two layers with interrupted sutures. Negative control group animals were subjected to the same procedure without pylorus ligation. Five hours after pylorus ligation, animals were sacrificed and stomachs were dissected out and cut open along the greater curvature. The volume and pH value of gastric juice were detected according to reported methods.[28] Briefly, gastric content was collected, measured, centrifuged and the pH was measured using pH meter (Jenway pH meter 3505).

Peptic activity was determined using hemoglobin as substrate and was expressed as μg of tyrosine/ml.[29] Acid hemoglobin was prepared by dissolving hemoglobin in 0.15 N NaCl to a final concentration of 2.5 g/100 ml and mixing with 0.16 N HCl (4:1) to pH 2.2. The reaction mixture consisted of 2.9 ml of acid hemoglobin and 0.1 ml of sample. After incubation for 30 min at 37 °C, peptic activity was stopped by adding 5 ml of ice cold 0.6 M trichloracetic acid. The mixture was centrifuged and the liberated amino acids in the supernatant were determined by the method of Lowry.[30]

5.3.3. Estimation of H^+/K^+-ATPase activity

The H^+/K^+-ATPase was determined as described before.[31] In summary, the isolated stomach was scrapped then the scrapped contents were homogenized in 20 mM Tris–HCl (pH 7.4) and centrifuged for 20 min at 5000*g*. The supernatant was used to determine H^+/K^+-ATPase activity. One milliter of the reaction mixture contained an aliquot of enzyme in Tris–HCl (20 mM, pH 7.4), $MgCl_2$ (2 mM) and KCl (2 mM). Reaction was started with the addition of ATP (2 mM, Sigma–Aldrich) then incubated at 37 °C for 30 min. To stop the reaction, 1 ml of ammonium molybdate and trichloroacetic acid mixture was added. The specific activity of the enzyme was expressed in μM of inorganic phosphorus liberated/min/mg of protein (μmol Pi/h/mg prot).

5.4. Acute toxicity

The median lethal doses (LD_{50}) of all compounds were investigated according to the method of Smith.[32] All the compounds were studied for acute toxicity; LD_{50} values were found to be >1000 mg/kg po.

5.5. Statistical analysis

Results are expressed as mean ± SEM or mean ±SD. Statistical analysis was carried out using One-Way ANOVA followed by Dunnett's test. Results were considered highly significant if p <0.01 and less significant if p <0.05.

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