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SYNTHESIS AND EVALUATION OF NOVEL PYRIMIDYLTHIOMETHYL AND PYRIMIDYL-SULFINYLMETHYL BENZIMIDAZOLES DERIVATIVES FOR THEIR ANTIULCER ACTIVITY

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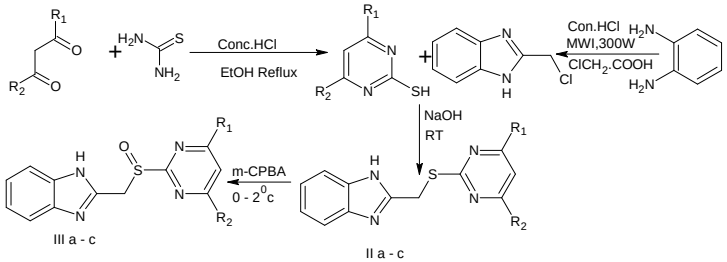
Abstract: A series of novel pyrimidylthiomethyl benzimidazole (IIa-c) and pyrimidylsulfinylmethyl benzimidazolles (III a-c) have been synthesized and evaluated for their antiulcer activity, by the pylorus ligation of rats (say method). Compound IIa and IIIa when evaluated significantly decreased the gastric acid secretion, free acidity, as well as gastric ulcer in the pylorus ligated rats and the effects are dose dependent and comparable to omeprazole of the two compounds, the sulfinyl derivative IIIa is more effective then the thio analog IIa.

Keywords: Antiulcer agents, H/K ATPase inhibitors, Pyridylmethylsulfinyl benzimidazole, Pyrimidylthiomethyl benzimidazole, Pyrimidylsulfinylmethyl benzimidazole.

INTRODUCTION

Pyridylmethylsulfinyl benzimidazoles derivatives such as omeprazole, rebeprazole, lansoprazole, pantoprazole, esomptazole are the drug of choice for the acid related gastrointestinal disorders. These drugs act by inhibiting the proton pump (H/K ATPase) which is involved in the acid secretion in the stomach[1]. The proton pump is responsible for the exchange of K ions with the H of the parietal cells in the stomach[2]. The proton pump inhibitors bind covalently to the cysteine 813 and cystine -822 residues of the H/ K ATPase, which leads to the inhibition of acid secretion[3]. Acid catalyzed activation of pyridylmethylsulfinyl benzimidazole with in the acidic medium in the parietal cells leads to the formation of a reactive intermediate, sulfonamide that irreversibly binds to the thiol group of the enzyme present in the apical membrane of parietal cells[4]. We here in report two novel series pyrimidylmethyl thio/ sulfinyl benzimidazoles IIa-c and III a-c as potent reversible proton pump inhibitors.

The target compounds were prepared from the appropriate 4, 6- disubstituted-2-mercaptopyrimidines and their subsequent condensation with 2-chloromethylbenzimidazole, followed by controlled oxidation of the condensation products (IIa-c) to the corresponding sufinyl derivatives (IIIa-c). The 2-chloromethylbenzimidazole has been prepared in excellent yield and purity through the microwave irradiation based condensation of o- phenyldiamine and chlorlacetic acid.



SCHEME

a=R_1=R_2=CH_3
b=R_1=CH_3,R_2=C_6H_5
c=R_1=OH,R_2=CH_3

MATERIALS AND METHODS

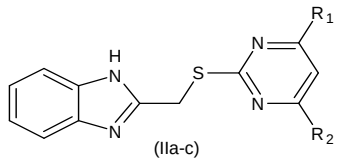
All the chemicals used in the synthesis were of laboratory grade (Loba chem., Mumbai). The melting points were determined in open capillary on veego (VMP) electronic apparatus and uncorrected. The IR spectra of the entire synthesized compound were recorded on Perkin Elmer BX2 FTIR Spectrometer in potassium bromide (anhydrous IR grade) pellets. ^{1}H NMR spectrum was recorded in DMSO- d_6 NMR Varian-Mercury 300 MHz with super conducting magnet. Mass spectra were obtained on an electron impact mass spectrometer at 70ev ionizing beam and using direct insertion probe Shimadzu GCMS- QP- 2010. Progress of the reaction were monitored TLC, performed on microscopic glass slides coated with silica gel-G, using benzene-methanol (4.5:0.5) or hexane, ethyl acetate and glacial acetic acid (3:2:2 drops) as the solvent system and the spot were visualized by expose to iodine vapors or under uv light.

Step-I: The synthesis of 2- chloro methyl benzimidazolw[5]: Monochloroacetic acid 7.5 gm (0.08 mol) and o- phenylenediamine (1) 7.57 gm (0.07mol) were irradiated in 60 mol of 5N HCl for 25 min with stirring in a microwave oven after the reaction mixture was cooled to 0-5°c, it was neutralized with aqueous ammoniumhydroxide. The precipited was collected by vacuum filtration, washed with water and dried in air.
Yield: 79.82%, MP 150-152° c.

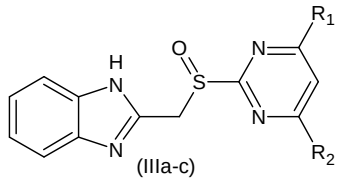
Step-II: The synthesis of 4, 6- disubstituted-2-mercaptopyrimidines[6-8]: To a suspension of finely powdered thiourea (4) (0.1) and appropriate 1, 3-dicarbonyl compound (3a-c) (0.12 mol) in ethanol, 250ml; was added con Hcl, 25 ml. The reaction mixture was refluxed for 2 hrs. After cooling, the yellow needles of 2- mercapto 4, 6- disubstitutedpyrimidine hydrochloride were collected by filtration and dissolved in 2M sodium hydroxide (100ml) and insoluble material was removed by filtration. The filtrate was acidified to P^H 6 with 5M H_2SO_4 and chilled. The precipitated product was filtered, washed with cold water and dried. Crude product was recrystalized from ethanol.

Step-III: The synthesis of pyrimidylthiomethyl benzimidazoles (IIa-c): The appropriate 4, 6-disubstituted-2- mercaptopyrimidine (0.01mol) was dissolved in 25ml of aqueous sodium hydroxide solution (0.015mol) by stirring at room temperature. To these clear solution added a pinch of TEBA chloride (Triethylbenzylammonium chloride) and stirring was further continued for 10 min. To this a solution of 2-chloromethylbenzimidazole (0.01mol) in methanol (25ml) was added over a period of 15-20 min. The reaction mixture was further stirred for 2 hrs. The reaction mixture was cooled to 5- 10°c for 0.5 hrs and the separated solid was filtered, washed with cold water and dried and the crude product on recrystallization from chloroform and methanol.

Step-IV: The synthesis of 2- (4, 6-disubstitutedpyrimidin -2- yl sulfinylmethyl) – 1H- benzo (d) imidazoles (IIIa-c): An appropriate 2- (4, 6 – disubstitutedpyrimidin -2- ylthiomethyl-1H- benzo (d) imidazole (IIa-c) (0.01mol) was dissolved in 20ml iso-propanol by stirring at room temperature. The reaction mixture was chilled thereafter in an ice salt bath and maintaining its temperature between 0-2°c, to this a solution of metachloroperbenzoic acid (m- CPBA) (0.02mol) added with stirring. The stirring was continued for 2-3 hrs. After completion of the reaction, the mixture was washed with 10% sodium bicarbonate and extracted with methylene dichloride (30ml). The organic layer was dried with anhydrous sodium sulfate and solvent was distilled of under reduced pressure at room temperature. The crude product on recrystallization from hexane- chloroform afforded colorless crystal.



(IIa-c)



(IIIa-c)

Table No.-1: Physical characterization data of compound (IIa-c) and (IIIa-c)

S.NO	Compound	R_1	R_2	M.P	Mol. Formula	Mol. Weight	%Yield
1	IIa	CH_3	CH_3	162	$C_{14}H_{14}N_4S$	270	55
2	IIb	CH_3	C_6H_5	170	$C_{19}H_{16}N_4S$	332	60
3	IIc	OH	CH_3	300	$C_{13}H_{12}N_4S$	272	55
4	IIIa	CH_3	CH_3	140	$C_{14}H_{14}N_4OS$	286	50
5	IIIb	CH_3	C_6H_5	110	$C_{14}H_{16}N_4OS$	348	80
6	IIIc	OH	CH_3	85	$C_{13}H_{12}N_4O_2S$	288	50

Antiulcer Activity: Wister albino rats of either sex (200-250gm) were kept in the departmental animal house at room temperature 25-30°c. Rats were divided in to various group (n=5) as follows.

- **Group I**: Treated with 1% CMC (0.4 ml/ Kg, p,o,): Control Group.
- **Group II**: Treated with Omeprazole (10 ml/ Kg, p,o,): Standard Group (10mg).
- **Group III**: Treated with Omeprazole (30 ml/ Kg, p,o,): Standard Group (30mg)
- **Group IV**: Treated with Compound IIa (10mg / Kg, p,o,)
- **Group I**: Treated with Compound IIa (30mg / Kg, p,o,)
- **Group I**: Treated with Compound IIIa (10mg / Kg, p,o,)

Group I: Treated with Compound IIIa (30mg/Kg, p, o,) Omeprazole (10 and 30 mg/kg) and pure compound IIa and IIIa (10 and 30 mg/ kg), were suspended in 1% suspension of CMC in distilled water and administered by oral route. The animals were fasted for 48 hrs prior the experimental, but had free access to water. After the fasting period, the animals were given the drug samples p.o, 1hrs prior the ligation. Thereafter, the rats were anaesthetized with anesthetic ether. An incision of 1 cm length in the abdomen just below the sternum was made. The stomach was exposed. A thread was passed around the pyloric sphincter and a light knot was applied. Then incision was closed by stitching the abdominal wall by a thread. An antiseptic cream was applied over the wound. Thereafter, the animal was kept in separate cage and allowed to recover. Nineteen hours later these animals were sacrificed and the stomach of each of the animals was isolated and cut open through its greater curvature. The gastric contents were carefully removed. Following parameters were noted:

1. **Volume of gastric juice (in ml):** The stomach contents were drained in to graduated centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min at room temperature and centrifuged samples were decanted and volume was measured.

2. **Determination of total acidity:** An aliquot of 1 ml of gastric juice was taken was taken in a 50 ml conical flask and 2 drops of phenolphthalein indicator to it. It was further titrated with 0.01 N NaOH until a permanent pale pink color was developed. The volume of alkali consumed was noted. The total acidity is expressed as mEq/lt by the following formula:

Total Acidity= N* 0.01*40*1000

Where; N=volume of NaOH consumed 40=Eq. wt. of NaOH 0.01= Normality of NaOH 1000= factor to convert the value in ml/lt.

3. **The ulcer score**: The gastric mucosa was examined for ulcers by magnifying lens and the ulcer scored according to its severity in comparison with that of the ulcer in the standard group. Ulcer score was recorded as follows;

   0= Normal; no ulcer
   1= Isolated hemorrhagic spot
   2= Dense hemorrhagic spot
   3= Small ulcer
   4= large ulcer
   5= Perforation

4. **Statistical analysis of data**: Result are expressed as mean±SEM. The statistical difference between the mean volume of gastric juice, mean total acidity and mean ulcer score of the treated group were calculated by using the Students t test.

RESULTS

The result expressing the total volume of gastric juice, total acidity and ulcer score of the control, standard and Treated groups reveal significant difference. Compound IIa (10 and 30 mg/kg) and compound IIIa (10 and 30mg/kg) reduced the ulcer formation significantly ($p<0.001$) comparable to the standard, Omeprazole treated group along with significant decrease in volume and total acidity of gastric juice. Especially, the effect of compound IIIa is more pronounced than IIa.

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally considered that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To re-establish the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to enhance the mucosal defense by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis[12].

Effect of proton pump inhibitor compounds IIa and IIIa (10mg/Kg and 30mg/kg, p.o.) on the volume of gastric juice (ml) in pylorus ligated rats.

n=5, Values are expressed as mean ± SEM
p<0.001 compared to control group (Students t- test)
p< 0.001 compound to control group (Students t- test)

Effect of proton pump inhibitor compounds IIa and IIIa (10mg/kg and 30mg/kg, p.o.) on total acidity (mEq/lit) in pylorus ligated rats.

n=5, Values are expressed as mean ± SEM
P<0.001 compared to control group (Students t- test)
P<0.001 compared to control group (Students t- test)

Effect of proton pump inhibitor compounds IIa and IIIa (10mg/kg and 30mg/kg,p.o.) on ulcer score on gastric mucosa in pylorus ligated rates.

n= 5, Values are expressed as mean ± SEM
P<0.001 compound to control group (Students t- test)
P<0.01 compound to control group (Students t- test)

The causes of ulceration in the gastric mucosa after pyloric ligation are believed to be due to either stress induced increase in the gastric HCl secretion and /or accumulation of acid. According to Shay et al[10], the volume of the secretion is also an important factor in formation of ulcer due to the exposure of the unprotected lumen of the stomach to the accumulating acid. The proton pump inhibitor omeprazole, irreversibly inhibits the gastric acid (proton) pump which is the final common pathway for acid secretion in response to all stimuli. It produces virtual anti acidity in vivo. In the present study the results show that compounds IIa (2-(4,6-dimethylpyrimidin-2-ylthiomethyl)-1H-benzol(d) imidazole) and IIIa(2-(4,6-dimethylpyrimidin-2-ylsulfinylmethyl 1Hbenzo(d) (imidazole) decreased significantly the gastric acid secretion, free acidity as well as gastric ulcers in the pylorus ligated rates and the effect are dose dependent. Of the two compounds, the sulfinyl(oxidized) derivative IIIa, is more effective than the thio (unoxidized) analogue, IIa. This may be due to the inhibitory effect of these compounds on the acid secretary capacity of the stomach in pylorus ligation induced gastric ulcers, which may be mediated by suppressing aggressive factor like gastric acid secretion, analogous to that established in case of the pyridine methyl sulfinyl benzimiazole (omeprazole analogs).However, further specific studies are needed to establish the mechanism involved in the antiulcer action of these compounds, and also if they are reversible or irreversible PPIs.

Table No.2: Spectral Data of the Synthesized Compounds.

S. no.	Comd.	IR Spectra	^{1}H NMR (ppm)
1	IIa	2951(C-Hstr), 1621(C=Nstr), 768(C-Sstr).	2.3 (S,6H,CH_3);4.5(S,2H,CH_2);7.2-7.5(m,5H,ArH);10.2(S, 1H,NH) ; 12.5(S, 1H, NH).
2	IIb	2918(C-Hstr), 1524(C=Cstr), 744(C-Sstr).	2.4 (S,3H,CH_3);4.7(S,2H,CH_2);7.2-8.0(m,10H,ArH);10.5(S, 1H,NH) ; 11.9(S, 1H, NH).
3	IIc	295 (2934(C-Hstr), 1577(C=Nstr), 740(C-SStr).	----
4	IIIa	2930(C-Hstr), 1593(C=Nstr), 1052(S=O), 770(C-Sstr).	2.4 (S,6H,CH_3);4.8(S,2H,CH_2);7.2-7.6(m,5H,ArH);10.9(S, 1H,NH) ; 12.1(S, 1H, NH).
5	IIIb	2918(C-Hstr),1577(C=Nstr), 1064(S=O), 746(C-Sstr).	2.6 (S,3H,CH_3);4.8(S,2H,CH_2);7.2-8.0(m,10H,ArH);10.9(S, 1H,NH) ; 12.3(S, 1H, NH).
6	IIIc	3201(OHStr),2950(C-Hstr),1045(S=Ostr), 1560(C=N), 755(C-Sstr).	-----

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