

Synthesis and Antiulcer Activity Studies of 2-(1'-Iminothioimido Substituted)-1'-Substituted Phenylbenzoic acids

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Subudhi, *et al.*: Antiulcer Activity of Substituted Phenyl Benzoic acids

Certain 2-(1'-iminothioimido substituted)-1'-substituted phenylbenzoic acids (P_{1-9}) were synthesized by reaction of phthalic anhydride with benzotriazole, 2-mercapto benzothiazole and 2-p-amino phenyl benzimidazole, respectively (A_{1-3}) followed by imine formation with Schiff bases of thiourea with salicylaldehyde, furfuraldehyde and 1-phenyl-3-methyl-5-pyrazolone. Antiulcer activity was evaluated using reduction in total acidity, free acidity and ulcer index as parameters. Compounds P_3 , P_6 , P_7 and P_9 (100 mg/kg) showed significant ($P < 0.001$) antiulcer action compared to control and omeprazole (40 mg/kg).

Key words: Benzothiazole, benzotriazole, benzimidazole, total acidity, free acidity, ulcer index

The long-term acid suppression by irreversible H^+ / K^+ -ATPase inhibitors because of covalent binding to the specific enzyme is reported to increase *C. difficile* infection¹, improper absorption of Vit-B₁₂², calcium³ and other opportunistic infections⁴. SCH 28080⁵ is the prototype of a reversible proton pump inhibitor, which is effective in ulcer healing yet avoid prolonged achlorhydria. Derivatives of imidazole⁶ quinoline⁷, isoquinoline⁸, quinazoline⁹, pyrazine¹⁰ and thiazole¹¹ that lack the sulfinyl group but contain a basic group have been reported as reversible proton pump inhibitors. Keeping in view this structural flexibility and capitalizing on the basic structural feature, we thought to explore several benzimidazole, benzotriazole and benzothiazole derivatives to find some alternatives to the present irreversible proton pump inhibitors.

With the above aim, different 2-(1'-iminothioimido substituted)-1'-substituted phenyl benzoic acids (P_{1-9}) were prepared. Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked on silica gel-G coated plates using iodine as visualizing agent. Infrared spectra were recorded on Perkin Elmer model 600 spectrophotometer using KBr pellet. ¹H NMR (DMSO d₆) spectra of title compounds were recorded on a Bruker DRX-300 NMR spectrophotometer (300 MHz) using TMS as internal standard. The C, H

and N elemental analysis was carried out using a Perkin Elmer-2400 elemental analyser. The animal experiments were carried out following the protocols approved by the Institutional Animal Ethics Committee of the University Department of Pharmaceutical Sciences, Utkal University (Registration no-990/c/06/CPCSEA). The title compounds (P_{1-9}) at a dose of 100 mg/kg were tested for antiulcer activity¹² using reduction in total acidity, free acidity and ulcer index as parameters. Omeprazole (40 mg/kg) was used as the standard drug.

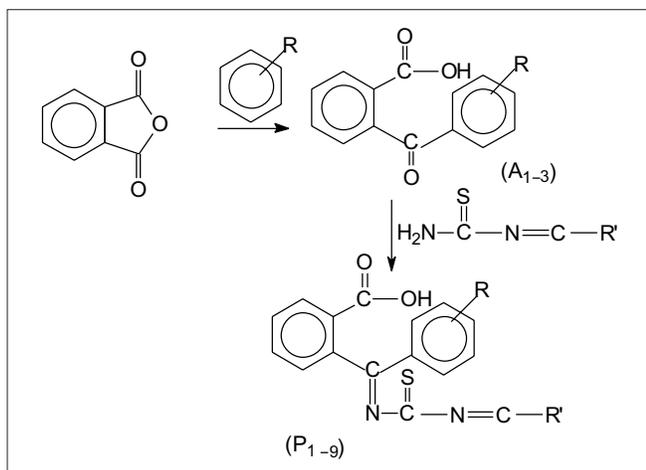
The compounds (A_{1-3}) were prepared according to standard procedure (fig. 1)¹³. Schiff bases of thiourea with salicylaldehyde, furfuraldehyde and 1-phenyl-3-methyl-5-pyrazolone were synthesized as per the standard laboratory method¹⁴. A mixture of compound A (0.015 mol) and corresponding Schiff base (0.015 mol) were taken in 25 ml of ethanol. Few drops of glacial acetic acid were added to it. The mixture was refluxed for 2 h. It was allowed to cool and then mixed with ice-cold water. The precipitate was filtered, dried, and recrystallised with 1, 4-dioxan. Nine such compounds $P_{(1-9)}$ were synthesized and characterized (Table 1). Following the above procedure compound 2-(1'-imino thioimido-o-hydroxy benzyl)-1'-(2''-mercaptobenzthiazolyl) benzoic acid (P_5) was obtained as a crystalline product. IR (KBr, cm⁻¹) spectrum of compound P_5 exhibited bands at 3366 cm⁻¹, (O-H), 1469 cm⁻¹ (C=C, ring of benzene), 1632 cm⁻¹ (C=O) and 1511 cm⁻¹ (C=N). ¹H NMR (DMSO d₆, δ ppm) spectra of P_5 exhibited peaks at δ 7.0-7.5(m,

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TABLE 1: PHYSICAL AND ANALYTICAL DATA OF COMPOUNDS P₍₁₋₉₎

Compd	Mol. Formula	Mol. Wt.	Yield %	mp	% of C, H, N calculated (found)		
					C	H	N
P ₁	C ₃₂ H ₂₅ N ₇ O ₂ S	571.1	51.59	167	67.25(68.12)	4.37(4.28)	17.15(17.89)
P ₂	C ₂₉ H ₂₁ N ₅ O ₃ S	519.1	54.23	162	67.03(67.45)	4.04(4.26)	13.48(13.22)
P ₃	C ₂₇ H ₁₉ N ₅ O ₃ S	493.1	48.82	179	65.70(66.1)	3.85(4.21)	14.19(14.32)
P ₄	C ₂₆ H ₁₉ N ₅ O ₂ S ₃	529.3	47.72	155	58.94(59.12)	3.58 (4.23)	13.22(13.20)
P ₅	C ₂₃ H ₁₅ N ₃ O ₃ S ₃	477.3	53.16	176	57.82(57.80)	3.14(3.28)	8.79(9.21)
P ₆	C ₂₁ H ₁₃ N ₃ O ₃ S ₃	451.3	46.98	173	55.83(55.13)	2.88(2.67)	9.30 (9.54)
P ₇	C ₂₅ H ₁₉ N ₇ O ₂ S	481.1	51.49	159	62.35(63.08)	3.94(4.01)	20.36(20.81)
P ₈	C ₂₂ H ₁₅ N ₅ O ₃ S	429.1	55.48	170	61.52(61.29)	3.49(3.45)	16.31(16.95)
P ₉	C ₂₀ H ₁₃ N ₅ O ₃ S	403.1	58.74	184	59.53(59.24)	3.22(3.14)	17.36(17.69)

**Fig. 1: Scheme of the synthesized compound.**

For compound P₁, R is p-amino phenyl-2-imidazole and R' is 1-phenyl-3-methyl-pyrazole; for compound P₂, R is p-amino phenyl-2-imidazole and R' is 2-hydroxy phenyl; for compound P₃, R is p-amino phenyl-2-imidazole and R' is furfuryl; for compound P₄, R is 2-mercapto thiazole and R' is 1-phenyl-3-methyl-pyrazole; for compound P₅, R is 2-mercapto thiazole and R' is 2-hydroxy phenyl; for compound P₆, R is 2-mercapto thiazole and R' is furfuryl; for compound P₇, R is triazole and R' is 1-phenyl-3-methyl-pyrazole; for compound P₈, R is triazole and R' is 2-hydroxy phenyl and for compound P₉, R is triazole and R' is furfuryl.

4H, Ar-H), δ 11.5(s, 1H, -OH), δ 8.1(s, 1H, CH=N). Other compounds of the series P₁₋₉ displayed broad IR absorption bands corresponding to Ar-OH superimposed on that of NH in the range of 3285-3368 cm⁻¹. Another sharp band appeared at 1645-1687 cm⁻¹ due to carbonyl function. Bands assigned to stretching vibrations of carbon-carbon double bonds of aromatic ring were observed at 1442-1489 cm⁻¹. ¹H NMR (DMSO d₆, δ ppm) spectral studies exhibited a broad singlet at δ 11.7-13.2 due to proton of aromatic carboxylic acid group. Another broad singlet appeared at δ 4.8-5.45 due to N-H proton. The aromatic protons appeared as multiplets in the range δ 7.05-7.65.

Male rats weighing between 140 and 175 g were selected for pyloric ligation ulcer model¹². Rats were divided into eleven groups consisting of six animals

each and were fasted overnight. One group received normal saline 2 ml/kg (negative control). The second group received omeprazole 40 mg/kg (positive control) and the other groups received test compounds (100 mg/kg) by oral route 30 min prior to pyloric ligation. Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N sodium hydroxide solution using Topfer's reagent as indicator to the end point when the solution turned to orange color. The volume of sodium hydroxide consumed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink color. The volume of sodium hydroxide solution required was noted and total acidity calculated. After opening the stomach the ulcer index was calculated. The results are expressed as mean \pm SEM. The difference between groups was determined using the one way analysis of variance (ANOVA) followed by Dunnett's test and $p < 0.05$ was considered significant.

From the data in Table 2 it can be inferred that all the compounds at the tested dose level exhibited varying degree of antiulcer activity. The compound P₃, P₆, P₇ and P₉ exhibited good gastro protective actions as indicated by their very low ulcer index, free acidity and total acidity values. The inhibition of gastric acid secretion was better for benzotriazole and benzothiazole derivatives than substituted benzimidazoles. However benzimidazole derivatives exhibited significant capacity for neutralization of gastric acid compared to control. The present study indicates the necessity of a basic group but shows structural flexibility among the compounds to exert the antiulcer effect. Presence of iminothioimido derivatives of substituted pyrazole, furfuryl and o-hydroxy phenyl does not seem to affect activity significantly.

TABLE 2: ANTIULCER ACTIVITY DATA OF COMPOUNDS

Compound	Total acid (mEq/l)	Free acid (mEq/l)	Ulcer index
Control	11.4± 1.6	3.18±0.29	4.4±0.35
Omeprazole	3.2± 1.25*	0.42±0.25*	0.33±0.06*
P ₁	8.69±0.21*	2.52±0.42*	3.66±0.81*
P ₂	8.31±0.29*	2.18±0.38*	3.36±0.65*
P ₃	6.4±0.75*	2.11±0.30*	2.33±0.22*
P ₄	9.04±0.34*	2.92±0.12*	3.4±0.29*
P ₅	7.3±0.57*	2.41±0.17*	2.83±0.34*
P ₆	4.8±0.55*	1.79±0.08*	1.41±0.14*
P ₇	5.02±0.35*	1.77±0.23*	2.33±0.30*
P ₈	8.21±0.34*	2.62±0.27*	3.17±0.27*
P ₉	6.04±0.15*	2.13±0.02*	1.75±0.51*

Each value represents the mean± SEM. No of animals in each group were 6. *p<0.05 as compared to control (Dunnett's test)

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