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ISSN: 0975-7538
Research Article

Design, synthesis, characterization and anti-inflammatory activity of some novel Benzimidazole derivatives

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ABSTRACT

A novel series of benzimidazole derivatives were synthesized through modifying the N-1 hydrogen of 2-substituted benzimidazoles with primary and/or secondary amines in ethanol yielded the corresponding N-Mannich bases (**SR**$_{4-9}$). The synthesized compounds were elucidated by UV, IR, ^{1}H NMR and Mass spectral analysis. In IR spectra, all the compounds were showed above 3100 cm^{-1} due to N-H stretching vibration and in **SR**$_{4-6}$ showed the region of 1312 – 1320 cm^{-1} due to presence SO_2stretching of sulphonamide group. In ^{1}H NMR spectra of N-mannich bases showed well resolved peaks at 7.10-7.64 ppm as a result of Ar-H, at 4.57-4.60 ppm due to two protons in SO_2NH_2 group and at 1.13-1.15 ppm due to one proton in NH group of piperazine. The final compounds were evaluated for their anti-inflammatory activity by HRBC membrane stabilization method and Carrageen in induced rat hind paw edema. The synthesized derivative (**SR$_9$**) exhibited very good significant anti-inflammatory activity.

Keywords: Anti-inflammatory; Benzimidazole; HRBC membrane; Mannich bases.

INTRODUCTION

Benzimidazole is a fused heterocycle, containing a benzene ring attached with one face of the imidazole ring. It is key structure in numerous compounds of therapeutic importance. Benzimidazoles were exhibits to possess antimicrobial, anti-inflammatory, anti-viral, anticonvulsant, anti-protozoal and anthelmintic activities. It has been observed that the presence of two (or) more heterocyclic moieties fused or linked enhance the biological profile of drug molecules by many folds (J. T. Leonard *et al*., 2007; K. F. Ansari *et al*., 2009; Periyasamy Selvam *et al*., 2010; K. Anandarajagopal *et al*., 2010; G. Mariappan *et al*., 2011).

In view of above reports we have synthesized a series of N-substituted benzimidazoles **SR**$_{4-9}$ (Scheme-1) by Mannich reaction of various 2-substituted benzimidazoles with sulphanilamide and piperazine groups as primary and secondary amines. The required starting material 2-substituted benzimidazoles **SR**$_{1-3}$ were prepared by reacting with appropriate organic carboxylic acids like acetic acid, propionic acid and butyric acid. The structural assignments of **SR**$_{4-9}$ were confirmed by spectral data. In IR spectra, all the compounds were showed above 3100 cm^{-1} due to N-H stretching vibration and in **SR**$_{4-6}$ showed the region of 1312 – 1320 cm^{-1} due to the presence SO_2 stretching of sulphonamide group. The ^{1}H NMR spectra of N-mannich bases showed well resolved peaks at 7.10-7.64 ppm as a result of Ar-H, at 4.57-4.60 ppm due to two protons in SO_2NH_2 group and at 1.13-1.15 ppm due to one proton in NH group of piperazine. The mass spectra were confirmed the molecular weight of the synthesized compounds.

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Received on: 02-06-2015
Revised on: 19-06-2015
Accepted on: 25-06-2015

MATERIALS AND METHODS

Reagents & Chemicals

The reagents and solvents were commercially available (Rankem, SD fine, Loba and Fluka) and of synthetic grade. Glassware was oven or flame - dried for moisture sensitive reactions. Melting points of all the synthesized compounds were determined by open capillary tube method and are uncorrected. The purity of all compounds was checked by pre-coated Silica gel-G TLC (Loba Chemie, Mumbai) using methanol and water (8:2) as solvent system and iodine vapours as visualising agent. Elemental analysis was carried out on C, H, N elemental analyser (Thermo Finnegan Flash EA 1112).

Instrumentation

The UV spectra were recorded on a SHIMADZU – 1700 spectrometer. IR spectra were recorded on a Thermo Fisher – Nicolet is5 spectrometer (cm^{-1}). ^{1}H NMR spectra were recorded on a Bruker – NMR 500 MHz spectrometer in DMSO-d_6 or $CDCl_3$ using tetra methyl silane (TMS) as an internal standard (chemical shift in δ ppm). Mass spectra were recorded on a JEOL GC mate mass spectrometer.

Synthesis of 2–substituted benzimidazole (SR_{1-3})

O-phenylene diamine **(**0.125 mol) and appropriate aliphatic carboxylic acids (0.17 mol) were refluxed at 100° C for 6-8 hr, cooled and added 10% sodium hydroxide solution slowly until the mixture was just alkaline to litmus. The crude benzimidazole derivative was filtered and then washed with ice cold water. The crude product was recrystallised from boiling water; 2 gm of decolorizing carbon was added and digested for 15 min. The product was filtered, cooled and dried at 100° C and weighed (Sheela Joshi *et al*., 2005; Rita Bamnela *et al*., 2011; Geol P.K *et al* 2007).

General procedure for the synthesis of N-Mannich bases (SR_{4-9})

To a solution of 2-substituted benzimidazoles (0.01 mol), in 15 ml of ethanol, (0.01 mol) of respective amine and (0.01 mol) of formaldehyde solution (37% v/v) were added. The reaction mixture was adjusted to the pH of 3.5 with hydrochloric acid slowly with constant stirring under rigorous ice cold condition for half an hour to avoid losses of formaldehyde and then it was refluxed on water bath up to 1-3 hr. On cooling at 0° C for 2-3 days in deep freeze, the product was obtained, filtered, dried in vacuum and recrystallized through different suitable solvents viz., dry distilled ethanol and DMF (Murugesan Sugumaran *et al*., 2011).

Adopting the above methods, six derivatives **SR_{4-9}** were prepared, and their physical data are described in Table 1.

RESULTS AND CONCLUSION

Anti-inflammatory activity

Study of anti-inflammatory effects by membrane stabilizing property (Ghosh MN *et al*., 1984)

The anti-inflammatory activity the synthesized compounds **SR_{4-9}** were determined by HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of (2% dextrose, 0.8% sodium citrate, 0.05% citric acid & 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) & 10% v/v suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in Table 1). 1 ml of phosphate buffer (0.15M, pH7.4), 2ml of hyposaline (0.36%), 0.5 ml of HRBC suspension were added. Diclofenac was used as the reference drug. Instead of hyposaline, 2 ml of distilled water was used as control. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged. The haemoglobin content in the supernant solution was estimated using colorimeter at 560 nm. The percentage hemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the following formula.

$$\text{Percentage inhibition of Heamolysis} = 100\,X\,\frac{OD_1 - OD_2}{OD_1}$$

Where $OD_1$and OD_2 are the absorbance of Diclofenac and SR_9 respectively

From the results of synthesized compound SR_9 at concentration range of 6-100 µg /ml protects the human erythrocyte membrane against lysis induced by hypotonic solution. At concentration 100 µg/ml, the compound produced 36.14% inhibition of RBC haemolysis as compared with 48.14% produced by Diclofenac sodium in (Table 2). Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. The results obtained demonstrate that SR_9 can significantly and dose-dependently inhibits RBC haemolysis.

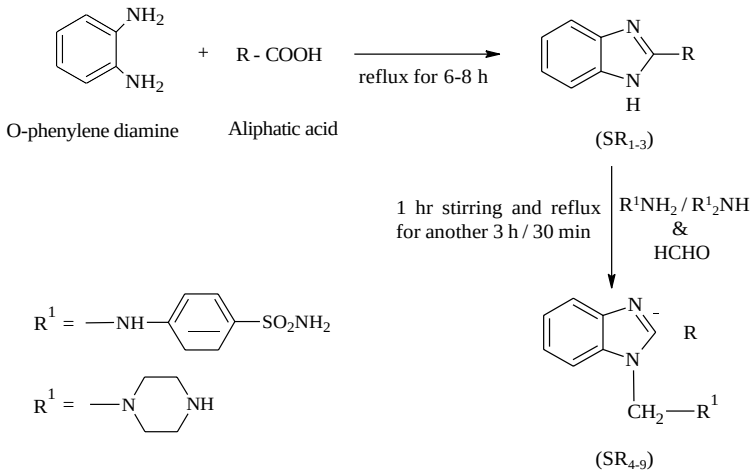
Study of anti-inflammatory effects by Carrageenin induced rat hind paw edema (Winter CA *et al*., 1962)

The rats were divided into 4 groups comprising of six animals in each. Group 1 served as control and received 2 ml/kg of normal saline; Group 2 served as standard reference and received indomethacin (10 ml/kg), while Group 3 received SR_6 (250 mg /kg) and Group 4 received SR_9 (250 mg /kg) by oral route. An injection of 0.1ml of 1% carrageen (in water) suspension into the right hid paw of each rat in the subplantar region was made. The paw volume was measured at 0 hour and 3 hours after injection of carrageenin. The plethysmograph apparatus was used for the measurement of rat paw volume based on Haris and Spencer method. Drug pre treatment was given 1 hour before the injection of carrageenin. The percentage of edema inhibition was calculated. All the results were analyzed for the significance using Students 't' test.

The synthesized compound, SR_9 (250 mg/kg, p.o.) significantly inhibited carrageenin-induced rat paw edema ($p<0.001$). The inhibition at 3 hours was greater than at 1 hour after induction of edema in (Table 3). Carrageenin-induced paw edema was taken as prototype of exudative phase of inflammation. The results obtained demonstrate that SR_9 can significantly active than SR_6. Further works are in progress to find out its exact mechanism of action.

SPECTRAL data

SR_4: UV (MeOH): 265.5 nm. IR (KBr) cm^{-1}: 3376 (1° N-H stretch in sulphonamide), 3263 (2° N-H stretch in sulphonamide), 1595 (C=N stretch), 1386 (C-N stretch of aromatic tertiary amine), 1312 (SO_2 stretch). ^{1}H NMR (δ, ppm): 7.10 – 7.64 (8H, m, Ar-H – C_4, C_5, C_6, C_7 and 4-phenyl protons), 6.06 (2H, s, -CH_2), 4.57 (2H, s, -SO_2NH_2), 5.82 (1H, s, -NH), 2.53, (3H, s, -CH_3). EIMS



SCHEME-1

Figure 1: Schematic representation of novel benzimidazole derivatives

Table 1: Physical data of the synthesized compounds (SR4-9)

Compound	R	R^1	M.P. (° C)	Yield (%)	R_f Value ($CH_3OH:H_2O$) 8:2
SR_4	CH_3	Sulphanilamido	142	45.58	0.93
SR_5	CH_2CH_3	Sulphanilamido	123	31.61	0.81
SR_6	$CH_2CH_2CH_3$	Sulphanilamido	149	28.45	0.82
SR_7	CH_3	Piperazino	148	71.17	0.86
SR_8	CH_2CH_3	Piperazino	161	92.70	0.84
SR_9	$CH_2CH_2CH_3$	Piperazino	174	69.10	0.87

Table 2: Effect of synthesized compound (SR9) on Human Erythrocyte Haemolysis

Compound/Drug	% Prevention of lysis
SR_9 100 µg/ml	36.14
SR_9 50 µg/ml	33.31
SR_9 25 µg/ml	21.05
SR_9 12.5 µg/ml	19.52
SR_9 6.3 µg/ml	14.28
Diclofenanc Sodium 100µg/ml	48.14

Table 3: Effect of synthesized compounds (SR6 & SR9) on carrageenin-induced paw edema

Treatment	Dose (mg/kg)	Increase in Paw volume (ml)	% Inhibition of edema
Normal Saline	-	0.62 ± 0.03	-
Indomethacin	10	0.24 ± 0.02	61.29
SR_6	250	**0.27** ± 0.04*	28.42
SR_9	250	**0.36** ± 0.03*	41.93

N=6, values are expressed as mean ± SEM

*p<0.001 when compared to control based on a Students't' test

(m/z): 316 (M^+). Elemental analysis: (C, 56.94; H, 5.10; N, 17.71; O, 10.11; S, 10.14).

SR_5: UV (MeOH): 240.0 nm, 293.0 nm. IR (KBr) cm^{-1}: 3378 (1° N-H stretch in sulphonamide), 3263 (2° N-H stretch in sulphonamide), 1597 (C=N stretch), 1387 (C-N stretch of aromatic tertiary amine), 1313 (SO_2 stretch). ^{1}H NMR (δ, ppm): 7.12 – 7.64 (8H, m, Ar-H – C_4, C_5, C_6, C_7 and 4-phenyl protons), 6.14 (2H, s, -CH_2), 5.83 (1H, s, -NH), 4.57 (2H, s, -SO_2NH_2). EIMS (m/z): 330 (M^+). Elemental analysis: (C, 58.16; H, 5.49; N, 16.96; O, 9.68; S, 9.70).

SR_6: UV (MeOH): 263.0 nm. IR (KBr) cm^{-1}: 3385 (1° N-H stretch in sulphonamide), 3289 (2° N-H stretch in sulphonamide), 1561 (C=N stretch), 1383 (C-N stretch of aromatic tertiary amine), 1320 (SO_2 stretch).). ^{1}H NMR (δ, ppm): 7.10 – 7.64 (8H, m, Ar-H – C_4, C_5, C_6, C_7 and 4-phenyl protons), 6.18 (2H, s, -CH_2), 5.76 (1H, s, -NH), 4.60 (2H, s, -SO_2NH_2). EIMS (m/z): 344 (M^+). Ele-

mental analysis: (C, 59.28; H, 5.85; N, 16.27; O, 9.29; S, 9.31).

SR_7: UV (MeOH): 273.5 nm. IR (KBr) cm^{-1}: 3409 (2° N-H stretch in piperazine), 1622 (C=N stretch), 1346 (C-N stretch of aromatic tertiary amine). ^1H NMR (δ, ppm): 7.15 – 7.64 (4H, m, Ar-H – C_4, C_5, C_6 and C_7), 5.47 (2H, s, -CH_2), 2.37 – 2.93 (8H, m, CH_2 of piperazine), 1.13 (1H, s, -NH). EIMS (m/z): 230 (M^+). Elemental analysis: (C, 67.80; H, 7.88; N, 24.33)

SR_8: UV (MeOH): 274.5 nm. IR (KBr) cm^{-1}: 3416 (2° N-H stretch in piperazine), 1622 (C=N stretch), 1347 (C-N stretch of aromatic tertiary amine). ^1H NMR (δ, ppm): 7.15 – 7.64 (4H, m, Ar-H – C_4, C_5, C_6, C_7), 5.61 (2H, s, -CH_2), 2.37 - 2.93 (10H, m, CH_2 of piperazine and CH_2 of ethyl group), 1.13 (1H, s, -NH). EIMS (m/z): 244 (M^+). Elemental analysis: (C, 68.82; H, 8.25; N, 22.93)

SR_9: UV (MeOH): 274.0 nm. IR (KBr) cm^{-1}: 3385 (2° N-H stretch in piperazine), 1631 (C=N stretch), 1347 (C-N stretch of aromatic tertiary amine). ^1H NMR (δ, ppm): 7.15 – 7.64 (4H, m, Ar-H – C_4, C_5, C_6 and C_7), 5.47 (2H, s, -CH_2), 2.64 – 2.93 (8H, m, CH_2 of piperazine), 1.15 (1H, s, -NH). EIMS (m/z): 258 (M^+). Elemental analysis: (C, 69.73; H, 8.58; N, 21.69)

CONCLUSION

From the results it suggests that the benzimidazole ring is an important pharmacophore in modern drug discovery and the tested derivatives of benzimidazoles have excellent scope for further development as commercial anti-inflammatory agents in the chemotherapeutic approach in human. Our findings will prove useful to those chemists, pharmacists, medicinal chemists who are interested in the synthesis of potential Mannich bases as drugs with minimum side effects and also have comparatively low cost.

ACKNOWLEDGMENTS

The author is great thankful to Management and Principal, Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor, Andhra Pradesh and School of Advanced Sciences, VIT University, Vellore for providing research facilities and spectral analysis. I very thank, University Grants Commission (UGC), New Delhi, India for providing the financial support for my research in the form of grant under Minor Research Project for college teachers.

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