

**SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF
SOME BENZIMIDAZOLE-2-CARBOXYLIC ACIDS**

Prasad A. Thakurdesai^{1,*}, Sudhir G. Wadodkar², Chandrabhan T. Chopade²

¹ Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune 411 038, India.

² Department of Pharmaceutical Sciences, Nagpur University Campus, Nagpur-10

Summary

Benzimidazole is one of the most promising heteroaryl moiety that yielded many successful drugs like omeprazole and mebendazole. Benzimidazole moiety with carboxylic acid substitution at 2 position, fulfills the minimum and desirable structural requirements that are common in most of the marketed anti-inflammatory drugs and so some benzimidazole-2-carboxylic acid derivatives (3a-1 and 4a-c) were synthesized. They were tested for acute anti-inflammatory activity against carrageenan induced rat paw edema model. The test compounds were found to be safe upto 2000 mg/kg, p.o. doses and exhibited good anti-inflammatory activity at 100 mg/kg p.o. and higher doses. Their activity largely depends on substituents at position 5 and chain length at position 2 of benzimidazole moiety. With 1-benzyl substitution, activity was found to increase. The anti-inflammatory effect against carrageenan edema suggests inhibition of prostaglandin synthesis as their probable mechanism of action.

Keywords: Benzimidazole-2-carboxylic acids, acute anti-inflammatory activity, Carrageenan induced rat paw edema model

*Corresponding author –

Dr. Prasad A. Thakurdesai
Assistant Professor, Department of Pharmacology,
Bharati Vidyapeeth University, Erandawane, Paud Road, Pune-38
Tel: +91-20-25437237 Ext-29, Fax: +91-20-25439383,
E-mail: prasad.thakurdesai@gmail.com

Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used drug category against inflammation, mild to moderate pain, and fever. Specific uses also include the treatment of headaches, arthritis, sports injuries, and menstrual cramps. Their use is mainly restricted by their well known and serious adverse gastrointestinal side effects (1-5) such as gastroduodenal erosions and ulcerations. NSAID-induced gastropathy are estimated to affect up to half of chronic NSAID users, with major world health implications (6). Therefore, search for better and safer anti-inflammatory agents is always going on at a rapid pace.

Majority of potent and therapeutically useful *non-steroidal antiinflammatory drugs* (NSAIDs) are *aryl- or hetero-aryl acids*. According to hypothetical receptor models for common structural features of marketed NSAIDs (7-9) aryl- or heteroaryl alkanolic ring structure with an acidic (carboxy) group and presence of an additional center for lipophilicity in the form of either alkyl chain or an additional aromatic ring (10) are necessary for the anti-inflammatory effect.

Benzimidazole is one of the most promising heteroaryl moiety that yielded many successful drugs like omeprazole and mebendazole (11). Wide variety of pharmacological activities have been reported by benzimidazole moiety itself (12) and its derivatives (11). Benzimidazole moiety fulfills the minimum structural requirements that are common for anti-inflammatory compounds (7-9) after carboxylic acid substitution at 2 position. The pseudoacidic nature of benzimidazole and its derivatives is reflected in the ability of such compounds to form metallic salts (13). Furthermore, pKa of benzimidazole (5.5) moiety (11) falls within desirable pKa range of 5.3 to 7.9 for acidic NSAIDs (9). Hence, benzimidazole carboxylic acids seem to have good promise towards anti-inflammatory activity. In the past, many benzimidazole derivatives showed potential for anti-inflammatory and analgesic activity in animal models of inflammation and pain (14-22). However, benzimidazole-2-carboxylic acids were not been studied towards anti-inflammatory activity. Therefore, it was thought worthwhile to explore anti-inflammatory potential of carboxylic acids derivatives of benzimidazole moiety by synthesizing some benzimidazole-2-carboxylic acid derivatives and evaluating them for the anti-inflammatory activity using standard animal models of acute inflammation.

Material and Methods

Drugs and chemicals

All the chemicals used for the present study were of Fluka, B.D.H., Merck, or LOBA-Chemie and were of synthetic grade. The melting points of the compounds were determined using melting point apparatus (Toshniwal) by open capillary tubes and are uncorrected. The homogeneity and purity of the compounds were conformed by thin layer chromatography (TLC). . These starting materials (substituted o-phenylenediamines, OPDs) were synthesized in laboratory by well reported procedures (23) except OPD and 4-nitro OPD, which were obtained commercially.

For the pharmacological testing, the suspensions of test compounds were freshly prepared in 50% w/v simple syrup and were administered orally to rats in a volume not more than 0.3 ml./rat unless mentioned otherwise.

Experimental animals

Sprague-Dawley male rats (150-200 g. body weight) bred in the animal house of the department of Pharmaceutical sciences, Nagpur University were used. They were kept in polycarbonate cages in a centrally air-conditioned room at an ambient temperature with 12 hr. light/dark cycle (lights on 6.30 a.m. to 6.30 p.m.) The animals were maintained on commercial palleted food (Gold Mohur, Hindustan lever) and water was given *ad libitum*. All the experiments were conducted between 9.00 a.m. and 3.00 p.m. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Synthesis of benzimidazole-2-acids

The test compounds (3a-1 and 4a-c) were prepared from *o*-phenylenediamine (OPD) or 4-substituted OPD as shown in the Figure 1. Yield (in %) and melting points were presented in Table 1. The compounds were characterized by standard methods using spectroscopic techniques of UV, IR, and ¹H NMR and also by elemental (C, H, N) analysis. The details of the procedures are presented below.

Synthesis of 2-hydroxymethylbenzimidazoles (2a-e) from o-phenylenediamines (2a)

o-phenylenediamine, OPD (1a) or 4-substituted OPD (1b-e) (0.2 mole), glycolic acid (0.3 mole), hydrochloric acid (80 ml., 4 N.), 80 ml. water and activated charcoal (1 g.) were refluxed in all glass standard joint assembly for 45 minutes. The reaction mixture was cooled to room temperature, filtered and neutralized cautiously with aqueous ammonia to yield corresponding 2-hydroxymethylbenzimidazoles (2a-e). The product was recrystallized from 50% ethanol.

Synthesis of benzimidazole-2-carboxylic acids (3a-e) from 2-hydroxymethylbenzimidazoles (2a-e) (24)

Corresponding 2-hydroxymethyl benzimidazoles (2a-e) (0.1 mole) and sodium carbonate (13 g) were dissolved in minimum amount of boiling water separately and then mixed together. The resultant mixture was heated on boiling water bath and saturated solution of 14 g. of potassium permanganate was added in small portions to the warm solution with stirring over a period of one hour. The hot solution was filtered at the pump and precipitate of MnO₂ was washed several times with hot water (6 x 100 mL.). The combined filtrate was cooled and neutralized carefully with dilute acetic acid. The precipitated benzimidazole-2-carboxylic acids were filtered at the pump and dried at 60°C. The product were recrystallized from ethanol.

Synthesis of 2-cyanomethylbenzimidazoles (2f-h) from 4-substituted OPD (1a-e) (24)

In a conical flask, OPD (1a) or 4-substituted OPD (1b-e) (0.3 M.) and ethyl cyanoacetate (0.45 M.) were placed in the reaction tube and heated in oil bath at 185 °C. for 20 minutes. After

cooling the residue was broken up and washed several times with ether. The product was then decolorized using activated charcoal, crystallized in hot water and finally recrystallized with ethanol.

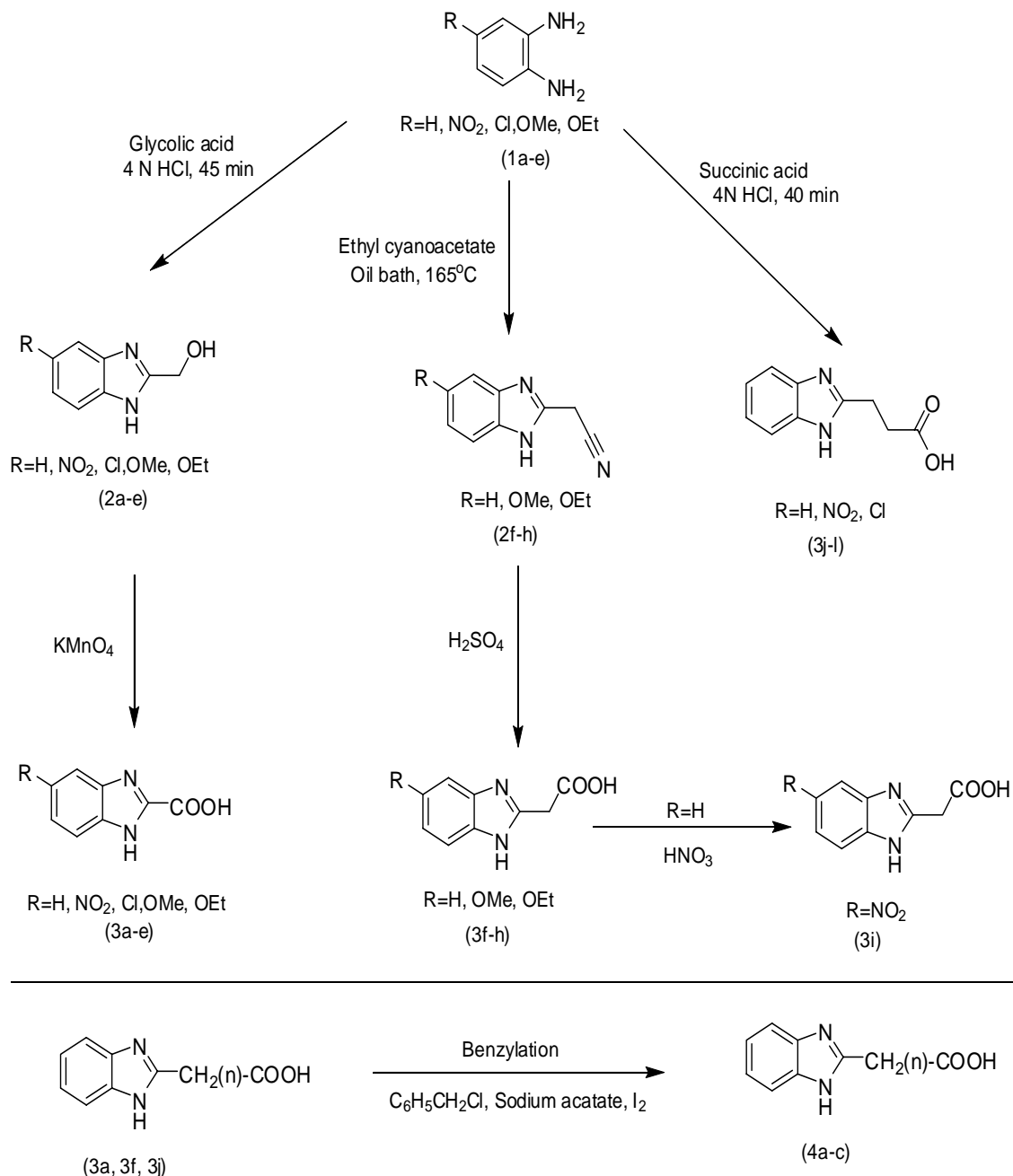
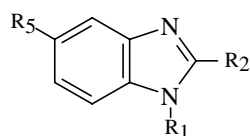


Figure 1: Synthetic scheme for benzimidazole-2-acids (3a-1, 4a-c)

Table 1: Structures, Molecular weights, formulae, % Yield, and melting points of benzimidazole-2-acids



Sr. No.	R-1	R-2	R-5	Mol. Wt.	Mol. Formula	% Yield	Melting point (°C)		Reference
							Obs.	Lit.	
3a	H	COOH	H	162.14	C ₈ H ₆ N ₂ O ₂	75.32	171-72	169-71	(24)
3b	H	COOH	NO ₂	207.14	C ₈ H ₅ N ₃ O ₄	69.73	248-51	> 235	(25)
3c	H	COOH	Cl	196.59	C ₈ H ₅ N ₂ O ₂ Cl	73.18	159-160	160	(44)
3d	H	COOH	OCH ₃	190.17	C ₉ H ₈ N ₂ O ₃	69.65	152-154	-	
3e	H	COOH	OC ₂ H ₅	206.20	C ₁₀ H ₁₀ N ₂ O ₃	66.60	156-158	-	
3f	H	CH ₂ COOH	H	176.17	C ₉ H ₈ N ₂ O ₂	62.45	118-120	116	(24)
3g	H	CH ₂ COOH	OCH ₃	206.20	C ₁₀ H ₁₀ N ₂ O ₃	68.20	> 300	-	
3h	H	CH ₂ COOH	OC ₂ H ₅	220.22	C ₁₁ H ₁₂ N ₂ O ₃	76.01	285	-	
3i	H	CH ₂ COOH	NO ₂	221.17	C ₉ H ₇ N ₃ O ₄	69.40	181-182	-	
3j	H	CH ₂ CH ₂ COOH	H	190.20	C ₁₀ H ₁₀ N ₂ O ₂	33.00	226-228	228	(45)
3k	H	CH ₂ CH ₂ COOH	NO ₂	235.19	C ₁₀ H ₉ N ₃ O ₄	31.71	144-145	-	
3l	H	CH ₂ CH ₂ COOH	Cl	224.64	C ₁₀ H ₉ N ₂ O ₂ Cl	40.06	187-89	190-91	(46)
4a	CH ₂ -Ar	COOH	H	252.27	C ₁₅ H ₁₂ N ₂ O ₂	49.27	196-98	-	
4b	CH ₂ -Ar	CH ₂ COOH	H	266.29	C ₁₆ H ₁₄ N ₂ O ₂	46.31	240-41	-	
4c	CH ₂ -Ar	CH ₂ CH ₂ COOH	H	280.32	C ₁₇ H ₁₆ N ₂ O ₂	35.05	200-201	-	

Synthesis of benzimidazole-2-acetic acids (3f-h) from 2-cyanomethylbenzimidazoles (2f-h) (24)

2-Cyanomethylbenzimidazole (0.128 M.) was refluxed with 1:1 sulphuric acid (130 ml.) for 2 hours. On cooling, colourless needles of sulphates were separated. These separated crystals were collected on a buckner funnel and dissolved in minimum quantity of water. This solution was made alkaline with aqueous ammonia and filtered. Filtrate was made just acidic to litmus with dilute acetic acid. Small needles of benzimidazole-2-acetic acids (3f-h) were separated. These needles were filtered and crystallized with ethanol.

Synthesis of 2-(5-nitro-1H-benzimidazol-2-yl)acetic acid (3i) (25)

Attempts to synthesize title compound (3i) from the substituted 2-cyanomethyl benzimidazole by using above procedure was failed. Therefore, the title compound (3i) was prepared by direct nitration procedure adopted (25). Benzimidazole-2-acetic acid (3f) (0.03 M.) was added portionwise to 12 ml. concentrated sulphuric acid. The mixture of 2.4 ml. concentrated nitric acid and 3.6 ml. concentrated sulphuric acid was prepared, cooled and gradually added to reaction mixture with constant mechanical stirring for 30 minutes. The reaction mixture is then poured on crushed ice and allowed to stand for 20 minutes. Then 7.2 ml. of 2:1 nitric acid was added with stirring. The reaction mixture was carefully neutralized with liquid ammonia. The precipitated product (3i) was filtered, washed with water and crystallized from ethanol.

Synthesis of benzimidazole-2-propionic acids (3j-l) (26)

A mixture of OPD (1a) or 4-substituted OPD (1b-e) (0.2 M.), succinic acid (0.25 M.) and 4 N. hydrochloric acid (180 ml.) was refluxed for 40 minutes, allowed to cool and precipitated solid was separated. The filtrate was basified with liquid ammonia and small amount of amorphous precipitate were filtered. The resultant filtrate on careful neutralization with dilute acetic acid gave corresponding benzimidazole-2-propionic acid (3j-l) which were crystallized from water. However, methoxy and ethoxy substituted benzimidazole-2-propionic acids could not be prepared by above procedure or its modification.

Synthesis of 1-benzyl-benzimidazole acids (4a-c)

Benzimidazole-2-carboxylic (3a) or -acetic (3f) or -propionic (3j) acid along with freshly fused sodium acetate (1.5 g.) and freshly distilled benzyl chloride (6 ml.) were refluxed with speak of iodine crystals for 20 minutes. The reaction mixture were then poured to excess of water with stirring. Excess of water were decanted and solid mass left were triturated with small quantity of ethanol. Products (4a-c) were crystallized from ethanol-water mixture

Acute oral Toxicity testing and gross behavioral studies

The different doses of various test compounds were administered orally to different groups of eight rats each as per OECD (27) guideline No. 425. The animals were observed for gross changes in behavior at every hour for 4 hr. and mortality if any after 48 hr. Vehicle treated control group was also maintained simultaneously.

Anti-inflammatory activity of test compounds

The tests compounds were evaluated for their acute anti-inflammatory activity against carrageenan induced rat paw edema (28). Male albino rats were fasted overnight and divided in different groups of five animals in each. They were treated orally either with test compounds (25, 50, 100, 200 and 400 mg/kg), standard anti-inflammatory drug, indomethacin (5 mg/kg) or vehicle (1 ml/kg); one hour before the subplanter injection of 0.05 ml. of 1 % w/v carrageenan (Sigma-Aldrich, USA). Paw volumes were measured using plethysmometer (Ugo Basile, Italy) immediately (measured within 30 sec. and referred as initial paw volume) and 3 hours (final volume) after carrageenan injection. The difference between these two observations gave the amount of edema developed. The percent inhibition of edema for the treated groups was calculated by following formula

$$\% \text{ Inhibition} = 100 \times \left[1 - \frac{V_t}{V_c} \right]$$

Where V_t and V_c are the mean changes of paw volume in the treated and control group respectively.

Statistical analysis

The results were expressed as mean changes of paw volume (mL.) \pm SEM and as percent inhibition of edema (Table 3). The data was analyzed by two-way Analysis of Variance (ANOVA) followed by unpaired “t” test for statistical significance. Separate graphs of Log (dose) v/s probit (% Inhibition of edema) were plotted for each treatment and relative potencies in terms of ED₅₀ (mg and Molar conc.) was calculated by regression analysis. (29) and presented as Figure 2.

Results

The test compounds were synthesized in good percentage of yield and characterized by standard methods (Table 1 and Table 2). In the acute oral toxicity study, no mortality was noticed within 48 hours by any of the compounds upto 2000 mg/kg, p.o. dose level in the present study. However, some behavioral changes were noticed depending at the higher dose (400 mg/kg or more). In general, all compounds at dose of 400 mg/kg and above, decreased alertness, grooming and motor activity, grip strength, pinna and corneal reflexes. Doses lower than 400 mg/kg did not exhibit such effects. A marked inhibition of response to touch and pain was noticed by all the doses higher than 100 mg/kg.

Table 2: Characterization of synthesized benzimidazole-2-acids

Sr. No.	Elemental analysis % Found (% Calculated)			UV (λ_{max} , nm.)	R_f^*	IR. (KBr) cm^{-1} ,	1H NMR (DMSO- d_6 , 300MHz) \pm , J(Hz);
	C	H	N				
3a	59.48 (59.26)	3.42 (3.7)	17.71 (17.27)	214.5	0.32	3200-2400 (aryl C-H stretch,, > NH stretch), 1650 (>C=O stretch)	5.0 (1H, -NH), 12.74 (1H, -COOH), 7.22, 7.59 (2H, Ar)
3b	45.78 (46.38)	2.27 (2.42)	20.47 (20.27)	225.7	0.28	3100-2100 (aryl C-H stretch,, > NH stretch), 1514 (aryl C-NO ₂), 1650 (> C=O stretch), 1350 (aryl C-NO ₂)	5.0 (1H, -NH); 12.74 (1H, -COOH); 7.66, 8.10 (2H, Ar-NO ₂); 8.39 (1H, Ar)
3c	48.53 (48.87)	3.02 (2.54)	14.62 (14.24)	217.8	0.34	3400-2200 (aryl C-H stretch,, > NH stretch), 1480 (2-substituted benzimidazole,) 1650 (> C=O stretch), 800 (chloro -C-Cl)	5.0 (1H, -NH); 12.74 (1H, -COOH); 7.14, 7.53 (2H, Ar-Cl); 8.36 (1H, Ar)
3d	57.1 (56.84)	4.86 (4.21)	14.78 (14.72)	217.5	0.35	3100-2800 (aryl C-H stretch,, > NH stretch), 1550 (2-substituted benzimidazole), 1700 (> C=O stretch), 1250 1150, and 2850 (-O-CH ₃ of methoxy)	5.0 (1H, -NH); 12.74 (1H, -COOH); 6.93, 7.48 (2H, Ar); 3.83 (1H, Ar-OCH ₃)
3e	58.84 (58.22)	4.24 (4.86)	13.13 (13.48)	285.5	0.34	3400-2200 (aryl C-H stretch,, > NH stretch), 1650 (> C=O stretch), 1225, 1150 (-O-CH ₃ of ethoxy)	5.0 (1H, -NH); 12.74 (1H, -COOH); 6.93, 7.48 (2H, Ar), 7.14 (1H, Ar); 4.09 (4H, Ar-O-CH ₂), 1.32 (2H, CH ₃ of ethoxy)
3f	61.12 (61.36)	4.67 (4.54)	15.56 (15.89)	266.0	0.38	3100-2200 (aryl C-H stretch,, > NH stretch), 1644 (>C=O stretch)	12.18 (1H, -NH); 12.34 (1H, -COOH); 7.22 (m, Ar), 7.59 (2H, Ar); 3.49 (1H, -COOH)
3g	58.6 (58.24)	4.48 (4.85)	13.75 (13.58)	220.8	0.41	3100-2800 (aryl C-H stretch,, > NH stretch), 1525 (2-substituted benzimidazole), 1650 (> C=O stretch), 1250 1150, and 2850 (-O-CH ₃ of methoxy)	12.18 (1H, -NH); 12.34 (1H, -COOH); 7.28, 6.93 (2H, Ar), 7.14 (1H, Ar); 3.83 (1H, Ar-OCH ₃), 3.49 (1H, -CH ₂)
3h	60.42 (59.99)	5.8 (5.45)	12.62 (12.71)	301.5	0.43	3200-2600 (aryl C-H stretch,, > NH stretch), 1650 (> C=O stretch), 1225, 1150 (-O-CH ₃ of ethoxy)	12.18 (1H, -NH); 12.34 (1H, -COOH); 6.93, 7.48 (2H, Ar), 7.14 (1H, Ar); 4.09 (4H, -OCH ₃), 3.49 (1H, -CH ₂), 1.32 (2H, CH ₃ of ethoxy)
3i	49.14 (48.87)	3.55 (3.16)	18.74 (18.99)	211.2	0.34	3700-2500 (aryl C-H stretch,, > NH stretch), 1550 (2aryl C-NO ₂), 1650 (> C=O stretch), 1345 (aryl C-NO ₂)	12.18 (1H, -NH); 12.34 (1H, -COOH); 8.10, 7.66 (2H, Ar), ; 8.39 (1H, Ar), 3.49 (1H, -CH ₂)
3j	63.65 (63.14)	5.83 (5.25)	14.60 (14.72)	214.5	0.40	3100-2000 ((aryl C-H stretch,, > NH stretch), 1406 (>C=O stretch displaced)	12.18 (1H, -NH, 1H, -COOH); 7.22 (m, Ar), 7.59 (2H, Ar); 2.82 (3H, Ar-CH ₂), 2.51 (3H, Ar-CH ₂ -CH ₂)

Sr. No.	Elemental analysis % Found (% Calculated)			UV (λ_{max} , nm.)	R_f^*	IR. (KBr) cm^{-1} ,	1H NMR (DMSO- d_6 , 300MHz) \pm , J(Hz);
3k	50.92 (51.07)	4.14 (3.83)	17.38 (17.86)	314.2	0.41	3200-2600 (aryl C-H stretch, > NH stretch), 1550 (2-substituted benzimidazole, aryl C-NO ₂), 1400 (> C=O stretch, displaced), 1350 (aryl C-NO ₂)	12.18 (1H, -NH, 1H, -COOH); 8.39 (1H, Ar), 8.10, 7.66 (2H, Ar); 2.82 (3H, Ar-CH ₂), 2.51 (3H, Ar-CH ₂ -CH ₂)
3l	53.89 (53.46)	4.41 (4.01)	12.34 (12.46)	214.5	0.42	3200-2200 (aryl C-H stretch, > NH stretch), 1400 (> C=O stretch, displaced), 800 (chloro -C-Cl)	12.18 (1H, -NH, 1H, -COOH); 8.36 (1H, Ar), 7.14, 7.56 (1H, Ar); 2.82 (3H, Ar-CH ₂), 2.51 (3H, Ar-CH ₂ -CH ₂)
4a	74.3 (74.41)	4.47 (4.76)	11.72 (11.1)	225.7	0.65	3100-2800 (aryl C-H stretch), 1550 (2-substituted benzimidazole,) 1650 (> C=O stretch), 1339 (> N-C of 1-benzyl), 3350 (-O-H stretch)	12.74 (1H, -COOH); 7.22 7.59 (m, Ar); 7.23, 7.26, 7.33 (m, Ar-CH ₂); 5.46 (1H, CH ₂ of 1-benzyl)
4b	71.97 (72.16)	5.01 (5.26)	10.7 (10.51)	217.5	0.69	3200-2800 (aryl C-H stretch), 1650 (> C=O stretch), 1372 (> N-C of 1-benzyl), 3450 (-O-H stretch)	12.74 (1H, -COOH); 7.22 7.59 (m, Ar); 7.23, 7.26, 7.33 (m, Ar-CH ₂); 5.49 (1H, CH ₂ of 1-benzyl), 3.79 (1H, Ar-CH ₂ of acid)
4c	73.11 (72.83)	5.93 (5.71)	9.89 (9.93)	214.5	0.73	3100-2800 (aryl C-H stretch), 1410 (> C=O stretch, displaced), 1350 (> N-C of 1-benzyl), 3400 (-O-H stretch)	12.74 (1H, -COOH); 7.22 7.59 (m, Ar); 7.23, 7.26, 7.33 (m, Ar-CH ₂); 5.49 (1H, CH ₂ of 1-benzyl), 3.49 (1H, Ar-CH ₂ of prop. acid)
* Solvent system for TLC studies was chloroform: methanol (8:2).							

Carrageenan induced rat paw edema model was used as initial screen. The results of present study indicated that all the compounds possess remarkable anti-inflammatory activity but they were less potent than standard drug, indomethacin (observed ED₅₀ = 3 mg/kg). The order of potency as assessed from their oral ED₅₀ values was found to be 3c > 4b > 4a > 3f > 3a > 4c > 3d > 3l > 3g > 3h > 3j > 3e > 3b > 3i > 3k. However, 1-benzyl derivatives (4a, 4b, 4c) were more active than corresponding compounds with no substitution at position-1 (3a, 3f and 3j). The order of activity with respect to substitution at position 5 (R-5) was in order chloro > methoxy > unsubstituted > ethoxy > nitro (Table 3).

Table 3: Effect of test compounds against carrageenan induced rat paw edema

Treat ment	Mean change in paw volume in ml. \pm S.E.M. (% inhibition) at dose (mg/kg, p.o)				
	25	50	100	200	300
3a	0.84 \pm 0.052 (29.27)	0.71 \pm 0.061 (39.59)	0.52 \pm 0.027 (55.67)	0.45 \pm 0.039 (62.27)	0.40 \pm 0.020 (65.82)
3f	0.67 \pm 0.058 (42.98)	0.68 \pm 0.048 (42.47)	0.58 \pm 0.063 (50.76)	0.50 \pm 0.037 (57.53)	0.43 \pm 0.050 (63.62)
3j	0.80 \pm 0.096 (32.66)	0.69 \pm 0.040 (41.79)	0.66 \pm 0.045 (44.20)	0.63 \pm 0.062 (46.36)	0.58 \pm 0.071 (50.93)
3b	0.88 \pm 0.048 (25.72)	0.83 \pm 0.018 (29.95)	0.77 \pm 0.037 (35.19)	0.68 \pm 0.043 (42.30)	0.64 \pm 0.034 (45.52)
3i	1.06 \pm 0.026 ^{ns} (10.66)	0.97 \pm 0.022 (18.10)	0.84 \pm 0.037 (28.60)	0.82 \pm 0.035 (30.80)	0.78 \pm 0.033 (33.84)
3k	0.99 \pm 0.074 (16.24)	0.92 \pm 0.032 (22.34)	0.86 \pm 0.046 (27.58)	0.80 \pm 0.058 (32.74)	0.59 \pm 0.048 (50.43)
3c	0.70 \pm 0.050 (40.44)	0.49 \pm 0.041 (58.54)	0.41 \pm 0.043 (65.65)	0.36 \pm 0.040 (69.37)	0.27 \pm 0.028 (77.16)
3l	0.79 \pm 0.022 (33.50)	0.71 \pm 0.025 (39.76)	0.62 \pm 0.053 (47.72)	0.47 \pm 0.063 (60.24)	0.42 \pm 0.033 (64.81)
4a	0.76 \pm 0.053 (35.53)	0.58 \pm 0.071 (50.93)	0.56 \pm 0.037 (52.96)	0.46 \pm 0.074 (60.74)	0.38 \pm 0.018 (67.85)
4b	0.65 \pm 0.038 (45.35)	0.60 \pm 0.018 (49.58)	0.56 \pm 0.040 (52.79)	0.50 \pm 0.058 (57.70)	0.44 \pm 0.020 (62.44)
4c	0.70 \pm 0.049 (40.95)	0.65 \pm 0.024 (45.35)	0.60 \pm 0.048 (48.90)	0.54 \pm 0.059 (54.15)	0.48 \pm 0.038 (59.73)
3d	0.79 \pm 0.048 (32.99)	0.68 \pm 0.026 (42.64)	0.63 \pm 0.039 (46.70)	0.50 \pm 0.049 (57.53)	0.41 \pm 0.031 (65.48)
3g	0.97 \pm 0.030 (18.10)	0.87 \pm 0.032 (26.06)	0.72 \pm 0.029 (39.42)	0.53 \pm 0.045 (55.16)	0.43 \pm 0.023 (63.96)
3e	0.99 \pm 0.038 (16.58)	0.91 \pm 0.039 (23.18)	0.78 \pm 0.068 (34.18)	0.76 \pm 0.069 (35.87)	0.50 \pm 0.116 (57.87)
3h	1.05 \pm 0.030 ^{ns} (11.34)	0.76 \pm 0.143 (36.04)	0.69 \pm 0.107 (41.79)	0.62 \pm 0.063 (47.38)	0.57 \pm 0.069 (51.61)

Difference of paw volume of control group was 1.18 ± 0.087 (mL.). Percent inhibition of edema in indomethacin treated group was 30.96, 48.05, 56.18, 62.61 and 77.16 % in the doses of 1, 3, 5, 8 and 10 mg/kg p.o. respectively. Data was analyzed by Two way ANOVA (With followed by unpaired Student 't' test as compared to vehicle control group and significance was calculated. Non-significant values are expressed as ns. All other values are significant at $p < 0.05$ as compared with vehicle control group. Number of animals in each group = 5

Discussion

In this study, we have synthesized 15 benzimidazole-2-carboxylic acids by the scheme depicted in Figure 1. The test compounds were synthesized in good percentage of yield and characterized by standard methods (Table 1 and Table 2). Then, they were tested for the acute anti-inflammatory activity using carrageenan induced rat paw edema model, which is standard screening model for the drugs that might act through prostaglandin synthesis inhibition. (Table 3). All the compounds exhibited good anti-inflammatory activity at 100 mg/kg p.o. or more and their activity largely depends on substituents at position 2, 5 and 1 of benzimidazole moiety.

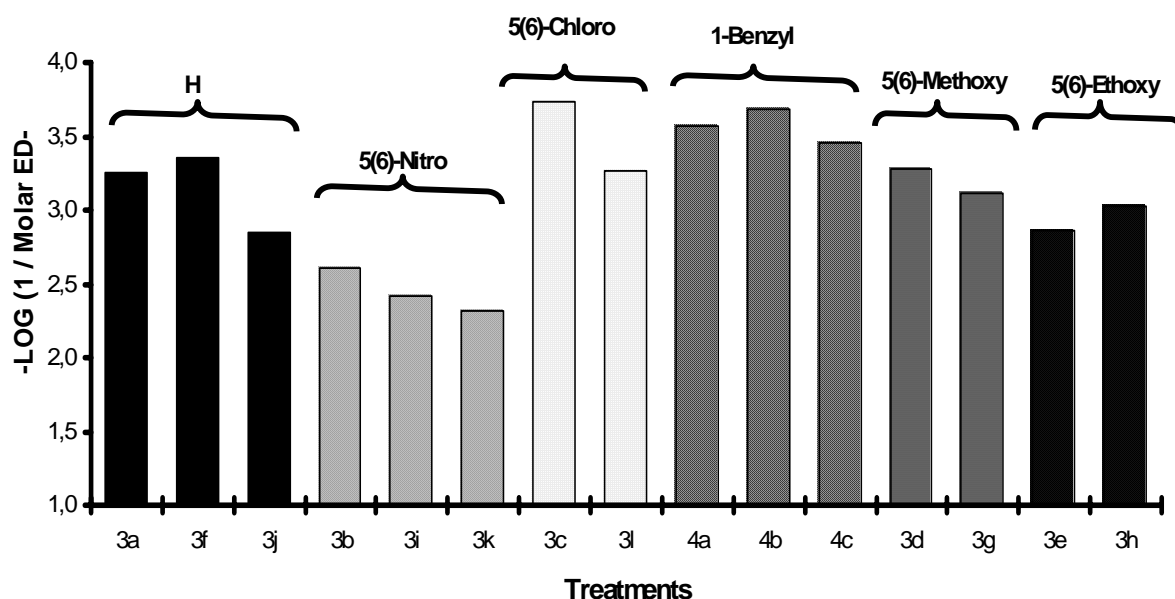


Figure 2: Relative potencies of test compounds in Molar ED₅₀ (C)

The relative anti-inflammatory potencies (as shown in Figure 2) against CPE model be can be explained on the basis of proposed anti-inflammatory receptor models by Nicholson et al (8). The order of activity also depends on the type of substitution at position 5 (R-5). Chloro is being most lipophilic amongst substituents tested, might comply highly electropositive area in hypothetical receptor model of Nicholson et al (8) to maximum extend and so exhibit highest activity. In fact, the compound 3c (5-chloro-1*H*-benzimidazole-2-carboxylic acid) is most potent amongst tested compound (ED₅₀ = 36.48 mg/kg) which is comparable with many marketed drugs. With the increase in length (and bulk) of substituents at 5-position of moiety, it may go beyond hypothetical receptor model and so might have resulted in reduced activity (as in case of ethoxy substituted compounds as compared to other substituents at position 5). However, with the increased separation of moiety and -COOH group (2-carboxylic acids > 2-acetic acids > 2-propionic acids), anti-inflammatory activity was found to decrease. In case of propionic acid derivatives, -COOH group may go beyond the receptor and hence, propionic acids were less potent than their carboxylic acid counterparts. Furthermore, 1-benzyl derivatives of benzimidazole acids were more active than corresponding compounds without any substitution at position-1. The trough in the hypothetical receptor model as suggested by Nicholson et al (8) may be partially occupied by aromatic ring of benzyl group and responsible for this increase in activity.

Our test compounds were effective at third hour of carrageenan edema suggesting inhibition of prostaglandins as their probable mechanism of action. This accelerating phase of carrageenan edema is attributed to release of prostaglandins (7). Our results are in line with reports of mechanism of action of some previously reported anti-inflammatory benzimidazole derivatives (30). In the past, many benzimidazole derivatives showed promise for anti-inflammatory and analgesic activity (14-16). Another benzimidazole compound, 3-(p-chlorophenyl)thiazolo-[3,2-a] benzimidazole-2-acetic acid (Wy-18,251) was showed promise for against arthritis (17) mainly due to its dual effects as immunomodulatory and anti-inflammatory activity (18).

Inhibition of prostaglandin synthesis (31) through inhibition of key enzymes, cyclooxygenase (COX) (32-34) as well as lipo-oxygenase (LO) (35-38) was suggested to be mechanism of many anti-inflammatory drugs after long research. Benzimidazole derivatives were shown to have inhibitory effects on prostaglandin and/or leukotriene biosynthesis (30). Moreover, specific inhibitory effects against cyclooxygenase (COX) (39) were reported for many benzimidazole derivatives. However, many benzimidazole derivatives have demonstrated anti-inflammatory activity that appears to have a mechanism distinct from typical cyclo-oxygenase inhibiting nonsteroidal anti-inflammatory drugs (Lazer et al, 1987). The possibility of mechanism other than COX inhibition is also strengthened by many prior reports on anti-inflammatory benzimidazoles. Some oxoalkanoic acid esters which include some benzimidazole acids esters were found to be inhibitors of rat polymorphonuclear leukocyte 5-lipoxygenase (LO) and leukotriene D₄ (LTD₄) *in vitro* (40). Because of suggested role of LO in the mechanism and safety of anti-inflammatory drugs (41-43), this possibility of mechanism of anti-inflammatory action of our test compounds cannot be ruled out. Yet another possible mechanism of these compounds can be inhibition of leukocyte emigration and exudation of proteins into site of injury. A potent benzimidazole derivative (KB-1043) was shown to produce strong anti-inflammatory effects mainly by such mechanism (15).

Conclusions

In conclusion, benzimidazole-2-carboxylic acid derivatives possess good anti-inflammatory activity against acute inflammation and so can provide new option for the treatment of inflammatory diseases like arthritis and gout. However, they need to be tested for chronic effects as well as for safety profile to explore their therapeutic potential.

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