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Analgesic and Anti-inflammatory Activity of Some Newly Synthesized Novel Pyrazole Derivatives of Benzimidazole

Rashmi Arora, Amandeep Kaur and Naresh Singh Gill

Department of Pharmaceutical Chemistry, Rayat Institute of Pharmacy, S.B.S. Nagar, Railmajra, 144533, Punjab, India

Corresponding Author: Rashmi Arora, Department of Pharmaceutical Chemistry, Rayat Institute of Pharmacy, S.B.S. Nagar, Railmajra, 144533, Punjab, India

ABSTRACT

A novel series of pyrazole derivatives of benzimidazole was synthesized by the reaction between o-phenylenediamine and equimolar quantities of lactic acid. The products obtained were treated with phenylhydrazine in the presence of ethanolic sodium acetate to synthesize various pyrazole derivatives of benzimidazole i.e., 2-[5-(4-chloro-phenyl-2H-pyrazol-3-yl)-1H-benzimidazole (1a), 2-[5-(3-bromo-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole (1b), 4-[5-(1H-benzimidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl]-phenyl}-dimethyl-amine (1c), 2-[5-(3-Chloro-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole (1d) and 3-[5-(1H-benzimidazol-2-yl)-1-phenyl-1h-pyrazol-3-yl]-phenol (1e) having %age yield 55, 62, 80, 58, 66, respectively. The structures of these compounds were established by IR, ¹H-NMR and elemental analysis. The synthesized Compounds was evaluated for analgesic as well as anti-inflammatory activity. The compound 1a-1e showed significant analgesic activity at 7.36±0.05, 7.50±0.08, 8.43±0.12, 7.88±0.02 and 7.64±0.08 at 90 min when compared with standard i.e., 9.45±0.28 at 90 min, respectively. Further the compounds 1a-1e were evaluated for anti-inflammatory activity and showed significant % inhibition of edema i.e., 59.09, 63.63, 62, 60.60 and 57.57% at a dose of 200 mg mL⁻¹ as compared to standard drug i.e., 69.6% at 3 h, respectively. The compound 1b showed significant anti-inflammatory where as compound 1c showed potent analgesic activity and the other compounds showed moderate activity. This comprehensive study summarizes the different pyrazole derivatives of benzimidazole were showed excellent significant analgesic and anti-inflammatory activities.

Key words: Benzimidazole, pyrazole, analgesic activity, anti-inflammatory activity, conventional synthesis

INTRODUCTION

Heterocyclic chemistry is crucial to the recent drug development especially at the pharmacophore optimization phase, but methods for implemented for the synthesis of bioactive molecules has remained same (Rao *et al.*, 2004). Benzimidazole is a versatile lead compound for designing highly therapeutic bioactive agents in pharmaceutical research. The benzimidazole and pyrazole nucleus represent an important class of heterocyclic compounds occurring naturally or synthetically compounds and in medicinal chemistry (Kalirajan *et al.*, 2010). Numerous attempts have been made to develop new structural prototypes to search for more effective analgesic and anti-inflammatory agents. Literature reveals that the benzimidazole play a vital role in pharmaceutical research and their derivatives exhibited a wide variety of pharmacological activities

like antitumor, antiviral, analgesic, antioxidant, antiamebic, antihistaminic, anti-inflammatory (Thakurdesai *et al.*, 2007), antipyretic, anthelmintic (Sreena *et al.*, 2009), antiulcer (Patil *et al.*, 2008), antifungal (Ghorab *et al.*, 2011) and anti hypertensive activities. In addition, it is well documented that the pyrazole nucleus is an important pharmacophore in modern drug development which is not only theoretically important scaffold, but also associated with a wide spectrum of biological activities. In recent years, considerable attention has been made to the pyrazole and their derivatives as antimicrobial (Mohamed *et al.*, 2006), anti-inflammatory (Arunkumar *et al.*, 2009) and analgesic activities (Turan-Zitouni *et al.*, 1999). The effectiveness of the benzimidazole and pyrazole moieties towards various microbes prompted the synthesis of some new benzimidazole derivatives bearing the pyrazole nucleus and the screening of their potential biological activities. In continuation of studies on biologically active benzimidazole derivatives (Ansari and Lal, 2009) the results of the synthesis of some new benzimidazole derivatives having a pyrazole nucleus are reported herein. Based on literature it has been found that pyrazole substituted benzimidazole could have potential pharmacological activity. Based on the literature survey our present objective is to synthesize and characterize pyrazole derivatives of benzimidazole and explore for their analgesic and anti-inflammatory activities.

MATERIALS AND METHODS

Starting materials and reagents used were procured from commercial suppliers. The confirmatory establishment of structure of all the newly synthesized compounds was done by melting point, TLC, IR, ¹H-NMR and Elemental analysis. Purity of the compounds was checked by TLC using benzene and acetone in the ratio of 9:1. TLC was performed on silica gel G plates of SD-Fine and spots were located in iodine chamber. All melting points were recorded on a Buchi melting point apparatus in open capillary tubes. IR spectra were recorded on FT-IR spectrometer (BRUKER) using KBR disc method in the range of 4000-400 cm⁻¹. ¹H-NMR spectra were recorded on a BRUKER AVANCE II 400 NMR spectrometer using DMSO as solvent and tetramethylsilane (TMS) as internal reference standard and the chemical shifts were reported in parts per million downfield from tetramethylsilane (TMS) and its values were expressed in ppm. Physical properties of the synthesized compounds are listed in Table 1 whereas scheme of synthesis is given in Fig. 1.

Synthesis of 1-(1H-benzo [D] imidazol-2-yl) ethanol: O-phenylenediamine 0.1 mol (10 g) was placed in 250 mL of round bottom flask and added lactic acid 0.1 mL (9 mL). The mixture was refluxed in water bath at 100°C for 12 h, cool and add 10% NaOH solution slowly with constant rotation of the flask until the mixture is just alkaline to litmus.

Table 1: Physical properties of newly synthesized compounds 1a-1e

Compound	R ₁	R ₂	Molecular formula	Molecular weight	Yield (%)	MP (°C)
1a	Cl	H	C ₂₂ H ₁₅ N ₄ Cl	370	55	214-218
1b	H	Br	C ₂₂ H ₁₅ N ₄ Br	414	62	209-213
1c	N(CH ₃) ₂	H	C ₂₄ H ₂₁ N ₅	379	80	212-215
1d	H	Cl	C ₂₂ H ₁₅ N ₄ Cl	370	58	201-206
1e	OH	H	C ₂₂ H ₁₆ N ₄ O	362	66	184-187

MP (°C): Melting point

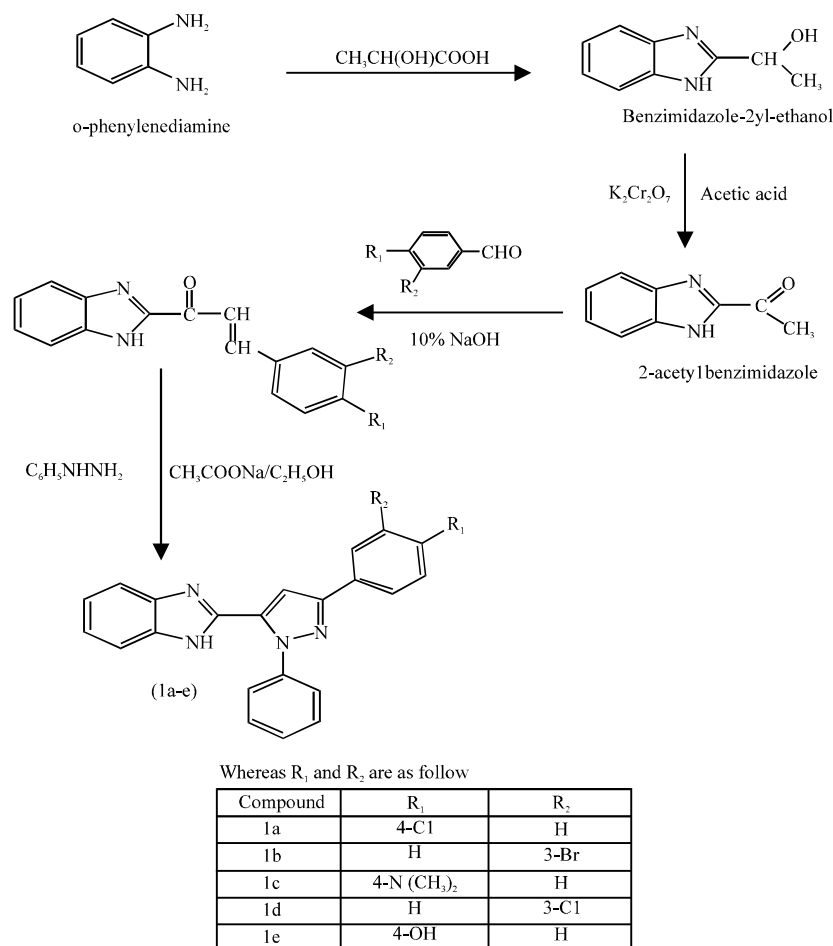


Fig. 1: Scheme of target compounds synthesis

Crude product was filtered off, washed with ice cold water and recrystallised with hot water to obtained light orange color crystals. The solvent system used for TLC was chloroform: ethanol (7:3).

Synthesis of 2-acetylbenzimidazole: To a solution of the 1-(1H-Benzo [D] Imidazol-2-yl) ethanol (10 mmol) added aqueous acetic acid (5% v/v, 10 mL) at room temperature with the solution of potassium dichromate (10 mmol) then, the mixture was stirred for 2 h and the separated product was Filtered off, washed with water and dried. The dried product was recrystallized with ethanol to obtained brown color crystals. The solvent system used for TLC was chloroform: ethanol (9:1).

Synthesis of aromatic aldehydes derivatives of benzimidazole: To a solution of 2-acetylbenzimidazole (10 mmol), aq. NaOH (10 %, 30 mL) was added with respective aldehydes (10 mmol) at room temperature. The reaction mixture was stirred for 4 h and the separated solid was filtered off, washed with water and the crude product was recrystallized from ethanol to obtain brown color crystals. The solvent system used for TLC was benzene: acetone (9:1).

Synthesis of pyrazole derivatives of benzimidazoles : (Compound 1a-1e)

Synthesis of 2-[5-(4-chloro-phenyl)-2H-pyrazol-3-yl]-1H-benzimidazole, 1a: A mixture of 1-(H-benzoimidazol-2-yl)-3-(4-chloro-phenyl)-propenone (0.03 mol), phenylhydrazine (0.03 mol) were refluxed with ethanolic sodium acetate (25 mL) in different conditions for 6 h. Then the mixture was concentrated in water bath and poured into ice cold water. The precipitate obtained was filtered off, washed and recrystallized from ethanol to obtain brown color crystals. The solvent system used for TLC was benzene: acetone (9:1).

Synthesis of 2-[5-(3-bromo-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole, 1b: A mixture of 1-(1H-benzoimidazol-2-yl)-3-(3-bromo-phenyl)-propenone (0.03 mol), phenylhydrazine (0.03 mol) were refluxed with ethanolic sodium acetate (25 mL) for 9 h. Then the mixture was concentrated in water bath and poured into ice cold water, then the precipitate obtained was filtered off, washed and recrystallized from ethanol to obtain color crystals. The solvent system used for TLC was benzene: acetone (9:1).

Synthesis of {4-[5-(1H-benzoimidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl]-phenyl}-dimethyl-amine, 1c: A mixture of 1-(1H-benzoimidazol-2-yl)-3-(4-dimethylamino-phenyl)-propenone (0.03 mol), phenylhydrazine (0.03 mol) were refluxed with ethanolic sodium acetate (25 mL) for 11 h. Then the mixture was concentrated in water bath and poured into ice cold water, then the precipitate obtained was filtered off, washed and recrystallized from ethanol to obtain brown color crystals. The solvent system used for TLC was benzene: acetone (9:1).

Synthesis of 2-[5-(3-chloro-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole, 1d: A mixture of 1-(1H-benzoimidazol-2-yl)-3-(3-chloro-phenyl)-propenone (0.03 mol), phenylhydrazine (0.03 mol) were refluxed with ethanolic sodium acetate (25 mL) different for 6 h. Then the mixture was concentrated in water bath and poured into ice cold water, then the precipitate obtained was filtered off, washed and recrystallized from ethanol to obtain brown color crystals. The solvent system used for TLC was benzene: acetone (9:1).

Synthesis of 3-[5-(1H-benzimidazol-2-yl)-1-phenyl-1h-pyrazol-3-yl]-phenol, 1e: A mixture of 1-(1H-benzoimidazol-2-yl)-3-(4-hydroxy-phenyl)-propenone (0.03 mol), phenylhydrazine (0.03 mol) were refluxed with ethanolic sodium acetate (25 mL) in different conditions for 4 h. Then the mixture was concentrated in water bath and poured into ice cold water and then the precipitate obtained was filtered off, washed and recrystallized from ethanol to obtain brown color crystals. The solvent system used for TLC was benzene: acetone (9:1).

Pharmacology: The animals of either sex were procured from the Punjab agriculture university Ludhiana (India) and were maintained in colony cages at $25\pm 3^{\circ}\text{C}$, relative humidity 45-55%, under a 12 h light and dark cycle; they were fed standard rat pellet diet and water *ad libitum*. All the animals were acclimatized for a period of 2 weeks in our laboratory environment before use. Albino rats (Sprague-Dawley), weighing 150-180 g, were fasted for 12 h and used for determining the anti-inflammatory activity. Albino mice (Sprague-Dawley), weighing 20-25 g, were fasted for 12-24 h and used for determining the analgesic activity.

Analgesic activity

Eddy hot plate method: The method of Eddy and Leimbach (1953) was adopted for the study of newly synthesized pyrazole derivatives of benzimidazole. The pain threshold of the animals was measured on a hot plate before treatment of the test and reference compounds and the animals that showed more than 10 sec of reaction time were not considered (Bhaskar and Mohite, 2010). Albino mice were divided into 7 groups of six mice each. One group served as control and was administered 0.5 mL distilled water. Diclofenac sodium was given p.o. at a dose of 5 mg kg⁻¹ to other group as a reference drug for comparison. Gum acacia suspensions (1% v/v) of test compounds (1a-1e) was administered p.o. at a dose of 100 and 200 mg kg⁻¹ to the remaining groups, respectively, 30 min before the evaluation of analgesic activity by hot plate method:

- **Group 1: Control:** -0.5 mL of distilled water
- **Group 2: Standard:** -Diclofenac sodium (5 mg kg⁻¹, p.o.)
- **Group 3: Test:** -Suspension of test compounds 1a to 1e, respectively (100 mg kg⁻¹) in 1% Gum acacia (p.o.)
- **Group 4: Test:** -Suspension of test compounds 1a to 1e, respectively (200 mg kg⁻¹) in 1% Gum acacia (p.o.)

The reaction time (sec) was noted for all the groups on Eddy's hot plates at 30, 60 and 90 min (Anandarajangopal *et al.*, 2010). After the treatment of test and reference compounds, the pain threshold results of the animals are presented in Table 2.

Anti-inflammatory activity

Carrageenan-induced rat paw edema: The anti-inflammatory activity of newly synthesized Pyrazole derivatives of benzimidazole were carried out using Carrageenan induced rat hind paw edema method (Dubois *et al.*, 2004):

Method : Inhibition of Carrageenan induced rat paw edema
Animals used : Albino Rats
No. of animals used : 6 in each group
Dose of compound : 100 mg, 200 mg kg⁻¹
Dose of standard drug : 10 mg kg⁻¹ (Diclofenac sodium)

Table 2: Spectral data of newly synthesized compounds 1a-1e

Compound	IR (KBr cm ⁻¹)	¹ H-NMR (DMSO)	Elemental analysis (%)
1a	3363 (N-NH), 1604 (C=N), 1500 (C=C), 906 (CH=CH), 759 (Ar C-H)	5.0 (s, NH), 7.22-7.62 (m, Ar C-H), 8.05 (m, C-H)	C: 71.25, H: 4.06, N: 15.13, Cl: 9.57
1b	3336 (N-NH), 1600 (C=N), 1504 (C=C), 900 (CH=CH), 754 (Ar C-H)	5.0 (s, NH), 7.21-7.64 (m, Ar C-H), 8.05 (C-H)	C: 71.27, H: 3.96, N: 18.12
1c	3368 (N-NH), 1608 (C=N), 1478 (C=C), 795 (CH=CH), 759 (Ar C-H)	5.0 (s, NH), 7.21-7.63 (m, Ar C-H)	C: 70.24, H: 4.01, N: 16.13
1d	3363 (N-NH), 1608 (C=N), 1506 (C=C), 840 (CH=CH), 756 (Ar C-H)	5.3 (s, NH), 7.23-7.65 (m, Ar C-H)	C: 71.25, H: 4.06, N: 15.13, Cl: 9.57
1e	3386 (N-NH), 1600 (C=N), 1478 (C=C), 865 (CH=CH), 758 (Ar C-H)	5.2 (s, NH), 7.22-7.63 (m, Ar C-H), 5.0 (OH)	C: 69.27, H: 4.96, N: 18.20, O: 8.57

IR (KBr cm⁻¹): Infrared potassium bromide, ¹H-NMR (DMSO): Nuclear magnetic resonance (dimethyl sulfoxide)

Route of administration

Oral (suspended in 1% gum acacia solution): Rats were assigned into 7 groups of 6 animals each. Rats were selected by random sampling technique. They were marked with picric acid for individual animal identification and the animals were fasted overnight (allowed free access to water *ad libitum*). Diclofenac sodium (10 mg kg⁻¹) was administered as a reference drug and the newly synthesized compounds were administered at dose of 100 and 200 mg kg⁻¹ p.o. once before 30 min the injection of Carrageenan. After 30 min of test compound administration, 0.1 mL of 1%w/v of Carrageenan in normal saline was injected by subplantar injection into the plantar tissue of one hind paw.

Immediately after the Carrageenan injection, the volume of its displacement was measured using plethysmometer. The reading was recorded at 60, 120 and 180 min, then the %age inhibition of edema was calculated at the end by using the formula (Soni *et al.*, 2011).

$$\text{Inhibition of drug (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

where, V_c is the inflammatory increase in paw volume of control group of animals and V_t is the inflammatory increase in paw volume of drug-treated animals. After the treatment of test and reference compounds, the %age inhibition of paw edema is presented in Table 3.

Statistical analysis: Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The $p < 0.05$ was considered statistically significant.

RESULTS

The synthesis of the Pyrazole derivatives of benzimidazole (1a, 1b, 1c, 1d, 1e) were carried out according to the Scheme (Fig. 1). Characterization data of the synthesized compounds were shown in Table 2 and physical properties of compounds were shown in Table 1. Biological activities like analgesic as well as anti-inflammatory activity was performed on the newly synthesized compounds and their result (analgesic) were shown in Table 3 and anti-inflammatory was shown in Table 4.

Table 3: Analgesic activity by Eddy's hot plate method

Groups	Treatment	Dose (mg kg ⁻¹)	Mean reaction time (sec)			
			0 min	30 min	60 min	90 min
I	Control	0.5 mL gum acacia	3.300±0.08	3.323±0.190	3.403±0.062	3.397±0.07
II	Diclofenac sodium	5	3.30±0.17 ^a	5.77±0.069 ^a	7.45±0.12 ^a	9.45±0.28 ^a
III	1a	100	3.30±0.08 ^{ab}	4.71±0.01 ^{ab}	6.39±0.05 ^{ab}	7.38±0.12 ^{ab}
		200	3.30±0.08 ^{ab}	4.79±0.01 ^{ab}	6.68±0.02 ^{ab}	7.36±0.05 ^{ab}
IV	1b	100	3.30±0.08 ^{ab}	4.86±0.01 ^{ab}	6.75±0.02 ^{ab}	7.54±0.06 ^{ab}
		200	3.30±0.08 ^{ab}	4.96±0.01 ^{ab}	6.84±0.02 ^{ab}	7.50±0.08 ^{ab}
V	1c	100	3.30±0.08 ^{ab}	4.82±0.01 ^{ab}	7.79±0.01 ^{ab}	8.40±0.12 ^{ab}
		200	3.30±0.08 ^{ab}	4.88±0.01 ^{ab}	7.93±0.01 ^{ab}	8.43±0.12 ^{ab}
VI	1d	100	3.30±0.08 ^{ab}	4.78±0.02 ^{ab}	6.86±0.05 ^{ab}	7.84±0.03 ^{ab}
		200	3.30±0.08 ^{ab}	4.86±0.08 ^{ab}	6.87±0.03 ^{ab}	7.88±0.02 ^{ab}
VII	1e	100	3.30±0.08 ^{ab}	4.75±0.01 ^{ab}	6.86±0.05 ^{ab}	7.41±0.06 ^{ab}
		200	3.30±0.08 ^{ab}	4.92±0.01 ^{ab}	6.93±0.04 ^{ab}	7.64±0.08 ^{ab}

Values are Mean±SEM of 6 animals in each group, ^a $p < 0.05$ vs. control, ^b $p < 0.05$ vs. diclofenac sodium

Table 4: Anti-inflammatory activity by carrageenan induced rat paw edema method

Groups	Treatment	Dose (mg kg ⁻¹) orally	Mean paw volume (mL)			Inhibition of edema (%)
			60 min	120 min	180 min	
I	Control	1 % CMC	0.52±0.06	0.62±0.05	0.66±0.008	----
II	Diclofenac sodium	10	0.37±0.03 ^a	0.33±0.12 ^a	0.20±0.008 ^a	69.6
III	1a	100	0.59±0.01 ^{ab}	0.43±0.17 ^{ab}	0.31±0.08 ^{ab}	53.03
		200	0.60±0.02 ^{ab}	0.48±0.17 ^{ab}	0.25±0.01 ^{ab}	59.09
IV	1b	100	0.47±0.08 ^{ab}	0.44±0.11 ^{ab}	0.33±0.02 ^{ab}	50.00
		200	0.42±0.17 ^{ab}	0.43±0.17 ^{ab}	0.24±0.00 ^{ab}	63.63
V	1c	100	0.60±0.17 ^{ab}	0.41±0.17 ^{ab}	0.32±0.01 ^{ab}	51.51
		200	0.61±0.02 ^{ab}	0.51±0.15 ^{ab}	0.23±0.01 ^{ab}	62.00
VI	1d	100	0.45±0.02 ^{ab}	0.53±0.03 ^{ab}	0.30±0.01 ^{ab}	54.54
		200	0.46±0.00 ^{ab}	0.50±0.01 ^{ab}	0.26±0.01 ^{ab}	60.60
VII	1e	100	0.43±0.01 ^{ab}	0.56±0.08 ^{ab}	0.29±0.01 ^{ab}	56.06
		200	0.59±0.00 ^{ab}	0.48±0.08 ^{ab}	0.28±0.01 ^{ab}	57.57

CMC: Carboxymethyl cellulose, Values are Mean±SEM of 6 animals in each group, ^ap<0.05 vs. control, ^bp<0.05 vs. diclofenac sodium

Spectral data of compound 2-[5-(4-chloro-phenyl-2H-pyrazol-3-yl)]-1H-benzimidazole, 1a:

The derivative 2-[5-(4-chloro-phenyl-2H-pyrazol-3-yl)]-1H-benzimidazole were synthesized by the reaction of ortho phenylene diamine with lactic acid which resulted in the formation of benzimidazole-2-yl-ethanol which on acetylation gives 2-acetylbenzimidazole, further on cyclisation with 4-chlorobenzaldehyde and phenylhydrazine resulted in the formation of the compound (1a) which was characterized by IR, ¹H-NMR and Elemental analysis as:

- Brown colour crystals, Mol. formula. C₂₂H₁₅N₄Cl, solvent used for TLC was benzene: acetone (9:1), m.p. 214-218°C, yield (%): 55
- IR (KBr cm⁻¹) 3363 (N-NH), 1604 (C=N), 1500 (C=C), 906 (CH=CH), 759 (Ar C-H)
- ¹H-NMR (DMSO-d₆) 5.0 (s, NH), 7.22-7.62 (m, Ar C-H), 8.05 (m, C-H)

Elemental analysis: C, 71.26; H, 4.05; N, 15.12; Cl, 9.56; Reported: C, 71.25; H, 4.06; N, 15.13; Cl, 9.57%.

The compound was evaluated for analgesic as well as anti-inflammatory activities: The analgesic activity of the synthesized compounds was evaluated in albino mice by eddy's hot plate method. The compound 1a showed mild activity at a dose of 100 and 200 mg kg⁻¹ in the range of 7.38±0.12 and 7.36±0.05 at 90 min when compared with standard drug Diclofenac sodium at a dose of 5 mg kg⁻¹ in the range of 9.45±0.28 at 90 min. The compound showed slightly better activity at dose of 100 mg kg⁻¹ in the range of 7.38±0.12 than 200 mg kg⁻¹ (Table 3).

Further the compound was evaluated for anti-inflammatory activity in albino rats by carrageenan induced rat paw edema method. The compound 1a showed slightly less moderate activity in the range of 53.03 and 59.09% at a dose of 100 and 200 mg kg⁻¹ at time interval of 3 h of carrageenan challenge when compared to compound 1c (63.63%) as well as when compared with standard Diclofenac sodium at the same time period exhibited 69% of activity, respectively at a dose of 10 mg kg⁻¹. The compound 1a showed moderate activity at dose of 200 mg kg⁻¹ in the range of 59.09% than 100 mg kg⁻¹ (Table 4).

2-[5-(3-bromo-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole, 1b: The derivative 2-[5-(3-bromo-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole were synthesized by the reaction of ortho phenylene diamine with lactic acid which resulted in the formation of benzimidazole-2-yl-ethanol which on acetylation gives 2-acetylbenzimidazole, further on cyclisation with 3-Bromobenzaldehyde and phenylhydrazine resulted in the formation of the compound (1b) which was characterized by IR, ¹H-NMR and Elemental analysis as:

- Brown color crystals, Mol. formula. C₂₂H₁₆N₄Br, solvent used for TLC was benzene: acetone (9:1), m.p. 209-213°C, yield (%): 62
- IR (KBr cm⁻¹) 3336 (N-NH), 1600 (C=N), 1504 (C=C), 900 (CH=CH), 754 (Ar C-H)
- ¹H-NMR (DMSO-d₆) 5.0 (s, NH), 7.21-7.64 (m, Ar C-H), 8.05 (C-H)

Elemental analysis: C: 71.28, H: 3.98, N: 18.11, Reported; C: 71.27, H: 3.96, N: 18.12%. The compound was evaluated for analgesic as well as anti-inflammatory activities.

The analgesic activity of the synthesized compounds was evaluated in albino mice by Eddy's hot plate method. The compound 1b showed moderate activity at a dose of 100 and 200 mg kg⁻¹ in the range of 7.54±0.06 and 7.50±0.06 at 90 min when compared with standard drug Diclofenac sodium at a dose of 5 mg kg⁻¹ in the range of 9.45±0.28 at 90 min. The compound 1b showed slightly better activity at dose of 100 mg kg⁻¹ in the range of 7.54±0.06 than 200 mg kg⁻¹ (Table 3).

Further the compound was evaluated for anti-inflammatory activity in albino rats by carrageenan induced rat paw edema method. The compound 1b showed potent activity in the range of 63.63 and 50% at a dose of 200 and 100 mg kg⁻¹ at time interval of 3 h of carrageenan challenge, when compared with standard Diclofenac sodium at the same time period exhibited 69% of activity respectively at a dose of 10 mg kg⁻¹. The compound 1b showed highly potent activity at dose of 200 mg kg⁻¹ in the range of 63.63% than 100 mg kg⁻¹ (Table 4).

{4-[5-(1H-benzoimidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl]-phenyl}-dimethyl-amine, 1c: The derivative {4-[5-(1H-benzoimidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl]-phenyl}-dimethyl-amine were synthesized by the reaction of ortho phenylene diamine with lactic acid which resulted in the formation of benzimidazole-2-yl-ethanol which on acetylation gives 2-acetylbenzimidazole, further on cyclisation with p-dimethylaminobenzaldehyde and phenylhydrazine resulted in the formation of the compound (1c) formed was characterized by IR, ¹H-NMR and Elemental analysis as:

- Brown color crystals, Mol. Formula. C₂₄H₂₁N₅, solvent used for TLC was benzene: acetone (9:1), m.p. 212-218°C, yield (%): 80
- IR (KBr cm⁻¹) 3368 (N-NH), 1608 (C=N), 1478 (C=C), 795 (CH=CH), 759 (Ar C-H)
- ¹H-NMR (DMSO-d₆) 5.0 (s, NH), 7.21-7.63 (m, Ar C-H)

Elemental analysis: C: 70.26, H: 3.99, N: 16.18, Reported; C: 70.24, H: 4.01, N: 16.13%. The compound was evaluated for analgesic as well as anti-inflammatory activities.

The analgesic activity of the synthesized compounds was evaluated in albino mice by eddy's hot plate method. The compound 1c showed more significant analgesic activity at a dose of 100 and

200 mg kg⁻¹ in the range of 8.40±0.12 and 8.43±0.12 at 90 min when compared with standard drug Diclofenac sodium at a dose of 5 mg kg⁻¹ in the range of 9.45±0.28 at 90 min. The compound 1c showed highly significant activity at dose of 200 mg kg⁻¹ in the range of 8.43±0.12 than 100 mg kg⁻¹ (Table 3).

Further the compound was evaluated for anti-inflammatory activity in albino rats by carrageenan induced rat paw edema method. The compound 1c showed slightly less potent activity in the range of 62 and 51.51% at a dose of 200 and 100 mg kg⁻¹ at time interval of 3 h of carrageenan challenge, when compared with compound 1b as well as when compared with standard Diclofenac sodium at the same time period exhibited 69% of activity respectively at a dose of 10 mg kg⁻¹. The compound 1c showed potent activity at dose of 200 mg kg⁻¹ in the range of 62%^{ab} than 100 mg kg⁻¹ (Table 4).

2-[5-(3-chloro-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole, 1d: The derivative 2-[5-(3-chloro-phenyl)-2-phenyl-2H-pyrazol-3-yl]-1H-benzimidazole, were synthesized by the reaction of ortho phenylene diamine with lactic acid which resulted in the formation of benzimidazole-2-yl-ethanol which on acetylation gives 2-acetylbenzimidazole, further on cyclisation with 3-chlorobenzaldehyde and phenylhydrazine resulted in the formation of the compound (1d) which was characterized by IR, ¹H-NMR and Elemental analysis as:

- Brown color crystals, Mol. Formula. C₂₂H₁₅N₄Cl, solvent used for TLC was benzene: acetone (9:1), m.p. 201-206°C, %age yield (%): 58
- IR (KBR cm⁻¹) 3363 (N-NH), 1608(C=N), 1506 (C=C), 840 (CH=CH), 756 (Ar C-H)
- ¹H-NMR (DMSO-d₆) 5.3 (s, NH), 7.23-7.65 (m, Ar C-H)

Elemental analysis: C: 71.26, H: 4.05, N: 15.12, Cl: 9.56, Reported; C: 71.25, H: 4.06, N: 15.13, Cl: 9.57%. The compound was evaluated for analgesic as well as anti-inflammatory activities.

The analgesic activity of the synthesized compounds was evaluated in albino mice by Eddy's hot plate method. The compound 1d showed slightly less significant activity at a dose of 100 mg kg⁻¹ and 200 mg kg⁻¹ in the range of 7.84±0.02 and 7.88±0.02 at 90 min when compared with compound 1a as well as with standard drug Diclofenac sodium at a dose of 5 mg kg⁻¹ in the range of 9.45±0.28 at 90 min. The compound 1d showed slightly more significant activity at dose of 200 mg kg⁻¹ in the range of 7.88±0.02 than 100 mg kg⁻¹ (Table 3).

Further the compound was evaluated for anti-inflammatory activity in albino rats by carrageenan induced rat paw edema method. The compound 1d showed moderate activity in the range of 60.60 and 54.54% at a dose of 200 and 100 mg kg⁻¹ at time interval of 3 h of carrageenan challenge, when compared with compound 1b as well as when compared with standard Diclofenac sodium at the same time period exhibited 69% of activity respectively at a dose of 10 mg kg⁻¹. The compound 1d showed moderate activity at dose of 200 mg kg⁻¹ in the range of 60.60% than 100 mg kg⁻¹ (Table 4).

3-[5-(1H-benzimidazol-2-yl)-1-phenyl-1h-pyrazol-3-yl]-phenol, 1e: The derivative 3-[5-(1H-benzimidazol-2-yl)-1-phenyl-1h-pyrazol-3-yl]-phenol were synthesized by the reaction of ortho phenylene diamine with lactic acid which resulted in the formation of benzimidazole-2-yl-ethanol which on acetylation formed 2-acetylbenzimidazole, further on cyclisation with

4-hydroxybenzaldehyde and phenylhydrazine resulted in the formation of the compound (1e) which was characterized by IR, ¹H-NMR and Elemental analysis as:

- Brown color crystals, Mol. formula. C₂₂H₁₆N₄O, solvent used for TLC was benzene: acetone (9:1), m.p. 184-187°C, yield (%): 66
- IR (KBr cm⁻¹) 3386 (N-NH), 1600 (C=N), 1478 (C=C), 865 (CH=CH), 758 (Ar C-H)
- ¹H-NMR (DMSO-d₆) 5.2 (s, NH), 7.22-7.63 (m, Ar C-H), 5.0 (OH)

Elemental analysis: C: 69.29, H: 4.95, N: 18.22, O: 8.56, Reported; C: 69.27, H: 4.96, N: 18.20, O: 8.57%. The compound was evaluated for analgesic as well as anti-inflammatory activities.

The analgesic activity of the synthesized compound was evaluated in albino mice by Eddy's hot plate method. The compound 1e showed slightly less moderate activity at a dose of 100 mg kg⁻¹ and 200 mg kg⁻¹ in the range of 7.41±0.06 and 7.64±0.06 at 90 min when compared with compound 1b as well as with standard drug Diclofenac sodium at a dose of 5 mg kg⁻¹ in the range of 9.45±0.28 at 90 min. The compound 1e showed moderate activity at dose of 200 mg kg⁻¹ in the range of 7.64±0.06 than 100 mg kg⁻¹ (Table 3).

Further the compound was evaluated for anti-inflammatory activity in albino rats by carrageenan induced rat paw edema method. The compound 1e showed slightly less moderate activity in the range of 57.57 and 56.06% at a dose of 200 and 100 mg kg⁻¹ at time interval of 3 h of carrageenan challenge, when compared with compound 1d as well as when compared with standard Diclofenac sodium at the same time period exhibited 69% of activity, respectively at a dose of 10 mg kg⁻¹. The compound 1e showed moderate activity at dose of 200 mg kg⁻¹ in the range of 57.57% than 100 mg kg⁻¹ (Table 4).

DISCUSSION

The benzimidazole and pyrazole moiety and their novel derivatives studied frequently in the past time and found potent in various pharmacological and pathological conditions. All the newly synthesized novel pyrazole derivatives of benzimidazole were evaluated for their analgesic as well as anti-inflammatory activities. Previous studies showed that the chloro substituent at different position, particularly methoxy, nitro, 2-methoxy-4-hydroxy substituents which showed anticancer, antimicrobial, antitubercular activities (Kaliranjani *et al.*, 2011). The newly synthesized compound 1c and 1b have dimethylamino and bromo at meta and ortho position of the phenyl ring which showed excellent analgesic as well as anti-inflammatory activity compared to substitution at any other positions.

The compound 1b and 1e showed high yields because 1b and 1e has been synthesized by conventional method. This type of synthesis gives high yield. The compound 1b showed excellent anti-inflammatory activity due to the presence of ortho substituted Br group and compound 1c showed potent analgesic activity due to the presence of para substituted N(CH₃)₂ group. Whereas, the synthesized compound 4-chloro and 4-hydroxy substitution showed less significant activity. This concluded that with increase in dose there is increase in analgesic as well as anti-inflammatory activity, for this we used two different doses 100 and 200 mg kg⁻¹. Previously this type of activity i.e., analgesic and anti-inflammatory activity has not been evaluated on this moiety. The present study deals with combination of benzimidazole and pyrazole moiety and evaluate for its analgesic and anti-inflammatory activity. A novel series of novel pyrazolo[3, 4-d]pyrimidines were

synthesized by Kota *et al.* (2011) which shows that derivatives with 4-chloro substitution showed excellent anti-inflammatory activity. 1-substituted-3, 5-dimethyl-4-[(substituted phenyl)diazenyl] pyrazole derivatives synthesized by Hussain *et al.* (2010) which shows that the derivative 1-(5-chloro-6-fluoro-1, 3-benzothiazole-2-yl) thiocarbamoyl-3, 5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles showed significant anti-inflammatory activity and all others compounds showed moderate analgesic activity. Other different benzimidazole derivatives with chloro, hydroxy, dimethylamino, 3,4-dichloro anilino and dipropylamine substitutions showed excellent anti-inflammatory and analgesic activity (Mohan *et al.*, 2011; Mariappan *et al.*, 2011; Achar *et al.*, 2010; Shukla, 2012). These researches were support the current research work i.e., Newly Synthesized novel series of pyrazole derivatives of benzimidazole. In current research we used different substitution i.e., chloro, bromo, p-dimethylamino and hydroxy which showed good analgesic as well as anti-inflammatory activity.

CONCLUSION

The heterocyclic molecules i.e., benzimidazole and pyrazole was synthesized and characterized further these compounds was evaluated for its therapeutic activities i.e., analgesic as well as anti-inflammatory activity. By choosing proper experimental conditions, we made to synthesize pyrazole derivatives of benzimidazole which was obtained, when these two molecules was fused together, which were screened for analgesic as well as anti-inflammatory activity. The present results showed that the pyrazole derivatives of benzimidazole served as a lead molecule for future investigation and can be a useful source for the exploration of new molecules by fusing other heterocyclic molecules.

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