

# Synthesis and antinociceptive activity of novel mannich base derivatives of some new fused 3,5-pyrazolidinedione

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## ABSTRACT

In the present study, a novel series of mannich bases 1-((1-substituted ethyl-1H-benzo[d]imidazol-2-yl) methyl)-2-substituted phenylpyrazolidine-3,5-dione 3(a-l) were synthesized and evaluated as antinociceptive agents in mice by Eddy's hot plate and acetic acid-induced writhing models. The structures attributed to compounds 3a-3l were elucidated by using IR, <sup>1</sup>H-NMR, and Mass spectroscopic techniques. Some compounds showed promising analgesic activity when compared with the standard drug Diclofenac sodium. Results of analgesic activity via hot plate model showed that compounds 3d and 3f were found to be more active than standard drug. Results of analgesic activity via acetic acid-induced writhing model showed that compounds 3a, 3c, 3k, and 3l showed activity which is comparable with the standard drug.

**Key words:** Antinociceptive activity, diclofenac sodium, Eddy's hot plate, mannich bases

## INTRODUCTION

Despite an ever-growing body of knowledge about endogenous nociceptive and antinociceptive systems, clinical treatment of pain today is dominated by two main groups of analgesics: The opioids such as morphine and codeine and the non-steroidal anti-inflammatory drugs including aspirin and ibuprofen. Given the reluctance to use opiates because of their liability toward physical dependence, tolerance, respiratory depression, and constipation, and the limitations in efficacy of the peripheral analgesics associated to classical drawbacks, i.e., gastrointestinal lesions, the quest is to develop new

potent analgesic agents with the efficacy of morphine without the undesired and use-limiting side effects. Because of this, there has been a renewed interest for anti-inflammatory agents endowed with either more selective mechanisms (COX-1 *vs* COX-2 inhibition) or novel modes of action.<sup>[1]</sup> Pyrazoles represent one of the most active classes of compounds possessing wide spectrum of biological activities.<sup>[2,3]</sup> Many of the therapeutically useful compounds such as phenylbutazone,<sup>[4]</sup> oxyphenbutazone, and celecoxib,<sup>[5]</sup> belonging to pyrazoles exhibited anti-inflammatory, anti-pyretic, and analgesic properties. Moreover, pyrazoline derivatives also constitute an interesting class of organic compounds with diverse chemical and pharmacological applications.<sup>[6-8]</sup> In continuation of our work on the synthesis of biologically important heterocyclic compounds,<sup>[9-12]</sup> we reported the synthesis of pyrazolidine 3,5 dione derivatives substituted with benzimidazoles.<sup>[13]</sup> Mannich bases 1 (a-f) were synthesized by reaction of 2-chloro methyl benzimidazole with formaldehyde and secondary amine. The prepared mannich bases<sup>[14-18]</sup> were further treated with phenyl hydrazines 2 (a-l), which on reaction with diethyl malonate gave 1H-benzimidazol-2-yl methyl-2-phenylpyrazolidine-3,5-dione derivatives 3 (a-l). All the synthesized compounds were evaluated for their antinociceptive activity in mice by Eddy's hot plate model and acetic acid-induced writhing response. Some of these compounds showed promising analgesic activity when compared with the standard drug Diclofenac sodium (Scheme 1).

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## MATERIALS AND METHODS

Figure 1 shows the scheme protocol for synthesis. Substituents used in mannich base reaction are shown in the Table 1. All the chemicals were purchased from Merck (India), Loba Chem (India), and Central Drug House Pvt. Ltd. India and were used without further purification. The melting points of the synthesized compounds were determined by open capillary tube method and are uncorrected. The homogeneity of the synthesized compounds were monitored by ascending thin-layer chromatography on silica gel G-coated glass plates, visualized by Iodine vapors. Developing solvents were toluene and ethyl acetate (5:4) and benzene, acetone (3:2). The IR spectra were recorded on Shimadzu FTIR 8400S using potassium bromide pellet technique. The <sup>1</sup>H-NMR spectra were recorded at 400 MHz at BRUKER NMR spectrophotometer in CDCl<sub>3</sub> and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Mass spectra were recorded on a thermo Finnegan LCQ advantage max ion trap mass spectrometer for title compounds. Spectral data were consistent with the assigned structures. Spectral data were consistent with the assigned structures. 2-Chloromethyl benzimidazole was prepared using Phillips condensation, by condensing o-phenylene diamine with carboxilic acid in 4N HCl.<sup>[12]</sup>

### Synthesis

(a) General procedure for synthesis of 2-(chloromethyl)-1-substituted ethyl-1H-benzo[d]imidazoles (1a-l): A mixture of 2-chloromethyl benzimidazole (8.3 g, 0.05 mol), formaldehyde (1.5 ml, 0.05 mol), substituted amine (0.05 mol) and conc. HCl (2 ml) were refluxed in methanol for 3 hours.<sup>[19,20]</sup> The hot mixture was filtered and the filtrate obtained was cooled in cold water. Crystals obtained were separated by filtration and recrystallized by absolute ethanol. The completion of reaction was checked by TLC and further purified by column chromatography.

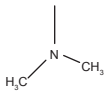
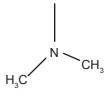
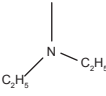
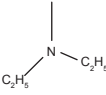
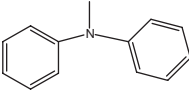
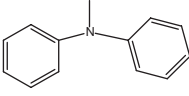
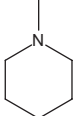
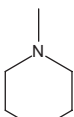
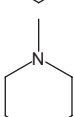
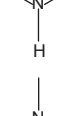
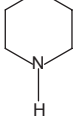
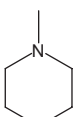
(b) General procedure for synthesis of 2-[(2-phenylhydrazinyl) methyl]-1H substituted benzimidazole 2(a-l)

A mixture of the 2-(chloromethyl)-1-(substituted)-1H-benzo[d]imidazole 1(a-l) (0.02 mol) and phenylhydrazine (10.8, 0.1 mol) were refluxed in methanol for 3 hours. The hot mixture was poured in crushed ice with constant stirring<sup>[13]</sup>; the solid was filtered, dried, and recrystallized from ethanol. The completion of reaction was checked by TLC.

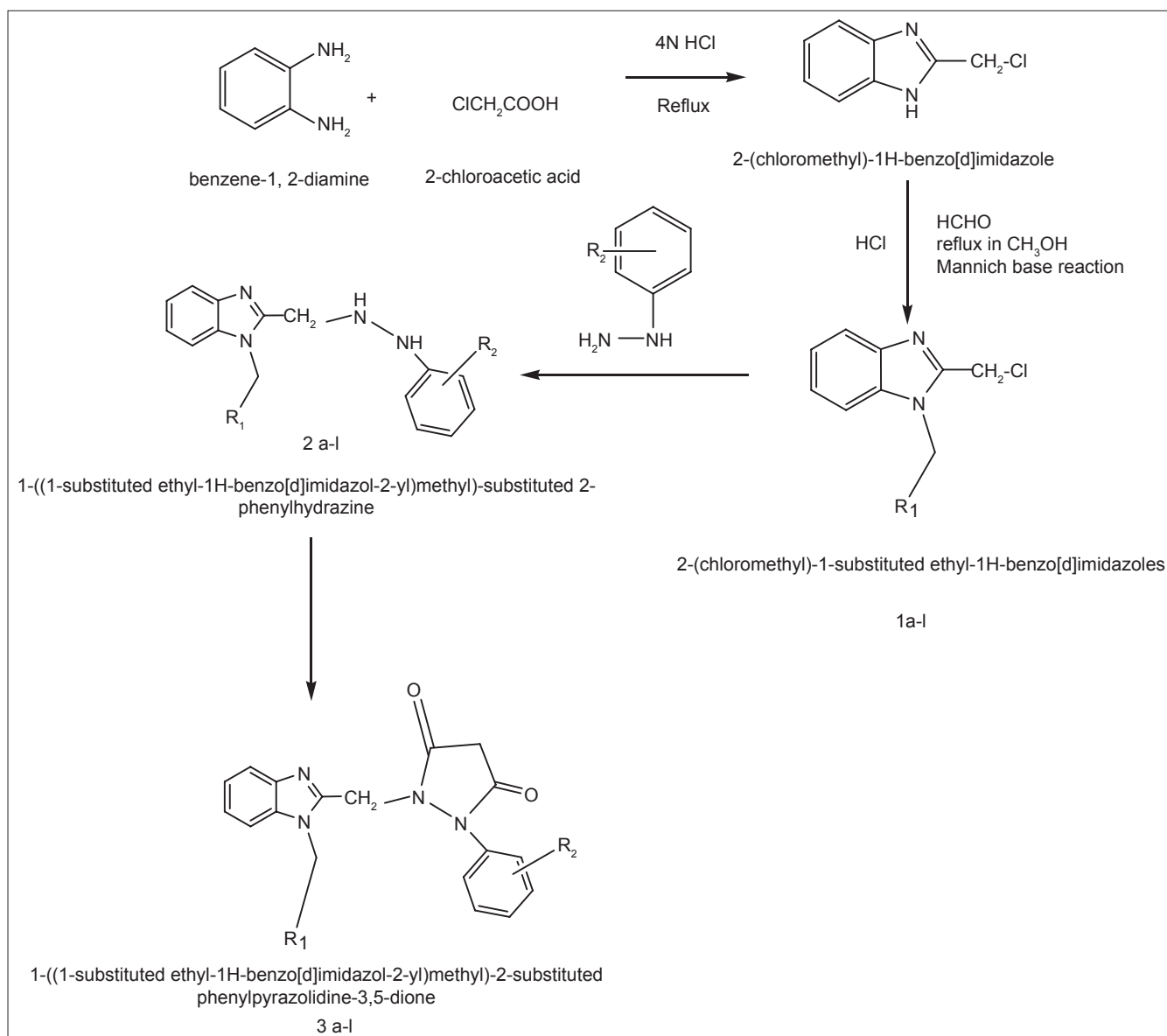
(c) General procedure for synthesis of 1-((1-substituted ethyl-1H-benzo[d]imidazol-2-yl) methyl)-2-substituted phenylpyrazolidine-3,5-dione 3(a-l)

To a mixture of 2-[(2-phenylhydrazinyl) methyl]-1H substituted benzimidazole 2(a-l) (0.01 mol) and diethyl malonate (3 ml, 0.015 mol) added ethanol (90 ml) and acetic acid (1 ml) and refluxed for 5 hours<sup>[13]</sup>. The reaction mixture was left in open dish for 2 to 3 hours. The solid precipitate

**Table 1: Substituents used in mannich base reaction**

Compounds	Secondary amines	Substituent R <sub>2</sub>	Substituent R
3a	Dimethyl amine	H	
3b	Dimethyl amine	2-CH <sub>3</sub>	
3c	Diethyl amine	H	
3d	Diethyl amine	2-CH <sub>3</sub>	
3e	Diphenyl amine	H	
3f	Diphenyl amine	2-CH <sub>3</sub>	
3g	Piperidine	H	
3h	Piperidine	2-CH <sub>3</sub>	
3i	Piperazine	H	
3j	Piperazine	2-CH <sub>3</sub>	
3k	Morpholine	H	
3l	Morpholine	2-CH <sub>3</sub>	

formed was filtered, dried, and recrystallized from ethanol. The completion of reaction was checked by TLC.



**Figure 1:** Scheme mannich base derivatives of 3,5-pyrazolidinedione

## Biological screenings

### Animals

Albino-Swiss mice of either sex weighing (20-25 g) were used for studying *in vivo* analgesic activity. Animals were maintained under standard laboratory conditions ( $24 \pm 2^\circ C$ ; relative humidity 60-70%). Study protocol was approved by the institutional Animal Ethics Committee for the Purpose of Control and Supervision on Experiments on Animals (IAEC, Approval No. 1452/PO/a/11/CPCSEA) before experiment. Albino-Swiss mice from Laboratory Animal House Section, Department of Pharmacology, Devsthal Vidyapeeth College of Pharmacy, Lalpur, Rudrapur (U. S. Nagar) were used in the study. The animals were procured from IVRI, Bareilly (U.P.). Minimum of 6 animals were used in each group. The animals were divided into 14 groups with five mice in each group. Group 1 animals received vehicle (1%

tween 80 in water, 10 ml/kg), animals of Group 2 received Diclofenac sodium (50 mg/kg) while animals of Group 3 to Group 14 were treated with the prepared compounds 3(a-l).

### Acute toxicity studies

The acute oral toxicity studies were performed to study the acute toxic effects and to determine safest dose of the synthesized compounds. Swiss albino mice of either sex weighing 20 to 25 g were used for the study. The aqueous solution of compounds was administered orally to different groups of overnight-fasted mice at the doses of 30, 100, 300, 1,000, and 3,000 mg/kg body weight. After administration of the compounds, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hours. Furthermore, the animals were under investigation up to

a period of one week. 500 mg/kg body weight was found to be safe. So dose was decided as  $1/10^{\text{th}}$  of the safe dose, i.e. 50 mg/kg body weight. Subsequent to administration of compounds, animals were observed closely for the first three hours, for any toxic manifestation (increased motor activity, sedation, acute convulsion, coma, and death). Thereafter, the observations were made at regular intervals for 24 hours. The animals were under further observation for one week.

### Analgesic activity

#### Method 1 (Hot plate method)

Heat is used as a source of pain. Animals were individually placed on the hot plate maintained at constant temperature ( $55^{\circ}\text{C}$ ) and the reaction of animals, such as paw licking or jump response, was taken as the end response. Analgesic drugs/compounds increase the reaction time. The method was first described by Eddy and Leimbach (A cut-off period of 15 sec was observed to avoid damage to the paw)<sup>[21-23]</sup>. The compounds were dissolved in the Carboxy Methyl Cellulose (0.5% suspension). Control (CMC 0.5% suspension), standard, and test compounds were given per orally to the animals and the reaction of time of animals at 15, 30, 60, and 120 min interval was noted on the hot plate after drug administration. The method of Eddy and Leimbach using techno-heated plat analgesic apparatus was used. Diclofenac Sodium (50 mg/kg) was used with reference drug for comparison. The result was tabulated in Table. Results were expressed as means  $\pm$  S.E.M. Statistical significance was analyzed using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where  $P < 0.05$  was accepted to be a significant difference.

#### Method 2 (Analgesic activity by acetic acid-induced writhing test)

The analgesic activities of compounds were also studied using acetic acid-induced writhing model in mice. Synthesized compounds, control, and standard drug were administered orally 30 min before intraperitoneal administration of 0.6% v/v acetic acid. All compounds (3 (a-l)) were administered intraperitoneally (0.5 ml) as a suspension in sterile 0.9% DMSO solution as vehicle. Diclofenac (10 mg/kg) was used as the standard drug under same conditions. After an interval of 5 min, the mice were observed for specific contraction of body referred to as "writhing" for the next 10 min. In the present study, Diclofenac Sodium was used as the standard drug. Results of percentage analgesic activity of compounds were calculated using the following formula and the results are shown in Table.

$$\text{Analgesic activity} = \frac{N_c - N_t}{N_c} \times 100$$

$N_c$  - No. of writhings for control

$N_t$  - No. of writhings for test compound

## RESULTS AND DISCUSSION

All the synthesized compounds were characterized by their IR,  $^1\text{H}$ NMR, and mass data.

#### 1-((1-((dimethylamino) methyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-phenylpyrazolidine-3,5-dione 3a

IR (KBr):  $3,086\text{ cm}^{-1}$  (Ar, C-H),  $2,943\text{ cm}^{-1}$  (alkenes),  $2,856\text{ cm}^{-1}$  (alkane),  $1,675\text{ cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 1.76 to 1.85 (6H,  $\text{CH}_3$ ,  $J = 4.8\text{ Hz}$ ), 3.14 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione), 3.89 (2H,  $\text{CH}_2$ , benzimidazole), 6.9 to 7.16 (4H, phenyl, pyrazolidine 3,5 dione,  $J = 7.0\text{ Hz}$ ), 7.56 to 7.85 (4H, Ar - benzimidazole,  $J = 7.5\text{ Hz}$ ) MS (ESI):  $m/z$  (%) = 363 ( $\text{M}^+$ ), 364 ( $\text{M} + \text{H}^+$ ).

#### 1-((1-((dimethylamino) methyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-o-tolylpyrazolidine-3,5-dione 3b

IR (KBr):  $3,023\text{ cm}^{-1}$  (Ar, C-H),  $2,945\text{ cm}^{-1}$  (alkenes),  $2,823\text{ cm}^{-1}$  (alkane),  $1,685\text{ cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 1.51 to 1.63 (6H,  $\text{CH}_3$ ,  $J = 4.8\text{ Hz}$ ), 3.12 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione), 6.59 to 6.99 (4H, phenyl, pyrazolidine 3,5 dione,  $J = 7.0\text{ Hz}$ ), 2.35 (3H  $\text{CH}_3$ , phenyl), 4.46 (2H,  $\text{CH}_2$ , benzimidazole,  $J = 7.5\text{ Hz}$ ), 7.26 to 7.70 (4H Ar- benzimidazole). MS (ESI):  $m/z$  (%) 377 ( $\text{M}^+$ ).

#### 1-((1-((diethylamino) methyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-phenylpyrazolidine-3,5-dione 3c

IR (KBr):  $3,456\text{ cm}^{-1}$  (NH, Piprazine),  $3,079\text{ cm}^{-1}$  (Ar, C-H),  $2,956\text{ cm}^{-1}$  (alkenes),  $2,847\text{ cm}^{-1}$  (alkane),  $1,689\text{ cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 1.12 to 2.75 (10H,  $\text{C}_2\text{H}_5$ ,  $J = 3.2\text{ Hz}$ ), 3.12 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione), 4.6 (2H, NH piprazine), 6.59 to 6.99 (4H, phenyl, pyrazolidine 3,5 dione,  $J = 7.0\text{ Hz}$ ), 2.35 (3H,  $\text{CH}_3$ , phenyl), 4.46 (2H,  $\text{CH}_2$ , benzimidazole), 7.26 to 7.70 (4H, Ar- benzimidazole,  $J = 7.5\text{ Hz}$ ). MS (ESI):  $m/z$  (%) = 391 ( $\text{M}^+$ ), 392 ( $\text{M} + \text{H}^+$ ).

#### 1-((1-((diethylamino) methyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-o-tolylpyrazolidine-3,5-dione 3d

IR (KBr):  $3,476, 3,389\text{ cm}^{-1}$  (NH, Piprazine),  $3,042\text{ cm}^{-1}$  (Ar, C-H),  $2,941, 2,945\text{ cm}^{-1}$  (alkenes),  $2,876, 2,863\text{ cm}^{-1}$  (alkane),  $1,696\text{ cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 1.12-2.75 (10H,  $\text{CH}_3$ ,  $J = 3.2\text{ Hz}$ ), 3.12 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione), 4.6 (2H, NH piprazine), 6.59- 6.99 (4H, phenyl, pyrazolidine 3,5 dione,  $J = 7.0\text{ Hz}$ ), 2.35 (3H,  $\text{CH}_3$ , phenyl), 4.46 (2H,  $\text{CH}_2$ , benzimidazole,  $J = 7.5\text{ Hz}$ ), 7.26-7.70 (4H, Ar- benzimidazole). MS (ESI):  $m/z$  (%) 405 ( $\text{M}^+$ ).

#### 1-((1-((diphenylamino) methyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-phenylpyrazolidine-3,5-dione 3e

IR (KBr):  $3,052, 3,012\text{ cm}^{-1}$  (Ar, C-H),  $2,956, 2,913\text{ cm}^{-1}$  (alkenes),  $2,874, 2,856\text{ cm}^{-1}$  (alkane),  $1,705\text{ cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 3.12 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione), 6.59 to 6.99 (4H, phenyl,



pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 6.43 to 7.14 (10 H, Phenyl,  $J = 6.7$  Hz), 4.46 (2H,  $\text{CH}_2$ , benzimidazole,  $J = 7.5$  Hz), 7.26 to 7.70 (4H, Ar- benzimidazole). MS (ESI):  $m/z$  (%) 487 ( $\text{M}^+$ ), 488 ( $\text{M} + \text{H}$ ) $^+$ .

*1-((1-((diphenylamino) methyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-o-tolylpyrazolidine-3,5-dione 3f*

IR (KBr): 3,073  $\text{cm}^{-1}$  (Ar, C-H), 2,945, 2,912  $\text{cm}^{-1}$  (alkenes), 2,856  $\text{cm}^{-1}$  (alkane), 1,663  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 3.12 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione), 6.59 to 6.99 (4H, phenyl, pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 6.43 to 7.14 (10 H Phenyl,  $J = 6.7$  Hz), 4.46 (2H  $\text{CH}_2$ , benzimidazole), 7.26 to 7.70 (4H Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 501 ( $\text{M}^+$ ), 502 ( $\text{M} + \text{H}$ ).

*1-phenyl-2-((1-(piperidin-1-ylmethyl)-1H-benzo[d] imidazol-2-yl) methyl) pyrazolidine-3,5-dione 3g*

IR (KBr): 3,012  $\text{cm}^{-1}$  (Ar, C-H), 2,947 (alkenes), 2,812  $\text{cm}^{-1}$  (alkane), 1,647  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 1.76 to 2.59 (10H,  $\text{CH}_2$ , piperidine,  $J = 2.7$  Hz), 3.12 (2H,  $\text{CH}_2$  pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 6.59 to 6.99 (4H, phenyl, pyrazolidine 3,5 dione), 4.46 (2H,  $\text{CH}_2$ , benzimidazole), 7.26 to 7.70 (4H, Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 403 ( $\text{M}^+$ ).

*1-((1-(piperidin-1-ylmethyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-o-tolylpyrazolidine-3,5-dione 3h*

IR (KBr): 3,085, 3,056  $\text{cm}^{-1}$  (Ar, C-H), 2,996, 2,945 (alkenes), 2,874  $\text{cm}^{-1}$  (alkane), 1,655  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 1.76 to 2.59 (10H  $\text{CH}_2$ , piperidine,  $J = 2.7$  Hz), 3.12 (2H  $\text{CH}_2$  pyrazolidine 3,5 dione), 6.59 to 6.99 (4H phenyl, pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 4.46 (2H  $\text{CH}_2$ , benzimidazole), 7.26 to 7.70 (4H Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 417 ( $\text{M}^+$ ), 418 ( $\text{M} + \text{H}$ ) $^+$ .

*1-phenyl-2-((1-(piperazin-1-ylmethyl)-1H-benzo[d] imidazol-2-yl) methyl) pyrazolidine-3,5-dione 3i*

IR (KBr): 3,103  $\text{cm}^{-1}$  (Ar, C-H), 2,989 (alkenes), 2,875  $\text{cm}^{-1}$  (alkane), 1,695  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 2.65 to 2.78 (4H  $\text{CH}_2$ , piperazine), 2.98 to 3.12 (4H  $\text{CH}_2$ , piperazine), 4.6 (2H NH piperazine,  $J = 1.6$  Hz), 3.12 (2H  $\text{CH}_2$  pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 4.6 (2H NH piperazine), 6.59 to 6.99 (4H phenyl, pyrazolidine 3,5 dione), 2.35 (3H  $\text{CH}_3$ , phenyl), 4.46 (2H  $\text{CH}_2$ , benzimidazole), 4.80 (2H N- $\text{CH}_2$ ), 7.26 to 7.70 (4H Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 404 ( $\text{M}^+$ ).

*1-((1-(piperazin-1-ylmethyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-o-tolylpyrazolidine-3,5-dione 3j*

IR (KBr): 3,078  $\text{cm}^{-1}$  (Ar, C-H), 2,943 (alkenes), 2,856  $\text{cm}^{-1}$  (alkane), 1,693  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.65 to 2.78 (4H  $\text{CH}_2$ , piperazine), 2.98 to 3.12 (4H  $\text{CH}_2$ , piperazine), 4.6 (2H NH piperazine,  $J = 1.6$  Hz), 3.12 (2H  $\text{CH}_2$  pyrazolidine 3,5 dione), 4.6 (2H NH piperazine), 6.59 to 6.99 (4H phenyl, pyrazolidine

3,5 dione,  $J = 7.0$  Hz), 2.35 (3H  $\text{CH}_3$ , phenyl), 4.46 (2H  $\text{CH}_2$ , benzimidazole), 4.80 (2H N- $\text{CH}_2$ ), 7.26 to 7.70 (4H Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 418 ( $\text{M}^+$ ), 419 ( $\text{M} + \text{H}$ ) $^+$ .

*1-((1-(morpholinomethyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-phenylpyrazolidine-3,5-dione 3k*

IR (KBr): 3,045, 3,015  $\text{cm}^{-1}$  (Ar, C-H), 2,978 (alkenes), 2,845, 2,841  $\text{cm}^{-1}$  (alkane), 1,689  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.37 (4H tetrahydro-1,4-oxazine), 3.12 (2H  $\text{CH}_2$  pyrazolidine 3,5 dione), 3.67 (4H tetrahydro-1,4-oxazine), 6.59 to 6.99 (4H phenyl, pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 2.35 (3H  $\text{CH}_3$ , phenyl), 4.46 (2H  $\text{CH}_2$ , benzimidazole), 7.26 to 7.70 (4H Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 405 ( $\text{M}^+$ ).

*1-((1-(morpholinomethyl)-1H-benzo[d] imidazol-2-yl) methyl)-2-o-tolylpyrazolidine-3,5-dione 3l*

IR (KBr): 3,040  $\text{cm}^{-1}$  (Ar, C-H), 2,972 (alkenes), 2,895  $\text{cm}^{-1}$  (alkane), 1,712  $\text{cm}^{-1}$  ( $>\text{C}=\text{O}$ , pyrazolidinedione).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 2.37 (4H tetrahydro-1,4-oxazine), 3.12 (2H  $\text{CH}_2$  pyrazolidine 3,5 dione), 3.67 (4H tetrahydro-1,4-oxazine), 6.59 to 6.99 (4H phenyl, pyrazolidine 3,5 dione,  $J = 7.0$  Hz), 2.35 (3H  $\text{CH}_3$ , phenyl), 4.46 (2H  $\text{CH}_2$ , benzimidazole), 4.80 (2H N- $\text{CH}_2$ ), 7.26 to 7.70 (4H Ar- benzimidazole,  $J = 7.5$  Hz). MS (ESI):  $m/z$  (%) 419 ( $\text{M}^+$ ).

### Analgesic activity

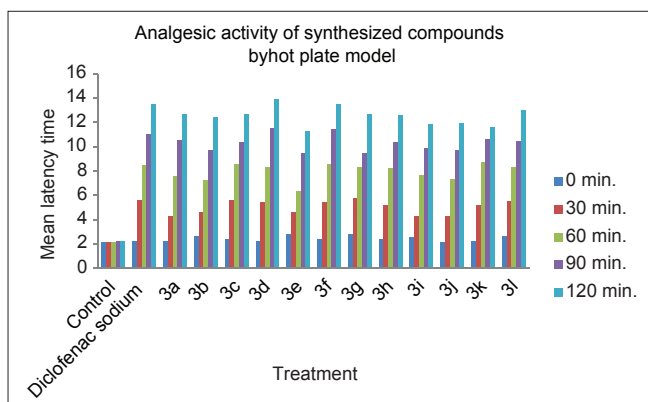
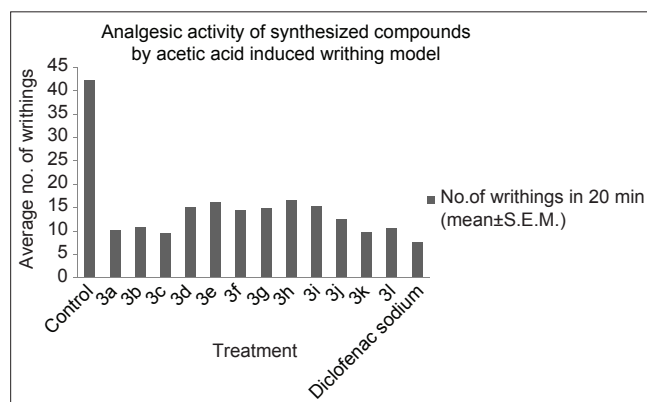
The present work has clearly demonstrated that 2-chloromethyl benzimidazole moiety can be successfully used to synthesize a wide variety of pyrazolidine 3,5 dione derivatives of pharmaceutical interest. A novel series of compounds 3a-l were synthesized and characterized by their IR and NMR data and mass data. All the synthesized compounds were subjected to the acute toxicity studies and their analgesic activity.

The activity is done by hot plate method which is further confirmed by acetic acid-induced writhing method<sup>[24]</sup>. Results of analgesic activity via hot plate model showed that compound 3d and 3f showed activity more than standard drug [Table 2, Figure 2]. Compounds 3c, 3h, and 3l were found to possess the significant analgesic activity which is comparable with the standard [Table 1]. Hot plate method is an acute spinally mediated reflex to noxious thermal stimuli and it is known to be selective for centrally acting analgesics. Mechanism of action of compounds could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of diclofenac sodium and other NSAIDs. This is because NSAIDs can inhibit COX in peripheral tissues and, therefore, interfere with the mechanism of transduction of primary afferent nociceptors.

**Table 2: Effect of synthesized compounds on latency to hot plate test in mice**

Treatments	Mean latency(s) before and after drug administration(s)				
	0 min	30 min	60 min	90 min	120 min
Control (CMC 0.5% suspension)	2.13±0.219	2.13±0.228	2.13±0.187	2.20±0.241	2.23±0.227
Diclofenac sodium	2.23±0.098***	5.64±0.678***	8.46±0.647***	11.04±0.507***	13.47±0.796***
3a	2.210±0.073***	4.32±0.164***	7.56±0.758***	10.53±0.419***	12.64±0.473***
3b	2.62±0.186*	4.63±0.685**	7.24±0.587**	9.75±0.087*	12.43±0.098**
3c	2.43±1.276**	5.63±1.47**	8.54±0.076**	10.36±0.250**	12.69±0.376***
3d	2.21±0.567***	5.43±0.660***	8.28±1.150***	11.50±0.107***	13.94±0.188***
3e	2.81±1.10*	4.58±0.402**	6.38±0.038**	9.46±0.123**	11.24±1.34***
3f	2.42±0.635*	5.47±1.02*	8.56±0.205**	11.46±1.29***	13.52±0.116**
3g	2.78±1.186*	5.75±0.632**	8.32±0.506**	9.45±0.026**	12.67±0.364***
3h	2.41±1.076**	5.17±0.724***	8.23±0.610**	10.36±0.036**	12.56±0.446***
3i	2.53±0.403**	4.26±0.610	7.65±0.814	9.86±0.664***	11.87±1.35
3j	2.12±0.765*	4.27±0.715**	7.32±0.846**	9.74±0.653**	11.96±0.146**
3k	2.23±0.823**	5.23±0.542***	8.72±0.365**	10.63±0.056**	11.62±0.531**
3l	2.62 ± 0.602**	5.52 ± 1.102	8.35 ± 0.406**	10.43 ± 0.312**	12.98 ± 0.092***

Two-way Analysis of Variance: \*\*\*P value found to be <0.0001 is considered extremely significant. The data were expressed as mean±S.E.M.; Tukey Kramer multiple comparison test: \*\*P<0.001 and \*P<0.05 (Compounds vs control), CMC: Carboxy methyl cellulose


**Figure 2:** Comparative effect of synthesized compounds on latency to hot plate test in mice

**Figure 3:** Comparative effect of synthesized compounds on acetic acid-induced writhing in mice

Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve endings. Since the writhing test usually lasts for less than one hour, it is too short to cover central prostaglandin-dependent sensitization, which requires spinal COX-2 induction. However, it primarily involves COX-1-derived prostanoids and thus non-selective COX inhibitors offer a better antinociceptive effect as compared to selective COX-2 inhibitors. All compounds have shown analgesic activity. Results of analgesic activity via acetic acid-induced writhing model showed that 3a, 3c, 3k, and 3l showed activity which is comparable with the standard drug [Table 3, Figure 3]. The results were evaluated by measuring the mean ± S.E.M and P value. Our study revealed that the antinociceptive potential of synthesized compounds QAA-03a and 3c produced significant inhibition in acetic acid writhings, p.o. suggesting peripheral analgesic activity.

**Table 3: Effect of synthesized compounds on acetic acid-induced writhing in mice**

Derivative	No. of writhings in 20 min (mean±S.E.M)	% Analgesic activity
Control	42.3±0.223607	0
3a	10.1±0.341565***	76.12
3b	10.8±0.288675***	74.46
3c	9.5±0.341565***	77.54
3d	15.1±0.74162**	64.30
3e	16.23±0.619139**	61.63
3f	14.5±1.204159**	65.72
3g	14.9±0.67082	64.77
3h	16.6±0.341565***	60.75
3i	15.2±0.465475	73.52
3j	12.5±1 0.204159**	70.44
3k	9.8±1.204159**	76.83
3l	10.6±1.204159**	74.94
Diclofenac sodium	7.6 ± 0.223607***	80.84

N = 6; student t test; \*P≤0.05; \*\*P≤0.01; \*\*\*P≤0.0001 when compared with control

## CONCLUSION

The present work clearly demonstrates that 2-chloromethylbenzimidazole moiety may be successfully used to synthesize a wide variety of benzimidazole derivatives of pharmaceutical interest. So, we synthesized and evaluated some 1-((1-substituted-1H-benzo[d]imidazol-2-yl) methyl)-2-phenyl pyrazolidine-3,5-diones 3(a-l) as analgesic agents. Twelve derivatives of 2-substituted benzimidazole were prepared, of which compounds 3c and 3c possess potent analgesic activity by both the models as shown in the hot plate model and acetic acid-induced writhing test. Hence, the focus is on the synthesis of potent 1,2-substituted benzimidazole derivatives as potential drug-like scaffolds for future development.

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