Synthesis and Evaluation of Analgesic and Antioxidant Activities of Substituted Benzimidazole Derivatives

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ABSTRACT: The present study describes the synthesis and pharmacological evaluation of a number of substituted benzimidazole derivatives designated by **3A-1**, **3A-2**, **3A-3**, **3B-1** and **3B-2** through condensation of different *o*-aryldiamine compounds with the corresponding aldehyde employing ammonium salt as a catalyst. All the compounds were characterized by IR and ^{1}H NMR spectroscopic analysis. The synthesized benzimidazole derivatives were investigated for analgesic and antioxidant activities using acetic acid-induced writhing inhibition in Swiss albino mice and DPPH free radical scavenging assay, respectively. Compounds **3A-3**, **3B-1** and **3B-2** at a dose of 50 mg/kg body weight reduced the number of writhings by 88.24%, 84.03% and 85.71%, respectively ($p<0.001$) in comparison with standard diclofenac (90.76% inhibition). The derivatives **3A-1**, **3A-2**, **3A-3** and **3B-2** showed prominent antioxidant activity with IC_{50} values of 0.038, 0.959, 8.834 and 7.519 µg/ml, respectively in comparison with the standard butylated hydroxytoluene (BHT) (14.44 µg/ml). Among the synthesized compounds, **3A-3** and **3B-2** emerged as the most promising analgesic and antioxidant agents and expressed their potential as lead compounds in future research.

Key words: Synthesis, Benzimidazole, Analgesic, Antioxidant, Writhing inhibition, IC_{50} value.

INTRODUCTION

Heterocyclic compounds carry special significance in drug design and medicinal chemistry owing to their wide range of biological activities. A large number of heterocycles have been explored in the last few decades for developing new therapeutic and pharmaceutical agents, among which the benzimidazole derivatives have occupied a very prominent place.[1] Benzimidazole nucleus, principally formed as a five-membered ring system shows notable basic characteristics because of its nitrogen content and thus forms the active substances of several commercially available drugs. Benzimidazole derivatives encompass various pharmacological activities such as antimicrobial and antibacterial[2], anthelmintics (albendazole)[3], antiviral (enviradine)[4], fungicidal (carbendazim)[5], anti-neoplastic (bendamustine)[6], antihistaminic (mizolastine)[7], analgesic (bezitramide)[8], antihypertensive (telmisartan)[9], anticoagulant (dabigratan)[10], proton pump inhibitors (omeprazole)[11] and calcium sensitizer (pimobendan).[12,13] Therefore, benzimidazole moiety is considered as a 'privileged structure' in heterocyclic chemistry and has been of great interest to synthetic and medicinal chemists.

As per recent literature reviews, the substitution at the 1, 2 and 5 positions of the benzimidazole ring is crucial for exhibiting numerous pharmacological activities.[14-17] Specifically, 2-substituted analogs of benzimidazole are known to be potent biologically active compounds.[18-20] In view of the extensive applications of benzimidazole moiety in medicinal chemistry, an attempt was made to produce a number of 2-substituted benzimidazole derivatives bearing different functional groups at various positions. A simple and easily reproducible procedure was employed involving the condensation of diamine

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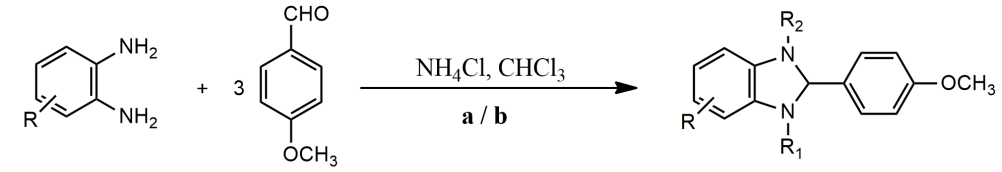
compounds, specifically the derivatives of *o*-aryldiamine with the corresponding aldehydes using ammonium salts as catalysts. Recently, benzimidazole derivatives substituted at 1 and 2 positions have been found to possess prominent antioxidant activity.[21-23] Moreover, benzimidazole nucleus with different functional groups attached to 1, 2, 5 and 6 positions has been reported as the pharmacophore of choice for developing new molecules with analgesic and antioxidant properties.[3,24,25] Encouraged by these findings published in the literature and considering the importance of synthetic drugs and medications in pain relief and management of different disease conditions, our present work was aimed to synthesize several 2-substituted benzimidazole derivatives with varied substituents at different positions of benzimidazole nucleus and explore their analgesic and antioxidant potential.

MATERIALS AND METHODS

Chemicals and reagents. All reactions were carried out in well-dried glassware using a nitrogen atmosphere. Chemicals and reagents, i.e. *o*-phenylenediamine, 2,3-diaminotoluene, anisaldehyde, chloroform, ammonium chloride, *n*-hexane, ethyl acetate, sodium sulfate, sodium chloride, acetic acid, *tert*-butyl-1-hydroxytoluene or simply butylated hydroxytoluene (BHT) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, USA. *n*-hexane and ethyl acetate were distilled over calcium hydride before use. Silica gel 60 (0.06-0.2 mm, ROTH) was used for column chromatography (CC). IR spectra were recorded on a SHIMADZU FTIR-8400S spectrometer (Shimadzu Corporation, Japan).

Acquisition of spectral data and monitoring of reactions. ^{1}H NMR spectra were acquired on a JEOL Alpha 400 spectrometer (400 MHz) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal reference standard. The chemical shift (δ) values are reported in parts per million (ppm) and spin-spin coupling constants (*J*) were expressed in Hz. To monitor the reactions, thin layer chromatography (TLC) was performed on precoated plates of silica gel (silica gel 60 GF_{254}, Sigma-Aldrich).

General procedure for the synthesis of benzimidazole derivatives. The synthetic pathway is depicted in Scheme 1. Two derivatives of *o*-aryldiamine (**1**) i.e. *o*-phenylenediamine (**1A**) and 2,3-diaminotoluene (**1B**) along with anisaldehyde (**2**) were chosen as starting materials, and ammonium chloride was selected as a catalyst for the experiment based on their commercial availability and reported toxicity profiles. The substituted benzimidazole derivatives were synthesized by modifying a method



o-Aryldiamine **(1)** Anisaldehyde **(2)**

1A, R = H

1B, R= CH_3

Substituted benzimidazole **(3A-1 to 3B-2)**

R_1 = H or substituted benzyl derivative

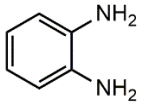
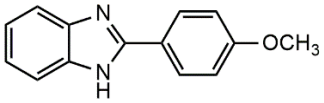
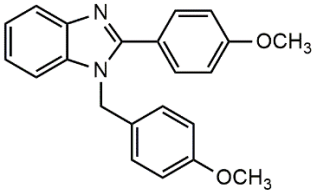
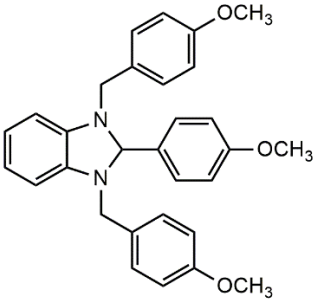
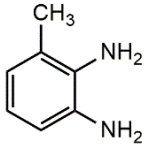
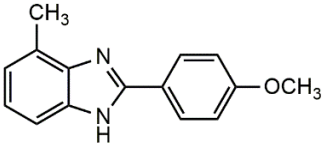
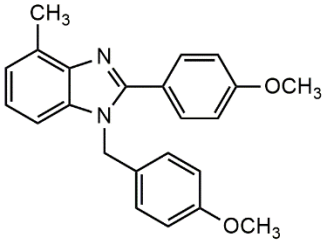
R_2 = Ring nitrogen or substitution of benzyl derivative

Scheme 1. Synthesis of substituted benzimidazole derivatives. **a**: Room temperature, 6 hrs. **b**: 40°C, 12 hrs.

previously reported in the literature.[26] Initially, 1.0 equivalent of diamine compounds, 3.0 equivalents of anisaldehyde and 4.0 equivalents of ammonium chloride were taken in a round bottom flask and the resultant mixture was stirred for five min at required temperature as per the starting material (Table 1). Afterwards, 5 ml of chloroform was added to the solution and stirring was continued for a certain hour

in accordance with each reaction (Table 1). The completion of the reaction was monitored by TLC using *n*-hexane: ethyl acetate (1 : 1) as the mobile phase. The solvent was subsequently removed under reduced pressure with the help of a rotary evaporator. The resultant residue was extracted with 20 ml of ethyl acetate and the organic layer was washed with 10 ml of brine (sodium chloride) solution. In this way, the layers were separated and the organic layer was dried over sodium sulfate. Following the removal of the solvent under reduced pressure, the product was subjected to column chromatography using ethyl acetate and *n*-hexane in different ratios. Several compounds, designated as **3A-1** to **3B-2** were obtained (Table 1) which was taken for characterization using analytical tests.

Table 1. Summary of the reactions.

Name of derivatives *o*-aryldiamine (1) reacting with Anisaldehyde (2)	Reaction temperature and reaction period	Name of products	Total yield (%)	Individual yield (%)
 *o*-Phenylenediamine **(1A)**	Room temperature, 6 hrs **(a)**	 **3A-1**  **3A-2**  **3A-3**	70	3A-1=15% 3A-2=35% 3A-3=25%
 2,3-Diaminotoluene **(1B)**	40°C, 12 hrs **(b)**	 **3B-1**  **3B-2**	75	3B-1=40% 3B-2=35%

2-(4-Methoxyphenyl)-1H-benzimidazole (3A-1). IR (KBr, cm^{-1}): 3437 (C-H stretching of aromatic ring), 2963 (C-H stretching of aliphatic ring), 1631 (C=N stretching of imidazole ring), 1503, 1452 (C=C stretching of aromatic ring); ^{1}H NMR (400 MHz, δ/ppm): 4.10 (*s*, 3H, OCH_3), 6.97 (*d*, *J* =8 Hz, 2H), 7.65 (*s*, 2H), 7.75 (*s*, 2H), 7.85 (*s*, 1H); Yield: 15%.

1-(4-Methoxybenzyl)-2-(4-methoxyphenyl)-1H-benzimidazole (3A-2). IR (KBr, cm^{-1}): 3462 (C-H stretching of aromatic ring), 2964 (C-H stretching of aliphatic ring), 1608 (C=N stretching of imidazole ring), 1513, 1442 (C=C stretching of aromatic ring), 1244 (C-N stretching of imidazole ring), 1171 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 3.80 (*s*, 3H, OCH_3), 3.90 (*s*, 3H, OCH_3), 5.40 (*s*, 2H, -CH_2-), 6.85 (*d*, *J* =8 Hz, 2H), 6.97 (*d*, *J* =8 Hz, 2H), 7.04 (*d*, *J* =8 Hz, 2H), 7.20 (*m*, 2H), 7.25-7.35 (*m*, 1H), 7.64 (*d*, *J* =8 Hz, 2H), 7.85 (*d*, *J* =8 Hz, 1H); Yield: 35%.

1-(4-Methoxybenzyl)-2-(4-methoxyphenyl)-3-(4-methoxybenzyl)-1H-benzimidazole (3A-3). IR (KBr, cm^{-1}): 3053 (C-H stretching of aromatic ring), 2962 (C-H stretching of aliphatic ring), 1501, 1435 (C=C stretching of aromatic ring), 1254 (C-N stretching of imidazole ring), 1179, 1125 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 3.78 (*s*, 3H, OCH_3), 3.83 (*s*, 3H, OCH_3), 3.88 (*s*, 3H, OCH_3), 5.40 (*s*, 2H, -CH_2-), 6.85 (*d*, *J* =8 Hz, 2H), 6.95-7.05 (*m*, *J* =8 Hz, 6H), 7.20 (*d*, *J* =8 Hz, 2H), 7.25-7.30 (*m*, 2H), 7.63 (*d*, *J* =8 Hz, 3H), 7.85 (*d*, *J* =8 Hz, 1H), 8.00 (*d*, *J* =8 Hz, 1H); Yield: 25%.

2-(4-Methoxyphenyl)-4-methyl-1H-benzimidazole (3B-1). IR (KBr, cm^{-1}): 3244 (C-H stretching of aromatic ring), 2964 (C-H stretching of aliphatic ring), 1613 (C=N stretching of imidazole ring), 1511, 1480, 1467 (C=C stretching of aromatic ring), 1248 (C-N stretching of imidazole ring), 1171 (-OCH_3), 3393 (-NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 3.87 (*s*, 3H, OCH_3), 2.60 (*s*, 3H, -CH_3), 6.95-7.05 (*m*, 3H), 7.25 (*t*, *J* =8 Hz, 1H), 7.50 (*s*, *J* =8 Hz, 1H), 7.95-8.05 (*d*, *J* =8 Hz, 2H); Yield: 40%.

1-(4-Methoxybenzyl)-2-(4-methoxyphenyl)-4-methyl-1H-benzimidazole (3B-2). IR (KBr, cm^{-1}): 3390 (C-H stretching of aromatic ring), 2964 (C-H stretching of aliphatic ring), 1261 (C-N stretching of imidazole ring), 1098, 1024 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 3.75 (*s*, 3H, OCH_3), 3.85 (*s*, 3H, OCH_3), 2.75 (*s*, 3H, -CH_3), 5.35 (*s*, 2H, -CH_2-), 6.70-7.60 (*m*, 11H, aromatic); Yield: 35%.

Screening for analgesic activity. The *in vivo* analgesic activity of the synthesized compounds was evaluated by acetic acid-induced writhing method (peripheral analgesia) using Swiss albino mice (*Mus musculus*).[27,28]

Experimental animal. Swiss albino mice of either sex (25-30 g, aged 4-5 weeks) were collected from the animal house of Jahangirnagar University, Bangladesh. They were housed in polyvinyl cages under standard environmental conditions of 25±2°C temperature, 65-75% relative humidity and natural (12 hrs) light and dark schedule[29] in the animal house of the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh, and provided with icddr,b (The International Center for Diarrheal Diseases and Research, Bangladesh) formulated rodent food and water *ad libitum*. As these animals were very sensitive to environmental changes, they were kept for at least 3-4 days before the test in the environment where the experiment was carried out. All ethical manners involving the use of experimental animals were maintained carefully.

Experimental design. Sixty Swiss albino mice were randomly selected and divided into twelve groups, each consisting of 5 mice. Every group received a particular treatment as shown in Table 2, i.e. control, standard, and lower and higher doses (50 and 100 mg/kg body weight, respectively) of each synthesized sample at zero hour. Each mouse was weighed properly before any treatment and the doses of the standard, control and test samples were adjusted accordingly. Analgesic agent diclofenac was chosen as standard and was given orally at a dose of 50 mg/kg body weight. To produce pain sensation, acetic acid (0.7%) was administered intra-peritoneally after 40 min at a dose of 0.1 ml/10 g of body weight to each animal of all the groups. After 5 min of its administration, the number of writhing responses or squirms was counted for each mouse for 15 min. The responses of the sample and diclofenac

treated groups were compared with the control group. The percentage inhibition of writhing in comparison with the control group was taken as an index of analgesia and calculated by using the following formula:

$$\text{Inhibition (\%)} = [(Wc\text{-}Wt) \times 100)] / Wc$$

where Wc is the average number of writhing reflexes in the control group and Wt is the average number of writhing reflexes in the test group.

Table 2. Screening of peripheral analgesic activity of benzimidazole derivatives by acetic acid-induced writhing response in mice.

Sample code	Writhing count					Number of writhing (Mean ± SEM)[a]	Writhing (%)	Inhibition (%)
	M-1	M-2	M-3	M-4	M-5			
CS	23	21	31	19	25	23.8 ± 2.059***	100.00	-
SS	2	1	3	4	1	2.2 ± 0.583***	9.24	90.76
3A-1 (d_1)	18	14	11	7	19	13.8 ± 2.223**	57.98	42.02
3A-1 (d_2)	10	10	5	19	4	9.6 ± 2.657***	40.34	59.66
3A-2 (d_1)	10	4	6	9	6	7.0 ± 1.095***	29.41	70.59
3A-2 (d_2)	11	10	5	7	4	7.4 ± 1.364***	31.09	68.91
3A-3 (d_1)	2	3	2	1	6	2.8 ± 0.860***	11.76	88.24
3A-3 (d_2)	7	15	10	6	9	9.4 ± 1.568***	39.50	60.50
3B-1 (d_1)	1	4	3	5	6	3.8 ± 0.860***	15.97	84.03
3B-1 (d_2)	10	10	12	15	10	11.4 ± 0.979***	47.90	52.10
3B-2 (d_1)	5	3	1	6	2	3.4 ± 0.927***	14.29	85.71
3B-2 (d_2)	4	14	13	3	14	9.6 ± 2.502***	40.34	59.66

[a]Each value represents Mean ± SEM, (n=5); ***p<0.001; **p<0.01; *p<0.01 compared with control (One-way ANOVA followed by Dunnett's test); CS = Control sample; SS = Standard sample (Diclofenac, 50 mg/kg b.w.); (d_1) = Lower dose (50 mg/kg b.w.); (d_2) = Higher dose (100 mg/kg b.w.).

Statistical analysis. All values are expressed as the mean ± standard error of the mean (SEM). The results were statistically analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test by using IBM SPSS Statistics 19 software. Here, $p < 0.05$ was considered to be statistically significant.

Screening for antioxidant activity. The synthesized compounds were assessed for *in vitro* antioxidant potential by DPPH free radical scavenging method described by Brand-Williams *et al.*[30] with minor modifications. In this method, DPPH (2,2-diphenyl-1-picrylhydrazyl) was utilized to assess the free radical scavenging activity of various compounds[31], and *tert*-butyl-1-hydroxytoluene (BHT), a potential antioxidant, was used as the positive control. Briefly, 2.0 ml of methanol solution of the test samples prepared in different concentrations (0.977, 1.953, 3.906, 7.813, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml) was mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The solutions were kept for 30 min at room temperature in a dark place. Afterwards, the absorbance was measured at 517 nm against methanol as blank by using UV-Spectrophotometer. DPPH, consisting of an unstable free radical, traps any radicals produced by the test compounds resulting in a visible color change ranging from violet to pale yellow. This decolorization is indicative of the reducing or free radical scavenging ability of the compounds which is also specified by changes in absorbance of the solutions of different concentrations measured at 517 nm. Inhibition of free radical DPPH in percent (I %) was calculated using the following equation:

$$(I\ \%) = (1\text{-}\ A_{sample}/A_{blank}) \times 100$$

Where, A_{blank} is the absorbance of control reaction (containing all reagents except the test materials) and A_{sample} is absorbance of sample.

The concentration of the compound providing 50% inhibition (IC_{50}) was calculated from the graph

having the % inhibitions plotted against respective concentrations of the samples.

RESULTS AND DISCUSSION

The substituted benzimidazole derivatives were synthesized through the condensation of *o*-aryldiamine derivatives and aromatic aldehyde (Scheme 1). For the reaction, 1.0 equivalent of diamine compounds, (**1A** and **1B**) was allowed to condense with 3.0 equivalents of anisaldehyde (**2**) in the presence of ammonium chloride as a catalyst and chloroform as solvent. Based on temperature and reaction time (Table 1), five substituted benzimidazole derivatives identified as 2-(4-Methoxyphenyl)-1*H*-benzimidazole (**3A-1**), 1-(4-Methoxybenzyl)-2-(4-methoxyphenyl)-1*H*-benzimidazole (**3A-2**), 1-(4-Methoxybenzyl)-2-(4-methoxyphenyl)-3-(4-methoxybenzyl)-1*H*-benzimidazole (**3A-3**), 2-(4-Methoxyphenyl)-4-methyl-1*H*-benzimidazole (**3B-1**) and 1-(4-Methoxybenzyl)-2-(4-methoxyphenyl)-4-methyl-1*H*-benzimidazole (**3B-2**) were obtained. The synthesis of substituted benzimidazole derivatives has been reported in some previous studies, however, in different reaction conditions.[32-36] Some of these reactions involved the use of expensive catalysts and high temperature.[34-37] In order to overcome these limitations and identify the most suitable condition for obtaining higher yields of substituted benzimidazoles, we employed a simple condensation reaction with minor modification of a previously reported method.[26] The reaction involved ammonium chloride, an inexpensive, commercially available and environment-friendly catalyst. Apart from minimizing the overall production cost, the reagent also contributed to the individual yield of our products.

The reaction of *o*-phenylenediamine (**1A**) with anisaldehyde produced a mixture of compounds **3A-1, 3A-2** and **3A-3** in 15, 35 and 25% yield respectively (total yield 70%). The mono-, di- and trisubstituted derivatives, named **3A-1**[26,33,35], **3A-2**[34-37] and **3A-3**[38] respectively, have been reported in the literature. On the other hand, the reaction between 2,3-diaminotoluene (**1B**) and anisaldehyde resulted in the formation of compounds **3B-1** and **3B-2** with 40 and 35% individual yield respectively (total yield 75%). The monosubstituted derivative **3B-1**[39,40] and the disubstituted derivative **3B-2**[40] were reported in previous studies. Unlike the first reaction, the trisubstituted compound could not be identified from the reaction between **1B** and anisaldehyde. The possible reason behind the lack of formation of trisubstituted derivative might be the presence of a -CH_3 group at 4-position of benzimidazole moiety which extended steric hindrance to the upcoming functional group at 3-position. All the compounds were characterized by careful analyses of their IR and ^{1}H NMR spectral data. The IR spectrum of benzimidazole derivatives displayed stretching bands of aromatic and aliphatic rings in the regions of 3462-3053 cm^{-1} and 2964-2962 cm^{-1}, respectively. The C=N signals were observed for compounds **3A-1**, **3A-2** and **3B-1** at 1631-1608 cm^{-1}. Further stretching vibrations (C=C, C-N, -OCH_3) were detected at 1513-1435 cm^{-1}, 1261-1244 cm^{-1} and 1179-1024 cm^{-1} respectively. In ^{1}H NMR spectra of disubstituted products **3A-2** and **3B-2**, the protons of two -OCH_3 groups showed two singlets at δ 3.80, δ 3.90 and δ 3.75, δ 3.85 respectively, and doublet and multiplets were seen in between δ 6.85-7.85 and 6.70-7.60, which indicated 12 and 11 aromatic protons of phenyl ring respectively. The formation of trisubstituted **3A-3** can be explained by three singlets for three -OCH_3 groups at δ 3.78, δ 3.83 and δ 3.88, and 16 aromatic protons in between δ 6.85-8.00 in the NMR spectrum.

In the evaluation of peripheral analgesic activity, all the synthesized benzimidazole derivatives reduced the number of acetic acid-induced abnormal constrictions or writhings in mice. Statistical evaluation of the data ($p<0.001$) demonstrated that the compounds **3A-3**, **3B-1** and **3B-2** depicted encouraging analgesic property (% of inhibition 88.24, 84.03 and 85.71, respectively) at a dose of 50 mg/kg compared to that obtained by standard diclofenac (% of inhibition 90.76). The synthesized compounds **3A-3**, **3B-1** and **3B-2** at a dose 100 mg/kg (% of inhibition 60.50, 52.10 and 59.66

respectively) and other derivatives at doses of both 50 mg/kg and 100 mg/kg showed moderate analgesic activity. The results are summarized in Table 2 and Figure 1.

The acetic acid promoted abdominal constriction response is a sensitive technique to establish nociception or pain in test animals. The response primarily involves the secretion of cytokines, such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and interleukin 8 (IL-8) by the local peritoneal macrophages and mast cells and is controlled by the prostaglandin pathways.[41] The standard diclofenac used in our experiment is a nonsteroidal anti-inflammatory drug (NSAID), which blocks the prostaglandin synthesis by inhibiting cyclooxygenase (COX) enzyme, precisely COX-2 and produces antinociceptive action.[42] Since the antinociceptive activity of our synthesized benzimidazole derivatives was comparable to that of diclofenac, they are likely to contain analgesic components capable of interfering with the prostaglandin pathways. Hence, the compounds bear the potential to be developed into NSAIDs and used in pain relief.

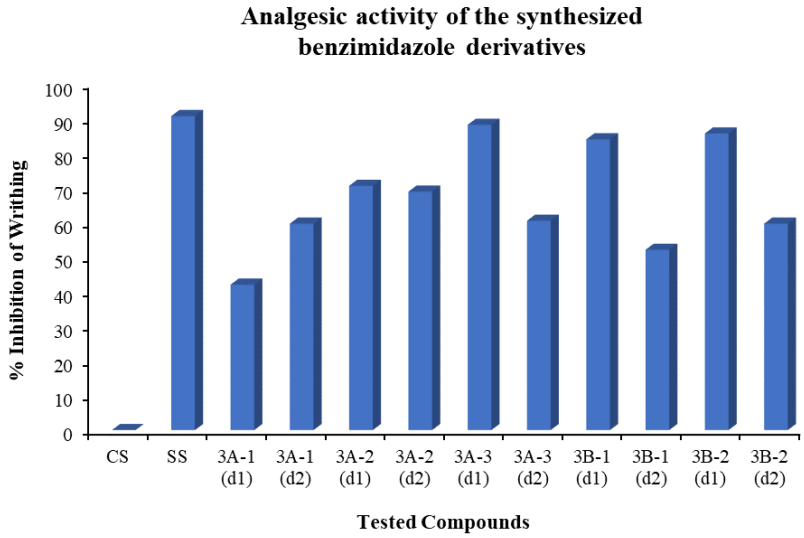


Figure 1. Peripheral analgesic activity of benzimidazole derivatives. CS: Control sample; SS: Standard sample (Diclofenac, 50 mg/kg b.w.); (d1): Lower dose (50 mg/kg b.w.); (d2): Higher dose (100 mg/kg b.w.).

Table 3. Screening of antioxidant activity of benzimidazole derivatives by DPPH free radical scavenging method.

Sample	Inhibition of DPPH free radical at different concentrations (%)										IC_{50} value (µg/ml)
	0.977 µg/ml	1.953 µg/ml	3.906 µg/ml	7.813 µg/ml	15.625 µg/ml	31.25 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	
BHT	13.16	14.74	17.63	22.89	43.95	93.95	94.47	83.42	90.79	94.05	14.44
3A-1	65.26	68.42	69.74	77.63	79.74	82.89	87.63	90.26	91.05	93.68	0.038
3A-2	27.63	43.42	80.00	85.79	82.39	83.68	84.74	86.84	88.95	92.11	0.959
3A-3	40.53	42.11	43.68	48.42	51.58	54.74	60.53	65.26	69.47	77.89	8.834
3B-1	18.16	20.79	21.58	26.05	30.26	32.11	33.94	41.32	48.68	66.32	254.29
3B-2	41.57	43.42	46.05	48.68	52.63	54.47	59.47	65.79	72.63	78.16	7.519

In the evaluation of antioxidant property, compounds **3A-1, 3A-2, 3A-3** and **3B-2** produced superior results than the standard BHT (IC_{50} =14.44 µg/ml) which is evident from their IC_{50} values (0.038, 0.959, 8.834 and 7.519 µg/ml respectively). The derivatives **3B-1** had mild free radical scavenging activity compared to the standard. The results of the antioxidant activity of the compounds are summarized in Table 3.

The *p*-methoxybenzyl group present on imidazole nitrogen of **3A-2, 3A-3** and **3B-2** might contribute to the improved antioxidant potential of these compounds compared to the standard and they emerged as the most promising antioxidant agents. The extended resonance produced by the radical formed from the benzyl group during the reaction may help in enhancing DPPH scavenging ability of the compounds.[43]

CONCLUSIONS

A simple, easy, and cost-effective method has been described for the synthesis of various substituted benzimidazole derivatives from two different diamine compounds and anisaldehyde with significant yields in the range of 70-75%. To the best of our knowledge, this is the first report of antioxidant and analgesic properties of the synthesized derivatives among which compounds **3A-3** and **3B-2** showed the most encouraging results. The presence of *p*-methoxy benzyl substituent appeared to be beneficial for their biological activities. We assume these derivatives as potential lead compounds to design and develop novel, safe and potent antioxidant and analgesic agents.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

AUTHORS' CONTRIBUTIONS

SMAR designed the experimental work and supervised all the research activities. SRB performed the synthesis work. ZAM and SRB designed and performed the experiments with laboratory animals and analyzed the experimental data. PS carried out the rest of the work related to biological evaluation. SRB and PS performed the literature survey and wrote the manuscript.

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