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Short communication

In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives

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ABSTRACT

A series of 2-methylaminobenzimidazole derivatives (**1–11**) were synthesized by the reaction of 2-(chloromethyl)-1*H*-benzimidazole derivatives with primary aromatic amines. All these compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, GC-MS and elemental analysis. The newly synthesized compounds were screened for analgesic and anti-inflammatory activities on acetic acid induced writhing in mice and carrageenan induced paw oedema in rats. Compounds (**7**) and (**2**) showed a potent analgesic (89% at 100 mg/kg b.w) and anti-inflammatory (100% at 100 mg/kg b.w) activities compared with standard drug Nimesulide (100% at 50 mg/kg b.w) respectively. The other compounds showed good analgesic and anti-inflammatory activities.

1. Introduction

Inflammation is a local reaction of the vascular and supporting elements of a tissue to injury resulting in the formation of a protein-rich exudates; it is a protective response of the nonspecific immune system that serves to localize, neutralize, or to destroy an injurious agent in preparation for the process of healing. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumor (swelling), and functio laesa (loss of function). Cause of inflammation includes physical agents, chemical agents, immunological reactions, and infection by pathogenic organism [1]. Inflammation is divided into acute and chronic patterns. The characteristics of acute inflammation are the exudation of fluid and plasma proteins (oedema) and the emigration of leukocytes, predominantly neutrophils. Chronic inflammation is considered to be inflammation of prolonged duration (weeks or months) in which active inflammation, tissue destruction, and attempts at repair are proceeding simultaneously. Chronic inflammation includes some of the most common and disabling human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases [2].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the choice treatment in various inflammatory diseases such as arthritis, rheumatisms as well as to relieve the aches and pain of everyday life [3]. Classical NSAIDs exhibit their action by restricting the biosynthesis of prostaglandin, some of which are pro-inflammatory. This is essentially brought about by inhibiting the rate limiting cyclooxygenase (COX) enzyme involved in the inflammatory cascade [4]. Among different types of NSAIDs, imidazole and fused imidazole with six-membered rings [5], occupy central position among those compounds that are used as analgesic and anti-inflammatory agents.

Fused imidazole derivatives have occupied a prominent place in medicinal chemistry because of their significant properties as therapeutics in clinical applications [6–9]. Thus, benzimidazole is being explored in the pharmaceutical industries and the substituted benzimidazole derivatives have also been found in the diverse therapeutic applications [10,11]. Because of the versatile core contained in several substances of benzimidazole derivatives are possess a broad spectrum of pharmacological activities [12–15]. In particular, it has been an important pharmacophore and privileged structure in medicinal chemistry [16,17], encompassing a diverse range of biological activities including anti-arrhythmic, HIV-RT inhibitor [18], anti-cancer, pesticide, anti-ulcer, anti-inflammatory, anthelmintical, inotropic, antihistamin, anti-microbial, anti-viral and cytotoxicity [19–22]. Therefore, the optimization of benzimidazole derivatives based on their structures have resulted various potent drugs that are now being currently practiced in the market, amongst Albendazole (inhibitor of Encephalitozoon Intestinalis infection in AIDS patients), Omeprazole (proton pump inhibitor), Pimobendan (ionodilator), and Mebendazole

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(anthelmintic). Therefore, preparation of benzimidazole has attracted considerable attention in recent years.

The aforesaid numerous pharmacological activities of benzimidazoles prompted us to study the *in-vivo* analgesic and anti-inflammatory activities of some important benzimidazole derivatives. Owing to the importance and in continuation of our ongoing research project on benzimidazole derivatives [23–26], now we wish to explore simple and the novel approach towards the synthesis of 2-methylaminobenzimidazole derivatives by readily obtainable materials [27–29] such as 2-(chloromethyl)-1*H*-benzimidazole derivatives [30,31] and *in-vivo* screening of the resultant compounds by analgesic and anti-inflammatory activities.

2. Chemistry

The synthetic strategy has been explored to obtain the title compounds in excellent yield as shown in Scheme 1. Condensation of 2-(chloromethyl)-1*H*-benzimidazole derivatives [32,33] with various substituted aromatic amines by refluxing in ethanol containing KI/KOH for 6 h gave 2-methylaminobenzimidazole derivatives [31] (**1–11**). Elemental analysis, yield and melting points were given in the Table 1. This protocol is very significant because of its specific generation of crystalline products. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, GC-MS and elemental analysis.

3. Pharmacological evaluation

All the compounds prepared herein were screened for their pharmacological activities such as *in-vivo* analgesic and anti-inflammatory activities. These activities were carried out by measuring the physiological responses of animals to the thermal and chemical stimuli. For analgesic activity, acetic acid induced writhing in mice [34,35] at 50 mg/kg body weight (b.w) was performed. The percent of protection was calculated compared to standard drug nimesulide. All compounds showed potent activity compared with Nimesulide. For anti-inflammatory activity, carrageenan induced inflammation on rat hind paw oedema method of Winter et al. [36], in mice at 50 mg/kg b.w. was performed. The percent of inhibition was determined for synthesized compounds as well as standard drug nimesulide.

4. Results and discussion

4.1. Chemistry

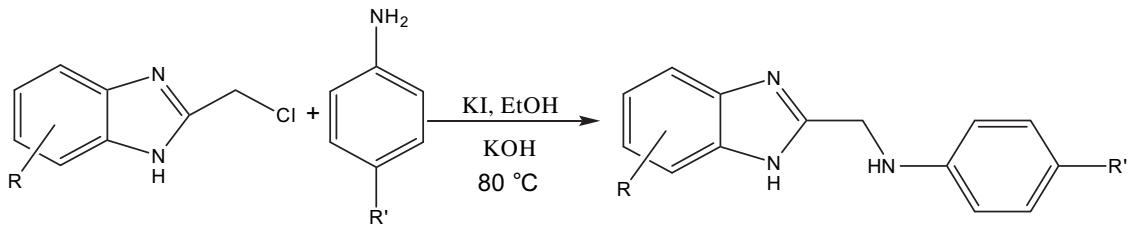
Condensation of 2-(chloromethyl)-1*H*-benzimidazole derivatives with various substituted aromatic amines by refluxing in ethanol containing KI/KOH for 6 h yielded substituted 2-methylaminobenzimidazole derivatives (**1–11**) according to literature method [31]. The maintenance of optimum temperature (80 °C) is very important as it led to the crystalline products. The corresponding yields (70–89%) were obtained in good agreement. The postulated structures of newly synthesized targeted benzimidazole derivatives are in accordance with the elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and GC-MS. IR spectra of all the compounds showed stretching bands at 3448–3424 cm^{-1} and 3327–3308 cm^{-1} of aromatic and aliphatic –NH respectively. Further (C═N) stretching bands appeared at 1596–1619 cm^{-1} respectively. The ^{1}H NMR spectral broad singlet signals were observed at δ 3.5–4.3 NH protons of aliphatic and NH protons of imidazole are merged with aromatic and which are D_2O exchangeable. In ^{13}C NMR spectra, the peaks at δ 115,116,122,129,137 and 141 confirm the formation of benzimidazole moiety. GC-MS spectra showed the corresponding molecular ion peaks for all the compounds. All the spectral results of 2-methylaminobenzimidazole derivatives (**1–11**) are tabulated in Table 2.

4.2. Analgesic activity

The analgesic activity of the synthesized compounds was assessed by the acetic acid-induced writhing method. According to the structure–activity relationship (SAR) studies, almost all the compounds have shown very potent analgesic activity when compared with standard nimesulide drug. Among the tested compounds *N*-[(5-bromo-1*H*-benzimidazol-2-yl) methyl]-3-chloroaniline (**7**) showed pronounced analgesic activity (89%, 100 mg/kg b.w). The remaining compounds **2**, **4**, **6**, **7**, **8**, **9**, **10** and **11** have also shown good activity because of bromo and nitro substituted benzimidazole with bromo, chloro, methyl and methoxy substituted aryl of *N*-[(5-substituted-1*H*-benzimidazol-2-yl)methyl]-substituted anilines. Within the same ring system i.e., 2-methylaminobenzimidazole, it was noticed that the introduction of bromine and nitro group in benzimidazole and bromo, chloro, methyl and methoxy in the aniline at different positions enhanced the analgesic activity. The results are summarized in Table 3 and Fig. 1.

4.3. Anti-inflammatory activity

The results of tested compounds as well as reference standard were measured before administration the carrageenan. After the administration of carrageenan inflammation was induced in rats, the effect was measured in the intervals of 30, 60, 120 and 180 min. The percent oedema inhibition was calculated as a regard to saline control group, as depicted in Table 4, Figs. 2 and 3. Most of the tested compounds have shown good results in comparison with standard nimesulide standard drug. Amongst all the compounds, compound (**1**) and (**2**) have shown potent anti-inflammatory activity. From a view of structure–activity relationship (SAR) studies, the unsubstituted benzimidazoles and chloro substituted



Where R = H, Br, NO_2
R'= H, Cl, Br, CH_3, OCH_3

Scheme 1. Schematic representation of 2-methylamino-1*H*-benzimidazole derivatives.

Table 1
Physical data of synthesized compounds.

Compound	R	R′	Molecular formula	Yield[a] (%)	m.p. (°C)	C (%)		H (%)		N (%)	
						Calcd	Found	Calcd	Found	Calcd	Found
1	H	H	$C_{14}H_{13}N_3$	85	138–140	75.31	75.37	5.87	5.79	18.82	18.68
2	H	Cl	$C_{14}H_{12}ClN_3$	84	143–145	65.25	65.05	4.69	4.54	16.30	16.17
3	H	Br	$C_{14}H_{12}BrN_3$	84	203–205	55.65	55.46	4.00	3.86	13.91	13.78
4	H	OMe	$C_{15}H_{15}N_3O$	80	153–155	71.13	71.07	5.97	5.99	16.59	16.45
5	Br	H	$C_{14}H_{12}BrN_3$	82	198–200	55.65	55.60	4.10	4.00	13.91	13.79
6	Br	Cl	$C_{14}H_{11}ClN_3$	81	195–198	49.95	49.85	3.29	3.15	12.48	12.35
7	Br	Cl	$C_{14}H_{11}ClN_3$	80	192–195	49.94	49.98	3.29	3.15	12.49	12.38
8	Br	Br	$C_{14}H_{11}Br_2N_3$	80	186–188	44.13	44.02	2.91	2.82	11.03	10.06
9	Br	OMe	$C_{15}H_{14}BrN_3O$	79	188–190	54.23	54.14	4.25	4.16	12.65	12.51
10	Br	Me	$C_{15}H_{14}BrN_3$	81	193–195	56.98	56.39	4.46	4.32	13.29	13.16
11	NO_2	Br	$C_{14}H_{11}BrN_4O_2$	82	186–188	48.43	48.35	3.19	3.05	16.14	16.04

[a] Isolated yield.

aniline have shown potent anti-inflammatory activity when compared with other substituted benzimidazole (–Br and $–NO_2$) with standard drug nimesulide. This has resulted new path in the synthesis of new class of benzimidazole derivatives.

5. Conclusion

In conclusion, we have described simple and efficient protocol for the preparation of 2-methylaminobenzimidazole derivatives with excellent yields. All the synthesized compounds have been screened for their *in-vivo* analgesic and anti-inflammatory activities. In the newly synthesized compounds, it is cleared that the highest analgesic activity in compound (**7**) and anti-inflammatory activity in compound (**1**) and (**2**) were observed. Apart from compound (**7**), remaining compounds have shown good analgesic activity almost equal to standard nimesulide drug. Compounds **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10** and **11** have shown moderate anti-inflammatory activity. The preliminary *in-vivo* studies of these compounds evidenced that, the chloro group in the meta position in aniline ring enhances the analgesic as well as anti-inflammatory activities, which might serve as new templates in the synthesis and development of potent therapeutics. Therefore, it can be concluded that such compounds exert their pharmacological effects. This has resulted good impact on chemists and biochemists for further investigations in the field of medicinal chemistry for search of analgesic and anti-inflammatory agents containing halo and methyl functional groups.

6. Experimental protocols

6.1. Materials and methods

The melting points of the products were determined by open capillaries on a Buchi apparatus and are uncorrected. The IR spectra were recorded on a *Nicolet Impact 410 FT-IR* Spectrophotometer, using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a *Bruker Avance-300F 300 MHz* spectrometer in $CDCl_3$ using TMS as an internal standard with ^{1}H resonant frequency of 300 MHz and ^{13}C resonant frequency of 75 MHz. D_2O exchange was applied to confirm the assignment of the signals of NH protons. The Mass spectra were recorded on a *GC-2010 Shimadzu Mass Spectrometer* (GCMS). The elemental analysis was carried out by using *Heraus CHN* rapid analyzer. All the compounds gave C, H and N analysis within ±0.5% of the theoretical values. The homogeneity of the compounds was described by TLC on aluminum silica gel 60 F_{254} (Merck) detected by U.V light (254 nm) and iodine vapors. All reagents were analytical graded or chemically pure.

6.2. General procedure for synthesis of 2-Arylaminomethylbenzimidazoles (**1–11**)

A mixture of 2-(chloromethyl)-1*H*-benzimidazole derivatives (10 mmol), substituted aniline (10 mmol) and KI (10 mmol) in 50 mL of ethanol was heated under reflux. After 6 h, KOH (10 mmol in 5 mL of water) was added with continuous stirring for 2 h. Finally the reaction mixture was left aside at r.t. and then poured into crushed ice water. The solid products that precipitated were filtered off and recrystallized from ethanol [31].

6.2.1. N-(1H-benzimidazol-2-ylmethyl) aniline (**1**)

Yellow crystal: mp. 138–140 °C (85%), IR (KBr): ν (cm^{-1}), 3429 (Ar-NH) and 3321(aliph-NH), 2919 (aliph-CH) and 1602 (C═N); ^{1}H NMR, (300 MHz, $CDCl_3$, δ ppm): 2.19(s, 2H, CH_2), 4.3(s, 1H, aliph-NH), 6.5(s, 1H, imidazole-NH), 6.3–7.56(m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 52, 112, 115, 116, 122, 129, 138,141, 143; MS: 224.09 [M + 1].

6.2.2. N-(1H-benzimidazol-2-ylmethyl)-3-chloroaniline (**2**)

Yellow crystal: m.p. 143–145 °C (85%), IR (KBr) ν (cm^{-1}): 3437(Ar-NH) and 3318(aliph-NH), 2927 (aliph-CH) and 1596 (C═N); ^{1}H NMR, (300 MHz, $CDCl_3$, δ ppm): 2.19(s, 2H, CH_2), 3.75(s, 1H, aliph-NH), 6.6(s, 1H, imidazole-NH), 6.4–7.7(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 52, 110, 112, 115, 117, 122, 130, 134, 137, 141, 145; MS: 258.1 [M + 1].

6.2.3. N-(1H-benzimidazol-2-ylmethyl)-3-bromoaniline (**3**)

Yellow crystal, m.p. 203–205 °C (85%), IR (KBr) ν (cm^{-1}): 3448 (Ar-NH) and 3321(aliph-NH), 2921 (aliph-CH) and 1612 (C═N); ^{1}H NMR, (300 MHz, $CDCl_3$, δ ppm): 2.18(s, 2H, CH_2), 4(s, 1H, aliph-NH), 6.62(s, 1H, imidazole-NH), 6.4–7.7(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 52, 110, 112, 115, 117, 122, 130, 134, 137, 144; MS: 303.08 [M + 1].

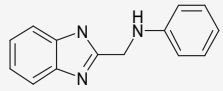
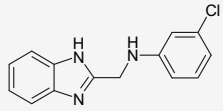
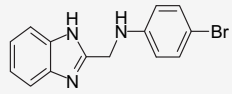
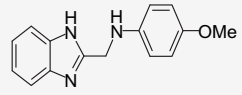
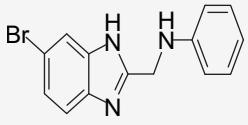
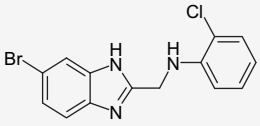
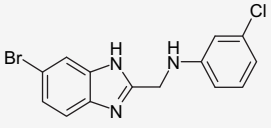
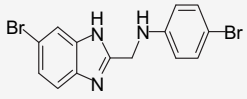
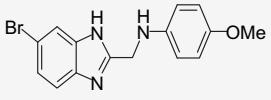
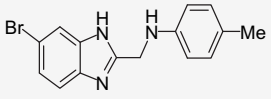
6.2.4. N-(1H-benzimidazol-2-ylmethyl)-4-methoxyaniline (**4**)

Yellow crystal: m.p. 153–155 °C (85%), IR (KBr), ν (cm^{-1}): 3427 (Ar-NH) and 3325(aliph-NH), 2936 (aliph-CH) and 1617 (C═N); ^{1}H NMR, (300 MHz, $CDCl_3$, δ ppm): 2.17(s, 2H, CH_2), 4.3(s, 1H, aliph-NH), 6.61(s, 1H, imidazole-NH), 6.4–7.9(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 52, 56, 113, 114, 117, 118, 126, 135, 136, 140, 141, 150; MS: 254.11 [M + 1].

6.2.5. N-[(5-bromo-1H-benzimidazol-2-yl) methyl] aniline (**5**)

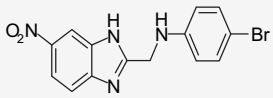
Light buff crystal: m.p. 198–200 °C (85%), IR (KBr) ν (cm^{-1}), 3433(Ar-NH) and 3308(aliph-NH), 2925 (aliph-CH) and 1619 (C═N); ^{1}H NMR, (300 MHz, $CDCl_3$, δ ppm): 2.2 (s, 2H, CH_2), 4(s, 1H, aliph-NH), 6.7(s, 1H, imidazole-NH), 6.4–7.8(m, 7H, Ar-H); ^{13}C NMR

Table 2
Spectral analysis.

Sl.No.	Compounds and Structures	Infrared cm^{-1} (KBr pallets)	^1H Nuclear Magnetic Resonance Values in ppm	^{13}C NMR Values in ppm	Mass At m/z (M + 1)
1	**2**	**3**	**4**	**5**	**6**
1.		3429 cm^{-1} –NH stretching, for benzimidazole ring NH 3321 cm^{-1} –NH bending for secondary amide 1602 cm^{-1} C=N stretching, for imidazole ring	2.19 (s, 2H, CH_2) 4.3 (s, 1H, NH, disappeared on D_2O addition) 6.5 (s, 1H, NH, disappeared on D_2O addition) 6.3–7.56 (m, Ar-H)	52, (Methylene carbon) 112–138 (Ar-Carbons) 141, (C=N carbon) 143, (N–C carbon)	224.09
2.		3437 cm^{-1} –NH stretching, for benzimidazole ring NH 3318 cm^{-1} –NH bending for secondary amide 1596 cm^{-1} C=N stretching, for imidazole ring	2.19 (s, 2H, CH_2) 3.75 (s, 1H, NH, disappeared on D_2O addition) 6.6 (s, 1H, NH, disappeared on D_2O addition) 6.4–7.7 (m, Ar-H)	52, (Methylene carbon) 110–137 (Ar-Carbons) 141, (C=N carbon) 145, (N–C carbon)	258.1
3.		3448 cm^{-1} –NH stretching, for benzimidazole ring NH 3321 cm^{-1} –NH bending for secondary amide 1612 cm^{-1} C=N stretching, for imidazole ring	2.18 (s, 2H, CH_2) 4.0 (s, 1H, NH, disappeared on D_2O addition) 6.62 (s, 1H, NH, disappeared on D_2O addition) 6.4–7.7 (m, Ar-H)	52, (Methylene carbon) 110–134 (Ar-Carbons) 137, (C=N carbon) 144, (N–C carbon)	303.08
4.		3427 cm^{-1} –NH stretching, for benzimidazole ring NH 3325 cm^{-1} –NH bending for secondary amide 1617 cm^{-1} C=N stretching, for imidazole ring	2.17 (s, 2H, CH_2) 4.3 (s, 1H, NH, disappeared on D_2O addition) 6.61 (s, 1H, NH, disappeared on D_2O addition) 6.4–7.9(m, Ar-H)	52, (Methylene carbon) 56, (Methoxy carbon) 113–140 (Ar-Carbons) 141, (C=N carbon) 150, (N–C carbon)	254.11
5.		3433 cm^{-1} –NH stretching, for benzimidazole ring NH 3308 cm^{-1} –NH bending for secondary amide 1619 cm^{-1} C=N stretching, for imidazole ring	2.2 (s, 2H, CH_2) 4.0 (s, 1H, NH, disappeared on D_2O addition) 6.7 (s, 1H, NH, disappeared on D_2O addition) 6.4–7.8 (m, Ar-H)	52, (Methylene carbon) 112–140(Ar-Carbons) 141, (C=N carbon) 143, (N–C carbon)	302.09
6.		3429 cm^{-1} –NH stretching, for benzimidazole ring NH 3321 cm^{-1} –NH bending for secondary amide 1616 cm^{-1} C=N stretching, for imidazole ring	2.2 (s, 2H, CH_2) 4.3 (s, 1H, NH, disappeared on D_2O addition) 6.72 (s, 1H, NH, disappeared on D_2O addition) 6.7–8.0 (m, Ar-H)	51, (Methylene carbon) 114–140(Ar-Carbons) 142, (C=N carbon) 144, (N–C carbon)	336.89
7.		3429 cm^{-1} –NH stretching, for benzimidazole ring NH 3327 cm^{-1} –NH bending for secondary amide 1616 cm^{-1} C=N stretching, for imidazole ring	2.18 (s, 2H, CH_2) 4.1 (s, 1H, NH, disappeared on D_2O addition) 6.6 (s, 1H, NH, disappeared on D_2O addition) 6.2–7.9 (m, Ar-H)	52, (Methylene carbon) 111–140 (Ar-Carbons) 141, (C=N carbon) 143, (N–C carbon)	337.04
8.		3430 cm^{-1} –NH stretching, for benzimidazole ring NH 3325 cm^{-1} –NH bending for secondary amide 1617 cm^{-1} C=N stretching, for imidazole ring	2.18 (s, 2H, CH_2) 4.1 (s, 1H, NH, disappeared on D_2O addition) 6.8 (s, 1H, NH, disappeared on D_2O addition) 6.32–7.87 (m, Ar-H)	52, (Methylene carbon) 111–140 (Ar-Carbons) 141, (C=N carbon) 142, (N-C carbon)	382.07
9.		3424 cm^{-1} –NH stretching, for benzimidazole ring NH 3323 cm^{-1} –NH bending for secondary amide 1602 cm^{-1} C=N stretching, for imidazole ring	2.19 (s, 2H, CH_2) 4.0 (s, 1H, NH, disappeared on D_2O addition) 6.7 (s, 1H, NH, disappeared on D_2O addition) 6.4–7.7 (m, Ar-H)	52, (Methylene carbon) 56, (Methoxy Carbon) 113–140(Ar-Carbons) 141, (C=N carbon) 148, (N–C carbon)	332.97
10.		3428 cm^{-1} –NH stretching, for benzimidazole ring NH 3324 cm^{-1} –NH bending for secondary amide 1616 cm^{-1} C=N stretching, for imidazole ring	2.18 (s, 2H, CH_2) 4.0 (s, 1H, NH, disappeared on D_2O addition) 6.7 (s, 1H, NH, disappeared on D_2O addition) 6.4–7.7 (m, Ar-H)	20, (Metyl Carbon) 52, (Methylene carbon) 112–136(Ar-Carbons) 140, (C=N carbon) 141, (N–C carbon)	316.92

(*continued on next page*)

Table 2 (*continued*)

Sl.No.	Compounds and Structures	Infrared cm^{-1} (KBr pallets)	^1H Nuclear Magnetic Resonance Values in ppm	^{13}C NMR Values in ppm	Mass At m/z (M + 1)
1	**2**	**3**	**4**	**5**	**6**
11.		3430 cm^{-1} –NH stretching, for benzimidazole ring NH 3321 cm^{-1} –NH bending for secondary amide 1612 cm^{-1} C═N stretching, for imidazole ring	2.19 (s, 2H, CH_2) 4.2 (s, 1H, NH, disappeared on D_2O addition) 6.8 (s, 1H, NH, disappeared on D_2O addition) 6.32–8.63 (m, Ar-H)	52, (Methylene carbon) 110–141(Ar-Carbons) 142, (C═N carbon) 144, (N–C carbon)	348.04

(75 MHz, $CDCl_3$, δ ppm): 52, 112, 116, 117, 118, 126, 129, 136, 140, 141, 143; MS: 302.09 [M + 1].

6.2.6. N-[(5-bromo-1H-benzimidazol-2-yl) methyl]-2-chloroaniline (**6**)

Buff crystal: m.p. 195–198 °C (85%), IR (KBr) ν (cm^{-1}), 3429 (Ar-NH) and 3321(aliph-NH), 2923 (aliph-CH) and 1616 (C═N); ^1H NMR, (300 MHz, $CDCl_3$, δ ppm) : 2.2(s, 2H, CH_2), 4.3(s, 1H, aliph-NH), 6.7(s, 1H, imidazole-NH), 6.7–8(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) : 51, 114, 117, 118, 119.2, 127, 130, 137, 140, 142, 144; MS: 336.89 [M + 1].

6.2.7. N-[(5-bromo-1H-benzimidazol-2-yl) methyl]-3-chloroaniline (**7**)

Buff crystal: m.p. 192–195 °C (85%), IR (KBr) ν (cm^{-1}), 3429 (Ar-NH) and 3327(aliph-NH), 2932 (aliph-CH) and 1616 (C═N); ^1H NMR, (300 MHz, $CDCl_3$, δ ppm): 2.18 (s, 2H, CH_2), 4.1(s, 1H, aliph-NH), 6.6(s, 1H, imidazole-NH), 6.2–7.9(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) : 52, 111, 113, 114, 117, 118, 119, 126, 130, 134, 136, 140, 141, 143; MS: 337.04 [M + 1].

6.2.8. 3-bromo-N-[(5-bromo-1H-benzimidazol-2-yl) methyl] aniline (**8**)

Black crystal: m.p. 186–188 °C (85%), IR (KBr) ν (cm^{-1}), 3430 (Ar-NH) and 3325(aliph-NH), 2921(aliph-CH) and 1617 (C═N); ^1H NMR, (300 MHz, $CDCl_3$, δ ppm) : 2.18(s, 2H, CH_2), 4.1(s, 1H, aliph-NH), 6.8(s, 1H, imidazole-NH), 6.32–7.87(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) : 52, 111, 114, 117, 118, 126, 136, 140, 141, 142; MS: 382.07 [M^+].

Table 3
Analgesic activity of tested compounds (100 mg/kg b.w) and Nimesulide (50 mg/kg b.w).

Compound	Mean writhing (X ± SE)	Protection (%)
Control	30.0 ± 1.55**	–
1	8.3 ± 3.33**	72.33
2	5.6 ± 1.85**	**81.33**
3	8.3 ± 1.45**	70.00
4	5.0 ± 2.51**	**83.33**
5	6.0 ± 2.00**	**80.00**
6	6.0 ± 0.57**	**80.00**
7	3.3 ± 1.66**	**89.00**
8	4.3 ± 2.02**	**85.66**
9	5.0 ± 2.08**	**83.33**
10	3.6 ± 2.33**	**88.00**
11	6.3 ± 1.45**	79.00
Nim	–	100.00

Data represent mean values ± SE of six mice per group, shown at the final value for each group (saline, nimesulide and tested compounds) after 3 h.
Data were analyzed using one-way ANOVA followed by Turkey–Krammer Multiple comparison test $^{**}p < 0.01$.
Percentage change was calculated from basal (pre-drug) values and post-drug values.
Protection was calculated as regards the percentage change of the Nimesulide.
SE, standard error; Nim., Nimesulide.
The active compounds are marked in bold letters.

6.2.9. N-[(5-bromo-1H-benzimidazol-2-yl) methyl]-4-methoxyaniline (**9**)

Buff crystal: m.p.189–190 °C (85%), IR (KBr) ν (cm^{-1}), 3424(Ar-NH) and 3323(aliph-NH), 2924 (aliph-CH) and 1617 (C═N); ^1H NMR, (300 MHz, $CDCl_3$, δ ppm) : 2.19(s, 2H, CH_2), 4(s, 1H, aliph-NH), 6.7(s, 1H, imidazole-NH), 6.4–7.7(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) : 52, 56, 113, 114, 117, 118, 126, 135, 136, 140, 141, 148; MS: 332.97 [M + 1].

6.2.10. N-[(5-bromo-1H-benzimidazol-2-yl) methyl]-4-methylaniline (**10**)

Buff crystal: m.p.193–195 °C (85%), IR (KBr) ν (cm^{-1}), 3428 (Ar-NH) and 3324(aliph-NH), 2921 (aliph-CH) and 1616 (C═N); ^1H NMR, (300 MHz, $CDCl_3$, δ ppm) : 2.18(S, 2H, CH_2), 4(s, 1H, aliph-NH), 6.7(s, 1H, imidazole-NH), 6.4–7.7(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) : 20, 52, 112, 117, 118, 126, 130, 136, 140, 141; MS: 316.92 [M + 1].

6.2.11. 4-bromo-N-[(5-nitro-1H-benzimidazol-2-yl) methyl] aniline (**11**)

Buff crystal: m.p.186–188 °C (85%), IR (KBr) ν (cm^{-1}), 3430 (Ar-NH) and 3321(aliph- NH), 2927 (aliph-CH) and 1612 (C═N); ^1H NMR, (300 MHz, $CDCl_3$, δ ppm) : 2.19(s, 2H, CH_2), 4.2(s, 1H, aliph-NH), 6.8(s, 1H, imidazole-NH), 6.32–8.63(m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 52, 110, 111, 114, 116, 118, 132, 138, 141, 142, 144; MS: 348.04 [M + 1].

6.3. Pharmacological assay

6.3.1. Animals used

Adult Swiss albino mice (20–25 g) and albino rats weighing (150–200 g) of either sex were used for studying acute toxicity. In each group six animals were housed individually in polypropylene cages with paddy husk bed. Animals were maintained at 25–27 °C and 30–70% relative humidity. Study protocol was approved by the

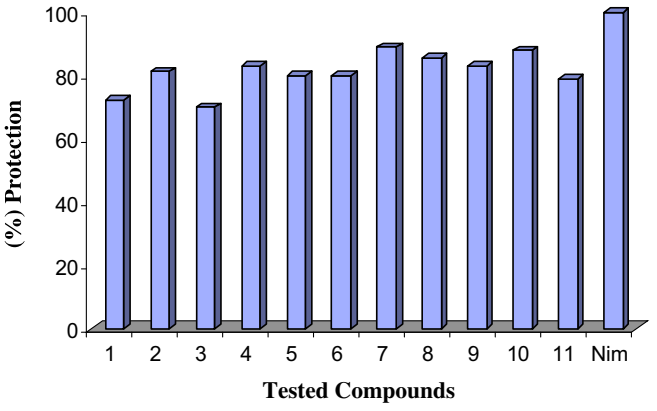


Fig. 1. Analgesic activity of tested compounds (100 mg/kg b.w) and standard drug Nimesulide (50 mg/kg b.w.).

Table 4
The anti-inflammatory activity of the tested compounds (100 mg/kg b.w) and Nimesulide (50 mg/kg b.w).

Compound	Paw oedema thickness (mm)							
	30 m (X ± SE)	% oedema inhibition	60 m (X ± SE)	% oedema Inhibition	120 m (X ± SE)	% oedema inhibition	180 m (X ± SE)	% oedema Inhibition
Control	1.3 ± 0.05	–	1.5 ± 0.03	–	1.7 ± 0.03	–	1.8 ± 0.03	–
1	1.2 ± 0.03	7.6	1.1 ± 0.00**	**26.6**	1.1 ± 0.03**	**41.1**	1.1 ± 0.05**	**38.8**
2	1.1 ± 0.03	**15.3**	1.1 ± 0.00**	**26.6**	1.1 ± 0.03**	**41.1**	1.0 ± 0.03**	**44.4**
3	1.2 ± 0.05	7.6	1.3 ± 0.03**	13.3	1.3 ± 0.03**	23.5	1.3 ± 0.06**	**29.4**
4	1.2 ± 0.06	7.6	1.1 ± 0.05**	**26.6**	1.2 ± 0.03**	**29.4**	1.2 ± 0.06**	**33.3**
5	1.2 ± 0.03	7.6	1.1 ± 0.03**	**26.6**	1.2 ± 0.05**	**29.4**	1.4 ± 0.05**	**22.2**
6	1.3 ± 0.05	–	1.2 ± 0.05**	20.0	1.3 ± 0.08**	23.5	1.4 ± 0.05**	**22.2**
7	1.2 ± 0.03	7.6	1.1 ± 0.06**	**26.6**	1.4 ± 0.03**	17.6	1.5 ± 0.05**	16.6
8	1.4 ± 0.00	–	1.2 ± 0.03**	20.0	1.3 ± 0.10**	23.5	1.5 ± 0.05**	16.6
9	1.4 ± 0.00	–	1.3 ± 0.00**	13.3	1.4 ± 0.00**	17.6	1.6 ± 0.06**	12.5
10	1.2 ± 0.05	7.6	1.2 ± 0.05**	20.0	1.3 ± 0.06**	23.5	1.5 ± 0.11**	16.6
11	1.1 ± 0.03	**15.3**	1.2 ± 0.03**	20.0	1.3 ± 0.05**	23.5	1.4 ± 0.03**	**22.2**
Nim	1.1 ± 0.05	15.3	1.1 ± 0.00**	26.6	1.0 ± 0.00**	41.1	1.1 ± 0.00**	44.4

Data represent mean values ± SE of six mice per group and the percent changes versus 30, 60, 120 and 180 m post-carrageenan injection.
Data were analyzed using one-way ANOVA followed by Turkey–Krammer Multiple comparison test **$p < 0.01$.
Percent oedema inhibition was calculated as regards saline control group.
** Significant difference from the control value at $p < 0.01$.
SE, standard error; Nim., Nimesulide.
The active compounds are marked in bold letters.

institutional Animal Ethics Committee (*IACE, Reg. No. 346/CPCSEA: Dated. 03-01-2001*) before experiment.

6.3.2. Analgesic activity screening

Acetic acid induced writhing model was used to evaluate analgesic activity of the synthesized compounds. Five groups of six Swiss albino mice, each 20–25 g b.w, were used. 0.6% acetic acid (dose = 10 ml/Kg) was injected intra-peritoneally. The numbers of writhes were counted for 20 min, after 5 min of injection of acetic acid into each mice. This reading was taken as a control. Next day, same groups of mice were used for evaluating analgesic activity. Each group was administered orally with the synthesized compounds. The dose of 100 mg/kg of animal was given 1 hour before injection of acetic acid. After 5 min of acetic acid injection, mice were observed for the number of writhings for the duration of 20 min. The mean value for each group was calculated and compared with control. Nimesulide was used as a standard drug for comparison of analgesic activity. Percent protection was calculated using the following formula:

$$(1 - Vc/Vt) \times 100$$

where Vt = Mean number of writhing in test animals and Vc = Mean number of writhing in control. Statistical significance was analyzed using one way ANOVA followed by Turkey–Krammer Multiple comparison test and $p < 0.01$ was considered significant.

6.3.3. Anti-inflammatory activity screening

All the synthesized compounds were tested for their anti-inflammatory activity using carrageenan induced rat hind paw oedema method of Winter et al. [36]. The oedema hind paw was induced by injection of 0.1 mL of 1% carrageenan solution into sub-planter region of right hind paw. The volume of the paw was measured plethysmographically immediately and 180 m after the injection of the irritant. The difference in volume gave the amount of oedema developed. Percent inhibition of the oedema between control group and the compound treated group was calculated and compared with the group receiving standard drug at 50 mg/kg b.w. The results are tabulated in Table 4.

6.3.4. Statistical analysis

In analgesic and anti-inflammatory study, data are expressed as Means ± SE. Differences between vehicle control and treatment groups were tested using one-way ANOVA followed by Turkey–Krammer Multiple comparison test. A probability value less than 0.01 was considered as statistically significant.

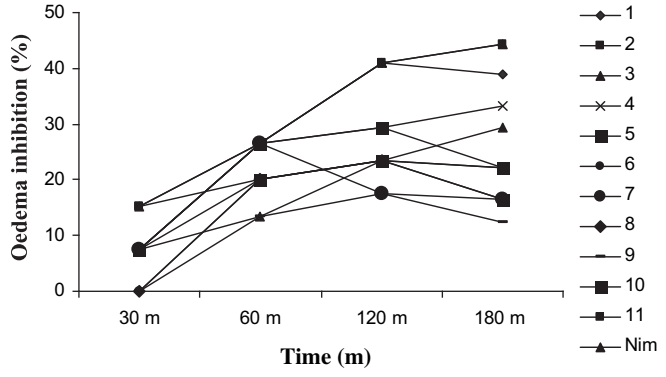


Fig. 2. The inhibition of anti-inflammatory activity of the tested compounds (100 mg/kg b.w.) and standard drug Nimesulide (50 mg/kg b.w.).

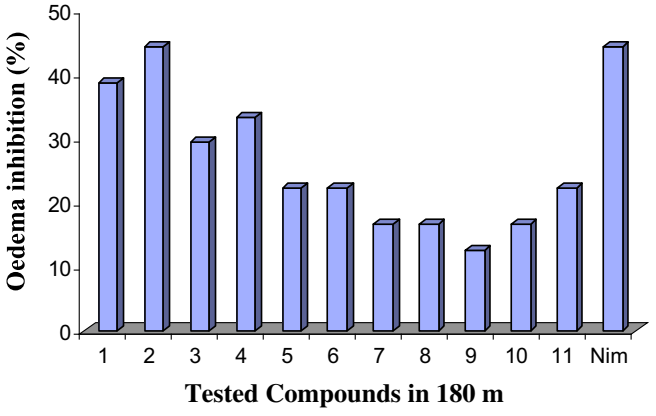


Fig. 3. The percentage of inhibition of anti-inflammatory activity of the tested compounds (100 mg/kg b.w) and standard drug Nimesulide (50 mg/kg b.w) in 180 m.

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