Research Article

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Synthesis, characterization and analgesic activity screening of some new benzimidazole carboxylate derivatives

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ABSTRACT

Benzimidazole 1-carboxylate derivatives were synthesized and investigated for analgesic activity in this study. After synthesis the compounds were taken for spectral analysis like FTIR, NMR and Mass spectroscopy. Of the benzimidazole compounds tested, 6b,6c and 6d showed remarkable analgesic activity by tail flick method. The analgesic activity of synthesized compounds were compare with standard drug Diclofenac. The % analgesic activity at 3hfor the compound 6a,6b,6c,6d and diclofenac were found to be 359.09, 159.09, 268.18, 204.54 and 368.18 respectively. All the 4 synthesized compound shows good analgesic activity compare with diclofenac. The compound **6a**that possess maximum potency. Acute toxicity of the compounds were 100 mg/kg i.p. mice. It was concluded that presence of CH_2CH_3,, $CH_2C(CH_3)_2$ and $CH_2C_6H_5$ but methoxy groups is important for analgesic activity by tail flick method.

Keyword: Analgesic activity, Diclofenac, Benzimidazole, Tail flick.

INTRODUCTION

Pain, which is associated with a number of different conditions, is the common symptom of many diseases. Although there are many drugs currently available for relieving pain, the treatment of pain is still a major problem due to the adverse effects accompanying the long-term use of these drugs[1,2].

Analgesics, which are most widely used drugs for the treatment of pain, can be divided into two groups: morphine and related drugs and nonsteroidal anti-inflammatory drugs (NSAIDs). The fear of addiction and tolerance associated with morphine and related drugs has led to the restriction and withdrawal of these drugs[1–5].

NSAIDs act primarily by inhibiting cyclooxygenase (COX) enzymes, which catalyze the first step in the prostaglandin biosynthesis. The long-term use of NSAIDs may also lead to severe gastrointestinal side effects, which limit the use of these drugs. The adverse effects accompanying the use of non-selective NSAIDs arise from the reduction of the levels of protective prostaglandins in the gastrointestinal (GI) tract due to the inhibition of COX-1. Although selective COX-2 inhibitors cause less GI adverse effects than nonselective NSAIDs, their use in the treatment is also limited due to their serious cardiovascular effects[1–5].

From the above discussion, it is clear that the search for new effective compounds has gained great importance. Acetamide derivatives have been found to possess analgesic activity. Paracetamol, which is one of the world's most widely used drugs, is an example of analgesic agents bearing acetamide group[6–9].

Medicinal chemists have carried out considerable research for novel analgesic agents which possess carboxylic acid moiety. The prominent compounds bearing carboxylic acid group are aspirin, ibuprofen and naproxen, all of which are widely used as over-the-counter drugs for the alleviation of pain.

Benzimidazole nucleus is a constituent of many bioactive heterocyclic compounds that are of wide interest because of

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their diverse biological and clinical applications [1]. This interest in benzimidazole chemistry has been increased by the discovery that the 5, 6-dimethylbenimidazole moiety is part of chemical structure of vitamin B12 [2]. This created interest for researchers who have synthesized variety of benzimidazole derivatives and screened them for their various biological activities [3-7]. Recent review revealed that compounds containing benzimidazole nucleus exert remarkable biological and pharmacological activities. Such activities include anticancer, antimicrobial, antitubercular, antimalarial, antiprotozoal, antiviral, antidiabetic, antihistaminic, anticonvulsant, anti-HIV, antihypertensive, antioxidant, and antiulcer [8]. Analgesic and anti-inflammatory activities of various benzimidazole derivatives were also reported in the literature [9-13].

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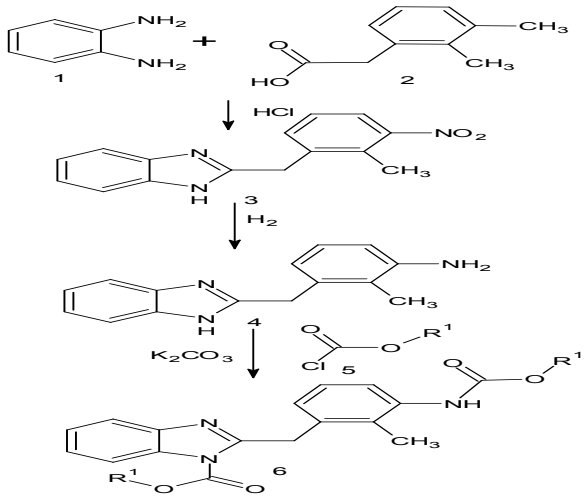
MATERIALS AND METHOD

Experimental

All chemicals and reagents were used are of analytical grade. Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-Fourier transform infra-red **(FTIR)-8400 Spectrophotometer** using **KBr** disc. 1H NMR spectra were recorded on a **Bruker DPX-400 MHz** spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. The mass spectral data were obtained with a **SHIMADZU-LCMS**

Working scheme



R^1= 6a(CH_3), 6b(CH_2CH_3), 6c($CH_2C(CH_3)_2$),6d($CH_2C_6H_5$)

General method of preparation

Preparation of 2-(2-methyl-3-nitrobenzyl)-1H-benzimidazole (3)

4N HCl solution (50 cm^3) was added to a round-bottom flask equipped with a magnetic stirring bar and reflux condenser. o-Phenylenediamine (1, 4.0 g, 0.036 mol) and 2-methyl-3-nitrophenylaceticacid (2, 6.6 g, 0.036 mol) were added and the solution was stirred for 30 minutes. There action mass was reflux at temperature 85°C for 3 hour. The product was precipitatedbyaddingammoniasolution.Theresultingprecipitated solid was filtered and washed with water. The compound was re- crystallized from water and ethanol.

Preparation of 3-(1H-benzimidazole-2yl-methyl)-2-methyl aniline (4)

2-(2-Methyl-3-nitrobenzy1)-lH-benzimidazole (3, 10 g, 0.03 mol) and methanol (100 cm') were added to 80 cm^3closed vessel flask equipped with a magnetic stirring bar. Ammonium formate (10 g, 0.16 mol) and 5% palladiumoncarbon 50% wet (1g,0.1volume) were added to the reaction mass and the flask was closed. The reaction mass was refluxattemperature90C-100°Cfor 2 hour After completion of there action, the catalyst was filtered off by using celite bed and the solvent was removed under vacuum below 60°C. The product obtained was dried in a desiccator to afford 3-(1H-benzimidazo1e-2-ylmethyl)-2-methylaniline(4).

Preparation of substituted benzimidazole carboxylate (6)

Acetone(15cm^3) was added to around-bottom glass flask equipped with a magnetic stirring bar. Potassium carbonate (8.28g,0.06mol) was added to there action mass followed by 3-(lH-benzimidazo1e-2- y1 methyl)-2-methylaniline (4, 11.3 g, 0.048 mol). Substituted chloroformate (7, 0.048 mol) was then added maintaining the temperature at 0 - 5°C. The precipitated solid was removed by vacuum filtration. The filtrate was collected and the acetone was removed under vacuum leaving the crude product. The compounds (6) were recrystallized from water and ethanol.

Analgesic activity

The analgesic activity of the synthesized compounds (6a-6d) was evaluated by tail-flick model as per standard experimental procedure[23]using male albino rats weighing between 125-150g as the experimental animals. The test compounds were administered by making suspension using Tween-40 and normal saline at a dose of 50mg/kg b.w. through i.p. route. The standard drug, Diclofenac sodium at a dose of 10mg/kg b.w. was used on a parallel manner for comparison. The basal reaction time will observe at 1, 2 & 3 hrs after administration of the synthesized compound by placing the tail tip of the rats 1-2cm on the radiant heat of analgesiometer, heated at 55°C ± 0.5°C. The percentage analgesic activity is calculated by observing the following formula:

$$\%\ of\ Analgesic\ \ activity\ = \frac{Reaction\ time\ of\ test}{Reaction\ time\ of\ solvent} \times 100$$

RESULTS AND DISCUSSION

Physicochemical characterization of synthesized compound

The physicochemical characterization like melting point(M.P), % of yield and elemental analysis of synthesized compound were done and the result were shown in table 1.

Compound	Molecular formula	M.P	% Yield	Elemental Analysis					
				Calculated			Found		
				%C	%H	%N	%C	%H	%N
6a	$C_{19}H_{19}N_3O_4$	107	78	64.58	5.42	11.89	64.66	5.51	11.95
6b	$C_{21}H_{25}N_3O_4$	115	75	66.13	6.08	11.02	66.33	6.4	11.33
6c	$C_{25}H_{31}N_3O_4$	90	70	68.63	7.14	9.60	68.70	7.32	9.89
6d	$C_{31}H_{27}N_3O_4$	140	72	73.65	5.32	12.66	73.75	5.48	12.79

Spectral analysis

Methyl-2{3[(methoxy carbonyl) amino]-2-methylbenzy1}-1H benzimidazole-l-carboxylate(6a)

IR (KBr, cm^{-1}): 3443.3 (C-H aromatic),3193.5-2959(-NH str.),1763.6 and1726.0(-O.CO.N str.),1455.0, 1443.5,1357.6,1239.0,1214.0,1126.2,1098.3,1038.5, 778.1,762.7, 749.2 (C-C aromatic); ^{1}H NMR (400 MHz, DMSO (d_6)): 2.10 (s, 3H, -CH3), 3.64 (s, 3H, $CO.OOH_3$), 4.01 (s, 3H, -$CO.OOH_3$), 4.50 (s, 2H, -CH_2), 6.83-7.22 (m, 4H, Ar-H), 7.30-7.63 (m, 3H, Ar-H), 8.92 (s, 1H, -NH); ^{13}C NMR (6 ppm, DMSO-d_6)13.84(CH_3),34.80(CH_3),51.61(OCH_3),54.55(OCH_3),114.84(ArC),119.30(ArC),124.15(ArC),124.38(ArC),125.39(ArC),126.09(ArC),131.39(ArC),132.64(ArC),136.32(ArC),136.45(ArC),141.82 (ArC), 150.46 (ArC), 154.23 (C=0), 155.09 (C=0); MS (ESI) [M^{+1}-1^1: m/z353.9.

Ethyl-2-{3-[(ethoxycarbonypamino]-2-methylbenzyl}-1H-benzimidazole-lcarboxylate(6b)
*IR (KBr, cm^{-1}): 3429.8(C-H aromatic),3211.9-2978.5 (C=N str.), 1744.3 and 1724.0 (-O.CO.N str.),1474.3,1456.0,1376.0,1358.6,1334.5,1283.4 1235.2, 1176.4,1132.0,1097.3,1047.2,1010.5, 762.7, 749.2 (C-C aromatic); ^{1}H NMR (400 MHz, DMSO (d_6)): 1.21-1.24(t, 3H, -CH_3), 1.32-1.36**(t,**3H,-CH_3), 2.11(s, 3H,-CH_3), 4.06-4.12 (q, 2H, -CH_2), 4.42-4.8(q,2H, -CH_2), 4.50 (s, 2H, -CH_2), 6.78-7.96 (m, 7H, Ar-H), 8.88 (s,1H, -NH); ^{13}C NMR (6 ppm, DMSO-d_6):13.74(CH_3),13.84(CH_3), 14.60 (CH_3), 34.93 (CH_3), 60.07 (OCH_3), 64.05 (OCH_3), 114.83(ArC),119.31(ArC),124.04(ArC),124.12(ArC),124.41(ArC),125.34(ArC),125.78(ArC),131.29(ArC),132.72 (ArC),136.41(ArC),136.51(ArC),141.85(A rC),149.81(ArC),154.17(C=0),154.64 (C=0).MS (ESI) [M^{+1}-1]: m/z381.9.*

2-methylpropy1-2-{3-[(2-methylpropoxycarbonyl)amino]-2-methylbenzyl}-1Hbenzimidazole-l-carboxylate(6c)
IR (KBr, cm^{-1}): 3444.2(C-H aromatic),3284.2-2875.3(-NH str.),1745 and1694.2(-O.CO.N str.),1472.4,1455.0,1295.9,1246.8,1204.3,1121.4,1047.2,982.6,763.7,742.5(C-Caromatic); ^{1}H NMR (400 MHz, DMSO (d_6)): 0.919-0.964 (d, 6H, -$C(CH_3)_2$), 0.971-0.976(d,6H,-$C(CH_3)_2$),1.87 -1.93(m,1H,-CH),2.05-2.10(m,1H,-CH), 3.83-3.85(d,2H, -CH_2), 4.23-4.26(d,2H, -CH_2), 4.51(s, 2H,-CH_2), 6.80-7.96 (m, 7H, Ar-H), 8.89 (s, 1H, -NH); ^{13}C NMR (6 ppm, DMSO-d_6):13.03(CH_3),13.83(CH_3), 18.84 (CH_3), 18.91 (CH_3), 27.14 (CH), 27.66 (CH), 35.00 (CH_3),70.03(OCH_2),73.69(OCH_2),114.66(ArC),119.38(ArC),124.14. (ArC),124.44(ArC),125.34 (ArC),125.97(ArC) 131.42(ArC),132.74(ArC),136.31(ArC),136.50(ArC),141.86(ArC), 150.00(ArC), 154.22 (C=O), 154.78 (C=O). MS (ESI) m/z [M+H] at 438.0.

Phenyl 2- {3-[(phenoxycarbonyl)amino]-2-methyl benzy1}-1H-benzimidazole-l carboxylate (6d)
IR (KBr, cm^{-1}):3444.2 (C-H aromatic), 3295.8-2955(-NH str.),1748.3 and1691.2(-O.CO.N str.),1533.1, 1455.0,1391.4,1339.3, 1299.8,1203.4,1121.4, 1086.7,1040.4,969.1,764.6,742.5,697.1 (C-C aromatic); ^{1}H NMR (400 MHz, DMSO (d_6)):2.06 (s, 3H, -CH3), 4.91(s, 2H,-CH2), 5.14 (s,2H, -OCH2), 5.4 (s, 2H, -OCH2), 6.78-7.91(m,17H, Ar-H), 9.05 (s, 1H, -NH).^{13}C NMR (6 ppm, DMSO-d_6):13.80(CH_2),34.93(CH_2),65.66(OCH_2),69.26(OCH_2),114.76(ArC),119.39(ArC),124.03(ArC),124.21(ArC),124.46(ArC) 125.41(ArC),125.93(ArC),127.86(ArC),128.40(ArC),128.65(ArC),131.31 (ArC),132.67(ArC),134.62(ArC),136.35(ArC),136.39(ArC),136.92(ArC), 141.87 (ArC), 149.76 (ArC), 154.19 (C=0), 154.54 (C=0); MS (ESI) m/z [M+H] at 506.

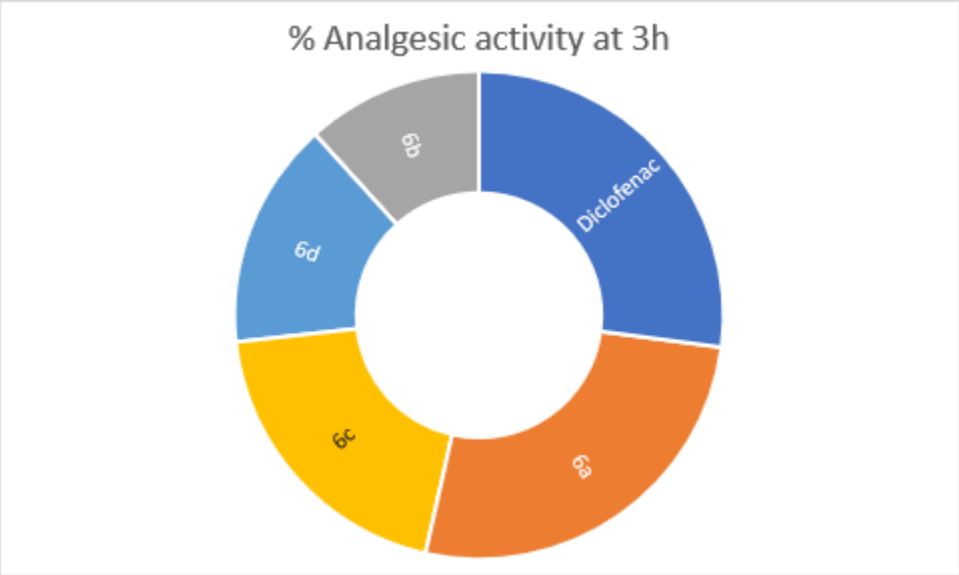
Analgesic activity

The study of analgesic activity by tail flick method showed that the percentage analgesic activity ranges between 159 and 359 at the end of 3 hrs, where as the standard drug Diclofenac sodium registered 368 (Table –2).

Table 2: Analgesic activity of benzimidazole carboxylate derivatives (6a, 6b, 6c and 6d) in Rats by Tail-flick method

Sl. No.	Compound. No.	Reaction time (sec)			% Analgesic activity at 3h
		1h	2h	3h	
1.	Solvent	2.3±0.11	2.5±0.08	2.2±0.06	–
2.	Diclofenac (10mg/kg)	3.9±0.17***	6.8±0.12***	8.1±0.08***	368.18
3.	6a	3.6±0.04***	5.5±0.06***	7.9±0.15***	359.09
4.	6b	2.8±0.08**	3.1±0.13***	3.5±0.11***	159.09
5.	6c	3.2±0.11***	4.1±0.14***	5.9±0.09***	268.18
6.	6d	2.9±0.13**	3.5±0.23**	4.5±0.08***	204.54

Values expressed as Mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, Dose of the test compound: 50 mg/kg. (ANOVA followed by Dunnet's t-test).



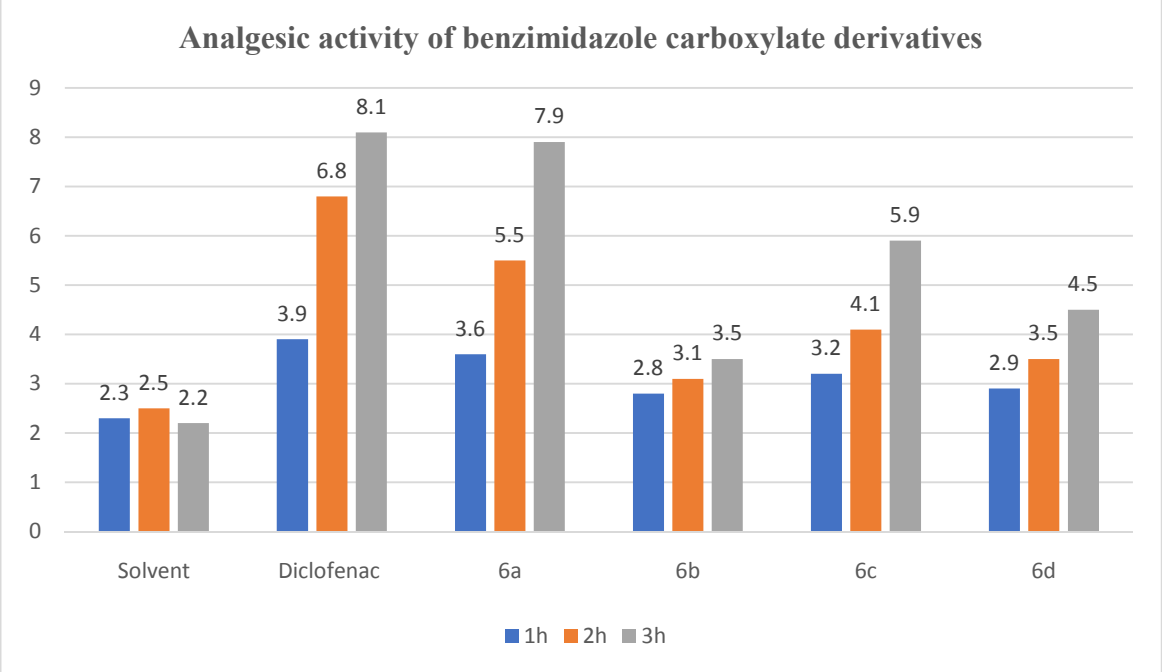


Figure 1 Analgesic activity of benzimidazole carboxylate derivatives

DISCUSSION

The results of this present study exhibited the significant analgesic activities for all of the test compounds (6a–6d). Analgesic activities observed in tail-flick tests clearly showed the pharmacological effects of these compounds on mechanical nociceptive pathways.As test compounds showed significant analgesic activities in tail-flick tests, it may be suggested that analgesic activities observed in the present study are related to both supraspinal and spinal mechanisms.It has been observed that the maximum analgesic activity was recorded by the compound 6a having 359 % analgesic activity followed by compound 6c (268 %), 6d(204 %)& 6b (159%) analgesic potential. It has been found that the reaction time increases significantly from $p<0.05$ to $p<0.001$ when compared with solvent control. The compounds 6ashowed nearly similar percentage analgesic activity compared to standard drug Diclofenac sodium, whereas 6c,6d & 6b registered percentage analgesic activity relatively less than standard drug. The compound 6athat possess maximum potency.

CONCLUSION

The analgesic activity evaluations of some benzimidazole-1-carboxylase were demonstrated in the present study. The compounds (6a–6d) exhibited statistically significant analgesic activities. Based on these findings, it can be concluded that all the compounds (6a–6d) act on nociceptive mechanisms.

The findings of this present investigation prove the hypothesis that benzimidazole-1-carboxylase possess antinociceptive activities. These newly synthesized benzimidazole carboxylate derivatives seemed to have analgesic like activity, so further investigations are needed to identify the mechanism of action. New N-substituted benzimidazole derivatives were synthesized and tested for analgesic activity. These newly synthesized benzimidazole derivatives seemed to have NSAIDs like activity, so further investigations are needed to identify the mechanism of action. Also studying the anti-inflammatory activity of these compounds possibly could lead to positive results as many drugs share both analgesic and anti-inflammatory activity New N-substituted benzimidazole derivatives were synthesized and tested for analgesic activity. These newly synthesized benzimidazole derivatives seemed to have NSAIDs like activity, so further investigations are needed to identify the mechanism of action. Also studying the anti-inflammatory activity of these compounds possibly could lead to positive results as many drugs share both analgesic and anti-inflammatory activity

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