Research Article

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Synthesis, characterization and biological evaluation of benzimidazole carboxylate derivatives

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ABSTRACT

The present investigation deals with preparation of total of four compounds of benzimidazole carboxylate derivatives, characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy and assayed biological test for analgesic activity. The analgesic activity of the synthesized compounds was evaluated by tail-flick model as per standard experimental procedure. 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&Aspirin at a dose of 50mg/kg b.w. was used on a parallel manner for comparison. The compound **6a**that possess maximum potency. The compounds **6c, 6d&6b** which showed 2nd,3rd& 4th most potent analgesic activity respectively.

Keyword: Analgesic activity, 2-(2-methyl-3-nitrobenzyl)-1H-benzimidazole,

INTRODUCTION

Inflammation is a complex defensive mechanism of the body to any noxious stimulus; this process may vary from a localized to a generalized response characterized by the accumulation of fluids and leukocytes leading to edema and pain [1] This inflammatory response seems to be mediated by different physiological and immunological mediators that play a role in acute and chronic inflammation[2]. Acute inflammation occurs as the initial response to tissue injury, being mediated by the release of autacoids, for example, serotonin, thromboxane, histamine and leukotrienes[3]. On the other hand, the chronic inflammatory process involves the release of diverse mediators, as interleukins, interferon and tumour necrosis factor a (TNF–a), and a cytokine that plays a major role in this kind of inflammatory process[4,5]
Benzimidazoles and its derivatives fulfil the minimum structural requirements for analgesic[7,8]. Encouraged by the aforementioned findings and in continuation of an ongoing program aiming at finding new gastroprotective leads with potential anti-inflammatory, analgesic activities, it was rationalized to explore novel benzimidazole derivatives. In the present research work, benzimidazole derivatives have been synthesized and bio-evaluated for their analgesic. Moreover, the drug-likeness properties of the resulted compounds are also presented.benzimidazole heterocyclic compounds with substituted carboxylate derivatives was being proposed.benzimidazole carboxylate derivatives weresynthesized and tested for analgesic activity.

RESULTS AND DISCUSSION

Chemistry

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

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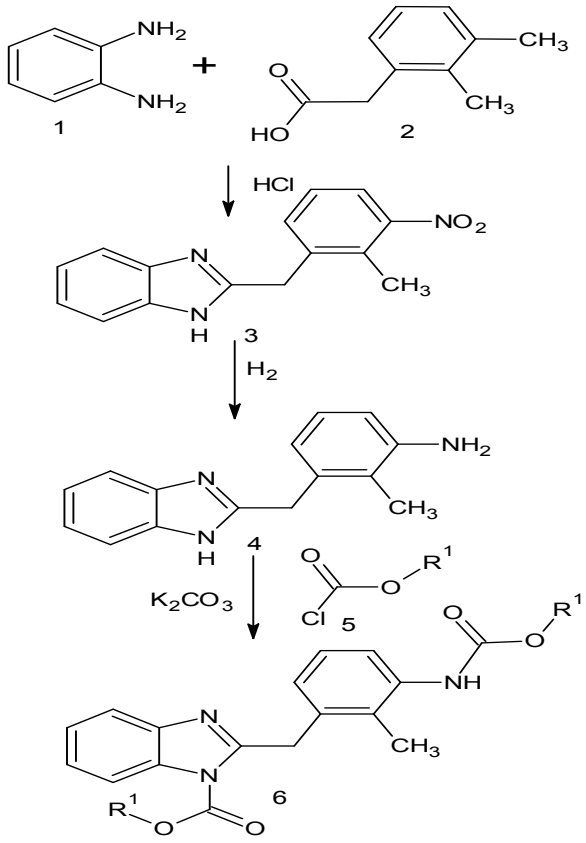
with an iodine vapor. IR spectra were recorded on a Shimadzu-Fourier transform infra-red (FTIR)-8400 Spectrophotometer using KBr disc. 1H NMR spectra were recorded on a Bruker DPX-400 MHz spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. The mass spectral data were obtained with a SHIMADZU-LCMS

Working scheme



Biological evaluation
Analgesic activity

The analgesic activity of the synthesized compounds (6a-6d) was evaluated by tail-flick model as per standard experimental procedure[67]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^{o}C ± 0.5^{o}C. The percentage analgesic activity is calculated by observing the following formula:

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

Biological results

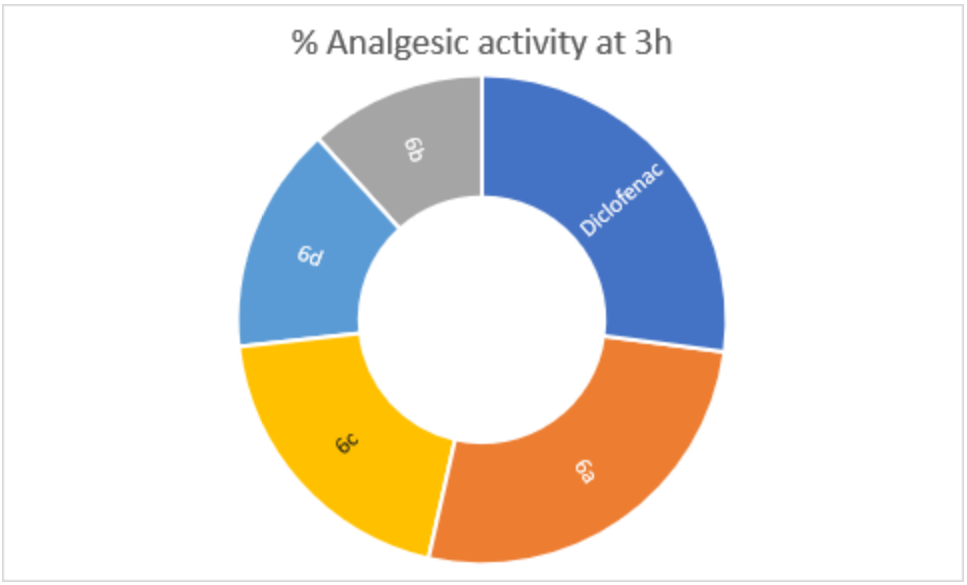
The study of analgesic activity by tail flick method showed that the percentage analgesic activity ranges between 159 and 359 at the end of 3 hrs, where as the standard drug Diclofenac sodium registered 368 (Table –2). It has been observed that the maximum analgesic activity was recorded by the compound **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 **6a**showed 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 **6a**that possess maximum potency. The compounds **6c,6d&6b** which showed 2nd, 3rd& 4th most potent analgesic activity.[13-15]

Table 1: 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.	7a1	3.6±0.04***	5.5±0.06***	7.9±0.15***	359.09
4.	$7a_2$	2.8±0.08**	3.1±0.13***	3.5±0.11***	159.09
5.	7b1	3.2±0.11***	4.1±0.14***	5.9±0.09***	268.18
6.	7b2	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).



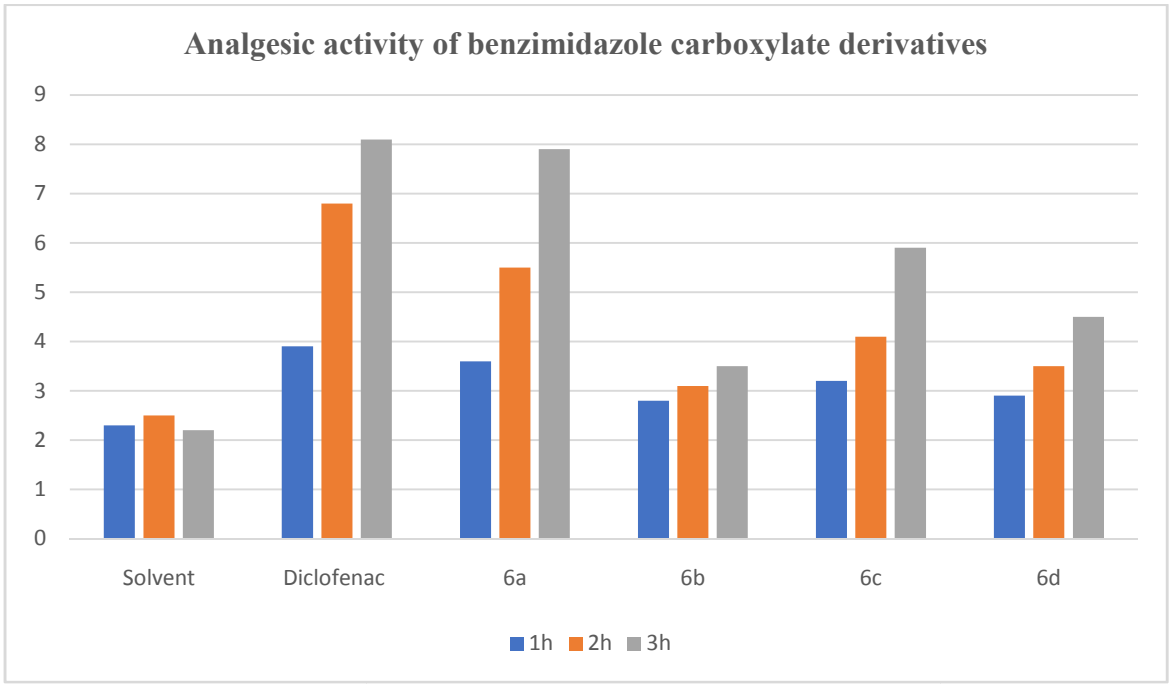


Figure 1 Analgesic activity of benzimidazole carboxylate derivatives

Experimental

General method of preparation

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

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.Thereactionmasswasrefluxat temperature 85°C for 3 hour. The product was precipitated by adding ammonia solution. There sulting precipitated solid was filtered and washed with water. The compound was re- crystallized from water and ethanol.[10,12]

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

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 reflux at temperature 90C-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).

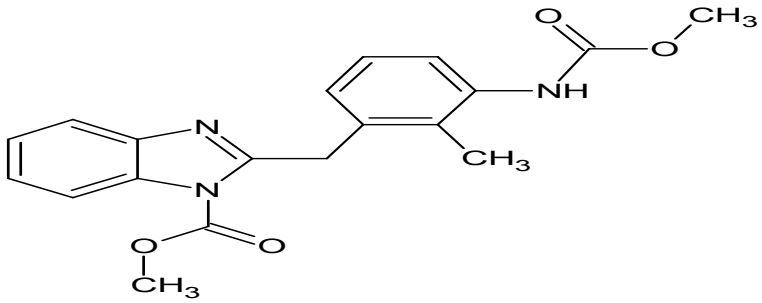
Preparation of substituted benzimidazole carboxylate

Acetone (15cm^3) was added to around-bottom glass flask equipped with a magnetic stirring bar. Potassium carbonate (8.28g,0.06mol) wasaddedtothereactionmassfollowedby3-(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[16-18].

Table:2 Characterization of synthesized compounds

Compound	R^1	Molecular formula	M.P	% Yield	Elemental Analysis					
					Calculated			Found		
					%C	%H	%N	%C	%H	%N
6a	CH_3	$C_{19}H_{19}N_3O_4$	107	78	64.58	5.42	11.89	64.66	5.51	11.95
6b	CH_2CH_3	$C_{21}H_{25}N_3O_4$	115	75	66.13	6.08	11.02	66.33	6.4	11.33
6c	$CH_2C(CH_3)_2$	$C_{25}H_{31}N_3O_4$	90	70	68.63	7.14	9.60	68.70	7.32	9.89
6d	$CH_2C_6H_5$	$C_{31}H_{27}N_3O_4$	140	72	73.65	5.32	12.66	73.75	5.48	12.79

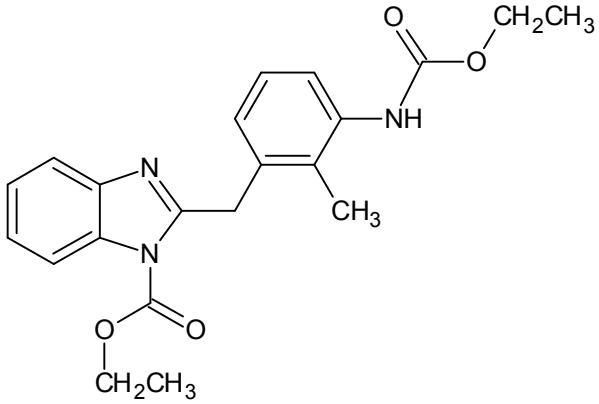
4.1Compound 6a (Methyl-2-{3-[(methoxy carbonyl) amino]-2methylbenzy1}-1H benzimidazole-l-carboxylate**)**
Molecular structure



Spectral Analysis of synthesized compounds

The IR spectrum of the compound **6a** obtained in KBr phase and expressed in cm^{-1} showed peaks at 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).The ^{1}H NMR spectrum of the compound **6a** in DMSO-d6 is interpreted in 6 ppm and showed peaks at 2.10 (s, 3H, -CH3), 3.64 (s, 3H, CO.00H3), 4.01 (s, 3H, -CO.00H3), 4.50 (s, 2H, -CH2), 6.83-7.22 (m, 4H, Ar-H), 7.30-7.63 (m, 3H, Ar-H), 8.92 (s, 1H, -NH).
The ^{13}C NMR spectrum of the compound **6a** in 6 ppmand obtained in DMSO-d6 phase showed the following peaks :13.84(CH_3),34.80(CH3),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).The mass spectrum of the compound **6a** in ESI mode showed the peak molecular ion m/z [M+1-1] at 353.9.

Compound 6b (ethyl 2- {3-[(ethoxycarbonypamino]-2-methylbenzyl} -1H-benzimi dazol e-l-carboxylate.)
Molecular structure

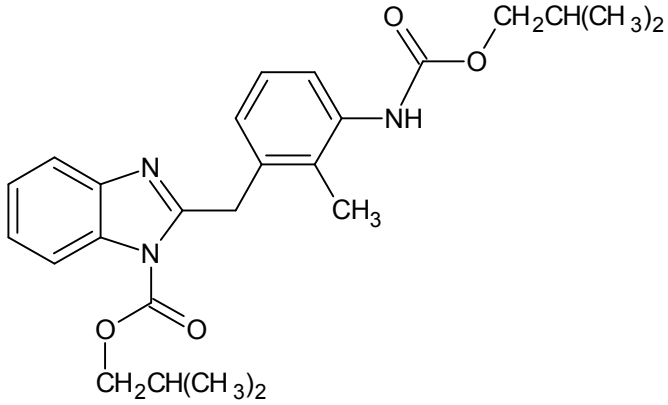


Spectral Analysis of synthesized compounds

The IR spectrum of the compound **6b** obtained in KBr phase andexpressed in cm^{-1} showed peaks at3429.8(C-H aromatic),3211.9-2978.5 (C=N str.), 1744.3 and 1724.0 (-0.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).The ^{1}H NMR spectrum of the compound **6b** in 6 ppm and obtained in DMSO-d6 phase showed the following peaks :1.21-1.24(t, 3H, -CH3), 1.32-1.36**(t,**3H,-CH3), 2.11(s, 3H,-CH3), 4.06-4.12 (q, 2H, -CH2), 4.42-4.8(q,2H, -CH2), 4.50 (s, 2H, -CH2), 6.78-7.96 (m, 7H, Ar-H), 8.88 (s,1H, -NH).The ^{13}C NMR spectrum of the compound **6b** in 6 ppm and obtained inDMSO-d6 phase showed the following peaks:13.74(CH3),13.84(CH3), 14.60 (CH3), 34.93 (CH3), 60.07 (OCH3), 64.05 (OCH3), 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(ArC),149.81(ArC),154.17(C=0),154.64 (C=0).The mass spectrum of the compound **6b** in ESI mode showed the peak molecular ion m/z [M+H] at 381.9.

Compound 6c (2-methyl propy1-2 - {3- [(2 methylpropoxycarbonyl) amino]2-methyl benzyl} -1H-benzimidazole-l-carboxylate.)

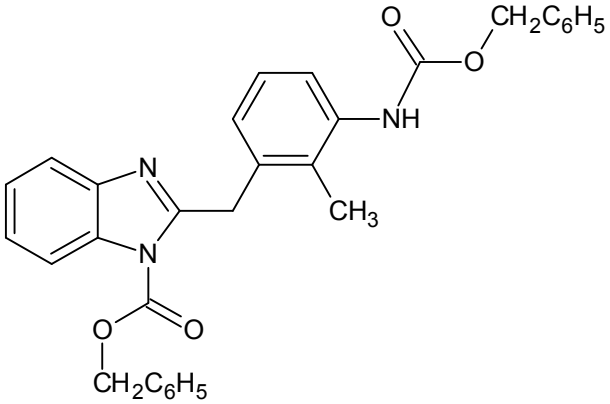
Molecular structure



Spectral Analysis of synthesized compounds

The IR spectrum of the compound 6c obtained in KBr phase and expressed in cm^{-1} showed peaks at3444.2(C-H aromatic), 3284.2-2875.3(-NH str.),1745 and1694.2(-0.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).The ^{1}H NMR spectrum of the compound 6c in DMSO-d6 is interpreted in 6 ppm and showed peaks at 0.919-0.964 (d, 6H, -C(CH3)2), 0.971-0.976(d, 6H,-C(CH3)2),1.87 -1.93(m,1H,-CH), 2.05-2.10 (m,1H,-CH), 3.83-3.85(d,2H, -CH2), 4.23-4.26(d,2H, -CH2), 4.51(s, 2H,-CH2), 6.80-7.96 (m, 7H, Ar-H), 8.89 (s, 1H, -NH).The ^{13}C NMR spectrum of the compound 6c in 6 ppm and obtained inDMSO-d6 phase showed the following peaks: 13.03(CH3),13.83(CH3), 18.84 (CH3), 18.91 (CH3), 27.14 (CH), 27.66 (CH), 35.00 (CH3),70.03(OCH2),73.69(OCH2),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=0), 154.78 (C=0). The mass spectrum of the compound **6c** in ESI mode showed the peak molecular ion m/z [M+H] at 438.0.

Compound 6d (Phenyl 2- {3-[(phenoxycarbonyl)amino]-2-methyl benzy1}-1H-benzimidazole-l carboxylate)
Molecular structure



Spectral Analysis of synthesized compounds

The IR spectrum of the compound **6d** obtained in KBr phase and expressed in cm-1 showed peaks at 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).
The ^{1}H NMIR spectrum of the compound **6d** in 6 ppm and obtained in DMSO-d6 phase showed the following peaks : 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).The ^{13}C NMR spectrum of the compound **6d** in 6 ppm and obtained inDMSO-d6 phase showed the following peaks:13.80(CH2),34.93(CH2),65.66(OCH2),69.26(OCH2),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).The mass spectrum of the compound **6d** in ESI mode showed the peak molecular ion m/z [M+H] at 506.

CONCLUSION

A total of 4 compounds of benzimidazole carboxylate derivatives were prepared, characterized by IR, ^{1} H NMR, ^{13} C NMR and Mass spectroscopy and assayed biological test for analgesic activity. The analgesic activity of the synthesized compounds was evaluated by tail-flick model as per standard experimental procedure. 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&Aspirin at a dose of 50mg/kg b.w. was used on a parallel manner for comparison. The compound **6a**that possess maximum potency. The compounds **6c, 6d&6b** which showed 2nd,3rd& 4th most potent analgesic activity respectively.New benzimidazole carboxylate derivatives weresynthesized and tested for analgesic activity. These newlysynthesized benzimidazole carboxylate derivatives seemed to have analgesic like activity, so further investigations are needed toidentify 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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