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Synthesis and Biological Evaluation of Benzimidazole derivatives

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Abstract
Benzimidazole derivatives are an important class of drugs because of their characteristic chemical and biological properties. They have been the centre of interest for medicinal chemists and pharmacologists. These derivatives are the basis of a vast array of drug compounds that can be used to fight a wide variety of diseases, from things like bacterial and fungal infections to long-term and complex diseases like cancer and high blood pressure. These are structurally versatile molecules with wide-ranging biochemical activity that makes them capable of interacting efficiently with many biological systems, allowing them to serve as multitarget drugs.The synthesis of benzimidazole derivatives has attracted much attention from chemists and numerous articles on the synthesis of this class of heterocyclic compound have been reported over the years. Present abstract deals with synthesis and biological activity evaluation of benzimidazole derivatives. All the compounds were characterized by UV, IR, [1]H NMR, mass spectral data and CHN elemental analysis.

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I. Introduction

Heterocyclic compounds are cyclic organic molecules in which one or more carbon atoms in the ring are replaced by a heteroatom [1-3]. The most common heteroatoms are nitrogen, oxygen, and sulphur [4-5].

Several biologically significant molecules, including enzymes, pharmaceuticals, proteins, alkaloids, and natural colouring agents, belong to this class of compounds. Based on the degree of saturation in the ring, heterocyclic compounds are broadly divided into saturated and unsaturated types. Saturated heterocyclic compounds are those in which the ring contains only single bonds and often show modified steric characteristics when compared with their acyclic counterparts. Tetrahydrofuran and piperidine are notable examples of saturated heterocycles

In recent times, benzimidazole has been acknowledged as the choice of moiety due to their role in different disease. Furthermore, it has been characterised as the main lead against the survivability of the different gram-positive and gram-negative strains and even acts as an effective therapy against the antibacterial agents that causes bacterial resistance. Taking all these influences into consideration and their emergence in further validation and repurposing of benzimidazole, the study is targeted to explore the present evidence on benzimidazole and its derivatives for their respective reported pharmacological activities. The present paper deals with synthesis and analgesic activity of synthesized benzimidazole derivatives.

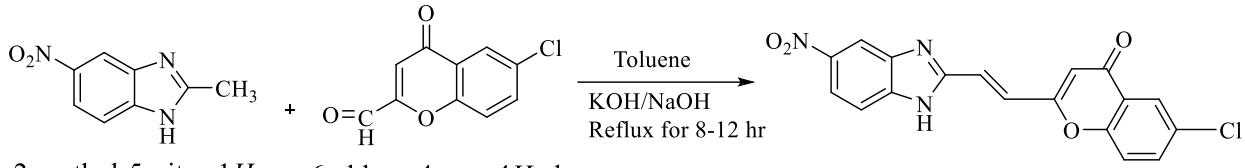
Experimental section

Melting point were determined in open capillary tubes and are uncorrected**.** The time required for the completion of the reaction was monitored by TLC using Silica gel G plates and spots were exposed in Iodine chamber.IR spectra were recorded on Perkin Elmer 1800(FTIR) spectrophotometer. IH NMR spectra (DMSO) were taken on a DRX – 300 spectrometer 300 MHz using TMS as internal standard and chemical shifts are expressed in ᵹ ppm.

II. Methodology:

Materials and Methods.

Synthesis



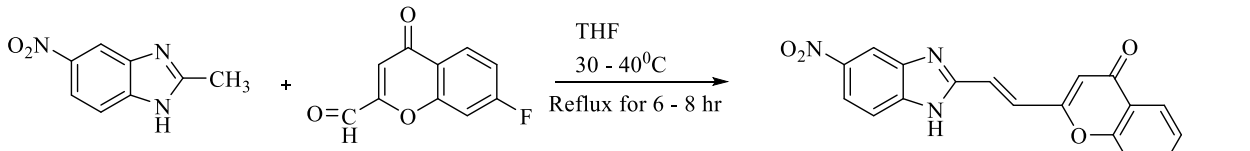
2-methyl-5-nitro-1*H*-benzo[*d*]imidazole
Chemical Formula: $C_8H_7N_3O_2$
Molecular Weight: 177

6-chloro-4-oxo-4*H*-chromene-2-carbaldehyde
Chemical Formula: $C_{10}H_5ClO_3$
Molecular Weight: 209

(*E*)-6-chloro-2-(2-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4*H*-chromen-4-one
Chemical Formula: $C_{18}H_{10}ClN_3O_4$
Molecular Weight: 368

Common Synthetic Routes
Method:
Reactants: Toluene, 2-methyl-5-nitro-1H-1,3-benzimidazole and 6-chloro-4-oxochromene-2-carbaldehyde.
Reaction: Take toluene in round bottom flask, then add 2-methyl-5-nitro-1H-1,3-benzimidazole. Stir for 5 minutes, then add 6-chloro-4-oxochromene-2-carbaldehyde slowly over 1 hour. After completion of addition, stir for 5 minutes, then add a catalytic amount of base, KOH, or NaOH and heat the reaction mass slowly up to reflux for 8-12 hrs. Check TLC or HPLC for the absence of the starting material if itis not present in the reaction mass. Heat the reaction mixture for 2-3 hrs. more under reflux condition. When the reaction is complete, cool the reaction mass to 20-300 °C, add water, stir for 5 minutes, settle the reaction mass, and separate the organic layer. Extract the water layer with toluene (2 x 10 ml), settle the reaction mass, and separate the organic layer. Collect all organic layers, dry over sodium sulphate, and remove excess solvent. The compound was purified by recrystallisation at 0-5 °C. filter and wash with chilled toluene at 0-5 °C, dry material in a vacuum oven for 3-5 hrs. at 40-50^{0}C.
Mechanism Methodology:
The reaction would likely be a Knoevenagel condensation or a similar base-catalyzed condensation reaction. The reaction between 2-methyl-5-nitro-1H-1,3-benzimidazole and 6-chloro-4-oxo-4H-1- benzopyran-2-carbaldehyde is a condensation reaction. The process begins with the deprotonation of the acidic methyl group on the benzimidazole ring by a base, creating a carbanion. This nucleophilic carbanion then attacks the electrophilic carbonyl carbon of the carbaldehyde. The resulting intermediate undergoes dehydration to form a final product containing a carbon-carbon double bond between the two ring systems. This type of reaction is an example of a Knoevenagel condensation.

3.4.2 Compound SC_2
Methodology:



2-methyl-5-nitro-1*H*-benzo[*d*]imidazole

7-fluoro-4-oxo-4*H*-chromene-2-carbaldehyde

(*E*)-7-fluoro-2-(2-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4*H*-chromen-4-one

Common Synthetic Routes
Method:
Reactants: 2-methyl-5-nitro-1H-1,3-benzimidazole and fluoro-4-oxo-4H-1- benzopyran-2-carbaldehyde.
Reaction: Take THF in a RB flask and add 2-methyl-5-nitro-1H-1,3-benzimidazole stir for 15 minute at room temperature slowly raise the temperature 30-40^{0}C then start slow addition of 7-fluoro-4-oxo-4H-1- benzopyran-

2-carbaldehyde to it, if temperature raise stop addition when temperature at controlled condition start again addition of reacting compound slowly after completion of the reaction stir the mass for 15 minute then raise temperature slowly up to reflux and maintain reflux temperature for 6-8 hrs, check TLC and HPLC to monitor unreacted compound if reaction completed cool reacting mass and take for workup add water in reaction mass stir for 5 minute to settle. Separate the organic layer, wash the aqueous layer with THF and settle and separate the organic layer. Remove excess solvent from the organic layer at 30-50 °C under vacuum distillation to an oily mass, then charge ethyl acetate into the oily mass, stir for 15 minutes at 40-50 °C, stop heating, and cool the reaction mass to 20-30 °C.
Cool the reaction mass to 0-50 °C for recrystallisation, stir for 1 hour, filter the mass under vacuum and dry in a vacuum oven at 30-450 °C for 3-4 hrs.

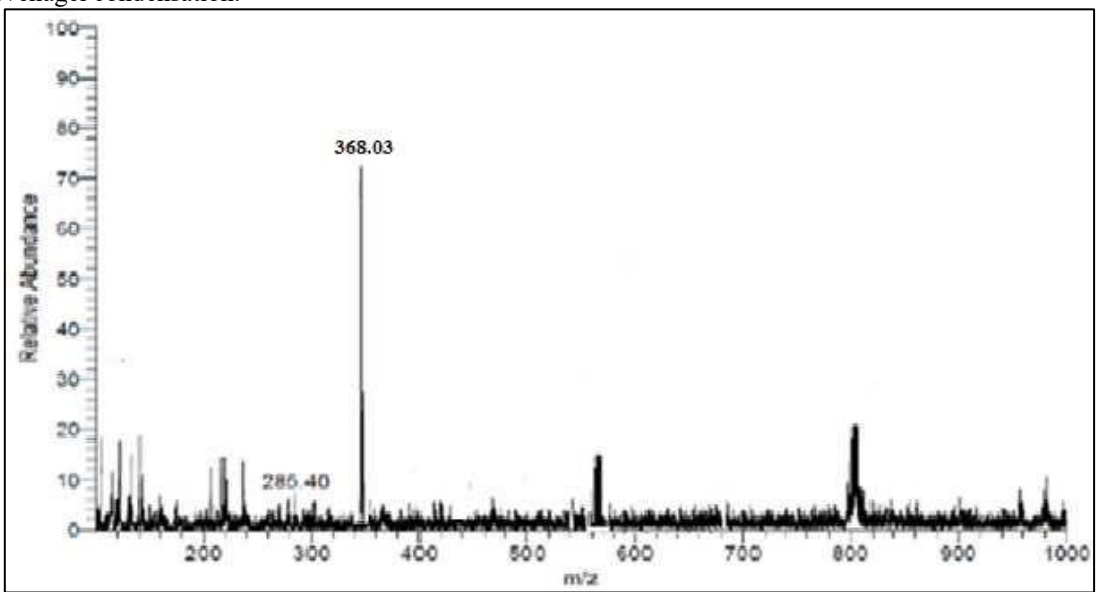
Mechanism Methodology: The final product of this reaction is a compound that links the benzimidazole and benzopyran rings through a vinyl group (−CH=CH−). The process is a Knoevenagel condensation, which is a type of dehydration reaction.

Characterisation of Synthesized Compounds
4.4.1 Compound SC_1

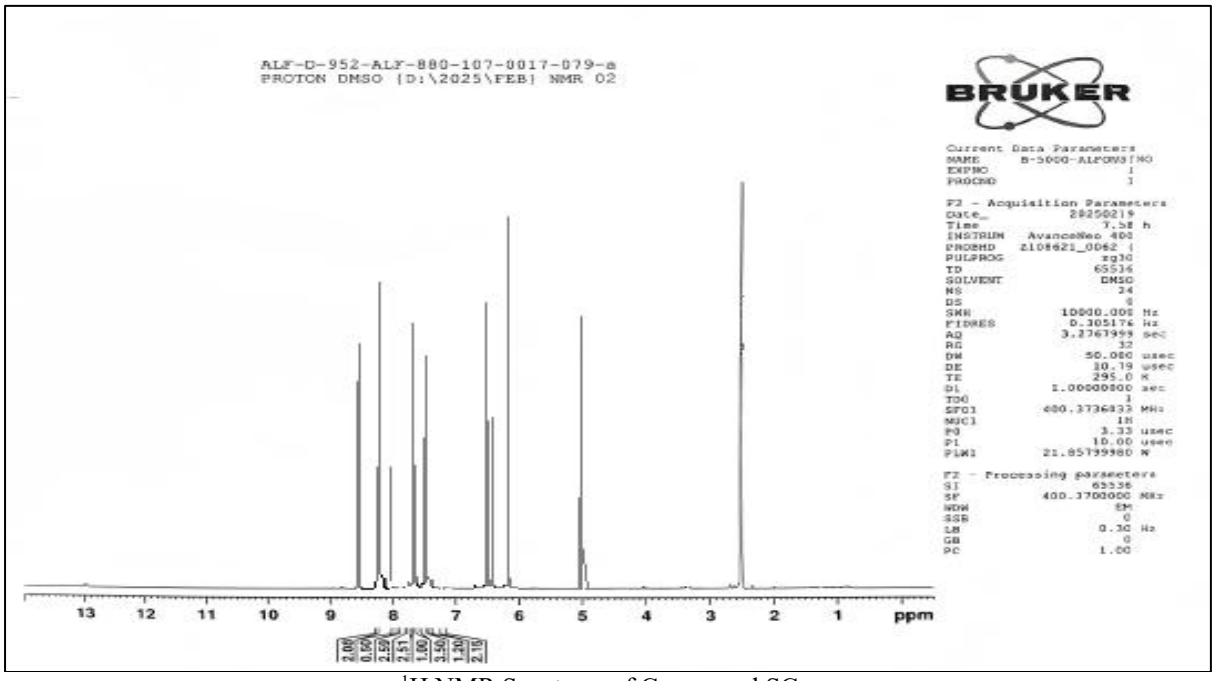
Physical Appearance	Crystalline orange solid
Chemical Formula	$C_{18}H_{10}ClN_3O_4$
Molecular weight	368g
M.P.	218-220°C
Density	1.1-1.3 g/cm^3
Elemental Analysiss	C-58.79; H-2.74; Cl-9.64; N-11.43; O-17.40
Pka	5-6
Yield	39%
Mass Fragmentation	368 $M^{+\cdot}$ 280,199,147,137

IR(KBr) Cm^{-1}:3158,3095,1634,1644,724.
ES - MS:m/z368.03.
^{1}HNMR(400MHz,DMSO, δ ppm): 8.39(s,1H),8.07(d,*J*=7.1Hz,1H),8.10(m,2H),7.86–7.77(m,3H),6.39–6.37(d,1H),6.65(s,1H),5.0(s,NH).

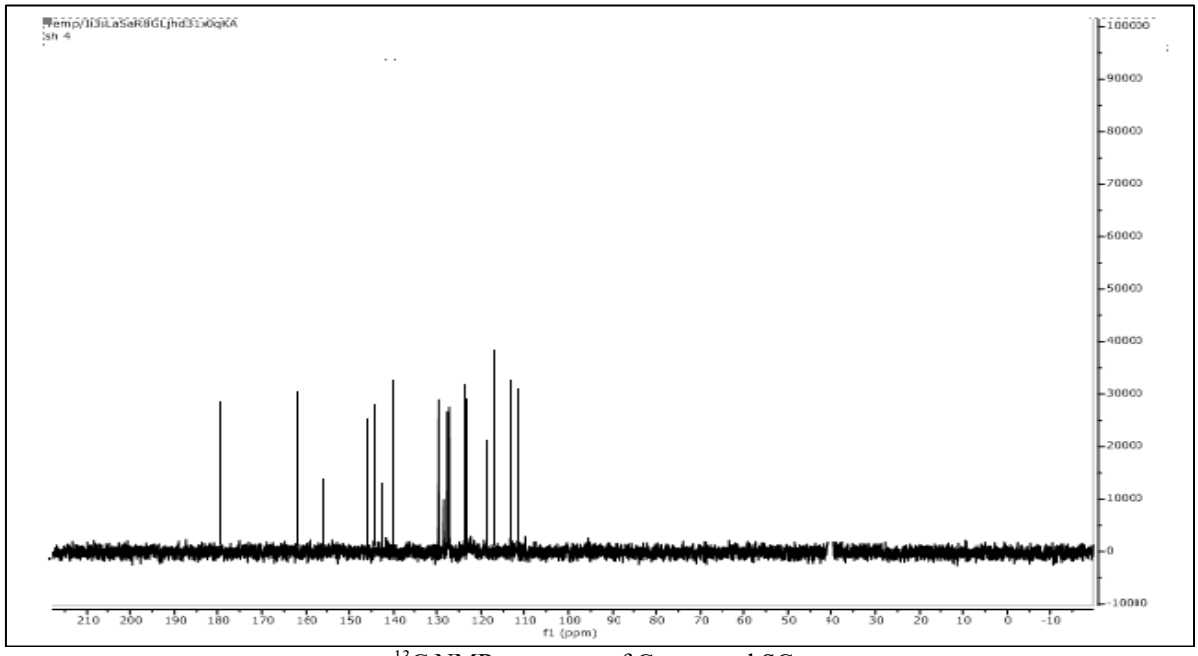
^{13}NMR (100MHz, DMSO, δ ppm). Check: 178.5,163.2,155.3, 144.3,145.0, 141.5, 139.8, 139.4, 145.0,130. 6, 129.0,128.8,125.5, 125.3, 119.6, 118.6,116.1, 112.2.
Knoevenagel condensation.



Mass Spectrum of Compound SC_1



^{1}H NMR Spectrum of Compound SC_1

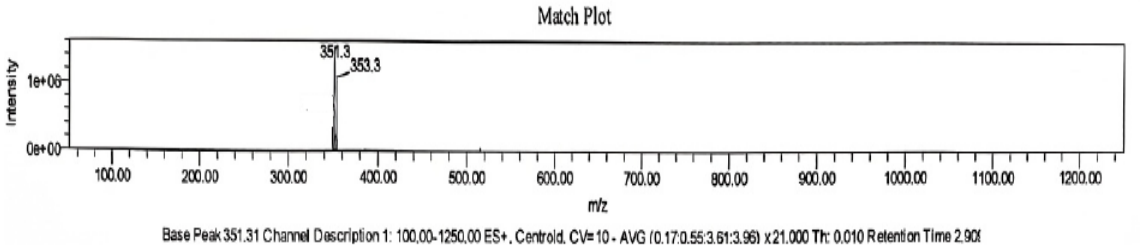


^{13}C NMR spectrum of Compound SC_1

4.4.2 Compound SC_2

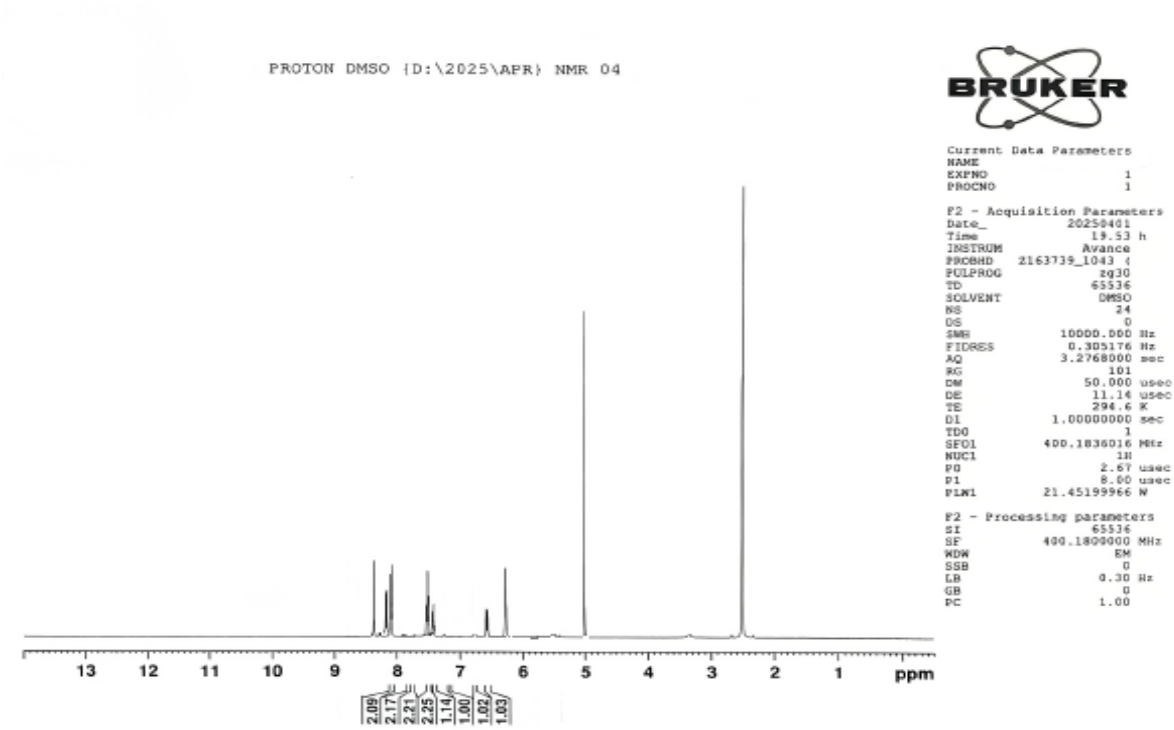
Physical Appearance	Crystalline Yellow solid
Chemical Formula	$C_{18}H_{10}FN_3O_4$
Molecular weight	351g
M.P.	210-212°C
Density	1.40 - 1.55 g/cm^3
Elemental Analysis	C-61.54; H-2.87; F-5.41; N-11.96; O-18.22
Pka	9-11
Refractive Index	0.41 - 0.40
Yield	42%
Mass Fragmentation	351 M^+ ,353 $M+2H^+$

IR(KBr) Cm^{-1}:3157,3095,1644,1317,1124,824,726.
GC/MS: (m/z)351

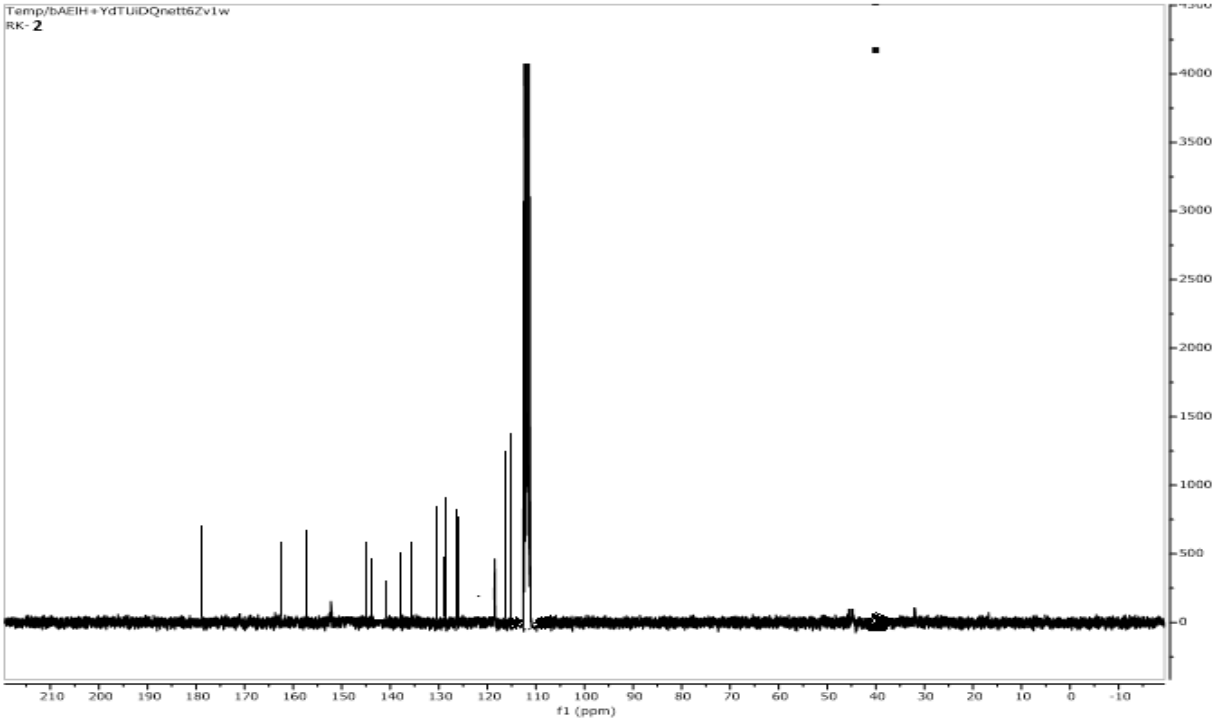


Mass Spectrum of Compound SC_2

^{1}HNMR(400MHz,DMSO, δ ppm): 8.39(s, 1H);8.17(d,*J*=7.1Hz,1H);8.1(m,2H); 7.56 -7.67(m,3H);6.36 -6.31(d,1H); 6.65(s,1H);5.0(s,NH).
^{13}NMR (100MHz, DMSO, δ ppm): 178.5,163.2,155.3, 144.3,145.0,141.5, 139.8, 136.4, 145.0,130. 6, 128.7,128.8,125.5, 125.3, 119.6, 117.6,116.1, 111.2.



^{1}H NMR Spectrum of Compound SC_2



^{13}C NMR spectrum of Compound SC_2

Aanalgesic activity

The analgesic activity was carried out by Tail-flick method using Swiss albino mice. In this method, heat is used as a source of pain. Overnight fasted healthy and adult male Swiss albino mice weighing between 20 g and 25 g, in a group of six each were taken for the investigation. The animals were kept into a small cage with an opening for the tail at the rear wall. The tail was held gently and a light beam exerting radiant heat was directed to the proximal third of the tail. The tip of the tail of the mice was individually placed on the radiant heat source at constant temperature 55 °C [6]. The cut-off reaction time was fixed at 15 s to avoid tissue damage. The tail flick response was measured at 0- 5 hours after treatment of test compounds by digital analgesiometer (INCO, Ambala, India). The drug pentazocine (3.9 mg/kg, i.p.) was used as standard drug for comparison and test groups received synthesized benzimidazole derivatives at 100 mg/kg p.o.

The analgesic activity

The analgesic activity revealed that almost all the compounds showed very potent analgesic activity when compared with standard pentazocine. Among the tested compounds **SC1 and SC2**showed profound analgesic activity. The rest of the compounds **s**howed moderate activity when compared with the control.

Table 1. Analgesic activity of benzimidazole derivatives on mice by using tail-flick method.

Compounds code	TW time in second (Mean ± SEM)				
	0 h	1 h	2 h	3 h	4 h
Control	1.76 ± 0.13	1.98 ± 0.14	2.53 ± 0.119	2.63 ± 0.20	2.89 ± 0.52
Standard	1.86 ± 0.56	4.5 ± 0.44	7.13 ± 0.21##	8.12 ± 0.27#	9.53 ± 0.29##
SC_1	2.4 ± 0.31	5.37 ± 0.19	4.19 ± 0.48#	6.13 ± 0.30#	5.16 ± 0.28##
SC_2	2.19 ± 0.27	4.22 ± 0.32	5.6 ± 0.41#	4.36 ± 0.27#	3.89 ± 0.45#

n = 6 animals in each group.

# $p < 0.05$ vs control.
$p < 0.01$ vs control

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