SYNTHESIS OF SUBSTITUTED 2-(4-BROMOPHENYL)-1H-BENZO [D] IMIDAZOLE DERIVATIVES AND THEIR EVALUATION FOR ANTIDEPRESSANT ACTIVITY

Kanchan Yadav[*1], Preeti Yadav[2], Muskan Bhardwaj[3]

Abstract

The current study was based upon the synthesis of derivatives of substituted 2-(4-Bromophenyl)-1h-Benzo [D]Imidazole and the evaluation for antidepressant effect of the synthesized benzimidazole derivatives. The synthesized derivatives were evaluated for melting point determination, TLC, infrared, mass, NMR and Docking. Albino rats of either sex weighing 130-160g were obtained from the Animal House, Department of Pharmacy, IIMT University, Meerut (UP) India. The animals are maintained in proper conditions, at room temperatures of 25 ± 1°C with 12-hour light/dark cycle. Rats were divided into 4 group (n=6) and treated for 21 days i.e., group 1 was administered normal saline, group 2 administered fluoxetine (20mg/kg, orally), group 3 and group 4 administered all the novel derivatives of benzimidazole at 100mg/kg, orally and 200mg/kg, orally, respectively. Thin layer chromatography is used in synthetic chemistry to confirm the production of a molecule based on its Rf value, which varies depending on the compound. Rf value was obtained as 0.79, 0.73, 0.71, 0.83, and 0.76 of C1, C2, C3, C4, and C5, respectively. Antidepressant activity was evaluated through parameters including Tail Suspension Test, Motor coordination test and Locomotion Activity. In results, it showed that mass spectroscopy showed near molecular weight as estimated. TST and Rota-rod models exhibited highest level of decrease in time of motion that indicated for their anti-depressant potential. Actophotometer also shown moderate anti-depressant action while in the models, effect was observed in dose dependent manner. In conclusion, depression can be treated using synthesized substituted benzimidazole derivatives, which alleviate symptoms and boost mental health to help you in elevated mood. Its mode of action must be explained in detail before it can be considered for treatment of various mental health problems.

Keywords: benzimidazole derivatives, synthesis, melting point, antidepressant, TST.

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1. Introduction

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest (Wang, 2015). The common features of all the depressive disorders are sadness, emptiness, or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual's capacity to function (Ormel et al. 2019). As per WHO, depression is a prevalent mental illness. According to estimates, 5 percent of adults worldwide experience depression. The largest cause of disability in the world today is depression, which also significantly contributes to the overall burden of sickness on the planet. Depression affects more women than males. Suicide can result from depression (Charney, 2003; WHO, 2022). Common depression vulnerabilities include those related to cognition, interpersonal relationships, and personality traits (National Academies Press, 2009). Many studies have shown that stress hormones and psychosocial induced-stress, and neurotransmitters such as serotonin, noradrenaline, DA, Glutamate & GABA, neurotrophic causes & circadian rhythms are leading cause behind depression (Hasler, 2010; Thibaut, 2017). Persons with depression are generally mis-diagnosed and treated with antidepressants in countries of all income levels (Evans-Lacko et al. 2018; Gorman et al. 2000).

Benz imidazole: Benz imidazole is an organic molecule with a heterocyclic aromatic ring. The two aromatic rings of benzene and imidazole can be fused together to form this bicyclic molecule. It's a white solid that crystallizes in tabular shapes. Heterocyclic compounds are essential for life and extensively distributed in nature. A significant role has been played by heterocyclic compounds in the metabolism of all living cells. The nitrogen based heterocyclic compound play important role for mankind. Particularly benzimidazole has an immense importance not only biologically but also industrially among the entire nitrogen based heterocyclic compound.

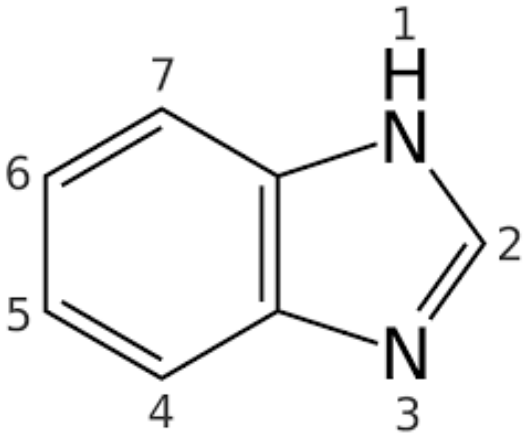


Fig 1. Benz imidazole

IUPAC name: 1H-benzimidazole
Molecular formula: C7H6N2
Molar mass: 118.14 g/mol

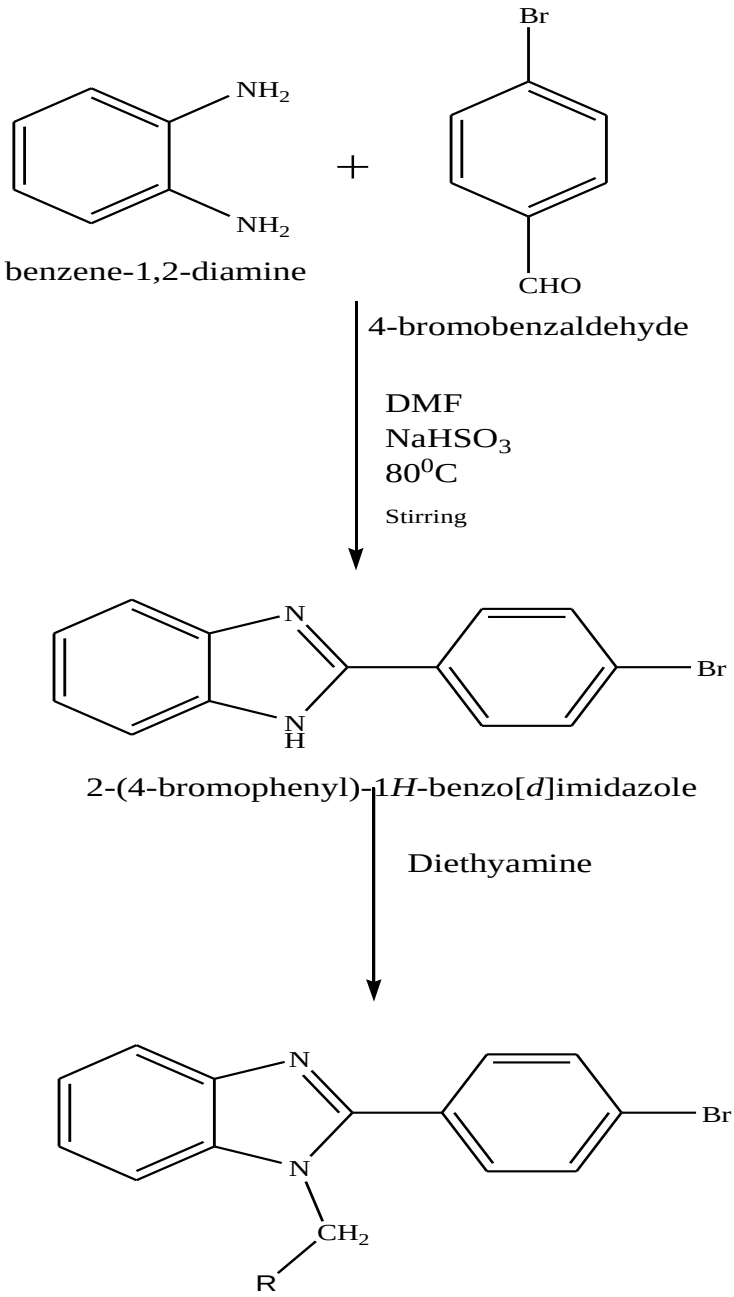
First benzimidazole derivative synthesized by Hobrecker in 1872. The first research paper on pharmacological properties of benzimidazole published by Goodman and Nancy Hart in 1943. Then Woolley reported the antibacterial activity of some benzimidazole derivatives in 1944. Afterward from the acid hydrolysis of Vitamin B-12, Norman GB and Karl Folker in 1949 reported 5, 6-dimethyl benzimidazole as a degradation product. After long research, it concluded that benzimidazole is important heterocyclic system because it exhibits biological activity against a number of pathogens and physical disorders (Yusuf et al. 2010; Anand 7 Wakode, 2017).

The current study was based upon the synthesis of derivatives of substituted 2-(4-Bromophenyl)-1h-Benzo[D]Imidazole and the evaluation for antidepressant effect of the synthesized benzimidazole derivatives.

2. Materials and Methodology

Experimental Requirements: Benzene-1,2-diamine, 4-bromobenzaldehyde, distilled water, ethanol, methanol, weighing balance, digital pH meter, hot air oven, laboratory thermometer, round bottom flask.

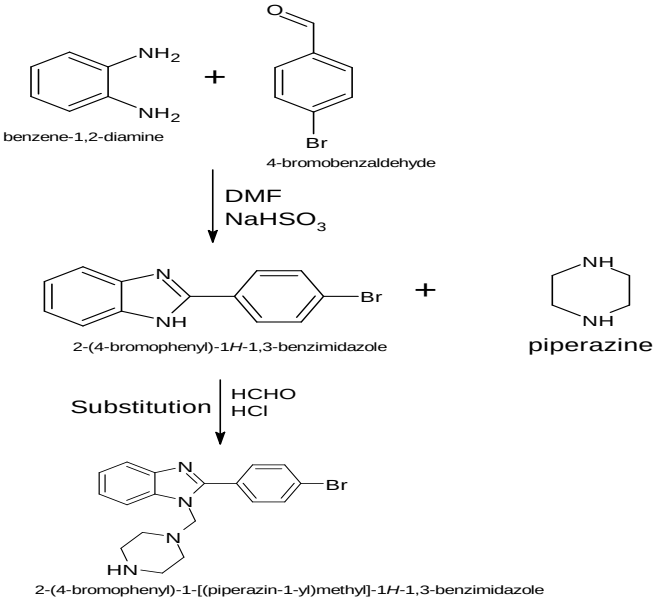
Synthesis of substituted 2-(4-Bromophenyl)-1h-Benzo [D] Imidazole



Scheme for substituted benzimidazole synthesis

The Benzene-1,2-diamine and 4-bromobenzaldehyde were made reacted in the presence of Sodium bisulfite at the temperature of 80ºC. Thus, intermediate was obtained as 2-(4-bromophenyl)-1H-benzo[d]imidazole. The produced intermediate upon reaction with diethylamine produced the derivative compound 2-(4-bromophenyl-1-Substituted ethyl-1H-benzo[d]imidazole

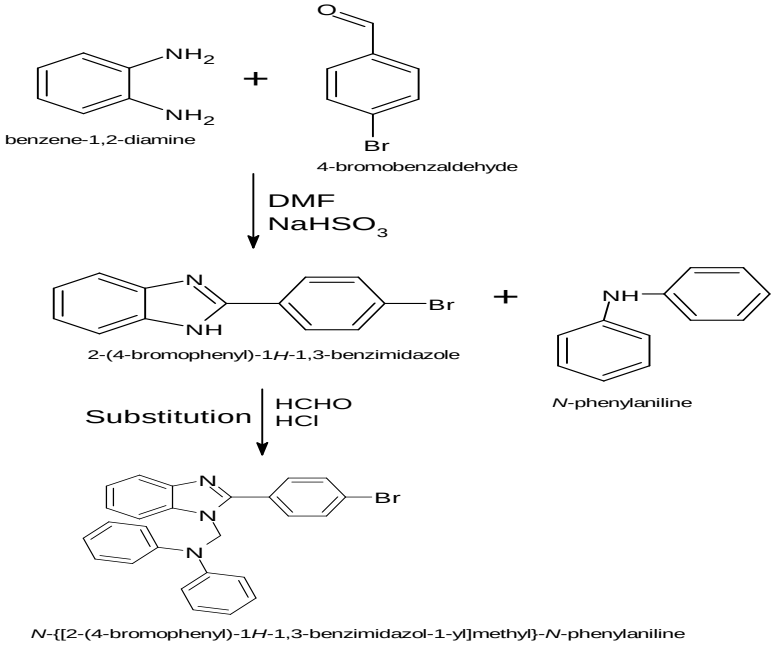
Procedure of synthesis (C1)



Synthesis of benzimidazole derivative (C1)

Upon reaction of the Benzene-1, 2-diamine with 4-bromobenzaldehyde in the presence of Sodium bisulfite at the temperature of 80ºC. Thus, intermediate was obtained as 2-(4-bromophenyl)-1H-1,3benzimidazole. The produced intermediate upon reaction with piperazine the derivative compound 2-(4-bromophenyl)-1-[(piperazin-1-yl) methyl]-1H-1, 3-benzoimidazole.

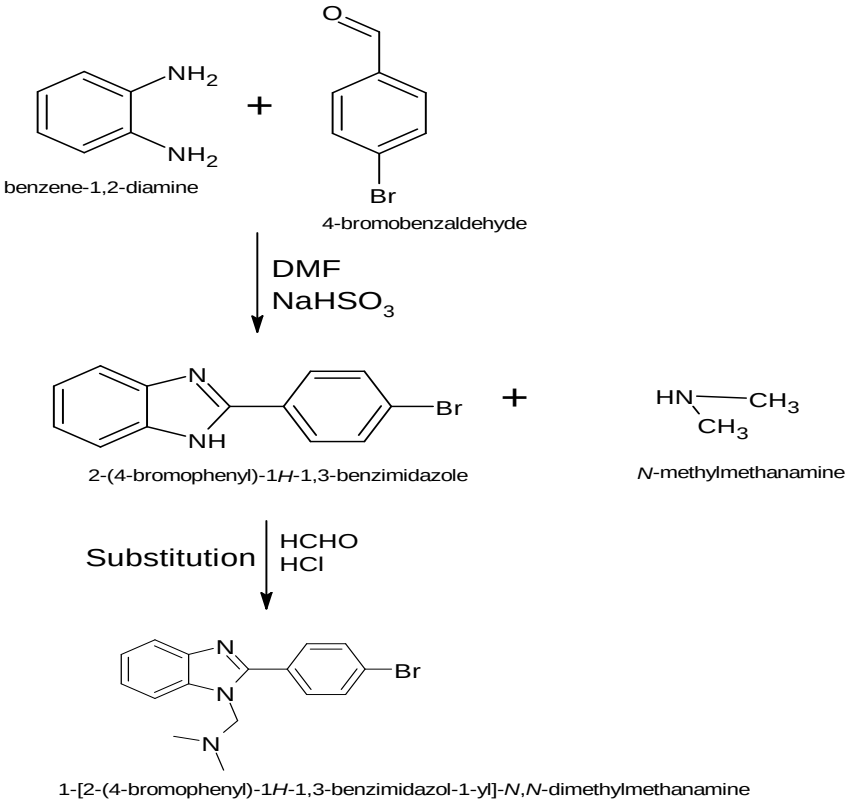
Procedure of synthesis (C2)



Synthesis of benzimidazole derivative (C2)

The Benzene-1,2-diamine made reacted with 4-bromobenzaldehyde in the presence of Sodium bisulfite at the temperature of 80ºC that produced intermediate as ate was obtained as 2-(4-bromophenyl)-1H-1,3benzimidazole. The produced intermediate upon reaction with N-phenylaniline produced the final compound N-{[2-(4-bromophenyl)-1H-1, 3-benzoimidazole-1-yl] methyl}-N-phenylaniline.

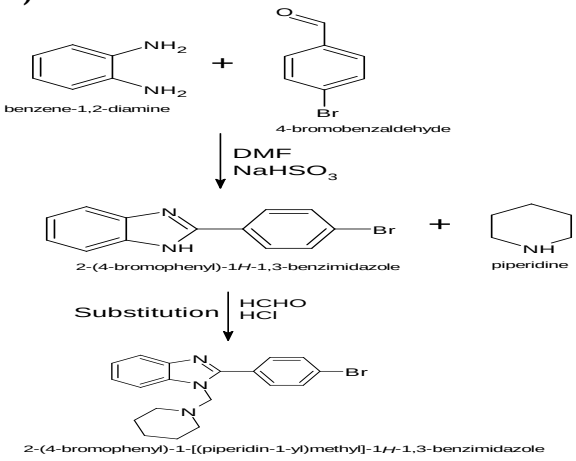
Procedure of synthesis (C3)



Synthesis of benzimidazole derivative (C3)

The Benzene-1, 2-diamine made reacted with 4-bromobenzaldehyde in the presence of Sodium bisulfite at the temperature of 80ºC that produced intermediate as 2-(4-bromophenyl)-1H-1,3benzimidazole. The produced intermediate upon reaction with N-methylmethanamine produced the final compound 1-[2-(4-bromophenyl)-1H-1, 3-benzoimidazol-1-yl]-N, N-methylmethanamine.

Procedure of synthesis (C4)

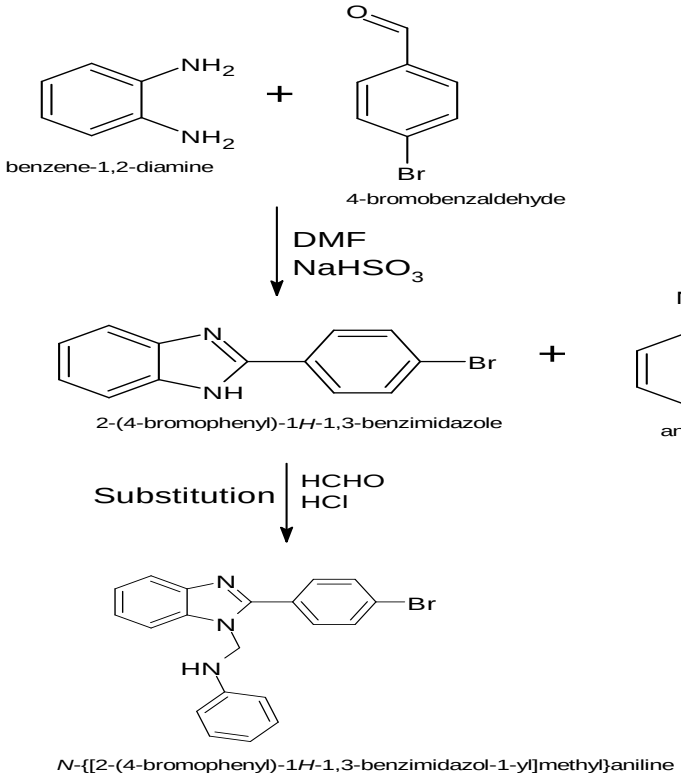


Synthesis of benzimidazole derivative (C4)

Upon reaction of the Benzene-1, 2-diamine with 4-bromobenzaldehyde in the presence of Sodium bisulfite at the temperature of 80ºC. Thus, intermediate was obtained as 2-(4-bromophenyl)-1H-1,3benzimidazole. The produced intermediate upon reaction with piperidine produced the final compound 2-(4-bromophenyl)-1-[(piperidin-1-yl) methyl]-1H-1, 3-benzoimidazole.

Procedure of synthesis (C5)

By same process and reactants, the intermediate I was produced. Thus, intermediate was obtained as 2-(4-bromophenyl)-1H-1,3benzimidazole. The produced intermediate upon reaction with aniline produced the derivative compound N-{[2-(4-bromophenyl)-1H-1, 3-benzimidazol-1-yl] methyl} aniline.



Synthesis of benzimidazole derivative (C5)

Characterization Parameters

Melting point determination: Melting point tube was used to determine the melting point of an organic compound (capillary tube method). The most important and straightforward means of distinguishing one compound from another is to determine its melting point.

Thin Layer Chromatography TLC stands for thin layer chromatography and is used in synthetic chemistry to infer the production of a molecule based on its RF value, which varies depending on the compound. It also aids in confirming the reaction's progress.

Infrared Spectroscopy: Infrared spectroscopy is one of the most essential methods for determining different functional groups and probable chemical structures. The main benefit of IR over other techniques is that it easily produces fingerprints (1300-650/cm) of molecules' structure (functional group, associating with one other). There are no two compounds with the same fingerprint region. This method is based on the molecular vibration of the chemical, which causes each bond to vibrate at a particular frequency, which corresponds to the IR frequency. As a result, IR spectra of each bond was created. On a Jasco V410, FTIR spectra were obtained in KBr powder.

NMR Spectroscopy: By exposing a substance to two magnetic forces, one fixed and the other fluctuating at a radio frequency, the interaction between matter and electromagnetic forces can be seen. The sample detects energy at a certain combination of fields, and absorption is detected as a change in single developed by a radio frequency detector and amplifier. The magnetic dipolar character of a spinning nucleus can be linked to this absorption energy. Nuclear Magnetic Resonance is the name for this technology. This method is beneficial for determining the molecule's structure. A Bruker Ultraspec 500MHz/

AMX400MHz spectrometer was used to measure 1H- NMR spectra in CDCl3 and d6-DMSO.

Mass Spectroscopy: In this method, a beam of powerful electrons is used to repeatedly strike individual molecules. After being ionised, the molecules disintegrate into a plethora of pieces, some of which are positive ions. The mass-to-charge ratio, or m/e, is unique for each ion type. Most ions have a single charge, making their m/e ratio equal to their molecular mass. Mass spectra are obtained by detecting and recording signals from moving ions as they go through a system of magnetic and electric fields to a detector.

Molecular docking: Molecular Docking calculations of benzimidazole derivatives (C1-C6) was done on serotonin protein site of the cells performed using SwissDock (http://swissdock.vital-it.ch/) web service based on the docking software EADock DSS. This web-based service was selected because it has user friendly interface with the facility to input desired protein and ligand structures directly from databases, modify docking parameters, and visualize most favorable clusters online. The structure of compounds was drawn in Chemsketch and subject to energy minimization.

Preparation of animals: Albino rats of either sex weighing 130-160g were obtained from the Animal House, Department of Pharmacy, IIMT University, Meerut (UP) India. The animals are maintained in proper conditions, at room temperatures of 25±1°C with 12-hour light/dark cycle. The relative humidity is maintained at 44-56%, and are fed with standard rodent diet and water ad libitum. Animals will keep on fasting but free access to water up to 1 h before initiation of study (Siddiqui et al. 2008).

Experimental protocols
Rats were divided into 4 group (n=6) and treated for 21 days.
Group 1- rats are administered normal saline (vehicle) daily.
Group 2- rats are administered fluoxetine (20mg/kg, orally).
Group 3- rats are administered all the novel derivatives of benzimidazole (100mg/kg, orally).
Group 4- rats are administered all the novel derivatives of benzimidazole (200mg/kg, orally).

Evaluation Protocols
Tail Suspension Test: The TST was conducted as described in detail earlier (Kendler et al. 2001). In this experiment, mice were dangled 30cm in the air by an adhesive tape attached to the base of their tails. When animals stopped making any attempts to escape and froze, we considered them to be immobile. The final four minutes of the six-minute test session were used to determine the total length of the immobile behavior. Mice that tried to escape the testing by climbing their tails were thrown out (Gamze et al. 2018).

Motor coordination test in this experiment, we employed a horizontal spinning rod that made 20 revolutions per minute. Only mice that lasted longer than 180 seconds on the rod were selected. Diazepam was administered to the mice 15 minutes prior to the experiment, and the rodents were placed on it for 180 seconds half an hour after receiving the vehicle or medication. After dropping from the rotating pole, the researchers timed how long it took each mouse to reach the ground (Silva et al. 2006).

Locomotion Activity: For reliable readings, the actophotometer must first be turned on and checked to ensure that all photocells are operational. Every rat spends 10 minutes every session in an activity cage. Until 10 minutes have passed, a rat's activity level is recorded. After all that, we check in on the movers and see how they stack up against the gold standard, diazepam (Stahl et al. 2004).

3. Results and Discussion

Synthesized derivatives
Novel substituted 2-(4-Bromophenyl)-1h-Benzo [D] Imidazole derivatives were developed specified scheme. The procedure was followed as conventional tool for the benzimidazole synthesis as mentioned in materials and methods section. After synthesis,

all the derivatives were characterized i.e., physical parameters, % yield, melting point, and molecular weight.

Identification of Physical Properties

Melting point determination: For substituted 2-(4-Bromophenyl)-1h-Benzo[D]Imidazole derivative, the melting point was determined as 201ºC, 241ºC, 227ºC, 204ºC, and 243ºC for compounds C1, C2, C3, C4, and C5, respectively.

Thin Layer Chromatography: Thin layer chromatography is used in synthetic chemistry to confirm the production of a molecule based on its Rf value, which varies depending on the compound. Rf value was obtained as 0.79, 0.73, 0.71, 0.83, and 0.76 of C1, C2, C3, C4, and C5, respectively. Benzimidazole derivatives were tested for their physical properties i.e., percentage yield, melting point, molecular weight, and functional groups attached with were tested. C1 and C4 were demonstrated for its highest % yield as 73.49% and 70.53%. Lowest % yield was seen in C3 as 66.20%. The highest melting point was found in compound C4 as 251ºC. Highest melting point indicates about the strongest density of the compound. Molecular weight was also found significant in the analogues of benzimidazole developed. Molecular weight was found as 351.32, 364.45, 371.17 and 354.11 for C1, C2, C3 and C4 respectively. The following table summarized physical properties of all the compounds.

Table 1. Physical properties of synthesized substituted 2-(4-Bromophenyl)-1h-Benzo[D]Imidazole derivatives

Compound	Yield (%)	Rf Value	Melting point	Molecular weight
C1	73.49%	0.79	201ºC	351.32
C2	68.24	0.73	241ºC	364.45
C3	66.20%	0.71	227ºC	371.17
C4	70.53%	0.83	204ºC	354.11
C5	67.38	0.76	243ºC	372.20

Infrared spectroscopy

Infrared interpretation of different derivatives was showed as below-

Table 2. Interpretation of infrared spectra of C1

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	3120.2	3000-3700
2.	1509.3	1400-1700
3.	1528.4	1500-1700
4.	636	600-700

Table 3. Interpretation of infrared spectra of C2

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm_{-1}) (theoretical)
1.	1839.4	1600-1900
2.	3641.3	3000-3700
3.	1554.2	1500-1700
4.	3249.4	3100-3300

Table 4. Interpretation of infrared spectra of C3

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1647.2	1600-1900
2.	3261.2	3000-3700

3.	1585.3	1500-1700
4.	3249.6	3200-3300
5.	684.2	600-700
6.	3462.3	3400-3500

Table 5. Interpretation of infrared spectra of C4

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1757.2	1600-1900
2.	3329.3	3000-3700
3.	1579.2	1500-1700
4.	3186.3	3100-3300
5.	645.2	600-700
6.	1132.4	900-1300

Table 6. Interpretation of infrared spectra of C5

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1664.4	1600-1900
2.	3430.2	3000-3700
3.	1578.4	1500-1700
4.	3238.3	3100-3300
5.	1687.2	1600-1700
6.	1046.2	1000-1100

NMR Spectroscopy

Interpretation of NMR is depicted as below-

Table 7. Interpretation of NMR spectra of C1

S. No.	Chemical shift (ppm)	Proton
1.	1.14-1.16	4
2.	4.28-4.38	2
3.	7.30-7.38	4
4.	7.42	1

Table 8. Interpretation of NMR spectra of C2

S. No.	Chemical shift (ppm)	Proton
1.	2.38	2
2.	4.14-4.24	2
3.	7.71-7.75	4

Table 9. Interpretation of NMR spectra of C3

S. No.	Chemical shift (ppm)	Proton
1.	7.32	4
2.	7.32-8.36	14
3.	8.38	1

Table 10. Interpretation of NMR spectra of C4

S. No.	Chemical shift (ppm)	Proton

1.	3.84	4
2.	7.49	2
2.	7.37-8.46	12
3.	8.26	2

Table 11. Interpretation of NMR spectra of C5

S. No.	Chemical shift (ppm)	Proton
1.	7.36-7.44	4
2.	7.38-8.46	14
3.	7.29	2

Mass spectra

Interpretation of mass is as follows-

C1-MS: m/z (%) $[M]^+$, 274.9(100), 272.9(84), 284.3(32), 270.2(24), 257(9).

C2-MS: m/z (%) $[M]^+$, 274.9(100), 272.9(84), 284.3(32), 270.2(24), 257(9).

C3-MS: m/z (%) $[M]^+$, 566.9(100), 568.9(76), 564.9(35), 569.9(29), 570.2(14).

C4-MS: m/z (%) $[M]^+$, 274.9(100), 272.9(82), 275.9(13), 270.2(11), 557.2(6).

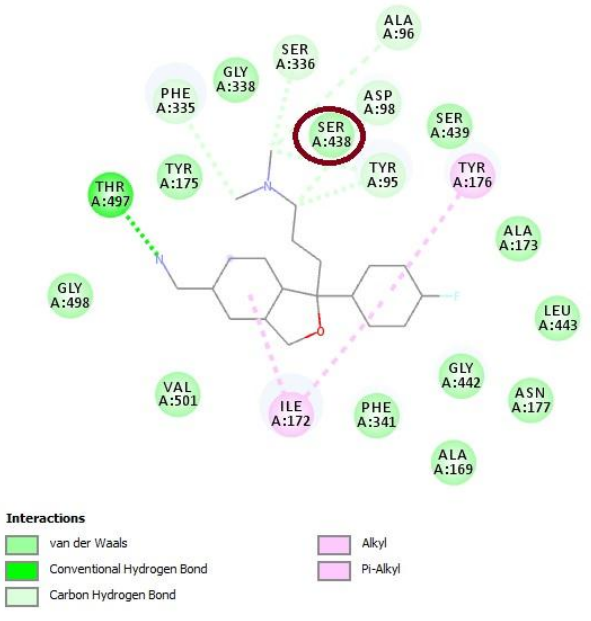
C5-MS: m/z (%) $[M]^+$, 274.9(100), 272.9(84), 284.3(33), 270.2(24), 257(9).

Docking

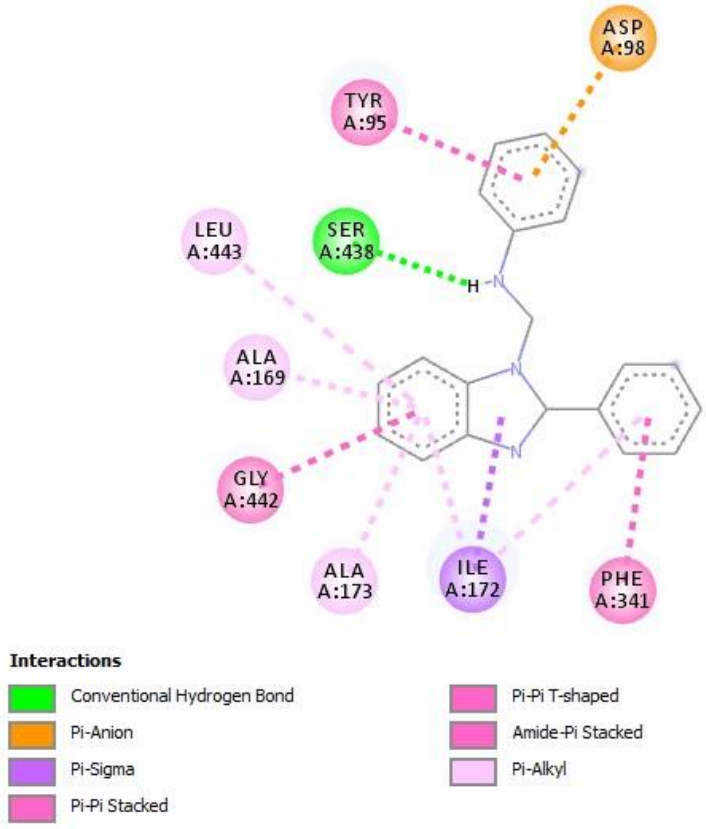
The 5 compounds have shown the successful docking inside the active site of serotonin protein with a binding energy of -8.8 to -11 Kcal/mol. We compared the predicted docking data with known serotonin protein inhibitors Citalopram having binding energy of **-9.5** Kcal/mol.

Table 12. Molecular docking of synthesized derivatives

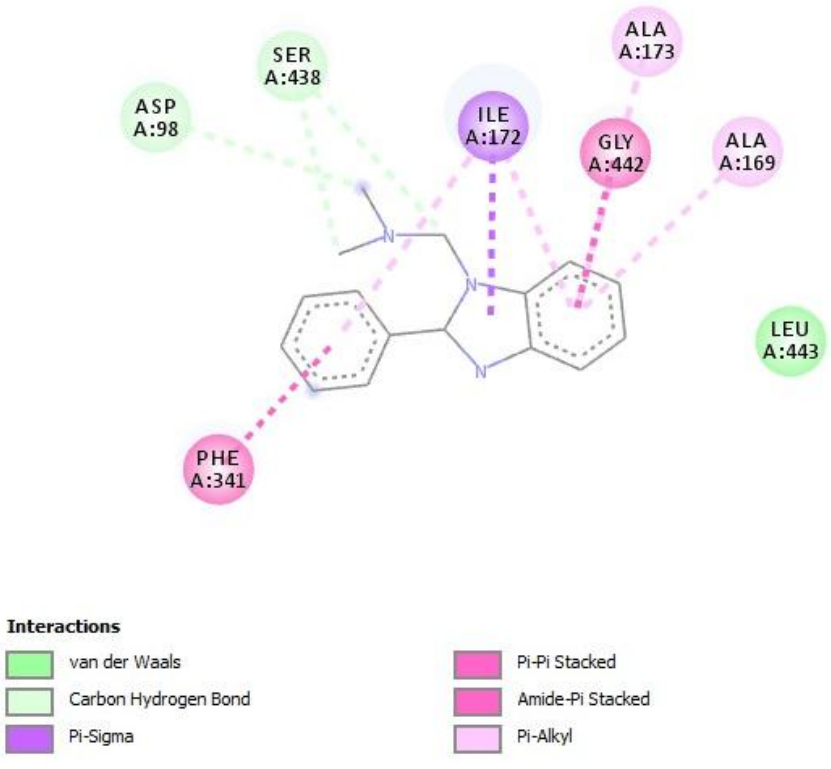
Compound No	Binding Energy (ΔG) (Kcal/mol)
Citalopram	-9.5
C1	-10.3
C2	-8.8
C3	-11
C4	-9.3
C5	-9.3



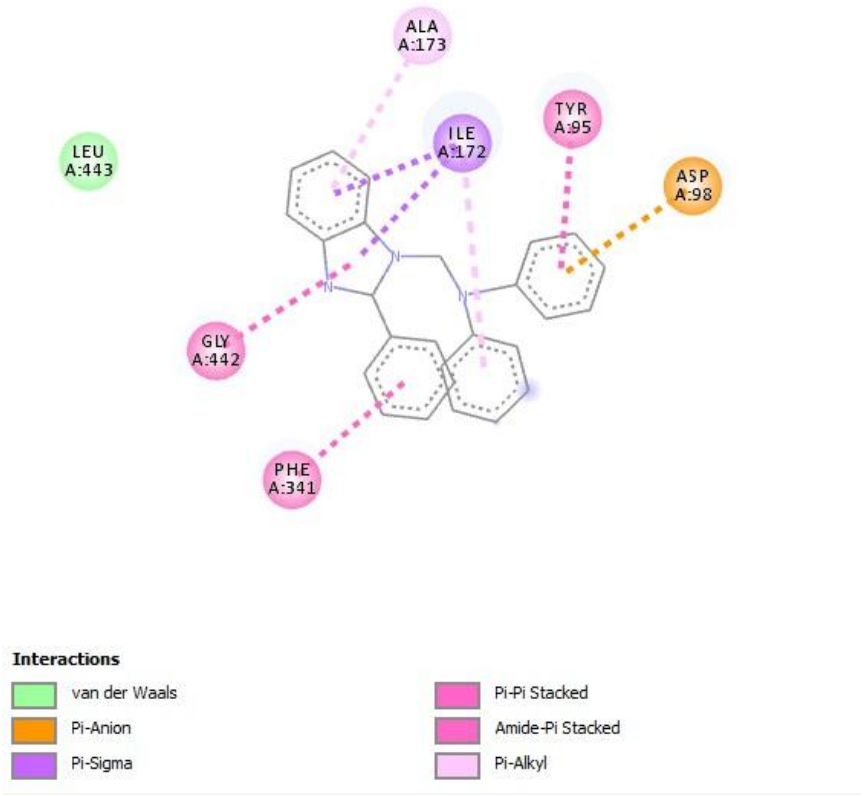
Docking of Citalopram



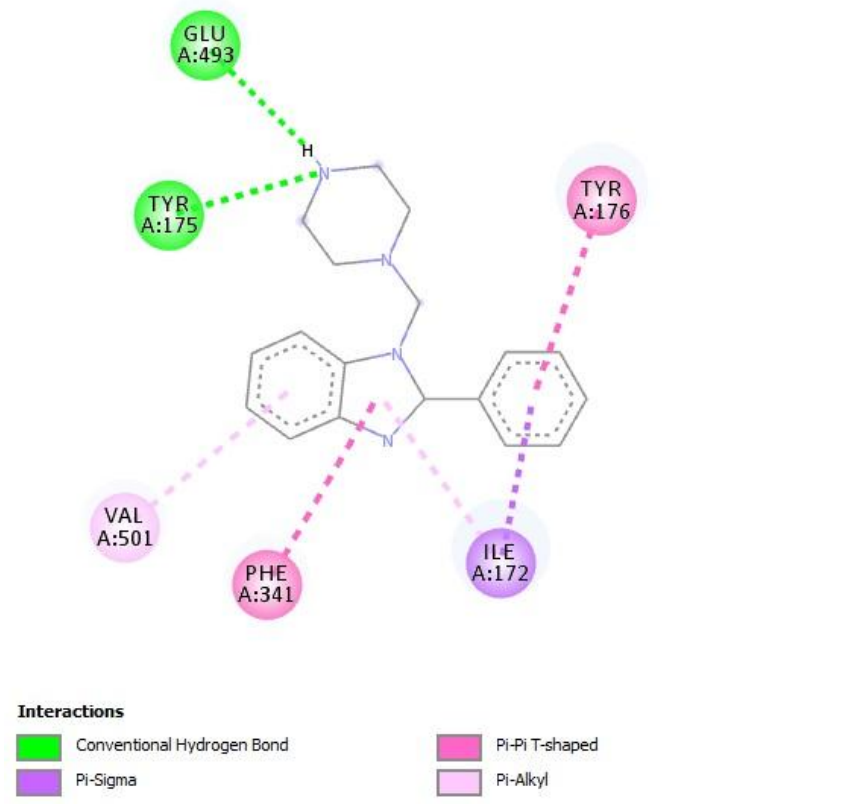
Docking of C1



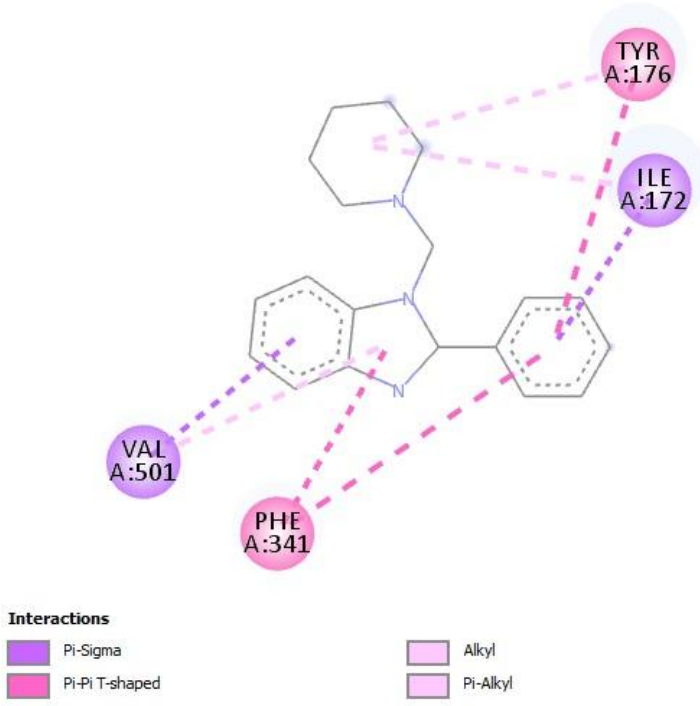
Docking of C2



Docking of C3



Docking of C4



Docking of C5

Evaluation Protocols

Tail suspension test

In this TST model, immobility time in seconds were evaluated in rats. In control group, immobility time was estimated as 173± 2.54**sec. In fluoxetine treated rats, it was observed as 71± 2.17**sec. The substituted benzimidazole derivatives demonstrated immobility time as 101± 1.30** sec, 106± 1.32** sec, 104± 1.11** sec and 103± 2.26*** sec in compounds C1, C2, C3 and C4, respectively at the dose of 100mg/kg.

Moreover, immobility time was obtained as 105± 2.74*** sec in C5 at the dose of 100mg/kg of substituted benzimidazole derivatives. When the response was compared with control group, C1 and C4 were much effective in depression alleviation with near response to standard group rats. When the responses of treated rats were compared, it showed that C4 has greater response.

Table 13. TST of vehicle, fluoxetine and substituted benzimidazole derivatives (100mg/kg)

Treatment	Immobility time (sec)
Vehicle	173± 2.54**
fluoxetine (20mg/kg, p. o.)	71± 2.17**
C1 (100mg/kg)	101± 1.30**
C2 (100mg/kg)	106± 1.32**
C3 (100mg/kg)	104± 1.11**
C4 (100mg/kg)	103± 2.26***
C5 (100mg/kg)	105± 2.74***

At $P<0.05$ values were found significant
n=6 & values were given in Mean± SEM

Same procedure was performed for higher dose (200mg/kg) too. In this model, immobility time in seconds was record and the same was estimated as 173± 2.54***sec in control, 71± 2.17** in fluoxetine treated group. The substituted benzimidazole derivatives demonstrated immobility time as 86± 1.25***sec, 92± 1.20**sec, 96± 1.28**sec and 84± 1.25**sec in compounds C1, C2, C3 and C4, respectively at the dose of 200mg/kg.

In C5, immobility time was found as 93± 1.12** in C5 at the dose of 200mg/kg of benzimidazole derivatives. When the response was compared with control group, C1 and C4 were much effective in modulation of immobility and thus depression. While C4 was much prominent.

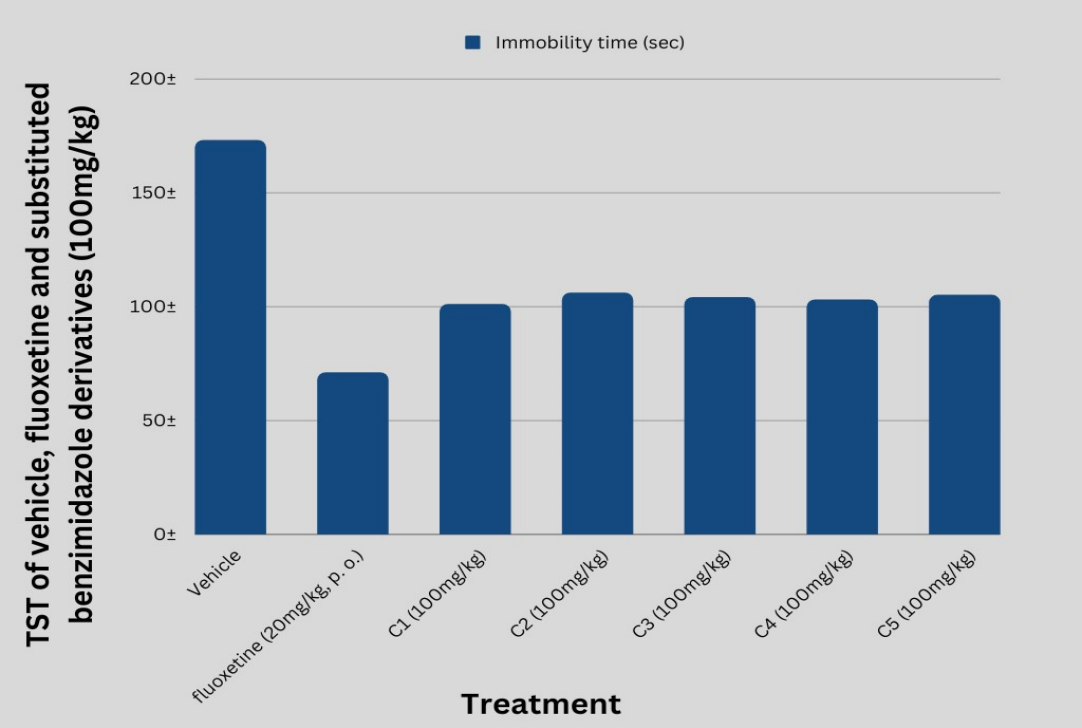


Fig 2. TST of vehicle, fluoxetine and substituted benzimidazole derivatives (100mg/kg)

Table 14. TST of vehicle, fluoxetine and substituted benzimidazole derivatives (200mg/kg)

Treatment	**Immobility time (sec)**
Vehicle	173± 2.54***
fluoxetine (20mg/kg, p. o.)	71± 2.17**
C1 (200mg/kg)	86± 1.25***
C2 (200mg/kg)	92± 1.20**
C3 (200mg/kg)	96± 1.28**
C4 (200mg/kg)	84± 1.25**
C5 (200mg/kg)	93± 1.12**

At P<0.05 values were found significant
n=6 & values were given in Mean± SEM

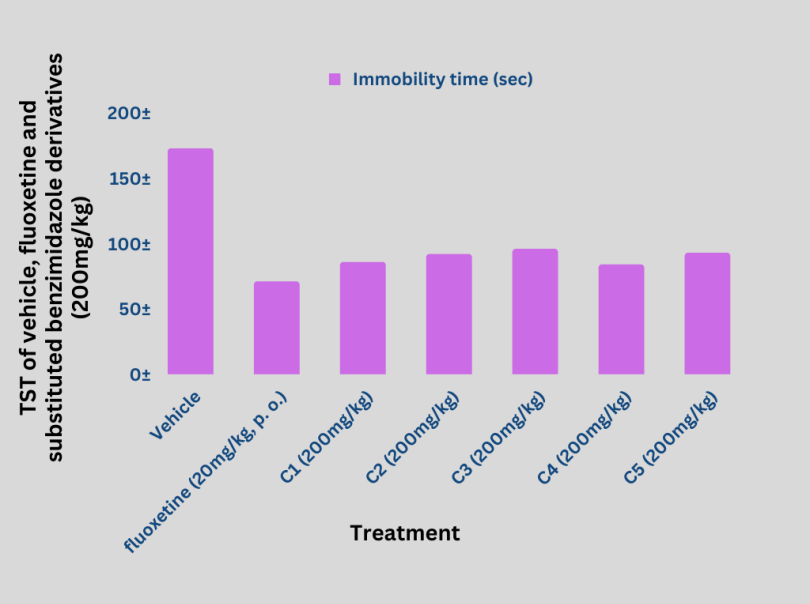


Fig 3. TST of vehicle, fluoxetine and substituted benzimidazole derivatives (200mg/kg)

The benzimidazole derivatives affirm for their antidepressant role in all the doses when compared with control group. The pharmacological response was observed in dose-dependent manner. Substituted benzimidazole derivatives at the dose of 200mg/kg exhibited optimum response.

Motor Co-ordination determination

Motor co-ordination using Rota rod is a rational protocol for evaluation of anti-depressant drugs. Motor coordination was found as 129± 1.38**seconds in the control group, and 23± 1.34** seconds in the standard group. Whereas, the substituted benzimidazole derivatives at dose of 100mg/kg exhibited the motion as 73± 1.43**sec, 82± 1.54**sec, 76± 1.34**sec and 76± 1.24**sec in C1, C2, C3 and C4, respectively.

However, motion in Rota rod was obtained as 83± 1.35**sec in C5 at the dose of 100mg/kg of substituted benzimidazole derivatives. When the response was compared with control group, C1 and C4 were much effective in depression with near response to standard treated rats.

Table 15. Motor Coordination test of vehicle, fluoxetine and substituted benzimidazole derivatives (100mg/kg)

Treatment	Motion in Rota rod (sec)
Vehicle	129± 1.38**
fluoxetine (20mg/kg, p. o.)	23± 1.34**
C1 (100mg/kg)	73± 1.43**
C2 (100mg/kg)	82± 1.54**
C3 (100mg/kg)	76± 1.34**
C4 (100mg/kg)	76± 1.24**
C5 (100mg/kg)	83± 1.35**

At $P<0.05$ values were found significant
n=6 & values were given in Mean± SEM

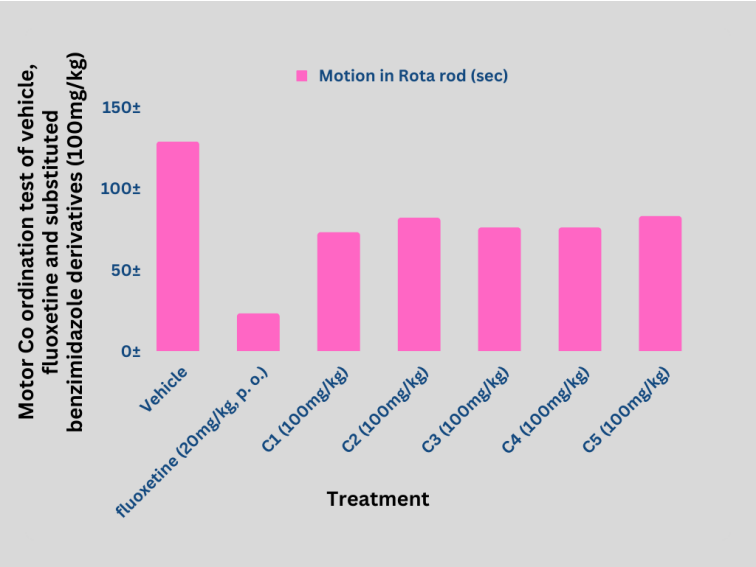


Fig 4. Motor Coordination test of vehicle, fluoxetine and substituted benzimidazole derivatives (100mg/kg)

The anti-depressant role of substituted benzimidazole derivatives was also noted in terms of decreased motion in Rota-rod apparatus. This action was observed in dose-dependent manner as substituted benzimidazole derivatives showed much potent action when observed at 200mg/kg.

At 200mg/kg, the benzimidazole derivatives (200mg/kg) exhibited the motor coordination as 54± 1.36**sec, 63± 1.40**sec, 68± 1.41**sec and 51± 1.32**sec in C1, C2, C3 and C4, respectively. However, motion in Rota rod was obtained as 63± 1.48**sec in C5 at the dose of 200mg/kg of substituted benzimidazole derivatives.

When the response was compared with control group, C1 and C4 were much effective in decreasing motion in test groups. When the responses of treated rats were compared in b/w, it showed that C4 has greater response.

Table 16. Motor Coordination test of vehicle, fluoxetine and substituted benzimidazole derivatives (200mg/kg)

Treatment	**Motion in Rota-rod (sec)**
Vehicle	129± 1.38**
fluoxetine (20mg/kg, p. o.)	23± 1.34**
C1 (200mg/kg)	54± 1.36**
C2 (200mg/kg)	63± 1.40**
C3 (200mg/kg)	68± 1.41**
C4 (200mg/kg)	51± 1.32**
C5 (200mg/kg)	63± 1.48**

At P<0.05 values were found significant
n=6 & values were given in Mean± SEM

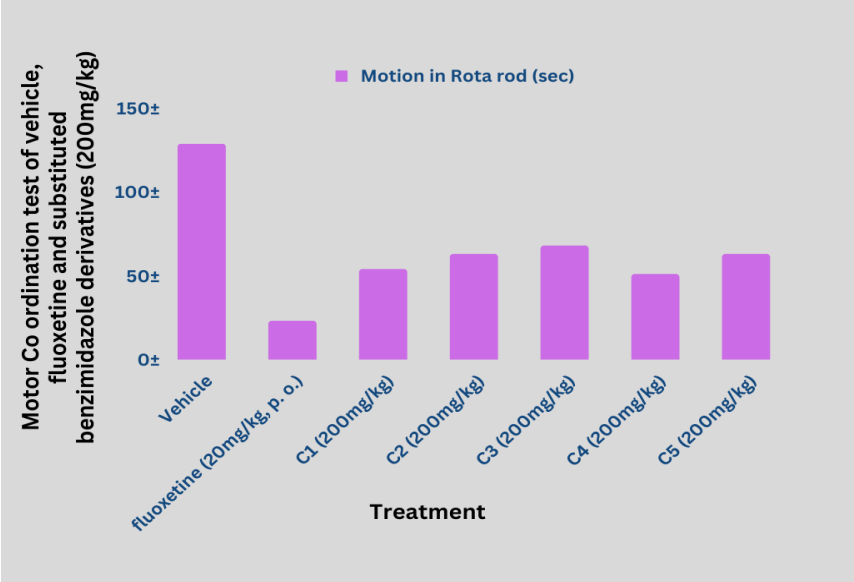


Fig 5. Motor Coordination test of vehicle, fluoxetine and substituted benzimidazole derivatives (200mg/kg)

Locomotion activity

Locomotor behavior of animals using actophotometer was recorded in experiment duration of 10 min. The control group showed locomotor score as 173±0.16* while the fluoxetine treated group had the lowest activity at 87±0.11*.

Substituted benzimidazole derivatives demonstrated locomotor response as 119±0.13**, 131±0.23**, 129±0.13** and 112±0.65** in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 100mg/kg.

Table 17. Locomotion response of vehicle, fluoxetine and substituted benzimidazole derivatives (100mg/kg)

Treatment	**Locomotor response**
Vehicle	173±0.16*
fluoxetine (20mg/kg, p. o.)	87±0.11*
C1 (100mg/kg)	119±0.13**
C2 (100mg/kg)	131±0.23**
C3 (100mg/kg)	129±0.13**
C4 (100mg/kg)	112±0.65**
C5 (100mg/kg)	126±0.23**

At P<0.05 values were found significant
n=6 & values were given in Mean± SEM

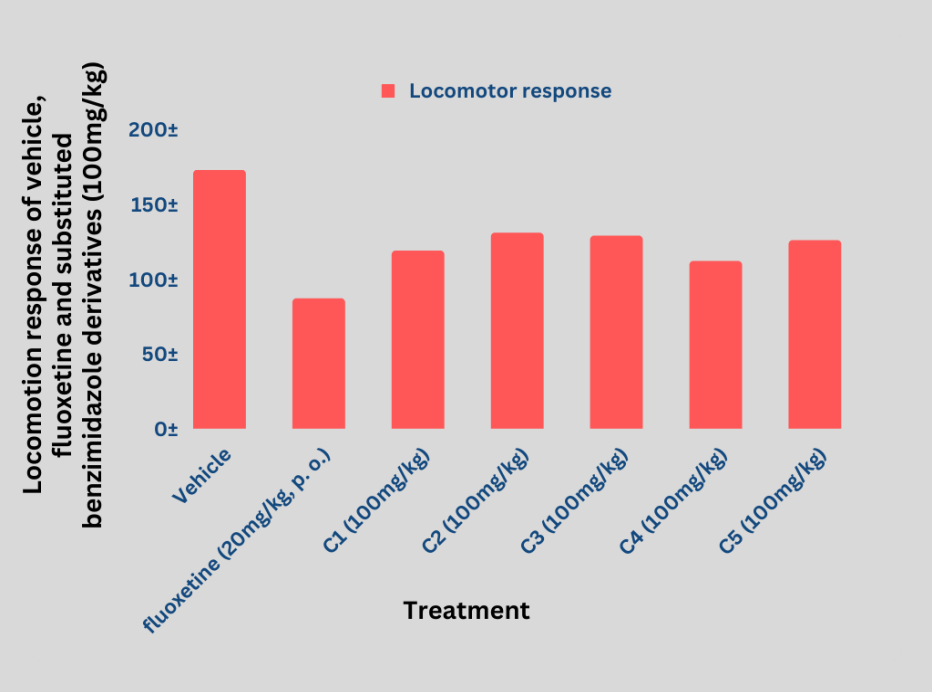


Fig 6. Locomotion response of vehicle, fluoxetine and substituted benzimidazole derivatives (100mg/kg)

At 200mg/kg, the locomotor response was observed as 108±0.61***, 104±0.25***, 109±0.37*** and 112±0.18*** in C1, C2, C3 and C4, respectively. When the response was compared with control group, C1 and C4 were much effective in movement with near response to fluoxetine treated rats. When the responses of treated rats were compared, it showed that C4 has greater locomotor response at higher dose of substituted benzimidazole derivatives.

Table 18. Locomotion response of vehicle, fluoxetine and substituted benzimidazole derivatives (200mg/kg)

Treatment	**Locomotor response**
Vehicle	173±0.16*
fluoxetine (20mg/kg, p. o.)	87±0.11*
C1 (200mg/kg)	108±0.61***
C2 (200mg/kg)	104±0.25***
C3 (200mg/kg)	109±0.37***
C4 (200mg/kg)	112±0.18***
C5 (200mg/kg)	114±0.15***

At P<0.05 values were found significant
n=6 & values were given in Mean± SEM

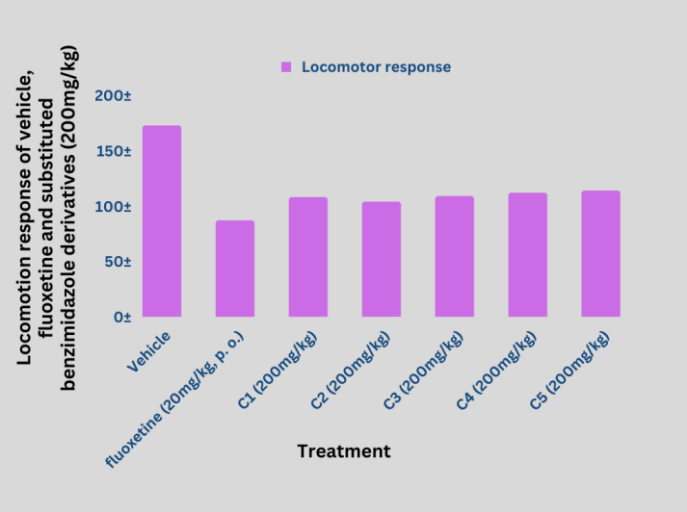


Fig 7. Locomotion response of vehicle, fluoxetine and substituted benzimidazole derivatives (200mg/kg)

In addition to natural chemical moieties, synthetic derivatives have been shown to be beneficial in treating a variety of pharmacological conditions, such as depression, insomnia, and neurodevelopmental disorders. For the antidepressant potential of several substituted benzimidazole derivatives, the TST model suggests that Serotonin reuptake inhibition may be necessary. C1 and C4 stand out as the most potent antidepressants among numerous tested substituted benzimidazole derivatives. It may work by inhibiting neurotransmitter release or speeding up the breakdown of catecholamines. The decrease in biogenic amines contributed to the improved mental clarity.

In results, it showed that mass spectroscopy showed near molecular weight as estimated. TST and Rota-rod models exhibited highest level of decrease in time of motion that indicated for their anti-depressant potential. Actophotometer also shown moderate anti-depressant action while in the models, effect was observed in dose dependent manner.

4. Conclusion

It may promote the inhibition of serotonin transporter protein. The GABA, an inhibitory neurotransmitter, which in turn promotes the inward movement of Cl- ions, hyperpolarization, and the subsequent suppression of neurotransmitter release. Therefore, this investigation confirms the anti-depressant potential of benzimidazole derivatives.

In conclusion, depression can be treated using synthesized substituted benzimidazole derivatives, which alleviate symptoms and boost mental health to help you in elevated mood. Its mode of action must be explained in detail before it can be considered for treatment of various mental health problems. It suggests to isolate and identify the much effective derivatives and determine the mode of action of managing depression by which it might be used in diverse mental disorders like Alzheimer's disease.

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