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DEVELOPMENT OF DIFFERENT BENZIMIDAZOLE DERIVATIVES AND THEIR EVALUATION FOR SEROTONIN TRANSPORTER PROTEIN INHIBITION AS ANTIDEPRESSANT AGENT

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ABSTRACT

Depression is a prevalent mental illness. According to estimates, 5 percent of adults worldwide experience depression. Benzimidazole 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. The present study was based upon the development of different benzimidazole derivatives and their evaluation for serotonin transporter protein inhibition as antidepressant agent. The phenylenediamine and 4-nitrobenzoyl chloride were used as initial components for the synthesis of Benzimidazole derivatives. Total 6 derivatives were synthesized and characterized in terms of melting point, molecular weight, TLC, FTIR, Mass, NMR analysis and Molecular docking. Albino rats of either sex weighing 130–160g were obtained from the Animal House, Department of Pharmacy, IIMT University, Meerut (UP) India. Rats were divided into 5 group (n=6); including group 1 administered normal saline (vehicle), group 2 administered p-chlorophenylalanine methyl ester (PCPA) (100mg/kg, i. p.), group 3 administered fluoxetine (20mg/kg, orally), group 4 administered all the novel derivatives (100mg/kg, orally) and group 5 administered all the novel derivatives (200mg/kg, orally) for 15 days. Novel derivatives of benzimidazole were evaluated for anti-depressant action through parameters i.e., TST, Motor coordination (Rota rod), Light/dark arena and Locomotor activity (Actophotometer). In results, benzimidazole derivatives (200mg/kg) demonstrated immobility time as 96± 1.15***sec, 89± 2.16***sec, 90±1.73***sec and 98±.16***sec in C1, C2, C3 and C4, respectively in TST. The benzimidazole derivatives (100mg/kg) exhibited the motion as 98± 2.21***sec, 102± 2.27***sec, 88± 2.36***sec and 92± 2.53***sec in C1, C2, C3 and C4, respectively. However, motion in Rota rod was obtained as 106± 4.10***, and 83± 1.37*** in C5 and C6, respectively Thus, it indicates that serotonin reuptake might be inhibited for the anti-depressant potential of diverse benzimidazole derivatives. In conclusion, depression can be treated using new benzimidazole derivatives, which alleviate symptoms and boost mental health to help you in elevated mood. The synthesized derivatives might be tested for their toxicity and evaluated for their possible mechanism of action through which it showed anti-depressant potential.

Keywords: Phenylenediamine, Benzimidazole derivatives, Serotonin protein inhibition, Anti-depressant activity, TST.

INTRODUCTION

Depression is a prevalent mental illness. According to estimates, 5 percent of adults worldwide experience depression [1]. The largest cause of disability in the world today is depression, which also significantly contributes to the overall burden of sickness on the planet [2]. Depression affects more women than males. Suicide can result from depression [3]. Major depression is influenced by a number of important elements, including genetic, neurological, hormonal, immunological, and neuroendocrinological mechanisms [4]. The recipient experiences stress and emotional behaviour as a result of these circumstances. Gender and progressive factors may affect etiological processes differently [5]. Depression is brought on by stressful events in those who are more susceptible owing to biological and behavioural characteristics. Chronic stress, traumatic childhood experiences, and recent life events are examples of environmental factors linked to depression [6]. Common depression vulnerabilities include those related to cognition, interpersonal relationships, and personality traits. It is the main danger factor for self-destruction, a main source of death around the world, particularly in teenagers, young adults and the older people [7][8]. 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 [9-10].

CRH come-out from hypothalamus against psychological stress through cortical brain region. This hormone facilitates the secretion of corticotropin that excite the adrenal gland to secrete cortisol into the blood plasma [11]. Rise cortisol level may act as a factor for major depression and its chronic consequences i.e., coronary artery disease, Diabetes mellitus- Type 2 & osteoporosis [12][13]. Approx. 7 lacs people die by suicide, each year. Suicide is the 4th highest cause of death among those 15-29 years old [14].

Benzimidazole

Benzimidazole 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 is a white solid that crystallizes in tabular shapes [15].

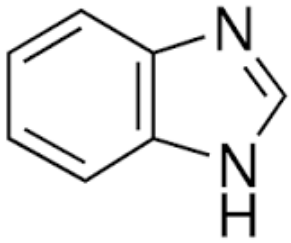


Fig 1. Benzimidazole structure (IUPAC name: 1H-benzimidazole)

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.

Benzimidazole has been utilized for various pharmacological activities including anticancer [16], antihypertensive, anti-inflammatory, aantimicrobial [17], antioxidant, anticoagulants. antidiabetic agents [18], anthelmintic, analgesic, antispasmodic [19].

Previous studies shows that benzimidazole is potent agent in the treatment of various medical conditions. But synthesis and evaluation of novel derivatives for antidepressant action as serotonin transporter protein inhibition has not been done yet. Therefore, this study was based upon the pharmacological evaluation of development of different benzimidazole derivatives and their evaluation for serotonin transporter protein inhibition as antidepressant agent.

MATERIALS AND METHODOLOGY

Experimental Requirements

Phenylenediamine, p-nitrobenzaldehyde, distilled water, ethanol, methanol, weighing balance, digital pH meter, hot air oven, laboratory thermometer, round bottom flask.

Scheme for benzimidazole synthesis

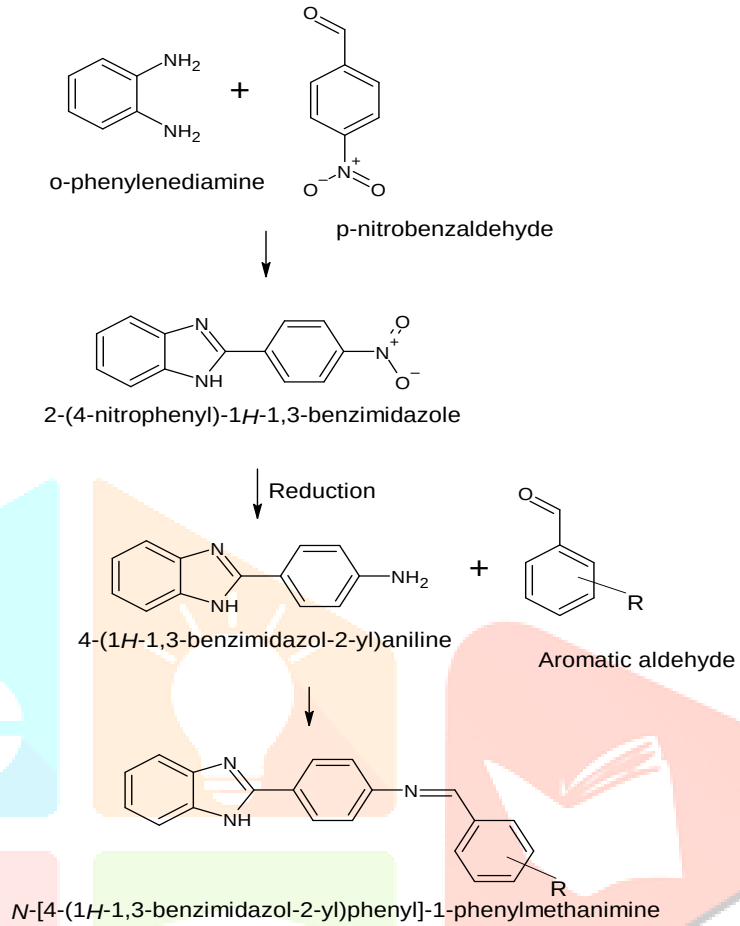


Fig 2. Scheme for benzimidazole synthesis

The o-phenylenediamine made reacted with p-nitrobenzaldehyde to produce 2-(4-nitrophenyl)-1H-1,3-benzimidazole that on reduction converted into 4-(1H-1,3benzimidazole-2yl)aniline which upon reaction with aromatic aldehyde produced the final benzimidazole derivatives as N-[4-(1H-1,3benzimidazole-2yl)phenyl]-1-phenylmethanimine.

Derivatives:

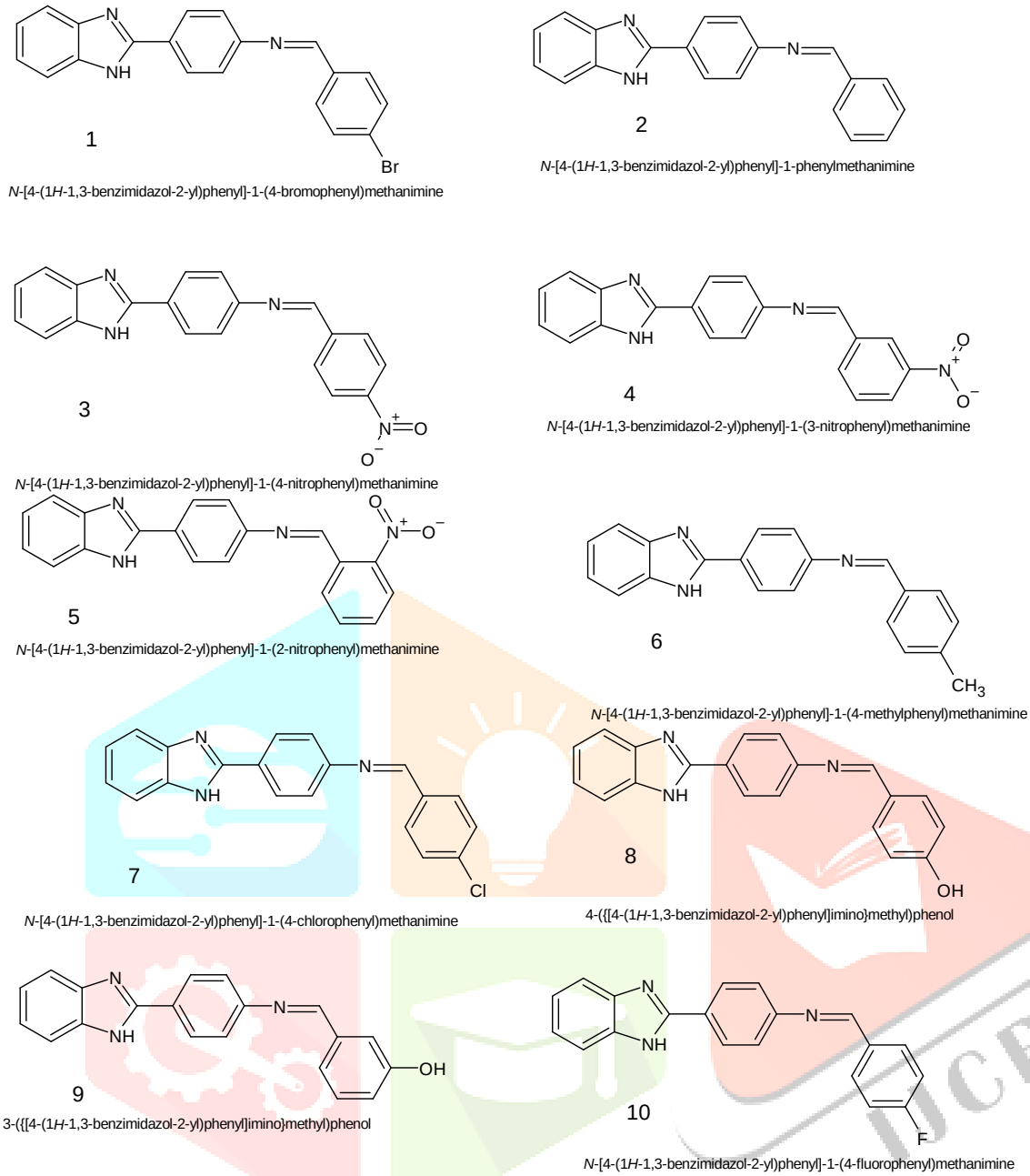


Fig 3. Derivatives of benzimidazole

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 (Rf value)

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. Binding modes were scored using their FullFitness and clustered. Clusters were then ranked according to the average FullFitness of their elements

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.

Experimental protocols

Rats were divided into 5 group (n=6) as follows-

Group 1- rats are administered normal saline (vehicle) daily for 15 days.

Group 2- rats are administered p-chlorophenylalanine methyl ester (PCPA) (100mg/kg, i. p.) for 15 days.

Group 3- rats are administered fluoxetine (20mg/kg, orally) for 15 days.

Group 4- rats are administered all the novel derivatives (100mg/kg, orally) for 15 days.

Group 5- rats are administered all the novel derivatives (200mg/kg, orally) for 15 days.

Protocol

Tail Suspension Test

The TST was conducted as described in detail earlier. In this experiment, rats were dangled 30 centimeters 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 time of the immobile behaviour. Animals that tried to escape the testing by climbing their tails were thrown out [22].

Motor co ordination 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 [23].

Light-Dark Arena model

A 100W bulb is suspended 30cm above the base of the box in the light-dark arena type. The rats spend 5 minutes exposed in the box's bright center. We keep track of how many people enter the light arena and how long they stay there for up to five minutes. Each time a new rat is introduced, it is thoroughly cleaned [23].

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 [23].

RESULTS AND DISCUSSION

Synthesized derivatives

Novel benzimidazole derivatives (C1-C10) 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 benzimidazole derivative, the melting point was determined as 176ºC, 165ºC, 205ºC, 210ºC, 217ºC and 170ºC for compounds C1, C2, C3, C4, C5 and C6, respectively. Moreover, C7 and C9 showed highest melting points as 210ºC and 200ºC, respectively.

Thin Layer Chromatography (Rf value)

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, 0.72, 0.74, 0.82, 0.71 and 0.68 of C1, C2, C3, C4, C5 and C6, respectively. While C9 showed Rf value as 0.73 and C10 as 0.69.

Benzimidazole derivatives were tested for their physical properties i.e., percentage yield, melting point, molecular weight, and functional groups attached with were tested. C2 and C4 were demonstrated for its highest % yield as 71.24% and 71.11%. Lowest % yield was seen in C3 as 63.19%. The highest melting point was found in compound C4 as 239ºC. 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 119.24, 142.53 and 121.39 for C2, C3 and C4 respectively. The following table summarized physical properties of all the compounds.

Table 1. Physical properties of synthesized benzimidazole derivatives

Compound	Yield (%)	Rf Value	Melting point	Molecular weight
C1	64.37	0.71	120-180 ºC	332.45
C2	71.24	0.78	175 ºC	456.808
C3	63.19	0.76	150 ºC	316
C4	71.11	0.64	180 ºC	314
C5	65.38	0.70	260 ºC	312
C6	69.17	0.68	120 ºC	297
C7	67.54	0.72	210ºC	343
C8	71.28	0.68	190ºC	343
C9	66.49	0.73	200ºC	314

C10	68.12	0.69	180ºC	314

Infrared Spectroscopy

Compound from C1-C6 were analyzed for infrared spectroscopy and these spectra confirmed for the physical characteristics of benzothiazole analogues.

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.	1721.1	1600-1900
2.	3428.2	3000-3700
3.	1610.3	1600-1700
4.	1564.2	1500-1700
5.	761	600-700

Table 3. Interpretation of infrared spectra of C2

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm_{-1}) (theoretical)
1.	1721.2	1600-1900
2.	3429.2	3000-3700
3.	1603.3	1500-1700
4.	3145.2	3100-3300
5.	696.3	600-700
6.	1609.2	1640-1667

Table 4. Interpretation of infrared spectra of C3

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1724.6	1600-1900
2.	3427.3	3000-3700
3.	1603.4	1500-1700
4.	3141.2	3200-3300
5.	762.3	600-700
6.	3425.47	3400-3500

Table 5. Interpretation of infrared spectra of C4

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1723.3	1600-1900
2.	3427.2	3000-3700
3.	1559.3	1500-1700
4.	3135.5	3100-3300
5.	693.3	600-700
6.	1607.5	1640-1667
7.	1013.2	900-1300

Table 6. Interpretation of infrared spectra of C5

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1724.2	1600-1900
2.	3437.3	3000-3700
3.	1562.6	1500-1700
4.	3139.2	3100-3300
5.	702.5	600-700
6.	1652.4	1640-1667
7.	1094.3	1096-1089

Table 7. Interpretation of infrared spectra of C6

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1724.3	1600-1900
2.	3423.5	3000-3700
3.	1564.2	1500-1700
4.	3136.5	3100-3300
5.	761.3	600-700
6.	1612.2	1640-1667
7.	1093.3	1096-1089

Table 8. Interpretation of infrared spectra of C7

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1739.2	1600-1900
2.	3320.7	3000-3700
3.	1619.2	1500-1700
4.	3149.6	3200-3300
5.	769.2	600-700
6.	3429.4	3400-3500

Table 9. Interpretation of infrared spectra of C8

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1729.1	1600-1900
2.	3434.3	3000-3700
3.	1563.2	1500-1700
4.	3138.4	3100-3300
5.	697.2	600-700
6.	1602.3	1640-1667
7.	1019.4	900-1300

Table 10. Interpretation of infrared spectra of C9

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1727.5	1600-1900
2.	3442.1	3000-3700
3.	1565.2	1500-1700
4.	3136.4	3100-3300
5.	704.2	600-700
6.	1657.3	1640-1667
7.	1097.6	1096-1089

Table 11. Interpretation of infrared spectra of C10

S. No.	Frequency (cm^{-1}) (observed)	Frequency (cm^{-1}) (theoretical)
1.	1728.2	1600-1900
2.	3435.	3000-3700
3.	1572.3	1500-1700
4.	3139.2	3100-3300
5.	759.2	600-700
6.	1627.3	1640-1667
7.	1097.4	1096-1089

5.2.4 NMR Spectroscopy

Interpretation of NMR is depicted as below-

Table 12. Interpretation of NMR spectra of C1

S. No.	Chemical shift (ppm)	Proton
1.	1.10-1.12	4
2.	4.17-4.28	2
3.	7.29-7.36	4
4.	7.68	1

Table 13. Interpretation of NMR spectra of C2

S. No.	Chemical shift (ppm)	Proton
1.	2.17	3
2.	4.10-4.12	2
3.	7.69-7.73	4

Table 14. Interpretation of NMR spectra of C3

S. No.	Chemical shift (ppm)	Proton
1.	7.263	2
2.	7.284-8.236	12
3.	8.353	1

Table 15. Interpretation of NMR spectra of C4

S. No.	Chemical shift (ppm)	Proton
1.	3.93	3
2.	7.24	4
2.	7.29-8.24	11
3.	8.73	2

Table 16. Interpretation of NMR spectra of C5

S. No.	Chemical shift (ppm)	Proton
1.	7.28-7.37	2
2.	7.37-8.35	12
3.	8.42	1

Table 17. Interpretation of NMR spectra of C6

S. No.	Chemical shift (ppm)	Proton
1.	7.38	2
2.	7.24-8.19	14
3.	8.27	1

Table 18. Interpretation of NMR spectra of C7

S. No.	Chemical shift (ppm)	Proton
1.	7.27	3
2.	7.20-8.27	11
3.	8.34	1

Table 19. Interpretation of NMR spectra of C8

S. No.	Chemical shift (ppm)	Proton
1.	3.84	2
2.	7.21	2
2.	7.23-8.38	12
3.	8.68	2

Table 20. Interpretation of NMR spectra of C9

S. No.	Chemical shift (ppm)	Proton
1.	7.39-7.42	1
2.	7.37-8.45	14
3.	8.59	1

Table 21. Interpretation of NMR spectra of C10

S. No.	Chemical shift (ppm)	Proton
1.	7.22	1
2.	7.25-8.39	12
3.	8.42	2

Mass Spectroscopy

Interpretation of mass is as follows-

C1-MS: m/z (%) $[M]^+$, 210.09(100), 211.09(56), 240.06(8), 212.09(4).

C2-MS: m/z (%) $[M]^+$, 210.09(100), 211.09(56), 240.06(8), 212.09(4).

C3-MS: m/z (%) $[M]^+$, 315.15(100), 334.10(74), 336.10(28), 316.16(20), 282.27(6).

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

C5-MS: m/z (%) $[M]^+$, 210.09(100), 211.09(56), 240.06(8), 212.09(4).

C6-MS: m/z (%) $[M]^+$, 315.15(100), 334.10(74), 336.10(28), 316.16(20), 282.27(6).

C7-MS: m/z (%) $[M]^+$, 210.09(100), 211.09(56), 240.06(8), 212.09(4).

C8-MS: m/z (%) $[M]^+$, 274.9(100), 272.9(82), 275.9(13), 270.2(11), 557.2(6).

C9-MS: m/z (%) $[M]^+$, 210.09(100), 211.09(56), 240.06(8), 212.09(4).

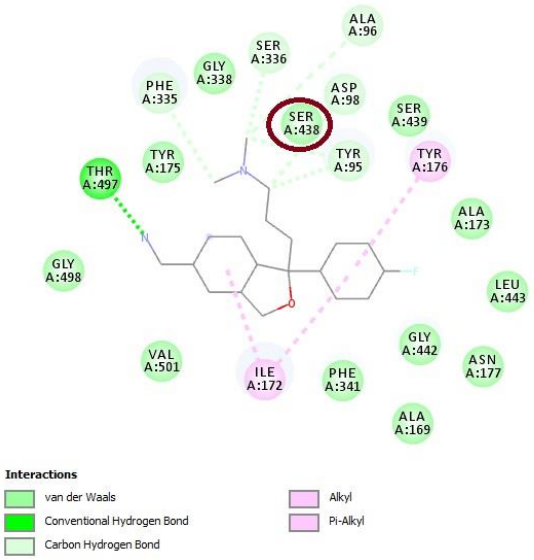
C10-MS: m/z (%) $[M]^+$, 315.15(100), 334.10(74), 336.10(28), 316.16(20), 282.27(6).

Molecular docking

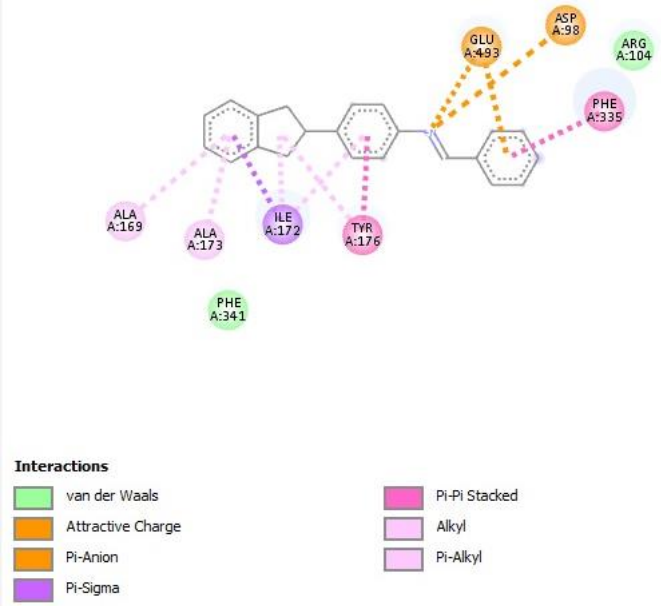
The 10 compounds have shown the successful docking inside the active site of serotonin protein with a binding energy of -9.6 to -11.2Kcal/mol. We compared the predicted docking data with known serotonin protein inhibitors Citalopram having binding energy of **-9.6** Kcal/mol.

Table 22. Molecular docking of synthesized derivatives

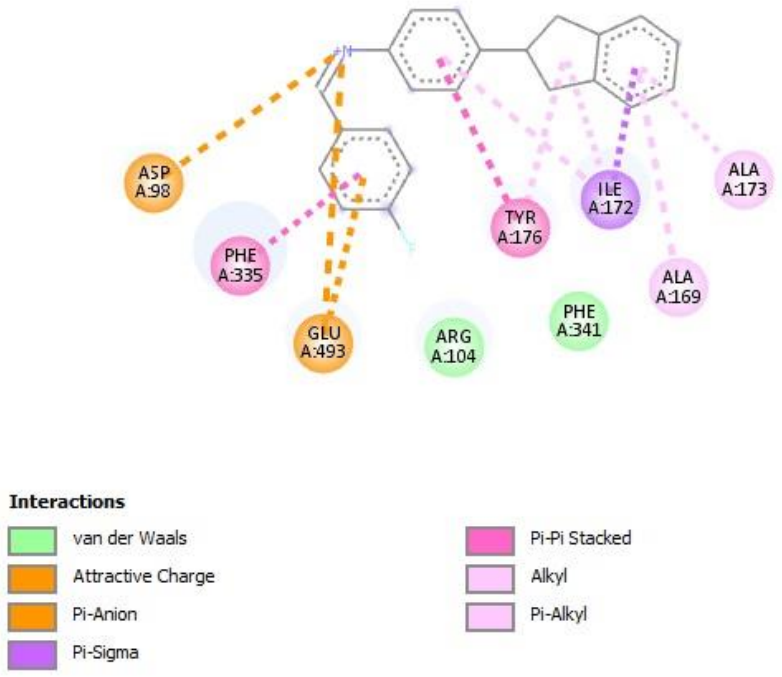
Compound No	Binding Energy (ΔG) (Kcal/mol)
Citalopram	-9.6
C1	-10.5
C2	-10.6
C3	-10.7
C4	-10.8
C5	-10.6
C6	-10.9
C7	-10.5
C8	-11.1
C9	-10.6
C10	-11.2



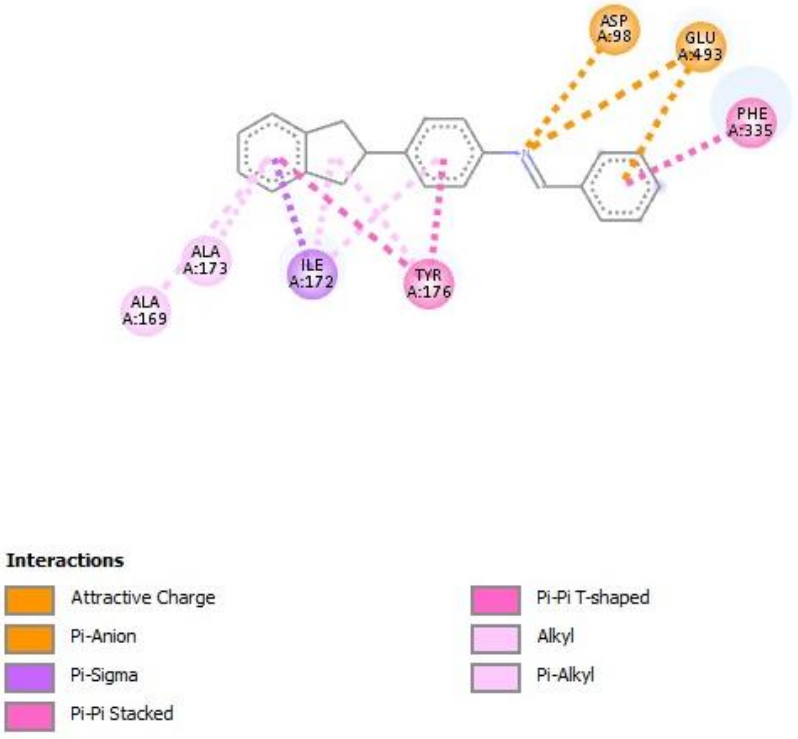
Docking of Citalopram



Docking of C1



Docking of C2



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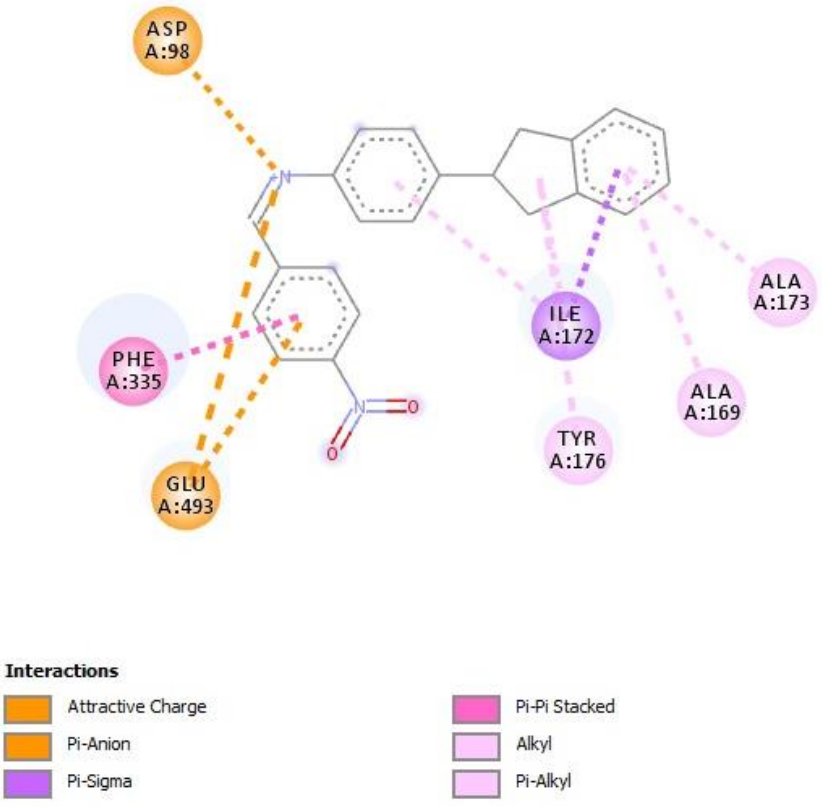

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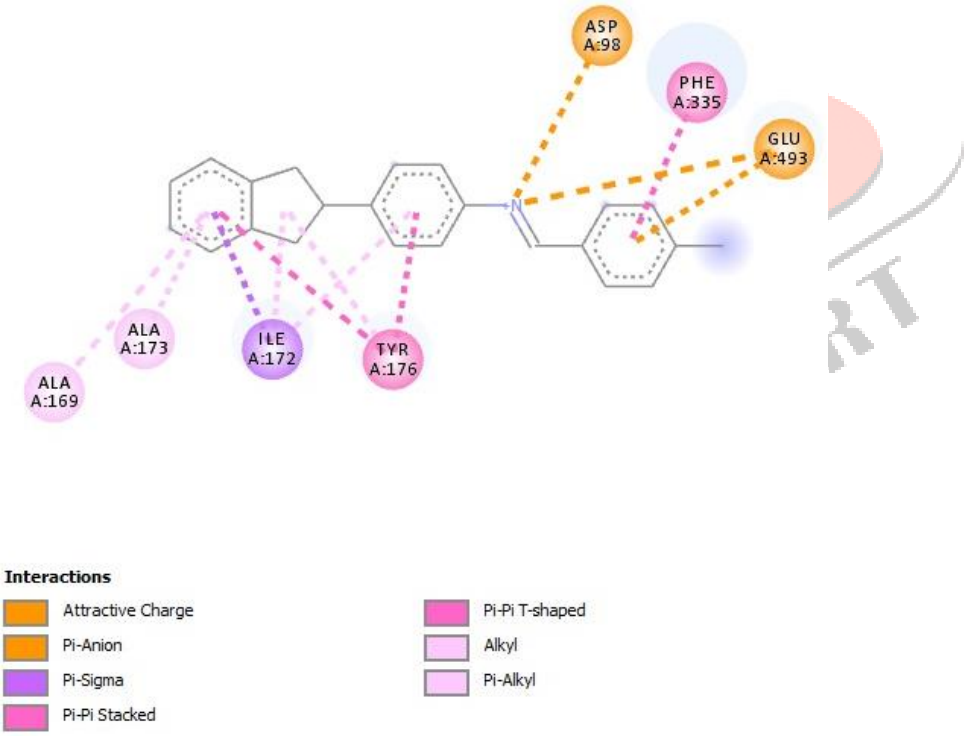
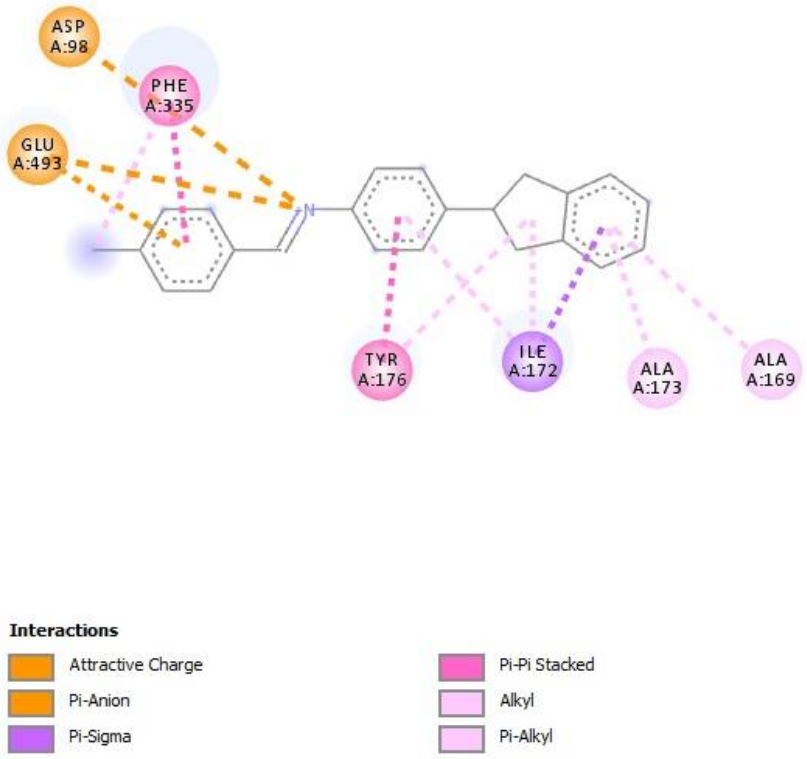


Docking of C5



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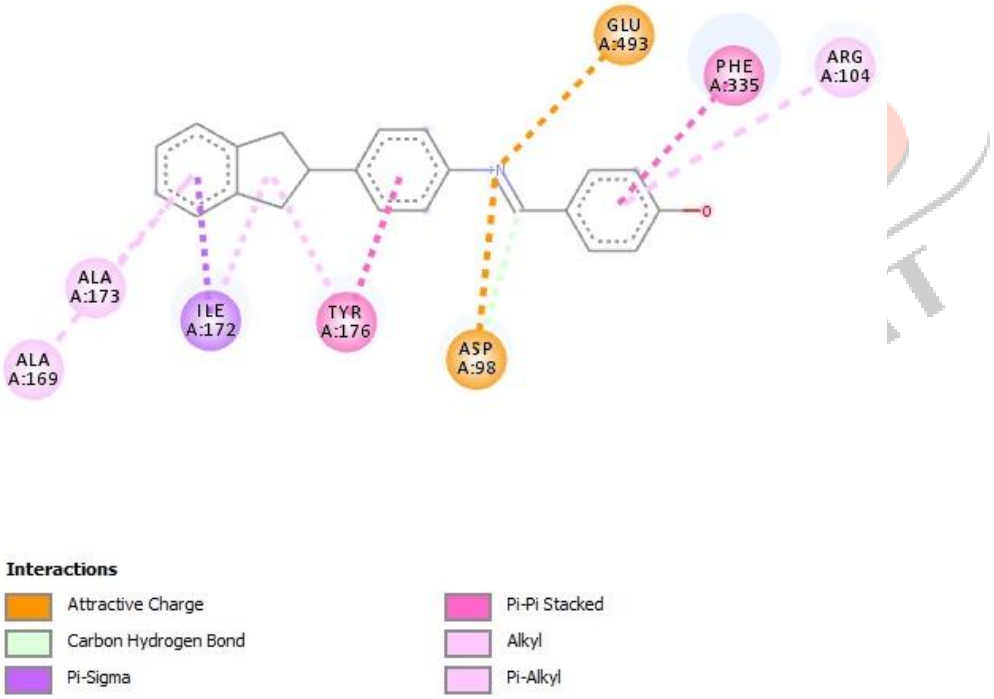
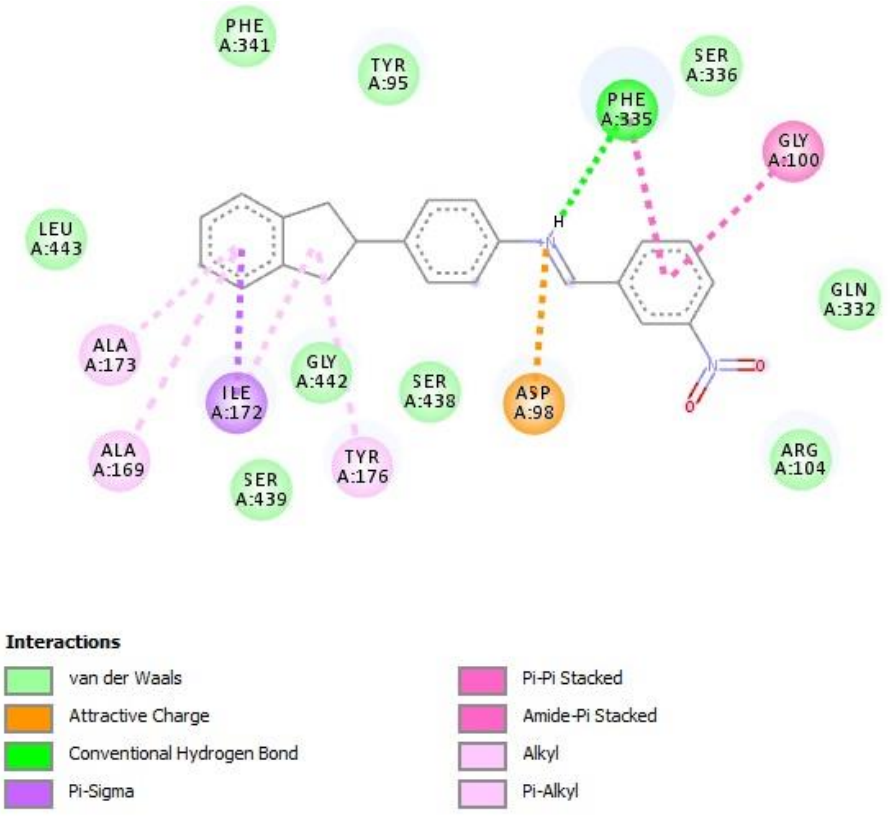
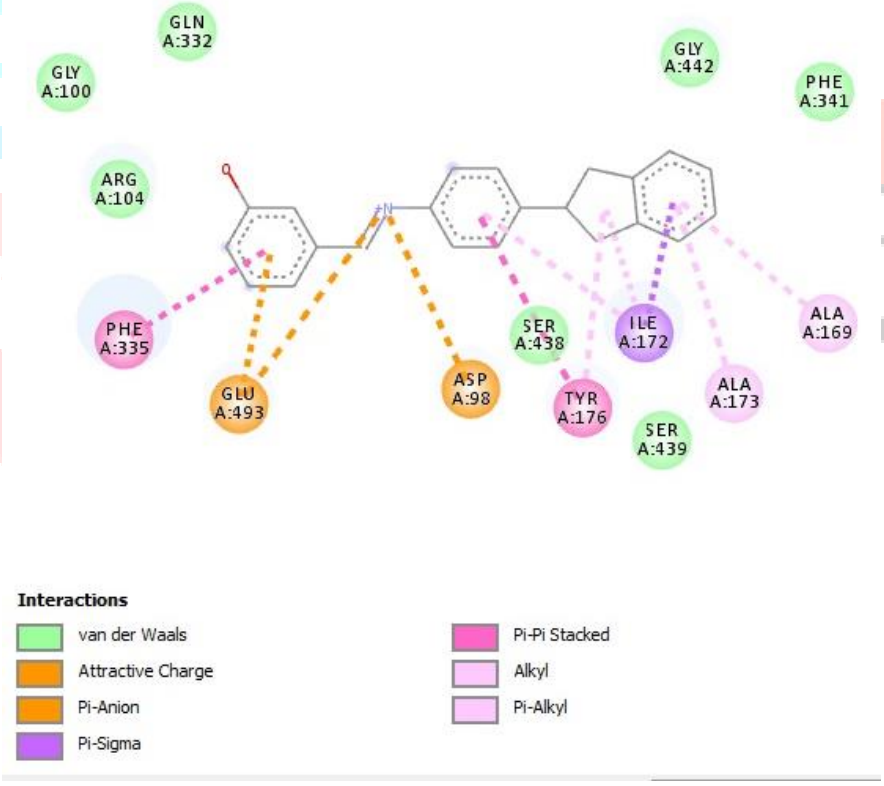


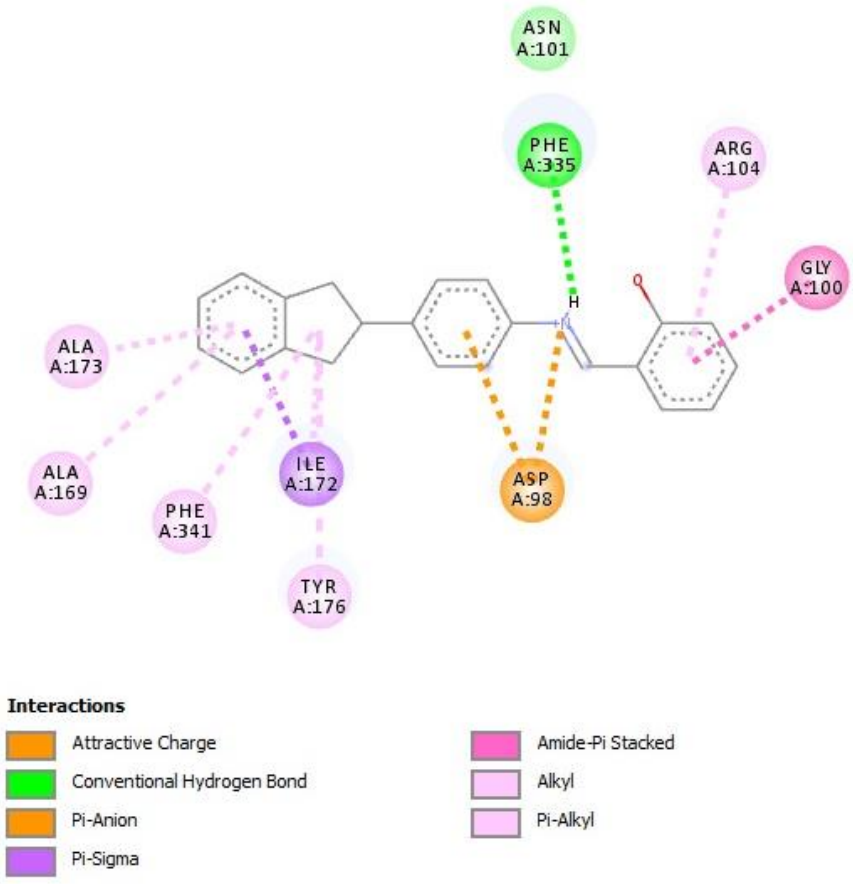
Docking of C7



Docking of C8



Docking of C9



Docking of C10

Fig 4. Molecular docking of synthesized compounds

5.3 Evaluation of antidepressant potential

Tail suspension test

In this test, immobility time in seconds were evaluated in rats- a most significant model. Immobility time was estimated as 168± 1.20*** in control, 138± 1.49*** in PCPA and 74± 1.18*** in fluoxetine treatment group. The benzimidazole derivatives demonstrated immobility time as 108± 1.15***, 102± 2.16***, 104± 1.73***and 104± 3.16*** in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 100mg/kg.

However, immobility time was obtained as 109± 2.74***, 98±4.17*** in C5 and C6, respectively at the dose of 100mg/kg of benzimidazole derivatives. When the response was compared with control group, C2 and C6 were much effective in modulation of sleep with near response to standard- diazepam treated rats. When the responses of treated rats were compared in b/w, it showed that C6 has greater response at higher dose than lower dose.

Table 23. TST of PCPA, Fluoxetine and benzimidazole derivatives (100mg/kg)

Treatment	Immobility time (sec)
Vehicle	168± 1.20***
PCPA (100mg/kg, i. p.)	138± 1.49***
fluoxetine (20mg/kg, p. o.)	74± 1.18***
C1 (100mg/kg)	108± 1.15***
C2 (100mg/kg)	102± 2.16***
C3 (100mg/kg)	104± 1.73***
C4 (100mg/kg)	104± 3.16***
C5 (100mg/kg)	109± 2.74***
C6 (100mg/kg)	98±4.17***
C7 (100mg/kg)	101±2.20***

C8 (100mg/kg)	97±3.19***
C9 (100mg/kg)	103±1.14**
C10 (100mg/kg)	106±2.18***

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

In this model, immobility time in seconds were evaluated in rats- a most significant model. Immobility time was estimated as 168± 1.20*** in control, 138± 1.49*** in PCPA and 74± 1.18*** in fluoxetine treatment group. The benzimidazole derivatives demonstrated immobility time as 96± 1.15***, 89± 2.16***, 90± 1.73*** and 98± 3.16*** in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 200mg/kg.

However, immobility time was obtained as 87± 2.74*** and 83±4.17*** in C5 and C6, respectively at the dose of 100mg/kg of benzimidazole derivatives. When the response was compared with control group, C2 and C6 were much effective in modulation of immobility near response to standard treated rats. When the responses of treated rats were compared, it showed that C6 has greater response at higher dose than lower dose.

Table 24. TST of PCPA, Fluoxetine and benzimidazole derivatives (200mg/kg)

Treatment	Immobility time (sec)
Vehicle	168± 1.20***
PCPA (100mg/kg, i. p.)	138± 1.49***
fluoxetine (20mg/kg, p. o.)	74± 1.18***
C1 (200mg/kg)	96± 1.15***
C2 (200mg/kg)	89± 2.16***
C3 (200mg/kg)	90± 1.73***
C4 (200mg/kg)	98± 3.16***
C5 (200mg/kg)	87± 2.74***
C6 (200mg/kg)	83±4.17***
C7 (200mg/kg)	96±2.19**
C8 (200mg/kg)	97±1.39***
C9 (200mg/kg)	88±2.28***
C10 (200mg/kg)	84±2.73***

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

The benzimidazole derivatives affirm for their antidepressant role in all the doses when compared with control group. Moreover, their effects were almost near to standard group, at dose of (200mg/kg) when compared. Benzimidazole derivatives at the dose of 200mg/kg exhibited optimum response.

Motor Co-ordination determination

Animals' responses to tests of motor coordination were verified using a Rota-rod in this model. Motor coordination was timed at 132± 2.17*** seconds in the control group, 128± 2.19*** in PCPA and 25± 1.28***seconds in the standard group. Whereas, the benzimidazole derivatives (100mg/kg) exhibited the motion as 98± 2.21***sec, 102± 2.27***sec, 88± 2.36***sec and 92± 2.53***sec in C1, C2, C3 and C4, respectively.

However, motion in rota rod was obtained as 106± 4.10***, and 83± 1.37*** in C5 and C6, respectively at the dose of 100mg/kg of benzimidazole derivatives. When the response was compared with control group, C2 and C6 were much effective in depression with near response to standard treated rats.

In this model, C6 showed highest decreased level of motion when observed in Rota rod apparatus.

Table 25. Motor Co ordination test of PCPA, Fluoxetine and benzimidazole derivatives (100mg/kg)

Treatment	Motion in Rota rod (sec)
Vehicle	132± 2.17***
PCPA (100mg/kg, i. p.)	128± 2.19***
fluoxetine (20mg/kg, p. o.)	25± 1.28***
C1 (100mg/kg)	98± 2.21***
C2 (100mg/kg)	102± 2.27***
C3 (100mg/kg)	88± 2.36***
C4 (100mg/kg)	92± 2.53***
C5 (100mg/kg)	106± 4.10***
C6 (100mg/kg)	83± 1.37***
C7 (100mg/kg)	97± 1.13***
C8 (100mg/kg)	102± 1.29***
C9 (100mg/kg)	104± 1.55***
C10 (100mg/kg)	95± 2.29***

At P<0.05 values were found significant

n=6 & values were given in Mean± SEM

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

Rodents' behavior tests of motor coordination were verified using a Rota-rod in this model. Motor coordination was timed at 132± 2.17*** seconds in the control group, 128± 2.19*** in PCPA and 25± 1.28***seconds in the standard group. Whereas, the benzimidazole derivatives (200mg/kg) exhibited the motion as 74± 2.31***sec, 69± 2.47***sec, 67± 2.26***sec and 43± 2.18***sec in C1, C2, C3 and C4, respectively.

However, motion in rota rod was obtained as 67± 2.10*** and 72± 2.16*** in C5 and C6, respectively at the dose of 200mg/kg of benzimidazole derivatives. When the response was compared with control group, C2 and C6 were much effective in depression with near response to standard treated rats. When the responses of treated rats were compared in b/w, it showed that C6 has greater response at higher dose than lower dose.

Table 26. Motor Co ordination test of PCPA, Fluoxetine and benzimidazole derivatives (200mg/kg)

Treatment	Motion in Rota-rod (sec)
Vehicle	132± 2.17***
PCPA (100mg/kg, i. p.)	128± 2.19***
fluoxetine (20mg/kg, p. o.)	25± 1.28***
C1 (200mg/kg)	74± 2.31***
C2 (200mg/kg)	69± 2.47***
C3 (200mg/kg)	67± 2.26***
C4 (200mg/kg)	63± 2.18***
C5 (200mg/kg)	67± 2.10***
C6 (200mg/kg)	72± 2.16***
C7 (200mg/kg)	68± 2.10***
C8 (200mg/kg)	66± 1.14**
C9 (200mg/kg)	72± 1.28***
C10 (200mg/kg)	69± 2.43***

At P<0.05 values were found significant

n=6 & values were given in Mean± SEM

Light-dark arena model

No. of entries was estimated as 4.52±0.32* in control, 4.32±0.64** in PCPA and 8.91±0.27*** in fluoxetine treatment group. The benzimidazole derivatives demonstrated no. of entries as 5.83±0.19**, 5.69±0.10**, 5.58±0.34** and 5.27±0.30** in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 100mg/kg.

However, time spent in light arena was observed as 93.48±0.30, 96.20±0.47, 102.13±0.35 and 94.63±0.11 in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 100mg/kg which was much prominent as compared with control. When the response was compared with control group, C2 and C6 were much effective in movement with near response to standard drug treated rats. When the responses of treated rats were compared, it showed that C6 has greater response at higher dose than lower dose.

Table 27. Light/ dark arena test

Treatment	**No. of entries-** ***light arena***	**Time spent-** ***light arena (sec)***	**% of time spent-** ***light arena (sec)***
Vehicle	4.52±0.32*	67.26± 0.72	24.66±0.37*
PCPA (100mg/kg, i. p.)	4.32±0.64**	65.53±0.32**	23.23±0.12**
fluoxetine (20mg/kg, p. o.)	8.91±0.27***	153.42± 0.38	54.38±0.22**
C1 (100mg/kg)	5.83±0.19**	93.48±0.30	37.25±0.12***
C2 (100mg/kg)	5.69±0.10**	96.20±0.47	38.27±0.39***
C3 (100mg/kg)	5.58±0.34**	102.13±0.35	40.11±0.64***
C4 (100mg/kg)	5.27±0.30**	94.63±0.11	43.46±0.62**
C5 (100mg/kg)	5.87±0.46**	98.47±0.40	38.63±0.10***
C6 (100mg/kg)	5.82±0.13**	102.64±0.34	42.35±0.11***
C7 (100mg/kg)	5.78±0.23**	98.24±0.30	41.10±0.28***
C8 (100mg/kg)	5.80±0.27**	104.21±0.11	40.28±0.33***
C9 (100mg/kg)	5.67±0.32**	101.12±0.28	39.45±0.27***
C10 (100mg/kg)	5.63±0.19**	96.24±0.29	40.27±0.64***

At P<0.05 values were found significant

n=6 & values were given in Mean± SEM

Moreover, same procedure was estimated for higher dose of 200mg/kg. The no. of entries was estimated as 4.52±0.32* in control, 4.32±0.64** in PCPA and 8.91±0.27*** in fluoxetine treatment group. The benzimidazole derivatives demonstrated no. of entries as 6.83±0.20**, 6.69±0.64**, 6.58±0.53** and 6.27±0.13** respectively at the dose of benzimidazole derivatives at the dose of 200mg/kg.

However, time spent in light arena was observed as 102.48±0.30, 114.20±0.47, 107.13±0.35 and 99.63±0.11 in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 200mg/kg which was much prominent as compared with control group. When the response was compared with control group, C2 and C6 were much effective in depression with near response to standard drug treated rats. When the responses of treated rats were compared, it showed that C6 has greater response at higher dose than lower dose.

Table 28. Light/ dark arena test

Treatment	No. of entries- *light arena*	Time spent- *light arena (sec)*	% of time spent- *light arena (sec)*
Vehicle	4.52±0.32*	67.26± 0.72	24.66±0.37*
PCPA (100mg/kg, i. p.)	4.32±0.64**	65.53±0.32**	23.23±0.12**
fluoxetine (20mg/kg, p. o.)	8.91±0.27***	153.42± 0.38	54.38±0.22**
C1 (200mg/kg)	6.83±0.20**	102.48±0.30	45.25±0.18***
C2 (200mg/kg)	6.69±0.64**	114.20±0.47	48.27±0.34***
C3 (200mg/kg)	6.58±0.53**	107.13±0.35	42.11±0.61***
C4 (200mg/kg)	6.27±0.13**	99.63±0.11	46.46±0.68**
C5 (200mg/kg)	6.87±0.46**	104±0.40	34.63±0.14***
C6 (200mg/kg)	7.82±0.86**	110.64±0.34	48.35±0.18***
C7 (200mg/kg)	7.18±0.20**	98.29±0.19	41.27±0.29***
C8 (200mg/kg)	7.27±0.11**	102.29±0.30	38.30±0.39***
C9 (200mg/kg)	7.34±0.38**	97.65±0.32	36.25±0.10**
C10 (200mg/kg)	7.31±0.29***	107.21±0.44	48.28±0.17***

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

For a period of 5 minutes, data on the number of entries, total time, and percentage of time spent in the light arena were collected. The number of participants was lowest in the light category. The rats given fluoxetine spent the most time in the light arena (153.42± 0.38) and had the highest number of entries (8.91±0.27***).

Locomotion activity

In a 10-minute actophotometer test, the control group had the maximum activity at 163±0.20** while the fluoxetine treated group had the lowest activity at 95±0.37**.

The locomotor response was estimated as 163±0.20** in control, 158±0.54** in PCPA and 95±0.37** in fluoxetine treatment group. The benzimidazole derivatives demonstrated locomotor response as 132±0.74***, 129±0.16***, 123±0.23*** and 124±0.65*** in C1, C2, C3 and C4, respectively at the dose of benzimidazole derivatives at the dose of 100mg/kg.

Table 29. Locomotion response

Treatment	Locomotor response
Vehicle	163±0.20**
PCPA (100mg/kg, i. p.)	158±0.54**
fluoxetine (20mg/kg, p. o.)	95±0.37**
C1 (100mg/kg)	132±0.74***
C2 (100mg/kg)	129±0.16***
C3 (100mg/kg)	123±0.23***
C4 (100mg/kg)	124±0.65***
C5 (100mg/kg)	119±0.45***
C6 (100mg/kg)	114±0.40***
C7 (100mg/kg)	107±0.28***
C8 (100mg/kg)	108±0.19***
C9 (100mg/kg)	107±0.37***
C10 (100mg/kg)	111±0.29***

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

Similarly, 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 at the dose of benzimidazole derivatives at the dose of 100mg/kg which was much prominent as compared with control. When the response was compared with control group, C2 and C6 were much effective in movement with near response to fluoxetine treated rats. When the responses of treated rats were compared, it showed that C6 has greater locomotor response at higher dose of benzimidazole derivatives.

Table 30. Locomotion response

Treatment	Locomotor response
Vehicle	163±0.20**
PCPA (100mg/kg, i. p.)	158±0.54**
fluoxetine (20mg/kg, p. o.)	95±0.37**
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)	109±0.15***
C6 (200mg/kg)	102±0.43***
C7 (200mg/kg)	100±0.27***
C8 (200mg/kg)	107±0.26***
C9 (200mg/kg)	103±0.19***
C10 (200mg/kg)	101±0.32***

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

Drug evaluation include verifying its identity, determining its quality, purity, and identifying the type of adulteration. Proximate analysis is helpful in establishing the sample's authenticity and purity, and these values are crucial qualitative benchmarks.

Synthetic derivatives are another promising chemical moiety effective in different pharmacological properties including depression, insomnia, and other neurodevelopmental disorders.

In TST model, it indicates that Serotonin reuptake might be inhibited for the anti-depressant potential of diverse benzimidazole derivatives. Among the several benzimidazole derivatives, the results show that C2 and C6 are the most effective in treating depression and its symptoms. Its mode of action may involve either an increase in the breakdown of catecholamines or a decrease in the release of neurotransmitters. Additionally, the concentration of biogenic amines was reduced, leading to a more refreshed state of mind.

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 new benzimidazole derivatives, which counter 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.

Future prospectives

Depression and stress are two of the most common causes of mental incapacity, and they may have economic and pharmaceutical effects on people's behavior. The synthesized derivatives might be tested for their toxicity and evaluated for their possible mechanism of action through which it showed anti-depressant potential.

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Nil.

CONFLICT OF INTEREST

None.

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