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Synthesis of Different Benzoxazole Derivatives for The Evaluation of Anti-Anxiety/Anti-Depressants

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ABSTRACT

Anxiety and depression are among the most prevalent mental health disorders, often co-occurring and presenting significant challenges to current pharmacological and psychotherapeutic interventions. Existing treatments such as SSRIs and cognitive behavioral therapy are limited by delayed therapeutic effects, variable patient responses, and undesirable side effects, highlighting the urgent need for novel therapeutic agents. Benzoxazole derivatives, due to their heterocyclic structure and ability to interact with biomolecules, offer promising potential as psychoactive compounds. This research focused on the synthesis, characterization, and evaluation of novel benzoxazole derivatives using various synthetic methods, including formylation, cyclization, Suzuki coupling, and nucleophilic substitution reactions. The compounds were characterized through TLC, NMR, IR, and GC-MS analyses to confirm structural integrity and purity. Preclinical studies were conducted using Wistar albino rats, where antidepressant activity was assessed through forced swim and tail suspension tests. Results demonstrated that all synthesized derivatives exhibited significant antidepressant activity, with high-dose derivatives, particularly D2, showing superior efficacy compared to the standard drug, imipramine hydrochloride. These findings suggest that benzoxazole derivatives may represent an effective class of compounds for the treatment of depressive disorders. The study supports further investigation into their mechanism of action and potential clinical application in mental health therapeutics

Key Words:

Benzoxazole Derivatives, **Antidepressant** Activity, Synthetic Methods, **Behavioral** Pharmacology, **Mental** Health Therapeutics.

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1.INTRODUCTION

Mental health conditions, especially anxiety and depression, are major public health problems worldwide. Anxiety disorders are excessive fears or worries that disrupt the ability to function on a day-to-day basis, and depression is defined by constant sad feelings, loss of interest in normal activities, or loss of pleasure from normal life. Due to symptom overlap, a high frequency of co-occurrence exists amongst these disorders, often complicating their diagnoses and treatments . Anxiety is among the most common mental disorders in the population, with approximately 31% of U.S. adults experiencing an anxiety disorder at some point in their

lifetime . Indeed, depression is one of the most common mental disorders and is a very crucial cause of disability internationally, as over 264 million people of all ages face depressive disorders according to the World Health Organization.[1,2,3,4]

This enormous treatment burden highlights the need for effective treatments, which continue to rely on pharmacotherapy, psychotherapy, or both. Today, treatment can involve medication, like selective serotonin reuptake inhibitors (SSRIs), and therapy, like cognitive behavioral therapy (CBT). Although pharmacological treatments yield diverse responses and the requisite, trial-and-error nature in deciding the effective treatment regimen is patient dependent, an important part of the physiological variability among individuals, these compounds often fall short . Furthermore, SSRIs often take weeks to reach full therapeutic effect, leading patients to abandon treatment plans. Furthermore, several patients either do not respond to current treatments or suffer from adverse effects associated with them; thus, the development of new agents with improved effectiveness and tolerability has been sought. [5,6,7,8]

These limitations highlight the need for new agents for therapy, which is represented in the research landscape of mental health. The need for more effective compounds with the ability to modulate these neurotransmitter systems, compared with standard medications, has increased . For example, their compounds are promising to have anxiolytic [10] and antidepressant activity due to their ability to modulate serotonin and norepinephrine that are mood-related neurotransmitters . Exciting work in preclinical models for the treatment of mental health disorders is also being explored with new compounds. Studies of animals have shown that it can increase the resilience to stress and change the emotion-related neural pathways by drugs . Additionally, the data indicate that such molecules affect neuropeptide systems (neuropeptide Y) that may be involved in anxiety and depression. [9,10,11,12]

The potential combination of these novel agents with current therapies may expand upon treatment regimens, especially for patients with clinically significant comorbidity, where treatment is more complicated . Furthermore, new therapies aim at targeting the biological basis of anxiety and depression, treating the disorder and not just symptoms to the underlying neurobiology that leads to these disorders. Non-invasive neuromodulation techniques, for example, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), have been investigated as treatments for anxiety and depression, even in treatment-resistant populations.This reflection marks a broader transition that moves away from pharmacological or psychotherapeutic-only approaches and toward an integrated mindset. The acknowledgement of common neurobiological paths of anxiety and depressive disorders highlights the need for new therapeutic agents.[13,14,15,16]

The overlap in symptoms common to both conditions gives rise to research and treatment approaches focused on one disorder that can apply to the other . If a wider lens is used in clinical research, this allows treatments to more holistically represent the networked biopsychosocial model of emotional disorders. As such, exploring new compounds is a potential approach to address therapeutic voids for mental health, suitable for the current practice environment . Benzoxazole is one of the important members of heterocyclic aromatic compounds, and it is composed of a fused benzene and an oxazole ring. Its large melting point range suggests that its derivatives have biologically active processes, which further emphasizes it in medicinal chemistry. The planar structure of benzoxazole enables π-stacking interactions

as well as hydrophobic interactions with several biomolecules, especially proteins, and so it is commonly used in drug development.[1,2]

2. MATERIAL AND METHODS

2.1 SYNTHETIC METHODS

2.1.1 Formylation reaction

In a suitable reaction vessel equipped with a stirrer and a reflux condenser, combine 2,5-dimethylphenol and urea in the desired molar ratio (commonly 1:1.2 to ensure complete conversion). Add a catalytic amount of phosphoric acid (typically 10–20 mol% relative to the phenol) to the mixture . After that, the resulting solution is heated under reflux to 160–180°C while being constantly stirred. Ammonia gas is a byproduct of the heating process, which is kept up for a number of hours (usually 4-8 hours). The solid chemical derivative is precipitated by pouring the raw substance into ice-cold water after the reaction mixture has cooled to its original state. Filtration is used to collect the precipitate, which is then cleaned with water to get rid of any remaining urea and acid subsequently dried at lower pressure. If higher purity is required, the crude product can be recrystallized from ethanol or another suitable solvent.[1,20]

2.1.1.1 Reaction Mechanism

The mechanism of the reaction begins with the oxidative addition of an aryl iodide (Ar-I) to a palladium(0) complex in the presence of a ligand (L) [21] This step forms a palladium(II) intermediate. In the next step, the palladium(II) complex undergoes coordination with carbon monoxide (CO), resulting in the formation of an arylpalladium(II) carbonyl complex . The palladium-carbonyl complex then proceeds to a reductive elimination step, where the aryl group and a proton are released as a carboxylic acid product (HCOOH), and the palladium(0) catalyst is regenerated. This allows the reaction to cycle and repeat, with the palladium catalyst facilitating the insertion of CO and the formation of the desired carboxylic acid. DABCO (a base) and the ligand (L) stabilize the palladium complex and promote the overall reaction efficiency.[22,23]

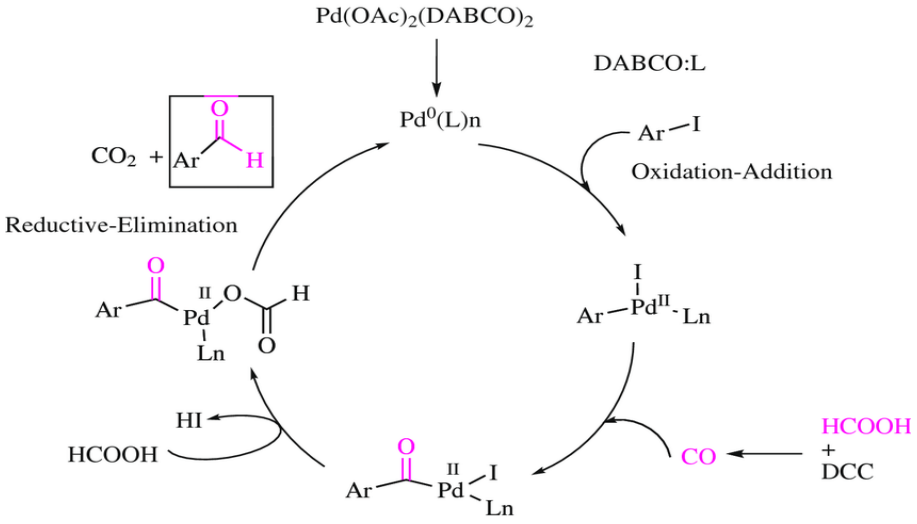

Fig .1 Reaction mechanism of formylation reaction

2.1.2 Cyclization reaction

A mixture of the appropriate precursor, commonly 2-aminophenol , is placed in a reaction vessel. Phosphorus oxychloride is then added, often in excess, to serve both as the dehydrating agent and the reaction medium . The mixture is stirred at room temperature for a short period (typically 30 minutes) to ensure thorough mixing and dissolution. The reaction temperature is then gradually increased, often to reflux (typically between 90–120 °C), and the mixture is maintained under these conditions for several hours (commonly 6–12 hours), allowing cyclization and dehydration to proceed, forming the benzoxazole ring system. In some protocols, a catalyst such as dimethylaniline, pyridine, or its derivatives may be added to enhance the reaction rate and yield. After completion, as monitored by TLC or other analytical methods, the reaction mixture is cooled, and the excess $POCl_3$ is removed by distillation under reduced pressure. The crude product is then purified, typically by recrystallization or column chromatography, to afford the desired benzoxazole derivative in good yield .[24,25]

2.1.2.1 Reaction Mechanism

The mechanism involves a catalytic cycle with a metal-ligand complex. Initially, the boron-containing substrate ($B(C_6F_5)_3$) reacts with an amine (NR_3) to form an intermediate complex (A), where the amine coordinates to the metal center. This enhances the reactivity of the metal-ligand complex . In step A, the metal center undergoes nucleophilic attack on the carbonyl group of the substrate, resulting in the formation of intermediate complex B. This step involves the formation of a metal-carbon bond. In step C, the intermediate undergoes a transformation that leads to proton release (H^+), resulting in the formation of a new species. Finally, in step D, the complex undergoes another transformation that leads to the formation of the final product, regenerating the metal-ligand complex and completing the catalytic cycle. This mechanism is characteristic of reactions like hydrogenation or other metal-catalyzed transformations.[26,27]

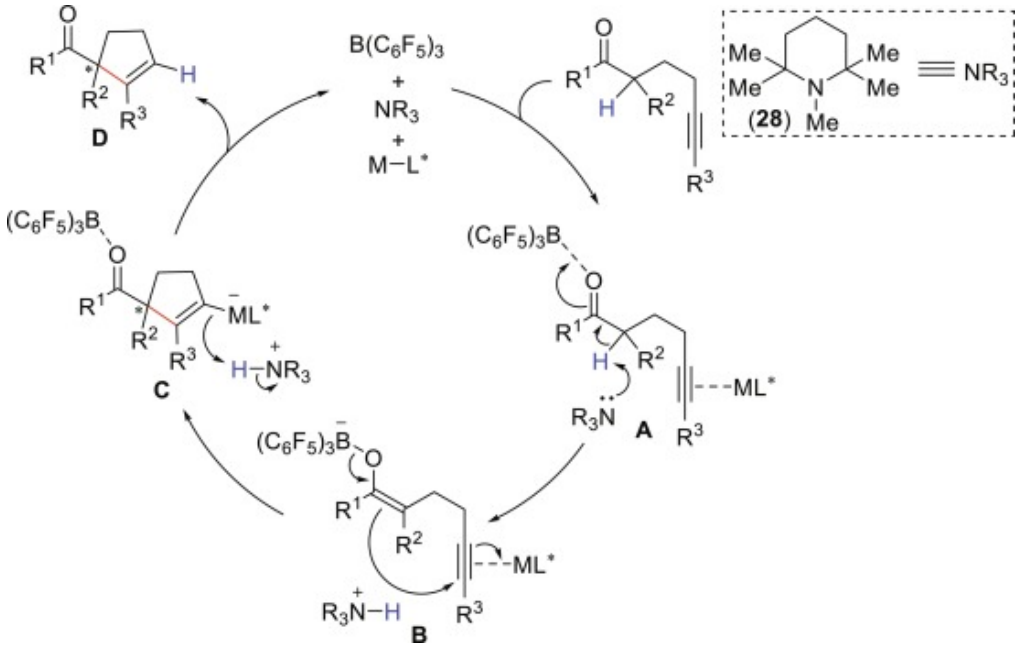

Fig 2 Reaction mechanism of Cyclization Reaction

2.1.3 Suzuki coupling reaction using a palladium catalyst.

To synthesize a benzoxazole derivative via the Suzuki coupling reaction using a palladium catalyst, begin by preparing the appropriate halogenated benzoxazole substrate (2-bromobenzoxazole) and the desired aryl or heteroaryl boronic acid. In a dry round-bottom flask, add the halogenated benzoxazole (1.0 equivalent), the boronic acid (1.2–1.5 equivalents), a palladium catalyst such as $Pd(PPh_3)_4$ (typically 2–5 mol%), and a suitable base like potassium carbonate (K_2CO_3, 2–3 equivalents). Add a solvent system such as a 10:1 mixture of 1,4-dioxane and water or DMF, ensuring the total reaction volume allows for efficient stirring.Seal the flask and purge with nitrogen to maintain an inert atmosphere. Heat the reaction mixture to 80–100 °C and stir for 3–18 hours, monitoring the progress by TLC. Upon completion, cool the mixture to room temperature, dilute with water, and extract the product into an organic solvent such as ethyl acetate. Wash the organic layer with brine, dry over anhydrous sodium sulfate, and concentrate under reduced pressure. Purify the crude product by column chromatography using an appropriate eluent system. For final purification and to remove residual palladium, treat the product with activated carbon if necessary, then recrystallize or further purify as required. This protocol yields the desired benzoxazole derivative with high efficiency and functional group tolerance.[28,29]

2.1.3.1 Reaction Mechanism

Several crucial phases are involved in the Suzuki and Miyaura cross-coupling response process facilitated by a palladium catalyst. Initially, the palladium catalyst (Pd^0) undergoes oxidative addition with an aryl halide (red substrate), where the palladium inserts into the carbon-halide bond, forming a palladium(II) complex. In this step, the palladium is oxidized from the 0 to the +2 oxidation state . The next step, transmetallation, involves the exchange of the palladium from the aryl halide to the boronic acid or ester (blue substrate). This step is facilitated by a base such as Na_2CO_3, which helps in transferring the aryl group from the boron atom to the palladium, forming a new palladium-boron bond. Finally, in the reductive elimination step, the palladium(II) complex releases the coupled product—an aryl-aryl bond—regenerating the palladium(0) catalyst, which can enter another catalytic cycle. This efficient process enables the formation of carbon-carbon bonds between aryl or vinyl compounds and is widely used in organic synthesis.[30]

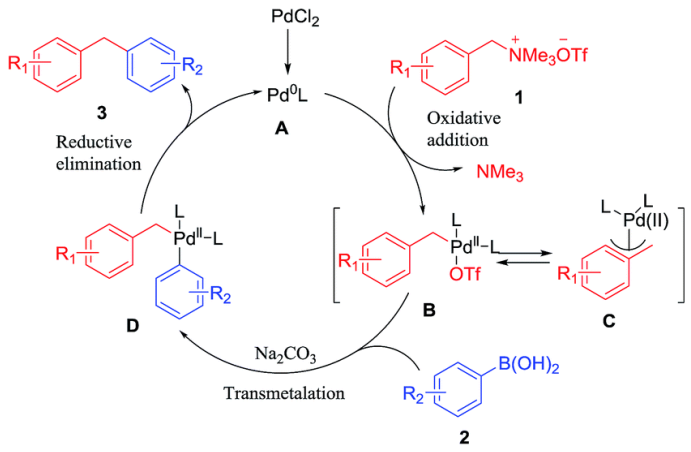

Fig 3 Reaction Mechanism of Suzuki Coupling Reaction

2.1.4 Nucleophilic substitution reaction

To perform a nucleophilic substitution reaction for the synthesis of a benzoxazole derivative in the laboratory, begin by preparing a suitable 2-halo (typically 2-chloro) benzoxazole as the electrophilic substrate. In a dry round-bottom flask, dissolve the 2-halo benzoxazole (e.g., 1 mmol) in an aprotic polar solvent such as dimethylformamide (DMF) or acetonitrile under a nitrogen atmosphere to prevent moisture interference. Add the chosen nucleophile (such as an aryloxy, alkoxy, or thiolate compound, 1.1–1.5 mmol) to the reaction mixture, followed by a base like potassium carbonate (K_2CO_3) or sodium hydride (NaH) to deprotonate the nucleophile and enhance its reactivity. Stir the mixture at an elevated temperature (typically 80–120 °C) for several hours, monitoring the progress by thin-layer chromatography (TLC). After the process is finished, let it cool to room temp and then add chilled water to the mixture to cause the result to precipitate. Use an appropriate solvent (e.g., ethyl acetate) to extract the organic material, then rinse with a brine solution, dry over anhydrous potassium sulphate, and concentrated under low pressure. Use column chromatography with an appropriate eluent method to purify the raw material.[31,32]

2.1.4.1 Reaction Mechanism

The process begins with the departure of the chloride ion (Cl^-) from the substrate, forming a planar carbocation intermediate. This carbocation is achiral, making it capable of undergoing nucleophilic attack from either the left or right side.The iodide ion (I^-) attacks the carbocation, and because the intermediate is planar, the nucleophile can approach from either direction, resulting in the formation of two possible enantiomers. If the iodide attacks from the left side, the product is the R-enantiomer, while an attack from the right side results in the S-enantiomer. This leads to a racemization of the product, typical of SN1 reactions, where both enantiomers are formed due to the planar nature of the carbocation intermediate.[33,34]

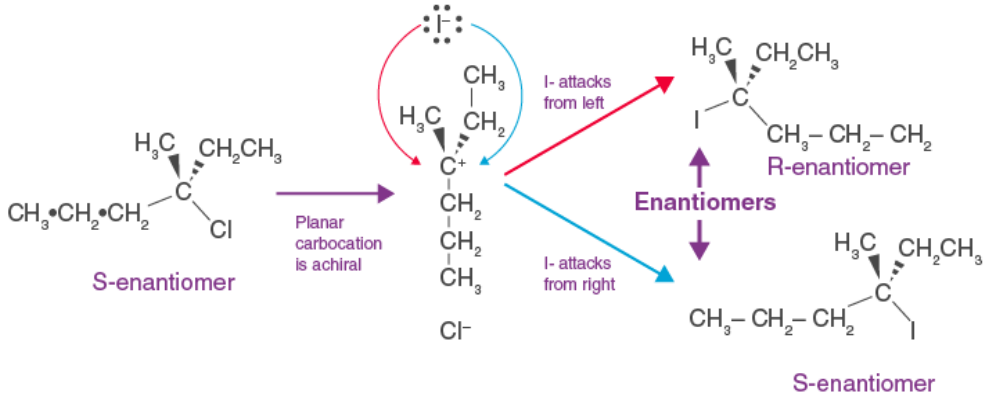

Fig 4 Reaction Mechanism of Nucleophilic Substitution Reaction

2.2 Characterization of the different synthesized benzoxazole derivatives

2.2.1 Thin Layer Chromatography (TLC)

The characterisation of synthesised benzoxazole derivatives through thin-layer chromatography (TLC) is a pivotal technique commonly employed in organic synthesis to monitor reaction progress and assess the purity of products. For instance, the work by Łukowska-Chojnacka et al. demonstrated the importance of TLC for monitoring reactions

producing benzoxazole derivatives, confirming the necessity of analyzing products alongside ongoing processes to ensure high purity levels before further purification steps . Similarly, Kakkar et al. highlighted the use of TLC in evaluating the synthesis of various benzoxazole derivatives, successfully confirming the presence of desired compounds through effective separation and analysis . Additionally, Alheety emphasized the effectiveness of TLC not only in characterizing benzoxazole derivatives but also in establishing the identity and purity through comparative visualization against standard samples. Thus, TLC remains an essential method for the characterization of benzoxazole derivatives in both qualitative and quantitative contexts.[35,36,37]

2.2.2 Nuclear Magnetic Resosnance (NMR)

The synthesized benzoxazole derivatives were characterized using Nuclear Magnetic Resonance (NMR) spectroscopy, which provided detailed insights into their structural features. The proton NMR (^1H NMR) spectra typically exhibited distinct signals corresponding to the aromatic protons, with peaks in the 6.5-8.5 ppm range, characteristic of the benzene ring. Substituent groups on the benzoxazole ring would influence the chemical shifts and multiplicity of these signals, depending on their electronic effects and positions. In addition, the ^13C NMR spectra revealed the chemical environments of the carbon atoms within the benzoxazole ring, with the carbon signals appearing in the range of 100-160 ppm. Any additional functional groups, such as halogens or alkyl groups, would result in shifts specific to those environments. The integration of the peaks allowed for quantification of the different protons and carbons, confirming the purity and composition of the synthesized derivatives. Additionally, coupling constants (J values) were used to determine the spin-spin interactions between neighboring protons, helping to confirm the molecular structure of the compounds.[38,39,40]

2.2.3 Infrared Spectroscopy (IR)

Infrared (IR) spectroscopy is an efficient analytical method for the identification of functional groups and investigation of the molecular structure of compounds. IR spectroscopy can give significant information about the molecular characteristics of a benzoxazole derivative. The characteristic bands of absorption in the IR spectrum of a benzoxazole derivative are usually the N-H stretching vibration between 3300-3500 cm^{-1} (in case the compound has an amine or an amide group), the aromatic ring C-H stretching vibrations around 3000 cm^{-1}, and the C=N stretching vibration, which also occurs around 1600-1650 cm^{-1} . The benzene ring also causes several absorption bands between 1450-1600 cm^{-1} corresponding to the C=C stretching vibrations. The existence of the oxygen atom within the heterocyclic framework usually gives rise to a C-O stretching band in the 1200-1300 cm^{-1} range. These diagnostic IR absorptions serve to identify and confirm the structure of the benzoxazole derivative and to differentiate it from other related compounds.[41,42]

2.2.4 Gas Chromatography – Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is a method of analysing chemical compounds in mixtures. In the case of searching for benzoxazole derivatives, GC-MS offers a sensitive and effective means of detecting the compounds. The procedure starts with the sample being vaporised and injected into the gas chromatograph, where it is separated into components according to their volatility. As every compound comes out of the chromatograph, it is scanned by the mass spectrometer, which breaks up the molecules and finds their mass-to-charge ratio. In the case of benzoxazole derivatives, GC-MS can provide useful data regarding molecular structure, functional groups, and fragmentation patterns of such compounds. Benzoxazole derivatives may possess characteristic mass spectral peaks through which they can be identified even from complex mixtures. With the application of a known compound database, the identification of the benzoxazole derivative is confirmed by comparison of the fragmentation pattern with that of the reference standards.[43,44]

2.2.5 Experimental study for Anti-depressant activity for derivatives

Chemicals: The investigation made use of imipramine hydrochloride. Standard imipramine hydrochloride and prepared derivatives were dissolved in distilled water and given orally (p.o.) or intraperitoneally (i.p.). The vehicle was made of distilled water.
Animals: For the experiment, Wistar albino rats weighing between 50 and 100 g of either sex were used. The animal was housed under standard settings, which included a 12-hour light/dark cycle, a room temperature of 26 ± 2°C, and a relative humidity of 45–55%, in an animal home approved by CPCSEA, the Committee for Control & Supervision of Experiments on the Animal. The animal had unfettered access to food and drink while housed in polypropylene cages.

2.2.6 Groups for study:

There were 8 groups of rats (female) (n=6). The animals were given drugs or a vehicle 60 minutes before the research began.
Group I= Saline administration (Negative Control) i.e., 2ml/kg

Group II= Received Standard Drug (10 mg/kg)

Group III= Received D1 (low dose 250 mg/kg)

Group IV= Received D1 (high dose 500 mg/kg)

Group V= Received D2 (low dose 250 mg/kg)

Group VI= Received D2 (high dose 500 mg/kg)

Group VII= Received D3 (low dose 250 mg/kg)

Group VII= Received D4 (low dose 250 mg/kg)

Group VIII= Received D4 (high dose 500 mg/kg)

2.7 Experiment

- **Forced Swim Test**

As component of the forced swim test (FST), rats were compelled to swim separately in an open circular container that was 10 cm in diameter and 25 cm in height. The container was

filled with 19 cm of water that was at 25±1°C. The therapy was given 60 minutes before to the start of the trial, per the study design. Each animal was forced to swim for a total of six minutes, and the duration of immobility during the last four minutes of the test was recorded. Each mouse was deemed immobile when it gave up trying and remained immobile within water with only moving to maintain its head under the surface. A decrease in the duration of immobility suggests an inverse antidepressant effect.[45]

Fig 5 Forced Swim Test

- **Tail Suspension Test**

This study's tail suspension technique was comparable to those outlined by Steru et al. (1985) . According to the study design, the treatment was administered 60 minutes before the study began. With the aid of sticky tape positioned about 1 centimeter from the tip of the tail, rat were suspended on the edge of the table, 50 cm above the floor. For six of the ten minutes, the complete amount of immobility brought on by tail suspension was noted. When an animal could not move its body and was suspended passively and motionless, it was deemed immobile.[46,47]

Fig 6 Tail Suspension Test

2.8 Statistical Analysis

The data were statistically examined using a one-way ANOVA and the Dunett Multiple Comparison Test; $P<0.05$ was deemed significant. All values were presented as Mean ± S.E.M.

3.Result & Discussion

3.1 Formylation reaction

In a 100 mL round-bottom flask, 15.27 g of 2, 5-dimethylphenol was measured and placed in 6.8 mL of urea to create the desired benzoxazole derivative. The reaction liquid was then carefully stirred to guarantee complete mixing after 1.1 mL of phosphorous acid was introduced to the beaker as a catalyst. The mixture had been heated in reflux on a bath of water for 6–8 hours after a condenser for reflux was connected to the flask. Thin Layer Chromatography, or TLC, was used to monitor the reaction's progress on a regular basis. The reflux was continued until the starting materials' spots on the TLC plate vanished, indicating that the reaction had been completely consumed. Following the completion of the reaction, the liquid was given time to cool to ambient temperature before being gradually poured, while being continuously stirred, into a beaker filled with crushed ice. The substance precipitated as a solid as a result of this cooling and diluting procedure. After collecting the solid via a vacuum system using a funnel made by Buchner, it was repeatedly rinsed with cold water to get rid of any soluble contaminants and unreacted acetic acid. The material was recrystallised from alcohol or an alcohol-petroleum ether combination in order to cleanse the crude product. In order to get pure 2-substituted benzoxazole, the purified crystals were ultimately baked in an oven at 40–50°C.

Fig .7 Reflux Condensor

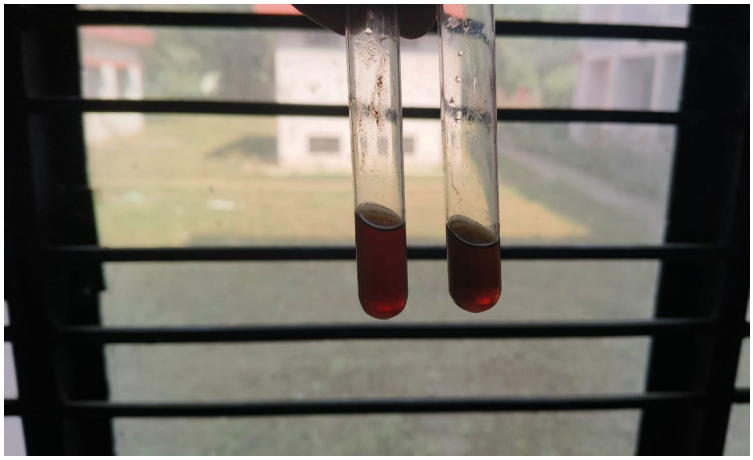

Fig 8 Synthesized Product

3.2 Characterization of the 1st synthesized product

- **Thin Layer Chromatography (TLC)**

The thin layer chromatography (TLC) analysis of the product was conducted using a suitable solvent system. The developed TLC plate revealed a prominent spot corresponding to the product, which travelled a distance close to the solvent front. The calculated retention factor (Rf) value for the compound was 0.96, indicating that the compound is relatively non-polar under the employed conditions. This high Rf value confirms the successful formation of the product and suggests efficient separation with minimal impurities visible on the plate.

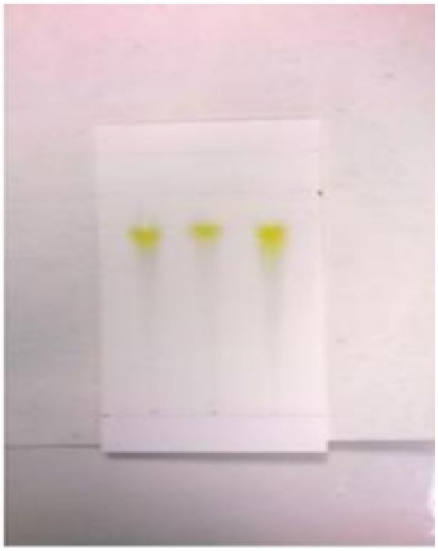

Fig 9 TLC of the Synthesized Benzoxazole Derivative

- **Infrared Spectroscopy (IR)**

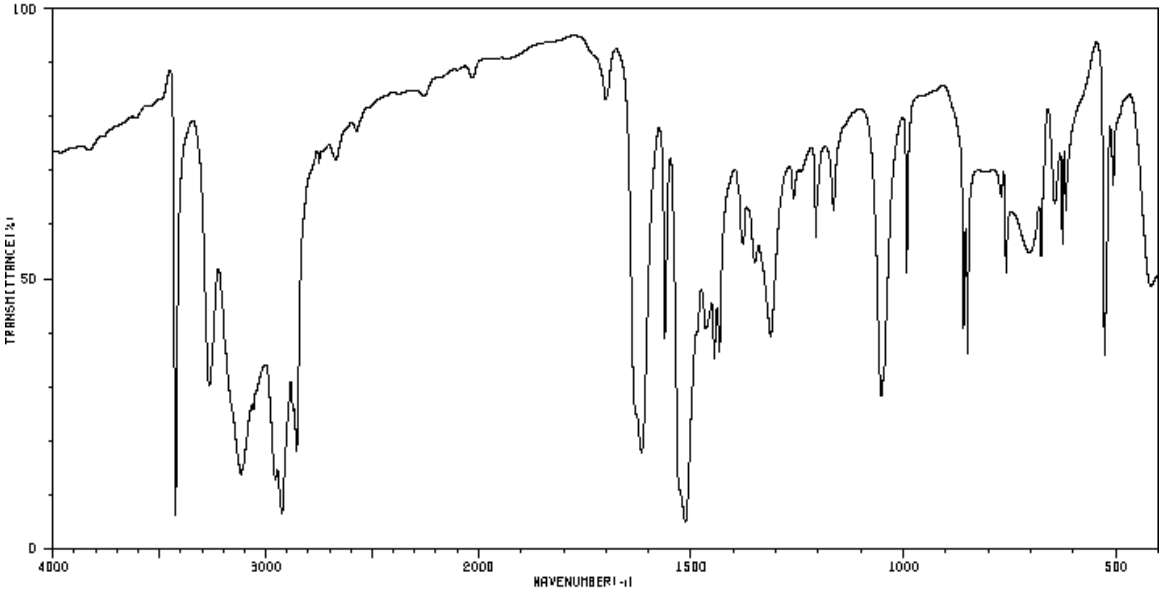

Fig 10 .IR data of the synthesized of derivative 1

Table 1 Interpretation of the IR data

Wavenumber (cm^{-1})	Intensity (%)	Functional Group / Assignment	Interpretation
~3300–3500	Strong, Broad	O–H stretch	Broad signal suggests –OH
~2950–2850	Medium	C–H stretch (sp^3)	Typical of alkanes
~1700	Strong	C=O stretch	Carbonyl group (could be ester, acid, ketone)
~1600–1500	Medium	C=C stretch (aromatic or alkene)	Indicates possible aromatic ring
~1250–1050	Medium	C–O stretch	Supports ester

- **NMR Data**

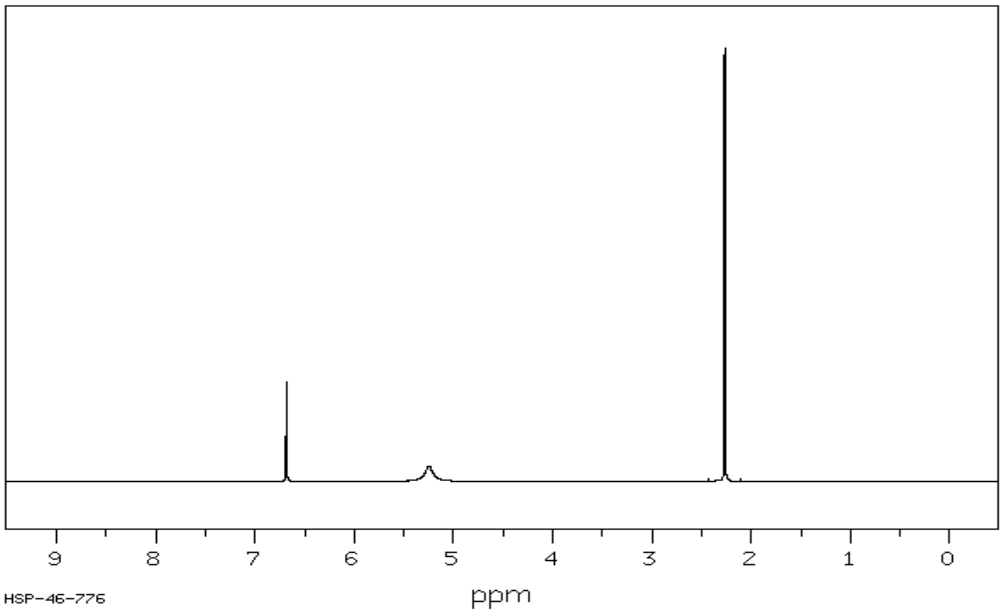

Fig 11 NMR data of the synthesized derivative 1

Table 2 Interpretation of the NMR data of derivative

Chemical Shift (ppm)	Integration (Relative Area)	Multiplicity (Splitting)	Possible Proton Type	Possible Environment
~7.2	1	Singlet or narrow multiplet	Aromatic or vinyl H	Likely aromatic ring
~5.2	1	Doublet or multiplet	vinylic H or near electronegative atom	Possibly -CH= or -CH(O)-
~2.1	3	Singlet	Methyl group (CH_3)	Possibly -CH_3 adjacent to carbonyl or aromatic

- **GC MS Data**

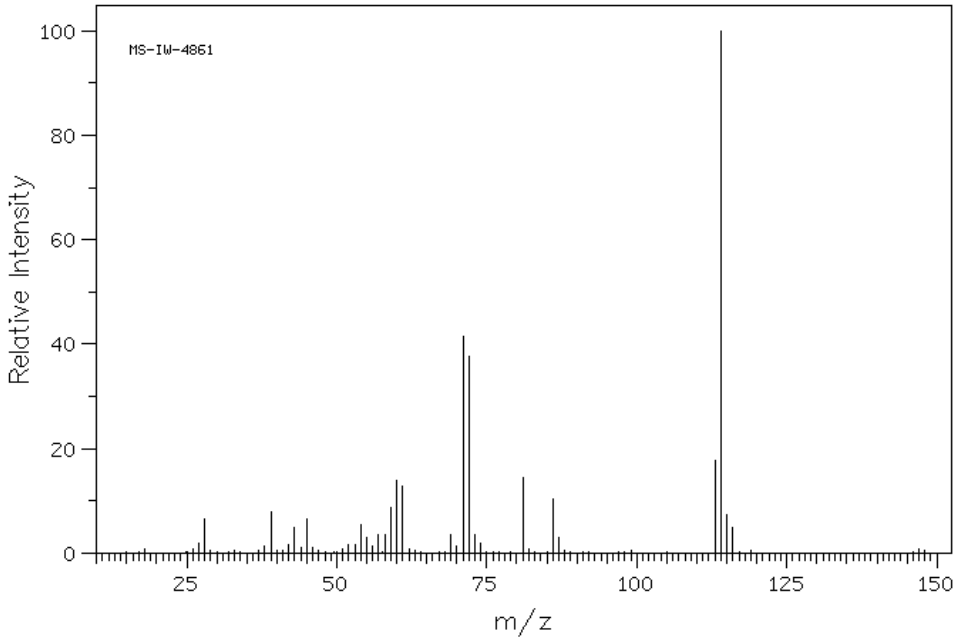

Fig 12 GC MS data of the synthesized derivative 1

Table 3. GC MS Data Interpretation of the 1st synthesized derivative

m/z	Relative Intensity (approx.)	Possible Fragment	Interpretation
112	100 (base peak)	M^+ or stable fragment ion	Likely molecular ion (M^+), MW ≈ 112

85–90	~30–40	M^+ – CH_3/CH_2=CH_2	Loss of alkyl group
72–75	~20–30	Common alkyl/aromatic fragments	Possibly tropylium or substituted ring systems
43–57	~10–20	CH_3^+, $C_2H_5^+$, CO^+	Low-mass alkyl/carbonyl fragments

3.3 Cyclization reaction

A 100 mL cylindrical-bottom flask containing 1.09 g (10 mmol) of two-aminophenol was filled with 4.6 mL of phosphorous oxychloride ($POCl_3$), which served as the reaction's medium and dehydrating agent. As an optional catalyst, 80 µL of quinoline was used for a greater yield and faster reaction rate. To guarantee even mixing and reactant dissolution, the mixture was allowed to agitate for half an hour at ambient temperature. The chemical benzoxazole ring system was then produced by cyclisation and dehydration, which were made possible by refluxing the reaction to 90–120 °C and maintaining it there for 6–12 hours. After the reaction was finished, the remainder of $POCl_3$ was vacuum-extracted and the mixture that resulted was given time to cool to the ambient temperature. If necessary, a completely saturated solution of sodium bicarbonate was used to neutralise the resultant combination after the residue was gradually cooled with ice or frigid water while being stirred. Ethyl acetate was used to extract the product, and anhydrous sodium sulphate was used to dry the mixed organic layers. To obtain the required benzoxazole derivative, the crude product was either refined by column chromatography or recrystallised from ethanol after the solvent was filtered off.

Fig 13 Synthesized Derivative via Cyclization Reaction

3.4 Characterization of the 2nd synthesized product

- **TLC**

In the experiment, a thin-layer chromatography (TLC) plate was observed under UV light, as shown in the image. The distance traveled by the spot from the baseline and the distance traveled by the solvent front were measured. The spot had moved 6.5 cm from the baseline, while the solvent front had reached 10 cm. Using these measurements, the Rf value was calculated by dividing the distance travelled by the spot by the distance travelled by the solvent front, resulting in a positive Rf value of 0.65. This indicated that the compound had shown moderate movement with respect to the stationary phase under the experimental conditions.

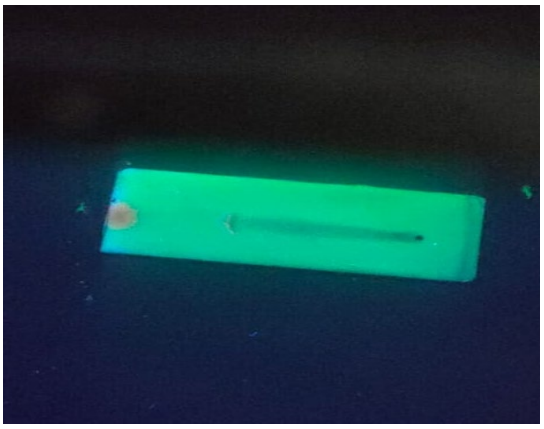

Fig 14 TLC **of the 2nd Synthesized Derivative**

- **IR Data**

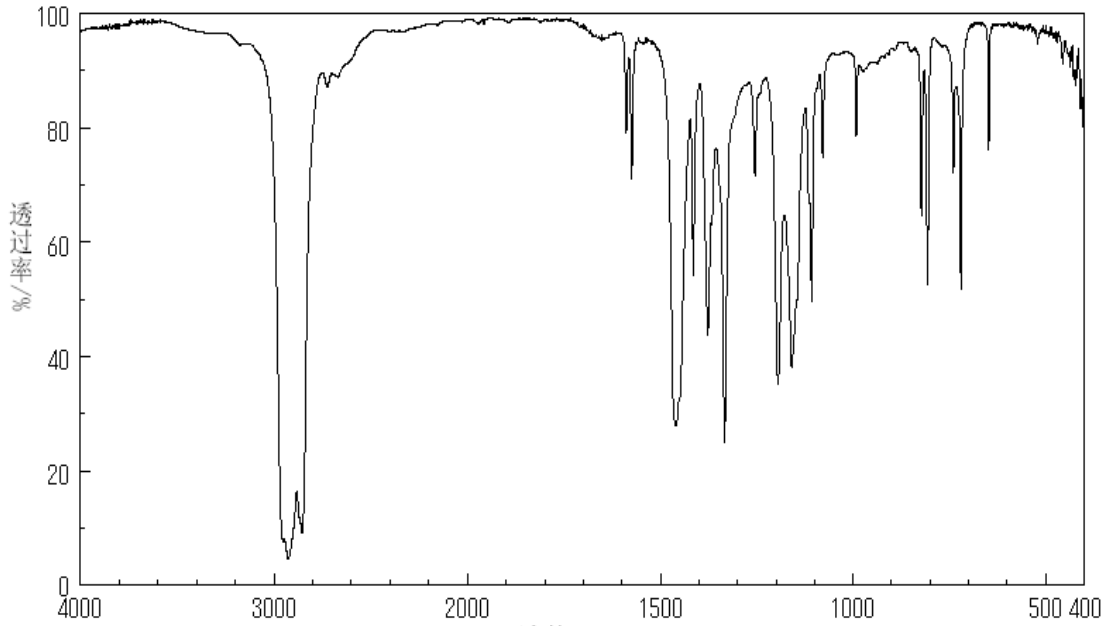

Fig. 15 IR Data of the 2nd Synthesized Derivative

Table 4 IR data Interpretation of the 2nd derivative

Wavenumber (cm^{-1})	**Functional Group**	**Intensity / Peak Description**
~3300	O–H (alcohol/phenol)	Very broad, strong
~2950	C–H (alkane)	Medium, sharp
~1700	C=O (carbonyl)	Strong, sharp
~1600	C=C (aromatic/alkene)	Medium, sharp
~1450	C–H (alkane bending)	Medium
~1250–1000	C–O (alcohol, ester, ether)	Strong, sharp
~900–700	Aromatic C–H (out-of-plane)	Medium to strong, multiple sharp peaks

- **NMR Data**

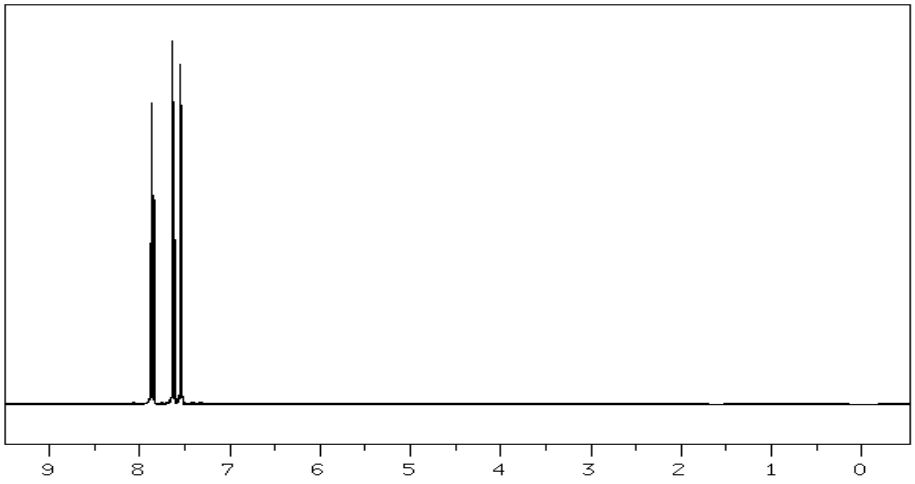

Fig 16 NMR Data of the 2nd synthesized derivative

Table .5 Interpretation of NMR Data of the 2nd Derivative

Chemical Shift (ppm)	Functional Group / Proton Type	Intensity / Peak Description
7.0 – 8.5	Aromatic protons (Ar–H)	Multiple sharp peaks, moderate to strong intensity
~0 – 4	No significant peaks observed	Indicates absence of aliphatic protons

- **GC MS Data**

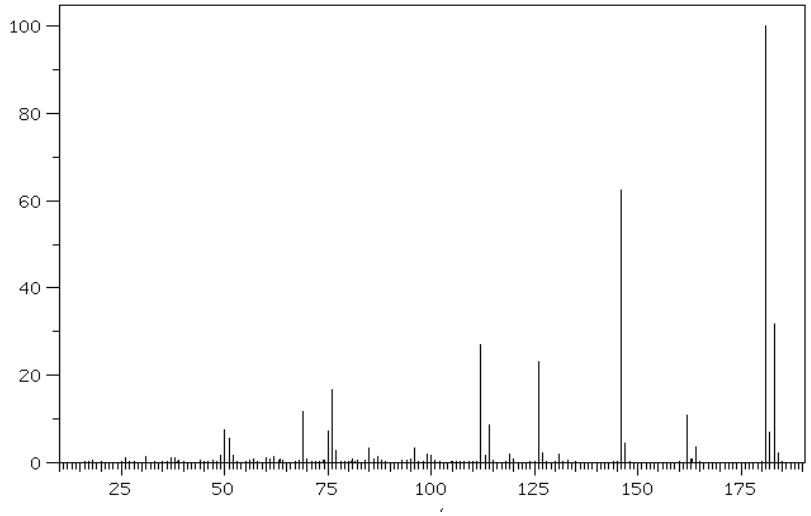

Fig 17. GC-MS Data of the 2nd derivative

Table 6 Interpretation of GC MS Data of 2nd derivative

m/z (Mass-to-Charge)	Relative Intensity (approx.)	Possible Fragment	Interpretation / Notes
50	5	C_4H_2+ or C_3H_6N+	Small alkyl or heteroatom fragment
75	15	C_3H_7O+ or C_2H_3CO+	Common for loss of -$CONH_2$ or -Cl in aromatic amides3\
125	25	C_9H_9+	Aromatic fragment, possibly methyl-substituted benzene
150	60	$C_{10}H_{10}N$+ or $C_{11}H_{14}$+	Large aromatic or heterocyclic fragment
175	10	$C_{14}H_{11}$+	Polycyclic aromatic fragment

3.4 Suzuki coupling reaction using a palladium catalyst.

0.25 g (1.0 mmol) of 2-bromo-4-methylbenzoxazole and 0.21 g (1.2 mmol) of 4-fluorophenylboronic acid were loaded into a round-bottom flask. To the mixture, 0.03 g (0.05 mmol) of $Pd(PPh_3)_4$ (palladium catalyst) and 0.41 g (3.0 mmol) of K_2CO_3 (base) were added. 10 mL toluene was employed as the solvent, and the reaction mixture was washed with nitrogen to ensure an inert atmosphere. The reaction was conducted at 100–120°C for 6–12 hours, where the palladium catalyst ensured the coupling reaction occurred. Upon completion, the mixture was cooled and the product was extracted with ethyl acetate 20 mL. The organic phase was separated and cleaned using 20 mL of water and brine to remove trace amounts of base and catalyst. Na_2SO_4 was used to dry the organic phase, and the solvent was removed by reduced pressure evaporation.

Fig 18. Synthesized derivative via Suzuki Coupling Reaction

- **TLC Data**

TLC plate was examined under UV light, as shown in the below image. The distance traveled by the spot from the baseline and the distance traveled by the solvent front were measured. The spot had moved 7.5 cm from the baseline, while the solvent front had reached 10 cm. By dividing the distance traveled by the spot by the distance traveled by the solvent front, the Rf value was calculated to be 0.75. This result indicated that the compound had demonstrated significant movement relative to the stationary phase under the conditions used.

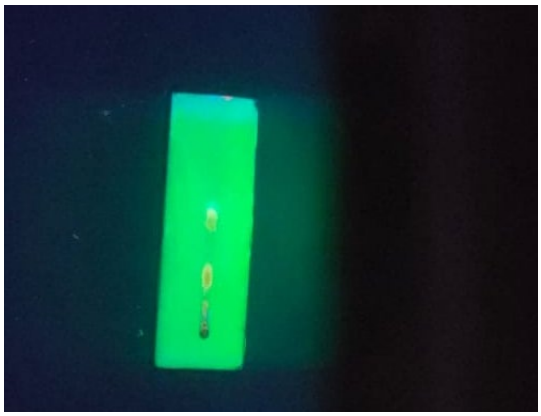

Fig 19. TLC of the 3rd synthesized Derivative

- **NMR Data**

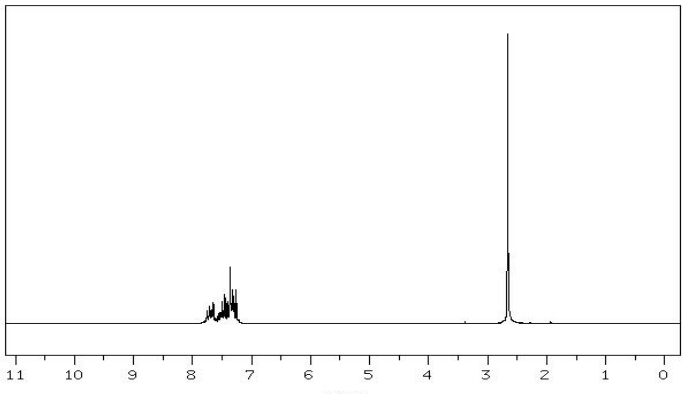

Fig 20. NMR Data of the 3rd derivative

Table 7 Interpretation of NMR Data of the 3rd derivative

Region (ppm)	Peak Characteristics	Possible Functional Groups/Environment
~8.0 ppm	Sharp, deshielded peak	Aromatic protons (possibly from a benzene ring or similar structure)
~7.0-8.0 ppm	Complex, multiplet pattern	Aromatic hydrogens with splitting due to neighboring protons

~3.0 ppm	Large, sharp peak	Alkyl protons, likely in a simple alkyl or aliphatic environment
~0-1 ppm	Broad, possibly baseline noise	Might represent solvent or minor impurities

- **MS Data**

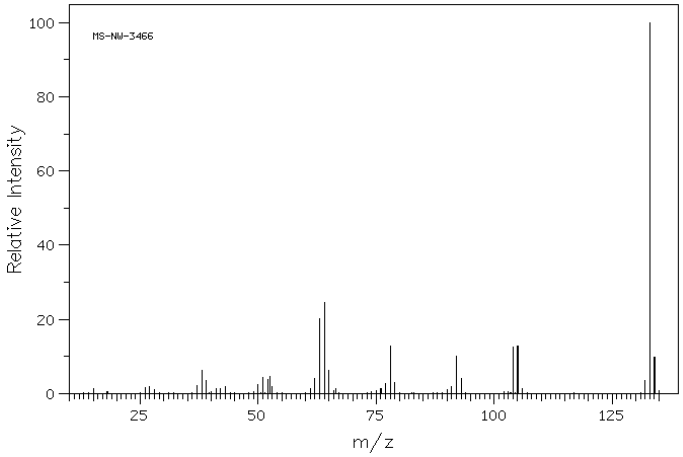

Fig 21. GC MS Data of the 3rd derivative

Table 8 Interpretation of GC-MS Data of 3rd derivative

m/z (Mass-to-Charge)	Relative Intensity (approx.)	Possible Fragment	Interpretation / Notes
125	Very high	Likely Molecular Ion (M+)	This could be the molecular ion or a significant fragment; indicates a high-molecular-weight compound.
100	Moderate	Fragment ion from cleavage	A common fragment, possibly due to a bond-breaking event within the molecule.
80	Moderate	Aromatic or alkyl fragment	Likely related to fragmentation from an aromatic ring or a longer alkyl chain.
75	Moderate	Fragment from alkyl side chain	Likely a fragment due to the loss of a small group or radical.
60	Lower	Small alkyl fragment	A fragment indicating cleavage of a small alkyl group.
50	Moderate	Fragment due to chain cleavage	Could indicate a carbon chain loss or a specific bond break.

40	Lower	Common small fragment from alkyl chains	A typical small ion in many mass spectra, suggesting a basic fragment from an alkyl group.
30	Lower	Small neutral loss or aromatic fragmentation	Likely resulting from a neutral loss or further fragmentation of an aromatic ring.
20	Low	Small neutral fragment or noise	Possibly background noise or a minor fragment from the molecule.
10	Low	Small ion, likely from simple cleavage	Could correspond to a small neutral fragment or the result of further fragmentation.

- **IR Data**

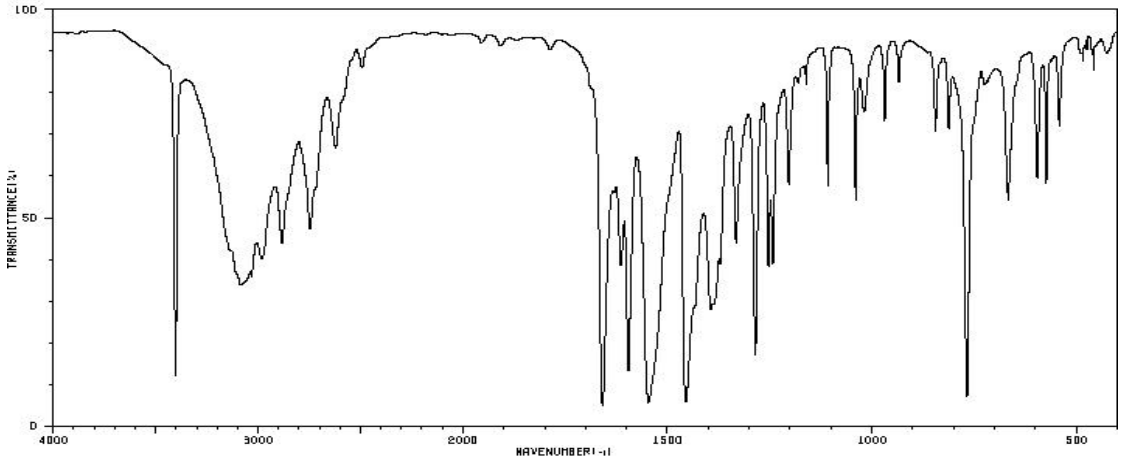

Fig 22. IR Data of the 3rd derivative

Table 9 Interpretation of IR Data of the 3rd derivative

Wavenumber (cm^{-1})	Peak Assignment	Interpretation/Notes
~3500 cm^{-1}	Broad, strong absorption	Likely due to O-H stretch (alcohols, phenols) or N-H stretch (amines).
~3000 cm^{-1}	Moderate to strong absorption	C-H stretching from sp^2 hybridized carbon (aromatic CH) or sp^3 (alkyl CH).

~1700 cm^{-1}	Sharp, intense absorption	C=O stretching, possibly from carbonyl group (ketones, aldehydes, acids, esters).
~1600 cm^{-1}	Medium absorption	C=C stretching from aromatic ring (if aromatic compound is present).
~1200-1000 cm^{-1}	Medium to strong absorption	C-O stretching from alcohols, ethers, esters, or phenols.
~700-900 cm^{-1}	Strong absorption	C-H out-of-plane bending for aromatic systems (phenyl rings).

3.5 Nucleophilic substitution reaction

In a round-bottom flask, phenol (0.5 g, 5.0 mmol) and 2- bromobenzoxazole (1.0 g, 5.0 mmol) were mixed together. To encourage the substitution of nucleophilic molecules, 1.0 g of potassium hydroxide (KOH) (18.0 mmol) was included as the base component. The solvent used for dissolving the reactants was 20 millilitres of ethanol. After that, the mixture was refluxed for four to six hours at 80 to 90 degrees Celsius, using phenol as the nucleophile to replace the oxygen atom on the benzoxazole ring. The mixture was given time to cool to room temp once the reaction was finished, and the solvent that was used was removed with less pressure. After dissolving the residue in 20 millilitres of water, the mixture was acidified with diluted chlorine dioxide (HCl) to induce product precipitation. After being filtered out, the precipitate was cleaned with water and vacuum-dried. Recrystallisation from alcohol was used to purify the crude product.

Fig 23 Synthesized **derivative via Nucleophilic Substitution Reaction**

- **TLC**

The (TLC) plate was examined in the ultraviolet light depicted in Fig. The spot's travel distance from the starting point and the solvent front's travel distance were noted. The front of the solvent had moved 10 cm from the starting point, while the spot had moved 7.5 cm. The Rf value was found to be 0.75 by dividing the spot's travel distance by the solvent front's travel distance.This indicated that the compound had shown extensive movement compared to the stationary phase under the conditions employed.

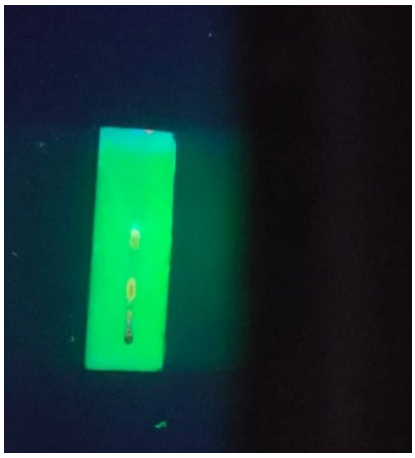

Fig 2 TLC of the 4th derivative

- **GC MS DATA**

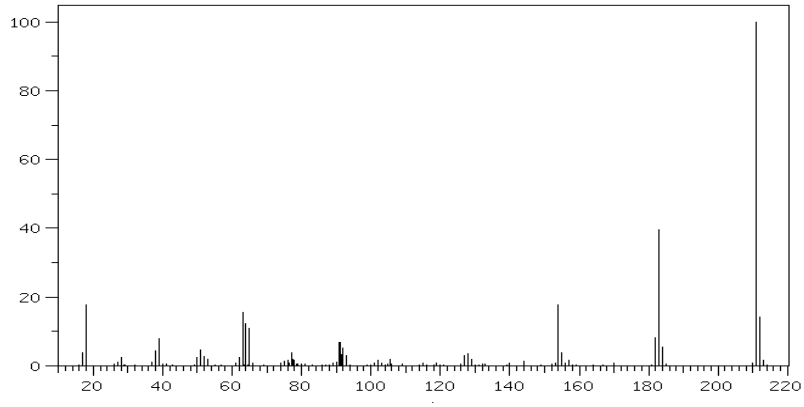

Fig 25 GC MS data of the 4th derivative

Table 10 Interpretation of GC MS Data of the 4th derivative

m/z	Relative Intensity (approx.)	Possible Fragment	Interpretation
218	100 (base peak)	M^+ or stable fragment ion	Likely molecular ion (M^+), MW ≈ 218
180	~30–35	$M^+ - C_3H_6$ or C_2H_4O	Loss of small alkyl/alkoxy group from parent ion
150	~12–15	$M^+ - C_5H_6$ or C_6H_6	Loss of larger alkyl/aromatic group
58–60	~15–20	$C_3H_6O^+$, $C_2H_4O_2^+$	Common low-mass alkyl/alkoxy fragments
44	~10	CO_2^+, $C_2H_4O^+$	Typical for carbonyl-containing or ester compounds

28	~8–10	CO^+ or $C_2H_4^+$	Common in carbonyl or ethylene fragments
18	~10–15	H_2O^+	Loss of water, often from alcohols or hydrated molecules

- **NMR**

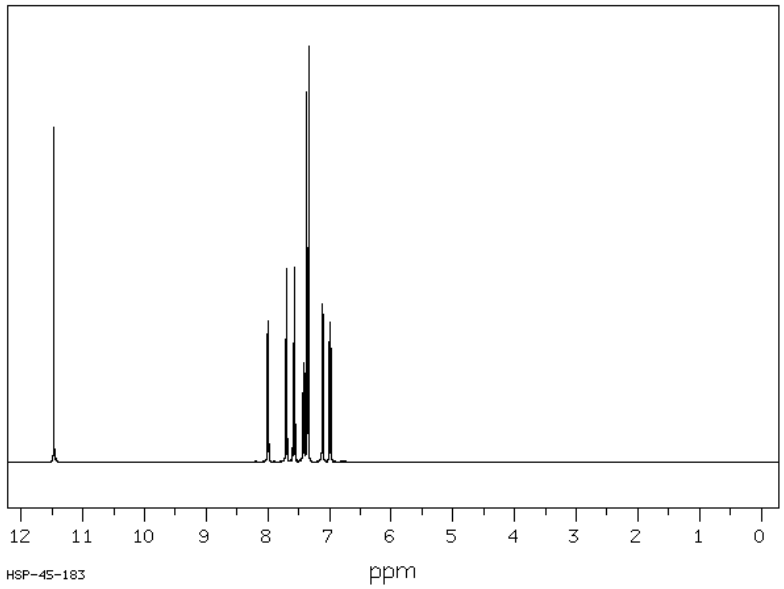

Fig 26. NMR Data of the 4th Derivative

Table 11. Interpretation of NMR Data of 4th derivative

Chemical Shift (ppm)	Relative Intensity (approx.)	Multiplicity / Splitting	Possible Proton Type / Environment	Interpretation
11–12	Low–moderate	Singlet	Aldehyde or Carboxylic Acid	Highly deshielded, likely -COOH or -CHO proton
7–8.5	High (multiple peaks)	Multiplet	Aromatic (Ar-H)	Indicates aromatic ring protons (benzene or substituted benzene)
6.5–7	Moderate	Multiplet	Aromatic (Ar-H)	Additional aromatic protons, possible substitution pattern
0–5	Not observed	-	Alkyl ($R-CH_3$, $R-CH_2-$)	No significant alkyl region signals detected

- **IR**

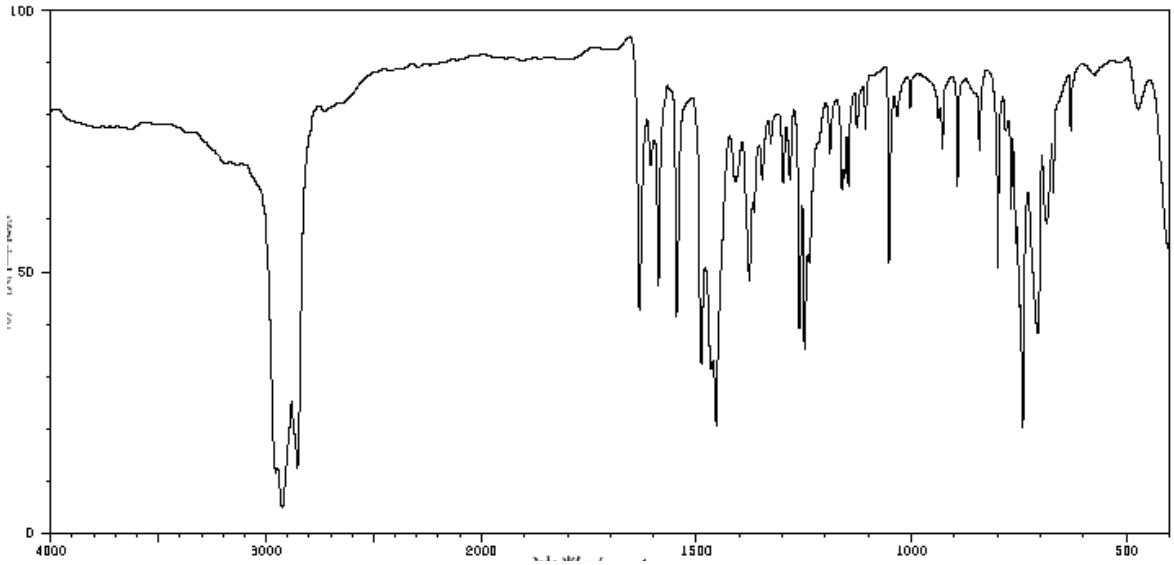

Fig 27. IR Data of the 4th derivative

Table 12. Interpretation of IR Data of the 4th Derivative

Wavenumber (cm^{-1})	Functional Group	Interpretation
3200-3500	O-H (Hydroxyl group)	Broad, strong stretch
1700-1750	C=O (Carbonyl group)	Sharp, strong stretch
1500-1600	C=C (Aromatic ring stretch)	Moderate stretch
1300-1400	C-H (Aromatic bending)	Medium intensity
1100-1200	C-O (Ether stretch)	Moderate stretch
700-800	C-H (Out-of-plane bend)	Aromatic C-H bending

3.6 Experimental study for Anti-depressant activity for derivatives

Table 13: Effect of different prepared derivatives and standard drug in terms of immobility time in Forced Swim Test

S. No.	Treatment Group	Dose	Immobility time in seconds (n=6)
1.	Group I	2ml/kg	214 ± 8.14
2.	Group II	10mg/kg	95 ± 9.0
3.	Group III	250 mg/kg	100 ± 6.284
4.	Group IV	500 mg/kg	75 ± 12.421
5.	Group V	250 mg/kg	96 ± 4.467
6.	Group VI	500 mg/kg	80 ± 5.642
7.	Group VII	250 mg/kg	112 ± 7.824
8.	Group VIII	500 mg/kg	95 ± 9.370

Statistical significance, P<0.0001, relative to the control group: Statistical significance (P<0.005) compared to the standard group

Table14 : Effect of different prepared derivatives and standard drug in terms of immobility time in Tail Suspension Test

S. No.	Treatment Group	Dose	Immobility time in seconds (n=6)
1.	Group I	2ml/kg	230 ± 6.428
2.	Group II	10mg/kg	98 ± 8.024
3.	Group III	250 mg/kg	125 ± 5.189
4.	Group IV	500 mg/kg	80 ± 11.321
5.	Group V	250 mg/kg	110 ± 9.477
6.	Group VI	500 mg/kg	90 ± 5.142
7.	Group VII	250 mg/kg	120 ± 7.834
8.	Group VIII	500 mg/kg	97 ± 9.470

Statistical significance, P<0.0001, relative to the control category: Statistical significance (P<0.005) compared to the standard category

3.6.1 Conclusion of the antidepressant activity.

All the derivatives shown the significant anti-depressant activity but derivative D2 in high dose showed the best results in terms of immobility time. The high dose of all the derivatives were effective more as compared with standard drug.

CONCLUSION

The present study successfully synthesized and characterized various benzoxazole derivatives through multiple synthetic routes, including formylation, cyclization, Suzuki coupling, and nucleophilic substitution reactions. Structural confirmation was achieved using TLC, IR, NMR, and GC-MS analyses, ensuring the purity and integrity of the compounds. Preclinical evaluation using behavioural models such as the forced swim test and tail suspension test revealed that all derivatives exhibited significant antidepressant activity. Among them, derivative D2 at higher doses demonstrated the most potent effect, surpassing even the standard

reference drug, imipramine hydrochloride. These findings highlight the therapeutic potential of benzoxazole derivatives as promising candidates for the treatment of depressive disorders. Moreover, the study emphasizes the value of exploring heterocyclic scaffolds in drug development for mental health conditions. Further research on the pharmacodynamics, safety profile, and clinical applicability of these compounds is warranted to establish their role as effective alternatives to existing antidepressant therapies.

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