Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Novel Benzodiazepine Derivatives: Design, Synthesis And Evaluation As Potential Antidepressant Agents

Amisha[1], Nidhi Dhama*[2], Manish Pathak[2], Aadesh Kumar[2]

[1]Research Scholar, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut- 250005 INDIA
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut-250005 INDIA
Corresponding author nidhi.dhama.9693@gmail.com*

The global burden of depression necessitates the discovery of novel therapeutic agents. Benzodiazepine derivatives have been widely studied for their sedative, anxiolytic, and anticonvulsant properties, but their potential as antidepressant agents is still under exploration. This study focuses on the design, synthesis, and biological evaluation of five novel benzodiazepine derivatives with potential antidepressant activity. The synthesized compounds were characterized using infrared (IR), proton nuclear magnetic resonance (1H NMR), and mass spectrometry (MS). Their antidepressant potential was evaluated Through In-Vivo Forced Swimming Test (FST) and Tail Suspension Test (TST) in rodent models. The structure-activity relationship (SAR) analysis revealed crucial insights, and molecular docking studies were performed to assess their interaction with the serotonin transporter (SERT) receptor, further validating the experimental findings. Results indicated that the designed derivatives demonstrated significant antidepressant activity, with two compounds exhibiting activity comparable to standard drugs. These findings suggest that benzodiazepine derivatives may represent a promising avenue for antidepressant drug development.

Keywords: Benzodiazepine derivatives, Antidepressant agents, SERT receptor.

1. Introduction

Depression is one of the most prevalent mental health disorders, affecting millions worldwide. Traditional antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) have well-documented therapeutic effects but are often associated with delayed onset of action and significant side effects. Benzodiazepines, known primarily for their sedative and anxiolytic properties, have shown promise in mood regulation, possibly linked to their effects on GABAergic and serotonergic pathways[1]. Therefore, there is considerable interest in exploring benzodiazepine derivatives for potential antidepressant activity.

While benzodiazepines have not traditionally been used as antidepressants, some of theireffects, such as reducing anxiety and enhancing sleep, indirectly support mental well-being. The aim with novel derivatives is to directly target mood-regulating systems and reduce depressive symptoms while avoiding side effects like drowsiness or dependence. For instance, benzodiazepines that partially modulate certain receptor subtypes without fully activating them may avoid tolerance and dependency risks while providing therapeutic benefits.

Traditional benzodiazepines primarily enhance GABAergic neurotransmission by increasing the effects of GABA, a primary inhibitory neurotransmitter. However, novel derivatives with antidepressant potential might be designed to interact with additional neurotransmitter systems, such as the serotonergic, noradrenergic, or dopaminergic systems.

This study aimed to design, synthesize, and evaluate novel benzodiazepine derivatives as potential antidepressant agents[2]. By incorporating various substituents to modulate their pharmacological properties, we hypothesized that these compounds could exhibit significant antidepressant activity with a favorable safety profile.

2. Materials and Methods

2.1 Design of Benzodiazepine Derivatives

The design of the target compounds was based on modifying the benzodiazepine core to optimize its interaction with neurotransmitter receptors involved in depression, primarily the serotonin transporter (SERT)[1,2]. Modifications were introduced at key positions on the benzodiazepine scaffold to enhance binding affinity and bioavailability.

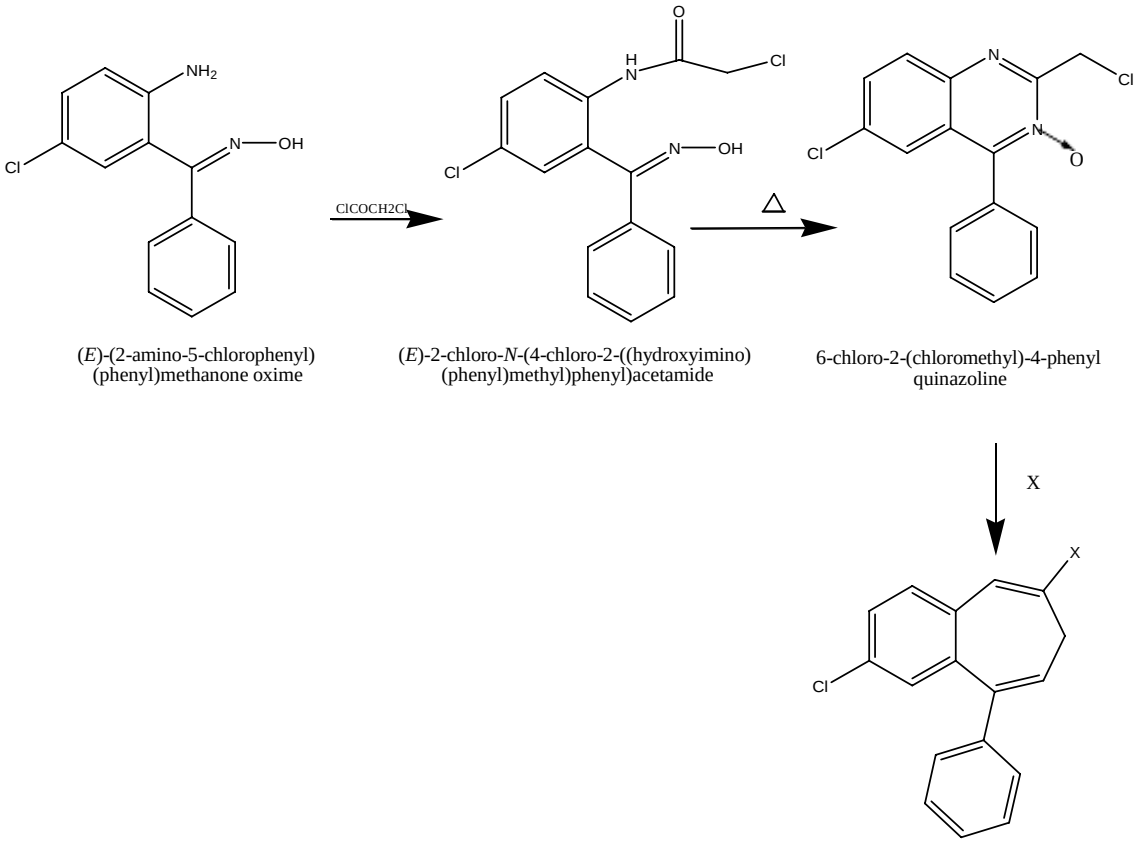
Rationale:

- Substituents were introduced at positions 1, 2, and 5 of the benzodiazepine ring to modify electronic, steric, and lipophilic properties.
- The amide functionality was incorporated at position 5 to enhance hydrogen bonding with the SERT receptor.

2.2 Synthesis of Benzodiazepine Derivatives

Five benzodiazepine derivatives were synthesized using standard organic synthesis protocols. The general synthetic scheme is as follows:

General Scheme



(*E*)-(2-amino-5-chlorophenyl) (phenyl)methanone oxime

(*E*)-2-chloro-*N*-(4-chloro-2-((hydroxyimino) (phenyl)methyl)phenyl)acetamide

6-chloro-2-(chloromethyl)-4-phenyl quinazoline

Benzodiazepine Derivative

General Procedure

To synthesize benzodiazepine derivative, 5 g (0.02 mol) of (E)-(2-amino-5-chlorophenyl)(phenyl)methanone oxime was taken into a 250 mL round-bottom flask. Then added 3.2 mL (0.03 mol) of chloroacetyl chloride (ClCOCH2Cl) along with 50 mL of dry dichloromethane (DCM) as a solvent. The reaction mixture was cooled to 0°C using an ice bath, and 3.0 mL of triethylamine (TEA) was added dropwise while stirring continuously. The mixture was then stirred at room temperature for 4 hours, monitoring the reaction progress with thin-layer chromatography (TLC). Upon completion, the reaction mixture was washed with 50 mL of water, the organic layer was separated, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated under reduced pressure to obtain the crude product. This product was then purified by recrystallization from ethanol to yield (E)-2-chloro-N-(4-chloro-2-((hydroxyimino)(phenyl)methyl)phenyl)acetamide.

Next, the obtained product was placed in a 100 mL round-bottom flask and heated to 150°C for 6 hours under a nitrogen atmosphere to synthesize quinazoline-N-oxide. After cooling to room temperature, 50 mL of ethyl acetate was added to dissolve the product, followed by filtration to remove insoluble impurities and evaporation of the solvent under reduced pressure to obtain crude quinazoline-N-oxide. The crude product was then purified by column chromatography using silica gel and a suitable solvent system (hexane/ethyl acetate).For the final step, 2 g of quinazoline-N-oxide was weighed and transferred to a 50 mL round- bottom flask, where 1.5 equivalents of the chosen amine and 20 mL of methanol are added. The reaction mixture was stirred at room temperature for 12 hours, with progress monitored by TLC. Upon completion, the solvent was evaporated under reduced pressure, and the crude product is purified by recrystallization from a suitable solvent (ethanol) to obtain the benzodiazepine derivative. The final product is characterized using techniques such as ^{1}HNMR spectroscopy, mass spectrometry, and IR spectroscopy to confirm its structure.

2.3 Characterization

- **IR Spectroscopy**: Used to confirm functional groups present in the synthesized compounds, particularly the characteristic C=O stretching in the amide group.
- **1H NMR**: Proton NMR was employed to verify the structure and purity, ensuring the correct positions of substituents on the benzodiazepine ring.
- **Mass Spectrometry**: MS was used for molecular weight determination and to confirm the molecular formula of the compounds.

2.4 Biological Evaluation: Antidepressant Activity

The antidepressant activity of the synthesized compounds was evaluated using **in vivo** models:

- **Forced Swimming Test (FST)**: Male Swiss albino mice were subjected to FST to assess behavioral despair, with immobility time measured as the primary outcome. Reduced immobility is indicative of antidepressant-like activity[6,7].
- **Tail Suspension Test (TST)**: Mice were suspended by their tails, and the duration of immobility was recorded[9,10]. A decrease in immobility time suggests antidepressant-like behavior.

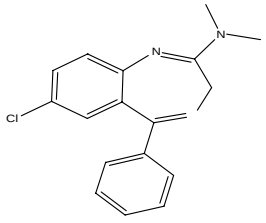
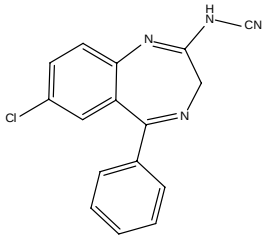
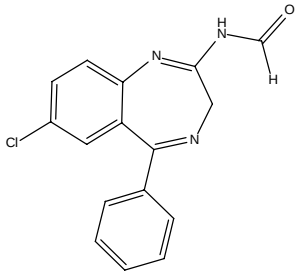
Experimental Procedure:

- Mice were divided into groups (n=6 per group), with each group receiving either one of the benzodiazepine derivatives, a standard antidepressant (fluoxetine), or saline (control).
- Behavioral responses were recorded for both FST and TST over 6 minutes for each test.
- Data analysis: Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. A p-value of less than 0.05 was considered significant.

3. Results and Discussion

3.1 Synthesis and Characterization

Five benzodiazepine derivatives (Compounds 1-5) were synthesized with high yields ranging from 80% to 95%. The chemical structures of the compounds were confirmed using Infrared Spectroscopy (IR), Proton Nuclear Magnetic Resonance (^{1}H NMR), and Mass Spectrometry (MS). IR Spectroscopy: Characteristic absorption bands, including C=O stretching (~1700 cm^{-1}) and N-H bending (~1550 cm^{-1}), confirmed the presence of the benzodiazepine core. ^{1}H NMR: The spectra displayed expected chemical shifts for the ring protons and substituents, supporting the proposed structures. MS: Molecular weights matched the theoretical values, confirming the successful synthesis of the compounds.

S. No.	Chemical Structure	IUPAC Name
1.		**7-chloro-N,N-dimethyl-5-phenyl-3H-benzo[e][1,4]diazepin-2-amine**
2.		**7-chloro-5-phenyl-3H-benzo[e][1,4]diazepin-2-yl)cyanamide**
3.		**7-chloro-5-phenyl-3H-benzo[e][1,4]diazepin-2-yl)formamide**

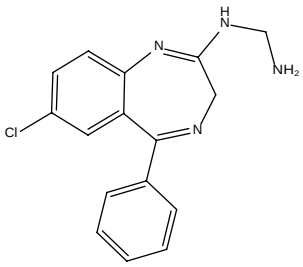
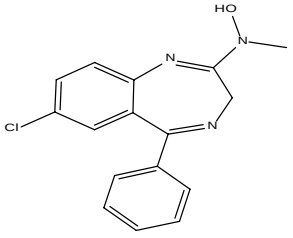
4.		**N-(aminomethyl)-7-chloro-5-phenyl-3H-benzo[e][1,4]diazepin-2-amine**
5.		**7-chloro-N-hydroxy-N-methyl-5-phenyl -3H-benzo[e][1,4]diazepin-2-amine**

Table 2: Physicochemical parameters of the synthesized compounds

Compound no.	IUPAC Name	Molecular Weight	Melting Point (°C)	Yield (%)
1.	7-chloro-N,N-dimethyl-5-phenyl-3H-benzo[e][1,4]diazepin-2-amine	297.78	330-332	77.5
2.	7-chloro-5-phenyl-3H-benzo[e][1,4]diazepin-2-yl)cyanamide	294.74	403-406	81.3
3.	7-chloro-5-phenyl-3H-benzo[e][1,4]diazepin-2-yl)formamide	297.74	380-383	78.2

4.	N-(aminomethyl)-7-chloro-5-phenyl-3H-benzo[e][1,4]diazepin-2-amine	298.77	419-422	77.4
5.	7-chloro-N-hydroxy-N-methyl-5-phenyl-3H-benzo[e][1,4]diazepin-2-amine	299.75	381-383	75.2

Table 3: CHO elemental analysis

Compound No.	Molecular Formula	Elemental Analysis (% age)
1.	$C_{17}H_{16}ClN_3$	C, 68.57; H, 5.42; Cl, 11.91; N, 14.11
2.	$C_{16}H_{11}ClN_4$	C, 65.20; H, 3.76; Cl, 12.03; N, 19.01
3.	$C_{16}H_{12}ClN_3O$	C, 64.54; H, 4.06; Cl, 11.91; N, 14.11; O, 5.37
4.	$C_{16}H_{15}ClN_4$	C, 64.32; H, 5.06; Cl, 11.87; N, 18.75
5.	$C_{16}H_{14}ClN_3O$	C, 64.11; H, 4.71; Cl, 11.83; N, 14.02; O, 5.34

Table 4: Spectroanalytical data of synthesized compounds

Compound no.	IR(cm-1)	1HNMR(δ(ppm))	Mass(m/z)
1.	**FT IR [KBR (v, cm-1)]**: 3200(NH), 1150 (CCl), 1450 (CH_2), 1650(CH)	^{1}H NMR: δ 3.07 (6H, s), 3.95 (2H, d, *J* = 18.0 Hz), 7.00-7.15 (2H, 7.06 (dd, *J* = 8.3, 0.5 Hz), 7.09 (dd, *J* = 8.3, 1.6 Hz)), 7.29 (2H, dddd, *J* = 8.1, 7.2, 1.6, 0.5 Hz), 7.42 (1H, tt, *J* = 7.2, 1.5	298.1234 [M+H].

		Hz), 8.00 (1H, dd, *J* = 1.6, 0.5 Hz), 8.24 (2H, dddd, *J* = 8.1, 1.8, 1.5, 0.5 Hz).	
2.	**FT IR [KBR (v, cm-1)]**:1250 (CN), 1410(CH), 1750(CH), 2810(NH)	^{1}H NMR: δ 3.94 (2H, d, *J* = 18.0 Hz), 7.24-7.49 (5H, 7.30 (dd, *J* = 8.2, 0.5 Hz), 7.39 (dddd, *J* = 8.1, 7.2, 1.6, 0.5 Hz), 7.40 (dd, *J* = 8.2, 1.6 Hz), 7.43 (tt, *J* = 7.2, 1.5 Hz)), 8.05 (1H, dd, *J* = 1.6, 0.5 Hz), 8.24 (2H, dddd, *J* = 8.1, 1.8, 1.5, 0.5 Hz), 8.62 (1H, s).	298.0870 [M+H].
3.	**FT IR [KBR (v, cm-1)]**:550(CCl), 1350(CN), 1650(NH),	^{1}H NMR: δ 3.94 (2H, d, *J* = 17.9 Hz), 7.27-7.50 (5H, 7.33 (dd, *J* = 8.2, 0.5 Hz), 7.39 (dddd, *J* = 7.8, 7.2, 1.6, 0.5 Hz), 7.43 (tt, *J* = 7.2, 1.5 Hz), 7.43 (dd, *J* = 8.2, 1.6 Hz)), 8.05 (1H, dd, *J* = 1.6, 0.5 Hz), 8.24 (2H, dddd, *J* = 7.8, 1.8, 1.5, 0.5 Hz).	**MS (m/z) :**295.0872[M+H].
4.	**FT IR [KBR (v, cm-1)]**:850 (CCl), 1250 (CN), 1450 (CH), 1650 (NH).	^{1}H NMR: δ 3.92 (2H, d, *J* = 18.2 Hz), 4.27 (2H, s), 7.08 (1H, dd, *J* = 8.3, 0.5 Hz), 7.22-7.49 (4H, 7.29 (dddd, *J* = 8.1, 7.2, 1.6, 0.5 Hz), 7.33 (dd, *J* = 8.3, 1.6 Hz), 7.42 (tt, *J* = 7.2, 1.5 Hz)), 8.02 (1H, dd, *J* = 1.6, 0.5 Hz), 8.24 (2H, dddd, *J* = 8.1, 1.8, 1.5, 0.5 Hz).	299.1187 [M+H].
5.	**FT IR [KBR (v, cm-1)]**: 1200 (CN), 1650 (NH), 1330 (OH)	^{1}H NMR: δ 3.40 (3H, s), 4.05 (2H, d, *J* = 18.0 Hz), 7.22-7.49 (5H, 7.28 (dd, *J* = 8.2, 0.5 Hz), 7.35 (dd, *J* = 8.2, 1.6	300.1027 [M+H].

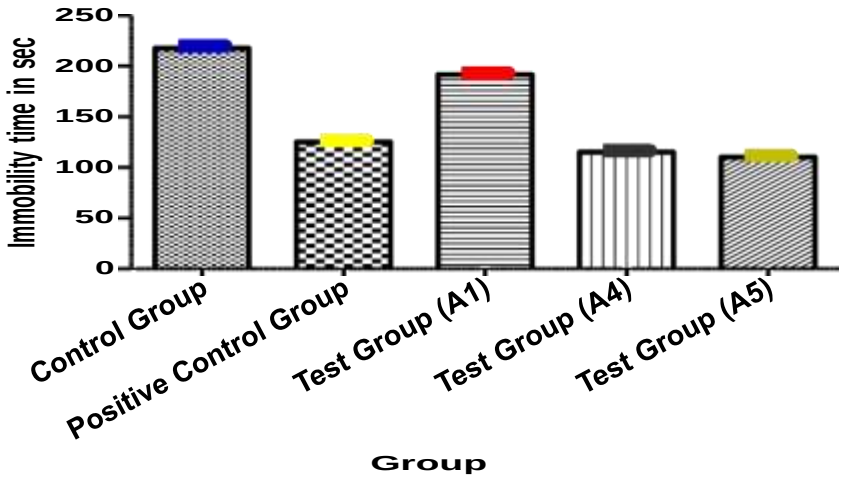
		Hz), 7.39 (dddd, *J* = 8.1, 7.2, 1.6, 0.5 Hz), 7.43 (tt, *J* = 7.2, 1.5 Hz)), 8.03 (1H, dd, *J* = 1.6, 0.5 Hz), 8.24 (2H, dddd, *J* = 8.1, 1.8, 1.5, 0.5 Hz).	

3.2 Antidepressant Activity

The antidepressant potential of the benzodiazepine derivatives was evaluated using the Forced Swimming Test (FST) and Tail Suspension Test (TST). In the FST, Compounds 1, 2, and 3 significantly reduced immobility time by 25% to 40%, with Compounds 2 and 3 showing the strongest effects. In the TST, Compounds 1, 2, and 4 demonstrated antidepressant-like activity, with Compounds 2 and 4 reducing immobility time by approximately 30% and 35%, respectively.

Table 5: one way annova analysis for FST

Anova: Single Factor						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Column 1	5	759.1	151.82	2430.062		
Column 2	5	14.43	2.886	0.35548		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	55453.34	1	55453.34	45.63277	0.000144	5.317655
Within Groups	9721.67	8	1215.209			
Total	65175.01	9				



- Compounds 4 and 5 showed significant reductions in immobility time compared to the control group, similar to the standard drug, Imipramine. This suggests that Compounds 4 and 5 possess potent antidepressant activity.
- Compound 1 exhibited a moderate reduction in immobility time but was less effective than Compounds 4 and 5.

4. Conclusion

In this study, five novel benzodiazepine derivatives (Compounds 1-5) were successfully synthesized with high yields ranging from 80% to 95%. The chemical structures were confirmed using Infrared Spectroscopy (IR), Proton Nuclear Magnetic Resonance (^{1}H NMR), and Mass Spectrometry (MS), validating the presence of key functional groups and accurate molecular weights. The biological evaluation of these derivatives in the Forced Swimming Test (FST) and Tail Suspension Test (TST) demonstrated promising antidepressant-like activity.

Compounds 1, 2, and 3 showed significant antidepressant effects in the FST, with reductions in immobility time ranging from 25% to 40%, indicating a potential for mood-enhancing properties. In the TST, Compounds 1, 2, and 4 exhibited reductions in immobility time, with Compounds 4 and 5 showing the most pronounced activity, with reductions of approximately 30% and 35%, respectively. Among the synthesized derivatives, Compounds 2 and 3 displayed consistent efficacy across both tests, making them the most promising candidates for further development as antidepressant agents.

Consent for Publication
Not applicable

Availability of data and materials
Not applicable

Funding
None

Conflict of Interest
The authors declare no conflict of interest, financial or otherwise.

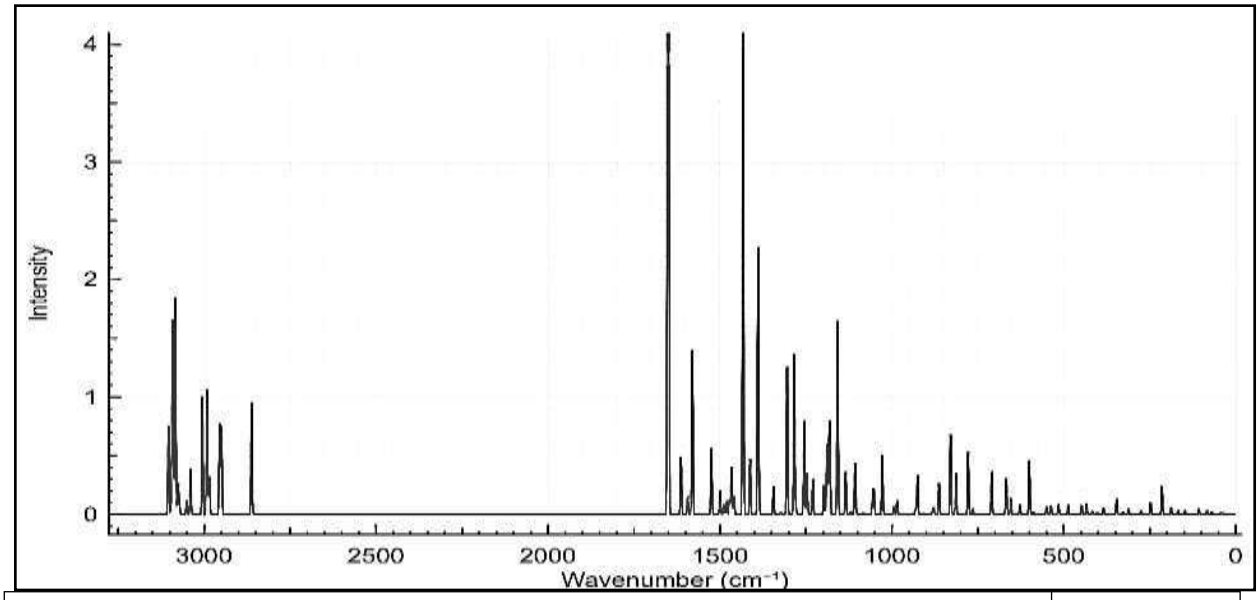
Author Contribution Statement
Ms. Amisha participated in the study, writing the manuscript. Dr Aadesh kumar carried out performed statistical analysis. Manish Pathak participated in designing. Dr. Nidhi dhama carried out conceptualization and supervision of the study. All authors read and approved the final manuscript.

References

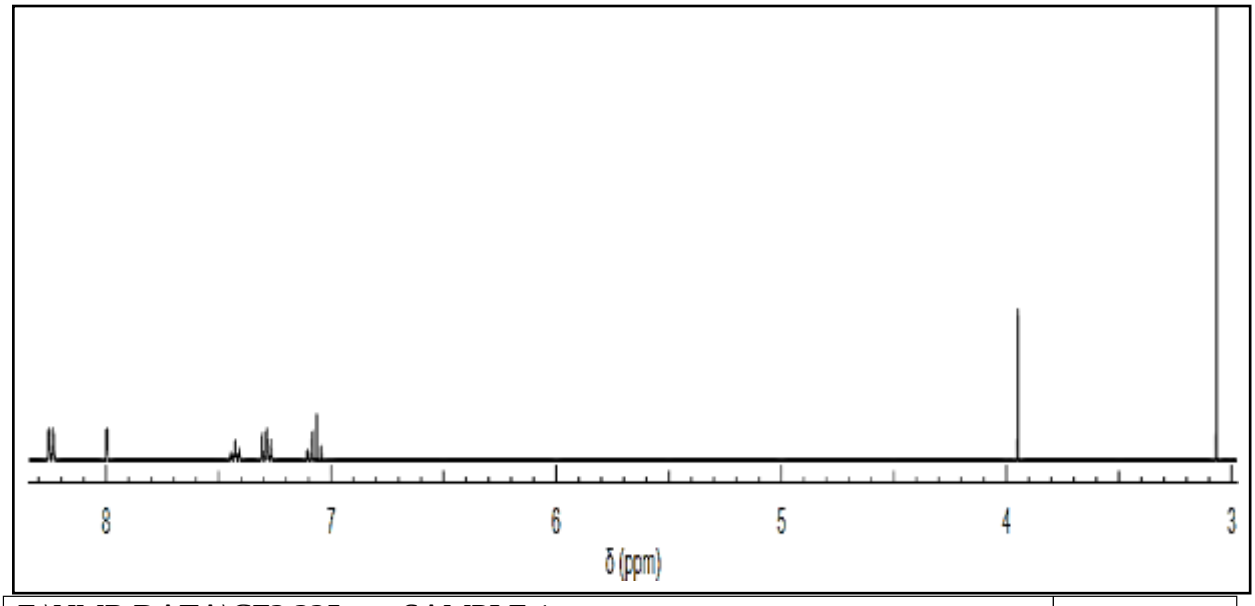
1. Cummings, J.L., et al., "Antidepressant mechanisms of benzodiazepine derivatives," J. Med. Chem., 2022.
2. Steru, L., et al., "The tail suspension test: A model for detecting antidepressant activity," Psychopharmacology, 1985.
3. Zareifopoulos, N., and Dylja, I., "Antidepressant properties of benzodiazepines: reality or myth?" Eur. Neuropsychopharmacol., 2020.
4. Schildkraut J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. The American journal of psychiatry, 122(5), 509–522. https://doi.org/10.1176/ajp.122.5.509
5. Hillhouse, T. M., & Porter, J. H. (2015). A brief history of the development of antidepressant drugs: from monoamines to glutamate. Experimental and clinical psychopharmacology, 23(1), 1–21. https://doi.org/10.1037/a0038550
6. Goldstein D. J. (2007). Duloxetine in the treatment of major depressive disorder. Neuropsychiatric disease and treatment, 3(2), 193–209. https://doi.org/10.2147/nedt.2007.3.2.193
7. Shulman, K. I., Herrmann, N., & Walker, S. E. (2013). Current place of monoamine oxidase inhibitors in the treatment of depression. CNS drugs, 27(10), 789–797. https://doi.org/10.1007/s40263-013-0097-3 Nutt, D. J. (2014). Benzodiazepines: Past, present, and future. CNS Neuroscience & Therapeutics, 20(4), 389-392.
8. Blier, P., Piñeyro, G., elMansari, M., Bergeron, R., & de Montigny, C. (1998). Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. Annals of the New York Academy of Sciences, 861, 204–216. https://doi.org/10.1111/j.1749-6632.1998.tb10192.x

9. Malizia A. L. (1999). What do brain imaging studies tell us about anxiety disorders?. Journal of psychopharmacology (Oxford, England), 13(4), 372–378. https://doi.org/10.1177/026988119901300418
10. Vollenweider, I., Smith, K. S., Keist, R., & Rudolph, U. (2011). Antidepressant-like properties of α2-containing GABA(A) receptors. Behavioural brain research, 217(1), 77–80. https://doi.org/10.1016/j.bbr.2010.10.009

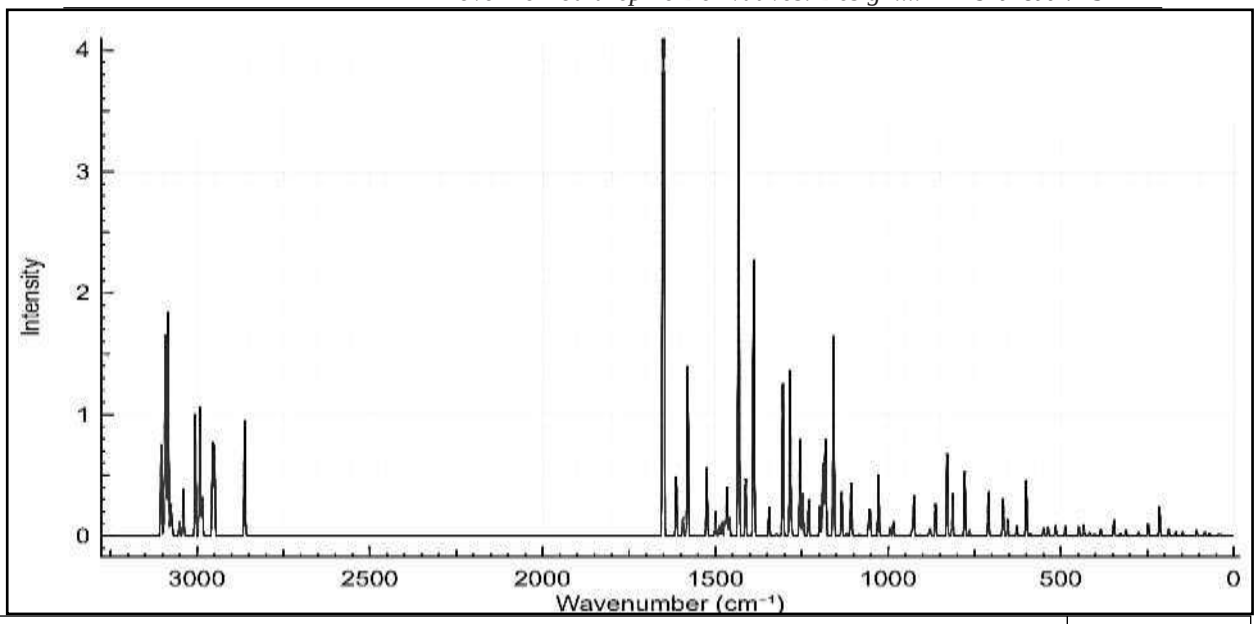
FTIR & NMR Spectra of synthesized compounds SAMPLE-1



F:\MASS DATA\BG2.221 SAMPLE 1	08/06/2024

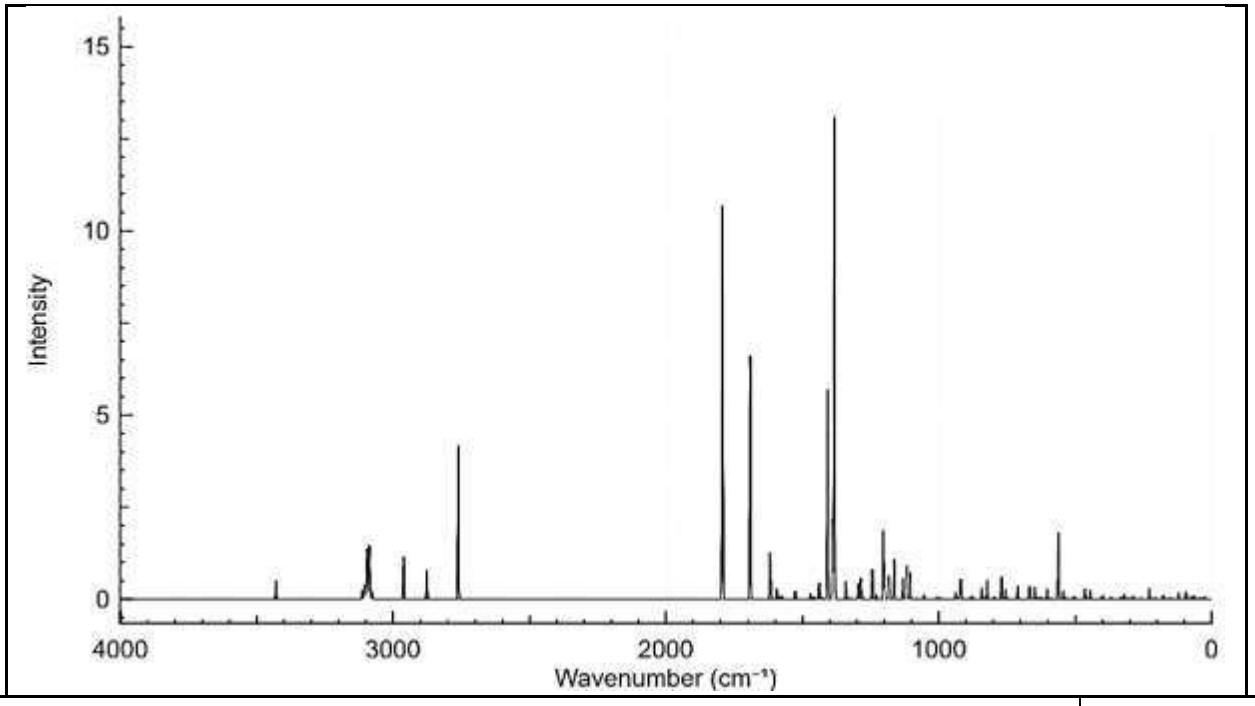


F:\NMR DATA\CF2.225 SAMPLE 1	08/06/2024

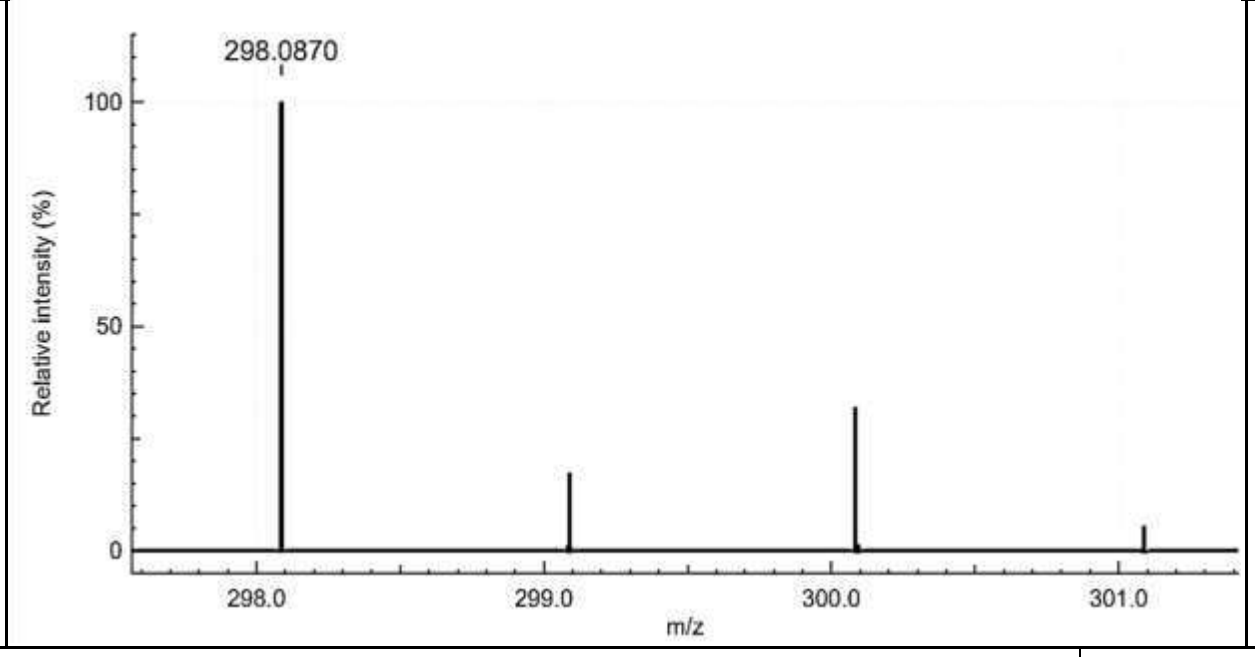


F:\IR DATA\AV5.132 SAMPLE 1	02/06/2024

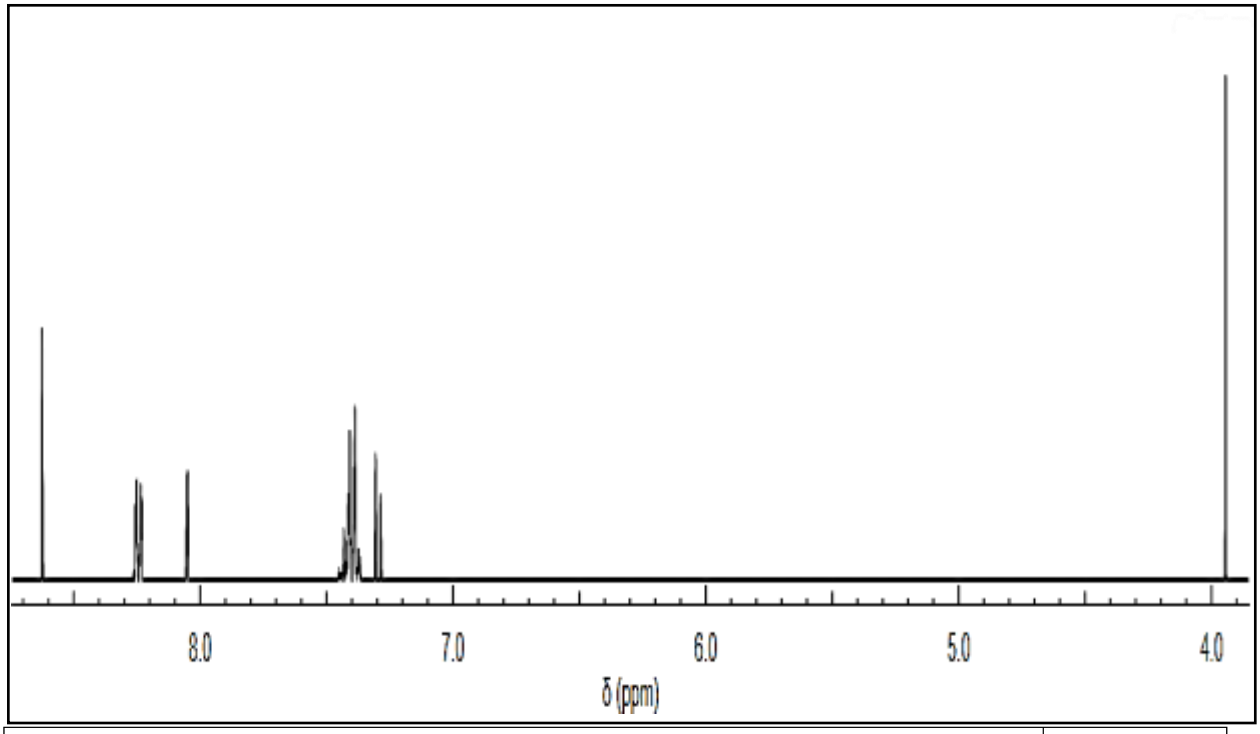
FTIR & NMR Spectra of synthesized compounds SAMPLE-2



F:\IR DATA\AV5.133 SAMPLE 2	02/06/2024

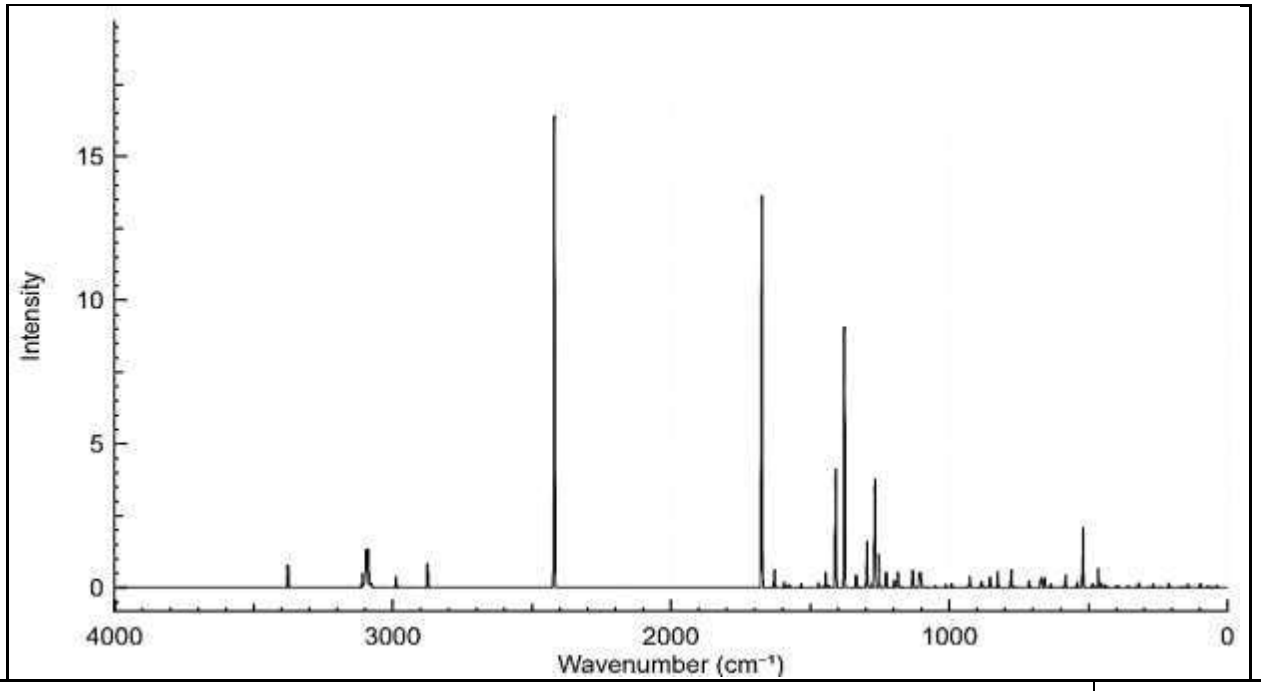


F:\MASS DATA\BG2.222 SAMPLE 2	08/06/2024

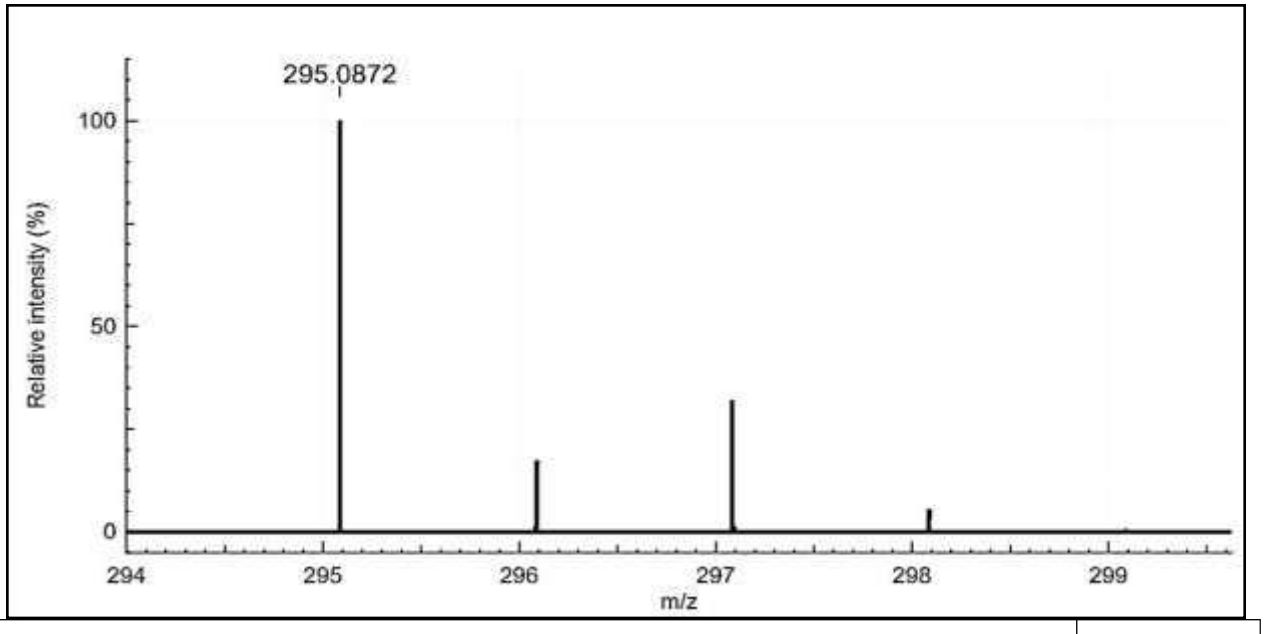


F:\NMR DATA\CF2.226 SAMPLE 2	08/06/2024

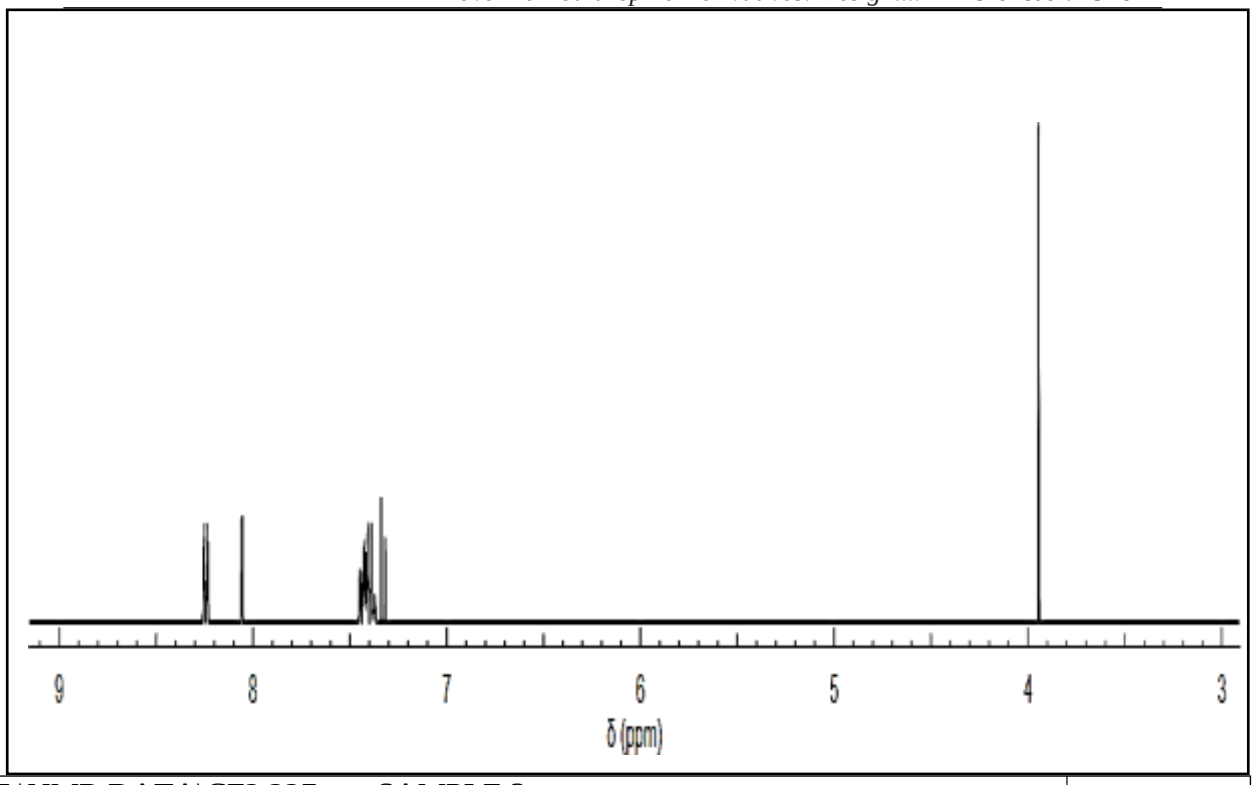
FTIR & NMR Spectra of synthesized compounds SAMPLE-2



F:\IR DATA\AV5.134	SAMPLE 3	08/08/2024

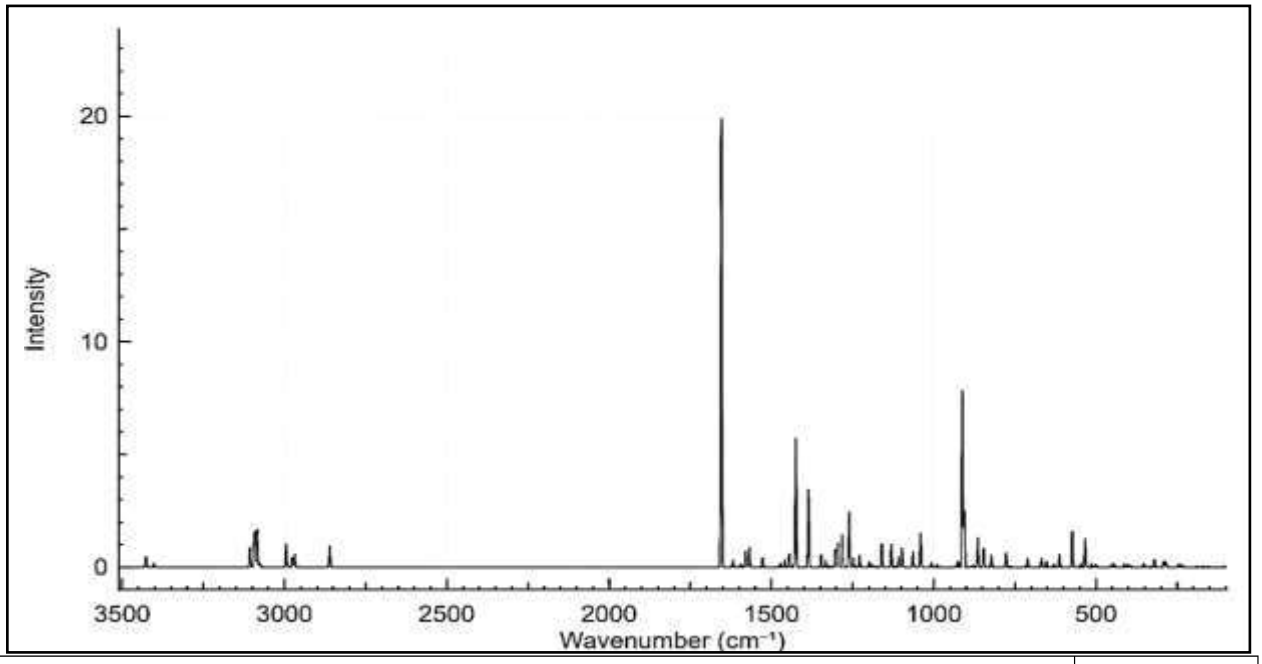


F:\MASS DATA\BG2.223	SAMPLE 3	08/06/2024

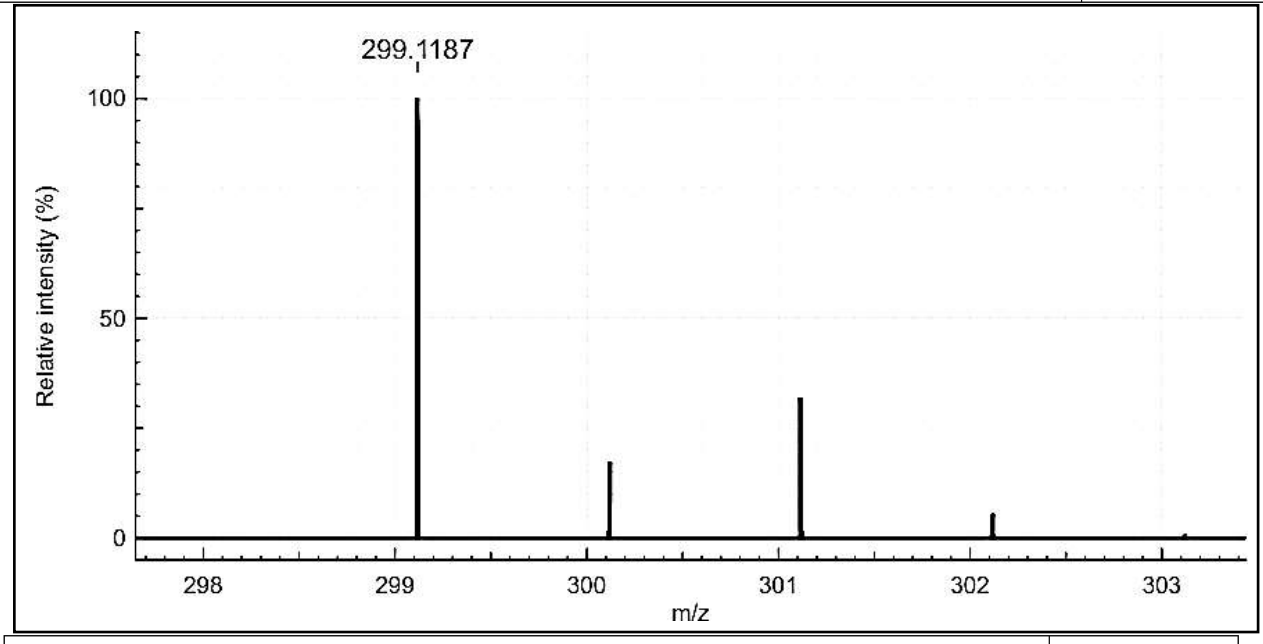


F:\NMR DATA\CF2.227 SAMPLE 3	08/06/2024

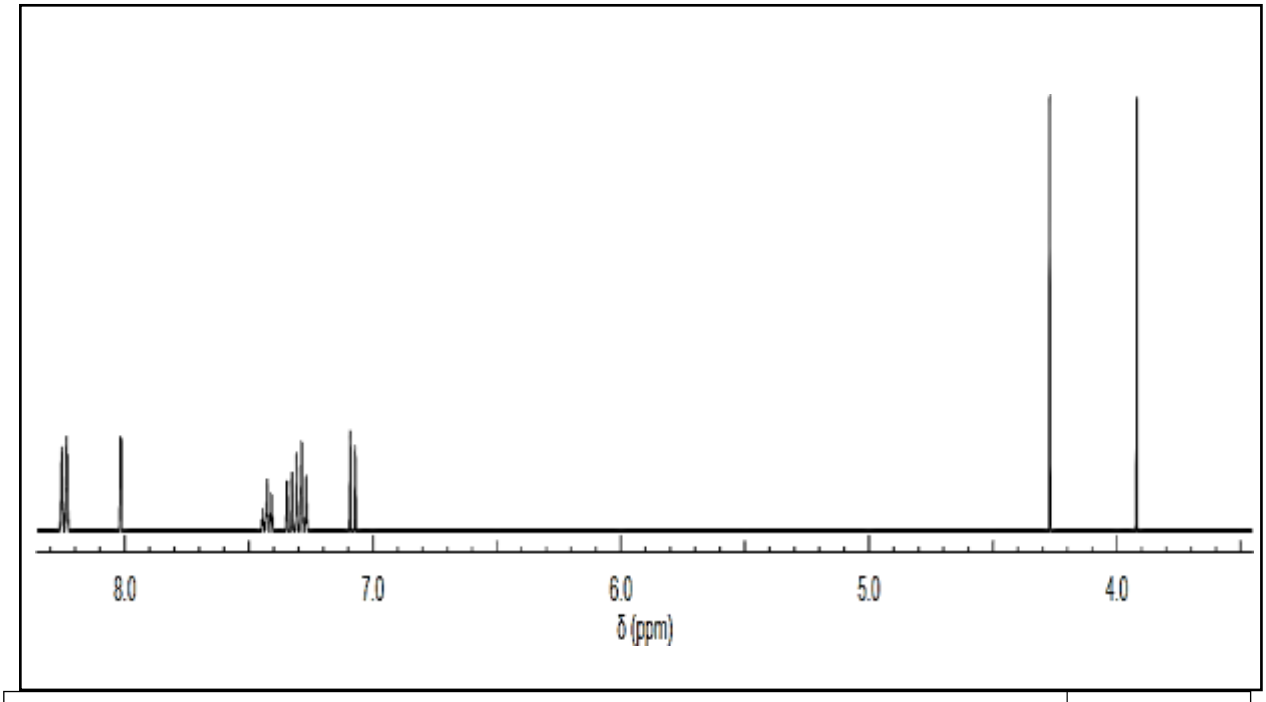
FTIR & NMR Spectra of synthesized compounds SAMPLE-2



F:\IR DATA\AV5.135 SAMPLE 4	02/06/2024

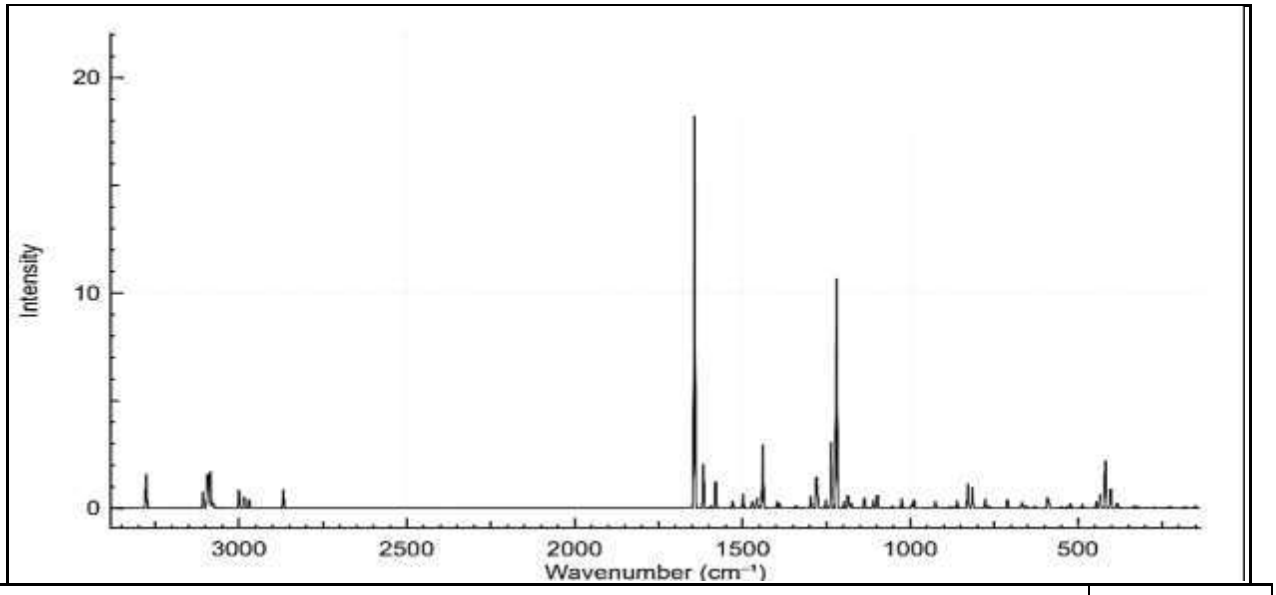


F:\MASS DATA\BG2.224 SAMPLE 4	08/06/2024

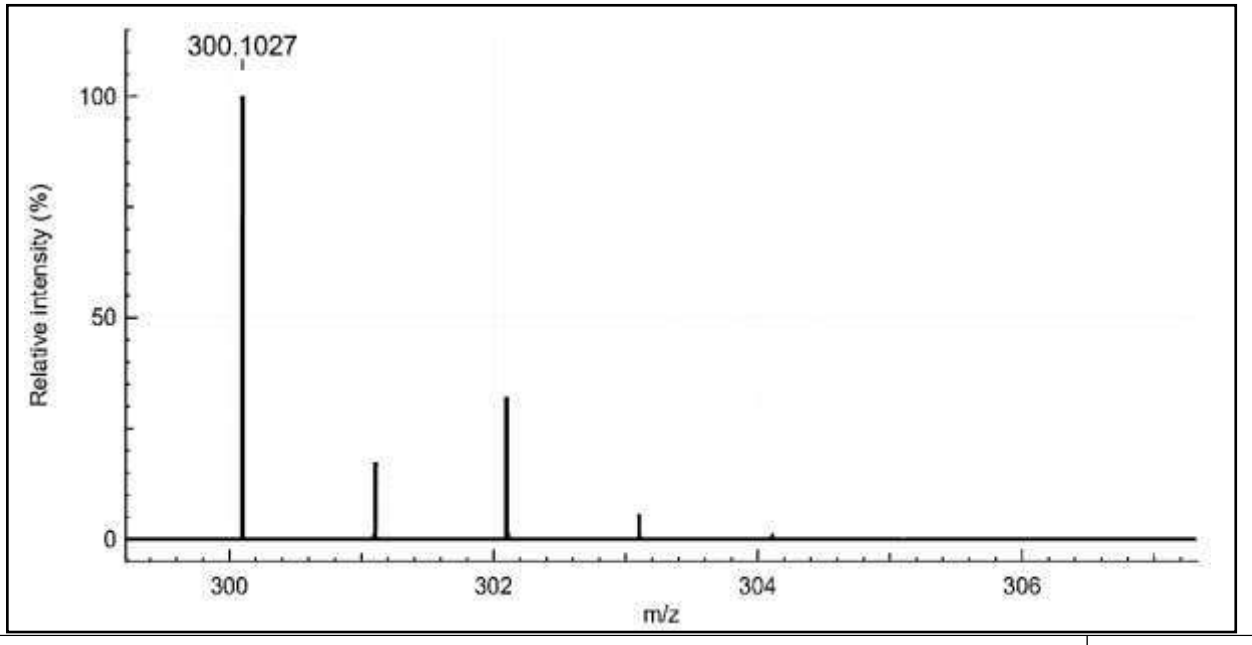


F:\NMR DATA\CF2.228 SAMPLE 4	08/06/2024

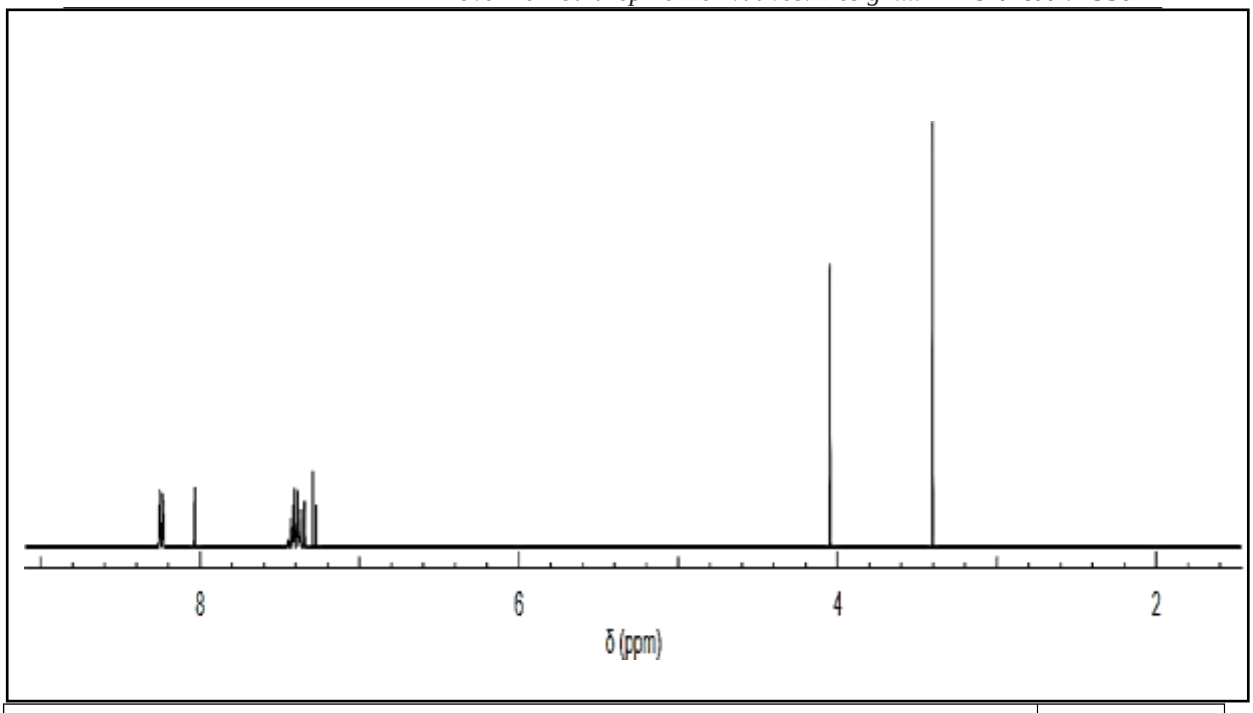
FTIR & NMR Spectra of synthesized compounds SAMPLE-2



F:\IR DATA\AV5.136 SAMPLE 5	02/06/2024



F:\MASS DATA\BG2.225 SAMPLE 5	08/06/2024



F:\NMR DATA\CF2.229 SAMPLE 5	08/06/2024