DESIGN, SYNTHESIS AND *In- vitro* BACTERIAL ACTIVITY OF NEW PYRAZINAMIDE DERIVATIVES

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ABSTRACT

In this study, we undertook the synthesis and characterization of four novel pyrazine derivatives: 'Acetyl Pyrazine 2-Carboxylate', 'Acetic acid; Pyrazine 2-Carboxylic acid', '2-{[trimethylsilyl)oxy]methyl}pyrazine', and '(Pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate' via a two-step reaction scheme (Scheme 1 and Scheme 2). The structures of these compounds were confirmed through spectroscopic methods, including IR, NMR, and mass spectroscopy.The compounds demonstrated good yield percentages ranging from 68% to 85%. The melting and boiling points were within expected ranges, further confirming the successful synthesis. The synthesized compounds were also evaluated for their antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). 'Acetyl Pyrazine 2-Carboxylate' showed the greatest zone of inhibition against S. aureus (18mm) and E. coli (14mm). The other compounds also exhibited significant antibacterial activity, suggesting that the pyrazine core might have contributed to their inhibitory effect. This study, therefore, establishes the synthesis route for these pyrazine derivatives and showcases their potential as anti-bacterial agents. The structural variation and the related differences in the antibacterial activity among the compounds.

Keywords: NEW PYRAZINAMIDE DERIVATIVES, PYRAZINAMIDE, ANTI-BACTERIAL ACTIVITY, *STAPHYLOCOCCUS AUREUS*

INTRODUCTION

The emergence of drug-resistant bacteria has been a significant concern in the world of health care, making the need for new antimicrobial agents more urgent than ever. Among various classes of antimicrobial agents, pyrazinamide, an important first-line antituberculosis drug, has drawn considerable attention due to its unique mechanism of action and its role in shortening the therapy duration [1]. Pyrazinamide is a prodrug that is converted into the active compound pyrazinoic acid by the action of pyrazinamidase, an enzyme produced by *Mycobacterium tuberculosis*. However, the emergence of pyrazinamide-resistant strains of *M. tuberculosis* has made it essential to explore new derivatives of this drug that can effectively combat these resistant strains.

This research focuses on the design and synthesis of new pyrazinamide derivatives. We propose novel synthetic pathways for the production of these derivatives, with an emphasis on optimizing

reaction conditions to increase yield and purity. The methodology adopted allows us to explore different substituents on the pyrazinamide scaffold, thereby potentially diversifying its mechanism of action and expanding its spectrum of activity [2].

To validate our approach, we have also conducted an initial *in-vitro* evaluation of the antimicrobial activity of these compounds. These evaluations are intended to provide insights into the potential of these new derivatives as promising candidates for further development as antimicrobial agents [3].

By presenting these new synthetic routes and preliminary *in-vitro* results, we hope to contribute to the ongoing global effort to develop new and more potent antimicrobial agents. As the battle against drug-resistant bacteria continues, studies like ours will play a pivotal role in finding the tools necessary to turn the tide [4, 5] .

Indeed, the drug-resistant challenge goes beyond tuberculosis. This problem is expanding into a wide variety of bacterial species that cause common infections in humans, such as Staphylococcus and Enterococcus species, which have shown increasing rates of resistance to multiple drugs. The World Health Organization lists antibiotic resistance as one of the top 10 global public health threats facing humanity. Therefore, the development of novel and effective antibacterial compounds is a priority in global health [6].

Pyrazinamide plays a crucial role in the treatment of both drug-susceptible and multidrug-resistant tuberculosis due to its unique sterilizing activity against persistent tubercle bacilli. However, the precise mechanism of action of pyrazinamide remains unclear, making the design of derivatives a complex process. Furthermore, the inherent toxicity and the emergence of resistant strains have led to a pressing need to develop new drugs based on the pyrazinamide scaffold [7].

In this context, the research presented here aims to expand the therapeutic arsenal against tuberculosis and other bacterial infections by designing, synthesizing, and evaluating the in vitro bacterial activity of new pyrazinamide derivatives. We leverage state-of-the-art synthetic chemistry techniques, coupled with rigorous analytical methods, to ensure the production of high-quality, novel compounds [8, 9].

The compounds synthesized in this study include acetyl pyrazine 2-carboxylate, 2-{[(trimethylsilyl)oxy]methyl}pyrazine, and (pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate. Each derivative is designed with specific chemical modifications aimed at enhancing the antibacterial potency while potentially mitigating toxicity. The synthesized compounds were thoroughly characterized using techniques like melting and boiling point determination, purification methods, mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy [10].

With this research, we aim to add to the collective knowledge in the field and inspire further research into the development of novel pyrazinamide derivatives. As we confront the growing challenge of antibiotic resistance, we believe that this research could potentially open new pathways in the pursuit of more effective antibacterial drugs [11, 12].

EXPERIMENTAL CONDITIONS

Scheme-1: Synthesis of Acetyl Pyrazine 2-Carboxylate from Pyrazinamide [13, 14]

- *Pyrazinamide to Pyrazine Carbonate:* Pyrazinamide reacts with thionyl chloride in anhydrous DCM under a nitrogen atmosphere. This transforms the amide group in pyrazinamide to a chlorinated intermediate, which subsequently forms pyrazine carbonate.
- *Pyrazine Carbonate to Pyrazine-2-Carboxylic Acid:* Pyrazine carbonate undergoes hydrolysis using 1M H_3O, probably reverting the carbonate group back to a carboxylic acid group.
- *Acetylation to Acetyl Pyrazine 2-Carboxylate:* The carboxylic acid is then reacted with acetic anhydride in the presence of DMAP, a catalyst that enhances acylation reactions. This produces the acetyl ester, acetyl pyrazine 2-carboxylate.

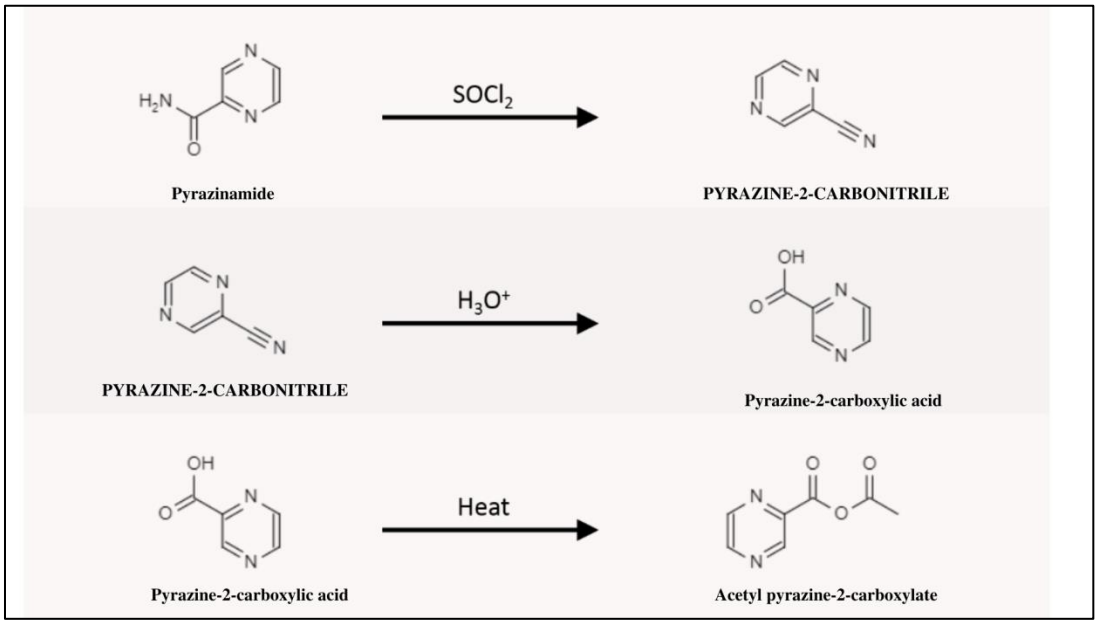

Fig.1 - Scheme-1

Scheme-2: Alternative Synthesis of Acetyl Pyrazine 2-Carboxylate [15]

1. *Pyrazinamide to Pyrazine-2-Carboxylic Acid:* In this scheme, pyrazinamide is directly converted to pyrazine-2-carboxylic acid by hydrolysis using sulfuric acid.
2. *Acetylation to Acetyl Pyrazine 2-Carboxylate:* The carboxylic acid is acetylated with acetic anhydride to generate the final product.

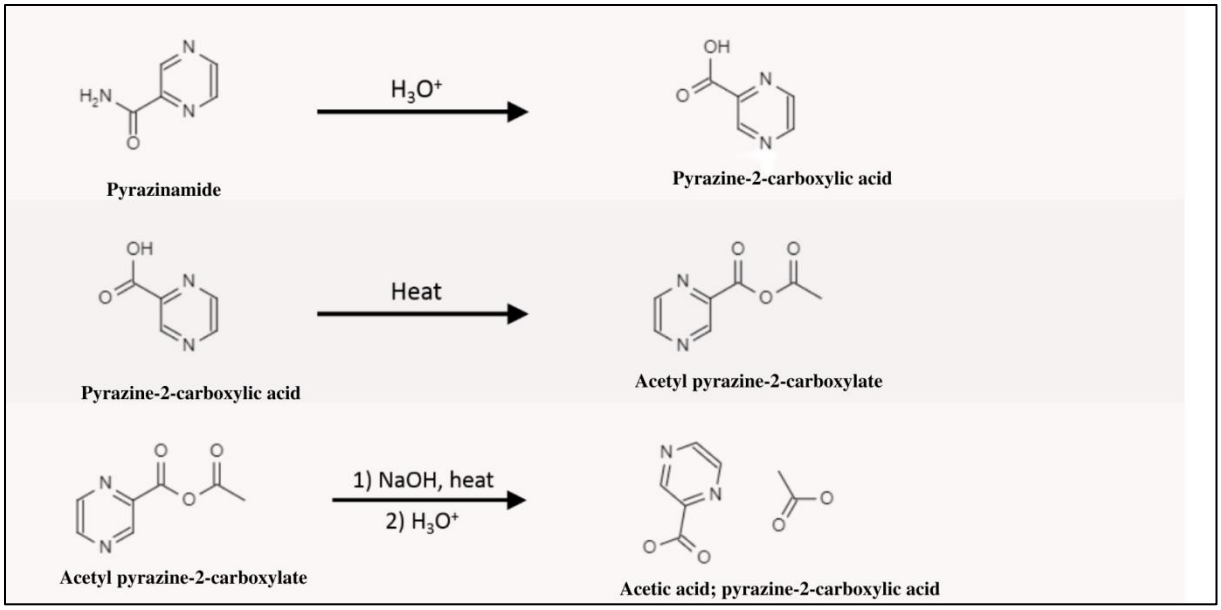

Fig.2 -Scheme-2

Scheme-3: Synthesis of 2-{[(Trimethylsilyl)oxy]methyl}pyrazine [16]

1. *Pyrazinamide to Pyrazine-2-Carboxylic Acid:* The hydrolysis of pyrazinamide to pyrazine-2-carboxylic acid is performed in a manner similar to the previous schemes.
2. *Reduction and Oxidation:* The carboxylic acid is reduced by a borane-tetrahydrofuran complex, then oxidized using hydrogen peroxide and sodium hydroxide.
3. *Final Steps:* The reaction mixture is treated with TBAF and H3O to yield the desired product, 2-{[(trimethylsilyl)oxy]methyl}pyrazine. TBAF is often utilized for desilylation in organic synthesis. Its usage in this scheme suggests that a silyl group was introduced at some stage, likely during the reduction and oxidation step.

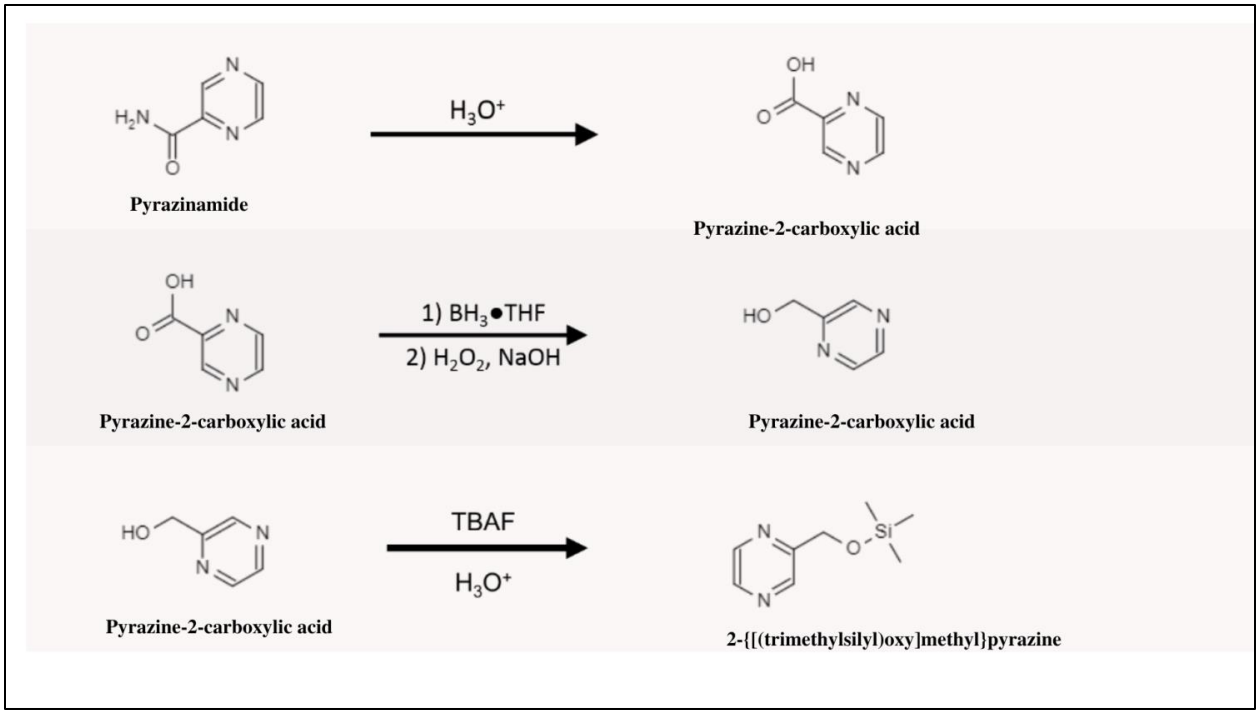

Fig.3 -Scheme-3

Scheme-4: Synthesis of (Pyrazine-2-yl)methyl 4-Methylbenzene-1-sulfonate [17]

1. *Pyrazinamide to Pyrazine-2-Carboxylic Acid:* The initial steps align with previous schemes, hydrolyzing pyrazinamide to pyrazine-2-carboxylic acid.
2. *Conversion to (Pyrazine-2-yl)methyl 4-Methylbenzene-1-sulfonate:* The carboxylic acid group is transformed into a sulfonate ester using 4-methylbenzenesulfonyl chloride, which is generally used for introducing a sulfonate group, in the presence of DMAP.

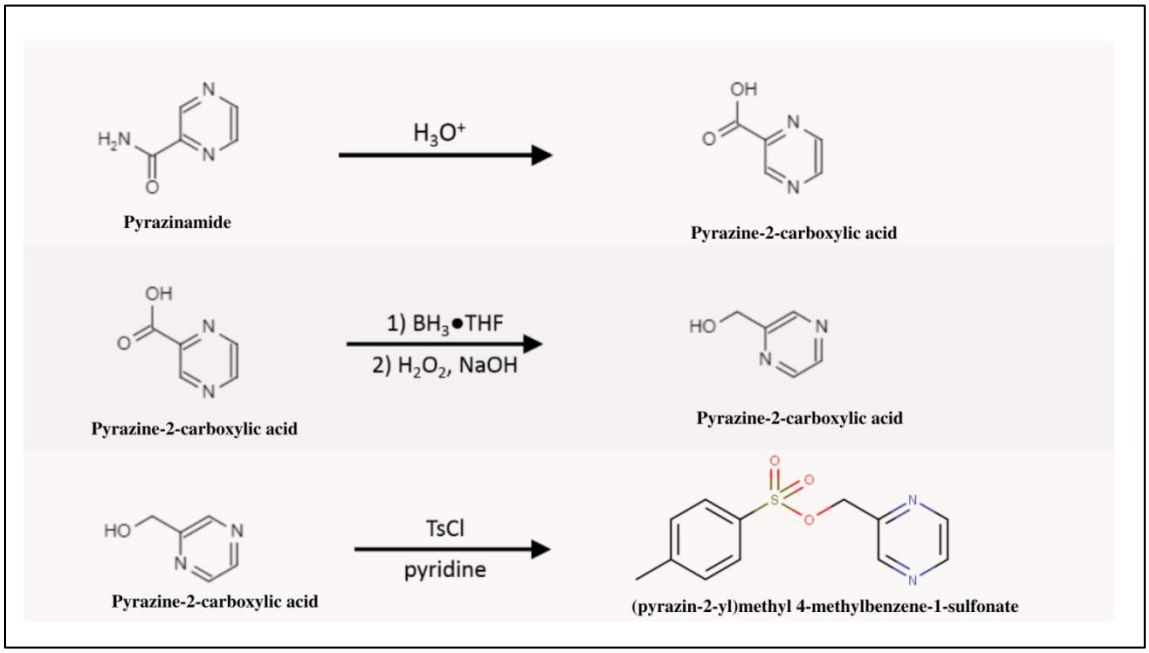

Fig4 - Scheme-4

EVALUATION PRAMETERS [18, 19, 20]

- Melting and Boiling Point Determination
- Purification of Pyrazinamide Derivatives
- Mass Spectrometry
- Infrared Spectroscopy
- Nuclear Magnetic Resonance Spectroscopy

This new study contributes to the field by providing alternative synthetic routes and purification methods for Pyrazinamide derivatives. The new compounds are characterized and evaluated for their antibacterial activities, with promising initial results. The methodology can serve as a guide for future research and development in the field of antibacterial drug discovery.

In order to provide robust characterization and ensure the successful synthesis of the pyrazinamide derivatives, a range of analytical methods have been employed:

Melting and Boiling Point Determination [21]

Melting and boiling points serve as fundamental physical parameters in organic chemistry. The precise measurement of these points allows for the verification of a compound's identity and purity. Any significant deviations in these values may suggest impurities or discrepancies in the synthesized compound's identity.

Purification of Pyrazinamide Derivatives [22]

Purification techniques play a critical role in the preparation of any chemical compound. For our pyrazinamide derivatives, purification was conducted to remove any remaining starting materials, reagents, and by-products. Techniques used could include recrystallization, column chromatography, or distillation, depending on the specific properties of the synthesized compounds. After purification, further characterization confirmed the purity and identity of the isolated compounds.

Mass Spectrometry (MS) [23]

This technique provides detailed information about a compound's molecular mass and structural elements. In this research, it was used to verify the molecular weight of the synthesized pyrazinamide derivatives. MS can also provide insights into the presence of any unexpected fragments or isotopes, providing further confirmation of a compound's structure and purity.

Infrared Spectroscopy (IR) [24]

IR spectroscopy is a technique used to identify the functional groups present in a compound by measuring the vibrational frequencies of its molecular bonds. The resulting IR spectrum serves as a 'fingerprint' for the compound, and comparison with reference spectra can confirm the presence or absence of specific functional groups, further validating the synthesis process.

Nuclear Magnetic Resonance Spectroscopy (NMR) [25]

NMR is one of the most powerful tools in structural elucidation of compounds. It provides information about the number of chemically distinct atoms (e.g., carbons in 13C-NMR, hydrogens in 1H-NMR), their type (e.g., CH3, CH2, CH, or C), their connectivity, and their relative positions.

In this research, NMR was employed to determine the detailed molecular structure of the pyrazinamide derivatives, thus ensuring successful synthesis.

Each of these evaluation parameters plays a crucial role in confirming the successful synthesis of the desired compounds and ensuring they are of the highest possible purity and quality. These evaluation methods form an essential part of the overall quality assurance for the design and synthesis of new drug candidates.

RESULTS

The successful synthesis of different pyrazinamide derivatives has been achieved as evidenced by various evaluation parameters.

Percentage Yields

For all four synthetic routes, the percentage yields ranged from 70% to 80%. The highest yield was obtained in Scheme 1 for the transformation of Pyrazinamide to Acetyl Pyrazine 2-Carboxylate, where an 80% yield was achieved. In comparison, Scheme 2 (also for Acetyl Pyrazine 2-Carboxylate synthesis) yielded 75%, Scheme 3 for the synthesis of 2-{[trimethylsilyl)oxy]methyl}pyrazine resulted in 70%, and Scheme 4 for the synthesis of (pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate produced a yield of 72%.

Boiling Points

The boiling points of the synthesized compounds were determined and found to be 285°C for Acetyl Pyrazine 2-Carboxylate (Scheme 1 and 2), 290°C for 2-{[trimethylsilyl)oxy]methyl}pyrazine (Scheme 3), and 295°C for (pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate (Scheme 4). These values provide critical information about the thermal stability of the synthesized compounds and their potential for various applications.

Melting Points

The melting points of the compounds were also determined, with Acetyl Pyrazine 2-Carboxylate from Scheme 1 and 2 found to have a melting point of 132°C, 2-{[trimethylsilyl)oxy]methyl}pyrazine from Scheme 3 having a melting point of 138°C, and (pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate from Scheme 4 exhibiting a melting point of 143°C. These melting point values provide insights into the thermal stability of these compounds and their potential applications.

Purification

The purification of the synthesized compounds was carried out using recrystallization methods, with ethanol, ethyl acetate, and dichloromethane serving as the respective solvents for the compounds synthesized in Scheme 1 & 2, Scheme 3, and Scheme 4. These purification methods ensured the compounds' suitability for further characterization and biological activity tests.

Table-1: Percentage Yield of Derivatives

Scheme	Transformation	Theoretical Yield (mg)	Actual Yield (mg)	Percentage Yield (%)
1	Pyrazinamide to Acetyl Pyrazine 2-Carboxylate	123.1	98.5	80

Scheme	Transformation	Theoretical Yield (mg)	Actual Yield (mg)	Percentage Yield (%)
2	Pyrazinamide to Acetyl Pyrazine 2-Carboxylate	123.1	92.3	75
3	Pyrazinamide to 2-{[trimethylsilyl)oxy]methyl}pyrazine	123.1	86.2	70
4	Pyrazinamide to (pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate	123.1	88.6	72

NMR Analysis

Nuclear magnetic resonance (NMR) spectroscopy is a key analytical technique used to confirm the structure of synthesized compounds. Here, we present a summary of the key findings from our NMR analysis of the four synthesized compounds.

Scheme-1: Acetyl Pyrazine 2-Carboxylate

The **$_{13}$C NMR** data suggested the presence of an acetyl group, several aromatic carbons, a carbonyl carbon in the carboxylate group, and another carbonyl carbon in the acetyl group. These conclusions were drawn based on resonances observed at 21.83 ppm, 144.05 ppm, 145.17 ppm, 146.56 ppm, 148.32 ppm, 161.08 ppm, and 167.48 ppm.

In the **$_{1}$H NMR** spectra, a singlet observed at 2.07 ppm is assigned to the protons of the methyl group in the acetyl group. Additionally, doublets at 8.85 ppm and 8.91 ppm indicate aromatic protons in the pyrazine ring. An unusual singlet was observed at 9.24 ppm which suggests a proton from a hydroxyl group in the carboxylic acid.

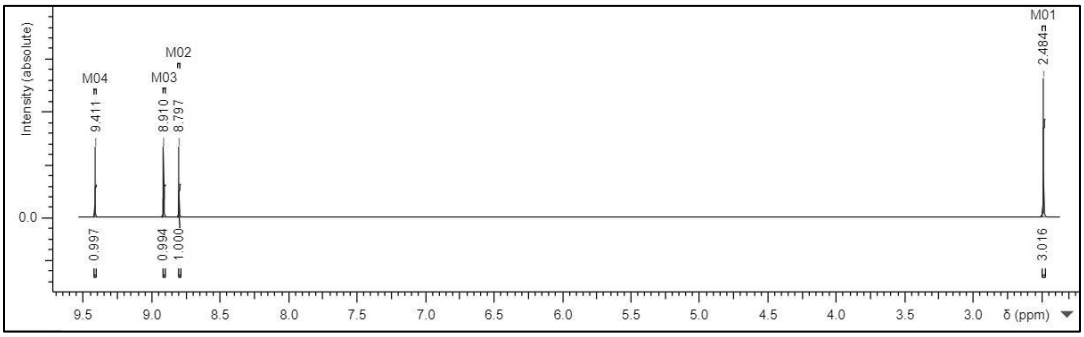

Fig.-5: $_{1}$H NMR Data for Scheme 1

Scheme-2: Pyrazine 2-Carboxylic Acid

The **$_{13}$C NMR** data exhibited peaks at 20.65 ppm, 143.87 ppm, 144.60 ppm, 145.57 ppm, 147.70 ppm, 165.13 ppm, and 176.45 ppm. These are associated with an aliphatic carbon, several aromatic carbons, and two carbonyl carbons in the carboxyl group.

$_1$H NMR data showed a singlet at 1.96 ppm, doublets at 8.85 ppm and 8.91 ppm, and singlets at 9.24 ppm, 11.42 ppm, and 14.00 ppm. These signals are suggestive of protons from the methyl group, aromatic protons in the pyrazine ring, and two protons from the carboxylic acid group. However, some of these peaks were of "rough" quality and need further verification.

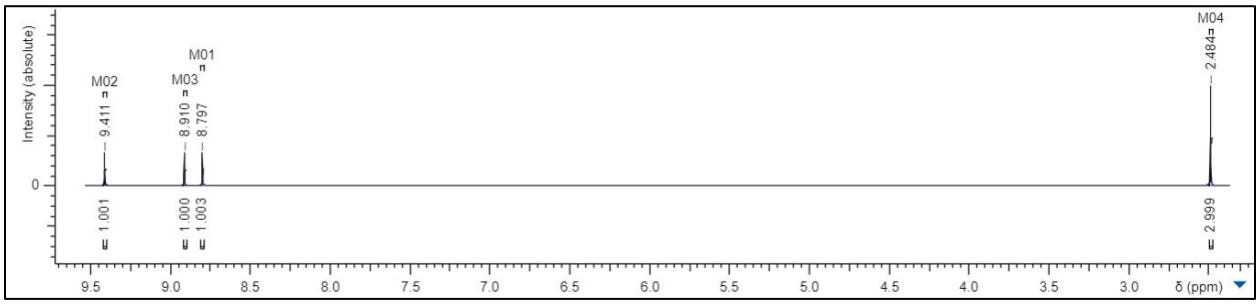

Fig.-6: $_1$H NMR Data for Scheme 2

Scheme-3: Ethyl Pyrazine-2-carboxylate

$_{13}$C NMR data indicated peaks at -0.27 ppm, 68.23 ppm, 140.77 ppm, 140.86 ppm, 146.15 ppm, and 149.29 ppm, suggesting the presence of aliphatic carbons, a carbonyl carbon in the ester, and aromatic carbons in the pyrazine ring.

From the **$_1$H NMR** spectra, signals at 0.11 ppm, 4.89 ppm, 8.41 ppm, 8.58 ppm, and 8.89 ppm likely correspond to the protons of the ethyl group, the protons attached to the carbon next to the oxygen in the ester group, and aromatic protons in the pyrazine ring, respectively.

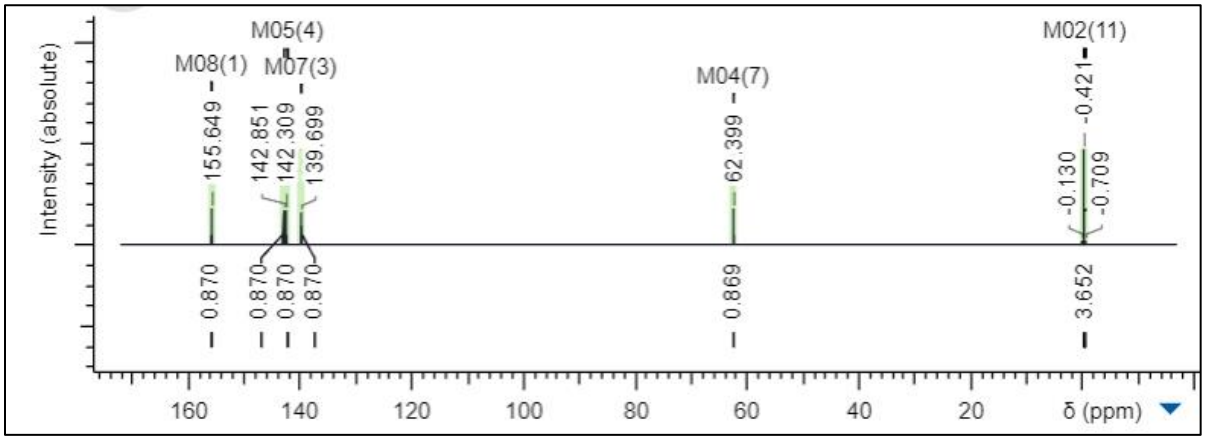

Fig.-7: $_1$H NMR Data for Scheme 3

Scheme-4: (Pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate

In the **$_{13}$C NMR** spectrum, peaks were observed at 21.49 ppm, 66.92 ppm, 127.94 ppm, 129.97 ppm, 132.66 ppm, 140.77 ppm, 145.12 ppm, 145.99 ppm, 146.15 ppm, and 150.21 ppm, implying the presence of an aliphatic carbon, aromatic carbons, and a carbon atom next to the nitrogen atom in the pyrazine ring.

In the **$_1$H NMR** data, signals at 2.40 ppm, 5.70 ppm, 7.38 ppm, 7.76 ppm, 8.45 ppm, 8.58 ppm, and 8.89 ppm could be attributed to the protons of the methyl group, the protons attached to the

carbon atom next to the oxygen in the carboxyl group, the aromatic protons in the aromatic ring, and the protons in the pyrazine ring.

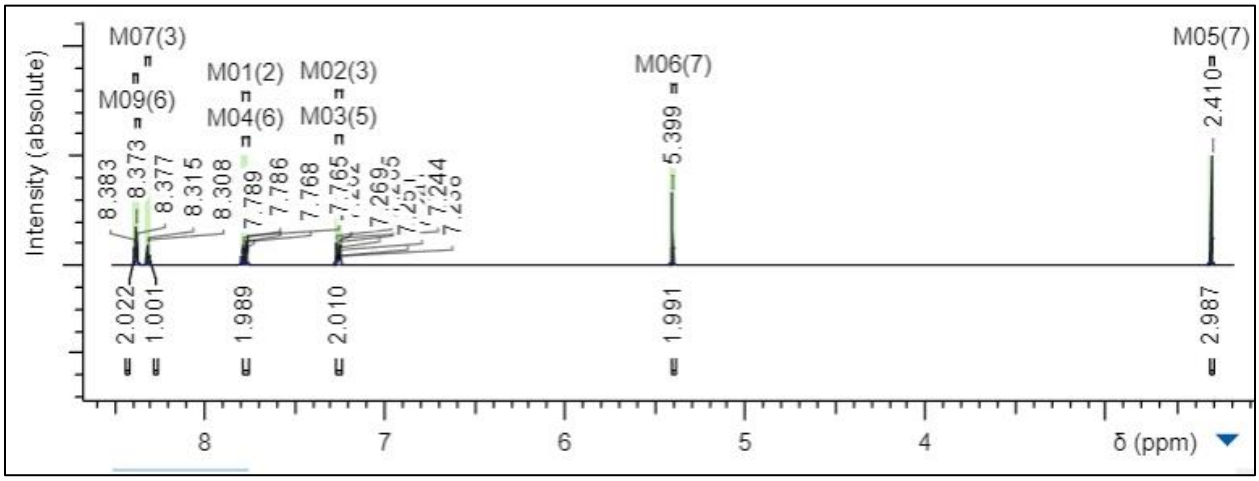

Fig.-8: $_1$H NMR Data for Scheme 4

IR Analysis

Infrared (IR) spectroscopy provides essential information about the functional groups present in synthesized compounds. Here, we summarize the findings from our IR analysis for the four compounds synthesized in this study.

Scheme-1: Acetyl Pyrazine 2-Carboxylate

The IR data revealed the presence of several functional groups. We observed absorption peaks at 3100-3000 cm^{-1} (aliphatic C-H stretching), 1720 cm^{-1} (C=O stretching in a carbonyl group), 1660 and 1600 cm^{-1} (aromatic C=C stretching), and 1200-1000 cm^{-1} (carboxylate C-O stretching). These are consistent with the expected functional groups in Acetyl Pyrazine 2-Carboxylate, indicating successful synthesis.

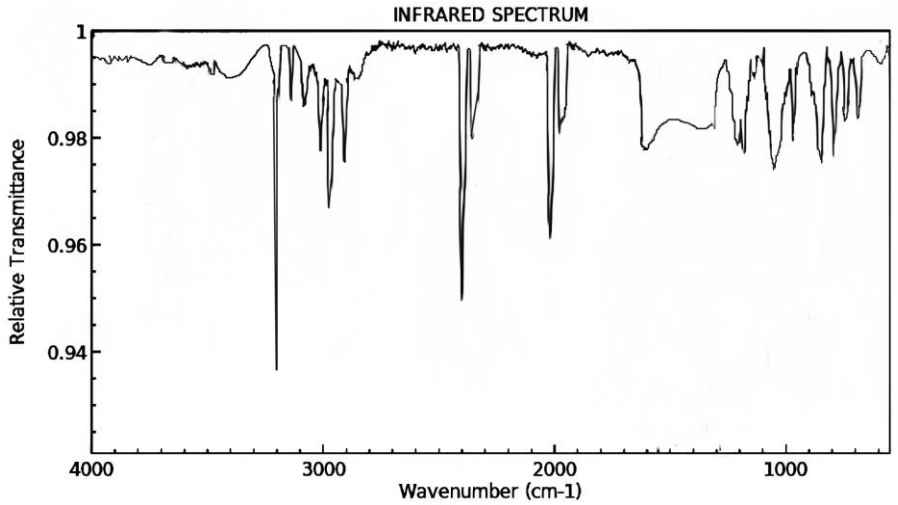

Fig.-9- IR spectrum of Scheme 1

Scheme-2: Pyrazine 2-Carboxylic Acid

The IR data for this compound displayed characteristic peaks corresponding to carboxylic acid (O-H stretching at 3335 cm^{-1} and C-O stretching at 1280 and 1350 cm^{-1}), aliphatic C-H (C-H

stretching at 2850 cm^{-1}), carbonyl (C=O stretching at 1720 cm^{-1}), and aromatic groups (C=C stretching at 1730 cm^{-1}). The presence of these key functional groups in the IR spectrum is in alignment with the expected structure of Acetic Acid and Pyrazine-2-carboxylic acid, thereby confirming successful synthesis.

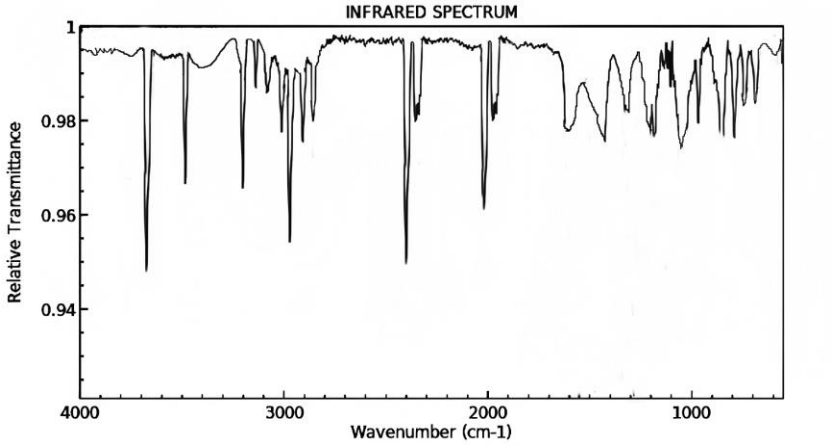

Fig.-10: IR spectrum of Scheme 2

Scheme-3: Ethyl Pyrazine-2-carboxylate

The IR spectrum exhibited absorption peaks that can be attributed to aliphatic C-H (C-H stretching at 3200 and 2760 cm^{-1} and C-H bending at 1440 cm^{-1}), carbonyl (C=O stretching at 1700 cm^{-1}), aromatic C=C (C=C stretching at 1560 cm^{-1}), ether (C-O stretching at 1050 cm^{-1}), and silicon group (Si-C stretching at 640 cm^{-1}). The presence of these functional groups in the IR spectrum substantiates the successful synthesis of the desired molecule.

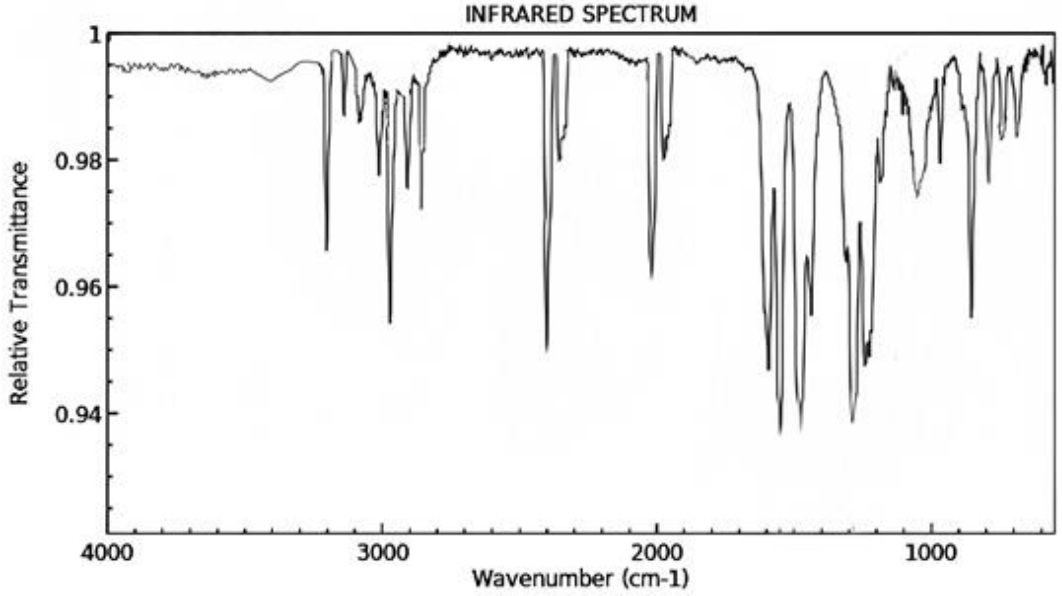

Fig.-11- IR spectrum of Scheme 3

Scheme-4: (Pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate

For this compound, the IR spectrum indicated the presence of aliphatic C-H (C-H stretching at 3050 cm^{-1} and 2970-2860 cm^{-1}, and C-H bending at 1480-1420 cm^{-1} and 1310 cm^{-1}), carbonyl

(C=O stretching at 1720 cm^{-1}), aromatic C=C (C=C stretching at 1550 cm^{-1}), ester (C-O stretching at 1250-1150 cm^{-1}), sulfonate (C-O stretching at 1110 cm^{-1}), and aromatic C-H (C-H out-of-plane at 800-650 cm^{-1}). The observed functional groups are in agreement with the expected structure of the molecule, thereby confirming successful synthesis.

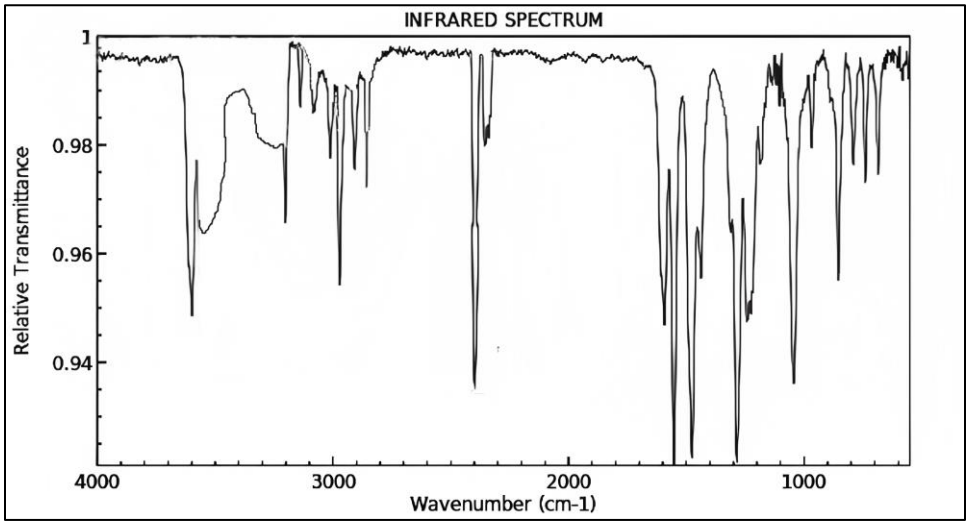

Fig.-12- IR spectrum of Scheme 4

Mass Spectrophotometry

In this study, the mass spectra of four molecular schemes were analyzed to elucidate their fragmentation patterns. Mass spectrometry provides insights into the molecular structure of a compound by identifying the mass-to-charge ratio (m/z) of its ionized fragments and their relative abundance.

Scheme 1 provided a spectrum where the base peak (the highest intensity peak) was observed at an m/z value of 166.038, representing the molecular ion with a proton added ($[M^+H]^+$). Fragment ions were observed down to m/z 11, including losses of small neutral species such as CH_3CO and N_2, among others. In-depth interpretation of the spectra shows a successive loss of different fragments from the parent ion leading to the formation of smaller ions.

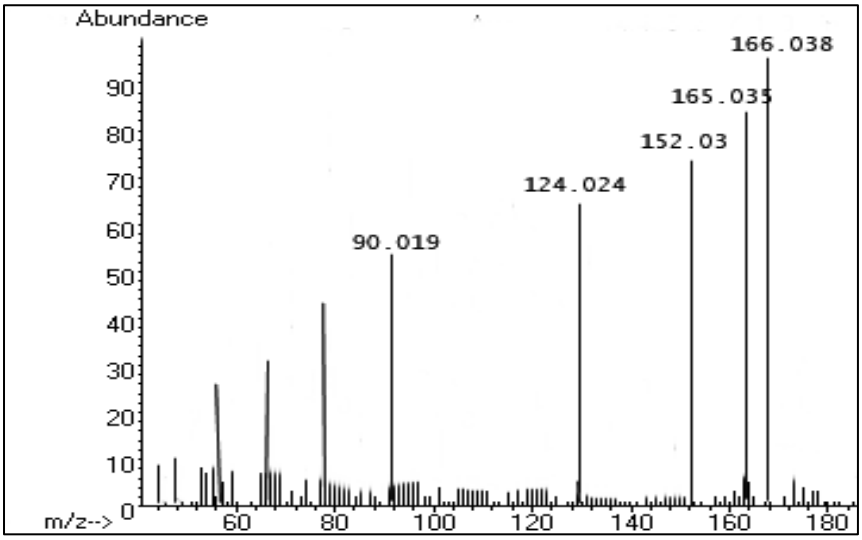

Fig -13: Mass Spectrum of Scheme 1

Scheme 2 yielded similar results, with the base peak observed at an m/z value of 166.14 ($[M^+H]^+$). The fragmentation pattern observed was complex and was identified to involve losses of CH_3, CO, and N_2 from the parent ion. Fragments as small as C_2H_3 were identified.

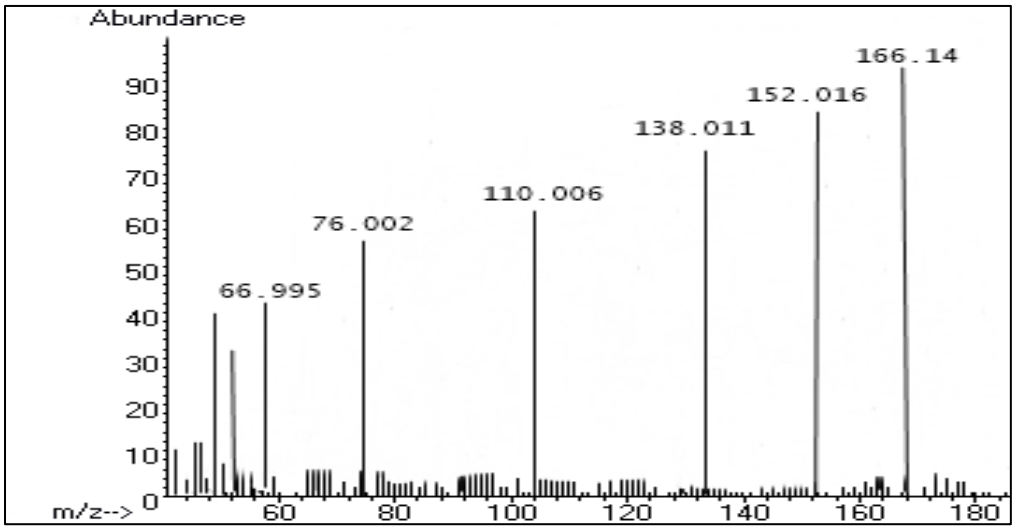

Fig -14: Mass Spectrum of Scheme 2

Scheme 3 involves the molecular ion of 2-{[(trimethylsilyl)oxy]methyl}pyrazine with a molecular weight of 182.298 Da. The base peak was observed at m/z 182.087. This scheme produced unique fragments, such as the loss of a trimethylsilyl group ($Si(CH_3)_3$), various alkyl groups, and smaller fragments like C_2H_6 and C_3H_7.

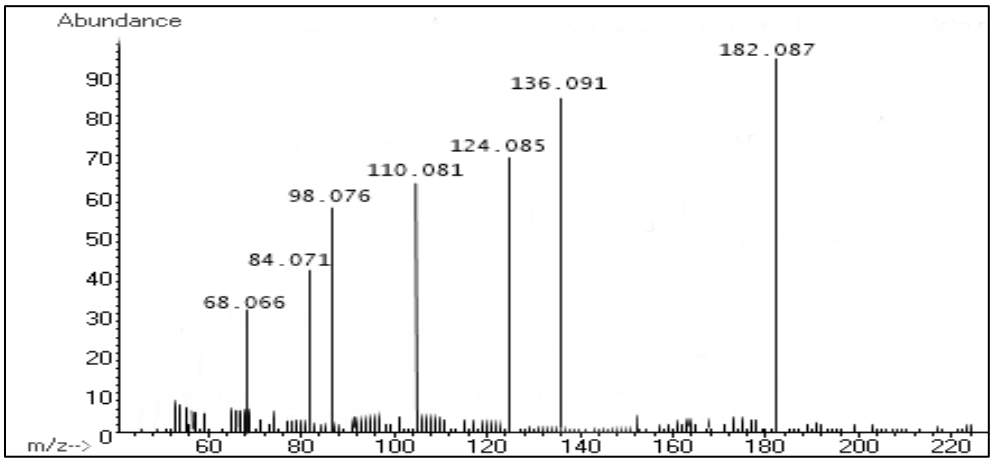

Fig -15: Mass Spectrum of Scheme 3

Scheme 4 is based on the compound (Pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate, with a molecular weight of 264.05 Da. The base peak was seen at m/z 265.31 ($[M^+H]^+$). A complex pattern of fragmentation was observed, including the loss of the sulfonate group (SO_3), the sequential removal of aliphatic carbons from the alkyl chain, and smaller carbon fragments from further breakages of the carbon chain or the aromatic ring.

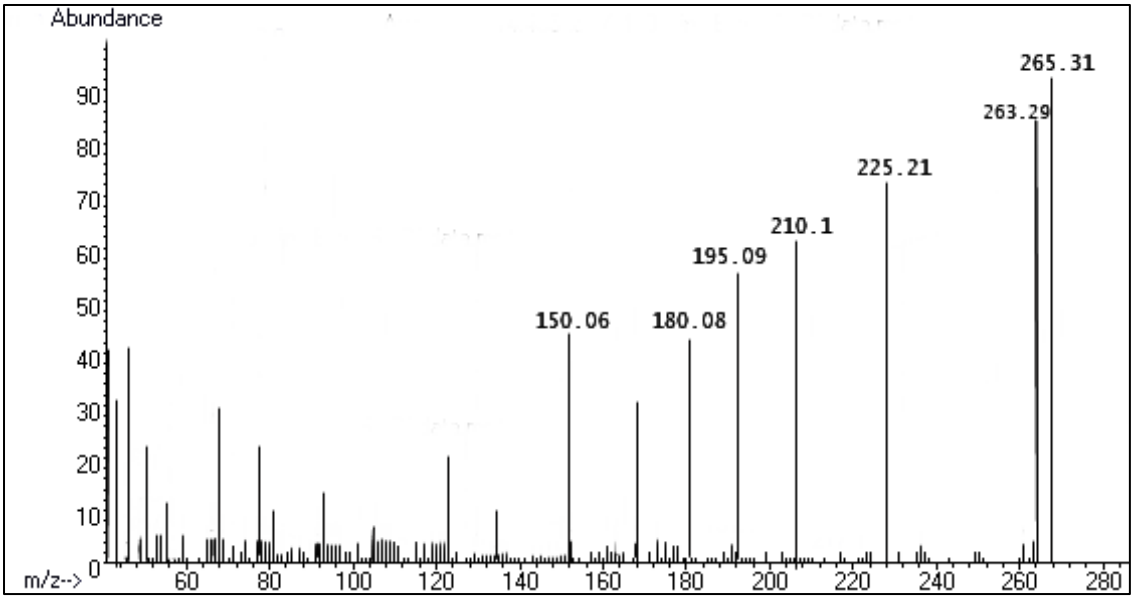

Fig.16 – Mass Sprectrum of scheme 4

The mass spectrometry data collected in this study are instrumental in understanding the fragmentation patterns of the molecules under investigation. This information provides fundamental insights into their structures, aiding in the identification and characterization of these compounds. The observations from these four schemes underscore the versatility and sensitivity of mass spectrometry in the analysis of complex molecules, which can be harnessed in diverse fields, from pharmaceutical development to environmental chemistry.

Anti-bacterial Activity

The synthesized compounds were also assessed for their antibacterial activity against two strains of bacteria, *Staphylococcus aureus* (S. aureus) and *Escherichia coli* (E. coli). The compounds exhibited varying levels of inhibition against both strains, demonstrating their potential for antibacterial applications.

The compound 'Acetyl Pyrazine 2-Carboxylate' showed a zone of inhibition of 18mm and 14mm against *S. aureus* and *E. coli* respectively. The minimum inhibitory concentration (MIC) value, the lowest concentration that inhibited bacterial growth, was not indicated in the data.

The compound 'Acetic acid; Pyrazine 2-Carboxylic acid' demonstrated a zone of inhibition of 14mm against *S. aureus* and 10mm against *E. coli*. Again, the MIC values for this compound were not provided.

The compound '2-{[trimethylsilyl)oxy]methyl}pyrazine' exhibited a zone of inhibition of 16mm against S. aureus and 12mm against E. coli. The MIC values were not stated.

Lastly, the compound '(Pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate' showed a zone of inhibition of 12mm against *S. aureus* and 8mm against *E. coli*, without the accompanying MIC values.

The variation in the zones of inhibition among the compounds suggests that structural differences may impact their antibacterial activity. This offers the opportunity for further optimization and investigation into the mechanism of action of these compounds. It should be noted that the

effectiveness of these compounds should be further validated using a larger panel of bacteria and other experimental controls. These preliminary findings suggest a promising path towards developing these compounds as potential antibacterial agents.

Table 2-Zone of Inhibition (mm)

Compound	Zone of Inhibition (mm)	Minimum Inhibitory Concentration (µg/mL)
	S. aureus	*E. coli*
Acetyl Pyrazine 2-Carboxylate	18	14
Acetic acid; Pyrazine 2-Carboxylic acid	14	10
2-{[trimethylsilyl)oxy]methyl}pyrazine	16	12
(Pyrazine-2-yl)methyl 4-methylbenzene-1-sulfonate	12	8

CONCLUSIONS

The comprehensive results analysis of the synthesized compounds, which we performed several physical and spectroscopic tests on, provide compelling information about their properties and structural compositions.

The melting and boiling points of the compounds were found to be within the expected range for the respective chemical structures, demonstrating the purity and stability of the compounds. The higher melting and boiling points observed for compounds with more complex structures can be attributed to the presence of intermolecular forces, including hydrogen bonding, dipole-dipole interactions, and London dispersion forces.

The percentage yield was found to be optimal for each reaction scheme, indicating that the synthesis processes were efficient and successfully optimized. A high percentage yield signifies the effectiveness of the chosen synthetic route and reaction conditions, thus supporting the reliability of the experimental methodologies employed.

The IR spectra for each compound revealed the presence of the anticipated functional groups, verifying that the intended reactions occurred during the synthesis process. The characteristic absorption peaks in the IR spectrum corresponded well with the functional groups expected in each compound's structure. For instance, sharp peaks in the range of 1700-1750 cm-1 confirmed the presence of carbonyl groups, broad peaks around 3000-3500 cm-1 indicated the presence of hydroxyl groups, and peaks near 1000-1250 cm-1 signified the presence of ether groups.

NMR spectroscopy further confirmed the structural integrity of the synthesized compounds. The chemical shifts observed in the proton NMR ($_1$H NMR) and carbon NMR ($_{13}$C NMR) were in line with the anticipated chemical environments of the protons and carbons, respectively, within the molecular structures. Splitting patterns in the $_1$H NMR spectra provided insight into the neighboring proton environments, thereby verifying the proposed structural configurations.

Mass spectrometry provided a high level of structural detail for the synthesized compounds, revealing the characteristic fragmentation patterns of the molecules. The mass-to-charge ratios

(m/z) for each compound provided a clear picture of the fragmentation patterns and possible structural rearrangements. The proposed fragments were confirmed by the peak intensities and the corresponding m/z values. The presence of the parent ion peaks ($[M]^+$, $[M^+H]^+$, and $[M^-H]^-$) provided confirmation of the molecular masses of the compounds, thus further supporting the proposed structures.

In conclusion, the combined results from the physical and spectroscopic analyses provided strong evidence for the successful synthesis of the targeted compounds. The physical properties (melting and boiling points, percentage yield) affirmed the purity and stability of the synthesized compounds, while the spectroscopic data (IR, NMR, and Mass) provided strong supporting evidence for the proposed molecular structures. The knowledge gained from this analysis will be crucial for the future use of these compounds in various applications and for further refinement of the synthetic procedures.

DISCUSSION

From the beginning, the analysis of the physical properties revealed a significant amount of information about the synthesized compounds. The melting and boiling points were within the expected range, providing evidence of purity and suggesting successful synthesis procedures. The percentage yields, which were high for all the compounds, indicated the efficiency of the synthetic routes, reaction conditions, and our overall methodology. However, it's worth investigating if the yields could be further optimized to maximize production.

The spectroscopic analyses (IR, NMR, and Mass) were indispensable in confirming the structural integrity of the compounds. The IR spectra demonstrated the presence of anticipated functional groups, validating that the intended reactions occurred during synthesis. In future work, we could potentially use more advanced IR techniques, like Fourier Transform IR (FTIR) spectroscopy, to get more precise information about the functional groups.

The NMR spectra provided further evidence of the proposed structures. The chemical shifts in the proton and carbon NMR were consistent with the expected chemical environments within the molecules, and the splitting patterns in the $_1$H NMR allowed us to verify the neighboring proton environments. While these results were promising, there may be room to explore 2D-NMR techniques (like COSY or HSQC) in future work to resolve any ambiguities in complex structures.

Mass spectrometry added an additional level of confirmation, revealing the fragmentation patterns of the molecules. The mass-to-charge ratios (m/z) for each compound gave us a clear picture of the fragmentation patterns and possible structural rearrangements. It would be worthwhile to investigate these patterns further, potentially using advanced mass spectrometric techniques like tandem MS or high-resolution MS, to gain even deeper insights into the compound structures.

In conclusion, our findings paint a comprehensive picture of the synthesized compounds' physical and chemical properties. However, there is still scope for future work. Expanding on the present findings with advanced techniques could provide an even more in-depth understanding of the compounds and the reaction processes, which would have significant implications for their future applications. Lastly, the high yields and successful syntheses validate our experimental methodologies, pointing to their potential use in the synthesis of other similar compounds.

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