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Synthesis, Characterization and Biological Evaluation of Piperazine Derivatives

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ABSTRACT

A new series of piperazine analogs containing benzimidazole segments was successfully synthesized with favorable yields. The structures of these four piperazine analogs were verified using various analytical techniques including 1H-nuclear magnetic resonance, liquid chromatography-mass spectrometry, and FTIR. These newly created derivatives were then evaluated for their effectiveness in combatting tuberculosis. Notably, compound 1 demonstrated the highest yield at 74.86%. The synthesized compounds were assigned Rf values in the range of 0.5 to 0.82, and they displayed excellent solubility in both methanol and water. The melting points of the compounds varied from 168°C to 288°C. The experimental results revealed that the compounds exhibited promising minimum inhibitory concentration (MIC) values against the tuberculosis-causing agent. Encouragingly, compounds 1, 3, and 4 exhibited MIC values comparable to those of standard drugs. Impressively, compound 2 displayed the most potent anti-tubercular activity, with a MIC of 0.1µg/ml. To confirm their identity and purity, all synthesized compounds underwent rigorous checks, and spectral analysis provided confirmation of their structural integrity. In conclusion, several of these compounds exhibited substantial anti-tubercular activity, marking a significant advancement in the field.

1. INTRODUCTION

The primary goal of organic and medicinal chemistry revolves around creating, identifying the qualities of, and assessing the pharmacological potential of molecules with strong therapeutic effects. In our current era, as many organisms are becoming more resistant, there is a growing necessity to design novel compounds that exhibit heightened potency against resistant microorganisms, particularly bacteria, viruses, and fungi. A crucial domain within antimicrobial research is dedicated to developing and analyzing biologically active compounds aimed at combating drug-resistant bacteria and fungi [1]. A notable focus of this research lies in the synthesis and characterization of substituted piperazine derivatives, which have been recognized for their diverse pharmacological attributes, encompassing antimicrobial and insecticidal properties [2], as well as antitumor and antifungal effects [3].

The combination of microbial resistance progression and economic motivations has driven intensive research and innovation aimed at discovering novel antibiotics. This pursuit is essential to ensure a consistent arsenal of efficacious medications. Although the emergence of resistant variants is unavoidable, the careless practices surrounding the prescription and utilization of antibiotics have significantly intensified this phenomenon [4]. Despite the extensive array of antibiotics and therapeutic compounds accessible for medical purposes, the rise of both previously known and novel antibiotic-resistant bacterial strains in recent years underscores a pressing requirement for a fresh category of antibacterial agents [5].

Advancements in the design of biologically active heterocyclic compounds have been facilitated not only by refining the methodologies for examining these substances but also by substantial progress in their synthesis techniques. The piperazine ring stands as a versatile foundational structure [5], extensively explored in medicinal chemistry due to its significant pharmacological attributes. This core structure exhibits a diverse array of biological effects such as antimicrobial, anti-tubercular, anti-malarial, anti-hypertensive, antidepressant, antipyretic, analgesic, and anesthetic properties [6-10].

The process of synthesizing, characterizing, and assessing the biological properties of piperazine derivatives constitutes a pivotal endeavor in the realm of medicinal chemistry. By systematically modifying the piperazine structure, researchers aim to enhance its therapeutic potential and explore its diverse pharmacological activities. This multifaceted approach involves the creation of novel compounds, their structural analysis, and rigorous evaluation of their efficacy in various biological contexts. The intricate interplay between synthesis,

characterization, and biological assessment contributes significantly to advancing our understanding of these derivatives and their potential applications in the development of innovative pharmaceutical agents [10].

Hence, in order to expand the potential applications of piperazine derivatives as important foundational structures for therapeutic purposes, and recognize the significant need for identifying fresh chemical compounds with promising biological properties, we directed our focus towards the creation of innovative anti-tubercular agents. Our objective encompassed the synthesis and assessment of the biological effects of recently developed piperazine derivatives that incorporate Benzimidazole components. This article documents the process of synthesis, characterization, and investigation of the antimicrobial capabilities of novel piperazine analogs with the potential to serve as potential anti-tubercular agents.

2. MATERIAL AND METHOD

2.1 Materials

Benzaldehyde, formic acid, acetic acid, and propionic acid were acquired from reputable commercial sources including Across, Merck, and Fisher. These substances were subsequently purified using established procedures. Methanol and orthophenylene diamine were also obtained from commercial suppliers and refined as required following accepted protocols.

2.2 Experimental Procedure

The synthesis of Benzimidazole involved dissolving 0.025 moles of o-phenylenediamine in a 250 ml round-bottom flask (RBF). To this solution, 0.034 moles of 90% formic acid were added. The resulting mixture was heated in a water bath for two hours while gradually introducing a 10% sodium hydroxide (NaOH) solution. The addition of NAOH continued until the mixture reached a slightly alkaline level according to the litmus test. The resulting mixture was then filtered to obtain crude benzimidazole. This crude product was washed with 5 ml of ice-cold water, drained, and subjected to an additional wash. The crude material was then dissolved in 40 ml of boiling water and mixed with 0.2 grams of decolorizing carbon. After digestion for 15 minutes, the mixture was filtered to separate the Benzimidazole. The obtained product was rinsed with 5 cc of cold water and dried at 100°C.

Synthesis of Compound 1 (1-[(piperazin-1-yl) methyl]-1H-1,3-benzimidazole)

0.01mol of benzimidazole in 20ml of methanol was stirred at room temperature with 0.01 mol of piperazine, 1 ml formaldehyde solution [40% w/v] and 1 ml HCl. Then the reaction mixture was refluxed for 10 hrs at 70-75℃. The hot mixture was filtered and kept in the refrigerator overnight. The product was filtered, dried and recrystallized from ethanol to give solid compound.

Synthesis of Compound 2 (2-phenyl-1-[(piperazin-1-yl) methyl]-1H-benzimidazole)

0.01mol of 2-methyl benzimidazole in 20ml of methanol was stirred at room temperature with 0.01 mol of piperazine, 1 ml formaldehyde solution [40% w/v] and 1 ml HCl. Then the reaction mixture was refluxed for 10 hrs at 70-75℃. The hot mixture was filtered and kept in the refrigerator overnight. The product was filtered, dried and recrystallized from ethanol to give a solid compound.

Synthesis of Compound 3 (2-ethyl-1-[(piperazin-1-yl)methyl]-1H-benzimidazole)

0.01mol of 2-ethyl benzimidazole in 20ml of methanol was stirred at room temperature with 0.01 mol of piperazine, 1 ml formaldehyde solution [40% w/v] and 1 ml HCl. Then the reaction mixture was refluxed for 10 hrs at 70-75℃. The hot mixture was filtered and kept in the refrigerator overnight. The product was filtered, dried and recrystallized from ethanol to give solid compound.

Synthesis of Compound 4 (2-phenyl-1-[(piperazin-1-yl)methyl]-1H-benzimidazole)

0.01mol of 2-phenyl benzimidazole in 20ml of methanol was stirred at room temperature with 0.01 mol of piperazine, 1 ml formaldehyde solution [40% w/v] and 1 ml HCl. Then the reaction mixture was refluxed for 10 hrs at 70-75℃. The hot mixture was filtered and kept in the refrigerator overnight. The product was filtered, dried and recrystallized from ethanol to give a solid compound.

Characterization Studies

The newly synthesized compounds were identified through a combination of physical and chemical attributes, including factors like melting and boiling points, solubility, chemical tests, and elemental composition analysis. In addition to these methods, the compounds were characterized using various analytical techniques such as Thin Layer Chromatography (TLC),

Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) [11-13].

Thin layer chromatography (TLC)

Thin-layer chromatography is an analytical method in which a solid stationary phase is spread as a thin layer on a rigid plate, and a liquid mobile phase is allowed to move across it. This technique is commonly employed to identify chemicals with distinct Rf values. It is also useful for tracking the progress of reactions and assessing the purity of the final product. For this purpose, silica gel plates were prepared as the stationary phase, and various mixtures like methanol to chloroform (1:9), acetone to chloroform (1:1), toluene to ethyl acetate, petroleum ether to ethyl acetate, etc., were used as mobile phases. To visualize the separated compounds, the plate was placed in an iodine chamber after the chromatogram's development. The Rf values of each compound were calculated using the formula: RF = Distance traveled by the compound front was used to compute Rf values for each compound.

Infrared spectral studies (FTIR)

Infrared spectroscopy is a vital approach for discerning diverse functional groups and plausible chemical arrangements. Its primary advantage over alternative methods lies in its ability to effortlessly offer distinctive data (ranging from 1300 to 650 cm^{-1}) that characterizes the structure, including functional groups and interactions with other molecules, of various compounds. This uniqueness stems from the fact that the vibrations of each bond within a molecule occur at a specific frequency, aligning with the infrared frequency. Consequently, an infrared spectrum is generated for each bond.

Mass Spectral Studies

Mass spectroscopy plays a crucial role in determining the molecular weight of unfamiliar compounds. The Mass Spectrometry instrument utilized for this purpose was a Varian Inc. apparatus, model 410 Prostar Binary LC with 500 MS IT PDA Detectors, and the mass spectra were recorded by the "Sophisticated Analytical Instrumentation Facility" at Punjab University, Chandigarh, India.

Nuclear magnetic resonance spectra (NMR)

By simultaneously subjecting a substance to two magnetic fields, where one is static while the other varies at a radio frequency, it becomes possible to explore the interplay between matter

and electromagnetic forces. The sample absorbs energy under specific field conditions, and this absorption is manifested as a modification in the signal generated by a radio frequency detector and amplifier. This energy absorption is linked to the magnetic dipolar nature of spinning nuclei and proves valuable in deducing molecular structure. The analysis of synthesized compounds involved studying their ^{1}H-NMR spectra using the "BRUKER AVANCE II 400 NMR spectrometer."

Biological Activity

Anti-tubercular Activity

Microbes at a 0.5 McFarland standard will be appropriately diluted as per the study's protocol. Specific tubes were prepared, containing 10µl of treatment dilutions at varying concentrations (as indicated in the Excel sheet) and 500µl of bacteria (M. tuberculosis, MTCC 300) log cultures that had been diluted. These prepared tubes were then kept in incubation for a duration of 15 days. After the initial 24 hours of incubation, the complete contents from these tubes were moved to a 96-well plate and combined with MTT Solution at a final concentration of 250µg/ml. Following the incubation period, measurements were taken at both 490 nm and 595 nm using an Elisa Plate Reader (iMarkBiorad). The positive control utilized was 100µg of ciprofloxacin [14, 15].

3. RESULT AND DISCUSSION

Characterization Studies

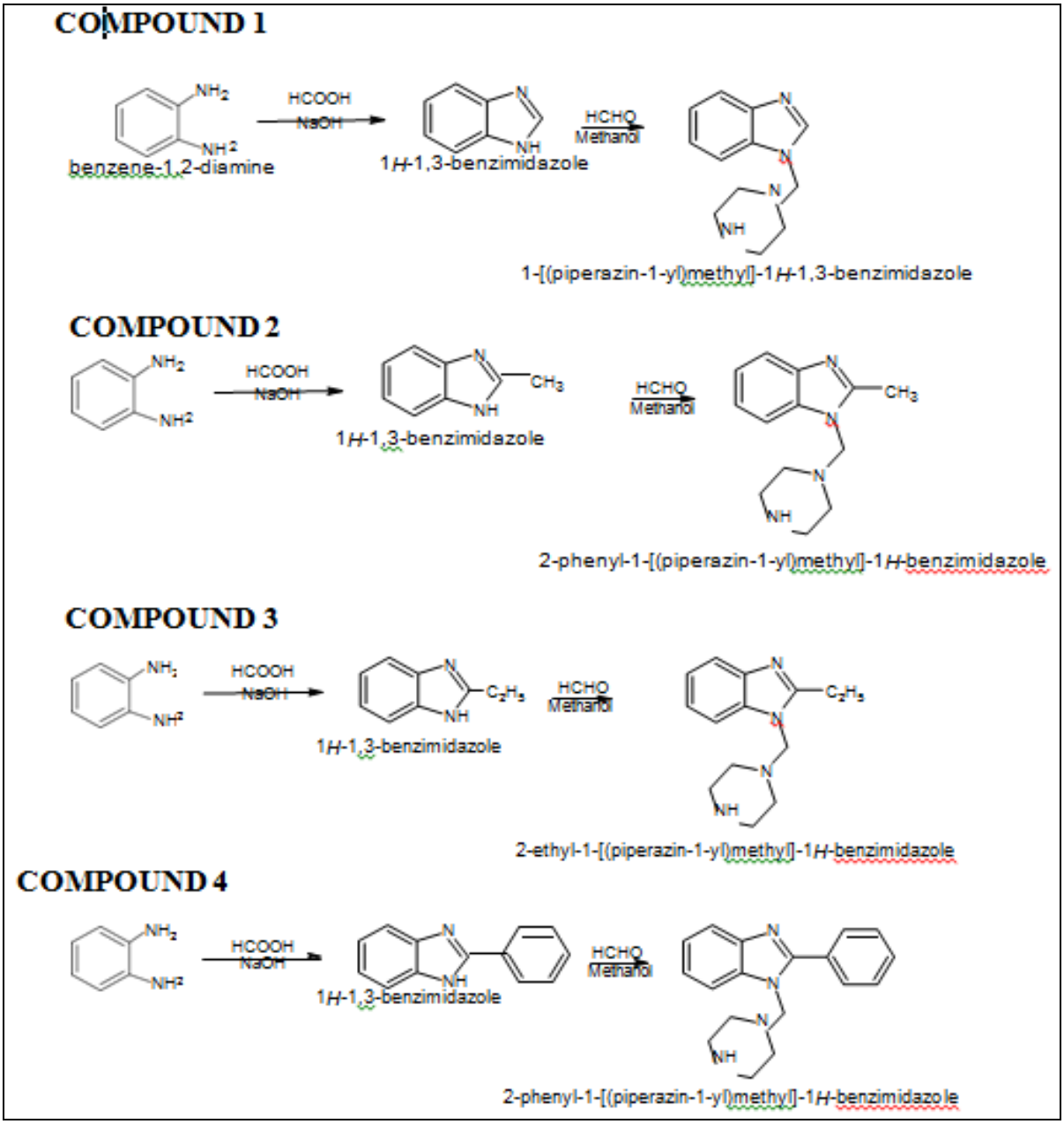

Figure 1: Schematic representation of novel piperazine derivatives

Table 1: Physicochemical information about synthetic piperazine derivatives

Sr.No	Comp. code	Nature	Color	Solubility	Molecular formula	Molecular weight
1	1	Solid	White solid	Methyl alcohol	C7H6N2	118.14
2	2	solid	Light beige to brown	Methyl alcohol	C8H8N2	132.17
3	3	solid	White to light yellow	Methyl alcohol	C9H10N2	146.29
4	4	solid	White	water	C13H10N2	194.23

Table 2: Physicochemical data of synthesized piperazine derivatives

Sr. No	Compound code	Rf value	Melting Point (°C)	Yield (%)
1	1	0.52	168-170	56.17 %
2	2	0.56	172-174	37.20 %
3	3	0.59	170-172	46.91 %
4	4	0.61	286-288	74.86 %

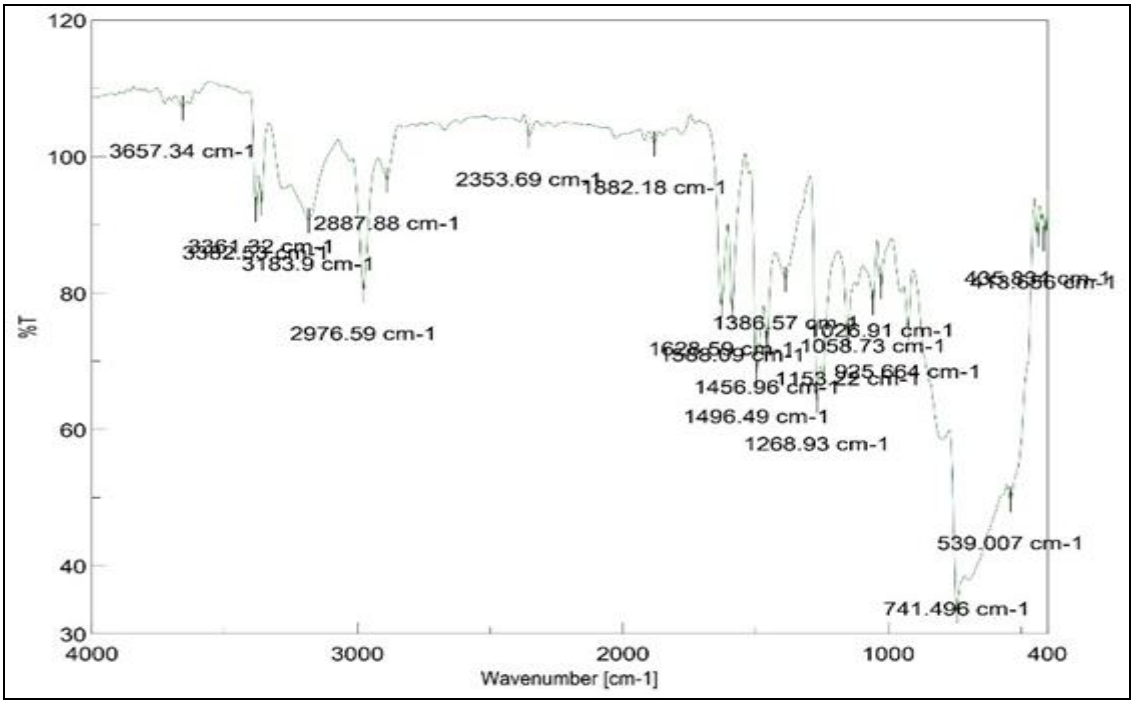

Figure 2: FTIR of Compound 1

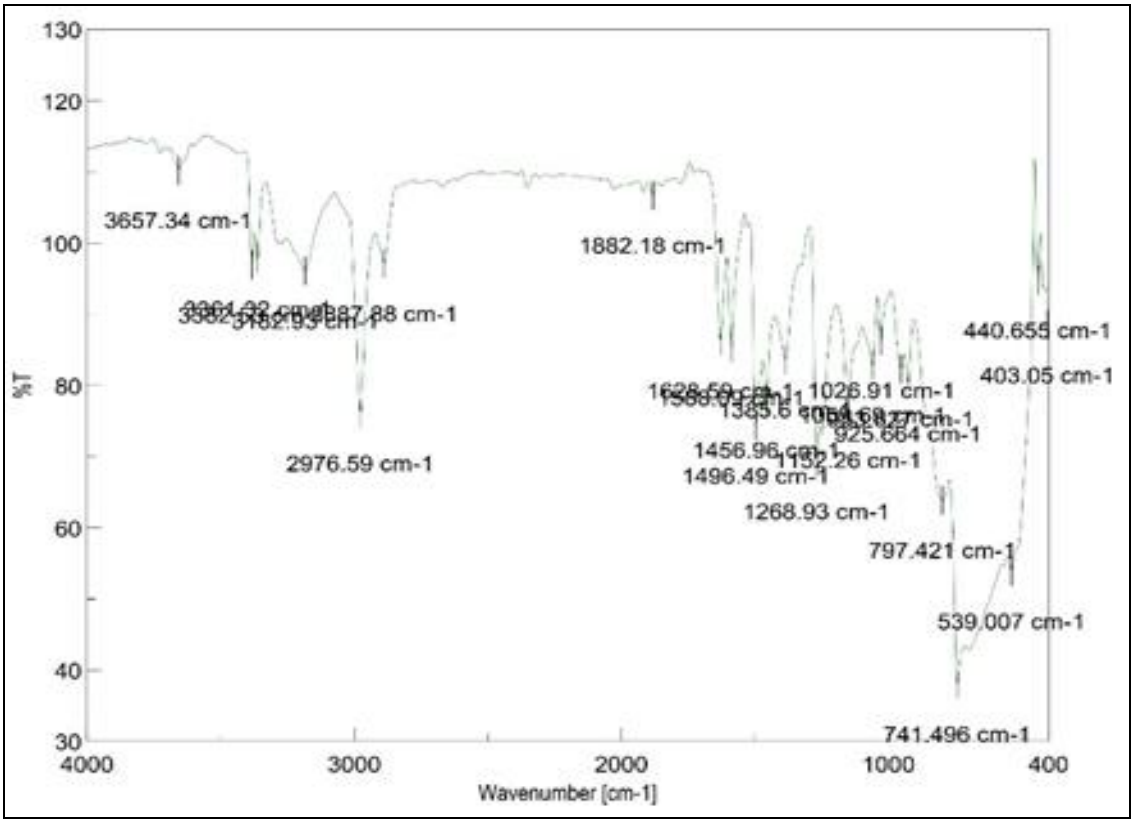

Figure 3: FTIR of Compound 3

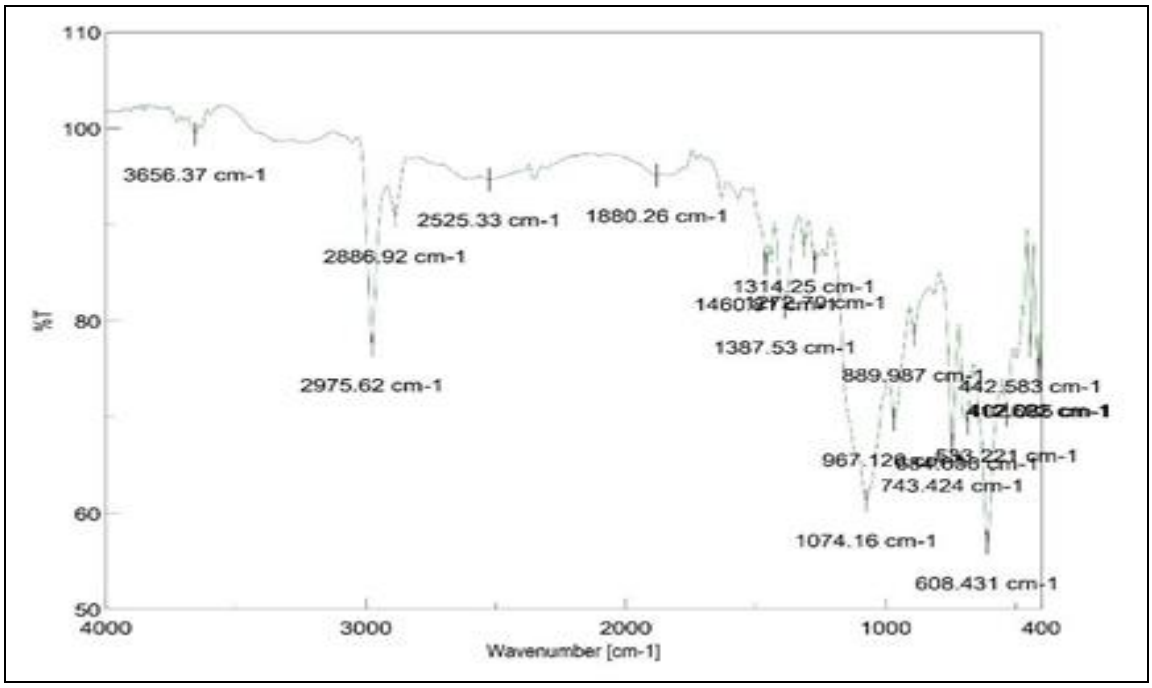

Figure 4: FTIR of Compound 4

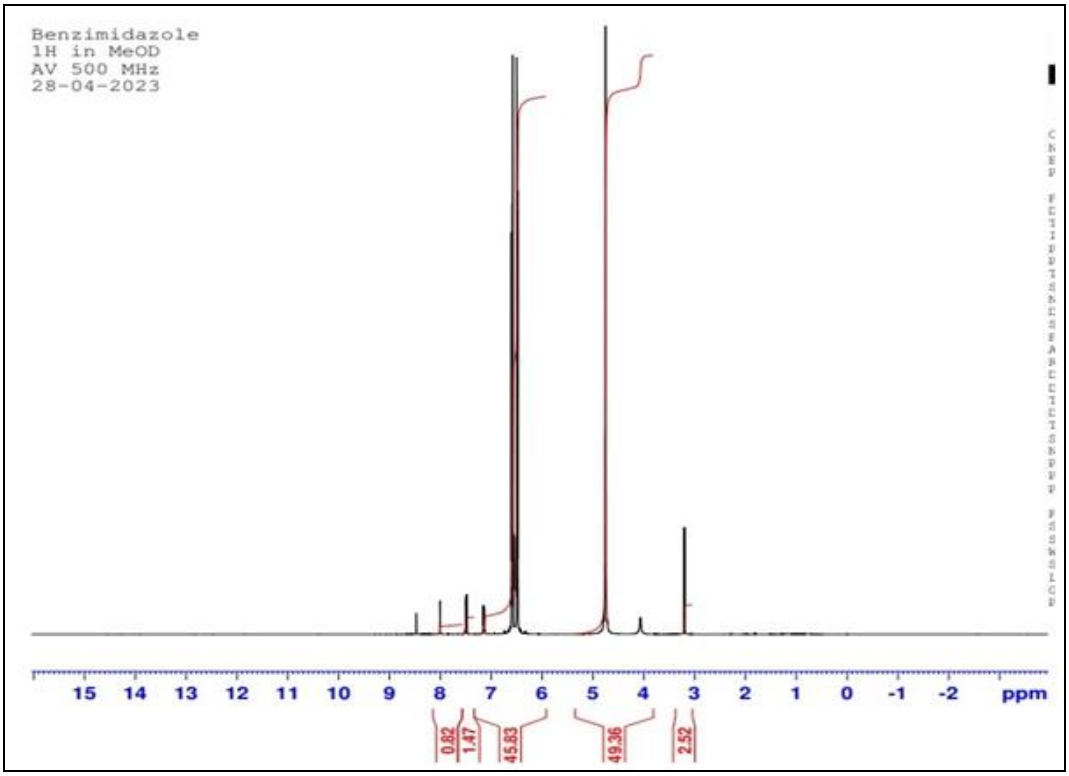

Figure 5: NMR spectra of Compound 1

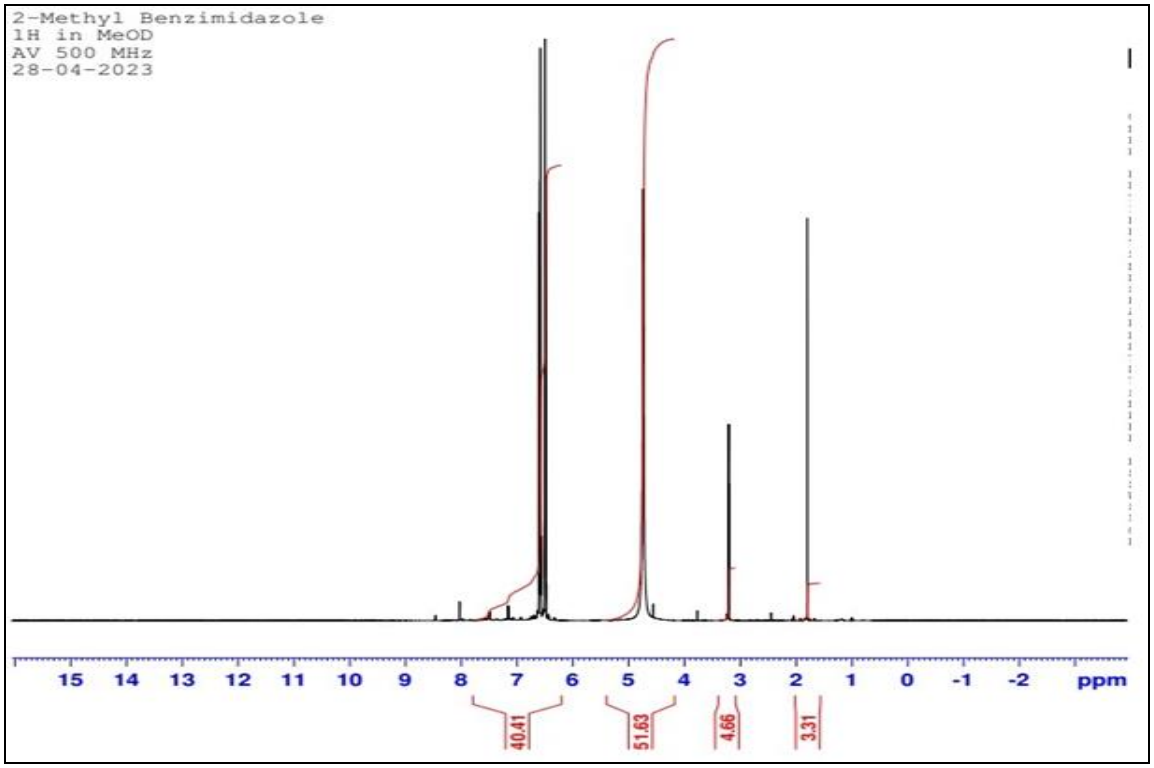

Figure 6: NMR spectra of Compound 2

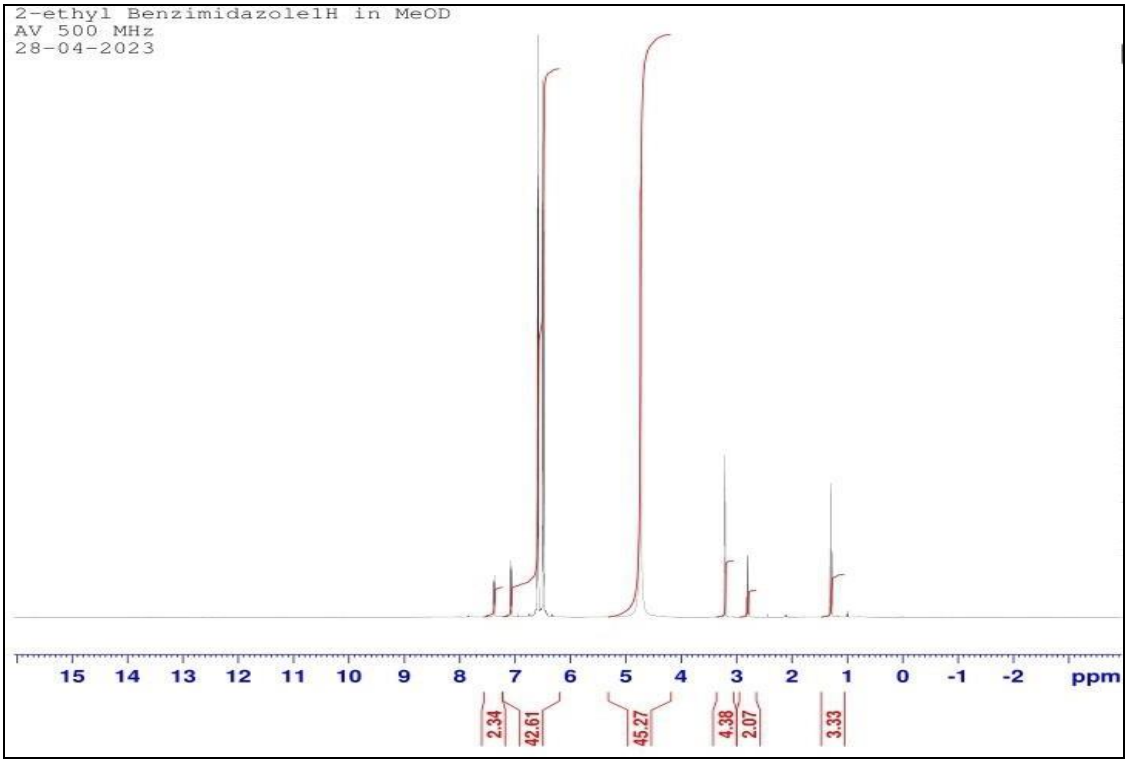

Figure 7: NMR spectra of Compound 3

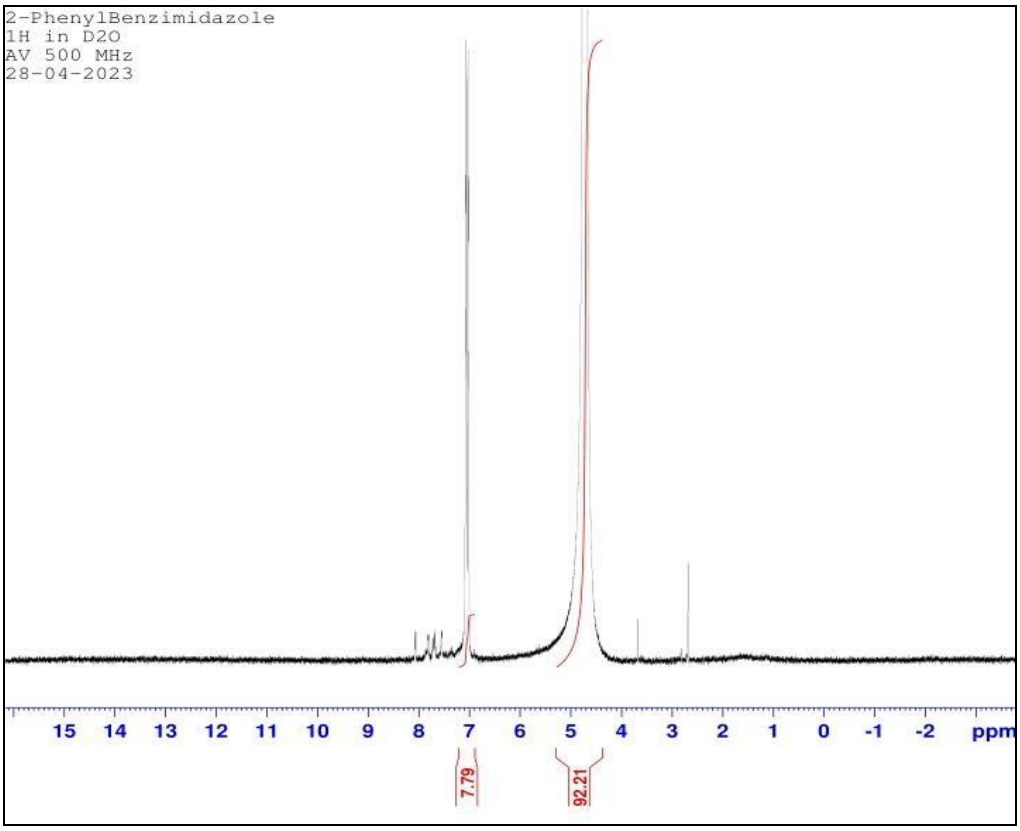

Figure 8: NMR spectra of Compound 4

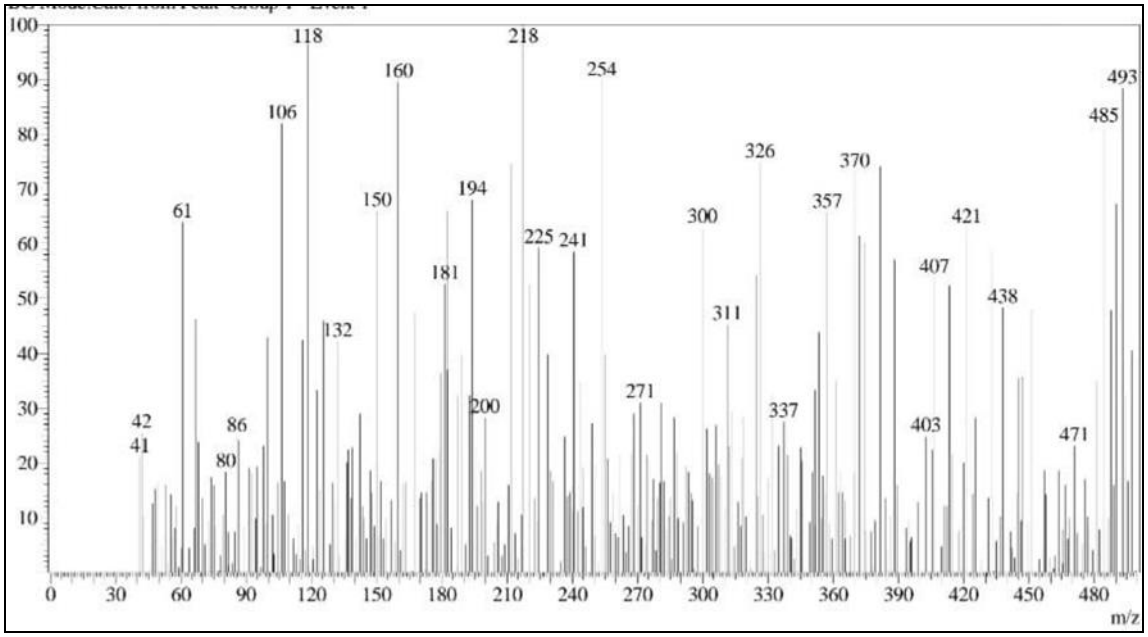

Figure 9: Mass spectra of Compound 1

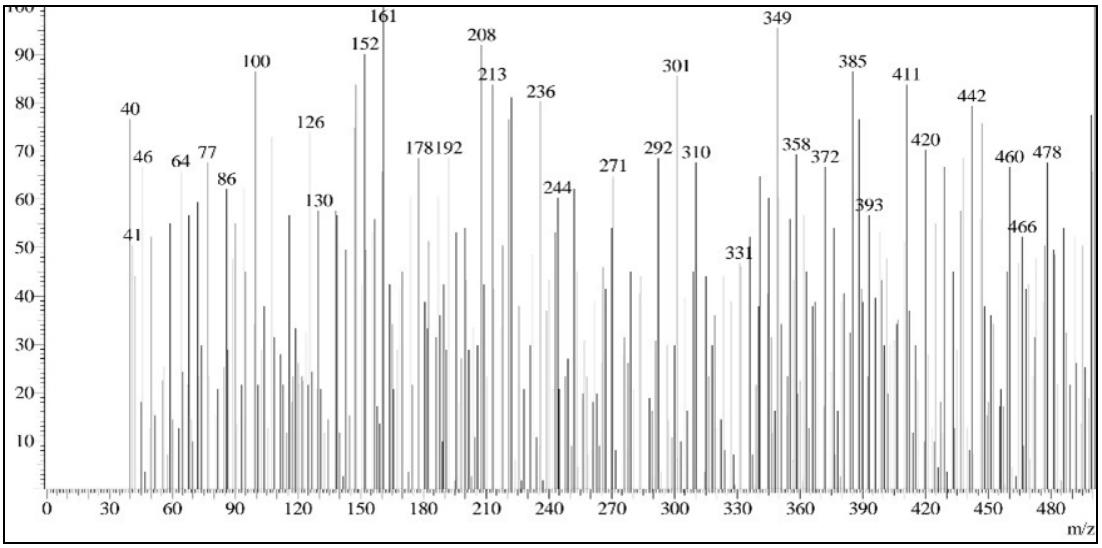

Figure 10: Mass spectra of Compound 2

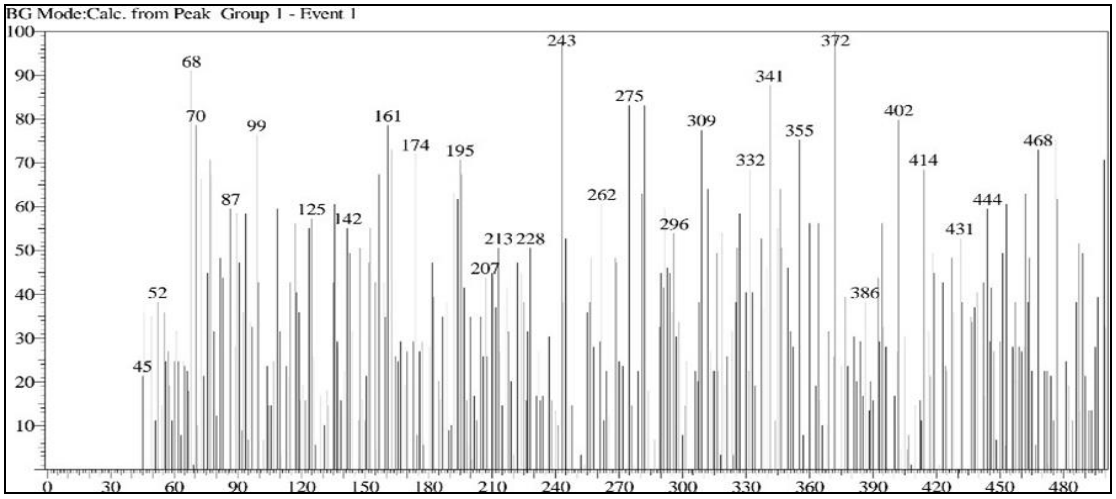

Figure 11: Mass spectra of Compound 3

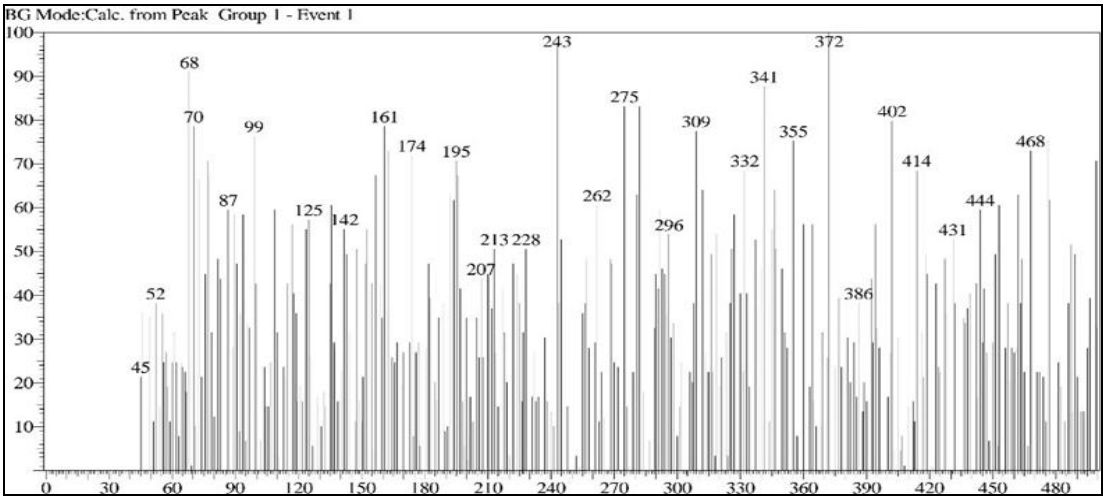

Figure 12: Mass spectra of Compound 4

The primary objective of this current study is to synthesize new variations of piperazine molecules that meet the specific structural prerequisites for unique biological activities. A strategic plan was devised to generate four original piperazine derivatives **[16]**. The synthesis procedure involved producing distinct piperazine derivatives by conducting a condensation reaction between commercially available orthophenylene-diamine and formic acid, utilizing NaOH (sodium hydroxide) as a base within a methanol solvent. The reaction was initiated through the interaction of o-phenylene diamine with formic acid, resulting in water removal and product formation. This process led to the formation of benzimidazole as a white solid crystal. To enhance purity, the compound underwent recrystallization using suitable solvents like methanol **[17]**. The synthesized compound's authentication was carried out through multiple techniques, including TLC (thin layer chromatography), melting point determination,

FTIR (Fourier Transform Infrared spectroscopy), NMR (Nuclear Magnetic Resonance), and Mass Spectroscopy as shown in Tables 1 and 2.

In the FTIR spectrum of compound number 1 (Figure 2), distinct vibrations were identified at 1496.49 cm^{-1} (indicative of aromatic C=C stretching), 2887.88 cm^{-1} (corresponding to C-H stretch), and 2976.59 cm^{-1} (related to C-H stretching). In the ^{1}H-NMR spectra (Figure 5), clearly resolved resonance peaks appeared within the range of 2.52-49.56, denoting Ar-H signals, and at 51.63-40.41, representing alkene signals. The molecular ion peak of compound number 1 (Figure 9) was detected at 118. Therefore, based on the analyses of FTIR, NMR, and MASS spectra, the legitimacy of compound 1 was verified, and a similar confirmation process can be applied to compounds 2, 3, and 4. In the FTIR of compound 2, distinct vibrations were detected at 1496.49 cm^{-1} (reflecting aromatic C=C stretching) and 2976.59 cm^{-1} (associated with C-H stretching). In the ^{1}H-NMR spectra (Figure 6), clearly, distinguished resonance peaks were visible within the range of 3.31-4.66, indicating R-CH signals, and at 51.63-41.40, representing Ar-CH signals. The molecular ion peak of compound 2 (Figure 10) was identified at 132.17. Therefore, relying on the analyses of IR, NMR, and MASS spectra, we have verified the legitimacy of compound 2.

In the FTIR spectrum of compound 3 (Figure 3), distinct vibrations were identified at 723.424 cm^{-1} (indicating C=C stretching), 1387.53 cm^{-1} (associated with NO_2 stretching), 2525.33 (related to C-H stretching), and 2975.62 cm^{-1} (reflecting C-H stretching). In the ^{1}H-NMR spectra (Figure 7), clearly, distinguishable resonance peaks were present within the range of 45.27-42.61, representing Al-CH signals, and at 3.33-2.07, corresponding to Ar-H signals. The molecular ion peak of compound 3 (Figure 11) was detected at 146.27. In the FTIR spectrum of compound 4 (Figure 4), distinct vibrations were observed at 797.421 cm^{-1}, indicating aromatic stretching. In the ^{1}H-NMR spectra (Figure 9), clearly distinguishable resonance peaks were visible within the range of 92.21-7.79, representing R-CH signals. The molecular ion peak of compound 4 (Figure 12) was identified at 194.23.

Anti-tubercular Activity

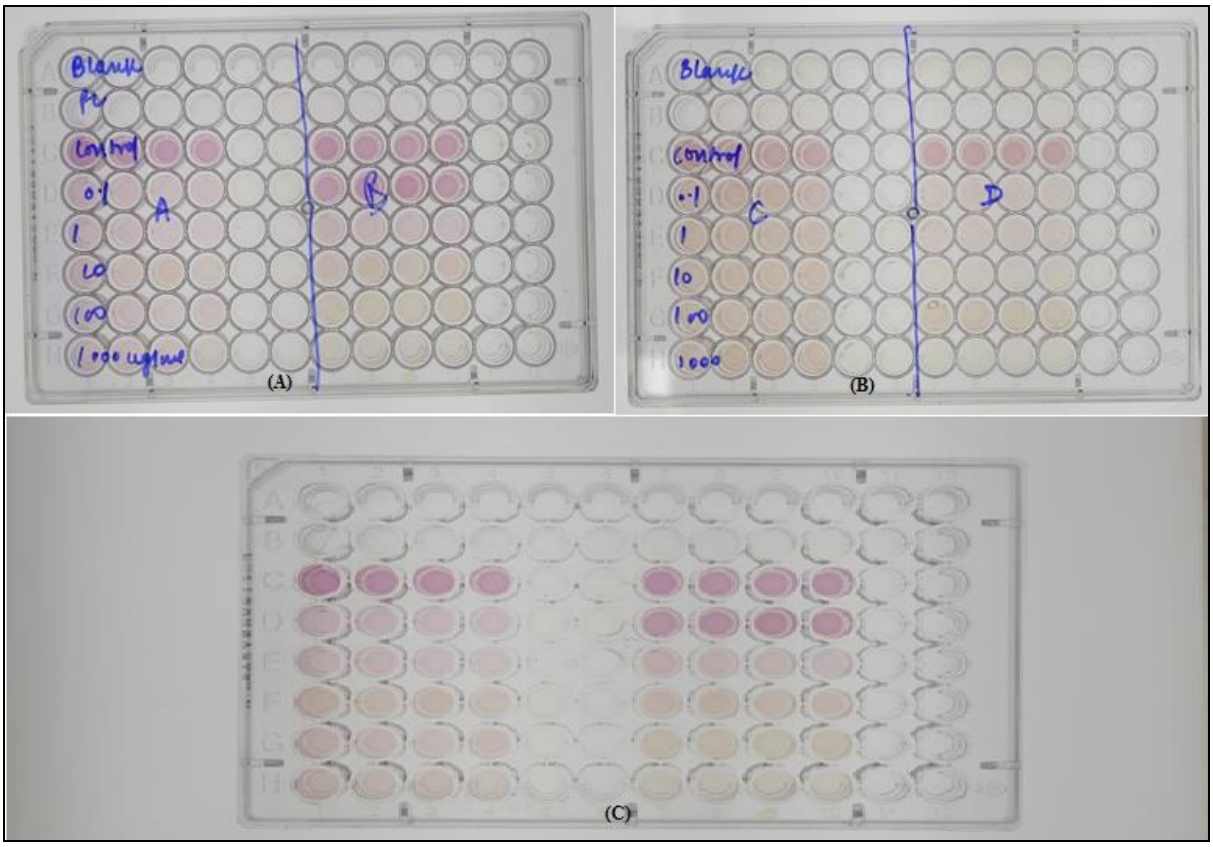

Figure 13: Anti-tubercular image of (A): Compounds 1 and 2, (B): Compounds 3 and 4, (C): standard drug Ciprofloxacin

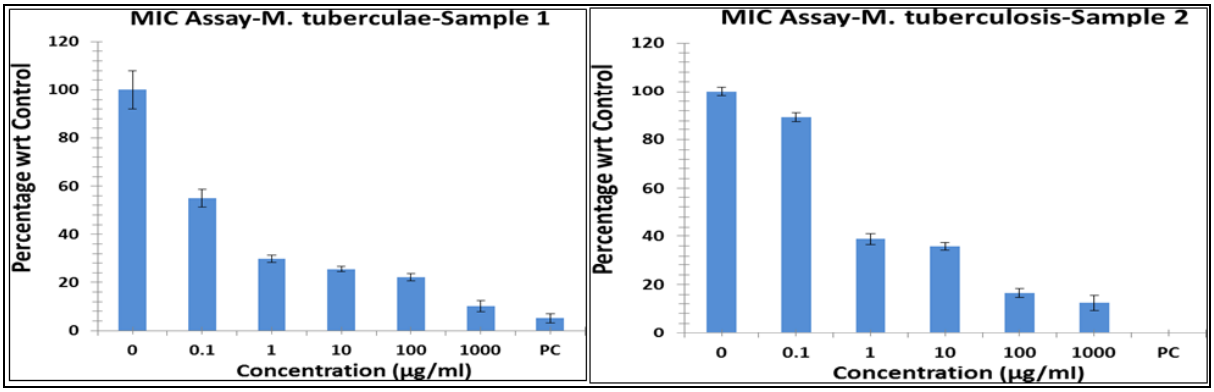

Figure 14: Anti-tubercular activity of Compound 1 and 2

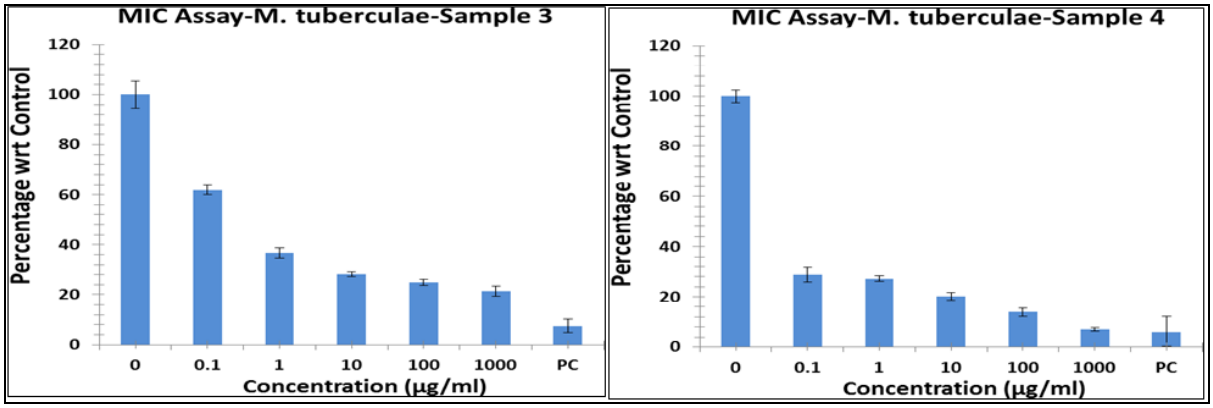

Figure 15: Anti-tubercular activity of Compounds 3 and 4

Results (Table 3, Figure 13-15) show that compounds displayed acceptable MIC values. It was encouraging to see that some compounds (1, 3, and 4) showed MIC values equivalent to or close to that of standard drugs. Compound 2 stands out as the most potent compound with a MIC of 0.1µg/ml [14, 15].

Table 3: Result of Anti-tubercular Activity of All Compounds

Sr. No.	Compounds	MIC (µg/ml) Value
1	1	Less than 0.1
2	2	0.1
3	3	0.1
4	4	0.05

4. CONCLUSION

A total of four compounds were synthesized through condensation under alkaline conditions. The resulting piperazine derivatives were refined through recrystallization, and all reaction processes during synthesis were tracked using Thin Layer Chromatography (TLC) with a solvent system of Ethyl acetate: Hexane, using varying proportions. Among the synthesized compounds, compound 1 exhibited the highest yield percentage (74.86%). The Rf values for all synthesized compounds ranged from 0.5 to 0.82. All compounds exhibited excellent solubility in both methanol and water. The melting points of the synthesized compounds ranged between 168°C and 288°C. Characterization of the synthesized piperazine derivatives was achieved through FTIR, 1H-NMR, and MASS spectroscopy. The anti-tubercular potential of the synthesized piperazine derivatives was assessed in vitro against M. Tuberculosis

through culture incubation. The outcomes indicate that certain synthesized piperazine derivatives possess notable anti-tuberculosis properties. Moreover, there exists an opportunity for further enhancing their structure to achieve compounds that are even more potent and safe.

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