

Molecular Docking, Synthesis, Characterization of Novel Pyridine/Piperidine N-Mannich Base Derivatives and their Bactericidal Studies

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ABSTRACT

OBJECTIVE This study aims to synthesize and estimate the anti-bacterial, anti-fungal and anti-tubercular activity of novel N-pyridine/piperidine Mannich base derivatives (M1-M6).

METHODS The novel N-pyridine/piperidine Mannich base derivatives were prepared through condensation reactions between pyridine/piperidine, furfuraldehyde and various aromatic/ aliphatic amines. The structures of the synthesised compounds were characterized using FTIR, ¹³C-NMR, ¹H-NMR, and mass spectroscopy. These derivatives were assessed for anti- bacterial, anti-fungal and anti-tubercular activity using in vitro methods such as paper disc diffusion and agar well diffusion methods.

RESULTS The antimicrobial and antitubercular activities of the Schiff base Mannich derivatives (M1-M6) were systematically evaluated. Antimicrobial screening against bacterial strains (*S. aureus*, *E. coli*, *S. epidermidis*, and *P. aeruginosa*) and fungal strains (*Aspergillus fumigatus* and *A. niger*) demonstrated clear concentration-dependent activity. At a concentration of 100 µg/disc, compound M2 exhibited notable inhibition zones of 10.25 mm (*S. aureus*), 9.76 mm (*E. coli*), and 10.43 mm (*A. fumigatus*), while M4 showed comparable activity with 10.03 mm, 8.56 mm, and 9.87 mm, respectively. In contrast, M3 displayed the lowest activity, especially against *P. aeruginosa* and fungal strains, whereas M5 and M6 showed moderate effects. Standard drugs, ciprofloxacin and fluconazole, exhibited superior potency. In antitubercular assays, M2 and M4 showed dose-dependent inhibition of *mycobacterium*, with M2 demonstrating higher efficacy. Docking studies further supported these findings, as M2 and M4 showed strong binding affinities toward InhA and EthR proteins, correlating well with their biological activity.

CONCLUSION These findings emphasize the therapeutic promise of Mannich base derivatives, particularly piperidine-containing compounds (M2, M4), as potential antimicrobial and antitubercular agents. Their higher basicity enhances molecular interactions with microbial targets. Further structural optimization of these scaffolds could improve potency, selectivity, and effectiveness against resistant bacteria, fungi, and *Mycobacterium tuberculosis*.

KEYWORDS pyridine, piperidine, Mannich base, in vitro anti-microbial activity, in vitro anti-tubercular activity

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INTRODUCTION

Pyridine derivatives have been extensively studied for their potential diverse biological activities (1). Novel pyridine derivatives are being designed and synthesized to combat microbial infections, particularly those caused by multi-drug-resistant bacteria (2). They have emerged as promising anti-microbial agents due to their versatile chemical structure and ability to interact with various biological targets (3). The nitrogen atom in the pyridine ring enhances binding affinity with microbial enzymes and proteins, contributing to their potent anti-bacterial and anti-fungal activities (4). In addition to that, pyridine derivatives have shown promise as potential anti-tubercular agents, exhibiting inhibitory activity against *Mycobacterium tuberculosis* enzymes and pathways (5). Tuberculosis (TB) is a bacterial illness caused by *Mycobacterium tuberculosis*, mainly targeting the lungs (6, 7). It remains a major global health issue, resulting in millions of new infections and deaths annually (8). TB is transmitted through airborne droplets released by an infected individual (9). Common symptoms include a constant fever, cough and unintentional loss of body weight (10). Without proper treatment, the disease can be fatal. While TB is curable with antibiotics, the rise of drug-resistant strains creates a significant challenge (11). Efforts to combat TB include developing new treatments, improving diagnosis, and increasing access to healthcare, particularly in high-burden countries (12). Early detection and treatment are crucial (13). The development of novel anti-tubercular agents is essential to combat this disease (14). Pyridine derivatives can disrupt essential cellular processes such as DNA replication, protein synthesis, and cell wall formation (15, 16). Pyridine derivatives have garnered attention for their potential therapeutic applications, including anti-tubercular activity (17). Structural modifications of the pyridine ring have led to the development of compounds with broad-spectrum anti-microbial activity, including efficacy against resistant strains (18). Recent studies have highlighted the promise of pyridine-based compounds as anti-microbial and anti-tubercular agents, demonstrating their potential to target specific bacterial enzymes and pathways (19, 20). As a result of a continuous search for the above-mentioned area, we synthe-

sized and evaluated novel N-Mannich base derivatives as potential anti-bacterial, anti-fungal and anti-tubercular agents. The results reveal that the biological activities of N-Mannich derivatives are similar to that of standard drugs.

METHODS

Chemicals and reagents

The chemicals employed in this study were purchased from reputable pharmaceutical companies, including Sigma, Aldrich, Ranbaxy, and Dr. Reddy's, and were of laboratory reagent grades. The synthesized Mannich base derivatives were characterized using various analytical techniques. A Perkin Elmer FT-IR spectrophotometer (spectrum RX1) was employed to identify functional groups while examining from 4000 cm^{-1} to 400 cm^{-1} . A Bruker 400 MHz spectrometer (Bruker Avance DRX 400) was employed to determine the environmental protons, using deuterated chloroform as a solvent. A Shimadzu mass spectrometer (Q-TOF) was used to determine the molecular weight of the compounds. A Perkin Elmer analyzer (2400 CHNS) was used to identify the atoms present in the synthesized Mannich base derivatives (21). All the instruments were in proper working condition during the study.

Molecular docking

CB-Dock2 is a user-friendly molecular docking tool used to predict the binding interaction between ligands and bacterial enzymes such as *Mycobacterium tuberculosis* InhA/ EthR regulatory proteins. It automatically identifies potential binding sites on the enzyme and docks the ligand without requiring prior knowledge of the active site. The most stable 3D structure of each drug was selected and docked with the target enzyme. Binding affinity assessed using a docking score, with lower values indicating stronger interactions. This helps evaluate and compare the potency of different drug candidates (22).

Preparation of pyridine mannich base derivatives (M1, M3, M5)

A mixture of 0.79 g of pyridine (0.01 mole), 1.69 g of diphenyl amine (0.01 mole)/diethyl amine (0.01 mole)/ethyl methyl amine (0.01 mole) and 0.30 mL of furfuraldehyde was placed in 30 mL of ethyl alcohol. The obtained solution was blended

was refluxed for five hours, after which the warm solution was allowed to cool and then poured into ice water. Precipitated mass was filtered and crystallized from ethyl alcohol (Figure 1).

Preparation of piperidine mannich base derivatives (M2, M4-M6)

A mixture of 0.85 g of piperidine (0.01 mol), 1.69 g of diphenyl amine (0.01 mole)/0.59 g of diethyl amine (0.01 mole)/ethyl methyl amine (0.01 mole) and 0.30 mL of furfuraldehyde was placed in 30 mL of ethyl alcohol. The resulting solution was mixed thoroughly and refluxed for five hours. It was then allowed to cool and poured into cold water. The precipitated product was collected by filtration and recrystallized from ethyl alcohol (23, 24). (Figure 2).

Biological activities

Anti-bacterial activity:

The anti-microbial effects of Mannich base derivatives were estimated by using the paper

disc diffusion method. Anti-microbial efficacy was estimated by measuring the zones of inhibition on Mueller–Hinton agar (anti-bacterial study) and Sabouraud dextrose agar (anti-fungal study). Sterilized culture media filled into plates of 4 mm width, allowed to harden, and then inoculated with various microorganisms: *S. epidermidis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *A. fumigatus*, and *A. niger*. Mannich base derivatives were prepared in dimethylformamide at concentrations of 50 µg and 100 µg. Paper discs soaked in these solutions were placed on the inoculated media and incubated at 37°C for 24 and 48 hours for bacterial and fungal organisms, respectively. Ciprofloxacin (100 µg/disc) and fluconazole (100 µg/disc) served as standard references for anti-bacterial and anti-fungal activity, respectively. A solvent control (dimethylformamide alone) was also included. Antimicrobial activity was assessed by measuring the zones of inhibition produced by the Mannich base derivatives and comparing them with those produced by the corresponding standard drugs (25).

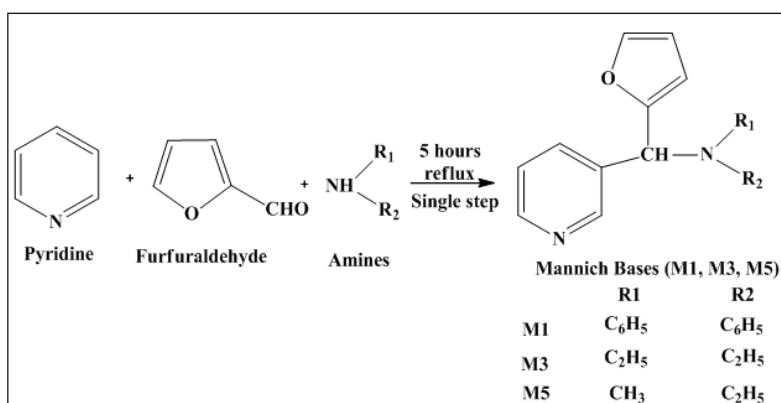


Figure 1. Synthetic scheme of Mannich base derivatives (M1, M3, M5)

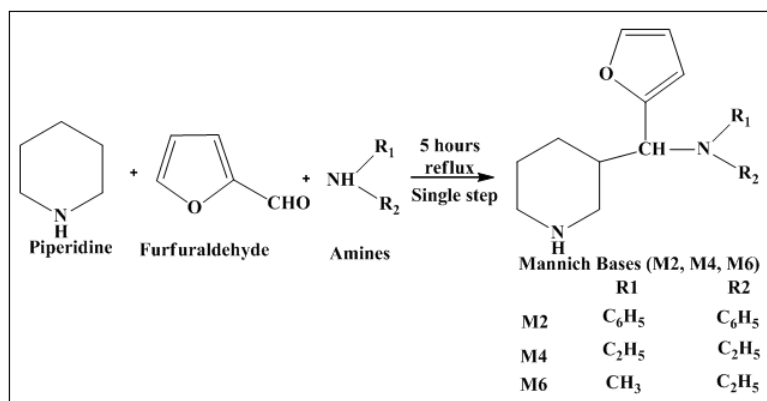


Figure 2. Synthetic scheme of Mannich base derivatives (M2, M4, M6)

In vitro anti-tubercular activity

Preparation of Lowenstein-Jensen medium: 37.24 g of LJ medium base was dissolved in 600 mL of distilled water containing 12 mL of glycerol, with gentle heating applied if necessary to ensure complete dissolution. The solution was then sterilized by autoclaving at 121°C under 15 lb pressure for 15 minutes. Meanwhile, 1,000 mL of whole egg emulsion was prepared under aseptic conditions. After the base medium cooled slightly, the egg emulsion was aseptically added and mixed thoroughly. The combined medium was then sterilized by autoclaving at 85°C for 45 minutes. After sterilization, the medium was inoculated with a culture of *Mycobacterium tuberculosis* obtained from Rontgen's Medical Centre, Thanjavur, Tamil Nadu. The inoculated LJ medium was incubated at 37°C for seven days to facilitate bacterial growth (26).

Agar well diffusion technique

The plates filled with Mueller-Hinton Agar (MHA) were inoculated with a *Mycobacterium tuberculosis* culture adjusted to a 0.5 McFarland standard. Bore wells of 6 mm diameter were produced in the medium using a sterile cork borer. Different concentrations of Mannich base derivatives (100, 500, and 1,000 µg/mL) were prepared for the assay. A volume of 0.3 mL of each concentration was carefully introduced into the wells and allowed to diffuse for 5–10 minutes at room temperature before incubation. The plates were then incubated at 37°C for 48 hours. Each concentration was tested in triplicate to ensure reproducibility and reduce experimental error. After incubation, the diameters of the zones of inhibition (IZD) were measured and recorded for further analysis. Rifampicin was used as the standard control (27).

RESULTS

Molecular docking

The binding affinities of six N-Mannich base derivatives (M1–M6) were evaluated against *Mycobacterium tuberculosis* InhA and EthR regulatory proteins (Table 1). The results indicate that all derivatives exhibited moderate to strong interactions with both protein targets. M2 showed the highest binding affinity, with –8.30 kcal/mol for InhA and –8.91 kcal/mol for EthR, suggesting its potential as the most effective inhibitor among

the tested compounds. M4 also demonstrated significant binding (–8.02 kcal/mol for InhA and –8.54 kcal/mol for EthR). These results highlight the influence of structural variations among the derivatives on their protein-binding capabilities, which may guide further optimization for anti-tuberculosis activity. Rifampicin exhibited the highest binding affinities toward both *M. tuberculosis* InhA (–8.92 kcal/mol) and EthR (–9.34 kcal/mol) proteins (Table 1 and Figure 3–4).

Chemistry

N-(furan-2-yl)(pyridin-3-yl) methyl-N-phenylaniline-(M1): Yield (%): 78; MP.: 293–295°C; Shade: off white; FTIR($\nu_{\max}$, cm^{-1}): 3408 (str., ArH), 1518 (str., ArC), 1596 (CN), 1774 (CO); ^1H NMR (CDCl_3 , δ ppm): 5.43 (s, 1H, CH-H), 6.45 (d, 1H, J = 10.2 Hz, ArH), 6.52–6.71 (m, 1H, ArH), 6.82–6.89 (m, 2H, ArH), 7.34–7.51 (m, 9H, ArH), 7.74–7.79 (m, 1H, ArH); 7.91–7.96 (m, 1H, ArH), 8.51 (d, 1H, J = 9.6 Hz, ArH), 8.64 (s, 1H, ArH); ^{13}C -NMR ($\text{DMSO}-d_6$, δ ppm): 80.6 (C-6), 107.5 (C-8), 112.1 (C-9), 119.3 (C-12,16, 18,22), 121.4 (C-14,20), 123.7 (C-4), 129.6 (C-13,15, 19,21), 132.2 (C-4), 134.2 (C-2), 142.3 (C-10), 147.5 (C-5), 149.6 (C-11,17), 150.9 (C-1), 153.4 (C-7); MS (m/z , %): 326 (42) [M^+], 259 (45), 168 (100), 78 (32), 53 (28), 40 (55), 26 (27); Anal. calcd. (found) % for $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}$ (M.W326): C, 80.96 (81.03); H, 5.56 (5.62); O, 4.90 (4.92); N, 8.58 (8.43).

N-(furan-2-yl) (piperidin-3-yl)methyl-N-phenylaniline (M2): Yield (%): 83; MP.: 301–303°C; Shade: Slight Yellow; FTIR: 3476 (str., Ar-proton), 1518 (str., Ar-C), 1594 (CN), 1772 (CO); ^1H NMR: 2.16 (s, 2H, NH-proton), 5.67 (s, 1H, CH-H), 5.75 (s, 1H, OH-proton), 6.31–6.59 (m, 1H, ArH), 6.76–6.89

Table 1. Binding affinity of N-Mannich base derivatives against *Mycobacterium tuberculosis* InhA/ EthR regulatory proteins

Compounds	<i>Mycobacterium tuberculosis</i> InhA protein (kcal/mol)	EthR regulatory protein (kcal/mol)
M1	-7.46	-7.52
M2	-8.30	-8.91
M3	-7.83	-8.10
M4	-8.02	-8.54
M5	-7.03	-7.22
M6	-7.59	-7.76
Rifampicin	-8.92	-9.34

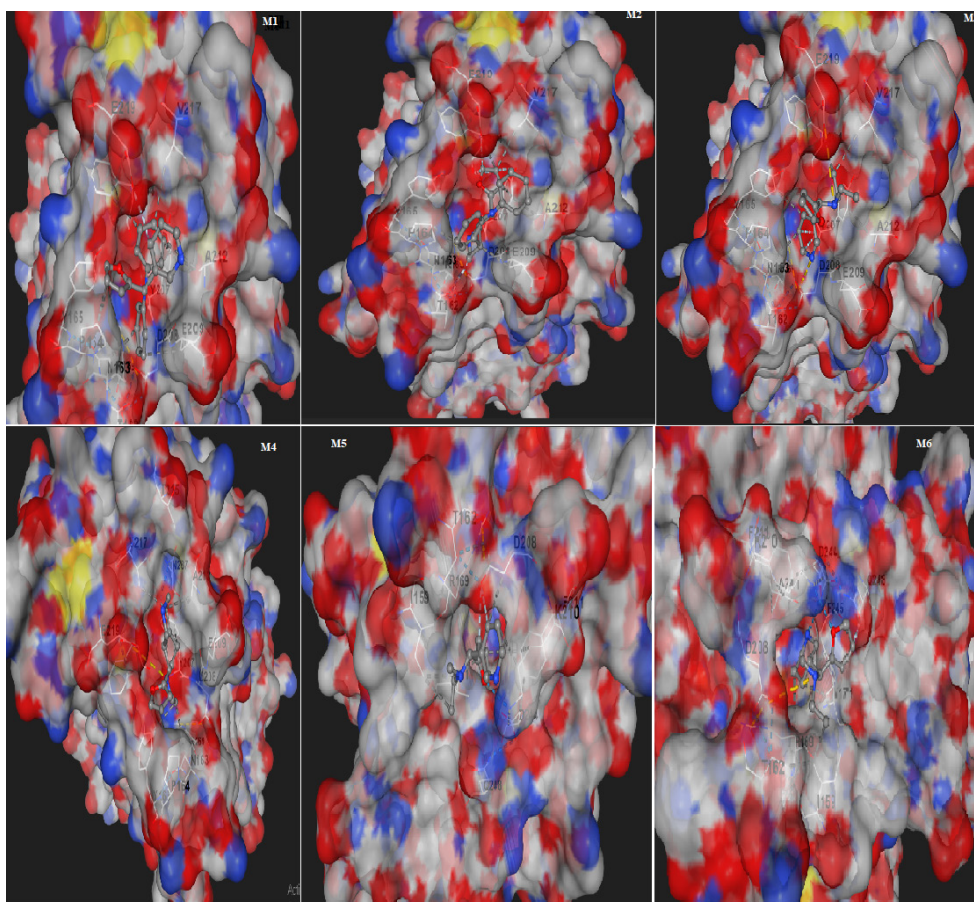


Figure 3. Docking images of Mannich base derivatives against *Mycobacterium tuberculosis* InhA complex

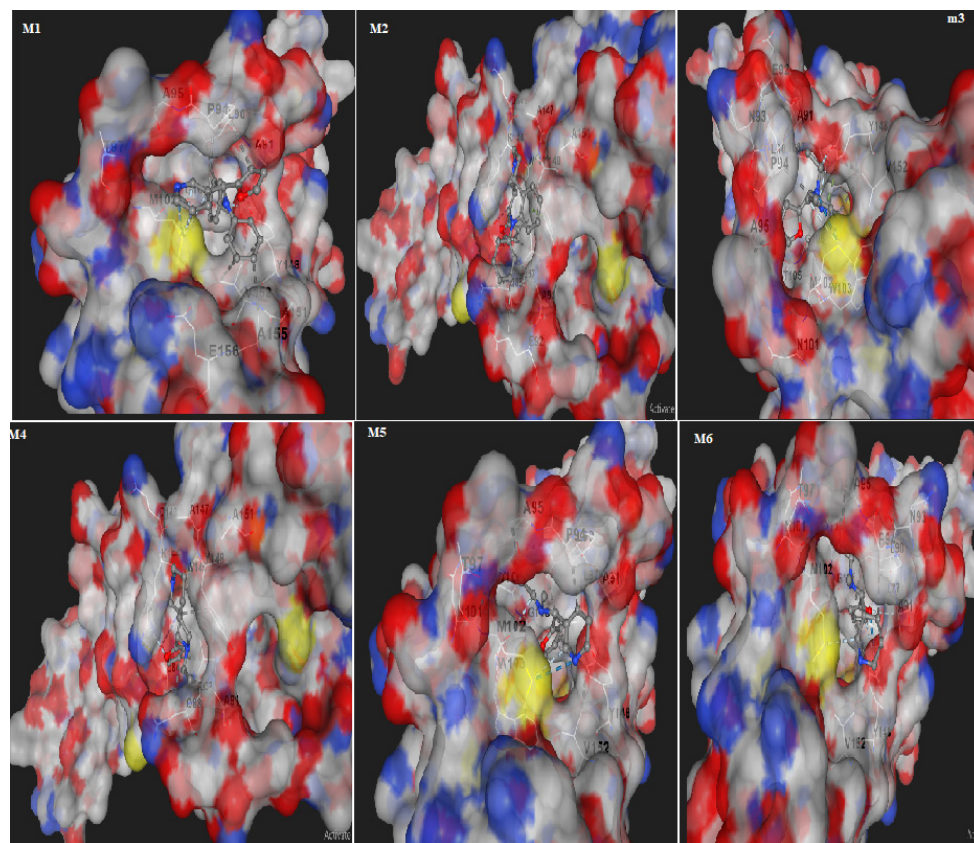


Figure 4. Docking images of Mannich base derivatives against *Mycobacterium tuberculosis* EthR complex

(m, 1H, Ar-proton), 6.82-7.05 (m, 2H, ArH), 7.14-7.39 (m, 1H, Ar-proton), 7.44-7.69 (m, 1H, Ar-proton), 7.72-7.84 (m, 1H, ArH), 8.64 (s, 2H, NH₂-H); ¹³C-NMR (DMSO-*d*₆, δ ppm): 24.3 (C-3), 25.8 (C-4), 34.6 (C-2), 46.7 (C-8), 48.2 (C-1), 73.9 (C-5), 108.4 (C-6), 111.2 (C-12), 119.2 (C-9,16, 18,22), 121.7 (C-14,20), 129.2 (C-13,15, 19,21), 141.7 (C-10), 149.1 (C-11,17), 150.7 (C-7); MS (*m/z*, %): 336 (46) [M⁺], 249 (100), 158(52), 91(48), 78 (26), 40 (37), 38 (25), 27 (28); Anal. calcd. (found) % for C₂₂H₂₄N₂O, (*m.w.* 332): C, 79.48 (79.15); H, 7.28 (7.53); O, 4.81 (4.76); N, 8.43 (8.57).

N-ethyl-*N*-(furan-2-yl(pyridin-3-yl)-methyl)ethanamine (M3): Yield (%): 87; MP.: 214-216 °C; Shade: light brown; FT-IR: 3452 (*str.*, Ar-H), 1521 (*str.*, ArC), 1586 (CN), 1656 (CO); ¹H NMR: 1.16 (m, 6H, CH₃-proton), 2.48 (d, 4H, *J* = 8.7 Hz, CH₂-H), 5.53 (s, 1H, CH-H), 6.35 (d, 1H, *J* = 9.6 Hz, ArH), 6.55-6.67 (m, 1H, Ar-proton), 7.49-7.59 (m, 1H, Ar-proton); 7.71-7.78 (m, 1H, ArH), 7.92-7.97 (m, 1H, ArH), 8.63-8.69 (m, 1H, Ar-proton), 8.72 (s, 1H, Ar-H); ¹³C-NMR: 13.5 (C-12,14), 46.5 (C-11,13), 72.8 (C-6), 106.3 (C-8), 110.5 (C-9), 123.7 (C-4), 132.5 (C-3), 134.3 (C-2), 142.8 (C-10), 147.3 (C-5), 150.6 (C-1), 152.4 (C-7); MS (*m/z*, %): 230 (37) [M⁺], 201 (65), 172 (100), 105 (38), 78 (42), 40 (29), 29 (22); Anal. calcd. (found) % for C₁₄H₁₈N₂O, (*m.w.* 230): C, 73.01 (73.14); H, 7.88 (7.79); O, 6.95 (6.75); N, 12.16 (12.32).

N-ethyl-*N*-(furan-2-yl(piperidin-3-yl)-methyl)ethanamine (M4): Yield (%): 69; MP.: 223-225°C; Shade: Off white; FTIR: 3438 (*str.*, ArH), 1535 (*str.*, ArC), 1572 (CN), 1614 (CO); ¹H NMR: 1.12-1.23 (m, 6H, CH₃-proton), 1.69-1.78 (m, 4H, CH₂-proton), 2.10-2.15 (m, 1H, NH-proton), 2.21-2.30 (m, 1H, CH-proton), 2.45-2.52 (m, 4H, CH₂-proton), 2.67-2.74 (m, 2H, CH₂-proton), 2.79-2.85 (m, 4H, CH₂-proton), 4.25 (d, 1H, CH-H), 6.62-6.73 (m, 2H, ArH), 7.74-7.81 (m, 1H, ArH); ¹³C-NMR: 13.8 (C-12,14), 24.2 (C-3), 25.5 (C-4), 35.3 (C-2), 46.3 (C-1), 47.8 (C-11,5), 48.3 (C-13), 68.1 (C-6), 108.7 (C-8), 110.4 (C-9), 141.3 (C-10), 150.2 (C-7); MS (*m/z*, %): 236 (54) [M⁺], 207 (52), 178 (100), 164 (45), 138 (32), 84 (27), 29 (22); Anal. calcd. (found) % for C₁₄H₂₄N₂O, (*m.w.* 236): C, 71.14 (72.02); H, 10.23 (9.92); O, 6.77 (6.89); N, 11.85 (11.17).

N-(furan-2-yl(pyridin-3-yl)-methyl)-*N*-methyl ethanamine (M5): Yield (%): 77; MP.: 207-209°C; Shade: colourless; FTIR:(3452 (*str.*, ArH), 1526 (*str.*, ArC), 1513 (CN), 1647 (CO); ¹H NMR: 1.08 (m, 3H, CH₃-proton), 2.35 (s, 3H, CH₃-proton), 2.53-2.64 (m, 2H, CH₂-H), 5.40 (s, 1H, CH-H), 6.39 (d, 1H,

J = 9.8 Hz, Ar-H), 6.43-6.51 (m, 1H, ArH), 7.26-7.38 (m, 1H, ArH); 7.68-7.75 (m, 1H, ArH), 7.90-7.98 (m, 1H, ArH), 8.43-8.51 (m, 1H, ArH), 8.69 (s, 1H, Ar-H); ¹³C-NMR: 12.5 (C-13), 37.9 (C-11), 49.4 (C-6), 75.6 (C-12), 106.3 (C-8), 110.1 (C-2), 123.5 (C-3), 132.1 (C-4), 134.4 (C-9), 142.8 (C-10), 147.3 (C-5), 150.2 (C-1), 152.7 (C-7); MS (*m/z*, %): 216 (38) [M⁺], 201 (37), 172 (100), 105 (42), 78 (18), 52 (56), 29 (34); Anal. calcd. (found) % for C₁₃H₁₆N₂O, (MW. 216): C, 72.19 (71.84); H, 7.46 (7.65); O, 7.40 (7.67); N, 12.95 (12.84).

N-(furan-2-yl(piperidin-3-yl)-methyl)-*N*-methyl ethanamine (M6): Yield (%): 79; MP.: 216-218 °C; Shade: colourless; FT-IR: 3429 (*str.*, ArH), 1540 (*str.*, ArC), 1518 (CN), 1635 (CO); ¹H NMR: 1.05 (t, 3H, CH₃-proton), 1.62-1.76 (m, 4H, CH₂-proton), 2.02-2.10 (m, 1H, NH-proton), 2.15-2.26 (m, 1H, CH-proton), 2.32 (s, 3H, CH₃-proton), 2.45-2.53 (m, 2H, CH₂-proton), 2.61-2.74 (m, 2H, CH₂-proton), 2.84-2.91 (m, 2H, CH₂-proton), 4.19 (d, 1H, CH-H), 6.51-6.58 (m, 2H, ArH), 7.48-7.55 (m, 1H, ArH); ¹³C-NMR: 12.8 (C-13), 24.6 (C-3), 25.5 (C-4), 35.8 (C-2), 41.7 (C-11), 46.2 (C-1), 48.7 (C-5), 49.2 (C-10), 71.8 (C-8), 106.8 (C-6), 110.4 (C-9), 141.6 (C-6), 150.2 (C-12); MS (*m/z*, %): 222 (48) [M⁺], 207 (37), 178 (100), 111 (32), 84 (52), 56 (32), 28 (38); Anal. calcd. (found) % for C₁₃H₂₂N₂O, (*m.w.* 222): C, 70.23 (69.24); H, 9.97 (10.56); O, 7.20 (7.89); N, 12.60 (12.31).

Anti-microbial activity

The anti-microbial evaluation of Schiff base derivatives (M1-M6) was conducted against a range of bacterial (*S. aureus*, *E. Coli*, *S. epidermidis* and *P. aeruginosa*,) and fungal (*A. fumigatus* and *Aspergillus niger*) strains. The study revealed a concentration-dependent increase in activity for all tested compounds, with larger zones of inhibition observed at 100 µg compared to 50 µg. Among the tested compounds, M2 and M4 consistently exhibited the highest anti-microbial activity across both bacterial and fungal strains. At 100 µg, M2 demonstrated strong inhibition zones, particularly against *E. coli* (9.76 mm), *S. aureus* (10.25 mm), and *A. fumigatus* (10.43 mm), approaching the efficacy of standard drugs. Similarly, M4 showed comparable inhibition, with notable activity against *E. coli* (8.56 mm), *S. aureus* (10.03 mm), and *A. fumigatus* (9.87 mm). In contrast, M3 exhibited the weakest activity, especially against *P. aeruginosa* and fungal strains, with

Table 2. Zone of inhibition of Mannich base derivatives (M1-M6) against different microorganism (mm)

Compounds	<i>S. aureus</i>		<i>S. epidermidis</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>A. niger</i>		<i>A. fumigatus</i>	
	50	100	50	100	50	100	50	100	50	100	50	100
M1 (µg/disc)	7.94	9.57	7.23	8.98	8.98	8.45	5.23	8.03	5.98	8.45	5.76	9.54
M2 (µg/disc)	8.79	10.25	7.42	9.56	9.56	9.76	5.68	8.43	6.34	9.76	6.89	10.43
M3 (µg/disc)	6.82	8.63	6.03	8.11	8.11	7.90	4.23	7.41	4.90	7.90	4.34	7.65
M4 (µg/disc)	8.61	10.03	7.30	9.30	9.30	8.56	5.59	8.35	6.21	8.56	6.56	9.87
M5 (µg/disc)	7.50	9.23	6.56	8.76	8.76	8.17	4.13	7.56	5.35	8.17	5.28	8.82
M6 (µg/disc)	7.65	9.42	6.97	8.91	8.91	8.31	4.89	7.87	5.72	8.31	5.63	9.23
Ciprofloxacin (100 µg/disc)	13.85		10.30		9.60		10.35		-			
Fluconazole (100 µg/disc)	-		-		-		-		10.65		10.90	

zones of inhibition significantly lower than those of the reference antibiotics. Compounds M5 and M6 demonstrated moderate anti-microbial activity, with improved performance at higher concentrations but not reaching the levels of M2 and M4. Standard antibiotics Ciprofloxacin and Fluconazole served as positive controls. Ciprofloxacin showed superior anti-bacterial activity with zones ranging from 9.60 to 13.85 mm, indicating higher potency than the Mannich base derivatives at both test concentrations. Fluconazole showed strong anti-fungal activity against *A. fumigatus* (10.90 mm) and *A. niger* (10.65 mm), again outperforming the test compounds. Overall, the data suggest that compounds M2 and M4 possess promising broad-spectrum anti-microbial properties, particularly at higher concentrations. Their activity, while not exceeding that of standard drugs, highlights their potential as scaffolds for further structural optimization and development into more potent anti-microbial agents (Table 2).

Anti-tubercular activity

The compounds M2 and M4 exhibited the highest anti-tubercular activity in the in vitro study. The compounds M2 and M4 showed a significant zone of inhibition on *Mycobacterium* at different concentrations, as shown in Table 3. Piperidine nucleus-based Mannich base derivatives demonstrated greater anti-tubercular activity than pyridine nucleus-based Mannich base derivatives. The anti-TB activity was directly proportional to the concentration. The highest concentration (1,000 µg/mL) of piperidine activities nearest to the standard. M2 Mannich base de-

rivative was more potent than M4 in all the concentrations, with larger inhibition zones ranging from 5.10 mm to 10.35 mm, while M4 ranged from 4.55 mm to 9.20 mm. Rifampicin produced the largest inhibition (12.90 mm), indicating higher potency.

DISCUSSION

The novel Mannich base derivatives (M1-M6) successfully synthesised through a reflux method using ethyl alcohol as the solvent. The compounds were characterized using ¹HNMR, ¹³CNMR, FTIR and mass spectrometry. The FTIR spectra of the compounds demonstrated characteristic absorption bands for aromatic C-H stretching, CN stretching, and CO stretching vibrations. The ¹H NMR spectra revealed the existence of aromatic protons, CH-protons, and NH-protons, while the ¹³CNMR spectra confirmed the presence of aromatic carbons, CH-carbons, and C = O carbons (22). The bactericidal activity of M1-M6 was evaluated

Table 3. In vitro anti-tubercular activity against *Mycobacterium tuberculosis*

Compounds	Inhibition zone diameters (mm)		
	100 µg/mL	500 µg/mL	1,000 µg/mL
M1	3.41	6.53	7.03
M2	5.10	9.80	10.35
M3	3.26	6.02	6.55
M4	4.55	7.30	9.20
M5	3.24	5.93	6.17
M6	3.92	7.14	8.38
Rifampicin (30 µg/mL)	12.90		

against various microorganisms, including bacteria (*S. aureus*, *E. coli*, *P. Aeruginosa*, *S. epidermidis*) and fungi (*A. fumigatus* and *A. niger*). The zone of inhibition (mm) was measured at two concentrations (50 µg and 100 µg) (22). All compounds showed increased activity at the higher concentration (100 µg). The microbicidal evaluation discovered that derivatives M2 and M4 exhibited the highest activity against both bacterial and fungal strains. M2 showed strong inhibition zones against *S. aureus* (10.25 mm), *E. coli* (9.76 mm), and *A. fumigatus* (10.43 mm), while M4 demonstrated comparable inhibition. These findings suggest that the piperidine nucleus in M2 and M4 contributes to their enhanced anti-microbial activity (19, 22). Ciprofloxacin exhibited superior bactericidal activity, with zones of inhibition ranging from 9.60 to 13.85 mm. Fluconazole exhibited strong anti-fungal activity, with zones of inhibition of 10.90 mm and 10.65 mm against *A. fumigatus* and *A. niger*, respectively. M2 and M4 showed significant activity, with zones of inhibition comparable to standard drugs (19, 22). In addition to that, the Mannich base derivatives M1-M6 exhibited significant anti-tubercular activity against *Mycobacterium tuberculosis*. The study revealed piperidine-containing Mannich base derivatives (M2, M4, M6) showed higher anti-TB activity compared to pyridine-containing derivatives (M1, M3 and M5). The anti-tubercular activity assessment showed that M2 and M4 exhibited significant activity against *Mycobacterium tuberculosis*. M2 displayed higher potency, with inhibition zones ranging from 5.10 mm to 10.35 mm, while M4 ranged from 4.55 mm to 9.20 mm. The standard drug, Rifampicin, showed higher potency than both compounds. The observed antitubercular activity is consistent with previous studies demonstrating that nitrogen-containing heterocyclic scaffolds, including pyridine-containing compounds, can serve as promising frameworks for the development of antitubercular agents (5, 19). The study suggests that the piperidine nucleus in Mannich base derivatives enhances their anti-microbial and anti-tubercular activity. The existence of specific functional groups, such as the furan-2-yl group, may also contribute to the observed activity. The synthesised Mannich base derivatives, particularly M2 and M4, showed promising anti-microbial and anti-tubercular activity. The binding

affinities of the N-Mannich base derivatives (Table 1) were compared with their in vitro antitubercular activity against various microorganisms (Table 3). Compounds exhibiting stronger binding affinities, particularly M2 (InhA: -8.30 kcal/mol; EthR: -8.91 kcal/mol), showed higher zones of inhibition against *M. tuberculosis*, especially at 1000 µg concentrations. Similarly, M4 demonstrated both favorable docking scores and notable anti-tubercular activity. Conversely, derivatives with weaker binding affinities, such as M5 and M3, displayed comparatively lower inhibitory zones. Molecular docking has been widely used to investigate the predicted interactions and binding modes of heterocyclic antimicrobial compounds with potential biological targets (19, 22). This correlation indicates that the computational docking results align well with experimental anti-tubercular activity, validating docking as a predictive tool for assessing the potential efficacy of N-Mannich base derivatives against *M. tuberculosis*. It also highlights M2 as the most promising compound for further optimization. These findings provide valuable insights into the development of novel anti-bacterial, anti-fungal and anti-TB therapies. Further structural optimization and development of these compounds may lead to more potent agents against microbial infections.

CONCLUSIONS

The novel Mannich base derivatives (M1-M6) were successfully synthesized via a reflux method and thoroughly characterized using FTIR, ¹H NMR, ¹³C NMR, and mass spectrometry. Anti-microbial evaluation revealed that all compounds exhibited enhanced activity at higher concentrations, with M2 and M4 demonstrating the most potent bactericidal and fungicidal effects. Notably, M2 showed strong inhibition against *S. aureus*, *E. coli*, and *A. fumigatus*, suggesting a crucial role of the piperidine nucleus in biological activity. Anti-tubercular studies further confirmed the superior efficacy of piperidine-containing derivatives (M2, M4, M6) compared to pyridine analogues, with M2 showing the highest potency against *Mycobacterium tuberculosis*. These findings highlight the therapeutic potential of Mannich base derivatives, especially those containing the piperidine moiety, as promising candidates for future anti-microbial and anti-TB drug development.

Structural optimization of the piperidine-based scaffolds (M2, M4) may enhance potency and selectivity against resistant bacterial and fungal strains, as well as *Mycobacterium tuberculosis*. In vivo evaluations, pharmacokinetic profiling, and eventually clinical trials, are essential to validate the therapeutic potential of these Mannich base derivatives. This highlights the translational relevance of the work and underscores their promise as leads for future anti-microbial and anti-tubercular drug development.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

C.G.: conceptualization, methodology, investigation, data curation, data analysis, writing – original draft; M.D.: validation, data curation, visualization; V.M.: writing – original draft; P.C.: methodology and investigation; L.K.: writing – review & editing.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human or animals.

INFORMED CONSENT STATEMENT

Not applicable. This study did not involve human or animals.

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