Synthesis, Characterization and Biological Studies of Pyridine-Based Schiff Bases as Potential Antimicrobial and Antitubercular Agents

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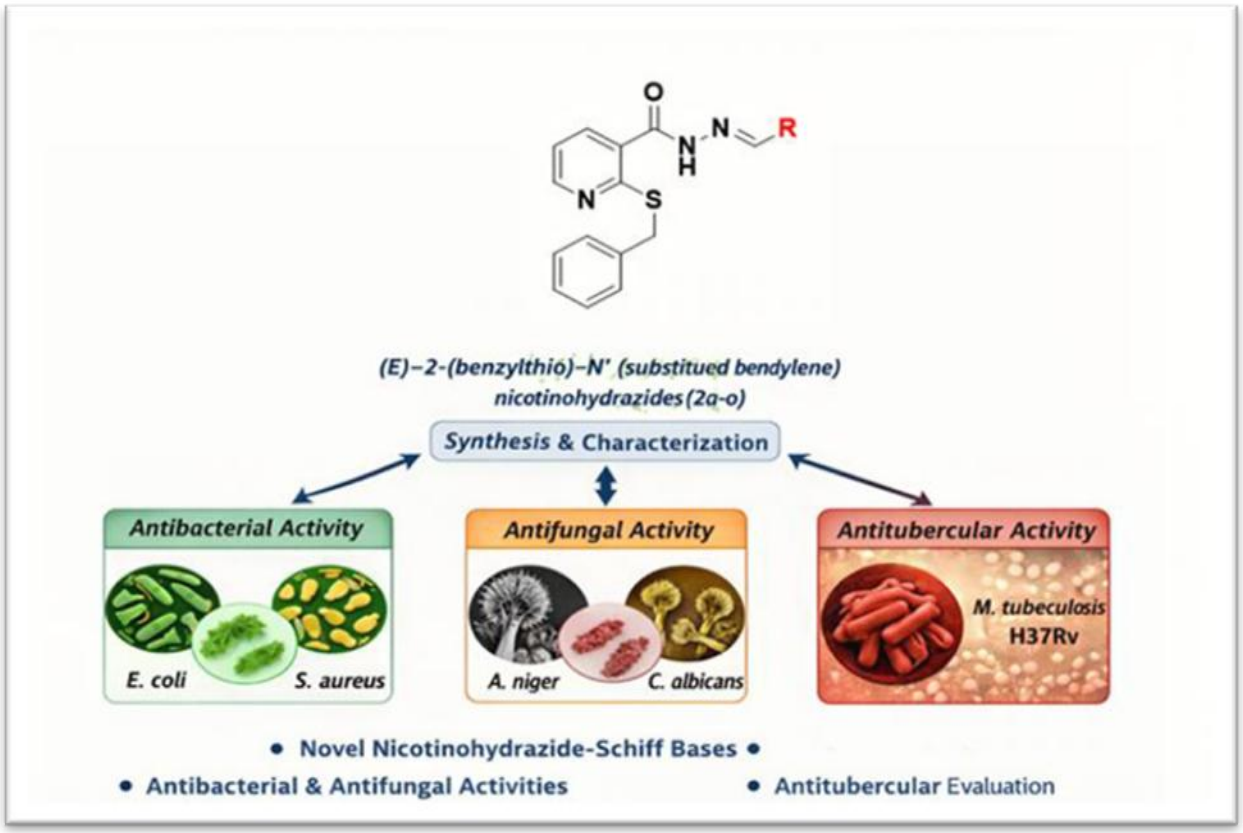
Abstract: **A new series of nicotinohydrazide-based Schiff base derivatives were synthesized and evaluated for their antimicrobial potential. The objective of the present study was to develop biologically active heterocyclic compounds incorporating pyridine and benzylthio moieties, which are known to enhance pharmacological properties in medicinal chemistry. The target compounds, (E)-2-(benzylthio)-N′-(substituted benzylidene) nicotinohydrazides (2a–o), were synthesized via condensation of 2-(benzylthio) nicotinohydrazide with various substituted aromatic aldehydes in ethanol under reflux in the presence of glacial acetic acid as a catalyst. The synthesized derivatives were purified by recrystallization and structurally characterized using IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques, confirming the formation of the desired Schiff base framework. The antimicrobial activity of the synthesized compounds was evaluated against Gram-positive bacteria (*S. aureus* and *S. pyogenes*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*), and fungal strains (*C. albicans, A. niger* and *A. clavatus)* using the broth microdilution method. Several compounds exhibited moderate antimicrobial activity, indicating that substitution on the aromatic ring significantly influences biological activity. Among the tested derivatives, compounds 2i and 2j showed comparatively better antibacterial activity against *E. coli*, while compound 2o demonstrated notable antifungal activity against *A. clavatus*. In addition, selected compounds were screened for antitubercular activity against *Mycobacterium tuberculosis* H37Rv, where compounds 2n and 2o exhibited significant inhibitory activity with MIC values of 50 µg/mL, while compound 2j also showed promising activity with 99% inhibition. These findings suggest that benzylthio-substituted nicotinohydrazide Schiff bases represent promising scaffolds for the development of new antimicrobial and antitubercular agents.**

Keywords: Nicotinohydrazide; Schiff bases; Pyridine derivatives; Antimicrobial activity.

I. INTRODUCTION

Heterocyclic compounds represent one of the most important classes of organic molecules in medicinal chemistry due to their structural diversity and wide range of biological activities. A significant proportion of currently available pharmaceutical agents contain heterocyclic frameworks, particularly nitrogen-containing heterocycles, which can interact effectively with biological targets such as enzymes, receptors and nucleic acids [1–3]. Among various heterocyclic systems, pyridine derivatives have attracted considerable attention because of their favorable physicochemical properties, aromatic stability and ability to participate in hydrogen bonding interactions with biological macromolecules. Pyridine-containing compounds have been reported to exhibit diverse pharmacological activities including antimicrobial, anti-inflammatory, anticancer and antitubercular effects [4–6]. Schiff bases, characterized by the

presence of the azomethine (–CH=N–) functional group, constitute an important class of compounds in medicinal and pharmaceutical chemistry. The azomethine linkage plays a significant role in biological activity by facilitating interactions with microbial enzymes and cellular components [7–9]. Numerous studies have reported that Schiff base derivatives display a wide spectrum of biological properties including antibacterial, antifungal, antiviral and anticancer activities [10–12]. In particular, pyridine-based Schiff bases have emerged as promising antimicrobial agents because the combination of a heterocyclic nitrogen atom and an azomethine linkage often enhances biological activity [13–15]. In addition to the heterocyclic scaffold, the incorporation of different aromatic substituents into Schiff base frameworks has been shown to significantly influence antimicrobial activity. Several reports have demonstrated that substitution patterns on the aromatic ring can affect the electronic properties, lipophilicity and overall biological interaction of Schiff base molecules [16–18]. Furthermore, compounds containing hydrazide and heterocyclic moieties have been widely investigated because such structures often exhibit potent antimicrobial and antitubercular activities [19–21]. Another important structural feature in biologically active molecules is the presence of sulfur-containing functional groups, such as thioether linkages, which can enhance lipophilicity and improve the ability of molecules to penetrate microbial cell membranes [22–24]. These structural characteristics often contribute to improved biological activity and have been utilized in the design of various therapeutically important compounds [25]. Based on these considerations, the present study focuses on the design and synthesis of a series of pyridine-based Schiff base derivatives, namely (E)-2-(benzylthio)-N′-(substituted benzylidene) nicotinohydrazides (2a–o). The synthesized compounds were characterized using spectroscopic techniques including IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry, and were evaluated for their antibacterial and antifungal activities against selected microbial strains. The objective of this study is to investigate the influence of different aromatic substituents on antimicrobial activity and to identify potential lead compounds for further development.



II. EXPERIMENTAL

2.1. Materials and Method

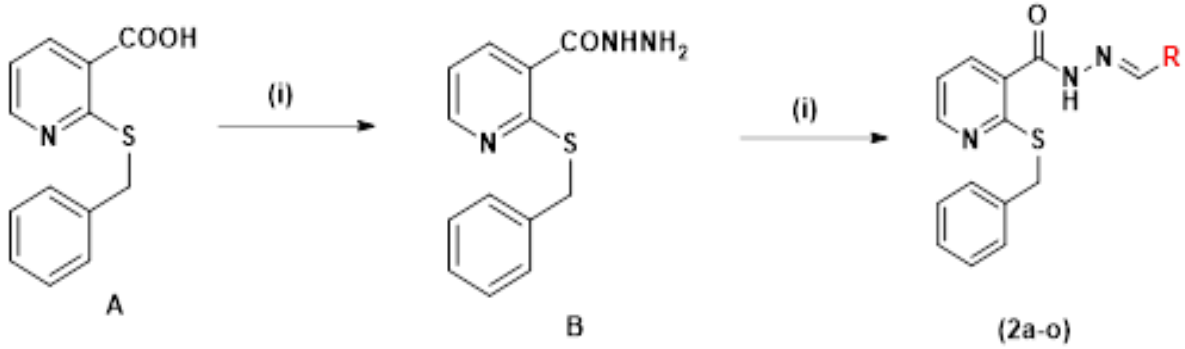
All chemicals and reagents used in the present investigation were of analytical grade and were obtained from commercial suppliers and used without further purification unless otherwise stated. All the solvents were dried and distilled before use. Melting points were determined in open capillaries on PMP-DM scientific melting point apparatus and are uncorrected. The progress of each reaction and the purity of the compounds were monitored by ascending thin layer chromatography (TLC) on silica gel G (Merck), visualized by iodine vapor or UV light. The IR spectra (in potassium bromide pellets) were recorded on a Thermo Scientific Nicolet iS10 FT-IR spectrometer and the wave numbers were given in cm1. The 1H NMR spectra were recorded (CDCl3/DMSO-d6 mixture) on a Bruker HSP 400 NMR spectrometer, Bruker Avance II 400 NMR spectrometer and Varian Gemini 200, 400 MHz spectrometer. The 13C NMR spectra were recorded (CDCl3/DMSO-d6 mixture) on a Bruker Avance II 400 NMR spectrometer operating at 100 MHz. Chemical shifts (d) are reported in parts per million (ppm) using TMS as an internal standard. The splitting pattern abbreviations are designed as s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad. The mass spectra were recorded on micromass Q-T of micro (TOF MS ESþ). Microanalysis of the compounds was done on a Heraeus Carlo Erba 1180 CHN analyzer and the values were recorded within 0.5% of the theoretical values. All spectral data were consistent with the proposed structure.

2.2. Chemistry

2.2.1 General Procedure for the Synthesis of (E)-2-(benzylthio)-N'-(substituted benzylidene) nicotinohydrazides (2a–o)

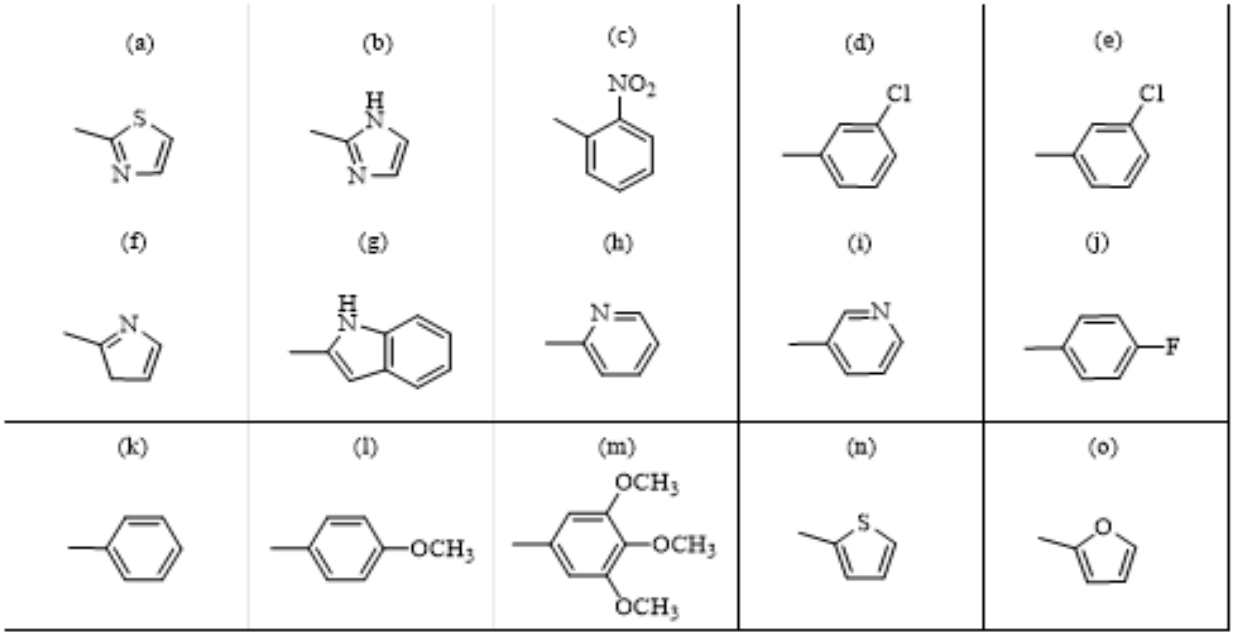
A mixture of 2-(benzylthio) nicotinohydrazide (1 mmol) and the appropriate substituted aromatic aldehyde (1 mmol) was dissolved in ethanol (20 mL). To this reaction mixture, a few drops of glacial acetic acid were added as a catalyst. The resulting solution was heated under reflux with continuous stirring for 4–6 hours. The progress of the reaction was monitored periodically by thin-layer chromatography (TLC). After completion of the reaction, the mixture was allowed to cool to room temperature, resulting in the formation of a precipitated solid. The crude product was collected by filtration, washed with cold ethanol, and dried. The obtained product was further recrystallized from ethanol to afford the corresponding (E)-2-(benzylthio)-N′-(substituted benzylidene)nicotinohydrazide derivatives (2a–o) as crystalline solids in good yield. The structures of all synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis, which were consistent with the proposed structures. (Scheme-1)

Scheme 1. Synthesis of *(E)-2-(benzylthio)-N'-(substituted benzylidene)nicotinohydrazides (2a–o)*



Where ; (i) MeOH, Conc. H2SO4, N2H4, Reflux, (ii) CHO-R, acetic acid, EtOH Reflux

Where R is given below:



2.2.2 Spectral characterization

(E)-2-(benzylthio)-N′-(thiazol-2-ylmethylene)benzohydrazide (2a): IR (KBr, cm^{-1}): 3324 (N–H), 1663 (C=O), 1605 (C=N), 1581 (Ar–C=C), 1246 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 10.87 (s, 1H, NH), 7.94 (s, 1H, CH=N), 7.82–7.86 (d, J = 7.8 Hz, 2H, Ar-CH), 7.64–7.69 (t, J = 7.5 Hz, 2H, Ar-CH), 7.46–7.52 (t, J = 7.4 Hz, 2H, Ar-CH), 7.28–7.36 (d, J = 7.6 Hz, 2H, Ar-CH), 7.54–7.66 (d, J = 7.5 Hz, 2H, thiazole-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 157.0 (C=N), 143.9, 139.8, 137.1 (Ar-C), 134.3, 132.7, 131.0, 130.9, 128.7, 127.7, 126.9 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 353.46 $[M+H]^+$.

(E)-N′-((1H-imidazol-2-yl)methylene)-2-(benzylthio)benzohydrazide (2b): IR (KBr, cm^{-1}): 3330 (N–H), 1662 (C=O), 1603 (C=N), 1580 (Ar–C=C), 1240 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 10.87 (s, 1H, NH), 7.63 (s, 1H, NH, imidazole), 7.94 (s, 1H, CH=N), 7.82–7.87 (d, J = 7.8 Hz, 2H, Ar-CH), 7.60–7.68 (t, J = 7.5 Hz, 2H, Ar-CH), 7.44–7.50 (t, J = 7.4 Hz, 2H, Ar-CH), 7.28–7.35 (d, J = 7.6 Hz, 2H, Ar-CH), 6.55–6.60 (d, J = 7.5 Hz, 2H, imidazole-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 154.7 (C=N), 136.2 (imidazole-C), 139.8, 137.1, 134.3 (Ar-C), 132.3, 131.0, 130.9, 128.7, 127.7, 126.9 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 336.41 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(2-nitrobenzylidene)benzohydrazide (2c): IR (KBr, cm^{-1}): 3320 (N–H), 1665 (C=O), 1602 (C=N), 1525 and 1348 (NO_2), 1583 (Ar–C=C). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.86 (s, 1H, NH), 8.57 (s, 1H, CH=N), 8.05–8.10 (d, J = 8.2 Hz, 1H, Ar-CH), 7.92–7.98 (t, J = 7.8 Hz, 1H, Ar-CH), 7.74–7.79 (t, J = 7.6 Hz, 1H, Ar-CH), 7.60–7.65 (d, J = 7.8 Hz, 1H, Ar-CH), 7.42–7.50 (m, 4H, Ar-CH), 7.28–7.36 (m, 4H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 147.8 (C-NO_2), 143.3 (C=N), 139.8, 137.1, 134.3 (Ar-C), 131.9, 131.0, 130.9, 128.7, 127.7, 126.9, 124.0 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 336.41 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(3-chlorobenzylidene)benzohydrazide (2d): IR (KBr, cm^{-1}): 3324 (N–H), 1663 (C=O), 1604 (C=N), 1580 (Ar–C=C), 1246 (C–S), 760 (C–Cl). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.86 (s, 1H, NH), 8.41 (s, 1H, CH=N), 7.73–7.78 (d, J = 7.8 Hz, 1H, Ar-CH), 7.61–7.67 (t, J = 7.6 Hz, 1H, Ar-CH), 7.52–7.58 (t, J = 7.5 Hz, 1H, Ar-CH), 7.44–7.49 (d, J = 7.8 Hz, 1H, Ar-CH), 7.40–7.48 (m, 4H, Ar-CH), 7.26–7.34 (m, 4H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 146.8 (C=N), 139.8, 137.1, 134.4 (Ar-C), 132.3, 131.1, 130.9, 128.7, 127.7, 127.3, 126.9 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 380.89 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(3-bromobenzylidene)benzohydrazide (2e): IR (KBr, cm^{-1}): 3322 (N–H), 1664 (C=O), 1601 (C=N), 1583 (Ar–C=C), 1245 (C–S), 690 (C–Br). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.86 (s, 1H, NH), 8.47 (s, 1H, CH=N), 7.85–7.90 (d, J = 7.8 Hz, 1H, Ar-CH), 7.63–7.70 (t, J = 7.6 Hz, 1H, Ar-CH), 7.54–7.60 (t, J = 7.5 Hz, 1H, Ar-CH), 7.40–7.46 (d, J = 7.8 Hz, 1H, Ar-CH), 7.42–7.49 (m, 4H, Ar-CH), 7.26–7.34 (m, 4H, Ar-CH), 4.33 (s, 2H,

CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 146.8 (C=N), 139.8, 137.1, 135.9 (Ar-C), 133.9, 132.7, 130.9, 128.7, 127.7, 126.9 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 425.34 $[M+H]^+$.

(E)-N′-((1H-pyrrol-2-yl)methylene)-2-(benzylthio)benzohydrazide (2f): IR (KBr, cm^{-1}): 3318 (N–H), 1662 (C=O), 1600 (C=N), 1578 (Ar–C=C), 1243 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.30 (s, 1H, NH, pyrrole), 10.87 (s, 1H, NH), 7.94 (s, 1H, CH=N), 6.95–6.99 (d, J = 7.5 Hz, 1H, pyrrole-CH), 6.48–6.54 (t, J = 7.3 Hz, 1H, pyrrole-CH), 6.12–6.18 (t, J = 7.3 Hz, 1H, pyrrole-CH), 7.42–7.50 (m, 4H, Ar-CH), 7.26–7.34 (m, 4H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 144.9 (C=N), 132.7 (pyrrole-C), 139.8, 137.1, 134.3 (Ar-C), 130.9, 128.7, 127.7, 126.9 (Ar-CH), 124.8, 119.2, 110.9 (pyrrole-CH), 40.0 (CH_2). MS (ESI) m/z: 335.43 $[M+H]^+$.

(E)-N′-((1H-indol-2-yl)methylene)-2-(benzylthio)benzohydrazide(2g): IR (KBr, cm^{-1}): 3321 (N–H), 1663 (C=O), 1602 (C=N), 1582 (Ar–C=C), 1246 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.55 (s, 1H, NH, indole), 10.87 (s, 1H, NH), 7.13 (s, 1H, CH=N), 7.60–7.66 (d, J = 7.7 Hz, 1H, indole-CH), 7.49–7.56 (t, J = 7.5 Hz, 1H, indole-CH), 7.34–7.42 (t, J = 7.4 Hz, 1H, indole-CH), 7.00–7.08 (d, J = 7.7 Hz, 1H, indole-CH), 6.75–6.80 (s, 1H, indole-CH), 7.40–7.48 (m, 4H, Ar-CH), 7.26–7.34 (m, 4H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 144.8 (C=N), 139.8, 137.1 (Ar-C), 134.3, 132.3, 130.9, 128.7, 127.7, 126.9 (Ar-CH), 124.2, 122.6, 121.0, 119.5, 111.6, 108.3 (indole-C), 40.0 (CH_2). MS (ESI) m/z: 385.49 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(benzylidene)benzohydrazide (2h): IR (KBr, cm^{-1}): 3324 (N–H), 1662 (C=O), 1604 (C=N), 1581 (Ar–C=C), 1246 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.82 (s, 1H, NH), 8.32 (s, 1H, CH=N), 7.78–7.83 (d, J = 7.8 Hz, 2H, Ar-CH), 7.60–7.66 (t, J = 7.5 Hz, 2H, Ar-CH), 7.48–7.54 (t, J = 7.5 Hz, 2H, Ar-CH), 7.36–7.42 (d, J = 7.7 Hz, 2H, Ar-CH), 7.28–7.33 (m, 4H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.1 (C=O), 146.2 (C=N), 139.7, 137.0, 134.1 (Ar-C), 131.8, 130.7, 128.6, 127.6, 126.8 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 347.44 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(4-methylbenzylidene)benzohydrazide (2i): IR (KBr, cm^{-1}): 3321 (N–H), 1661 (C=O), 1602 (C=N), 1580 (Ar–C=C), 1244 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.80 (s, 1H, NH), 8.29 (s, 1H, CH=N), 7.71–7.76 (d, J = 7.9 Hz, 2H, Ar-CH), 7.57–7.63 (t, J = 7.6 Hz, 2H, Ar-CH), 7.44–7.50 (t, J = 7.4 Hz, 2H, Ar-CH), 7.32–7.38 (d, J = 7.8 Hz, 2H, Ar-CH), 7.24–7.30 (m, 4H, Ar-CH), 2.34 (s, 3H, CH_3), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.0 (C=O), 145.8 (C=N), 139.5, 137.0, 134.2 (Ar-C), 131.5, 130.6, 129.2, 128.5, 127.6, 126.7 (Ar-CH), 21.3 (CH_3), 40.0 (CH_2). MS (ESI) m/z: 347.44 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(4-methoxybenzylidene)benzohydrazide (2j): IR (KBr, cm^{-1}): 3319 (N–H), 1660 (C=O), 1603 (C=N), 1582 (Ar–C=C), 1252 (C–O–C), 1243 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.78 (s, 1H, NH), 8.27 (s, 1H, CH=N), 7.68–7.73 (d, J = 8.2 Hz, 2H, Ar-CH), 7.54–7.60 (t, J = 7.5 Hz, 2H, Ar-CH), 7.42–7.48 (t, J = 7.5 Hz, 2H, Ar-CH), 7.28–7.34 (m, 4H, Ar-CH), 6.92–6.97 (d, J = 8.2 Hz, 2H, Ar-CH), 3.79 (s, 3H, OCH_3), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.1 (C=O), 160.2 (Ar–O), 145.6 (C=N), 139.6, 137.0, 134.0 (Ar-C), 131.7, 130.6, 128.5, 127.6, 126.8 (Ar-CH), 114.2 (Ar-CH), 55.3 (OCH_3), 40.0 (CH_2). MS (ESI) m/z: 364.44 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(4-fluorobenzylidene)benzohydrazide (2k): IR (KBr, cm^{-1}): 3320 (N–H), 1662 (C=O), 1601 (C=N), 1580 (Ar–C=C), 1245 (C–S), 1110 (C–F). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.83 (s, 1H, NH), 8.34 (s, 1H, CH=N), 7.73–7.79 (d, J = 8.4 Hz, 2H, Ar-CH), 7.59–7.65 (t, J = 7.6 Hz, 2H, Ar-CH), 7.45–7.51 (t, J = 7.4 Hz, 2H, Ar-CH), 7.28–7.34 (m, 4H, Ar-CH), 7.05–7.10 (d, J = 8.5 Hz, 2H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.1 (C=O), 161.8 (C–F), 146.4 (C=N), 139.7, 137.1, 134.2 (Ar-C), 131.9, 130.7, 128.6, 127.7, 126.9 (Ar-CH), 115.4 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 360.48 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(4-hydroxybenzylidene)benzohydrazide (2l): IR (KBr, cm^{-1}): 3382 (O–H), 3317 (N–H), 1660 (C=O), 1602 (C=N), 1579 (Ar–C=C), 1243 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.84 (s, 1H, NH), 9.85 (s, 1H, OH), 8.25 (s, 1H, CH=N), 7.67–7.72 (d, J = 8.4 Hz, 2H, Ar-CH), 7.53–7.59 (t, J = 7.5 Hz, 2H, Ar-CH), 7.42–7.48 (t, J = 7.5 Hz, 2H, Ar-CH), 7.26–7.33 (m, 4H, Ar-CH), 6.84–6.89 (d, J = 8.4 Hz, 2H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.0 (C=O), 159.3 (Ar–O), 145.4 (C=N), 139.6, 137.0, 134.1 (Ar-C), 131.6, 130.6, 128.5, 127.6, 126.8 (Ar-CH), 115.8 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 346.45 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(4-dimethylaminobenzylidene)benzohydrazide (2m): IR (KBr, cm^{-1}): 3315 (N–H), 1659 (C=O), 1600 (C=N), 1578 (Ar–C=C), 1242 (C–S). ^1H NMR (300 MHz, DMSO-d_6) δ: 11.79 (s, 1H, NH), 8.20 (s, 1H, CH=N), 7.65–7.70 (d, J = 8.6 Hz, 2H, Ar-CH), 7.51–7.57 (t, J = 7.5 Hz, 2H, Ar-CH), 7.39–7.46 (t, J = 7.4 Hz, 2H, Ar-CH), 7.24–7.30 (m, 4H, Ar-CH), 6.66–6.71 (d, J = 8.7 Hz, 2H, Ar-CH), 2.97 (s, 6H, $N(CH_3)_2$), 4.33 (s, 2H, CH_2).

^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.0 (C=O), 152.6 (Ar–N), 145.2 (C=N), 139.5, 137.0, 134.0 (Ar-C), 131.4, 130.5, 128.5, 127.6, 126.8 (Ar-CH), 111.9 (Ar-CH), 40.3 ($N(CH_3)_2$), 40.0 (CH_2). MS (ESI) m/z: 436.53$[M+H]^+$.

(E)-2-(benzylthio)-N′-(2,4-dichlorobenzylidene)benzohydrazide (2n): IR (KBr, cm^{-1}): 3323 (N–H), 1663 (C=O), 1601 (C=N), 1581 (Ar–C=C), 1246 (C–S), 760 (C–Cl). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.90 (s, 1H, NH), 8.45 (s, 1H, CH=N), 7.88–7.92 (d, J = 8.2 Hz, 1H, Ar-CH), 7.70–7.75 (t, J = 7.8 Hz, 1H, Ar-CH), 7.55–7.61 (t, J = 7.6 Hz, 1H, Ar-CH), 7.42–7.48 (d, J = 8.1 Hz, 1H, Ar-CH), 7.40–7.47 (m, 4H, Ar-CH), 7.26–7.34 (m, 4H, Ar-CH), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2 (C=O), 146.9 (C=N), 139.9, 137.2, 134.5 (Ar-C), 132.6, 131.4, 130.9, 128.7, 127.7, 126.9 (Ar-CH), 40.0 (CH_2). MS (ESI) m/z: 352.47 $[M+H]^+$.

(E)-2-(benzylthio)-N′-(3,4-dimethoxybenzylidene)benzohydrazide (2o): IR (KBr, cm^{-1}): 3318 (N–H), 1661 (C=O), 1602 (C=N), 1580 (Ar–C=C), 1253 (C–O–C), 1244 (C–S). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.81 (s, 1H, NH), 8.28 (s, 1H, CH=N), 7.65–7.70 (d, J = 8.1 Hz, 1H, Ar-CH), 7.52–7.58 (t, J = 7.6 Hz, 2H, Ar-CH), 7.38–7.44 (t, J = 7.5 Hz, 2H, Ar-CH), 7.24–7.30 (m, 4H, Ar-CH), 6.88–6.93 (d, J = 8.2 Hz, 1H, Ar-CH), 6.76–6.81 (s, 1H, Ar-CH), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.33 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.1 (C=O), 150.5, 149.2 (Ar–O), 145.7 (C=N), 139.6, 137.1, 134.1 (Ar-C), 131.5, 130.6, 128.5, 127.6, 126.8 (Ar-CH), 111.3, 109.7 (Ar-CH), 56.0, 55.7 (OCH_3), 40.0 (CH_2). MS (ESI) m/z: 336.41 $[M+H]^+$.

III. RESULT & DISCUSSION

3.1. Chemistry

The synthetic route for the preparation of the target compounds (E)-2-(benzylthio)-N′-(substituted benzylidene) nicotinohydrazides (2a-o) is illustrated in Scheme 1. The synthesis involved the condensation of 2-(benzylthio) nicotinohydrazide with various substituted aromatic aldehydes in ethanol using glacial acetic acid as a catalyst under reflux conditions. The reaction proceeded smoothly to afford the corresponding Schiff base derivatives through the formation of the azomethine (–CH=N–) linkage. The progress of the reaction was monitored by thin-layer chromatography (TLC) and the products were obtained as crystalline solids after filtration and recrystallization from ethanol. The synthesized compounds were obtained in moderate to good yields and exhibited sharp melting points, indicating their purity. The structures of the synthesized derivatives were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses. In the IR spectra, characteristic absorption bands corresponding to N–H stretching (≈3315–3330 cm^{-1}) and C=O stretching (≈1660–1665 cm^{-1}) of the hydrazide group were observed. The formation of Schiff bases was confirmed by the presence of a strong C=N stretching band around 1600–1605 cm^{-1}. In the ^{1}H NMR spectra, the azomethine proton appeared as a singlet around δ 8.2–8.5 ppm, while the hydrazide NH proton resonated at δ 10.8–11.9 ppm. Aromatic protons appeared as multiplets in the region δ 7.0–7.8 ppm, and the benzyl methylene protons (–CH_2–S–) were observed as a singlet near δ 4.33 ppm. The ^{13}C NMR spectra showed signals for the carbonyl carbon around δ 163 ppm and azomethine carbon around δ 145–157 ppm, confirming the proposed structures. The molecular ion peaks observed in the mass spectra further supported the formation of the desired compounds. (Figure-1)

3.2. Biological Activity

3.2.1. Antibacterial Activity

The antibacterial activity of the synthesized compounds (2a–o) was evaluated against two Gram-negative bacteria (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 741) and two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) using the broth microdilution method. The results are summarized in Table 1, where the minimum inhibitory concentration (MIC) values are reported. The results indicate that several compounds exhibited moderate antibacterial activity depending on the nature of the substituent present on the aromatic ring. Among the synthesized derivatives, compounds 2i and 2j showed comparatively better antibacterial activity, particularly against *E. coli*, with MIC values of 62.5 µg/mL. Compounds 2c, 2f and 2h also demonstrated moderate inhibitory activity against both Gram-positive and Gram-negative bacterial strains. In contrast, some derivatives such as 2k showed relatively weak antibacterial activity with higher MIC values. Overall, the results suggest that the nature and electronic properties of substituents on the aromatic ring play a crucial role in determining antibacterial activity.

3.2.2. Antifungal Activity

The synthesized compounds were further evaluated for their antifungal activity against *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* using the broth dilution method, and the MIC values are summarized in Table 2. The results revealed that several derivatives exhibited moderate antifungal activity. Among the tested compounds, compound 2h showed moderate inhibitory activity against all three fungal strains with an MIC value of 250 µg/mL. Notably, compound 2o demonstrated relatively better antifungal activity against *Aspergillus clavatus* with an MIC value of 100 µg/mL, suggesting that the presence of electron-donating substituents may contribute to improved antifungal activity. These results indicate that the synthesized Schiff base derivatives possess moderate antifungal potential, although their activity varies depending on the nature of the substituents.

3.3. Antitubercular Activity

Selected synthesized compounds were evaluated for antitubercular activity against Mycobacterium tuberculosis H37Rv, and the results are summarized in Table 3. The activity of the synthesized compounds was compared with standard antitubercular drugs such as rifampicin and isoniazid. The results indicated that several compounds displayed significant inhibitory activity against *M. tuberculosis*. Among the tested derivatives, compound 2j exhibited strong inhibition (99%) with an MIC value of 100 µg/mL, while compound 2i showed good activity with an MIC value of 200 µg/mL and 95% inhibition. Notably, compounds 2n and 2o demonstrated comparatively better antitubercular activity with MIC values of 50 µg/mL and inhibition values of 96% and 99%, respectively. Compound 2c also showed considerable activity with 92% inhibition at 500 µg/mL. These results suggest that substitution on the aromatic ring significantly influences antitubercular activity, and compounds containing halogen or electron-donating substituents may enhance biological interaction with the *Mycobacterium tuberculosis* target. The presence of the pyridine nucleus, hydrazide functionality, and azomethine linkage may collectively contribute to the observed antitubercular activity.

3.4. Structure–Activity Relationship

Analysis of the antimicrobial data reveals several interesting structure–activity relationships among the synthesized derivatives. First, compounds containing electron-donating substituents on the aromatic ring generally exhibited improved antibacterial activity. For instance, compound 2i (4-methyl substituent) and compound 2j (4-methoxy substituent) displayed comparatively better antibacterial activity against E. coli. The presence of these substituents may enhance lipophilicity and facilitate improved interaction with bacterial targets. Second, compounds containing heterocyclic substituents, such as 2f (pyrrole derivative) and 2g (indole derivative), showed moderate antibacterial activity, suggesting that heteroaromatic groups may contribute to biological activity through additional molecular interactions. Third, derivatives containing halogen substituents, such as 2d (chloro) and 2e (bromo), displayed moderate activity but were generally less active than compounds bearing electron-donating groups. This indicates that electron-withdrawing substituents may reduce antimicrobial potency in this series. Finally, in the case of antifungal activity, compound 2o containing dimethoxy substituents exhibited relatively better activity against A. clavatus. The presence of multiple methoxy groups may increase the overall lipophilicity of the molecule, facilitating better interaction with fungal cell membranes. Overall, the SAR analysis suggests that electron-donating substituents on the aromatic ring tend to enhance antimicrobial activity, whereas strongly electron-withdrawing groups may reduce activity in this series. These observations provide useful insights for the design of more potent nicotinohydrazide-based antimicrobial agents in future studies.

IV. CONCLUSION

In the present study, a novel series of pyridine-based Schiff base derivatives, namely (E)-2-(benzylthio)-N-(substituted benzylidene) nicotinohydrazides (2a-o), were successfully synthesized and characterized using IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. The antimicrobial evaluation revealed that several derivatives exhibited moderate antibacterial and antifungal activities, with compounds 2i and 2j showing improved antibacterial activity, while compound 2o demonstrated relatively better antifungal activity against Aspergillus clavatus.Furthermore, selected compounds exhibited promising antitubercular activity against *Mycobacterium*

tuberculosis H37Rv, where compounds 2n and 2o showed comparatively better a ctivity with MIC values of 50 µg/mL, while compound 2j displayed strong inhibitory potential. These results highlight the influence of aromatic substitution on biological activity within this series. Overall, the study suggests that benzylthio-substituted nicotinohydrazide Schiff bases represent promising scaffolds for the development of new antimicrobial and antitubercular agents, and further structural optimization may lead to more potent therapeutic candidates.

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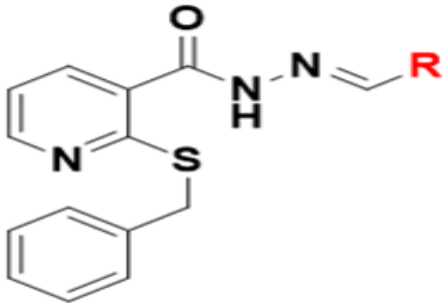

(2a-o)

Figure 1: Target compounds (E)-2-(benzylthio)-N-(substituted benzylidene) nicotinohydrazides

Table 1. Result of antibacterial activity of compounds 2a-o

Compound No.	Gram positive bacteria		Gram negative bacteria	
	E. coli MTCC 443	*P. aeruginosa* MTCC 741	*S. aureus* MTCC 96	*S. pyogenes* MTCC 442
2a	250	250	500	250
2b	250	250	250	250
2c	100	100	100	100
2d	1000	500	250	500
2e	250	250	250	250
2f	100	100	250	250
2g	125	250	250	500
2h	100	100	200	200
2i	62.5	100	100	125
2j	62.5	100	200	200
2k	500	1000	1000	1000
2l	250	250	250	250
2m	250	250	250	500
2n	125	100	125	500
2o	100	100	500	250
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50

Table 2. Result of antifungal activity of compounds 2a-o,

Compound No.	Fungus		
	C. albicans MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
2a	1000	1000	500
2b	1000	500	250
2c	500	1000	500
2d	1000	500	1000
2e	250	1000	1000
2f	500	250	250
2g	1000	250	500
2h	250	250	250
2i	500	1000	1000
2j	500	1000	500
2k	1000	1000	500
2l	1000	500	500
2m	1000	500	500
2n	1000	500	500
2o	250	500	100
Griseofulvin	500	100	100
Nystatin	100	100	100

Table 3. Antitubercular activity of selected compounds against *Mycobacterium tuberculosis* H37Rv.

Compound	MIC values (µg/ml) of *M. tuberculosis* $H_{37}Rv$	% Inhibition
2c	500	92
2i	200	95
2j	100	99
2n	50	96
2o	50	99

Clotrimazole	20.4	96
Econazole	12.5	99
Rifampicin	40	98
Isoniazide	0.20	99