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From Isoniazid to 2-Pyrazolines: Synthesis, *In silico* Behaviour and Antimicrobial Activity

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This study explores the synthesis, *in silico* behaviour and antimicrobial activity of isoniazid derived 2-pyrazolines as potential drugs for tuberculosis and bacterial infections. The investigation encompasses the synthesis, computational studies for molecular behaviour and antimicrobial efficacy assessment. Incorporating established drugs like isoniazid is explored to enhance their pharmacological properties. The synthesized compounds were characterized by analytical techniques such as IR, NMR, mass spectral analysis and elemental analysis. Synthesized pyrazolines **4a-j** were evaluated for *in vitro* antitubercular and antibacterial activities against various biological strains. *In silico* analysis provides valuable insights into ADMET descriptors, confirming good pharmacokinetic properties. This suggests these compounds as templates for developing new anti-mycobacterial agents, guiding the design of novel compounds with improved therapeutic potential.

Keywords: Isoniazid, 2-Pyrazolines, Chalcone synthons, ADMET descriptors, Antitubercular activity, Antibacterial activity.

INTRODUCTION

Drug-resistant strains of infectious pathogens, such as *Mycobacterium tuberculosis* and other harmful bacteria, constitute an increasing hazard to public health in contemporary medicines. These resistant strains render pharmaceutical interventions less effective, requiring an early response to the increased complexity of infectious diseases. The urgency of this situation stems from the potential consequences of uncontrolled infections, not only in terms of individual patient outcomes but also in the broader context of public health. The prolonged and ineffective treatment of drug-resistant infections can lead to increased morbidity, mortality and the spread of resistant strains within communities and healthcare settings [1,2]. Due to these challenges, new and effective medications must be developed frequently. Infectious diseases evolve, thus innovative treatments are needed to keep up with these tough pathogens. This emphasizes the importance of research on novel chemicals, methods of action and treatment options, which could change infectious disease management and protect global public health [3,4].

2-Pyrazolines consist of five-membered heterocyclic structure, are versatile compounds with diverse biological activities. Synthesized through various methods, these molecules exhibit anticancer [5], antitubercular [6], antimicrobial [7], anti-Alzheimer [8], anti-inflammatory [9], antiparasitic [10] properties and agents for neuropsychiatric and neurodegenerative disorders [11]. Of particular interest is their potential in combating tuberculosis and bacterial infections, critical global health concerns. The synthetic adaptability, particularly through chalcone synthons, enhances structural adjustments, making 2-pyrazolines compelling for drug development [12,13].

Isoniazid, a mainstay in tuberculosis therapy for over 40 years, remains a critical element of the treatment regimen. Its

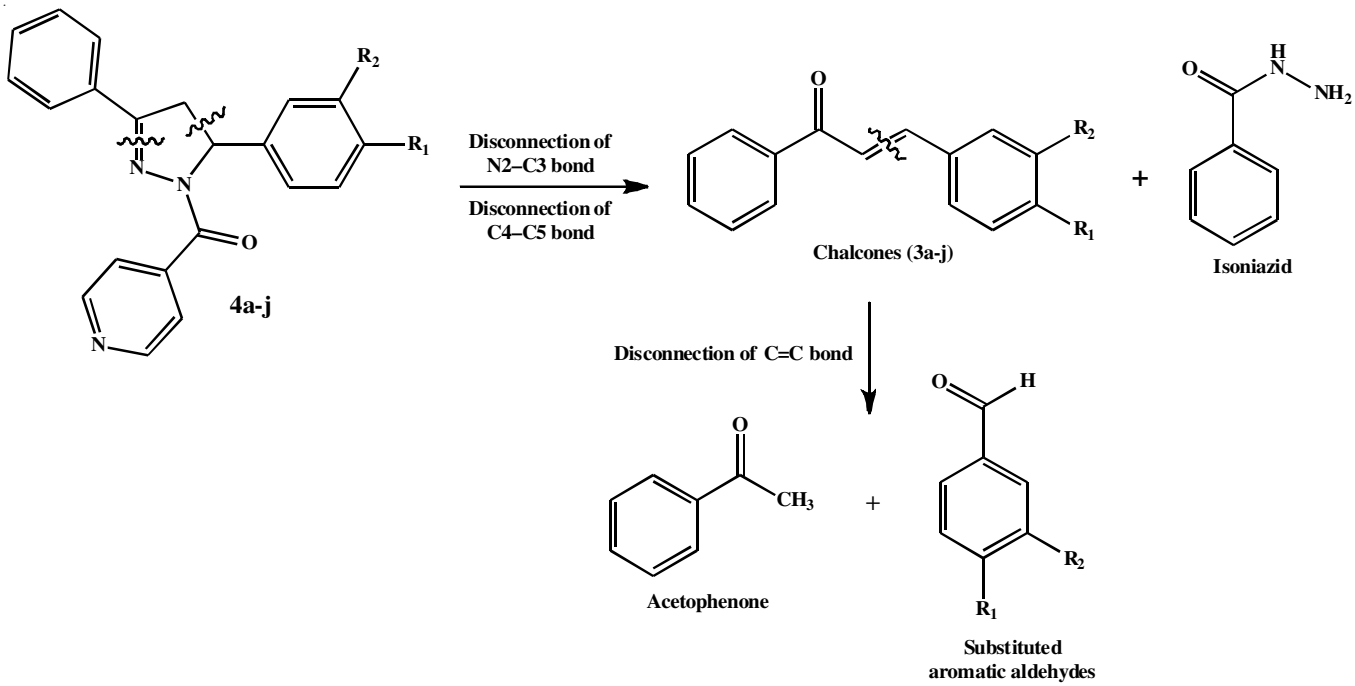
significant role has spurred ongoing research to develop new isoniazid derivatives that offer enhanced efficacy, reduced toxicity and minimized side effects [14]. Moreover, these derivatives have been associated with a broad spectrum of pharmacological activities [15,16], highlighting their potential beyond tuberculosis treatment. Recent investigations have focused on enhancing the structural complexity of isoniazid by integrating hydrophobic moieties. This strategy not only diversifies the biological activity of isoniazid derivatives [17,18] but also aims to improve their penetration into the lipid-rich cell walls of tuberculosis bacteria. Such advancements are thought to potentially boost the effectiveness of isoniazid, broadening its therapeutic reach [19-21].

The strategic incorporation of isoniazid into pyrazoline compounds underscores this innovative approach. Utilizing pyrazolines, known for their versatile pharmacological profiles, as scaffolds for INH derivatives enables the prediction and enhancement of their biological activities. This synthesis method creates new compounds with improved bioavailability, reduced toxicity and enhanced antimicrobial capabilities, paving the way for next-generation antitubercular agents. In this context, the study employs *in silico* analysis to gain a deeper understanding of the molecular interactions, pharmacokinetics and safety profile of isoniazid derived 2-pyrazolines. This approach helps in the rational design of compounds with optimal binding affinities and improved therapeutic outcomes. The combination of synthetic, computational and pharmacological approaches in this research contributes to the development of novel drugs with potential clinical applications in the fight against tuberculosis and bacterial infections.

EXPERIMENTAL

The chemicals and reagents used in this study were procured from commercial suppliers such as Qualigens Fine Chemicals, Himedia Laboratories Pvt. Ltd., Sigma-Aldrich Co., USA and Merck, USA. Melting points of the synthesized compounds were determined on open capillary tube and are uncorrected. To assess compound purity, thin layer chromatography was conducted on precoated silica gel strips with a hexane: ethyl acetate (2:1) solvent system. Spot detection was accomplished using an ultraviolet chamber. Infrared spectra were recorded utilizing a Shimadzu FT-IR 4000 instrument with KBr disks. Elemental analysis for carbon, hydrogen, nitrogen and oxygen (CHNO) was performed using the Perkin-Elmer Series II 2400 CHNS/O Elemental Analyzer. Mass spectra were generated employing a Jeol GC mate II GC-Mass spectrometer at 70 eV, utilizing the direct insertion probe method. The NMR spectra were acquired on a Bruker AVIII-500 MHz FT NMR spectrometer. Tetramethylsilane (TMS) served as the internal standard and dimethyl sulfoxide (DMSO) was the solvent of choice for NMR analyses.

Retrosynthetic analysis: Retrosynthetic analysis (RSA) is a method involving the deconstruction of a target molecule's structure into simpler precursor structures (synthons). This process follows a pathway leading to readily available starting materials for chemical synthesis. By working backward from the desired product, chemists can identify feasible routes for synthesis. In this study, retrosynthetic analysis was applied to the target molecules 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**) (**Scheme-I**). Disconnection of the N2-C3 and C4-C5 bonds in the pyrazoline nucleus yielded a chalcone compound and a isoniazid fragment as initial precursors. Further disconnection of the C=C bond (α,β unsaturated bond) in the chalcone compound resulted in acetophenone and substituted aromatic aldehydes as simpler precursor structures. This systematic disconnection and simplification process helps in identifying appropriate starting materials and synthetic pathways for the chemical synthesis of the target molecule.



Scheme-I: Retro-synthetic analysis of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**)

General synthesis of chalcones (3a-j): All the chalcone derivatives were synthesized through a base-catalyzed Claisen Schmidt condensation reaction [22]. A mixture consisting of acetophenone (**1**, 0.01 mol) and aromatic aldehydes (**2**, 0.01 mol) in 50 mL of ethanol was cooled to 5-10 ºC. Aqueous NaOH (70%, 5 mL) was added dropwise with continuous stirring. The resulting reaction mixture underwent magnetic stirring for 2 h and left undisturbed overnight. Subsequently, neutralization was achieved using conc. HCl. The resulting solid product was collected and subjected to additional purification through the crystallization process using ethanol. The confirmation of the purity of all synthesized compounds was carried out through thin-layer chromatography (TLC), utilizing a mobile phase comprising *n*-hexane and ethyl acetate.

General synthesis of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (4a-j): In the synthesis process, a mixture of substituted chalcone (0.003 mol), isoniazid (0.0045 mol) and pyridine (0.3 mL) as catalyst was subjected to reflux in 50 mL of ethanol for 5 h. The advancement of the reaction was monitored through TLC employing a hexane:ethyl acetate (2:1) solvent system as the mobile phase. Following the completion of the reaction, the reaction mixture was cooled and gradually introduced into crushed ice with continuous stirring. The resulting solid precipitate was collected *via* filtration, thoroughly washed with cold water, dried and subsequently subjected to recrystallization from ethanol (**Scheme-II**).

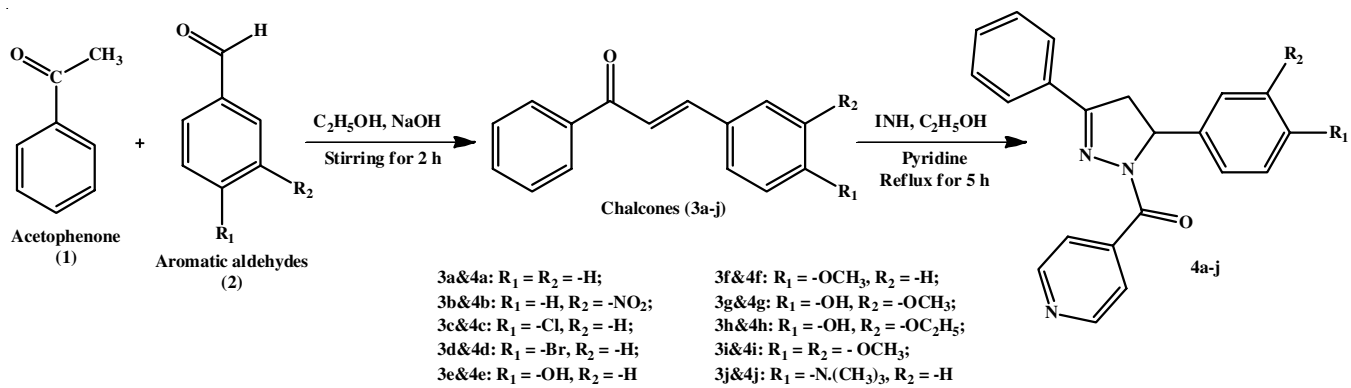
3,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 68%, m.p.: 201-203 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3364 (N-H), 2873 (C-H), 1568 (C=N), 1512 (C=C), 1748 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 3.16-3.31 (2H, 3.23 (dd), 3.24 (dd)), 5.73 (1H, dd), 7.08 (2H, dd), 7.17-7.50 (6H, 7.23 (tt), 7.28 (tt), 7.30 (dd), 7.43 (dd)), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 121.0 (2C, s), 127.0 (2C, s), 127.3 (3C, s), 127.8 (2C, s), 128.3-128.5 (4C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 327.13 (M+); Anal. calcd. (found) % for $C_{21}H_{17}N_3O$: C, 77.04 (77.08); H, 5.23 (5.21); N, 12.84 (12.86): O, 4.89 (4.90).

5-(3-Nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 52%, m.p.: 165-167 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3418 (N-H), 2907 (C-H), 1536 (NO_2), 1572 (C=N), 1502 (C=C), 1726 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 3.03 (1H, dd), 3.27 (1H, dd), 5.72 (1H, dd), 7.28 (1H, tt), 7.36-7.50 (4H, 7.43 (dd), 7.44 (dd)), 7.88-8.05 (4H, 7.94 (dd), 7.99 (dd)), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 120.8 (2C, s), 121.0 (2C, s), 127.3 (3C, s), 127.8 (1C, s), 128.4 (2C, s), 128.6 (2C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.3 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 372.16 (M+); Anal. calcd. (found) % for $C_{21}H_{16}N_4O_3$: C, 67.73 (67.71); H, 4.33 (4.32); N, 15.05 (15.07): O, 12.89 (12.90).

5-(4-Chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 72%, m.p.: 160-162 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3396 (N-H), 2852 (C-H), 1584 (C=N), 1508 (C=C), 1731 (C=O), 742 (C-Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 3.03 (1H, t), 3.22 (1H, dd), 5.70 (1H, dd), 7.28 (1H, tt), 7.36-7.71 (6H, 7.43 (dd), 7.52 (dd), 7.64 (dd)), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 121.0 (2C, s), 127.3-127.3 (3C, s), 127.3 (s)), 127.8 (1C, s), 128.4 (2C, s), 128.6-128.8 (4C, s), 133.7 (1C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 361.12 (M+); Anal. calcd. (found) % for $C_{21}H_{16}N_3OCl$: C, 69.71 (69.74); H, 4.46 (4.43); N, 11.61 (11.63); O, 4.42 (4.44); Cl, 9.80 (9.82).

5-(4-Bromophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 62%, m.p.: 170-172 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3382 (N-H), 2858 (C-H), 1579 (C=N), 1512 (C=C), 1734 (C=O), 638 (C-Br); ^{1}H NMR (DMSO-d_6, δ ppm): 3.04 (1H, t), 3.23 (1H, dd), 5.69 (1H, dd), 7.22-7.35 (3H, 7.28 (dd), 7.28 (tt)), 7.36-7.53 (4H, 7.43 (dd), 7.47 (dd)), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 121.0 (2C, s), 122.3 (1C, s), 127.3 (3C, s), 127.8 (1C, s), 128.6 (4C, s), 131.7 (2C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 406.07 (M+); Anal. calcd. (found) % for $C_{21}H_{16}N_3OBr$: C, 62.08 (62.10); H, 3.97 (3.99); N, 10.34 (10.33); O, 3.94 (3.92); Br, 19.67 (19.70).

5-(4-Hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 68%, m.p.: 212-214 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3346 (N-H), 3218(O-H), 2836 (C-H), 1585 (C=N), 1504 (C=C), 1728 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.83 (1H, dd), 3.07 (1H, dd), 5.64 (1H, dd), 6.65 (2H, dd), 7.15-7.34 (3H, 7.22 (dd), 7.28 (tt)), 7.43 (2H, dd), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 115.7 (2C, s), 121.0 (2C,



Scheme-II: Synthetic scheme of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**)

s), 127.3 (5C, s), 127.8 (1C, s), 128.4 (2C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 157.4 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 343.16 (M+); Anal. calcd. (found) % for $C_{21}H_{17}N_3O_2$: C, 73.45 (73.43); H, 4.99 (4.98); N, 12.24 (12.27): O, 9.32 (9.34).

5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 62%, m.p.: 185-187 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3362 (N-H), 2907 (C-H), 1574 (C=N), 1502 (C=C), 1738 (C=O), 1285 (C-O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.82 (1H, dd), 3.08 (1H, dd), 3.74 (3H, s), 5.60 (1H, dd), 6.89 (2H, dd), 7.11-7.34 (3H, 7.17 (dd), 7.28 (tt)), 7.43 (2H, dd), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 56.0 (1C, s), 59.2 (1C, s), 114.3 (2C, s), 121.0 (2C, s), 127.3 (5C, s), 127.8 (1C, s), 128.4 (2C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 159.8 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 357.18 (M+); Anal. calcd. (found) % for $C_{22}H_{19}N_3O_2$: C, 73.93 (73.95); H, 5.36 (5.38); N, 11.76 (11.79): O, 8.95 (8.97).

5-(4-Hydroxy-3-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 56%, m.p.: 192-194 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3405 (N-H), 3226 (O-H), 2874 (C-H), 1559 (C=N), 1524 (C=C), 1716 (C=O), 1268 (C-O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.83 (1H, dd), 3.12 (1H, dd), 3.79 (3H, s), 5.51 (1H, dd), 6.59-6.84 (3H, 6.65 (dd), 6.78 (dd), 6.77 (dd)), 7.28 (1H, tt), 7.43 (2H, dd), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 56.0 (1C, s), 59.2 (1C, s), 109.9 (1C, s), 115.8 (1C, s), 121.0 (2C, s), 127.3 (4C, s), 127.8 (1C, s), 128.4 (2C, s), 138.9 (1C, s), 142.9 (1C, s), 146.7 (1C, s), 147.5 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 373.15 (M+); Anal. calcd. (found) % for $C_{22}H_{19}N_3O_3$: C, 70.76 (70.78); H, 5.13 (5.12); N, 11.25 (11.28): O, 12.85 (12.87).

5-(3-Ethoxy-4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 65%, m.p.: 164-166 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3392 (N-H), 3248 (O-H), 2896 (C-H), 1583 (C=N), 1508 (C=C), 1704 (C=O), 1154 (C-O); ^{1}H NMR (DMSO-d_6, δ ppm): 1.25 (3H, t), 2.88 (1H, dd), 3.12 (1H, dd), 4.02-4.14 (2H, 4.08 (q), 4.08 (q)), 5.52 (1H, dd), 6.60-6.85 (3H, 6.66 (dd), 6.78 (dd), 6.78 (dd)), 7.28 (1H, tt), 7.43 (2H, dd), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 14.2 (1C, s), 46.1 (1C, s), 59.2 (1C, s), 64.3 (1C, s), 109.9 (1C, s), 115.8 (1C, s), 121.0 (2C, s), 127.3 (4C, s), 127.8 (1C, s), 128.4 (2C, s), 138.9 (1C, s), 142.9 (1C, s), 146.7 (1C, s), 147.3 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 387.18 (M+); Anal. calcd. (found) % for $C_{23}H_{21}N_3O_3$: C, 71.30 (71.32); H, 5.46 (5.45); N, 10.85 (10.87): O, 12.39 (12.42).

5-(3,4-Dimethoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 68%, m.p.: 155-157 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3376 (N-H), 2905 (C-H), 1564 (C=N), 1522 (C=C), 1741 (C=O), 1292 (C-O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.82 (1H, dd), 3.07 (1H, dd), 3.68-3.85 (6H, 3.73 (s), 3.80 (s)), 5.55 (1H, dd), 6.71-6.84 (3H, 6.76 (dd), 6.77 (dd), 6.78 (dd)), 7.28 (1H, tt), 7.43 (2H, dd), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 46.1 (1C, s), 56.0 (2C, s), 59.2 (1C, s), 109.9 (1C, s), 111.2 (1C, s), 121.0 (2C, s), 127.2 (4C, s), 127.8 (1C, s), 128.4 (2C, s), 138.9 (1C, s), 142.9 (1C, s), 148.2 (1C, s), 148.4 (1C, s), 149.9 (2C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 387.43 (M+); Anal. calcd. (found) % for $C_{23}H_{21}N_3O_3$: C, 71.30 (71.32); H, 5.46 (5.48); N, 10.85 (10.83): O, 12.39 (12.41).

5-(4-Dimethylamino)phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanone: Yield: 60%, m.p.: 174-176 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3329 (N-H), 2976 (N-C), 2864 (C-H), 1573 (C=N), 1502 (C=C), 1748 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.66-2.81 (7H, 2.73 (dd), 2.74 (s)), 2.99 (1H, dd), 5.60 (1H, dd), 6.58-6.79 (4H, 6.65 (dd), 6.73 (dd)), 7.28 (1H, tt), 7.43 (2H, dd), 7.94 (2H, dd), 8.36 (2H, dd), 8.74 (2H, dd); ^{13}C NMR (DMSO-d_6, δ ppm): 40.3 (2C, s), 46.1 (1C, s), 59.2 (1C, s), 112.0 (2C, s), 121.0 (2C, s), 127.3 (3C, s), 127.8 (1C, s), 128.4 (2C, s), 128.6 (2C, s), 139.5 (1C, s), 142.9 (1C, s), 149.9 (2C, s), 150.9 (1C, s), 152.5 (1C, s), 164.9 (1C, s); MS (*m/z*, %): 370.21 (M+); Anal. calcd. (found) % for $C_{23}H_{22}N_4O$: C, 74.57 (74.56); H, 5.99 (5.97); N, 15.12 (15.13): O, 4.32 (4.31).

Prediction of activity spectra for substances-PASS: The synthesized compounds underwent pharmacological activity prediction utilizing the online program PASS. This tool employs the structural formula of chemical compounds to predict their biological activity spectra. The predictions encompass a diverse range of pharmacological effects, offering valuable insights into the potential activities of the synthesized compounds. Through the analysis of the chemical structure of these compounds, the PASS tool generates predictions for various biological activities [23].

Swiss ADME: The pharmacokinetic and medicinal chemistry properties of the synthesized compounds **4a-j** were evaluated using the Swiss ADME online tool. This computational tool predicts essential absorption, distribution, metabolism and excretion (ADME) parameters for small molecules based on their chemical structures. The predictions include key factors such as lipophilicity, solubility and bioavailability, offering valuable information to assess the compounds' drug-like characteristics [24].

Syntelly: Syntelly, an artificial intelligence platform specializing in organic and medicinal chemistry, was employed to swiftly access experimental data and predict properties for a vast database of over 150 million organic compounds. This platform incorporates a visual module designed for exploring chemical space including RSA and generating structures with predefined properties. The central database of Syntelly serves as a comprehensive repository, allowing efficient molecule searches through input in various formats. Utilizing a traffic light system on the molecule card, safety parameters, including toxicity, physicochemical attributes, biological characteristics and environmental properties, were systematically compared [25]. This approach provided a robust framework for evaluating and comparing the safety profiles of the synthesized compounds.

Antitubercular activity: For the assessment of antitubercular activity, the synthesized 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**) underwent screening using the microplate Alamar blue assay method (MABA). The compounds were tested against the *M. tuberculosis* H37 RV strain, with isonicotinic acid hydrazide

serving as the standard for comparison. For the assay setup, the outermost wells of a sterile 96-well plate received 200 µL of sterile deionized water to prevent medium evaporation. Subsequently, 100 µL of Middlebrook 7H9 (MB 7H9) broth was added to the wells and the synthesized compounds were serially diluted directly on the plate. Antitubercular activity was assessed at final drug concentrations ranging from 0.2 to 100 µg/mL.

The plates were covered, sealed with parafilm and incubated at 37 ºC for 5 days. Following incubation, 25 µL of freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween 80 was added to each well, followed by an additional 24 h incubation. Interpretation of the results relied on the observed colour change: a blue colour indicated no mycobacterial growth, while a pink colour signified mycobacterial growth [26].

Antibacterial activity: For antibacterial activity assessment, the synthesized compounds **4a-j** underwent testing using the agar cup plate method against a spectrum of bacteria, including Gram-positive organisms (*Staphylococcus epidermatitis* and *Bacillus subtilis*) and Gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*). The minimum inhibitory concentration (MIC) method was employed for evaluation, with ciprofloxacin as the reference standard. The procedure involved bringing brain heart infusion agar to room temperature and transferring colonies to the plates, adjusting their turbidity to match a 0.5 McFarland turbidity standard. Swabbing the agar plate surface uniformly, a 5 mm hollow tube was pressed to develop wells, into which 75, 50, 25, 10 and 5 µL of the synthesized compounds were added. Incubation at 37 ºC for 24 h followed compound application and the inhibition zone diameter was measured to the nearest millimeter. The MIC procedure included serial dilution up to a 10^{-9} dilution for each synthesized compound [27].

RESULTS AND DISCUSSION

The synthetic route outlining the contemporary strategy for the synthesis of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**) is depicted in **Scheme-II**. The process commenced with the reaction between acetophenone (**1**) and aromatic aldehydes (**2**), resulting in the formation of chalcones (**3a-j**) [28]. This was followed by cyclization with isoniazid, ultimately leading to the formation of a pyrazoline ring. To confirm the purity of all the synthesized compounds, thin-layer chromatography (TLC) was employed, utilizing a mobile phase composed of a hexane and ethyl acetate mixture. Furthermore, each synthesized compound exhibited distinct peaks in both FT-IR and NMR spectra. Mass spectra analysis provided additional confirmation, substantiating the presence of the anticipated molecular ion peak (M+) fragments for the synthesized compounds.

The PASS predictions presented in Table-1 suggest the diverse pharmacological activities for the synthesized compounds, including potential therapeutic applications in various medical conditions such as neuropathy, neurologic disorders, cancer and microbial infections. These predictions provide a foundation for further indepth experimental investigations to validate and explore the multifaceted pharmacological profiles of these compounds.

TABLE-1
PREDICTED ACTIVITY SPECTRUM FOR
5-(SUBSTITUTED PHENYL)-3-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL(PYRIDIN-4-YL)METHANONES (**4a-j**)

Compd.	Pa	Pi	Activity
4a	0.435	0.019	Diabetic neuropathy treatment
	0.353	0.043	Antialcoholic
	0.251	0.118	Antimycobacterial
	0.120	0.073	Lanosterol 14 alpha demethylase inhibitor
4b	0.532	0.065	Acute neurologic disorders treatment
	0.346	0.057	Antimycobacterial
	0.231	0.132	Antituberculosic
	0.129	0.056	Lanosterol 14 alpha demethylase inhibitor
4c	0.484	0.006	Diabetic neuropathy treatment
	0.407	0.019	Antialcoholic
	0.244	0.125	Antimycobacterial
	0.155	0.026	Lanosterol 14 alpha demethylase inhibitor
4d	0.349	0.044	Antialcoholic
	0.303	0.023	Antineoplastic (non-small cell lung cancer)
	0.323	0.067	Antimycobacterial
	0.115	0.087	Lanosterol 14 alpha demethylase inhibitor
4e	0.436	0.035	Menopausal disorders treatment
	0.330	0.054	Antialcoholic
	0.264	0.107	Antimycobacterial
	0.113	0.092	Lanosterol 14 alpha demethylase inhibitor
4f	0.402	0.040	Diabetic neuropathy treatment
	0.336	0.017	Antineoplastic (non-small cell lung cancer)
	0.257	0.111	Antiinflammatory, intestinal
	0.254	0.115	Antimycobacterial
4g	0.418	0.042	Menopausal disorders treatment
	0.357	0.099	Diabetic neuropathy treatment
	0.273	0.099	Antimycobacterial
	0.133	0.081	Antineoplastic (gastric cancer)
4h	0.340	0.176	Antiviral (Rhinovirus)
	0.338	0.091	Vasodilator, coronary
	0.281	0.029	Antineoplastic (non-small cell lung cancer)
	0.271	0.100	Antimycobacterial
4i	0.364	0.005	Phosphodiesterase inhibitor
	0.363	0.014	Antineoplastic (non-small cell lung cancer)
	0.360	0.022	Antinephritic
	0.238	0.131	Antimycobacterial
4j	0.327	0.019	Antineoplastic (non-small cell lung cancer)
	0.296	0.123	Menopausal disorders treatment
	0.290	0.077	Antiprotozoal (Amoeba)
	0.189	0.189	Antimycobacterial

Table-2, presenting Swiss ADME predictions, serves as a valuable tool to assess the pharmacokinetic properties of the synthesized compounds. These predictions offer insights into crucial parameters related to absorption, distribution, metabolism and excretion. Parameters such as BBBP and P-gpS provide information on the compounds' potential to penetrate the blood brain barrier and their interaction with P-glycoprotein, respectively. Additionally, LogKp, Log P and Log S offer valuable data on skin permeability, lipophilicity and water solubility, impacting drug distribution and bioavailability. The SA values indicated the synthetic accessibility of the compounds. Collectively, these predictions contribute to a comprehensive under-

TABLE-2
PREDICTION OF PHARMACOKINETIC AND MEDICINAL CHEMISTRY PROPERTIES OF 5-(SUBSTITUTED PHENYL)-3-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL(PYRIDIN-4-YL)METHANONES (**4a-j**) USING SWISS ADME

Compound	GIA[a]	BBBP[b]	P-gpS[c]	LogKp (cm/s)[d]	Log P	Log S	SA[e]
4a	High	Yes	No	-5.96	3.25	-4.21	3.36
4b	High	No	No	-6.36	2.67	-4.26	3.53
4c	High	Yes	No	-5.72	3.80	-4.80	3.38
4d	High	Yes	No	-5.95	3.89	-5.11	3.42
4e	High	Yes	No	-6.31	2.87	-4.06	3.37
4f	High	Yes	No	-6.17	3.26	-4.27	3.45
4g	High	Yes	No	-6.51	2.88	-4.13	3.56
4h	High	Yes	No	-6.34	3.21	-4.37	3.69
4i	High	Yes	No	-6.37	3.22	-4.34	3.67
4j	High	Yes	No	-6.14	3.25	-4.43	3.65

a = Gastrointestinal absorption, b = Blood brain barrier permeant, c = P-gp substrate, d = Skin permeant, e = Synthetic accessibility

standing of the pharmacokinetic profiles of the compounds, providing guidance for further experimental investigations.

The Syntelly predictive toxicity properties of the synthesized compounds **4a-j** are summarized in Table-3. The Blood-Brain Barrier Permeability (BBBPa) results suggest that all the compounds exhibited permeability, allowing them to pass through the blood-brain barrier. Regarding cardiotoxicity (CTX), mutagenicity (MUT) and carcinogenicity (CAR), the synthesized compounds were generally categorized as non-toxic, except for hepatotoxicity (HEP), where most of the compounds exhibited toxicity. Specifically, the hepatotoxicity (HEP) results indicated that all compounds, except forcompound **4g**, were predicted to be toxic. Reproductive toxicity (RT) predictions suggested toxicity for all the compounds. In comparison, the standard drugs isoniazid and ciprofloxacin displayed impermeability through the blood-brain barrier and showed similar trends in toxicity predictions. These findings provide valuable insights into the potential safety profiles of the synthesized compounds, contributing to a better understanding of their pharmacological characteristics.

The Syntelly RSA prediction for the synthesized compounds yielded four routes (RSA 1-4) with scores ranging from 0.98 to 0.92, as illustrated in Fig. 1. RSA 1 synthons involve 5-substituted phenyl-3-phenyl-4,5-dihydro-1*H*-pyrazole (A) + pyridine-4-carbonyl chloride (B), RSA 2 synthons involve 5-substituted phenyl-3-phenyl-4,5-dihydro-1*H*-pyrazole (A) + pyridine-4-carboxylic acid (C), RSA 3 synthons involve 3-phenyl-1-(pyridin-4-yl)prop-2-en-1-one (D) + 4-[2-bromo-1-hydrazinylideneethyl]pyridine (E) and RSA 4 synthons involve 3-phenyl-1-(pyridin-4-yl)prop-2-en-1-one (D) + 1-(pyridin-4-ylcarbonyl)triaza-1,2-dien-2-ium (F). However, none of these routes were deemed feasible when compared with the RSA route, which suggests that carbonyl compounds, chalcones and isonicotinic acid hydrazide (INH) are more practical synthons. This is attributed to their easy availability or simpler methods of preparation for these synthons.

The results of the minimum inhibitory concentration (MIC) testing for the antitubercular activity of compounds **4a-j** are summarized in Table-4. The MIC values indicate the concentration of each compound required to inhibit the growth of *Mycobacterium tuberculosis*. Compound **4a** exhibited resistance (R) across all tested concentrations (6.75, 12.5, 25, 50 and 100 µg/mL). Compounds **4b** and **4j** also showed resistance at lower concentrations but demonstrated sensitivity (S) at higher concentration (100 µg/mL). Compounds **4g**, **4h** and **4i** exhibited sensitivity at concentrations of 25 µg/mL and above, while all synthesized compounds displayed resistance across all concentrations below 25 µg/mL.

The results of the minimum inhibitory concentration (MIC) testing for antitubercular activity revealed varying degrees of sensitivity among the synthesized compounds, whereas the standard drug isoniazid demonstrated sensitivity across all the

TABLE-3
PREDICTIVE TOXICITY PROPERTIES 5-(SUBSTITUTED PHENYL)-3-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL(PYRIDIN-4-YL)METHANONES (**4a-j**) USING SYNTELLY

Compound	Blood brain barrier permeability	Cardiotoxicity	Mutagenicity	Carcinogenicity	Hepatotoxicity	Reproductive toxicity
4a	Permeable	Non-toxic	Non-toxic	Non-toxic	Toxic	Toxic
4b	Permeable	Non-toxic	Toxic	Toxic	Toxic	Toxic
4c	Permeable	Non-toxic	Non-toxic	Non-toxic	Toxic	Toxic
4d	Permeable	Non-toxic	Non-toxic	Non-toxic	Toxic	Toxic
4e	Permeable	Non-toxic	Non-toxic	Non-toxic	Toxic	Toxic
4f	Permeable	Non-toxic	Non-toxic	Non-toxic	Toxic	Toxic
4g	Permeable	Non-toxic	Non-toxic	Non-toxic	Non-toxic	Toxic
4h	Permeable	Non-toxic	Non-toxic	Non-toxic	Non-toxic	Toxic
4i	Permeable	Non-toxic	Non-toxic	Non-toxic	Toxic	Toxic
4j	Permeable	Non-toxic	Non-toxic	Non-toxic	Non-toxic	Toxic
Isoniazid	Impermeable	Non-toxic	Toxic	Non-toxic	Toxic	Toxic
Ciprofloxacin	Impermeable	Non-toxic	Toxic	Non-toxic	Toxic	Toxic

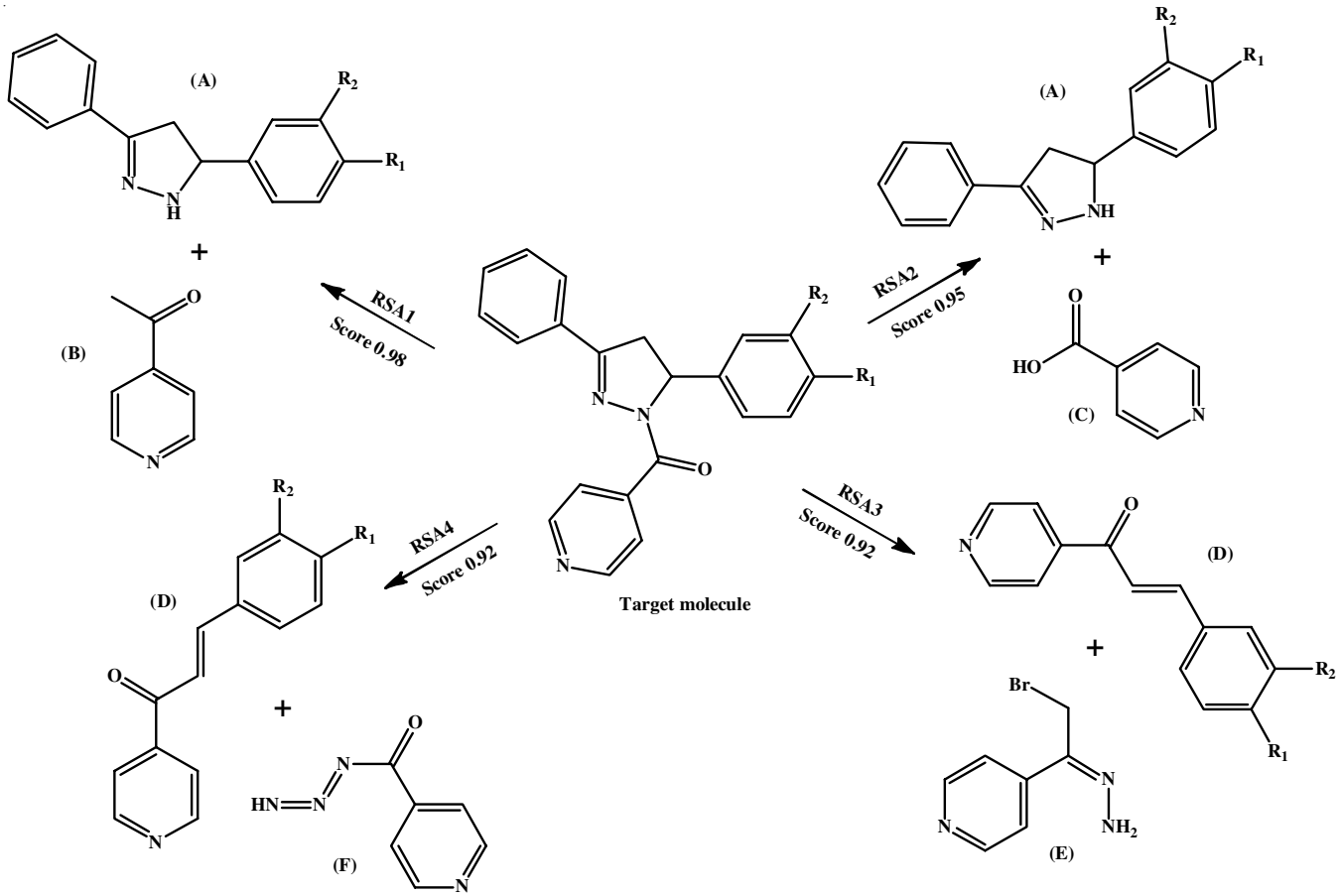


Fig. 1. Retrosynthetic analysis of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**) predicted by Syntelly

TABLE-4
ANTITUBERCULAR ACTIVITY OF 5-(SUBSTITUTED PHENYL)-3-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL(PYRIDIN-4-YL)METHANONES (**4a-j**)

Compd.	Minimum inhibitory concentration (µg/mL)				
	6.75	12.5	25	50	100
4a	R	R	R	R	R
4b	R	R	R	R	S
4c	R	R	R	S	S
4d	R	R	R	S	S
4e	R	R	R	S	S
4f	R	R	R	S	S
4g	R	R	S	S	S
4h	R	R	S	S	S
4i	R	R	S	S	S
4j	R	R	R	R	S
Isoniazid	S	S	S	S	S

concentrations. This divergence in activity highlights distinct antitubercular profiles among the synthesized compounds, which include the incorporation of isoniazid in their structures, with some showing remarkable sensitivity. These findings underscore the importance of additional structural requirements beyond isoniazid as a pharmacophore, emphasizing the multifaceted nature of the compounds' interactions with the target.

A plausible explanation for these differences lies in the structural requirements, specifically the need for hydroxyl or lower alkoxy groups (1-2 carbons) or a combination of both at the *meta*- and *para*-positions of phenyl ring located at the 5th position of the pyrazoline nucleus. These functional groups likely contribute to molecular interactions, including hydrogen bonding, hydrophobic and polar interactions and steric effects. These interactions can influence the compounds' binding affinity to the target, potentially enhancing their antitubercular activity.

Fig. 2 displays the results of the antibacterial activity testing, as determined by the agar plate method. The concentrations used for the synthesized compounds **4a-j** and the reference drug ciprofloxacin were 100 µg/0.1 mL. For ciprofloxacin, the inhibition zone diameters (in mm) against different organisms were observed as *S. epidermatitis*: 22 mm, *B. subtilis*: 20 mm, *E. coli*: 20 mm and *P. aeruginosa*: 19 mm.

The synthesized compounds **4a-j** exhibited varying degrees of antibacterial activity against the tested organisms. The results indicate that compound **4g** demonstrated the highest inhibitory effect among the synthesized compounds, with inhibition zone diameters of 18 mm against *S. epidermatitis* and *B. subtilis*, 15 mm against *E. coli* and 14 mm against *P. aeruginosa*. Compounds **4i** and **4h** also displayed notable antibacterial activity, with inhibition zones ranging from 16 to 18 mm against different organisms.

Comparatively, the reference drug ciprofloxacin exhibited strong antibacterial activity, with wide inhibition zones against

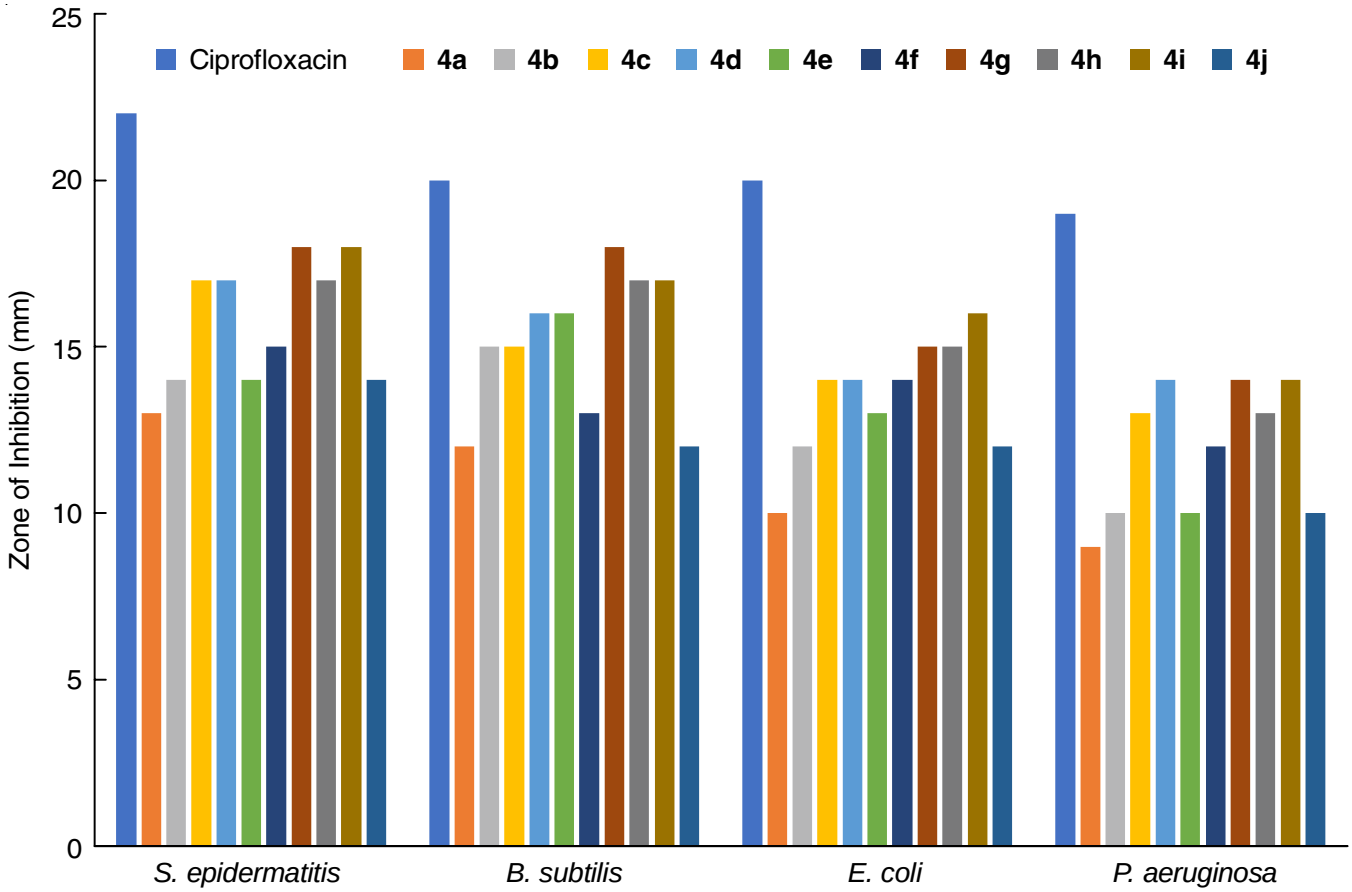


Fig. 2. Antibacterial activity of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**)

all tested bacteria. These findings suggest that the synthesized compounds possess antibacterial properties, although their efficacy varies across different strains. Further comprehensive analysis and discussion will be conducted to elucidate the implications of these findings for the development of potential antitubercular and antibacterial agents. This will include an in-depth exploration of the structure-activity relationship (SAR) and the mechanistic insights into how specific substituents affect the compounds' interactions with the target, guiding future optimization strategies for enhanced efficacy.

Conclusion

In conclusion, this work represents a multifaceted exploration into the synthesis, *in silico* behaviour and antimicrobial impact of 5-(substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl(pyridin-4-yl)methanones (**4a-j**) derived from isoniazid. The compounds were successfully synthesized using a carefully controlled reaction involving acetophenone, aromatic aldehydes, chalcones and isoniazid, leading to the formation of a pyrazoline ring. Rigorous characterization using analytical techniques confirmed the purity of the synthesized compounds. Pharmacological predictions from PASS and Swiss ADME tools provided valuable insights into the diverse potential activities and pharmacokinetic properties of the compounds. The safety profile, assessed through Syntelly predictive toxicity properties, indicated permeability through the blood-brain barrier and minimal toxicity, highlighting the favourable attribution of the synthesized compounds. The rational design strategy, incorporating isoniazid into pyrazoline compounds, highlighted an innovative approach to enhance pharmacological properties. Syntelly RSA prediction for synthons suggested the practicality of carbonyl compounds, chalcones and isoniazid as building blocks. The antibacterial efficacy showed varied levels of sensitivity among the synthesized compounds, while some of the compounds exhibited significant antibacterial activity, however, further analysis is needed to explore the structure-activity relationship and mechanistic insights. Overall, this study contributes significantly to the ongoing efforts to address the challenges posed by drug-resistant strains in the context of infectious diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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