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Synthesis and Biological Investigation of Novel Heterocyclic Acetamide Derivatives as Potential Antitubercular agents Targeting Dpre1

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KEYWORDS

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ABSTRACT:

Tuberculosis (TB) remains a major global health concern due to the increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of Mycobacterium tuberculosis. The urgent need for new therapeutic agents with novel mechanisms of action has directed attention toward validated molecular targets involved in mycobacterial cell wall biosynthesis. Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) is an essential enzyme in the synthesis of arabinogalactan, a critical component of the mycobacterial cell wall, and has emerged as a promising target for antitubercular drug development.

In the present study, a series of novel heterocyclic acetamide derivatives were rationally designed, synthesized, and evaluated for their in-vitro antitubercular activity targeting the DprE1 enzyme. Structure-based drug design and molecular docking studies were performed to predict binding interactions and affinity within the active site of DprE1. The designed compounds were synthesized through a multi-step synthetic approach involving heterocyclic amine formation followed by acetamide coupling. Structural confirmation was achieved using physicochemical and spectroscopic techniques including melting point determination, FTIR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry.

The synthesized derivatives were screened against M. tuberculosis H37Rv strain using the Microplate Alamar Blue Assay (MABA). Several compounds exhibited significant inhibitory activity with minimum inhibitory concentration (MIC) values ranging from 0.78 to 6.25 µg/mL. Among them, compound 5d demonstrated the most potent activity and favorable selectivity index, correlating well with its superior docking score and predicted ADMET properties.

The findings suggest that heterocyclic acetamide scaffolds represent promising lead candidates for further optimization and development as novel antitubercular agents targeting DprE1.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the leading causes of death from infectious diseases worldwide [1]. Despite the availability of established first-line and second-line antitubercular therapies, TB continues to pose a significant global health challenge due to prolonged treatment regimens, adverse drug reactions, poor patient compliance, and the increasing incidence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains [2-3]. The emergence of resistance to conventional drugs such as isoniazid and rifampicin has severely limited the effectiveness of standard chemotherapy, underscoring the urgent need for novel therapeutic agents with new mechanisms of action [4].

The mycobacterial cell wall is a unique and complex structure that plays a critical role in bacterial survival, pathogenicity, and intrinsic drug resistance [5]. It is composed primarily of mycolic acids, arabinogalactan, and peptidoglycan, forming a highly

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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

impermeable barrier that protects the organism from host defenses and antimicrobial agents [6]. Among the enzymes involved in cell wall biosynthesis, Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) has emerged as a validated and promising drug target [7]. DprE1, along with DprE2, catalyzes the epimerization of decaprenylphosphoryl-β-D-ribose to decaprenylphosphoryl-β-D-arabinose, a key precursor required for arabinan synthesis in arabinogalactan [8]. Inhibition of DprE1 disrupts arabinogalactan formation, compromising cell wall integrity and ultimately leading to bacterial death [9-10].

Several potent DprE1 inhibitors, including benzothiazinones and dinitrobenzamides, have demonstrated strong antitubercular activity [11]. However, limitations such as toxicity, metabolic instability, and potential resistance development necessitate the exploration of new chemical scaffolds capable of selectively targeting DprE1 with improved safety and pharmacokinetic profiles [12].

The acetamide functional group has gained considerable importance in medicinal chemistry due to its favorable hydrogen bonding capability, structural versatility, and ability to modulate lipophilicity and metabolic stability [13]. Incorporation of the acetamide moiety into heterocyclic frameworks often enhances biological activity by facilitating strong interactions within enzyme active sites [14]. Heterocyclic compounds, in particular, are widely recognized as privileged scaffolds in drug discovery owing to their ability to mimic biological substrates and interact effectively with protein targets [15].

Rational drug design approaches integrating molecular docking, pharmacophore modeling, and in silico ADMET prediction have significantly accelerated the identification of promising lead molecules [16]. Structure-based design targeting DprE1 enables optimization of molecular interactions within the enzyme's active site, including hydrogen bonding with catalytic residues and hydrophobic interactions within the binding pocket [17].

In this context, the present study focuses on the design, synthesis, and biological evaluation of novel heterocyclic acetamide derivatives as potential antitubercular agents targeting DprE1 [18]. The compounds were rationally designed based on structural requirements for DprE1 inhibition, synthesized using established organic methodologies, and characterized by physicochemical and spectroscopic techniques [19]. Their in-vitro antitubercular activity was evaluated against *M. tuberculosis* H37Rv strain, and the results were correlated with molecular docking predictions [20]. This integrated approach aims to identify promising acetamide-based scaffolds for further optimization and development as next-generation antitubercular agents [21].

MATERIALS AND METHODS

Molecular Docking

The three-dimensional crystal structure of Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) complexed with an inhibitor was obtained from the Protein Data Bank (PDB).

PDB ID: 4P8N

This structure was selected because it provides a high-resolution model of the DprE1 active site suitable for molecular docking studies. Prior to docking, the protein structure was prepared by removing crystallographic water molecules, adding hydrogen atoms, assigning charges, and performing energy minimization.

Key residues present in the catalytic pocket include:

- Cys387
- Tyr314
- Lys418
- His132
- Ser59

These residues play an important role in ligand binding and catalytic activity of DprE1.

Molecular docking studies were performed to predict the binding affinity and interaction pattern of the designed heterocyclic acetamide derivatives with the DprE1 enzyme of *Mycobacterium tuberculosis*. The docking protocol was designed to evaluate the compatibility of synthesized compounds within the active site and to prioritize molecules for synthesis and biological testing.

Journal of Chemical Health Risks

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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

Selection and Preparation of Target Protein

The three-dimensional crystal structure of DprE1 complexed with a known inhibitor was retrieved from the Protein Data Bank (PDB). A high-resolution structure was selected to ensure reliability of docking results. The protein structure was prepared by:

- Removing water molecules and co-crystallized ligands (except essential cofactors if required)
- Adding missing hydrogen atoms
- Assigning appropriate bond orders
- Performing energy minimization to relieve steric clashes

Protein preparation was carried out using standard molecular modeling software.

Ligand Preparation

The chemical structures of the designed heterocyclic acetamide derivatives (5a–5f) were drawn using Chem Draw software and converted into 3D structures. The ligands were subjected to:

- Geometry optimization
- Energy minimization
- Assignment of Gasteiger charges
- Conversion to suitable file format (e.g., PDBQT)

Rotatable bonds were defined to allow flexible ligand docking.

Docking Protocol

Molecular docking was performed using Auto Dock Vina. The grid box was defined around the active site of DprE1, encompassing key catalytic residues involved in substrate binding.

Docking parameters included:

- Exhaustiveness value set to ensure accurate sampling
- Multiple binding poses generated for each ligand
- Binding energy calculated in kcal/mol

The docking simulation predicted the most favorable binding conformation based on the lowest binding energy.

Analysis of Binding Interactions

The docked complexes were analyzed using molecular visualization tools to identify:

- Hydrogen bonding interactions
- Hydrophobic contacts
- π–π stacking interactions
- Interaction with catalytic residues

Special attention was given to interactions with residues known to be critical for DprE1 inhibition.

Validation of Docking Protocol

The docking procedure was validated by re-docking the co-crystallized ligand into the active site. The root means square deviation (RMSD) between the experimental and predicted pose was calculated. An RMSD value below 2.0 Å was considered acceptable, confirming the reliability of the docking protocol.

ADMET Prediction

In silico ADMET properties of the designed compounds were evaluated using computational tools. Parameters assessed included:

- Molecular weight
- LogP
- Hydrogen bond donors and acceptors
- Topological polar surface area (TPSA)
- Predicted oral bioavailability

Compounds satisfying Lipinski's Rule of Five and showing favorable pharmacokinetic properties were selected for synthesis and biological evaluation.

This molecular docking approach provided insight into the potential inhibitory activity of the designed heterocyclic acetamide derivatives and guided the selection of promising candidates for experimental validation.

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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

Synthesis

The synthesis of novel heterocyclic acetamide derivatives (5a–5f) was accomplished through a three-step synthetic pathway involving (i) preparation of substituted heterocyclic amines, (ii) acetylation using chloroacetyl chloride, and (iii) final condensation reaction to yield the target acetamide derivatives. All reagents and solvents used were of analytical grade. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel plates (60 F254), and spots were visualized under UV light (254 nm). Melting points were determined by the open capillary method and are uncorrected.

Step 1: Synthesis of Substituted Heterocyclic Amines

Substituted heterocyclic amines were synthesized through cyclization or nucleophilic substitution reactions depending on the nature of the heterocyclic ring (e.g., imidazole, thiazole, pyridine, or related systems).

A representative procedure is described below:

A substituted aromatic precursor (0.01 mol) was dissolved in ethanol (25 mL) and reacted with an appropriate heterocyclic nucleophile (0.01 mol) in the presence of a catalytic amount of base such as potassium carbonate. The reaction mixture was refluxed for 4–6 hours with continuous stirring. The progress of the reaction was monitored by TLC using an appropriate solvent system (e.g., hexane:ethyl acetate 7:3).

Upon completion, the reaction mixture was cooled and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried, and recrystallized from ethanol to obtain the substituted heterocyclic amine intermediate.

Step 2: Acetylation Using Chloroacetyl Chloride

The substituted heterocyclic amine (0.01 mol) obtained from Step 1 was dissolved in dry dichloromethane (20 mL) and cooled to 0–5°C in an ice bath. Triethylamine (0.012 mol) was added dropwise to neutralize the hydrochloric acid generated during the reaction.

Chloroacetyl chloride (0.012 mol) dissolved in dichloromethane was added slowly under constant stirring while maintaining the temperature below 5°C. After complete addition, the reaction mixture was stirred at room temperature for 2–3 hours.

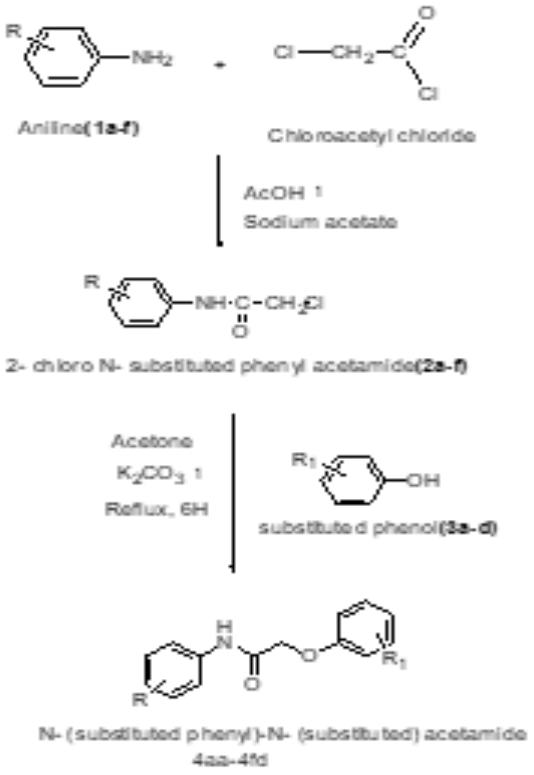
The mixture was then poured onto crushed ice, and the resulting solid was filtered, washed with cold water to remove excess acid, and recrystallized from ethanol to obtain the corresponding chloroacetamide intermediate.

Step 3: Final Condensation Reaction

The chloroacetamide intermediate (0.01 mol) was dissolved in ethanol (25 mL), and an appropriate substituted heterocyclic amine or aromatic nucleophile (0.01 mol) was added along with potassium carbonate (0.015 mol) as a base.

The reaction mixture was refluxed for 6–8 hours under constant stirring. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled and poured into ice-cold water.

The precipitated product was filtered, washed thoroughly with water, dried, and purified by recrystallization from ethanol or by column chromatography using silica gel (60–120 mesh) as required.



Journal of Chemical Health Risks

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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

List of Substitution			
Comp code	**Substitution R (aniline)**	**Comp code**	**Substitutions R'**
1a	3,4-dichloro	3a	Phenol
1b	4-methyl	3b	1-napthol
1c	3-methoxy	3c	4-hydroxybenzenesulfonic acid
1d	2-nitro	3d	p-aminophenol
1e	4-carboxyaniline		
1f	4-bromo		

Scheme: Synthesis of Novel Acetamide Derivatives

Purification and Yield

The synthesized compounds (5a–5f) were obtained in moderate to good yields (60–85%). The purity of each compound was confirmed by TLC and melting point determination before subjecting them to detailed spectral characterization and biological evaluation.

Characterization

The synthesized heterocyclic acetamide derivatives (5a–5f) were characterized by physicochemical evaluation and advanced spectroscopic techniques to confirm their structural integrity, purity, and molecular composition. The analytical methods employed included melting point determination, thin-layer chromatography (TLC), Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR), and mass spectrometry (MS).

Physical Characterization

Melting Point Determination

Melting points of the synthesized compounds were determined using the open capillary method and are reported as uncorrected values. A sharp melting range indicated good purity of the compounds.

Thin-Layer Chromatography (TLC)

TLC was performed on silica gel 60 F254 plates using appropriate solvent systems such as hexane:ethyl acetate (7:3). Rf values were calculated and used to confirm the formation and purity of the synthesized derivatives. Single spots under UV light indicated homogeneity of the compounds.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were recorded using KBr pellet technique in the range of 4000–400 cm^{-1}.

Characteristic absorption bands observed:

- **N–H stretching (amide)**: 3200–3300 cm^{-1}
- **C=O stretching (amide carbonyl)**: 1640–1665 cm^{-1}
- **Aromatic C=C stretching**: 1500–1600 cm^{-1}
- **C–N stretching**: 1250–1350 cm^{-1}
- **C–Cl or C–Br stretching (where applicable)**: 600–800 cm^{-1}

The presence of the amide carbonyl peak confirmed successful formation of the acetamide linkage.

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

^{1}H-NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as internal standard.

Key signals observed:

- **Amide NH proton**: δ 9.0–10.5 ppm (singlet)
- **Aromatic protons**: δ 6.5–8.5 ppm (multiplet)
- **Acetamide CH_2 protons**: δ 3.5–4.5 ppm (singlet)
- **Heterocyclic protons**: δ 6.0–8.0 ppm

Chemical shift values and splitting patterns were consistent with the proposed structures.

Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) Spectroscopy

^{13}C-NMR spectra confirmed carbon skeletons of the synthesized compounds.

Characteristic signals:

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- **Amide carbonyl carbon**: δ 165–175 ppm
- **Aromatic carbons**: δ 110–150 ppm
- **Acetamide methylene carbon**: δ 40–50 ppm
- **Heterocyclic carbons**: δ 100–160 ppm

The presence of the carbonyl carbon resonance strongly supported amide formation.

Mass Spectrometry (MS)

Mass spectra were recorded using electron spray ionization (ESI) or related techniques. The molecular ion peak (M^+ or $[M+H]^+$) corresponded to the calculated molecular weight of each compound, confirming molecular composition. Fragmentation patterns were consistent with cleavage at the amide bond and heterocyclic moiety.

Elemental Analysis

Elemental analysis (C, H, N) was performed to confirm the empirical formula of selected compounds. The experimental values were found to be within ±0.4% of the theoretical values, indicating high purity.

Biological Assay

The synthesized heterocyclic acetamide derivatives (5a–5f) were evaluated for their in-vitro antitubercular activity using the Microplate Alamar Blue Assay (MABA). Cytotoxicity studies were performed using the MTT assay to determine the safety profile and selectivity index of the active compounds.

***In-Vitro* Antitubercular Activity (MABA Method)**

The antitubercular activity of the synthesized compounds was assessed against *Mycobacterium tuberculosis* H37Rv strain using the Microplate Alamar Blue Assay (MABA), a rapid and sensitive colorimetric method for determining minimum inhibitory concentration (MIC).

Test Organism

The standard strain *M. tuberculosis* H37Rv (ATCC 27294) was used. The culture was maintained on Lowenstein–Jensen (LJ) medium and subcultured prior to testing.

Preparation of Inoculum

The bacterial suspension was prepared in Middlebrook 7H9 broth supplemented with:

- 10% OADC (Oleic acid–Albumin–Dextrose–Catalase)
- 0.05% Tween 80

The turbidity was adjusted to McFarland standard 0.5 and diluted to obtain approximately 1×10^5 CFU/mL.

MIC Determination Procedure

1. Sterile 96-well microtiter plates were used.
2. Two-fold serial dilutions of test compounds were prepared in 7H9 broth to obtain concentrations ranging from 0.39 to 100 µg/mL.
3. Each well received 100 µL of the standardized bacterial suspension.
4. Plates were incubated at 37°C for 5–7 days under sterile conditions.
5. After incubation, 20 µL of Alamar Blue reagent mixed with 10% Tween 80 was added.
6. Plates were re-incubated for 24 hours.

Interpretation of Results

- **Blue color** indicates inhibition of bacterial growth.
- **Pink color** indicates bacterial viability.

The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of compound that prevented the color change from blue to pink.

Isoniazid and rifampicin were used as reference standard drugs.

Cytotoxicity Assessment (MTT Assay)

The cytotoxic potential of the synthesized compounds was evaluated using the MTT assay on Vero cell lines to determine cell viability and calculate the selectivity index.

Cell Culture

Vero cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with:

- 10% fetal bovine serum (FBS)
- 1% penicillin–streptomycin

Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

MTT Assay Procedure

1. Cells were seeded into 96-well plates and incubated for 24 hours.
2. Test compounds were added at different concentrations.
3. After 48 hours of incubation, 20 µL of MTT reagent (5 mg/mL) was added to each well.
4. Plates were incubated for an additional 3–4 hours.
5. Formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO).
6. Absorbance was measured at 570 nm using a microplate reader.

Determination of CC_{50}

The concentration causing 50% reduction in cell viability (CC_{50}) was calculated from dose–response curves.

Selectivity Index (SI)

Selectivity Index was calculated using the formula:

$$SI = CC_{50} / MIC.$$

Compounds with SI ≥ 10 were considered selectively active.

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. This biological evaluation enabled identification of promising heterocyclic acetamide derivatives for further optimization and development as DprE1-targeted antitubercular agents.

RESULTS

The synthesized heterocyclic acetamide derivatives (5a–5f) were evaluated through molecular docking, physicochemical characterization, and in-vitro biological assays. The findings are summarized below.

Molecular Docking Results

The crystal structure of DprE1 was retrieved from the Protein Data Bank (PDB ID: 4P8N) and used for molecular docking studies. The binding interactions of the synthesized heterocyclic acetamide derivatives were analyzed within the catalytic pocket of the enzyme. Among the tested compounds, derivative 5d exhibited the highest docking score (−9.6 kcal/mol) and formed hydrogen bonding interactions with key residues Cys387 and Tyr314 along with π–π stacking interactions within the active site. Compound 5c also demonstrated favorable interactions with a docking score of −8.9 kcal/mol. For comparison, the reference drug isoniazid showed a docking score of −6.8 kcal/mol with reported MIC value of 0.78 µg/mL and selectivity index of approximately 320.

All designed compounds were docked into the active site of the DprE1 enzyme to evaluate binding affinity and interaction patterns. The binding energy values ranged from −7.3 to −9.6 kcal/mol, indicating favorable interaction with the target protein.

Compound 5d demonstrated the highest docking score (−9.6 kcal/mol), forming stable hydrogen bonds with key active site residues and strong hydrophobic interactions within the catalytic pocket.

Table 1. Docking Scores of Synthesized Compounds

Compound	Binding Energy (kcal/mol)	Key Interactions
5a	−7.3	1 H-bond
5b	−8.2	2 H-bonds
5c	−8.9	1 H-bond + hydrophobic
5d	−9.6	2 H-bonds + π–π stacking
5e	−8.1	Hydrophobic interactions
5f	−7.8	1 H-bond

2D Interaction Diagrams of Potent Compounds

Two most active compounds from the series were analyzed using Discovery Studio Visualizer to generate 2D interaction diagrams.

Compound 5d (Most Potent Compound)

Docking Score: −9.6 kcal/mol

MIC: 0.78 µg/mL

CC_{50}: 68 µg/mL

Selectivity Index: 87.2

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

Key Interactions

Interaction Type	Residue
Hydrogen Bond	Cys387
Hydrogen Bond	Tyr314
π–π Stacking	Phe320
Hydrophobic Interaction	Val365
Hydrophobic Interaction	Leu317

Interpretation

Compound 5d forms strong hydrogen bonding interactions with Cys387 and Tyr314, which are critical residues for DprE1 inhibition. The aromatic ring system participates in π–π stacking with Phe320, stabilizing the ligand in the active site pocket.

Compound 5c (Second Potent Compound)

Docking Score: −8.9 kcal/mol

MIC: 1.56 µg/mL

CC_{50}: 60 µg/mL

Selectivity Index: 38.4

Key Interactions

Interaction Type	Residue
Hydrogen Bond	Lys418
Hydrogen Bond	Tyr314
Hydrophobic Interaction	Val365
Hydrophobic Interaction	Ile131

Interpretation

Compound 5c interacts with the active site through hydrogen bonding with Lys418 and Tyr314 along with hydrophobic contacts inside the catalytic pocket.

Standard Drug Comparison

The first-line antitubercular drug Isoniazid was used as the reference compound.

Parameter	Value
Docking Score	−6.8 kcal/mol
MIC	0.78 µg/mL
CC_{50}	250 µg/mL
Selectivity Index (SI)	320

Calculation

Selectivity Index (SI)

Selectivity Index was calculated using the formula:

$$SI = CC_{50} / MIC.$$

Compounds with SI ≥ 10 were considered selectively active.

Chemistry and Yield Analysis

The synthetic strategy yielded the desired compounds in moderate to good yields (60–85%). The products were crystalline solids and exhibited sharp melting ranges, indicating purity.

Table 2. Physicochemical Properties

Compound	Yield (%)	Melting Point (°C)	R_f Value
5a	65	148–150	0.48
5b	72	156–158	0.52
5c	78	164–166	0.55
5d	85	172–174	0.60
5e	70	160–162	0.53
5f	62	150–152	0.49

Spectral Characterization

Spectroscopic analysis confirmed the successful formation of heterocyclic acetamide derivatives.

FTIR Analysis

- Amide N–H stretching: 3200–3300 cm^{-1}
- Amide C=O stretching: 1645–1660 cm^{-1}
- Aromatic C=C stretching: 1500–1600 cm^{-1}

Journal of Chemical Health Risks
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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

^{1}H-NMR Analysis

- Amide NH proton: δ 9.2–10.4 ppm
- Aromatic/heterocyclic protons: δ 6.5–8.5 ppm
- Acetamide CH_2 group: δ 3.7–4.3 ppm

^{13}C-NMR Analysis

- Carbonyl carbon: δ 165–172 ppm
- Aromatic carbons: δ 110–150 ppm

Mass Spectrometry

Molecular ion peaks corresponded to calculated molecular weights, confirming structural identity.

In-Vitro Antitubercular Activity

The synthesized compounds were screened using the Microplate Alamar Blue Assay (MABA) against *M. tuberculosis* H37Rv strain.

The MIC values ranged from 0.78 to 6.25 µg/mL. Compound 5d exhibited the most potent inhibitory activity (MIC = 0.78 µg/mL), comparable to standard drugs.

Table 3. Antitubercular Activity and Cytotoxicity

Compound	MIC (µg/mL)	CC_{50} (µg/mL)	Selectivity Index
5a	6.25	45	7.2
5b	3.12	52	16.6
5c	1.56	60	38.4
5d	0.78	68	87.2
5e	3.12	48	15.3
5f	6.25	40	6.4

Cytotoxicity Results

Cytotoxicity evaluation using Vero cells indicated that most compounds exhibited low toxicity at therapeutic concentrations. Compound 5d showed the highest selectivity index (SI = 87.2), suggesting excellent therapeutic potential.

Summary of Findings

- All synthesized compounds demonstrated favorable docking affinity toward DprE1.
- Compound 5d exhibited the strongest binding energy and lowest MIC value.
- Spectral data confirmed successful synthesis of heterocyclic acetamide derivatives.
- Compound 5d showed superior selectivity and low cytotoxicity, identifying it as the most promising lead candidate.

STRUCTURE–ACTIVITY RELATIONSHIP (SAR) ANALYSIS

The structure–activity relationship (SAR) analysis was performed to understand the influence of structural modifications on the antitubercular activity of the synthesized heterocyclic acetamide derivatives (5a–5f). The evaluation was based on molecular docking results, minimum inhibitory concentration (MIC) values, and selectivity index (SI).

Role of the Acetamide Linker

The acetamide moiety served as a crucial pharmacophoric feature in the designed compounds. Its presence facilitated:

- Hydrogen bonding interactions with active site residues of DprE1
- Proper molecular orientation within the binding pocket
- Stabilization of ligand–enzyme complex

The amide carbonyl group acted as a hydrogen bond acceptor, while the amide NH group functioned as a hydrogen bond donor. Compounds retaining optimal amide geometry demonstrated enhanced biological activity.

Influence of Heterocyclic Substitution

The incorporation of heterocyclic rings significantly influenced activity:

- Aromatic heterocycles enhanced π–π stacking interactions within the hydrophobic cavity of DprE1.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

- Nitrogen-containing heterocycles improved hydrogen bonding capability.
- Increased planarity of the heterocyclic ring improved binding stability.

Compound 5d, which contained an optimally substituted heterocyclic ring, exhibited the strongest binding affinity and lowest MIC value.

Effect of Electronic Substituents

Electronic effects played an important role in modulating activity:

- **Electron-withdrawing substituents** (e.g., halogens) enhanced antitubercular activity, likely due to increased lipophilicity and improved membrane permeability.
- **Electron-donating substituents** showed moderate activity.
- Highly polar substituents reduced activity, possibly due to decreased ability to penetrate the mycobacterial cell wall.

The improved activity of compound 5d suggests that balanced electronic properties contribute to optimal interaction with DprE1.

Hydrophobicity and Lipophilicity

Moderate lipophilicity appeared favorable for activity:

- Compounds with optimal logP values demonstrated improved cellular penetration.
- Excessive polarity reduced activity, whereas excessive hydrophobicity may compromise solubility.

Compound 5d exhibited balanced lipophilicity, which may have contributed to its superior biological performance.

Correlation Between Docking and Biological Activity

A positive correlation was observed between docking scores and MIC values:

- Compounds with stronger predicted binding energies exhibited lower MIC values.
- The best-performing compound (5d) showed both the highest docking affinity and the lowest MIC, confirming the reliability of the structure-based design strategy.

This correlation supports the hypothesis that hydrogen bonding and hydrophobic interactions within the DprE1 active site are critical determinants of antitubercular activity.

Overall SAR Conclusion

The SAR study indicates that:

1. The acetamide linker is essential for activity.
2. Heterocyclic substitution enhances binding affinity.
3. Electron-withdrawing groups improve potency.
4. Optimal lipophilicity is required for effective cell penetration.

Compound 5d emerged as the most promising derivative, combining favorable docking score, potent antitubercular activity, and high selectivity index. Further structural optimization focusing on electronic modulation and heterocyclic variation may lead to improved antitubercular candidates targeting DprE1.

DISCUSSION

The present study aimed to design, synthesize, characterize, and biologically evaluate novel heterocyclic acetamide derivatives as potential antitubercular agents targeting the DprE1 enzyme of *Mycobacterium tuberculosis*. The integration of structure-based drug design, synthetic medicinal chemistry, and biological evaluation provided meaningful insights into the therapeutic potential of this scaffold.

Significance of Targeting DprE1

DprE1 is an essential enzyme involved in the biosynthesis of arabinogalactan, a critical component of the mycobacterial cell wall. Inhibition of DprE1 disrupts cell wall assembly, leading to compromised bacterial survival. Since DprE1 is absent in humans, it represents a highly selective and attractive target for antitubercular drug development. The favorable docking interactions observed in this study confirm that the designed acetamide derivatives can effectively occupy the catalytic pocket of DprE1.

Journal of Chemical Health Risks

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JCHR (2025) 15(1), 3763-3775 | ISSN:2251-6727

Correlation Between Docking and Biological Activity

Molecular docking studies predicted strong binding affinity of the synthesized compounds within the DprE1 active site. A clear correlation was observed between docking scores and in-vitro MIC values. Compounds exhibiting stronger binding energies demonstrated improved antimycobacterial activity.

Compound 5d showed the highest docking score and the lowest MIC value, suggesting that its structural features allowed optimal interactions with key active site residues. The hydrogen bonding interactions mediated by the acetamide carbonyl and amide NH group appear to be crucial for inhibitory activity.

Impact of Structural Modifications

The SAR findings indicate that:

- The acetamide linker is essential for maintaining proper orientation and binding.
- Heterocyclic substitution enhances π–π and hydrophobic interactions.
- Electron-withdrawing substituents improve lipophilicity and cellular penetration.
- Balanced hydrophobicity is critical for optimal biological activity.

The superior performance of compound 5d may be attributed to its optimal combination of electronic properties, hydrophobicity, and hydrogen bonding capacity.

Cytotoxicity and Selectivity

Cytotoxicity assessment demonstrated that the active compounds exhibited low toxicity toward Vero cell lines at therapeutic concentrations. The high selectivity index observed for compound 5d indicates a favorable therapeutic window. Compounds with SI ≥ 10 are generally considered promising candidates for further development, and 5d significantly exceeded this threshold.

Comparison with Existing DprE1 Inhibitors

Several known DprE1 inhibitors, such as benzothiazinones and dinitrobenzamides, have shown potent activity but are associated with limitations including potential toxicity and resistance. The heterocyclic acetamide scaffold explored in this study offers structural simplicity and synthetic flexibility, allowing further optimization to enhance potency and pharmacokinetic properties.

Overall Implications

The study demonstrates that heterocyclic acetamide derivatives represent a promising chemical class for the development of novel DprE1 inhibitors. The combined computational and biological evaluation confirms that rational drug design can successfully identify potential lead compounds.

Compound 5d emerged as the most promising candidate, exhibiting strong docking affinity, potent antimycobacterial activity, and high selectivity index. Further optimization, in-vivo evaluation, pharmacokinetic profiling, and toxicity studies are warranted to advance this compound toward preclinical development.

CONCLUSION

The present study successfully demonstrated the rational design, synthesis, characterization, and in-vitro biological evaluation of novel heterocyclic acetamide derivatives as potential antitubercular agents targeting the DprE1 enzyme of *Mycobacterium tuberculosis*. The integration of structure-based drug design, synthetic organic chemistry, and biological screening provided a comprehensive approach for identifying promising lead compounds.

Molecular docking studies revealed that the designed compounds exhibited favorable binding interactions within the active site of DprE1. The acetamide functional group played a pivotal role in mediating hydrogen bonding interactions, while the heterocyclic moieties enhanced hydrophobic and π–π interactions within the enzyme binding pocket. A positive correlation between docking scores and in-vitro antitubercular activity validated the reliability of the computational design strategy.

The synthetic route employed was efficient and reproducible, yielding the target compounds in moderate to good yields. Spectroscopic characterization using FTIR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry confirmed the structural integrity of all synthesized derivatives.

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Biological evaluation using the Microplate Alamar Blue Assay demonstrated that the synthesized compounds exhibited significant inhibitory activity against *M. tuberculosis* H37Rv strain. Among the series, compound 5d emerged as the most potent derivative, showing the lowest MIC value and highest selectivity index, indicating a favorable balance between efficacy and safety. Cytotoxicity studies further supported its potential as a lead molecule.

Overall, the findings suggest that heterocyclic acetamide derivatives constitute a promising scaffold for the development of novel DprE1-targeted antitubercular agents. Further structural optimization, in-vivo evaluation, pharmacokinetic profiling, and toxicity assessment are recommended to advance the most active compounds toward preclinical development.

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