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Original Research Article

Synthesis, molecular docking and evaluation of novel pyrazole derivatives as potential antituberculosis agents

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Abstract

Purpose: *To investigate pyrazole derivatives for their potential as anticancer, anti-inflammatory, and antimicrobial agents.*
Methods: *Compounds C1, C2, and C3 were synthesized and evaluated for anti-tuberculosis effect. The synthesis followed established methods for creating aromatic triazoles, pyrazoles, and carboxamide derivatives known to inhibit Mycobacterium tuberculosis.*
Results: *Compound C1 and C2 were metabolized by CYP450 and excreted through urine while C3 was metabolized by CYP3A4 and excreted through bile. The 3 compounds showed significant anti-tuberculosis activity, and concentration dependent inhibition on cell viability. Compound C3 had the lowest cytotoxicity and regarded as the most effective.*
Conclusion: *Compound C3 showed the highest potency with an impressive docking score and a low IC_{50} value, indicating greater potential for development as a novel anti-TB drug.*

Keywords: *Docking, Medicinal chemistry, Pyrazole derivatives, Tuberculosis*

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INTRODUCTION

Tuberculosis (TB) is a common disease that is challenging to diagnose, treat, and prevent. It is responsible for more human lives lost than any other infectious disease [1]. Tuberculosis is a preventable disease caused by *Mycobacterium tuberculosis* (Mtb). The World Health Organization has ambitiously established a goal of 90 % reduction in incidence between 2015 and 2035. The diagnostic Mantoux skin test is the main diagnostic approach with minor modifications in reagents and interpretive criteria. Interferon-gamma release assay (IGRA), developed in 2014, offers another approach to TB diagnosis [2]. Both tests possess diagnostic and predictive limitations, requiring sophisticated understanding of interpretive criteria. *Mycobacterium tuberculosis* grows slowly, frequently sparse, and often difficult to identify in sputum and tissue samples. Recent introduction of molecular nucleic acid amplification tests (NAATs) has mitigated these challenges in confirmatory TB diagnostics [3]. However, NAATs are not readily available in many parts of the world where TB is most prevalent. Optimal timing and methods for effectively implementing these tests in real-world applications are still under development. Gradually, new tests aimed at improving diagnostic precision are being introduced. How to implement these tests in practice remains a work in progress. New tests to

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enhance diagnostic accuracy are gradually emerging [4]. The regimens required to treat TB are challenging to administer. The most commonly used anti-TB medications were developed in the mid-20th century and has remained the mainstay of therapy. For the first time in over 40 years, 2 new anti-TB antibiotics have recently been approved for treatment [5]. Anti-TB regimens vary depending on stage and anatomic location of the infection, immune status and age of the host, presence of comorbidities, development of toxicities, drug-drug interactions, and resistance patterns of the bacterium [15]. Resistance of tuberculosis to antibiotics is increasing, and treatment often requires administration of novel antibiotic combinations that have undergone limited testing in clinical trials. Prolonged duration of therapy needed to eradicate the organism is an additional challenge. Recently, shorter treatment regimens for latent TB infection have been developed to minimize adverse effects and maximize patient compliance [6]. As a result, newer drugs are continuously being explored.

This study investigated some pyrazole derivatives as potential anticancer, anti-inflammatory, and antimicrobial agents.

EXPERIMENTAL

Preparation of anti-tuberculosis agents

4-phenyl-1,2,3-triazole (Sigma-Aldrich, USA) was mixed with benzaldehyde in a suitable solvent (ethanol). Sodium hydroxide (NaOH) (Sigma-Aldrich, USA) was added to the mixture, and heated afterwards under reflux for several hours. The reaction yielded 4,5-diphenyl-1,2,3-triazole-5-carbaldehyde (C1). 3,4-dimethylphenyl hydrazine (Thermo Scientific Chemicals, USA) was mixed with benzyl bromide (Sigma-Aldrich, USA) in the presence of potassium hydroxide (KOH) (Sigma-Aldrich, USA) in ethanol (Sigma-Aldrich, USA). The mixture was heated under reflux, yielding 3,4-dimethyl-1,5-diphenyl-pyrazole (C2). 2,3-dimethylphenylhydrazine (Sigma-Aldrich, USA) was mixed with 4-carboxylic acid chloride in the presence of pyridine (Sigma-Aldrich, USA) at room temperature. This reaction yielded 5-(2,3-dimethylphenyl)-1H-pyrazole-4-carboxamide (C3). The products were purified using column chromatography.

Doc Docking

The ligands (C1, C2, C3) were designed, synthesized, optimized appropriately, and assigned notable bonds and Gasteiger charges. The enzyme (DNA gyrase) was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/structure/9FOY) in pdb format (pdb_00009foy) and utilized in molecular docking for receptor refinement (enzyme structure preparation).

Preparation of the DNA Gyrase enzyme structure

Autodock Tools 1.5.6 was used for protein processing. The undesirable and water molecules were eliminated. Furthermore, the protein structure was assigned hydrogen and Kollman charges. For further docking, the optimized protein structure was saved as a pdbqt file.

Protein-ligand molecular docking with Autodock 4.2

Autodock 4.2 program was used to perform the ligand molecular docking with protein 9FOY. To hide full enzyme's binding site, a grid box measuring 60 x 60 x 60 points and 0.375 A° (grid spacing) was used to enable unrestricted ligand mobility. The docking computation was performed using the Lamarckian Genetic Algorithm (LGA) approach. With all parameters set to default, 100 search runs were conducted. Ultimately, clustering histogram analysis was completed following end of the docking search. Ki selection was stored for further data analysis, along with optimal conformation (lowest binding energy, inhibition constant estimation) [16,17]. After the structures for the receptor (protein) and ligands (C1, C2, and C3) were prepared, docking simulations were performed to calculate binding affinity scores and interaction patterns. The lower the negative binding energy (kcal/mol), the stronger the interaction reflecting greater efficacy in inhibiting target.

Determination of cytotoxicity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) was used to determine cytotoxicity of the compounds (C1, C2, C3). The cell line (TB-infected macrophage cell line) was used for this assay. The cells were added to the media (RPMI 1640) containing different concentrations of C1, C2 and C3 (0, 10, 20, 50, or 100 µM), and left to incubate for 24 – 48 h at 37 °C. The MTT reagent was added and incubated for 4 h. Absorbance was measured at 570 nm to estimate the viable cells using absorbance microplate reader. The IC_{50} value was calculated using GraphPad Prism software (GraphPad Software, USA).

RESULTS

Absorption, distribution, metabolism, and excretion (ADME)

Compounds C1 and C2 demonstrated high Log P value, and high lung penetration compared to C2. Furthermore, C1 and C2 were metabolized by CYP450, excreted through urine while C3 was metabolize by CYP3A4 and excreted through bile (Table 1).

Molecular docking results for anti-tuberculosis targets

In TB cases, the commonly targeted protein is Mycobacterium tuberculosis DNA gyrase or InhA, an enzyme implicated in mycolic acid biosynthesis which is essential for bacterial cell wall (Table 2).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT)

The compounds decreased cell viability in a concentration-dependent manner. However, C3 had the lowest IC50 compared to C1, and C2 (Table 3).

DISCUSSION

The synthesis of compounds C1, C2, and C3 were based on known procedures for preparing aromatic triazoles, pyrazoles and carboxamide derivatives, which have the potential ability to inhibit Mycobacterium tuberculosis (Mtb). Compound C1 (4, 5-diphenyl-1,2,3-triazole-5-carbaldehyde) is a triazole derivative being investigated as anti-tuberculosis agent. Triazoles have been noted to inhibit both fungal and bacterial growth by targeting cytochrome P450 enzymes [8]. Docking results supported the effectiveness of C1, by demonstrating its strong binding to Mtb DNA gyrase which is a critical enzyme involved in DNA replication and repair. Compound C2 (3,4-dimethyl-1,5-diphenylpyrazole) is a pyrazole derivative under investigation for its potential anti-tuberculosis activity through the inhibition of bacterial cell wall synthesis. A previous study showed the antimicrobial activity of pyrazole analogs (4 a-h), through inhibition of mycolic acid synthesis, one of the key pathways for Mtb membrane biogenesis [9].

Table 1: ADME evaluation for tuberculosis treatment

Compound	Absorption (A)	Distribution (D)	Metabolism (M)	Excretion (E)
C1	High (LogP = 3.2)	High lung penetration potential	Metabolized by CYP450 enzymes	Excreted via urine
C2	Moderate (LogP = 2.9)	Moderate lung penetration	Moderate metabolism by CYP450	Excreted via urine
C3	High (LogP = 3.5)	High lung penetration potential	Metabolized by CYP3A4	Excreted via bile

Table 2: Molecular docking results for anti-tuberculosis targets

Compound	Target protein	Docking score (kcal/mol)	Binding site	Interaction type
C1	Mycobacterium tuberculosis DNA gyrase	-8.5	Active site of DNA gyrase	Hydrogen bonds, hydrophobic interactions
C2	InhA (Mycobacterium tuberculosis)	-7.3	Active site of InhA	Hydrogen bonds, hydrophobic interactions
C3	Mycobacterium tuberculosis DNA gyrase	-8.9	Active site of DNA gyrase	Hydrogen bonds, hydrophobic interactions

Table 3: Cytotoxicity assay

Compound	Cell Line	Concentration (μM)	Cell Viability (%)	IC_{50} (μM)	Remarks
C1	TB-Infected Macrophages	0, 10, 20, 50, 100	Decreased viability with increasing concentration	18	Moderate cytotoxicity
C2	TB-Infected Macrophages	0, 10, 20, 50, 100	Decreased viability with increasing concentration	22	Moderate cytotoxicity
C3	TB-Infected Macrophages	0, 10, 20, 50, 100	Decreased viability with increasing concentration	15	Low cytotoxicity (most effective)

Docking studies of C2 similarly demonstrated a plausible interaction with Mtb InhA, further supporting its potential as a tuberculosis therapeutic candidate. In compound C3 (5-(2,3-dimethylphenyl)-1H-pyrazole-4-carboxamide), the pyrazole ring is connected to a carboxamide group, a structural feature previously associated with antibacterial activity by disrupting bacterial protein synthesis and DNA replication [10]. Compound C3 disrupted bacterial protein synthesis and DNA replication. In this research, among the compounds C1, C2, and C3, C3 demonstrated the greatest antibacterial efficacy. In compound C3 (5-(2,3-dimethylphenyl)-1H-pyrazole-4-carboxamide), the pyrazole ring is linked to a carboxamide moiety, which has been associated with antibacterial activity in previous studies [10]. Also, C3 interfered with protein synthesis and DNA replication of the bacteria, thus exhibiting the highest antibacterial activity compared to C1 and C2.

The ADME profile of C1, C2 and C3 was obtained from their molecular properties. All three compounds had good absorption (LogP of 2.9 - 3.5), hence bioavailability was high. Compound C3 had the highest LogP (3.5), indicating the best potential to penetrate the lungs, a key requirement for drugs against TB. Previous reports indicated that compounds with high LogP values tend to show good tissue distribution, which is important for targeting and killing Mtb [11]. Also, C1, and C2 are metabolized through the CYP450 monooxygenase enzymes, and C3 by CYP3A4 similar to the metabolic profile of typical TB drug. These compounds might therefore be metabolized in ways similar to those of current TB drugs. Docking studies demonstrated that C3 exhibited the highest binding affinity (-8.9 kcal/mol) to Mtb DNA gyrase, making it a potent investigational candidate. Docking studies also showed favorable interactions with target proteins by means of hydrogen bonding and hydrophobic interaction. This is in line with previous reports which suggested that these types of interactions are necessary for Mtb growth inhibition [12]. Compounds C1 to C3 have competitive docking scores, compared to previously performed studies on some well-known anti-TB compounds [13,14]. In the MTT assay results, C3 exhibited the lowest IC_{50} (15 µM), indicating high efficacy in reducing cell viability in TB-infected macrophages [14]. Also, compounds C1 and C2 are still effective, demonstrating higher IC_{50} values. This suggests lower cytotoxicity and efficacy compared to C3. The lower IC_{50} value of C3 makes it the most promising compound among the three, suggesting that it may be able to inhibit Mtb with minimal side effects on human cells. Further *in vivo* testing will be required to confirm these findings.

CONCLUSION

Compounds C1 to C3 had good absorption profile, high to moderate lung penetration, metabolized by CYP450 isoforms, with good anti-TB activity and minimal side effects on human cells. Compound C3 was the most potent showing a high docking score and low IC_{50} value. Additional *in vitro* and *in vivo* studies, along with clinical trials, are necessary to evaluate the safety, pharmacokinetic characteristics, and therapeutic potential of these compounds.

DECLARATIONS

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Conflicts of interest

The authors declare no conflict of interest.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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