Synthesis, Antitubercular Activity, and Molecular Docking Studies of Benzyl-modified 8-hydroxyquinolines

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RESEARCH ARTICLE

Abstract

Background: Infection with *Mycobacterium tuberculosis,* the causative agent of TB, is responsible for one of the global epidemics. Thus, new drugs are needed that do not confer cross-resistance with currently administered front-line therapeutics. Quinoline-based natural products and synthetic derivatives have been extensively explored for antitubercular activity.
Objective: The main goal of this study was to prepare a collection of benzylated 8-hydroxyquinoline derivatives through synthesis and assess their antitubercular activity along with a molecular docking study to clarify their biological mechanism of action.
Methodology: The benzylated 8-hydroxyquinoline derivatives were synthesized using Williamson synthesis methods. Antitubercular activity was assessed against fast replicating *M. tuberculosis* $H_{37}Rv$ using Microplate Alamar Blue Assay (MABA) and non-replicating cultures using Low-Oxygen Recovery Assay (LORA). Molecular docking studies were carried out against enoyl-acyl carrier protein reductase (InhA).
Results: Five benzylated 8-hydroxyquinoline derivatives were synthesized in moderate yields and characterized using NMR spectroscopy. MABA and LORA assays indicate compounds **3–5** as the most inhibitory derivatives with MIC_{90}'s ranging from 6.38 to 54.28 µM. Molecular docking against InhA showed modest binding energies for compounds **4** (-8.5 kcal/mol) and **5** (-8.6 kcal/mol).
Conclusion: Findings suggest a rationale for the further evolution of this promising series of antitubercular quinoline small molecules. Structure-activity analysis shows that an 8-benzyl moiety with chlorine atom/s is important for improved activity against replicating and non-replicating *M. tb*. $H_{37}Rv$. This is also supported by our *in silico* studies.

Keywords: *Antitubercular, Mycobacterium tuberculosis, quinolines, molecular docking, enoyl-acyl carrier protein reductase.*

Introduction

Pulmonary tuberculosis (TB) is one of the most common infectious diseases that continue to cause major morbidity and mortality worldwide. It is a severe contagious disease caused by *Mycobacterium tuberculosis* that most often affects the lungs but can also affect other sites of the body [1]. Currently, it is the leading infectious disease in the world [2]. Despite the availability of tuberculosis treatment regimens, approximately 9 million new cases of TB are reported annually, and 1.5 million cases were found to cause morbidity [3]. Worst epidemiological scenarios have been noted in Third World countries including the Philippines where the infections range from 100–300 cases per 100,000 inhabitants [4]. The emergence of multi-drug resistant (MDR) strains, which are insensitive to one or more of the first-line drugs, Isoniazid, and Rifampicin, has worsened the problem

and created an urgent search for alternative drug treatments for *M. tuberculosis* infections. Moreover, mycobacterial resistance to Rifampicin, Isoniazid, quinolones, and aminoglycosides has increased and is labeled extensively drug-resistant tuberculosis (XD-TB) [5]. Another problem arising is the bad synergistic effect between antiviral drugs used in patients infected with the HIV virus and antitubercular drugs [6]. Thus, an intensive search for new and effective antimycobacterial drugs is currently an interest for medicinal chemists involved in TB drug discovery.

The identification of TMC207, an anti-TB compound under Phase II clinical trials with a novel biological action, has gained attention to investigate quinoline as a promising scaffold to discover and develop new antimycobacterial drugs (Figure 1) [7]. This further heightened the interest of uncovering new quinoline-based antimycobacterial drugs and has spurred the discovery of numerous quinoline derivatives and analogs through synthesis and assessment of their efficacy against drug-sensitive and multi-drug-resistant mycobacterial species. Interestingly, a number of quinoline alkaloid natural products has been observed to exhibit antituberculosis activity [8]. For example, three quinoline alkaloids from the Philippine endemic plant, *Lunasia amara* Blanco, displayed low MIC against *Mycobacterium tuberculosis* $H_{37}Rv$ *in vitro* [9,10,11].

As part of efforts to discover new antituberculosis compounds through diversity-oriented synthesis and natural products isolated from Philippine medicinal plants, this study aimed to perform the synthesis, characterization, and *in vitro* evaluation against replicating and non-replicating *Mycobacterium tuberculosis* $H_{37}Rv$ of a series of benzylated 8-hydroxyquinoline derivatives [12,13]. Molecular docking study was also performed against mycobacterial enoyl-acyl carrier protein reductase (InhA), a drug target of Isoniazid and some quinoline-based antituberculars and an integral enzyme in mycolic acid biosynthesis of mycobacterial cell wall [14]. It envision that the attachment of a benzyl group will provide new congeners with enhanced anti-TB activity as previously predicted to bind to hydrophobic catalytic triads of mycobacterial ketoacyl synthase carrier proteins [15].

Methodology

General Considerations

All chemicals used for the synthesis were purchased from Sigma-Aldrich and used without further purification. The ^1H NMR spectra were recorded on a Bruker Avance AMX-500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) in $CDCl_3$. The chemical shifts are given in δ (ppm) scale using tetramethylsilane (TMS) as the internal standard reference. The homogeneity of the newly obtained compounds was confirmed by TLC on silica gel 60 F_{254} plates (Merck) with UV visualization (254 and 365 nm) and Dragendorff's reagent spray staining. Compounds **3** and **5** have been published previously [16,17].

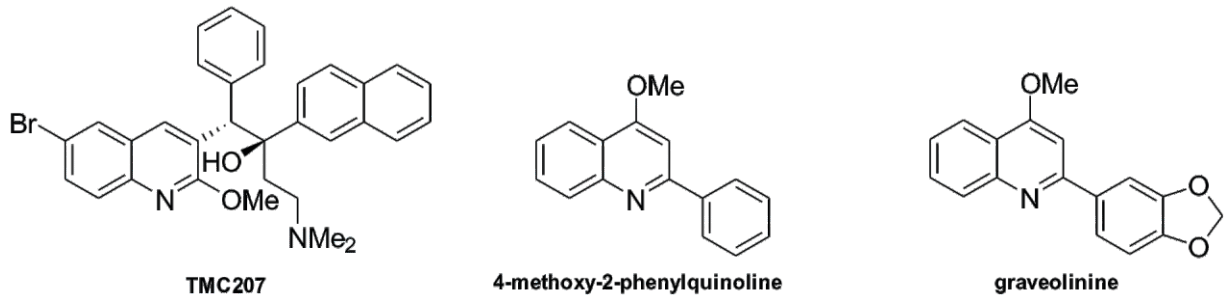

Figure 1. *Structure of antitubercular TMC207 and Lunasia amara alkaloids.*

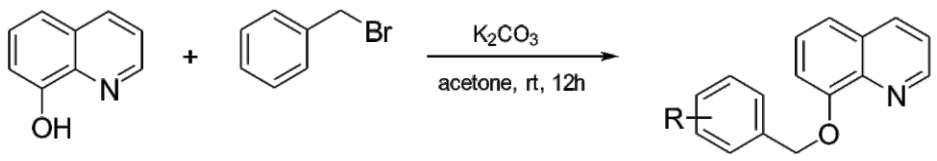

Figure 2. *Reaction scheme for the synthesis of benzylated 8-hydroxyquinolines **1–5**.*

General Procedure for the Synthesis of Benzylated 8-hydroxyquinoline Derivatives

A mixture of 8-quinolinol (1 equiv), benzyl halide (1.2 equiv), potassium carbonate (2 equivs), and acetone (6 mL/equiv of 8-quinolinol) was added to a round-bottom flask equipped with a magnetic stirring bar and stirred in a water bath at room temperature for 12 hours. The reaction mixture was monitored by thin-layer chromatography (2:1 hexane-ethyl acetate). After the reaction was completed, the solution was filtered and the residue was rinsed with ethyl acetate. The organic solution was evaporated using a rotary evaporator (45 °C) and the crude organic reaction mixture was dissolved with 1:1 ethyl acetate-hexanes. The concentrated residue was passed through a column of silica gel 60 (Merck 1.07734) and eluted with 4:1 ethyl acetate-hexane. The product was concentrated and subjected to ^{1}H NMR spectroscopic analysis as follows:

4-[(quinolin-8-yloxy) methyl] benzonitrile **(1)**. 24% isolated yield. ^{1}H NMR (500 MHz, Chloroform-*d*) d 8.89 (m, 1H), 8.10 (m, 1H), 7.31-7.45 (m, 3H), 7.23-7.29 (m, 2H), 7.10-7.19 (m, 3H), 5.47 (s, 2H).

8-{[4-(methylsulfanyl) benzyl]oxy} quinoline **(2)**. 11% isolated yield. ^{1}H NMR (500 MHz, Chloroform-*d*) d 9.00 (s, 1H), 8.17 (d, J = 8.0 Hz, 1H), 7.64 – 6.88 (m, 8H), 5.41 (s, 2H), 2.47 (s, 3H).

8-[(3-chlorobenzyl) oxy] quinoline **(4)**. 22% isolated yield. ^{1}H NMR (500 MHz, Chloroform-*d*) d 8.88 (br s, 1H), 8.02-8.05 (m, 1H), 7.32-7.44 (m, 5H), 7.15-7.20 (m, 4H), 6.91-6.95 (m, 1H), 5.30 (s, 2H).

Determination of Minimum Inhibitory Concentration (MIC_{90}) using Microplate Alamar Blue Assay (MABA) and Low-Oxygen Recovery Assay (LORA)

The benzylated 8-hydroxyquinoline derivatives **1–5** were subjected to MABA and LORA assays against *M. tuberculosis* [18]. RMP, INH, and TMC were used as positive drug standards. A primary screen was conducted at 64 μg/mL against *M. tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay. Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested against *M. tuberculosis* H_{37}Rv at lower concentrations in order to determine the actual MIC_{90} in the MABA. The MIC_{90} was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Screening for the activity of the compounds against bacteria in the non-replicating state that models clinical persistence used an 11-day high-throughput luminescence-based low-oxygen-recovery assay (LORA), where *M. tb* containing a plasmid with an acetamidase promoter driving a bacterial luciferase gene was first adapted to low oxygen conditions by extended culture. The MIC_{90} was determined as in the MABA method.

Determination of 50% Inhibitory Concentrations (IC_{50}) versus VERO cells

Concurrent with the determination of MIC_{90}s, compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations less than or equal to 64 μg/mL. After 72 hours of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the PromegaCellTiter 96 Non-radioactive Cell Proliferation Assay. The Selectivity Index (SI = IC_{50}/MIC_{90}) was also determined [18].

Molecular Docking Studies

The benzylated 8-hydroxyquinoline derivatives **1-5** were subjected to molecular docking simulations with the enoyl-acyl carrier protein reductase (InhA) (PDB ID: 2PR2) to assess their binding characteristics. The enzymes were fetched from the RCSB protein data bank. USCF Chimera was used to facilitate the removal of bound residues and minimization of structures. Both ligand and protein structures were prepared using Antechamber and molecular docking was performed using the BFGS algorithm of AutoDock Vina, setting up a grid at the binding site of the positive control, Isoniazid-NAD adduct [19,20]. The control was likewise docked onto InhA for validation of the docking protocol. The resulting conformational protein-ligand structure was visualized and analyzed using Biovia Discovery Studios®.

Results

The compounds were prepared following the Williamson ether synthesis reaction using 8-hydroxyquinoline and benzyl halide derivatives as starting materials. The structures of benzylated 8-hydroxyquinoline derivatives **1–5** are shown in Figure 3. The benzylated 8-hydroxyquinoline derivatives are 4-[(quinolin-8-yloxy) methyl] benzonitrile **(1)**, 8-{[4-(methylsulfanyl) benzyl]oxy} quinoline **(2)**, 8-[(4-methoxybenzyl)oxy] quinoline **(3)**, 8-[(3-chlorobenzyl)oxy] quinoline **(4)**, and 8-[(2, 6-dichlorobenzyl)oxy] quinoline **(5)** [16,17].

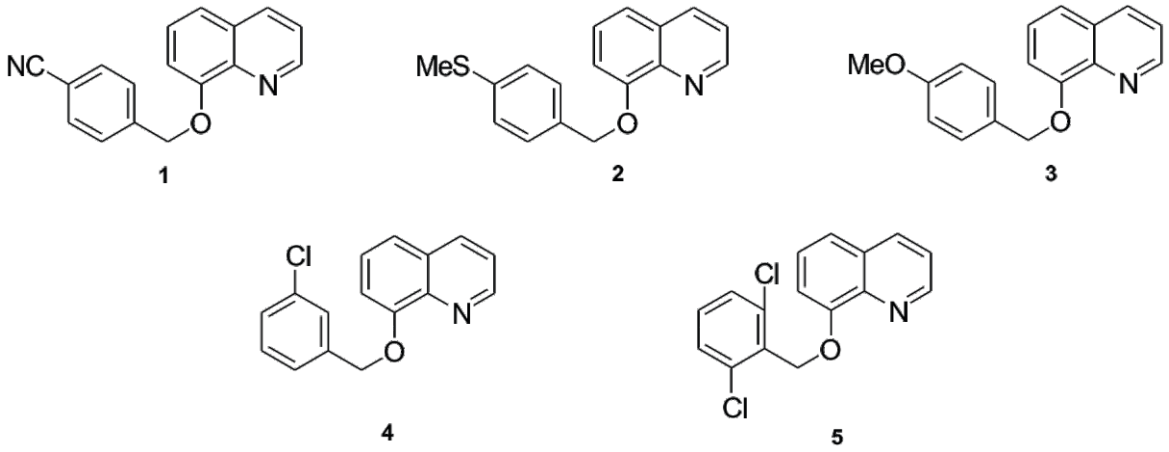

Figure 3. *Structure of benzylated 8-hydroxyquinolines **1–5**.*

These compounds were subjected to Microplate Alamar Blue Assay (MABA) and Low Oxygen Recovery Assay (LORA) [18] to test their growth inhibition against *Mycobacterium tuberculosis* H_{37}Rv. The MICs of benzylated 8-hydroxyquinoline derivatives **1–5** in inhibiting *M. tuberculosis* H_{37}Rv are shown in Table 1. All derivatives exhibited potent inhibitory activity ≥ 92% at 64 µg/mL. Compound **5** showed the most potent inhibitory activity among the other benzylated 8-hydroxyquinoline derivatives with MABA MIC_{90} of 6.38 µM. The LORA MIC_{90} also showed quinoline derivative **5** as the most inhibitory congener at 12.49 µM.

Quinolines **1–5** were screened for their cytotoxicity (IC_{50}) against VERO cells (Table 2). Based on the cytotoxicity screening results, the benzylated 8-hydroxyquinoline derivatives, **3** and **5**, were determined to have Selectivity Indices (SI's = IC_{50}/MIC_{90}) of 12.34 and 10.93 (based on MABA), respectively.

A molecular docking approach was done to simulate and visualize the protein-ligand interactions of all the synthesized quinoline derivatives against enoyl-acyl carrier protein reductase (InhA, PDB ID: 2PR2). Table 3 shows the various

Table 1. *Minimum inhibitory concentration* (MIC_{90}) *of **1–5** versus Mycobacterium tuberculosis H_{37}Rv.*

Compound	MABA MIC_{90} (µg/mL)[a]	LORA MIC_{90} (µg/mL)[a]
1	58.40	56.86
2	101.29	83.52
3	14.51	54.28
4	14.46	24.99
5	6.38	12.49
INH[b]	3.50	2.48
RMP[c]	0.04	>311
TMC207	0.09	0.40

[a]n = 3
[b]INH = Isoniazid; [c]RMP = Rifampin.

Table 2. *Cytotoxicity and selectivity indices (SI) of benzylated 8-hydroxyquinolines **1–5**.*

Compound	Ic_{50} vs. Vero cell (µM)[a]	SI vs. MABA	SI vs. LORA
1	>245	4.20	4.32
2	159.51	1.58	1.91
3	179.04	12.34	3.30
4	74.44	5.15	2.98
5	69.15	10.93	5.58

[a]n = 3

Table 3. *Summary of ligand interactions and docking scores of benzylated 8-hydroxyquinolines **1–5** against enoyl-acyl carrier protein reductase (PDB ID: 2PR2)*

Interactions	Interacting amino acids of enoyl-acp reductase (PDB ID: 2PR2)				
	1	2	3	4	5
π-π stacking	Phe^{41}	Phe^{41}	Phe^{41}	Phe^{41}	Phe^{41}
π-σ	Ile^{16} Ile^{95} Ile^{122}	Ile^{16} Ile^{95}	Ile^{16} Ile^{95}	Phe^{41} Ile^{95} Ile^{122}	Phe^{41} Ile^{95} Ile^{122}
π-alkyl/alkyl	Ile^{95}	Val^{65} Ile^{95} Ile^{122}	Val^{65} Ile^{95} Ile^{122}	Ile^{16} Ile^{47} Val^{65} Ile^{95}	Ile^{16} Val^{65} Ile^{95}
conventional hydrogen bond	Arg^{43}				
binding energy (kcal/mol)	-8.3	-8.0	-7.0	-8.5	-8.6

protein-ligand interactions of the quinoline derivatives upon docking with InhA. The 2D binding diagrams for each docked complex are shown in Figure 4. The docking scores from the best ligand pose for each compound in the enzyme's binding region are also reported, with binding energies ranging from -7.0 kcal/mol to -8.6 kcal/mol.

Discussion

Heterocyclic systems bearing the quinoline nuclei represent privileged structural moieties in medicinal chemistry and are ubiquitous sub-structures associated with many biologically active natural products [21]. Quinoline derivatives are known to

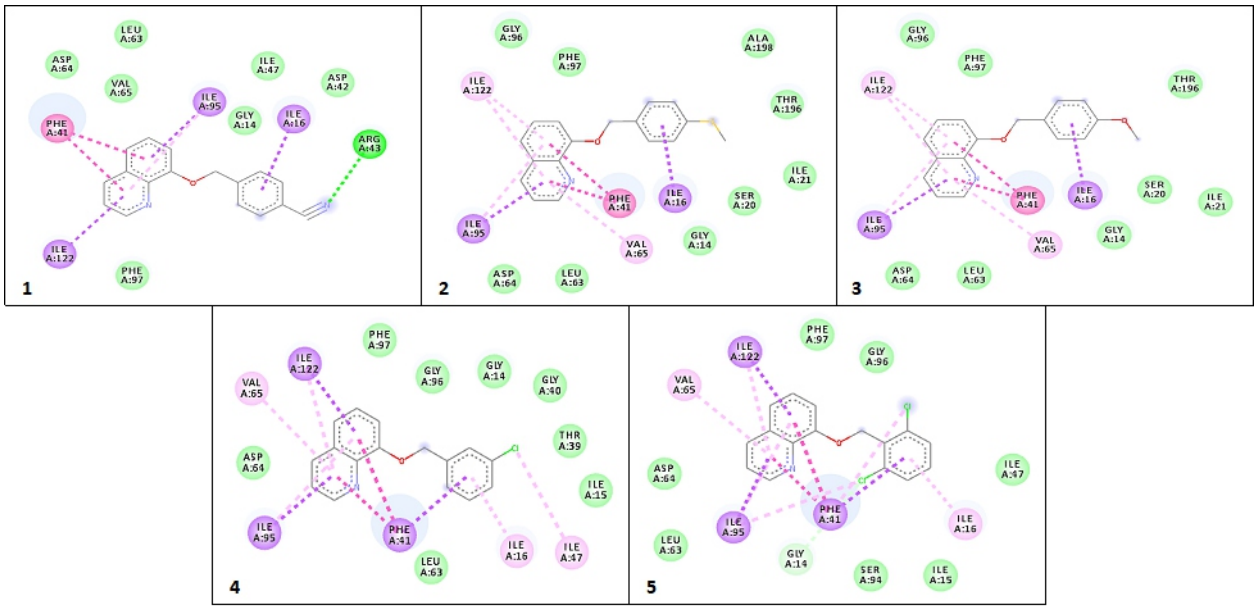

Figure 4. *2D Binding diagram of compounds **1 – 5** against enoyl-acp reductase (PDB ID: 2PR2)*

exhibit a wide range of biological activities and represent interesting rigid models for pharmacological studies, including studies for developing new TB drugs. They have been considered a pharmacophore for the design of anti-TB agents [14].

Compounds **1–5** were screened for inhibitory activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) using Microplate Alamar Blue Assay (MABA) for fast replicating strains and Low Oxygen Recovery Assay (LORA) for non-replicating cultures. These assays are non-toxic, uses thermally stable reagents, and shows good correlation with BACTEC radiometric methods [22]. In the LORA assay, determination was performed against low oxygen adapted *M. tuberculosis* $H_{37}Rv$ luxAB carrying a luciferase reporter gene following 10 days incubation under low oxygen followed by 28 hours of normoxic recovery. The identification of inhibitors of the non-replicating state provides tools that can be used to probe the hypothesis that during *Mycobacterium tuberculosis* infection, a population of bacteria is thought to exist in a non-replicating state, refractory to antibiotics, which may contribute to the need for prolonged antibiotic therapy. The development of such inhibitors also has the potential to shorten the duration of antibiotic therapy required [23].

Quinoline derivatives such as 8-hydroxyquinoline, 4-methoxy-2-phenylquinoline, and an alkaloid from *Lunasia amara*, are known to display potent antitubercular activities *in vitro* [9-11]. 8-Hydroxyquinoline was reported to possess a strong antimycobacterial activity more potent than the antibiotic nitroxoline (5-nitro-8-hydroxyquinoline) against the growth of *Mycobacterium bovis* BCG [24].

Among the benzylated 8-hydroxyquinoline derivatives, quinoline **5** exhibited the most potent activity with MABA MIC_{90} of 6.38 µM while compound **2** showed weakest anti-TB activity (MABA MIC_{90} = 101.29 µM). The MIC data clearly shows that antimycobacterial activity of benzylated 8-hydroxyquinoline derivatives is significantly affected by the type of substituents present in the aromatic ring. The presence of chlorine atoms significantly enhances anti-TB activity as shown by the low MIC_{90}'s observed for compounds **4** and **5** in both *in vitro* assays. Interestingly, the attachment of two chlorine at the C-2 (or *ortho)* position in the benzene ring of the benzyl moiety has amplifying effects. Several studies have shown that antimycobacterial quinolines incorporating chlorine atoms on a benzyl moiety are essential for antimycobacterial activity especially that they interact with the catalytic triad of ketoacyl synthetase carrier proteins [15]. Comparing the activity of compounds **1** and **3**, the presence of a *p*-methoxy group in the benzyl group enhances inhibition against fast replicating *M. tb* $H_{37}Rv$ over a *p*-cyano group – but not in non-replicating species. Overall, the presence of a thiomethyl ether group in the para position significantly weakens activity.

The Selectivity Index ($SI = IC_{50}/MIC_{90}$) was also determined based on the cytotoxicity screening results. An SI greater than 10 is considered significant [25]. Derivatives with antimycobacterial SI's higher than 10 and MICs less than 10 µg/mL would be considered promising leads for further investigations in the development of new antitubercular drugs. Thus, compounds **3** and **5**, are remarkable scaffolds for drug development due to their strong antimycobacterial activity, low toxicity against VERO cells, and antimycobacterial selectivity indices higher than 10.

The NADH-dependent InhA is a known target for the frontline anti-tubercular pro-drug, Isoniazid which requires activation through formation of an adduct with NAD. Several drug prototypes containing the quinoline nucleus have been investigated as InhA inhibitors and are thought to proceed without the requirement for activation [26]. Thus, the synthesized benzylated 8-hydroxyquinoline derivatives were evaluated for *in silico* binding with InhA to show the various ligand interactions and their corresponding estimated binding energy. Interestingly, the results revealed that the compounds were bound to a different binding site of the enzyme and not to its catalytic site (Tyr^{158} and Lys^{165}) [27,28]. Contrary to the binding characteristics of Isoniazid, compounds **1-5** were interestingly attached to the NADH binding region of the enzyme which may still be a possible site for enzyme inhibition as the reductive catalytic mechanism of InhA is NAD-dependent [29,30]. In terms of ligand interactions, the quinoline core of all the derivatives similarly interacted with Ile^{95} and Ile^{122} through π-σ/π-alkyl and with Phe^{41} through π-π-stacking. Also, the benzyl ring of each derivative was stabilized by either π-σ or π-alkyl interaction with Ile^{16}. The presence of various substituents in the benzyl ring led to non-hydrophobic interactions unique to each compound. Among the derivatives, 3-chloro- **(4)** and 2,6-dichlorobenzylated **(5)** 8-hydroxyquinolines were the highest scoring ligands, with binding energies of -8.5 kcal/mol and -8.6 kcal/mol, respectively. This finding, correlates to the results of the MABA and LORA assays highlighting the importance of chlorine atoms on the benzyl moieties to boost antitubercular activity. The binding energy of the Isoniazid-NAD adduct (-10.9 kcal/mol) was found, however, to be higher compared to the tested compounds.

In conclusion, short and simple benzylation derivatization approaches on 8-quinolinol enabled the preparation of

antituberculosis, low-molecular weight quinoline derivatives. The study established the discovery of new types of quinoline analogues with significant and promising antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$. Structure-activity analysis revealed that an 8-benzyl moiety with chlorine atom/s is important for improved activity against replicating and non-replicating *M. tb.* $H_{37}Rv$. In addition, the strongly inhibiting compounds **3** and **5** were observed non-toxic on mammalian cell model with good selectivity indices. The *in silico* results are consistent with *in vitro* assays in terms of corroborating the hypothesized and observed improved activity of chloro-substituted benzyl quinoline derivatives as antituberculosis agents. Optimization of antitubercular activity of the two most active compounds is ongoing in our laboratories.

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