


(2Z)-3-Hydroxy-3-(4-R-Phenyl)-Prop-2-Enedithioic Acids as New Antituberculosis Compounds

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Background: Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. Compounds including a sulfur-containing scaffold have been shown to be key scaffolds in various antituberculosis agents. Interestingly, the 3-hydroxy-3-phenyl-prop-2-enedithioic acids **11a-j** have, to the best of our knowledge, not been previously described as antituberculosis agents.

Purpose: In the present study, we investigated the role of substituents attached to the phenyl ring of a 3-hydroxy-3-phenyl-prop-2-enedithioic acid scaffold (compounds **11a-j**) in inhibiting the growth of *M. tuberculosis* strain H37Rv.

Methods: (Z)-3-hydroxy-3-(4-R-phenyl)-prop-2-enedithioic acids **11b-j**, with R groups including various electron-donating or electron-withdrawing groups, were designed by structurally modifying the lead compound **11a**. The syntheses of **11a-j** involved each one-step procedure starting from the corresponding substituted acetophenone. Compounds **11a-j** were tested against *M. tuberculosis* strain H37Rv to evaluate their bacterial growth inhibitory activities. ADMET profiles were predicted by employing three different methods. In addition, molecular docking studies were carried out, based on the molecular similarities of the synthesized compounds with ethionamide (**5**), on the active site of the *M. tuberculosis* H37Rv (3R)-hydroxyacyl-ACP (HadAB) dehydratase heterodimer.

Results: The antituberculosis activities of compounds **11a-j** could be explained in terms of the presence of electron-donating or electron-withdrawing substituents on the aromatic ring of the substituted 3-hydroxy-3-phenyl-prop-2-enedithioic acid core. The activity and selectivity index (SI) value of (Z)-3-hydroxy-3-(4-nitrophenyl)-prop-2-enedithioic acid **11e** suggested that this compound could be used for the design of novel antituberculosis agents. Most of the synthesized molecules showed an acceptable ADME profile and a low probability of being toxic. Docking studies of **11d** and **11e** showed them forming hydrogen bonds with the ACys61 residue of the HadAB enzyme.

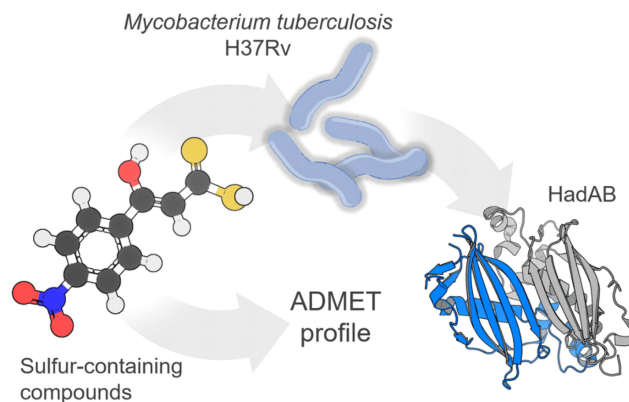
Conclusion: Our results suggested that the antituberculosis compound **11e** could be used for the design of novel antituberculosis agents.

Keywords: synthesis, 3-hydroxy-3-(4-R-phenyl)-prop-2-enedithioic acids, antituberculosis agents, docking, theoretical ADME

Introduction

Mycobacterium tuberculosis is a pathogenic bacterium that is well known to be the causative agent of tuberculosis. The World Health Organization (WHO) has pinpointed this pathogen as one of the two leading causes worldwide of higher mortality resulting from an infectious agent. The increase in the number of cases of tuberculosis, the presence of *M. tuberculosis* strains showing resistance to first-line drugs, and the adverse effects or long treatment durations of current

Graphical Abstract



tuberculosis medications have increased concerns about the ability to find a safe and effective treatment for this disease but at the same time have led researchers in medicinal chemistry to search for new compounds that are more effective against this disease.¹ Most drugs launched in the last decade were derived by modifying known drugs or lead structures, and structural modification has been shown to be an effective approach for understanding the mechanism of drug action and for designing better drugs.^{2,3}

A sulfur-containing scaffold, for example, is a key structural feature present in most compounds displaying antituberculosis activity (Figure 1). Several drugs targeting various diseases contain this scaffold, including the anticoagulant agent rivaroxaban **1**,⁴ the prostate cancer drug enzalutamide **2**,⁵ and disulfiram **3**, which is used for the treatment of alcohol abuse.⁶ Compounds containing a sulfur-containing scaffold and displaying antituberculosis activity, however, have not been reported on much, but with examples that have been reported including

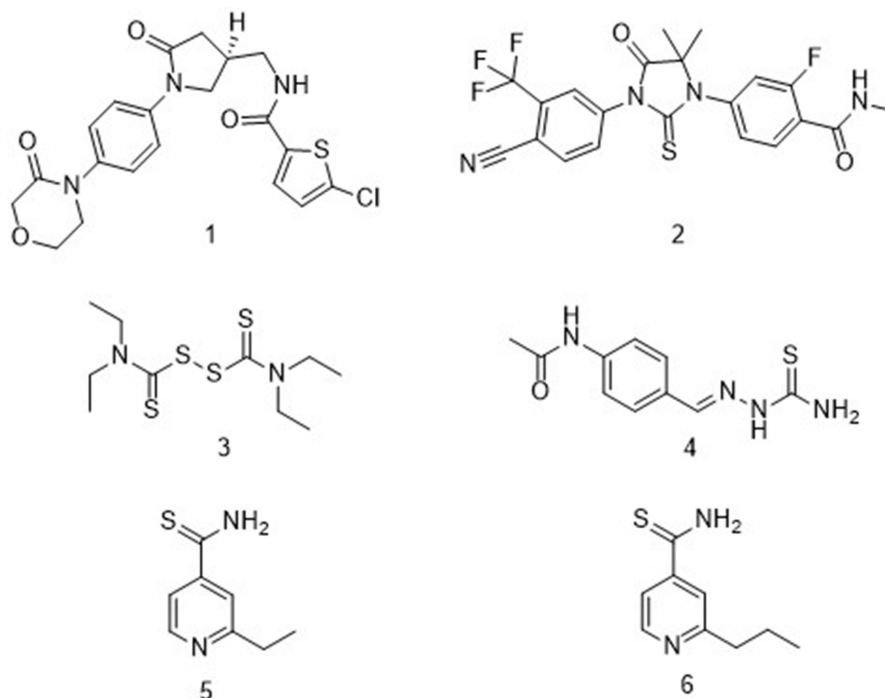


Figure 1 Examples of compounds that both contain a thio scaffold and exhibit biological activity.

thioacetazone **4**, ethionamide **5**, and prothionamide **6** as prodrugs.⁷ In the present study, we investigated the role of substituents attached to the phenyl ring of a 3-hydroxy-3-phenyl-prop-2-enedithioic acid scaffold (to form compounds **11a–j**) in inhibiting the growth of *M. tuberculosis* strain H37Rv. Compounds **11a–j** have, to the best of our knowledge, not been previously described as antituberculosis agents—but some of them were found in the current work to display antituberculosis activity.

Materials and Methods

Melting points were measured for the compounds in open capillaries using a Mel-Temp apparatus. ¹H-NMR spectra were recorded using a 300 MHz Jeol Eclipse spectrometer with the samples dissolved in deuterated chloroform (CDCl₃) solutions containing tetramethylsilane (TMS) as an internal standard ($\delta = 0$ ppm), and ¹³C-NMR spectra were recorded at 75 MHz using the same instrument. Regarding these NMR spectra, the chemical shift (δ) values are reported in parts per million (ppm), and the peak shapes are indicated as s for singlet, d for doublet, t for triplet, q for quartet, m for multiplet, and bs for broad signal. The coupling constant (*J*) values are reported in hertz (Hz). IR spectra were obtained using a Magna-IR spectrometer. Mass spectra were recorded using a Jeol AccuTOF Direct Analysis in Real Time (DART) spectrometer with time-of-flight detection for low- and high-resolution measurements. Flash column chromatography was carried out using silica gel 60 (230–400 mesh ASTM) from Macherey–Nagel GmbH & Co. Reactions were monitored using thin-layer chromatography (TLC). The TLC plates were visualized using a dual short-wavelength/long-wavelength UV lamp or by staining them with an ethanol solution of potassium permanganate, vanillin, or *p*-anisaldehyde. The reagents **12a–j**, sodium hydride, and carbon disulfide were used as obtained from Sigma-Aldrich, St. Louis, MO, USA, as commercial raw materials.

Chemistry

General Procedure for Preparing (Z)-3-Hydroxy-3-(4-R-Phenyl)-Prop-2-Enedithioic Acids **11a–j**

Ten samples of ice-bath-cooled NaH (12.0 mmol) suspensions in pre-dried THF (5.0 mL) were prepared, and to each sample was added, dropwise, a THF solution (2.0 mL) of a different acetophenone **12a–j** (4 mmol). Each resulting mixture was stirred for 30 minutes, and then to each mixture was added, dropwise, a THF solution (1.0 mL) of CS₂ (6 mmol)). Each resulting mixture was

stirred for over 12–15 hours at room temperature. In each case, after the starting material was consumed (as monitored using TLC), the crude reaction product was poured into a water–ice suspension and extracted three times with ethyl acetate. The resulting aqueous phase was acidified with 2 N sulfuric acid to a pH of 2 and then extracted three times with ethyl acetate, while the resulting organic layer was dried with Na₂SO₄. Removal of the solvent under reduced pressure gave an amorphous solid that was crystallized from an appropriate solvent.⁸ Spectroscopic data collected from each of the resulting compounds **11a–j** fully supported their expected structures (see Spectral Data in the [Supplementary Information](#)).^{9,10}

Antitubercular Activity Evaluation

Preparations of Stock Solutions

DMSO (Sigma-Aldrich, St. Louis, MO, USA) stock solutions of the synthesized compounds at a concentration of 10 mg/mL were prepared for carrying out in each case the resazurin microtiter assay (REMA). Each compound was diluted in a Middlebrook 7H9 broth medium (Difco, Sparks, MD, USA). Stock solutions for each of the reference compounds at 64 μ g/mL were prepared and filtered employing a 0.22 μ m-diameter pore membrane (Millipore, Darmstadt, Germany). The prepared stock solutions were stored at –20°C prior to their evaluation.

Cell Culture

The Vero cell line (African green monkey kidney) from American Type Culture Collection (ATCC) was employed to carry out the cytotoxicity assays. RPMI 1640 medium (Gibco, Grand Island, NE, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco) and nonessential amino acids (Gibco) was used to culture these cells.

Cytotoxicity Assay

A total of 10,000 Vero cells were placed in a 96-well plate and then incubated in an RPMI medium (100 μ L) for 24 h. Then, the plate was washed and fresh medium containing the synthesized compounds at different concentrations was added to it. Each compound was incubated under a 5% CO₂ atmosphere for 48 h at 37°C. A 10 μ L MTT (Sigma-Aldrich) solution (5 mg/mL in sterile PBS) was added to each well, and the resulting mixtures were incubated for another 4 h. The medium was then removed, and a volume of 100 μ L of DMSO was used to solubilize the formazan (Sigma-Aldrich, St. Louis, MO, USA). The formazan absorbance

(ABS) at a wavelength of 570 nm was measured, and cytotoxicity was calculated as $\% \text{toxicity} = (1 - (\text{ABS experimental}/\text{ABS control})) \times 100$. Controls were cells without treatment but following the same procedures as described above.¹¹

M. tuberculosis Inocula Preparation

A 7H9–glycerol–10% ADC–0.01% tyloxapol medium was used to cultivate *M. tuberculosis* strains H37Rv, H37Ra (virulent and non-virulent reference strains, respectively) and 209 (clinical isolated strain, rifampicin resistant) at 37°C until an OD_{600nm} of 0.4 was reached.

Antimicrobial Susceptibility Test Using the Resazurin-Based Microtiter Plate Assay (REMA)

The REMA procedure used in the current work was developed based on a microplate alamar Blue assay (MABA) used in a previous work for determining antimycobacterial activities of various compounds.¹² In brief, the outer wells of a 96-well plate were each filled with 200 µL of sterile PBS to prevent dehydration from occurring during the long incubation period (8 days). Here, rifampicin (RIF) (Sigma-Aldrich, St. Louis, MO, USA) was included in each plate as a reference drug (16–0.001 µg/mL serial two-fold dilutions), DMSO, DMSO+*Mtb*, medium, media +*Mtb*, and compound alone were added as control to validate the plate. The compounds were evaluated at various concentrations, ranging from 0.98 µg/mL to 250 µg/mL and in triplicate. After 6-day incubations of the plates, a volume of 30 µL of 0.01% (weight/volume) resazurin (Sigma-Aldrich, St. Louis, MO, USA) instead of MABA was added to each well, and the plates were incubated for additional 2 days. Visual inspection was done to determine the color of each well, with blue interpreted as a lack of cells growth and pink as growth of cells. The MIC value for each experiment was defined as the lowest concentration of the compound in those wells where the blue color was observed.

Selectivity Index (SI)

This index was calculated by comparing the IC₅₀ values obtained in Vero cells with the bacteria MIC₁₀₀ determined by performing the REMA.

Minimal Bactericidal Concentration (MBC)

Following the methodology described in a previous study, some of the compounds were selected to determine their respective MBCs. Here, MBC was defined as the minimum extract concentration that did not cause a color shift

in cultures reincubated in fresh medium. Briefly, 5 µL volumes of REMA duplicate bacteria suspensions were transferred to a new microplate containing 195 µL of fresh culture medium in each well. Subsequently, the microplates were incubated as described previously for the REMA.¹³

Physicochemical Properties and ADMET Prediction

The ADME-Tox filtering tool FAF-Drugs4 was used to evaluate the physicochemical properties of the synthesized compounds.¹⁴ The ADMET profiles were predicted by employing the consensus of the results of vNN-ADMET,¹⁵ admetSAR 2.0 (unrestricted applicability domain),¹⁶ and pkCSM.¹⁷

In silico Molecular Docking Studies

Validation of the Docking Procedure

The complex of 4RLU with 2',4,4'-trihydroxychalcone (HCC) was selected for docking studies and done so due to 4RLU having been identified as the protein receptor for HadAB.³³ To validate the docking process, the co-crystallized ligand (HCC) was re-docked into the active site, and an RMSD value of 3.0 Å between the co-crystallized structure and the redocked structure was obtained.

Ligand Preparation

The structures of compounds **11a–j** were each modeled in their two tautomeric forms (keto and enol) as 2D structures by using the software ChemBio Draw Ultra 12.0 and were converted into 3D structures in MDL format.¹⁸ Their protonated states were then modeled using the online tool Chemicalize.¹⁹ The geometries of the compounds and co-crystallized ligand were calculated at the density functional B3LYP level using the 6-31G (d, p) basis set in the Gaussian 16 software package.²⁰ Finally, using Autodock Tools, the ligands were prepared by including in them polar hydrogens and Gasteiger charges as well as rotatable (ie, single) bonds that were assigned by default, and a PDBQT file was generated.²¹

Receptor Preparation

The X-ray coordinates of the HadAB receptor were retrieved from the Protein Data Bank (PDB code:4RLU).²² Molecular water was removed from the crystallographic structure, and the final preparation and minimization of the receptor structure was carried out by deploying the Dock Prep module of Chimera software.²³

using the AMBER-ff14SB force field. Lastly, Kollman charges were added to the obtained structure by using Autodock Tools, and a PDBQT file was generated.

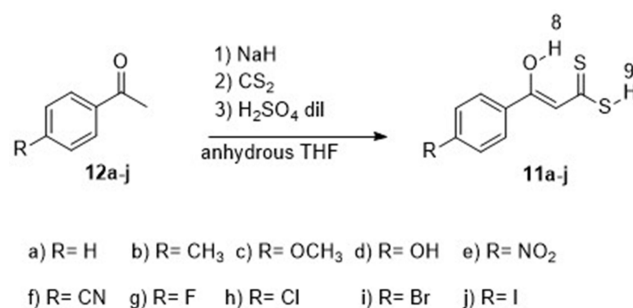
Docking Calculations

Rigid receptor molecular docking was carried out in Autodock4 using the Lamarckian genetic algorithm.²⁴ We used grid maps with $70 \times 70 \times 70$ points in the active site of the receptor with the coordinates $x=0.272$, $y=22.253$, $z=-30.833$, and a grid-point spacing of 0.375 Å. AD4.dat parameters were applied to all the ligands. The parameters used were 10 runs, a population size of 100, and a run-termination criterion of a maximum of 27,000 generations or a maximum of 250,000 energy evaluations. The visualization and analysis of the nonbonded interactions as hydrogen bonds of the best poses were carried out using Discovery Studio Visualizer software.²⁵

Results and Discussion

Chemistry

The desired 3-hydroxy-3-(4-R-phenyl)-prop-2-enedithioic acids **11a–j** (Scheme 1) were prepared with good yields by carrying out respective coupling reactions between carbon disulfide and the substituted acetophenones **12a–j** in the presence of sodium hydride in dry tetrahydrofuran.⁸ The spectroscopic data of the **11a–j** products were fully consistent with their expected structures. Note that the ¹H-NMR and ¹³C-NMR spectra of these products showed the signals characteristic for their enol and not keto forms.^{9,10}



Scheme 1 Synthetic route to compounds **11a–j**.

Antitubercular Activity Evaluation

Compounds **11a–j** were evaluated as *M. tuberculosis* strain H37Rv growth inhibitors in 250 µg/mL–0.98 µg/mL serial two-fold dilutions (Table 1). These activities were compared with those of RIF, which is usually indicated for the treatment of *Mycobacterium* infections, including tuberculosis (TB).^{11–}

¹³ First, we synthesized compound **11a** (R=H) and measured its antituberculosis minimal inhibition concentration (MIC) to be 62.5 µg/mL. Then, to examine the influence of electron-donating and electron-withdrawing groups on the antituberculosis activity, we synthesized compounds **11b** (R=CH₃), **11c** (R=OCH₃), and **11d** (R=OH). Surprisingly, **11b** did not inhibit the growth of *M. tuberculosis* cells (MIC=250 µg/mL). The compound resulting from replacing the 4-CH₃ substituent with the 4-OCH₃ group (**4c**) exhibited inhibition levels similar to those of **11a** (R=H) but with a lower SI [SI (**11a**)= 3.46 vs SI (**11c**)= 1.60].

Compound **11d**, generated by incorporating an -OH group at position 4 of the phenyl ring of compound **11a**,

Table 1 Anti-*M. tuberculosis* Effects and Cytotoxicity Levels of Compounds **11a–j**

Compound	R	REMA-Determined MIC ₁₀₀ (µg/mL)	REMA-Determined MBC (µg/mL)	MTT-Determined IC ₅₀ , in Vero Cells (µg/mL)	Selectivity Index (SI)
11a	-H	62.5	ND	216	3.46
11b	-CH ₃	250	ND	221	0.88
11c	-OCH ₃	62.5	ND	100	1.60
11d	-OH	15.6	ND	101	6.47
11e	-NO ₂	7.8	31.25	255	32.69
11f	-CN	62.5	ND	>50	0.80
11g	-F	62.5	ND	443	7.09
11h	-Cl	15.6	ND	110	7.05
11i	-Br	62.5	ND	ND	ND
11j	-I	500	ND	381	0.762
Rifampin	–	0.06	ND	>1000	>16,666

Notes: Mtb: *M. tuberculosis* H37Rv ATCC 27294 reference strain. SI= IC₅₀/MIC₁₀₀.

Abbreviations: MIC₁₀₀, minimal inhibition concentration; REMA, resazurin microtiter assay; MBC, minimal bactericidal activity; IC₅₀, inhibitory concentration 50 (MTT assay in Vero cells).

showed an inhibitory activity four times that of **11a** and a toxicity level lower than that of **11a**; and compound **11e**, generated by incorporating an $-NO_2$ group at position 4 of the phenyl ring of compound **11a**, showed an even greater inhibitory activity – eight times that of **11a** – and was observed to be much less toxic [SI (**11a**)= 3.46 vs SI (**11e**) = 32.69]. Because the reduction in the aromatic nitro group in vivo produces hydroxylamine and nitrosamine,²⁶ both highly reactive and carcinogenic species, this group was replaced with an isosteric group, namely $-CN$, which produces fewer toxic compounds than to nitro compounds.²⁷ Unexpectedly, when the 4- NO_2 substituent was replaced with 4-CN (**11f**), the new compound exhibited inhibition levels similar to those of **11a** (R=H) but with a lower SI [SI (**11a**)=3.45 vs SI (**11f**) =0.80]. We also investigated the effects of halogen substituents on the antituberculosis activities by synthesizing compounds containing fluorine, chloro, bromo, and iodine groups at the 4 position of the phenyl ring. Compound **11g**, having a fluorine at position 4 of the phenyl group, exhibited an antituberculosis activity essentially the same as that of **11a** (MIC=62.50 $\mu\text{g/mL}$) but with lower toxicity [SI (**11a**)= 3.46 vs SI (**11g**)=7.09]. The inhibitory activity of compound **11h**, generated by the incorporation of a chlorine substituent at position 4 of the phenyl ring, was four times that of **11a**, and the compound was essentially equally toxic. In contrast, when 4-H was replaced with 4-Br, the generated compound **11i** exhibited inhibition levels similar to those of **11a** (R=H), and with a lower SI [SI (**11a**)=3.46 vs SI (**11i**)=1.60]. And compound **11j**, having an iodine substituent, showed no inhibitory activity.

All told, none of the compounds with halogen substituents showed an antituberculosis activity as good as that of **11e**. Based on our results, derivatization of the phenyl ring with substituents such as $-OH$ and especially $-NO_2$ offered a suitable approach for optimizing the antituberculosis activities of the 3-hydroxy-3-(4-R-phenyl)-prop-2-enedithioic acids. In both cases, the presence of hydrogen bonds apparently influenced the antituberculosis activity. The presence of a nitro group has been proposed to increase the number of hydrogen bond interactions with the target receptor.²⁸ In vitro activity against *M. tuberculosis* strains that are resistant to a single TB drug (eg, isoniazid or RIF) has been used as a key criterion to determine the reliability of a compound to be considered as an anti-TB drug.²⁹ Therefore, we also evaluated compound **11e** with non-virulent *M. tuberculosis* H37Ra

Table 2 REMA-Determined MIC₁₀₀ Values of **11e** Against Virulent, Non-Virulent and RIF-Resistant *M. tuberculosis* Strains

Compound	MIC ₁₀₀ $\mu\text{g/mL}$ in H37Rv 27294	MIC ₁₀₀ $\mu\text{g/mL}$ in H37Ra	MIC ₁₀₀ $\mu\text{g/mL}$ in Mtb-209
11e	7.8	7.8	15.6
Rifampin	0.06	0.008	320

Notes: *M. tuberculosis* H37Rv ATCC 27294 reference strain; *M. tuberculosis* H37Ra: non-virulent strain. *M. tuberculosis*-209: RIF-resistant clinical isolate of *M. tuberculosis*.

bacteria, which are more susceptible to lower concentrations of drugs than are virulent strains. Specifically, compound **11e** activities against *M. tuberculosis* H37Ra and 209 (an RIF-resistant strain) were evaluated using 250 $\mu\text{g/mL}$ –0.98 $\mu\text{g/mL}$ serial two-fold dilutions (Table 2). Table 2 also lists the MIC₁₀₀ values of RIF. Compound **11e** was found to be active against *M. tuberculosis* strains H37Ra and 209.

ADMET Predictions

Table 3 shows the determined physicochemical properties of the ten synthesized compounds. Assessment of these molecules using the Lipinski,³⁰ Veber,³¹ and Egan³² rules suggested that they each had a high probability of exhibiting good oral bioavailability. An absorption, distribution, metabolism, excretion, and toxicity (ADMET) consensus analysis indicated that none of the compounds could inhibit P-glycoprotein and that only compounds **11b** and **11d** could be pumped out of the cell through this multidrug-resistant protein (Table 3).

The prediction also suggested that all the compounds could lower CYP1A2 activity, and that only **11c**, **11h**, **11i**, and **11j** could inhibit CYP2C19, while **11b** and **11h** would be metabolized by CYP2C9 (Table 3). The compounds were concluded to have a low probability of exerting mutagenicity, cardiotoxicity, mitochondrial toxicity, and cytotoxicity, but have a high risk of damaging the liver and the skin (see Table 4). Therefore, it will apparently be necessary to modify the structure at the hit-to-lead stage to reduce its hepatotoxicity.

Docking Studies

To investigate the possible mode or modes of interaction of compounds **11a-j** with *M. tuberculosis*, molecular docking studies were carried out, based on the molecular similarity of the synthesized compounds with ethionamide (**5**), on the active site of the (3R)-hydroxyacyl-ACP (HadAB)

Table 3 Calculated Physicochemical Properties of Compounds **IIa-j**

Compound	MW	logP	logD	logSw	tPSA	RB	HBD	HBA	HA	PAINS
IIa	196.29	2.42	1.64	-2.67	72.22	2	1	1	12	NO
IIb	210.32	2.78	2.16	-2.96	72.22	2	1	1	13	NO
IIc	226.32	2.39	1.49	-2.74	81.45	3	1	2	14	NO
IId	212.29	2.06	1.33	-2.53	92.45	2	2	2	13	NO
IIe	241.29	2.25	4.97	-2.76	113.76	3	1	4	15	NO
IIf	221.3	2.13	1.5	-2.63	96.01	2	1	2	14	NO
IIg	214.28	2.52	1.79	-2.85	72.22	2	1	1	13	NO
IIh	230.73	3.04	2.25	-3.28	72.22	2	1	1	13	NO
IIi	275.19	3.11	2.41	-3.6	72.22	2	1	1	13	NO
IIj	322.19	3.07	2.57	-3.86	72.22	2	1	1	13	NO

Abbreviations: Cmpd, compound; MW, molecular weight; logP, logarithm of the partition coefficient between n-octanol and water; logD, logP of the physiological pH; logSw, logarithm of the water solubility of the compound; RB, numbers of rotatable bonds; HBD, hydrogen bond donors; HBA, hydrogen bond acceptors; HA, heavy atoms in the compound; PAINS, pan-assay interference compounds.

Table 4 Predicted Toxicities of the Synthesized Compounds **IIa-j**

Compound	Mutagenicity (AMES Test)	Cardiotoxicity (hERG Blocker)	Mitochondrial Toxicity (MMP)	Hepatotoxicity	DILI	Cytotoxicity	AO
IIa	-	-	-	-	+	-	III
IIb	-	-	-	-	+	+	III
IIc	-	-	-	-	-	-	III
IId	-	-	-	-	-	-	II
IIe	+	-	-	+	+	-	III
IIf	-	-	-	+	+	-	II
IIg	-	-	-	-	+	-	III
IIh	-	-	-	+	+	-	III
IIi	-	-	-	-	+	+	III
IIj	-	-	-	-	+	-	III

Notes: no risk (-); high risk (+).

Abbreviations: Cmpd, compound; hERG, human ether-à-go-go-related gene; MMP, mitochondrial membrane potential; DILI, drug-induced liver injury; AO, acute oral toxicity category.

dehydratase heterodimer (PDB: 4RLU).³³ It is important to note that each of compounds **IIa-j** can be found in a tautomeric ketone or enol form. Although the enol form of each of them was found according to ¹H-NMR observations to be predominant in organic media (see above), the relative amounts of the two forms of each compound may differ when the compound is placed in aqueous media or when interacting with a receptor biomolecule. Therefore, we decided to carry out in silico studies of the interactions of both the keto and enol forms of each compound with the HadAB enzyme. The predicted free energy and affinity constant values are listed in [Table 5](#).

Molecular Dockings of Compounds **IIa-j**

The above-described docking protocol was implemented to analyze the binding interactions between each of the

Table 5 Calculated Binding Free Energy (ΔG) from Molecular Docking Results of the Two Tautomeric Forms (Keto and Enol) of **IIa-j** Compounds Within HadAB Active Site

Compound	ΔG (kcal/mol)	
	Keto	Enol
IIa	-5.96	-5.74
IIb	-6.36	-6.62
IIc	-5.78	-5.91
IId	-6.02	-5.91
IIe	-6.03	-5.45
IIf	-7.24	-6.55
IIg	-5.90	-6.10
IIh	-6.40	-6.72
IIi	-6.69	-6.52
IIj	-6.61	-6.94
HCC	-8.32	

compounds **11a–j** and HadAB. To compare these results with that for a leading compound known to interact with the same receptor, an anchoring study involving the co-crystallized enzyme complex and the 2',4,4'-trihydroxychalcone (HCC) inhibitor was carried out under the same conditions as those used with **11a–j**. Here, the free energy of the binding of HCC was found to be -8.32 kcal/mol, within about 2 kcal/mol of the values obtained for the tested compounds. All compounds were analyzed in their best pose with the lowest binding free energy with the nonbonding interactions at the active site. While **11f**, **11h**, **11i**, **11j**, and **11b** in both tautomeric forms showed better-than-predicted free energy values, only **11e** in its keto and enol forms and **11d** in its enol form showed nonbonding interactions with ACys61 (Figure 2). Similar free energy values were predicted for the ketonic tautomers of **11d** and **11e**, but quite different ones for their enolic forms, with **11d** predicted to have the lowest binding energy. This difference may have been due to their making different numbers of hydrogen bonds with the enzyme according to the docking studies—with **11d** predicted to make four hydrogen bonds with the enzyme, specifically two with ACys61 and at the

same time two with AGln86, and the **11e** enolic form predicted to only make two hydrogen bonds, one with ACys61 and the other with BAsp36. Moreover, each of the two tautomeric forms of **11e** was predicted to make hydrogen bond interactions with ACys61. Thus, the molecular docking studies indicated that the experimentally measured anti-tuberculosis activity of **11d** could be explained by its forming hydrogen bonds with HadAB.

Conclusions

The present results indicated that derivatizing the phenyl group of 3-hydroxy-3-phenyl-prop-2-enedithioic acids with electron-donating and electron-withdrawing groups modified its antituberculosis activity. We investigated the role of electron-donating groups in the antituberculosis activity of this type of acid by synthesizing compounds **11b–d**. Our results showed that the 4-hydroxy derivative of **11a** (ie, **11d**) was the most active of these compounds. The data presented here were found to be inconclusive regarding the relationship between the electronegativity of the halogen substituent on the phenyl group and the inhibition of the growth of *M. tuberculosis* cells, as well as

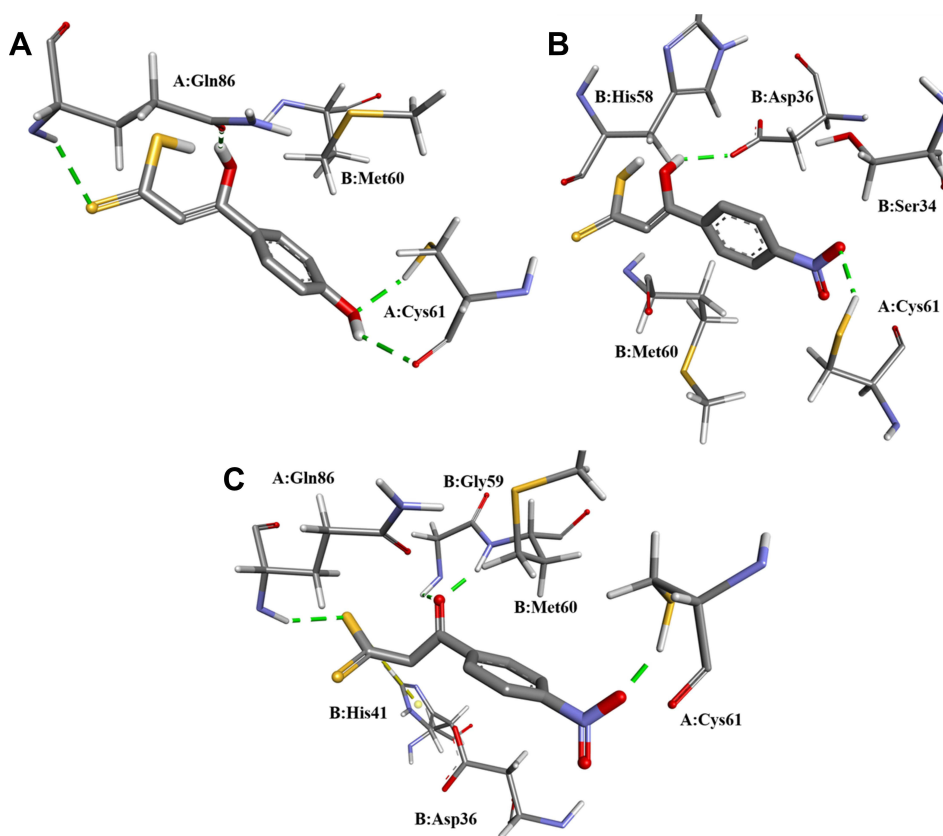


Figure 2 Nonbonding interactions between the active site of HadAB and (A) the **11d** enolic tautomer, (B) **11e** enolic tautomer, and (C) **11e** ketonic tautomer.

regarding the relationship between the size of the halogen substituent and this inhibition (compounds **11g–j**).

The role of the electron-withdrawing groups in the antituberculosis activity was investigated by synthesizing and measuring the activity levels of compounds **11e** and **11f**; in these experiments, compound **11e** (R=NO₂) was more active and showed a remarkable selectivity index (SI=32.69). All of the tested molecules were also shown to not be pan-assay interference compounds (PAINS), so the antiproliferative activities reported in this study could be due to a target-specific interaction. In our docking studies, **11d** and **11e** made hydrogen bond interactions with ACys61 of the HadAB enzyme, leading us to think that HadAB should be considered as a possible therapeutic target for these compounds and suggesting that the development of new compounds based on these structures should be pursued. Accepted criteria for developing new drugs against infectious diseases such as tuberculosis include an SI higher than 10, an MIC lower than 10 μM, and activity against a mono-resistant strain.²⁹ Therefore, our results suggested that the antituberculosis compound **11e** might be useful for the design of novel antituberculosis agents. Further optimization of this hit compound is expected to provide information about the structural requirements for achieving high antitubercular activity. Overall, this study represents an important attempt towards the development of novel tuberculosis treatments.

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Disclosure

The authors declare no conflicts of interest, financial or otherwise.

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