

Synthesis and molecular docking of isonicotinohydrazide derivatives as anti-tuberculosis candidates

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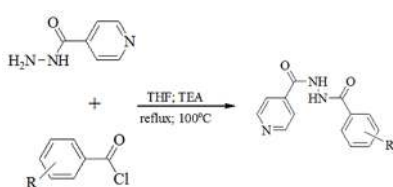
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Graphical abstract



Abstract

Tuberculosis (TB) is a chronic disease as a result of *Mycobacterium tuberculosis*. It can affect all age groups, and hence, is a global health problem that causes the death of millions of people every year. One of the drugs used in tuberculosis treatment is isonicotinohydrazide (Isoniazid). In this study, N'-benzoylisonicotinohydrazide derivative compounds (**a-l**) were prepared using acylation reactions between isonicotinohydrazide and benzoyl chloride derivatives, employing the reflux method. Molecular docking studies suggested that all of the compounds had better interaction with *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase (InhA) than isonicotinohydrazide. It can be concluded that N'-benzoylisonicotinohydrazide derivatives (**a-l**) can be used as anti-tuberculosis candidates. The docking results obtained revealed that all of the compounds were interacted well with InhA, with compound **g** exhibiting the best interaction.

Keywords: N'-benzoylisonicotinohydrazide, inhibin Alpha Subunit (InhA) inhibition, anti-tuberculosis, synthesis, docking

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INTRODUCTION

Tuberculosis (TB) is a chronic disease as a result of *Mycobacterium tuberculosis*. The disease is more common now than in previous cases, and is associated with high rates of morbidity and mortality. The most common cause of death of HIV-AIDS patients is tuberculosis (WHO, 2016). A large number of new TB cases is increasingly resistant to drugs; this is a detrimental effect of first and second line anti-TB drugs. Because of the increased incidence of TB as a result of viral infections, there has been increased research interest aimed at the discovery of new anti-TB compounds (Lougheed *et al.*, 2009). Specifically, the most resistant strains are resistant to the two most routinely used anti-TB drugs, rifampicin and isoniazid, and this has produced serious impacts on epidemiology and disease control (Kochi and Varelzdis, 1992).

Isoniazid, also named as isonicotinohydrazide (INH), is a hydrazide compound derived from isonicotinic acid compound (pyridine-4-carboxylic acid), and is one of the first-line drugs for tuberculosis. INH enables KatG to form the INH-nicotinamide adenine dinucleotide (NAD) adduct. This adduct inhibits the acyl carrier protein (ACP) and InhA reductase, resulting in the synthesis of type II fatty acids, which in turn will synthesize mycolic acid, causing cell death (Rodrigues *et al.*, 2013).

Previous studies have modified isoniazid with amino azetidines (Thomas and Harindran, 2016), pyrazoles (Vidya, 2014), oxadiazole (Malhotra *et al.*, 2012), gelatine (Cassano *et al.*, 2012), heteroaromatic and chloroquinoline (Ferreira *et al.*, 2010), thiosemicarbazide (Sharma, Nagda and Talesara, 2006), 3-methoxysalicylaldehyde (Pahlavani, Kargar and Sepehri Rad, 2015), anilines (Rychtářková *et al.*, 2014), via the carbonyl group, methyl salicylate (Alea, Laguna and Caparas, 2014), acylated with acyl compound (Hearn and Cynamon, 2003), azetidine (Asundaria, Patel and Patel, 2011), fatty acid hydrazide (Rodrigues *et al.*, 2013), and using molecular hybridization with phenyl derivatives (Chollet *et al.*, 2015).

In the study of structure-activity relationships, Ragno *et al.* (2000) showed that high partition coefficient (logP) values play an important role in antimicrobial activity because lipophilic compounds have more potent antimycobacterial potential. High logP values represent an increase in drug permeability through the lipid-rich mycobacterial cell wall.

In this study, we designed and synthesized isoniazid compounds which were acylated with benzoyl chloride derivatives, thereby increasing the logP value of the compounds. The interaction of the compounds with *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase (InhA) was computationally studied.

EXPERIMENTAL

Materials

All of the chemical reagents used in synthesis and characterization were reagent grade. The N'-benzoylisonicotinohydrazide derivatives were synthesized from the reaction of isonicotinohydrazide with the benzoyl chloride derivatives (Fig. 1). The structures of the compounds were identified using Infrared (IR) spectroscopy, ¹H-nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry. Their purity was determined by melting point and thin layer chromatography (TLC) analysis.

General experiments

All reagents and solvents were purchased from standard commercial suppliers. Melting points were measured with an electrothermal melting point apparatus without correction. Infra red spectra were recorded in KBr on a Jasco FT-IR 5300, and the major absorption was recorded in cm⁻¹. ¹H-NMR spectra were taken on an NMR Agilent system consol DD2 spectrometer (¹H-NMR 500MHz; ¹³C-NMR 125 MHz). MS spectra were measured with a high resolution mass spectrometry (waters LCT Premier XE, detector ESI TOF, solvent aceton+0.1% formic acid in acetonitrile water (1:1)).

TLC was carried out on aluminium plates coated with silica gel F254 (E Merck), using an ultra violet (UV) lamp with a wavelength of 254 nm for spot detection.

Synthesis of N'-benzoylisonicotinohydrazide derivatives (a-l)

Isonicotinohydrazide (0.05 mol) was mixed with triethylamine (3 mL) in tetrahydrofuran (20 mL), and few drops of 0.025 mol benzoyl chloride in tetrahydrofuran (THF) were added slowly at room temperature. The temperature was raised to 100°C and the mixture was refluxed for five hours. The mixture was concentrated by evaporating THF from the mixture, and the product was washed with distilled water and then saturated with sodium bicarbonate. The resulting solid was recrystallized with hot ethanol to give the N'-benzoylisonicotinohydrazide derivatives (Ruswanto et al., 2015).

N'-benzoylisonicotinohydrazide (a)

The compound was acquired with a yield of 51.71%. The compound was a white crystal; m.p. range 233-233.6°C; UV-Vis (DMSO) λ_{\max} (nm): 296. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.50-7.53 (t, 2H, $J = 7.5$ Hz, 2CH-aromatic.); 7.59-7.62 (t, 1H, $J = 7.25$ Hz, CH-aromatic); 7.721-7.73 (dd, 4H, $J = 5$ Hz, 4CH-aromatic); 7.87-7.88 (dd, 2H, $J = 8$ Hz, 2CH-aromatic); 8.82-8.83 (dd, 2H, $J = 5.5$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3208.96; 3004.55 (N-H), 1650.77 (C=O amide), 1484.92; 1292.7 (C=C Ar), 748.25; 701.60 (C-H mono substitution). MS m/z ESI: 242.09 (M+H)⁺.

N'-(2-chlorobenzoyl)isonicotinohydrazide (b)

The product was obtained with a yield of 9.79%. The product was a yellow powder; m.p. range 98-100°C; UV-Vis (DMSO) λ_{\max} (nm): 365.5. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.42-7.46 (m, 1H, CH-aromatic.); 7.48-7.54 (m, 2H, 2CH-aromatic); 7.67-7.69 (d, 1H, $J = 9.05$ Hz, CH-aromatic); 7.87-7.88 (dd, 4H, $J = 6.146$ Hz, 4CH-aromatic); 8.74-8.75 (dd, 2H, $J = 6.15$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3456.44; 3379.29 (N-H), 1658.78 (C=O amide), 1519.91; 1489.05 (C=C Ar), 1126.43; 1072 (C-N amine). 756 (C-Cl). MS m/z ESI: 276.01 (M+H)⁺.

N'-(2-fluorobenzoyl)isonicotinohydrazide (c)

The product was obtained with a yield of 7.07%. The product was a yellow powder; m.p. range 198-200°C; UV-Vis (DMSO) λ_{\max} (nm): 356.6. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.24-7.29 (m, 1H, CH-aromatic.); 7.3096-7.3504 (m, 1H, CH-aromatic); 7.57-7.63 (m, 3H, CH-aromatic); 7.87-7.88 (dd, 2H, $J = 6.15$ Hz, 2CH-aromatic); 8.73-8.74 (dd, 2H, $J = 6.1$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3217.27 (N-H), 1597.06 (C=O amide), 1473.62; 1404.18 (C=C Ar), 1288.45; 1219.01 (CH of aromatic disubstitution). MS m/z ESI: 258.47 (M-1).

N'-(4-fluorobenzoyl)isonicotinohydrazide (d)

The product was obtained with a yield of 13.84%. The product was a white crystal; m.p. range 222-224°C; UV-Vis (DMSO) λ_{\max} (nm): 297. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.23-7.27 (t, 2H, $J = 7.9$ Hz, CH-aromatic); 7.88-7.89 (d, 2H, d, $J = 7.7$ Hz, 2NH); 7.98-8.01 (m, 4H, CH-aromatic); 8.72-8.73 (d, 2H, d, $J = 4.55$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹): 3464.15 (N-H), 1666.5 (C=O amide), 1515.18; 848.68 (C=C ar), 748.38; 648.08; 617.22 (C-H ar.). MS m/z ESI: 258.30 (M-1).

N'-(3-bromobenzoyl)isonicotinohydrazide (e)

The product was obtained with a yield of 2.09%. The product was a light yellow powder; m.p. range 200-202°C; UV-Vis (DMSO) λ_{\max} (nm): 365.5. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.44-7.47 (t, 1H, $J = 7.9$ Hz, CH-aromatic.); 7.76-7.79 (1H, d, $J = 11.1$, CH-aromatic); 7.87-7.88 (m, 4H, 4CH-aromatic); 8.09 (s, 1H, CH-aromatic); 8.74-8.76 (d, 2H, $J = 6.1$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3410.15; 3224.98 (N-H), 1643.35 (C=O amide), 1558.48 (C=C Ar). MS m/z ESI: 319.141 (M-1).

N'-(3-chlorobenzoyl)isonicotinohydrazide (f)

The product was obtained with a yield of 7.23%. The product was a white powder; m.p. range 208-210°C; UV-Vis (DMSO) λ_{\max} (nm): 357. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.49-7.53 (t, 2H, $J = 9.75$ Hz, CH-aromatic); 7.61-7.64 (d, 1H, $J = 11.35$ Hz, CH-aromatic); 7.85-7.88 (m, 3H, 3CH-aromatic); 7.95 (s, 2H, 2CH-aromatic); 8.74-8.76 (dd, 2H, $J = 6.15$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹): 3433.29 (N-H), 1643.35 (C=O amide), 1543.10 (C=C Ar), 756.10 (C-Cl). MS m/z ESI: 274.41 (M-1).

N'-(3-nitrobenzoyl)isonicotinohydrazide (g)

The product was obtained with a yield of 59.11%. The product was a white crystal; m.p. range 194.5-195.7°C; UV-Vis (DMSO) λ_{\max} (nm): 365.5. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.73-7.76 (t, 1H, $J = 7.25$ Hz, CH-aromatic.); 8.04-8.05 (d, 2H, $J = 4.5$ Hz, 2CH-aromatic); 8.46-8.47 (dd, 2H, $J = 7.5$ Hz, 2CH-aromatic); 8.54-8.55 (dd, 1H, $J = 6.5$ Hz, 2CH-aromatic); 8.76 (s, 1H, CH-aromatic); 8.84-8.85 (d, 1H, $J = 4.5$ Hz, CH-aromatic); 8.89-8.90 (d, 2H, $J = 5$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3220.54 (N-H), 1646.91 (C=O amide), 1531.2; 1349.93 (C=C Ar), 856.24; 713.53; 593.96 (disubstitution of aromatic). MS m/z ESI: 286.0702 (M+H)⁺.

N'-(4-bromobenzoyl)isonicotinohydrazide (h)

The product was obtained with a yield of 53.25%. The product was a light yellow powder; m.p. range 230-232°C; UV-Vis (DMSO) λ_{\max} (nm): 364.5. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.68-7.71 (dd, 2H, $J = 11.05$ Hz, 2CH-aromatic.); 7.83-7.84 (dd, 2H, $J = 4.5$ Hz, 2CH-aromatic); 7.85-7.85 (dd, 2H, $J = 1.9$ Hz, 2CH-aromatic); 7.87-7.88 (dd, 2H, $J = 6.2$ Hz, 2CH-aromatic); 8.74-8.75 (dd, 2H, $J = 6.15$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3425.58; 3186.40 (N-H), 1597.06 (C=O amide), 1458.18 (C=C Ar). MS m/z ESI: 318.49 (M-1).

N'-(4-tert-butylbenzoyl)isonicotinohydrazide (i)

The product was obtained with a yield of 12.95%. The product was a white powder; m.p. range 202-204°C; UV-Vis (DMSO) λ_{\max} (nm): 365.5. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 1.36-1.37 (s, 9H, 3(CH₃)); 7.54-7.57 (m, 4H, 4CH-aromatic); 7.87-7.89 (m, 4H, 4CH-aromatic); 8.74-8.75 (dd, 2H, $J = 6.15$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹): 3425.58 (N-H), 1635.54 (C=O amide), 1481.33 (C=C Ar). MS m/z ESI: 296.73 (M-1).

N'-(4-methylbenzoyl)isonicotinohydrazide (j)

The product was obtained with a yield of 9.6%. The product was a white-yellowish powder; m.p. range 200-202°C; UV-Vis (DMSO) λ_{\max} (nm): 370. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 2.42 (s, 3H, -CH₃); 7.32-7.34 (dd, 2H, $J = 7.95$ Hz, 2CH-aromatic); 7.82-7.84 (dd, 2H, $J = 8.25$ Hz, 2CH-aromatic); 7.86-7.87 (dd, 2H, $J = 1.7$ Hz, 2CH-aromatic); 7.87-7.88 (dd, 2H, $J = 1.7$ Hz, 2CH-aromatic); 8.73-8.75 (dd, 2H, $J = 6.15$ Hz, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 3425.58; 3194.12 (N-H), 1651.07 (C=O amide), 1543.05 (C=C Ar). MS m/z ESI: 254.17 (M-1).

N'-(4-chlorobenzoyl)isonicotinohydrazide (k)

The product was obtained with a yield of 8.63%. The product was a yellow powder; m.p. range 198-200°C; UV-Vis (DMSO) λ_{\max} (nm): 364.5. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.52-7.55 (dd, 2H, $J = 13.6$ Hz, 2CH-aromatic); 7.87-7.88 (dd, 2H, $J = 6.2$ Hz, 2CH-aromatic); 7.90-7.91 (dd, 2H, $J = 4.5$, 2CH-aromatic); 7.92-7.93 (dd, 2H, $J = 4.6$ Hz, 2CH-aromatic); 8.74-8.75 (dd, 2H, $J = 6.2$, 2NH); IR (KBr): ν_{\max} (cm⁻¹) 371.57; 3278.99 (N-H), 1681.93 (C=O amide), 1597.6 (C=C Ar), 1226.73; 1095.57 (C-N amine). 745.35 (C-Cl). MS m/z ESI: 274.15 (M-H).

N'-(3,5-dinitrobenzoyl)isonicotinohydrazide (l)

The product was obtained with a yield of 11.88%. The product was a light brown crystal; m.p. range 222-224°C; UV-Vis (DMSO) λ_{\max} (nm): 359. ¹H-NMR (CHCl₃-DD2, 500 MHz): δ (ppm) 7.91-7.92 (dd, 2H, $J = 6.1$ Hz, 2CH-aromatic.); 8.68-8.69 (dd, 2H, $J = 6.15$, 2CH-

aromatic); 8.97 (s, 1H, CH-aromatic); 9.22-9.23 (dd, 4H, $J = 2.15$ Hz, 2NH/2CHH-aromatic); IR (KBr): ν_{max} (cm^{-1}) 3546.6 (N-H), 1597.8 (C=O amide), 1532.3 (N=O stretch), 1348.8 (N=O bending). MS m/z ESI: 332.0633 (M+H)⁺.

Molecular docking

Prepared docking input files and molecular docking were performed using Autodock Tools version 1.5.6rc3. The ligands were treated as flexible, while the protein was kept rigid. Grid boxes were situated around the binding site using 5-Hexyl-2-(2-methylphenoxy)phenol (TCU) as the grid box centre (-20.088, -4.456, and -31.202). The box size was set to 26, 30, 32 Å (x, y, and z, respectively) and the grid spacing to 0.375 Å. Docking calculations were performed using Lamarckian Genetic Algorithm (LGA) (Morris and Huey, 2009). LigPlot⁺ v.1.4.5 was used for two dimensional

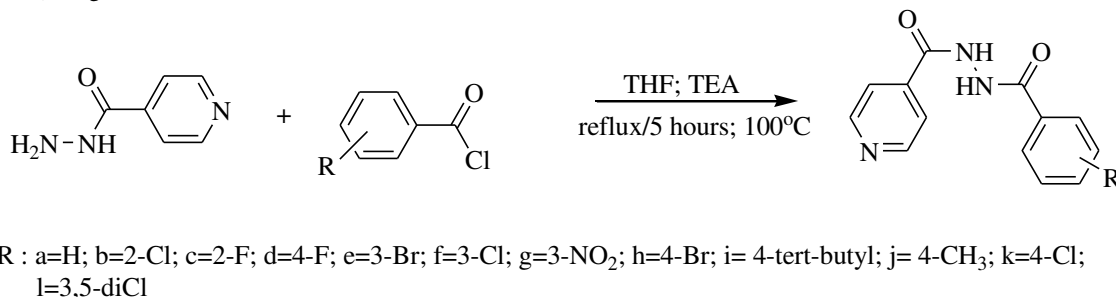


Fig. 1 Synthesis of the N'-benzoylisonicotinohydrazide derivatives.

The N'-benzoylisonicotinohydrazide derivatives were produced by nucleophilic acylation between benzoyl chloride derivatives and isonicotinohydrazide; the nucleophile was isonicotinohydrazide. The structural change of isonicotinohydrazide to N'-benzoylisonicotinohydrazide derivatives was characterized by the conversion of the -NH₂ moiety of isonicotinohydrazide to 2 (-N-C=O) (amide). This change could be observed in the ¹H-NMR spectra, which showed a proton peak at the -NH region, and was supported by the IR spectra with NH bands.

The characteristic $\nu(\text{N-H})$ stretching vibrations of N'-benzoylisonicotinohydrazide derivatives appeared in the 3004.55-3546.6 cm^{-1} range. The difference between the $\nu(\text{N-H})$ stretching frequencies is due to intramolecular hydrogen bonding between H atom (-NH) with O atom (-C=O) (Coelho *et al.*, 2012; Pahlavani, Kargar and Sepehri Rad, 2015). Strong absorption of the $\nu(\text{C=O})$ amide bands in the IR spectra of the compounds were observed in the 1597.06-1681.93 cm^{-1} region; this is in a lower frequency range compared with ordinary carbonyl absorption (1700 cm^{-1}). In addition to the infrared spectrum, the synthesized compounds were also confirmed by ¹H-NMR spectroscopy and mass spectrometry.

Molecular docking results

The RpoB, InhA, ATP synthase, DprE1, and DNA Gyrase are some of drug targets for tuberculosis; for tuberculosis treatment has found the drugs which inhibited of these targets (Ghorab *et al.*, 2017; Vilchère and Jacobs, Jr., 2007). The most potent treatment targeting the InhA enzyme is isoniazid. Isoniazid is found to interfere with NAD-utilizing enzymes, primarily the enoyl-ACP reductase encoded by the InhA gene, leading to the arrest of mycolic acid synthesis, which is essential to *M. tuberculosis* (Seifert *et al.*, 2015; Vilchère and Jacobs, Jr., 2007). The InhA enzyme was chosen based on its hydrophobic properties, which would interact favourably with thioamide or thiourea moieties (Chollet *et al.*, 2015).

Recently, in order to explore the possible binding modes, molecular docking of all compounds to the active site of InhA is performed to determine the possible binding site of the compounds. For this purpose, InhA is co-crystallized with 5-hexyl-2-(2-methylphenoxy)phenol (TCU) (PDBid: 2X23) (Luckner *et al.*, 2010). The Autodock Tools version 1.5.6rc3 was used for all docking procedures. The docking protocol was tested and verified by re-docking of the co-crystallized

visualization (Luscombe, Laskowski and Thornton, 1997; Laskowski *et al.*, 2011). The X-ray crystallographic structure of Mycobacterium tuberculosis enoyl-acyl carrier protein reductase (InhA) was obtained from the Protein Data Bank with PDBid: 2X23 (Luckner *et al.*, 2010)

RESULTS AND DISCUSSION

Synthesis of N'-benzoylisonicotinohydrazide derivatives

The N'-benzoylisonicotinohydrazide derivatives (**a-l**), as shown in Figure 1, were synthesized from isonicotinohydrazide and benzoyl chloride in one step. The twelve compounds were white, yellow, and brown, and all of them were water insoluble substances. The structures of the compounds were identified using UV-visible, FT-IR, and ¹H-NMR spectroscopies, and mass spectrometry as follow:

ligand in the binding pocket of the enzyme binding affinity = -7.52 kcal/mol and root mean standard deviation (RMSD) = 1.2285 (Fig. 2).

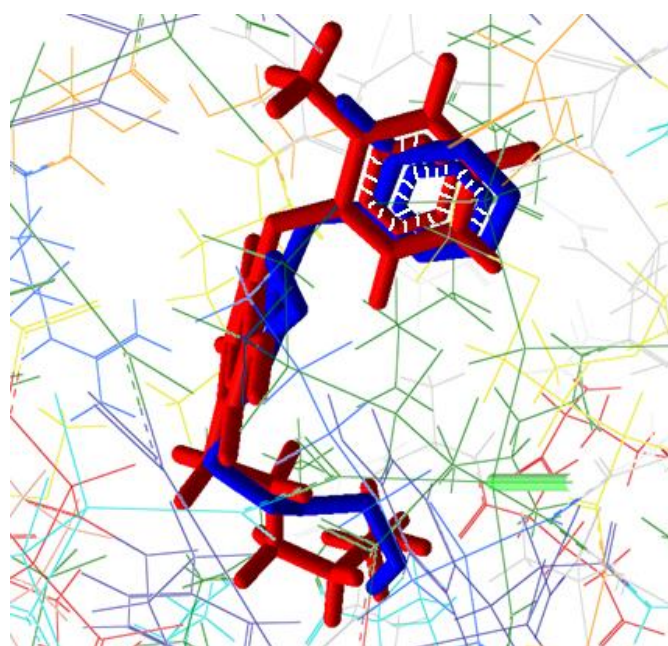


Fig. 2 Superimposition of the co-crystallized ligand (red) and the docked ligand (blue).

The 2D and 3D ligand interactions of compound **g** (Fig. 3) demonstrated that the compound binds to the amino acid of the active site, Ile 194 through one hydrogen bond (3.28 Å), Lys 165 through one hydrogen bond (2.72 Å), and Tyr 158 through one hydrogen bond (2.63 Å); it has a binding affinity of -8.17 kcal/mol. All binding affinity data can be found in Table 1.

Based on Table 1, it was evident that all of the N'-benzoylisonicotinohydrazide derivatives (**a-l**) have binding affinities and predicted inhibition constant better than isoniazid. Thus, the compounds (**a-l**) are predicted to have good interaction with the InhA active site, and the best predicted binding is observed for compound **g** (Kartasmita *et al.*, 2009; Khokra *et al.*, 2016).

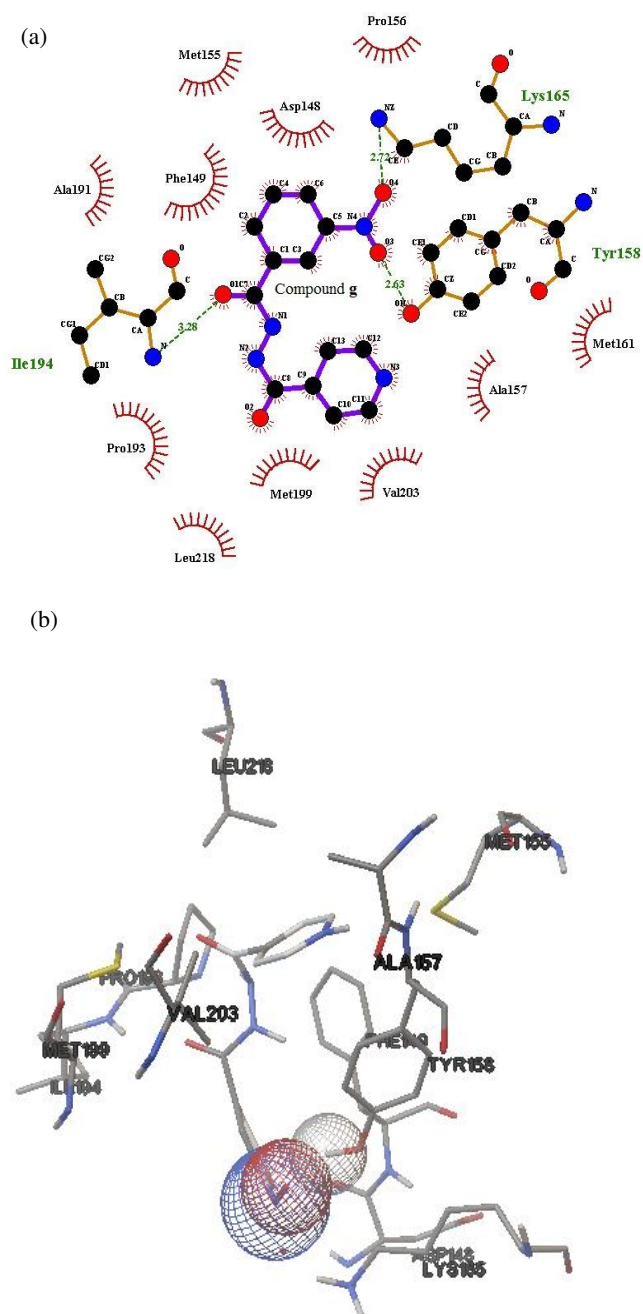


Fig. 3 The 2D visualization (a) and 3D visualization (b), of compound **g** in the InhA active site.

Table 1. Docking results of the compounds inside the InhA active site.

Compound	Binding affinity (kcal/mol)	Predicted inhibition constant (μM)
TCU	-7.52	3.06
	-5.91	46.33
	-6.13	32.08
	-5.72	64.12
	-6.20	28.31
	-6.76	11.06
	-6.92	8.54
	-8.17	1.02
	-6.64	13.67
	-6.84	9.61
	-6.25	26.43
-6.44	19.15	
-7.61	2.63	
Isoniazid	-4.64	398.64

Note: TCU = 5-Hexyl-2-(2-methylphenoxy)phenol

CONCLUSION

New series of *N*'-benzoylisonicotinohydrazide compounds (**a-l**) were synthesized by acylating reactions between isonicotinohydrazide and benzoyl chloride derivatives, using the reflux method. The target compounds were designed and synthesized as potential anti-tuberculosis compounds. Docking results revealed that all of the compounds were bound well to InhA, with compound **g** exhibited the best binding affinity (-8.17 kcal/mol) and inhibition constant value (1.02 μM). Therefore, compound **g** can be used as an anti-tuberculosis candidate.

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