RESEARCH ARTICLE

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Computational Test of Isoniazid Derivatives as Anti-*Mycobacterium tuberculosis* Sensitive and Resistant Types

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ABSTRACT: The development of antimicrobial drugs through structure modification of existing compounds are aimed at improving therapeutic effects while reducing side effects, toxicity and resistance potential. Isoniazid (INH) is still the first-line choice for TB treatment and isoniazid resistance has been reported in several regions in the world include Indonesia, so develop isoniazid derivatives are needed. This study aimed to predict the anti-tuberculosis potential of isoniazid derivatives using molecular docking method. The research materials were digital data of isoniazid derivatives in SMILES and 3D formats of 18 isoniazid derivatives and Catalase-Peroxidase (KatG) receptor targets of Mycobacterium tuberculosis sensitive and KatG resistant downloaded through www.rscb.org. The implementation of this research includes collecting ligand data from the literature, converting to SMILE and 3D formats using ChemOffice 10, affinity observation using Arguslab 4.01 and Biovia Discovery 2016, and evaluating the pharmacokinetic and toxicity profiles of selected ligands. The results of this study showed that 13 of the 18 INH derivatives had stronger affinity and the same interaction pattern with isoniazid through the formation of hydrogen bonds with Asp 137 against sensitive KatG receptors, and 7 of them did not show the same interaction pattern with isoniazid against resistant KatG receptors through the formation of hydrogen bonds with Arg 104. While evaluating the pharmacokinetic and toxicity profiles of 7 selected INH derivatives, only 3 showed better potential than INH. Thus, several INH derivative candidates are predicted to have better potential anti-tuberculosis effects through an in-silico approach.

KEYWORDS*: ADMET, anti-TB, derivative isoniazid, molecular docking.*

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INTRODUCTION

Tuberculosis (TB) is a highly infectious disease in the world. There are more than 50 types Mycobacterium which has been reported [1], but kind Mycobacterium tuberculosis (MTB) is considered the main cause of TB disease with the lungs as the main organ that is its habitat [2]. Mycobacterium tuberculosis as the main bacterial agent that causes tuberculosis (TB), it is reported that it causes the deaths of ± 3 million people every year and 8 million new cases occur. Based on the WHO report 2014 [3], Indonesia is one of the countries that contributes the largest TB in the world with a number of new cases of ± 539,000 with a very high number of deaths (± 101,000 per year). In fact, TB disease is reported to contribute to the highest death rate in cases of the infectious disease group and is the third highest number of deaths after cardiovascular disease and respiratory tract disease [4].

One of the drugs that is the first line of TB therapy is Isoniazid (INH), which has been used since 1970 [5]. However, the use of INH either as a single therapy or as a combination therapy has reported a prevalence of resistance reaching 10 - 20% [6]. The cause of resistance by genetic changes in the form of mutations in at least five genes such as KatG, InhA, ahpC [7-10]. The resistance to KatG is reported to occur due to gene mutations in several codons that make up the KatG amino acid, causing a change in the type of amino acid that makes up the enzyme. Among the amino acids that undergo these changes are the change from the amino acid

arginine to glycine at the 418th position, tryptophan to glycine at the 321st amino acid position, serine to threonine at the 315th amino acid position [11]. Mutations at the 315th amino acid position lead to changes in the interaction of isoniazid with the KatG enzyme. In KatG which has not undergone a mutation, there is an interaction between isoniazid and KatG, thus forming a hydrogen bond with the amino acid asparagine (Asp 137). Meanwhile, for KatG, which has undergone a mutation, a hydrogen bond between INH and KatG occurs at the amino acid position arginine 104 (Arg 104) [12]. This change causes a decrease in the sensitivity of INH as an anti-TB agent [13].

The problem of TB drug resistance is still the main issue, the reason the number of deaths is difficult to overcome. So, it is necessary to find and develop effective anti-TB drugs. However, in developing a drug, a lot of money is required and a long time is needed. Therefore, a practical approach is needed that can be considered in drug discovery in a fast time period, namely in silico. In Silico is a computational technique for predicting the interaction of a compound with a target receptor. This technique can be used to discover and develop new drugs by designing, discovering and optimizing a bioactive compound. Technical advantages in in silico, namely minimizing costs due to not using test animals, and getting fast results, the mechanism for selecting compounds as drug candidates can be observed visual by virtual screening [14]. In addition, the selection of candidate drug compounds can be done by modifying the structure of an existing drug whose complete biological activity is known. Several literatures state that hydrophilic INH derivatives with hydrophobic or lipophilic side chains can increase antibacterial potential against MTB types [15,16].

This research aims to study the interactions of modified INH compounds and refers to several INH derivatives that have been carried out by previous researchers. This research was carried out using an approach in silico or use software integrated with a computing system. Method in silico used as an initial step to guide work before clinical trials so that it is more effective and efficient. In addition, it predicts the interaction strength between INH derivatives through techniques molecular docking. Next, an analysis of its potential as a selected drug candidate was carried out through pharmacokinetic and toxicity profile analysis

MATERIALS & METHODS

Materials

Several isoniazid (INH) derivative compounds were selected as test ligands based on literature screening. The receptor used is the Catalase-Peroxidase (KatG) protein Mycobacterium tuberculosis sensitive (pdb id. 1SJ2) and Catalase-Peroxidase (KatG) resistant (pdb id. 2CCD) downloaded via www.rscb.org. Test in silico using hardware with specifications Intel® Core™ i7-4720HQ CPU @2.60Ghz, RAM (Random Access Memory) 8 gigabyte, And Graphic Card (NVIDIA GeForce GTX 950M) 4055 megabyte with operating system Windows10 equipped with software Arguslab 4.01 (FreeWare), ChemOffice 12.0 for 2 and 3 dimensional structural design, Biovia Discovery 2016 for visualization and supported by several web servers such as https://mordred.phs.osaka-u.ac.jp/, http://www.scbdd.com/cdk_desc/index/, https://biosig.lab.uq.edu.au/pkcsm.

Methods

1. Screening for INH Derivative Compounds That Have the Potential to Act as Antituberculosis
Identification of INH derivatives was traced through several Science Direct, Scopus and Google Scholar articles.

2. Preparation of 2 and 3 Dimensional Structures
Making 2 and 3 dimensional structures of INH and its 18 derivatives using the ChemOffice 12.0 program by copying and pasting the canonical SMILES structure of each compound from PubChem, then saving in format pdb.

3. Ligand Preparation
Ligands were drawn in 2D form using ChemDraw Ultra 12 software, then converted to 3D structures using Chem3D Pro 12 and after that saved in .pdb form. All ligands (INH and its 18 derivative compounds) were input into the Arguslab 4.01 window, then added protons if they were still incomplete, gave them a charge and adjusted the structure geometry so that it reached optimum conditions. Next, each ligand was arranged

into a ligand that was suitable for the Arguslab application system by clicking the "Make a ligand group from this residue" option. Meanwhile, in setting the receptor, it was first cleaned of water molecules and other non-standard molecules. Then proceed with the addition of hydrogen atoms.

4. Docking Using ArgusDock

The sensitive KatG (1SJ2) and resistant KatG (2CCD) receptors did not possess native ligands; therefore, the binding sites were selected based on the reports by (12,17), which stated that the antituberculosis activity of the sensitive KatG receptor occurred when INH formed a hydrogen bond with the amino acid Asp137, while in the resistant KatG receptor, a hydrogen bond was formed between INH and the amino acid Arg104. Each test compound was docked into the respective receptor binding site using a grid resolution of 0.4 Å. The binding free energy was calculated using the Ascore function. The binding sites were designed to be as flexible as possible to allow the ligand to move around Asp137 in the sensitive KatG and Arg104 in the resistant KatG, with the grid box axes adjusted accordingly. Docking was performed with 10 replications for each compound. All docking procedures were carried out under conditions in which water molecules and non-standard molecules that could interfere with the docking process were removed.

5. Binding Free Energy Value (ΔGbinding)

The binding free energy resulting from molecular docking was stored in format pdb. The selected enzyme-ligand complex was the complex that had the smallest binding free energy value for further analysis.

6. Hydrogen Bonding

The hydrogen bonds that occur in the best enzyme-ligand complex resulting from docking were identified and analyzed in a 2-dimensional structure using software *Biovia* Discovery 2016 (Free).

7. Visualization of Molecular Docking Results

The interaction model resulting from molecular docking of each ligand was explained descriptively

Table 1 Toxicity class levels are based on Globally Harmonized System (GHS)

Level	Category	LD_{50} value
Class 1	Fatal is swallowed	(LD50 ≤ 5 mg/kg)
Class 2	Fatal is swallowed	(5 < LD50 ≤ 50 mg/kg)
Class 3	Toxic is swallowed	(50 < LD50 ≤ 300 mg/kg)
Class 4	Dangerous if swallowed	(300 < LD50 ≤ 2000 mg/kg)
Class 5	Maybe dangerous if swallowed	(200 < LD50 ≤ 5000 mg/kg)
Class 6	Non-Toxic	(LD50 > 5000 mg/kg)

8. ADMET Profile Predictions

Prediction of absorption, distribution, metabolism, excretion and toxicity profiles was carried out using the pkCSM program (https://biosig.lab.uq.edu.au/pkcsm/prediction) and protox ii (http://tox.charite.de/protox_II), was done by entering the SMILES list or *the Simplified Molecular Input Line Entry Specification* in the entry box provided. ADME showed various predictive profiles of absorption, distribution, metabolism, and excretion of INH derivatives. ADME parameters included observations of absorption capacity in the human intestine (human intensive absorption, HIA), distribution of the blood brain barrier (BBB), plasma protein binding (PPB). P-glycoprotein (Pgp) inhibition parameters and substrates, carcinogenicity potential and hepatotoxic. LD50 or median lethal dose is the dose that shows that 50% of the test subjects died due to exposure to the treatment compound. Toxicity prediction results were based on 2D similarity analysis (SMILE input). The toxicity level referred to classification globally harmonized system of classification of labelling of chemicals (GHS) (Table 1) Pharmacokinetic and toxicity profile data were tabulated in tables and analyzed descriptively.

Contain brief but sufficiently complete description of procedures and materials in order to allow the experiment to be repeated. Only new procedures should be described. Previous published procedures should be referenced. Significance materials should be described in detail.

RESULTS

1. Analysis Lipinski Rule of Five

There were 18 INH derivatives collected from literatures. The analysis of Lipinski's rule was done to predict the compatibility of oral route administration of these compounds. Apart from that, the Lipinski Rule is also used to predict the bioavailability of an active substance, this is because it is related to the solubility and permeability of a compound in the gastrointestinal tract. Results *Lipinski rule of five* can be seen on Table 2

Table 2. The Results of Analysis Lipinski's Rule of INH Derivatives

Compound	Molecular Weight (<500g/mol)	Molar Refractivity (40-130)	Log P (<5)	Hydrogen Bond		Satisfies Lipinski's rule
				Donor (<5)	Acceptor (<10)	
Isoniazid	137	36.85*	0.8	2	3	Yes
Derivat 1	424	88.72	2.7	2	6	Yes
Derivat 2	180	44.42	0.9	3	6	Yes
Derivat 3	196	52.89	1	3	4	Yes
Derivat 4	249	59.07	0.8	2	7	Yes
Derivat 5	259	67.11	1	2	6	Yes
Derivat 6	179	45.52	0.6	2	5	Yes
Derivat 7	253	71.13	2.0	1	5	Yes
Derivat 8	253	45.52	2.0	1	5	Yes
Derivat 9	255	71.67	1.7	2	5	Yes
Derivat 10	255	71.67	1.7	2	5	Yes
Derivat 11	203	57.60	2.1	1	4	Yes
Derivat 12	293	86.79	4.0	1	4	Yes
Derivat 13	283	77.08	2.0	1	6	Yes
Derivat 14	317	92.57	4.0	1	5	Yes
Derivat 15	291	82.46	3.8	2	5	Yes
Derivat 16	179	45.52	0.9	2	5	Yes
Derivat 17	295	86.28	3.9	2	4	Yes
Derivat 18	167	43.70	0.8	4	6	Yes

Description: (*) There are deviations *rule of five lipinski*

Based on Table 2, it showed that there were 5 derivatives that have molecular weights in the range of 101- 199 g/mol. Meanwhile, 11 derivatives have molecular weights in the range of 201 to 299 g/mol. Apart from that, there is 1 compound which has a molecular weight of more than 300 g/mol, and 1 other derivative has a molecular weight of more than 400 g/mol. This shows that none of the INH derivative compounds has a molecular weight greater than that all INH derivative compounds have a molecular weight ≤ 500 g/mol. Meanwhile, INH has a molecular weight of 137g/mol. So, INH and all its derivatives can be absorbed and have high permeability in the intestinal tract. This is in accordance with the rules RO5 that is, the smaller the molecular weight of a compound, the greater the chance of absorption. So that INH and all its derivatives meet the molecular weight criteria.

The next parameter to look at is: *molar refractivity* (*MR*), there are 4 derivatives that have *MR* in the 41-50 range. Meanwhile, there are 3 derivatives that have scores *MR* in the 51-60 range. 1 other derivative has a score *MR* in the 61-70 range. Then there are 5 derivatives that have a score *MR* in the 71-80 range. Furthermore, there are 4 derivatives that have scores *MR* with a range of 81-90, and 1 other derivative has a score *MR* with a range of 91-100. Meanwhile INH has a score *MR* namely 37.8. In the results listed on Table 2 explained that INH has poor solubility properties so it is necessary to modify the INH compound to increase its solubility properties. This is in accordance with Table 2 All INH derivatives of this compound have good solubility properties so they can be absorbed orally.

The next criterion is the assessment of the LogP score. There are 7 derivatives that have LogP scores in the range 0.1-1. Furthermore, there are 5 derivatives with LogP scores in the range 1.1-2.0. Then there are 2 INH derivatives with LogP scores in the range 2.1-3.0. And 4 other derivatives have Log P scores of 3.1-4.0. Meanwhile, INH has a Log P score of 0.78. This shows that INH and all its derivatives have the ability to penetrate the plasma membrane, distribute and have an affinity for plasma proteins.

On Table 2**,** 7 derivatives were obtained that had a hydrogen donor with a score of 1. Then there were 9 derivatives that had a hydrogen donor with a score of 2. There were 2 derivatives that had a hydrogen donor with a score of 3, and 1 derivative that had a hydrogen donor with a score of 4. Meanwhile, INH had a hydrogen donor. with a score of 2. Based on this data, not a single derivative was found that had a hydrogen donor with a score of more than 5, so that INH and its 18 derivatives do not require a lot of energy to carry out the absorption process and are stable in binding to biological targets. So that from Table 2 The potential of compounds can be used as potential effective drugs. Based on Table 2, 4 derivatives were obtained that had a hydrogen acceptor with a score of 4. There were 8 derivatives that had a hydrogen acceptor with a score of 5. Then there were 5 derivatives that had a hydrogen acceptor with a score of 6, and 1 other derivative had a hydrogen acceptor with a score of 7. Meanwhile, INH got an acceptor hydrogen with a score of 3. Based on Table 2 not a single compound was found that was more than a hydrogen acceptor. Thus, INH and INH derivatives have the ability to penetrate cell membranes and do not require a lot of energy in the absorption process so that the compounds obtained have the potential to become effective drugs.

2. Analysis Molecular Docking With 1SJ2 (Cat sensitive)
All derivatives are then processed *molecular docking* on Sensitive KatG receptor (targeting amino acid Asp 137) is unprocessed redocking because it does not bind to the native ligand so the process molecular docking what is done is blind docking oriented (directed). Instead of mold binding site targeting processes docking using the reference amino acid Asp 137.

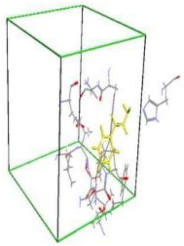
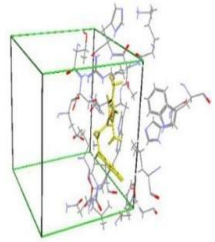
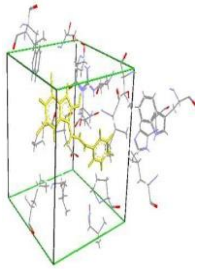
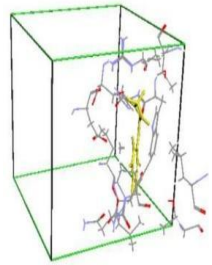
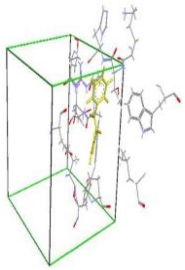
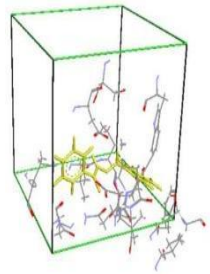
The docking results of all INH derivatives on the KatG receptor are sensitive to bacteria *M. tuberculosis.* It can be seen that the affinity value is stronger than INH. On Table 3 shows INH derivatives that have a score ΔG *binding* the lowest is derivative 12. The lower Δ*Gbinding* then the interaction becomes stronger. However, the affinity is not enough to make it a derivative that has greater potential than INH. Therefore, it is necessary to analyze derivatives that interact with Asp 137 hydrogen bonds as amino acid targets [12].

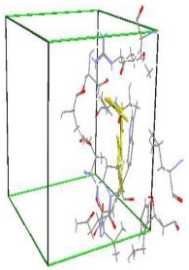
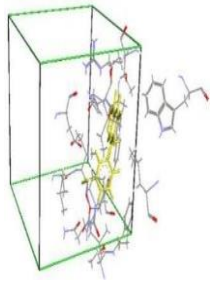
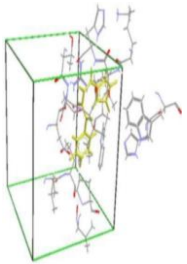
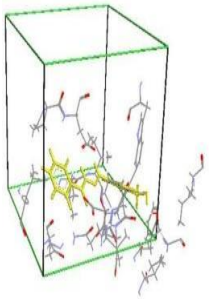
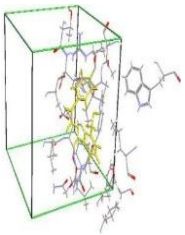
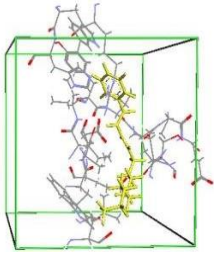
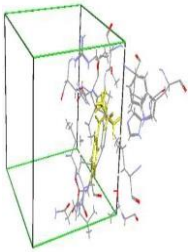
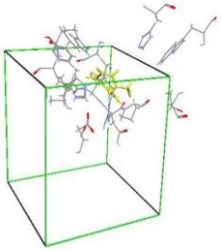
Based on Table 3, There are 13 derivatives that have interactions with Asp 137 and have score Δ*Gbinding*: derivative 12, derivative 18, derivative 8, derivative 5, derivative 1, derivative 13, derivative 15, derivative 9, derivative 11, derivative 7, derivative 17, derivative 4, and derivative 6 interact with Asp 137. Meanwhile there are 5 Derivatives that do not have hydrogen bond interactions with Asp 137 are derivative 10, derivative 14, derivative 16, derivative 2 and derivative 3. So, they do not have an anti-TB effect, this is because they are not found to interact with Asp 137 hydrogen bonds.

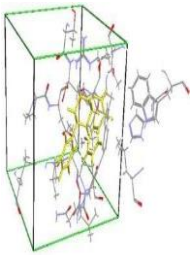
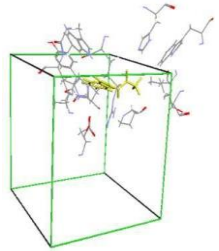
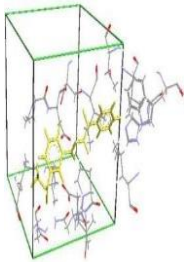
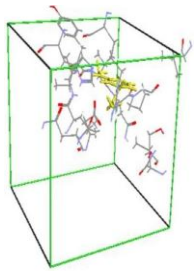
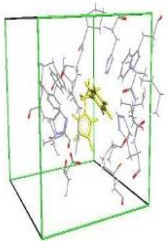
3. Analysis Molecular Docking With 2CCD (KatG Resistant)
Thirteen derivatives which had better potential than INH were continued with screening methods molecular docking on Cat resistant. In this process use binding site and has a target on the amino acid Arginine 104 (Arg 104). Results molecular docking can be seen on Table 4.

Analysis of INH derivatives against sensitive KatG was then carried out molecular docking with a target of 2CCD in 3x repetitions to identify derivatives that do not have the potential to experience resistance, namely by looking at the INH derivative indicator that does not interact with the Arg 104 hydrogen bond. Based on Table 4**,** 7 INH derivatives were obtained that did not interact with the Arg 104 hydrogen bond, namely derivative 11, derivative 4, derivative 7, derivative 9, derivative 13, derivative 1, derivative 12, and derivative 13.

Table 3. Analysis of results molecular docking best 19 compounds with 1SJ2 (sensitive KatG) in 3x repetitions

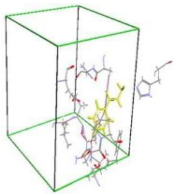
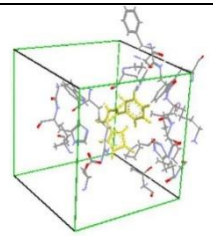
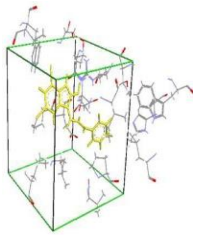
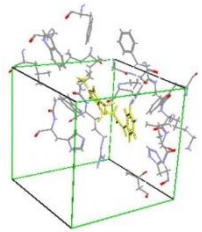
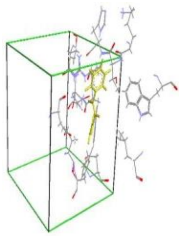
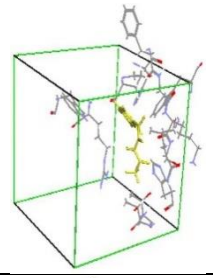
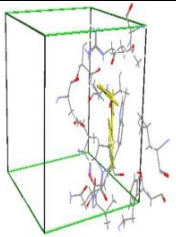
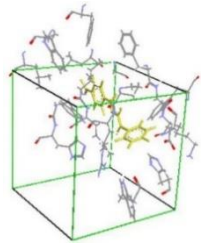
Compound	$\Delta G_{binding}$	Hydrogen Bonding	3 dimensions	Compound	$\Delta G_{binding}$	Hydrogen Bonding	3 dimensions
Isoniazid	-6.87	**Asp 137**, Val 230, His 270		Derivative 5	-7.88	Val 230, His 108, **Asp 137**, Arg 104	
Derivative 1 *	-7.86	**Asp 137**, Ser 315		Derivative 6	-7.06	Tyr 229, Trp 107, **Asp 137 (2)**	
Derivat 2	-6.89	Val 230, His 108, His 270, Trp 107		Derivative 7*	-7.43	His 270, **Asp137**	

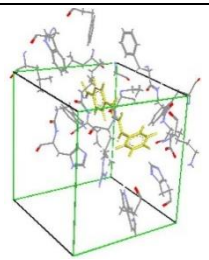
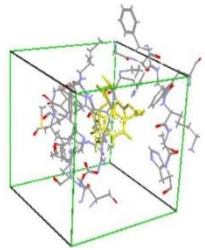
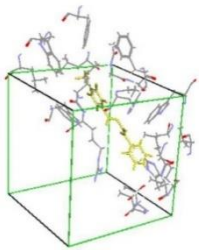
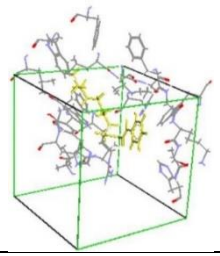
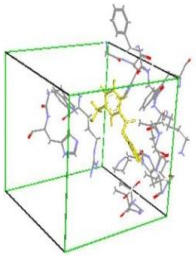
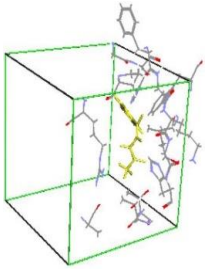
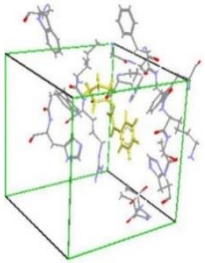
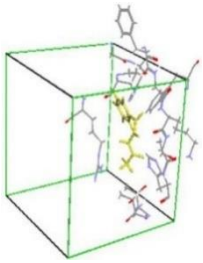
Derivat 3	-7.02	His 108, Arg 104, Val 230		Derivative 8	-8.11	**Asp 137**, Trp 108, His 108	
Derivat 4 *	-7.07	**Asp 137** (2), Arg 104 (2), His 108		Derivative 9	-7.65	**Asp 137**	
Derivative 10	-7.72	Ser 315, Trp 107, His 108		Derivative 15*	-7.83	His 108, Pro 232, Val 230 (2), **Asp 137 (2)**	
Derivative 11*	-7.48	**Asp 137**, Thr 229, His 108		Derivative 16	-7.19	Val 230, Trp 107, Arg 104, His 108 (2)	

Derivat 12*	-9.40	Arg 104, **Asp 137,** His 108, Val 230 (2)		Derivative 17*	-7.08	**Asp 137**, Trp 107, His 108 (2)	
Derivat 13*	-7.83	**Asp 137**, Thr 314, Arg 104, His 108, Trp 107 (2)		Derivative 18*	-8.36	**Asp 137**, Tyr 229, His 108, Arg 104, Val 230	
Derivat 14	-7.52	Val 230, Trp 107					

Information: * INH derivatives that have better potential compared to INH. It is denoted by $\Delta G_{binding}$ lower and binds to asp 137

Table 4. Analysis of results molecular docking best 19 compounds with 2CCD receptors (*KatG* resistance) in 3 repetitions

Compound	$\Delta G_{binding}$	Hydrogen Bonding	3 dimensions	Compound	$\Delta G_{binding}$	Hydrogen Bonding	3 dimensions
Isoniazid	-6.87	**Asp 137**, Val 230, His 270		Derivative 4	-7.86	Thr 315, His 270 (2), Pro 100	
Derivative 1 *	-7.86	**Asp 137**, Ser 315		Derivative 5	-7.67	Val 230, His 108, Asp 137, Arg 104	
Derivat 2	-6.89	Val 230, His 108, His 270, Trp 107		Derivative 6	-7.54	Trp 107, His 108, His 270, Lys 274, Arg 104	
Derivat 3	-7.02	His 108, Arg 104, Val 230		Derivative 7	-8.00	His 108, Trp 107, Val 230, His 270	

Derivative 8	-8.29	His 108, Arg 104		Derivative 14	-9.26	His 276, His 108, Val 230, Trp 107	
Derivative 9	-8,86	Val 230, His 108, Trp 107, His 270, Pro 100		Derivative 15	-11.43	Lys 274, Arg 104, Trp 107, His 270	
Derivative 10	-8.66	Thr 315, His 270 (2), Pro 100, Arg 104		Derivative 16	-7.34	Trp 107, Lys 274, Arg 104	
Derivative 11	-7.62	Lys 274, His 270		Derivative 17	-7.58	His 270, Gly 269 (2), Phe 272, Lys 274, Arg 104	

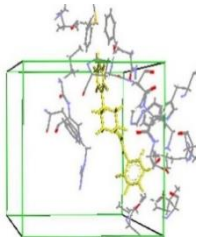
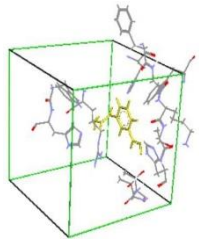
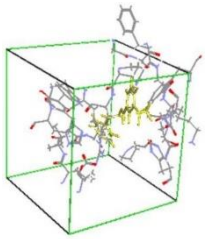
Derivative 12	-10,30	His 270 (2), Thr 315		Derivative 18	-8.91	Trp 107, Arg 104, His 108, His 276, Lys 274, His 270	
Derivative 13	-8.95	Lys 274, His 276, Val 230, His 270, Trp 107, His 108					

Table 6. The results of Pharmacokinetic Parameters of Isoniazid and 4 derivatives using the PkCSM website

Compound	Absorption	Distribution		Excretion	Metabolism (CYP 450)				
	HIA (%)	PPB (%)	BBB (%)	Total Clearance	3A4 substrate	1A2 inhibitior	2C19 inhibitor	2C9 inhibitor	3A4 inhibitor
INH	75,65	63,5	-0,117	0.782	No	Yes	No	No	Yes
Derivat 1*	92,53	14,1	-0,733	-0,378	No	No	No	No	No
Derivat 4*	78,25	38,4	-1,023	-0,336	No	No	No	No	No
Derivat 7	95,84	19,2	-0,333	0,668	Yes	Yes	Yes	No	Yes
Derivat 9 *	93,57	21,7	-0,331	0,707	Yes	No	No	No	No

Note: (*) Derivatives that have better effectiveness compared to INH

Based on Table 5, there are 2 derivatives that have predicted LD_{50} with a range of 301 – 399 in compound derivative 11 and derivative 12, then there are 3 derivatives that have predicted LD_{50} the same, namely derivative 7, derivative 9, and derivative 13 with a value of 710 mg/kg. Apart from that, there is 1 derivative that has a predicted LD $value_{50}$ 1080 mg/kg is derivative 4, and there is one other derivative that has an LD $value_{50}$ amounting to 3900 mg/kg. Meanwhile, INH has LD $predictions_{50}$ amounted to 133 mg/kg. Based on this data, INH is a class of compounds that are toxic and have a high risk of toxic poisoning. One way that can be done to increase the LD $value_{50}$ by modifying the INH structure. Therefore, it is proven by the existence of 7 derivatives listed in Table 5 LD $value_{50}$ has increased and is in a safer group compared to isoniazid.

On Table 5, unique data was obtained, namely 6 derivatives belonging to the same category (category IV). These derivatives are derivative 4, derivative 7, derivative 9, derivative 11, derivative 12, derivative 13. And we obtained 1 compound which is included in category V, namely derivative 1. Meanwhile, INH is included in category III, which means INH is included in the "Toxic" category. If swallowed" so things that must be done include making isoniazid derivatives and administering the drug via intramuscular injection based on pharmaceutical preparations circulating on the market. Therefore, these 7 potential isoniazid derivatives have a safe level of safety to be given compared to INH.

On parameters carcinogenicity looks on Table 5 All isoniazid derivatives do not have the potential to be carcinogenic, while isoniazid has been found to be potentially carcinogenic (causing the growth of cancer cells). So, the solution that can be provided so that isoniazid does not have the potential to be carcinogenic is by modifying the structure of isoniazid. Proven on Table 5 It was found that all isoniazid derivatives were not indicated to have carcinogenic potential. So, the isoniazid derivative is safe to administer.

On parameters hepatotoxicity which is written on Table 5 It was found that 4 derivatives did not have the potential to be hepatotoxic, and 3 other derivatives had the potential to be hepatotoxic (disrupting and damaging the function of the liver). Meanwhile, it was found that isoniazid does not have the potential to be hepatotoxic. So, from toxicity testing it was found that 4 compounds (derivative 1, derivative 4, derivative 7, and derivative 9) were potentially safer than INH.

5. Prediction of Absorption, Distribution, Metabolism, and Excretion (ADME)

Potential derivatives and better security levels, namely derivative 1, derivative 4, derivative 7, and derivative 9 are continued towards the ADME process. The ADME process is important to determine the effectiveness of a compound and avoid unwanted failures in drug development. The ADME process is carried out through a site-based website web namely pkCSM. ADME results can be seen at Table 6.

Based on Table 6, there are 3 derivatives that have a % HIA in the range of 91% - 99%, namely derivative 1, derivative 7 and derivative 9, and 1 other derivative has a % HIA of 78.25%, namely derivative 4. Meanwhile, INH has a % HIA of 75.65%. No compounds were found that had a % HIA value < 70%. No literature has been found that consistently mentions % HIA. If based on data on Table 6 INH and its derivatives have good absorption capacity. If the HIA % is less than 70% then INH therapy and its derivatives experience a decrease in bioavailability thereby reducing the level of therapeutic effect.

Then the profile Plasma Protein Binding (PPB) on Table 6, 2 derivatives were obtained which had a concentration of 11% -20%, namely derivative 1 and derivative 7. There was 1 derivative which had a PPB concentration of 21%, and 1 other derivative had a concentration of 38%. Meanwhile, INH has a PPB level of 63.5%. Specific levels of PPB have not yet been found. But if you look at Table 6. Isoniazid and its derivatives have PPB levels < 90%. This means that INH and its derivatives are weakly bound to plasma proteins so that all compounds can be distributed well to their working targets. If the test compound is strongly bound to PBB, it will reduce the bioavailability levels of INH and its derivatives.

Other distribution parameters are Blood Brain Barrier (BBB). On Table 6, it was found that 3 derivatives had BBB levels in the range -0.1 to -0.9, and 1 other derivative, namely derivative 4, had a BBB level of -1.023. Meanwhile, isoniazid has a BBB level of -0.117. Based on the literature, specific BBB levels have not been found. However, if you refer to Table 6, INH and its derivatives do not have levels > 0.3. So, isoniazid and its derivatives have difficulty penetrating the brain barrier. If INH and its derivatives have the ability to easily penetrate the barrier, there is a big risk of causing side effects, and BBB levels must be monitored during

treatment.

Based on Table 6 with total clearance parameters, it was found that 2 derivatives had a total clearance with a range -301 to -399, namely in derivative 1 and derivative 4. Then there is 1 derivative which has a total clearance value of 0.668, namely in derivative 7, and 1 other derivative has a total clearance value of 0.778. Meanwhile, INH has a total clearance value of 0.782. A good total clearance value has not been found. Based on data obtained in Table 6 showed that INH derivatives had total clearance levels lower than INH. Thus, the range of repeated use of INH derivatives with low total clearance values must be adjusted to avoid the risk of increasing side effects and toxicity.

Another substrate parameter that needs to be considered is the CYP3A4 substrate. Can be seen at Table 6, there are two derivatives that interact with CYP3A4 substrates, namely derivative 7 and derivative 9. The other two derivatives, namely derivative 1 and derivative 4, do not interact with CYP3A4 substrates. Meanwhile, INH does not interact with CYP3A4 substrates. However, there was no literature that stated that INH interacts with the CYP3A4 substrate. The two derivatives interacting with CYP3A4 require monitoring of levels, especially if they are to be combined with other drugs, INH drug levels are required for dose adjustment. This is because it can increase the level of INH drug to be excreted so that the effectiveness of the drug decreases because the drug level is easily excreted [19].

The next parameter is CYP1A2 inhibitor. Can be seen at Table 6 there are 3 derivatives that do not interact with CYP1A2 inhibitor metabolism, namely derivative 1, derivative 4 and derivative 9. Meanwhile, derivative 7 and INH interact with CYP1A2 inhibitors. There has been no literature that states that INH interacts with the metabolism of CYP1A2 inhibitors. But based on Table 6 What you need to pay attention to is the INH drug level in the plasma, and if you want to combine it with other drugs you need to monitor the levels of the combination drug. Because it interacts with CYP1A2 inhibitors, it causes INH drug levels to be inhibited, and increases combination drug levels and results in side effects [20].

The next inhibitor parameter is CYP2C19 inhibitor. Can be seen at Table 6, there are INH and 3 derivatives that do not interact with CYP2C19 inhibitor metabolism, namely derivative 1, derivative 4 and derivative 9. Meanwhile, derivative 7 interacts with CYP2C19 inhibitors. Literature was found which states that INH interacts with the metabolism of CYP2C19 inhibitors. This is in accordance with the data obtained in Table 6, thus causing INH levels to remain in the blood plasma longer. If you want to combine it with other drugs, it is necessary to monitor the levels of the combination drug in the blood plasma, and make a 50% dose adjustment for the combination drug so that the concentration in the plasma does not increase significantly. Provide education if there are side effects in the form of nausea and vomiting to report to health care workers [21].

The next inhibitor parameter is CYP2C9 inhibitor. Can be seen at Table 6, the results showed that INH and all derivative do not interact with CYP2C9 inhibitor metabolism (derivative 1, derivative 4, derivative 9) along with INH did not interact with CYP2C9 inhibitors. However, in Desta's journal [22] stated that INH interacts with the metabolism of CYP2C9 inhibitors, and the results showed that there was no significant increase in plasma drug levels. So, if you want to combine it with other drugs, you are required to monitor the levels of the drug in the plasma, so that it doesn't experience a spike and cause side effects such as nausea, vomiting and even hepatotoxicity [22].

The final inhibitor parameter is CYP3A4 inhibitor. Based on Table 6, it was found that 3 derivatives did not interact with CYP3A4 inhibitors, namely derivative 1, derivative 4, and derivative 9. Meanwhile, derivative 7 and isoniazid interact with CYP3A4 inhibitors. This is in accordance with the research by Zhuang et al. (2022) [23] which causes the interaction of INH with rifampicin because INH has inhibitory properties. The results showed that there was toxicity in INH, this was because INH inhibited the drug rifampicin, thereby increasing drug levels in plasma, and causing toxicity. Thus, things that must be considered are adjusting the dose to minimize the level of rifampicin in the plasma, as well as monitoring liver function to avoid hepatotoxicity [19].

This based on the ADME parameters listed on Table 6, it was found that INH derivatives had better effectiveness, namely derivative 1, derivative 4, and derivative 9. Meanwhile, derivative 7 had the same

pharmacokinetic profile as the pharmacokinetic profile of INH. So, 3 derivatives were selected (derivative 1, derivative 4, derivative 9) which had better anti-TB effectiveness than isoniazid

CONCLUSION

Based on the research results that have been obtained, it can be concluded that of the 18 INH derivatives, 3 INH derivatives (derivative 1, derivative 4, and derivative 9) have the potential to have better anti-TB effects and are safer than INH. This is because it has the same target as isoniazid and has the potential to be more effective and safer than isoniazid.

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