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RESEARCH ARTICLE

Chemoinformatics approach to the screening and development of quassinoids from *Brucea javanica* as antituberculosis drugs

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Abstract

Background: The morbidity and mortality of tuberculosis (TB) remain high in various countries as a result of pharmacological intervention failures, such as incomplete treatment regimens and inadequate doses, triggering resistance of *Mycobacterium tuberculosis* strains to anti-TB drugs used. This phenomenon requires innovation to explore and develop novel anti-TB drugs so that the problem of resistance is overcome and treatment of TB is more optimal. **Objective:** In this study, chemoinformatics investigations were carried out on quassinoids derived from *Brucea javanica* to be developed as anti-TB drugs. **Method:** Evaluation of drug-likeness with the SwissADME online tool, prediction of toxicity with the pkCSM online tool, and molecular docking studies with AutoDock Vina software were performed on 18 quassinoids *from Brucea javanica*. **Result:** The findings showed that Bruceine A, Bruceine, and Bruceine D, met the drug-likeness criteria, showed a good toxicity profile, and had better binding energy (-7.5; -7.5; and -7 kcal/mol, respectively) than isoniazid (-5.8 kcal/mol) which is a first-line anti-TB drug on enoyl acyl carrier protein reductase (InhA; PDB ID: 2NSD). **Conclusion:** This study found several quassinoids from *Brucea javanica* with potentials to be developed as anti-TB drugs.

Introduction

Tuberculosis (TB) is a respiratory infectious disease caused by the invasion of *Mycobacterium tuberculosis* (Qiang *et al*., 2021). A global investigation of TB surveillance data in 2019 estimated that no less than nine million individuals had TB, and at least one point four million TB-related deaths were found worldwide (Fukunaga *et al*., 2021). The prevalence rate of individuals infected with TB is highest in Africa and Southeast Asia (including Indonesia) (Chakaya *et al*., 2021; Fukunaga *et al*., 2021). The high number of TB cases, morbidity, and mortality cannot be separated from the failure of pharmacological interventions caused by incomplete treatment regimens and inadequate doses that lead to increased resistance to anti-TB drugs (Dartois & Rubin, 2022).

The phenomenon of resistance to at least two types of first-line anti-TB drugs (isoniazid and rifampicin) or known as multi-drug-resistant (MDR)-TB, has prompted the development of second-line anti-TB drugs (thiocarlide, thioacetazone, prothionamide, ethionamide, amikacin, kanamycin or capreomycin, as well as fluoroquinolone derivatives) (Prestinaci *et al*., 2015). However, second-line anti-TB drugs also have various disadvantages, including being less potent, more toxic, and more expensive than first-line anti-TB (Aaina *et al*., 2021). In addition, various studies have also revealed the phenomenon of resistance followed by extensive drug-resistant (XDR)-TB, namely;

resistance to rifampicin, isoniazid, fluoroquinolones, and one of amikacin, kanamycin, or capreomycin, while total-drug-resistant (TDR)-TB which is a phenomenon of resistance to all anti-TB drugs both first line and second line are also widely found (Chauhan *et al*., 2021). Additionally, the emergence of the COVID-19 pandemic is a new challenge in TB control, and it is estimated that the number of MDR-TB cases will increase (Tiberi *et al*., 2021; Caren *et al*., 2022). The various emergency conditions above indicate that efforts are still needed to explore and develop new anti-TB drugs. The recent development on bedaquiline, delamanid, pretomanid, second-generation oxazolidinones (TB-223, delpazolid, sutezolid), and diarylquinolines (TBAJ-876 and TBAJ-587), may show that need for an emphasis on TB (Black & Buchwald, 2021).

Brucea javanica, an evergreen shrub, is broadly distributed in Southeast Asia and Australia (Ye *et al*., 2015). Several studies revealed that *Brucea javanica* contains compounds with various pharmacological effects ranging from anticancer, antidiabetic, antioxidant, anti-inflammatory, and antimalarial (Ye *et al*., 2015; Ablat *et al*., 2017; Huang *et al*., 2017; Guo *et al*., 2022). Uniquely, no studies have explored the potential of *Brucea javanica*-derived compounds as anti-TB or the underlying signalling pathway, so this opens a wide window for further research. Thus, in this study, exploration and screening of quassinoids from *Brucea javanica* were carried out to be developed as anti-TB drugs through a chemoinformatic approach.

Methods

Ligands selection and preparation

In this study, we carried out an in-silico approach from February 11th 2022 to June 29th 2022. The selected ligands were 18 quassinoids from *Brucea javanica*, which were found and isolated in previous studies (Ye *et al*., 2015). Isoniazid, a first-line anti-TB drug, was chosen as a comparator ligand in this study. Three-dimensional structures of all ligands were created and optimised using ChemDraw and Chem3D version 21.0.0 software (PerkinElmer). Ligands were prepared by removing water molecules, protonating them, and adding gasteiger charge using AutoDockTools-1.5.7 software (Gani *et al*., 2021).

Protein selection and virtual elucidation

In this study, the protein chosen was enoyl acyl carrier protein reductase (InhA). Protein structure with PDB ID: 2NSD containing the reference ligand N-(4-methyl-benzoyl)-4-benzyl-piperidine downloaded from the Protein Data Bank (https://www.rcsb.org/). Protein preparation was carried out by removing water molecules, adding hydrogen along with Kollman charges, and lost atoms were repaired using AutoDockTools-1.5.7 software (Gani *et al*., 2021).

Protein binding sites determination and validation

The selection of the InhA protein chain as the target for the molecular docking study was determined based on the pocket binding sites on the protein chain, which resulted in the highest drug score. Evaluation of pocket binding sites was carried out using the DoGSiteScorer (http://dogsite.zbh.uni-hamburg.de). A high drug score at a pocket-binding site indicates a high tendency of drugs to occupy the pocket-binding site (Nurhan *et al*., 2022). Protein binding sites in the selected chain were validated by docking using a reference ligand three times. Furthermore, the root mean square deviation (RMSD) of the docked form of the reference ligand was evaluated using PyMOL version 2.3.4 software. The grid box occupied by the reference ligand is ideal and valid to be targeted as a protein binding site if it produces an RMSD value < 2 Å.

Molecular docking

Molecular docking was performed using AutoDock Vina software. Docking results are interpreted as binding energy values. The lower binding energy value indicates a good interaction between the functional groups of ligands and the amino acid residues of the protein. In this study, the binding energy values of the tested ligands (eighteen quassinoids from *Brucea javanica*) were compared to the binding energy values of isoniazid.

Drug-like properties and toxicity profiles prediction

Prediction of drug-like properties of eighteen quassinoids from *Brucea javanica* and isoniazid was performed using the SwissADME online tool. The toxicity profiles, including AMES toxicity, hepatotoxicity, oral rat acute toxicity (median lethal dose; LD_{50}), and oral rat chronic toxicity (lowest-observed-adverse-effect level; LOAEL), were evaluated using the pkCSM online tool (Nurhan *et al*., 2021).

Results

Determined protein binding sites

Evaluation of the protein showed that the pocket binding site with the highest drug score (0.82) was located in chain A. Validation of the protein binding site in the selected chain (chain A) showed an average ± standard deviation of the RMSD of 0.07 ± 0.01 (RMSD <

2 Å) after three docking replications of the reference ligand. Thus, the solution formed is categorised as a good solution so that the grid box occupied by the reference ligand is valid and ideal to be used as a docking target. The structure of the InhA protein is visualised in Figure 1, and the selected grid boxes used in this study are shown in Table I.

Table I: Selected grid boxes of *InhA* protein as targeted docking

Target protein	Grid centre coordinates (Å)	Grid box size (Å)
InhA (PDB ID: 2NSD)	Centre x = 49.806	Size x = 40
	Centre y = 51.767	Size y = 40
	Centre z = 37.329	Size z = 40

Interaction prediction of quassinoids from Brucea javanica with InhA protein

Based on the docking analysis of the tested ligands for InhA protein, it was shown that all quassinoids from *Brucea javanica* produced lower binding energy values than isoniazid (< -5.8 kcal/mol). The range of binding energy values of the eighteen compounds ranged from -9.3 to -6.9 kcal/mol. Overall binding energy values of each ligand tested against InhA are shown in Figure 2.

Drug-like properties and toxicity profiles prediction of quassinoids from Brucea javanica

Based on the analysis of drug-likeness properties, it was found that bruceine A, bruceine, and bruceine D met Lipinski's rule of five criteria (Lipinski *et al.,* 2012) (see Table II). Prediction of the Ames toxicity profile showed that none of the quassinoids from *Brucea javanica* tested in this study was positive. Meanwhile, the hepatotoxicity evaluation showed that one quassinoid from *Brucea javanica* (bruceantinol) was predicted to be hepatotoxic. The assessment of LD_{50} and LOAEL of the test compounds showed that all quassinoids from *Brucea javanica* tested in this study showed both LD_{50} and LOAEL values were higher than the comparator ligand (isoniazid).

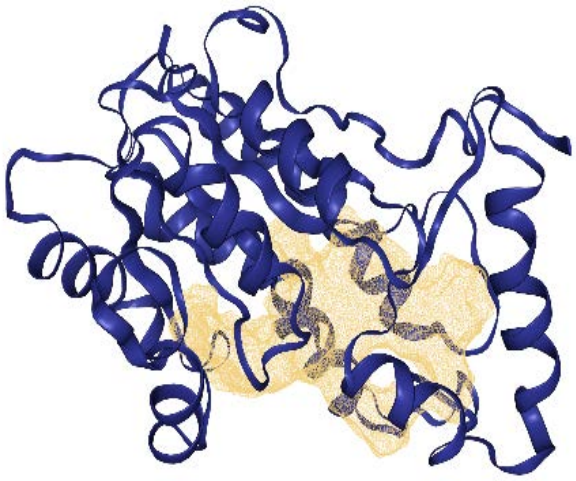

Figure 1: Visualisation of InhA protein with its pocket binding site as a targeted docking. Blue ribbons represent InhA protein, and the pocket binding site is identified by the light gold

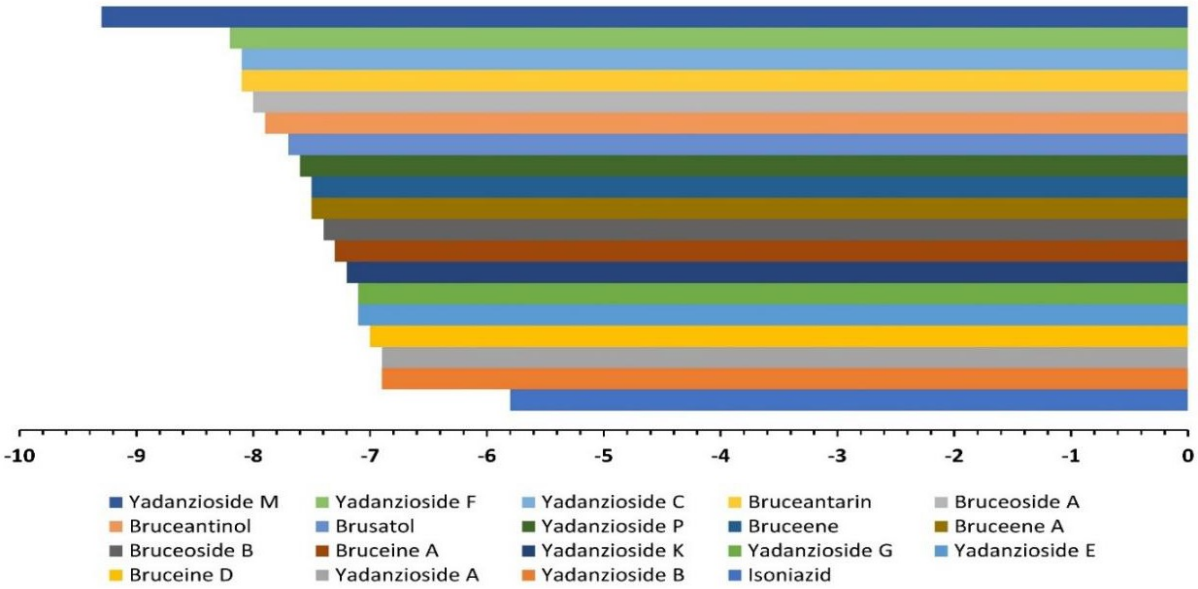

Figure 2: Binding energy of quassinoids from *Brucea javanica* compared with isoniazid to InhA protein

Table II: Drug-likeness properties and toxicity profiles prediction of quassinoids from *Brucea javanica*

Common name	Formula	Drug-likeness (Lipinski's rule of five)	AMES toxicity	Hepatotoxicity	Oral rat acute toxicity (LD_{50}*, mol/kg)	Oral rat chronic (LOAEL**, log mg/kg BW/day)
Bruceene A	$C_{20}H_{26}O_9$	Meets the criteria	No	No	2,700	4,948
Bruceene	$C_{20}H_{26}O_8$	Meets the criteria	No	No	2,777	4,100
Bruceine D	$C_{20}H_{26}O_9$	Meets the criteria	No	No	2,931	4,482
Yadanzioside E	$C_{32}H_{44}O_{16}$	Does not meet the criteria	No	No	3,205	5,602
Brusatol	$C_{26}H_{32}O_{11}$	Does not meet the criteria	No	No	3,301	3,643
Bruceoside B	$C_{32}H_{42}O_{16}$	Does not meet the criteria	No	No	3,481	4,988
Bruceantinol	$C_{30}H_{38}O_{13}$	Does not meet the criteria	No	Yes	3,640	3,563
Yadanzioside K	$C_{36}H_{48}O_{18}$	Does not meet the criteria	No	No	3,354	4,712
Yadanzioside P	$C_{34}H_{46}O_{16}$	Does not meet the criteria	No	No	3,280	5,611
Bruceine A	$C_{26}H_{34}O_{11}$	Does not meet the criteria	No	No	3,623	3,697
Yadanzioside B	$C_{32}H_{44}O_{17}$	Does not meet the criteria	No	No	2,891	6,287
Bruceantarin	$C_{28}H_{30}O_{11}$	Does not meet the criteria	No	No	4,097	3,679
Bruceoside A	$C_{32}H_{42}O_{16}$	Does not meet the criteria	No	No	3,340	4,948
Yadanzioside C	$C_{34}H_{46}O_{17}$	Does not meet the criteria	No	No	3,265	4,754
Yadanzioside G	$C_{36}H_{48}O_{18}$	Does not meet the criteria	No	No	3,239	4,731
Yadanzioside F	$C_{29}H_{38}O_{16}$	Does not meet the criteria	No	No	3,090	4,627
Yadanzioside A	$C_{32}H_{44}O_{16}$	Does not meet the criteria	No	No	3,320	5,341
Yadanzisoide M	$C_{34}H_{40}O_{16}$	Does not meet the criteria	No	No	3,090	5,513
Isoniazid (Control)	$C_6H_7N_3O$	Meets the criteria	Yes	No	2,364	2,824

*LD_{50} is the amount of a compound given all at once that causes the death of 50% of a rat.
**LOAEL is the lowest dose of a compound that results in an observed adverse effect.

Discussion

Nowadays, the treatment of TB is a complicated health process. Therefore, the exploration and development of new anti-TB drugs are intensively carried out (Dartois & Rubin, 2022). In this *in-silico* study, enoyl-acyl-carrier-protein reductase (InhA) was selected as the target protein. Mutations to InhA protein alone are adequate to cause resistance to isoniazid, so searching for new compounds that inhibit InhA protein is an appropriate strategy to improve TB treatment (Unissa *et al*., 2016).

In a molecular docking study, it was found that all quassinoids from *Brucea javanica* tested in this study (eighteen compounds) interacted well with InhA protein, as evidenced by the binding energy value obtained was better than isoniazid (<-5.8 kcal/mol). The better binding energy value demonstrated the higher potential efficacy of all quassinoids from *Brucea javanica* than isoniazid. However, further confirmation through *in vitro*, *in vivo*, and clinical trials are still needed.

Based on an investigation of approximately 2000 drug compounds obtained from the World Drugs Index (WDI) data, it was concluded that the absorption or permeability profile of a compound is said to be good if it meets at least three of four rules: MW ≤ 500; calculated octanol/water partition coefficient ≤ +5; H-bond donor ≤ 5; and H-bond acceptor ≤ 10. The above criteria are now also known as Lipinski's rule of five. This study found that three out of eighteen quassinoids from *Brucea javanica* (bruceine A, bruceine, and bruceine D) met Lipinski's rule of five criteria (Lipinski *et al*., 2012).

Furthermore, the toxicity analysis showed that bruceine A, bruceine, and bruceine D had a better Ames toxicity profile than isoniazid. Ames toxicity test is an approach to predict the mutagenic capability of a compound. Along with these findings, the LD_{50} and LOAEL values of bruceene A, bruceene, and bruceine D were better (higher) than isoniazid. The hepatotoxicity evaluation showed similar results to isoniazid, which was not hepatotoxic (Pires *et al*., 2015).

Conclusion

In conclusion, the present results demonstrate that three quassinoids from *Brucea javanica* (bruceine A, bruceine, and bruceine D) have the potential to be developed as anti-TB drugs. Interestingly, no studies have evaluated the potential of bruceine A, bruceine, or bruceine D as anti-TB. Thus, this study opens a wide window for further exploration of bruceine A, bruceine, and bruceine D as anti-TB drugs.

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