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MOLECULAR DOCKING AND ADMET OF BRUCEA JAVANICA QUASSINOIDS ON DRUG-RESISTANT TUBERCULOSIS TARGETS

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ABSTRACT

Tuberculosis remains the infectious disease with the highest global burden, and its control is complicated by drug-resistant cases; resistance to the two first-line drugs is largely attributable to the katG S315T and rpoB S531L mutations. Quassinoids of Brucea javanica (L.) Merr. are reported to be antimicrobial, but comparative studies of their interaction with wild-type and mutant target proteins remain limited. To evaluate B. javanica quassinoids as early antitubercular drug candidates through molecular docking and ADMET prediction against KatG and RpoB in both wild-type and mutant forms. An exploratory and predictive in silico study with a comparative design. Twenty-five quassinoids and two controls (isoniazid, rifampicin) were retrieved from PubChem and ChEMBL; target structures were 2CCA (wild-type KatG), 2CCD (KatG-S315T), 5UAC (wild-type RpoB) and 5UAL (RpoB-S531L). Ligands were minimised with the UFF force field and docked with AutoDock Vina in PyRx (exhaustiveness 8, nine poses per combination). The RpoB search space was centred on the co-crystallised rifampicin and validated by redocking, whereas the KatG search space covered the whole enzyme. ADMET was predicted with ADMET-AI against the ATC J04 reference set. On wild-type RpoB all 25 quassinoids bound between −6.7 and −8.6 kcal/mol (mean −7.61 ± 0.52) and 15 matched or exceeded re-prepared rifampicin (−7.5 kcal/mol), although none surpassed the native ligand (−9.2 kcal/mol). S531L weakened every compound (mean ΔE +0.57 kcal/mol), the largest loss occurring in the native ligand itself (+1.20 kcal/mol). On wild-type KatG affinities were weaker and widely dispersed (mean −3.34 ± 3.02 kcal/mol) and none exceeded isoniazid (−6.1 kcal/mol); S315T improved scores (mean ΔE −2.04 kcal/mol) but abolished every contact with the distal heme pocket. Only bruceine D, bruceine H and bruceine I met all four Lipinski criteria, with intestinal absorption ⩾ 0.96 and low hERG and cytochrome P450 liability, whereas all sixteen glycosides violated three criteria simultaneously. RpoB is the more promising route for this scaffold; bruceine I is the priority candidate and bruceoside C the glycoside lead. All values are predictive and await experimental confirmation.

Keywords: ADMET; brucea javanica; drug resistance; molecular docking; quassinoid; tuberculosis

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INTRODUCTION

Tuberculosis (TB) remains one of the infectious diseases with the highest health burden worldwide, and Indonesia is among the countries carrying the largest number of cases. The global case count for 2023 was estimated at 10.8 million with about 1.25 million deaths, and nearly half of those cases arose in South-East Asia (Dall, 2024). Beyond the burden of drug-susceptible disease, the more pressing challenge is the rise of drug-resistant TB, particularly multidrug-resistant tuberculosis (MDR-TB) and rifampicin-resistant TB, for which detection and treatment success rates remain low (World Health Organization, 2023). Resistant disease forces a shift from the standard six-month regimen to longer, more toxic and more costly second-line therapy, so it is a public-health problem rather than an individual clinical one (Dheda et al., 2024; Farhat et al., 2024).

Isoniazid and rifampicin form the backbone of first-line therapy, and resistance to them is concentrated in two mutations. Isoniazid is a prodrug that must be activated oxidatively by the catalase-peroxidase KatG before the isonicotinoyl radical it yields can form the IN-NAD adduct

that inhibits mycolic acid biosynthesis (Barozi et al., 2022). The S315T substitution narrows the access channel leading to the heme group from roughly 6 Å to 4.7 Å, so isoniazid reaches the oxidative centre far less efficiently while the enzyme retains most of its physiological activity (Barozi et al., 2022). The combination of retained fitness and lost drug activation makes S315T the dominant isoniazid-resistance mechanism in clinical isolates (Seid et al., 2022).

Rifampicin resistance is almost exclusively linked to mutations in rpoB, which encodes the β subunit of RNA polymerase, and the S531L substitution inside the rifampicin resistance-determining region is the most frequently reported worldwide (Kabir et al., 2021; Seid et al., 2022). Rifampicin binds a pocket adjacent to the DNA/RNA channel and blocks extension of the nascent transcript (Sudzinová et al., 2023); replacing the serine hydroxyl with a leucine side chain removes a hydrogen-bond node and reorganises the binding interface, so the drug can no longer engage the pocket optimally (Molodtsov et al., 2017). Together, katG S315T and rpoB S531L constitute the molecular basis of most MDR-TB.

Most experimental and computational drug-discovery work nevertheless still uses the wild-type protein as its target (Amusengeri et al., 2022; Ugbaja et al., 2025). For a candidate intended to work against resistant strains this is a substantive gap: a compound may bind the wild-type enzyme strongly and yet depend on precisely the residue that mutates, so screening against the wild type alone cannot show whether affinity survives the mutation. Docking against matched wild-type and mutant structures addresses that question directly and, when combined with contact-residue analysis, distinguishes a lost interaction from displacement of the ligand out of the pocket.

Brucea javanica (L.) Merr. is used traditionally across Asia for dysentery, malaria and parasitic infection, and its seeds are rich in quassinoids, a class of highly oxygenated degraded triterpenes with antimalarial, antitumour, anti-inflammatory and antimicrobial activity (Chen et al., 2023). Quassinoids of this species have been reported to inhibit InhA in silico (Sulistyowaty et al., 2023), and natural-product libraries remain a productive route to leads against isoniazid-resistant TB (Madhana et al., 2025). What has not been examined is how the same scaffold behaves once the target carries a clinically dominant resistance mutation, and whether the compounds that bind best are also developable. This study therefore evaluated 25 B. javanica quassinoids as early antitubercular candidates by docking them against KatG and RpoB in wild-type and mutant forms and by predicting their ADMET profile, with the specific aims of describing their molecular interaction with each target, comparing that interaction between wild-type and mutant forms, and assessing the ADMET compliance of the selected compounds.

METHOD

This was an *in silico* study, exploratory and predictive in character, with a comparative design applied at two levels: test compounds against reference drugs, and wild-type targets against their resistant mutant forms. All outputs, both binding affinity and pharmacokinetic and safety parameters, are computational estimates rather than measurements. Because the study used only publicly available structural and chemical data and involved neither human subjects nor animals, ethical approval was not required. Twenty-five quassinoids (codes L01–L25), comprising nine aglycones (L01–L09) and sixteen glycosides (L10–L25), were selected from literature published between 2022 and 2024 together with searches of PubChem and ChEMBL. Isoniazid (C01) and rifampicin (C02) were included as controls for the KatG and RpoB routes respectively, giving a final panel of 27 ligands. Four receptor structures were downloaded from the Protein Data Bank (Table 1). Each wild-type/mutant pair was taken from a single deposition series, 2CCA and 2CCD and 5UAC and 5UAL, which removes most of the technical variability that otherwise confounds such comparisons. The RpoB structures are *Escherichia coli* RNA polymerase engineered to carry the clinical mutation; the resistance-determining region is highly conserved between bacteria and S531L in *E. coli* numbering corresponds to S450L in *M. tuberculosis* numbering, so absolute

affinities against these structures cannot be claimed as affinities for the *M. tuberculosis* enzyme, and only the direction and pattern of the mutation-induced change were interpreted.

Table 1.
Target protein structures and docking search-space parameters

Target protein and form	PDB ID	Organism	Resolution (Å)	Search space (centre basis; size)	Validation
KatG, wild type	2CCA	*M. tuberculosis*	2.41	Whole receptor structure; 77.9 × 65.9 × 75.7 Å	Procedural
KatG, S315T mutant	2CCD	*M. tuberculosis*	2.00	Whole receptor structure; 77.8 × 64.7 × 75.9 Å	Procedural
RpoB, wild type	5UAC	*E. coli* K-12	3.80	Co-crystallised rifampicin; 21.7 × 21.2 × 23.7 Å	Redocking
RpoB, S531L mutant	5UAL	*E. coli* K-12	3.89	Co-crystallised rifampicin; 20.3 × 23.0 × 22.8 Å	Redocking

Note: WT = wild type. Search-space values were read from the AutoDock Vina configuration file of each target. Source: Protein Data Bank and research data (2026).

Ligand three-dimensional structures were retrieved and their identity and stereochemistry verified programmatically in Python. Energy minimisation with the Universal Force Field, conversion to PDBQT format, definition of the search space and docking itself were carried out in PyRx, which integrates Open Babel and AutoDock Vina in one workspace (Eberhardt et al., 2021). Water molecules and non-essential heteroatoms were removed, whereas the heme group was retained as part of the KatG receptor. Search-space dimensions affect both pose accuracy and ranking quality (Agarwal & Smith, 2023); for RpoB the box was centred on the co-crystallised rifampicin, whereas for KatG, which offers no co-crystallised drug to anchor the box, the box covered the whole enzyme surface, making the docking blind. Exhaustiveness was set to 8 and the program defaults of nine poses and a 3 kcal/mol energy range were retained. Each of the 108 combinations was run once without a fixed random seed, and the value reported is the affinity of the top-ranked pose.

The protocol on RpoB was validated by redocking the separated co-crystallised rifampicin into its parent structure with identical parameters, judged on agreement of the contact residues with the resistance-determining region, the rank of the native ligand among all entries, and reproduction of the direction of mutation-induced weakening; because the top-ranked pose is not always the closest to the experimental structure, the full set of poses was inspected (Agu et al., 2023). Redocking was not possible on KatG because neither structure contains a suitable co-crystallised drug, so protocol adequacy there was judged procedurally from coverage of the active pocket, uniformity of parameters between the two structures and the orientation of isoniazid.

Ligand–receptor interactions were mapped as two-dimensional interaction diagrams and three-dimensional visualisations, with particular attention to residue 315 on KatG, residue 531 on RpoB and the residues surrounding the heme cofactor. Wild-type and mutant forms were compared through $\Delta E = \Delta G(\text{mutant}) - \Delta G(\text{wild type})$, where positive values denote predicted weakening; changes were classified as very limited ($|\Delta E| \leq 0.2$ kcal/mol), moderate (0.3–0.5 kcal/mol) or substantial (> 0.5 kcal/mol) and complemented by set operations on the contact-residue lists, sorting residues into retained, lost and newly formed. ADMET was predicted once per ligand from SMILES notation using ADMET-AI (Swanson et al., 2024), with percentiles computed against the 20 molecules of ATC class J04 rather than the whole drug database, since antitubercular drugs as a class deviate from conventional drug-like chemical space. Drug-likeness was assessed with the Lipinski criteria (Lohit et al., 2024), topological polar surface area (Fawibe et al., 2025) and the quantitative estimate of drug-likeness (Li et al., 2024). Affinities were summarised descriptively and compared as relative ranks rather than absolute values.

RESULT

Redocking of the co-crystallised rifampicin returned −9.2 kcal/mol on 5UAC and −8.0 kcal/mol on 5UAL, the strongest values among all entries on each structure. The pose contacted Ser512, Phe514, Arg529, Leu533, Arg540 and Asn568 on 5UAC and Arg143, Ser509, Gln510, Ser512, Gln513, Phe514 and Arg529 on 5UAL, all within the rifampicin resistance-determining region. On 5UAC the second pose lay 1.204 Å from the first with only 0.3 kcal/mol between them, indicating that the search converged on a single solution; the wider pose spread on 5UAL is consistent with the rearrangement of the binding interface reported for the mutant structure (Molodtsov et al., 2017). The KatG protocol was internally consistent but not geometrically validated, so the two target pairs do not carry equal interpretive weight.

Table 2.

Binding energy (ΔG, kcal/mol) of 25 quassinoids and controls against the four target structures and the energy difference ΔE = ΔG(mutant) − ΔG(wild type)

Code	Compound	2CCA (WT)	2CCD (S315T)	ΔE KatG	5UAC (WT)	5UAL (S531L)	ΔE RpoB
L01	Bruceine A	−5.2	−5.2	0.00	−7.1	−6.3	+0.80
L02	Bruceine B	−5.3	−6.0	−0.70	−6.8	−6.2	+0.60
L03	Bruceine C	−5.5	−5.8	−0.30	−7.9	−6.9	+1.00
L04	Bruceine D	−5.2	−6.0	−0.80	−7.0	−6.7	+0.30
L05	Bruceine E	−5.3	−6.1	−0.80	−7.1	−6.9	+0.20
L06	Bruceine H	−4.9	−6.0	−1.10	−6.7	−6.4	+0.30
L07	Bruceine I	−5.6	−6.2	−0.60	−7.1	−6.8	+0.30
L08	Bruceine J	−5.5	−5.9	−0.40	−6.8	−6.4	+0.40
L09	Bruceantarin	−5.5	−6.5	−1.00	−7.4	−7.2	+0.20
L10	Bruceoside A	−3.0	−4.7	−1.70	−7.3	−6.9	+0.40
L11	Bruceoside B	−2.8	−6.7	−3.90	−8.4	−7.4	+1.00
L12	Bruceoside C	−4.8	−5.3	−0.50	−7.9	−7.8	+0.10
L13	Bruceoside D	−6.0	−5.9	+0.10	−7.8	−7.1	+0.70
L14	Bruceoside E	−5.4	−5.6	−0.20	−7.8	−7.2	+0.60
L15	Bruceoside F	+2.2	−4.2	−6.40	−7.9	−7.4	+0.50
L16	Yadanzioside A	−5.8	−5.2	+0.60	−7.8	−7.1	+0.70
L17	Yadanzioside B	−1.8	−5.5	−3.70	−8.1	−7.4	+0.70
L18	Yadanzioside C	−1.9	−5.4	−3.50	−7.8	−7.3	+0.50
L19	Yadanzioside E	−2.9	−5.4	−2.50	−7.9	−7.2	+0.70
L20	Yadanzioside F	−5.9	−6.0	−0.10	−7.4	−7.1	+0.30
L21	Yadanzioside G	−0.2	−4.7	−4.50	−7.7	−7.1	+0.60
L22	Yadanzioside K	+4.2	−4.1	−8.30	−8.0	−6.9	+1.10
L23	Yadanzioside L	−4.5	−4.6	−0.10	−7.6	−7.4	+0.20
L24	Yadanzioside M	−0.1	−3.8	−3.70	−8.6	−7.5	+1.10
L25	Yadanzioside P	+3.2	−3.6	−6.80	−8.4	−7.4	+1.00
	Mean of 25 quassinoids	−3.34	−5.38	−2.04	−7.61	−7.04	+0.57
C01	Isoniazid (control)	−6.1	−4.9	+1.20	−4.7	−4.6	+0.10
C02	Rifampicin (control)	–	–	–	−7.5	−7.1	+0.40
–	Native ligand (rifampicin)	–	–	–	−9.2	−8.0	+1.20

Note: More negative values indicate a more favourable predicted interaction within the same protocol. A positive ΔE denotes predicted weakening on the mutant form. Rifampicin was not docked against KatG because its mechanism targets RNA polymerase. Source: research data (2026).

On wild-type RpoB all 25 quassinoids gave negative binding energies within a narrow range of −6.7 to −8.6 kcal/mol (mean −7.61 ± 0.52). The most negative value belonged to yadanzioside M (−8.6 kcal/mol), followed by bruceoside B and yadanzioside P (−8.4 kcal/mol each) and yadanzioside B (−8.1 kcal/mol); the upper ranks were dominated by the large glycosides. Fifteen of the 25 compounds matched or exceeded the re-prepared rifampicin control (−7.5 kcal/mol), but

none approached the native ligand (−9.2 kcal/mol). On the mutant the range shifted to −6.2 to −7.8 kcal/mol (mean −7.04 ± 0.40), with bruceoside C the most negative (−7.8 kcal/mol). The mutation weakened every compound without exception (Table 2): mean ΔE was +0.57 kcal/mol across a narrow spread of +0.10 to +1.10 kcal/mol, and 13 compounds weakened substantially. The largest single loss in the entire table belonged to the native ligand itself (+1.20 kcal/mol), exactly what would be expected of the molecule most dependent on residue 531, and it serves as an internal control that the protocol captures the effect of the mutation correctly.

KatG behaved differently. On the wild type, 22 of 25 quassinoids gave negative energies while three gave positive ones, namely yadanzioside K (+4.2), yadanzioside P (+3.2) and bruceoside F (+2.2 kcal/mol), producing a very wide distribution (−6.0 to +4.2 kcal/mol; mean −3.34 ± 3.02). The nine aglycones occupied a far tighter band (−5.6 to −4.9 kcal/mol), and the dispersion was contributed almost entirely by the bulky glycosides, several of which showed unfavourable steric contacts with Asp137, Pro232 and the heme group. No quassinoid exceeded isoniazid (−6.1 kcal/mol). On the mutant, all 25 compounds gave negative energies within a narrower band (−6.7 to −3.6 kcal/mol; mean −5.38 ± 0.84) while isoniazid weakened to −4.9 kcal/mol, so that 18 quassinoids now scored more negatively than the control.

That reversal does not indicate an advantage against the resistant enzyme. Geometric inspection of the top-ranked poses showed eight ligands within 6 Å of the heme iron on the wild type, the deepest penetrating to 2.8 Å, whereas on the mutant no ligand came closer than 8.5 Å; isoniazid itself relocated 12.1 Å to a peripheral pocket around Thr112, Trp198, Arg209 and Gln224. In the residue profile, every contact with the distal heme pocket vanished on the mutant, with Arg104, Trp107, His108, Val230 and the heme group all falling to zero frequency, while outward-lying residues gained: Trp300 rose from 5 to 11 compounds, Asn231 from 7 to 10 and Glu233 from 12 to 16. Residue 315 remained the most frequent partner on both forms, contacting 19 compounds as Ser315 and 16 as Thr315. The improved scores therefore reflect relocation to the roomier vestibule in front of the narrowed channel, where the steric penalty disappears, rather than stronger engagement with the catalytic machinery.

Across targets, every one of the 25 compounds scored more negatively on RpoB than on KatG in both wild-type and mutant forms, a mean difference of 4.27 kcal/mol for the wild-type pair and 1.66 kcal/mol for the mutant pair. On RpoB, Arg529 and Arg687 acted as hydrogen-bond anchors for almost every ligand, supported by Gln513, Asn568, Asp516 and Asn518, the same region that binds the native ligand (Table 3). Ser531 was directly involved in the hydrogen-bond network of four compounds on the wild type, namely bruceine I, bruceoside E, bruceoside F and yadanzioside G, and formed no interaction at all once mutated to Leu531, its place taken by a strengthened role for Phe514, Arg143 and Gln761. Four compounds, bruceine J, bruceoside C, yadanzioside F and yadanzioside L, kept $|\Delta E| \leq 0.5$ kcal/mol on both target pairs.

Table 3.
Representative contact residues of selected quassinoids and controls on wild-type and mutant RpoB

Compound	Key residues, wild type (5UAC)	Key residues, mutant (5UAL)
Bruceine I (L07)	Gln513, Arg529, Ser531	Asp516, Asn518, Arg687
Bruceoside C (L12)	Asn518, Arg529, Asn568, Arg687	Arg529, Asn568, Arg687
Bruceoside F (L15)	Asp516, Asn518, Arg529, Ser531	Ser509, Ser512, Phe514, Arg529
Yadanzioside G (L21)	Asp516, Arg529, Ser531, Arg687	Arg529, Gln688, Gln761
Yadanzioside M (L24)	Gln513, Arg529, Arg687	Arg143, Ser512, Arg529
Rifampicin (C02)	Gln513, Arg540, Asn568	Ser509–Phe514
Native ligand	Phe514, Arg529, Arg540, Asn568	Ser509, Gln513, Phe514, Arg529

Note: Residues listed are those forming hydrogen bonds or hydrophobic contacts with the top-ranked pose. Source: research data (2026).

ADMET prediction separated the panel sharply along the aglycone–glycoside divide (Table 4). Only bruceine D, bruceine H and bruceine I satisfied all four Lipinski criteria, joining isoniazid, whereas all sixteen glycosides violated three criteria simultaneously, as rifampicin does, because of molecular weights of 643–769 g/mol, up to 18 hydrogen-bond acceptors and TPSA above 245 Å². The same divide reappeared in absorption: aglycones showed intestinal absorption of 0.85–1.00 and PAMPA permeability of 0.33–0.76, glycosides only 0.35–0.81 and 0.16–0.55 respectively. Safety markers were comparatively favourable. Predicted cytochrome P450 inhibition was low, generally below 0.01 for CYP1A2, CYP2C19, CYP2C9 and CYP2D6 and at most 0.163 for CYP3A4 in bruceine C, while hERG probabilities ranged from 0.05 to 0.50 and Ames mutagenicity from 0.03 to 0.24, all below both controls. The high predicted liver-injury probabilities of the controls, 0.769 for isoniazid and 0.971 for rifampicin, match their established hepatotoxicity and act as an internal validity check on the model.

Table 4.
Physicochemical, drug-likeness and selected ADMET parameters of the priority candidates and controls

Compound	MW (g/mol)	LogP	TPSA (Å²)	QED	Lipinski violations	HIA	hERG	DILI
Bruceine I (L07)	436.46	0.42	139.59	0.525	0	0.997	0.134	0.312
Bruceine D (L04)	410.42	−1.95	153.75	0.287	0	0.971	0.129	0.270
Bruceine H (L06)	410.42	−1.95	153.75	0.310	0	0.963	0.118	0.200
Bruceine J (L08)	508.52	0.51	176.89	0.389	1	0.851	0.049	0.509
Bruceantarin (L09)	542.54	0.86	165.89	0.366	2	0.982	0.311	0.615
Bruceoside C (L12)	682.67	−2.22	245.04	0.099	3	0.688	0.223	0.434
Yadanzioside M (L24)	704.68	−2.02	245.04	0.138	3	0.752	0.310	0.554
Isoniazid (control)	137.14	−0.31	68.01	0.317	0	1.000	0.123	0.769
Rifampicin (control)	822.95	4.34	220.15	0.109	3	0.940	0.701	0.971

Note: MW = molecular weight; TPSA = topological polar surface area; QED = quantitative estimate of drug-likeness; HIA = human intestinal absorption; hERG = human ether-à-go-go related gene inhibition; DILI = drug-induced liver injury. HIA, hERG and DILI are probabilities (0–1). Source: research data (2026).

Integrating affinity, stability against the resistance mutations and developability placed bruceine I first, followed by bruceine D and bruceine H, the only three compounds in the panel with no Lipinski violation, intestinal absorption of at least 0.96 and hERG probability below 0.14. Bruceine I combined RpoB affinities of −7.1 and −6.8 kcal/mol with the highest drug-likeness index of the panel (0.525, the 60th percentile against approved antitubercular drugs) and displayed no unfavourable interaction on any of the four structures. Bruceoside C was retained as a glycoside lead because it produced the most negative affinity on mutant RpoB (−7.8 kcal/mol) together with the smallest |ΔE| on both target pairs. The shared weakness of the three priority compounds is a strongly hydrophilic character that limits passive diffusion, with Caco-2 values of −5.58 to −5.92, and a medium-to-high predicted hepatocyte clearance of 73.9–96.5.

DISCUSSION

Of the two routes examined, RpoB offers the more promising basis for this scaffold. The rifampicin site on the β subunit is a deep cleft that accommodates a molecule as large as rifampicin (822.95 g/mol) and is lined with charged polar residues, particularly Arg529 and Arg687, which offer many hydrogen-bond partners to the hydroxyl and glycosidic oxygens that quassinoids carry in abundance. The region probed on KatG, by contrast, is a narrow access channel that naturally serves a small substrate such as isoniazid (137.14 g/mol), so a panel weighing 410–769 g/mol was largely held at the mouth of the channel and some members even received positive energies. Two caveats attach to this cross-target comparison. The AutoDock Vina scoring function is not calibrated for comparing different proteins and its pairwise-additive form favours larger ligands, so the difference should be read as a qualitative tendency rather than an equivalent affinity comparison (Agu et al.,

2023); and the RpoB protocol alone was validated by redocking, so it bears the greater interpretive weight.

The two mutations act through fundamentally different mechanisms even though both end in reduced drug effectiveness. On KatG, S315T works as a steric gate: narrowing of the heme access channel pushed the whole panel out of the distal pocket, and the resulting improvement in score is an artefact of relocation to a roomier vestibule where the steric penalty disappears, not evidence of better binding. This reading is decisive for interpretation, because isoniazid is a prodrug that requires oxidative activation at the distal heme pocket; a more negative score is meaningful only if the compound occupies a position relevant to that mechanism. Since no quassinoid reached the distal pocket on the mutant, there is no basis for proposing this scaffold as acting through the peroxidative activation route in S315T-bearing strains. On RpoB, S531L instead deletes a recognition node: the serine hydroxyl is replaced by a leucine side chain that can neither donate nor accept, so the hydrogen bond is lost without displacing the ligand from the pocket. The change is smaller in magnitude but carries the greater pharmacological consequence.

Weakening on RpoB cannot be attributed solely to loss of direct contact, since all 25 compounds weakened including the 21 that never contacted residue 531. The more likely explanation is the overall rearrangement of the binding interface that accompanies the substitution, as reported for the source structures (Molodtsov et al., 2017) and consistent with the conformational analyses of clinical rpoB mutants (Amusengeri et al., 2022). The behaviour of the native ligand supports this reading and, being the largest loss in the table, functions as an internal control. The principle that emerges is directly relevant to anti-resistance design and applies to both targets: what determines effectiveness against a mutant strain is not binding strength alone but whether the compound depends on the residue that mutates. Compounds whose contact network is distributed over several conserved residues are better placed to survive, which is why bruceine J, bruceoside C, yadanzioside F and yadanzioside L are of interest despite not being the strongest binders. A comparable strategy of screening natural-product libraries against paired wild-type and mutant RpoB structures has been reported for marine metabolites (Alsulais et al., 2024), and the present results extend that approach to a plant scaffold across two resistance targets simultaneously.

The ADMET results show that binding strength and developability pull in opposite directions within this panel. The highest affinities on RpoB belong to the glycosides, yet the sugar conjugation that supplies the extra hydrogen-bond partners simultaneously raises molecular weight, TPSA and hydrophilicity, the three quantities that jointly suppress passive membrane diffusion. The small aglycones are correspondingly the most plausible oral candidates but sit below most glycosides on affinity, so bruceine I is a development priority rather than the strongest inhibitor. The apparently favourable safety profile also requires caution, because quassinoids are long known to be strongly cytotoxic to mammalian cells, the very property that underpins anticancer interest in the class, and the endpoints available in ADMET-AI do not include cellular cytotoxicity. Low Ames, hERG and carcinogenicity probabilities therefore cannot be read as overall safety, and the selectivity index against mammalian cells is the parameter that will most determine whether any member of this class advances. The glycosides are best positioned as leads for targeted deglycosylation or prodrug development rather than discarded.

Several limitations bound these conclusions. All findings are predictive: docking assesses geometric and energetic complementarity in a static state, does not demonstrate enzyme inhibition, and does not address whether a compound reaches its target inside a bacterium protected by a mycolic-acid-rich cell wall. The scoring function carries a standard error in the order of 1–2 kcal/mol, treats the receptor as rigid, models no explicit water and handles conformational entropy inadequately (Agu et al., 2023), so small differences between neighbouring compounds should not be treated as a firm ranking. The KatG protocol could not be validated by redocking; the RpoB structures are an E. coli

surrogate at 3.80 Å resolution; ligands were prepared as single UFF-minimised conformers without exploring protonation or tautomeric states; only two targets and one mutation each were examined, leaving inhA, ahpC, other rpoB codons and efflux mechanisms unaddressed; and the J04 percentile reference contains only 20 molecules. Molecular dynamics simulation with MM-PBSA or MM-GBSA free-energy calculation, minimum inhibitory concentration testing against H37Rv and drug-resistant strains, enzyme inhibition assays and mammalian cytotoxicity testing are the natural next steps.

CONCLUSION

Brucea javanica quassinoids interact favourably with M. tuberculosis targets, most clearly with RpoB, where all 25 compounds gave negative binding energies and 15 matched or exceeded re-prepared rifampicin, although none surpassed the co-crystallised native ligand. The two resistance mutations act differently: S315T on KatG displaces the panel out of the distal heme pocket while improving scores, whereas S531L on RpoB weakens every compound modestly but uniformly without displacing them, so the smaller change carries the greater mechanistic consequence. Bruceine J, bruceoside C, yadanzioside F and yadanzioside L were the most stable against both mutations. On ADMET grounds only bruceine D, bruceine H and bruceine I met all Lipinski criteria with favourable absorption and toxicity markers, and the integrated assessment designates bruceine I as the priority candidate and bruceoside C as the glycoside lead.

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