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Anti-tuberculosis potential of bruceine: An *in silico* approach

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Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. The bacterial enzyme pantothenate synthetase (PS) catalyzes the synthesis of pantothenate, a precursor of coenzyme A. Hence, targeting PS is a potential mechanism in the development of anti-tuberculosis drugs. Bruceine, a highly oxygenated natural quassinoid molecule, is isolated from plants of the *Simaroubaceae* family. The anti-tuberculosis potential of eleven bruceine (A, B, C, D, E, G, H, I, J, K and L) has been investigated by *in silico* approach. The molecular docking (AutodockVina) and drug-likeness (Lipinski's rule of five) analyses identified bruceine D as a potential inhibitor. Further, it has shown six hydrogen bond interactions with the key amino acids residues of the target protein, Tyr82, His135, Lys160 and Asp161. The ring-A and -D has contributed two hydrogen bonds, while one each from ring-C and -E. The results reveal that bruceine D possesses drug-likeness property and binding energy of -9.3 kcal/mol. The binding score similar to pantoyl adenylate suggests chemical modifications to enhance the protein inhibition potency. Bruceine D has a great potential to inhibit PS and could contribute to the tuberculosis drug discovery process.

Keywords: Bruceine, Docking, Pantothenate synthetase, Quassinoids, Tuberculosis

Coenzyme A is a cofactor required in a wide range of cellular reactions and bacterial pathogenicity in *Mycobacterium tuberculosis*. The primary precursor of coenzyme A is pantothenate (vitamin B5). The biosynthesis of pantothenate is catalyzed by four enzymes encoded by the mycobacterial genome i.e., *panB*, *panC*, *pan D* and panE. The *panC* gene encodes thepantothenate synthetase (PS) enzyme which catalyzes the final step of pantothenate biosynthesis. The PS enzyme is a homodimer that catalyzes the condensation of pantoate and β-alanine in an ATP-dependent reaction. The key residues of PS involved in substrate interaction and the intermediate (pantoyl adenylate) stabilization are His44, His47, Asn69, Gln72, Lys160 and Gln164[1]. In fact, the absence of pantothenate biosynthesis in mammals is beneficial for the design and development of chemotherapeutic agents which will purely target bacteria and offers an efficient strategy to combat tuberculosis. Considering these facts, the researchers selected PS as a potential therapeutic target and its inhibition is shown to counteract the mycobacterial pathogenicity. In this context, various synthetic anti-TB drugs designed against PS have gained significant attention in recent times[2–5]. These drugs engage in chemical interactions with the active amino acids of the PS and offer an excellent inhibition strategy.

Bruceine is a natural quassinoid found in the plant species of the family *Simaroubaceae*. It is a highly oxygenated molecule and comprises four rings: ring-A is α,β-unsaturated cyclo-hexa-none, ring-B and -C are cyclo-hexane, ring-D is six-membered lactone and ring-E is tetra-hydro-furan[6–12]. The rich bioactivity of quassinoids is correlated to their structural aspects. The structural importance of α,β-unsaturated keto group in ring-A and the oxymethylene bridge is reported in a wide range of biological applications[13,14]. At the molecular level, quassinoids interact with important targets and exhibit diverse therapeutic properties[15–19]. For example, bruceine A and D are shown to have a potential anticancer activity[20–22], bruceine D and E is shown to exhibit glucose lowering response[14] and bruceine D and H is shown to demonstrate antimalarial activity[13]. However, the pharmacological property of quassinoids is unexplored in anti–TB research. Hence, the objective of this work is to evaluate the anti-tuberculosis potentials of eleven brucein equassinoids (A, B, C, D, E, G, H, I, J, K and L) using molecular docking and drug-likeness (Lipinski's rule of five). The results suggest that bruceinemight be a potential drug candidate for the management of pulmonary tuberculosis.

Experimental Section

Protein and ligand preparation

The Autodock1.5.6 was used in the preparation of the protein and ligands. The crystal structure of pantothenate synthetase from *Mycobacterium tuberculosis* in complex with pantoyl adenylate (PDB ID: 1N2H) was retrieved from the RCSB protein data bank (www.rcsb.org). The substances and molecules associated with protein were removed and the hydrogen atoms and charges were assigned. Further, domain A of the homodimer protein was selected for the study. The ligand structure of pantoyl adenylate (reaction intermediate) and the eleven bruceine were obtained from the PubChem database and their respective PubChem ID are shown in Table 1.

Molecular docking

The molecular docking study was performed using Auto Dock Vina. The grid size was set as 30x40x50 Å and grid centres as center_x = 31.59, center_y = 35.27 and center_z = 42.53 with a point spacing of 0.375 Å, which spans the active site of the target protein. Further, the exhaustiveness value was set at 8[23]. The binding affinity of the bruceine was represented in kcal/mol. The docking results were analyzed using PyMol visualization software, discovery studio and Ligplot.

Lipinski's rule of five

The drug-likeness property of bruceine was evaluated by Lipinski's rule of five. The parameters, molecular weight, Log P, hydrogen bond donor and hydrogen bond acceptor were obtained from (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp).

Table 1 — Pubchem ID of Pantoyl adenylate.

Bruceine	PubChem ID
Pantoyl adenylate	447261
A	160006
B	161496
C	5315509
D	441788
E	5315510
G	102059835
H	101600138
I	21126551
J	23656476
K	101549286
L	101549287

Results and Discussion

Molecular docking using pantoyl adenylate

The initial molecular docking study was carried out with the reaction intermediate, pantoyl adenylate, which forms a stable complex with the PS. The docking results showed a binding affinity of -9.3 kcal/mol. It formed seven hydrogen bonds with key amino acid residues of the target protein, which include Met40, His47, Gln72, Gly158 and Asp161 with the bond distance of 2.96, 3.31, 3.13, 3.22 and 2.82 Å, respectively[1,24,25]. The result highlight that it is desirable to design a drug molecule that binds to these conserved residues with an exceptional docking score compared to pantoyl adenylate. Such drugs help to prevent the catalytic action of protein and result in functional inhibition.

Molecular docking using bruceine

The molecular docking results of eleven bruceine, their hydrogen bond interaction and the bond distance are presented in Table 2. The common observation noticed in all the ligands (except bruceine J, K and L) is hydrogen bonding between Asps161 amino acid residue and the hydroxyl group at C12 of ring-C. The bruceine was categorized based on the structure as type I, II and III. The docking results of each category were interpreted to elucidate the structural significance, which could further render a structural clue in the design and development of synthetic anti-tuberculosis drugs.

Table 2— Molecular docking results of bruceine.

Bruceine	Docking score (kcal/mol)	Amino acids involved in interaction (bond distance in Å)
A	-9.6	MET40 (3.23), HIS47 (3.05), GLN72 (3.19), HIS135 (3.19), ASP161 (3.23), GLN164 (3.19)
B	-9.0	MET40 (3.24), HIS47 (3.05), GLN72 (3.22), GLU128 (2.98), HIS135 (3.23), ASP161 (3.18), GLN164 (3.13)
C	-9.8	HIS47 (3.09), GLN72 (3.25), TYR82 (2.90), HIS135 (3.06), GLY158 (2.99), ASP161 (2.93)
D	-9.3	TYR82 (2.86 & 3.07), HIS135 (3.33 & 3.01), LYS160 (3.20), ASP161 (2.77)
E	-9.0	TYR82 (2.83 & 3.11), HIS135 (3.29), LYS160 (3.13), ASP161 (2.76)
G	-9.1	HIS47 (3.02), GLN72 (3.24), TYR82 (2.50), HIS135 (3.13), GLY158 (3.19), ASP161 (2.88)
H	-9.2	HIS47 (2.91), TYR82 (2.87), GLY158 (3.07), ASP161 (2.92), SER197 (3.26)
I	-9.0	GLU128 (2.69), HIS135 (2.89), LYS160 (3.16), ASP161 (2.68)
J	-9.7	MET40 (2.97), HIS47 (3.14), GLN72 (3.18), HIS135 (3.04), GLN164 (3.26)
K	-8.6	HIS47 (2.81 & 3.03), TYR82 (3.09), GLU128 (3.01), HIS135 (2.89), GLN164 (3.09), SER197 (2.96)
L	-8.8	MET40 (3.22), TYR82 (2.88), GLN164 (2.77), SER197 (2.97 & 3.15)

Type I Bruceine (A, B, C, I and J)

Figure 1 shows the general structure of type I bruceine which comprises bruceine A, B, C, I and J. Bruceine A, B and J showed docking scores of -9.6, -9.0 and 9.7 kcal/mol, respectively. Due to the structural homology of the bruceine, a similar pattern of hydrogen bonding and hydrophobic interactions is observed. They interacted with Met40, His47, Gln72, Glu128, His135, Asp161 and Gln164 amino acid residues of the target protein through hydrogen bonds. For example, bruceine A, B and J interacted with target protein through six, seven and five hydrogen bonds, respectively. In bruceine A, rings-A contributed one hydrogen bond and five from rings-C.

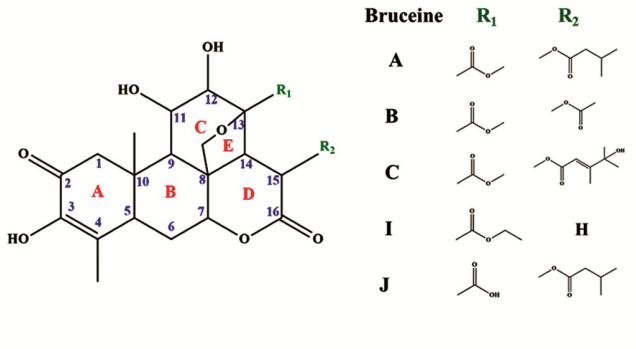


Fig. 1 — Structure of type I bruceine (A, B, C, I and J).

In bruceine B, the ring-A contributed two hydrogen bonds and five from ring-C. In bruceine J, ring-A contributed one hydrogen bond and four from ring-C.

The bruceine C and I showed docking score of -9.8 and -9.0 kcal/mol, respectively. The high (564.58 g/mol) and low molar mass (436.5g/mol) of the respective bruceine might accommodate perfectly or imperfectly into the active site. As a result, bruceine C formed six hydrogen bonds with the target protein, whereas bruceine I formed four hydrogen bonds. In bruceine C, the ring-A, -C and -D contributed to one, three and two hydrogen bonds, respectively. Further, it interacted with His47, Gln72, Tyr82, His135, Gly158 and Asp161 amino acid residues of the target protein. In bruceine I, the ring-A contributed two hydrogen bonds and one each from ring-C and -D. It formed hydrogen bonds with Glu128, His135, Lys160 and Asp161 amino acid residues. Figure 2 shows the 2D interaction between type I bruceine ligands and target protein.

Type II Bruceine (D, E, G and H)

Figure 3 shows the general chemical structure of type II bruceine which includes bruceine D, E, G and H. The respective bruceine showed the docking score of -9.3, -9.0, -9.1 and -9.2 kcal/mol. The reason for the nearly equal docking score is due to their

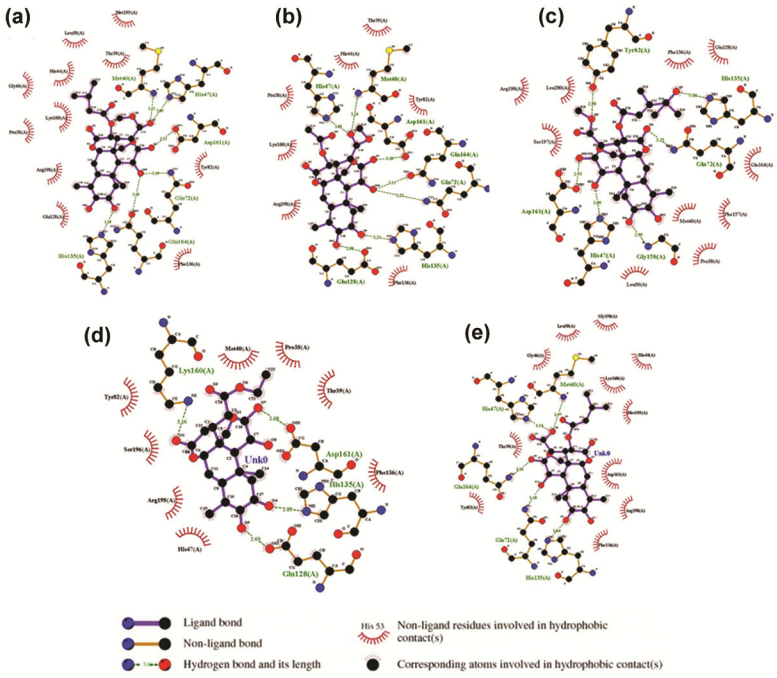
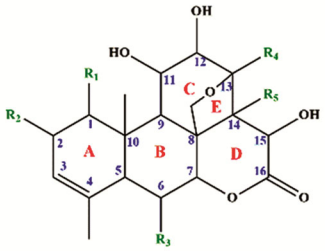


Fig. 2 — Two-dimensional interaction between pantothenatesynthetase and type I bruceine (a) bruceine A; (b) bruceine B; (c) bruceine C; (d) bruceine I and (e) bruceine J.



Bruceine	R_1	R_2	R_3	R_4	R_5
D	OH	=O	H	CH_3	OH
E	OH	OH	H	CH_3	OH
G	H	=O	OH	CH_3	H
H	OH	=O	H	CH_2OH	OH

Fig. 3 — Structure of type II bruceine (D, E, G and H).

structural similarity. A common observation noticed among bruceine D, E and H is their hydrogen bond interaction with Tyr82. The hydroxyl group attached at C-14 site engages in hydrogen bonding with Tyr82. Additionally, the Tyr82 forms a second hydrogen bond with the oxygen atom of the oxymethylene bridge. However, in the case of bruceine G, the absence of hydroxyl group at C-14 site did not show similar interaction, but the presence of hydroxyl group at C-6 site showed a hydrogen bond with the Gln72. Narihiko *et al.* reported that the bioactivity of the quassinoids was influenced by several factors such as the presence of oxymethylene bridge, the nature of the side chain at C-15 site and the nature of modification in ring-A[26]. Chumkaew *et al.* described the structural importance of α,β-unsaturated ketone group at C-2 site and the oxymethylene bridge between C-8 and C-13 for high antiplasmodial activity[13]. Shahida *et al.* reported less toxicity of bruceine E compared to bruceine D owing to the presence of hydroxyl moiety at C-2 site of the former[14].

The bruceine D and G formed six hydrogen bonds, whereas bruceine E and H formed five hydrogen bonds with the target protein. In bruceine D, ring-A and -D contributed two hydrogen bonds and one each from ring-C and -E. In bruceine E, ring-D contributed two hydrogen bonds and one each from ring-A, -C and -E. In bruceine G, ring-C contributed two hydrogen bonds and one each from ring-A, -B, -D and -E. In bruceine H, ring-C contributed three hydrogen bonds and one each from ring-A and -E. The type II bruceine interacted with His47, Gln72, Tyr82, His135, Gly158, Lys160, Asp161 and Ser197 amino acid residues of the target protein. Figure 4 shows the 2D interaction between type II bruceine ligands and target protein residues.

Type III Bruceine (K and L)

Figure 5 shows the structure of type III bruceine which includes bruceine K and L. The chemical

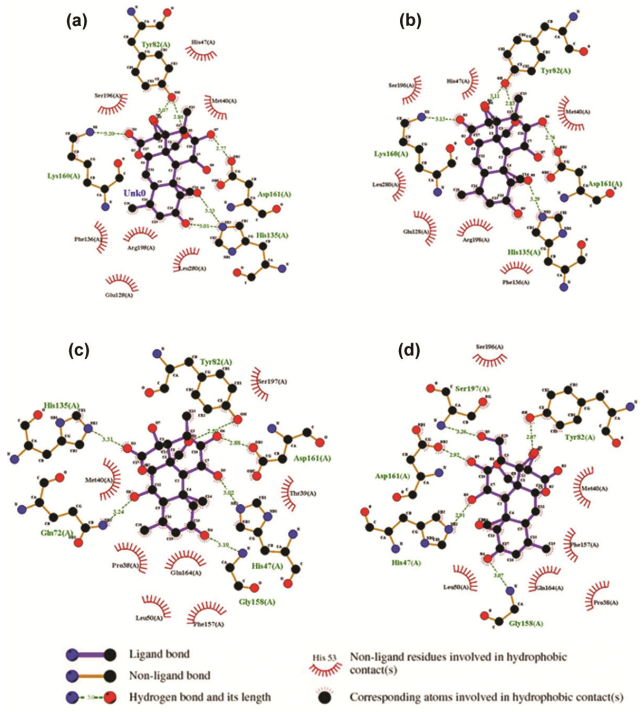


Fig. 4 — Two-dimensional interaction between pantothenate synthetase and type II bruceine (a) bruceine D; (b) bruceine E; (c) bruceine G and (d) bruceine H.

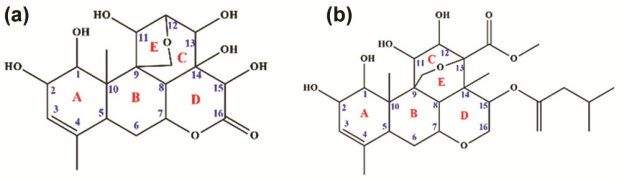


Fig. 5 — Structure of type III bruceine (a) bruceine K and (b) bruceine L.

structure of bruceine III differs from type I and II with respect to the oxymethylenebridge[27]. Type I and II have an oxygen bridge between C8 and C13, but category III is between C9 and C12 (bruceine K) or between C9 and C13 (bruceine L). Narihiko *et al.* study indicated that compounds containing C8 to C-

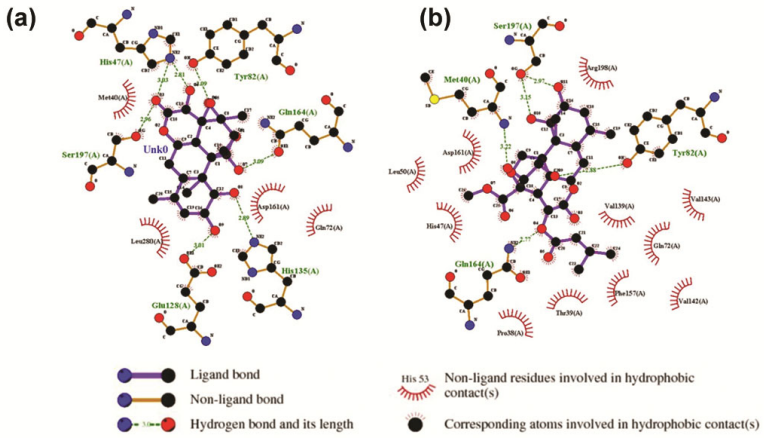


Fig. 6 — Two-dimensional interaction between pantothenate synthetase and type III bruceine (a) bruceine K and (b) bruceine L.

Table 3 — Drug-likeness property of bruceine.

Bruceine	Molecular weight (g/mol)(< 500)	Log P(< 5)	HBD(< 5)	HBA(< 10)	Violation	Drug-likeness
A	522.54	0.59	3	11	2	No
B	480.46	-0.43	3	11	1	Yes
C	564.58	0.27	4	12	2	No
D	410.42	-1.95	5	9	0	Yes
E	412.43	-2.16	6	9	1	Yes
G	394.42	-1.07	4	8	0	Yes
H	426.41	-1.95	6	10	1	Yes
I	436.45	0.42	3	9	0	Yes
J	508.51	0.51	4	11	2	No
K	412.43	-2.16	6	9	1	Yes
L	522.54	-0.61	4	11	2	No

13 or C8 to C11 oxymethylene bridge are active inhibitors of protein synthesis compared to the compounds lacking the bridge[26]. The bruceine K and L showed docking score of -8.6 and -8.8 kcal/mol, respectively. The low docking score of type III infers the significance of the oxygen bridge. Specifically, the oxygen bridge between C8 and C13 is favourable for the interaction with the key amino acids of the target protein. The bruceine K interacted with His47, Tyr82, Glu128, His135, Gln164 and Ser197 amino acid residues through seven hydrogen bonds. On the other hand, bruceine L formed four hydrogen bonds with Met40, Tyr82, Gln164 and Ser197 residues. Figure 6 shows the 2D interactions between type III bruceine ligands and pantothenate synthetase.

Lipinski rule of five

The drug-likeness score of eleven selected bruceine was determined by the Lipinski rule of five and shown in Table 3. The rule of five considers the important parameters such as molecular weight, solubility, hydrogen bond donor and hydrogen bond acceptor. A drug is regarded to follow the rule when the violation is not more than one. Among the eleven selected bruceine, seven followed the rule, while the four violated it. Although the bruceine A, C and J showed exceptional docking scores but violated the Lipinski rule of five due to their high molecular weight (>500 g/mol) and the high number of hydrogen bond acceptors (> 10). Further, among the seven bruceine which obeyed the rule, bruceine D showed a better docking score. Thus, the study results conclude that bruceine D with docking energy of -9.3 kcal/mol against the pantothenate synthetase and its adherence to Lipinski rule of five, is a potential drug candidate in the treatment of tuberculosis.

Conclusion

In the present study, the anti-tuberculosis potentials of eleven selected bruceine (A, B, C, D, E, G, H, I, J, K and L) have been investigated using molecular docking and Lipinski's rule of five. The abundant oxygen functional groups in bruceine formed hydrogen bonds with the key amino acid residues of the target protein, pantothenatesynthetase. The results revealed the drug-likeness of bruceine D with a docking score of -9.3 kcal/mol. The findings have

also shown the importance of the oxymethylenebridge between C-8 to C-13 and the nature of the side chain at C-15 site of ring-D for the favourable stabilizing of the target protein. However, the docking score of bruceine D is comparable to pantoyl adenylate and it provides an understanding of the chemical modification of ligand to enhance its protein inhibition potency. Further, the study recommends *in vitro* and *in vivo* experimental evidence to offer a satisfactory conclusion.

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