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Computational Analysis Reveals 10-Acetyl-9,10-dihydroacridine as a Novel Biomolecule from *Bacillus licheniformis* (MW301654) Possessing Nematicidal Property against Banana Root Knot Nematode *Meloidogyne incognita*

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ABSTRACT: Root knot nematodes are devasting plant pathogens that affect the agricultural crops all over the world. The recent efforts to identify biorational pesticides to counteract the harmful effects of synthetic pesticides necessitated the development of an efficient and environmentally sound biomolecule for the management of nematodes. In the present study, secondary metabolites produced by *Bacillus licheniformis* (MW301654) during the ditrophic interaction with *Fusarium oxysporum f.* sp. cubense (Foc) were screened for its nematicidal property. Molecular Docking was done for the biomolecules10-acetyl-9,10-dihydroacridin, valeric acid and formic acid with the protein targets of *M. incognita* such ascytochrome c oxidase subunit, calreticulin, venom allergen-like protein and endoglucanase. Modelled structure of protein targets was docked with biomolecules using the PyRx 0.8 server's AutoDock Vina module for predicting the binding energy of ligand and target protein. Among the chosen targets, docking analysis revealed that 10-acetyl-9,10-dihydroacridin exhibited the highest binding affinity of -9.4 kcal/mol with the target cytochrome c oxidase subunit, binding affinity of calreticulin was -6.7 kcal/mol, endoglucanase had -4.5 kcal/ binding affinity and the binding affinity of venom allergen-like protein was -7.2 kcal/mol in comparison with nematicide carbofuran 3G. Besides, increased binding affinity of 10-acetyl-9,10-dihydroacridin with the protein target sites facilitated to explore it as a novel nematicidal molecule for the management of banana root knot nematode *M. incognita*. Thus, present study confirmed that the small molecule 10-acetyl-9,10-dihydroacridin can be utilised for its nematicidal activity against M. incognita.

Keywords: Banana, Meloidogyne incognita, Bacillus licheniformis, 10-Acetyl-9,10-dihydroacridin,Molecular modelling, Molecular docking and Nematicidal activity.

INTRODUCTION

Root-knot nematodes (RKNs; *Meloidogyne* spp.) are sedentary endoparasitic worms that can infect a variety of plant species globally. This causes annual crop losses of about $70 billion (Caboni *et al*., 2012). The four most significant crop-damaging species of root knot nematode are *M. incognita*, *M. arenaria, M. hapla,* and *M. javanica*, which are among the top 10 most economically devastating plant-parasitic nematodes (Jones *et al*., 2013). Banana yields are reduced by 20–30% as a result of *M. incognita* (Liu *et al*., 2005). Besides having a brief biological cycle, they also produce root lesions, which helps in secondary pathogen invasion (Caboni *et al*., 2016). Conventionally nematode management was mostly accomplished through the application of nematicides (Caboni *et al*., 2016). However, numerous synthetic nematicides have lost their effectiveness over time, and their use has been connected to a variety of harmful effects, including soil and groundwater contamination, as well as animal, farmer, and consumer health

concerns (López-Lima *et al*., 2013). Beneficial bacteria and fungi limit the population dynamics of plant parasitic nematodes (PPN) in soils, halting their growth either by trapping or releasing toxins, or by interacting with tiny compounds produced as secondary metabolites by hostile bacteria. Some of the microorganisms associated in nematode suppression are *Bacillus subtilis, B. velezensis, B. amyloliquefaciens, Pseudomonas fluorescens,* and *Pasteuria penetran*s (Davies *et al.,* 2015; Silva *et al.,* 2019). Secondary metabolites produced by bacterial endophytes have been found to have antinemicactivity against a range of plant parasitic nematodes (Yadav *et al.,* 2021). Cefazolin is a secondary metabolite released by *B. velenzensis* which is having antifungal activity (Nayana *et al*., 2022). Furthermore, recent bioinformatics advances have led to the identification of nematode target sites and their interactions with biomolecules produced by various plant growth-promoting rhizobacteria. Thus, availability of annotated protein sequence of root knot and lesion nematode has allowed researchers to look into the most important proteins that play a vital role in the nematode's survival and invasion to host as potential therapeutic targets. An *in-silico* approach aids in the screening of secondary metabolites produced by bacterial endophytes to elucidate their inhibitory activity towards PPN (Terstappen and Reggiani, 2001). Further, Molecular docking facilitates virtual screening of diverse novel small molecules in short time for facilitating the structure-based drug design and to reduce the screening time (Amaro and Mulholland, 2018). Thus, based on the advances in the field of molecular docking, molecular simulation and release kinetics, novel biomolecules from bacterial origin could be identified for the management of plant parasitic nematodes infecting banana.

MATERIALS AND METHODS

Selection and molecular modelling of protein targets. Based on literature review, the potential protein targets of root knot nematode *Meloidogyne incognita*, were identified as cytochrome c oxidase subunit 1 (COX1) (Aditi Kundu *et al.,* 2021), calreticulin (CRT) (Li *et al.,* 2015; Jaouannet *et al.,* 2013), venom allergen proteins (VAP) (Li *et al.*, 2021 and Wilbers *et al.,* 2018) and β-1,4 -endoglucanase (Smant *et al.,* 1998). The Uni Prot database was utilised to retrieve the protein target sequences of the root knot nematode *M. incognita*.

The chosen virulent protein targets for root knot nematode were lacking experimentally and computationally solved structures. SWISS-MODEL software was used to produce homology modelling for COX 1, endoglucanase, CRT and VAP (Waterhouse *et al.*, 2018). The protein targets CRT,COX 1, endoglucanase, and VAP were blasted against Protein Data Bank (PDB) and followed homology modelling methodology. The parameters like Global Mean Quality Estimation (GMQE) score around 1, sequence identity percentage (30-50 %), and maximal query coverage were used to ensure the excellent quality of modelled structures.

Model Validation of protein targets. From the Structural Analysis and Verification of protein (SAVES) server, the PROCHECK programme (https://saves.mbi.ucla.edu/) was used for analysing energy and stereo-chemical property of the modelled protein structures. Ramachandran plot was built for each target using the PROCHECK tool to find whether the residues are in the energetically favoured region. Energy minimisation along with the loop building for residues in the disallowed regions of Ramachandran plot was executed with the help of SWISS-PDB Viewer (Guex *et al.*, 1997)

Preparation of the Ligand. Structures of the ligands 10-acetyl-9,10-dihydroacridin valeric acid and formic acidwere all obtained in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Kim *et al*.,2016). The commercial nematicide carbofuran 3G was used as a positive check. For the conversion of SDF to PDB file format Open Babel software was used (O'Boyle *et al.*, 2011).

Molecular docking analysis. To accomplish molecular docking, PyRx 0.8's AutoDock vina module was used (Dallakyan and Olson, 2015). The "make macromolecule" option in PyRx programme was used to prepare the structure of proteins. For the energy minimization of ligands conjugate gradient, first-order derivatives of an optimization procedure with 200 steps, and commercial molecular mechanics parameters-Unified Force Field (UFF)-were used. To determine binding site pockets for the targets, the Computed Atlas Topography of Proteins CASTp 3.0 server was used (Tian *et al.*, 2018). AutoDock4 and autogrid4 parameter files were used for docking and grid configuration respectively. BIOVIA Discovery studio client 2021 (https://www.3ds.com/products-services/biovia/) was used to visualise interactions of docked conformations of protein-ligand complexes. To distinguish between the receptor, ligand, and interacting atoms, different colours were assigned to them (Design, 2014).

RESULTS AND DISCUSSION

Modelling of protein targets. Modelling for CRT was done using SWISS-MODEL software with the template protein (PDB ID-6ENY) of 66.05 percent identity,91 percent query coverage, and a GMQE (Global Model Quality Estimation) score of 0.75. Template protein for COX1 (PDB ID-3ABM) had 64.86 percent identity, 99 percent query coverage, and a GMQE score of 0.77, β-1,4 -endoglucanase was modelled using template structure with (PDB ID-5IHS) had 49.17 percent identity,59 percent query coverage and 0.47 GMQE score, while VAP had 34.76 percent identity, 64 percent coverage, and a GMQE score of 0.45 with template protein (PDB ID-6ANY) (Fig.1).

Model validation. Based on Ramachandran plot, the models were validated and was revealed that COX1 had 94.5 percent of the residues in the most preferred region (Fig. 2). The allowable percentage of CRT residues was found to be 87.3 percent (Fig. 3). 85.7 percent of residues of the target β-1,4-endoglucanase was found to be in allowed region(Fig. 4). Similarly, for the target

VAP 82.3 percent of residues were found in most preferred region (Fig. 5).

Molecular docking of biomolecules with theprotein targets of *M. incognita.* Virtual screening methods such as molecular docking contributed significantly to the discovery of a potential new small molecule with a broad range of mode of action. To use the advantage of molecular docking so that to find a molecule with the highest binding affinity against *M. incognita* protein target, we investigated the biomolecules, for their potential nematicidal activity against the potential effector and virulent protein targets of *M. incognita*. Docking studies were used to investigate the binding affinity of modelled protein structures with the compounds. The binding affinity of 10-acetyl-9,10-dihydroacridin with the target COX 1 was -9.4 kcal/mol (H-bonds: 0), binding affinity of calreticulin was -6.7 kcal/mol, β-1,4-endoglucanase had -4.6 kcal/mol as binding affinity (H-bonds: LYS 285, ALA 286) and VAP has a binding affinity of -8.3 kcal/mol. (H-bonds: TYR 172) exhibiting intermolecular interactions towards the binding pocket that provides stability to the complex (Fig. 6, Table 1 and 2).

Carbofuran is a nematode-control agent used commercially by farmers. Hence, it was utilised to compare the affinity values as a positive check. The binding energy value of carbofuran found with the targets for COX 1 -4.2 kcal/mol (H-bonds: GLU 1), for β- 1,4 -endoglucanase -5.8 kcal/mol (H-bonds: LYS 241), for CRT–5.8 kcal/mol (H-bonds: ASN 166) and -6.6 kcal/mol for the target VAP (H-bonds: GLN 145, GLU 88). Two types of hydrogen bonds were found one with backbone and other with side chain. Other than hydrogen bonds there were other interactions also which are known as weak interaction like van der Waals interactions, pi-pi stacked interactions, alkyl, and pi-alkyl interactions (Fig. 7, Table 1 and 2).

Among the selected protein targets of *M. incognita*, 10-acetyl-9,10-dihydroacridin exhibited the highest binding energy in relation to target sites COX-1, VAP, β-1,4- endoglucanase, CRT than the commercial nematicide carbofuran 3G.The maximum binding affinity (-9.4 kcal/mol) for the 10-acetyl-9,10-dihydroacridin with the protein target cytochrome c oxidase subunit-1 might inhibit the oxidative phosphorylation pathway and ultimately will cause mortality of nematodes while for the same target carbofuran shows binding energy of (-7.5 kcal/mol) which is less than 10-acetyl-9,10-dihydroacridin (Aditi Kundu *et al.*, 2021). Likewise, maximum binding energy (-7.1 kcal/mol) of 10-acetyl-9,10-dihydroacridin with VAP may blocks these proteins which are involved in the establishment of persistent infections of nematodes in plants (Li *et al.,* 2021). Further the maximum binding (-4.5 kcal/mol) of β- 1,4 -endoglucanase with ligand 10-acetyl-9,10-dihydroacridincould restrict the nematode access into plant tissues by inhibiting the nematode's ability to hydrolyse β-1,4 linkage in cellulose polymer (Smant *et al*., 1998). Subsequently 10-acetyl-9,10-dihydroacridinalso had the maximum binding energy (-6.7 kcal/mol) with protein target calreticulin. It indicated that binding of 10-acetyl-9,10-dihydroacridin with CRT might have blocked Ca^{2+} multifunctional protein and thus suppressed pathogenesis, reproduction, parasitism and aided in blocking evasion from immunity of host against root knot nematode (Li *et al.,* 2015). However, no reports on the nematicidal action of 10-acetyl-9,10-dihydroacridin have been found. in comparison to carbofuran 3G, 10-acetyl-9,10-dihydroacridin showed maximum binding affinity and this suggests the possibility of 10-acetyl-9,10-dihydroacridin to have a nematicidal property better than the nematicidecarbofuran 3G which is commercially available. As a result, the current study is unique in that 10-acetyl-9,10-dihydroacridin can be used to create a formulation for the control of the banana root knot nematode.

Table 1: Binding affinity values of ligands with the protein targets.

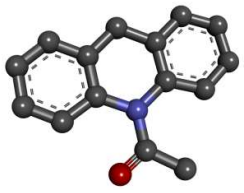
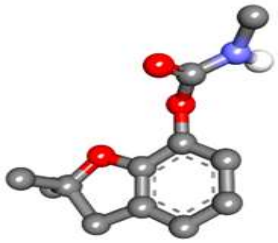
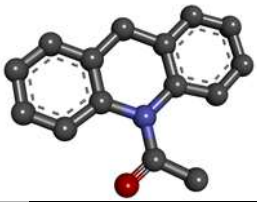
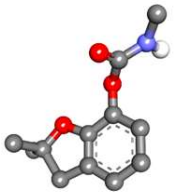
Targets	Binding affinity (kcal/mol) of 10-Acetyl-9,10-dihydroacridin and carbofuran on different targets	
	10-Acetyl-9,10-dihydroacridin 	Carbofuran 3G 
cytochrome c oxidase subunit 1	-9.4	-7.5
β 1,4 endoglucanase	-4.5	-4.2
Calreticulin	-6.7	-5.8
Venom allergen-like protein	-7.1	-6.6

Table 2: Hydrogen bonds formed by ligands with the protein targets.

Targets	H-bonds formed	
	10-Acetyl-9,10-dihydroacridin 	Carbofuran 3G 
cytochrome c oxidase subunit 1	HIS 54	GLU 1
β 1,4 endoglucanase	LYS 28, ALA 286	LYS 241
Calreticulin	ASP 240	ASN 166
Venom allergen-like protein	TYR 172	GLN 145, GLU 88

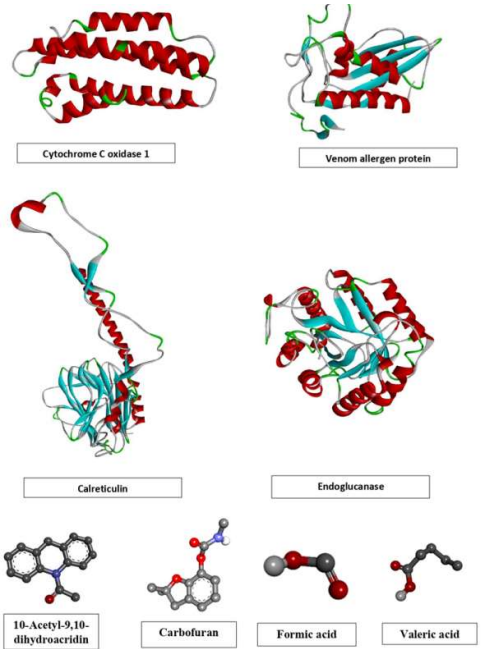


Fig. 1. The 3D structure of the target proteins and ligands.

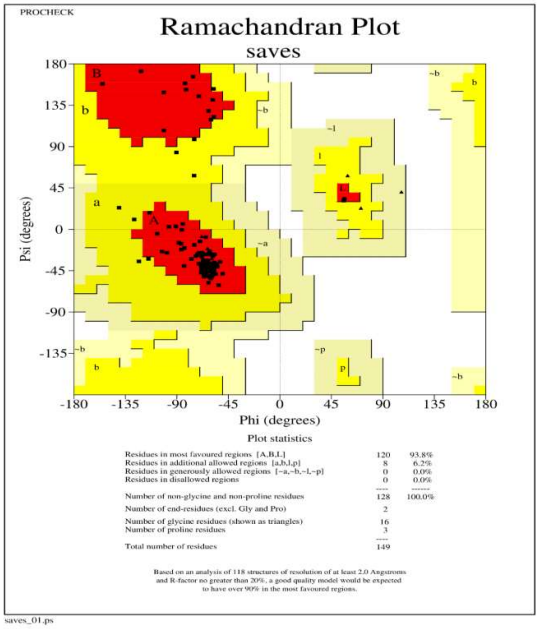


Fig. 2. Ramachandran plot for cytochrome c oxidase subunit 1.

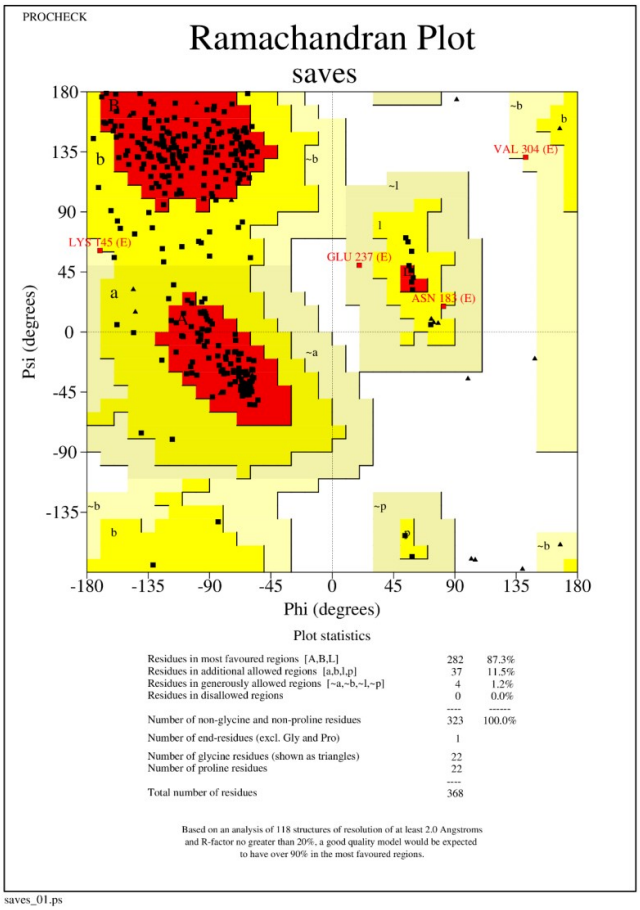


Fig. 3. Ramachandran plot for calreticulin.

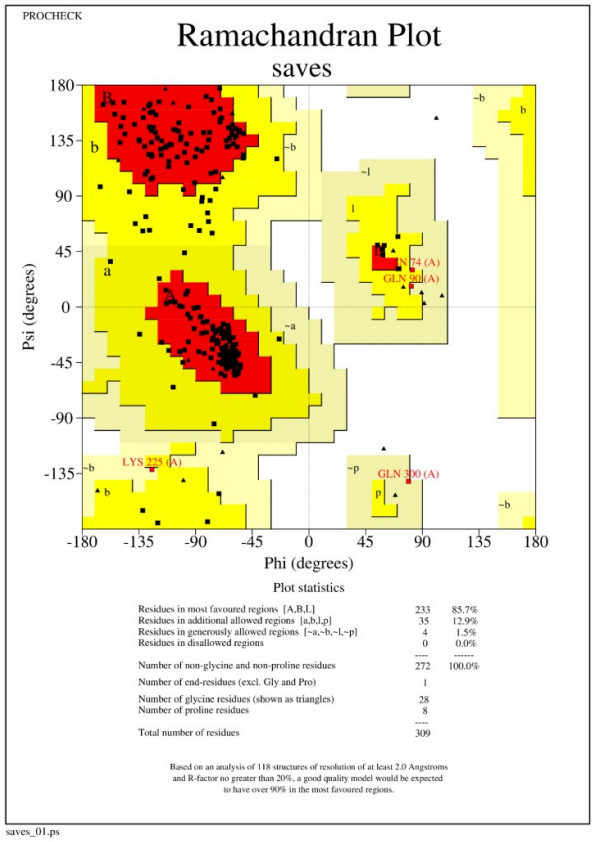


Fig. 4. Ramachandran plot for endoglucanse.

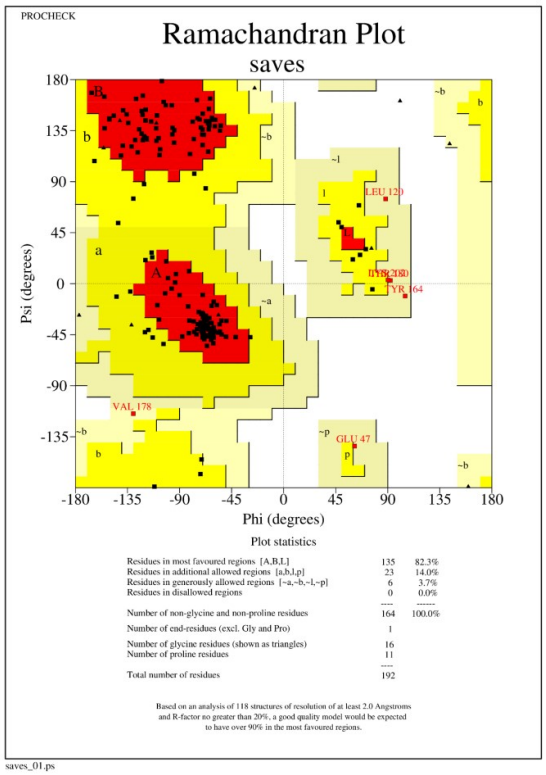


Fig. 5. Ramachandran plot for venome allergen protein.

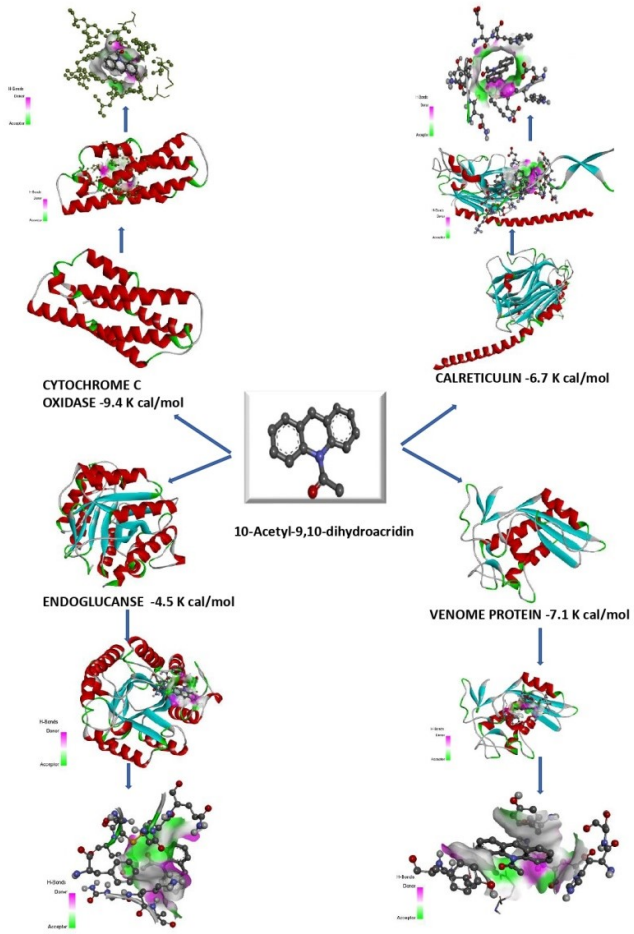


Fig. 6. Interaction of 10-Acetyl-9,10-dihydroacridin with the protein targets of *M. incognita.*

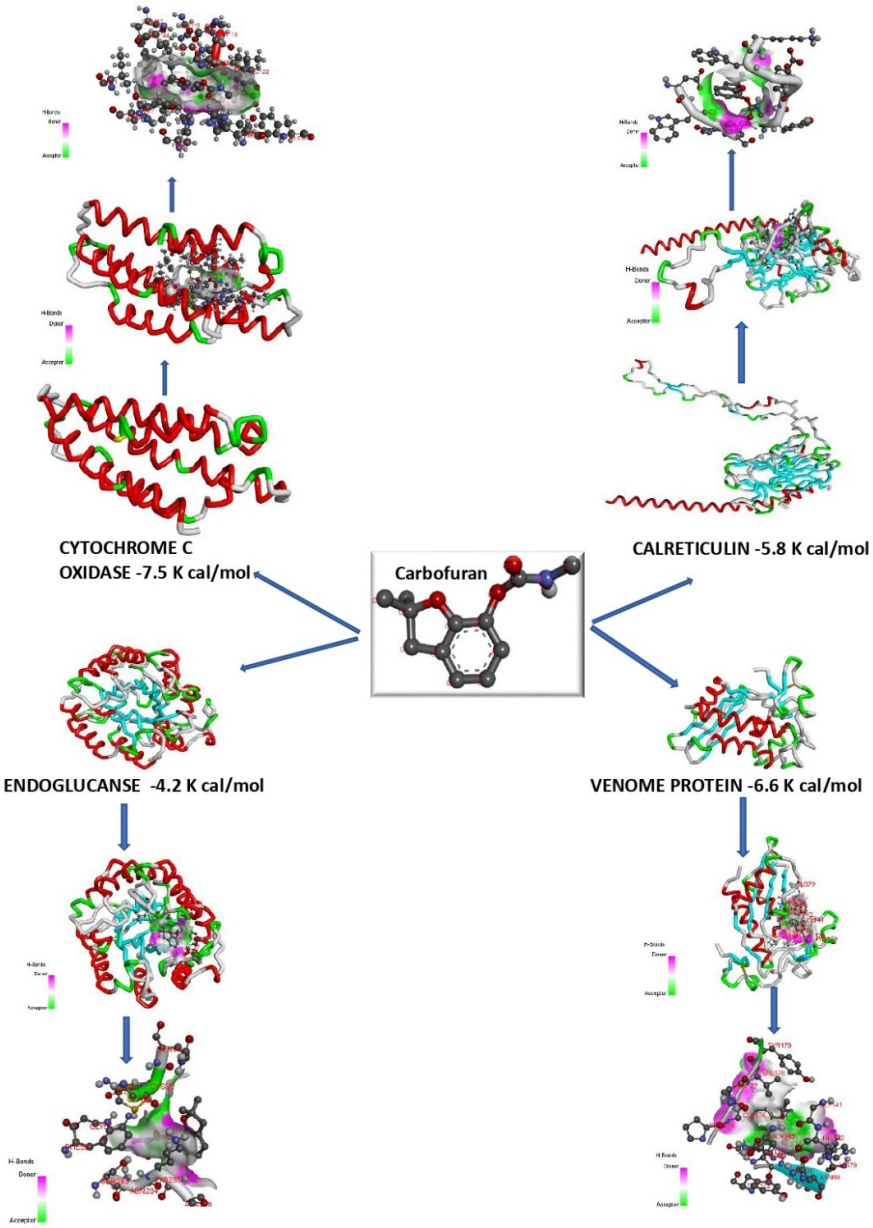


Fig. 7. Interaction of carbofuran 3G with protein targets of *M.incognita.*

CONCLUSION

The current study used computational strategy to explain the diverse modes of action of endophytic bacterial generated biomolecules.10-acetyl-9,10-dihydroacridin was having highest binding affinity with the various protein targets of the banana root knot nematode than the carbofuran 3G. Thus, the nematicidal biomolecule 10-acetyl-9,10-dihydroacridin can act as a possible inhibitor of the target sites involved in disrupting the functions of β- 1,4 -endoglucanase, COX-1, CRT, and VAP. As a result, 10-acetyl-9,10-dihydroacridin can be used as a nematicidal compound to combat the banana root knot nematode.

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Conflict of Interest. None.

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