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Original Article

COMPUTATIONAL INTERACTION OF ENTOMOPATHOGENIC FUNGAL SECONDARY METABOLITES WITH PROTEINS INVOLVED IN HUMAN XENOBIOTIC DETOXIFICATION

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ABSTRACT

Objective: Entomopathogenic fungi are rich source of secondary metabolites which posses both pharmacological and insecticidal activity. It is essential to assess metabolite toxicity of chemically diverse toxic metabolites of entomopathogenic fungus. Human acetylcholine esterase, cytochrome p450 and glutathione S-transferase are important enzymes involved in human xenobiotic detoxification.

Methods: In this study, *in silico* interaction of 13 selected secondary metabolites of entomopathogenic fungi with the target human proteins were carried out using Molegro Virtual Docker 4.0.2.

Results: This study reveals serinocyclin-A, have shown highest binding energy (176.07 KJ mol^{-1}) with glutathione S-transferase followed by helvolic acid, cytochalasin B and beauverolide H have shown considerable inhibition among the metabolites tested.

Conclusion: The study concludes that serinocyclin-A, helvonic acid, cytochalasin B and beauverolide among 13 secondary metabolites tested were found to be more toxic and may inhibit the human metabolic pathways.

Keywords: Entomopathogenic fungi, Xenobiotic detoxification, *In silico* interaction, Serinocyclin-A, Glutathione S-transferase, Molegro virtual Docker, Helvolic acid, Cytochalasin B, Beauverolide H.

INTRODUCTION

Entomopathogenic fungi are promising source for bioactive molecules of pharmacological and insecticidal interest [1], [2], [3]. Some of them can be used for food and medicine development [4]. There is cumulative interest in the utilization of entomopathogenic fungi (EPF) for the biological control of crop pests and diseases [5], [6]. Globally, a number of entomopathogenic fungal based commercial products were registered for the use as insect pest control agents in crop protection [7], while many other biocontrol fungal strains or formulations are estimated to be placed on the global market in the next few years. These metabolites serve diverse functions, depending on the ecological niche of the fungus, therefore assessment of environmental risks composed by these microbial pest control agents is essential [8]. Hence, based on metabolite toxicology of entomopathogenic fungal biological control agents, considerations must be made as for the possible presence of toxins in the formulated products [9].

In response to xenobiotic exposures mammals have generally evolved counter-defense mechanisms to induce proteins involved in xenobiotic detoxification. In the perspective of human biochemistry, detoxification can be described as a specific metabolic pathway, active throughout the human body by which unwanted chemicals are eliminated. This metabolic detoxification involves a series of enzymatic reactions which neutralize, solubilise toxins and transport them to the liver or kidneys, so that they can be eliminated from the body. This process is also known as xenobiotic metabolism. The detoxification enzymes namely: glutathione S-transferase [10], cytochrome p450 [11], acetylcholine esterase [12] were reported to play a significant role in this process.

Purification of any secondary metabolite of EPF is time consuming and requires the use of several analytical methods. Only a few among the several possible metabolites produced by these organisms are isolated. Therefore, a risk assessment investigation based on entomopathogenic fungal secondary metabolites is not economically feasible.

However some reports are available regarding assessment of few fungal metabolites (destruxin A and oosporein) toxicity using whole organism assays using *Artemica salina* and *Daphnia magna* [13]. Strasser et al. [9] proposed risk assessment of metabolites produced by microbials in crop protection products. It was proved that all secondary metabolites of EPF have biological activity (Table 1). But there were no reports about interaction of these thirteen secondary metabolites of EPF with the target proteins that are involved in detoxification.

To understand the effect of fungal secondary metabolites on humans in the current investigation using molecular docking studies were done on selected proteins (enzymes) of human. Hence, the current hypothesis focuses on a comparison and interaction study of three detoxification enzymes glutathione S-transferase (PDBID: 3LII), cytochrome p450 (PDBID: 3NXU), acetylcholine esterase (PDBID: 4GTU) in *Homo sapiens* using Molegro virtual Docker (MVD 2010.4.0.2). The current investigation is to study the interaction of secondary metabolites with target proteins and predict which one is more toxic.

MATERIALS AND METHODS

Preparation of proteins

The three dimensional crystal structures of acetylcholine esterase (PDB ID: 3LII), cytochrome p450 (PDB ID: 3NXU) and glutathione S-transferase (PDB ID: 4GTU) were retrieved from Protein Data bank (http://www. rcsb. org/). The imported protein structures were loaded in Molegro Virtual Docker 2010.4.0.2 (http://www. clcbio. com/products/clc-drug-discovery-workbench/). Optimization was done by removing water molecules and co-crystallized ligands. Further preparation of proteins were done by assigning bonds, bond order, hybridization, charges and tripos atom types, creating explicit hydrogens.

Preparation of Ligands

The secondary metabolites of EPF considered having potential pharmacological and pesticidal activities (Table 1).

These metabolites were identified from the *Beauveria*, *Metarhizium* and *Lecanicillium* sps were collected from various literatures [1], [2], [3]. The three dimensional structures of 9 metabolites of EPF namely aphidicolin (CID-457964), aurovertin- B (CID-6441012), beauverolide H (CID-194155), cytochalasin-B (CID-5311281), dipicolonic acid (CID-10367), helvolic acid (CID-3002143), serinocyclin-A (CID-24762344), swainsonine(CID-51683), tenellin (CID-54704235) were downloaded from Pubchem compound search of NCBI database as *. sdf (dot sdf) file format(http://pubchem. ncbi. nlm. nih. gov/). The structures of 4 fungal metabolites namely, bassionalides CID-163065, beauvericin (CID-105014), destruxin-E (CID-107863) and oosporein (CID-5359404) were drawn using Accelrys Draw 4.1(http://accelrys. com/) and ACD/ChemSketch software (http://www. acdlabs. com/). Further optimization was done by converting into three dimensional structures of these ligands using ACD chemsketch – 3D structure optimization option. Three dimensional structures of three organophosphorus pesticides namely, parathion (CID-991), phosmet (CID-12901) and azinphosmethyl (CID-2268) which were found to have highest interaction (toxicity) towards the test proteins 3LII, 3NXU and 4GTU respectively [14] were taken from existing databases and used as controls. All ligands were imported in Molegro Virtual Docker for optimization by removing water molecules and further preparation of proteins were done automatically by assigning bonds, bond order, hybridization, charges and tripos atom types, creating explicit hydrogens and detect flexible torsions in ligands.

Table 1: Pharmacological and pesticidal activities of 13 secondary metabolites from different entomopathogenic fungi

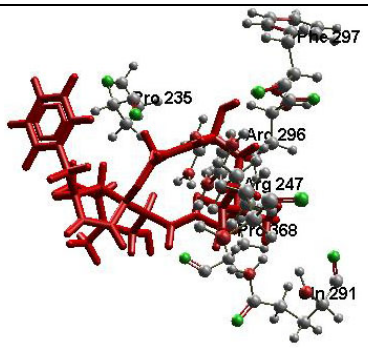
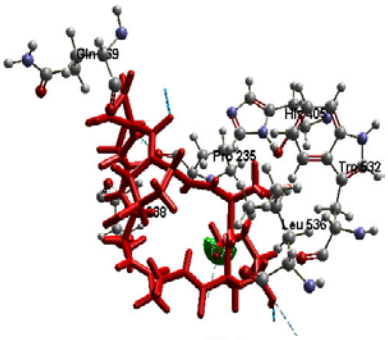
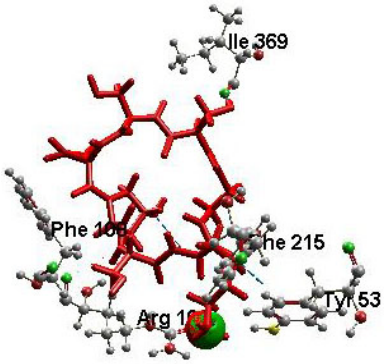
Secondary metabolite	Source Entomopathogenic fungi	Pharmacological/pesticidal activity
Aurovertin B (CID-6441012)	*Metarhizium anisopliae* [22]	Inhibits proliferation of breast cancer cells *in vivo* [23]
Aphidicolin (CID-457964)	*Lecanicillium* Sp [24]	Antiviral activity [25]
Bassionalide (CID-163065)	*Beauveria bassiana* [26]	Insecticidal activity [27]
Beauvericin (CID-105014)	*B. bassiana* [28]	Insecticidal activity, Antitumor activity and Antimicrobial activity [29]
Beauverolide (CID-194155)	*Beauveria sps* [30]	Antiatherogenic activity[31]
Cytochalasin D (CID-5311281)	*M. anisopliae* [32]	Antibiotic and antiviral activity [33]
Destruxin-E (CID-107863)	*M. anisopliae* [3]	Insecticidal activity, antituberculotic activity [34], [35]
Dipicolinic acid (CID-10367)	*Verticillium lecanii* [36]	Insecticidal activity[37]
Helvolic acid (CID-3002143)	*M. anisopliae* [38]	Antimicrobial activity [20], [38]
Oosporein (CID-5359404)	*Beauveria brongniartii* [39], [9]	Ativiral activity [40]
Serinocyclin A (CID-24762344)	*M. anisopliae* [16]	Sub lethal effect on mosquito larvae [16]
Swainsonine (CID-51683)	*M. anisopliae* [9]	Antitumor activity [41], [42], [43]
Tenellin (CID-54704235)	*B. brongniartii* [44]	Toxic towards erythrocyte membranes ATPases [45]

Table 2: Details of Energy scores and H-bond interactions of the top docking hits of ligands with target proteins.

Protein	Ligand	MolDock[a]	Rerank[b]	Interaction[c]	Internal[d]	HBond[e]	LE1[f]	LE3[g]
3LII(A)	5311281	-149.57	-81.81	-133.18	-16.38	-3.93	-4.27	-2.33
3LII(A)	991	-96.92	-79.91	-101.97	5.04	-4.85	-5.38	-4.43
3LII(B)	24762344	-153.69	-88.09	-146.86	-6.83	-13.02	-3.27	-1.87
3LII(B)	991	-98.04	-78.98	-104.52	6.48	-2.5	-5.44	-4.38
3NXU(A)	24762344	-176.07	-118.24	-169.07	-6.99	-6.50	-3.74	-2.51
3NXU(A)	12901	-102.34	-81.74	-111.08	8.73	-7.63	-5.38	-4.30
3NXU(B)	24762344	-167.15	-104.26	-158.23	-8.92	-5.94	-3.55	-2.21
3NXU(B)	12901	-101.94	-80.26	-106.39	4.44	-6.30	-5.36	-4.22
4GTU(A)	3002143	-150.46	-9.03	-162.15	11.68	-7.73	-3.66	-0.22
4GTU(A)	2268	-106.48	-78.54	-122.16	15.68	-1.74	-5.60	-4.13
4GTU(B)	24762344	-151.14	-27.89	-144.23	-6.90	-14.00	-3.21	-0.59
4GTU(B)	2268	-91.40	-77.85	-100.64	9.24	-2.5	-4.81	-4.09
4GTU(C)	194155	-135.52	-103.51	-147.80	12.27	-6.51	-3.87	-2.95
4GTU(C)	2268	-82.61	-64.96	-91.45	8.84	-2.87	-4.34	-3.41
4GTU(D)	24762344	-136.72	34.30	-130.14	-6.58	-10.71	-2.90	0.72
4GTU(D)	2268	-83.84	-58.79	-89.40	5.56	-4.77	-4.41	-3.09
4GTU(E)	24762344	-162.84	32.01	-156.72	-6.12	-13.29	-3.46	0.68
4GTU(E)	2268	-86.92	-60.31	-98.97	12.04	-11.49	-4.57	-3.17
4GTU(F)	24762344	-162.38	46.02	-154.97	-7.41	-16.54	-3.45	0.97
4GTU(F)	2268	-92.41	-77.98	-103.29	10.88	-2.02	-4.86	-4.10
4GTU(G)	24762344	-171.01	-59.91	-162.77	-8.24	-12.69	-3.63	-1.27
4GTU(G)	2268	-92.66	-79.55	-105.04	12.38	-3.56	-4.87	-4.18
4GTU(H)	24762344	-155.64	3.32	-151.41	-4.22	-10.69	-3.31	0.07
4GTU(H)	2268	-78.73	-62.84	-88.65	9.92	-1.66	-4.14	-3.30

[a]Moldock score is resulting from the PLP scoring functions through a new hydrogen bonding expression and new charge schemes. (Thomsoen and Christensen 2006). [b]Rerank score is a linear grouping of E-inter (steric, Van der Waals, hydrogen bonding, electrostatic) between ligand and the protein, and E-intra, (torsion, sp2-sp2, hydrogen bonding, electrostatic) of the ligand biased by pre-defined coefficients. (Thomsen and Christensen 2006). [c]Total contact energy between the pose and the protein(kJ mol^{-1}). [d]Internal energy of the pose. [e]Hydrogen bonding energy (kJ mol^{-1}). [f] Ligand Efficiency 1: MolDock Score divided by Heavy Atoms count. [g]Ligand Efficiency 3: Rerank Score divided by Heavy Atoms count.

Table 3: Binding modes of best ligands (secondary metabolites) of entomopathogenic fungi towards enzymes involved in human xenobiotic detoxification. The maximum volume for the cavities of pose 1 was taken into concern in all the cases for superior docking with proximal amino acids in the cavities.

Ligand	Protein	Mol Dock Score (kJ mol^{-1})	Binding mode	Cavity Volume (A°)	Amino acid Proximity value	Amino acid Sequence
5311281	3LII[A]	-149.57		207.752	1.56	Arg-247; Gln-291; Phe-297; Pro-368
24762344	3LII[B]	-153.69		156.16	1.67	Gln-369; His-405; Leu-536; Pro-235
24762344	3NXU(B)	-167.15		781.824	1.56	Arg-106; Ile-369; Phe-215; Tyr-53

Molecular docking

Docking studies of optimized three dimensional structures of acetylcholine esterase (PDB ID: 3LII), cytochrome p450 (PDB ID: 3NXU) and glutathione S-transferase (PDB ID: 4GTU) were done by creating surface and detection of cavities in protein surface were done using Molegro Virtual Docker.

Acetylcholine esterase possess two protein chains (3LII [A] & [B]), while Cytochrome p450 possess two protein chains (3NXU [A] & [B]) and finally glutathione S-transferase possess eight protein chains (4GTU [A], [B], [C], [D], [E], [F], [G] and [H]). The optimized three dimensional ligands of aphidicolin (CID-457964), aurovertin-B (CID-6441012), bassionalides CID-163065, beauverolide H (CID-194155), beauvericin (CID-105014), cytochalasin-B (CID-5311281), destruxin-E (CID-107863), dipicolonic acid (CID-10367), helvolic acid (CID-3002143), oosporein (CID-5359404), serinocyclin-A (CID-24762344), swainsonine(CID-51683), tenellin (CID-54704235) along with three organophosphorus pesticides namely, parathion (CID-991), phosmet (CID-12901) and azinphosmethyl (CID-2268) were docked into these cavities having highest volume of individual protein chains (Table 3). They were saved individually into Molegro Virtual Docker software on 'mol' format.

RESULTS AND DISCUSSION

Docking of 13 EPF metabolites namely aphidicolin, aurovertin- B, bassionalides, beauverolide H, beauvericin, cytochalasin-B, destruxin-E, dipicolonic acid, helvolic acid, oosporein, serinocyclin-A, swainsonine, tenellin along with three organophosphorus pesticides namely, parathion, phosmet and azinphosmethyl were done within the cavities of acetylcholine esterase, cytochrome p450 and glutathione S-transferase generated five poses individually with unique chemical arrangement. The MolDock scores and cavity volume of best pose of each metabolite was selected for the subsequent protein-ligand interaction energy analysis (Table 2). The binding activity of secondary metabolites of EPF were found to be higher than the control ligands (organophosphorus pesticides) namely, parathion (CID-991), phosmet (CID-12901) and azinphosmethyl (CID-2268) towards the test proteins 3LII, 3NXU and 4GTU respectively (Table 2).

In our docking studies, the Mol Dock score of highest interaction energy calculated was -176.07 relative units. It was interesting to note that among the 13 metabolites used in this study, serinocyclin-A has shown highest interaction with nine protein chains tested namely, 3LII[B], 3NXU[A], 3NXU[B], 4GTU[B], 4GTU[D], 4GTU[E],

4GTU[F], 4GTU[G] and 4GTU[H] which includes maximum MolDock score of -176.07 with 3NXU [A] and the proximal amino acid residues involved are Arg105, Leu482, Phe108, Tyr53 (Table 2). In case of 4GTU [G] the highest MolDock score was -171.01 and involved proximal amino acid residues includes Asn58, Ile9, Thr209, Tyr115 (Table 3). 3NXU[B] shows that third highest binding energy is -167.15 and the involved amino acid residues are Arg106, Ile369, Phe215, Tyr53 (Table 3). Similarly, the fourth and fifth highest binding energy with serinocyclin-A were found with 4GTU [E] and 4GTU [F] are -162.84 and -162.38 respectively. It was interesting to note that the secondary metabolites of *Beauveria brongniartii* induced the glutathione S-transferase [15]. Alternation in glutathione S-transferase was found in the hosts when treated with the secondary metabolites of *Beauveria bassiana*. These serinocyclins were found to inhibit the swimming ability of mosquito larvae [16] and may play role in their control [17]. It was interesting to note that serinocyclins do not function as virulence factors in *Spodoptera exigua* [18], [19] and Colorado potato beetle [18]. However functional roles of these metabolites have been not yet identified. After serinocyclin-A, it was helvolic acid which has shown highest binding affinity towards the protein side chain of (human glutathione S-transferase) 4GTU [A] with a MolDock score of -150.46 and proximal amino acid residues involved are Asp161, Leu207, Ser107, Tyr115 (Table 3).

Helvolic acid was known for its strong antimicrobial activity [20]. Cytochalasin B has shown higher binding affinity towards protein chain 3LII[A] with a MolDock score of -149.57 with proximal amino acid residues involved are Arg247, Gln291, Phe297, Pro368. Cytochalasin B is known for its inhibition of actin filament formation [21]. Beauverolide H was found to have MolDock score-135.52 towards 4GTU[C] having a proximal amino acid residues include Arg112, Leu12, Thr109, Tyr115 (Table 3). The docking results indicated that serinocylin A as the most toxic entomopathogenic fungal secondary metabolites towards all the protein chains tested followed by helvolic acid, cytochalasin B and beauverolide H. However, further analysis can be carried out in wet lab to determine whether these findings are reflective to *in vivo* conditions.

CONFLICT OF INTERESTS

Declared None

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Table 3: Contd.

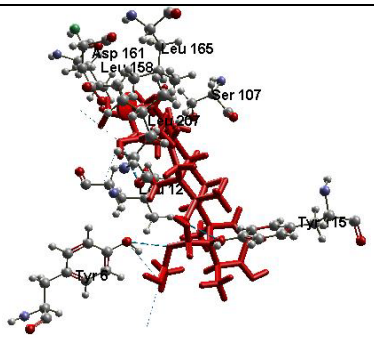
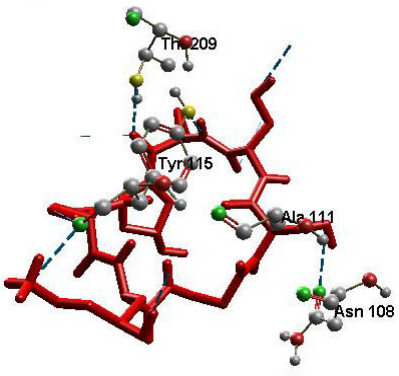
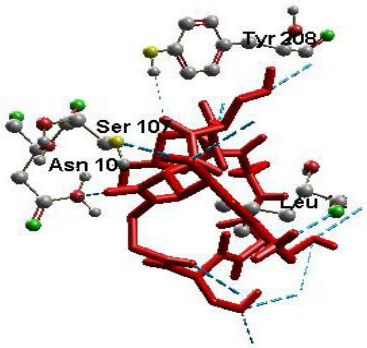
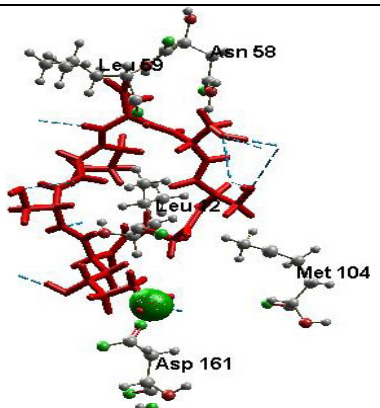
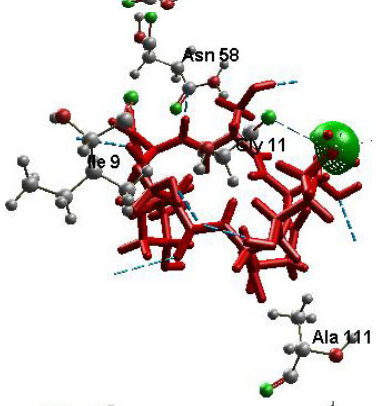
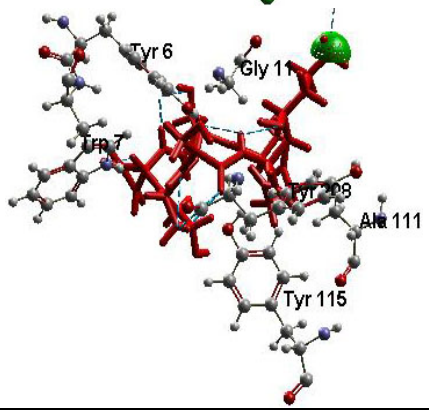
Ligand	Protein	Mol Dock Score (kJ mol^{-1})	Binding mode	Cavity Volume (A^{o})	Amino acid Proximity value	Amino acid Sequence
3002143	4GTU[A]	-150.46		68.608	1.89	Asp-161; Leu-207; Ser-107; Tyr-115
24762344	4GTU[B]	-151.14		61.952	1.75	Ala-111; Asn-108; Thr-209; Tyr-115
24762344	4GTU[D]	-136.72		34.816	1.40	Asn-108; Leu-12; Ser-107; Tyr-208

Table 3: Contd.

Ligand	Protein	Mol Dock Score (kJ mol^{-1})	Binding mode	Cavity Volume (A°)	Amino acid Proximity value	Amino acid Sequence
24762344	4GTU[E]	-162.84		45.568	1.46	Asn-58; Asp-161; Leu-12; Met-104
24762344	4GTU[F]	-162.38		53.248	1.42	Ala-111; Asn-58; Gly-11; Ile-9
24762344	4GTU[H]	-155.64		46.592	1.58	Ala-111; Gly-11; Trp-7; Tyr-115

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