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High-Throughput Virtual Screening of Affine Interactions of a Fluorescent Oleylamine Derivative with Protein Targets of Several Insects †

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Abstract

Computer-aided docking combined with AlphaFold-based protein modeling revealed strong binding affinities of the ligand DOLA ((Z)-5-(dimethylamino)-N-(octadec-9-en-1-yl)naphthalene-1-sulfonamide) to metabolic proteins in insect species *Tenebrio molitor*, *Tribolium castaneum*, *Locusta migratoria*, *Lucilia cuprina*, *Drosophila melanogaster*. Significant interactions were observed with cytochrome P450 families CYP6 and CYP4, involved in detoxification, hormone regulation, and fatty acid metabolism, with binding energies ranging from −10.4 to −8.5 kcal/mol. DOLA also showed affinity for enzymes related to fatty acid metabolism and transport, including long-chain fatty acid-CoA ligase, fatty acyl-CoA reductases, CYP18a1, lipocalins, and fatty acid desaturases. These in silico findings highlight DOLA as a promising ligand for studying insect metabolic pathways and as a potential agent for population control, warranting further experimental validation.

Keywords: molecular docking; Alphafold; oleylamine derivative; fluorophore; fatty acid metabolism; cytochrome P450; insects; pest control; *Tenebrio*; *Tribolium*; *Locusta*; *Lucilia*; *Drosophila*

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1. Introduction

The search for novel and effective methods for controlling insect populations remains a pressing challenge for agriculture and public health. Traditional insecticides often prove ineffective due to the development of resistance in insects and possess detrimental effects on the environment and human health. In this context, the development of targeted insecticides, aimed at specific biochemical processes within insect organisms, represents a promising avenue. The present work is dedicated to employing molecular docking to identify potential inhibitors or substrates of proteins involved in xenobiotic detoxification, metabolism, transport, and the reception of fatty acids and their derivatives, and which constitute promising targets for the development of new tools for studying metabolism or insect population control agents.

2. Materials and Methods

Molecular docking was performed using AutoDock Vina 1.1.2 with the following parameters: a docking area of 6 × 6 × 6 nm centered on the protein, a step size of 0.1 nm, an exhaustiveness parameter of 15, and the calculation of five models. Visualization of the results was performed using BIOVIA Discovery Studio v16.1.0.15350. The original FYTdock assistant program [1] was used to automate the organization, execution of calculations, and analysis of the resulting data. The structure of compound DOLA ((Z)-5-(dimethylamino)-N-(octadec-9-en-1-yl)naphthalene-1-sulfonamide) was selected as the ligand. To create a library of protein structures from the UniProt and AlphaFold databases, 383 structures of chemosensory, cuticular, transport, metabolic, and fatty acid/fatty acid derivative receptor proteins from the insect genera *Lucilia*, *Locusta*, *Tenebrio*, and *Tribolium* were selected, along with 229 cytochrome P450 structures from the insect genera *Drosophila*, *Lucilia*, *Locusta*, *Tenebrio*, and *Tribolium*. Results for the obtained ligand-protein model complexes with binding energy (Ebind) values no greater than −8.2 kcal/mol were considered in the discussion.

3. Results

Computational modeling revealed the most affine interactions with the structures of insect CYP6 and CYP4 family cytochrome P450s (Table 1).

Table 1. DOLA binding energies in modeled complexes with the structures of insect CYP6 and CYP4 families cytochrome P450s.

UniProt Code	Protein Name	Organism	Binding Energy (kcal/mol)
A0A139WKY9	CYP6a23-like	*Tribolium castaneum*	−10.4
A0A0L0CAR5	Cyp4aa1	*Lucilia cuprina*	−10.1
A0A0L0CBH0	CYP6g1	*Lucilia cuprina*	−9.3
Q9V7G5	Cyp4aa1	*Drosophila melanogaster*	−8.6
Q9VRI9	CYP6t1	*Drosophila melanogaster*	−9.5
L0CRC5	CYP6t3	*Drosophila melanogaster*	−9.3
L0CR17	CYP6t3	*Drosophila melanogaster*	−9.1
L0CPB6	CYP6t3	*Drosophila melanogaster*	−9.0
L0CQN3	CYP6t3	*Drosophila melanogaster*	−8.9
A0A0L0BXP5	CYP4d2	*Lucilia cuprina*	−8.5

The ligand was positioned in proximity to the heme group of the cytochrome P450s, with a distance no greater than 0.50 nm. Cytochrome P450s of the CYP6 family may participate in the metabolism of insect hormones and the detoxification of synthetic insecticides and other xenobiotics, while members of the CYP4 family are involved in the metabolism of fatty acids, as well as various foreign compounds, which underscores the significance of searching for substrates and inhibitors of these enzymes for studying metabolism and discovering new insect control agents [2,3].

The DOLA compound was found to bind to structure of CYP6t3, with the double bond of its alkyl fragment oriented near the heme iron atom (Figure 1).

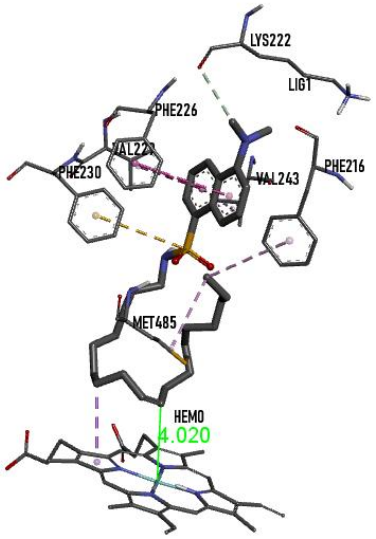
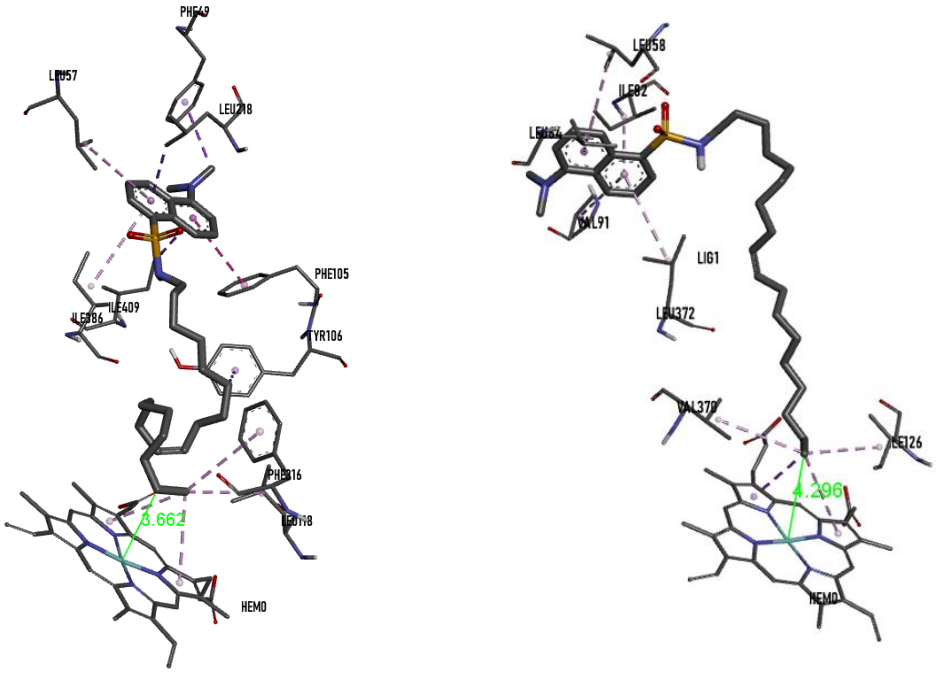


Figure 1. The calculated position of DOLA in proximity to the heme group of CYP6t3 (UniProt code—L0CRC5). Distances in the figures are indicated in Angstroms (Å).

CYP6t3 may be involved in the metabolism of insect hormones and the breakdown of insecticides, but its precise role remains largely uncharacterized. Nevertheless, some evidence suggests its potential participation in insect development [4,5].

Computational modeling revealed that DOLA also bound to CYP4d2, an enzyme involved in the detoxification of insecticides [6,7] (e.g., β-cypermethrin [6]), with the methylene group at the ω-1 position of its alkyl chain positioned in close proximity to the heme iron atom (Figure 2a).

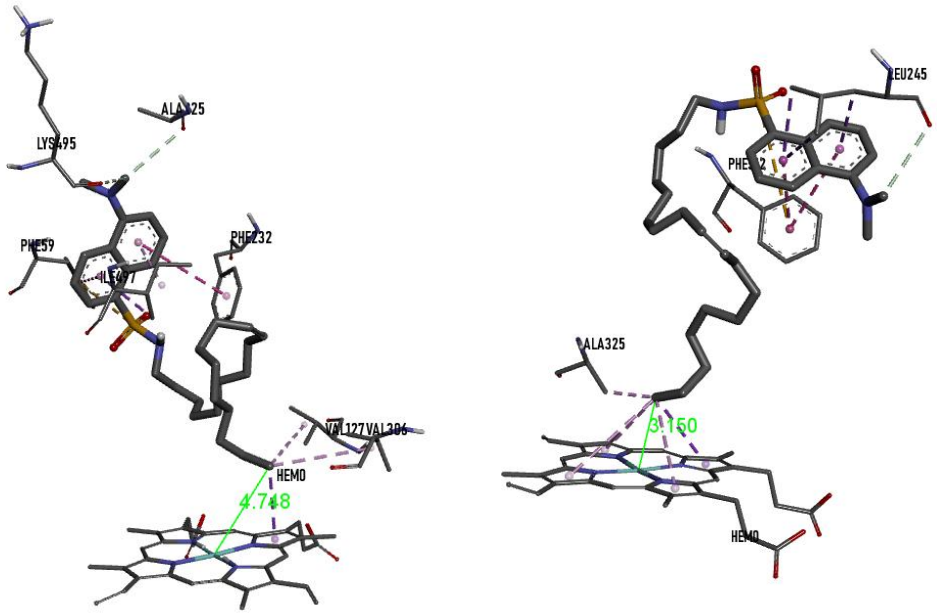


(**a**) (**b**)

Figure 2. (**a**) The calculated position of DOLA in proximity to the heme group of CYP4d2 (UniProt code—A0A0L0BXP5); (**b**) The calculated position of DOLA near the heme group of the ω-hydroxylase CYP4aa1 (UniProt code—A0A0L0CAR5). Distances in the figures are indicated in Angstroms (Å).

Notably, literature suggests that CYP4aa1 may possess ω-hydroxylase activity [8]. Our computational modeling indicated that ligand DOLA oriented its terminal methyl group of its alkyl fragment towards the heme group of Cyp4aa1, suggesting that ω-hydroxylation of this ligand could potentially occur (Figure 2b). Therefore, DOLA, possessing a fluorescent moiety within its structure, may serve as a valuable tool for investigating this interaction and the enzyme's function.

The DOLA ligand also was oriented with the terminal methyl group of its alkyl fragment towards the heme of cytochromes Cyp6t1 and Cyp6g1 (Figure 3).



(**a**) (**b**)

Figure 3. (**a**) The calculated position of DOLA near the heme group of the CYP6g1 (UniProt code—A0A0L0CBH0); (**b**) The calculated position of DOLA near the heme group of the CYP6t1 (UniProt code—Q9VRI9). Distances in the figures are indicated in Angstroms (Å).

Notably, Cyp6t1 is one of the P450 enzymes most closely related to Cyp6g1 [9]. CYP6g1 and CYP6g2 confer resistance to the neonicotinoids imidacloprid and nitenpyram in *Drosophila melanogaster* [10].

According to the protein-ligand interaction modeling, the ligand DOLA also bound to the structures of long-chain fatty acid-CoA ligase, fatty acyl-CoA reductases, CYP18A1, lipocalins, as well as fatty acid desaturase domain-containing proteins (Table 2).

Table 2. DOLA binding energies in modeled complexes with the structures of insect fatty acid metabolism proteins.

UniProt Code	Protein Name	Organism	Binding Energy (kcal/mol)
A0A0K1YW92	CYP18A1	*Tenebrio molitor*	−9.2
A0A0L0C0L6	Fatty acyl-CoA reductase	*Lucilia cuprina*	−8.7
A0A0L0CFW6	Lipocalin	*Lucilia cuprina*	−8.6
A0A0L0CII7	Lipocalin	*Lucilia cuprina*	−8.6
A0A0L0CLB5	Fatty acid desaturase domain-containing protein	*Lucilia cuprina*	−8.5
A0A0L0BY47	Fatty acyl-CoA reductase	*Lucilia cuprina*	−8,4
A0A0L0BTW8	Fatty acyl-CoA reductase	*Lucilia cuprina*	−8,2
A0A0L0BPB5	Lipocalin	*Lucilia cuprina*	−8.2

CYP18A1 is a cytochrome P450 (26-hydroxylase) known to be involved in ecdysone (molting hormone) metabolism in insects, and is also an ω-1-fatty acid hydroxylase involved in the synthesis of caste-specific pheromones in bees [8,11]. Fatty acyl-CoA reductases catalyze the reduction of fatty acyl-CoAs to fatty alcohols. Fatty alcohols are important components of waxes and lipids required for insect cuticle construction, and can also be used for pheromone synthesis [12–15]. Fatty acid desaturases catalyze the formation of double bonds in fatty acids, which is necessary for the synthesis of unsaturated fatty acids and, consequently, for maintaining the structure of cell membranes and signal transduction, and adaptation to various environmental conditions [16–18]. Lipocalins are a family of proteins that bind hydrophobic compounds, such as lipids. In insects, they may be involved in the transport, storage, and metabolism of fatty acids, as well as in chemical communication and immune response [19,20].

4. Conclusions

Based on in silico evaluation, the ligand DOLA was identified as a potential substrate for a number of important metabolic proteins in insects of the genera *Drosophila*, *Lucilia*, *Locusta*, *Tenebrio*, and *Tribolium* (cytochrome P450s, transport proteins, and proteins involved in the metabolism of fatty acids and their derivatives). The obtained data will serve as a basis for further experimental in vitro investigations of this compound as a tool for studying insect metabolism or as a potential regulator of their populations.

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Abbreviations

The following abbreviations are used in this manuscript:

DOLA	(Z)-5-(dimethylamino)-N-(octadec-9-en-1-yl)naphthalene-1-sulfonamide

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