In Silico Identification of Protein Targets Associated to the Insecticide Activity of Eugenol Derivatives †

Tatiana F. Vieira 1, Maria F. Araújo 1, Maria José G. Fernandes 2, David M. Pereira 3, A. Gil Fortes 2, Elisabete M. S. Castanheira 4, M. Sameiro T. Gonçalves 2 and Sérgio F. Sousa 1,*

1 UCIBIO/REQUIMTE, BioSIM—Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal; tatianafvieira@gmail.com (T.F.V.); mffaraujo@live.com.pt (M.F.A.)
2 Centre of Chemistry, Department of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; mjfernandes@quimica.uminho.pt (M.J.G.F.); gill@quimica.uminho.pt (A.G.F.); msameiro@quimica.uminho.pt (M.S.T.G.)
3 REQUIMTE/LAQV, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; dpereira@ff.up.pt
4 Centre of Physics, Department of Physics, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; ecoutinho@fisica.uminho.pt
* Correspondence: sergiofsousa@med.up.pt
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Abstract: The control of insect pests and the need for increased food production due to the world population growth, together with environmental issues associated with synthetic pesticides, has stimulated the development of new and “greener” alternatives, based on natural compounds. Eugenol is a natural compound that is the major component of clove oil. It has demonstrated antimicrobial and antioxidant activity, being also a powerful insecticide. Recently, new eugenol derivatives have been developed, with some molecules displaying increased insecticide activity. One of the difficulties associated with the rational development of new eugenol derivatives with enhanced insecticidal activity lies in the lack of knowledge of the specific protein target responsible for its activity and to the binding conformation of these molecules. Here, we report the application of an integrated molecular modeling—inverted virtual screening protocol of a collection of eugenol derivatives with confirmed insecticide activity against a molecular library of protein targets typically associated with the insecticide activity of natural compounds. The protocol included six different scoring functions from popular docking software alternatives. The results consistently show a marked preference for interaction of the eugenol derivatives with the odorant binding proteins (OBPs) in insect species. Interestingly, OBPs have been regarded as promising targets in the insect periphery nerve system for environmental-friendly approaches in insect pest management. The present results provide clues for the rational development of new eugenol derivatives as bioinsecticides targeting OBPs.

Keywords: biopesticides; eugenol; odorant binding proteins; inverted virtual screening

1. Introduction

The increase in population has caused a strain in agriculture due to rising demand and decreased land availability. Crops need to become resistant to damage and disease and the use of pesticides, fungicides, and herbicides has allowed for crop protection and long-term storage [1,2]. However, when these chemicals are used extensively or incorrectly, they become hazardous to the environment and to human health [3]. Thus, the search for new natural, safe, and ecofriendly alternatives, i.e., biopesticides, is being encouraged. Plants, animals, and bacteria produce metabolites that can exhibit
insecticidal activity. Using those metabolites to protect crops may lead to higher specificity, lower toxicity, and even a decrease in pest resistance [4].

Eugenol, the major component of clove oil, has established insecticidal and antimicrobial activity against a variety of targets [5–7]. Consequently, the search for new eugenol derivatives with higher efficiency was boosted to find additional alternatives to known insecticides. However, there is still significant work to be done to find out their correct binding conformation and, most importantly, their exact target(s) and mechanism(s) of action. This is precisely where computational chemistry can provide valuable insight.

In this study, it is presented the application of an integrated molecular modeling—an inverted virtual screening protocol for the identification of potential protein targets for a series of eugenol derivatives with confirmed insecticide activity. The protocol included the study of protein targets typically associated with the insecticide activity and included six different scoring functions from popular docking software alternatives.

2. Methods

A search on Scopus was performed for papers describing virtual screening (VS) studies, involving targets and molecules with insecticidal/herbicide activity. The selection criteria placed relevance of the target and year of publication. In the eighteen studies found, fourteen targets were identified (listed in Table 1).

### Table 1. List of targets selected for the inverted virtual screening study.

| Target | Organism | PDB Target | Resolution (Å) | Description | Reference |
|--------|----------|------------|----------------|-------------|-----------|
| Ecdysone receptor | Heliothis virescens | 1R20 | 3.00 | VS based on 1R20 bound to an agonist as a model for the development of a receptor-based pharmacophore model. | [8] |
| | | 1R1K | 2.90 | VS of 2 million compounds against 1R1K, an ecdysone receptor structure bound to its known ligand Ponasterone A. | [9] |
| Chitinase | Ostrinia furnacalis | 3WL1 | 1.77 | Pharmacophore-based screening using two crystal structures of chitinases: 3WL1 bound to its reaction product and 3WQV bound to an inhibitor. | [10] |
| | | 3WQV | 2.04 | | |
| beta-N-acetyl-D-hexosaminidase OfHex1 | | 3NSN | 2.10 | VS of the ZINC database to identify OfHex1 inhibitors using 3NSN crystal structure bound to a known inhibitor. | [11] |
| | | 3OZP | 2.00 | VS of the ZINC database targeting 3OZP, a crystal structure of OfHex1 bound to an inhibitor. | [12] |
| N-Acetylglucosamine-1-phosphate uridylyltransferase (GlmU) | Xanthomonas oryzae | 2V0K | 2.30 | Homology model built for docking using 2V0K and 2VD4 as templates. 2V0K crystal structure is bound to its known ligand and 2VD4 is bound to a possible inhibitor. | [13] |
| | | 2VD4 | 1.90 | | |
| Acetylcholinesterase | Aedes aegypti | 1QON | 2.72 | Search for new molecules with insecticidal activity against Ae. Aegypti using acetylcholinesterase crystal structures 1QON and 4EY6 as targets, both bound to possible inhibitors. | [14] |
| | | 4EY6 | 2.40 | | |
| | Drosophila melanogaster | 1DX4 | 2.70 | Homology 3D model built for vs. using 1DX4 as template. 1DX4 crystal structure is bound to a potent inhibitor. | [15] |
| Polyphenol oxidase | Ipomoea batatas | 1BUG | 2.70 | Docking simulations using the homologous polyphenol oxidase crystal structure of sweet potato in complex with phenylthiourea, a commonly used pesticide. | [16] |
| p-hydroxyphenylpyruvate dioxygenase | Arabidopsis thaliana | 6ISD | 2.40 | Development of a receptor-ligand pharmacophore model based on the crystal structure 6ISD bound to a commonly used | [17] |
pesticide. The best model created was then used for VS studies.

| Protein | Organism | PDB ID | Resolution |
|---------|----------|--------|------------|
| Oxidoreductase | Spinacia oleracea | 1YVE | 1.65 |
| Voltage-gated sodium channel | Periplaneta americana | 6A95 | 2.60 |
| Octopamine receptor | Blattella germanica | 4N7C | 1.75 |
| Sterol carrier protein-2 (HaSCP-2) | Helicoverpa armigera | 4UEI | Solution NMR |
| Peptide deformylase | Xanthomonas oryzae | 5CY8 | 2.38 |
| Alpha-esterase-7 (αE7) | Lucilia cuprina | 5TYP | 1.88 |
| Odorant Binding Protein | Drosophila melanogaster | 2GTE | 1.40 |
| | Anopheles gambiae | 3N7H | 1.60 |
| | Aedes aegypti | 3K1E | 1.85 |

Eugenol and eleven derivatives (Figure 1 EU1–EU3e) were selected as new potential insecticides. These molecules have been previously synthesized and validated experimentally with good insecticidal activity.

Each Protein Databank (PDB) structure was prepared for docking using the AutoDock Vina plugin for PyMOL [26]. Crystallographic waters and cofactors were removed. The ligands were extracted and saved in separate files to be used for the re-docking and as a reference site for the docking coordinates. When there were no crystallographic ligands present, a selection based on the most important active site residues was made. Re-docking was used to evaluate the ability of the docking software to reproduce the geometry and orientation of the crystallographic pose as well as the quality of the docking protocol, and to optimize the docking protocol.

The docking programs/scoring functions used were GOLD [27] (PLP, ASP, ChemScore, and GoldScore scoring functions), AutoDock Vina [28], and LeDock [29]. With each docking program/scoring function, the protocol was optimized for each protein target, to minimize the rmsd in the docking predictions of the reference ligand in redocking, by comparison with the crystallographic structure of the corresponding complex.
Figure 1. Eugenol and derivatives used in this study.

The optimized parameters for each program/scoring function were: Vina—docking box position, docking box dimension, exhaustiveness; LeDock—docking box position, docking box dimension; GOLD (PLP, ASP, ChemScore, GoldScore)—binding pocket center, docking region radius, search efficiency, number of runs. The final optimized conditions were used for the subsequent stages. Eugenol and derivatives were prepared for docking using Datawarrior [30] and OpenBabel [31] and were docked into each structure with the optimized protocol with all the six scoring functions. A ranked list was prepared based on the average scores of each target.

3. Results and Discussion

Table 2 presents the average scores obtained for all the eugenol derivatives for each potential target with each scoring function. The score for all of the GOLD scoring functions is dimensionless, and the higher the score, the better the binding affinity. Vina and LeDock scoring functions, on the other hand, use a metric that is a more precise approximation of binding free energy, so a more negative value means better affinity.

| Target                                      | PDB   | PLP  | ASP  | ChemScore | GoldScore | Vina  | LeDock |
|---------------------------------------------|-------|------|------|-----------|-----------|-------|--------|
| Ecdysone receptor                           | 1R20  | 57.3 | 27.5 | 28.1      | 52.5      | −6.4  | −4.7   |
|                                             | 1R1K  | 59.3 | 26.4 | 28.3      | 54.5      | −7.1  | −5.2   |
| Chitinase                                   | 3WL1  | 63.0 | 40.8 | 30.1      | 60.0      | −6.9  | −4.8   |
|                                             | 3WQV  | 63.4 | 40.7 | 30.6      | 55.7      | −6.5  | −4.3   |
| beta-N-acetyl-D-hexosaminidase OfHex1       | 3NSN  | 66.7 | 46.7 | 29.1      | 62.8      | −6.1  | −4.4   |
|                                             | 3OZP  | 63.3 | 43.7 | 28.3      | 58.7      | −7.1  | −4.3   |
| N-Acetylglucosamine-1-phosphate uridylyltransferase (GlmU) | 2V0K  | 55.0 | 24.1 | 23.3      | 54.3      | −5.9  | −4.6   |
|                                             | 2VD4  | 46.9 | 22.2 | 21.6      | 43.8      | −5.2  | −3.7   |
| Acetylcholinesterase                        | 1QON  | 73.3 | 48.2 | 35.3      | 62.2      | −7.6  | −5.0   |
|                                             | 4EY6  | 72.6 | 41.2 | 32.4      | 55.2      | −7.1  | −5.0   |
|                                             | 1DX4  | 70.0 | 43.2 | 32.2      | 55.3      | −7.2  | −4.9   |

Table 2. Average eugenol derivative scores obtained for all PDB structures with the six different scoring functions.
Overall, the results show good consistency, with odorant binding proteins, acetylcholinesterases, octopamine receptors, and chitinases yielding better scores. On the other hand, targets, such as voltage-gated sodium channels, sterol carrier protein-2 (HaSCP-2), and N-Acetylglucosamine-1-phosphate uridyltransferase (GlmU), are consistently presenting lower scores for all scoring functions.

The structure with the best score was selected for each potential target and they were ranked from the best target to worst, according to the predictions of the different docking programs/scoring functions. The results are listed in Table 3. Globally, considering the results obtained with the several scoring functions, odorant binding proteins are the target with the highest affinity towards eugenol derivatives, followed closely by acetylcholinesterase, chitinases, and octopamine receptors. Enan in 2001 [5] suggested that the insecticidal activity of eugenol was mediated by octopamine receptors. Our study implies that there might be other targets involved as well, as the binding affinity of eugenol derivatives was higher for odorant binding proteins (OBPs) and acetylcholinesterase.

Some variations between the predictions of different scoring functions exist. For example, for the PLP and ChemScore scoring function, odorant binding proteins, and acetylcholinesterase come in first and second as preferable targets for eugenol derivatives. However, for ASP and Vina, the preferable target is the acetylcholinesterase, and for both Vina and LeDock, odorant binding proteins are the second preferable targets. The discrepancy is even higher for GoldScore, with odorant binding proteins coming in third place and octopamine receptors presenting the highest binding affinity for eugenol derivatives. This may be explained by the own nature of each scoring function, as they consider different aspects of protein-ligand binding.

| Odorant Binding Protein | PLP | ASP | ChemScore | GoldScore | Vina | LeDock | Overall Ranking |
|-------------------------|-----|-----|-----------|-----------|------|--------|-----------------|
| Odorant Binding Protein | 1   | 4   | 1         | 3         | 2    | 2      | 1               |
| Acetylcholinesterase    | 2   | 1   | 2         | 5         | 1    | 5      | 2               |
| Chitinase               | 4   | 2   | 5         | 2         | 6    | 7      | 3               |
| Octopamine receptor     | 3   | 5   | 3         | 1         | 5    | 10     | 4               |
| Peptide deformylase     | 6   | 11  | 12        | 4         | 7    | 1      | 5               |
| Oxidoreductase          | 5   | 12  | 4         | 6         | 11   | 4      | 6               |
| β-N-acetyl-D-hexosaminidase OffHex1 | 7 | 3 | 9 | 7 | 3 | 13 | 7 |
| Ecdysone receptor       | 9   | 9   | 8         | 10        | 4    | 3      | 8               |
| α-esterase-7            | 8   | 6   | 7         | 12        | 9    | 8      | 9               |
| Sterol carrier protein-2 (HaSCP-2) | 13 | 8 | 6 | 14 | 8 | 6 | 10 |
| p-hydroxyphenylpyruvate dioxygenase | 10 | 7 | 11 | 13 | 10 | 12 | 11 |
| Polyphenol oxidase (PPO) | 11 | 10 | 10       | 9         | 14   | 14     | 12              |
| N-Acetylglucosamine-1-phosphate uridylyltransferase (GlmU) | 12 | 13 | 13 | 11 | 12 | 9 | 13 |
| Voltage-gated sodium channel | 14 | 14 | 14 | 8 | 13 | 11 | 14 |

Table 3. Ranking of targets obtained with the different docking programs/scoring functions.
The consistency of the results was visually confirmed by the analysis of the corresponding poses. The hypothesis formed is that eugenol and eugenol derivatives can be used as repellents because they can bind to odorant binding proteins or be used as pesticides, inhibiting insect acetylcholinesterase. As observed in Figure 2, they are very different targets, both in size and in function.

Figure 2. Docking-predicted binding mode of EU3e to OBPs (a) and docking-predicted binding modes of EU3e to Acetylcholinesterase (b) with PLP scoring function.

Odorant binding proteins (OBPs) are a large family of insect proteins that are crucial for species survival and reproduction, as they use pheromones, plant volatiles, and other odorant molecules to mate, find food, and avoid predators [32]. OBPs are present in a variety of organisms, are highly expressed and highly divergent in sequence. They do, however, present a few common features, such as their small size and the presence of six conserved cysteines [33]. These features also make them good targets for rapid screenings. There is not enough consensus regarding the specificity of these proteins and further studies must be performed to better understand the sensitivity of OBPs [2].

Acetylcholinesterase (AChE) is one of the most common targets of synthetic pesticides, such as organophosphates and carbamate [34], and has been a target of reference for over 50 years. This enzyme is a serine hydrolase and is responsible for regulating the levels of acetylcholine in a variety of organisms, from mammals to insects [35]. Due to its extensive “attack”, some pests have become resistant to organophosphates, and the search for new and effective alternatives is currently being promoted [36].

Interestingly, during a search in the Protein Data Bank for eugenol, a structure of an odorant binding protein was found complexed with eugenol found. It is an OBP of Apis mellifera (PDB: 3S0E) that exhibits high affinity for eugenol [37]. This reinforces the proposed theory that eugenol and derivatives can, in fact, bind to OBPs and could potentially work as repellents. Still, additional computational and experimental studies need to be performed to further optimize and develop this hypothesis.

4. Conclusions

In the present study, we report the application of an integrated molecular modeling—an inverted virtual screening protocol of a collection of eugenol derivatives in order to find possible protein targets in which they present insecticidal activity.

First, we explored the literature for other virtual screening studies performed on known targets to minimize the candidate pool. Of 18 studies found, 14 targets were selected to continue the study. After careful optimization of the VS protocol, the eugenol derivatives were docked into each target with six different scoring functions (PLP, ASP, ChemScore, GoldScore, Vina and LeDock). The consistency of the scores was evaluated and a ranked list of most likely targets was created.
Eugenol derivatives showed an increased binding affinity for odorant binding proteins and acetylcholinesterases. Since there is, already in the PDB database, a structure of an OBP bound to eugenol not considered in the VS, it reinforces the proposal that eugenol derivatives can potentially be used as repellents.

This work presents a simple approach for the application of inverted virtual screening in identification of possible targets for new insecticides.

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