Virtual screening of potential biofungicide candidate for sustainable fungal disease control

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Abstract: Captafol is widely used as a fungicide compound. However, it is banned in Thailand due to its carcinogen risk level. This study aimed to search for a potential biofungicide candidate via a computational approach to compensate the using of chemical fungicide. Based on the goal, natural compounds showing structures and properties similar to those of captafol were retrieved from various databases. The fungicide-likeness properties were screened. The binding pocket of chitin synthase I was identified, after which virtual screening was performed by AutoDock Vina, and interaction patterns were analysed by Discovery Studio. Finally, kaurane-16,18-diol 18-acetate (NPC132839) extracted from plants was selected as a potential biofungicide candidate with -7.0 kcal/mol of binding energy. The outcomes of this study could be utilised as a highly useful resource to increase the successful exposure of bioactive compounds of plant extracts without damaging the environment.

1. Introduction

Commonly, chemical fungicides are applied to inhibit the progression of fungal disease. Among them, captafol (C$_{10}$H$_9$Cl$_4$NO$_2$S) is widely used as a surface protectant for many crops for over 50 years [1]. It is a broad-spectrum non-systemic fungicide, and it belongs to the phthalimide class [1-2]. Unfortunately, captafol is an extremely hazardous substance, as categorised by WHO [1]. It is considered to be probably carcinogenic to humans (group 2a) [2]. Captafol has been banned in Thailand since 1987 based on the risk level of its carcinogenic effect [3]. However, in vitro tests show that captafol is very effective in inhibition to mycelia and zoospores and hugely decreases the creation of sporangia at low quantity [4]. As the aforementioned, the author believes that captafol could be used as a scaffold compound to find a potential natural product for controlling the fungal disease. Regarding funding, infrastructure, and time savings during the discovery process, virtual screening is a potential in silico method to obtain the bioactive compound [5]. It consists of two steps, including molecular docking and the analysis of the enzyme-inhibitor complex [6]. Many successful studies have used this technique [5]. This confirms that virtual screening is credible for biofungicide discovery.

Based on the aforementioned, the objectives of this study were (1) to search for natural molecules having structural and property similarities to captafol from natural product databases, (2) to filter the fungicide-likeness properties via FungiPad, (3) to identify and select the binding pocket using PrankWeb and AutoDock Vina, respectively, and (4) to evaluate the potential of a biofungicide candidate using AutoDock Vina to compute the binding energy and Discovery Studio to analyse the binding interaction pattern.

2. Methodology

2.1. Hardware

All computational studies were performed on a laptop with the following specifications: Intel (R) Core(TM) i7-10750H CPU at 2.60 GHz of processing speed.

2.2. Ligand and receptor retrieval

The 2D chemical structure of captafol was obtained from PubChem in the SMILE file [7], acting as a query compound. Compounds were retrieved from database of SuperNatural_3 based on the 2D structure and properties search [8], database of NPASS based on property search [9], and database of Kyoto Encyclopedia of Genes and Genomes [10] by structure 2D similarity search via SIMCOMP web tool [11]. The property criteria included 348-350 g/mol of molecular weight, 0-4 hydrogen bond donors, acceptors, and rotatable bonds, and 15-20 heavy atoms. The compounds were kept in the SMILE format. The 3D structure of chitin synthase I with PDB_ID: 7WJM [12] was downloaded from the RSCB protein database [13] in a pdb file as a receptor.

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2.3. Fungicide-likeness property filtration

All candidates were changed to a sdf type via OpenBabel GUI 3.1.1 [14]. The fungicide-likeness properties were analysed using the FungiPAD web tool [15]. The criteria for fungicide properties were fungicide-likeness, relative drug likelihood, and Gaussian score function, acting as QEF, RDL, and GAU, respectively. Only candidates passing the fungicide criteria were selected as biofungicide candidates.

2.4. Binding pocket identification and selection

The receptor structure was repaired by Discovery Studio [16]. The binding pockets were identified by PrankWeb (https://prankweb.cz/) [17]. To choose the best binding pocket, captafol was docked into each binding pocket using AutoDock Vina [18]. The captafol structure was changed from the SMILE format to a pdb format via Discovery Studio. The captafol, receptor, and grid parameters were prepared by AutoDock Tool [19]. The pdbqt file for ligand was generated by adding the polar hydrogen and the Gasteiger charges. The pdbqt files of each binding pocket were prepared by adding the polar hydrogen and Kollman charge. The config file needed to be generated. The grid centre was set on each binding pocket, and the grid size covered all residues in each pocket. Receptor-ligand interaction on a 2D diagram was analysed by Discovery Studio. The perfect pocket was chosen based on the lowest energy of binding and interaction pattern, which are similar to those of captafol.

2.5. Virtual screening

Biofungicide candidates were loaded into the best binding pocket by AutoDock Vina. The pdbqt files for biofungicide candidates were prepared utilizing captafol preparation. The pdbqt file of the selected binding pocket was used. The grid centre of the selected binding pocket was 33.559, 33.731, 8.924 Å, the grid size was 60 x 60 x 60 Å, and 20 docking runs. The effectiveness value was 16. The other parameters were set to default. The potential biofungicide candidate was selected based on having binding energy and interaction patterns that were similar to those of captafol.

3. Results and Discussion

3.1. Biofungicide candidate compounds

From the SuperNatural_3 database, 30 compounds were obtained via similarity structure search, and 22 compounds were obtained via property search. From the NPASS and KEGG databases, 11 and 5 natural compounds were obtained, respectively. Based on the similarity search, Tanimoto coefficient ($\tau$) is calculated as shown in equation (1).

$$\tau = \frac{A \cap B}{A \cup B}$$

where each letter represents the number of ON bits: $A$ shared by compound 1 and 2, $B$ found in compound 1, and $C$ found in compound 2 [20].

The values for fungicide criteria are higher than 0.28 (QEF), 1.21 (RDL), and 4.62 (GAU). This means that compounds with high scores have a higher chance of becoming fungicides [15]. A total of 29 compounds were obtained as biofungicide candidates due to meet those fungicide criteria.

3.2. Binding pocket identification and selection

A total of 15 binding pockets were identified. The pocket scores were in the range of 0.73 to 24.90. This score is computed by Jensen-Shannon divergence method from a multiple sequence alignment [17]. The lowest free binding energies for captafol in each binding pocket were in the range of -2.2 to -5.8 kcal/mol. Four binding pockets, namely R2, R3, R4, and R5, were selected based on their similar binding energy (-5.8 to -5.6 kcal/mol). The interaction patterns for captafol in each of the selected binding pockets are shown in figure 1. Only the R4 pocket (Figure 1C) was selected based on triple hydrogen bonds with His514 and His394, whereas the remaining pockets were discarded due to the disappearance of hydrogen bonds. The interactions between enzyme and inhibitor depend critically on hydrogen bonds, as a facilitator of binding [21]. Furthermore, it is not expected for a single hydrogen bond to support a complex because it is weak. Whereas multiple hydrogen bonds have significant stability for binding [22]. Importantly, the thiol group of captafol forms pi-sulfur with His514 residue. This interaction involves charge transfer, which aids in the drug's intercalation at the receptor's binding site [23].

3.3. Potential biofungicide candidate

A total of 29 biofungicide candidates were docked into the R4 pocket. The binding energy of captafol was -5.7 kcal/mol, acting as the reference value. A total of 22 candidates were chosen based on their binding score, which was better than that of captafol. Their binding affinity values was -5.7 to -7.6 kcal/mol. The best biofungicide candidates, including C00050068 and NPC132839, were selected for binding interaction analysis. The interaction patterns are revealed in figure 2. Although both compounds form hydrogen bonds with Asn402 and Tyr849, an aromatic ring of NPC132839 also forms an alkyl interaction with Ile850 (Figure 2B), similar to captafol (Figure 1C).
C00050068 is found in sponges, which is difficult to study [10-11]. Meanwhile, NPC132839 is found in plants (i.e. Anthoxanthum nitens, Sabal causiarum and Rubia schumanniana), metazoans (Cereus dumortieri), and fungi (Plenodomus tracheiphilus) [9]. Thus, the potential biofungicide candidate was NPC132839 due to the similarity of captafol properties and the possibility of application. Plant extracts are the focus of this study for a variety of reasons, including the following: 1) a wide variety of natural substances possessing antifungal properties are produced by plants, 2) plant extracts are non-toxic and have the potential to be efficacious in controlling fungal diseases, 3) plant extracts can inhibit the progression of pathogen resistance, and 4) numerous reports have demonstrated the antifungal activity of plant extracts [24]. NPC132839 is kaurane-16,18-diol-18-acetate as shown in figure 3. Its molecular formula is C_{22}H_{36}O_{3} and it has 348.27 g/mol of molecular weight. It has physicochemical properties as follows: 3 rotatable bonds and hydrogen bond acceptors, as well as a hydrogen bond donor. It is classified as a kaurane diterpenoid [9]. Kaurane diterpenoids have been recognized for antimicrobial agents including antibacterial activity (inhibits Staphylococcus aureus ssp. aureus with a minimum inhibitory concentration 50 value of 19.35 μg/ml), antifungal activity (inhibits some plant-causing fungi), and antiprotozoal activity (inhibits Trypanosoma cruzi) [25].
However, the mechanism of NPC132839 was expected to inhibit chitin synthase I (EC 2.4.1.16), which is a promising target [12]. Inhibition of chitin synthase I leads to (1) a decrease in cuticular chitin content at the cell and tissue level, (2) an increase in premature molting, and (3) an increase in fungi mortality, respectively [26]. For further experiment, the chitin synthase inhibition assay and antifungal assay should be performed according to the method of Shi and his college [27]. The inhibition rate will be calculated as shown in equation (2).

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\text{Growth inhibition (\%)} = \frac{(A-B)}{(A-C)} \times 100
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where each letter represents the average of colony diameter: A belonging to control, B belonging to the compounds, and C belonging to the inoculum plug (millimeters) [27].

4. Conclusion

In this study, a natural compound NPC132839 with potential fungicidal effect was successfully screened by computational biology method. This provides a strong candidate for the development of new, environmentally friendly biocides. In the future, the author will further study the fungicidal effect and mechanism of NPC138239 in order to provide scientific basis for its promotion in practical applications.

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References


