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Virtual screening of natural meroterpenoids towards SARS-CoV-2 main protease

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Abstract – The term meroterpenoid states hybrid secondary metabolites which are partially derived from terpenoids and synthesized in terrestrial plants, marine animals, and fungi. So far, myriads of meroterpenoids with unique chemical structures and promising bioactivities were documented. Virtual screening is a widely used, relatively fast, and economical simulation tool for predicting *in silico* the binding modes and affinities of molecular recognition processes. In the present study, we tested on SARS-CoV-2 main protease inhibitory activity of some selected meroterpenoids [(stachybotrylactone (**1**), stachybotrylactone acetate (**2**), and 2α-stachybotrylactone (**3**)] by distinct algorithms which has been one of the most basic and important strategies for drug design. Our findings showed that stachybotrylactone acetate (**2**) had a significant free binding of energy towards the viral main protease at the value of -6.34 kcal/mol.

Keywords – Meroterpenoids, Main Protease, Molecular Docking, Virtual Screening, Stachybotrylactone(S)

I. INTRODUCTION

Meroterpenoids are naturally occurring products of mixed biosynthetic origin in which the Greek prefix *mero-* states 'part, fragment'. The expression 'meroterpenoid' was initially used by J.W. Cornforth, in 1968, to define natural occurring compounds of mixed biochemical origin which partially originated from terpenoid pathways [1]. They are hybrid secondary metabolites which are commonly produced different sources such as the animals, plants, bacteria, and fungi [2, 3]. Among them, fungi are one of the most significant producers, but others can metabolize different kinds of the meroterpenoid compounds. Fungal-derived meroterpenoids are widely distributed in marine organisms with diverse molecular scaffolds which are assembled by terpene units with other precursors including polyketide moiety by several biosynthetic pathways (Figure 1).

Even though many secondary metabolites can be described as meroterpenoids, they can be categorized into two main classes depend on their biosynthetic origins as polyketide-terpenoids and non-polyketide-terpenoids [2]. Polyketides are synthesized by natural sources through condensation of carboxylic acids which are catalyzed multifunctional enzyme complexes, termed as polyketide synthases [2].

Several secondary metabolites can be classified as meroterpenoids such as phenylpropanoid compounds containing isoprenoid side chains and cytokinins. Additionally, vinca alkaloids such as anticancer drugs vincristine and vinblastine are also classified as merotepenoidal compounds [2].

Of note, some of them display distinct pharmacological properties, such as mitochondrial respiratory chain inhibitory (terretonins E and F) [4], BACE1 inhibitory (asperterpenes A and B) [5], anti-inflammation [6], lipid-lowering effect [7].

Stachybotrylactone (**1**), stachybotrylactone acetate (**2**), and 2α-stachybotrylactone (**3**) were firstly isolated from aspen fungus *Stachybotrys cylindrospora* [8-9].

Chemical structures of meroterpenoids tested are schematically shown in Figure 2.

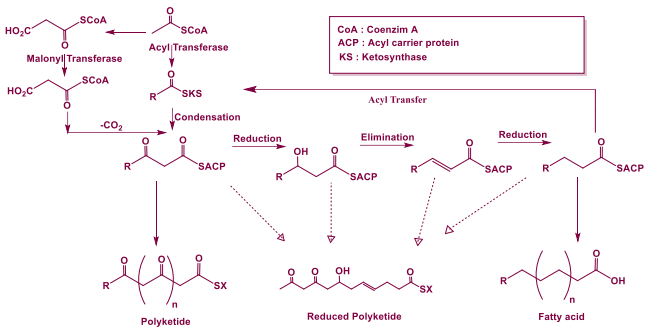
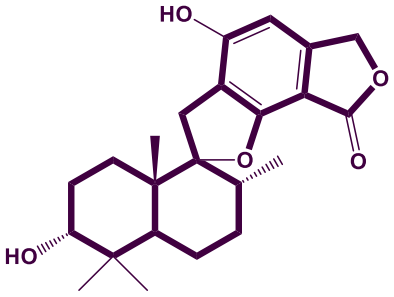
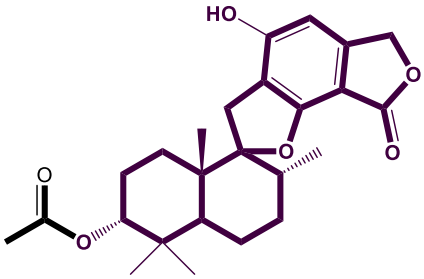


Fig. 1 The assembly of fatty acids, polyketides, and reduced polyketides [2]

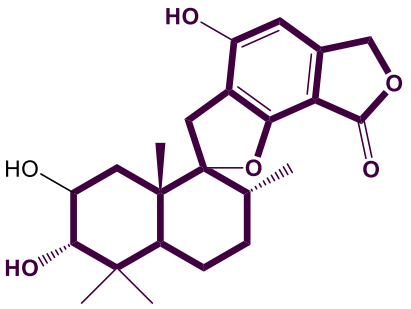
Interestingly, stachybotrylactones **1**, **2**, **3** which have mixed polyketide-terpenoid structures which are not common in microbial metabolites. In a recent study, selected meroterpenoids were reported to inhibit the PAK1 proteins by molecular docking methods [8].



Stachybotrylactone (1)



Stachybotrylactone acetate (2)



2α–hydroxystachybotrylactone (3)

Figure 2. Chemical structures stachybotrylactone (1), stachybotrylactone acetate (2) and 2α-stachybotrylactone (3) with diverse molecular architectures

Molecular docking is a technique which predicts the preferred orientation of one molecule to a second when a ligand and a target are bound to form a stable complex [10]. Knowledge of the preferred orientation in turn might be applied to predict the strength of association or binding affinity between two molecules. Molecular docking simulation is one of the most often used methods in structure-based drug discover/design, due to its ability to predict the binding-conformation of ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes [11].

However, protein–ligand docking has been an active area of research for more than 25 years, causing a wide variety of available docking well-established softwares such as AutoDock.

This proceeding deals with the molecular docking analysis of stachybotrylactone (1), stachybotrylactone acetate (2) and 2α-stachybotrylactone (3) as meroterpenoid compounds with the SARS-CoV-2 main protease.

II. Materials and Method

A. *Ligand selection*

As ligand molecules, the natural meroterpenoids stachybotrylactone, stachybotrylactone acetate, and 2α-stachybotrylactone were selected.

The 2D chemical structures of ligand molecules were drawn using the ChemDraw Professional 16.0.1 (PerkinElmer). The illustrated structures were saved in the file format of '.mol2', and then converted to the .pdb file format (3D) using the

Open Babel GUI: The Open Source Chemistry Toolbox.

B. *Targeted protein selection*

The crystal structure of the targeted protein of SARS-CoV-2 main protease (PDB ID: 6LU7) [13] was downloaded from RCSB protein data bank. The data file was saved in the file format of .pdb.

The protein of main protease was prepared with the protein preparation wizard in Schrodinger suite. All organic molecules or inorganic residues were removed from structures and then loop segments were completed.

C. *Molecular docking*

To determine the molecular interaction of represented compounds with the main protease at the active site, *in silico* molecular docking method was applied.

In silico simulations were performed using AutoDock 4.2, an automated docking tool [14]. In this technique, both Gasteiger partial charges and polar hydrogen atoms were incorporated into the three-dimensional structure of the targeted enzymatic protein. All protein structures were initially converted into the file format of '.pdbqt' for further analysis. On the other hand, the grid size for simulation of SARS-CoV-2 main protease and ligands **1**, **2**, and **3** were set at 60Å×60Å×60 Å force field, followed by 0.375 Å spacing centred and grid centres x (-26.283), y (12.599), and z (58.965). Receptor grids were produced before docking with active site determined by the position of co-crystal ligand.

Additionally, the Lamarckian Genetic Algorithm 4.2 was applied in the docking analysis [15], while the protein macromolecules were kept rigid throughout the docking simulation. The genetic algorithm runs were set at 400, while default settings were maintained for the other parameters for docking simulations. The best protein-ligand conformations were selected from the AutoDock 4.2 scoring function which ranked the results according to their estimated free binding of energy (kcal/mol).

Free energy of binding was calculated as follow:

Final Intermolar Energy *1*
Final Total Internal Energy *2*
Torsional Free Energy *3*
Unbound System's Energy *4*
Estimated Free Energy of Binding = [(1)+(2)+(3)-(4)]

Moreover, the inhibition constant (K_i value) (μM) which is an indication of how potent an inhibitor for each ligand were recorded. Obtained docking results were also analysed using the Discovery Studio Visualizer 4.1 client. Our findings are summarized in Table 1.

III. RESULTS

Although the effective vaccine and drug treatment progress against the virus, Covid-19 disease is a still major health problem and with the latest deaths reported to WHO now exceeding 3.3 million, based on the excess mortality calculates produced for 2020, peoples are probably facing an important undercount of total deaths directly and/or indirectly attributed to Covid-19 [15]. The promising treatment strategies are still needed for the knockout treatment of Covid-19 and

Table 1. Interactions between ligands and targeted protein

Protein	Ligands	Estimated free binding of energy (kcal.mol^{-1})	Inhibition constant (K_i) (μM)
6LU7	**1**	-5.49	94.73
	2	-6.34	22.45
	3	-5.26	138.42

As it can be easily understood in the Table 1, the inhibition constant values are directly proportional the binding energy data.

We found that ligand 2 had the highest binding energy towards targeted protein with a reported value of -6.34 kcal/mol. In addition, 2 had an inhibition constant value of 22.45 μM. The lowest binding affinity was detected between ligand 3 and targeted protein with the value of -5.26 kcal/mol and an inhibition constant of 138.42 μM.

Although the chemical structures of 1 and 2 are extremely similar, the bond between C-1 and C-2 has significantly changed because of being single/double.

IV. DISCUSSION

Molecular docking is a widely used, relatively fast, and economical computational tool for predicting in silico the binding modes and affinities of molecular recognition events [16].

To the best of our knowledge, there is no scientific studies on theoretical and experimental trials linked to the inhibition of SARS-CoV-2 main protease enzyme by meroterpenoids. Herein,

stachybotrylactone (**1**), stachybotrylactone acetate (**2**) and 2α-stachybotrylactone (**3**) are hybrid natural products which have polyketide-terpenoid origin were selected as ligand molecules. Meroterpenoids demonstrated distinct binding grades of privileged interactions with targeted protein. Table 1 displays the inhibition constant values and estimated free binding of energy (kcal.mol^{-1}). On the other hand, the low free binding of energy recorded by us between the interaction shows that the pairing has high binding affinity and strong interaction [17].

V. CONCLUSION

Virtual screening techniques are widely used, relatively faster, and economical simulation algorithms for predicting *in silico* the binding approaches and affinities of molecular recognition events. Recent years, development and application of the theoretical/computational techniques are significant and indispensable because they might be relatively less laborious and more economic than experimental techniques and they could facilitate the commentary of the existing practical throughputs and direct the design of novel models. Although the protein-ligand docking is less accurate in calculating the free energy of binding than mathematical calculation approaches, it can forecast correct bound conformations and interactions and thus, are especially appropriate for practical high-throughput virtual drug screening [16].

This proceeding concludes that meroterpenoids 1, 2, and 3 revealed to have relatively strong binding affinities to viral main protease. However, further studies would be needed to compare the various protein structures using different software and experimental models. Furthermore, the selected meroterpenoids should be evaluated further using *in vitro* and *in vivo* studies as well.

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