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ORIGINAL RESEARCH

IN SILICO STUDY OF ELASTASE ENZYME WITH NAPHTHOQUINONE DERIVATIVES AS LIGAND

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Abstract. We simulated 17 molecules classified as Naphthoquinones derivatives with the Enzyme Elastase to observe data regarding energies produced after bonding. These 17 Molecules were Eleutherin, Isoeleutherin, Elacanacin, Eleutherinone, Eleutherol A, Eleutherol B, Eleutherol C, Eleuthinones B, Eleuthinones C, Eleutherine A, Eleutherine B, Eleutherine C, Eleutherine D, Eleutherine E, Eleutherine F, Eleutherine G, Eleucanainones A. To prepare the ligand and protein for docking, we used the Discovery Studio application. For the molecular docking itself, we used the Pyrx application. Regarding interpreting the result, first, we chose the lowest rmsd/ub or rmsd/lb, and then we analyzed the energy result in which the lowest rmsd occurred. The docking results data indicated that all the ligand-enzyme bonding had negative binding affinity energy, but Eleucanainones A produced the lowest energy (Binding Affinity -7.7, mode 1, Rmsd/ub 1.787, Rmsd/lb 3.54), meaning it bound most easily with the enzyme Elastase. This study was only an initial or foundational step and further studies were highly needed for the development of the correlation between the ligands and the enzyme mentioned above.

Keywords: Elastase, Molecular Docking, Naphthoquinone Derivatives.

1. INTRODUCTION

Elastase belongs to the chymotrypsin family of proteases, and it is responsible for the breakdown of elastin and other proteins, such as collagen and fibronectin, which are fundamental for the ECM elastic properties (Imokawa & Ishida, 2015). Misregulations of this enzyme are involved in skin aging processes. The excessive hydrolysis of the dermal elastin fiber network leads to the loss of skin elasticity and consequent skin sagging (Thring et al., 2009). Therefore, the inhibition of elastase enzyme is highly needed in skin rejuvenation. As mentioned above, an excess of this enzyme can lead to premature aging, so it can be simply concluded that inhibiting this enzyme will help rejuvenate the skin.

However, besides the excess of elastase enzyme, other factors contribute to the loss of skin elasticity, both internal and external. Inhibiting elastase enzyme is part of internal treatment, and it is important to note that internal factors involve more than just the excess of this

enzyme; there are several other factors to consider. Additionally, external factors such as photoaging and others play a role as well. Repetitive UVB irradiation elicits a marked alteration in the three-dimensional structure of elastic fibers, which is closely associated with a subsequent reduction in the elastic properties of the skin. UVB irradiation stimulates the activity of fibroblast elastases in the dermis (Imokawa, 2009). Figure 1 below shows the crystal structure that makes up the compound known as Elastase – 1b0f.

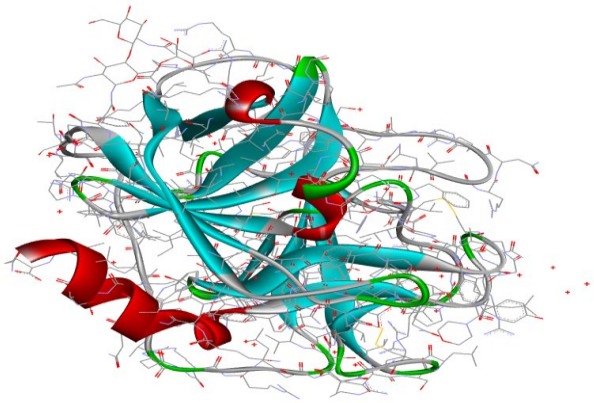


Figure 1: Crystal Structure of Elastase – 1b0f (Protein Data Bank)

Elastase enzyme represents many aspects of the human body; an excess or deficiency of this enzyme is an indication of an imbalance occurring in the human body. As mentioned below, several studies have linked this enzyme to various diseases, such as: Sputum neutrophil elastase activity is a biomarker of disease severity and future risk in adults with bronchiectasis (Chalmers et al., 2017), A growing body of evidence indicates that neutrophil elastase (NE) is involved in the pathogenesis of respiratory infectious diseases, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Karampoor et al., 2021), The HNE-TACE signalling pathway has an important role in the process of MUC5AC overexpression in chronic rhinosinusitis (CRS).

Eleutherin is a natural compound. The major presence of eleutherin is found in the Eleutherine bulbosa plant (Silva et al., 2024). This compound possesses intriguing biological activities and has garnered attention in scientific research. Eleutherin has been studied for its potential pharmacological activities, including its anticancer and antioxidant properties. However, it is important to note that further research may be necessary to fully understand the potential and usage of this compound in specific medical treatments or applications. Also If we talk about Eleutherine, one plant called Eleutherine americana Merr a plant from Borneo forest that is commonly used by the local tribe as an herb or a traditional medicine that can cure various diseases. The bulbus of this plant which came from Eleutherine genus have been scientifically proven by several studies to contain the secondary metabolite compound in the type of naftokuinon (elecanacin, eleutherin, elutherol, eleutherinon) (The Effect of Bulb Extract from Dayak Onion (Eleutherine Americana Merr) to the Cholesterol Level and the Trigliresida Level in Mice (Mus

Musculus), 2018). From the Elutherin Americana bulb, Eleutherine derivatives, such as Eleutherin A, Eleutherine B, Eleutherine C, Eleutherin D, Eleutherine F, Eleutherine G also found form the extraction (Chen et al., 2019). A novel new naphtoquinone called elacanacin were isolated from a bulb of eleutherine Americana (NII-Electronic Library Service, n.d.). also this elacanacin has the potential for HIV Virus Inhibitor. Also Two naphthoquinone-derived heterodimers with unprecedented carbon skeletons, eleucanainones A (1) and B (2),were isolated from the bulbs of Eleutherine americana. Their structures were elucidated by comprehensive spectroscopic methods.The structures of 1 and 2 were determined to be the first examples of dibenzofuran- and naphthalenone-containing naphthoquinone dimers. Compound1exhibited significant anti-MRSA activity in vitro with minimum inhibitory concentration (MIC) values of 0.78μg/mL by downregulation of basal expression ofagrA,cidA,icaA andsarA in methicillin-resistantS. aureus(MRSA) (Chen et al., 2020a) Isoeleutherin can be found in the extraction of several types of plants, one of which is by extracting from Eleutherine plicata (Albuquerque et al., 2023). Molecular docking studies showed that the compounds have good complementarity in the active site with important hydrogens bonds. Eleutherine plicata Herb. (Iridaceae) is a native American plant, with reports of oc.currence in other tropical countries and regions such as Southern Africa, the eastern Mediterranean, and Central and South America. In Brazil, it is popularly known as maru-pari, marupazinho, marupá-piranga, palmeirinha, lírio-folha-de-palmeira and wáro. It presents itself in the form of a clump with red bulbs similar to onions, features whole, simple and pleated leaves with colorful flowers from white to pink. This species is widely used in Brazilian popular herbal medicine (Prameela et al., 2018)

2. METHODOLOGY

Preparation and Method

The preparation and method section serves as a crucial foundation for our study, elucidating the methodology employed for enzyme preparation and the synthesis of naphthoquinone derivatives with the Avogadro and Discovery Studio application. Moreover, this section provides a detailed insight into the experimental procedures, facilitating a comprehensive understanding of the ligand-enzyme interaction and the potential therapeutic applications of these compounds.

Preparation of Constituents

First, we prepared the protein by downloading it from the Protein Data Bank with the protein code 1b0f. After it was downloaded, we prepared the elastase protein using the Discovery Studio Visualizer application. The preparation involved removing heteroatoms from the protein and adding polar hydrogens. This leads to the protein being ready for the docking process.

For the ligand (in this case 17 Derivatives of naphthoquinone), we designed it using the Avogadro application, because only the 2D structure of this compound is available based on the literature. However, for the molecular docking,

we are required to obtain a 3D structure. Therefore, to acquire 3D results, we redesigned these ligands. After the molecular redesigning, we optimized the energy with UFF-Force Field and Steps Per Update = 4.

Methodology

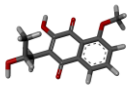
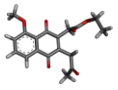
After the preparation of the elastase protein was completed, the protein was uploaded to the Pyrx application, which is further transferred to the AutoDock, and converted into a macro-molecular structure. Subsequently, the ligands that had been prepared were also uploaded to Pyrx through the Open Babel menu, followed by minimization. The next step involved converting them into AutoDock ligands. In the Vina Wizard menu, we run the protein docking with all the molecules at once. One of the advantages of the Pyrx application is that we can run all the ligands to the protein simultaneously, without the need to repeat the process one by one.

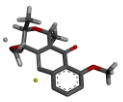
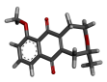
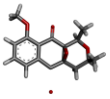
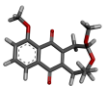
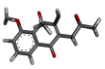
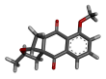
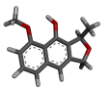
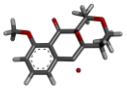
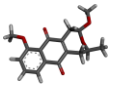
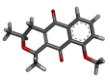
3. RESULTS AND DISCUSSION

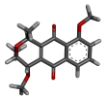
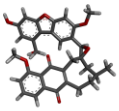
After successfully running the docking between Elastase and the 17 naphthoquinone derivatives, the results are obtained as shown in the following Table 1.0. From **Table 1** above, we can see that out of the 17 molecules docked to the elastase protein, eleucanainones A exhibits the lowest binding affinity energy. In other words, the protein binds most easily to this molecule following the laws of thermodynamics.

Furthermore, a low RMSD value indicates a closer resemblance of the simulation to the original value. Other compounds also exhibit negative binding affinity, which implies a potential for binding to the protein. This could serve as an alternative for further studies involving these ligands on the elastase protein. In the next steps, we will focus solely on eleucaniones A due to its lowest binding affinity. Our next objective is to visualize the position of the ligand and the protein, as shown in **Figure 2** below.

Table 1. Experimental Results based on the Methodology.

No.	Molecules	Binding Affinity	Mode	Rmsd/ub	Rmsd/lb	Structure
1	Eleuthinones c	-5.1	5	2.883	5.237	
2	Eleuthinones B	-5.5	3	1.268	4.426	
3	Eleutherinone B	-5.4	2	1.092	3.212	

4	Eleutherol B	-5.3	2	1.05	3.613	
5	Eleutherine C	-5.1	7	1.634	3.878	
6	Eleutherine G	5.2	8	7.768	9.632	
7	Eleutherol C	-5.5	5	1.378	3.867	
8	Eleutherine A	-5.6	4	3.596	7.638	
9	Eleutherine E	-5.6	5	2.024	3.566	
10	Eleutherine	-5.6	3	3.094	5.433	
11	Elacanacin	-5.8	1	1.956	4.142	
12	Eleutherol A	-5.1	6	2.2	5.626	
13	Eleutherine B	-5.7	1	4.237	6.247	
14	Eleutherine F	-5.7	4	1.062	2.108	
15	Isoeleutherine	-6.2	1	1.259	5.488	

16	Eleutherine D	-5.8	1	1.593	6.431	
17	Eleucanainones A	-7.7	1	1.787	3.54	

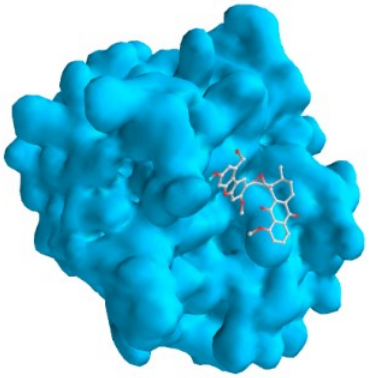


Figure 2.0:

4. CONCLUSION

We have confined this study to only the 17 molecules mentioned above, even though the number of molecules that can be docked to the elastase protein is infinite. Further studies are greatly needed to understand the effects that occur upon the binding of these ligands to the protein. This study is expected to serve as an initial step in learning about elastase enzyme inhibitors, which can be applied in various industrial sectors as per human need, such as the cosmetic industry. As we know, one of the causes of skin elasticity loss is attributed to this elastase enzyme. Therefore, if its activity can be inhibited, it may help to delay premature aging, where skin elasticity is a key indicator. Additionally, some diseases requiring elastase enzyme inhibition can benefit from this preliminary study. These ligands mentioned above can be sourced from various places, for example Eleutherin Americana and E. Plicata . The isolation of a mass of bioactive anthraquinones, naphthoquinones, and naphthanes from the bulbs of E. Americana can be used to obtain the ligands (Chen et al., 2020b). Several studies have been conducted on E. Americana, leading to the discovery of these molecules. And we also observe how eleucaniones A becomes the most negative ligand among the 17 other ligands, in other words, eleucaniones a have the highest potential to be an inhibitor against elastase enzyme among the other ligands.

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