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Molecular docking analysis of natural unusual triterpenoids towards PAK1 protein

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Abstract **–**Triterpenoids are secondary metabolites produced by different natural sources and present in natural sources in the form of free acid or aglycones for triterpenoid saponins in nature. Stellettin B, rhabdaprovidine B, and neviotine A are unusual triterpenoids with their unique skeletal backbone. The serine/threonine p21-activating PAK kinases which play an important pivotal role in intracellular signaling pathways as mediator and join the biochemical process in a cell, such as cytoskeleton dynamics, in cell adhesion, polarity, motility, migration, proliferation, apoptosis, cell division, and in vesicle-mediated transport processes. In this study, molecular docking technique was used for detecting free binding of energy and inhibition constant values of selected triterpenoids towards PAK1 protein. Results showed that that ligand 1 (stellettin B) significantly inhibited the PAK1 enzyme at the value of -8.69 kcal/mol.

Keywords – Meroterpenoids, Main Protease, Molecular Docking, Virtual Screening, Terreulactone

I. INTRODUCTION

Triterpenoids are distinguished by their essential structural variety and represent a wide-spread class of complex metabolites with carbon skeleton based on six isoprene moieties produced in many organisms by rearrangement of the 30-C intermediate squalene epoxide. They present in natural sources in the form of free acid or aglycones for triterpenoid saponins [1-2]. The primary source of structural diversity based on skeleton formation, catalyzed by biosynthetic enzymes through distinct chemistries, such as aldol and Claisen condensations in order to produce C-C bonds, alkenes undergo electrophilic carbocation addition, and so on [3].

Many triterpenoids are produced by terrestrial plants, marine flora and fauna, and microbes to optimize nature. The maximum triterpenoids are 6-6-6-5 tetracyclic, 6-6-6-6-5 pentacyclic, or 6-6-6-6-6 pentacyclic; however, unusual hexacyclic, butacyclic, tricyclic, bicyclic, and monocyclic triterpenoids have also been isolated from natural resources [4].

Of note, many unusual triterpenoids exhibit fascinating biological properties, such as stimulating apoptotic cell death [5], NO production in LPS stimulated BV2 cells [6], and anti-angiogenic [7].

Stellettin B (1) was reported to induce apoptotic cell death by inhibition of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway in human cancers, as shown in glioblastoma cancer SF295 cells, human non-small-cell lung cancer cells, and human chronic myeloid leukemia cells indicated anticancer action [5].

Moreover, stellettin B (1) also affects filamentous actin rearrangement by reducing phosphor-Girdin cross-linkage, blocking glioblastoma cell invasion, and the synthesis and vascular endothelial growth factor (VEGF), a critical proangiogenic factor. Additionally, 1 inhibits human umbilical vein endothelial cells from growing angiogenic tubules [7].

Rhabdastrella sponges are rich in source of rhabdaprovidines A-K and particularly

rhabdaprovidine B (2) has been reported to reduce nitric oxide generation in LPS induced BV2 cells with IC50 value of 17.5 μM (dung et al 2018) [6].

Neviotine A (3), a new new sipholane type triterpenoid, has recently been isolated from the pipe sponge Siphonochalina siphonella (Haliconidea) However, neviotine A was reported to inhibit receptor activator of nuclear factor- κB ligand (RANKL)-induced osteoclastogenesis with IC50 value of 32.8 μM, suggesting Thus, neviotine A could be introduced as a new chemical scaffold for the prospective development of new treatments of osteoclast associated malfunctions including osteoporosis and bone metastasis [8].

Stellettin B (1), rhabdaprovidine B (2), and neviotine A (3) are unusual triterpenoids tested and their unique chemical structures are shown in Figure 1.

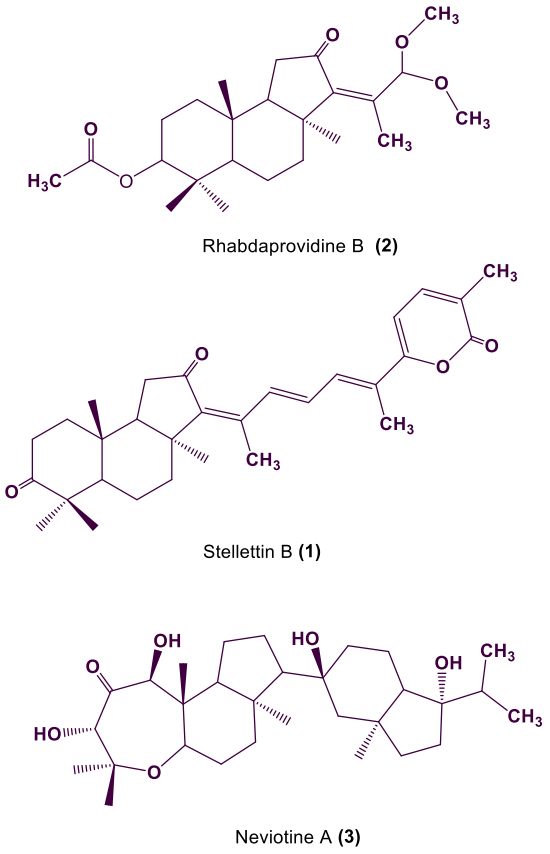

Figure 1. Chemical structures of stellettin B (1), rhabdaprovidine B (2), and neviotine A (3) with diverse molecular architectures

The serine/threonine p21-activating PAK kinases which play an important pivotal role in intracellular signaling pathways as mediator and join the biochemical process in a cell, such as cytoskeleton dynamics, in cell adhesion, polarity, motility, migration, proliferation, apoptosis, cell division, and in vesicle-mediated transport processes [9]. Interestingly, PAK1 was reported to be an effector for progressing of RAS cancers that represent approximately 90% of pancreatic cancer, 50% of colon cancer and 30% of lung cancer and Covid-19 for causing the inflammation [10-11]. It is well-documented that the most deaths are due to pulmonary inflammation. In our previous study, we reported that N-triterpene saponins-based PAK1 inhibitors as promising agents for the struggle towards SARS-CoV-2 infections [12]. Doubtlessly, we have to know correctly how covid-19 virus replicates and finally lead to pulmonary fibrosis to fight disease.

We hypothesized that as PAK1 inhibitors ligands tested by activating PTEN, a phosphatase enzyme, which down-regulates PAK1, ligands tested would block pulmonary fibrosis.

Protein-ligand molecular docking is a method which predicts the preferred affinity orientations based on the strength of the possible bond(s) in an attempt to make stable complex between ligand and protein [13]. Additionally, molecular docking simulation is one of the most frequently applied techniques in functional structure-based drug design, because of its capacity to estimate the binding-conformation of ligands to the convenient binding site on protein. Definition of the binding behavior contributes to develop new drugs and to explain basic biochemical pathways as well [14].

Further, protein–ligand docking has been a research platform more than 25 years to predict how drug candidate molecules bind to a protein of known 3D structure. Over the years, molecular docking platforms have been modified and improved to add new functionalities, and multiple search engine facilities. AutoDock is a suite of automated docking tools and its v4.2 version uses Lamarckian genetic algorithm which employs a variation of genetic algorithm in contrast to a deterministic technique. On the other hand, AutoDock has various limitations, for example, it often leads to unreliable results when small molecules are docked into flexible binding sites. Also, it is unable to account for flexibility of cyclic and macrocyclic ligands [14-15].

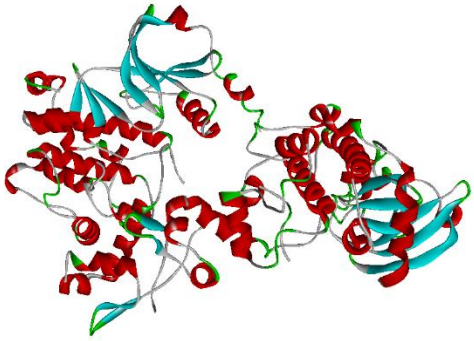

Fig. 2 3D protein structures of human PAK1 (PDB ID: 1F3M) [16]

This proceeding deals with the molecular docking analysis of selected unusual triterpenoids towards human PAK1 protein whose 3D structure is shown in Figure 2 [16].

II. Materials and Method

A. *Ligand selection*

Unusual triterpenoids stellettin B (1), rhabdaprovidine B (2), and neviotine A (3) were selected as ligand molecules by literature survey and considering their molecular size and functionalities. The 2D chemical structures of ligands were drawn using the ChemDraw Professional 16.0.1 (PerkinElmer). Initially, the 2D structures were saved in the file format of '.mol2', and then converted to the .pdb file format (3D) using the The Open Babel Graphical User Interface: The Open Source Chemistry Toolbox.

B. *Targeted protein selection*

The targeted enzymatic protein structure of PAK1 (PDB ID: 1F3M) [16] was downloaded from the Protein Data Bank web page (RCSB PDB). Initially, the data file was saved in the file format of '.pdb' and then the water, inorganic substances and pre-existing ligand molecule were removed from the protein structure through the BIOVIA Discovery Studio Visualizer 4.1 Client software.

C. *Molecular docking*

Molecular docking analyses were performed using AutoDock 4.2.6, an automated docking tool [17]. During the molecular docking, both Gasteiger partial charges and polar hydrogen atoms were incorporated into the three-dimensional structure of the PAK1 protein. All protein structures and selected ligands were converted into the file format of '.pdbqt' for further analysis on tool interface. On the other hand, the grid size for simulation of PAK1 and ligands 1, 2, and 3 were set at 60×60×60 points, followed by 0.375 Å spacing centred and grid centres x (1.739), y (26.410), z (46.186). However, minimum coordinates in grid = (-9.511, 15.160, 34.936) and maximum coordinates in grid = (12.989, 37.660, 57.436) were recorded.

The grid boxes were determined according to the amino acid active sites on PAK1 protein. Additionally, the Lamarckian Genetic Algorithm 4.2 was applied in the docking analysis [18], 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 analyses. 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).

However, the inhibition constant (K_i value) (μM) which is an indication of how potent an inhibitor for each ligand was also recorded. All docking results were analysed using the Discovery Studio Visualizer 4.1 client. Results obtained from molecular docking analyses are given in Table 1.

III. Results

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 which also allow virtual screening of large databases of naturally occurring products to discover drug candidates [19].

To the best of our knowledge, there is no report regarding the inhibitory effects of unusual triterpenoids tested towards PAK1 protein. In this study, the unusual triterpenoids stellettin B (1), rhabdaprovidine B (2), and neviotine A (3) were selected as ligands by considering their chemical structure and functionalities. They have unusual skeletal backbone and unique structure which are significant to bind to targeted protein. Table 1 shows the free binding of energy and inhibition constant values of ligands tested. Results revealed that all of them showed different binding potency towards PAK1 protein. However, ligand 1 had the highest binding energy with the value of -8.69 kcal/mol and

inhibition constant (Ki value) with the value of 0.427 (μM). Ligand 3 was found to be the lowest binding energy with the value of -6.80 kcal/mol and inhibition constant (Ki value) with the value of 10.39 (μM).

Table 1. Interactions between ligands and targeted protein

Protein target	Ligand	Free binding of energy (kcal.mol^{-1})	Inhibition constant (K_i value) (μM
PAK1	1	-8.69	0.427
	2	-6.99	7.56
	3	-6.80	10.39

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

The inhibitory constant (Ki value) represents the equilibrium binding affinity for a ligand which reduces the activity of its binding protein. Ki also represents the concentration at which the inhibitor ligand occupies 50% of the receptor sites when no competing ligand is present. The smaller the Ki the greater the binding affinity and the smaller the amount of ligand is needed to inhibit its binding partners activity. In our study, ligand 1 had the smallest Ki with the value of 0.427 (μM) which means 1 the greatest binding affinity towards PAK1. Herein, both free binding of energy and Ki data displayed that ligand 1 is the best candidate for suppressing the PAK1 protein.

IV. CONCLUSION

In silico molecular docking is a widely used, relatively fast, and partially economical simulation tool for predicting the binding approaches and affinities of molecular recognition events [19].

This proceeding study revealed that ligand **1** and partially **2** and **3** had the significant binding affinity to PAK1 protein. To protect pulmonary inflammation natural products particularly unusual triterpenoids may be a key molecule by activating PTEN and/or inhibiting PAK1 pathway and this approach may be a logical way to prevent lung fibrosis. Today, as throughout history, natural products become a major reservoir of drug candidates and unusual triterpenoids with distinct functionalities are an important class of naturally occurring products.

As a result, ligand 1 should be further studied *in vitro* and *in vivo* models to show its inhibitory potency towards PAK1 protein.

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