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In Silico Screening of Hesperetin and Naringenin Ester Derivatives as Anticancer Against Phosphoinositide 3-Kinase

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Abstract : Objective: Study the in silico Phosphoinositide 3-Kinase (PI 3-K) inhibition activity of hesperetin and naringenin ester derivatives. Acyl group substituent was different in the length of the carbon atom chain (propionyl, butyryl and valeryl).
Methods: Partition coefficient was predicted by the Chem Draw Ultra program. In silico docking using PLANTS program and visualized by Yasara program. The model of three dimension enzyme structures used in this research was Phosphoinositide 3-Kinase (PI 3-K) binding pocket with the Protein Data Bank (PDB) code 4KZC. Two dimension and three dimension conformation models of hesperetin and naringenin ester derivatives and idelalisib as the standard PI 3-K inhibitor generated by using the Marvin Sketch program.
Results: Hesperetin and naringenin have a lower partition coefficient than idelalisib. Tributyryl hesperetin, trivaleryl hesperetin, tributyryl naringenin and trivaleryl naringenin have a higher partition coefficient than idelalisib. It means that hesperetin and naringenin derivatives solubility in the oil phase to cross the cell membrane was higher than idelalisib. Docking score of hesperetin and naringenin as the lead compound and their derivatives was lower than idelalisib as the PI 3-K inhibitor standard compound. Ester derivatives of hesperetin and naringenin with the increasing the length of the acyl carbon atom chain substituted on hesperetin and naringenin will increase the PI 3-K inhibition activity. Butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin shows the lower docking score than hesperetin and naringenin as the lead compound.
Conclusion: Increasing of the length of the acyl carbon atom chain substituted on hesperetin and naringenin it will increase the Phosphoinositide 3-Kinase (PI 3-K) inhibition activity. Trivaleryl hesperetin has the best activity in this study and thus to be a good compound to be synthesized and to be combined with anticancer drug.
Keywords: In Silico, Hesperetin, Naringenin, Ester Derivatives, Phosphoinositide 3-Kinase (PI 3-K).

Introduction

Phosphoinositide 3-Kinase (PI3Ks) are among the most frequently activated signaling pathways in cancer[1]. Molecular and genomic analysis of human cancers that the phosphoinositide 3-Kinase (PI 3-K) pathway is deregulated in malignant progression. Prototype of PI 3-K inhibitors show evidence of anticancer activity in vitro and in vivo animal models. The recent development of isoform-selective inhibitors shows

considerable promise for cancer treatment[2]. Signaling of PI 3-K pathway plays an important role in the regulation of cell biological behaviors, such as the proliferation and the apoptosis, in which this pathway is an active state in tumors. Chemotherapeutic drugs which exert their anti tumors effects by blocking this pathway can inhibit the proliferation, induce apoptosis and cause cell cycle arrest in cancer cell[3]. PI 3-K pathway is a key signal transduction system that links oncogenes and is perhaps the most commonly activated signalling pathway in human cancer. This pathway therefore presents both an opportunity and a challenge for cancer therapy[4].

Idelalisib is a PI 3-K inhibitor with specific isoforms. Nowadays idelalisib widely investigated in a variety of malignant diseases. It is an oral drug, selective PI 3-K inhibitor that induces apoptosis in cancer cell from patients with malignant cancer cell. In a clinical study it was observed that idelalisib is an effective agent in relapsed indolent lymphoma both as a single agent and in combination with first line therapy[5]. The PI 3-K pathway is important for cancer therapy. inhibition of the PI 3-K pathway was very important. It is known that many flavonoids are inhibitors of PI 3-K[6].

Flavonoids are phenol compounds present in the pigments of fruits and vegetables. A few molecular mechanisms through which flavonoids exert their anti-cancer action are presented. One of the molecular mechanisms on which their anti-cancer action is based is their anti-oxidant activity. Another is that by which flavonoids interact with the pathways signaling cell growth and apoptosis. Flavonoids interact with the signaling pathways for PI 3-K. Flavonoids consist of several subclasses such as: catechin, flavanone, flavone, isoflavone, flavonol, dihydroflavonol, chalcone, dihydrochalcone and aurone[7]. Figure 1 below shows the basic structure of various flavonoid derivatives.

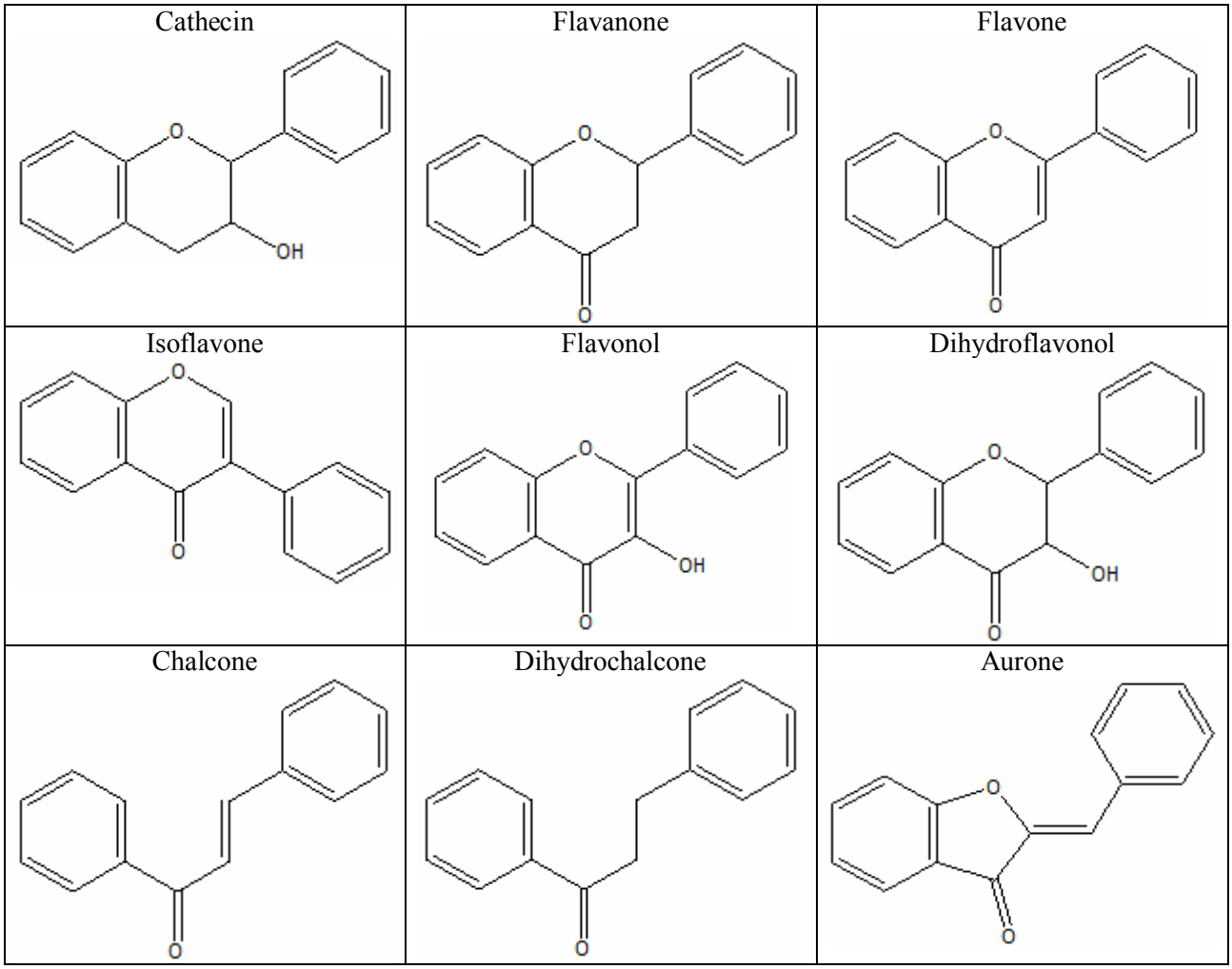


Fig. 1: Basic structure of various flavonoid derivatives

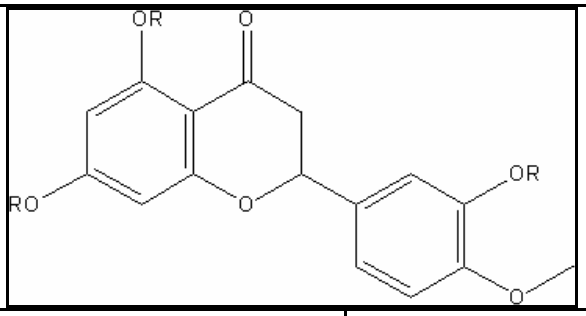
Flavonol widely available in abundance at the various types of fruit, while the flavanone and flavone more limited and often found in groups of citrus. Flavone found in citrus fruit (including oranges and grapefruit,

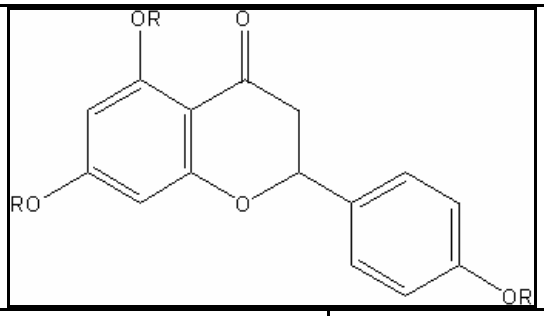
as well as tomatoes and cherries) in a relatively low amount compared to flavanone. Hesperidin and naringin are a form of flavanone glycones, while hesperetin and naringenin are a form of flavanone aglycones; generally found in citrus fruits such as oranges, lemons, limes, and are also present in tomatoes[8]. The use of a combination of flavonoids and anticancer drug allows beneficial effects[9].

Flavonoids are widely used in the pharmaceutical field, the use of flavonoids is limited due to the hydrophilic nature of flavonoids, which resulted in flavonoid compounds have low solubility and low stability in lipophilic media. Increased lipophilic properties of flavonoids it will be followed by an increase in biological activity of flavonoids. Because compounds that are lipophilic will more easily penetrate cell membranes and intracellular work on. Compounds derived flavonoids in the form of esters will lead to the enhancement of the lipophilic (hydrophobic) and allow it to provide better penetration into cancer cells. Techniques to improve the lipophilic properties of flavonoid compounds are by esterification (acylation) hydroxyl group of flavonoids[10]. Semisynthesis flavonoid derivatives by esterification (acylation) flavonoids with several kinds of acyl group substituent to the hydroxyl group will increase the overall lipophilicity properties of lead compound flavonoids[11].

Lead discovery was the main components of today's early pharmaceutical research. The aim of target discovery is the identification and validation of suitable drug targets for therapeutic intervention. Computational methods are being developed to predict the drug likeness of compounds. Thus, drug discovery is already on the road towards electronic research & development. In silico approaches contribute significantly to early pharmaceutical research and are especially important in target discovery and lead discovery. The need for timely adaptation and application of in silico approaches in pharmaceutical research has clearly been recognized and is expected to improve further the overall efficiency of drug discovery[12]. Flavonoids as antidiabetic and anticancer have been studied by in silico docking[13,14].

The longer the carbon atom chain in the acyl group substituted flavonoids is enhanced lipophilic properties and more easily penetrate across the cell membranes. Lipophilic compounds that enter into the intracellular of cancer cells are expected to inhibit PI 3-K. Thus, increasing the anticancer drug accumulation in cancer cells. The purpose of this research is to study the in silico PI 3-K inhibition activity of hesperetin and naringenin derivative lipophilic compounds obtained through esterification (acylation) overall hydroxyl groups. Increasing of the length of the acyl carbon atom chain substituted on hesperetin and naringenin (propionyl, butyryl and valeryl) will be observed. Figure 2 below shows the structure of hesperetin and naringenin derivatives obtained through esterification (acylation) overall hydroxyl groups.





Hesperetin	R = H	Naringenin	R = H
Tripropionyl Hesperetin	R = C_2H_5 – CO –	Tripropionyl Naringenin	R = C_2H_5 – CO –
Tributyryl Hesperetin	R = C_3H_7 – CO –	Tributyryl Naringenin	R = C_3H_7 – CO –
Trivaleryl Hesperetin	R = C_4H_9 – CO –	Trivaleryl Naringenin	R = C_4H_9 – CO –

Fig. 2: Structure of hesperetin and naringenin derivatives obtained through esterification (acylation) overall hydroxyl groups

Methods

Fujitsu T Series (T4310) operated by Windows 7 Home Premium, Intel® Core™ 2 Duo CPU T660 @ 2.20 GHz, 32-bit, hard disk drive 320 GB, and RAM memory 4.00 GB was used to run the molecular docking process. Partition coefficient was predicted by the Chem Draw Ultra program. In silico docking using PLANTS program and visualized by Yasara program. Connector for Windows Operation System to Linux Operation

System was done by Co Pen Drive Linux KDE program. The model of three dimension enzyme structures used in this research was Phosphoinositide 3-Kinase (PI 3-K) binding pocket with the Protein Data Bank (PDB) code 4KZC obtained through the website http://www.rcsb.org/pdb. Two dimension and three dimension conformation models of hesperetin and naringenin ester derivatives and idelalisib as the standard PI 3-K inhibitor generated by using the Marvin Sketch program.

Results and Discussion

N-{6-[6-amino-5-(trifluoromethyl)pyridin- 3-yl]imidazo[1,2-a]pyridin-2-yl}acetamide (1UK) which was crystallized in the structure of 4KZC Phosphoinositide 3-Kinase (PI 3-K) binding pocket was extracted and docked again into its original PI 3-K binding pocket. The Root Mean Square Deviation (RMSD) values resulted from these ligand docking was 0.9530 Å. The RMSD obtained was less than 2.0000 Å, a value typically used in evaluating the success of docking algorithms, indicating the docking methods were valid[15]. Figure 3 shows the docking of 1UK into the 4KZC PI 3-K binding pocket.

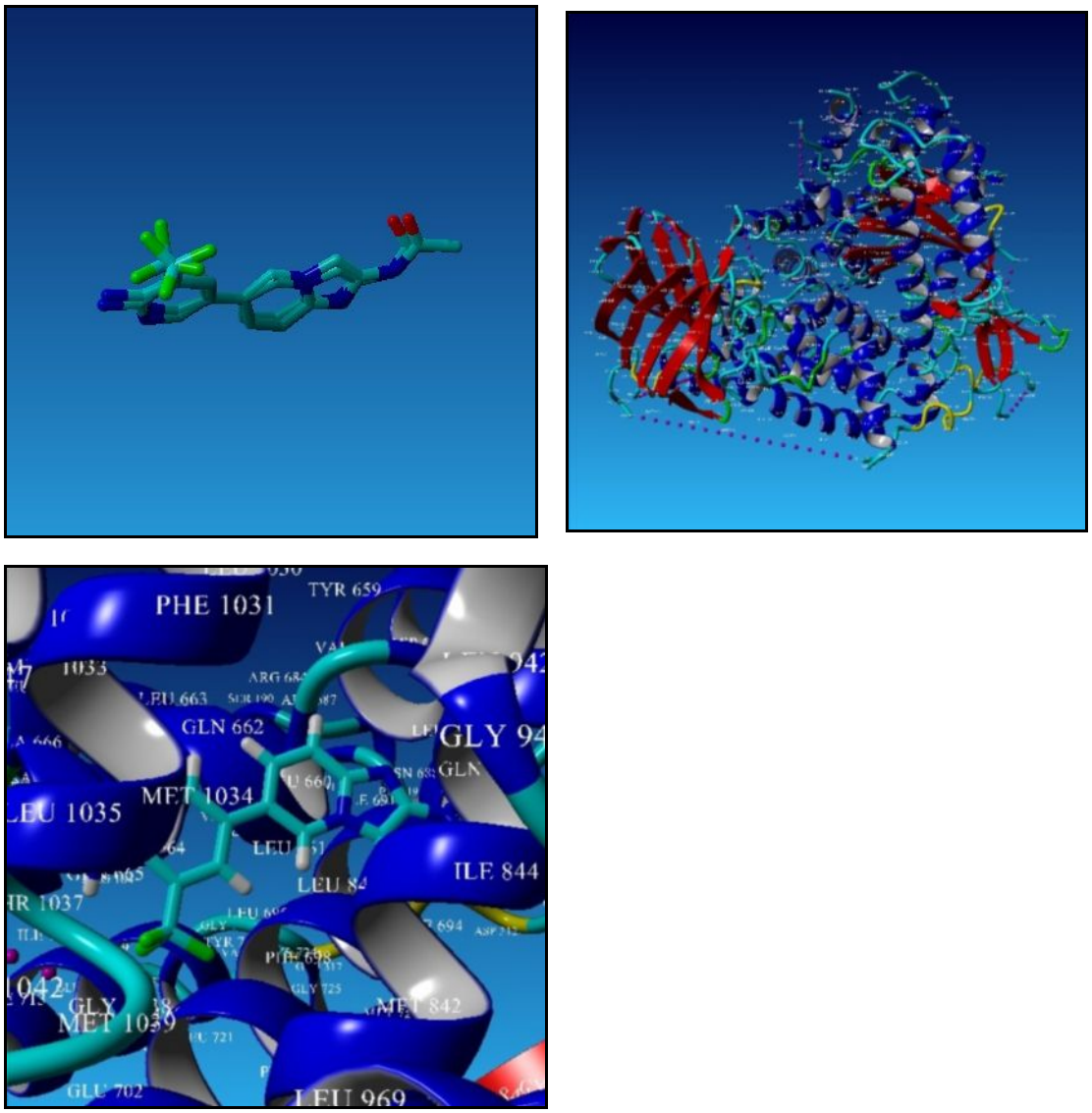


Fig. 3: Docking of 1UK into the 4KZC PI 3-K binding pocket

In silico docking between hesperetin and naringenin ester derivative compounds into the 4KZC PI 3-K binding pocket is resulting the docking score. Table 1 show the docking score result of the ligand into the 4KZC PI 3-K binding pocket.

Table 1: Docking score of the ligand into the 4KZC PI 3-K binding pocket

Number	Ligand Name	Molecular Formula	Length of Acyl Carbon Atom Chain Substituent	Partition Coefficient	Docking Score
1	Idelalisib	$C_{22}H_{18}FN_7O$	-	3.88	– 88,8258
2	Hesperetin	$C_{16}H_{14}O_6$	0	1.78	– 79,1049
3	Tripropionyl Hesperetin	$C_{25}H_{26}O_9$	3	3.67	– 84,8385
4	Tributyryl Hesperetin	$C_{28}H_{32}O_9$	4	4.92	– 90,5424
5	Trivaleryl Hesperetin	$C_{31}H_{38}O_9$	5	6.17	– 110,1400
6	Naringenin	$C_{15}H_{12}O_5$	0	1.90	– 82,0865
7	Tripropionyl Naringenin	$C_{24}H_{24}O_8$	3	3.79	– 83,7670
8	Tributyryl Naringenin	$C_{27}H_{30}O_8$	4	5.04	– 86,5673
9	Trivaleryl Naringenin	$C_{30}H_{36}O_8$	5	6.30	– 91,9636

Hesperetin and naringenin have a lower partition coefficient than idelalisib. It means that their solubility in the oil phase to cross the cell membrane was lower than idelalisib. Tributyryl hesperetin, trivaleryl hesperetin, tributyryl naringenin and trivaleryl naringenin have a higher partition coefficient than idelalisib. It means that their solubility in the oil phase to cross the cell membrane was higher than idelalisib. Partition coefficient only means the ability of the drug to soluble in the oil phase (cell membrane) to reach the inside of the cell, but the activity of the drug to bind with the binding pocket not depend to the partition coefficient but depend on the structure.

The docking score represents the binding affinity of the ligand to the target protein, smaller docking score value shows stronger interaction. Docking score of hesperetin and naringenin as the lead compound and their derivatives was lower than idelalisib as the PI 3-K inhibitor standard compound. It means that hesperetin, naringenin and their derivatives have a stronger interaction to target protein than idelalisib[16]. Esterification (acylation) of hesperetin and naringenin with propionyl to overall hydroxyl groups it will increase the lipophilicity property (higher partition coefficient) and will easier to penetrate across the cell membrane, but the PI 3-K inhibition activity was based on the structure. Docking score results of esterification (acylation) of hesperetin and naringenin with propionyl to overall hydroxyl groups also caused their docking score lower than hesperetin and naringenin as the lead compound. It means that tripropionyl hesperetin and tripropionyl naringenin have a stronger interaction to target protein than hesperetin and naringenin. Based on the results it is also known that an increase in the length of acyl carbon atoms chain substituted on hesperetin and naringenin it will be decreasing the docking score, thus increase the PI 3-K inhibition activity. Increase in the length of acyl carbon atoms chain substituted on hesperetin and naringenin it will also be increasing the lipophilicity property (lower partition coefficient) and will eassier to penetrate across the cell membrane. Butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin shows the lower docking score than hesperetin and naringenin as the lead compound. It means that butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin have a stronger interaction to target protein than hesperetin and naringenin. This results was supported by the previous research which the longer acyl carbon atoms chain substituted on hesperetin and naringenin it will also be increasing the stronger interaction to P-Glycoprotein (P-GP)[17].

Increasing costs of drug development and reduced number of new chemical entities have been a growing concern for new drug development in recent years. Therefore, there is a need for the use of alternative tools to get answers on the efficacy and safety faster, with more certainty and at lower cost. One such alternative tool is the in silico drug design or the computer aided drug design (CADD). In silico drug design can play a significant role in all stages of drug development from the preclinical discovery stage to late stage clinical development[18]. The results obtained in silico screening have shown that it represents the best way to get accurate results in a very short time period and saving manner[19].

From the in silico screening results known that trivaleryl hesperetin was the most potential drug to inhibit the PI 3-K. Trivaleryl hesperetin is expected to be combined with anticancer drugs to increase the effectiveness of therapy, because the inhibition of the PI 3-K will sensitizes cancer cell both death receptor and

chemotherapy induce apoptosis cancer cell. Figure 4 below shows the visualization of interaction between interaction between trivaleryl hesperetin and PI 3-K as the target protein. Although the application of docking and scoring has led to some remarkable successes, there are still some major challenges ahead[20].

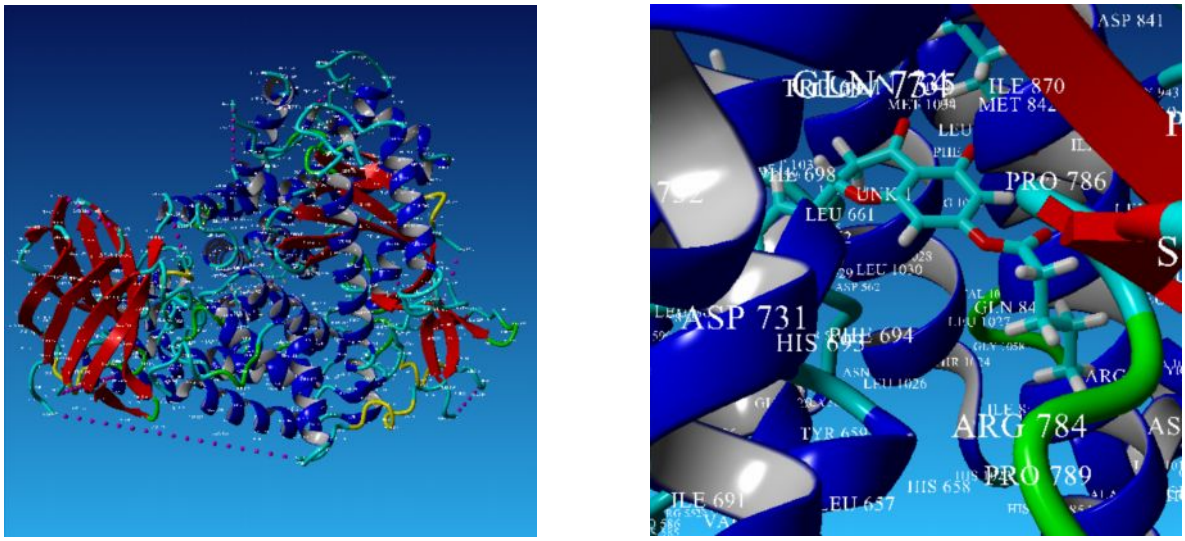


Figure 4. Visualization of interaction between interaction between trivaleryl hesperetin and PI 3-K as the target protein

PI 3-K has been reported to correlate with adverse clinical outcome in human cancer cell in vivo. PI 3-K which is a serious obstacle in chemotherapy, has also been implicated in causing inhibition of tumor cells apoptosis. PI 3-K inhibition broadly primes cancer cells for apoptosis, rationale for using PI 3-K inhibitors in combination regimens to enhance chemotherapy induced apoptosis in cancer cell[21]. Improved knowledge of the PI 3-K pathway will be useful in understanding of tumor development and for identifying ideal targets of anticancer therapy for cancer[22]. Thus very important to find the new PI 3-K inhibitor; combination of PI 3-K inhibitor with the anticancer drug can enhance the efficacy of anticancer drug by accumulating the anticancer drug in the intracellular of cancer cell and inhibit the anticancer drug resistance.

Conclusions

Hesperetin and naringenin were flavonoid compound with the flavanone subclasses. Hesperetin and naringenin are abundantly contained in citrus fruits. They show the higher PI 3-K efflux pump inhibition activity than idelalisib as the standard PI 3-K inhibition drug. With modification of the hydroxyl group with the acyl substituted by esterification will be increasing the PI 3-K inhibition activity. Increasing of the length of the acyl carbon atom chain substituted on hesperetin and naringenin it will increase the PI 3-K inhibition activity. Butyryl and valeryl be substituted as the acyl substituent to hesperetin and naringenin shows better partition coefficient than idelalisib. Trivaleryl hesperetin has the best activity in this study and thus to be a good compound to be synthesized and to be combined with anticancer drug. Stronger PI 3-K inhibition activity might sensitizes cancer cell to chemotherapy agent and induces apoptosis of cancer cell, the treatment also might be more successful.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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