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Der Pharma Chemica, 2013, 5(6):303-311
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Selective phosphoinositide 3-kinase inhibition by natural products: A molecular docking study

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ABSTRACT

Phosphoinositide 3-kinase (PI3K) is responsible for a large number of signaling pathways involved in a range of conditions ranging from allergic response to tumor growth, and much research is currently underway to identify novel selective inhibitors for the different isoforms of PI3K. In this work, a molecular docking analysis of 80 anti-inflammatory natural products was carried out with three isoforms of PI3K in order to identify potential selective inhibitors from natural sources. Seven natural products with "drug-like" properties were found to dock strongly and selectively with PI3K. These include the alkaloids berberine, chelerythrine, and isaindigotone; the flavonoids malvidin, isovitexin, and vitexin; and the triterpenoid cucurbitacin B.

Keywords: PI3K, molecular docking, berberine, chelerythrine, cucurbitacin B, isaindigotone, isovitexin, malvidin, vitexin.

INTRODUCTION

Chronic inflammation has been implicated in a number of chronic diseases, including cancer [1], diabetes [2], atherosclerosis [3], and Alzheimer's disease [4]. A promising new direction is the targeting of signaling molecules that participate in inflammatory activation. Phosphoinisitide 3-kinases (PI3Ks) are lipid kinases that are expressed in leukocytes and participate in their function and activation. There are several forms of PI3K that modulate a wide range of cellular activities and research is underway to identify selective PI3K inhibitors to treat chronic inflammation, cardiovascular diseases and cancer [5].

PI3Kα and PI3Kβ are found in all cell types. Mice that are lacking either isoform do not survive embryonic development [6]. Both PI3Kγ and PI3Kδ are mainly found in leukocytes. Mice with nonfunctional γ and δ isoforms are able to survive development and are even fertile. These modified γ and δ mice show a lowered reaction to allergic stimuli [7]. PI3Kγ's role in inflammation is well understood. The tissue resident mast cells signal for a migration of effector cells including mast cell precursors, neutrophils, and monocytes [8,9]. Activation of PI3Kγ produces phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 leads to the activation of Bruton's tyrosine kinase (Btk) and phospholipase Cγ (PLCγ). Ca^{2+} channels are then opened in the plasma membrane and mast cell granules release mediators including histamine and heparin [10]. Mast cells produced in mice with null PI3Kγ have a lowered degranulation response when compared to wild type mast cells. This leads to PI3Kγ null mice having a complete resistance to systemic anaphylaxis [11]. PI3Kδ "knock-in" mice show only a partially retarded response in mast cells. PI3Kδ "knock-in" mice are only partially protected against systemic anaphylaxis which points towards PI3Kγ playing the largest role in allergic response [12]. PI3Kγ and PI3Kδ will serve as possible targets for a wide

variety of inflammatory disorders such as rheumatoid arthritis [13], systemic lupus erythematosus [14], and atherosclerosis [15].

Overexpression of PI3Kα in mice leads to an enlarged heart. This size increase is due to an increase in cell size, not number of cells [16]. The overall architecture and function of the hearts were not affected. Mice that have a base expression of protein kinase B (PKB/Akt) encoded have an even larger heart size [17]. Inactivation of the phosphatase and tensin (PTEN) regulator also led to an increased heart size along with reduced contractility [18]. This shows that PI3Kα's function is directly accomplished through PKB. PI3Kγ is expressed in the heart in low levels. Mice that are PI3Kγ deficient do not show any change in heart size or contractility. By targeting PI3Kγ in mice that have a PTEN deficiency, the mice show a restoration of contractility [5]. Class 1A PI3Ks control heart size while PI3Kγ impacts contractility [19]. An active form of PI3Kγ is not required for heart contractility, but the protein structure serves as a scaffolding for cAMP breakdown [20]. Selective inhibitors of PI3Kγ and PI3Kδ have been shown to diminish the inflammatory response after ischemia. Tissue repair and endothelial cell mitosis were not affected [21]. This promising discovery might lead to the creation of novel treatments for patients with myocardial infarctions.

PI3K activity is often modified in various cancer cells. Due to PI3K's control of cell growth, survival, and proliferation, a change in the activation or regulation of PI3K can lead to tumor formation and metastasis. Elevated levels of PIP3 lead to over-activity of the cellular processes regulated by PI3K [22]. Mutations affecting activation and expression have been reported in several pathway participants including the p85 subunit, PDK1, and PKB/Akt [23]. Mutations have also been found in known PI3K activators including several growth factor receptors [24]. Several tumors have shown changes in the level of expression for PTEN, the PI3K regulating phosphatase. Other tumors show methylations of the PTEN locus or even loss of heterozygosity [25]. Hyper-methylation of PTEN promoters is a common method of down-regulation found in more than 50% of breast, prostate, colorectal, and endometrial cancers [26]. A loss of PTEN function is found in late tumor progression and in advanced tumor stages [27]. Several different tumors show overexpression of the catalytic subunit of PI3Kα including ovarian, lung, and cervix cancer. These mutant species display elevated lipid kinase activity when compared to the wild type. These cells with modified PI3Kα have increased survivability in poor growth conditions when compared to wild type cells [28]. Modified cells show an increased ability to migrate in a variety of conditions. This suggests that cells with an over-activation of PI3Kα have an increased ability to grow and migrate in suboptimal conditions contributing to tumor formation and metastasis [5].

Higher plants have served as a source of medicinal agents throughout human history [29], and plant-derived natural products are a rich source of anti-inflammatory compounds [30], including alkaloids [31], flavonoids [32], and triterpenoids [33]. In this work, we present a molecular docking approach to identify anti-inflammatory natural products from plants that may serve as selective PI3K inhibitors.

MATERIALS AND METHODS

Protein-ligand docking studies were carried out based on the crystal structures of three PI3K isoforms: PI3Kα (PDB 3zim [34], PDB 3t8m [35] PDB 4fa6 and 4fad [36]), PI3Kγ (PDB 2chw and 2chx [37], PDB 3ps6 [38], and PDB 4g11 [39]), and PI3Kδ (PDB 2wxi [40], PDB 4gb9 [41], PDB 4ezj and 4ezl [42]). Prior to docking all solvent molecules and the co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds with each of the proteins were undertaken using Molegro Virtual Docker v. 6.0 [43], with a sphere large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. The binding site chosen for each structure was the site of the co-crystallized inhibitor or substrate. Standard protonation states of the proteins based on neutral pH were used in the docking studies. Each protein was used as a rigid model structure; no relaxation of the protein was performed. Assignments of charges on each protein were based on standard templates as part of the Molegro Virtual Docker program; no other charges were necessary to be set. Each ligand structure was built using Spartan '10 for Windows [44] and the lowest-energy conformation determined. Flexible ligand models were used in the docking and subsequent optimization scheme. As a test of docking accuracy and for docking energy comparison, co-crystallized ligands were re-docked into the protein structures (see Table 1). Different orientations of the ligands were searched and ranked based on their energy scores. The RMSD threshold for multiple cluster poses was set at < 1.00Å. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50 and a minimum of 100 runs for each

ligand. The collection of poses generated was sorted by re-rank score. The pose with the most negative re-rank score was selected for each ligand and are summarized in Table 1.

Table 1. Molegro docking energies (re-rank scores, kJ/mol) for anti-inflammatory natural products with phosphoinositide 3-kinase isoforms

	PI3Kα				PI3Kγ				PI3Kδ			
PDB Code	4FA6	3ZIM	3T8M	4FAD	2CHW	2CHX	3PS6	4G11	4EZJ	4GB9	4EZL	2WXI
Ligand												
Co-crystallized Ligand	-103.2	-125.6	-138.3	-105.8	-127.6	-106.0	-100.5	-110.2	-120.7	-125.4	-109.0	-148.7
Alkaloids												
Berbamine	-78.8	-87.3	-99.9	-89.6	-76.2	-89.0	-89.5	-89.6	-81.9	-70.9	-65.7	-78.9
Berberine	-77.9	-108.9	-102.5	-79.0	-78.5	-98.0	-97.3	-83.9	-94.7	-79.2	-99.6	-90.5
Boldine	-88.5	-84.7	-77.5	-86.5	-83.1	-88.2	-83.9	-80.6	-88.3	-79.0	-90.9	-74.1
Castanospermine	-57.3	-59.7	-55.0	-60.6	-69.6	-64.7	-72.0	-55.2	-60.8	-58.6	-62.1	-62.3
Cepharanthine	-95.7	-72.1	-93.0	-66.0	-91.1	-74.3	-85.5	-86.5	-86.2	-76.5	-76.4	-68.8
Chelerythrine	-83.4	-93.5	-95.4	-92.9	-77.8	-94.5	-108.5	-79.9	-103.2	-77.7	-102.2	-82.6
Crotalaburine	-65.7	-68.8	-71.6	-70.4	-74.2	-63.4	-84.0	-78.6	-63.9	-20.5	-64.2	-75.1
Cryptolepine	-78.3	-82.3	-72.8	-77.3	-73.1	-78.4	-81.8	-67.7	-76.2	-79.5	-79.4	-69.6
Evodiamine	-76.7	-65.2	-61.8	-86.8	-87.9	-65.6	-87.0	-86.4	-72.2	-67.8	-64.0	-89.8
Halofuginone	-76.1	-96.5	-84.9	-78.7	-93.5	-96.6	-88.4	-81.5	-88.6	-92.0	-83.7	-95.2
Harmine	-73.7	-73.6	-72.5	-72.3	-69.2	-72.1	-85.1	-78.2	-71.5	-77.2	-76.4	-66.2
Indirubin 3-monoxide	-73.6	-92.4	-90.9	-93.7	-87.2	-96.1	-94.4	-79.3	-94.7	-84.0	-97.6	-92.1
Isaindigotone	-86.5	-119.7	-99.9	-98.7	-85.8	-99.7	-94.8	-92.3	-108.7	-88.5	-107.9	-89.9
Lycorine	-79.6	-94.1	-88.1	-80.3	-81.6	-92.6	-84.5	-79.3	-97.5	-90.5	-97.6	-78.6
Matrine	-62.8	-72.5	-68.2	-68.8	-69.8	-68.1	-81.2	-65.6	-69.0	-72.1	-68.1	-68.6
Norisboldine	-78.2	-90.6	-75.9	-78.0	-84.4	-88.4	-87.8	-83.6	-87.5	-80.4	-92.9	-83.9
Noscapine	-86.1	-83.8	-80.9	-100.5	-101.3	-86.2	-90.9	-91.7	-85.4	-91.3	-95.0	-97.5
Piperine	-84.5	-97.8	-86.2	-89.2	-82.1	-90.1	-89.0	-84.6	-81.7	-81.7	-82.9	-83.2
Pseudocoptisine	-88.1	-101.0	-95.0	-93.6	-79.5	-100.0	-99.3	-91.1	-98.7	-97.3	-99.6	-77.6
Rutaecarpine	-75.5	-93.2	-82.3	-80.4	-77.6	-90.5	-79.5	-79.8	-86.9	-74.3	-79.0	-59.8
Sanguinarine	-85.1	-95.6	-83.9	-87.1	-76.4	-94.9	-93.9	-89.4	-85.4	-88.1	-88.1	-71.7
Sinomenine	-62.0	-51.5	-57.2	-49.9	-55.3	-57.7	-58.7	-68.6	-55.5	-60.0	-53.0	-63.7
Tetrandrine	-72.5	-67.6	-95.4	-88.8	-71.6	-89.7	-91.8	-95.3	-80.6	-95.5	-74.7	-80.8
Thaliporphine	-78.3	-72.8	-84.5	-76.9	-74.9	-82.7	-86.1	-90.8	-82.9	-77.8	-83.0	-79.9
Theacrine	-67.4	-70.3	-64.4	-67.1	-66.7	-68.5	-76.5	-66.1	-65.4	-69.8	-68.2	-63.9
Triptanthrin	-78.7	-83.3	-77.2	-81.8	-73.9	-83.3	-88.3	-69.1	-83.3	-83.5	-83.5	-70.6
Flavonoids												
Acacetin	-85.1	-98.2	-84.9	-92.8	-79.2	-91.4	-96.4	-88.6	-95.4	-86.7	-95.4	-84.8
Apigenin	-78.2	-93.5	-82.7	-88.5	-77.5	-87.9	-91.7	-80.3	-92.5	-86.6	-90.1	-79.4
Baicalein	-78.7	-93.7	-81.3	-87.5	-77.7	-89.4	-88.4	-79.3	-92.5	-84.0	-91.3	-78.2
Biochanin A	-89.8	-95.8	-85.0	-90.5	-83.1	-89.4	-97.1	-82.7	-97.3	-77.7	-96.9	-91.3
Chrysin	-74.4	-87.9	-80.5	-83.1	-75.7	-79.2	-92.0	-73.2	-85.7	-79.3	-85.0	-74.1
Cyanidin	-83.3	-91.9	-85.5	-90.9	-81.3	-89.6	-103.2	-83.9	-91.5	-98.5	-94.7	-84.5
Deguelin	-65.0	-65.5	-73.6	-65.8	-83.7	-74.6	-76.4	-83.8	-81.2	-77.4	-74.9	-82.1
Delphinidin	-78.5	-94.9	-84.4	-91.9	-82.5	-90.7	-100.7	-83.6	-95.1	-91.5	-98.4	-88.4
Diosmetin	-86.2	-103.8	-91.2	-95.4	-83.6	-93.6	-101.0	-92.6	-100.7	-89.6	-102.8	-91.8
Equol	-68.4	-65.3	-65.5	-74.7	-76.7	-67.4	-78.8	-78.9	-72.0	-70.7	-71.3	-82.2
Eridictyol	-77.9	-70.1	-66.8	-68.9	-79.0	-72.0	-70.9	-72.8	-74.2	-72.6	-82.3	-80.7
Fisetin	-88.6	-93.7	-82.2	-92.9	-86.2	-93.2	-98.8	-86.2	-93.1	-89.7	-96.6	-84.8
Formononetin	-84.0	-85.7	-83.4	-83.0	-75.5	-79.6	-88.8	-78.4	-90.9	-80.4	-88.9	-85.9
Geninstein	-87.8	-94.7	-82.3	-85.3	-81.2	-89.4	-88.6	-77.6	-94.3	-88.4	-89.4	-83.0
Glabridin	-73.3	-96.8	-82.8	-83.1	-89.1	-85.5	-96.3	-82.1	-86.0	-87.1	-80.5	-92.4
Glyceitein	-90.1	-93.9	-85.3	-87.1	-76.9	-87.8	-91.6	-86.4	-89.5	-91.0	-91.9	-77.7
Gossypetin	-89.5	-101.7	-89.6	-99.3	-88.6	-97.1	-104.9	-89.3	-99.4	-96.6	-103.0	-91.1
Gossypin	-116.2	-126.2	-110.8	-123.6	-105.9	-122.3	-124.9	-120.4	-114.5	-121.8	-116.8	-125.3
Hesperetin	-77.3	-69.3	-74.8	-76.5	-80.1	-77.5	-78.8	-81.5	-74.9	-72.3	-80.5	-79.0
Isovitexin	-101.5	-98.6	-99.2	-100.8	-104.9	-105.2	-114.5	-98.6	-88.6	-101.5	-103.6	-95.8
Kaempferol	-82.6	-90.0	-77.6	-88.1	-78.4	-88.4	-89.3	-78.8	-90.6	-90.6	-90.6	-80.6
Luteolin	-86.4	-99.0	-88.8	-93.2	-82.0	-94.5	-101.9	-86.9	-100.2	-91.5	-98.3	-86.2
Malvidin	-92.3	-103.7	-93.5	-100.5	-90.4	-100.1	-99.5	-93.8	-107.3	-93.7	-103.6	-90.7
Morin	-80.7	-95.5	-80.5	-86.9	-78.6	-89.3	-97.4	-83.7	-93.9	-90.4	-93.6	-81.2
Naringenin	-76.2	-69.9	-66.3	-64.2	-74.8	-66.7	-64.7	-71.6	-68.6	-69.9	-79.6	-76.3
Naringin	-111.5	-121.9	-100.5	-117.0	-112.7	-123.6	-127.1	-120.6	-117.5	-115.1	-125.0	-100.1
Nobiletin	-75.8	-93.3	-96.2	-88.4	-85.9	-83.6	-100.8	-81.2	-91.1	-100.1	-101.2	-81.2
Pentamethoxyflavone	-87.9	-106.1	-93.5	-99.8	-95.1	-96.1	-101.1	-99.1	-94.6	-99.8	-96.8	-79.5
Peonidin	-82.4	-98.4	-87.1	-94.7	-86.6	-91.7	-100.3	-88.9	-100.0	-95.0	-95.6	-89.0
Petunidin	-89.6	-99.8	-88.1	-96.0	-90.4	-95.2	-102.2	-90.7	-99.8	-92.1	-100.6	-94.5
Quercetin	-87.9	-98.3	-87.0	-95.7	-83.9	-93.8	-105.6	-87.4	-98.2	-94.2	-97.9	-87.3
Tangeretin	-78.5	-90.2	-95.1	-95.3	-89.3	-83.2	-101.1	-85.7	-88.8	-77.2	-92.8	-86.0
Taxifolin	-88.1	-99.6	-87.1	-96.6	-82.6	-94.7	-100.5	-86.4	-98.8	-94.1	-100.0	-87.0
Vitexin	-100.7	-105.1	-100.1	-95.6	-103.0	-109.6	-117.1	-106.0	-94.5	-104.4	-98.4	-101.2
Wogonin	-80.7	-93.0	-85.1	-86.7	-81.0	-87.0	-90.1	-80.3	-89.9	-83.7	-94.3	-82.0
Triterpenoids												
α-Amyrin	-56.3	-41.4	-70.5	-60.9	-60.4	-53.0	-70.3	-70.1	-56.8	-71.7	-62.8	-43.2
Asiatic Acid	-83.3	-61.2	-66.1	-62.1	-77.7	-79.5	-70.9	-85.5	-67.5	-59.3	-64.7	-57.9

	PI3Kα				PI3Kγ				PI3Kδ			
PDB Code Ligand	4FA6	3ZIM	3T8M	4FAD	2CHW	2CHX	3PS6	4G11	4EZJ	4GB9	4EZL	2WXI
Betulin	-85.4	-67.4	-72.5	-68.0	-76.3	-82.9	-80.3	-74.4	-69.5	-83.6	-68.9	-77.1
Betulinic Acid	-92.2	-80.8	-72.1	-67.0	-74.1	-81.7	-75.7	-76.9	-72.4	-78.2	-40.7	-58.0
Celastrol	-76.0	-66.0	-74.7	-50.0	-70.8	-89.0	-82.2	-75.7	-81.4	-79.8	-69.7	-65.7
Cucurbitacin B	-103.3	-72.6	-96.9	-93.2	-90.5	-117.4	-96.5	-94.6	-96.0	-113.4	-92.7	-80.6
Diosgenin	-71.7	-73.5	-48.0	-65.3	-71.1	-77.6	-85.1	-86.1	-71.6	-76.9	-65.4	-75.9
Ganoderic Acid Df	-78.0	-73.2	-89.2	-90.6	-79.3	-86.4	-100.7	-99.1	-82.0	-81.3	-74.5	-66.3
Glycyrrhetinic Acid	-71.9	-72.6	-66.2	-69.3	-77.2	-70.4	-71.5	-91.4	-69.7	-81.2	-67.2	-73.7
Lupeol	-75.9	-52.8	-23.7	-66.4	-75.1	-81.1	-76.3	-39.1	-64.6	-81.8	-63.6	-75.3
Madecassic Acid	-81.6	-63.5	-77.4	-49.3	-79.1	-78.1	-65.4	-80.8	-60.5	-60.0	-66.9	-62.1
Maslinic Acid	-76.7	-7.5	-79.0	-49.6	-73.1	-71.1	-73.1	-80.9	-70.4	-59.9	-57.3	-49.0
Oleandrin	-106.1	-88.8	-92.9	-95.0	-98.9	-87.6	-102.5	-88.3	-81.6	-91.2	-86.8	-86.8
Oleanic Acid	-74.3	-58.7	-62.4	-65.5	-70.6	-68.0	-69.9	-77.4	-66.3	-52.0	-69.0	-38.0
Pristimerin	-72.5	-62.8	-67.2	-54.5	-71.9	-78.0	-61.6	-72.4	-74.9	-88.0	-82.9	-62.8
Soyasaspogenol B	-68.6	-16.0	-67.2	-49.7	-65.6	-64.0	-52.8	-70.2	-56.4	-68.2	-1.6	-52.7
Sumaresinolic Acid	-58.2	-60.7	-68.5	-56.1	-68.7	-62.1	-63.7	-76.9	-66.7	-52.0	-59.4	-47.5
Ursolic Acid	-87.0	-75.3	-63.7	-66.1	-68.7	-65.5	-76.9	-83.0	-59.3	-30.0	-40.3	-73.9
Uvaol	-62.8	-43.1	-70.3	-56.6	-72.9	-73.6	-51.6	-74.6	-61.8	-57.2	-64.6	-38.3
Withanolide	-92.8	-90.1	-102.6	-78.0	-100.7	-97.0	-104.3	-106.2	-89.3	-96.8	-93.2	-102.5

RESULTS AND DISCUSSION

A total of 80 anti-inflammatory phytochemicals, including 26 alkaloids, 34 flavonoids, and 20 triterpenoids were docked with four crystal structures each of PI3Kα, PI3Kγ, and PI3Kδ. The docking energies are summarized in Table 1. Two alkaloid ligands (Figure 1) showed notably strong docking (docking energies < -107 kJ/mol, i.e., less than the average – 1.5 × standard deviation) with PI3Kα; isaindigotone (E_{dock} = -119.7 kJ/mol) and berberine (E_{dock} = -108.4 kJ/mol). Additionally, both isaindigotone and berberine selectively docked with PI3Kα over PI3Kγ or PI3Kδ. These alkaloid ligands docked in a hydrophobic pocket formed by Ile932, Tyr836, Asp933, Ile848, Val850, and Val851 (see Figure 2).

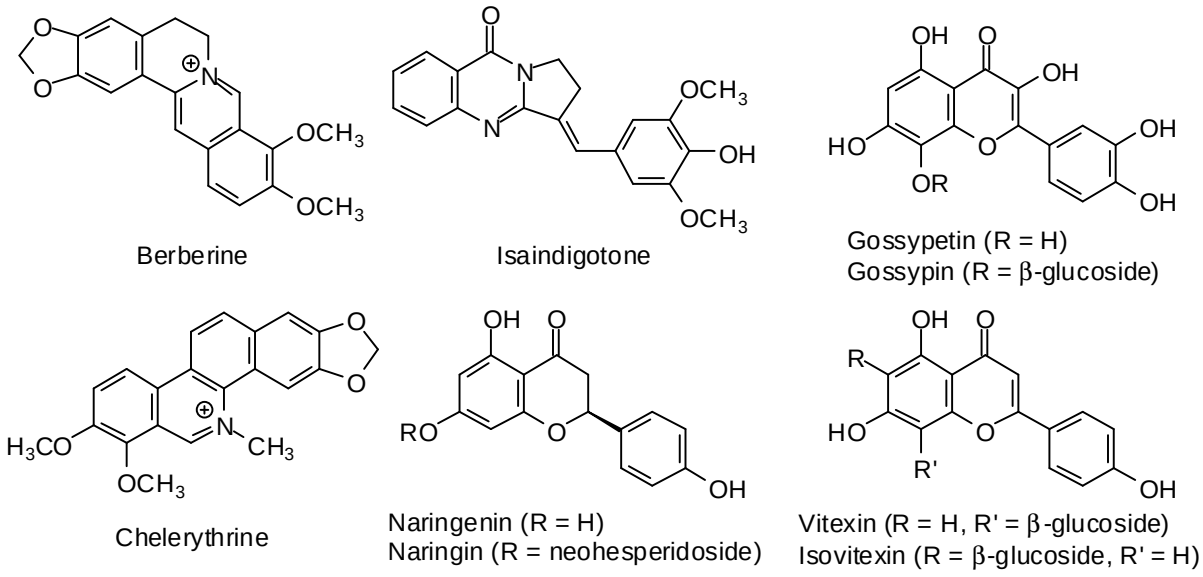

Figure 1. Phytochemical ligands discussed in this work

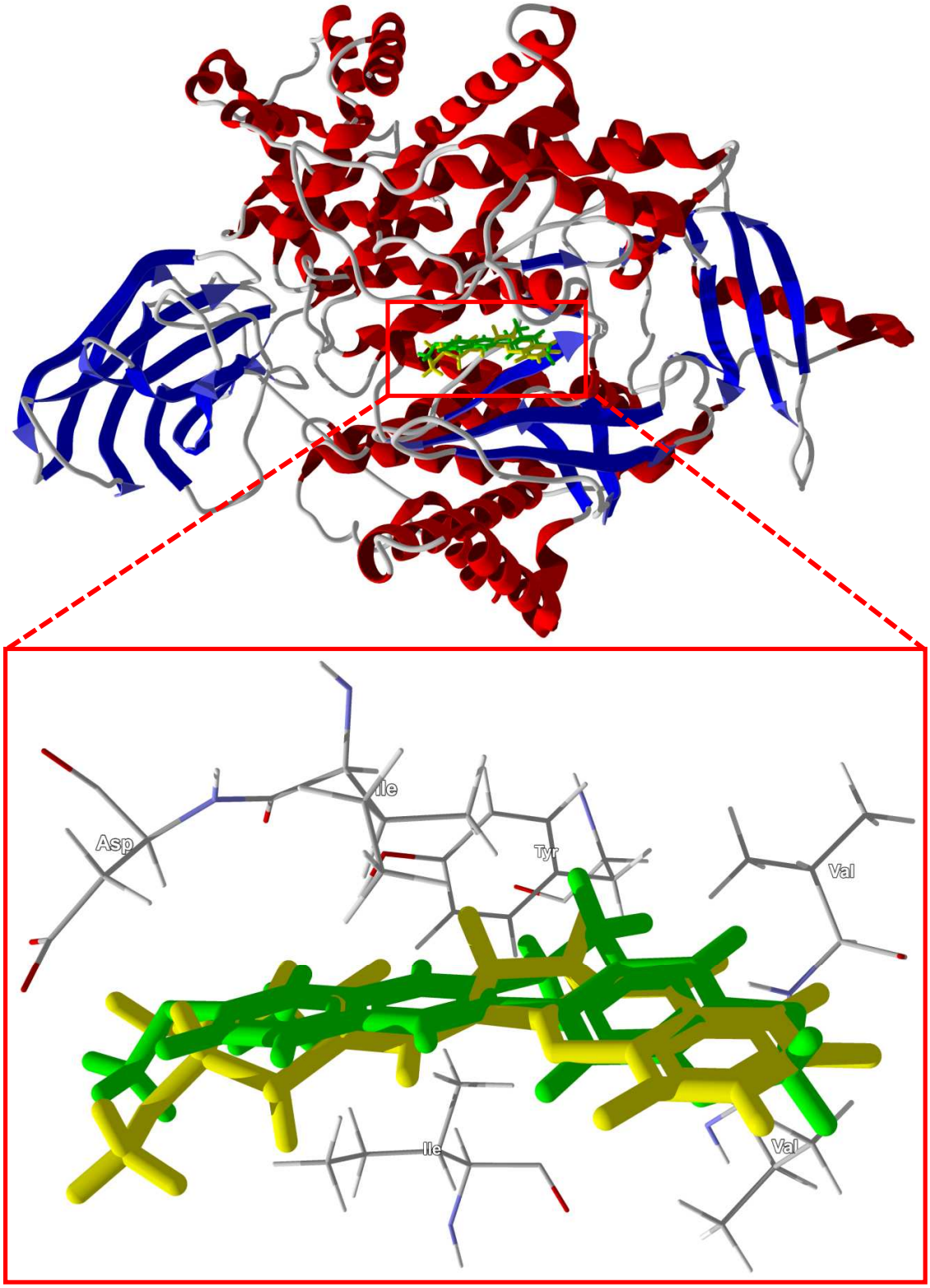

Figure 2. Berberine (green) and isaindigotone (yellow) docking with PI3Kα (PDB 3zim)

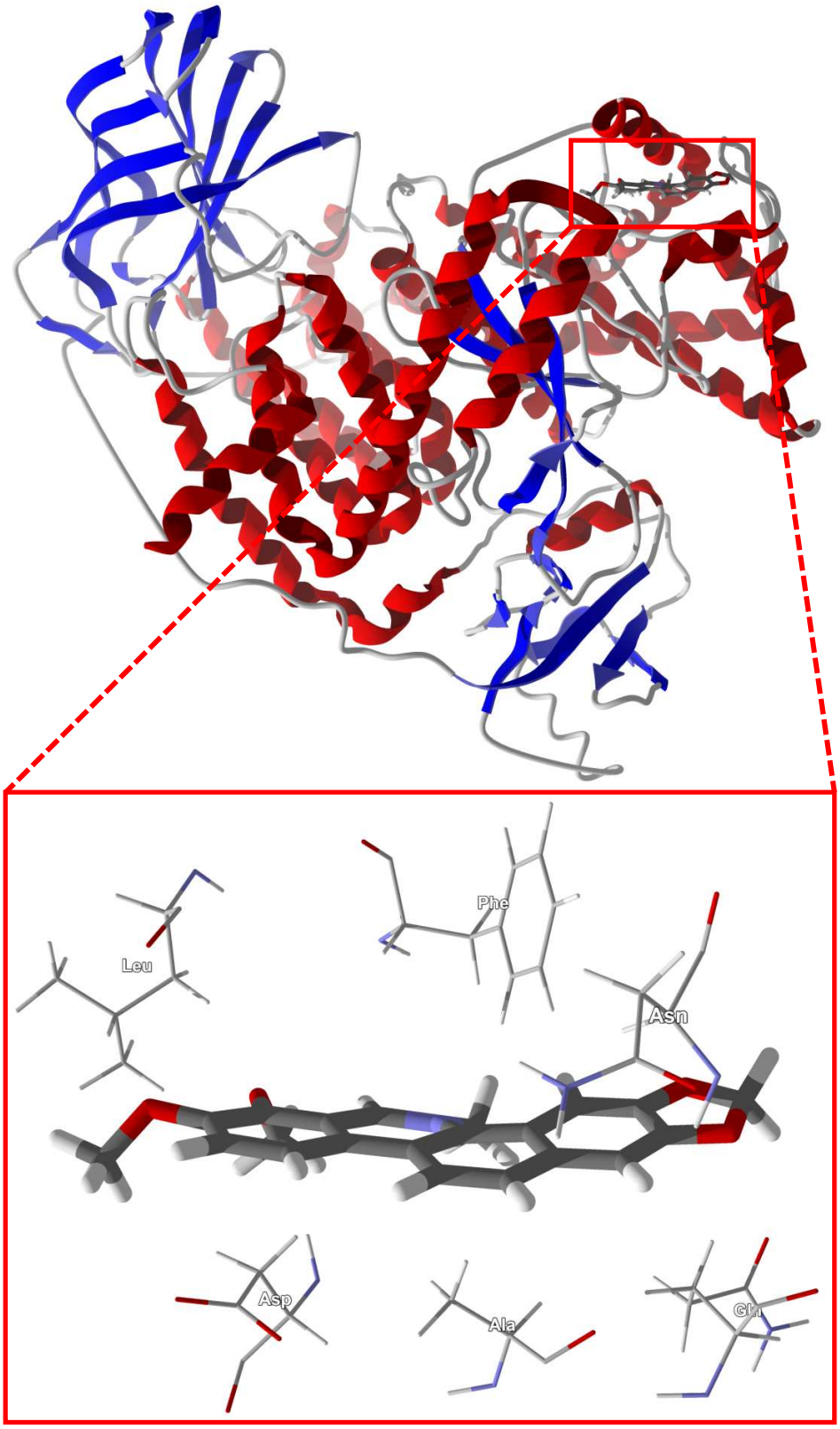

Figure 3. Lowest-energy pose of chelerythrine with the active site of PI3Kγ (PDB 3ps6)

Berberine has demonstrated anti-inflammatory activity [45]. Although the exact mechanism for the anti-inflammatory activity of berberine is unknown, the compound has demonstrated reduced cyclooxygenase-2 expression and prostaglandin E_2 production *in vitro* and *in vivo* [46], significantly reduced expression of pro-inflammatory cytokines such as tumor necrosis factor α, interleukin-1β, interleukin-6, C-reactive protein, and haptoglobin [47,48]. Berberine has been shown to inhibit the PI3K/Akt signaling cascade in breast cancer cells that overexpress human epidermal growth factor receptor 2, analogous to the PI3K-specific inhibitor LY294002, leading to apoptosis [49]. In apparent contrast, however, berberine has been shown to activate the PI3K/Akt pathway in ischemia models, preventing hypoxia-induced apoptosis, and that the anti-apoptotic effects were blocked by the PI3K inhibitor LY294002 [50,51].

The flavonoid glucoside gossypin also docked strongly with PI3Kα (E_{dock} = -126.2 kJ/mol). This ligand, however, did not show docking selectivity, docking strongly also with PI3Kγ (E_{dock} = -124.9 kJ/mol) and PI3Kδ (E_{dock} = -125.3 kJ/mol). In addition, the compound violates Lipinski's "rule of 5" [52]. The "rule of 5" was coined to describe the criteria which must be maintained to function well *in vivo*. In order to have good bioavailability and absorption into the cell, the compounds must have fewer than five H-bond donors, fewer than 10 H-bond acceptors, a molecular weight of less than 500, as well as a calculated partition coefficient (CLogP) of less than 5. Gossypin has nine H-bond donors, 13 H-bond acceptors, and a CLogP of -4.49, and does not, therefore, exhibit good "drug-like" characteristics. Since gossypin is a flavonoid glucoside, it may be expected to undergo hydrolysis *in vivo* to release the aglycone gossypetin. Gossypetin has one "rule of 5" violation with six H-bond donor atoms, but does dock selectively with PI3Kγ (E_{dock} = -104.9 kJ/mol).

The alkaloid chelerythrine (E_{dock} = -108.5 kJ/mol), flavonoid glycoside naringin (E_{dock} = -127.1 kJ/mol), and the glucosylflavonoids vitexin (E_{dock} = -117.1 kJ/mol) and isovitexin (E_{dock} = -114.5 kJ/mol) exhibited strong selective docking to PI3Kγ. The three flavonoids violate the "rule of 5", however. The aglycone of naringin, naringenin, docked relatively weakly with PI3K (PI3Kα, E_{dock} = -76.2 kJ/mol; PI3Kγ, E_{dock} = -75.8 kJ/mol, and PI3Kδ, E_{dock} = -79.6 kJ/mol). The flavonoid malvidin, on the other hand, showed selective docking to PI3Kδ with a docking energy of -107.3 kJ/mol. The lowest-energy pose of chelerythrine with PI3Kγ shows the ligand to occupy a hydrophobic pocket formed by Asp950, Phe1087, Ala889, Leu1090, Asn898, and Gln892 (see Figure 3). The triterpenoid cucurbitacin B also showed strong and selective docking with PI3Kγ with a docking energy of -117.4 kJ/mol.

Both naringin and naringenin have shown anti-inflammatory activity [53]. Naringin has been shown to mediate the release of interleukin-6 and -8, apparently repressing the PI3K/Akt pathway in vascular smooth muscle cells [54], and naringenin has inhibited nuclear factor-κB (NF-κB) in activated macrophages [55]. Naringenin has been shown to be an inhibitor of PI3K activity [56]. Cucurbitacin B has shown anti-inflammatory activity [57], inhibition of NF-κB, as well as inhibition of PI3K/Akt [58].

Apparently isaindigotone, chelerythrine, malvidin, and gossypetin have not been reported to show evidence of PI3K inhibition. Isaindigotone does inhibit 5-lipoxygenase activity as well as leukotriene B_4 [59], malvidin inhibited phospholipase A_2 [60] and NF-κB, but enhanced the PI3K/Akt pathway [61]. The molecular docking results presented in this work suggest that berberine, isaindigotone, gossypetin, malvidin, and chelerythrine, in addition to several other modes of action, may also selectively block one or more PI3K isoforms as part of their anti-inflammatory activities.

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