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RESEARCH ARTICLE

In Silico Approach for Identification of PI3K/mTOR Dual Inhibitors for Multiple Myeloma Treatment

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ABSTRACT

Objective: Multiple myeloma is a hematologic malignancy in which targeting phosphoinositide 3 kinase (PI3K) and/or the mammalian target of rapamycin (mTOR) individually has been shown to have anti-proliferative effects, however, inhibiting both proteins simultaneously has been reported to have more effective results for its treatment. The aim of this study is to determine the molecular interactions and predicted inhibitory effects of 40 different dual inhibitors on mTOR, PI3Kδ, and PI3Kγ to propose potentially the most effective dual inhibitor that targets the PI3Kδ and PI3Kγ isoforms as well as the mTOR proteins since those isoforms are known to be predominant in multiple myeloma patients. Therefore, the focus in this study is built around the specific targeting of the PI3Kδ and PI3Kγ isoforms from the multiple myeloma perspective.

Materials and Methods: *In silico* docking experiments were conducted to determine the binding energies for different ligands that target mTOR, PI3Kδ, and PI3Kγ. Protein-dual inhibitor complexes and the amino acids and bond types were visualized to identify molecular interactions. The absorption, distribution, metabolism, and excretion properties of dual inhibitors were analyzed and evaluated.

Results: The binding affinity values were found to be between -7 and -9.9 kcal/mol. The toxicity prediction values of the selected dual inhibitors were obtained from the Pro-Tox-II web tool and classified according to the globally harmonized system of classification of labeling of chemicals.

Conclusion: Correspondingly, among all dual inhibitors, Vistusertib is determined to be a promising compound against multiple myeloma cells by inhibiting both PI3Kδ and PI3Kγ as well as mTORC1/2.

Keywords: *In silico* search, docking, dual inhibition, PI3K/mTOR pathway

INTRODUCTION

Phosphatidylinositol 3-kinases (PI3Ks) are a type of lipid kinases which are responsible for the regulation of various cellular activities. The PI3K pathway is important in cancer proliferation and one of the most promising therapeutic targets due to its activities over other downstream effectors such as Akt serine-threonine kinase (Akt) and mTOR (mammalian target of rapamycin).[1] Multiple PI3K isoforms are found, which can be classified into three groups based on structural similarity, substrate selectivity, and regulatory mechanism. Class I PI3Ks are the isoforms which significantly contributes to driving oncogenesis and are split into two classes as receptor tyrosine kinases activated Class IAs and GPCR activated Class IBs.[2]

mTORC1 and mTORC2 are two multiprotein mTOR complexes made up of separate proteins and partners. Abnormal activation of the mTOR signaling pathway has been commonly observed in many cancers. As a result, it's gotten a lot of attention as a potential target for oncology drug discovery.

Targeting both PI3K and mTOR has been the most common approach to dual inhibition, by taking advantage of structural similarities between the catalytic site of mTOR and ATP-binding domain of p110.[2] The PI3K/Akt/mTOR is one of the hub pathways in multiple myeloma (MM) because it is abnormally activated in a significant portion of MM patients. mTOR is an Akt downstream target that is important in MM progression, proliferation and gene and protein synthesis. Because the PI3K/Akt/mTOR pathway is a large signaling network and engaged with other pathways, inhibiting an upstream element such as Akt or mTOR may not be sufficient to inhibit downstream effectors.[3,4] Targeting two critical sites of the same pathway can result in more efficacy, overcome feedback inhibition caused by blocking mTOR activity, and reduce the possibility of the generation of chemoresistance that would emerge if

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only one p110 isoform was targeted. Indeed, dual PI3K/mTOR inhibitors have performed better than targeting PI3K isoform inhibitors, all PI3K isoform inhibitors, and mTOR inhibitors in the preclinical context.[5]

MM is a heterogeneous disease that complicates the diagnosis and detection of the molecular origin of the disease. In MM progression, naive B cells, before they get fully developed into plasma cells, proliferate in an uncontrolled manner and overproduce antibodies.[6,7] Although the mortality of the disease has decreased with newer strategies, more effective treatments are still needed in order to reach higher survival rates.[8]

Proteasome inhibitors have been used to treat cancer for over 20 years. Bortezomib (Velcade, PS-341) is a proteasome inhibitor that was approved first for multiple myeloma treatment which advanced quickly from bench to initial approval in 2003 It is approved for the use as a first-line treatment as well as in patients who have relapsed or are resistant to prior therapies. Bortezomib causes immunogenic stress and cell death in multiple myeloma cells; which is the mechanism of its therapeutic efficacy.[9] Although Bortezomib was a significant advancement in the treatment of multiple myeloma, about 20% of individuals have primary resistance, and a lot of patients relapsed after using it alone or in combination, which results in a lack of response to treatment.[10–12] Using data from prior research, the combination therapy of Bortezomib has been demonstrated to be promising. In the combined treatments of Bortezomib, Gedatolisib, Omipalisib and Dactolisib, Panulisib and Vistusertib have been investigated with very promising results in preclinical leukemia models, including acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).[12–15]

There is a continuously growing need to discover novel dual PI3K/mTOR inhibitors to be developed into therapeutic possibilities for cancer treatment. The demand for new dual inhibitors of PI3K/mTOR to be converted into therapeutic options for cancer therapy is constantly growing.

The objective in this study is to search for the most potent suitable dual inhibitor that would target mTOR and PI3K isoforms PI3Kδ, and PI3Kγ since those two isoforms have been detected in multiple myeloma patients. Although there are generic dual inhibitors against PI3K and mTOR, the main goal here is to potentially identify the most effective inhibitor that could be proposed to use for multiple myeloma treatment.

This study is organized into two main parts: theoretical and *in silico* experimental to achieve potentially the most promising candidate to be proposed for therapeutic purposes. The theoretical section gives a quick rundown of the most recent scientific results. The second alf, the experimental component, is devoted to a holistic view and comparative analysis of the aforesaid dual inhibitors in PI3K/mTOR inhibitors for multiple myeloma treatment utilizing *in silico* methodologies starting with docking. In addition, *in silico* the absorption, distribution, metabolism, and excretion (ADME) parameters, physicochemical descriptors, lipophilicity, solubility, pharmacokinetic properties, drug-like nature, and suitability for medicinal chemistry were examined using the SwissADME online tool to predict dual inhibitor compatibility and behavior, while toxicity was investigated using the ProTox II web server. These preliminary results, which provide pharmacological information, are indicative of designing and developing new treatment agents. Acute toxicity, cytotoxicity, hepatotoxicity, mutagenicity, immunotoxicity, carcinogenicity, adverse outcomes pathways, and toxicity targets of the candidate chemicals were predicted with Pro-Tox-II according to molecular similarity, fragment propensities, pharmacophores, and machine-learning models. The PyRx software was used for insertion experiments for the target proteins PI3Kδ and PI3Kγ, and mTOR, whereas Discovery Studio (Accelrys, San Diego, CA), a powerful simulation tool, was employed for imaging. The hypothesis in the project had the possibility to be successfully analyzed in numerous ways with the help of these tools.[16]

MATERIALS AND METHODS

Compound Selection and Preparation

The identified compounds were downloaded in SDF format for 40 dual inhibitors with 3-dimensional structure as well as their CAS numbers in the PubChem database. All of the compounds were used as dual inhibitors and had a 3D structure. 40 dual inhibitors that target PI3K delta and gamma isoforms and mTOR were selected according to literature (Table 1).[12] ArgusLab was used to remove bound ligands and water molecules, insert hydrogen atoms, merge non-polar hydrogens, and Gasteiger charges were included to prepare the structure of the protein for docking.[17]

Docking Studies

Molecular docking was conducted to calculate the ligand library's binding energy in PI3K isoform proteins and mTOR. 3D macromolecular structures of the mammalian target of rapamycin (mTOR) (PDB ID: 4JT6), phosphoinositide 3 kinase delta (PI3Kδ) (PDB ID: 4GB9) and phosphoinositide 3 kinase gamma (PI3Kγ) (PDB ID: 6C1S) were obtained from Protein Data Bank (www.pdb.org) which is an online database used to search for molecule structures as pdb format. In the protein preparation step, all crystallographic water molecules are removed unless they are known to be tightly bound to the protein.[18] *In silico* docking calculations were performed with the PyRx (PyRx-Python Prescription 0.8) software's AutoDock Vina option which is open-source software for computer-aided drug design. Energy minimization was performed for the loaded proteins, which is essential for determining the appropriate molecular arrangement in space. Initially, the active sites to which the proteins will bind to the ligand were determined. A 25-angstrom grid box was defined for the X, Y, and Z dimen-

Table 1. PIK3/mTOR dual pathway.

Ligand	Target	Ligand	Target
Dactolisib	PI3K (α, β, γ + δ) + mTOR	TORKinib	mTOR
Omipalisib	PI3K (α, β, γ + δ) + mTOR	Copanlisib	PI3K (α, δ)
Duvelisib	PI3K (δ, γ)	PQR-530	PI3K (α, β, γ + δ) + mTOR
MK-2206	AKT 1,2+3	Bimiralisib	PI3K (α, β, γ + δ) + mTOR
Miransertib	AKT 1,2+3	Onatasertib	PI3K α + mTOR
Pictilisib	PI3K (α, β, γ + δ)	Gdc 0084	PI3K (α, β, γ + δ) + mTOR
Pilaralisib	PI3K (α, β + δ)	GSK-2636771	PI3Kβ
PKI-402	PI3K α + mTOR	PF-04691502	PI3K (α, β, γ + δ)
Gedatolisib	PI3K (α + γ) + mTOR	AZD 8186	PI3Kβ
Vistusertib	PI3K (α, β, γ + δ) + mTOR	Uprosertib	AKT 1,2+3
Taselisib	PI3K (α, γ + δ)	PKI 179	PI3K (α, β, γ + δ) + mTOR
Alpelisib	PI3K α	SN-32976	PI3K (α, β, γ + δ) + mTOR
Panulisib	PI3K +mTOR	PWT-33597	PI3K α + mTOR
BGT-226	PI3K (α, β, γ + δ) + mTOR	Buparlisib	PI3K (α, β, γ + δ)
Fimepinostat	PI3K (α, β, δ)	VS-5584	PI3K (α, β, γ + δ) + mTOR
Apitolisib	PI3K (α, β, γ + δ) + mTOR	Sapanisertib	PI3K (α, γ + δ) + m TOR
Ipatasertib	AKT 1,2+3	Voxtalisib	PI3K (mostly γ) + mTOR
PI-103	PI3K (α, β, γ + δ) + m TOR	GNE-477	PI3Kα + mTOR
LY 294002	PI3K (α, β + δ)	Capivasertib	AKT 1,2+3
Samatolisib	class I PI3K isoforms + mTOR	PF-04979064	PI3K (α, γ + δ) + m TOR

sions. The refined low-energy structures of the proteins were coupled with each ligand. The binding affinities of the proteins were then calculated. Significant identifiers and relevant pharmaceutical properties were predicted for the compounds.

In silico ADME and Toxicity Screening Predictions

A drug candidate must be measurably effective in order to be approved. The candidate molecule has to have an adequate concentration when it reaches its target in the body and stays there actively so that it can actually do the work it is assigned to. The approach to the development of the drug involves ADME. The molecules were analyzed and evaluated with the SwissADME web tool according to their physicochemical properties, pharmacokinetics, lipophilicity, water-solubility, drug-likeness, and medicinal chemistry.[19] The prediction of the toxicity of the compounds is performed in the Pro-Tox-II web tool. Pro-Tox-II offers data on molecular similarity, fragment propensities, frequently observed features, and machine learning, based on a total of 33 models, for the prediction of various toxicity endpoints, including acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways, and toxicity targets. Toxicity classes are classified in 6 levels, all defined by a globally accepted system of classification of labeling of chemicals (GHS).[20]

Visualizing Protein-Ligand Complexes

mTOR (PDB ID: 4JT6), PI3Kδ (PDB ID: 4GB9), PI3Kγ (PDB ID: 6C1S) proteins and ligand (Dactolisib, Gedatolisib, Panulisib, Pki-179, Omipalisib, Vistusertib) complexes were visualized with Discovery Studio. As an outcome of the docking combination of three proteins and six ligands, a total of 18 samples were accessible in Discovery Studio. The 2D Show of each of the 18 samples revealed amino acid interactions between the protein structures and ligands.

RESULTS

Docking Calculations

A library of 40 dual inhibitors was docked with selected PI3Kδ, PI3Kγ and mTOR proteins and their binding affinities with one another were obtained in order to select the best possible drug candidate in a way where previous literature is conducted upon.[21] The first 16 ligands with binding values of -7 kcal/mol and lower (down to -9.9) were selected, while significant interactions were found to ensure the highest binding affinity and efficiency. This selection adjusted the number of ligands used for further analysis. The results of the molecular docking experiments were represented as binding affinities of the evaluated

Table 2. Ligand-protein binding energies of selected 16 ligand molecules and 3 identified target proteins.

Ligand	Binding Affinity with PI3Kγ (kcal/mol)	Binding Affinity with PI3Kδ (kcal/mol)	Binding Affinity with mTOR (kcal/mol)
Dactolisib	-9.9	-9.8	-9.5
Omipalisib	-9.4	-7.7	-9.7
Duvelisib	-8.5	-7.7	-9.1
MK-2206	-8.6	-7.5	-8.6
Miransertib	-8.3	-8.9	-9.2
Pictilisib	-8.3	-7	-8.9
Pilaralisib	-8.4	-8.1	-9.2
PKI-402	-8.8	-7.5	-9.5
Gedatolisib	-8.3	-8	-9.9
Vistusertib	-8.4	-7.7	-9.3
Taselisib	-8.2	-7.2	-8.8
PKI-179	-8.8	-7.6	-9.7
Panulisib	-9.8	-9.4	-9.1
BGT-226	-8.8	-7.8	-8.9
Fimepinostat	-8.7	-7.4	-8.3
Apitolisib	-8.5	-7.3	-9.3

ligands on mTOR, PI3Kδ, and PI3Kγ, and are listed in Table 2 Results reported as mean square deviation (RMSD) lower bound and upper bound values were used to compare displacement and conformational changes, based on results with the highest conformation (0 Angstrom). Also, aspirin, which is not known to be a compound that targets PI3Kδ, PI3Kγ or mTOR, was selected as a negative control and molecular docking results have been presented in Table 1.

In silico ADME and Toxicity Screening Predictions

The canonical SMILES formats of the formerly selected 16 dual inhibitors, according to the results of the dockings, were listed on the online tool of SwissADME. *In silico* physicochemical properties and predictions have to be checked in order for a drug to be investigated efficiently. Properties such as molecular weight, number of heavy atoms, Fraction Csp3, number of aromatic heavy atoms, number of rotatable bonds, number of H-bond acceptors, number of H-bond donors, molar refractivity, and TPSA (Topological Polar Surface Area), and lipophilicity provide information of log Po/w values and help to determine the access of a potential drug. Water solubility, the pharmacokinetics of the compounds according to GI absorption, BBB permanent, P-gp substrate, CYP inhibitors (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), log Kp (skin permeation) were analyzed whiledrug-likeness was scored with respect to Lipinski, Ghose, Veber, Egan, Muegge filters* and the bioavailability score of the compound (Tables 3 and 4). Medicinal chemistry properties such as lead-likeness and synthetic accessibility are also checked to investigate how difficult it is for drug candidates' molecular fragments to be obtained with also the relationship of the molecules' synthesis taken into consideration. The Egan BOILED-Egg (Brain or IntestinaL EstimateD) permeation predictive model diagram is used for the visualization of the 16 dual inhibitors as in vivo prediction as well as passive human gastrointestinal absorption, blood-brain barrier permeation, and the presence or absence of P-glycoprotein parameters are checked (Figure 1). The molecules represented in the BOILED-Egg model predicted that 10 of the selected compounds can be passively absorbed by the gastrointestinal tract while 6 of the further selected molecules are not expected to be absorbed by the gastrointestinal tract or permeated through the blood brain barrier (Figure 1).[22]

The elimination of the dual inhibitors was conducted with respect to the drug-likeness filters. There are several expert criteria that are used in drug design for how "drug-like" a substance is with respect to factors like bioavailability, such as Lipinski, Ghose, Veber, and Egan rules, etc. While 5 filters

Table 3. Selected physicochemical and pharmacokinetic properties of the analyzed compounds.

	Chemical-Physical Properties								
Compound	**Heavy atoms**	**Aromatic heavy atoms**	**Fraction Csp3**	**Rotatable bonds**	**H-bond acceptors**	**H-bond donors**	**MW (g/mol)**	**MR**	**TPSA**
Gedatolisib	45	18	0.47	10	8	2	615.73	182.07	128.29
Omipalisib	36	28	0.04	6	9	1	505.5	129.52	115.34
Dactolisib	36	29	0.13	3	4	0	469.54	143.88	76.5
Panulisib	39	25	0.19	4	9	1	527.5	138.46	134.49
Vistusertib	34	16	0.44	5	6	1	462.54	136.83	92.71
PKI-179	36	18	0.4	7	7	2	488.54	140.73	117.63
Compound	**Pharmacokinetic**								
	GI	**BBB**	**P-gp substrate**	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**CYP3A4**	**Log Kp (cm/s)**
Gedatolisib	High	No	Yes	No	No	Yes	Yes	Yes	-8.34
Omipalisib	Low	No	No	No	No	Yes	Yes	Yes	-7.03
Dactolisib	High	No	No	No	Yes	Yes	No	No	-5.43
Panulisib	Low	No	Yes	No	No	Yes	No	No	-6.87
Vistusertib	High	No	Yes	No	No	Yes	Yes	Yes	-7.15
PKI-179	High	No	Yes	No	No	Yes	Yes	Yes	-7.92

Table 4. Drug-likeness predictions of the analyzed compounds.

	Drug-likeness					
Compound	**Lipinski # violations**	**Ghose # violations**	**Veber # violations**	**Egan # violations**	**Muegge # violations**	**Bioavailability score**
Gedatolisib	2	3	0	0	1	0.17
Omipalisib	1	2	0	1	0	0.55
Dactolisib	0	2	0	1	1	0.55
Panulisib	1	3	0	2	0	0.55
Vistusertib	0	1	0	0	0	0.55
PKI-179	1	2	0	0	0	0.55
	Medicinal Chemistry					
Compound	**PAINS**	**Brenk**	**Leadlikeness**	**Synthetic Accessibility**		
Gedatolisib	0	0	2	4.65		
Omipalisib	0	0	1	3.57		
Dactolisib	0	0	2	3.39		
Panulisib	0	1	2	3.93		
Vistusertib	0	0	1	4.4		
PKI-179	0	0	1	4.95		

were identified according to the values of molecular weight, Log P, number of H-bond donors, and number of H-bond acceptors, the ones that violated the rules could not serve as a great candidate. Therefore, while deciding on the most proper dual inhibitor for this study, the number of violations of the Lipinski, Ghose, Veber, Egan, and Muegge rules are evaluated as well as bioavailability scores and synthetic accessibility are decided to be chosen as parameters. After the dual inhibitors' ADME results were obtained and analyzed, 6 dual inhibitors were found to be the most effective among the 16, which are Omipalisib, Vistusertib, Panulisib, Gedatolisib, PKI-179 and Dactolisib (Figure 2). After ADME *in vivo* predictions, the toxicity of the possible drugs was analyzed. Toxicity estimates, shown as inactive (green) and/or active (red), also help decide which dual inhibitor would be most beneficial to use and classify drug candidates according to lethal doses. The predicted LD50 values, the predicted toxicity classes, average similarity, and prediction accuracy of the dual inhibitors were evaluated as well as the radar chart that analyzes the probabilities for activity. From the data, it was obtained that Omipalisib and Panulisib were predicted to be active for hepatotoxicity, immunotoxicity, hepatotoxicity, carcinogenicity, and cytotoxicity respectively. For this reason, the candidate dual inhibitor number decreased to 4. While deciding on one dual inhibitor only, the data obtained both from ADME and toxicity were evaluated in combination, and Vistusertib was decided to be the most promising for the inhibition of PI3Kδ, PI3Kγ, and mTOR complexes in multiple myeloma treatment.

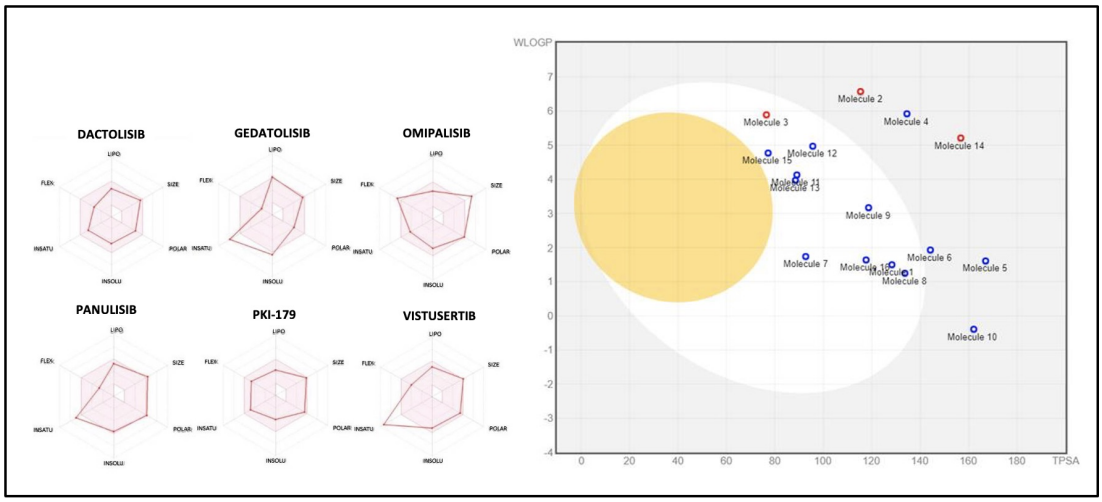

Figure 1. (A) Bioavailability radar (pink area exhibits optimal range of particular property) for studied compounds [LIPO lipophilicity as in XLOGP3; SIZE indicates size as molecular weight; POLAR means polarity as TPSA (topological polar surface area); INSOLU is insolubility in water by log S scale; INSATU means insaturation as per fraction of carbons in the sp3 hybridization and FLEX indicates the flexibility as per rotatable bonds]. (B) Egan-BOILED-Egg model of the candidate dual inhibitors. The molecules represented as circles located in the yellow region (yolk) is the ones to be expected to passively permeated through blood-brain barrier (BBB), on the other hand, other molecules within the white region are the molecules predicted to be passively absorbed by the gastrointestinal tract (HIA). [To estimate the toxicity of the candidate compounds, the Pro-Tox-II web server is used, which classifies the chemicals according to the LD50 (the median lethal dose) values [mg/kg], Class I being the fatal dose (LD50 ≤ 5) and Class VI being the non-toxic (LD50 > 5000). By selecting additional models to predict, Organ toxicity (Hepatotoxicity), Toxicity endpoints (Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity), Tox21 Nuclear receptor signaling pathways (Aryl hydrocarbon Receptor (AhR), Androgen Receptor (AR), Androgen Receptor Ligand Binding Domain (AR-LBD), Aromatase, Estrogen Receptor Alpha (ER), Estrogen Receptor Ligand Binding Domain (ER-LBD), Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)) and Tox21 Stress response pathways (Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE), Heat shock factor response element (HSE), Mitochondrial Membrane Potential (MMP), Phosphoprotein (Tumor Suppressor) p53, ATPase family AAA domain-containing protein 5 (ATAD5)) can be further computed.

Amino Acid Interactions Between Indicated Protein-Ligand Complexes

Ligand-protein complexes were categorized according to their amino acid interactions. Hydrogen bonds count, interactions in the amino acids with the ligands, hydrophobic amino acids that include the interactions in the complexes were determined. Discovery Studio 2D shows that Vistusertib is positioned at 2423 of the TYR, with a single hydrogen bond only to the 4JT6 protein in Supplementary Table 2. Omipalisib ligand appears to be located at LYS 890, LYS 756, SER 806, and VAL 882 with 4 hydrogen bonds to the 4GB9 protein and 2 hydrogen bonds to the 6C1S protein at LYS 833 and TYR 867, as shown in Table 2 As seen, the Dactolisib ligand appears to be positioned at TYR 1776, with a single hydrogen bond to the 4JT6 protein. The Panulisib ligand has been observed to be located in GLN 2499 and ILE 2498 with 2 hydrogen bonds to 4JT6 protein, 2 hydrogen bonds to 4GB9 protein in TYR 867, and LYS 833, 2 hydrogen bonds to 6C1S protein in TYR 867 and ASP 964 It has been observed that the Gedatolisib ligand is located in GLN 1901, GLU 1799, and ARG 1905 with 3 hydrogen bonds to the 4JT6 protein, in LYS 890 with a single hydrogen bond to the 4GB9 protein, and in ALA 805 with a single hydrogen bond to the 6C1S protein. PKI-179 possesses four hydrogen bonds in its ligand to the 4GB9 protein in LYS 890, MET 804, LYS 833 and ASP 964, and three hydrogen bonds to the 6C1S protein in ASP 950, ASP 964, and LYS 890 The Discovery Studio's visualization tool showed any amino acid residues implicated in hydrophobic interactions in addition to the formation of hydrogen bonds between ligands and proteins (Figure 3). Table 3 lists the amino acid residues that reacted with the ligand.

DISCUSSION

Many cancers, including different types of leukemia, lymphoma, and multiple myeloma, are known to have dysregulation of the PI3K/Akt signaling pathway. Overactivation of PI3K/Akt results in chemoresistance and poor outcomes, whereas knocking down PI3K or Akt results in cancer cell death. As a result, the PI3K/Akt pathway has been regarded as a promising candidate to be used in cancer treatment since its inhibition was shown to trigger apoptosis in MM cells. Therefore, many PI3K/Akt signaling pathway inhibitors, such as CAL-101, NVP-BKM120, and Perifosine, have been developed and tried as treatment agents for MM and are in the ongoing clinical trials. However, there still is a high demand for new PI3K inhibitors to be developed with more potent and efficient effects.[23]

The PI3K/Akt/mTOR pathway is a key regulator of various cancer cell activities including their survival, proliferation and drug resistance. The route is especially critical for lymphoma cells, and blocking it with drugs has demonstrated to be beneficial to patients with various lymphoproliferative neoplasms.[12] Many intracellular and extracellular myeloma

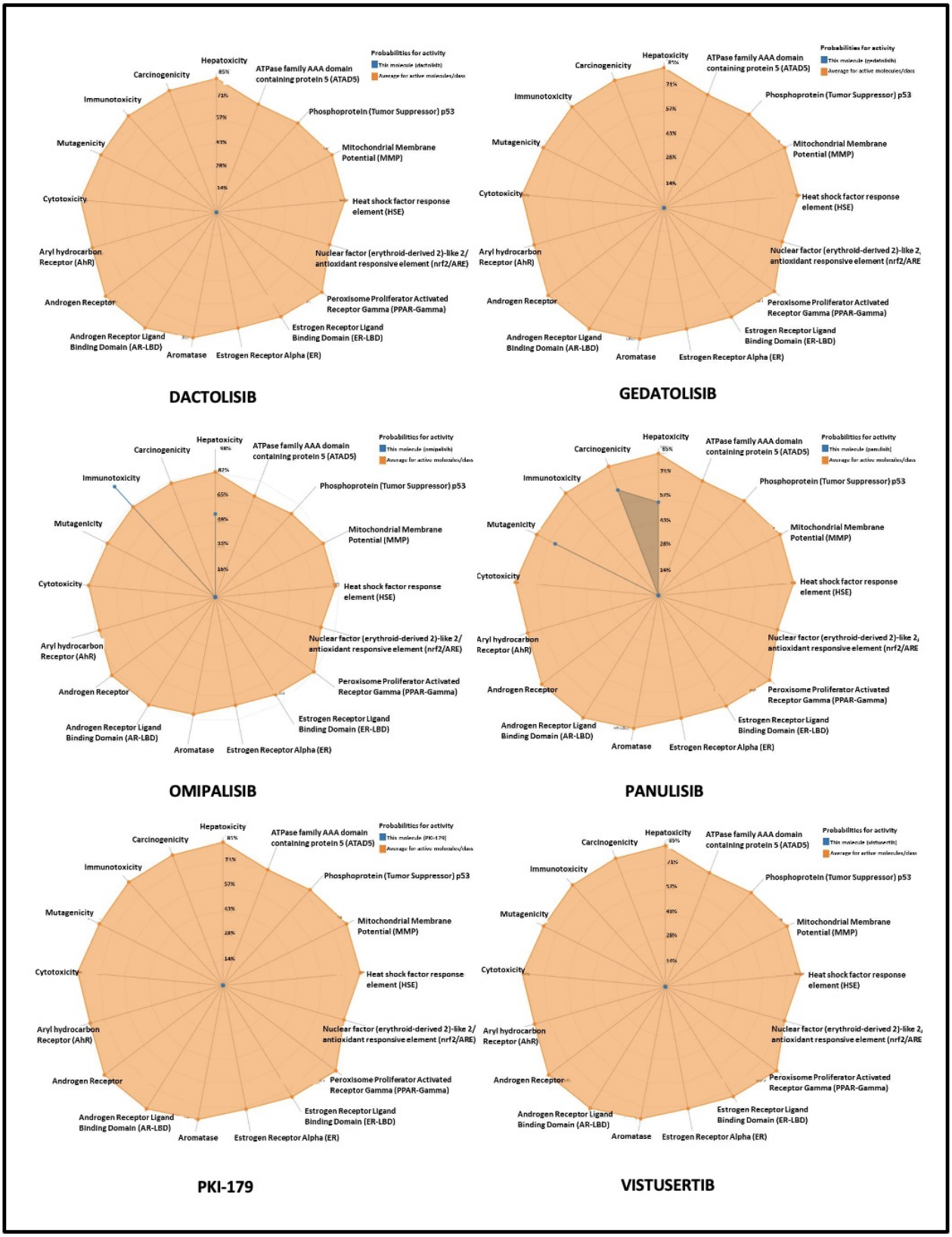

Figure 2. ProTox radar plots for selected 6 compounds. ProTox-II classification is represented in the scheme; as orange dots/lines show the average probability of the compound's active class, acquired by computing from the trained model.

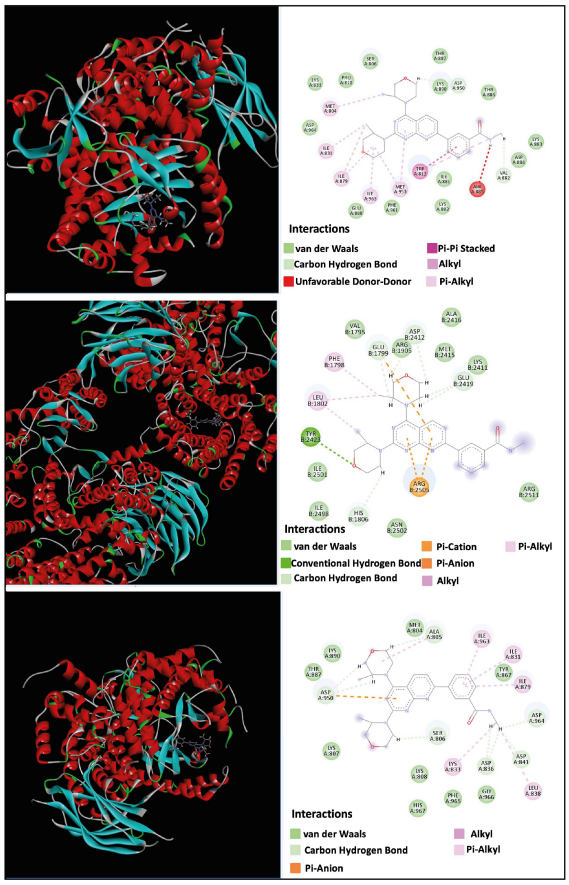

Figure 3. 2D representation of intermolecular interactions depicted using Discovery Studio Visualizer (A) 4GB9 protein with Vistusertib, (B) 4JT6 protein with Vistusertib, (C) 6C1S protein with Vistusertib. Dashed lines represent the different interactions and line color represents the interaction type. Amino acid residue numbers are shown in colored circles with their three letter code.

growth cytokines activate the PI3K/Akt pathway, concluding the probability that inhibiting PI3K will improve anti myeloma effects even further.[24]

Among the different classes of PI3K, Class I PI3Ks are the only ones shown to be correlated with cancer, and no confirmed study was found that shows the involvement of Class II PI3Ks or Class III PI3K (Vsp34p) in cancer progression. Class I PI3K is a dimeric formed enzyme, together with one catalytic and one regulatory subunit. The catalytic subunit is formed and can be found in four different isoforms designated as p110α, p110β, p110γ, and p110δ. It is thought to be related to the fact that PIP2 is used to generate PIP3, and PIP3 is a molecule that is shown to have a role in cell growth and cell replication, which only Class I PI3K can achieve. This molecule has the ability to confer tumorigenic capacity to lipid kinase. p110γ and p110δ are leukocyte-specific, and their genetic inactivation leads to defective immune responses. Constant overexpression of p110δ has been detected in acute myeloblastic leukemia, and p110δ inhibitors prevent the proliferation of leukemic cells, suggesting the role of p110δ as an oncoprotein. Also in different studies, increased expression levels of p110γ were shown in chronic myeloid leukemia.[25]

The involvement of p110δ and p110γ in hematological malignancies and cellular signaling has been shown to be important especially for studying the inhibitors targeting these two isoforms simultaneously, which turned to be a success and led to clinical trials for B- and T-cell lymphomas. In a study, p110γ inhibition was shown to inhibit myeloid cell migration into the tumor area which results in decreased malignancy through being able to target tumor microenvironment. Another study pointed that inhibition of p110δ suppressed tumor progression by interfering regulatory T-cell mediated immune response. These findings indicate novel approaches for targeting p110δ or p110γ selectively could be discussed as new treatment options in cancer.[26] In the light of these studies, it is thought that inhibiting both isoforms PI3Kδ and PI3Kγ, as well as mTOR complexes, might lead to a significant inactivation of the tumor cells' irrepressible proliferation. This is why, in this study, a dual inhibitor that targets these proteins is decided to be used against multiple myeloma cell lines.

PI3K activation is induced by IL-6 and many other cytokines and growth factors, which activates Akt and consequently mTOR. Akt signaling activation is assumed to be responsible for MM cell survival and proliferation. mTOR activation in MM has a reducing effect on apoptosis in myeloma cells.[24] Thus, it can be deduced that both PI3K and mTOR are suitable targets in MM. Here, we describe the prominent inhibitors when describing PI3K/mTOR dual inhibitors for multiple myeloma treatment.[27]

Molecular docking data of the 40 best-known mTOR inhibitors was defined by extensive binding interactions within the same active site in research targeting mTOR inhibitors for the treatment of breast cancer, and SF1126 was identified as the best protein-ligand complexes in this study. The docking score of this chosen inhibitor was -8,705 kcal/mol, and the free binding energy was found to be -36.926.[28]

In another study, the PI3K/Akt/mTOR axis was targeted with selected Olea europaea phenolic compounds in PIK3CA mutant colorectal cancer. Luteolin, which gave the best results, was found to have a binding energy of -9.4 kcal/mol to the PI3K protein, -8.1 kcal/mol to the Akt protein, and -8.8 kcal/mol to the mTOR protein.[29]

In a study conducted on the COVID-19 disease, Atranorin was the ligand with the best binding affinity (-7.0 kcal/mol) among other ligands, while the binding energies in our research range from -7 to -9.9 kcal/mol.[30]

In a study conducted with *in silico* drug screening analysis in the literature evaluated the selected final compounds using the ProTox-2 server depending on the toxicity of candidates' compounds in order to assess their drug-like characteristics.

The selected molecules were subjected to various toxicity elements. Researchers checked parameters of hepatotoxicity, cytotoxicity, mutagenicity, and Oral LD50 value. Predicted toxicity classes were obtained as Class IV, which is also the same for the compounds we analyzed.[21]

Dactolisib (BEZ235) is a dual pan-PI3K/mTOR inhibitor that is known to have oral activity against MM in animal models.[14] It was found to induce cell cycle arrest and death in HER2-overexpressed, PIK3CA-mutated, or other PI3K pathway mutations in breast cancer cells as well. Dactolisib has recently completed approximately 10 clinical trials in which it was combined with trastuzumab, everolimus, paclitaxel, and newer agents such as MEK162 and Buparlisib.[9,31]

Both *in vitro* and *in vivo*, Gedatolisib (PKI-587/PF-05212384) has shown promising outcomes in breast cancer tissues. Lower activity on phosphorylation of p70S6K and 4EBP1 residues in Akt and mTOR showed a link between tumor growth inhibition and PI3K pathway signaling inhibition.[9] When PI3K/mTOR signal inhibitors were observed, Gedatolisib was investigated in acute lymphoblastic leukemia and resulted in long-term animal survival and almost extinction, and thus, its use in other advanced cancer patients is being discussed.[32] In the light of these data, the ADME results indicate that Gedatolisib violated more rules than the other compounds, and the bioavailability score is lower than the other 5 inhibitors as well, which is why it was not a suitable candidate for MM. PKI-179 was structurally studied previously using computational approaches, and the inhibitory mechanism of an active PI3K/mTOR dual inhibitor was explored by checking its binding modes to PI3Kγ and mTOR.[33] The *in vitro* effects of dual inhibitor Omipalisib (GSK458) and its inhibition on PI3K/Akt/mTOR pathway were investigated in chemotherapy resistant and sensitive Burkitt lymphoma (BL) cell line models. Inhibition of PI3K and mTOR by the dual inhibitor Omipalisib suppressed the activation of the PI3K/Akt/mTOR pathway, leading to disruption of BL cell proliferation by induction of G1 cell cycle arrest and altering apoptosis in chemotherapy-resistant cell line models of Burkitt lymphoma.[34] Panulisib was unsuitable for use in leukemia models due to insufficient data.

Vistusertib was found to be effective in diffuse large B-cell lymphoma patients after the phase 1/2 trials when used with Acalabrutinib (Bruton tyrosine kinase inhibitor). Studies on the TMD8 tumor model also show that the combination of Acalabrutinib and Vistusertib succeeds in promoting tumor regression.[35] Considering studies using Vistusertib in combination, a study with the non-allosteric mTORC1/2 inhibitor Vistusertib showed that a combination therapy of Vistusertib and paclitaxel resulted in a significant regression in tumor growth and an elevated level of apoptosis.[36] Also, Vistusertib previously showed synergistic effects with some inhibitors in acute myeloid leukemia cells.[13]

Summing up, based on previous combination studies, Vistusertib provides tumor regression, has a short half-life, and is in phase II trials for lymphoma models. Although Vistusertib has not been used for the treatment of blood cancer on its own up to date, *in silico* modeling and testing the physicochemical nature of the dual inhibitor with tools like ADME and Pro-Tox-II indicate that it is an important candidate for inhibiting myeloma cell growth, believed to be able to fight multiple myeloma cells in the long run.

In silico prediction and toxicity analysis studies have shown that certain parameters need to be achieved for an agent to be proposed for *in vitro* tests. The four parameters that should be correlated with solubility and permeability are molecular weight, Log P, number of H-bond donors, and number of H-bond acceptors. The cutoff values for these parameters were close to 5, leading to a simple mnemonic called the "rule of 5".[37] The "rule of 5" regarding solubility and permeability poor absorption or permeability is more likely if: more than 5 H bond donors, MWT above 500, Log P above 5 (or MLogP above 4.15), There are more than 10 H-bond acceptors. Substrates are the exception to this rule.[37] Based on these pharmacokinetic scores and analyses, this study revealed the suitability of Vistusertib to be tested on multiple myeloma cells based on its selective efficiency on PI3K isoforms and mTOR.

All in all, PI3K/mTOR is an important pathway for hematological malignancies that are being studied to develop new therapeutic approaches. The negative consequences of this pathway are high toxicity rates observed in solid tumors, low clinical activity levels, and the numerous unknown targets for inhibition. Based on these results, dual PI3K/mTOR inhibitors are valued, which are part of ongoing research and make it possible to avoid toxicity. This study revealed the binding properties and molecular interactions of ranked 6 dual inhibitors with PI3Kδ, PI3Kγ, and mTOR using molecular docking analyses. Potent inhibitors that are specific and have lower toxicity can be optimized further based on the docking results. Recent information on Vistusertib *in silico* action is promising, and clinical trial needs are accumulating. More translational research into its action and toxicity is expected to lead to the development and clinical success.

CONCLUSION

In conclusion, the PI3K/Akt/mTOR pathway plays a significant role in cancer cell activities, including survival, proliferation, and drug resistance. The Class I PI3K isoforms, p110δ and p110γ, have been found to be involved in hematological malignancies, cellular signaling, and tumor progression, making them a target of interest for selective inhibition. The inhibition of PI3K and mTOR has shown promising results in the treatment of MM. Several inhibitors have been developed, and their binding interactions with the target proteins have been studied

using molecular docking. The use of dual inhibitors that target both PI3K and mTOR could lead to a significant inactivation of tumor cells' irrepressible proliferation. Future studies could evaluate selected final compounds using the ProTox-2 server, depending on the toxicity of candidate compounds, to assess their drug-like characteristics.

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REFERENCES

1. Wang L, Lin N, Li Y. The PI3K/AKT signaling pathway regulates ABCG2 expression and confers resistance to chemotherapy in human multiple myeloma. *Oncol Rep.* 2019;41:1678-1690.
2. McKenna M, McGarrigle S, Pidgeon GP. The next generation of PI3K-Akt-mTOR pathway inhibitors in breast cancer cohorts. *Biochim Biophys Acta Rev Cancer.* 2018;1870:185-197.
3. John L, Krauth MT, Podar K, Raab MS. Pathway-directed therapy in multiple myeloma. *Cancers.* 2021;13(7):1668.
4. Ramakrishnan V, Kumar S. PI3K/AKT/mTOR pathway in multiple myeloma: from basic biology to clinical promise. *Leuk Lymphoma.* 2018;59(11):2524-2534.
5. Tarantelli C, Lupia A, Stathis A, Bertoni F. Is there a role for dual PI3K/mTOR inhibitors for patients affected with lymphoma? *Int J Mol Sci.* 2020;21(3):1060. doi: 10.3390/ijms21031060.
6. Jourdan M, Caraux A, De Vos J, et al. An in vitro model of differentiation of memory B cells into plasmablasts and plasma cells including detailed phenotypic and molecular characterization. *Blood.* 2009;114(25):5173-5181.
7. Corre J, Munshi NC, Avet-Loiseau H. Risk factors in multiple myeloma: is it time for a revision?*Blood.* 2021;137(1):16-19.
8. Clinical pathways to address the challenges of treatment resistance and relapse in multiple myeloma. *J Clin Pathw.* Accessed March 16, 2023. https://www.journalofclinicalpathways.com/white-paper/clinical-pathways-address-challenges-treatment-resistance-and-relapse-multiple
9. Zitvogel L, Kroemer G. Bortezomib induces immunogenic cell death in multiple myeloma. *Blood Cancer Discov.* 2021;2(4):405-407.
10. Robak P, Szemraj J, Mikulski D, Drozdz I, Robak T. Prognostic value of resistance proteins in plasma cells from multiple myeloma patients treated with bortezomib. *J Clin Med.* 2021;10(21):5028. doi:10.3390/jcm10215028
11. Teicher BA, Tomaszewski JE. Proteasome inhibitors. *Biochem Pharmacol.* 2015;96:1-9.
12. Painuly U, Kumar S. Efficacy of bortezomib as first-line treatment for patients with multiple myeloma. *Clin Med Insights Oncol.* 2013;7:53-73.
13. Mologni L, Marzaro G, Redaelli S, Zambon A. Dual kinase targeting in leukemia. *Cancers.* 2021;13. doi:10.3390/cancers13010119.
14. Abramson HN. Kinase inhibitors as potential agents in the treatment of multiple myeloma. *Oncotarget.* 2016;7:81926-81968.
15. Musiol R. An overview of quinoline as a privileged scaffold in cancer drug discovery. *Expert Opin Drug Discov.* 2017;12:583-597.
16. BIOVIA Discovery Studio - BIOVIA - Dassault Systèmes®. Dassault Systèmes. Accessed July 18, 2022.
17. Thompson MA. molecular docking using ArgusLab, an efficient shape-based search algorithm and the a score scoring function. ACS meeting, Philadelphia. Scientific Research Publishing. Accessed July 18, 2022.
18. Wong SE, Lightstone FC. Accounting for water molecules in drug design. *Expert Opin Drug Discov.* 2011;6:65-74.
19. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 2017;7:42717. doi:10.1038/srep42717
20. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res.* 2018;46:W257-W263.
21. Fareed MM, El-Esawi MA, El-Ballat EM, et al. *In silico* drug screening analysis against the overexpression of PGAM1 gene in different cancer treatments. *Biomed Res Int.* 2021;2021:5515692. doi: 10.1155/2021/5515692
22. Bojarska J, Remko M, Breza M, et al. A supramolecular approach to structure-based design with a focus on synthons hierarchy in ornithine-derived ligands: review, synthesis, experimental and *in silico studies.Molecules.* 2020;25(5):1135. doi: 10.3390/molecules25051135
23. Han K, Xu X, Chen G, et al. Identification of a promising PI3K inhibitor for the treatment of multiple myeloma through the structural optimization. *J Hematol Oncol.* 2014;7:9. doi: 10.1186/1756-8722-7-9
24. Harvey RD, Lonial S. PI3 kinase/AKT pathway as a therapeutic target in multiple myeloma. *Future Oncol.* 2007;3(6):639–647.
25. Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. *Oncogene.* 2008;27(41):5486–5496.
26. Thorpe LM, Yuzugullu H, Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer.* 2015;15(1):7–24.
27. Baumann P, Schneider L, Mandl-Weber S, Oduncu F, Schmidmaier R. Simultaneous targeting of PI3K and mTOR with NVP-BGT226 is highly effective in multiple myeloma. *Anticancer Drugs.* 2012;23(2):131–138.
28. Patidar K, Panwar U, Vuree S, et al. An *in silico* approach to identify high affinity small molecule targeting m-TOR inhibitors for the clinical treatment of breast cancer. *Asian Pac J Cancer Prev.* 2019;20(4):1229–1241.

29. Sain A, Kandasamy T, Naskar D. *In silico* approach to target PI3K/Akt/mTOR axis by selected Olea europaea phenols in PIK3CA mutant colorectal cancer. *J Biomol Struct Dyn.* 2021:1–16.
30. Putra PP, Abdullah SS, Rahmatunisa R, Junaidin J, Ismed F. Structure, activity, and drug-likeness of pure compounds of Sumatran lichen (Stereocaulon halei) for the targeted ACE2 protein in COVID-19 disease. *Pharmaciana.* 2020;10(2):135–146.
31. Schult C, Dahlhaus M, Glass A, et al. The dual kinase inhibitor NVP-BEZ235 in combination with cytotoxic drugs exerts antiproliferative activity towards acute lymphoblastic leukemia cells. *Anticancer Res.* 2012;32:463–474.
32. Tasian SK, Teachey DT, Li Y, et al. Potent efficacy of combined PI3K/mTOR and JAK or ABL inhibition in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood.* 2017;129:177–187.
33. Mohd Rehan. A structural insight into the inhibitory mechanism of an orally active PI3K/mTOR dual inhibitor, PKI-179 using computational approaches. *J Mol Graph Model.* 2015;62:226–234.
34. Ippolito T, Tang G, Mavis C, Gu JJ, Hernandez-Ilizaliturri FJ, Barth MJ. Omipalisib (GSK458), a novel Pan-PI3K/mTOR inhibitor, exhibits in vitro anti-lymphoma activity in chemotherapy-sensitive and -resistant models of burkitt lymphoma. *Blood.* 2016;128:5376. doi: 10.1182/blood.V128.22.5376.5376
35. Collins GP, Clevenger TN, Burke KA, et al. A phase 1/2 study of the combination of acalabrutinib and vistusertib in patients with relapsed/refractory B-cell malignancies. *Leuk Lymphoma.* 2021;62:2625–2636.
36. Rinne N, Christie EL, Ardasheva A, et al. Targeting the PI3K/AKT/mTOR pathway in epithelial ovarian cancer, therapeutic treatment options for platinum-resistant ovarian cancer. *Cancer Drug Resist.* 2021;4:573–595.
37. Lipinski CA, Lombardo ational approaches to estimate solubility and permeability in drug discovery and development settings1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery *Reviews 23 (1997) 3–25.1. Adv Drug Deliv Rev.* 2001;46:3–26.

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