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Computational Identification of Azole Derivatives Targeting the mTOR Rapamycin Binding Domain for Therapeutic Development

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Abstract
This study investigates the potential of eight azole-derived compounds (KR1–KR8) as inhibitors of the mammalian target of rapamycin (mTOR), a critical protein involved in regulating autophagy and associated with diseases such as cancer, obesity, and aging. Using in silico molecular docking, we evaluated the interaction of these compounds with the rapamycin binding (FRB) domain of mTOR. Drug-likeness assessments revealed that all compounds adhered to Lipinski's rule, indicating favorable oral bioavailability, with optimal values for molecular weight, LogP, and total polar surface area (TPSA). In addition, the compounds demonstrated low toxicity profiles and high absorption potential, with minimal interactions with cytochrome P450 enzymes, suggesting a favorable pharmacokinetic profile. Among the tested compounds, KR4 showed the most promising results, exhibiting a strong binding affinity to mTOR with a docking score of -7.61 kcal/mol. KR4 specifically interacted with key residues of the FRB domain (LEU-2031, SER-2035, PHE-2039, TRP-2101, TYR-2105, and PHE-2108), closely resembling the binding mode of rapamycin. Further Prime MM-GBSA analysis confirmed KR4's stable binding within the mTOR binding site, with a predicted binding energy of -3.00 kcal/mol. These findings suggest that KR4 holds significant potential as a lead compound for developing selective mTOR inhibitors, which could provide new avenues for targeted therapies in cancer and other mTOR-related diseases.

Key words: Autophagy, Ligand, Receptor, mTOR, Binding energy.

1. Introduction

Autophagy, a cellular mechanism found across eukaryotic organisms, plays a crucial role in maintaining cellular balance by breaking down macromolecules and organelles through the lysosomal pathway. It safeguards cells, aiding in their survival during starvation or when deprived of growth factors (Lu et al., 2005). Additionally, it contributes to both innate and adaptive immune responses. Cells generally employ two main degradation pathways: proteasome-mediated degradation, handling short-lived proteins, and autophagy, activated during nutrient scarcity and cellular stress, responsible for breaking down long-lived proteins, protein clusters, and entire organelles. Autophagy induction due to starvation has been observed in various eukaryotic organisms, spanning fungi, plants, slime mold, nematodes, fruit flies, mice, rats, and humans (Levine & Klionsky, 2004). In essence, autophagy is a self-degrading process crucial for survival, differentiation, development, disease management, cell death, and maintaining equilibrium, existing widely among eukaryotes from yeast to humans (Reggiori & Klionsky, 2002, Aman et al 2021). Many cytotoxic drugs prompt cell proliferation control through necrotic cell death, causing heightened breakdown of cell components, potentially impacting normal cells negatively. However, drugs capable of inducing autophagy in proliferating cells not only facilitate safe cell elimination but also trigger an anti-inflammatory gene response in phagocytes (Yamauchi, Izumi, Yamamoto, & Nomori, 2014). Consequently, the quest to develop pharmacologically effective agents from natural products with reduced toxicity and fewer side effects has become a focal point in drug research.

The mTOR pathway is crucial in both cancer development and regular cell functions like growth, survival, and specialization. It stands out as a prime target for designing therapeutic drugs due to its frequent dysregulation in cancer, making it a central focus for potential treatments. mTOR, a serine/threonine protein kinase related to PI3K, governs various cellular functions such as cytoskeleton arrangement, metabolism, cell survival, proliferation, and autophagy. It acts as a pivotal player at the core of multiple cellular signaling pathways, integrating growth factor-triggered responses and nutrient-sensing pathways (Chiarini et al., 2015). It binds to various regulatory subunits, forming two distinct complexes: mTORC1 and mTORC2. Dysregulation of both upstream and downstream elements of mTORC1 and mTORC2 is implicated in aging, autoimmune disorders, neurodegenerative diseases, diabetes, obesity, and cancer (Cornu, Albert, & Hall, 2013). The negative feedback exerted by mTORC1 on PI3K and mTORC2 underscores the potential for targeting mTOR in drug development across various diseases (Saxton & Sabatini, 2017).

Azole rings have a reputation for being efficient against cancer (Mahmoud et al., 2020; Ahmad et al., 2018), bacteria (Al-Hussain et al., 2021; Muhammad et al., 2021), fungi and numerous incurable diseases (Ahmad et al., 2018). It has

been demonstrated that heterocycles have an azole pharmacophore, which is a preferred structure in both medicinal chemistry and the pharmaceutical sector (Kaur et al., 2015; Khan et al., 2016). This area of research has provided opportunities for biologists and chemists to design novel entities with improved efficacy through chemical transformations and development of new moieties with broad spectrum therapeutic implications on the basis of existing synthetic approaches and the anticancer properties of such heterocycles (Ahmad et al., 2018). An appreciable fraction (approximately 50%) of globally approved anticancer medications stems from either natural products or their derivatives, often crafted based on existing knowledge of small or macromolecules. The noteworthy efficacy of several azole derivatives in the realm of anticancer exploration has priorly surfaced (Jabir et al., 2016).

Computational methods have consistently played a crucial role in expediting drug discovery by streamlining the identification of promising drug targets. Structural bioinformatics investigations, such as molecular protein–ligand docking, offer highly accurate forecasts regarding potential drug targets for lead or drug-like molecules (Lokesh et al., 2020). Ligand-protein interaction studies facilitate the prediction of the binding mode between a known 3-dimensional structure of a ligand and protein (Cross et al., 2009). These proteins possess active sites that become functional upon encountering external compounds. Consequently, understanding the binding orientation of drug candidates to their protein targets aids in predicting the affinity and activity of these small molecules through molecular docking. Hence, docking assumes a critical role in the strategic development of drugs. Molecular docking primarily aims to achieve a relative orientation of both the protein and ligand, optimizing their confirmation to minimize the free energy of the entire system (Mohanty, 2023).

We proposed conducting *in silico* studies, molecular docking analyses and MM-GBSA analysis on pivotal signaling proteins implicated in regulating autophagy, specifically mTOR. These pathway inhibitors are currently undergoing clinical assessment and development for treating a range of conditions, including cancer, obesity, and aging. Our focus is on screening eight newly identified azole-derived compounds against these three primary target proteins. The objective of this study is to identify an azole-based compound that can be tailored as a potent inhibitor for mTOR protein, potentially serving as an effective drug to modulate autophagy.

2. Materials and Methods

2.1. Hardware and Software used

The computational analyses were conducted using a PC running Windows 8.1 Ultimate, equipped with an Intel Core i5 microprocessor, 2 GB memory, and a 64-Bit operating system. For accessing biological data, we utilized databases like the Protein Data Bank (PDB) and Chemspider. Additionally, online tools such as Swiss ADME, admetSAR, ProTox-II and CASTp were employed. Various software packages like Autodock 4.2, Chemdraw Ultra 6.0, Open Babel, Prime 4.11.2, PyMOL 2.3.1 and Discovery Studio Visualizer 3.5 were utilized throughout the study.

2.2. Preparation of Ligand

The azole derivative compounds (KR1- KR8) used in the present study were obtained from the previous study in Immuno Pharmacology Laboratory, CMST, MSU. The 2D structure of these eight ligand molecules was crafted using Chemdraw Ultra 6, while the native ligands' structures were obtained from the Chemspider database in SDF format. To prepare for docking experiments, the ligand molecules were converted from SDF to PDB format using Open Babel. Subsequently, these structures were visualized using Discovery Studio to facilitate the docking studies.

2.3. Drug-likness of the ligands

The molecular properties of the eight azole compounds in our study were assessed using the online tool SwissADME (http://www.swissadme.ch/index.php). These properties were screened based on Lipinski's rule of five (Lipinski, 2000), which helps gauge oral bioavailability issues in molecules that violate more than one of these rules. Parameters such as molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, molar refractivity, topological polar surface area (TPSA), and the partition coefficient between n-octanol and water (log Po/w) were calculated utilizing the Swiss ADME tool (Daina, Michielin, & Zoete, 2017).

2.4. *In silico* Pharmacokinetic Studies (Analysis of ligand)

The pharmacokinetic profiles encompassing Absorption, Distribution, Metabolism, Excretion, and Toxicity of the compounds were assessed using the admetSAR online server (http://www.admetexp.org). This platform employs web-based query tools equipped with a built-in molecular interface, allowing queries within the database via SMILES (simplified molecular-input line-entry system) and structural similarity searches. It provides up-to-date and meticulously curated data on various chemicals associated with known ADMET profiles (admetSAR@LMMD) (F. Cheng et al., 2012).

2.5. Retrieval of Protein structure and preparation

The target structure for mTOR (PDB ID: 4DRI) were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) and visualized using Discovery Studio Visualizer 3.5. These proteins were

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resolved using the X-ray diffraction method, yielding resolution factor of 1.45 Å, with corresponding R values of 0.177, respectively. Upon obtaining the proteins in PDB format, preprocessing involved removing the native ligand, unwanted chains, and crystalline water from the structure to prepare them for subsequent docking studies.

2.6. Active Site prediction

Using the Computed Atlas of Surface Topography of proteins (CASTp) server (http://cast.engr.uic.edu), we identified the active sites within the target proteins. CASTp allowed us to analyze the area and volume of each pocket, examining cavity characteristics in both Richard's Solvent Accessible Surface and Connolly's Molecular Surface. This analysis detects feasible pockets within the protein structure (Dundas et al., 2006). Among these, the primary pocket predicted by CASTp was selected as the biologically most promising active site for our docking studies.

2.7. Prediction of Toxicity

The ProTox-II server, accessible at https://tox-new.charite.de/protox_II/, was utilized to forecast toxicity parameters (Banerjee, Eckert, Schrey, & Preissner, 2018). Canonical SMILES representations of the ligands were employed as input for the ProTox-II server (Drwal, Banerjee, Dunkel, Wettig, & Preissner, 2014).

2.8. *In silico* molecular docking

We conducted molecular docking using Autodock 4.2 software (Morris et al., 2009) with the Lamarckian Genetic Algorithm. This algorithm integrates energy assessment through grids of affinity potential, pinpointing the optimal binding position for a ligand within a specific protein. Initially, polar hydrogen atoms were added to the protein targets, and Kollman united atomic charges were computed. Subsequently, hydrogen atoms were added to the ligands, Gastiger partial charges were assigned, and bond orders were validated. Grid points were established along the X, Y, and Z axes, and a grid box was appropriately positioned within the target, ensuring the inclusion of the active residue at the center. The default docking algorithms were set according to the standard docking protocol. Subsequently, a hundred independent docking runs were executed for each ligand, resulting in binding energies. These outcomes were ranked based on increasing docking energies, and the representative result within each cluster was selected based on the lowest binding energy (Morris et al., 2009).

2.9. Protein- Ligand Interaction Visualization

The results generated in protein ligand complex were visualized via Accelrys Discovery Studio visualizer 3 (SYSTÈMES, 2016). The analysis focused on understanding the interactions in relation to the minimum binding energy (Kcal/mol), Inhibition constant (Ki) value (μM), and the count of hydrogen bonds and stacking interactions that occurred between the active site residues of the macromolecule and the ligand. These parameters shed light on the strength of binding and the nature of interactions within the protein-ligand complex. The docked complex was then superimposed on to the reference co-crystallized complex using PyMOL 2.3.1.

2.10. Prime Molecular mechanics-generalized born surface area MM-GBSA

The relative binding-free energy (G bind) of compound KR4 with mTOR was determined using the prime MMGBSA method (Prime Version 4.11.2).
The G bind was calculated according to the formula:
$\Delta G(bind) = \Delta G(solv) + \Delta E(MM) + \Delta G(SA)$
where:

- The solvation energy of the unliganded mTOR- KR4 complex in the GBSA as compared to the total of the solvation energies of the unliganded mTOR and inhibitor KR4 is known as ΔGsolv.
- ΔEMM is the difference between the minimised energies of the unliganded mTOR- KR4 complex and the total of the energies of the unliganded mTOR and inhibitor KR4.
- ΔGSA is the difference between the surface area energies of the unliganded mTOR- KR4 complex and the sum of the surface areas of the unliganded mTOR and inhibitor KR4 when they are not liganded.

The energy of optimal free receptors, free ligands, and ligand-receptor complexes are all calculated by prime MM-GBSA. The ligand is also placed in a solution that was generated automatically by the VSGB 2.0 suit, and the ligand strain energy is calculated. An energy visualisation was shown using the prime energy visualizer.

3. Result and Discussion

Azole compounds represent nitrogen-containing heterocyclic structures, possessing unique electron-rich aromatic characteristics. These nitrogen-based heterocycles serve as crucial structural elements in various bioactive compounds (Vennila et al., 2019). Azole derivatives find extensive applications in medicinal, chemical, supramolecular, agricultural, and material sciences, primarily due to their capacity for weak interactions (Cannalire et al., 2013). Through noncovalent interactions, azoles readily engage with enzymes and receptors in medicinal biology, showcasing

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promising biological activities (Zhan et al., 2011). In this study, the structure of eight azole-derived compounds, obtained from ChemDraw, was designated as KR1-KR8 (Figure: 1). The receptor mTOR retrieved from RCSB-PDB were minimized by removing unwanted chains and HETATOMS using Discovery Studio (Figure: 2). Molecular docking analyses were conducted *in silico* on these key signaling protein involved in autophagy modulation mTOR. These pathway inhibitor are currently undergoing clinical evaluations for the treatment of conditions such as cancer, obesity, and aging. The utilization of eight novel azole-derived compounds aimed to screen their interactions with these three primary target proteins. Recent years have witnessed a surge in the development of mTOR pathway inhibitors, many of which are undergoing clinical trials (Dienstmann et al., 2014). The multifaceted approach is gaining traction due to concerns about side effects, off-target effects, and the efficacy of new drugs. Our aim was to demonstrate the binding mode with mTOR kinases through molecular docking studies conducted with our synthesized azole derivatives.

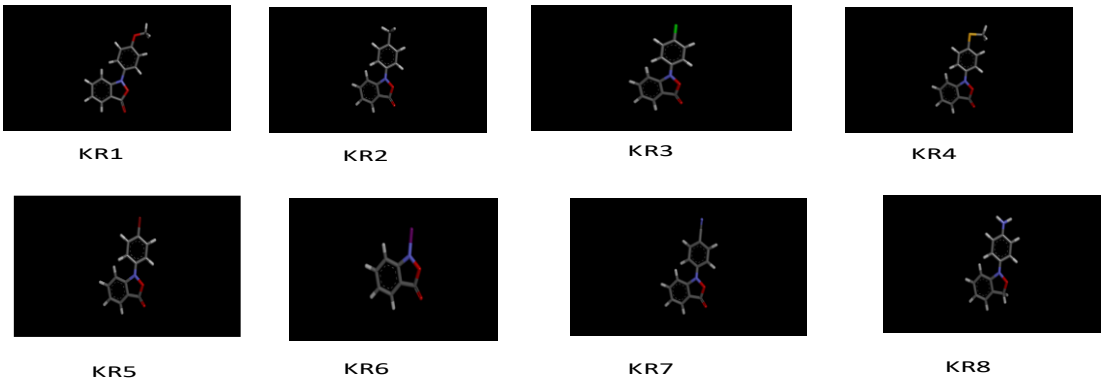

Figure 1: Structure of azole derivative compounds

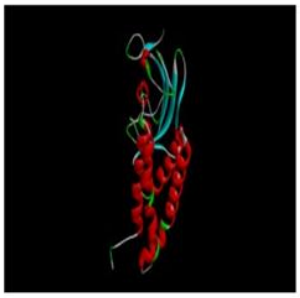

Figure 2: The three-dimensional structure of selected target proteins

3.1. Drug- likeness of the ligand

The SwissADME tool was utilized to calculate the molecular characteristics of selected compounds. These compounds, listed in Table 1, underwent scrutiny for drug suitability based on Lipinski's filter, assessing eight molecular descriptors. The criteria encompassed molecular weight ≤ 500 Dalton, Log P ≤ 5, H-bond acceptors ≤ 10, H-bond donors ≤ 5, and Molar Refractivity between 40 and 130 (Vennila et al., 2019; Yasmin et al., 2017). Notably, all compounds examined in this study exhibited high potential for optimal oral bioavailability. Their calculated LogP and TPSA values adhered to Lipinski's rule of five (Cheng, 2004). Intriguingly, the compounds in this study fell within the molecular weight range of 212-290 (<500). Molecules with a molecular weight under 500 Daltons tend to be more easily transported, diffused, and absorbed by membranes compared to heavier molecules (Ahmed, 2019; Srimai et al., 2013). Moreover, these compounds satisfied the Lipinski rule by maintaining fewer than 10 hydrogen bond acceptors (O and N atoms) and fewer than 5 hydrogen bond donors (NH and OH). Their LogP values, essential for permeability analysis, ranged between 2.54 and 4.05 (<5), falling within an acceptable limit for membrane penetration in drug delivery. Total Polar Surface Area (TPSA), calculated from oxygen and nitrogen atoms and their attached hydrogen atoms, is closely linked to a compound's hydrogen bonding capability (Clark, 1999; Paramashivam et al., 2015). In this study, all tested compounds exhibited absorption rates between 35% and 60%, indicating favorable oral bioavailability. Their

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bioavailability score was deemed good due to possessing fewer than 10 rotatable bonds and a TPSA of less than 140 Å (Veber et al., 2002). Remarkably, all compounds investigated in this study adhered to Lipinski's rule, signaling their favorable drug-like properties.

Table 1 : Druglikness property of the test compounds as deduced from Swiss ADME

Properties	Compound1	Compound2	Compound3	Compound4	Compound5	Compound6	Compound7	Compound8
Formula	C14H11NO3	C14H11NO2	C13H8C1NO2	C14H11NO2S	C13H8BrNO2	C7H4INO2	C14H8N2O2	C13H12N2O
Molecular Weight	241.24g/mol	225.24g/mol	245.66g/mol	257.31g/mol	290.11g/mol	261.02g/mol	236.23g/mol	212.25g/mol
Num.heavy atoms	18	17	17	18	17	11	18	16
Num.rotatable bonds	2	1	1	2	1	0	1	1
Num.H-bond acceptors	3	2	2	2	2	2	3	1
Num.H-bond donors	0	0	0	0	0	0	0	1
Molar Refractivity	68.30	66.78	66.82	73.53	69.51	49.89	66.53	67.36
TPSA	44.37Å	35.14Å	35.14Å	60.44Å	35.14Å	35.14Å	58.93Å	38.49Å
Log $P_{o/w}$ (XLOG P3)	3.39	3.79	4.05	3.93	4.11	2.59	3.14	2.54
Log S(ESOL)	-3.96	-4.21	-4.50	-4.40	-4.81	-3.70	-3.83	-3.25
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes;
Bio availabiliy Score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

3.2 In silico pharmacokinetic studies

The ADMET properties of the compounds were assessed using admetSAR, following the method outlined by Cheng et al. in 2012. Key factors like Blood-Brain Barrier (BBB) penetration, Human Intestinal Absorption (HIA), Caco-2 cell permeability, and AMES data were computed and compiled in Table 2. Predictive values indicated that all tested compounds possessed the ability to penetrate the BBB and be absorbed by the human intestine. With the exception of compound KR6, all compounds demonstrated penetration into Caco-2 cells. Understanding whether the tested compounds act as substrates for P-glycoprotein (P-gp) is crucial, as P-gp is responsible for effluxing drugs and various compounds, potentially impacting their therapeutic efficacy due to lower than anticipated drug concentrations (Amin, 2013; Levin, 2012). Interestingly, in our study, none of the tested compounds were confirmed as substrates for P-gp, suggesting a favorable profile in this regard. The cytochrome P450 superfamily, particularly isoforms such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, plays a pivotal role in drug metabolism and clearance within the liver (Vasanthanathan et al., 2009). Interaction with these isoforms can lead to metabolic biotransformation and subsequent drug elimination (Krämer & Testa, 2009). Inhibiting certain cytochrome P450 isoforms may result in drug-drug interactions where co-administered drugs fail to metabolize, potentially accumulating to toxic levels (Lynch & Price, 2007, Zhao et al 2021). Notably, a few of the tested compounds displayed inhibition of specific cytochrome P450 isoforms, indicating the possibility of such interactions. Additionally, the majority of the compounds did not exhibit acute toxicity or mutagenic effects based on the AMES test data, which is promising in terms of safety assessment.

Table 2: ADMET prediction by using admetSAR

Absorbtion								
Properties	**Compound 1**	**Compound 2**	**Compound 3**	**Compound 4**	**Compound 5**	**Compound 6**	**Compound 7**	**Compound 8**
BBB	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
HIA	Positive	Positive	Positive	Positive	Posoitive	Positive	Positive	Positive
Caco-2	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive
P-gp substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
P-gp inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor
ROC transporter	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Distribution and Metabolism								
CYP450 substrate								
CYP450	Non-	Non-	Non-	Non-	Non-	Non-	Non-	Non-

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2C9	substrate	substrate	substrate	substrate	substrate	substrate	substrate	substrate
CYP450 2D6	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4	Substrate	Substrate	Substrate	Substrate	Substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 inhibitor								
CYP450 1A2	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
CYP450 2C9	Inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor
CYP450 2D6	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Inhibitor	Inhibitor
CYP450 3A4	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP IP(IP)#	High	High	High	High	High	Low	High	High
Excretion and Toxicity								
HERG inhibitor	Weak	Weak	Weak	Weak	Weak	Weak	Weak	Weak
Inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
AMES toxicity	toxic	Non- toxic	Non -toxic	Non - toxic	Non- toxic	Non - toxic	toxic	toxic
Carcinogen	Noncarcinogens	Noncarcinogens	Noncarcinogens	Noncarcinogens	Noncarcinogens	Noncarcinogens	Noncarcinogens	Noncarcinogens
Fish toxicity	High	High	High	High	High	High	High	High
T.P toxicity	High	High	High	High	High	High	High	High
H.B toxicity	Low	Low	Low	Low	Low	High	Low	Low
Biodegradation	NRB	NRB	NRB	NRB	NRB	NRB	NRB	NRB
Acute Oral Toxicity	Category 3	Category 3	Category 3	Category 3	Category 3	Category 3	Category 3	Category 3

Key: BBB: Blood Brain Barrier, HIA: Human Intestinal Absorption, P-gp: P Glycoprotein, ROC: Renal Organic Cation, IP: inhibitory promiscuity, HERG: Human Ether-a-go-go- Related Gene, TP: Tetrahymena Pyriformis, NRB: Non ready Biodegradable H.B: Honey Bee

3.3. Prediction of Toxicity

The assessment of toxicity holds significant importance in the selection of compounds for drug development (P. Mondal et al., 2020). Utilizing the Protox-II server, we examined the toxic properties of the ligands, specifically focusing on hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity (Lounkine et al., 2012). According to Table 3, all compounds are weakly active on hepatotoxic, yet they exhibit non-cytotoxic characteristics. However, apart from KR2 and KR7, the compounds demonstrate inactivity in terms of carcinogenicity and immunogenicity. KR1, KR3, KR4, KR5, and KR8 are reported to have immunogenic effects, while KR2, KR6, and KR7 are inactive in terms of immunogenicity. One effective approach to mitigate toxicity levels in compounds involves structural modifications. Recent chemical strategies include applying structural alerts within molecules to reduce toxicity. This can be achieved through partial or complete replacement, altering electronic density, or introducing structural elements that influence metabolism, thereby reducing toxicity (Limban et al., 2018).

Table 3: Predicted toxicities for compounds

Compounds	Hepatotoxicity	Carcinogenicity	Immunogenicity	Mutagenicity	Cytotoxicity
KR1	Yes (0.69)	No (0.62)	Yes (0.96)	No (0.97)	No (0.93)
KR2	Yes (0.61)	Yes (0.66)	No (0.99)	Yes (0.55)	No (0.67)
KR3	Yes (0.69)	No (0.62)	Yes (0.96)	No (0.97)	No (0.93)
KR4	Yes (0.69)	No (0.62)	Yes (0.96)	No (0.97)	No (0.93)
KR5	Yes (0.69)	No (0.62)	Yes (0.96)	No (0.97)	No (0.93)
KR6	Yes (0.53)	No (0.51)	No (0.99)	No (0.61)	No (0.68)

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KR7	Yes (0.58)	Yes (0.61)	No (0.99)	Yes (0.57)	No (0.67)
KR8	Yes (0.69)	No (0.62)	Yes (0.96)	No (0.97)	No (0.93)

Probability: (Yes, active) or (No, inactive)

3.4. Active site identification

The CASTp server was utilized with optimized parameters to identify the primary active sites within the targeted receptor protein mTOR. Through CASTp calculations, distinct surface-accessible pockets and interior inaccessible cavities within these proteins were delineated. The mTOR protein, concerning 23 amino acid residues from LEU2031 to SER2112 were crucial in the composition of binding pockets (Figure: 3). The amino acid residues involved in these targeted proteins were visually highlighted using blue-colored boxes, emphasizing their significance in the binding site configurations.

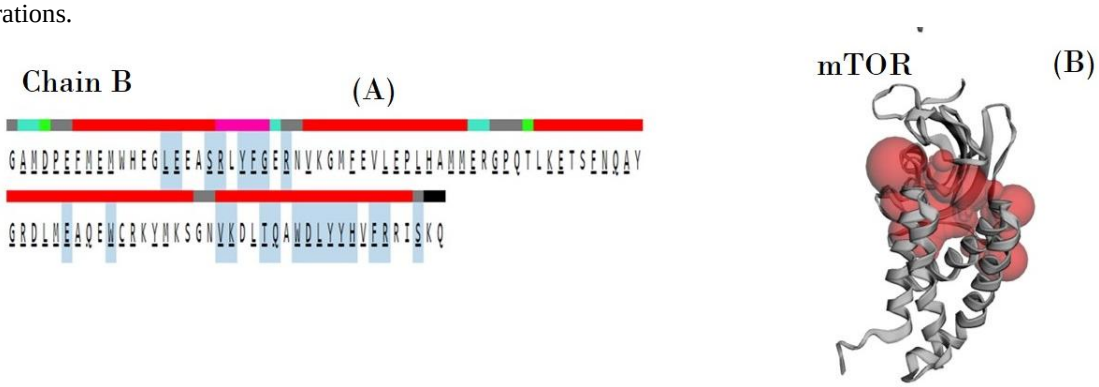

Figure 3: Binding pocket identification by CASTp server

A) Blue colour boxes highlight the amino acid residues present in the binding site.

B) Shows the binding sites of receptor

3.5. *In silico* molecular docking studies

In the realm of drug discovery, *in silico* molecular docking serves as a pivotal tool, enabling a comprehensive understanding of the interactions between a compound (ligand) and its target receptor (protein). This method, cited by Anderson in 2003, significantly expedites the identification of potential new drug candidates (Li, 2001; Patrick Walters et al., 1998). Ligand-protein interaction studies, such as those conducted by Cross et al. in 2009, allow the prediction of binding modes between known 3D structures of ligands and proteins. By examining the binding orientations of drug candidates with their protein targets, molecular docking aids in forecasting the affinity and activity of small molecules. The primary objective of molecular docking is to establish a favorable orientation between the protein and ligand, optimizing their confirmation to minimize the overall free energy of the system (Monika et al., 2010). Computational tools like AutoDock offer significant advantages by facilitating the discovery of new lead compounds more rapidly and cost-effectively (Gilbert, 2004; Warren et al., 2006). This approach streamlines the exploration of potential interactions between compounds and target proteins, offering insights crucial for drug development and design.

In Table 4, the results of the docking study was summarized, focusing on binding confirmations and energies. Analyzing the docking results highlighted that compound KR4 secured a top position with displaying favorable docking scores (-7.61) with the target mTOR. The analysis of how KR4 docks with mTOR showed that the drug attaches to the rapamycin binding site, specifically interacting with residues LEU-2031, SER-2035, PHE-2039, TRP-2101, TYR-2105, and PHE-2108 of mTOR (as depicted in Figure: 4). These six amino acids play a crucial role in mTOR's catalytic activity. While KR4 exhibited decent binding affinity and inhibitory concentration with mTOR, it was observed to be lower than that of the natural ligand rapamycin, a compound derived from a soil bacterium known to inhibit tumor growth by binding to mTOR (Pinanti et al., 2021). In our investigation, the amino acids interacting with mTOR appeared more akin to KR4 and rapamycin. When comparing the binding of KR4 with mTOR to rapamycin, all amino acids, except Glu-2032 and Tyr-2104, showed a close alignment with the interacting residues of rapamycin at the mTOR-FRB domain. Previous research indicated that carvacrol had a binding energy of -7.5 Kcal/mol with the mTOR-FRB domain, and our compound KR4 showed a similar pattern, with a binding energy of -7.61 Kcal/mol with the mTOR-FRB domain. The interacting amino acids, including Leu-2031, Ser-2035, Trp-2101, Tyr-2105, and Phe-2108 of carvacrol, overlapped with the interacting residues of KR4 with the mTOR-FRB domain (Herrera-Calderon et al., 2020). Furthermore, when comparing KR4's binding with the FRB domain to other studies, it was observed that the interacting residues Trp-2101, Tyr-2105, Phe-2108, and Phe-2039 overlapped with the interacting residues of asiaticoside at the FRB domain of mTOR (Zulkipli et al., 2020). These findings suggest that KR4 can strongly bind to the FRB domain, potentially hindering mTOR's function by employing similar binding interactions to rapamycin, thereby impeding mTOR's catalytic activity. The protein structures before and after molecular docking was then compared by

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superimposing the structures. Moreover, the superimposed binding pose of KR4 with mTOR shown in Figure: 5 are similar to those reported previously for X-ray crystallographic structure.

Table 4: The binding strengths of azole derived compound with mTOR compared with the native ligand from co-complex structures from PDB

Target	Compound	Binding Energy	Inhibitory constant	Number of hydrogen bond interacted	Residues involved in H bonding
mTOR	KR1	-6.92	8.53 µM	-	-
	KR2	-7.32	4.34 µM	1	SER2035
	KR3	-7.42	3.62 µM	1	SER2035
	KR4	-7.61	3.24 µM	1	SER2035
	KR5	-7.49	2.66 µM	1	SER2035
	KR6	-6.1	33.82 µM	-	SER2035
	KR7	-7.38	3.92 µM	1	SER2035
	KR8	-6.75	11.37 µM	1	ASP2102
	Native Ligand	-13.35	162.97 pM	1	SER2035

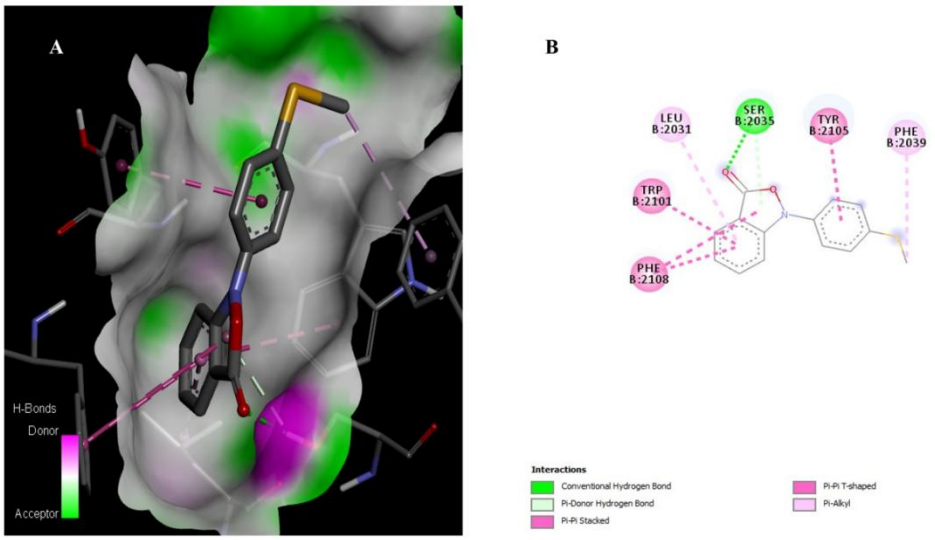

Figure 4: A) Shows the 3D diagram of the compound KR4 and mTOR interaction. B) Shows the 2D diagram of the compound KR4 and mTOR interactions.

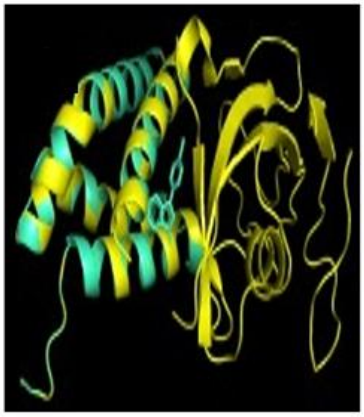

Figure: 5 mTOR's crystalline structure (shown as a yellow ribbon) and the docked mTOR-KR4 complex are superimposed (shown as blue ribbon).

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3.6. Prime MM-GBSA analysis

One effective way to pinpoint the correct binding position of a drug amidst various potential positions is by docking the molecule into the protein's binding site. This process enhances the accuracy of binding energy calculations through prime MM-GBSA analysis compared to relying solely on molecular docking energies (Pattar et al., 2020). The calculated values offer estimations of binding free energies, with lower numbers indicating stronger binding affinities. This highlights the anticipated binding energies of the ligand within the receptor's binding site. In Table 5, compound KR4 exhibited a predicted binding energy of -3.00 kcal/mol in the rapamycin binding site of mTOR. Our investigation revealed that, compound KR4 displays stronger binding tendencies toward mTOR. Hence, there's clear potential for molecule KR4 to serve as a lead compound in the development of inhibitors targeting mTOR.

Table 5: The relative binding-free energies (kcal/mol) obtained by Prime MM–GBSA

Target	ΔG_{bind} (kcal/mol)	$\Delta E_{Coulomb}$ (kcal/mol)	$\Delta E_{Covalent}$ (kcal/mol)	$\Delta E_{H\text{-}bond}$ (kcal/mol)	ΔE_{vdw} (kcal/mol)	Lipo	Sol-GB
mTOR	-3.00	5.00	-1.00	0	2.00	-1.649196436	0

4. Conclusion

In this study, we successfully explored the potential of azole-derived compounds as inhibitors of the mTOR pathway, focusing on the FRB domain, a critical site for therapeutic intervention. Through comprehensive in silico molecular docking studies, we identified key binding interactions between the azole compounds and mTOR, with compound KR4 demonstrating the strongest binding affinity. The favorable drug-likeness properties, low toxicity, and promising pharmacokinetic profiles of these compounds suggest their potential as viable candidates for further development as mTOR inhibitors. KR4, in particular, exhibited interactions with critical residues in the rapamycin binding site, similar to that of the natural inhibitor rapamycin, highlighting its ability to potentially disrupt mTOR's catalytic activity. These findings underscore the importance of azole derivatives as lead compounds in drug discovery and provide valuable insights for the design of targeted therapies for mTOR-associated diseases, including cancer, obesity, and aging. Future experimental studies are needed to validate the binding interactions and therapeutic efficacy of these compounds, paving the way for the development of novel mTOR-targeted therapies.

References

1. Ahmad, K., Khan, M. K. A., Baig, M. H., Imran, M., & Gupta, G. K. (2018). Role of azoles in cancer prevention and treatment: present and future perspectives. *Anti-Cancer Agents in Medicinal Chemistry* (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), *18*(1), 46-56.
2. Ahmed, A. H. (2019). Insilico pharmakinetics and molecular docking- studies of lead compounds derived from Diospyros mespiliformis. *Pharmatutor*, *7*(3), 31–37.
3. Al-Hussain, S. A., Alshehrei, F., Zaki, M. E., Harras, M. F., Farghaly, T. A., & Muhammad, Z. A. (2021). Fluorinated hydrazonoyl chlorides as precursors for synthesis of antimicrobial azoles. Journal of Heterocyclic *Chemistry*, *58*(2), 589-602.
4. Aman, Y., Schmauck-Medina, T., Hansen, M., Morimoto, R. I., Simon, A. K., Bjedov, I., ... & Fang, E. F. (2021). Autophagy in healthy aging and disease. *Nature aging*, *1*(8), 634-650.
5. Amin, M. L. (2013). P-glycoprotein inhibition for optimal drug delivery. *Drug Target Insights*, (7), 27–34. https://doi.org/10.4137/DTI.S12519
6. Anderson, A. C. (2003). The Process of Structure-Based Drug Design. *Chemistry & Biology*, *10*, 787–797. https://doi.org/0.1016/j .c he m bi ol . 20 03 . 09 .0 0
7. Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: A webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, *46*(W1), W257–W263. https://doi.org/10.1093/nar/gky318
8. Cannalire, R., Barreca, M. L., Manfroni, G., & Cecchetti, V. (2016). A Journey around the Medicinal Chemistry of Hepatitis C Virus Inhibitors Targeting NS4B: From Target to Preclinical Drug Candidates. *Journal of Medicinal Chemistry*, *59*(1), 16–41. https://doi.org/10.1021/acs.jmedchem.5b00825
9. Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., … Tang, Y. (2012). AdmetSAR: A comprehensive source and free tool for assessment of chemical ADMET properties. *Journal of Chemical Information and Modeling*, *52*(11), 3099–3105. https://doi.org/10.1021/ci300367a
10. Cheng, L. C. W. (2004). 2,4,6-trisubstituted pyrimidines as a new class of selective adenosine A1 receptor antagonists. *Journal of Medicinal Chemistry*, *47*(26), 6529–6540. https://doi.org/10.1021/jm049448r
11. Chiarini, F., Evangelisti, C., McCubrey, J. A., & Martelli, A. M. (2015). Current treatment strategies for inhibiting mTOR in cancer. *Trends in Pharmacological Sciences*, *36*(2), 124–135. https://doi.org/10.1016/j.tips.2014.11.004
12. Clark, D. E. (1999). Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. *Journal of Pharmaceutical Science*, *88*(8), 807–814. https://doi.org/10.1021/js9804011

13. Cornu, M., Albert, V., & Hall, M. N. (2013). MTOR in aging, metabolism, and cancer. *Current Opinion in Genetics and Development*, *23*(1), 53–62. https://doi.org/10.1016/j.gde.2012.12.005
14. Cross, J. B., Thompson, D. C., Rai, B. K., Baber, J. C., Fan, K. Y., Hu, Y., & Humblet, C. (2009). Comparison of several molecular docking programs: Pose prediction and virtual screening accuracy. *Journal of Chemical Information and Modeling*, *49*(6), 1455–1474. https://doi.org/10.1021/ci900056c
15. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(October 2016), 1–13. https://doi.org/10.1038/srep42717
16. Dienstmann, R., Rodon, J., Serra, V., & Tabernero, J. (2014). Picking the point of inhibition: A comparative review of PI3K/AKT/mTOR pathway inhibitors. *Molecular Cancer Therapeutics*, *13*(5), 1021–1031. https://doi.org/10.1158/1535-7163.MCT-13-0639
17. Drwal, M. N., Banerjee, P., Dunkel, M., Wettig, M. R., & Preissner, R. (2014). ProTox: A web server for the insilico prediction of rodent oral toxicity. *Nucleic Acids Research*, *42*(W1), 53–58. https://doi.org/10.1093/nar/gku401
18. Dundas, J., Ouyang, Z., Tseng, J., Binkowski, A., Turpaz, Y., & Liang, J. (2006). CASTp: Computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Research*, *34*(WEB. SERV. ISS.), 116–118. https://doi.org/10.1093/nar/gkl282
19. Gilbert, D. (2004). Bioinformatics software resources. *Briefings in Bioinformatics*, *5*(3), 300–304. https://doi.org/10.1093/bib/5.3.300
20. Herrera-Calderon, O., Yepes-Pérez, A. F., Quintero-Saumeth, J., Rojas-Armas, J. P., Palomino-Pacheco, M., Ortiz-Sánchez, J. M., … Andía-Ayme, V. (2020). Carvacrol: An insilico approach of a candidate drug on HER2, PI3Kα, mTOR, HER-α, PR, and EGFR receptors in the breast cancer. *Evidence-Based Complementary and Alternative Medicine*, https://doi.org/10.1155/2020/8830665
21. Jabir NR, Firoz CK, Bhushan A, Tabrez S, Kamal MA (2016). The use of Azoles Containing Natural Products in Cancer Prevention and Treatment: An Overview. Anticancer Agents *Med Chem*, *18*: 6–14. [PMID: 27198985 DOI: 10.2174/1871520616666160520112839]
22. Kaur, K., Kumar, V., & Gupta, G. K. (2015). Trifluoromethylpyrazoles as anti-inflammatory and antibacterial agents: A review. *Journal of Fluorine Chemistry*, 178, 306-326.
23. Khan, M.F.; Alam, M.M.; Verma, G.; Akhtar, W.; Akhter, M.; Shaquiquzzaman, M (2016). The therapeutic voyage of pyrazole and its analogs: A review. *Eur. J. Med. Chem*., *120*, 170-201.
24. Krämer, S. D., & Testa, B. (2009). The Biochemistry of Drug Metabolism - An Introduction. *Chemistry & Biodiversity*, *6*(7), 1144–1144. https://doi.org/10.1002/cbdv.200990008
25. Levin, G. M. (2012). P-glycoprotein: Why this drug transporter may be clinically important. *Current Psychiatry*, *11*(3), 38–40.
26. Levine, B., & Klionsky, D. J. (2004). Development by self-digestion: Molecular mechanisms and biological functions of autophagy. *Developmental Cell*, *6*(4), 463–477. https://doi.org/10.1016/S1534-5807(04)00099-1
27. Li, J. (2001). *for Discovering Leads*. *40*(2).
28. Limban, C., Nuţă, D. C., Chiriţă, C., Negreș, S., Arsene, A. L., Goumenou, M., … Sarigiannis, D. A. (2018). The use of structural alerts to avoid the toxicity of pharmaceuticals. *Toxicology Reports*, *5*, 943–953. https://doi.org/10.1016/j.toxrep.2018.08.017
29. Lipinski, C. A. (2000). Drug-like properties and the causes of poor solubility and poor permeability. *Journal of Pharmacological and Toxicological Methods*, *44*(1), 235–249. https://doi.org/10.1016/S1056-8719(00)00107-6
30. Lokesh R, Jeyakanthan J, Kannabiran K. (2020) Targeting VEGFR2 protein by marine Streptomyces globosus VITLGK011-derived compound BECA: An in vitro and in silico analysis. *J Basic Microbiol*.1–11. https://doi.org/10.1002/jobm.202000461
31. Lounkine, E., Keiser, M. J., Whitebread, S., Mikhailov, D., Hamon, J., Jenkins, J. L., … Urban, L. (2012). Large-scale prediction and testing of drug activity on side-effect targets. *Nature*, *486*(7403), 361–367. https://doi.org/10.1038/nature11159
32. Lu, Z., Dono, K., Gotoh, K., Shibata, M., Koike, M., Marubashi, S., … Monden, M. (2005). Participation of autophagy in the degeneration process of rat hepatocytes after transplantation following prolonged cold preservation. *Archives of Histology and Cytology*, *68*, pp. 71–80. https://doi.org/10.1679/aohc.68.71
33. Lynch, T., & Price, A. (2007). The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *American Family Physician*, *76*(3), 391–396.
34. Mahmoud, H. K., Farghaly, T. A., Abdulwahab, H. G., Al-Qurashi, N. T., & Shaaban, M. R. (2020). Novel 2-indolinone thiazole hybrids as sunitinib analogues: Design, synthesis, and potent VEGFR-2 inhibition with potential anti-renal cancer activity. *European Journal of Medicinal Chemistry*, *208*, 112752.
35. Mohanty, M., & Mohanty, P. S. (2023). Molecular docking in organic, inorganic, and hybrid systems: a tutorial review. *Monatshefte für Chemie-Chemical Monthly*, 1-25.
36. Mondal, P., Natesh, J., Abdul salam, A. ajees, Thiyagarajan, S., & Meeran, S. M. (2020). Traditional medicinal plants against replication, maturation and transmission targets of SARS-CoV-2: computational investigation. *Journal*

of Biomolecular Structure and Dynamics, *40*(6), 2715–2732. https://doi.org/10.1080/07391102.2020.1842246
37. Monika, G., Punam, G., Sarbjot, S., & Gupta, G. D. (2010). An overview on molecular docking. *International Journal of Drug Development & Research*, *2*(2).
38. Morris, G. M., Ruth, H., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256
39. Muhammad, Z. A., Farghaly, T. A., Althagafi, I., Al-Hussain, S. A., Zaki, M. E., & Harras, M. F. (2021). Synthesis of antimicrobial azoloazines and molecular docking for inhibiting COVID-19. *Journal of heterocyclic chemistry*, *58*(6), 1286-1301.
40. Paramashivam, S. K., Elayaperumal, K., Natarajan, B., Ramamoorthy, M., Balasubramanian, S., & Dhiraviam, K. (2015). Insilico pharmacokinetic and molecular docking studies of small molecules derived from Indigofera aspalathoides Vahl targeting receptor tyrosine kinases. *Bioinformation*, *11*(2), 73–84. https://doi.org/10.6026/97320630011073
41. Patrick Walters, W., Stahl, M. T., & Murcko, M. A. (1998). Virtual screening - An overview. *Drug Discovery Today*, *3*(4), 160–178. https://doi.org/10.1016/s1359-6446(97)01163-x
42. Pattar, S. V., Adhoni, S. A., Kamanavalli, C. M., & Kumbar, S. S. (2020). In silico molecular docking studies and MM/GBSA analysis of coumarin-carbonodithioate hybrid derivatives divulge the anticancer potential against breast cancer. Beni-Suef *University Journal of Basic and Applied Sciences*, *9*(1), 1-10.
43. Pinanti, H. N., Nafisah, W., Christina, Y. I., Widodo, W., Rifa'i, M., & Djati, M. S. (2021). Molecular docking studies of biflavonoids from Selaginella doederleinii hieron as anticancer agents to inhibit mTOR. *AIP Conference Proceedings*, *2353*(May). https://doi.org/10.1063/5.0052704
44. Reggiori, F., & Klionsky, D. J. (2002). Autophagy in the eukaryotic cell. *Eukaryotic Cell*, *1*(1), 11–21. https://doi.org/10.1128/EC.01.1.11-21.2002
45. Saxton, R. A., & Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell*, *168*(6), 960–976. https://doi.org/10.1016/j.cell.2017.02.004
46. Srimai, V., Ramesh, M., Satya Parameshwar, K., & Parthasarathy, T. (2013). Computer-aided design of selective Cytochrome P450 inhibitors and docking studies of alkyl resorcinol derivatives. *Medicinal Chemistry Research*, *22*(11), 5314–5323. https://doi.org/10.1007/s00044-013-0532-5
47. SYSTÈMES, D. (2016). *Dassault Syst mes BIOVIA, Discovery Studio Modeling Environment, Release 2017*. Dassault Syst mes. Retrieved from http://accelrys.com/products/collaborative-science/biovia-discovery-studio/
48. Vasanthanathan, P., Taboureau, O., Oostenbrink, C., Vermeulen, N. P. E., Olsen, L., & Jorgensen, F. S. (2009). Classification of cytochrome P450 1A2 inhibitors and noninhibitors by machine learning techniques. *Drug Metabolism & Disposition*, *37*(3), 658–664. https://doi.org/10.1124/dmd.108.023507
49. Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, *45*(12), 2615–2623. https://doi.org/10.1021/jm020017n
50. Vennila, K. N., Prabha, K., Sunny, D., Madhuri, S., & Elango, K. P. (2019). Preparation and biological evaluation of quinoline amines as anticancer agents and its molecular docking. *Medicinal Chemistry Research*, *28*(8), 1298–1307. https://doi.org/10.1007/s00044-019-02374-w
51. Warren, G. L., Andrews, C. W., Capelli, A. M., Clarke, B., LaLonde, J., Lambert, M. H., … Head, M. S. (2006). A critical assessment of docking programs and scoring functions. *Journal of Medicinal Chemistry*, *49*(20), 5912–5931. https://doi.org/10.1021/jm050362n
52. Yamauchi, Y., Izumi, Y., Yamamoto, J., & Nomori, H. (2014). Coadministration of erlotinib and curcumin augmentatively reduces cell viability in lung cancer cells. *Phytotherapy Research*, *28*(5), 728–735. https://doi.org/10.1002/ptr.5056
53. Yasmin, S., Mhlongo, N. N., Soliman, M. E., GR, S., & Jayaprakash, V. (2017). Comparative Design, Insilico Dockingand Predictive ADME/ TOX Properties of Some Novel 2, 4-hydroxy Derivatives of Thiazolidine-2, 4-diones as PPARÎ^3 Modulator. *Journal of Pharmaceutical Chemistry*, *4*(2), 11–19. https://doi.org/10.14805/jphchem.2017.art74
54. Zhan, P., Chen, X., Li, D., Fang, Z., De Clercq, E., & Liu, X. (2011). HIV-1 NNRTIs: structural diversity, pharmacophore similarity, and impliations for drug design. *Medicinal Research Reviews*, *1*(E1), 72. https://doi.org/10.1002/med.20241
55. Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., ... & Qin, S. (2021). Cytochrome P450 enzymes and drug metabolism in humans. *International journal of molecular sciences*, *22*(23), 12808.
56. Zulkipli, N. N., Zakaria, R., Long, I., Abdullah, S. F., Muhammad, E. F., Wahab, H. A., & Sasongko, T. H. (2020). Insilico Analyses and Cytotoxicity Study of Asiaticoside and Asiatic Acid from Malaysian Plant as Potential mTOR Inhibitors. *Molecules*, *25*(17), 1–15. https://doi.org/10.3390/molecules25173991.